Genetic Variation in the Selected Populations of *Hovenia dulcis* var. *koreana* Nakai. Based on RAPD Analysis

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ABSTRACT

This study used RAPD markers to assume genetic diversity and variation in selected populations of *Hovenia dulcis* var. *koreana*. Ratio of polymorphic RAPD markers were 93.4% in selected populations of *Hovenia dulcis* Thunb., difference of genetic structure among populations and within populations showed 16.45%, 83.55%, respectively in amount of total genetic variation of 4 populations. Total gene diversity(H_T) that show genetic diversity appeared 0.313 and coefficient of gene differentiation(G_{ST}) that compare genetic differentiation of populations appeared 0.1645, analysis of AMOVA for variation among populations and within populations was significantly different ($P < 0.001$). Genetic diversity of whole populations showed that 12.44% difference among population and 87.56% difference within populations. As a result, difference within populations was larger than difference among populations in genetic diversity. Nei's genetic distance and cluster analysis appeared that mean genetic distance among populations was 0.076, thus dividing two main groups and geographic relationship did not show in populations.

Key words : Genetic variation, Selected population, RAPD analysis, Genetic diversity

INTRODUCTION

Hovenia. dulcis var. *koreana* is a deciduous hardwood tree in dahurian buckthorn family and that height and diameter at breast height grow to 20m and 80cm, respectively. *H. dulcis* var. *koreana* is registered as an endemic species in Korea because that is distinguished by petiole size, seed and color of flower from *H. dulcis* and *H. tomentosa* which belong to same genus in north east Asia(Lee, 1985). *H. dulcis* var. *koreana* has strong freezing resistance, shade tolerance, sprout ability and mainly grows at fertile location where is slope or valley at elevations of 700∼900m above the sea level in south of Kyunggi or Kangwon.

Reported efficacy of fruit which was counteraction of alcoholic poison, lifeblood, urination, reducing thirst and recently, that tree was focused on their extracts which had effect for hangover relief, liver activity and protecting(Na, 2000).

In general, there is the need for improvement of the characteristic forest form and the selection of excellent individuals for management of genetic variation through analysis of genetic variation within a forest which contains a variety of genetic variations. It is necessary to carry out research successively and continuously for a dominant population or mass

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selection, with tree selection and progeny testing from a natural populations. To promote this research, systematic and basic researches are needed for understanding the level of variation within selected population and individual variation among populations.

It is known as that a genetic variation of plants has much more diversity in allogamy plants which has wide range and long life than others which has opposite conditions(Hamrick *et al.*, 1992) and the plants like *H. dulcis* var. *koreana* which has narrow range or rare variety have much less genetic diversity because of inbreeding and genetic immobility(Lee *et al.*, 2002).

This study was conducted to select superior tree for high petiole productivity through genetic diversity analysis using RAPD marker in selected *H. dulcis* var. *koreana* population. This study will provide basic information for tree breeding and genetic resources protecting through research of genetic characteristic in natural population.

MATERIALS AND METHODS

Materials

Leaf samples were collected 2∽3g per tree from clone bank where was established to preserve superior tree for high productivity of *H. dulcis* var. *koreana*. There was composed grafting trees which were proliferated from 4 regions 60 selected sample trees.

RAPD PCR

Total DNA was extracted from collected leaf samples by adjusted Huff's(1993) method. Amplification reactions were performed in 25$\mu\ell$ reaction buffer ; 10mM Tris-Hcl(pH 8.3), 1.5mM $MgCl_2$, 0.025% BSA, 50mM Kcl, 200μM each dNTPs, 60ng Random primer, 1 unit Taq. DNA polymerase, 1X Amplification buffer and 25ng Template DNA. 60 random primers and 10 primers(OPS #11, OPV #6, #7, #8, #12, #16, #20, OPW #3, #12, #16) which were selected according to number and definition of polymorphism amplification products used to analysis of genetic variation. PCR cycle was forty-four times repeated under 94℃/5sec, 36℃/1min and 72℃/70sec condition by PTC-200 thermal cycler(MJ-Research Inc). After that process, there were amplification complete after one more treated under 94℃/5sec, 36℃/1min and 72℃/5min. The amplified products were confirmed RAPD band by electrophoresis on 2% agarose gels and each RAPD band was given a score of 1 for presence or 0 for absence.

Data analysis

Amount of genetic variation was analyzed into AMOVA(Analysis of Molecular Variance) method of Excoffier(1992) and Huff(1993). AMOVA belongs to a nested design and that uses genetic distance among individual trees for variate. This study used Excoffier's(1992) method to know genetic distance(formula 1).

$$\text{Excoffier's distance} = n \times \left(1 - \frac{n_{11}}{n}\right) \text{-------(formula 1)}$$

※ n : total number of polymorphism band positions

n_{11} : the number of positions where x=1 and Y=1

Also, genetic diversity and distance among populations were analyzed into POPGEN(ver. 3.1; Yeh *et al.*, 1999) and RAPDistance(ver 1.04; Amstrong *et al.*, 1994), respectively, and AMOVA(ver 1.55; Excoffier, 1995) program used to AMOVA(Table. 1).

RESULTS AND CONCLUSIONS

RAPD PCR

For genetic variation analysis of selected *H. dulcis* var. *koreana* populations, 60 random primers were used and 10 primers were selected by polymorphism and reproducibility(Table. 2).

The selected 10 primers used to PCR process about

Table 1. General design for hierarchical analysis of molecular variance(AMOVA)

Source of variation	d.f.	Mean square deviation	E(MSD)
Among populations	ΣI-1	MSD/(AP)	$\sigma_b^2+n\,\sigma_a^2$
Among individuals within populations	N*-ΣI	MSD/(WP)	σ_b^2
Total	N-1		

* N : total number of individuals.

60 individual trees from 4 regions(Kangwon, Kyunggi, Jeonnam, Chungbuk) and 196 bands of amplification products(UBC primer #11: 21ea, #06: 23ea, #07: 21ea, #08: 21ea, #12: 23ea, #16: 14ea, #20: 16ea, #3: 24ea, #12: 17ea, #16: 16ea) were obtained from that process. 183 of 196 amplification products (93.4%) showed polymorphism and the average number of amplification products was 18.3 per primer(Table. 2, Fig. 1). Also, 13 amplification products (6.6%) showed monomorphism and the average number was 1.3 per primer. This study's values appeared as similar as results of *Populus tremuloides* Michx.(90.2%; Yeh *et al.*, 1995) and *Pinus sylvestris*(90.9%; Szmidt *et al.*, 1996). However, those values were higher than Kim's(1998) RAPD marker analysis of collected *Acanthopanax senticosus*(57.3%, the average number was 5.7 per primer). In general, it is known as that RAPD marker showed higher polymorphism than isozyme marker(Vicario *et al.*, 1995). The selected population of *H. dulcis* var. *koreana* has a high level of genetic variation, because natural habitat of that tree is geographically limited in korea and isozyme maker's average rate of polymorphism loci was shown 71.7% in gymnosperm (Hamrick *et al.*, 1992).

Genetic diversity

The results of genetic diversity and specialized level in selected populations of *H. dulcis* var. *koreana*

Table 2. Attributes of oligonucleotide primers used for generating RAPD markers of 60 individuals of *H. dulcis* var. *koreana* sampled from four populations

Primer	Nucleotide sequance 5' to 3'	G+C content(%)	Number of polymorphic markers	Number of monomorphic markers within all populations
OPS-11	AGT CGG GTG G	70	20	1
OPV-06	ACG CCC AGG T	70	23	0
OPV-07	GAA GCC AGC C	70	19	2
OPV-08	GGA CGG CGT T	70	21	0
OPV-12	ACC CCC CAC T	70	23	0
OPV-16	ACA CCC CAC A	60	12	2
OPV-20	CAG CAT GGT C	60	13	3
OPW-03	GTC CGG AGT G	70	13	3
OPW-12	TGG GCA GAA G	60	16	1
OPW-16	CAG CCT ACC A	60	23	1

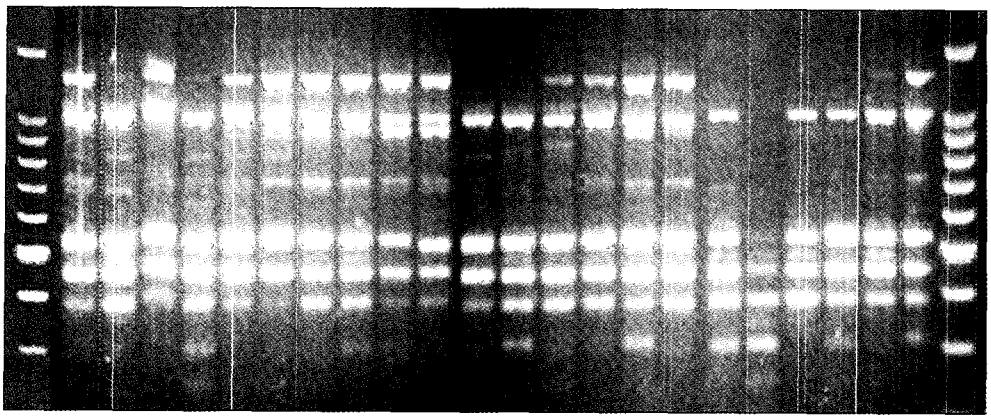

Fig. 1. Result of RAPD band pattern with 25ng/ul template DNA in *H.dulcis* var. *koreana*(The number of Individuals: 1-8; Gwangwon, 9-10; Gyunggi, 11-16; Cheonnam, 17-22; Chungbuk, M; 100bp size marker).

showed like Table 3 and Nei's(1987) total gene diversity values (H_T) was 0.313. H_T means expected mean heterozygosity when all individuals would be random breeding in the gene pool(Guries and Ledig, 1982). Though, H_T of this study was less than result of RAPD method in red pine(Kim, 1995; 0.442), that showed same genetic diversity of isozyme method(Kim and Lee, 1992; 0.291). However, that was higher than result of RAPD method in *Populus davidiana*(Hong *et al.*, 1998; 0.274).

In general, the genetic diversity of woody plants is known as that tree which has broad habitat or pollinated by anemophilous cross-pollination is higher than tree which has opposite condition(Hamrick *et al.*, 1992). For that reason, although, *H. dulcis* var. *koreana* doesn't have continuous geographic habitat, that tree seems to show genetic diversity by entomophilous crosspollination and environmental change.

Coefficient of gene differentiation(G_{ST}) which use for genetic specialization of population was 0.1645(Table 3). That conclusion meant that 16.45% of total amount of genetic variation was caused by genetic difference among populations. That is higher rate of genetic specialization than mean of G_{ST}(8.4%) by isozyme method (Hamrick *et al.*, 1992) and lower than G_{ST} of *Populus davidiana* by RAPD method(Hong *et al.*, 1998; 0.274). Therefore, total amount of genetic variation of 4 populations seems to be composed of difference among populations(16.45%) and genetic structure difference among individuals within population(83.55%). In general, tree population has higher genetic variation among individuals within population than other plant species and conclusion of this study was alike.

Genetic structure and relationship.

Total 183 RAPD bands from 60 individuals within 4 populations in *H. dulcis* var. *koreana* were analyzed into AMOVA and that showed significantly difference ($P < 0.001$) about variation among populations and individuals within population(Table 4). In genetic

Table 3. Estimate of Nei's gene diversity and genetic differentiation with null band frequencies as homozygote's from 183 RAPD bands in 4 natural populations of *H. dulcis* var. *koreana*

Populations	Sample size	H_T	H_S	G_{ST}
4	60	0.3129	0.2615	0.1645

Table 4. Analysis of molecular variance(AMOVA) of *H. dulcis* var. *koreana* 60 individuals from 4 populations using 183 RAPD bands

Source of variance	d.f.	SSD	MSD	Variance component	% Total	P-value	Φ-statistics
Among popualtions	3	233.09	77.69	3.79	12.44		
Within populations	56	1492.43	26.65	26.65	87.56	< 0.001	Φ_{st}=0.124

diversity of total populations, 12.44% was difference among populations but, 87.56% was difference among individuals within population. Thus, difference among individuals within population showed higher trend than difference among populations in genetic diversity. The most of previous studies, about genetic variation among populations showed below 20% in tree population by isozyme method (Hamrick *et al.*, 1992) and those of this study showed lower than result of *Populus davidiana*(Hong, 1998; 35.1%) and *Abies koreana*(Kim and Hyun, 1999; 19.8%) by RAPD method. However, result of this study was higher value than genetic variation among populations in *Torreya nucifera* by ISSR marker(Hong *et al.*, 2000; 9.4%). Also, Φ_{st} value, a degree of genetic specialization among populations, was 0.124 and that was not high value. The tree population is known as that they has higher genetic variation among individuals within population than other plant species and animals. Therefore, there's can consideration that tendency as typical genetic variation distribution of the tree population through other previous researches (Hamrick *et al.*, 1992).

Nei's genetic distance(Nei's, 1987) and unbiased genetic distance were used coefficient in order to cluster analysis by unweighted pair group method(Table 5, Fig. 2). Mean genetic distance among 4 populations was 0.076 and that was classified into mainly two groups. Kyunggi population was clustered one group and Chungbuk, Kangwon and Jeonnam populations were clustered another. Also, the second group was clustered small groups. Chungbuk population was clustered small group and Kangwon and Jeonnam populations were clustered another small group. Thus, that results did not show geographic tendency and the value of mean genetic distance was lower than red pine population(Kim, 1995; 0.095) and Abies population(Vicario *et al.*, 1995; 0.1) but higher than *Populus davidiana* population (Hong *et al.*, 1998; 0.014). The results of UPGMA analysis did not show geographic tendency in population of *H. dulcis* var. koreana but Kyunggi population was clustered external population.

Table 5. Matrix of Nei's genetic distance with 4 populations of *H. dulcis* var. *koreana*

Population	Kwangwon	Kyunggi	Chungnam	Chungbuk
Kwangwon	---	0.9162	0.96.1	0.9372
Kyunggi	0.0875	---	0.9014	0.9172
Chonnam	0.0407	0.1038	---	0.9274
Chungbuk	0.0649	0.0864	0.0754	---

Above diagonal : Nei's(1978) unbiased genetic identity,

Below diagonal : Nei's(1978) unbiased genetic distance.

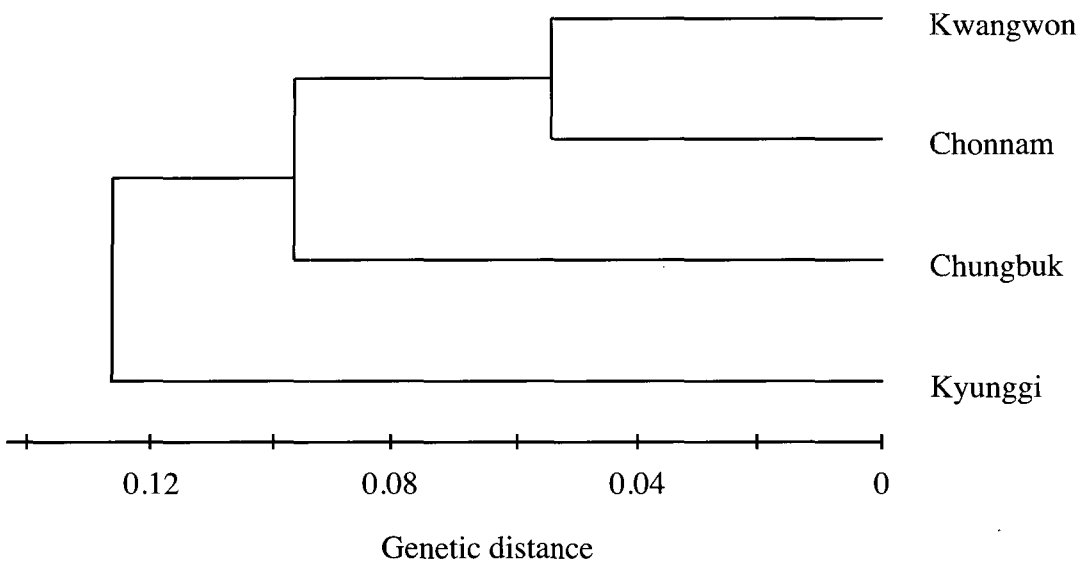

Fig. 2. Dendrogram showing the clustering of *H. dulcis* var. *koreana* 60 individuals from 4 populations using 183 RAPD bands based on Nei's(1978) unbiased genetic distance coefficient

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