

外源山梨醇对桃苗叶片基因表达网络的影响

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摘要:【目的】研究外源山梨醇对桃叶片基因表达网络的影响。【方法】采用 $100 \text{ mg} \cdot \text{L}^{-1}$ 山梨醇溶液喷施桃幼苗, 以清水喷施为对照。转录组测序, 分析山梨醇处理样品中成熟叶(喷施山梨醇)和幼叶中(未喷施山梨醇)相关基因表达的变化。【结果】转录组测序表明, 成熟叶(喷施山梨醇)中差异表达的基因数多于幼叶(未喷施山梨醇)。成熟叶中与胁迫相关的基因表达上调, 次级代谢物代谢通路发生变化。幼叶中碳水化合物代谢相关基因表达发生改变。一些与环境应激相关的基因在成熟叶和幼叶中表达均上调, 与离子平衡、细胞内脂质代谢相关的一些基因表达均下调。【结论】山梨醇喷施后, 成熟叶中胁迫响应机制启动, 影响次级产物代谢。当相关信号传递到幼叶后, 碳水化合物代谢平衡发生相应变化, 响应相关刺激。基本明确了桃对高浓度外源山梨醇溶液喷施的响应过程及涉及的代谢通路。

关键词: 桃; 山梨醇; 转录组

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Effects of exogenous sorbitol spray on gene expression networks of peach leaves

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Abstract:【Objective】In many fruit trees of the Rosaceae family, sorbitol serves as the end products of photosynthesis and transporter carbohydrates, as well as an important signal substance in tissue development. Some previous studies had indicated that the accumulation of sorbitol might have a positive influence on soluble carbohydrates content and growth development. To understand how exogenous sorbitol affect metabolic pathways in source and sink leaf tissues, we investigated the transcriptional regulation of the gene expression networks in metabolic pathways after spraying the sorbitol on mature leaves in peach using RNAseq analysis.【Methods】The 6-month-old peach seedlings were sprayed by exogenous sorbitol solution ($100 \text{ mg} \cdot \text{L}^{-1}$), and the clean water treatment was employed as a control. Before spraying, the young leaves on the top of seedling were temporarily covered with a plastic bag, then removed 2 hours later. 48 hours after the spray, total RNA was extracted using Tritol reagent from at least 3 pieces of leaves mixture in each peach seedling groups respectively. The transcriptome sequencing library (300-400 bp DNA insertion) was constructed using NEB approaches, then the sequencing was performed on Illuminar Hiseq 2500 platform. The final sequencing reads were processed to trim off adaptors sequence and low-quality bases for following analysis. Finally all the filtered clean reads were aligned to the peach reference genome (Ppersica_298_v2.1, Phytozome) using TopHat2 and statistical analyses for differential gene expression were performed by cufflink script to analyze the changes of gene expression in mature leaves (with sorbitol spray) and young leaves (without sorbitol spray).【Re-

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sults】The reads were mapped to the peach genome. The percentages of unique reads pairs showed a perfect match from 74.8% to 80.7% in the young and the mature leaves under different spraying treatment. The results of transcriptome sequencing showed that the number of DEGs(Differentially Expressed Genes) in the mature leaves treated with sorbitol was more than that of the young leaves without sorbitol spray. A total of 665 DEGs were identified in the mature leaves and control samples. Among these DEGs, 408 (61.4%) genes were up-regulated, and 257 (38.6%) were down-regulated, while the number of that in the young leaves without sorbitol spraying were 253 (in total), 90 (up-regulated) and 163 (down-regulated) respectively. 5 genes responding to environmental stress remarkably increased at transcription level in both the mature leaves and the young leaves. These genes encode a Ribulose bisphosphate carboxylase(Pp.1G311400), Homeodomain-like superfamily protein (Pp.2G200400), ferretin (Pp.6G283700), TIR-NBS-LRR(Pp.8G023900) and TIR-NBS-LRR(Pp.8G027100) respectively. Meanwhile, 3 genes related to ionic equilibrium (oligopeptide transporter, Pp.4G004600; Aldolase-type TIM barrel protein, Pp.4G129800; natural resistance-associated macrophage protein, Pp.6G225900) and 2 genes involved in intracellular lipid metabolism (lipoxygenase, Pp.2G005500; terpene synthase, Pp.3G222200) were inhibited. Especially in the mature leaves, more stress genes identified and related secondary metabolites pathways were affected. Geneontology enrichment analysis showed that ATPase family (GO:0042626, 12 genes of DEGs, coupling to transmembrane movement of substances), hydrolase family (GO:0016820, 12 genes of DEGs, catalyzing transmembrane movement of substances), calcium ion transmembrane transporter family (GO:0015085, 7 genes of DEGs, stimulation-induced cellular transmembrane signal transduction) and primary active transmembrane transporter family (GO:0015399, 12 genes of DEGs) were crucial for osmolytes accumulation, transmembrane transportation and ion uptake under stress condition in the mature leaves after sorbitol spraying. Using Kyoto Encyclopedia of Genes and Genomes database, we annotated 52 differentially expressed genes participated in the biosynthesis of plant secondary metabolites regulating response to exogenous sorbitol, which resulted in the detected changes in gene expression of Linoleic acid metabolism, diterpenoid biosynthesis, propanoate metabolism, phenylpropanoid biosynthesis and amino sugar and nucleotide sugar metabolism. Unlike in the mature leaves, the expression of genes associated with carbohydrates metabolism was primarily modulated in the young leaves. It was noted that hydrolase family (GO:0004553, 7 genes of DEG) which hydrolyze O-glycosyl compounds and the special transcription factor(GO:0001071, GO:0003700, 6 genes of DEGs) played an acute role in response to endogenous stimulus from mature leaves after sorbitol spraying. The transcriptome evidence confirmed that the spraying treatment indirectly influenced carbohydrates metabolism in sink tissue, such as starch and sucrose metabolism, galactose metabolism, pentose phosphate metabolism, fructose and mannose metabolism, amino sugar and nucleotide sugar metabolism. 【Conclusion】After the application of sorbitol, the stress-response mechanisms in the mature leaves of peach initiated and affected secondary product metabolism. When the corresponding stress signals were transmitted to the young leaves, carbohydrates metabolism showed unbalance accordingly, responding to the relevant stimulus.

Key words: Peach; Sorbitol; Transcriptome

山梨醇是一种糖醇,又名山梨糖醇,与单糖的结构相似,是重要的化工原料。目前工业生产山梨醇是通过葡萄糖上的醛基还原为羟基来制得,价格成本低廉,规模生产容易。山梨醇最早从植物 moun-

tain strawberry 的果实中分离发现。现在已知它在蔷薇科木本植物中普遍存在。从微观的分子结构上看,带有6个羟基的山梨醇亲水性极强,是有效的渗透调节物质,可以有效地保持细胞内的水势。低浓度的

山梨醇可以帮助植物细胞在胁迫条件下尽可能地保持水势、稳定蛋白质的构象和内在膜结构的完整性。对蔷薇科果树来说,山梨醇更是不可缺少的重要碳水化合物,它是成熟叶中输出的主要光合产物之一,是稳定的运输物质和贮藏物质,在蔷薇科果树的生长和代谢中有重要作用。

前人研究表明,山梨醇是影响桃果实糖酸组分的因素之一^[1]。外源喷施山梨醇提高了苹果砧木——平邑甜茶(*Malus hupenensis* Rhed.)的抗旱性,在一定程度上缓解了干旱胁迫下的生长抑制^[2]。对苹果喷施2%(*w*)的山梨醇溶液可改善果实内在品质,提高可溶性固形物含量^[3]。对苹果果肉、叶圆片饲喂山梨醇可以诱导糖代谢酶活性变化,从而改变糖组分平衡^[4-5]。对桃喷施山梨醇后,成熟果实中蔗糖积累,葡萄糖和果糖含量下降^[6]。还有研究表明蔷薇科果树果实的风味在一定程度上取决于输入的山梨醇在果肉细胞液泡和细胞质中转变为其他类型糖的比例。除此之外,在其他非蔷薇科植物中,过表达山梨醇代谢基因,积累山梨醇也能提高植物的抗逆性。可见,山梨醇在植物抗逆和糖代谢进程中都起到重要的作用。但一直以来,对山梨醇外源喷施对蔷薇科果树内在影响的分子机制没有深入、系统地分析和研究,特别是很多研究只局限于特定的代谢产物和代谢基因,对整个基因网络和代谢网络的影响没有清晰的认识。低浓度与高浓度外源施用是否会造成结果差异不得而知,喷施带来的影响和持续时间以及造成的长远影响也需进一步评估和研究。故笔者拟采用转录组测序技术,通过分析山梨醇喷施后短期内桃叶片基因表达网络的变化,从基因转录水平重新认识外源高浓度山梨醇喷施对桃应激反应和代谢途径的短期影响。

1 材料和方法

1.1 材料

以桃实生苗为供试材料,其亲本为当地野生普通桃自交后代。由于不能保证实生苗之间的遗传背景完全一致,因此在试验设计中尽可能囊括一定数量的后代实生苗,形成混合池样品,在整体上做到遗传背景尽量接近。具体操作如下:选取穴盘种植的生长健壮、约30 cm高的半年生桃幼苗40株,划分为试验组和对照组,其中试验组和对照组各20株,置于人工气候室中培养(16 h光照,室温20 °C)。2016

年7月开展试验。

1.2 外源山梨醇处理

在前期的研究中,发现100 mg·L⁻¹的山梨醇溶液喷施树体可以改变桃成熟果实中可溶性糖组分的浓度^[6],故在本试验中继续采用该浓度山梨醇溶液处理幼苗。具体处理方法为:使用透明PE封口袋小心套住桃苗顶端幼叶。对试验组幼苗喷壶喷施100 mg·L⁻¹的山梨醇溶液,在保证PE封口袋中幼叶不沾染喷液的情况下,喷施下端成熟叶至全部湿润、药液滴落为止。对照组喷清水。喷施处理后48 h内未观察到明显的表型变化。

1.3 RNA的提取与转录组测序

喷施山梨醇和清水48 h后,采摘试验组和对照组桃苗幼叶混合样和成熟叶混合样进行试验(分2次采集对照组20株桃苗不同小枝不同叶位上的相应叶片,混合,获得对照组1和对照组2;相同操作采集试验组20株桃苗的相应叶片,混合,获得试验组1和试验组2)。Trizol法提取总混合叶RNA。采用Ultra RNA Library Prep Kit for illuminar (NEB, E7530L)构建测序文库:参考说明书起始RNA量1 μg,磁珠吸附富集mRNA,逆转mRNA合成双链cDNA;末端修复补平缺口加A,连接特定接头(adapter);筛选300~400 bp片段;利用特异序列对目的片段进行PCR扩增,富集产物构成高通量测序文库。

转录组测序在福建农林大学基因组与生物技术中心完成:Qubit标定测序文库DNA浓度,Agilent 2100 DNA芯片检测测序文库大小,并检测最终文库质量。Q-PCR精确定量文库浓度,按比例稀释文库至2 nmol·L⁻¹,cBOT成簇反应,利用 Illuminar Hiseq2500 PE100快速模式双端测序。

1.4 测序数据分析

对得到的原始数据进行过滤,Trim_galore(http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/)去除数据中index和adaper序列,保留测序质量Q20以上的数据,得到最终Clean Reads。利用TopHat^[7-8]比对Reads至桃参考基因组(Ppersica_298_v2.1, Phytozome, <https://phytozome.jgi.doe.gov/pz/portal.html>)。Cufflink^[9]分析生成的bam比对文件,cuffdiff^[9]检测样品间差异基因表达。在线GO(Gene Ontology)、KEGG(Kyoto Encyclopedia of Genes and Genomes)数据库对相关基因功能、类别、代谢通路富集情况进行注释。代谢通路的KEGG富集使用

hypergeometric test方法统计差异计算P值,BH FDR校正获得校正P值。

2 结果与分析

2.1 测序数据的产出与质量控制

所得12个样本文库Raw Reads的Q20值均大于93%,测序数据量为2.5~3.0 Gb。过滤低质量片

段获得Clean Reads比对桃参考基因组序列,各样品测序数据与所选参考基因组的比对率均超过81.0%,表明大部分测序reads可以定位到桃基因组上。且测序所得pair reads比对到基因组唯一位置比率最低为74.8%,说明大约超过74.8%的reads是特定基因的转录片段,可以明确相应的基因(表1)。

表1 转录组数据质量分析

Table 1 Data quality analysis in transcriptome

样本 Sample	Clean Reads Clean Reads number	比对到参考基因组 的reads总数 Clean Reads number mapping to reference genome	比对率 Rate of mapping/%	比对到参考基因组唯一 位置的pair reads数 Unique mapping PE reads	pair reads比对到基因组 唯一位置比例 Rate of unique mapping PE reads in clean reads/%
幼叶(对照组1) Young leaf (Control 1)	24 299 467	19 674 465	81.0	18 176 000	74.8
幼叶(对照组2) Young leaf (Control 2)	26 568 018	22 520 694	84.8	20 723 054	78.0
幼叶(试验组1) Young leaf (Treatment 1)	18 923 862	16 134 509	85.3	14 893 078	78.7
幼叶(试验组2) Young leaf (Treatment 2)	25 871 447	21 658 425	83.7	20 024 500	77.4
成熟叶(对照组1) Mature leaf (Control 1)	23 392 131	20 461 311	87.5	18 877 450	80.7
成熟叶(对照组2) Mature leaf (Control 2)	22 002 680	18 215 411	82.8	16 700 034	75.9
成熟叶(试验组1) Mature leaf (Treatment 1)	22 335 663	18 477 065	82.7	17 019 774	76.2
成熟叶(试验组2) Mature leaf (Treatment 2)	29 869 761	25 317 195	84.8	23 507 500	78.7

2.2 差异表达基因(DEGs)的筛选

使用cuffdiff检测差异表达基因转录本,筛选试验组与对照组表达量差异显著的基因,顶端幼叶与成熟叶样本中表达上调与下调基因数如表2所示。结果表明,喷施山梨醇的成熟叶中有665个注释基因的表达量较对照组成熟叶发生了显著的变化,其中表达上调基因数(408个)高于表达下调基因数(257个)。而未喷施到山梨醇溶液的顶端幼叶

中,与对照组幼叶相比只有253个基因表达量发生变化,表达上调基因数(90个)低于表达下调基因数(163个)。总体上看,与相应的对照组叶片相比,喷施了山梨醇的成熟叶中基因表达量明显多于未喷施到山梨醇的顶端幼叶。

进一步分析这些表达量差异基因可发现:山梨醇处理后,与相对对照组相比,有20个基因在成熟叶和幼叶中表达均上调,35个基因表达均下调;有12个基因在成熟叶中表达下调而在幼叶中上调,4个基因在成熟叶中表达上调而在幼叶中下调(图1)。

2.3 差异表达基因的GO注释

对处理组和对照组成熟叶差异表达基因进行GO注释,发现差异表达基因在新陈代谢这个功能小类中所占比例最高,其次为生物活性调节,胁迫应激响应、碳水化合物代谢反应也占相对较高的比例;而在幼叶中同样也是新陈代谢反应差异表达基因比例最高,碳水化合物代谢反应次之(图2)。

表2 叶中差异表达基因

Table 2 Differentially expressed genes (DEGs) in leaf tissue

试验组样本 Samples	表达上调基因数 Up-regulated genes	表达下调基因数 Down-regulated genes	差异基因 总数 DEG
幼叶(未喷施山梨醇) Young leaves (without sorbitol spraying)	90	163	253
成熟叶(喷施山梨醇) Mature leaves (with sorbitol spraying)	408	257	665

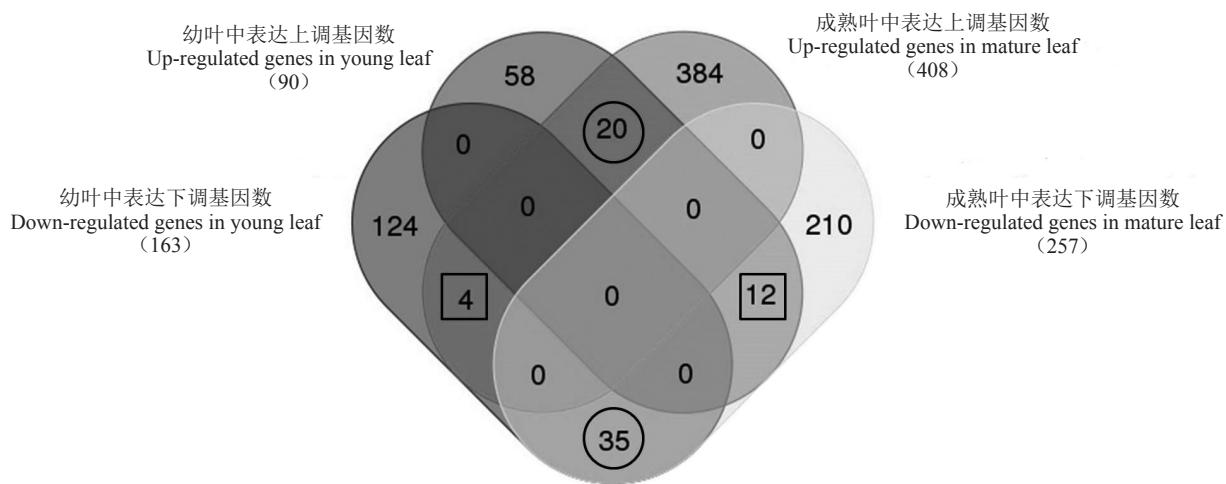
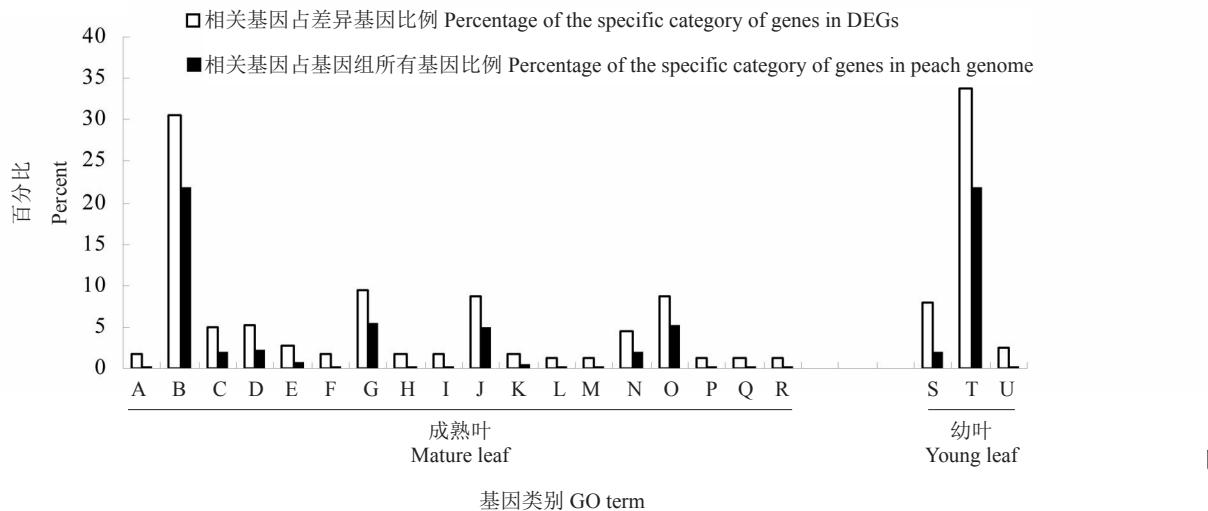


图1 外源山梨醇喷施后不同样品中差异表达基因数 Venn 图

Fig. 1 The Venn diagrams show the number of DEGs in variable samples spraying sorbitol solution



A. 生物刺激响应; B. 代谢过程; C. 胁迫响应; D. 刺激响应; E. 防卫反应; F. 细胞中物质动态平衡; G. 生物调节; H. 平衡调节过程; I. 糖代谢过程; J. 细胞代谢调节; K. 生物学质量调节; L. 胞内葡聚糖代谢; M. 葡聚糖代谢; N. 碳水化合物代谢; O. 生物过程调控; P. 胞内糖代谢; Q. ATP 代谢过程; R. ATP 生物合成; S. 碳水化合物代谢; T. 代谢过程; U. 碳水化合物分解代谢过程。

A. Response to biotic stimulus; B. Metabolic process; C. Response to stress; D. Response to stimulus; E. Defence response; F. Cellular homeostasis; G. Biological regulation; H. Homeostasis process; I. Polysaccharide metabolic process; J. Regulation of cellular process; K. Regulation of biological quality; L. Cellular glucan metabolic process; M. Glucan metabolic process; N. Carbohydrate metabolic process; O. Regulation of biological process; P. Cellular polysaccharide metabolic; Q. ATP metabolic process; R. ATP biosynthetic process; S. Carbohydrate metabolic process; T. Metabolic process; U. Carbohydrate catabolic process.

图2 差异表达基因的 GO 分类统计

Fig. 2 GO classification of differentially expressed genes

分类统计表达上调和下调基因,成熟叶中307个表达上调基因得到GO注释,富集到147个分类;表达下调基因中有222个得到GO注释,富集于77个GO分类中。这其中,成熟叶中与物质跨膜运输相关的ATP酶类(GO:0042626、GO:0043492)、水解

酶类(GO:0016820)、钙离子跨膜转运蛋白(GO:0015085)以及其他跨膜转运蛋白(GO:0015399)表达上调明显,一些裂解酶类(GO:0016835、GO:0016829、GO:0016836)、铁离子结合蛋白(GO:0005506)、血红素结合蛋白(GO:0020037)表达量下

调。而未喷施山梨醇溶液的幼叶中51个表达上调基因得到GO注释,富集到23个GO分类;表达下调基因中有118个得到GO注释,富集于35个GO分类中。糖苷水解酶(GO:0004553、GO:0016798)、淀粉水解酶(GO:0016161)、转录因子类蛋白(GO:0001071、GO:0003700)基因表达上调;一些催化酶

类(GO:0003824)、裂解酶类(GO:0016835、GO:0016829)、萜内酯合成酶(GO:0010333)、氧化还原酶(GO:0016491)基因表达下调(表3)。

与对照组相应叶片相比,喷施山梨醇后成熟叶和幼叶中表达量均上调的20个基因中,6个基因与应激反应有关,根据GO注释可知它们可以响应低

表3 差异表达基因GO富集分析(前5位)
Table 3 Go enrichment analysis of DEGs

差异表达基因 DEG	GO ID	GO基因分子功能注释 GO molecular function annotation	基因数 Gene number	P-value	q-value
下端成熟叶中表达上调基因 Up-regulated genes in mature leaf	GO:0042626	ATPase activity, coupled to transmembrane movement of substances	12	2.1e-07	3.041e-04
	GO:0016820	hydrolase activity, acting on acid anhydrides, catalyzing transmembrane movement of substances	12	3.1e-07	3.041e-04
	GO:0043492	ATPase activity, coupled to movement of substances	12	4.9e-07	3.041e-04
	GO:0015085	calcium ion transmembrane transporter activity	7	7.4e-07	3.041e-04
	GO:0015399	primary active transmembrane transporter activity	12	7.7e-07	3.041e-04
下端成熟叶中表达下调基因 Down-regulated genes in mature leaf	GO:0016835	carbon-oxygen lyase activity	12	1.1e-07	2.607e-04
	GO:0016829	lyase activity	15	9.8e-07	1.161e-03
	GO:0016836	hydro-lyase activity	7	6.5e-06	5.135e-03
	GO:0005506	iron ion binding	15	7.5e-05	4.444e-02
	GO:0020037	heme binding	15	0.000 13	6.162e-02
顶端幼叶中表达上调基因 Up-regulated genes in young leaf	GO:0004553	hydrolase activity, hydrolyzing O-glycosyl compounds	7	0.000 16	2.054e-01
	GO:0016161	beta-amylase activity	2	0.000 25	2.054e-01
	GO:0016798	hydrolase activity, acting on glycosyl bonds	7	0.000 26	2.054e-01
	GO:0001071	nucleic acid binding transcription factor activity	6	0.000 86	3.397e-01
	GO:0003700	transcription factor activity, sequence-specific DNA binding	6	0.000 86	3.397e-01
顶端幼叶中表达下调基因 Down-regulated genes in young leaf	GO:0003824	catalytic activity	67	2.3e-06	5.451e-03
	GO:0016835	carbon-oxygen lyase activity	6	0.000 17	2.015e-01
	GO:0016829	lyase activity	7	0.001 11	7.782e-01
	GO:0010333	terpene synthase activity	3	0.002 22	7.782e-01
	GO:0016491	oxidoreductase activity	18	0.002 68	7.782e-01

温胁迫、渗透胁迫的应答,响应茉莉酸、脱落酸刺激。成熟叶和幼叶中表达量均下调的35个基因中,5个基因与离子平衡、细胞内脂质代谢有关(表4)。

2.4 差异表达基因的KEGG注释与富集分析

对差异基因的代谢通路进行富集分析,当在 $p < 0.05$ 水平时,在对照组和处理组成熟叶间,可富集665个差异表达基因至12条代谢通路,多涉及次生代谢物生成与代谢。而在幼叶间,由于差异表达基因数目较少,可富集到6条代谢通路,涉及淀粉和蔗糖、半乳糖等碳水化合物代谢、光合碳固定、氮素代谢等(表5)。

2.5 碳代谢基因网络表达分析

成熟叶喷施山梨醇后,幼叶中相应糖代谢酶基因表达量出现不同变化,如以尿苷二磷酸葡萄糖为底物催化生成海藻糖的海藻糖磷酸合酶(2.4.1.15)和

海藻糖磷酸酶(3.1.3.12)基因表达上调;催化蔗糖水解为葡萄糖和果糖的转化酶(3.2.1.20)表达量上调,这符合植物响应渗透胁迫时提高可溶性单糖浓度,生成海藻糖的趋势。糖代谢网络基因表达量变动参见图3。

3 讨 论

3.1 山梨醇处理成熟叶引发胁迫响应和次级代谢物合成的改变

对山梨醇处理桃苗幼叶和成熟叶片中基因表达网络的变化进行比较分析,结果表明,成熟叶直接接触山梨醇溶液后,表达量发生变化的基因数目超过未接触山梨醇的幼叶。一定程度上说明喷施山梨醇后,成熟叶细胞产生了更多途径的防御机制来响应外界胁迫,并给成熟叶带来了更多代谢途径的改变。

表 4 幼叶、成熟叶中 6 个均上调基因、5 个均下调基因的 GO 注释
Table 4 Go annotation of 6 up-regulated expressed and 5 down-regulated expressed genes in co-regulated genes

		GO 注释 GO annotation								
差异基因 DEG		低温响应 Response to cold	盐胁迫响应 Response to salt stress	渗透胁迫响应 Response to osmotic stress	辐射响应 Response to radiation	金属离子响应 Response to metal ion	茉莉酸响应 Response to jasmonic acid	脱落酸响应 Response to abscisic acid	非生物刺激响应 Response to abiotic stimulus	内源刺激响应 Response to endogenous stimulus
表达上调基因 Up-regulated genes	■Pp.1G311400 二磷酸核酮糖羧化酶 Ribulose bisphosphate carboxylase	▲	▲	▲	▲	▲	▲	▲	▲	▲
	■Pp.2G200400 同源异型家族蛋白 Homeodomain-like superfamily protein	▲				▲				▲
	■Pp.6G283700 铁蛋白 Ferritin 1	▲		▲		▲		▲		▲
	■Pp.7G259000 糖基水解酶 Glycosyl hydrolase family protein	▲	▲	▲	▲	▲	▲	▲	▲	▲
	■Pp.8G023900 R 蛋白 TIR-NBS-LRR				▲					
	■Pp.8G027100 R 蛋白 TIR-NBS-LRR				▲					
差异基因 DEG	■Pp.2G005500 脂氧化酶 Lipoxygenase 2	铁离子平衡 Iron ion homeostasis	过渡金属离子平衡 Transition metal ion homeostasis	金属离子平衡 Metal ion homeostasis	阳离子平衡 Cation homeostasis	无机离子平衡 Inorganic ion homeostasis	离子平衡 Ion homeostasis	离子平衡 Cellular lipid metabolic	细胞脂质代谢 Cellular lipid metabolic	▲
	■Pp.3G222200 萜类合成酶 Terpene synthase	▲	▲	▲	▲	▲	▲	▲	▲	▲
	■Pp.4G004600 寡肽转运蛋白 Oligopeptide transporter	▲	▲		▲	▲	▲	▲	▲	▲
	■Pp.4G129800 TIM 桶道蛋白 Aldolase-type TIM barrel family protein	▲	▲		▲	▲	▲	▲	▲	▲
	■Pp.6G25900 天然抵抗力相关蛋白 Natural resistance-associated macrophage protein	▲	▲	▲	▲	▲	▲	▲	▲	▲

注: ■后为基因名, ▲表示该基因归属于相应的基因 GO 类群。

Note: ■ indicated gene names, ▲ indicated that the gene was defined to the related GO groups.

表5 差异基因KEGG注释与通路富集分析
Table 5 KEGG annotation and analysis of DEGs ($p < 0.05$)

Sample	KEGG Term	DEGs number	Corrected p -value
成熟叶(喷施山梨醇)	次生代谢物合成 Biosynthesis of secondary metabolites	52	2.55e-06
Mature leaves (with sorbitol spraring)	α -亚麻酸代谢 Alpha-Linolenic acid metabolism	7	0.000 371
	新陈代谢通路 Metabolic pathways	62	0.005 905
	单萜合成 Monoterpeneoid biosynthesis	3	0.007 363
	亚油酸代谢 Linoleic acid metabolism	3	0.011 579
	双萜合成 Diterpenoid biosynthesis	4	0.012 654
	谷胱甘肽代谢 Glutathione metabolism	7	0.025 523
	丙酸酯代谢 Propanoate metabolism	4	0.034 583
	苯丙烷合成 Phenylpropanoid biosynthesis	9	0.038 354
	苯丙氨酸、酪氨酸、色氨酸合成 Phenylalanine, tyrosine and tryptophan biosynthesis	5	0.041 795
	氨基糖、核酸糖代谢 Amino sugar and nucleotide sugar metabolism	8	0.044 934
幼叶(未喷施山梨醇)	蔗糖、淀粉代谢 Starch and sucrose metabolism	7	0.000 381
Young leaves (without sorbitol spraring)	新陈代谢通路 Metabolic pathways	32	0.000 703
	光合碳固定 Carbon fixation in photosynthetic organisms	6	0.001 242
	碳代谢 Carbon metabolism	9	0.004 647
	氮素代谢 Nitrogen metabolism	4	0.007 898
	半乳糖代谢 Galactose metabolism	4	0.013 630

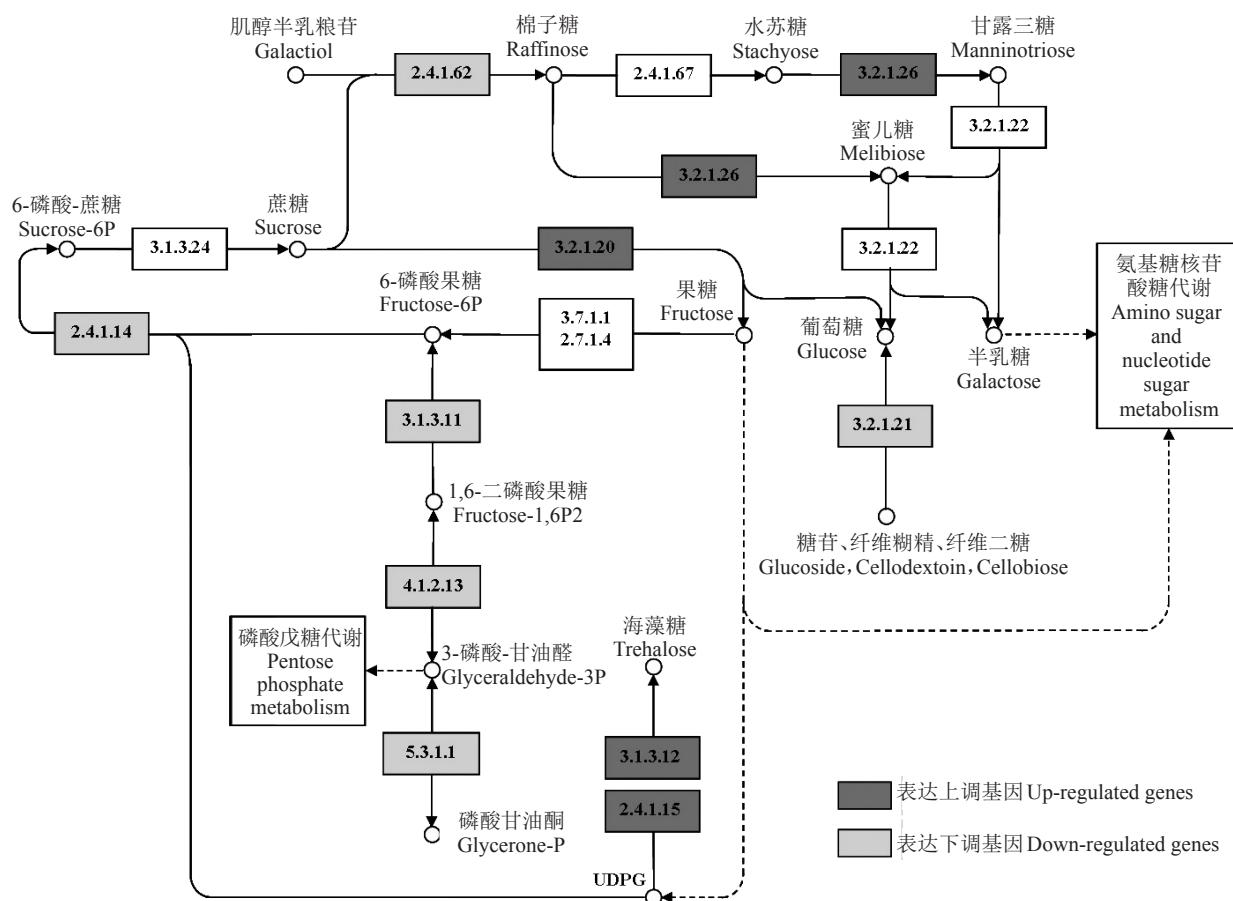


图3 幼叶中碳水化合物新陈代谢通路相关基因表达变化
Fig. 3 Related gene expression changes of carbohydrate metabolism

从差异表达基因的GO聚类分析和KEGG通路富集分析来看,成熟叶对外源山梨醇的响应包括了细胞内无机离子的积累。GO分析表达量上调基因表明主要是一些ATP酶和膜转运蛋白基因,这可能与无机离子的转运有关。因为现有的研究表明植物细胞发生渗透胁迫后,开始累积无机离子以调节渗透势,如旱生、盐生植物主要靠细胞内无机离子的累积来进行渗透调节^[10-12]。在过表达离子通道蛋白的植物中,离子浓度的提高能够增强植物对渗透胁迫的抗性^[13]。而植物对无机离子的吸收是主动运输过程,细胞中积累高浓度无机离子提高渗透水势抵御外界介质中胁迫物质。植物对无机离子的吸收和积累是耗能过程,需要大量ATP酶的水解。这些无机离子通过细胞膜和液泡膜上的膜转运蛋白进入细胞累积在液泡中,成为细胞中重要的渗透调节物质。因此对成熟叶喷施山梨醇后表现出了ATP酶和膜转运蛋白基因表达量上调的趋势。在本试验中,还观察到钙离子通道蛋白基因的上调,这可能是由于钙离子的摄入加强了植物对钠、钾无机离子的吸收,以抵御渗透失水胁迫^[14]。

此外,在植物中,有很多类酚类物质系通过苯丙烷途径(phenylpropanoid biosynthesis)产生,是植物体内重要的次生代谢产物,广泛参与胁迫应激保护、抵御病原菌等生物过程^[15-23]。在对成熟叶喷施山梨醇后,同样也发现了这些代谢通路相关基因表达的改变,表明次生代谢物的代谢也是桃对山梨醇应激反应的重要环节。

外源高浓度山梨醇的施用对桃叶形成了一定的渗透胁迫,植物主体上表现出胁迫应激反应,没有观察到与山梨醇代谢和糖代谢相关基因表达的大范围变化,说明短期内桃对高浓度山梨醇的反应是以应激反应为主。

3.2 幼叶通过改变碳水化合物代谢平衡响应外源山梨醇刺激

笔者设计了未沾染山梨醇溶液的幼叶比较试验,方便研究山梨醇处理对远端幼叶(库器官)的影响。结果表明,幼叶与成熟叶表现不同,由于没有直接接触高渗溶液,其差异表达基因数明显少于成熟叶。对这些差异基因进行GO聚类分析和KEGG通路富集聚集,发现主要是一些糖代谢网络中的基因表达发生改变。这很有可能是因为渗透胁迫的信号传导至幼叶后,细胞内双糖(蔗糖)、三糖(棉子糖)、

四糖(水苏糖)被催化水解为果糖、葡萄糖的趋势增强,以提高渗透势响应胁迫;同时机体内倾向于合成海藻糖,在恶劣环境条件下保护和稳定生物膜和生物大分子,有效地防止膜质层和蛋白质分子破坏失活。由此推测,库器官感应信号刺激后总体上倾向于多糖水解为单糖,提高渗透势。

综上所述,对桃喷施外源溶液后,直接接触山梨醇的成熟叶(光合源器官)快速启动胁迫响应机制,影响次级产物代谢,48 h内保持胁迫响应。与此同时相关信号传递到未直接接触山梨醇的顶端幼叶(光合库器官)后,碳水化合物代谢酶基因表达量发生一定变化,推测此时细胞内多糖倾向于水解为单糖,以响应刺激。通过本研究基本上可以明确桃对高浓度外源山梨醇溶液喷施的响应过程及涉及的代谢通路,但由于取样时间点只选取了48 h,在此之前以及之后所发生的复杂信号传导和生理代谢过程仍需进一步检测,特别是胁迫解除后对相关器官的影响有待进一步研究。

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