果 树 学 报 2023,40(6):1109-1120
Journal of Fruit Science

DOI:10.13925/j.cnki.gsxb.20220233

苹果花脸症状相关基因差异表达的分析

郑朋飞[1],张学勇[2],孙 骞[3],王楚堃[1],杨钰莹[1],
芮 麟[1],宋来庆[2],张振鲁[1*],由春香[1*]

([1]山东农业大学园艺科学与工程学院•作物生物学国家重点实验室,山东泰安 271018;
[2]烟台市农业科学研究院,山东烟台 265500;[3]广饶县林业发展服务中心,山东广饶 257399)

摘 要:【目的】苹果花脸病主要由苹果锈果类病毒(apple scar skin viroid,ASSVd)引起,是一种严重危害苹果产量和果实品质的病害,通过分析苹果花脸症状相关基因表达,探索花脸症状形成的潜在机制。**【方法】**削取无症状且无ASSVd侵染的果皮和表现花脸症状的弘前富士苹果果皮,提取果皮总RNA,通过高通量测序并进行转录组数据分析。**【结果】**利用RT-PCR方法从表现花脸症状的苹果果皮中得到两条ASSVd序列,其基因组长度分别为331 nt和330 nt,与已公布的ASSVd山东烟台苹果分离物SDYT-1(MW302328.1)和SDYT-3(MW315909.1)完全一致。转录组测序结果表明,与无症状果皮相比,表现花脸症状的果皮中共有差异表达基因6938个,其中有3331个基因显著上调,3607个基因显著下调。通过差异表达基因功能注释分析,表明这些差异基因参与到植物激素(茉莉酸、水杨酸和生长素)合成和信号转导、苯丙烷生物合成等代谢途径。此外,转录组数据分析发现与植物防御反应相关转录因子的编码基因,如*MdWRKY18-like*、*MdWRKY71*、*MdNAC29*、*MdWRKY70*等在表现花脸症状的苹果果皮中显著上调,而*MdERF61*等则显著下调。利用实时荧光定量PCR验证了显著差异表达基因变化情况。**【结论】**转录组分析结果表明,苹果花脸病果实与正常果实差异表达基因主要参与植物激素信号转导和苯丙烷合成途径。此外,植物抗病相关转录因子及防御相关激素信号可能参与调控苹果花脸病的抗病反应。

关键词:苹果;ASSVd;转录组分析;差异表达基因;转录因子

中图分类号:S661.1 **文献标志码:**A **文章编号:**1009-9980(2023)06-1109-12

Transcriptome sequencing analysis of differentially expressed genes involved in the formation of dapple symptoms in apple fruits

ZHENG Pengfei[1], ZHANG Xueyong[2], SUN Qian[3], WANG Chukun[1], YANG Yuying[1], RUI Lin[1], SONG Laiqing[2], ZHANG Zhenlu[1*], YOU Chunxiang[1*]

([1]College of Horticulture Science and Engineering, Shandong Agricultural University/State Key Laboratory of Crop Biology, Tai' an 271018, Shandong, China; [2]Yantai Academy of Agricultural Sciences, Yantai 265500, Shandong, China; [3]Forestry Development Service Center of Guangrao, Guangrao 257399, Shandong, China)

Abstract:【Objective**】** Apple (*Malus domestica*) is one of the most important fruit crops worldwide, especially in China. As perennial woody plants, apple trees are vulnerable to multiple pathogens, including fungi, bacteria, viruses, and viroids. Among them, apple scar skin disease is a common apple disease induced by apple scar skin viroid (ASSVd). Apple scar skin disease has two types of symptoms that may be related to the apple cultivar. One type is scar skin symptom, which usually appears on cultivars such as Golden Delicious, Indo, and Ralls Janet. The other type is dapple symptom that commonly appears on red-skin cultivars, such as Red Fuji, Red Gold, and so on. The red-skin Red Fuji is the major apple cultivar in China, the disease has caused severe losses due to the unmarketable fruits and attenuated yield. ASSVd, the major causal agent of the disease, is mainly transmitted by grafting among apple trees. The disease with dapple symptom has been reported in some of the apple cultivated area around

收稿日期:2022-05-13 **接受日期:**2023-01-13

基金项目:国家自然科学基金项目(31901988);中国博士后科学基金项目(2019M662413;2020T130388)

作者简介:郑朋飞,男,在读博士研究生,研究方向为果树生物技术和分子生物学。Tel:17863805322,E-mail:1159332359@qq.com

*通信作者 Author for correspondence. E-mail:zhangzhenluwenchao@163.com;E-mail:youchunxiang@sdau.edu.cn

the world, such as China, Japan, the republic of Korea, the United States, Canada, and *etc*. Although this disease has been identified many years ago, the mechanism of the dapple symptom formation is still unclear. In this sutdy, we used high-throughput sequencing technology to determine the differentially expressed genes that are related to the dapple symptom to investigate the behind mechanism of how the symptom is formed in apple fruits.【Methods】The apple peels from the apple fruits without symptom or with dapple symptom were obtained and stored in freezer (−80 ℃). The total RNA was subsequently extracted using an RNA extraction kit, and cDNA was synthesized by reverse transcriptase. The specific primers were designed to detect the presence of ASSVd and other possible viruses and viroids by RT-PCR. The full-length ASSVd genome was amplified by two rounds RT-PCR. Then, the RNA samples were delivered to company for high-throughput sequencing. The GO analysis and KEGG analysis were performed to analyze the annotation the differentially expressed genes. Moreover, differentially expressed genes involved in plant defence-related hormone signaling and transcription factors were also analyzed through the transcriptome data. Finally, qRT-PCRexperiments were utilized to verify the reliability of the transcriptome data.【Results】After RT-PCR analysis with specific primers, we found that ASSVd indeed present in the apple peel with dapple symptom, but not in the healthy apple fruit. Moreover, other viruses and viroids, including apple necrosis mosaic virus (ApNMV), apple chlorotic leafspot virus (ACLSV), apple stem grooving virus (ASGV), and apple dimple fruit viroid (ADFVd), that are commonly infectious to apple were not detected when performing RT-PCR assay using specific primers. We next obtained the full-length genome sequence of ASSVd *via* two round RT-PCR assay. Specifically, a new pair of primers were designed based on the previously obtained sequence, and the resulted sequences of the two-round PCR were assembled. We finally got two distinct isolates from the apple fruits with dapple symptom. The length of the two isolates were 331 nt and 330 nt, which are identical to the sequences of ASSVd Shandong Yantai apple isolate SDYT-1 (MW302328.1) and SDYT-3 (MW315909.1), respectively. The transcriptome sequencing assay was performed using Illumina platform, and the high quality of transcriptome sequencing data were obtained. By $|\log_2 \text{Fold Change}| > 1$ & $p < 0.05$ screening standard, we screened out 6938 differentially expressed genes. Among them, 3331 were significantly up-regulated and 3607 were significantly down-regulated in dapple apple peels compared with those of healthy apple. GO analysis revealed that the differentially expressed genes were mainly concentrated in the functions of cellular processes, metabolic processes, cellular components, binding and catalytic activities. The KEGG analysis showed that the differentially expressed genes were mainly enriched in plant hormone signal transduction, phenylpropanoid biosynthesis, photosynthesis-antenna proteins, photosynthesis, and other pathways. Furthermore, the analysis of the hormonal pathways showed thatmetabolism and signal transduction of jasmonic acid (JA), salicylic acid (SA), auxin, and other hormones might be involved in the dapple symptom appearance. The expression levels of the SA-related gene *NPR4a* (MD05G1256300), the JA-related gene *TIFYs* (MD13G1127100, MD09G1178600, MD02G1096100, MD16G1127400, and MD17G1164400), and the ethylene-related gene *ERS1* (MD03G1292200) were down-regulated, while the expression level of *EIN3* (MD08G1245800) was up-regulated. In addition, a large number of genes involved in auxin pathway-relatedwere were also down-regulated in dapple symptom fruits. Among the auxin-related genes, most of them were key enzymes and receptor proteins encoding IAA. The multiple transcription factor-encoding genes, including the *WRKY*, *MYB*, *ERF* and *NAC* were also identified in the differentially expressed genes. Specifically, the *MdWRKY18-like*, *MdWRKY71*, *MdNAC29*, and *MdWRKY70* were up-regulated in dapple apple fruits, while genes such as *the MdERF61* were down-regulated. Finally, quantitative real-time PCR assay was

utilized to confirm the reliability of the transcriptome data.【Conclusion】Transcriptome analysis showed that multiple cellular pathways, especially phenylpropanoid biosynthesis, and hormonal pathways were involved in the dapple symptom formation in apple fruits. Moreover, defensive transcription factor-encoding genes might also be related to the plant defense responses during the course of interactions between ASSVd and apple plants.

Key words: *Malus domestica*; ASSVd; Transcriptome analysis; Differentially expressed gene; Transcription factor

苹果（*Malus domestica*）是世界上种植最广泛的经济果树之一。作为典型的多年生木本植物，在其生长过程中极易受到病毒或类病毒的侵染[1]。目前常见的侵染苹果的病毒包括苹果茎沟病毒（apple stem grooving virus，ASGV）[3]、苹果花叶病毒（apple mosaic virus，ApMV）[4]、苹果褪绿叶斑病毒（apple chlorotic leaf spot virus，ACLSV）、苹果锈果类病毒（apple scar skin viroid，ASSVd）[5-6]、苹果凹果类病毒（apple dimple fruit viroid，ADFVd）[7-9]，以及新近鉴定的与中国苹果花叶病紧密相关的苹果坏死花叶病毒（apple necrotic mosaic virus，ApNMV）[4,10-11]。

由类病毒引发的苹果锈果病是苹果生产中最具破坏性的病害之一。目前，该病害在世界各苹果主产区均有发生，特别是在中国、日本、韩国，以及美国、印度和伊朗[12-15]。在苹果生产中，该病害根据在不同品种上的表型可分为3种症状：锈果型、花脸型和锈果-花脸混合型症状[16]。锈果型的典型症状是在果实上生有与心室顶部对应的五条规则的木栓化斑纹，主要发生在金冠等品种上。花脸型症状主要表现在果皮表面形成圆形的黄绿色或红色斑点，主要发生在着色品种上，如富士系[16]。而锈果-花脸混合型是上述两种症状的复合症状[1]。该病害主要影响果实的品质，包括硬度、风味等内在品质和果实着色等外观品质，使果实失去商业价值，造成严重经济损失[1]。

诱导苹果锈果病发生的主要病原是ASSVd，其属于马铃薯纺锤形块茎类病毒科（Pospiviroidae），苹果锈果类病毒属（*Apscarviroid*）[17-18]。苹果植株被ASSVd侵染后将终生带毒，严重影响苹果果实的产量和品质。ASSVd的基因组为环状单链RNA，长度约为330个核苷酸[17]。其基因组RNA不编码任何蛋白质，完全依靠寄主的转录机制进行复制和增殖[17]。

尽管苹果锈果病已有30多年的研究历史，但目前主要集中于其病原物ASSVd的检测及分离方面。关于该病害发生的内在机制研究尚不多见。笔者在本研究中利用转录组测序技术对山东烟台地区的表现花脸症状富士苹果检测，通过分析差异表达基因的功能，探究与花脸症状形成的可能代谢途径，为后续深入探究苹果花脸症状形成的内在机制提供重要依据。

1　材料和方法

1.1　试验材料

对照和感病呈现花脸症状的富士果实样品（图1）于2018年9月采自山东省烟台市烟台农业科学研究院果树基地（121.39° E，37.52° N），样本分别来自树龄11年、长势相对一致的健康且无花脸症状和有花脸症状的弘前富士苹果树，随机取样后削取果皮，混样后设置3次生物学重复，液氮冷冻后置于-80 ℃用于后续RNA的提取及转录组测序分析。

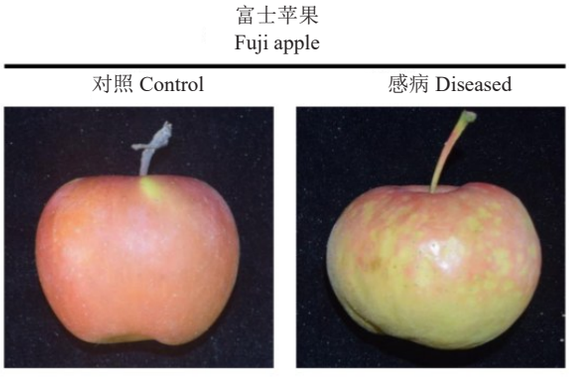


图1　健康和花脸症状的富士果实

Fig. 1　Healthy and dapple Fuji apple samples

1.2　ASSVd检测及克隆

参照GenBank中已公布的ASSVd（AY972082.1）序列设计引物ASSVd-F1/R1用以检测富士苹果中的ASSVd。根据扩增到的序列设计基因特异性引物ASSVd-F2/R2进行第二轮PCR扩增，通过序列组装获得ASSVd全基因序列。

1.3 转录组测序

1.3.1 cDNA文库构建及测序质量控制 委托青岛欧易生物公司提取果皮总RNA，经过Agilent 2100 Bioanalyzer质量检测后进行样本转录组测序分析。在原始数据Raw reads中去接头（Adaptor），去除低质量Reads，从3'端及5′端以不同方式去除低质量碱基，得到clean data用于后续数据分析。

1.3.2 测序数据比对和表达分析 利用Hisat2以金冠苹果基因组（GDDH13）为参考基因组对clean data进行序列比对。采取序列相似性比对的方法鉴定出各蛋白编码基因在各样本中的表达丰度。使用htseq-count软件获取每个样本中比对到蛋白编码基因上的reads数，cufflinks软件来计算蛋白编码基因的表达量FPKM值。

1.3.3 差异基因筛选及功能分析 利用DESeq软件进行样本间的差异基因分析，对各个样本基因的counts数目进行标准化处理（采用basemean值来估算表达量），计算差异倍数，并采用NB（负二项分布检验的方式）对reads数进行差异显著性检验，最终根据差异倍数及差异显著性检验结果来筛选差异蛋白编码基因。筛选标准为|log2 FoldChange|＞1且p＜0.05。利用基因本体数据库（GO）和京都基因与基因组百科全书（KEGG）对基因进行功能注释、分析以及统计。

1.4 总RNA的提取及实时定量PCR（qRT-PCR）验证

采用天根生化公司的植物RNA提取试剂盒进行RNA提取；通过Clontech SMART™ Library试剂盒进行反转录，合成用于定量分析的cDNA链。康为世纪公司的Ultra SYBR Mixture（Low ROX）试剂盒用于实时荧光定量PCR，以18S核糖体RNA基因作为内参基因[17]，引物序列见表1。

1.5 数据分析

所有试验设置3次生物学重复，使用SPSS 19.0软件进行差异显著性检验。

表1 RT-PCR克隆ASSVd及qRT-PCR检测转录因子表达量所用引物

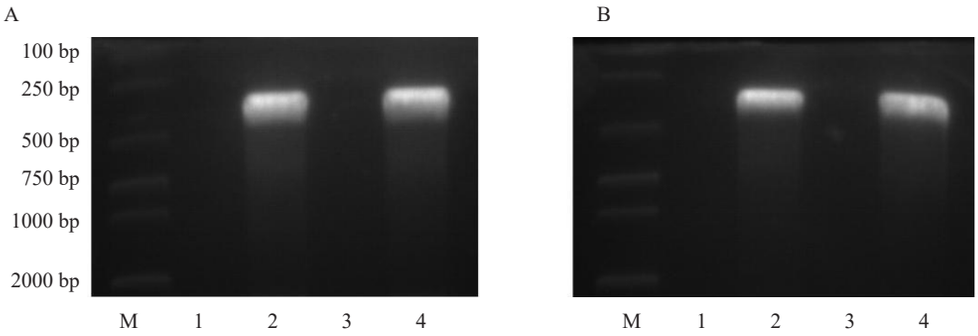
Table 1 Primers for cloning ASSVd by RT-PCR and for testing the expression levels of transcription factors using qRT-PCR

引物名称 Primer name	序列（5’→3’） Sequence (5’→3’)
ASSVd-F1（RT-PCR）	CCGGTGAGAAAGGAGCTGCCAGCA
ASSVd-R1（RT-PCR）	CCTTCGTCGACGACGACAGGTGAGT
ASSVd-F2（RT-PCR）	CCGGACGGCGCCCTCGCACCAGTTCCGCTGTGG
ASSVd-R2（RT-PCR）	CTTAGTGCTGGCAGCTCCTTTCTCACCGGCCTTCG
18s-F（qRT-PCR）	AGGCGCGAAATTACCAATCC
18s-R（qRT-PCR）	GCCCTCCAATTGTTCCTCGTTAAG
MdWRKY18-like-F（qRT-PCR）	CTCGTACGCCTAACAAACAAAAGGTTGTAGA
MdWRKY18-like-R（qRT-PCR）	CTTTAGAGTCGGTTTTTACTAGGAACTGTG
MdWRKY71-F（qRT-PCR）	GAAAAATCCTTACCAGTACGATCCTTTCGA
MdWRKY71-R（qRT-PCR）	CTTCATTAGATGAAGAAGATATTGAGGAAT
MdNAC29-F（qRT-PCR）	TCAGCTTCCTCGCAGTCATCTTCAGCGTCCT
MdNAC29-R（qRT-PCR）	CAGCATTGTGTTTGAAGTCACCATTCGGCAG
MdWRKY70-F（qRT-PCR）	GACTGATCGGAGAGCTACATGAAGGCCAGA
MdWRKY70-R（qRT-PCR）	TTTTGGCCATATTTTCTCCAAGCCTGTCCAT
MdERF61-F（qRT-PCR）	ATGCATGAAAACAACCTTGCTTTTGGGTTC
MdERF61-R（qRT-PCR）	CTCGGCGACCCATTTGCCCCAATGCCTCTG
MdMYB34-like-F（qRT-PCR）	GATCACCGTGCTGTGACAAGGTGGGTTTGA
MdMYB34-like-R（qRT-PCR）	CGTATTCCAGTAGTTTTTTATCTCATTGTC
MdWRKY72-F（qRT-PCR）	GGGAGTGAACTGGATGATGCCAAAGCTGAA
MdWRKY72-R（qRT-PCR）	TTCCAGCTTCTGCACGATCCTTATTAGGTTC

2 结果与分析

2.1 ASSVd检测及序列克隆

利用引物ASSVd-F1/R1进行PCR检测，从表现花脸的苹果果皮中扩增得到约300 bp左右的条带，而在健康果皮中则没有该条带（图2-A）。经过DNA测序并将所得序列在NCBI数据库（https://www.ncbi.nlm.nih.gov/）进行BLASTn比对，发现该序列与ASSVd山东烟台苹果分离物SDYT-1（MW302328.1）高度相似。为进一步获得ASSVd基因组全序列，根据已经获得的序列设计基因特异性引物ASSVd-F2/



A. 利用引物 ASSVd-F1/R1 检测 ASSVd；B. 利用引物 ASSVd-F2/R2 扩增 ASSVd 全长序列；泳道 1. RT-PCR 检测的健康富士苹果果皮样品；泳道 2. RT-PCR 检测表现花脸富士苹果果皮样品；泳道 3. 水作为阴性对照；泳道 4. 阳性对照；M. DNA Marker.

A. Using primer ASSVd-F1/R1 to detect ASSVd; B. Using prime ASSVd-F2/R2 to amplify ASSVd sequence. Lane 1. The RT-PCR product amplified using the peel of healthy Fuji apple sample; Lane 2. The RT-PCR product amplified using the peel of dapple Fuji apple sample; Lane 3. Negative control by using ddH_2O as template; Lane 4. Positive control; M. DNA Marker.

图 2　表现花脸症状果皮中 ASSVd 的 RT-PCR 检测及序列扩增

Fig. 2　RT-PCR detection and sequence amplification of ASSVd

R2。用该引物进行PCR扩增，获得第二轮ASSVd序列（图2-B）。最终，通过序列比对和组装获得2条ASSVd全长序列，全长分别是331 nt和330 nt。通过比对，发现2条序列与GenBank数据库中ASSVd山东烟台苹果分离物SDYT-1（MW302328.1）和SDYT-3（MW315909.1）序列完全一致。

2.2　花脸症状果皮中差异表达基因分析

将对照组（CK_1, CK_2, and CK_3）和表现花脸症状果皮样品（Diseased_1, Diseased_2, and Diseased_3）经Illumina测序后共获得43.47 G原始测序数据（表2）。质控检测结果表明，clean data覆盖度超过89%，各样本的Q30均超过92%，GC含量约为48%，说明测序质量较高，符合后续的数据分析要求。

为探究感染苹果花脸病后果皮中的基因表达情况，对差异表达基因（DEGs）进行分析。以$|\log_2 \text{Fold Change}|>1$ & $p<0.05$为筛选标准，两组样品中共筛选出6938个DEGs，其中上调DEGs共有3331个，下调DEGs共有3607个。

表 2　转录组测序质控后的质量统计

Table 2　Quality statistics of filtered transcript group sequencing data

样品 Sample	原始序列 Raw reads/M	原始碱基 Raw bases/G	过滤序列 Clean reads/M	过滤碱基 Clean bases/G	有效碱基 Valid bases/%	Q30/%	GC/%
Sample_Diseased_1	49.42	7.41	46.55	6.62	89.34	93.57	48.09
Sample_Diseased_2	49.42	7.41	47.15	6.64	89.51	94.26	48.09
Sample_Diseased_3	49.42	7.41	46.89	6.64	89.55	93.98	48.24
Sample_CK_1	42.80	6.42	40.89	5.84	91.03	92.71	47.76
Sample_CK_2	49.42	7.41	47.19	6.73	90.84	92.86	48.10
Sample_CK_3	49.42	7.41	46.93	6.65	89.74	94.01	48.05

2.3　ASSVd侵染苹果果实DEGs的GO富集分析

为探究上述差异表达基因的功能，笔者对DEGs进行GO富集分析，结果表明，上述DEGS涉及生物进程（biological process，BP）、细胞组分（cellular component，CC）和分子功能（molecular function，MF）3个大类。在DEGs富集数目的前30个亚类中（图3），生物进程注释到17个亚类，其中主要集中在细胞过程（cellular process）、代谢过程（metabolic process）、单有机体过程（single-organism process）和刺激反应（response to stimulus）等过程（图3）。富集在细胞组分的DEGs主要分为9个亚类，集中在细胞（cell）、细胞部分（cell part）、细胞器（organelle）和膜（membrane）等组分（图3）。在分子功能的4个亚类中，DEGs主要涉及结合（binding）和催化活性（catalytic activity）（图3）。

2.4　表现花脸症状苹果果皮中DEGs的KEGG富集分析

为了进一步研究DEGs的生物学功能，笔者对筛

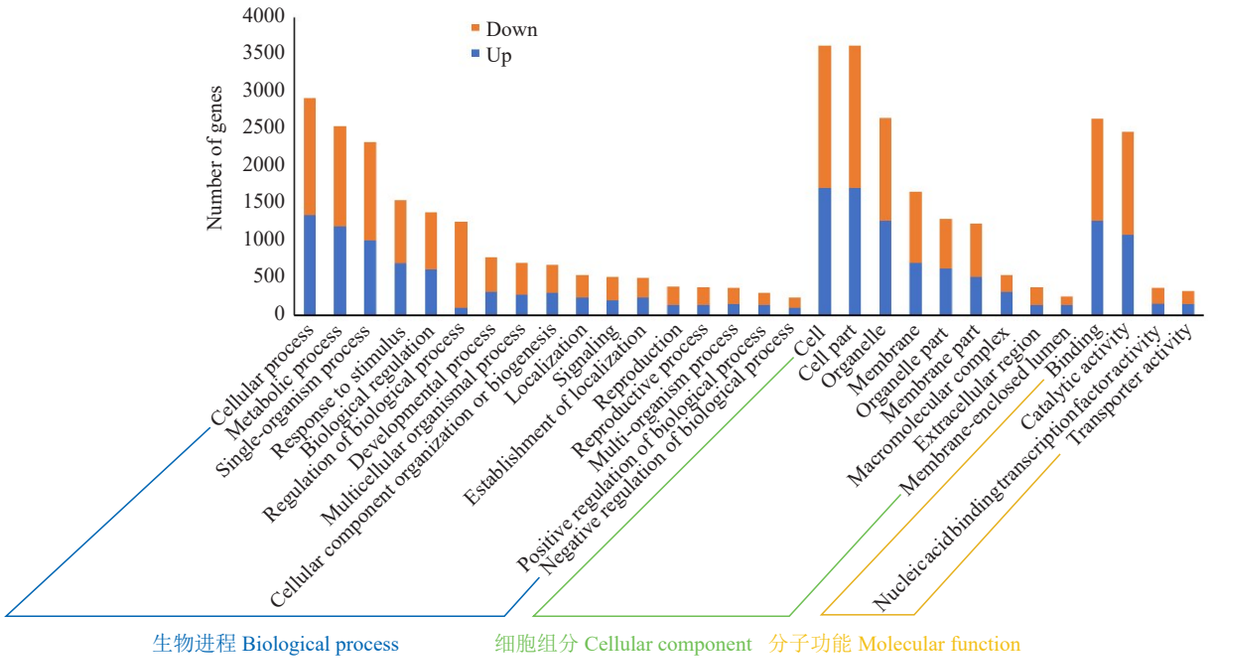


图3 GO分析中DEGs在不同功能分类中的数目及比例

Fig. 3 The amount and ratio of DEGs in different categories of GO analysis

选出的DEGs进行KEGG富集分析。结果表明，共有2432个DEGs富集到了191条通路中（图4）。其中富集显著的前20条代谢通路包括植物激素信号转导（plant hormone signal transduction）、苯丙烷生物合成（phenylpropanoid biosynthesis）、光合作用-触角蛋白（photosynthesis-antenna proteins）、光合作用（photosynthesis）、脂肪酸降解（fatty acid degradation）、不饱和脂肪酸的生物合成（biosynthesis of unsaturated fatty acids）（图4）。

2.5 表现花脸症状苹果果皮的激素相关DEGs分析

植物激素是调控植物生长发育和应对环境变化的重要调节因子。在KEGG富集分析中，植物激素信号转导是DEGs富集数目最多的代谢通路之一，共有90个DEGs得到富集（图5）。上述与植物激素

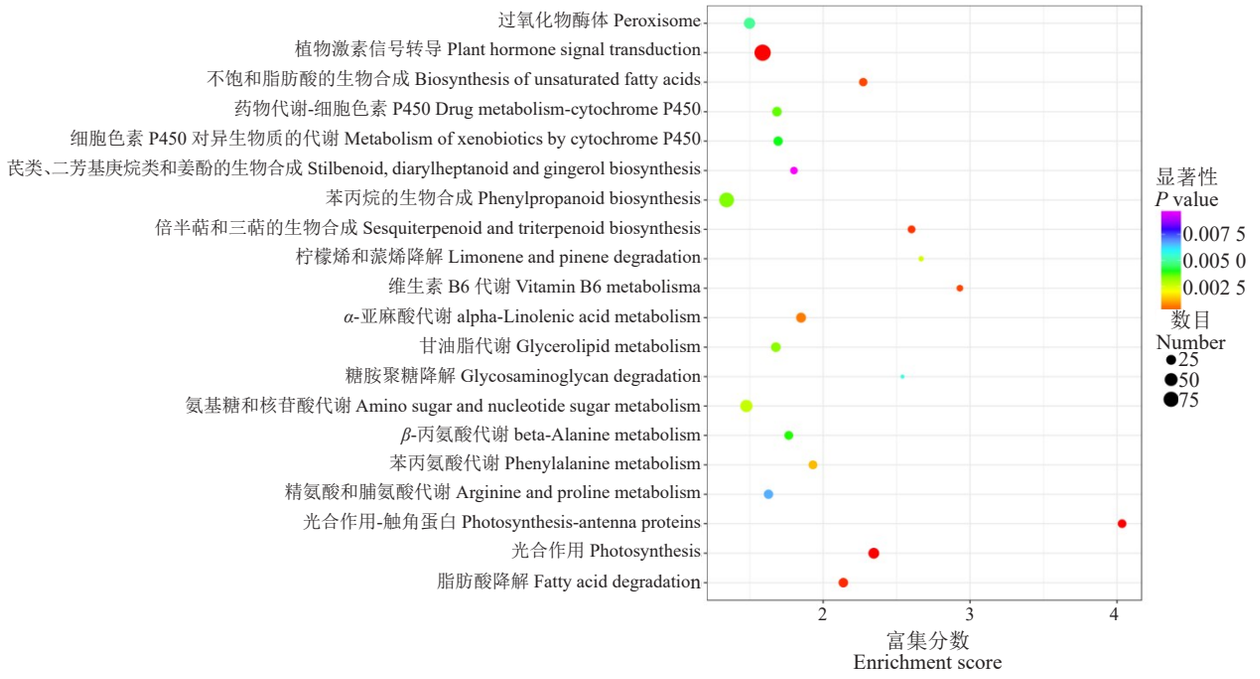
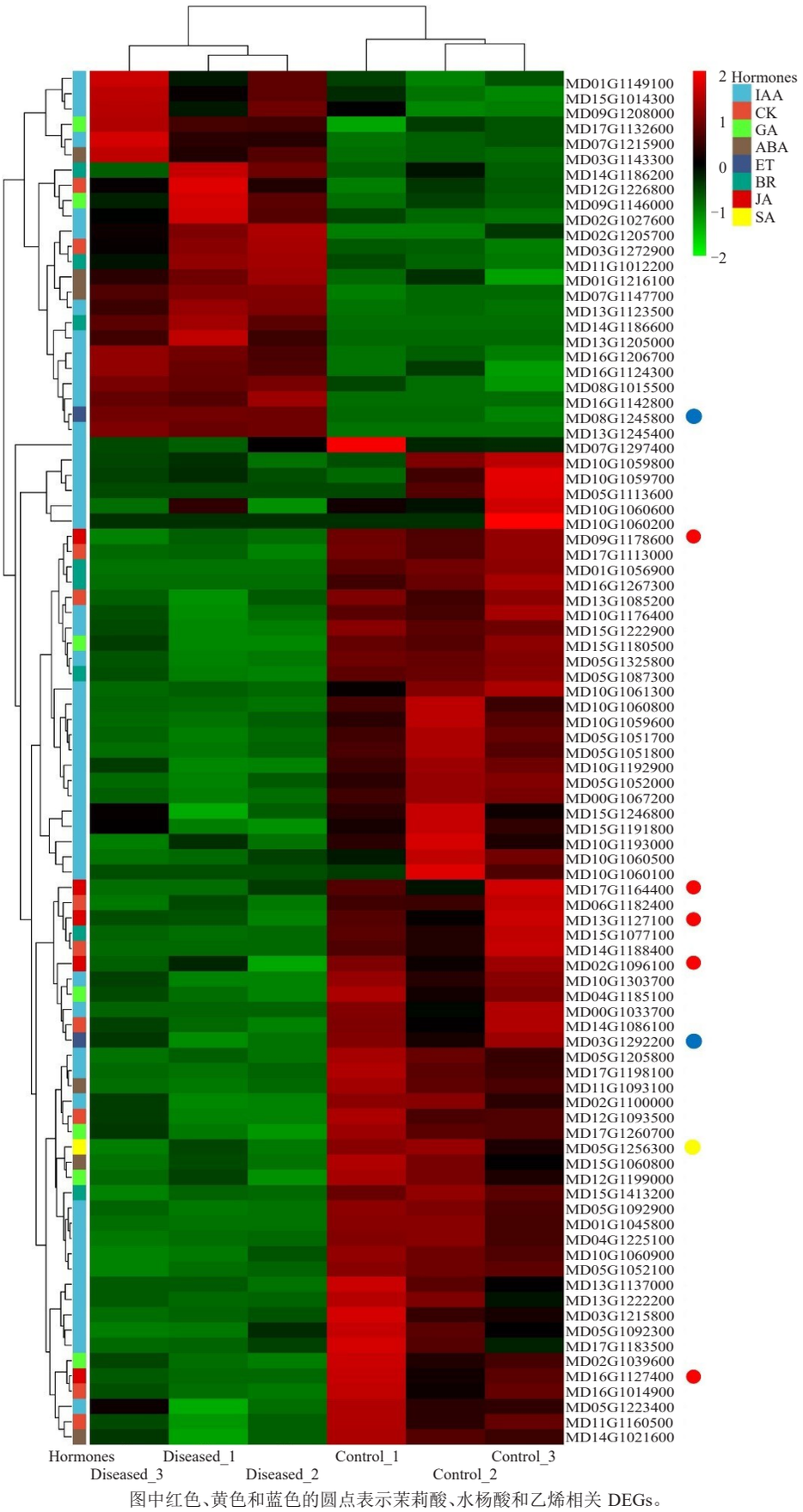


图4 表现花脸症状苹果果皮中DEGs的KEGG富集分析

Fig. 4 Analysis of the KEGG enrichment of DEGs in dapple apple fruit



图中红色、黄色和蓝色的圆点表示茉莉酸、水杨酸和乙烯相关 DEGs。

The red, yellow and blue dots in the figure represent DEGs related to JA, SA and ET.

图 5　植物激素相关 DEGs 的表达水平热图

Fig. 5　Heat map of the expression level of plant hormone-related DEGs

信号转导相关的90个DEGs中，有51个生长素（auxin，IAA）相关的DEGs，10个细胞分裂素（cytokinin，CK）相关的DEGs，7个赤霉素（gibberellins，GAs）相关的DEGs，6个脱落酸（abscisic acid，ABA）相关的DEGs，2个乙烯（ethylene，ET）相关的DEGs，8个油菜素内酯（brassinosteroid，BR）相关的DEGs，5个茉莉酸（jasmonic acid，JA）相关的DEGs和1个水杨酸（salicylic acid，SA）相关的DEGs。

对上述90个植物激素信号转导相关DEGs的表达量分析发现，其中24个DEGs相比于对照组表达水平是上调的，66个DEGs是下调的（图5），笔者选取表达倍数差异显著的前10个基因进行分析，发现其多数为生长素信号相关基因。通过设计基因特异性引物进行qRT-PCR验证，发现其与转录组测序结果一致，所有基因表达量均显著下调（图6，表3）。

2.6 表现花脸症状苹果果皮中的转录因子表达分析

转录因子在调控植物生长发育及抗病反应中发挥重要作用。对DEGs中编码转录因子的基因进行

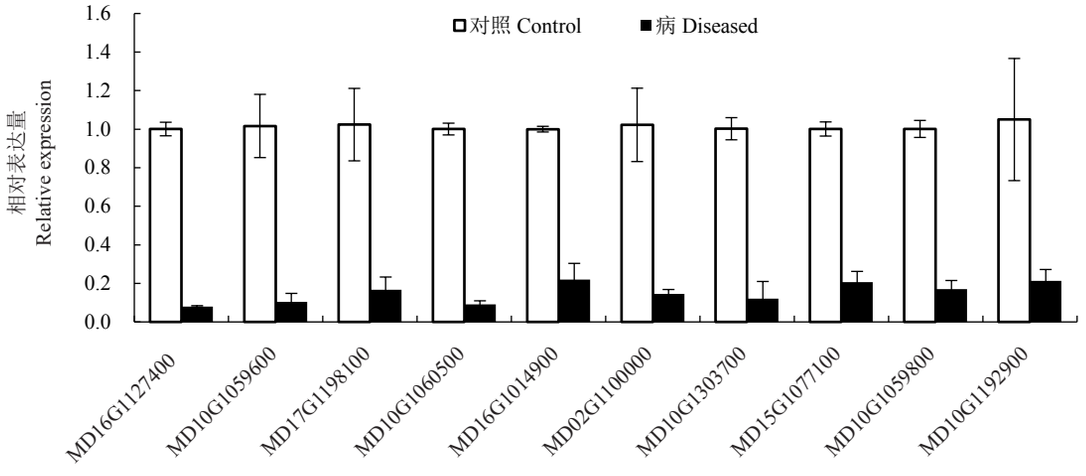


图6 实时定量PCR检测植物激素相关DEGs基因表达量

Fig. 6 Determination for the expression of plant hormonal-related DEGs using qRT-PCR

表3 植物激素相关DEGs信息及实时定量PCR引物

Table 3 Plant hormone related DEGs information and qRT-PCR primers

基因ID Gene ID	注释 Annotation	引物序列（5’→3’） Primer sequence (5’→3’)
MD16G1127400	茉莉酸-锌-结构域蛋白10 Jasmonate-zim-domain protein 10	F：ACCGTTGAGCTTGATTTTTTCGGCA R：CTGGCATTGCCGGAAGCGATGACGG
MD10G1059600	SAUR-like生长素响应蛋白家族 SAUR-like auxin-responsive protein family	F：CCTGGTATTTTACTTGCCAAGAAAA R：CTACTTATACACCAAAGCTGAGGTGA
MD17G1198100	吲哚-3-乙酸诱导19 Indole-3-acetic acid inducible 19	F:CTTGAAATTACAGAGCTGAGGTTGG R:GCCATAATAACCAAATAACTTCTCC
MD10G1060500	SAUR-like生长素响应蛋白家族 SAUR-like auxin-responsive protein family	F：ATGGGTTTCCGTCGTCCAAGTGTAA R：CTGGCAGAGGAAAGATATGGGAACCA
MD16G1014900	包含组氨酸激酶蛋白的CHASE结构域 CHASE domain containing histidine kinase protein	F：CAAGAAGAAGAATCAAAGAAATTGG R：GGGTTCTTGTAGTAATGGAAGGTGGA
MD02G1100000	SAUR-like生长素响应蛋白家族 SAUR-like auxin-responsive protein family	F：AAGTTGACCGGAATCAGGCAGATTG R：CCCTTGGGGGACATCAGGTGGCGGT
MD10G1303700	F-box/RNI-like亚家族蛋白 F-box/RNI-like superfamily protein	F：CGGGTTGCACGACCCGGAAGACGAA R：CCGCGAACCGGGGCTTCCCCTTCAG
MD15G1077100	细胞周期-D3-1-like Cyclin-D3-1-like	F：TAGAATCAAGTGAAGAACAAAACCC R：GCACCGCCGTGAGGGCGGAGAAAGAG
MD10G1059800	SAUR-like生长素响应蛋白家族 SAUR-like auxin-responsive protein family	F：ATGGGGTTCCGTCTGCCTTCTGTAA R：TCACTGACACAAATTTTCAAGAATAGT
MD10G1192900	吲哚-3-乙酸7 Indole-3-acetic acid 7	F：GCCGGGTGGTGGTGGCGGCGGCGTC R：TTGGCTAGGGCATCAGAGAGCTCAG

分析，发现共有194个转录因子的编码基因被显著诱导表达，并筛选出其中与植物抗病相关的转录因子家族编码基因：14个WRKY家族蛋白编码基因、14个MYB家族蛋白编码基因、12个ERF家族蛋白编码基因和5个NAC家族蛋白编码基因。与对照组相比，25个转录因子表达上调，20个转录因子表达下调（图7）。为验证转录组数据的可靠性，笔者利用qRT-PCR验证发现，*MdWRKY70*（MD01G1168600）、*MdWRKY71*（MD17G1138100）、*MdWRKY18- like*（MD15G1039600）、*MdNAC29*（MD13G1063900）基因表达量显著上调，而*MdERF61*（MD14G1127700）、*MdMYB34-like*（MD17G1051700）、*MdWRKY7*（MD1-7G10-48400）基因的表达量显著下调，与转录组检测结果一致（图8）。

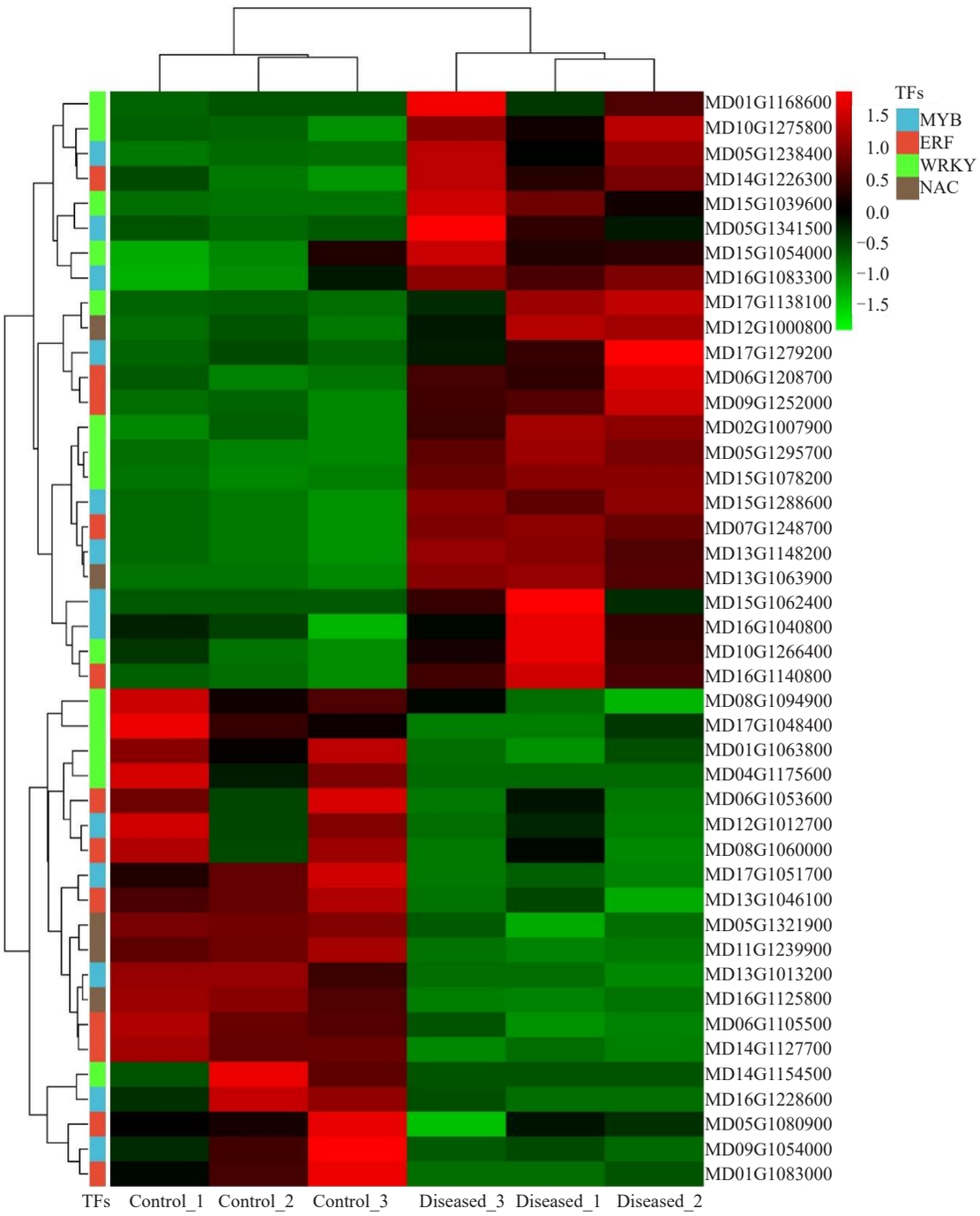


图7　转录因子相关 DEGs 的表达水平热图

Fig. 7　Heat map of the expression level of transcription factor-related DEGs

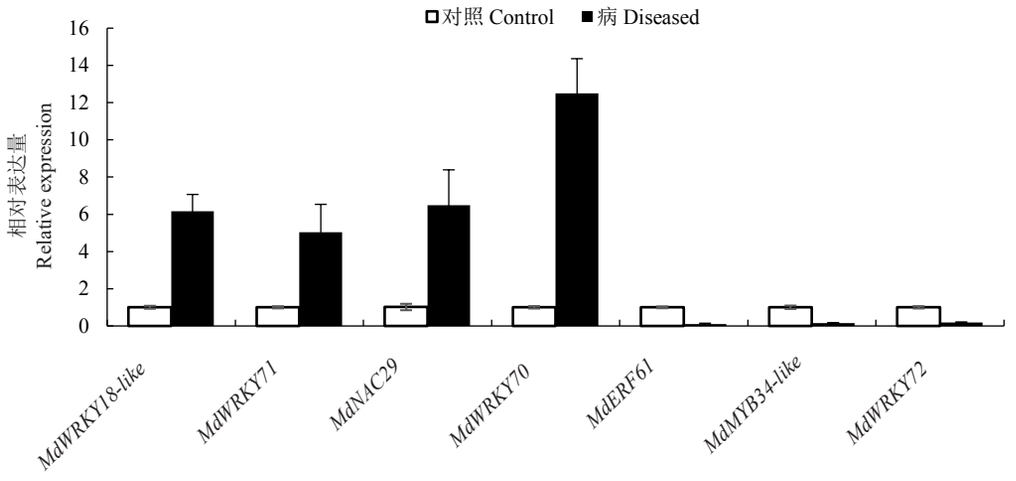


图 8 实时荧光定量 PCR 检测转录因子编码基因的表达量差异

Fig. 8 Determination of transcription factor-encoding genes using qRT-PCR

3 讨 论

近年来，在中国苹果主产区，如山东、辽宁、陕西、甘肃、新疆、河北等地，苹果锈果类病毒引发的病害逐年加重，已成为限制苹果产业健康发展的重要因素。但目前关于该病害的研究主要集中于ASSVd的检测及序列分析，关于病害发生的机制等问题尚未见报道。笔者在本研究中借助RT-PCR和转录组测序技术，对表现花脸症状的苹果果皮进行分析，共获得了6938个差异表达基因。并对差异表达基因的功能注释进行分析总结，为后续研究苹果花脸病害发生的机制及相关代谢途径提供了参考依据。

笔者在本研究中获得的2条ASSVd序列与GenBank中已收录的来自烟台地区富士苹果上的ASSVd分离物SDYT-1（MW302328.1）和SDYT-3（MW315909.1）完全一致，未发现碱基变异。有研究表明ADFVd与ASSVd同为Pospiviroidae科*Apscarviroid*属成员，其基因组RNA长度为306~310 nt，且与ASSVd序列高度相似，含有ASSVd序列的整个保守区域。

GO富集分析结果表明，表现花脸症状的苹果果皮中的DEGs主要集中在细胞过程、代谢过程、细胞、细胞组分、结合和催化活性等功能；同时，KEGG分析发现DEGs富集程度最高的代谢通路是植物激素信号转导，其次是苯丙烷生物合成和光合作用等通路，这说明花脸病对上述调控及反应途径有重要影响。

病原菌入侵会触发植物体内激素信号途径而激活植物免疫反应，提高植物抵御病原菌侵染的能力[19]。一般认为，SA、JA和ET是植物抗病反应中重要防御激素，SA通常参与植物对活体营养型和半活体营养型病菌防卫反应的激活，JA和ET则负责激活对死体营养型病菌的抗性，其他激素如IAA、ABA、GA等通过相互作用直接或间接地参与调节植物的抗病性。笔者在本研究中发现表现花脸症状的苹果果皮中SA信号途径相关基因*NPR4a*（MD05G1256300）、JA信号途径相关基因*TIFYs*（MD13G1127100、MD09G1178600、MD02G1096100、MD16G1127400、MD17G1164400）和ET信号途径相关基因*ERS1*（MD03G1292200）表达水平下降，而*EIN3*（MD08G1245800）表达水平上调。然而，不同于典型的抗病反应激素，表现花脸症状的苹果果皮中IAA途径相关DEGs数目最多，且多为编码IAA信号途径中的关键酶和受体蛋白。IAA信号途径的相关基因的表达模式与对照组相比多呈现下调趋势，表明IAA信号可能参与调控果实抗病性。同时基于IAA对植物生长发育的影响，笔者推测花脸病的发生可能通过影响IAA途径相关基因，减缓了IAA的生物合成，这可能是果实变小的原因之一。

当植物受到外界胁迫刺激时，转录因子对于传递胁迫信号及启动特定基因的表达具有重要作用[20]。研究表明，WRKY、MYB、ERF和NAC类转录因子广泛参与植物生长发育及防御过程，特别是WRKY类转录因子[19-20]。当植物受到细菌、病毒等病原物侵染时，WRKY基因的转录水平、转录后翻

译水平以及结合活性都会发生明显变化[21]，从而调节植物抗性反应。杨树*PsnWRKY70*基因与MARK级联成员结合增强植物对叶枯病的抗性[22]；过表达苹果*MdWRKY100*基因正调控苹果植株对炭疽病菌（*Colletotrichum*）的抗性[23]。MYB类转录因子主要通过调节植物的过敏反应（hypersensitive response，HR）和系统性获得抗性（systemic acquired resistance，SAR）来增强植物对病原菌的抗性[24]。此外，ERF和NAC家族蛋白也在植物抗病反应中发挥重要作用，如过表达*MdERF11*增强苹果对轮纹病菌（*Botryosphaeria dothidea*）的抗性[25]。水稻*OsNAC6*的表达受稻瘟菌所诱导，过量表达水稻*OsNAC6*基因提高了水稻对稻瘟病的抗性。笔者在本研究中发现，表现花脸病的果皮中大量WRKY、MYB、ERF类转录因子的编码基因表达水平发生显著变化。其中，*MdWRKY70*、*MdWRKY71*、*MdWRKY18- like*和*MdNAC29*表达受到显著诱导，而*MdERF61*、*MdMYB34-like*和*MdWRKY72*表达则受到显著抑制。推测转录因子可能通过调控下游结构基因的表达，参与寄主对病原菌的抗病反应。但关于其准确的内在机制还有待进一步验证。

4　结　论

笔者在本研究中对表现花脸症状的富士苹果果皮进行分析，分离得到2个ASSVd序列。转录组测序分析共获得6938个差异表达基因。利用基因功能注释分析发现这些差异表达基因多参与植物激素信号途径及苯丙烷代谢途径。此外，与抗病相关的WRKY、MYB、ERF类转录因子可能参与到苹果对花脸病的防御过程。

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