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**Title: Rapid Screening of Muscadine Grapes for Browning and Pigment Profile and Identification of Key Anthocyanins in Selections for Breeding for Stable Juice Color**

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**Objectives:**

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
3. Develop a model that can predict juice stability based on total anthocyanin, percent malvidin pigments, and percent peonidin pigments.

**Materials and methods:**

Plant material. Fruit from purple/black muscadine seedling populations containing some degree of *V. vinifera* or *V. munsoniana* (NC and SC), and selections of Southern Home background (AR) will be used. Fruit from standard cultivars, including 'Supreme' and 'Noble' from the three locations (NC, SC, AR), and 'Southern Home' from AR, will be used as controls since there is published information on the pigment profiles of these fruit. Since relative percentages of pigments can change with ripeness (Flora, 1978; Lamikanra, 1988), fruit of a soluble solids content of 13 to 16% (where the relative pigment content becomes stabilized) will be used. Samples for 2015 are at about 180; the targeted number of samples to run for 2016 is 150-200. Differences among years in pigment profile have been reported (Conner and MacLean, 2013), so two years of harvest is needed to verify differences.

Extraction of pigments and HPLC. Total pigment content and amounts of the 3,5 diglucosides and/or mono glucosides will be determined by utilizing the muscadine peel. In frozen or fresh muscadines, peel will be removed by hand by 'popping' out the inside flesh in the slip skin types or peeled if the crisp-textured types. The saved peel will be frozen for browning experiments or freeze dried for pigment analysis (held at -20 or -80 °C). Peel will be extracted with acidified methanol and pigments separated by HPLC as described in Conner and MacLean, 2013. The soluble solids content and pH will be measured for all samples from the remaining juice to make sure fruit are fully ripe (SSC>13%).

Browning method. Using a water bath with acidified methanol became problematic when larger sample numbers were used due to difficulty in preventing evaporation, lack of room for samples, and the relatively long incubation time. An alternative browning test using citric acid buffered with sodium hydroxide and bleaching with hydrogen peroxide was tried with muscadines (S. Talcott via P. Conner, personal communication). With this method, and using only a few grapes, frozen and partially thawed muscadine peel was cut with razor blades, spun in a microcentrifuge to recover juice, and juice mixed with citric acid at pH 3.0 to achieve an absorbance at 520 nm of 0.7 to 0.9. Hydrogen peroxide was added and samples placed for 10,20, and 30 min at 60 °C in a convection oven. Resulting absorbance at 520 nm was determined by UV-vis spectrophotometer.

**Timeline (year 2):**

Winter and spring 2016. Determine browning in selections from Arkansas, Georgia, and North Carolina. Do HPLC analysis of anthocyanins on 180 samples.
Summer, 2016. Summarize results.

Late summer, Fall, 2016. Collect more fruit for assays.

**Results**

Table 1 shows the species composition of muscadine selections from the NC and AR programs that were included in this study. HPLC profiles of a total of 180 selections and varieties from the Arkansas, Georgia, and North Carolina programs were done in 2015. Of these, 56 were found to be high in total pigment (over 1000 mg/100 g dwt) and of this group, 13 were found to have over 30% of pigments as malvidin 3,5 diglucoside (Table 2). Theoretically, these varieties should impart color stability in juices and wines. Five selections were found to have small amounts of pelargonidin 3,5 diglucoside (up to 9% of total pigment).

About 150 samples from the 2016 harvest have been prepared for analysis, with 50% done. While relative profiles were generally similar, the total amount of pigment varied widely for some selections from year to year, despite screening for soluble solids content to make sure grapes met at least 14% SSC (Table 3). These differences present a challenge in data evaluation if screening for high pigment grapes over several harvest seasons and extra protocols, such as choosing only darkest colored, unshriveled berries from samples may need to be done to reduce variability. Looking at 'Noble' fruit collected from AR and NC test plots, there was a 20% variation in total anthocyanin content and even profile values, representing both year and location effects. Although 20% sounds like a lot of variability, when profiles of the sample selections are studied, distinct differences are apparent, such as in delphinidin 3,5 diglucoside and cyaniding 3,5 diglucoside between Noble and NC 74 CO49-10, as well as in malvidin 3,5 diglucoside.

The citric acid/hydrogen peroxide test for resistance to bleaching was not related to percentages of malvidin or peonidin 3,5 diglucosides or to total pigment content (data not shown). Some of this seems to be due to different results for the same grapes when measured on different days. Modifications are being made to the protocol to allow sampling of larger sample numbers per day, and also to identify and correct points of weakness in reproducibility in order to get the system to work and to simplify it. Inclusion of certain grapes in each test run as markers that should be more resistant (such as Sunbelt) or less resistant (such as Black Beauty) should be done. It is also possible that the resistance to bleaching is imparted by phenolic compounds, other than or in addition to, specific anthocyanins. Polymeric anthocyanin content was checked in several selections in addition to monomeric anthocyanins, and was found to be very low.

Table 1. *Vitis* species composition of different selections.

| Genotype | *Euvitis* | *V. munsoniana* | *V. popenoei* | *V. aestivalis* |
|---|---|---|---|---|
| UCD 6-38 | 50 | 0 | 0 | |
| NC CH11-25:64 | 50 | 0 | 0 | |
| Marsh | 0 | 100? | 0 | |
| NC 74 CO 49-10 | 62 | 0 | 0 | |
| Fennel's 3 way | 0 | 25 | 50 | |
| DVIT 2970 | 0 | 0 | 100 | |
| DRX 60-40 | 12 | 0 | 0 | |
| FL H 17-66 | 0 | 50 | 0 | |
| NC CH11-26:45 | 50 | 0 | 0 | |
| NC CH 11-26:116 | 50 | 0 | 0 | |
| Olmo U67-2 | 0 | 100 | 0 | |
| A1575 | 100 | 0 | 0 | |
| Lenoir | 50 | 0 | 0 | 50 |

Table 2.  Anthocyanin content and profile of grapes (primarily of muscadine background) harvested in 2015 from Georgia (GA), Arkansas (AR) and North Carolina (NC).

| Genotype | Source | Total anthocyanin (mg CDG/100 g DW)[z] | %DDG | %CDG | %PTDG | %PGDG | %PNDG | %MDG | %C3G | %M3G | other (mono glucosides or acylated glucosides) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 11-6-49 | GA | 1303.6 | 66.7 | 8.1 | 18.8 | 0.0 | 2.7 | 3.5 | 0.0 | 0.0 | |
| 11-6-90 | GA | 1018.7 | 1.4 | 1.8 | 4.8 | 0.0 | 52.2 | 39.8 | 0.0 | 0.0 | |
| 11-6-92 | GA | 1109.0 | 1.4 | 0.1 | 9.6 | 0.0 | 6.6 | 82.3 | 0.0 | 0.0 | |
| 12-10-4 | GA | 1296.5 | 49.6 | 4.5 | 25.8 | 0.2 | 2.3 | 10.9 | 1.1 | 0.5 | 5.0 |
| 12-18-3 | GA | 1491.6 | 72.7 | 8.2 | 15.0 | 0.7 | 1.4 | 2.0 | 0.0 | 0.0 | |
| 12-20-4 | GA | 1068.0 | 48.9 | 10.8 | 26.6 | 0.4 | 2.8 | 10.5 | 0.0 | 0.0 | |
| 12-20-7 | GA | 1358.5 | 41.6 | 16.3 | 20.3 | 8.7 | 6.1 | 0.0 | 2.5 | 0.3 | 4.2 |
| 9-6-135 | GA | 1318.1 | 15.0 | 3.3 | 42.4 | 0.0 | 7.6 | 31.6 | 0.0 | 0.0 | |
| 9-6-37 | GA | 1132.3 | 10.7 | 2.1 | 38.2 | 0.0 | 13.0 | 35.9 | 0.0 | 0.0 | |
| 9-6-60 | GA | 1020.0 | 57.0 | 4.8 | 28.0 | 5.2 | 5.0 | 0.0 | 0.0 | 0.0 | |
| 9-6-67 | GA | 1273.5 | 60.7 | 8.4 | 23.1 | 0.0 | 4.8 | 3.0 | 0.0 | 0.0 | |
| 9-6-79 | GA | 1145.9 | 15.5 | 3.0 | 31.9 | 0.0 | 17.3 | 32.3 | 0.0 | 0.0 | |
| 9-6-85 | GA | 1246.7 | 11.0 | 2.6 | 43.3 | 0.0 | 6.6 | 36.4 | 0.0 | 0.0 | |
| A1575 | AR | 4855.3 | 0.0 | 0.0 | 0.0 | 0.0 | 9.6 | 0.0 | 3.0 | 34.6 | 52.8 |
| A1665 | AR | 1406.5 | 0.0 | 0.0 | 0.0 | 0.0 | 2.7 | 29.7 | 0.0 | 9.5 | 58.1 |
| AM132 | AR | 1673.4 | 47.2 | 25.9 | 15.0 | 0.0 | 8.0 | 3.8 | 0.0 | 0.0 | |
| AM43 | AR | 1698.1 | 44.1 | 24.5 | 17.5 | 0.0 | 9.7 | 4.2 | 0.0 | 0.0 | |
| AM48 | AR | 3038.8 | 35.5 | 46.2 | 9.1 | 0.0 | 8.1 | 1.2 | 0.0 | 0.0 | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| AM61 | AR | 2509.9 | 51.2 | 6.8 | 28.9 | 0.0 | 4.2 | 9.0 | 0.0 | 0.0 | |
| AM67 | AR | 2233.8 | 50.1 | 26.2 | 14.6 | 0.0 | 7.1 | 2.0 | 0.0 | 0.0 | |
| AM77 | AR | 2002.4 | 59.4 | 8.6 | 22.6 | 0.0 | 3.7 | 5.7 | 0.0 | 0.0 | |
| AM83 | AR | 3924.3 | 56.1 | 7.4 | 26.0 | 0.0 | 3.4 | 7.1 | 0.0 | 0.0 | |
| BLACK FRY | NC | 1033.0 | 49.5 | 31.1 | 11.7 | 0.0 | 5.7 | 1.9 | 0.0 | 0.0 | |
| BLACK BEAUTY | NC | 965.8 | 42.5 | 39.5 | 9.6 | 0.0 | 6.9 | 1.5 | 0.0 | 0.0 | |
| DRX 60-40 | NC | 1633.2 | 24.0 | 12.9 | 15.8 | 0.0 | 15.4 | 8.0 | 6.6 | 0.0 | 17.4 |
| DVIT 2970 | NC | 1026.0 | 15.5 | 3.0 | 21.7 | 0.0 | 6.2 | 53.5 | 0.0 | 0.0 | |
| FAMU 014-15-1 | NC | 1444.7 | 56.9 | 10.5 | 21.9 | 0.0 | 3.8 | 6.9 | 0.0 | 0.0 | |
| FAMU 028-22-5 | NC | 1780.3 | 46.5 | 5.7 | 29.2 | 0.0 | 5.0 | 13.6 | 0.0 | 0.0 | |
| FARRER 31 | NC | 3307.2 | 65.1 | 5.5 | 22.3 | 0.3 | 1.9 | 4.9 | 0.0 | 0.0 | |
| FENNELS | NC | 1060.3 | 11.7 | 2.6 | 20.3 | 0.0 | 7.9 | 57.5 | 0.0 | 0.0 | |
| FL 66 | NC | 5955.7 | 58.8 | 7.6 | 24.2 | 0.5 | 2.6 | 6.2 | 0.0 | 0.0 | |
| GA 1937 | NC | 3101.0 | 64.1 | 5.0 | 23.1 | 0.0 | 1.9 | 5.8 | 0.0 | 0.0 | |
| GA 12-12-2 | NC | 1181.0 | 24.4 | 2.1 | 23.0 | 0.0 | 7.8 | 32.3 | 1.0 | 0.0 | 9.4 |
| GOLDY NO MONO | NC | 1394.1 | 50.8 | 20.7 | 16.9 | 0.0 | 8.3 | 3.3 | 0.0 | 0.0 | |
| ISON NC | NC | 1415.2 | 55.6 | 8.6 | 23.1 | 0.0 | 5.1 | 7.6 | 0.0 | 0.0 | |
| JUMBO NC | NC | 2288.2 | 69.6 | 7.4 | 18.1 | 0.0 | 1.7 | 3.2 | 0.0 | 0.0 | |
| LANE | GA | 2778.3 | 64.1 | 8.6 | 20.4 | 0.0 | 2.4 | 4.5 | 0.0 | 0.0 | |
| LENOIR | NC | 1633.9 | 7.7 | 6.4 | 6.1 | 0.0 | 30.4 | 10.7 | 15.4 | 4.4 | 18.8 |
| LOOMIS | NC | 1063.3 | 32.7 | 53.2 | 6.6 | 0.0 | 6.7 | 0.8 | 0.0 | 0.0 | |
| MARSH | NC | 1587.4 | 47.3 | 6.2 | 24.1 | 0.0 | 10.9 | 7.5 | 1.1 | 0.6 | 2.2 |
| NC 028-22-58 | NC | 3654.3 | 43.3 | 5.7 | 30.2 | 0.0 | 5.3 | 15.5 | 0.0 | 0.0 | |
| NC 18-29 | NC | 2627.7 | 43.2 | 9.6 | 27.2 | 0.0 | 7.3 | 12.6 | 0.0 | 0.0 | |
| NC 22-47 | NC | 1983.2 | 39.9 | 9.0 | 27.6 | 0.0 | 9.2 | 14.3 | 0.0 | 0.0 | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| NC 67A105-26 | AR | 2338.4 | 53.8 | 12.7 | 22.2 | 0.0 | 4.8 | 6.5 | 0.0 | 0.0 | |
| NC 74 CO 49-10 | NC | 1652.8 | 18.8 | 5.5 | 17.0 | 0.0 | 20.2 | 28.8 | 0.0 | 1.2 | 8.5 |
| NC CH 11-26:116 | NC | 5245.3 | 54.2 | 3.4 | 29.6 | 0.0 | 2.5 | 10.3 | 0.0 | 0.0 | |
| NC CH 11-26:15 | NC | 3131.5 | 62.1 | 5.2 | 24.2 | 0.0 | 2.7 | 5.7 | 0.0 | 0.0 | |
| NC CH 11-26:45 | NC | 2078.3 | 63.6 | 5.0 | 23.6 | 0.0 | 2.5 | 5.3 | 0.0 | 0.0 | |
| NC CH 11-23:111 | NC | 2422.7 | 57.7 | 15.3 | 18.4 | 0.0 | 3.7 | 4.9 | 0.0 | 0.0 | |
| NOBLE | AR | 2355.1 | 29.2 | 27.0 | 18.3 | 0.0 | 19.2 | 6.3 | 0.0 | 0.0 | |
| OLMO U67-2 | NC | 4235.7 | 66.2 | 3.9 | 23.7 | 0.0 | 1.6 | 4.7 | 0.0 | 0.0 | |
| SOUTHERN HOME | NC | 2195.9 | 49.3 | 27.7 | 14.2 | 0.0 | 6.5 | 2.4 | 0.0 | 0.0 | |
| SOUTHLAND | NC | 1352.2 | 35.0 | 13.2 | 24.6 | 0.0 | 14.0 | 13.2 | 0.0 | 0.0 | |
| SUPREME | NC | 1542.7 | 40.8 | 38.2 | 10.6 | 0.0 | 8.5 | 1.9 | 0.0 | 0.0 | |

[z]Total anthocyanin as equivalents of cyanidin 3,5 diglucoside

[x]DDG=delphinidin 3,5 diglucoside; CDG=cyanidin 3,5 diglucoside; PTDG=petunidin 3,5 diglucoside; PNDG=peonidin 3,5 diglucoside; MDG=malvidin 3,5 diglucoside; C3G=cyanidin 3-glucoside; M3G=malvidin 3-glucoside

Table 3. Comparison of anthocyanin profiles between years of sampling.

| Year | Genotype | | Total anthocyanin (mg/100 g dw)[z] | %DDG[x] | %cCDG | %PTDG | %PNDG | %MDG | %C3G | %M3G |
|---|---|---|---|---|---|---|---|---|---|---|
| 2015 | 11-6-49 | GA | 1303.6 | 66.7 | 8.1 | 18.8 | 2.7 | 3.5 | 0.0 | 0.0 |
| 2016 | 11-6-49 | GA | 1172.4 | 60.1 | 12.50 | 19.9 | 3.7 | 3.8 | 0.0 | 0.0 |
| | | | | | | | | | | |
| 2015 | 11-6-96 | GA | 866.9 | 58.3 | 5.2 | 26.6 | 3.0 | 6.8 | 0.0 | 0.0 |
| 2016 | 11-6-96 | GA | 1340.1 | 46.1 | 7.9 | 28.5 | 6.1 | 11.4 | 0.00 | 0.0 |
| | | | | | | | | | | |
| 2015 | AM43 | AR | 1698.1 | 44.1 | 24.5 | 17.5 | 9.7 | 4.2 | 0.0 | 0.0 |
| 2016 | AM43 | AR | 1276.5 | 45.6 | 20.4 | 18.9 | 9.6 | 5.5 | 0.0 | 0.0 |
| | | | | | | | | | | |
| 2015 | AM83 | AR | 3881.8 | 55.8 | 7.2 | 26.4 | 3.35 | 7.20 | 0.0 | 0.0 |
| 2016 | AM83 | AR | 3695.8 | 59.2 | 6.2 | 25.50 | 2.77 | 6.29 | 0.0 | 0.0 |
| | | | | | | | | | | |
| 2015 | NC 74 C049-10 | NC | 1652.8 | 18.8 | 5.5 | 17.0 | 20.2 | 28.8 | 0.0 | 1.2 |
| 2016 | NC 74 C0 49-10 | NC | 1480.9 | 11.4 | 2.9 | 14.7 | 20.7 | 44.4 | 0.02 | 1.4 |
| | | | | | | | | | | |
| 2015 | Noble | NC | 2949.1 | 34.8 | 17.0 | 22.1 | 16.2 | 9.8 | 0.0 | 0.0 |
| 2016 | Noble | CH | 2453.2 | 31.3 | 20.6 | 21.7 | 18.2 | 8.3 | 0.0 | 0.0 |
| 2016 | Noble | AR | 2713.9 | 35.1 | 22.1 | 20.9 | 14.2 | 7.6 | 0.06 | 0.0 |
| 2015 | Noble | AR | 2355.1 | 29.2 | 27.0 | 18.3 | 19.2 | 6.3 | 0.0 | 0.0 |

[z]Total anthocyanin as equivalents of cyanidin 3,5 diglucoside

[x]DDG=delphinidin 3,5 diglucoside; CDG=cyanidin 3,5 diglucoside; PTDG=petunidin 3,5 diglucoside; PNDG=peonidin 3,5 diglucoside; MDG=malvidin 3,5 diglucoside; C3G=cyanidin 3-glucoside; M3G=malvidin 3-glucoside