A Spontaneous Eggplant (*Solanum melongena* L.) Color Mutant Conditions Anthocyanin-free Fruit Pigmentation

Carmina Gisbert

Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Camino de Vera 14, 46022 Valencia, Spain

Judith M. Dumm

Genetic Improvement of Fruits and Vegetables Laboratory, Beltsville Agricultural Research Center, Agriculture Research Service, United States Department of Agriculture, Beltsville, MD 20705-2325

Jaime Prohens and Santiago Vilanova

Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Camino de Vera 14, 46022 Valencia, Spain

John R. Stommel¹

Genetic Improvement of Fruits and Vegetables Laboratory, Beltsville Agricultural Research Center, Agriculture Research Service, United States Department of Agriculture, Beltsville, MD 20705-2325

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Abstract. We identified a single plant in a grow out of the eggplant (*Solanum melongena* L.) variety ‘Black Beauty’ bearing green fruit. ‘Black Beauty’ normally produces violet/black pigmented fruit attributed to anthocyanin accumulation. We selected the green-fruited true-breeding genotype E13GB42 from the S2 generation obtained from selfing of the S0 green-fruited color mutant. Characterization of 12 simple sequence repeat (SSR) markers, eight fruit morphological attributes and fruit yield support E13GB42 arising as a spontaneous mutant of ‘Black Beauty’. With the exception of fruit calyx prickliness, E13GB42 was not significantly different from ‘Black Beauty’ for fruit morphological attributes and yield. E13GB42 exhibited an SSR marker profile identical to that of ‘Black Beauty’ but polymorphic with that of eight violet/black-fruited modern eggplant hybrids, older open-pollinated varieties and landraces. Transcript levels of key anthocyanin biosynthetic genes (*Chs*, *Dfr*, and *Ans*) and regulatory genes (*Myb*_C, *Myc*, and *Wd*) were significantly lower in the green-fruited E13GB42 mutant in comparison with the black-fruited variety ‘Black Beauty’ at various stages of fruit development ranging from small post-anthesis fruit to full-size marketable fruit. Progeny obtained from selfing of the original mutant and reciprocal crosses with ‘Black Beauty’ produced violet, green, and green with violet striped color classes that together were not compatible with one or two gene inheritance models, suggesting that the mutation responsible for the E13GB42 phenotype influences multiple genetic factors that control fruit pigmentation.

Mutants affecting flavonoid synthesis have been characterized in numerous plant species. Alterations in flower and seed

pigmentation facilitated establishment of maize (*Zea mays*), snapdragon (*Antirrhinum majus*), and petunia (*Petunia ×hybrida*) as early models for flavonoid genetics (Dooner et al., 1991; Griesbach, 2005; Mol et al., 1996). More recent studies in *Arabidopsis thaliana*, grape (*Vitis vinifera*), and numerous other crop species add to our knowledge of the molecular genetic aspects of flavonoid biosynthesis (Dubos et al., 2010; Jaakola, 2013; Matus et al., 2008). The flavonoids can be subdivided into anthocyanins (colored) and flavonol copigments (colorless). The anthocyanin biosynthetic pathway has been elucidated and considerable research has focused on structural and

regulatory genes responsible for tissue- and developmental-specific gene expression. Anthocyanin structural gene transcription is dependent on the expression of at least one member of each of three transcription factor families—MYB, MYC, and WD. The function of this regulatory protein complex is well accepted for its role in anthocyanin biosynthesis (Koes et al., 2005).

Anthocyanins have varied functions in plants. The most well known of these include pollinator attraction, seed dispersal, feeding deterrents, and protection against ultraviolet and oxidative light stress. In eggplant, anthocyanins and chlorophylls together are responsible for the dark violet to black pigmentation characteristic of many eggplant varieties. Delphinidin-3-*p*-coumaroylrutinoside-5-glucoside or delphinidin-3-rutinoside are the anthocyanins commonly reported in eggplant (Ichihara et al., 2005; Wu and Prior, 2005). If anthocyanins are absent or present at very low concentration, fruit are green pigmented and if chlorophyll concentration is also very low, fruit color is white.

Anthocyanin biosynthetic and regulatory gene duplication and subsequent sequence divergence are postulated to account for genetic variation in anthocyanin pigmentation and tissue specificity of pigmentation (Purugganan and Wessler, 1994). Recently published reports on diverse species demonstrate that different anthocyanin gene expression profiles can also elicit comparable anthocyanin pigmentation phenotypes. Similar anthocyanin pigmentation phenotypes resulting from regulation by one or several anthocyanin MYBs and MYCs have been reported in Solanaceous species including pepper (Stommel et al., 2009; Zhang et al., 2015) and tomato (Gonzali et al., 2009) and rosaceous crops including apple and pear (Jaakola, 2013). We previously demonstrated downregulation of biosynthetic and regulatory gene transcript levels in violet anthocyanin pigmented eggplant fruit vs. a white-fruited variety with very low anthocyanin concentration (Stommel and Dumm, 2015). We have discovered a green-fruited variant in a grow out of the violet/black pigmented eggplant variety ‘Black Beauty’. Here, our objective was to determine if biosynthetic and regulatory gene expression is similarly downregulated and whether this fruit color variant is likely a spontaneous mutant of ‘Black Beauty’.

Material and Methods

Plant material. The violet-fruited *S. melongena* cultivar Black Beauty (B & T World Seeds, Aigues Vives, France) and a green-fruited genotype designated E13GB42 that was derived from a green-fruited variant which we identified in a grow out of ‘Black Beauty’, have been used for the morphological characterization and gene expression experiments. E13GB42 was obtained after

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¹Corresponding author. E-mail: john.stommel@ars.usda.gov.

Table 1. Alleles for 12 genomic SSR markers (Vilanova et al., 2012) in E13GB42, 'Black Beauty', and eight other black varieties. For heterozygous loci the two alleles are indicated.

SSR locus	Linkage group ^y	E13GB42 and 'Black Beauty'				Other black varieties ^z							
		E13GB42		'Black Beauty'		Modern hybrids	Old varieties			Landraces			
		Sample 1	Sample 2	Sample 1	Sample 2		'Florida High Bush'	'Prosperosa'		AFR-S-1	ASI-S-1	B-S-5	'Rami' SUD-S-5
CSM12	6	229	229	229	229	227	227	227		226/228	226	227	227
CSM27	3	212	212	212	212	225	225	213		208/225	229	216	213
CSM29	12	258	258	258	258	257/259	257	255		254	256	257	255
CSM32	4	264	264	264	264	263	263	263		267	263	258	263
CSM36	9	376	376	376	376	370/376	374	378		380	397	385	399
CSM40	4	310	310	310	310	308/318	308	308		308	310	310	308
CSM44	Unknown	265	265	265	265	267	267	265		267	259	259	269
CSM52	Unknown	256	256	256	256	254/256	255	255		255	255	255	253
CSM54	9	250	250	250	250	252	249	249		251	264	244	249
CSM7	Unknown	228	228	228	228	223/256	226	226		226/228	225	226	225
CSM74	12	203	203	203	203	203	203	203		203	205	203	203
CSM78	10	297	297	297	297	317	337	296		312	296	317	312

SSR = simple sequence repeat.

^zSee Muñoz-Falcón et al. (2009) for the origins of these violet/black materials.

^yAccording to the eggplant genetic map of Gramazio et al. (2014).

Table 2. Mean values $\pm$ SE of fruit morphological traits for E13GB42 (n = 24) and for 'Black Beauty' (n = 36), as well as for yield of individual plants (n = 15 for both varieties) and *P* value for the *t* test for comparison of means.

Trait	E13GB42	'Black Beauty'	<i>P</i> value
Fruit weight (g)	364 $\pm$ 13	354 $\pm$ 9	0.5355
Fruit length (cm)	11.6 $\pm$ 0.2	12.1 $\pm$ 0.2	0.0741
Fruit breadth (cm)	9.7 $\pm$ 0.2	9.8 $\pm$ 0.1	0.7464
Fruit length/breadth ratio	1.21 $\pm$ 0.03	1.25 $\pm$ 0.03	0.3123
Fruit curvature ^z	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.0000
Fruit cross section ^y	3.58 $\pm$ 0.25	4.33 $\pm$ 0.32	0.0957
Relative fruit calyx length ^x	4.00 $\pm$ 0.21	3.78 $\pm$ 0.16	0.4034
Fruit calyx prickles ^w	4.67 $\pm$ 0.41	3.19 $\pm$ 0.36	0.0108
Yield (g/plant)	4,709 $\pm$ 709	4,888 $\pm$ 397	0.8267

^zMeasured in a 1 (none) to 9 (U shaped) scale.

^yMeasured in a 1 (circular) to 9 (very irregular) scale.

^xMeasured in a 1 (very short; <10%) to 9 (very long; <75%) scale.

^wMeasured in a 0 (none) to 9 (very many; >30) scale.

Table 3. Mean values $\pm$ SE for fruit color parameters (L*, a*, and b*; n = 54 for both varieties) and *P* value for the *t* test for comparison of means.

Trait	E13GB42	'Black Beauty'	<i>P</i> value
L*	43.06 $\pm$ 0.65	21.15 $\pm$ 0.57	<0.0001
a*	-12.83 $\pm$ 0.13	6.06 $\pm$ 0.18	<0.0001
b*	16.41 $\pm$ 0.30	0.87 $\pm$ 0.23	<0.0001

two generations of selfing from an anthocyaninless green-fruited sport of 'Black Beauty'. Plants of 'Black Beauty' and E13GB42 were cultivated in the open field at Valencia (Spain) for the morphological characterization experiments and in the open field at Beltsville (MD) for gene expression experiments. Eight other violet/black varieties (Table 1) corresponding to a modern hybrid, old varieties, and landraces were also used in the molecular fingerprinting experiment. Full details on the origin of these violet/black control varieties can be found in Muñoz-Falcón et al. (2009).

The original mutant plant (S0) from which the E13GB42 genotype was derived, as well as clonal replicates of it (S0), first selfings (S1), selfings of three green plants from the S1 generation (S2), as well as reciprocal hybrids between 'Black Beauty' and the original mutant plant have been grown at Valencia. Open field and greenhouse conditions and different years have been used

to study segregation data. Cultivation conditions were performed using standard cultivation practices. For further details on cultivation conditions, see Stommel et al. (2015).

Molecular characterization. 'Black Beauty', E13GB42, and the eight violet/black-fruited controls were screened with 13 genomic SSR markers (Table 1). These markers were selected for their high degree of polymorphism in an eggplant collection (Vilanova et al., 2012). Full details on the SSR repeated sequence, primers sequence, and annealing temperature can be consulted in Vilanova et al. (2012). For 'Black Beauty' and E13GB42, two independent samples, each consisting of a pool of DNA from five plants was used. For the rest of the varieties a single pool of DNA from five plants was used. SSRs were tested following the procedure and conditions described by Vilanova et al. (2012). The polymerase chain reaction (PCR) products after amplification were separated in an ABI Prism 3100 (Applied Biosystems, Foster City, CA) genetic analyzer and resolved using GeneScan 500 LIZ molecular size standards with GenoTyper 3.7 software (Applied Biosystems).

Morphological characterization. Thirty-six plants of 'Black Beauty' and 24 plants of E13GB42 cultivated in Valencia in the open field were used for the morphological characterization. Eight fruit traits as well as yield

per plant (Table 2) were measured for each plant. For fruit traits, several fruits per plant were measured and the data averaged to obtain the mean value of each plant. In addition, for 54 fruits of each variety, the L*, a*, and b* Hunter color coordinates of the skin color of market quality fruits (evaluated by the size and glossiness of the skin) were assessed with a CR-300 (Minolta, Osaka, Japan) chromameter. Skin color measurements were made at middistance between the proximal part of the fruit and the equatorial part of the fruit and at middistance between the distal part of the fruit and the equatorial part of the fruit. Average skin color values were obtained for each fruit. Mean values and standard errors for morphological traits and chromameter measures were calculated for each trait and variety and the significance of differences was assessed with a *t* test.

Individual plants of generations S0, S1, S2, and reciprocal hybrids of the S0 original plant with 'Black Beauty' (Table 3) were classified in three categories depending on the fruit color: violet (fruits presenting anthocyanins uniformly distributed in the skin of the fruit), green with violet striping (fruits having a variable percentage of the fruit skin covered by violet stripes, generally greater in the parts more exposed to the solar radiation, in a green background), and green (fruits lacking anthocyanins and presenting only the green background color).

Gene expression. Fruits of 'Black Beauty' and E13GB42 were harvested at four different stages, ranging from small fruit enclosed in the calyx (stage w) to commercial maturity stage, i.e., market quality fruit (stage z). Mean fruit weights for 'Black Beauty' and E13GB42 were stage w = 2.4 g, 3.0 g; stage x = 15.0 g, 15.2 g; stage y = 101.4 g, 87.9 g; and stage z = 401.5 g, 479.3 g, respectively. Each developmental stage was represented by four replicates obtained from individual fruit of each cultivar. Total RNA for individual samples was isolated from $\approx$ 100 mg of frozen tissue using the protocol described in Stommel and Dumm (2015). Real-time PCR

was used to compare flavonoid gene expression (*Chs*, *Dfr*, *Ans*, *Myb*, *Myc*, and *Wd*) between ‘Black Beauty’ and E13GB42 fruits. Degenerate primer sets from *Petunia ×hybrida* (Griesbach and Beck, 2005) were used to generate PCR products. The protocols, primers, and procedure used for the amplification can be consulted elsewhere (Stommel and Dumm, 2015). Results were normalized to the expression of tubulin (*Tub*). Standard deviation of *Tub* values across development stages and cultivars was ≤ 1.0 .

Results

Molecular and morphological characterization. Replicate samples of ‘Black Beauty’ and E13GB42 analyzed with 13 SSR markers exhibited the same profile, with all SSR loci presenting a single allele in both varieties (Table 1). In contrast, these same 13 SSR markers were polymorphic when considering the eight control dark violet to black-fruited varieties represented by modern hybrids, older open pollinated varieties and landraces. SSR profiles demonstrated that ‘Black Beauty’ and E13GB42 were distinct from the other dark violet to black-fruited varieties evaluated.

Morphological characterization of ‘Black Beauty’ and E13GB42 for eight fruit traits and for yield resulted in nonsignificant ($P > 0.05$) differences between varieties for all traits evaluated, with the exception of fruit calyx prickliness (Table 2). The calyx of E13GB42 exhibited significantly greater prickliness (4.67) than that of ‘Black Beauty’ (3.19). Consistent with green vs. violet fruit color for respective E13GB42 and ‘Black Beauty’ varieties (Figs. 1 and 2), chromameter color parameters (L^* , a^* , and b^*) were significantly different between varieties. Skin of E13GB42 fruit had greater luminosity (higher L^* values), were greener (lower a^* values), and more yellow (higher b^* values) in comparison with ‘Black Beauty’ fruit (Table 3).

Segregation data. The mutant plant and its clonal replicates (S0) had green fruits. Progeny of the first selfed generation (S1) arising from the original mutant plant segregated for fruit color. Three fruit color classes were evident for S1 plants scored in the open field; violet, green with violet striping, and green fruit color classes. Two color classes, violet or green fruit were identified when S1 plants were produced under greenhouse conditions (Table 4). Plants of three S2 lines obtained from the selfing of individual green-fruited S1 plants produced exclusively green fruit when cultivated in greenhouse (Table 4). Progeny obtained from reciprocal crosses between the original mutant plant (S0) and ‘Black Beauty’ yielded similar fruit color segregation patterns, although, green-fruited progeny were not identified in the smaller sample size available for the S0 $\times$ ‘Black Beauty’ cross. The collective data were not consistent with one or two gene inheritance models (chi-square data not shown).

Gene expression. Transcript levels of key biosynthetic genes (*Chs*, *Dfr*, and *Ans*) in the anthocyanin biosynthetic pathway and transcript of regulatory genes (*Myc*, *Myb_B*, *Myb_C*, and *Wd*) that comprise the MYB-MYC-WD transcription factor complex were measured to assess genetic mechanism underlying

differences observed in pigmentation of E13GB42 and ‘Black Beauty’ fruit. Transcript levels for *Chs*, *Dfr*, and *Ans* in violet epidermal tissue of ‘Black Beauty’ fruit increased 2.5- to 3.4-fold between stage w to stage x of small developing fruit and declined with increasing fruit size (Table 5). E13GB42

Fig. 1. Plants of ‘Black Beauty’ (left) and E13GB42 (right).

Fig. 2. Longitudinal and distal view of fruits of E13GB42 (left) and ‘Black Beauty’ (right). Background green color in ‘Black Beauty’ can be observed in the area surrounding the stylar scar.

Dfr and *Ans* transcript levels declined or were unchanged in developing fruit whereas *Chs* transcript level increased as fruit size increased but was still 10-fold lower than that measured in 'Black Beauty' market size fruit. Structural gene transcript levels were significantly lower at all stages of fruit development in E13GB42 relative to those measured in 'Black Beauty' and were unchanged between stage w and stage x when expression was maximal in 'Black Beauty'. Differences in structural gene transcript levels between the two cultivars were large at stage x ranging from 7200-fold lower for *Dfr* to 727,000-fold lower for *Ans* in E13GB42 relative to 'Black Beauty' when transcript levels were maximal.

Myb products with sequence homology to flavonoid related *Myb* clones from potato and tomato were identified in developing eggplant fruit and designated *Myb_B* and *Myb_C*, respectively. Consistent with structural gene expression, transcript levels for *Myb_C* and

Myc regulatory genes were significantly greater in fruit of 'Black Beauty' relative to that observed in E13GB42 at all stages of fruit development (Table 6). Levels of *Myb_C* and *Myc* transcript were up to 95- and 31-fold greater, respectively in 'Black Beauty' vs. E13GB42. 'Black Beauty' *Myb_C* transcript level increased 2.2-fold in small developing fruit coincident with structural gene expression and subsequently declined in large market size fruit. *Myc* transcript in this cultivar increased 3.1-fold between stage w and stage y and maintained elevated transcript level through stage z. *Wd* transcript levels in E13GB42 and 'Black Beauty' were relatively constant throughout fruit development but increased $\approx$ 10-fold in market size fruit (stage z). Transcript levels between cultivars were comparable at stage w and stage y but were $\approx$ 2-fold greater in 'Black Beauty' relative to E13GB42 in larger fruit (stage y and stage z). Relative to *Myb_C*, *Myb_B* transcript levels were low and similar in both cultivars.

Discussion

Based on SSR marker profiles and morphological data, our results suggest that E13GB42 and 'Black Beauty' are identical, except for fruit color and calyx prickliness. E13GB42 likely arose as a spontaneous mutant of 'Black Beauty' and is not the result of seed contamination or hybridization of 'Black Beauty' with other varieties. Changes in anthocyanin pigmentation require genetic or epigenetic changes to occur that influence the anthocyanin biosynthetic pathway. Genetic changes may be caused by cis-regulatory changes to enzyme-coding genes that directly affect enzymatic expression, coding-sequence mutations in enzyme-coding genes, cis-regulatory changes to anthocyanin-regulating transcription factors, or by coding-sequence mutations to anthocyanin-regulating transcription factors (Streisfeld and Rausher, 2011). DNA methylation, histone modification, and noncoding RNA associated gene silencing can result in epigenetic changes (Egger et al., 2004). In flowers, extensive work on spontaneous mutations demonstrated that most flower color changes attributed to anthocyanin pigmentation result from loss of function mutations in coding regions that abolish enzyme function or mutations in regulatory regions that reduce or abolish protein expression (Rausher, 2008). Changes in regulatory elements are more often better tolerated in comparison with changes in biosynthetic pathway enzymes due to negative pleiotropic effects of these mutations in structural genes.

Green fruit color is a distinguishing characteristic of E13GB42. Our results demonstrate that anthocyanin structural and regulatory genes are differentially expressed in violet/black fruit of 'Black Beauty' vs. E13GB42. Transcript of both structural and regulatory genes were elevated in 'Black Beauty' coincident with violet/black pigmentation conditioned by anthocyanin accumulation. In contrast, transcript levels were very low in green fruit characteristic of E13GB42. Downregulation of three key genes in the anthocyanin biosynthetic pathway, *Chs*, *Dfr*, and *Ans* occurred coincident with reduced expression of *Myb_C* and *Myc* transcription factors in E13GB42. Chalcone synthase is the first and key regulatory enzyme of flavonoid biosynthesis. Dihydroflavonols are subsequently converted to a colorless leucoanthocyanidin by dihydroflavonol 4-reductase, the first committed enzyme of anthocyanin

Table 4. Segregation data for full sized fruit for the different generations tested in two different environments, open field and greenhouse, in Valencia, Spain.

Generation	Environment	N	Violet	Green with violet striping ^z	Green
Mutant (S0)	Open field	1	0	0	1
Clonal replicates of mutant (S0)	Greenhouse	8	0	0	8
Selfing of mutant (S1)	Open field	16	5	2	9
	Greenhouse	11	2	0	9
Selfing of selected green S1—line 2 (S2)	Greenhouse	28	0	0	28
Selfing of selected green S1—line 4 (S2)	Greenhouse	43	0	0	43
Selfing of selected green S1—line 10 (S2)	Greenhouse	28	0	0	28
'Black Beauty' $\times$ Mutant (S0)	Greenhouse	40	3	31	6
Mutant (S0) $\times$ 'Black Beauty'	Greenhouse	25	4	21	0

^zPlants having variable percentage of parts of the fruit skin covered by green and violet (striped) colors. Violet coloration is generally greater on fruit surfaces with direct sun radiation.

Table 5. Relative expression for E13GB42 and 'Black Beauty' anthocyanin structural genes in anthocyanin pigmented ('Black Beauty') and nonpigmented (E13GB42) fruit peels. Expression values are normalized to *Tub* for each of four developmental stages. Data are presented as the mean of four biological replicates.

Stage	Gene					
	<i>Chs</i>		<i>Dfr</i>		<i>Ans</i>	
	E13GB42	'Black Beauty'	E13GB42	'Black Beauty'	E13GB42	'Black Beauty'
	Relative expression (mean $\pm$ SE)					
w	5E-5 $\pm$ 1E-5	0.370 $\pm$ 0.055	3E-4 $\pm$ 8E-5	0.836 $\pm$ 0.150	1E-6 $\pm$ 8E-7	0.213 $\pm$ 0.031
x	5E-5 $\pm$ 1E-5	1.030 $\pm$ 0.122	3E-4 $\pm$ 9E-5	2.160 $\pm$ 0.635	1E-6 $\pm$ 2E-7	0.727 $\pm$ 0.126
y	0.003 $\pm$ 0.003	0.358 $\pm$ 0.091	2E-4 $\pm$ 9E-5	1.302 $\pm$ 0.410	nd ^z	0.246 $\pm$ 0.099
z	0.001 $\pm$ 6E-4	0.010 $\pm$ 0.070	6E-4 $\pm$ 1E-4	0.388 $\pm$ 0.155	1E-6 $\pm$ 4E-7	0.446 $\pm$ 0.261

^zExpression not detected in 50 polymerase chain reaction cycles.

Mean fruit weights for E13GB42 and 'Black Beauty' are stage w = 3.0 g, 2.4 g; x = 15.2 g, 15.0 g; y = 87.9 g, 101.4 g; and z = 479.3 g, 401.5 g, respectively.

Chs = chalcone synthase; *Dfr* = dihydroflavonol reductase; *Ans* = anthocyanidin synthase.

Table 6. Relative expression for E13GB42 and 'Black Beauty' anthocyanin regulatory genes in anthocyanin pigmented ('Black Beauty') and nonpigmented (E13GB42) fruit peels. Expression values are normalized to *Tub* for each of four developmental stages. Data are presented as the mean of four biological replicates.

Stage	Gene							
	<i>Myb_B</i>		<i>Myb_C</i>		<i>Myc</i>		<i>Wd</i>	
	E13GB42	'Black Beauty'	E13GB42	'Black Beauty'	E13GB42	'Black Beauty'	E13GB42	'Black Beauty'
	Relative expression (mean $\pm$ SE)							
w	2E-6 $\pm$ 1E-6	2E-6 $\pm$ 7E-7	4E-4 $\pm$ 1E-4	0.038 $\pm$ 0.006	0.004 $\pm$ 7E-4	0.050 $\pm$ 0.011	0.246 $\pm$ 0.028	0.235 $\pm$ 0.025
x	9E-6 $\pm$ 5E-6	3E-6 $\pm$ 7E-7	0.001 $\pm$ 4E-4	0.083 $\pm$ 0.011	0.004 $\pm$ 1E-4	0.124 $\pm$ 0.004	0.296 $\pm$ 0.037	0.298 $\pm$ 0.037
y	1E-5 $\pm$ 7E-6	1E-5 $\pm$ 2E-6	0.001 $\pm$ 6E-4	0.055 $\pm$ 0.019	0.008 $\pm$ 0.004	0.157 $\pm$ 0.018	0.101 $\pm$ 0.027	0.234 $\pm$ 0.037
z	3E-5 $\pm$ 2E-5	5E-5 $\pm$ 4E-5	0.002 $\pm$ 0.001	0.032 $\pm$ 0.013	0.016 $\pm$ 0.014	0.167 $\pm$ 0.071	0.985 $\pm$ 0.285	2.094 $\pm$ 0.584

Mean fruit weights for E13GB42 and 'Black Beauty' are stage w = 3.0 g, 2.4 g; x = 15.2 g, 15.0 g; y = 87.9 g, 101.4 g; and z = 479.3 g, 401.5 g, respectively.

biosynthesis. Leucoanthocyanidins are subsequently converted to colored anthocyanidins by anthocyanidin synthase. The current results are in agreement with expression profiles characterized in prior studies wherein transcript levels were measured in different eggplant genotypes with violet/black vs. white colored fruit (Stommel and Dumm, 2015; Zhang et al., 2014). Zhang et al. (2014) demonstrated increased transcript for all six biosynthetic enzymes required for production of colored anthocyanins and upregulation of *Myb* but not *Myc* in anthocyanin pigmented vs. nonpigmented eggplant fruit. The results from all of these studies suggest that loss of anthocyanin structural gene function is influenced by MYB and/or MYC transcription factors.

Multiple mechanisms may account for reduced anthocyanin biosynthesis. MicroRNAs (miRNA) can serve as negative regulators by cleaving or suppressing translation of complementary coding mRNAs. miRNAs have been demonstrated to reduce expression of *Mybs* that have a positive effect on anthocyanin biosynthesis, thus reducing expression of key enzymes in the anthocyanin biosynthetic pathway (Jia et al., 2015; Yang et al., 2013). In addition to *Mybs* that are positive regulators of anthocyanin biosynthesis, *Mybs* that function as part of the MYB-MYC-WD complex and repress transcription have been described (Albert et al., 2014; Yoshida et al., 2015). Both mechanisms may account for downregulation of the anthocyanin pathway and reduced anthocyanin pigmentation phenotypes.

The segregation ratios obtained for fruit pigmentation suggests that epigenetics may be involved in the presence or lack of anthocyanin-influenced pigmentation characterized in segregants obtained from selfing of the green-fruited mutant and reciprocal backcrosses to the mutant. Although true-breeding green-fruited segregants were identified, overall, the segregation ratios obtained for simple presence and absence of violet/black pigmentation or more complex uniform violet/black, violet/black striped, and uniform green classes were not compatible with simple Mendelian inheritance models, even when considering several genes, epistatic interactions, or variable penetration and expressivity. Similar to the violet/black-fruited segregants that we obtained from the selfing of the green-fruited mutant, Tatebe (1944) identified violet F₁ and violet as well as green-fruited segregants from a cross between two green-fruited varieties.

The complexity of eggplant color genetics was illustrated by Tigchelaar et al. (1968) who reported oligogenic control of eggplant fruit pigmentation by three complementary and up to six independent multiallelic genes that influenced anthocyanin pigmentation. More recent results using molecular markers report varying results, likely reflecting the genetic background of parental genotypes used in mapping studies. Barchi et al. (2012) identified markers distributed in three linkage groups that influenced anthocyanin pigmentation but were not fruit specific. Using a small population descended from an interspecific cross, Frary et al. (2014) reported two quantitative

trait loci (QTL) on chromosomes 11 and 12 that explained 86% and 69% of the variation for fruit anthocyanin intensity in one environment, but were not detected when the population was grown at a second location. Two additional QTL on chromosome 10 were associated with the presence of anthocyanin and explained 87% and 100% of the variability for the trait but again were detected in one environment but not in a second environment. Using association mapping, Cericola et al. (2014) identified 56 SNP markers associated with anthocyanin pigmentation, ten of which were associated with fruit color and distributed over five chromosomes. Despite, synteny between anthocyanin associated markers in eggplant and genomic regions in related Solanaceous species coding for several anthocyanin biosynthetic pathway genes and two anthocyanin related MYB transcription factors, efforts to ascribe fruit anthocyanin pigmentation to a major gene have been frustrated by putative environmental effects.

Multiple anthocyanin-related MYB-encoding genes have been characterized in numerous species and may be redundant with other members of the functionally diverse MYB gene family (Dubos et al., 2010). The role of MYB proteins in regulation of epidermal cell fate and identity is well documented (Feller et al., 2011). Interaction between multiple MYB transcription factors influences trichome initiation, extension, and branching. Chen et al. (2014) has demonstrated differential expression of several MYB-encoding genes during cucumber fruit spine/trichome development in a spined genotype and a glabrous mutant. Similarly, the increase in calyx prickliness observed in E13GB42 relative to 'Black Beauty' might be influenced by a member of the *Myb* gene family. Differential responsiveness of MYB proteins to biotic and abiotic stress (Dubos et al., 2010) may further suggest the action of a *Myb* variant in E13GB42. Eggplant plants develop a greater degree of prickliness when subjected to biotic and abiotic stresses (Gisbert et al., 2011; Prohens et al., 2004). Hence, reduced anthocyanin content in E13GB42 and resultant decreased antioxidant properties and protection of developing fruit from solar radiation might influence prickliness (Prohens et al., 2004).

We identified a green-fruited eggplant variant of the violet/black-fruited 'Black Beauty'. Our results demonstrate that the variant from which we selected E13GB42 likely arose as a spontaneous mutant of 'Black Beauty'. Loss of anthocyanin structural gene function in E13GB42 was associated with reduced levels of *MybC* and/or *Myc* regulatory gene transcript. These results, together with morphological data for calyx prickliness and segregation data for fruit color, suggest that the putative discreet mutation responsible for the E13GB42 phenotype is likely influenced by multiple genetic factors associated with the anthocyanin transcription factor complex. This genetic variant provides a valuable resource for future studies required to characterize these genetic effects.

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