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·研究报告·

拟南芥转录因子GRAS家族基因群响应渗透和干旱胁迫的初步探索

郭华军[1, 3†], 焦远年[2†], 邸超[2], 姚冬霞[2], 张盖华[2], 郑雪[2], 刘岚[2], 张群莲[2], 郭蔼光[1*], 苏震[2*]

[1]西北农林科技大学生命科学学院, 杨凌 712100; [2]中国农业大学生物学院, 北京 100193; [3]山西农业大学农学院, 太谷 030801

摘要 GRAS家族是一类植物特有的转录调控因子, 已有报道表明该家族基因在植物生长发育和光信号转导过程中具有重要作用。目前在拟南芥(*Arabidopsis thaliana*)基因组中已鉴定了33个GRAS家族基因。利用功能基因组学和生物信息学手段, 通过基因芯片数据挖掘和基因功能预测, 对拟南芥GRAS家族基因在渗透和干旱胁迫过程中的应答模式进行了初步探索, 提出了一类响应渗透胁迫和干旱胁迫的拟南芥GRAS家族基因。以*SCL13*为例, 利用基因芯片相关性和GO分析, 对其在渗透胁迫信号转导过程中可能的调控机制进行了预测和分析。这一研究将为阐明GRAS家族基因参与水分胁迫的分子机制提供新的思路, 同时也为植物抗逆分子育种提供候选基因。

关键词 干旱胁迫, 表达谱分析, GRAS家族基因, 渗透胁迫, SCL13

郭华军, 焦远年, 邸超, 姚冬霞, 张盖华, 郑雪, 刘岚, 张群莲, 郭蔼光, 苏震 (2009). **拟南芥转录因子GRAS家族基因群响应渗透和干旱胁迫的初步探索**. **植物学报 44**, 290–299.

转录因子也称反式作用因子, 是能够与真核基因启动子区域中顺式作用元件发生特异性相互作用的DNA结合蛋白, 通过它们之间以及与其它相关蛋白之间的相互作用, 激活或抑制转录。在转录水平上与DNA发生相互作用的蛋白质分子中, 最具有多样性的便是转录因子。植物感受外界干旱、高盐、激素、病害及体内细胞发育等信号, 通过一系列传递(钙含量变化、第二信使、磷酸化和脱磷酸化等)激发转录因子(即反式作用因子), 反式作用因子与顺式作用元件结合后, 激活RNA聚合酶转录复合物, 从而启动基因的转录表达, 最后通过基因产物的作用对外界信号在生理生化等方面作出适合的调节反应 (Chen et al., 2002; Singh et al., 2002; Davuluri et al., 2003; Matys et al., 2003; Chen and Zhu, 2004; Broun, 2004; Gray, 2005; Guo et al., 2005; Kurata et al., 2005; Tsunoyama, 2005; Qu and Zhu, 2006; Nardmann and Werr, 2007; Balazadeh et al., 2008)。转录因子与植物生长发育和形态建成、与植物抗逆及在高等植物改良上的应用均证明其在基因的表达调控以及高等植物的整个生命过程中都起着关键作用。通过遗传学和分子生物学研究, 表明许多转录因子及其基因家族在响应外界环境胁迫时起重要调控作用。改变这些转录因子的表达可以大大提高植物的抗性, 说明转录水平调控是保护植物免受外界环境影响的一个重要调控机制。在转录水平调控基因表达可以影响许多生命活动过程, 不同的基因表达常常通过不同的转录因子来控制, 因此转录因子成为许多科学家研究的重点。植物各类转录因子的作用主要与抗逆性和生长发育调控有关 (Martin and Paz-Ares, 1997; Chen et al., 2002; Singh et al., 2002; Chen and Zhu, 2004; Guo and Ecker, 2004; Broun, 2004; Chen et al., 2005; Etheridge et al., 2005; Gray, 2005; Kurata et al., 2005; Braam, 2005; Tsunoyama, 2005; Maizel, 2006; Nardmann and Werr, 2007; Tran et al., 2007; Balazadeh et al., 2008)。从转录层面上来说,同一种胁迫可能会同时激活多条途径(多个

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† 共同第一作者。
* 通讯作者。E-mail: zhensu@cau.edu.cn; guoaiguang@yahoo.com.cn

转录因子)。例如低温、干旱可以诱导拟南芥(*Arabidopsis thaliana*)的CBF1 (Stockinger et al., 1997)和DREB1/2 (Thomashow, 1999; Vogel et al., 2005; Novillo et al., 2007)、水稻(*Oryza sativa*)的OsDREB (Dubouzet et al., 2003)、玉米(*Zea mays*)的DBF1/2 (Kizis and Pages, 2002; Saleh et al., 2006)、JAZ家族基因 (Chini et al., 2007; Santner and Estelle, 2007; Thines et al., 2007; Turner, 2007; Chico et al., 2008; Chung et al., 2008; Katsir et al., 2008a, 2008b; Melotto et al., 2008; Staswick, 2008)、JERF基因 (Riechmann and Meyerowitz, 1998; Guo and Ecker, 2004; Chen et al., 2005; Etheridge et al., 2005)、WRKY基因 (Ulker and Somssich, 2004; Xie et al., 2005; Eulgem and Somssich, 2007)和MYB基因 (Martin and Paz-Ares, 1997; Chen et al., 2006)。同一转录因子可能会被多种胁迫激活。从功能基因层面上来说, 同一转录因子也可能会同时激活多种功能基因。大量的组织和细胞类型、发育阶段和不同的环境胁迫使我们不能通过特定的单一实验把这些不同的种类全部结合起来, 于是, 高通量基因表达分析已经成为一个功能强大的生物学研究工具, 大量的基因芯片被用来鉴别基因的表达变化。目前已经有很多基因芯片数据库, 例如: NASCArrays (Craigon et al., 2004)、ArrayExpress at European Bioinformatics Institute (Brazma et al., 2006; Parkinson et al., 2009)、NCBI GEO of NCBI (Barrett et al., 2009)、GENEVESTIGATOR (Zimmermann et al., 2004)和中国水稻cDNA芯片数据库 (RIFGP-CDMD, http://plantbiol.genetics.ac.cn/) 等。利用基因芯片来研究基因的表达, 能够很好地预测并设计实验。

GRAS家族是一类植物特有的转录调控因子, 该家族基因已被报道在植物生长发育(如赤霉素信号转导、根的发育、分生组织的形成、光敏色素A信号转导以及雄配子发育等)方面具有重要作用 (Pysh et al., 1999; Bolle et al., 2000; Day et al., 2003; Morohashi et al., 2003; Bolle, 2004; Gao et al., 2004; Sena et al., 2004; Tian et al., 2004; Torres-Galea et al., 2006; Heckmann et al., 2006; Murakami et al., 2006; Sanchez et al., 2007; Laajanen et al., 2007; Lee et al., 2008; Fode et al., 2008; Gallagher and Benfey, 2008; Sole et al., 2008)。其中最具代表性的与植物生长发育密切相关的GRAS家族基因是*MOC1*。水稻分蘖控制基因*MOC1*是水稻分蘖的关键调控因子, 这一发现是水稻分蘖分子调控机理研究的突破性进展(Li et al., 2003)。近年来, 关于拟南芥转录因子GRAS家族基因的研究已有不少报道。Lim研究组采用生物信息学的方法, 在拟南芥基因组中鉴定了33个GRAS家族基因, 并利用基因芯片数据分析、qRT-PCR、反向遗传学和蛋白-蛋白互作等研究手段发掘GRAS家族基因组织特异性表达以及其在植物生长发育过程中的作用 (Lee et al., 2008)。另外, GRAS蛋白PAT1和SCL13均被发现参与了光敏色素信号转导途径 (Torres-Galea et al., 2006)。 关于*SCL14*基因 (一个GRAS家族成员) 也有了最新研究进展, 该GRAS家族基因与*TGA*基因蛋白互作, 在拟南芥受外界有毒物质侵入时具有广谱性的解毒功能 (Fode et al., 2008)。但对于拟南芥GRAS家族基因参与干旱和渗透等水分胁迫的研究报道较少。

植物对渗透和干旱等水分胁迫存在两大类逆境响应的信号通路, 即脱落酸依赖途径和脱落酸不依赖途径 (Thomashow, 1999)。水分是影响作物产量的一个关键的环境因子, 研究植物应答水分胁迫的分子机制, 对农业生产具有非常重要的实践意义 (Thomashow, 1999; Xiong et al., 2002; Zhang et al., 2004; Seki et al., 2007)。本研究利用功能基因组学手段, 通过基因芯片数据挖掘及基因功能预测, 探索拟南芥GRAS家族基因参与渗透和干旱等水分胁迫的作用机理, 以期为植物抗逆的分子育种提供候选基因, 也为GRAS家族基因参与水分胁迫的分子机制研究奠定基础。

1 材料和方法

1.1 拟南芥GRAS家族基因数据获取

拟南芥转录因子GRAS家族的信息来源于国际专业植物转录因子数据库, 分别是中国的DATF(http://datf.cbi.pku.edu.cn/)、德国的PlnTFDB(http://plntfdb.bio.uni-

potsdam.de/v2.0/)和美国的AtTFDB (http://arabidopsis.med.ohio-state.edu/AtTFDB/)。GRAS家族基因的最新注释信息以及其所对应的拟南芥ATH1基因芯片探针组的信息来源于TAIR数据库(http://www.arabidopsis.org/)的最新版本。拟南芥全基因组表达谱数据来源于NASCArrays (Craigon et al., 2004)和TAIR数据库, 以上数据包括在正常条件、渗透胁迫处理和干旱处理下不同时间点的拟南芥幼苗茎叶部和根部的表达谱数据。每个样品设2个重复。

1.2 表达谱数据分析

利用GCOS软件的MAS5算法对拟南芥在正常条件、渗透胁迫处理和干旱处理下的全基因组表达谱数据进行归一化处理, 从中提取与GRAS家族成员相对应的表达数据。聚类分析采用Cluster软件(http://rana.lbl.gov/)中的分层聚类(hierarchical cluster)算法, 聚类分析结果通过Treeview软件(http://rana.lbl.gov/)来展现。在特定处理条件下, 与某个GRAS家族成员共表达基因的选取采用我们自己开发的基因芯片数据浏览器(http://bioinformatics.cau.edu.cn/cgi-bin/gbrowse/arabidopsis/)。

1.3 Gene Ontology功能分类分析

Gene Ontology(GO)是一套标准的基因属性描述词汇, 其设计目的是为了将各个物种中细胞分子生物学研究成果综合起来。GO独立于任何生物物种或细胞类型, 是对基因属性的客观描述。GO词汇系统能够帮助从实验数据中发掘生物学知识, 我们采用EasyGO (Zhou and Su, 2007), 对与GRAS家族成员共表达基因进行了GO功能分类分析。以*ABI1*(At4g26080)为例, 将与其在渗透胁迫条件下地上部分中具有相似表达趋势的基因进行GO功能分类分析(http://bioinformatics.cau.edu.cn/ZhenSuLab/lw_AtSCL13.htm)。结果显示, 在这些基因中, 应答ABA、水分胁迫和渗透胁迫的类别有明显的富集, 而这些描述与*abi1*突变体的表型相吻合, 从而证明GO功能分类分析的结果具有很高的可信度, 可以用于对未知基因功能的挖掘。

2 结果与讨论

2.1 拟南芥GRAS家族基因在渗透胁迫和干旱胁迫时的基因芯片表达谱分析

通过生物信息学分析发现, 在拟南芥基因组中共有33个GRAS家族成员。我们对已发表的与渗透胁迫和干旱胁迫相关的拟南芥ATH1芯片数据进行挖掘, 发现在芯片中, 27个GRAS家族基因有探针组。然后利用分层聚类算法分别分析了GRAS基因在茎叶和根部响应渗透和干旱的芯片数据(具体的芯片表达数据见http://bioinformatics.cau.edu.cn/ZhenSuLab/lw_AtSCL13.htm), 并通过Treeview展现分层聚类结果(图1)。

图1A是利用茎叶在渗透和干旱胁迫下的表达谱数据的聚类结果。研究发现有一组基因在茎叶中因受渗透或/和干旱胁迫诱导而聚集在一起, 包括10个GRAS家族基因: *SCL1*、*SCL3*、*SCL5*、*SCL8*、*SCL9*、*SCL11*、*SCL13*、*SCL14*、*SCL31*和*SCL33*。其中4个基因(*SCL8*、*SCL11*、*SCL13*和*SCL31*)在茎叶中同时受渗透和干旱胁迫诱导。另外, 通过对根中的渗透和干旱胁迫下不同时间的表达谱数据的聚类分析(图1B), 发现GRAS家族基因在根部也受渗透和干旱胁迫诱导, 它们是*SCL1*、*SCL5*、*SCL6*、*SCL8*、*SCL9*、*SCL13*、*SCL14*、*SCL15*、*SCL26*和*SCL33*。这些GRAS基因中的大部分主要在渗透胁迫时反应明显, 但*SCL13*在渗透胁迫时表达明显上调, 并且在早期的干旱处理时明显被诱导, *SCL15*在根部干旱胁迫时变化尤为明显。通过聚类分析, 我们初步确定共有13个GRAS家族基因在茎叶和根部受不同程度的渗透和干旱胁迫诱导, 其中7个基因同时在茎叶和根部受诱导, *SCL3*、*SCL11*和*SCL31*只在茎叶中受诱导, 而*SCL6*、*SCL15*和*SCL26*只在根部受诱导。

进一步以二倍变化为域值, 表1显示在不同时间的渗透胁迫时, 有5个GRAS家族基因(*SCL5*、*SCL7*、*SCL13*、*SCL14*和*SCL26*)诱导随时间呈明显上升趋势, 尤其是*SCL13* (At4g17230)和*SCL14* (At1g07530)在苗期茎叶和根部均受渗透胁迫诱导。在茎叶和根部的干旱处理芯片实验中, 有3个GRAS家族基因受干旱

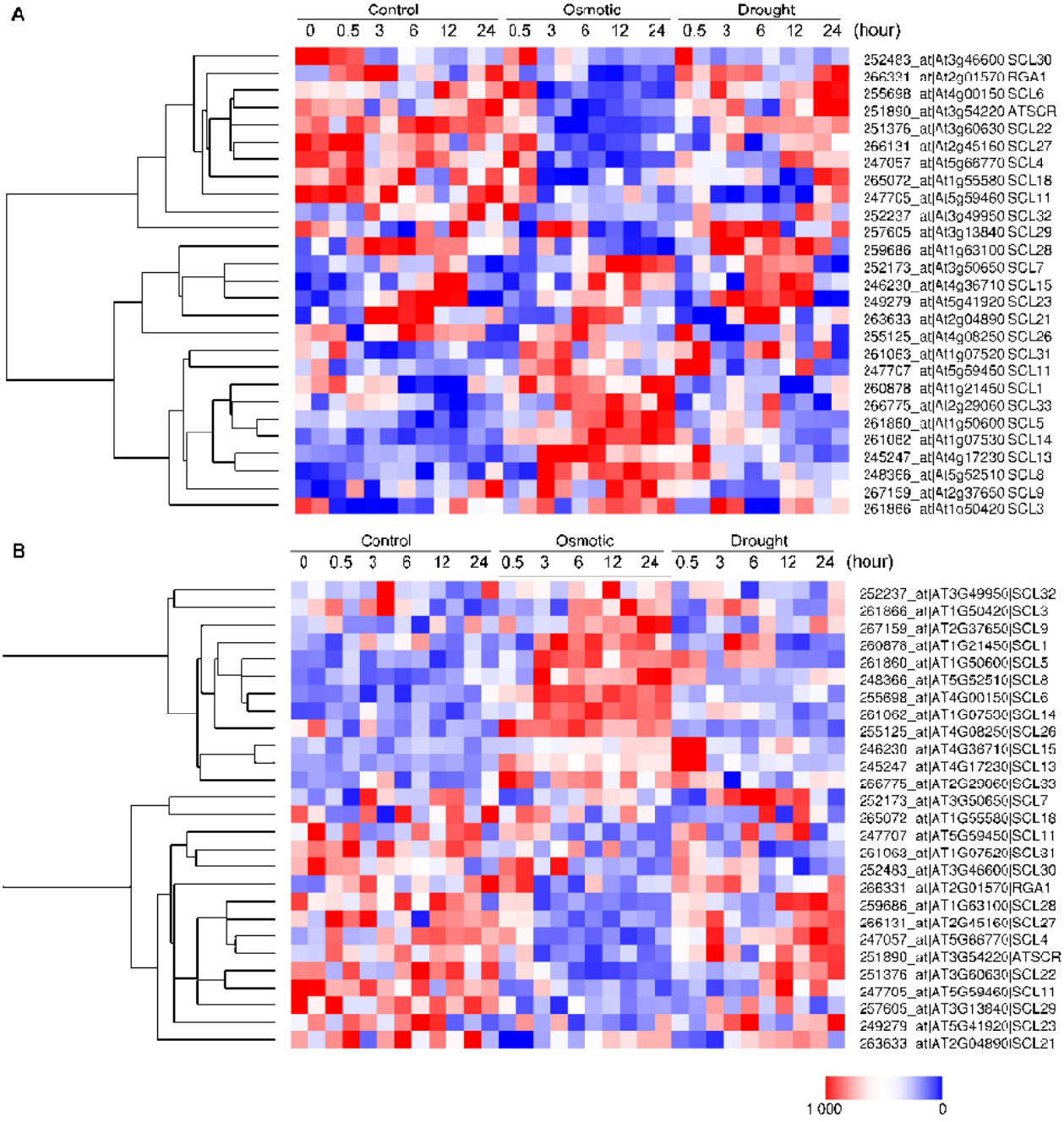

图 1　在正常、渗透胁迫处理和干旱处理条件下拟南芥 GRAS 家族基因在拟南芥幼苗中的表达模式

(A) 茎叶部; (B) 根部

Figure 1　The expression pattern of Arabidopsis GRAS family genes in seedling plant under normal condition, osmotic stress, and drought stress

(A) Shoot tissues; **(B)** Root tissues

表1 受渗透胁迫诱导的拟南芥GRAS家族基因的表达模式

Table 1 The expression pattern of Arabidopsis GRAS family genes induced by osmotic stress

Probe set ID	Locus ID	Gene name	Osmotic/CK in shoot					Osmotic/CK in root				
			0.5 h	3 h	6 h	12 h	24 h	0.5 h	3 h	6 h	12 h	24 h
261860_at	AT1G50600	*SCL5*	0.98	1.10	2.04	2.33	2.36	1.15	1.53	1.67	1.93	1.54
252173_at	AT3G50650	*SCL7*	0.57	0.83	1.30	1.77	4.18	0.89	0.71	1.14	0.86	0.95
248366_at	AT5G52510	*SCL8*	0.85	1.42	1.46	1.59	1.66	1.02	1.61	1.82	1.62	1.80
247707_at	AT5G59450	*SCL11*	1.40	1.45	1.14	1.18	0.97	1.09	1.02	0.74	0.65	0.61
245247_at	AT4G17230	*SCL13*	1.43	4.81	4.44	4.06	8.41	2.27	1.26	1.87	2.12	1.81
261062_at	AT1G07530	*SCL14*	1.36	1.21	1.77	1.78	2.02	1.18	1.30	1.73	2.14	1.56
246230_at	AT4G36710	*SCL15*	0.93	0.90	1.18	0.84	0.72	1.35	1.14	1.33	1.41	1.73
255125_at	AT4G08250	*SCL26*	0.95	1.09	0.90	0.78	0.83	2.40	1.38	2.32	2.12	2.15

表2 受干旱胁迫诱导的拟南芥GRAS家族基因的表达模式

Table 2 The expression pattern of Arabidopsis GRAS family genes induced by drought stress

Probe set ID	Locus ID	Gene name	Drought/CK in shoot					Drought/CK in root				
			0.5 h	3 h	6 h	12 h	24 h	0.5 h	3 h	6 h	12 h	24 h
261860_at	AT1G50600	*SCL5*	0.89	1.17	1.74	1.19	1.01	1.70	1.18	1.32	1.06	0.84
252173_at	AT3G50650	*SCL7*	0.97	1.00	1.59	1.01	1.02	0.67	1.09	1.83	0.95	0.85
248366_at	AT5G52510	*SCL8*	1.64	1.10	1.11	1.07	1.19	1.37	1.10	1.27	1.00	1.00
247707_at	AT5G59450	*SCL11*	2.73	0.86	0.97	1.44	0.86	1.17	0.98	1.00	1.08	0.78
245247_at	AT4G17230	*SCL13*	6.69	1.34	1.36	1.35	2.43	4.02	1.26	1.32	1.01	0.98
261062_at	AT1G07530	*SCL14*	1.22	1.24	1.32	1.06	1.07	1.12	1.17	1.15	0.99	0.88
246230_at	AT4G36710	*SCL15*	1.16	0.96	1.18	1.00	1.18	3.94	1.42	1.04	1.23	0.91
255125_at	AT4G08250	*SCL26*	1.51	0.65	0.63	0.80	0.94	0.91	0.76	0.92	0.88	0.82

诱导, 分别是*SCL11*、*SCL13*和*SCL15*, 其中*SCL13*在苗期茎叶和根部的干旱处理后其表达呈明显上升, *SCL11*只在茎叶中受干旱诱导, *SCL15*只在根部受干旱诱导(表2)。综合以上结果, 以二倍变化为域值, 共有7个GRAS家族基因(*SCL5*、*SCL7*、*SCL11*、*SCL13*、*SCL14*、*SCL15*和*SCL26*)受渗透或干旱胁迫的诱导。另外, *SCL8*在渗透胁迫时尽管变化不到二倍, 但不管在茎叶中还是在根中均有1.6倍以上的诱导。

2.2 利用生物信息学手段对受渗透胁迫诱导的基因进行功能预测(以*SCL13*为例)

在拟南芥苗期茎叶和根的渗透和干旱胁迫中, 有10个左右GRAS家族基因受到不同程度的表达诱导, 对于其作用的分子机制还需进一步探讨和研究。*SCL13*曾被发现作为正调控因子参与了依赖于光敏色素的红光信号转导途径, 并且对光敏色素A的反应有调节作用。同时, 通过*SCL13*-promoter-5'-UTR-*GUS*转基因植株的GUS染色, 发现*SCL13*基因的表达受盐和渗透胁迫诱导, 但*SCL13*的反义RNA转基因植株和野生型(WT)在盐和渗透胁迫下未发现明显表型差异 (Torres-Galea et al., 2006)。芯片数据挖掘结果显示, *SCL13*在苗期茎叶和根部经渗透和干旱处理后其表达明显上升(表1, 表2)。现以*SCL13*为例, 对GRAS家族基因在渗透和干旱胁迫中参与调控的可能分子机制进行预测。首先, 利用我们自己开发的基因芯片的Genome Browse数据服务器, 搜寻*SCL13*在拟南芥苗期茎叶渗透胁迫时基因芯片中的表达方式(图2A), 发现该基因与205个探针组存在0.8以上的统计学相关性(图2B), 这些探针组ID和它们相关的基因名详见 http://bioinformatics.cau.edu.cn/ZhenSuLab/lw_AtSCL13.htm, 这些基因可能存在相似的信号调控分子机制。我们利用EasyGO进一步对这些基因进行Gene Ontology(GO)分类 (Zhou and Su,

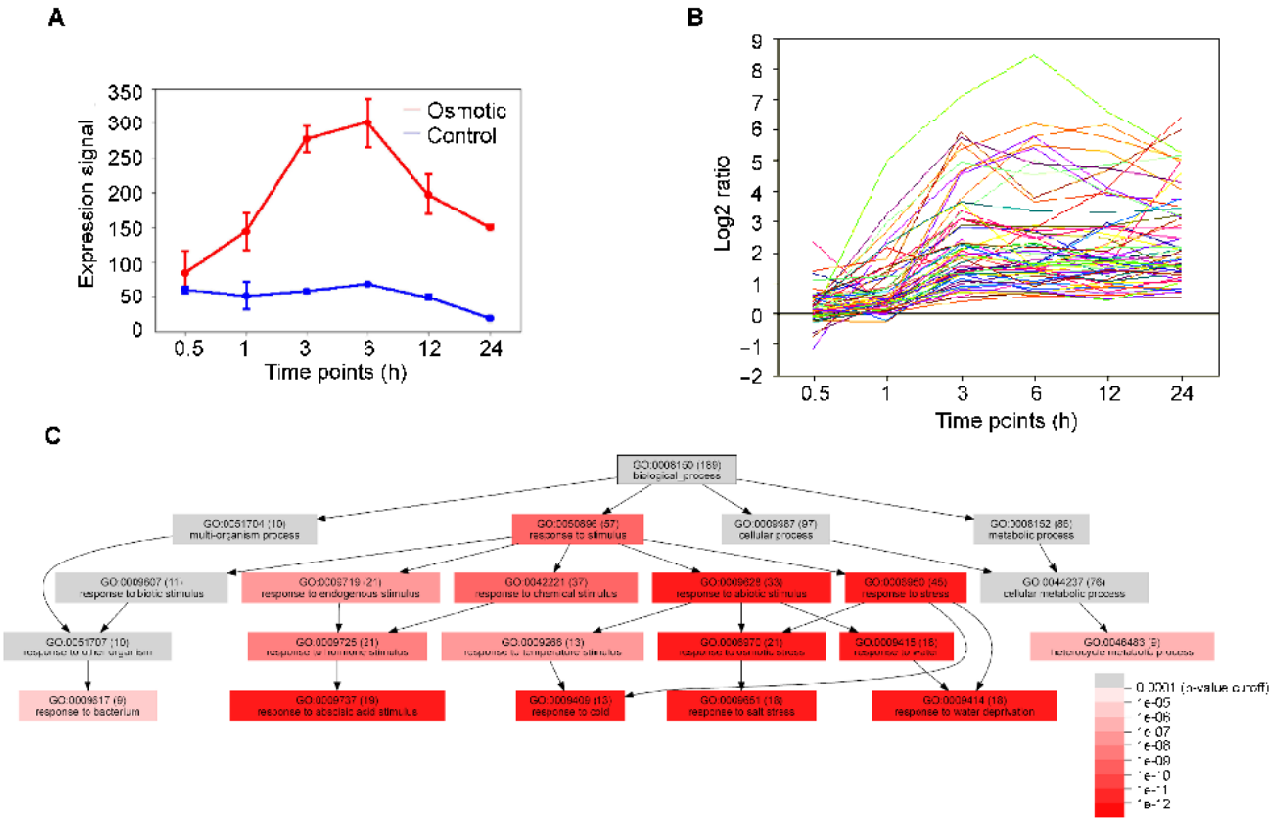

图2　拟南芥*SCL13*基因在渗透胁迫下茎叶部共表达和GO功能分类分析

(A) 拟南芥*SCL13*基因在渗透胁迫下茎叶部的表达模式; (B) 渗透胁迫下拟南芥的茎叶部与*SCL13*具有共表达趋势的基因; (C) 对(B)中所示与*SCL13*具有共表达趋势基因的GO功能分类分析

Figure 2　Co-expression and GO analysis of *SCL13* in shoot tissue of *Arabidopsis thaliana* under osmotic stress

(A) Expression pattern of *SCL13* in shoot tissue under osmotic stress; **(B)***SCL13* co-expression genes in shoot tissue under osmotic stress; **(C)** GO analysis of the *SCL13* co-expression genes shown in **(B)**

2007)。

通过GO注释富集度(annotation enrichment)的显著性计算分析(图2C), 发现*SCL13*及其密切相关基因与以下生物学过程密切相关, 包括失水反应(16个基因, FDR p-value为1.42e-42)、脱落酸(ABA)反应(19个基因, FDR p-value为3.12e-31)、渗透胁迫(14个基因, FDR p-value为1.36e-17)和冷胁迫(12个基因, FDR p-value为7.00e-13)等水分胁迫, 生物胁迫如对细菌的反应(7个基因, FDR p-value为2.72e-06)以及吲哚类似物的合成(6个基因, FDR p-value为4.77e-21)等。19个ABA反应相关的基因包括*RD29A*、*ABF3/DPBF5*、*COR15A*、*ERD14*、*ERD10*、*HVA22D*、*KIN1*、*KIN2*和*ABI1*等。这些ABA和水分胁迫相关基因可能在拟南芥受渗透和干旱胁迫时存在与*SCL13*类似的分子调控机制。

我们利用生物信息学进行基因芯片数据挖掘, 通过全基因组芯片分析, 发现了将近10个GRAS家族基因在渗透和干旱胁迫时其表达呈现显著上升趋势。进一步利用功能基因组平台, 以*SCL13*为例利用基因表达谱的相关性和基因功能分类(GO)手段, 对GRAS基因家族进行功能预测, 发现*SCL13*与干旱和渗透胁迫存在密切的相关性。*SCL13*基因在应答渗透和干旱胁迫时可能参与了依赖于ABA的信号转导途经。下一步我们将进行反向遗传学研究, 从该基因敲除的T-DNA插入突变体

中, 筛选出纯合体, 进行表型鉴定。关于与渗透和干旱胁迫相关的GRAS家族基因的研究, 仅见少量报道。本文对拟南芥GRAS家族基因响应干旱和渗透胁迫进行了初步探索, 关于这些GRAS家族基因参与抗逆性反应的分子调控机制还需进一步研究。

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Discovery of Arabidopsis GRAS Family Genes in Response to Osmotic and Drought Stresses

Huajun Guo[1, 3†], Yuannian Jiao[2†], Chao Di[2], Dongxia Yao[2], Gaihua Zhang[2], Xue Zheng[2], Lan Liu[2], Qunlian Zhang[2], Aiguang Guo[1*], Zhen Su[2*]

[1]*College of Life Sciences, Northwest Agricultural and Forestry University, Yangling 712100, China*
[2]*College of Biological Sciences, China Agricultural University, Beijing 100193, China*
[3]*College of Agronomy, Shanxi Agricultural University, Taigu 030801, China*

Abstract The GRAS family is a class of plant specific transcription factors, playing essential roles in plant development and light signal transduction pathways. 33 GRAS family genes were identified in Arabidopsis genome. In this study, we found a group of GRAS family genes in response to osmotic and/or drought stresses through Arabidopsis GeneChip data mining. Meanwhile, we conducted co-expression analysis and gene ontology (GO) analysis and predicted that SCL13 was possibly involved in the response to osmotic stress. Our study will be helpful to elucidate some GRAS family genes related in signal transduction pathways during water stress, and be beneficial to crop molecular breeding in the future.

Key words drought stress, expression analysis, GRAS family gene, osmotic stress, SCL13

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† These authors contributed equally to this work.
* Author for correspondence. E-mail: zhensu@cau.edu.cn; guoaiguang@yahoo.com.cn

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