ISSR Markers of Authentication for Korean and Chinese Platycodon Grandiflorum

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Platycodon grandiflorum is a long-lived herbaceous and one of the very important herbal medicine and foods. P. grandiflorum is called do-ra-ji in Korea. Inter-simple sequence repeats (ISSR) markers were performed in order to analyse the phenetic relationships of four accessions of P. grandiflorum. Wild groups had higher expected diversity, 0.164 for Korean and 0.157 for Chinese accessions than those of cultivated groups, 0.079 for Korea and 0.059 for China. The total genetic diversity in P. grandiflorum was 0.268 across species and the value was lower than average values for species with similar life history traits. The patchy distribution and domestication are proposed as possible factors contributing to low genetic diversity. An assessment of the proportion of diversity within species, HAccession/HSpecies, indicated that about 57.1% the total genetic diversity was among species. Thus, the majority of genetic variation (42.9%) resided within accessions. The estimated Nm (the number of migrants per generation) was very low among four accessions (mean Nm = 0.376). The low estimate of Nm indicated that gene flow was not extensive among four accessions. ISSR01-02 locus can be recognized as an unique locus of Korean groups (wild and cultivated accessions). Thus the locus can be used to distinguish Korean accessions from Chinese accessions. ISSR04-06 locus was found specific to Chinese groups (wild and cultivated accessions) and was not shown in Korean accessions. Although the size of sampling was not large enough for P. grandiflorum, the analyses of ISSRs will certainly provide an enhanced view on the phylogeny of accessions.

Key words : platycodon grandiflorum, Inter-simple sequence repeats

Introduction

Platycodon grandiflorum (Jacq.) A. DC. (Campanulaceae) is a very important traditional medicinal plant and called do-ra-ji in Korea. The plant is the principal herb in Chinese medicine for diseases of the lungs and throat, and is commonly used for inflammatory conditions of the eyes, ears, and sinuses[1]. The herb has a strong expectorant and antitussive effects and is included in many cough formulas, usually in combination with licorice[2]. The active component of P. grandiflorum is saponin, which makes its roots to have a bitter taste and unique scent[3]. Its root contains an abundant amount of saponin glycosides and platycodins of which platycodin D is one of the major components[4]. Recently, the rapid expansion of industries producing foods for health promotion and materials for oriental medicine caused an increase in the utilization of P. grandiflorum as herbal medicine[3]. Before domestication, the roots for domestic consumers were supplied by gathering from the mountainous regions in Korea. Since then, imports of Chinese roots have increased greatly along with the increasing demand. P. grandiflorum is also cultivated as a medicinal crop in Korea and China. Roots of do-ra-ji have been used as ingredients in Korean cooked potherbs for a long time. The levels of the active components can be different between these regions of two nations, but these medicines are sold in some Korean herbal markets without discrimination.

Recently, as the amounts of cheap Chinese medicines imported to Korea increases, the distribution of inferior and mixed herbal medicines at Korean herbal market is expected.

The domestic do-ra-ji is currently being sold at a price 10 times higher than the Chinese one. Although these two accessions can be identified by root morphology, it is impossible to distinguish between the two accessions when the roots are sliced. Moreover, in herbal markets the Chinese

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do-ra-ji is illegally sold either without the correct label or by mixing with domestic do-ra-ji. Therefore, finding a rapid mean to discriminate between the two accessions when they were dried and sliced is very important in Korea.

During the last decade several types of novel DNA-marker have emerged, which have been rapidly used as a routine laboratory tool for genome analysis. Since their introduction, random amplified polymorphic DNA (RAPD) markers have become very popular and have been used for a variety of purposes in plant genetic analysis, mainly because of the easy of analysis[5]. In addition, inter-simple sequence repeats (ISSR) markers are a potentially useful tool for species discrimination[6]. Especially ISSR assay has been useful in determining genetic relationships among closely related species[7]. ISSR analysis is quick, robust and requires minimal preliminary work[8,9]. Cultivar identification, parentage determination, genetic relationship evaluation, and estimation of population genetic variability are some examples of the multiple usages of the ISSR technique[10,11].

Thus, it is indispensably important issue to establish the distribution system on the basis of discrimination of the herbal medicines and quality control. The first step in this process is to develop efficient markers. This study was carried out to develop a method that could be used to discriminate between Korean and Chinese P. grandiflorum based on molecular markers.

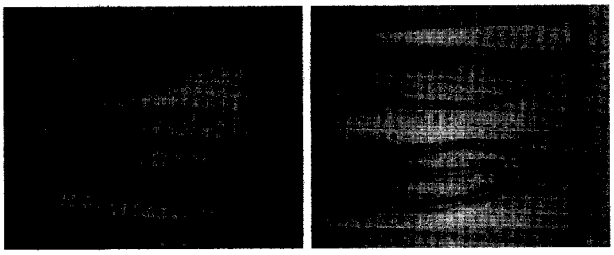

Fig. 1. Roots morphology of Korean do-ra-ji (left) and Chinese do-ra-ji (right).

Materials and Methods

1. Sampling Procedure

All of the four accessions of P. grandiflorum were collected from populations in Korea and China. Wild and domestic accessions of P. grandiflorum were collected from Mt. Giri and Andong Province in Korea, respectively. The imported cultivated accession of P. grandiflorum from China was purchased from market and Chinese wild P. grandiflorum was provided from National Crop Experiment Station, Suwon, Gyonggi Province. Fifteen plants were randomly collected from each accession.

2. DNA Extraction and ISSR analysis

The genomic DNA of the 270 samples including outgroup (Codonopsis lanceolata) was extracted from fresh roots using the plant DNA Zol Kit (Life Technologies Inc., Grand Island, New York, U.S.A.) according to the manufacturer's protocol. Eleven arbitrarily chosen primers of Bioneer Technoligies (Korea) were used. All the reactions were repeated twice and only reproducible bands were scored for analyses(Table 1).

Amplification reactions were performed in 2.5 μl of the reaction buffer, 10 mM Tris-HCl (pH 8.8), 1.25 mM each of dATP, dCTP, dGTP, dTTP, 5.0 pM primer, 2.5 units Taq DNA polymerase, and 25 ng of genomic DNA. A 100 bp ladder DNA marker (Pharmacia) was used for the estimation of fragment size. The amplification products were separated by electrophoresis on 1.5% agarose gels, stained with ethidium bromide, and photographed under UV light using Alpha Image TM (Alpha Innotech Co., USA).

Table 1. List of decamer oligonucleotides utilized as primers, their sequences, and associated fragments

No	Sequence (5' to 3')	No. of fragments
ISSR01	-AGAGAGAGAGAGAGAGG-	4
ISSR02	-CTCTCTCTCTCTCTCTG-	8
ISSR03	-CACACACACACACACAG-	10
ISSR04	-GCGAACACACACACACACAC-	6
ISSR05	-GGAGAGGAGAGGAGA-	2
ISSR06	GAGAGAGAGAGAGAGAGAGT	5
ISSR07	-GCGAACACACACACACACAC-	4
ISSR08	-GAGAGAGAGAGAGATC-	3
Total		42

3. Statistical analyses

All ISSR bands were scored manually and only unambiguously scored bands were used in the analyses. Because ISSRs are dominant markers, it was assumed that each band corresponded to a single character with two alleles, presence (1) and absence (0) of the band.

The following genetic parameters were calculated using a POPGENE computer program (ver. 1.31) developed by Yeh et al.[12]: the percentage of polymorphic loci (Pp), mean numbers of alleles per locus (A), effective number of alleles per locus (Ae), and gene diversity (H)[13].

The estimation of genetic similarity (GS) between genotypes was based on the probability that an amplified fragment from one individual will also be present in another[13]. GS was converted to genetic distance (1-GS).

Homogeneity of variance among accessions was tested by Bartlett's statistics. A phenetic relationship was constructed by the neighborjoining (NJ) method[14] using the NEIGHBOR program in PHYLIP version 3.57[15].

Results

From the 11 decamer primers used for a preliminary ISSR analysis, eight primers produced good amplification products for P. grandiflorum both in quality and variability, while the remaining primers did not amplified sucessfully or showed smear banding pattern. Overall, 42 fragments were generated among the tested do-ra-ji array(Table 1). The fragments ranged from 2-10 per primer.

Table 2. Measurements of genetic variation for accessions of do-ra-ji (Platycodon grandiflorum)

Accession	Np	Pp	A	AE	H	I
Korean wild	17	40.5	1.405	1.298	0.164	0.239
Korean cultivated	9	21.4	1.214	1.138	0.079	0.117
Chinese wild	16	38.1	1.381	1.285	0.157	0.228
Chinese cultivated	7	16.7	1.167	1.099	0.059	0.088

The number of polymorphic loci (Np), percentage of polymorphism (Pp), mean number of alleles per locus (A), effective number of alleles per locus (AE), gene diversity (H), and Shannon's information index (I).

In a simple measure of intraspecies variability by the percentage of polymorphic bands, the Chinese cultivated exhibited the lowest variation(16.7%). The Korean wild accession showed the highest(40.5%)(Table 2). Mean number of alleles per locus (A) ranged from 1.167 to 1.405 with a mean of 1.292. The effective number of alleles per locus (Ae) ranged from 1.099 to 1.298.

The phenotypic frequency of each band was calculated and used for estimating genetic diversity (H) within accessions.

As the typical populations of wild do-ra-ji were small, isolated, and patchily distributed for natural populations, they maintained a low level of genetic diversity for eight polymorphic primers. Wild groups had higher expected diversity, 0.164 for Korea and 0.157 for China than those of cultivated groups, 0.079 for Korea and 0.059 for China. The total H was 0.268 across species. Shannon's index of phenotypic diversity (I) of Korean wild was highest(0.239) of all accessions and Chinese wild was the second(0.228).

As both cultivated groups were found to have fewer alleles per locus, lower percent polymorphic locus, and lower diversity than two wild groups. The both groups showed significant difference in genetic variability except Ae(paired t test).

An assessment of the proportion of diversity present within species, HAccession/HSpecies, indicated that about 57.1% of the total genetic diversity was among species(Table 3).

Thus, the majority of genetic variation(42.9%) resided within accessions. The estimated Nm was slightly low among four accessions(mean Nm = 0.376).

A similarity matrix based on the proportion of shared fragments(GS) was used to evaluate the relatedness among species. The estimate of GS ranged from 0.660 to 0.838(Table 4).

Table 3. Estimates of genetic diversity of do-ra-ji. Total genetic diversity (HT), genetic diversity within populations (HS) proportion of total genetic diversity partitioned among accessions (GST), and gene flow (Nm).

Species	HT	HS	GST	Nm
Total	0.268	0.115	0.571	0.376

Table 4. Genetic identity (above diagonal) of four accessions of do-ra-ji based on ISSR and genetic distances (below diagonal)

Accession	Korean wild	Korean cultivated	Chinese wild	Chinese cultivated
Korean wild	-	0.838	0.660	0.794
Korean cultivated	0.176	-	0.758	0.809
Chinese wild	0.416	0.278	-	0.769
Chinese cultivated	0.231	0.213	0.263	-

ISSR01-02 locus can be recognized as an unique locus of Korean groups (wild and cultivated accessions)(Fig. 2). Thus the locus can be used to distinguish Korean accessions from Chinese accessions. While ISSR04-06 locus was found specific to Chinese groups(wild and cultivated accessions) and did not shown in Korean accessions.

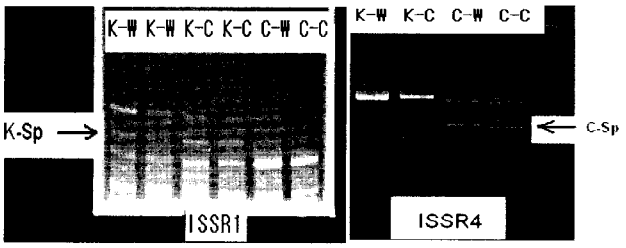

Fig. 2. A portion of ISSR patterns in four accessions of Platycodon grandiflorum using primer ISSR01 and ISSR04. K-W: Korean wild, K-C: Korean cultivated, C-W: Chinese wild, and C-C: Chinese cultivated. K-Sp: Specific band to Korean group. C-Sp: Specific band to Chinese group.

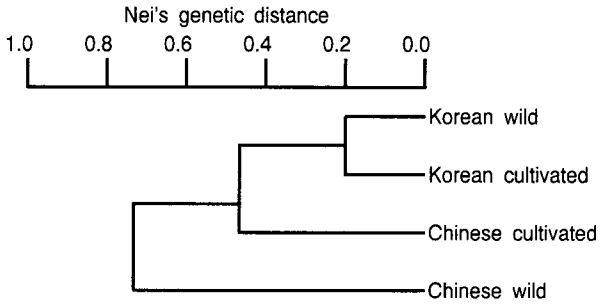

Fig. 3. A phenogram showing the relationships among four accessions of do-ra-ji, based on data of genetic distance obtained by ISSR.

Clustering of accessions was performed based on the matrix of calculated distances using the NJ algorithm(Fig. 3).

The phenetic tree showed the distinct groups Korean and Chinese accessions. The tree also shows genetic differentiation

between wild and cultivated accessions for Korean species.

Discussion

The roots of P. grandiflorum have been used as a food material and have been used in Asia as a traditional folk remedy for diseases such as bronchitis, asthma, pulmonary tuberculosis, hyperlipidemia, diabetes and inflammatory disease. Recently, several studies on the extracts of the P. grandiflorum root revealed that it contains a wide variety of compounds with immunopharmacological effects[16] and preventive effects against hypercholesterolemia, hyperlipidemia[17], and antioxidant activity[18].

Periodical removal of roots have often been moved from hillside to nearby farmhouse for the purpose of medicine during the past several decades of years. Small populations tend to have fewer multilocus genotypes and genetic diversity than large populations. It is not expected that P. grandiflorum has the ability to regenerated by roots sprouting when harvested for medicine. Smaller populations probably exhibit more inbreeding than larger populations by virtue of the limited opportunities for outcrossing during any flowering season.

At multilocus analysis, the allelic composition of the cultivated accessions was a subset of that for the wild accessions. Thus, the domestication processes via artificial selection have eroded the levels of genetic diversity in cultivated P. grandiflorum. It is in good agreement with the concept that most inland crops show a reduced level of polymorphisms as compared to their presumed progenitors[19].

Ultimately high levels of variability of the wild species are expected because they were not subject to any of the selection pressures of domestication, and the maintenance of higher genetic variability would favor their survival under natural conditions[20].

Although the size of sampling was not large enough for P. grandiflorum, the analyses of ISSRs will certainly provide an enhanced view on the phylogeny of accessions. In addition, additional molecular experiments such as AFLP(amplified fragment length polymorphism) and microsatellites are necessary to identify accessions.

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