Flower Color and Pigments of Intersubgeneric Hybrid between White-flowered Evergreen and Yellow-flowered Deciduous Azaleas

Ikuo Miyajima¹, Kenji Ureshino¹, Nobuo Kobayashi² and Masaru Akabane³

¹ Laboratory of Horticultural Science, Faculty of Agriculture, Kyushu University 46-01, Fukuoka 812-8581

² Tatebayashi Azalea Research Station, Tatebayashi, Gunma 374-0005

³ 2469-5, Mibu-machi, Tochigi 321-0201

Summary

Flower color and floral pigments were evaluated on an intersubgeneric hybrid (9027-2), obtained by crossing *R. kiusianum* Makino cv. Shirobana-miyamakirishima × *R. indicum* (L.) Sweet cv. Hakata-jiro, and *R. japonicum* f. *flavum*. The flowers of the 9027-2 were pale yellow at full bloom. The absorption peaks detected in the extracts from the petals of *R. japonicum* f. *flavum* and 9027-2 are considered to be β -carotene and that this pigment was inherited by 9027-2 from *R. japonicum* f. *flavum*. The 9027-2 was confirmed as the hybrid between (*R. kiusianum* cv. Shirobana-miyamakirishima × *R. indicum* cv. Hakata-jiro) hybrid No. 1 and *R. japonicum* f. *flavum* No. 1 by isozyme analysis.

Key Words: azalea, carotenoid, isozyme, *Rhododendron japonicum* f. *flavum*, yellow flower.

Introduction

Flower colors of evergreen azaleas have been restricted to red, pink, white, reddish-purple, and purple. A yellow-flowered evergreen azalea has been the dream and goal of many azalea breeders. The deep yellow-flowered azalea, *Rhododendron japonicum* (A. Gray) Suringar f. *flavum* Nakai is a native Japanese deciduous azalea; its flower color is attributed to the presence of a carotenoid (Spethmann, 1978). Thus, this species is one of the most valuable parents for breeding of yellow-flowered evergreen azalea.

In 1993, Akabane (1993) obtained intersubgeneric hybrids from crosses between white-flowered hybrid (*R. kiusianum* Makino cv. Shirobana-miyamakirishima × *R. indicum* (L.) Sweet cv. Hakata-jiro) and yellow-flowered azalea (*R. japonicum* f. *flavum*); their anthesis was observed in 1995. The morphological characteristics, except flower color of the 15 intersubgeneric hybrids, and their hybridity confirmation by RAPD analysis have already been reported (Kobayashi et al., 1996).

In this study, the floral pigments of another intersubgeneric hybrid obtained from the same cross combination were characterized and the isozyme analyses were conducted to confirm its hybridity.

Materials and Methods

Plant materials

The flowers and leaves from (*R. kiusianum* cv. Shirobana-miyamakirishima × *R. indicum* cv. Hakata-jiro) hybrid No.1, *R. japonicum* f. *flavum* No.1 and their intersubgeneric hybrid 9027-2 were analyzed. The details of these crossings were described by Kobayashi et al. (1996).

Measurement of flower color and absorption spectrum

Fully expanded fresh flowers were collected at random from each plant and five portions of lower wings of the flowers were measured by the colorimeter (NR-3000, Nippon Denshoku Co., Japan). The petals were homogenized with 10 ml of ethanol and the extracts filtered; the absorption spectra of the filtrate were measured with a spectrophotometer (UV-160A, Shimadzu Co., Japan).

To eliminate the interference by chlorophyll and other leaf constituents, the procedure for extraction and purification of pigments from the petals of 9027-2 were as follows; the fresh petals of the 9027-2 were homogenized and extracted with 10 ml of acetone. The homogenate was filtered under reduced pressure, and the residue washed thoroughly with an acetone-hexane (75:60, v/v) mixture until the filtrate became colorless. The filtrate was then transferred to a separatory funnel and washed with distilled water and then with 85% methanol; the lower phase was discarded. For

saponification, 20% KOH in 85% methanol was added and the sample kept in dark at room temperature for overnight. After the hexane phase was repeatedly washed with 85% methanol, its absorption spectrum (from 200 to 700nm) was measured on a spectrophotometer.

Isozyme analysis

The extraction, purification, and gel staining for electrophoresis followed the protocols of Wendel and Parks (1982) and Wendel (1983). Isozyme analysis was conducted using glucosephosphate isomerase (GPI, EC 5.3.1.9) and phosphoglucumutase (PGM, EC 2.7.5.1).

Results and Discussion

The petals of F_1 hybrid No.1 of (*R. kiusianum* cv. Shirobana-miyamakirishima $\times$ *R. indicum* cv. Hakata-jiro) were white, and those of *R. japonicum* f. *flavum* No.1 were deep yellow. The flowers of the 9027-2 were yellowish-green at bud stage, but turned pale yellow at full bloom (Fig. 1).

The absorption peaks of 340, 325, and 327nm in the ethanol extracts from petals of the F_1 hybrid No.1 of (*R. kiusianum* $\times$ *R. indicum*), *R. japonicum* f. *flavum* No.1 and their hybrid 9027-2 seem to match those of colorless flavones or flavonols (Fig. 2). However, ethanolic petal extracts of *R. japonicum* f. *flavum* had the absorption peaks of 450 and 475nm, whereas those of 9027-2 peaked in the region of 400 to 500nm (Fig. 2). In addition, the absorption spectrum of the hexane phase of petals from 9027-2 had peaks of 425, 448, and 475nm (Fig. 2).

That β -carotene is the main component of the deep yellow color of the petals of *R. japonicum* f. *flavum* was reported by Spethmann (1978); this pigment is absent in petals of white-flowered azaleas. The absorption maxima of β -carotene is 427, 449, and 475nm in ethanol and 425, 450, and 477nm in hexane (Davies,

Fig. 1. The flower of 9027-2 obtained from the crossing between (*R. kiusianum* cv. Shirobana-miyamakirishima $\times$ *R. indicum* cv. Hakata-jiro) hybrid No.1 $\times$ *R. japonicum* f. *flavum* No.1.

Fig. 2. Absorption spectra of ethanol extracts from the petals of (*R. kiusianum* cv. Shirobana-miyamakirishima $\times$ *R. indicum* cv. Hakata-jiro) hybrid No.1, *R. japonicum* f. *flavum* No.1 and their hybrid 9027-2 (upper), and those of hexane phase extracted and purified from the petals of 9027-2 (lower).

1976). Accordingly, the absorption peaks, detected in the extracts from the petals of *R. japonicum* f. *flavum* and 9027-2, are considered to be β -carotene which was inherited by 9027-2 from *R. japonicum* f. *flavum*.

Electrophoretic gels of 9027-2 exhibited parental bands of both GPI and PGM isozymes which confirms that it is truly a progeny between (*R. kiusianum* cv. Shirobana-miyamakirishima $\times$ *R. indicum* cv. Hakata-jiro) hybrid No.1 and *R. japonicum* f. *flavum* No.1 (Fig. 3).

Many breeders have tried to produce yellow-flowered evergreen azaleas by using interspecific crosses between evergreen species and *R. japonicum* f. *flavum* (Akabane et al., 1971; Heursel, 1981; Yamaguchi et al., 1985). Most of these crosses, however, failed to yield viable

Fig. 3. Schematic illustration of GPI and PGM isozymes of parents and their hybrid.

Lane 1: (*R. kiusianum* cv. Shirobana-miyamakirishima × *R. indicum* cv. Hakata-jiro) hybrid No.1, Lane 2: *R. japonicum* f. *flavum* No.1, Lane 3: 9027-2.

progenies because they were mostly albinos. The 9027-2 was obtained from the three-way crossing between evergreen azaleas and *R. japonicum* f. *flavum* which may be a useful means for breeding yellow-flowered evergreen azaleas (Ureshino et al., 1998). There is a possibility of developing progenies that contain larger amounts of carotenoid in their petals and produce deep yellow flowers in the future.

Literature Cited

- Akabane, M. 1993. On the cross compatibility F_1 -plants (*Rhododendron kiusianum* Mak. × *R. indicum* Sweet) × *R. japonicum* Suringar. J. Japan. Soc. Hort. Sci. 62 (Suppl. 1): 344-345 (In Japanese).
- Akabane, M., A. Yamanaka, D. Takashima, T. Nakatsue and Y. Nakamura. 1971. On the fertility of interspecific crossing and the growth of F_1 seedlings in rhododendron species. Bull. Tochigi Pref. Agr. Expt. St. 15: 95-102 (In Japanese).
- Davies, B. H. 1976. Carotenoids. p. 108-124. In: T. W. Goodwin (ed). Chemistry and biochemistry of plant pigments. vol. 2. Academic Press, London.
- Heursel, J. 1981. Diversity of flower colors in *Rhododendron simsii* Planch. and prospects for breeding. Euphytica 30: 9-14.
- Kobayashi, N., M. Akabane, T. Handa and K. Takayanagi. 1996. Inheritance of morphological characters and RAPD markers in intersubgeneric hybrids of azalea, (*Rhododendron kiusianum* Makino × *R. indicum* (L.) Sweet) × *R. japonicum* (A. Gray) Suringar f. *flavum* Nakai. J. Japan. Soc. Hort. Sci. 65: 145-153.
- Spethmann, W. 1978. Flavonoids and Carotenoids of rhododendron flowers and their significance for the classification of the genus *Rhododendron*. p. 247-276. In: J. L. Luteyn and M. E. O'Brien (eds.). Contributions toward a classification of rhododendron. New York Bot. Garden, New York.
- Ureshino, K., I. Miyajima and M. Akabane. 1998. Effectiveness of three-way crossing for the breeding of yellow-flowered evergreen azalea. Euphytica 104: 113-118.
- Wendel, J. F. 1983. Electrophoretic analysis of genetic variation in wild and cultivated *Camellia japonica* L. Ph. D. Thesis, University of North Carolina, Chapel Hill, North Carolina.
- Wendel, J. F. and C. R. Parks. 1982. Genetic control of isozyme variation in *Camellia japonica* L. Hered. 73: 197-204.
- Yamaguchi, S., M. Kunishige and T. Tamura. 1985. Interspecific compatibility in Japanese rhododendrons. Bull. Veg. and Ornam. Crop Res. Stn. Japan, Ser. B. 8: 87-97 (In Japanese with English summary).

常緑性白色花ツツジと落葉性黄色花ツツジとの 亜属間雑種個体における花色と花色素

宮島郁夫¹・嬉野健次¹・小林伸雄²・赤羽 勝³

¹ 九州大学農学部園芸学教室 812-8581 福岡市東区箱崎

² 館林市つつじ研究所 374-0005 群馬県館林市花山町

³ 321-0201 栃木県下都賀郡壬生町安塚

摘 要

(ミヤマキリシマ '白花ミヤマキリシマ' × サツキ '博多白') × キレンゲツツジの交配で得られた亜属間雑種個体 9027-2 の花色を調査し花色素分析を行うとともに、同個体の雑種性をアイソザイム分析で確認した。満開時の 9027-2 の花色は淡黄色であった。花卉抽出液の吸収特性からキレンゲツツジと 9027-2 の花卉にはいずれも β -カロチンが含まれており、同色素がキレンゲツツジから 9027-2 に遺伝したことが示唆された。アイソザイム分析の結果、9027-2 は (ミヤマキリシマ '白花ミヤマキリシマ' × サツキ '博多白') No.1 とキレンゲツツジ No.1 との雑種であることが確認された。