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International Journal of Current Research
Vol. 7, Issue, 07, pp.17683-17688, July, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

THE BIOLOGICAL PECULIARITIES COLORATION OF THE *JAPANESE CAMELLIA* FLOWER

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ARTICLE INFO

Article History:
Received 22nd April, 2015
Received in revised form
07th May, 2015
Accepted 09th June, 2015
Published online 28th July, 2015

Key words:

Camellia,
Flavonoid,
Flower,
Cyanidine - 3 –glucoside

ABSTRACT

The variety of *Japanese camellia* is to perform qualitative and quantitative analyzesof anthocyanins pigments in the flowers of various colors are reviewed. Four variety of *Japanese camellias* of different colors were taken to analyze from the Batumi Botanic Garden *Camellia japonica*,, Margaret Walker"; *Camellia japonica*,, Marchioness of Salisbury"; *Camellia japonica*,, Anemonaeflora"; and *Camellia japonica*,, Takayama". Therefore, based on the research results it could be concluded that anthocyanins composition plays dominant role in flower colorarion of Japanese camellia. The highest amount of them is detected in Camellia variety Takayama (760 mg / kg); The lowest amount is found in variety Margaret Walker - A (30 mg / kg) and Margaret Walker - B (60 mg/kg). The anthocyanins cyanidin-3-diglucosideis dominant one in both variation (A and B) (85 and 75 %, respectively).

Citation: Dali Kamadadze, Davit Barata Svili, Elza Kamadadze, Aleko Kalandia and Mariam Metreveli, 2015. "The biological peculiarities coloration of the Japanese camellia flower", *International Journal of Current Research,* 7, (7), 17683-17688.

INTRODUCTION

The flowering periods, duration, flower structure, formation and coloration are the most important issues of the Japanese breeding. The Camellia in the specific soil and climatic conditions of the Subtropical zone is characterized with the certain peculiarities regarding development of the vegetative and reproductive organs. It is well adapted to the local environment, as smooth winter flowering proves. The species and forms of Japanese camellia are characterized with red, pink, white, purple and other colors. A combination of various colors is also genetically peculiar for them. Yellow flower that has been breeding for half a century has not been cultivated yet (Park, 2000; Shinichi *et al*., 2004 and Tanaka *et al*., 2008). Study of a flower pigmentation is very significant in vegetation breeding and is the key in the flower color variation in Genetic engineering (Gou *et al*., 2011). It is well known that the pigments and flavonoids determine the flower pigmentation. They are characterized with diverse and universal function in the plant world and have the main input in synthesis of anthocyanins, playing the key role in colouration (Buer *et al*., 2010). Anthocyanins or coloured glycosides are found in petals, stamens, fruit and other tissues of other organs. The review of the scientific literature on Camellias revealed informationabout high content of flavonoidsin different types of camellias (Camellia reticulate, C. Japonica, C. Nitidissima, C.salienensis, C.hongkongensis, Camellia sinensis). Its composition is in peak when the flower budis fully open (Xing-Wen Zneu *et al*., 2013; Ikuo Miyajima *et al*., 1985; Terahara *et al*., 2001; Yueh-Jiang Hwang *et al*., 1992; Li *et al*., 2007 and Xi-Feng Teng *et al*., 2008). Two basic anthocyanins were identified in the petals of the wild ancestor of Japanese camellia, mainly cyanidin-3-glucoside and cyanidin-3-galactoside, which which participate in the formation of the red color of the flower (Nobumine TATEISHI *et al*., 2010).

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It is to be an interesting challenge to identify the chemical composition of the Japanese Camellia variety flower of intense coloration and to study biological peculiarities of coloration formation. Based on the above stated information, we decided to experimentally identify the color formation and biological features of the most interesting flowers in the Japanese camellia variety.

Experimental

The aim of our study is to perform qualitative and quantitative analyzesof anthocyanins pigments in the flowers of various colors of the Japanese camellia species. Four variety of Japanese camellias of different colors were taken to analyze from the Batumi Botanic Garden *Camellia japonica*„ Margaret Walker"; *Camellia japonica*„ Marchioness of Salisbury"; *Camellia japonica*„ Anemonaeflora"; and *Camellia japonica* „Takayama";

The following color variations of the research species were included in the study:

1. The variety, „ Margaret Walker" (four variations):

A- White Petals with light red spots;
B- White Petals with dark red spots;
C- Light red petals
D- Red petals.

2. The variety „ Marchioness of Salisbury"–Red Petals with white spots;
3. The variety „ Anemonaeflora"- Petls of light pink colour;
4. The variety „Takayama"-Petals with red flowers.

Frozen samplers were blended to a puree using commercial blender. Subsamples (5 g) of puree were then homogenized for 1 min in 20 ml of extraction solution containing by 70 %-ethanol (the ratio of sample and extracting agent is 1:15), first at room temperature, and then in the boiling chamber with the same solvent. Extracts were filtered and the filtrates were centrifuged for 5 min at 5000 rpm. All samples were passed through o,45 µm filters (Acrodist LC PVDF Syringe Filters Waters) prior to HPLC analysis.

In cases of flavonols extraction of samples was carried out with 70 % ethanol. For qualitative analysis following reagents have been used, and their composition has been defined by spectric method by setting standard caliber curve. Flavonols quantified as - rutin (400 nm), anthocyanins quantified as – cyanidine-3-glucoside (510 nm). The results are shown in Tables 1. HPLC analysis of flavonoids Samples (10 µL) were analyzed using a Waters HPLC system equipped with a model 525 pump, UV/Vis detector. Separation was carried out using a 4,6 X 150 Symmetry C 18 column (Waters Corp, Milford, MA, USA) with a 3,9 mm X 20 mm C 18 guard column. The mobile phase was a linear gradient of 1 % Phosphoric acid (A) and Acetonitril (B) from 0 % to 10 % B for 0-10 min, 10 % - 14B for 10-25 min, 14-20 % B for 25-40 min, 20-0 % B for 40-45 min, at 1,0 ml min-1. The system was equilibrated for 10 min at the initial gradient prior to each injection. Detection wavelengths used were 510 nm for anthocyanins and 370 nm for flavonols. Total anthocyanin derivatives were calculated as the sum of individual anthocyanin. Flavonols were quantified as rutin equivalents.

RESULTS AND DISCUSSION

During the study of anthocyanins of the *Japanese camellia* flowers at least four compounds were recognized in all research species. At this stage of the research two dominant compounds were identified: cyanidin-3-glucoside (retention time 24.6 min) and cyanidin-3-diglucoside (retention time 27.3 min). The composition of these compounds determines the coloration of a flower, which ranges according to the species and the variations. According to coloration of a petal these variations were conditionally subdivided into four groups:

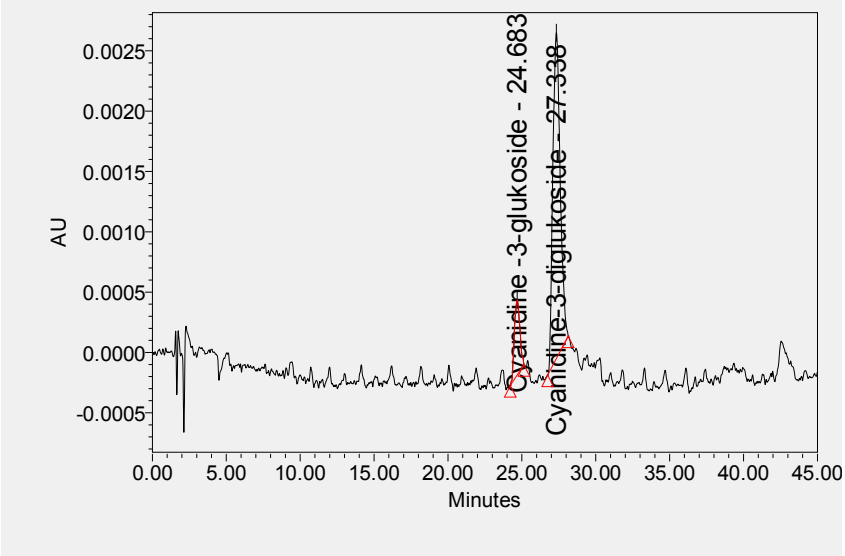
Margaret Walker"-A petals are white with red spots. The flower of this variation contains low amount of anthocyanins (30 mg/kg). The cyanidin-3-diglucoside is dominant (up to 85 %) as shown in Picture 1.1; Table 1.1; The petals of the of „Margaret Walker"-B flower are white with dark read spots. The composition of anthocyanins is higher – 60 mg/kg. the cyanidin-3-diglucoside is still dominant there (up to 75 %) as shown in Picture 1.2 Table 1.2. The composition of anthocyanins in the flower of „Margaret Walker"-C Creaches 410 mg/kg. The dominant anthocyanin is cyanidin-3-diglucoside - 88 % as shown in Picture 1.3; Table 1.3. „Margaret Walker"-D Dcontains 560 mg/kg anthocyanin and The dominant anthocyanin is still cyanidin-3-diglucoside - 88 % as shown in Picture 1.4; Table 1.4.

The variety „Marchioness of Salisbury"- petals are red. Composition of anthocyanins – 510 mg/kg. Dominant is still cyanidin-3-diglucoside – 65 % as shown in Picture 2, Table 2.

The variety „Anemonaeflora" contains less amount of anthocyanins (360 mg/kg); the dominant compound is altered there and it is cyanidin-3-glucoside (65 %). The petals are red as shown in Picture 3; Table 3.

Particularly large amount of anthocyaninsare found in the petals of the „Takayama" variety (730 mg/kg) as shown in Picture 4; Table 4. The composition of cyanidin-3-glucosideand cyanidin-3-diglucosideis 36 & and 57 %, respectively. Petals are purple red color.

Therefore, based on the research results it could be concluded that anthocyanins composition plays dominant role in flower colorarion of *Japanese camellia*. The highest amount of them is detected in Camellia variety „Takayama" (760 mg/kg); The lowest amount is found in variety „Margaret Walker"-A (30 mg/kg) and „Margaret Walker" B (60 mg/kg). The anthocyanins cyanidin-3-diglucosideis dominant one in both variation (A and B) (85 and 75 %, respectively). Composition and spectrum of anthocyanins in camellia variety is genetically programmed process. Camellia as a cross-pollinating plant and compound heterozygote is highly tend to impact of the environmental factors (temperature, nutrition, chemical and radio toxics, etc).

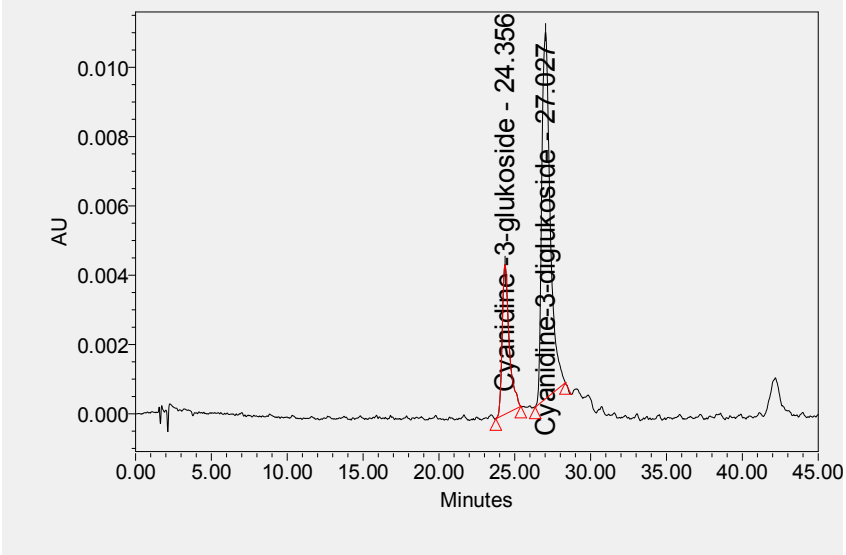


Picture 1.1. The flower anthocyanins chromatogram of the Margaret Walker-A variety

Table 1.1. The chromatographic characteristics of the Margaret Walker-A flower anthocyanins

	SampleName	Acq Method Set	Injection Volume	Channel Description	ColumnType
3	Margaret Walker -A	Anthociane CH3CN	20.00	W2489 ChB 524nm	C 18, 5 µm

	Name	Retention Time	Area	% Area	Amount	Amount б.ð.	Units
1	Cyanidine -3-glukoside	24.683	15991	14.57	43,8	4,38	mg/kg
2	Peak2	25.610					
3	Cyanidine-3-diglukoside	27.338	93738	85.43	256	25,6	mg/kg
	Total Anthocyans				310	30	mg/kg

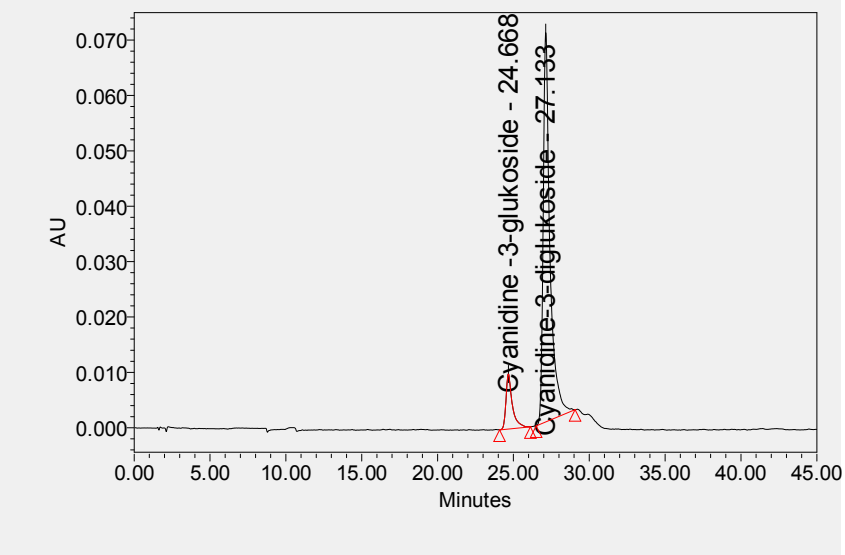


Picture 1.2 – the flower anthocyanins chromatogram of the Margaret Walker-B variety

Table 1.2 -chromatographic characteristics of the Margaret Walker-B flower anthocyanins

	Sample Name	Acq Method Set	Injection Volume	Channel Description	ColumnType
2	Margaret Walker-B	Anthociane CH3CN	20.00	W2489 ChB 524nm	C 18, 5 µm

	Name	Retention Time	Area	% Area	Amount	Amount б.ð	Units
1	Cyanidine -3-glukoside	24.356	138723	25.20	150	15	mg/kg
2	Peak2	25.610					
3	Cyanidine-3-diglukoside	27.027	411867	74.80	450	45	mg/kg
	Total Anthocyans				600	60	mg/kg

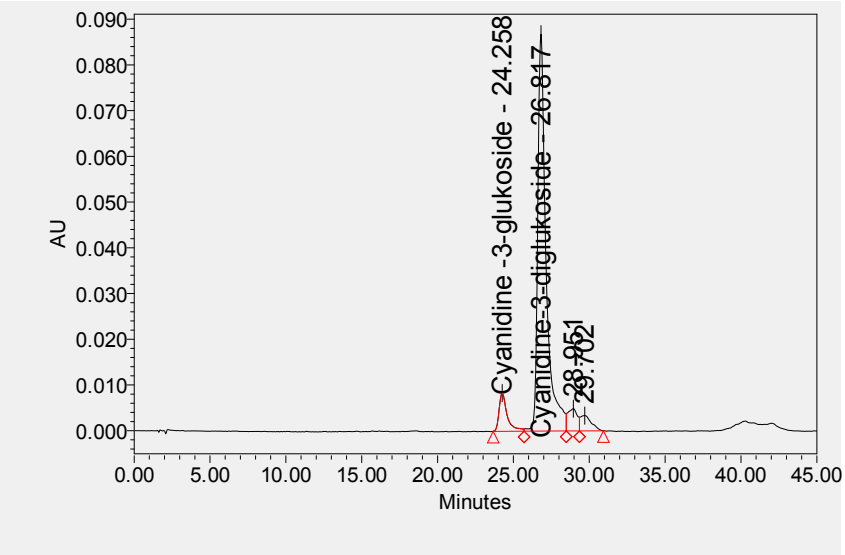


Picture 1.3. The flower anthocyanins chromatogram of the Margaret Walker-C variety

Table 1.3. The chromatographic characteristics of the Margaret Walker-C flower anthocyanins

	SampleName	Acq Method Set	Injection Volume	Channel Description	ColumnType
4	Margaret Walker-C	Anthociane CH3CN	20.00	W2489 ChB 524nm	C 18, 5 µm

	Name	Retention Time	Area	% Area	Amount	Amount б.ð.	Units
1	Cyanidine -3-glukoside	24.668	289074	11.61	457,8	45,7	mg/kg
2	Peak2	25.610					
3	Cyanidine-3-diglukoside	27.133	2201321	88.39	3624,4	362,4	mg/kg
	Total Anthocyans				4100	410	mg/kg

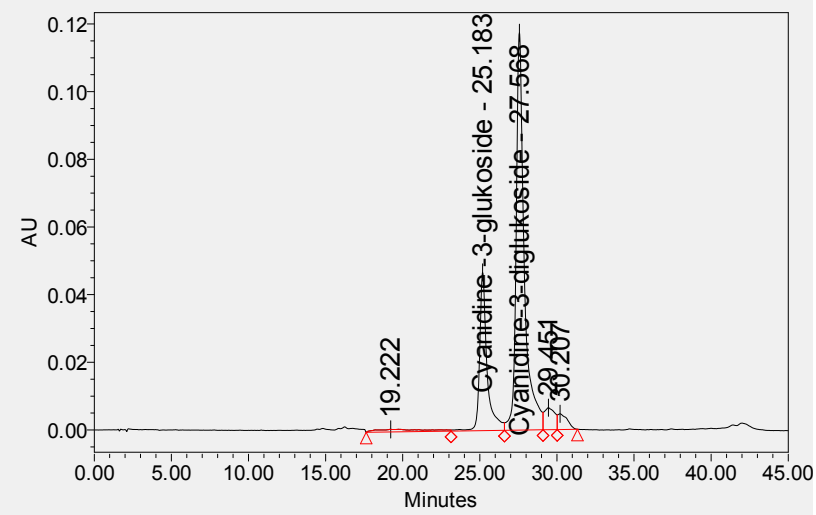


Picture 1.4. The flower anthocyanins chromatogram of the Margaret Walker-D variety

Table 1.4. The chromatographic characteristics of the Margaret Walker-D flower anthocyanins

	SampleName	Acq Method Set	Injection Volume	Channel Description	ColumnType
5	Margaret Walker-D	Anthociane CH3CN	20.00	W2489 ChB 524nm	C 18, 5 µm

	Name	Retention Time	Area	% Area	Amount	Amount б.ð.	Units
1	Cyanidine -3-glukoside	24.258	311251	7.72	347,4	43,2	mg/kg
2	Peak2	25.610					
3	Cyanidine-3-diglukoside	26.817	3333439	82.69	3722	463,1	mg/kg
4		28.951	214374	5.32			
5		29.702	172200	4.27			
	Total Anthocyans				4500	560	mg/kg

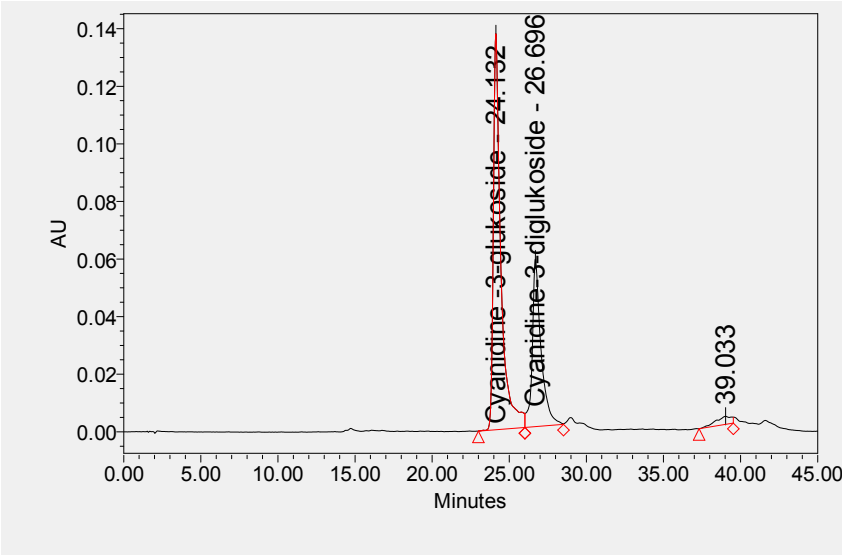


Picture 2. The flower anthocyanins chromatogram of the Marchioness of Salisbury variety

Table 2. The chromatographic characteristics of the Marchioness of Salisbury flower anthocyanins

	SampleName	Acq Method Set	Injection Volume	Channel Description	ColumnType
7	Marchioness of Salisbury	Anthociane CH3CN	20.00	W2489 ChB 524nm	C 18, 5 µm

	Name	Retention Time	Area	% Area	Amount	Amount б.д.	Units
1		19.222	169784	2.57			
2	Cyanidine -3-glukoside	25.183	1570076	23.81	1381	121,4	mg/kg
3	Peak2	25.610					
4	Cyanidine-3-diglukoside	27.568	4343903	65.88	3816,4	336,1	mg/kg
5		29.451	308749	4.68			
6		30.207	201437	3.05			
	Total Anthocyans				5800	510	mg/kg

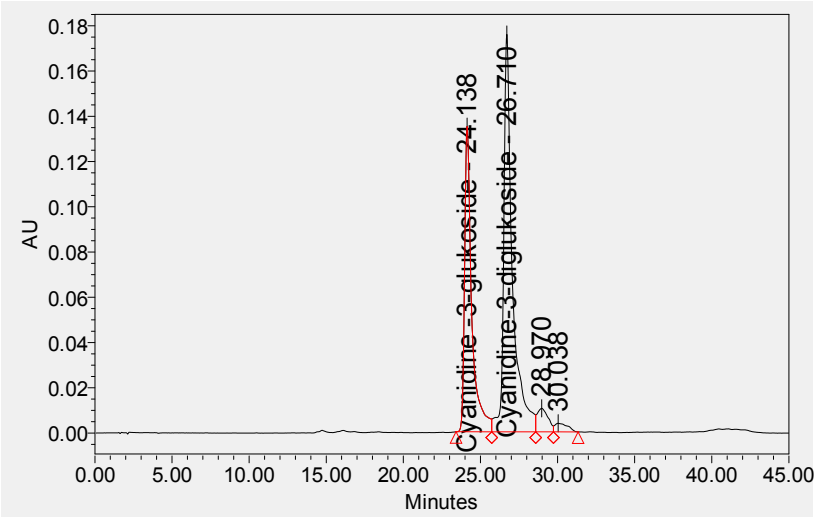


Picture 3. The flower anthocyanins chromatogram of the Anemonaeflora variety

Table 3. The chromatographic characteristics of the Anemonaeflora flower anthocyanins

	SampleName	Acq Method Set	Injection Volume	Channel Description	ColumnType
9	Anemonaeflora	Anthociane CH3CN	20.00	W2489 ChB 524nm	C 18, 5 µm

	Name	Retention Time	Area	% Area	Amount	Amount б.д.	Units
1	Cyanidine -3-glukoside	24.132	4701546	65.20	2999,2	234,7	mg/kg
2	Peak2	25.610					
3	Cyanidine-3-diglukoside	26.696	2308862	32.02	1473,0	115,2	mg/kg
4		39.033	200791	2.78			
	Total Anthocyans				4600	360	mg/kg



Picture 4. The flower anthocyanins chromatogram of the Takayama species

Table 4. The chromatographic characteristics of the Takayama flower anthocyanins

	SampleName	Acq Method Set	Injection Volume	Channel Description	ColumnType
6	Takayama	Anthociane CH3CN	20.00	W2489 ChB 524nm	C 18, 5 µm

	Name	Retention Time	Area	% Area	Amount	Amount б.д.	Units
1	Cyanidine -3-glukoside	24.138	4374551	36.34	2871	265,3	mg/kg
3	Cyanidine-3-diglukoside	26.710	6926159	57.53	4545	420,1	mg/kg
4		28.970	514632	4.27			
5		30.038	222844	1.85			
	Total Anthocyans				7900	730	mg/kg

Therefore, as a result of mutation th anthocyaninsare changed as well (both quntitavely and qualitatively) that is finally refcleted in the flower coloration.

Acknowledgments

We are grateful to “Regional Chromatographic Center” under the grant AP/96/13 for their support.

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