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Flower Colors and Pigments of Interspecific Hybrids between Camellia japonica L. and C. *chrysantha* (Hu) Tuyama

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The colors and the yellow pigment components of the interspecific hybrids obtained by crossing C. *japonica cvs.* Shiratama and Chochidori with C. *chrysantha* and those of their parents were investigated and analyzed. The white flowered seed parents contained only one common major flavonols and the yellow flowered pollen parent had three major flavonols and some carotenoids. The yellow flavonols in both parents and carotenoids in pollen parent were detected in the hybrids. Therefore, it is deducible that the transmission of the yellow pigment genes from both parents to the F_1 hybrids was successful.

INTRODUCTION

The ornamental value of *Camellia japonica* L. has long been recognized, and it is cultivated worldwide nowadays. The flower colors encountered in C. *japonica* are ranged from white to pink and red. Studies of flower color in C. *japonica* have been confined to inheritance of red and white colors based on anthocyanin constituents (Parks, 1966, 1968; Hanson, 1978; Sakata et al., 1980, 1981; Sakata and Arisumi, 1983, 1985).

To develop yellow flowers of garden camellias has been the dream and goal of many camellia breeders. The yellow flowered camellia, C. *chrysantha* (Hu) Tuyama, containing yellow flavonoids and carotenoids (Miyajima *et al.*, 1985; Scogin, 1986; Parks and Scogin, 1987) has been used to cross with C. *japonica*. Due to the incompatibility between these two species, only a few hybrids have been reported (Yamaguchi et al., 1987; Uemoto *et al.*, 1988; Yamaguchi, 1990). To investigate the transmission of yellow pigment from C. *chrysanthu* to the hybrids, the petals of two pale yellow flowered hybrids of C. japonica X *C. chrysanthu* were analyzed.

MATERIALS AND METHODS

Fully expanded fresh petals of the hybrids of *C. japonica cv.* Shiratama X *C. chrysantha* and of C. *japonica cv.* Chochidori x *C. chrysanthu* were used to obtain single or interpolated values best describing color phenotype. Nominal descriptions of petal color were based on visual observations and nominal equivalents to values published with the RHS Colour Chart (1966).

Pigments type and location were determined from transverse sections of fresh petals. Petals were free-hand-sectioned with a razor blade and sections were mounted in water and examined using light microscopy (Nikon BIOPHOT VD). Presence or absence of chromoplasts and vacuolar pigments in the upper epidermis, ground parenchyma, and lower epidermis were observed.

After the spectral absorption of fresh intact petals was measured by a Shimadzu multi-purpose spectrophotometer MPS-5000, the petals were lyophilized and stored in desiccators until further analysis.

The lyophilized samples were extracted with 100 % methanol, and then filtered by filter paper. A small portion of the filtrate was passed through millipore (Millex-SR) 0.5 μm filter again, and the filtrates were analyzed by high performance liquid chromatography (HPLC) (Shimadzu LC-6A pump, SPD-6AV spectrophotometric detector) using cosmosil-C_{18} column (4.6 mm i.d. **x** 250 mm). The chromatographic conditions were given as follows: the flow rate was 1.0 $mlmin^{-1}$; solvent B (acetonitrile) was gradually increased from 15 to 25 % in the mixture with solvent A (0.1 M acetic acid) in the first 20 min, and solvent B was fixed at 25 % in the following 10 min; samples were detected at 370 nm with 0.01 absorbance unit full scale.

Another portion of the filtrate was concentrated, then resuspended and partitioned in petroleum ether and distilled water. The occurrence of carotenoids was determined both by the spectral absorption of fresh intact petals and the absorption peak of petroleum ether fraction at 490 nm. The solutes in the water fraction (flavonoids) were collected and concentrated by a Sep-Pak C_{18} cartridge (Waters). The flavonoids in the cartridge were eluted by methanol after being washed with water twice to eliminate the water-soluble or hydrophilic contaminants. Two-dimensional thin layer chromatography (TLC) was adopted for isolation and purification of the flavonoids. The eluates were spotted on 20 **x** 20 cm glass plates coated with Avicel microcrystalline cellulose powder and developed with n-butanol: acetic acid: water (BAW, 4:1:5, by vol., upper layer) for the first dimension and 15 % acetic acid for the second. To identify the flavonoids, the air-dried chromatograms on the plates were viewed in transmitted long wave UV light (365 nm) with and without the presence of ammonia vapor and after spraying with 5 % ethanolic aluminum chloride (Harborne, 1959).

RESULTS AND DISCUSSION

The petal color of both two seed parents, cvs. Shiratama and Chochidori, is white (Figs. 1-A and C), and that of pollen parent, C. *chrysantha*, is golden yellow. The petals of the hybrid from cv. Shiratama is creamed center shading outwardly to pink at the margin (Fig. 1-B); that of the hybrid from cv. Chochidori is pale yellow and only the outermost petals with slightly pink edge (Fig. 1-D).

Yellow-colored vacuoles and chromoplasts were observed in petals of C. *chrysantha* by Miyajima ***et*** *al.*(1985). In this experiment, yellow pigments were observed not only in the upper and lower epidermis but also in internal parenchyma cells of C. *chrysantha* and the two F_1 hybrids. Vacuolar pigmentation of upper epidermis was the most intense when compared with that of lower epidermis and parenchyma cells. Chromoplasts were also present in most cells, especially parenchyma, of all petals observed. Pigmentation of petals of the hybrid from cv. Shiratama consisted of

Fig. 1. Flowers of *C. japonica* cv. Shiratama (A), *C. japonica* cv. Chochidori (C), F_1 hybrid of *C. japonica* cv. Shiratama × *C. chrysantha* (B), and F_1 hybrid of *C. japonica* cv. Chochidori × *C. chrysantha* (D).

Table 1. Color and spectral data of fresh petals.

Species and F_1 hybrids	Petal color[z]	RHS[y] Colour Chart group	RHS Colour Chart code number	Absorbance max. (nm)
C. chrysantha	Yellow	Yellow	11A	385,490s[x]
C. *japonica* *cv.* Shiratama	White	White	158D	355
F_1	Ivory/pink	Yellow/ red	11D/ 56A	380,454s,490s
C. japonica *cv.* Chochidori	White	White	155D	355
F_1	Pale yellow	Yellow	10D	370,460s,490s

[z] Based on visual observations.
[y] Based on comparisons with the Royal Horticultual Society Colour Chart.
Interpolated values (/) were required when no single color chip best described petal color.
[x] s; shoulder.

carotenoid-containing chromoplast in the cytoplasm and yellow and pink flavonoids in the vacuoles although pink flavonoids were absent in its seed and pollen parents.

Spectral curve of the petals of cvs. Shiratama and Chochidori exhibited only one maximum, in 340-380nm region, which indicated the presence of the yellow flavonoids (Table 1). ***Camellia chrysantha*** showed an absorption maximum of yellow flavonoids at 385 nm and a slight shoulder of carotenoids at 490 nm. The hybrid from cv. Shiratama presented two carotenoid absorption shoulders (454 and 490 nm) with one flavonoid maximum at 380 nm. The hybrid from cv. Chochidori presented absorption pattern as that of the hybrid from cv. Shiratama, one flavonoid maximum and two carotenoid shoulders.

Spots which showed on chromatograms of cv. Shiratama were designated as JS1, JS2, . . . JSn; those of cv. Chochidori were as JC1, JC2, . . . JCn; and those of C. *chysanthu* were as Cl, ***C2,*** . ..and Cn (Fig. 2). The chromatograms of TLC and HPLC revealed that cvs. Shiratama and Chochidori contained only one and the same major yellow flavonoid constituent (Figs. 2 and 3). The spot C6 also occurred in cvs. Shiratama and Chochidori, while its quantity was so small and its retention time was near that of JC1(JS1) that it was covered by JC1(JS1) on TLC chromatograms. All the major spots, JSl(JCl), Cl, C6, and Cl4 were postulated to be flavonols from the change of the colors under visible or UV light when the TLC plates were fumed with ammonia vapor or sprayed with ethanolic aluminum chloride (Table 2).

In the hybrid of cv. Shiratama × C. *chrysantha* the major spot of seed parent, JS1, was detected in relatively high amount; yet, the major spots of C. ***chrysantha,*** Cl and C6, were trace, and C14, was none. In the hybrid of cv. Chochidori X ***C. chrysantha,*** although the amount of spot Cl was not so high as spot JS1, it is considerable one. The spot C6 was also present in a small amount in the hybrid but from which parents it was inherited was not known. Besides these flavonols, the carotenoids were also transferred from C. ***chrysantha*** to the two hybrids (Table 3).

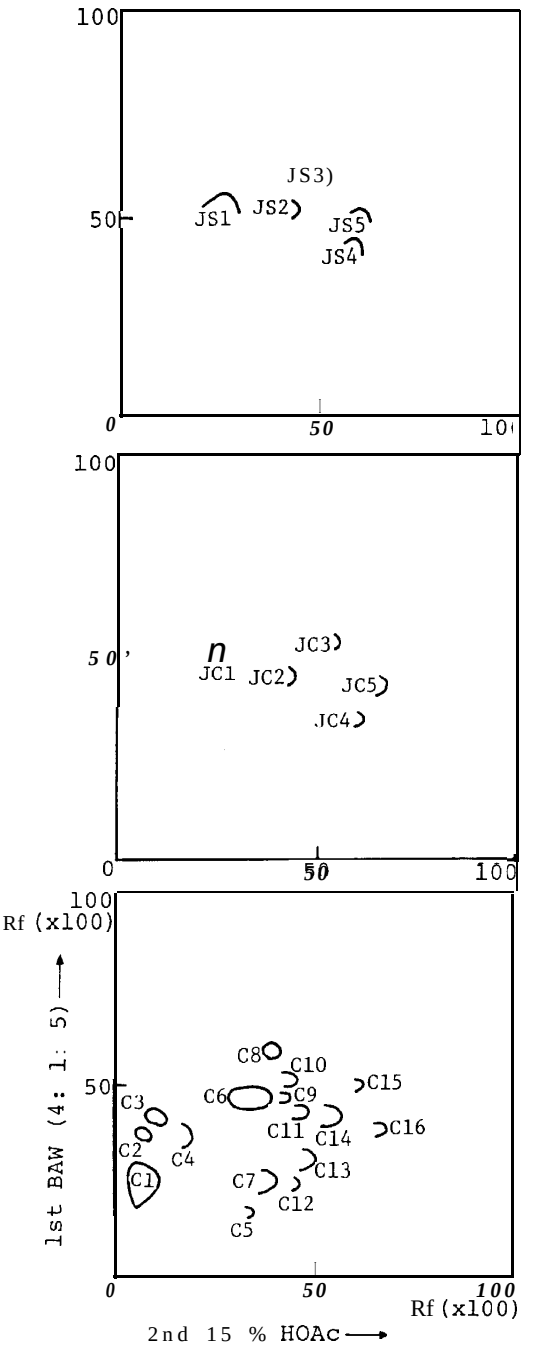

Fig. 2. Two dimensional thin layer chromatograms of yellow flavonoids from petals of C. *japonica* ***cvs.*** Shiratama (above) and Chochidori (middle) and C. ***chysantha*** (below).

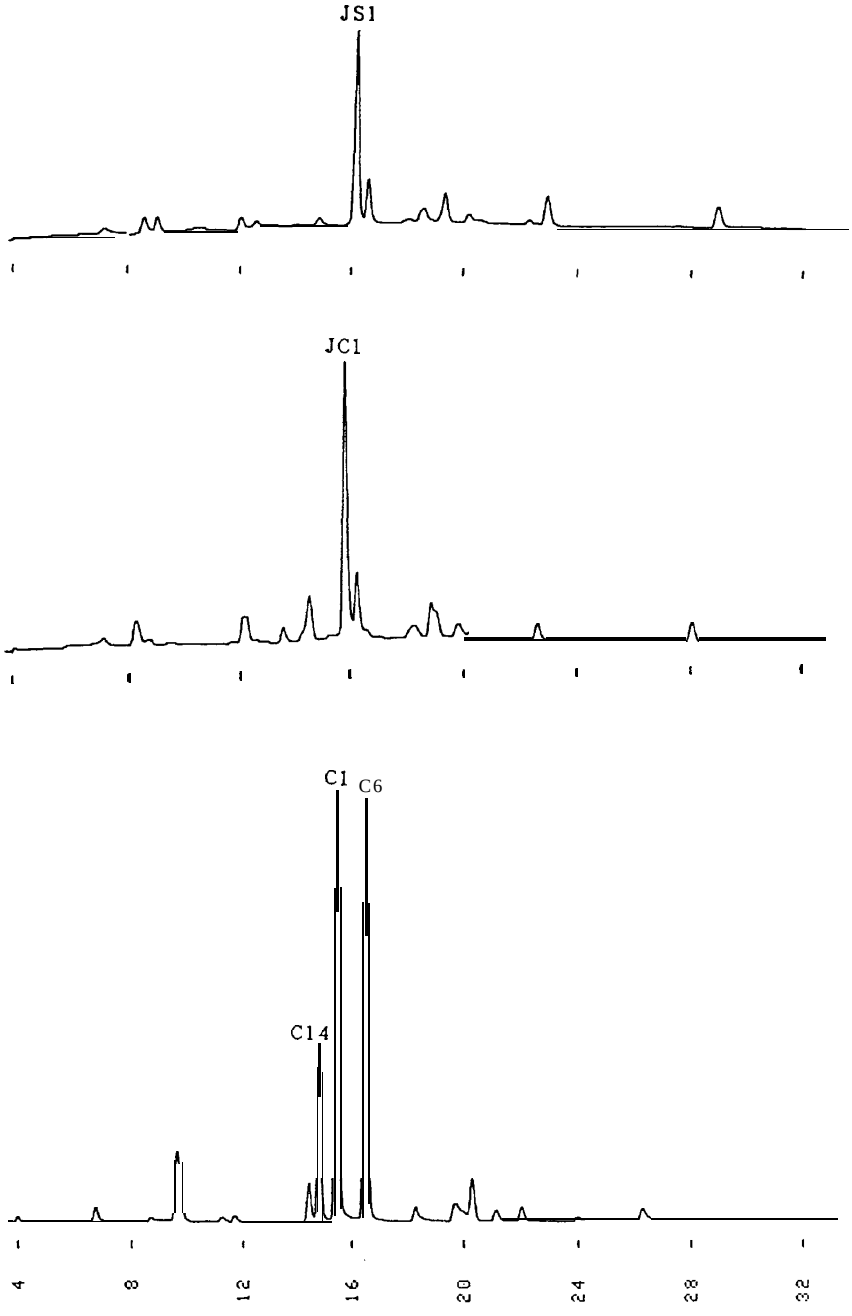

Fig. 3. Selected traces from HPLC analyses of C. *japonica cvs.* Shiratama (above) and Chochidori (middle) and C. *chrysantha* (below).

Table 2. Properties of the main spots of C. japonica cvs. Shiratama and Chochidori, C. ***chysantha*** and their F, hybrids on thin layer plates.

Spot[z] No.	Rf(x 100)		Color					
	BAW[y]	15% HOAc	None		$+NH_3$		$+AlCl_3$[x]	
			Visible	u v	Visible	UV	Visible	u v
C 1	25	6	weak yellow	yellow(f[w])	yellow	orangeish-yellow	greenish-yellow	yellowish-green(f)
C 6	50	32	pale yellow	brown	yellow	ochre	yellow	deep yellow
C14	41	70	pale yellow	dull brown	yellow	ochre	yellow	yellow
JS1	55	25	pale yellow	dull brown	yellow	ochre	yellow	yellow
JC1	53	27	none	dull brown	pale yellow	ochre	pale yellow	yellow

[z] Correspond to the numbers in Fig. 2.
[y] n-butanol: acetic acid: water (4:1:5, by vol., upper phase)
[x] 5% ethanolic aluminum chloride.
[w] Fluorescence.

Table 3. Pigment distribution in floral extracts.

Species and F_1 hybrids	Carotenoid occurrence[z]	Distribution of flavonol glycoside[y]			
		Cl[x]	C6	C14	JS1(JC1)[w]
C. chysantha	P		++++	++	–
C. japonica ***cv.*** Shiratama	A		+	–	
F_1	P	+	+	–	
C. japonica ***cv.*** Chochidori	A		+		
F_1	P	++	+		

[z] P, presence; A, absence.
[y] –, none; +, trace; ++, low; + + +, intermediate; + + ++, high.
[x] Correspond to the numbers in Fig. 2.
[w] JS1 and JC1 were considered to be the same spot.

It is regrettable that the pollen of both the two hybrids, especially the one from cv. Shiratama, was a little and fertile. However, they could be used as seed parents for their pistils seemed to be fertile.

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