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Genetic Variation in Sucrose Metabolizing Enzymes among Six Muscadine Varieties

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Accumulation of sugars is an important process that occurs during grape berry development. Sugars are transported into the berry mainly in the form of sucrose and are broken down into glucose and fructose by the enzymes invertase and sucrose synthase. In this study we have determined changes in the soluble sugar content, and invertase and sucrose synthase enzyme activity levels during different stages (30, 60, 90 d, and ripe) of berry development among selected muscadine varieties. Soluble sugar content varied among muscadine genotypes, with the highest being in 'African Queen' and lowest in 'Carlos'. Expression of invertase enzyme was highest in 90-d-old berry as well as in ripened berry among the muscadine varieties tested. Sucrose synthase activity was highest in 90-d-old berries among 'Summit', 'Scarlet', and 'African Queen' cultivars. In contrast, 'Noble', 'Carlos', and 'Welder' contained the highest sucrose synthase activity in 60-d-old berries. These data suggest that in muscadine grape berry invertase and sucrose synthase activities are positively correlated with high berry sugar content.

Muscadine (*Muscadinia rotundifolia* Michx.) grapes are native to the southeastern United States, and are widely used to make wine and eaten as fresh fruit, but their wine quality is perceived to be inferior to the wines made from bunch grape. One of the reasons for their poor wine quality is believed to be their low berry sugar content. Sugar accumulation is an important event in berry ripening physiology of grapevine and is a vital characteristic essential for superior enological characteristics and product value. Sugars are synthesized in leaves as a result of photosynthesis and imported into the berry in the form of sucrose. Upon reaching the berry, sucrose is split into glucose and fructose by the enzymes invertase and sucrose synthase (Dantas et al., 2005; Patrick et al., 2001; Quick, 1996). Any limitations or restrictions in sugar transport and breakdown can adversely affect the sugar content and composition of grape berry as well as its quality. Despite the importance of natural sugars on determining berry quality, their synthesis, transport and fate within the berry are poorly understood in muscadine grape. Hence, to understand the sucrose metabolism in muscadine grape, the function of enzymes sucrose synthase and invertase should be known during berry development to understand the constraints involved with low sugar content of muscadine berry.

In this regard, we have determined changes in Brix, sugars and enzyme activities of six muscadine cultivars with varying sugar content during berry development and ripening. This information will provide a basis for identifying enzymes involved in carbohydrate metabolism in muscadine grape, which will help select molecular targets to enhance sugar content of muscadine grape berry.

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Materials and Methods

Chemicals and reagents. All reagents and samples used in this study were of analytical grade and used without further purification. Chemicals for preparation of buffers and reagents were purchased from Sigma (St. Louis, MO) unless otherwise stated.

Plant material. Muscadine (*Muscadinia rotundifolia*) grape berries were collected from the vineyard at the Center for Viticulture and Small Fruit Research, Florida A&M University. Twenty muscadine grape cultivars, 'African Queen', 'Alachua', 'Albermarle', 'Carlos', 'Cowart', 'Darlene', 'Dixie Red,' 'Doreen', 'Farrer', 'Fry', 'Higgins', 'Jumbo', 'Noble', 'Regale', 'Scuppernong', 'Southland', 'Summit', 'Scarlet', 'Sweet Jenny', and 'Welder', which differ in their sugar content, were used in the study. The selected cultivars included both the table and wine varieties. Berry samples were collected in plastic bags and brought to the lab on ice for analysis. The fresh berries were frozen in liquid nitrogen, ground to a powder and stored at −80 °C until use.

Determination of total soluble solids and sugars content in developing berry. Total soluble solids content (Brix) of the berries from twenty muscadine cultivars was determined using a refractometer (Alago U.S.A., Inc., Bellevue, WA) and measuring the refractive value. A minimum of three readings were obtained for each genotype and the values were averaged to derive an average Brix value for each cultivar. Soluble sugars were extracted from fresh tissue (1.0 g) by homogenization using a Polytron homogenizer (Brinkman Instruments, Delran, NJ) and 5 mL of 80% ethanol. The supernatant containing soluble sugars was collected after centrifugation at 20,000 g_n for 10 min. The resulting pellet was re-extracted with another 5 mL of 80% ethanol, centrifuged and the supernatants were combined. The combined extracts were centrifuged for an additional 15 min at 20,000 g_n to remove any insoluble material. Soluble sugars concentration was determined following the anthrone-sulfuric acid method (Yemn and Wills, 1954). Glucose (0.01 to 0.1 mg) in 80% ethanol was used as the standard to determine sugar concentration of the

extracts. The samples were analyzed in replicates and the values were expressed as gram of sugar per 100 g of fresh tissue (or as a percentage of tissue fresh weight).

Extraction of invertase and sucrose synthase enzymes from developing berries. After removing the seed, the berry tissue was homogenized with ice cold buffer (1:5; W/V) containing 50 mmol/L HEPES (pH 7.5), 10 mmol/L EDTA, 1 mmol/L dithiothreitol (DTT), 10% (v/v) glycerol and 0.2% (v/v) Triton X 100. The homogenate was centrifuged at 15,000 g_n for 15 min at 4 °C and the supernatant was dialyzed at 4 °C overnight against 0.1 M sodium phosphate buffer pH 7.5 with gentle stirring. The dialysate was clarified by centrifugation and used as the enzyme source. Total protein content was determined according to the Bradford method (Bradford, 1976) using BSA as the standard.

Invertase enzyme assay. Invertases (acid, neutral, and basic) are known to break down sucrose into almost equal amounts of glucose and fructose. Preliminary studies using acidic (pH 3.0), neutral (pH 7) and basic (pH 10) reaction pH showed peak invertase activity at acidic pH (data not shown). Hence, comparative analyses of invertases among muscadine cultivars were conducted at pH 3.5. The invertase assay mixture (1 mL) consisted of 100 mM citrate-phosphate buffer, pH 3.5, 100 mM sucrose, and 500 µL of dialyzed berry extract. The reaction mixture was incubated at 37 °C for 1 h. The reaction was terminated by immersing in boiling water bath for 5 min. One milliliter of Dintro salicylic acid mixture (DNS) was added and incubated in boiling water bath for 10 min. The optical density of the reaction was read at 560 nm.

Sucrose synthase assay. The sucrose synthase assay was measured with the reduction of NAD^+ and measured as the change in absorbance at 340 nm in the presence of excess UDP glucose dehydrogenase. Reaction mixtures contained in a volume of 1 mL, 20 µmol HEPES-KOH buffer (pH 7.5), 100 µmol sucrose, 2 µmol UDP, 1.5 mmol NAD, 25 µg UDP glucose dehydrogenase, and an appropriate volume of enzyme.

Results and Discussion

Soluble solids (Brix). Differences in soluble solids level among twenty muscadine cultivars was determined by measuring the Brix value of ripe berries. Brix value ranged between 14% to 19% with the highest Brix being that of 'African Queen' and lowest 'Dixie Red' (Table 1). In addition, total soluble sugars content also varied among muscadine cultivars. Among the cultivars studied, 'African Queen' had the highest amount of sugars followed by 'Welder', 'Scarlet', 'Summit', 'Noble', and 'Carlos'. Since the Brix value of most of the cultivar studied was similar, the total sugar content data for only six cultivars that are distinct are shown in Figure 1 to avoid duplication. As seen in Table 1, the Brix value ranged between 14 and 19 among the cultivars. Although sugar accumulation has been studied extensively in *Vitis vinifera* (Conde et al., 2007; Davies and Robinson, 1996; Deluc et al., 2007; Pan et al., 2009), limited studies exist on sugar metabolism in muscadine grape. Earlier studies have shown varying levels of sugars in leaf and berry tissue (Jain et al., 2002) among the muscadine genotypes, indicating possible differences in their sugar metabolizing enzymes.

Enzyme activity. Enzyme analysis revealed that invertase activity varied among muscadine cultivars during berry development. Peak invertase activity was observed between 90-day-old and ripened berries in all the cultivars tested. Among them, 'African Queen' displayed higher invertase activity followed by 'Welder', 'Summit', 'Scarlet', 'Noble', and 'Carlos'. Invertase activity of only six cultivars is included in Fig. 2. to avoid duplication, which demonstrate distinct enzymes profiles observed among the cultivars. Invertase activity was found to correlate positively with increasing berry sugar content. Deficiency in invertase activity has been shown to result in reduced glucose and fructose levels, and increased sucrose levels in the fruit (Chetelate et al., 1995; Stommel, 1992; Yelle et al., 1991). These data indicate existence of genetic differences in invertase activity among the muscadine cultivars.

In addition to invertase, sucrose synthase activity also varied among the muscadine cultivars studied during the course of berry development. Among them, 'African Queen' displayed the highest sucrose synthase activity followed by 'Summit', 'Noble', 'Welder', 'Scarlet', and 'Carlos' (Fig. 3). Again the figure includes data for only six cultivars, which reveal overall enzyme expression pattern among the cultivars. Maximum sucrose synthase activity was observed at 90 d of berry development in 'Summit', 'Scarlet', and 'African Queen', while in 'Noble, 'Carlos', and 'Welder' the highest sucrose synthase activity was found in 60-d-old berry (Fig. 3).

Table 1. pH and Brix of muscadine juice grown at the Center for Viticulture and Small Fruit Research, Florida A&M University, and collected at 120 d after anthesis.

Muscadine genotypes	pH	Brix
African Queen	3.4	19
Alachua	3.7	17.0
Albermarle	3.4	16.0
Carlos	2.9	15.0
Cowart	3.5	18.0
Darlene	3.4	17.0
Dixie Red	3.3	14.0
Doreen	3.4	18.0
Farrer	3.5	15.0
Fry	3.8	15.0
Higgins	3.3	16.0
Jumbo	3.5	18.0
Noble	2.8	17.0
Regale	2.9	16.0
Scuppernong	3.4	17.0
Southland	3.6	17.0
Summit	3.2	18.0
Scarlet	3.8	18.0
Sweet Jenny	3.6	16.0
Welder	3.4	18.5

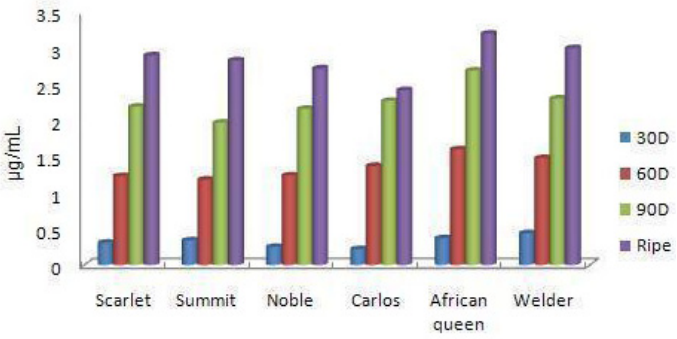

Fig 1. Total soluble sugar content among six muscadine cultivars.

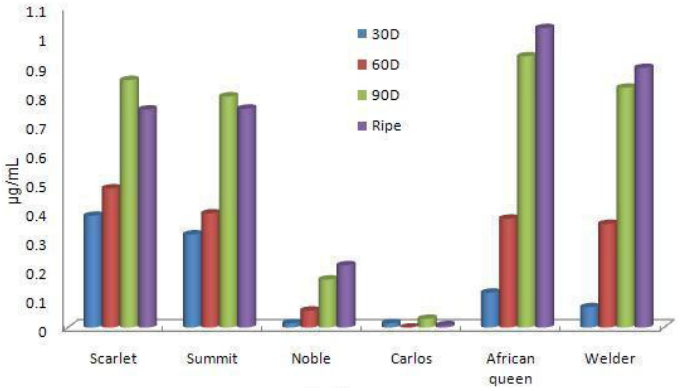

Fig. 2. Variation in invertase enzyme activity during the course of berry development among six muscadine cultivars.

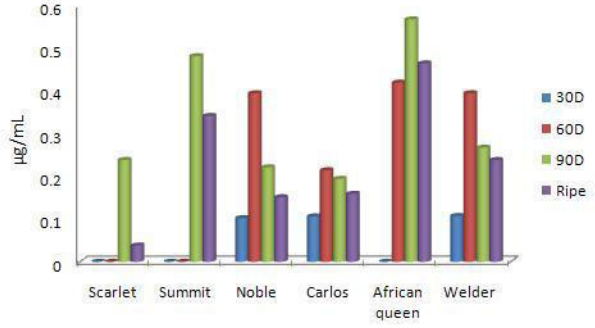

Fig. 3. Variation in sucrose synthase enzyme activity during the course of berry development among six muscadine cultivars.

Overall, the results showed that among the muscadine cultivars tested, 'African Queen' displayed the highest invertase and sucrose synthase activities while 'Carlos' showed the lowest invertase and sucrose synthase activities. Interestingly, in all the cultivars tested, sucrose synthase activity increased until berry maturation while invertase activity increased during maturation as well as ripening. These data suggest that muscadine cultivars that are sweeter contain higher levels of sucrose synthase and invertase activities than the less sweeter cultivars. 'Carlos', being low in both invertase and sucrose synthase activities, may be lower in hexose (glucose and fructose) content due to the lower breakdown of transported sucrose in the berries.

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