Analyses of Coloration-related Components in *Hydrangea* Sepals Causing Color Variability According to Soil Conditions

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In many *Hydrangea* cultivars, sepal color depends on soil conditions. The traditional concept is that different levels of absorption of aluminum ions from soil and its accumulation in sepal vacuoles changes *Hydrangea* sepal color. To investigate how sepal coloration can be stabilized, we examined the components that may contribute to color variability according to the traditional concept. Using 10 cultivars and lines with sepals of stable red or stable blue color plants or with sepals of variable color (red or purple) plants grown in acid soils and alkaline soils, we analyzed sepal pH and sepal contents of anthocyanin, aluminum ion, 5-O-caffeoylquinic acid, and 3-O-caffeoylquinic acid. Sepals of all cultivars became bluer when plants were grown in acid soil than when they were grown in alkaline soil, even if the change in stable color plants was milder than that of variable color plants. The same component changes probably happen in sepals of both stable and variable color plants in response to different soil conditions to cause the coloration change. When the two soil conditions were compared, a statistically significant difference was detected for delphinidin 3-glucoside, which is a major anthocyanin of *Hydrangea*, in the variable-color line ‘HH2’ and for 3-O-caffeoylquinic acid in the stable red line ‘HH19’, but not for any other compound examined, including aluminum ions. Although there is possibility that localization of aluminum ions in vacuoles of the colored cells changes, it is assumed that changes in contents of aluminum ion chelaters such as phosphoric acid affect the sepal color change in response to different soil conditions, as well as the coloration stability or variability. When cultivars were compared in terms of properties of sepal coloration, although contents of aluminum ions and 5-O-caffeoylquinic acid tended to be higher in stable blue cultivars than in other cultivars, these differences were not statistically significant. In agreement with previous reports, our data indicate that a lower content of 3-O-caffeoylquinic acid is essential for blue *Hydrangea* sepals.

Key Words: aluminum, delphinidin 3-glucoside, 3-O-caffeoylquinic acid, 5-O-caffeoylquinic acid.

Introduction

Typical sepal colors of *Hydrangea* cultivars are red, purple, and blue. The sepal color of many *Hydrangea* cultivars depends on cultivation conditions (Allen, 1943). Cultivation in soil with lower pH, which contains more soluble aluminum ions than soil with higher pH, results in generation of bluer sepals (Allen, 1943; Chenery, 1937). Based on these studies, it was deduced that the differences in the absorption levels of aluminum ions from soil and its accumulation in the sepal vacuoles cause the changes in *Hydrangea* sepal color (Allen, 1932, 1943).

In *Hydrangea* cultivars, the same anthocyanin, delphinidin 3-glucoside, is responsible for sepal colors of red, purple, and blue (Asen and Siegelman, 1957; Asen et al., 1956; Hayashi and Abe, 1953; Lawrence et al., 1938). Aluminum ions, 5-O-caffeoylquinic acid, and 3-O-caffeoylquinic acid interact with this anthocyanin and together with pH affect the color of delphinidin 3-glucoside solution (Kondo et al., 2005; Takeda et al., 1985a, b, 1990). At pH 3 or lower, delphinidin 3-glucoside solution is red; at pH 4 or higher, delphinidin 3-glucoside forms a stable blue-colored complex with aluminum ions and 5-O-caffeoylquinic acid (Ito et al., 2009; Kondo et al., 2005; Yoshida et al., 2003).
Because 3-O-caffeoylquinic acid interferes with the formation of this complex, its addition shifts the color to purple (Ito et al., 2009; Kondo et al., 2005; Takeda et al., 1985a, b, 1990; Yoshida et al., 2003). Based on analyses of sepal tissue or protoplast prepared from sepal cells, the same mechanism is thought to operate in the vacuoles of sepal cells, causing Hydrangea sepal color variation (Ito et al., 2009; Takeda et al., 1985a; Yoshida et al., 2003, 2009).

Stable sepal color is necessary for commercial production of Hydrangea plants (Hazu and Matsuda, 1972; Kodama et al., 2015; Matsuda et al., 1974; Nagamura et al., 1981; Nanzyo et al., 1976). Sepal color stabilization has been attempted by controlling soil composition and acidity, and by application of aluminum ions or phosphate fertilizer for aluminum insolubilization (Hazu and Matsuda, 1972; Matsuda et al., 1974; Nagamura et al., 1981; Nanzyo et al., 1976). The effect of soil conditions on the sepal color differs among cultivars (Allen, 1943; Hazu and Matsuda, 1972; Matsuda et al., 1974) and a more general and efficient technique to control sepal color is required.

Some cultivars have stable red or blue sepals regardless of soil conditions, which is an important characteristic for commercial cultivars. We tried to identify factors that affect the coloration stability or variability. In this study, we compared the contents of coloration-related compounds in red and blue sepals of stable color cultivars and red and purple sepals of variable color cultivars grown in acid soils and alkaline soils. Analyses by using protoplasts prepared from Hydrangea sepals were performed (Ito et al., 2009). These data are likely to be more precise than those obtained from analyses of whole sepals. However, considering the benefit of simple and effortless performance, we analyzed whole sepals to allow comparison of many cultivars in this study. Contrary to expectations, we found no association between aluminum ion content and sepal color change in response to differences in soil acidity.

**Materials and Methods**

**Plant materials**

Four cultivars (‘Ruby red’, ‘Blue sky’, ‘Frau Yoshiko’, and ‘Frau Yoshimi’) and six lines (‘HH2’, ‘HH9’, ‘HH11’, ‘HH12’, ‘HH13’, and ‘HH19’) were used. The cuttings were planted in June 2013, transplanted into 7.5 cm pots filled with soil (see Table 1 for soil composition) in July 2013, and grown in a glasshouse. They were retransplanted into 12 cm pots filled with alkaline or acid soil (Table 1) in February 2014. Liquid fertilizers used are listed in Table 1. Aluminum sulfate solution was injected into the acid soil (Table 1) in February 2014. Sepals were collected in May 2014.

**Measurement of sepal chromatic values, absorption spectra, and microscopy**

Chromatic values (L*, a*, b*, and h) were measured with a colorimeter (CR-200; Konica Minolta Co. Ltd., Tokyo, Japan). Visible absorption spectra from 400 nm to 700 nm were measured with a spectrophotometer (UV2450; Shimadzu Co. Ltd., Kyoto, Japan) attaching an integrating sphere (ISR-2200; Shimadzu Co. Ltd.), which is a device to measure absorption spectra of solid samples using a standard white board as a reference. Images of fresh sepals were taken under a microscope equipped with a CCD camera (VH-8000; Keyence Co. Ltd., Osaka, Japan).

**Measurement of sepal pH**

Four sepals were homogenized with no additions in a

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**Table 1. Components of alkaline and acid soils and fertilizers for cultivation of Hydrangea.**

<table>
<thead>
<tr>
<th>Soil composition (v/v)</th>
<th>Alkaline soil</th>
<th>Acid soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akadama soil</td>
<td>35%</td>
<td>Akadama soil</td>
</tr>
<tr>
<td>PRO-MIX BX*</td>
<td>35%</td>
<td>Peatmoss</td>
</tr>
<tr>
<td>Leaf mold</td>
<td>20%</td>
<td>Leaf mold</td>
</tr>
<tr>
<td>Pearlite</td>
<td>10%</td>
<td>Chaff mold</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pre-planting fertilizer</th>
<th>Magnesium multi-phosphate</th>
<th>3 g·L⁻¹</th>
<th>Potassium sulfate</th>
<th>2 g·L⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fused phosphate</td>
<td>2 g·L⁻¹</td>
<td>Fused phosphate</td>
<td>1 g·L⁻¹</td>
<td></td>
</tr>
<tr>
<td>Badguano</td>
<td>1 g·L⁻¹</td>
<td>Superphosphate</td>
<td>0.5 g·L⁻¹</td>
<td></td>
</tr>
<tr>
<td>Maganp II*</td>
<td>1 g·L⁻¹</td>
<td>NK ECOLONG 2038-100*</td>
<td>2 g·L⁻¹</td>
<td></td>
</tr>
<tr>
<td>LONG 413-70*</td>
<td>2 g·L⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| pH*                    | 6.5          | 5.5       |
| Fertilizer*            | Peters 15-30-15, diluted 1500x | Peters 20-10-20, diluted 2500x |

* PRO-MIX BX (Peatmoss mix) is produced by Premier Tech Ltd., Rivière-du-Loup QC Canada; Maganp II is produced by HYPONEX JAPAN Co. Ltd., Osaka, Japan; LONG 413-70 and NK ECOLONG are produced by JCAM AGRI Co. Ltd., Tokyo, Japan.

* pH was measured in soil mixed with distilled water (1:10); the mixture was shaken for 30 min before the measurement.

* Each fertilizer was applied twice a week.

* Aluminum sulfate solution (0.2%, 300 mL) was injected 10, 20, and 30 days after planting.
HPLC analysis of sepal components

Fresh sepals were extracted overnight at room temperature with 10% acetic acid, with a volume ratio of 2 mL per 700 mg fresh weight, and then extracted again for 3 h with 10% acetic acid with a volume ratio of 1 mL per 700 mg fresh weight. The pigment composition of the extracts was analyzed using an HPLC system (Agilent 1100 system combined with the pumps and a photodiode array detector of the Agilent 1200 system; Agilent Technologies, Santa Clara, CA, USA) and an Inertsil ODS-2 column (4.6 mm × 250 mm; GL Sciences Co. Ltd., Tokyo, Japan) at 40°C and a flow rate of 0.8 mL·min⁻¹. A linear gradient of 20%–100% solvent B (1.5% phosphoric acid, 20% acetic acid, 25% acetonitrile) in solvent A (1.5% phosphoric acid) was run over 40 min. Anthocyanins, 3-O-caffeoylquinic acid, and 5-O-caffeoylquinic acid were identified based on their absorption spectra and retention times by comparison with their standards (Funakoshi Co. Ltd., Tokyo, Japan) and quantified using absorbance at 530 nm for anthocyanins or 330 nm for 3-O-caffeoylquinic acid and 5-O-caffeoylquinic acid.

Aluminum analysis

Fresh sepals were dried for 12 h at 85°C; 2 mL of 60% HNO₃ was added to the sample (50 mg dry weight) and the sample was heated for 10 min in a microwave oven. After cooling, the solution was diluted to 10 mL with 1 N HNO₃ and aluminum concentration was measured with an inductively coupled plasma–atomic emission spectrometer (820-MS; Varian Co. Ltd., Walnut Creek, CA, USA).

Results

Sepal color

Sepals of ‘Ruby Red’, ‘HH9’, ‘HH13’, and ‘HH19’ were red regardless of whether the plants were grown in alkaline or acid soil (Table 2); the hue angles of sepals of these cultivars were 336.8°–359.5°, i.e. in the red area. For sepals of each cultivar except the line ‘HH19’ grown in acid soil, the a* value was 3–14 lower and the b* value was 4–11 lower than for sepals of the same cultivar grown in alkaline soil, resulting in a decrease of 4°–11°, which represents a change towards a blue color.

Sepals of ‘Blue Sky’, ‘HH11’, and ‘HH12’ were blue regardless of whether the plants were grown in alkaline or acid soil; the hue angles were 288.0°–303.7°, i.e. in the blue area. For sepals of each cultivar grown in acid soil, the a* value and hue angle were similar, but the b* value was 2–6 lower, in comparison with sepals of the same cultivar grown in alkaline soil.

Sepals of ‘Frau Yoshiko’, ‘Frau Yoshimi’, and ‘HH2’ were red with hue angles of 344.7°–357.2° when plants were grown in alkaline soil and their sepals were purple with hue angles of 307.2°–314.7° when plants were grown in acid soil. In acid soil, the a* value was 13–18 lower and b* value 14–18 lower than in alkaline soil, resulting in a decrease of 30°–45° in the hue angle, which represents a change towards a blue color.

Sepals of all cultivars were red regardless of soil conditions. Representative images of sepal cells for each cultivar group are shown in Figure 1.

Sepal absorption spectra

The absorption spectrum of the sepals of the stable red line ‘HH13’ showed λ_max at 535 nm when plants were grown in alkaline soil, and at 544 nm when they were grown in acid soil. A decrease in absorbance at 400–600 nm and an increase at 600–700 nm were observed in the sepals of plants grown in acid soil in comparison with those of plants grown in alkaline soil (Fig. 2). The absorption spectrum of the sepals of the stable blue line ‘HH11’ showed λ_max at 582 nm when plants were grown in alkaline soil, and at 584 nm when they were grown in acid soil. The absorption spectrum of the sepals of the variable-color cultivar ‘Frau Yoshiko’ showed λ_max at 543 nm when plants were grown in alkaline soil, and at 576 nm when plants were grown in acid soil. A strong increase in absorbance at 580–700 nm was observed when plants were grown in acid soil in comparison with those grown in alkaline soil. Representative absorption spectra of sepals of each cultivar group are shown in Figure 2.

Sepal pH

Regardless of the culture soil conditions, pH of sepal homogenates was 3.9–4.3 for stable red cultivars, 4.0–4.5 for stable blue cultivars, and 4.1–4.3 for variable-color cultivars (Table 2). There were no significant differences in pH values between different cultivar groups. There were also no significant differences in sepal pH between plants grown in acid soil and those grown in alkaline soil for any cultivar. Sepal pH was not related to sepal color, or to color stability or variability.

Contents of anthocyanin, 5-O-caffeoylquinic acid, and 3-O-caffeoylquinic acid

Delphinidin 3-glucoside was the major sepal pigment in each cultivar (data not shown). Regardless of soil conditions, anthocyanin content (in μmol g⁻¹ FW) was 0.16–0.59 in stable red cultivars, 0.08–0.35 in stable blue cultivars, and 0.25–0.74 in variable-color cultivars (Fig. 3A). There were no significant differences in anthocyanin content between plants grown in acid soil and those grown in alkaline soil for any cultivar except the variable-color line ‘HH2’, which was higher in plants grown in acid soil than in plants grown in alkaline soil. Anthocyanin content was not related to sepal color, or to color stability or variability.

Regardless of soil conditions, the content of 5-O-
Table 2. Images, chromatic values, and pH of the sepals of *Hydrangea* grown in alkaline and acid soils.

<table>
<thead>
<tr>
<th></th>
<th>Ruby Red HH9</th>
<th></th>
<th>HH13</th>
<th></th>
<th>HH19</th>
<th></th>
<th>Blue Sky HH11</th>
<th></th>
<th>HH12</th>
<th></th>
<th>Frau Yoshiko</th>
<th></th>
<th>Frau Yoshimi</th>
<th></th>
<th>HH2</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Sepal color</td>
<td><img src="ruby-red.png" alt="Image" /></td>
<td><img src="ruby-red.png" alt="Image" /></td>
<td><img src="ruby-red.png" alt="Image" /></td>
<td><img src="ruby-red.png" alt="Image" /></td>
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<td><img src="ruby-red.png" alt="Image" /></td>
<td></td>
</tr>
<tr>
<td>L*</td>
<td>50.0 ± 3.7</td>
<td>37.2 ± 3.1</td>
<td>39.6 ± 2.7</td>
<td>36.6 ± 1.4</td>
<td>46.3 ± 2.3</td>
<td>44.1 ± 2.3</td>
<td>49.5 ± 1.7</td>
<td>44.7 ± 1.4</td>
<td>58.9 ± 0.8</td>
<td>56.1 ± 0.3</td>
<td>63.5 ± 2.9</td>
<td>50.7 ± 1.7</td>
<td>44.5 ± 1.0</td>
<td>30.3 ± 0.6</td>
<td>52.2 ± 4.8</td>
<td>47.6 ± 0.6</td>
</tr>
<tr>
<td>a*</td>
<td>42.6 ± 1.7</td>
<td>39.0 ± 1.0</td>
<td>41.4 ± 1.3</td>
<td>35.1 ± 3.3</td>
<td>38.5 ± 2.3</td>
<td>35.8 ± 1.8</td>
<td>11.5 ± 0.7</td>
<td>15.2 ± 1.7</td>
<td>9.3 ± 0.1</td>
<td>11.4 ± 0.7</td>
<td>36.0 ± 3.3</td>
<td>22.7 ± 1.0</td>
<td>46.4 ± 1.2</td>
<td>28.0 ± 0.1</td>
<td>37.5 ± 2.8</td>
<td>27.0 ± 2.0</td>
</tr>
<tr>
<td>b*</td>
<td>−1.5 ± 2.5</td>
<td>−8.9 ± 1.1</td>
<td>−10.1 ± 1.5</td>
<td>−15.1 ± 1.8</td>
<td>−0.8 ± 0.9</td>
<td>−14.1 ± 1.4</td>
<td>−35.1 ± 0.3</td>
<td>−37.5 ± 0.5</td>
<td>−28.5 ± 0.8</td>
<td>−32.7 ± 0.4</td>
<td>−5.2 ± 0.6</td>
<td>−29.7 ± 1.5</td>
<td>−2.3 ± 0.8</td>
<td>−30.9 ± 2.3</td>
<td>−10.2 ± 0.6</td>
<td>−27.3 ± 1.9</td>
</tr>
<tr>
<td>Hue-angle (°)</td>
<td>358.0</td>
<td>347.2</td>
<td>346.3</td>
<td>336.8</td>
<td>359.5</td>
<td>338.5</td>
<td>288.1</td>
<td>292.1</td>
<td>288.0</td>
<td>289.2</td>
<td>351.7</td>
<td>307.2</td>
<td>357.2</td>
<td>312.2</td>
<td>344.7</td>
<td>314.7</td>
</tr>
<tr>
<td>pH</td>
<td>4.0 ± 0.1</td>
<td>4.0 ± 0.1</td>
<td>3.9 ± 0.1</td>
<td>4.4 ± 0.1</td>
<td>4.0 ± 0.1</td>
<td>4.1 ± 0.1</td>
<td>4.2 ± 0.0</td>
<td>4.3 ± 0.1</td>
<td>4.5 ± 0.1</td>
<td>4.3 ± 0.1</td>
<td>4.1 ± 0.1</td>
<td>4.3 ± 0.1</td>
<td>4.1 ± 0.1</td>
<td>4.1 ± 0.1</td>
<td>4.1 ± 0.1</td>
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caffeoylquinic acid (in μmol·g⁻¹ FW) was 1.0–4.0 in stable red cultivars, 2.5–7.0 in stable blue cultivars, and 1.5–3.0 in variable-color cultivars (Fig. 3B), and that of 3-O-caffeoylquinic acid (in μmol·g⁻¹ FW) was 25.0–38.0 in stable red cultivars, 8.0–11.0 in stable blue cultivars, and 22.0–38.0 in variable-color cultivars (Fig. 3C). There were no significant differences in the contents of 5-O-caffeoylquinic acid and 3-O-caffeoylquinic acid between plants grown in acid soil and those grown in alkaline soil for any cultivar except for the content of 3-O-caffeoylquinic acid in the stable red line ‘HH19’, which was higher in plants grown in acid soil than in plants grown in alkaline soil. In stable blue cultivars, the 3-O-caffeoylquinic acid content was obviously lower than in other cultivars. Although the 5-O-caffeoylquinic acid content tended to be higher in stable blue cultivars than in the other cultivars, the difference was not statistically significant. Neither 3-O-caffeoylquinic acid nor 5-O-caffeoylquinic acid content was related to color stability or variability.

Aluminum content
Regardless of soil conditions, the aluminum content (in μmol·g⁻¹ FW) was 7.0–13.0 in stable red cultivars, 8.0–20.0 in stable blue cultivars, and 8.0–10.0 in variable-color cultivars (Fig. 3D). There were no significant differences between plants grown in acid soil and those grown in alkaline soil. Although aluminum content tended to be higher in stable blue cultivars than in the other cultivars, the difference was not statistically significant. Aluminum content was not related to color stability or variability.

Discussion
At the start of this study, we confirmed that the soil conditions we used affected coloration of sepals of variable color cultivars. Sepal hue angles or b* values were lower in plants grown in acid soil than in plants grown in alkaline soil not only in variable-color cultivars but also in stable red and blue cultivars (Table 2). Shifts toward longer wavelengths of λmax in the absorption spectra were observed not only in variable-color cultivars, but also in stable red and blue cultivars (Fig. 2). Sepals of all cultivars analyzed in this study became bluer in plants grown in acid soil than in those grown in alkaline soil, even if the changes in stable color plants.
were milder than those of variable color plants; this seems a general feature of Hydrangea cultivars. We think that the same component changes which are concerned with the coloration change happen in sepals of both stable and variable color plants.

Because the sepals of all cultivars we studied were composed of nearly uniform coloration cells, we hoped that analysis of whole sepal tissue could reveal the

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**Fig. 3.** Contents of coloration-related components in *Hydrangea* sepals. (A) Delphinidin 3-glucoside, (B) 5-O-caffeoylquinic acid, (C) 3-O-caffeoylquinic acid, (D) Aluminum. White bars, stable red cultivars; black bars, stable blue cultivars; hatched bars, variable-color cultivars. For each cultivar, data for plants cultivated in alkaline and acid soil are shown. Values are the means ± SD (n = 3); different letters indicate significant differences (P < 0.01 by Tukey’s test).
mechanisms that underlie the differences in sepal color among Hydrangea cultivars. On the basis of a previous study (Ito et al., 2009; Takeda et al., 1985a; Yoshida et al., 2003, 2009), we selected pH, anthocyanins, 5-O-caffeoylquinic acid, 3-O-cafeoylquinic acid, and aluminum as factors that may affect sepal color, and analyzed their value or contents in sepals. No significant differences in the pH values or the content of any of these compounds were detected in any cultivar between plants grown in acid soil and those grown in alkaline soil; the only exceptions were the anthocyanin content in the variable-color line ‘HH2’ and 3-O-cafeoylquinic acid in the stable red line ‘HH19’ (Table 2; Fig. 3).

It is generally believed that the changes in Hydrangea sepal color under different soil conditions are caused by different levels of aluminum solubility in soil, which affects aluminum ion absorption by roots and accumulation in sepals (Allen, 1932, 1943; Okada and Funaki, 1967). The link with the aluminum ion content and the sepal coloration was supported by chemical and organic chemical studies (Ito et al., 2009; Takeda et al., 1985a; Yoshida et al., 2003, 2009). However, Nanzyo et al. (1976) reported that sepal aluminum content did not increase even when the sepal color of Hydrangea ‘Flambeau’ changed from red to grayish-blue because of decreased soil pH and an increased amount of aluminum sulfate in soil. Using 10 cultivars and lines of different color and color stability, we obtained results similar to those of Nanzyo et al. (1976) (Fig. 3D), where the aluminum content in whole sepal tissue was analyzed by atomic absorption spectrometry and the color of representative sepal was mentioned. Thus, these findings may be relevant to many Hydrangea cultivars. We think that localization of aluminum ion in vacuoles of the colored cells needs to be studied in order to clarify its contribution to the sepal coloration change in response to different soil conditions. Additionally, we assume that factors other than aluminum ion absorption and accumulation affect the sepal color change and also cause the coloration stability or variability of each cultivar.

Aluminum ions can be chelated by various compounds such as citric acid and phosphoric acid (Ma et al., 1997; Pierre and Stuart, 1933), the contents of which may be affected by soil conditions. Therefore, these compounds change the availability of aluminum ions to be chelated by anthocyanin. Okada and Okawa (1974) assumed that the concentration of soluble phosphoric acid is higher in alkaline soil, and the absorbed phosphoric acid insolubilizes aluminum ions in cells. Nanzyo et al. (1976) reported that application of phosphoric acid at the flower budding stage resulted in red sepals. Furthermore, we obtained preliminary data suggesting that contents of not aluminum ions, but rather phosphoric acid, are linked to the sepal coloration in another study analyzing whole sepals (Kodama and Tanabe, unpublished results). In a future study, we intend to analyze the phosphoric acid and aluminum ion contents in protoplasts to clarify their localization in the vacuoles of sepal colored cells to clarify the coloration mechanism of Hydrangea sepals.

Of all compounds examined, only the content of 3-O-cafeoylquinic acid was lower in stable blue cultivars than in other cultivars (Fig. 3C). The pH and the contents of aluminum ions and 5-O-cafeoylquinic acid were not significantly different, but tended to be higher in stable blue cultivars than in other cultivars (Table 2; Fig. 3A, B, D). Yoshida et al. (2003) reported that vacuolar pH is higher in blue sepals than in red sepals, while they also reported that the press-juice pH of blue and red sepals was around 4 (Toyama-Kato et al., 2003). Since homogenate pH may not correctly reflect vacuolar pH, further studies are necessary to clarify any role of vacuolar pH and the difference in sepal coloration. Takeda et al. (1985a) reported that sepals of blue cultivars contain more aluminum and 5-O-cafeoylquinic acid, and less 3-O-cafeoylquinic acid than those of red cultivars, whereas anthocyanin contents are similar in blue and red cultivars. Ito et al. (2009) compared protoplasts prepared from sepals of one blue and one red cultivar; they found that the aluminum ion content of blue cells was higher than that of red cells. As mentioned above, in our study, only 3-O-cafeoylquinic acid contents showed a significant difference (Fig. 3C). The difference in contents of aluminum ions and 5-O-cafeoylquinic acid could also be observed in some pairwise comparisons between cultivars by whole sepal analyses (Fig. 3B, D).

Takeda (2013) reported that 3-O-cafeoylquinic acid competitively inhibits the interaction of 5-O-cafeoylquinic acid with anthocyanins and aluminum ions; therefore, the content of 3-O-cafeoylquinic acid needs to be low for Hydrangea sepals to be blue. Sepals of the variable-color cultivars analyzed in this study were purple and not blue, even if the plants were grown in acid soil (Table 2). Therefore, a lower level of 3-O-cafeoylquinic acid is probably essential for the blue color in Hydrangea cultivars. Relatively high contents of aluminum ions and 5-O-cafeoylquinic acid favor blue sepals, and cultivars possessing these properties may be selected during breeding of blue cultivars.

Acknowledgements

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