Identification and Quantification of Anthocyanins in Transgenic Purple

Tomato

Xiaoyu Su[†], Jianteng Xu[†], Davina Rhodes[†], Yanting Shen[†], Weixing Song[‡], Benjamin Katz[‡],
John Tomich[‡], Weiqun Wang^{*†}

[†]Department of Food Nutrition Dietetics & Health, [‡]Department of Statistics, [‡]Department of
Biochemistry, Kansas State University, Manhattan, KS 66506, USA

* Corresponding author at: Department of Food Nutrition Dietetics & Health, Kansas State
University, Manhattan, Kansas 66506, USA. Tel.: +1-785-5320153; Fax: +1-785-532-3132; E-
mail: wwang@ksu.edu (W. Wang).

Abbreviations used: *Del*, Delila; *F3'5'H*, flavonoid 3'5'-hydroxylase; *PAL*, phenylalanine
ammonia-lyase; *Ros1*, Rosea1.

ABSTRACT: Anthocyanins are natural pigments derived from the phenylpropanoid pathway. Most tomatoes produce little anthocyanins, but the transgenic purple tomato biosynthesizes a high level of anthocyanins due to expression of two transcription factors (*Del* and *Ros1*). This study was to identify and quantify anthocyanins in this transgenic tomato line. Seven anthocyanins, including two new anthocyanins [malvidin-3-(p-coumaroyl)-rutinoside-5-glucoside and malvidin-3-(feruloyl)-rutinoside-5-glucoside], were identified by LC-MS/MS. Petunidin 3-(trans-coumaroyl)-rutinoside-5-glucoside and delphinidin 3-(trans-coumaroyl)-rutinoside-5-glucoside were the most abundant anthocyanins, making up 86% of the total anthocyanins. Compared to undetectable anthocyanins in the wild type, the contents of anthocyanins in the whole fruit, peel, and flesh of the *Del/Ros1*-transgenic tomato were 5.2 ± 0.5 , 5.1 ± 0.5 , and 5.8 ± 0.3 g/kg dry matter, respectively. Anthocyanins were undetectable in the seeds of both wide-type and transgenic tomato lines. Such novel and high levels of anthocyanins obtained in this transgenic tomato may provide unique functional products with potential health benefits.

KEYWORDS: *Anthocyanins, transgenic tomatoes, Delila; Roseal*

1. Introduction

The natural pigments produced in plants, including chlorophylls, carotenoids, and anthocyanins, are generally synthesized via phenylpropanoid and terpenoid pathways (Gonzali et al., 2009). Anthocyanins (derived from Greek *anthos* (flower) and *kyanos* (dark blue)) are one of the most important water-soluble plant pigments (Delgado-Vargas & Paredes-López, 2003). They are synthesized by the flavonoid branch of the phenylpropanoid pathway through secondary metabolism in higher plants. Among over 600 types of anthocyanins (Xu & Howard,

2012), the majority of anthocyanin aglycones found in nature consist of six anthocyanidins, i.e., cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin. They share a 2-phenylbenzopyrilium (flavyl-ium) skeleton hydroxylated in 3, 5, and 7 positions, with different substitutions at R1 and R2 (Fig. 1). In comparison with other flavonoids, anthocyanins possess a positive charge on its C-ring, which leads to different colors in response to various pH (Wang & Stoner, 2008).

Anthocyanins present in human foods have received considerable attention due to their possible health-promoting properties such as antioxidant and anti-inflammatory effects (Lim et al, 2013; Bowen-Forbes et al, 2010). Based on food intake data from NHANES 2001-2002, the daily intake of anthocyanins was estimated to be 12.5 mg/day/person in the United States (Xu et al., 2006). The predominant dietary anthocyanins are malvidin, delphinidin, and peonidin glycosides (Bognar et al., 2013), which can be found in many plant foods, including berries, purple sweet potatoes, grapes, and wine.

Tomato (*Solanum lycopersicum L.*) is one of the most important food crops in the world. Its rich red color is due to accumulation of the carotenoid pigments, i.e., lycopene and phytoene, in the peel and flesh (Pannellini et al., 2004; Khachik et al., 2002). However, when compared to anthocyanin-enriched plants, tomatoes generally produce little anthocyanins. Genetic engineering is a powerful approach to induce and enhance biosynthesis of anthocyanins in plants (Schijlen et al., 2004), which has been successfully applied in food crops such as potato and rice (Tanaka and Ohmiya 2008; Lukaszewicz et. al., 2004). Several transgenic tomatoes with increased flavonoid levels have also been developed. A transgenic tomato line created by expressing two maize regulatory genes, *Lc* and *Cl*, was reported to produce a high level of flavonols rather than anthocyanins (Bovy et al., 2002). Overexpression of the *ANT1* gene encoding a MYB

transcription factor was further reported to induce a purple spotting on the epidermis of tomatoes (Mathews et al., 2003). In addition to the *ANT1* gene, combining the *atv* gene with either *Aft* or *Abg* was found to generate anthocyanin petunidin-3-(p-coumaryl)-rutinoside-5-glucoside predominantly in the epidermis of tomatoes up to 0.1% in fresh weight (Peter et al., 2008). Furthermore, expression of two snapdragon (*Antirrhinum majus*) transcription factors, i.e., Delila (*Del*) and Rosea1 (*Ros1*), in the fruit of transgenic tomatoes activated multiple anthocyanin biosynthesis-related genes, including phenylalanine ammonia-lyase (PAL), and flavonoid 3'5'-hydroxylase (F3'5'H) (Butelli et al., 2008). The *Del/Ros1* transgenic tomato grew normally during the green stage and then started to accumulate purple pigments during the ripening stage, exhibiting an intense and uniform purple color both in the peel and flesh (Butelli et al., 2008). According to the reported methods by Butelli et al. (2008), the Park lab has engineered and produced the *Del/Ros1* transgenic tomato line (Lim et al. 2014). Figure 2 shows the whole, cross-section, and freeze dry of ripe wild-type and transgenic *Del/Ros1* fruits.

The objectives of this study were to identify and quantify the anthocyanin profile in this transgenic tomato, and to determine the distribution of anthocyanins in the peel, flesh, and seed of the fruits.

2. Materials and methods

2.1. Materials

Acetonitrile, methanol (MeOH), and formic acid used in this study at either HPLC grade or analytic grade were purchased from Thermal fisher Scientific (Suwanee, GA). Water used was purified through Barnstead E-Pure Deionization System (Dubuque, IA) and filtered by Millipore

0.45 µm membrane (Bedford, MA). A standard of Peonidin-3-glucoside chloride was purchased from Sigma-Aldrich (St. Louis, MO).

2.2. Sample preparation and extraction

Wild type (*Solanum lycopersicum* L. cv Rubion) and *Del/Ros1* transgenic purple tomatoes generated in previous studies (Lim et al. 2014) were harvested in the Kansas State University Department of Horticulture greenhouses. For each line, ripe tomatoes were washed with tap water, diced into approximately 0.5 cm cubes, freeze-dried (Labconco, FreeZone 2.5), and ground by a food processor into powder. Prepared powder was then stored at -80 °C until further extraction. For preparation of anthocyanin extracts, 0.05 g of the powder was extracted with 4 mL of acidified MeOH with 1N formic acid at 9:1 (v/v). The flasks containing powder/solvent mixture were wrapped with aluminum foil to avoid light exposure. After a 12-hour extraction, the samples were centrifuged at 2,800 rpm for 30min and then the supernatant was collected and dried by vacuum drier at 25 °C overnight. One mL of the acidified MeOH was added and then the dissolved extract was filtered by Whatman syringe filter (Whatman 0.45µm PVDF) for LC-MS/MS analysis.

2.3. Identification and analysis of anthocyanins by LC-MS/MS

LC coupled Electrospray Ionization tandem Mass Spectrometry (LC-MS/MS) was used to carry out anthocyanin identification and quantification. A Shimadzu HPLC system (Kyoto, Japan) was used for chromatographic analysis and separation. This system employed a DGU-20A3 built in degasser, a LC-20AB solvent delivery pump, a SIL-20AHT auto-sampler, a CTO-20AC column-holding oven, a CBM-20A communicator module, and a SPD-M20A Photodiode Array Detectors. A Waters (Milford, MA) C₁₈ reversed phase column (250 mm length, 4.6 mm diameter) was used for anthocyanin separation. Data was analyzed using LC

solution software (Kyoto, Japan). Elution was performed with mobile phase A (5% formic acid in de-ionized water) and mobile phase B (5% formic acid in acetonitrile/water 1:1 v: v). An optimum column temperature was set at 25 °C. At a flow rate of 0.8mL/min, the gradient conditions were set as follows: solvent B volume at 5-20% for 35min, 20-50% for 10min, and held at 50% for 10 min before returning to 5% at 60 min. The detector performed a full spectrum scan between 190-800nm, where 520 nm was used for monitoring anthocyanins. Peonidin-3-glucoside was used as an internal standard for quantitation of extraction recovery and the anthocyanin contents were expressed as peonidin 3-glucoside equivalent (PN3GE). Based on a signal-to-noise ratio of 3:1 and the standard deviation of the lowest concentration of PN3G/slope of the calibration line, the detection limit was estimated to be 2 pmol.

Mass spectrometric scan was performed on a Bruker Esquire 3000 in positive mode with a scanning interval 500-1200 m/z. Nebulization was conducted at 350 °C aided by concurrent N₂ flow at 10 psi; capillary and cone voltages were set at 3.5 kV and 40 V; drying gas flow rate was 5 L/min. Mass of precursor ions and reactions of fragments loss were evaluated. Data were analyzed using Bruker Hystar Post Processing software (Bruker Daltonics, GmbH, Billerica, MA). The ESI/MS data was used to confirm the mass of each anthocyanin HPLC peak. The mass spectrometry instrument was controlled by the esquire control 5.3 software (Bruker Daltonics, GmbH, Billerica, MA) and the data were processed with Data analysis 3.3 software (Bruker Daltonics, GmbH, Billerica, MA). Individual identification of each anthocyanin was accomplished by comparison of HPLC retention time, absorbance spectra, and MS spectra with our previously published anthocyanin data (Lim et al. 2013; Xu et al. 2015). The new anthocyanins were identified by matching the mass spectral data with those from the National

Institute of Standards and Technology Mass Spectra Library data (NIST08, National Institute of Standards and Technology, Gaithersburg, MD, USA).

2.4. Statistical analysis

Data were analyzed using SAS statistical software, version 9.3 (SAS Institute, Cary, NC, USA). Results were evaluated by one-way ANOVA using a general linear model procedure followed by Tukey's post-hoc test. The results were presented as means $\pm$ SD, and a probability at $p \leq 0.05$ was considered significant.

3. Results and discussion

3.1. Chromatographic separation

The objectives of this study were focused on characterizing the anthocyanin profile in a transgenic purple tomato and quantifying the anthocyanin content in each part of the tomato using HPLC-MS/MS. The profile of the anthocyanin peaks from transgenic tomato *Del/Ros1* was shown by HPLC chromatogram in Fig. 3. While no anthocyanin peaks were detectable in the wild type, a total of seven peaks were eluted at the retention times between 23 and 38 min in the transgenic *Del/Ros1* fruits. Of these, peaks 2 and 4 were the major anthocyanins and their peak areas appeared to be more than half of the total anthocyanin peak areas.

3.2. Mass spectrometric identification

Following HPLC separation, LC-MS/MS data were characterized by monitoring the molecular ion characteristics for each peak. The m/z ratio of each intact anthocyanin and its daughter fragments are listed in Table 1. As shown in Table 1, delphinidin (Dpd m/z 302), petunidin (Ptd m/z 316), and malvidin (Mv m/z 331) were the three anthocyanidin aglycones detected in the transgenic tomato line. Five of the seven anthocyanins including delphinidins

(peaks 1-3) and petunidins (peaks 4-5) were reported previously by Bultelli et al. (2008). However, two new malvidins (peaks 6-7) were found in the transgenic tomato line for the first time. As shown in Figure 4a, the ions of peak 6, i.e., malvidin-3-(p-coumaroyl)-rutinoside-5-glucoside (m/z 947), produced three fragments of m/z 785, 493, and 331. Transition 947 to 785 and 947 to 493 implied the loss of glucose (m/z 162) and p-coumaroyl (m/z 454), respectively. Transition 947 to 331 produced malvidin aglycone (m/z 331) caused by the loss of glucose and p-coumaroyl. In Figure 4b, malvidin-3-(feruloyl)-rutinoside-5-glucoside (peak 7) produced transitions of 977 to 815, 493, and 331 m/z . Transition 977 to 815 and 947 to 493 indicated the loss of glucose (m/z 162) and feruloyl (m/z 484), respectively, while transition 947 to 331 produced malvidin aglycone (m/z 331).

When compared with other glycosylated anthocyanidins, malvidins have been found to have stronger inhibitory effects on nitric oxide production in LPS/IFN- γ -activated RAW 264.7 mouse macrophage cells due to better absorption and better free radical scavenging activity (Wang and Mazza, 2002). In addition, possible health benefits of dietary malvidins have been reported because of anti-proliferative (Seeram & Zhang, 2003; Hyun & Chung, 2004) and anti-inflammatory activities (Jing et al. 2008; Wedick et al. 2012). The total content of anthocyanins in the *Del/Ros1* transgenic tomato is equally distributed, with 5.1 ± 0.5 g/kg DW in the peel and 5.8 ± 0.3 g/kg DW in the flesh. Total anthocyanin contents in *Del/Ros1* are near 5.2 ± 0.5 g PN3GE/kg DW, or 0.5% of dry weight, which is higher than some of the anthocyanin-enriched foods such as red raspberry (3.9 g/kg DW by Wang & Lin 2000), strawberry (3.2 g/kg DW by Wang & Lin 2000), and mulberry (2.1 g/kg DW by Bae & Suh, 2007).

The reason why two new anthocyanins could be detected in the transgenic line may relate to the modified extraction and HPLC method that allowed for a distinct peak separation. In this

study, 10% of formic acid was added to the methanol before extraction, creating a low pH environment for anthocyanin stabilization. The decrease of flow rate and solvent B ratio during the gradient elution might also provide better peak separation. Lastly, with a C18 stationary phase column, more polar solvent A and less polar solvent B mobile phase may carry out a better gradient elution.

3.3. Anthocyanin quantification in transgenic tomato

While anthocyanins were undetectable in the wild-type, the contents of anthocyanins in the whole fruit, peel, and flesh of the *Del/Ros1*-transgenic tomato were 5.2 ± 0.5 , 5.1 ± 0.5 , and 5.8 ± 0.3 g/kg dry matter, respectively. Anthocyanins were undetectable in the seeds of both wide-type and transgenic tomato lines.

Table 2 lists the content profile of individual anthocyanin in the whole, peel, and flesh of the transgenic tomato line. The predominant anthocyanins were delphinidin-3-(trans-coumaroyl)-rutinoside-5-glucoside and petunidin-3-(trans-coumaroyl)-rutinoside-5-glucoside, which contributed to nearly 86% of the total anthocyanins. The reason why they are the highest among all the anthocyanins is not clear, but it may be due to the transgenic *Del/Ros1*-induced overexpression of the genes that relate to the specific anthocyanin biosynthesis pathway in which delphinidin-3-(trans-coumaroyl)-rutinoside-5-glucoside is an immediate precursor for petunidin-3-(trans-coumaroyl)-rutinoside-5-glucoside (Holton & Cornish 1995). Two new anthocyanins, malvidin-3-(p-coumaroyl)-rutinoside-5-glucoside and malvidin-3-(feruloyl)-rutinoside-5-glucoside made up 6% of the total anthocyanins.

In conclusion, seven anthocyanins, including 2 new anthocyanins, have been identified in the *Del/Ros1* transgenic tomato. Compared to undetectable anthocyanins in the wild type, the *Del/Ros1* transgenic tomato produced a high level of anthocyanins that may provide unique

197 functional products with potential health benefits.

198

199 **Acknowledgments**

200 The authors greatly thank Dr. Sunghun Park (Kansas State University Department of
201 Horticulture) for providing wild type and transgenic tomato samples for anthocyanin analysis.

202 This study was supported in part by a USDA Cooperative Project KS511-1001903 from the
203 Agricultural Experiment Station, Kansas State University (Contribution #15-329-J from the
204 Kansas Agricultural Experiment Station).

205

References

- Bognar, E., Sarszegi, Z., et al. (2013). Antioxidant and Anti-Inflammatory Effects in RAW264.7 Macrophages of Malvidin, a Major Red Wine Polyphenol. *PLOS ONE*, 8(6), 1.
- Bovy, A., de Vos, R., Kemper, M., Schijlen, E. et al. (2002). High-flavonol tomatoes resulting from the heterologous expression of the maize transcription factor genes LC and C1. *Plant Cell*, 14(10), 2509-2526.
- Bowen-Forbes, C.S., Zhang Y. J., Nair, M. G. (2010). Anthocyanin content, antioxidant, anti-inflammatory and anticancer properties of blackberry and raspberry fruits. *Journal of Food Composition and Analysis*, 23(6), 554–560.
- Butelli, E., Titta, L., Giorgio, M., et al. (2008). Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. *Nature Biotechnology*, 26(11), 1301-1308.
- Bae, S. H., Suh, H. J. (2007). Antioxidant activities of five different mulberry cultivars in Korea. *LWT - Food Science and Technology*, 40(6), 955–962.
- Delgado-Vargas, F. O., P-L. (2003). Anthocyanins and betalains. In *Natural Colorants for Food and Nutritional Uses*, Ch.8, 167-219
- Etminan, M., Takkouche, B., Caamaño-Isorna, F. (2004). The role of tomato products and lycopene in the prevention of prostate cancer: a meta-analysis of observational studies. *Cancer Epidemiology, Biomarkers & Prevention*, 13(3), 340-345.
- Gonzalez, S., Mazzucato, A., Perata, P. (2009). Purple as a tomato: towards high anthocyanin tomatoes. *TRENDS in Plant Science*, 14(5), 237-241.
- Holton, T.A., Cornish, E.C. (1995). Genetics and biochemistry of anthocyanin biosynthesis. *Plant Cell*, 7, 1071-1083.

229 Hyun, J.W., Chung, H. S. (2004). Cyanidin and Malvidin from *Oryza sativa* cv. Heugjinjubyeo
 230 mediate cytotoxicity against human monocytic leukemia cells by arrest of G (2)/M phase
 231 and induction of apoptosis. *Journal of agricultural and food chemistry*, 52(8), 2213-2217.
 232 Jing, P., Bomser, J.A., Schwartz, S.J., He, J., Magnuson, B.A., Giusti, M.M. (2008). Structure-
 233 function relationships of anthocyanins from various anthocyanin-rich extracts on the
 234 inhibition of colon cancer cell growth. *Journal of Agricultural and Food Chemistry*,
 235 56(20), 9391-9398.
 236 Khachik, F., Carvalho, L., Bernstein, P.S., Muir, G.J., Zhao, D.Y., Katz, N.B. (2002). Chemistry,
 237 distribution, and metabolism of tomato carotenoids and their impact on human health.
 238 *Experimental Biology and Medicine*, 227(10), 845-851.
 239 Lim, S., Xu, J., Kim J, Chen, T. et al. (2013). Role of anthocyanin-enriched purple-fleshed sweet
 240 potato p40 in colorectal cancer prevention. *Molecular Nutrition & Food Research*,
 241 57(11), 1908-1917.
 242 Lim, W., Miller, R., Park, J., Park, S. (2014). Consumer sensory analysis of high flavonoid
 243 transgenic tomatoes. *Journal of Food Science*, 79:S1212–1217.
 244 Lukaszewicz, M., Matysiak-Kata, I., Skala, J., Fecka, I., Cisowski, W., Szopa, J. (2004).
 245 Antioxidant capacity manipulation in transgenic potato tuber by changes in phenolic
 246 compounds content. *Journal of Agricultural and Food Chemistry*, 52(6), 1526-1533.
 247 Mathews, H., Clendennen, S. K., Caldwell, C. G. et al. (2003). Activation Tagging in Tomato
 248 Identifies a Transcriptional Regulator of Anthocyanin Biosynthesis, Modification, and
 249 Transport. *Plant Cell*. 15(8), 1689–1703.

250 Mes, P. J., Boche, P., Myers, J. R. (2008). Characterization of Tomatoes Expressing
 251 Anthocyanin in the Fruit. *Journal of the American Society for Horticultural Science*,
 252 133(2), 262-269.

253 Pannellini, T., Iezzi, M., Liberatore, M. et al. (2010). A dietary tomato supplement prevents
 254 prostate cancer in TRAMP mice. *Cancer Prevention Research*, 3(10), 1284-1291.

255 Schijlen, E. G., Ric de Vos, C. H., van Tunen, A. J., Bovy A. G. (2004). Modification of
 256 flavonoid biosynthesis in crop plants. *Phytochemistry*, 65(19), 2631–2648.

257 Seeram, N. P., Zhang, Y., Nair, M. G. (2003). Inhibition of proliferation of human cancer cells and
 258 cyclooxygenase enzymes by anthocyanidins and catechins. *Nutrition and Cancer*, 46(1),
 259 101-106.

260 Tanaka, Y., Ohmiya, A. (2008). Seeing is believing: engineering anthocyanin and carotenoid
 261 biosynthetic pathways. *Current opinion in Biotechnology*, 19(2), 190-197.

262 Wang, J., Mazza, G. (2002). Inhibitory effects of anthocyanins and other phenolic compounds
 263 on nitric oxide production in LPS/IFN-gamma-activated RAW 264.7 macrophages.
 264 *Journal of Agricultural and Food Chemistry*, 50(4), 850-857.

265 Wang, L. S., Stoner, G. D. (2008). Anthocyanins and their role in cancer prevention. *Cancer*
 266 *Letters*, 269(2), 281-290.

267 Wedick, M.N., Pan, A., Cassidy, A., Rimm, E.B. (2012). Dietary flavonoid intakes and risk of
 268 type 2 diabetes in US men and women. *The American Journal of Clinical Nutrition*,
 269 95(4), 925-933.

270 Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., Prior, R. L. (2006).
 271 Concentrations of anthocyanins in common foods in the United States and estimation of
 272 normal consumption. *Journal of Agricultural and Food Chemistry*, 54(11), 4069-4075.

273 Xu J, Su X, Lim S, Griffin J, Carey E, Katz B, Tomich J, Smith JS, Wang W. (2015).
274 Characterization and stability of anthocyanins in purple-fleshed sweet potato P40. *Food*
275 *Chemistry*, 186(11), 90-96.
276 Xu, Z. Howard, L. (2012). Analysis Methods of Anthocyanins. *Analysis of Antioxidant-Rich*
277 *Phytochemicals*, Ch. 5, 149-180.
278

279 **Figure Legends**

280 **Figure 1.** Structures of common anthocyanidins and anthocyanins

281 **Figure 2.** Representative images of the whole, cross-section, and freeze dry of the ripe wild-type
282 (left column) vs. the transgenic *Del/Ros1* tomato fruit (right column).

283 **Figure 3.** Representative HPLC chromatograms of anthocyanins in the wild-type and the
284 transgenic *Del/Ros1* tomatoes (the peak number corresponding to anthocyanin name is shown in
285 Table 1).

286 **Figure 4.** Mass spectrometric data of two new malvidins detected in the transgenic *Del/Ros1*
287 purple tomato: a) Malvidin-3-(p-coumaroyl)-rutinoside-5-glucoside; b) malvidin-3- (feruloyl) -
288 rutinoside-5-glucoside (Mv, Malvidin; Glc, glucose; 3FR, 3-(feruloyl)-rutinoside; 3PR, 3-(p-
289 coumaroyl)-rutinoside).

Table 1. Mass Spectrometric Data of Anthocyanins in the Transgenic *Del/Ros1* Tomato

Peak #	Retention time (min)	Anthocyanins	[M+H] ⁺ (m/z)	Detected fragments*
1	24.67	Delphinidin-3-(caffeoyl)-rutinoside-5-glucoside	935	773(Dpd+3CR), 465 (Dpd+Glc), 302(Dpd)
2	28.17	Delphinidin-3-(trans-coumaroyl)-rutinoside-5-glucoside	919	757(Dpd+3TR), 465 (Dpd+Glc), 302(Dpd)
3	29.80	Delphinidin-3-(feruloyl)-rutinoside-5-glucoside	949	787(Dpd+3FR), 464 (Dpd+Glc), 303(Dpd)
4	31.77	Petunidin-3-(trans-coumaroyl)-rutinoside-5-glucoside	933	772 (Ptd+3TR), 479 (Ptd+Glc), 316(Ptd)
5	33.26	Petunidin-3-(feruloyl)-rutinoside-5-glucoside	963	800 (Ptd+3FR), 478 (Ptd+Glc), 316(Ptd)
6	34.39	Malvidin-3-(p-coumaroyl)-rutinoside-5-glucoside	947	785 (Mv+3PR), 493(Mv+Glc), 331(Mv)
7	36.81	Malvidin-3-(feruloyl)-rutinoside-5-glucoside	977	815 (Mv+3FR), 493(Mv+Glc), 331(Mv)

*Dpd, delphinidin; Ptd, petunidin; Mv, malvidin; Glc, glucose; 3CR, 3-(caffeoyl)-rutinoside; 3TR, 3-(trans-coumaroyl)-rutinoside; 3FR, 3-(feruloyl)-rutinoside; 3PR, 3-(p-coumaroyl)-rutinoside.

Table 2. The contents of individual anthocyanin (mg/kg dry matter) in the whole fruit, flesh and peel of the transgenic *Del/Ros1* tomato

	Whole	Peel	Flesh
Delphinidin3-(caffeoyl)-rutinoside-5-glucoside	136.7±10.9 ^a	84.9±7.0 ^b	153.6±11.5 ^c
Delphinidin3-(trans-coumaroyl)-rutinoside-5-glucoside	1434.6±144.2 ^a	1145.3±115.0 ^b	1624.3±104.5 ^a
Delphinidin3-(feruloyl)-rutinoside-5-glucoside	53.4±6.3 ^a	25.5±2.8 ^b	64.9±4.0 ^c
Petunidin3-(trans-coumaroyl)-rutinoside-5-glucoside	2849.6 ±303.7 ^a	3325.1±351.4 ^a	3021.6±156.5 ^a
Petunidin 3-(feruloyl)-rutinoside-5-glucoside	446.6±75.1 ^a	233.8±11.9 ^b	554.2±30.2 ^c
malvidin-3-(p-coumaroyl)-rutinoside-5-glucoside	240.0±15.4 ^a	270.6±23.0 ^a	259.0±27.7 ^a
malvidin-3-(feruloyl) -rutinoside-5-glucoside	76.2±15.5 ^a	34.1±2.6 ^b	107.0±13.3 ^c

*Data are expressed as mean ± SD (n=4-6). Values marked by different letters within same row indicate significant difference ($p<0.05$).

Figure 1

Figure 2

Figure 3

Figure 4