Aluminum Ions Are Involved in Purple Flower Coloration in *Camellia japonica* ‘Sennen-fujimurasaki’

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Flowers of wild *Camellia japonica* L. are usually red, but infrequently the flowering trees of this species may have purple flowers. Such purple flowers are a highly desired horticultural property, but the color expression is not fixed. Even if a tree has splendid purple flowers in the spring, they may revert back to the red color of a wild *C. japonica* flower the next year. We investigated the factors responsible for the purple coloration using red, purplish-red, and purple flowers of the cultivar ‘Sennen-fujimurasaki’. The epidermal cells of purplish-red and purple petals were composed of both red and purple colored cells, whereas those of the red petals were uniformly red. Many of the purple cells contained blue-black granules. Cyanidin 3-glucoside and cyanidin 3-p-coumaroylglyceroside, major pigments of red-flowered *C. japonica*, were the major anthocyanins of ‘Sennen-fujimurasaki’. The anthocyanin contents were not noticeably different among flowers of these different colors. Potential co-pigments such as flavones, flavonols, and cinnamic acid derivatives were negligibly detected. No significant differences were found in the Ca, Mg, Mn, Fe, Cu, and Zn ion contents or in the pH of petal homogenates; however, a significant difference was found in the Al ion content. The Al content of the purplish-red and the purple petals was 4–10 times higher and 14–21 times higher than that of red petals, respectively. A cyanidin 3-glucoside solution prepared at pH 4.8 was pale red with no precipitates. When Al ions were added to the cyanidin 3-glucoside solution, the solution became purple and produced blue-black precipitates similar to the blue-black granules observed in the purple colored cells. Differences in the spectral properties of the petals from those of the prepared solution could be caused by the co-occurrence of red and purple cells and may be influenced by other Al-chelating compounds and/or substantial Al concentrations in the vacuoles. We conclude that the purple flower color of ‘Sennen-fujimurasaki’ is generated by chelation of Al ions by anthocyanins. In other purple-flowered *C. japonica* exhibiting unstable flower coloration similar to that of ‘Sennen-fujimurasaki’, Al-anthocyanin chelation is also likely associated with the purple flower color.

**Key Words:** anthocyanin, camellia, flower color, metal ions.

**Introduction**

The genus *Camellia* contains more than 200 species (Chang and Bartholomew, 1984) that have white, pink, red, or yellow flowers, but purple-, violet-, and blue-flowered species have not been found in this genus. Camellias are one of the most popular woody ornamentals, and have great value as rare winter and early-spring blooming evergreen shrubs and trees in temperate areas. The most utilized species as an ornamental cultivar is *Camellia japonica* L. (Chang and Bartholomew, 1984; Hakoda, 2006). *Camellia japonica* is a native plant grown widely in Japan, except for Hokkaido. Flower pigments of *C. japonica* are simple structural anthocyanins. Cyanidin 3-glucoside is the main pigment, and cyanidin 3-galactoside and cyanidin 3-p-coumaroylglyceroside are also present at various ratios (Saito et al., 1987; Sakata, 1988). Flowers of the wild forms of *C. japonica* are usually red, and those of *C. japonica* cultivars are white, pink, and red based on the relative concentrations of the anthocyanin pigments. Occasionally, purple-flowered trees of *C. japonica* have been discovered. Because the purple flower color is so rare, these plants were brought into cultivation by propagation using cutting and grafting, and were given cultivar names. However, very regrettably, the purple flower...
color expression is very unstable. The flower color of propagated young trees often returns to the red of wild *C. japonica* flowers, even if the original tree had splendid purple flowers when it was discovered. Because of the unstable flower color, these purple-flowered cultivars have not been popularized.

Various factors are required for anthocyanins to develop a bluer color: an anthocyanin structure with two or more aromatic acyl residues, a higher vacuolar pH, the existence of co-pigment compounds (such as flavones, flavonols, and cinnamic acid derivatives), and complexation with metal ions (Yoshida et al., 2009). Anthocyanins form complexes with aluminum (Al) ions leading to bathochromic shifts in their absorption spectra (Dangles et al., 1994). Al ion is an important factor for developing the blue color of hydrangea flowers (Takeda, 2006; Yoshida et al., 2009). In the genus *Camellia*, tea plant, *C. sinensis* (L.) O. Kuntze is a famous Al-accumulating plant (Chenery, 1955; Matsumoto et al., 1976). *Camellia japonica* also accumulates Al (Yamada, 1980). Previously, we showed that the deep yellow flower color of *C. chrysantha* (Hu) Tuyama is generated by chelation of Al ions by quercetin derivatives, which are naturally pale yellow flavonols (Tanikawa et al., 2008). Thus, we hypothesized that Al ions may be involved in developing the purple color of *C. japonica* flowers.

The cultivar ‘Sennen-fujimurasaki’ was discovered among *C. japonica* trees grown in wild conditions in the Shimabara Peninsula of Nagasaki Prefecture (Yokoyama and Kirino, 2005). Its flowering period is from February to April (Yokoyama and Kirino, 2005). This cultivar had splendid purple flowers when it was discovered, but the flower color of the propagated tree has been unstable (Yokoyama and Kirino, 2005). In this study, we used red, purple, and the intermediate color (purplish-red) flowers of ‘Sennen-fujimurasaki’ and investigated the anthocyanins, co-pigment compounds, pH of the petal homogenate, and metal ions of these flowers in order to elucidate the cause of the purple coloration in *C. japonica*. We report the involvement of Al ions in the occurrence of purple flowers in *C. japonica*.

**Materials and Methods**

**Plant materials**

Flowers were collected from three individual ‘Sennen-fujimurasaki’ trees. Tree 1 (NIFS No. 637) was a pot-cultivated tree grown in an unheated plastic greenhouse at the NARO Institute of Floricultural Science (NIFS). Trees 2 and 3 were grown in a private garden, and were about 4 m high and about 30 years old. Flowers of *C. japonica* NIFS No. 1083 and No. 1088 were also used, which have typical wild-formed red flowers, and were grown in a field at the NIFS.

**Observation of petal epidermal cells**

In 2013, petal epidermal cells from red, purplish-red, and purple flowers of ‘Sennen-fujimurasaki’ were observed using a digital microscope VH-8000C with a VH-Z75 lens (KEYENCE Corporation, Osaka, Japan).

**Measurements of colorimetric values and absorption spectra of petals**

As indicators of color tones, colorimetric values of CIELAB (*L*, *a*, *b*), *C*, and *h*° for the adaxial side of petals were measured under 2° observer and illuminant C using a spectrophotometer (CD110; Yokogawa Meters & Instruments Corporation, Tokyo, Japan). The *L* value represents lightness. The *a*° value represents the red-purple/bluish-green hue component (positive *a*° indicates a hue of red-purple and negative *a*° indicates that of bluish-green), and the *b*° represents the yellow/blue hue component (positive *b*° indicates a hue of yellow and negative *b*° indicates that of blue). The *C*° value represents chroma. Hue angle *h*° (degree) is the value calculated from the arctangent of *b*/*a*, and angles from 0°, 90°, 180°, to 270° correspond to red-purple, yellow, bluish-green, to blue, respectively (McGuire, 1992). Two petals per flower were measured, and the average was used as the value for the flower. Three or more flowers for each sample were measured.

Visible absorption spectra with a wavelength range of 400–700 nm of red, purplish-red, and purple flowers of ‘Sennen-fujimurasaki’ were investigated in 2013. Petals were measured using a UV-Vis spectrophotometer (UV-2450) equipped with an integrating sphere (ISR-2200; Shimadzu Corporation, Kyoto, Japan). The absorption spectra were calculated using UVPProbe software (Ver. 2.31; Shimadzu Corporation). Absorption spectra for one petal from each of three red flowers, three purplish-red flowers, and four purple flowers were measured. The spectra from each flower color were subsequently averaged to obtain the average spectrum for each flower color.

**Measurement of pH from fresh petal homogenates**

One petal was homogenized, and the pH of the homogenate was measured using a compact pH meter (B-212; Horiba, Ltd., Kyoto, Japan). Two petals per flower were measured, and the average was used as the value for the flower. Three or more flowers for each sample were measured.

**Analysis of anthocyanins and related compounds**

Fresh petals were frozen in liquid nitrogen, and stored at −80°C. Petals were extracted twice with 50% aqueous (aq.) acetic acid (AcOH) (2 mL·g−1 FW), and the combined extract was analyzed by high performance liquid chromatography (HPLC). HPLC analysis was conducted using an HP1100 system with a photodiode array detector (220–600 nm; Agilent
Results

Visual flower color

Flowers from three individual trees of ‘Sennen-fujimurasaki’ were used in this investigation (trees 1, 2, and 3). Flowers from tree 1 were evaluated in the spring of 2013 (Fig. 1a), 2014, and 2015 and were visually red in every year. Flowers of tree 2 were evaluated only in 2013, and the flower color was purplish-red (Fig. 1b). Flowers of tree 3 were investigated in 2013 and 2014 (Fig. 1c, d), and the flower color was a beautiful purple in 2013 (Fig. 1d) and a more purplish-red color in 2014 (Fig. 1c). The petals of red flowers were uniformly red, but the petals of purplish-red and purple flowers had reddish portions and purplish portions. Flowers of *C. japonica* No. 1083 and No. 1088 were evaluated in 2014 and 2015, respectively, and both were red (Fig. 1e, f).

Observations of the petal epidermis

Epidermal cells of the red petals were uniformly red (Fig. 2a). In the purplish-red petals, both red- and purple-colored cells were observed (Fig. 2b). Purple cells were observed mainly along the vascular tissue, and blue-black granules were observed in many of the purple cells (Fig. 2b). In the purple petals, many more epidermal cells were purple and blue-black granules were also observed in many of the purple cells (Fig. 2c).

Colorimetric values, absorption spectra, and pH values

Colorimetric values (*L*, *a*, *b*, *C*, and *h*) corresponded well with their visual flower color tones (Fig. 1; Table 1). The reddish color intensity was reflected by the *a* value; the flower colors were more reddish, and the *a* values were more positive. The purplish color intensity of these flowers was reflected by the *b* value; the flowers were more bluish, and the *b* values were more negative. The *h* value also corresponded well with their visual color tones.

The visible absorption spectra of the three colors of ‘Sennen-fujimurasaki’ flowers were measured in 2013 (Fig. 3). The averaged spectra of red and purplish-red petals had absorption maxima around 529 nm. The spectrum of purplish-red petals had a higher absorbance over a longer wavelength region at about 560 nm compared with the spectrum from the red petals. The averaged spectrum from the purple petals had absorption maxima around 535 nm. The absorbance near the absorption maximum was lower for the purple petals than for the red and purplish-red petals, but the absorbances at wavelengths over about 570 and 580 nm were high compared with those of the red and purplish-red petals, respectively.

Homogenates of ‘Sennen-fujimurasaki’ red petals had pH values from 4.5 to 4.7 (Table 1). Homogenates from purplish-red petals had pH values of 4.6 and 4.7,
and homogenates from purple petals measured pH 4.8. Homogenates from No. 1083 and No. 1088 had pH values of 3.9 and 4.5, respectively.

**Anthocyanins and related compounds**

As a result of HPLC analyses, the same anthocyanins as those of No. 1083 and 1088 were detected in red, purplish-red, and purple petals of 'Sennen-
**Table 1.** Colorimetric values and pH of flowers of *C. japonica* ‘Sennen-fujimurasaki’, No. 1083, and No. 1088.

| Flower color   | ‘Sennen-fujimurasaki’ | No. 1083 | No. 1088 |
|---------------|------------------------|----------|----------|
|               | Tree 1 | Tree 2 | Tree 3 |               | Tree 1 | Tree 2 | Tree 3 |               |
| Year          | 2013   | 2014   | 2015   | 2013 | 2014 | 2013 | 2014   | 2015 |
| Sample number | n = 4  | n = 3  | n = 3  | n = 3 | n = 5 | n = 5 | n = 3  | n = 4 |
| L*            | 44.20±0.39 | 43.06±0.16 | 42.81±1.35 | 41.44±0.69 | 43.70±0.46 | 42.74±1.33 | 38.69±0.31 | 41.06±0.53 |
| a*            | 59.27±0.87 | 60.63±0.33 | 59.18±0.90 | 53.90±0.88 | 48.63±2.22 | 35.14±0.87 | 48.86±1.08 | 56.60±0.35 |
| b*            | 9.43±0.56 | 12.66±0.27 | 11.87±0.82 | 5.01±0.62 | -1.27±1.86 | -11.42±0.55 | 17.85±0.46 | 14.08±0.63 |
| C*            | 60.03±0.95 | 61.94±0.36 | 60.39±0.88 | 54.18±0.93 | 48.84±2.14 | 37.03±0.70 | 52.02±1.13 | 58.34±0.19 |
| h°            | 9.00±0.40 | 11.79±0.20 | 11.28±0.81 | 5.21±0.59 | 358.04±2.34 | 341.79±1.16 | 20.07±0.34 | 13.97±0.69 |
| pH            | 4.6±0.0  | 4.5±0.0  | 4.7±0.0  | 4.7±0.0  | 4.6±0.1  | 4.8±0.1  | 3.9±0.1  | 4.5±0.0  |

The averages± SE are indicated.

**Table 2.** Anthocyanin content (μmol·g⁻¹ FW) of flowers of *C. japonica* ‘Sennen-fujimurasaki’, No. 1083, and No. 1088.

| Flower color | ‘Sennen-fujimurasaki’ | No. 1083 | No. 1088 |
|--------------|------------------------|----------|----------|
|              | Tree 1 | Tree 2 | Tree 3 |               | Tree 1 | Tree 2 | Tree 3 |               |
| Year         | 2013   | 2014   | 2015   | 2013 | 2014 | 2013 | 2014   | 2015 |
| Sample number | n = 4  | n = 3  | n = 3  | n = 3 | n = 4 | n = 6 | n = 3  | n = 4 |
| Total Anthocyanin | 1.31±0.06 | 1.17±0.06 | 1.18±0.07 | 1.05±0.05 | 0.82±0.04 | 0.89±0.04 | 1.80±0.06 | 1.73±0.10 |
| Cyanidin 3-galactoside | 0.02±0.00 | 0.03±0.00 | 0.06±0.01 | 0.03±0.00 | 0.05±0.00 | 0.03±0.00 | 0.45±0.02 | 0.22±0.02 |
| Cyanidin 3-glucoside | 0.34±0.03 | 0.34±0.01 | 0.56±0.06 | 0.35±0.03 | 0.31±0.01 | 0.27±0.01 | 0.45±0.02 | 0.22±0.02 |
| Cyanidin 3-p-coumaroylglucoside | 0.64±0.02 | 0.53±0.04 | 0.34±0.02 | 0.44±0.02 | 0.29±0.02 | 0.41±0.02 | 0.08±0.00 | 0.23±0.02 |

The averages± SE are indicated.

**Metal ion content**

Anthocyanin chelation of divalent- and trivalent-type metal ions leads to blue coloration in several species with blue flowers (Takeda, 2006; Yoshida et al., 2009). In addition to Al ions, the concentrations of Ca, Mg, Mn, Fe, Cu, and Zn ions in petals of ‘Sennen-fujimurasaki’, No. 1083, and No. 1088 were measured as shown in Table 3. In ‘Sennen-fujimurasaki’, a significant difference was clearly found in the Al ion content of the red, purplish-red, and purple petals (Tukey’s test at *P* < 0.05). The average Al content of purplish-red petals from two trees ranged from 1.04 to 1.56 μmol·g⁻¹ FW, values that were 4–10 times higher than those of the red petals. The Al content of purple petals was 3.23 μmol·g⁻¹ FW, a value 14–21 times higher than that of the red petals. The ratios of the Al content to the total anthocyanin content in the red, purplish-red, and purple petals were 0.1 to 0.2, 1.0 to 1.9, and 3.6, respectively.
The absence of Al ions, the color intensity of cyanidin 3-glucoside decreased immediately after being dissolved in 0.1 M AcOH buffer at pH 4.8, which was the same pH as the purple petal homogenate of ‘Sennen-fujimurasaki’. After 24 h, the solution was a pale red color with an absorption maximum around 521 nm, and produced no precipitates (Fig. 4). By increasing the molar ratio of Al ions to cyanidin 3-glucoside from 0.1:1, 0.2:1, 0.3:1, 0.4:1, 0.5:1, to 1:1, the absorption maximum shifted from around 528, 536, 544, 547, 550, to 554 nm, respectively (Fig. 4b). At ratios of 1:2 and 1:3, the absorption maxima remained around 555 nm (Fig. 4b). When Al ions were added, blue-black precipitates appeared, even when adding Al ions at a 0.1 molar ratio of Al ions to cyanidin 3-glucoside (Fig. 4a).

**Effect of aluminum ions on anthocyanin solutions**

Because a significant difference was found in the Al content among the red, purplish-red, and purple flowers of ‘Sennen-fujimurasaki’ (Table 3), we investigated the effect of Al ions on the coloration of anthocyanins using cyanidin 3-glucoside, one of the major anthocyanins present in ‘Sennen-fujimurasaki’ and a compound readily available as a highly purified standard. In the absence of Al ions, the color intensity of cyanidin 3-glucoside decreased immediately after being dissolved in 0.1 M AcOH buffer at pH 4.8, which was the same pH as the purple petal homogenate of ‘Sennen-fujimurasaki’. After 24 h, the solution was a pale red color with an absorption maximum around 521 nm, and produced no precipitates (Fig. 4). By increasing the amount of Al ions added to the solutions, the color of the solutions became more purple and deeper (Fig. 4a). By increasing the molar ratio of Al ions to cyanidin 3-glucoside from 0.1:1, 0.2:1, 0.3:1, 0.4:1, 0.5:1, to 1:1, no difference in the anthocyanin components was found among the red, purplish-red, and purple petals of ‘Sennen-fujimurasaki’.

### Table 3. Metal ion content (μmol·g−1 FW) of flowers of C. japonica ‘Sennen-fujimurasaki’, No. 1083, and No. 1088.

| Flower color | Year | Sample number | Al | Ca | Mg | Mn | Fe | Cu | Zn |
|--------------|------|---------------|----|----|----|----|----|----|----|
| ‘Sennen-fujimurasaki’ | Tree 1 | Red | 2013 n=4 | 0.15±0.03 | 4.85±0.26 | 5.34±0.32 | 0.035±0.006 | 0.007±0.001 | 0.0022±0.0003 | 0.0071±0.0007 |
|                | Red | 2014 n=3 | 0.23±0.04 | 3.80±0.09 | 4.10±0.18 | 0.052±0.001 | 0.005±0.000 | 0.0014±0.0002 | n.d. |
|                | Red | 2015 | 0.18±0.03 | 5.52±0.44 | 4.59±0.15 | 0.082±0.014 | 0.007±0.002 | 0.0039±0.0007 | 0.0049±0.0005 |
| Tree 2 Purplish-red | 2013 n=3 | 1.04±0.06 | 5.12±0.20 | 4.74±0.21 | 0.086±0.009 | 0.029±0.002 | 0.0042±0.0002 | 0.0054±0.0002 |
| Tree 3 Purplish-red | 2014 n=4 | 1.56±0.15 | 4.20±0.41 | 3.12±0.28 | 0.070±0.004 | 0.010±0.003 | 0.0041±0.001 | n.d. |
|                | Purple | 2013 n=5 | 3.23±0.23 | 4.33±0.13 | 4.03±0.24 | 0.122±0.006 | 0.019±0.004 | 0.0053±0.0003 | 0.0048±0.0002 |
| No. 1083 | Red | 2014 n=4 | 1.58±0.11 | 5.20±0.46 | 2.63±0.11 | 0.121±0.005 | 0.013±0.004 | 0.0036±0.0004 | n.d. |
| No. 1088 | Red | 2015 n=4 | 1.02±0.04 | 5.81±0.25 | 4.60±0.32 | 0.184±0.007 | 0.023±0.003 | 0.0074±0.0002 | 0.0126±0.0015 |

The averages ± SE are indicated. “n.d.” means “not detected”. Values with different letters are significantly different according to Tukey’s test at \( P<0.05 \).

### Table 4. The molar ratios of Al content (Table 3) to total anthocyanin content (Table 2).

| Flower color | No. 1083 | No. 1088 |
|--------------|----------|----------|
| ‘Sennen-fujimurasaki’ | Tree 1 | Tree 2 | Tree 3 |
| Year | Red | Red | Red | Purplish-red | Purple | Red | Red |
| 2013 | 0.1 | 0.2 | 0.2 | 1.0 | 3.6 | 0.9 | 0.6 |
| 2014 | 0.1 | 0.2 | 0.2 | 1.0 | 3.6 | 0.9 | 0.6 |
| 2015 | 0.1 | 0.2 | 0.2 | 1.0 | 3.6 | 0.9 | 0.6 |

To elucidate the cause of the rare purple flower coloration found in C. japonica, we investigated flowers with different color tones from red to purple of the C. japonica cultivar ‘Sennen-fujimurasaki’ (Fig. 1; Table 1). In red flowers, the petal tissues were uniformly colored and the epidermal cells were composed of only red colored cells, and contained no granules (Figs. 1a and 2a). In purplish-red flowers, both red and purple portions existed, most notably the purple color surrounded the vascular tissues (Fig. 1b, c). Microscopically, both red and purple epidermal cells were observed, and blue-black granules were observed in many of the purple cells (Fig. 2b). In purple flowers, many more epidermal cells were purple, and blue-black granules were also observed in many of the cells (Fig. 2c). These observations indicated that the purple color of ‘Sennen-fujimurasaki’ did not occur by uniformly transforming all of the epidermal cells to become purple, but by increasing the frequency of purple cells as a result of the cells acquiring some factor/component from the vascular tissues.

No difference in the anthocyanin components was found among the red, purplish-red, and purple petals of ‘Sennen-fujimurasaki’.
‘Sennen-fujimurasaki’, and these components were the same as those detected in *C. japonica* No. 1083 and No. 1088. Slight differences in the concentration of each component and the total anthocyanin content were found among each sample of ‘Sennen-fujimurasaki’, but these differences did not correspond to the difference in petal color (Table 2). Compounds such as flavones, flavonols, and cinnamic acid derivatives were detected negligibly in each sample of ‘Sennen-fujimurasaki’ by HPLC, suggesting that the purple coloration was not caused by co-pigmentation due to these compounds. Differences in the pH among the petal homogenates of ‘Sennen-fujimurasaki’ were too small to regard pH as the cause for the color changes (Table 1).

A significant difference was found in the Al ion content among the red, purplish-red, and purple flowers of ‘Sennen-fujimurasaki’ (Table 3). The Al ion content of the purplish-red petals was 4 to 10 times higher than that of the red petals, and the Al ion content of the purple petals was 14 to 21 times higher than that of the red petals. In tree 3 of ‘Sennen-fujimurasaki’, the flower color was purple in 2013 (Fig. 1d) and purplish-red in 2014 (Fig. 1c). Corresponding to the flower color difference, the Al amounts decreased from 3.23 to 1.56 μmol·g$^{-1}$ FW (Table 3). The Ca, Mg, Mn, Fe, Cu, and Zn ion contents were similar among the red, purplish-red, and purple flowers, or the differences did not correspond to the color of ‘Sennen-fujimurasaki’ (Table 3).

We investigated the effect of Al ions on the coloration of anthocyanins using solutions of commercial cyanidin 3-glucoside. Anthocyanins exist in the vacuoles of flower cells, cellular compartments that are usually weakly acidic or neutral, but the simple structure anthocyanins rapidly lose their color under weakly acidic or neutral conditions *in vitro* (Cabrita et al., 2000; Goto and Kondo, 1991). The color intensity of cyanidin 3-glucoside decreases to its weakest point around pH 5 when monitored between pH 1.0 to 10.6 (Cabrita et al., 2000). The mechanism that preserves the red color of anthocyanins in *C. japonica* remains a mystery. Therefore, it would have been difficult to reproduce correctly the exact color of ‘Sennen-fujimurasaki’ *in vitro*. As we expected, the color intensity of the cyanidin 3-glucoside solution decreased immediately after being dissolved in 0.1 M AcOH buffer at pH 4.8, the same pH as the pur-
ple petal homogenate of ‘Sennen-fujimurasaki’. However, we first verified that the solution had a pale red color with no precipitates when Al ions were absent (Fig. 4). Next, the color of the solution became more purple with increasing amounts of Al ions (Fig. 4a), and the absorption spectra had bathochromic shifts as the absorption shifted to longer wavelengths (Fig. 4b). Moreover, blue-black precipitates resulting from the chelation of Al ions by cyanidin 3-glucoside that were similar to the granules observed in many of the purple cells of ‘Sennen-fujimurasaki’ were also observed (Fig. 4a). The observation that the purple cells spread out from the veins could be interpreted as the cell color change being induced by Al ions probably supplied from the vascular bundles. The blue-black granules in the purple cells were likely the products of anthocyanin chelation of Al ions. These results strongly suggest that Al ions are involved in the production of purple flowers in ‘Sennen-fujimurasaki’.

There were some discrepancies between the absorption spectra of the cyanidin 3-glucoside solutions to which Al was added and the absorption spectra of ‘Sennen-fujimurasaki’ petals. In the Al-cyanidin 3-glucoside solution experiment, only when the molar ratio of Al ions to anthocyanin was 0.1 did the solution produce precipitates (Fig. 4a). When the amount of Al was greater than an equal molar ratio to the anthocyanin concentration, the solution spectra showed hyperchromic shifts, whereas the absorption maximum remained around 555 nm (Fig. 4b). On the other hand, in the red petals of ‘Sennen-fujimurasaki’, the ratio of the Al content to the anthocyanin content was 0.1 (Table 4), but granules were not observed (Fig. 2a). The absorption maximum of the purplish-red petals that contained a molar ratio of Al content to the total anthocyanin content of 1.0 was around 529 nm, a value that was almost the same as the absorption maximum of the red petals (Fig. 3; Table 4). The molar ratio of the Al content to the total anthocyanin content in the purple petals was as much as 3.6, but the absorption maximum was around 535 nm (Fig. 3). One possibility to explain these discrepancies may be that because various organic acids in vacuoles compete with anthocyanins, larger numbers of Al ions may be required to produce the purple color than the experimental values. Organic acids such as citric acid readily form complexes with Al ions (Hue et al., 1986; Ma, 2000). Another possibility is that only a small fraction of Al ions present in petals are localized in the vacuoles of epidermal cells. Al is mainly localized in the cell walls of epidermal cells in C. sinensis leaves, a strategy proposed to be a mechanism for tolerating Al toxicity (Tolra et al., 2011). Moreover, the absorption spectra recorded for purplish-red and purple petals should reflect a mixture of red and purple cells possessing varying levels of Al ions in the cells. Therefore, the bathochromic shifts of the absorption maxima recorded for the purplish-red and purple petals were probably attenuated by co-occurrence of the red and purple color cells; and instead, the increases in the purple colored cells ratios were reflected by increases in absorbance at the longer wavelength region over 560 nm (Fig. 3). Integrating these ideas, we conclude that the production of purple flowers in ‘Sennen-fujimurasaki’ results from the chelation of Al ions by anthocyanins. The cause of the differences in flower colors in ‘Sennen-fujimurasaki’ is derived from differences in the amount of Al accumulated in the flowers. Instability of flower coloration of ‘Sennen-fujimurasaki’ is also interpreted as being derived from this cause.

Even though similar amounts of Al ions were present in the petals of No. 1083, No. 1088 and the purplish-red petals of ‘Sennen-fujimurasaki’ (Tables 3 and 4), the petals of No. 1083 and No. 1088 were red (Fig. 1; Table 1). ‘Sennen-fujimurasaki’ may contain lower amounts of other compounds that can chelate Al, such as organic acids in the vacuoles, and/or it may have acquired the ability to accumulate high levels of Al ions in flowers and the capacity to transport more Al ions into vacuoles compared with typical red-flowered wild C. japonica.

Metal ion-anthocyanin complexes have been found to cause the blue flower coloration in Hydrangea macrophylla, Commelina communis, Centaurea cyanus, Salvia patens (Takeda, 2006; Yoshida et al., 2009), Meconopsis grandis (Yoshida et al., 2006), and Tulipa gesneriana (Shoji et al., 2007). In these flowers, flavones, flavonols, or cinnamic acid derivatives are also involved in the metal-complex pigments. Since negligible amounts of flavones, flavonols, and cinnamic acid derivatives were detected in the petals of ‘Sennen-fujimurasaki’, these compounds seem not to be involved in the Al-anthocyanin complex in ‘Sennen-fujimurasaki’, unlike the above flowers. The presence of the blue-black granules in many of the purple-colored epidermal cells may also support the concept that a simple Al-anthocyanin complex is formed in ‘Sennen-fujimurasaki’ since it was demonstrated that similar blue-black precipitates occurred by mixing of only Al ions and cyanidin 3-glucoside in vitro.

Pigment aggregates, described as “anthocyanic vacuolar inclusions (AVIs)” and “blue spherules”, have been found to affect flower color. They were reported to be involved in expression of bluer and/or dusky flower colors in carnations (Markham et al., 2000; Okamura et al., 2013), Japanese morning glory (Morita et al., 2005), and roses with a bluing phenomenon (Yasuda, 1970), so the blue-black granules in ‘Sennen-fujimurasaki’ may also have such effects on purple flower coloration.

We have reported that chelation of Al ions with flavonols causes a deep yellow coloration of flowers in another camellia species, C. chrysantha (Tanikawa et al., 2008). Both anthocyanins and flavonols are types of flavonoids. Our studies indicate that chelation of Al by flavonoids leads to these unique flower colorations
in *Camellia* spp. In other purple-flowered *C. japonica* with unstable coloration similar to ‘Sennen-fujimurasaki’, Al-anthocyanin chelation is likely responsible for their purple flower color.

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