遗传学报 2006;33(5):441-448 ISSN:0379-4172 CN:11-5458/R

植物遗传学

水稻质体葡萄糖-6-磷酸脱氢酶基因的克隆与表达研究

侯天云1，黄耀1，杨海飞2，王雨飞1，张红生1
1. 南京农业大学作物遗传与种质创新国家重点实验室,南京 210095;
2. 扬州大学农学院，扬州225009

收稿日期 2005-4-23 修回日期 2005-9-13 网络版发布日期 2006-5-15 接受日期

摘 要

葡萄糖磷酸脱氢酶是高等植物中重要的代谢途径，主要生理功能是产生NADPH以及质体糖代谢的糖酸受酶，葡萄糖-6-磷酸脱氢酶（G6PDH）是这酶糖磷酸脱的关键酶。广泛存在于高等植物糖酸酶糖酸质和质体中。本研究百克从水稻（Oryza sativa L.）幼苗中分离了胞编码的质体G6PDH基因为OsG6PDH2。序列分析表明OsG6PDH2编码一个具有500个氨基酸残基的多肽，等电点为6.5，分子量66 kDa。OsG6PDH2的N端有1个70个氨基酸的信号肽，推测的裂解位点为Cys-55和Ala-56，表明OsG6PDH2编码产物可能定位于质体。多序列比较的结果表明OsG6PDH2与拟南芥、微管、马铃薯质体G6PDH的一致性分别达81%、87%、83%，进化关系证明水稻OsG6PDH2与拟南芥（AtG6PD3）、马铃薯（StG6PD1）处于高等植物同型质体G6PD分支上。给出了OsG6PDH2可能是一个Z型的质体蛋白。用Inspector程序分析表明，OsG6PDH2在起始密码子上游含有一个KZF序列和四个上游引物位点，一个AbaRI位点，一个CRT/DRE元件和1个W-box元件，予定量RT-PCR分析表明，OsG6PDH2在水稻根、茎、叶和幼穗组织中都呈低 平度组成型表达，在根部表达较高。在水稻幼苗中的表达显著受暗处理的诱导，将OsG6PDH2的完整开放阅读框和建到大肠杆菌表达载体pET30a(+)中，pET30a(+)-OsG6PDH2在大肠杆菌中得到了有效表达，酶活性测定证明，OsG6PDH2的编码产物具有葡萄糖-6-磷酸脱氢酶的功能。

关键词

水稻; 葡萄糖-6-磷酸脱氢酶; 基因克隆; 表达分析

分类号

Isolation and Expression Analysis of Plastidic Glucose-6- phosphate Dehydrogenase Gene from Rice (Oryza sativa L.)

HOU ,Fu-Yun1, ,HUANG ,Yi1, ,LU ,Ju-Fei2, ,WANG ,Zhou-Fei1, ,ZHANG ,Hong-Sheng1

1. State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210095, China;
2. College of Agriculture, Yangzhou University, Yangzhou 225009, China

Abstract

<P>Glucose-6-phosphate dehydrogenase is a rate-limiting enzyme of pentose phosphate pathway, existing in cytosolic and plastidic compartments of higher plants. A novel gene encoding plastidic glucose-6-phosphate dehydrogenase was isolated from rice (Oryza sativa L.) and designated OsG6PDH2 in this article. Through semiquantitative RT-PCR approach it was found that OsG6PDH2 mRNA was weakly expressed in rice leaves, stems, immature spikes or flowered spikes, and a little higher in roots. However, the expression of OsG6PDH2 in rice seedlings was significantly induced by dark treatment. The complete opening reading frame (ORF) of OsG6PDH2 was inserted into pET30a (+), and expressed in Escherichia coli strain BL21 (DE3). The enzyme activity assay of transformed bacterial cells indicated that OsG6PDH2 encoding product had a typical function of glucose-6-phosphate dehydrogenase.</P>

Key words

oryz; glucose-6-phosphate; dehydrogenase; gene; cloning; expression; analysis

DOI:

通讯作者 张红生 hsshang@njau.edu.cn

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侯天云
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王雨飞
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