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Characterization and Inheritance of the *Anthocyanin fruit* (*Aft*) Tomato

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Abstract

Tomato (*Lycopersicon esculentum*) accession LA1996 with the *Anthocyanin fruit* (*Aft*) gene has dark green foliage, elevated anthocyanin expression in the hypocotyls of seedlings, and anthocyanin in the skin and outer pericarp tissues of the fruit. Interest in the health benefits and antioxidant capacity of anthocyanins led to this study of the genetic potential for increased levels of this important class of phytonutrients in tomato fruit. In order to conform to tomato gene nomenclature rules, we propose changing the symbol *Af* for *Anthocyanin fruit* to *Aft*. Segregation ratios of anthocyanin expression in F_2 and BC_1 populations of a cross between the processing tomato UC82B and LA1996 were consistent with a single dominant gene hypothesis. Anthocyanin expression was reduced in backcross populations compared to F_2 populations. Anthocyanin concentration, as measured by the pH differential method, of pigment-rich pericarp and skin tissues from LA1996 was estimated to be 20.6 mg/100 g and 66.5 mg/100 g, respectively. Anthocyanidin composition was characterized by high-performance liquid chromatography (HPLC). Fruit of accession LA1996 contained predominantly petunidin, followed by malvidin and delphinidinin. Lycopene, β-carotene, phytoene, and phytofluene levels were similar to those of normal tomatoes and lower than those found in *high pigment* tomatoes.

Anthocyanins are the most common class of purple, red, and blue plant pigments. More than 300 different anthocyanin compounds have been identified in plants. They are planar molecules with a C6-C3-C6 carbon structure typical of flavonoids. The compounds are separated into distinct classes based on (1) substitution by hydroxyls at positions 3′, 4′, or 5′ of the phenyl "B" ring, (2) the type of glycosidic subunits, and (3) acyl substitution of the sugar hydroxyl (Mazza and Miniati 1993). Anthocyanins have received particular attention because of their very strong antioxidant activity as measured by the oxygen radical absorbing capacity (ORAC) assay. Grapes (Wang et al. 1996), blueberries, blackberries, raspberries, and cherries (Wang et al. 1997) all have high antioxidant capacity in comparison to other fruits and vegetables. Dietary phytonutrients and antioxidants provided by fruits and vegetables are considered to be of primary importance in the prevention of disease and aging (Ames 1983; Ames et al. 1993). Anthocyanins and related polyphenolic and flavonoid compounds are increasingly considered important phytonutrient contributors (Hollman et al. 1996; Lazarus and Schmitz 2000; Lean et al. 1999; Proteggente et al. 2002; Soleas et al. 1997).

Anthocyanin in Tomato Fruit

Tomato (*Lycopersicon esculentum*) fruit are not usually reported to contain anthocyanin. The red color in tomatoes is from the carotenoid, lycopene (Rick and Stevens 1986). The fruit of several closely related species—*Lycopersicon chilense, Lycopersicon hirsutum, Lycopersicon cheesmanii*, and *Solanum lycopersicoides*—do, however, contain anthocyanins (Giorgiev 1972; Rick 1964; Rick et al. 1994). *Anthocyanin fruit* (*Aft*) from *L. chilense, Aubergine* (*Abg*) from *S. lycopersicoides*, and *atroviolacium* (*atv*) from *L. cheesmanii* cause anthocyanin expression in tomato fruit.

A single paragraph note reported the presence of the dominant gene, *Af* (*Aft*) for *Anthocyanin fruit*, derived from a *L. esculentum* × *L. chilense* cross (Giorgiev 1972). Giorgiev (1972) reported that the purple color was caused by anthocyanins, though scant evidence was presented to confirm the pigment was indeed anthocyanin or verify inheritance of the trait. It is important to note that while *Af* has been used to represent *Anthocyanin fruit*, the *af* gene symbol was already in use for an independent gene, the recessive *anthocyanin free*, which lacks anthocyanin in vegetative tissues (Burdick 1958; Clayberg 1960). In order

to conform to tomato gene nomenclature rules (Clayberg 1970), we propose the gene symbol *Aft* for *Anthocyanin fruit*.

The allelic relationships between *Aft, Abg*, and *atv* remain unclear. *Abg* resulted from the intergeneric cross *S. lycopersicoides* × *L. esculentum* and is linked to a random amplified polymorphic DNA (RAPD) marker on chromosome 10 (Rick et al. 1994). It is possible that *Abg* lies in the chromosomal inversion recently identified between *S. lycopersicoides* and *L. esculentum* on chromosome 10 (Pertuze et al. 2002). Homozygous *S. lycopersicoides* introgressions of this region were not achieved using a marker-assisted selection approach (Canady 2002). Stable homozygotes of *Abg* have not been obtained. The instability of *Abg* has impeded traditional allele tests with *Aft*. *L. chilense*, the donor genome for *Aft*, is not known to contain any chromosomal inversions in relation to *L. esculentum*. It is possible that *Aft* is an allele of *Abg* and resides on chromosome 10, however, six restriction fragment length polymorphism (RFLP) loci surveyed along chromosome 10 of LA1996 failed to uncover a *L. chilense* introgression (Jones C, unpublished data). *Aft* and *Abg* show differential expression of anthocyanin in and beneath the epidermal cell layer. Plants with the gene *atv* (derived from a Galapagos island accession described in the original publication as *L. pimpinellifolium* but now considered to be *L. cheesmanii*) also express anthocyanin (Rick 1964). Pigment expression in *atv* is distinct from *Aft*. Expression in vegetative tissues is more intense in *atv*, while expression in the fruit is not as great and appears to be expressed in different cell layers not visually detectable (Mes P, unpublished observations) as in *Aft*.

Reports of pigments attributed to anthocyanins in tomato fruit are confusing. Many heirloom varieties of tomatoes whose names contain "purple" and "black" do not contain anthocyanins. Rather, the *green flesh* (*gf*) gene(s) is probably involved (Butler 1962; Kerr 1956), and the muddy brown fruit colors are attributed to persistent green chlorophyll combined with red from lycopene (Jones 2000). (During normal tomato ripening, chlorophyll breaks down concurrent with the increase in carotenoid formation.) However, no allelism tests have been reported between these heirloom varieties and the *green flesh* gene. The functionally homologous gene *cl*, inhibiting chlorophyll breakdown, is responsible for green and brown mature fruit color in peppers (Smith 1950).

Anthocyanin in Tomato Vegetative Tissues

Normal tomato genotypes routinely have anthocyanin in vegetative tissues. Previously identified genes (*a, aa, ae, ai, al, af, afr, ah, aw, bls, hp-1*, and *hp-2*) are known to affect anthocyanin levels in vegetative tissues. Bovy et al. (2002) identified petunidin 3-(*p*-coumaryl rutinoside)-5-glucoside and malvadin 3-(*p*-coumaryl rutinoside)-5-glucoside as the principal anthocyanins in tomato vegetative tissue. This is consistent with vegetative anthocyanins identified by Gromer (2000). Petunidin 3-(*p*-coumaryl rutinoside)-5-glucoside was tentatively identified in extracts from wild-type seedlings, while peonidin-3-(*p*-coumaryl rutinoside)-5-glucoside was tentatively identified in tomato mutants *a, ag, ai, al, atv*, and *hp-1* (Von Wettstein-Knowles 1968).

Anthocyanins in Related Solanaceae

Petunidin 3-(*p*-coumaryl-rutinoside)-5-glucoside is the principal anthocyanin found in garden huckleberry (*Solanum scabrum*), purple-fleshed potatoes (*Solanum tuberosum*), as well as the fruit of purple tomatillos (*Physallis ixocarpa*) (Mazza and Gao 1994; Price and Wrolstad 1995). In contrast, red-fleshed potatoes are characterized as containing predominantly pelargonidin 3-(*p*-coumaryl rutinoside)-5-glucoside (Rodriguez-Saona et al. 1998). Eggplant (*Solanum melongena*) contains principally delphinidins (Jackman and Smith 1996).

Rational

High antioxidant activity associated with anthocyanins in other species suggests that increasing anthocyanin content in tomato fruit may increase their antioxidant capacity. Recent attempts to enhance flavonoid and antioxidant levels through transgenic expression of chalcone isomerase (Muir et al. 2001; Verhoeyen et al. 2002) and maize transcription factors *LC* and *C1* (Bovy et al. 2002) have dramatically increased flavonoid levels, though not anthocyanins, in tomato fruit.

Plants containing the *Aft* gene have phenotypic similarities to tomatoes with the *high pigment* genes. The *high pigment* tomato fruits have high levels of carotenoids, flavonoids, and vitamin C due to a mutation that exaggerates phytochrome response (Barker and Tomes 1964; Kerckhoffs et al. 1997; Kerr 1957, 1965; Minoggio et al. 2003; Palmieri et al. 1978; Peters et al. 1992). The homologous phenotypes include deeper green foliage and stems, as well as intense anthocyanin expression in the hypocotyls and roots of germinating seedlings. Pigment production in *Aft* fruit is stimulated by light exposure (Giorgiev 1972) and phytochromes are known to act in light-mediated regulation of anthocyanin production (Adamse et al. 1989; Kerckhoffs and Kendrick 1993). Consequently phytochrome action may be involved in the *Anthocyanin fruit* phenotype, and other physiological traits may be affected as they are in *high pigment* tomatoes.

The objectives of this study were to verify the inheritance of *Aft*, describe its expression, and measure total anthocyanin and carotenoid levels.

Materials and Methods

Germplasm Sources

The accession LA1996 containing *Aft* (Figure 1) was obtained from the C. M. Rick Tomato Genetics Resource Center, Davis, CA. LA1996 was originally contributed and annotated as containing *Anthocyanin fruit* by Giorgiev (1972). UC82B, a red-fruited open-pollinated processing tomato, was provided by Lockhart Seed Company, Stockton, CA.

Anthocyanin Concentration

Ripe tomatoes were harvested from five plants grown at the Seeds of Change Research Farm, Corvallis, OR, in 1998. We

chose fruit expressing medium to high levels of anthocyanin for extraction in order to represent potential, not average, anthocyanin production. Areas of skin containing anthocyanin were removed with a scalpel and pericarp tissue containing anthocyanins was separated from the rest of the fruit with a vegetable peeler. Pericarp and skin were kept separate and frozen at –23°C until extraction.

We extracted anthocyanins according to the procedures of Giusti and Wrolstad (1996). Tissues were frozen with liquid nitrogen and powdered in a mill prior to extraction. A total of 17.64 g of pericarp tissue powder and 6.42 g of skin powder were extracted separately with 100% acetone and filtered with a Buchner funnel. Filter cake was reextracted with 70% acetone. We partitioned the extractant with chloroform in a separatory funnel and allowed it to stand at 1°C for 12 h. The chloroform fraction was discarded and the aqueous fraction was rotoevaporated at 40°C until dry and brought to total volume of 50 ml with distilled water. Total anthocyanin content was measured by the pH differential method (Fuleki and Francis 1968) as described by Wrolstad (1976) using a Varian DMS double-beam spectrophotometer. This method estimates total anthocyanin content based on the reversible conversion of anthocyanins from the oxonium to the hemiketal form, but does not determine the individual compounds present. Samples were diluted 5:1 in pH 1.0 and pH 4.5 buffer. The difference in absorption between the ultraviolet (UV)-visible adsorption maxima (540 nm for skin tissue and 535.5 for pericarp) and adsorption at 700 nm was determined at pH 1.0 and pH 4.5. The difference between these two values was used to determine the total anthocyanin concentration according to the formula (Abs pH 1.0 – Abs pH 4.5)/$\varepsilon L \times 10^3 \times MW \times$ dilution factor. The results are expressed as petunidin-3-(*p*-coumaroyl rutinoside)-5-glucoside based on 17,000 as the extinction coefficient (ε) and 934, the molecular weight (*MW*) (Price and Wrolstad 1995). *L* is the path length (in centimeters) of the spectrophotometer.

High-Performance Liquid Chromatography (HPLC) Anthocyanidin Analysis

We extracted anthocyanins from fruit of accession LA1996 using the method described by Rodriguez-Saona et al. (1998). This method isolates anthocyanins based on the preferential solubility of anthocyanins in acetone versus chloroform. Twelve grams of skin and pericarp tissue containing anthocyanin were excised from the fruit and homogenized in liquid nitrogen for subsequent anthocyanin isolation. Tubes of isolated anthocyanins were stored at –23°C. Anthocyanidins were produced from the isolated anthocyanin solution using the acid hydrolysis protocol for preparation of anthocyanidins (Durst and Wrolstad 2001). Forty milliliters of isolated anthocyanins were thawed and filtered through a 0.45 μm filter attached to an activated C_{18} SPE cartridge. The SPE cartridge was rinsed twice with 5 ml of acidified water. The anthocyanin was then eluted into a rotoevaporation boiling flask using two washes with 5 ml methanol. The solution was evaporated until a small amount of liquid remained. The solution was placed in a screw-top test tube, to which 10 ml 2 N HCl was added. The tube was flushed with nitrogen, sealed loosely, and placed in a boiling water bath for 30 min. The tube was then placed in an ice bath until cool. The sample was reapplied to a C_{18} SPE cartridge, rinsed, and rotoevaporated to remove methanol. Two milliliters of 4% phosphoric acid was added and the aliquot was analyzed promptly by HPLC. A sample of the isolated anthocyanins was also saponified using the method described by Durst and Wrolstad (2001) to remove acylation. A subsample of the saponified anthocyanins was subjected to acid hydrolysis and then analyzed by HPLC.

HPLC Carotenoid Analysis

High-performance liquid chromatography analysis was performed using a rapid HPLC method developed for tomato carotenoid analysis (Jones 2000). Homogenized tomato samples were extracted with tetrahydrofuran, filtered, and combined 1:1 with eluent A (85% acetonitrile, 10% methanol, 2.5% hexane, and 2.5% dichloromethane, v:v). Samples were microfiltered with a 0.2 μm nylon syringe filter (Alltech Inc., Deerfield, IL) and 50 μl was injected into the HPLC system. All extractions were carried out under subdued ambient light. HPLC was performed with a Beckman model 334 gradient liquid chromatograph (Beckman Instruments Inc., Berkeley, CA) equipped with a Waters 991 photodiode array detector (Waters Inc., Milford, MA). Analysis was carried out using a reverse phase Spheri-5 RP-18 column (220 mm × 4.6 mm, 5 μm; Perkin Elmer Brownlee, Norwalk, CT) coupled to an ODS-5S guard column (3.0 cm × 4.6 mm; Bio-Rad, Richmond, CA). Gradient conditions employed for the mobile phase consisted of eluent A (85% acetonitrile, 10% methanol, 2.5% hexane, and 2.5% dichloromethane, v:v) and eluent B (45% acetonitrile, 10% methanol, 22.5% hexane, and 22.5% dichloromethane v:v). The chromatographic conditions at a flow rate of 1 ml/min were: 0–1 min, 100% isocratic eluent A; 1–7 min, linear gradient from 0–100% eluent B; and 7–20 min, 100% eluent B.

Genetic Studies

Single plants of LA1996 and UC82B were crossed in the greenhouse during the winter of 1999 by standard emasculation and crossing techniques. F_1 plants were grown and observed at the Seeds of Change Research Farm in Corvallis, OR, in 1999. Plants were allowed to self-pollinate and were crossed to UC82B to produce the F_2 and the BC_1F_1 populations. F_2 and backcross populations were grown and scored in the greenhouses of Oregon State University (OSU) in the winter/spring of 2000 or at the OSU Vegetable Research Farm in Corvallis, OR, in the summer of 2000 (F_2 population only). Greenhouse plants were grown in 4 L pots with a soil-free potting mix (Rexius Forest Products, Eugene, OR) and fertilized with Osmocote 14-14-14 slow release fertilizer (Scott Sierra, Marysville, OH). Natural light was supplemented with high-intensity metal halide and sodium

Figure 1. Fruit from tomato accession LA1996 showing anthocyanin conditioned by *Aft*. Epidermis has been peeled to reveal anthocyanin expression in the pericarp. Color image is available at http://tgrc.ucdavis.edu/images/Aft-figure1.

lamps suspended 1.3 m above the bench. Supplementary lighting was provided 10 h/day and temperatures were maintained at a minimum of 24°C during the day and 18°C at night. We evaluated segregating populations in a green fruit stage on a scale of 1–4 (1 represents no anthocyanin and 2, 3, and 4 represent low, medium, and high levels of expression, respectively; see Figure 1). Anthocyanin expression is correlated in the green and red stages; however, we scored in the green fruit stage because low expression levels in some plants are more difficult to score in the presence of mature fruit color. All fruit on the plant was inspected and the plant was scored according to the maximum anthocyanin expression in the fruit.

We analyzed data by using chi-square tests of goodness-of-fit, with the Yates continuity correction recommended for data with one degree of freedom (Little and Hills 1978; Yates 1934). Data from separate populations were pooled for analysis based on a chi-square test of homogeneity (Little and Hills 1978).

Results and Discussion

Phenotypic Description

Vegetative tissues of *Aft* tomato plants are distinctive. Leaves are darker green and stems contain visibly more purple speckling than do wild-type plants. The plants are vigorous and do not have the reduced growth associated with *high pigment* genotypes (Sayama 1979; Sayama and Tigchelaar 1985; Wann 1996; Wann et al. 1985). With the exception of anthocyanin expression, LA1996 is phenotypically a normal *L. esculentum* plant, suggesting most of the *L. chilense* genome has been eliminated. Fruit anthocyanin expression was strongest in areas exposed to light. For example, anthocyanin was absent in areas shaded by the calyx. Anthocyanin expression was strongest in the skin and pericarp tissues beneath the skin (Figure 2). The fruit interior did not contain visible anthocyanin.

Anthocyanidin Pigments and Anthocyanin Concentration

The purple color of LA1996 (Figures 1 and 2) is produced by the glycosylated anthocyanidins of petunidin, malvidin, and delphinidin, as determined by comparison of elution times of anthocyanidins compared to known standards (Figure 3). Of these, petunidin and malvidin are predominant. The three major peaks of the saponified anthocyanins were not positively identified, but were consistent with the typical elution time of a 3,5-diglycoside, such as the petunidin 3-(*p*-coumaryl)rutinoside-5-glucoside reported in tomato vegetative tissues (Von Wettstein-Knowles 1968). This would suggest that the anthocyanins present in *Aft* fruit are petunidin, malvidin, and delphinidin 3-(*p*-coumaryl-rutinoside)-5-glucoside, though analysis by liquid chromatography-mass spectrophotometry will be necessary to confirm this. Petunidin 3-(*p*-coumaryl-rutinoside)-5-glucoside is the dominant anthocyanin in garden huckleberry (*S. scabrum*) fruit (Francis and Harborne 1966; Price and Wrolstad 1995), potatoes (Mazza and Gao 1994), and tomatillo fruit (Price and Wrolstad 1995), which indicates that this compound is widely distributed in the Solanaceae.

The total monomeric anthocyanin concentration of pigment-rich tissues separated from whole LA1996 fruits was estimated by the pH differential method to be 20.6 mg/100 g in the pericarp tissue and 66.5 mg/100 g in the skin expressed as petunidin 3-(*p*-coumaryl-rutinoside)-5-glucoside. This demonstrates the anthocyanin production potential from pigment-rich tissues, not the average values for LA1996 fruit, as only pigment-rich tissues were extracted. Concentrations of anthocyanins measured by this method may be affected by copigmentation with other flavonoids (Asen 1975; Asen et al. 1972). Very little copigmentation occurs at pH 2.0 or less, but increases with pH (Asen et al. 1972). We measure absorbance at pH 1.0 and subtract the pH 4.5 value; therefore if copigmentation occurs, greater absorbance at pH 4.5 would cause us to underestimate actual concentrations.

Inheritance of *Aft*

The hypothesis that a single dominant gene confers the *Anthocyanin fruit* phenotype is consistent with our data. F_1 progeny of 12 separate pollinations to LA1996 were evaluated in the field in 1999, and all F_1 plants expressed anthocyanin in the fruit. We subjected the three F_2 populations (derived from three separate F_1 plants representing different pollination events) to a homogeneity test ($P = .601$)

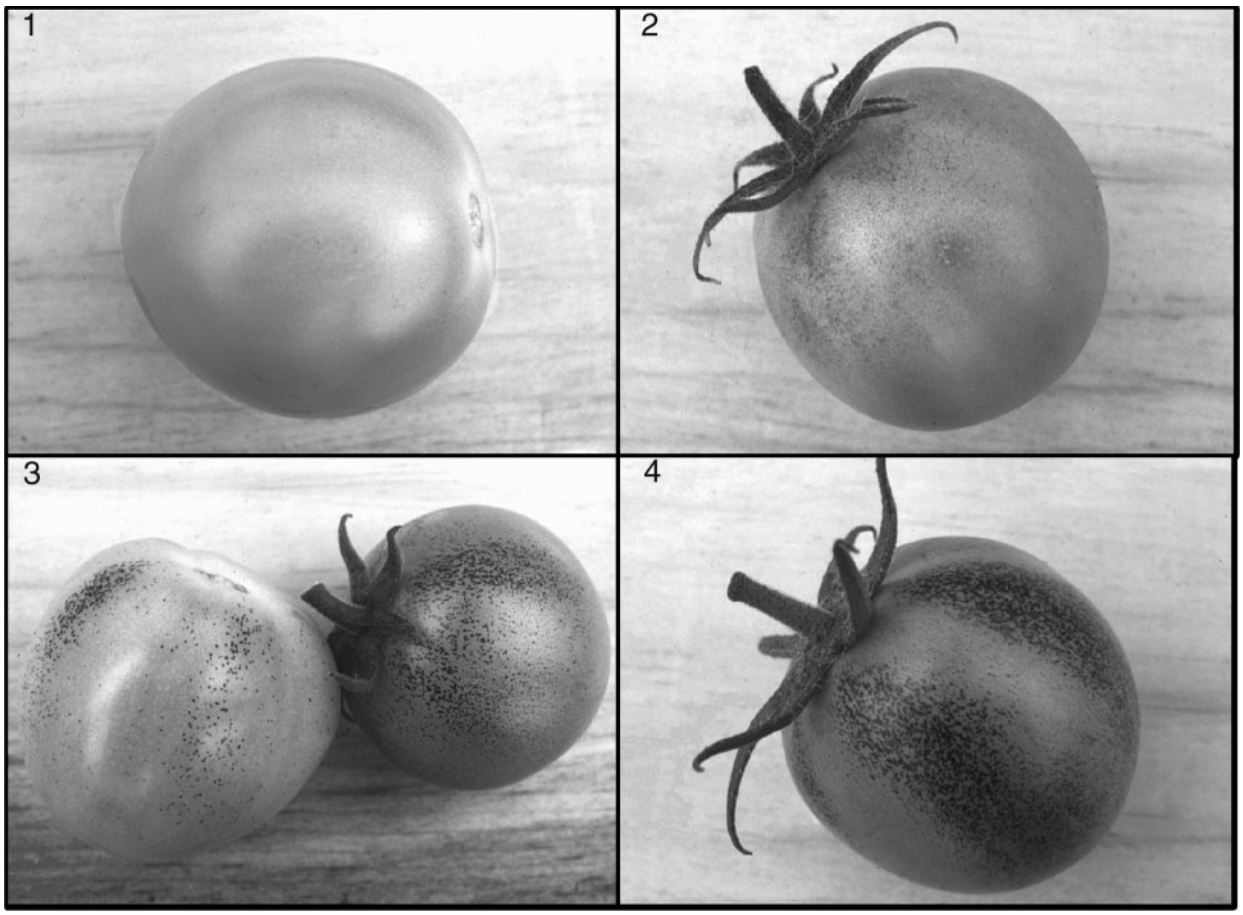

Figure 2. Visual rating scale used to evaluate anthocyanin in F_2 and backcross progeny: 1, no expression; 2, low expression; 3, medium expression; 4, high expression. Color image is available at http://tgrc.ucdavis.edu/images/Aft_figure2.

and pooled the data (Table 1). F_2 progeny of a cross between LA1996 (*Aft*) and the processing cultivar UC82B were consistent with the hypothesis of a single dominant gene for *Anthocyanin fruit* ($P = .190$). We tested two gene ratios of 9:7 and 13:3 and found the data fit neither model. Progeny of two different backcrosses to UC82B segregated in a manner consistent with a single dominant gene hypothesis when analyzed separately (Table 1). However, after a test of homogeneity and pooling of data from progeny of both backcross populations, there were fewer than expected

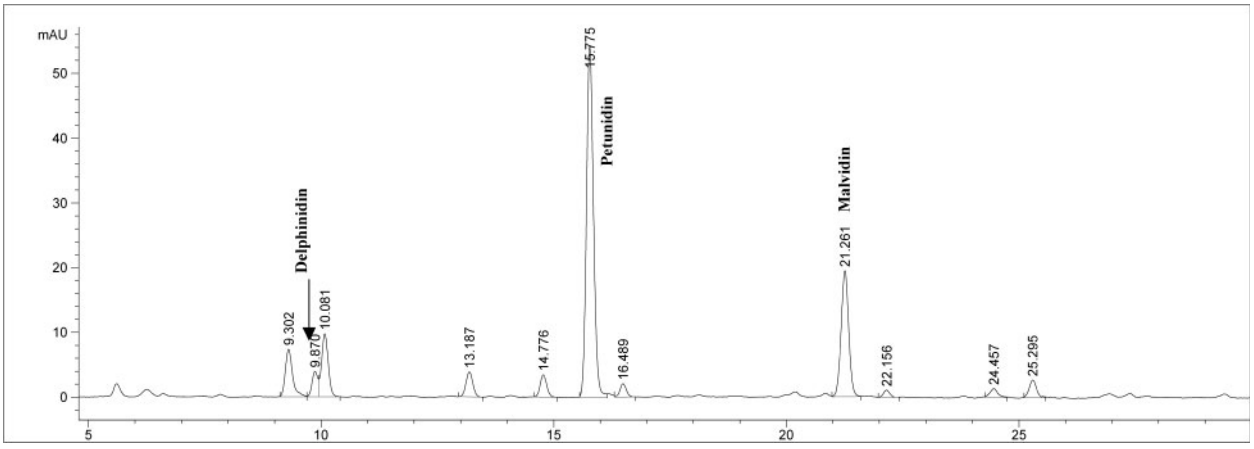

Figure 3. Chromatogram of LA1996 *Aft* fruit upon extraction and acid hydrolysis.

Table 1. Chi-square test of single dominant gene hypothesis for *Aft* in F_2 and BC_1 populations grown in the greenhouse in the winter/spring 2000 and in the field in the summer of 2000

Location	Cross	Number of plants		Expected ratio	χ^2	*P*
		Anthocyanin present	Anthocyanin absent			
F2 populations						
Greenhouse	UC82B × LA1996	40	20	3:1	1.80	.18
Field	UC82B × LA1996	87	33	3:1	0.28	.60
Field	UC82B × LA1996	32	12	3:1	0.03	.86
Crosses combined		159	65	3:1	1.72	.19
Heterogeneity					0.81	.37
Backcross populations (greenhouse)						
Backcross S-2	(UC82B × LA1996) × UC82B	16	28	1:1	2.75	.10
Backcross S-1	(UC82B × LA1996) × UC82B	7	13	1:1	1.25	.26
Backcrosses combined		23	41	1:1	4.52	.03
Heterogeneity					0.01	1.00

Anthocyanin fruit (*Aft aft*) plants ($P = .034$). This may have resulted from *Aft* deriving from an interspecific cross between *L. esculentum* and *L. chilense* for which significant fertility barriers exist (Rick and Stevens 1986). Elimination of an allele through chromosome loss could be involved. Alternatively, lower expression of anthocyanin in the backcross population than in the F_2 could result from reduced expression in heterozygous BC_1F_1 plants or result from lower light levels in the greenhouse environment where the backcross populations were grown. Consequently some heterozygous plants could have displayed such limited expression that they were incorrectly scored.

Chi-square tests are presented (Table 1) based on the presence or absence of anthocyanin. Levels of anthocyanin expression were variable and were scored from 1 (no anthocyanin) to 4 (high anthocyanin). The *Aft* phenotype differed between the F_2 population and the backcross populations, with very little medium or high anthocyanin expression in the backcrosses (Figure 4). While the low anthocyanin levels in the backcross populations as compared to the F_2 generation may suggest reduced expression in the heterozygotes, we did not grow F_3 plants from putative heterozygotes to address the possibility of incomplete dominance.

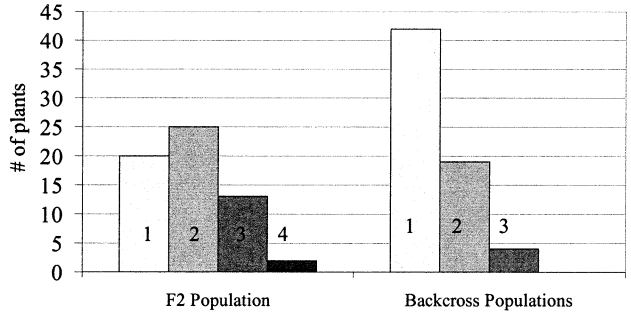

Figure 4. Anthocyanin intensity in greenhouse grown F_2 and backcross populations of *Aft* crosses: 1, no expression; 2, low expression; 3, medium expression; 4, high expression.

Normal tomato genotypes routinely contain anthocyanin in the vegetative parts of the plants but not in the fruit. The cuticles of *L. esculentum* fruit contain chalconaringenin, the pigment believed to be responsible for the yellow color of tomato skin (Hunt and Baker 1980). Chalcones are biosynthetic precursors to the anthocyanins. These facts indicate that all the structural genes necessary for anthocyanin production are already present in the tomato genome. Consequently we believe *Aft* is more likely a regulatory gene or promoter region of a structural gene that up-regulates fruit anthocyanin expression. Bovy et al. (2002) found that transgenic fruit with high levels of flavonoids did not produce anthocyanins due to low fruit expression of flavonoid 3′-hydroxylase and flavonoid 3′5′-hydroxylase. It is likely that these genes are up-regulated in *Aft* fruit.

While *Aft* has phenotypic similarities to the *hp* phytochrome response mutants, *Aft* may be unrelated to the phytochrome response mechanism. While the vegetative and fruit characteristics share similarities, the deep vegetative and fruit color of the *hp* mutants is considered to be principally the result of increased chlorophyll in the leaves and unripe fruit and carotenoid content in ripe fruit (Barker and Tomes 1964; Jarret et al. 1984; Wann et al. 1985). In contrast, we attribute the dark foliage and dark ripe fruit characteristic of LA1996 to the presence of anthocyanins. In addition, unripe fruit of LA1996 are not dark green like that of the phytochrome mutants, and we demonstrate in this article no carotenoid increase in the ripe fruit. Also lacking in the *Anthocyanin fruit* tomato plants are the brittle stems and characteristic shortened seedling hypocotyls found in the *hp* mutants (Jarret et al. 1984).

Carotenoids of the *Anthocyanin fruit* Tomato

The phenotypic similarities between the *Aft* tomato plants and *high pigment* tomato plants led us to investigate the possibility that LA1996 may also have heightened carotenoid levels (Jones 2000). Carotenoid levels observed in LA1996 in

three separate trials were not statistically different from normal tomatoes. The mean carotenoid content of LA1996 was 1.93 mg/100 g FW phytoene, 1.31 mg/100 g FW phytofluene, 0.64 mg/100 g FW β-carotene, and 5.15 mg/100 g FW lycopene. *Aft* fruit were significantly lower in lycopene and β-carotene than *hp-1, hp-2*, and *dg* genotypes. While nearly isogenic lines with *Aft* are not yet available, our results indicate that it is unlikely that *Aft* substantially alters carotenoid quantity or carotenoid ratios.

Conclusion

We have established the presence and quantity of anthocyanins in *Aft* tomatoes. We have identified petunidin, followed by malvidin and delphinidinin as the principal anthocyanidins in *Anthocyanin fruit*. We have shown that the *Anthocyanin fruit* phenotype introgressed into *L. esculentum* from *L. chilense* is inherited in a manner consistent with a single dominant gene. *Aft* plants have shared phenotypic traits with other tomato photomorphogenic mutants (*hp-1, hp-2*, and *dg*). In contrast to these *high pigment* tomatoes, no increase in carotenoid content was associated with the presence of *Aft*. While intense anthocyanin expression is needed for strong antioxidant activity, the introduction of the *Anthocyanin fruit* characteristic into carotenoid-rich tomatoes provides the opportunity to develop new cultivars rich in water- and lipid-soluble antioxidants. The simple inheritance of *Aft* makes utilization of this gene in existing tomato germplasm feasible.

Acknowledgments

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