Anthocyanin Content of Wild Black Raspberry Germplasm

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Abstract

Because of its intense anthocyanin pigments, black raspberry (*Rubus occidentalis* L.) has a long history of use as a natural colorant and dye. Recent studies showing black raspberries to be a rich source of anthocyanins and other dietary phytochemicals have led to renewed interest in breeding better adapted cultivars that meet the demands of these markets. Anthocyanin content is a critical indicator of fruit quality for fresh and processed markets. Previous studies characterizing black raspberry anthocyanins have focused on existing cultivars comprising a narrow genetic base; however, progress in breeding new cultivars with better adaptability and disease resistance will rely on the use of new germplasm sources. Using high performance liquid chromatography/diode array detector/ion trap mass spectrometer, we examined anthocyanin content and profiles in the juice of fruit from black raspberry seedlings representing 78 wild populations from across the species' native range over a two year period. Anthocyanin profiles were similar to those previously reported, however total anthocyanin content varied widely. Total anthocyanins in individual clones ranged from 39 to 996 mg/100 ml (expressed as cyanidin-3-glucoside) and averaged slightly higher in 2010 than in 2009. Black raspberry cultivars fell in the middle of this range, with individual wild clones ranging from less than one fourth to nearly three times the anthocyanin concentration of the industry standard, 'Munger'. Genetic diversity for anthocyanin content is present in recently collected wild black raspberry germplasm and should be carefully evaluated when using this material for breeding improved cultivars.

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 (Lee and Slate, 1954; Hong and Wrolstad, 1990). Because of these historical markets for black raspberry fruit, anthocyanin content is a critical indicator of fruit quality for processed markets, and new cultivars will need anthocyanin concentrations similar to or better than current cultivars.

Studies characterizing the types of anthocyanins present in black raspberry fruit date back to at least the 1960s (Nybom, 1968). Levels of anthocyanins and total phenolics in black raspberry fruit have been found to compare favorably to a variety of other small fruits such as elderberries, blueberries, black currants, blackberries, etc. (Moyer et al., 2002; Wu et al., 2006a; Lee and Finn, 2007) and studies linking the high anthocyanin values of black raspberry with potential health benefits have led to increasing interest in black raspberry, from various functional food and nutraceutical markets in recent years.

Despite the renewed interest in black raspberry anthocyanins, relatively little is known about the variability present in black raspberry germplasm available for breeding. Dossett et al. (2008, 2010) found that progeny of a wild selection from North Carolina (NC 84-10-3) and 'Mac Black' had the highest anthocyanin concentrations among crosses primarily between black raspberry cultivars, indicating potential for developing new cultivars with increased anthocyanin levels. The black raspberry industry is in need of new cultivars with better disease resistance and durability. The use of new germplasm will be the key to meeting this objective; however, the range of variability in anthocyanin content in wild germplasm that may be used for future breeding has not been examined. The purpose of this study was to survey fruit anthocyanins found in seedlings from wild black raspberry populations collected from across North America so that the impact of using this germplasm for breeding can be better gauged, and so that germplasm with superior anthocyanin concentrations could be identified and saved.

MATERIALS AND METHODS

During the summer of 2006, friends and colleagues living in eastern North America, within the native distribution of *R. occidentalis*, were solicited to send seed or fruit from wild plants in their area. Through this effort, seeds were obtained from more than 110 locations across the range, including 18 US states and two Canadian provinces. Upon arrival in the lab, seeds were extracted from the fruit, dried, and stored in a cool dry place until being treated to promote germination as described by Dossett and Finn (2010).

In September of 2007, seedlings were planted in the field in a randomized complete block design with four replications representing 78 wild populations (Fig. 1) at the USDA-ARS North Farm (Corvallis, OR, USA). Wild populations were represented by four plants per replication as were plants of 'Jewel', 'Mac Black', and 'Munger' for comparison. Plants were spaced 0.9 m apart and trained to a three-wire trellis system with a lower wire at 0.5 m and two parallel wires hung 0.15 m apart at 0.9 m. In early June, primocane tips were pruned at 1 m to induce branching. In late winter, floricanes from the previous fruiting season were removed and branches on new floricanes were pruned to approximately 30 cm in length.

In 2009, two seedlings from each plot were randomly selected and approximately 100 g of fruit from each were harvested for further evaluation. Initial analysis of this data (not shown) indicated that variation between plots was generally greater than variation from within a plot. This, combined with disease pressure limiting the availability of fruit in many plots, led to the decision to harvest a bulk sample of approximately 100 g of fruit from each plot for analysis in 2010. Collection, handling, and extraction of fruit samples was otherwise performed as described by Dossett et al. (2008).

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), and the protocol for identifying and quantifying anthocyanins was described by Dossett et al. (2008, 2010, 2011). Total anthocyanins were determined by summing the amounts of the individual anthocyanins detected.

RESULTS AND DISCUSSION

Overall, total anthocyanins averaged higher in 2010 than in 2009 (465.1 mg/100 ml vs. 350.1 mg/100 ml, $p<0.0001$ (data not shown). Total anthocyanins also varied widely between the wild populations, ranging from 39.1 mg/100 ml of juice (Greene County, IN, USA 2009) to 996.2 mg/100 ml of juice (DeKalb County, TN, USA 2010) in individual samples indicating tremendous variability. Across years, mean anthocyanin concentrations ranged from 211.9 mg/100 ml (Dutchess County, NY, USA) to 645.0 mg/100 ml (DeKalb County, TN, USA). This is a somewhat wider range than the 244.8-541.3 mg/100 ml of juice found by Dossett et al. (2010) in a black raspberry diallel consisting mostly of cultivars, and is a strong indication of variability for increased anthocyanin content in wild germplasm. While a few populations showed large differences in their relative ranking of total anthocyanin content between years, most of the best and worst performers were the same. Four of the five populations with the lowest anthocyanin concentration ranked in the bottom five in both years and 12 of the 15 populations with the highest average anthocyanins ranked among the 15 highest in both years (data not shown).

Black raspberry cultivars fell near the middle of the range of observed values; 'Munger', 'Jewel', and 'Mac Black' averaged 350.6 mg/100 ml, 387.3 mg/100 ml, and 405.9 mg/100 ml respectively (Table 1). While not statistically different from each other, 'Munger' had the lowest anthocyanin concentration among cultivars in both years, and 'Mac Black' had the highest. Individual wild clones ranged from less than half to nearly three times the concentration of the industry standard, 'Munger', in both years, further indicating the potential for breeding progress from using this germplasm.

Five anthocyanins (in order of elution, cyanidin-3-sambubioside, cyanidin-3-glucoside, cyanidin-3-xylosylrutinoside, cyanidin-3-rutinoside, and pelargonidin-3-rutinoside) were detected in every sample. Trace amounts of peonidin-3-rutinoside were also detected in most samples (example in Fig. 2). In addition, trace levels of pelargonidin-3-glucoside were detected in roughly 25% of the samples. This is not entirely surprising; pelargonidin-3-glucoside is a precursor to pelargonidin-3-rutinoside (detected in all samples) and has been observed in black raspberry in two other studies (Wu et al., 2006b; Dossett et al., 2011). Representative chromatograms for the anthocyanins detected in black raspberry fruit under these analytical conditions are shown by Dossett et al. (2008, 2011).

Proportions of the two major anthocyanins also varied. Dossett et al. (2008) noted that progeny of the wild selection NC 84-10-3 had a strong tendency toward fruit with an increased proportion of cyanidin-3-xylosylrutinoside relative to cyanidin-3-rutinoside as compared to crosses between black raspberry cultivars. In this study, the three cultivars all had proportions of cyanidin-3-rutinoside greater than cyanidin-3-xylosylrutinoside, while this proportion was reversed in about two-thirds of the wild populations sampled. This appears to be common in wild germplasm and studies examining the suitability of different anthocyanin profiles for different end-uses may be needed to determine whether this is an important consideration.

CONCLUSIONS

Total anthocyanin content in wild black raspberries varies widely, and new germplasm should be carefully evaluated before use in breeding to avoid introducing inferior genotypes. The range of diversity present also indicates that it should be possible to breed cultivars with superior anthocyanin concentrations using this germplasm. Bulked screening of seedlings from wild populations can be used to narrow the search for populations of interest, but may risk losing the best clones. While anthocyanin concentrations were significantly different from one year to the next, seedling populations with the lowest and highest concentrations generally were among the highest and lowest across years, indicating that initial selection of populations or clones of interest from a single year may be successful if standards for comparison are used.

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Tables

Table 1. Anthocyanin concentration across two seasons for the five populations with the highest and lowest mean total anthocyanin content, as well as the standard cultivars ‘Munger’, ‘Jewel’, and ‘Mac Black’.

Population	2009 Concentration (mg/100 ml juice)	2010 Concentration (mg/100 ml juice)	Mean
ORUS 3842	137.5	286.3	211.9 a[Z]
ORUS 3780	132.2	305.1	232.6 a
ORUS 3866	215.9	290.9	233.2 a
ORUS 3811	179.4	373.4	262.1 ab
ORUS 3777	260.0	343.9	294.2 abc
Munger	247.9	454.9	350.6 bc
Jewel	284.4	477.5	387.3 c
Mac Black	301.3	529.0	405.9 c
ORUS 3904	467.5	648.9	544.8 d
ORUS 3817	714.8	559.2	581.8 de
ORUS 3931	586.1	586.6	586.4 de
ORUS 3804	291.8	810.6	588.2 de
ORUS 3868	423.2	778.0	645.0 e
Mean	350.1	465.1	402.4

[Z]Mean separation by Duncan’s multiple range test $p\leq0.05$.

Figures

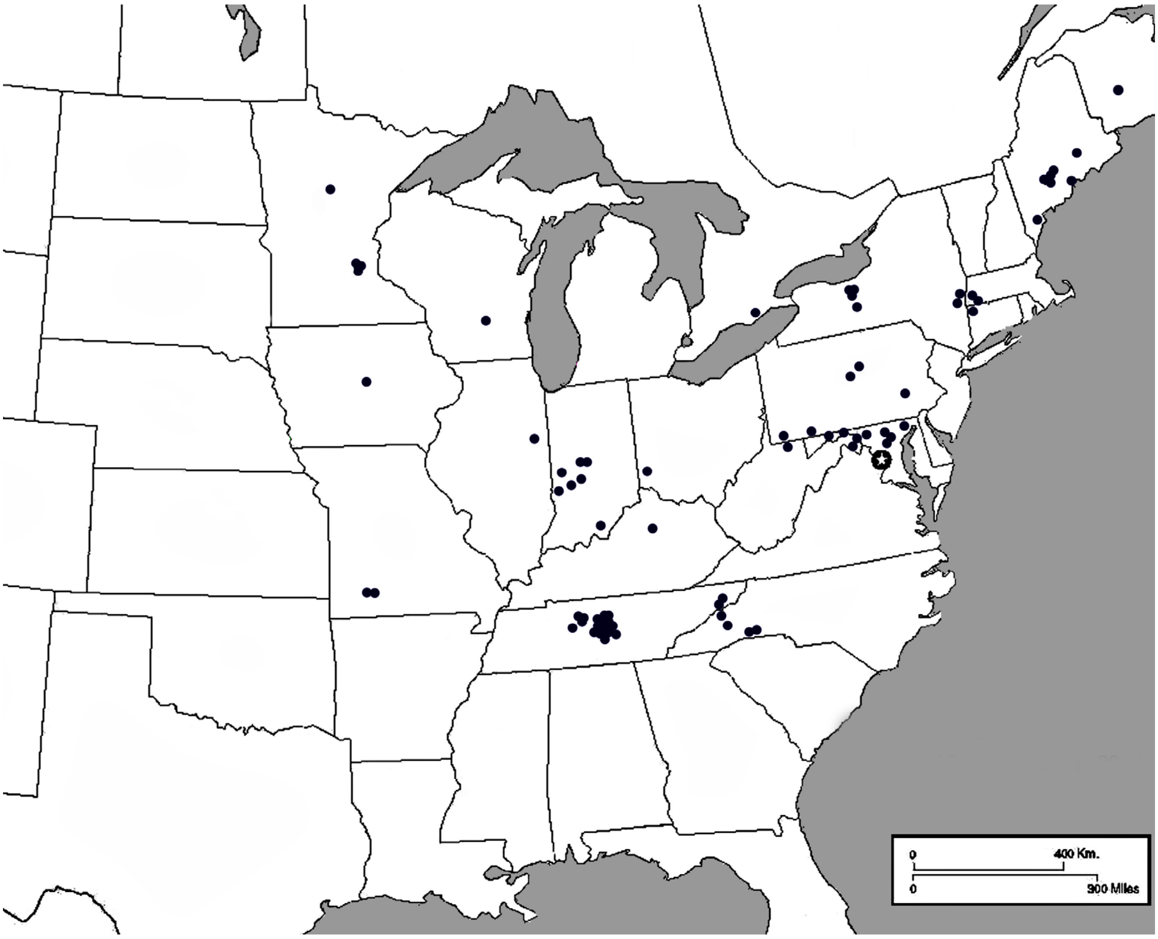

Fig. 1. Geographic origin of populations sampled in this study.