Ⅲ- 5

단순 내부 반복 서열에 의한 헛개나무의 유전적 다양성과 집단구조

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Genetic Diversity and Population Structure of *Hovenia dulcis* in Korea Using ISSR

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Objectives

Hovenia dulcis is a long lived woody species and very important herbal medicine in Korea. We evaluated a representative populations of this species in Korea with inter simple sequence repeats (ISSR) markers to estimate genetic diversity and population structure in these populations. The genus *Hovenia* is comprised of about four or five taxa in Korea (Lee, 1997). The taxonomy of *Hovenia* has processed mainly through morphological characteristics. However morphological characteristics are restricted their resolving power mainly because of the small number of variables available.

The aims of this study were; 1) to estimate how much total genetic diversity is maintained in the *Hovenia* species, 2) to describe how genetic variation is distributed within and among species, and to elucidate the suitability and efficiency of the ISSR analyses assess the phenetic relationships among the related species in Korea. 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.

Materials and Methods

○ Materials

All of the ten populations of *Hovenia dulcis* Thunberg were collected from natural populations in Korea (Table 1). To analyze the proportion of genetic diversity among and within populations, twenty plants were randomly collected from each population.

○ Methods

The genomic DNA of the 200 samples including outgroup (*Rhammus davurica*) was extracted from fresh leaves (1.2 g). DNA was extracted using the plant DNA Zol Kit.

All the reactions were repeated twice and only reproducible bands were scored for analyses. From the eleven decamer primers used for a preliminary ISSR analysis, six primers of them produced good amplification products both in quality and variability.

A phenetic relationship was constructed by the neighborjoining (NJ) method using the NEIGHBOR program in PHYLIP version 3.57.

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Results

From the fifteen decamer primers used for a preliminary ISSR analysis, eleven primers of them produced good amplification products for ten populations of H. dulcis in quality and variability, while the remaining primers (ISSR12, ISSR13, ISSR14, ISSR15) did not amplified or showed smear. For ten populations of H. dulcis, the primers ISSR04 and ISSR05 revealed the seven and nine distinct fragments, respectively. The both primers, ISSR02 and ISSR03 revealed the nine fragments. The primer ISSR03 was shown the highest percentage of polymorphic fragments (88.9%), while the lowest percentage was generated by the primer ISSR-04 (50%). Overall, 70 reliable ISSR markers were produced with sizes ranging from 600 to 2100 bp. A total of 54 (77.1%) of these bands were polymorphic. The remaining fragments were monomorphic in all populations.

In a simple measure of intraspecies variability by the percentage of polymorphic bands, KG2 exhibited the lowest variation (22.9%). CN1 showed the highest (35.7%). An mean of 28.7% of the loci was polymorphic within taxa. The genetic similarities between species ranged from a minimum value of 0.730 between CB1 and KN1 and the maximum value of 0.991 between KW1 and KW2. The mean genetic distance was 0.167 across populations, varying from 0.009 to 0.315.

The dendrogram showed distinct four clades. One includes KB1, KB2, CN1, and KN1. this clade is sister with KG1 and KG2. Another includes two populations, KW1 and KW2. The distinct clade contained CB1 and CB2.

Table 3. Measures of genetic variation for ten populations of *Hovenia dulcis* in Korea. The number of polymorphic loci (Np), percentage of polymorphism (Pp), mean number of alleles per locus (A), effective number of alleles per locus (A_E), gene diversity (H), and Shannon's information index (I)

Population	Np	Pp	A	A_E	H	I
KB1	17	24.3	1.243	1.200	0.107	0.153
KB2	19	27.1	1.271	1.185	0.105	0.154
KG1	17	24.3	1.243	1.162	0.092	0.136
KG2	16	22.9	1.229	1.166	0.093	0.136
CB1	23	32.9	1.329	1.310	0.159	0.222
CB2	24	34.3	1.343	1.251	0.137	0.199
CN1	25	35.7	1.357	1.265	0.145	0.210
KN1	23	32.9	1.329	1.226	0.127	0.186
KW1	19	27.1	1.271	1.224	0.120	0.171
Kw2	18	25.7	1.257	1.200	0.110	0.158
Mean	20.1	28.7	1.287	1.219	0.120	0.173
Species	54	77.1	1.771	1.385	0.240	0.370