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Mining and comparison of the genes encoding the key enzymes involved in sugar biosynthesis in apple, grape, and sweet orange

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ABSTRACT

Fruit taste depends on the contents and types of soluble sugars and organic acids. The large-scale identification and comparison of genes involved in sugar biosynthesis in apple, grape, and sweet orange based on the genome and expressed sequence tag information have not been reported. In this study, we found that *sorbitol-6-phosphate dehydrogenase* (*S6PDH*) was one of the most important genes in sorbitol biosynthesis in apple leaf, and was decomposed by *NAD*$^+$*-dependent sorbitol dehydrogenase* (*NAD*$^+$*SDH*) in apple fruit. The high expression level of *soluble acidic invertase* (*AIV*) gene possibly caused the high soluble sugar content (i.e., glucose and fructose) in grape. The expression level of *sucrose synthase* (*SUS*) in grape and sweet orange was also significantly higher than that in apple. The results show that *SUS* was involved in the resynthesis of fructose and UDP-glucose into sucrose in grape and sweet orange. By contrast, sucrose was transported into a vacuole and decomposed into glucose and fructose by *AIV*. Thus, *ADP-glucose pyrophosphorylase* (*AGP*), *granule-bound starch synthase* (*GBSS*), *isoamylase 1* (*ISA1*), and *isoamylase 2* (*ISA2*) could be the key genes in starch biosynthesis, whereas *α-amylase* (*AMY3*), *isoamylse 3* (*ISA3*), and *pullulanase* (*PUL*) could be the key genes in starch degradation in apple. This study provides new insight into the isolation and comparison of candidate genes involved in the sugar biosynthetic pathway among multiple plants.

1. Introduction

Fruit ripening is a complex, genetically programmed process that culminates in dramatic changes in color, texture, flavor, and aroma of the fruit flesh (Alexander and Grierson, 2002). Soluble sugars and organic acids are important components of fruit taste, and aroma can affect the overall organoleptic quality of fruit. Fruit taste depends on the content and type of soluble sugars and organic acids (Pangborn, 1963). In most fleshy fruits, the main soluble sugars are sucrose (Suc), fructose (Fru), and glucose (Glu), whereas the main organic acids are malic and citric acids but sorbitol and quinic acid are also detected at low levels (Moing et al., 1998).

Different fleshy fruits have different contents and types of soluble sugars and organic acids. For example, Fru and Glu are the major sugars that accumulate in mature grape berry, whereas Suc is the major sugar that accumulates in mature peach fruit. The predominant organic acids in ripe peach fruit, grape berries, and citrus fruit are malic and citric acids, malic acid, and citric acid, respectively (Chapman et al., 1991; Diakou et al., 2000; Harker and Maindonald, 1994; Moing et al., 1998; Or et al., 2000; Sadka et al., 2000a,b; Seymour et al., 1993). During the different fruit development stages, the contents and types of soluble sugars also differ in some fruits. Banana has 20% to 25% of starch in the fruit pulp in the early stage of fruit development. Once the climacteric stage occurs, the accumulated polysaccharide is rapidly degraded and most polysaccharides are converted into soluble sugars (Cordenunsi and Lajolo, 1995). The same starch metabolic process is also found in apple, in which starch accumulates in 35 d to 87 d after anthesis (DAA) and declines in 87 DAA to 132 DAA, whereas sugar increases in 87 DAA to 132 DAA; thus, fruit ripening begins slightly earlier than 132 DAA (Janssen et al., 2008). In grape, the accumulation of Glu and Fru commences only at véraison and continues throughout ripening (Davies and Robinson, 1996).

Sugar metabolism and accumulation in plants were discovered and named 'futile recycles' or 'Suc–Suc cycle' (Li et al., 2012; Nguyen-Quoc and Foyer, 2001). In this system, Suc is transported from leaf to fruit, and is converted into Fru and Glu by neutral invertase (**NINV**, EC 3.2.1.26) or into Fru and UDP-glucose (UDPG) by sucrose synthase (**SUSY**, EC 2.4.1.13). The resulting Glu and Fru are then phosphorylated to glucose-6-phosphate (G6P) and fructose-6-phosphate (F6P) by hexokinase (**HK**, EC 2.7.1.1) and fructokinase (**FK**, EC 2.7.1.4). The interconversions among F6P, G6P, glucose-1-phosphate (G1P), and UDPG are catalyzed by phosphoglucoisomerase (**PGI**, EC 5.3.1.9), phosphoglucomutase

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(**PGM**, EC 5.4.2.2), and UDPG-pyrophosphorylase (**UGP**, EC 2.7.7.9) in readily reversible reactions. The F6P produced in sugar metabolism enters glycolysis and the tricarboxylic acid cycle to generate energy and intermediates for other processes. G1P is used for starch synthesis, whereas both F6P and UDPG can be combined to resynthesize Suc via sucrose phosphate synthase (**SPS**, EC 2.4.1.14) and sucrose phosphate phosphatase (**SPP**, EC 3.1.3.24) (Rolland et al., 2006). Starch is degraded into Glu and maltose mainly by α-amylase (**AMY3**, EC 3.2.1.1), isoamylase (**ISA3**, EC 3.2.1.68), β-amylase (**BAM**, EC 3.2.1.2), and disproportionating enzyme (**DPE1**, EC 2.4.1.25), and then exported to the cytosol to be used either for Suc synthesis or as an energy source (Pop, 2009). Most of the Suc, Glu, Fru, and other soluble sugars that have not been metabolized are transported into the vacuole by special transporter proteins located on the vacuole membrane. Once inside the vacuole, Suc can also be converted to Glu and Fru by vacuolar acid invertase (Rolland et al., 2006). The key enzyme-encoding genes in sugar biosynthetic pathways have been isolated, analyzed, or cloned in many plants, such as *Arabidopsis thaliana*, *Malus domestica*, and *Vitis vinifera* (Davies et al., 1999; Fillion et al., 1999; Kanayama et al., 1992; Karve et al., 2012; Manning et al., 2001; Vargas et al., 2008; Yamada et al., 1998; Zheng et al., 2011).

Two sequence resources are important for functional and comparative genomic studies. Whole genome sequencing projects (http://www.mgrc.com.my/list_eukaryotic_genomes.shtml) have been accomplished for more than 30 plants, and the genome sequence and predicted gene information of fruits are freely available to researchers. Another important online resource of these plants is expressed sequence tags (EST), which are widely used in gene identification, genetic linkage map construction, genome sequence annotation, DNA marker development, gene expression profile analysis, etc. (Chen et al., 2010; Graham et al., 2004; Huang et al., 2012; Johnson et al., 2010; May et al., 2008; Murray et al., 2005; Toulza et al., 2010; Wongsurawat et al., 2010). As of January 1 2013, 74,186,692 ESTs have been deposited in the dbEST of the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html). A large number of ESTs (>100,000 ESTs) have been reported in plants, such as *V. vinifera* (446,664), *M. domestica* (325,020), and *Citrus sinensis* (214,598).

Genome sequencing, ESTs, and bioinformatic tools provide new techniques in discovering differences in sugar biosynthetic pathways among apple, grape, and sweet orange. The aim of this study is to identify and analyze the differences among genes that encode key enzymes in sugar biosynthetic pathways in four fruits using genome and EST information.

2. Materials and methods

2.1. Data and tool preparation

Two sequence formats of ESTs (EST-GenBank format and EST-FASTA format) for apple (*M. domestica*), grape (*V. vinifera*), and sweet orange (*C. sinensis*) were downloaded from the dbEST of NCBI (http://www.ncbi.nlm.nih.gov/nucest/).

The predicted gene sequences (protein and nucleotide sequences) were downloaded from the Orange (*C. sinensis*) Genome Annotation Project Database (http://citrus.hzau.edu.cn/orange/), Genome Database for *Rosaceae* (http://www.rosaceae.org/), and Genoscope (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/) (Jaillon et al., 2007; Velasco et al., 2010; Xu et al., 2012). The target sequences of enzyme-encoded genes were downloaded from The Arabidopsis Information Resource database (TAIR, http://www.arabidopsis.org/) and NCBI nucleotide database (http://www.ncbi.nlm.nih.gov/nuccore). BLAST (2.2.27) was downloaded from the NCBI FTP server (ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/).

2.2. Isolation of homologous genes in sugar biosynthetic pathways in apple, grape, and sweet orange

To identify the homologous genes in sugar biosynthetic pathways in apple, grape, and sweet orange, we used the gene sequences of *A. thaliana* and other species as queries to search whole genome protein datasets using the BLASTp program. The searching parameters were: *E*-value >1×10^{-10} and score >100. By manual correction and analysis of conserved domains, the predicted genes of the three fruits were isolated.

2.3. EST analysis of related genes in the sugar biosynthetic pathway

The protein sequences of candidate genes in apple, grape, and sweet orange were used as queries to search the sugar biosynthesis related-EST sequences in each plant EST database. The alignment parameters were: score >100 and *E*-value >1×10^{-20}. The tissue types of selected ESTs were isolated from the EST-GenBank format file, which was downloaded from NCBI dbEST.

3. Result

3.1. Identification of genes involved in the sugar biosynthetic pathway in apple, grape, and sweet orange

Based on previous reports (Li et al., 2012; Loescher, 1987; Lytovchenko et al., 2007; Ohdan et al., 2005; Thitisaksakul et al., 2012; Yamaki, 1995), Suc and sorbitol are synthesized in leaf, transported to fruit by transport proteins, and synthesized or decomposed to Fru, Glu, and starch by a series of key enzymes (Fig. 1). In these three plants, more than 86,000 genes were annotated from the genome sequences in the three fruits, namely, 30,294 genes in apple, 26,346 genes in grape, and 29,445 genes in sweet orange. A total of 77 *A. thaliana*, *M. domestica*, and *Pyrus pyrifolia* genes involved in the sugar biosynthetic pathway were downloaded from TAIR and NCBI database, and used to search for homologous genes in the three fruits (Table 1, details in Table S1). In *Rosaceae* plant leaves, sorbitol was found to be synthesized by sorbitol-6-phosphate dehydrogenase (**S6PDH**, EC 1.1.1.200) and sorbitol-6-phosphate phosphatase (**SorPP**, EC 3.1.3.50) enzymes.

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.scienta.2013.11.026.

In this study, two S6PDH enzyme-encoding genes were identified in apple (MDP0000408705 and MDP0000312001). Only one S6PDH enzyme-encoding gene was discovered in grape (GSVIVT01024164001) and sweet orange (Cs8g07590.1), respectively. No SorPP enzyme-encoding gene was identified in these three plants because the SorPP enzyme-encoding gene has not been identified in a model plant in the previous report, and cannot be used as the query in BLASTp search (Table 1, details in Table S1).

In the sucrose biosynthetic pathway, **PGM**, **UGP**, **SPS**, and **SPP** have vital functions in plant leaves. Through BLASTp search, two PGM enzyme-encoding genes, three UGP enzyme-encoding genes, five SPS enzyme-encoding genes, and one SPP enzyme-encoding gene were identified in apple in this study. Two PGM enzyme-encoding genes and two UGP enzyme-encoding genes were identified in grape, whereas one PGM enzyme-encoding gene and one UGP enzyme-encoding gene were identified in sweet orange. Four SPS enzyme-encoding genes were identified in grape and sweet orange. Two SPP enzyme-encoding genes were