Title: Methods for Rapid Screening of Muscadine Grapes for Browning and Pigment Profile

Progress Report
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Research Proposal

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Objective:

1. Develop a rapid and simplified method to detect juice browning in muscadine selections
2. Determine pigment profiles of purple/black muscadine grape selections and seedling populations and test browning of juice from these grapes

Justification and description

Muscadine (*Vitis rotundifolia*) grapes are native to the southern U.S. These grapes are unique in that they contain pigments that are almost all diglucosides, in contrast to the monoglucoside-type pigments found in *V. vinifera*. These pigments, consisting of 3,5 diglucosides of malvidin, delphinidin, peonidin, petunidin, and cyanidin, can result in browning of muscadine juices and wine over time. Work done by Ballinger et al. (1974), Flora (1978), and recently by Conner and MaClean (2013) indicate that the relative amount of malvidin 3,5 diglucoside is at least part of the solution to prevent browning; total anthocyanin content needs to be above 150 mg/100g peel dry weight as well (bronze grapes contain less than 150 mg/100g). The presence of malvidin 3,5-diglucoside as at least 4% of total pigments in purple/black grapes, and possibly presence of monoglucosides (such as malvidin 3-glucoside) are also needed to slow browning, as found in 'Noble' grapes (Ballinger et al., 1974). Muscadines can contain 0 to 60% malvidin 3,5-diglucoside, and the presence of *V. munsoniana* in the varietal background appears to be critical for increased malvidin content, as shown in data from Fennel's three-way hybrid. Peonidin 3,5-diglucoside may also play a role in stabilizing muscadine juice color (Talcott and Lee, 2002). Additionally, the cultivar Southern Home was developed by the University of Florida, and although considered a muscadine type, contains other species in its background as Fennel's three-way hybrid is its grandparent (Mortensen et al., 1994). The investigation of selections derived from 'Southern Home' are of interest also for pigment identification relative to other fresh market varieties and for potential health benefit.

Extraction and identification of the anthocyanin pigments is relatively simple, but requires high performance liquid chromatography for high through- put screening. This is a problem for breeding programs where access to HPLC or mass spectrophotometer technology may be limited or expensive. Use of a simple juice browning system would offer an alternative option for screening of potential lines where stable juice is needed, and would reduce the number of samples where HPLC analysis is required

Materials and methods:

Plant material. Fruit from purple/black muscadine seedling populations containing some degree of *V. vinifera* or *V. munsoniana* (NC), and selections of Southern Home background (AR) were obtained from NC, GA, and AR breeding programs in 2014 and 2015. Fruit from standard cultivars, including 'Supreme' and 'Noble' from the three locations (NC, GA, AR), and 'Southern Home' from AR, will be used as controls since there is published information on the pigment profiles of these frui. To minimize ripeness effects on pigment content, fruit of soluble solids content of 13 to 20% were used. To date, about 80 of 130 selections from the 2015 harvest have been run on high performance liquid chromatography (HPLC). Differences among

years in pigment profile have been reported (Conner and MacLean, 2013), so two years of harvest are needed to verify differences.

Extraction of pigments and HPLC. Total pigment content and amounts of the 3,5 diglucosides was determined by removing the muscadine peel from berries by hand, freeze dried, and ground to a fine powder using a genogrinder. Peel was extracted with acidified methanol and pigments separated by HPLC as described in Conner and MacLean, 2013, but anthocyanin expressed in equivalents of cyanidin 3-glucoside.

Browning method. Peel of frozen berries was removed and used for browning by crushing using a pestle and heated or not heated at 80 C for 1 h to stimulate browning. A 0.2 to 0.4 g of material was extracted in 10 ml of acidified methanol and water. The pH differential method of Giusti and Wrolstad was used to measure total anthocyanin pigment and the percent difference between values of heated or unheated samples was used to indicate browning.

Results

Selections from the 2014 harvest season from North Carolina were run on HPLC. About 1/3 of the 2015 samples from the University of Georgia, University of Arkansas, and North Carolina State have been run with HPLC (Table 1). Additionally, four 2014 samples (DVIT 2970, UCD6-38, NCCH4910, and DRX 60-40) that appeared to have malvidin 3-glucoside and acetylated glucosides were sent for identification and quantification of acetyl- and coumaroyl-glucoside and galactoside derivatives by mass spectrophotometry.
Among the 2015 samples run on HPLC to date, three patterns of results appear. First are those with total anthocyanins high in percent delphinidin 3,5 diglucoside. Second are those high in malvidin 3,5 diglucoside, and the third group are those where malvidin 3-glucoside and other mono glucosides appear, and the presence of acetylated glucosides may also be present. While most of the samples tested were high in delphinidin 3,5 diglucoside and exhibited the five common diglucosides spectra, typical of many muscadine selections, ten of the GA selections had more than 50% of pigment present as malvidin 3,5 diglucoside (Table 1). Thirteen samples had a trace or more of malvidin 3-glucoside (Table 1). One sample, A1575, is a bunch grape selection and showed a high proportion of monoglucosides, as reported for Euvitis. A1665 was much lower in monoglucosides than A1575 but also much higher than the other selections. NC74C049-10 and UCD6-38 had small amounts of malvidin 3-glucoside compared to A1575 or A1665 (Table 2). Muscadine juice or wine browning is thought to be due in part to low levels of malvidin or peonidin glucoside or lower total anthocyanin.

A summary of results of browning experiments and anthocyanin identification for these 2014 samples can be found in the SFRC Newsletter, 15(3) 13-18 (http://www.smallfruits.org/Newsletter/SmallFruitNews.htm). Determination of browning has been difficult as there is considerable variation among replications. This may be due to the fact that only peel tissue is used, rather than crushed berries, reducing relative amounts of juice. Browning experiments are slowly being completed and we do not yet have enough samples run to do correlations with pigment content. However, from what is present in the selections that have had pigments identified, we do have a good range of pigment contents in our material.

Impact Statement: Muscadine selections from three breeding programs show a wide range of differences in pigment composition, and 10 selections were found to have a pigment shift from delphinidin to malvidin 3,5 diglucoside as the predominant pigment, offering a possibility of more stable juice products.

References:

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Table 1. Anthocyanin pigments in freeze dried peel of grape selections and cultivars determined by high performance liquid chromatography and expressed as total amount and as percent of total anthocyanins.

Cultivar/selection	Source of berries	Total anthocyanin	Malvidin 3,5 DG	Peonidin 3,5 DG	Delphinidin 3,5 DG	Petunidin 3,5 DG	Cyanidin 3,5 DG
		mg/100 g C3G DWT		%			
AM49	AR	833.43	0.6	10.5	25.2	7.2	56.6
LOOMIS	NC	1063.25	0.8	6.7	32.7	6.6	53.2
AM48	AR	2966.69	1.2	8.2	34.8	8.9	47.0
ALBEMARLE	NC	322.80	1.6	12.8	25.8	8.0	51.8
AM67	AR	2233.76	2.0	7.1	50.1	14.6	26.2
AM97	AR	1823.57	2.2	5.3	53.5	14.7	24.2
SHOME	AR	2195.85	2.4	6.5	49.3	14.2	27.7
NC 1006	NC	857.58	3.0	10.5	29.3	9.5	47.6
AM72	AR	659.86	4.1	12.2	33.9	13.3	36.5
AM43	AR	1698.15	4.2	9.7	44.1	17.5	24.5
LANE (GA)	GA	2778.31	4.5	2.4	64.1	20.4	8.6
GA 11-6-10	GA	667.78	4.5	3.3	57.9	25.8	8.4
OLMO U67-2	NC	4235.67	4.7	1.6	66.2	23.7	3.9
GA 12-24-1	GA	457.5	4.6	4.5	62.5	23.0	5.4
NCCH 23:111	NC	2386.79	4.9	3.7	57.5	18.5	15.3
GA 11-6-50	GA	846.5	5.0	7.8	41.8	25.0	20.4
GA 9-6-60	GA	1020.0	5.0	57.0	5.2	28.0	4.8
FARRAR	NC	3217.27	5.0	1.9	65.3	22.2	5.4
NCCH 26:45	NC	2078.29	5.3	2.5	63.6	23.6	5.0
GA 9-6-47	GA	410.4	5.5	13.8	40.5	27.4	12.8
GA 11-6-16	GA	841.80	5.6	4.3	53.6	27.4	9.1
AM77	AR	2002.42	5.7	3.7	59.4	22.6	8.6

GA 11-6-1	GA	905.09	5.7	2.6	61.6	25.0	5.1
NC 67A105-26	AR	2338.37	6.5	4.8	53.8	22.2	12.7
LANE (NC)	NC	1583.82	6.5	2.0	62.6	24.1	4.8
FAMU 014-15-1	NC	1367.59	6.9	3.8	57.0	21.9	10.4
AM83	AR	3881.78	7.2	3.4	55.8	26.4	7.2
MARSH	NC	1582.57	7.5	10.8	47.5	24.1	6.3
AM61	AR	2509.88	9.0	4.2	51.2	28.9	6.8
GA 11-6-14	GA	455.74	11.4	8.3	34.4	38.4	7.6
SOUTHLAND	NC	1298.20	13.2	14.1	35.0	24.6	13.0
FAMU 028-22-5	NC	1780.27	13.6	5.0	46.5	29.2	5.7
NCCH 22:47	NC	1924.14	14.3	9.2	39.8	27.6	9.0
GA 12-18-12	GA	481.87	16.6	5.6	27.0	31.2	19.6
GA 9-6-78	GA	583.84	27.4	7.7	16.4	47.5	1.0
GA 11-6-90	GA	1018.75	39.8	52.2	1.4	4.8	1.8
GA 11-6-102	GA	483.85	54.0	33.2	1.8	9.1	1.9
GA 11-6-74	GA	350.00	56.5	38.6	0.2	4.0	0.6
GA 11-6-110	GA	592.21	63.2	25.6	2.2	7.7	1.3
GA 11-6-20	GA	667.77	68.0	15.5	3.3	12.4	0.7
GA 11-6-12	GA	395.66	69.0	26.2	0.1	4.4	0.3
GA 11-6-30	GA	534.99	72.4	17.4	1.3	8.4	0.6
GA 11-6-19	GA	479.23	74.2	18.4	0.3	6.6	0.4
GA 11-6-108	GA	813.32	74.6	14.5	1.0	9.0	0.9
GA 11-6-15	GA	756.09	76.6	14.2	1.7	7.2	0.2
GA 11-6-100	GA	712.69	76.9	11.9	2.6	8.2	0.4

G=glucoside; DG=diglucoside

Table 2. Anthocyanin profiles for muscadine grape selections (A1575 is bunch grape) with traces of malvidin 3-glucoside. GA 12-20-7, A1665 and A1575 were from 2015 samples and remainder were from 2014.

Selection	Total anthocyanin	Delphinidin 3,5 DG[x]	Malvidin 3,5 DG	Peonidin 3,5 DG	Cyanidin 3,5 DG	Petunidin 3,5 DG	Malvidin 3G
	mg/100 g C3G dwt			%			
FL H 17-66	2208.73	64.9	3.1	1.9	8.2	18.9	0.1
Olmo U67-2	3626.17	61.2	5.4	1.6	5.1	25.3	0.0
GA 12-20-7[y]	1576.31	35.8	5.2	7.5	14.1	17.5	0.3
DRX 60-40[z]	1696.08	23.2	7.9	0.5	18.8	15.4	1.6
NC CH 11-26:116	4270.78	51.5	9.5	1.4	2.4	29.3	0.5
Marsh	1503.22	29.3	10.5	4.2	4.8	30.0	2.0
NC CH11-26:45	2835.18	49.1	13.5	2.7	3.3	30.2	0.0
NC CH11-25:64	5127.20	44.6	17.2	1.6	1.8	33.5	0.1
UCD 6-38	1040.61	5.5	20.5	16.8	6.6	6.9	7.6
DVIT 2970	4312.90	0.2	24.0	74.0	0.5	0.7	0.4
NC 74 CO49-10	2775.02	23.4	31.3	11.9	4.5	21.3	1.1
Fennels 3 way	994.34	12.5	56.3	8.0	2.5	20.3	0.2
A1665	1406.55	0.0	29.7	2.7	0	0	9.4
A1575	5123.14	0.0	0.0	8.9	0	0	32.4

[x]G=glucoside; DG=diglucoside, C3G=cyanidin 3-glucoside equivalents.

[y]GA 12-20-7 contained 13% peonidin, petunidin, and cyanidin 3-glucoside with the remainder as glucosides and galactosides with acetyl or coumaryl groups.

A1665 contained 22% peonidin and petunidin 3-glucoside with the remainder as glucosides and galactosides with acetyl or coumaryl groups.

A1575 contained 25% peonidin, petunidin, and cyanidin 3-glucoside with the remainder as glucosides and galactosides with acetyl or coumaryl groups.

[z] DRX 60-40 and NC 74CO49-10 are quasi F1's between V. vinifera and muscadine (V. rotundifolia). UCD 6-38 is an F1 between V. vinifera and muscadine.