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Country: Belgium

Subject Area: Agricultural and Biological Sciences | Medicine

Subject Category: Horticulture Q4, Medicine (miscellaneous)

Publisher: International Society for Horticultural Science. **Publication type:** Book Series. **ISSN:** 05677572

Coverage: 1976, 1988, 1996, 1998, 2002, 2006-2011

H Index: 13

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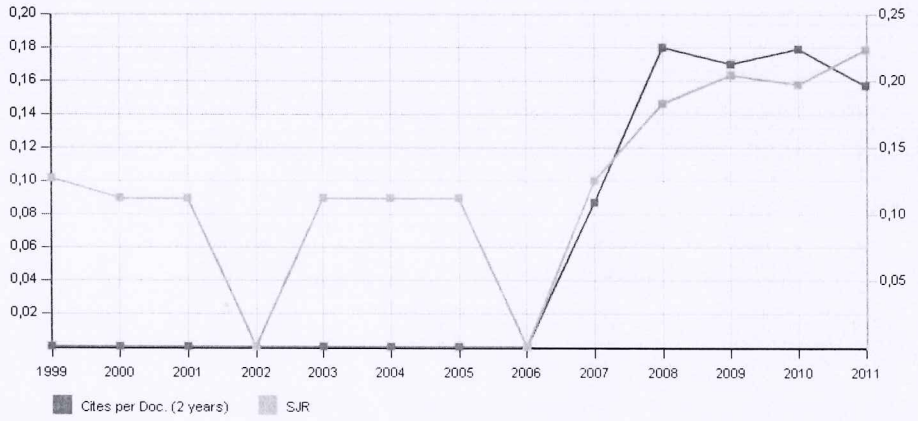

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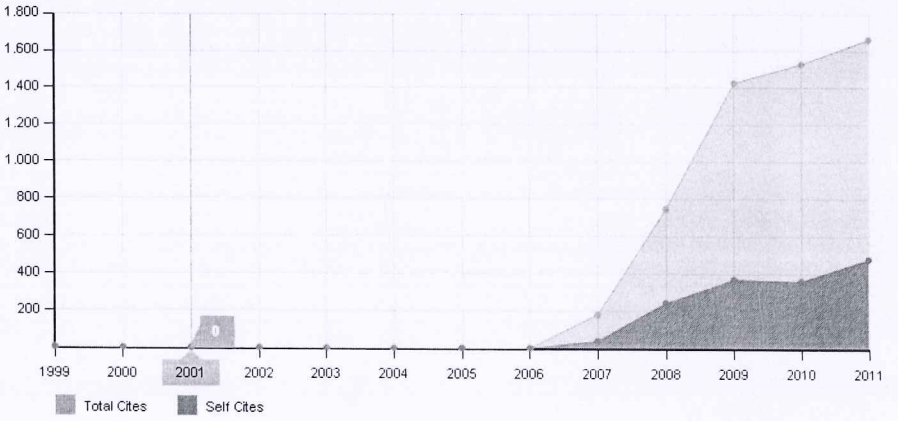

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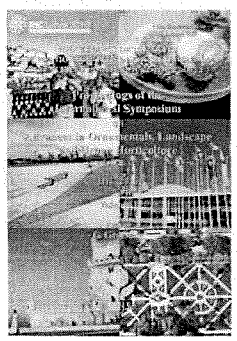

Publicaton date
September 2012

Number of articles
160

Volumes
2

Pages
1300

Symposium venue
Lisbon (Portugal)

Symposium date
August 22, 2010

Symposium
Ornamentals: Diversity and Opportunities in Ornamental Horticulture (XXVIII International Horticultural Congress - IHC2010)

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ANALYSIS OF PIGMENTS IN YELLOW-FLOWERED *PELARGONIUM* SECTION *HOAREA*

Authors
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Abstract
The flowers of *Pelargonium* section *Hoarea* were pale yellow and yellow based on the Royal Horticulture Society Colour Chart. The chromaticity of fresh petals was measured with a colorimeter which was expressed by the L*, a*, and b* values indicating lightness, redness, and yellowness, respectively. The absorption spectra were analyzed by a UV-VIS spectrophotometer. Yellow pigments were extracted with acetone followed by evaporation until dry. The pigments were re-dissolved in methanol and directly analyzed. The solution absorbed light wavelengths between 400 to 500 nm. Chromatographic analysis was carried out by HPLC. The portion of pigments extracted for spectroscopic analysis was dissolved in diethyl ether and saponified with 5% KOH/CH_3OH. The solution was washed with a NaCl saturated solution and distilled water. The diethyl ether fraction was dried over Na_2SO_4 and evaporated to dryness. The hydrolyzed pigments were dissolved in methanol and filtered through 0.45 µm prior to HPLC analysis. Carotenoids in yellow petals of *Pelargonium* section *Hoarea* were identified as lutein and β-carotene. Carotenoid composition, content, and the relationship between petal colors and carotenoid compositions are also discussed.

Citation
Sukhumpinij, P., Kakihara, F., Hondo , K. and Kato, M. 2012. ANALYSIS OF PIGMENTS IN YELLOW-FLOWERED *PELARGONIUM* SECTION *HOAREA* .

Acta Horticulturae 937
XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on Advances in Ornamentals, Landscape and Urban Horticulture

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Acta Hort. (ISHS) 937:711-716
http://www.actahort.org/books/937/937_86.htm

Keywords
carotenoids, anthocyanidins, red blotches, HPLC, *Geraniaceae*

Language
English

Full text
http://www.actahort.org/members/showpdf?booknrarnr=937_86

Article number
937_86

Pages
711-716

Groups
- Commission Landscape and Urban Horticulture
- Section Ornamental Plants
- Workgroup Urban Horticulture
- Workgroup Protea

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Analysis of Pigments in Yellow-Flowered *Pelargonium* Section *Hoarea*

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Keywords: carotenoids, anthocyanidins, red blotches, HPLC, *Geraniaceae*

Abstract

The flowers of *Pelargonium* section *Hoarea* were pale yellow and yellow based on the Royal Horticulture Society Colour Chart. The chromaticity of fresh petals was measured with a colorimeter which was expressed by the L*, a*, and b* values indicating lightness, redness, and yellowness, respectively. The absorption spectra were analyzed by a UV-VIS spectrophotometer. Yellow pigments were extracted with acetone followed by evaporation until dry. The pigments were re-dissolved in methanol and directly analyzed. The solution absorbed light wavelengths between 400 to 500 nm. Chromatographic analysis was carried out by HPLC. The portion of pigments extracted for spectroscopic analysis was dissolved in diethyl ether and saponified with 5% KOH/CH_3OH. The solution was washed with a NaCl saturated solution and distilled water. The diethyl ether fraction was dried over Na_2SO_4 and evaporated to dryness. The hydrolyzed pigments were dissolved in methanol and filtered through 0.45 µm prior to HPLC analysis. Carotenoids in yellow petals of *Pelargonium* section *Hoarea* were identified as lutein and β-carotene. Carotenoid composition, content, and the relationship between petal colors and carotenoid compositions are also discussed.

INTRODUCTION

Pelargonium is a genus within the family *Geraniaceae*, of which some species or hybrids are horticulturally used as potted or bedding plants of considerable economic importance (Mithila et al., 2001). The flower colors of *Pelargonium* cultivars are usually white, pink through scarlet, violet, or purple. Breeding aims to develop new flower cultivars in which yellow flower species in section *Hoarea* are important for breeding new colored pelargonium cultivars. *Pelargonium* section *Hoarea* have underground tubers which are often turnip-or carrot-shaped. The inflorescence often branched, usually has many flowers and each flower may have three to four or as many as fifty to sixty flowers. Most of flowers are cream or yellow, rarely pink or magenta. In natural habitat, these species grow mainly in the arid area. They adapted to withstand the hot dry summer by dying down immediately after flowering and passing most of the year underground in a dormant state. The foliage appears after the winter rain but the flowers do not develop until the leaves have died down, it is rarely possible to examine the flowers and leaves at the same time (Craib, 2001).

Color is attributed to several pigments, including the chlorophylls, carotenoids, flavonoids, and betalains. The yellow through orange of flowers are typically due to the carotenoid pigment, whereas blue to red are typically attributed to anthocyanins. The wide range of petal colors in various plants originates mainly from combinations of these pigments (Kashimoto et al., 2007). There are a few reports in yellow pigments analysis of pelargonium. It has been reported that the pale yellow color of *Pelargonium quinquelobatum* in section *Ciconium* was flavonoid by Thin Layer Chromatography (TLC) (Denis-Peixoto et al., 1997). Mitchell et al. (1998) has reported a paper in *Pelargonium* section *Hoarea*, carotenoids were found as main pigments of pale yellow-flowered *Pelargonium appendiculatum* which was analyzed using spectroscopic analyses. This is a first report presented carotenoids composition on yellow-flowered *Pelargonium* section *Hoarea* analyzed using HPLC. The objectives of our study were to investigate the yellow-

flowered characteristic and identify the yellow pigment in *Pelargonium* section *Hoarea* for its carotenoid composition and content. Meanwhile, the findings can also provide guidelines for selecting potential parents for breeding new cultivars with novel colors by crossing.

MATERIALS AND METHODS

Plant Materials

Pelargonium section *Hoarea* (Fig. 1.) were obtained from the University of Stellenbosch, South Africa and grown in a Laboratory of Plant Breeding, Ehime University, glasshouse under natural light. Petals of fully-opened flower were harvested and stored at -80°C until analysis.

Measurement of Color Parameters

The Royal Horticulture Society colour chart was used, which is based on measurements of human visual responses to color. The chromaticity of fresh petals was measured with a colorimeter (Konica Minolta CM-2600d) and was expressed by the CIELAB (CIE 1976) method: L*, a* and b* values indicate lightness, redness, and yellowness, respectively (Robertson, 1977). Color saturation and hue were expressed as chroma (C) and hue-angle (h), respectively, which were calculated from a* and b* values. The calculation formulas used are as follows:

$$C = \sqrt{(a^*)^2+(b^*)^2}$$

$$h = \tan^{-1}\left(\frac{b^*}{a^*}\right)$$

Analysis of Carotenoids

Carotenoid pigments were extracted from yellow petals with acetone and evaporated to dryness. The pigments were re-dissolved in methanol and directly analyzed using a spectrophotometer. The absorption spectra were analyzed by UV-VIS spectrophotometer (Jasco V-570). The portion of pigment extract for spectroscopic analysis was dissolved in 5% KOH/CH_3OH at room temperature for 3 h. The solution was added to a NaCl saturated solution. The yellow pigments were extracted with diethyl ether and then washed with distilled water. The diethyl ether fraction was dried over Na_2SO_4 and evaporated to dryness. The hydrolyzed pigments were dissolved in methanol and filtered through 0.45 μm Whatman filters prior to HPLC analysis under the following conditions: column, Inertsil OSD-3 (4.6x250 mm) GL-Sciences; solvent A, 90% CH_3CN; solvent B, ethyl acetate; gradient, 0/100, 10/99, 35/35, 45/30, 50/0 (min/%A); flow rate, 1.0 ml/min; column temperature, 35°C. They were quantified by their absorbance at 450 nm wavelength.

Analysis of Anthocyanidins

Anthocyanin pigments were extracted in acidified methanol (1% HCl). Nonacid hydrolyzed extracts were added to methanol and analyzed using a UV-VIS spectrophotometer (Jasco V-570). The portion of pigment extract was acid hydrolyzed at 100°C in 6N HCl for 50 min. The acid hydrolyzed pigments were added to methanol and filtered through 0.45 μm Whatman filters prior to HPLC analysis under the following conditions: column, Inertsil OSD-3 (4.6 x 250 mm) GL-Sciences; solvent A, 1.5% H_3PO_4; solvent B, 1.5% H_3PO_4: 20% acetic acid: 25% CH_3OH in H_2O; gradient, 0/50, 30/0 (min/%A); flow rate, 1.0 ml/min; column temperature, 35°C, monitored at 520 nm. The pigments were identified by comparison with the authentic pigments. Authentic pigments were obtained from *Pelargonium* ×*domesticum* Bailey 'Dark Venus' (Fujioka et al., 1991).

RESULTS AND DISCUSSION

Measurement of Color Parameters

The yellow petals of *Pelargonium* section *Hoarea* had yellow and yellow-orange with conspicuous blotches of red and red-purple on the upper petals. The human visual responses to colour are expressed by the Royal Horticulture Society colour chart. The yellow petals of *Pelargonium* section *Hoarea* had yellow (color chart code no. 2D, 4D, 5D, 8C, 10B, 10C, 11B, 12C and 13D) and yellow-orange (color chart code no. 22C) (Table 1). Red blotches on the yellow petals were also observed which varied from red (44B and 53A), red-purple (59A and 64B) to grey-purple (187A) (Table 3). The chromaticity was measured with a colorimeter, which was expressed by the L*, a* and b* values indicating lightness, redness, and yellowness, respectively. The L* values ranged from 55.85 to 89.25 in the yellow area and 28.53 to 76.12 in the red blotches. The a* value ranged from -3.03 to 6.92 in the yellow area and 7.48 to 34.97 in the red blotches, and the b* values were from 9.04 to 31.77 in the yellow area and -7.18 to 24.79 in the red blotches. The hue-angles (h) of yellow-flowered *Pelargonium* section *Hoarea* ranged from 74.74 to 103.26 degrees in the yellow area and 1.86 to 359.18 degrees in the blotches.

Spectroscopic Analysis

The pigments extracted from the yellow petals and red blotches were recorded with absorption maxima in Tables 2 and 4.

HPLC Analysis of Pigments

The difference between yellow and red blotch in the yellow petals of *Pelargonium* section *Hoarea* were due to the concentration of carotenoids and anthocyanins, respectively. Carotenoids in the petal extracts were identified by their retention times in HPLC. The determination of the individual carotenoid concentration was carried out by comparing the peak area of the standard carotenoids. The results of analysis of the component carotenoids are shown in Table 2. The major carotenoids of all species were lutein (64.71 to 91.13 percent of pigment content) together with β-carotene (0.55 to 13.86 percent of pigment content) as a minor component and the unidentified carotenoids were also present whereas a minor β-carotene was not detected in STEU 3557. The red blotches were analyzed by comparing with the authentic pigments of *Pelargonium* ×*domesticum* Bailey 'Dark Venus'. The main anthocyanidins were found as delphinidin, cyanidin, petunidin, pelargonidin, peonidin and malvidin with apparent differences in the components of anthocyanidins in each species (Table 4). The various flower colors of the crossings between two species seem to be derived from various pigment combinations: yellow was caused by carotenoids, pink, reddish purple and purple flowers by anthocyanin, and orange flowers from the coexistence of anthocyanins and carotenoids. The difference in color (yellow or orange) appeared to be due to the variation in the total carotenoid concentration and small changes in the concentration of the minor carotenoids. Thus subtle changes in carotenoid content can result in more obvious visual changes in flower color. The ratio of the amount of anthocyanins and carotenoids are also important factors in determining flower color, and by selecting potential parents for cross breeding new cultivars, various novel colors can be obtained.

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Tables

Table 1. Chromaticity in yellow-flowered *Pelargonium* section *Hoarea* species.

Species	RHS[z] code	Chromaticity				
		L*	a*	b*	h	C
STEU[y] 3162	11B	78.79	1.52	28.12	86.91	28.16
STEU 3209	11B	88.31	2.82	26.44	83.91	26.59
STEU 3418 a	22C	70.51	6.92	25.37	74.74	26.30
STEU 3418 b	10B	86.96	5.19	28.82	79.79	29.28
STEU 3424	10C	55.85	-0.40	16.36	91.40	16.36
STEU 3557	12C	71.76	0.23	19.86	89.34	19.86
STEU 4216	13D	77.63	-0.18	12.16	90.85	12.16
STEU 4218	13D	88.88	1.51	15.83	84.55	15.90
P. appendiculatum	8C	89.25	-0.33	31.77	90.60	31.77
P. caroli-henrici	10B	79.01	0.39	26.25	89.15	26.25
P. oblongatum	5D	77.32	-1.88	25.89	94.15	25.96
P. rapaceum	2D	70.42	-3.03	12.86	103.26	13.21
P. vinaceum	4D	84.42	-0.20	9.04	91.27	9.04

[z] Royal Horticulture Society colour chart; [y] University of Stellenbosch accession number.

Table 2. Carotenoid composition in yellow petals of *Pelargonium* section *Hoarea* species.

Species	Absorption maxima (nm)	Pigment composition
STEU[z] 3162	418, 446, 472	Lt[y], β-carotene, UN[x]
STEU 3209	418, 446, 472	Lt, β-carotene, UN
STEU 3418 a	416, 448, 472	Lt, β-carotene, UN
STEU 3418 b	422, 448, 474	Lt, β-carotene, UN
STEU 3424	422, 448, 474	Lt, β-carotene, UN
STEU 3557	418, 448, 472	Lt, UN
STEU 4216	420, 446, 472	Lt, β-carotene, UN
STEU 4218	420, 446, 472	Lt, β-carotene, UN
P. appendiculatum	424, 444, 474	Lt, β-carotene, UN
P. caroli-henrici L[w]	418, 446, 472	Lt, β-carotene, UN
P. caroli-henrici U[v]	420, 444, 470	Lt, β-carotene, UN
P. oblongatum	420, 442, 468	Lt, β-carotene, UN
P. rapaceum	424, 444, 464	Lt, β-carotene, UN
P. vinaceum	418, 446, 472	Lt, β-carotene, UN

[z] University of Stellenbosch accession number; [y] Lutein; [x] Unidentified; [w] Lower petal; [v] Upper petal.

Table 3. Chromaticity of red blotches in yellow-flowered *Pelargonium* section *Hoarea* species.

Species	RHS[z] code	Chromaticity				
		L*	a*	b*	h	C
STEU[y] 3162	64A	51.25	32.15	-4.05	352.82	32.40
STEU 3209	44B	67.02	34.97	24.79	35.33	42.86
STEU 3418 a	45B	58.87	12.20	17.65	55.35	21.46
STEU 3418 b	64A	72.92	13.79	-7.18	332.50	11.77
STEU 3424	187A	37.40	10.16	2.80	15.41	10.54
STEU 3557	47B	45.72	14.17	4.15	16.32	14.77
STEU 4216	53A	58.97	10.46	5.12	26.08	11.65
STEU 4218	59A	69.09	19.07	0.62	1.86	19.08
P. appendiculatum	55B	76.12	15.31	24.14	57.62	28.59
P. caroli-henrici L[x]	53A	66.67	8.23	18.16	65.62	19.94
P. caroli-henrici U[w]	53A	53.36	13.17	12.25	42.93	17.99
P. oblongatum	59A	54.91	7.48	11.16	56.17	13.43
P. rapaceum	59A	28.53	11.23	0.43	2.19	11.24
P. vinaceum	187A	29.22	11.87	-0.17	359.18	11.87

[z] Royal Horticulture Society colour chart; [y] University of Stellenbosch accession number; [x] Lower petal; [w] Upper petal.

Table 4. Anthocyanidins identified from red blotches in yellow petals of *Pelargonium* section *Hoarea* species.

Species	Absorption maxima (nm)	Pigment composition
STEU[z] 3162	540	Dp[y], Pt[x], Pn[w], Mv[v]
STEU 3209	512	Pg[u], Pn, Mv
STEU 3418 a	530	Dp
STEU 3418 b	536	Dp, Pt, Pg, Pn, Mv
STEU 3424		Dp, Pt, Pg, Pn, Mv
STEU 3557		Dp, Cy[t], Pt, Pe, Mv
STEU 4216		Dp, Pt, Pg, Mv
STEU 4218		Dp, Pt, Mv
P. appendiculatum		Dp, Mv
P. caroli-henrici L[s]	532	Dp, Pt
P. caroli-henrici U[r]	540	Dp, Pt, Mv
P. oblongatum	540	Dp, Pt, Mv
P. rapaceum		Dp, Pt, Pg, Pn, Mv
P. vinaceum	536	Dp, Pt, Pn, Mv

[z] University of Stellenbosch accession number; [y] Delphinidin; [x]Petunidin; [w]Peonidin; [v] Malvidin; [u] Pelargonidin; [t] Cyanidin; [s] Lower petal; [r] Upper petal.

Figures

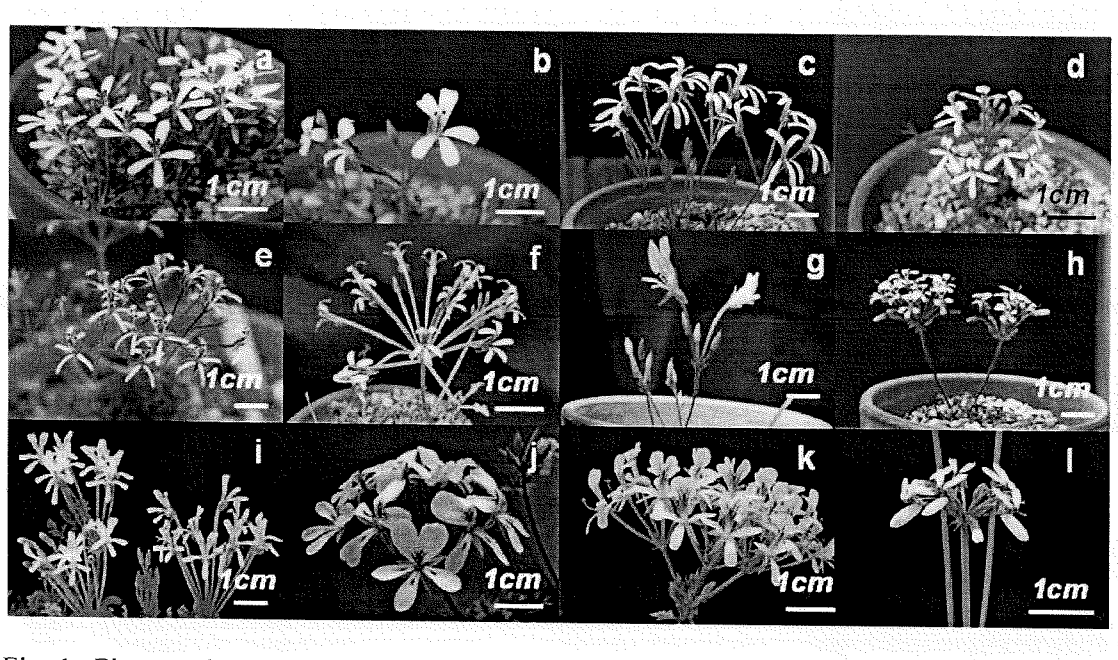

Fig. 1. Flower characteristic and flower color of *Pelargonium* section *Hoarea* species; (a) STEU3162; (b) STEU3209; (c) STEU3418a; (d) STEU3418b; (e) STEU3424; (f) STEU3557; (g) STEU4216; (h) STEU4218; (i) *Pelargonium appendiculatum*; (j) *Pelargonium caroli-henrici*; (k) *Pelargonium oblongatum*; and (l) *Pelargonium rapaceum*.