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Research Article

Gene Expression and Molecular Architecture Reveals UDP-Glucose: Flavonoid-3-O-Glucosyltransferase *UFGT* as a Controller of Anthocyanin Production in Grapes

Phillip Corbiere[1], Anthony Ananga[*1], Joel W. Ochieng[2], Ernst Cebert[3], Violeta Tsolova[1]

[1]*Center for Viticulture and Small Fruit Research, College of Agriculture and Food Science, Florida A&M University, 6505 Mahan Drive, Tallahassee Fl 32317, USA*

[2]*Centre for Biotechnology and Bioinformatics, College of Agriculture and Veterinary Sciences, University of Nairobi, P.O. Box 29053 Nairobi, 00625 Kenya*

[3]*Department of Biological and Environmental Sciences, Alabama A&M University, 4900 Meridian Street, Normal AL 35762, USA*

**Corresponding author:* *Dr. Anthony Ananga, Center For Viticulture and Small Fruit Research, Florida A&M University, 6505 Mahan Drive, Tallahassee Florida, 32317, USA, Tel:+1-850-412-7393; Fax: +1-850-412-561-2617;*

E-mail: anthony.ananga@gmail.com or Anthony.ananga@famu.edu

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Abstract

Anthocyanin pigments from the flavonoid biosynthesis pathway in grapes (*Vitis sp.*) are gaining increasing popularity in the wine industry as a mark for quality, preventive medicine in the natural products sector, food colorants and raw materials for cosmetics. Grape skins, the primary source of these pigments cannot meet increasing market demand, therefore, this study was undertaken to explore *in-vitro* cell culture of grape as a mean to redirect and control the expression of genes in the anthocyanin biosynthetic pathway. This study examined levels of expression of UDP-Glucose: Flavonoid-3-O-Glucosyltransferase (UFGT), which is a gene responsible for the last stages of anthocyanin production between unripe grape berries, ripening (véraison) berries and fully ripe (physiologically mature) berries. *In-vitro* red cell cultures were carried out to establish the optimal stage of harvesting cells for culturing to maximize production of anthocyanin and to determine feasibility of using cell culture over fresh grape tissues. Clones of the UFGT gene from muscadine grapes were isolated, sequenced, and compared to other known species to examine variations of functional significance. Changes in functional regions which could affect accumulation of anthocyanins including substrate binding sites, methyl donor binding sites and catalytic sites were examined. Results from this study indicated that, cell cultures produced significantly higher amount of anthocyanin over fresh tissues, and véraison was the optimum stage for harvesting cells for culturing. This work demonstrated that, the UFGT gene can be a viable candidate for shifting the metabolic flux of anthocyanins in grapes.

Keywords: Anthocyanin; Glucosyltransferase; Muscadine Grape

Introduction

Anthocyanins are plant secondary metabolites that originate from the flavonoid biosynthesis pathway. They are the most widely distributed group of water-soluble plant pigments in nature and produce a diverse assortment of hues including lavender, red, blue and purple colors in flowers, fruits, leaves, seeds and other organs in flowering plants. Anthocyanins have gained acceptance in numerous manufacturing sectors including food processing, pharmaceuticals and in cosmetics as natural alternatives to artificial colorants, complementary therapies and preventive medicine. The use of anthocyanins as an antioxidants by pharmaceutical industries and complementary products in medicine have been reviewed [1,2].

Currently, anthocyanins are obtained from whole plant extracts [3] with the most common source being grape skins. Limitations of obtaining anthocyanins directly from fresh plant materials include low metabolite yield, inconsistency in quality, seasonal availability of raw materials, and pigment degradation caused by storage and extraction process [4]. Plant variety, growing region and cultural practices also influence the level of anthocyanin and the profile of different pigments [5, 6]. Advanced optimization and cultivation strategies for cell culture have redirected attention to *in-vitro* approaches for product development. The application of these new technologies has allowed improved redirection and controlled expression of genes in the anthocyanin and flavonoid biosynthetic pathway. However, biosynthetic instabilities, variability, low yield and abundance of metabolites in some cell cultures limit their use by industry. Genetic engineering strategies for over-expression of structural or regulatory genes in the biosynthetic pathway are required to scale-up these new approaches for plant cell suspensions and bioreactor design. Significant differences exist between vine varieties as determined by the presence or absence of anthocyanins in their berries. Such variances as well as compositions of different anthocyanin in colored berries have not been fully studied at the molecular level. Different species or varieties of grapes have unique sets of anthocyanin pigments [7]. The diversity of these anthocyanins usually reflects the modification of common aglycones by hydroxylation, glycosylation, methylation, and acylation. Some studies have reported that some *V. vinifera* L. cultivars produce only nonacylated anthocyanidin-3-O-monoglucosides, while *V. rotundifolia* (Michx.) Small accumulates only nonacylated anthocyanidin-3,5-O-diglucosides [8-12]. These modifications and their consequences can be understood at the molecular level when the structural and regulatory genes of the biosynthetic pathway are characterized.

Although genes of the anthocyanin biosynthetic pathway have been characterized in some plant species, available data show that the control of anthocyanin pathway differs among species. It has been reported that in maize regulation of anthocyanin starts at CHS, while in snapdragon it is further down in the pathway at F3H and in petunia the control anthocyanin production starts at DFR [13, 14]. Comparison of temporal changes in expression profiles for seven structural genes of this pathway (PAL, CHS, CHI, F3H, DFR, LDOX, UFGT) [6] showed UDP-Glucose: Flavonoid-3-O-Glucosyltransferase (UFGT) to be regulated independently of the other genes, suggesting UFGT as the major control point for anthocyanin production. UFGT catalyzes the transfer of the glucosyl moiety from UDP-glucose to the 3-hydroxyl group of anthocyanidins, producing the first stable anthocyanins [12]. In this study, we characterized both genomic and expressional differences within *Vitis* grape varieties at UFGT to determine the mechanisms in biosynthetic stabilities, variability, and quantity of anthocyanins. Previous studies have confirmed that the UFGT gene is present but not expressed in white grape cultivars [5, 6]. Elucidation of molecular mechanisms for pigments will aid in the transfer of desirable quantitative traits found in some of the white grapes into other economically important grape varieties.

Materials and Methods

Plant materials

Skins of berries and *in vitro* red cell lines of muscadine grapes ("Noble" var.) were used in this study. Berries were harvested from the Florida A&M University vineyard at three different development stages (green, véraison and physiologically mature) (Figure 1 A, B, C, D). Injury free berries of similar size were selected at every stage for mRNA extraction. Harvested grapes were washed with distilled water; skins were peeled off and immediately placed in liquid nitrogen and stored at -80°C. The established protocols (patent no. US 2011/0054195 A1) for *In vitro* red cell cultures from super-epidermal cells of red berries were used in this study. The cells were cultured in a growth chamber at 23°C under a white light (150 µE m−2S−1) with a 16 h light/8 h dark cycle.

Maintenance of cell cultures

Solid and liquid culture media were used to grow and maintain the cells, while grape cell cultures were maintained in B-5 media. The cells in solid media were sub-cultured every 30 days and cell suspensions were transferred to fresh liquid media every 12 days. For the liquid medium, approximately 2.5 ml of the cell suspension were transferred into a 25 ml Erlenmeyer flask with B-5 liquid medium and placed on a shaker (135 rpm) in a growth room. All cells were maintained at room temperature.

RNA extraction, gel electrophoresis and cDNA synthesis

Samples from all three stages of harvest as well as cell lines of 'Noble' grape were prepared for total RNA isolation, using the RNeasy Plant Mini Kit (Qiagen, CA, USA) according to the manufacturer's protocol. RNA was quantified using Nano drop

3300 (Thermo Scientific, USA), and the inactivity was inspected by formaldehyde agarose gel electrophoresis. Purified RNA was treated with RNase-free DNAse 1 and immediately frozen to -20˚C. Formaldehyde gel electrophoresis (1% agarose; W:V) was used to evaluate the quality of RNA, which were then used in primary gene expression profiling. The SuperScript first-strand synthesis system for RT-PCR (Invitrogen, USA) was used to synthesize cDNA in a 20 mL reaction containing 1 mg of DNase I-treated total RNA, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$, 10 mM dithiothreitol, 0.5 mg oligo (dT), 0.5 mM of each dNTPs, and 200U SuperScript II reverse transcriptase. RNA, dNTPs, and oligo (dT) were mixed first, heated to 65˚C for 5 min and placed on ice. The reaction was incubated at 50˚C for 50 min and terminated by heat inactivation at 85 ˚C for 5 min. The cDNA product was treated with 1 µl of Rnase H (Invitrogen) for 20 min at 37˚C. An identical reaction without the reverse transcriptase was performed to verify the absence of genomic DNA (no-RT control). The cDNA was stored at -20˚C until use.

Analysis of gene expression patterns by RT-PCR

The expression levels of *V. rotundifolia* UFGT gene from three stages of muscadine berry skins were determined by qRT-PCR using SYBR green method on a CFX96 real-time cycler (BIO-RAD, USA). Relative-quantitative real-time PCR reactions were performed in a 96-well plate to monitor cDNA amplification according to the manufacturer's protocol. For a control, a parallel amplification reaction of Actin (a housekeeping gene) was also performed. Each primer set was designed based on the 3'-end cDNA sequence of the corresponding gene. Primers used were: UFGT, 5'-CGCCGGAGAGCTTTAGGCAG-3' (Forward) and 5'-CCAAAACGGCAGCCAAGCCAC-3'(Reverse), with an expected product of 200 bp; Actin 5'-TAGAAGCACTTCCTGTGGAC-3' (Forward) and 5'-GGAAATCACTGCACTTGCTC-3' (Reverse), with expected product of 120 bp. Each PCR reaction (20 µl) contained 0.6 µl primer F, R (10 µM), 1 µl cDNA (10 ng), and 10 µl SsoAdvancedTM SYBR® Green Supermix (BIO-RAD, USA). The qRT-PCR conditions were: 1 cycle at 95˚C for 3 min; 35 cycles at 95˚C for 10 s and 60˚C for 30 s, followed by a melt cycle from 65˚C to 95˚C. All qRT-PCR reactions were carried out in three replicates for each sample. Relative expression values were calculated as 2-ΔCT, normalizing against the internal control - actin. The highest expression level of each gene observed served as a calibrator (1.0), while lower range of expressions were expressed as ratios in relation to the calibrator (relative expression ratio).

Isolation and sequencing of *Vitis rondutifolia* UFGT gene

Amino acid sequences of published UFGT sequences were aligned and a primer pair designed at conserved regions: forward - 5'- CACCATGTCTCAAACCACCACCA -3' and reverse - 5'-CTAGACATCCTTTGGTTTTG-3'. For PCR, cDNA synthesized from mRNA of the veriason berry skins was used as a template. PCR was performed with high fidelity polymerase (Promega) using the following thermocycling program: 95˚C for 5 min, then 35 cycles of 95˚C for 50s, 55˚C for 50s, and 72˚C 90s; followed by elongation at 72˚C for 10 min. PCR products were separated on 1% agarose gel, followed by purification of a slice containing a strong band corresponding to the DNA fragment of interest by DNA gel extraction kit (Qiagen, CA) according to the operator's manual. Purified PCR fragment or the full length of UFGT gene was subsequently cloned into pGEM-T Easy Vector (Promega, USA). Vectors and PCR-amplified products were mixed and ligated overnight at 4˚C and transformed into *Escherichia coli* strain JM109. The putative recombinant plasmid-pGEM-UFGT was extracted for PCR analysis. The pGEM-UFGT plasmid was sequenced from both ends at Eurofins mwg/Operon (Huntsville, AL, USA).

Identification, verification and alignment of sequences

Sequenced regions were confirmed by comparing recovered sequences with those in GenBank database using BLAST program from National Center for Biotechnology Information (NCBI). *V. rondutifolia* UFGT sequence was used to search through Basic Local Alignment Tool (BLAST), homology, and searches in public domains, namely GenBank [15] and the grape genome browser [16]. The GenBank UFGT protein sequence of *V. rotundifolia* (Accession no. AGS57502.1) was used for BLAST and homology searches against other plants. The deduced amino acid sequences were analyzed using the program DNAMAN. Multiple alignment of the putative amino-acid sequence of *V. rotundifolia* UFGT was performed using the T-Coffee program [17]. The alignment of 16 UFGT protein sequences from different plants was summarized using the Plotcon similarity graph [18], which indicates similarity along the set of aligned sequences.

Protein three-dimensional structure prediction

The *V. rotundifolia* UFGT structural model was obtained from its amino-acid sequence using the SWISS MODEL [19] and Protein Homology/analogy Recognition Engine (PHYRE) prediction servers [20]. The model obtained was classified according to identity percentage. Predicted substrate binding sites, methyl donor binding sites and catalytic sites were inferred according to *V. rotundifolia* UFGT crystal structure analysis.

Results and Discusssion

Expression of the gene UFGT responsible for the last stages of anthocyanin production between unripe (green) grape berries, ripening (véraison) berries, and those that are fully ripe (physiologically mature), as well as *in-vitro* cell cultures from red berries are shown in Figure 1. The full length UFGT gene was cloned, sequenced and deposited in the GenBank (Accession number KC936148) (Figure 2), and its sequences were compared to those of related grape species to examine variations of functional significance in anthocyanin production in grapes.

Examination for differences in functional regions which could affect accumulation of anthocyanins based on protein three-dimensional/crystal structure of *V. rotundifolia* UFGT as shown in Figure 3, was predicted in-silico to infer substrate binding sites, methyl donor binding sites and catalytic sites. These results were obtained using the SWISS MODEL [21] and protein homology/analog Recognition Engine (PHYRE) [22] prediction servers. The data showed cell cultures to be a superior source for anthocyanin production, while the véraison development stage was optimal for harvesting grapes for cell culturing.

Cell culturing as a reliable source of anthocyanin:

Analysis of relative expression of UDP-Glucose: Flavonoid-3-O-Glucosyltransferase (UFGT transcripts) in muscadine berry skins and *in-vitro* red cell lines (Noble Variety) by quantitative real-time PCR (qRT-PCR) showed *V. rotundifolia* UFGT expressed at the highest level in the cell lines (RE = 110), followed by skins of véraison stage (RE = 20), then physiologically mature red berries (RE = 17), while no expression was observed in the skins of green berries (Figure 1). Expression level in cell cultures was more than five-fold compared to either véraison or physiologically mature red berries. This finding validated that obtaining anthocyanins directly from fresh plant extracts has limitations in low metabolite yields, and therefore demonstrated the superiority of *in-vitro* grape cell culture engineering as a more reliable cultivation strategy for anthocyanins.

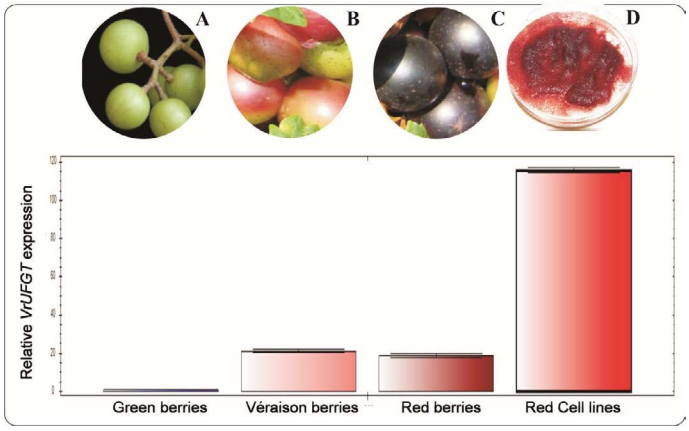


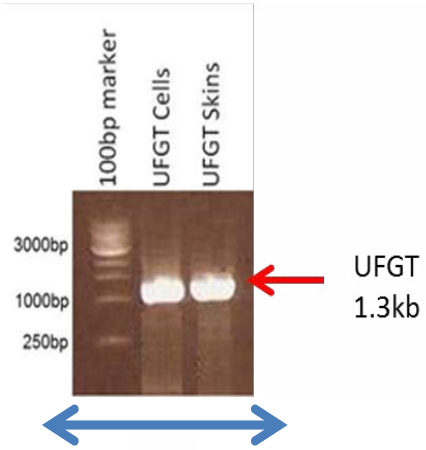
Figure 1. Relative expression of UDP-Glucose: Flavonoid-3-O-Glucosyltransferase (UFGT) in muscadine berry skins and In-vitro Red cell lines (Noble Variety). Expression is displayed as relative quantity (ΔΔCt) with skin tissue as the calibrator sample and Ubiquitin as the reference gene. A-Green berries, B-Véraison berries, C-Red berries, D-Red Cell lines.

UFGT transcripts comparison in unripe muscadine berry (green) skins, those at véraison, and skins of physiologically mature berries showed greater expression in véraison compared to physiologically mature berries. The quantity and quality of color in grape berries at harvest are crucial in wine making. A study on expression of seven genes in the biosynthesis pathway [6] indicated the onset of anthocyanin accumulation began at eight weeks post flowering, which coincides with véraison stage. In the current study, anthocyanin accumulation was quantified at two different timescales: véraison and physiologically mature berries, and with data indicating more accumulation at the former, thus harvesting grapes for cell culturing can be done as early as véraison and avoid poor fruit qualities at the later stages.

UFGT gene best candidate for manipulation of anthocyanins in grape

In this and previous studies [6, 21], the expression of UFGT was found to be crucial in the development of "colored" phenotype in grape skin, with appearance of anthocyanins at véraison correlating the detectability of UFGT mRNA. These findings confirmed that UFGT plays a controlling role in anthocyanin biosynthesis. Considering the many medicinal properties of these compounds, manipulation of the anthocyanin biosynthetic pathway in muscadine grape cell cultures would provide an alternative to control the overall metabolic flux of the targeted products and maximize the benefits of this economically important plant. In fact, UFGT expression has been observed in white grapes that unexpectedly acquired the ability to accumulate anthocyanins [6]. Evidence has been provided that two adjacent transcription factors, VvMybA1 and VvMybA2 are able to induce the *V. vinifera* UFGT transcription needed for berry pigmentation [23]. White/colored variation in grape co-segregates as a monogenic locus with the VvMybA1 locus [24]. The white grape phenotype has been linked to the homozygous presence of a transposable element Gret1 in the promoter of the VvMybA1 locus [25]. Genetic engineering remains the most plausible improvement tool as it is very difficult to cross muscadine and European bunch grapes due to variations in chromosome numbers. Chromosome numbers of Muscadines or *V. rotundifolia* are 2n = 40 compared to 2n = 38 for European bunch grape *V. vinifera* L. Other genes in the flavonoid biosynthetic pathway play multiple roles during berry development while UFGT may only be concerned with catalytic transfer of the glucosyl moiety, therefore, producing stable anthocyanins. As such, its manipulation is expected to affect only anthocyanin accumulation, the target end product. Molecular manipulation of other genes (PAL, CHS, CHI, F3H, DFR, LDOX) have been found to express at multiple stages of berry development in addition to other regulatory roles, developmental and quantitative traits.

Amplification of UDP-Glucose: Flavonoid-3-O-Glucosyltransferase (UFGT) in the red skins and *in vitro* cell lines of muscadine grapes was effective, with agarose gel indicating presence of a band at expected size of 1300 bases (Figure 2A). On sequencing, a 1368 bp long cDNA encoding the putative UFGT was isolated (Figure 2B). The cDNA nucleotide sequences of

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1       ATGTCTCAAACCACCACCAACCCCCATGTGGCCGTCCTGGCCTTCCCCTTCTCCACCCAT
1        M  S  Q  T  T  T  N  P  H  V  A  V  L  A  F  P  F  S  T  H

61      GCAGCCCCCCTCCTTGCCGTCGTTCGCCGCCTTGCTGCCGCTGCCCCTCATGCAGTCTTC
21       A  A  P  L  L  A  V  V  R  R  L  A  A  A  A  P  H  A  V  F

121     TCCTTCTTGAGCACCAGCGAATCCAACGCCTCCATCTTCCACGACTCCATGCATGCCATG
41       S  F  L  S  T  S  E  S  N  A  S  I  F  H  D  S  M  H  A  M

181     CAATGTAACATCAAGTCCTATGATGTGTCCGACGGTGTGCCTGAGGGGTATGTGTTCGCC
61       Q  C  N  I  K  S  Y  D  V  S  D  G  V  P  E  G  Y  V  F  A

241     GGGCGGCCCCAGGAGGATATTGAGCTGTTCATGAGGGCTGCGCCGGAGAGCTTTAGGCAG
81       G  R  P  Q  E  D  I  E  L  F  M  R  A  A  P  E  S  F  R  Q

301     GGGATGGTGATGGCTGTGGCCGAGACAGGGCGGCCAGTGAGCTGCCTGGTGGCTGACGCA
101      G  M  V  M  A  V  A  E  T  G  R  P  V  S  C  L  V  A  D  A

361     TTCATTTGGTTTGCTGCAGATATGGCAGCAGAGATGGGGGTGGCTTGGCTGCCGTTTTGG
121      F  I  W  F  A  A  D  M  A  A  E  M  G  V  A  W  L  P  F  W

421     ACTGCAGGGCCTAACTCACTCTCCACCCATGTTTACACTGATGAAATCAGAGAAAAGATT
141      T  A  G  P  N  S  L  S  T  H  V  Y  T  D  E  I  R  E  K  I

481     GGAATTTTAGGCATTCAAGGCCGTGAAGACGAGCTGCTCAATTTCATTCCCGGAATGTCT
161      G  I  L  G  I  Q  G  R  E  D  E  L  L  N  F  I  P  G  M  S

541     AAAGTACGTTTTCGCGACCTGCAGGAAGGCATCGTGTTCGGAAACCTAAACTCGCTCTTC
181      K  V  R  F  R  D  L  Q  E  G  I  V  F  G  N  L  N  S  L  F

601     TCACGCATGCTCCATCGGATGGGCCAAGTGCTACCTAAGGCGACTGCAGTTTTCATAAAC
201      S  R  M  L  H  R  M  G  Q  V  L  P  K  A  T  A  V  F  I  N

661     TCCTTCGAGGAGCTCGACGATTCCCTAACCAATGATCTCAAATCCAAGCTCAAGACGTAC
221      S  F  E  E  L  D  D  S  L  T  N  D  L  K  S  K  L  K  T  Y

721     CTCAATATCGGTCCATTTAACCTAATAACCCCACCGCCGGTTGTACCCAACACAACCGGC
241      L  N  I  G  P  F  N  L  I  T  P  P  P  V  V  P  N  T  T  G

781     TGCCTCCAATGGCTCAAAGAAAGAAAACCCACCTCGGTCGTGTACATTAGCTTTGGCACC
261      C  L  Q  W  L  K  E  R  K  P  T  S  V  V  Y  I  S  F  G  T

841     GTCACGACACCACCCCCAGCCGAGCTTGTAGCCCTAGCTGAGGCATTGGAGGCAAGCCGG
281      V  T  T  P  P  P  A  E  L  V  A  L  A  E  A  L  E  A  S  R

901     GTACCGTTTATATGGTCCCTAAGGGACAAGGCAAGGGTGCATTTGCCAGAAGGGTTCTTG
301      V  P  F  I  W  S  L  R  D  K  A  R  V  H  L  P  E  G  F  L

961     GAGAAGACCAGAGGGTACGGAATGGTTGTTCCATGGGCACCTCAGGCGGAGGTCCTAGCA
321      E  K  T  R  G  Y  G  M  V  V  P  W  A  P  Q  A  E  V  L  A

1021    CATGAGGCAGTTGGGGCTTTTGTAACACATTGTGGTTGGAACTCATTGTGGGAAAGCGTG
341      H  E  A  V  G  A  F  V  T  H  C  G  W  N  S  L  W  E  S  V

1081    GCCGGTGGGGTACCCTTGATTTGCAGGCCCTTTTTTGGGGACCAAAGGCTCAATGGGAGG
361      A  G  G  V  P  L  I  C  R  P  F  F  G  D  Q  R  L  N  G  R

1141    ATGGTGGAGGATGTTTTGGAGATTGGAGTGAGAATTGACGGTGGGGTTTTCACAAAGAGT
381      M  V  E  D  V  L  E  I  G  V  R  I  D  G  G  V  F  T  K  S

1201    GGGCTAATGAGTTGCTTTGATCAAATTCTCTCACAAGAAAAAGGGAAGAAACTGAGGGAA
401      G  L  M  S  C  F  D  Q  I  L  S  Q  E  K  G  K  K  L  R  E

1261    AATCTGAGAGCCCTAAGAGAGACTGCAGACAGGGCAGTTGGTCCTAAAGGGAGTTCTACT
421      N  L  R  A  L  R  E  T  A  D  R  A  V  G  P  K  G  S  S  T

1321    GAGAATTTCAAAACCATGGTGGATTTAGTGTCAAAACCAAAGGATGTCTAG
441      E  N  F  K  T  M  V  D  L  V  S  K  P  K  D  V
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Figure 2. PCR amplified UFGT (A) and (B) the complete cDNA sequence and amino acid sequence of the protein encoded by UFGT (GeneBank accession number: KC936148.

V. rondutifolia UFGT was 98% homologous with that of *V. vinifera* and contained a 1368bp open reading frame (ORF) which encodes a protein of 456 amino acid residues (Figure 2B). This nucleotide sequence has been deposited in the GenBank database (Accession No. KC936148). Protein translation showed UFGT has considerable similarity in its amino acid throughout the entire coding region when compared with other plant-derived UFGTs, with search from NCBI PSI-BLAST revealing high identity and similarity with *V. vinifera* (99%) and *V. lambrusca* (98%). This included the UDP-binding domain of 44 amino acid residues often located in the C-terminal region [27]. Comparison with other grape species showed that there were 30 variable nucleotides. The diversity of anthocyanins usually reflects the modification of common aglycones by hydroxylation, glycosylation, methylation, and acylation [2]. Methylation in plants typically occurs at CpG or CpNpG sites (i.e., where Cy-

tosine is directly followed by a Guanine). Only two mutations at potential methylation sites were identified, with one at base 375 and the other at 1179. The first involved at CT variation (*V. vinifera* = C; *V. rotundifolia* and *V. labrusca* = T), while the other an AC variation (*V. rotundifolia* = C; *V. vinifera* and *V. labrusca* = A). Two-dimensional structural prediction showed *V. rondutifolia* UFGT has 42.32% alpha-helix, 15.13% extended strand, 5.48% beta turn and 37.06% random coil. The alpha-helix and random coil are arranged with interlaced domination of the main part of the secondary structure. The three-dimensional (3D) structure of *V. rondutifolia* UFGT (Figure 3) shared 85.6% similarity with the template, which further facilitated the positive identification of muscadine UFGT.

Figure 3. Three-dimensional (3D) analysis of the UDP-glucose:flavonoid 3-O-glucosyltransferase (UFGT) protein structure.

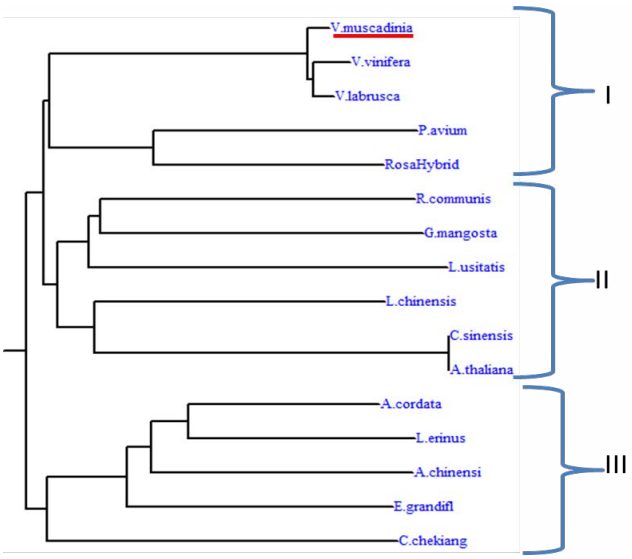

Figure 4. Molecular phylogenetic tree of the deduced amino acid sequences of UDP-glucose:flavonoid 3-O-glucosyltransferases (UFGTs) from different plants. The branches represent the bootstrap support for 1,000 replicates. The muscadine UFGT protein is underlined in red.

Phylogenetic relationships of UFGTs

Phylogenetic tree was constructed using the predicted amino sequence of the putative UFGT proteins from muscadine as well as other plant species (Figure 4). The UFGTs from different plant species were divided into three subgroups: I, II, and III (Figure 4). The cloned UFGT belongs to the subgroup I, close to *V. vinifera* and *V. labrusca*. Part of the branch forming subgroup I are members of the *Vitis* spp. Phylogenetic tree confirmed a close relationship between muscadine UFGT and *V. vinifera* UFGT (Figure 4). These results correspond to previous findings that suggest UFGT is well conserved among plants of different groups and has distinct species specificity.

Conclusion

In this study, the full length cDNA of UFGT from muscadine grapes was cloned and characterized for the first time. Phylogenetic analysis places UFGT from muscadine grapes with UFGT from the *Vitis* subgroup (Figure 4). Spatial and temporal mRNA expression profiling among different tissues and developmental stages suggest that UFGT is most abundant in véraison, and physiologically mature berry skins and is highly expressed in *in-vitro* red cell lines. Berry development and maturity, significantly influence the expression of UFGT in *V. rotundifolia*, thereby, associating the involvement of UFGT in the production of anthocyanin. However, future research involving RNAi-based functional characterization is warranted to establish this link.

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Conflict of Interest

The authors declare no conflicts of interest with this article and content therein.

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