Korean J. Crop Sci. 44(2) : 112~116(1999)

Evaluation of Genetic Relationship and Fingerprinting of Rice Varieties using Microsatellite and RAPD Markers

Soo-Jin Kwon*[1], Sang-Nag Ahn*, Hae-Chune Choi*, and Huhn-Pal Moon*

ABSTRACT

Genetic diversity of 31 rice varieties including 25 *japonica* and 6 *indica* varieties was evaluated using a combination of 19 microsatellite or simple sequence repeats (SSRs) and 28 random decamer oligonucleotide primers. All 19 microsatellite primer sets representing 19 loci in the rice genome showed polymorphisms among the 31 varieties and revealed 91 alleles with an average of 4.80 bands per primer. Also all 28 random decamer primers used were informative and generated 114 non-redundant bands with a mean of 4.07 bands. Microsatellite markers detected higher number of alleles than random primers although the mean difference was not statistically significant. A cluster analysis based on Nei's genetic distances calculated from the 205 bands resolved the 31 varieties into two major groups that correspond to *indica* and *japonica* subspecies, which is consistent with the genealogical information. As few as six random decamer primers or a combination of one microsatellite and four random decamer primers were sufficient to uniquely differentiate all 31 varieties. These combinations would be potentially useful in rice variety protection and identification considering that 25 out of 31 varieties used in this study are *japonica* rices with high grain quality and have close make up.

Keywords : fingerprinting, genetic diversity, microsatellites, RAPDs, rice, variety protection.

Cultivated rice consists of two subspecies, *indica* and *japonica* (Oka, 1988). The *japonica* rices are predominantly grown in the temperate regions whereas the *indica* rices are cultivated widely in the tropics and subtropics. The narrow genetic diversity within intra-subspecific group is reflected by a low level of polymorphism for isoenzymes and several types of DNA markers (Glaszmann, 1987; Ranjhan et al., 1988; Wang et al., 1992). An exception is the relatively high level of polymorphism with randomly amplified polymorphic DNAs (RAPDs) and microsatellite markers, as demonstrated by Mackill (1995) and Olufowote et al. (1997).

* Rice Breeding Division, National Crop Experiment Station, Rural Development Administration, Suweon 441-100, Korea. *[1] Corresponding author:(E-mail) sjkwon@nces.go.kr (Phone)+82-331-290-6782. Received 13 Jan. 1999.

RAPDs obtained via PCR have been extensively employed as molecular markers for tagging genes (Chunwongse et al., 1994; Martin et al., 1991), and constructing molecular maps (Lefebvre et al., 1995). RAPDs were also used for variety fingerprinting (Ahn et al., 1996; Fukuoka et al., 1992; Mackill, 1995; Yu and Nguyen, 1994) and these studies suggested that RAPDs would be useful in studying genetic diversity and variety fingerprinting of rice.

Microsatellites or simple sequence repeat (SSR) are DNA sequences consisting of arrays of a basic repeat unit of 2-8 base pairs (Wang et al., 1994). These repeats are highly polymorphic, even among closely related varieties, due to variation in the number of repeat units. Wu and Tanksley (1993) demonstrated in rice that microsatellites show much more polymorphism than RFLP markers. Olufowote et al. (1997) also showed that microsatellites were able to discriminate between even genetically close varieties more efficiently than RFLP. This suggests that microsatellites would be ideally suited for studying genetic diversity in rice. This study was carried out to investigate the genetic diversity and fingerprint of 25 *japonica* and 6 *indica* varieties widely used in rice breeding programs in Korea using RAPDs and microsatellite markers.

MATERIALS AND METHODS

A total of 31 rice varieties were used for this study; 25 were *japonica* rices from Korea, Japan, U. S. A., Australia, China, North Korea, and the remaining six were *indica* rices (Table 1). Genomic DNA was extracted from rice leaves and purified by the method as described by Causse et al. (1994).

A set of 28 random decamer primers (Operon Tech., Inc., U.S.A) known to amplify rice DNA based on previous studies was used or RAPDs analysis (Table 2). Amplification procedure was as described by Ahn et al. (1996). Amplified products were resolved by electrophoresis in 2% agarose gels

19 microsatellites were used to amplify simple sequence repeat polymorphism (SSR) of genomic DNA from the 31 varieties (Table 2). The polymerase chain reactions were performed in a total volume of 50 μl containing 40 ηg of genomic DNA, 0.2 μM of each primer, 200 μM each of dATP, dTTP, dGTP and dCTP, 1.0 unit of *Taq* DNA polymerase, 10 mM Tris-

Table 1. List of rice varieties used in this study.

No.	Designation	Ecotype	Origin	No.	Designation	Ecotype	Origin
1	Odaebyeo	*Japonica*	Korea	17	Amaroo	*Japonica*	Australia
2	Sobaegbyeo	〃	〃	18	Langz	〃	〃
3	Ilpumbyeo	〃	〃	19	Jobae 2	〃	China
4	Jinmibyeo	〃	〃	20	Jobae 16	〃	〃
5	Hwaseongbyeo	〃	〃	21	Hongbeon 1	〃	〃
6	Dongjinbyeo	〃	〃	22	Pyungyang 8	〃	N. Korea
7	Akitagomachi	〃	Japan	23	Pyungyang 15	〃	〃
8	Hitomebore	〃	〃	24	Pyungyang 18	〃	〃
9	Koshihikari	〃	〃	25	Seohaechal	〃	〃
10	Kinuhikari	〃	〃	26	Namcheonbyeo	*Indica*	Korea
11	S-201	〃	U.S.A.	27	Dasanbyeo	〃	〃
12	M-201	〃	〃	28	Milyang 23	〃	〃
13	Calrose	〃	〃	29	IR 36	〃	IRRI
14	L-202	〃	〃	30	IR 66	〃	〃
15	Jarrh	〃	Australia	31	IR 72	〃	〃
16	Pelde	〃	〃				

Table 2. Number of alleles as detected by individual markers.

No.	Marker type	Marker	Chr. No.	No. of alleles	PIC value	No.	Marker type	Marker	Chr. No.	No. of alleles	PIC value
1	SSR	CT158	1	4	0.612	25	RAPD	OPC-09	n.d*	3	0.831
2	〃	CT550	1	7	0.801	26	〃	OPC-13	〃	5	0.781
3	〃	GA12	1	5	0.703	27	〃	OPD-08	〃	4	0.558
4	〃	GA580	3	2	0.329	28	〃	OPE-01	〃	2	0.471
5	〃	CT206	4	4	0.418	29	〃	OPE-04	〃	4	0.816
6	〃	CT404	4	5	0.722	30	〃	OPE-07	〃	2	0.383
7	〃	CT500	4	5	0.722	31	〃	OPE-11	〃	6	0.568
8	〃	GA41	5	2	0.175	32	〃	OPE-14	〃	9	0.772
9	〃	RM164	5	7	0.795	33	〃	OPF-05	〃	4	0.481
10	〃	CT469	7	5	0.545	34	〃	OPF-13	〃	3	0.761
11	〃	CT6	9	4	0.653	35	〃	OPF-14	〃	3	0.471
12	〃	CT22	9	5	0.699	36	〃	OPP-01	〃	4	0.606
13	〃	CT522	9	8	0.831	37	〃	OPP-11	〃	4	0.614
14	〃	CT531	10	2	0.312	38	〃	OPP-16	〃	3	0.524
15	〃	CT14	11	6	0.621	39	〃	OPQ-07	〃	4	0.731
16	〃	CT199	11	5	0.468	40	〃	OPS-17	〃	3	0.554
17	〃	GA275	11	5	0.749	41	〃	OPT-07	〃	4	0.568
18	〃	RM167	11	5	0.701	42	〃	OPU-06	〃	6	0.576
19	〃	CT462	12	6	0.689	43	〃	OPU-07	〃	1	0.383
20	RAPD	OPA-02	n.d*	1	0.383	44	〃	OPU-09	〃	4	0.771
21	〃	OPA-07	〃	7	0.849	45	〃	OPU-13	〃	10	0.566
22	〃	OPA-10	〃	7	0.807	46	〃	OPW-02	〃	4	0.658
23	〃	OPB-02	〃	3	0.537	47	〃	OPV-12	〃	3	0.335
24	〃	OPB-03	〃	1	0.351						

n.d* : not determined

HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin. Samples were amplified through 45 cycles of 1 min at 94℃, 1 min at 55℃, 2 min at 72℃, followed by a final extension at 72℃, for 5 min in a Perkin Elmer Thermal Cycler. After amplification, 25 μl of stop solution was added to the PCR products, and they were denatured at 94℃, for 2 min. Six microliters of each reaction were run on a 4% polyacrylamide denaturing gel containing 7 M urea. A silver-staining procedure was used as described by Panaud et al. (1996).

Band patterns for respective RAPD and microsatellite marker were recorded for each variety. Band profiles for each variety were given with 1 or 0 indicating the presence or the absence of a specific

band, respectively. Genetic distances among all 31 varieties were estimated by the Nei's distance equation (Nei, 1987). The unweighed pair group method (UPGMA) in the computer program NTSYS-pc was used to carry out cluster analysis. For both RAPDs and microsatellite markers, the polymorphism information content (PIC) value was used to refer to the relative value of each marker with respect to the amount of polymorphism exhibited. PIC value was estimated as $PIC_i = 1 - \sum_{j=1}^{n} P_{ij}^2$

where P_{ij} is the frequency of the jth pattern for marker i and the summation extends over n patterns (Anderson et al., 1993).

RESULTS AND DISCUSSION

The number of alleles or bands that can be identified at a single locus might determine the ability of different kinds of marker systems in terms of detecting diversity. Table 2 summarizes the number of alleles detected per marker. The number of bands amplified by each random primer ranged from 1 (OPA-02) to 10 (OPU-13). All of 28 RAPD primers were informative and generated bands which differentiated at least one from the other varieties. These RAPD primers generated a total of 114 non-redundant polymorphic bands with an average of 4.07 bands. Also all 19 microsatellite markers representing 19 loci in the rice genome detected polymorphism among the 31 varieties and revealed 91 loci with a mean of 4.80 loci (Table 2). The PIC value was computed for each marker (Table 2). The 28 RAPDs markers had an average PIC value of 0.597 with a range from 0.335 (OPV-12) to 0.849 (OPA-07). The PIC values of mircosatellite markers ranged from 0.175 (GA41) to 0.831 (CT522) averaging 0.607. Microsatellite markers detected higher number of alleles than RAPDs markers (4.80 vs 4.07) although the mean difference was not statistically significant ($t = 1.412$, $P = 0.165$). This seems to indicate that microsatellite markers provide a greater resolution than RAPDs markers in detecting genetic diversity considering that RAPDs markers used in this study had been selected to be informative based on the previous studies (Ahn et al., 1996; Jeon et al., 1999).

Table 3. Combination of RAPDs and mocrosatellite markers which differentiated all the 31 varieties tested.

Combination	Markers
1	OPA-07, OPC-09, OPE-04, OPF-13, OPP-11, OPP-16
2	OPA-07, OPC-09, OPE-04, OPF-13, OPP-11, OPT-07
3	OPA-07, OPC-09, OPE-04, OPE-14, OPF-13, OPP-11
4	OPA-07, OPE-04, OPP-11, GA12*, OPP-16
5	OPA-07, OPE-04, OPP-11, RM164, OPP-16
6	OPA-07, OPE-04, OPP-11, CT22, OPP-16
7	OPA-07, OPE-04, OPP-11, CT522, OPP-16

* Underlined are microsatellite markers

Thirty one varieties used in this study are widely employed as crossing parents for various breeding purposes such as high quality, high yielding, earliness, and so on. Especially, 10 *japonica* varieties from Korea and Japan are high quality rices and are closely related (Kim et al., 1994). For example, Odaebyeo and Sobaegbyeo are sister varieties developed from the cross between 'Fuji 269' and 'Akitsuho'. Ilpumbyeo and Jinmibyeo share the parent, 'Inabawase' in common which was developed from a cross using 'Koshihikari' as the parent. In this regard it would also be interesting to find which individual or combination of RAPDs or SSR is the most informative and useful for fingerprinting these closely related varieties for the purpose of variety protection and identification. Three RAPDs primers (OPA-07, OPC-09 and OPF-13) and two microsatellite markers (CT6 and CT550) showed polymorphism between Odaebyeo and Sobaegbyeo. Polymorphism between Koshihikari and Kinuhikari was detected only with OPP-11. Seven (CT6, CT14, CT22, CT206, CT522, CT531, and GA12) out of 19 microsatellite primers generated subspecies-specific alleles. Additional experiment to confirm whether these polymorphisms generated by seven microsatellite primers represent alleles present either at a higher allele frequency or exclusively in *indica* or *japonica* subspecies is underway with larger samples of rice varieties and the question will be clarified soon. Thirty one cultivars were uniquely distinguished from another using the RAPDs patterns generated by as few as selected six random primers or using a combination of one microsatellite and four random primers (Table 3). These combinations would be potentially useful in rice variety protection and identification considering that *japonica* rices used in this study are closely related with high grain quality.

Genetic distances were calculated for all 465 combinations between the 31 varieties based on 205 non-redundant bands. The distance ranged from 0.0117 (Hitomebore vs Kinuhikari) to 0.3242 (Langz vs Pyungyang 18) with a mean of 0.1604 for the 465 pairwise combinations (data not shown). The average genetic distances between 25 *japonica* varieties per the origin were computed and are presented in Table

Table 4. Genetic distance among *japonica* varieties by the origin.

Origin	Genetic distance			No. of varieties	No. of pairwise combination
	RAPDs	SSR	Total		
Korea	0.052	0.184	0.100	6	15
Japan	0.051	0.082	0.063	4	6
U.S.A.	0.153	0.201	0.171	4	6
Australia	0.128	0.176	0.145	4	6
China	0.078	0.151	0.104	3	3
N. Korea	0.219	0.168	0.201	4	6

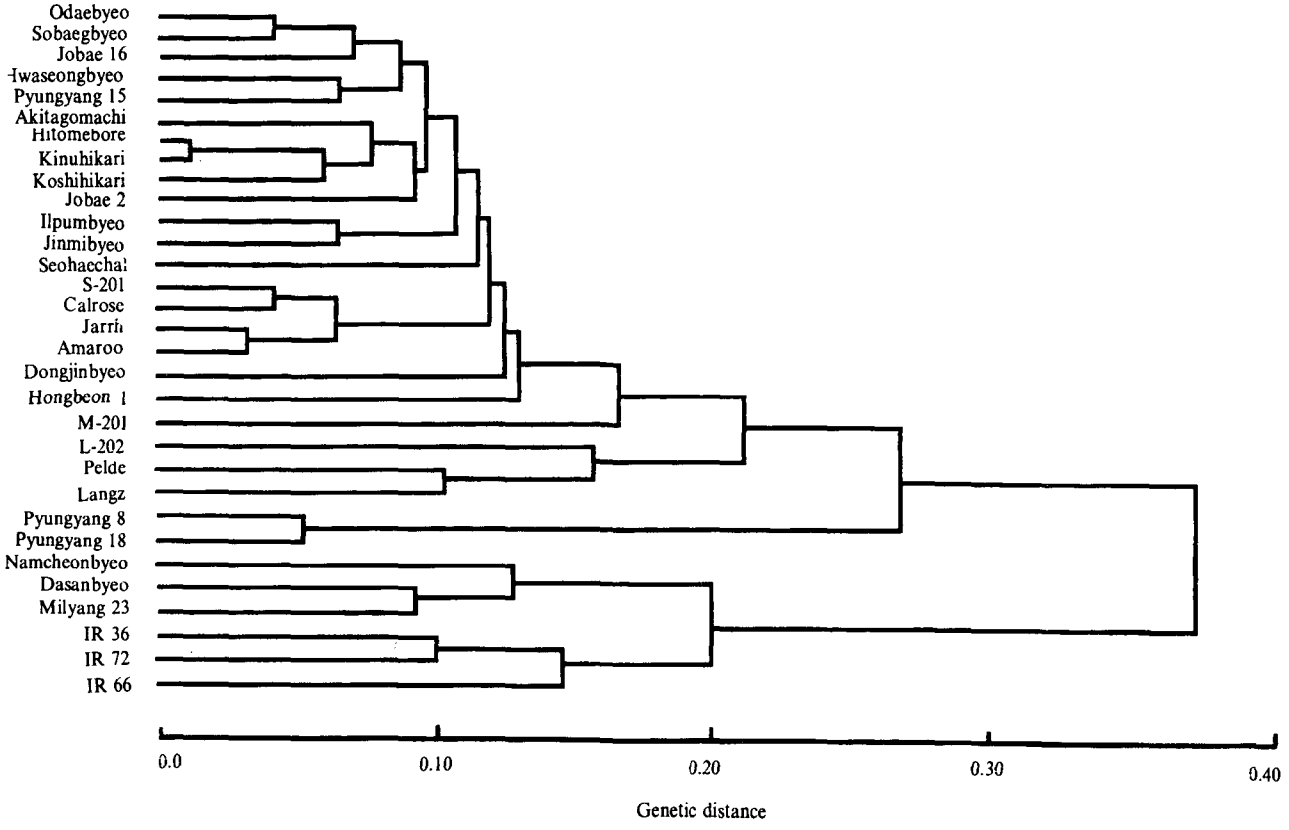

Fig. 1. Cluster diagram for thirty-one cultivars classified by 205 polymorphic bands.

4. With the entire data set of RAPDs and microsatellites the distance with four varieties from North Korea showed the highest value (0.201) among six groups and this value was three times as high as that with four varieties from Japan (0.063). This difference might be attributable to the fact that Pyungyang 8 and Pyungyang 18 derived from a cross between an *indica* and *japonica* cross (personal comm.) were included in samples for North Korea whereas four varieties representing Japan in this study are temperate *japonica* with similar genetic background. The genetic distances within varieties from U.S.A. and Australia were 0.171 and 0.145, respectively and were relatively higher compared with those of Korea (0.100) and China (0.104). The use of both temperate and tropical *japonica* varieties in samples of U.S.A. and tropical *japonica* varieties in Australia might explain the relatively wider genetic diversity whereas only temperate *japonica* varieties were included for Korean and Chinese rice samples in this study. The result that the genetic distance of four varieties from Australia was larger than those of Korean, Japanese and Chinese rice materials seems to indicate that the tropical *japonica* varieties are more divergent than the temperate *japonica* varieties in this study and this needs to be tested using larger samples of rice germ-

plasm.

Cluster analysis based on genetic distances classified 31 varieties into two major groups, *indica* and *japonica* (Fig. 1). The *indica* group was apparently divided into two subgroups of three Tongil-type varieties (Milyang 23, Dasanbyeo, and Namcheonbyeo) and three IRRI bred varieties (*IR 36, IR 66, and IR 72*). In the *japonica* cluster, 25 varieties were divided largely into three subgroups. The first subgroup comprising two varieties (Pyungyang 8 and Pyungyang 18) was loosely connected to the *japonica* cluster. Three tropical *japonica* varieties formed the second subgroup: two from Australia (Pelde and Langz), and one from U.S.A. (L-202). The third subgroup contained six Korean (Odaebyeo, Sobaegbyeo, Ilpumbyeo, Jinmibyeo, Hwaseongbyeo, and Dongjinbyeo), four Japanese (Akitagomachi, Hitomebore, Koshihikari, and Kinuhikari), three Chinese (Jobae 2, Jobae 16, and Hongbeon 1), and two North Korean (Pyungyang 15 and Seohaechal), two Australian varieties (Jarrh, and Amaroo) and three American varieties (S-201, Calrose, and M-201) (Fig. 1). Overall the groupings of the 31 varieties based on RAPDs and microsatellite markers were in good agreement with the genealogical information and this demonstrates that these markers can be useful in assigning rice germplasm into appropriate subspecies.

REFERENCES

Ahn, S. N., H. W. Park, H. C. Choi, and H. P. Moon. 1996. Fingerprinting of *japonica* rice cultivars using RAPD markers. Korean J. Breeding 28(2): 178-183.

Anderson, J. A., G. A. Churchill, J. E. Autrique, and S.D. Tanksley. 1993. Optimizing parental selection for genetic linkage maps. Genome 36: 181-186.

Causse, M. A., T. M. Fulton, Y. G. Cho, S. N. Ahn, J. Chunwongse, K. S. Wu, J. H. Xiao, Z. H. Yu , P. C. Ronald, S. E. Harrington, G. Second, S.R. McCouch, and S. D. Tanksley. 1994. Saturated molecular map of the rice genome based on an intraspecific backcross population. Genetics 138: 1251-1274.

Chunwongse, J., T. B. Bunn, C. Crossman, J. Jiang, and S. D. Tanksley. 1994. Chromosomal localization and molecular marker tagging of the powdery mildew resistance gene (*lv*) in tomato. Theor. Appl. Genet. 89: 76-79.

Fukuoka, S., K. Hosaka, and O. Kamijima. 1992. Use of randomly amplified polymorphic DNAs (RAPDs) for identification of rice accessions. Japan. J. Genet. 67: 243-252.

Glaszman, J. C. 1987. Isozyme and classification of Asian rice cultivars. Theor. Appl. Genet. 74: 21-30.

Jeon, Y. H., S. N. Ahn, H. C. Choi, T. R. Hahn, and H.P. Moon. 1999. Identification of RAPD marker linked to brown planthopper resistance gene in rice. Euphytica (in press).

Kim, K. H., S. Y. Cho, H. P. Moon, and H. C. Choi. 1994. Breeding strategy for improvement and diversification of grain quality in rice (in Korean). Korean J. Breeding 26 (Supp. 2): 3-19.

Lefebvre, V., A. Palloix, C. Caranta, and E. Pochard. 1995. Construction of an interspecific integrated linkage map of pepper using molecular markers and doubled-haploid progenies. Genome 38: 112-121.

Mackill, D. J. 1995. Classifying *japonica* rice cultivars with RAPD markers. Crop Sci. 35: 889-894.

Manual for Standard Evaluation Method in Agricultural Experiment and Research 1995. Rural Development Administration Press (in Korean), Suweon, Korea.

Martin, G. B., J. G. K. Williams, and S. D. Tanksley. 1991. Rapid identification of markers linked to a *Pseudomonas* resistance gene in tomato by using random primers and near-isogenic lines. Proc. Natl. Acad. Sci. USA 88: 2336-2340.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Oka, H. I. 1988. Functions and genetic bases of reproductive barriers. In: Oka HI (ed) Origin of cultivated rice. Japan Scientific Societies Press, Tokyo, pp 181-209.

Olufowote, J. O., Y. Xu, X. Chen, W. D. Park, H. M. Beachell, R. H. Dilday, M. Goto, and S. R. McCouch. 1997. Comparative evaluation of within-cultivar variation of rice (*Oryza sativa* L.) using microsatellite and RFLP markers. Genome 40: 370-378.

Panaud, O., X. Chen, and S. R. McCouch. 1996. Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.). Mol. Gen. Genet. 252: 597-607.

Ranjhan, S., J. L. Glaszman, D. A. Ramirz, and G. S. Khush. 1988. Chromosomal location of four isozyme loci by trisomic analysis in rice (*Oryza sativa* L.). Theor. Appl. Genet. 75: 541-545.

Wang, Z. Y., G. Second, and S. D. Tanksley. 1992. Polymorphism and phylogenetic relationships among species in the genus *Oryza* as determined by analysis of nuclear RFLPs. Theor. Appl. Genet. 83: 565-581.

———. and S. D. Tanksley. 1989. Restriction fragment length polymorphism in *Oryza sativa* L. Genome 32: 1113-1118.

———, J. L. Weber, G. Zhong, and S. D. Tanksley. 1994. Survey of plant short tandom DNA repeat. Theor. Appl. Genet. 88: 1-6.

Wu, K. S., and S. D. Tanksley. 1993. Abundance, polymorphism and genetic mapping of microsatellites in rice. Mol. Gen. Genet. 241: 225-235

Yu, L. X., and H. T. Nguyen. 1994. Genetic variation detected with RAPD markers among upland and lowland rice cultivars (*Oryza sativa* L.). Theor. Appl. Genet. 87: 668-672.