基因芯片技术分析转*SlNAC10*基因拟南芥非生物胁迫相关差异表达基因

焦 杨 于静洋 李倩倩 李秋莉*

(辽宁师范大学生命科学学院, 辽宁省植物生物技术重点实验室, 大连 116081)

摘要 NAC(NAM、ATAF1/2和CUC2)转录因子是植物特有的转录调控因子, 在植物的器官建成、生长发育以及抵御非生物胁迫等方面发挥着至关重要的作用。该文利用基因芯片技术筛选转*SlNAC10*基因拟南芥和野生型拟南芥非生物胁迫抗性相关差异表达基因, 并通过实时荧光定量PCR对部分差异表达基因进行验证。芯片结果显示, 差异表达2倍以上的基因有4 054个, 其中与非生物胁迫相关基因有15个, 与非生物胁迫相关的转录因子基因有14个, 这些基因参与应答渗透胁迫、响应高盐、冷、热、高光强等胁迫。对差异表达2倍以上的基因进行GO(Gene Ontology)分析和KEGG(Kyoto Encyclopedia of Genes and Genomes)分析, 发现这些基因在非生物胁迫相关的13个注释中富集, 涉及相关代谢途径96个, 其中包括植物激素信号转导、精氨酸和脯氨酸代谢、吲哚生物碱合成、谷胱甘肽代谢等。以上结果表明, SlNAC10可直接或间接调控多种下游基因的表达, 提高植物抵御非生物胁迫的能力。

关键词 SlNAC10; 基因芯片; 非生物胁迫; 转基因拟南芥

Microarray Analysis of Differentially Expressed Genes Related to Abiotic Stress in *SlNAC10*-Transgenic *Arabidopsis*

Jiao Yang, Yu Jingyang, Li Qianqian, Li Qiuli*

(*College of Life Science, Liaoning Normal University, Key Laboratory of Plant Biotechnology of Liaoning Province, Dalian 116081, China*)

Abstract NACs (NAM、ATAF1/2 and CUC2) were plant specific transcription factors and played essential roles in plant morphogenesis, growth and development, and abiotic stress responses. In this study, microarray was used to analyze differentially expressed genes related abiotic stress in the wild-type (WT) and *SlNAC10*-transgenic *Arabidopsis* (L1), and the expression patterns of selected differentially expressed genes were further identified by quantitative Real-time PCR. A total of 4 054 differentially expressed genes were found, 15 of them were related to abiotic stress, and 14 were abiotic stress-related TFs. These genes were involved in responding to osmotic stress, high salinity, cold, heat, high light intensity respectively. The differentially expressed genes were subjected to GO (Gene Ontology) analysis and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways enrichment analysis. These genes enriched in 13 annotations related to abiotic stress and involved in 96 metabolic pathways, including plant hormone signal transduction, arginine and proline metabolism, indole alkaloid biosynthesis and glutathione

收稿日期: 2016-01-11 接受日期: 2016-06-16
国家自然科学基金(批准号: 31340052)资助的课题
*通讯作者。Tel: 0411-85827078, E-mail: skyliqiuli@163.com
Received: January 11, 2016 Accepted: June 16, 2016
This work was supported by the National Natural Science Foundation of China (Grant No.31340052)
*Corresponding author. Tel: +86-411-85827078, E-mail: skyliqiuli@163.com
网络出版时间: 2016-08-31 16:13:09 URL: http://www.cnki.net/kcms/detail/31.2035.Q.20160831.1613.006.html

metabolism. These results indicated that SlNAC10 regulated the expression of downstream genes directly or indirectly and enhanced plant resistance to abiotic stress

Keywords SlNAC10; microarray; abiotic stress; transgenic *Arabidopsis*

植物在生长发育过程中会遭受到各种非生物胁迫(低温、高盐、干旱等), 在长期进化过程中, 植物形成了诸多机制适应这些胁迫, 转录因子在植物适应逆境胁迫中起着重要作用。NAC(NAM、ATAF1/2和CUC2)转录因子是植物特有的转录因子[1], 迄今为止已经有超过100个家族成员在不同的物种中被陆续发现。NAC转录因子参与植物生长发育的各个方面, 能促进侧根发育[2]、参与调节衰老[3]和形成次生壁[4]等。NAC转录因子还参与植物适应非生物胁迫。海岸松(*Pinus pinaster*)中的*PpNAC2*和*PpNAC3*响应高盐、茉莉酸甲酯和机械损伤诱导[5]。二穗短柄草(*Brachypodium distachyon*)中的*BdNAC*响应干旱、盐、低温、高温和植物激素诱导[6]。锦鸡儿(*Caragana sinica*)中的*CiNAC*响应于盐、渗透胁迫和ABA诱导, 可促进侧根的形成以提高植株耐盐性[7]。*TaNAC67*定位于细胞核, 响应干旱、盐、冷和ABA胁迫, 过表达*TaNAC67*显著增强抗旱、抗盐和抗冻能力[8]。过表达*SNAC1*可促进棉花(*Gossypium spp*)根系发育且减少蒸腾速率, 提高抗旱和耐盐能力[9]。过表达*OsNAC5*的水稻(*Oryza sativa*)在开花期能扩大中柱和通气组织、增大根的直径, 从而提高水稻的抗旱能力[10]。一个*NAC*基因往往响应多个压力信号, 其蛋白质产物参与不同的调节过程, NAC可通过自身调节或交叉调节发挥抗逆作用。目前, 关于NAC响应非生物胁迫的文章很多, 大部分研究尚处于基因克隆、结构鉴定和表达分析等层面上, 对于NAC抵御非生物胁迫机制研究还处于起步阶段。NAC016可直接与脱落酸反应元件AREB1结合抑制AREB1转录, 参与干旱胁迫响应[11]。过表达基因芯片分析发现, ANAC092调控拟南芥中多个盐胁迫下衰老相关基因的表达[12]。在玫瑰(*Rosa hybrid*)中, RhNAC3可增强脱水耐受性; 拟南芥中, 过表达*RhNAC3*发现其参与ABA信号通路, 可提高其抗旱能力[13]。本实验前期克隆了辽宁碱蓬(*Suaeda liaotungensis*)*SlNAC10*基因, 通过蘸花法转化拟南芥, 获得了转*SlNAC10*基因拟南芥, 过表达*SlNAC10*可提高转基因拟南芥在干旱、低温和高盐环境下的存活率。本研究利用基因芯片技术对转*SlNAC10*拟南芥的基因表达进行分析, 旨在探究NAC转录因子调节的下游基因, 进一步揭示其参与植物逆境条件下的信号调节网络。

1 材料与方法

1.1 实验材料

拟南芥(*Arabidopsis thaliana* L.)(Columbia)野生型(WT)为本实验室保存, 转*SlNAC10*基因拟南芥(L1)为实验室筛选获得的转基因纯合株系。

1.2 幼苗培养和取样

L1和WT种子经无菌水浸泡后, 用70%酒精及10%次氯酸钠洗涤, 无菌水冲洗, 完成拟南芥种子的消毒。将经过消毒处理的种子播种于MS培养基上, 置于黑暗条件下春化3 d, 转移至培养箱(23 ℃, 16 h光照/8 h黑暗)培养10 d, 待幼苗长出3~4片叶子时转移至营养土中培养, 每周浇水1次。培养2周后, 分别取L1和WT幼苗叶片, 迅速置于液氮中速冻保存。

1.3 RNA提取和检测

采用TaKaRa RNA提取试剂盒(RNAiso Plus)提取样品总RNA, NanoDrop ND-2000分光光度计及Agilent Bioanalyzer 2100检测样品总RNA质量。

1.4 基因芯片

本实验所用芯片为拟南芥全基因组表达谱4×44K芯片, 由安捷伦科技有限公司提供。

1.5 RNA的放大和标记

样品RNA采用Agilent表达谱芯片试剂盒(Low Input Quick Amp Labeling Kit, One-Color)经标准操作流程对样品进行放大和标记, 并用RNeasy Mini Kit纯化标记后的cRNA。

1.6 芯片杂交

使用Agilent标准杂交试剂盒(Gene Expression Hybridization Kit)在分子杂交炉(Hybridization Oven)中滚动杂交17 h(65 ℃, 10 r/min), 在洗缸中洗片, 所用的试剂为Gene Expression Wash Buffer Kit。

1.7 芯片扫描

完成杂交的芯片采用Agilent扫描仪进行图像扫描, 扫描分辨率为5 μm, PMT 100%。用Feature Extraction Software 10.7读取数据, 最后采用Gene Spring Software 12.6.1进行归一化处理, 所用的算法为Quantile。

1.8　芯片数据分析

对归一化处理的数据进行差异基因分析, 计算得出两样品之间差异倍数, 筛选差异表达基因(fold change>2或<0.5)。对差异基因进行GO分析, 筛选相关GO terms, 寻找与非生物胁迫抗性相关的基因。对差异基因进行KEGG分析, 筛选相关Pathway。

1.9　实时荧光定量PCR(quantitative Real-time PCR, qRT-PCR)验证基因芯片结果

利用*Actin 2*基因(NM_112764)为内参, 从芯片分析结果中选择8个差异表达的基因进行实时荧光定量PCR。引物由生工生物工程(上海)股份有限公司设计并合成。分别取两样品RNA采用TaKaRa反转录试剂盒(PrimeScript™ RT reagent Kit with gDNA Eraser)合成cDNA, 利用TaKaRa公司Thermal Cycler Dice Real time分析荧光信号值, 获得各样品基因Ct值, 以$2^{-\Delta\Delta Ct}$方法计算目的基因相对表达量。

2　结果

2.1　RNA提取和检测

样品RNA检测结果显示, RNA条带清晰, 无降解, 28S和18S比值接近2:1(图1)。两个样品浓度均大于100 ng/μL, 样品总量大于1.5 μg, D_{260}/D_{280}比值在1.8~2.0之间。提取的RNA完整性好、纯度较高, 可以进行后续基因芯片实验。

2.2　芯片扫描

对芯片进行荧光扫描, 扫描图中可见杂交信号及背景清晰, 结果可靠(图2)。对芯片数据进行散点图分析, 散点图中每个点代表芯片上的探针点, 横坐标为WT的信号值, 纵坐标为L1信号值。落在图形中灰色区域的点表示差异倍数小于2, 灰色区域两侧的点表示差异倍数大于2。越偏离y=x直线的点差异倍数越大, 黑色上调表达, 灰色为下调表达。散点图中大部分基因的表达是没有差异的, 部分基因表达有差异, 差异倍数不同(图3)。SlNAC10调控了部分基因的表达。

2.3　差异表达的基因统计

对表达倍数小于2差异表达基因进行统计分析。结果显示, 在43 604个基因中, 差异表达基因4 054个, 基因差异表达倍数分布情况见表1, 其中上调表达50倍以上的基因有10个(表2)。在2倍以上差异表达基因中, 通过关键词检索找到与非生物胁迫相关基因有15个(表3), 它们参与渗透胁迫, 响应高盐、冷、热、高光强等胁迫。除此之外, 还找到了与胁迫相关的转录因子14个, 其表达倍数也有不同程度的改变(表4)。

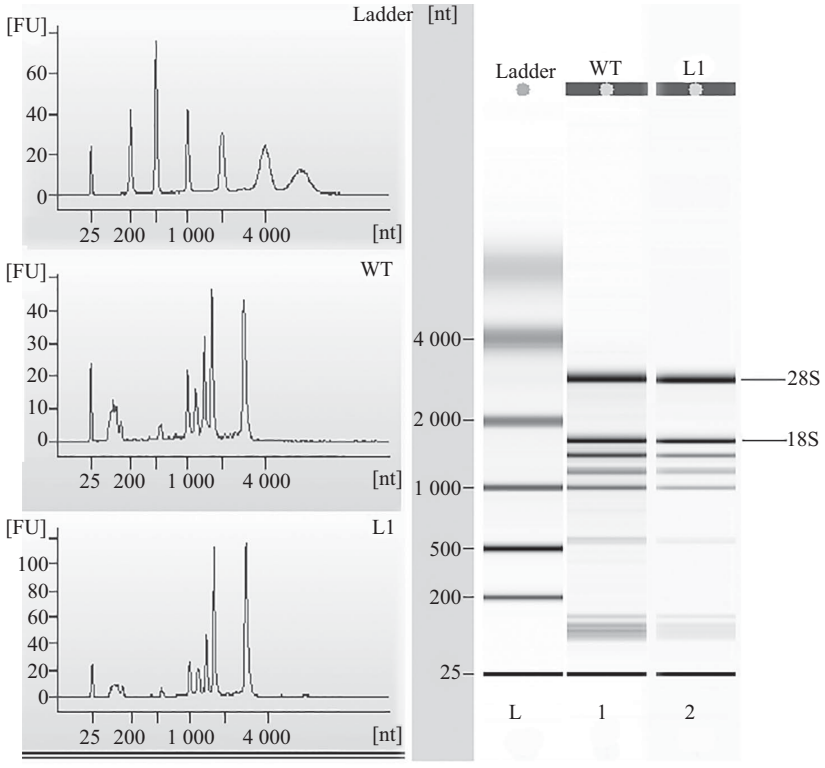

图1　RNA Agilent Bioanalyzer 2100检测结果图

Fig.1　The results of RNA test by Agilent Bioanalyzer 2100

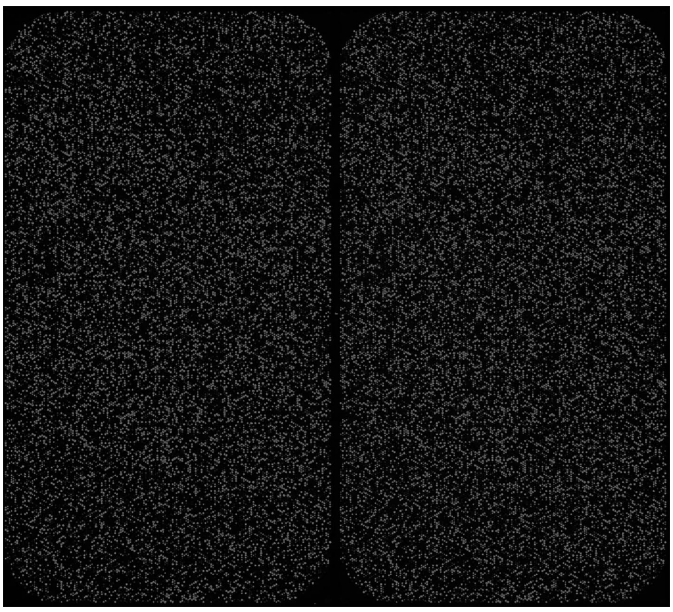

图2 WT(左)与L1(右)拟南芥基因芯片扫描图

Fig.2 The results of chip scanning of WT (left) and L1 (right) *Arabidopsis*

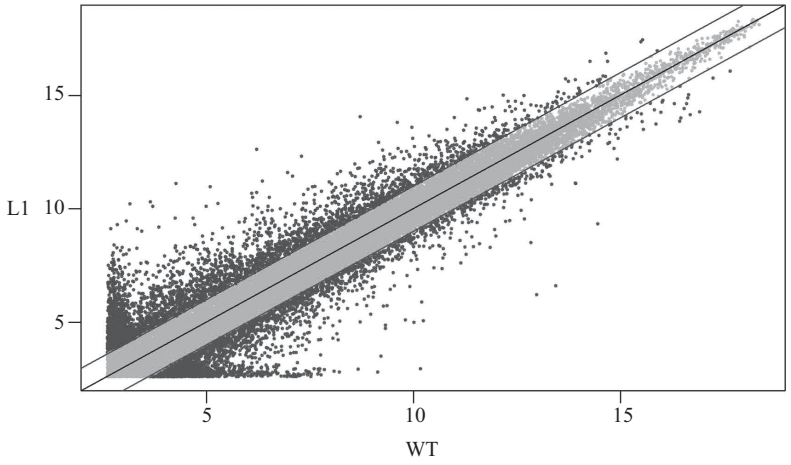

图3 芯片数据散点图

Fig.3 Scatter plot of microarray data

表1 差异表达基因上调和下调倍数分级分析

Table 1 The grading analysis of genes down-regulated and up-regulated

差异倍数 Fold change (FC)	上调基因数 The number of up-regulated genes	下调基因数 The number of down-regulated genes
FC>100	3	3
100≥FC>50	7	2
50≥FC>40	8	4
40≥FC>30	13	7
30≥FC>20	29	30
20≥FC>10	140	44
10≥FC>5	407	244
5≥FC≥2	1 747	1 366
Total	2 354	1 700

表2　上调表达50倍以上的基因

Table 2　The genes up-regulated more than 50 times

探针名称 Probe name	差异倍数 Fold change	基因代码 Gene symbol	基因名称 Gene name	描述 Description
A_84_P551808	135.057 710 9	AT3G01345	Hypothetical protein	*Arabidopsis thaliana* uncharacterized protein mRNA
A_84_P21341	114.754 617 8	ATPMEPCRB	Probable pectinesteraseinhibitor 41	*Arabidopsis thaliana* probable pectinesterase inhibitor 41 mRNA
A_84_P807679	101.666 509 4	AT5G15780	Pollen Ole e 1 allergen and extensin family protein	*Arabidopsis thaliana* pollen Ole e 1 allergen and extensin family protein mRNA
A_84_P576622	88.263 331 64	AT2G32200	Hypothetical protein	*Arabidopsis thaliana* uncharacterized protein mRNA
A_84_P22911	86.324 729 3	AT2G14900	Gibberellin-regulated protein	Gibberellin-regulated protein (*Arabidopsis thaliana*)
A_84_P193394	84.951 340 05	AT3G16670	Pollen Ole e 1 allergen and extensin family protein	*Arabidopsis thaliana* pollen Ole e 1 allergen and extensin family protein mRNA
A_84_P16974	84.893 147 48	*GER3*	Germin-like protein subfamily 3 member 3	*Arabidopsis thaliana* germin-like protein subfamily 3 member 3 mRNA
A_84_P12704	84.656 632 7	AT3G27200	Plastocyanin-like domain-containing protein	*Arabidopsis thaliana* plastocyanin-like domain-containing protein mRNA
A_84_P19115	59.176 542 43	AT2G43590	Chitinase family protein	*Arabidopsis thaliana* chitinase family protein mRNA
A_84_P867334	55.606 229 1	AT3G27200	Plastocyanin-like domain-containing protein	Plastocyanin-like domain-containing protein

表3　非生物胁迫相关的差异表达基因

Table 3　The differentially expressed genes related to abiotic stress

探针名称 Probe name	差异倍数 Fold change	基因代码 Gene symbol	描述 Description
A_84_P13733	4.160 208 977	*SZF1*	SZF1 (SALT-INDUCIBLE ZINC FINGER 1); transcription factor
A_84_P597426	0.459 241 524	*HSA32*	HEAT-STRESS-ASSOCIATED 32; catalytic
A_84_P11914	5.857 243 613	AT4G12400	Stress-inducible protein, putative
A_84_P835451 A_84_P14164	2.109 934 188 0.183 796 551	*HOS10*	HIGH RESPONSE TO OSMOTIC STRESS 10; DNA binding/transcription regulator
A_84_P19231	8.508 546 501	*HSFA2*	Heat stress transcription factor A-2 (*Arabidopsis thaliana*)
A_84_P10923	0.487 197 285	AT3G62550	Universal stress protein (USP) family protein
A_84_P810987	2.230 673 71	*ERD15*	Dehydration-induced protein ERD15 (*Arabidopsis thaliana*)
A_84_P13852	0.147 608 018	*HSP21*	HSP21 (HEAT SHOCK PROTEIN 21)
A_84_P819143	2.154 416 972	*CPHSC EAT SHOCK PROTEIN_70-2*	CPHSC70-2 EAT SHOCK PROTEIN 70-2 (CHLOROPLAST HEAT SHOCK PROTEIN 70-2); ATP binding
A_84_P23171	2.596933372	*HSFA7A*	AT-HSFA7A; DNA binding; transcription factor
A_84_P12521	2.061 642 81	*HSP60-2*	HSP60-2 (HEAT SHOCK PROTEIN 60-2); ATP binding
A_84_P584092	5.860 044 012	AT4G28088	Hydrophobic protein, putative; low temperature and salt responsive protein, putative
A_84_P226439	0.274 929 572	*HDG11*	HDG11 (HOMEODOMAIN GLABROUS 11); DNA binding; transcription factor
A_84_P21570	2.946 769 673	*GORK*	GORK (GATED OUTWARDLY-RECTIFYING K+CHANNEL); cyclic nucleotide binding; inward rectifier potassium channel; outward rectifier potassium channel
A_84_P582947	2.775 622 179	*NHX4*	NHX4 (SODIUM HYDROGEN EXCHANGER 4); sodium ion transmembrane transporter; sodium: hydrogen antiporter

表4 差异表达的转录因子

Table 4 The differential expression of transcription factors

探针名称 Probe name	差异倍数 Fold change	基因代码 Gene symbol	描述 Description
A_84_P16608	7.709 073 591	*MYB55*	myb domain protein 55; DNA binding; transcription factor
A_84_P17156	12.672 766 52	*AtMYB103*	DNA binding; transcription activator; transcription factor
A_84_P16918	17.113 590 86	*MYB119*	DNA binding; transcription factor
A_84_P10033	20.847 735 79	*MYB98*	DNA binding; transcription factor
A_84_P16606	5.794 585 638	*WRKY22*	Transcription factor
A_84_P221516	7.847 244 219	*WRKY59*	Transcription factor
A_84_P20925	6.070 050 84	*WRKY67*	Transcription factor
A_84_P22607	3.399 43268	*ERF104*	Transcription activator; transcription factor
A_84_P820417	3.861 964 76	*ERF6*	DNA binding; transcription activator
A_84_P11410	12.666 886 05	*ERF14*	DNA binding; transcription activator
A_84_P15068	2.503 011 119	*bZIP3*	DNA binding; transcription factor
A_84_P257180	2.320 787 773	*bZIP34*	Basic helix-loop-helix domain-containing protein
A_84_P826599	2.548 006 574	*bZIP9*	DNA binding; protein heterodimerization; transcription factor
A_84_P10906	3.684 860 491	*bZIP61*	DNA binding; transcription activator; transcription factor

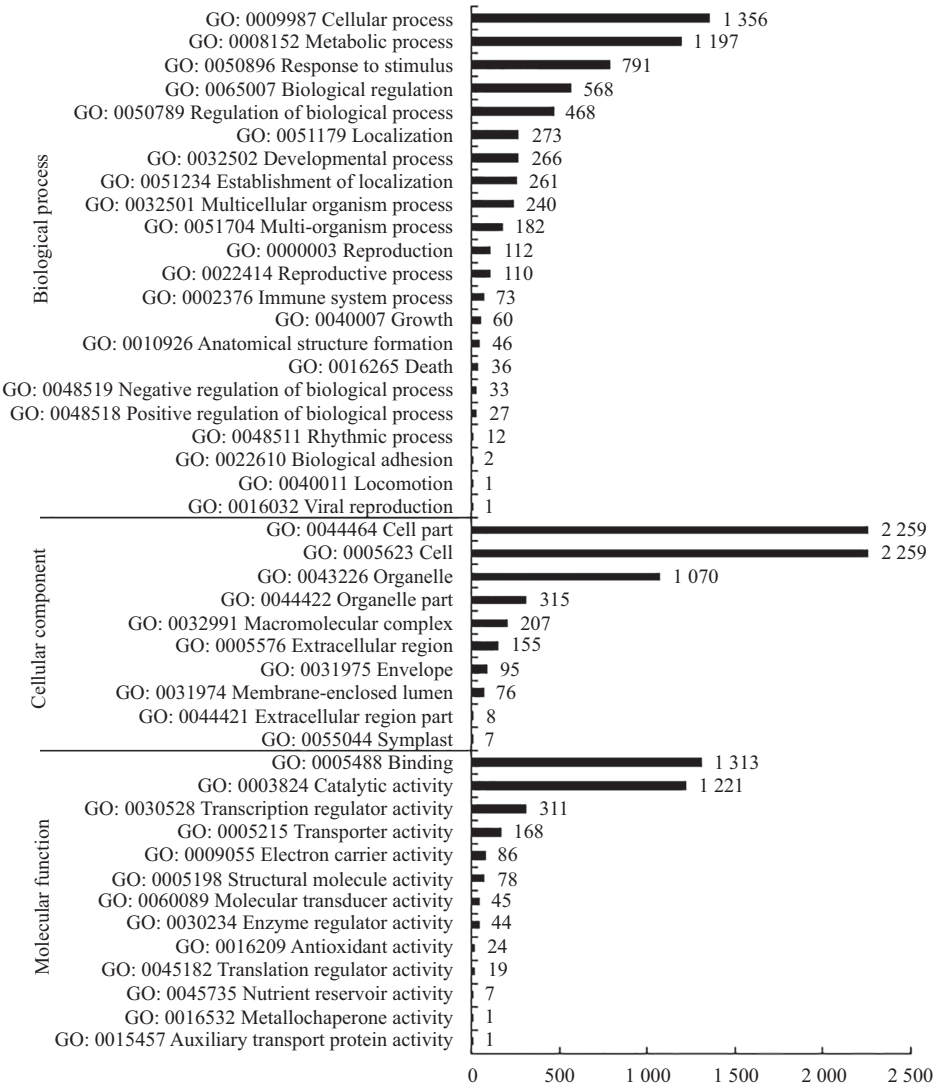

图4 差异基因GO注释

Fig.4 The GO analysis of differentially expressed genes

2.4 差异基因GO注释

GO富集度统计学分析表明, 差异表达基因中生物学过程(biological process)相关基因3 243个, 占33.16%; 细胞组分(cellular component)相关基因3 210个, 占32.82%; 分子功能(molecular function)相关基因3 327个, 占34.02%。生物学过程相关基因中主要涉及细胞过程(22.17%)、新陈代谢过程(19.57%)、响应刺激(12.94%)、生物调节过程(9.28%)和生物过程调节(7.65%)等。细胞组分相关基因中包括细胞和细胞成分(35.02%)、组织(16.59%)、组织成分(4.88%)、大分子复合物(3.21%)等。分子功能相关基因主要涉及结合(39.57%)、催化活性(36.80%)、转录调节活性(9.37%)和转录活性(5.06%)等(图4)。差异表达基因在响应渗透胁迫、氧化胁迫和激素刺激等非生物胁迫注释中富集(表5)。

2.5 差异基因KEGG分析

KEGG分析表明, 差异表达基因涉及的相关代谢途径共有96个, 其中包括植物激素信号转导、精氨酸和脯氨酸代谢、吲哚生物碱合成、谷胱甘肽代谢等(表6)。植物激素在种子萌发、根系生长、延时或促进衰老、抵御生物和非生物胁迫等方面起作用, ABA介导的信号途径是植物响应非生物胁迫的信号

表5　非生物胁迫相关差异基因GO注释

Table 5　The GO analysis of differentially expressed genes associated with abiotic stress

GO代码 GO ID	名称 Name	数量 Hits	总数 Total	基因 Genes	*P*值 *P* value	Q值 Q value
GO: 0009628	Response to abiotic stimulus	316	1 992	*GORK*, *LTP*, *MYB96*, *MYB51*, *DREB1A*, *ERD15*, *P5CS1*	0.000 0	0.000 0
GO: 0006970	Response to osmotic stress	118	660	*MYB96*, *MYB7*, *GAI*, *AtMYB32*, *HOS10*, *JR1*, *ABS1*	0.000 0	0.000 0
GO: 0006979	Response to oxidative stress	72	422	*HSP21*, *MYC2*, *ERD5*, *APX5*, *P5CS1*	0.000 0	0.000 0
GO: 0080134	Regulation of response to stress	2	5	*BT2*	0.096 7	0.214 8
GO: 0009266	Response to temperature stimulus	115	648	*GORK*, *DREB1A*, *AT3G16450*, *AT3G16460*, *HOS10*, *CBF1*	0.000 0	0.000 0
GO: 0009409	Response to cold	83	458	*GORK*, *DREB1A*, *HOS10*, *JR1*, *CA1*, *AT5G24090*	0.000 0	0.000 0
GO: 0009408	Response to heat	39	205	*HSP21*, *ABI2*, *ATHSFA2*, *AT2G27140*	0.000 0	0.000 1
GO: 0009414	Response to water deprivation	54	303	*GORK*, *LTP3*, *MYB96*, *MYB2*, *DREB1A*, *CBF1*, *ERD15*, *P5CS1*	0.000 0	0.000 0
GO: 0009788	Negative regulation of abscisic acid mediated signaling	5	23	*CIPK15*, *ABI2*, *TMAC2*, *MIR159B*, *ATAF1*	0.060 9	0.157 9
GO: 0009787	Regulation of abscisic acid mediated signaling	7	44	*MYB101*, *CIPK15*, *GPCR*, *ABI2*, *TMAC2*, *ATAF1*	0.096 3	0.214 8
GO: 0009753	Response to jasmonic acid stimulus	66	240	*GORK*, *MYB113*, *MYB30*, *ERF2*, *ERF1*, *MYB95*, *MYB59*, *MYB34*	0.000 0	0.000 0
GO: 0009751	Response to salicylic acid stimulus	58	225	*MYB96*, *WERK40*, *WRKY18*, *MYB7*, *WRKY38*, *MYB59*, *MYB30*	0.000 0	0.000 0
GO: 0009850	Auxin metabolic process	15	84	*DFL1*, *AAO1*, *HCT*, *ASB1*, *AT5G17300*, *PIN5*	0.009 0	0.035 4

表6　差异表达基因KEGG分析

Table 6　KEGG analysis of differentially expressed genes

通路名称 Pathway name	数量/总数 Hits/total	基因 Genes	P值 *P* value	Q值 Q value
Plant hormone signal transduction	51/232	*ERF1*, *ERF2*, *ABI2*, *AHBP-1B*, *MYC2*	0.000 0	1.0E–4
Porphyrin and chlorophyll metabolism	15/47	*GSA2*, *PORA*, *PORB*, *ATFER1*, *CLH2*	3.0E–4	0.012 2
alpha-linolenic acid metabolism	10/30	*AOC1*, *AOC3*, *OPR1*, *HPL1*, *AOS*	0.002 3	0.045 9
Phenylpropanoid biosynthesis	22/109	*4CL3*, *PAL4*, AT3G28200, AT4G11290	0.003 1	0.049 4
Cysteine and methionine metabolism	18/84	*ACS11*, *ACS6*, *ATMS1*, *BUD2*, *ATMS2*	0.004 2	0.054 8
Nitrogen metabolism	10/43	*ACA1*, *ATGSR1*, *CA1*, *PAL4*, *GUD2*	0.018 5	0.161 3
Arginine and proline metabolism	11/66	*P5CS1*, *ERD5*, *ATGSR1*, *BUD2*, *GDH2*	0.083 2	0.297 0
Glutathione metabolism	10/62	*APX5*, *ATGSTF2*, *ATGSTF3*, *GGT3*	0.110 2	0.374 2
Indole alkaloid biosynthesis	2/7	*MES5*, *MES9*	0.191 0	0.466 9

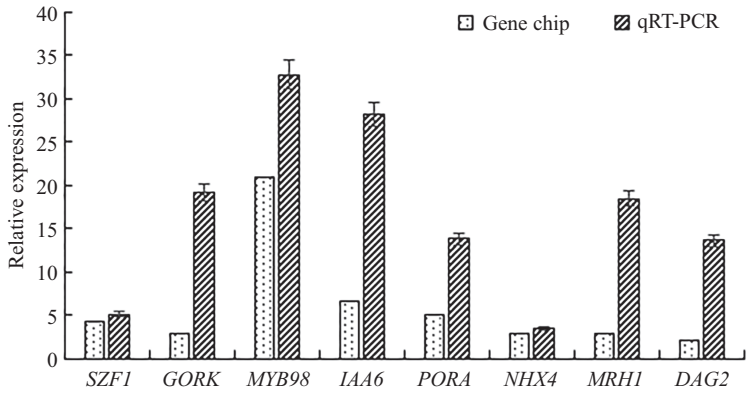

图5 芯片与荧光定量PCR结果对比

Fig.5 The comparisons on analysis result of microarray and qRT-PCR

转导途径之一。脯氨酸作为渗透调节剂和渗透保护剂, 在植物体内积累是适应低温、干旱和盐渍化等逆境的一个很重要的机制, 在植物抵御非生物胁迫方面有重要作用。

2.6 差异表达基因的实时荧光定量PCR结果

为验证基因芯片所筛选的差异表达基因, 本文选取了8个基因, 以*Actin 2*作为参照基因, 进行实时荧光定量PCR分析。结果显示, 两者所得基因表达差异的趋势一致(图5), 表明基因芯片结果是可靠的。

3 讨论

转*SlNAC10*基因拟南芥中, *ATPMEPCRB*上调114.75倍(表2)。*ATPMEPCRB*是细胞壁合成过程中的果胶酯酶抑制剂, 在植物抵御冷害、冻害中起重要作用。过表达同亚家族的*AtPME41*基因发现, 其可依赖BR信号转导途径提高转基因拟南芥抵御低温胁迫的能力[14], 推测SlNAC10可能通过调控*ATPMEPCRB*的表达提高转基因拟南芥的抗冻性。

非生物胁迫相关基因中, *AT4G28088*(5.86倍)、*AT4G12400*(5.86倍)、*SZF1*(4.16倍)上调表达倍数较高(表3)。目前, 没有对于响应低温和盐诱导蛋白质AT4G28088、结构蛋白质AT4G12400功能的研究。SZF1是受盐胁迫诱导的锌指结构蛋白质, 参与植物盐胁迫响应。盐处理下可快速诱导*AtSZF1*和*AtSZF2*表达, *atszf1*/*atszf2*双突变体与野生型相比显示出了更高的盐敏感性[15]。推测*SlNAC10*通过调控*SZF1*表达提高转基因拟南芥的抗盐性。

差异基因中存在多个转录因子家族(MYB、ERF、WRKY等)(表4)。*MYB98*上调20.84倍, 在抵御各种胁迫、响应激素信号以及许多植物代谢和发育过程有调节作用。通过系统发育和基因芯片数据分析, 水稻(*Oryza sativa*)中的OsMYB可被干旱胁迫诱导, 参与抗旱调节[16]。在干旱胁迫下, MYB被显著诱导表达, 酵母双杂交(Y2H)分析显示, 1R-MYB转录因子可调节鹰嘴豆(*Cicer aretinum* L.)耐旱机制的能力[17]。差异基因中有18个*WRKY*基因上调表达, WRKY结构蛋白质是转录因子超家族, 在拟南芥中存在高达100种, 参与防御病原体、机械损伤和毛状体发育过程中的基因表达, 在植物衰老期间起调节作用[18]。*WRKY53*依赖过氧化氢参与信号转导特异性调节衰老基因表达[19]。*ERF14*上调12.66倍, 且*ERF1*、*ERF2*涉及“plant hormone signal transduction”途径。ERF是一个大的转录因子基因家族, 通过特定功能结构域与下游基因启动子上的特定序列结合, 调节下游基因的表达, 在植物的生长、发育及抵御各种生物和非生物胁迫中有重要意义[20]。过表达JERF3增强烟草种子萌发和幼苗发育过程中抵御干旱、冷冻和渗透胁迫能力, 调节氧化基因表达, 提高超氧化物歧化酶活性, 减少ROS积累, 提高抗旱、抗冻和抗盐能力[21]。我们推测, NAC可能是一个比较上游的转录因子, 可以通过调控其他下游的转录因子, 如MYB、WRKY、ERF等的表达, 从而提高植物非生物胁迫抗性。

差异基因GO注释表明, *GORK*基因在“response to water deprivation”、“response to temperature stimulus”等多个GO注释中富集(表5)。GORK是唯一存在于保卫细胞的外向整流K^+通道, 调节K^+外流, 控制气孔关闭, 抑制KAT1通道活性, 同时提高ABA含量[22]。拟南芥中GORK功能被破坏, 导致气孔关闭,

GORK在干旱适应过程中起着重要的作用[23]。推测*SlNAC10*通过调控*GORK*表达提高转基因拟南芥的抗旱性。

综上所述, NAC对植物抵抗非生物胁迫的调节过程是十分复杂的, 既包括直接对参与生理生化途径基因的调节, 也包括与其他转录因子相互作用参与调控。本研究以野生型和转*SlNAC10*基因拟南芥为材料, 通过过表达*SlNAC10*基因, 利用基因芯片技术寻找NAC调控的下游基因及相关的代谢途径。下一阶段我们将从中选择部分基因进行后续的研究, 希望能够完善NAC在植物抵御非生物胁迫过程中调控的下游基因网络。

参考文献 (References)

1 Aida M, Ishida T, Fukaki H, Fujisawa H, Tasaka M. Genes involved in organ separation in *Arabidopsis*: An analysis of the cup-shaped cotyledon mutant. Plant Cell 1997; 9(6): 841-57.

2 Li J, Guo G, Guo W, Guo G, Tong D, Ni Z, *et al*. miRNA164-directed cleavage of ZmNAC1 confers lateral root development in maize (*Zea mays* L.). BMC Plant Biol 2012; 12: 220.

3 Balazadeh S, Siddiqui H, Allu AD, Matallana-Ramirez LP, Caldana C, Mehrnia M, *et al*. A gene regulatory network controlled by the NAC transcription factor ANAC092/AtNAC2/ORE1 during salt-promoted senescence. Plant J 2010; 62(2): 250-64.

4 Zhong R, Lee C, Ye ZH. Evolutionary conservation of the transcriptional network regulating secondary cell wall biosynthesis. Trends Plant Sci 2010; 15(11): 625-32.

5 Pascual MB, Cánovas FM, Ávila C. The NAC transcription factor family in maritime pine (*Pinus Pinaster*): Molecular regulation of two genes involved in stress responses. BMC Plant Biol 2015; 15: 254.

6 You J, Zhang L, Song B, Qi X, Chan Z. Systematic analysis and identification of stress-responsive genes of the NAC gene family in Brachypodium distachyon. PLoS One 2015; 10(3): e0122027.

7 Han X, Feng Z, Xing D, Yang Q, Wang R, Qi L, Li G. Two NAC transcription factors from *Caragana intermedia* altered salt tolerance of the transgenic Arabidopsis. BMC Plant Biol 2015; 15: 208.

8 Mao X, Chen S, Li A, Zhai C, Jing R. Novel NAC transcription factor TaNAC67 confers enhanced multi-abiotic stress tolerances in *Arabidopsis*. PLoS One 2014; 9(1): e84359.

9 Liu G, Li X, Jin S, Liu X, Zhu L, Nie Y, *et al*. Overexpression of rice NAC gene SNAC1 improves drought and salt tolerance by enhancing root development and reducing transpiration rate in transgenic cotton. PLoS One 2014; 9(1): e86895.

10 Jeong JS, Kim YS, Redillas MC, Jang G, Jung H, Bang SW, *et al*. OsNAC5 overexpression enlargesroot diameter in rice plants leading to enhanced drought tolerance and increased grain yield in the field. Plant Biotechnol J 2013; 11(1): 101-14.

11 Sakuraba Y, Kim YS, Han SH, Lee BD, Paek NC. The *Arabidopsis* transcription factor NAC016 promotes drought stress responses by repressing AREB1 transcription through a trifurcate feed-forward regulatory loop involving NAP. Plant Cell 2015; 27(6): 1771-87.

12 Balazadeh S, Siddiqui H, Allu AD, Matallana-Ramirez LP, Caldana C, Mehrnia M, *et al*. A gene regulatory network controlled by the NAC transcription factor ANAC092/AtNAC2/ORE1 during salt-promoted senescence. Plant J 2010; 62(2): 250-64.

13 Jiang G, Jiang X, Lü P, Liu J, Gao J, Zhang C. The rose (*Rosa hybrida*) NAC transcription factor 3 gene, RhNAC3, involved in ABA signaling pathway both in rose and *Arabidopsis*. PLoS One 2014; 9(10): e109415.

14 Qu T, Liu R, Wang W, An L, Chen T, Liu G, *et al*. Cryobiology. Brassinosteroids regulate pectin methylesterase activity and AtPME41 expression in *Arabidopsis* under chilling stress. Cryobiology 2011; 63(2): 111-7.

15 Sun J, Jiang H, Xu Y, Li H, Wu X, Xie Q, *et al*. The CCCH-type zinc finger proteins AtSZF1 and AtSZF2 regulate salt stress responses in Arabidopsis. Plant Cell Physiol 2007; 48(8): 1148-58.

16 Smita S, Katiyar A, Chinnusamy V, Pandey DM, Bansal KC. Transcriptional regulatory network analysis of MYB transcription factor family genes in rice. Front Plant Sci 2015; 6: 1157.

17 Ramalingam A, Kudapa H, Pazhamala LT, Garg V, Varshney RK. Gene expression and yeast two-hybrid studies of 1R-MYB transcription factor mediating drought stress response in chickpea (*Cicer arietinum* L.). Front Plant Sci 2015; 6: 1117.

18 Eulgem T, Rushton PJ, Robatzek S, Somssich IE. The WRKY superfamily of plant transcription factors. Trends Plant Sci 2000; 5(5): 199-206.

19 Miao Y, Laun T, Zimmermann P, Zentgraf U. Targets of the WRKY53 transcription factor and its role during leaf senescence in Arabidopsis. Plant Mol Biol 2004; 55(6): 853-67.

20 Sakuma Y, Liu Q, Dubouzet JG, Abe H, Shinozaki K, Yamaguchi-Shinozaki K. DNA-binding specificity of the ERF/AP2 domain of *Arabidopsis* DREBs, transcription factors involved in dehydration- and cold-inducible gene expression. Biochem Biophys Res Commun 2002; 290(3): 998-1009.

21 Wu LJ, Zhang ZJ, Zhang HW, Wang XC, Huang RF. Transcriptional modulation of ethylene response factor protein JERF3 in the oxidative stress response enhances tolerance of tobacco seedlings to salt, drought, and freezing. Plant Physiol 2008; 148(4): 1953-63.

22 Eisenach C, Papanatsiou M, Hillert EK, Blatt MR. Clustering of the K^+ channel GORK of *Arabidopsis* parallels its gating by extracellular K^+. Plant J 2014; 78(2): 203-14.

23 Hosy E, Vavasseur A, Mouline K, Dreyer I, Gaymard F, Porée F, *et al*. The *Arabidopsis* outward K^+ channel GORK is involved in regulation of stomatal movements and plant transpiration. Proc Natl Acad Sci USA 2003; 100(9): 5549-54.