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‘赣南早’脐橙早熟性状回复型突变体的生理与转录组分析

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摘 要：【目的】明确早熟品种‘赣南早’脐橙（wild type，WT）与其早熟性状回复型突变体（mutant type, MT）在生理和转录水平的差异，探究柑橘果实成熟的调控机制。**【方法】**测定MT和WT的果实品质及成熟相关生理指标，采用RNA-Seq分析MT和WT果实的转录差异。**【结果】**MT具有稳定的早熟性状回复现象。MT和WT果皮的各可溶性糖含量差异显著。MT和WT有机酸含量在果肉间及果皮间差异均显著。MT果皮的赤霉素（gibberellin，GA）、吲哚乙酸（indole acetic acid，IAA）和茉莉酸（jasmonic acid，JA）显著高于WT，脱落酸（abscisic acid，ABA）则相反。转录组测序分析表明，MT与WT果皮间DEGs数量为980个，果肉间为289个。在果皮DEGs中，有38种GO分类、6个KEGG代谢通路被显著性富集，而果肉中未见GO分类和KEGG代谢通路的显著性富集。ABA合成基因*CsNCED1*在MT果皮和果肉中均下调表达，而分解基因*CsCYP707A1*在MT果皮中上调表达。**【结论】**MT成熟期比WT推迟约30 d。MT果皮GA含量及GA合成基因*CsCPS1*、*CsKAO*表达量均高于WT，可能与果皮的褪绿延迟相关。MT果皮ABA积累抑制可能受合成基因*CsNCED1*下调表达及分解基因*CsCYC707A1*上调表达的影响。MT和WT果皮的生理和转录组水平差异均比果肉间差异大，说明果皮在柑橘果实成熟过程中具有重要作用。

关键词：‘赣南早’脐橙；回复型突变；果实成熟；果实品质；转录组

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Physiological and transcriptome analysis of the restorative mutant from the early-ripening ‘Gannanzao’ navel orange

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Abstract:【Objective】Selection of early or late ripening varieties is an important target of citrus breeding. The regulation mechanism of citrus fruit ripening is of great significance for breeding early or late ripening varieties. Previous studies regarding ripening mechanism of citrus fruits mainly focused on the flesh, little attention was paid on the pericarp. Herein, both flesh and pericarp of ‘Gannanzao’ navel orange (Wild type, WT), and its restorative mutant (Mutant type, MT) were investigatedin order to explore the regulation mechanism of the ripening of navel orange through comprehensive comparison of the physiological and transcriptional differences between the WT and the MT.【Methods】The fruit qualities and physiological properties, including the contents of soluble sugar, organic acid and phytohormones, in both flesh and pericarp of the WT and the MT were determined. Transcriptome data of both flesh and pericarp of the WT and the MT were obtained and analyzed by high-throughput sequenc-

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ing.【Results】MT featured an obvious and stable late-maturing character. The soluble sugar content was significantly different only in the pericarp of the WT and the MT, while the organic acid content was significantly different in both of the flesh and the pericarp. The MT had much lower contents of both malic acid and citric acid, and higher content of quinic acid compared with those of the WT. At 200DAF, the contents of GA, IAA and JA in the pericarp of the MT were significantly higher than those of the WT, whereas the content of ABA in the pericarp of the MT was obviously lower. than that of the WT. Meanwhile, the comparison of transcriptome sequencing between the MT and the WT showed that the number of differentially expressed genes (DEGs) were 980 and 289 in the pericarp and the flesh respectively, and 94 DEGs were the common DEGs of the pericarp and the flesh. Interestingly, the significant enrichment ($p \leqslant 0.05$) of GO terms and KEGG pathways were only found in the pericarp. A total of 38 GO terms were significantly enriched in the pericarp. Furthermore, a total of 6 KEGG pathways being involved in photosynthesis, photosynthesis-antenna proteins, peroxisome, cutin, suberine and wax biosynthesis, protein digestion and absorption, and ubiquinone and other terpenoid-quinone biosynthesis were significantly enriched ($p \leqslant 0.05$) in the pericarp. In ABA synthesis signal transduction pathway, the key limiting gene *CsNCED1* of ABA synthesis was downregulated in both of the pericarp and the flesh in the MT, and the decomposition gene *CsCYP707A1* was up-regulated in the MT pericarp.【Conclusion】The ripening date of the mutant (MT) was 30 days later than that of the WT. The delay of both chlorisis and color transition might be related to the increase of GA accumulation and the up-regulated expression of *CsCPS1* and *CsKAO*. The down-regulated and up-regulated expression of *CsNCED1* and *CsCYC707A1* in the MT pericarp might lead to the inhibition of ABA accumulation. The differences of the physiological and transcriptome levels in the pericarp between the WT and the MT were greater than those in the flesh, indicating that the pericarp may play an important role in citrus fruit ripening. It seems that the investigation of the physiology and transcriptome both in the flesh and the pericarp are essentially necessary for studying the mechanism of the citrus fruit ripening.

Key words: ‘Gannanzao’navel orange; The mutant with traits restoration; Fruit ripening; Fruit quality; Transcriptome

柑橘（*Citrus* L.）是世界第一大水果，随着我国柑橘产业的快速发展，柑橘产业已成了柑橘主产区区域经济发展的重要支柱。但是我国柑橘品种单一、成熟期集中，大量鲜果在短期内集中上市，制约了我国柑橘产业持续稳定发展。选育早、晚熟新品种是柑橘育种的重要目标之一。

果实成熟调控机制研究是果实发育生理和分子生物学研究热点[1]。果实成熟是一个复杂的生理生化过程，色素合成及降解、糖代谢、激素水平、反转录因子等均是果实成熟的调控因子，凡是能够调控成熟相关基因时空表达的各种因子及产物都会对果实成熟产生影响[2]。脱落酸（abscisic acid，ABA）参与番茄[3]、草莓[4]、葡萄[5]及覆盆子[6]的果实成熟调控。Jia等[7]发现，蔗糖可以作为信号分子参与ABA互作调控草莓果实的成熟。茉莉酸（jasmonic acid，JA）可通过调节花青素积累、细胞壁修饰及乙烯合成参与草莓果实的发育成熟[8]。乙烯在非跃变型和跃变型果实的合成调控路径和成熟生理调控具有相似之处[9]，调控方式不仅依赖于乙烯浓度，且与果实对乙烯的敏感性相关[10]。但是关于果实成熟调控的信息主要来自于对拟南芥、番茄和烟草等突变体的研究。柑橘属于非跃变型果实，其成熟机制有别于跃变型果实，关于柑橘果实熟期研究报道较少。Liu等[11]研究了脐橙晚熟突变体与原品种果实的着色差异，造成着色延迟的原因主要是果皮前期叶绿素含量减少和后期类胡萝卜素积累进程推迟。‘锦橙’晚熟突变体[12]和‘奉节晚橙’[13]研究揭示了甜橙果实成熟相关的因素，但其成熟机制还不清楚。Zhang等[12]从‘锦橙’晚熟突变体与对照的转录组中发现，ABA途径和蔗糖代谢途径变化对柑橘果实成熟具有重要

作用。Wu等[14]研究了‘奉晚’脐橙与对照‘奉节’脐橙在转录组和蛋白组的差异，发现基因差异主要集中在植物激素代谢通路。

‘赣南早’脐橙（*Citrus sinensis* L. Osbeck）系‘纽荷尔’脐橙的早熟芽变品种[14]。在‘赣南早’脐橙推广试验中，发现了其早熟性状回复的芽变。与‘赣南早’脐橙相比，该芽变果实成熟期延迟，但其他性状有别于‘纽荷尔’脐橙。‘赣南早’脐橙早熟性状回复型的获得为研究柑橘果实成熟机制提供了宝贵的试验材料。在前人的研究中，果实成熟机制研究主要以果肉为对象，而柑橘果皮作为果实重要组成部分，其作用还知之甚少。笔者以‘赣南早’脐橙及其早熟性状回复型突变体为试验材料，将果皮与果肉分离后独立研究，通过分析‘赣南早’脐橙及早熟性状回复型突变体果皮及果肉的可溶性糖、有机酸和内源激素含量，并对果肉和果皮进行RNA-seq测序分析，以明确2个材料在生理和转录水平的差异，探究柑橘果实成熟调控机制。

1　材料和方法

1.1　植物样本采集

早熟性状回复型突变体材料（mutant type，MT）是在‘赣南早’脐橙（wild type，WT）果树上发现的芽变。MT和WT转录组测序，可溶性糖、有机酸和激素含量测定的材料取自芽变母树，选取花后200和220 d这2个果实发育关键时期取样，花后200 d时WT处于完熟期，而MT还未完全转色，花后220 d时MT着色完成。每个时期分别取10个果实，取样后立即将果皮和果肉分离，液氮速冻，保存于−80 ℃冰箱。将WT和MT材料高接于甜橙。果实常规品质试验材料取自高接的WT和MT果实，取样时间为花后110、140、155、170、185、200、215、235和250 d。

1.2　果实品质和激素测定

可溶性固形物含量用手持式糖量计测定，可滴定酸含量用NaOH滴定法测定。可溶性糖和有机酸含量参照米兰芳[15]的气相色谱法测定。ABA、JA、赤霉素（gibberellin，GA）和吲哚乙酸（indole acetic acid，IAA）含量参照Pan等[16]的方法测定。以上试验均为5次生物学重复。

1.3　转录组测序与验证

转录组测序所用材料为芽变母树花后200 d时MT和WT的果皮及果肉组织，3次生物学重复。总RNA提取参照刘永忠等[17]的方法。测序工作委托上海美吉生物医药科技有限公司完成。测序数据与甜橙数据库（http://citrus.hzau.edu.cn/orange/）比对[18]。以WT为对照，MT差异表达基因（differentially expressed genes，DEGs）筛选条件为：FDR≤0.05，|logFC|≥1。

采用实时定量反转录PCR（qRT-PCR）的内参基因归一化$2^{-\Delta\Delta Ct}$法进行转录组数据的验证，3个生物学重复。验证基因和内参基因ACTB引物序列如表1所示，引物设计运用Primer Premier 5软件。

2　结果与分析

2.1　MT和WT果品品质及部分生理指标的比较

通过对MT和WT果实品质及特性连续2 a（年）的观测及分析，结果显示，MT果实成熟期迟于WT，具有早熟性状回复现象，且性状稳定。MT在花后170 d时完成褪绿，比WT推迟约15 d，转色期在花后185~215 d，比WT延长约15 d（图1）。MT果实的单果质量和固酸比均显著低于WT，而可滴定酸含量相反（图2）。MT可溶性固形物含量在花后235 d时出现峰值，比WT延迟约35 d（图2）。参照《脐橙》国家标准[19]，花后185 d后，MT果肉固酸比达到特级指标（固酸比≥10），花后200 d后果肉可溶性固形物含量达到特级指标（可溶性固形物含量≥11%），而WT在花后170 d时果肉固酸比和可溶性固形物含量已达特等指标。

如图3所示，MT和WT果皮中各可溶性糖含量差异显著，而果肉中无显著差异。果皮中，花后200 d和220 d，MT果糖含量显著低于WT，而花后220 d蔗糖含量则相反，花后200 d MT总可溶性糖含量极显著低于WT。MT和WT的有机酸含量在果肉间及果皮间均存在显著差异。果皮中，花后220 d时MT的苹果酸、柠檬酸和总有机酸含量显著低于WT；果肉中，花后200和220 d时MT的柠檬酸和总有机酸含量极显著高于WT，而奎宁酸相反。激素含量测定显示，MT果皮的GA含量在花后200和220 d时显著高于WT，果肉中因含量低未检出；花后200 d时，MT果皮的GA、IAA和JA含量显著高于WT，而ABA含量显著低于WT；在果肉中，MT的ABA含量在花后200和220 d时显著高于WT，JA含量在花后200 d时极显著高于WT，而220 d时相反（图4）。

表 1 qRT-PCR 验证引物

Table 1 Primer sequences for qRT-PCR

基因ID Gene ID	引物序列(5'→3') Primer sequences (5' to 3')	
	正向序列 Forward sequences	反向序列 Reverse sequences
Cs5g14370	GCCGGAATGGTGAACAGAA	AGGGCTAAGTACGCGAAACG
Cs2g03270	TGGCTGCTTCTACTAC	TTCTTCAAGGCTATGG
Cs8g14150	TCTTGGTGAGTCCGAT	AAGTCTCCTTTGTGGT
Cs8g18780	CCAGGAACTCCGTATCAC	GACAGCGTCCAGCAGA
Cs8g20420	GCGATTGTCGCGCTGTTAT	GCCGGTGGTCTGAAGTGAGT
Cs9g18020	CTGCTGGAGGAAGGGTCATA	CACCATCACATCTGGCTTTG
orange1.1t00478	CAGGACCGAACTGAACCAATC	CTACAAGAAGCAAGCCACTAAAGAA
Cs2g23870	CTGCCCACTACAACTGC	GAGCGAGGACCGATAA
Cs9g18030	GGATGCCAGAAGACTATG	CCACCCAGAAACAACA
Cs3g16400	CAACTTCATAATCCCTCCG	GAAAGCCGTGATAGTCCTG
Cs2g20590	TTTCTATGTGCGTCACCTCCC	TTCCTTCATCACCTTCCTGTATTC
Cs4g17960	CTCTCTGGCCGTGATTGGA	TGGCAGATCACTCAGGACAAGA
Cs5g29870	TGAGCTTCTGTTCAGCCACAA	CGTTTCCTCTGAGTCGTTGATG
Cs5g08360	TTATGCGGTGCTGGATTCAGT	TCAGTCCAGTCTCCGATTCGT
Cs1g07950	GTCTGGCTCGGCACCTT	TCGTTTGGCGTTGGGA
Cs9g16810	CGTGGTGTTGTTGGCGT	GACTTCTTGTTCGGCTCCC
Cs5g05240	TGCTATGAGGCGTATT	TCTGTTGCCCAATCTA
Cs6g07990	CCATCACTGAAACCCT	AAATCCAGCACACATC
Cs7g08080	GAACGAGGAACAGGTGC	CAACGCCAGTGGGATA
Cs1g22140	TCGCTCTGCCATCCTG	GCCCTTCCCTTTGTCC
Cs3g25900	CGCTGCTAGGGTACTTGCTT	TGGCCGGGTTAGATTTACTG
orange1.1t03773	TGGAAATCCTTTCTCGAAAAACC	TGAAACTGTGCAGGGAAGCA
Cs1g17210	CGCCTCCAATGTGATG	GGTATGGTGCTCTTGC
Cs6g15930	CTGTTGTTATGCCTCCAC	TATGCTCCGAAATCCACT
orange1.1t03668	ACTTGGCAGATAGAGGG	TCACGGGTGTTACGAG
Cs8g17370	GGAGCAGTTCCCTACCA	CCATCTGAGCACCAAAG
Cs1g26330	ACTAATCCGACTCCCGTAC	CCAGCCACCTGTAGTTTC
Cs9g02570	GGCTGACGAGGAGCTTGAGA	CTGCACCCTGACATGAATCG
Cs2g14920	CGGGCAGCCATACATT	CGGCACATCGTCCACT
Cs5g01740	GGGCATGCAATATGCTCTTTAAT	TGACACATGATACTGGGCAGTTG
ACTB	CCAATTCTCTCTTGAACCTGTCCTT	GAAGACCGTCAAGAGTAGTCAGT

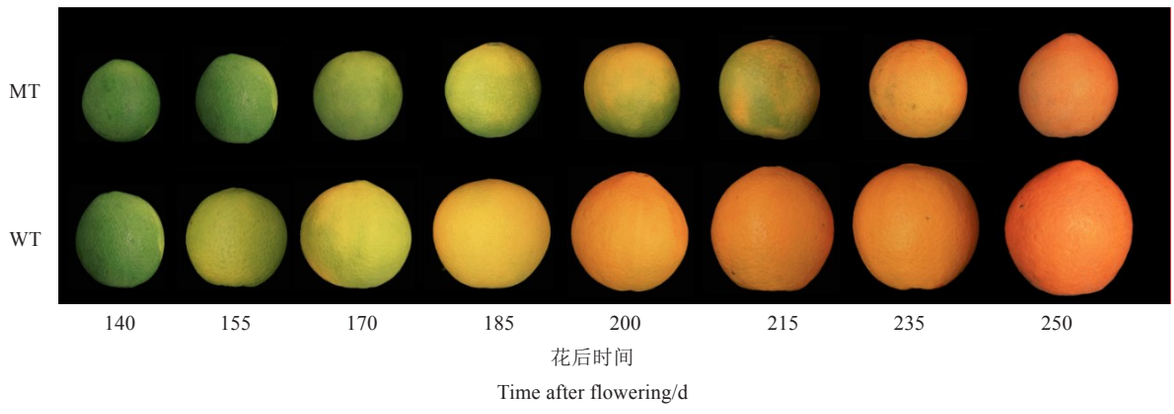
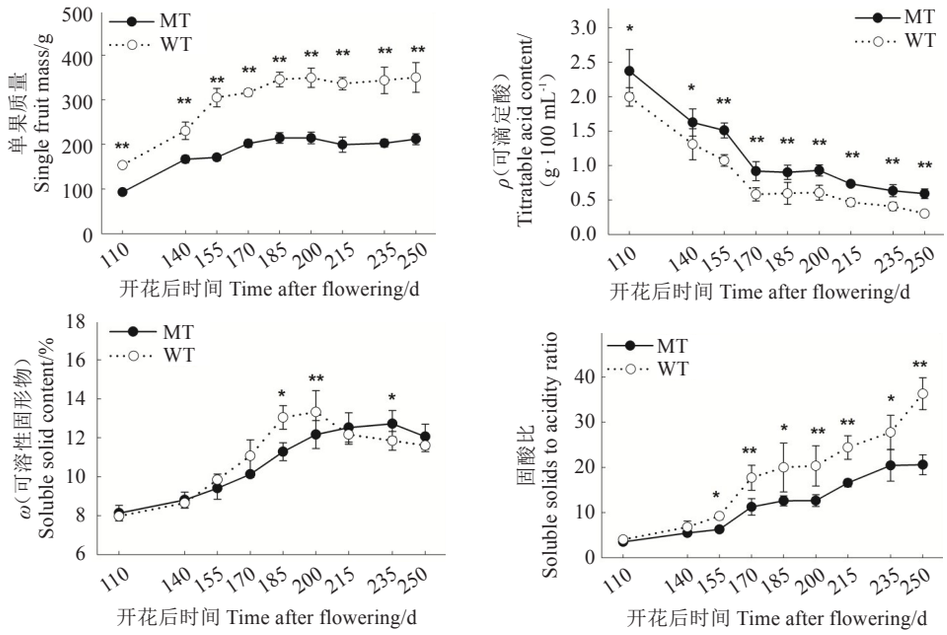


图 1 MT 和 WT 果实发育表型特征

Fig. 1 Phenotypic characterization of fruit development in MT and WT



*表示在 $p \leqslant 0.05$ 差异显著，**表示在 $p \leqslant 0.01$ 差异显著。下同。

* and ** indicate significant difference at $p \leqslant 0.05$ and $p \leqslant 0.01$. The same below.

图 2　MT 和 WT 果实发育过程中品质差异

Fig. 2 The differences of quality during fruit development between MT and WT

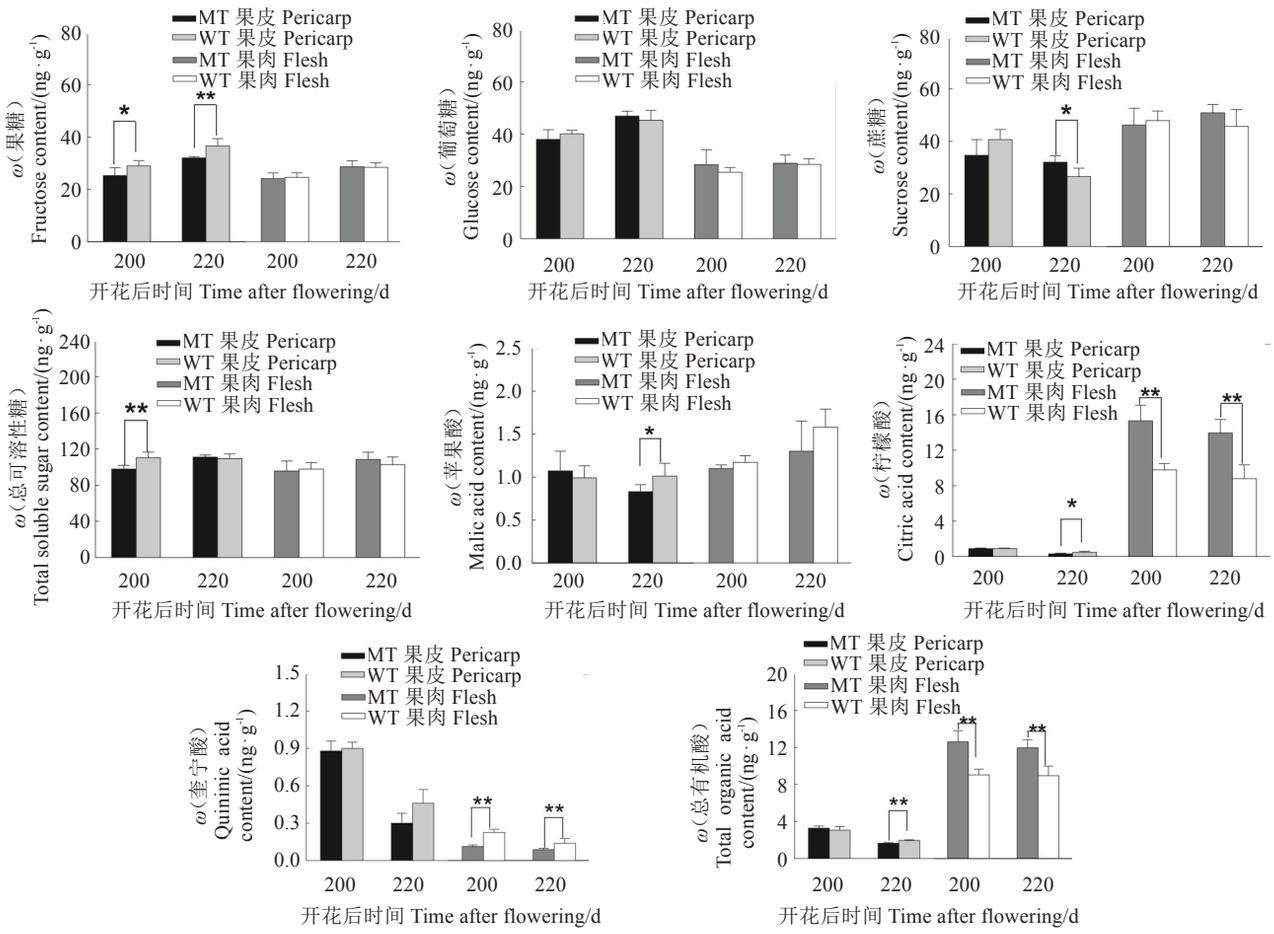


图 3　MT 和 WT 果皮及果肉中可溶性糖和有机酸含量比较

Fig. 3 Comparisons of the content of soluble sugar and organics acid in pericarp and flesh between MT and WT

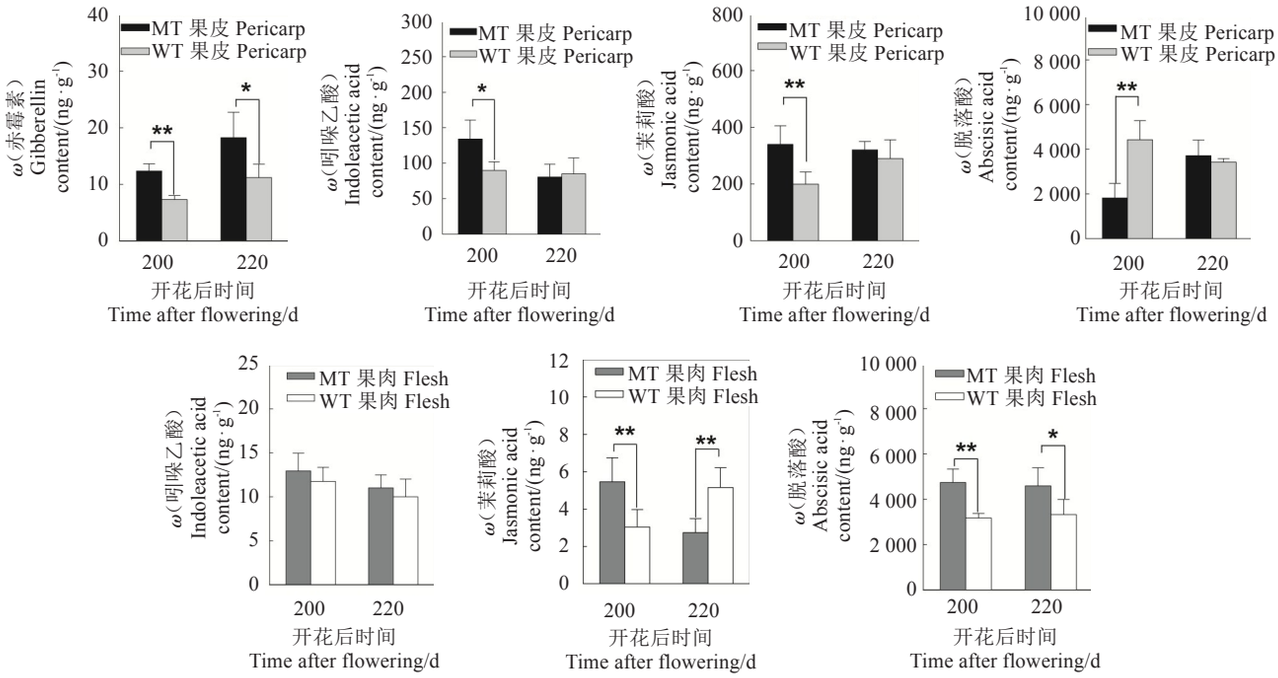


图4 MT和WT果皮及果肉中激素含量比较

Fig. 4 Comparisons of phytohormone contents in pericarp and flesh between MT and WT

2.2 RNA-Seq数据验证结果

采用qRT-PCR技术对RNA-Seq数据进行验证，RNA-Seq与qRT-PCR数据线性回归分析显示，果皮中决定系数R^2=0.856，相关系数R=0.925（图5-A）；果肉中决定系数R^2=0.717，相关系数R=0.847（图5-B）。结果表明，果皮和果肉组织的RNA-Seq与qRT-PCR数据均高度线性相关，RNA-Seq数据准确可信。

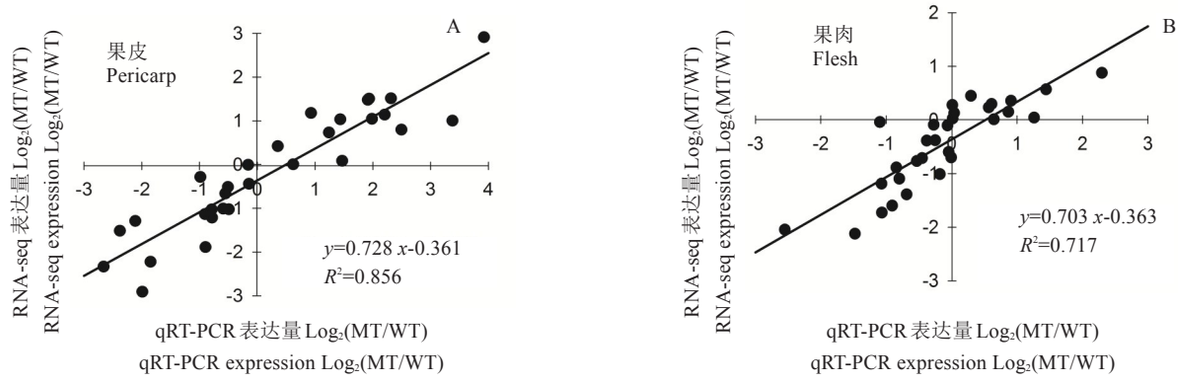


图5 果皮和果肉RNA-Seq与q-PCR数据的线性回归分析

Fig. 5 Regression linear analysis of RNA-Seq data and qRT-PCR data in pericarp and flesh

2.3 MT和WT在转录组水平的差异

转录组测序结果显示，MT和WT果皮间DEGs数量为980个，果肉间为289个；其中有94个DEGs为果皮与果肉共有。DEGs的GO分类和KEGG通路富集分析发现，GO分类富集数量为果皮84个，果肉15个；KEGG通路富集数量为果皮115个，果肉63个。在果皮中有38种GO功能分类被显著性富集（$p \leqslant 0.05$），其中5个分类包含着大量的DEGs，分别为分子功能565个、生物过程480个、催化活性417个、代谢过程371个和单体代谢过程314个，而在果肉中未见GO功能分类被显著性富集。果皮中的DEGs在KEGG代谢通路显著性富集（$p \leqslant 0.05$），包括光合作用、光合天线蛋白、过氧化物酶体、角质-软木脂-蜡的生物合成、蛋白质的消化与吸收和CoQ-其他萜醌类生物合成6个通路（表2），在果肉中未见KEGG通路被显著性富集。

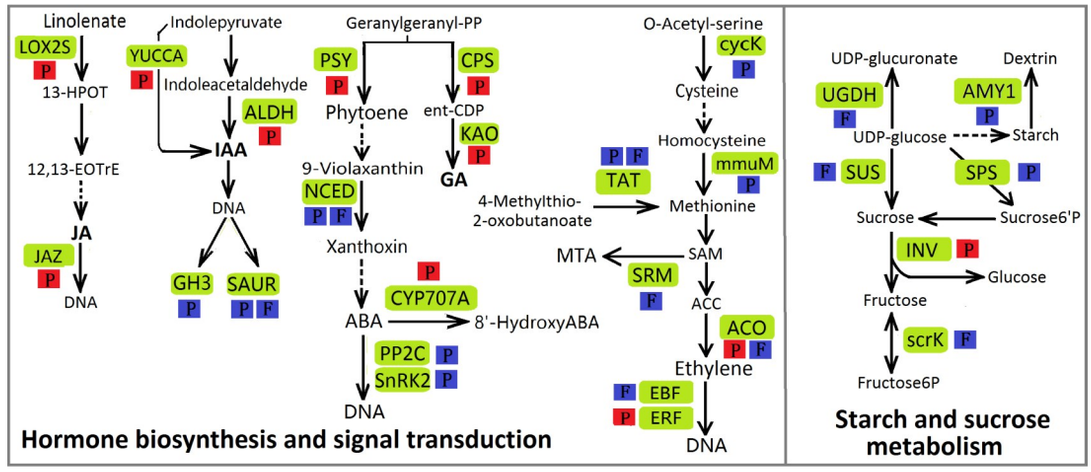
表 2　果皮中显著性富集的 KEGG 通路（$p \leqslant 0.05$）

Table 2 The significant enrichment of KEGG pathways in pericarp（$p \leqslant 0.05$）

通路ID Pathway ID	通路注释 Pathway annotation	差异基因数 Number of DEGs	背景基因数 Background gene number
ko00195	光合作用 Photosynthesis	13	45
ko00196	光合-天线蛋白 Photosynthesis - antenna proteins	6	15
ko04146	过氧化物酶体 Peroxisome	12	80
ko00073	角质、软木脂和蜡的生物合成 Cutin, suberine and wax biosynthesis	6	21
ko04974	蛋白质的消化与吸收 Protein digestion and absorption	7	38
ko00130	CoQ与其他萜醌类生物合成 Ubiquinone and other terpenoid-quinone biosynthesis	9	63

2.4　植物激素合成-信号转导和淀粉-蔗糖代谢通路相关基因的转录调控

选取果实成熟调控重要通路激素合成-信号转导和淀粉-蔗糖代谢通路进行差异基因表达分析（图6）。在ABA合成-信号转通路中，合成关键限速酶基因 *CsNCED1* 在MT果皮和果肉下调表达，而分解关键酶基因 *CsCYP707A1* 在MT果皮上调表达。ABA信号转导中 *CsPP2C8* 和 *CsSnRK2* 在MT果皮下调表达。乙烯通路中，MT果皮中 *CsACO* 和 *CsERF* 上调表达，而 *CscycK*、*CsmmuM* 和 *CsTAT* 下调表达；果肉中 *CsTAT*、*CsSRM*、*CsACO* 和 *CsEBF* 下调表达。MT果皮中JA合成基因 *CsLOX2S* 和 *CsAOS*、IAA合成基因 *CsYUCCA* 和 *CsALDH*、GA合成基因 *CsCPS1* 和 *CsKAO* 均为上调表达。在淀粉-蔗



绿色方块表示 MT 果皮(P)和果肉(F)中 DEGs(以 WT 为对照)，红色为上调表达，蓝色为下调表达。LOX2S. 脂氧合酶；JAZ. 茉莉酸 ZIM 结构域蛋白质；ALDH. 醛脱氢酶(NAD+)；YUCCA. 吲哚 3 丙酮酸氧化酶；GH3. 吲哚乙酸酰胺合成酶；SAUR. 生长素上调小 RNA 家族蛋白；PSY. 八氢番茄红素合酶；NCED. 9-顺式环氧类胡萝卜素双加氧酶；CYP707A. ABA8’-羟化酶；PP2C. 蛋白质磷酸酶 2C；SnRK2. 丝氨酸/苏氨酸蛋白激酶 SRK2；cycK. 半胱氨酸合成酶；mmuM. 同型半胱氨酸甲基转移酶；TAT. 酪氨酸转氨酶；SRM. 亚精胺合成酶；ACO. 1-氨基环丙烷-1-羧酸氧化酶；EBF. EIN3 结合的 F-box 蛋白；ERF. 乙烯应答转录因子；CPS. 内部柯巴基焦磷酸合酶；KAO. 内部异贝壳杉烯酸羟化酶；UDGH. UDP-葡萄糖脱氢酶；AMY1. α-淀粉酶 1；SUS. 蔗糖合酶；SPS. 蔗糖磷酸合酶；INV. 转化酶；scrK. 果糖激酶。

Green boxes indicate DEGs of the pericarp (P) and flesh (F) in MT comparing with WT, the red boxes indicate DEGs that were up-regulated, and the blue boxes indicate DEGs that were down-regulated. LOX2S. Lipoxygenase; JAZ. Jasmonate ZIM domain-containing protein; ALDH. Aldehyde dehydrogenase (NAD+); YUCCA. Indole-3-pyruvate monooxygenase; GH3. Indole-3-acetic acid amido synthetase; SAUR. Small auxin upregulated RNA family protein; PSY. Phytoene synthase; NCED. 9-*cis*-epoxy-carotenoid dioxygenase; CYP707A. ABA8’-hydroxylase; PP2C. Protein phosphatase 2C; SnRK2. Serine/threonine protein kinase SRK2; cycK. Cysteine synthase; mmuM. Homocysteine S-methyltransferase; TAT. Tyrosine aminotransferase; SRM. Spermidine synthase; ACO. 1-amino-cyclopropane-1-carboxylate oxidase; EBF. Ein3-binding f-box protein; ERF. Ethylene responsive transcription factor; CPS. Ent-copalyl diphosphate synthase; KAO. Ent-kaurenoic acid hydroxylase; UDGH. UDP-glucose dehydrogenase; AMY1. Alpha-amylase 1; SUS. Sucrose synthase; SPS. Sucrose phosphate synthase; INV. Invertase; scrK. Fructokinase.

图 6　参与果实成熟的激素合成-信号转导通路和淀粉-蔗糖代谢通路 DEGs 表达情况

Fig. 6 Summary of DEGs in the hormone biosynthesis and signal transduction, and starch-sucrose metabolism pathway involved fruit ripening

糖代谢通路中，MT果肉中*CsUDGH*、*CsSUS5*和*CssscrK*下调表达，果皮中*CsAMY1*和*CsSPS1*下调表达而*CsINV*上调表达。

3 讨 论

糖不仅是果实主要风味物质，也是果实器官发育和物质代谢的物质基础。MT果皮中总可溶性糖含量显著性低于WT，这可能与MT果皮褪绿和转色延迟有关。MT果肉中柠檬酸含量显著高于WT，而奎宁酸相反，这与续丽红等[20]在晚熟品种中的结果相同。Zhang等[12]在晚熟柑橘中发现JA和IAA含量高于早熟品种，这与本试验中MT果皮JA和IAA含量显著高于WT结果一致。Enriqueta等[21]研究认为，GA可抑制脱镁叶绿体加氧酶表达使柑橘褪绿延迟。本研究中，GA含量在MT果皮中积累增加，MT果皮褪绿延迟可能与GA积累增加有关。WT和MT果皮间DEGs数量多于果肉，且GO分类和KEGG通路仅在果皮DEGs中被显著性富集，说明2个材料果皮间的生理及转录组水平的差异比果肉间大。因此，在柑橘果实成熟机制研究中，果皮作为重要研究对象，有必要将其与果肉分离开独立研究。

非跃变型果实成熟调控中ABA占主导地位，ABA积累量受其生物合成和分解共同调控，合成基因*NCED*和分解基因*CYP707A*发挥着限速作用[12-13,22-23]。ABA信号转导机制为当ABA存在时，受体PYR/PYL与PP2Cs结合，PP2Cs释放SnRK2s激活下游转录因子[24]。Wu等[13]发现在晚熟脐橙果肉中，*CsNCED1*下调表达可使ABA积累峰值延迟。本研究中，ABA合成关键基因*CsNCED1*在MT果皮及果肉中下调表达，分解基因*CsCYP707A1*在果皮中上调表达，信号转导中*CsPP2C8*和*CsSnRK2*在果皮中下调表达，使果皮和果肉中ABA含量显著改变。Fujii等[25]在柑橘转录组研究中揭示了乙烯抑制光合作用、叶绿体生物合成和糖代谢相关基因的转录，同时可诱导与抵抗、防卫、胁迫、氨基酸合成、蛋白质降解和次生代谢相关基因的转录。Rodrigo等[26]发现，钝化乙烯诱导的类胡萝卜素合成使柑橘褪绿延迟。笔者发现，晚熟突变体中乙烯合成和信号转导基因的大量下调表达可能与KEGG通路中如光合作用、光合天线蛋白、过氧化物酶体、角质-软木脂-蜡的生物合成和蛋白质的消化吸收通路显著性富集有一定相关性。本研究中，JA、IAA和GA合成相关的DEGs均为上调表达，这与同时期MT果皮和果肉中IAA、JA和GA含量均高于WT的结果相对应。

植物合成的光合产物主要以蔗糖的形式供应和运输，而蔗糖磷酸合成酶（SPS）作为蔗糖利用所必需的关键酶，其基因*CsSPS1*在成熟度高的柑橘组织中表达量更高[27]。笔者发现*CsSPS1*在MT果皮中下调表达，反映了MT果皮的成熟度低于WT。本研究中晚熟材料MT果皮总可溶性糖含量极显著减少可能与*CsSPS1*下调表达相关。

4 结 论

MT成熟期比WT推迟约30 d。MT果皮GA含量及GA合成基因*CsCPS1*、*CsKAO*表达量均高于WT，可能与果皮的褪绿延迟相关。MT果皮ABA积累抑制可能受合成基因*CsNCED1*下调表达及分解基因*CsCYC707A1*上调表达的影响。MT和WT果皮的生理和转录组水平差异均比果肉间大，说明果皮在柑橘果实成熟过程中具有重要作用。

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