果 树 学 报 2019,36(11):1453-1462
Journal of Fruit Science

DOI:10.13925/j.cnki.gsxb.20190148

苹果6-磷酸葡萄糖酸脱氢酶*Md6PGDH6*基因的功能鉴定

朱小平,孙美红,安建平,路 静,齐晨辉,由春香,郝玉金*

(山东农业大学园艺科学与工程学院•作物生物学国家重点实验室,山东泰安 271018)

摘 要:【目的】探究苹果6-磷酸葡萄糖酸脱氢酶*Md6PGDH6*基因的性质及其生物学功能。**【方法】**利用PCR技术得到MDP0000235230(*Md6PGDH6*)基因的全长,进行相关生物信息学分析,利用qRT-PCR技术研究其时空表达模式,利用农杆菌介导的转化方法获得*Md6PGDH6*转基因'王林'愈伤组织,以及异源表达*Md6PGDH6*基因的拟南芥和烟草。**【结果】**MDP0000235230基因与拟南芥AT4G29120.1(*At6PGDH6*)归为一类,将MDP0000235230命名为*Md6PGDH6*基因。*Md6PGDH6*基因包含一个942 bp开放阅读框。在线网站预测发现,Md6PGDH6蛋白整体表现为亲水性。启动子分析发现,其含有分生组织、激素响应、环境因子和非生物胁迫响应顺式作用元件。*Md6PGDH6*基因在茎、果皮、果肉和种子中表达量较高,在根、叶和花中表达量较低。*Md6PGDH6*基因促进'王林'愈伤组织、拟南芥和烟草的生长。**【结论】***Md6PGDH6*在果肉中表达量较高,*Md6PGDH6*基因有助于'王林'愈伤组织的生长。

关键词:苹果;6-磷酸葡萄糖酸脱氢酶;功能鉴定

中图分类号:S661.1 **文献标志码:**A **文章编号:**1009-9980(2019)11-1453-10

Functional identification of 6-phosphogluconate dehydrogenase *Md6PGDH6* gene in apple

ZHU Xiaoping, SUN Meihong, AN Jianping, LU Jing, QI Chenhui, YOU Chunxiang, HAO Yujin*

(*College of Horticulture Science and Engineering, Shandong Agricultural University/State Key Laboratory of Crop Biology, Tai' an 271018, Shandong, China*)

Abstract:【Objective】The catabolism of saccharides includes glycolysis pathway and pentose phosphate pathway. 6-phosphogluconate dehydrogenase is a key enzyme and rate-limiting enzyme in the pentose phosphate pathway. Previous studies have found that 6-phosphogluconate dehydrogenase could respond to biotic and abiotic stresses, and promoted the growth of straw mushroom (*Volvariella volvacea*) and needle mushroom (*Flammulina velutipes*). At present, there is a little research on 6-phosphogluconate dehydrogenase in woody plants, especially in apple (*Malus domestica* Borkh.). In order to explore the function of 6-phosphogluconate dehydrogenase gene in apple, the 6-phosphogluconate dehydrogenase gene (MDP0000235230) was cloned from 'Gala' apple and was named *Md6PGDH6* by comparing with the phylogenetic tree of 6-phosphogluconate dehydrogenase family in *Arabidopsis thaliana*. The bioinformatics were analyzed and the biological functions were studied by transforming 'Orin' calli, *Arabidopsis thaliana* and tobacco.【Methods】The full length of MDP0000235230 gene was obtained by RT-PCR (reverse transcription-PCR) and PCR. A variety of software and online sites were used to analyze its properties. The physical and chemical properties of Md6PGDH6 protein were analyzed with ExPASyProtParam tool. *Arabidopsis thaliana* 6-phosphogluconate dehydrogenase gene family was obtained with TAIR website. The phylogenetic tree was structured with software MEGA-X. The conservative functional domains, secondary and tertiary structures of the protein, subcellular local-

收稿日期:2019-04-09 接受日期:2019-07-05

基金项目:山东省科技厅科技领军人才工作室(鲁科字[2016]206号);NSFC-山东联合基金(重点项目)(U1706202)

作者简介:朱小平,男,在读硕士研究生,研究方向为果树分子生物学。Tel:18763823701,E-mail:haoyujin@sdau.edu.cn

*通信作者 Author for correspondence. Tel:13953888025,E-mail:haoyujin@sdau.edu.cn

izations and promoter *cis*-elements were analyzed using the NCBI CDD tool, SOPMA and Phyre^2, the WoLF PSORT online website, and PlantCARE online database. The temporal and spatial expression patterns were studied by quantitative real-time PCR (qRT-PCR). Md6PGDH6 protein was obtained by using the prokaryotic expression system of *Escherichia coli*. Pfu DNA polymerase was used to amplify the target gene. Purpose fragment was recycled, and connected to *pEASY*® - Blunt E1. Then, the recombinant was transformed into DH5 competent cells. Positive clones were screened by PCR, and several positive clones were selected and sent for sequencing. A small number of sequenced correct positive clones was dipped and shaken, and extracting plasmids were transformed into *Trans* BL21 competent cells. *Trans* BL21 recombinant cells were cloned and screened using PCR, and then the positive clones were used for *in vitro* protein induction experiments. "A" was added before the forward and reverse primers of the target gene as the forward primer and reverse primer connecting the overexpression vector pCXSN-Myc. High fidelity DNA polymerase HiFi was used to clone target fragments. The product was connected to pCXSN-Myc at 16 ℃ overnight, and then transformed into agrobacterium. 'Orin' calli transgenic with *Md6PGDH6* and *Arabidopsis thaliana* and tobacco with heterogenously expressed *Md6PGDH6* were obtained by agrobacterium-mediated transformation. The phenotypes of the obtained transgenic calli and wild calli were observed by measuring the fresh weight of different calli. A similar experiment was carried out in *Arabidopsis thaliana* and transgenic tobacco to verify the conserved function of the gene in different species. Observations were made by measuring the length of the primary root and the fresh weight of the shoot of *Arabidopsis thaliana*. In addition, the activity of 6-phosphogluconate dehydrogenase in *Arabidopsis thaliana* was also detected to indicate that *Md6PGDH6* indeed promoted the growth of *Arabidopsis thaliana* by increasing the activity of 6-phosphogluconate dehydrogenase in *Arabidopsis thaliana*.【Results】Phylogenetic tree analysis showed that MDP0000235230 gene was classified as At4g29120.1 in *Arabidopsis thaliana*, and MDP0000235230 was named *Md6PGDH6* gene. *Md6PGDH6* gene contained a 942 bp complete open reading frame, encoding 313 amino acids. Protein molecular weight was 37.68 kDa and its theoretical isoelectric point was 8.28. Md6PGDH6 protein had a conserved structural domain of MmsB, belonging to NAD binding 11 superfamily, and its overall performance was hydrophilic. By SOPMA analysis, the secondary structure of Md6PGDH6 protein in apple was predicted to consist of 42.81% α-helix, 32.59% random coil, 16.29% extended strand and 8.31% β-turn. Therefore, α-helix and random coil were the largest structural elements of Md6PGDH6 protein in apple, while the extended strand and β-turn were scattered in the whole polypeptide chain. The Phyre^2 prediction also proved this outcome. TMHMM analysis of the amino acid sequence of Md6PGDH6 in apple showed no transmembrane domain. WoLF PSORT was used to predict the location of the protein in chloroplast. The promoter of *Md6PGDH6* gene was found to contain CAT-box, a *cis*-acting element in meristem. There were also environmental response elements, such as the light response element Box 4, and abiotic stress response elements, such as the low temperature response element LTR and the drought response element MBS. In addition, there were also a variety of regulatory elements related to hormone response, such as ABA response element ABRE, ethylene response element ERE, gibberellin response element P-box and salicylic acid response element TCA-element. The qRT-RCR showed that *Md6PGDH6* gene was expressed in different tissues of apple, with higher expression in stem, peel, pulp and seed and lower expression in root, leaf and flower. Md6PGDH6 protein with correct prokaryotic expression was obtained. *Md6PGDH6* gene promoted the accumulation of biomass in calli of 'Orin'. *Md6PGDH6* gene promoted the primary root length and shoot growth of *Arabidopsis thaliana*. *Md6PGDH6* gene promoted the growth of primary root length in tobacco.【Conclusion】

Md6PGDH6 was highly expressed in stem, peel, pulp and seed. *Md6PGDH6* contributed to the growth of 'Orin' calli, and such a conservative mechanism also existed in *Arabidopsis thaliana* and tobacco.

Key words: Apple; 6-phosphogluconate dehydrogenase; Functional identification

植物在长期进化过程中，形成了一系列糖响应和调节机制。植物体内糖代谢分为合成代谢和分解代谢，分解代谢又可分为糖酵解途径、三羧酸循环途径和磷酸戊糖途径[1]。在正常生长条件下，磷酸戊糖途径所占比例较低，在胁迫条件下，磷酸戊糖途径比例有所增加，甚至在糖酵解途径受到抑制的条件下，磷酸戊糖途径仍能正常进行，由此可见磷酸戊糖途径的重要性。磷酸戊糖途径不仅能为植物提供高还原力物质NADPH，该过程的中间产物还能为核苷酸等重要物质的生物合成提供原料[2]。

6-磷酸葡萄糖酸脱氢酶（6-phosphogluconate dehydrogenase，6PGDH，EC1.1.1.44）是催化磷酸戊糖途径第三步反应的酶。6-磷酸葡萄糖酸脱氢酶催化6-磷酸葡萄糖酸（gluconate-6-phosphate，Glc-6-P），氧化脱酸生成5-磷酸核酮糖（ribulose-5-phosphate，Ru-5-P），以及高还原力物质NADPH。5-磷酸核酮糖不仅是核苷酸合成的原料，而且还是ATP、CoA等物质的组成部分[3]。细胞内存在许多还原性生物合成过程，如长链脂肪酸、类固醇和四氢叶酸的合成等都需要NADPH作为还原剂[3]，NADPH同时还参与无机氮的同化[4]，和莽草酸途径相联系[5-7]，并保持必要的氧化还原电位，以防止氧化应激[8]。在硝酸盐同化过程中，植物组织中磷酸戊糖途径代谢增加，增强对硝酸盐和亚硝酸盐还原的能力[9]。此外，NADPH是降解奇异不饱和脂肪酸所必需的，这主要发生在高等植物过氧化物酶体中[10]。已从大豆[11]、番茄[12]、玉米[13]和甜菜[14]、紫花苜蓿[15]、菠菜[16]、水稻[17]、黄瓜[18]、拟南芥[19]和苹果[20]等植物中克隆或纯化得到6-磷酸葡萄糖酸脱氢酶。

6-磷酸葡萄糖酸脱氢酶是高度保守的酶。6-磷酸葡萄糖酸脱氢酶在植物胞质和叶绿体中的同工酶与其蓝藻同源物的相似性最高，高于来自其他原核生物或非光合真核生物胞质的同源物。这表明植物的6-磷酸葡萄糖酸脱氢酶基因是通过来自蓝藻的叶绿体前体的内共生基因转移而进入细胞核的[16]。有研究表明6-磷酸葡萄糖酸脱氢酶不仅能参与多种非生物胁迫[21]，还能影响植物生长。在玉米中的研究发现，6-磷酸葡萄糖酸脱氢酶等位基因的剂量与它的酶活存在正相关[22]。玉米双突纯合子（6pgd1-null；6pgd2-null）中6-磷酸葡萄糖酸脱氢酶活性较低，在田间条件下足以维持正常生长和繁殖能力，但双突纯合子生长相对缓慢[23]。在研究草菇杂交育种时发现，分离的多数单孢菌株PYd21和PYd15生长缓慢，但这些菌株配对后，得到的异核体菌株H1521却生长旺盛。通过对草菇基因组、转录组和表达谱的分析，发现异核体菌株H1521中6-磷酸葡萄糖酸脱氢酶基因表达量明显高于单孢菌株，这说明6-磷酸葡萄糖酸脱氢酶基因表达量与草菇生长速度呈正相关[24]。在金针菇的研究中也发现了同样的结论[25]。

笔者发现了一个影响苹果生长的6-磷酸葡萄糖酸脱氢酶基因，组成型过表达6-磷酸葡萄糖酸脱氢酶基因加快了苹果'王林'愈伤组织的生长。首次在苹果中发现促进苹果愈伤组织生长的6-磷酸葡萄糖酸脱氢酶基因，对于后续研究磷酸戊糖途径具有一定的参考价值。6-磷酸葡萄糖酸脱氢酶是磷酸戊糖途径的限速酶，6-磷酸葡萄糖酸脱氢酶基因的表达水平变化对于磷酸戊糖途径速率具有重要作用，该研究为进一步展开有关磷酸戊糖途径在苹果中的功能研究奠定一定的基础。

1　材料和方法

1.1　材料

取山东省泰安市山东农业大学试验田的'嘎拉'苹果（*Malus*×*domestica* Borkh.'Gala'）4月中旬的生长根、一年生茎、幼叶和花，以及花后60 d的幼果，利用液氮将材料研磨成粉后，提取总RNA。'王林'（'Orin'）苹果愈伤组织保存在山东农业大学作物生物学国家重点实验室-2层植物生长室。26 ℃暗室培养，继代周期为15 d左右。放置于继代培养基（MS+1.5 mg·L^{-1} 2,4-D+0.5 mg·L^{-1} 6-BA）中。哥伦比亚野生型（Columbia-0 ecotype）拟南芥（*Arabidopsis thaliana*）和本生烟草（*Nicotiana benthamiana*）于26 ℃，光周期为16 h/8 h条件下培养。

1.2　RNA的提取和实时荧光定量PCR（qRT-PCR）

利用试剂盒法提取样品RNA。采用植物总RNA提取试剂盒（RNAplant Plus Reagent，TIANGEN，DP437）提取样品总RNA，使用Takara公司编

号为RR047A的反转录试剂盒[PrimeScript® RT reagent Kit with gDNA Eraser(Perfect Real Time)]中的SYBR® Green分析法去除基因组DNA，按试剂盒附带说明书进行操作获得cDNA，将cDNA稀释后为模板，进行实时荧光定量PCR。采用苹果的18sRNA为内参基因，进行差异表达分析。18sRNA正向引物序列：5'-ACACGGGGAGGTAGTGACAA-3'，反向引物序列：5'-CCTCCAATGGATCCTCGTTA-3'。根据Md6PGDH6基因序列，采用Primer Premier 5.0设计其qRT-PCR引物。目的基因正向引物序列：5'-TGCTTGAAGGAGTGCCAGAAGATG-3'，反向引物序列：5'-CCGCTCCACCGCCACAATAAG-3'。参照Qi等[26]的方法进行qRT-PCR分析。qRT-PCR反应完成后，利用$2^{-\Delta\Delta CT}$方法计算目的基因相对表达水平。

1.3 苹果*Md6PGDH6*基因的克隆、过表达载体的构建及转基因材料的获得

从GDR(https://www.rosaceae.org/)检索苹果6-磷酸葡萄糖酸脱氢酶基因(MDP0000235230)序列，采用Primer Premier 5.0设计引物，扩增目的片段。正向引物序列：5'-ATGGCCACCGTCGCGTCAT-3'，反向引物序列：5'-TTATTTTGCAGGAGCGTATTCAAGTGACAC-3'。pCXSN-Myc正向引物序列：5'-AATGGCCACCGTCGCGTCAT-3'，反向引物序列：5'-ATTATTTTGCAGGAGCGTATTCAAGTGACAC-3'。

使用全式金(TransGen)公司编号为AP131的高保真DNA聚合酶(TransTaq® DNA Polymerase High Fidelity(HiFi))克隆目的基因。反应体系为50 μL，模板DNA 1 μL，正向引物和反向引物各2 μL，10×TransTaq® HiFi Buffer Ⅱ 5 μL，2.5 mmol·L^{-1} dNTPs 4 μL，TransTaq® HiFi DNA Polymerase 0.5 μL，ddH_2O 35.5 μL。PCR反应条件：94 ℃ 5 min，94 ℃ 30 s，56 ℃ 30 s，72 ℃ 30 s，72 ℃ 10 min。反应过程中的变性、退火和延伸过程循环35次。提前配制1.3%琼脂糖DNA凝胶，电泳检测PCR产物。利用TaKaRa公司编号为9762的琼脂糖DNA凝胶回收试剂盒(TaKaRa MiniBEST Agarose Gel DNA Extraction Kit Ver.4.0)回收DNA。

将纯化回收的目的片段，16 ℃过夜连接载体pMD18-T，连接体系：回收产物5 μL，pMD18-T 1 μL，Solution Ⅰ 4 μL。连接产物转化大肠杆菌感受态DH5α(Trans5α Chemically Competent Cell，CD201，TransGen)。氨苄青霉素抗性筛选阳性克隆，送公司测序鉴定。挑选阳性克隆，摇菌，利用天根公司(TianGen)编号为DP103的质粒小提试剂盒(TIANprep Mini Plasmid Kit)提取菌液中的质粒。利用目的基因正向和反向引物前加"A"为连接过表达载体pCXSN-Myc的正向引物和反向引物，利用高保真DNA聚合酶HiFi克隆目的片段。将产物进行16 ℃过夜连接。连接体系：目的片段5 μL，pCXSN-Myc 3 μL，10×T4 Buffer 1 μL，T4 DNA Ligase 1 μL。利用冻融法转化农杆菌感受态细胞LBA4404，再用相应青霉素筛选出阳性克隆。参考Hu等[27]的方法侵染苹果王林愈伤组织及拟南芥，获得转基因愈伤组织和拟南芥。参照Zhao等[28]的方法侵染烟草，获得转基因烟草株系。提取植物RNA，利用qRT-PCR技术检测植物材料目的基因表达量。本实验所采用植物材料为拟南芥和烟草的第三代纯合株系。

1.4 苹果Md6PGDH6蛋白生物信息学分析

利用TAIR(https://www.arabidopsis.org/)获得拟南芥中的6-磷酸葡萄糖酸脱氢酶家族所有的基因，利用MEGA-X软件进行氨基酸序列比对，并构建系统进化树；利用在线工具Protscale(http://web.expasy.org/protscale/)分析蛋白亲/疏水性；利用NCBI的CDD工具(http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi)预测蛋白保守功能域；利用在线数据库PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/)预测启动子顺式作用元件。

1.5 转基因材料的长势观测

取生长状态一致的转基因愈伤组织及野生愈伤组织置于愈伤继代培养基上，观测20 d左右的长势，测量其鲜质量，3个为一组测其鲜样质量，测3组(为3个重复)，计算其平均值；待拟南芥种子在MS培养基上萌发4 d后，挑选生长一致的转基因拟南芥和野生型拟南芥置于含MS培养基的13 cm×13 cm的方形培养皿上，观测生长20 d左右的拟南芥主根长度和地上部鲜质量，转基因拟南芥和野生型拟南芥株系各取20株计算其平均值，并且检测其酶活。利用转基因烟草和野生型烟草进行相同的实验。

1.6 6PGDH酶活测定

利用6PGDH催化6-磷酸葡萄糖酸和$NADP^+$生成NADPH，NADPH在340 nm处有特征吸收峰，而$NADP^+$没有的原理测定6PGDH酶活。通过测定

340 nm 处吸光度增加速率，计算 6PGDH 活性。利用苏州科铭生物技术有限公司（comin）编号为 6PGDH-2-W 的试剂盒进行测量。

1.7　统计学分析

使用 IBM SPSS Statistics 20 软件单因素 ANOVA 进行统计学分析。* $p < 0.05$ 表示差异显著；** $p < 0.01$ 表示差异极显著。

2　结果与分析

2.1　*Md6PGDH6* 的分离及表达载体的构建

在苏玲等[20]研究 6-磷酸葡萄糖酸脱氢酶（MDP0000279299）的基础上，我们找到了一个 6-磷酸葡萄糖酸脱氢酶基因（MDP0000235230），该基因比已报道的基因少 105 bp，含有 942 bp 的完整开放阅读框，编码 313 个氨基酸。利用网站 ExPASy（http://web.expasy.org/protparam/）预测该蛋白理论等电点 pI 为 8.28，表明该基因编码的蛋白为不稳定蛋白。将其蛋白序列与拟南芥中 6-磷酸葡萄糖酸脱氢酶（6PGDH）家族成员的蛋白序列进行进化树分析，发现该蛋白与 At4G29120.1（*At6PGDH6*）亲缘关系最近，因此命名为 *Md6PGDH6*（图 1）。

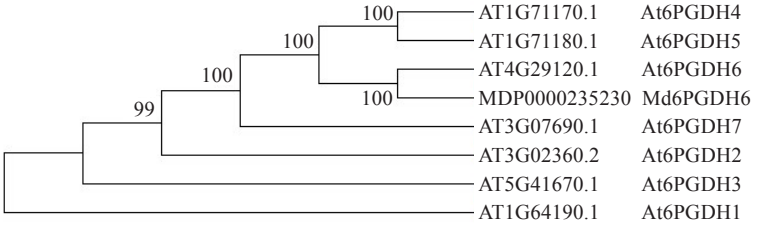

图 1　Md6PGDH6 的进化树分析

Fig. 1　Phylogenetic analysis of Md6PGDH6

将克隆得到的片段连接到 pMD18-T 载体上。利用目的基因正向和反向引物前加“A”为引物，扩增 *Md6PGDH6* 基因的片段，将该片段连接到过表达载体 pCXSN-Myc 上，连接产物转化大肠杆菌感受态 DH5α，挑取阳性克隆进行 PCR 检测，结果表明 *Md6PGDH6* 片段成功连接于过表达载体 pCXSN-Myc 上。

2.2　*Md6PGDH6* 的生物信息学分析

使用 NCBI 的 CDD 工具预测苹果 Md6PGDH6 蛋白的功能域和它的功能，结果表明苹果 Md6PGDH6 蛋白整体具有 MmsB 结构域（图 2）。

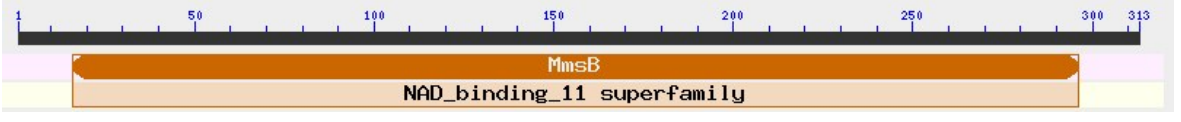

图 2　Md6PGDH6 蛋白的功能域分析

Fig. 2　Functional domain analysis of Md6PGDH6

利用 Protscale 在线工具分析苹果 Md6PGDH6 蛋白亲/疏水性，结果显示，多肽链第 157 位的天冬酰胺分值最低，为−1.800，该位点亲水性最强；第 195 位的赖氨酸分值最高，为 2.089，该位点疏水性最强。多肽链整体表现为亲水性（图 3）。

2.3　*Md6PGDH6* 启动子顺式作用元件分析

利用网站 PlantCARE 对苹果 *Md6PGDH6* 启动子上的顺式作用元件进行预测，发现该启动子具有多种激素响应相关的调控元件，如 ABA 响应元件 ABRE、乙烯响应元件 ERE、赤霉素响应元件 P-box 和水杨酸响应元件 TCA-element；环境因子响应元件，如光响应元件 Box 4；非生物胁迫响应元件，如

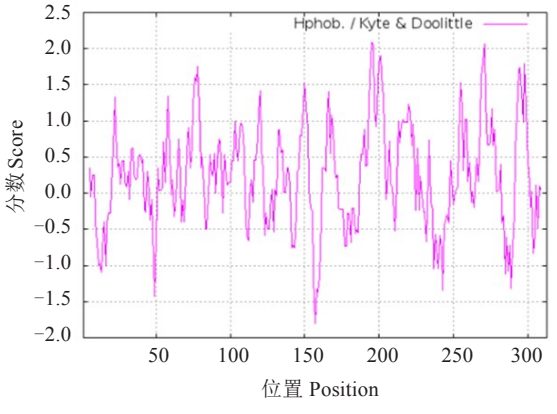

图 3　Md6PGDH6 蛋白的亲疏水性分析

Fig. 3　Hydrophilic and hydrophobic analysis of Md6PGDH6 protein

低温响应元件LTR和干旱响应元件MBS(表1);分生组织表达顺式作用元件CAT-box。根据以上结果,推测*Md6PGDH6*可能对光、低温、干旱和植物激素等多种外界条件作出响应。

表1 *Md6PGDH6*上游调控序列重要顺式作用元件

Table 1 Some important cis-acting regulatory elements in the upstream regulatory sequences of *Md6PGDH6*

顺式作用元件 *Cis*-acting regulatory elements	序列 Sequence	位点功能 Function of site	位置 Location
ABRE	ACGTGGC	脱落酸响应元件 *Cis*-acting element involved in the abscisic acid responsiveness	−1 807
Box 4	ATTAA	光响应元件 Part of a conserved DNA module involved in light responsiveness	+817
CAT-box	GCCACT	分生组织表达元件 *Cis*-acting regulatory element related to meristem expression	−777
CCAAT-box	CAACGG	MYBHv1绑定位点 MYBHv1 binding site	−1 833
ERE	ATTTCAAA	乙烯响应元件 Ethylene-responsive element	+1 840
LTR	CCGAAA	低温响应元件 *Cis*-acting element involved in low-temperature responsiveness	−1 060
MBS	CAACTG	干旱诱导MYB绑定位点 MYB binding site involved in drought-inducibility	−452
P-box	CCTTTTG	赤霉素响应元件 Gibberellin-responsive element	+1 638
TCA-element	CCATCTTTTT	水杨酸响应元件 *Cis*-acting element involved in salicylic acid responsiveness	−953

2.4 *Md6PGDH6*组织表达分析

为研究*Md6PGDH6*基因的功能,对*Md6PGDH6*基因进行组织表达分析。实时荧光定量PCR(qRT-PCR)分析表明,Md6PGDH6基因在茎、果皮、果肉和种子中表达量较高,尤其在果肉表达非常高,分别是根(对照组)中相对表达量的4,5,69,8倍;在根、叶和花中表达量较低,叶和花中的表达量分别是根中的1和2倍(图4)。

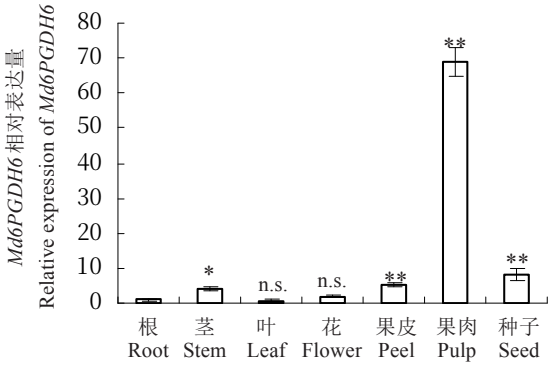

图4 *Md6PGDH6*的组织表达分析

Fig. 4 Tissue expression analysis of *Md6PGDH6*

2.5 *Md6PGDH6*在苹果愈伤组织过量表达提高愈伤组织生物量

将*Md6PGDH6*基因连接到过表达载体pCXSN-Myc上,转化LBA4404农杆菌,侵染'王林'愈伤组织,获得*Md6PGDH6*转基因苹果愈伤组织。选取生长时间(约15 d)、生长状态一致的王林愈伤组织和*Md6PGDH6*转基因苹果愈伤组织,在'王林'培养基上培养3~4周,观察比较愈伤组织的生长状况。在'王林'培养基上,观察到*Md6PGDH6*转基因苹果愈伤组织比野生型愈伤组织生长速度快,而且差异显著。在继代培养基上生长24 d后,通过测定不同愈伤组织的鲜质量,发现转基因苹果愈伤组织鲜质量为野生型愈伤组织的1.7倍(图5)。以上结果表明,在苹果愈伤组织中过量表达*Md6PGDH6*基因能够提高苹果愈伤组织的生物量。

2.6 拟南芥和烟草中异位表达*Md6PGDH6*提高其生物量

为进一步研究*Md6PGDH6*基因对植物的影响,实验分别获得*Md6PGDH6*拟南芥和烟草的转基因株系进行研究。待拟南芥种子在圆形培养皿上生长4 d时,挑选生长一致的拟南芥幼苗,转移到含MS培养基的方形培养皿中竖直培养,以观察较大拟南芥幼苗的生长情况。21 d后拍照,取样。从主根长度和地上部鲜重的测量数据看出转基因拟南芥长势优于野生型(图6-A)。拟南芥主根长度(图6-C)和地上部鲜质量(图6-D)与观察结果一致。野生型拟南芥主根长度平均值为3.4 cm,鲜质量平均值为0.004 g;从左

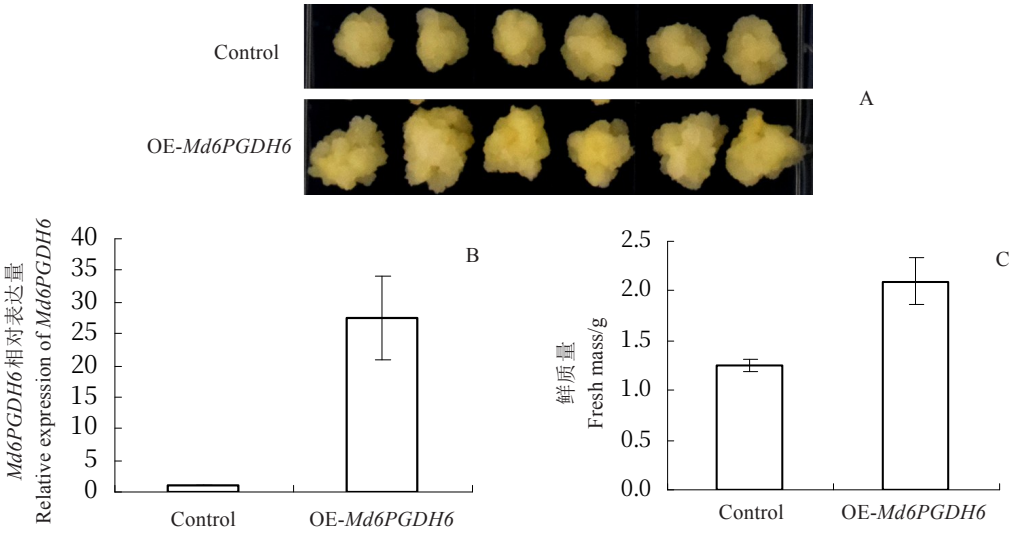

图 5　*Md6PGDH6* 促进愈伤组织生长

Fig. 5　*Md6PGDH6* promotes growth of calli

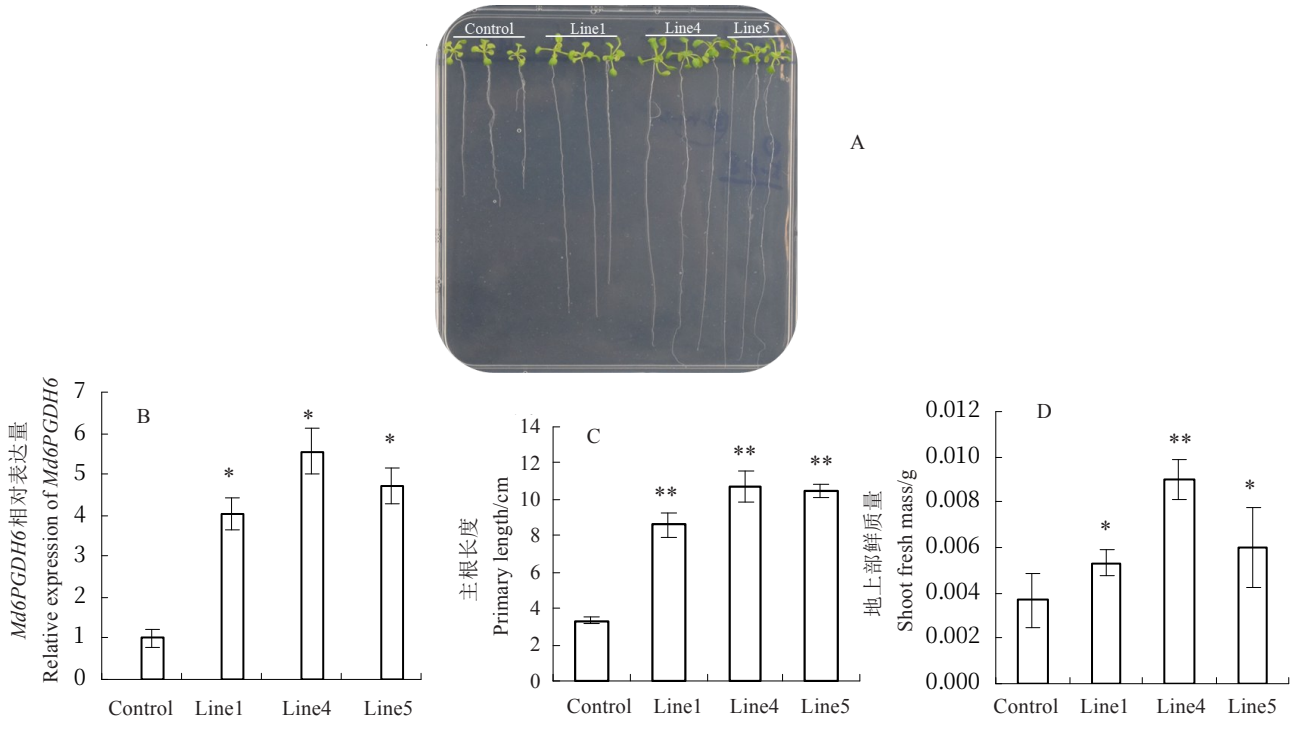

图 6　*Md6PGDH6* 促进拟南芥生长

Fig. 6　*Md6PGDH6* promotes growth of *Arabidopsis thaliana*

到右，3 个转基因株系主根长度平均值为 8.6，10.7，10.4 cm，鲜质量平均值为 0.005，0.009，0.006 g。

将生长 4 d 且生长一致的野生型和 *Md6PGDH6* 转基因烟草置于 13 cm×13 cm 方形培养皿上竖直培养，22 d 后观察表型。发现转基因烟草主根长度比野生型长（图 7），野生型烟草主根长度平均值为 2.5 cm，从左到右，转基因烟草主根长度平均值为 4.5，3.6 cm，进一步说明 *Md6PGDH6* 基因能促进植物生长。

2.7　*Md6PGDH6* 通过提高拟南芥酶活促进其生长

为进一步探究 *Md6PGDH6* 基因促进植物生长的原因，实验检测野生型和转基因拟南芥中 6-磷酸葡萄糖酸脱氢酶的活性。结果发现转基因拟南芥中 6-磷酸葡萄糖酸脱氢酶的活性高于野生型，从左到右，3 个转基因株系的酶活分别为野生型酶活的 2，3，3 倍（图 8）。猜测 *Md6PGDH6* 基因可能通过提高拟南芥磷酸戊糖途径中的 6-磷酸葡萄糖酸脱氢酶的

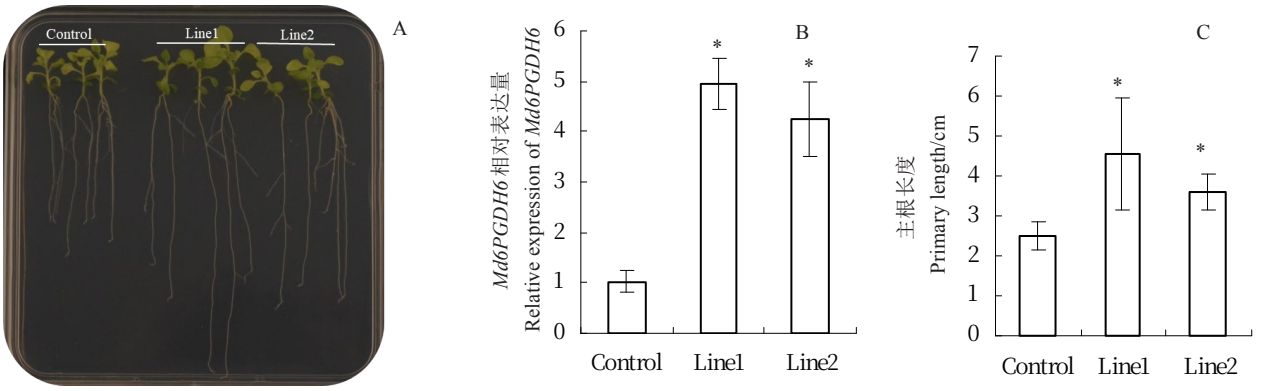

图 7 *Md6PGDH6* 促进烟草生长

Fig. 7 *Md6PGDH6* promotes growth of tobacco

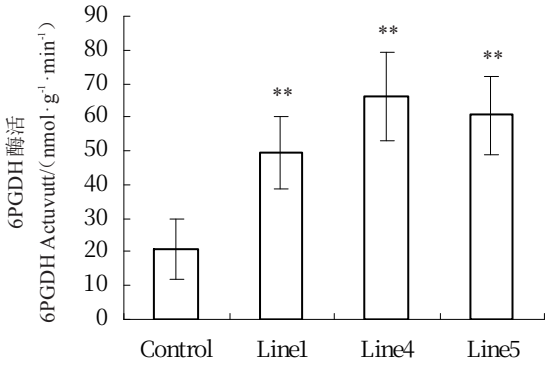

图 8 野生型及转基因拟南芥中 6-磷酸葡萄糖酸脱氢酶活性

Fig. 8 6PGDH activity in wild type and transgenic Arabidopsis thaliana

活性，加快6-磷酸葡萄糖酸生成5-磷酸核酮糖和NADPH的过程，从而加快磷酸戊糖途径的速度，促进植物的物质代谢和能量代谢，表现为植物生物量的积累。

3 讨 论

对*Md6PGDH6*的启动子分析发现，该基因响应多种植物激素，如ABA、茉莉酸甲酯、乙烯、赤霉素和水杨酸等等，激素可以调控植物的生长以及植物对多种非生物和生物胁迫的响应，推测*Md6PGDH6*可能参与多种激素对植物生长的调控过程；该基因还响应光等环境调节因子，以及分生组织的表达，猜测*Md6PGDH6*可能参与光对苹果生长的调控。保守结构域分析发现，该基因具有MmsB功能域，属于NAD结构域，属于6-磷酸葡萄糖酸脱氢酶典型结构域。苹果*Md6PGDH6*可能具有多种重要功能，对其进行充分而广泛的研究也将具有重要意义。

目前对于磷酸戊糖途径功能的研究主要是围绕葡萄糖-6-磷酸脱氢酶、6-磷酸葡萄糖酸内酯酶和6-磷酸葡萄糖酸脱氢酶等有关酶展开。6-磷酸葡萄糖酸内酯酶是催化磷酸戊糖途径第二步反应的酶，催化6-磷酸葡糖酸内酯的水解，后者是葡萄糖-6-磷酸脱氢酶氧化6-磷酸葡萄糖的产物。在拟南芥6-磷酸葡萄糖酸内酯酶突变体（pgl3）中，6-磷酸葡萄糖酸内酯酶基因表达水平和蛋白活性显著低于野生型拟南芥，表明PGL3是6-磷酸葡萄糖酸内酯酶基因的主效基因，并且PGL3插入突变敲除了大部分6-磷酸葡萄糖酸内酯酶活性。拟南芥6-磷酸葡萄糖酸内酯酶突变体株系的大小约为野生型拟南芥大小的一半[29]。过表达*Md6PGDH6*促进愈伤组织的生长，与拟南芥6-磷酸葡萄糖酸内酯酶突变体（pgl3）中的结果具有相似性。然而，在酵母细胞中，6-磷酸葡萄糖酸内酯酶的活性对于酵母细胞的正常生长和繁殖不是必需的[30]。

在草菇中发现，6-磷酸葡萄糖酸脱氢酶表达量高的菌株生长较快[24]。在金针菇的研究中也有类似的结论[25]，这与本研究结果一致。本研究通过转化愈伤组织、拟南芥和烟草三种材料，证明了*Md6PGDH6*促进生长的功能在不同物种中是保守存在的，在不同物种中可能存在一个或者多个6-磷酸葡萄糖酸脱氢酶促进植物生长。在早期有关6-磷酸葡萄糖酸脱氢酶的研究中人们发现，从植物中纯化的6-磷酸葡萄糖酸脱氢酶蛋白常常以二聚体的形式存在，6-磷酸葡萄糖酸脱氢酶蛋白的二聚体形式可能改变原有单体的定位和功能，例如，菠菜[16]，拟南芥[31]。Md6PGDH6蛋白也有可能通过形成同源和异源二聚体发挥作用。

目前对于苹果分子生物学的研究主要围绕抗逆

性、果实品质和遗传改良等方面展开，而有关影响苹果生长的分子机制研究较少。本研究发现了一个促进苹果愈伤组织生长的6-磷酸葡萄糖酸脱氢酶。lncRNA和mRNA的表达谱研究发现6-磷酸葡萄糖酸脱氢酶（MDP0000279299）在果实组织中高度表达[32]，组织表达分析结果显示，*Md6PGDH6*在果肉组织中表达较高，而*Md6PGDH6*能促进苹果生长，*Md6PGDH6*可能与果实生长发育有关。

6-磷酸葡萄糖酸脱氢酶是磷酸戊糖途径的限速酶，6-磷酸葡萄糖酸脱氢酶基因的表达水平变化对于磷酸戊糖途径速率具有重要作用，有必要对其功能进行深入研究。

4　结　论

*Md6PGDH6*在茎、果皮、果肉和种子中的表达明显高于在根、叶和花中的表达。*Md6PGDH6*能促进‘王林’愈伤组织、拟南芥和烟草的生长，表明磷酸戊糖途径的氧化部分是植物生长所必需的，在苹果、拟南芥和烟草中存在6-磷酸葡萄糖酸脱氢酶促进生长的保守机制。

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