

Genetic Diversity among Tea (*Camellia sinensis*) Accessions Based on Random Amplified Polymorphic DNA (RAPD) Patterns

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ABSTRACT

Genetic diversity of 45 tea accessions from Korea, Japan, China and Taiwan was investigated by using RAPD analysis. Out of the eighty primers screened, twenty primers generated 99 polymorphic bands with a polymorphic rate 87.0%. The size of the amplified fragments ranged from about 3,138 bp to 520 bp. By cluster analysis, all of the 45 accessions can be grouped into five groups. Over 90% of the 32 Korean accessions belonged to group II, III, IV and V. Moreover, newly developed Korean cultivars (accession no. 13, 14 and 15) belonged to very different group compared with any other Korean accessions. Among the Korean accessions, the minimum genetic similarity 0.500 was obtained between accession no. 17 and 37 and the largest genetic similarity 0.912 between no. 20 and 21.

Key words : *Camellia sinensis*, cluster analysis, genetic diversity, RAPD, tea

INTRODUCTION

Tea (*Camellia sinensis*) was discovered more than 2,000 years ago in China but is naturally distributed in the whole Asian Monsoon region (Banerjee, 1992). Tea is one of the most popular beverages consumed in Asian. Due to its high economic importance, numerous studies devoted to its botany and taxonomy (Sealy, 1958; Wight, 1992; Lee and Nou, 1995; Oh and Hong, 1995; Wachira *et al.*, 1995; Ueno *et al.*, 1999; Mondal *et al.*, 2000; Shiv *et al.*, 2000; Park *et al.*, 2002). Tea can be classified two geographical varieties based on leaf and growth characteristics: the small leaf, dwarf

and slow growing *Camellia sinensis* var. *sinensis* from China and the large leaf, tall and quick growing *Camellia sinensis* var. *assamica* (Masters) Kitamura from the Assam region in India. Korean tea was originated from mainland China and was brought by Buddhist monks as early as the seventh century. Though tea plants are distributed in only southern areas of Korea, genetic variability of the plants may be very diverse. Because tea plants have a strong self-incompatibility controlled by multi-alleles (de Nettancourt, 1997). And the genes responsible for pollination reactions would be heterogeneous in natural population.

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Molecular approach such as RFLP, RAPD and AFLP, provide an information to study the variability within plant germplasm (Welsh and McClelland, 1990; Williams *et al.*, 1990; Koller *et al.*, 1993). Genetic analysis using RFLP showed limitation in Japanese tea cultivars (Matsumoto *et al.*, 1994). As an alternative, the RAPD method described by Williams *et al.* (1990), which is similar to the arbitrarily primed PCR (Welsh and McClelland, 1990), is a fast and simple approach for exploring genetic polymorphism. RAPD methodology has been successfully applied in determining the relationships among clones, varieties and species of the genus *Camellia* (Matsumoto *et al.*, 1994; Lee and Nou, 1995; Oh and Hong, 1995; Wachira *et al.*, 1995).

In the present article, we report the use of RAPD markers to classify Korean, Japanese, Chinese and Taiwan tea accessions and to examine genetic diversity among them.

MATERIALS AND METHODS

Plant materials

A total of 45 tea (*Camellia sinensis*) accessions including 3 Taiwanese, 4 Chinese, 6 Japanese and 32 Korean accessions were used in this study. The Korean tea accessions were collected from southern regions of Korea. All of the tea accessions were obtained from the Korea Experimental Tea Station at Bosung. The origins of the accessions were listed in Table 1.

DNA extraction

DNA was extracted from 100mg fresh leaf power samples of all accessions using GMO DNA Extraction Kit (Bioneer). One hundred mg of leaf tissue was frozen in liquid nitrogen and ground into a fine powder using a pestle. The pulverized material was transferred to a 1.5ml centrifuge tube and add 400 μ l plant lysis buffer. After 10 minutes, centrifuge the tube at 12,000rpm for 5

minutes, then transfer the supernatant to a new centrifuge tube. And 100 μ l isopropanol was added and lightly vortex for about 5 seconds. The binding column was fit into the 2ml centrifuge tube and the liquid was transferred into the binding column. Then the 2ml tube with binding column was centrifuged for 1 minute at 8,000rpm and the binding column was transferred to a new 2ml centrifuge tube. Finally 100 μ l preheated elution buffer (70°C) was added into the binding column, centrifuged at 8000rpm for 1 minute and quantified DNA content by spectrophotometer (Ultraspec[®] 2000, Pharamacia Biotech).

DNA amplification

Eighty arbitrary 10-mer primers (kit A, B, C, D from Operon Technologies Inc.) were used for the PCR based on the protocol of Williams *et al.* (1990) with some modification. The reaction condition was: 50ng of genomic DNA, 1 units of *Taq* polymerase, 10 \times PCR reaction buffer (10mM Tris-HCl pH 8.3; 50mM KCl; 1.5mM MgCl₂), 0.2mM dNTP, 50pmol primer. The final volume per reaction was 25 μ l. The PCR reactions were run in a Gene Amp[®] 2700 (Applied Biosystem). DNA was amplified by the following procedure: pre-denature at 94°C for 2 min 1 cycle; denature at 94°C for 30 sec, annealing at 36°C for 1 min, extension at 72°C for 2 min, 15 cycles; denature at 94°C for 30 sec, annealing at 45°C for 1 min, extension at 72°C for 2 min, 25 cycles; post-extension at 72°C for 10 min, and then followed by soaking at 4°C. The PCR product were separated on 1.2% agarose gel and stained with ethidium bromide. The gels were visualized with a UV trans-illuminator and photographed.

Data analysis

Twenty primers of ten bases in length were selected from a pool of primers (Table 2) that gave reasonable numbers of strong amplification products under the PCR conditions described above.

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Table 1. Origin of the tea (*Camellia sinensis*) accessions used in this study

No.	Accession	Origin	Country
01	Taicha27	Seed selection cultivated	Taiwan
02	Taicha29	Seed selection cultivated	Taiwan
03	Cheongsimoeryong	Seed selection cultivated	Taiwan
04	Kukaengjong	Seed selection cultivated	China
05	Yabukita	Seed selection cultivated	Japan
06	Kurasawa	Yabukita seedling	Japan
07	Fujimidori	Seed selection cultivated	Japan
08	Keumgokro	Seed selection cultivated	Japan
09	Saemidori	Seed selection cultivated	Japan
10	Shunmei	Seed selection cultivated	Japan
11	Youngsang	Seed selection cultivated	China
12	Kukaengjong	Seed selection cultivated	China
13	Myungseon	Seed selection cultivated	Korea
14	Charmnok	Seed selection cultivated	Korea
15	Bohyang	Seed selection cultivated	Korea
16	4-1-12	Seed selection cultivated	Korea
17	3-25-5	Seed selection cultivated	Korea
18	3-27-4	Seed selection cultivated	Korea
19	4-2-3	Seed selection cultivated	Korea
20	4-4-5	Seed selection cultivated	Korea
21	4-6-1	Seed selection cultivated	Korea
22	4-25-12	Seed selection cultivated	Korea
23	2-8-3	Seed selection cultivated	Korea
24	4-11-45	Seed selection cultivated	Korea
25	4-21-1	Seed selection cultivated	Korea
26	3-7-11	Seed selection cultivated	Korea
27	2-31-12	Seed selection cultivated	Korea
28	2-8-20	Seed selection cultivated	Korea
29	1-26-4	Seed selection cultivated	Korea
30	2-23-11	Seed selection cultivated	Korea
31	4-22-8	Seed selection cultivated	Korea
32	2-12-11	Seed selection cultivated	Korea
33	2-2-4	Seed selection cultivated	Korea
34	3-6-14	Seed selection cultivated	Korea
35	2-24-33	Seed selection cultivated	Korea
36	3-7-17	Seed selection cultivated	Korea
37	3-22-3	Seed selection cultivated	Korea
38	3-2-6	Seed selection cultivated	Korea
39	2-8-13	Seed selection cultivated	Korea
40	2-9-3	Seed selection cultivated	Korea
41	2-2-2	Seed selection cultivated	Korea
42	4-1-10	Seed selection cultivated	Korea
43	1-4-4	Seed selection cultivated	Korea
44	3-7-19	Seed selection cultivated	Korea
45	Bokjeongdaebaek	Seed selection cultivated	China

Each plant and cultivar was scored for the presence (1) or absence (0) of the amplification products and the results were put into a binary data matrix. Common band analysis was conducted to make pairwise comparisons between the plants and cultivars, and to determine the values of genetic distance (Rogers, 1972) among them. The genetic distance was calculated as the Euclidian value of the total number of bands scored that each pair of plants and cultivars. Dendrograms were generated by cluster analysis of genetic distance values based on Ward method analysis (Manly, 1986). Factorial correspondence analysis was carried out on the 45 × 20 raw data matrix. Both cluster and factorial

correspondence analyses were performed using SAS 8.2 statistical software (SAS Institute Inc., USA).

RESULTS AND DISCUSSION

RAPD polymorphism pattern in the tea accessions

The genetic similarity among 45 cultivars was evaluated by RAPD analysis by using 80 arbitrary 10-mer primers. Among these primers 20 primers that gave amplification products in the initial screening were selected. And clear and scorable bands were obtained for data analysis.

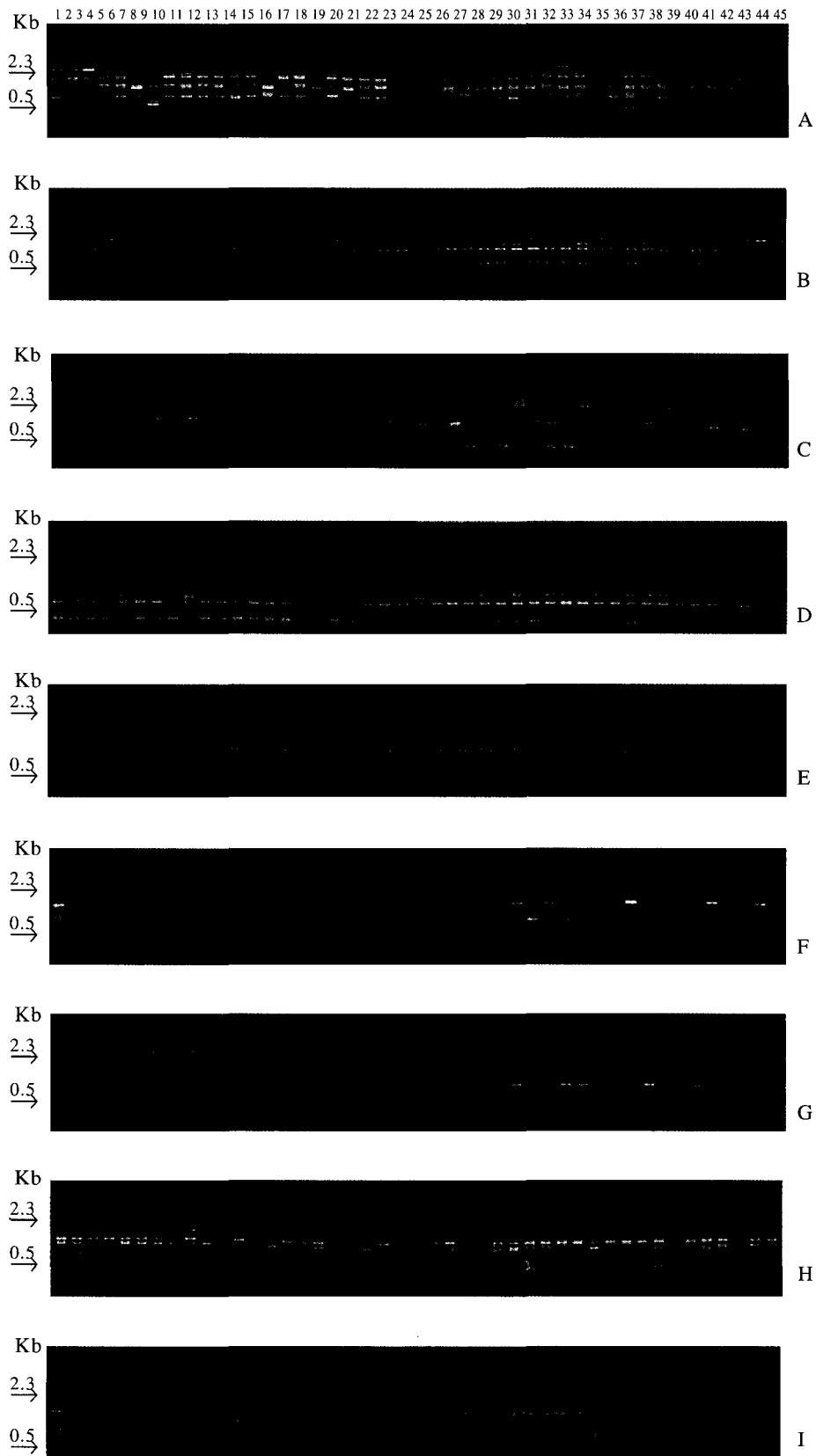
A total of 114 bands were scored, of which 99

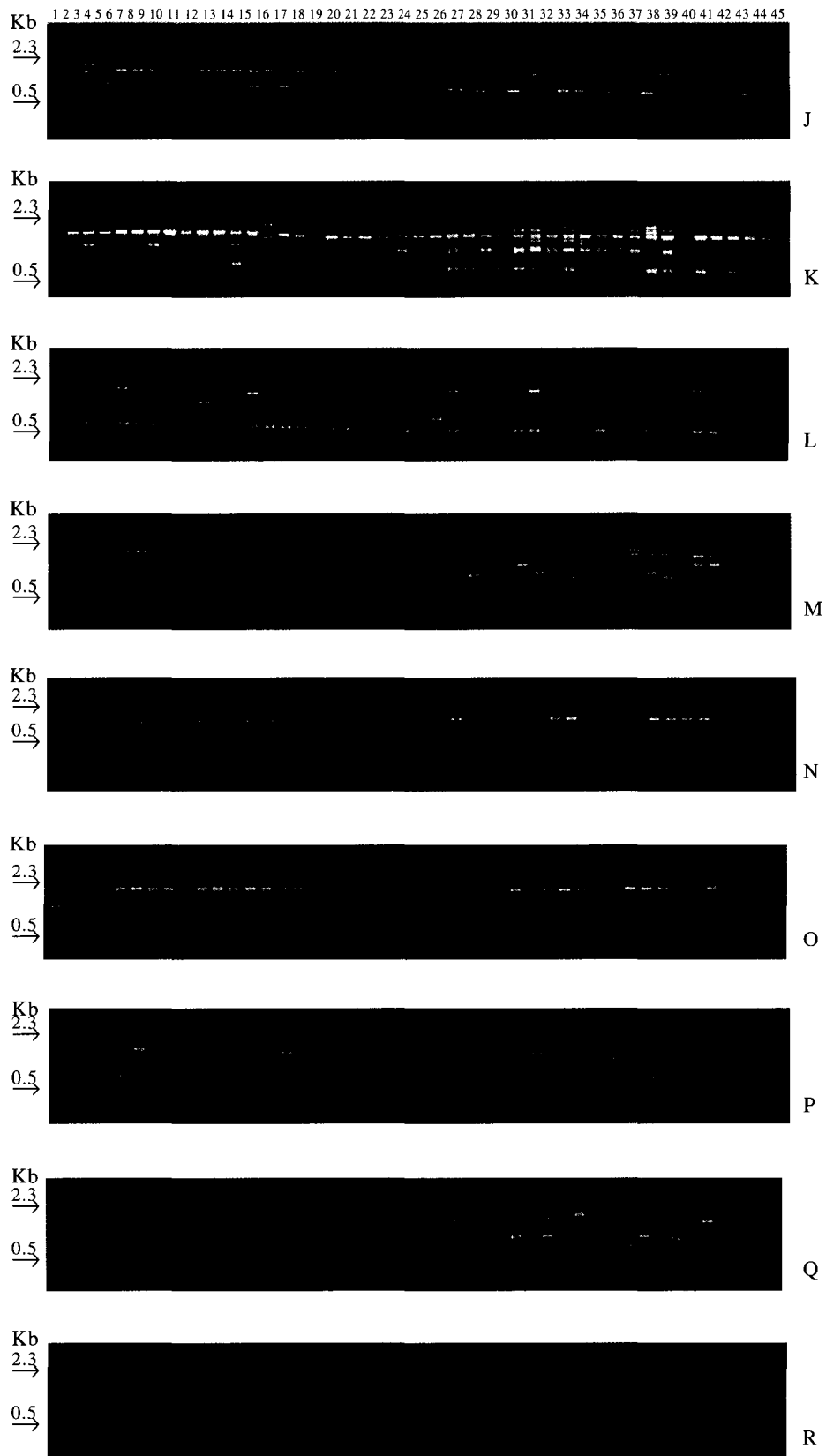
Table 2. Nucleotide sequences of random primer (Operon Technologies Inc.) and polymorphism rate (%)

Fig. no.	Primer	Sequence	G+C cont.(%)	Total band number	No. of polym*	Polymor- phism rate (%)
A	OPA-01	CAGGCCCTTC	70%	10	8	80.0
B	OPA-04	AATCGGGCTG	60%	6	4	66.6
C	OPA-09	GGGTAACGCC	70%	3	3	100
D	OPA-11	CAATCGCCGT	60%	4	3	75.0
E	OPA-13	CAGCACCCAC	70%	2	2	100
F	OPB-03	CATCCCCCTG	70%	8	8	100
G	OPB-05	TGCGCCCTTC	70%	5	4	80.0
H	OPB-06	TGCTCTGCCC	70%	4	3	75.0
I	OPB-08	GTCCACACGG	70%	8	7	87.5
J	OPB-10	CTGCTGGGAC	70%	8	7	87.5
K	OPB-12	CCTTGACGCA	60%	5	4	80.0
L	OPB-18	CCACAGCAGT	60%	6	5	83.3
M	OPC-06	GAACGGACTC	60%	8	8	100
N	OPC-09	CTCACCGTCC	70%	5	5	100
O	OPC-11	AAAGCTGCGG	60%	7	6	85.7
P	OPC-16	CACACTCCAG	60%	5	4	80.0
Q	OPC-18	TGAGTGGGTG	60%	4	3	75.0
R	OPD-03	GTCGCCGTCA	70%	7	6	85.7
S	OPD-11	AGCGCCATTG	60%	4	4	100
T	OPD-20	ACCCGGTCAC	70%	5	5	100
Total				114	99	87.0

*Number of polymorphism bands.

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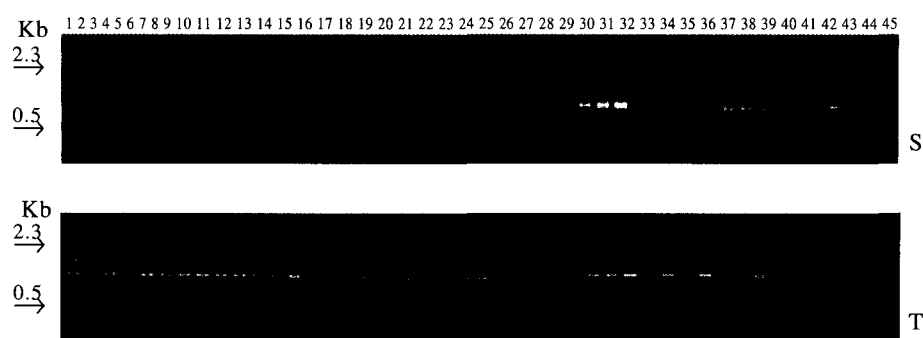


Fig. 1. Agarose gel electrophoresis patterns of RAPD-amplified *C. sinensis* DNA. DNA from each of the 3 Taiwanese, 4 Chinese, 6 Japanese and 32 Korean green tea accessions was amplified using primer A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T (Table 2) and separated on a 1.2% agarose gel as described in the methods. The numbers of the lane indicate the sample number as in Table 1.

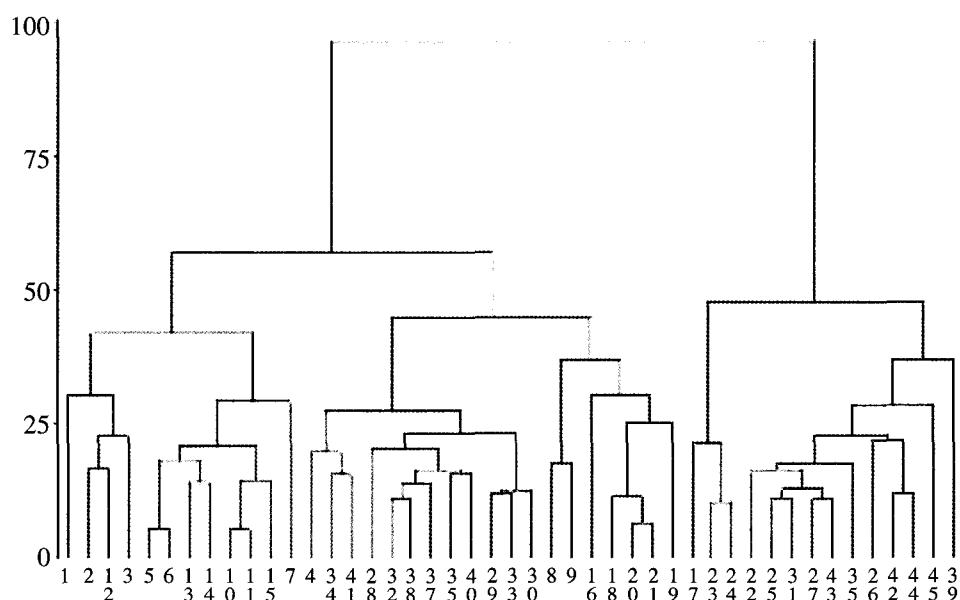


Fig. 2. Dendrogram generated by cluster analysis using Ward method. Relative branch lengths indicate relative genetic distances between cultivars.

(87.0%) were polymorphic by the 20 primers (Table. 2). This results is very similar to previous reports (Oh and Hong, 1995). The number of bands for each primer varied from 2 (OPA-13) to 10 (OPA-01), with an average of 5.7 bands per primer. In addition, the number of polymorphic bands per primer varied from 2 (OPA-13) to 8 (OPA-01, OPC-06) with an average of 5. The size of the amplified fragment ranged from about 520 bp to 3,138 bp (Fig. 1).

The total number of DNA bands generated in this study was very similar to that observed in 27 accessions which revealed 99 bands per 17 primers in intraspecific tea plants (Shiv *et al.*, 2000). The level of polymorphism observed by using RAPD primers indicates extensive amount of genetic variation among the tea samples. The result is consistent with that genetic diversity exists in the teas (Oh and Hong, 1995; Shiv *et al.*, 2000).

Specific DNA marker was identified only in an accession no. 1 by OPA-13 primer among the 45 accessions. Polymorphism information and line specific DNA bands obtained through RAPD analysis may be useful to identify polymorphic primers for further studies in the *Camellia* species.

Genetic similarity

Genetic similarities among the 45 tea accessions ranged between 0.500 and 0.939 (Table 3). Among the Korean accessions, the minimum genetic similarity 0.500 was obtained between accession no. 17 and 37 and the largest genetic similarity 0.912 between no. 20 and 21. The distance that was somewhat higher than that reported by Shiv *et al.* (2000) in intraspecific variations of tea accessions with a genetic distance ranging from 0.51 to 0.64. Among the Korean accessions, the minimum genetic similarity 0.500 was obtained between accession no. 17 and 37 and the largest genetic similarity 0.912 between no. 20 and 21. The genetic similarity of domestic tea accessions is similar to previous reports (Oh and Hong, 1995).

Cluster analysis of the genetic distance values was carried out to generate dendrograms indicating relationships among accessions. By ward method, the 45 samples could be divided into 5 groups at below about 42 of Euclidian distance value; group I, including 12 accessions (sample no. 1, 2, 12, 3, 5, 6, 13, 14, 10, 11, 15, 7), with 0.649~0.939 similarity indices; group II, including 12 accessions (sample no. 4, 34, 41, 28, 32, 38, 37, 36, 40, 29, 33, 30), with 0.675~0.833 similarity indices; group III, including 7 accessions (sample no. 8, 9, 16, 18, 20, 21, 19), with 0.649~0.912 similarity indices; group IV, including 3 accessions (sample no. 17, 23, 24), with 0.763~0.860 similarity indices; group V, including 11 accessions (sample no. 22, 25, 31, 27, 43, 35, 26, 42, 44, 45, 39), with 0.632~0.825 similarity indices (Fig. 2). The high similarity index arise between accession no. 10 and 11,

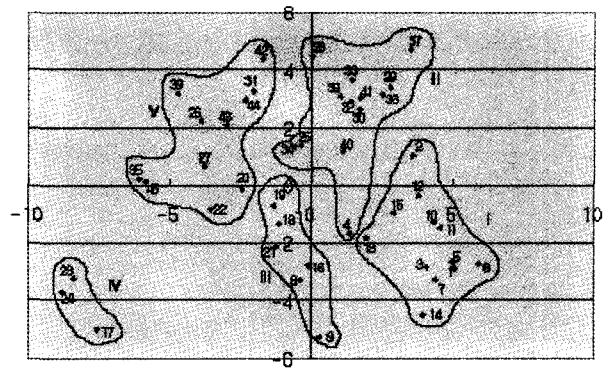


Fig. 3. Factorial correspondence analysis (first and second principal components) was carried out on 45 tea accessions and 20 RAPD bands.

because its origin is the same country. The index of the next arise between accession no. 5 and 6 which are Japanese cultivar, Yabukita and Kurasawa. Approximately 90.6% of Korean cultivars belonged to group II, II, IV and V. Interestingly, newly developed cultivars from the Korea Tea Experiment Station, accession no. 13, 14 and 15, belonged to group I. And most of the other countries' belonged to group I except for no. 8, 9 and 45.

Factorial correspondence analysis carried out on a 45 × 20 (accession × markers) matrix confirmed the relationships with cluster analysis (Fig. 3). The accessions no. 2, 4 and 9 were relatively distant from a homogeneous group. However, most of accessions except for accession no. 25 (Fig. 3) were very related with the groupings by cluster analysis (Fig. 2). The result with closed relationship between cluster analysis and correspondence analysis at RAPD level is consistent with the previous reports (Park *et al.*, 2002; Shiv *et al.*, 2000).

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