Postprint: The Relationship Between Flower Color and Key Intracellular Environmental Factors in Camellia sect. Chrysantha

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Abstract

To investigate the relationship between flower color and important intracellular environmental factors in Camellia chrysantha group plants, petal color, total flavonoid content, water content, cell pH, and concentrations of 7 metal ions were measured in 9 populations of 8 Camellia chrysantha group species with different flower colors. The results showed that the average flower color parameters of the measured Camellia chrysantha group plants were: lightness L = 80.82, hue a* = -2.88, hue b* = 53.97, chroma C = 54.10, and hue angle h = 93.19, indicating that the flower color is bright yellow with high lightness. Among these parameters, hue b* is the primary indicator for describing yellowness, based on which the measured plants could be classified into three categories: golden yellow, yellow, and light yellow. The petal total flavonoid content was 20.17%, and petal water content was 88.14%, both showing significant differences among species and both exhibiting weak correlations with flower color, indicating minor influence on yellow color expression. Petal cells were weakly acidic, with an average pH of 6.19, showing significant differences among species. Cell pH was significantly positively correlated with flower color, suggesting that a weakly acidic to neutral intracellular environment is conducive to yellow color expression in Camellia chrysantha petals. Among metal ion concentrations, K+ content was the highest (12.61 mg · g ^{1}), followed by Ca2+ (3.91 mg · g ^{1}), Mg2+ (1.28 mg · g ^{1}), Al3+ (0.98 mg · g ^{1}), Na+ (0.17 mg · g ^{1}), Fe3+ (0.07 mg · g ^{1}), and Cu2+ content was the lowest (0.0038 mg · g ^{1}). All 7 metal ions showed significant differences among the measured plants. Among them, Al3+, Fe3+, and Ca2+ exerted varying degrees of interference on yellow flower color formation in Camellia chrysantha, with yellowness decreasing and flower color becoming lighter as concentrations of these three metal ions increased. Therefore, lower concentrations of Al3+, Fe3+, and Ca2+ may be more conducive to yellow flower expression in Camellia chrysantha.

Full Text

Preamble

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Title: Relationship between Flower Color and Important Cellular Environmental Factors in Yellow Camellia

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Abstract

To investigate the relationship between flower color and intracellular environmental factors in yellow camellia, we examined nine populations from eight species of *Camellia* sect. *Chrysantha* with varying yellow hues. Measurements included petal color parameters, total flavonoid content, water content, cellular pH, and concentrations of seven metal ions. The results showed that the average color parameters of the studied yellow camellias were: lightness L* = 80.82, hue a* = -2.88, hue b* = 53.97, chroma C* = 54.10, and hue angle h = 93.19°, indicating a bright yellow coloration. Among these, hue b* served as the primary indicator for yellow coloration, enabling classification into three categories: golden yellow, yellow, and light yellow. Total flavonoid content averaged 20.17% (dry weight), while water content averaged 88.14%, both showing significant interspecific variation but only weak correlation with flower color, suggesting minimal influence on yellow expression. Petal cells exhibited weak acidity with an average pH of 6.19, varying significantly among species. Cellular pH showed a significant positive correlation with flower color—higher pH values (more neutral) corresponded to more intense yellow coloration. Among metal ions, K exhibited the highest concentration (12.61 mg · g ^{1}), followed by Ca^{2} (3.91 mg · g ^{1}), Mg^{2} (1.28 mg · g ^{1}), Al^{3} (0.98 mg · g ^{1}), Na (0.17 mg · g ^{1}), and Fe^{3} (0.07 mg · g ^{1}), with Cu^{2} being the lowest (0.0038 mg · g ^{1}). All seven metal ions differed significantly among species. Notably, Al^{3} , Fe^{3} , and Ca^{2} interfered with yellow color formation to varying degrees; as their concentrations increased, yellow intensity decreased and flower color became lighter. Therefore, lower concentrations of Al^{3} , Fe^{3} , and Ca^{2} may be more conducive to the expression of yellow coloration in camellias.

Keywords: yellow camellia; petal color; total flavonoid content; cellular pH;

metal ion concentration

Introduction

Plants of *Camellia* sect. *Chrysantha* Chang belong to the family Theaceae and genus *Camellia* L., comprising evergreen shrubs or small trees up to 5 m in height with golden-yellow flowers of high ornamental value (Zhang and Ren, 1998). Within the genus *Camellia*, yellow-flowered species account for only 7.14% of the total. Among the nearly 30,000 registered camellia cultivars in the International Camellia Society, almost all are red or white varieties, making yellow-flowered types extremely rare and valuable for both ornamental and scientific research (Savige, 1993, 1997; Haydon, 2011). Flower color presentation fundamentally depends on specific pigments present in petal cells, such as flavonoids, carotenoids, and alkaloids, while being simultaneously influenced by various intracellular environmental factors including pH and metal ions (Lapidot et al., 1999; Yoshida, 2006). Previous research has explored the relationship between red coloration and cellular environment in *Camellia reticulata* (Xue et al., 2015).

Prior studies on yellow camellia have primarily focused on nutritional value, measuring total flavonoids (Huang et al., 2009; Su et al., 2014), total polyphenols (Tang et al., 2017; Su et al., 2014), macro- and micro-elements, and other nutritional components (Tang et al., 2017; Lin et al., 2010). However, systematic investigation of the intrinsic relationship between flower color and important cellular environmental factors remains unreported. This study represents the first comprehensive analysis of petal color, total flavonoid content, water content, cellular pH, and key metal ion concentrations in selected yellow camellia species, providing new insights into the physiological and biochemical mechanisms underlying golden-yellow flower color formation.

Materials and Methods

1.1 Plant Materials

In January 2018, fully opened petals were collected from the National Golden Camellia Germplasm Resource Bank at Nanning Golden Camellia Park in Guangxi (E108°20 53 , N22°49 11 , altitude 75 m). Samples were preserved with ice packs and air-shipped to the laboratory on the same day. Based on visual yellow coloration, nine populations from eight species were selected across three categories:

- **Golden yellow type:** *Camellia nitidissima* (Fenghshan population), *C. multipetala*, *C. nitidissima* (Fangcheng population)
- **Yellow type:** *C. phanii*, *C. hakodae*, *C. pubipetala*
- **Light yellow type:** *C. achrysantha*, *C. tunghinensis*, *C. impressinervis*

C. phanii and *C. hakodae* were introduced from Vietnam, while the remaining species were native. All trees were 20–30 years old and growing vigorously under a canopy of tall pines and broadleaf trees that provided partial shade. Standard cultivation practices for fertilization, irrigation, and pest management were applied.

1.2 Experimental Methods

Color Measurement: Petal color was measured from outer to inner petals using the Royal Horticultural Society Colour Chart (RHS, UK) and a colorimeter (NF555, Japan) with six biological replicates per species. Color parameters were quantified according to the CIE L*a*b* color space system established by the International Commission on Illumination (CIE), including lightness (L*), hue (a* and b*), chroma (C*), and hue angle (h). Qualitative classification using the color chart and quantitative three-dimensional coordinates from the colorimeter enabled categorization of the studied species (Gonnet, 1998, 1999).

Total Flavonoid Content: Following the method of Yang et al. (2011) with optimization, total flavonoid content was determined using rutin as a standard. The regression equation was y = 9.6862x - 0.0195 (R^2 = 0.9953). Fresh petals (1.0 g) were ground and extracted with 10 mL of 65% ethanol at 65°C for 12 h. After filtration, 1 mL of extract was subjected to color development and diluted to 10 mL with 65% ethanol. Absorbance was measured at 510 nm using a microplate spectrophotometer (Multiskan GO, USA), and flavonoid concentration ($mg \cdot mL^{1}$) was calculated using the regression equation.

Cellular pH: Following Xue et al. (2015) with minor modifications, fresh petals were cut into small pieces, immediately ground into homogenate in a clean, dry mortar, and measured directly using a pH meter (PHS-3C, Shanghai) with the probe embedded in the homogenate. Three biological replicates were performed per species.

Water Content: Determined according to the National Food Safety Standard GB 5009.3-2016 using an electric thermostatic drying oven (DHG-914385-III, Shanghai).

Metal Ion Concentrations: Determined according to the Forestry Industry Standard LY/T 1270-1999 using inductively coupled plasma mass spectrometry (NexION 300D, USA) and inductively coupled plasma optical emission spectrometry (ICP-OES 7400, USA) for Na , K , Ca^2 , Mg^2 , Al^3 , Fe^3 , and Cu^2 .

1.3 Data Analysis

Data analysis and graphing were performed using Microsoft Office Excel 2007. Statistical analyses, including correlation analysis, were conducted using SPSS Statistics 17.0.

Results

2.1 Flower Color Characteristics and Classification

Color chart measurements of fully opened petals from nine populations of eight camellia species [Figure 1: see original paper] ranged between Green-Yellow 1-C and Yellow 9-A. Colorimeter data revealed that hue a* values were near zero (at the red-green intersection) and varied by species, while hue b* values ranged from 40–70 (>0), indicating distinct yellow coloration. Lightness L* values of 70–90 indicated bright coloration, chroma C* values of 40–70 indicated vivid coloration, and hue angle h values near 90° confirmed yellow coloration.

Correlation analysis showed a highly significant positive correlation between chroma C* and hue b* ($r = 0.955$, $P < 0.01$), indicating that chroma in yellow camellias is primarily influenced by yellow intensity. Thus, hue b* serves as the main descriptor of yellow coloration.

Based on hue a* and b* coordinates, the studied species were classified into three categories: golden yellow (*C. nitidissima* Fenghshan population, *C. multipetala*, *C. nitidissima* Fangcheng population), yellow (*C. phanii*, *C. hakodae*, *C. pubipetala*), and light yellow (*C. tunghinensis*, *C. achrysantha*, *C. impressinervis*), consistent with visual observations .

2.2 Variation in Total Flavonoid and Water Content

Total flavonoid content varied significantly among species. On a fresh weight basis, *C. multipetala* showed the highest content (4.09%), while *C. tunghinensis* showed the lowest (0.55%), with an average of 2.42%. On a dry weight basis, *C. impressinervis* exhibited the highest content (35.28%) and *C. tunghinensis* the lowest (6.09%), averaging 20.17%—approximately 8.3 times the fresh weight value. The ranking of species by total flavonoid content (dry weight) was: *C. impressinervis* > *C. multipetala* > *C. pubipetala* > *C. phanii* > *C. achrysantha* > *C. nitidissima* (Fenghshan) > *C. hakodae* > *C. nitidissima* (Fangcheng) > *C. tunghinensis*.

Water content ranged from 84.89% (*C. multipetala*) to 91.04% (*C. tunghinensis*), averaging 88.14%. Despite high water content in early spring, significant interspecific differences were observed.

Correlation analysis with yellow intensity (hue b*) revealed weak negative correlations between flower color and both total flavonoid content (dry weight) ($r = -0.222$, $P = 0.566$) and water content ($r = -0.241$, $P = 0.532$), indicating minimal influence on yellow coloration.

2.3 Cellular pH Analysis

Cellular pH values ranged from 6.06 (*C. tunghinensis*) to 6.55 (*C. nitidissima* Fenghshan population), with an average of 6.19, indicating weak acidity. Significant interspecific variation was observed [Figure 3: see original paper].

Correlation analysis with yellow intensity (hue b*) demonstrated a significant positive correlation (r = 0.819, P = 0.007), revealing that higher pH values (more neutral) corresponded to more intense yellow coloration, while increased acidity resulted in lighter yellow color.

2.4 Metal Ion Concentrations

Concentrations of seven metal ions are presented in . K exhibited the highest concentration (9.21–18.40 mg · g ^{1}, mean 12.61 mg · g ^{1}), 1.97 times the sum of the other six ions. Ca^{2} ranked second (1.75–6.14 mg · g ^{1}, mean 3.91 mg · g ^{1}), followed by Mg^{2} (1.05–1.50 mg · g ^{1}, mean 1.28 mg · g ^{1}). The remaining ions showed lower concentrations: Al^{3} (0.49–2.29 mg · g ^{1}, mean 0.98 mg · g ^{1}), Na (0.02–0.42 mg · g ^{1}, mean 0.17 mg · g ^{1}), Fe^{3} (0.03–0.14 mg · g ^{1}, mean 0.07 mg · g ^{1}), and Cu^{2} (0.0021–0.0062 mg · g ^{1}, mean 0.0038 mg · g ^{1}). All seven metal ions differed significantly among species.

Correlation analysis with yellow intensity (hue b*) revealed complex relationships. Al^{3} (r = -0.511), Fe^{3} (r = -0.607), and Ca^{2} (r = -0.639) showed negative correlations, indicating that increased concentrations of these ions resulted in lighter yellow coloration. Conversely, K , Mg^{2} , Na , and Cu^{2} showed only very weak correlations with yellow intensity.

2.5 Correlation Analysis

Stepwise regression analysis at the 0.05 significance level eliminated non-significant variables, yielding the following regression equation for flower color (hue b*):

b* = -270.291 + 52.356x , R = 0.819, P = 0.007 (x : cellular pH)

Only cellular pH was retained in the final model, with all other factors excluded.

Discussion

3.1 Impact of Total Flavonoid Content on Yellow Camellia Coloration

Total flavonoid content showed only a weak, non-significant correlation with petal color (hue b*), consistent with previous reports. Huang et al. (2009) measured total flavonoid content in five yellow camellia species, finding golden-yellow* C. nitidissima* and *C. euphlebia* contained 0.64% and 0.50% (fresh weight), respectively, while lighter-yellow *C. impressinervis* and *C. pubipetala* contained 0.86% and 2.70%, and the palest *C. pingguoensis* contained 0.72%. Although these values were slightly lower than our results, no clear correlation with flower color was observed. Similarly, Su et al. (2014) reported dry-weight flavonoid contents of 4.80% in golden-yellow *C. nitidissima* versus 13.78% and 6.12% in light-yellow *C. pubipetala* and *C. achrysantha*, respectively—values somewhat higher than ours but again showing no significant color correlation.

Yellow flower coloration primarily depends on yellow pigments within flavonoids. However, total flavonoids also include colorless compounds such as catechins that may interfere with yellow expression (Tanaka et al., 2010). The specific composition and relative proportions of these pigments require more detailed investigation. Water content showed extremely weak correlation with coloration, likely because all studied petals contained high moisture levels sufficient for saturating soluble flavonoids. Additionally, dissolved sugars may interfere with color expression, as demonstrated in red-flowered *Camellia reticulata* (Xue et al., 2015). We observed that despite their golden-yellow appearance, ground petal powder and extracts from *C. nitidissima* showed relatively light coloration, suggesting high concentrations of colorless or white compounds that reduce total flavonoid content—hypotheses requiring further verification.

3.2 Impact of Cellular pH on Yellow Camellia Coloration

This study revealed that petal cellular pH in yellow camellias ranges from 6.06-6.55 (weakly acidic), with higher pH values (more neutral) corresponding to more intense yellow coloration. These findings establish a foundation for understanding pH effects in yellow flowers, suggesting that a neutral-to-weakly-acidic environment may provide stable conditions for yellow pigment expression.

Among factors influencing flower color, cellular pH most strongly affects pigment compounds like flavonoids and carotenoids by altering their molecular forms. Generally, red flowers exhibit lower pH than blue flowers; acidic pH promotes red coloration. Red-flowered *C. reticulata* showed cellular pH of 3.6-4.0 (Xue et al., 2015), significantly lower than our yellow camellias. Deep-red rose petals also exhibit vacuolar pH around 4 (Tanaka et al., 2010). In contrast, increased cellular pH deepens blue coloration in *Petunia hybrida* (Yoshida et al., 2009), and red-flowered *Hydrangea macrophylla* has pH approximately 0.8 units lower than blue-flowered forms (Ito et al., 2009). Other studies indicate anthocyanins appear red in strongly acidic conditions, blue in alkaline environments, and reddish-purple in weakly acidic to neutral conditions (Faraco et al., 2009). Collectively, these findings demonstrate that while pH varies considerably among species and has minimal variation within species, its impact on flower color is highly significant.

3.3 Impact of Metal Ion Concentrations on Yellow Camellia Coloration

Although K showed the highest concentration in yellow camellia petals, its effect on color formation was not significant. Mg^{2} varied little among species and showed no significant correlation with color, exerting weak influence on yellow expression. Cu^{2} and Na occurred at low concentrations with minimal association with color formation. In contrast, Al^{3}, Fe^{3}, and Ca^{2} inhibited yellow color formation to varying degrees; as their concentrations increased, yellow intensity decreased. Therefore, lower concentrations of these three ions appear more favorable for yellow color expression.

Metal ions primarily influence flower color through complexation with flavonoids, enhancing pigment stability. In blue flowers, Al^{3} , Mg^{2} , and Fe^{3} form blue complexes with flavonoids: e.g., the blue pigment commelinin from *Commelina* flowers is a Mg^{2} -delphinidin complex (Hayashi et al., 1958); blue *Centaurea cyanus* coloration results from cyanidin-3,5-diglucoside-Fe^{3} complexation with flavonols (Takeda & Tominaga, 1983); and blue *Hydrangea macrophylla* coloration involves Al^{3} complexation with delphinidin glycosides and 5-caffeoylquinic acid (Ito et al., 2009). In red chrysanthemum cultivars, Fe^{3} , Mg^{2} , Al^{3} , and Ca^{2} enhanced anthocyanin coloration but had no effect on flavonoids in white cultivars (Bai, 2007). In yellow *Gerbera hybrid*, Fe^{2} and Ca^{2} significantly affected coloration while Mg^{2} did not (Razieh et al., 2013). Additionally, the shift from purple to blue in wild-type *Ipomoea tricolor* resulted from increased vacuolar pH mediated by Na /K antiporters (Verweij et al., 2008). These findings demonstrate that the same metal ion can have different effects across species, color types, or even within different color types of the same species. Therefore, the specific complexation mechanisms between metal ions and flavonoids in yellow camellias require further investigation.

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Note: Figure translations are in progress. See original paper for figures.