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甘蓝型油菜烯脂酰-CoA还原酶基因*BnECR*的克隆及功能分析

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Cloning and Functional Analysis of Enoyl-CoA Reductase Gene *BnECR* from Oilseed Rape (*Brassica napus* L.)

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摘要 反式烯脂酰-CoA还原酶(trans-2, 3-enoyl-CoA reductase, ECR)是催化超长链脂肪酸(VLCFAs)合成的脂肪酰-CoA延长酶之一。根据已报道拟南芥等的ECR基因设计引物，采用RACE (rapid amplification of cDNA ends)方法从甘蓝型油菜中克隆ECR的全长cDNA序列和对应的基因组序列，命名为*BnECR* (GenBank登录号分别为FJ899705和FJ899706)。序列分析结果显示，*BnECR*的全长cDNA序列为1 328 bp，对应的基因组序列为2 093 bp，由4个外显子组成，在ORF的上、下游分别有一个163 bp的5′ UTR和一个232 bp的3′ UTR。根据编码区预测BnECR前体蛋白为一个310个氨基酸残基的多肽链，包含ECR蛋白的重要功能位点K_{144}、R_{145}及一个NAD(P)H结合基序G_{225}SGGYQIPR/HG_{234}。NCBI Blastn、氨基酸序列多重比对及保守域分析表明，该基因与拟南芥*AtECR*基因的同源性最高，是对应拟南芥*AtECR*的垂直同源基因。RT-PCR分析表明，*BnECR*基因在甘蓝型油菜根、茎、叶、花及角果中均有表达，其中在茎中的表达量最高。*BnECR*在高芥酸材料种子发育中后期的表达量显著高于低芥酸种子，表明*BnECR*可能参与甘蓝型油菜芥酸的合成。将*BnECR*克隆到酿酒酵母的穿梭表达载体中，分别转化野生型酵母By4743和突变体菌株YDL015c，添加半乳糖诱导表达。气相色谱分析表明，*BnECR*在酿酒酵母中有效表达，转化菌株中的芥酸(C22:1)占总脂肪酸含量的1.34%，比对照增加了52%；对突变体的转化结果表明芥酸含量恢复到野生型水平。

关键词： 甘蓝型油菜 烯脂酰CoA还原酶 克隆 芥酸 酿酒酵母

Abstract: Very-long-chain fatty acids (VLCFAs) are critical components found in cuticular waxes, sphingolipids and triacylglycerols in higher plants. Biosynthesis of VLCFAs is catalyzed by the fatty acyl-CoA elongase, a membrane-bound enzymatic complex containing 3-ketoacyl-CoA synthase (KCS), 3-ketoacyl-CoA reductase (KCR), 3-hydroxacyl-CoA dehydratase (HCD), and trans-2, 3-enoyl-CoA reductase (ECR). In this research, primers were designed based on multiple alignments of trans-2,3-enoyl-CoA reductase (ECR) gene sequences from *Arabidopsis thaliana* and other plants, and the full- length cDNA, here designated *BnECR,* and the corresponding genomic sequenceswere isolated from *Brassica napus* by using rapid amplification of cDNA ends (RACE) method (GenBank Accession No. FJ899705 and FJ899705). The sequence of *BnECR* cDNA was 1 328 bp (excluding the poly dA tail), and the corresponding genomic sequence was 2093 bp. *BnECR* was composed of four exons and contained a 163 bp 5' untranslated region (5' UTR) and a 233 bp 3' UTR. The deduced BnECR protein was 310 amino acid in length, with a MW of 735.78 kD and a pI of 9.52. The critical functional sites K_{144}, R_{145} in AtECR were unchanged in BnECR. The G_{225}SGGYQIPR/HG_{234} which presented a non-classical NADPH-binding motif was found in C-terminal of BnECR. NCBI Blastn, multiple alignments and conserved domain search showed that *BnECR* had the highest homology to *A. thaliana AtECR*. RT-PCR analysis showed that *BnECR* was ubiquitously expressed in *B. napus* and preferentially expressed in the stem. The transcript level of *BnECR* at middle and late stages of seed development in low erucic acid rapeseed cultivar was obviously lower than that in high erucic acid rapeseed cultivar, suggesting that *BnECR* was involved in biosynthesis of erucic acid. The 933 bp *BnECR*ORF was subcloned into the yeast-*E. coli* shuttle vector pYES2.0. And then the recombinant plasmid was transformed into *Saccharomyces cerevisiae* wild type strain By4743 and mutant strain YDL015c, respectively. With galactose as inducer, the transformant was cultured to induce the expression of *BnECR*. The GC result indicated that *BnECR* was overexpressed effectively in *S. cerevisiae*, and the content of erucic acid reached to 1.34% of the total fatty acid in recombinant strain, an increase of 52% over the control. Functional complementation of *BnECR* in a *ECR*-deficient mutant yeast demonstrated that *BnECR* mediated the biosynthesis of VLCFAs. Our results suggest that *BnECR* should be functional orthologue of *AtECR*.

Keywords: *Brassica napus* L Enoyl-CoA reductase Cloning Erucic acid *Saccharomyces cerevisiae*

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