

Fruit skin color and the role of anthocyanin

Emrul Kayesh · Lingfei Shangguan · Nicholas Kibet Korir ·
Xin Sun · Nadira Bilkish · Yanping Zhang · Jian Han ·
Changnian Song · Zong-Ming Cheng · Jinggui Fang

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Abstract Fruit skin coloration is a unique phase in the life cycle of fruiting plants and is mainly attributed to anthocyanin pigments. Anthocyanins are the largest and most diverse group of plant pigments derived from the phenyl propanoid pathway. They are water-soluble phenolic compounds that form part of a large and common group of plant flavonoids. Coloration encompasses several physiological and biochemical changes that happen through differential expression of various developmentally regulated genes. Due to research importance and economic value, *Arabidopsis thaliana* (chromosome no. = 5) and *Vitis vinifera* (chromosome no. = 19) have been used for investigations of the structural genes involved in anthocyanin biosynthesis. Thus for this review, *V. vinifera* is used as a model crop. In anthocyanin biosynthesis, a wide range of constructive genes including phenylalanine ammonia lyase, chalcone synthase and anthocyanidin synthase that are regulated by *MYB* transcription factors are involved. These genes are coordinately expressed and their levels of expression are positively related to the anthocyanin concentrations. Expression or suppression of

the constructive genes contributes to a variety of changes that make fruits visually attractive and edible. Transgenic approaches also have discovered a strong relationship between phenyl propanoid/flavonoid gene expressions for fruit skin coloration. In this study, various developments that have taken place in the last decade with respect to identifying and altering the function of color-related genes are described.

Keywords Anthocyanin · Fruits · Skin color · Biosynthesis · Structural genes · Transgenic

Introduction

Fruits have vital importance in the human diet and the quality of fruit is determined by a wide range of desirable characteristics such as nutritional value, shelf life and fruit skin color. Fruit skin color is one of the most important traits for both commercial and esthetic value of fruit and is mainly determined by the content and composition of anthocyanins (Pomar et al. 2005; Torres et al. 2010). Anthocyanins are the largest and most diverse group of water-soluble phenolic plant pigments derived from the phenyl propanoid pathway (Tunen et al. 1991) with the genetics and biochemistry of the anthocyanin biosynthesis pathway, which is a major well-characterized branch of flavonoid metabolism (Holton and Cornish 1995; Winkel-Shirley 2001; Kim et al. 2003; Martens et al. 2010). During the last decade, many structural genes involved in the anthocyanin biosynthetic pathway and various transcription factors (*MYB*, *bHLH*, and *WD40*) have been identified and functionally characterized in fruit crops (Boss et al. 1996; Honda et al. 2002; Koes et al. 2005; Cultrone et al. 2010).

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E. Kayesh · L. Shangguan · X. Sun · N. Bilkish · Y. Zhang ·
J. Han · C. Song · Z.-M. Cheng · J. Fang (✉)
College of Horticulture, Nanjing Agricultural University,
1 Weigang, Nanjing 210095, China
e-mail: 2010204002@njau.edu.cn; fanggg@njau.edu.cn

E. Kayesh
Department of Horticulture, BSMR Agricultural University,
Gazipur, Bangladesh

N. K. Korir
Department of Agricultural Science and Technology, Kenyatta
University, Nairobi, Kenya

Color development in fruits is an important evolutionary trait and a major factor contributing to fruit quality and subsequent market value. Anthocyanin biosynthesis in fruits has become an interesting and useful aspect of research due to the need of better understanding its mechanism and development of fruit cultivars with higher anthocyanin contents. There is no clear study reported on anthocyanin biosynthesis mechanism in fruit crops apart from that in apple and grape (Matus et al. 2008), probably due to some genes involved in anthocyanin biosynthesis having pleiotropy and being affected by many internal and external factors. Despite this drawback, many regulatory genes have been newly identified in grape, apple and other fruit crops (Hugueneu et al. 2009; Cultrone et al. 2010; Feng et al. 2010). In this review, we describe different aspects of the gene involved in the regulation of anthocyanins and their functions in plants.

Economics of colored fruits

The share of colored fruits in the total world fruit production is very significant with the largest contributing several billion dollars annually. We compiled data based on FAO statistics (<http://www.faostat.fao.org>) for the year 2009 on major colored fruits for this review. Fruits produced by the 20 top countries have been referred as the total world production of that fruit and therefore the figures quoted herein follow this assumption. The total world production of the 22 major colored fruits is at least 473,679,743 MT, out of which tomato occupies the top position with 29.9 % (141,400,629 MT); banana 20 %, 95,595,965 MT; apple 15 % (71,736,938 MT); grapes 14 % (66,935,199 MT); mangoes and mangosteens 7.40 % (35,035,641 MT) (Table 1). On the extreme end, cranberries and gooseberries have the least acreage harvested (0.14 %) and percentage of total production (0.12 %) (Fig. 1).

In terms of market value, fleshy fruits generate more than 1.2×10^{12} international dollars (Int. \$) worldwide, out of which grape tops the list (2.7×10^{10}), followed by tomato (2.5×10^{10}). While considering export potential, it may be realized that apple, banana, tomato and grape fetch maximum value as compared to other fruits (Bapat et al. 2010).

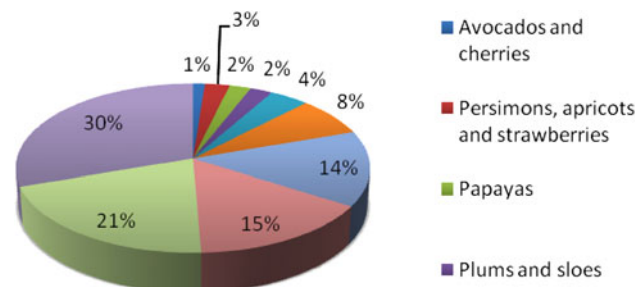
Anthocyanin biosynthesis

Fruit skin color is an appealing feature of many fruits, with anthocyanins being the most prominent pigment imparting red, blue and black hues to the fruits in which they accumulate (Wheelwright and Janson 1985). The anthocyanin

Table 1 Area and production of major colored fruits

Fruit	Area harvested (Ha)	Production (Metric tonnes)
Apples	4,957,192	71,736,938
Apricots	520,455	3,831,823
Avocados	438,325	3,585,156
Bananas	4,843,595	95,595,965
Blueberries	72,554	306,383
Cherries	369,766	2,150,107
Cranberries	22,591	409,523
Currants	114,617	631,108
Gooseberries	26,083	120,996
Grapes	7,437,141	66,935,199
Kiwi fruit	85,983	1,285,553
Mangoes, mangosteens, guavas	4,745,782	35,035,641
Papayas	413,227	10,213,069
Peaches and nectarines	1,655,285	18,579,393
Persimmons	784,252	3,807,843
Plums and sloes	2,525,048	10,679,206
Quinces	68,420	497,237
Raspberries	91,103	483,620
Sour cherries	259,605	1,358,326
Strawberries	253,900	4,132,352
Tomato	4,980,424	141,400,629
Total	34,665,348	472,776,067

Source FAOSTAT Agriculture 2009

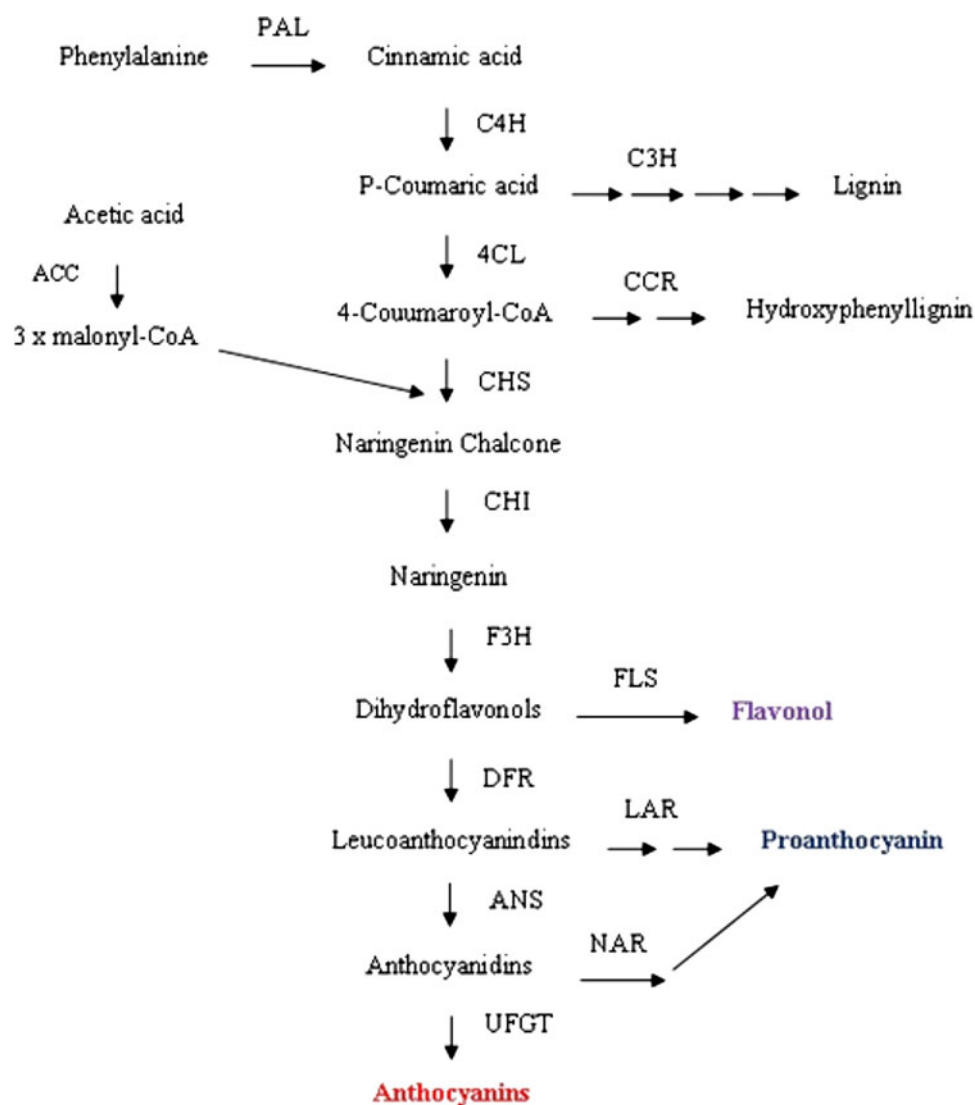


Source: FAOSTAT Agriculture, 2009

Fig. 1 Percent share of major colored fruit out of total world production in MT (color figure online)

biosynthesis pathway is fairly complex and a variety of metabolites are responsible for synthesis of this pigment in fruit crops (Boss et al. 1996). There are a wide range of constructive genes involved in anthocyanin biosynthesis and include phenylalanine ammonia lyase (*PAL*), chalcone synthase (*CHS*) and anthocyanidin synthase (*ANS*) (Fig. 2). Among these genes, *PAL* plays a critical role in anthocyanin synthesis, but when precursors are sufficient anthocyanin synthesis does not depend on *PAL* activity (Boss et al. 1996; Salvatierra et al. 2010). A series of enzyme-

Fig. 2 Simplified anthocyanin biosynthesis pathway and its branches. *PAL* p-aminobenzoate lyase, *C4H* cinnamate 4-hydroxylase, *4CL* 4-coumarate-CoA ligase, *CHS* chalcone synthase, *CHI* chalcone isomerase, *F3H* flavanone 3-hydroxylase, *DFR* dihydroflavonol 4-reductase, *ANS* anthocyanidin synthase, *UFGT* UDPG-flavonoid-3-O-glucosyltransferase, *ANR* anthocyanidin reductase, *LAR* leucoanthocyanidin reductase, *FLS* flavonol synthase, *ACC* acetyl-CoA carboxylase, *CCR* cinnamyl-CoA reductase, *C3H* 4-coumarate 3-hydroxylase



catalyzed reactions are involved in the conversion of phenylalanine to anthocyanins. Initially, phenylalanine is transformed to *trans*-cinnamic acid through *trans*-elimination of ammonia as catalyzed by *PAL* followed by *trans*-cinnamic acid changing into 4-coumaroyl-CoA mediated by *C4H* and *4CL*. One molecule of 4-coumaroyl-CoA together with three molecules of malonyl-CoA are catalyzed by *CHS* to form naringenin chalcone which is rapidly and stereo specifically isomerized to naringenin by *CHI*. After hydroxylation at the 3-position by *F3H*, naringenin converts to dihydroflavonols, which are subsequently reduced to leucoanthocyanidin by *DFR*. *ANS* catalyzes the synthesis of corresponding colored anthocyanidins using leucoanthocyanidin as substrate. Finally, the hydroxyl group at C₃ of anthocyanidins is glycosylated by glycosyl

moieties from *UDP*-activated sugar donor molecule by the action of *UFGT* to yield resultant anthocyanins.

Anthocyanin concentration and distribution in the fruit

Anthocyanins are present in different plant organs (fruits, flowers, stem, leaves and roots) and are normally dissolved uniformly in the vacuolar solution of epidermal cells (Brouillard 1982). In certain plant species, anthocyanins are however localized in discrete regions of the cell vacuole, called anthocyanoplasts (Peckert and Small 1980). The content of anthocyanin (Table 2) may vary between fruits of the same type due to different external and internal factors such as genetic and agronomic factors, intensity and

Table 2 Anthocyanin concentration in some colored fruits

Fruit	Anthocyanin (mg/100 g)
Red grape	30–750
Apple, red delicious	1.7
Blackberry	82.5–325.9
Blueberry	25–495
Bilberry	300–698
Cherry	2–450
Chokeberry	410–1,480
Cranberry	67–140
Crowberry	360
Gooseberry	2.0–43.3
Grapefruit	5.9
Peach	4.2
Pear	5–10
Pomegranate (juice)	600–765
Raspberry	20–687
Red apple	1.3–12
Rowanberry	14
Saskatoon berry	234
Strawberry	19–55

type of light, temperature, processing, handling and storage. Anthocyanin concentrations in red grapes, for example, are distinctively variable with a capacity of reaching values of up to 250 mg/100 g. The most common anthocyanidins in higher plants are: delphinidin, cyanidin, petunidin, pelargonidin, peonidin and malvidin. The glycosides of the three non-methylated anthocyanidins (delphinidin, cyanidin and pelargonidin) are the most abundant in nature, representing 80, 69 and 50 % of leaf, fruit and flower pigments, respectively. The six most common anthocyanidins distributed in the edible parts of plants are cyanidin (50 %), pelargonidin (12 %), peonidin (12 %), delphinidin (12 %), petunidin (7 %) and malvidin (7 %). The most widespread anthocyanin in most fruits is cyanidin-3-glucoside (Kong et al. 2003).

Structural genes identified in fruit crops

Most of the structural genes in fruit crops have been isolated and well characterized (Boss et al. 1996; Piero et al. 2005; Ubi et al. 2006; Matus et al. 2010). The structural genes (Table 3) involved in anthocyanin synthesis are coordinately expressed and their levels of expression positively related to the degree of anthocyanin concentration (Honda et al. 2002; Piero et al. 2005; Matus et al. 2010; Crifó et al. 2011).

PAL, *CHS*, *CHI*, *F3H*, *F3'H*, *DFR*, *ANS*, *LODX*, *UFGT*, *OMT* and *GST* structural genes are correlated with loci for anthocyanin synthesis in grapevine (Table 3), while the transcript levels of *UFGT* can be higher in red compared to white fruits (Kobayashi et al. 2001; Jeong et al. 2004; Cultrone et al. 2010; Niu et al. 2010; Zhang et al. 2011; Xin et al. 2012). In strawberry the genes *DFR*, *ANS* and *UFGT* have been shown to be positively correlated with anthocyanin synthesis, while *C4H* and *4CL* did not seem to have a determining role in the differences in fruit pigmentation, but had close relation to synthesis of lignin monomers (Salvatierra et al. 2010).

CHS, *ANS* and *UFGT*, and *GmDFR*, *GmUFGT* and *GmDOX* were found to be responsible for anthocyanin synthesis in citrus and magosteen, respectively, while in apple, coordinated expression of *MdCHS*, *MdF3H*, *MdANS*, *pDFR* and *pUFGluT* was correlated with anthocyanin synthesis (Honda et al. 2002), though *MdCHI* is yet to be isolated (Tacos et al. 2006a, b). Different structural genes have been isolated from fruit crops and analyzed as shown in Table 3.

Homologous genes involved in anthocyanin biosynthesis in *Arabidopsis* and grapevine

The anthocyanin biosynthesis pathway is an important mechanism in plants and has been studied in many plants, ranging from potato, *Arabidopsis*, grape and maize, among others. Due to the research importance and the economic value of *Arabidopsis thaliana* (chromosome no. = 5), and *V. vinifera* (chromosome no. = 19), these two model plant species have been widely used for investigation of the structural genes involved in the anthocyanin biosynthesis pathway. These genes include *phenylalanine ammonia lyase (PAL)*, *cinnamate 4-hydroxylase (C4H)*, *4-coumarate-CoA ligase (4CL)*, *chalcone synthase (CHS)*, *chalcone isomerase (CHI)*, *flavanone 3-hydroxylase (F3H)*, *flavanone 3'-hydroxylase (F3'H)*, *flavanone 3'5'-hydroxylase (F3'5'H)*, *dihydroflavonol reductase (DFR)*, *anthocyanidin synthaselleucoanthocyanidin dioxygenase (ANS/LODX)*, and *UDP-flavonoid glucosyl transferase (UFGT)*.

After blast search and protein domain analysis, the homologous genes involved in the anthocyanin biosynthesis pathway were isolated (Table 4). *PAL* is the first structural gene in the pathway and 4 copies (*AtPAL1*, *AtPAL2*, *AtPAL3* and *AtPAL4*) have been reported in *A. thaliana* and 11 in *V. vinifera*. *AtC4H* is located in chromosome 2 of *A. thaliana*, and three copies of *C4H* have been isolated in *V. vinifera* with two copies of *C4H* being in chromosomes 6 and 11. *Vv4CL5* was located in

Table 3 Regulatory genes identified in different fruit crops and the targeting structural genes involved in anthocyanin biosynthesis

Fruit species	MYB	bHLH	Structural genes	Fruit tissue	Accession no.	References
Grapes	<i>VvMYB5a</i>		<i>UFGT, C4H, ANS, F3H</i>	Berry skin	AY555190	Deluc et al. (2006)
	<i>VvMYB5b</i>	<i>MYCA1</i>	<i>VvLAR and VvANR</i>	Berry skin	AY899404	Deluc et al. (2008)
	<i>VvMYBA1</i>		<i>CHI, CHS, 4CL, DFR</i>	Berry skin	DQ886418	Walker et al. (2007)
	<i>VvMYBA2</i>		<i>UFGT, ANR</i>		DQ886419	Walker et al. (2007)
	<i>VlmybA1-1</i>		<i>UFGT</i>		AB073010	Kobayashi et al. (2002)
	<i>VlmybA1-2</i>		<i>UFGT, OMT, GST</i>		AB073012	Kobayashi et al. (2002)
	<i>VlmybA2</i>		<i>UFGT</i>		AB073013	Kobayashi et al. (2002)
	<i>VlmybB1-1</i>		Unknown		AB073016	Kobayashi et al. (2002)
	<i>VlmybB1-2</i>		Unknown		AB073017	Kobayashi et al. (2002)
	<i>VlmybC</i>		Unknown		AB073014	Kobayashi et al. (2002)
	<i>VlmybD</i>		Unknown		AB073015	Kobayashi et al. (2002)
	<i>VvmybA1</i>		<i>UFGT</i>		AB097923	Kobayashi et al. (2004)
	<i>VvmybA2</i>		<i>UFGT</i>		AB097924	Kobayashi et al. (2004)
	<i>VvmybA3</i>		Unknown		AB097925	Kobayashi et al. (2004)
	<i>VvMybA1</i>		<i>UFGT</i>		DQ886417	Walker et al. (2007)
	<i>VvMybA2r</i>		<i>UFGT</i>		DQ886419	Walker et al. (2007)
	<i>VvMybA2w</i>		Unknown		DQ886420	Walker et al. (2007)
	<i>VvMybA3</i>		Unknown		DQ886421	Walker et al. (2007)
	<i>VvMybPA1</i>		<i>CHI, F3'5'H, ANR, LAR</i>		AM259485	Bogs et al. (2007)
	Apple	<i>MdMYB1</i>	<i>MdHLH3</i>	<i>ANS</i>	Peel	DQ886414
<i>MdMYBA</i>		<i>HLH33</i>		Peel	AB279598	Ban et al. (2007)
<i>MdMYB10</i>				Peel/flesh/leaf	EU518250	Espley et al. (2009)
Strawberry	<i>FaMYB1</i>		<i>ANS, UFGT</i>	Flesh	AF401220	Aharoni et al. (2001)
	<i>FaMYB10</i>		<i>DFR</i>		EU155162	Wang et al. (2010)
	<i>FvMYB10</i>		<i>DFR</i>		EU155163	Wang et al. (2010)
Pear	<i>PyMYB10</i>		<i>DFR</i>	Peel	GU253310	Feng et al. (2010)
	<i>PcMYB10</i>			Peel	HM775223	Pierantoni et al. (2010)
	<i>PbMYB10</i>				EU153577	Wang et al. (2010)
	<i>PpyMYB10</i>				EU153576	Wang et al. (2010)
Peach	<i>PprMYB10</i>		<i>DFR</i>	Unknown	EU155160	Wang et al. (2010)
Bilberry	<i>VmTDR4</i>		<i>VmMYB2</i>	Peel/flesh	FJ418852	Jaakola et al. (2010)
Red raspberry	<i>RiMYB10</i>		<i>DFR</i>	Unknown	EU155165	Wang et al. (2010)
Chinese bayberry	<i>MrMYB1</i>		<i>AtDFR</i>	Flesh	GQ340767	Niu et al. (2010)
Mangosteen	<i>GmMYB10</i>		<i>GmDFR, GmUFGT, GmLDOX</i>	Pericarp	FJ197137	Palapol et al. (2009)
Loquat	<i>EjMYB10</i>			Unknown	EU153572	Wang et al. (2010)
Quince	<i>CoMYB10</i>			Unknown	EU153571	Wang et al. (2010)
Apricot	<i>ParMYB10</i>		<i>DFR</i>	Unknown	EU153178	Wang et al. (2010)
Cherry	<i>PavMYB10</i>		<i>DFR</i>	Unknown	EU153581	Wang et al. (2010)
	<i>PcrMYB10</i>		<i>DFR</i>	Unknown	EU153582	Wang et al. (2010)
	<i>PcfMYB10</i>			Unknown	EU153583	Wang et al. (2010)
Almond	<i>PdMYB10</i>		<i>DFR</i>	Unknown	EU155159	Wang et al. (2010)
Plum	<i>PiMYB10</i>			Unknown	EU153579	Wang et al. (2010)
	<i>PdmMYB10</i>			Unknown	EU153580	Wang et al. (2010)
	<i>PsMYB10</i>			Unknown	EU155161	Wang et al. (2010)

chromosome 1_random, while *VvC4H3*, *Vv4CL8* and *VvCH3* were linked to chromosome unknown_random. In total, 11 grapevine structural genes (18.18 %) were located

in random sequences, with 8 being located in chromosome unknown_random, 2 in chromosome 16_random and 1 in chromosome 1_random.

Table 4 Homologous genes involved in anthocyanin biosynthesis in Arabidopsis and grape

Arabidopsis gene	Gene ID	Chr	Start	End	Grape gene	Gene ID	Chr	Start	End
<i>AtPAL1</i>	AT2G37040	2	15,557,376	15,560,363	<i>VvPAL1</i>	GSVIVP00013939001	chr16	700,746	703,220
<i>AtPAL2</i>	AT3G53260	3	19,744,051	19,746,780	<i>VvPAL2</i>	GSVIVP00013930001	chr16	648,662	651,184
<i>AtPAL4</i>	AT3G10340	3	3,204,013	3,208,022	<i>VvPAL3</i>	GSVIVP00013943001	chr16	724,118	727,239
<i>AtPAL3</i>	AT5G04230	5	1,160,831	1,163,775	<i>VvPAL4</i>	GSVIVP00013928001	chr16	618,476	621,190
					<i>VvPAL5</i>	GSVIVP00013947001	chr16	779,795	782,254
					<i>VvPAL6</i>	GSVIVP00013922001	chr16	597,226	599,843
					<i>VvPAL7</i>	GSVIVP00023211001	chr8	11,718,432	11,721,087
					<i>VvPAL8</i>	GSVIVP00018175001	chr13	5,969,524	5,991,084
					<i>VvPAL9</i>	GSVIVP00024561001	chr6	3,222,841	3,226,347
					<i>VvPAL10</i>	GSVIVP00013927001	chr16	612,547	614,853
					<i>VvPAL11</i>	GSVIVP00013924001	chr16	606,754	608,798
<i>AtCAH</i>	AT2G30490	2	12,993,663	12,995,770	<i>VvCAH1</i>	GSVIVP00023932001	chr6	8,813,071	8,816,574
					<i>VvCAH2</i>	GSVIVP00017017001	chr11	10,158,634	10,160,529
					<i>VvCAH3</i>	GSVIVP00007155001	chrUn_random	45,548,882	45,550,610
<i>At4CL1</i>	AT1G51680	1	19,158,752	19,161,552	<i>Vv4CL1</i>	GSVIVP00031383001	chr11	12,980,141	12,983,730
<i>At4CL2</i>	AT3G21240	3	7,454,269	7,457,379	<i>Vv4CL2</i>	GSVIVP00031385001	chr11	13,004,990	13,010,232
<i>At4CL3</i>	AT1G65060	1	24,167,202	24,171,502	<i>Vv4CL3</i>	GSVIVP00014031001	chr16	2,123,569	2,128,535
<i>At4CL5</i>	AT3G21230	3	7,448,040	7,452,000	<i>Vv4CL4</i>	GSVIVP00022179001	chr6	22,071,551	22,079,236
<i>At4CL4</i>	AT5G63380	5	25,387,411	25,390,063	<i>Vv4CL5</i>	GSVIVP00002799001	chr1_random	48,50,822	4,853,094
<i>At4CL6</i>	AT4G05160	4	2,664,385	2,666,707	<i>Vv4CL6</i>	GSVIVP00018101001	chr13	7,219,492	7,221,432
<i>At4CL7</i>	AT4G19010	4	10,411,490	10,414,249	<i>Vv4CL7</i>	GSVIVP00015553001	chr18	1,226,323	1,230,351
<i>At4CL8</i>	AT5G38120	5	15,213,765	15,216,205	<i>Vv4CL8</i>	GSVIVP00003591001	chrUn_random	26,173,966	26,179,603
<i>At4CL9</i>	AT1G20510	1	7,103,454	7,105,881	<i>Vv4CL9</i>	GSVIVP00020555001	chr14	15,331,127	15,333,537
<i>At4CL10</i>	AT1G62940	1	23,310,535	23,312,747	<i>Vv4CL10</i>	GSVIVP00020554001	chr14	15,323,828	15,330,405
<i>At4CL12</i>	AT1G20500	1	7,100,502	7,102,915					
<i>At4CL14</i>	AT1G20480	1	7,094,833	7,097,114					
<i>AtCHS</i>	AT5G13930	5	4,488,688	4,490,264	<i>VvCHS1</i>	GSVIVP00037969001	chr14	13,875,792	13,877,198
					<i>VvCHS2</i>	GSVIVP00037967001	chr14	13,889,324	13,890,882
					<i>VvCHS3</i>	GSVIVP00006341001	chrUn_random	37,798,575	37,800,731
<i>AtCHI</i>	AT3G55120	3	20,430,114	20,431,470	<i>VvCHI</i>	GSVIVP00029513001	chr13	2,128,014	2,129,693
<i>AtF3H</i>	AT3G51240	3	19,025,264	19,026,939	<i>VvF3H1</i>	GSVIVP00036784001	chr4	14,790,912	14,792,727
					<i>VvF3H2</i>	GSVIVP00014419001	chr18	12,160,108	12,162,146
					<i>VvF3H3</i>	GSVIVP00036782001	chr4	14,832,092	14,835,848
<i>AtF3'H</i>	AT5G07990	5	2,560,394	2,563,109	<i>VvF3'H1</i>	GSVIVP00016217001	chr17	8,112,032	8,114,427
					<i>VvF3'H2</i>	GSVIVP00016215001	chr17	8,135,555	8,137,842
<i>AtF3'5'H1</i>	AT4G12300	4	7,307,737	7,309,755	<i>VvF3'5'H1</i>	GSVIVP00007272001	chr2	4,774,838	4,777,086
<i>AtF3'5'H2</i>	AT4G12320	4	7,314,778	7,316,669	<i>VvF3'5'H2</i>	GSVIVP00007269001	chr2	4,754,383	4,756,418
<i>AtF3'5'H3</i>	AT4G12310	4	7,310,416	7,312,577	<i>VvF3'5'H3</i>	GSVIVP00010339001	chrUn_random	63,649,713	63,651,747
<i>AtF3'5'H4</i>	AT4G12330	4	7,317,560	7,319,739	<i>VvF3'5'H4</i>	GSVIVP00007267001	chr2	4,745,715	4,747,542
<i>AtF3'5'H5</i>	AT5G44620	5	17,997,779	17,999,558	<i>VvF3'5'H5</i>	GSVIVP00010337001	chrUn_random	63,642,459	63,644,286
<i>AtF3'5'H6</i>	AT4G22690	4	11,929,359	11,931,693	<i>VvF3'5'H6</i>	GSVIVP00010332001	chrUn_random	63,611,190	63,613,352
<i>AtF3'5'H7</i>	AT4G22710	4	11,934,969	11,936,755	<i>VvF3'5'H7</i>	GSVIVP00010336001	chrUn_random	63,635,888	63,637,834
					<i>VvF3'5'H8</i>	GSVIVP00007266001	chr2	4,738,982	4,741,051
					<i>VvF3'5'H9</i>	GSVIVP00038447001	chr16_random	1,531,571	1,533,743
					<i>VvF3'5'H10</i>	GSVIVP00038441001	chr16_random	1,444,657	1,446,826
					<i>VvF3'5'H11</i>	GSVIVP00038443001	chr16_random	1,473,707	1,475,916
					<i>VvF3'5'H12</i>	GSVIVP00001045001	chr2	4,336,836	4,339,311
					<i>VvF3'5'H13</i>	GSVIVP00026276001	chr15	6,313,161	6,315,563

Table 4 continued

Arabidopsis gene	Gene ID	Chr	Start	End	Grape gene	Gene ID	Chr	Start	End
					<i>VvF3'5'H14</i>	GSVIVP00015660001	chr2	9,317,991	9,320,950
					<i>VvF3'5'H15</i>	GSVIVP00001048001	chr2	4,317,518	4,319,383
					<i>VvF3'5'H16</i>	GSVIVP00026282001	chr15	6,260,387	6,262,922
					<i>VvF3'5'H17</i>	GSVIVP00022287001	chr6	20,475,674	20,477,909
					<i>VvF3'5'H18</i>	GSVIVP00005727001	chr3	9,985,993	9,987,872
					<i>VvF3'5'H19</i>	GSVIVP00026277001	chr15	6,309,081	6,311,743
					<i>VvF3'5'H20</i>	GSVIVP00016437001	chr11	832,958	835,061
					<i>VvF3'5'H21</i>	GSVIVP00003596001	chrUn_random	26,288,189	26,290,502
					<i>VvF3'5'H22</i>	GSVIVP00015613001	chr2	10,830,659	10,833,635
					<i>VvF3'5'H23</i>	GSVIVP00016433001	chr11	811,943	813,571
					<i>VvF3'5'H24</i>	GSVIVP00015616001	chr2	10,779,040	10,785,958
					<i>VvF3'5'H25</i>	GSVIVP00026289001	chr15	6,166,643	6,169,581
					<i>VvF3'5'H26</i>	GSVIVP00034070001	chr9	824,462	826,981
					<i>VvF3'5'H27</i>	GSVIVP00021704001	chr8	17,059,699	17,061,345
					<i>VvF3'5'H28</i>	GSVIVP00026288001	chr15	6,180,928	6,183,234
					<i>VvF3'5'H29</i>	GSVIVP00017654001	chr17	3,820,820	3,823,244
					<i>VvF3'5'H30</i>	GSVIVP00025511001	chr8	20,193,957	20,195,681
<i>AtDFR</i>	AT5G42800	5	17,164,141	17,165,918	<i>VvDFR</i>	GSVIVP00014584001	chr18	10,768,605	10,771,679
<i>AtANS/ AtLDOX</i>	AT4G22880	4	12,004,768	12,006,209	<i>VvANS/ AtLDOX</i>	GSVIVP00001063001	chr2	4,220,998	4,222,539
<i>AtUFGT1</i>	AT5G17050	5	5,607,788	5,609,492	<i>VvUFGT</i>	GSVIVP00014047001	chr16	2,334,697	2,341,263
<i>AtUFGT2</i>	AT5G17030	5	5,603,133	5,604,738					
<i>AtUFGT3</i>	AT1G30530	1	10,814,659	10,816,583					
<i>AtUFGT4</i>	AT5G17040	5	5,605,284	5,606,970					

Grapevine as a model crop for anthocyanin studies in fruit crops

Grapevine (*V. vinifera* L.) is the most important fruit crop and has been widely cultivated in the world, with a long history of physiological, biochemical and molecular investigations and advances in the development of genetic and molecular tool kits for this species. The grapevine species is a representative of the genus *Vitis* and has a disomic inheritance with $2n = 38$ and a relatively small genome of 475 Mbp (Lodhi and Reisch 1995; Aradhya et al. 2003).

Grape skin color is an important factor both biologically and economically, since the quality of grape berries greatly depends on skin color, which greatly affects market prices and the wine industry (Koshita et al. 2011). Fruit skins of grapes have the highest amounts of volatile and polyphenolic compounds compared to other parts of the fruit. The color of grape berries is determined by the quantity and composition of anthocyanins in their skins (Pomar et al. 2005; Torres et al. 2010).

Though earlier studies were focused more on the molecular biology of fruit skin color, genomics approaches have recently been used to reveal insights into control of primary coloration upstream of anthocyanin, coloration-

related signal transduction systems and downstream metabolic networks. Grape pleiotropic coloration mutations have added greatly to the understanding of fruit skin color.

Genes of anthocyanin production and pigment biosynthesis enzymes were among the first anthocyanin-responsive genes isolated from fruits of grape and anthocyanin biosynthesis in grape skin has been extensively studied (Boss et al. 1996). In grapevine, anthocyanin biosynthesis depends on *Myb*-related genes, which play a crucial role in the different varieties that exhibit an assortment of biosynthesis modes. In American hybrid grapevine, *VlmybA1-2*, *VlmybA2* and *VlmybA3* are located in the pericarp, though the exact location is unknown, and also regulate the anthocyanin biosynthetic gene cluster (dashed box in Fig. 3). Gene clusters can encode the production of the *Myb* protein, thus contributing to the expression of the anthocyanin biosynthetic pathway in *UFGT*-turned grape skin color. Grape color loci are mainly divided into three cases (Fig. 3): (1) *VvmybA1* and *VvmybA2* can be mutated, *VvmybA1a* and *VvmybA2w* have no function, and *VvmybA1b*, *VvmybA1c* and *VvmybA2r* have a function. In addition, some red white mutant varieties *VvmybA1* and *VvmybA2* are lost and cannot synthesize anthocyanins, though *VvmybA2r* and *VvmybA1c*, *VvmybA2w* and *VvmybA1b*, and *VvmybA2w* and *VvmybA1c*

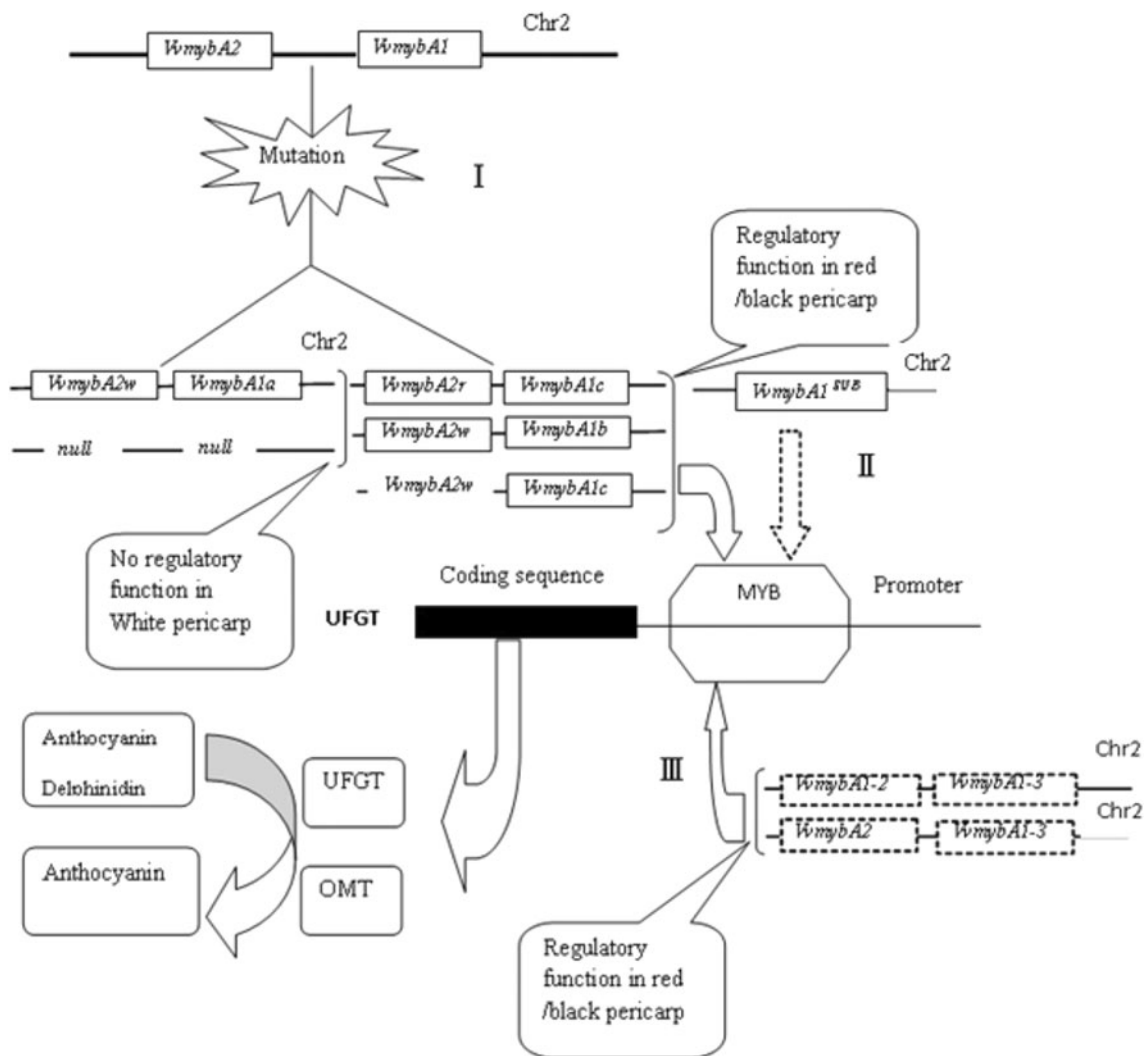


Fig. 3 Schematic diagrams of the regulation of skin color locus of grape on anthocyanin biosynthesis

can synthesize anthocyanins in Europe and American hybrid grapes. (2) The European Oriental species has only *VvmybA1^{SUB}* and lacks *VvmybA2*, *VlmybA1-2*, *VlmybA2*- and *VlmybA3*. Whether *VvmybA1^{SUB}* can regulate anthocyanin biosynthesis in grapevine is still unknown (Fig. 3, dashed arrows). (3) In European and American hybrid grapes, the *VlmybA1-3* gene cluster can be encoded separately, and *VlmybA1-2* and *VlmybA2* composition generating the *Myb* protein induced *UFGT* to express anthocyanins in the skin.

Anthocyanins from fruit to human health

Anthocyanins act as powerful antioxidants, water-soluble vacuolar pigments and also possess a multitude of biological roles, including protection against solar exposure and ultraviolet radiation, free radical scavenging and antioxidative

capacity, defense against many different pathogens, attraction of predators for seed dispersal, as well as the newly proposed modulation of signaling cascades (Bornsk et al. 2012). Anthocyanins are able to prevent oxidative damage to DNA, proteins, lipids and other macromolecules caused by reactive oxygen species (ROS). They have systemic action, since they are absorbed and circulate in the blood, and it is in this circulating form that they act upon different target tissues in the human body. Anthocyanins may also act locally, in the gut, if they are not absorbed by intestinal mucosa. Finally, they may also act as topical agents, for example, by protecting the skin from UV radiation. Free radical damage contributes to the etiology of many chronic diseases (Bobe et al. 2006; Chen et al. 2006; Valcheva-Kuzmanova and Belcheva 2007) and thus antioxidants may have beneficial effects on human health at different levels. Within the assayed anthocyanins, the 3-glucoside of delphinidin, which

is the only anthocyanin containing the 30-, 40- and 50-hydroxy group (gallo catechol structure) in the B-ring, has shown the highest antioxidant capacity (Garcia-Alonso et al. 2005). Although some researchers have cast doubts on their bioavailability, considerable attention has been focused on the potential beneficial effects of anthocyanins on human health and their derived compounds in fruits in recent years. On the other hand, with no literature reports of anthocyanin toxicity, their safety has been extensively demonstrated by the widespread and incidence-free consumption of food products that contain anthocyanins. Because of their brilliant color, high water solubility and beneficial biological properties, anthocyanins are considered as a class of potential natural pigments for replacing synthetic colorants in many kinds of food.

Transgenic plants developed for anthocyanin

Transgenic approaches have discovered a strong relationship between phenylpropanoid/flavonoid gene expression and a plant's defense response levels. In view of the potential of the flavonoid pathway in plant defense response, attempts are being made in several laboratories to genetically engineer plants for increased flavonoid levels by over-expressing the structural and/or the regulatory genes of the pathway. Transgenic lines revealed an insight into the regulation of the flavonoid pathway and the possibilities and limitations of engineering metabolic pathways in general and the flavonoid pathway in particular. The results can be used to design novel strategies for engineering plants with an optimal content of flavonoids, and with minimal pleiotropic side effects. In addition, identified key genes involved in fruit ripening and/or flavonoid production can be used as targets for marker development in breeding programs. Since the production of

the first transgenic plants in the 1980s, a wide diversity of patents have been sought, and granted, on all aspects of the process, ranging from the underlying methods for tissue culture to the means of introducing the heterologous DNA, and to the composition of the DNA construct so introduced (Dunwell 2005; Pray and Naseem 2007). The amount of patent information available in the area of plant transformation alone can be judged by the fact that a search of the US application database (<http://www.bios.net/daisy/bios/patentlens.html>) alone for “transgenic plant” and “method” returns substantial results. Summaries of relevant recently granted patents and patent applications in the USA are given in Tables 5 and 6.

Conclusion and future prospects

Anthocyanins are major factors in fruit skin color. A linear set of events can be described; fruit skin color variation is strongly associated with genetic variation and a combination of bioinformatics aspects (Borevitz et al. 2000; Xie et al. 2006). Anthocyanin biosynthesis is one of the most active research areas due not only to visual appeal, but also to the nutritional value of colored fruits. To develop an anthocyanin-rich cultivar, a better understanding of the regulatory network controlling anthocyanin biosynthesis in fruit crops is a prerequisite. Although the pathway itself is well understood, its regulation appears to be under a hierarchy of complex events, which are slowly being deciphered. The identification of new transcription factors involved in anthocyanin biosynthesis should be conducted together with the investigation of the parameters controlling their expression. This review established a clear relationship between fruit pigmentation, differential transcriptional factors in the anthocyanin-related genes of phenylpropanoid and flavonoid biosynthesis pathways and the accumulation of

Table 5 US patents (granted) on transgenic plants developed for fruit skin color published from 2010 to 1 April 2011

Patent no.	Date	Named inventors	Applicant	Area
7678393	16 Mar 2010	Bradford Ray et al.	DB laboratories LLC, Stites, ID (USA)	Anthocyanin mixture useful for topical and internal application
7682637	23 Mar 2010	Gerald T et al.	Phenolics, LLC, Omaha, NE (USA)	Compositions enriched in total phenols
7727564	1 Jun 2010	Kenneth A et al.	K2A LLC. Apringville, UTC (USA)	Fruit-based dietary supplements
7750211	6 Jul 2010	Richard A et al.	The Samuel Roberts Noble Foundation, Ardmore, OK (USA)	Production of flavonoid and isoflavonoid
7767416	3 Aug 2010	Spangenberg et al.	Agriculture Victoria Service Pty Ltd, Altwood (AU) and Agresearch Limited Hamilton (NZ)	Flavonoid biosynthesis in plants
7855319	21 Dec 2010	Rommens et al.	J.R. Simplot Company, Boise, ID (USA)	Antioxidant-containing food
7880059	1 Feb 2011	Richard A et al.	The Samuel Roberts Noble Foundation, Ardmore, OK (USA)	Proanthocyanins to improve forage quality

Table 6 US patent applications on transgenic plants developed for fruit color published from 2010 to 1 April 2011

Patent no.	Date	Named inventors	Applicant	Area
20100016565	21 Jan 2010	Connors et al.	Exelixis Plant Science, Inc.	Anthocyanin mutant (ANT1) in tomato
20100015065	21 Jan 2010	Matsumoto et al.	–	Anthocyanin composition in food
20100021614	28 Jan 2010	Nishijima et al.	–	Bioabsorption of flavonoid
20100041877	18 Feb 2010	Tamura et al.	–	Purified anthocyanin
20100107277	29 Apr 2010	Bruguera et al.	International Flower Developments PTY. Ltd	Flavonoid 3'-5' hydroxylase gene
20100216729	30 Apr 2010	Gutierrez-uribe et al.	–	Cancer cell growth inhibition
20100199370	5 Aug 2010	Levin et al.	–	Fruits with high level of anthocyanin and flavonols
20110072539	24 Mar 2011	Espley et al.	–	Pigment production in plant

representative anthocyanins during fruit developmental process. Furthermore, using mutated genes controlling anthocyanin biosynthesis, we can develop functional molecular markers to carry out marker-assisted selection in fruit crops. This promising work needs numerous explorations in the future.

The latest achievements in plant genomics have enabled scientists to identify the entire array of genes that sustain a plant. The best example is publication of whole genome sequence of grape providing a valuable tool and resources to the fruit plant community. This progress in genomic research has fostered new approaches to address the understanding of physiological and biochemical pathways. However, the functional relationships between this genetic element and transcriptional activity are still unknown and more studies are needed to better characterize the genetic bases of fruit skin coloration. Future expansions in this area of research will depend strongly on solving these problems.

Author contribution EK and JGF conceived and designed the experiments. EK, LFSG, XS, NB and NKK contributed conceptual framework, tables and figures. EK and NB wrote the paper. EK, LFSG, NKK and XS participated in the design and coordination of the study. EK, NKK, LFSG, NB and JGF revised the manuscript. EK, LFSG, NKK, XS, NB, YZ, JH, CS, ZMC and JGF read and approved the final manuscript.

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