Chapter 8

Four Long-Chain Acyl-Coenzyme: A Synthetase Genes that Might be Involved in the Biosynthesis of Lipids in Brassica Napus

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ABSTRACT

Long chain acyl-coenzyme A synthetases (LACs) activate free fatty acid to acyl-CoA thioesters, and play important roles in the biosynthesis and degradation of lipids. In this study, four cDNAs (Complementary DNA) encode long chain fatty acyl-CoA synthetase activity has been found in Brassica napus. Sequence analysis indicated that the four LACs possessed typical molecular characteristics of LAC. Compared with low oil content varieties seed, the four genes are strongly expressed in high oil content varieties seeds at 35 days after pollination (DAP). The expression pattern suggested that the four LACs might be involved in the biosynthesis of lipids and oil accumulation in rapeseed.

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**INTRODUCTION**

Rapeseed is a main oil crop, providing about 50% of the edible oil in China and rapeseed oil is widely applied in edible oil as well as in industry such as detergent, lubricant, paint, biodegradable plastic and bioenergy, etc. An effective way to promote the economic value of rapeseed is to increase the seed oil content.

Seed oil is a major storage compound and has significant economic value. Lipid is the main component of rapeseeds oil (Shen et al., 2006). Long-chain fatty acyl-coenzyme A (CoA) synthetases (LACSs) play a crucial role in both anabolism and catabolism of lipid. These enzymes activate free fatty acid to form acyl-CoA, a key esterification step necessary for the utilization of fatty acids in lipid metabolism (Iijima et al., 1996).

In higher plants, the LACSs play an important role in lipid catabolism. During the biosynthesis of lipid, LACSs could play a key role by providing fatty acyl-CoA, and thus link fatty acid de novo synthesis and TAG assembly (Ohlrogge & Jaworski, 1997). LACSs could provide sufficient acyl-CoA pool for TAG synthesis and are also believed to be correlated with the content and variety of TAG (Ichihara et al., 2003). The sum of CoA and acetyl-CoA levels plays an important role in plant growth, salt/osmotic stress resistance, and seed lipid storage (Rubio et al., 2008). LACS genes have been cloned from several plant species and most LACS enzymes display highest levels of activity with the fatty acids that make up the common structural and storage lipids in the plant (He, 2007). In *Arabidopsis thaliana*, AtLACS2 gene (At1g49430) is expressed in young, rapidly expanding tissues, and in leaves expression is limited to cells of the epidermal layers, suggesting that the AtLACS2 enzyme may act in the synthesis of very-long-chain fatty acids (VLCFA) (Schnurr et al., 2004). AtLACS2 has previously been shown to be involved in tolerance to biotic and abiotic stress and in the pathogenesis of *B. cinerea* (Tang et al., 2007). AtLACS1 has overlapping functions with AtLACS2 in plant’s very-long-chain fatty acid (VLCFA) synthesis (Shiyou et al., 2009). AtLACS9 participates in glycerolipid synthesis in the developing seed (Shockey et al., 2002).

In *B. napus*, 4 cDNAs encoding the putative ACSs were isolated but their detail functions were still unknown (Fulda et al., 1997). In our report, we describe a bioinformatics assessment of the four *Brassica napus* LACSs (BnLACSs) genes. The investigation of potential relationship between rapeseed oil-content and BnLACSs was performed by RT-PCR.

**MATERIALS AND METHODS**

**Bioinformatics Analysis**

**Tools and Methods**

DNA and amino acid sequence manipulation were performed with DNAMAN. WU-BLAST Network Service in TAIR (http://www.arabidopsis.org) and Blast in NCBI (http://www.ncbi.nlm.nih.gov/) were used for searching genes. Phylogenetic tree was constructed using MEGA 4.0.

**Accession Numbers**

*Brassica napus* LACSs: BnLACS1: ABY77761.1 BnACS6: CAC19877.1 X94624: CAA64327.1 Z72153: CAA96523.1

*Arabidopsis thaliana* LACSs: AtLACS1: AF503751 AtLACS2: NP_175368.2 AtLACS3: NM_114758 AtLACS4: AAM28621.1 AtLACS5: NP_197141.1 AtLACS6: AF503765 AtLACS7: NP_188316.1 AtLACS8: AAM28625.1 AtLACS9: NP_177882.1

**Plant Materials and Growth Conditions**

*Brassica napus* cv. Ningyou16 was utilized for expression analysis of the 4 BnLACSs at repro-
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