GENETIC VARIATION OF JAPANASE ELM (*ULMUS DAVIDIANA* VAR. *JAPONICA)* EMPLOYING ISSR MARKERS

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We studied genetic variation of Japanese elm calling medicinal tree, *Ulmus davidiana* var. *japonica* (Rehder) Nakai, to establish the strategy for the conservation of its genetic resources. A total of 171 individual samples were collected from seven populations and their DNAs were used for inter simple sequence repeat-PCR amplification with 47 ISSR primers. Eight out of the 47 primers were selected and yielded 55 clear fragments to be scored. The percentage of polymorphic loci (*P*) ranged from 87.27% to 98.18% with a mean of 93.25%. The gene diversity (*h*) averaged over all was 0.33. The Shannon's information index (*S.I.*) ranged between 0.455 and 0.515 with an average of 0.494. The AMOVA showed that most of the genetic variation (96.1%) was allocated among individuals within populations. The dendrogram showed no clear association between the clustering of population and their geographical origin.

Introduction

Japanese elm belongs to the family Ulmaceae and the genus *Ulmus* L. and distributes in northeastern Asia including China, Japan and Korea. This tree is considered to be one of the most useful medicinal tree species because the extracts from the bark enhance splenocyte proliferation and cell viability. Furthermore, the extracts from the roots have an activity to prevent reactive oxygen species in human cells. In spite of these useful effects, to our best knowledge, only a few genetic studies using molecular markers have been performed for this species. The objectives of this study are to investigate the genetic variation of Japanese elm and to provide fundamental information for the conservation of its genetic resources.

Materials and Methods

Plant materials of the 171 individuals were collected from seven natural populations, *Ulmus davidiana* var. *japonica,* in South Korea (Figure 1). The genomic DNA was extracted from 80㎎ leaf using DNeasy plant mini kit. PCR amplification was performed with seven primers out of 47 UBC ISSR primers. For each primer, amplified fragments with the same molecular weight (bp) were recovered as present (1) or absent (0), and the resulting binary matrix was used in the statistical analysis. The computer program POPGENE version 1.31 was used to estimate genetic diversity parameters (Yeh et al. 1999) and Shannon's index (Table 2). The analysis of molecular variance (AMOVA) was carried out to measure the degree of genetic differentiation. A dendrogram was derived from the unweighted-pair group method with arithmetic mean (UPGMA) clustering based on basis of pair-wise Manhattan distance (Wright 1978)

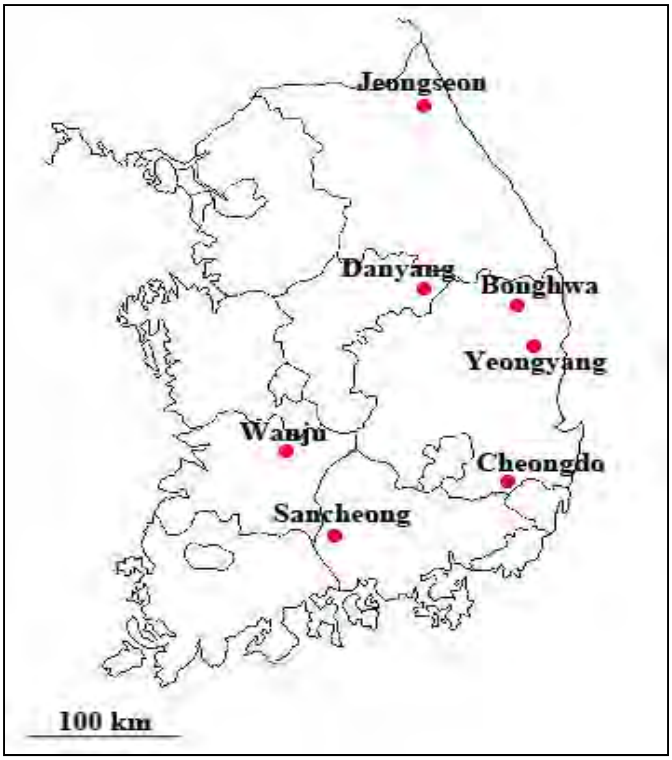

Figure 1. Natural distributions of population of *Ulmus davidiana* var. *japonica.*

Results and Discussion

The average number of the loci per primer was 6.9. Nevertheless the primer UBC #822 had only 2 bands to score, they were scored because of their clear patterns. Two markers such as UBC #807 and UBC #811 had 10 scored bands. The markers usually had (AG) or (GA) motifs (Table 1). The results from analysis of genetic variability to seven populations, Japanese elm, the Shannon's information index (*S.I.*) indicating genetic diversity ranged from 0.455 to 0.519, with an average of 0.494 at the population level (Table 2). These degrees of genetic diversity were higher than other deciduous trees such as *Oplopanax elatus*, its degree of genetic diversity was 0.187 (Lee et al. 2002) in Korea and *Kirengeshoma palmate*, its degree of genetic diversity was 0.259 (Zhang et al. 2006) in China. This is because of that endangered plant species have low genetic diversity due to the genetic drift and gene flow (Karron 1991).

Table 1. ISSR markers used for genetic variation analysis.

Primer name	Repeat region 5' → 3'	Scored bands
UBC807	$(AG)_8T$	10
UBC810	$(GA)_8T$	6
UBC811	$(GA)_8C$	10
UBC812	$(GA)_8A$	7
UBC813	$(CT)_8T$	7
UBC822	$(TC)_8A$	2
UBC855	$(AC)_8YT$	6
UBC873	$(GACA)_4$	7
Total	8	55

Y=C or T

Therefore, Japanese elm's genetic variation is higher than that species. However, other tree species showed similar or slightly lower genetic diversity such as Smile rosebay was 0.395 (Hong *et al*., 2003), *Taxus cuspidata* was 0.478 (Kwon et al., 2002), Japanese red pine was 0.453 (Hong et. al., 2007), *Camellia sinensis* had 0.343 (Yang *et al*., 2010), and *Torreya nucifera* was 0.353 of biodiversity (Hong *et al*., 2000). This result means that Japanese elm has been adapting similar environment changes with these tree species. Sancheong was the highest at 0.492 in Shannon's index (*S.I.*), while Wanju was the lowest at 0.455. But there was no significant difference among populations.

Table 2. Genetic variability of *U. davidiana* var. *japonica* from ISSR analysis.

Population	*N*	A_o	A_e	*h*	*P* (%)	*S.I.*
Bonghwa	23	1.946 (0.229)	1.571 (0.336)	0.330(0.161)	94.55	0.492(0.210)
Cheongdo	18	1.909 (0.290)	1.587 (0.321)	0.340(0.155)	90.91	0.503(0.207)
Danyang	26	1.982 (0.135)	1.573(0.2830)	0.342(0.128)	98.18	0.515(0.160)
Jeongseon	25	1.964 (0.189)	1.535 (0.310)	0.320(0.146)	96.36	0.485(0.186)
Sancheong	25	1.946 (0.229)	1.622 (0.331)	0.353(0.158)	94.55	0.519(0.206)
Wanju	24	1.873 (0.336)	1.522 (0.346)	0.305(0.174)	87.27	0.455(0.236)
Yeongyang	30	1.910 (0.290)	1.558 (0.332)	0.326(0.158)	90.91	0.486(0.210)
Average	24.43	1.933	1.567	0.331	93.25	0.494

N, sample sizes; A_o, observed number of alleles per locus; A_e , Effective number of alleles per locus; *h* , Nei's (1973) gene diversity; *P* , percentage of polymorphic loci; *S.I.*, Shannon's information index (1948). Standard deviations are given in parentheses.

The results of the AMOVA analysis to find out genetic structure in seven populations, Japanese elm, showed, 96.16% of total genetic variation that exists in individual variations within groups and among difference of populations was 13.3% (Table 3). This genetic variation of Japanese elm was lower than *Oplopanax elatus* (Φ_{ST}=0.109, G_{ST}=0.155; Lee et al. 2002), *Camellia sinensis* L. (Φ_{ST}=0.132, Yang et al., 2010), and *Alnus hirsute* (G_{ST}=0.087; Huh and Huh, 1999) in Korea. Furthermore, White elm showed Φ_{ST}=0.290 with 20 allozyme loci analysis in Finland (Vakkari et al. 2009).

Table 3. Analysis of molecular variance (AMOVA) within/among populations.

Source of variation	d.f.	Sum of squares	Variance component	Percentage of variance
Among populations	6	119.42	0.40	3.84
Within populations	164	1654.61	10.09	96.16

In the results of AMOVA, 3.84% of total genetic variation was caused by the difference among populations and 96.16% of the others were caused by between the individuals within populations. According to this the results, for effective conservation of this genetic resources to increasing genetic diversity, it is proper that conservation by select many individuals within a population rather than select many populations for *ex-situ* conservation and conservation by select a few populations rather than select many populations for *in-situ* conservation. In order to identify relationship among populations, as the results of UPGMA cluster analysis (Figure 2) in seven populations, were grouped in three parts according to the genetic distance. The first group includes Danyang, Bonghwa, and Yeongyang population. The second group is Wanju, Jeongseon, and Sancheong population. The last group is only Cheongdo population. Based on the genetic diversity and UPGMA cluster, Danyang and Sancheong represent their group, respectively because Danyang and Sancheong population showed more genetic diversity in their groups.

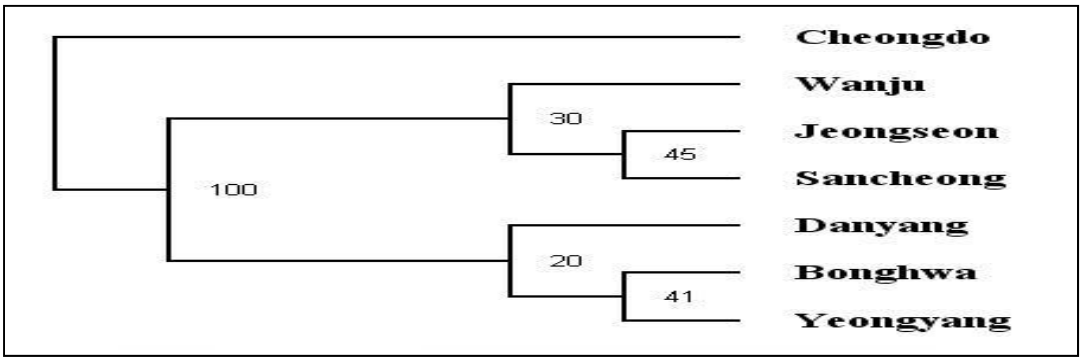

Figure 2. A dendrogram based on the Manhattan distance among seven populations of *U. davidiana* var. *japonica* generated by UPGMA clustering.

According to the above results and geographic distribution of these populations, Japanese elm (Figure 1), we found that the second group was not related to geographic distance. Because three populations within the second group were geographically distant from each other. Especially, Jeogseon and Sancheong populations are the farthest away among total populations (Figure 1) We should be more study to the origin of the species and biological evolutionary process for explain of this grouping.

Conclusions

In conclusion, in order to conserve effectively, after searching and investigating natural populations of target species, we should be decide to the optimal number and size of target species for conservation by analyzing the genetic structure and genetic variation using the appropriate genetic markers for effective conservation. Genetic studies of population on target species of conservation. In addition, these materials can be used to develop new varieties.

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