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# 翠冠梨大果芽变果实组织切片、激素变化及转录组分析

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**摘 要:【目的】**芽变材料是果树育种中的重要资源。前期发现1个翠冠梨的大果芽突变体,果实明显大于普通翠冠,对其变异来源和分子机制进行探究。**【方法】**通过石蜡切片、内源激素测定以及转录组测序解析大果芽变机制。**【结果】**石蜡切片结果显示,大翠冠果肉细胞横切面积大于翠冠,说明大翠冠的细胞体积增大。大翠冠中反式玉米素和赤霉素$A_3$的含量分别在开花后10和20 d高于翠冠。花后40 d内分5个时间点取样,利用RNA-seq高通量测序评估幼果发育期间的基因表达水平,共鉴定出2015个差异表达基因,其中302个基因在2个以上时间点发生差异表达。在花后0和10 d时,一些植物激素信号转导相关基因发生差异表达。在大翠冠中3个细胞分裂素相关基因(*AHP1*、*ORR10*和*ARR17*)花后10 d上调表达,在大翠冠中赤霉素负调控相关基因*GA3ox1*和*GA2ox1*分别在花后0和10 d下调表达。在大翠冠中发现1个长链非编码*lncRNA*在所有时间点均下调表达。在大翠冠中晚期胚胎富集蛋白基因*D29*、锌指蛋白基因*dof2.1*和伸展蛋白基因*extensin*等在一些时间点发生表达上调,表明这些基因可能在控制果实细胞大小中起重要作用。**【结论】**该研究结果从生理生化和分子水平揭示了翠冠梨大果芽变可能的变异原因,细胞分裂素和赤霉素可能参与调控果实的大小,转录组分析为解析翠冠梨大果芽变的分子机制奠定了基础。

**关键词:**翠冠梨;芽变;果实大小;发育;转录

**中图分类号:**S661.2 **文献标志码:**A **文章编号:**1009-9980(2022)10-1737-11

## Tissue sections, hormone changes, and transcriptomes analysis of the large-fruited bud mutation of *Pyrus pyrifolia* 'Cuiguan'

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**Abstract:**【Objective】Fruit size is a very important agronomic trait, and large fruit cultivars are more appreciated in the market. Genetic background, cultivation measures, and chemicals could regulate fruit size. A large-fruited bud mutant of the Cuiguan pear (*Pyrus pyrifolia*) was found previously. The mechanism of mutation of this mutant were detected by studying the physiology, biochemistry and gene expression of the mutant.【Methods】A large-fruited bud mutant from Cuiguan pear (BCG) and normal Cuiguan pear (CG) were studied. All trees were grafted on seedling rootstocks. The fruit flesh of CG and BCG were sampled on 0, 10, 20, 30, 40 days after blossoming (DAB). Three independent biological replicates were analyzed per treatment (each replicate was composed of a pool of 5 fruits). The histological observation of the pulp tissue of the cross section was obtained from fruit samples. Three pears in each sample were used for slicing. The anatomical images were observed using a microscopic imaging system of Eclipse Ti-S (Nikon). The plant hormones were determined by liquid chromatography - mass spectrometry. A total of 100 mg of sample were extracted by ethyl acetate. The supernatants were evaporated to dryness. The dry matter at the bottom of the tube was dissolved with methanol. The super-

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natant was injected into a LC-MS (AB Qtrap5500). The content of trans-zeatin (tZT) (Parent ion: m/z = 218.0; quantitative ion: m/z =134.0) and gibberellin $A_3$ ($GA_3$) (Parent ion: m/z =345.2; quantitative ion: m/z =239.1) were analyzed. The standards were tZT (Z0876) and $GA_3$ (48880) (Sigma-Aldrich). The calibration curves were plotted using 0, 2.5, 5.0, 10, 12.5, 25 and 50 ng·mL$^{-1}$ of each standard. The total RNA was extracted using the CTAB method. The RNA purity and concentration were assessed using a NanoDrop 2000 (Thermo). The cDNA libraries were constructed using NEBNext Ultra RNA Library Prep Kit (NEB) according to the manufacturers instructions. The cDNA fragments of preferentially 250−300 bp in length were purified. Subsequently, USER Enzyme (NEB) was used with size-selected, adaptor-ligated cDNA. Then PCR was performed, and the PCR products were purified and library quality was assessed by the Agilent Bioanalyzer 2100 system. Each library (approximately 10 ng) was used for Paired-End sequencing using Illumina HiSeq™ 4000 (Illumina). The raw sequence data were filtered to remove low-quality reads. The clean reads were mapped to the *Pyrus* reference genome (NCBI, GCF_000315295.1) using Hisat2 software. The unique mapped reads were used in subsequent analyses. The gene expression level was calculated using the method of fragments per kb per Million reads (FPKM) by RSEM. The raw counts were analyzed using DESeq2 software based on negative binomial distribution. The genes/transcripts with differential expression between groups were selected by *p*-adjust ≤ 0.05 and |$Log_2$Foldchange| ≥ 1.【Results】The median value of mature fruit weight of BCG and CG was 425.8 g and 269.0 g respectively. The mature fruit of BCG was 59.4% larger than that of CG. The Paraffin section results showed that the cross-section area of cell of BCG was bigger than that of CG. The content of tZT decreasing in both of CG and BCG after blossoming. On 10 DAB, tZT content was of BCG higher than that of CG. At other time points, the difference of tZT content was not significant. The $GA_3$ content exhibited the trend of down-up-down. On 20, 30 and 40 DAB, the content of $GA_3$ of BCG was higher than that ofCG. The significant difference was only found on 20 DAB. BCG and CG fruits were sampled at five stages and subjected to RNA-Seq analysis. Between 42.7 and 61.1 million paired-end reads (raw data) were obtained from each library. After a stringent quality filtering process, the counts of clean reads per library ranged from 42.33 to 60.52 M, with a Q30 percentage ≥ 93.4%. The SRA data of RNA-seq was deposited in Genome Sequence Archive in the National Genomics Data Center (https://ngdc.cncb.ac.cn/) (accession number, CRR327161-CRR327190). The differentially expressed genes (DEGs) were identified by pairwise comparisons of the libraries from CG and BCG at each sample time. A high number of DEGs (264 upregulated and 345 downregulated DEGs) were found on 10 DAB, and a low number of DEGs (52 upregulated and 62 downregulated DEGs) were found on 20 DAB. 609 and 639 DEGs were found on 0 and 30 DAB respectively. After combining the DEGs in all five stages, 2015 DEGs were found in the young fruit development in pear. The Venn diagram showed that a large number of DEGs were specific at each stage. 302 DEGs were shared in more than two stages. It is generally believed that DEGs shared in different periods might be the key genes. Among the 302 DEGs, only one gene was found to be differentially expressed at all stages, which was a *long non-coding RNA* (*lncRNA*, LOC103927682). It was down-regulated in BCG and presumed to be a negative regulatory gene. Six genes were significantly differentially expressed at the four stages. A *late embryo enrichment protein D-29* (LOC103944693) was significantly up-regulated in BCG at the last four stages. Three genes were related to metal elements. The *Metallothionein 1* (LOC103944922), *metal nicotinamide transporter YSL2* (LOC103951610) and *iron superoxide dismutase* (LOC103953698) were down-regulated in BCG at the last four stages. A gene for *RNA-dependent RNA polymerase 1* (LOC103943088) was found to be up-regulated in BCG at most stages. An *extensin* gene

(LOC103937946) was significantly up-regulated in BCG on 10 and 20 DAB. In addition to analyzing the differential genes shared by different periods, we analyzed the differential genes on 0 and 10 DAB. On 0 DAB, the expression of *auxin-binding proteins ABP19a*, *KLUH* and *gibberellin regulatory protein 6* was significantly up-regulated in BCG. The expression of *gibberellin 3-β-dioxygenase* was down-regulated. On 10 DAB, the expression of the *expansin* was significantly up-regulated, and two members of *gibberellin 2-beta-dioxygenase* were significantly down-regulated in BCG. The three cytokinin-related genes (*AHP1*, *ARR17* and *ORR10*) were significantly up-regulated in BCG.【Conclusion】This study revealed the possible reasons for the large fruit bud sport of Cuiguan pears in the physiological, biochemical and molecular levels. The cytokinins and gibberellins might be involved in the control of fruit size. The transcriptome analysis provided a relatively complete molecular platform for future studies on the difference of pear fruit size.

**Key words:** Cuiguan Pear; Bud mutation; Fruit size; Development; Transcript

果实大小是非常重要的农艺性状，果实大的品种在市场上更受欢迎。遗传背景、栽培措施和化学物质喷施都能决定果实大小[1]。在相同生长条件下，有的梨属种果实非常小。如野生梨中的川梨、豆梨和杜梨等，它们的果实直径为1~2 cm，质量不到10 g[2]。这些野生梨的果实发育期非常短暂，果实膨大仅发生在花后2周内，随后果实生长异常缓慢，而栽培砂梨大多数品种的果实发育期长，单果质量超过200 g。在相同梨属种内，通常砂梨晚熟品种的果实比早熟品种的果实大，差异主要取决于果实中的细胞数量，而不是细胞大小[3]。在苹果[4]、甜瓜[5]和甜樱桃[6]中，果实大小不同的品种之间细胞数量存在显著差异。还有一些研究表明，细胞数量和大小共同决定果实大小[7]。由于细胞增殖减少和细胞变小，海棠的果实比栽培苹果小[8]。Grand Gala是从Gala苹果突变而来的大果品种，其果实大小与细胞数量和细胞大小有关[9]。栽培措施和化学品也可以调控果实大小。疏果施肥可以有效增大果个[10-11]。细胞分裂素和赤霉素等激素的喷施也可以显著增大果个[12]。

很多基因参与了果实大小的调控。在番茄中，果实大小相关基因已有大量的研究[13]。*fw2.2*、*fw3.2*和*fw11.3*被鉴定为与单果质量相关的数量性状基因座（quantitative trait locus，QTL）[14]。*fw2.2*负调控细胞分裂，*fas*能调控心室的数量，进而影响果实大小[15]。除果实大小直接相关基因外，还有很多基因参与调节细胞分裂和增大[16]，如控制细胞分裂的细胞周期蛋白基因[17]。细胞周期蛋白激酶抑制剂基因*KRP2*可以降低细胞数量、减少叶面积和改变叶形[18]。在拟南芥中，转录因子*ANT*[19]、生长素诱导基因*ARGOS*[20]和*BIG BROTHER*[21]可以调节器官生长和器官大小的变化。番茄中赤霉素信号转导相关基因*DELLA1*的沉默和*GA20ox1*的过表达会产生小而长的果实[22]。这些大量参与调节果实大小的基因表明，许多途径通过影响细胞数量和大小进而影响果实的大小。

笔者课题组前期发现了1个翠冠梨（*Pyrus pyrifolia*）的芽变材料，其成熟果实大小比普通翠冠大50%以上。研究翠冠梨的大果芽变材料的生化特性和基因表达情况将有助于深入了解调控砂梨果实大小的分子机制。

## 1　材料和方法

### 1.1　试验材料

研究使用的翠冠梨大果芽变（简称大翠冠）和正常翠冠梨材料生长于上海市农业科学院金山果树试验站。每个材料各10株嫁接树，嫁接后树龄为6 a（年）。分别在开花后0、10、20、30、40 d取样。0 d的果实为去除花瓣后的花托。在每个取样点，均设置3个生物学重复，每个生物学重复采集5个果实。

### 1.2　幼果组织切片

在每个采样点分别采集翠冠和大翠冠3个果实，从中间进行横切，选择横切面的果肉组织进行石蜡切片，采样后立即用甲醛-乙酸-乙醇固定液固定，4 ℃保存。将果肉组织在分级乙醇（$\varphi$）系列中脱水（70%、80%、90%和100%，每级45 min），并用二甲苯清洗1 h，然后包埋在石蜡中。使用Leica RM2265切片机获取薄切片。将切片在玻璃基板上干燥并再水化，然后用番红（1%，$w$）染色1 h。用水和乙醇洗涤后，切片用固绿（1%，$w$）染色。使

用Eclipse Ti-S显微成像系统观察解剖图像。使用Image-pro Plus软件对细胞横切面面积进行测量，每个果实测量30个细胞，每个处理共计90个细胞。

### 1.3 植物激素测定

在10 mL离心管中用4 mL乙酸乙酯萃取总共100 mg样品，将离心管涡旋1 min，然后在4 ℃的黑暗中浸泡过夜（12 h）。将样品在4 ℃、10 000 r·min$^{-1}$下离心10 min。将上清液转移到新的10 mL离心管中，然后将3 mL乙酸乙酯加入到残留物中。将试管震荡1 min并在4 ℃、10 000 r·min$^{-1}$下离心10 min。合并上清液，30 ℃真空离心浓缩至1 mL。剩余液体用0.22 μm有机滤膜过滤，转移至1.5 mL离心管中，30 ℃真空离心浓缩器继续蒸干至无液体。用250 μL 50%甲醇（$\varphi$）溶解管底的干物质。用0.22 μm有机滤膜过滤上清液，转移至250 μL衬管中，装入棕色样品瓶中。将10 μL上清液注入液相色谱-质谱仪（LC-MS，AB Qtrap5500）。对反式玉米素（trans-zeatin，tZT，母离子：m/z =218.0；定量离子：m/z =134.0）和赤霉素$A_3$（gibberellin $A_3$，$GA_3$，母离子：m/z =345.2；定量离子：m/z =239.1）的含量分别进行分析。流动相由溶解在再蒸馏水中的甲醇和0.5%（$\varphi$）乙酸组成。标准品为tZT（Z0876）和$GA_3$（48880）（Sigma）。使用0、2.5、5.0、10、12.5、25.0和50.0 ng·mL$^{-1}$的标准品绘制标准曲线。

### 1.4 RNA提取和转录组测序

使用十六烷基三甲基溴化铵法（Cetyltrimethylammonium Bromide，CTAB）分别提取30个样本的总RNA。使用NanoDrop 2000评估RNA纯度和浓度。使用NEBNext Ultra RNA Library Prep Kit构建cDNA文库。通过Oligo(dT)磁珠富集总RNA中带有polyA结构的mRNA，采用离子打断的方式，将RNA打断到长度为300 bp左右的片段，纯化长度为250~300 bp的cDNA片段。以mRNA为模板，用六碱基随机引物合成第一条cDNA链，然后加入缓冲液、dNTPs、RNase H和DNA polymerase Ⅰ合成第二条cDNA链，利用AMPure XP beads纯化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。最后通过PCR富集得到cDNA文库并在Agilent Bioanalyzer 2100系统上评估文库质量。每个文库（约10 ng）用于使用Illumina HiSeq™ 4000（Illumina，San Diego，CA，USA）进行双末端测序。过滤原始序列数据以去除低质量reads。转录组的SRA数据保存在国家基因组科学数据中心（https://ngdc.cncb.ac.cn/，登录号：CRR327161~CRR327190）。

### 1.5 差异表达基因的鉴定

使用Hisat2软件将过滤后的Reads比对到梨参考基因组（NCBI，GCF_000315295.1），允许不超过2个碱基的错配。使用RSEM软件运用每千个碱基转录每百万映射读取片段数（FPKM）的方法计算基因表达水平。使用基于负二项分布的DESeq2软件对raw counts进行分析，设置$p$-adjust≤0.05，|$Log_2$Fold-change|≥1的筛选条件比较组间表达差异的基因/转录本。不同阶段共有的差异基因数量用维恩图表示。利用京都基因与基因组百科全书（kyoto encyclopedia of genes and genomes，KEGG）通路分析将所有差异基因映射到KEGG数据库，计算每个代谢通路的基因数。

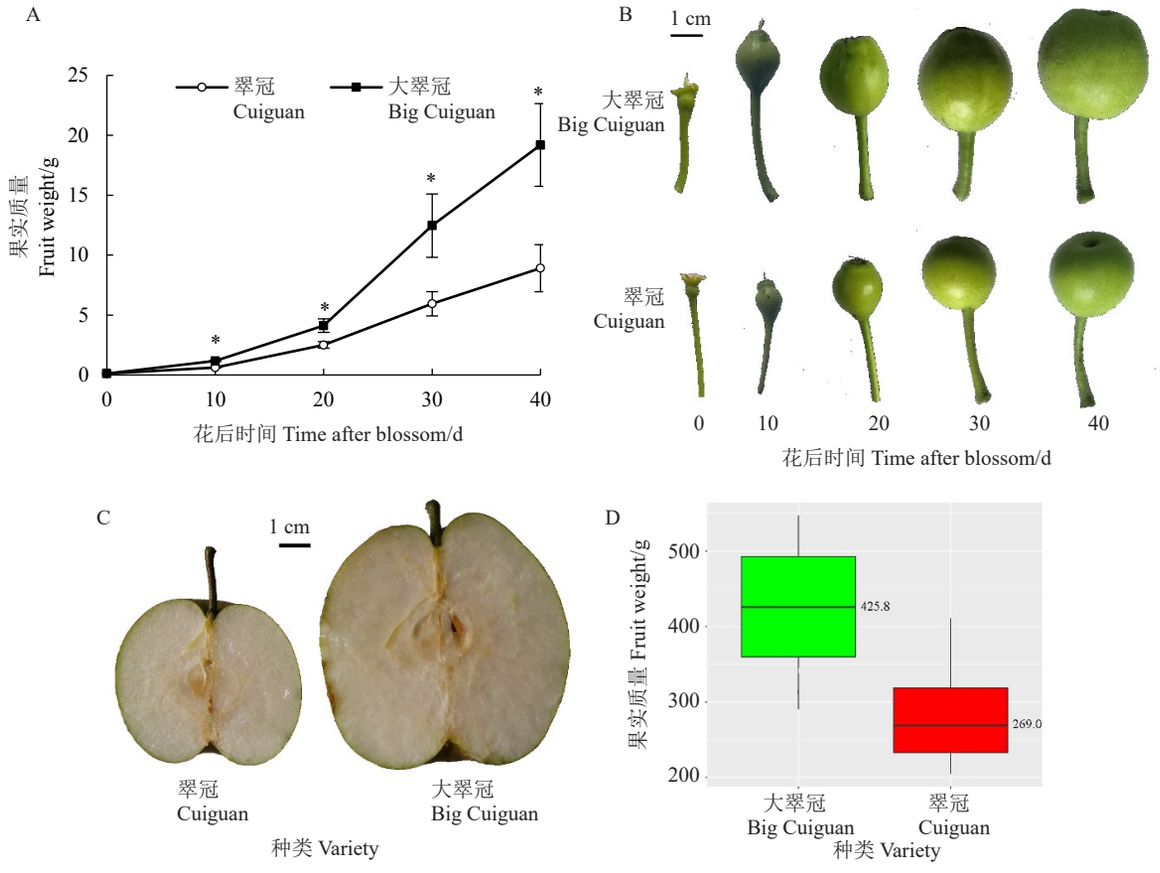
## 2 结果与分析

### 2.1 翠冠及其芽变体开花后40 d果实发育状况

在花后0、10、20、30和40 d，翠冠和大翠冠单果质量逐渐增加（图1-A）。从花后10 d开始，两者之间的果实大小存在显著差异，在各时期，大翠冠的果实均更大（图1-A~B）。花后120 d的成熟果实，大翠冠的横向直径和纵向直径都大于翠冠（图1-C）。两者成熟果实单果质量的中位数分别为425.8和269.0 g（图1-D），大翠冠单果质量比翠冠果实大59.4%。石蜡切片结果显示，大翠冠果实细胞的横截面积大于翠冠（图2）。在5个阶段中，大翠冠和翠冠的细胞大小均增加（图3），芽变后果实的细胞更大。

### 2.2 翠冠和大翠冠中的反式玉米素tZT和赤霉素$A_3$含量测定

对果肉中tZT和$GA_3$的含量进行分析，结果（图4）显示大部分阶段tZT和$GA_3$的含量差异不显著。在翠冠和大翠冠中，随着果实发育tZT含量均逐渐降低。在花后10 d时，大翠冠中的tZT含量显著高于翠冠。而在花后0、20和30 d时，虽然翠冠中的tZT含量略高于大翠冠，但差异并不显著。$GA_3$的含量在2个样本中均呈现下降-上升-下降的趋势。在花后20、30和40 d时，大翠冠中$GA_3$含量高于翠冠，但仅在花后20 d时差异显著。

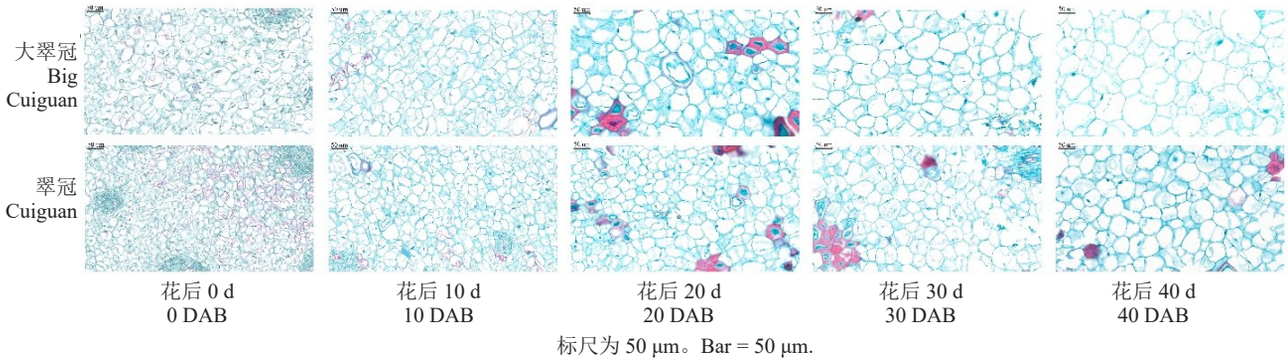



A. 幼果；B. 果实发育；C. 成熟果实；D. 成熟果实。*表示差异显著（$t$ 检验，$p<0.05$），误差线表示标准差。下同。

A. Young fruit weight; B. The fruit development; C. The mature fruit; D. The mature fruit weight. * indicates significance at $p<0.05$ using a student's $t$-test. Bars indicate standard error. The same below.

**图 1　翠冠和大翠冠开花后 40 d 内果实发育情况**

**Fig. 1　Development of the fruit of Cuiguan and Big Cuiguan at 40 days after blossom**



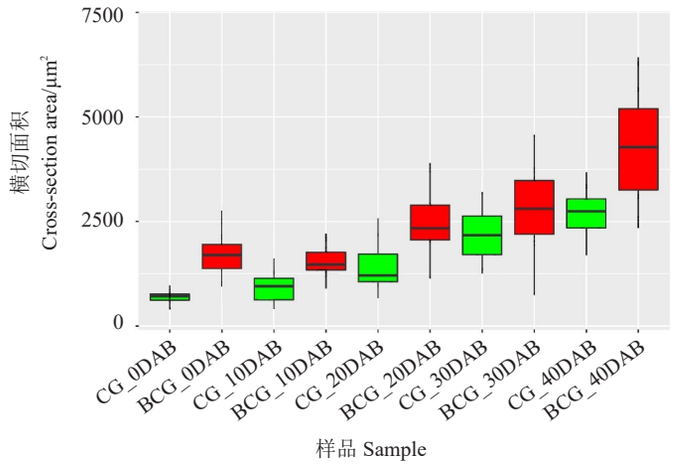

标尺为 50 μm。Bar = 50 μm.

**图 2　翠冠和大翠冠开花后 40 d 内果肉细胞横切面**

**Fig. 2　Histological sections of young fruit cell of Cuiguan and Big Cuiguan in 40 days after blossom (DAB)**

### 2.3　果实发育中差异表达基因分离

为了进一步分析大翠冠和翠冠果实发育之间的分子差异，对两者花后5个时间点进行转录组分析。在30个文库中，获得双末端raw reads 42.7万~61.1万条。经过严格的质量过滤过程后，每个文库的clean reads数范围为42.33~60.52 M，Q30百分比≥93.4%。在每个采样时间点对翠冠和大翠冠果实内基因表达进行差异分析（图5），在花后10 d时发现了大量的差异基因（264个上调和345个下调基因），随后花后20 d时差异基因数量下降，分别有52个上调和62个下调的差异基因。在花后0和30 d中分别发现了609和639个差异基因。综合所有5个阶段的差异




CG_0DAB 表示花后 0 d 的翠冠，BCG_0DAB 表示花后 0 d 的大翠冠，以此类推。

CG_0DAB represents the fruits of Cuiguan at 0 days after blossom, BCG_0DAB represents the fruits of Big Cuiguan at 0 days after flowering, and so on.

**图 3 翠冠（CG）和大翠冠（BCG）开花后 40 d 内果肉细胞横切面积**

**Fig. 3 The cross-section area of cell size in Cuiguan (CG) and Big Cuiguan (BCG) in 40 days after blossom**

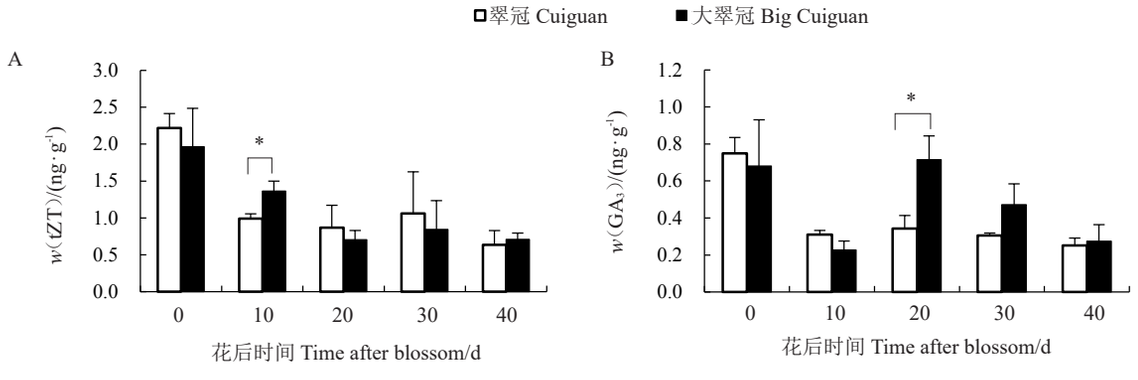
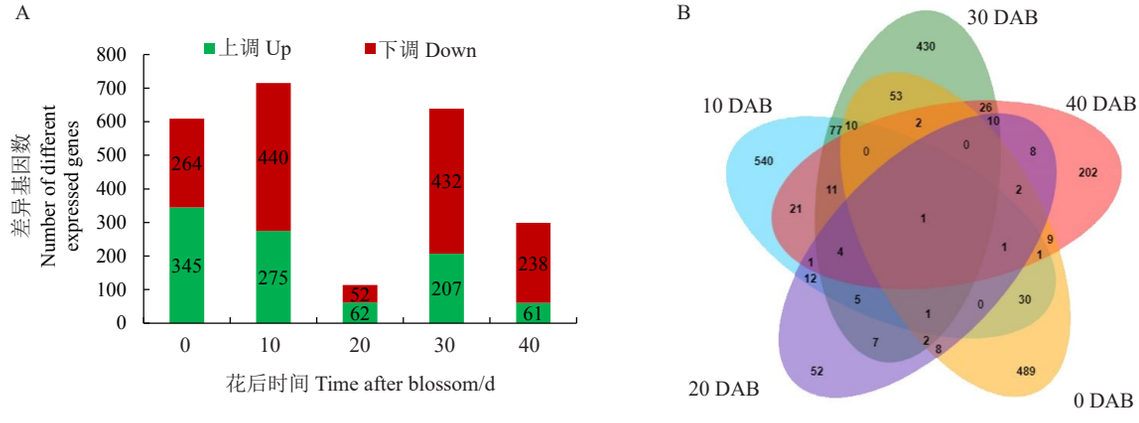



**图 4 翠冠和大翠冠果实发育过程中 tZT 和 $GA_3$ 的含量**

**Fig. 4 Content of trans-zeatin and gibberellin $A_3$ during fruit developmentof Cuiguan and Big Cuiguan**




A. 差异基因数量；B. 5 个时期差异基因维恩图。

A. The number of differentially expressed genes; B. The Venn diagram of differentially expressed genes.

**图 5 翠冠和大翠冠开花后 40 d 内差异基因数量**

**Fig. 5 The different expressed genes between Cuiguan and Big Cuiguan in 40 days after blossom (DAB)**

基因，在梨果实发育早期共发现了2015个差异表达基因。维恩图显示大量的差异基因为1个时期特有。302个基因在2个以上的时期发生差异表达，51个基因在3个以上的时期发生差异表达。

### 2.4　各时期差异基因注释和表达分析

一般认为不同时期共有的差异基因可能为关键基因。在302个差异表达基因中，只发现了1个基因在所有5个时期均发生差异表达。此基因为1个长链非编码*RNA*（*lncRNA*，LOC103927682），在大翠冠中发生下调表达，推测为负调控基因。6个基因在4个时期显著差异表达，均为晚期胚胎富集蛋白基因*D-29*（LOC103944693），在最后4个时期在大翠冠中显著上调表达。3个基因与金属元素有关，包括金属硫蛋白基因1（LOC103944922）、金属烟胺转运蛋白基因*YSL2*（LOC103951610）和铁超氧化物歧化酶基因（LOC103953698），最后4个阶段在大翠冠中下调表达。RNA依赖性RNA聚合酶1的1个基因（LOC103943088）大多数时期在大翠冠中上调表达，并且发现抗病反应蛋白206基因（LOC103927296）在翠冠中上调表达。在2~3个时期中共有的差异基因中，1个液泡铁转运蛋白基因（LOC103935577）和1个锌指蛋白基因*dof2.1*（LOC103943779）在大翠冠中显著上调表达。*WAT1*相关蛋白的2个基因（LOC103939120和LOC103948386）在花后0和10 d时在大翠冠中显著上调表达。1个*extensin*基因（LOC103937946）在花后10和20 d时在大翠冠中显著上调表达。

大翠冠和翠冠之间的果实大小差异起始于花后10 d，所以除了分析不同时期共有的差异基因外，对花后0和10 d这2个时期的差异基因进行了分析（表1）。根据表达量差异倍数和基因注释挑选出部分可能与果实大小相关的基因。在花后0 d，生长素结合蛋白基因*ABP19a*、*KLUH*和赤霉素调控蛋白6基因在大翠冠中显著上调表达，而赤霉素3-*β*-双加氧酶基因下调表达。在花后10 d，扩展蛋白*expansin*基因在大翠冠中显著上调表达。赤霉素2-*β*-双加氧酶基因的2个成员显著下调表达。细胞分裂素相关的3个基因（*ARR17*、*ORR10*和*AHP1*）在大翠冠中均显著上调表达。

### 2.5　细胞分裂素和赤霉素相关基因表达分析

细胞分裂素和赤霉素能够显著影响果实的大小，通过匹配KEGG数据库，结果显示花后0和10 d有27个差异基因富集在植物激素信号转导通路中，推测细胞分裂素和赤霉素相关基因在翠冠梨大果芽变材料幼果期发挥重要作用。在花后10 d鉴定出3个细胞分裂素和4个赤霉素相关的差异表达基因（图6）。细胞分裂素相关的差异基因分别是含组氨酸的磷酸转移蛋白1基因*AHP1*、双组分反应调节剂

**表1　翠冠和大翠冠梨果实在花后0 d和10 d时果实大小相关的差异表达基因**

**Table 1　The differentially expressed genes related to fruit size between Cuiguan and Big Cuiguan at 0 and 10 days after blossom**

| 花后时间<br>Time after blossom/d | 基因登录号<br>Gene accession number | 基因注释<br>Gene description | $Log_2$差异倍数（大翠冠/翠冠）<br>$Log_2$FC(Big Cuiguan/Cuiguan) | 调控<br>Regulation |
|---|---|---|---|---|
| 0 | NW_008988303.1 | 生长素结合蛋白基因ABP19a<br>Auxin-binding protein 19a | 1.04 | 上调<br>Up |
| | NW_008988249.1 | 细胞色素CYP78A5 (KLUH)<br>Cytochrome P450 78A5 (KLUH) | 1.68 | 上调<br>Up |
| | NW_008988744.1 | 赤霉素3-*β*-双加氧酶GA3ox1<br>Gibberellin 3-beta-dioxygenase 1 | −1.37 | 下调<br>Down |
| | NW_008988051.1 | 赤霉素调控蛋白GRP6<br>Gibberellin-regulated protein 6 | 1.16 | 上调<br>Up |
| 10 | NW_008988145.1 | 扩展蛋白<br>Expansin | 2.45 | 上调<br>Up |
| | NW_008988189.1 | 赤霉素2-*β*-双加氧酶GA2ox1-1<br>Gibberellin 2-beta-dioxygenase 1 | −1.17 | 下调<br>Down |
| | NW_008988339.1 | 赤霉素2-*β*-双加氧酶GA2ox1-2<br>Gibberellin 2-beta-dioxygenase 1 | −1.14 | 下调<br>Down |
| | NW_008988145.1 | 双组分响应调节基因ARR17<br>Two-component response regulator ARR17 | 1.32 | 上调<br>Up |
| | NW_008988583.1 | 双组分响应调节基因ORR10<br>Two-component response regulator ORR10-like | 1.32 | 上调<br>Up |
| | NW_008988277.1 | 含组氨酸磷酸转移蛋白AHP1<br>Histidine-containing phosphotransfer protein 1 | 1.26 | 上调<br>Up |

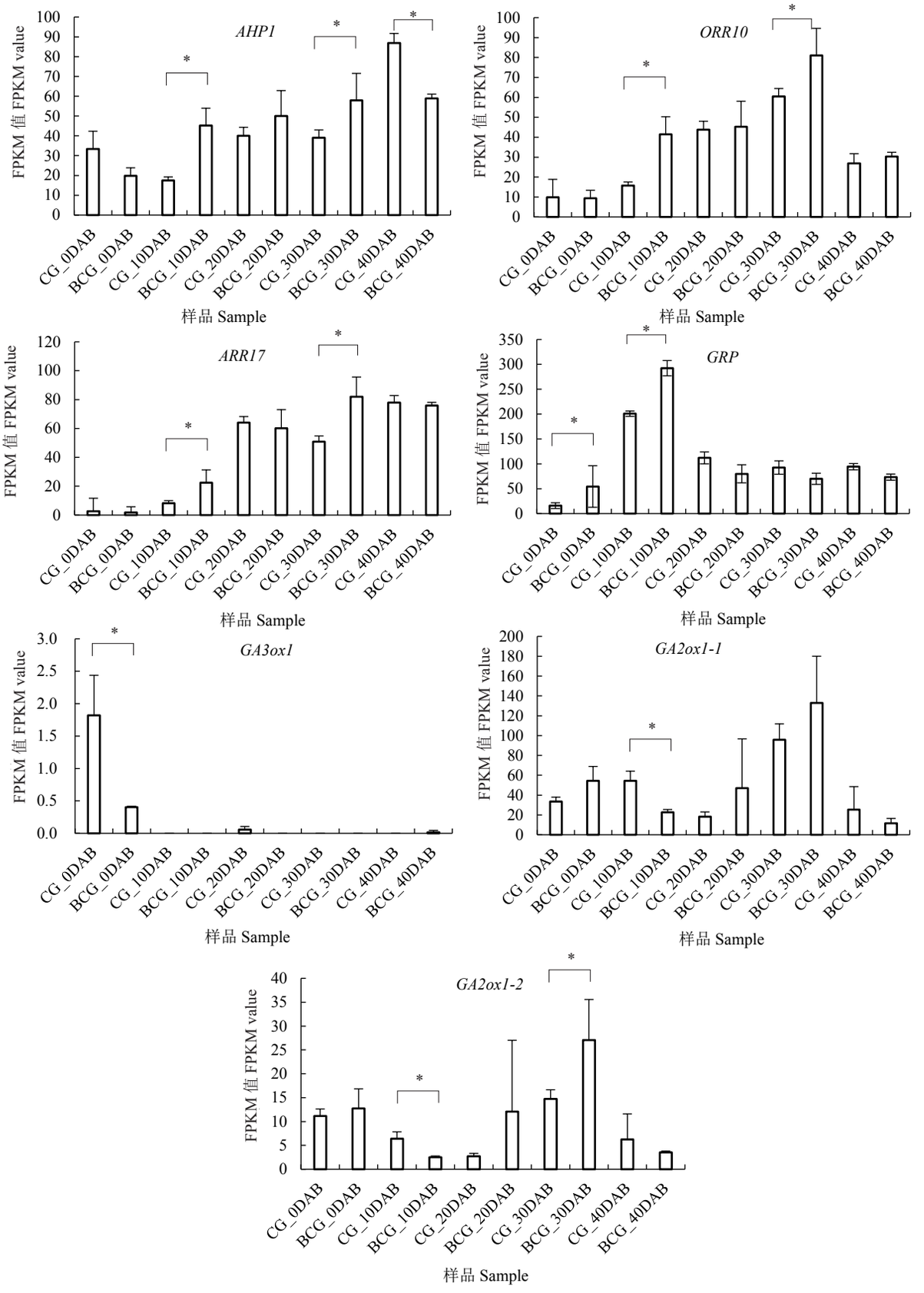



图 6 7 个细胞分裂素和赤霉素相关的基因表达量

**Fig. 6 The transcriptional level of seven differentially expressed genes related to cytokinin and gibberellin**

基因*ORR10*和*ARR17*。这3个基因在大翠冠中均在花后10 d时显著上调，同时在花后30 d时也显著上调表达。赤霉素相关基因共有4个，其中赤霉素调控蛋白基因*GRP6*属于赤霉素正调控基因，其在花后0和10 d在大翠冠中显著上调表达。其余3个基因属于赤霉素氧化酶基因，都是赤霉素信号过程中的负调控因子。其中*GA3ox1*在花后0 d时在翠冠梨中显著上调表达，而*GA2ox1*的2个成员在花后10 d时在翠冠梨中上调表达。

## 3　讨　论

果树芽变选种是果树培育新品种的重要途径。在梨属植物中，褐皮芽变突变体锈酥梨选自砀山酥梨[23]。在西洋梨品种La France中发现了1个大果突变体[24]。本研究中，在翠冠梨中发现了1个大果芽变突变体，是不可多得的大果育种材料。果实大小受许多因素影响，例如遗传背景等。在果树品种驯化过程中，由于人类的选择，筛选后育成的品种比野生种要大。先前的研究报道显示，砂梨果实大小的演变主要取决于细胞分裂而不是细胞大小[3]。本研究中，大翠冠细胞的横截面积大于普通翠冠，这表明大翠冠的1个变异来源是细胞增大。由于梨果肉细胞排列无序，加上石细胞的干扰，笔者无法准确测量梨横切面上果肉细胞的层数。根据过去5 a的物候期记录，翠冠和大翠冠的花期一致，这表明变异并非来自开花的早晚。翠冠和大翠冠的果实发育表明，花后果实大小差异逐渐变大。这表明一些重要基因可能从开花后即发挥作用。

细胞分裂素和赤霉素可以调节果实的大小和质量。细胞分裂素苄基腺嘌呤能增加Spadona和Coscia梨的果实大小[25]。反式玉米素是一类嘌呤类的植物细胞分裂素[26]。本研究中，在花后10 d时，大翠冠中tZT的含量高于翠冠，暗示高含量tZT可能促进了梨果肉细胞的分裂。赤霉素的喷施可以导致Akca梨的单果质量增加20.2%[12]。赤霉素能够控制细胞的伸长[27]，其含量与最终果实大小、果皮细胞数量呈正相关[28]。本研究中在后期的3个时间点，大翠冠果肉中$GA_3$的含量始终高于翠冠。这表明大翠冠果实大小的变异来源可能与赤霉素含量的变化相关。大翠冠和翠冠之间细胞分裂素和赤霉素含量的不同表明这2种激素可能都参与了果实大小的控制。

芽变材料变异来源单一，可能是基因组中某个碱基或者某个小片段的插入缺失导致，这使得从DNA层面找到变异位点比较难以实现。笔者在本研究中从基因表达入手，分析了大翠冠和翠冠之间的差异表达基因，共发现了2015个差异表达基因。仅有1个差异基因存在于所有5个采样时期，有302个差异基因存在于2个以上采样时期。在柑橘无核突变体[29]和梨果皮突变体[30]的转录组分析中，也只发现了少量的差异表达基因。这表明芽变材料中基因表达差异不大。由于细胞分裂素和赤霉素在芽变材料中有差异，笔者重点分析了相关基因的表达情况。含组氨酸的磷酸转移蛋白基因*AHP*参与细胞分裂素的信号转导机制，其功能很可能与反应调节因子基因*RRs*一致[31]。双组分响应调节基因*ORR*和*ARR*可由细胞分裂素诱导并调节生长发育[32]。本研究中这3个基因在花后10 d时在大翠冠中显著上调表达，而此时大翠冠中tZT的含量也较高，表明这3个基因可能受到细胞分裂素诱导，参与果实增大调控。赤霉素双加氧酶参与了赤霉素的信号转导，能够催化赤霉素失去生物活性[33]。本研究中*GA3ox1*和*GA2ox1*在大翠冠果实发育初期均表现出表达量下降，暗示具有生物活性的赤霉素含量上升，促进果实的增大。

除了激素相关基因外，笔者在本研究中还鉴定了一些其他基因。1个长链非编码*lncRNA*在所有5个时间点在大翠冠中均发生显著下调表达。该基因的功能目前尚不明确。晚期胚胎富集蛋白质基因*D-29*后4个时间点在大翠冠中上调。脱落酸胁迫处理后，晚期胚胎富集蛋白质在根尖分生组织和侧根原基中高度积累，导致根系扩张增强[34]。晚期胚胎富集蛋白质基因*D-29*的上调可能会促进果实发育。此外，笔者鉴定出一些与金属相关的差异基因，例如金属硫蛋白样蛋白和金属-烟胺转运蛋白基因*YSL2*等，在后4个阶段在翠冠中上调表达。这些金属相关的蛋白是否参与了果实大小的调控还有待进一步研究。同时，笔者发现了1个RNA聚合酶基因*RDR1*和1个锌指蛋白基因*dof2.1*在3个以上时间点于大翠冠中上调。*RDR*主要通过基因沉默调节植物的生长发育[35]。DOF蛋白参与多种植物过程，包括发育和抗逆性[36]。大翠冠中*RDR1*和*dof2.1*基因的上调表达可能与果实大小相关。在花后0和10 d时，2个*WAT1*基因在大翠冠中显着上调。*WAT1*基

因由生长素诱导并促进细胞壁厚度增加[37]，可能参与了果实发育，细胞色素P450蛋白基因*CYP78A5/KLUH*主要以非细胞自主性的方式调控了拟南芥器官大小的发育，该基因在花后0 d时，在大翠冠中显著上调表达。这些基因的功能以及在果实发育中的作用还需要进一步研究。

## 4 结 论

对翠冠梨大果芽变进行了组织切片、激素变化研究及转录组分析，初步明确了变异来源。大翠冠在果实发育初期细胞大小更大。反式玉米素和赤霉素含量在大翠冠果实发育的一些时期高于翠冠。通过转录组分析分离了一些可能的关键差异基因，例如*lncRNA*、*AHP1*和*GA3ox1*等，为梨果实大小调控的分子机制研究提供了参考。

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