

RuGT1, A New Candidate Proanthocyanidin-related Glycosyltransferase?

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Abstract

Upon investigating anthocyanin and proanthocyanidin (PA) biosynthesis in blackberry fruits, we newly isolated an UDP-glucose: flavonoid 3-O glycosyltransferase (UFGT) gene (Accession No. JF764808), designated as *RuGT1*. It contained the typical signature motif of glycosyltransferases and with high homology to those of UDP-glucose: anthocyanidin 3-O glycosyltransferases. However, its expression patterns were coordinated with PA content along the maturation of blackberry fruits. Together with other recent advances in the plant PA metabolite research, *RuGT1* could be deduced as a candidate PA-related glycosyltransferase.

Keywords: *RuGT1*, proanthocyanidin, glycosyltransferase

1. Introduction

Glycosylation is one of the mechanisms for plants in perceive environmental changes both in and outside plant tissues. This is achieved by a group of enzymes named glycosyltransferases (GTs) (Bowles et al., 2006). They are capable of transferring sugars to a wide range of acceptors, such as hormones, secondary metabolites, biotic or abiotic chemical or toxins in the environment. The best-characterized GTs are those that catalyze the first 3-O-glucosylation event common to the biosynthetic pathway of all anthocyanins. In the building blocks of the flavonoid pathway (Figure 1A, Chen et al., 2012a), glycosylation by UDP-glucose: flavonoid 3-O-glycosyltransferase or other GTs could increase stability or water solubility of the anthocyanins (Bowles et al., 2006). The deficiency of GTs in the white grape berry skins resulted in no anthocyanin accumulations (Boss et al., 1996), while down-regulation *FaGT1*, tremendously decreasing of anthocyanin were observed in strawberry (Griesser et al., 2008).

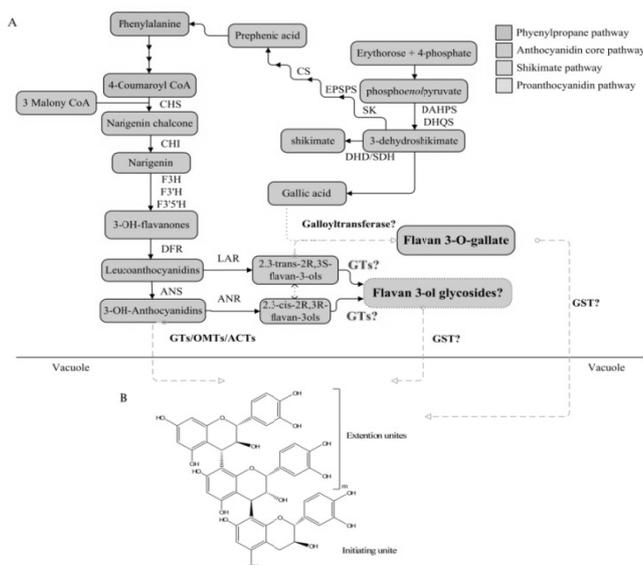


Figure 1. Schematic diagram of anthocyanin and proanthocyanidin biosynthesis pathway

By contrast, limited information was known in the proanthocyanidin branch. In 2008, Pang et al., (2008) isolated a GT gene, *UGT72L1*. It was shown to be induced in *Medicago truncatula* hairy roots in parallel with PA biosynthesis, and could specifically transfer sugar units to epicatechin (Pang et al., 2008). Khater et al. (2012) also isolated three PA-related GTs, which were strongly expressed at the beginning of the green stage and decreased thereafter until reaching very low values after grape veraison. However, the exact substrates, in the forms of glycosylated or not, which were finally condensed to PA (Figure 1B) were still unclear. Here, we briefly introduced our newly isolated RuGT1, which might also be a proanthocyanidin-related glycosyltransferase in blackberry fruits, thus further our standing in the PA biosynthesis pathway.

2. Method

2.1 Plant Materials

The plant material used in the current study was collected from the scientific research base of Sichuan Agricultural University. Fruits of blackberry cultivar 'Arapaho' at different maturation stage as indicated by Chen et al (2012) were used for DNA and RNA isolation. Followed the procedure described by Lodhi et al., (1994) and Jones et al., (1997), pure and integrate nucleic acids were obtained and assessed by gel electrophoresis and UV spectrum scanning.

2.2 Gene Isolation

A pair of degenerate primers was designed referring to the GTs genes in the Rosacea family: GT-F: 5'-CCA GCC ACA GTG AGT CAC A-3' and GT-R: GAC GCG TTN TTG TGG TNT TCG. Genomic DNA was first used to amplify the candidate GTs in blackberry. Standard PCR reaction conditions were used to amplify the responding gene, including 1 × super PCR mastermix (Takara, Dalian, China), 10 μM of each primer and 10 ng DNA or cDNA template. The expected amplicons were cut and cloned into pMD19-T vector (Takara, Dalian, China), sequenced in an ABI 3730 sequencing system (Applied Biosystems, USA). The obtained results were confirmed by BlastN or BlastX against nr or pdb database at NCBI to get at least 75% similarities to the released corresponding GTs. Finally, primers for quantitative PCR (qPCR) were picked out in primer express 3.0.

2.3 Real-time qPCR for Expression Analysis

First strand cDNA was synthesized by using 1 μg total RNA and 1 μl Revert-aid reverse transcriptase (Fermenters, USA) following the protocol of the supplier. qPCR primers qGT-F: 5'-GGA GCT GAA GAA AAG ACT CCA GAA-3' and QGT-R: 5'-ACT GGC CGG ACC AGA TGT AG-3' were picked out in primer express 3.0 avoiding cross homology to other sequences in the blackberry EST library (Lewers et al., 2008). Expression analysis of the RuGT was done by real-time qPCR, using SYBR green method on a CFX96 real-time cycler (BIO-RAD). Each PCR reaction (20 μl) contained 0.3 μM primer (each), 1 μl cDNA (diluted 1:5), and 1 × Takara SYBR Premix. The thermal cycling conditions were 95°C 30s followed by 95°C for 5s, 60°C for 30s for 40 cycles, followed by a melt cycle from 50°C to 96°C. With all cDNAs used, the primer set gave single PCR products, which were verified by determining the melting curves for the products at the end of each run. The efficiency of the primers was tested in preliminary experiments with dilutions of the cDNA and amplified both the gene of interest and internal control beta-actin gene (Forward 5'-TGA CAA TGG GAC TGG AAT GGT-3', Reverse 5'-GCC CTG GGA GCA TCA TCA-3'). Samples were considered positive if they had a cycle threshold (Ct) value < 40 and characteristic amplification plots. All samples were measured triplicate. Every run included the β-actin gene as an internal control for each sample. Gene expression differences were presented as $2^{-\Delta\Delta CT}$ ($\Delta CT = CT_{\text{target}} - CT_{\text{actin}}$) (Schmittgen and Livak., 2008).

3. Results

RuGT1 fragment was amplified via PCR based on the conserved domain of Rosacea GTs amino acids. It was 796 bp in length, containing an 84 bp intron, encoding 238 amino acids. Further screening of other blackberry cDNA libraries (Lewers et al., 2008) did not come up with any EST sequences with significant similarities. Blast analysis revealed the relationship between RuGT1 and other GTs as well as biochemically characterized plant glycosyltransferases. RuGT1 was most similar to the UDP-Glucose: flavonol 3-glycosyltransferase from *Rosa hybrida* cultivar (max identity 82%). Similarities to glycosyltransferases from *Vitis vinifera* (Accession No. xp 002271236), *Aralia cordata* (BAD06514.1), and *Medicago truncatula* (XP 003610163) were also found, all of which transfer either UDP-Glc or UDP-Gal to anthocyanidins or flavonols. Conserved domain search (Figure 2) showed that the RuGT1 was a typical glycosyltransferase GTB type superfamily member, with signature amino acids.

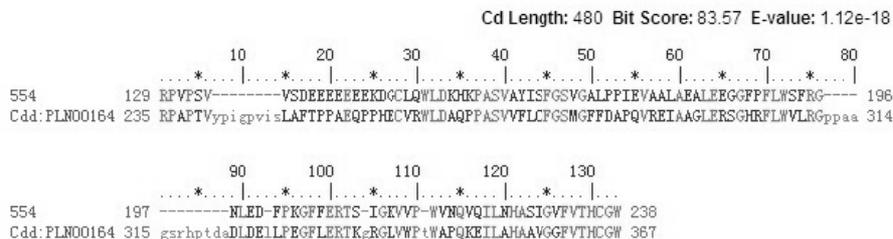


Figure 2. Conserved amino acids hit to the glycosyltransferase PLN00164 domain

At this time point, we believe that RuGT1 was an anthocyanin glycosyltransferase and further our exploring its expression in the blackberry fruits. Because in the most recent years, blackberry fresh fruits or its products were demonstrated to be rich in polyphenol compounds (Cuevas-Rodriguez et al., 2010; Dai et al., 2007), which were healthy-promoted in terms of anti-cancer (Landis-Piwowar et al., 20010), anti-inflammation (Dai et al., 2007) and protecting against age-related neurological disorders (Shin et al., 2006). But the molecular information of this species was still lacking. Quantitative PCR technique was utilized in accession RuGT1 expression patterns at 5 different stages: green, green-to-red, red, red-to-black and ripen black fruit. Interestingly, the expression patterns of RuGT1 were beyond our expectations. From the literature, anthocyanidin glycosyltransferases expression levels were always concomitant to the anthocyanin biosynthesis in plants. For example, FaGT1 in strawberry fruits was strictly expressed and increased its transcript levels when fruits turned red (Almeida et al., 2007). Nevertheless, RuGT1 exhibited a gradually decreasing pattern, with highest transcript levels early in the green stage. When the fruit dramatically accumulated anthocyanin in the turning black stage (red/black in Figure 3), the lowest level was observed (decreased more than 100 fold).

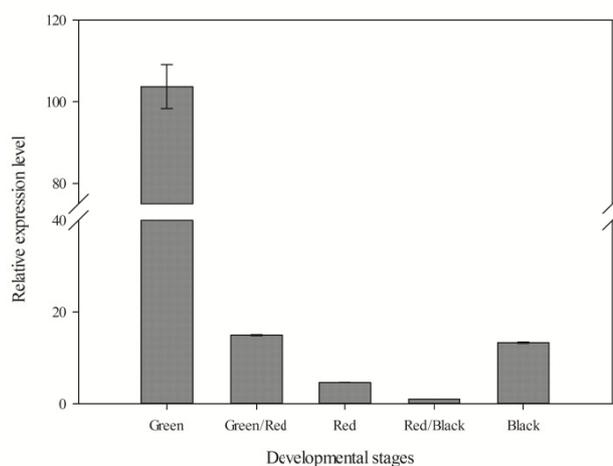


Figure 3. Relative expression levels of RuGT1 in developmental blackberry fruits

Noteworthy, this pattern was coordinated to our past investigations of proanthocyanidins, which were also key compounds in the flavonoid pathway (Chen et al., 2012b). Meanwhile, the pattern was much similar to that of UGT72L1, in *Medicago truncatula* (Pang et al., 2008), which showed highest expression level at early stages, when PA largely increased in quantity, and diminished to a minimum level when PA degraded in the latter developmental stages. Their enzymatic assay revealed that UGT72L1 was capable of transferring sugars to (-)-epicatechin, which was a dominant precursor for PA condensations. It was also the first report of glycosylation on the 4'-OH of PAs related products in plants. However, no more instances had been reported ever since. From the expression styles of our newly *RuGT1*, it might be another PA related glycotransferase. Further studies were in the way in hoping that novel insight could be shed into this field.

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