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研究论文

水稻谷蛋白的一个新基因克隆及表达分析

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摘要
与其他禾本科作物以醇溶蛋白为主不同，水稻种子含有醇溶蛋白和谷蛋白两种主要蛋白质贮藏形式。其中，谷蛋白约占胚乳蛋白总量的70%~80%。水稻谷蛋白是由多基因家族编码合成的，到目前为止至少已克隆获得了9个全长cDNA，根据这些cDNA编码的氨基酸序列同源性可将谷蛋白分为A、B两个亚家族。B亚族谷蛋白成员富含赖氨酸等人体必需氨基酸，与稻米的营养品质直接相关，因此挖掘、利用B亚族基因成员对于改良稻米蛋白品质性状至关重要。本文报道了以^{32}P标记的谷蛋白基因*GluB-2* cDNA片段为探针筛选水稻胚乳cDNA文库获得1个新的水稻谷蛋白基因全长cDNA。序列分析显示该基因核苷酸序列共1588 bp，含有1个由495个氨基酸残基组成的开放阅读框，编码蛋白分子量约为56 kD。推导的氨基酸序列与其他已知谷蛋白基因家族成员间序列相似性介于57.8%~97.8%之间，并与B亚族谷蛋白基因的同源性更高，因此命名为*GluB-7*（GenBank注册号AY987390）。Northern杂交显示，*GluB-7*具有高度的胚乳表达特性。

关键词 水稻 谷蛋白 基因克隆
分类号

Cloning and Expression Analysis of a New Glutelin Gene cDNA in Rice

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Abstract Rice seeds with rich reserve of starch and protein are a major food source in many countries. Unlike the seeds of other plants, which typically accumulate one major type of storage protein, rice seeds contain two major proteins, prolamines and globulin-like glutelins. Glutelin, which accounts for 70% – 80% of the total proteins of rice seeds, is coded by a multi-gene family, and to date at least nine different cDNAs have been isolated. After transcription in the cell nucleus, rice glutelin mRNA is targeted to a specific subdomain of the cortical endoplasmic reticulum (ER) where it is translated to synthesize a larger precursor with a signal sequence which is cotranslationally processed during translocation to the ER lumen whereupon correct folding and disulfide bond formation occur. The glutelin precursor is then transported to vacuolar protein bodies (PB-Ⅱ) presumably by way of the Golgi complex. At PB-Ⅱ, glutelin is proteolytically processed into acidic (a) and basic (h) polypeptides. Accoding to the primary sequence comparisons, glutelin can be classified into A and B types. Rice seed proteins are deficient in the essential amino acid, lysine. Therefore, nutritional improvement in the amino acid composition of rice proteins is needed. B type glutelin is superior to A type in terms of nutritional value since B type has more of the first limiting amino acid, lysine. For this reason, B type glutelin should be a noteworthy genetic resource to improve rice protein quality.

Here we reported the cloning and characterization of cDNA for a new rice glutelin gene from a local cultivar (Oryza sativa L). After screening the rice endosperm cDNA library by 32P-labeled GluB-2 cDNA probes (1 389 bp), we cloned a new glutelin gene cDNA, named GluB-7 (GenBank accession number AY987390). DNA sequence analysis showed that the size of the cloned cDNA was 1 588 bp, and carried entire coding sequences, which encode a 495 amino acid protein, corresponding to the size of the glutelin protein family. Signal peptide prediction with software found that GluB-7 included a 24-residue signal peptide with the cleavage site between arginine and glutamine and a 471-residue mature protein. Homology analysis showed that the deduced amino acid sequence of GluB-7 shared 57.8% – 97.8% identity with others of rice glutelin gene family. Southern blot analysis of the genomic DNA showed the presence of multiple copies in the rice genome. Northern blot analysis using GluB-7 cDNA partial sequence as a probe showed that the GluB-7 was expressed specifically in rice endosperm, and the largest accumulation of mRNA occurred in 12 DAA, while no corresponding band was found in roots, stems, and leaves. The cloning of GluB-7 cDNA provides the basis for future studies on glutelin gene expression, the identification of the molecular mechanism of rice seed storatge protein biosynthesis, and especially the improvement of protein quality in ric

e breeding.

Key words Rice Glutelin Gene cloning

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