

1-MCP处理采后不同成熟度桃果实 生理效应及转录组分析

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摘要:【目的】挖掘响应1-MCP处理并且参与果实成熟软化进程的相关基因,为桃果实采后生理效应及作用机制研究提供新的思路。【方法】以溶质桃品种‘中油桃13’为研究对象,使用1-MCP分别处理呼吸跃变前期和呼吸跃变后期桃果实,测定1-MCP处理呼吸跃变前期和呼吸跃变后期果实乙烯和硬度的变化,进行转录组测序,筛选1-MCP处理呼吸跃变前期和呼吸跃变后期桃果实中的差异表达基因。【结果】1-MCP处理呼吸跃变前期桃果实,抑制桃果实贮藏期间乙烯释放和软化进程,1-MCP处理呼吸跃变后期桃果实刺激乙烯的释放。1-MCP处理1 d后,呼吸跃变前期和呼吸跃变后期果实与对照果实之间差异基因分别有1 365和346个,其中58个基因既存在于呼吸跃变前期差异基因,又存在于呼吸跃变后期差异基因中,288个基因只在呼吸跃变后期果实中差异表达。对288个基因进行功能富集分析,表明差异基因主要富集于激素合成和生物胁迫途径中。筛选到11个多肽类基因,可能在桃果实成熟软化过程中发挥重要的作用。【结论】1-MCP处理呼吸跃变前期和呼吸跃变后期桃果实对其采后贮藏过程中乙烯释放及软化作用不同。呼吸跃变后期桃果实中乙烯和生长素等激素信号传导相关基因响应1-MCP处理,更新颖的是发现一类多肽基因,其在呼吸跃变后期响应1-MCP处理,在桃果实成熟过程中发挥重要的作用,可能为以后桃果实采后保鲜的研究提供新的思路。

关键词:桃;1-MCP;乙烯;果实成熟;转录组分析

中图分类号:S662.1

文献标志码:A

文章编号:1009-9980(2020)12-1798-13

Physiological effects and transcriptome analysis of peach fruit with different maturity after 1-MCP treatment

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Abstract: 【Objective】1-methylcyclopropene (1-MCP), known to block ethylene receptors, which has improved application potential in fruit ripening and in cut flowers. Melting flesh peach is a typical respiratory climacteric fruit which softens quickly and susceptible to infections by pathogenic bacteria, thus causing severe economic losses. Limited studies related to 1-MCP treatment on postharvest physiological effects during peach fruit by high-throughput sequencing technology were reported. In this study, to identify the differentially expressed genes (DEGs) including the genes involved in fruit ripening, hormones and polypeptide under 1-MCP treatment, the molecular mechanisms of 1-MCP treatment on peach fruit by high-throughput sequencing was explored. 【Methods】The melting flesh ‘CN13’ was grown in Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences. ‘CN13’ fruits were collected at 80 and 85 days after flowering (DAF) (designated S4 I and S4 II, respective-

收稿日期:2020-03-31 接受日期:2020-11-14

基金项目:国家重点研发计划(2019YFD1000200);国家自然科学基金(31872085);中国农业科学院科技创新工程(CAAS-ASTIP-2019-ZFRI)

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ly), S4 I represents the stage corresponding to class 0 (pre-climacteric), S4 II represents the stage corresponding to class 1 (onset of climacteric), and was treated with 1-MCP at the concentration of $10 \text{ mmol} \cdot \text{L}^{-1}$ for 24 h. Ethylene and firmness were measured by gas chromatography and penetrometer respectively. The cDNA library was constructed and analyzed using Illumina HiSeq 2500. Align and annotate reference genome sequences with software Tophat. The software HTSeq was used to analyze the difference level; the software DEGSeq was used to analyze the differential gene expression. The software Goseq and KOBAS were used to perform GO enrichment analysis and KEGG enrichment analysis of differentially expressed gene. Predictive analysis of transcription factors was done by using iTAK software. 【Results】In pre-climacteric, 1-MCP was effective in both reducing ethylene emission and fruit firmness, while in onset of climacteric, 1-MCP effect was intermediate, the inhibitor induced ethylene production but was able to delay fruit softening. The transcriptome of 24 h treatment with 1-MCP was emphatically analyzed. 1 384 differentially expressed genes were found in pre-climacteric, including 1 067 up-regulated genes and 317 down-regulated genes; 346 differentially expressed genes in onset of climacteric, with 143 genes were up-regulated, and 203 genes were down-regulated. Only 288 differentially expressed genes were found between the fruits under 1-MCP treatment in pre-climacteric and in onset of climacteric. The clustering heat map analysis of the 288 genes included 124 genes were significantly down-regulated after 1-day treatment with 1-MCP, and 164 genes were significantly up-regulated after 1-day treatment with 1-MCP. GO enrichment analysis have shown that the differential expression genes were mainly enriched in hormone synthesis and biological stress pathways. Transcription factor analysis has shown the genes mainly belong to WARK, ETR and ERF family. Functional annotation of unknown genes revealed that 11 out of 40 unknown genes were polypeptide, accounting for 27.5% of unknown genes. RT-PCR analysis found that the aging-related gene *Prupe.4G214900* and ethylene-related gene *Prupe.4G095400* were down-regulated after 1-MCP treatment, and the polypeptide genes *Prupe.1G472800*, *Prupe.4G270100*, *Prupe.5G072500*, *Prupe.7G256100*, and *Prupe.5G059100* were up-regulated. 【Conclusion】1-MCP treatment had distinct effects on ethylene release and softening process in pre- and onset climacteric peach fruits. Transcriptome analysis indicated that 1-MCP treatment affects differential gene expression depend on different development stage. In onset climacteric peach fruits, 1-MCP treatment induces the expression of genes related to ethylene and auxin signaling, and the genes may accerlate fruit softening process during postharvest storage. Furthermore, some new peptide genes were observed, which may play an important role in peach fruit ripening. These findings will provide new insight for future study on postharvest preservation of peach fruits.

Key words: Peach (*Prunus persica*); 1-MCP; Ethylene; Fruit ripening; Transcriptome analysis

桃是世界大宗果品之一,果实营养丰富,风味鲜美独特,消费者需求大,收益可观^[1]。根据软化程度,桃被分为溶质型、不溶质型和硬质型桃^[2]。溶质型桃属于典型的呼吸跃变型果实,果实生长曲线呈双S型,果实采后成熟期间伴随着乙烯跃变高峰的出现,果实迅速软化,不耐储运并且易受病原菌的感染,造成严重的经济损失^[2]。研究桃果实的成熟软化机制以及采后生理效应,具有重要的应用价值。

乙烯在桃果实成熟软化进程中起着至关重要的

调控作用。乙烯是通过杨氏循环中两个关键酶[ACC合成酶(ACC synthase, ACS)和ACC氧化酶(ACC oxidase, ACO)]的催化产生的。乙烯合成后由位于细胞膜上的乙烯受体(ETR)感知,并将乙烯信号传递至细胞核,最后由乙烯响应因子(ERFs)调控相关基因的表达。乙烯合成或信号转导相关基因的沉默会直接影响果实的成熟。番茄中,沉默乙烯合成途径中关键基因 *ACS2* 和 *ACO1*,果实成熟进程被显著抑制或推迟,说明乙烯对跃变型果实的成熟过程起着重要的作用^[3]。

1-甲基环丙烯(1-MCP)作为一种乙烯受体竞争性抑制剂,能够与乙烯受体 ETR1 和 ERS1 蛋白互作进而阻断乙烯受体与乙烯的正常结合,抑制果实后熟衰老^[4]。1-MCP 无毒、无明显气味、稳定性好且作用浓度低,在果品保鲜和贮运中应用范围广泛^[5]。近年来研究发现,在果实发生呼吸跃变,大量释放乙烯前,使用 1-MCP 熏蒸能明显抑制苹果、香蕉、梨、猕猴桃等多种呼吸跃变型果实成熟过程中乙烯与受体的结合进而抑制乙烯的释放,使得果品贮藏期和货架期大大延长^[6]。田蓉等^[7]运用 1-MCP 处理苹果品种‘富金’发现,1-MCP 处理可使‘富金’苹果保持较高的贮藏品质;Jiang 等^[8]研究表明,1-MCP 不仅能够显著降低香蕉的乙烯产量和呼吸速率,还不同程度地延迟乙烯和呼吸峰的出现,延缓果实衰老,延长果实的贮藏期和货架寿命。李富军等^[9]运用 1-MCP 对采后肥城桃进行熏蒸处理,发现 1-MCP 能显著降低桃果实乙烯释放量,提高冷藏中果实的硬度,减轻失重及冷害发生,延缓果实衰老。使用 1-MCP 处理‘秦王’桃^[10-11]、‘青州’蜜桃^[12]、‘中华’寿桃^[13-14]、蟠桃^[15]、‘丽江’蜜桃^[16]、‘八月脆’桃^[17]、蜜桃^[18]、‘菊黄’桃^[19]和‘艳红’水蜜桃^[20]等,均可以抑制果实内源乙烯的合成和呼吸强度,推迟呼吸高峰出现,并且延缓果实软化。但是 1-MCP 对果蔬保鲜效果受果蔬成熟度的影响。Harris 等^[21]研究发现,1-MCP 处理绿熟香蕉果实可维持较长的处理效应;Fan 等^[22]和 Baritelle 等^[23]分别对杏与梨研究表明,1-MCP 的效果随成熟度增加而减弱;Tadiello 等^[24]采用 1-MCP 分别处理溶质桃呼吸跃变前期和呼吸跃变后期果实发现:1-MCP 处理呼吸跃变前期的桃果实,能够显著抑制贮藏过程中乙烯的产生,使果实硬度下降速率减慢;相反地,1-MCP 处理跃变后期的桃果实在贮藏过程中刺激乙烯的释放。

以上研究表明,1-MCP 处理对呼吸跃变前期和呼吸跃变后期的桃果实乙烯释放效果不同,针对这一问题,笔者采用 1-MCP 分别处理呼吸跃变前期和呼吸跃变后期的桃果实,对采后生理效应进行分析;对 1-MCP 处理的呼吸跃变前期和呼吸跃变后期的桃果实进行转录组测序,通过对测序结果进行基因功能注释、差异基因分析以及代谢途径分析等,进一步挖掘响应 1-MCP 处理并且参与果实成熟软化进程的相关基因,为桃果实采后生理效应及作用机制研究提供新的思路。

1 材料和方法

1.1 试验材料及处理

以中国农业科学院郑州果树研究所桃育种圃保存的溶质桃品种‘中油桃 13’为试材,‘中油桃 13’属于典型的呼吸跃变型桃果实,成熟后期果实迅速软化并伴有乙烯释放高峰出现,不耐贮运。跃变前期果实硬度下降缓慢,无乙烯释放,跃变后期桃果实硬度迅速下降,出现乙烯释放高峰。采取处于跃变前期(花后 80 d)与跃变后期(花后 90 d)的‘中油桃 13’果实各 60 个,各时期果实均随机分为两组,每组 30 个果。1-甲基环丙烯(1-MCP)为安喜培牌泡腾片,有效成分含量为 0.18%,室温贮藏。处理组和对照组桃果实置于收纳箱中分别用清水和 10 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP 处理 24 h,在 20 $^{\circ}\text{C}$ 条件下保存^[25]。

1.2 ‘中油桃 13 号’果实乙烯和硬度的测定

对实试组和对照组的呼吸跃变前期桃果实连续取样 6 d,呼吸跃变后期桃果实取样 5 d,对每个时间点分别随机取 3~5 个果实,用津岛 GC-2010 气相色谱仪和手持式数字硬度仪(300N 30kg 4401b)测定乙烯释放量和果实硬度,测定方法参照丁义峰^[26]的方法。随后将样品立即分样冻存在液氮中,保存于-80 $^{\circ}\text{C}$ 冰箱中备用。

1.3 总 RNA 提取,文库构建及转录组 Illumina HiSeq 测序

按常规 Trizol 法提取 1-MCP 处理‘中油桃 13’呼吸跃变前后及对照组果实的总 RNA。运用 1%琼脂糖凝胶电泳检测 RNA 降解程度以及是否有污染;运用 Nanodrop 检测 RNA 的纯度, OD_{260}/OD_{280} 为 1.8~2.2;Qubit 对 RNA 浓度进行精确定量;Agilent 2100 bioanalyzer 精确检测 RNA 的完整性。样品检测合格后,用带有 Oligo(dT)的磁珠,通过 A-T 互补配对与 mRNA 的 polyA 尾结合方式富集真核生物 mRNA。随后加入 fragmentation buffer 将 mRNA 打断成短片段,以 mRNA 为模板,用六碱基随机引物(random hexamers)合成一链 cDNA,然后加入缓冲液、dNTPs 和 DNA polymerase I 合成二链 cDNA,随后利用 AMPure XP beads 纯化双链 cDNA。纯化的双链 cDNA 再进行末端修复、加 A 尾并连接测序接头,然后用 AMPure XP beads 进行片段大小选择,最后进行 PCR 富集得到最终的 cDNA 文库。随后使用 Illumina HiSeq 2500 进行测序及分析。

1.4 转录组序列比对及注释

对测序得到的原始测序序列进行过滤处理,去除带接头(adapter)的 reads、N(表示无法确定碱基信息)的比例大于 10%的 reads 以及低质量 reads (Qphred ≤ 20 的碱基数占整个 read 长度的 50%以上的 reads),运用软件 Tophat v2.0.12^[27]对参考基因组序列进行比对并注释。

1.5 差异表达分析及差异富集分析

采用 readcount 数据,运用软件 HTSeq v0.6.1^[28]进行差异水平分析;运用软件 DESeq^[29]进行差异基因表达分析,设定阈值为 $|\log_2\text{foldchange}| > 1$ 且 $p \leq 0.05$ 。利用 1-MCP 处理呼吸跃变后期‘中油桃 13’的 288 个差异基因的 FPKM 值绘制聚类热图,运用软件 Goseq Release2.12^[30]和 KOBAS v2.0^[31]进行差异表达基因的 GO 富集分析和 KEGG 富集分析。

1.6 差异表达基因转录因子与未知功能基因分析

使用转录因子预测软件 iTAK,利用 database 中分类定义好的 TF(transcription factor) family 及规则,通过 hmmscan 鉴定 TF。对未能在基因组中进行功能注释的差异表达基因进行氨基酸序列比对、进化分析、文献查找及功能注释。

将呼吸跃变后期桃果实 1-MCP 处理放置 0~3 d 及对照果实提取总 RNA,反转录 cDNA,使用 Light-Cycler 480 II 荧光定量仪进行 qRT-PCR,对 40 个未知功能基因的转录组结果进行验证,具体方法参照王雁等^[32]的方法。

1.7 qPCR 检测转录组数据

运用 Primer 3 (v. 0.4.0)设计荧光定量引物,在核酸系列保守区内设计并具有特异性,引物长度在 20 bp 左右,G+C 含量在 40%~60%,引物 Tm 值在 58~62 °C,引物避免发夹结构和引物二聚体。定量引物见表 1。

2 结果与分析

2.1 1-MCP 处理跃变前后期果实乙烯和硬度的变化

使用 1-MCP 处理呼吸跃变前期和呼吸跃变后期‘中油桃 13’桃果实,运用气相色谱仪和硬度计分别测定对照与处理的乙烯与硬度变化(图 1,图 2)。与对照相比,1-MCP 处理呼吸跃变前期桃果实抑制乙烯的释放,果实硬度下降速度慢,延缓果实的成熟(图 1)。1-MCP 处理呼吸跃变

后期桃果实硬度下降较慢,但是刺激乙烯的释放(图 2)。

2.2 跃变前后差异基因查找与热图分析

为进一步验证 1-MCP 处理呼吸跃变前期和呼吸跃变后期果实是否存在差异基因表达,分别取呼吸跃变前期桃果实采后处理放置 0~3 d 的 1-MCP 处理与对照样品和呼吸跃变后期桃果实采后 0~3 d 的 1-MCP 处理与对照样品进行转录组测序。呼吸跃变后期共检测到 26 874 个转录本信息,呼吸跃变前期共检测到 17 587 个转录本信息。根据筛选标准($p \leq 0.05$ 且 $|\log_2\text{Ratio}| \geq 1$),筛选差异表达基因。重点分析 1-MCP 处理 1 d 时转录组信息,呼吸跃变前期共有 1 384 个差异表达基因,其中上调表达基因 1 067 个,下调表达基因 317 个;呼吸跃变后期共有 346 个差异表达基因,其中上调表达基因 143 个,下调表达基因 203 个。根据 1-MCP 处理前后差异基因绘制维恩图(图 3)。由于 1-MCP 处理 1 d 时已经对桃果实基因表达产生了显著影响,接下来将重点对 1-MCP 处理 1 d 后与对照果实差异表达基因进行分析。维恩图分析显示,‘中油桃 13’呼吸跃变前期和呼吸跃变后期共表达的差异基因有 58 个,仅在 1-MCP 处理 1 d 呼吸跃变后期‘中油桃 13’果实中差异表达的基因有 288 个。由于 1-MCP 处理跃变后桃果实刺激乙烯的释放,因此将只在呼吸跃变后期桃果实中差异表达的 288 个基因作为研究重点。

根据 1-MCP 处理呼吸跃变后期‘中油桃 13’的 288 个差异基因的 FPKM 值绘制聚类热图(图 4),结果发现,这 288 个基因聚为两大类,一部分基因在 1-MCP 处理果实 1 d 后表达显著下调,另一部分基因在 1-MCP 处理果实 1 d 后显著上调表达。

2.3 1-MCP 处理跃变后期桃果实与对照果实差异基因 GO 富集分析

对 288 个差异基因进行 GO 富集分析(图 5)。由 GO 富集分析结果可以看出,差异基因主要富集在激素合成和生物胁迫途径中。

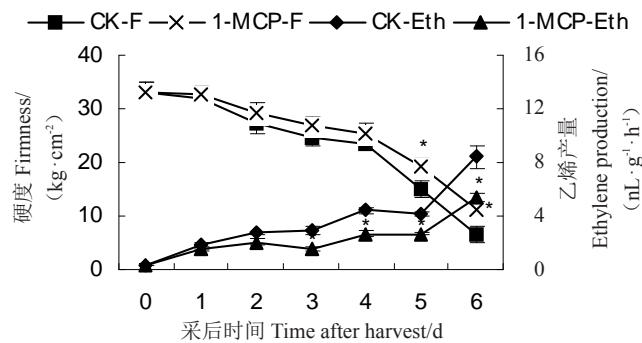
2.4 1-MCP 处理跃变后期桃果实与对照果实差异基因 KEGG 富集分析

对 288 个差异基因与 KEGG 数据库比对(图 6),由 KEGG 富集分类图可以看出,1-MCP 处理呼吸跃变后期桃果实差异基因的富集通路主要集中在

表 1 实时定量 PCR 引物

Table 1 Primers used for quantitative RT-PCR

基因名称 Gene name	引物 Primers (forward/reverse)
<i>Prupe.1G038200</i>	5'-ACCACCTGTTCTCTTGCTGA-3'/5'-TGCTGCCTCATTATCAAGCG-3'
<i>Prupe.1G042300</i>	5'-CTCTGAAGCAATCCAAGCCC-3'/5'-GTCACAGACGCCTTGTGAC-3'
<i>Prupe.1G233600</i>	5'-ATCCCAACACTTCCAGCCTT-3'/5'-CGGCTGTGGAGGATTGAGTA-3'
<i>Prupe.1G253100</i>	5'-GGTTTCGGTGCTGTCTTTGT-3'/5'-GCTGCGGGTTAGTGTCAAT-3'
<i>Prupe.1G280200</i>	5'-ATCGGCACCTCTAAACCTGT-3'/5'-TGGAGTTGTTGGGGATGGT-3'
<i>Prupe.1G322100</i>	5'-TGCCACCTCTTTGCTTCT-3'/5'-TGGGGATTGTGCAGTCTCTT-3'
<i>Prupe.1G379600</i>	5'-GCCTCAGAAAACGAAGGAGC-3'/5'-AGCTTCTTCTGTTTGTGGTGT-3'
<i>Prupe.1G411600</i>	5'-GGAATCTGTCTCAATTTGGGCT-3'/5'-CCTTGCTGTGGTTTTGTGG-3'
<i>Prupe.1G415300</i>	5'-AACTTCACTCGTGGCTCTCA-3'/5'-GAGATGAACGACGGGGAGAT-3'
<i>Prupe.1G472800</i>	5'-AGACATCAGGGGCACAGAAG-3'/5'-GGCCTCATTTCCAAGTCTAACT-3'
<i>Prupe.1G577800</i>	5'-TCATCTTCAAAGCTGCCTCA-3'/5'-TGAACAACCAAGTCCCTCA-3'
<i>Prupe.2G282900</i>	5'-CACCCACACCCAAACAAACC-3'/5'-CTTCGAGGGAGATGGAGTCG-3'
<i>Prupe.2G301600</i>	5'-GGGCTTGAAGGGCTTGATTC-3'/5'-CTTCTCCTTTTGTGTTGCC-3'
<i>Prupe.3G073700</i>	5'-CCGTCTCCCAAATAGCGG-3'/5'-GCCACGGACTTGAGTTGAGT-3'
<i>Prupe.4G037000</i>	5'-GCGAGTCTTATGATGTGCCAAT-3'/5'-CCTGTTATGATCGGCACTGG-3'
<i>Prupe.4G082100</i>	5'-GTTACAGTCACCAAAAACAAAG-3'/5'-TTAATAGTGCTTGGTGG-3'
<i>Prupe.4G095400</i>	5'-AATTTCCCAATTCGTCGCCG-3'/5'-CGGGTTATTCTTCTTCTGTTCT-3'
<i>Prupe.4G161400</i>	5'-TCTTGTCTCCTCTTTGCTCT-3'/5'-GTCACCCAGATCAGAAAAGC-3'
<i>Prupe.4G214900</i>	5'-TCTTGGGAAAGTTGTGGTGC-3'/5'-ACCTCCCAAACATCCTCTTCC-3'
<i>Prupe.4G270100</i>	5'-AGAAATGGGTTGCTGCCAAG-3'/5'-GCACAGCTTGATGTCGTC-3'
<i>Prupe.5G059100</i>	5'-ATGCTCACCCTCGTATGTT-3'/5'-GGTGGTTGATGGAGGTGGT-3'
<i>Prupe.5G072500</i>	5'-GCTGAAACATGACCCATGAAAG-3'/5'-GCATGCCCTTGTTCCTCAA-3'
<i>Prupe.5G109500</i>	5'-ACCAAAGAAGTGATGCAGAGTC-3'/5'-ATGTTGCCTAGCCTGAACCT-3'
<i>Prupe.5G177800</i>	5'-TTGGTCTGTGAGTGCAAGTG-3'/5'-ATTGTTGTTGGGATTGGAGGG-3'
<i>Prupe.5G188200</i>	5'-AAAGCTTCTTCGGTATGCGC-3'/5'-GCTTGTCTTGCCAGAGAGA-3'
<i>Prupe.6G063000</i>	5'-AAAATGGCGTGCTGTGGG-3'/5'-TCGACGTATTGAGATGATGTGT-3'
<i>Prupe.6G112200</i>	5'-AGCATGGAAGAGTTGAGGCA-3'/5'-TGTGCTTCATCTTTTCTCTGA-3'
<i>Prupe.6G285700</i>	5'-GTTCTGGTACGTGCTTCTT-3'/5'-TCAGGCAGAGACAAGAAGCA-3'
<i>Prupe.6G329100</i>	5'-AGCTGGAGGTTATGGATCTGA-3'/5'-TCCCAATCACAGTCACCATCC-3'
<i>Prupe.7G061500</i>	5'-TTCTACTGCCACAAACTG-3'/5'-TGCCTTGACACCGCAACT-3'
<i>Prupe.7G091900</i>	5'-TGAAGATGATGAGGTGGCGT-3'/5'-GACCTTCGATCCCTTCCACA-3'
<i>Prupe.7G120800</i>	5'-TGCATCAAACACAAAACCC-3'/5'-TGTTTGGACTTGGTGGCAAA-3'
<i>Prupe.7G204200</i>	5'-GCCATCATCACCTTCTTCT-3'/5'-CGAAGATCCCGCAGAGAATC-3'
<i>Prupe.7G256100</i>	5'-TTTCTGGGGCTCAAGGGATT-3'/5'-ACTCTCCACCATCTTTCGAGA-3'
<i>Prupe.8G014200</i>	5'-TCTTGGGATCTTTGGCATCTG-3'/5'-ACTGTTGGTGTGTTTCCGGT-3'
<i>Prupe.8G031200</i>	5'-AGCGACGGAATATCAAGGGT-3'/5'-CGACGTGGGTTTGGAAAAGAG-3'
<i>Prupe.8G065400</i>	5'-AATCCCCTACATCGTCACC-3'/5'-GAGAAGGTGGAGAGACTGCG-3'
<i>Prupe.8G065500</i>	5'-GGCGTCGTTTTGGTGCTAAT-3'/5'-TTTTGGGAGAGAACACAGGG-3'
<i>Prupe.8G119300</i>	5'-CCCTGTGGACTTCAACTTCG-3'/5'-GAGAGGTGCTTGAAGTGGCT-3'
<i>Prupe.8G270300</i>	5'-TGCGTCAGTGATACATGGGA-3'/5'-CTTGTGAGCCGTGGTTGTAG-3'



CK-F 和 CK-Eth 分别代表对照桃果实硬度和乙烯释放量, 1-MCP-F 和 1-MCP-Eth 分别代表 1-MCP 处理桃果实硬度和乙烯释放量。
 CK-F and CK-Eth represent the firmness and ethylene release of the control peach fruit, 1-MCP-F and 1-MCP-Eth represent the firmness and ethylene release of the 1-MCP-treated peach fruit, respectively.

图 1 1-MCP 处理跃变前期 ‘中油桃 13’ 桃果实采后乙烯和硬度的变化

Fig. 1 Changes of ethylene and firmness in the pre-climacteric of ‘CN13’ peach fruit by 1-MCP treatment

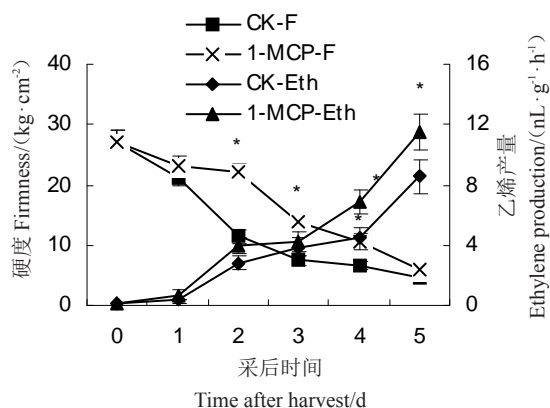


图2 1-MCP处理跃变后期‘中油桃13’桃果实采后乙烯和硬度的变化

Fig. 2 Changes of ethylene and firmness in the post-climacteric of ‘CN13’ peach fruit under 1-MCP treatment

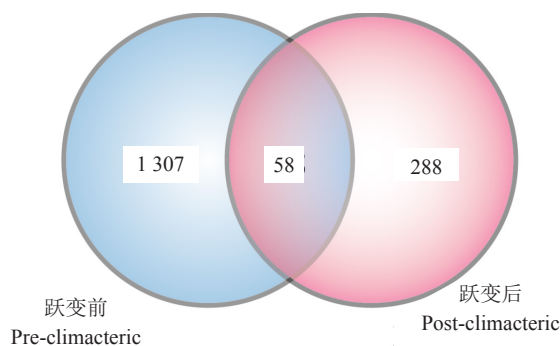
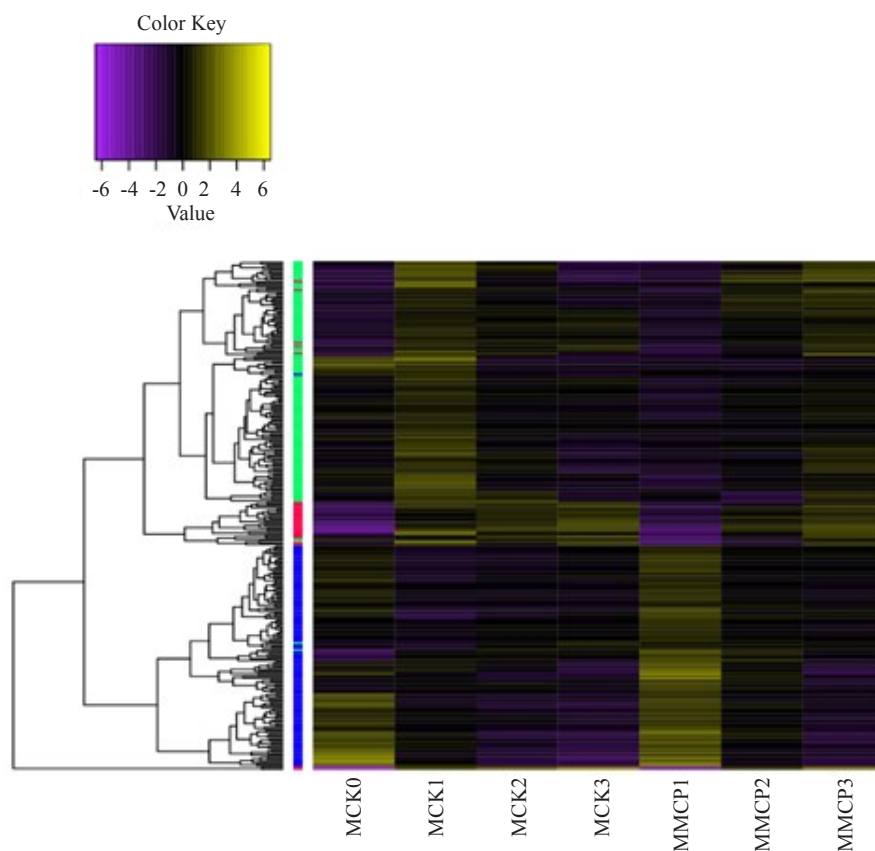


图3 差异基因维恩图

Fig. 3 Venn diagram of differential genes



MCK0、MCK1、MCK2、MCK3、MMCP1、MMCP2、MMCP3 分别代表呼吸跃变后期桃果实采后 0 d、1 d、2 d、3 d 对照与 1-MCP 处理样品。
MCK0, MCK1, MCK2, MCK3, MMCP1, MMCP2, MMCP3 represent the 0 d, 1 d, 2 d, 3 d control and 1-MCP treated samples of the post-harvest peach fruit in the post-climacteric.

图4 差异基因表达热图

Fig. 4 Heatmap of differential gene expression

新陈代谢(metabolism)部分,富集基因条数最多的通路是植物激素信号转导(plant hormone signal transduction),有13条,占有被KEGG注释的差异基因的23.21%。

2.5 1-MCP处理跃变后期桃果实与对照果实差异基因转录因子分析

1-MCP处理呼吸跃变后期桃果实差异基因的转录因子查找结果见表2。共有11类转录因子,其

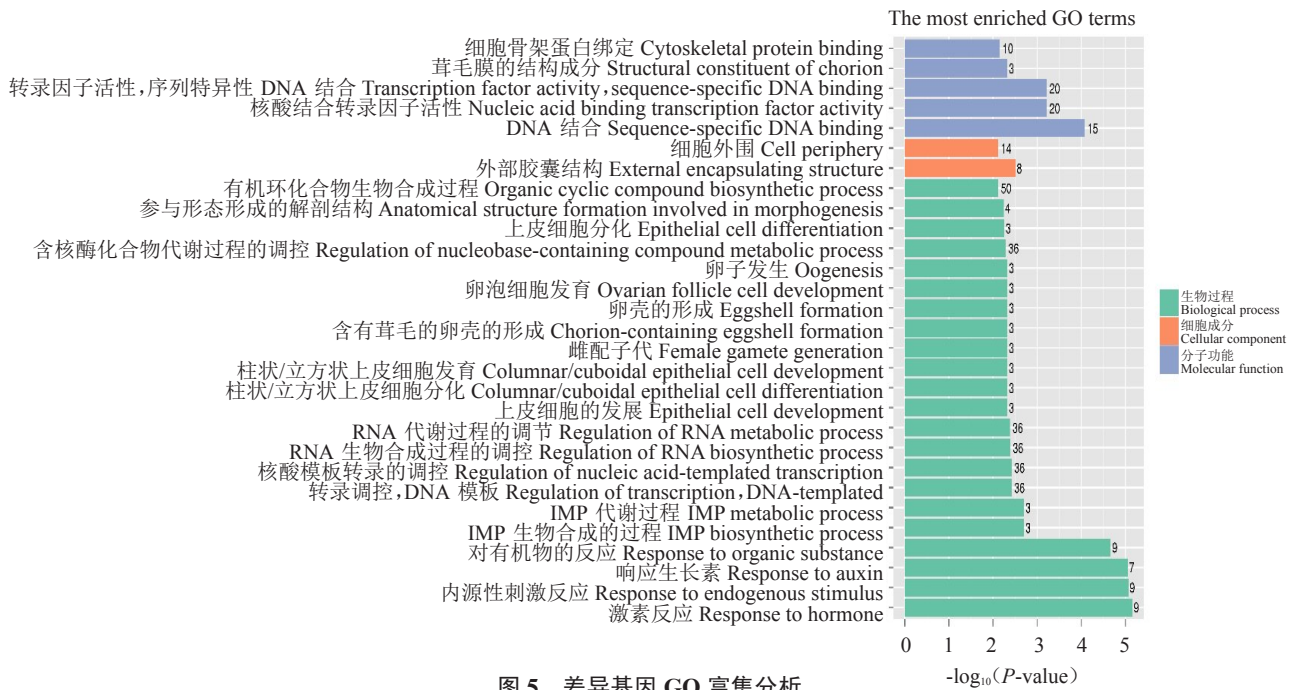


图 5 差异基因 GO 富集分析

Fig. 5 GO enrichment analysis of differential genes

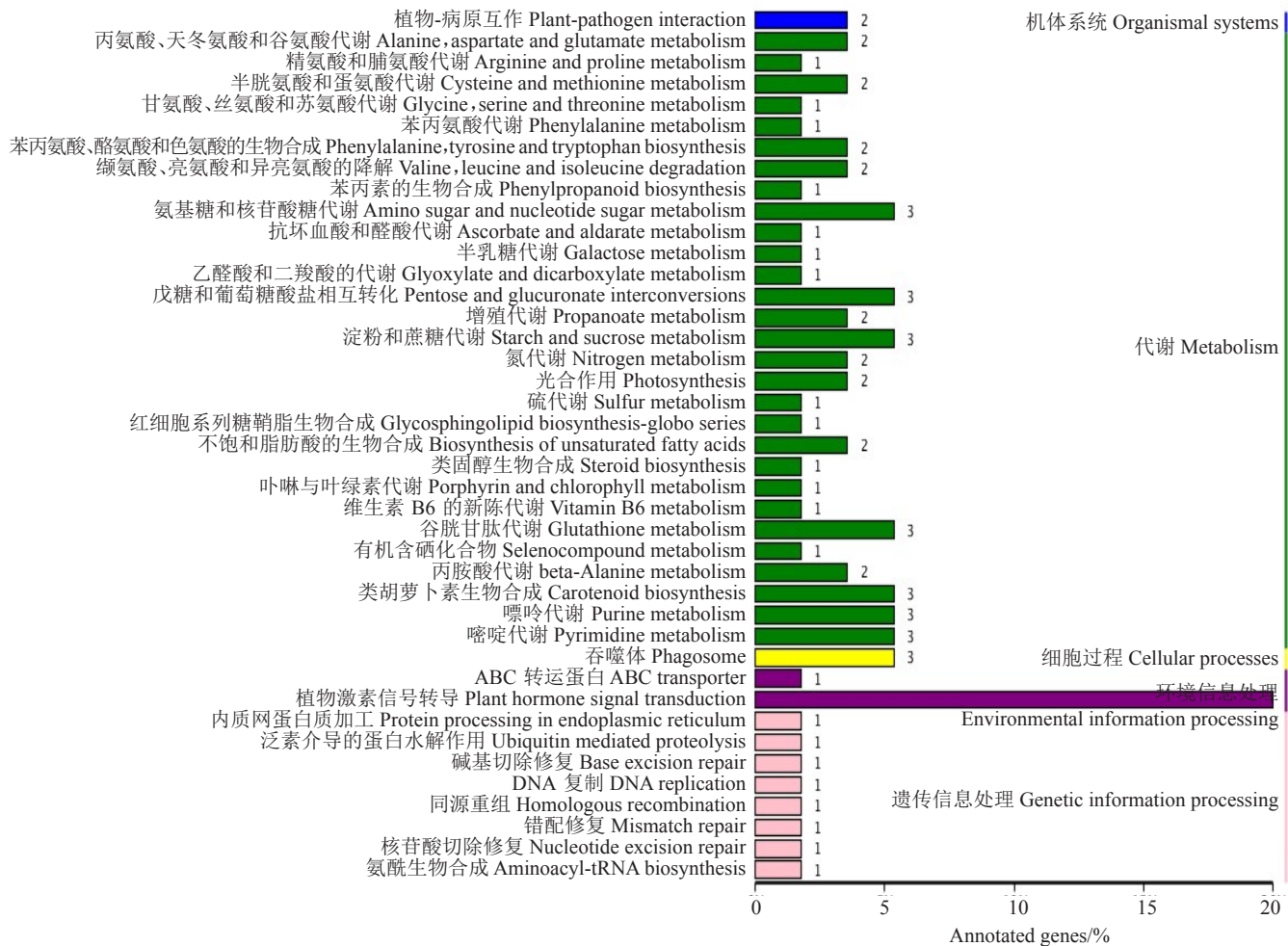


图 6 差异基因 KEGG 富集分析

Fig. 6 KEGG enrichment analysis of differential genes

表2 差异基因转录因子分析

Table 2 Transcription factor analysis of differential genes

基因名称 Gene name	转录因子类型 Transcription factor type	基因功能注释 Gene function annotation
<i>Prupe.2G035400</i>	PTR	NRT1/ PTR 蛋白家族 NRT1/ PTR protein family
<i>Prupe.5G205700</i>	HAT	复制蛋白 A 70 kDa DNA 结合亚基 B Replication protein A 70 kDa DNA-binding subunit B
<i>Prupe.5G064300</i>	HAT	亮氨酸拉链蛋白质 HAT4 Homeobox-leucine zipper protein HAT4
<i>Prupe.1G416800</i>	HAT	亮氨酸拉链蛋白质 HAT2 Homeobox-leucine zipper protein HAT2
<i>Prupe.1G130300</i>	RAP	乙烯应答转录因子 RAP2-3 Ethylene-responsive transcription factor RAP2-3
<i>Prupe.2G289500</i>	DRE1D	脱水反应元件结合蛋白 1D Dehydration-responsive element-binding protein 1D
<i>Prupe.5G061800</i>	EF	乙烯应答转录因子 IA Ethylene-responsive transcription factor 1A
<i>Prupe.8G224600</i>	ERF	乙烯应答转录因子 IB Ethylene-responsive transcription factor 1B
<i>Prupe.3G240000</i>	RAVL	AP2/ERF 和 B3 结构域-转录抑制因子 TEM1 AP2/ERF and B3 domain-containing transcription repressor TEM1
<i>Prupe.3G108700</i>	HFA4B	热应激转录因子 A-4b Heat stress transcription factor A-4b
<i>Prupe.5G117800</i>	ERF	乙烯应答转录因子 ERF061 Ethylene-responsive transcription factor ERF061
<i>Prupe.1G034300</i>	ETR	乙烯受体 2 Ethylene receptor 2
<i>Prupe.1G071400</i>	WRKY	WRKY 转录因子 11 Probable WRKY transcription factor 11
<i>Prupe.1G114800</i>	WRKY	WRKY 转录因子 48 Probable WRKY transcription factor 48
<i>Prupe.1G277500</i>	NAC	NAC 转录因子 29 NAC transcription factor 29
<i>Prupe.1G431100</i>	WRKY	WRKY 转录因子 7 WRKY transcription factor 7
<i>Prupe.2G185100</i>	WRKY	WRKY 转录因子 WRKY transcription factor
<i>Prupe.2G307400</i>	WRKY	WRKY 转录因子 53 WRKY transcription factor 53
<i>Prupe.3G098100</i>	WRKY	WRKY 转录因子 40 WRKY transcription factor 40
<i>Prupe.5G117000</i>	WRKY	WRKY 转录因子 41 WRKY transcription factor 41

中数量最多的是 WRKY 转录因子,说明 1-MCP 处理对于果实的抗逆性可能具有调控作用。与乙烯相关的转录因子有 3 个,其中包括 ETR 和 ERF,说明 1-MCP 调控呼吸跃变后期果实中乙烯的合成和信号传导。

2.6 未知功能基因注释、多肽基因的确定及表达分析

对未知基因进行功能注释,发现 40 个未知基因中 11 个为多肽基因(表 3),占未知基因总数的 27.5%,多肽基因分别为:*Prupe.1G472800*、*Prupe.1G322100*、*Prupe.1G415300*、*Prupe.1G038200*、*Prupe.4G16140*、*Prupe.4G270100*、*Prupe.5G059100*、*Prupe.5G072500*、*Prupe.7G256100*、*Prupe.8G065400*、*Prupe.8G065500*。11 个多肽基因参与的功能包括生长因子调控、细胞膜组分、编码糖蛋白、电子转移等。

运用转录组数据分析 40 个未知基因在 1-MCP 处理呼吸跃变后期桃果实与对照之间的基因表达情况,运用 FPKM 值绘制表达热图(图 7),发现其中有 16 个基因表达上调,24 个基因下调表达。1-MCP 处理之后衰老相关基因 *Prupe.4G214900* 和乙烯相关基因 *Prupe.4G095400* 下调表达。多肽基因 *Prupe.1G472800*、*Prupe.4G270100*、*Prupe.5G072500*、*Prupe.7G256100*、*Prupe.5G059100* 上调表达(图 7)。

对转录组数据进行 qRT-PCR 验证,绘制表达热图(图 8),与转录组数据一致,qRT-PCR 结果显示,1-MCP 处理 1 d 后下调表达基因 24 个,上调表达基因 16 个,并且衰老相关基因 *Prupe.4G214900* 和乙烯相关基因 *Prupe.4G095400* 下调表达,多肽基因 *Prupe.1G472800*、*Prupe.4G270100*、*Prupe.5G072500*、*Prupe.7G256100* 上调表达。将转录组数据与 qRT-PCR 结果进行相关性分析,Pearson 相关系数为 0.89(图 9),显示两者趋势呈正相关。

3 讨论

1-MCP 能有效抑制乙烯产生,在果蔬贮藏和保鲜方面具有潜在的应用价值^[33]。1-MCP 对桃果实采后贮藏保鲜效果受品种、成熟度等影响,因此深入研究 1-MCP 与桃果实采后生理效应及作用机制具有重要的应用价值。

Tadiello 等^[24]研究发现,1-MCP 的作用受果实发育期的影响,1-MCP 处理呼吸跃变前期桃果实,抑制乙烯的释放,果实硬度下降缓慢;1-MCP 处理呼吸跃变后期桃果实,促进乙烯的释放,果实硬度下降变化不明显。Nicola 等^[35]通过芯片技术进一步挖掘到多肽类基因 *CTG134* 维持果实成熟过程中生长素与乙烯的动态平衡,在果实成熟过程中发挥重要的作用。

表 3 未知功能基因分析
Table 3 Analysis of unknown functional gene

未知基因 Unknown genes	名称 Name	功能描述 Functional description
<i>Prupe.1G038200</i>	信号肽 Signal peptide	早期结瘤蛋白-75 LOC18788671; early nodulin-75
<i>Prupe.1G042300</i>		转导蛋白/WD40 含域蛋白样蛋白 Transducin/WD40 domain-containing protein-like proteins1
<i>Prupe.1G233600</i>	含 TLC 结构域的蛋白 TLC domain-containing protein	DNA 结合相关蛋白 DNA-binding protein-related
<i>Prupe.1G253100</i>		未知功能蛋白 Protein of unknown function (DUF1645)
<i>Prupe.1G280200</i>	膜组分 Integral component of membrane	PTHR33133:SF7-F26K24.10 蛋白 PTHR33133:SF7-F26K24.10 protein
<i>Prupe.1G322100</i>	信号肽 Signal peptide	未表征蛋白 LOC18793001 Incharacterized protein LOC18793001
<i>Prupe.1G379600</i>		
<i>Prupe.1G411600</i>	响应脱落酸 Response to abscisic acid	棉纤维表达蛋白 Cotton fibre expressed protein(DUF761)
<i>Prupe.1G415300</i>	信号肽 Signal peptide	膜组分 Integral component of membrane
<i>Prupe.1G472800</i>	信号肽 Signal peptide	未知功能蛋白 PF04862-Protein of unknown function
<i>Prupe.1G577800</i>		SF13 -乳糖谷胱甘肽裂解酶乙二醛酶 I SF13-lactoylglutathione lyase glyoxalase I
<i>Prupe.2G282900</i>	At5g51670 样蛋白 At5g51670-like protein	SF4 -未知相似蛋白 SF4 unknown protein
<i>Prupe.2G301600</i>	金属离子转运 Metal ion transport	重金属合成相关蛋白 SF77-heavy-metal-associated domain-containing protein
<i>Prupe.3G073700</i>	着丝点、着丝点 DNA 结合、着丝点装配 Kinetochore、centromeric DNA binding、 kinetochore assembly	着丝粒蛋白 C Centromere protein C
<i>Prupe.4G037000</i>		
<i>Prupe.4G082100</i>	假定蛋白 Hypothetical protein	
<i>Prupe.4G095400</i>		乙烯调节的核蛋白 ERT2 样相关蛋白 Ethylene-regulated nuclear protein ert2-like
<i>Prupe.4G161400</i>	信号肽 Signal peptide	
<i>Prupe.4G214900</i>	衰老调节子 Senescence regulator	衰老调节子、RNA 聚合酶主因子、富亮氨酸重复变异 Senescence regulator、RNA Polymerase Primary Sigma Factor、Leucine-rich Repeat Variant
<i>Prupe.4G270100</i>	信号肽 Signal peptide	膜电子转移组成成分 Integral component of membrane electron transfer activity
<i>Prupe.5G059100</i>	信号肽 Signal peptide	经典阿拉伯半乳糖蛋白 4 Classical arabinogalactan protein 4
<i>Prupe.5G072500</i>	信号肽 Signal peptide	
<i>Prupe.5G109500</i>		
<i>Prupe.5G177800</i>		未知功能 Domain of unknown function
<i>Prupe.5G188200</i>		
<i>Prupe.6G06300</i>		
<i>Prupe.6G112200</i>	蛋白质启动同源物 Protein enabled homolog	未知功能蛋白 Protein of unknown function
<i>Prupe.6G285700</i>	膜组分 Integral component of membrane	甾醇-24- c -甲基转移酶/酶甾醇-24-甲基转移酶 Sterol 24-c-methyltransferase / Zymosterol-24-methyltransferase
<i>Prupe.6G329100</i>	金属离子结合 Metal ion binding	重金属相关的异戊二烯化植物蛋白 Heavy metal-associated isoprenylated plant protein 34
<i>Prupe.7G061500</i>	假设蛋白 Hypothetical protein	
<i>Prupe.7G091900</i>	未描述蛋白 Uncharacterized protein	PTHR31182:SF2-F26K24.5 蛋白 PTHR31182:SF2-F26K24.5 protein
<i>Prupe.7G120800</i>	MYB-DNA 结合 4 MYB_DNA-bind_4	MYB-DNA 结合 4 MYB_DNA-bind_4
<i>Prupe.7G204200</i>	DUF761	棉纤维表达蛋白 PF05553-Cotton fibre expressed protein (DUF761)
<i>Prupe.7G256100</i>	信号肽 Signal peptide	生长活性因子 Growth factor activity
<i>Prupe.8G014200</i>	膜组分 Integral component of membrane	PTHR33133:SF5 -表达蛋白 PTHR33133:SF5-expressed protein
<i>Prupe.8G031200</i>	DUF793	未知功能蛋白 Protein of unknown function
<i>Prupe.8G065400_v2.0.a1</i>	信号肽 Signal peptide	假定蛋白 Hypothetical protein
<i>Prupe.8G065500_v2.0.a1</i>	信号肽 Signal peptide	经典阿拉伯半乳糖蛋白 1 Classical arabinogalactan protein 1
<i>Prupe.8G119300_v2.0.a1</i>		未知功能蛋白 PF07816-Protein of unknown function (DUF1645)
<i>Prupe.8G270300_v2.0.a1</i>	蛋白酶体内肽酶复合物 Proteasome endopeptidase complex	蛋白质水解蛋白 alpha/beta-hydrolases superfamily protein

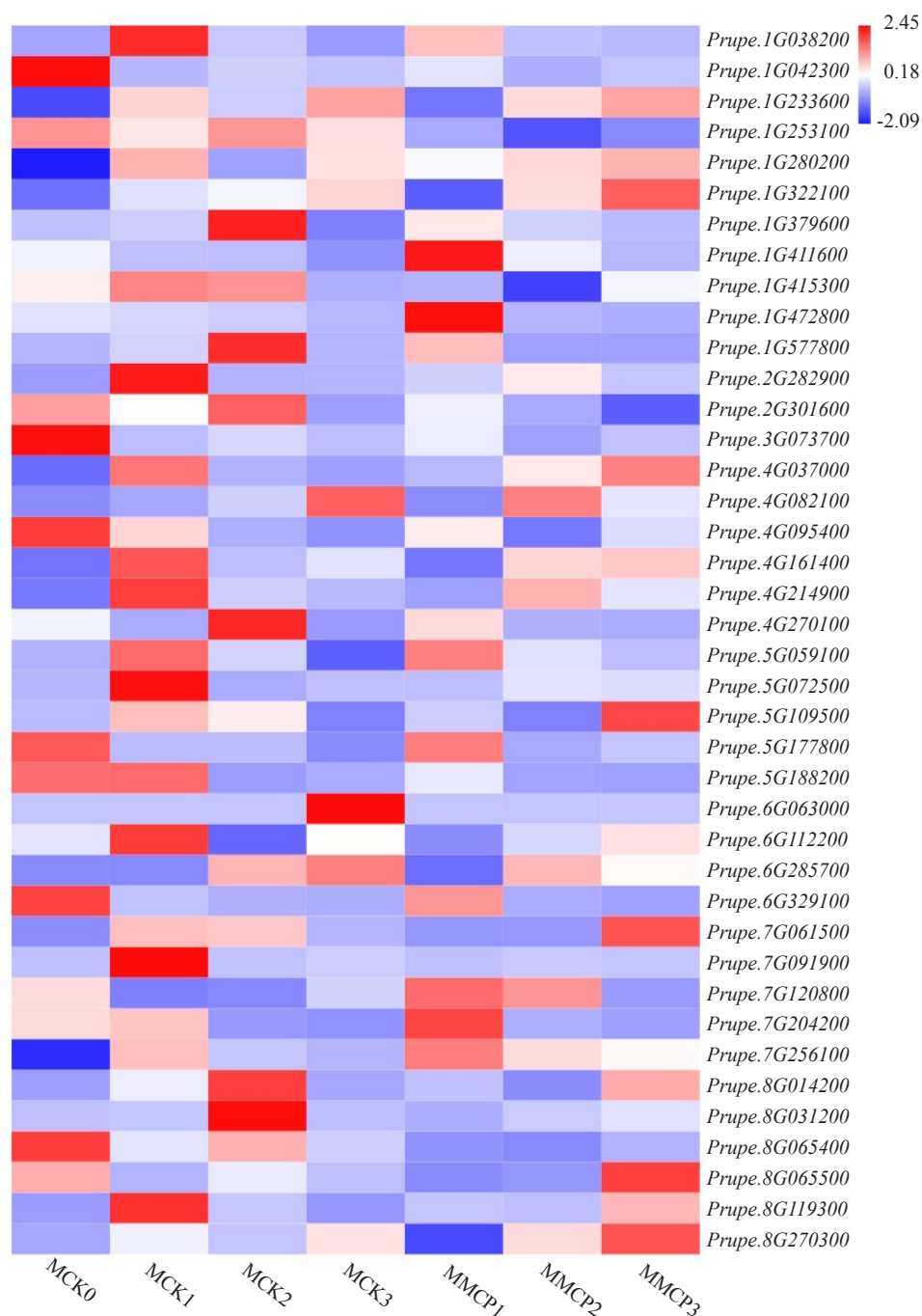


图7 未知功能基因表达热图

Fig. 7 Heat map of unknown functional gene expression

笔者使用 1-MCP 分别处理‘中油桃 13’呼吸跃变前期和呼吸跃变后期桃果实,观察处理与对照之间乙烯与硬度的变化,结果与前人研究相似,1-MCP 处理呼吸跃变前期桃果实抑制乙烯的释放,果实硬度下降速度慢,延缓果实的成熟;1-MCP 处理呼吸跃变后期桃果实硬度下降较慢,但是刺激乙烯的释放,说明 1-MCP 对不同成熟度的‘中油桃 13’果实采后发挥不同的作用。前人对不同肉质类型桃的研究发现,溶质桃呼吸跃变后期生长素合成关键基因

PpYUC11、乙烯合成关键限速酶基因 *PpACS1* 均上调表达^[34]。1-MCP 处理呼吸跃变前期桃果实抑制乙烯相关基因 *PpACS1* 的表达,说明果实呼吸跃变前期 1-MCP 通过抑制乙烯相关基因的表达抑制了乙烯的产生,达到了采后保鲜的效果。1-MCP 处理跃变后期桃果实发现多肽基因 *Prupe.1G472800*、*Prupe.4G270100*、*Prupe.5G072500*、*Prupe.7G256100*、*Prupe.5G059100* 上调表达,最终促进呼吸跃变后期乙烯的产生^[35],解释 1-MCP 处理呼吸跃变前期和呼

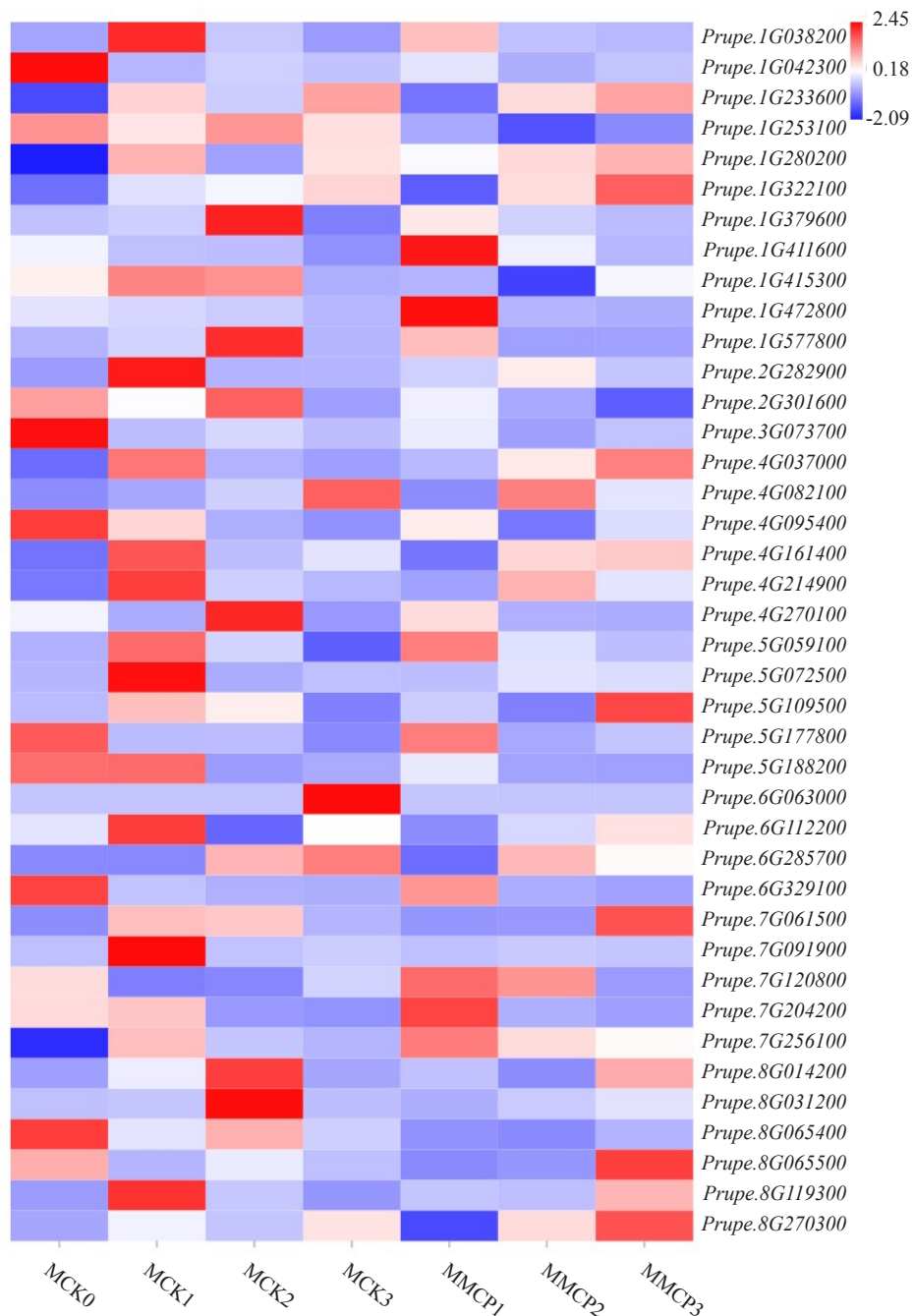


图 8 qRT-PCR 验证未知功能基因表达分析

Fig. 8 Expression of unknown functional genes verified by qRT-PCR

吸跃变后期效果不同的可能原因。

笔者对 288 个差异基因进行深入研究,发现上调表达的基因包含多个生长素合成及信号转导相关基因、糖代谢相关基因、多肽合成基因等,下调表达的基因包含多个乙烯合成与信号传导相关基因、逆境相关基因等。1-MCP 可能刺激呼吸跃变后期生长素和多肽的合成,诱导乙烯的生物合成。1-MCP 可能干扰乙烯下游的信号途径,从而增加果实硬度。

通过对转录因子分析发现,除乙烯相关转录因

子 ETR 和 ERF,还存在多个 WRKY 转录因子,说明 1-MCP 处理可能提高果实的抗逆性。马书尚等^[10]发现 1-MCP 处理的桃果实冷害症状减轻,说明 1-MCP 提高了桃果实采后贮藏抗冷性。

综上所述,1-MCP 处理呼吸跃变前期和呼吸跃变后期桃果实对乙烯释放及软化进程的作用不同,原因可能是由于 1-MCP 处理呼吸跃变前期和呼吸跃变后期桃果实诱导差异基因表达。呼吸跃变后期桃果实中乙烯和生长素等激素信号传导相关基因响

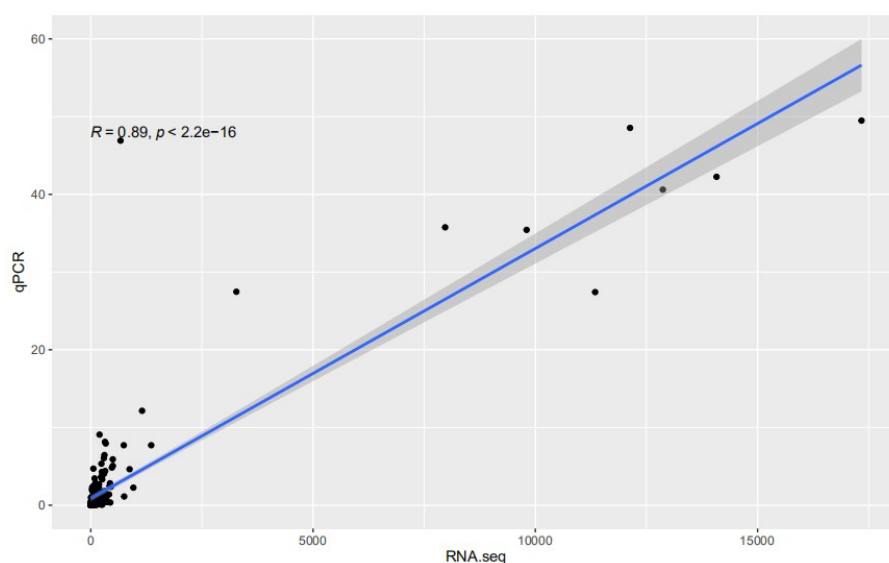


图9 皮尔逊相关系数

Fig. 9 Pearson correlation coefficient

应 1-MCP 处理,更新颖的是,发现一类多肽基因,其在呼吸跃变后期响应 1-MCP 处理,可能在桃果实成熟过程中发挥重要的作用,为以后桃果实采后保鲜的研究提供新的思路。

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