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Research article

Transcriptome analysis reveals the effects of grafting on sugar and α-linolenic acid metabolisms in fruits of cucumber with two different rootstocks

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ABSTRACT

Flavor quality in cucumber is affected by different rootstocks, but the molecular mechanism is largely unclean. To clarify the differences of sugar and aromatic compounds, cucumber (*cucumis sativus*) fruits from plants of self-grafted (SG) or grafted onto figleaf gourd (*Cucurbita ficifolia*; G1) or 'Weisheng No.1' rootstock (*Cucurbita moschata* x*Cucurbita moschata* hybrids; G2) were performed the transcriptome analysis. We obtained 1013 and 920 differentially expressed genes (DEGs) from G1 and G2 compared to SG respectively, in which 453 genes were co-expressed. Functional annotations showed many DEGs were involved in glycolysis/gluconeogenesis metabolism, fructose metabolism and α-Linolenic acid metabolisms, 20 DEGs were selected from the 3 pathways to validate sequencing accuracy by quantitative real-time PCR. The gene relative expression levels were concurrent with RNA-seq results and sugar and aromatic compounds content phenotypes. Moreover, some vital transcript factors and transport proteins were analyzed. These findings indicate that different rootstocks could induce significantly changes in the physiological profiling and transcripts of sugar- and aromatic flavor-related genes. This study provides a novel insight into the molecular mechanisms of fruit quality regulated by candidate genes.

1. Introduction

Cucumber (*Cucumis sativus* L.) is important vegetables worldwide, and its excellent cultivation greatly depends on grafting by increasing resistance to abiotic stresses, soil-borne pests and pathogens (Xia et al., 2009; Xin et al., 2017). Although grafting is an environmentally friendly way to improve the growth and resistance of cucumber, the fruit quality significantly decreased (Kyriacou et al., 2017). Changes in fruit quality of grafted cucumber are mainly determined by selecting appropriate combinations of scion and rootstock (Colla et al., 2013). Several rootstocks for cucumber grafting have been applied, such as *Cucurbita* maximax*C. moschata*, *C. ficifolia*, *C. moschata*, *C. argyrosperma*, *Lagenaria siceraria*, *Benincasa hispida*, *Luffa cylindrica* (L.) M. Roem., *Momordica charantia* L., *Sicyos angulatu* (Rouphael et al., 2010, 2012). To date, the key regulatory molecular pathway in fruit quality of grafting cucumber with different rootstocks in unknown.

Abbreviations: G1, fruit obtained from cucumber seedlings grafted onto figleaf gourd (*Cucurbita ficifolia*) rootstock; G2, fruit obtained from cucumber seedlings grafted onto 'Weisheng NO.1' (*Cucurbita moschata* x*Cucurbita moschata* hybrids) rootstock; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; RNA-Seq, transcriptome sequencing; DEGs, differentially expressed genes; DAA, days after anthesis; SSC, soluble sugar content; HPLC, high performance liquid chromatography; HP-SPME-GC-MS, high performance (HP)-solid phase micro extraction (SPME)-gas-chromatography-mass spectrometry (GC-MS); FPKM, fragments per kb per million reads; ALDH, aldehyde dehydrogenase; ADH, alcohol dehydrogenase; AOS, hydroperoxide dehydratase; AOC, allene oxide cyclase; ORPs, 12-oxophytodienoic acid reductase; LOX, lipoxygenase; PFK1, 6-phosphofructokinase 1; RFOs, raffinose family oligosaccharides

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The fruit quality of cucumber is becoming increasingly important to consumers around the world (Condurso et al., 2012; Verzera et al., 2014). Sweetness and flavor are the two most prominent elements that determine fruit quality. Fructose, glucose and sucrose are considered to be the main soluble sugars existing in fruit. Sucrose is the only soluble sugar that serves as a basic material for the synthesis of other crucial quality components, such as aromatic compounds and pigments (Proietti et al., 2008). The degree of sweetness is generally depended on the relative proportions of the three main sugars. Previous study has shown the contents of glucose and fructose are higher than that of sucrose in both mesocarp and endocarp tissue of cucumber fruit (Handley et al., 1983), cucumber is RFOs (raffinose family oligosaccharides)-transporting plant and sucrose rather than stachyose or raffinose is the transport sugar from the peduncle to fruit (Hu et al., 2009). More than 70 volatile substances have been characterized in cucumber fruit, with aldehydes and alcohols being the main sources of its aromatic flavor (Hao et al., 2013). Aldehydes are largely attributed to providing the fresh taste in cucumber, and *trans*-2, *cis*- 6-nonadienal was identified as contributing a pleasant note to the overall flavor (Forss et al., 1962; Chen et al., 2015). The aldehydes content in cucumber varies with the developmental stages of fruit and breeds.

As to changes of fruit quality after grafting, some controversies are mostly about the three profiles, i.e., improvement, reduction and constant status. Five *Cucurbita* rootstocks and two genotypes of melons were shown to be compatible, and had a symbiotic relationship and no negative effects on fruit quality (Condurso et al., 2012). Grafting could potentially affect the aroma compounds in the peel and flesh from cucumbers grafted with bottle gourd rootstock, and this analysis showed grafting could increase total alcohols content and decrease the main aldehyde levels, while no significant differences in ketones, terpenes and hydrocarbons. Many experiments have indicated that the combination of rootstock and scion contributes to the influence of aroma (Martínez-Ballesta et al., 2010; López-Marín et al., 2017) and sweetness profiles (Rouphael et al., 2010) in cucumber fruit. However, the molecular mechanism by which grafting regulates sweetness and aroma synthesis is not clear.

The genome-wide transcriptome analysis could help to elucidate the specific and underling molecular mechanisms of grafting-depended biochemical processes. In a transcriptome analysis of grafted apple, growth and development of the rootstock were found to be modulated by physical mechanisms, including sugar metabolic process and auxin and cytokinin signaling pathways, while sugar content was not affected in MB- grafted roots (root of M. robusta rootstock grafted with the scion of more branching) (Li et al., 2016). Many studies have shown that gain-of-function transcripts had much effect on tissue biological activities, impacting greatly on certain characters such as leaf formation and root structure (Mahajan et al., 2012). A previous study evaluated the molecular aspects of microRNAs and mRNAs and RNA-regulated mechanisms in grafted watermelon seedlings. Using high-throughput sequencing, 20 and 47 miRNAs, 787 and 3485 genes were differently expressed in watermelon grafted onto bottle gourd and squash rootstocks, respectively, compared with self-grafted watermelon without any stress treatments (Liu et al., 2013, 2016). The grafting-responsive microRNAs and mRNAs may play prominent roles in mediating diverse biological and metabolic processes. The availability of cucumber wide genome sequence and the advance of high-throughput sequencing technique, it could be beneficial to further research metabolic pathways in fruit quality formation of grafting cucumber (Huang et al., 2009; Shang et al., 2014).

In this study, cucumber was grafted onto two rootstocks of figleaf gourd (*C. ficifolia Bouché*) and Weisheng No.1 rootstock (*C. moschata* x *C. moschata* hybrids), and self-grafted was used as the control. In order to explore gene functions and genes-regulated networks in fruit quality of cucumber grafted onto different rootstocks, we combined the analysis of fruit phenotype dynamics and overall transcriptome sequence to perform. Moreover, we observed the secondary metabolisms and screen for some differentially expressed genes (DEGs) involved in the biosynthesis of sugar and aromatic flavor to validate the sequencing accuracy by quantitative real-time PCR (qRT-PCR). The overall transcriptome analysis of fruit quality in cucumber grafted onto two rootstocks in our study offered a reasonable foundation for deeply digging the functions of candidate genes and regulatory metabolisms.

2. Materials and methods

2.1. Plant materials and grafting treatments

Cucumber (*Cucumis Sativus* cv.'Jindi No.1', obtained from Jinzhou Jindi Seedings Company, Jinzhou, Liaoning, China) was grafted onto two different squash rootstocks: figleaf gourd rootstock(*Cucurbita ficifolia*, obtained from Horticulture Research Institute, Yunnan Academy of Agricultural Science, Kunming, China, G1) and 'Weisheng No.1' rootstock(*Cucurbita moschata* x*Cucurbita moschata* hybrids, obtained from Vegetable Research Institute, Liaoning Academy of Agricultural Science, Shenyang, China, G2). Cucumber seedlings grafted onto cucumber seedlings were used as the control (SG).

The grafted seedlings were cultured in a greenhouse in Vegetable Research Institute, Liaoning Academy of Agricultural Science, Shenyang, Liaoning, China(41°49′N, 123°32′E). Tapped and recorded the numbers of nodes and days after anthesis (DAA) carefully, and cucumbers were harvested on the 9 DAA from the 10th to 12th node (the first node is described as the first true leaf at the basal part of cucumber plant).

We determined soluble solids content (SSC), sugar and volatile compound profiles from at least 30 fruits of G1, G2, and SG. For transcriptome analysis, the mesocarp in the middle part of cucumber fruit was cut into small pieces, immediately wrapped in aluminum foil, frozen in liquid nitrogen, and stored at −80 °C until they were used for RNA extraction.

2.2. Soluble solid contents (SSC), soluble sugar and volatile compounds profiles

The SSC was measured by dripping freshly extracted juice onto a digital refractometer (DBR45, Huixia, Fujian, China) (Mendoza et al., 2012). The soluble sugar content was performed by a HPLC instrument (Waters 600E, USA), a Prevail carbohydrate ES 5u column (250 mmx4.6 mm) and an evaporative light scattering detector (Alltech 2000 ES, USA) according to the method described in Guo (Guo et al., 2017). The volatile compounds content and types were measured using headspace (HP)-solid-phase-micro extraction (SPME)-gas-chromatography-mass spectrometry (GC-MS) according to the published methods (Guo et al., 2017), and 10 μl of a 59.5 mg/l 1-octanal was used as the internal standard. The values were measured by three biological replicates.

2.3. RNA extraction, cDNA library preparation and RNA sequence

Total RNA was isolated with TRIzol Reagent (Takara, Japan) following the manufacturer's manuals and each sample was performed by three biological triplicates. DNase I (Promega, USA) was used to remove contaminating genomic DNA. Transcriptome sequencing was conducted after evaluating the quality, concentration and integrity of the total RNA samples using Agilent 2100 Bioanalyzer (Agilent RNA 6000 Nano Kit, USA) and ABI Step One Plus Real-Time PCR System (Takara, Japan). The purified mRNA was fragmented, first-strand cDNA was synthesized using Reverse Transcriptase, and second-strand cDNA was then synthesized with DNA polymerase and RNase H. cDNA libraries were constructed, clustered and sequenced. The data was sequencing using the Illumina HiSeq™ 4000 system at InGene Biological Technology Co., Ltd. (Shenzhen, China). The raw reads were filtered using cutadapt (version1.13) software and the clean reads were saved