

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*, Ryukyu azalea (*R. × mucronatum*) and 'Oomurasaki' (*R. × pulchrum*) are all included in this *R. ripense* plot group. These results of this study suggested the important genetic contribution of *R. ripense* to the development of these azalea cultivars.

Keywords: Ancestral parent; Breeding history; Evergreen azalea; Genetic resources

Introduction

In the genus *Rhododendron* (*Ericaceae*), the species in the subgenus Tsutsusi, section Tsutsusi, are important genetic resources for evergreen azaleas used as ornamental shrubs or pot azaleas in the world. Among the native azalea species in Japan, such as *R. kaempferi*, *R. kiusianum*, *R. indicum*, *R. eriocarpum*, *R. macrosepalum* and *R. ripense* have the high ornamental value. By means of mutation and hybrid selection from the natural populations of these wild azaleas, hundreds of azalea cultivars have been developed since the Edo era (1603-1867) [1]. The development of each cultivar groups, such as Edo Kirishima, Ryukyu, Hirado, Satsuki and Kurume are the fruits of Japanese horticulture history [2,3].

Rhododendron ripense Makino belongs to subgenus Tsutsusi, section Tsutsusi subsection Scabra [4], distributes in rocky riverside areas of the Chugoku and Shikoku areas of West Japan [5]. This Japanese endemic species has been considered to be the important ancestral parent of evergreen azalea cultivars, especially, Ryukyu azalea (*R. × mucronatum*) and 'Oomurasaki' (*R. × pulchrum*) [6]. And also, this large flowered azalea is one of the best ornamental performance species with high adaptability of environmental conditions [7,8].

In this study, the genetic contributions of *R. ripense* to the development of Japanese azalea cultivars were evaluated by using SSR (Simple sequence repeat) markers. With the comparison of the genetic distance calculated by SSR genotypes, the genetic relationship of *R. ripense* to azalea cultivars and also to other species was investigated.

Materials and Methods

Plant materials

Azalea species and cultivars used in this study were collected from wild habitat in Japan and/or the collection of Niigata Prefectural Botanical Garden, and the Plant Breeding Laboratory of Shimane University. They included 73 individuals of *R. ripense* from 23 wild habitats of 12 rivers in the Chugoku (47) and Shikoku (26) areas (Figure 1). And also 45 individuals of *R. kaempferi*, 19 individuals of *R. kiusianum*, 15 individuals of *R. eriocarpum* and 25 individuals of *R. macrosepalum* were used. Furthermore, 53 cultivars from the cultivar groups of Ryukyu azalea (*R. × mucronatum*), Oookirishima (*R. × pulchrum*), Kurume azalea (*R. × obtusum*), *R. macrosepalum* and *R. ripense* were analyzed.



Figure 1: *R. ripense* Makino in the natural habitat of Shimane prefecture, Chugoku area, Japan.

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 (ASTECH, PC320). The size of fragments was determined by ABI PRISM 310 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 GenAEx 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 GenAEx 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 among species, as indicated by the expected heterozygosity (HE) which ranged from the Chugoku area of *R. ripense* (0.539) to *R. macrosepalum* (0.898). Among five wild species, *R. ripense*, *R. macrosepalum* and *R. kaempferi* harbored from 3 to 14 private alleles.

PCoA using SSR data of wild species and cultivars made the plot distributions which would reflect the taxon and genetic relationship between cultivar groups and their ancestral species (Figure 2). Individuals of *R. kaempferi* and *R. kiusianum* which classified in subsection Tsutsusi series *Kaempferia* made overlap distribution, and most of Kurume azalea cultivars were also overlapped in this group. The breeding history of this small flower cultivar group which had developed from these two species was corresponding to these distribution patterns. *R. eriocarpum* classified in subsection Tsutsusi and *R. macrosepalum* classified in subsection *Scabra* also located in the vicinity of these groups.

All individuals of *R. ripense* which classified in subsection *Scabra* makes distribution group apart from other species, and the population of the Shikoku area was gathered inside of the population of Chugoku area. *R. ripense* 'Wakasagi', 6 cultivars of Ryukyu azalea, 4 cultivars of Ookirishima and 2 presumed cultivars of *R. macrosepalum* are all included in this large flowered *R. ripense* group. These results back up the important genetic contribution of *R. ripense* to the development of Ryukyu azalea (*R. × mucronatum*) and 'Oomurasaki' (*R. × pulchrum*) cultivars. And the results of AFLP marker analysis also supported these genetic relationship [14,15]. Six cultivars of Kurume azalea groups plotted inside or the vicinity of *R. ripense* distribution area. These distributions also support the genetic relationship between *R. ripense* and some of Kurume azalea cultivars because of the similarity leaf surface morphology [16].

As a result of this study, all cultivars of Ryukyu azalea and Ookirishima groups and a part of Kurume azalea cultivars were included in the *R. ripense* plot distribution of PCoA using SSR data.

The genetic contribution of *R. ripense* to the development of some of Japanese azalea cultivars was suggested by SSR marker analysis.

		N	NA	HO	HE	PA
<i>R. ripense</i>	Chugoku area	47	9.33	0.376	0.539	3*
	Shikoku area	26	8.00	0.705	0.589	3*
<i>R. macrosepalum</i>		25	16.33	0.800	0.898	14
<i>R. kaempferi</i>		45	16.33	0.733	0.890	7
<i>R. kiusianum</i>		19	10.33	0.702	0.747	0
<i>R. eriocarpum</i>		15	5.67	0.511	0.627	0

Table 1: Population genetic parameters estimated from 3 SSR markers for 5 azalea species. N total number of analysed samples, NA means number of alleles per locus, HO observed heterozygosity, HE expected heterozygosity, PA total number of private alleles. *PA was calculated in the combined two areas of *R. ripense*.

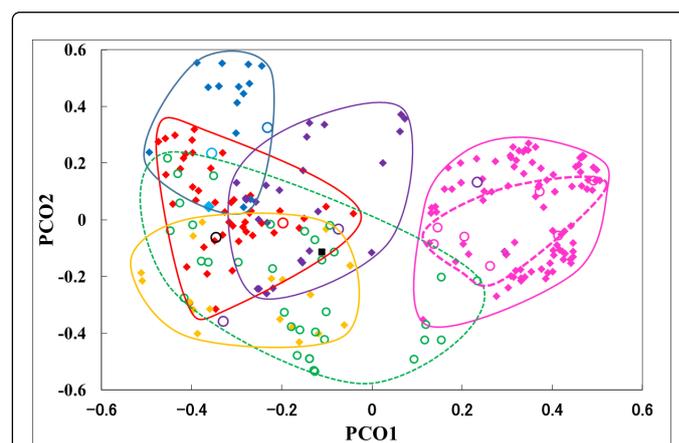


Figure 2: Principal co-ordinate analysis (PCoA) of 177 wild azalea individuals and 53 cultivars by means of three SSR markers.

◆Diamond showed wild species of *R. ripense* (Chugoku; Pink and Shikoku; Orange); *R. macrosepalum* (Purple); *R. kaempferi* (Red); *R. kiusianum* (Yellow); *R. eriocarpum* (Blue). ○Opened circle showed azalea cultivars of Ryukyu (Light blue), Kurume azalea (Green); *R. macrosepalum* (Purple). △Ookirishima (Light blue). □*R. ripense* (Light blue)

The detail of the genetic relationship between species and cultivars will be explicated by the future investigation with using other SSR markers reported by De Keyser [17]. And the investigation of functional gene related to the important ornamental trait; flower color and flower shape [18] will be informative for the study of development history of cultivars from wild azaleas.

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