Southern Region Small Fruit Consortium

Research Progress Report

Title: Can early berry color be used as a proxy to select for improved anthocyanin composition of mature blackberry fruit?

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Public Abstract

Anthocyanins are the compounds responsible for the characteristic dark purple/black color of blackberry fruit. These compounds have nutraceutical properties and their degradation during harvest and cold storage is associated with the red drupelet reversion postharvest disorder. Significant genotypic variation in total anthocyanin content and composition of specific anthocyanins has been previously documented in University of Arkansas System Division of Agriculture (UA) blackberry breeding germplasm; however, all cultivars appear fully black at harvest and it is impossible to discern anthocyanin content in the field based on visual observation. Interestingly, field observations have shown that blackberry genotypes have strikingly different flower color and berry color early during the fruit development period. The goal of this study was to assess whether there is a relationship between berry color early in development and total anthocyanin content or the composition of individual anthocyanins in blackberry. If such a relationship exists, breeders could very easily visually select for high anthocyanin content and potentially increased health benefits and reduced RDR based on immature berry color. Preliminary data from 12 UA blackberry breeding selections and cultivars in 2023 confirms that there are significant genotypic differences in flower color and immature berry color at 14 and 28 days after bloom. Anthocyanin quantification is currently being conducted. It should be possible to determine where to determine whether flower color or berry color 14 or 28 days after flowering can serve as a useful proxy to select for total anthocyanin content or individual anthocyanin composition in mature blackberry fruit based on this data when anthocyanin analysis is completed.

Introduction:

Anthocyanins are the compounds responsible for the characteristic dark purple/black color of blackberry fruit (Wang et al., 2009). These compounds are polyphenolic antioxidants with potential benefits for human health (Kaume et al., 2012). These health benefits have been a significant driver of increased consumer demand and growth of the blackberry industry (Clark et al., 2007).

Anthocyanin degradation is also the cause of red drupelet reversion, a postharvest disorder of blackberries that occurs when black drupelets on ripe berries turn red during and after cold storage. Whole shipments of blackberries can be rejected if over 10% of produce is not fully black or blue colored according to United States Department of Agriculture (USDA) marketing standards (USDA-AMS, 2016). According to an online survey, the 72.9% of consumers prefer of prefer solid black fruit with no reverted drupelets (Threlfall et al., 2020). When presented with three randomized clamshells filled with berries having varying levels of RDR, only 18.5% of consumers preferred the clamshell with the highest RDR in a consumer preference study conducted in person (Threlfall et al., 2021).

Red drupelet reversion occurs when anthocyanins sequestered in the vacuole spill out into the cytoplasm following by intracellular damage to the cell wall and vacuolar membranes (Edgley et al., 2019). The anthocyanins in the cytosol are then susceptible to biochemical reactions that favor changes to their structural moiety. A 39.1-43.4% decrease in total anthocyanin content was found in red drupelets from 'Apache', 'Ouachita', and 'Triple Crown' after a week in cold storage (Kim et al., 2019). Edgley et al. (2019) also found a 58.2% decrease in total anthocyanins and a 59.7% average decrease in cyanidin-3-glucoside between black and red drupelets in a similar analysis performed with 'Ouachita' blackberries. This reduction in anthocyanins is suspected as the reason black drupelets turn red during storage.

Cultural management practices, including time of harvest, mechanical damage during harvest and transport, and nitrogen rate, can all impact the incidence and severity of red drupelet reversion (Edgley et al., 2018; Pérez-Pérez et al., 2018; Armour et al., 2021). There are also pronounced genotypic differences in susceptibility to red drupelet reversion, with firm and 'crispy' textured blackberries experiencing much less pronounced reversion than softer genotypes (Salgado and Clark, 2016; Segantini et al., 2017; Felts et al., 2018; Armour et al., 2021). Other factors beyond firmness may also contribute to genotypic differences in RDR. Armour et al. (2021) found that only 28.4% and 12.7% of genotypic variation in RDR was explained by firmness in 2018 and 2019, respectively. 'Osage' and 'Ouachita' had lower RDR in both years than anticipated based on berry firmness.

The anthocyanin content and composition of different genotypes may also impact their susceptibility to RDR. Genotypes with higher total anthocyanin content at harvest may be able to retain a black or dark maroon color even with some degradation of anthocyanins in damaged drupelets during harvest and shipping, while those with lower anthocyanin content at the shiny black stage of ripeness may turn a more pronounced red. Anthocyanins also vary in their stability depending on the sugars and other functional groups attached to the anthocyanidin (Welch et al., 2008). Cyanidin-3-glucoside is the dominant anthocyanin in blackberry and it is suspected to encounter the most chemical changes during color reversion as polymeric anthocyanin

derivatives are created (Pérez-Pérez et al., 2018). Edgley et al. (2019) found that cyanidin-3-rutinoside and two of the acylated anthocyanins [cyanidin-3-dioxalylglucoside and cyanidin-3-(6"-malonylglucoside)] were not significantly reduced in red drupelets compared to black drupelets in 'Ouachita' blackberries, suggesting that these compounds may be somewhat protected from degradation during RDR.

It is impossible to visually assess the total anthocyanin content or the composition of individual anthocyanins of a blackberry at maturity. There is significant variation in total anthocyanin content among UA blackberry genotypes (e.g. 55.4 to 247 mg /100 g in 11 genotypes evaluated by Threlfall et al. 2016), yet these genotypes all appear fully black. Similarly, blackberries vary widely in their cyanidin-3-rutinoside content (Cho et al., 2004), yet these differences are indistinguishable to the naked eye. Interestingly, field observations have shown that blackberry genotypes have strikingly different flower color and berry color early during the fruit development period. Some genotypes, including 'Von' and 'Prime-Ark® Traveler', have very green fruit 14-21 days after pollination, while others, including 'Osage' and 'Apache' have bright red fruit 14 days after pollination. These differences in berry color do not seem to be related to differences in the length of the fruit development period. These differences in early color have also been noted by other berry researchers (Gina Fernandez, personal communication; https://teamrubus.blogspot.com/search?q=flower+to+fruit). Some genotypes with bright red color early during ripening have been demonstrated to have very high anthocyanin content [e.g. 'Apache', (Cho et al., 2004)] or less RDR than anticipated based on fruit firmness [e.g. 'Osage', (Armour et al., 2021)].

The goal of this study was to assess whether there is a relationship between berry color early in development and total anthocyanin content or the composition of individual anthocyanins in blackberry. If such a relationship exists, breeders could very easily visually select for high anthocyanin content and potentially increased health benefits and reduced RDR based on immature berry color. The specific objectives of the project were: 1) to measure flower and berry color during fruit development for breeding selections and cultivars in the UA blackberry breeding program, and 2) to determine whether flower color or berry color 14 or 28 days after flowering can serve as a useful proxy to select for total anthocyanin content or individual anthocyanin composition in mature blackberry fruit/

Materials and Methods:

Plant material

Twelve fresh-market blackberry genotypes representing the range of immature berry color in the UA blackberry breeding program ('Ouachita', 'Von', 'Natchez', A-2845TN, A-2796TN, APF-276TN, APF-479TN, A-2790TN, A-2575TPF, APF-448, A-2687T, and A-2717T) were evaluated in this study. The selections were grown on 6-m plots at the UA Fruit Research Station (Clarksville AR, lat. 35°31'5" N, long. 93°24'12" W).

Flower tagging and harvest

Twenty fully open flowers from each selected genotype were tagged on four days during a two-week period during peak flowering for a total of 80 tagged flowers per genotype. The flowers were tagged with color-coded yarn tied to the pedicel indicating the date the flower was fully

open. The color of the flowers and developing fruit from each of the flowers was non-destructively phenotyped at 0, 14 and 28 days after tagging without harvesting or damaging the fruit. The color of each developing flower and fruit was assessed using The Royal Horticultural Society color chart in combination with a Pico paint matching device (Palette, Fitzroy, Australia) and its diameter and length were measured with digital calipers. Color was recorded as L* a* b* coordinates and transformed into chroma (C*) and hue angle (h°) using the equations: C* = (a*2 + B*2)1/2 and h° = tan–1(b*/a*) (McGuire 1992). The Royal Horticulture Society color code was also recorded as a check to ensure the color chips and L*a*b* coordinates were recorded correctly by the Pico device.

The tagged fruit was then harvested at the shiny black stage of ripeness. The number of days between the date of tagging and harvest was recorded and any damaged fruit was discarded. The harvested fruit was phenotyped for color again with the Pico paint matching device (Palette, Fitzroy, Australia). The average berry weight was measured with a digital balance and the diameter and length of each fruit was measured with digital calipers. The fruit from each sample was placed in quart sized zip-style freezer bags and frozen (–10 °C) until processing for composition and anthocyanins.

Composition

Frozen fruit from each sample (12 genotypes x 4 flowering/harvest dates = 48 samples) was slightly thawed and pureed using with a Magic Bullet blender (MBR-1101, Los Angeles, CA) within 24 hours prior to evaluation. Thawed samples were placed in cheesecloth to extract the juice from the puree for analysis of basic composition attributes (soluble solids, pH, and titratable acidity). Soluble solids (expressed as %) of the juice were measured with a Bausch & Lomb Abbe Mark II refractometer (Scientific Instruments, Keene, NH), and the pH of juice was measured using a pH700 Benchtop pH meter (APERNA Instruments, Columbus, OH). Titratable acidity was measured with a Metrohm 862 Compact Titrosampler (Metrohm AG, Herisau, Switzerland). Titratable acidity was determined using 3 g of juice diluted with 50 mL of deionized, degassed water by titration with 0.1 N sodium hydroxide (NaOH) to an endpoint of pH 8.2; with results expressed as g/L citric acid.

Anthocyanins

Anthocyanins have not yet been measured in the samples collected in 2023 because the Speed Vac concentrator needed to conduct the analysis was broken. A new machine has been procured and we are preparing to complete this analysis following the protocol described below during December 2023 or January 2024

A subsample of the fruit from each of the 48 samples will be utilized to extract phenolics through a series of solvent rinses. Three milliliters of the solution from each sample will be dried using a Speed Vac concentrator (ThermoSavant, Holbrook, NY) and resuspended in 1 mL of 3% formic acid. The samples will be put through 0.45-µm polytetrafluoroethylene (PTFE) syringe filters (Varian, Inc., Palo Alto, CA) before HPLC analysis. The analysis will be performed following Cho et al. (2004). A Waters HPLC System® (Waters Corporation, Milford, MA) containing a 600 pump, a 717 Plus autosampler, and a 996-photodiode array detector will be used. Separation will be performed using a 4.6 mm x 250 mm Symmetry® (Waters Corporation, Milford, MA) C18 column with a 3.9 mm x 20 mm Symmetry® C18 guard column. Anthocyanins (cyanidin-3-glucoside, cyanidin-3-rutinoside, cyanidin-3-xyloside, cyanidin-3-malonylglucoside, and

cyanidin-3-dioxalylglucoside) will all be quantified as cyanidin-3-glucoside equivalents (C3GE). Total monomeric anthocyanin results will be expressed as mg C3GE•100 mL^{-1} berry juice.

Statistical analysis

Data were analyzed using an ASReml linear mixed model approach in RStudio v. 4.2.3 (PBC, Boston, MA) with genotype as a fixed effect and tagging date and the interaction of tagging date and genotype as random effects. Batches of fruit tagged on the same day during flowering acted as the experimental units. Mean separation was performed with Tukey's Honestly Significant Difference (HSD) ($\alpha = 0.05$). Pearson's correlation coefficient will be used to test the significance of the correlation between berry color during fruit development and final anthocyanin content and composition.

Preliminary results

The effect of genotype was significant for each coordinate of the L*a*b* values collected at each evaluation date during development (Table 1). Genotypes had different berry color at 14 and 28 days after bloom, although all berries appeared fully black at harvest. significant differences in bloom color were also observed among genotypes. L*, a*, and b* values all differed significantly among genotypes at each date.

Most genotypes had flowers in the RHS white color group (Figures 1 and 2), though A-2790TN and especially A-2717T had pink flowers in the RHS red-purple group (Figure 2c, 2d). By 14 days after flowering, some genotypes were still very green (Figure 1, Figure 2e), while others were dark red (Figure 1, Figure 2g, Figure 2h). A-2575TPF, 'Von', and 'Natchez' were particularly green 14 days after flowering, while A-2687T had the darkest red color. Twenty-eight days after flowering A-2575TPF and 'Von' were still very green, while other genotypes had turned more brown or red (Figure 1).

There was no discernable difference in berry color at harvest among the genotypes evaluated and anthocyanin content is still being measured. Significant differences found in bloom and early berry color should allow for an analysis to find associations between early berry/bloom color and end anthocyanin content if one exists.

Table 1. ANOVA output values for the linear models describing the effect of genotype on the individual L*a*b* values at days 0, 14, and 28. Genotype was found to have a significant effect on each L*a*b* component.

		L*		**a***		**b***	
	Day Count	F-value	P-value	F-value	P-value	F-value	P-value
Bloom color	0	30.751	<0.0001	28.713	<0.0001	38.747	<0.0001
Berry color	14	206.33	<0.0001	119.43	<0.0001	188.04	<0.0001
Berry color	28	218.07	<0.0001	147.67	<0.0001	436.1	<0.0001

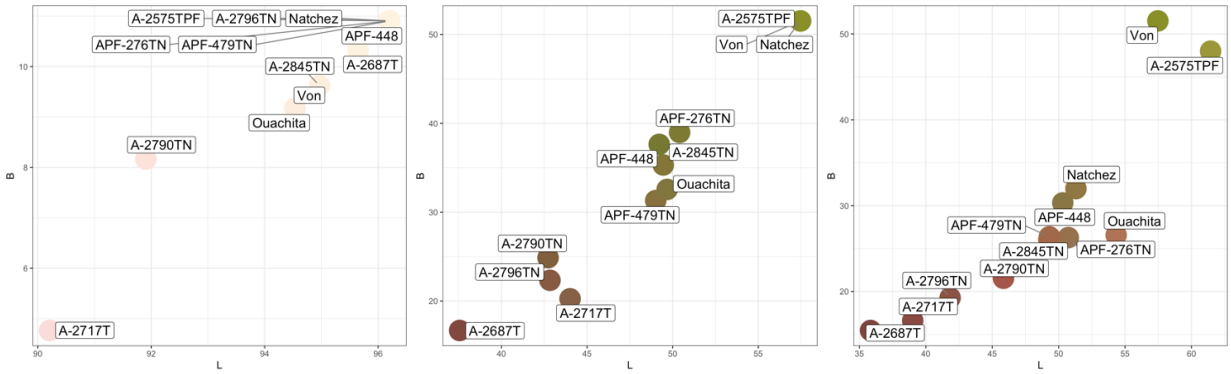

Figure 1. Plot of average L* and b* values for all genotypes at Day 0 (left), Day 14 (center), and Day 28 (right). The color of the plotted points reflect the average color for each genotype from all four flagging dates.

Figure 2. Examples of bloom and developing fruit color assessment using RHS color chips. (a) Ouachita, Day 0 (b) Ouachita, Day 0 (c) A-2717T, Day 0 (d) A-2790TN, Day 0 (e) A-2845TN, Day 14 (f) A-2845TN, Day 28 (g) A-2796TN, Day 14 (h)A-2687T, Day 14.

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