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Miyajima, Ikuo
Laboratory of Horticultural Science, Faculty of Agriculture, Kyushu University

Miyahara, Kayo
Laboratory of Horticultural Science, Faculty of Agriculture, Kyushu University

Ureshino, Kenji
Laboratory of Horticultural Science, Faculty of Agriculture, Kyushu University

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Contribution of *Rhododendron eriocarpum* Nakai to the flower color variation in *R. sataense* Nakai

Ikuo Miyajima, Kayo Miyahara* and Kenji Ureshino

Laboratory of Horticultural Science, Faculty of Agriculture, Kyushu University 46–01, Fukuoka 812–8581, Japan
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The origin of flower color variation in *R. sataense* was discussed by means of the comparison of some morphological characteristics of flowers and petal pigmentation in *R. sataense* with those of *R. eriocarpum*, *R. kaempferi* and their F_1 progenies. Flower color variations were very wide in *R. sataense*, whereas those in the two other wild species were extremely narrow. Although there were no F_1 the individuals having vermilion or red flowers, the flower color variations of them were wider than those of *R. eriocarpum*.

The frequency of individuals having 5 stamens were highest in *R. sataense* and the number of stamens of *R. kaempferi* and *R. eriocarpum* were only 5 and 10, respectively. The number of stamens in the F_1 was different in the cross combinations.

Almost all individuals of *R. eriocarpum* contained a high ratio of methylated flavonols in their petals, whereas *R. kaempferi* contained few amount. However, there were two groups identified in the F_1 hybrids regarding to the constitutions of the flavonols in their petals; one group was formed with individuals containing high percentage of methylated flavonols and another containing low.

From these findings, it was possibly suggested that the appearance of the progenies with wide flower color variations, 5 stamens and containing few methylated flavonols in their petals such as *R. sataense* were able to be obtained from the crossing between *R. eriocarpum* and *R. kaempferi*.

INTRODUCTION

Rhododendron sataense Nakai distributes in the low altitude mountains and plateaus in southern Kyushu such as Mt. Taka–Toge, Mt. Sakurajima, Mt. Tsujigatake in Osumi peninsula and Mt. Kaimondake and surrounding of Ikeda lake in Satsuma peninsula. It is one of the wild evergreen azaleas and has a wide range of flower color variations (Abe, 1969). Based on its similarities in the morphological and pigmental characteristics to ornamental Kurume azalea, *R. sataense* has been regarded as one of the parents of Kurume azalea (Kunishige, 1978).

R. sataense has also been regarded as a local variety of *R. kaempferi* Planch. because of its similarity in petal size, blotch expression, and the growth habit of branches (Kunishige and Tamura, 1961). The morphological characters of the flowers of *R. sataense* are similar to those of *R. kaempferi* except for flower color variations.

Kunishige and Kobayashi (1980) reported that the wild evergreen azalea populations of which the flower colors range from vermilion through red, pink to purple are usually the progenies of natural crossings between the species having the anthocyanin of

* Present address: Oita Junior College, Oita 870–8658
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delphinidin series (purple flowers) and that of cyanidin series (red flowers). The extremely wide variation of flower colors in *R. sataense*, therefore, suggests that it is the result of interspecific crossing of wild evergreen azaleas.

In the previous report (Miyajima *et al.*, 1997), we pointed out that the flower color variations of *R. sataense* do not seem to be derived from the natural crossing between *R. kiusianum* Makino and *R. kaempferi* from the constitutions of flavonol pigments in their petals.

In this report, we discuss the origin of flower color variation in *R. sataense* by means of the comparison with some morphological characteristics of flowers and petal pigmentation in *R. sataense* and F_1 progenies of *R. eriocarpum* Nakai×*R. kaempferi*.

MATERIALS AND METHODS

Plant materials used in this study are listed in Table 1. The number of stamen were counted with five flowers in each species and F_1 progenies. Fully expanded petals were collected and the colors were identified according to the Royal Horticultural Society Colour Chart (RHSCC). Two ml of 2 N hydrochloric acid were added directly to the fresh petals and the flavonols in the petals were hydrolyzed for 90 min at 90 °C. After hydrolyzation, 2 ml of distilled water were added and held on Sep–Pak cartridge (C_{18}, Millipore). The flavonol pigments on the cartridge were washed with distilled water to eliminate the water–soluble hydrophilic contaminants and eluted with 2 ml of methanol. The methanol eluents were filtrated with membrane filter (0.45 μm) and injected into high performance liquid chromatography (HPLC) developed with LC–6A pump (Shimadzu), 4.6 mm I.D.×250 mm Cosmosil $5C_{18}$–AR column (Nakarai Tesque) and SPD–6AV detector (Shimadzu) set at 360 nm. A flow rate of 1.0 ml/min was maintained and a mixture of acetonitrile–0.1 M acetic acid (30:70, v/v) was employed as the eluant. The percentages of methylated flavonols were calculated as the ratios of 5–O–methylmyricetin and 5–O–methylquercetin in total flavonol aglycones.

Table 1. Number of individuals used in this investigation.

Species and cross combination	Collection site	Number of individuals
R. eriocarpum	Tokara Islands, Kagoshima	26
R. kaempferi	Sasaguri town, Fukuoka	12
R. kaempferi	Tarumizu city, Kagoshima	25
R. sataense	Mt. Taka–Toge, Kagoshima	125
R. eriocarpum No. 1[z]×*R. kaempferi* No. 1		104
R. kaempferi No. 1×*R. eriocarpum* No. 1		93
R. kaempferi No. 2×*R. eriocarpum* No. 2		86

[z] No. 1, No. 2; individual numbers.

RESULTS AND DISCUSSION

Figure 1 shows the results of HPLC separation and structures of the four flavonol aglycones found in the petals of evergreen azaleas. The order of the elution was 5–O–methylmyricetin, 5–O–methylquercetin (Azaleatin), myricetin and quercetin under our analytical conditions.

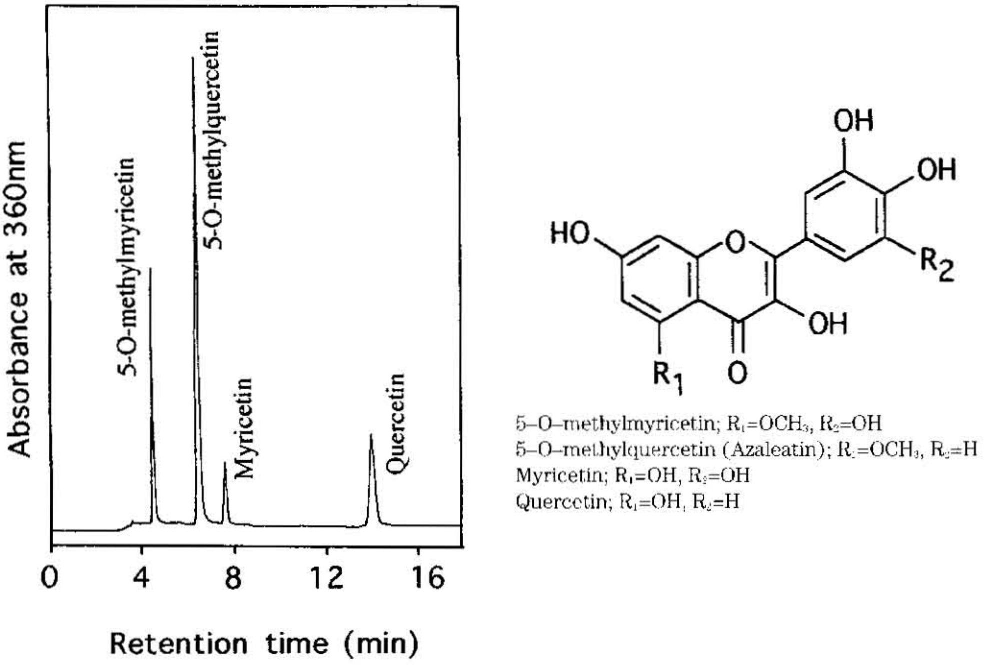

Fig. 1. HPLC profiles and structure of four flavonol aglycones in the petals of evergreen azaleas. The analytical conditions of HPLC are shown in the text.

Flower colors widely varied in *R. sataense*, whereas they were extremely narrow in other two species; almost all *R. eriocarpum* and *R. kaempferi* flowers have pale purple (RHSCC No. 75<) and vermilion (RHSCC No. 45>) petals, respectively (Fig. 2).

Although there were no individuals that have vermilion or red flowers in F_1 progenies, the flower color variations of the both F_1 progenies obtained from the crossings between *R. kaempferi* No. 1 (RHSCC No. 48) and *R. eriocarpum* No. 1 (RHSCC No. 84), and between *R. kaempferi* No. 2 (RHSCC No. 51) and *R. eriocarpum* No. 2 (RHSCC No. 84) were wider than those of *R. eriocarpum*.

The frequency of individuals having 5 stamens were highest in *R. sataense*, and *R. kaempferi* and *R. eriocarpum* had only 5 and 10 stamens, respectively (Fig. 3). The number of stamens in the F_1 hybrids between *R. eriocarpum* and *R. kaempferi* were dependent in the cross combinations. The progenies that had 5 stamens were most

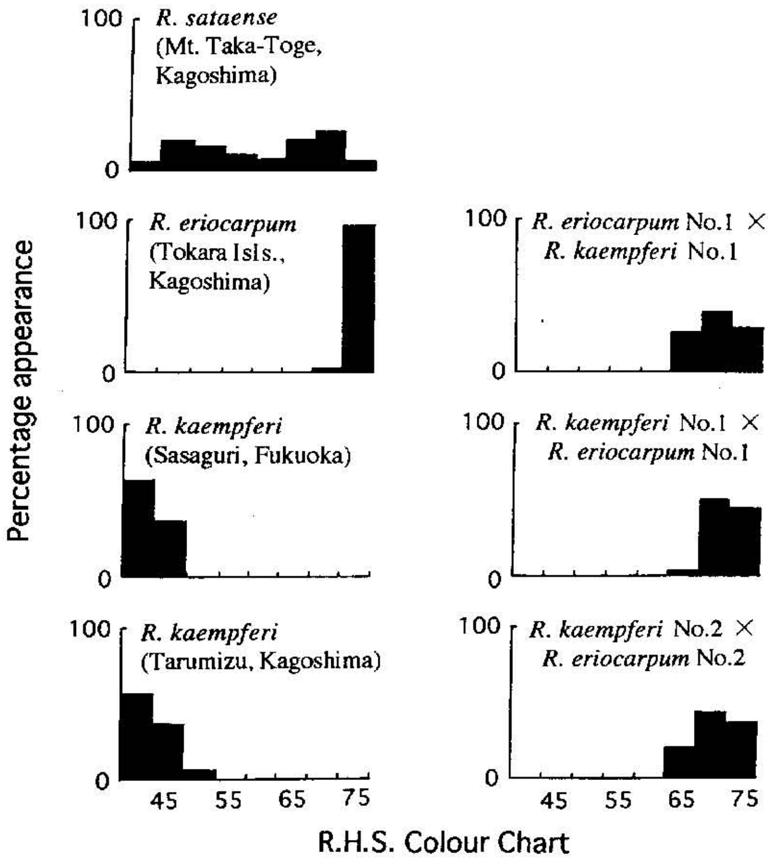

Fig. 2. Frequency of individuals based on the flower colors of wild evergreen azaleas and their interspecific hybrids.

observed in the crossing between *R. kaempferi* No. 1 and *R. eriocarpum* No. 1. However, in the crossing between *R. kaempferi* No. 2 and *R. eriocarpum* No. 2, the percentage of appearance of F_1 hybrids that had 5 stamens was about 25 % and almost all other individuals had more than 5 stamens.

Almost all individuals of *R. eriocarpum* contained a high ratio of methylated flavonols in their petals, whereas *R. kaempferi* did a few amount (Fig. 4). The percentages of methylated flavonols in the petals of the F_1 hybrids between *R. kaempferi* No.1 and *R. eriocarpum* No. 1 were different from that of *R. sataense*. The F_1 hybrids contained 30 to 70% methylated flavonols, whereas only three out of 125 individuals analyzed in the populations of *R. sataense* contained the methylated flavonols (Fig. 4). The F_1 hybrids between *R. kaempferi* No. 2 and *R. eriocarpum* No. 2, however, classified into two groups based on the constitutions of the petal flavonols; one group containing high percentage of methylated flavonols and another one containing low (Fig. 4).

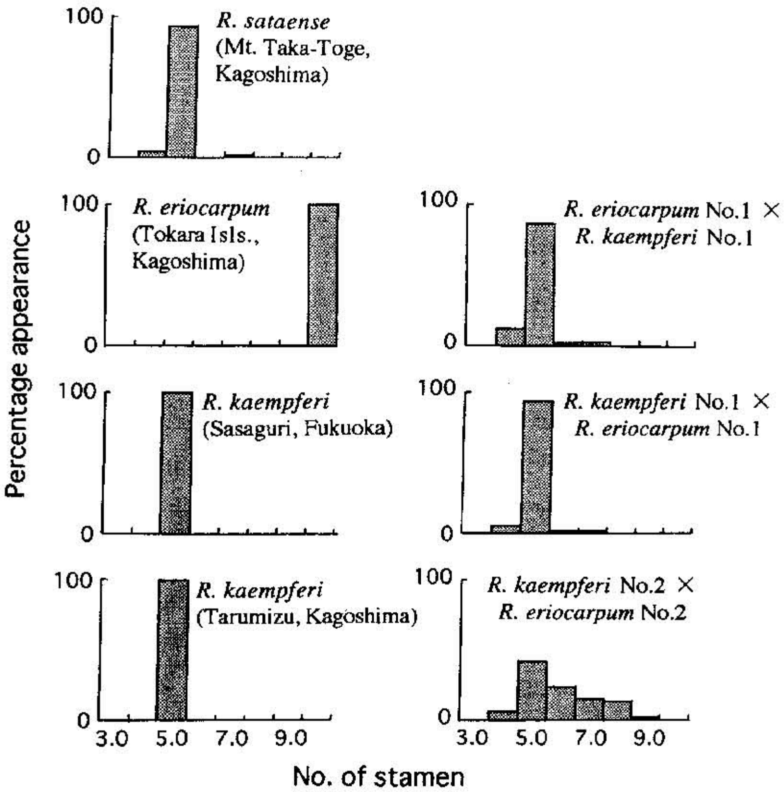

Fig. 3. Frequency of individuals based on number of stamen in wild evergreen azaleas and their interspecific hybrids.

It is possibly suggested from these findings that appearance of the progenies with wide flower color variations and 5 stamens and containing few methylated flavonols in their petals such as *R. sataense* were able to be obtained from the crossing between *R. eriocarpum* and *R. kaempferi*.

On Kirishima and Unzen mountain mass, we investigated the variation of morphological and pigmental characteristics of *R. kiusianum*, *R. kaempferi* and some wild azaleas which have been considered to be their natural hybrids (Sakata *et al.*, 1993; Miyajima *et al.*, 1995). Kunishige and Kobayashi (1980) reported that the populations in Amakusa district, Kyushu island have wide flower color variation and seemed to be derived from the natural crossing between *R. reticulatum* D. Don ex G. Don and *R. weyrichii* Maxim. In addition, the populations which have also flower color variation and thought as the result of the natural crossing between *R. eriocarpum* and *R. indicum* (L.) Sweet were found in Yakushima island. In these cases, the wide flower color variations were observed at the areas where the distribution of purple and red flowered species were overlapped. Therefore, the extremely wide variation of flower colors in *R. sataense* suggests that it is

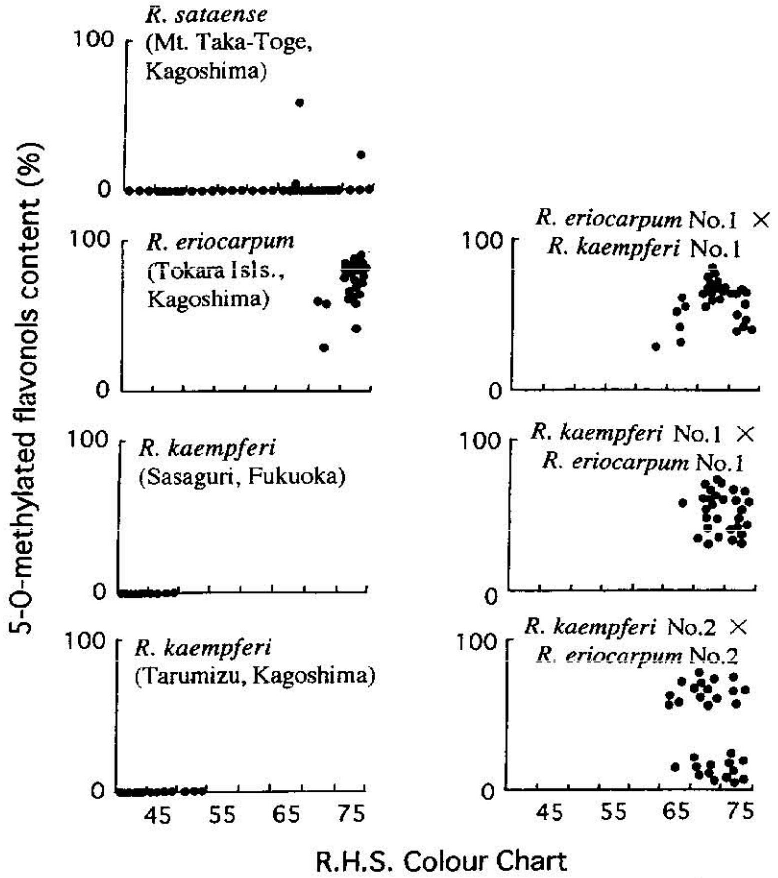

Fig. 4. Distribution of individuals based on the occurrence of 5-O-methylated flavonols in the petals of wild evergreen azaleas and their interspecific hybrids.

an interspecific hybrid between two wild evergreen azaleas that have purple and red flowers.

In previous report (Miyajima *et al.*, 1997), we pointed out that the contribution of *R. kiusianum* to the flower color variation in *R. sataense* seems to be low from the results of the inheritance pattern of flavonol pigments, although this species has purple flowers. And we suggested that the morphological and pigmental characteristics of *R. sataense* should be compared with those of *R. eriocarpum*.

R. sataense and *R. eriocarpum* have a similar leaf shape (Hatsushima, 1958) and both species distribute closely in southern Kyushu. From these facts, it is suggested that the progenies which have 5 stamens and contained few methylated flavonols in their petals such as *R. sataense* were able to become into existence from the crossing between *R. eriocarpum* and *R. kaempferi*.

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