2016 Southern Region Small Fruit Consortium Progress Report

Title: Separation of Sugars and Organic Acid Components in Blackberries for Further Characterization of Flavor and Quality

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Objectives:

1. To use high performance liquid chromatography (HPLC) analysis to identify and quantify the main sugars and organic acids in juice extracted from University of Arkansas (UA)-developed blackberry varieties, advanced selections, and seedlings.
2. To utilize this information in genetic analysis of soluble solids and other fruit quality traits in the Specialty Crop Research Initiative Grant "RosBreed 2: Combining Disease Resistance with Horticultural Quality In New Rosaceous Cultivars".

Justification and Description:

Blackberry (*Rubus* L. subgenus *Rubus* Watson) is cultivated globally, with North America being the largest producer by weight (Kaume et al., 2012). Interest in blackberry with consumers has increased in recent years due in part to several studies documenting high in vitro antioxidant activity in comparison to other fruits (Kaume et al., 2012).

One of the most important characteristics of improvement for further expansion of the blackberry industry in the Southeast is flavor, which is multiplex and generally divided into acidity, aromatic content, astringency, and sweetness (Clark and Finn, 2011). Clark (2005) suggested that meeting consumer preferences, especially by increasing sweetness, is important in expanding the blackberry market. Because both nutraceutical content and sweetness are driving factors that increase demand of blackberry, it is important to investigate the nutrient composition of the fruit and better understand the particular ratios that constitute high quality products.

Sweetness is a primary component of flavor, and sweet berries that are not highly acidic are most desired by consumers. Sweetness is usually measured as soluble solids (SSC), which provides a measure of sweetness but does not identify the individual sugars present in the fruit. Previous work utilizing high performance liquid chromatography (HPLC) showed that glucose, fructose, and sucrose were the main sugars in blackberry, while malic acid is the primary organic acid (Kaume et al., 2012; Mikulic-Petkovsek et al., 2012). This work was not done on Arkansas or southern US blackberry cultivars, however, and there is no information on the relative levels of these individual components for southern-US blackberries. It is notable that in berry fruits perceptible sweetness does not accurately reflect actual sugar content, but rather lower levels of organic acids, particularly malic acid (Mikulic-Petkovsek et al., 2012).

The University of Arkansas has a large blackberry breeding program, and the southern US industry is based largely on Arkansas-developed cultivars. A major emphasis in the program is on fruit flavor, particularly in increasing sweetness and overall flavor in new cultivars.

An example of where flavor has also been a major focus is the Washington State University (WSU) apple breeding program, where individual sugar and organic acid analyses were conducted with commercial cultivars, advanced selections, and seedlings as part of the SCRI grant "RosBREED: Enabling marker-assisted breeding in *Rosaceae*", the initial RosBREED grant. In this effort, measurements of sugars and sugar alcohols that contributed most significantly to total sugars and SSC were identified and quantified, and the ratios of individual sugars to malic acid were calculated (Guan, 2013). The ratio of sugar to malic acid concentration was highly correlated with sensory sweetness, which is in agreement with findings in blackberry (Mikulic-Petkovsek et al., 2012).

The 2014-funded Specialty Crop Research Initiative Grant "RosBreed 2: Combining Disease Resistance with Horticultural Quality In New Rosaceous Cultivars." includes blackberry among its crops, and UA is one of two blackberry breeding programs involved. The grant provides funding for basic phenotyping and genetic analysis of parents and seedlings including measurements of SSC, titratable acidity (TA), firmness and several other traits. However, it does not provide funding for measurement or characterization of individual sugars and acids. This project is aimed towards identifying and quantifying the main organic acids and sugars found in southern US, particularly UA blackberries. This information will then be added to other phenotypic data and used in molecular analysis of these plants with the hope that molecular markers can be developed specific for quality components of blackberries. This could be essential to establishing genetic markers for use in marker-assisted selection in blackberry breeding, leading to increased breeding efficiency and ultimately to improved quality cultivars.

Methodology

Shiny black fruits were collected from individuals from six seedling populations and 12 parental genotypes between June and Aug. of 2015. Fruits were immediately analyzed for fresh fruit characteristics, frozen, and stored at -20 °C. Frozen fruits were brought to room temperature, processed into juice and analyzed for SSC, pH, and titratable acidity (TA) between Sept. and Dec. of 2015. The remaining juice volumes were retained in a -20 °C freezer until HPLC analysis.

For HPLC analysis, juice samples were brought to room temperature and homogenized by inverting the tubes repeatedly. The samples were then diluted to a 1:5 ratio by adding 500 µL of juice to 2500 µL of deionized, degassed water. Each dilution was then mixed and transferred using a disposable transfer pipet into a 3 mL Luer-Lok® syringe with a 0.45 µm nylon filter (Pall® Acrodisc® 13 mm syringe filters, 0.45 µm nylon membrane) attached, and then filtered into a 1 mL glass shell vial and capped. The filtered samples were then analyzed with a Waters® HPLC system which included: Waters® 996 Photodiode Array (PDA) detector, 2414 Refractive Index (RI) detector, 717 Plus Autosampler, 515 HPLC pump, and In-Line degasser AF as well as a BioRAD Aminex® HPX-87H for organic acid separation. The solvent used was 0.08 M sulfuric acid in water with a flow rate of 0.65 mL/min at approximately 6.89 MPa. For each sample vial, the injection volume was 10 uL and the run time was set to 20 min.

To determine the organic acids and sugars present in diluted juice samples, standards for quinic, succinic, shikimic, fumaric, malic, isocitric, phytic, citric, isocitric lactone, and ascorbic acids and glucose, fructose, and sucrose were prepared and analyzed. The resulting peaks were used to determine retention times and concentrations for each standard and then used to identify peaks for the chromatograms generated from the filtered samples. Using standard peak retention times and areas, sample chromatograms were quantified using Waters® Empower Pro™ software.

Results and Discussion

The main organic acids detected consistently in both seedling and parent samples were isocitric lactone, isocitric and citric acids, and malic acid. Isocitric and citric acid had retention times of 10.5 min and 10.3 min respectively. As a result, peaks often overlapped and could not be distinguished and so were calculated as one peak: isocitric and citric acids. While glucose, fructose, and sucrose were identified in some samples, glucose and fructose were the predominant sugars in all samples, with sucrose peaks appearing to be negligible or unidentifiable in most samples.

Preliminary data analysis suggests that malic acid is the main organic acid in these six seedling populations, followed by citric and isocitric acids, and then isocitric lactone (Table 1). For sugars, glucose appears to be found in greater concentration than fructose in all populations (Table 1).

Table 1. Mean values of sugar and organic acid content for UA blackberry seedling populations per 100 g of undiluted juice.

		Population						
	Compound	1222	1229	1236	1250	1253	1261	Parents
Acids (mg/100 g juice)	Citric + Isocitric	23.807	20.302	16.398	21.997	18.486	22.935	24.012
	Isocitric Lactone	3.780	3.510	3.419	5.711	4.417	6.185	2.460
	Malic	57.824	59.472	58.171	98.566	60.769	77.718	51.093
Sugars (g/100 g juice)	Glucose	0.694	0.771	0.650	0.543	0.544	0.513	0.648
	Fructose	0.599	0.657	0.561	0.469	0.477	0.448	0.546

Canonical variate analysis (CVA) suggested that approximately 76% of variability amongst individuals could be explained by the first two dimensions: canonical variate (CV) 1 was most significantly affected by SSC, glucose, and fructose and CV 2 was most significantly affected by isocitric lactone and malic acid. Though 2015 data shows a great deal of variability amongst individuals, most population means could be

separated at 95% confidence intervals when considering the 76% of variability explained by CV 1 and 2. The CVA plot suggests that populations 1253 and 1261 were slightly overlapping, populations 1222 and 1229 had similar responses, and that populations 1250 and 1236 were most dissimilar (Fig. 1).

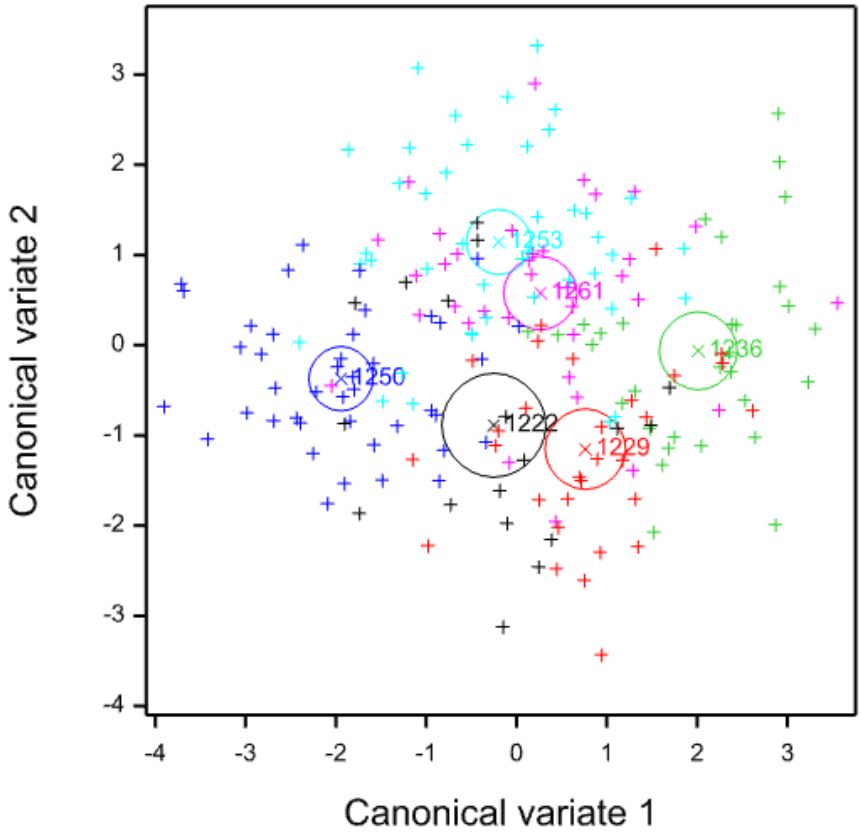

Fig. 1. Canonical variate analysis plot of CV 1 and 2; circles indicate 95% confidence intervals for each population highlighted in the same color.

Conclusion

Preliminary analysis of 2015 data indicate that southern US (UA) blackberry cultivars, advanced selections, and seedlings have similar sugar and organic acid profiles as found in previous work on other blackberry accessions. The principal organic acid appears to be malic acid, while glucose and fructose are the main sugars found in UA blackberries. Additional analysis of 2015 HPLC and phenotypic data suggests that SSC and individual sugars contribute the most to variability amongst individuals in UA blackberry populations.

A second year of data collected from the 2016 season should be considered to determine if the results from 2015 are reproducible and consistent. Once two years of data have been compiled, the results will be shared with collaborators at USDA-ARS NCGR in Corvallis, OR to add to a phenotypic dataset that will be analyzed for the development of molecular markers for blackberry.

Impact Statement

The data collected from this study will help characterize blackberry fruit quality in relation to future breeding objectives and also help understand flavor perception quantitatively. Additionally, when merged with additional phenotypic data, this study will assist in developing molecular markers in the US blackberry breeding effort, increasing the efficiency of breeding programs.

Literature Cited

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