Scientia Horticulturae 195 (2015) 145–153

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Flower color and pigments in yellow-flowered hybrid progeny raised from the interspecific cross *Pelargonium quinquelobatum* × white-flowered geraniums

Kana Hondo[a], Pornpan Sukhumpinij[b], Fumika Kakihara[a,*]

[a] *Laboratory of Plant Breeding, Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama 790-8566, Japan*
[b] *Faculty of Agricultural Technology, Rambhai Barni Rajabhat University, Muang, Tachang, Chantaburi 22000, Thailand*

ARTICLE INFO

Article history:
Received 21 April 2015
Received in revised form 1 September 2015
Accepted 9 September 2015
Available online 24 September 2015

Keywords:
Flavonol
Geranium
Interspecific cross
Pelargonium quinquelobatum
Quercetin
Yellow flower

ABSTRACT

The aim of this study was to produce a yellow-flowered geranium (*Pelargonium* × *hortorum* Bailey). Interspecific crosses between white-flowered geraniums and *Pelargonium quinquelobatum* Hochst. ex A. Rich. were performed. Unilateral incompatibility was observed, such that normal seeds were obtained when *P. quinquelobatum* was used as the seed parent, whereas no seeds were obtained when *P. quinquelobatum* was used as the pollen parent. The F_1 progeny produced sterile pollen; therefore, genome doubling of the F_2 progeny was induced by colchicine treatment to ensure fertile pollen; F_3 progeny were also produced. The F_3 progeny were crossed with tetraploid geraniums to produce BC_1 and BC_1F_2 progeny. The flower color of the hybrid progeny were pale yellow, ranged from green–yellow 1D to yellow 3C, according to the Royal Horticultural Society Color Chart, and became increasingly the value of the colorimetric parameter b* with advancing generations. The pigmentation of the pale yellow flowers involved a large amount of flavonols and a small amount of carotenoids: the main pigments were kaempferol or quercetin. The quercetin contents were significantly correlated with the b* value ($r = 0.82$, $P < 0.001$), whereas no correlation was found between the kaempferol or carotenoids contents and the b* value. Furthermore, the b* value was unaffected by the aluminum content or the pH value in the petals.

1. Introduction

Pelargonium × *hortorum* Bailey, generally known as geraniums, is a perennial plant belonging to the genus *Pelargonium.* Geraniums are one of the most economically important floriculture crops for bedding, pots, and hanging baskets in North America and Europe. Especially in the United States, the annual value at wholesale exceeded US$137 million (USDA, 2014). Geraniums have many desirable characteristics, including ease of growth, flowering through all four seasons, and propagation by both seedling and cutting. Geraniums have many florets to each umbel, which comprise many individual florets arising from the same point, and show a long flowering time with various flower colors.

Geraniums had been developed from several wild species of the section *Ciconium*, such as *P. zonale*, *P. inquinans*, *P. frutetorum*, *P. acetosum*, and *P. salmoneum*, which were introduced to Europe from South Africa in the 18th century (Clifford, 1958; Harney, 1966). The color pallet of geranium flowers is wide, from white, pale pink to deep red, coral, salmon, magenta, and lavender, which likely reflects the flower color of their ancestors; however, there are no yellow geraniums. Some pale yellow-flowered commercial cultivars such as 'Botham's Surprise', 'Creamery', and 'Fast Yellow', which is likely to be the most yellow cultivar available at present, are on the market. These cultivars, however, are unpopular, possibly because of their difficult cultivation (Brawner, 2003; Taylor, 1990). Brawner (2003) presumed that these pale yellow cultivars were developed from hybrids between *P.* × *hortorum* and *P. quinquelobatum* Hochst. ex A. Rich., which is a wild species with pale yellowish-green to grayish-green–blue flowers within the section *Ciconium* (Miller, 1996). However, there were no details in the report regarding the development of these cultivars. Both geraniums and *P. quinquelobatum* are classified into the section *Ciconium* and their basic chromosome number is the same ($2n = 18$, $x = 9$) (Clifford, 1958; Gibby and Westfold, 1986). Moreover, Denis-Peixoto et al. (1997) obtained hybrid plants with pale pink flowers that had blotches of pale yellow from the interspecific cross

Abbreviations: FC, Flow cytometry; LS, Linsmaier and Skoog(1965).

* Corresponding author. Fax: +81 89 946 9757.
E-mail addresses: hondo.kana.mf@ehime-u.ac.jp (K. Hondo), sukhumpinij@hotmail.com (P. Sukhumpinij), kakihara@agr.ehime-u.ac.jp (F. Kakihara).

http://dx.doi.org/10.1016/j.scienta.2015.09.014
0304-4238/

between *P. quinquelobatum* and *P.* × *hortorum* via ovule culture. Therefore, the presumption that these pale yellow-flowered geraniums were developed from a cross between *P. quinquelobatum* and *P.* × *hortorum* was reasonable.

Expansion of characteristics, especially flower color, will generally have a significant beneficial economic effect on the flower business. In the present study, we aimed to produce desirable cultivars of geraniums with true yellow flowers. Therefore, we carried out interspecific crosses between geraniums with white flowers and *P. quinquelobatum* and further produced F_2, F_3, BC_1, and BC_1F_2 progeny. We describe the variation of morphological characteristics, especially the color and pigments of flowers, among the interspecific hybrid progeny, and discuss the expression of the yellow pigmentation.

2. Materials and methods

2.1. Plant materials

White- or yellow-flowered geraniums were used for crossing (Table 1). Three strains of geranium (H1–3) were developed by crossing among several cultivars of geranium in the Plant Breeding Laboratory of the Faculty of Agriculture, Ehime University, and another three horticultural cultivars were purchased at a nursery. The seeds of *P. quinquelobatum* were obtained from Floranova Ltd., Dereham, UK.

2.2. Production of hybrid progeny

As *Pelargonium* is protandrous, stamens were removed before anthesis and pollination was carried out 1–2 days after anthesis when the stigmas had split open. F_1 plants obtained from the cross between *P. quinquelobatum* and diploid geraniums had sterile pollen. Therefore, a part of F_1 seeds were treated with colchicine and the fertility-restored F_1 plants were self-pollinated to produce F_2 and F_3 plants. BC_1 plants were produced from reciprocal crosses between F_3 plants and tetraploid geraniums; BC_1 plants were self-pollinated to produce BC_1F_2 plants. To sow the hard seeds of *Pelargonium*, the seed coats were scarified with a knife, sown in petri dishes on filter paper soaked in distilled water, and then incubated under 25 °C and a 16 h light/8 h dark photoperiod until germination. For colchicine treatment, seeds were scarified before sterilization, absorbed in a petri dish of sterilized water for 1–2 days and then sown in LS medium (Linsmaier and Skoog, 1965) supplemented with 20 g l^{-1} sucrose and 2 g l^{-1} gellan gum (pH 5.8), and incubated at 25 °C with a 16 h light/8 h dark photoperiod. When one true leaf emerged from the seeds, colchicine solution (0.2%) with 1% gellan gum was swabbed on the base part of the true leaf. After cultivation for 1–4 days under the low temperature (5 °C) in continuous darkness, they were cultured again for approximately 1 month under the same conditions (25 °C, a 16 h light/8 h dark photoperiod). All seedlings, following acclimatization, were transplanted into pots containing a mixture of vermiculite and perlite (1:1), cultured for 2–3 months, and then grown in a greenhouse at the Ehime University, Faculty of Agriculture.

2.3. Pollen stainability

Mature pollen grains were collected at anthesis and stained with 1% acetocarmine. Pollen stainability (%) was calculated as the proportion of stained pollen grains in a sample of at least 1000 pollen grains. Stained pollen grains were considered fertile.

2.4. Ploidy estimation

The ploidy level of the progeny plants was estimated using flow cytometry (FC). FC analysis was carried out according to Jadrná et al. (2009), with some modifications, using an Accuri C6 flow cytometer (BD Biosciences, Tokyo, Japan). The propidum iodide (PI) dye (50 μg mL^{-1}) was excited with a 488 nm (blue) laser and PI emission was detected with a 585 ± 20 nm bandpass filter (FL2). The results were acquired using FlowJo X 10.0.7r2 software (Tree Star Inc., San Carlos, CA, USA) for ploidy analysis. The ploidy level was determined by comparing the major flow cytometry peak positions in the progeny samples with those of standard diploid and tetraploid *Primula sieboldii*.

2.5. Characteristics of flowers

To assess flower color, we used the Royal Horticultural Society Color Chart. The colorimetric parameters L*, a*, and b* (CIE 1976) were measured using a colorimeter (CM-2600d, Konica Minolta Sensing Inc., Tokyo, Japan). Five fresh petals per individual were used. The absorption spectra of fresh petals were analyzed using an ultraviolet–visible spectrophotometer (V 570, JASCO, Tokyo, Japan) in the wavelength range of 200–700 nm. Flavonols and carotenoids in petals were determined by using a high-performance liquid chromatography system (LC–20AD, Shimadzu System, Kyoto, Japan) with an Inertsil OSD-3 (4.6 × 250 mm, GL–Sciences, Tokyo, Japan) column. For flavonol analysis, frozen petals (200 mg) were soaked in methanol and dried. After the addition of 0.5 mL of 2N hydrochloric acid, the petals were hydrolyzed for 120 min at 95 °C. The acid hydrolysate was passed through Waters Sep-pak C18 cartridges. Aglycones trapped on the cartridge were washed with distilled water, and then eluted with 1.5 mL of methanol. The methanol eluates were injected into the high-performance liquid chromatography apparatus. A flow rate of 1.0 mL min^{-1} was maintained and a mixture of acetonitrile–water–phosphoric acid (35:65:0.2 v/v) was employed as the eluent. The flavonol aglycones were quantified by their absorbance at 350 nm. For identification, kaempferol (Kanto Chemical, Tokyo, Japan), myricetin (Acros Organics, Geel, Belgium), and quercetin dihydrate (EMD Bioscience, San Diego, CA, USA) were used as authentic standards. Carotenoid and anthocyianidin analysis was carried out according to Sukhumpinij et al. (2012), and β-carotene (Sigma–Aldrich, St. Louis, MO, USA) and lutein (Extrasynthese, Lyon, France) were used as standards. The concentration of each pigment in the petals was calculated from a standard curve.

Petal pH was determined using a compact pH meter (B-712, Horiba Ltd., Kyoto, Japan). Fresh petals (1 g) were ground with a mortar and the pH of the homogenate was measured. The measurements were conducted three times for each plant and the means were calculated.

The aluminum concentration was measured using an inductively coupled plasma mass spectrometer (ICP–MS 7700X, Agilent Technology, Tokyo, Japan). Petals were dried at room temperature, and 50 mg of dried petal was used for the measurements. The assays were outsourced to A-KIT (Atmosphere Knowledge Information Technology Corporation, Gifu, Japan).

Petal size (length and width) was measured for the middle petal out of lower three ones by counting 10 flowers for an individual plant. The number of florets was measured in more than five peduncles of each plant.