

基于转录组测序的宁夏枸杞不同品种果实活性成分合成差异表达基因分析

刘雪霞¹, 范文强¹, 焦慧慧¹, 高寒¹, 唐建宁², 朱金忠³, 岳思君¹, 郑蕊^{1*}

1 宁夏大学生命科学学院 西部特色生物资源保护与利用教育部重点实验室 宁夏优势特色作物现代分子育种重点实验室, 宁夏 银川 750021

2 宁夏枸杞产业发展中心, 宁夏 银川 750021

3 中宁县杞鑫枸杞苗木专业合作社, 宁夏 中宁 755100

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摘 要: 为探究不同品种宁夏枸杞果实活性成分生物合成相关基因的表达水平, 筛选关键差异表达基因(differentially expressed genes, DEGs), 揭示宁夏枸杞品种间活性成分含量差异的分子机制, 本研究采用 Illumina NovaSeq 6000 高通量测序技术, 对宁夏枸杞‘宁杞1号’和‘宁杞7号’青果期、转色期及成熟期果实进行转录组测序, 比较2个品种果实不同发育期相关基因表达谱的变化。结果显示: 转录组测序共获得 811 818 178 条 clean reads, 有 121.76 Gb 有效数据。‘宁杞1号’和‘宁杞7号’在青果期、转色期和成熟期差异表达基因分别有 2 827、2 552 和 2 311 个; 分别有 2 153、2 050 和 1 825 个差异基因在基因本体论(gene ontology, GO)、京都基因与基因组百科全书(Kyoto encyclopedia of genes and genomes, KEGG)富集分析和同源蛋白簇(clusters of orthologous groups of proteins, KOG)分析等6个数据库中被成功注释。青果期、转色期和成熟期果实的差异表达基因, 在GO数据库分别有 1 307、865 和 624 个被富集到生物学过程、细胞组分及分子功能3个部分中; KEGG 通路富集结果均集中在代谢途径、次生代谢物生物合成和植物-病原互作过程; 在KOG数据库, 3个发育期分别注释了 1 775、1 751 和 1 541 个差异表达基因。对注释的基因进行PubMed数据库检索, 在青果期、转色期和成熟期分别筛选到与枸杞活性成分合成相关的差异表达基因 18、

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*Corresponding author. E-mail: xlzheng@126.com

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26 和 24 个, 这些基因主要参与类胡萝卜素、类黄酮、萜类、生物碱和维生素等代谢途径。选取 7 个差异表达基因进行 RT-qPCR 验证, 结果与转录组测序数据表达趋势一致。本研究从转录水平为不同品种宁夏枸杞活性成分含量差异提供了初步证据, 为进一步挖掘枸杞活性成分生物合成的关键基因及解析其表达调控机制提供了研究基础。

关键词: 宁夏枸杞; 果实发育; 转录组; 差异表达基因; 活性成分

Comparative analysis of differentially expressed genes for biosynthesis of active ingredients in fruits of different cultivars of *Lycium barbarum* L. based on transcriptome sequencing

LIU Xuexia¹, FAN Wenqiang¹, JIAO Huihui¹, GAO Han¹, TANG Jianning², ZHU Jinzhong³, YUE Sijun¹, ZHENG Rui^{1*}

¹ Key Laboratory of Modern Molecular Breeding for Dominant and Special Crops in Ningxia, Key Laboratory of Ministry of Education for Protection and Utilization of Special Biological Resources in Western China, College of Life Science, Ningxia University, Yinchuan 750021, Ningxia, China

² Wolfberry Industry Development Center, Yinchuan 750021, Ningxia, China

³ Qixin Wolfberry Seedling Professional Cooperatives of Zhongning County, Zhongning 755100, Ningxia, China

Abstract: To explore the differentially expressed genes (DEGs) related to biosynthesis of active ingredients in wolfberry fruits of different varieties of *Lycium barbarum* L. and reveal the molecular mechanism of the differences of active ingredients, we utilized Illumina NovaSeq 6000 high-throughput sequencing technology to conduct transcriptome sequencing on the fruits of ‘Ningqi No.1’ and ‘Ningqi No.7’ during the green fruit stage, color turning stage and maturity stage. Subsequently, we compared the profiles of related gene expression in the fruits of the two varieties at different development stages. The results showed that a total of 811 818 178 clean reads were obtained, resulting in 121.76 Gb of valid data. There were 2 827, 2 552 and 2 311 DEGs obtained during the green fruit stage, color turning stage and maturity stage of ‘Ningqi No. 1’ and ‘Ningqi No. 7’, respectively, among which 2 153, 2 050 and 1 825 genes were annotated in six databases, including gene ontology (GO), Kyoto encyclopedia of genes and genomes (KEGG) and clusters of orthologous groups of proteins (KOG). In GO database, 1 307, 865 and 624 DEGs of green fruit stage, color turning stage and maturity stage were found to be enriched in biological processes, cell components and molecular functions, respectively. In the KEGG database, the DEGs at three developmental stages were mainly concentrated in metabolic pathways, biosynthesis of secondary metabolites and plant-pathogen interaction. In KOG database, 1 775, 1 751 and 1 541 DEGs were annotated at three developmental stages, respectively. Searching the annotated genes against the PubMed database revealed 18, 26 and 24 DEGs related to the synthesis of active ingredients were mined at the green fruit stage, color turning stage and maturity stage, respectively. These genes are involved in carotenoid, flavonoid, terpenoid, alkaloid, vitamin metabolic pathways, etc. Seven DEGs were verified by

RT-qPCR, which showed consistent results with transcriptome sequencing. This study provides preliminary evidences for the differences in the content of active ingredients in different *Lycium barbarum* L. varieties from the transcriptional level. These evidences may facilitate further exploring the key genes for active ingredients biosynthesis in *Lycium barbarum* L. and analyzing their expression regulation mechanism.

Keywords: *Lycium barbarum* L.; fruit development; transcriptome; differentially expressed genes; active ingredients

宁夏枸杞(*Lycium barbarum* L.)是茄科(Solanaceae)枸杞属(*Lycium* L.)多年生灌木,广泛种植于我国西北地区,是一种具有良好保健功效的药食同源功能性特色植物资源,被《中国药典》收录为药用枸杞^[1]。宁夏枸杞果实作为一种名贵中药材,含有多种生物活性物质,如多糖、黄酮、类胡萝卜素、生物碱、花青素、VC、甜菜碱等^[1-2],具有滋肝补肾、益精明目、增强免疫力、抗衰老、抗氧化、抗疲劳、降血糖血脂、保护心血管等多种功效^[1-2],开展枸杞果实活性成分相关研究,对于合理有效利用这一宝贵资源具有重要意义。

宁夏枸杞品种多,其果实品质参差不齐。‘宁杞1号’枸杞是宁夏农林科学院选育的高产、优质、适应性强的枸杞品种,为宁夏乃至周边省区多年主栽品种。‘宁杞7号’为‘宁杞1号’园中发现的变异优株,综合经济性状优良^[3]。2个枸杞品种不仅在果形、重量、大小及质地等表型上存有差异^[4](图1),其风味品质和营养成分也不尽相同。据报道,‘宁杞7号’多糖、甜菜碱和还原型维C含量分别为0.078 4 mg/g、0.011 2 g/g和9.494 2 mg/g,比‘宁杞1号’分别增加了约6.8%、8.7%和10.6%;类胡萝卜素和总糖含量分别为0.403 3 mg/g和0.097 2 g/g,比‘宁杞1号’分别减少了约5.0%和10.2%^[4],这些差异可能与果实活性成分合成相关基因的差异性表达有关。

转录组学是在整体水平上研究基因表达及

转录调控规律,是研究细胞表型和基因功能关系的有效手段。对于缺乏基因组信息的药用植物开展基因功能相关研究,通过转录组测序及生物信息学分析,可挖掘相关的功能基因,进而揭示特定生物学过程的分子机制。高通量测序技术因其具有数据量大、快速便捷、准确性好等优点,近年来被广泛应用于发掘和鉴定药用植物在生长发育及次生代谢产物生物合成方面的相关功能基因。包雪梅等^[5]比较黑果枸杞和白果枸杞转录组,筛选到调控黑果枸杞花青素合成代谢的候选基因 *CL8159.Contig5_All*。Wang等^[6]通过转录组分析,鉴定了黑枸杞果实发育过程中差异表达的38个MYB转录因子的编码基因,其中 *LrMYB1* 通过表达激活黄酮合成相关结构基因,并提高了黄酮含量,表明RNA-seq技术在识别植物发育、品质形成等表型性状相关候选基因等相关研究方面的重要性。

果实成熟过程是果实品质与功能形成的基础,是由一系列相互作用的基因和信号通路协同作用的复杂的发育过程,伴随着果实中糖类、氨基酸、脂肪酸及黄酮、类胡萝卜素、生物碱等次生代谢物、营养物质等的变化。果实发育过程受遗传、基因表达和环境等因素的影响,这是果实品质存在差异性的原因。枸杞果实品质有外观品质和内在品质,外观品质的评价指标主要是果实大小、色泽等,内在品质的评价指标主要有多糖、黄酮、甜菜碱和类胡萝卜素

等的含量。目前对不同种质枸杞质量评价的报道较多^[7],但从转录水平上解析宁夏枸杞不同品种活性成分存在差异的研究较少,而这部分基因表达的信息对于研究枸杞遗传进化的分子机制以及枸杞品种的进一步改良具有重要指导意义。此外,枸杞活性物质基础研究薄弱,活性成分的分子结构、生物功效与药理作用机制尚不明确,使得我国枸杞产业发展滞后,制约农民增收、产业增效,使枸杞加工业远远落后于世界发达国家的药食两用资源加工业的发展水平,因此,枸杞活性成分基础研究是促进其产业发展的关键。本研究通过高通量测序技术,对宁夏枸杞品种‘宁杞 1 号’和‘宁杞 7 号’青果期、转色期及成熟期 3 个不同发育期果实进行转录组测序,挖掘活性成分合成相关差异表达基因(differentially expressed genes, DEGs),解

析‘宁杞 1 号’和‘宁杞 7 号’两个枸杞品种果实在成熟过程中活性成分存在差异的分子基础,为枸杞果实品质的形成及优良株系选育提供基础数据。

1 材料与方法

1.1 实验材料

本研究选用宁夏枸杞‘宁杞 1 号’和‘宁杞 7 号’不同发育期果实为研究材料,于 2021 年 6–7 月采自中宁县杞鑫枸杞资源圃(105.723 569°E, 37.535 386°N)。采用对角线取样法,分别采摘无病害、长势良好的‘宁杞 1 号’和‘宁杞 7 号’青果期、转色期及成熟期枸杞果实(图 1)。每个时期样品取 3 个生物学重复,样品及其编号信息见表 1。果实采摘后立即置于液氮中带回,–80 °C 冰箱保存备用。

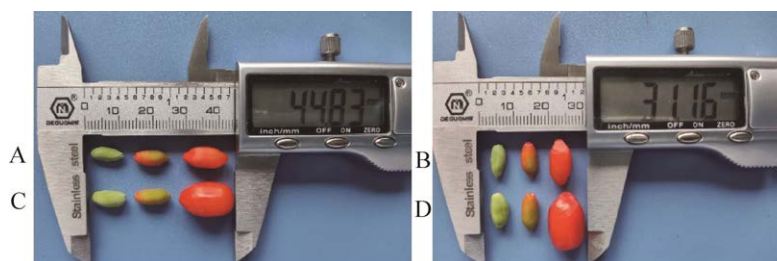


图 1 不同发育时期枸杞果实 A 和 B 从左到右依次为 YA、ZA 和 XA; C 和 D 从左到右依次为 YC、ZC 和 XC

Figure 1 Fruit morphology of *Lycium barbarum* L. in different developmental stages. A and B: From left to right, it's YA, ZA and XA. C and D: From left to right, it's YC, ZC and XC.

表 1 样品编号

Table 1 Sample number

Varieties of <i>Lycium barbarum</i> L.	Developmental stages	Sample number	Duplicate group number
‘Ningqi No. 1’	Green fruit stage	YA	N1-1, N1-2, N1-3
	Color turning stage	ZA	N1-4, N1-5, N1-6
	Maturity stage	XA	N1-7, N1-8, N1-9
‘Ningqi No. 7’	Green fruit stage	YC	N7-1, N7-2, N7-3
	Color turning stage	ZC	N7-4, N7-5, N7-6
	Maturity stage	XC	N7-7, N7-8, N7-9

1.2 方法

1.2.1 枸杞果实总 RNA 的提取及转录组测序分析

将枸杞果实样品干冰条件下送至武汉迈维代谢生物公司进行总 RNA 的提取和转录组测序。提取的总 RNA, 检测其完整性、浓度和纯度, 以保证 RNA 符合建库质量要求。待 cDNA 文库构建以及库检合格后, 通过 Illumina NovaSeq 测序平台进行测序, 将获得的原始测序数据(raw data)过滤, 去除低质量序列(adapter reads、未知碱基 N 含量大于 10% 的 reads), 获得高质量的 clean data。利用 HISAT2 2.1.0^[8]软件将每个样品的 clean reads 与枸杞参考基因组(尚未公布)进行序列比对, 获取在参考基因组或基因上的位置信息, 以及测序样品特有的序列特征信息。根据比对上基因组的 reads 的位置信息, 使用 StringTie 1.3.4d^[9]将 reads 组装成转录本(transcripts)。

1.2.2 差异表达基因(differentially expressed genes, DEGs)分析与功能注释

以‘宁杞 1 号’3 个发育时期的枸杞果实为对照, 利用高通量测序技术对‘宁杞 1 号’和‘宁杞 7 号’3 个发育时期果实的转录组数据进行比较分析。使用 DESeq2 1.22.1^[10-11]软件分析差异表达基因, 用每千个碱基的转录每百万映射读取的片段数(fragments per kilobase of transcript per million fragments mapped, FPKM)值表示基因的

表达量, 以 $|\log_2 \text{fold change}| \geq 1.5$, 且 $\text{FDR} < 0.05$ 作为筛选标准, 满足此筛选标准的基因即为 DEGs。将筛选到的 DEGs 分别在 KEGG、GO、KOG、NR、Swiss-Prot 和 Pfam 数据库进行比对, 获得与 DEGs 对应的蛋白功能注释信息。进而对 DEGs 进行 KOG 分类、GO 功能富集分析和 KEGG 代谢通路富集分析。

1.2.3 实时荧光定量 PCR 分析

以枸杞组成型表达基因 *LbActin* 为内参 (GenBank 登录号 HQ415754.1), 选取筛选到的参与类胡萝卜素代谢途径的 2 个 DEGs (marker00046333、marker00029155)、参与花青素代谢途径的 3 个 DEGs (marker00005595、marker00027109、marker00027724) 和 2 个差异表达的转录因子基因 (marker00040298、marker00008029) 进行 RT-qPCR 验证, 设计 RT-qPCR 特异引物(表 2), DEGs 的相对表达量采用 $2^{-\Delta\Delta C_t}$ 法计算, 实验设 3 次生物学重复, 使用 Origin 2022 作图。

2 结果与分析

2.1 转录组测序数据质量分析

‘宁杞 1 号’和‘宁杞 7 号’3 个发育时期的果实进行转录组测序, 共获得 811 818 178 条 clean reads, 有 121.76 Gb 的有效数据, GC 含量为

表 2 实时荧光定量 PCR 引物序列

Table 2 Primers for real-time fluorescence quantitative PCR

Gene	Forward primer sequence (5'→3')	Reverse primer sequence (5'→3')
marker00046333	TCCTGATGCTGATGAAGATGATGGC	GCACACGATGAGGCAATTTGACAG
marker00029155	GGAAGTGAAGTCTTTGGAGGAACCC	GTAGGCGAGAGTCAAGCATTCAAGG
marker00005595	TGGCACTGTTCTTGCTGCTTCAC	AGGGAAGTGGACATTGAGAGGAGTC
marker00027109	CGGCTCGTTATGGAATCTGACCTG	CGTTGATCTCACAGCTCTCGGATG
marker00027724	CTTTCTTTGAGAGCCTGAGGGTGTC	TCTGAAGTCTGGAATTGGCTGGTTG
marker00040298	CTTTCAAGATTGCTGCGTGGGATTC	GCCTCGGTCTGAATTGTGTAGAGTG
marker00008029	TCTCAGTGACAGCGTTAGGAAGG	TCACAGTATCCTCCCTCAGCTTCG
<i>LbActin</i>	CTTCCAGCCATCCATGATCGGTATG	AGCCACCACTGAGCACAATGTTAC

40.42%–43.59%；各样品 Q20>95.52%；Q30>89.92%，测序数据质量较高。样品过滤数据与枸杞参考基因组序列对比，除 N7-9 样品比对效率为 70.49%外，其余样品比对效率均在 88.64%及以上，实验数据可进行后续分析。

2.2 DEGs 分析

青果期(YA vs. YC)筛选到 2 827 个 DEGs，在‘宁杞 7 号’有 1 372 个上调表达，1 455 个下调表达；转色期(ZA vs. ZC)有 2 552 个 DEGs，上调表达 1 666 个，下调表达 886 个；成熟期(XA vs. XC)有 1 416 个 DEGs 上调，895 个下调。

表 3 枸杞果实 DEGs 注释

Table 3 Annotation of DEGs in wolfberry fruits

Stage of fruit development	DEGs annotated in each database						Total number of DEGs annotated
	KEGG	GO	NR	Swiss-Prot	Pfam	KOG	
Green fruit stage	1 508	1 766	2 142	1 526	1 712	1 775	2 153
Color turning stage	1 476	1 697	2 044	1 520	1 674	1 751	2 050
Maturity stage	1 363	1 518	1 817	1 308	1 478	1 541	1 825

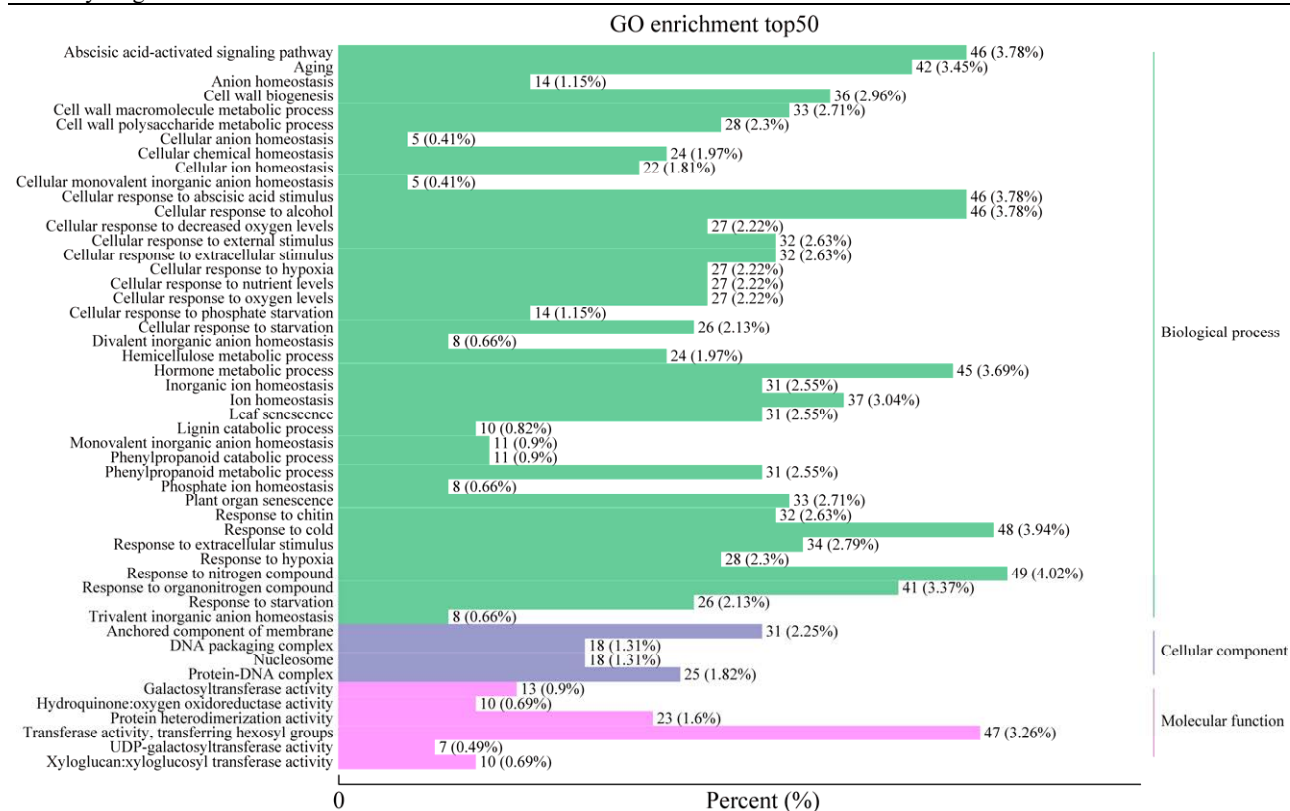


图 2 枸杞果实青果期 DEGs 的 GO 富集

Figure 2 GO enrichment of DEGs at green fruit stage of wolfberry.

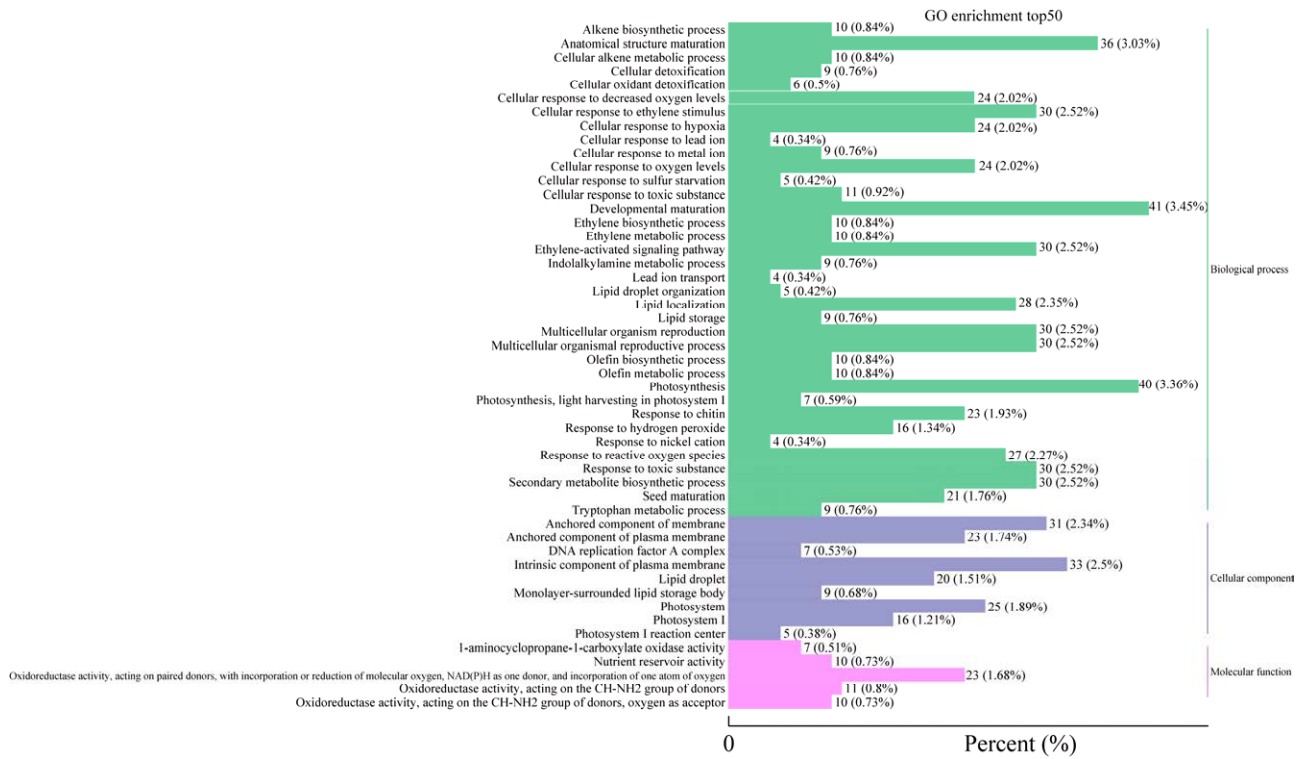


图3 枸杞果实转色期 DEGs 的 GO 富集

Figure 3 GO enrichment of DEGs at color turning stage of wolfberry.

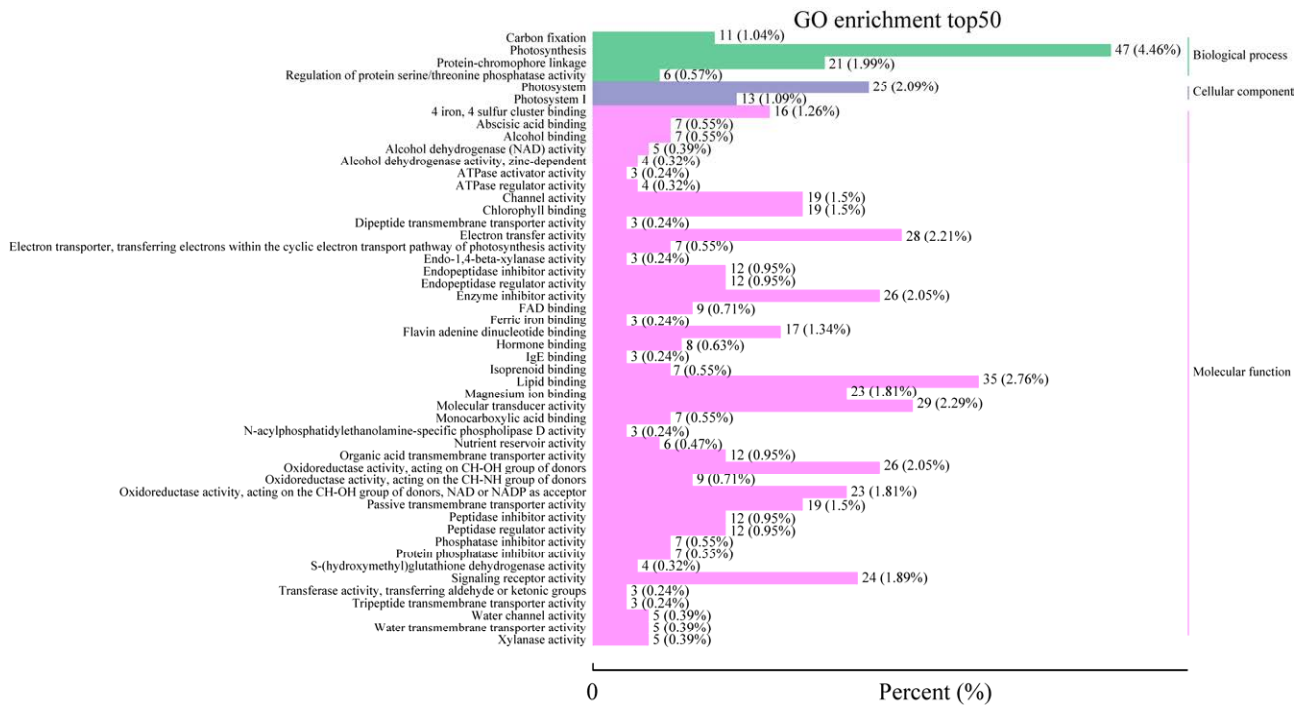


图4 枸杞果实成熟期 DEGs 的 GO 富集

Figure 4 GO enrichment of DEGs at maturity stage of wolfberry.

在 BP 中, 青果期、转色期和成熟期分别富集了 1 105、635、85 个 DEGs, 其中 YA vs. YC 富集最多的是对氮化合物的响应(response to nitrogen compound, 49)和对寒冷的反应(response to cold, 48); ZA vs. ZC 在发育成熟(developmental maturation, 41)和光合作用(photosynthesis, 40)中所占比例最高; XA vs. XC 主要富集在光合作用(photosynthesis, 47)中。在 CC, 青果期、转色期和成熟期富集到的 DEGs 分别有 92、169 和 38 个, 其中 YA vs. YC 富集最多的是膜锚定组件(anchored component of membrane, 31); ZA vs. ZC 在质膜的固有成分(intrinsic component of plasma membrane, 33)中所占比重最大; XA vs. XC 仅富集了光系统(photosystem, 25)和光系统 1 (photosystem 1, 13)。在 MF 中, 青果期、转色期和成熟期分别富集到 DEGs 有 110、61

和 501 个, 其中 YA vs. YC 主要富集在转移酶活性, 转移己糖基(transferase activity, transferring hexosyl groups, 47)中; ZA vs. ZC 和 XA vs. XC 分别在氧化还原酶活性, 作用于配对供体, 结合或还原分子氧, NAD(P)H 作为一个供体, 并结合一个氧原子(oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, NAD(P)H as one donor, and incorporation of one atom of oxygen, 23)和脂质结合(lipid binding, 35)中富集最多。

2.2.3 DEGs 的 KEGG 注释和富集分析

KEGG 可将基因组信息和其功能信息有机地结合起来, 能够对各基因产物在细胞内的代谢途径进行系统分析, 进而综合分析这些基因产物的功能。KEGG 代谢通路分析发现(图 5、图 6、图 7), ‘宁杞 1 号’和‘宁杞 7 号’

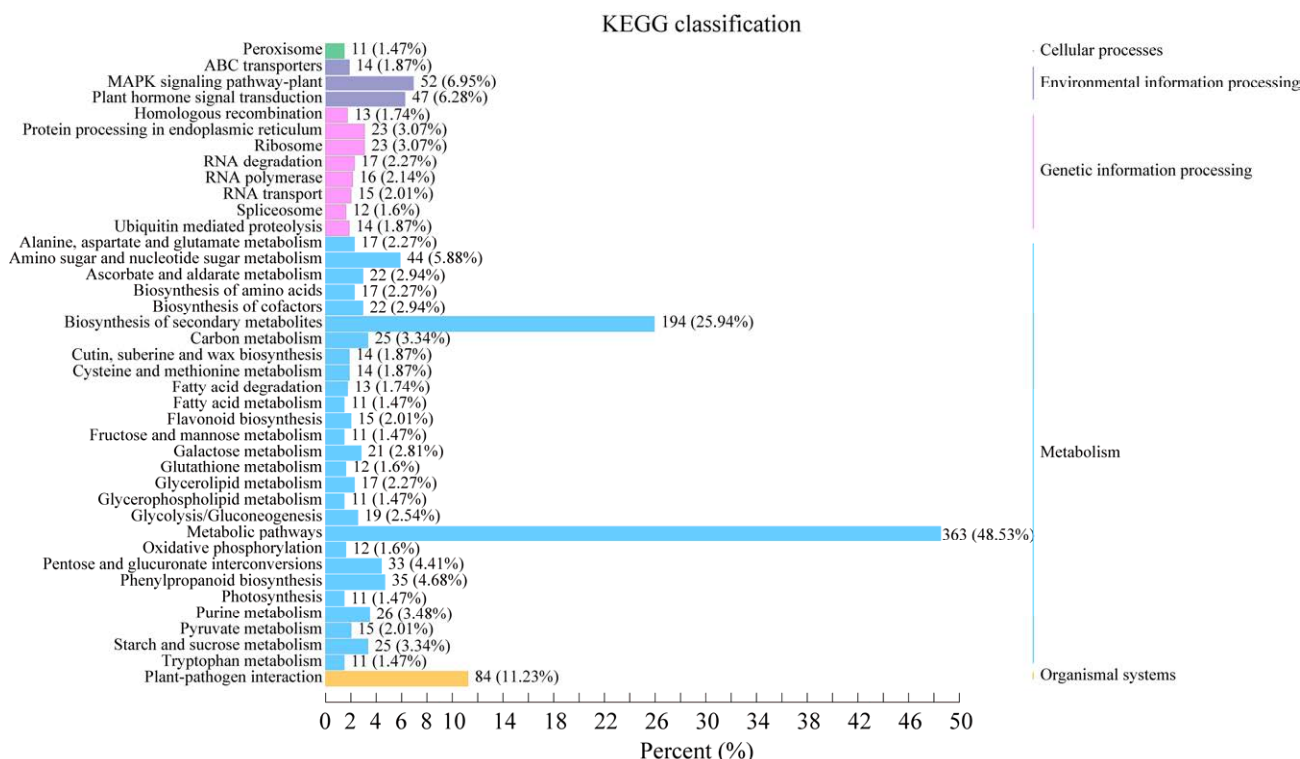


图 5 枸杞果实青果期 DEGs 的 KEGG 分类

Figure 5 KEGG classification of DEGs at green fruit stage of wolfberry.

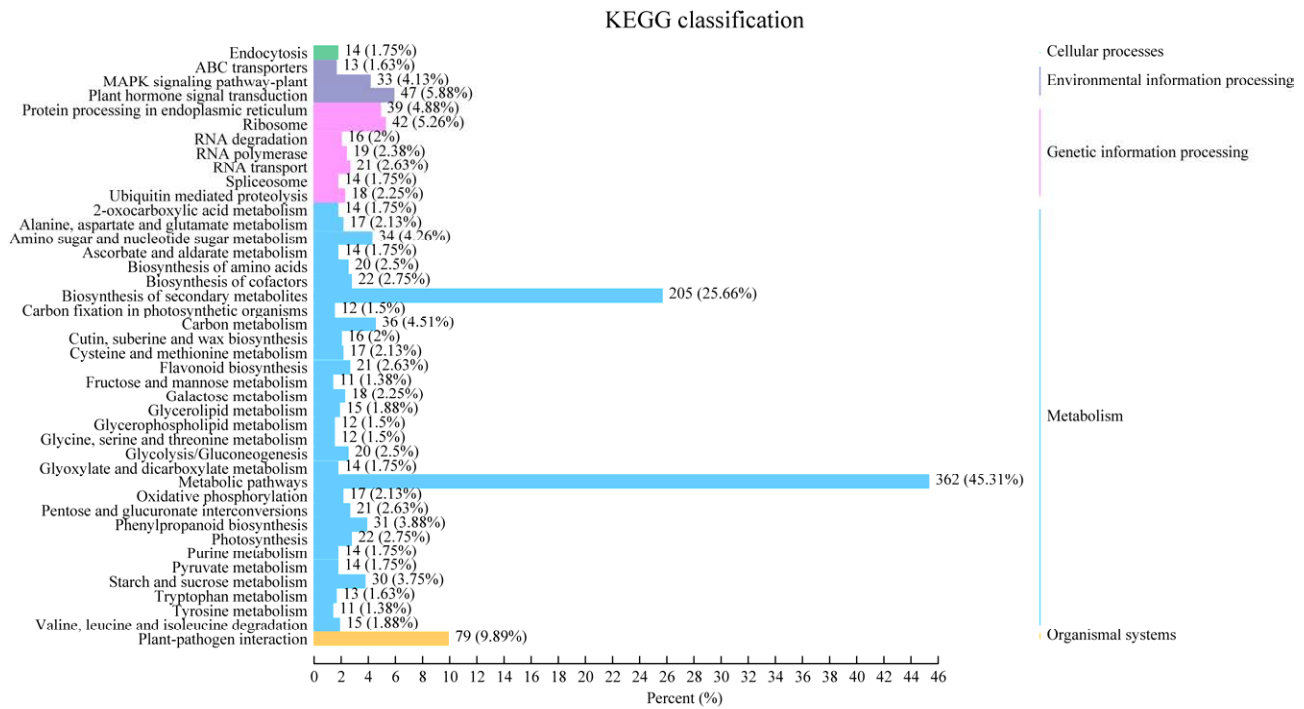


图 6 枸杞果实转色期 DEGs 的 KEGG 分类

Figure 6 KEGG classification of DEGs at color turning stage of wolfberry.

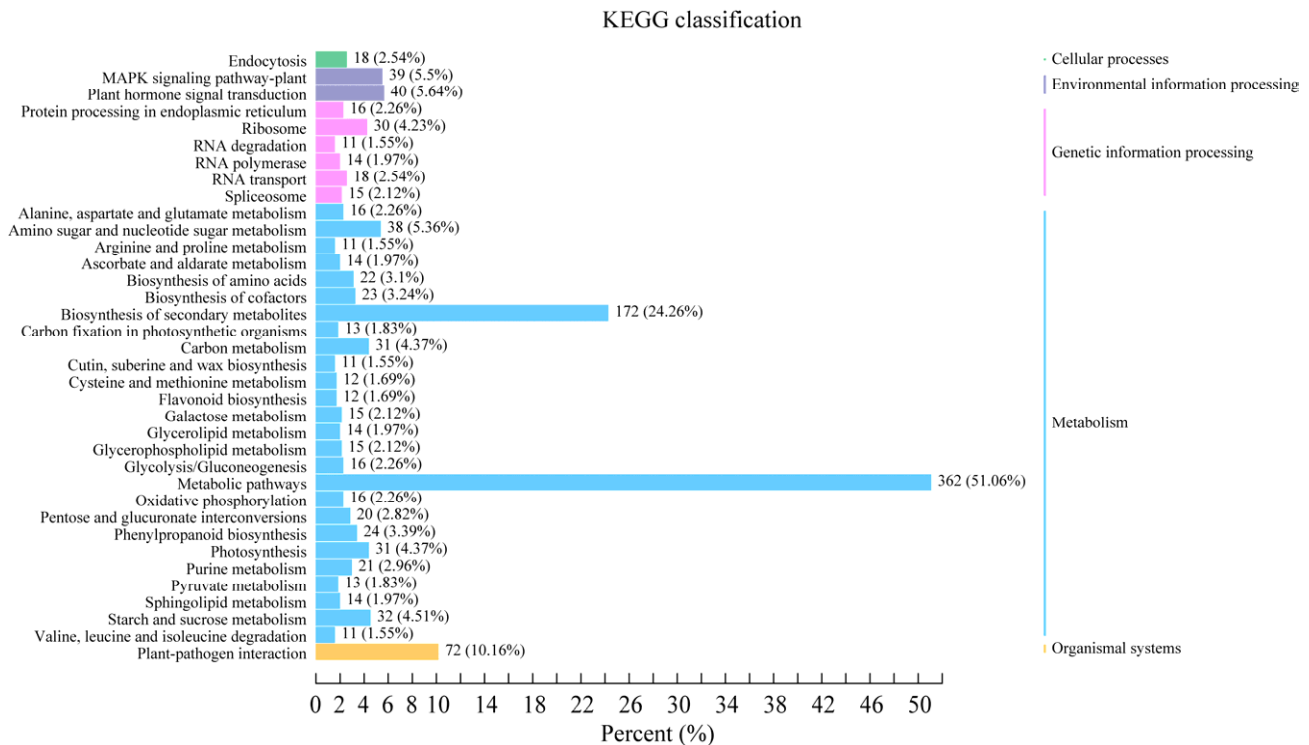


图 7 枸杞果实成熟期 DEGs 的 KEGG 分类

Figure 7 KEGG classification of DEGs at maturity stage of wolfberry.

的青果期有 1 371 个 DEGs 参与 5 大类 40 条代谢通路, 转色期有 1 435 个 DEGs 参与 42 条代谢通路, 成熟期有 1 252 个 DEGs 参与 36 条代谢通路。其中, YA vs. YC、ZA vs. ZC 和 XA vs. XC 均在代谢途径(metabolic pathways)富集的 DEGs 数最多, 分别有 363、362 和 362 个; 其次是次生代谢物生物合成(biosynthesis of secondary metabolites), 分别有 194、205 和 172 个; 植物-病原互作(plant-pathogen interaction)富集到的 DEGs 次之, YA vs. YC、ZA vs. ZC 和 XA vs. XC 分别有 84、79 和 72 个。

对 3 个发育时期的 DEGs 进行 KEGG 功能富集, 分别筛选出显著性富集的 20 条代谢通路(图 8、图 9、图 10), 青果期、转色期和成熟期

的 DEGs 分别在牛磺酸和亚牛磺酸代谢(taurine and hypotaurine metabolism)、甜菜红色素生物合成(betalain biosynthesis)和 C5-支链二元酸代谢(C5-branched dibasic acid metabolism)中富集程度最大。此外, YA vs. YC 和 ZA vs. ZC 分别在牛磺酸和亚牛磺酸代谢(taurine and hypotaurine metabolism)和甜菜红色素生物合成(betalain biosynthesis)中 DEGs 富集水平最显著; XA vs. XC 的 DEGs 富集水平最显著的则在代谢途径(metabolic pathways)以及光合作用(photosynthesis)。YA vs. YC 和 XA vs. XC 的 DEGs 数目均在代谢途径中富集最多, 而 ZA vs. ZC 的 DEGs 富集数目最多的则在次生代谢物生物合成(biosynthesis of secondary metabolites)中。

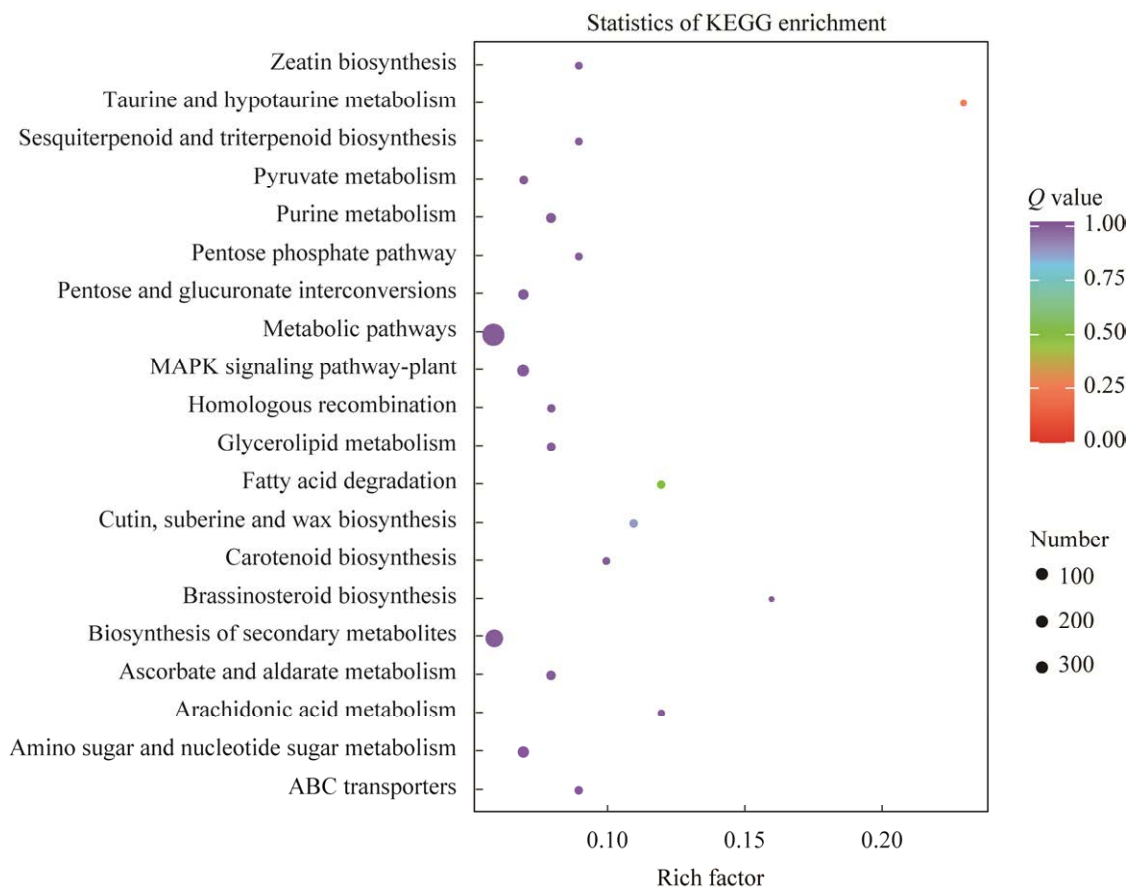


图 8 枸杞果实青果期 DEGs 的 KEGG 富集

Figure 8 KEGG enrichment of DEGs at green fruit stage of wolfberry.

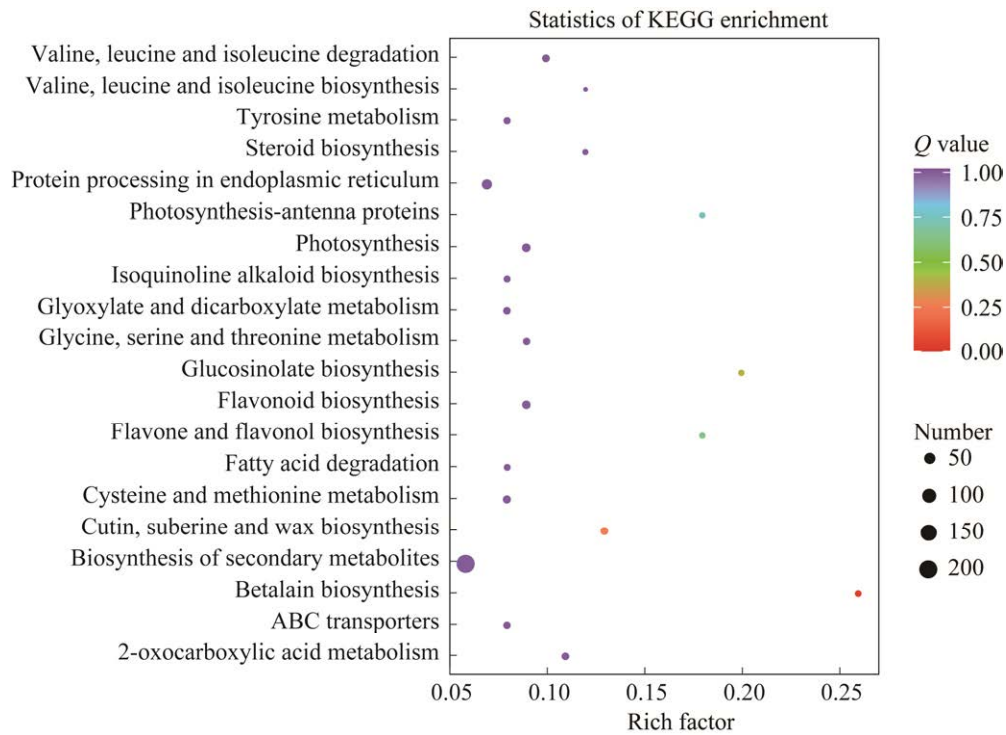


图9 枸杞果实转色期 DEGs 的 KEGG 富集

Figure 9 KEGG enrichment of DEGs at color turning stage of wolfberry.

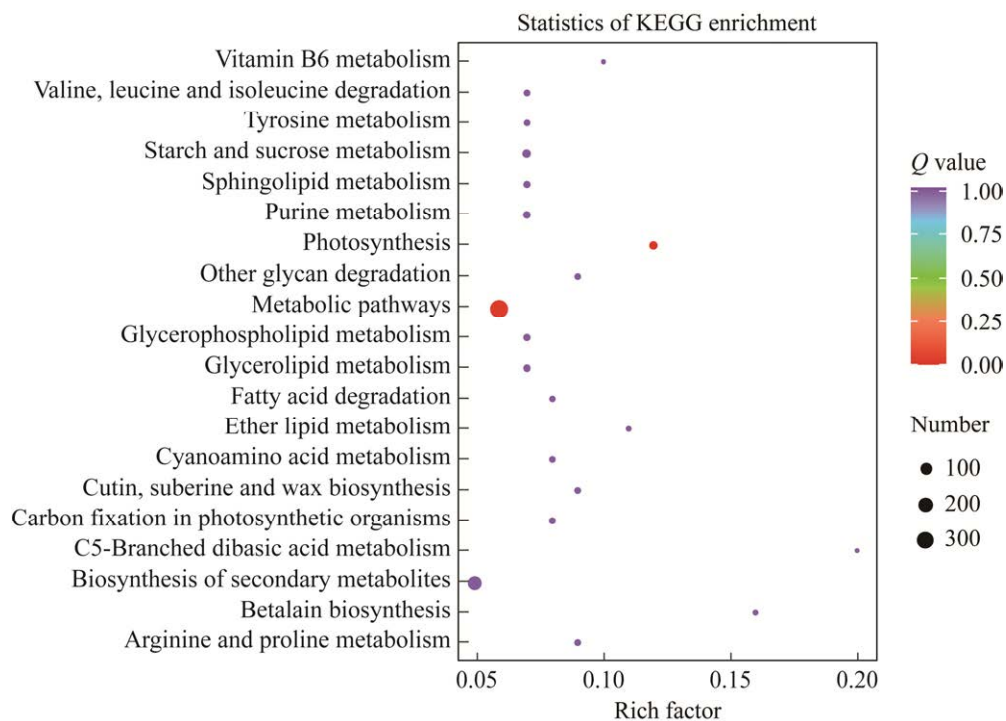


图10 枸杞果实成熟期 DEGs 的 KEGG 富集

Figure 10 KEGG enrichment of DEGs at maturity stage of wolfberry.

2.2.4 DEGs 的 KOG 注释及功能分类

根据 KOG 注释结果,统计了每个 KOG 功能分类所包含的 DEGs 数量, YA vs. YC、ZA vs. ZC 和 XA vs. XC 的 DEGs 均参与 24 类代谢途径(图 11、图 12、图 13)。其中 YA vs. YC 的 DEGs 在仅一般功能预测(general function prediction only)中注释数目最多,有 324 个;信号转导机制(signal transduction mechanisms) DEGs 注释数目次之,有 104 个。ZA vs. ZC 和 XA vs. XC 的 DEGs 均在仅一般功能预测(general function prediction only)和翻译后修饰、蛋白质流转及分子伴侣(posttranslational modification, protein turnover, chaperones)中得到较多注释,其中, ZA vs. ZC 和 XA vs. XC 的 DEGs 在仅一般功能预测中分别注释 350 个和 319 个,其次在翻译后修饰、蛋白质流转及分子伴侣中各注释了 112 个和 72 个;且 YA vs. YC、ZA vs. ZC

和 XA vs. XC 的 DEGs 均在核结构(nuclear structure)中注释最少,分别注释了 1、1 和 2 个。

2.3 ‘宁杞 1 号’和‘宁杞 7 号’果实活性成分合成相关基因挖掘

宁夏枸杞作为天然无毒的药食两用中药材,其中含有黄酮、类胡萝卜素、花色苷、生物碱等多种活性成分,在医疗保健方面占有重要地位,受到人们的广泛关注。结合 NR 数据库注释信息和 PubMed 数据库文献,分别从‘宁杞 1 号’和‘宁杞 7 号’青果期、转色期和成熟期的果实转录组数据筛选到的 2 827、2 552、2 311 个 DEGs 中,筛选到青果期(YA vs. YC)活性成分合成相关的 18 个 DEGs,转色期(ZA vs. ZC) 26 个,成熟期(XA vs. XC) 24 个,分别参与类胡萝卜素、类黄酮、萜类、生物碱、和维生素等代谢途径。其中 YA vs. YC 有 15 个与活性成

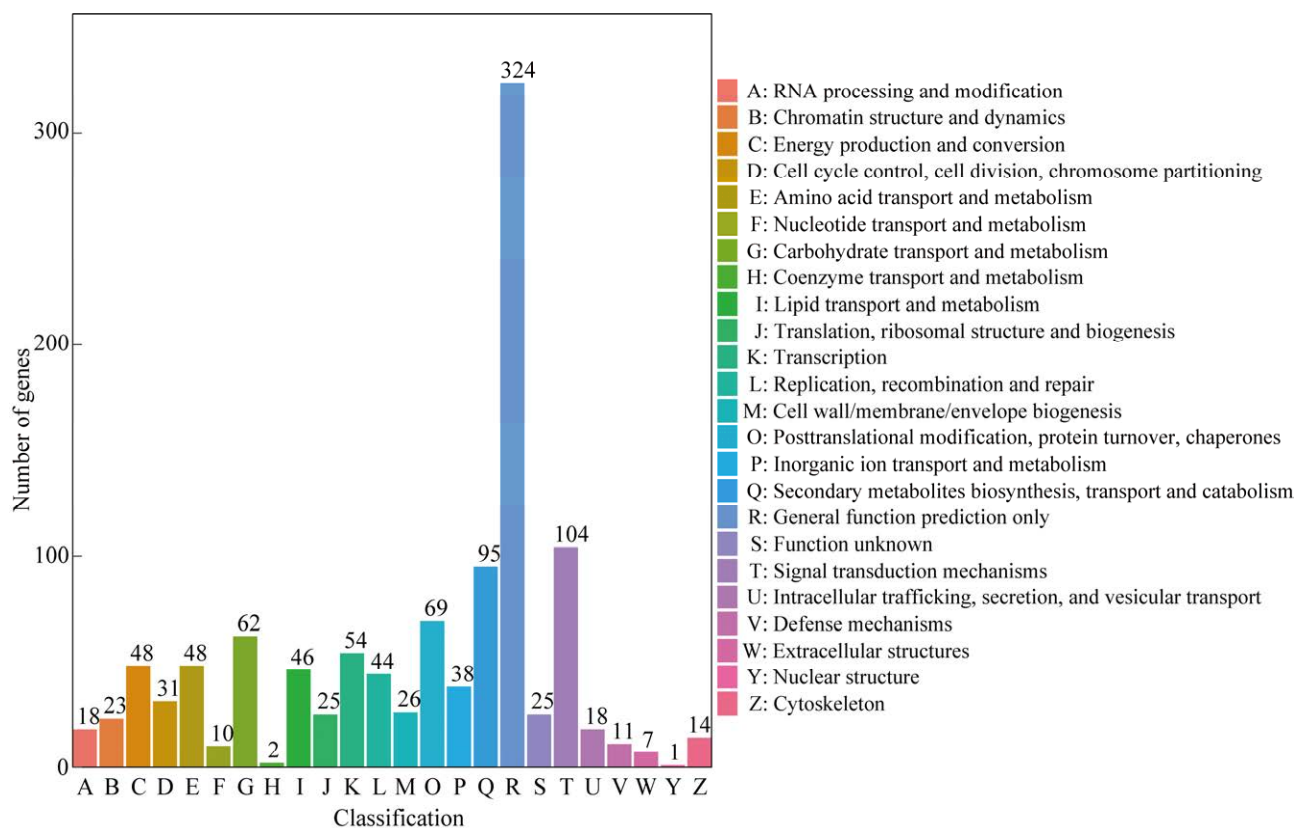


图 11 枸杞果实青果期 DEGs 的 KOG 分类

Figure 11 Histogram of KOG classification of DEGs at green fruit stage of wolfberry.

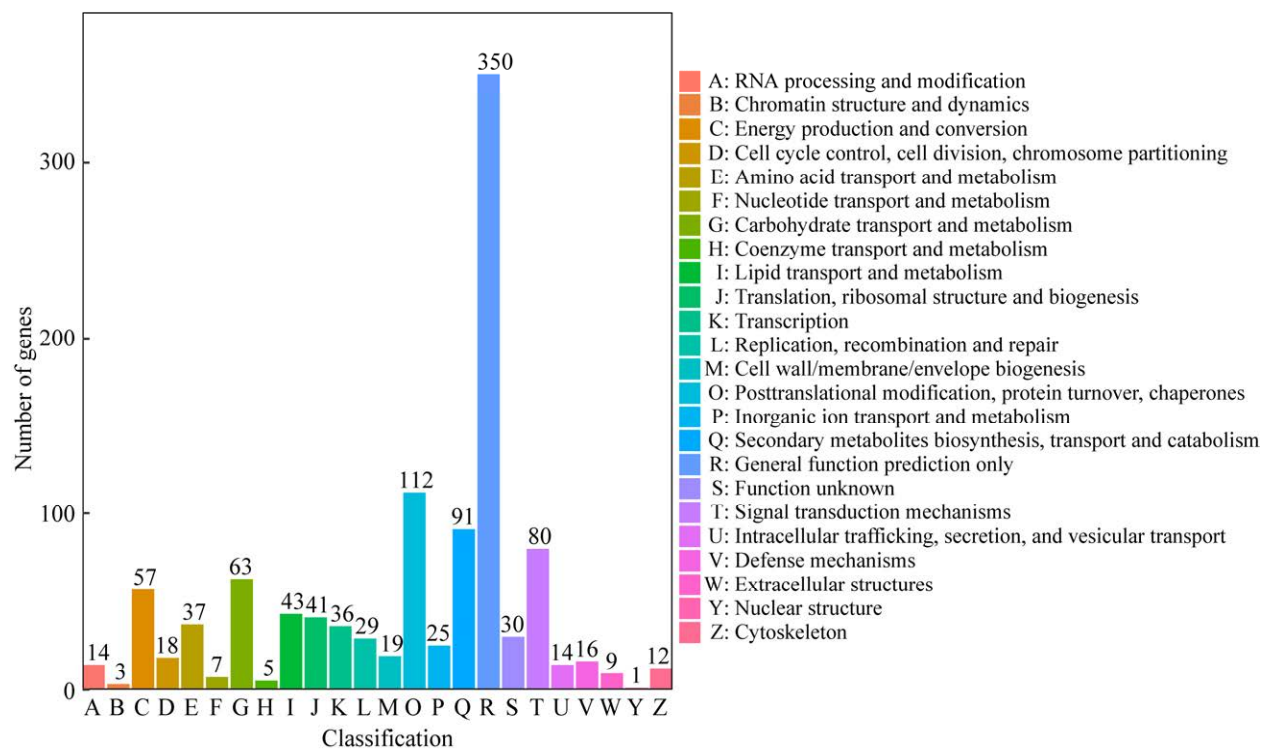


图 12 枸杞果实转色期 DEGs 的 KOG 分类
Figure 12 Histogram of KOG classification of DEGs at color turning stage of wolfberry.

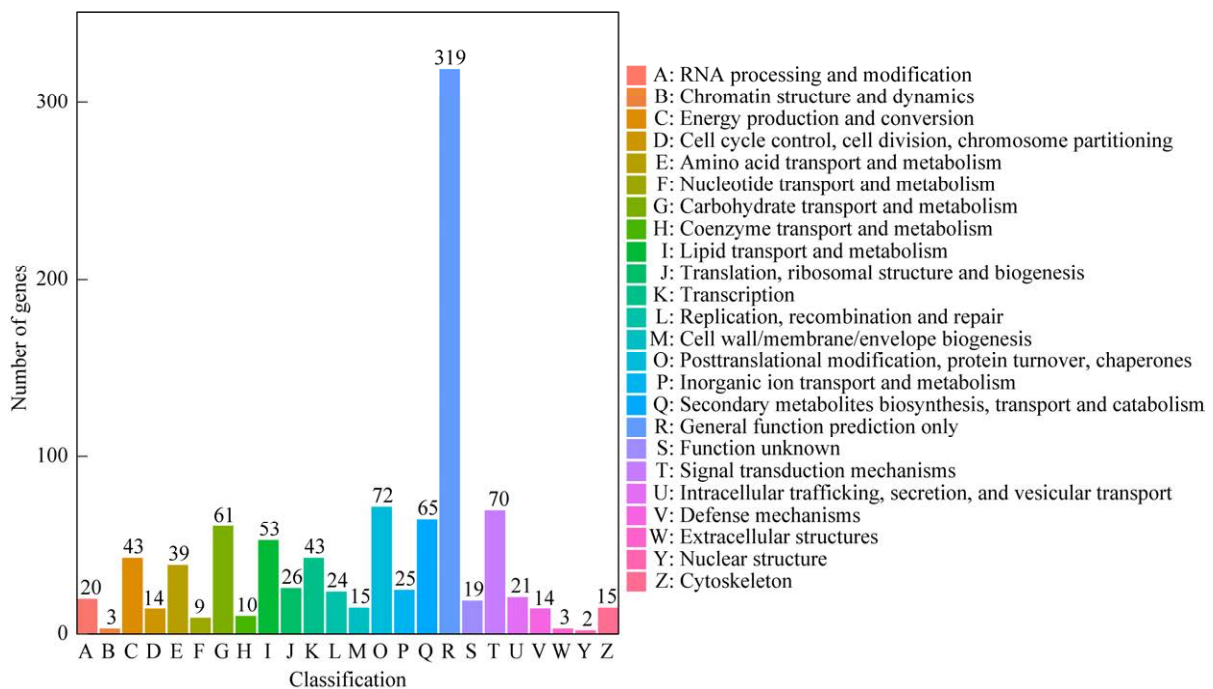


图 13 枸杞果实成熟期 DEGs 的 KOG 分类
Figure 13 Histogram of KOG classification of DEGs at maturity stage of wolfberry.

分合成相关的 DEGs 在‘宁杞 7 号’中下调表达, DEGs 分别有 13 个和 14 个, 上调 DEGs 各有 3 个上调表达; ZA vs. ZC 和 XA vs. XC, 下调 13 和 10 个(表 4)。

表 4 果实活性成分合成相关 DEGs 及其功能注释

Table 4 DEGs related to the synthesis of active ingredients and functional annotations

Development stage	Gene ID	NR database annotation	Gene function	log ₂ (fold change)
Green fruit stage	marker00012475	Phytoene synthase 1	Carotenoid biosynthesis	-1.952
	marker00035118	PREDICTED: phytoene synthase2, chloroplastic-like	Carotenoid biosynthesis	-1.916
	marker00025871	15-cis- ζ -carotene isomerase	Carotenoid biosynthesis	-1.575
	marker00027654	Carotenoid beta-ring hydroxylase 2	Functional lutein biosynthesis	-1.573
	marker00005595	PREDICTED: anthocyanidin reductase-like	Proanthocyanidins (PA) biosynthesis	1.762
	marker00034314	Anthocyanidin synthase	Anthocyanin biosynthesis	-2.966
	novel.16929	PREDICTED: laccase-14-like	Relate to the degradation of anthocyanins	-2.541
	marker00016307	Chalcone synthase, partial	A key enzyme in the flavonoid pathway	-1.954
	marker00022688	Chalcone synthase, partial	A key enzyme in the flavonoid pathway	-3.724
	marker00027109	Flavonoid 3'-hydroxylase	Involve in the flavonoid pathway	-2.250
	marker00038026	Flavonoid-3',5'-hydroxylase	Involve in the flavonoid pathway	-2.385
	marker00018617	3-hydroxy-3-methylglutaryl coenzyme A synthase	Terpenoid biosynthesis	-1.548
	novel.21847	Terpene synthase	Terpene biosynthesis	-8.197
	marker00044724	Glycosyltransferase	Ginsenoside biosynthesis	-1.597
	marker00040598	PREDICTED: beta-amylase	Carbohydrate metabolism	1.879
	marker00045838	PREDICTED: laccase-4-like	Relate to lignin biosynthesis	-4.276
	marker00028451	PREDICTED: laccase-4-like	Relate to lignin biosynthesis	3.666
Color turning stage	marker00012475	Phytoene synthase 1	Carotenoid biosynthesis	-1.552
	marker00048068	Phytoene desaturase	Carotenoid biosynthesis	-1.566
	marker00005595	PREDICTED: anthocyanidin reductase-like	Proanthocyanidins (PA) biosynthesis	2.603
	marker00012448	PREDICTED: anthocyanidin reductase	Proanthocyanidins (PA) biosynthesis	2.516
	marker00021870	PREDICTED: flavonol Synthase/flavanone 3-hydroxylase-like	A key enzyme for flavonol synthesis	-1.507
	marker00034314	Anthocyanidin synthase	Key step of anthocyanin biosynthesis	2.910
	marker00015647	Caffeoyl-CoA O-methyltransferase	Polymethoxylated flavones biosynthesis	3.047
	marker00016307	Chalcone synthase, partial	A key enzyme in the flavonoid pathway	1.570
	marker00022688	Chalcone synthase, partial	A key enzyme in the flavonoid pathway	3.437
	marker00022157	Putative transcription factor KAN4	Regulate the seed flavonoid biosynthesis	-1.612
	marker00027109	Flavonoid 3'-hydroxylase	Involve in the flavonoid pathway	-3.247
	marker00038026	Flavonoid-3',5'-hydroxylase	Involve in the flavonoid pathway	5.319
	novel.3707	PREDICTED: 2-hydroxyisoflavanone dehydratase-like	Isoflavone biosynthesis	2.537
	marker00044724	Glycosyltransferase	Ginsenoside biosynthesis	-1.724
	marker00014229	Glycosyltransferase	Ginsenoside biosynthesis	-7.018
	novel.21847	Terpene synthase	Terpene biosynthesis	-8.335
	marker00030850	PREDICTED: Beta-amyrin synthase	Terpenoid biosynthesis	-1.703
	novel.2667	PREDICTED: Tropinone reductase homolog	The branch point of tropane alkaloid metabolism	2.603
	marker00040095	PREDICTED: Vinorine synthase-like	A central role in the biosynthesis of the antiarrhythmic monoterpenoid indole alkaloid ajmaline	-5.001
	marker00024878	PREDICTED: Acetylajmalan esterase-like	Play an essential role in the late stage of ajmaline biosynthesis	2.656

(待续)

(待续 1)

Development stage	Gene ID	NR database annotation	Gene function	log ₂ (fold change)
Maturity stage	marker00030846	RecName: Full=Sucrose synthase; AltName: Full=SS16; AltName: Full=Sucrose-UDP glucosyltransferase AAA33841.1 sucrose synthase (EC 2.4.1.13)	Sucrose metabolism	5.013
	marker00044067	PREDICTED: Alpha-amylase-like	Carbohydrate metabolism	4.109
	novel.11679	PREDICTED: Beta-amylase 1, chloroplastic-like	Degrade transitory starch during drought stress	-9.041
	marker00022295	Riboflavin synthase	Vitamin biosynthesis	-1.937
	marker00008586	PREDICTED: Monodehydroascorbate reductase	Key enzyme in the conversion of oxidized ascorbate (AsA) back to reduced AsA in plants	2.346
	marker00001335	PREDICTED: Squalene synthase-like	Sterol biosynthesis	-2.074
	marker00040298	Transcription factor bHLH36-like	A homolog of bHLH36 involved in maize carotenoid metabolism	-7.691
	marker00046333	Carotenoid cleavage dioxygenase 4	Catalyzes the production of β -ionone (carotenoid derivatives)	-1.685
	marker00029155	15-cis- ζ -carotene isomerase	Carotenoid biosynthesis	-1.563
	marker00005595	PREDICTED: Anthocyanidin reductase-like	Proanthocyanidins (PA) biosynthesis	4.890
	marker00027109	Flavonoid 3'-hydroxylase	Involved in flavonoid pathway	-2.711
	marker00027724	PREDICTED: Gibberellin 2-beta-dioxygenase 8-like	Cause the accumulation of anthocyanins	1.862
	marker00015647	Caffeoyl-CoA O-methyltransferase	Polymethoxylated flavones biosynthesis	-2.731
	novel.3707	PREDICTED: 2-hydroxyisoflavanone dehydratase-like	A critical determinant of isoflavone productivity	1.621
	marker00028104	PREDICTED: 1-deoxy-D-xylulose 5-phosphate reductoisomerase, chloroplastic	Isoprenoids biosynthesis	-1.613
	marker00041897	PREDICTED: Geranylgeranyl pyrophosphate synthase, chloroplastic-like	A key enzyme in the synthesis pathway of diterpenoid.	-1.985
	novel.21847	Terpene synthase	Terpenoids biosynthesis	-7.791
	novel.21848	Sesquiterpene synthase	Sesquiterpenoids biosynthesis	1.862
	novel.16162	PREDICTED: Beta-amyrin synthase-like	Influence the oleanane-type ginsenoside level	4.963
	marker00040095	PREDICTED: Vinorine synthase-like	Involved in the biosynthesis of the antiarrhythmic monoterpene indole alkaloid ajmaline	-4.472
	marker00030846	RecName: Full=Sucrose synthase; AltName: Full=SS16; AltName: Full=Sucrose-UDP glucosyltransferase AAA33841.1 sucrose synthase (EC 2.4.1.13)	Key enzyme in plant sucrose catabolism	-4.472
	marker00044067	PREDICTED: alpha-amylase-like	Carbohydrate metabolism	2.856
	novel.11679	PREDICTED: Beta-amylase 1, chloroplastic-like	Degrade transitory starch during drought stress	-8.414
	marker00008511	Glycoside hydrolase, family 32	Fructan biosynthesis	4.025
	marker00014059	glycosyltransferase	Involve in the biosynthesis of xyloglucans and mannans	1.533
	marker00028696	PREDICTED: Beta-fructofuranosidase, insoluble isoenzyme 1-like	Involve in the hydrolysis of sucrose	-1.510
	marker00022295	Riboflavin synthase	Vitamin biosynthesis	-2.636
	marker00024231	GDP-mannose 3',5'-epimerase	Vitamin C biosynthesis	-1.679
	marker00026923	PREDICTED: Pyridoxal reductase, chloroplastic-like	Vitamin B6 salvage pathway	-2.272
	marker00008029	MYB family transcription factor isoform X1	A key factor in controlling development and metabolism	1.506

2.4 ‘宁杞 1 号’和‘宁杞 7 号’中活性成分合成相关差异基因的表达模式

基于转录组数据中 FPKM 值, XA vs. XC 活性成分合成相关的 24 个 DEGs 在‘宁杞 1 号’和‘宁杞 7 号’中的表达特征热图见图 14, 24 个基因可分为 2 种表达模式: 第 1 种模式(cluster I) 中有 14 个基因, 相对于‘宁杞 1 号’, 在‘宁杞 7 号’中低表达; 第 2 种模式(cluster II) 包含 10 个

基因, 在‘宁杞 7 号’中呈现高表达。

为了进一步明确这些基因在‘宁杞 1 号’和‘宁杞 7 号’中的表达模式, 选择成熟期参与类胡萝卜素代谢途径的 2 个 DEGs、参与花青素代谢途径的 3 个 DEGs 和 2 个差异表达的转录因子基因进行 RT-qPCR 分析, 结果表明这 7 个 DEGs 在不同枸杞品种中的表达水平与转录组测序结果基本一致(图 15), 表明转录组数据真实可靠。

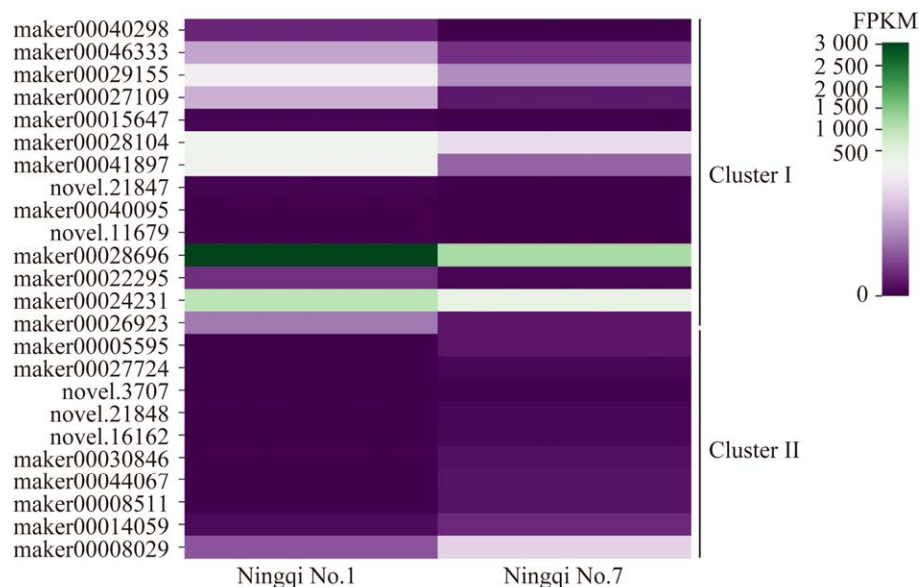


图 14 24 个活性成分合成相关 DEGs 表达模式

Figure 14 Heat map of 24 DEGs related to synthesis of active ingredients.

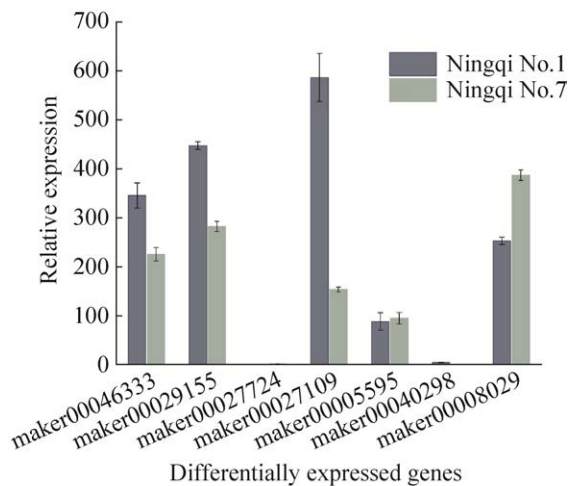


图 15 RT-qPCR 分析 DEGs

Figure 15 RT-qPCR analysis of DEGs.

3 讨论与结论

枸杞果实中的黄酮、多酚、类胡萝卜素等成分, 具有增强免疫力和抗氧化等功效^[12], 是理想的药食两用医疗保健品。枸杞多糖、籽油、黄酮等活性物质的提取也成为开发枸杞附加产品的热点^[13]。但不同品种宁夏枸杞果实品质参差不齐, 故为从分子层面解析枸杞果实所含活性成分的差异, 本研究对‘宁杞 1 号’和‘宁杞 7 号’果实不同发育期进行比较转录组分析, 筛选到与类胡萝卜素、花青素等活性成分代谢相关的若干差异表达基因。

类胡萝卜素属脂溶性维生素,人体无法自身合成,必须从食物中摄取^[14],作为天然抗氧化剂,对人类健康起着重要的作用^[12]。枸杞类胡萝卜素是其果实活性成分之一,既影响枸杞果实的口味,同时也是色素合成的关键成分,是枸杞品质评价的重要指标之一^[2,15]。目前,枸杞类胡萝卜素相关研究多集中在含量及其代谢途径关键基因的功能验证上^[12,16],对宁夏枸杞不同品种间类胡萝卜素合成相关 DEGs 的研究鲜有报道。本研究从‘宁杞 1 号’和‘宁杞 7 号’青果期、转色期和成熟期果实转录组数据中,分别筛选到类胡萝卜素代谢相关的 4、2 和 3 个 DEGs,涉及编码八氢番茄红素合酶 1 (phytoene synthase 1, *Psy1*)、八氢番茄红素合酶 2 (phytoene synthase 2, *Psy2*)、15-顺- ζ -胡萝卜素异构酶 (15-*cis*- ζ -carotene isomerase, *Z-ISO*)、八氢番茄红素去饱和酶(phytoene desaturase, *PDS*)、类胡萝卜素裂解双加氧酶 4 (carotenoid cleavage dioxygenase 4, *CCD4*)等 5 种酶,这 8 个基因在‘宁杞 7 号’果实相应的发育阶段均下调表达,这可能是不同枸杞品种类胡萝卜素积累差异的原因,可推测‘宁杞 7 号’果实中类胡萝卜素含量较‘宁杞 1 号’低。张晓娟等^[4]发现成熟期‘宁杞 7 号’类胡萝卜素含量低于‘宁杞 1 号’的结果,支持了本研究的推测。这些 DEGs 在枸杞类胡萝卜素合成中的功能,有待进一步验证。

基因表达受转录因子的调控,相继有 bHLH 和 MYB 类转录因子参与调控类胡萝卜素代谢的报道^[17-18]。本研究在‘宁杞 7 号’成熟期果实中识别到 bHLH 转录因子家族基因 *bHLH36-like* (marker00040298)下调表达。前人研究报道 *bHLH36* 转录因子的同源物参与玉米类胡萝卜素代谢^[19],据此推测 *bHLH36-like* 在宁夏枸

杞类胡萝卜素代谢过程中可能具有重要的调控作用。另外,在果实成熟期发现的 *CCD4* 和 *Z-ISO* 两个 DEGs 与 *bHLH36-like* 表达趋势相同。据报道 *CCD4* 在桃果肉中的低表达与类胡萝卜素和类胡萝卜素衍生挥发物的积累相关^[20]。苹果中类胡萝卜素含量与 ζ -carotene isomerase 的表达量呈正相关^[21]。*CCD4* 和 *Z-ISO* 在枸杞类胡萝卜素代谢中的具体功能及其表达是否受 *bHLH36-like* 的调控? 若二者的表达与该转录因子无关,那么 *bHLH36-like* 作用的靶基因及调控模式是什么(图 16)? 这些问题尚需进一步深入研究,以确定相关转录因子的靶基因及其功能,进而解析其可能在枸杞类胡萝卜素代谢途径中的调控机制。

MYB 类转录因子家族调控植物类胡萝卜素合成的相关研究已有大量报道, *MYB7* 通过转录激活猕猴桃类胡萝卜素生物合成途径番茄红素 β -环化(*AdLCY-B*)基因的启动子,调节类胡萝卜素的积累^[22]。木瓜转录因子 *CpMYB1* 和 *CpMYB2* 可与类胡萝卜素生物合成基因 *CpPDS2*、*CpPDS4* 和 *CpCHY-b* 的启动子结合,抑制其活性,影响类胡萝卜素积累^[23]。Wu 等^[24]发现 *SIMYB72* 与 *PSY*、*Z-ISO*、*LCY-B* 基因结合,调控番茄类胡萝卜素的生物合成。据此推测本研究在‘宁杞 7 号’成熟期果实中发现的上调表达基因 *MYB X1* (marker00008029),可能参与枸杞类胡萝卜素代谢途径的调控(图 16)。*MYB X1* 和 *bHLH36-like* 转录因子是否调控枸杞类胡萝卜素的积累,是单独调控还是协同调控,其调控模式等相关问题,尚需开展相关研究进行验证。

花青素是一种天然水溶性植物色素,参与果实品质的形成,影响果实的口感、颜色和光泽^[25],且具有较高的抗氧化活性,能猝灭人

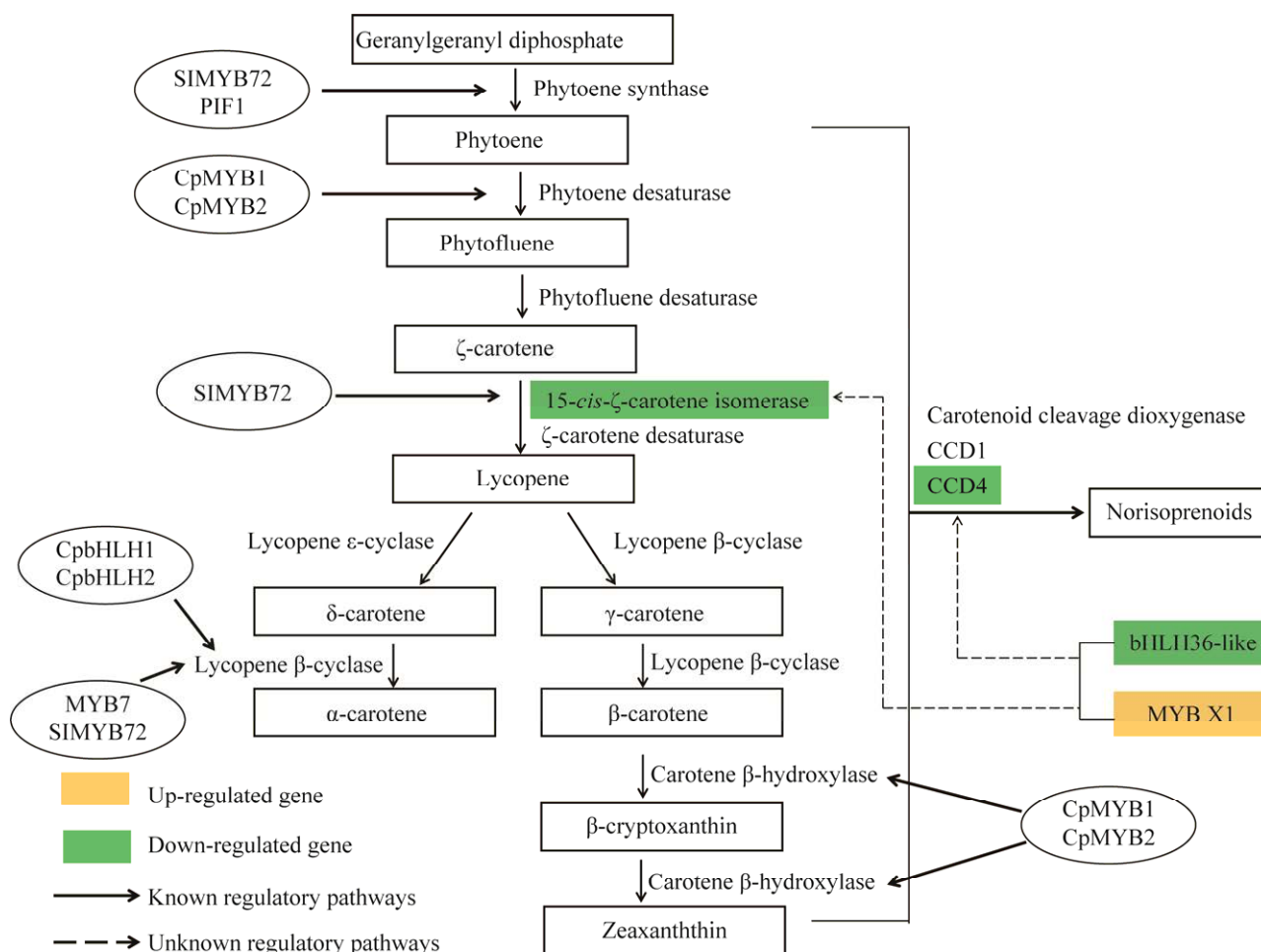


图 16 类胡萝卜素生物合成途径

Figure 16 Carotenoid biosynthesis pathway. *CpbHLH1* and *CpbHLH2*^[17]; *PIF1*^[18]; *MYB7*^[22]; *CpMYB1* and *CpMYB2*^[23]; *SIMYB72*^[24].

体自由基, 具有抗癌、抗衰老、降血糖和保肝明目等功效^[25-26], 故植物花青素的生物合成已成为众多研究者关注的热点。本研究识别到类黄酮代谢途径中编码花青素合酶(anthocyanin synthase, ANS)、花青素还原酶(anthocyanidin reductase, ANR)、查尔酮合酶(chalcone synthase, CHS)、类黄酮 3'-羟化酶(flavonoid 3'-hydroxylase, F3'H)、类黄酮 -3',5'-羟化酶(flavonoid-3',5'-hydroxylase, F3',5'H)、黄酮醇合酶(flavonol synthase, FLS)、咖啡酰辅酶 A-O-甲基转移酶

(caffeoyl-CoA-O-methyltransferase, OMT)、2-羟基异黄酮酮脱水酶(2-hydroxyisoflavanone dehydratase, HID)等 8 种酶的 14 个基因在‘宁杞 1 号’和‘宁杞 7 号’枸杞果实中差异表达。这些 DEGs 可能是不同枸杞品种黄酮类化合物积累差异的原因。Lin 等^[27]通过比较 Bluecrop 和 Northblue 两个蓝莓品种果实花青素和酚类化合物含量及其转录组差异, 发现 *CHI*、*DFR*、*F3'H*、*FLS*、*CHS*、*OMT*、*UGT*、*ANS* 和 *F3H* 等基因的表达水平与花青素含量变化趋势一致。本研

究中参与花青素代谢途径的关键酶基因 *ANS* 基因、*F3',5'H* 基因和 *CHS* 基因,均在‘宁杞 7 号’青果期下调表达,在转色期又上调表达,而在成熟期时差异不显著。推测 *ANS*、*F3',5'H* 和 *CHS* 基因并不是造成‘宁杞 1 号’和‘宁杞 7 号’成熟时花青素含量差异的主要原因。而 *F3'H* 基因在‘宁杞 7 号’枸杞果实 3 个发育阶段相对表达量均低于‘宁杞 1 号’,推测 *F3'H* 基因的差异表达可能是‘宁杞 1 号’和‘宁杞 7 号’花青素生物合成存在差异的重要原因,这些差异基因在枸杞中的确切功能有待进一步研究证明。

原花青素是枸杞最主要的活性成分之一,在植物外观、风味和品质方面具有重要作用,其合成途径的研究一直是作物性状改良的热点^[28]。原花青素前体的生物合成与花青素具有相同的上游途径,共享相同的酶。位于下游的原花青素特异性途径包括无色花青素还原酶(leucoanthocyanidin reductase, LAR)参与的 LAR 途径和花青素还原酶(anthocyanidin reductase, ANR)参与的 ANR 途径^[29]。本研究在‘宁杞 1 号’和‘宁杞 7 号’枸杞果实 3 个发育阶段的比较转录组中,仅发现 *ANR* 基因差异表达,且其在‘宁杞 7 号’3 个发育阶段均高于‘宁杞 1 号’,未筛选到参与 LAR 途径的相关 DEGs,故 *ANR* 基因的差异表达可能是造成‘宁杞 1 号’和‘宁杞 7 号’原花青素代谢存在差异的重要原因。

双子叶植物原花青素生物合成由早期生物合成基因(early biosynthetic genes, EBGs),如 *CHS*、*CHI*、*F3H* 和 *F3'H* 基因和晚期生物合成基因(lateral biosynthetic genes, LBGs),如 *DFR*、*ANS*、*ANR* 和 *UFGT* 基因^[30]共同催化完成。这些结构基因的时空表达受调节基因所编码的转录因子调控。众所周知,参与花青素合成相关

的转录因子主要有 3 类: MYB 类转录因子、bHLH 类转录因子和 WD40 类转录因子。MYB 基因既可以活跃表达调控^[31],也可以负调控花青素的合成^[32]。bHLH 类转录因子 *GL3::GL3* 转化番茄,转基因植株花青素的积累量高于野生型,且 *GL3::GL3* 转基因番茄中 *PAL*、*CHS*、*DFR* 和 *ANS* 的表达水平均显著高于野生型^[32]。众多研究表明 MYB 和 bHLH 转录因子二者相互作用,共同调控花青素的合成^[33-34]。本研究在‘宁杞 1 号’和‘宁杞 7 号’成熟期果实中发现两个转录因子差异表达,分别是 *bHLH36-like* 和 *MYB X1*,其中 *bHLH36-like* 在‘宁杞 7 号’中下调表达,与 *F3'H* 基因的表达趋势一致; *MYB X1* 在‘宁杞 7 号’中上调表达,与 *ANR* 基因的表达变化一致。初步推测这两个转录因子可能单独或者协作参与调控花青素合成途径。若二者参与调控花青素代谢途径,它们的转录与哪个结构基因相互作用,又如何调控其表达,二者之中哪一个基因是枸杞中花青素积累或减少的关键原因,是否二者协同调控等问题(图 17),有待进一步深入研究,以解析‘宁杞 1 号’和‘宁杞 7 号’果实花青素含量差异的分子机制,为培育富含花青素的枸杞品种提供重要的研究基础。

‘宁杞 1 号’和‘宁杞 7 号’果实活性成分相关 DEGs 的识别,将有助于揭示枸杞果实活性成分生物合成及其调控机制。然而,物种特异性和遗传多样性使得相关活性成分的生物合成途径及相关基因的表达调控极其复杂。本研究识别的果实活性成分相关候选基因的功能验证,还有待于进一步深入研究,这将为提升枸杞果实品质,改善枸杞果实的风味营养品质,同时也为探索多靶点改良品种指明新方向。

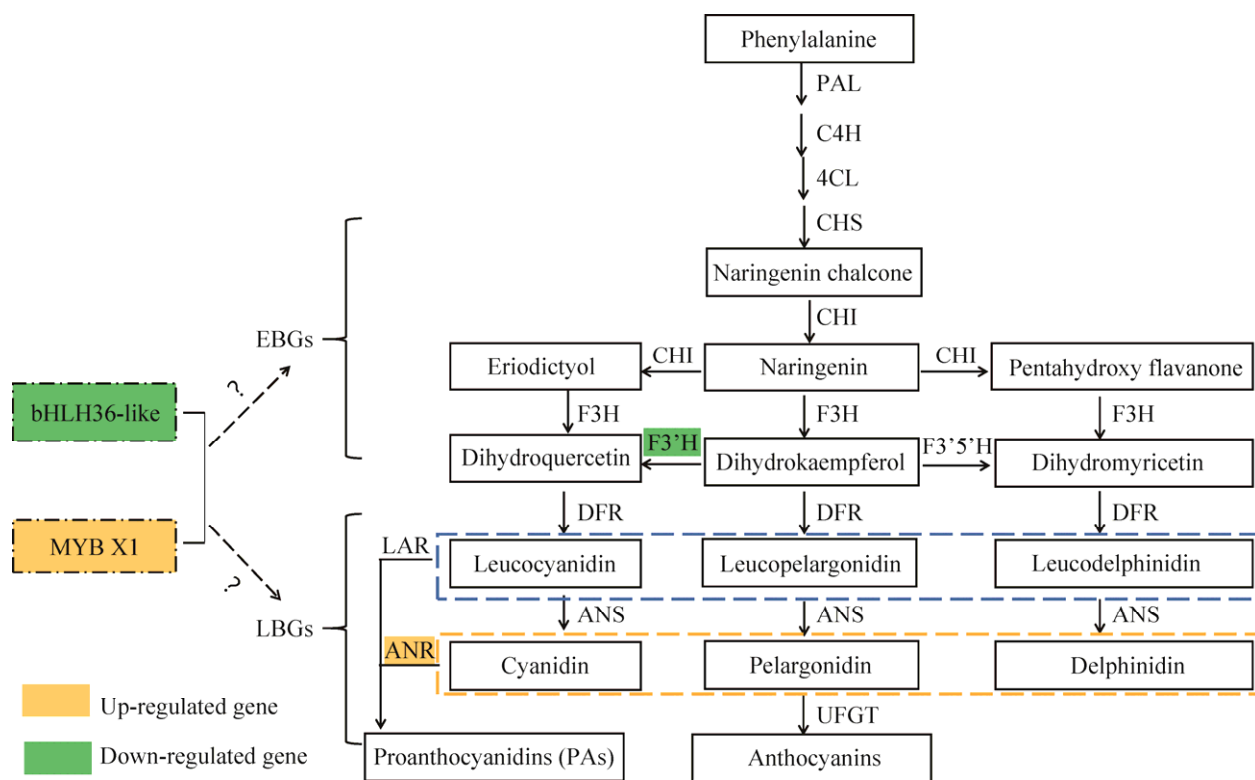


图 17 黄酮类化合物生物合成途径 PAL: 苯丙氨酸氨裂解酶; C4H: 肉桂酸-4-羟化酶; 4CL: 4-香豆酸: 辅酶 A 连接酶; CHI: 查尔酮异构酶; F3H: 黄烷酮-3-羟化酶; DFR: 二氢黄酮还原酶; UFGT: UDP 类黄酮葡萄糖基转移酶

Figure 17 Flavonoid biosynthesis pathway. PAL: Phenylalanine ammonia lyase; C4H: Cinnamic acid-4-hydroxylase; 4CL: 4-coumarate-CoA ligase; CHI: Chalcone isomerase; F3H: Flavanone-3-hydroxylase; DFR: Dihydroflavonol reductase; UFGT: UDP flavonoid glucosyltransferase.

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