Flavonoid Components of Different Color Magnolia Flowers and Their Relationship to Cultivar Selections

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Abstract. Magnolia (Magnoliaceae) is widely cultivated for its beauty; however, despite this, the components of the different flower colors in Magnolia have not been elucidated. In this study, the color parameters of 10 Magnolia petals with different colors were measured by the Royal Horticultural Society Color Chart (RHSCC) and a color reader CR-10. The composition and content of the flavonoids in the petals were analyzed by high-performance liquid chromatography coupled with diode array detection (HPLC-DAD) as well as HPLC with electrospray ionization and mass spectrometry (HPLC-ESI-MS). All results showed that the 10 petals were divided into four color groups. Regarding the flavonoid composition, four types of anthocyanins, including Cyanidin-glucosyl-rhamnoside (Cy-GR), Cyanidin-glucosyl-rhamnosyl-glucoside (Cy-GRG), Peonidin-glucosyl-rhamnoside (Pn-GR), and Peonidin-glucosyl-rhamnosyl-glucoside (Pn-GRG), were identified, as well as 10 types of flavonols. The flavonols included isorhamnetin, quercetin, kaempferol, and their glycosides, which included rutinoside, rhamnose, and glucoside. Cyanidin and peonidin make Magnolia petals appear red-purple and purple, respectively, and the flavonols perform as evident auxiliary pigments, particularly quercetin. The Magnolia cultivar flower phenotypes sampled in this study differed by changes in their existing flavonoid content rather than by the appearance of new flavonoids. Consequently, this study provides a reference for further revealing the basis of Magnolia flower color and provides clues for color breeding.

Materials and Methods

Plant materials. Ten plant materials, including six species and four cultivars (Fig. 1), were planted in Zhejiang Agriculture and Forestry University, Lin’an, China (located at long. 118°51′ to 119°52′ E, lat. 29°56′ to 30°23′ N). All plant materials were chosen from one tree, and three flowers were picked from the trees at random. One petal from each flower was selected. The outermost petals of the flowers at the full-bloom stage were used as materials in this study. The flowers were cut off with their branches and placed in water in the laboratory. The color parameters of some flowers were measured immediately, whereas some petals were quickly frozen in liquid nitrogen and stored at –80 °C for future analysis.

Flower color parameter measurements. The use of the Royal Horticultural Society Color Chart (RHSCC) gave the ability to classify colors. The RHSCC values were assigned by comparing the base of the fresh petals (the darkest part) under the same external lighting conditions. At the same time, the color parameter lightness (L*) and chromatic component (a* and b*) values of the CIE (French Commission internationale de l’éclairage) L*a*b* color coordinates were
measured using the color reader CR-10 (Konica Minolta Optics, Inc., Sakai, Japan). \( L^* \) represents the lightness of the color, with the value ranging from low to high and the color from lighter to darker. The \( a^* \) value represents the red (positive) and green (negative) values. The \( b^* \) value represents yellow (positive) and blue (negative) values (Bioley and Jay, 1993).

**Extraction of the flavonoids.** Fresh petals (0.5 g) were ground into a powder with liquid nitrogen, and then 1.5 mL of 2% formic acid (Shanghai, China) and 70% methyl alcohol (Ohio, USA) were added. The mixture was placed into a 2-mL centrifuge tube and centrifuged at 10,000g for 10 min at 4 °C to obtain the supernatant. The flavonoid extract solution was obtained after passing the mixture through a 0.22-μm syringe filter into the sample bottles (Shi et al., 2015).

**Qualitative and quantitative analysis of the flavonoids.** A quantitative analysis of the flavonoids was conducted using a Shimadzu HPLC System (Kyoto, Japan) consisting of an LC-20AT pump, an SPD-M20A DAD detector, a CTO-10AS VP column oven, a SIL-20A auto injector. A 10-mL syringe filter was used to filter the sample solutions. The reference substances were cyanidin 3-O-rutinoside chloride, quercetin 3-O-glucoside, and kaempferol 3-O-rutinoside, which were purchased from Sigma-Aldrich (St. Louis, MO). By drawing a standard curve for the semiquantitative measurement, cyanidin chloride was used as the anthocyanin standard, whereas quercetin 3-O-rutinoside was used as the flavonol standard.

**Statistical analysis.** Data are expressed as the means ± the SD of the three replicates. Microsoft Office Excel 2016 and IBM SPSS Statistics 19.0 (SPSS, Chicago, IL) were used for data processing and analysis. Origin 9 was used to draw diagrams. A Pearson correlation analysis was used to analyze the relationship among the color parameters (\( L^* \), \( a^* \), and \( b^* \) values), anthocyanins, and flavonoids (C, P, I, S, Qu, K, total anthocyanin content (TA), and total flavonol content (TF)) across 10 plant materials.

**Results**

*Flower phenotype determination of the Magnolia.* According to the RHSCC and the \( L^* \), \( a^* \), and \( b^* \) values, the 10 plant materials were categorized into different groups based on their flower phenotype.
were divided into four color groups: the white group (w1), the yellow-green group (y1), the red-purple group (r1–r6), and the purple group (p1 and p2). In three-dimensional space distribution, the b* and L* values of the white group and the yellow-green group were significantly higher than those of the red-purple group and the purple group, and the L* value of the red-purple group was significantly higher than that of the purple group. The a* value of the red-purple group was significantly higher than those of the three other groups (Fig. 1, Table 1).

Identification of the anthocyanins in Magnolia. The anthocyanins had a characteristic absorption peak at 520 nm, with four components isolated from the flavonoid extraction solutions. Components P1 and P3 had fragment ions 287[Y0]+, corresponding to cyanidin, and components P2 and P4 had fragment ions 301[Y0]+, corresponding to peonidin, thus showing that P1 and P3 are cyanidin derivatives, whereas P2 and P4 are peonidin derivatives. MS showed that P1 had molecular ion m/z 757[M]+ and fragment ions m/z 595[M-162]+, 449[M-162-146]+, and 287[M-162-146-162]+, thus indicating that P1 is a cyanidin-glucosyl-rhamnosyl-glucoside (Cy-GR). P2 had molecular ion m/z 771[M]+ and fragment ions m/z 609[M-146]- and 301[M-146-162]-a, and the retention time of F10 was the same as that of the reference substance, quercetin 3-O-rutinoside. F11 and F12 were kaempferol derivatives. F11 had quasi molecular ion m/z 593[M-H]- and fragment ions m/z 447[M-146]- and 285[M-146-162]-, and the retention time of F10 was the same as that of the reference substance, kaempferol 3-O-rutinoside. It was therefore speculated that F12 was kaempferol 3-O-rutinoside (Km3G) (Sokkar et al., 2014) (Table 3, Supplemental Fig. 2).

Composition analysis of the anthocyanins and flavonols in Magnolia. All 10 materials contained isorhamnetin (Is), quercetin (Qu), and kaempferol (Km), but the specific flavonol composition in each plant material was different. There were no anthocyanins in the white and yellow-green groups. The red-purple group and the purple group both had anthocyanins. The main anthocyanin in Magnolia × soulangeana ‘Fu Rong’, M. × soulangeana ‘Dan Xin’, and Magnolia cylindrica was peonidin (Pn). Magnolia sinostellata had only cyanidin (Cy), mainly Cy-GR. For Magnolia stellata ‘Chrysanthemumiflora’, the main

<table>
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<tr>
<th>Peak</th>
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<th>λmax (nm)</th>
<th>[M]+ (m/z)</th>
<th>Fragment (m/z)</th>
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Table 1. Identification of flavonoids in Magnolia with high-performance liquid chromatography with electrospray ionization and mass spectrometry.
pigment was Cy, mainly Cy-GRG. Last, in *M. ×soulangeana*, Cy and Pn were almost the same. In the purple group, the main pigment in *Magnolia liliiflora* was Pn-GR, whereas it was Pn-G in *M. liliflora* "Hong Yuanbao".

Compared with the other groups, the TA in the purple group was significantly higher, and the TF was significantly lower. The TF in *M. liliiflora* "Hong Yuanbao" (1238.28 µg·g⁻¹ FW), followed by *M. liliflora* (1273.96 µg·g⁻¹ FW) (Fig. 2, Table 4).

Relationship between the color parameters and the flavonoids. The correlation analysis results showed that TA and Pn had a highly significant negative correlation with L*. The accumulation of TA could reduce the lightness of the petal, especially Pn. L* showed a significantly positive correlation with TF and had a positively significant correlation with QA. The accumulation of flavonols could increase the lightness of the petal, especially QA. However, the anthocyanins or flavonols showed no significant correlation with a* and b* (Table 5).

The auxiliary effect index CI value (Copigmentation index=TA/TA) was greater than 5 in 10 plant materials, suggesting that flavonols played an evident auxiliary pigment function.

**Discussion**

Relationship between the flavonoids and the formation of new cultivars of Magnolia. *M. denudata* was the only species with pure white flowers. Yellow flowers in *Magnolia* are rare outside of the North American cucumber magnolia (*Magnolia acuminata*). *M. denudata* "Fei Huang" is a cultivar of *M. denudata* selected because of its unusual yellow flowers.

In some yellow-orange from flowers, yellow-to-orange flower colors are attributed to carotenoids, chlorophylls, or other flavonoids, such as flavones, flavonols, and chalcones (Lewis et al., 1998; Mizuno et al., 2015). This study showed that there was no anthocyanin in *M. denudata* or *M. denudata* "Fei Huang", and that the components and contents of the flavonoids were similar in *M. denudata* and *M. denudata* "Fei Huang". The results suggest that flavonoids were probably not responsible for the yellow flower color in *Magnolia*. Carotenoids or chlorophyll might exist in the petals of *M. denudata* "Fei Huang", resulting in the yellow flower color. Nonetheless, further research is needed.

Red-purple is the most common color in *Magnolia*, and thus in this experiment, we chose six typical species with red-purple flowers of *Magnolia*. Among them, *M. ×soulangeana* is a hybrid species obtained by crossbreeding *M. denudata* and *M. liliiflora*. The composition of anthocyanin in *M. ×soulangeana* flowers is the same as that in *M. liliiflora*, as both have four anthocyanin components, whereas the TA content in *M. ×soulangeana* is nearly two-fifths that of *M. liliiflora*. The formation of this new variety is due to the changes in TA.

*M. ×soulangeana* "Fu Rong" and *M. ×soulangeana* "Dan Xin" are cultivars of *M. ×soulangeana*. The flower color is from a moderate purplish-red to a strong reddish-purple. In contrast to *M. ×soulangeana*, the TA in *M. ×soulangeana* "Fu Rong" and *M. ×soulangeana* "Dan Xin" was reduced, especially Cy, whereas TF had no obvious changes. The difference between *M. ×soulangeana* "Fu Rong" and *M. ×soulangeana* "Dan Xin" was the scale of Pn-GR and Pn-GR. Thus, it can be concluded that the formation of these two new varieties is caused by the change in the anthocyanin composition and content.

*M. sinostellata* and *M. stellata* "Chrysanthemum" were once considered to be one species (Fan et al., 2018). According to this experiment, the composition of anthocyanins is very different between *M. sinostellata* and *M. stellata* "Chrysanthemum". In addition, they are not the same species at the pigment level. Furthermore, *M. sinostellata* is an endangered *Magnolia* shrub species. We hope that the endangering mechanism of *M. sinostellata* can be studied in future research based on our findings (Bradshaw and Schemske, 2003; Nakatsuka et al., 2008).

*M. liliflora* is a typical species of *Magnolia* with purple flowers, whereas *M. liliflora* "Hong Yuanbao" is a cultivar of *M. liliflora*, with a flower color from dark purple to grayish-purple. The main pigment in *M. liliflora* is Pn-GR, whereas it is Pn-GR in *M. liliflora* "Hong Yuanbao". The contents of TA and TF were slightly different. The glycosylation of the anthocyanins resulted in reddening (Honda and Saito, 2002), which can explain why the a* value of p1 is higher than that of p2. The formation of this new variety is caused by different glycosides in the same anthocyanins.

**Genetic engineering breeding methods of Magnolia based on the flavonoids.** Traditional breeding can improve flower color based on existing colors, such as within-genus crossbreeding. However, a new flower color can be achieved by genetic engineering breeding, as opposed to traditional breeding.

The anthocyanin biosynthesis pathway belongs to the flavonoid biosynthesis pathway, and includes pelargonidin, cyanidin, and delphinidin biosynthesis branches (Martin et al., 2016). Thus, there are several ways to improve Magnolia flower colors depending on the type of genetic engineering breeding. First, by introducing the corresponding structural genes, one can add a biosynthesis branch other than the cyanidin biosynthesis branch to increase the pigment types in the *Magnolia* petals to adjust the flower color, such as the inexistence of blue Magnolia flowers; by synthesizing delphinidin for the blue hues by incorporating F3’-5’H (flavonoid 3’, 5’-hydroxylase), one could obtain blue flowers (Jin et al., 2016; Wu et al., 2016). Second, it is possible to change the contents of
the existing pigment components to improve flower color. For example, OMT (O-methyltransf erase) could catalyze Cy into Pn (Akita et al., 2011; Du et al., 2015). We speculate that the regulation of OMT could adjust the proportion of Cy and Pn, with the final result being a change in Magnolia flower color.

Conclusions

The types of flavonoids in Magnolia petals are relatively few, with only two types of anthocyanin aglycones and three flavonol aglycones. There are no other flavonoids, such as flavone, and only three glycosides, including rhamnoside, glucoside, and rutinoside, exist in Magnolia petals. Cyanidin and peonidin make Magnolia petals appear red-purple and purple, respectively, whereas flavonols evidently serve as auxiliary pigments, particularly quercetin.

The formation of the cultivars of Magnolia used in this study was caused only by changes in the existing flavonoid composition rather than in the appearance of new flavonoids. The results will be valuable for flower color breeding of Magnolia.

Literature Cited


Supplemental Fig. 1. HPLC chromatogram of anthocyanins in Magnolia.
Supplemental Fig 2. HPLC chromatogram of flavonoids in *Magnolia.*