

Genetic Relationship of *Rhododendron ripense* Makino to Japanese Evergreen Azalea Cultivars Evaluated by SSR Markers

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Abstract

Evergreen azalea cultivars as the important ornamental shrubs and pot plants have been developed based on the genetic resources of Japanese wild azaleas. *Rhododendron ripense* Makino is one of the representative wild azalea species endemic to West Japan and the ancestral parent of evergreen azalea cultivars. In this study, we focused on this large flowered riverside azalea and the genetic contribution to azalea cultivars was evaluated by means of SSR markers. The results of PCoA using SSR data made plot distributions, which would reflect the taxon and genetic relationship between cultivar groups and their ancestral species. All individuals of *R. ripense* which classified in subsection Scabra makes distribution group apart from *R. kaempferi* and *R. kiusianum* group which classified in subsection Tsutsusi. The cultivars of *R. ripense*

SSR marker analysis

Total genomic DNA was extracted from -80°C freeze fresh leaves of each plant by modified CTAB methods [9]. Each DNA samples were genotyped by using 3 pairs of SSR primers (AZA002, AZA003 and AZA008) [10,11]. PCR amplification was carried out with a thermal cycler (ASTEC, PC320). The size of fragments was determined by ABI PRISM310 genetic analyzer and GeneScan™ programs. To evaluate the characteristics of each wild azalea species, the mean number of alleles per locus (NA), observed heterozygosity (HO), expected heterozygosity (HE), and total number of private alleles (PA) were calculated using GenAlEx 6.503 [12]. Genetic relationships between all individuals of the wild species and cultivars were evaluated by a principal co-ordinate analysis (PCoA) of codominant genotypic distance [13] using GenAlEx 6.503 to visualize the relationship among the accessions in a scatter-plot.

Results and Discussion

In SSR analysis using 3 SSR primer sets, totally 78 alleles including polymorphic patterns were obtained in all samples. With the primer set of AZA002, 34 alleles including 5 common alleles between species were detected in the range of 139-295bp. And the primer sets of AZA003 and AZA008 detected 17 alleles in the range of 144-184bp, 23 alleles in the range of 152-239bp, respectively. The genetic diversity parameters are shown in Table 1. The genetic diversity observed in the five wild species differed

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