

砂糖橘和砂糖灯笼橘性状差异的转录组分析

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摘要:【目的】通过比较砂糖橘和砂糖灯笼橘性状差异及利用转录组测序数据, 筛选砂糖橘和砂糖灯笼橘叶、果肉、橘络、橘皮之间的差异基因, 探究砂糖橘自然变异品种砂糖灯笼橘橘皮、叶中与蜡质生物合成相关的显著差异基因。【方法】分别取同一生长时期的砂糖灯笼橘和砂糖橘进行观察记录, 取叶、果肉、橘络、橘皮4个组织样本, 液氮速冻, 设立3个生物学重复进行转录组测序。分析2个物种间的转录组测序结果, 寻找关键差异基因。【结果】通过砂糖灯笼橘与砂糖橘对比, 砂糖灯笼橘叶较大、卷曲, 边缘性状不规则; 果实偏大, 果皮表面布满沟壑, 存在较多点状突起, 同时叶和果皮部位存在蜡质缺失。砂糖灯笼橘叶存在差异基因数量最多, 其次为果肉、皮、橘络, 差异基因主要参与的功能为蛋白结合、分子结合、ATP结合。砂糖灯笼橘叶、果皮分别存在12个、9个与蜡质生物合成相关的显著差异基因, 其中包含5个相同的下调基因, 为 *CER1*、*CER3*、*HHT*、*P45086A8*、*P45086A22*, 这些基因极有可能是导致砂糖灯笼橘表面蜡质排布不均的关键基因。【结论】砂糖橘与砂糖灯笼橘性状差异明显, 转录组数据显示叶的差异基因数量最多, 其中叶和果皮中存在5个表达趋势一致的相同蜡质合成基因, 可能是导致砂糖灯笼橘表面蜡质缺失的关键基因。

关键词: 砂糖灯笼橘; 砂糖橘; 性状; 转录组; 蜡质

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Transcriptome analysis based on the characteristic difference between Shatang tangerine and Shatang Lantern tangerine

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Abstract: 【Objective】 In order to investigate the reason for characteristic differences between Shatang tangerine (*Citrus reticulata*) and Shatang Lantern tangerine, we compared the size of plants, waxy arrangement and transcriptome information. By using transcriptome sequencing data, we screened out the differential genes among the leaves, pulp, tangerine pith and peel of Shatang tangerine and Shatang Lantern tangerine, and explored the significant differential genes related to wax biosynthesis in the peel and leaves. 【Methods】 Four types of tissue samples including leaf, pulp, tangerine pith and peel were taken at the same growth period and frozen in liquid nitrogen immediately for transcriptome sequencing. Three biological replicates were set up for every experiment. Characteristic analysis and toluidine blue staining experiment were observed and recorded by naked eyes. The transcriptome sequencing results between the two samples were analyzed to find out the key differential genes. The change threshold set as the absolute value of \log_2FC (fold change) was greater than or equal to 1 (FC greater than or equal to 2) and q value was less than 0.05. Besides, we used <https://www.omicstudio.cn/index> as a tool to analyze difference genes, like GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis. 【Results】 Compared with Shatang tangerine, the leaves of Shatang Lantern tangerine were larger and curly, and had irregular edge. The fruit was larger and had a coarse and roughly textured rind. The peel in Shatang Lantern tangerine showed obvious gully, and the surface of the peel was

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full of dots, like a “lantern”. After toluidine blue staining, the peel and leave of Shatang Lantern tangerine was covered with dye, indicating that the waxy composition in these parts was loosely distributed. After filtering raw sequencing data of transcriptome, the effective data size was 6.32–8.00 G, respectively. Besides, the effective data accounted for more than 96% of the original data, among which the bases with mass value ≥ 30 accounted for more than 97% (Q 30%), indicating that the quality of the measured transcriptomic data was good and the results were reliable. When compared with the reference genome of *Citrus clementina*, the matching rate was 93.32%–95.17%, which could meet the conditions of subsequent bioinformatics analysis. Through comparative analysis of transcriptome data, there were more differential genes in the leave of Shatang Lantern tangerine, followed by pulp, peel and tangerine pith. The main functions of differential genes were protein binding, molecular binding and ATP binding. The number of differential genes ranked in descending order was leaf, pulp, pericarp and tangerine pith, the number of up-regulated genes ranked in descending order was leaf, pulp, pericarp and tangerine pith, which was the same as the number of down-regulated genes. The number of differential genes in leaves were much higher than that of other tissue parts, and the number of down-regulated genes was the largest (3239), followed by the number of up-regulated genes (2443). Tangerine pith had the lowest number of differential genes. **【Conclusion】** These results indicated that the differences between Shatang tangerine and Shatang Lantern tangerine were mainly reflected in leaves, pulp and peel, which was consistent with the results observed by naked eyes. According to the differences in wax distribution of the two cultivars, the genes involved in the keratin, suberin and waxy biosynthesis pathway (map00073) were analyzed. There were 12 and 9 different genes related to wax biosynthesis in the leaf and peel of Shatang Lantern tangerine, respectively, including 5 identical down-regulated genes, named as *CER1*, *CER3*, *HHT*, cytochrome *P45086A8* and cytochrome *P45086A22*. These genes were highly likely to be the key genes causing uneven waxy distribution on the surface of Shatang Lantern tangerine. Most of the differential genes were down-regulated except *FACR2* in pericarp and cytochrome *P45086B1* and cytochrome *P45096A1* in leaf. Therefore, the characteristics of Shatang tangerine and Shatang Lantern tangerine were significantly different including morphology and waxy distribution. The transcriptome data showed that the number of differentially expressed genes was largest in the leaves, and there were five identical wax synthesis genes with the same expression trend in the middle leaves and the peel, which may be the key genes leading to the loss of wax on the surface of Shatang Lantern tangerine.

Key words: Shatang Lantern tangerine; Shatang tangerine; Character; Transcriptome; Wax

砂糖灯笼橘(Shatang Lantern tangerine)为砂糖橘^[1]的自然变异品种,其表皮布满沟壑,酷似灯笼,故而得名。砂糖灯笼橘产地为广西,形态独特美观,口感较砂糖橘好,糖度高,深受广大消费群体欢迎。对该变异进行品质相关研究具有重要意义。

依靠目前RNA-Seq技术获得的转录组数据在植物功能基因挖掘、植物性状分析、辅助植物育种方面已获得较多成果。如葛卉等^[2]通过对金枝黄花柳亲本及回交子代进行转录组测序,发现两者叶绿素合成酶相关基因表达量出现差异,从而使代谢通路发生变化,进而导致枝条颜色存在明显差异。蔡宇等^[3]通过建立沟叶结缕草的Illumina高通量测序平

台,以野生型和多穗突变体为样本进行转录组测序,挖掘与花序发育相关的重要基因,结合WGCNA分析认为EHD1、MADS14为影响花序发生的关键基因,为沟叶结缕草的植物育种提供了关键的技术支撑。转录组数据结合植物形状分析往往能提供更多的参考证据,如郑雪梦等^[4]通过对京欣3号、甜王2种硬度不同的西瓜进行转录组数据测序,分析后发现硬度较大的甜王与碳水化合物代谢、脂质代谢途径相关的基因与京欣3号具有显著性差异,通过生物信息学分析为品种筛选提供了参考依据。通过对草莓白肉突变体小白及亲本草莓红颜的转录组测序,范小青等^[5]发现小白草莓中存在参与苯丙素、类

黄酮、黄酮醇生物合成途径的差异基因,这些基因与花青素代谢具有密切的关联,该研究为草莓花色苷形成提供了理论依据,也为白草莓的育种提供了一定的支持。刘恋等^[6]针对同一遗传背景下的3个金柑品种的果皮性状差异,选取了同一时期的滑皮金柑、融安金柑、脆蜜金柑的果皮进行转录组测序,发现融安金柑在苯丙烷生物合成途径中的关键基因的表达与其他两者存在明显差异,并且木质素合成相关基因的表达量明显降低,进而果皮表现出韧性差、易失水的特征。由此可见,通过转录组测序技术可以从分子水平上对植物性状进行探究,可以挖掘重要的特征基因,有助于更好地利用基因工程技术实现品种的高产及优质生产,对植物育种的发展具有显著意义。

柑橘类物种果实表面布满蜡质,是植物抵御外界病虫害的第一层保护屏障,同时有研究发现,蜡质对果实保水、延缓衰老及贮藏保鲜等具有重要作用^[7],目前果实蜡质生物合成途径已经基本被解析清楚,蜡质排布及蜡质含量对于植物的品质极其重要^[8-9]。同时,干旱、盐胁迫等环境对于植物表面蜡质生物合成也极其重要,吕慧等^[10]通过测定不同土壤含水量、不同钠盐环境下生长的辣椒转录组数据,发现干旱、盐胁迫主要影响苯丙烷的生物合成途径,适度的胁迫会使辣椒素积累更多。Tanaka等^[11]利用甲苯胺蓝染液对拟南芥叶片进行染色,发现对于表面蜡质缺失或排列分布不均的拟南芥叶片,甲苯胺蓝能够快速将其染色,大大加快了蜡质分布的检测速度,因此该方法广泛应用于植物中。

目前砂糖灯笼橘相关的研究报道较少,笔者以同一产地、同一生长时期的砂糖橘、砂糖灯笼橘为研究材料,对其表型进行观察对比,同时取叶、果实、橘络、果肉4个组织部位分别进行转录组测序,进行对比分析,重点关注与蜡质生物合成相关的关键基因,研究结果可为探究砂糖灯笼橘表型特异性原因及蜡质生物合成的变化提供分子水平上的理论支持及参考。

1 材料和方法

1.1 试验材料

砂糖灯笼橘及砂糖橘(*Citrus reticulata* ‘Shiyue Ju’)产地均为广西,砂糖灯笼橘样本为自然变异品种,均为3年生植株,采收于2021年10月下旬。选

择成熟程度相近、大小相仿的果实,按照果肉、橘络、果皮、叶4个部位进行分离,果肉保留囊衣,果皮保留白皮层,分别取3株砂糖灯笼橘、砂糖橘植物,设立3个生物学重复,用锡箔纸包裹,依次编号,迅速放置于液氮速冻,将样本送至杭州联川生物技术股份有限公司进行转录组测序。对测序得到的原始数据进行质控、差异基因分析及筛选。

1.2 性状分析

分别取相同生长发育时期的砂糖灯笼橘及砂糖橘果实、叶,对其表观形态进行观察记录,比较性状差异。

1.3 蜡质排布分析

分别取砂糖灯笼橘、砂糖橘的橘皮、叶,置于甲苯胺蓝染色液(1%)中浸泡2~4 h,清水冲洗0.5 h,比较染色部位的差异。

1.4 转录组数据分析

委托联川生物技术公司对样品进行RNA提取及转录组测序。

通过Cutadapt等^[12]将含接头、polyA、polyG的reads、N>5%的reads、低质量reads等去除,获得有效数据。以柑橘属克里曼丁橘(*Citrus* ‘Clementina’)参考基因组数据为参照,使用HISAT2(<https://ccb.jhu.edu/software/hisat2>)^[13-15]进行比对,使用StringTie软件(<https://ccb.jhu.edu/software/hisat2>)^[14,16-17]对转录本进行组装并用FPKM^[15,18]定量。分别对砂糖灯笼橘、砂糖橘4个组织部位的转录组数据进行比较分析。针对物种间性状差异,筛选可能引起表型差异的关键基因。使用R包edgeR(<https://bioconductor.org/packages/release/bioc/html/edgeR.html>)对样本之间的差异基因进行分析,设定变化阈值为 \log_2FC (fold change)绝对值 ≥ 1 (差异倍数 $FC \geq 2$),同时 q 值 < 0.05 作为筛选两者差异基因的标准。对筛选得到的差异基因进行统计分析,利用联川生物云平台(<https://www.omicstudio.cn/index>)的在线绘图工具绘制GO富集分析^[19-20]、KEGG通路富集分析^[21]图。

2 结果与分析

2.1 砂糖灯笼橘、砂糖橘的性状特征

对同一时期的砂糖灯笼橘、砂糖橘叶及成熟果实外观形态进行观察对比(图1),发现砂糖灯笼橘叶卷曲程度比砂糖橘明显,砂糖灯笼橘叶偏大,且叶边缘呈现不规则形态,而砂糖橘边缘较平滑;砂糖灯

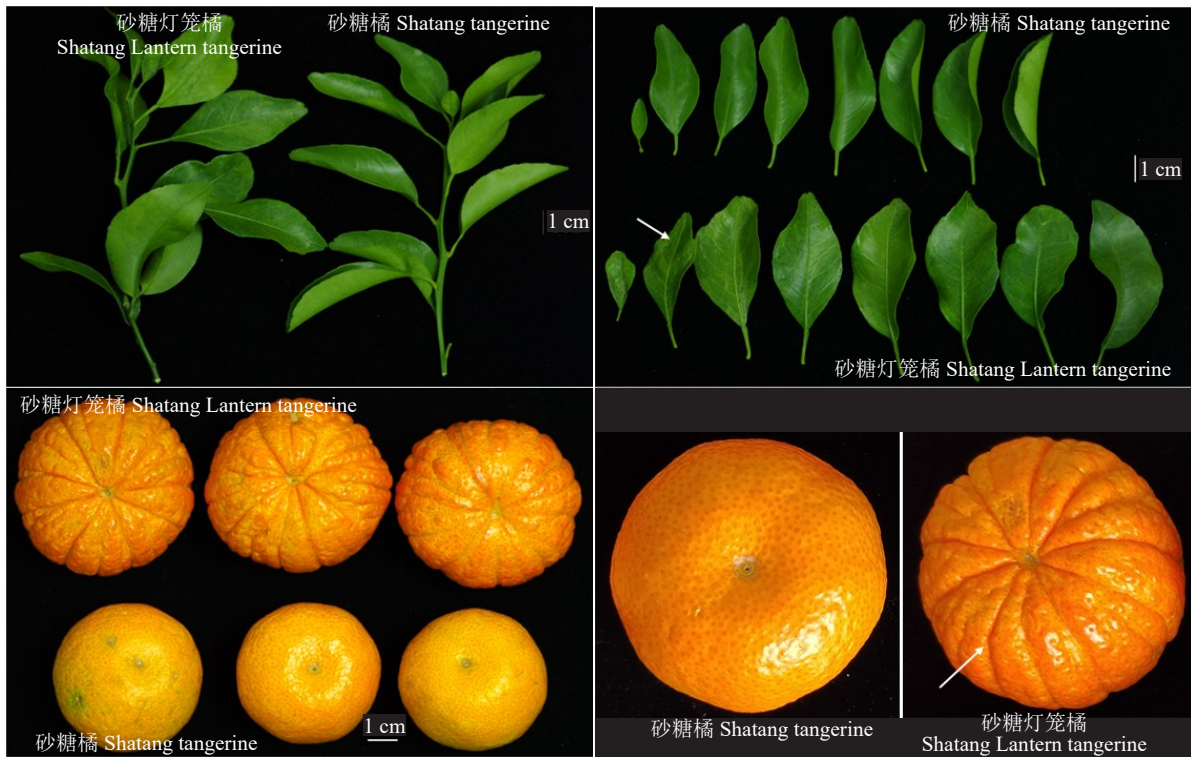


图 1 砂糖灯笼橘、砂糖橘性状特征及对比

Fig. 1 Comparison of characters and characteristics of Shatang Lantern tangerine and Shatang tangerine

灯笼果实较砂糖橘偏大,且果皮呈现明显的沟壑,果皮表面布满点状突起,形似“灯笼”。

2.2 砂糖灯笼橘、砂糖橘蜡质分布

如图2所示,甲苯胺蓝染料能对植物表面蜡质排布不均匀的地方进行染色,通过对砂糖灯笼橘、砂糖橘果皮及叶的染色实验,发现蜡质分布不均匀且

主要存在于果脐附近,砂糖橘整个外果皮能被均匀染色,而砂糖灯笼橘仅果实表面突起的位置极易被染色,这部分蜡质缺失现象较明显,说明在自然变异过程中,砂糖灯笼橘中存在的某些与蜡质生物合成相关基因在转录水平表达量发生变化,进而导致这种现象发生。

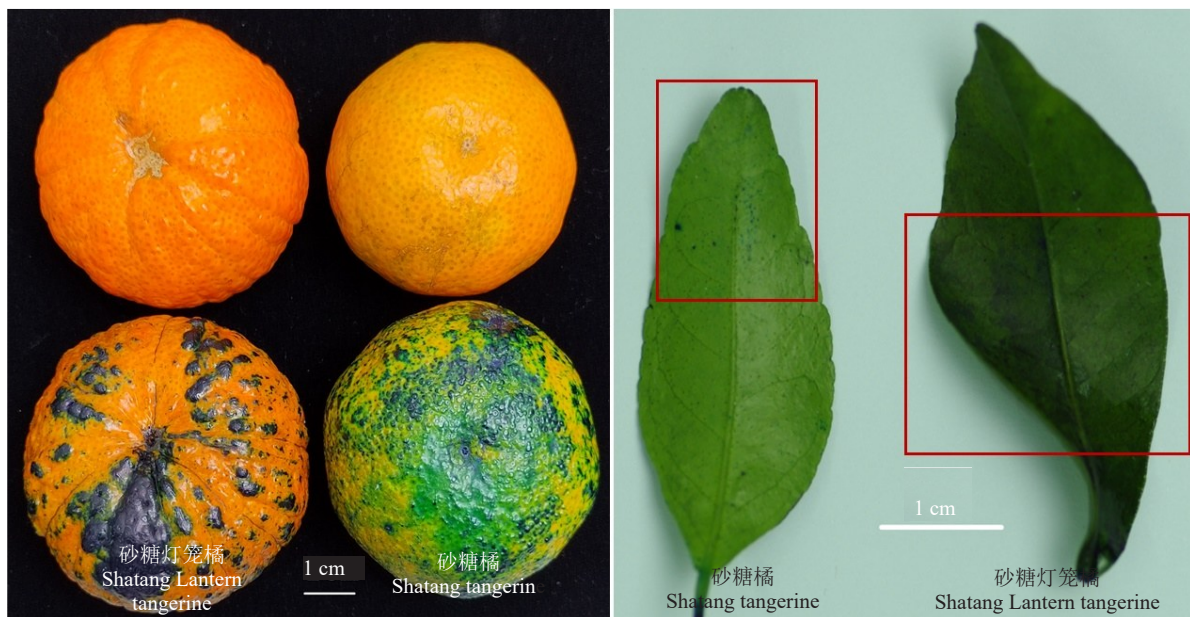


图 2 砂糖灯笼橘、砂糖橘蜡质染色对比

Fig. 2 Comparison of wax staining of Shatang Lantern tangerine and Shatang tangerine

2.3 砂糖灯笼橘、砂糖橘转录组测序质量评估

将高通量测序后的原始数据去除接头、未识别数据等获得有效数据。通过基因组比对,对各基因的表达量进行分析。

经过过滤后有效数据大小为6.32~8.00 G,从表

1中可以看出,有效数据占原始数据的比例均为96%以上,而其中质量值 ≥ 30 的碱基所占的比例(Q30%)皆大于97%,说明测得的转录组数据质量较好、结果真实可信。与克里曼丁橘参考基因组进行比对,其匹配率为93.32%~95.17%,能够满足后续生

表1 砂糖灯笼橘、砂糖橘转录组测序数据信息

Table 1 Transcriptome sequencing data information of Shatang Lantern tangerine and Shatang tangerine

组织 Tissue	命名 Name	有效数据比例 Valid data/%	质量值 ≥ 30 的碱基所占百分比 Q30/%	GC含量 GC content/%	比对到参考基因组比例 Reads mapped reads/%
果肉 Pulp	STJ-GR DLJ-GR	96.60~97.03 96.80~97.57	97.48~97.80 97.80~97.87	44.50 44.50	94.88~95.17 94.74~94.85
橘络 Tangerine	STJ-JS DLJ-JS	96.94~97.38 97.48~97.54	97.59~97.63 97.84~97.96	43.50 43.50	94.44~94.50 93.96~94.02
果皮 Pericarp	STJ-GP DLJ-GP	96.62~97.79 97.12~97.56	97.46~97.69 97.74~97.78	44.00 44.00	94.43~94.84 94.44~94.50
叶 Leaf	STJ-Y DLJ-Y	96.41~96.67 97.02~97.32	97.70~97.74 97.74~98.02	43.50 43.50	93.32~93.58 93.90~94.12

物信息学分析的条件。

2.4 砂糖灯笼橘、砂糖橘差异表达基因分析

分别对砂糖灯笼橘、砂糖橘中提取到的有效基因进行统计分析,对叶、果肉、橘络、果皮4个组织部位的差异基因进行比较(图3)。发现差异基因数量总数排列为叶>果肉>果皮>橘络,上调基因数量排列为叶>果肉>果皮>橘络,下调基因数量排列为叶>果肉>果皮>橘络;砂糖灯笼橘叶的上、下调差异基因数量远高于其他组织部位,下调基因的数量最多,为3239个,其次是上调基因的数量,为2443个;橘络的差异基因数量最少。这些结果说明

砂糖灯笼橘、砂糖橘的差异主要体现在叶、果肉、果皮这3个部位,这与肉眼观察到的结果一致。

同时,对不同组织的差异基因分别进行GO、KEGG分析。结果发现,叶内显著差异基因主要参与的KEGG通路包括植物-病原互作、核糖体、ABC转运蛋白、半乳糖代谢、磷酸肌醇代谢。GO分析显示,差异基因主要参与的生物学过程为抗逆、生物反应、DNA复制的转录调控;差异基因主要位于细胞核、质膜、膜整体组件;参与的分子功能为蛋白结合、分子结合、ATP结合。果肉显著差异基因参与的KEGG通路主要为植物激素信号传导、类苯基丙烷

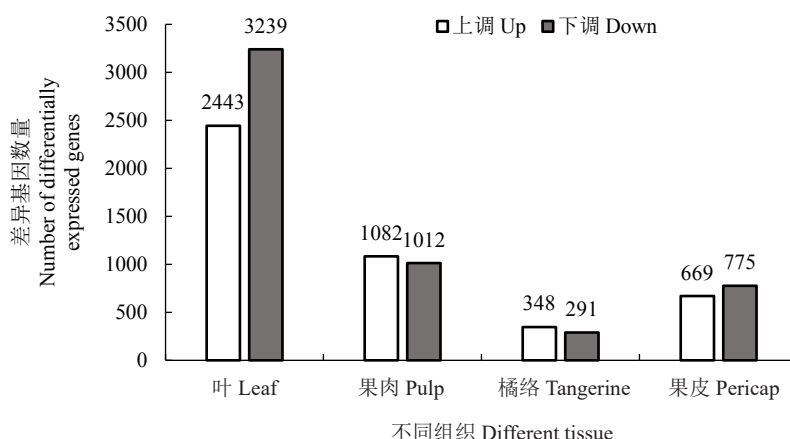


图3 砂糖灯笼橘、砂糖橘不同组织部位的差异基因数量

Fig. 3 The number of different genes in different tissue parts of Shatang Lantern tangerine and Shatang tangerine

生物合成、淀粉和蔗糖代谢、黄酮类生物合成、半乳糖代谢。GO 分析显示其参与的生物学过程包括 DNA 复制的转录调控、DNA 复制的转录、生物反应;这些基因的分子功能为蛋白结合、分子结合、ATP 结合;与叶子 GO 分析结果相同的是,果肉的差异基因同样位于细胞核、质膜、膜整体组件。对果皮的显著差异基因进行 KEGG 分析后,发现其参与的通路主要为类苯基丙烷生物合成、光合作用、黄酮类生物合成、芪类、双苯庚烷及姜辣素生物合成、光合生物体的固碳作用。GO 分析结果显示,果皮差异基因参与的生物学过程、基因定位及分子功能与果肉完全一致,但从具体的基因功能来看,果肉组织的差异基因主要在细胞壁、胞外区、质膜部位参与作用,其主要功能为 DNA 结合转录因子活性;而果皮的差异基因主要位于细胞膜、叶绿体类囊体及膜,其功能主要为氧化还原酶活性、参与次生代谢生物合成过程。对橘络的显著差异基因进行 KEGG 分析后发现,其主要参与类苯基丙烷的生物合成、黄酮类生物合成、过氧化物酶体、芪类、双苯庚烷、姜辣素的生物合成、氨基糖、核苷酸糖的代谢。GO 分析显示橘络中的大部分差异基因功能为黄酮类生物合成、黄酮类糖脂化、木质素生物合成、氧化还原酶、植物型次生细胞壁生物合成等。橘络差异基因的生物学过程包括 DNA 复制的转录调控、生物反应、氧化还原过程;这些基因主要位于细胞核、质膜、膜整体组件;其分子功能为蛋白结合、DNA 结合转录因子活性、分子结合。

砂糖灯笼橘果皮在外观形态上与砂糖橘明显不同,为了对其差异性性状进行更深入的解析,筛选果皮内 $p < 0.01$ 的显著差异基因,进行富集分析。

砂糖灯笼橘果皮上调基因共 669 个,从 GO 富集结果(图 4)可以看出,大部分差异基因为生物反应类,其次是分子功能,差异数量最多的基因其功能为氧化还原作用、次生代谢产物合成及 DNA 复制。从通路富集结果可以看出,差异数量最多的基因与苯丙素生物合成相关,其次是黄酮类生物合成、芥子油苷生物合成。

砂糖灯笼橘果皮下调基因共 775 个,超过一半的差异基因为细胞组分 1 类,主要与细胞膜、细胞器膜有关。在生物反应 1 类中,差异基因主要参与了氧化还原反应、水杨酸响应及 DNA 复制。从 KEGG 通路分析可知,差异基因参与了光反应、苯丙素的生

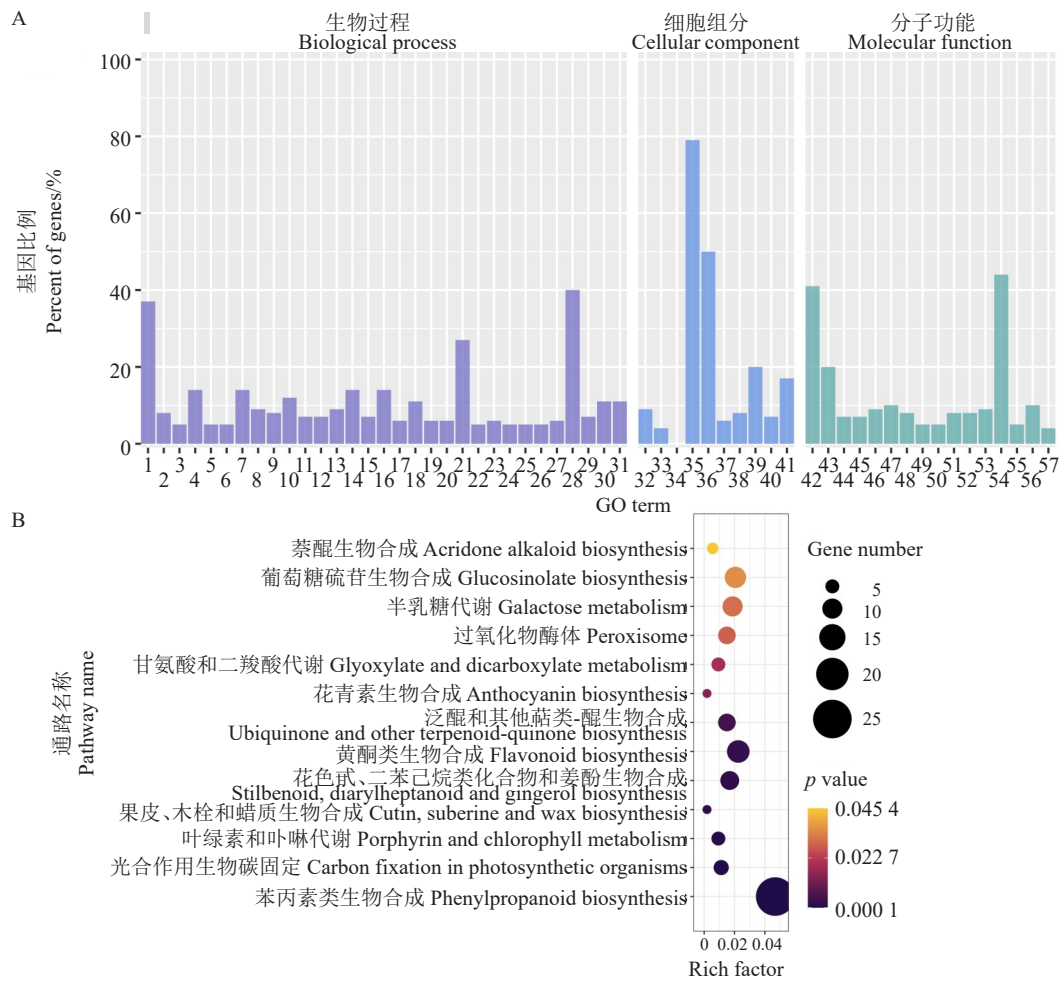
物合成、半乳糖代谢等过程(图 5)。值得注意的是,砂糖灯笼橘中与角质层、蜡质合成相关基因的表达量呈现明显变化,这可能是砂糖灯笼橘呈现出表型差异的重要原因。

2.5 砂糖灯笼橘、砂糖橘调控蜡质生物合成相关基因筛选

根据前期的甲苯胺蓝染色实验结果,初步推测砂糖灯笼橘、砂糖橘在蜡质生物合成基因表达上存在着明显的差异,因此重点对果皮和叶的蜡质生物合成相关基因进行统计分析。初步筛选砂糖橘、砂糖灯笼橘果皮差异基因中参与到角质、软木脂、蜡质生物合成途径(map00073)的基因,共得到 40 个基因。以 \log_2FC 的绝对值 ≥ 1 且 q 值 < 0.05 作为标准筛选具有显著性差异的基因,得到了 9 个基因,分别为 LOC18055362 (*CER3 isoform X1*)、LOC18038381 (*FACR2*)、LOC18048646 (*cytochrome P45086A22*)、LOC18035687 (*HHT*)、LOC18049733 (*CER1 isoform X2*)、LOC18046005 (*cytochrome P45086A8*)、LOC18039129 (*CER1*)、LOC18039665 (*hypothetical protein CICLE_v10013412mg*)、LOC18044180 (*TAT*),除 *FACR2* 基因上调外,其余基因表达量均降低。

对叶片参与蜡质生物合成途径的差异基因进行筛选,获得了 40 个差异基因,以 \log_2FC 的绝对值 ≥ 1 且 q 值 < 0.05 为原则筛选其中的显著差异基因,共获得 12 个基因,分别为 LOC18055362 (*CER3 isoform X1*)、LOC18049733 (*CER1 isoform X2*)、LOC18037666 (*alkane hydroxylase MAH1-like*)、LOC18037897 (*cytochrome P45096A1*)、LOC18049734 (*CER1*)、LOC18035687 (*HHT*)、LOC18052048 (*omega-hydroxypalmitate O-feruloyl transferase*)、LOC18034580 (*hypothetical protein CUMW_152270*)、LOC18046005 (*cytochrome P45086A8*)、LOC18048646 (*cytochrome P45086A22*)、LOC18044337 (*cytochrome P45086B1*) 和 LOC18036471 (*ERF011*)。砂糖灯笼橘叶的上调基因为 LOC18037897、LOC18044337,其余基因皆下调。

对叶片、果皮筛选与蜡质合成相关的显著差异基因绘制韦恩图(图 6),其中 2 个组织部位存在 5 个相同的基因,分别为 LOC18055362、LOC18048646、LOC18035687、LOC18049733、LOC18046005,与砂糖橘相比,这些基因呈现明显的下调,提示这些基因可能与砂糖灯笼橘蜡质的生物合成密切相关;同时,

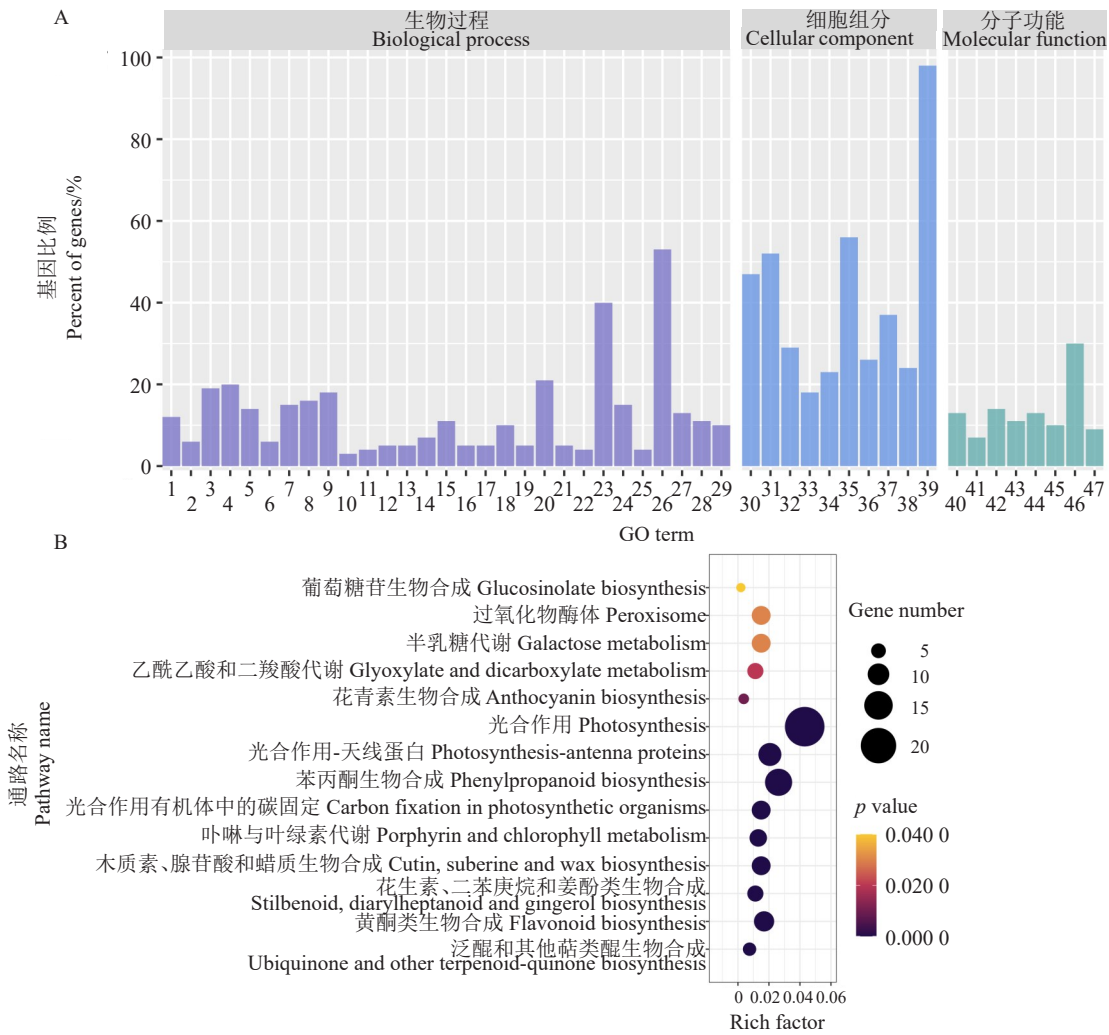


A. GO 富集分析; B. KEGG 通路分析。1. 次生代谢产物生物合成过程; 2. 对光刺激的响应; 3. 尿酸分解代谢; 4. 黄酮类葡萄糖醛酸化; 5. 尿素代谢过程; 6. 嘌呤核碱基分解代谢过程; 7. 黄酮类生物合成过程; 8. 对真菌的防御反应; 9. 对损伤的反应; 10. 参与细胞蛋白降解的蛋白酶解过程; 11. 多糖生物合成过程; 12. 对卵菌的反应; 13. 木质素生物合成过程; 14. 细胞壁组织; 15. 防御反应的调节; 16. 对水分缺乏的反应; 17. 代谢过程; 18. 对脱落酸的响应; 19. 类植物型主细胞壁生物合成; 20. 不饱和脂肪酸生物合成; 21. 氧化还原过程; 22. 对蓝光的响应; 23. 对氧化应激的反应; 24. 脂溶性维生素生物合成过程; 25. 维生素 E 生物合成过程; 26. 韧皮部蔗糖载荷; 27. 芳香化合物生物合成过程; 28. 转录, DNA 模板; 29. 纤维素生物合成过程; 30. 对寒冷的响应; 31. 脱落酸激活信号通路; 32. 细胞外间隙; 33. 锚定的质膜组分; 34. 质膜; 35. 膜; 36. 细胞外区域; 37. 叶绿体膜; 38. 溶酶体; 39. 细胞内有膜的细胞器; 40. 叶绿体包膜; 41. 细胞壁; 42. 氧化还原酶活性, 作用于成对供体, 伴有分子氧的融合或还原, NAD(P)H 作为一个供体, 结合一个氧原子; 43. 槲皮素 3-O-葡萄糖苷转移酶活性; 44. 纤维素合成酶活性; 45. 纤维素合成(UDP 形成)活性; 46. 半胱氨酸型内切蛋白酶活性; 47. RNA 聚合酶 II 调控区域序列特异性 DNA 结合; 48. O-甲基转移酶活性; 49. 转移酶活性, 转移烷基或芳基(甲基除外)基团; 50. 霍姆吉酚酸植物酰基转移酶活性; 51. 蛋白二聚化活性; 52. 碳水化合物结合; 53. 双氧酶活性; 54. S-腺苷甲硫氨酸-同半胱氨酸 S-甲基转移酶活性; 55. 蛋白质丝氨酸/苏氨酸激酶活性; 56. 异戊烯基转移酶活性; 57. S-腺苷甲硫氨酸依赖的甲基转移酶活性。

A. GO enrichment bar plot; B. KEGG enrichment scatter plot. 1. Secondary metabolite biosynthetic process; 2. Response to light stimulus; 3. Allantoin catabolic process; 4. Flavonoid glucuronidation; 5. Ureide catabolic process; 6. Purine nucleobase catabolic process; 7. Flavonoid biosynthetic process; 8. Defense response to fungus; 9. Response to wounding; 10. Proteolysis involved in cellular protein catabolic process; 11. Polysaccharide biosynthetic process; 12. Response to oomycetes; 13. Lignin biosynthetic process; 14. Cell wall organization; 15. Regulation of defense response; 16. Response to water deprivation; 17. Metabolic process; 18. Response to abscisic acid; 19. Plant-type primary cell wall biogenesis; 20. Unsaturated fatty acid biosynthetic process; 21. Oxidation-reduction process; 22. Response to blue light; 23. Response to oxidative stress; 24. Fat-soluble vitamin biosynthetic process; 25. Vitamin E biosynthetic process; 26. Phloem sucrose loading; 27. Aromatic compound biosynthetic process; 28. Transcription, DNA-templated; 29. Cellulose biosynthetic process; 30. Response to cold; 31. Abscisic acid-activated signaling pathway; 32. Extracellular space; 33. Anchored component of plasma membrane; 34. Plasma membrane; 35. Membrane; 36. Extracellular region; 37. Chloroplast membrane; 38. Lysosome; 39. Intracellular membrane-bounded organelle; 40. Chloroplast envelope; 41. Cell wall; 42. Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, NAD(P)H as one donor, and incorporation of one atom of oxygen; 43. Quercetin 3-O-glucosyltransferase activity; 44. Cellulose synthase activity; 45. Cellulose synthase (UDP-forming) activity; 46. Cysteine-type endopeptidase activity; 47. RNA polymerase II regulatory region sequence-specific DNA binding; 48. O-methyltransferase activity; 49. Transferase activity, transferring alkyl or aryl (other than methyl) groups; 50. Homogentisate phytyltransferase activity; 51. Protein dimerization activity; 52. Carbohydrate binding; 53. Dioxygenase activity; 54. S-adenosylmethionine-homocysteine S-methyltransferase activity; 55. Protein serine/threonine kinase activity; 56. Prenyltransferase activity; 57. S-adenosylmethionine-dependent methyltransferase activity.

图4 砂糖灯笼橘上调差异基因的 GO、KEGG 分析

Fig. 4 GO and KEGG analysis of up-regulated differential genes in Shatang Lantern tangerine



A. GO 富集分析; B. KEGG 通路分析。1. 光合作用, 光系统 I 中的光捕获; 2. 次生代谢物生物合成过程; 3. 光合作用; 4. 对光刺激的反应; 5. 类黄酮葡萄糖酰化; 6. 蜡质生物合成过程; 7. 类黄酮生物合成过程; 8. 对真菌的防御反应; 9. 对损伤的反应; 10. 茉莉酸代谢过程; 11. 光合作用, 光反应; 12. 光合作用电子传递链; 13. 木质素生物合成过程; 14. 叶绿素生物合成过程; 15. 细胞壁组织; 16. 蜡质生物合成过程; 17. 光合作用中光系统 I 的电子传递; 18. 对水分缺乏的反应; 19. 代谢过程; 20. 对脱落酸的反应; 21. 光系统 II 的组装; 22. 海藻糖生物合成过程; 23. 氧化还原过程; 24. 对氧化应激的反应; 25. 氧脂生物合成过程; 26. 转录, DNA 模板; 27. 对寒冷的反应; 28. 脱落酸激活的信号传导途径; 29. 对几丁质的反应; 30. 叶绿体类囊膜; 31. 膜; 32. 叶绿体类囊; 33. 胶体; 34. 类囊体; 35. 胞外区域; 36. 胞内膜包围的细胞器; 37. 叶绿体包膜; 38. 细胞壁; 39. 细胞膜; 40. 槲皮素 3-O-葡萄糖基转移酶活性; 41. 氧化还原酶活性, 作用于成对供体, 通过分子氧的插入或还原, NAD(P)H 作为一个供体, 并且插入一个氧原子; 42. 叶绿素结合; 43. 色素结合; 44. 槲皮素 7-O-葡萄糖基转移酶活性; 45. RNA 聚合酶 II 调控区序列特异性 DNA 结合; 46. 蛋白质丝氨酸/苏氨酸激酶活性; 47. 依赖于 S-腺苷甲硫氨酸的甲基转移酶活性。

A. GO enrichment bar plot; B. KEGG enrichment scatter plot. 1. Photosynthesis, light harvesting in photosystem I; 2. Secondary metabolite biosynthetic process; 3. Photosynthesis; 4. Response to light stimulus; 5. Flavonoid glucuronidation; 6. Cutin biosynthetic process; 7. Flavonoid biosynthetic process; 8. Defense response to fungus; 9. Response to wounding; 10. Jasmonic acid metabolic process; 11. Photosynthesis, light reaction; 12. Photosynthetic electron transport chain; 13. Lignin biosynthetic process; 14. Chlorophyll biosynthetic process; 15. Cell wall organization; 16. Wax biosynthetic process; 17. Photosynthetic electron transport in photosystem I; 18. Response to water deprivation; 19. Metabolic process; 20. Response to abscisic acid; 21. Photosystem II assembly; 22. Trehalose biosynthetic process; 23. Oxidation-reduction process; 24. Response to oxidative stress; 25. Oxylipin biosynthetic process; 26. Transcription, DNA-templated; 27. Response to cold; 28. Abscisic acid-activated signaling pathway; 29. Response to chitin; 30. Chloroplast thylakoid membrane; 31. Membrane; 32. Chloroplast thylakoid; 33. Plastoglobule; 34. Thylakoid; 35. Extracellular region; 36. Intracellular membrane-bounded organelle; 37. Chloroplast envelope; 38. Cell wall; 39. Plasma membrane; 40. Quercetin 3-O-glucosyltransferase activity; 41. Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, NAD(P)H as one donor, and incorporation of one atom of oxygen; 42. Chlorophyll binding; 43. Pigment binding; 44. Quercetin 7-O-glucosyltransferase activity; 45. RNA polymerase II regulatory region sequence-specific DNA binding; 46. Protein serine/threonine kinase activity; 47. S-adenosylmethionine-dependent methyltransferase activity.

图 5 砂糖灯笼橘下调差异基因的 GO、KEGG 分析

Fig. 5 GO and KEGG analysis of down-regulated differential genes in Shatang Lantern tangerine

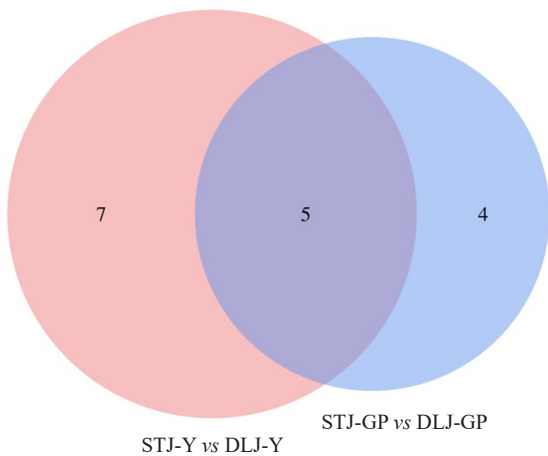


图6 砂糖灯笼橘和砂糖橘叶、果皮与蜡质合成相关的显著差异基因韦恩图

Fig. 6 Venn diagram of significantly different genes related to wax synthesis in the leaves and skins of Shatang Lantern tangerine and Shatang tangerine

叶中独立存在7个与蜡质合成相关的显著差异基因,果皮中存在4个与蜡质合成相关的显著差异基因,说明不同组织部位与蜡质生物合成相关基因的表达存在独特性。

3 讨论

植物自然突变体^[22]是植物在生长发育过程中,其基因组内某些基因突变,从而导致其出现性状或代谢物成分变化从而形成的新的个体。对于突变体的研究通常是从植物的表型、遗传物质角度出发,与野生型植株进行性状及转录组数据的比较分析,找到突变的分子机制。

基于的Illumina的测序技术^[23-24]已被证明转录组学适用于植物基因注释、基因表达分析以及通路研究。本研究中,以自然突变的砂糖灯笼橘、其同源砂糖橘为样本进行二代转录组测序,筛选后发现不同组织部位差异基因数量各不相同,趋势为叶>果肉>果皮>橘络。经过GO分析后发现,叶、果皮、橘络的差异基因主要位于细胞核、质膜、膜整体组件,果肉组织中的差异基因主要分布于细胞壁、胞外区、质膜部位。注意到果肉中显著差异基因参与的KEGG通路主要为植物激素信号传导、类苯基丙烷类生物合成、黄酮类生物合成等。根据文献报道,柑橘果肉中的乙烯、赤霉素、脱落酸、吲哚乙酸、玉米素等内源性激素^[25]对于果实的甜度、成熟落果^[26]、色泽^[27]等特征至关重要。而苯丙烷类及黄酮类生物合

成基因主要参与到植物的生长发育及抗逆等生物功能,许多MYB类转录因子可以调控苯丙烷类^[28]、黄酮类化合物生物^[29-30]合成中相关基因的表达,进而增强植物的抗逆性。因此果肉中筛选得到的差异基因极有可能对砂糖灯笼橘的果实甜度及生长发育至关重要。在对差异基因数量最多的叶子转录组数据分析后发现,其差异基因主要参与到植物-病原互作、半乳糖代谢、转运蛋白运输等过程。柑橘类果皮富含多种化学成分^[31-32],在食品、医药方面具有巨大的应用潜力,同时,果皮作为柑橘抵抗自然环境、病虫害的第一道防线^[33],其组成结构极其重要,转录组数据显示,果皮中的差异基因主要参与到抗逆、转录调控、生物反应等过程。柑橘橘络中富含多种类黄酮类化合物,如橙皮苷、槲皮素^[34],GO分析显示,砂糖灯笼橘橘络差异基因主要参与到黄酮类化合物的生物合成及糖脂化作用,对抵抗外界恶劣环境、生长发育具有重要意义。

通过甲苯胺蓝染色实验,发现砂糖灯笼橘叶片、果皮表面存在蜡质缺失的现象。植物表面的蜡质是由脂肪酸及其衍生物混合构成的一道屏障^[35],主要用于抵抗外界辐射、抵抗气温胁迫、防止病虫害危害及水分散失^[36-37]。根据转录组差异基因筛选的数据,砂糖灯笼橘果皮中存在9个与蜡质生物合成相关的差异基因,叶片中存在12个差异基因,果皮中只有*FACR2*为上调基因,其余基因表达量皆下调。*FACR2*为柑橘的脂肪酰辅酶A还原酶2异构体X2,参与到蜡质生物合成的第一步,即还原酶蛋白编码。目前对于该基因的具体功能未见报道,但对小麦中超长链脂肪酰辅酶A还原酶*TaFAR*的研究^[38]表明,*FAR*能够促进叶片表皮蜡质晶体的合成,存在正向调控的作用。因此可以推测柑橘中的*FACR2*基因极有可能正向调节蜡质晶体的合成,但由于砂糖灯笼橘体内饱和脂肪酸底物不足,即使其表达量高,也很难转化为脂肪醇。叶片中存在2个与蜡质合成相关的上调基因,皆为细胞色素P450酶。*CYP450*为单加氧酶,广泛参与植物中多种次生代谢产物的生物合成^[39-40],其可能参与到砂糖灯笼橘蜡质合成相关化合物的代谢通路中。叶片、果皮与蜡质生物合成相关的差异基因韦恩图显示2个组织样本中存在5个共同基因,分别为*CER1*、*CER3*、*HHT*、细胞色素*P45086A8*、细胞色素*P45086A22*,表达量皆显著下调。*CER1*、*CER3*为拟南芥烷烃合成

酶同源基因^[41-42]。拟南芥中 *CER3* 等基因^[43]可以参与到花粉内油脂类化合物合成与转运;小麦中的 *CER1*^[42]能够调控其表面蜡质合成,正向调控韧皮部防卫反应及应对麦长管蚜的抗性。因此 *CER* 系列基因对于调控砂糖灯笼橘表面果皮、叶片蜡质成分的合成和积累起着至关重要的作用,其下调可能会直接导致砂糖灯笼橘蜡质缺失。*HHT* 编码阿魏酸辅酶 A 转移酶,从而参与木脂素生物合成。基于花花柴高温环境下的表达谱筛选到与蜡质合成相关基因 *KcHHT*,能够明显提升花花柴蜡质合成,进而增强其抗逆作用^[44],因此可以推测出筛选到的砂糖灯笼橘中 *HHT* 基因也能够直接参与到蜡质生物合成,其表达量降低也会导致砂糖灯笼橘表面蜡质缺失。目前砂糖灯笼橘中筛选的蜡质合成相关基因的功能研究尚未见报道,后续对这些基因进行具体深入的研究有助于砂糖灯笼橘的定向优质育种。

4 结 论

砂糖灯笼橘为砂糖橘自然变异品种,其叶大且叶片边缘不规则;果实较砂糖橘大,果皮有明显沟壑,表面布满点状突起,形似“灯笼”。甲苯胺蓝染色说明叶片、果实表面蜡质排布不均。转录组测序发现叶片差异基因数量最多,其次为果肉、果皮、橘络。筛选叶片、果皮中与蜡质生物合成相关的显著差异基因,发现叶片中存在 12 个,果皮中存在 9 个,且有 5 个共同的显著下调基因,分别为 *CER1*、*CER3*、*HHT*、细胞色素 *P45086A8*、细胞色素 *P45086A22*,这些基因可能是砂糖灯笼橘表面蜡质生物合成的关键基因。进一步对这些基因的功能进行深入挖掘,可以为定向培育优质的砂糖灯笼橘种质资源提供理论依据。

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