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Genetic Diversity and Genetic Relationships of Japonica Rice Varieties in Northeast Asia Based on SSR Markers

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Abstract: Genetic diversity and the relationship among 9 Japonica rice groups consisting of 288 varieties in Northeast Asia with different geographical origins (Heilongjiang province, Jilin province and Liaoning province of China; Japan; Korea; Democratic People's Republic of Korea; the Russian Far East district of Russian Federation) and types (landraces and improved varieties) were evaluated with 154 SSR markers. A total of 823 alleles were detected. The observed number of alleles (*Na*) per locus, the Nei's gene diversity (*He*) and the polymorphism information content (*PIC*) ranged from 2 to 9, 0.061 to 0.869, and 0.060 to 0.856, with an average of 5.344, 0.624 and 0.586. RM1350, RM1369, RM257, RM336 and RM1374 are suitable for studying the difference on genetic diversity of rice cultivars in Northeast Asia. Analysis of molecular variance showed that the variation within group and among groups was about 8.40% and 91.60%. *He* and *PIC* of the 9 groups could be ranked in a descending order: Heilongjiang landraces, Jilin landraces, Japanese improved varieties, Heilongjiang improved varieties, Russian Far East district of Russian Federation improved varieties, Liaoning improved varieties, Jilin improved varieties, Korean improved varieties, Democratic People's Republic of Korean improved varieties. The dendrogram based on UPGMA method divided these 9 groups into 3 clusters and 288 varieties into 2 clusters. Research results showed that the level of genetic diversity in Northeast Asia is low and genetic information exchange happened frequently among the 9 groups. This study may also contribute to parent selection aiming at broaden genetic base of Japonica rice germplasm in Northeast Asia.
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Keywords: Japonica rice; genetic diversity; genetic relationships; simple sequence repeat (SSR); Northeast Asia

1. Introduction

Rice (*Oryza sativa L.*) is one of the most important crops in the world. The Northeast Asia includes the vast areas of Heilongjiang Province, Jilin Province and Liaoning Province of China; Japan; Korea; Democratic People's Republic of Korea; Mongolia and the Russian Far East district of Russian Federation (Hippel et al., 2011). In 2010, the area planted to rice in this region, except for Mongolia, reached 7.617 million hectares, accounting for 4.96% of the world record and the total production reached 48.09 million tons, accounting for 7.23% of the world production (FAO, 2010).

The study of genetic diversity is of both academic importance and useful as a guide to the effective conservation and optimal use of the vast gene resources. In recent years, simple sequence repeats (SSRs), with its advantages of allele-specific and co-dominant, have been used extensively to assess genetic diversity and the relationships of subspecies among rice cultivars (Latif et al., 2011; Liu & Zhang, 2010; Kobayashi et al., 2006; Zeng et al., 2004).

Research on genetic diversity and genetic structure of rice cultivars in Korea, Japan and China using SSR markers have been published (Zhao et al., 2009). However, researches on genetic affinity and genetic structure of japonica rice cultivars of different types from different places in Northeast Asia are rarely seen till now.

In this research, 154 SSR markers randomly located at rice chromosomes were used to analyze the genetic diversity of 288 accessions of rice from Northeast Asia to investigate the genetic diversity and the genetic affinity among different groups (landraces and improved varieties) with different geographical distributions (Heilongjiang Province, Jilin Province and Liaoning Province of China; Japan; Korea; Democratic People's Republic of Korea and the Russian Far East district of Russian Federation).

Table 1. The situation of varieties tested in this experiment.

No.	cultivars	origins	type	No.	cultivars	origins	type	No.	cultivars	origins	type
1	Jingzu	HLJ[b]	L[g]	52	Putongludao	HLJ[b]	L[g]	103	Longjing 28	HLJ[b]	IV[h]
2	Qingniante	HLJ[b]	L[g]	53	Wumangziyedao	HLJ[b]	L[g]	104	Longdao 9	HLJ[b]	IV[h]
3	Honggu	HLJ[b]	L[g]	54	Hejiang 1	HLJ[b]	IV[h]	105	Hongmao	JL[c]	L[g]
4	Longhuadahonggu	HLJ[b]	L[g]	55	Hejiang 3	HLJ[b]	IV[h]	106	Hongmaodao	JL[c]	L[g]
5	Baidadu	HLJ[b]	L[g]	56	Mudanjiang 1	HLJ[b]	IV[h]	107	Yuanzier	JL[c]	L[g]
6	Wumingzhu	HLJ[b]	L[g]	57	Hejiang 11	HLJ[b]	IV[h]	108	Huangjianguangtouluyu	JL[c]	L[g]
7	Tangyuan 6	HLJ[b]	L[g]	58	Mudanjiang 2	HLJ[b]	IV[h]	109	Dahongmaogailiangbeihai	JL[c]	L[g]
8	Wumingdao	HLJ[b]	L[g]	59	Fengchan 9	HLJ[b]	IV[h]	110	Erjie	JL[c]	L[g]
9	Hongmaozi	HLJ[b]	L[g]	60	Hejiang 18	HLJ[b]	IV[h]	111	Tainan	JL[c]	L[g]
10	Gailiangguoguang	HLJ[b]	L[g]	61	Mudanjiang 5	HLJ[b]	IV[h]	112	Dangdibeihai	JL[c]	L[g]
11	Hongniandao	HLJ[b]	L[g]	62	Mudanjiang 8	HLJ[b]	IV[h]	113	Xiangyanghongmang	JL[c]	L[g]
12	Laotoudao 1	HLJ[b]	L[g]	63	Mudanjiang 12	HLJ[b]	IV[h]	114	Luwei	JL[c]	L[g]
13	Dahuangjingzi	HLJ[b]	L[g]	64	Hejiang 17	HLJ[b]	IV[h]	115	Hongmao	JL[c]	L[g]
14	Hongmaodaozi	HLJ[b]	L[g]	65	Taiyang 3	HLJ[b]	IV[h]	116	Jinzao	JL[c]	L[g]
15	Caizhongpu	HLJ[b]	L[g]	66	Heijing 2	HLJ[b]	IV[h]	117	Hongmaodao	JL[c]	L[g]
16	Qishitianhuanjia	HLJ[b]	L[g]	67	Puxuan 10	HLJ[b]	IV[h]	118	JIngou	JL[c]	L[g]
17	Laoguangtou 83	HLJ[b]	L[g]	68	Hejing 22	HLJ[b]	IV[h]	119	Baidaduxingya	JL[c]	L[g]
18	Xiaobaijingzi	HLJ[b]	L[g]	69	Songjing 1	HLJ[b]	IV[h]	120	Laoguangtou	JL[c]	L[g]
19	Heimangdao	HLJ[b]	L[g]	70	Hejiang 23	HLJ[b]	IV[h]	121	Xiaobaimao-2	JL[c]	L[g]
20	Laotoudao	HLJ[b]	L[g]	71	Songjing 2	HLJ[b]	IV[h]	122	Hongjian	JL[c]	L[g]
21	Wuchangbaimao	HLJ[b]	L[g]	72	Dongnong 415	HLJ[b]	IV[h]	123	Yuanzinian	JL[c]	L[g]
22	Guangtounuo	HLJ[b]	L[g]	73	Longjing 3	HLJ[b]	IV[h]	124	Xiaobaipitiantai	JL[c]	L[g]
23	Panxudao	HLJ[b]	L[g]	74	Dongnong 418	HLJ[b]	IV[h]	125	Luyu 132-1	JL[c]	L[g]
24	Hongmaodaozi	HLJ[b]	L[g]	75	Mudanjiang 20	HLJ[b]	IV[h]	126	Xiaobaimao	JL[c]	L[g]
25	Daxingguo	HLJ[b]	L[g]	76	Songnian 1	HLJ[b]	IV[h]	127	changchunwumang	JL[c]	L[g]
26	Erbaimao	HLJ[b]	L[g]	77	Longjing 8	HLJ[b]	IV[h]	128	Xiaobaijingzihuadianbai	JL[c]	L[g]
27	Gonghedao	HLJ[b]	L[g]	78	Wuyoudao 1	HLJ[b]	IV[h]	129	Jilinriluo	JL[c]	L[g]
28	Dalidao	HLJ[b]	L[g]	79	Kendao 8	HLJ[b]	IV[h]	130	Songliao 1	JL[c]	IV[h]
29	Guangtou	HLJ[b]	L[g]	80	Beidao 1	HLJ[b]	IV[h]	131	Songliao 2	JL[c]	IV[h]
30	Hongfujianguoguang	HLJ[b]	L[g]	81	Longdao 1	HLJ[b]	IV[h]	132	Changbai 5	JL[c]	IV[h]
31	Hongmang	HLJ[b]	L[g]	82	Longdun 102	HLJ[b]	IV[h]	133	Jijing44	JL[c]	IV[h]
32	Jinxiandao 1	HLJ[b]	L[g]	83	Dongnong 422	HLJ[b]	IV[h]	134	Jijing46	JL[c]	IV[h]
33	Hongniandao	HLJ[b]	L[g]	84	Longdun 104	HLJ[b]	IV[h]	135	Changbai6	JL[c]	IV[h]
34	Hailunguangjian	HLJ[b]	L[g]	85	Longjing 13	HLJ[b]	IV[h]	136	Shuangfeng8	JL[c]	IV[h]
35	Baidadu	HLJ[b]	L[g]	86	Longdao 3	HLJ[b]	IV[h]	137	Jiudao6	JL[c]	IV[h]
36	Fuguo	HLJ[b]	L[g]	87	Suijing 7	HLJ[b]	IV[h]	138	Jijing61	JL[c]	IV[h]
37	Niandaozi	HLJ[b]	L[g]	88	Mudanjiang 26	HLJ[b]	IV[h]	139	Jiudao8	JL[c]	IV[h]
38	Guangtouzijian	HLJ[b]	L[g]	89	Dongnong 424	HLJ[b]	IV[h]	140	Changbai7	JL[c]	IV[h]
39	Zhumaodao	HLJ[b]	L[g]	90	Songjing 10	HLJ[b]	IV[h]	141	Jijing63	JL[c]	IV[h]
40	Liushuzhong	HLJ[b]	L[g]	91	Beidao 3	HLJ[b]	IV[h]	142	Jiyujing	JL[c]	IV[h]
41	Xiaohongmang	HLJ[b]	L[g]	92	Longjing 16	HLJ[b]	IV[h]	143	Tongyu211	JL[c]	IV[h]
42	Binxianludao	HLJ[b]	L[g]	93	Longdao 7	HLJ[b]	IV[h]	144	Changbai9	JL[c]	IV[h]
43	Longjiangguangtou	HLJ[b]	L[g]	94	Mudanjiang 29	HLJ[b]	IV[h]	145	Chaochan1	JL[c]	IV[h]
44	Huodaozi	HLJ[b]	L[g]	95	Kendao 12	HLJ[b]	IV[h]	146	Tongnian2	JL[c]	IV[h]
45	Baidadu	HLJ[b]	L[g]	96	Longdao 5	HLJ[b]	IV[h]	147	Jiudao33	JL[c]	IV[h]
46	Guangtouhong	HLJ[b]	L[g]	97	Songjing 6	HLJ[b]	IV[h]	148	Jiudao46	JL[c]	IV[h]
47	Heimangdao	HLJ[b]	L[g]	98	Longjing 20	HLJ[b]	IV[h]	149	Jijing88	JL[c]	IV[h]
48	Tonghejingzu	HLJ[b]	L[g]	99	Longjing 24	HLJ[b]	IV[h]	150	Jijing502	JL[c]	IV[h]
49	Youmangziyedao	HLJ[b]	L[g]	100	Suijing 9	HLJ[b]	IV[h]	151	Tongyu403	JL[c]	IV[h]
50	Baimangdao	HLJ[b]	L[g]	101	Suijing 11	HLJ[b]	IV[h]	152	Jite 639	JL[c]	IV[h]
51	Xinkaizhong	HLJ[b]	L[g]	102	Dongnong 428	HLJ[b]	IV[h]	153	Changbai 17	JL[c]	IV[h]

Table 1 continued

No.	cultivars	origins	type	No.	cultivars	origins	type	No.	cultivars	origins	type
154	Jijing 106	JL[c]	IV[h]	199	yamakata 86	Japon	IV[h]	244	tohoku 45	Japon	IV[h]
155	Changbai 16	JL[c]	IV[h]	200	rikuu	Japon	IV[h]	245	woondoobyeo	Korea	IV[h]
156	Weiguo	LN[d]	IV[h]	201	sachiminori	Japon	IV[h]	246	hoabongbyeo	Korea	IV[h]
157	Weiguo 7	LN[d]	IV[h]	202	iwanishki	Japon	IV[h]	247	yeunghaebyeo	Korea	IV[h]
158	Aifeng 2	LN[d]	IV[h]	203	wainishiki	Japon	IV[h]	248	gancheukbyeo	Korea	IV[h]
159	Gongzi 1	LN[d]	IV[h]	204	fukei 168	Japon	IV[h]	249	nonganbyeo	Korea	IV[h]
160	Liaojing 5	LN[d]	IV[h]	205	fukei 180	Japon	IV[h]	250	woonbongbyeo	Korea	IV[h]
161	Liaoyannuo	LN[d]	IV[h]	206	fukei 144	Japon	IV[h]	251	jinbooolbyeo	Korea	IV[h]
162	Liaojing 421	LN[d]	IV[h]	207	shimahikari	Japon	IV[h]	252	taeseungbyeo	Korea	IV[h]
163	Shennong 91	LN[d]	IV[h]	208	isaomochi	Japon	IV[h]	253	geuroobyeo	Korea	IV[h]
164	Liaoyan 282	LN[d]	IV[h]	209	akihikari	Japon	IV[h]	254	doonnaebyeo	Korea	IV[h]
165	Shennong 129	LN[d]	IV[h]	210	fuchiminori	Japon	IV[h]	255	mananbyeo	Korea	IV[h]
166	Liaoyan 241	LN[d]	IV[h]	211	sinei	Japon	IV[h]	256	moonzangbyeo	Korea	IV[h]
167	Liaoyan 283	LN[d]	IV[h]	212	reisio	Japon	IV[h]	257	sambaekbyeo	Korea	IV[h]
168	Liaoyan 16	LN[d]	IV[h]	213	noaze	Japon	IV[h]	258	sangmibyeo	Korea	IV[h]
169	Shennongxiangnuo	LN[d]	IV[h]	214	sasanishiki	Japon	IV[h]	259	sangzoochal	Korea	IV[h]
170	Liaonong 938	LN[d]	IV[h]	215	sasashigure	Japon	IV[h]	260	sinsangzoo	Korea	IV[h]
171	Danjing 8	LN[d]	IV[h]	216	akiyutaka	Japon	IV[h]	261	sinwoonbong	Korea	IV[h]
172	Liaoyou 7	LN[d]	IV[h]	217	sasaminori	Japon	IV[h]	262	otaebyeo	Korea	IV[h]
173	Kaijing 2	LN[d]	IV[h]	218	akisio	Japon	IV[h]	263	woondoo	Korea	IV[h]
174	Kaijing 3	LN[d]	IV[h]	219	kiyonishiki	Japon	IV[h]	264	woonbong	Korea	IV[h]
175	Qianchonglang 1	LN[d]	IV[h]	220	akimine	Japon	IV[h]	265	woonzang	Korea	IV[h]
176	Liaoxing 6	LN[d]	IV[h]	221	fuchisaka 5	Japon	IV[h]	266	inwoul	Korea	IV[h]
177	Liaoxing 10	LN[d]	IV[h]	222	rikuuakihonami	Japon	IV[h]	267	hongzinzoo	Korea	IV[h]
178	Liaoyan 166	LN[d]	IV[h]	223	koshiminori	Japon	IV[h]	268	zaorim	Korea	IV[h]
179	Liaojing 912	LN[d]	IV[h]	224	wasekogane	Japon	IV[h]	269	oljinboo	Korea	IV[h]
180	Shendao 9	LN[d]	IV[h]	225	rikuukaori	Japon	IV[h]	270	jinboochal	Korea	IV[h]
181	Shendao 3	LN[d]	IV[h]	226	sinsei	Japon	IV[h]	271	taebong	Korea	IV[h]
182	Dan 137	LN[d]	IV[h]	227	kansannishiki	Japon	IV[h]	272	huadong	Korea	IV[h]
183	Liaoxing 12	LN[d]	IV[h]	228	kitakogane	Japon	IV[h]	273	pyeongyang 1	DPRK	IV[h]
184	Liaoxing 13	LN[d]	IV[h]	229	rikuukomachi	Japon	IV[h]	274	pyeongyang 10	DPRK	IV[h]
185	etsuu 334	Japon	IV[h]	230	sugaruasahi	Japon	IV[h]	275	pyeongyang 15	DPRK	IV[h]
186	tohoku 15	Japon	IV[h]	231	yamakata 26	Japon	IV[h]	276	hamnam 1	DPRK	IV[h]
187	toyonishki	Japon	IV[h]	232	aokei 98	Japon	IV[h]	277	hamnam 16	DPRK	IV[h]
188	kansan	Japon	IV[h]	233	etsuyo	Japon	IV[h]	278	hamnam 23	DPRK	IV[h]
189	kinuhikari	Japon	IV[h]	234	akitakomachi	Japon	IV[h]	279	yeomzoo 1	DPRK	IV[h]
190	kinuhikari	Japon	IV[h]	235	satonouta	Japon	IV[h]	280	yongseong 14	DPRK	IV[h]
191	chiyonishiki	Japon	IV[h]	236	hitomebore	Japon	IV[h]	281	zaoseonbyeo	DPRK	IV[h]
192	yamahikari	Japon	IV[h]	237	fuchihikari	Japon	IV[h]	282	ДТР6217	RFER	IV[h]
193	tentai	Japon	IV[h]	238	kuiku 131	Japon	IV[h]	283	ЗПР6213	RFER	IV[h]
194	koshihikari	Japon	IV[h]	239	yumeake	Japon	IV[h]	284	Свиревщаника	RFER	IV[h]
195	waseaikoku 3	Japon	IV[h]	240	kakehashi	Japon	IV[h]	285	Мрдиаскуокрони	RFER	IV[h]
196	etsuu 394	Japon	IV[h]	241	waswaomori	Japon	IV[h]	286	Сабалмо	RFER	IV[h]
197	fukei 198	Japon	IV[h]	242	kotentai 5	Japon	IV[h]	287	Скороспегна	RFER	IV[h]
198	aokei 138	Japon	IV[h]	243	hashiribozu	Japon	IV[h]	288	Аропщанй	RFER	IV[h]

[a] BY = Breeding year. [b] HLJ = Heilongjiang province of China. [c] JL = Jilin province of China. [d] LN = Liaoning province of China. [e] DPRK = Democratic People's Republic of Korean. [f] RFER = Russian Far East district of Russian Federation. [g] L = landraces. [h] IV = improved variety.

Hopefully this research will help to deepen people's understanding on present situation, characteristics, developing trend and improvement emphasis of germplasm resource in Northeast Asia, and furthermore to provide references for protecting genetic

diversity, broadening genetic bases and the effective utilization of germplasm resource.

2. Material and Methods

2.1 Materials

In this study, 288 accessions preserved and provided by the Crop Science Research Institute of Chinese Academy of Agricultural Sciences, Liaoning Provincial Academy of Agricultural Sciences, Heilongjiang Provincial Academy of Agricultural Sciences and Northeast Agricultural University were selected. Based on geographical distribution and variety type, those accessions were divided into nine groups: Heilongjiang landraces (HL), Jilin landraces (JL), Heilongjiang improved varieties (HIV), Jilin

improved varieties (JLIV), Liaoning improved varieties (LIV), Japanese improved varieties (JIV), Korean improved varieties (KIV), Democratic People's Republic of Korean improved varieties (DPRKIV) and the Russian Far East district of Russian Federation improved varieties (RFERIV). Each group consisted of 53, 51, 25, 26, 29, 60, 28, 9 and 7 accessions, respectively (Table 1).

2.2 Total genomic DNA extraction

Total genomic DNA was extracted and purified from the young leaves by a modified CTAB method described by Edwards *et al* (1991). The DNA extracts were checked for DNA concentration on 0.8% agarose mini-gel in 1×TBE buffer (0.09 M Tris–borate and 0.5 M EDTA) at 80 V for 90 min with ethidium bromide staining.

2.3 Primer screening

Based on primer information at http//www.gramene.org, 600 primers, randomly located on rice chromosomes, were designed and synthesized by the Sangon Biotech (Shanghai) Co., Ltd. 12 varieties of different groups (Laotoudao 1, Baidadu, Kendao 12, Xiaobaijingzihuadianbai, Jijing 61, Danjing 8, Shennong 91, Fuchihikari, kuiku 131, Woonbongbyeo, Jinbooolbyeo, and Pyeongyang 15) were selected for testing the polymorphism of primers. 154 out of 600 primers, with high amplification rate and distinct polymorphism, were selected and used as markers (Table 2).

2.4 PCR amplification

SSR PCR reaction was set in 20 ul mixes containing 2ul of genomic DNA (25ng/ul), 1.5 ul $MgCl_2$ (25mM), 0.3 ul dNTP mixtures (10mM), 2 ul 10 × PCR buffer, 2ul SSR primer (2 uM), 0.2ul Taq polymerase enzyme (10 units/ul), 12ul ddH_20. The amplification temperature profiles were as follows: 2 min at 94℃, followed by 35 cycles of 30 sec at 94℃, 30 sec at 47℃, 30 sec at 72 ℃, then 5 min at 72 ℃.

2.5 PCR products separation and detection

After the PCR reaction, PCR products were added with loading buffer (2.5mg/ml bromophenol blue, 2.5mg/ml diphenylamine blue, 10mM EDTA, 95% formamide), which were denatured for 5 min at 94 ℃ and then put on ice for 5 min. Then the PCR products were separated on 6% denaturing polyacrylamide gel and were directly detected after rapidly silver straining (Trigiano & Caetano-Anollés, 1998).

2.6 Data analysis

Each SSR primer detects 1 locus, and each amplified polymorphism band makes 1 allele. All clearly detectable polymorphic bands were scored for the analysis, and band presence as score 1 and band absence as score 0 in each accession. POPGENE 1.32 (Yeh et al., 1999) was used to calculate the genetic identity (Nei, 1972), genetic distance (Nei, 1972), the coefficient of genetic differentiation (Nei, 1987), and gene flow (Nei, 1987) between the nine groups. *Na*, *He* and *PIC* (Nei et al., 1983) were calculated with the program PowerMaker 3.25 (Liu & Muse, 2005). Data matrices were entered into the NTSYS-pc 2.1 software (Rohlf, 2000) was used to calculate the SM similarity coefficient, and UPGMA was used for cluster analysis. The data were analyzed with the qualitative routine to generate Jaccard's similarity coefficients. Similarity coefficients were used to construct dendrogram using the UPGMA (unweighted pair group method with arithmetic average) and the SHAN (sequential, hierarchical, and nested clustering) routine in the NTSYS program. The molecular variance within and among nine groups were calculated using AMVOA under GenAlEx6.2 (Peakall & Smouse, 2006).

3. Results

3.1 SSR polymorphism

823 allelic variations were detected using 154 SSR primers (Table 2). *Na* ranged from 2 (RM272, RM292, RM345, RM346 and RM1210, locating at chromosomes 1, 6 and 7) to 9 (RM1347, RM1350, RM1369, RM336, RM1306, RM1353, RM257, and RM1374, locating at chromosomes 2, 3, 6, 7, 9, and 10). *He* ranged from 0.061 to 0.869, with an average of 0.624. *PIC* ranged from 0.060 to 0.856, with an average of 0.586. *I* varied from 0.042 to 2.070, with the average 1.144. RM 1350, RM1369, RM257, RM336 and RM1374 on chromosomes 3, 6, 9, 7 and 10 ranked the top five on *He, PIC* and *Na,* indicating that these primers are suitable for the comparison on genetic diversity in Northeast Asia. The 6^{th} to the 10^{th} largest *PIC* was RM207, RM264, RM1306, RM501 and RM1379; the 6^{th} to the 10^{th} largest *He* was RM207, RM264, RM1306, RM501 and RM1379; the 6^{th} to the 10^{th} largest *Na* was RM1306, RM1353, RM1347, RM207 and RM264.

Table 2 Genetic diversity information of different SSR primers.

No.	primer	C[a]	*Na*[b]	*He*[c]	*PIC*[d]	*I*[e]	No.	primer	C[a]	*Na*[b]	*He*[c]	*PIC*[d]	*I*[e]
1	RM220	1	7	0.591	0.558	1.198	52	RM241	4	6	0.717	0.681	1.289
2	RM237	1	5	0.316	0.299	0.601	53	RM273	4	4	0.493	0.408	0.740
3	RM243	1	4	0.457	0.398	0.770	54	RM303	4	6	0.337	0.326	0.708
4	RM246	1	5	0.735	0.689	1.382	55	RM307	4	3	0.446	0.406	0.456
5	RM265	1	3	0.565	0.499	0.717	56	RM349	4	4	0.700	0.644	0.982
6	RM272	1	2	0.061	0.060	0.042	57	RM471	4	5	0.712	0.674	1.302
7	RM292	1	2	0.154	0.144	0.271	58	RM473	4	6	0.648	0.616	1.288
8	RM302	1	4	0.350	0.335	0.543	59	RM518	4	6	0.764	0.731	1.458
9	RM306	1	6	0.647	0.601	1.058	60	RM551	4	6	0.676	0.631	1.277
10	RM476	1	4	0.741	0.694	1.133	61	RM1205	4	4	0.648	0.606	1.055
11	RM486	1	3	0.602	0.521	0.796	62	RM1272	4	5	0.555	0.509	0.979
12	RM488	1	3	0.530	0.445	0.837	63	RM1354	4	5	0.622	0.565	1.060
13	RM562	1	4	0.658	0.596	0.829	64	RM249	5	8	0.772	0.749	1.756
14	RM579	1	7	0.813	0.791	1.744	65	RM405	5	5	0.481	0.449	0.665
15	RM580	1	7	0.810	0.784	1.728	66	RM430	5	5	0.767	0.729	1.513
16	RM583	1	7	0.640	0.609	1.255	67	RM470	5	4	0.307	0.279	0.481
17	RM594	1	5	0.758	0.722	1.393	68	RM480	5	6	0.713	0.669	1.226
18	RM1244	1	4	0.604	0.531	0.985	69	RM593	5	4	0.241	0.232	0.461
19	RM1254	1	5	0.665	0.604	1.121	70	RM1200	5	5	0.649	0.586	1.028
20	RM1282	1	5	0.657	0.599	1.106	71	RM1271	5	6	0.754	0.720	1.433
21	RM1287	1	3	0.551	0.461	0.769	72	RM1366	5	6	0.369	0.356	0.643
22	RM1297	1	6	0.690	0.646	1.289	73	RM225	6	5	0.679	0.619	1.149
23	RM1320	1	6	0.602	0.571	1.086	74	RM253	6	6	0.617	0.549	1.076
24	RM1360	1	6	0.817	0.791	1.669	75	RM276	6	7	0.648	0.614	1.299
25	RM207	2	8	0.852	0.835	1.894	76	RM345	6	2	0.263	0.245	0.200
26	RM208	2	4	0.585	0.518	0.969	77	RM412	6	7	0.675	0.648	1.340
27	RM213	2	3	0.557	0.468	0.865	78	RM510	6	5	0.463	0.437	0.673
28	RM233	2	4	0.126	0.123	0.288	79	RM527	6	5	0.763	0.725	1.345
29	RM236	2	3	0.456	0.391	0.604	80	RM539	6	5	0.775	0.741	1.358
30	RM290	2	4	0.799	0.767	1.386	81	RM540	6	5	0.704	0.670	1.290
31	RM327	2	4	0.611	0.530	1.000	82	RM541	6	7	0.814	0.791	1.659
32	RM406	2	6	0.439	0.424	0.825	83	RM584	6	5	0.763	0.725	1.420
33	RM525	2	7	0.736	0.702	1.452	84	RM586	6	5	0.803	0.775	1.505
34	RM530	2	5	0.767	0.734	1.444	85	RM589	6	8	0.781	0.751	1.582
35	RM1255	2	5	0.673	0.639	1.131	86	RM1340	6	7	0.810	0.784	1.637
36	RM1267	2	5	0.530	0.494	1.013	87	RM1369	6	9	0.865	0.851	2.039
37	RM1307	2	6	0.651	0.592	1.192	88	RM10	7	4	0.472	0.431	0.522
38	RM1313	2	6	0.652	0.593	1.188	89	RM234	7	5	0.258	0.247	0.484
39	RM1347	2	9	0.824	0.804	1.815	90	RM298	7	3	0.481	0.401	0.646
40	RM1367	2	8	0.783	0.754	1.648	91	RM336	7	9	0.863	0.848	2.027
41	RM1379	2	8	0.838	0.817	1.818	92	RM346	7	2	0.565	0.503	0.579
42	RM200	3	5	0.374	0.342	0.709	93	RM418	7	4	0.684	0.637	1.180
43	RM283	3	5	0.750	0.708	1.269	94	RM440	7	4	0.482	0.430	0.683
44	RM411	3	3	0.260	0.245	0.263	95	RM478	7	3	0.367	0.338	0.533
45	RM426	3	8	0.667	0.642	1.401	96	RM501	7	8	0.843	0.824	1.820
46	RM514	3	3	0.607	0.557	0.821	97	RM505	7	3	0.487	0.444	0.738
47	RM1284	3	4	0.519	0.452	0.813	98	RM542	7	4	0.650	0.577	0.742
48	RM1324	3	7	0.795	0.764	1.595	99	RM560	7	4	0.472	0.441	0.650
49	RM1350	3	9	0.869	0.856	2.070	100	RM1210	7	2	0.219	0.204	0.317
50	RM1352	3	6	0.645	0.605	1.149	101	RM1243	7	8	0.816	0.792	1.774
51	RM1371	3	8	0.832	0.812	1.797	102	RM1306	7	9	0.844	0.827	1.974

Table 2 continued

No.	primer	C[a]	*Na*[b]	*He*[c]	*PIC*[d]	*I*[e]	No.	primer	C[a]	*Na*[b]	*He*[c]	*PIC*[d]	*I*[e]
103	RM1353	7	9	0.836	0.816	1.803	129	RM1374	10	9	0.852	0.835	1.985
104	RM1357	7	8	0.786	0.757	1.648	130	RM1375	10	7	0.817	0.794	1.732
105	RM1362	7	7	0.834	0.813	1.786	131	RM21	11	5	0.565	0.487	0.995
106	RM1364	7	7	0.825	0.802	1.672	132	RM144	11	5	0.761	0.727	1.461
107	RM1365	7	7	0.819	0.795	1.739	133	RM224	11	5	0.550	0.522	1.083
108	RM1377	7	8	0.626	0.597	1.335	134	RM229	11	6	0.692	0.641	1.273
109	RM25	8	4	0.566	0.516	0.683	135	RM254	11	6	0.699	0.659	1.308
110	RM152	8	4	0.506	0.451	0.879	136	RM286	11	8	0.742	0.709	1.588
111	RM223	8	5	0.776	0.740	1.450	137	RM287	11	5	0.239	0.228	0.383
112	RM264	8	8	0.849	0.831	1.855	138	RM332	11	3	0.649	0.579	1.058
113	RM281	8	5	0.657	0.605	1.135	139	RM536	11	3	0.543	0.463	0.798
114	RM284	8	4	0.677	0.626	1.114	140	RM1219	11	4	0.565	0.523	0.925
115	RM407	8	4	0.422	0.399	0.637	141	RM1355	11	6	0.452	0.428	0.832
116	RM515	8	6	0.792	0.761	1.540	142	RM101	12	3	0.648	0.588	1.031
117	RM547	8	7	0.788	0.763	1.604	143	RM270	12	4	0.681	0.621	1.102
118	RM1309	8	4	0.302	0.281	0.502	144	RM309	12	3	0.646	0.597	0.926
119	RM1345	8	5	0.761	0.723	1.386	145	RM415	12	6	0.813	0.788	1.617
120	RM201	9	3	0.442	0.368	0.618	146	RM1226	12	3	0.424	0.363	0.712
121	RM215	9	4	0.697	0.642	1.243	147	RM1227	12	6	0.706	0.680	1.440
122	RM219	9	6	0.791	0.759	1.586	148	RM1246	12	5	0.663	0.623	1.170
123	RM242	9	5	0.257	0.247	0.487	149	RM1261	12	4	0.448	0.421	0.607
124	RM257	9	9	0.864	0.849	2.028	150	RM1300	12	5	0.745	0.704	1.339
125	RM288	9	3	0.276	0.259	0.333	151	RM1302	12	6	0.750	0.716	1.519
126	RM566	9	5	0.519	0.452	0.798	152	RM1310	12	6	0.721	0.670	1.357
127	RM1328	9	6	0.604	0.525	1.090	153	RM1337	12	8	0.798	0.773	1.702
128	RM228	10	6	0.445	0.425	0.871	154	RM1381		8	0.773	0.752	1.658
						mean				5.344	0.624	0.586	1.144

[a] C = Chromosome. [b] *Na* = Allele number. [c] *He* = Nei's genetic diversity index. [d] *PIC* = Polymorphism information content. [e] *I* = Shannon's Information index.

3.2 Genetic diversity among different groups

Molecular variance analysis showed (Table 3) that significant difference existed both among different groups and within each group ($P<0.01$). The variation within a group accounted for 8.40% and among different groups accounted for 91.60%. Therefore, it is necessary to make further analysis in this case.

Table 3 Analysis of molecular variance (AMVOA) among different groups and within one group.

Source	Df[a]	SS[b]	MS[c]	% Var.[d]	P
Among Pops	8	5471.8	684.0	8.40	＜0.01
Within Pops	279	49920.3	178.9	91.60	＜0.01
Total	287	55392.1		100.00	

[a] df = Degrees of freedom. [b] SS = Sum of square. [c] MS = Mean square. [d] %Var. = Percentage of total variance.

From Table 4 it can be seen that according to *He* and *PIC* the 9 groups could be ranked in a descending order: HL, JL, JIV, HIV, RFERIV, LIV, JLIV, KIV, DPRKAL. It also can be calculated that the mean *He* and *PIC* of landraces (HL and JL) is 0.624 and 0.577, of improved varieties (HIV, JLIV, and LIV), 0.540 and 0.495. Apparently, the mean *He* and *PIC* of landraces (HL and JL) are higher than that of improved varieties (HIV, JLIV, and LIV), indicating that landraces bear the richest genetic diversity among all tested materials.

3.3 The genetic affinity among different groups

Compared with the genetic identity and genetic distance (Table 5) among different groups, it can be found that JIV bore the highest genetic identity (0.921, 0.922, and 0.923) but the smallest genetic distance (0.082, 0.082, and 0.080) with HIV, JLIV and LIV. Except for HL, the other 7 groups bore the smallest genetic identity and the largest genetic distance with RFERIV.

To make clear why there are differences in genetic identity and genetic distance among those nine groups, a comparison was made on coefficient of differentiation (*Fst*) and gene flow (*Nm*) (Table 6). Result showed that the *Fst* among groups ranged from 0.036 to 0.164, with an average of 0.0811, indicating that 8.11% genetic variation happened among different groups, while 91.89% variation happened within a group. This is almost the same as the results of molecular variance analysis. *Nm* ranged from 1.272 to 6.678, with mean value being 3.299, indicating that there were frequent genetic information exchange among the 9 groups, i.e. rice varieties were frequently introduced and exchanged in this region.

Table 4 The Genetic diversity of groups.

Group	*Na*[a]	*He*[b]	*PIC*[c]
HL	4.435	0.641	0.596
HIV	4.013	0.570	0.527
JL	3.994	0.606	0.559
JLIV	3.377	0.522	0.476
LIV	3.630	0.528	0.483
JIV	4.506	0.572	0.530
KIV	3.481	0.513	0.469
DPRKIV	2.532	0.501	0.441
RFERIV	2.708	0.551	0.488

[a] *Na* = the observed number of alleles.
[b] *He* = Nei's genetic diversity index.
[c] *PIC* = Polymorphism information content.

A similar conclusion can be drawn from Table 6 that JIV bore the highest *Nm* (6.678, 6.446, and 5.890) but the lowest *Fst* (0.036, 0.037, and 0.041) with HIV, LIV, and JLIV. Except for HL, the other 7 groups bore the smallest *Nm* and the largest *Fst* with RFERIV.

From Table 5 and Table 6, it can be concluded that JIV had the most frequent genetic information exchange with HIV, JLIV and LIV, and especially had remarkable influence on HIV. RFERIV had the least exchange with other groups, follow by KIV and DPRKIV. Compared with other groups, RFERIV and HL had the smallest genetic distance and the most frequent genetic information exchange with each other.

The following facts can be concluded according to the *Nm* of one group vs. every other eight groups (Table 6): the *Nm* of HL vs. JL is the largest, and vice versa; the *Nm* of RFERIV vs. HL and JIV vs. HIV is the largest; the *Nm* of JLIV, LIV, KIV, and DPRKIV vs JIV are the largest. The *Nm* of HL and RFERIV vs. DPRKIV are the smallest, except for this, the *Nm* of other 7 groups vs. RFERIV are the smallest.

Different *Nm* values reflect the difference in genetic information exchange among different groups in Northeast Asia: HL and JL groups are greatly influenced by each other; RFERIV is greatly influenced by HL; HIV, JLIV, LIV, KIV and DPRKIV bear the largest influence by JIV, but the least by RFERIV; JL and JIV bear the least influence by RFERIV; HL and RFERIV bear the least influence by DPRKIV.

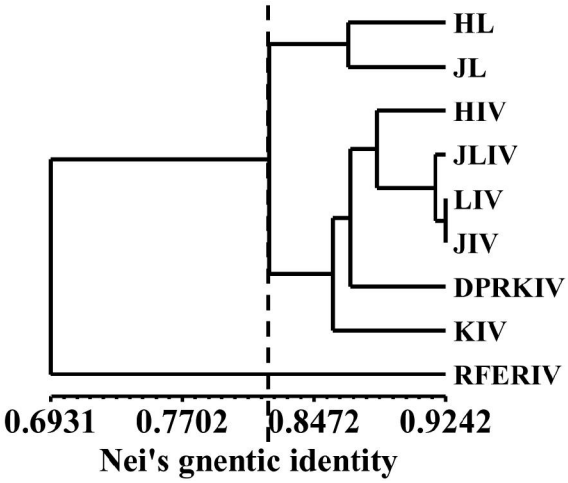

Figure 1. UPGMA dendrogram based on Nei's (1972) genetic identity among nine groups.
The vertical dot line indicates the genetic identity value, 0.8212, dividing the 9 groups into 3 clusters.

3.4 Cluster

Based on genetic identity, UPGMA was used to divide those nine groups into three clusters with a threshold of 0.8212. The 1st cluster includes HL and JL; the 2nd cluster includes JLIV, HIV, LIV, JIV, DPRKIV and KIV; the 3rd cluster is solely RFERIV. In the 2nd cluster, HIV, JLIV and LIV are closely related to JIV, especially for LIV (Fig. 1).

Based on coefficient of genetic similarity, UPGMA was used to divide the 288 varieties into two clusters with a threshold of 0.6925 (Fig. 2). The 1st cluster (I) included 216 varieties, and the 2nd cluster (II) included 72 varieties. The 1st cluster consisted of 45.3%HL, 84.3% HIV, 20.0% JL, 100% JLIV, 93.1% LIV, 85.5% JIV, 96.4% KIV, 100% DPRKIV and 42.9% RFERIV. It is obvious that 84.3~100% HIV, JLIV, LIV, JIV, KIV and DPRKIV existed in cluster I; while 54.7~80.0% HL, JL, and RFERIV existed in cluster II. This indicates that landrace varieties tend to be in one cluster, so does improved varieties. Such situation is in accord with the above-mentioned result that HL, JL, RFERIV and other improved varieties bear a relationship of far genetic distance, large Fst, small coefficient of genetic similarity, and small gene flow.

When the threshold was set at 0.7490, those varieties can be further divided into 13 subclusters, which were I-1-1, I-1-2, I-2-1-1, I-2-1-2, I-2-1-3, I-2-2, I-3-1-1, I-3-1-2, I-3-2, II-1-1-1, II-1-1-2, II-1-2 and II-2, consisting 75, 34, 39, 14, 15, 4, 18, 13, 4, 22, 31, 17 and 2 varieties respectively. Except for I-III-I-I, each subcluster was made up of varieties from more than two groups (Table 7), revealing a complicate genetic information exchange among them. RFERIV existed in I-1-2 with 23 HL and 2 JL; it also existed in II-1-1-2 with other 12 JL. Those 35 HL accounted for 66.0% of all HL varieties, and 14 JL accounted for 56.0% of all JL varieties. This is coherent with the relationship between RFERIV vs. HL and JL that has been described in above text. 58.8% HIV and 56.7% JIV existed in I-1-1, indicating that JIV had the largest influence on HIV.

Table 5 genetic identity(above diagonal) and genetic distance(below diagonal) between groups.

	HL	HIV	JL	JLIV	LIV	JIV	KIV	DPRKIV	RFERIV
HL	****	0.853	0.871	0.813	0.810	0.860	0.788	0.788	0.830
HIV	0.159	****	0.871	0.882	0.848	0.921	0.843	0.833	0.753
JL	0.138	0.138	****	0.843	0.818	0.871	0.763	0.794	0.746
JLIV	0.207	0.126	0.170	****	0.914	0.922	0.866	0.884	0.688
LIV	0.210	0.164	0.202	0.090	****	0.923	0.852	0.877	0.694
JIV	0.151	0.082	0.138	0.082	0.080	****	0.883	0.882	0.744
KIV	0.239	0.171	0.270	0.144	0.161	0.124	****	0.851	0.673
DPRKIV	0.238	0.183	0.230	0.123	0.131	0.126	0.161	****	0.672
RFERIV	0.187	0.284	0.293	0.374	0.365	0.296	0.395	0.398	****

Table 6 Coefficient of differentiation (*Fst*, below diagonal) and Gene flow (*Nm*, above diagonal) between groups.

	HL	HIV	JL	JLIV	LIV	JIV	KIV	DPRKIV	RFERIV
HL	****	4.136	5.338	2.826	2.991	4.449	2.442	2.335	3.274
HIV	0.057	****	4.458	3.902	3.223	6.678	2.884	2.619	2.072
JL	0.045	0.053	****	3.177	2.908	4.534	2.146	2.266	2.091
JLIV	0.081	0.060	0.073	****	5.112	5.890	3.060	3.414	1.491
LIV	0.077	0.072	0.079	0.047	****	6.446	2.902	3.289	1.594
JIV	0.053	0.036	0.052	0.041	0.037	****	3.961	3.528	2.003
KIV	0.093	0.080	0.104	0.076	0.079	0.059	****	2.626	1.417
DPRKIV	0.097	0.087	0.099	0.068	0.071	0.066	0.087	****	1.272
RFERIV	0.071	0.108	0.107	0.144	0.136	0.111	0.150	0.164	****

Table 7 The accessions number of every subcluster.

	HL	HIV	JL	JLIV	LIV	JIV	KIV	DPRKIV	RFERIV	Total
I-I-I		30	3	2	6	34				75
I-I-II	23	1	2			3	1	1	3	34
I-II-I-I				20	6	2	5	6		39
I-II-I-II				1	13					14
I-II-I-II					1	11	2	1		15
I-II-II	1					2		1		4
I-II-I-I							18			18
I-II-I-II		12					1			13
I-II-II				3	1					4
II-I-I-I	3	8	4		1	6				22
II-I-I-II	12		12		1	2			4	31
II-I-II	14		3							17
II-II			1				1			2
Total	53	51	25	26	29	60	28	9	7	288

4.Discussion

It is essential to understand genetic diversity for the effective conservation and utilization of rice germplasm. Many molecular studies on genetic diversity of natural rice populations of improved varieties and landraces have been reported. Zhao et al. (2009) used 29 SSR primers to analyze the genetic diversity of 150 accessions of cultivated rice from Korea, China, and Japan. The *Na* obtained was 12.9, the mean *PIC* was 0.6683, the mean *He* was 0.7001. Sixty-nine accessions were surveyed with 26 SSR markers to reveal the genomic relationship among cultivars in Argentina. The *Na* obtained was 8.4, the mean *PIC* was 0.69 (Giarrocco at al., 2007). Thomson et al. (2007) characterized 330 rice accessions using 30 microsatellite markers. The *Na* obtained was 13, the mean *PIC* was 0.66. Shu et al. (2009) studied the genetic diversity of 313 improved japonica varieties from 20 countries with 34 SSR primers. The *Na* obtained was 12.9, the mean *He* was 2.8471. Obviously, results of some parameters in this study (such as *Na* and *PIC*) were smaller than that of above-mentioned studies, indicating that the level of genetic diversity in Northeast Asia is comparatively low.

Xu et al. (2012) and Sun et al. (2001) found that there was mismatch in parametric relationship among different groups. For example, in Xu et al.'s study, *He* of indica cultivars was higher than that of japonica cultivars, although the number of accessions and *Na* of indica accessions were less than those of japonica cultivars (Xu et al., 2012). Another example, in Sun et al.'s study, although the average gene diversity of the South Asian common wild rice was higher than that of the Southeast Asian common wild rice, its percentage of polymorphic per loci, *Na* and number of genotypes were all smaller (Sun et al., 2001). The same parametric relationship emerged in this study. JIV had higher number of accessions and *Na* than HL, but *He* and *PIC* were lower than that of HL. This indicates that comparing to number of varieties, *He* and *PIC* are more easily affected by variety improvement status. Since HL is less improved, it resembles more genetic diversity. Comparatively, JIV is highly improved so it bears less genetic diversity.

Many researchers have compared the genetic diversity of improved varieties, landraces and common wild rice among different countries and regions. The Results of Shu et al. indicated that genetic similarity

(GS) of the varieties for north of China with Korea, DPRK and Japan were higher. The second GS was between Korea and DPRK (Shu et al., 2009). The Results of Zhao et al. indicated the genetic diversity of the Korean and Chinese cultivars was higher than that of the Japanese cultivars (Zhao et al., 2009). In this study, the 9 groups can be ranked in a descending order according to *He* and *PIC*, from which it can be concluded that HL and JL have the richest genetic diversity, JIV is higher than that of HIV, LIV, JLIV and KIV.

Genetic background of the parent must be fully considered during the practices of genetic base broadening and new variety breeding. Zhao et al. (2008) studied the genetic variation of japonica rice cultivars in Yunnan of China and Korea using SSR markers. Results showed that there was great difference in genetic variation between Yunnan and Korea rice cultivars. And it was suggested to utilize Korean varieties to explore Yunnan varieties' genetic base and improve rice quality. Base on the genetic relationship of those 9 groups it is suggested that: firstly, HL and JL should be adopted more frequently to improve HIV, JLIV and LIV; secondly, unfavorable influence caused by similar genetic background must be fully considered when using JIV to improve LIV, JLIV and LIV, especially for HIV; thirdly, RFERIV should be adopted to broaden the genetic base of rice cultivars of other countries in Northeast Asia. When hybridization barrier are encountered, HL could be considered as bridge parents.

In conclusion, this research studied the level of genetic diversity and the relationship among nine japonica rice groups of different geographical origins and types in Northeast Asia. Research results will be useful to appropriately identify and select parent in breeding practices, explore genetic variation, broaden genetic foundation and protect genetic diversity of japonica rice germplasm in Northeast Asia.

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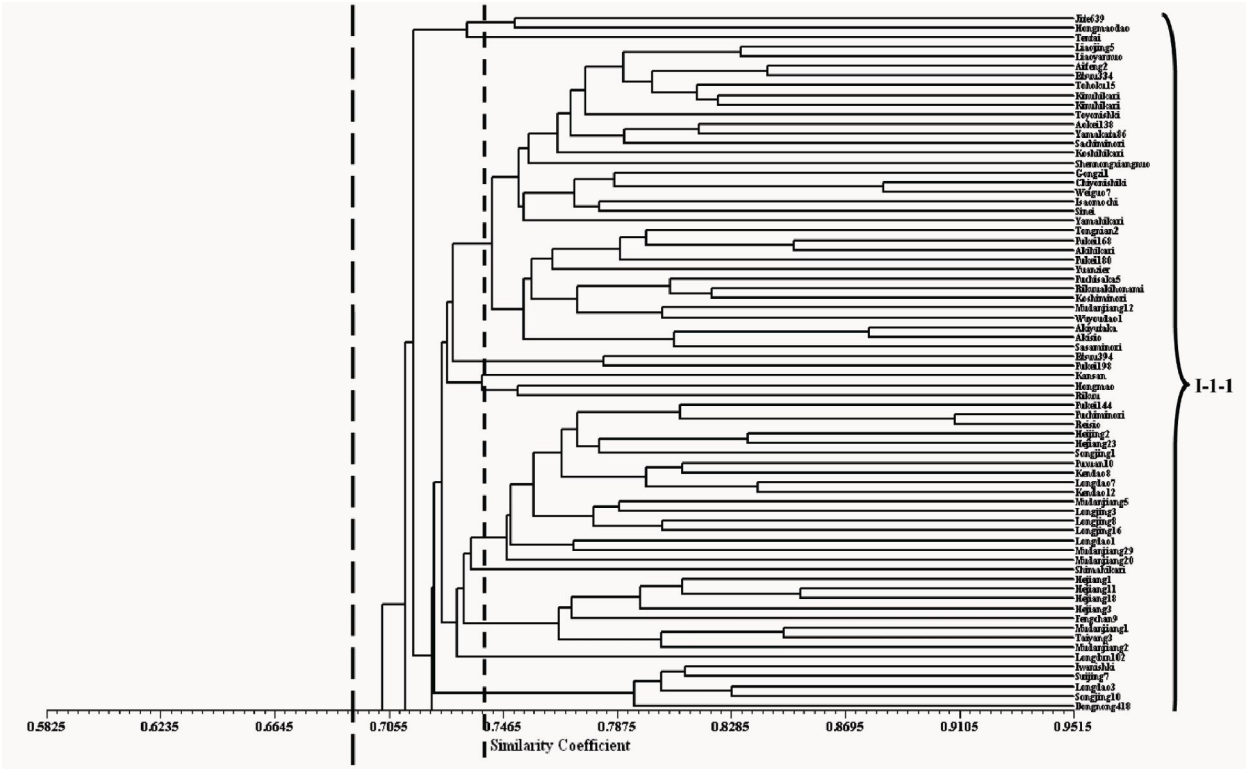

Figure 2. UPGMA dendrogram based on the Nei's (1972) coefficient of genetic similarity among 288 varieties. There are the dendrogram of 288 accessions based on similarity coefficient using the UPGMA and SHAN routine in the NTSYS 2.1 program. The vertical dash line indicates the coefficient of genetic similarity value, 0.6925, dividing the 288 varieties into 2 clusters; and the vertical dot line indicates the coefficient of genetic similarity value, 0.7490, dividing the 288 varieties into 13 subclusters.

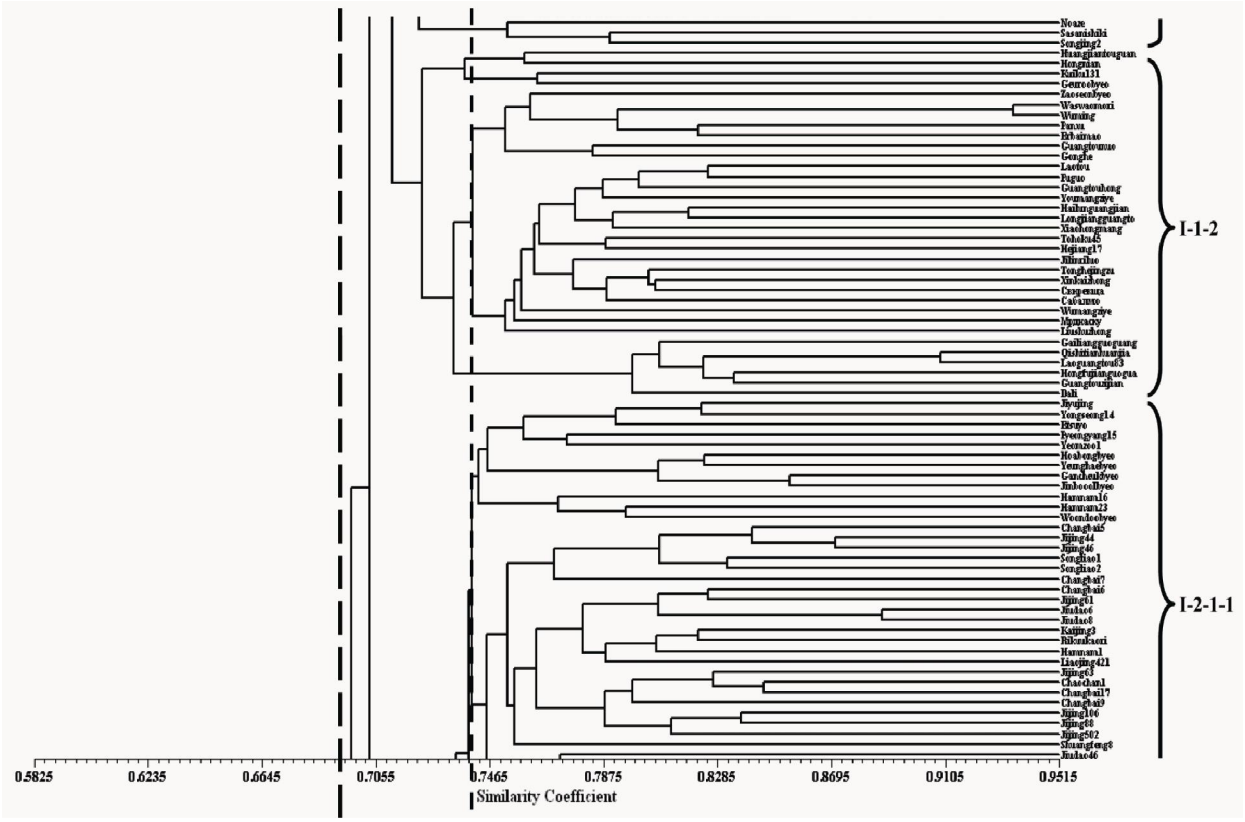

Figure 2 continued.

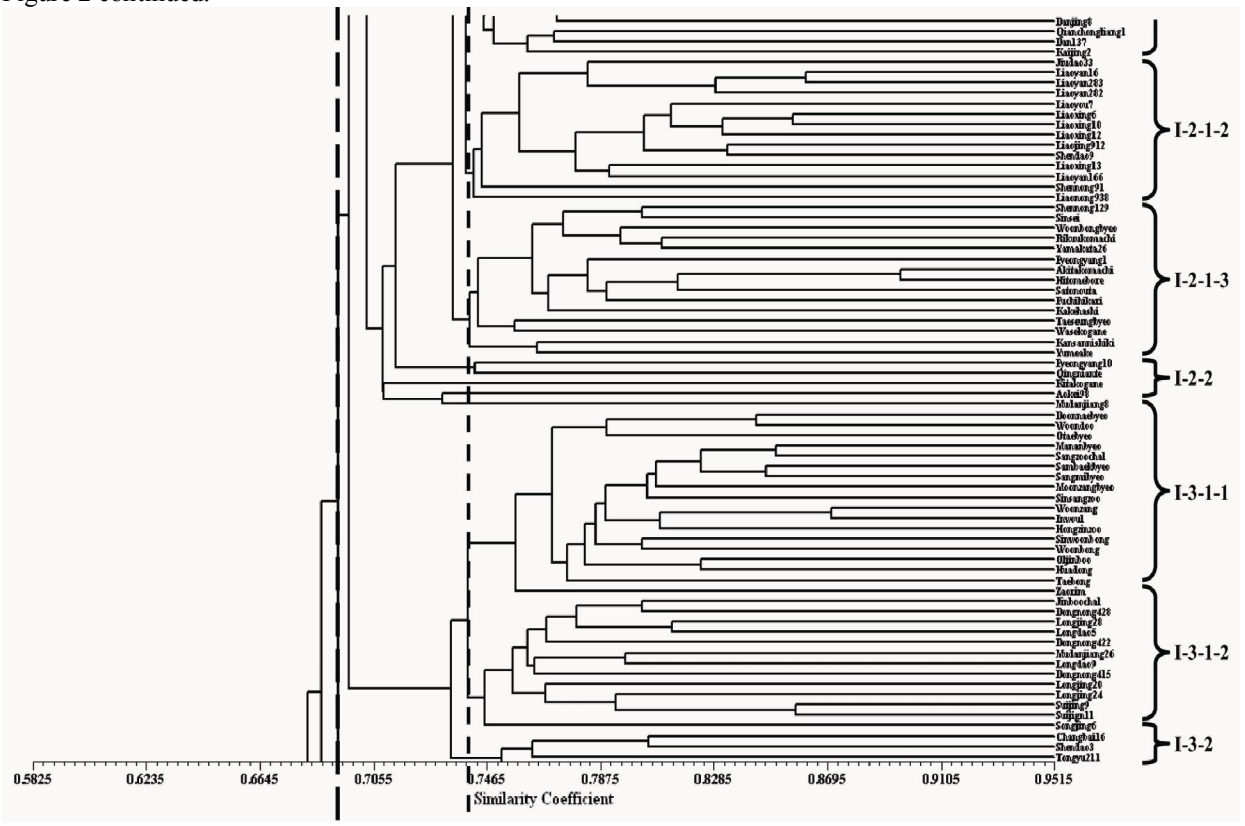

Figure 2 continued.

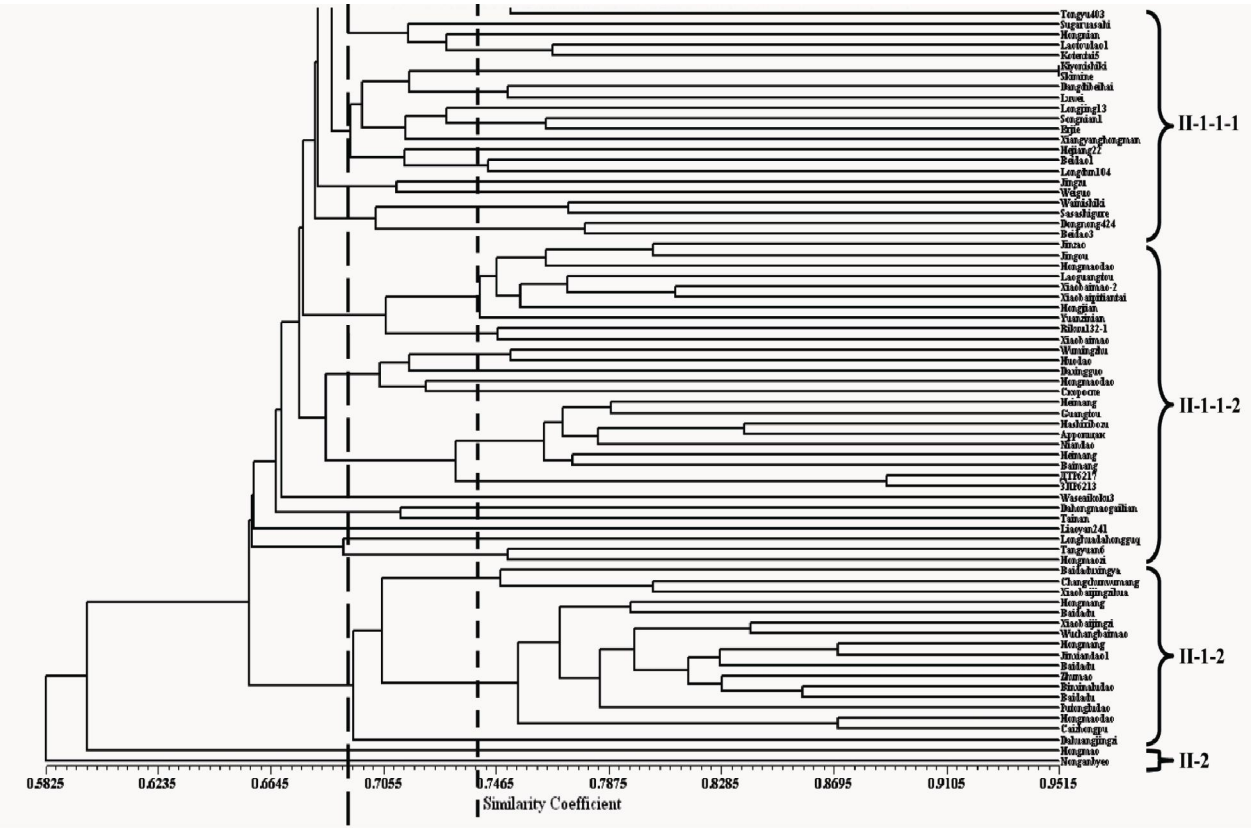

Figure 2 continued.