J-蛋白AtJ8在拟南芥渗透胁迫适应方面的作用

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摘要 AtJ8是拟南芥J-蛋白家族成员。已有报道, AtJ8定位于叶绿体中, 参与光合作用的调节。该文主要研究AtJ8在适应盐和水分胁迫方面的作用。实时定量PCR结果表明, 盐、水分胁迫及脱落酸(abscisic acid, ABA)诱导*AtJ8*基因的表达。反向遗传学研究显示, 在正常生长条件下, *AtJ8*突变体(*atj8-1*)的种子萌发率及绿色子叶出现率与野生型(WT)和突变体恢复株系(R4-1)没有明显差别; 在盐和水分胁迫条件下, *atj8-1*种子萌发延迟, 绿色子叶出现率明显低于WT和R4-1株系, 表明AtJ8可能在调节种子及幼苗对渗透胁迫的适应性方面发挥重要作用。此外, 实时定量PCR结果显示, *AtJ8*基因突变改变了ABA反应基因*ABI1*、*ABI2*、*RAB18*、*RD29A*和*RD29B*的表达, 说明AtJ8调节植物对渗透胁迫的适应性可能是通过影响ABA信号途径来完成的。

关键词 AtJ8; 拟南芥; 盐胁迫; 水分胁迫

The Roles of J-protein AtJ8 in Adapting to Osmotic Stress in *Arabidopsis thaliana*

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Abstract AtJ8 is a member of the *Arabidopsis thaliana* J-protein family. It is reported that AtJ8 is targeted to the chloroplast and involved in optimization of photosynthetic reactions in *A. thaliana*. Herein, the roles of AtJ8 in adapting to saline and water stresses were studied in *A. thaliana*. The result of Real-time PCR analysis showed that *AtJ8* expression was induced through salinity, dehydration and abscisic acid (ABA) in young seedlings. The result of reverse genetic analysis showed that seed germination rate and cotyledon greening rate of *AtJ8* mutant plants (*atj8-1*) were not clearly different from those of wild-type (WT) and the rescued mutant plants (R4-1) under standard culture conditions. However, under saline or water stress condition, *atj8-1* seeds broke dormancy after the WT and R4-1 seeds, and cotyledon greening rate for *atj8-1* seedlings was clearly lower than that for the WT and R4-1 seedlings, which suggested that AtJ8 played important roles in regulating the responses of seeds and seedlings to osmotic stress. Moreover, Real-time PCR analysis indicated that *AtJ8* gene knockout altered the expression levels of several ABA-responsive genes, *ABI1*, *ABI2*, *RAB18*, *RD29A* and *RD29B*, which suggested that AtJ8 modulated the adaptability of plants to osmotic stress likely through its effects on ABA signaling pathways.

Keywords AtJ8; *Arabidopsis thaliana*; saline stress; water stress

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盐和干旱胁迫严重地影响了农业生产并降低了农作物的产量。最近十多年, 植物适应盐和干旱的分子和细胞学机制方面的研究取得了重要的进展[1-10]。植物激素脱落酸(abscisic acid, ABA)在适应盐和干旱胁迫方面有重要的作用[11-15]。盐和水分胁迫能诱导ABA的产生。ABA能改变细胞内的多种生理过程, 如使气孔快速关闭以减少水分的丧失并诱导许多抗盐及抗旱基因的表达。至今, 人们已发现了多种ABA信号组分, 包括ABA受体、磷脂酶Dα1(phospholipase Dα1, PLDα1)、磷脂酸(phosphatidic acid, PA)、G蛋白、NADPH氧化酶、第二信使[Ca^{2+}、H_2O_2、NO和DAG(diacylglycerol)]、蛋白激酶、蛋白磷酸酶和离子通道蛋白[16-24]。一个新颖的ABA信号途径模型为: ABA受体、PP2C/ABI1/ABI2(protein phosphatases 2C/ABA-insensitive 1/ABA-insensitive 2)(作为负调节剂)和SnRK2/OST1(sucorse nonfermenting 1-related protein kinase 2/open stomata 1)(作为正调节剂)共同决定下游的ABA信号组分的激活或钝化[25]。

J-蛋白是一类包含J-结构域的蛋白质大家族。J-蛋白广泛存在于生物界的多种有机体中。J-蛋白独立或与Hsp70(heat shock protein 70)结合发挥分子伴侣的功能。它们在多种生物学过程中发挥作用, 如帮助新生蛋白质的折叠、组装、转运及防止未折叠的蛋白发生聚集。在胁迫条件下, 它们能将变性蛋白质恢复成有功能的天然蛋白质, 维持蛋白质的稳态, 使细胞免于危害[26]。基因组序列分析表明, 拟南芥(*Arabidopsis thaliana*)基因组中存在120种J-蛋白基因[27-28]。已报道J-蛋白在植物生长发育及信号转导过程中发挥作用[29-31]。J-蛋白在适应环境胁迫方面的作用也有报道。2007年, Li等[32]报道了拟南芥J-蛋白AtDjA2和AtDjA3在改善拟南芥耐热性方面的作用; 2009年, Yang等[33]证明TMS1在花粉管耐热性方面发挥作用; 2010年, Yang等[34]报道在高pH值环境下, *AtDjA3*突变体显示敏盐的特性。

已有报道拟南芥J-蛋白AtJ8定位于叶绿体中, 光负调节*AtJ8*基因的表达[35], AtJ8参与光合作用的调节[36]。至今, AtJ8在植物的环境胁迫适应方面的功能还未见报道。本文中, 笔者利用*GUS*报告基因和实时定量PCR的方法研究了AtJ8的组织特异性表达, 利用定量PCR方法分析了盐、水分胁迫和ABA对*AtJ8*基因表达的影响。进一步利用反向遗传学的方法研究了种子萌发过程中及萌发后发育阶段AtJ8在调节盐及水分胁迫适应中的作用及机制。

1 材料与方法

1.1 拟南芥种植及培养

以Columbia(Col)生态型拟南芥为材料。种子经75%(v/v)乙醇消毒30 s, 无菌水冲洗3次, 5%(w/v)次氯酸钠消毒5 min, 无菌水冲洗3次, 然后播种在无菌的MS培养基[含1%(w/v)蔗糖, 0.8%(w/v)琼脂, pH5.8]上, 避光4 ℃春化3 d, 转至22 ℃, 在16 h/8 h(光/暗)光周期下培养。10~14 d(幼苗长至4叶期)后将幼苗移栽至浸透Hoagland营养液的蛭石和营养土的混合物中, 在相同温度和光照条件下继续培养, 每周浇灌一次Hoagland营养液。

1.2 反转录PCR(RT-PCR)

通过RT-PCR扩增*AtJ8*基因编码区。将0.1 g的拟南芥幼苗于液氮中充分研磨, 待液氮自然挥发后将粉末转移到事先加有1 mL RNArose的1.5 mL EP管中, 立即用移液器反复吹打以彻底混匀, 然后按常规方法提取总RNA。以RNA为模板通过SYBR ExScript™ RT-PCR反转录试剂盒(TaKaRa公司)合成cDNA。反转录程序为: 42 ℃, 15 min; 85 ℃, 30 s。向反转录得到的cDNA中加入*AtJ8*基因(At1g80920)特异引物(F: 5′-TCT AGA ATG ACA ATT GCT TTA ACG AT-3′; R: 5′-GAG CTC TCA AGC GTA AGG ATT AAC GT-3′)和Taq DNA聚合酶后, 在PCR仪上进行PCR扩增反应, 程序为: 94 ℃预变性5 min; 94 ℃变性30 s, 62 ℃退火1 min, 72 ℃延伸30 s, 共29个循环。同时以*Actin*(At2g37620)基因作为内参(F: 5′-AGG CAC CTC TTA ACC CTA AAG C-3′; R: 5′-GGA CAA CGG AAT CTC TCA GC-3′)。

1.3 实时定量PCR分析

通过实时定量PCR(Real-time PCR)方法检测不同组织中及胁迫条件下*AtJ8*基因的表达水平、转基因恢复株系中*AtJ8*基因的转录水平及野生型和突变体中ABA反应基因的表达水平。以不同材料中提取的RNA为模板, 用PrimeScript RT Reagent试剂盒(TaKaRa公司)进行反转录合成cDNA, 程序为: 42 ℃ 15 min, 然后95 ℃ 2 min。以cDNA为模板, 各自基因特异性引物见表1。用SYBR Premix Ex Taq试剂盒(TaKaRa公司)进行定量PCR分析, 程序为: 95 ℃预变性10 s; 然后95 ℃ 5 s, 60 ℃ 30 s, 共40个循环。 以

表1 用于定量PCR的引物

Table 1 Primers used for the Real-time PCR amplifications

基因名称 Gene names	序列号 Accession no.	引物序列(5′→3′) Primer sequences (5′→3′)
AtJ8	At1g80920	F: GGA AGG GAC GGA GGA ATT TG; R: ACG TGT CGC AAT CTG GAT CA
ABI1	At4g26080	F: TGT GGT GGT GGT TGA TTT GAA GCC; R: GCC TCA GTT CAA GGG TTT GCT CTT
ABI2	At5g57050	F: AAG TGT GCG ATT TGG CTC GGA AAC; R: TCC GGC CAT CGC GTT CTT CTT AT
OST1	At4g33950	F: TGG AGG AAG ACT TAG AGA GCG ACC TT; R: TGC GTA CAC AAT CTC TCC GCT ACT
RAB18	At5g66400	F: GCA GTC GCA TTC GGT CGT TGT ATT; R: ACA ACA CAC ATC GCA GGA CGT ACA
RD29A	At5g52310	F: GTG CCG ACG GGA TTT GAC; R: CGC CGG AAA TTT ATC CTC TTC
RD29B	At5g52300	F: ACA ATC ACT TGG CAC CAC CGT T; R: AAC TCA CTT CCA CCG GAA TCC GAA
Actin	At2g37620	F: TGT GCT CAG TGG TGG AAC CA; R: GGA GCC AAA GCA GTG ATC TCT T

*Actin*基因作为内参。定量PCR仪使用ABI Prism 7000 Sequence Detection System(Applied Biosystems)产品。

1.4 β-葡糖苷酸酶(β-glucuronidase, GUS)组织化学染色

GUS染色参照Jefferson等[37]的方法。转基因苗浸入GUS染液[50 mmol/L Na_3PO_4, pH7.0, 10 mg/mL X-Gluc和0.02%(v/v) Triton X-100]中, 于37 ℃染色12 h, 吸去反应液, 然后用70%(v/v)的乙醇脱去叶绿素, 用体视显微镜(Motic, SMZ-168)观察并照相。

1.5 *AtJ8*基因突变体的分离及鉴定

*AtJ8*基因的T-DNA插入突变体(SALK_024617)种子从拟南芥资源中心(the *Arabidopsis* Biological Resource Center, ABRC)获得。根据Salk机构基因组分析实验室的方法(http://signal.salk.edu/T-DNA_Genotyping_Procedure.ppt)鉴定纯合突变体。取0.05 g 10 d龄的由ABRC获得的T2代苗, 加入400 μL DNA提取液(100 mmol/L Tris-HCl, pH8.0; 20 mmol/L EDTA, 1.4 mol/L NaCl)研磨至匀浆, 然后按常规方法提取基因组DNA。以基因组DNA为模板, 以*AtJ8*基因特异性引物(F: 5′-CGA AGC AAG AGA AAG ACA TGG-3′, R: 5′-TTT CAG ACA ACT CGC TAA AAA GG-3′)和T-DNA左边界引物LBb1进行PCR反应。通过PCR产物测序鉴定T-DNA插入位点。通过RT-PCR检测野生型和突变体中*AtJ8*基因的转录本水平。

1.6 载体构建及遗传转化

取0.05 g 10 d龄的拟南芥苗, 加入400 μL DNA提取液(100 mmol/L Tris-HCl, pH8.0; 20 mmol/L EDTA, 1.4 mol/L NaCl)研磨至匀浆, 然后按常规方法提取基因组DNA。以基因组DNA为模板, 根据拟南芥基因组序列(http://*Arabidopsis*.org)设计*AtJ8*基因启动子特异的引物(F: 5′-CTG CAG GCT TGC TGT CTC CAG ACT CCA-3′; R: 5′-TCT AGA ATT TTC TTA TGC CGG TGG TTC A-3′), 通过PCR方法扩增1 214 bp的*AtJ8*启动子序列。将*AtJ8*启动子在*Pst* I和*Xba* I(TaKaRa公司)酶切位点连接到包含*GUS*报告基因的双元载体pCAMBIA1300(www.cambia.org/daisy/cambia/585.html)上, 构建了pCAMBIA1300-J8 promoter:GUS(PAtJ8:GUS)。

以从拟南芥幼苗中提取的RNA为模板, 反转录获得cDNA。以获得的cDNA为模板, 以*AtJ8*基因特异性引物(同1.2)通过PCR方法扩增492 bp的*AtJ8*编码区序列, 连接到PUC-T载体上后送上海生工生物工程技术服务有限公司测序。将前面获得的PAtJ8:GUS载体用*Xba* I和*Sac* I进行酶切, 回收大片段。将测序正确的*AtJ8*基因从PUC-T载体上酶切(*Xba* I和*Sac* I)下来后用T4连接酶与大片段进行连接, 获得了pCAMBIA1300-J8 promoter:J8(*PAtJ8:AtJ8*)构建。

将构建好的双元载体*PAtJ8:GUS*和*PAtJ8:AtJ8*分别转化农杆菌GV3101, 然后通过农杆菌介导的浸花法转化拟南芥野生型或*atj8-1*突变体, 经潮霉素(25 μg/mL)抗性筛选获得纯合的PAtJ8:GUS株系用于组织定位研究和纯合的PAtJ8:AtJ8突变体恢复株

系用于基因功能的研究。

1.7 胁迫处理方法

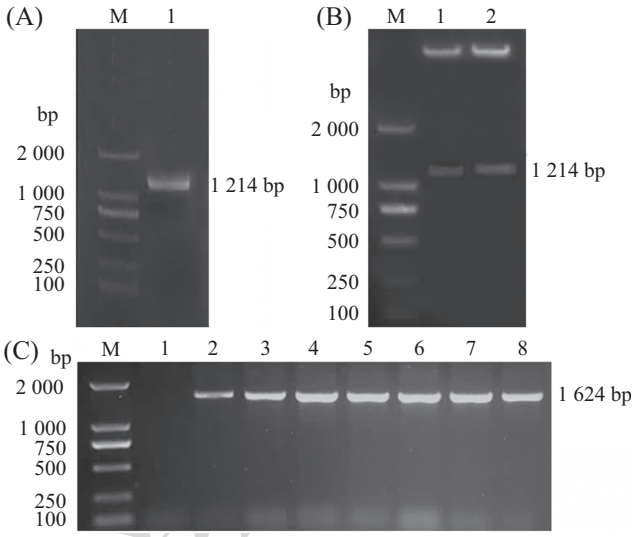
胁迫诱导*AtJ8*基因表达: 将22 °C培养10 d的拟南芥幼苗转移到含有200 mmol/L NaCl或400 mmol/L甘露醇的MS培养基上, 分别处理0, 2, 4, 6, 8 h或0, 6, 12, 24, 36 h后取材0.1 g, 放入液氮中速冻, 用于RNA的提取。对于ABA处理, 以100 μmol/L的ABA溶液处理22 °C培养10 d的拟南芥幼苗0, 15, 30, 60 min, 取材0.1 g于液氮中速冻, 用于RNA的提取。

种子萌发率和绿色子叶出现率分析: 不同基因型的种子播种在包含1%蔗糖和200 mmol/L NaCl或400 mmol/L甘露醇的同一个MS板的不同区域。种子在4 °C黑暗中培养3 d后转移到22 °C培养箱中培养6 d, 适时统计种子萌发率和绿色子叶出现率。

2 结果

2.1 *AtJ8*基因组织专一性表达分析

2.1.1 *GUS*报告基因方法分析AtJ8的组织定位 以拟南芥基因组DNA为模板, 以*AtJ8*启动子特异性引物通过PCR扩增1 214 bp的*AtJ8*启动子序列, PCR扩增结果如图1A所示。将测序正确的*AtJ8*启动子连接到包含*GUS*的pCAMBIA1300中, 获得了*PAtJ8:GUS*

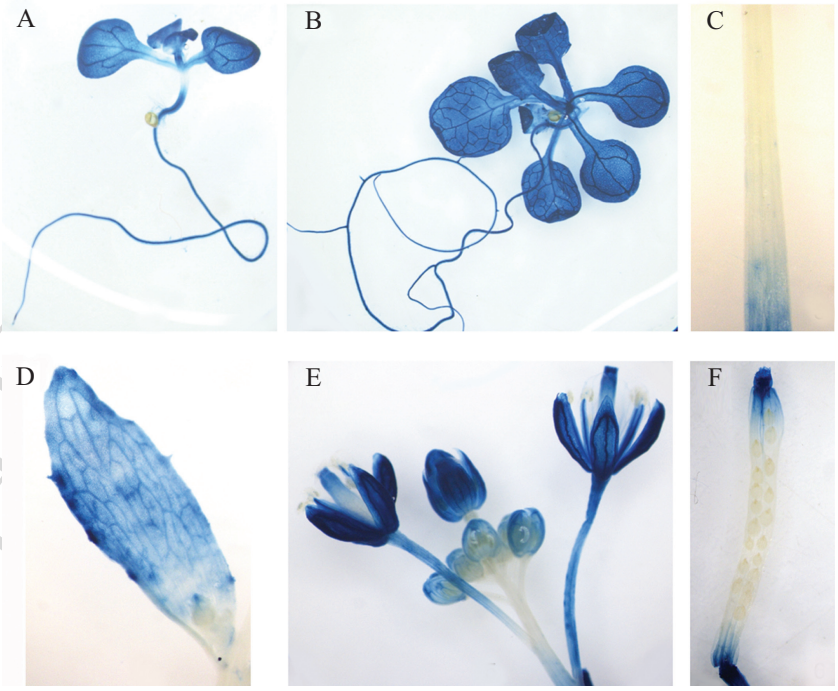


A: PCR扩增*AtJ8*启动子, 1: *AtJ8*启动子的PCR产物; B: 构建转基因载体的酶切鉴定, 1和2: 用*Pst* I和*Xba* I双酶切PAtJ8:GUS的产物; C: 转基因株系DNA水平的鉴定, 1: 野生型中没有扩出*GUS*; 2~8: 7个转基因株系均扩出*GUS*。M: 2 000 bp的DNA分子量标准。

A: amplification of *AtJ8* promoter by PCR, 1: *AtJ8* promoter obtained by PCR; B: identification of constructed transgenic vector, 1 and 2: digestion of PAtJ8:GUS by *Pst* I and *Xba* I; C: identification of transgenic lines, 1: no *GUS* sequence was amplified in WT, 2~8: *GUS* sequence was amplified in 7 transgenic lines. M: 2 000 bp DNA marker.

图1 *PAtJ8:GUS*转基因株系的构建及鉴定

Fig.1 Construction and identification of transgenic lines harboring *PAtJ8:GUS*

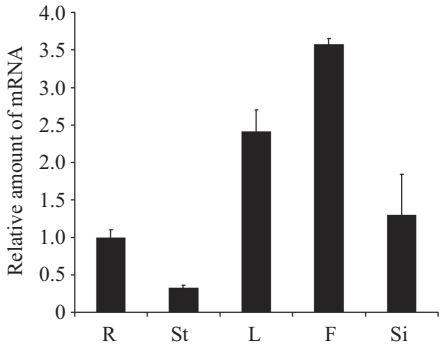


A: 7 d龄苗; B: 14 d龄苗; C: 茎; D: 茎叶; E: 花序; F: 长角果。

A: 7 d seedling; B: 14 d seedling; C: stem; D: stem leaf; E: inflorescence; F: mature silique.

图2 *PAtJ8:GUS*转基因植株的GUS染色

Fig.2 GUS staining in transgenic *PAtJ8:GUS* plants



根中*AtJ8*的表达水平设为1。*Actin*作为内参。数据为3个生物学重复的平均值。R: 根; St: 茎; L: 叶; F: 花; Si: 长角果。

AtJ8 expression level of roots was used for normalization, setting to 1. *Actin* was used as an internal control. Data were mean from three biological replicates. R: root; St: stem; L: leave; F: flower; Si: silique.

图3 实时定量PCR分析*AtJ8*基因在拟南芥中的表达模式

Fig.3 Expression pattern of the *AtJ8* gene in *Arabidopsis thaliana* analyzed by Real-time PCR

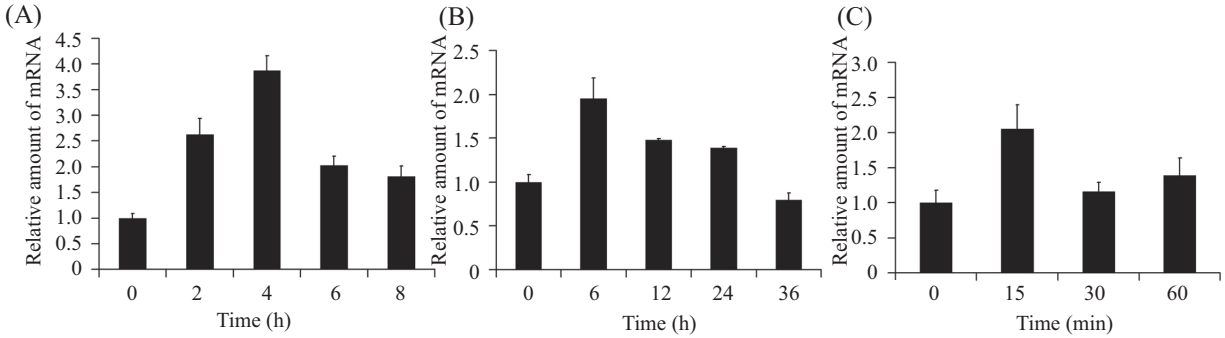
转基因双元载体, 酶切鉴定结果正确(图1B)。将构建好的双元载体转化拟南芥野生型, 在潮霉素抗性板上筛到了7个阳性株系。以从T1代转基因植株叶片中提取的基因组DNA为模板, 以*GUS*基因的特异引物(F: 5′-TGT AGA AAC CCC AAC CCG TGA-3′; R: 5′-CCA GCC ATG CAC ACT GAT ACT CT-3′)进行PCR扩增, 7个阳性株系均能扩出约1 624 bp的*GUS*条带(图1C), 而野生型对照不能扩出*GUS*条带, 说明获得的转基因株系是正确的。继续在潮霉素抗性板上筛选2代, 得到了纯合的转化*PAtJ8:GUS*的株系。在*PAtJ8:GUS*转基因植株中, *GUS*基因的表达是受*AtJ8*基因启动子控制的。因此, *GUS*基因的表达模式即代表*AtJ8*基因的表达模式。

对*PAtJ8:GUS*株系的不同时期的幼苗或同一时期植株的不同器官进行GUS染色, 结果表明*AtJ8*基因在根、莲座叶、茎叶、花丝、花萼和雌蕊中大量表达, 在茎和成熟的长角果中表达量较少(图2)。

2.1.2 实时定量PCR方法分析*AtJ8*的组织定位 为了进一步研究*AtJ8*基因的组织定位, 我们通过实时定量PCR的方法检测*AtJ8*基因在不同器官组织中的表达水平。结果表明, *AtJ8*基因在花中表达量最高, 在叶、根和长角果中次之, 在茎中的表达量最低(图3)。PCR的结果与GUS染色的结果基本相符。

2.2 渗透胁迫及ABA对*AtJ8*基因表达的影响

为了研究渗透胁迫对*AtJ8*基因表达的影响, 我们分别用200 mmol/L NaCl或400 mmol/L甘露醇处理10 d龄的野生型拟南芥苗0, 2, 4, 6, 8 h或0, 6, 12, 24, 36 h, 然后通过PCR方法检测*AtJ8*转录水平的变化。结果表明, 盐或脱水胁迫均可使*AtJ8*基因表达量明显增加。200 mmol/L NaCl处理2 h后*AtJ8*表达量开始增加, 4 h后表达量达到峰值, 为对照的4倍(图4A)。400 mmol/L甘露醇处理6 h后*AtJ8*表达量达到峰值, 约为对照的2倍(图4B)。以上结果表明, *AtJ8*基因表达可能受渗透胁迫的诱导。为了了解ABA对*AtJ8*基因表达的影响, 我们用100 μmol/L的ABA处理培养10 d的幼苗0, 15, 30, 60 min。结果显示, ABA能快速诱导*AtJ8*基因表达, 100 μmol/L的ABA处理

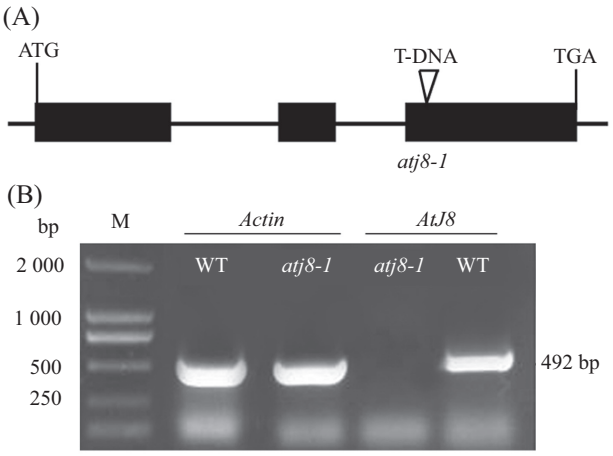


以*AtJ8*基因特异引物(表1), 通过定量PCR方法分析不同处理的材料的总RNA。*Actin*作为内参。未经处理的苗中*AtJ8*基因的表达水平设为1。数据为3次实验的平均值。A: 用200 mmol/L NaCl处理10 d龄的拟南芥苗; B: 用400 mmol/L甘露醇处理10 d龄的拟南芥苗; C: 用100 μmol/L的ABA处理10 d龄的拟南芥苗。

Total RNA was analyzed by Real-time PCR with *AtJ8* gene-specific primers (Table 1). *Actin* was used as the internal control. The *AtJ8* gene expression level in untreated seedlings was set to 1 and used for normalization. The values were the mean from three independent experiments. A: 10-d-old *Arabidopsis* seedlings were treated with 200 mmol/L of NaCl; B: 10-d-old *Arabidopsis* seedlings were treated with 400 mmol/L of mannitol; C: 10-d-old *Arabidopsis* seedlings were treated with 100 μmol/L of ABA.

图4 盐、脱水或ABA处理的拟南芥苗中*AtJ8*基因的表达

Fig.4 Expression of the *AtJ8* gene in *A. thaliana* seedlings treated with salinity, dehydration and ABA



A: *AtJ8*编码区的内含子和外显子结构及T-DNA插入位点。实心黑块: 外显子; 线: 内含子; 三角: T-DNA插入位点; B: 野生型和突变体中*AtJ8*全长转录本的RT-PCR分析。WT: 野生型; *atj8-1*: *AtJ8*突变体; M: 2 000 bp DNA分子量标准。

A: intron/exon organization of the *AtJ8* coding region and T-DNA insertion location. Solid boxes: exons; lines: introns; triangle: T-DNA insertion position; B: RT-PCR analysis of *AtJ8* full transcripts in WT and mutants. WT: wild-type plants; *atj8*-1: an *AtJ8* mutant; M: 2 000 bp DNA marker.

图5 突变体中*AtJ8*转录本的检测

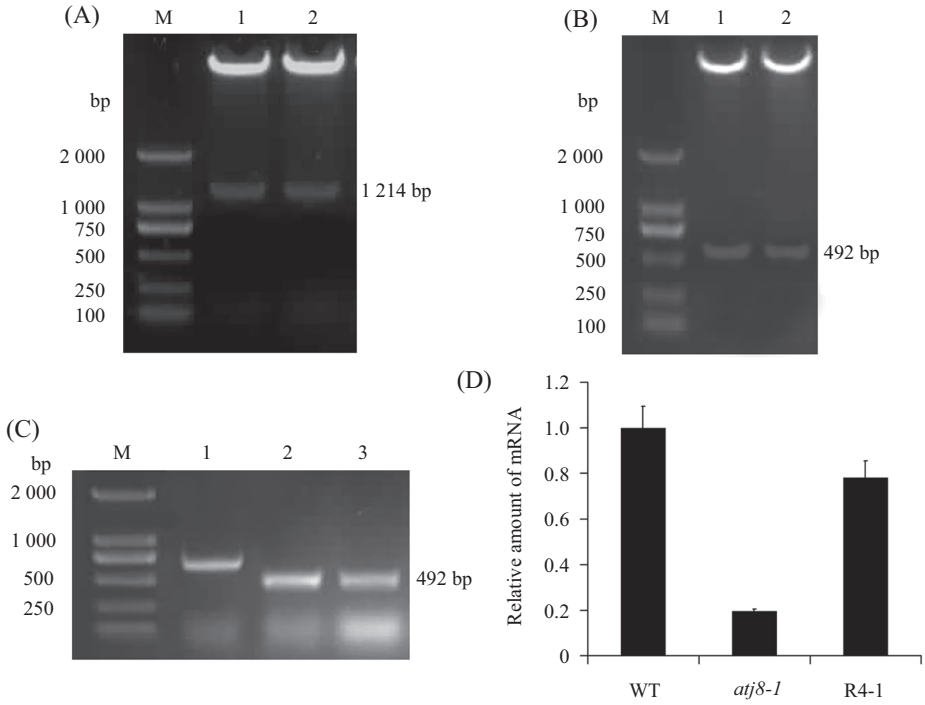
Fig.5 Detection of *AtJ8* transcript in mutant

15 min就能诱导*AtJ8*基因的表达升高2倍(图4C), 暗示AtJ8可能也涉及ABA信号途径。

2.3 *AtJ8*敲除对植物盐和水分胁迫反应的影响

2.3.1 *AtJ8*突变体及突变体恢复株系的获得 为了研究拟南芥AtJ8在渗透胁迫适应方面的功能, 我们以*AtJ8*基因特异性引物和T-DNA左边界引物LBb1筛选并鉴定了1个纯合的*AtJ8*基因的T-DNA插入突变体(*atj8-1*)。T-DNA侧翼区序列分析表明, T-DNA插入*AtJ8*基因的第3个外显子上(图5A)。RT-PCR结果表明, *atj8-1*纯合突变体中没有*AtJ8*基因的全长转录本(图5B)。

我们进一步构建了双元载体*PAtJ8:AtJ8*, 酶切鉴定结果正确(图6A和图6B)。将*PAtJ8:AtJ8*转化到*atj8-1*中, 获得了一个突变体恢复株系*atj8-1/AtJ8*(R4-1)。取纯合的R4-1植株的叶片提取基因组DNA, 以*AtJ8*基因特异引物进行PCR扩增。结果显示, R4-1中能扩出492 bp的目的条带(图6C), 说明已经获得了*AtJ8*突变体的恢复株系。然后通过定量PCR方



A、B: 构建的*PAtJ8:AtJ8*转基因载体的鉴定。M: 2 000 bp DNA分子量标准; 1和2: *Pst* I和*Xba* I双酶切的产物(A), *Xba* I和*Sac* I双酶切的产物(B); C: 转基因株系DNA水平的鉴定。M: 2 000 bp DNA分子量标准; 1: 野生型; 2和3: R4-1株系; D: 野生型、*atj8-1*和R4-1苗中*AtJ8*转录本的定量PCR分析。野生型样品中*AtJ8*的表达水平作为标准, 设为1。定量PCR使用*Actin*和*AtJ8*基因特异引物(表1)。数据来自3个生物学重复。WT: 野生型; *atj8-1*: *AtJ8*突变体; R4-1: 一个突变体恢复株系。

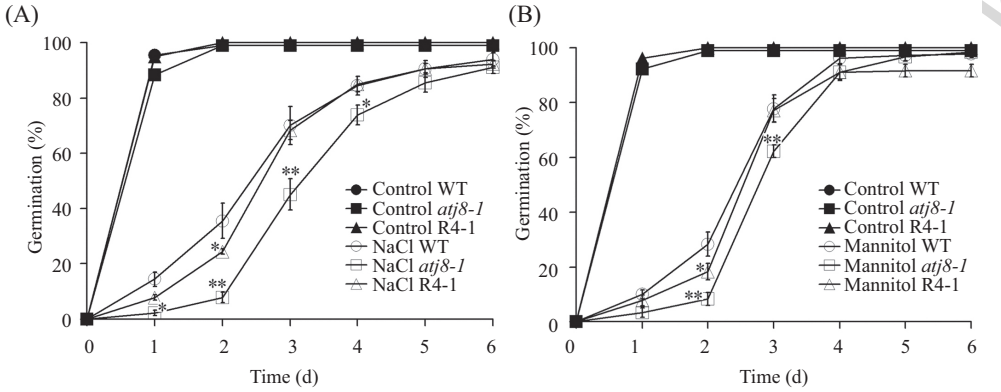
A,B: identification of constructed transgenic vector *PAtJ8:AtJ8*. M: 2 000 bp DNA marker; 1 and 2: digestion of *PAtJ8:AtJ8* by *Pst* I and *Xba* I (A), by *Xba* I and *Sac* I (B); C: identification of a transgenic line. M: 2 000 bp DNA marker; 1: wild type; 2 and 3: R4-1 line; D: Real-time PCR analysis of *AtJ8* transcript in WT, *atj8-1* and R4-1 seedlings. The expression level of the WT sample was used as the calibrator, setting to 1. Real-time PCR was performed using *Actin*- or *AtJ8*-specific primers (Table 1). Data are mean of three biological replicates.

图6 *AtJ8*突变体恢复株系的鉴定

Fig.6 Identification of the rescued *AtJ8* mutant line

法检测了野生型、*atj8-1*和R4-1植物中*AtJ8*基因的转录水平。结果表明, R4-1植物中*AtJ8*基因的表达水平明显高于*atj8-1*, 与野生型的接近(图6D)。

2.3.2 盐和水分胁迫条件下*AtJ8*敲除对种子萌发率的影响 我们以野生型、*AtJ8*突变体和突变体恢复株系为材料研究了种子萌发过程中AtJ8在适应盐和水分胁迫方面的功能。在正常培养条件下, 突变体*atj8-1*种子的萌发率与WT和恢复株系R4-1没有明显的差异。当种子被播种在含200 mmol/L NaCl的MS培养基中时, 播种后3 d内*atj8-1*种子的萌发率明显低于WT和R4-1株系, 4 d后3种不同基因背景种子的萌发率差异逐渐缩小(图7A), 表明盐胁迫下*atj8-1*

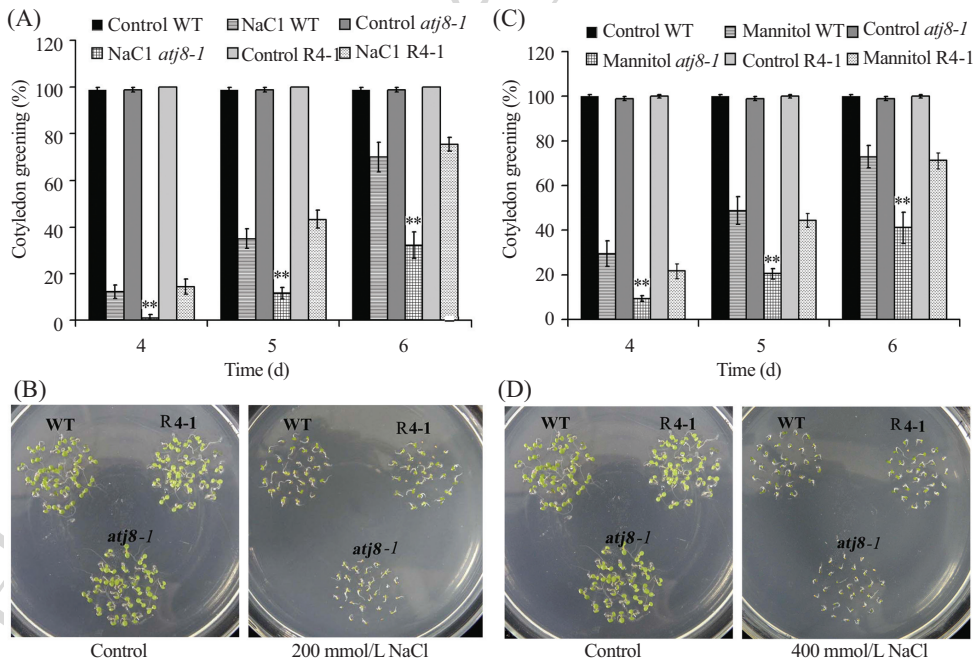


A: 200 mmol/L NaCl处理下不同基因型种子萌发率的比较; B: 400 mmol/L甘露醇处理下不同基因型种子萌发率的比较。每次实验检测90粒种子, 数据是3次独立实验的平均值(n=270)。*P<0.05, **P<0.01, 与NaCl处理的野生型比较。

A: comparison of germination rate among different genotypic seeds under 200 mmol/L NaCl treatment; B: comparison of germination rate among different genotypic seeds under 400 mmol/L mannitol treatment. Ninety seeds per genotype were measured in each experiment. The data were the means from three individual experiments (n=270). *P<0.05, **P<0.01 *vs* NaCl WT group.

图7 渗透胁迫条件下不同基因型株系种子萌发率的比较

Fig.7 The comparison of germination rate among different genotypic seeds under osmotic stress

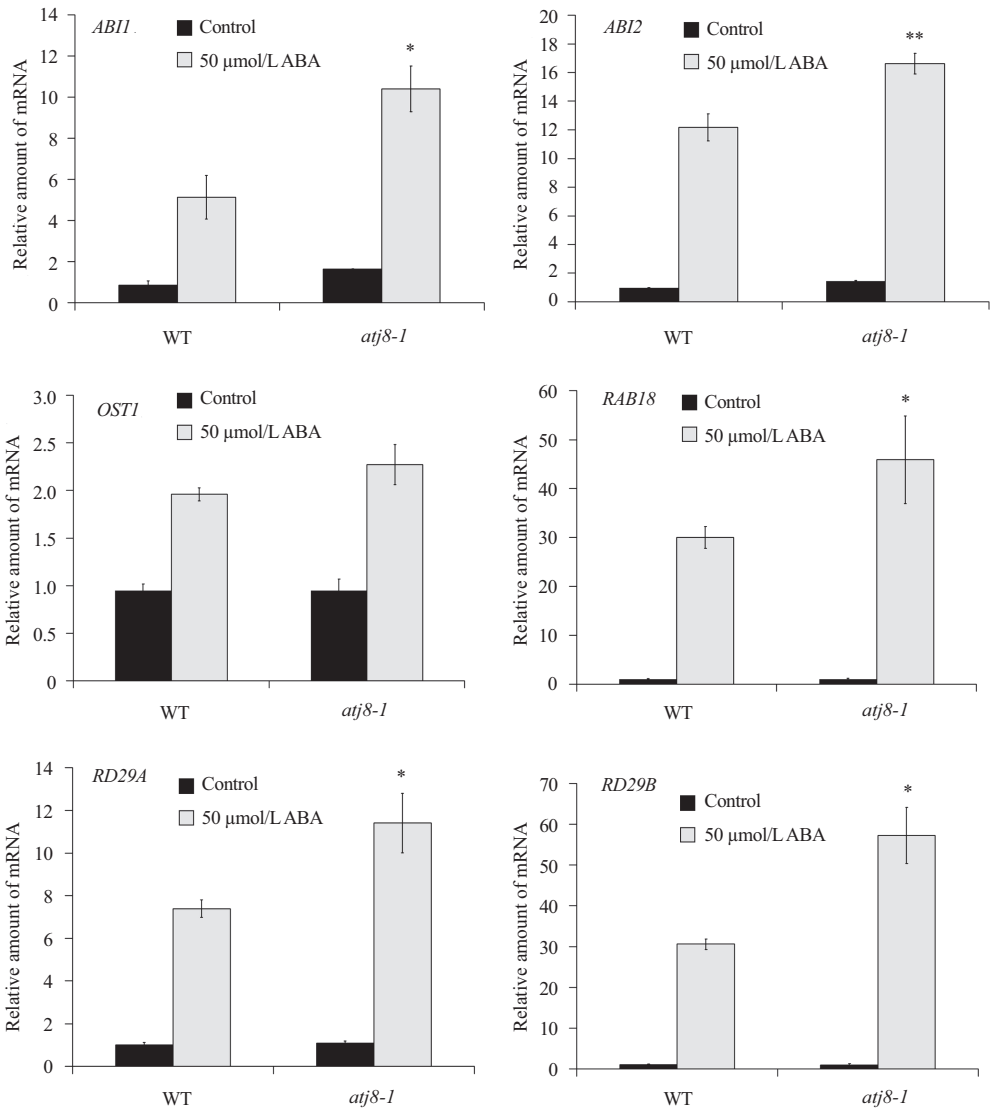


A、B: 拟南芥苗在含200 mmol/L NaCl的MS培养基上生长6 d; C、D: 拟南芥苗在含400 mmol/L甘露醇的MS培养基上生长6 d。每次实验检测30株幼苗, 数据是9次独立实验的平均值(n=270)。*P<0.05, **P<0.01, 与NaCl处理的野生型比较。

A,B: *Arabidopsis* seedlings were cultured for 6 d in MS medium including 200 mmol/L of NaCl; C,D: *Arabidopsis* seedlings were cultured for 6 d in MS medium including 400 mmol/L of mannitol. Thirty seedlings per genotype were measured in each experiment. The data were the mean from nine individual experiments (n=270). *P<0.05, **P<0.01 *vs* NaCl WT group.

图8 渗透胁迫条件下不同基因型幼苗绿色子叶出现率的比较

Fig.8 The comparison of cotyledon greening among different genotypic seedlings under osmotic stress



以水处理的野生型苗中基因的表达水平设为1。数值是3次独立实验的平均值。*P<0.05, **P<0.01, 与野生型比较。

The gene expression levels in water-treated WT plants were set to 1 and used for normalisation. The values are the mean from three independent experiments. *P<0.05, **P<0.01 *vs* WT group.

图9 *AtJ8*敲除改变了ABA反应基因的表达

Fig.9 *AtJ8* knockout altered the expression of ABA-responsive genes

种子萌发延迟。为了研究AtJ8在盐胁迫反应中的作用是否属于渗透调节, 我们将不同基因型的种子播种在包含400 mmol/L甘露醇的MS培养基上。结果显示, 脱水胁迫下*atj8-1*种子萌发同样比WT和R4-1株系延迟(图7B), 表明种子萌发过程中AtJ8可能在渗透胁迫调节方面发挥一定的作用。

2.3.3 盐和水分胁迫条件下*AtJ8*敲除对绿色子叶出现率的影响 我们以野生型、*AtJ8*突变体和突变体恢复株系为材料, 进一步研究了AtJ8在萌发后发育阶段适应盐及水分胁迫方面的功能。在正常培养条件下, *atj8-1*突变体的绿色子叶出现率与WT和恢复株系R4-1没有明显的差异。当种子被播种在含200 mmol/L NaCl的MS培养基中时, 播种4 d后*atj8-1*的绿色子叶出现率明显低于WT和R4-1株系(图8A和图8B), 说明*AtJ8*基因可能涉及萌发后发育阶段的盐适应性的调节。同时, 我们将不同基因型的种子播种在包含400 mmol/L甘露醇的MS培养基上。结果显示, 脱水胁迫下*atj8-1*突变体苗的绿色子叶出现率同样比WT和R4-1株系低(图8C和图8D), 表明AtJ8可能在萌发后发育阶段的渗透胁迫适应性调节方面发挥一定的作用。

2.4 AtJ8影响ABA反应基因的表达

为了了解AtJ8在植物对ABA反应中的作用, 我们通过定量PCR方法比较了野生型和*atj8-1*突

变体中几种ABA反应基因在外源ABA处理后的表达情况。在50 μmol/L ABA处理4周龄的植株24 h后, *atj8-1*植物中*ABI1*、*ABI2*、*RAB18*(response to ABA 18)、*RD29A*(response to desiccation 29A)和*RD29B*(response to desiccation 29B)基因的表达水平明显高于野生型, *OST1*基因的表达与野生型没有明显差异(图9), 表明AtJ8可能调节一些ABA反应基因的表达。

3 讨论

J-蛋白是一个超基因家族, 它们在植物的生命活动中行使了各种各样的功能, 这可能与它们不同的时空表达特性密切相关。基因的组织特异性表达特性能为蛋白质功能研究提供重要的信息和线索。本研究首先通过*GUS*报告基因的方法检测*AtJ8*基因的组织特异性表达, 结果表明, *AtJ8*在拟南芥中的表达是广泛的, 没有明显的组织表达特异性。然后, 通过定量PCR的方法比较了各个器官组织中*AtJ8*基因的表达, 结果显示, *AtJ8*的表达水平从高到低依次为花、叶、幼嫩长角果、根和茎。*AtJ8*基因在植物体内的广泛表达暗示该基因可能在细胞的生命活动中发挥重要的作用。Chen等[36]报道, AtJ8定位于叶绿体中, 参与光合作用的调节。本研究表明, *AtJ8*基因的表达受盐和水分胁迫的诱导, 暗示AtJ8可能参与非生物胁迫信号途径的调节。我们进一步以野生型、*AtJ8*突变体和突变体恢复株系为材料来研究AtJ8在盐和水分胁迫适应中可能发挥的功能, 结果显示, 在3种不同基因型植物中, 盐胁迫抑制种子萌发和子叶变绿, *AtJ8*突变体植物中这种抑制作用更明显。水分胁迫条件下, 在种子萌发及萌发后发育阶段*AtJ8*突变体的表型与盐胁迫条件下是一致的, 表明AtJ8可能是在渗透胁迫适应的调节方面发挥作用。此外, 我们也检测了成熟植物体对盐和水分胁迫的反应, 结果显示, *AtJ8*突变体与野生型的抗盐性和抗旱性没有明显的差异, 表明AtJ8可能是在植物的早期生长阶段调节植物对渗透胁迫的适应。

盐和水分胁迫诱导ABA的合成并激活ABA依赖的信号途径[7,12]。本研究发现, *AtJ8*基因表达受ABA的快速诱导, 暗示AtJ8调节非生物胁迫信号途径可能是通过ABA依赖的途径。ABA通过复杂的信号网络诱导许多参与适应性反应的基因表达的改变。微阵列数据库分析表明, 拟南芥中有2 900种基因参与对ABA的反应[38], 其中包括*RD29A*、*RD29B*、*ABI1*、*ABI2*、*OST1*和*RAB18*基因。这些基因可作为监测植物中胁迫适应及ABA反应途径的标记[39-40]。为了确定AtJ8在ABA信号途径中的作用, 我们通过定量PCR方法检测了*AtJ8*敲除对前面提及的6种ABA反应基因表达的影响。50 μmol/L的ABA处理后, *AtJ8*突变体中*ABI1*、*ABI2*、*RAB18*、*RD29A*和*RD29B*基因的表达明显高于野生型。这一结果暗示, AtJ8可能负调节以上ABA反应基因的表达, 且胁迫条件下*AtJ8*突变体中*ABI1*、*ABI2*、*RAB18*、*RD29A*和*RD29B*较高的表达水平可能抑制种子萌发和萌发后的发育。已有报道, 盐胁迫条件下拟南芥*RD29A*和*RD29B*突变体在根的生长速率、光合作用及水分利用效率方面均高于野生型[41]; ABI1和ABI2是ABA信号途径的负调节剂[42-43]。这些研究支持笔者的结论, 即渗透胁迫条件下*AtJ8*突变体的种子萌发及萌发后发育受抑制可能与*ABI1*、*ABI2*、*RD29A*和*RD29B*较高的表达水平相关。

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