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沃柑果皮响应柑橘锈螨危害的转录组分析

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摘 要：【目的】揭示柑橘抗柑橘锈螨危害的分子机制，筛选出在柑橘锈螨危害条件下的相关应答基因。**【方法】**研究以接种柑橘锈螨后危害3、5、7、9 d的沃柑果实为试材，健康果实为对照（CK），采用Novaseq6000平台进行转录组双向测序，并对数据进行生物信息学相关性分析。**【结果】**共获得了55.63 Gb clean bases，去除低质量碱基和接头序列后，CK3、CK5、CK7和CK9获得Clean reads分别是42 115 939、45 501 683、58 118 008和40 715 511条，WG3d、WG5d、WG7d和WG9d获得Clean reads分别是46 807 089、49 185 798、44 437 798和44 035 730条。柑橘锈螨危害3、5、7、9 d后引起上调差异表达基因数量分别为2764、1812、3217、2778个，下调差异表达基因数量分别为3036、1387、3350、2347个。GO功能富集分析结果显示，差异表达基因在不同的柑橘锈螨危害时间段，主要集中在生物学过程、细胞组分和分子功能中。KEGG注释分析结果显示，差异表达基因主要参与二苯乙烯、二芳基庚烷和姜辣素的生物合成、核糖体、磷酸戊糖、光合作用、碳代谢等途径。**【结论】**植物激素信号转导、植物病原互作通路、类黄酮生物合成、苯丙氨酸代谢、戊糖磷酸代谢5条通路在沃柑响应柑橘锈螨胁迫过程中起到了重要的调控作用。

关键词：沃柑；柑橘锈螨；转录组；差异表达基因

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Transcriptome analysis of citrus rustmite damage to the peel of Orah fruits

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Abstract:【Objective】In order to reveal the molecular mechanism of citrus rust mite damage to citrus fruits, the related response genes were screened, and the varieties resistant to citrus rust mite were improved by using the related resistance gene resources, so as to breed new varieties with stronger resistance to citrus rust mite.【Methods】Sixty citrus rust mites were inoculated on the same size fruits without diseases and insect pests in this experiment. During the experiment, no other diseases and insect pests were found. Three, five, seven and nine days after inoculation, three replicates of each sample were taken. 5 g peel with control (CK) and citrus rust mites groups (WG) were rinsed with sterile water, wrapped with tin foil, and treated with liquid nitrogen before they were put in the refrigerator at −80 ℃ for standby. The two-way transcriptome sequencing was performed on novaseq6000 platform, and the bioinformatics correlation analysis was carried out.【Results】A total of 55.63 gb clean bases were obtained. After removing low-quality bases and linker sequences, 42 115 939, 45 501 683, 58 118 008 and 40 715 511 clean reads were obtained from CK3, CK5, CK7 and CK9, and 46 807 089, 49 185 798, 44 437 798 and 44 035 730 clean reads were obtained from WG3d, WG5d, WG7d and WG9d, respectively. The number of up-regulated differentially expressed genes was 2764, 1812, 3217 and 2778 in treatment 3、5、7、9 d，and the number of down regulated differentially expressed genes was 3036,

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1387, 3350 and 2347 respectively. The results of GO enrichment analysis showed that the DEGs of 3 d were mainly enriched in the process of organic nitrogen metabolism, biological process, metabolic process and redox; the cellular components were enriched in ribosome, ribonucleoprotein complex and molecular complex; the molecular functions were mainly enriched in the structural composition, structural molecular activity and oxidoreductase of ribosome. In addition, the catalytic activity and other functions were also found. The DEGs of 5 d were mainly enriched in the redox process, monomer metabolism process, biological process, metabolic process and other processes in the biological process; the molecular functions were mainly enriched in heme binding, tetrapyrrole binding, iron ion binding, oxidoreductase activity, electron carrier activity, catalytic activity and so on. The DEGs of 7 d were mainly enriched in the metabolic process, biological process, redox process, organic nitrogen compounds metabolic process, organic nitrogen compounds biosynthesis process and so on; the cell components were enriched in the cytoplasmic part, ribonucleoprotein complex, ribosome, polymer complex, cytoplasm and so on; the molecular functions were mainly enriched in the catalytic activity, oxidation activity, reductase activity and so on. The DEGs of 9 d were mainly enriched in the biological process, metabolic process and single organism metabolic process, and the molecular function was mainly enriched in the activity of oxidoreductase and catalytic activity. KEGG annotation analysis showed that the DEGs of 3 d were mainly annotated to vitamin B6 metabolism, biosynthesis of stilbene, diarylheptane and gingerol, photosynthesis, phenylpropanoid biosynthesis, pentose phosphate and other pathways. The DEGs of 5 d were mainly enriched in vitamin B6 metabolism, biosynthesis of stilbene, diarylheptane and gingerol, selenium complex metabolism, photosynthesis antennae, photosynthesis, phenylalanine biosynthesis, phenylalanine, tyrosine and tryptophan biosynthesis. The DEGs of 7 d were mainly enriched in the biosynthesis of stilbene, diarylheptane and gingerol, ribosome, endoplasmic reticulum protein processing, proteolytic enzyme complex, porphyrin and chlorophyll metabolism, photosynthesis antennae, photosynthesis, phenylpropanoid biosynthesis, pentose phosphate pathway, oxidative phosphorylation and other pathways. The DEGs of 9 d were mainly enriched in the biosynthesis of stilbene, diarylheptane and gingerol, photosynthesis antennae, photosynthesis, phenylalanine biosynthesis, phenylalanine, tyrosine and tryptophan biosynthesis pathways.【Conclusion】High throughput transcriptome sequencing technology was used to sequence and analyze Orah fruits damaged by citrus rust mite, and multiple differentially expressed genes were screened, which were involved in plant hormone signal transduction, plant pathogen interaction pathway, flavonoid biosynthesis, phenylalanine metabolism, pentose phosphate and other pathways in different damage periods. The research on the molecular mechanism of Orah responding to the damage of citrus rust mite can provide not only abundant gene resources, but also a theoretical basis for the research on the gene regulatory network of Citrus germplasm resources under the mite damage conditions.

Key words：Orah; Citrus rust mite; Transcriptome; Differentially expressed genes

柑橘皱叶刺瘿螨[*Phyllocoptruta oleivora*（Ashmead）]隶属于蜱螨亚纲瘿螨科，是世界性害螨，我国柑橘园区多有分布[1-3]。柑橘锈螨通过将自身口器刺入柑橘果皮中吸取营养物质来维持自身生长发育，从而导致果皮细胞受损破裂，溢出的油脂被空气氧化成褐色或深黑色，最终形成黑皮果，致使树势衰弱和果实商品价值严重降低[4-10]。柑橘锈螨个体微小、繁殖周期短、传播途径多样化，果园管理容易忽视，极易暴发成灾[11-14]。

沃柑（Orah）系以色列选育的丹西红橘和坦普尔橘橙杂交品种[15-16]，由中国农业科学院柑桔研究所在2004年从韩国引入国内，具有产量高、品质好的特点[17-18]，适宜作为特色杂柑良种进行大面积推广[19-20]。沃柑是我国近年来发展速度最快的柑橘品种，广西为主产区并大力打造沃柑地域品牌[21]，但其果实极易受到柑橘锈螨危害，产生黑皮果，严重影响沃柑商品价值和生产效益。

近年来，转录组测序已经成为研究植物功能基

因组的重要手段，从分子水平上探究虫害所诱导的植物防御反应中涉及的识别机制、基因表达、信号通路、危害虫治理和抗虫植物培育提供了新的思路[22-24]。Artico等[25]研究棉花蕾受棉铃象虫侵染时转录组数据的变化情况，发现443个差异表达基因，通过对酶通路和植物激素信号转导途径的转录变化研究，为目标基因的筛选和遗传工程的数据库建立提供支持。植物遭受虫害胁迫后，体内氧化酶系、病程相关蛋白和苯丙烷类代谢途径、激素分子等物质在应对胁迫的防御过程中发挥了关键作用[26-32]。邢雪霞[33]通过对南方根结线虫侵染不同抗性的烟草进行转录组测序，研究表明289个差异基因富集在细胞壁修饰、激素信号转导和转录因子等通路上，为抗虫基因筛选和烟草抗线虫的分子机制研究提供了一定的基础。Dubey等[34]分析粉虱和蚜虫胁迫下毛金丝桃的转录组数据时发现，植物抗性相关的标记基因过表达，差异表达基因大部分与水解酶、激酶、生物胁迫等表达途径有关。植物体受害虫胁迫会激活一系列的分子信号途径，启动相关下游基因表达来调控胁迫响应，以降低自身伤害程度，而从分子水平上探究虫害所诱导的植物防御反应中涉及的识别机制、基因表达和信号通路，为害虫治理和抗虫植物的培育提供了新的途径[35-38]。因此，通过挖掘抗柑橘锈螨的相关基因，阐明其在应答柑橘锈螨危害过程中的分子调控机制，并利用相关抗性基因资源进行沃柑抗柑橘锈螨品种改良，对培育出抗虫性更强的沃柑新品种具有十分重要的意义。

1　材料和方法

1.1　材料

试验材料为沃柑，由广西大学农学院提供。选取树势一致、果实直径约4 cm、健康无病虫害的青果，按照60头/果实的虫口密度，用带微钩的昆虫针将柑橘锈螨成螨轻轻接种至沃柑果实上（WG），以未接种柑橘锈螨的健康沃柑果实作为空白对照组（CK），3次重复，每次重复4颗沃柑。在果柄处涂抹凡士林避免柑橘锈螨爬出实验果实，实验期间确保材料无其他病虫危害，生长温度控制在28 ℃，湿度调节在60%。接螨3、5、7、9 d进行采样，分别取对照组（CK）和柑橘锈螨危害组（WG）的果皮，使用无菌水进行冲洗，再用吸水纸吸干水分，锡纸包裹果皮放入液氮壶中迅速转运至-80 ℃储存。

1.2　RNA提取、文库构建及测序

采用南宁壹棵松生物科技有限公司生产的植物RNA提取试剂盒和反转录试剂盒，严格按照使用说明书进行RNA的提取和反转录，文库的构建和测序工作由北京诺禾致源生物信息科技有限公司处理完成，使用Novaseq6000为该样品的测序平台。

1.3　测序数据的过滤与组装

将通过测序平台Novaseq6000、使用某个碱基错误识别的概率为0.001的标准得到的Raw reads进行质量分析，去除带接头的（adapter）的reads，去除无法确定碱基信息且比例大于10%的reads，去除$Q_{phred} \leqslant 20$的碱基数占整个read长度的50%以上的低质量reads。利用Trinity软件对测序数据进行de novo拼接组装，得到最终的单基因簇。

1.4　差异表达基因筛选

采用DEGseq2软件进行基因表达水平分析。具体参数设置如下：$|\log_2(\text{FoldChange})| > -1$，$P$ value < 0.005。

1.5　差异表达基因GO富集分析

通过GOseq软件对差异基因进行GO富集分析，获得分布于GO功能组的差异基因，设定阈值为FDR $\leqslant 0.05$。

1.6　差异表达基因KEGG富集分析

KEGG富集分析所用的软件是KOBAS，Rich factor和Q value的大小以及富集到这个通路上基因个数的多少能够用来衡量KEGG的富集程度的大小。其中Rich factor表示的含义为该pathway中富集得到的差异表达基因的个数（Sample number）与注释到该pathway中所有基因个数（Background number）的比值。因此，Rich factor值越大，则表示该pathway的富集程度越大。参数设置：Corrected P-Value < 0.05。

2　结果与分析

2.1　转录组数据质量分析

通过对接种柑橘锈螨后3、5、7、9 d后的样品进行转录组测序，每个样品3个重复，所得数据取均值得表1。从错误率以及碱基含量等方面对原始测序数据进行了质量分析，共获得了55.63 Gb clean bases，其中CK3、CK5、CK7和CK9获得Raw Reads分别是42 820 929、46 412 337、59 316 621和41 563 010条，去除低质量碱基和接头序列后获得

表1 柑橘锈螨不同危害时段处理的转录组测序数据质量分析

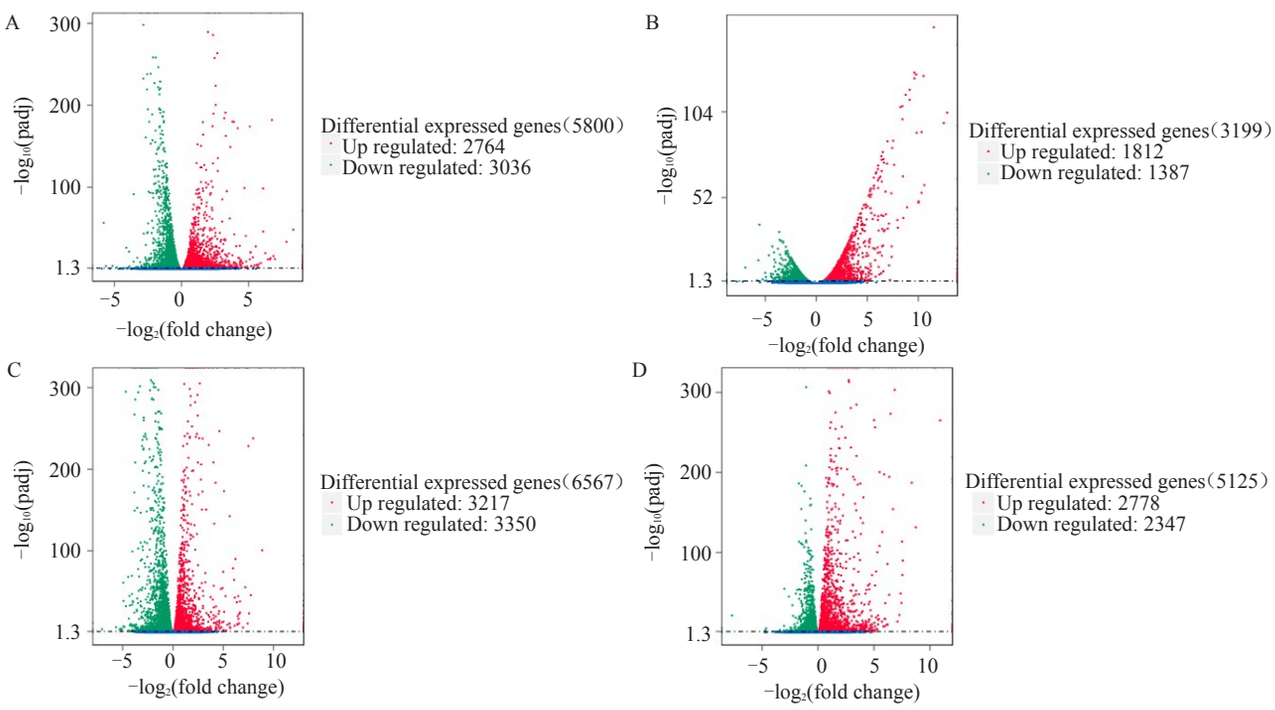
Table 1 Quality analysis of transcriptome sequencing data treated with different damage time periods of citrus rusting mite

样本 Sample	处理时间 Treatment time/d	原始读取 Raw reads	过滤后数据 Clean reads	比对率 Contrast ratio/%	错误率 Error rate/%	Q20含量 Q20 content/%	Q30含量 Q30 content/%	GC含量 GC content%
对照 CK	3	42 820 929	42 115 939	92.13	0.03	97.82	93.35	44.37
	5	46 412 337	45 501 683	92.00	0.03	98.14	94.07	43.96
	7	59 316 621	58 118 008	91.97	0.03	97.83	93.36	44.10
	9	41 563 010	40 715 511	92.82	0.03	98.23	94.41	44.21
处理 Treatment	3	47 916 087	46 80 7089	92.11	0.03	98.08	93.92	44.08
	5	50 286 368	49 185 798	92.47	0.03	97.96	93.65	43.99
	7	45 630 198	44 43 7798	92.42	0.03	98.13	94.06	44.19
	9	44 988 691	44 035 730	92.73	0.03	98.05	93.84	44.21

Clean reads分别是42 115 939、45 501 683、58 118 008和40 715 511条。WG3d、WG5d、WG7d和WG9d获得Raw Reads分别是47 916 087、50 286 368、45 630 198和44 988 691条，Clean reads分别是46 807 089、49 185 798、44 437 798和44 035 730条。各样品GC含量约为44%，Q20、Q30分别达到97%和93%以上，碱基错误率为0.03%，均低于0.05%，且Clean reads与甜橙基因组的比对率约为92%，说明转录组测序质量符合要求，所得数据可以用于后续分析。

2.2 差异表达基因数量分析

通过将对照组与柑橘锈螨危害组的转录组数据进行统计分析，火山图显示了差异基因的整体分布情况。柑橘锈螨危害3 d后统计结果显示（图1-A），DEGs总数达到5800个，其中表达水平上调和下降的DEGs数量分别为2764、3036个。柑橘锈螨危害5 d后统计结果显示（图1-B），DEGs总数达到3199个，其中表达水平上调和下降的DEGs数量分别为1812、1387个。柑橘锈螨危害7 d后统计结果显示（图1-C），DEGs总数达到6567个，其中表达水平上调和下降的DEGs数量分别为3217、3350个。柑橘锈螨危害9 d后统计结果显示（图1-D），DEGs总数达到5125个，其中表达水平上调和下降的DEGs数量分别为2778、2347个。



A. 柑橘锈螨危害3 d；B. 柑橘锈螨危害5 d；C. 柑橘锈螨危害7 d；D. 柑橘锈螨危害9 d。下同。

A. After 3 d of citrus rust mite damage; B. After 5 d of citrus rust mite damage; C. After 7 d of citrus rust mite damage; D. After 9 d of citrus rust mite damage. The same below.

图1 差异基因火山图

Fig. 1 Volcano map of differential genes

2.3　差异表达基因韦恩图

对read count进行标准化之后，筛选DEGs的标准为$p < 0.05$，对各组差异基因进行韦恩图分析，可知共有1181个DEGs在柑橘锈螨危害后3、5、7、9 d时均有显著差异（图2）。

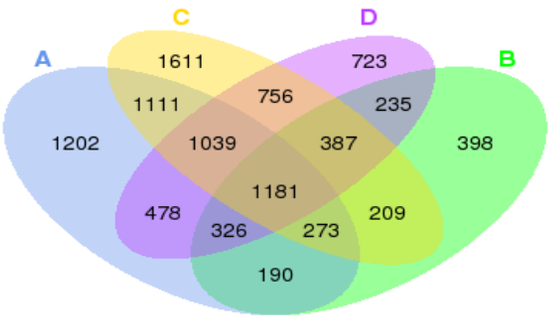


图2　差异表达基因维恩图

Fig. 2　Venn diagram of differentially expressed genes

2.4　差异表达基因GO功能富集分析

筛选出DEGs后，对其进行GO功能富集分析。生物学过程、细胞组分和分子功能富集情况能够通过GO富集柱状图直观地表现出来。柑橘锈螨危害3 d后的GO功能富集分析结果显示（图3-A），DEGs主要富集到生物学过程中的有机氮化合物代谢过程、生物学进程、代谢过程、氧化还原等过程中；细胞组分富集在核糖体、核糖核蛋白复合体、分子复合物等上；分子功能主要富集在核糖体的结构组成、结构分子活性、氧化还原酶活性、催化活性等功能上。柑橘锈螨危害5 d后的GO功能富集分析结果显示（图3-B），DEGs主要富集到生物学过程中的氧化还原过程、单体代谢过程、生物学过程、代谢过程等过程中；分子功能主要富集在血红素结合、四吡咯结合、铁离子结合、氧化还原酶活性、电子载流子活性、催化活性等上。柑橘锈螨危害7 d后的GO功能富集分析结果显示（图3-C），DEGs主要富集到生物学过程中的代谢过程、生物学过程、氧化还原过程、有机氮化合物代谢过程、有机氮化合物生物合成过程等过程中；细胞组分富集在细胞质部分、核糖核蛋白复合体、核糖体、高分子复合物、细胞质等上；分子功能主要富集在催化活性、氧化还原酶活性等上。柑橘锈螨危害9 d后的GO功能富集分析结果显示（图3-D），DEGs主要富集到生物学过程中的生物学过程、代谢过程、单个有机体代谢过程等过程中；分子功能主要富集在氧化还原酶活性、催化活性等上。

2.5　差异表达基因KEGG注释分析

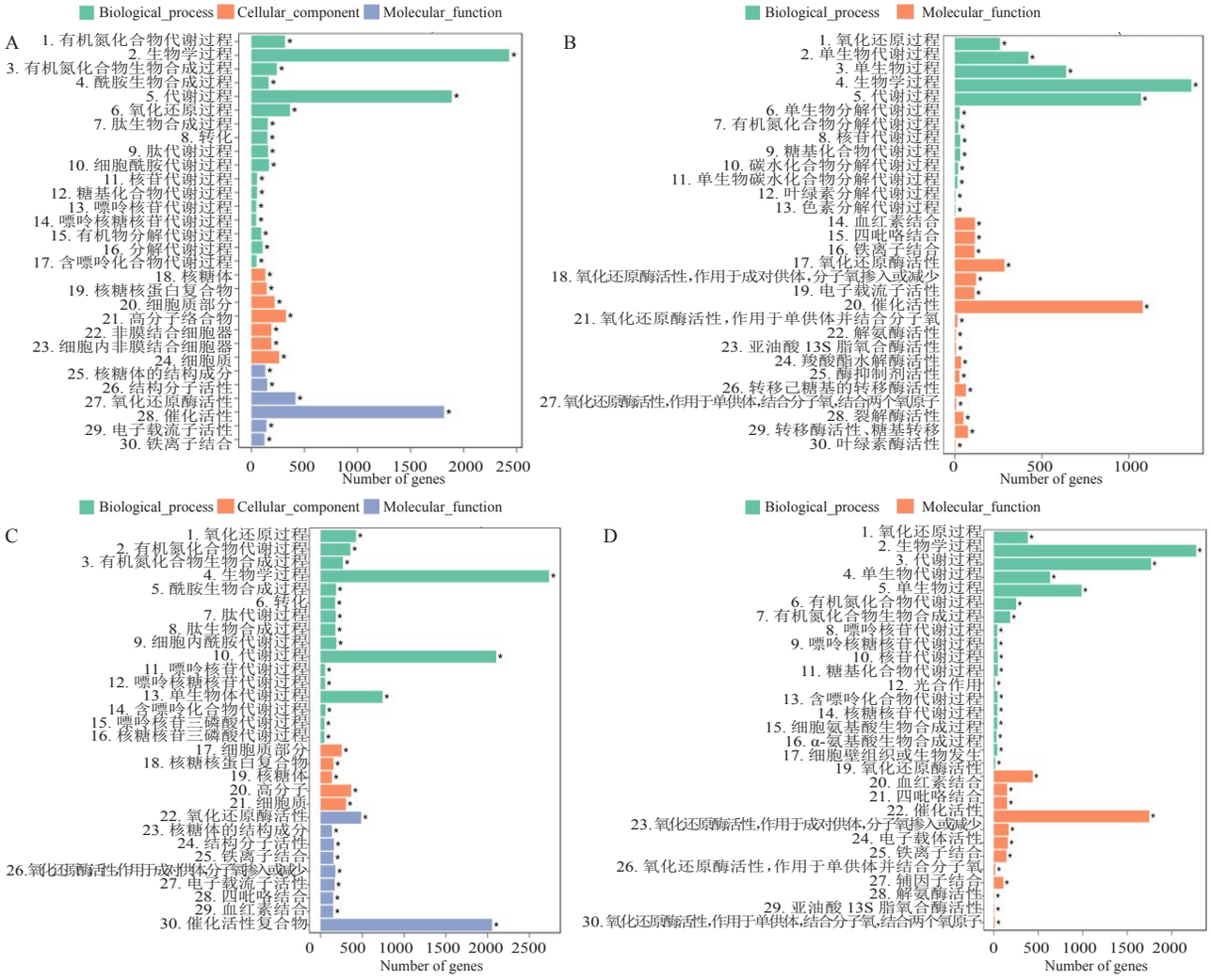
通过KEGG功能富集分析可以判断DEGs主要参与的代谢和转导途径，有助于对基因的功能和表达信息进行系统的研究。柑橘锈螨危害3 d后的KEGG富集分析结果显示（图4-A），DEGs主要注释到维生素B6代谢、二苯乙烯、二芳基庚烷和姜辣素的生物合成、光合作用、苯丙素生物合成、戊糖磷酸等通路中。柑橘锈螨危害5 d后的KEGG注释分析结果显示（图4-B），DEGs主要富集到维生素B6代谢、二苯乙烯、二芳基庚烷和姜辣素的生物合成、硒复合代谢、光合作用-触角蛋白、光合作用、苯丙素生物合成、苯丙氨酸、酪氨酸和色氨酸的生物合成等通路中。柑橘锈螨危害7 d后的KEGG注释分析结果显示（图4-C），DEGs主要富集到二苯乙烯、二芳基庚烷和姜辣素的生物合成、核糖体、内质网蛋白质加工、蛋白水解酶复合体、卟啉与叶绿素代谢、光合作用-触角蛋白、光合作用、苯丙素生物合成、磷酸戊糖途径、氧化磷酸化等通路中。柑橘锈螨危害9 d后的KEGG注释分析结果显示（图4-D），DEGs主要富集到二苯乙烯、二芳基庚烷和姜辣素的生物合成、光合作用-触角蛋白、光合作用、苯丙素生物合成、苯丙氨酸、酪氨酸和色氨酸的生物合成等通路中。

2.6　差异表达基因的功能鉴定

通过KEGG通路的进一步分析，发现DEGs主要涉及5个与抗虫相关的通路，分别是植物激素信号传导途径、植物病原互作通路、类黄酮合成途径、苯丙氨酸代谢途径、戊糖磷酸途径。

2.6.1　*植物激素信号转导途径差异基因分析*　通过对植物激素信号传导途径中的DEGs进一步分析，茉莉酸Zim结构域蛋白（JAZ）编码的4条基因（XM_006474637.3、XM_006465821.2、XM_006474477.3、XM_006465820.2）在柑橘锈螨危害3、5、7、9 d时均显著上调，诱导植物激素相关的基因表达；水杨酸调节因子（NPR1）在危害后期被激活，其中7 d和9 d分别编码了2、3条基因，除两个时段共同的基因（XR_371294.3）显著上调外，其余基因下调，可推断其在危害后期参与了沃柑的抗虫响应。

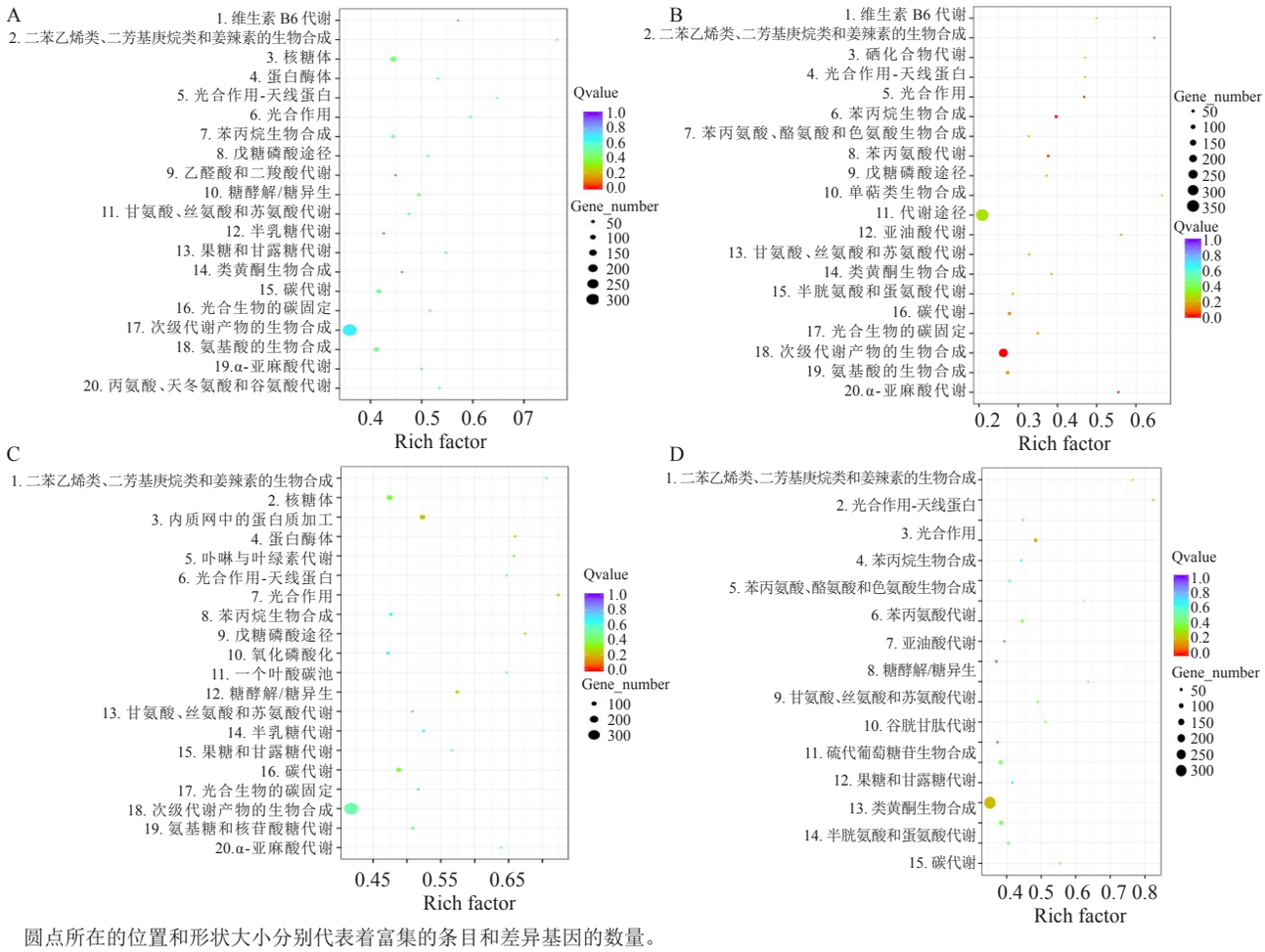
2.6.2　*植物病原互作通路差异基因分析*　通过对植物病原互作通路中的DEGs进一步分析，氧化酶同源酶（RBOH）编码的2条基因（XM_006472017.3、XM_006484987.3）在柑橘锈螨危害3、5、7、9 d时均显著上调，基因（XM_006476418.3）从5 d开始显著



A -1. Organonitrogen compound metabolic process; 2. Organonitrogen compound biosynthetic process; 3. Amide biosynthetic process; 4. Metabolic process; 5. Oxidation-reduction process; 6. Peptide biosynthetic process; 7. Translation; 8. Peptide metabolic process; 9. Cellular amide metabolic process; 10. Cellular amide metabolic process; 11. Nucleoside metabolic process; 12. Glycosyl compound metabolic process; 13. Purine nucleoside metabolic process; 14. Purine ribonucleoside metabolic process; 15. Organic substance catabolic process; 16. Catabolic process; 17. Purine-containing compound metabolic process; 18. Ribosome; 19. Ibonucleoprotein complex; 20. Cytoplasmic part; 21. Macromolecular complex; 22. Non-membrane-bounded organelle; 23. Intracellular non-membrane-bounded organelle; 24. Cytoplasm; 25. Structural constituent of ribosome; 26. Structural molecule activity; 28. Catalytic activity; 29. Electron carrier activity; 30. Iron ion binding. B-1. Oxidation-reduction process; 2. Single-organism metabolic process; 3. Single-organism process; 4. Biological_process; 5. Metabolic process; 6. Single-organism catabolic process; 7. Organonitrogen compound catabolic process; 8. Nucleoside metabolic process; 9. Glycosyl compound metabolic process; 10. Carbohydrate catabolic process; 11. Single-organism carbohydrate catabolic process; 12. Chlorophyll catabolic process; 13. Pigment catabolic process; 14. Heme binding; 15. Tetrapyrrole binding; 16. Iron ion binding; 17. Oxidoreductase activity; 18. Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen; 19. Electron carrier activity; 20. Catalytic activity; 21.Oxidoreductase activity, acting on single donors with incorporation of molecular oxygen; 22.Ammonia-lyase activity; 23. Linoleate 13S-lipoxygenase activity; 24.Carboxylic ester hydrolase activity; 25. Enzyme inhibitor activity; 26.Transferase activity, transferring hexosyl groups; 27. Oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen; 28. Lyase activity; 29. Transferase activity, transferring glycosyl groups; 30. Chlorophyllase activity. C-1. Oxidation-reduction process; 2. Organonitrogen compound metabolic process; 3. Organonitrogen compound biosynthetic process; 4. Biological_process; 5.Amide biosynthetic process; 6. Translation; 7. Peptide met abolic process; 8. Peptide biosynthetic process; 9. Cellular amide metabolic process; 10. Metabolic process; 11. Purine nucleoside metabolic process; 12. Purine ribonucleoside metabolic process; 13. Single-organism metabolic process; 14. Purine-containing compound metabolic process; 15. Purine nucleoside triphosphate metabolic process; 16. Ribonucleoside triphosphate metabolic process; 17. Cytoplasmic part; 18. Ribonucleoprotein complex; 19. Ribosome; 20. Macromolecular complex; 21. Cytoplasm; 22. Oxidoreductase activity; 23. Structural constituent of ribosome; 24. Structural molecule activity; 25. Iron ion binding; 26. Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen; 27. Electron carrier activity; 28. Tetrapyrrole binding; 29. Heme binding; 30. Catalytic activity. D-1. 1. Oxidation-reduction process; 2. Biological_process; 3. Metabolic process; 4. Single-organism metabolic process; 5. Single-organism process; 6. Organonitrogen compound metabolic process; 7. Organonitrogen compound biosynthetic process; 8. Purine nucleoside metabolic process; 9. Purine ribonucleoside metabolic process; 10. Nucleoside metabolic process; 11. Glycosyl compound metabolic process; 12. Photosynthesis, light harvesting; 13. Purine-containing compound metabolic process; 14. Ribonucleoside metabolic process; 15. Cellular amino acid biosynthetic process; 16. Alpha-amino acid biosynthetic process; 17.Cell wall organization or biogenesis; 18. Purine nucleoside biosynthetic process; 19. Oxidoreductase activity; 20.Heme binding; 21.tetrapyrrole binding; 22.Catalytic activity; 23. Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen; 24. Electron carrier activity; 25. Iron ion binding; 26. Oxidoreductase activity, acting on single donors with incorporation of molecular oxygen; 27.Cofactor binding; 28.Ammonia-lyase activity; 29. linoleate 13S-lipoxygenase activity; 30. Oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen.

图 3 差异基因 GO 富集柱状图

Fig. 3 Histogram of GO enrichment of differentially expressed genes



圆点所在的位置和形状大小分别代表着富集的条目和差异基因的数量。

The location and size of the dots represent the number of enriched entries and the number of different genes, respectively. A-1. Vitamin B6 metabolism; 2. Stilbenoid, diarylheptanoid and gingerol biosynthesis; 3. Ribosome; 4. Proteasome; 5. Photosynthesis-antenna proteins; 6. Photosynthesis 7. Phenylpropanoid biosynthesis; 8. Pentose phosphate pathway; 9. Glyoxylate and dicarboxylate metabolism; 10. Glycolysis/Gluconeogenesis; 11. Glycine, serine and threonine metabolism; 12. Galactose metabolism; 13. Fructose and mannose metabolism; 14. Flavonoid biosynthesis; 15. Carbon metabolism; 16. Carbon fixation in photosynthetic organisms; 17. Biosynthesis of secondary metabolites; 18. Biosynthesis of amino acids; 19. Alpha-Linolenic acid metabolism; 20. Alanine, aspartate and glutamate metabolism. B-1. Vitamin B6 metabolism; 2.Stilbenoid, diarylheptanoid and gingerol biosynthesis3. Selenocompound metabolism; 4. Photosynthesis - antenna proteins; 5. Photosynthesis; 6. Phenylpropanoid biosynthesis; 7. Phenylalanine, tyrosine and tryptophan biosynthesis; 8. Phenylalanine metabolism; 9. Pentose phosphate pathway; 10. Monoterpenoid biosynthesis; 11. Metabolic pathways; 12. Linoleic acid metabolism; 13. Glycine, serine and threonine metabolism; 14. Flavonoid biosynthesis; 15. Cysteine and methionine metabolism; 16. Carbon metabolism; 17. Carbon fixation in photosynthetic organisms; 18. Biosynthesis of secondary metabolites; 19. Biosynthesis of amino acids; 20. Alpha-Linolenic acid metabolism; C-1. 1.Stilbenoid, diarylheptanoid and gingerol biosynthesis; 2. Ribosome; 3. Protein processing in endoplasmic reticulum; 4. Proteasome; 5. Porphyrin and chlorophyll metabolism; 6. Photosynthesis - antenna proteins; 7. Photosynthesis; 8. Phenylpropanoid biosynthesis; 9. Pentose phosphate pathway; 10. Oxidative phosphorylation; 11. One carbon pool by folate; 12. Glycolysis / Gluconeogenesis; 13. Glycine, serine and threonine metabolism; 14. Galactose metabolism; 15. Fructose and mannose metabolism; 16. Carbon metabolism; 17. Carbon fixation in photosynthetic organisms; 18. Biosynthesis of secondary metabolites; 19. Amino sugar and nucleotide sugar metabolism; 20. Alpha-Linolenic acid metabolism. D-1. 1. Stilbenoid, diarylheptanoid and gingerol biosynthesis; 2. Photosynthesis - antenna proteins; 3. Photosynthesis; 4. Phenylpropanoid biosynthesis; 5. Phenylalanine, tyrosine and tryptophan biosynthesis; 6. Phenylalanine metabolism; 7. Linoleic acid metabolism; 8. Glycolysis / Gluconeogenesis; 9. Glycine, serine and threonine metabolism; 10. Glutathione metabolism; 11. Glucosinolate biosynthesis; 12. Fructose and mannose metabolism; 13. Flavonoid biosynthesis; 14. Cysteine and methionine metabolism; 15. Carbon metabolism; 16. Carbon fixation in photosynthetic organisms; 17. Biosynthesis of secondary metabolites; 18. Biosynthesis of amino acids; 19. Amino sugar and nucleotide sugar metabolism; 20. alpha-Linolenic acid metabolism.

图4　差异基因 KEGG 富集图

Fig. 4　KEGG enrichment diagram of differentially expressed genes

上调，并进一步调控相关生物合成基因的表达；钙依赖蛋白激酶（CDPK）在9 d时被激活，并编码了1条下调和3条显著上调的基因（LOC102607727、XM_006466146.3、LOC102626879），可推断其通过调节下游蛋白来降低柑橘锈螨对沃柑的危害程度。

2.6.3　类黄酮合成途径差异基因分析　通过对类黄酮合成途径中的DEGs进一步分析，莽草酸-羟基肉桂酰基转移酶（HCT）编码的1条基因（XM_006464582.3）在柑橘锈螨危害3、5、7、9 d时均显著上调；咖啡酰辅酶A在5 d被激活并编码了1条下调

和1条显著上调的基因，7 d和9 d分别编码了3、2条显著上调的基因，其中基因（XM_006480524.3、XM_006466523.2）为两个时段共同的显著上调基因。

2.6.4 苯丙氨酸代谢途径差异基因分析 通过对苯丙氨酸代谢途径中的DEGs进一步分析，苯丙氨酸脱氨酶（PAL）在柑橘锈螨危害3、5、7、9 d时均编码了相同的4条显著上调基因（XM_006481431.3、XM_006485585.3、XM_006481430.3、XM_006488000.3），4-香豆蔻-辅酶A（4CL）编码的基因除3 d和5 d各有1条下调外，其余基因均上调，其中基因（XM_006494749.3）为4个时段共同的显著上调基因。

2.6.5 戊糖磷酸途径差异基因分析 通过对戊糖磷酸途径中的DEGs进一步分析，6-磷酸葡萄糖（6PGDH）在柑橘锈螨危害5、7、9 d时均编码了1条显著上调基因（XM_006482280.3），推测戊糖磷酸途径被激活并参与了沃柑对柑橘锈螨的胁迫响应。

3 讨 论

植物激素作为一种低分子化合物，不仅能对植物正常的生长起到调控作用，还可以影响植物的抗病虫害能力[39-40]。植物受到虫害时，茉莉酸Zim结构域蛋白（JAZ）可以与SCFCOI1复合体结合，激活茉莉酸信号转导和调控生物分子合成途径，提高植物的抗虫性[41]。水杨酸通过参与植物的系统获得性抗性（SAR），提高植物对螨害和植食性昆虫的防御能力，而水杨酸调节因子（NPR1）在SAR中起到核心调控的作用，是重要的调控因子[42-43]。本研究发现沃柑受到柑橘锈螨的危害后，JAZ编码的基因XM_006474637.3、XM_006465821.2、XM_006474477.3、XM_006465820.2在3、5、7、9 d时均显著上调；NPR1在危害后期被激活，其中7 d和9 d编码的共同基因XR_371294.3显著上调，进而影响激素分子的生物合成，由此推测茉莉酸、水杨酸的信号转导通路被激活并参与了沃柑对柑橘锈螨的胁迫响应。

植物在与害虫的相互作用中，形成了一系列与生物合成相关的防御机制。活性氧可以作为信号分子，诱导植物抗性基因的表达，而氧化酶同源酶（RBOH）则是作为必要的催化酶催化活性氧的产生[44]。植物受到外源胁迫会激活钙依赖蛋白激酶（CDPK）引起下游靶标蛋白磷酸化，从而降低胁迫伤害，提高自身抗性能力[45]。本研究发现沃柑受到柑橘锈螨的危害后，RBOH编码的基因XM_006472017.3、XM_006484987.3在3、5、7、9 d时均显著上调；CDPK在危害后期被激活，9 d编码了1条下调和3条显著上调的基因LOC102607727、XM_006466146.3、LOC102626879，通过调节下游蛋白来降低柑橘锈螨对沃柑的危害，推测柑橘锈螨胁迫激活了植物病原互作通路，响应基因可以作为抗螨候选基因。

次生代谢物质通过直接或间接的方式参与植物抗虫性物质的合成，影响害虫的发育和繁殖。莽草酸-羟基肉桂酰基转移酶（HCT）可以催化莽草酸与咖啡酰辅酶A生成绿原酸的反应，而绿原酸会引起幼虫食欲减弱、发育迟缓，在受到螨类危害时，茶树的绿原酸合成反应会变得活跃[46]。植物体内类黄酮化合物具有很好的杀螨功效，其效果随含量的提高而提高[47]。苯丙氨酸脱氨酶（PAL）和4-香豆蔻-辅酶A（4CL）是类黄酮合成途径的必需酶，植物受到外源胁迫后，该类酶基因的表达量会发生上调[48]。同时，苯丙氨酸脱氨酶（PAL）也会参与用于加固植物细胞壁的次生代谢物质合成途径，其基因的表达强度也可以作为植物的抗虫性指标[49]。本研究发现沃柑受到柑橘锈螨的危害后，HCT编码的基因XM_006464582.3在3、5、7、9 d时均显著上调；咖啡酰辅酶A在7 d和9 d编码的基因XM_006480524.3、XM_006466523.2为两个时段共同的显著上调基因。PAL编码的基因XM_006481431.3、XM_006485585.3、XM_006481430.3、XM_006488000.3在3、5、7、9 d时均显著上调；4CL编码的基因除3 d和5 d各有1条下调外，其余基因均上调，其中基因XM_006494749.3为4个时段共同的显著上调基因，推测沃柑通过合成具有抗螨性作用的次生代谢物质参与防御柑橘锈螨的过程中。

戊糖磷酸途径（PPP）参与植物体内的葡萄糖合成，同时在植物体的正常生长发育和环境胁迫中起到重要的作用[50]。Sindelar等[51]研究发现烟草组织受到马铃薯Y病毒侵染后，6-磷酸葡萄糖（6PGDH）的活性显著增强，参与烟草组织的抗病毒反应机制。本研究发现沃柑受到柑橘锈螨的危害后，6PGDH编码的基因XM_006482280.3在3、5、7、9 d时均显著上调，推测戊糖磷酸途径被激活并参与了沃柑对柑橘锈螨的胁迫响应。

4 结　论

笔者在本研究中采用高通量转录组测序技术对柑橘锈螨危害后的沃柑果皮进行测序和分析，筛选得到不同危害时段参与植物激素信号转导、植物病原互作通路、类黄酮生物合成、苯丙氨酸代谢、戊糖磷酸代谢等途径的多个差异表达基因，为深入研究沃柑响应柑橘锈螨危害的分子机制提供了丰富的基因资源，为柑橘类种质资源在螨害条件下的基因调控网络研究提供了一定的理论依据。

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