研究论文

受油菜素内酯调控的水稻幼苗地上部膜蛋白的鉴定及功能分析

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摘要 油菜素内酯(brassinosteroids, BRs)对水稻株高、叶夹角等重要农艺性状具有明显的调控作用, 鉴定水稻中受BR调控的蛋白质对揭示BR调控水稻生长发育特定生化过程的潜在机制具有重要的意义。该研究以日本晴水稻(*Oryza sativa* L. ssp. *japanica cv.Nipponbare*)为研究材料, 用明显影响水稻地上部生长的BR浓度处理, 提取地上部膜蛋白, 经双向电泳及质谱分析, 鉴定到7个受BR调控的蛋白质: 光系统II稳定因子HCF136、PMRP(putative membrane related protein, gi|113565516)、ATP synthase(gi|113611230)、gi|113594641、gi|22831029、gi|47497322和gi|56784135, 对这些蛋白的功能鉴定可以为阐明BR调控水稻生长发育的机理提供新的思路和途径。其中功能未知的膜蛋白PMRP受BR下调, 经亚细胞定位, PMRP定位在细胞质膜上, 经生物信息学分析, PMRP具有磷脂酰胆碱结合位点, 可能影响膜的组分进而参与水稻抗逆性调控; PMRP RNAi转基因拟南芥对冷害的抵抗能力增强, 说明BR可通过调控PMRP的表达提高植物的抗冷性。

关键词 油菜素内酯(BR); 膜蛋白; 双向电泳; 水稻

Identification and Functional Analysis of Rice Seedling Shoot Membrane Protein Regulated by Brassinosteroids

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Abstract Brassinosteroids (BR) has obvious effect on important agronomic traits of rice, such as plant height and leaf angle. The identification of the proteins regulated by BR was important for revealing the potential mechanisms by which BR regulates specific biochemical and cellular processes in rice. In this study, *Oryza sativa* L. ssp. *japanica cv.Nipponbare* was used as research material, and the rice shoot was treated with BR which could induce obvious effect on rice shoot growth. Then we extracted the shoot membrane protein and analysed by two-dimensional electrophoresis and mass spectrometry. 7 proteins regulated by BR were identified: Photosystem II stability/assembly factor HCF136, PMRP (putative membrane related protein, gi|113565516), ATP synthase (gi|113611230), gi|113594641, gi|22831029, gi|47497322 and gi|56784135. PMRP was down regulated by BR.

收稿日期: 2014-08-21 接受日期: 2014-10-30

河北省自然科学基金项目(批准号: C2013204106)资助的课题

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Received: August 21, 2014 Accepted: October 30, 2014

This work was supported by the Natural Science Foundation of Hebei Province (Grant No. C2013204106)

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网络出版时间: 2014-12-25 11:07 URL: http://www.cnki.net/kcms/detail/31.2035.Q.20141225.1107.007.html

PMRP was mainly localized on cytoplasma membrane by subcellular localization analysis. Bioinformatics analysis showed that PMRP had a phosphatidylcholine binding site, so PMRP might influence the membrane components and play a role in the process of plant resistance. The PMRP RNAi transgenic *Arabidopsis* plants had enhanced resistance to chilling injury. These results prove that BR can improve the cold resistance of plants by regulating the expression of PMRP.

Keywords　brassinosteroid (BR); membrane protein; two-dimensional electrophoresis; rice

水稻是我国三大粮食作物之一, 也是单子叶植物基因组学研究的模式材料, 在生产实践和科学研究中都占有极其重要的地位。油菜素内酯(brassinosteroids, BRs)是国际上公认的活性最高、广谱、无毒的植物生长激素[1-2], 能促进作物生长和增加作物产量[3-5], 提高作物的抗逆性[6-9]。但BR调控水稻生长发育的机制还不是很清楚。目前, 水稻中BR信号转导途径的研究大都仅限于拟南芥信号转导途径组分同源基因的克隆及相应突变体的分析上, 而且已知的BR信号转导组分还很难组成一个完整的信号转导途径, 从BR信号的感知到信号在胞质的传递, 再到在核内引起特异基因的表达和酶活性等各个层次上都还有一些问题尚待阐明。

几个BR相关突变体的相继鉴定为BR调控水稻生长发育机制和信号转导的研究打开了突破口。第一个被克隆的BR信号转导相关基因是*OsBRI1*, *OsBRI1*的突变体*d61*与野生型相比较具有矮小、叶片直立、种子小、第二节间缩短等特点[10]。相继被鉴定的几个突变体是BR合成缺失突变体*brd1*、*brd2*、*brd11*、*brd2*[11-14]。这些BR合成缺失突变体表现为生长受抑制、株型矮小、叶片直立、叶色暗绿等。Bai等[15]利用反向遗传学研究了OsBZR1的功能并鉴定了一些与OsBZR1有相互作用的蛋白, 利用RNAi干涉降低植物体内OsBZR1的表达可导致植株矮小、叶片直立。*OsGSK1*是拟南芥*BIN2*的同源基因, 过表达全长*OsGSK1*的转基因植株表现为株型矮小, 推测*OsGSK1*可能是水稻BR信号转导途径中的负调节因子。敲除*OsGSK1*后的转基因植株对冷、热、盐、干旱的忍耐能力增强, 说明*OsGSK1*在逆境响应中起着非常重要的作用[16]。借助基因芯片结果, 鉴定到受BR调节的基因*OsBLE1*和*OsMPD1*, 把反义*OsBLE1*转入水稻中会抑制水稻的生长, *OsMPD1*在BR信号传递中起负调节作用[17-18]。*OsMADS22*和*OsMADS55*是*OsMPD1*的同源基因, 被证明也在BR信号传递中起负调节作用[19]。

综上所述, 以往主要采用分子遗传学手段对BR信号转导组分进行研究, 然而, 基因组中广泛存在的冗余性是限制遗传手段功能基因研究的巨大障碍。而蛋白质组学作为一个技术手段, 不受冗余性限制, 尤其是在对激素调节生长发育中的功能基因研究中, 鉴定受激素调控的蛋白可以有效地找到新的参与信号转导和生长调节的重要基因, 进而为阐明生长调节的分子机制并最终改进作物产量提供理论基础和分子手段。

1　材料与方法

1.1　材料

1.1.1　*供试植物和菌株*　水稻日本晴(*Oryza sativa* L. ssp. *japanica cv.Nipponbare*), 烟草(*Nicotiana Benthmianan)*、大肠杆菌(DH5α)、根癌农杆菌(*Agrobacterium tumefaciens*) EHA105和RNAi表达载体pTCK309、Cam35S-GFP融合表达载体均由本实验室保存。

1.1.2　*试剂与仪器*　RNA提取试剂盒(Cat# CW0588)、反转录试剂盒(Cat#CW0744)、PCR mix、限制性内切酶均购于北京百泰克生物技术有限公司。

1.2　方法

1.2.1　*水稻的培养方法*　水稻种子经过30 ℃浸种24 h后, 在28 ℃下萌发, 萌发2 d后转至木村B营养液中, 把不同浓度(0, 1×10^{-10}, 1×10^{-9}, 1×10^{-8}, 1×10^{-7}, 1×10^{-6} mol/L)的eBL(表油菜素内酯)加入营养液中处理水稻幼苗, 观察水稻幼苗表型。

选定含有1×10^{-6} mol/L eBL的营养液处理生长7 d的水稻幼苗6 h, 收集水稻地上部, 液氮速冻后, –80 ℃冰箱保存, 用于蛋白质、RNA和DNA的提取。

1.2.2　*地上部膜蛋白的提取*　参照Sandelius[20]的方法。取8 g材料, 液氮研磨至碎末, 加50 mL匀浆液(15 mmol/L Tris-HCl(pH7.8), 0.4 mol/L Sucrose, 1 mmol/L EDTA, 1 mmol/L蛋白酶抑制剂

苯甲基磺酰氟(phenylmethanesulfonylfluoride or phenylmethylsulfonyl fluorid, PMSF), 3 mmol/L DTT, 0.6%(W/V)抗氧化剂聚乙烯吡咯烷酮(polyvinyl pyrrolidone, PVP), mirocloth过滤, 4 ℃, 10 000 ×*g*离心15 min; 取上清液, 4 ℃, 20 000 ×*g*离心30 min, 弃上清; 用500 μL磷酸缓冲液(5 mmol/L, pH6.8)悬浮沉淀, 得到质膜的粗提物。

粗提质膜加入8 g样品系统液, 上下翻转混匀; 4 ℃, 1 500 ×*g*离心10 min; 上相移入同样离心分相且弃去上相的第一份洗涤系统液中, 混匀, 4 ℃ 1 500 ×*g*离心10 min; 再将上相移入同样离心分相且弃去上相的第二份洗涤系统液中, 混匀, 4 ℃, 1 500 ×*g*离心10 min; 收集上相; 用稀释液稀释3~5倍; 4 ℃ 120 000 ×*g*离心30 min; 纯化的质膜沉淀用100 μL裂解缓冲液裂解(7 mol/L尿素, 2 mol/L硫脲, 4%表面活性剂3-[3-(胆酰胺丙基)二甲氨基]丙磺酸内盐[3-((3-cholamidopropyl)dimethylammonium)-1-propanesulfonate, CHAPS], 13 mmol/L DTT, 2% Pharmalyte 3-10), 室温摇动1 h, 室温, 20 000 ×*g*离心20 min; 取上清液用于双向电泳。

样品系统液: 20%(W/W) Dextran T-500(2.48 g); 40%(W/W) PEG 4000(1.24 g); 1 mol/L Sucrose(2 mL); 200 mmol/L Potassium phosphate, pH6.8(187.5 μL); 加Milli Q纯水至7.5 g, potassium phosphate, pH6.8(0.5 g)。

洗涤系统液: 20%(W/W) Dextran T-500(2.48 g); 40%(W/W) PEG 4000(1.24 g); 1 mol/L Sucrose(2 mL); 200 mmol/L Potassium phosphate, pH6.8(200 μL); 加Milli Q纯水至8.0 g。

稀释液: 0.25 mmol/L Sucrose; 0.1 mmol/L DTT; 5 mmol/L Tris-HCl, pH7.2。

1.2.3 蛋白质浓度测定 按照Bradford法[21](1976)测定蛋白质提取液中的蛋白质含量。

1.2.4 双向电泳及质谱分析 参照本实验室以前的方法[22]进行。

1.2.5 PMRP的生物信息学分析 PMRP的保守域、氨基酸组成、具有的结合位点分析通过NCBI(http://www.ncbi.nlm.nih.gov)及TAIR(http://www.Arabidopsis.org)网站查询; PMRP蛋白质空间结构分析应用I-TASSER软件进行预测。

1.2.6 PMRP的亚细胞定位载体构建及观察 根据*PMRP*基因的cDNA序列和Cam35S-GFP融合表达载体的多克隆位点, 利用Primer 5.0软件设计基因特异引物(表1)。以野生型日本晴水稻幼苗cDNA作为模板, 扩增*PMRP*基因的CDS序列。酶切连接法转化*E. coli* DH5α, 重组质粒命名为PMRP-GFP, 转化农杆菌, 采用注射法把含有PMRP-GFP质粒的农杆菌转入烟草叶片, 48 h后用激光共聚焦显微镜, 激发波长为488 nm, 检测报告基因*GFP*的分布情况, 从而确定PMRP蛋白的亚细胞定位。

1.2.7 PMRP RNAi转基因拟南芥的获得 利用高保真Taq酶从水稻cDNA扩增出*PMRP*, 为1 443 bp的cDNA片段, 通过两步酶切法将520 bp(起始位点ATG开始第21个碱基至第540碱基)长的*PMRP*正向片段和反向片段连入RNAi载体pTCK309, 中间被一个463 bp的玉米内含子分开, 整个片段利用玉米的泛素启动子(Ubi promoter)驱动表达。将构建好的载体转入农杆菌EHA105用于转化拟南芥[23]。

1.2.8 PMRP RNAi转基因拟南芥的抗冷性实验 参照的Chinnusamy等[24]的方法, 将生长15 d的拟南芥先放在4 ℃下14 d, 然后放入–7 ℃的恒温箱中3 h, 再放在正常环境(16 h光照、21 ℃)下生长。

2 结果

2.1 BR对水稻幼苗生长的影响

BR在水稻的生长、发育过程中都起着非常重要的作用。水稻对BR的反应呈现剂量效应。为明确BR对水稻生长的影响, 用不同浓度BR处理水稻幼苗, 观察水稻幼苗的生长情况。选用的外源BR是人工合成的24-表油菜素内酯(epioBL, 简称eBL), 浓度依次为0, 1×10^{-10}, 1×10^{-9}, 1×10^{-8}, 1×10^{-7}, 1×10^{-6} mol/L, 处理

表1 PCR扩增和基因表达所用引物序列

Table 1 Primers sequence of PCR amplification and gene expression

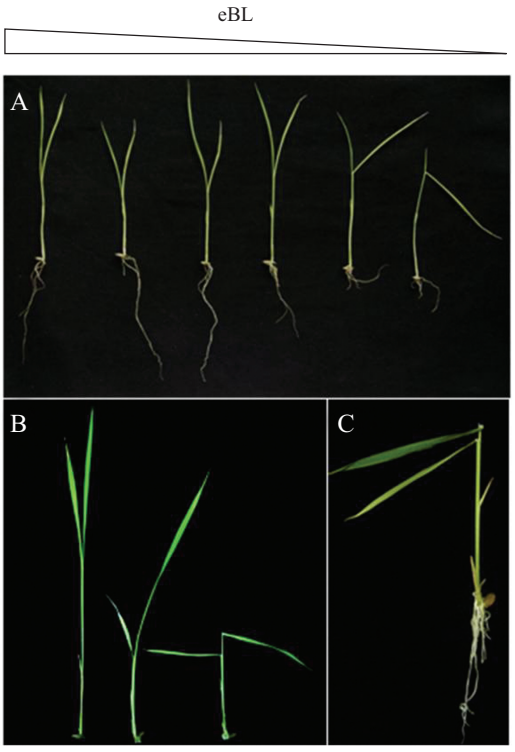
基因名称 Gene name	引物序列(5′→3′) Primer sequence (5′→3′)	用途 Using
PMRP	F: CGG GAT CCA TGA ATA GTG GTG ATA CCT TAT C R: CGG AAT TCT TAA ATA CCA GCG TTT TGG	The gene cloning
PMRP	F: CGA GCT CAT GAA TAG TGG TGA TAC CTT ATC R: GCT CTA GAA ATA CCA GCG TTT TGG GAA G	The subcellular localization of PMRP

5 d后观察, 发现随着eBL浓度的增加, 水稻幼苗的叶夹角依次变大(图1A)。1×10^{-6} mol/L eBL处理10 d后, 大多数水稻幼苗两片叶子近似平行(图1B), 个别可增大至接近270°(图1C)。

2.2　BR处理后水稻幼苗地上部膜蛋白的双向电泳及质谱鉴定

本研究利用两相分离法对0和1×10^{-6} mol/L eBL处理3 h后水稻幼苗地上部的质膜蛋白进行双向电泳分析, IPG胶条的pH范围为4~7, 电泳凝胶通过UMAX扫描仪获得图像, 扫描参数设置为256阶灰度、600dpi透射扫描(图2)。扫描后的图像用ImageMaster 2D Platinum软件(Amersham Biosciences)进行分析, 选定差异蛋白(表达丰度相差2倍以上), 样品送华大基因科技服务有限公司用AXIMA-CFR Plus MALDI-TOF质谱仪(Shimadzu, Japan)进行分析。得到肽质量指纹图谱(peptide mass fingerprinting, PMF)数据, 利用Mascot软件(Matrix science, London)在NCBI数据库中进行检索, 鉴定蛋白共得到7个蛋白(表2)。

从实验结果(表2)可以看出: 在鉴定的7个蛋白中, 光系统II稳定因子HCF136[25]被BR上调, 表明BR处理有利于光合系统的稳定性, 一个和膜的生物发生有关的蛋白质(gi|113565516)及ATP合成酶均受BR诱导, 表明BR在水稻的细胞建成方面具有重要作用; 几个功能未知的蛋白(gi|113594641、gi|22831029、gi|47497322、gi|56784135)受BR下调, 对这些蛋白的功能鉴定,

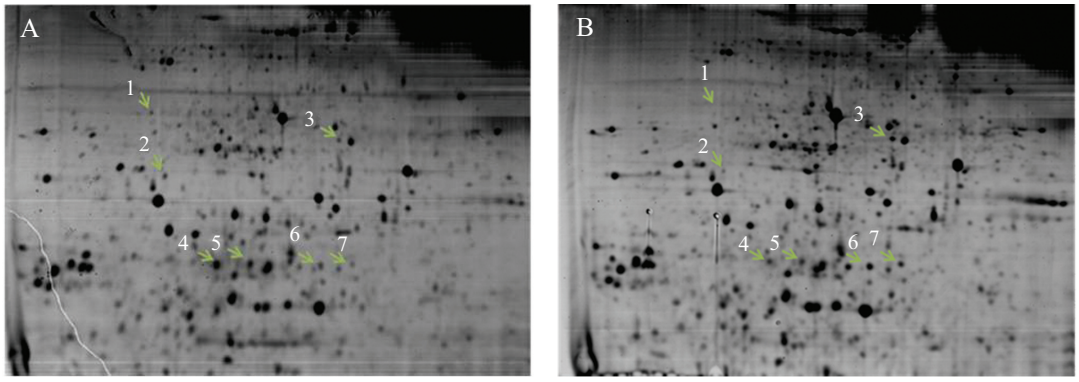


A: 不同浓度eBL处理5 d后水稻幼苗生长情况(从左到右eBL的处理浓度依次为0, 1×10^{-10}, 1×10^{-9}, 1×10^{-8}, 1×10^{-7}, 1×10^{-6} mol/L); B: 不同浓度eBL处理10 d后水稻幼苗生长情况(从左到右eBL的处理浓度依次为0, 1×10^{-10}, 1×10^{-6} mol/L); C: 示1×10^{-6} mol/L eBL处理10 d后, 个别水稻幼苗叶夹角呈近乎270°的情况。

A: 5 days rice seedlings after eBL treatment of different concentrations (from left to right, the concentrations of eBL were 0, 1×10^{-10}, 1×10^{-9}, 1×10^{-8}, 1×10^{-7}, 1×10^{-6} mol/L); B: 10 days rice seedlings after eBL treatment of different concentrations (from left to right, the concentrations of eBL were 0, 1×10^{-10}, 1×10^{-6} mol/L); C: individual rice seedlings 10 days after 1×10^{-6} mol/L of eBL treatment, the leaf angle was near 270°.

图1　不同浓度eBL处理后水稻幼苗生长情况

Fig.1　The rice seedlings treated by different concentrations of eBL



A: 0 mol/L eBL处理生长20 d的野生型水稻幼苗3 h后地上部膜蛋白的双向电泳图谱(箭头所示为BR处理后表达有差异的蛋白点); B: 1×10^{-6} mol/L eBL处理生长20 d的野生型水稻幼苗3 h后地上部膜蛋白的双向电泳图谱(箭头所示为BR处理后表达有差异的蛋白点)。

A: 2D-PAGE of the shoot membrane protein of 20 days rice seedlings after 0 mol/L of eBL treatment for 3 hours (the different expressed protein spots after BR treatment were indicated by arrows); B: 2D-PAGE of the membrane protein in shoot of 20 days rice seedlings after 1×10^{-6} ofmol/L eBL treatment for 3 hours (the different expressed protein spots after BR treatment were indicated by arrows).

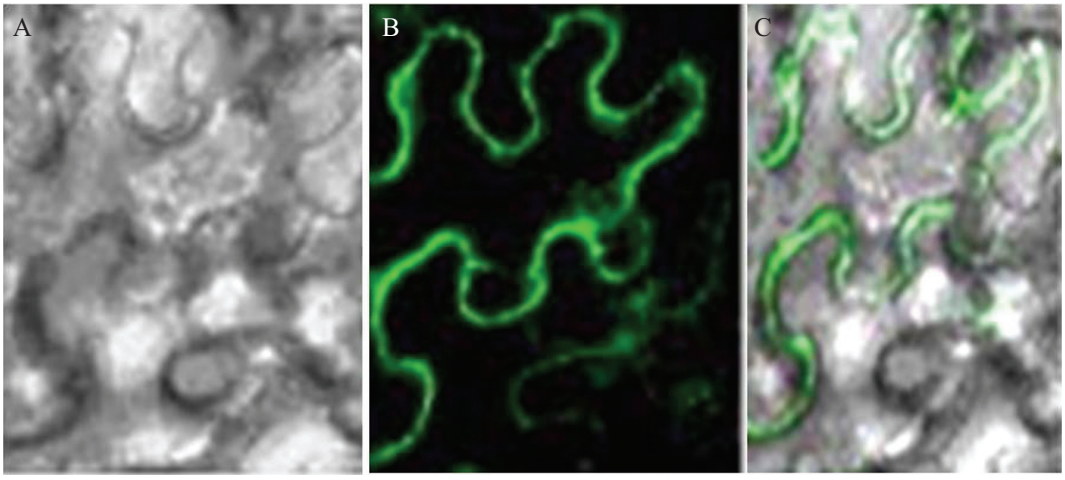
图2　eBL处理3 h后生长20 d的水稻幼苗地上部膜蛋白的双向电泳分析

Fig.2　2D-PAGE of the shoot membrane protein of the 20 days rice seedlings after eBL treatment for 3 hours

表2 水稻幼苗地上部膜蛋白中受BR调控的蛋白质

Table 2 The proteins regulated of BR in membrane proteins in shoot of rice seedlings

点序号 Spot No.	编码 Code	注释 Annotation	受BR调控效果 Regulated effect of BR
1	gi|113594641	Unknown protein	Down
2	gi|22831029	Unknown protein	Down
3	gi|75252730	Photosystem II stability/assembly factor HCF136[25]	Up
4	gi|47497322	Putative membrane related protein (PMRP)	Down
5	gi|56784135	Putative Y1 protein	Down
6	gi|113565516	Outer membrane biogenesis	Up
7	gi|113611230	ATP synthase	Up



A: 白光下烟草叶片细胞; B: 488 nm下PMRP-GFP融合蛋白的定位观察; C: A和B的合并图。

A: cells of tobacco leaf in white light; B: localization of PMRP-GFP observed at 488 nm wavelength; C: the merge of A and B.

图3 PMRP在烟草叶片中的亚细胞定位(4 000×)

Fig.3 Sub-cellular localization study of PMRP in tobacco leaf (4 000×)

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  1 magetdstpm aagravpppp eaaaprllll gggaelwrpv argggwataa alllllashl
 61 svlllrrlrl rrrlrpadav ssssaaaaav vtadsapgsa agmdglvteg dlrelvgnlg
121 vaarepereg wqqvvakgnd dvsyrvwcdk pmegpprylg vttyercste llrdfymdne
181 yemewdnevi kheqlqfden sgieigrtik kfplltprey illawrvwegn dksfyclvke
241 cehpvaprqr kfvrvgllrs gwcirkipgr dacritvlhh edngmniema klafakgiwn
301 yickqnsalr rypqrnissi siltmqrltk kfpqaletdv danhhpqgnt ranvvpthfa
361 rtssrqqpgk kssratiasg llligsivcl srgrsnlgaq lamafflkka fkqdkgsssq
421 rsisrtdvte prhle
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图中棕色部分为磷脂酰胆碱结合位点。

The brown parts of the figure were phosphatidylcholine binding sites

图4 PMRP的氨基酸组成及磷脂酰胆碱结合位点

Fig.4 The amino acid composition and phosphatidylcholine binding sites in PMRP protein

可以为阐明BR调控水稻生长发育的机理提供新的思路和途径。

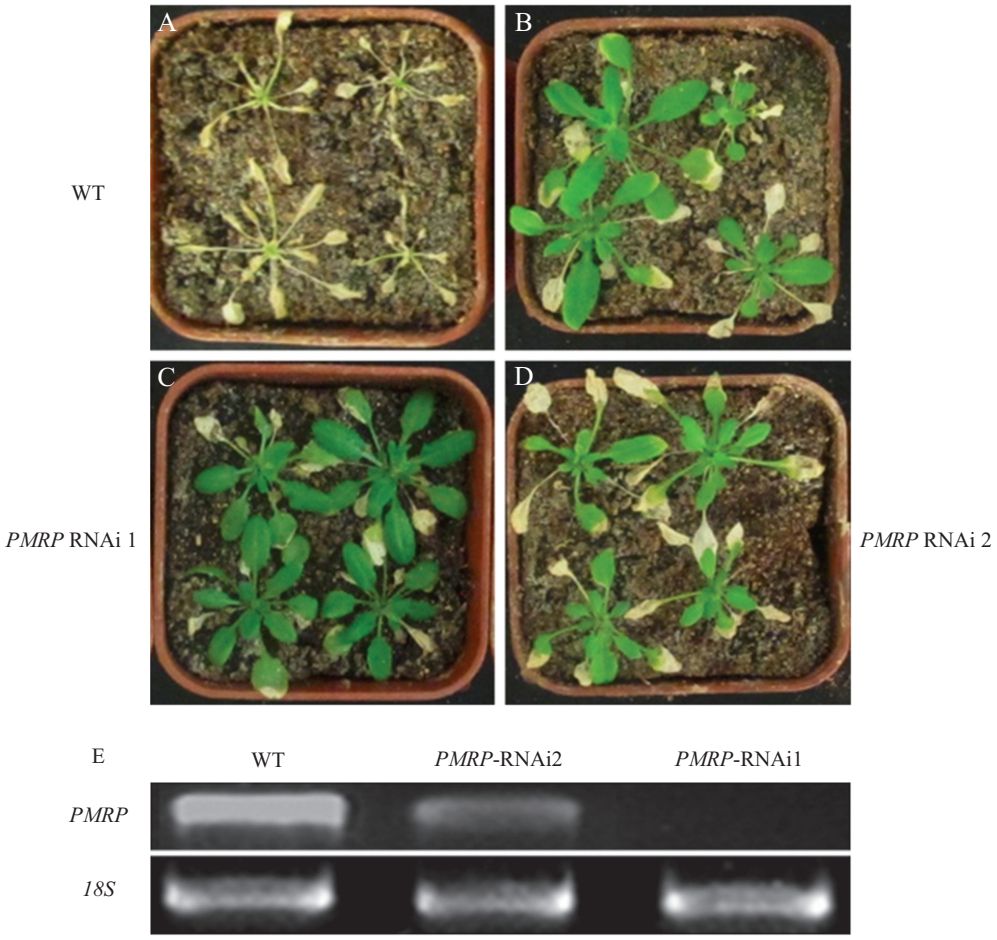
本研究中对PMRP(gi|47497322)的表达特性及功能进行了进一步的分析, 为阐明BR调控水稻生长发育机理提供基础。

2.3 BR调控蛋白PMRP的亚细胞定位

PMRP是435个氨基酸组成的功能未知的膜蛋白, 本研究验证了PMRP的亚细胞定位, 把PMRP-YFP定位载体转化烟草叶片, 在共聚焦显微镜下观察, 发现其确实定位在细胞膜上(图3)。

2.4 PMRP的生物信息学分析

通过NCBI网站(http://www.ncbi.nlm.nih.gov)查询, PMRP是共435个氨基酸、具有跨膜片段的功能未知的蛋白质, 含有START(the lipid/sterol-binding



A: 生长12 d的野生型拟南芥(Col-0)在4 ℃下放置14 d, –7 ℃下放置3 h, 转至22 ℃下4 d的生长情况; B: 生长12 d的野生型拟南芥(Col-0)喷施100 nmol/L BR 3 d后, 4 ℃下放置14 d, –7 ℃下放置3 h, 转至22 ℃下4 d的生长情况; C: 生长12 d的*PMRP* RNAi转基因株系1在4 ℃下放置14 d, –7 ℃下放置3 h, 转至22 ℃下4 d的生长情况; D: 生长12 d的*PMRP* RNAi转基因株系2在4 ℃下放置14 d, –7 ℃下放置3 h, 转至22 ℃下4 d的生长情况; E: *PMRP* RNAi转基因株系1和2中*PMRP*表达量的RT-PCR分析, *18S*为内参。

A: 12 d wild type *Arabidopsis* (Col-0) grown with cold acclimation (14 d at 4 °C, 3 h at –7 °C, and then 4 d at 22 °C); B: 12 d wild type *Arabidopsis* (Col-0) treated by 100 nmol/L of BR 3 d later and grown with cold acclimation (14 d at 4 °C, 3 h at –7°C, and then 4 d at 22 °C); C: 12 d *PMRP* RNAi transgenic *Arabidopsis* line 1 grown with cold acclimation (14 d at 4 °C, 3 h at –7 °C, and then 4 d at 22 °C) ; D: 12 d *PMRP* RNAi transgenic *Arabidopsis* line 2 grown with cold acclimation (14 d at 4 °C, 3 h at –7 °C, and then 4 d at 22 °C); E: the expression levels analysis of *PMRP* gene in *PMRP* RNAi transgenic *Arabidopsis* lines, *18S* was the loading control.

图5　*PMRP* RNAi转基因拟南芥的耐寒性实验

Fig.5　The freezing tolerance of *PMRP* RNAi transgenic *Arabidopsis*

StAR-related lipid transfer protein domains)保守域(第125-312aa)、*PMRP*基因编码的蛋白, 属于脂类运输超蛋白家族, 具有磷脂酰胆碱(PtdCho biding site)结合位点(图4)。由于磷脂酰胆碱是细胞膜的主要组成成分, 推测PMRP可能影响膜的组成进而影响植物的抗逆性。因此, 本研究构建了*PMRP* RNAi载体, 转化野生型拟南芥(Col-0), 进一步分析转基因拟南芥的抗寒性。

2.5　*PMRP* RNAi转基因植株的抗寒性分析

利用pTCK309质粒, 构建*PMRP* RNAi载体。用花絮浸泡法转化Columnbia野生型拟南芥, 获得*PMRP* RNAi转基因拟南芥, RT-PCR分析*PMRP*的表达量, 确认*PMRP* RNAi转基因拟南芥阳性苗(图5)。把生长12 d的*PMRP* RNAi转基因拟南芥在4 ℃下放置14 d, 然后在–7 ℃放置3 h, 分析拟南芥幼苗的生长情况。结果表明, *PMRP* RNAi转基因拟南芥(图5C和图5D)及外源喷施BR的野生型拟南芥(图5B)均比野生型拟南芥(图5A)耐寒, 表明PMRP在拟南芥的耐寒性过程中起负调控作用。

3　讨论

BR在植物对一些逆境的抵抗过程中起着至关

重要的作用, 包括盐、干旱、高温、氧胁迫、病虫草害等过程, 但是BR如何影响上述诸多生理生化过程仍不明确[26]。很多研究数据表明, BR信号途径的成员直接参与到上述过程中, BR信号转导激酶5(brassinosteroid signaling kinase 5, BSK5)被BR和脱落酸(abscisic acid, ABA)上调, 被非生物胁迫盐害和干旱诱导表达, 是盐胁迫和ABA介导的干旱抗性所必需的[27]。在非生物胁迫下, BR能促进或激活很多酶的活性, 例如超氧化物歧化酶的酶、抗坏血酸、谷胱甘肽还原酶及热休克蛋白等, 提高类胡萝卜素、抗坏血酸、谷胱甘肽、植物螯合肽的浓度, 提高ABA、乙烯和水杨酸(SA)的合成[28]。很多被SA、JA(茉莉酸)、乙烯和ABA调控的基因也被BR上调, 说明多种激素协同作用提高植物的抗逆性。据报道, BR提高植物的耐盐性是通过乙烯信号途径进行的; BR与ABA协同作用调控气孔的关闭, BR促进NO的积累, NO促进ABA的合成, ABA提高植物的抗旱性[26]。

BR调控蛋白的鉴定是深入阐明BR提高植物抗逆机理的基础。蛋白质是生命活动的执行者, 鉴定BR调控蛋白及进行生物学功能分析, 便可揭示BR可能参与调控的生理生化过程。双向电泳是鉴定激素调控蛋白的有效技术, 但总蛋白的双向电泳分析, 一些低丰度的蛋白质往往不会被检测到, 而低丰度蛋白恰恰是一些在信号转导过程中起关键作用的蛋白。因此, 本研究利用提取水稻幼苗地上部膜蛋白的策略, 目的在于鉴定细胞膜上的受BR调控的蛋白质, 明确BR调控水稻发育的机制。本研究鉴定到7个受BR调控的可能定位在膜上的蛋白质, 其中蛋白点1和2为未知蛋白质。为检验所鉴定的蛋白质确实为膜蛋白, 我们选取蛋白点4(PMRP)进行亚细胞定位, 结果表明, PMRP确实定位在细胞质膜上(图3); 蛋白点3(photosystem II stability/assembly factor HCF136)为位于类囊体膜上的蛋白质[25], 蛋白点5(putative Y1 protein)位于细胞质膜上[29]; 蛋白点6(outer membrane biogenesis)位于内质网膜上[30]; 蛋白点7(ATP synthase)[31]位于线粒体膜上。以上数据表明, 本方法确实是鉴定膜蛋白的有效方法。

PMRP是我们鉴定到的位于细胞质膜上的受BR调控且功能未知的蛋白质。经过*PMRP* RNAi转基因拟南芥的抗冷性分析, 结果表明PMRP参与了植物抗寒性调控, 于是我们也提出了BR通过调控PMRP的表达进而调控水稻抗冷性过程的新机制, 但PMRP与已知抗寒信号转导途径组分的关系还需要进一步实验证实。

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