GENETIC DIVERSITY OF JAPANESE WILD EVERGREEN AZALEAS IN KYUSHU (SOUTH MAIN ISLAND OF JAPAN) CHARACTERIZED BY AFLP

T. Handa, J. Eto, K. Kita and N. Kobayashi
Institute of Agriculture and Forestry
University of Tsukuba
Tsukuba, Ibaraki 305
Japan.
thanda@sakura.cc.tsukuba.ac.jp

Keywords: Rhododendron kiusianum, Rhododendron kaempferi, population genetics, introgressive hybridization

Abstract

The genetic diversity of wild evergreen azaleas in Kyushu, the south main island of Japan, was characterized with amplified fragment length polymorphism (AFLP). Kyushu is an important genetic resource center for breeding of the Japanese evergreen azalea cultivars. Rhododendron kiusianum Makino growing above 1000m alt. on the volcanic mountains and R. kaempferi Planch. distributed below 600m alt. are the most important wild species in Kyushu. Putative natural hybrid populations of these two species are found in the intermediate region. AFLP analysis has revealed the genetic variation within and among these populations. Some species specific bands were detected in R. kiusianum and R. kaempferi, and the genetic constitution of each population was identified by AFLP polymorphisms.

Abbreviations
AFLP: Amplified Fragment Length Polymorphism, PCR: Polymerase Chain Reaction, RFLP: Restriction Fragment Length Polymorphism

1. Introduction

Evergreen azalea is one of the important ornamental shrubs in Japan (Chamberlain and Rae, 1990). Kyushu, the south main island of Japan, is a gene center of Japanese evergreen azaleas. Several wild evergreen azalea species are native to Kyushu and they might be a genetic resource for Japanese evergreen azalea cultivars. Rhododendron kiusianum Makino, which inhabits above 1000m, has a dwarf phenotype with small pink-purple flowers and small elliptical leaves. R. kaempferi Planch., which is distributed below 600m, is a loosely branched shrub with big red-orange flowers and large oblong leaves (Sakata et al., 1993). Putative natural hybrid populations, which grow in the intermediate region of the mountain, exhibit varying combinations of traits of the two species. These hybrid populations have been thought to be the origin of some Japanese evergreen azalea cultivar groups such as Edo-Kirishima and Kurume azaleas. Kobayashi et al. (2000) found cytoplasmic introgressive hybridization among the populations in Kirishima mountains of Kyushu by PCR-RFLP analysis using 16SrDNA region of cpDNA. But no nuclear DNA data were reported on these populations.

Recently, the AFLP analysis was carried out in azalea cultivars and their close related species (De Riek et al., 1999). They found this technique could assess the genetic conformity within a breeder’s collection of evergreen azalas.

In this study, we tried to apply the AFLP analysis to evaluate the introgressed genotypes of hybrid populations of two native evergreen azalea species in Kirishima mountains of Kyushu, Japan.
2. Materials and methods

2.1 Plant material

A total of 46 native individuals of evergreen azaleas including 13 of *R. kiusianum*, 9 of *R. kaempferi* and 24 of introgressed hybrids on Kirishima mountain were used for plant materials. They were morphologically identified by the observation of tree height, leaf size, leaf shape, flower size, flower color and blotch on upper lobe of corolla. Fresh leaves were collected from these plants and stored in a freezer for DNA extraction.

2.2 DNA extraction

Total genomic DNA was extracted from frozen leaf samples by a modified CTAB method (Kobayashi *et al.*, 1998).

2.3 AFLP protocol

The AFLP procedure was performed using a commercially available kit from Perkin-Elmer Biosystems for fluorescent fragment detection (Perkin-Elmer, 1995). *Eco*RI and *Mse*I were used for DNA digestion. Selective amplification was done using fluorescent-labelled *Eco*RI-*Mse*I primer sets with 6 selective bases. Four primer sets (PS1-PS4) used in this study were indicated in Table 1. PCR amplifications were carried out in a Perkin-Elmer 9600 thermal controller. Amplification products were separated on 6% denaturing PAGE with a ABI Prism 377 DNA Sequencer.

2.4 Band scoring

The AFLP amplification products were designated by Genescan 3.1.2 software. Only clear AFLP bands were scored as present(1) or absent(0).

3. Results and discussion

A total of 470 unique fragments among the 46 genotypes were scored (Table 2). Of the 470 bands, 129 were monomorphic and 341 were polymorphic. Within 341 polymorphic bands, 25 were *R. kiusianum* specific and 15 were *R. kaempferi* specific bands. These 40 species-specific bands were considered further as species-specific introgressed markers.

All of 24 hybrid genotypes contained both groups of specific AFLP markers (Fig. 1). The number of specific markers of *R. kiusianum* and *R. kaempferi* varied from 6 to 19 and from 1 to 11, respectively. Of the 24 hybrid genotypes, which resembled to the *R. kaempferi* phenotype (C in Fig. 1) possessed *R. kaempferi* cpDNA (2 in Fig. 1) and they included 6-11 *R. kaempferi* specific AFLP markers. The hybrids which resembled to the *R. kiusianum* phenotype (A in Fig. 1) possessed either cpDNA and 9-19 *R. kiusianum* specific AFLP bands were observed.

The AFLP analysis can reveal the introgressive nature of each natural hybrid genotype between *R. kiusianum* and *R. kaempferi*. The morphological characteristics of each hybrid somewhat reflect the composition of the genome. More investigations using a wide range of native populations in Kyushu will further clarify the origin of Japanese evergreen azalea cultivars.

References

De Riek J., Dendauw J., Mertens M., De Loose M., Heursel J., and Van Bockstaele E.,
Table 1. Primer sets used in this study

<table>
<thead>
<tr>
<th>Primer Set</th>
<th>EcoRI-</th>
<th>MseI-</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS1</td>
<td>ACT</td>
<td>CTA</td>
</tr>
<tr>
<td>PS2</td>
<td>AAG</td>
<td>CAT</td>
</tr>
<tr>
<td>PS3</td>
<td>ACT</td>
<td>CAT</td>
</tr>
<tr>
<td>PS4</td>
<td>AAG</td>
<td>CTA</td>
</tr>
</tbody>
</table>

Table 2. Numbers of polymorphic AFLP bands

<table>
<thead>
<tr>
<th>Band polymorphism</th>
<th>PS1</th>
<th>PS2</th>
<th>PS3</th>
<th>PS4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monomorphic bands</td>
<td>23</td>
<td>40</td>
<td>29</td>
<td>37</td>
<td>129</td>
</tr>
<tr>
<td><em>R. kiusianum</em> specific bands</td>
<td>3</td>
<td>4</td>
<td>7</td>
<td>11</td>
<td>25</td>
</tr>
<tr>
<td><em>R. kaempferi</em> specific bands</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>Other polymorphic bands</td>
<td>97</td>
<td>88</td>
<td>61</td>
<td>55</td>
<td>301</td>
</tr>
<tr>
<td>Total</td>
<td>128</td>
<td>136</td>
<td>99</td>
<td>107</td>
<td>470</td>
</tr>
</tbody>
</table>

Figure 1. Numbers of species-specific markers in 24 natural hybrids.
Morphological type: A, *R. kiusianum*; B, hybrid; C, *R. kaempferi*
cpDNA type: 1, *R. kiusianum*; 2, *R. kaempferi*