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

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.

Table 1
Characteristics of *P. quinquelobatum*, geraniums, and their progeny.

Species or strains	Designation	Flower color					Absorption maxima of petals (nm)	Petal pH	Flower size[c]			No. of florets per peduncle	Flower types[d]	Pollen stainability (%)	Ploidy levels[e]
		RHSCC[a]		CIELAB coordinates[b]					PL	PW	PL/PW				
		Group name	No.	L*	a*	b*			(mm)	(mm)		(mean ± SE)			
P. quinquelobatum[f]	Q	Greyed-yellow	160C	73.02	−12.01	27.34	422, 578	4.8	18.2	7.9	2.3	4.8 ± 1.1f[g]	Single	99.5	2x
Geraniums															
Diploid															2x
H1		White	–	–	–	–	–	–	–	–	–	–	Single	MF[h]	
H2		White	–	–	–	–	–	–	–	–	–	–	Single	MF	
H3		White	–	83.13	−0.48	0.89	342,372	3.1	18.8	13.8	1.4	–	Double	MF	
'White King'	WK	White	–	–	–	–	–	–	–	–	–	–	Double	90.9	
Tetraploid															4x
'Glacis'	GL	White	–	86.19	−0.29	0.33	348,372	3.4	23.4	18.0	1.3	21.4 ± 10.4a	Double	MF	
'First Yellow'[i]	FY	Yellow	3C	81.58	−10.70	30.53	422	4.5	17.8	10.8	1.7	5.7 ± 0.9ef	Double	91.1	
F_1															2x
Q × WK -1		Green–yellow	1D	86.01	−5.54	17.70	–	–	16.3	9.9	1.6	–	Single	MS	
Q × WK -2		Green–yellow	1D	84.85	−4.88	21.93	–	–	–	–	–	–	Double	MS	
Q × WK -3		Green–yellow	1D	86.06	−5.34	22.91	–	–	–	–	–	–	Double	MS	
Q × WK -4		Green–yellow	1D	81.67	−5.58	16.39	–	–	–	–	–	–	Double	MS	
Q × WK -5		Green–yellow	1D	87.79	−6.81	21.71	–	–	–	–	–	–	Single	MS	
Q × H1-2		Green–yellow	1D	83.36	−6.44	17.40	–	–	–	–	–	–	Single	MS	
Q × H1-1		Yellow	2D	83.53	−6.23	17.33	–	3.8	16.8	9.8	1.7	10 ± 2.3cdef	Single	MS	
Q × H2 (1–8)[j]		White	155B	84.57	−2.95	5.44	–	–	–	–	–	–	Single	MS	
Q × H3		Yellow–green	150D	85.53	−9.02	21.68	–	–	14.0	9.3	1.5	–	Double	MS	
Colchicine-treated F_1															–
Q × H1Co-1	QH1Co-1	Green–yellow	1D	82.52	−4.45	14.74	–	–	19.4	11.8	1.7	–	Single	12.9	
Q × H1Co-2	QH1Co-2	Yellow	2D	83.61	−5.82	16.28	–	–	20.8	12.8	1.6	10.0 ± 2.3cdef	Single	93.8	
F_2															4x
QH1	F_2QH1	Green–yellow	1D	85.40	−7.82	21.18	–	–	20.5	12.5	1.6	8.5 ± 2.4cdef	Single	90.9	
QH3	F_2QH3	Green–yellow	1D	85.51	−8.31	21.38	–	–	20.4	13.0	1.6	11.1 ± 3.3cdef	Single	90.3	
QH4	F_2QH4	Green–yellow	1D	85.02	−7.46	19.60	–	–	20.6	13.9	1.5	9.0 ± 3.1cdef	Single	87.4	
QH5	F_2QH5	Green–yellow	1D	84.38	−7.87	20.32	–	–	21.0	13.1	1.6	9.8 ± 2.0cdef	Single	77.4	
QH2	F_2QH2	Yellow	2D	81.63	−3.82	18.95	–	–	20.9	12.6	1.7	10.4 ± 3.1cde	Single	83.5	
F_3															4x
QH1-3	F_3QH1-3	Green–yellow	1D	85.61	−9.05	22.19	–	–	19.3	13.3	1.5	–	Single	MF	
QH1-5	F_3QH1-5	Green–yellow	1D	85.58	−7.65	19.85	–	–	–	–	–	–	Single	MF	
QH1-6	F_3QH1-6	Green–yellow	1D	84.85	−8.19	21.73	–	–	20.5	13.5	1.5	6.9 ± 1.3def	Single	MF	
QH2-2	F_3QH2-2	Green–yellow	1D	89.99	−6.60	17.00	–	–	19.8	12.0	1.7	8.3 ± 3.4cdef	Single	MF	
QH2-4	F_3QH2-4	Green–yellow	1D	83.89	−6.28	21.38	–	–	20.4	12.6	1.6	7.0 ± 1.3def	Single	MF	
QH2-6	F_3QH2-6	Green–yellow	1D	86.65	−8.42	21.59	–	–	20.0	14.0	1.4	12.6 ± 2.7bcde	Single	MF	
QH3-5	F_3QH3-5	Green–yellow	1D	85.13	−7.62	18.20	–	–	–	–	–	–	Single	MF	
QH3-6	F_3QH3-6	Green–yellow	1D	84.51	−8.22	20.44	–	–	20.2	12.6	1.6	–	Single	MF	
QH4-4	F_3QH4-4	Green–yellow	1D	85.52	−8.54	21.15	–	–	20.3	14.0	1.4	–	Single	MF	
QH4-6	F_3QH4-6	Green–yellow	1D	84.63	−8.93	22.01	–	–	23.1	15.5	1.5	9.8 ± 2.6cdef	Single	MF	
QH1-2	F_3QH1-2	Yellow	2D	85.56	−7.29	18.10	–	–	21.8	13.2	1.7	–	Single	MF	
QH1-4	F_3QH1-4	Yellow	2D	–	–	–	–	–	–	–	–	–	Single	MF	
QH1-7	F_3QH1-7	Yellow	2D	85.65	−7.45	20.00	–	–	22.3	14.0	1.6	–	Single	MF	
QH2-8	F_3QH2-8	Yellow	2D	83.61	−6.64	21.06	–	–	–	–	–	–	Single	MF	
QH3-4	F_3QH3-4	Yellow	2D	86.02	−8.06	20.33	–	–	20.0	12.1	1.7	–	Single	MF	
QH4-1	F_3QH4-1	Yellow	2D	85.71	−7.30	18.24	–	–	20.3	13.7	1.5	13.2 ± 3.8bcd	Single	MF	
QH5-3	F_3QH5-3	Yellow	2D	85.10	−8.11	20.09	–	–	20.3	13.8	1.5	–	Single	MF	
QH2-1	F_3QH2-1	Yellow	4D	84.44	−5.18	16.08	–	–	20.3	14.0	1.5	11.4 ± 4.4cde	Single	MF	

Table 1 (*Continued*)

Species or strains	Designation	Flower color					Absorption maxima of petals (nm)	Petal pH	Flower size[c]			No. of florets per peduncle	Flower types[d]	Pollen stainability (%)	Ploidy levels[e]
		RHSCC[a]		CIELAB coordinates[b]					PL	PW	PL/PW				
		Group name	No.	L*	a*	b*			(mm)	(mm)		(mean ± SE)			
QH2-9	F_3QH2-9	Yellow	9D	82.96	−5.21	22.68	–	–	21.4	14.0	1.5	10.2 ± 1.3cdef	Single	MF	
QH2-3	F_3QH2-3	Orange	29D	79.43	−0.97	19.09	–	–	19.5	12.3	1.6	–	Single	MF	
QH2-7	F_3QH2-7	Red	54D	77.47	−8.08	8.60	–	–	18.4	11.8	1.6	–	Single	MF	
QH2-5	F_3QH2-5	–	–	–	–	–	–	–	–	–	–	–	Single	MF	
QH4-5	F_3QH4-5	–	–	85.69	−7.49	18.82	–	–	–	–	–	–	Single	MF	
BC_1															4x
QH1-6 × FY-2	BC_1FY-2	Green–yellow	1D	84.00	−9.64	23.73	422	4.2	19.5	12.7	1.5	7.3 ± 0.8def	Semi-double	77.4	
QH1-6 × FY-1	BC_1FY-1	Yellow	2C	84.53	−10.18	26.73	422	–	19.8	13.6	1.5	7.2 ± 0.9def	Semi-double	89.5	
QH1-6 × FY-4	BC_1FY-4	Yellow	2C	84.83	−10.14	26.81	–	4.2	19.9	12.8	1.6	7.5 ± 1.2def	Semi-double	MF	
QH1-6 × FY-5	BC_1FY-5	Yellow	2C	84.46	−9.79	25.36	422	4.2	20.2	12.7	1.6	10.5 ± 1.6cde	Semi-double	MF	
QH1-6 × FY-6	BC_1FY-6	Yellow	2C	83.24	−10.16	25.89	422	4.2	–	–	–	–	Semi-double	MF	
QH1-6 × FY-9	BC_1FY-9	Yellow	2C	84.56	−9.96	25.76	422	4.2	19.4	12.9	1.5	6.3 ± 0.9def	Semi-double	MF	
GL × QH5-3-1	$GLBC_1$-1	Yellow	<4D[j]	85.01	−4.57	10.90	422	3.9	23.2	17.9	1.3	14.8 ± 5.0bc	Semi-double	MS	
GL × QH5-3-2	$GLBC_1$-2	Yellow	<4D[j]	87.39	−3.64	7.94	–	–	22.4	17.9	1.3	19.0 ± 4.5ab	Single	MS	
GL × QH2-5-1	$GLBC_1$-3	–	–	–	–	–	–	–	19.0	15.2	1.3	–	Single	MS	
GL × QH2-5-2	$GLBC_1$-4	–	–	–	–	–	–	–	–	–	–	–	Semi-double	MS	
BC_1F_2															4x
BC_1FY-5-2	BC_1F_2-2	Green–yellow	1D	84.67	−9.43	24.33	–	3.7	21.3	13.7	1.6	11.3 ± 1.3cde	Single	MF	
BC_1FY-5-3	BC_1F_2-3	Green–yellow	1D	85.89	−10.12	25.64	–	3.9	20.8	13.8	1.5	11.6 ± 2.5cde	Single	MF	
BC_1FY-5-7	BC_1F_2-7	Green–yellow	1D	–	–	–	–	3.9	–	–	–	10.4 ± 2.1cde	Single	MF	
BC_1FY-5-9	BC_1F_2-9	Green–yellow	1D	–	–	–	–	–	21.4	14.0	1.5	–	Double	MF	
BC_1FY-5-10	BC_1F_2-10	Green–yellow	1D	–	–	–	–	–	–	–	–	7.6 ± 2.0def	Semi-double	MF	
BC_1FY-5-11	BC_1F_2-11	Green–yellow	1D	–	–	–	–	–	–	–	–	–	Semi-double	MF	
BC_1FY-5-12	BC_1F_2-12	Green–yellow	1D	–	–	–	–	–	–	–	–	–	Double	MF	
BC_1FY-5-4	BC_1F_2-4	Yellow	2C	83.25	−10.75	29.9	–	3.7	24.0	16.6	1.4	8.4 ± 2.1cdef	Single	MF	
BC_1FY-5-5	BC_1F_2-5	Yellow	2C	84.64	−10.70	28.4	–	–	22.8	14.6	1.6	10.5 ± 1.4cdef	Semi-double	MF	
BC_1FY-5-6	BC_1F_2-6	Yellow	2C	82.93	−8.92	26.1	–	–	–	–	–	–	Double	MF	
BC_1FY-5-8	BC_1F_2-8	Yellow	2C	–	–	–	–	4.0	21.9	13.0	1.7	–	Double	MF	
BC_1FY-5-1	BC_1F_2-1	Yellow	3C	84.36	−11.40	32.5	422	3.8	23.1	14.3	1.6	8.5 ± 1.6cde	Single	MF	

[a] Royal Horticultural Society Color Chart.

[b] L*: lightness, a*: redness/greenness, b*: yellowness/blueness.

[c] PL: petal length, PW: petal width.

[d] Single: five petals, Double: above eight petals, Semi-double: six to seven petals per flower.

[e] Ploidy levels were estimated by flow cytometry.

[f] The average data of some individuals.

[g] Different letters indicate a significant difference ($P < 0.05$, Tukey–Kramer's multiple range test).

[h] MF: male fertility, MS: male sterility.

[i] The characteristics were nearly same in eight flowering plants.

[j] ' <4D ' means lighter color than ' 4D ', inclining to white. ' – ' means no data.

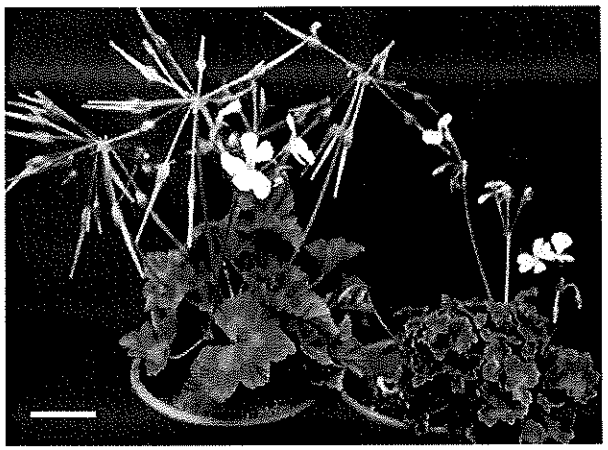


Fig. 1. Phenotypes of F_1 and F_2 plants derived from the cross *P. quinquelobatum* × geranium (H1). Left; F_2 progeny (4x, F_2QH2), right; F_1 progeny (2x, Q × H1–2). Scale bar: 5 cm.

3. Results

3.1. Production of hybrid progeny

For the cross between *P. quinquelobatum* and diploid geraniums, no seeds were obtained when *P. quinquelobatum* was used as the pollen parent, whereas seeds were obtained when *P. quinquelobatum* was used as the seed parent (2.7 seeds per fruit, Table 2). In these F_1 plants, many individuals grew slowly and died before flowering. As a result, only 16 surviving F_1 individual plants flowered, but they were completely pollen sterile, despite their parents being pollen fertile (Table 1). Therefore, for fertility restoration, a part of F_1 seeds obtained from the cross using 'White King' (WK) and H1 (42 and 13 seeds, respectively) were treated with colchicine. As a result, only two of the fertile F_1 plants derived from the cross with H1 were obtained (Table 1). Although one of them died and the other showed low pollen stainability (12.9%, Table 1), selfing of QH1Co-1 produced many F_2 seeds. Compared with the F_1 progeny plants, the F_2 progeny showed desirable characteristics such as high pollen stainability (over 77.4%), high seed production by open pollination, as well as great vigor (Table 1, Fig. 1). According to FC analysis, the histogram peaks of the F_1 or F_2 progeny were located in the same relative position as those of diploid or tetraploid in *Primula sieboldii*, respectively. Therefore, these F_1 or F_2 progeny plants were estimated to be diploid and tetraploids, respectively. F_3 plants produced by selfing of F_2 plants, and two tetraploid cultivars ('First Yellow'; FY and 'Glacis'; GL) were crossed reciprocally. Seeds could be obtained regardless of which seed parent was used (Table 2). BC_1 plants (BC_1FY), obtained by backcrossing using FY as the pollen parent, produced fertile pollen, and many progeny plants (BC_1F_2) were obtained (Table 1). Meanwhile, BC_1 plants ($GLBC_1$) obtained by backcrossing using GL as the seed parent, were pollen sterile.

3.2. Flower color and pigments

All F_1 plants produced pale yellow flowers (green–yellow, yellow and yellow–green D, according to RHSCC), except for progeny derived from the cross with H2 whose flowers were off-white (white 155B) in eight flowering plants (Table 1, Fig. 2a). The b* values of F_1 flowers ranged between those of the *P. quinquelobatum* and white-flowered geranium parents and the b* values were nearly the same in the F_2 and F_3 flowers (Table 1, Fig. 2b). As the generations advanced, the b* values of the progenies were increased gradually and the yellowish tone of the flowers deepened. In some BC_1F_2 plants, the b* values were the highest among all generations, such that the yellowish tone was the same as FY. By contrast, $GLBC_1$ flowers were very pale yellow inclining to white (yellow, < 4D), with b* values approximately half those of F_3 flowers (Table 1, Fig. 2c).

Petals of *P. quinquelobatum* contained a large amount of flavonols, and a small amount of carotenoids (β-carotene and lutein), chlorophyll and anthocyanidins (delphinidin, petunidin, and peonidin). As shown in Fig. 3a, the main flavonol pigments in the petals of *P. quinquelobatum* were quercetin (4.9 mg FW^{-1}) and myricetin (3.6 mg FW^{-1}); kaempferol (0.2 mg FW^{-1}) and unidentified No. 5 were isolated as minor peaks. High levels of quercetin were contained in the petals of FY and two plants of BC_1F_2 (Fig. 3 b). The b* value increased as the quercetin content increased, such that they were significantly correlated ($r = 0.82$, $P < 0.001$) (Fig. 4). Unidentified No. 5 showed the same tendency ($r = 0.84$, $P < 0.001$). Although kaempferol was detected as the main pigment in all progeny (Fig. 3b), no correlation was found with the b* value (Fig. 4). Furthermore, a small amount of β-carotene and lutein were detected in the pale yellow petals of hybrid progeny plants and FY; however, there was no correlation between the b* value and total carotenoid content.

The absorption maximum was detected around 422 nm in the fresh petals of *P. quinquelobatum*, FY, and yellow-flowered progeny plants (Table 1). The aluminum content of the flowers was highest in the white geranium (H3) (60.0 ppm), and became low in the order FY (21.9 ppm), BC_1FY-5 (18.3 ppm), and *P. quinquelobatum* (16.9 ppm), suggesting no clear relationship between aluminum content and the b* value of the petals. The petal pH of yellow-flowered plants, which ranged from 3.7 to 4.8 (Table 1), was not correlated with the b* value ($r = 0.23$).

3.3. Flower morphology

The petals of *P. quinquelobatum* are teardrop shaped; in contrast, geranium petals are round to oval. Flowers of the F_1 progeny were smaller than those of both parents (Table 1, Figs. 5 a and 6). However, the petals became larger in subsequent generations such as in the BC_1 and BC_1F_2 progeny (Table 1, Figs. 5 b and 6). The petal width of two $GLBC_1$ progenies was almost the same as that of GL. The number of florets per peduncle was not significantly different through all generations (Table 1). The flower type of FY was a double, where the flower was formed by more than eight petals, and sometimes included several small and slender petals (Table 1, Fig. 6). F_3 plants had a single flower, and BC_1FY plants showed a semi-double flower type. Furthermore, BC_1F_2 plants were segregated into single, semi-double, and double flower types.

4. Discussion

We attempted to produce yellow-flowered geraniums by interspecific crossing between *P. quinquelobatum* and white-flowered geraniums. Many normal seeds were obtained when *P. quinquelobatum* was used as the seed parent (Table 2), but an even fruit set was not observed when *P. quinquelobatum* was used as the pollen parent. Denis-Peixoto et al. (1997) also attempted an interspecific cross between *P. quinquelobatum* and geraniums, and hybrid seeds were also produced only when *P. quinquelobatum* was used as the seed parent. Although Denis-Peixoto et al. (1997) used the embryo rescue method, we obtained many seeds without using that method, which might reflect differences among the materials used. Such unilateral incompatibility in interspecific crossing using *P. quinquelobatum*, also occurred in the cross between *P. quinquelobatum* and ivy geraniums (Denis-Peixoto et al., 1997; Hondo et al., 2015). It was thought to reflect the genetic distance among the parents: *P. quinquelobatum* is classified before in the section *Eumorpha* (Gibby

Table 2
Crosses between geraniums and *P. quinquelobatum*, and backcrosses between their F_3 progeny and geraniums.

Cross combination[a]		No. of crosses	No. of fruit set	No. of seed set	Seed fertility	
Seed parents	Pollen parents	a	b	c	c/a	c/b
Geraniums[b] (2x)	*P. quinquelobatum*	62	0	0	0.0	
P. quinquelobatum	Geraniums (2x)					
	WK	51	41	114	2.2	2.8
	H1	39	19	39	1.0	2.1
	H2	4	4	11	2.8	2.8
	H3	20	12	43	2.2	3.6
	Total/Mean	114	76	207	1.8	2.7
F_3QH	Geraniums (4x)					
	FY	13	7	16	1.2	2.3
	GL	13	8	16	1.2	2.0
	Total/Mean	26	15	32	1.2	2.1
Geraniums (4x)	F_3QH					
FY		7	0	0	0.0	
GL		47	11	16	0.3	1.5
	Total/Mean	54	11	16	0.3	1.5

[a] Designations are the same as Table 1.
[b] Several strains and cultivars of geraniums were used for the crossing. The number indicates the total.

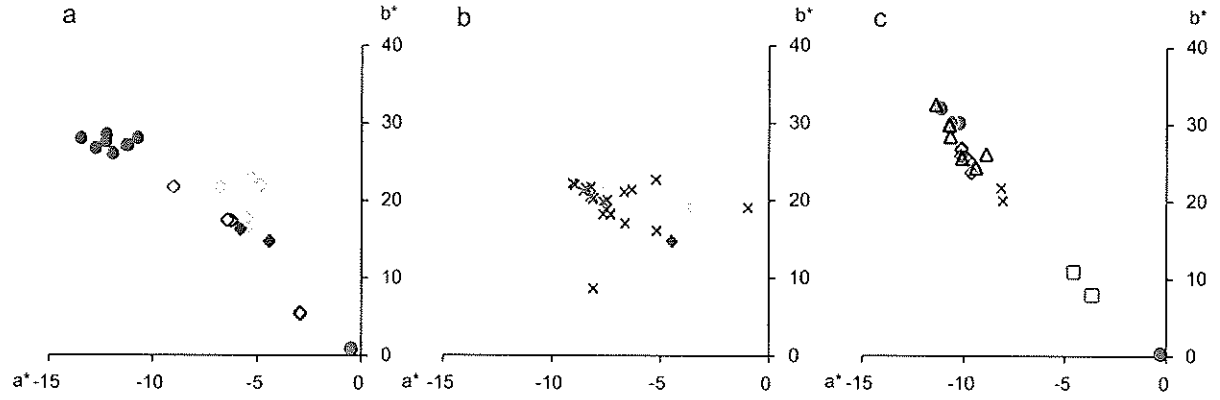


Fig. 2. Distribution of a* and b* based on CIELAB coordinates for *P. quinquelobatum*, geraniums, and their hybrid progeny. (a) ○ Q, ○ H3, △ F_1: Q × WK, ▽ F_1: Q × H1, ◇ F_1: Q × H2, □ F_1: Q × H3, ◆ Colchicine-treated F_1: QH1Co. (b) ◆ QH1Co-1, × F_2, × F_3. (c) ○ GL, ○ FY, × F_3QH1–6, F_3QH5-3, △ BC_1FY, △ GLBC$_1$, △ BC_1F_2. Designations are the same as Table 1. a* and b* values indicate redness–greenness and yellowness–blueness, respectively.

and Westfold, 1986; James et al., 2004), and molecular investigations (Bakker et al., 2004; James et al., 2004) showed a small genetic distance between *P. quinquelobatum* and the ancestors of geraniums.

Both flavonol and anthocyianidin are synthesized in the flavonoid biosynthesis pathway, and the white color of petals is caused by a blockage in the pathway. In *Dianthus caryophyllus*, *Petunia hybrida*, and *Matthiola incana*, white flowers are caused by a deficiency of flavanone 3-hydroxylase, dihydroflavonol-reductase, or anthocyanidin synthase, respectively (Heller et al., 1985; Kho et al., 1977; Mato et al., 2000; Mol et al., 1983). As for the development of yellow-flowered geraniums, Denis-Peixoto et al. (1997) suggested the use of white-flowered geraniums lacking dihydroflavonol-reductase activity as the crossing parent. Furthermore, the yellowness of petals might be promoted in geraniums when white-flowered strains with high activity of flavonoid 3'-hydroxylase, which hydroxylates dihydrokaempferol to dihydroquercetin, are used for crossing. Although in this study, the enzyme participating in white coloration for petals was not examined, we used five strains of white-flowered geraniums for crossing with *P. quinquelobatum*, and found that F_1 progeny plants segregated into two groups of flower color; one was the pale yellow group, which derived from the cross using WK, H1, and H3; and the other was the off-white group, which derived from the cross using H2, depending on their parent strains. From this result, we concluded that at least two genotypes exist in white-flowered geraniums.

The yellow pigmentation of flowers is derived from carotenoids or flavonoids. In geraniums and *P. quinquelobatum*, pale yellow petals contain a large amount of flavonol glycosides (Denis-Peixoto et al., 1997; Mitchell et al., 1998). In agreement with a previous report (Denis-Peixoto et al., 1997), we detected myricetin, quercetin, and kaempferol in pale yellow petals of geraniums and *P. quinquelobatum*. In addition, unidentified flavonol glycoside (No. 5) and a small amount of carotenoids were also detected. Consequently, only quercetin and unidentified No. 5 were correlated with the b* value (yellowness). This result agreed with previous reports (Miyajima et al., 1985; Tanikawa et al., 2008, 2010) in which quercetin derivatives were the main contributors to the pale yellow color of *Camellia*. However, quercetin is a "colorless" flavonoid. In contrast, "yellow" flavonoids, such as aurone and chalcone, produce brighter yellow-colored flowers, as shown in dahlia, snapdragon, and yellow cosmos (Harborne, 1963; Shimokoriyama and Hattori, 1953; Nordström and Swain, 1956). Yellow-flowered

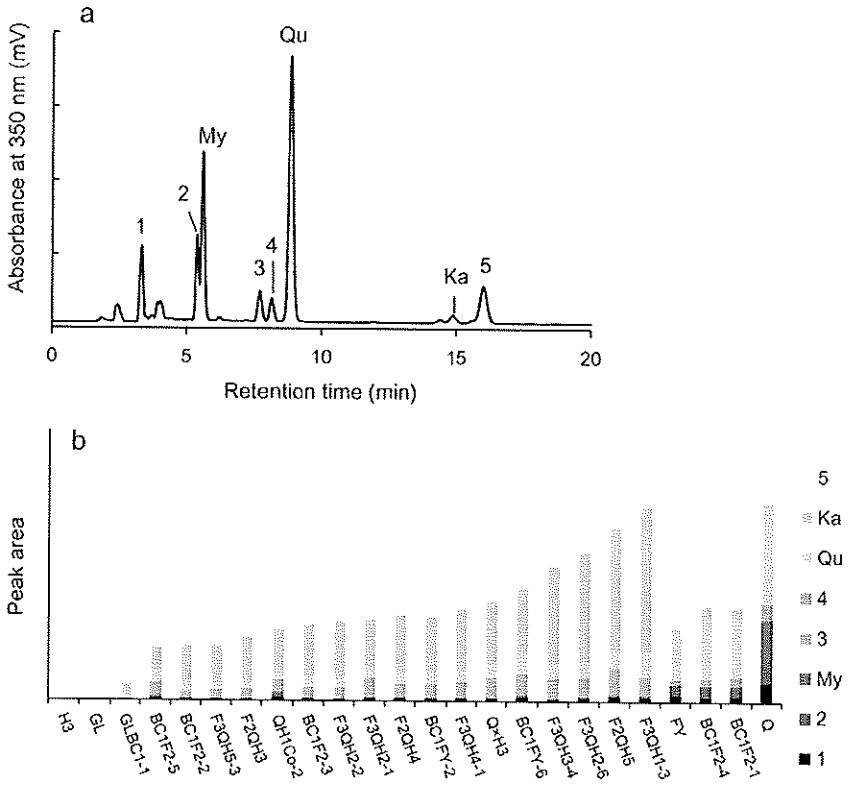


Fig. 3. High-performance liquid chromatography profiles of flavonols in the petals of *P. quinquelobatum*, geraniums, and their hybrid progeny. My: myricetin, Qu: quercetin, Ka: kaempferol. (a) Chromatogram of *P. quinquelobatum*. (b) Distribution of each peak to the total peak areas. Designations are the same as Table 1.

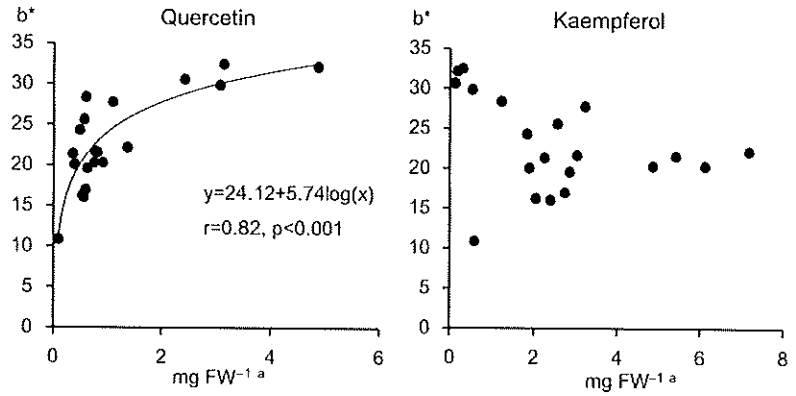


Fig. 4. Correlation between b* and quercetin or kaempferol from the petals of *P. quinquelobatum*, geraniums, and their hybrid progeny.
[a] Each flavonoid content (mg g^{-1} freezed petal weight) was calculated from the standard curve of standard pigments. b* value indicates yellowness.

Torenia hybrida, whose yellow flowers accumulate aurone, were obtained from blue-flowered plants into which *Antirrhinum majus* genes were introduced using a transgene approach (Ono et al., 2006). A transgenic procedure has been established in geraniums (Hassanein et al., 2005; Winkelmann et al., 2005). Therefore, brighter yellow flowers might be obtained if the aurone or chalcone synthetic genes were introduced into pale yellow geraniums.

Besides pigments, metal ion content and vacuolar pH cause alterations in flower color. Tanikawa et al. (2008) found that the deep yellow color of the *C. chrysantha* petal was caused by the interaction between quercetin derivatives and aluminum ions, and the absorption maximum of fresh petal was detected at 420 nm. Because in the present study, the absorption maximum of fresh petals was detected at 422 nm in *P. quinquelobatum*, FY, and the hybrid progeny with pale yellow flowers, their aluminum contents were examined. The aluminum contents, however, were lower in the yellow petals than in the white petals. In this study, the petal pH ranged from 3.1 to 4.8, regardless of the degree of yellowness of the petals. Mitchel et al. (1998) reported that in the red petals of geraniums, the pH ranged from 3.0 to 3.9 and had little effect on their color. Furthermore, Markham et al. (2001) investigated the yellow coloration of *Lathyrus chrysanthus* was not affected by their vacuolar pH, but by flavones aggregation. Such mechanisms might control the yellow coloration of geranium petals.

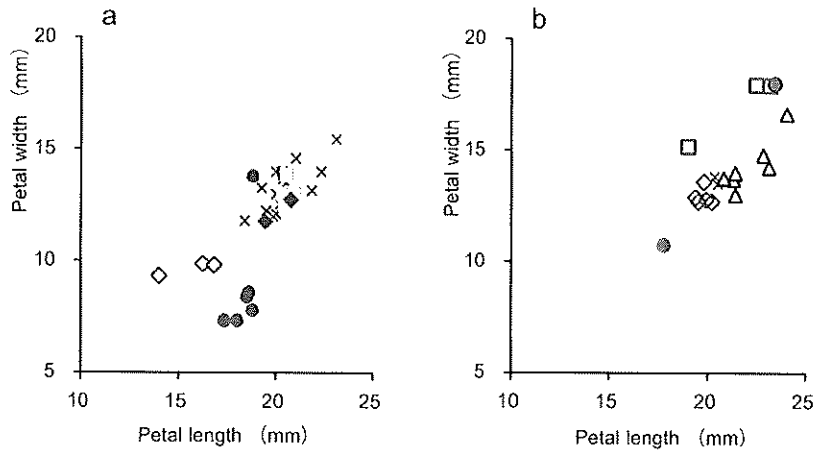


Fig. 5. Variations of petal size in *P. quinquelobatum*, geraniums, and their hybrid progeny. (a) ○ Q, ● H3, ▽ F_1: Q × WK, ▽ F_1: Q × H1, ▽ F_1: Q×H3, ◆ Colchicine-treated F_1: QH1Co, F_2, × F_3. (b) ● GL, ● FY, × F_3QH1–6, F_3QH5-3, ◇ BC_1FY, ▽ $GLBC_1$, △ BC_1F_2. Designations are the same as Table 1.

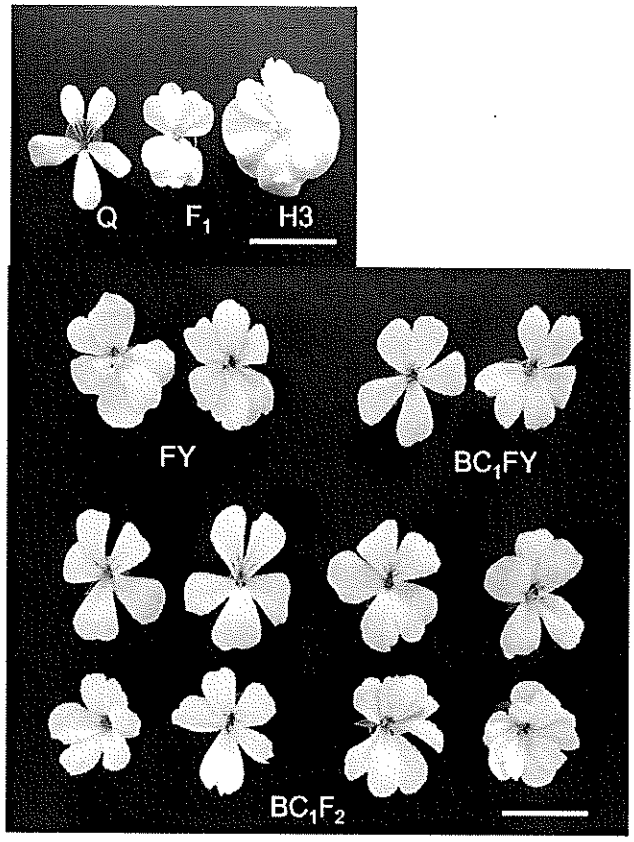


Fig. 6. Flowers of *P. quinquelobatum*, geraniums, and their hybrid progeny. Designations are the same as Table 1. Scale bars: 3 cm.

References

Bakker, F.T., Culham, A., Hettiarachi, P., Touloumenidou, T., Gibby, M., 2004. Phylogeny of *Pelargonium* (Geraniaceae) based on DNA sequences from three genomes. Taxon 53, 17–31.

Brawner, F., 2003. Geraniums. In: The Complete Encyclopedia. Schiffer Publishing Ltd., Atglen, pp. 138.

Clifford, D., 1958. Pelargoniums, Including The Popular 'Geranium'. Blandford Press, London.

Denis-Peixoto, L., Cadic, A., Renou, J.P., 1997. Interspecific crosses between *Pelargonium* × *hortorum* and *P. quinquelobatum* using embryo rescue and molecular characterization of hybrids by an endogenous *chs* probe. Plant Breed. 116, 177–180.

Gibby, M., Westfold, J., 1986. A cytological study of *Pelargonium* sect *Eumorpha* (*Geraniaceae*). Pl. Syst. Evol. 153, 205–222.

Harborne, J.B., 1963. Plant polyphenols X. Flavone and aurone glycosides of *Antirrhinum*. Phytochemistry 2, 327–334.

Harney, P.M., 1966. A chromatographic study of species presumed ancestral to *Pelargonium* × *hortorum* Bailey. Can. J. Genet. Cytol. 8, 780–787.

Hassanein, A., Chevreau, E., Dorion, N., 2005. Highly efficient transformation of zonal (*Pelargonium* × *hortorum*) and scented (*P. capitatum*) geraniums via *Agrobacterium tumefaciens* using leaf discs. Plant Sci. 169, 532–541.

Heller, W., Britsch, L., Forkmann, G., Grisebach, H., 1985. Leucoanthocyanidins as intermediates in *anthocyanidin biosynthesis* in flowers of *Matthiola incana* R. Br. Planta 163, 191–196.

Hondo, K., Yoshikawa, M., Kakihara, F., 2015. Flower color of hybrids raised from interspecific crosses between ivy-leaved geranium (*Pelargonium peltatum* (L.) L'Hér. ex Ait.) and related species. Hort. Res. 14, 17–24 (Japanese with English abstract).

Jadrná, P., Kobza, F., Plavcová, O., 2009. Polyploidization of *Pelargonium* × *hortorum* L. H. Bailey in greenhouse conditions. Hort. Sci. 36, 31–37.

James, C.M., Gibby, M., Barrett, J.A., 2004. Molecular studies in *Pelargonium* (Geraniaceae): A taxonomic appraisal of section *Ciconium* and the origin of the 'Zonal' and 'Ivy-leaved' cultivars. Plant Syst. Evol. 243, 131–146.

Kho, K.F., Bolsman-Louwen, A.C., Vuik, J.C., Bennink, G.J., 1977. Anthocyanin synthesis in a white flowering mutant of Petunia hybrida: II. Accumulation of dihydroflavonol intermediates in white flowering mutants; uptake of intermediates in isolated corollas and conversion into anthocyanins. Planta 135, 109–118.

Linsmaier, E.M., Skoog, F., 1965. Organic growth factor requirements of tobacco tissue cultures. Physiol. Plant. 18, 100–127.

Markham, K.R., Gould, K.S., Ryan, K.G., 2001. Cytoplasmic accumulation of flavonoids in flower petals and its relevance to yellow flower colouration. Phytochemistry 58, 403–413.

Mato, M., Onozaki, T., Ozeki, Y., Higeta, D., Itoh, Y., Yoshimoto, Y., Ikeda, H., Yoshida, H., Shibata, M., 2000. Flavonoid biosynthesis in white-flowered Sim carnations (*Dianthus caryophyllus*). Sci. Hortic. 84, 333–347.

Miller, D., 1996. Pelargoniums. Timber Press, Inc., Portland, pp. 50–51.

Mitchell, K.A., Markham, K.R., Boase, M.R., 1998. Pigment chemistry and colour of Pelargonium flowers. Phytochemistry 47, 355–361.

Miyajima, I., Uemoto, S., Sakata, Y., Arisumi, K., Toki, K., 1985. Yellow Pigment of *Camellia chrysantha* Flowers. J. Fac. Agr. Kyushu Univ. 29, 257–266.

Mol, J.N.M., Schram, A.W., de Vlaming, P., Gerats, A.G.M., Kreuzaler, F., Hahlbrock, K., Reif, H.J., Veltkamp, E., 1983. Regulation of flavonoid gene expression in *Petunia hybrida*: description and partial characterization of a conditional mutant in chalcone synthase gene expression. Mol. Gen. Genet. 192, 424–429.

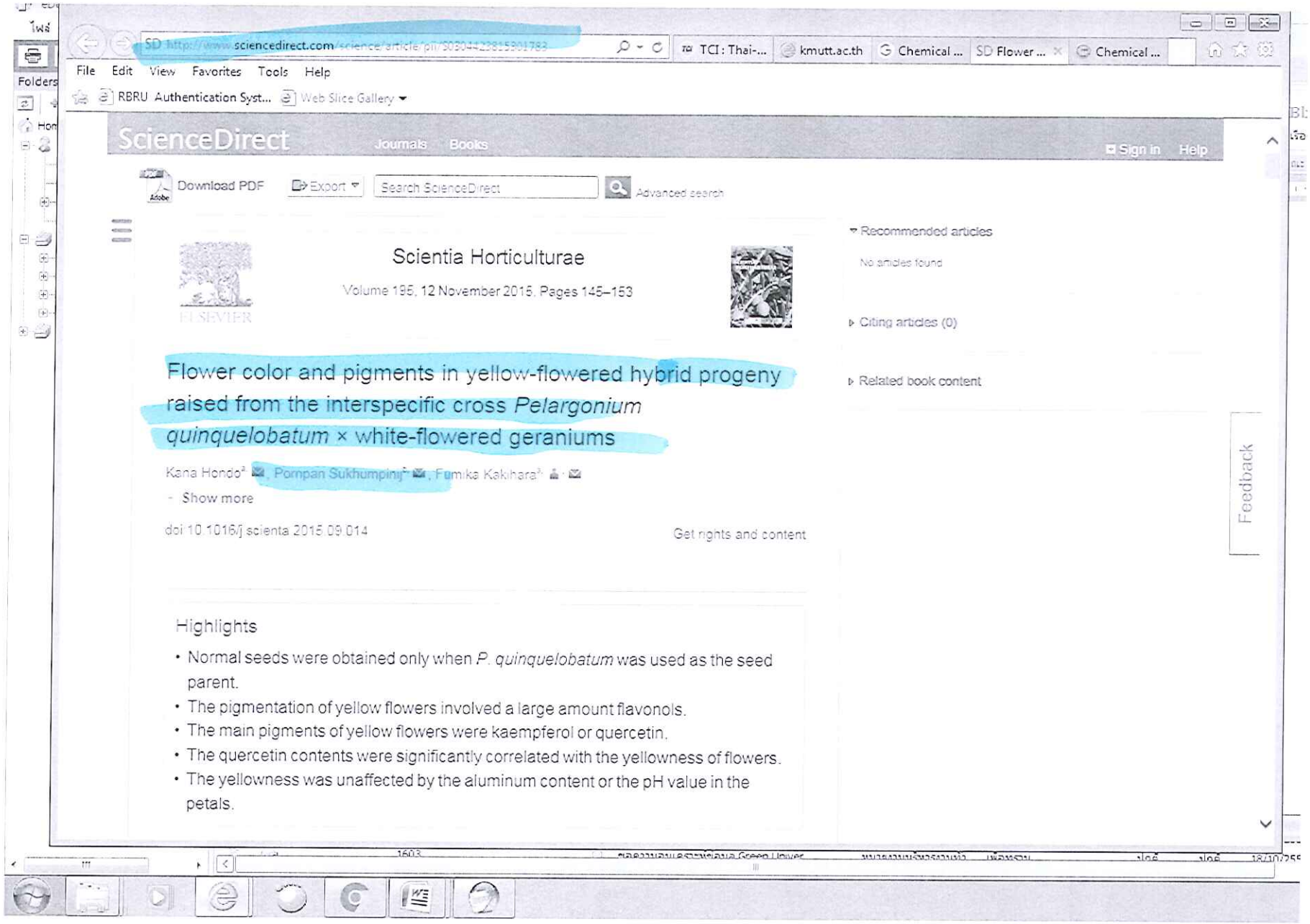
Nordström, C.G., Swain, T., 1956. The flavonoid glycosides of *Dahlia variabilis*: II. Glycosides of yellow varieties Pius IX and Coton. Arch. Biochem. Biophys. 60, 329–344.

Ono, E., Fukuchi-Mizutani, M., Nakamura, N., Fukui, Y., Yonekura-Sakakibara, K., Yamaguchi, M., Nakayama, T., Tanaka, T., Kusumi, T., Tanaka, Y., 2006. Yellow flowers generated by expression of the aurone biosynthetic pathway. PNAS 103, 11075–11080.

Shimokoriyama, M., Hattori, S., 1953. Anthochlor Pigments of *Cosmos sulphureus*, *Coreopsis lanceolata* and *C. saxicola*. J. Am. Chem. Soc. 75, 1900–1904.

Sukhumpinij, P., Kakihara, F., Hondo, K., Kato, M., 2012. Analysis of pigments in yellow-flowered *Pelargonium* section *Hoare*. Acta Hortic. 937, 711–716.

Tanikawa, N., Yamamizo, C., Ohmiya, A., 2010. Carotenoid components in petals of yellow *Camellia* species. Bull. Natl. Inst. Flor. Sci. 10, 75–79.
Tanikawa, N., Kashiwabara, T., Hokura, A., Abe, T., Shibata, M., Nakayama, M., 2008. A peculiar yellow flower coloration of camellia using aluminum-flavonoid interaction. J. Japan. Soc. Hortic. Sci. 77, 402–407.
Taylor, J., 1990. Pelargoniums. For Colour And variety, 117. The Crowood Press Ltd., Ramsvury, pp. 117.
USDA, 2014. United States Department of Agriculture, Floriculture crops 2013 summary 2014. National Agricultural Statistics Service. pp. 25–26.
Winkelmann, T., Kaviani, K., Serek, M., 2005. Development of a shoot regeneration protocol for genetic transformation in *Pelargonium zonale* and *Pelargonium peltatum* hybrids. Plant Cell Tiss. Org. Cult. 80, 33–42.


sciencedirect.com
File Edit View Favorites Tools Help
ScienceDirect
Journals Books
Sign in Help
Download PDF
Export
Search ScienceDirect
Advanced search
Scientia Horticulturae
Volume 195, 12 November 2015, Pages 145–153
Recommended articles
No articles found
Citing articles (0)
Related book content
Flower color and pigments in yellow-flowered hybrid progeny raised from the interspecific cross Pelargonium quinquelobatum × white-flowered geraniums
Kana Hondo, Pornpan Sukhumpiny, Fumika Kakihara
Show more
doi 10.1016/j.scienta.2015.09.014
Get rights and content
Highlights
• Normal seeds were obtained only when P. quinquelobatum was used as the seed parent.
• The pigmentation of yellow flowers involved a large amount flavonols.
• The main pigments of yellow flowers were kaempferol or quercetin.
• The quercetin contents were significantly correlated with the yellowness of flowers.
• The yellowness was unaffected by the aluminum content or the pH value in the petals.
Feedback

เอกสารแนบท้ายประกาศ

วารสารทางวิชาการที่เป็นไปตามหลักเกณฑ์ที่ ก.พ.อ. กำหนด ได้แก่ วารสารที่มีชื่ออยู่ในฐานข้อมูลที่เป็นที่ยอมรับในระดับชาติและระดับนานาชาติ ดังต่อไปนี้

๑. ฐานข้อมูลระดับนานาชาติ

- Academic Search Premier (http://www.ebsco.com/home)
  (select ebscohost and then academic search premier)
- Agricola (http://agricola.nal.usda.gov)
- BIOSIS (http://www.biosis.org)
- CINAHL (http://www.ebscohost.com/academic/cinahl-plus-with-full-text)
- EiCOMPENDEX (http://www.ei.org)
- ERIC (http://www.eric.ed.gov/)
- H.W.Wilson (http://www.ebscohost.com)
  (select ebscohost and then H.W.Wilson)
- Infotrieve (http://www.infotrieve.com)
- Ingenta Connect (http://www.ingentaconnect.com)
- INSPEC (http://www.theiet.org/publishing/inspec)
- MathSciNet (http://www.ams.org/mathscinet)
- MEDLINE/Pubmed (http://www.ncbi.nlm.nih.gov/pubmed/)
- PsycINFO (http://www.apa.org/pubs/databases/psycinfo/index.aspx)
- Pubmed (http://www.ncbi.nlm.nih.gov/pubmed/)
- ScienceDirect (http://www.sciencedirect.com)
- SciFinder (https://scifinder.cas.org/)
- Scopus (http://www.info.scopus.com)
- Social Science Research Network
  (http://papers.ssrn.com/sol3/DisplayAbstractSearch.cfm)
- Web of Knowledge (http://wokinfo.com)

๒. ฐานข้อมูลระดับชาติ ได้แก่ ศูนย์ดัชนีการอ้างอิงวารสารไทย (Thai Journal Citation Index -- TCI) เฉพาะวารสารที่มีชื่ออยู่ในกลุ่มที่ ๑ และกลุ่มที่ ๒ (http://www.kmutt.ac.th/jif/public_html/list%20journal.php)

หมายเหตุ กรณีที่มีการเปลี่ยนแปลงชื่อฐานข้อมูล ก.พ.อ. จะได้มีการประกาศเพิ่มเติมต่อไป