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Genetic Diversity of Korean Rice Breeding Parents as Measured by DNA Fingerprinting with Simple Sequence Repeat (SSR) Markers

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ABSTRACT

Molecular markers are useful tools for evaluating genetic diversity and determining cultivar identity. Present study was conducted to evaluate the genetic diversity within a diverse collection of rice accessions used for Korean breeding programs. Two hundred eighty-seven rice cultivars, composed of temperate japonica, tropical japonica, indica, and Tongil-type of Korean crossing parents were evaluated by means of 15 simple sequence repeat (SSR) markers. A total of 99 alleles were detected, and the number of alleles per marker ranged from 4 to 11, with an average of 6.6 per locus. Polymorphism information content (PIC) for each of the SSR markers ranged from 0.2924 to 0.8102 with an average of 0.5785. These results, with the result that use of only 15 SSR markers made all rice cultivars examined could be uniquely distinguished, imply the efficiency of SSR markers for analysis of genetic diversity in rice. Cluster analysis was performed on similar coefficient matrics calculated from SSR markers to generate a dendogram in which two major groups corresponding to japonica (Group I) and indica and Tongil type rice (group II) with additional subclasses within both major groups. The narrowness of the Korean breeding germplasm was revealed by the fact that most of the Korean-bred and Japan-bred temperate japonica cultivars were concentrated into only 2 of the sub-group I-1 (143 cultivars) and I-2 (58 cultivars) among six sub-groups in major group of japonica. This is because of the japonica accessions used in this study was a very closely related ones because of frequent sharing of the crossing parents with similar genetic background with synergy effect of the inherited genetic difference between indica and japonica. A rice breeding strategy with the use of molecular markers was discussed for overcoming of genetic vulnerability owing to this genetic narrowness.

Key words : rice, simple sequence repeat, genetic diversity, DNA fingerprinting

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\INTRODUCTION

Genetic diversity plays a significant role on improvement of crop plants. It has been realized that genetic diversity in Korean rice cultivars has been narrowing due to frequent use of limited japonica genotypes as well as repeated use of premium quality cultivars as crossing parents. For a extreme case, five leading rice cultivars grown over 50 % of Korean paddy field were shared the same premium quality cultivar as a crossing female parent (Song et al., 2002). Breeding for resistance to biotic and abiotic stress is difficult in Korea due to this narrow genetic diversity.

The cultivated rice species, O. sativa, is composed of two subspecies, indica and japonica (Oka, 1988). Indica is the predominant tropical subspecies. The japonica subspecies, consisting of temperate and tropical types, is mostly grown in East Asia area, and accounts for about 20% of world rice production (Mackill, 1995). In beginning of 1970s, a new type of rice was developed in Korea from the crosses between subspecies with wide genetic differences, japonica and indica, which was named as Tongil type rice (Park et al., 1990). This wide hybridization program ensured indica germplasm to be introduced in Korean germplasm, broadening genetic diversity of existing germplasm. However, recent changes in consumer's preference for the palatability for japonica rice to indica rice, only the temperate-japonica rice was grown in Korea presently.

Although the wide hybridization between different subspecies seems to be an ideal strategy to introduce novel genes, the use of wide crosses often results in sterility problems in the hybrids and their progenies, disruption of favorable linkage blocks and gene combinations, and linkage drag (Ikehashi and Araki, 1986). The reduced recombination and distorted segregation resulting from wide hybridization may cause difficulties in selection for desired recombinants during the breeding process (Pham and Bougerol, 1993). From the viewpoint of plant breeders, it is preferable to identify and use donors of important traits from within the same subspecies or cultivar group, if possible, although there are still a possibility to reduce the genetic diversity within existing breeding population (Ni et al., 2001). The information on genetic diversity within existing breeding germplasm is important for this point of view. When a guideline for setting up a crossing plan is established, a balance between genetic diversity for assuring the genetic diversity and difficulties in wide hybridization is considered (Song et al., 2002)

When grouping of germplasm or assessment of genetic diversity within a species, molecular markers are a powerful tool when considering the reports that only 10 to 15 markers are enough to distinguish the whole accessions examined (Jeong et al., 1999). Among molecular markers to be used for evaluating genetic diversity of germplasm, microsatellite or simple sequence repeat (Tautz, 1989) is present choice that are both technically efficient and cost-effective to use and are available for rice (Chen et al., 1997; Temnykh et al., 2000). Compared with Restriction fragment length polymorphism (RFLPs), SSR markers detect a significantly higher degree of polymorphism in rice (Wu and Tanksley, 1993; Yang et al., 1994), and are especially suitable for evaluating genetic diversity among closely related rice cultivars (Akagi et al., 1996).

The objectives of present study were to evaluate the genetic diversity within Korean rice crossing parents. This information may provide rice breeders a better understanding of the genetic materials to be used for breeding programs and be served as a guidelines to set up a crossing plan for the rice improvement program

MATERIALS AND METHODS

Plant materials

The accessions used in the present study included

287 rice cultivars used for crossing parents for Korean rice breeding program recently. Most of the accessions were classified as temperate japonica while a small portion of tropical japonica, Tongil and indica were included (Table 1). Although the genetic information, origin and ecotype of most of the accessions were well defined, those of some accessions were mistakenly defined because the information was traced from the breeder's experience rather than from the systematic analysis of genetic diversity.

DNA extraction and SSR analysis

Genomic DNA was isolated from green leaves of young seedlings according to method of Song et al. (2002). Fifteen SSR markers were used in this experiment as appeared in Table 2. The original sources and motifs for these markers can be found in the Gramene database addressed to http://www.gramene.org. The SSR marker positions on chromosome were known by previous study (Panaud et al., 1996). For the representation of SSR markers for whole genome, originally 100 SSR markers were screened with 4 rice cultivars representing each of the 4 types of tropical, temperate japonica, Tongil and indica rice. Among them, 15 SSR markers used in this study were selected because they showed different alleles in all the 4 ecotypes. For SSR amplification, each 20-μl amplification reaction mixer consisted of 10 mM of Tris-HCl (pH 9.0), 50 mM of KCl, 0.1% Triton-100, 2 mM of $MgCl_2$, 0.1 mM of dNTPs, 4 pM of primers, 0.5 units of Taq polymerase, and 20 ng of genomic DNA was used. All fragments were amplified using the following PCR profile: 5 min at 95℃, followed by 45 sec. at 94℃, 1 min. at 55℃, and 2 min. at 72℃ for 35 cycles, and 5 min. at 72℃ for a final extension. PCR fragments were separated on denaturing polyacrylamide gels consisting of 10% polyacrylamide (AA:BIS = 19:1) and 7 M urea in 0.5 M TBE buffer. To this end PCR reactions were mixed with equal volumes of loading buffer (formamide containing 0.8 mM EDTA and traces of bromophenol blue and xylene cyanol), denatured at 95℃ for 5 min and snap cooled on ice. Afterwards, the samples were loaded on pre-heated Sequi-Gen GT Sequencing Cells (Bio Rad, Munich), that were run at 1,800 V for 2.5 up to 4 h, depending on the fragment sizes to be separated. After the run, the fragments were visualized by silver staining. For this, the gel was fixed for 5 min in fixative (10% ethanol, 5% acetic acid), rinsed in deionized water, stained for 15 min in 0.3% (w/v) silver nitrate. Rinsing was followed again in deionized water, and developed for approximately 15 min until the bands became visible in 1.5 g/l of NaOH, 4 ml/l of 37% formaldehyde and 85 g/l of NaBH. Scoring was done by visual inspection.

Data analysis

Each amplified fragment was treated as a unit character and scored as a binary code 1 for presence or 0 for absence. Similarity between accessions was based on the algorithm of Nei and Li (1979); $Sxy = 2Nxy/(Nx+Ny)$, where Nxy means the number of shared bands between two individuals x and y. The Nx and Ny denote the total number of bands in individual x and y, respectively. Then, the phylogenetic dendrograms were constructed by UPGMA (unweighted pair-group method with arithmetic average) in the NTSYS-pc program (Roholf 1989). The number of alleles per locus was based on an evaluation of the total accessions evaluated. The term polymorphism information content (PIC) was originally introduced into human genetics by Botstein et al. (1980). It refers to the value of a marker for detecting polymorphism within a population, depending on the number of detectable alleles and the distribution of their frequency. In present study, PIC value of a marker was calculated according to a simplified version after Anderson et al. (1993):

$$PIC_i = 1 - \sum_{j=1}^{n} P_{ij}^2$$

where Pij is the frequency of the jth allele for the ith marker, and summed over n alleles.

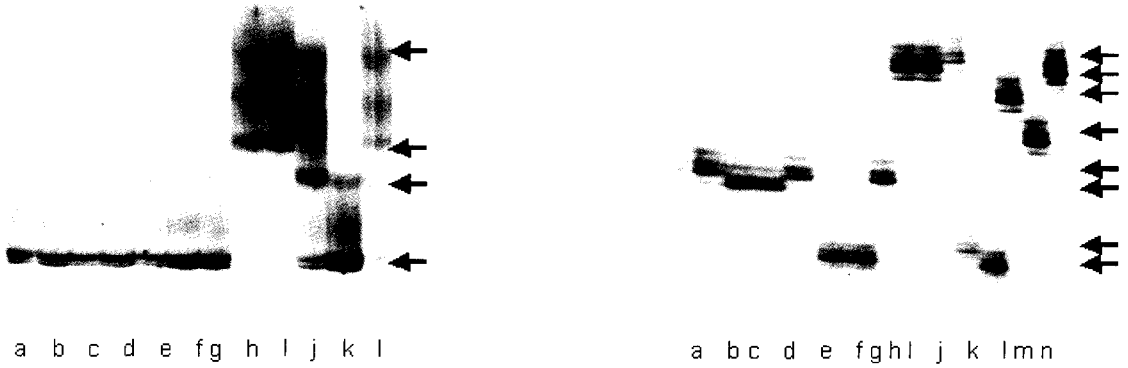
RESULTS

SSR polymorphism between sub-species

A total of 287 rice accessions was amplified using fifteen SSR markers. Most SSR markers produced multiple amplified products with a conspicuous distinction between subspecies of indica and japonica. A typical gel separation profile of amplified products is presented in Fig. 1; two SSR markers with 12 representative cultivars among 287 cultivars. Accessions shown here were representative varieties of each subspecies: entries labeled from "a" to "g" belonged to temperate japonica, from "h" to "i" to Tongil, "j" to tropical japonica, and from "k" to "n" to indica, respectively. For RM153, a clear distinction in band patterns between indica and japonica was observed with non-separation within subspecies. However, in the case of RM210 (Fig. 1. B), with the clear distinction among these four sub species, different band profiles were observed within subspecies; for example, within japonica (varieties "a" to "g"), RM210 produced three different products while RM153 did not have a band distinction within groups. All together the tendency with a clear distinction between subspecies and a less distinction within subspecies were observed for most of the primers examined in present study.

Allele numbers and PIC values of individual SSR markers

The number of alleles reflects the abundance of genes in a population. Of the 287 accessions studied, there are 99 alleles at 15 loci or 15 SSR markers investigated (Table 2). The maximum of eleven alleles in RM201 and the minimum of 4 alleles in RM153 with the average number of 6.6 alleles per loci were observed. A PIC value of 0.579 was calculated for the SSR markers based on data from 287 cultivars. The comparison was not made with other molecular markers. However, former study reported that comparison of PIC of SSR and RFLP markers on 20 rice accessions showed that the PIC values of each SSR markers are far greater than that of the RFLP markers (Panaud et al., 1996). This clearly demonstrates the greater power of resolution offered by SSR markers and



(A) RM153 (B) RM210

Fig. 1. Multiple allele detection of SSR markers (RM 153 and 210), illustrating typical band patterns observed in crossing parent of Korean rice. Arrow indicates the allele position in the polyacrylamide gel. Cultivars from "a" to "g" belonged to temperate japonica, from "h" and "I" to Tongil, "j" to tropical japonica, and "k" to "n" to indica.

their usefulness for rice genome analysis. Interestingly, there are a high correlation between numbers of the polymophic bands and PIC values (R2=0.62***), which again contributed this high PIC values of SSR markers compared to other DNA markers such as RFLPs with 2-4 alleles per locus. Compared to other experiments, the PIC values for SSR markers used in this experiment is lower than those of other experiments (Panaud et al., 1996; Ni et al., 2001). This is due to the fact that they use the genetically distant accession than the accession used in present study, mostly, consisted in temperate japonica of premium quality rice cultivars.

Cluster analysis

Cluster analysis was performed on similar coefficient matrix calculated from SSR marker data to generate a full dendrogram, covering all 287 accessions (appeared as group No. in Table 1). A simplified diagram of full dendrogram was shown in Fig. 2. All 287 accessions could be easily distinguished even though some accessions were very closely related. The UPGMA cluster diagram showed two major groups corresponding to japonica (group I) and indica and Tongil (group II) with additional subclasses within both major groups. The average genetic distance for the indica and Tongil group was significantly greater than that for the japonica group. This is because of the japonica accession used in this study is a very closely related ones because of frequent sharing of the crossing parents having similar genetic background with synergy effect of inherited genetic difference between indica and japonica.

With genetic distance (GD) < 0.79 as the standard for a sub-cluster, the japonica cluster could be divided into 6 sub-groups. Most of the Korean-bred and Japan-bred japonica cultivars belonged to sub-group I-1 (143 cultivars) and I-2 (58 cultivars). Other 4 sub-groups include the mixture of temperate japonicas from other countries such as U.S.A. and China. Tropical japonica belonged to this category with the distribution from sub-group I-3 to I-6. The non-distinction between tropical japonica and some of the Korean temperate

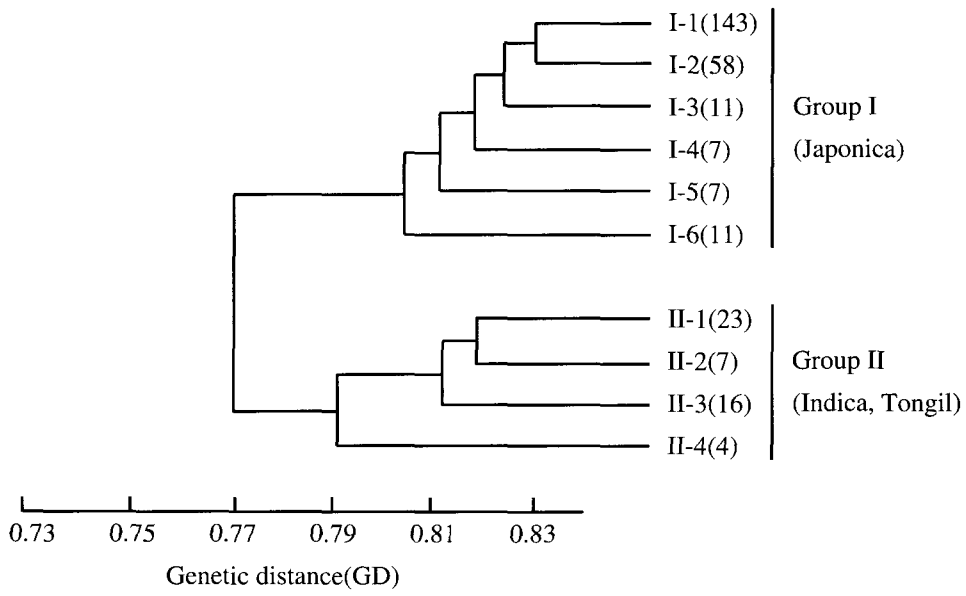


Fig. 2. Simplified UPGMA dendrogram of crossing parent of Korean rice based on SSR marker fingerprinting. The number in parenthesis indicates the numbers of cultivars clustered in the subgroup and each accessions belonged to respective groups is denoted as group no. in Table 1.

Table 1. List of rice accessions used in present study.

Group No.*	Accessions	Origin	Eco-type**	Group No.	Accessions	Origin	Eco-No.	Group type	Accessions	Origin	Eco-type
I-1	Aikawa1	Japan	J	I-6	Graldo	ND	ND	II-1	IR71604	IRRI	I
I-1	Akitsuho	Japan	J	I-1	Gru	Korea	J	II-2	IR8	IRRI	I
I-1	Amaroo	Australia	J	I-1	Gwehwa	Korea	J	II-3	IRAT254	ND	ND
II-1	Anda	Korea	T	I-1	Gyehwa19	Korea	J	I-4	IRAT267	ND	ND
I-2	Anjung	Korea	J	I-1	Gyehwa20	Korea	J	I-4	IRAT269	ND	ND
I-1	Ansan	Korea	J	I-1	Gyehwa21	Korea	J	I-4	IRAT300	ND	ND
I-1	Anseong	Korea	J	I-1	Gyehwa22	Korea	J	II-1	IRGA440	ND	ND
I-1	Aranghwangchal	Korea	J	I-1	Haepyeong	Korea	J	II-2	IRGA659	ND	ND
II-1	Areum	Korea	T	I-1	Hamju3	Korea	J	I-6	Iri346	Korea	J
I-5	ArrozdaTerra	Portugal	I	II-3	Hangangchaol	Korea	J	I-5	Jagwangdo	Korea	J
I-2	Asominori	Japan	J	II-3	Hanyangjo	Korea	J	I-1	Jangan	Korea	J
I-1	Banshinrikihenshu	Japan	J	I-2	HaoZhao5	China	J	II-1	Jangseong	Korea	J
II-3	BL122	Japan	J	I-2	Hapcheon1	China	J	I-4	Jasmine85	USA	I
I-3	BL245	Japan	J	II-1	Heibao	China	I	I-1	Jeogjinju	Korea	J
I-1	Bongkwang	Korea	J	I-3	Heughyang	Korea	J	I-1	Jinbong	Korea	J
I-6	Bunketsuto	Japan	J	I-2	Heugjinju	Korea	J	I-1	Jinbu	Korea	J
I-2	Calrose	USA	J	I-2	Heugnam	Korea	J	I-2	Jinbu22	Korea	J
I-1	Cheolweon63	Korea	J	I-2	Hoan	Korea	J	I-1	Jinbu32	Korea	J
I-1	Cheolweon64	Korea	J	I-2	Hojin	Korea	J	I-2	Jinbu34	Korea	J
I-1	Cheolweon65	Korea	J	I-2	Hwaan	Korea	J	I-1	Jinbu35	Korea	J
II-1	Cheongcheong	Korea	T	I-1	Hwabong	Korea	J	I-1	Jinbuchal	Korea	J
I-1	Cheongmyeong	Korea	J	I-1	Hwadong	Korea	J	I-6	Jinbuol	Korea	J
II-3	China1039	China	J	I-1	Hwajin	Korea	J	I-1	Jinmi	Korea	J
I-1	Chucheong	apan	J	I-1	Hwajung	Korea	J	I-1	Jinpum	Korea	J
I-1	Chungbukheugmi	Korea	J	I-1	Hwamyeong	Korea	J	I-1	Joryeong	Korea	J
II-3	Congshengla	ND	ND	I-1	Hwanam	Korea	J	I-1	Juan	Korea	J
I-1	Daean	Korea	J	I-1	Hwasam	Korea	J	I-1	Junam	Korea	J
I-1	Daecheong	Korea	J	I-1	Hwaseonchal	Korea	J	I-1	Jungan	Korea	J
I-1	Daejin	Korea	J	I-1	Hwaseong	Korea	J	I-1	Junghwa	Korea	J
I-2	Daerip1	Korea	J	I-1	Hwayeong	Korea	J	I-1	Jungsan	Korea	J
I-2	Daesan	Korea	J	II-1	Hyangmi1	Korea	T	II-1	Kariat3	India	I
I-1	Daeya	Korea	J	II-1	Hyangmi2	Korea	T	II-2	KataktaraDa2	India	I
II-1	Dasan	Korea	T	I-1	Hyangnam	Korea	J	I-2	KikeiBanshinriki	Japan	J
I-1	Dongan	Korea	J	I-2	Iksan444	Korea	J	I-2	Kinuhikari	Japan	J
I-1	Dongjin	Korea	J	I-1	Iksan448	Korea	J	I-2	Koshihikari	Japan	J
I-2	Dongjinchal	Korea	J	I-1	Iksan450	Korea	J	I-1	KotakeTamanisiki	Japan	J
I-2	DoradoPrecose	Portugal	J	I-2	Iksan454	Korea	J	I-1	Kwangan	Korea	J
II-4	Dular	India	I	I-1	Iksan455	Korea	J	I-5	Lacrosse	USA	ND
I-1	Dunnae	Korea	J	I-1	Iksan456	Korea	J	I-1	M201	USA	J
I-2	FeiWan9	China	J	I-1	Iksan457	Korea	J	I-1	M202	USA	J
I-2	Fujihikari	Japan	J	I-1	Iksan458	Korea	J	I-1	Magog1	Korea	J
I-3	Gancheog	Japan	J	I-5	Iksan459	Korea	J	I-1	Manan	Korea	J
II-1	Gaya	Korea	T	I-1	Iksan460	Korea	J	I-1	Mangeum	Korea	J
I-1	Geummo	Korea	J	I-2	Ilmi	Korea	J	I-4	Manongbalay	ND	ND
I-1	Geummo1	Korea	J	I-1	Ilpum	Korea	J	I-3	Manpung	Korea	J
I-2	Geummo2	Korea	J	I-2	Inabawase	Japan	J	I-4	Mawan11	China	J
I-1	Geumnam	Korea	J	I-2	Inweol	Korea	J	I-2	MaWan9	China	J
I-2	Ginggalagsale	Korea	J	II-3	IR50	IRRI	I	I-2	Mercury	USA	I
I-2	Goamy	Korea	J	I-4	IR57893	IRRI	I	I-1	Mihyang	Korea	J
I-6	Giza177	Egypt	I	I-1	IR68373	IRRI	I	I-1	Milyang169	Korea	J

* No indicates the groups that individual accessions is belonged by cluster analysis

* * J, japonica; I, indica; T, Tongil type; -, unknown ; ND, not defined.

Continued

Group No.*	Accessions	Origin	Eco-type**	Group No.	Accessions	Origin	Eco-No.	Group type	Accessions	Origin	Eco-type
I-1	Milyang173	Korea	J	I-1	Samcheon	Korea	J	I-1	Suweon473	Korea	J
I-1	Milyang176	Korea	J	II-3	Samgang	Korea	T	I-1	Suweon474	Korea	J
I-1	Milyang177	Korea	J	I-3	Samjiyeon4	Korea	J	I-3	Suweon475	Korea	J
I-1	Milyang178	Korea	J	II-3	Samjiyeon5	Korea	J	II-1	Suweon476	Korea	T
I-1	Milyang180	Korea	J	I-1	Sampyeong	Korea	J	I-1	Suweon477	Korea	J
II-1	Milyang181	Korea	I	I-1	Sanghaehyanghyella	China	J	I-1	Suweon478	Korea	J
I-1	Milyang182	Korea	J	I-1	Sangju	Korea	J	II-2	Suweon496	Korea	J
I-2	Milyang183	Korea	J	I-1	Sangju24	Korea	J	I-6	Syareo982114	ND	ND
I-1	Milyang184	Korea	J	I-1	Sangju25	Korea	J	II-1	Taebaeg	Korea	T
I-1	Milyang185	Korea	J	I-1	Sangju26	Korea	J	I-1	Tamjin	Korea	J
I-2	Milyang186	Korea	J	I-2	Sangjuchal	Korea	J	II-1	Tatsumimochi	Japan	J
I-1	Milyang187	Korea	J	I-1	Sangmi	Korea	J	I-2	TNI	Taiwan	J
I-1	Milyang188	Korea	J	I-2	Sangnambat	Korea	J	II-2	Tohoku144	Japan	J
II-2	Milyang23	Korea	I	I-1	Sangsan	Korea	J	II-3	Tongil	Korea	T
I-2	Milyang95	Korea	J	II-3	Sathi	India	I	I-4	Toro2	USA	I
II-3	Mirim67	Korea	T	I-6	Sekitorihen	Japan	J	I-1	Unbong	Korea	J
I-1	Miyagaori	Japan	J	I-1	Seoan	Korea	J	I-1	Unbong26	Korea	J
I-6	Mogkyo86	ND	ND	I-1	Seohyangchal	Korea	J	I-2	Unbong28	Korea	J
I-1	Moonjang	Korea	J	I-2	Seojin	Korea	J	I-1	Unbong29	Korea	J
I-4	Moroberekan	Africa	TJ	I-2	Shennong265	China	J	I-1	Undoo	Korea	J
I-1	Naepung	Korea	J	I-5	Shennong9660	China	J	I-1	Unjang	Korea	J
I-1	Nagdong	Korea	J	I-1	Shindongjin	Korea	J	I-1	Weiyou989	China	I
I-2	Namgang	Korea	J	I-6	Shinkaneoogaikoku	Japan	J	I-1	Weonhwang	Korea	J
II-1	Nampung	Korea	J	I-1	Shinseonchal	Korea	J	I-1	Weonsan67	Korea	J
I-3	Nampyeong	Korea	T	I-1	Shinunbong	Korea	J	I-1	Yaemugura	Japan	J
I-1	Namyang29	Korea	J	I-1	Shoryushinrikihen	Japan	J	I-1	Yangjo	Korea	J
I-1	Namweon	Korea	J	I-2	Siminori	Japan	J	I-3	Yeomju14	Korea	J
I-1	Namyang26	Korea	J	I-1	Sobaeg	Korea	J	I-3	Yeomju5	Korea	J
I-1	Namyang28	Korea	J	I-1	Sobee	Korea	J	I-2	Yeongdeog31	Korea	J
I-1	Namyang7	Korea	J	I-6	Songjeon	Japan	J	I-1	Yeongdeog32	Korea	J
II-4	NewBonet	USA	TJ	I-1	Soryuto	Japan	J	I-1	Yeongdeog33	Korea	J
II-1	Namcheon	Korea	T	I-1	Stejaree45	Russia	J	I-1	Yeonghae	Korea	J
II-3	Nongan	Korea	T	I-2	Sujin	Korea	J	I-1	Yeongnam	Korea	J
I-4	Nongho	Korea	J	I-1	Sura	Korea	J	II-1	Yongju	Korea	T
I-2	Nonglimna1	Japan	J	I-5	Suweon345	Korea	J	II-1	Yongmoon	Korea	T
II-4	NP125	Japan	J	I-3	Suweon365	Korea	J	II-3	Yushin	Korea	T
I-1	Obong	Korea	J	I-2	Suweon403	Korea	J	II-2	Zenith	USA	I
I-1	Odae	Korea	J	I-2	Suweon432	Korea	J				
I-2	OmachiSenshutsu	Japan	J	II-1	Suweon441	Korea	T				
I-1	Palgong	Korea	J	II-1	Suweon450	Korea	J				
I-6	Pyeongbuk5	Korea	J	I-5	Suweon459	Korea	J				
II-3	Pyeongdo1	Korea	J	I-1	Suweon460	Korea	J				
II-3	Pyeongyang18	Korea	J	I-1	Suweon461	Korea	J				
I-3	Pyeongyang21	Korea	J	I-1	Suweon462	Korea	J				
I-2	Pyeongyang4	Korea	J	I-1	Suweon463	Korea	J				
II-3	Pyeongyang41	Korea	J	I-1	Suweon464	Korea	J				
I-1	Raebong	Korea	J	I-1	Suweon466	Korea	J				
I-1	Rokkoku	Japan	J	I-1	Suweon468	Korea	T				
I-2	Saechuchucheong	Korea	J	I-1	Suweon470	Korea	T				
I-1	Sambaeg	Korea	J	I-2	Suweon472	Korea	J				

Table 2. Genetic diversity of SSR markers used in this study.

Marker	Parameters of genetic diversity	
	No. of alleles	Polymorphic information content (PIC)
RM 1	8	0.7113
RM 3	5	0.6611
RM 17	6	0.4172
RM 153	4	0.3649
RM 164	7	0.6815
RM 201	11	0.7281
RM 207	7	0.7399
RM 210	8	0.6542
RM 213	5	0.5879
RM 214	10	0.8102
RM277	4	0.2924
RM 340	6	0.3959
OSR 27	4	0.3052
OSR 29	8	0.8097
OSR 32	6	0.5174

japonica is explained by the fact that the boundary between tropical and temperate japonica types is not firm (Glaszmann and Arraudeau, 1986; Mackill, 1995). For indica and Tongil group, with the same standard (GD < 0.79), 50 cultivars could be divided into 11 sub-clusters. For the convenience of the expression, the lower level genetic diversity in the indica cultivars, GD < 0.76 was used so the group divided into 4 sub-groups as shown in simplified diagram of Fig. 2. There are no conspicuous distinction between subspecies of Tongil and indica. Instead, mixture of indica and Tongil was clustered in three subgroups (II-1, 2, and 3). Only sub-group II-4 contained indica cultivars only.

DISCUSSION

As observed in this study, the usual two main groups, divided by subspecies of indica and japonica, were observed in other studies (Mackill, 1995; Virk et al., 2000; Kwon et al., 1999a; Kwon et al., 1999b; Ni et al., 2001). And overall grouping and orders of genetic similarity in group of the 287 cultivars based on SSR markers were in good agreement with genealogical information and other DNA fingerprinting studies (Kwon et al., 1999b). However, a minor discrepancy to other study should be noticed that the sub-group within indica and Tongil group (Group II) is not well divided into distinctive clustering of indica and Tongil as observed by Ahn et al. (1996) and Kwon et al. (1999a; 1999b) in rice germplasm clustering. This inconsistency maybe arose from two factors. One is the numbers of the cultivars the previous studies used was not enough to show the distinction between these two subspecies. So small and very representative as 2-3 cultivars in a indica and Tongil were used in their studies so the cultivars divided into the adjacent sub-groups that the representative indica cultivars clustered in a group as happened in the sub-group II-4 of this study. The other

explanation is the number of SSR markers used in this study is not enough to represent the whole genomic diversity in rice although a considerable care was taken to choose the SSR markers. Although Jeong et al. (1999) reported 10 to 15 RAPD markers are enough to make resolution of individual cultivars in 100 cultivars, there are no evidence the genetic diversity or genetic distance within groups and subgroups were well reflected in the clustering. More of SSR markers and comparison of the results maybe needed in future evidence.

The principal goal of this study is to evaluate the genetic diversity of the crossing parents used for Korean rice breeding program to be used for the guideline for choosing the crossing combination for future breeding. Although there were several previous study to evaluate genetic diversities in Korean rice cultivars (Ahn et. al., 1996; Kwon et al., 1999a; Kwon et al., 1999b), this is the first study to examine a large number of full-set cultivars in crossing parents used for Korean breeding program. For that reason, the results from this study can be a useful guideline for decision making on choosing crossing materials, marker-assisted selection (MAS) procedures and core collection evaluation. For example, through this study, the narrow genetic diversity of Korean premium quality cultivars is again revealed to give rise concerns on genetic vulnerability of existing germplasm used for rice breeding. A novel breeding strategy combined with use of molecular marker could be suggested using the data. To overcome repeated use of premium quality cultivars as crossing parents for elite cultivar development leading to slow progress of rice breeding, the widening of the genetic background for japonica rice improvement program is needed. Introduction of new genes from the distant relatives and ecospecies (Jena and Khush 1990; Khush 1998) as well as wild relatives to the existing japonica accessions was suggested in spite of difficulties lying in genetic drags and high sterility in wide hybridization. Alternative is to introduce new germplasm to the existing breeding programs thorough search for new gene sources from very close relatives such as landlace varieties or japonica germplasm from foreign countries (Cho et al. 2002). Considering that most of the ancestors of Korean accession were Japan-origin, M202 and Mercury, temperate japonica rice from United States and Australia, were successful cases in introduction of genes from close relatives. The Manchuria, Russia, Italy, and North Korean could be candidate places to search for desirable traits for improving japonica rice breeding if continuous study on the genetic diversity following this study would provide additional information on collected crossing parents.

Additionally, there is more application of this work to determine the feasibility of mapping genes within the temperate japonica group, to which most of Korean cultivars belong. As observed in this experiment that a reasonably high polymorphism between genetically similar accessions such as between premium Korean cultivars existed, it is possible to map traits of interest easily using SSR markers. This is suggested by Ni et al. (2001) for American rice cultivar improvement and by Song et al (2002) for Korean rice improvement. They suggested that if enough polymorphism were detected in the mapping population derived from cross between genetically close parents, the marker for targeted traits would be easily scored and applicable to MAS breeding programs.

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