

论文  
甘蓝型油菜Cu/ZnSOD和FeSOD基因的克隆及菌核病菌诱导表达

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摘要:

依据拟南芥、芥菜型油菜和白菜已知超氧化物歧化酶(SOD)保守序列设计引物,用同源序列法和RT-RACE技术克隆甘蓝型油菜Cu/ZnSOD和FeSOD基因,经序列分析和基因片段拼接,得到Cu/ZnSOD和FeSOD基因的全长cDNA,分别为756 bp (GenBank登录号AY970822)和1 037 bp (GenBank登录号EF634058)。以cDNA序列设计引物,获得1 322 bp的Cu/ZnSOD基因组DNA (GenBank登录号DQ431853)和1 659 bp的FeSOD基因组DNA (GenBank登录号EF634057)。生物信息学分析表明,Cu/ZnSOD基因ORF框长459 bp,编码152个氨基酸残基的蛋白质,在基因组序列结构上具有7个外显子和6个内含子。而FeSOD基因ORF框长792 bp,编码263个氨基酸残基的蛋白质,在基因组序列结构上具有8个外显子和7个内含子。二者外显子和内含子交接处完全符合GT/AG规则。利用获得的Cu/ZnSOD的cDNA片段作探针,对菌核病菌诱导甘蓝型油菜叶片的mRNA进行Northern blotting分析,结果显示在同一品种(系)中菌核病菌诱导后Cu/ZnSOD mRNA表达量比诱导前升高,抗(耐)型油菜Cu/ZnSOD mRNA表达量明显高于感病型。油菜叶片SOD酶活性分析结果也获得了完全一致的结果。以上结果表明,甘蓝型油菜SOD基因与菌核病抗性相关。

关键词: Cu/Zn-超氧化物歧化酶 Fe-超氧化物歧化酶 甘蓝型油菜 菌核病菌 基因表达分析

Cloning of Cu/Zn-Superoxide Dismutase of *Brassica napus* and Its Induced Expression by *Sclerotinia sclerotiorum*

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Abstract:

By the primers designed from conserved homologous sequences of the genes for superoxide dismutase (SOD) among *Arabidopsis thaliana*, *Brassica juncea* and *B. rapa ssp. pekinensis*, Cu/ZnSOD and FeSOD genes in *B. napus* were cloned using the methods of homology-based candidate gene and RACE-PCR. After cloning and sequencing, the full lengths of cDNA of Cu/ZnSOD and FeSOD genes (accession no. AY970822 and EF634058 in GenBank) were 756 and 1 037 bp, respectively. Their corresponding genomic sequences were 1 322 bp for Cu/ZnSOD gene and 1 659 bp for FeSOD gene with accession nos. DQ431853 and EF634057 in GenBank, respectively. Bioinformatic analyses showed that the ORF of Cu/ZnSOD gene with 459 bp contained six introns with seven exons and a polyA tail and could express a protein of 152 amino acids; and the one of FeSOD gene with 792 bp contained seven introns with eight exons and could determine a protein of 263 amino acids. With cDNA of Cu/ZnSOD gene as probe, Northern blotting analysis showed that its mRNA expression in *B. napus* cultivars increased after the infection by *Sclerotinia sclerotiorum*, the expression amount in resistant or tolerant cultivars was higher than that in sensitive ones. The same trend was observed for the SOD activity in leaves. These results suggested that SOD genes were related with the resistance to the disease.

Keywords: Cu/ZnSOD FeSOD *Brassica napus* *Sclerotinia sclerotiorum* Gene expression analysis

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