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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^2). 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.**

Magnolia is an original genus belonging to Magnoliaceae and has a long history of cultivation in China given its beauty and fragrance. Therefore, *Magnolia* has been widely used in garden landscapes for its high ornamental value. Flower color is one of the main ornamental characteristics. White, pink, and purple are the main flower colors, whereas other flower colors, such as yellow and green, are uncommon (Wang et al., 2013; Zhang et al., 2011). An increase in the diversity of flower colors has become one of the main research directions of *Magnolia* specialists.

Flavonoids are secondary metabolites of water-soluble aromatics, with a series of two benzene rings connected by a central three-carbon chain, C6-C3-C6 (Zhao and Tao, 2015). Natural biological flavonoids are derivatives of their basic structures, and they often exist in the form of glycosides (Heiss et al., 2010). The different structural flavonoids can be divided into 10 types, including anthocyanin, flavone, flavonol, flavanone, flavanol, chalcone, isoflavone, dihydroflavonol, aurone, and proanthocyanidin (Hao et al., 2015; Tahara, 2007). Anthocyanins confer a diverse range of flower colors, including orange, red to purple, and violet (Tanaka et al., 2008), thus determining the flower color of ≈88% of the family angiosperms (Grotewold, 2006). Approximately 600 anthocyanins have been isolated and identified from nature, and their main derivatives are from six anthocyanin structures (Kong et al., 2003). In addition, colorless or light yellow flavonols and flavones, as copigments of anthocyanins, generally play a supplementary role in flower color (Asen et al., 1971; Ono et al., 2010). The diversity of flavonoids, which is determined by different metabolic pathways, is the fundamental cause of many flower colors (Tanaka and Brugliera, 2013). The targeted color modification of plants is conducted through the combination of the main pigment, the synthesis of the metabolic pathway, and genetic engineering (Chen et al., 2017; Tai et al., 2014).

In previous research, only one type of anthocyanin, cyanidin 3-*O*-glucoside chloride, was identified in *Magnolia sprengeri*, which was found to be the main pigment in this purple-flowered phenotype (Shi et al., 2015, 2014). Six types of flavonoids have been identified from the ethanol extracts of white-flowered *Magnolia grandiflora* petals, including apigenin 8-*C*-glucoside, luteolin 8-*C*-glucoside, quercetin 3-*O*-rutinoside, quercetin 3-*O*-galactoside, quercetin 3-*O*-glucoside, and kaempferol 3-*O*-rutinoside, whereas the flavonoids in the stamens have been identified as isorhamnetin 3-*O*-glucoside and isorhamnetin (Sokkar et al., 2014). However, there is no systematic study on the flavonoid composition and color of *Magnolia* petals.

The purpose of the present study was to integrate and compare the flavonoid composition formation patterns of *Magnolia* in purple, red, pink, yellow, and white flowers. Ten *Magnolia* specimens, including six typical species and four corresponding cultivars, were investigated to analyze their flavonoids 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^2). The results of the present study suggest a relationship between the components and the different flower colors in *Magnolia*. These results might provide some information for color breedings in Magnoliaceae.

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

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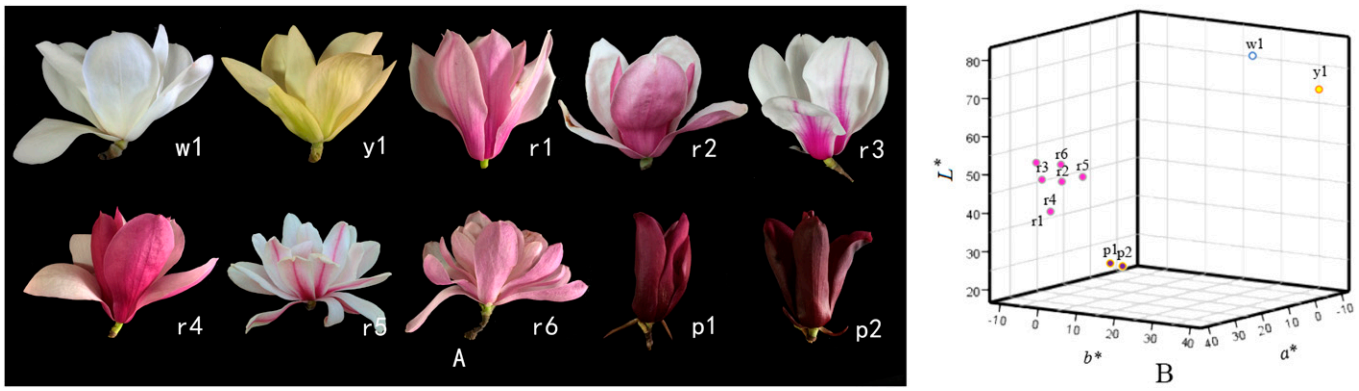

Fig. 1. (**A**) Ten plant materials (w1 *Magnolia denudata*; y1 *M. denudata* 'Fei Huang'; r1 *Magnolia* ×*soulangeana* 'Fu Rong'; r2 *M.* ×*soulangeana* 'Dan Xin'; r3 *Magnolia cylindrical*; r4 *M.* ×*soulangeana*; r5 *Magnolia sinostellata*; r6 *Magnolia stellata* 'Chrysanthemumiflora'; p1 *Magnolia liliiflora*; p2 *M. liliiflora* 'Hong Yuanbao'). (**B**) Color distribution in CIE *L* a* b** coordinate systems of trivariate.

Table 1. Color parameters of 10 plant materials.

Plant materials	RHSCC	Color group	Sample no.	CIE *L*a*b**		
				*L**	*a**	*b**
Magnolia denudata	155A	White	w1	77.83 ± 0.56 a	–3.10 ± 0.29 e	24.4 ± 2.19 b
M. denudate 'Fei Huang'	145C	Yellow-green	y1	70.67 ± 1.31 b	–6.23 ± 1.36 e	39.52 ± 4.99 a
Magnolia ×*soulangeana* 'Fu Rong'	72B	Red-purple	r1	50.50 ± 2.57 e	34.87 ± 427 a	–6.10 ± 1.25 d
M. ×*soulangeana* 'Dan Xin'	70B		r2	47.27 ± 1.72 c	29.60 ± 1.47 b	–3.07 ± 2.06 d
Magnolia cylindrica	67A		r3	49.10 ± 0.66 c	36.93 ± 3.30 a	–3.10 ± 0.60 d
M. ×*soulangeana*	70A		r4	40.13 ± 1.25 d	33.50 ± 4.82 ab	–3.33 ± 1.50 d
Magnolia sinostellata	61A		r5	48.67 ± 4.55 c	27.63 ± 3.14 b	1.20 ± 0.26 c
Magnolia stellata 'Chrysanthemumiflora'	71D		r6	50.70 ± 3.30 c	26.23 ± 1.01 b	–5.40 ± 1.18 d
Magnolia liliiflora	N79B	Purple	p1	25.17 ± 1.10 f	21.30 ± 0.87 c	4.00 ± 0.95 c
M. liliiflora 'Hong Yuanbao'	N77A		p2	23.63 ± 0.72 f	15.83 ± 2.31 d	3.20 ± 0.75 c

CIE = French Commission internationale de l'éclairage; RHSCC = Royal Horticultural Society Color Chart; *L** = lightness; *a**, *b** = chromatic components.

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 (Biolley 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 10,000*g* 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, and a SIL-20A auto injector. A 10-µL aliquot of each sample was eluted through a C18 column of Inertsil ODS-SP (4.6 mm × 250 mm, 5 µm) at a column temperature of 30 °C and a flow rate of 0.8 mL·min^{-1}. The mobile phase consisted of solvent A, 0.1% formic acid water, and solvent B, 0.1% formic acid acetonitrile (Ohio, USA). The gradient elution programs used were as follows: 0 to 5 min, 0% to 5% B; 5 to 15 min, 5% to 15% B; 15 to 25 min, 15% to 23% B; 25 to 35 min, 23% to 26% B; 35 to 52 min, 26% to 40% B; 52 to 57 min, 40% to 5% B; and 57 to 65 min, 5% to 0% B. The ultraviolet diode array detection spectra were scanned from 190 to 800 nm. Chromatograms were obtained at 350 nm and 520 nm simultaneously.

Table 2. Identification of anthocyanins in *Magnolia* with high-performance liquid chromatography with electrospray ionization and mass spectrometry.

Peak	t_R (min)	λ_{max} (nm)	$[M]^+$ (m/z)	Fragment (m/z)	Tentative compound
P1	16.43	515	757	595,449,287	Cyaindin-glucosyl-rhamnosyl-glucoside
P2	19.28	516	771	609,463,301	Peonidin-glucosyl-rhamnosyl-glucoside
P3	23.23	517	595	449,287	Cyanidin-glucosyl-rhamnoside
P4	26.44	518	609	463,301	Peonidin-glucosyl-rhamnoside

An HPLC-ESI-MS^2 analysis for the qualitative measurement of the flavonoids was performed using a Thermo Fisher ion trap mass spectrometer (Waltham, MA) equipped with an electrospray ionization (ESI) interface. The HPLC separation conditions were the same as those mentioned previously. The MS conditions were as follows: the anthocyanins were applied in positive ion (PI) modes, and the other flavonoids were applied in negative ion (NI) modes. The ESI source was as follows: N_2 was the drying gas and nebulizing gas, with a capillary temp of 300 °C; flow rate, 8.0 L·min^{-1}; ion spray voltage, 3500 V; the capillary offset and exit voltage were 4 V and 5 V, respectively, for PI and –33 V and –35 V, respectively, for NI; and the range of *m/z* was 100 to 1000 for the full-scan MS analysis.

The flavonoid composition of presumption depended on the MS databased on the method of Zhang et al. (2013). The determination of the content of the flavonoid components was carried out with an external standard method. 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 flavonols [Cy, Pn, Is, Qu, Km, 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

Table 3. Identification of flavonols in *Magnolia* with high-performance liquid chromatography with electrospray ionization and mass spectrometry.

Peak	t_R(min)	λ_{max}(nm)	$[M]^-$ (m/z)	Fragment (m/z)	Tentative compound
F1	11.65	244,323	353	191,179	No tentative identification
F2	17.80	243,324	353	191,179	No tentative identification
F3	23.04	244,329	947	785,623,461,315	Isorhamnetin-rhamnosyl-glucosyl-glucosyl-glucoside
F4	24.01	246,329	785	623,461,315	Isorhamnetin-rhamnosyl-glucosyl-glucoside
F5	24.96	246,329	931	769,623,461,315	Isorhamnetin-rhamnosyl-glucosyl-rhamnosyl-glucoside
F6	26.56	246,329	769	623,461,315	Isorhamnetin-rhamnosyl-glucosyl-rhamnoside
F7	27.00	246,329	805	769,623,461,315	Isorhamnetin-rhamnosyl-glucosyl-rhamnoside
F8	28.67	246,329	623	461,315	Isorhamnetin-rhamnosy-glucoside
F9	29.42	254,357	609	463,301	Quercetin 3-*O*-rutinoside
F10	30.30	253,352	463	301	Quercetin 3-*O*-glucoside
F11	32.04	246,330	753	617,285	Kaempferol derivative
F12	33.04	245,329	593	447,285	Kaempferol 3-*O*-rutinoside

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$[Y_0]^+$, corresponding to cyanidin, and components P2 and P4 had fragment ions 301$[Y_0]^+$, 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 cyanidan-glucosyl-rhamnosyl-glucoside (Cy-GRG). P2 had molecular ion m/z 771$[M]^+$ and fragment ions m/z 609$[M-162]^+$, 463$[M-162-146]^+$, and 301$[M-162-146-162]^+$, thus indicating that P2 is a peonidin-glucosyl-rhamnosyl-glucoside (Pn-GRG) (Fumi et al., 2012; Pedreschi and Cisneros-Zevallos, 2007; Zhang et al., 2012). P3 had molecular ion m/z 595$[M]^+$ and fragment ions m/z 449$[M-146]^+$ and 287 $[M-146-162]^+$, thus indicating that P3 was determined to be a cyanidin-glucosyl-rhamnoside (Cy-GR). P4 had molecular ion m/z 609$[M]^+$ and fragment ions m/z 463$[M-146]^+$ and 301 $[M-146-162]^+$, thus indicating that P4 was a peonidin-glucosyl-rhamnoside (Pn-GR) (Liu et al., 2013) (Table 2, Supplemental Fig. 1).

Identification of the flavonols in Magnolia. Under a wavelength of 350 nm, 12 components were isolated from the flavonoid extraction solution, of which 10 were identified, including six isorhamnetin derivatives, two quercetin derivatives, and two kaempferol derivatives. Using MS, components F1 and F2 had quasi molecular ion m/z 353$[M-H]^-$ and fragment ions m/z 191 and 179. There was no characteristic ion of flavonoids, so the accurate composition of F1 and F2 could not be inferred. However, through the similarity of the quasi molecular ions, a structural isomer was speculated. F3, F4, F5, F6, F7, and F8 had fragment ions 315$[Y_0]^-$ in the NI mode corresponding to the mass spectrum characteristics of isorhamnetin, thus indicating that they were isorhamnetin derivatives. The fragment ions indicated that the type of glycosides in isorhamnetin included rhamnoside and glucoside (Li et al., 2013). F9 and F10 had fragment ions 301 $[Y_0]^-$ and were thus speculated to be quercetin derivatives. F9 had quasi molecular ion m/z 609$[M-H]^-$ and fragment ions m/z 463$[M-146]^-$ and 301$[M-146-162]^-$, and the retention time of F9 was the same as that of the reference substance, quercetin 3-*O*-rutinoside. Thus, F9 was speculated to be quercetin 3-*O*-rutinoside (Qu3Ru) (Wang et al., 2016). F10 had quasi molecular ion m/z 463$[M-H]^-$ and fragment ion m/z 301$[M-162]^-$, and the retention time of F10 was the same as that of the reference substance, quercetin 3-*O*-glucoside. Therefore, F10 was speculated to be quercetin 3-*O*-glucoside (Qu3G) (Sokkar et al., 2014). F11 and F12 had fragment ion 285$[Y_0]^-$ in the reverse-phase HPLC and the same glycosides. The elution time of kaempferol was later than that of quercetin (Li et al., 2009), and according to the elution sequence, it was speculated that F11 and F12 were kaempferol derivatives. F11 had quasi molecular ion m/z 753$[M-H]^-$ and fragment ions m/z 617 and 285; thus, we could not infer its accurate composition. F12 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).

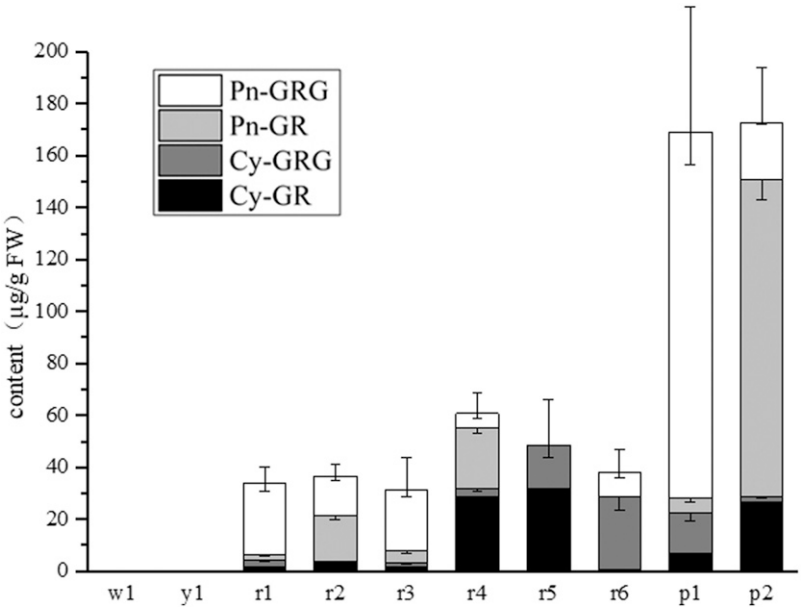

Fig. 2. Anthocyanin contents in *Magnolia.* Cy-GR = cyanidin-glucosyl-rhamnoside; Cy-GRG = cyanidin-glucosyl-rhamnosyl-glucoside; Pn-GR = peonidin-glucosyl-rhamnoside; Pn-GRG = peonidin-glucosyl-rhamnosyl-glucoside.

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 cylindrical* was peonidin (Pn). *Magnolia sinostellata* had only cyanidin (Cy), mainly Cy-GR. For *Magnolia stellata* 'Chrysanthemumiflora', the main

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-GRG, whereas it was Pn-GR in *M. liliiflora* 'Hong Yuanbao'.

Compared with the other groups, the TA in the purple group was significantly higher, and the TF was significantly lower. The TF [4836.99 μg·g^{-1} fresh weight (FW)], Qu (2115.48 μg·g^{-1} FW), and Km (330.50 μg·g^{-1} FW) in *Magnolia denudata* 'Fei Huang' were higher than those in the other groups. The highest Is was in *Magnolia cylindrical* (2813.67 μg·g^{-1} FW), whereas the lowest TF was in *M. liliiflora* 'Hong Yuanbao' (1283.28 μg·g^{-1} FW), followed by *M. liliiflora* (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 greatly positive correlation with Qu. The accumulation of flavonols could increase the lightness of the petal, especially Qu. However, the anthocyanins or flavonols showed no significant correlation with *a** and *b** (Table 5).

The auxiliary effect index CI value (Copigment index=TF/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 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 flavonols were similar in *M. denudata* and *M. denudata* 'Fei Huang'. The results suggest that flavonols were probably not responsible for the yellow flower color in *Magnolia*. Carotenoids or chlorophylls 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-GRG. 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* 'Chrysanthemumiflora' 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* 'Chrysanthemumiflora'. 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. liliiflora is a typical species of *Magnolia* with purple flowers, whereas *M. liliiflora* 'Hong Yuanbao' is a cultivar of *M. liliiflora*, with a flower color from dark purplish-red to grayish-purple. The main pigment in *M. liliiflora* is Pn-GRG, whereas it is Pn-GR in *M. liliiflora* '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., 1991). In this study, only the cyanidin biosynthesis branch was demonstrated in *Magnolia* (Boase et al., 2010; Jin et al., 2016; Wu 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

Table 4. Flavonoid contents of *Magnolia*.

Plant materials	Contents (μg·g^{-1} FW)										
	Is-RGGG	Is-RGG	Is-RGRG	Is-RGR	Is-RGR	Is-RG	Qu3Ru	Qu3G	Km derivative	Km3Ru	TF
w1	64.54 ± 1.27	986.24 ± 25.05	–	41.21 ± 9.73	–	646.02 ± 62.44	1371.03 ± 124.41	74.29 ± 8.53	94.75 ± 4.07	14.68 ± 1.39	3292.77 ± 236.90
y1	99.57 ± 8.82	1207.16 ± 24.50	–	27.78 ± 5.81	–	1056.49 ± 6.77	2115.48 ± 243.24	–	74.9 ± 2.64	255.6 ± 5.88	4836.99 ± 297.65
r1	228.9 ± 13.16	405.02 ± 22.33	230.67 ± 14.61	13.52 ± 2.64	103.59 ± 3.65	166.16 ± 2.83	1755.86 ± 163.52	82.51 ± 1.92	73.38 ± 7.67	26.12 ± 2.49	3085.74 ± 229.82
r2	107.63 ± 7.03	80.48 ± 7.05	–	201.14 ± 6.18	350.01 ± 9.59	207.17 ± 7.15	1221.86 ± 105.63	132.61 ± 36.86	115.68 ± 7.32	60.99 ± 5.09	2477.57 ± 188.89
r3	112.66 ± 9.88	806.32 ± 6.54	265.87 ± 11.74	62.78 ± 6.22	123.06 ± 6.67	1442.97 ± 157.92	1165.49 ± 114.91	202 ± 17.63	219.61 ± 6.75	58.97 ± 5.83	4459.74 ± 351.09
r4	1127.71 ± 90.52	540.95 ± 49.26	–	–	87.55 ± 23.60	280.05 ± 38.14	1195.5 ± 74.73	238.93 ± 18.27	78.17 ± 10.69	34.61 ± 2.78	3583.47 ± 307.99
r5	204.87 ± 24.58	450.32 ± 48.12	984.45 ± 75.43	12.96 ± 0.80	60.69 ± 5.02	93.83 ± 20.19	1085.32 ± 79.38	648.14 ± 83.13	57.54 ± 6.30	34.27 ± 2.48	3632.39 ± 345.43
r6	–	881.72 ± 52.81	150.73 ± 11.87	17.4 ± 1.85	57.18 ± 7.35	293.64 ± 52.13	1266.84 ± 172.98	103.25 ± 7.55	167.64 ± 5.12	–	2938.4 ± 311.68
p1	687.21 ± 74.98	94.36 ± 11.72	–	6.68 ± 2.28	16.53 ± 1.94	–	394.72 ± 43.09	–	53.32 ± 3.58	21.14 ± 1.07	1273.96 ± 138.65
p2	409.52 ± 41.71	–	–	43.43 ± 5.36	121.52 ± 8.17	–	427.67 ± 42.82	172.29 ± 14.98	64.14 ± 6.06	44.71 ± 3.72	1283.28 ± 122.82

– = not detected; FW = fresh weight; G = glucoside; Is = isorhamnetin; Km = kaempferol; Qu = quercetin; R = rhamnose; Ru = rutinoside; TA = total anthocyanin contents; TF = total flavonol contents

Table 5. Correlation analysis among color parameters, anthocyanins, and flavonols.

CIE *L*a*b** coordinate	Pearson correlation						
RHSCC	Cy	Pn	Is	Qu	Km	TA	TF
*L**	–0.539	–0.832[z]	0.563	0.765[z]	0.449	–0.903[z]	0.718[y]
*a**	0.322	0.075	–0.064	–0.123	–0.252	0.157	–0.113
*b**	–0.411	–0.215	0.316	0.321	0.453	–0.308	0.370

[z]Correlation is significant at the 0.01 level.
[y]Correlation is significant at the 0.05 level.
CIE = French Commission internationale de l'éclairage; Cy = cyanidin; Is = isorhamnetin; Km = kaempferol; Pn = peonidin; Qu = quercetin; RHSCC = Royal Horticultural Society Color Chart; TA = total anthocyanin contents; TF = total flavonol contents.

the existing pigment components to improve flower color. For example, OMT (*O*-methyltransferase) 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*.

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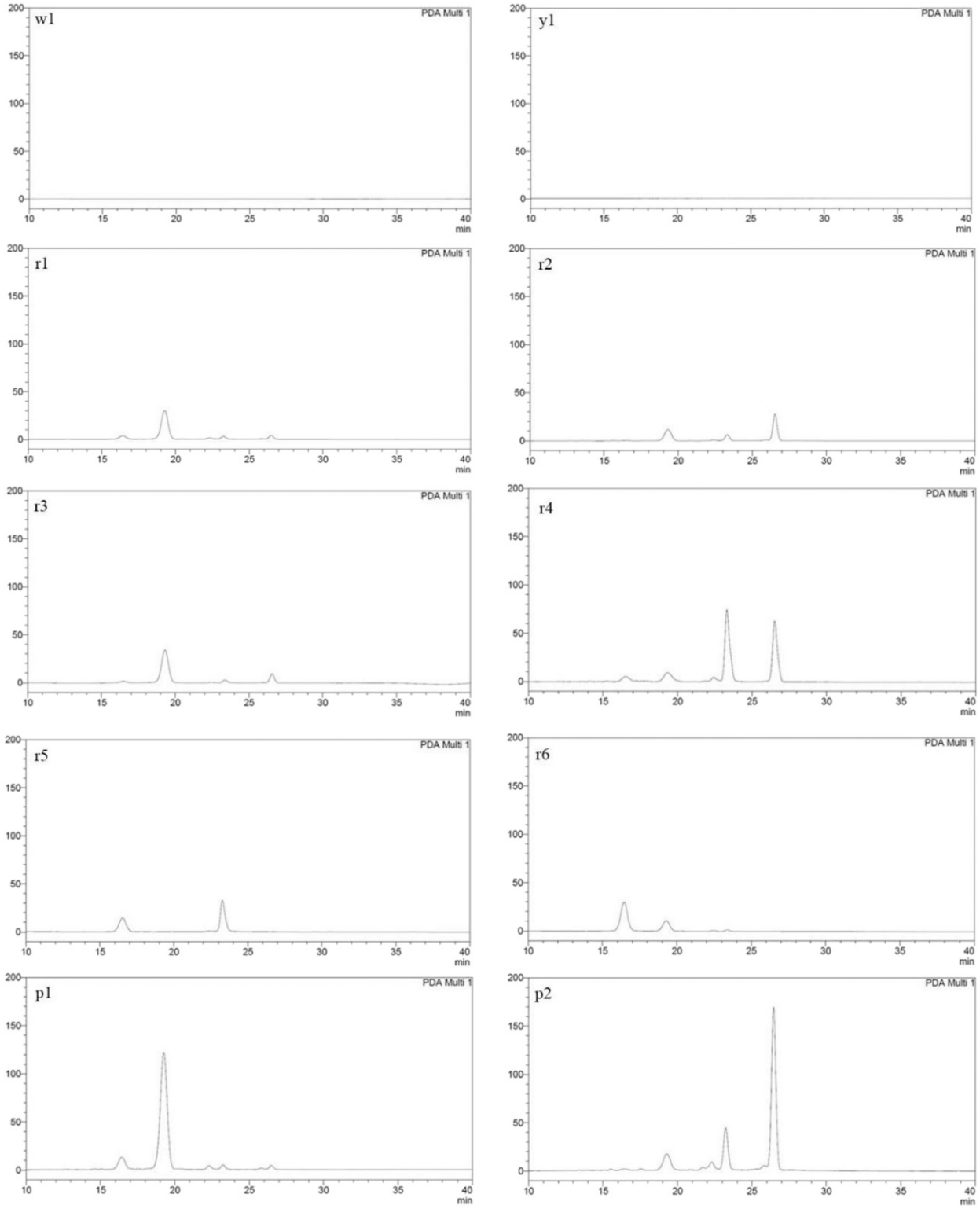
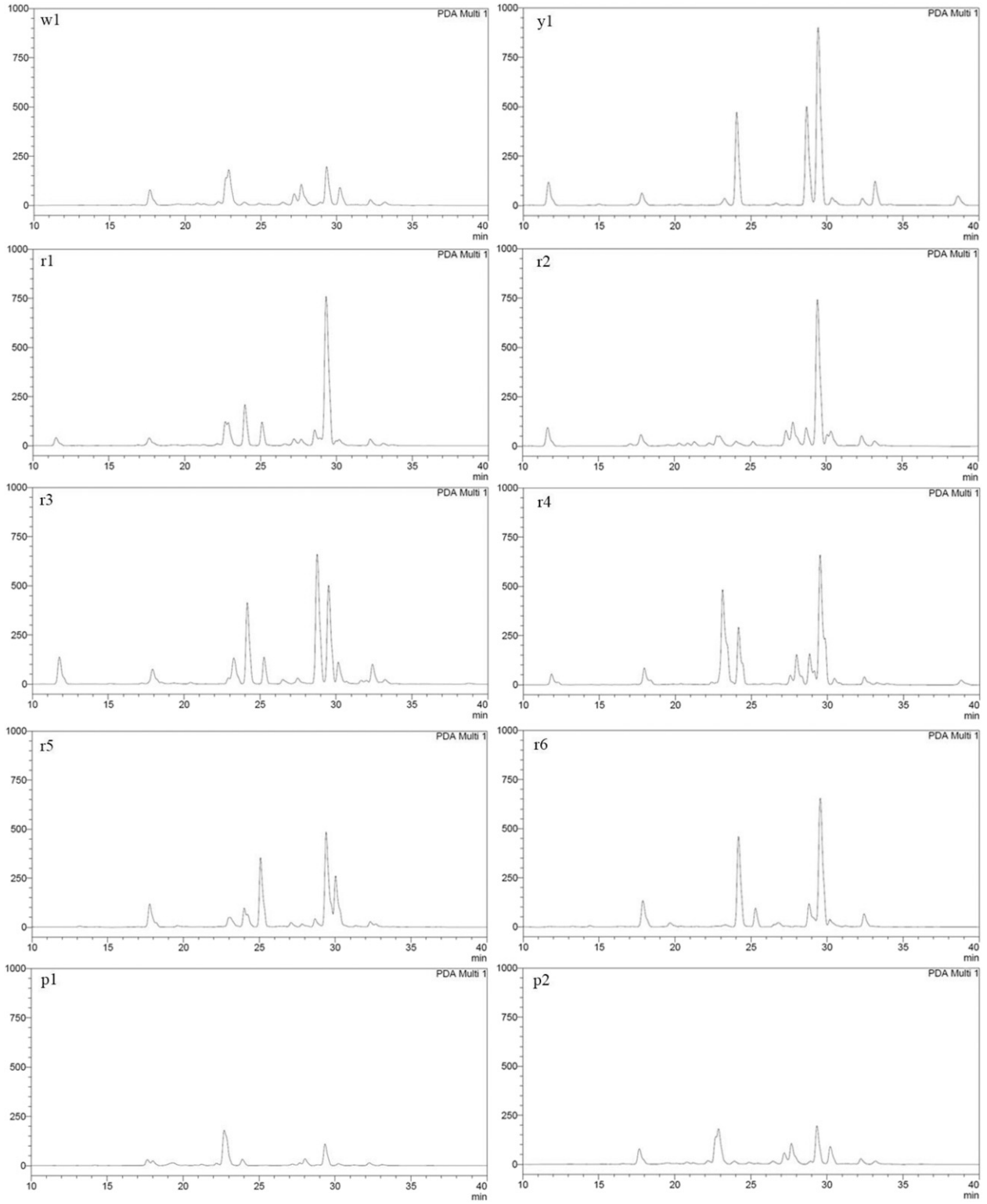


Supplemental Fig. 1. HPLC chromatogram of anthocyanins in *Magnolia.*



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