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Genetic Diversity and DNA Polymorphism in *Platycodon grandiflorum* DC. Collected from East-Asian Area

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ABSTRACT : Broadening the genetic base of *Platycodon grandiflorum* DC. cultivar to sustain improvement requires assessment of genetic diversity available in *P. grandiflorum* DC.. The objective of this study was to analyze the genetic variation, genetic relationship among 48 samples collected from East-Asian Area by means of RAPD-PCR (random amplified polymorphic DNA-polymerase chain reaction) markers. From the 18 primers tested, produced total 211 bands with an average of 11.7 bands per primer and obtained 103 polymorphic band with an average of 5.7 bands per primer,s revealed relatively high percentage of polymorphic bands (48.8%). The genetic similarities calculated from RAPD data varied from 0.688 to 0.994 and were clustered to six major groups on a criterion of 0.78 similarity coefficient. The present study has revealed the significant genetic similarity among the samples tested. The analysis of genetic relationships in *P. grandiflorum* using RAPD-PCR banding data can be useful for the breed improvement.

Key words : *Platycodon grandiflorum* DC., genetic diversity, RAPD-PCR

INTRODUCTION

P. grandiflorum (Jacq.) A. DC. (Pharmaceutical Name: Radix Platycodi) is a oriental medicinal plant and mainly distributed in Korea, China and Japan. The root contains many saponins such as platycodigenin (main saponin), small amount of polygalacic acid, platycogenic acid A, platycogenic acid BC, platycogenic acid C etc. and have the function of promote the dispersing of the lungs, resolve phlegm and expel pus, and widely used in treatment of rids of phlegm, inhibits cough, treats bronchitis, rids of puss, for tonsilitis, and sore throat (Chapmen & Hall, 1994). For the reason of wide usage of *P. grandiflorum*, recently it is cultured in large quantities in countries of East-Asian Area. But compare with the relatively importance of the usage and large quantities of cultivate, the effort and research is limited, especially in breeding programs compared with other crops. Until now, almost of the wide type of *P. grandiflorum* were cultured. It is limitate the improvement of the yield and purity of *P. grandiflorum*. For satisfaction the recently increasing demand of *P. grandiflorum*, it is necessary to develop the breeding program for the activation of the breed improvement.

Understanding the genetic similarity and diversity of frequently used germplasm is vital to any breeding program attempting to increase the genetic diversity of new cultivar. An accurate knowledge of the origin and genetic distance of parental germplasm may also lead to a better understanding of the inheritance of important genetic traits and helpful to correct selection of parent for conventional cross breeding program.

Genetic markers are a basic tool of plant breeders use for cultivar identification. Recently developed molecular marker techniques were widely used for analysis of the genetic similarity and diversity in many plant population. The RFLP markers have been used in rice and *Vigna* (Zhang *et al.*, 1992; Fatokun *et al.*, 1993), the AFLP markers have been used in cowpea (Fatokun *et al.*, 1997), the SSR markers have been used in wheat and maize (Plaschke *et al.*, 1995; Senior *et al.*, 1998) and the isozyme markers have been used in *Vigna unguiculata* (L.) Walp. and rice (Panella & Gepts, 1992; Second, 1985). Especially PCR-based RAPD markers (Williams *et al.*, 1990) has been a boon to plant breeders and geneticists in recent years. RAPD markers have the advantage of combining low technical input with almost unlimited marker numbers. RAPD markers have proven extremely useful in determining genetic relationships among breeding materials and fingerprinting cultivars in many crop plant, and have been used in coffee, flax, tea, *Coffea*, *Claviceps purpurea*, *Scleritinia homoeocarpa*, hordeum, cowpea, Mahogany, mate and flax

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(Orozco-Castillo *et al.*, 1994; Campbell *et al.*, 1995; Wachira *et al.*, 1995; Lashermes *et al.*, 1996; Jungehlsig & Tudzynski, 1997; Raina *et al.*, 1997; De Bustos *et al.*, 1998; Mignouna *et al.*, 1998; Gillies *et al.*, 1999; Gauer & Cavalli-Molina, 2000; Fu *et al.*, 2002). About the genetic variation and relationship analysis are limit in genetic resources of *P. grandiflorum*. In this paper we inverstigate genetic similarity of 48 cultivars of *P. grandiflorum* by using RAPD-PCR markers.

MATERIALS AND METHODS

Plant materials

The 48 cultivar of *P. grandiflorum* seeds collected from Korea, China and Japan were cultured in the filed of National Institute of Crop Science, RDA, Suwon, Korea and the DNAs were extracted from the leaves and applied in RAPD analysis. The samples used in the present study along with their collection site and country of origin are listed in Table 1.

DNA extraction

From the each cultivar, randomly selected five plants and the total genomic DNA were extracted with CTAB (cethyltrimethylammonium bromide) method according to the protocols (Doyle & Doyle, 1987), with minor modifications and pooled by cultivar. The young leaves (0.1~0.3 g) were ground to a fine powder in liquid nitrogen and mixed with extraction buffer (1% CTAB, 50 mM Tris-HCl pH 8.2, 10 mM EDTA, 0.7 M NaCl, 1% 2-mercaptoethanol) until it was homogenized. After incubation at 65°C for 10~30 minutes, equal volume of chloroform/isoamylalcohol (24:1) was added, and the mixture was shaken vigorously. The extract was centrifuged at about 12,000 rpm for 10 min at room temperature or until the aqueous phase was clear, and the supernatant transferred to a new microtube. The chloroform/isoamylalcohol extraction step was repeated twice. The aqueous phase containing the DNA was extracted and the DNAs were precipitated with 2/3 volumes of isopropanol. The nucleic acid precipitated was washed twice with 500 $\mu\ell$ of 70% ethanol and dried at room temperature. The pellet was dissolved with TE buffer solution containing 0.001 $\mu g/\ell$ RNase A. DNA was quantified on 0.8% agarose gels and diluted to uniform concentration (10 ng/ $\mu\ell$) for RAPD analysis and stored at –20°C until use.

RAPD analysis

A subset of three samples randomly selected from each of the three countries were used to screen the primers. Once the primers were selected, they were used to amplify DNA from all 48 samples. A total of 80 primers (OPA01-20, OPB01-20, OPC01-20, OPD01-20; Operon Technologies Inc., Alameda, CA) were applied in RAPD-PCR analysis. PCR reactions were performed according to the protocol of Jin *et al.* (2003). Briefly, PCR amplifications were carried out in a DNA Ther-

Table 1. List of samples used in the RAPD analysis of this study.

Entry no.	Sample name	Collection site	Color of flower
1	Jinke 1 hao	Anhui (China)	Purple
2	Taiji 1 hao	Anhui (China)	Purple
3	Sandonglensun	Sandong (China)	Purple
4	Neimeng 1 zhuofeng	Neimeng (China)	Purple
5	Neimeng 2	Neimeng (China)	Purple
6	Neimeng 3	Neimeng (China)	Purple
7	Liaoningxinbin	Liaoning (China)	Purple
8	Heilongjiaonghailin	Heilongjiang (China)	Purple
9	Heilongjiaongningan	Heilongjiang (China)	Purple
10	Miliza	Japan	
11	Samidale	Japan	
12	Kangwen	Kangwen (Korea)	Purple
13	South korea	Chuguangyyong (Korea)	White
14	North Korea	Pinglang (North Korea)	White
15	North Korea	Pinglang (North Korea)	White
16	Yongxin	Yongxin (China)	White
17	Jilin-1	Changchun (China)	Purple
18	Jilin-2	Tonghua (China)	Purple
19	Jilin-3	Tonghua (China)	Purple
20	Jilin-4	Dunhua (China)	Purple
21	Jilin-5	Dunhua (China)	Purple
22	Jilin-6	Wanbao (China)	Purple
23	Jilin-7	Wanbao (China)	Purple
24	Jilin-8	Techanzhanke (China)	Purple
25	Jilin-9	Jilin (China)	Purple
26	Gosun-1	Japan	Purple
27	Gosun-2	Japan	
28	Gosun-3	Japan	
29	Gosun-4	Japan	Purple
30	Gosun-5	Japan	Purple
31	Japan-1	Japan	Purple
32	Japan-2	Japan	Purple
33	Japan-3	Japan	
34	Japan-4	Japan	White
35	Samidale-1	Japan	
36	Samidale-2	Japan	Purple
37	Samidale-3	Japan	
38	5 weilwu	Japan	White
39	North korea-2	North Korea	
40	Hashida	China	White
41	Longjing-bai	Longjing	White
42	Longjing-zhi	Longjing	Purple
43	Antu-zhi	Antu	
44	Hamyang	South Korea	Purple
45	Jinan	South Korea	
46	Zhangbiak	South Korea	
47	Yinjea	South Korea	White
48	Yiseng	South Korea	White

mal Cycler (Perkin Elmer Cetus, USA), in a reaction volume of 20 $\mu\ell$ containing 1x PCR buffer (75 mM Tris-/HCl, pH 8.4, 50 mM KCl, 2.0 mM $MgCl_2$, and 20 mM $(NH_4)_2SO_4$), 0.2 mM each of dATP, dTTP, dCTP, and dGTP, 0.2 μm primer, 1 unit of *Taq.* Polymerase (Bioneer) and 40 ng of template DNA in sterile distilled water. Amplification reactions were carried out using the following cycle profile: initial denaturation at 94°C for 3 min followed by 2 cycles at 94°C for 30s, 36°C for 30s, 72°C for 3 min; and 20 cycles at 94°C for 30s, 36°C for 15s, 45°C for 15s, 72°C for 90s; and 19 cycles at 94°C for 20s, 36°C for 15s, 45°C for 15s, 72°C for 2 min; and a final 7 min for extension at 72°C PCR products were electrophoresised on a 1.5% (w/v) agarose gel in 1×TBE buffer (80 mM Tris-borate, 2 mM EDTA, pH 8.0) and stained with ethidium bromide. A 1.0 kb ladder DNA marker (Pharmacia) was used as a size standard. The gel image was recorded using a Gel Documentation System (UVP, UK). All PCR reactions were run in twice, and only bright and highly reproducible RAPD primers were chosen for RAPD analysis and the RAPD bands were calculated and the Dendrogram was constructed by UPGMA (unweighted pair-group method using arithmetic averages) method on the basis of RAPD data.

Data analysis

DNA banding patterns generated by RAPD-PCR were scored for the presence (1) or for absence (0) of each amplified band. All RAPD assays were repeated twice and only the reproducible bands were scored. For considering a marker as polymorphic, the absence of an amplified product in at least one species was used as a criterion. Using the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis for personal computer) software, version 2.1 (Rohlf, 2000), a dendrogram was constructed using the UPGMA method.

RESULTS AND DISCUSSION

RAPD-PCR analysis

RAPD-PCR is one of fast and simple research method to identify genetic difference and the polymorphism in various organisms that does not require the prior knowledge of the genomic DNA (Welsh *et al.*, 1991; Klinbunga *et al.*, 2000). In present study, a total of 48 DNA samples of *P. grandiflorum* DC. were applied in RAPD-PCR analysis. Eighteen primers (Table 2) out of 80 tested, were selected for the reliability and repeatability of banding patterns. Fig. 1 and 2 showed the two examples of RAPD-PCR patterns using Operon C07 and Operon C17 primer, respectively. The amplification of the genomic DNA generated total 211 bands with an average of 11.7 bands per primer and obtained 103 polymorphic band patterns with an average of 5.7 bands per primer. The percentage of polymorphic bands (48.8%) of RAPD-PCR in the species was higher than in other plants, e.g. *Lactoris fernandeziana* (Lactoridaceae) 24.5% (Crawford *et al.*, 1994), *Cathaya argyrophylla* 32% (Wang *et al.*, 1996), *Paeonia suffruticosa* 22.5% and *P. rockii* 27.6% (Pei *et al.*, 1995), and *Dacydium pierrei* 33.3% (Su *et al.*, 1999).

Table 2. Total number of amplified fragments and number of polymorphic bands generated by PCR using selected random decamers in 48 varictics of *P. grandiflorum*.

Name of primer	Sequence of primer	Total No. of amplification products (211)	No. of polymorphic products (103)	Size range (kb)
OPA 20	GTTGCGATCC	14	5	0.41~1.85
OPB 11	GTAGACCCGT	14	8	0.42~1.87
OPB 15	GGAGGGTGTT	13	7	0.45~2.20
OPB 17	AGGGAACGAG	10	5	0.47~1.80
OPB 18	CCACAGCAGT	8	4	0.54~2.00
OPC 07	GTCCCGACGA	11	5	0.30~1.60
OPC 08	TGGACCGGTG	13	7	0.33~1.60
OPC 10	TGTCTGGGTG	10	7	0.50~1.90
OPC 12	TGTCATCCCC	18	8	0.45~1.75
OPC 13	AAGCCTCGTC	7	3	0.45~1.22
OPC 16	CACACTCCAG	8	4	0.80~1.62
OPC 17	TTCCCCCCAG	10	5	0.54~2.20
OPC 19	GTTGCCAGCC	16	6	0.48~2.00
OPC 20	ACTTCGCCAC	17	8	0.40~2.10
OPD 01	ACCGCGAAGG	18	14	0.41~1.80
OPD 02	GGACCCAACC	8	2	0.40~1.60
OPD 03	GTCGCCGTCA	7	2	0.41~1.62
OPD 11	AGCGCCATTG	9	3	0.40~1.20

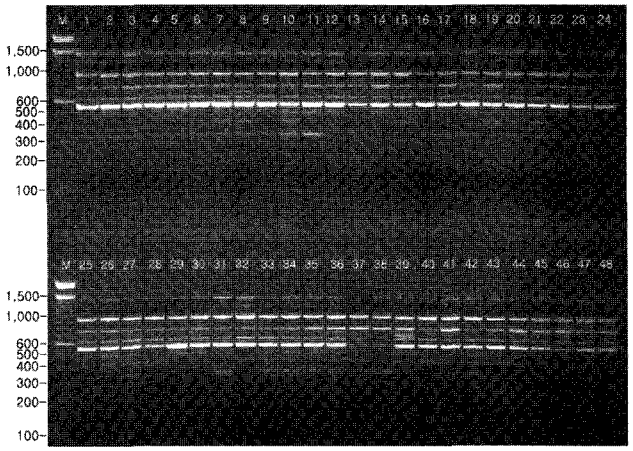

Fig. 1. Profiles of PCR products obtained from genomic DNA using the 10-based Operon C07 (OPC07) primer. Lane M, 1 kb DNA ladder; Lanes 1 to 48 correspond to series number for samples listed in Table 1.

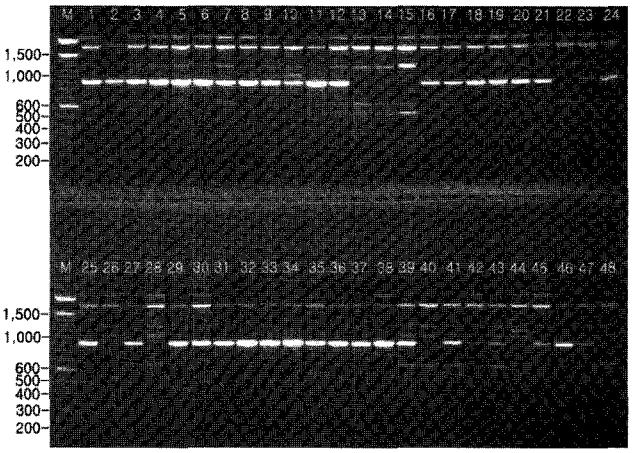

Fig. 2. Profiles of PCR products obtained from genomic DNA using the 10-based Operon C17 (OPC17) primer. Lane M, 1 kb DNA ladder; Lanes 1 to 48 correspond to series number for samples listed in Table 1.

Genetic relationships among samples

In present study, genetic similarities of all 48 cultivar reflected in the 103 RAPD loci were calculated. All the polymorphic bands were scored by presence vs. absence of specific amplification products and the data was used to calculate values of genetic distance between all the samples studied. By UPGMA cluster analysis, the genetic relationships among the 48 samples as revealed by genetic distance calculated from RAPD-PCR data varied from 0.688 to 0.994, and clustered into six major groups on a criterion of 0.78 similarity coefficient (Fig. 3). The cluster analysis indicates that 48 samples of *P. grandiflorum* can be grouped into six clusters based on similarity coefficient. The first major cluster (I) comprises two sub-minor cluster (A_1: Case No. 33, 32; A_2: Case No. 34, 31); the second major cluster (II) consist one minor cluster having one sample, only (B: Case No. 29); the third major cluster (III) consist two minor clusters, each minor cluster having one sam-

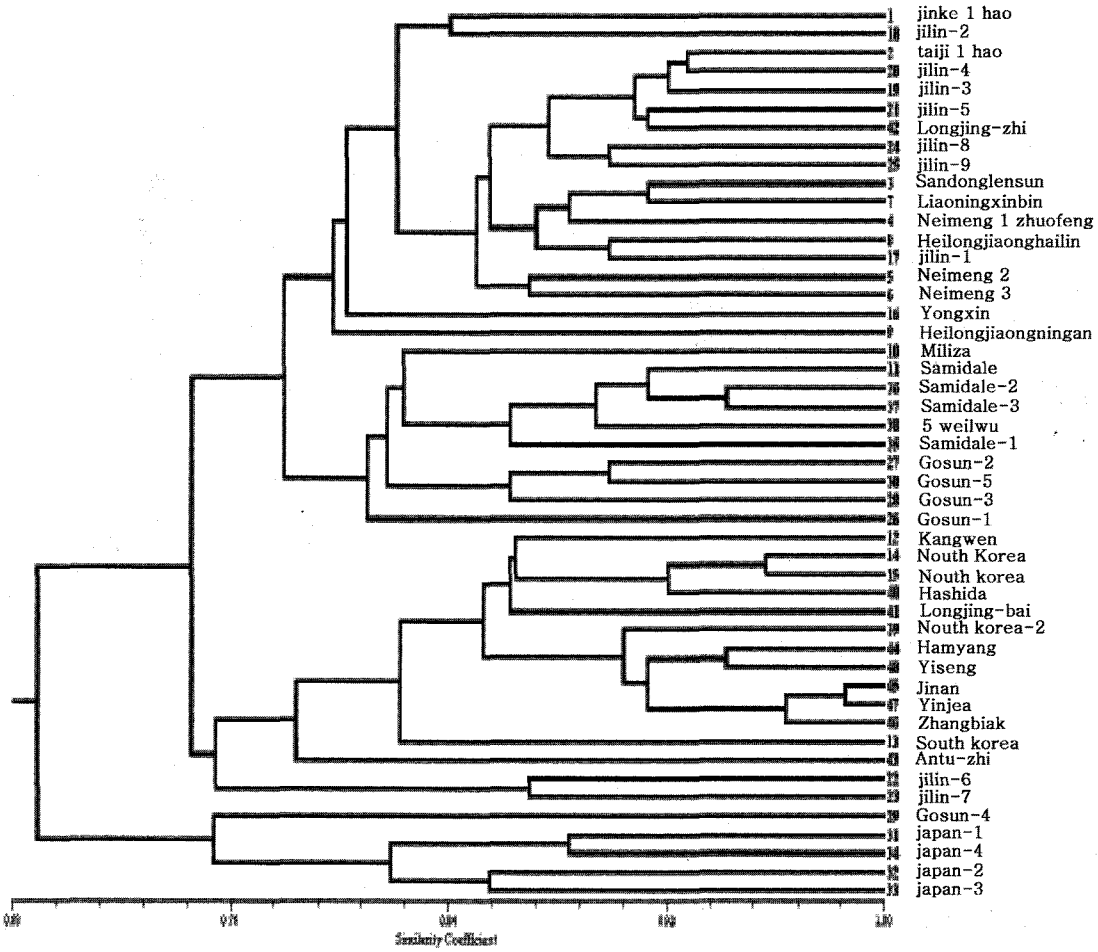

Fig. 3. Dendrogram illustrating genetic relationship among 48 *P. grandiflorum* DC. samples generated by UPGMA cluster calculated from 103 polymorphic RAPD markers amplified by 18 Operon primers as listed in Table 2. Scale at the bottom is genetic relatedness derived from Dice coefficient of similarity. The genotype was numbered and defined as in Table 1.

ple, only (C_1: Case No. 23; C_2: Case No. 22); the forth major cluster (IV) consist one minor cluster (D_1: Case No. 43) and one sub-major cluster (D_2: Case No. 13, 46, 47, 45, 48, 44, 39, 41, 40, 15, 14, 12); the fifth major cluster (V) consist one minor cluster having one sample, only (E_1: Case No. 26) and one sub-major cluster (E_2: Case No. 28, 30, 27, 35, 38, 37, 36, 11, 10); the sixth major cluster (VI) consist one minor cluster (F_1: Case No. 9) and one sub-major cluster (F_2: Case No. 16, 6, 5, 17, 8, 4, 7, 3, 25, 24, 42, 21, 19, 20, 2, 18, 1). Among the 48 samples, Case No. 47 and Case No. 45 showed the highest similarity coefficient (99.4%).

The results revealed that, First, the 48 samples were almost distinctly clustered geographically. All the samples clustered to group I, II, V were collected from Japan; and the samples clustered to the groups III and VI were collected from China. Almost samples clustered to the group IV were collected from South and North Korea. Second, inconsistent groups also existed. For example, in group IV, the 3 samples (Case No. 40, 41, 43) were collected from China, but they clustered to the same group collected from Korea. The sample, Case No. 43 showed 82.4% similarity to the sub-major cluster D_2; and the three samples, Case No. 40, 41 and 43 have relatively high genetic similarity (92%). These samples were collected from China, but they have more similarities with Korean samples. It is might due to the fact, although the three samples and the other samples (belong to the same group, IV) are cultivated on different countries, but they are distributed in geographically narrow area, nearby regions.

These results clearly indicate that the samples used in present study maintains the native wild type trait and are genetically conserved. Therefore RAPD-PCR markers have good potential for detection of genetic variation in *P. grandiflorum*. The genetic similarity values developed in this study provide breeders with a starting point for increasing the genetic diversity in their cross breeding program, and helpful for the breed improvement of *P. grandiflorum* DC..

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