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Characterization of a novel anthocyanin profile in wild black raspberry mutants: An opportunity for studying the genetic control of pigment and color

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ABSTRACT

The type and amount of anthocyanins in raspberries, and other small fruits, has recently received increased attention. Black raspberry (*Rubus occidentalis* L.), in particular, has long been recognized as a rich source of anthocyanins and has been the focus of many recent studies examining their potential health benefits. In this study, we characterized a novel anthocyanin profile found in seedlings of two wild black raspberry populations collected from South Dakota, USA. Seedlings from these populations lack pigments glycosylated with rutinoside in their fruit, have elevated levels of cyanidin-3-sambubioside, and contain a small but significant amount of pelargonidin-3-glucoside, a pigment reported only once previously in black raspberry. Affected fruit also have lower than typical total anthocyanins (77.5–134.4 mg 100 mL⁻¹). Based on the available evidence, we believe the plants have a mutation in the gene encoding anthocyanidin-3-glycoside rhamnosyltransferase (3RT), providing a unique opportunity to identify and study one of the major genes in the anthocyanin pathway and its effect on fruit anthocyanins and color.

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1. Introduction

In addition to traditional markets for consumption of fresh and processed black raspberry fruit, there is a long history of its use as a natural colorant and dye because of its high anthocyanin levels (Hong & Wrolstad, 1990a; Lee & Slate, 1954). Studies characterizing the types of anthocyanins present in black raspberry fruit date back to at least the 1960s (Nybom, 1968). A number of recent studies have examined the anthocyanin composition of black raspberry fruit using more sophisticated tools than those available 50 years ago and have consistently detected cyanidin-3-glucoside, cyanidin-3-sambubioside, cyanidin-3-rutinoside, cyanidin-3-xylo-

sylrutinoside, pelargonidin-3-rutinoside, and peonidin-3-rutinoside (Dossett, Lee, & Finn, 2008; Tian, Giusti, Stoner, & Schwartz, 2006a, 2006b; Tulio et al., 2008; Wyzgoski et al., 2010). Wu, Pittman III, and Prior (2006) also found trace levels of pelargonidin-3-glucoside in black raspberry fruit. The anthocyanins in black raspberry fruit are comprised of three anthocyanin aglycones: cyanidin, pelargonidin, and peonidin, glycosylated with a various combinations of three different sugars: glucose, rhamnose, and xylose. Anthocyanin biosynthesis has been well studied in a variety of plants and the biosynthetic pathway appears to be heavily conserved (Holton & Cornish, 1995). The six major anthocyanin aglycones are pelargonidin, cyanidin, peonidin, delphinidin,

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petunidin, and malvidin. Cyanidin-, pelargonidin-, and delphinidin-glucosides are the base anthocyanins, which then may undergo further glycosylation and/or B-ring modification (to produce petunidin, peonidin, and malvidin) in a series of stepwise modifications (Tanaka, Sasaki, & Ohmiya, 2008).

The anthocyanins of black raspberries, and other highly pigmented small fruits, have received increased attention in recent years because of interest in their potential health benefits. Black raspberries and other sources of dietary anthocyanins have been linked to many possible health benefits such as reducing eyestrain, improving night vision, helping to prevent macular degeneration, anti-inflammatory effects, protecting against DNA damage, and exhibiting anti-cancer activities (Afaq, Saleem, Krueger, Jess, & Mukhtar, 2005; Kresty et al., 2001, 2006; Lazze et al., 2003; Wang et al., 1999), and have been well reviewed (de Pascual-Teresa & Sanchez-Ballesta, 2008; Espin, Garcia-Conesa, & Tomas-Barberan, 2007; Rao & Snyder, 2010). Studies linking the high levels of anthocyanins in black raspberry with potential health benefits have led to increasing interest in black raspberry from consumers and from various functional food and nutraceutical markets (Espin et al., 2007).

Black raspberry fruit is dominated by cyanidin-3-rutinoside and cyanidin-3-xylosylrutinoside, which account for 80% or more of the total anthocyanins (Dossett, Lee, & Finn, 2010; Hong & Wrolstad, 1990a; Ozgen et al., 2008; Tian et al., 2006a, 2006b; Tulio et al., 2008; Wyzgoski et al., 2010). These two main anthocyanins are also more potent phenolic antioxidants (cyanidin-3-xylosylrutinoside > cyanidin-3-rutinoside) compared to the other anthocyanins present in black raspberry fruit (Tulio et al., 2008), though limited information is available about the potential bioactivity of individual cyanidin-based anthocyanins with different sugar moieties (Tian et al., 2006b; Tulio et al., 2008; Stintzing, Stintzing, Carle, Frei, & Wrolstad, 2002; Stoner et al., 2005) or their relative desirability for product development, food processing, natural colorant usage, and storability (Hager, Howard, Prior, & Brownmiller, 2008; Hong & Wrolstad, 1990a; Stintzing et al., 2002).

Aside from interest in their potential health benefits, anthocyanins in red and black raspberry play a more basic role as an indicator of fruit quality, suitability for different markets, and ultimately consumer acceptance. Fruit color is a function of not just the total amount but also the type of anthocyanins present, with different aglycones, glycosylation, and acylation each contributing to the overall color of the fruit in addition to pH and interactions with other fruit components (Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009; Giusti, Rodriguez-Saona, & Wrolstad, 1999; Stintzing et al., 2002; Tanaka et al., 2008). In black and red raspberry, color is a critical indicator of quality and suitability for processing with darker fruit generally being preferred over lighter colored fruit (Hall, Hummer, Jamieson, Jennings, & Weber, 2009). Studies have shown consumer perceptions of fruit freshness, ripeness, and flavor to be heavily influenced by color (Garber, Hyatt, & Starr, 2000; Hall et al., 2009; Zampini, Sanabria, Phillips, & Spence, 2007; Zellner & Durlach, 2003), underscoring the importance of color and color stability for fresh market fruit as well.

Despite the interest in black raspberry anthocyanins, little is known about the genetic control and regulation of their production in the fruit. While many of the enzymes involved in anthocyanin biosynthesis have been identified in model plant systems (*Petunia × hybrida* Hort., *Antirrhinum majus* L., and *Zea mays* L.), none of the genes involved in anthocyanin production have been mapped or identified in black raspberry. This is made difficult by the fact that, while anthocyanin deficient (yellow/orange-fruited) mutants have been recognized for more than a century (Card, 1898) and differences in the relative proportion of anthocyanins have been reported (Dossett et al., 2008, 2010), little variation in the types of anthocyanins present in black raspberry fruit has been found. While a major effort to identify and map genes involved in anthocyanin biosynthesis and fruit color is underway in red raspberry (Kassim et al., 2009; McCallum et al., 2010), the apparent lack of variation in cultivated black raspberry has limited similar work in this crop. The objective of this study was to identify black raspberry seedlings, collected from wild populations, that might contain a novel anthocyanin profile, and to examine their potential for use in further studies on the genetic control of black raspberry anthocyanin biosynthesis.

2. Materials and methods

2.1. Plant materials and sample preparation

Seeds of ORUS 4141 and ORUS 4143 were collected in July 2007 from wild black raspberry plants at Lewis and Clark State Park (Yankton, SD, USA) and Union Grove State Park (Beresford, SD, USA), respectively. These two collection sites are about 90 km apart. The seed was scarified, stratified, and germinated the following fall in the greenhouse using standard protocols (Finn & Hancock, 2008) and seedlings planted in the field in Corvallis, OR (USA) in May 2008 along with seedlings from 30 other wild black raspberry populations and the cultivars Munger, Jewel, and Mac Black in a randomized complete block design with four replicated plots of four plants each. Details of the field maintenance are described in Dossett et al. (2008). This project was part of a broader research effort to widen the genetic base of cultivated black raspberry.

Fruit were harvested from this plot in July 2010 for analysis of anthocyanins and other maturity traits (data not shown). Protocols used for fruit harvest and sample preparation were similar to those previously described (Dossett et al., 2008, 2010). Briefly, 25 berries from each plant (genotype) were picked, weighed, and added to a bulk fruit sample of the population for each plot. Due to disease [primarily *Verticillium* wilt (*Verticillium dahliae* Kleb. or *Verticillium albo-atrum* Reinke & Berthold)], only three of the four replicated plots of ORUS 4141 and ORUS 4143 had sufficient fruit for analysis (Table 1). As mentioned previously, this research was part of a larger project to identify useful wild germplasm for widening the genetic base of cultivated black raspberry; fruit from the 30 other wild populations were collected and analyzed, but fall outside the scope of this study, which focuses on ORUS 4141 and ORUS 4143. An entire replication (rep) was picked in a single day to minimize variation from the effects of differing irrigation status and weather at harvest. Harvested fruit was

Table 1 – Anthocyanin profiles and total anthocyanins by HPLC for juice (from fruit) from seedlings of wild black raspberry populations from Lewis and Clark State Park (ORUS 4141, Yankton, SD, USA) and Union Grove State Park (ORUS 4143, Beresford, SD, USA) and grown in Corvallis, OR, USA.

Population	Rep	Cyanidin-3-sambubioside ^a	Cyanidin-3-glucoside/cyanidin-3-xylosylrutinoside ^a	Cyanidin-3-rutinoside ^a	Pelargonidin-3-glucoside ^a	Pelargonidin-3-rutinoside ^a	Peonidin-3-rutinoside ^a	Total anthocyanins by HPLC/DAD ^a
ORUS 4141	2	45.6 (56)	33.0 ^b (41)	np ^c	2.8 (3)	np	np	81.3
ORUS 4141	3	23.9 (6)	282.2 ^d (67)	103.5 (24)	nd ^e	11.8 (3)	2.5 (1)	423.9
ORUS 4141	4	22.2 (15)	61.2 ^d (42)	58.1 (39)	2.3 (2)	3.5 (2)	1.0 (0.7)	147.2
ORUS 4143	1	90.7 (67)	39.4 ^b (29)	np	4.4 (3)	np	np	134.4
ORUS 4143	2	33.0 (43)	40.8 ^b (53)	np	3.7 (5)	np	np	77.5
ORUS 4143	3	92.9 (73)	30.9 ^b (24)	np	3.7 (3)	np	np	127.6

^a All units are in mg 100 mL⁻¹ followed by proportions of the individual anthocyanin in parenthesis (in %). Values shown represent fruit bulked by plot (rep, replication). Due to disease, plants from plots ORUS 4141 rep 1 and ORUS 4143 rep 4 did not produce sufficient fruit for analysis. Identification was performed by HPLC/DAD/ESI-MS/MS.

^b In ORUS 4141 rep 2 and in ORUS 4143 (all reps), cyanidin-3-glucoside only. No cyanidin-3-xylosylrutinoside was detected.

^c Not present.

^d Cyanidin-3-xylosylrutinoside is the major peak with coelution with a minor amount of cyanidin-3-glucoside.

^e Not detected.

packed on ice immediately after harvest and then frozen immediately (−23 °C) after arrival at the laboratory. Bulk samples were thawed and extracted as described by Dossett et al. (2008). An aliquot of each sample was centrifuged at room temperature at 2547g_n for 20 min to separate the juice from the pulp. The supernatant was then diluted with HPLC grade water, and filtered with a 0.45 μm syringe driven Millex-FH filter (Millipore, Bedford, MA, USA) prior to further analysis.

2.2. Analysis of anthocyanins

Total anthocyanins were determined by HPLC by summing the amounts of the individual anthocyanins detected. Anthocyanin profiles were determined by HPLC/diode array detector/ion trap XCT mass spectrometer (HPLC/DAD/ESI-MS/MS) on an Agilent 1100 series system (Agilent Technologies, Santa Clara, CA, USA). The guard and analytical columns, mobile phase composition, and the gradient program used for HPLC analysis are described by Lee and Finn (2007). Sample injection volume was 5 μL. Anthocyanins were monitored at 520 nm and quantified with a cyanidin-3-glucoside standard (Polyphenol As, Sandnes, Norway). Frozen strawberries (grown in USA; distributed by Western Family Foods Inc., Portland, OR, USA) were purchased at a local marketplace (Parma, ID, USA), extracted, filtered, and injected onto the HPLC/DAD/ESI-MS/MS to confirm the identity of pelargonidin-3-glucoside (Hong & Wrolstad, 1990b). Ultraviolet-visible (UV-Vis) absorption spectra (190–600 nm) were collected for all peaks. ESI-MS/MS parameters were set as described in Lee and Finn (2007). Individual peak assignments were made according to UV-Vis spectra, retention times, molecular ions mass-to-charge ratio (*m/z*), and fragmented ions *m/z*. Quantification was performed with HPLC/DAD results.

3. Results and discussion

Of the six samples from ORUS 4141 and ORUS 4143, only one (ORUS 4141, rep 2) had an anthocyanin profile closely resembling previous work under identical analytical conditions (Dossett et al., 2008, 2010). Six anthocyanins were detected in ORUS 4141, rep 2 (in elution order): cyanidin-3-sambubioside, cyanidin-3-glucoside, cyanidin-3-xylosylrutinoside, cyanidin-3-rutinoside, pelargonidin-3-rutinoside, and peonidin-3-rutinoside, with cyanidin-3-glucoside and cyanidin-3-xylosylrutinoside co-eluting (Table 1, Fig. 1). The relative proportions of these six anthocyanins were also similar to values previously reported for cultivated black raspberry (Dossett et al., 2010) with cyanidin-3-glucoside and cyanidin-3-xylosylrutinoside combining for approximately 67% of the total anthocyanins and cyanidin-3-rutinoside comprising another 24%. A second sample from this same population (ORUS 4141, rep 4) had a similar anthocyanin profile, containing the same six anthocyanins, as well as small amounts of pelargonidin-3-glucoside. This sample was also noted for having an elevated proportion of cyanidin-3-sambubioside and much lower total anthocyanins (Table 1). The remaining sample (rep 2) from ORUS 4141 and all three samples (reps 1, 2, and 3) from ORUS 4143 had significantly altered anthocyanin profiles (Fig. 1) with only three anthocyanins detected:

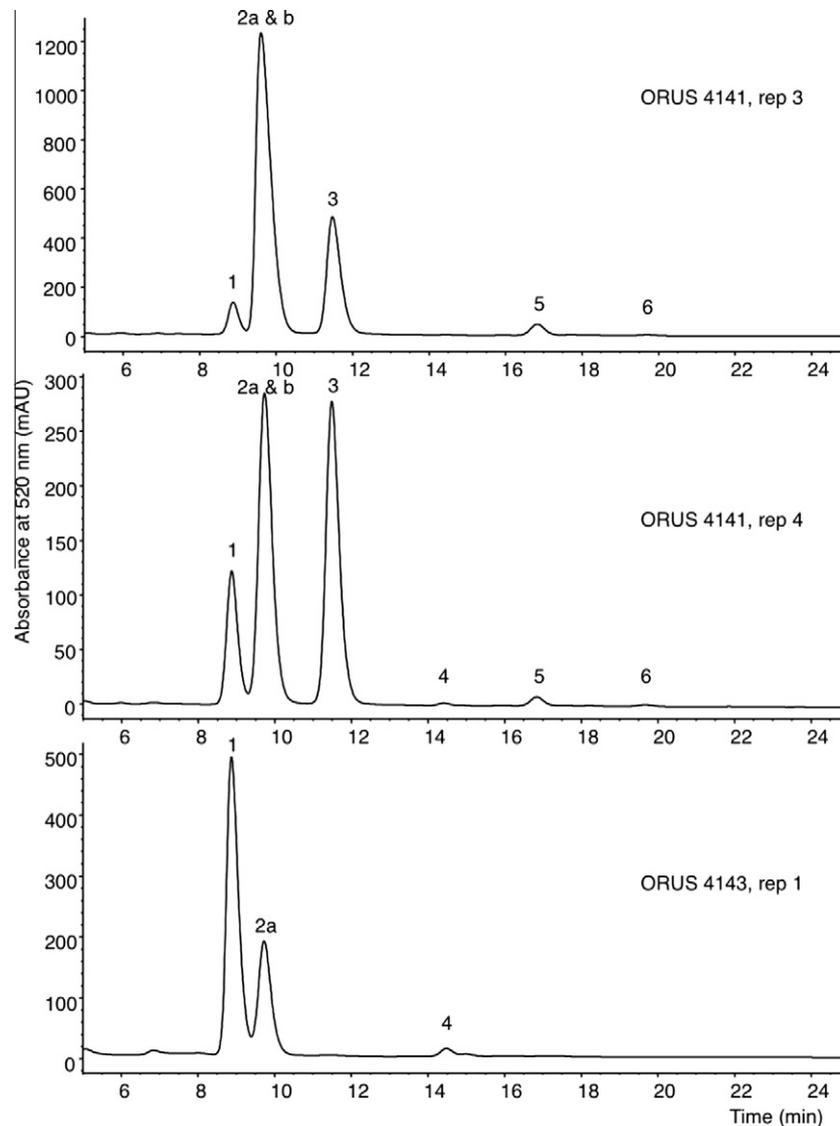


Fig. 1 – Chromatograms showing anthocyanin profiles of black raspberry juice from normal (ORUS 4141, rep 3), mixed (ORUS 4141, rep 4), and mutant (ORUS 4143, rep 1; *rr*) samples from seedlings grown in Corvallis, OR, USA and harvested in 2010. Peak numbers are identified as follows [*m/z* of molecular ions and their fragments]: (1) cyanidin-3-sambubioside (581, 287); (2) a, cyanidin-3-glucoside (449, 287; minor) and b, cyanidin-3-xylosylrutinoside (727, 581, 287); (3) cyanidin-3-rutinoside (595, 287); (4) pelargonidin-3-glucoside (433, 271); (5) pelargonidin-3-rutinoside (580, 271); and (6) peonidin-3-rutinoside (609, 301).

cyanidin-3-sambubioside, cyanidin-3-glucoside, and pelargonidin-3-glucoside. In addition to their notable lack of rhamnose containing anthocyanins, these four samples were also characterized by elevated proportions of cyanidin-3-sambubioside and relatively low total anthocyanins (Table 1).

While this is the first report of black raspberry fruit lacking anthocyanidin-3-rutinosides, variation for presence/absence of rutinoside pigments has been noted in red raspberry. Francis (1972), Mistic (1973), and Barritt and Torre (1975a) noted variation in the presence of cyanidin-3-rutinoside and pelargonidin-3-rutinoside in several red raspberry varieties. Barritt and Torre (1975b) studied the inheritance of rhamnose-containing pigments in red raspberry and concluded that it was controlled by a dominant gene, which they designated *R*. They also found segregation for xylose-containing pigments in red raspberry, which was further supported by

Jennings and Carmichael (1980) who designated the dominant gene controlling this trait as *Xy*. While gene *R* occurs in a range of red raspberry germplasm, gene *Xy* is more unusual in red raspberry and probably was introduced to red raspberry germplasm through inter-specific hybridization with black raspberry for other traits (Jennings & Carmichael, 1980).

The complete absence of anthocyanidin-3-rutinosides, but not their precursors, in fruit of some black raspberry genotypes is strong evidence for homozygous mutant or null alleles at the locus corresponding to gene *R* described in red raspberry (Barritt & Torre, 1975b). Kamsteeg, Van Brederode, and Van Nigtevecht (1979) identified UDP-rhamnose anthocyanidin-3-glucoside rhamnosyltransferase (3RT) as the enzyme responsible for catalyzing the addition of rhamnose to the 6-position of the glucose bound at the 3-position of the anthocyanidin skeleton, thereby forming anthocyanidin-

3-rutinosides from anthocyanidin-3-glucosides in *Silene L.* Brugliera et al. (1994) and Kroon et al. (1994) sequenced and cloned the gene for 3RT in *P. × hybrida*, and confirmed that it was responsible for catalyzing the formation of anthocyanidin-3-rutinosides. The absence of fruit with normal pigment composition in all samples from Union Grove State Park (ORUS 4143) suggests that a non-functional 3RT allele (*r*) is fixed in this population. The data also indicate that this allele segregated in the population from Lewis and Clark State Park (ORUS 4141). One sample from Lewis and Clark State Park closely resembled the three from Union Grove State Park, while another contained the normal pigment profile described in black raspberry. The composition of the third sample from Lewis and Clark State Park (ORUS 4141, rep 4) with pelargonidin-3-glucoside in addition to the expected anthocyanins, along with its lower total anthocyanin concentration and elevated proportion of cyanidin-3-sambubioside indicates that this is likely a mixed sample, representing bulked fruit from normal (*RR* or *Rr*) and homozygous recessive (*rr*) plants.

Schram, Jonsson, and Bennink (1984) and Tornielli, Koes, and Quattrocchio (2009) noted that homozygous mutations in 3RT in *P. × hybrida* resulted in the accumulation of anthocyanin-3-glucosides. It is difficult to confirm this result in the present black raspberry study, because cyanidin-3-glucoside could not be quantified in fruit from normally pigmented plants as a result of coelution with cyanidin-3-xylosylrutinoside. However, based on an extracted ion chromatogram of 449, only minor amounts of cyanidin-3-glucoside were detected in ORUS 4141, rep 3. It also appears that anthocyanidin-3-glucosides did not accumulate to a large degree in the fruit from *rr* plants, and certainly not by the amount expected to account for the missing proportions of cyanidin-3-rutinoside and its derivatives. The single normal sample in this study (ORUS 4141, rep 3) contained 103.5 mg 100 mL⁻¹ of cyanidin-3-rutinoside. This is only slightly less than the range of 113.6–257.3 mg 100 mL⁻¹ found by Dossett et al. (2010) in samples from 26 black raspberry seedling populations, but is 2.5–3.0 times the amount of cyanidin-3-glucoside detected in the fruit of *rr* plants (Table 1). Some excess cyanidin-3-glucoside may have been converted into cyanidin-3-sambubioside by the addition of a xylose to the 2-position on the glucose. This accounts for the substantial increase seen in the amount of cyanidin-3-sambubioside, which normally comprises only 2–6% of the total anthocyanins (Dossett et al., 2010). In the normal sample from this study, cyanidin-3-sambubioside accounted for 6% of the total anthocyanins, while 1.5 to nearly 4 times the amount of cyanidin-3-sambubioside was present in the *rr* fruit, accounting for up to 73% of the total anthocyanins (Table 1). This result suggests that in the absence of cyanidin-3-rutinoside as a substrate for xylosyltransferase (XyT), xylose is added to an increased amount of cyanidin-3-glucoside resulting in higher levels of cyanidin-3-sambubioside. Alternatively, cyanidin-3-sambubioside may be a substrate from which cyanidin-3-xylosylrutinoside is made and the accumulation of cyanidin-3-sambubioside is because it is no longer being used for this. While both of these routes of synthesis are illustrated in Fig. 2, further work examining the kinetics and substrate specificities of these enzymes is needed to determine which is responsible for production of cyanidin-3-xylosylrutinoside.

The presence of pelargonidin-3-glucoside in *rr* fruit was not surprising as this is a precursor for pelargonidin-3-rutinoside, which is normally observed (Kamsteeg et al., 1979). The identity of pelargonidin-3-glucoside was confirmed by comparison of peak retention times from pelargonidin-3-glucoside in strawberry (main strawberry anthocyanin; Hong & Wrolstad, 1990b) as well as by ESI-MS/MS (Fig. 1). Trace amounts of pelargonidin-3-glucoside were reported in black raspberry fruit by Wu et al. (2006), however, other studies (Dossett et al., 2008; Tian et al., 2006a, 2006b; Tulio et al., 2008; Wyzgoski et al., 2010) have not detected pelargonidin-3-glucoside in black raspberry, suggesting that most of it is normally converted to pelargonidin-3-rutinoside. Pelargonidin-3-glucoside levels were only slightly lower than the amount of pelargonidin-3-rutinoside that would otherwise be expected. In the normal sample from Lewis and Clark State Park, 11.8 mg 100 mL⁻¹ pelargonidin-3-rutinoside was detected, accounting for roughly 3% or the total anthocyanins. Dossett et al. (2010) found between 4.2 and 14.5 mg 100 mL⁻¹ pelargonidin-3-rutinoside in seedlings of black raspberry crosses. The sample of *rr* fruit with the highest pelargonidin-3-glucoside contained only 4.4 mg 100 mL⁻¹. Pelargonidin-3-glucoside comprised 3–5% of the total anthocyanins in these samples, a similar proportion to what would be expected, however the total amount was far less.

The data also allow us to infer the probable pathway for production of trace amounts of peonidin-3-rutinoside in black raspberry fruit. Peonidin is produced by methyltransferase (MT) activity on the 3' position of the cyanidin B-ring. Jonsson, de Vlaming, Wiering, Aarsman, and Schram (1983) showed that MT was the final step in the production of peonidin-3-(*p*-coumaroyl)-rutinoside-5-glucoside from cyanidin-3-(*p*-coumaroyl)-rutinoside-5-glucoside in *P. × hybrida*. The presence of peonidin-3-rutinoside in the two samples (reps 3 and 4) containing all or some normally pigmented fruit from ORUS 4141 is in contrast with the absence of peonidin-3-rutinoside in *rr* fruit from that population (rep 2) and all three *rr* samples from ORUS 4143. The absence of peonidin-3-glucoside in these samples as a precursor to peonidin-3-rutinoside synthesis suggests that peonidin-3-rutinoside is produced as a result of MT activity on cyanidin-3-rutinoside (Fig. 2).

While fruit of the normal black raspberry sample contained 423.9 mg 100 mL⁻¹ of total anthocyanins, fruit from the *rr* plants ranged from 77.5 to 134.4 mg 100 mL⁻¹. This is far less than the range of 244.8–541.3 mg 100 mL⁻¹ found by Dossett et al. (2010) and is also less than the rest of the >400 samples from other wild populations harvested in 2010 (data not shown). This reduction in total anthocyanins supports the observation that cyanidin-3-glucoside did not accumulate in proportion to the reduction in cyanidin-3-rutinoside and its derivatives in the 3RT mutants. It also indicates that there may be some feedback mechanism by which cyanidin-3-glucoside was no longer produced or was produced at a lower rate once it began to accumulate in the fruit. This could be because glucosyltransferase (GT) activity reaches equilibrium in raspberry fruit at a relatively low concentration of cyanidin-3-glucoside, or because of feedback to some limiting step at an earlier part of the pathway. Either way, it seems that in black raspberry fruit, it may be important for cyanidin-3-glucoside to be anabolized for synthesis of other anthocyanins if high

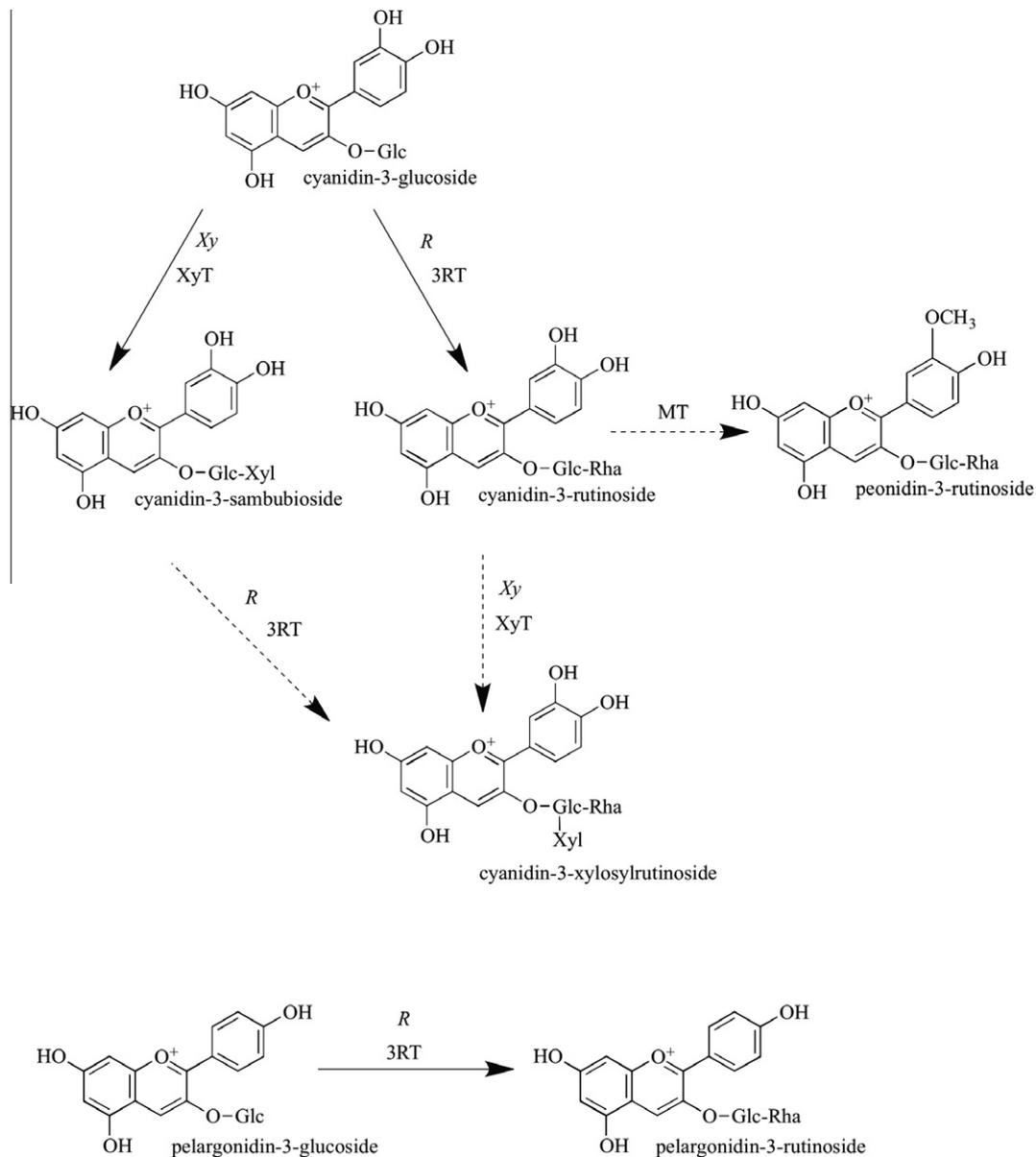


Fig. 2 – Pathway for anthocyanin modifications in black raspberry. Genes (*italicized*) follow the designations by **Barritt and Torre (1975b)** and **Jennings and Carmichael (1980)** while enzymes (**XyT = xylosyltransferase**, **3RT = UDP-rhamnose anthocyanidin-3-glucoside rhamnosyltransferase**, **MT = methyltransferase**) are in regular type. Hypothetical steps are illustrated by dashed arrows. Designation of sugars attached to aglycones is as follows: **Glc = glucose**, **Rha = rhamnose**, and **Xyl = xylose**.

total anthocyanin content is desired. If this is the case, selecting alleles for more efficient GT, RT, and XyT involved in anthocyanin biosynthesis, or for plants that produce more types of anthocyanins, may be a good strategy for breeding for high total anthocyanins. Alternatively, a decrease in cyanidin-3-glucoside and other anthocyanins may result in higher production of other phenolics that may be of interest to nutraceutical or other markets for fruit that do not require higher anthocyanin content but may be focused on the concentration of other compounds.

In addition to gaining insight regarding the biology of anthocyanin modifications in black raspberry, studying fruit from these 3RT mutants may also give researchers the oppor-

tunity to study the impact of raspberry anthocyanin composition on color of whole fruit and processed products. **Wiering and de Vlaming (1984)** indicated that anthocyanidin-3-rutinosides result in a bluer color in *P. × hybrida* flowers while flowers containing only anthocyanidin-3-glucosides appear redder. If this is also the case in black raspberry, it may be a reason for some of the color difference between black and red raspberry fruit besides differences in anthocyanin concentration. **Stintzing et al. (2002)** found that the addition of xylose to cyanidin-glycosides lowered the visual detectability threshold of anthocyanins. **Stintzing et al. (2002)** also noted that the color contribution of most anthocyanins was less than the percentage of their HPLC peak area. Cyanidin-3-glu-

coside accounted for 83% of the anthocyanins in fruit of *Rubus laciniatus* Willd., but only accounted for about 50% of the color. Fruit pH, as well as compositional and/or physical factors may also play a role in perceived color of whole fruit and processed products such as juice. While fruit of *rr* plants were not noted for unusual appearing fruit at the time of harvest, their lighter color, due presumably to lower anthocyanin concentration, was noted during the preparation of juice samples.

4. Conclusions

Anthocyanidin-3-rutinosides and their derivatives account for as much as 90% of black raspberry anthocyanins (Dossett et al., 2010; Tian et al., 2006a; Tulio et al., 2008; Wyzgoski et al., 2010). The identification of a black raspberry 3RT mutant offers an opportunity to identify a major gene responsible for anthocyanin modification in black raspberry as well as identifying other alleles with altered 3RT efficiency, affecting not only the amount of total anthocyanins but their proportions as well, and leading to a better understanding of raspberry anthocyanin synthesis and its effects on color and quality. Small quantities of seed of ORUS 4141 (PI 653314) and ORUS 4143 (PI 653316) are available by request from the USDA-ARS National Clonal Germplasm Repository in Corvallis, OR.

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