Quercetin 7-O-β-D glucoside is the key pigment in flower of yellow camellias while *CnFLS* is the main gene

Lina Jiang [1, 2], Zhengqi Fan [1, 2], Ran Tong [1], Jiyuan Li [1,2*], Hengfu Yin [1, 2*]

1. Research Institute of Subtropical Forestry, Chinese Academy of Forestry, Hangzhou, Zhejiang 311400, China

2. Key Laboratory of Forest Genetics and Breeding, Research Institute of Subtropical Forestry, Chinese Academy of Forestry, Hangzhou, Zhejiang 311400, China

* Authors for Correspondence: jiyuan_li@126.com or hfyin@caf.ac.cn

Abstract

Yellow camellias are a group with golden colored flowers in *Camellia*. In order to study the causes of its golden flower color, we selected six species of yellow camellia with different flower colors. We determined the color of the petals at five development stages. Meanwhile, we determined their pigment components by HPLC and determined the expression of related genes by quantitative PCR.

Key words: Yellow camellia, flower colour, pigment, gene

Introduction

Yellow Camellias belong to Sect. Chrysantha Chang in Camellia L. of Theaceae. They are evergreen shrubs up to 5m tall and beautiful form with golden flower color. In Camellia, yellow flower species account for only 7.14%. Among the more than 40,000 camellia cultivars registered by the International Camellia Society, almost all of them have red, pink, and white flowers. Yellow flower cultivars are very rare and precious, with high ornamental value and scientific research value.

Metabolites of flower color mainly include flavonoids, carotenoids and beet pigments. However, the reasons of how the yellow is regulated by floral pigmentation are still unknown. Studies reveal that the yellow coloration of yellow Camellia petals is mainly from the quercetin derivatives, but other researchers regard carotenoids as the main color pigment of the flowers. Zhou cloned genes related to the flower color of *Camellia nitidissima*. However, the specific floral pigment and key regulatory genes of yellow camellias are still unknown.

Here, we determined the color of flower petals of six different flower color species of yellow camellia -- *C.impressinervis. C.chuangtsoensis. C.tunghinensis. C.nitidissima. C .microcarpa.; C.achrysantha.*-- at five development stages. Meanwhile, we determined their pigment components by HPLC and determined the expression of related genes by Qrt-PCR. We tried to determine the main pigment and key regulatory gene of flower color formation of yellow camellias, and analyze the mechanism of flower color formation.

Results

The color of yellow Camellias

We used a colorimeter to measure the flower color data of petals of six yellow camellia species at five developmental stages (Figure 1a b). The hue b* value between 40 and 70 (>0) was yellow and deep. Luminance L* value was between 70-90, relatively bright color; Chromaticity C* was between 40-70, the flower color was more vivid; The hue Angle h was around 90° and was yellow. Through correlation analysis, we found that there was a very significant positive correlation between C* and b*, so the chromaticity of yellow camellias was mainly affected by yellowness. That is, with the increase of yellowness, the bright-colored degree of petals increased. So, the b* was the main index for describing the flower color of yellow Camellias. (Figure 1c)

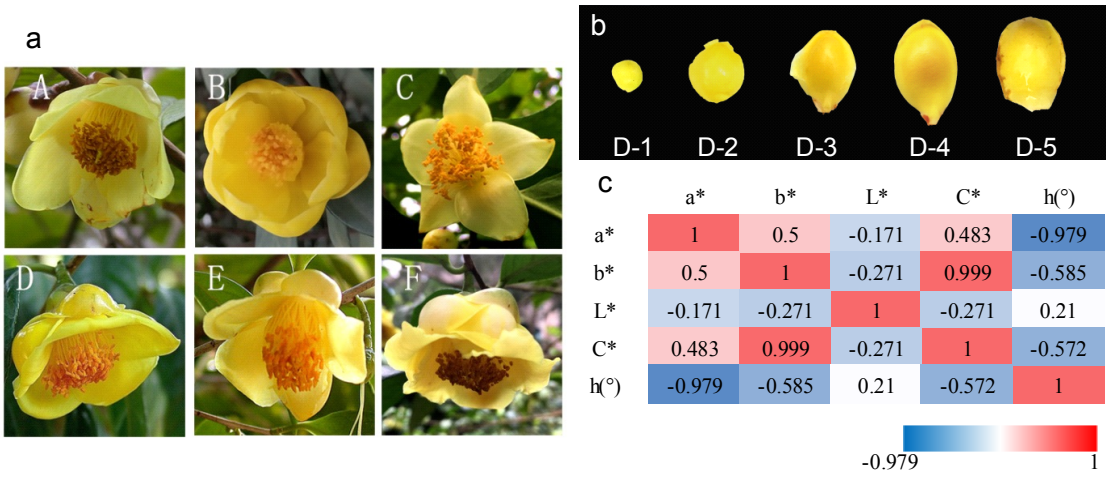

Figure 1 Sampling species and correlation between flower color indexs

a Sampling species. A, *C.impressinervis.* B, *C.chuangtsoensis.* C, *C.tunghinensis.* D, *C.nitidissima.* E, *C microcarpa.* F, *C.achrysantha.* **b,** Petals in five developmental stages. **c,** correlation between flower color indexes.

Determination of petal pigments

We determined the contents of flavonoids, polyphenols, carotenoids and anthocyanins in the petals by HPLC. We found that the main pigments in the petals of yellow camellias were flavonols, mainly Qu7G/ Qu3G etc.

Anthocyanin was mainly Cy3G and only found in some special species. The content of polyphenols in the petals was very high, mainly due to the competition of anthocyanin, which made the petals unable to form red. Carotenoid content was very low, which was not enough to affect the color (Table 1).

Table 1 Content of pigment in petals of yellow Camellias (mg/g)

Number	Ru	Qu	DHQ	DHK	Qu3R	Qu7G	Qu3G	Ka3G	TF	Cy3G	C	EC	ECG	CG	TP	Neo	Vio	Xan	Zea	β-Ca	TC
A-1	0.2768	0.0145	1.0170	0.0103	0.0000	0.1536	0.4446	0.0112	1.9281	0.0038	0.1239	3.8508	0.2392	1.1144	6.9530	0.0008	0.0000	0.0070	0.0000	0.0005	0.0083
A-2	0.2792	0.0139	0.9784	0.0288	0.0000	0.1511	0.4460	0.0111	1.9085	0.0043	0.1021	2.7559	0.1772	1.0463	5.3222	0.0006	0.0001	0.0038	0.0000	0.0010	0.0055
A-3	0.1993	0.0000	0.0205	0.0260	0.0000	0.0895	0.2771	0.0160	0.6283	0.0046	0.0693	1.8147	0.1156	0.6693	3.5908	0.0004	0.0000	0.0011	0.0000	0.0004	0.0019
A-4	0.1761	0.0094	0.0177	0.0263	0.0000	0.0812	0.2553	0.0058	0.5718	0.0057	0.0655	1.5798	0.1011	0.6114	3.0890	0.0004	0.0000	0.0038	0.0000	0.0008	0.0050
A-5	0.1455	0.0058	0.0149	0.0130	0.0000	0.0501	0.2106	0.0051	0.4451	0.0079	0.0503	1.2902	0.0743	0.5119	2.4265	0.0003	0.0001	0.0010	0.0000	0.0001	0.0015
B-1	0.4642	0.0041	0.0000	0.0141	0.0115	1.2759	0.5006	0.0207	2.2912	0.0000	0.1255	2.9467	0.2200	0.0695	4.7839	0.0004	0.0000	0.0034	0.0010	0.0026	0.0074
B-2	0.3973	0.0024	0.0000	0.0252	0.0249	1.5209	0.4988	0.0275	2.4971	0.0000	0.0920	1.6851	0.0367	0.0131	2.8188	0.0005	0.0000	0.0012	0.0011	0.0021	0.0048
B-3	0.2749	0.0021	0.0000	0.0659	0.0279	1.7345	0.5762	0.0443	2.7259	0.0000	0.0782	1.0290	0.0417	0.0515	1.8484	0.0004	0.0000	0.0009	0.0021	0.0009	0.0042
B-4	0.1620	0.0019	0.0000	0.0566	0.0346	1.0280	0.5658	0.0810	1.9300	0.0000	0.0621	0.7636	0.0537	0.0949	1.5120	0.0003	0.0004	0.0002	0.0017	0.0009	0.0035
B-5	0.0918	0.0011	0.0000	0.0475	0.0250	0.6159	0.4808	0.0406	1.3028	0.0000	0.0379	0.4867	0.0224	0.0965	1.0261	0.0002	0.0003	0.0001	0.0014	0.0004	0.0024
C-1	0.0546	0.0039	0.0219	0.1164	0.0245	0.2431	0.2641	0.0675	0.7959	0.0000	0.0352	0.4455	0.1860	0.1772	1.2114	0.0007	0.0010	0.0093	0.0000	0.0004	0.0114
C-2	0.0446	0.0018	0.0180	0.1206	0.0375	0.2526	0.2635	0.0668	0.8054	0.0000	0.0311	0.3061	0.1477	0.1296	0.8954	0.0005	0.0005	0.0055	0.0000	0.0004	0.0069
C-3	0.0283	0.0025	0.0101	0.1029	0.0416	0.2237	0.1713	0.0521	0.6325	0.0000	0.0329	0.2641	0.0685	0.1120	0.7283	0.0004	0.0005	0.0025	0.0000	0.0005	0.0038
C-4	0.0216	0.0030	0.0059	0.0769	0.0280	0.2426	0.1935	0.0412	0.6126	0.0000	0.0194	0.2265	0.0507	0.1019	0.5385	0.0003	0.0013	0.0021	0.0000	0.0003	0.0040
C-5	0.0191	0.0011	0.3038	0.0688	0.0211	0.2418	0.1725	0.0357	0.8639	0.0000	0.0234	0.2258	0.0437	0.0918	0.4937	0.0003	0.0013	0.0015	0.0000	0.0003	0.0034
D-1	0.6043	0.0037	0.0708	0.0116	0.0000	1.2596	0.3405	0.0081	2.2985	0.0000	0.1121	1.7593	0.2890	0.0498	2.6381	0.0005	0.0004	0.0012	0.0000	0.0002	0.0022
D-2	0.4636	0.0017	0.0337	0.0000	0.0000	1.4138	0.2311	0.0130	2.1569	0.0000	0.1088	1.6280	0.2211	0.0681	2.1918	0.0004	0.0003	0.0000	0.0000	0.0001	0.0008
D-3	0.3041	0.0000	0.0143	0.0268	0.0000	1.5726	0.1860	0.0193	2.1232	0.0000	0.0656	0.8405	0.0986	0.0545	1.1708	0.0003	0.0003	0.0000	0.0000	0.0001	0.0008
D-4	0.2552	0.0000	0.0143	0.0212	0.0000	0.3851	0.0843	0.0178	0.7779	0.0000	0.0402	0.7358	0.0608	0.0456	1.0175	0.0003	0.0005	0.0000	0.0000	0.0001	0.0009
D-5	0.1817	0.0000	0.0176	0.0187	0.0000	0.5278	0.0835	0.0130	0.8424	0.0000	0.0465	0.6996	0.0839	0.0072	1.0850	0.0003	0.0004	0.0000	0.0000	0.0001	0.0008
E-1	0.7199	0.0042	0.0779	0.0596	0.0000	1.0901	1.5175	0.0261	3.4953	0.0020	0.0784	1.0549	0.2272	0.0050	1.8609	-	-	-	-	-	0.0000
E-2	0.4535	0.0042	0.0510	0.0245	0.0000	1.0714	1.2275	0.0157	2.8479	0.0018	0.0490	0.6464	0.2029	0.0693	1.2066	-	-	-	-	-	0.0000
E-3	0.3783	0.0017	0.0405	0.0232	0.0000	0.5125	0.7144	0.0154	1.6859	0.0017	0.0000	0.5207	0.1988	0.1259	0.9394	-	-	-	-	-	0.0000
E-4	0.2707	0.0011	0.0229	0.0115	0.0000	0.4778	0.5735	0.0073	1.3649	0.0012	0.0000	0.5741	0.1453	0.2189	1.0114	0.0005	0.0003	0.0015	0.0000	0.0004	0.0027
E-5	0.1648	0.0007	0.0167	0.0095	0.0000	0.3661	0.4195	0.0054	0.9828	0.0011	0.0000	0.5432	0.1933	0.3773	1.1975	-	-	-	-	-	0.0000
F-1	0.4927	0.0083	0.0695	0.0810	0.0028	0.8435	0.8193	0.0484	2.3655	0.0000	0.1650	2.1140	0.1627	0.0632	3.5614	0.0009	0.0000	0.0029	0.0004	0.0003	0.0045
F-2	0.2761	0.0039	0.0290	0.0205	0.0000	0.4965	0.4886	0.0304	1.3450	0.0000	0.0961	1.3043	0.0765	0.0151	2.0463	0.0006	0.0000	0.0017	0.0006	0.0008	0.0038
F-3	0.2410	0.0027	0.0322	0.0199	0.0000	0.4557	0.4329	0.0274	1.2118	0.0000	0.0940	1.2534	0.0915	0.0346	2.0104	0.0006	0.0000	0.0010	0.0007	0.0009	0.0031
F-4	0.2368	0.0017	0.0339	0.0125	0.0000	0.4521	0.4297	0.0260	1.1926	0.0000	0.0928	1.1890	0.0720	0.0294	1.9037	0.0006	0.0000	0.0006	0.0006	0.0007	0.0026
F-5	0.2297	0.0030	0.0327	0.0518	0.0000	0.4556	0.4142	0.0274	1.2143	0.0000	0.0921	1.1538	0.0752	0.0158	1.9053	0.0006	0.0000	0.0012	0.0008	0.0003	0.0029

Note: - , the sample is insufficient and not tested.

In order to study the main pigment formed by the color of yellow camellias, the correlation between the color index b* and the pigment measured was analyzed. We found

that, among flavonoids, b* was significantly positively correlated with Qu7G and Qu3R and negatively correlated with Qu and DHQ. Anthocyanin and polyphenols were negatively correlated with b*. In carotenoids, Zea and β-Ca were positively correlated with b*, while the other pigments were negatively correlated (figure 2). At the same time, we calculated the multivariate progressive regression equation of b* and the measured pigment, which was b*=22.658*x*1-37.542*x*2+9367.039*x*3-32593.441*x*4+58.455, *R*=0.911. *x*1, Qu7G. *x*2, Ru. *x*3, β-Ca. *x*4, Neo. That signified that b* was positively correlated with Qu7G.

	b	Ru	Qu	DHQ	DHK	Qu3R	Qu7G	Qu3G	Ka3G	TF	Cy3G	C	EC	ECG	CG	TP	Neo	Vio	Xan	Zea	ß-Ca	TC
b	1	-0.012	-0.422	-0.319	0	0.485	0.628	-0.098	0.294	0.267	-0.412	-0.058	-0.134	-0.376	-0.372	-0.174	-0.533	-0.026	-0.384	0.67	0.465	0.005
Ru	-0.012	1	0.166	0.023	-0.413	-0.512	0.628	0.656	-0.377	0.833	-0.023	0.557	0.443	0.615	-0.159	0.341	0.34	-0.534	-0.202	0.108	0.298	-0.292
Qu	-0.422	0.166	1	0.778	-0.053	-0.212	-0.253	0.18	-0.175	0.183	0.53	0.502	0.722	0.366	0.726	0.785	0.581	-0.29	0.573	-0.178	0.162	0.48
DHQ	-0.319	0.023	0.778	1	-0.136	-0.188	-0.284	0.015	-0.222	0.133	0.357	0.294	0.626	0.308	0.774	0.689	0.396	-0.085	0.422	-0.244	0.033	0.342
DHK	0	-0.413	-0.053	-0.136	1	0.752	-0.157	-0.008	0.833	-0.158	-0.3	-0.201	-0.426	-0.207	-0.216	-0.342	0.124	0.514	0.461	0.042	-0.165	0.466
Qu3R	0.485	-0.512	-0.212	-0.188	0.752	1	0.055	-0.181	0.815	-0.137	-0.38	-0.276	-0.394	-0.414	-0.245	-0.331	-0.258	0.469	0.193	0.357	0.172	0.429
Qu7G	0.628	0.628	-0.253	-0.284	-0.157	0.055	1	0.348	0.05	0.801	-0.446	0.361	0.088	0.136	-0.506	-0.027	-0.142	-0.209	-0.385	0.526	0.353	-0.2
Qu3G	-0.098	0.656	0.18	0.015	-0.008	-0.181	0.348	1	0.007	0.747	0.032	0.181	0.089	0.375	-0.136	0.089	0.527	-0.506	0.055	0.61	0.442	-0.212
Ka3G	0.294	-0.377	-0.175	-0.222	0.833	0.815	0.05	0.007	1	-0.044	-0.488	-0.062	-0.367	-0.331	-0.357	-0.319	0.052	0.384	0.257	0.411	0.027	0.453
TF	0.267	0.833	0.183	0.133	-0.158	-0.137	0.801	0.747	-0.044	1	-0.189	0.494	0.349	0.435	-0.181	0.281	0.256	-0.385	-0.118	0.494	0.446	-0.124
Cy3G	-0.412	-0.023	0.53	0.357	-0.3	-0.38	-0.446	0.032	-0.488	-0.189	1	-0.01	0.364	0.142	0.762	0.455	0.001	-0.315	0.185	-0.33	-0.087	-0.033
C	-0.058	0.557	0.502	0.294	-0.201	-0.276	0.361	0.181	-0.062	0.494	-0.01	1	0.793	0.261	0.108	0.721	0.587	-0.621	0.052	0.209	0.356	0.262
EC	-0.134	0.443	0.722	0.626	-0.426	-0.394	0.088	0.089	-0.367	0.349	0.364	0.793	1	0.434	0.59	0.983	0.506	-0.601	0.276	-0.012	0.43	0.358
ECG	-0.376	0.615	0.366	0.308	-0.207	-0.414	0.136	0.375	-0.331	0.435	0.142	0.261	0.434	1	0.253	0.413	0.504	-0.139	0.483	-0.402	0.026	0.072
CG	-0.372	-0.159	0.726	0.774	-0.216	-0.245	-0.506	-0.136	-0.357	-0.181	0.762	0.108	0.59	0.253	1	0.701	0.252	-0.236	0.453	-0.343	-0.015	0.301
TP	-0.174	0.341	0.785	0.689	-0.342	-0.331	-0.027	0.089	-0.319	0.281	0.455	0.721	0.983	0.413	0.701	1	0.519	-0.569	0.375	-0.031	0.416	0.425
Neo	-0.533	0.34	0.581	0.396	0.124	-0.258	-0.142	0.527	0.052	0.256	0.001	0.587	0.506	0.504	0.252	0.519	1	-0.335	0.604	-0.115	0.118	0.565
Vio	-0.026	-0.534	-0.29	-0.085	0.514	0.469	-0.209	-0.506	0.384	-0.385	-0.315	-0.621	-0.601	-0.139	-0.236	-0.569	-0.335	1	0.148	-0.325	-0.355	0.105
Xan	-0.384	-0.202	0.573	0.422	0.461	0.193	-0.385	0.055	0.257	-0.118	0.185	0.052	0.276	0.483	0.453	0.375	0.604	0.148	1	-0.297	0.112	0.928
Zea	0.67	0.108	-0.178	-0.244	0.042	0.357	0.526	0.61	0.411	0.494	-0.33	0.209	-0.012	-0.402	-0.343	-0.031	-0.115	-0.325	-0.297	1	0.491	0.027
ß-Ca	0.465	0.298	0.162	0.033	-0.165	0.172	0.353	0.442	0.027	0.446	-0.087	0.356	0.43	0.026	-0.015	0.416	0.118	-0.355	0.112	0.491	1	0.406
TC	0.005	-0.292	0.48	0.342	0.466	0.429	-0.2	-0.212	0.453	-0.124	-0.033	0.262	0.358	0.072	0.301	0.425	0.565	0.105	0.928	0.027	0.406	1

-0.62 1

Figure 2 Correlation between b* and main pigment of petals

Expression levels of major functional genes

In order to further determine the regulatory genes of flower color in yellow camellias, we used quantitative PCR to determine the expression levels of main functional genes related to flavonol synthesis in petals of six species at five developmental stages.

We found that the expression of *FLS* was basically consistent with the change law of b*, the expression of *FLS* in different species tended to increase first then decrease or decrease all the time, which was close to the change trend of flower color. The yellower the flower color, the higher the expression of *FLS*. And *FLS* had a synergistic effect with *F3'H* and their expression patterns were similar. The change law of *DFR was* negatively correlated with b* and the relationship *F3'5'H* and b* was not obvious (Figure 3).

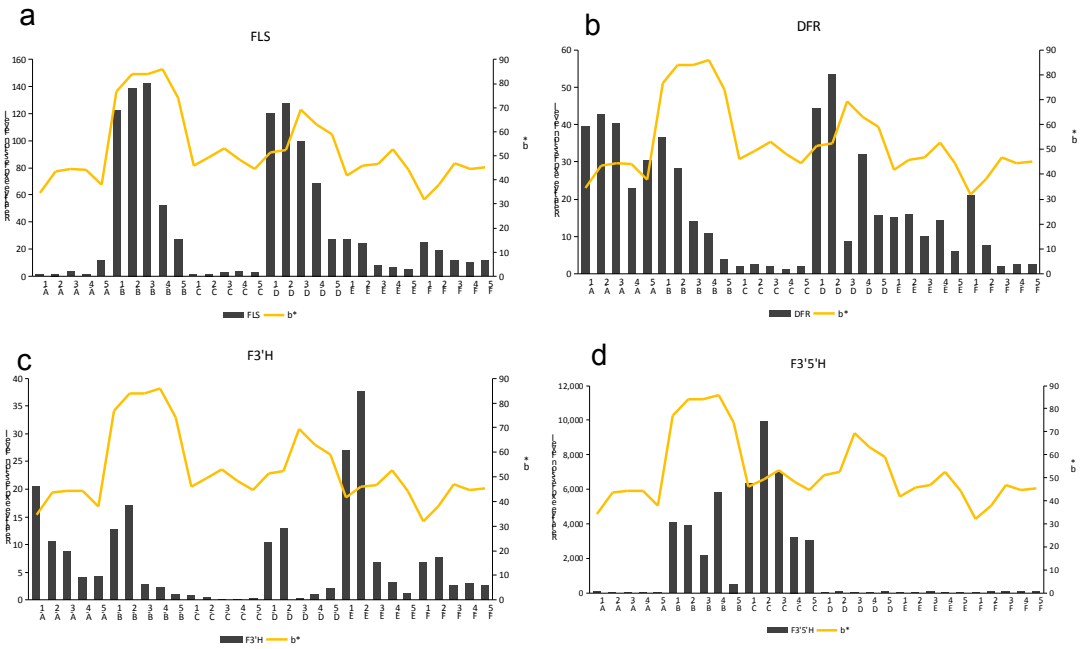

Figure 3 expression levels of key functional genes

Discussion

Our results show that the main color material in the petals of yellow camellias was flavonol, among which Qu7G is the most important pigment. The content of carotenoids was very low and not enough to affect the color (Table 1). This was consistent with the study of Tanikawa et al. (2008). Quercetin derivatives were the main constituent of the flower color of yellow camellias. But unlike the Zhou et al. (2017) study, Tanikawa believed carotenoids were responsible for the yellow color of yellow Camellias. At the same time, anthocyanin and polyphenols were negatively correlated with the yellow of the petals of yellow Camellias.

We used quantitative PCR to find that *FLS* gene was probably the key functional gene regulating the formation of flower color in yellow camellias (Figure 3), and its expression pattern is very similar to the change trend of b*. However, Zhou et al. (2012) believed that *FLS* was not the main regulatory gene, because *CnFLS* and *CnNSY* genes of yellow camellias were overexpressed in tobacco, *CnFLS* gene did not make tobacco flowers significantly yellow, while *CnNSY* formed significant yellow flowers. We determined the genes related to *FLS* and found that the expression of *F3'H* and *DFR* interfered with *FLS*. Therefore, overexpression of *FLS* alone may make it more difficult to obtain obvious yellow flowers.

Materials and methods

Plant materials and growth conditions

Yellow camellias tissues were collected from the National Camellia Germplasm Resource Bank (Guangxi, China, E 108°20'53", N 22°49'11", 75m above sea level) in Nanning, Guangxi province. The materials were frozen with liquid nitrogen and stored at -80□ for

later use.

We used the petals of six species (*C.achrysantha*, *C.chuangtsoensis, C.impressinervis, C.microcarpa, C.nitidissima,* and *C.tunghinensis*) at five development stages (small bud, big bud, half-open flowers, blooming flower and withering flowers） to conduct experiments.

Measuring petal color

We used the NF555 colorimeter (Nippon Denshoku Industries Co., Ltd, Japan) to detect the color indicator of petals. The flower color was measured from the outer petal to the inner petal, and six biological duplicates were set for each species. According to the L*, a* and b* expanded color system method developed by the international commission on illumination (CIE), the lightness of petals was determined by L*, hue a* and b*, chroma C* and hue Angle h.

High-performance liquid chromatography analysis

We did HPLC to analysis flavonoids, polyphenols and anthocyanins constituent. We ground fresh sample 0.6 g weight in liquid nitrogen, added 5 mL extraction solution (methanol: water: formic acid: trifluoroacetic acid =70:27:2:1). Then extracted in dark for 24 hours, shaking in the middle for a few times. After extraction, we filtered with absorbent cotton to remove residue and passed the samples through organic microporous filter membrane (0.22 cm) (ANPEL Laboratory Technologies (Shanghai) Inc., China). The filtrate was put through machine analysis.

We used Agilent Technologies 1260 Infinty (Agilent Technologies, Inc., Germany) and Waters SunFire C18 column (4.6×250 mm, 5μm) (Waters Co., America) for the HPLC. The column temperature was 30□. The flow rate was 1.0 mL/min, the injection volume was 10μL. The elution mobile phases were A: 2% formic acid solution and B: pure acetonitrile. The elution procedure for flavonoids: 0-5 min, 20% B; 5-15 min, 20% up to 40% B; 15-20 min, 40% up to 60% B; 20-20.2 min, 60% down to 20% B; 20.2-24 min, 20% B.

The detection wavelength of flavonoids was 350 nm. The elution procedure for polyphenols: 0-9 min, 98% down to 90.7% B; 9-15 min, 90.7% B; 15-20.5 min, 90.7% down to 85% B; 20.5-29.5 min, 85% down to 75% B; 29.5-30 min, 75% up to 98% B; 30-34 min, 98% B. The detection wavelength of polyphenols was 278 nm.

Quantitative PCR analysis

We chose 18S as the reference gene (Table 2) for quantitative PCR analysis. Using PrimeScript RT reagent Kit with gDNA Eraser (RR047, TaKaRa, Japan), we synthesized the cDNA first strand. According to the SYBR Prime Ex Tap □ (Tli RNaseH Plus) (RR420, TaKaRa, Japan), we built a quantitative PCR reaction system operation. The reaction was performed on QuantStudio® 7 Flex (Applied Biosystem, America) and the reaction procedure was as follows: pre-denaturation at 95□ for 30s; 98□ 5 s, 60□ 34s, 40 cycles; 95□ 15s, 60□ 1min,

95□ 15s. The relative expression quantity of *CnDFR* was measured in different organs and different development period of by $2^{(-\Delta\Delta CT)}$ method (Livak and Schmittgen, 2000).

Table 2 Primer list for quantitive PCR

Primer name	Sequence
18S RNA-F	GACTCAACACGGGGAAACTTACC
18S RNA-R	CAGACAAATCGCTCCACCAAC
FLS -F	TGGAAGGCAAGAAAGGATGGG
FLS -R	CCACCGGAACAACTTGTCTGC
DFR -F	ACTGTGGAAGGCGGATTTGA
DFR -R	CGTTGATTGTCGGCTTGATTAC
F3'H -F	ACAAGCGGCAGCAGTGAACC
F3'H -R	ACCGAACACCCTGTGACCCA
F3'5'H -F	GTGTGGGAAAGGCCATTAGA
F3'5'H -R	GCGAGTCCGAAACTCTCATC

Acknowledgements

This work was supported by the National key projects for international scientific and technological innovation cooperation among governments (2016YFE0126100) and the special funds for basic scientific research expenses of public welfare research institutes of the Chinese academy of forestry (CAFYBB2017ZF001).

References

Zhang H.D., Ren S.X., 1998. Flora of China [M], 49(3). Beijing: Science press.

Savige T., 1993. The International Camellia Register [M]. Vol one, INT CAME SCI. NSW, Australia: Fine Arts Press PTY: 1-1104.

Savige T., 1997. The International Camellia Register [M]. Supplement to Vol one and two, INT CAME SCI.Sydney, Australia: Fine Arts Press PTY: 1-386.

Haydon N., 2011. The International Camellia Register [M]. Second supplement, 1990-2010, INT CAME SCI. Wimborne Dorset, U.K: The Minster Press: 1-472.

Zhou L., Wang Y., Peng ZH.H., 2009. Advances in Study on Formation Mechanism and Genetic Engineering of Yellow Flowers [J]. SCIENTIA SILVAE SINICAE, 2009, 45(2): 111-119.

Tanaka Y., Ohmiya A., 2008. Seeing is believing: Engineering anrhocyanin and carotenoid biosynthetic pathways [J]. Curr Opin Bioteehnol, 19:190-197.

Hwang Y.J., Yoshikawa K., Miyajima I., Okubo H., 1992. Flower colors and pigments in hybrids with Camellia chrysantha. Sci. Hortic 51, 251-259.

Tanikawa N., Kashiwabara T., Hokura A., Abe T., Shibata M., Nakayama M., 2008. A Peculiar Yellow Flower Coloration of Camellia Using Aluminum-flavonoid Interaction. J. Japan. Soc. Hort. Sci. 77 (4): 402–407.

Zhou X., Li J., Yin H., Fan Z., Li X., 2013. Cloning of Camellia nitidissima flavonol synthase cDNA and construction of sense, RNA interference expression vectors. Bulletin of Botanical Research 33, 58-65.

Zhou X., Li J., Zhu Y., Ni S., Chen J., Feng X., Zhang Y., Li S., Zhu H., Wen Y., 2017. De novo assembly of the Camellia nitidissima transcriptome reveals key genes of flower pigment biosynthesis. Frontiers in Plant Science 8, 1545.

Zhou X., 2012. Clonging and function reasearch of pigment genes from Camallia nitidissma [D]. Beijing: Chinese Academy of Forestry Sciences: 39-58.

Livak K.; Schmittgen T., 2000. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-△△Ct Method. Methods 25.