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研究论文

小麦小G蛋白Tarab5B基因的全长cDNA克隆及表达特性的初步分析

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摘要 通过对白粉病菌诱导的小麦叶片cDNA文库的测序，获得一个与水稻Rab5B基因一致性达89%的序列。以该序列为信息探针，筛选小麦EST数据库并进行电子拼接。根据拼接结果设计引物，利用RT-PCR方法获得了一个小麦中尚未鉴定的全长cDNA克隆Tarab5B。Tarab5B基因编码的蛋白具有结合GTP/GDP的4个保守结构域以及Rab家族成员所特有的结构域。同源分析表明，该基因属于Rab5B亚族。麦类Rab5B蛋白的GDSGVGKS、DTAGQE、NKAD、ETSA和MGCSSS 5个结构域在进化上非常保守，而YYRGA结构域及其邻近的C端6个氨基酸残基在小麦材料间同源性很低。RT-PCR检测显示抗感两个材料Tarab5B基因在接种后24 h和未接种24 h的表达水平基本相同；在接种后1 h、4 h、7 h、12 h，抗感两个材料间Tarab5B基因的表达水平有一定的差异。

关键词 小麦 小G蛋白 基因克隆 结构域 白粉病

分类号 S512

Isolation，Primary Expression Analysis of a Full-length cDNA Clone Encoding Small GTP-binding Protein Gene Tarab5B in Wheat

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Abstract Rab proteins are monomeric small GTP-binding proteins. They exist in all eukaryotic cells and play an important role in vesicular transport. Vesicular transport is required for specialized phenomena as well as common house-keeping function in higher plants, for example, some Rab proteins are involved in development, morphosis and adversity resistance. To date, four Rab5B genes have been isolated respectively from Lotus japonicus, Mesembryanthemum crystallinum, Oryza sativa and Arabidopsis. Compared with yeast and mammalian, plant Rab5B proteins contain plant-unique domain in addition to possessing several conserved domains common to small GTP-binding protein suggesting that plants may well have developed plant-specific mechanisms of vesicular traffic. Rab5B protein functions have been studied extensively in yeast and mammalian systems. However, little information is available about the function of plant Rab5B proteins. In our study, a cDNA library was constructed using mRNA from leaves of Pm16 near isogenic line inoculation with powdery mildew. A partial sequence encoding Rab5B protein has been isolated by sequencing the cDNA library. In order to obtain the full-length cDNA sequence of Rab5B gene in wheat, silicon cloning was performed against wheat EST database based on this sequence. A contig was obtained which shared high similarity to Rab5B gene. According to the contig, a pair of primers was designed and a predicted fragment with 722 bp length was obtained via RT-PCR. PCR product was purified and ligated into vector. A positive cDNA clone was obtained, named Tarab5B, and sequenced. Judged by the characters of its nucleotide sequence, Tarab5B clone contains the full-length cDNA sequence of Rab5B gene. This protein encoded by this cDNA clone included four conserved domains for guanine nucleotide binding and GTPase activities and a domain specific to Rab family. Homologue analysis indicated that amino acid sequence deduced by Tarab5B clone showed high similarity to those of Rab5B genes from Oryza sativa, Arabidopsis thaliana, Mesembryanthemum crystallinum and Lotus japonicus. Domain comparison showed that GDSGVGKS, DTAGQE, NKAD, ETSA and MGCSSS were very conserved among six different materials. However, YYRGA and neighbouring six amino acid residues of its C ends showed significant differences. Expression profile analysis indicated that the expression level of the Tarab5B gene between resistant and susceptible materials at 24 hour after inoculation with powdery mildew and 24 hour control(no inoculating with powdery mildew). At 1 hour, 4 hour, 7 hour and 12 hour after inoculation with powdery mildew, expression level of the Tarab5B gene showed some difference between resistant and susceptible materials. Here it is the first time to report the full-length cDNA of Tarab5B gene in wheat. It includes complete coding sequence of Rab5B gene. The predicted protein belongs to Rab5B subfamily.

Key words Wheat Small G protein Gene cloning Domain Powdery mildew

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