

# 应用抑制性差减杂交技术 研究枣缩果病病程相关基因

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**摘要:**【目的】了解细交链孢菌侵染枣果实过程的差异表达基因。【方法】以白熟期‘蜂蜜罐’枣的果实为材料, 人工接种细交链孢菌, 分别在接种0.5、1、2、3和4 d后取样, 通过抑制性消减杂交技术(suppression subtractive hybridization, SSH)构建了病原菌诱导的差异表达的cDNA消减文库。【结果】PCR鉴定随机挑取的阳性克隆, 显示插入片段大小为200~900 bp。随机挑选200个阳性克隆进行测序, 利用BLASTx在GenBank Nr数据库进行序列比对分析, 共获得118个Unigenes。对这些ESTs进行功能分类发现细交链孢菌侵染下诱导表达基因涉及植物细胞内的多种代谢和应答过程, 其中包括抗病/防御类、信号传导途径类、新陈代谢类、蛋白质合成和加工类及细胞结构的组成等, 尤其以抗病/防御类基因所占比例最大(27.17%)。【结论】通过SSH研究了缩果病病原侵染果实过程的差异表达基因, 鉴定到一些与枣缩果病相关的基因, 为进一步研究相关基因及今后筛选防治枣缩果病提供理论基础。

关键词: 枣缩果病; 抑制差减杂交技术; 表达序列标签

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## Identification of differentially expressed genes related with *Jujube fruit shrink disease* by suppression subtractive hybridization

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**Abstract:**【Objective】Jujube fruit shrink disease caused by *Alternaria alternata* is one of main fruit diseases of *Ziziphus jujuba*. It could reduce the yields and quality of jujube severely. Although the disease has been investigated at the tissue and physiological level, the molecular response of jujube fruits to *A. alternata* infection is still unclear. Investigation on the differentially expressed genes can help us better understand the molecular processes involved in the interactions between pathogen and jujube fruits. In this study, a suppression subtractive hybridization (SSH) technique was used to identify the differentially expressed genes in jujube fruits infected by *A. alternata* in order to illustrated the molecular response of jujube fruits to the disease.【Methods】The physiological race (ZS091) of *A. alternata* was provided by Henan Academy of forestry, China. The isolate was cultured on PDA medium for one week and the proliferated spores were dissolved in Tween-80, and adjusted to  $10^8$  conidia per mL in distilled water as the inoculum. The inoculum was artificially inoculated on the fruits of *Z. jujuba* ‘Fengmiguan’ in the white mature stage in the Jujube Experimental Station of Northwest A & F University, Qingjian county, Shaanxi province, China. The fruits inoculated with distilled water were set as the reference. We picked three fruits 0.5, 1, 2, 3 and 4 d after inoculation, respectively. The fruit samples were immediately frozen in liquid nitrogen, and transported to laboratory in dry ice and stored at -80 °C before RNA extraction. Total RNA was extracted with the ‘MiniBEST Plant RNA Extraction Kit’ reagent (Takara) according to manu-

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facturer's instruction. cDNA subtractive library on differential expression induced by the infection of *A. alternata* by SSH was constructed with PCR-Select™ cDNA Subtraction Kit (Clontech). Double-stranded cDNAs of tester (the pooled samples of *A. alternata*-inoculated fruits) and driver (non-inoculated fruits) were synthesized from 1 μg of mRNA, respectively. The secondary PCR amplicons were purified with Advantage 2 PCR Kits. The subtracted products were cloned in pMD19-T vectors and transformed to DH5α competent cells (TaKaRa). Blue/white selection of transformants was carried out using X-gal and IPTG. Selected positive clones were subjected to sequence on ABI3730xl. The obtained sequences were edited manually using DNA Star Software for removing vector sequences and then aligned in the CAP3 to generate consensus sequences. The obtained consensus sequences were subjected to homology analysis on NCBI using BLASTn and BLASTx to search for homologous sequences in the non-redundant nucleotide and protein databases, respectively. **【Results】**SSH cDNA library was successfully constructed from the *A. alternata*-inoculated 'Fengmiguan' fruits and the non-inoculated fruits in the white mature stage. In total, 1 000 cDNA library clones were obtained, among which 200 positive clones were selected randomly for sequencing, and finally 182 high quality ESTs were obtained. And 118 Unigenes, including 7 Contigs and 111 Singlet, were obtained after cluster analyses. Homologous analysis revealed that 84 (86.44%) of the ESTs matched known proteins while 34 (13.56%) of the ESTs had no significant similarity with deposited sequences. Those best matched proteins mainly involves in protein synthesis, protein degradation, disease defense response, protein modification and transportation, signal transduction, cell structure, transcriptional regulation, metabolism and energy, etc. For example, serine/threonine protein kinase, C2 domain-containing protein, scarecrow-like protein and Zinc finger protein genes were supposed to function as signal transduction and transcription regulating genes in response of plant to infection of pathogens. Superoxide dismutase, thaumatin-like protein, metallothionein protein might be involved in the defense response of jujube fruits to the disease. Some abiotic stress induced genes, including the genes for wound-responsive family proteins and heat shock proteins, also occurred several folds, which indicated that there was cross-talk between biotic and abiotic stresses. Genes involved in photosynthesis and energy metabolism were also included in acquired ESTs, indicating primary metabolites might function in jujube resistance to *A. alternata*. **【Conclusion】**In this study, 118 differentially expressed genes were identified from the jujube fruits artificially inoculated by *A. alternata*, among which several genes were involved in the plant disease-resistance response. The expression profiles would provide a good starting point for understanding the molecular processes involved in the plant-pathogen interactions. This study may provide insights into the molecular pathogenesis mechanism of jujube fruit shrink disease.

**Key words:** *Jujube fruit shrink disease*; Suppression subtractive hybridization; Expressed sequence tag

枣(*Ziziphus jujuba* Mill.)是鼠李科(Rhamnaceae)枣属植物,是我国重要的经济林树种。枣果实营养丰富尤其是维生素C含量高,入药还可润心肺、益气养容<sup>[1]</sup>。枣缩果病于20世纪70年代开始正式报道<sup>[2]</sup>,其主要危害枣果,在果实白熟期发病。病原侵染枣果后,造成枣果变小,果皮变色干缩,果肉海绵状坏死味苦,果实早落。缩果病发病范围广,在我国的山西、陕西、河南、河北等省份的枣种植区均有发生,经常造成毁灭性灾害,严重影响枣果的产量和品质<sup>[3-4]</sup>。前人通过常规分离、形态学鉴定、分子鉴定

和接种试验表明,细交链孢菌[*Alternaria alternata* (Fr.) Keissler]是引起枣缩果病的主要病原<sup>[3,5-6]</sup>。目前对枣缩果病的研究主要集中在药剂防治、品种抗病性的筛选、生理生化机制等方面<sup>[7-9]</sup>,而对细交链孢菌侵染后枣缩果病的分子机制研究较少。随着EST相关技术的发展与完善,从分子水平上了解细交链孢菌侵染枣过程中基因的差异表达,对今后防治枣缩果病有重要意义。近年来,利用抑制消减杂交(suppression subtractive hybridization, SSH)研究差异表达基因是简便而有效的方法,具有速度快、效率

高、灵敏度高、假阳性率低等优点,目前已广泛用于植物抗逆基因筛选以及生长发育相关基因研究中<sup>[10-11]</sup>。王洋等<sup>[12]</sup>成功构建了植原体胁迫下的抗枣疯病品种‘星光’的SSH-cDNA文库,共获得了200条高质量的表达序列标签(EST),功能分类后发现抗病防御相关ESTs丰度较高。于秀梅等<sup>[13]</sup>利用抑制差减杂交技术构建了条锈菌(*Puccinia striiformis*)诱导的小麦叶片cDNA文库,共获得172条高质量的ESTs。对功能已知的contigs分类,能量和初级代谢类占32.5%,其中与光合作用相关的基因出现频率最高;参与膜及转运、抗病与防御的基因分别位于第2、3位;次生代谢、蛋白质合成加工和细胞结构建成的基因较少。

为了全面了解细交链孢菌侵染枣果实过程中的基因的表达情况,揭示其分子机制,笔者利用人工接种细交链孢菌侵染‘蜂蜜罐’枣果实,采用SSH文库技术,研究细交链孢菌侵染枣果实后的基因表达情况,获得与枣缩果病相关的差异表达基因,尝试从分子水平揭示枣缩果病的相关机制,进而为防治枣缩果病奠定了理论基础。

## 1 材料和方法

### 1.1 植物材料与病原菌

本研究所采用的枣品种为‘蜂蜜罐’白熟期枣果实,采自西北农林科技大学林学院苗圃,细交链孢菌为ZS091生理小种,来源于河南省林业科学院。

### 1.2 细交链孢菌人工接种方法

将细交链孢菌在PDA培养基上培养7 d后,用吐温80溶解病原菌孢子,并用无菌水稀释,经血球计数板计数后,配成浓度为 $1\times10^8$ 个·mL<sup>-1</sup>的孢子悬浮液。当枣果进入白熟期时,选用长势相似的健康‘蜂蜜罐’枣作为试验材料。用75%的酒精冲洗干净后用无菌水冲洗3遍,将孢子悬浮液均匀地喷洒在健康枣果表面作为实验组,同时以健康枣果喷洒无菌水作为对照组。接菌后0.5、1、2、3和4 d,2组同时取样。将样品带皮削成薄片,液氮速冻后,置于-80℃冰箱中保存备用。

### 1.3 总RNA提取

采用Takara公司的MiniBEST Plant RNA Extraction Kit来提取每个时期中的实验组和对照组中的总RNA。琼脂糖凝胶电泳检测总RNA的完整性,核酸蛋白检测仪测定其纯度和浓度。将0.5、1、2、3和

4 d的RNA等量混合,以此作为构建SSH文库的起始材料。

### 1.4 SSH文库构建

以未接种细交链孢菌的cDNA为对照组,病菌诱导后的cDNA为实验组,按照PCR-SelectTM cDNA Subtraction Kit(Clontech)操作说明进行2次差减杂交。纯化后的PCR产物连接到pGEM-T Easy Vector载体中,连接产物经热激法转化到大肠杆菌感受态Top10中,涂于含有X-gal(2%)及IPTG(20%)的氨苄LB平板表面,37℃过夜培养。利用蓝白斑反应检测文库克隆的重组率,并筛选阳性克隆。随机挑取白色单菌落培养扩增,提取质粒DNA进行PCR扩增,电泳检测插入片段长度。

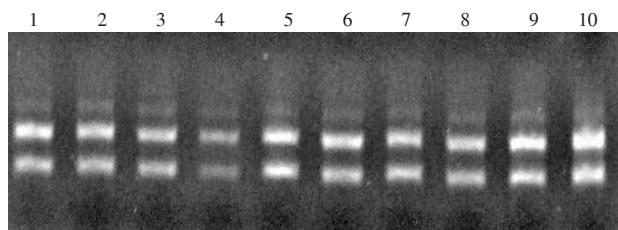
### 1.5 EST片段测序和序列生物信息学分析

随机挑取200个阳性克隆,提取并纯化其质粒DNA,送往上海生工有限公司进行测序。测序结果用DNASTar软件去除载体,将目的基因片段进行拼接整合及冗余序列的去除,对剩余序列进行与GenBank的蛋白数据库和核酸数据库进行BLASTx和BLASTn比对。以水稻<sup>[14]</sup>、拟南芥<sup>[15]</sup>功能分类为参照,进行筛选和功能注释。

## 2 结果与分析

### 2.1 总RNA质量检测

图1为试验和对照材料各时期果实总RNA电泳检测图,RNA有2条明显的带28 S和18 S,亮度在2:1左右。核酸蛋白检测仪检测后,A260/280的值为1.95~2.00,A260/230为2.00~2.10,说明总RNA完整且基本无降解,可用于构建SSH文库。



1~5. 枣果实接菌后0.5、1、2、3、4 d的RNA;6~10. 枣果实涂抹无菌水后0.5、1、2、3、4 d的RNA。

1~5. Total RNA extracted from jujube fruits 0.5, 1, 2, 3, 4 d after inoculated with *Alternaria alternate*, respectively; 6~10. Total RNA extracted from jujube fruits 0.5, 1, 2, 3, 4 d, after inoculated with sterile water, respectively.

图1 枣果实总RNA的电泳检测

Fig. 1 Total RNA from *Ziziphus*

## 2.2 两轮PCR产物扩增结果分析

将经过 *Rsa* I 酶切后的接种病原菌枣果的 cDNA 为实验组,以酶切后接种无菌水的枣果的 cDNA 为对照组,进行 2 次差减杂交。将未差减杂交的和差减杂交后的 cDNA 分别作为模板进行 2 次 PCR 扩增。如图 2 所示,未差减杂交的 cDNA 2 次 PCR 后的产物与差减后的第 2 次 PCR 产物相比较,差减后的第 2 次 PCR 产物有所下移,差减后一些片段消失,说明一些非特异性表达基因被去除,差减成功。

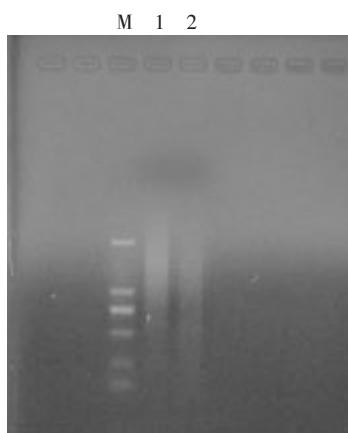
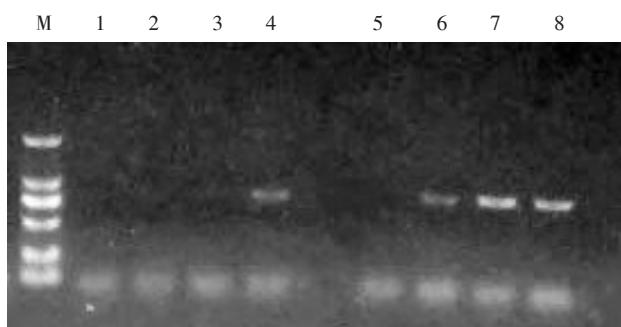


图 2 两轮 PCR 产物电泳分析

Fig. 2 Electrophoretic profile of second products

## 2.3 差减效率的检测

由图 3 可见,未差减的 cDNA 在扩增 20 个循环时就有条带产生,而差减后的 cDNA 在 30 个循环时候才有条带出现,10 个循环以上的差异说明大量组成型表达的基因已经被去除,进一步说明差减效率能达到建库要求。



M. Marker 2000;泳道 1~4. 以差减后的样品为模板扩增 15、20、25、30 个循环的 PCR 产物;泳道 5~8. 以未差减的样品为模板扩增 15、20、25、30 个循环的 PCR 产物。

M. Marker 2000; Lane 1~4. Secondary PCR products of subtracted cDNA of 15, 20, 25, 30 amplification cycles; Lane 5~8. Secondary PCR products of unsubtracted cDNA of 15, 20, 25, 30 amplification cycles.

图 3 差减效率的检测

Fig. 3 Evaluation of subtraction efficiency

## 2.4 文库质量的检测

从文库中随机挑取阳性克隆进行菌落 PCR 检测插入片段大小。由图 4 可知片段大小不同,基本分布在 200~900 bp,多数位于 500 bp 左右,说明本试验所构建的文库质量符合要求。

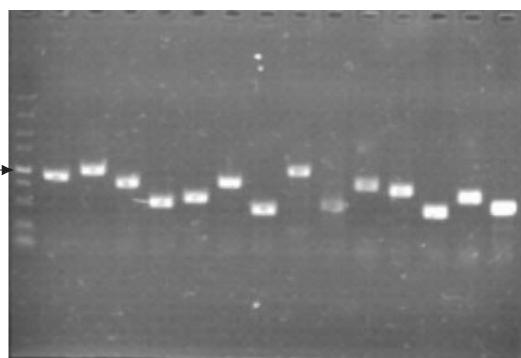


图 4 菌液 PCR 检测

Fig. 4 PCR amplification of bacteria from SSH-cDNA library

## 2.5 ESTs 序列分析

随机挑选的 200 个阳性克隆测序表明,去除低质量序列及重复序列后,共获得 118 条 EST。所获序列中最短的片段为 133 bp,最长的片段为 1 105 bp,平均长度为 430 bp。将测序结果到 GenBank 进行 BLASTx 和 BLASTn 比对,发现有 84 条 ESTs 可以找到已知的同源序列,占高质量数目的 86.44%。将已知同源序列按照拟南芥的功能分类为参照<sup>[16]</sup>,功能已知的 ESTs 主要包含参与蛋白质的降解、蛋白质合成修饰与运输、细胞结构组成、信号转导、抗病防御反应、新陈代谢与能量以及核酸的合成与代谢等相关的基因(表 1)。其中 3 类基因的含量较高,第 1 类是参与抗病与防御相关的基因,占 27.17%,第 2 类为新陈代谢与能量相关的基因,占 23.28%,第 3 类为蛋白质合成加工转运和分解的基因,占 22.34%。

## 3 讨 论

笔者为研究细交链孢菌侵染枣果实后的基因表达情况,构建了 SSH 文库。为监测细交链孢菌侵染枣果实的整个过程,分别于接种后 0.5、1、2、3 和 4 d 收集枣果实。为了避免低丰度差异表达基因的漏检,本文利用试剂盒法作为本试验提取 RNA 的方法,得到了适用于差减文库构建的高质量 RNA。阳性克隆测序后共组装成 118 条非冗余 ESTs,通过 Blastx 和 Blastn 比对,84 条获得同源性匹配,涉及枣

表1 抗病相关基因  
Table 1 The genes related with disease resistance

序列号 Clong	长度 Size/bp	描述 Description	种属 Organism	E值 E-Value	相似度 Identity/%
转录相关 Transcription					
3-3	989	SCL 蛋白 Scarecrow-like protein 8	枣 <i>Ziziphus jujuba</i>	4e-132	99
11-2	1 059	亮氨酸拉链 Basic leucine zipper	枣 <i>Ziziphus jujuba</i>	5e-18	82
13-9	396	WRKY 转录因子 35WRKY transcription factor 35	枣 <i>Ziziphus jujuba</i>	1e-49	95
10-3	306	WRKY 转录因子 31WRKY transcription factor 31	大豆 <i>Glycine max</i>	8e-32	78
2-3	530	反转录酶 Reverse transcriptase	生姜 <i>Zingiber officinale</i>	2e-20	82
蛋白降解相关 Protein degradation					
11-9	305	泛素聚合酶 E2Ubiquitin conjugating enzyme E2	鹰嘴豆 <i>Cicer arietinum</i>	8e-06	100
6-7	593	泛素结构域蛋白 Ubiquitin domain-containing protein 1-like	枣 <i>Ziziphus jujuba</i>	2e-74	94
18-1	394	泛素蛋白 Ubiquitin-like protein 5	桑树 <i>Morus notabilis</i>	1e-15	100
抗病/防御相关 Defense response					
16-1	418	类甜蛋白 Thaumatin-like protein 1b	枣 <i>Ziziphus jujuba</i>	8e-84	99
1	918	RING/U-box 超级蛋白 RING/U-box superfamily protein isoform 2	可可 <i>Theobroma cacao</i>	2e-149	83
7	244	F-BOX 蛋白 F-box family protein, putative isoform 1	可可 <i>Theobroma cacao</i>	1e-27	74
15-7	381	果胶酯酶抑制剂 Pectinesterase/pectinesterase inhibitor 34	枣 <i>Ziziphus jujuba</i>	3e-24	99
14-6	288	泛素连接酶 Ubiquitin-protein ligase	枣 <i>Ziziphus jujuba</i>	4e-07	100
17-4	285	半胱氨酸蛋白酶抑制剂 Cysteine proteinase inhibitor-like	枣 <i>Ziziphus jujuba</i>	1e-27	88
17-3	269	C2 结构域蛋白 C2 domain-containing protein	桑树 <i>Morus notabilis</i>	8e-41	86
15-9	336	甲硫氨酸硫还原酶 Peptide methionine sulfoxide reductase-like	枣 <i>Ziziphus jujuba</i>	8e-14	97
17-9	375	枣超氧化物歧化酶 Superoxide dismutase [Cu-Zn]	枣 <i>Ziziphus jujuba</i>	6e-70	99
4-3	373	金属硫蛋白 Metallothionein-like protein	覆盆子 <i>Rubus idaeus</i>	8e-21	73
19-5	303	病程相关蛋白 Pathogenesis-related protein 10	核桃 <i>Juglans regia</i>	2e-13	69
12-4	391	损伤响应家族蛋白 Wound-responsive family protein	可可 <i>Theobroma cacao</i>	3e-19	66
13-4	306	超敏反应蛋白 4Hypersensitive-induced response protein 4	枣 <i>Ziziphus jujuba</i>	5e-47	100
10-2	338	抗病蛋白 Protein enhanced disease resistance 2	枣 <i>Ziziphus jujuba</i>	3e-19	98
10-7	370	主要过敏原 Major allergen Pru av 1-like	枣 <i>Ziziphus jujuba</i>	4e-65	100
3-4	197	热激蛋白 Heat shock protein 21.4 (hsp 21.4)	报春 <i>Primula forrestii</i>	7e-04	100
蛋白质合成修饰与运输 Protein synthesis					
1-0	360	S3 核糖体蛋白 Ribosomal protein S3	榉树 <i>Zelkova serrata</i>	2e-45	95
11-5	364	40S 核糖体蛋白 Ribosomal protein 40S	大豆 <i>Glycine soja</i>	3e-26	98
11-6	884	组蛋白去乙酰化酶 Histone deacetylase 2	大豆 <i>Glycine soja</i>	4e-63	87
12-0	612	N-乙酰转移酶 Uncharacterized N-acetyltransferase YoaA-like	枣 <i>Ziziphus jujuba</i>	3e-116	100
11-4	685	抑制蛋白 Profilin-like	枣 <i>Ziziphus jujuba</i>	8e-47	74
3-5	319	60S 核糖体蛋白 L32-160S ribosomal protein L32-1-like	枣 <i>Ziziphus jujuba</i>	6e-66	98
18-5	263	40S 核糖体蛋白 S10-40S ribosomal protein S10	枣 <i>Ziziphus jujuba</i>	1e-102	95
6-4	246	KH 结构域蛋白 KH domain-containing protein	枣 <i>Ziziphus jujuba</i>	8e-89	98
15-2	639	WAT1 相关蛋白 WAT1-related protein	枣 <i>Ziziphus jujuba</i>	2e-55	97
19-1	178	肌醇转运蛋白 Probable inositol transporter 2, partial	枣 <i>Ziziphus jujuba</i>	2e-18	89
功能未知蛋白 Unknown					
3	186	假定蛋白 Hypothetical protein	苜蓿 <i>Medicago truncatula</i>	1e-16	95
11-8	253	假定蛋白 Hypothetical protein	桑树 <i>Morus notabilis</i>	4e-28	100
4-6	563	未知蛋白 Uncharacterized protein	枣 <i>Ziziphus jujuba</i>	1e-57	98
新陈代谢与能量相关 Metabolism process					
4-5	705	半胱氨酸合成酶 Cysteine synthase D1 isoform 4	可可 <i>Theobroma cacao</i>	2e-60	79
5-2	287	羧酸酯酶 Carboxylesterase 15	枣 <i>Ziziphus jujuba</i>	2e-30	99
5-5	749	$\beta$ -1,3-1,4 葡聚糖内切酶 Endo-1,3;1,4- $\beta$ -D-glucanase-like	枣 <i>Ziziphus jujuba</i>	5e-164	98
16-2	466	脱氢酶 X2Formate dehydrogenase, mitochondrial isoform X2	枣 <i>Ziziphus jujuba</i>	5e-06	100
2-7	143	谷氨酰胺合成酶 Glutamine synthetase	油菜 <i>Brassica napus</i>	1e-14	75
13-8	227	NADH 脱氢酶 Dehydrogenase [ubiquinone] iron-sulfur protein 7, mitochondrial	枣 <i>Ziziphus jujuba</i>	6e-40	98
14-5	380	单氧酶 Indole-3-pyruvate monooxygenase YUCCA10	枣 <i>Ziziphus jujuba</i>	7e-35	98
19-6	405	细胞色素 b6/f 复合物亚基 IV Cytochrome b6/f complex subunit IV (chloroplast)	大麻 <i>Cannabis sativa</i>	8e-32	97
3-8	929	叶绿素 a/b 结合蛋白 Chlorophyll a-b binding protein 8, chloroplastic	枣 <i>Ziziphus jujuba</i>	1e-161	99
4-9	319	3,4-羟基 2-丁酮激酶 Putative 3,4-dihydroxy-2-butane kinase	枣 <i>Ziziphus jujuba</i>	6e-136	99
10-6	309	半胱氨酸合成酶 Cysteine synthase, transcript variant X4	枣 <i>Ziziphus jujuba</i>	2e-16	99
11-1	246	O-酰基转移酶 O-acyltransferase WSD1-like, transcript variant X2	枣 <i>Ziziphus jujuba</i>	2e-60	100
13-2	284	腺苷酰硫酸还原酶 5'-adenylylsulfate reductase-like 7	枣 <i>Ziziphus jujuba</i>	7e-100	99
14-8	388	多聚腺苷二磷酸核糖聚合酶 Probable inactive poly [ADP-ribose] polymerase SR05	枣 <i>Ziziphus jujuba</i>	7e-171	98
17-2	159	$\beta$ -淀粉酶 Beta-amylase 1, chloroplastic	枣 <i>Ziziphus jujuba</i>	6e-43	94
细胞组成相关 Cell structure					
8-1	636	水通道蛋白 Aquaporin TIP1-1	桑树 <i>Morus notabilis</i>	1e-72	89
8-3	325	跨膜结构域蛋白 Transmembrane emp24 domain-containing protein 10	桑树 <i>Morus notabilis</i>	2e-11	66
10-0	937	叶绿体跨膜区蛋白 DUF21 domain-containing protein chloroplastic	枣 <i>Ziziphus jujuba</i>	7e-139	99
信号传导 Signal transduction					
3-9	250	丝氨酸/苏氨酸激酶 Serine/threonine-protein kinase receptor	木豆 <i>Cajanus cajan</i>	2e-45	95
6-0	753	组氨酸磷酸转移蛋白 Histidine-containing phosphotransfer protein 1-like	枣 <i>Ziziphus jujuba</i>	4e-10	99

抗病机制的各个方面。王洋等<sup>[12]</sup>利用植原体胁迫下的抗枣疯病品种‘星光’构建了SSH-cDNA文库,有89条序列与已知功能蛋白同源,本试验结果与之较为一致。对文库分析发现,某些基因在文库中的重复率偏高,如本试验获得的丝氨酸/苏氨酸激酶,在陆地棉接种黄萎病菌<sup>[17]</sup>和黄瓜接种尖孢镰刀菌后均有出现<sup>[18]</sup>。蛋白激酶是一类重要的信号分子,能及时对外界环境以及生长发育信号做出响应。丝氨酸/苏氨酸蛋白激酶是植物受体类蛋白激酶的主要形式,其作用是使蛋白质磷酸化,而蛋白磷酸化是最主要的细胞信号转导方式,几乎与植物的所有发育过程有关,已知在发育、转化、抗逆和抗病等生命活动中起重要的调控作用<sup>[19]</sup>。目前已经有很多植物上分离到蛋白激酶,这些蛋白激酶的磷酸化过程被证实参与到许多信号传导途径<sup>[20-21]</sup>。

在病原菌侵染初期,植物体内会出现短暂的活性氧的爆发,超氧化物歧化酶作为生物自由基的清除剂,具有清除逆境胁迫时体内过量的超氧化物自由基,维持活性氧代谢平衡的功能。研究表明SOD在参与植物抗病过程中起到重要作用<sup>[22]</sup>。在研究枣抗缩果病的生理生化机制时,发现SOD的活性随病菌的侵染在抗感品种体内均有不同程度的提高,且抗病品种的SOD活性上升早于感病品种<sup>[23]</sup>。以上结论与本研究中超氧化物歧化酶在SSH文库中大量出现这一结果是一致的。同时,植物在病原物诱导下的长期进化过程中会产生各种防御反应机制,如本试验分离的锌指蛋白,病程相关蛋白等基因均可参与植物的防御反应。锌指蛋白作为真核基因组中一类与Zn<sup>2+</sup>结合形成稳定的手指结构的转录因子,能够通过接受外界胁迫信号来激活或抑制某些基因的表达,使植物本身免于胁迫伤害,多属于植物的基础防御反应因子<sup>[19]</sup>。植物在受到真菌、细菌和病毒等病原体入侵或受到非生物胁迫时会产生和积累病程相关蛋白。PR10蛋白通常作为抵御病原入侵的标志,是植物自我防御机制中的可诱导组分<sup>[24]</sup>。类甜蛋白PR5,参与多种植物病原真菌侵染之后的主动防御反应,从而快速提高植物抗性。如当水稻纹枯病菌 *Rhizocotonia solani* 侵染水稻时,可诱导水稻TLPs基因表达,增强植物对该病原菌的抗性<sup>[25]</sup>。

通过对本试验中的基因表达分析可以看出,一些信号传导类、转录因子类、新陈代谢类以及蛋白质合成与降解与细胞结构类基因在病原菌侵染植物过

程中均有表达,说明植物对于外界环境胁迫的基础防御机制的一致性。本研究通过对SSH文库的EST进行序列分析,对获得的细交链孢菌侵染枣果中部分特异性表达的基因只进行了初步探讨。为了更系统、详尽地了解这些基因在细交链孢菌侵染枣果过程中表达模式和作用,需要对文库中筛选出的差异表达基因作进一步的定量分析和功能验证。且对于功能未知的基因可能与抗病相关的有重要研究价值的基因,尚有待于进一步研究。

## 4 结 论

本研究以‘蜂蜜罐’枣和细交链孢菌为材料,构建了细交链孢菌诱导12、24、48、72、96 h后的SSH-cDNA文库。经测序和生物信息学分析,发现可能参与细交链孢菌侵染枣果过程中的转录因子、信号传导和防卫基因等;这为进一步阐明细交链孢菌与枣互作机制、互作过程中相关基因的克隆和分离奠定了基础。本试验还找到一些未知功能的序列,对这些功能已知和未知的基因进一步研究,将对细交链孢菌侵染枣果实造成的生理生化变化具有指导意义。

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