Analysis of Genetic Variation and Diversity in *Nelumbo Nucifera* by RAPD and NIRS

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**ABSTRACT**

*Nelumbo nucifera* is a perennial aquatic plant. Tuber, nut, young leaf, embryo and stamen are edible. Since ancient times, humans have derived many benefits from natural plants and compounds. All parts of *N. nucifera* have been used for various medicinal purposes in oriental medicine.

Hampyeong accession of white lotus, Hampyeong accession of violet lotus, Hwasun accession of red lotus, Jangseong accession of red lotus, Muan accession of lotus, Naju Dongsanchon reservoir accession of lotus, Naju Sanpohwaji reservoir accession of lotus, and Hampyeong accession of red lotus were used as the experimental materials.

The RAPD assay was performed in a 20 ul volume containing 2 ul of 10x PCR buffer (100 mmol•1\(^{-1}\) Tris-HCl, 15 mmol•1\(^{-1}\) MgCl\(_2\), 400 mmol•1\(^{-1}\) KCl, pH 9.0) 1ul of 1 mmol•1\(^{-1}\) dNTPs (Bioneer, Seoul, Korea), 1 ul of 10 pmol•ul\(^{-1}\) primers, 0.2 ul of 5 unit of Taq DNA polymerase (Bioneer, Seoul, Korea), 14.8 ul of sterile ultrapure deionized water and 1 ul of 10 ng DNA template. For the RAPD analysis, nine 10-base primers (Bioneer; Korea) and six 10-base primers (Operon, USA) were used.

Genetic distance matrix was obtained by using Nei and Li (1979) coefficients, and clustering analysis was conducted by using UPGMA (unweighted pair-group method with arithmetic mean) with TREECON (ver. 1.3b) program (Van De Peer and De Wachter, 1993).

NIR spectrum showed lotus leaf tea of Muan and Yeonkkotnabi are different.

**KEYWORDS:** diversity, TREECON, leaf tea

**INTRODUCTION**

*Nelumbo nucifera* is a perennial aquatic plant. *N. nucifera* is androgynous and composes of about 300 stamen and about 40 pistil and receptacle. Tubers, nuts, young leaves, embryos and stamen are all edible. *N. nucifera* Gaertn. (Nymphaeaceae) also known as sacred lotus is a large aquatic herb with stout, creeping rhizome found throughout southern Asia, India and northern Australia. *N. nucifera* is a native of China, Japan and possibly India. Almost all parts of the lotus plant are eaten as vegetable and also used in the indigenous system of medicine. All parts of *N. nucifera* have been used for various medicinal purposes in oriental medicine. *N. nucifera* Gaertn., a traditional Chinese herb, is thought to be a useful medicinal plant.
Since ancient times, humans have derived many benefits from natural plants and compounds. It has been reported that rhizome extract showed anti-diabetic and anti-inflammatory effects (Mukherjee et al., 1997a,b), stalks extract showed anti-pyretic effect (Shinha et al., 2000), leave and stamens extracts showed anti-oxidant effect (Jung et al., 2003; Wu et al., 2003), and seeds extract showed hepatoprotective and free radical scavenging effects (Sohn et al., 2003). In particular, the leaves are known for diuretic and astringent properties, and are used to treat fever, sweating, and strangury and as a styptic. Through the ages, many herbal medicines in different oral formulations have been recommended for diabetes, and confident claims of cure are on record.

Lotus tea was made in Yeonkkotnabi (Butterfly of Lotus Flower) by Hampyeong accession of white lotus leave. The lotus is different from other regional lotus genetically and morphologically but could find hardly any scientific studies about. Therefore, in this study, we employed RAPD and NIR analysis because they can be used to assay in a short time and is sensitive enough to detect differences.

MATERIALS AND METHODS

1. PLANT MATERIALS

Hampyeong accession of white lotus, Hampyeong accession of violet lotus, Hwasun accession of red lotus, Jangseong accession of red lotus, Muan accession of lotus, Naju Dongsanchon reservoir accession of lotus, Naju Sanpohwaji reservoir accession of lotus, and Hampyeong accession of Red lotus were used as the experimental materials. All lotuses were grown the glass house of College of Agriculture and Life Science, Chonnam National University.

Young leaf samples were collected and stored in deep freezer at -70°C. The extraction of DNA in lotus leaf was carried out using DNeasy Plant Mini Kit (Quiagen, USA).

2. RAPD ANALYSIS

The RAPD assay was performed in a 20ul volume containing 2 ul of 10x PCR buffer (100 mmol l⁻¹ Tris-HCl, 15 mmol l⁻¹ MgCl₂, 400 mmol l⁻¹ KCl, pH 9.0) 1ul of 1 mmol l⁻¹ dNTPs (Bioneer, Seoul, Korea), 1 ul of 10 pmol ul⁻¹ primers, 0.2 ul of 5 unit of Taq DNA polymerase (Bioneer, Korea), 14.8 ul of sterile ultrapure deionized water and 1 ul of 10 ng DNA template.

For the RAPD analysis, nine 10-base primers (Bionner, Korea) and six 10-base primers (Operon, USA) were used.

Amplifications were performed with a PCT-200 DNA engine (MJ research, USA) programmed as follows: 3 min at 94°C for the first cycle, 40 cycles at a denaturation temperature of 92°C for 30 sec, annealing temperature 38°C for 40 sec, and extension temperature 72°C for 1 min. After the last cycle samples were incubated for 10 min at 72°C. Amplified fragments were separated on 0.8% agarose gel containing ethidium bromide (EtBr) 0.5ug/L in 1×TAE buffer at 140V for 1h. After electrophoresis PCR products were identified by UV transilluminator (Core Bio, Korea), the number of polymorphic and total band was analyzed. The size of amplified DNA fragments was identified by using 1Kb size marker (Bioneer, Korea).

3. DATA ANALYSIS

RAPD analyses were performed in duplicate for all materials to ensure the reproducibility. Presence and absence of amplified bands were scored into 1 and 0, respectively for the analysis of genetic diversity. Genetic distance matrix was obtained by Nei and Li (1979) coefficient, and clustering analysis was conducted by Unweighted Pair Group Method with Arithmetic mean (UPGMA) with TREECON (ver. 1.3b) program (Van De Peer and De Wachter, 1993).
4. NIR ANALYSIS

Lotus leaf, leaf tea and rhizome were ground and analyzed. Each sample was examined 10 times volume of 10g in 20 mL vial with Near infrared reflectance spectroscopy (NIRS). Spectra of twenty eight lotus leave and ten lotus leaf teas were obtained with NIRS and the data were collected after 3 point baseline correction. The collected data were classified by cluster analysis.

RESULTS AND DISCUSSION

Total of 192 bands were identified for polymorphic bands used by 16 RAPD primers (Table 1, Fig. 1). The primer produced 11 bands in average and it showed high polymorphism. Lotus lines are grouped in Large, Middle and small size species by RAPD data. Waterlily was classified as an out-group. White lotus of Hampyeong accession clustered with 04JL71 accession and grouped in middle size species (Fig. 2). It was classified in Large size species, White lotus of Muan accession, by the size of rhizome. Small size species, 04JL92 accession, were grouped individually.

Table 1. Primers with arbitrary sequence in the RAPD analysis

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 5′ → 3′</th>
<th>GC content (%)</th>
<th>Total bands</th>
<th>Polymorphic bands</th>
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<tr>
<td>N8038</td>
<td>GGTCCCTGAC</td>
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<td>13</td>
<td>12</td>
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<tr>
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<td>16</td>
<td>14</td>
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<td>10</td>
</tr>
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<td>60</td>
<td>14</td>
<td>14</td>
</tr>
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<td>15</td>
<td>11</td>
</tr>
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<td>7</td>
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<tr>
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<tr>
<td>OPG12</td>
<td>CAGCTCACC</td>
<td>60</td>
<td>13</td>
<td>11</td>
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</table>
Figure 1. RAPD patterns obtained from various accessions with RAPD primers

Figure 2. Dendrogram of 11 accessions constructed using UPGMA cluster analysis methods based on Nei and Li (1979) genetic distance value by TreeCon program

NIR spectrum showed lotus leaf tea of Muan and Yeonkkotnabi are different (Fig. 3). Clustering showed they were differently grouped. The lotus leaf of Yeonkkotnabi was grouped with White lotus of Hampyeong accession, Red lotus of Hwasun accession, White lotus of Kangjingeumjeong accession and 04JL40 accession and their elements are similar (Fig. 4). On the contrary, 04JL70 accession was clustered individually (Fig. 4). This results indirectly proved RAPD analysis data of the lotuses which are grouped large, middle and small size species by RAPD analysis.
Figure 3. Average of original NIR spectra for various leaf teas

Figure 4. Dendrogram derived from cluster analysis based on NIR data for 8 lotus accessions
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REFERENCES


