

## DEVELOPMENTAL CHANGES IN THE AMINO ACID CONTENT OF MUSCADINE GRAPE GENOTYPES

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**Abstract.** The present study was undertaken to determine developmental variation in the free amino acid content of leaves and berries of muscadine and Florida bunch grape genotypes. Leaf and berry samples were collected at six developmental stages from fifty-four grape genotypes with varying genetic background, and analyzed for total free amino acid content. Free amino acid content of the leaves varied from 0.7 mg·g<sup>-1</sup> to 13.7 mg·g<sup>-1</sup> fresh leaf at pre-flowering stage; 1.5 mg·g<sup>-1</sup> to 13.2 mg·g<sup>-1</sup> fresh leaf at flowering stage; 1.4 mg·g<sup>-1</sup> to 5.8 mg·g<sup>-1</sup> fresh leaf at young fruit stage; 1.5 mg·g<sup>-1</sup> to 7.5 mg·g<sup>-1</sup> fresh leaf at medium fruit stage; 1.5 mg·g<sup>-1</sup> to 10.4 mg·g<sup>-1</sup> fresh leaf at mature fruit stage; and 1.4 mg·g<sup>-1</sup> to 8.5 mg·g<sup>-1</sup> fresh leaf at ripe fruit stage. Accumulation of amino acids in berries varied from 0.8 mg·g<sup>-1</sup> to 4.5 mg·g<sup>-1</sup> fresh tissue at young fruit stage; 0.8 mg·g<sup>-1</sup> to 4.9 mg·g<sup>-1</sup> fresh tissue at medium fruit stage; 0.8 mg·g<sup>-1</sup> to 1.16 mg·g<sup>-1</sup> fresh tissue at mature fruit stage; and 1.2 mg·g<sup>-1</sup> to 17.0 mg·g<sup>-1</sup> fresh tissue at ripe fruit stage. The results showed wide variation in the leaf amino acid content among the genotypes and was highly significant ( $P > 0.05$ ) for different developmental stages and for the grape genotypes. The berry amino acid content was significantly different ( $P > 0.05$ ) between developmental stages but not among the genotypes. Based on the amino acid accumulation pattern in the leaves, the grape genotypes were categorized into six groups. Amino acid accumulation patterns at different developmental stages between these groups have been discussed. Determining the relationship between accumulation patterns of leaf amino acids with that of berry amino acids will help in understanding source and sink relationships, vine health, and juice and wine quality.

Nitrogen compounds have received much attention in the grape and wine industry, as they are certain to contribute to the process of fermentation (Bisson, 1991; Jiranek et al., 1991; and Kunkee, 1991). Of the nitrogen compounds found in grapes, free amino acids are reported to be present in the largest concentrations (Ough, 1992) and are of great importance in wine production (Lehtonen, 1996). Free amino acids act as a source of nitrogen for yeast during fermentation, are the primary source for yeast metabolism in addition to contributing to the flavor of wine (Drdak et al., 1993; Yokotsuka and Fukui, 2002). Accordingly, there have been a number of different reports on amino acids in grapes (Kliewer and Linder, 1970; Rapp, 1971; Winkler et al., 1974; Yokotsuka et al., 1975; 1993; Yokotsuka and Fukui, 2002).

Insufficient assimilable nitrogen (free amino acid and ammonium nitrogen) in grape musts is known to be a major

cause of stuck and sluggish fermentations during wine production (Henschke and Jiranek, 1983; Kunkee, 1991; Monteiro and Bisson, 1993). Laboratory studies on grape juices from *vinifera* genotypes conducted in Australia suggest a range of 328 to 467 mg·L<sup>-1</sup> of assimilable nitrogen is necessary for complete fermentation (Jiranek et al., 1995). Studies from California confirm these values (Monteiro and Bisson, 1993). Several factors which affect the demand of assimilable nitrogen include fermentation temperature, amount of air (oxygen) dissolved in the must, amount of utilizable amino acids present in the total amino acid pool in the must, the yeast strain being used for fermentation, competition from other microbes present and deficiency of some nutrients other than nitrogen (e.g. phosphate, Ough et al., 1991).

The accumulation of amino acids in leaf and their translocation into berries, the rate of amino acids imported into individual sink organs, and synthesis of amino acids in berries during berry development will have a major impact on juice and wine quality. Although the influence of amino acids on wine quality is well known, limited studies exist on the amino acid accumulation pattern of leaf and berry, and genetic variation in the amino acid accumulation pattern among the grape genotypes especially in muscadine and Florida bunch grape genotypes.

Because of the growing importance of the muscadine grape industry, and the influence of free amino acids on both vine health, and wine quality and flavor, the present study was undertaken to determine variation in the accumulation pattern of amino acids in the leaves and berries at different developmental stages, such as pre-flowering, flowering, and post-flowering (young, medium, mature and ripe fruit) stages of muscadine and Florida bunch grape genotypes.

### Materials and Methods

*Plant material.* Fifty-four grape genotypes that included 42 muscadine and 12 bunch germplasm accessions with varied origin and genetic diversity were used in this study (Tables 1 and 2). The leaf and fruit samples were collected from the vines grown at the Center for Viticulture and Small Fruit Research, Florida A&M University during 1999 and 2000 season. This is an established experimental vineyard located on the campus, with four vines of each cultivar planted randomly. The distance between rows is 15 ft and vine spacing within the row is 8 ft. The soil of the vineyard 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 temperature stress, tissue sampling was done between 8 AM. and 9 AM. To avoid location and shading effects, the plant tissues were collected randomly from 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, sealed in plastic bags, stored on ice, and brought

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to the laboratory for analyses. Several synchronously flowering branches of each genotype were labeled to collect samples at later developmental stages.

**Developmental stage and sample collection.** The growth period between bud-break to the fruit ripening stage was 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: 3 = young fruit, 4 = medium-mature fruit, 5 = fully developed/mature fruit and 6 = ripe fruit. Pre-flowering (stage 1) was considered to be 12-14 days after bud-break. Fifty percent bloom was considered to be the flowering (stage 2). For sampling during fruiting, synchronously flowering bunches were tagged and their development was followed up to fruit ripening. The young fruit stage (stage 3) was considered to be when berries attained a size of 3 to 4 mm in diameter, whereas the medium fruit stage (stage 4) was considered to be when berries were about 5 to 7 mm in diameter for small fruiting (< 4 g) varieties, and 6 to 9 mm diameter for medium to large fruiting varieties (4 to 10 g). The fruit that attained full size, firm and solid to touch, and with green skin were considered to be fully-developed/mature fruit (stage 5), which would be equivalent to the stage II of the growth pattern described earlier (Coombe, 1976). The red genotypes were very easy to distinguish at the mature fruit stage because with these genotypes anthocyanin pigments start appearing in the berry skin, a clear sign of the fully-developed/mature stage, and beginning of the ripening stage. When berries became soft and skin color changed to light green, yellowish green, red, brown, bronze or black (depending upon the genotype) they were considered to be ripe fruit (stage 6).

In the case of leaves, the oldest (fully expanded) leaf following bud-break represented the pre-flowering stage (stage 1). Fully expanded leaves were collected at the following reproductive stages: flowering, young fruit, medium-mature fruit, fully-developed/mature fruit, and ripe fruit, 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 during the analysis.

The samples were washed with 0.5% malic acid to remove the residual 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 then cut into two or four pieces, seeds were removed and pulp with skin was used for extraction of total free amino acids.

**Analysis of free amino acids.** Fresh leaf and/or berry (pulp with skin, no seeds) samples were used for total amino acids extraction. Fresh tissue (0.5 g) was weighed and homogenized using a Polytron homogenizer (Brinkman Instruments, N.J.) in 5 mL of 80% ethanol and the supernatant collected after centrifugation at 20000  $g_n$  for 10 min. The resulting pellet was re-extracted with another 5 mL of 80% ethanol, centrifuged and the supernatants were combined. The combined extract was centrifuged for an additional 15 min at 20000  $g_n$  to remove any insoluble material. Total free amino acid content of the ethanol-extracts was determined following the ninhydrin method (Yemm and Cocking, 1955). Two-hundred micro liters of ethanol-extract were transferred into a 30 mL glass test tube. One-hundred micro liters of ascorbic acid (0.25% solution) and 1 mL solution of ninhydrin (3% solu-

tion in piersolve) were added. The mixture was incubated in a boiling water bath for 10 min., cooled immediately, 3 mL of 80% ethyl alcohol were added and the absorbance at 570 nm was determined using a Spectrophotometer (Spectronic, Model Genosys 5). Leucine solutions (2.5  $\mu$ g to 50  $\mu$ g) in 80% ethanol were used as standards to determine the amino acid concentrations of leaf and berry tissues. The samples were analyzed in replicates of three or more, and the values were expressed as mg of total free amino acids per 1 g of fresh tissue.

**Statistical analysis.** The data were subjected to routine ANOVA procedures using SAS (version 8.2). Mean separation tests were done using Duncan's Multiple Range Test to determine significance differences in the observed amino acid levels among the genotypes at different developmental stages. Analysis of variance in SAS for two independent variables [developmental stage (pre-flowering to ripe) and tissue (leaf and berry)] was used and grape genotypes were grouped into six groups according to Duncan's method. The mean amino acid contents of the leaf and berry of each group over the developmental stages were plotted to represent the accumulation pattern among these groups.

## Results and Discussion

### Leaf Amino acid Content

**Pre-flowering.** Free amino acid content of leaf tissue varied significantly ( $P < 0.05$ ) at different developmental stages (Table 1) in both the bunch and muscadine genotypes. During pre-flowering (stage 1), the leaf amino acid content of the muscadine genotypes varied between 0.7  $mg \cdot g^{-1}$  and 13.7  $mg \cdot g^{-1}$  of fresh leaf, while in bunch grape it was between 0.8  $mg \cdot g^{-1}$  and 3.0  $mg \cdot g^{-1}$  fresh leaf. In bunch grape, the highest leaf amino acid content was found in 'Midsouth' and lowest in 'M4-83' while in muscadine grape the highest leaf amino acid content was recorded for the genotype 'GA3-3' and lowest for 'Farrer'.

**Flowering.** At flowering stage, the leaf amino acid content in muscadine genotypes varied between 1.8  $mg \cdot g^{-1}$  fresh leaf ('Southland') to 13.2  $mg \cdot g^{-1}$  fresh leaf ('CD8-81'). In Florida bunch grapes it varied between 1.5  $mg \cdot g^{-1}$  fresh leaf ('M4-83') to 4.7  $mg \cdot g^{-1}$  fresh leaf ('Lake Emerald').

**Post-flowering.** The leaf amino acid content of the bunch grape genotypes varied between 1.4  $mg \cdot g^{-1}$  fresh leaf ('Midsouth') and 3.1  $mg \cdot g^{-1}$  fresh leaf ('Stover') while in the muscadine genotypes it ranged between 2.4  $mg \cdot g^{-1}$  fresh leaf ('Scuppernong') and 5.8  $mg \cdot g^{-1}$  fresh leaf ('GA-23-45') at the young fruit stage (stage 3). The leaf amino acid content at the medium fruit stage (stage 4) ranged between 1.6  $mg \cdot g^{-1}$  fresh leaf ('M4-83') and 'Stover') and 3.5  $mg \cdot g^{-1}$  fresh leaf ('Lake Emerald') in bunch grape while it was between 1.5  $mg \cdot g^{-1}$  fresh leaf ('Black Fry') to 7.5  $mg \cdot g^{-1}$  fresh leaf ('Jumbo') in muscadine. At the mature fruit stage (stage 5), the leaf amino acid content in muscadine genotypes varied between 1.5  $mg \cdot g^{-1}$  fresh leaf ('GA-23-45') and 10.4  $mg \cdot g^{-1}$  fresh leaf ('Triumph') while in bunch grapes it was between 1.5  $mg \cdot g^{-1}$  fresh leaf ('M6-7E') and 6.1  $mg \cdot g^{-1}$  fresh leaf ('Lake Emerald'). For ripe fruit (stage 6), the leaf amino acid content in muscadine genotypes varied between 1.8  $mg \cdot g^{-1}$  fresh leaf ('GA-23-45') to 8.5  $mg \cdot g^{-1}$  fresh leaf ('Dixie Red') while in bunch grapes it varied between 1.4  $mg \cdot g^{-1}$  fresh leaf ('M6-7E') and 5.2  $mg \cdot g^{-1}$  fresh leaf ('Lake Emerald'). Differential accumulation of ami-

Table 1. Developmental changes in the leaf amino acid content of Florida bunch and muscadine grape genotypes (mg·g<sup>-1</sup> fresh leaf)

	Pre-Flowering	Flowering	Young	Medium	Mature	Ripe
<b>BUNCH</b>						
Blanc du Bois	2.1 <sup>f</sup>	2.4 <sup>gh</sup>	2.3 <sup>ef</sup>	2.5 <sup>e</sup>	3.8 <sup>g</sup>	2.5 <sup>gh</sup>
Blue Lake	1.6 <sup>f</sup>	2.5 <sup>gh</sup>	1.7 <sup>g</sup>	1.7 <sup>f</sup>	4.3 <sup>g</sup>	3.2 <sup>fg</sup>
Black Spanish	1.2 <sup>fg</sup>	1.9 <sup>h</sup>	1.5 <sup>g</sup>	2.2 <sup>e</sup>	1.6 <sup>i</sup>	2.9 <sup>gh</sup>
Herbemont	1.8 <sup>f</sup>	3.3 <sup>fg</sup>	2.1 <sup>ef</sup>	2.0 <sup>f</sup>	3.9 <sup>g</sup>	2.9 <sup>gh</sup>
Lake Emerald	2.1 <sup>f</sup>	4.7 <sup>d</sup>	2.9 <sup>e</sup>	3.5 <sup>d</sup>	6.1 <sup>e</sup>	5.2 <sup>cd</sup>
M4-83	0.8 <sup>g</sup>	<u>1.5</u> <sup>h</sup>	2.0 <sup>f</sup>	1.6 <sup>f</sup>	1.8 <sup>i</sup>	3.4 <sup>ef</sup>
M6-7E	1.3 <sup>fg</sup>	2.5 <sup>gh</sup>	2.6 <sup>ef</sup>	2.4 <sup>e</sup>	<u>1.5</u> <sup>i</sup>	<u>1.4</u> <sup>i</sup>
Midsouth	3.0 <sup>e</sup>	2.0 <sup>h</sup>	<u>1.4</u> <sup>g</sup>	1.9 <sup>f</sup>	4.2 <sup>g</sup>	2.0 <sup>hi</sup>
Orlando Seedless	2.1 <sup>f</sup>	2.3 <sup>gh</sup>	2.8 <sup>e</sup>	2.1 <sup>e</sup>	2.1 <sup>i</sup>	1.9 <sup>i</sup>
Stover	1.3 <sup>fg</sup>	2.2 <sup>gh</sup>	3.1 <sup>de</sup>	3.5 <sup>d</sup>	4.1 <sup>g</sup>	3.9 <sup>ef</sup>
Suwannee	1.3 <sup>fg</sup>	2.1 <sup>h</sup>	1.6 <sup>g</sup>	1.9 <sup>f</sup>	6.1 <sup>e</sup>	4.5 <sup>de</sup>
Tampa	1.2 <sup>fg</sup>	2.0 <sup>h</sup>	2.5 <sup>ef</sup>	1.7 <sup>f</sup>	1.7 <sup>i</sup>	3.2 <sup>fg</sup>
<b>MUSCADINE</b>						
African Queen	1.9 <sup>f</sup>	3.8 <sup>ef</sup>	4.7 <sup>b</sup>	1.7 <sup>f</sup>	8.4 <sup>bc</sup>	2.3 <sup>h</sup>
Alachua	1.1 <sup>g</sup>	3.6 <sup>f</sup>	4.1 <sup>bc</sup>	4.6 <sup>c</sup>	9.2 <sup>b</sup>	7.9 <sup>ab</sup>
Albemarle	8.8 <sup>b</sup>	3.2 <sup>fg</sup>	3.2 <sup>de</sup>	3.3 <sup>d</sup>	5.8 <sup>c</sup>	5.2 <sup>cd</sup>
Black Fry	1.3 <sup>fg</sup>	2.4 <sup>gh</sup>	4.4 <sup>bc</sup>	<u>1.5</u> <sup>f</sup>	3.9 <sup>g</sup>	2.3 <sup>h</sup>
Carlos	7.2 <sup>c</sup>	8.0 <sup>b</sup>	3.2 <sup>de</sup>	3.8 <sup>d</sup>	4.1 <sup>g</sup>	3.9 <sup>ef</sup>
CD8-81	13.5 <sup>a</sup>	<b>13.2</b> <sup>a</sup>	3.5 <sup>c</sup>	4.2 <sup>c</sup>	4.9 <sup>f</sup>	5.0 <sup>cd</sup>
Chowan	9.7 <sup>b</sup>	6.3 <sup>c</sup>	3.9 <sup>c</sup>	4.2 <sup>c</sup>	4.7 <sup>f</sup>	5.7 <sup>cd</sup>
Cowart	2.7 <sup>ef</sup>	3.8 <sup>ef</sup>	3.9 <sup>c</sup>	4.2 <sup>c</sup>	2.7 <sup>h</sup>	6.1 <sup>bc</sup>
Darlene	4.4 <sup>d</sup>	5.9 <sup>c</sup>	4.1 <sup>bc</sup>	2.4 <sup>e</sup>	4.8 <sup>f</sup>	3.6 <sup>ef</sup>
DB3-63	4.8 <sup>d</sup>	4.8 <sup>d</sup>	3.4 <sup>d</sup>	6.2 <sup>b</sup>	4.5 <sup>g</sup>	2.3 <sup>h</sup>
Digby	2.4 <sup>e</sup>	3.5 <sup>fg</sup>	3.8 <sup>c</sup>	4.4 <sup>c</sup>	4.2 <sup>g</sup>	1.9 <sup>i</sup>
Dixie	2.9 <sup>ef</sup>	3.1 <sup>fg</sup>	2.8 <sup>e</sup>	4.1 <sup>d</sup>	3.0 <sup>h</sup>	2.5 <sup>gh</sup>
Dixie Land	1.9 <sup>f</sup>	3.6 <sup>f</sup>	3.5 <sup>c</sup>	4.0 <sup>d</sup>	8.3 <sup>c</sup>	4.6 <sup>de</sup>
Dixie Red	1.9 <sup>f</sup>	3.7 <sup>f</sup>	5.2 <sup>b</sup>	3.9 <sup>d</sup>	10.1 <sup>a</sup>	<b>8.5</b> <sup>a</sup>
Doreen	3.3 <sup>e</sup>	4.0 <sup>ef</sup>	5.5 <sup>a</sup>	4.3 <sup>c</sup>	4.5 <sup>g</sup>	3.4 <sup>ef</sup>
Farrer	<u>0.7</u> <sup>g</sup>	2.2 <sup>gh</sup>	4.3 <sup>b</sup>	3.1 <sup>de</sup>	5.0 <sup>f</sup>	3.5 <sup>ef</sup>
Fry	2.3 <sup>f</sup>	2.4 <sup>gh</sup>	4.0 <sup>b</sup>	4.6 <sup>c</sup>	4.0 <sup>g</sup>	2.6 <sup>gh</sup>
GA-23-45	4.5 <sup>d</sup>	4.5 <sup>d</sup>	<b>5.8</b> <sup>a</sup>	6.8 <sup>b</sup>	<u>1.5</u> <sup>i</sup>	1.8 <sup>i</sup>
GA3-3	<b>13.7</b> <sup>a</sup>	8.1 <sup>b</sup>	3.5 <sup>c</sup>	5.2 <sup>b</sup>	<u>9.1</u> <sup>b</sup>	2.2 <sup>h</sup>
GA33-3-2	6.8 <sup>c</sup>	4.1 <sup>ef</sup>	3.5 <sup>c</sup>	4.5 <sup>c</sup>	7.9 <sup>c</sup>	6.1 <sup>bc</sup>
GA3-9-2	11.3 <sup>a</sup>	6.3 <sup>cd</sup>	5.4 <sup>ab</sup>	3.1 <sup>de</sup>	4.9 <sup>f</sup>	4.7 <sup>de</sup>
Higgins	7.5 <sup>c</sup>	4.1 <sup>ef</sup>	4.6 <sup>b</sup>	3.8 <sup>d</sup>	6.0 <sup>e</sup>	5.7 <sup>cd</sup>
Ison	4.0 <sup>d</sup>	4.0 <sup>ef</sup>	3.9 <sup>c</sup>	2.4 <sup>e</sup>	7.6 <sup>d</sup>	4.8 <sup>de</sup>
Janet	1.2 <sup>fg</sup>	1.9 <sup>h</sup>	3.9 <sup>c</sup>	2.2 <sup>e</sup>	5.1 <sup>f</sup>	3.3 <sup>fg</sup>
Jane Bell	5.8 <sup>c</sup>	4.4 <sup>de</sup>	2.9 <sup>e</sup>	3.2 <sup>de</sup>	7.6 <sup>d</sup>	5.7 <sup>cd</sup>
Jumbo	4.5 <sup>d</sup>	3.2 <sup>fg</sup>	4.8 <sup>b</sup>	<b>7.5</b> <sup>a</sup>	6.3 <sup>e</sup>	2.8 <sup>fg</sup>
Marsh	2.9 <sup>ef</sup>	3/4 <sup>g</sup>	3.6 <sup>c</sup>	3.9 <sup>d</sup>	4.8 <sup>f</sup>	1.9 <sup>i</sup>
Noble	7.7 <sup>c</sup>	4.5 <sup>d</sup>	4.5 <sup>b</sup>	5.3 <sup>b</sup>	5.5 <sup>e</sup>	7.2 <sup>b</sup>
Pam	3.2 <sup>e</sup>	2.8 <sup>g</sup>	2.8 <sup>e</sup>	5.3 <sup>b</sup>	5.4 <sup>ef</sup>	5.0 <sup>cd</sup>
Pink Hunt	6.4 <sup>c</sup>	6.4 <sup>c</sup>	4.5 <sup>b</sup>	5.0 <sup>c</sup>	3.7 <sup>g</sup>	3.7 <sup>ef</sup>
Regale	8.8 <sup>b</sup>	5.2 <sup>d</sup>	3.5 <sup>c</sup>	3.4 <sup>d</sup>	5.1 <sup>f</sup>	5.6 <sup>cd</sup>
Rosa	1.8 <sup>f</sup>	4.5 <sup>d</sup>	5.2 <sup>b</sup>	5.7 <sup>b</sup>	5.8 <sup>e</sup>	6.6 <sup>bc</sup>
Scuppernong	8.4 <sup>c</sup>	5.0 <sup>d</sup>	2.4 <sup>f</sup>	3.3 <sup>d</sup>	7.0 <sup>d</sup>	5.8 <sup>cd</sup>
Senoia	3.4 <sup>e</sup>	3.1 <sup>ef</sup>	4.9 <sup>b</sup>	2.8 <sup>e</sup>	7.1 <sup>d</sup>	4.8 <sup>de</sup>
Southers	4.2 <sup>d</sup>	4.2 <sup>ef</sup>	3.1 <sup>de</sup>	3.7 <sup>d</sup>	6.6 <sup>d</sup>	2.4 <sup>gh</sup>
Southland	1.1 <sup>g</sup>	1.8 <sup>h</sup>	3.1 <sup>de</sup>	6.0 <sup>b</sup>	4.2 <sup>g</sup>	6.5 <sup>bc</sup>
Summit	6.1 <sup>c</sup>	2.9 <sup>g</sup>	4.4 <sup>b</sup>	4.3 <sup>c</sup>	7.9 <sup>c</sup>	7.1 <sup>b</sup>
Sugar Pop	2.5 <sup>ef</sup>	3.3 <sup>fg</sup>	4.1 <sup>bc</sup>	5.5 <sup>b</sup>	5.2 <sup>f</sup>	3.0 <sup>fg</sup>
Sweet Jenny	1.5 <sup>f</sup>	2.7 <sup>gh</sup>	3.2 <sup>de</sup>	3.3 <sup>d</sup>	5.7 <sup>e</sup>	6.4 <sup>bc</sup>
Tarhee	5.3 <sup>d</sup>	5.4 <sup>c</sup>	3.2 <sup>de</sup>	3.3 <sup>d</sup>	2.8 <sup>h</sup>	5.8 <sup>cd</sup>
Triumph	1.6 <sup>f</sup>	4.2 <sup>ef</sup>	5.0 <sup>b</sup>	4.3 <sup>c</sup>	<b>10.4</b> <sup>a</sup>	4.1 <sup>de</sup>
Welder	5.3 <sup>d</sup>	3.5 <sup>fg</sup>	4.3 <sup>b</sup>	4.2 <sup>c</sup>	8.1 <sup>c</sup>	6.2 <sup>bc</sup>

Mean separation (in columns) by Duncan's multiple range test ( $P = 0.05$ ). Means with the same letter are not significantly different.

Numbers in bold indicate genotype showing highest amino acid content and numbers underlined indicate genotypes showing lowest amino acid content at a particular developmental stage.

no acids in the leaves during berry ripening indicates that different genotypes have varied genetic potential to accumulate a pool of free amino acids in their leaf tissue for possible translocation to berries.

#### Berry Amino acid Content

Variation in berry amino acid level was highly significant ( $P < 0.05$ ) for genotypes at all the developmental stages (Table 2).

Table 2. Developmental changes in the fruit amino acid content of Florida bunch and muscadine grape genotypes (mg·g<sup>-1</sup> fresh fruit).

	Young	Medium	Mature	Ripe
<b>BUNCH</b>				
Blanc du Bois	3.0 <sup>c</sup>	3.0 <sup>c</sup>	4.5 <sup>f</sup>	3.2 <sup>hi</sup>
Blue Lake	2.1 <sup>d</sup>	2.1 <sup>d</sup>	2.1 <sup>i</sup>	4.7 <sup>g</sup>
Black Spanish	1.1 <sup>e</sup>	1.9 <sup>e</sup>	<u>0.8</u>	2.4 <sup>i</sup>
Herbemont	3.0 <sup>c</sup>	3.7 <sup>b</sup>	1.5 <sup>i</sup>	3.1 <sup>hi</sup>
Lake Emerald	1.5 <sup>e</sup>	1.3 <sup>g</sup>	11.1 <sup>ab</sup>	14.7 <sup>b</sup>
M4-83	3.3 <sup>bc</sup>	2.8 <sup>c</sup>	2.1 <sup>i</sup>	6.0 <sup>e</sup>
M6-7E	3.4 <sup>bc</sup>	3.2 <sup>c</sup>	1.5 <sup>i</sup>	3.8 <sup>h</sup>
Midsouth	3.8 <sup>ab</sup>	1.6 <sup>e</sup>	2.5 <sup>h</sup>	4.8 <sup>g</sup>
Orlando Seedless	4.0 <sup>ab</sup>	3.7 <sup>b</sup>	4.8 <sup>f</sup>	4.6 <sup>g</sup>
Stover	3.9 <sup>ab</sup>	3.5 <sup>b</sup>	2.8 <sup>h</sup>	2.9 <sup>hi</sup>
Suwannee	2.7 <sup>c</sup>	3.9 <sup>b</sup>	2.0 <sup>i</sup>	4.0 <sup>h</sup>
Tampa	<b>4.5<sup>a</sup></b>	1.5 <sup>ef</sup>	3.2 <sup>h</sup>	6.8 <sup>d</sup>
<b>Muscadine</b>				
African Queen	2.4 <sup>c</sup>	3.6 <sup>b</sup>	9.0 <sup>b</sup>	6.6 <sup>d</sup>
Alachua	1.9 <sup>de</sup>	3.5 <sup>b</sup>	5.7 <sup>e</sup>	5.4 <sup>f</sup>
Albamarle	2.3 <sup>d</sup>	2.5 <sup>d</sup>	6.7 <sup>d</sup>	6.4 <sup>e</sup>
Black Fry	1.4 <sup>e</sup>	4.6 <sup>a</sup>	1.8 <sup>i</sup>	1.7 <sup>ji</sup>
Carlos	2.2 <sup>d</sup>	2.4 <sup>d</sup>	2.9 <sup>h</sup>	4.2 <sup>g</sup>
CD8-81	2.2 <sup>d</sup>	2.4 <sup>d</sup>	3.7 <sup>g</sup>	10.2 <sup>c</sup>
Chowan	2.8 <sup>c</sup>	<b>4.9<sup>a</sup></b>	5.8 <sup>d</sup>	7.2 <sup>d</sup>
Cowart	1.7 <sup>e</sup>	3.2 <sup>c</sup>	2.6 <sup>h</sup>	5.3 <sup>hi</sup>
Darlene	3.2 <sup>bc</sup>	<u>0.8<sup>h</sup></u>	4.1 <sup>g</sup>	4.5 <sup>g</sup>
DB3-63	1.9 <sup>de</sup>	2.2 <sup>d</sup>	7.9 <sup>c</sup>	4.3 <sup>g</sup>
Digby	1.5 <sup>e</sup>	1.1 <sup>g</sup>	3.5 <sup>g</sup>	1.6 <sup>ji</sup>
Dixie	<u>0.8<sup>f</sup></u>	1.9 <sup>e</sup>	5.4 <sup>e</sup>	4.4 <sup>g</sup>
Dixie Land	1.9 <sup>de</sup>	1.7 <sup>e</sup>	5.4 <sup>e</sup>	9.6 <sup>c</sup>
Dixie Red	1.7 <sup>e</sup>	1.1 <sup>g</sup>	3.4 <sup>g</sup>	5.1 <sup>g</sup>
Doreen	3.5 <sup>bc</sup>	1.1 <sup>g</sup>	<b>11.6<sup>a</sup></b>	3.8 <sup>h</sup>
Farrer	1.4 <sup>e</sup>	1.7 <sup>e</sup>	8.0 <sup>c</sup>	2.6 <sup>i</sup>
Fry	1.4 <sup>e</sup>	1.5 <sup>ef</sup>	3.7 <sup>g</sup>	2.9 <sup>hi</sup>
GA-23-45	1.9 <sup>de</sup>	3.0 <sup>c</sup>	0.9 <sup>j</sup>	2.5 <sup>i</sup>
GA3-3	1.6 <sup>e</sup>	2.3 <sup>d</sup>	7.2 <sup>c</sup>	3.9 <sup>h</sup>
GA33-3-2	1.4 <sup>e</sup>	1.5 <sup>ef</sup>	8.0 <sup>c</sup>	5.8 <sup>ef</sup>
GA33-9-2	1.9 <sup>de</sup>	1.7 <sup>e</sup>	3.7 <sup>g</sup>	8.8 <sup>c</sup>
Higgins	2.5 <sup>c</sup>	2.1 <sup>d</sup>	6.7 <sup>d</sup>	6.7 <sup>d</sup>
Ison	1.3 <sup>e</sup>	3.6 <sup>b</sup>	8.3 <sup>c</sup>	8.1 <sup>d</sup>
Janet	0.9 <sup>f</sup>	1.4 <sup>f</sup>	8.6 <sup>c</sup>	6.7 <sup>d</sup>
Jane Bell	1.3 <sup>e</sup>	2.3 <sup>d</sup>	4.5 <sup>f</sup>	7.2 <sup>d</sup>
Jumbo	1.8 <sup>e</sup>	0.30 <sup>e</sup>	0.56 <sup>e</sup>	0.23 <sup>i</sup>
Marsh	2.7 <sup>c</sup>	1.7 <sup>e</sup>	1.7 <sup>i</sup>	1.9 <sup>ji</sup>
Noble	2.5 <sup>c</sup>	3.2 <sup>c</sup>	4.9 <sup>f</sup>	4.3 <sup>g</sup>
Pam	0.9 <sup>f</sup>	1.6 <sup>ef</sup>	5.8 <sup>e</sup>	4.8 <sup>g</sup>
Pink Hunt	1.7 <sup>e</sup>	3.7 <sup>b</sup>	3.4 <sup>g</sup>	3.7 <sup>h</sup>
Regale	1.4 <sup>e</sup>	1.5 <sup>ef</sup>	2.8 <sup>h</sup>	3.3 <sup>hi</sup>
Rosa	2.8 <sup>c</sup>	2.4 <sup>d</sup>	5.3 <sup>f</sup>	6.0 <sup>e</sup>
Scuppermong	1.9 <sup>de</sup>	2.4 <sup>d</sup>	7.4 <sup>c</sup>	6.2 <sup>e</sup>
Senoia	1.4 <sup>e</sup>	1.5 <sup>ef</sup>	5.6 <sup>e</sup>	4.4 <sup>g</sup>
Southers	1.4 <sup>e</sup>	1.5 <sup>ef</sup>	10.5 <sup>ab</sup>	6.43 <sup>c</sup>
Southland	2.4 <sup>c</sup>	2.2 <sup>d</sup>	3.0 <sup>h</sup>	3.5 <sup>h</sup>
Summit	1.8 <sup>de</sup>	1.4 <sup>f</sup>	5.1 <sup>f</sup>	6.1 <sup>e</sup>
Sugar Pop	3.3 <sup>bc</sup>	1.2 <sup>g</sup>	3.5 <sup>g</sup>	<u>1.2<sup>j</sup></u>
Sweet Jenny	1.6 <sup>e</sup>	1.6 <sup>ef</sup>	5.5 <sup>e</sup>	7.5 <sup>d</sup>
Tarhee	1.6 <sup>e</sup>	1.6 <sup>ef</sup>	1.6 <sup>i</sup>	<b>17.0<sup>a</sup></b>
Triumph	2.8 <sup>c</sup>	1.0 <sup>g</sup>	5.3 <sup>f</sup>	3.1 <sup>hi</sup>
Welder	1.5 <sup>e</sup>	1.3 <sup>g</sup>	1.3 <sup>i</sup>	7.9 <sup>d</sup>

Mean separation (in columns) by Duncan's multiple range test  $p = 0.05$ . Means with the same letter are not significantly different. Numbers in bold indicate genotype showing highest amino acid content and numbers underlined indicate genotypes showing lowest highest amino acid content at a particular developmental stage.

In bunch grapes, the berry amino acid content for young fruit (stage 3) varied from 1.1 mg·g<sup>-1</sup> to 4.5 mg·g<sup>-1</sup> fresh tissue while in the muscadine grapes it was between 0.8 mg·g<sup>-1</sup> and 3.5 mg·g<sup>-1</sup> fresh tissue. The highest amino acid content was found in 'Tampa' (bunch) and the lowest in 'Dixie' (muscadine). For medium fruit (stage 4), the berry amino acid level varied from 1.3 mg·g<sup>-1</sup> ('Lake Emerald') to 3.9 mg·g<sup>-1</sup> fresh tissue ('Suwannee') in bunch grape and 0.8 mg·g<sup>-1</sup> ('Darlene') to 4.9 mg·g<sup>-1</sup> fresh tissue ('Chowan') in muscadine grape. For mature fruit (stage 5) the berry amino acid content varied between 0.8 mg·g<sup>-1</sup> ('Black Spanish') and 11.1 mg·g<sup>-1</sup> fresh tissue ('Lake Emerald') in bunch grape, and from 1.3 mg·g<sup>-1</sup> ('Welder') to 11.6 mg·g<sup>-1</sup> fresh tissue ('Doreen') in muscadine grape. For ripe fruit, the berry amino acid content in bunch grape varied between 2.4 mg·g<sup>-1</sup> ('Black Spanish') and 14.7 mg·g<sup>-1</sup> ('Lake Emerald') while in muscadine it ranged from 1.2 mg·g<sup>-1</sup> ('Sugar Pop') to 17.0 mg·g<sup>-1</sup> fresh tissue ('Tarhee').

#### *Amino acid accumulation pattern in leaves and berries*

The leaf and berry amino acid data of individual genotypes at six different developmental stages (stage 1 to stage 6) were compared to determine developmental profiles and amino acid accumulation patterns among the grape genotypes. The data showed major differences in the amino acid accumulation pattern among grape genotypes indicating existence of wide genetic variation. An analysis of variance was performed based on leaf and berry amino acid content. For the leaf amino acid content, the F-value was 6.09 ( $P = 0.05$ ). Genotypes in Group 1 had significantly higher amino acid content than did other genotypes belonging to any of the other groups. The berry amino acid levels showed significant variation between developmental stages however no significant variation was observed between groups. Grape genotypes were grouped in to six groups based on the Duncan's grouping that was determined by ANOVA for two independent variables. Table 3 represents the results of the Duncan test and the list of genotypes that belong to each group. The mean leaf and berry amino acid levels along with standard deviations for each group were plotted to determine differences in the amino acid accumulation pattern among these groups (Fig. 1).

The free amino acid content of the leaves differed significantly among the six groups. The eight genotypes that had higher leaf amino acid content (average 5.80 mg·g<sup>-1</sup> fresh leaf tissue) fall into the Group 1. Genotypes of Group 1 were not significantly different from that of Group 2; similarly genotypes of Group 2 were not significantly different from the genotypes of Group 3. However, the genotypes of Group 1 and Group 3 were significantly different from one another. Genotypes of Group 4 were not significantly different from that of Group 5; similarly the genotypes of Group 5 were not significantly different from the genotypes of Group 6. However, the genotypes of Group 4 and Group 6 were significantly different from each other. The leaf amino acid content was lowest (average 2.01 mg·g<sup>-1</sup> fresh leaf tissue) for the genotypes belonging to the Group 6. Genotypes of Group 1 and Group 2 showed decreasing levels of free leaf amino acid content. Genotypes of Group 1 maintained the free amino acid content in the leaves during the berry development whereas the genotypes of Group 2 had fluctuating leaf amino acid content. Genotypes of Group 3 showed increasing free amino acid content in the leaves, which increased rapidly to three-

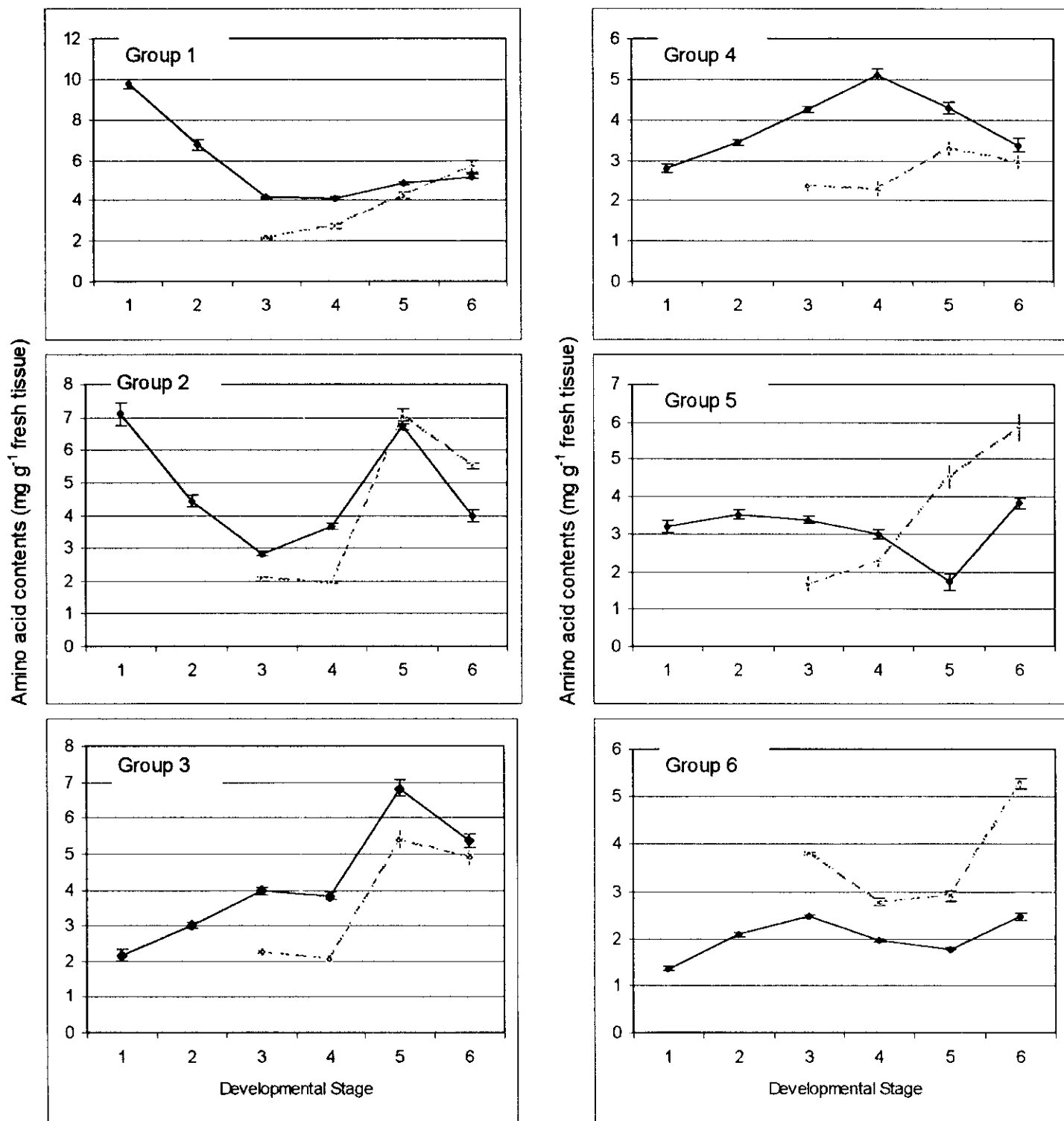


Fig. 1. Groups representing developmental profile of leaf and berry free amino acid accumulation patten. — Leaf, - - - Berry. Numbers on X-axis represent developmental stage: 1= Pre-flowering, 2 = Flowering, 3 = Young berry stage, 4 = Medium berry sage, 5 = Mature berry stage, and 6 = Ripe berry stage. error bar on Y-axis represent  $\pm$ SD.

fold (from 2.16 to 6.8 mg·g<sup>-1</sup> fresh leaf) between stages 1 and 5, then decreased to 5.36 mg·g<sup>-1</sup> fresh leaf at stage 6 (ripe berry). The genotypes of Group 4 showed about a 2-fold increase in the leaf free amino acid content during stage 1 through 4, then it slowly decreased during stages 5 and 6. The genotypes

of Group-5 and -6 showed less fluctuation in their leaf free amino acid content.

No unique amino acid accumulation pattern was found for either bunch or muscadine grape genotypes since the genotypes from both of these categories were scattered

Table 3. Results of the Duncan test and Grouping of grape genotypes based on the developmental profile and accumulation pattern of free amino acid in leaf and berry (Group with the same letter in Duncan's rank column indicates not significant difference).

Group	Duncan's Rank	Mean	Genotypes fall in the Group
Group 1	a	5.79	Carlos, CD-8-81, Chowan, GA-3-9-2, Higgins, Noble, Pink Hunt, Regale.
Group 2	ab	4.78	Lake Emerald, Midsouth, Albermarle, GA3-3, GA33-3-2, Scuppernong, Southers.
Group 3	b	4.19	Blanc du Bois, Suwannee, Alachua, Dixie Land, Dixie Red, Doreen, Farrer, Southland, Summit, Sweet Jenny, Triumph.
Group 4	c	3.90	Stover, Cowart, Digby, Fry, GA23-45, Jumbo, Marsh, Rosa, Sugar Pop.
Group 5	cd	3.21	Blue Lake, Black Spanish, Herbemont, African Queen, Black Fry, Darlene, DB3-63, Dixie, Ison, Janet, Jane Bell, Pam, Senoia, Tarhee, Welder.
Group 6	d	2.01	M4-83, M6-7E, Orlando Seedless, Tampa.

among different groups; however, the developmental pattern of the genotypes within each group was similar indicating physiological similarity within each group.

Results showed that the berry free amino acid content increased during development for most of the grape genotypes and was not significantly different among these groups. The present study indicates potential levels of free amino acid content in berry pulp. However, further studies are required to assay free amino acid content in the juice of muscadine and Florida bunch grapes to determine the availability of sufficient assimilable nitrogen for fermentation (Jiranek et al., 1995; Monteiro and Bisson, 1993). In addition, the qualitative amino acid analysis of juices from different cultivars will assist in determining their possible contribution to wine quality and aroma (Kliewer, 1969; 1970). Further studies in relation to genotypes with high and low leaf free amino acid content and disease occurrence during development will help in understanding the contribution of free amino acids to juice and wine quality, and overall vine health.

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