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VARIATION IN THE SUGAR ACCUMULATION PATTERN OF MUSCADINE GRAPE GENOTYPES

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Abstract. **The present study was undertaken to determine variation in the accumulation pattern of sugars in leaves and berries at different developmental stages of forty-two muscadine grape genotypes. In order to compare the sugar accumulation patterns and source sink relationships between bunch and muscadine grape genotypes, twelve-bunch grape genotypes were also studied. Leaf and berry sugar concentrations among the genotypes were significantly different ($P \leq 0.05$) at different developmental stages. Sugar concentrations in the leaves of muscadine genotypes varied from 1.94% (w/v) to 8.30% at the pre-flowering stage; 0.36% to 4.52% at the flowering stage; 2.19% to 4.10% at the young fruit stage; 2.25% to 6.05% at the medium fruit stage; 2.39% to 7.79% at the mature fruit stage; and 1.67% to 7.09% at the ripe fruit stage. Accumulation of sugars in berries varied from 0.61% to 2.25% at the young fruit stage; 0.38% to 3.18% at the medium fruit stage; 1.11% to 11.37% at the mature fruit stage; and 4.46% to 16.08% at the ripe fruit stage. The mean sugar concentrations over the developmental stages of the leaf and berry were tested using the RANK procedure that helped to assign the grape genotypes into seven distinct groups. Significantly higher leaf sugar content at fully developed/mature fruit stage (stage 5) and pre-flowering (stage 1), suggests that there are higher leaf sugar requirements after veraison (berry ripening) and during flowering. Change of grouping study shows that 17 genotypes that were in the lower leaf sugar group were moved to the upper group in terms of berry sugar concentration, 12 genotypes maintained their rank whereas 25 genotypes showed decreases in their rank. Further studies are suggested to study the impact of leaf sugar concentration on characteristics contributing to berry sugar such as leaf biomass, number of catkins per vine, number of berry cluster per vine, number of berries per cluster berry, size and levels of key enzymes involved in sucrose synthesis.**

The southeastern grape industry is based primarily on *Vitis* species native to the Gulf Plain of the United States, especially muscadine grapes (*Vitis rotundifolia* Michx). The muscadine genotypes are tolerant to most grape diseases, however, muscadine grapes are not desirable as a table grape (fresh fruit) because of sour taste, thick berry skin and seeded berries. Muscadine wines are gaining popularity because of their unique fruity flavor and full-body (Olien, 1990). Berry sugar concentration is an important characteristic that affects wine quality (Davies and Robinson, 1996). Sucrose is produced as a result of photosynthesis in the leaf and transported through phloem to the berries (Hawker et al., 1976; Swanson and El-Shishiny, 1958). The transported sugar is hydrolyzed to glucose and fructose in grape berries. Accumulation of sugars in the form of glucose and fructose within the vacuole is one of the main features of the ripening process in grape and continues through ripening. Photosynthetic capability, rate of import into individual sink organs, and levels of sucrose metabolizing enzymes such as invertase or sucrose phosphate synthase activity are very important components for sugar accumulation in grape berries (Hawker, 1969; Hubbard et al., 1991).

The sugar level in grape berries varies greatly among different genotypes. However, the sugar accumulation patterns in different muscadine genotypes have not been fully studied. The present study was undertaken to determine variation in

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the accumulation pattern of sugars in the leaves and fruits at different developmental stages, such as pre-flowering, flowering, and post-flowering (young, medium, mature and ripe fruit) stages of muscadine grape genotypes to understand the accumulation pattern of sugars in the source (leaf) and sink (berry) organs. Understanding the relationship of sugar accumulation patterns in source and sink tissue may help (i) identify developmental profiles leading to higher berry sugar concentrations (ii) grape breeders to predict berry sweetness of a desirable selection at early stages during the selection process of segregating progeny. For comparison, bunch grape genotypes were also included in the study to determine differences in sugar accumulation pattern between muscadine and bunch grape cultivars.

Materials and Methods

Plant Material. Fifty-four grape genotypes that included 42 muscadine and 12 bunch germplasm accessions, which varied in origin and genetic diversity, were used in the present study (Tables 1 and 2). The leaf and fruit samples were obtained (1999 and 2000 season) from the vines grown at the Center for Viticulture and Small Fruit Research, Florida A&M University. This is an established experimental vineyard located on the campus, with four vines of each cultivar and planted randomly. The distance between rows is 15 ft and vine spacing within the row is 8 ft. The soil is sandy clay with a gentle slope. The plants were irrigated as needed using drip irrigation, and were not exposed to drought stress. All the genotypes used in this study were grown at the same location with similar soil characteristics and were exposed to the same environmental conditions. To minimize the effect of environmental factors such as solar brightness and temperature stress, tissue sampling was done between 8:00 AM. and 9:00 AM Plant tissue was collected randomly from the exposed canopy along the length of the vine. The leaf (2 or 3 leaves ~5 g) and/or berry (2 or 3 berries from the top of the cluster) samples were collected from three separate plants. Samples were sealed in plastic bags, stored on ice, and brought to the lab for analyses. Several synchronously flowering branches of each genotype were labeled for sample collection at later stages of development. Since, season and cultural conditions are known to affect fruit characteristics such as berry size, weight of single berries, seed size, color of ripe berries and ripening period (Smart et el., 1990; Uhlig and Clingeleffer, 1998), these data were recorded to determine divergence in leaf and fruit characteristics among the genotypes during the study period.

Developmental Stage and Sample Collection. The growth curve of the grape berry is double-sigmoidal, like apples and peaches. The grape berry, however rapidly softens at the beginning of the second growth cycle, many weeks before ripeness. The onset of this rapid softening is quite distinct and is termed 'veraison' by viticulturist. After veraison, sugar accumulation, anthocyanin synthesis, reduction of acidity and the second growth cycle commence (Coombe, 1973; 1992; and Yakushiji et al., 2001). In this study, the growth periods of vines between bud-break and fruit ripening were divided into six categories based on vegetative, reproductive and fruiting status of the vine. These categories included: Before fruit-set: 1 = pre-flowering, 2 = flowering; after fruit-set but before 'veraison': 3 = young fruit, 4 = medium-mature fruit, 5 = fully developed/mature fruit and; after veraison: 6 = ripe fruit (equivalent to mature berries described by Yakushiji et al., 2001). Pre-flowering stage (stage 1) was considered 12-14 days after bud-break. Fifty percent bloom was considered to be flowering (stage 2). For sampling during fruiting, synchronously flowering bunches were tagged and their development was followed up to fruit ripening. Young fruit (stage 3) were considered to be when berries attained a size of 3 to 4 mm in diameter. Medium fruit (stage 4) were considered to be when berries were about 5 to 7 mm in diameter for small fruits (<4 g), and 6 to 9 mm diameter for medium to large fruits (4 to 10 g) as listed in Table 1. The fruit that attained full size and were still firm and solid to touch with green skin were considered as fully-developed/mature fruit (stage 5), which would be equivalent to the 'before veraison' stage of growth pattern described earlier (Coombe, 1973 and 1992; Yakushiji et al., 2001). The red genotypes were very easy to distinguish as mature fruit since in these genotypes the anthocyanin pigments start appearing in the berry skin, which was a clear sign of fully-developed/mature berries, and the beginning of the 'veraison' stage. When berries became soft and skin color changed completely to light green, yellowish green, red, brown, bronze or black (depending upon the genotype) they were considered to be ripe fruit (stage 6) and equivalent to the mature berry stage (Yakushiji et al., 2001). The fruit characteristics described in the Table 1 serve as a guide on variation in berry characteristics observed during the experimental period.

In the case of leaves, the oldest (fully expanded) leaf following bud-break represented the pre-flowering stage (stage 1). Fully expanded leaves at flowering, young fruit, medium-mature fruit, fully-developed/mature fruit, and ripe fruit stages were collected and represented stages 2, 3, 4, 5 and 6, respectively. Berries and leaves (approximately 5 g) from synchronous branches of three different vines were collected separately and used as replicates for the analyses.

Field collected samples were washed with 0.5% malic acid to remove the agricultural chemicals, rinsed with tap water to remove the malic acid, rinsed at least four times with deionized water, and allowed to dry on tissue paper. The berries were cut into two or four pieces, seeds were removed and pulp with skin was used for extraction of total sugars.

Analysis of Soluble Sugar. The fresh leaf and/or berry (pulp with skin without seeds) samples were used for sugar extraction. Fresh tissue (0.5 g) was weighed and homogenized using a Polytron homogenizer (Brinkman Instruments, NJ) in 5 mL of 80% ethanol and the supernatant was collected after centrifugation at 20000g for 10 min. The resulting pellet was re-extracted with another 5 mL of 80% ethanol, centrifuged and the supernatants were combined. The combined extract was centrifuged for an additional 15 min at 20000g to remove any insoluble material. Soluble sugar concentrations of the ethanol-extracts were determined following the anthrone-sulfuric acid method (Yemm and Wills, 1954). One-hundred micro liters of ethanol-extract were transferred into a 30 mL glass test tube and 2 mL solution of Anthrone-sulfuric acid was added. The mixture was incubated in a boiling water bath for 10 min., cooled immediately and the absorbance at 600 nm was determined using a Spectrophotometer (Spectronic, Model: Genosys 5). Glucose solutions (0.01 mg to 0.1 mg) in 80% ethanol were used as standards to determine the sugar concentrations of leaf and berry tissues. The samples were analyzed in replicates of three or more, and the values were expressed as g of sugars per 100 g of fresh tissue (or as a percentage of tissue fresh weight).

Table 1. List of grape genotypes, and their fruit characteristics (Average for 1999 and 2000 crop season).

Genotype	Ripening period[z]	Berry size[y]	Weight of single berry[x] (g)	Size of seeds[w]	Color of ripe berries
BUNCH GENOTYPES					
1. Blanc du Bois	Early	Small	2.78 ± 0.50	Medium	Light green
2. Blue Lake	Early	Small	1.40 ± 0.07	Medium	Radish black
3. Black Spanish	Early	Small	2.92 ± 0.37	Small	Brownish black
4. Herbemont	Early	Small	1.01 ± 0.08	Medium	Light green
5. Lake Emerald	Mid	Small	2.60 ± 0.62	Small	Wine red
6. M4-83	Early	Small	2.88 ± 0.16	Medium	Wine red
7. M6-7E	Early	Small	1.26 ± 0.11	Medium	Radish black
8. Midsouth	Early	Small	2.46 ± 0.19	Large	Radish black
9. Orlando Seedless	Early	Small	0.97 ± 0.12	Seedless	Yellowish green
10. Stover	Early	Small	2.20 ± 0.29	Large	Light green
11. Suwannee	Early	Small	2.43 ± 0.66	Medium	Light green
12. Tampa	Early	Small	0.91 ± 0.14	Medium	Greenish brown
MUSCADINE GENOTYPES					
1. African Queen	Mid	Medium	4.54 ± 1.05	Medium	Radish black
2. Alachua	Mid	Small	3.34 ± 1.24	Medium	Radish black
3. Albermarle	Mid/Late	Medium	5.85 ± 0.42	Large	Black
4. Black Fry	Late	Extra large	3.74 ± 1.95	Large	Black
5. Carlos	Mid/Late	Medium	6.16 ± 0.45	Large	Bronze
6. CD8-81	Late	Large	7.36 ± 0.91	Medium	Radish black
7. Chowan	Late	Medium	5.59 ± 1.09	Medium	Black
8. Cowart	Mid	Medium	5.15 ± 0.53	Large	Dark purple
9. DB3-63	Late	Extra large	11.21 ± 0.53	Large	Wine red
10. Darlene	Mid	Extra large	10.37 ± 1.86	Large	Bronze
11. Digby	Mid/Late	Large	8.63 ± 1.11	Small	Bronze
12. Dixie	Late	Medium	6.00 ± 0.72	Large	Bronze
13. Dixie Land	Late	Extra large	10.13 ± 1.49	Large	Bronze
14. Dixie Red	Mid	Large	7.44 ± 1.11	Large	Radish brown
15. Doreen	Late	Medium	5.92 ± 1.24	Large	Bronze
16. Farrer	Late	Extra large	11.01 ± 2.36	Large	Wine red
17. Fry	Late	Large	8.95 ± 1.64	Large	Brownish green
18. GA-23-45	Late	Medium	4.36 ± 0.27	Medium	Light green
19. GA3-3	Mid/Late	Extra Large	11.72 ± 1.83	Large	Black
20. GA33-3-2	Mid	Medium	6.45 ± 1.37	Large	Light green
21. GA3-9-2	Mid	Small	3.87 ± 0.37	Medium	Black
22. Higgins	Late	Large	7.42 ± 1.08	Large	Radish brown
23. Ison	Mid	Medium	5.70 ± 1.08	Large	Blackish purple
24. Janet	Mid	Medium	4.61 ± 0.73	Medium	Light brown
25. Jane Bell	Late	Large	8.13 ± 0.83	Large	Greenish brown
26. Jumbo	Early/Mid	Large	9.34 ± 1.68	Large	Dark purple
27. Marsh	Late	Small	3.78 ± 0.21	Large	Wine red
28. Noble	Mid	Small	3.82 ± 0.31	Medium	Black
29. Pam	Mid	Large	9.25 ± 0.92	Large	Bronze
30. Pink Hunt	Mid	Medium	5.04 ± 0.40	Small	Wine red
31. Regale	Mid/Late	Small	3.79 ± 0.33	Medium	Radish black
32. Rosa	Mid	Large	7.80 ± 0.25	Large	Radish green
33. Scuppernong	Late	Medium	6.34 ± 0.77	Large	Greenish brown
34. Senoia	Late	Large	7.12 ± 0.15	Large	Pinkish brown
35. Southers	Mid	Large	7.09 ± 0.56	Small	Wine red
36. Southland	Mid/Late	Medium	4.26 ± 0.59	Large	Radish black
37. Summit	Mid/ Late	Large	8.55 ± 1.02	Large	Greenish brown
38. Sugar Pop	Late	Extra large	10.35 ± 1.56	Large	Brownish green
39. Sweet Jenny	Mid	Large	8.17 ± 1.95	Large	Greenish brown
40. Tarheel	Late	Small	2.49 ± 0.26	Medium	Black
41. Triumph	Early	Small	2.98 ± 0.44	Small	Light brown
42. Welder	Late	Medium	6.85 ± 0.75	Large	Bronze

[z]Number of days to ripe more than 50% bunch after pruning (Early: 75 to 90 days; mid: 90-110 days; late above 110 days).
[x]Berry size classified based on berry appearance and weight (small less than 4 g; medium 4-7 g; large 7-10 g; extra large above 10 g)
[y]Average of 10 ripe berries (with seeds) ± standard error
[w]Seed size classified based on length (l) and width (w) [small less than 3 mm l & w; medium: 3-6 mm (l) 3-4 mm (w); large: above 6 mm (l), 4 mm (w)

Statistical analysis. Mean sugar concentrations for leaf or berry at different developmental stages for each cultivar were calculated for each year. Two-year pooled data were analyzed via the ANOVA procedure using SAS (version 8.2). Mean separations and testing for significant differences was done using Duncan's Multiple Range Test to determine significance in the observed sugar levels among the genotypes. For each genotype, the mean sugar concentrations over the developmental stages of leaves and berries were calculated. RANK procedure in SAS was used to assign the cultivars to seven groups based on the mean sugar content. In addition, the group differences were tested using ANOVA and the Duncan's grouping.

Results and Discussion

Because muscadine and bunch grape genotypes differ widely in their fruit characteristics, data were collected on berry size, weight of single berries, seed size, berry color and ripening period during both years to determine divergence in leaf and fruit characteristics among the genotypes during the study period (Table 1). As seen in the table, the mature berries of bunch genotypes were relatively small and weighed between 0.91 g ('Tampa') to 2.92 g ('Black Spanish'), while the muscadine berry size varied from 2.49 g ('Tarhee') to 11.72 g, ('GA3-3'). In addition, most of the bunch grapes matured early while the muscadine grapes matured later (Table 1). The color of the berries and size of the seed also varied widely in both the bunch and muscadine cultivars.

The leaf and berry sugar concentrations of these genotypes at different developmental stages were consistent for both seasons. Hence, data from both years were pooled and averaged to study the sugar accumulation pattern in leaves and berries.

Leaf Sugar Concentration

Pre-flowering. Soluble sugar concentration of the leaf tissue varied significantly ($P < 0.05$) at different developmental stages (Table 2) in both the bunch and muscadine genotypes. During pre-flowering (stage 1), the leaf sugar concentrations of the muscadine genotypes varied between 1.94% (w/v) to 8.30%, while in bunch grape they were between 2.97% to 6.39%. In bunch grape the highest leaf sugar concentration was found in 'Stover' (6.39%) and the lowest (2.97%) in 'Herbemont'. In muscadine grape, the highest leaf sugar concentration was recorded for the genotype 'DB3-63' (8.30%) and the lowest for 'Pam' (1.94%). Comparison of bunch and muscadine genotypes showed that leaf sugar concentrations of bunch as well as muscadine genotypes varied widely. However, the bunch grape genotypes appeared to contain generally higher levels of leaf sugars than the muscadine genotypes.

Flowering. At the flowering stage, the majority (47 out of 54) of the genotypes (bunch as well as muscadine) showed decreased levels of leaf sugars compared to their pre-flowering sugar levels. Nineteen genotypes showed more than a 50% decrease in their leaf sugar concentration. Maximum reduction occurred in 'Scuppernong' [5.04 $g \cdot 100g^{-1}$ fresh leaf to 0.36 $g \cdot 100g^{-1}$ fresh leaf (a 93% decrease)] followed by 'Southers' (an 88% decrease) and 'Midsouth' (an 84% decrease). Twenty genotypes showed a reduction in the range of 25% to 50% for their leaf sugar concentrations between pre-flowering to flowering stages in both seasons. Seven genotypes viz. 'Cowart', 'Digby', 'CD8-81', 'Dixie', 'Lake Emerald', and 'Noble' showed reduction in leaf sugar concentration below 15%. 'Ison', 'Herbemont' and 'African Queen' maintained their leaf sugar levels at flowering, as the leaf sugar concentration was not significantly different from their pre-flowering levels. Seven genotypes showed increased sugar levels at flowering in both years. The highest increase (47%) in leaf sugar was recorded for 'Senoia', and least (4%) for 'Higgins'. Reduction in leaf sugar concentration in most of the grape genotypes at flowering stage might be due to utilization of sugars to induce flowering (Coombe, 1992). Translocation of sugars and other metabolites may be critical for inducing uniform flowering. Further comparative understanding of physiological parameters and morphological traits such as flower type (female or male or perfect), number of inflorescence per plant, number of flowers per inflorescence may reveal information on uniform or irregular flowering and/or berry ripening characteristics of grapevines.

Post-flowering. The leaf sugar concentration of the bunch grape genotypes varied between 1.89% ('Midsouth') and 4.23% ('Orlando Seedless') at the young fruit set stage (Stage 3) while in the muscadine genotypes it ranged between 2.56% ('Marsh') and 4.10% ('Triumph'). Most of the genotypes showed increased leaf sugar concentrations at the young fruit stage compared to flowering (stage 2). Exceptions were 'GA3-9-2', 'Digby', 'Higgins', 'Cowart', 'Noble', 'Blue Lake', 'Tampa', 'Southland', 'Sugar Pop', 'Senoia', 'Lake Emerald', and 'Black Spanish'. The leaf sugar concentrations for medium fruit (stage 4) ranged from 2.77% ('Suwannee') to 4.66% ('Black Spanish') in bunch grape and from 2.25% ('Tarhee') to 6.05% ('Jumbo') in muscadine. Accumulation of leaf sugars continued in most of the grape genotypes until they reached the fully developed/mature berry stage (stage 5). For mature fruit (stage 5), the muscadine genotype 'Doreen' (7.79%) contained the highest leaf sugar level followed by 'Sweet Jenny' (6.89%) and 'Southland' (6.37%) while 'Pink Hunt' (2.47%), 'Tarhee' (2.42%) and 'GA-23-45' (2.39%) showed low sugar levels. For bunch grapes, 'M4-83' accumulated the highest amount (6.11%) of sugars while 'Blue Lake' contained the lowest amount (3.02%). For ripe fruit stage (stage 6), 'Darlene' (7.09%) and 'Sweet Jenny' (6.89%) contained highest level of sugars while 'GA3-3' had the lowest (1.67%). Most of the genotypes showed a decrease in their accumulated sugar levels between the mature fruit stage (stage 5) and the ripe fruit stage (stage 6). However, 'GA3-9-2', 'Tarhee', 'CD8-81', 'Noble', 'Regale', 'Darlene', 'Summit', 'Jane Bell', 'Triumph' and 'Blanc du Bois' showed increased leaf sugar concentrations between mature and ripe fruit stages. Differential accumulation of sugars in leaves during young and medium fruit stages indicates that different genotypes have varied genetic potential to accumulate sugar source for translocation to berries before and after 'veraison' (Coombe, 1992).

Berry Sugar Concentration

Variations in berry sugar levels were highly significant ($P < 0.05$) for genotypes at all developmental stages (Table 3). In bunch grapes, the berry sugar concentration for young fruit (stage 3) varied from 0.61% to 1.59% while in the muscadine grapes it was between 0.64% and 2.25%. The highest sugar concentration was found in 'Albemarle' (muscadine) and the lowest in 'Midsouth' (bunch). At medium fruit stage (stage 4),

Table 2. Developmental Changes in the Leaf Soluble Sugar Concentration of Grape Genotypes (g·100g^{-1} fresh leaf, average of 1999 and 2000 seasons).

Genotype	Pre-flowering	Flowering	Young	Medium	Mature	Ripe
BUNCH						
Blanc du Bois	5.09 ^{e}	2.28 ^{i}	2.17 ^{k}	3.12 ^{ij}	4.06 ^{ef}	**5.03** ^{cd}
Blue Lake	5.88 ^{c}	3.36 ^{f}	2.47 ^{j}	2.79 ^{kl}	3.02 ^{fg}	1.96 ^{i}
Black Spanish	5.03 ^{e}	2.64 ^{h}	2.14 ^{k}	**4.66** ^{d}	3.64 ^{ef}	2.66 ^{hi}
Herbemont	2.97 ^{hi}	2.98 ^{g}	2.45 ^{j}	4.43 ^{d}	4.15 ^{ef}	2.87 ^{hi}
Lake Emerald	3.75 ^{gh}	3.39 ^{f}	1.97 ^{l}	3.80 ^{fg}	4.32 ^{de}	4.18 ^{ef}
M4-83	3.09 ^{h}	2.20 ^{i}	2.37 ^{k}	3.23 ^{ij}	6.11 ^{b}	3.98 ^{fg}
M6-7E	3.07 ^{h}	1.88 ^{j}	3.56 ^{ef}	3.72 ^{gh}	4.03 ^{ef}	2.33 ^{hi}
Midsouth	4.97 ^{e}	0.79 ^{m}	1.89 ^{l}	3.68 ^{h}	3.94 ^{ef}	2.03 ^{i}
Orlando Seedless	5.61 ^{c}	3.74 ^{d}	**4.23** ^{a}	3.92 ^{fg}	4.22 ^{de}	3.72 ^{fg}
Stover	**6.39** ^{b}	**3.91** ^{cd}	3.87 ^{c}	3.74 ^{gh}	**5.53** ^{cd}	2.11 ^{i}
Suwannee	3.85 ^{gh}	1.98 ^{j}	2.27 ^{k}	2.77 ^{kl}	2.74 ^{fg}	4.62 ^{cd}
Tampa	5.48 ^{de}	3.20 ^{fg}	2.25 ^{k}	2.91 ^{kl}	3.97 ^{ef}	2.14 ^{i}
MUSCADINE						
African Queen	2.91 ^{hf}	2.89 ^{h}	3.80 ^{c}	2.71 ^{lm}	6.32 ^{b}	3.92 ^{fg}
Alachua	3.91 ^{g}	1.87 ^{j}	2.88 ^{h}	2.57 ^{lm}	4.04 ^{ef}	5.01 ^{cd}
Albermarle	5.21 ^{de}	3.32 ^{fg}	3.64 ^{de}	3.82 ^{fg}	6.14 ^{b}	4.18 ^{ef}
Black Fry	3.08 ^{h}	3.24 ^{fg}	3.70 ^{de}	5.36 ^{b}	3.84 ^{ef}	3.44 ^{fg}
Carlos	4.99 ^{e}	1.81 ^{jk}	2.82 ^{h}	3.13 ^{ij}	4.06 ^{ef}	2.63 ^{hi}
CD8-81	2.44 ^{ij}	2.15 ^{i}	3.21 ^{g}	3.53 ^{h}	3.43 ^{fg}	4.69 ^{de}
Chowan	3.05 ^{h}	1.87 ^{j}	2.84 ^{h}	3.82 ^{fg}	6.03 ^{bc}	4.80 ^{de}
Cowart	3.90 ^{g}	3.32 ^{fg}	2.64 ^{hi}	3.41 ^{h}	4.98 ^{de}	4.60 ^{de}
Darlene	2.08 ^{k}	1.18 ^{l}	3.60 ^{de}	3.54 ^{h}	4.18 ^{ef}	**7.09** ^{a}
DB3-63	**8.30** ^{a}	1.98 ^{j}	2.92 ^{h}	5.24 ^{c}	4.10 ^{ef}	2.34 ^{hi}
Digby	5.17 ^{de}	**4.52** ^{a}	2.55 ^{ij}	3.82 ^{fg}	3.75 ^{de}	3.32 ^{fg}
Dixie	3.25 ^{gh}	2.89 ^{h}	4.08 ^{b}	5.47 ^{b}	5.13 ^{cd}	3.34 ^{fg}
Dixie Land	2.70 ^{i}	1.06 ^{l}	3.78 ^{c}	3.07 ^{jk}	4.03 ^{ef}	3.06 ^{gh}
Dixie Red	3.09 ^{h}	3.42 ^{ef}	3.74 ^{de}	4.46 ^{d}	4.79 ^{de}	3.68 ^{fg}
Doreen	2.18 ^{k}	3.22 ^{h}	3.90 ^{c}	2.72 ^{lm}	**7.79** ^{a}	2.52 ^{hi}
Farrer	2.96 ^{hi}	2.03 ^{ij}	3.51 ^{ef}	2.34 ^{mn}	4.43 ^{de}	3.15 ^{gh}
Fry	4.57 ^{f}	3.36 ^{f}	3.62 ^{de}	3.93 ^{fg}	4.17 ^{ef}	3.26 ^{gh}
GA-23-45	4.79 ^{ef}	2.05 ^{i}	2.77 ^{h}	2.92 ^{jk}	2.39 ^{gh}	2.52 ^{hi}
GA3-3	4.00 ^{g}	1.08 ^{l}	2.75 ^{hi}	4.08 ^{e}	3.31 ^{fg}	1.67 ^{i}
GA33-3-2	4.40 ^{f}	2.86 ^{h}	3.14 ^{g}	4.18 ^{e}	4.17 ^{ef}	4.55 ^{de}
GA3-9-2	5.27 ^{de}	3.16 ^{g}	3.48 ^{fg}	2.37 ^{mn}	3.49 ^{ef}	5.47 ^{bc}
Higgins	4.08 ^{fg}	4.25 ^{b}	3.35 ^{fg}	5.29 ^{b}	4.67 ^{de}	4.08 ^{gh}
Ison	3.36 ^{gh}	3.20 ^{fg}	3.42 ^{fg}	2.94 ^{jk}	5.15 ^{cd}	4.08 ^{ef}
Janet	3.32 ^{gh}	3.53 ^{de}	3.17 ^{g}	3.62 ^{h}	5.69 ^{cd}	5.47 ^{bc}
Jane Bell	2.48 ^{ij}	1.83 ^{jk}	2.21 ^{k}	3.04 ^{jk}	4.94 ^{de}	5.53 ^{bc}
Jumbo	4.10 ^{fg}	1.71 ^{jk}	2.63 ^{hi}	**6.05** ^{a}	5.10 ^{cd}	3.20 ^{gh}
Marsh	4.05 ^{fg}	1.87 ^{j}	2.56 ^{ij}	2.79 ^{kl}	2.71 ^{fg}	2.01 ^{i}
Noble	4.06 ^{fg}	3.70 ^{d}	2.79 ^{h}	3.30 ^{h}	4.35 ^{de}	5.72 ^{bc}
Pam	1.94 ^{k}	0.63 ^{m}	3.53 ^{ef}	2.46 ^{mn}	3.72 ^{ef}	4.54 ^{de}
Pink Hunt	5.67 ^{c}	2.04 ^{ij}	3.22 ^{g}	2.47 ^{mn}	2.47 ^{gh}	2.14 ^{i}
Regale	3.56 ^{gh}	2.11 ^{i}	3.15 ^{g}	3.86 ^{mn}	3.98 ^{ef}	5.18 ^{cd}
Rosa	2.75 ^{i}	0.62 ^{m}	3.96 ^{b}	3.99 ^{e}	4.54 ^{de}	4.72 ^{de}
Scuppernong	5.04 ^{e}	0.36 ^{m}	3.49 ^{ef}	4.43 ^{d}	5.76 ^{bc}	4.61 ^{de}
Senoia	2.77 ^{i}	4.06 ^{b}	3.44 ^{fg}	4.15 ^{e}	5.43 ^{cd}	4.68 ^{de}
Southers	4.34 ^{f}	0.52 ^{m}	2.05 ^{l}	3.21 ^{ij}	4.26 ^{de}	2.43 ^{hi}
Southland	2.86 ^{i}	3.37 ^{f}	2.62 ^{ij}	5.15 ^{c}	6.37 ^{b}	6.00 ^{b}
Summit	5.04 ^{e}	2.17 ^{i}	3.52 ^{ef}	4.45 ^{d}	3.85 ^{ef}	4.21 ^{ef}
Sugar Pop	4.74 ^{ef}	3.31 ^{fg}	2.19 ^{k}	2.33 ^{mn}	4.34 ^{de}	3.53 ^{fg}
Sweet Jenny	3.04 ^{h}	1.84 ^{j}	2.60 ^{ij}	3.98 ^{fg}	6.23 ^{b}	6.89 ^{a}
Tarheel	5.73 ^{c}	1.99 ^{j}	3.37 ^{fg}	2.25 ^{mn}	2.42 ^{gh}	4.61 ^{de}
Triumph	4.37 ^{f}	2.05 ^{i}	**4.10** ^{b}	2.80 ^{kl}	4.26 ^{de}	5.99 ^{b}
Welder	4.11 ^{fg}	3.07 ^{g}	3.65 ^{de}	3.73 ^{gh}	6.03 ^{bc}	4.18 ^{ef}

Mean separation (in columns) by Duncan's multiple range test, 5% level, Means with the same letter are not significantly different.
Numbers in bold indicate genotype showing highest sugar levels and numbers underlined indicate genotypes showing lowest sugar levels at a particular developmental stage.

the berry sugar level varied from 0.38% ('Tampa') to 2.98% ('M4-83') in bunch and 0.53% ('Tarhee') and 3.18% ('Black Fry') in muscadine grape. In general the berry sugar concentrations in most of the genotypes either remained the same or decreased between young and medium berry development stages. Low or reduced sugar levels in soluble sugars during

Table 3. Developmental changes in the fruit soluble sugar concentration of grape genotypes (g·100g^{-1} fresh fruit, average of 1999 and 2000 season)

Genotype	Young	Medium	Mature	Ripe
BUNCH				
Blanc du Bois	**1.12** ^{ef}	0.86 ^{I}	8.38 ^{d}	9.08 ^{i}
Blue Lake	0.67 ^{h}	0.50 ^{j}	1.43 ^{k}	8.84^{i}
Black Spanish	0.82 ^{gh}	1.27 ^{fg}	2.64 ^{I}	5.55 ^{k}
Herbemont	1.00 ^{fg}	**1.56** ^{de}	2.00 ^{j}	4.46 ^{k}
Lake Emerald	0.76 ^{gh}	0.72 ^{I}	8.36 ^{d}	9.89 ^{i}
M4-83	0.73 ^{gh}	2.98 ^{b}	1.11 ^{k}	7.40 ^{j}
M6-7E	0.70 ^{gh}	0.81 ^{i}	2.19 ^{j}	5.65 ^{k}
Midsouth	0.61 ^{h}	0.48 ^{J}	3.38 ^{h}	5.60 ^{k}
Orlando Seedless	1.59 ^{b}	0.86 ^{i}	4.68 ^{g}	13.80 ^{d}
Stover	0.77 ^{gh}	0.78 ^{i}	1.77 ^{j}	5.63 ^{k}
Suwannee	1.04 ^{ef}	0.42 ^{j}	**10.13** ^{b}	**14.15** ^{d}
Tampa	0.67 ^{h}	0.38 ^{j}	1.67 ^{k}	5.56 ^{k}
MUSCADINE				
African Queen	1.08 ^{fg}	1.32 ^{ef}	11.35 ^{a}	12.83 ^{e}
Alachua	0.81 ^{gh}	1.29 ^{fg}	9.49 ^{c}	11.86 ^{fg}
Albermarle	**2.25** ^{a}	1.18 ^{fg}	5.86 ^{f}	11.59 ^{gh}
Black Fry	1.33 ^{de}	**3.18** ^{a}	4.04 ^{g}	7.10 ^{j}
Carlos	1.17 ^{ef}	0.99 ^{hi}	6.73 ^{e}	10.39 ^{h}
CD8-81	1.30 ^{ef}	0.89 ^{hi}	1.77 ^{j}	8.57 ^{i}
Chowan	1.25 ^{ef}	1.12 ^{gh}	7.05 ^{e}	8.54 ^{i}
Cowart	1.09 ^{fg}	1.06 ^{gh}	10.00 ^{b}	13.08 ^{e}
DB3-63	1.44 ^{cd}	0.92 ^{hi}	7.68 ^{e}	8.20 ^{i}
Darlene	1.84 ^{b}	1.26 ^{fg}	3.32 ^{h}	15.59 ^{b}
Digby	0.76 ^{gh}	1.22 ^{fg}	8.53 ^{e}	10.74 ^{h}
Dixie	1.79 ^{b}	1.75 ^{cd}	7.42 ^{d}	12.64 ^{e}
Dixie Land	1.75 ^{b}	1.53 ^{de}	7.26 ^{e}	11.83 ^{fg}
Dixie Red	1.48 ^{cd}	1.22 ^{fg}	3.28 ^{h}	12.74 ^{e}
Doreen	0.98 ^{fg}	1.31 ^{ef}	4.43 ^{g}	9.94 ^{i}
Farrer	1.37 ^{de}	1.29 ^{fg}	8.35 ^{d}	11.12 ^{h}
Fry	1.45 ^{cd}	1.84 ^{cd}	5.81 ^{f}	11.92 ^{fg}
GA-23-45	0.92 ^{fg}	0.60 ^{i}	1.31 ^{k}	7.02 ^{j}
GA3-3	1.09 ^{fg}	0.99 ^{hi}	3.96 ^{g}	8.50 ^{i}
GA33-3-2	1.01 ^{fg}	0.96 ^{hi}	8.33 ^{d}	11.30 ^{gh}
GA3-9-2	1.25 ^{ef}	0.99 ^{hi}	4.05 ^{g}	13.65 ^{d}
Higgins	1.24 ^{ef}	1.01 ^{gh}	8.66 ^{d}	12.42 ^{e}
Ison	1.12 ^{ef}	1.07 ^{gh}	9.76 ^{c}	10.89 ^{h}
Janet	1.19 ^{ef}	1.19 ^{fg}	8.40 ^{d}	11.23 ^{gh}
Jane Bell	1.27 ^{de}	1.84 ^{cd}	3.00 ^{h}	8.54 ^{i}
Jumbo	1.42 ^{c}	1.11 ^{gh}	7.71 ^{e}	10.80 ^{h}
Marsh	1.25 ^{ef}	1.03^{gh}	1.52 ^{k}	6.78 ^{j}
Noble	1.06 ^{fg}	0.99 ^{hi}	8.60 ^{d}	11.96 ^{fg}
Pam	1.74 ^{b}	1.47 ^{ef}	3.50 ^{h}	13.71 ^{d}
Pink Hunt	1.69 ^{b}	1.13 ^{gh}	3.41 ^{h}	9.89 ^{i}
Regale	1.29 ^{de}	1.25 ^{fg}	6.20 ^{f}	9.45 ^{I}
Rosa	1.31 ^{de}	1.61 ^{de}	**11.37** ^{a}	14.66 ^{C}
Scuppernong	1.23 ^{ef}	0.98 ^{hi}	5.69 ^{f}	10.61 ^{h}
Senoia	1.36 ^{de}	1.68 ^{cd}	5.33 ^{f}	8.54 ^{i}
Southers	1.33 ^{de}	0.89 ^{hi}	5.19 ^{f}	7.06 ^{j}
Southland	1.11 ^{ef}	1.39 ^{ef}	5.97 ^{f}	10.52 ^{h}
Summit	1.17 ^{ef}	1.47 ^{ef}	7.91 ^{e}	13.14 ^{e}
Sugar Pop	0.94 ^{fg}	1.31 ^{ef}	2.63 ^{I}	6.40 ^{j}
Sweet Jenny	1.27 ^{d}	1.64 ^{de}	10.34 ^{b}	**16.08** ^{a}
Tarheel	0.90 ^{fg}	0.53 ^{j}	4.25 ^{g}	8.54 ^{i}
Triumph	1.32 ^{de}	1.85 ^{c}	3.91 ^{g}	11.47 ^{gh}
Welder	0.64 ^{h}	0.92 ^{hi}	7.42 ^{e}	12.55 ^{e}

Mean separation (in columns) by Duncan's multiple range test, 5% level, Means with the same letter are not significantly different.
Numbers in bold indicate genotype showing highest sugar levels and numbers underlined indicate genotypes showing lowest sugar levels at a p.

young and medium (stages 3 and 4) stages might be due to accumulation of acids during this period (stage 3), which is the characteristic of the double-sigmoidal curve of berry development or rapid fruit growth resulting in sugar dilution. Maximum decrease in berry sugar concentration was recorded in 'Suwannee' (60%). However, 'African Queen', 'Alachua',

'Black Fry', 'Black Spanish', 'Digby', 'Doreen', 'Fry', 'Herbemont', 'Jane Bell', 'M4-83', 'Rosa', 'Senoia', 'Southland', 'Summit', 'Sugar Pop', 'Sweet Jenny', 'Triumph', and 'Welder' showed increased berry sugar concentrations at medium fruit stage (stage 4) during the two year study. Accumulation of sugars in berries past the medium fruit stage was recorded for all the genotypes. At mature fruit stage (stage 5) the berry sugar concentration varied between 1.11% ('M4-83') and 8.38% ('Blanc du Bois') in bunch grape, and from 1.31% ('GA-23-45') to 11.37% ('Rosa') in muscadine grape. 'African Queen' (11.35%) showed equally high sugar concentrations as 'Rosa' at the mature fruit stage. The deposition pattern of sugars during medium to mature fruit stage differed among genotypes. 'Sweet Jenny' showed a maximum (24- fold increase) deposition of sugars between the medium and mature fruit stages. Deposition of sugars in berries continued until the ripe fruit stage was reached (stage 6). At ripe fruit stage, the highest berry sugar concentration was recorded for 'Sweet Jenny' (16.08%) followed by 'Darlene' with 15.59% and 'Rosa' with 14.66%. Interestingly all of these genotypes happened to be muscadine genotypes. Two-year average data indicated that the lowest sugar concentration at ripe fruit stage was recorded for 'Herbemont' (4.46%), a bunch genotype. Highest sugar accumulation between mature (stage 5) and ripe (stage 6) fruit stages was recorded for 'M4-83' (a 7-fold increase) and 'Blue Lake' (a 6- fold increase). However, the final fruit sugar concentrations of these genotypes were only 7.4 and 8.83 g/100 g FW, respectively.

Relationship in the Accumulation Pattern of Sugars in Grape Leaf and Berries

The leaf and berry sugar data of individual genotypes at six different developmental stages (stage 1 to stage 6) were compared to determine developmental profiles and sugar accumulation patterns among the grape genotypes. The data showed major differences in the sugar accumulation patterns among grape genotypes indicating existence of wide genetic variation. The leaf sugar concentration was highest (with an average of 4.478%) at the fully developed/mature fruit stage (stage 5) followed by pre-flowering. Significantly higher leaf sugar levels at these stages suggest that the higher leaf sugar requirements at the fully developed/mature stage are required for changes after veraison (berry ripening) where accumulation of sugar in berries commence, and at pre-flowering stage for blooming (Coombe, 1973; 1992; and Yakushiji et al., 2001).

An analysis of variance was performed on the seven groups based on leaf and berry sugar concentration. For leaf sugar concentration, cultivars in Group 7 had significantly higher sugar content than any of the genotypes belonging to any of the other groups. For berry sugar concentration, genotypes in Group 7 had significantly higher sugar concentrations than any of the genotypes belonging to any of the other groups. Table 4 and Table 5 show the results of the Duncan's grouping test on the seven groups for leaf and berry.

Lack of distinct sugar accumulation pattern between the bunch and muscadine genotypes indicate that the bunch and muscadine genotypes do not posses unique sugar accumulation patterns but have similar developmental profiles. Thus, both bunch and muscadine genotypes are scattered among these groups.

High levels of sugar accumulation in berries of certain genotypes compared to others suggest that this may be due to the differences in the breakdown of apoplast and symplast compartmentalization (John and Dey, 1986; Lang and During, 1993). In developing grape leaves, the level of acid invertase has been reported to be similar (Davies and Robinson, 1996; Ruffner et al., 1990) and hence, variation in leaf sugar accumulation during leaf development appears to be due to changes in the specific activity of acid invertase during development (Takayanagi and Yokotsuka, 1997) or perhaps because the plasma membrane of the pericarp becomes leaky during ripening, resulting in the movement of phloem sap

Table 4. Grouping of grape genotypes based on leaf sugar concentration (same letter in Duncan grouping indicates means are not significantly different).

Leaf Group Number	Mean	Duncan Grouping	Genotypes fall in the group
7	4.26	a	Orlando Seedless, Stover, Albermarle, DB3-63, Higgins, Southland, Welder.
6	4.01	b	Dixie, GA33-3-2, Janet, Noble, Scuppernong, Senoia, Sweet Jenny Triumph
5	3.83	c	Black Fry, Cowart, Digby, Dixie Red, Fry, GA-3-9-2, Jumbo, Summit.
4	3.67	d	Blanc du Bois, Lake Emerald, African Queen, Chowan, Darlene, Doreen, Ison, Regale.
3	3.40	e	Black Spanish, M4-83, Tampa, Alachua, Jane Bell, Rosa, Sugar Pop, Tarhee.
2	3.16	f	Blue Lake, Herbemont, M6-7E, Suwannee, Carlos, CD-8-81, Farrer, Pink Hunt.
1	2.82	g	Midsouth, Dixie Land, GA23-45, GA3-3, Marsh, Pam, Southers,

Table 5. Grouping of Grape genotypes based on berry sugar concentration (Same letter in Duncan grouping indicates means are not significantly different).

Berry Group Number	Mean	Duncan Grouping	Genotypes fall in the group
7	6.54	a	Suwannee, African Queen, Cowart, Dixie, Rosa, Summit, Sweet Jenny.
6	5.65	b	Alachua, DB3-63, Dixie Land, Farrer, Higgins, Ison, Janet, Noble.
5	5.27	c	Orlando Seedless, Albermarle, Digby, Fry, GA33-3-2, Jumbo, Pam, Welder.
4	4.79	d	Blanc du Bois, Lake Emerald, Carlos, Dixie Red, GA-3-9-2, Scuppernong, Southland, Triumph.
3	4.20	e	Black Fry, Chowan, Darlene, Doreen, Jane Bell, Pink Hunt, Regale, Senoia.
2	3.16	f	Blue Lake, M4-83, CD-8-81, GA3-3, Marsh, Southers, Sugar Pop, Tarhee.
1	2.35	g	Black Spanish, Herbemont, M6-7E, Midsouth, Stover, Tampa, GA23-45.

into the berry due to differences in water potential between the source and the sink (Davies and Robinson, 1996; Lang and During, 1993).

This study shows that some of the genotypes with high leaf sugar concentrations didn't produce berries with high sugar levels. This may be due to differential levels of invertase associated with the cell wall as have been implicated in phloem unloading and source/sink regulation (Eschrich, 1980; Roitsch et al., 1995; Takayanagi and Yokotsuka, 1997) or it may be due to slower decreasing levels of berry acids (Kenellis et al., 1993; Kliewer, 1965). Further studies are required to analyze the quantity and activity of acid invertase and sucrose phosphate synthase levels at different developmental stages in the leaves and berries of contrasting genotypes within and between the groups. Also correlation of berry sweetness with leaf biomass (total leaf production), number of berry clusters per vine, number of berry per cluster and berry size may be helpful in understanding the genetics between these traits and the impact of leaf sugar content on berry sweetness (Coombe,1992; Yakushiji et al., 2001).

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