核农学报 2013, 27(7) 885-894 DOI: ISSN: 1000-8551 CN: 11-2265/S

植物诱变育种·农业生物技术

一个玉米Pht1家族磷转运蛋白基因克隆和功能分析

苏顺宗, 吴锋锴, 刘丹, 吴玲, 高世斌

四川农业大学玉米研究所, 四川 成都 611130

摘要:

从耐低磷玉米自交系178中分离和鉴定高亲和力磷转运蛋白质基因,为开展磷高效分子育种奠定理论基础。本研究以水稻和拟南芥中鉴定的磷转运蛋白基因为基础,运用生物信息学方法,对玉米进行全基因组预测及系统进化分析;并运用克隆、实时荧光定量PCR和亚细胞定位方法对其家族成员进行深入研究。结果表明,从玉米自交系B73全基因组序列中筛选出37个磷转运蛋白候选基因,并被聚类为五大家族。从耐低磷材料中扩增了一个属于Pht1家族成员*ZmPht*1;9的全长cDNA,该基因编码区长1620bp,编码539个氨基酸,含有典型的MFS超家族蛋白的保守结构域和12个跨膜结构;荧光定量PCR分析表明,在低磷胁迫下,该基因的相对表达量显著增加,且叶片中的表达量高于根系,同时在基因型之间也存在差异;原生质体转化的亚细胞定位结果显示,*ZmPht*1;9表达蛋白主要分布于细胞膜上。*ZmPht*1;9编码位于细胞膜上的高亲和力磷转运蛋白,对调节磷素的动态平衡起重要作用。

关键词: 玉米 磷转运蛋白 进化树 基因表达 亚细胞定位

Cloning and Characterization of a Phosphate Transporter Gene of Pht1 Family in Maize

SU Shun-zong, WU Feng-kai, LIU Dan, WU Ling, GAO Shi-bin

Maize Research Institute, Sichuan Agricultural University, Chengdu Sichuan 611130

Abstract:

To provide usefully theoretical information for improvement of phosphorus utilization, a high-affinity PHT gene from low-P tolerant maize inbred line 178 was isolated in this study. The PHT families of maize at whole genome-wide level were predicted based on the gene sequences of PHT orthologs in rice and Arabidopsis using bioinformatics approaches, and their phylogenetic relationship was also been analyzed based on amino acid sequence. The methods of clone, quantitative real-time PCR and subcellular localization were performed for further research of PHT family member. A total of 37 maize PHT genes were predicted from whole genome sequences of maize inbred line B73, which can be classed into five groups based on their characters of functional domains. A complete cDNA of *ZmPht*1;9, belongs to Pht1 family and encodes 539 putative amino acids with the complete coding region of 1 620bp in length, was further cloned from 178’s cDNA template. The protein of *ZmPht1;9* contains 12 transmembrane domains and has a typical MFS conservation structure domain, which shows highly conserved within plants. The relative expression levels of *ZmPht*1;9 detected by quantitative real-time PCR exhibited up-regulation under low phosphorus stress and was higher in the leaves than in the roots, and expression patterns were shown differently between two extreme maize lines with distinct tolerance to phosphorus deficiency. Subcellular localization analysis revealed that the *ZmPht*1;9 was expressed in cytoplasm membrane via protoplast-mediated. The results suggested that *ZmPht*1;9 was a high-affinity PHT gene and was possibly involved in maintaining phosphate dynamic homeostasis under phosphate deficiency.

Keywords: Maize Phosphate transporter Phylogenetic relationship Gene expression Subcellular localization

收稿日期 2013-02-22 修回日期 2013-06-16 网络版发布日期

DOI:

基金项目:

"973"计划(2009CB118400);"948项目"(2011-G15-2);国家自然科学基金(31171566)

通讯作者: 高世斌,男,教授,主要研究方向:玉米遗传育种。E-mail:shibingao@163.com

作者简介:

作者Email: shibingao@163.com

参考文献：

[1] Rausch C, Bucher M. Molecular mechanisms of phosphate transport in plants[J]. Planta, 2002, 216 (1): 23-37

[2] Lynch J. Root architecture and plant productivity[J]. Plant Physiology, 1995, 109: 7-13

[3] 孙海国, 张福锁, 杨军芳. 不同供磷水平小麦苗期根系特征与其相对产量的关系[J]. 华北农学报, 2001, 16(3): 98-104

[4] Raghothama K G. Phosphate acquisition [J]. Annual Review of Plant Physiology and Plant Molecular Biology, 1999, 50(1): 665-693

[5] Fang Z Y,Shao C, Meng Y J, Wu P, Cheng M. Phosphate signaling in *Arabidopsis* and *Oryza sativa*[J]. Plant Science, 2009, 176(2): 170-180

[6] Muchhal U S, Pardo J M, Raghothama K G. Phosphate transporters from the higher plant *Arabidopsis thaliana*[J]. Proceedings of the National Academy of Sciences, 1996, 93(19): 10519-10523

[7] Mudge S R, Rae A L, Diatloff E, Smith F W. Expression analysis suggests novel roles for members of the Pht1 family of phosphate transporters in *Arabidopsis*[J]. The Plant Journal, 2002, 31(3): 341-353

[8] Poirier Y, Bucher M. Phosphate transport and homeostasis in Arabidopsis[J]. The Arabidopsis Book, 2002, 1: e0024

[9] Daram P, Brunner S, Rausch C, Steiner C, Amrhein N, Bucher M. *Pht2;1* encodes a low-affinity phosphate transporter from Arabidopsis[J]. The Plant Cell, 1999, 11(11): 2153-2166

[10] Myoung R P, So-Hyeon B, Benildo R, Song Y. Overexpression of a high-affinity phosphate transporter gene from tobacco (*NtPT1*) enhances phosphate uptake and accumulation in transgenic rice plants[J]. Plant and Soil, 2007, 292(1): 259-269

[11] Rae A L, Cybinski D H, Jarmey J M, Smith F W. Characterization of two phosphate transporters from barley; evidence for diverse function and kinetic properties among members of the Pht1 family[J]. Plant molecular biology, 2003, 53(1): 27-36

[12] Rae A L, Jarmey J M, Mudge S R, Smith F W. Over-expression of a high-affinity phosphate transporter in transgenic barley plants does not enhance phosphate uptake rates[J]. Functional Plant Biology, 2004, 31(2): 141-148

[13] Takabatake R, Hata S, Taniguchi M, Kouchi H, Sugiyama T, Izui K. Isolation and characterization of cDNAs encoding mitochondrial phosphate transporters in soybean, maize, rice, and *Arabidopsis*[J]. Plant molecular biology, 1999, 40(3): 479-486

[14] Hamburger D, Rezzonico E, MacDonald-Comber Petétot J, Somerville C, Poirier Y. Identification and characterization of the Arabidopsis *PHO1* gene involved in phosphate loading to the xylem[J]. The Plant Cell Online, 2002, 14(4): 889-902

[15] Paszkowski U, Kroken S, Roux C, Briggs S P. Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis[J]. Proceedings of the National Academy of Sciences, 2002, 99(20): 13324-13329

[16] Ming F, Lu Q, Wang W, Zhang S, Guo B, Shen D. Cloning, expression and function of phosphate transporter encoded gene in *Oryza sativa* L.[J]. Science in China Series C: Life Sciences, 2006, 49(5): 409-413

[17] Hu B, Zhu C, Li F, Tang J, Wang Y, Lin A, Liu L, Che R, Chu C. *LEAF TIP NECROSIS1* plays a pivotal role in the regulation of multiple phosphate starvation responses in rice[J]. Plant Physiology, 2011, 156 (3): 1101-1115

[18] Secco D, Baumann A, Poirier Y. Characterization of the Rice *PHO1* Gene Family Reveals a Key Role for *OsPHO1;2* in Phosphate Homeostasis and the Evolution of a Distinct Clade in Dicotyledons[J]. Plant Physiology, 2010, 152(3): 1693-1704

[19] Nagy R, Vasconcelos M J, Zhao S, McElver J, Bruce W, Amrhein N, Raghothama KG, Bucher M. Differential regulation of five Pht1 phosphate transporters from maize (*Zea mays* L.) [J]. Plant Biology, 2006, 8(2): 186-197

[20] Alexandrov N N, Brover V V, Freidin S, Troukhan M E, Tatarinova T V, Zhang H, Swaller T J, Lu Y P, Bouck J, Flavell R B, Feldmann K A. Insights into corn genes derived from large-scale cDNA sequencing [J]. Plant molecular biology, 2009, 69(1): 179-194

[21] Paterson A H, Bowers J E, Bruggmann R, Dubchak I, Grimwood J, Gundlach H, Haberer G, Hellsten U, Mitros T, Poliakov A, Schmutz J, Spannagl M, Tang H, Wang X, Wicker T, Bharti A K, Chapman J, Feltus F A, Gowik U, Grigoriev I V, Lyons E, Maher C A, Martis M, Narechania A, Otillar R P, Penning B W, Salamov A A, Wang Y, Zhang L, Carpita N C, Freeling M, Gingle A R, Hash C T, Keller B, Klein P, Kresovich S, McCann M C, Ming R, Peterson D G, Mehboob-ur-Rahman, Ware D, Westhoff P, Mayer K F, Messing J, Rokhsar D S. The *Sorghum bicolor* genome and the diversification of grasses[J]. Nature, 2009, 457(7229): 551-556

[22] Wang Y, Secco D, Poirier Y. Characterization of the *PHO1* gene family and the responses to phosphate deficiency of Physcomitrella patens[J]. Plant Physiology, 2008, 146(2): 646-656

[23] 高妍, 姜佰文, 刘大森, 王春宏, 张迪, 刘学生. 不同种植年限黑土型蔬菜保护地磷素状况的研究[J]. 核农学报, 2011, 25(1): 121-126

[24] 侯彦楠, 杨俊诚, 姜慧敏, 张建峰, 吴庆钰, 李娟. 低磷胁迫下不同基因型玉米籽粒磷含量及品质性状分析[J]. 核农学报, 2009, 23 (2): 327-333

[25] 张吉海, 高世斌, 杨克诚, 张志明, 林海建, 黄宁, 郑溟, 徐克成, 陈义轩, 潘光堂. 玉米耐低磷种质资源的筛选与

鉴定[J]. 植物遗传资源学报, 2008, 9(3): 335-339
[26] Thompson J D, Gibson T J, Plewniak F, Jeanmougin F, Higgins D G. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools[J]. Nucleic acids research, 1997, 25(24): 4876-4882
[27] Tamura K, Dudley J, Nei M, Kumar S. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0[J]. Molecular biology and evolution, 2007, 24(8): 1596-1599
[28] Schultz J, Milpetz F, Bork P, Ponting C P. SMART, a simple modular architecture research tool: identification of signaling domains[J]. Proceedings of the National Academy of Sciences of the United States of America, 1998, 95(11): 5857-5864
[29] Yu C S, Chen YC, Lu C H, Hwang J K. Prediction of protein subcellular localization[J]. Proteins: Structure, Function, and Bioinformatics, 2006, 64(3): 643-651
[30] Pfaffl M W, Horgan G W, Dempfle L. Relative expression software tool (REST ?瘙 嚏) for group-wise comparison and statistical analysis of relative expression results in real-time PCR[J]. Nucleic acids research, 2002, 30(9): e36
[31] Raghothama K G. Phosphate transport and signaling[J]. Current Opinion in Plant Biology, 2000, 3 (3): 182-187
[32] Rausch C, Zimmermann P, Amrhein N, Bucher M. Expression analysis suggests novel roles for the plastidic phosphate transporter Pht2;1 in auto - and heterotrophic tissues in potato and *Arabidopsis*[J]. The Plant Journal, 2004, 39(1): 13-28
[33] Wohlrab H, Briggs C. Yeast mitochondrial phosphate transport protein expressed in Escherichia coli. Site-directed mutations at threonine-43 and at a similar location in the second tandem repeat (isoleucine-141) [J]. Biochemistry, 1994, 33(32): 9371-9375
[34] Stappen R, Krämer R. Kinetic mechanism of phosphate/phosphate and phosphate/OH- antiports catalyzed by reconstituted phosphate carrier from beef heart mitochondria[J]. The Journal of Biological Chemistry, 1994, 269(15): 11240-11246
[35] Karandashov V, Bucher M. Symbiotic phosphate transport in arbuscular mycorrhizas[J]. Trends in plant science, 2005, 10(1): 22-29
[36] Pinson B, Merle M, Franconi J M, Daignan-Fornier B. Low Affinity Orthophosphate Carriers Regulate *PHO* Gene Expression Independently of Internal Orthophosphate Concentration in *Saccharomyces cerevisiae*[J]. Journal of Biological Chemistry, 2004, 279(34): 35273-35280
[37] Hammond J P, Bennett M J, Bowen H C, Broadley M R, Eastwood D C, May S T, Rahn C, Swarup R, Woolaway K E, White P J. Changes in gene expression in Arabidopsis shoots during phosphate starvation and the potential for developing smart plants[J]. Plant Physiology, 2003, 132(2): 578-596
[38] Ai P, Sun S, Zhao J, Fan X, Xin W, Guo Q, Yu L, Shen Q, Wu P, Miller AJ, Xu G. Two rice phosphate transporters, OsPht1;2 and OsPht1;6, have different functions and kinetic properties in uptake and translocation[J]. The Plant Journal, 2009, 57(5): 798-809

本刊中的类似文章

1．张志勇, 陈梅, 李晚忱, 付凤玲.以玉米幼胚为受体转化海藻糖合成酶基因[J]. 核农学报, 2009,23(5): 743-746
2．周柱华, 徐立华, 王丽丽, 许方佐, 邢燕菊, 张凤云, 邱登林, 阴卫军, 韩金龙, 徐相波, 丁 一.玉米自交系鲁原92的选育及应用[J]. 核农学报, 2009,23(6): 986-989
3．曹墨菊,黄文超,潘光堂,荣廷昭,朱英国.首例航天诱变玉米细胞核雄性不育株与可育株的株高生长分析[J]. 核农学报, 2004,18(04): 261-264
4．王殿轩,李淑荣,温贤芳,原锴.电子束辐照谷物中玉米象不同虫态的生物效应[J]. 核农学报, 2004,18(02): 131-133
5．齐延芳,许方佐,周柱华,邢燕菊,徐立华,邱登林.种植密度对玉米鲁原单22光合作用的影响[J]. 核农学报, 2004,18(01): 14-17
6．左元梅,陈清,张福锁.利用~(14)示踪研究玉米/花生间作玉米根系分泌物对花生铁营养影响的机制[J]. 核农学报, 2004,18(01): 43-46
7．齐延芳,杨景成,周柱华,邢燕菊,徐立华,许方佐,邱登林.玉米自交系及F_2分离群体花药培养中的过氧化物同工酶分析[J]. 核农学报, 2003,17(03): 191-195
8．袁佐清,张怀渝,王化新,李晚忱,陈志渝.不同玉米自交系的抗旱力与超弱发光关系的研究[J]. 核农学报, 2003,17(01): 35-40
9．周柱华,齐延芳,许方佐,邢燕菊,徐立华,邱登林.辐照花粉对玉米F_1M_1结实及后代植株的影响[J]. 核农学报, 2002,16(06): 347-350
10．刘应红, 秦嘉岳, 黄小珍, 胡育峰, 黄玉碧.外源激素和糖类对玉米*zSs*1表达的影响[J]. 核农学报, 2011,25(3): 432-435,505
11．傅俊杰,冯风琴,包志毅,夏晓峰.甜玉米辐照保鲜研究[J]. 核农学报, 2002,16(03): 144-147
12．唐秀芝,张维强,任继明,刘志芳.粮饲兼用玉米中原单32号的育成与推广[J]. 核农学报, 2001,15(06): 360-364
13．潘家荣,巨晓棠,刘学军,张福锁,毛达如.高肥力土壤冬小麦/夏玉米轮作体系中化肥氮去向研究[J]. 核农学报, 2001,15(04): 207-212
14．周柱华,单成钢,朱斗北,许方佐,祝清俊,邢燕菊,齐延芳,徐立华.玉米自交系辐照效应的研究[J]. 核农学报, 2001,15(04): 213-218
15．李社荣,马惠平,谷宏志,朱保葛,刘根齐.返回式卫星搭载后玉米叶绿体色素变化的研究[J]. 核农学报, 2001,15

(02): 75-80