

纸皮核桃内果皮硬化期差异表达基因筛选及功能预测

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摘要:【目的】综合分析纸皮核桃内果皮硬化期转录本表达情况, 获得3个代表样本间差异表达基因, 为系统研究纸皮核桃内果皮木质化的分子机理打下基础。【方法】采用转录组测序技术对其内果皮硬化期3个样品测序, 利用生物信息学方法和软件筛选样本间的差异表达基因并进行生物学功能预测。【结果】通过Trinity软件拼接得到76 814条Unigene, 筛选出了609个差异表达基因, 利用Gene Ontology和KEGG数据库对差异表达基因注释, 发现差异表达基因显著富集在植物激素信号转导、苯丙烷类生物合成、苯丙氨酸代谢、ABC转运子合成、泛醌和其他萜类醌生物合成等途径中。【结论】筛选出了纸皮核桃内果皮硬化期差异表达基因显著富集的主要代谢途径, 为今后系统研究纸皮核桃内果皮木质化的分子机理打下了良好基础。

关键词:核桃内果皮; 差异表达基因; 表达模式分析; 功能显著性富集分析

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Screening and functional prediction of differential expression genes at lignification stage of endocarp in ‘Zhipi’ walnut

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Abstract:【Objective】The differential expression genes from the three representative samples were obtained by synthetically analyzing the expression of transcripts in the endocarp of walnuts, which laid a foundation for the comprehensive and systematic study on the molecular mechanism of lignification in walnut endocarp.【Methods】Walnut fruit was divided into two parts after sampling, one for observing lignin deposition in endocarp and the other for extracting RNA. With the former fruit, it was cut transversely and then longitudinally into thin slices. Finally, all the flakes were washed with flowing water and soaked in the modified Wiesner reaction solution for 5 minutes, and the image was photographed. With the later fruit, it was cut into pieces with a knife, and only the endocarp was preserved by removing the green peel(husk) and seed, and the endocarp (shell) was frozen with liquid nitrogen and preserved at -80 °C. The extraction of total RNA from walnut endocarp was carried out with pBIOZOL plant tissue RNA extraction kit (BioFlux). Specific operation was carried out according to the instruc-

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tion sheet. The samples of RNA extraction were sent to Beijing Genomics Institute in Shenzhen, China for quality and integrity detection. After qualified detection, mRNA was enriched with the magnetic beads of Oligo (dT) and then sequenced library was constructed before it was sequenced on computer. The sequenced image data was transformed into sequence data (raw reads) by base calling software, filtered by filter fq software to obtain the final needed data (clean reads), and then the clean reads was spliced and assembled. Finally, the required Unigene was used to screen out the differential expression genes among samples by bioinformatics and software and to predict the biological function of the Unigene.【Results】By Wiesner method, we found that the lignification of the endocarp began at 44 days after full bloom, and the process of lignification was basically completed by 80 days after full bloom. The lignin deposition was on the endocarp at the same time, but the amount of lignin deposition in the top of endocarp of the fruit was higher than that in other regions. A total of 76 814 Unigenes were obtained by using Trinity software to assemble reads, and 1 077 differential expression genes were obtained using $FDR < 0.001$ and $|\log_2\text{Ratio}| \geq 1$ as screening conditions. Three statistical significant gene expression profiles were obtained by further analysis of 1 077 selected differential expression genes in The Short Time-series Expression Miner (STEM) software. The gene expression profile was No.1 (0, -2, 1), No.6 (0, -1, 1), and No.10 (0, 1, 0) in turn. Through expression profile analysis, we finally selected 609 differential expression genes to be assigned into the salient model. In order to explore the biological function of 609 Unigenes, we first selected GO database to annotate 609 differential expression Unigenes. The results showed that Unigenes were significant enrichment in 42 functional terms under three major Gene Ontology, and in their biological process there were 17 terms, mainly including some cellular process, metabolic process, biological regulation, developmental process, signaling and reproduction and growth; there were 8 terms for cell components, including cell, cell part, organelle, membrane, membrane part, organelle part and cell junction and symplast, and there were only 11 enriched term numbers in molecular function. Secondly, KEGG annotation showed that differential expression genes were significantly enriched in protein processing in endoplasmic reticulum, ABC transporters, endocytosis, plant-pathogen interaction, phenylpropanoid biosynthesis, spliceosome, phenylalanine metabolism, plant hormone signal transduction, ubiquinone and other terpenoid-quinone biosynthesis. Among them, transcription factor BIM1 gene CL169.Contig7_All, serine/threonine-protein kinase SAPK3 gene Unigene28134_All, ethylene receptor 2 gene Unigene18324_All, auxin response factor 3 gene CL447.Contig4_All, histidine kinase 2 gene CL4024.Contig3_All and so on were involved in plant hormone signal transduction pathway, the gene CL2824.Contig3_All was involved in the synthesis of ATP-binding cassette transporter, the gene CL9312.Contig1_All, Unigene27245_All, and CL3278.Contig2_All were involved in the synthesis of ABC transporter A family member 2, ABC transporter B family member 11 and ABC transporter C family member 3 in the ABC transporter pathway and gene CL2405.Contig5_All, Unigene24904_All, CL2983.Contig6_All and Unigene7110_All were involved in phenylpropane biosynthesis pathway, phenylalanine catabolism pathway, phenylalanine lyase, coenzyme A ligase, acyltransferase, acyl-coenzyme A synthase and peroxidase.【Conclusion】We screened out the main metabolic pathways of differential expression genes in the endocarp of walnut, which laid a good foundation for the systematic study on the molecular mechanism of lignification in the endocarp of walnut in the future.

Key words: Walnut endocarp; Differential expression genes; Expression profile analysis; Functional significance enrichment analysis

核桃(*Juglans regia* L.)是我国分布最广泛的经济树种之一,主要分布于我国新疆、河北、云南等地^[1]。新疆是我国核桃的发源地和主产区之一,种植区主要集中在环塔里木盆地的喀什、和田和阿克苏等周边地区,截至2015年,新疆核桃种植面积约35.167 7万hm²,年产量达60.084 4万t,种植面积和产量较全国均居前列^[2-3]。新疆具有丰富的核桃优良种质资源,至今已选育出‘新新2号’‘新丰’‘新光’等26个优良核桃品种^[2]。在我国首批16个国家级核桃品种中,基于新疆核桃选育而成的品种约占80%^[4]。核桃富含不饱和脂肪酸、纤维素、蛋白质、维生素等营养成分,即可生食、炒食,也可榨油,不仅风味美,而且营养丰富,经济价值高。核桃仁含有益于人体健康的维生素B、生育酚、ω-3脂肪酸、抗氧化剂和褪黑激素等,可有效增强记忆力、防止细胞老化及延缓衰老和用于治疗高血压、神经衰弱、胃痛等病症^[5-6]。核桃品质包括营养品质和外观品质,核桃果壳的厚度是衡量核桃外观品质的一个重要指标,培育优质的薄壳核桃品种是目前的育种目标之一^[7]。

核桃果皮解剖结构研究表明核桃果皮分外、中、内3层,外果皮由靠近表皮的薄壁组织和表皮层组成,其薄壁组织由数层体积较小,排列紧密的细胞构成,表皮细胞在后期发育形成角质层及气孔;中果皮主要由最外的石细胞层和内层薄壁组织构成,薄壁组织中散生有数轮维管束,维管束相互交织形成复杂多样的维管束网络结构;内果皮发育前期由排列紧密且体积较小的细胞形成的薄壁组织组成,其内无维管组织分布,在发育后期薄壁组织木质化形成石细胞层(即坚硬的核桃果壳)^[8-9]。研究发现纤维素、半纤维素及木质素是构成核桃坚壳的主要化学组分,其所含纤维素成分较其他植物明显偏低,而酸不溶木素的含量比其他植物均较高;栽培地域和核品种的不同对组成核桃坚壳的化学组分含量有一定影响,但并不改变主要组分;核桃坚壳木质素并不是单一的,而是不同木质素单体交联而成的复杂的大分子物质,其中以愈创木基单元为主,紫丁香基型次之,并且分子中含有较多的甲氧基^[10]。

目前,对于核桃的研究主要集中在栽培模式^[11-13]、核桃丰产^[14-16]和核桃品质^[17-20]等普遍方向,对于核桃果皮的研究报道鲜见,而对于核桃种仁裸露现象的研究几乎没有。本团队致力于新疆核桃种质资源保存、评价及品种遗传改良研究,对核桃生产栽

培中出现的露仁问题已有初步的探究^[21-24]。新疆阿克苏地区种植的主栽核桃有‘纸皮’‘温138’‘温185’‘新新2号’‘新翠丰’‘新巨丰’等品种,其中‘温138’‘温185’等绝大部分栽培品种是薄皮品种,这些品种的硬壳平均厚度一般小于1.5 mm。纸皮核桃硬壳平均厚度为0.74 mm,明显小于1 mm,因而得名“纸皮”。纸皮核桃不但壳薄,且相对于其他品种而言,品质较好,在市场中具有优势地位。纸皮核桃内果皮发育是由遗传、营养和环境因素等多方面共同影响的,仅对已报到的木质素合成代谢途径进行研究显然是不全面的。因此,对纸皮核桃内果皮硬化期所有表达的基因进行综合分析,是研究纸皮核桃内果皮木质化分子机制更有效的方法。笔者利用高通量测序技术获得纸皮核桃内果皮硬化期的转录组数据,并对硬化期差异表达基因进行分析,以期为系统研究纸皮核桃内果木质化的分子机理提供参考。

1 材料和方法

1.1 试验材料

试验材料为新疆阿克苏地区温宿县核桃林场栽培的纸皮核桃,盛花后(Days After Full Bloom, DAFB)50 d左右核桃果实发育由膨大期进入硬化期,80 d左右硬化过程基本完成,50~80 d为核桃内果皮硬化期。因此,于51、66、80 d进行采样,每次从树体随机采取50个核桃果实,在低温条件将核桃果实切成片状,并小心去除外面的青皮及最里面的种皮和种仁部分,将分离出的内果皮混匀并迅速切至粉末状使之均匀化,用液氮速冻后于-80℃冰箱保存备用。

1.2 方法

1.2.1 核桃内果皮染色 核桃内果皮木质素沉积的检测方法用改进的Wiesner反应法^[25],改进后的办法更加便于实验操作,且具有更好的染色效果。具体步骤为:(1)用95%酒精和浓盐酸配制体积比为1:1的酒精-盐酸溶液;(2)以酒精-盐酸溶液溶解间苯三酚配制成3%的间苯三酚-酒精-盐酸染液;(3)将(材料)核桃果实切片置于染色液中染色5~6 min,木质素会被特异的染成粉红色,采用体视显微镜拍照或直接用相机拍照。配置后的溶液呈淡淡的亮黄色,溶液于棕色试剂瓶中密封保存可长达15 d。

1.2.2 核桃内果皮RNA提取 采用pBIOZOL的植

物RNA提取试剂盒(BioFlux)提取核桃内果皮中总RNA,具体提取操作流程参照试剂盒说明书。同一样品提取多份总RNA,将检测结果符合测序要求的总RNA取3份等体积混合,混合样作为最终的转录组测序样品。

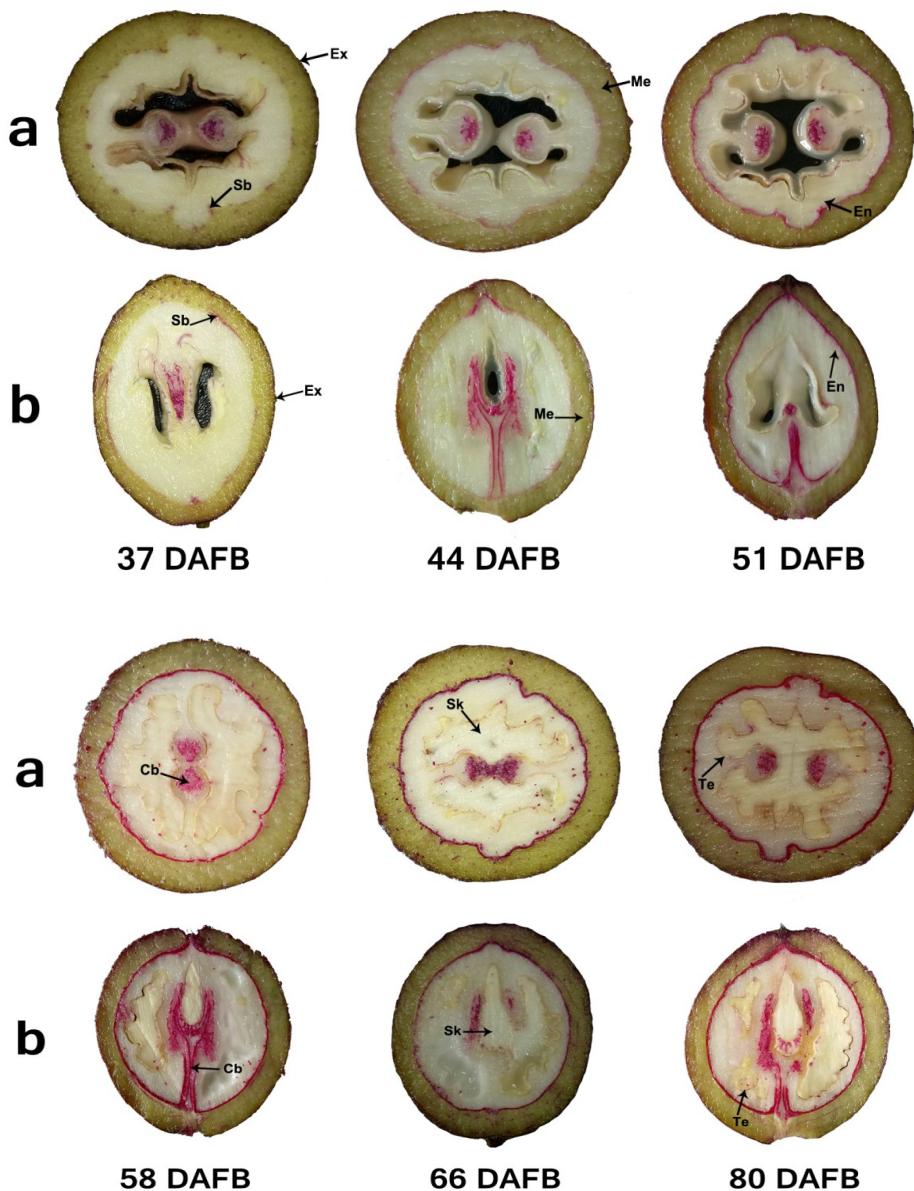
1.2.3 核桃内果皮转录组测序、数据组装及基因功能注释 对核桃内果皮硬化期转录组进行测序后,经base calling将测序图像数据转化为raw reads,再经filter_fq软件对raw reads过滤得到clean reads,并

对clean reads进行从头组装得到最终的Unigene,最后对获得的Unigene进行生物信息学分析及功能注释。转录组测序委托深圳华大基因完成。

2 结果与分析

2.1 纸皮核桃内果皮发育期木质素沉积变化

木质素在酸性条件下可以被间苯三酚特异的染成红色,因而可以用来观察核桃内果皮发育期木质素沉积的变化。如图1所示,花后37 d核桃外、中、



a. 核桃果实横切面;b. 核桃果实纵切面;Ex. 外果皮;Me. 中果皮;En. 内果皮;Sb. 萼片维管束;Cb. 心皮维管束;Sk. 种仁;Te. 种皮。

a. The cross-section of walnut fruit; b. The vertical section of walnut fruit; Ex. Exocarp; Me. Mesocarp; En. Endocarp; Sb. Sepal bundle; Cb. Carpellary bundle; Sk. Seed kernel; Te. Testa.

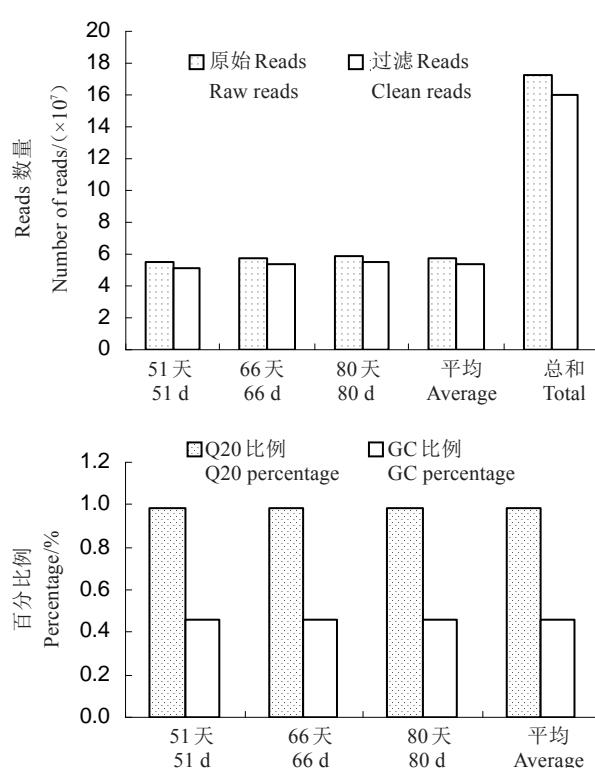
图1 不同发育时期核桃内果皮木质素沉积变化

Fig. 1 Lignification of endocarp of walnut during different development periods

内果皮均未被染色,而分布于中果皮及中、内果皮交界处的维管组织有极轻微的染色,此时期内果皮并没有木质素沉积。花后44 d内果皮最外面出现轻微染色,核桃果实的顶部和基部染色比较明显,说明木质素沉积已经开始。花后51 d相较花后44 d染色加深,横、纵切面均清晰可见,说明内果皮木质素沉积全面开始。花后58、66 d染色程度及染色厚度变化不明显,说明木质素沉积已达到顶峰,花后80 d内果皮的膈组织开始呈现淡淡的粉红色,并且大量失水而变得疏松,说明内果皮木质化过程接近尾声,内果皮木质化过程基本完成。

2.2 转录组测序组装结果评估

获得花后51、66和80 d三个时期的核桃内果皮样品,分别提取各样品中的总RNA,利用Illumina Hiseq 2000平台分别对各样品进行测序。共得到171 968 208条raw reads片段,利用filter_fq软件对reads进行过滤后共得到159 692 320条clean reads片段,其中包含有14 372 308 800个核苷酸信息,平均Q20(准确率为99%)为98.09%,平均GC%含量45.65%,说明测序数据质量较好,可用于后续的分析(图2)。利用Trinity软件对51、66和80 d三个时期的转录组测序数据进行De novo组装,总共获得76 814条Unigene,其中51、66和80 d分别获得74 630、72 810和74 561条序列,平均长度1 258个核苷酸(表1)。



Raw reads 为测序的原始读序列数量; Clean reads 为原始读序列过滤后的数量; Q20 percentage 为过滤后碱基质量不低于 20 的比例; GC percentage 为过滤后总碱基中碱基 G 和 C 的比例。

Raw reads represent the number of raw reads sequence of transcriptome sequencing; Clean reads is the number of raw reads sequence which is filtered; Q20 percentage is the percentage of the base that quality is no less than 20 after filtering; GC percentage is the percentage of base G and C in the total base after filtering.

图2 核桃内果皮测序数据统计

Fig. 2 Sequencing data statistics of walnut endocarp

表1 核桃内果皮转录组测序组装统计

Table 1 Assembly statistics of walnut endocarp transcriptome sequencing

样本 Sample	总组装序列 Total consensus sequences	序列总长度 Total length/nt	序列平均长度 Mean length/nt	N50	聚类Unigene数 Distinct clusters	非聚类Unigene数 Distinct singletons
51天 51 d	74 630	64 298 801	884	1 706	36 923	37 707
66天 66 d	72 810	49 633 755	819	1 535	36 279	36 531
80天 80 d	74 561	58 171 808	879	1 682	36 893	37 668
整体 All	76 814	96 656 456	1 258	1 983	37 966	38 848

注: Total consensus sequences 为组装出的 Unigene 个数; Total length 为所有 Unigene 序列的总碱基数; Mean length 为 Unigene 平均碱基数; N50 为所有 Unigene 依大小排列,从最小的 Unigene 开始累加计算碱基数,累加碱基数等于总碱基数一半时对应 Unigene 的碱基数即为 Unigene 的 N50; Distinct clusters 为聚类的 Unigene; Distinct singletons 为单独的 Unigene。

Note: Total consensus sequences is the number of unigene assembled; Total length is the total base number of all unigene sequences; Mean length is the average base number of unigene; N50 is the number of bases for all unigene in order of size, starting from the smallest unigene. When the number of bases is equal to half of the total, the number of bases for unigene is N50 of unigene; Distinct clusters are unigene of clustering; Distinct singletons is a separate gene.

2.3 核桃内果皮硬化期差异基因筛选

对3个时期样品中所比对上的基因使用FPKM(Fragments Per kb per Million fragments)法获得表达量(FPKM值),并计算基因在不同核桃样本间的

差异表达倍数。为了有效控制假阳性率,利用错误发现率(False Discovery Rate,FDR)法对计算结果做多重统计假设测验。因此,定义符合 $FDR \leq 0.001$ 且 $|\log_2\text{Ratio}| \geq 1$ 为筛选阈值的基因作为差异表达的

基因。对任意两个核桃样本中差异表达的基因进行比较,如a-vs-b,其中b是实验处理,a为实验控制,如果比较结果为上调,则说明基因在b中表达量高,如果比较结果为下调,则说明基因在a中表达量高。对纸皮核桃内果皮硬化的3个时期的表达基因进行两两比对,其中51 d-vs-zp-66 d组发现差异表达Unigene 11 360个,66 d-vs-80 d组共有差异表达Unigene 11 089个,而51 d-vs-80 d组仅有差异表达Unigene 6 883个,我们总共获得差异表达基因18 173条,其中有1 077条在3个时期均差异表达(图3)。花后51与66 d相比表达上调的Unigene为

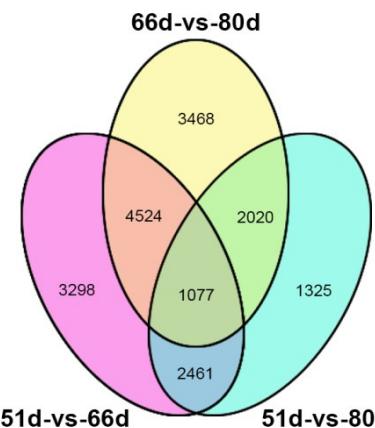


图3 核桃内果皮各时期差异表达基因数量关系

Fig. 3 The quantity relationship of differential expression genes in walnut endocarp

8 529条,80与66 d相比表达上调的Unigene为7 729条,51 d表达上调Unigene是66 d表达上调的3倍,而80 d表达上调Unigene是66 d表达上调的2.3倍(图4)。

2.4 基因表达的时序谱分析

为了进一步缩小上述已筛选的1 077个Unigene中具有重要意义的目标基因,利用The Short Time-series Expression Miner(STEM)软件^[26],选择使用16

个时序模型来分析基因的表达模式。如图4所示,在16个模式中确定了3个P值达到统计显著的基因表达模式,按照P值升序排列依次为1、6、10号。第1号图谱中355个基因和6号图谱中240个基因的表达量先降低再升高,第10号图谱中有14个基因的表达在核桃花后66 d达到最高(图5)。通过表达模式分析我们最终筛选出了609个差异表达基因(图6)。

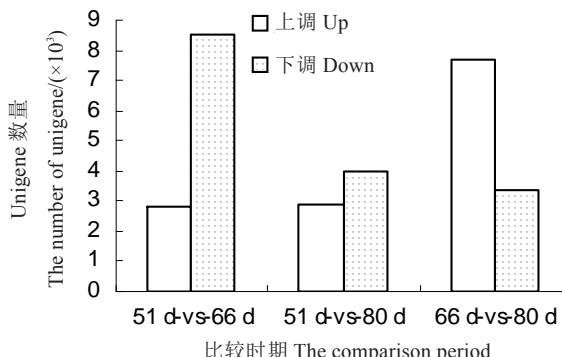


图4 核桃内果皮各时期差异基因数量统计

Fig. 4 The statistics on the number of differential expression genes in walnut endocarp

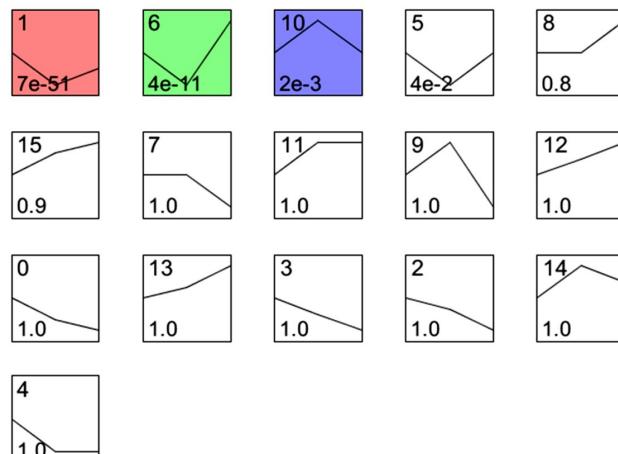


图5 1 077个基因的表达模式

Fig. 5 The expression patterns of 1 077 genes analyzed by model profile

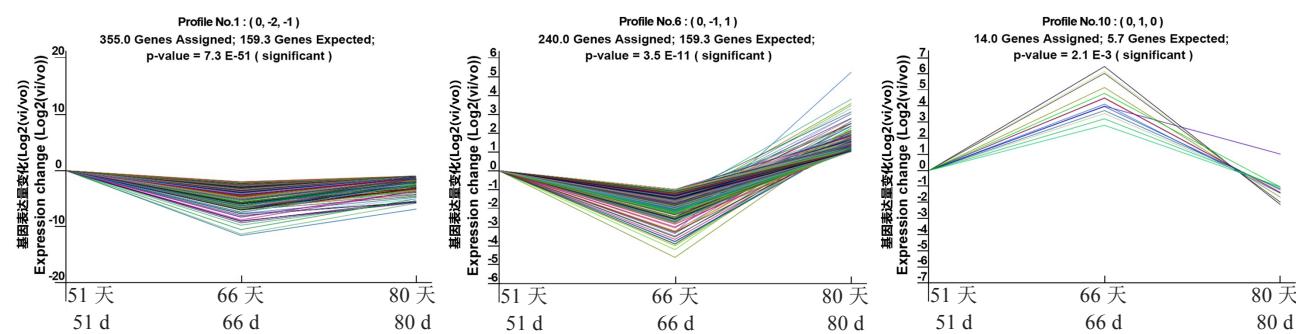


图6 核桃内果皮硬化期1,6,10号图谱中基因表达

Fig. 6 Gene expression of profile No.1, No.6, No.10 in the hardening period of walnut endocarp

2.5 差异表达基因功能注释

对本研究选中的609个差异表达的Unigene进行GO富集分析,如图7所示,左纵坐标表示富集的GO term中的Unigene数目,右纵坐标标示了该term下Unigene占所有Unigene的百分比,Unigene显著富集到GO的3大生物功能下的42个功能亚分组中。其中,生物学过程(biological process, BP)富集的term数最多,共有17个,主要有细胞过程(cellular process)、代谢过程(metabolic process)、生物调节(biological regulation)、信号传导(Signaling)、发育过程(developmental process)、繁殖(Reproduction)

和生长(Growth)7个功能亚分组;细胞组分(cellular component, CC)主要包含了细胞(cell)、细胞部分(cell part)、细胞器(organelle)、膜结构(membrane)、膜部分(membrane part)、细胞器部分(organelle part)、细胞连接(cell junction)和共质体(symplast)8个功能亚分组,总富集term数14个;分子功能(molecular function)中富集term数最少,仅有11个,其中转运活性(transporter activity)、催化活性(catalytic activity)和结合活性(binding)3个功能的Unigene在分子功能中共占88.56%(图7)。

KEGG由KEGG genes, KEGG Ligand, KEGG

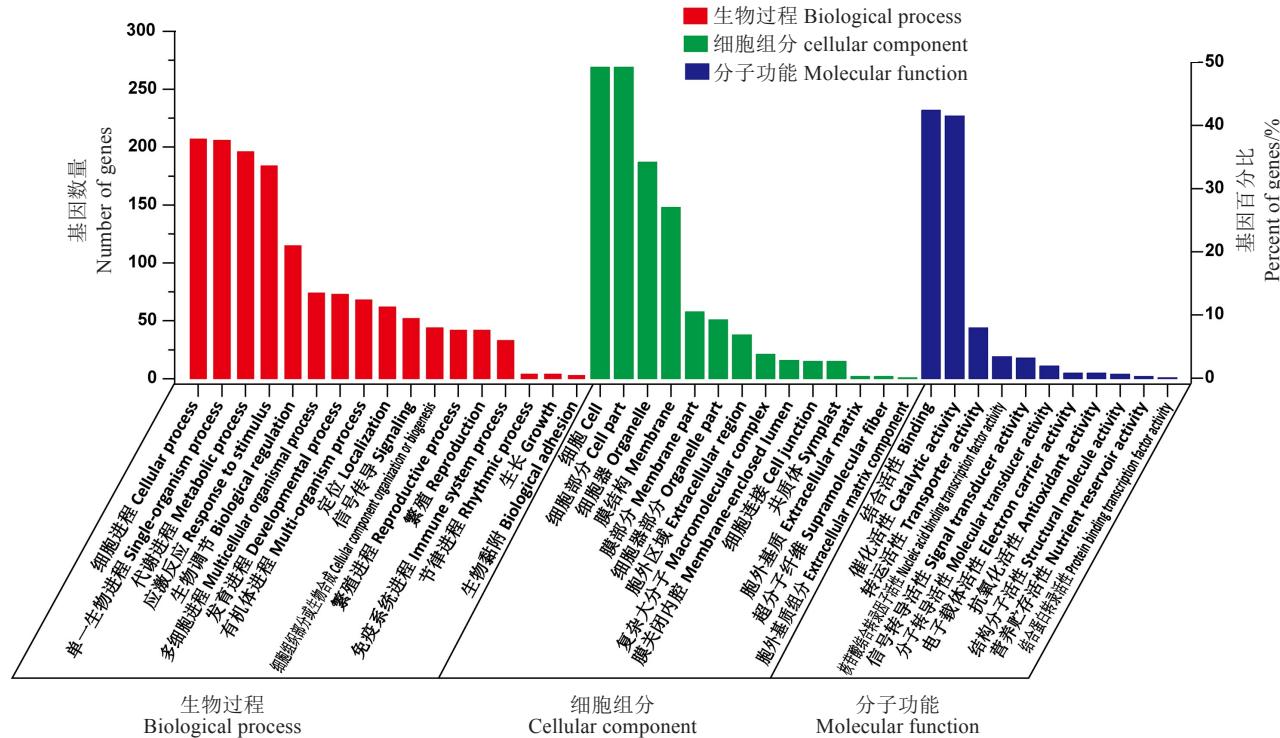


图7 差异基因的GO分类分析
Fig. 7 GO analysis of differential expression gene

pathway 和 KEGG BRITE 4个数据库共同构成,利用KEGG数据库可以研究细胞中基因、生化分子和蛋白质的功能、生化反应以及代谢途径。本研究中有191 Unigene富集到了79个Pathway中(图8),富集度排在前10的Pathway有植物激素信号转导(Plant hormone signal transduction),内质网蛋白加工(Protein processing in endoplasmic reticulum),ABC转运子(ABC transporters),胞吞作用(Endocytosis),植物-病原互作(Plant-pathogen interaction),剪接体(Spliceosome),苯丙烷类生物合成(Phenyl-

propanoid biosynthesis),苯丙氨酸代谢(Phenylalanine metabolism),角质、丝氨酸和蜡质生物合成(Cutin, suberine and wax biosynthesis),泛醌和其他萜类醌生物合成(Ubiquinone and other terpenoid-quinone biosynthesis)。其中,转录因子BIM1基因CL169.Contig7_All、丝氨酸/苏氨酸-蛋白激酶SAPK3基因Unigene28134_All、乙烯受体2基因Unigene18324_All、生长素响应因子3基因CL447.Contig4_All、组氨酸激酶2基因CL4024.Contig3_All等参与植物激素信号转导通路;ABC转运子通路中



图8 差异基因富集的KEGG pathway

Fig. 8 Enriched KEGG pathway of differential expression genes

基因CL2824.Contig3_All参与ATP结合盒转运因子合成,基因CL9312.Contig1_All、Unigene27245_All、CL3278.Contig2_All分别参与ABC家族A、B、C3类转录因子合成; CL2405.Contig5_All、Unige-

ne24904_All、CL2983.Contig6_All、Unigene7110_All等基因参与苯丙烷生物合成通路、苯丙氨酸代谢通路中苯丙氨酸裂解酶、辅酶A连接酶、酰基转移酶、酰基-辅酶A合成酶、过氧化物酶的合成(表2)。

表2 差异基因富集前10的代谢通路

Table 2 The top 10 enriched pathway of differential expression genes

富集通路 Pathway	差异基因代谢通路注释 DEGs genes with pathway annotation (191)	所有基因代谢通路注释 All genes with pathway annotation (19072)	P值 P-value	通路ID Pathway ID
内质网加工蛋白 Protein processing in endoplasmic reticulum	28 (14.66%)	818 (4.29%)	1.26E-08	ko04141
ABC 转运子 ABC transporters	10 (5.24%)	245 (1.28%)	0.000 188	ko02010
胞吞作用 Endocytosis	21 (10.99%)	884 (4.64%)	0.000 22	ko04144
植物-病原互作 Plant-pathogen interaction	29 (15.18%)	1 497 (7.85%)	0.000 453	ko04626
苯丙烷类生物合成 Phenylpropanoid biosynthesis	10 (5.24%)	296 (1.55%)	0.000 829	ko00940
剪接体 Spliceosome	20 (10.47%)	932 (4.89%)	0.001 109	ko03040
苯丙氨酸代谢 Phenylalanine metabolism	5 (2.62%)	110 (0.58%)	0.005 012	ko00360
角质、丝氨酸和蜡质生物合成 Cutin, suberine and wax biosynthesis	3 (1.57%)	58 (0.3%)	0.020 392	ko00073
植物激素信号转导 Plant hormone signal transduction	25 (13.09%)	1 693 (8.88%)	0.031 94	ko04075
泛醌和其他萜类醌生物合成 Ubiquinone and other terpenoid-quinone biosynthesis	4 (2.09%)	126 (0.66%)	0.038 013	ko00130

2.6 RNA-seq转录组的实时荧光定量PCR验证

为了验证转录组测序结果的可靠性,对选定的木质素生物合成通路中的10个处于重要节点上的差异表达基因进行实时荧光定量PCR验证。将qRT-PCR数据和转录组测序数据做相关性分析,其中有7个qRT-PCR结果与转录组测序结果显著相关,证明了转录组测序结果获得的基因表达信息是可靠的(表3)。

3 讨 论

本研究最先对纸皮核桃内果皮的发育历程中木质素沉积变化进行了初步的探究。在核桃内、中果皮交界面分布有大量维管组织,在果实基部发出在果实顶端汇集的主维管束通常较粗大,主维管束上有大量的次级维管束相互交织形成类似“橘络”的复杂维管网络。间苯三酚染色结果表明纸皮核桃木质素沉积出现在花后44 d(6月6日),该时期果实纵切染色显示除果实顶部内果皮区域染色鲜红明显外,在内果皮其他区域几乎无差别出现了较难辨识的粉红色,但在有维管组织的区域内果皮染色却比较明显。因此,推测纸皮核桃内果皮木质化可能在整个内果皮上几乎是同时发生的,并且内果皮木质化的

表3 转录组表达量与实时荧光定量PCR的相关性分析

Table 3 Correlation analysis of RNA-seq and qRT-PCR

木质素合成 Lignin biosynthesis related gene	回归方程 Regression equation	决定系数 Determination coefficient (R^2)	相关系数 Correlation coefficient (r)
ZJ-PAL	$y = 8.2716x + 1.8903$	$R^2 = 0.9591$	0.9793*
ZJ-C4H	$y = 25.828x + 35.629$	$R^2 = 0.9941$	0.9971**
ZJ-4CL	$y = 296.92x + 0.8512$	$R^2 = 0.9921$	0.9961**
ZJ-F5H	$y = 33.878x + 25.791$	$R^2 = 0.9972$	0.9986**
ZJ-CCR	$y = 259.2x + 147.64$	$R^2 = 0.9823$	0.9911**
ZJ-CAD	$y = 127.32x + 226.97$	$R^2 = 0.9006$	0.9490
ZJ-C3H	$y = 55.756x + 79.833$	$R^2 = 0.9902$	0.9951**
ZJ-LAC5	$y = 1628.6x - 16.419$	$R^2 = 0.9962$	0.9981**
ZJ-COMT	$y = -4.2478x + 61.426$	$R^2 = 0.13$	-0.3605
ZJ-POD	$y = -4902.9x + 1536.6$	$R^2 = 0.0443$	-0.2104

注:回归方程中y表示转录组,x表示qRT-PCR;*表示在 $p < 0.05$ 水平上显著,**表示在 $p < 0.01$ 水平上极显著相关。

Note: The y means transcriptional group and the x means qRT-PCR in the regression equation; * is significant at $p < 0.05$ level and ** indicates extremely significant correlation at $p < 0.01$ level.

程度与维管组织有一定联系,这与文菁等^[27]研究的硬壳木质化始于果顶端的结论不一致,具体原因还有待后续研究。推测可能的原因是文菁等^[27]6月2日的样本比本研究6月6日的样本的木质化过程早,因此仅能观察到果顶端出现染色,而6月16日的样

本由于采样时间间隔较长也未能观察到与本研究相同的现象。

为进一步深层次分析核桃内果皮硬化过程,我们对核桃内果皮硬化期样本进行了转录组测序,通过拼接、组装、聚类共获得 76 814 条 Unigene,其中 51、66 和 80 d 分别获得 74 630、72 810 和 74 561 条序列,平均长度 1 258 nt。张楠^[28]对‘香玲’核桃的脂肪积累期的胚组织进行转录组测序,通过数据拼接组装得到 44 503 条 Unigene,平均长度为 732 bp。本文获得的纸皮核桃内果皮硬化期转录组数据信息量与张楠测定的核桃脂肪积累期胚组织的转录组信息量相比,明显较大,说明此次的测序结果较好,较为可靠。我们使用 FPKM 法计算获得的 Unigene 表达量,并计算 Unigene 在不同样本间的差异表达倍数,分析得到 3 个时期差异表达基因 18 173 条,有 1 077 条在 3 个时期均差异表达。对 1 077 个 Unigene 进行基因表达模式分析,在建立的 16 个表达模型中确定了 3 个达到统计显著的基因表达模型。其中,1 号和 6 号图谱中的 595 个 Unigene 的表达呈现出“V”型,而 10 号图谱中的 14 个 Unigene 的表达与 1 号和 6 号图谱中 Unigene 表达恰好相反,呈现出倒“V”型,多次改变不同的建模参数,分析发现“V”型模型是纸皮核桃内果皮硬化期基因的主要表达模式,本团队研究发现纸皮核桃内果皮硬化期苯丙氨酸裂解酶和过氧化物酶活性变化也是呈“V”型^[21],同时进一步说明转录组测序数据的可信性。最后利用 KEGG 和 Gene Ontology 数据库对筛选出的 609 个差异表达基因进行功能注释及富集分析,发现了与本团队着重研究的木质素相关的苯丙氨酸代谢和苯丙烷类生物合成途径外,还获得了内质网蛋白质加工、ABC 转运因子、胞吞作用、植物激素信号转导、泛醌和其他萜类醌生物合成等几类重要途径,主要涉及生物代谢、生物调节、细胞发育、细胞通信、物质转运等主要生物学过程。

4 结 论

对纸皮核桃内果皮硬化期样品进行转录组测序,筛选出了内果皮硬化期差异表达的基因并进行了生物信息功能预测,发现候选基因参与内质网蛋白质加工、ABC 转运子合成、苯丙烷类生物合成、苯丙氨酸代谢、植物激素信号转导、泛醌和其他萜类醌生物合成等是核桃内果皮硬化进程中主要涉及的生

理生化过程。

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