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# Cloning and Functional Characterization of a Fatty Acyl-Acyl Carrier Protein Thioesterase Gene (*BnFatB*) in *Brassica napus* L.

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## ABSTRACT

**Plant fatty acyl-acyl carrier protein thioesterase (FAT) is a major enzyme regulating the amount and composition of fatty acids in lipids. In this study, one type of cDNA, corresponding to the fatty acyl-acyl carrier protein (acyl-ACP) thioesterase (Fat) enzyme, was isolated from the seed of *Brassica napus cv*. Ningyou12. BLAST results revealed that the cDNA identified highly with the *FatB* class of plant thioesterases. The cDNA contained a 1,245 bp open reading frame (ORF), encoding a protein that contained 414 amino acid residues. Subcellular localization results showed that the *BnFatB* protein was located in the chloroplast. The *BnFatB* (KC445243) gene was expressed in many tissues and was strongly expressed in seeds. Heterologous expression of the *BnFatB* gene in yeast cells was performed in order to ascertain the function of the *BnFatB* gene. Semi-quantitative RT-PCR results indicated that the expression level of the *BnFatB* gene in transformed yeast had significantly increased compared to the control. GC analysis of the fatty acid revealed that, when compared with the control, the content of C16:0 and C18:0 in yeast cells expressing *BnFatB* increased by 45.7 and 21.7%, respectively; while C16:1 and C18:1 decreased by 15.3 and 30.6%, respectively. This study demonstrated that the *BnFatB* gene had similar function as the FatB enzyme, preferentially releasing saturated fatty acid from the acyl carrier protein. It can therefore be used as a candidate target for fatty acid improvement in oilseed rape.**

**Keywords:** Fatty acid, GC analysis, Heterologous expression, Lipid, Yeast.

## INTRODUCTION

Rapeseed (*Brassica napus L.*) is an important oil and vegetable crop (Hemmat, 2009). Compared with animal oil, rapeseed oil has specific benefit on human health (Rahimi *et al*., 2011). In plants, *de novo* fatty acid synthesis in plastids can be terminated by the action of fatty acyl-acyl carrier protein thioesterases (Fat). They determine the amount and type of fatty acids that are exported from the plastids. The Fat enzyme hydrolyzes the acyl-ACP thioester bond, releasing free fatty acids and ACP. Plant Fat enzymes are encoded in the nucleus and are concentrated in the plastid (Harwood *et al*., 2005). Different plant tissues have different fatty acid demands. Therefore, Fat plays an extremely important role in cellular metabolism. There are two classes of Fats based on sequence identity and substrate (acyl-ACP) specificity: FatA and FatB (Jones *et al*., 1995). FatA has a substrate preference for unsaturated acyl-ACP, or more specifically C18:1-ACP, while FatB has a preference for saturated acyl-ACP, ranging from C8:0-ACP to C18:0-ACP (Jha *et al*., 2006; Salas *et al*.,

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2002). The Fat enzymes are involved in maintaining the compositional balance between the pools of saturated and unsaturated fatty acids in cells. Both FatA and FatB are considered to be housekeeping enzymes and are present in all plant tissues (Jones *et al*., 1995), but molecular evidence indicates that FatBs are predominantly expressed in developing seeds of plants, such as California bay, *Cuphea*, camphor, coconut, cotton and palm. There are, however, a few exceptions, such as *Arabidopsis* and *Brassica napus*, where FatA activity is more prominent than FatB (Dörmann *et al*., 1995; Dehesh *et al*., 1996; Jha *et al*., 2006).

This study identified a *BnFatB* gene using the homology based candidate gene method. Based on bioinformatics analysis and heterologous expression in yeast cells, we attempted to evaluate the function of *BnFatB* in the *de novo* fatty acid synthesis pathway of the oilseed rape. Subcellular localization results showed that *BnFatB* was located in chloroplasts. Heterologous expression of the *BnFatB* gene in *S. cerevisiae* changed the fatty acid composition of C16:n and C18:n. This study has shown that the *BnFatB* gene isolated from *B. napus* is involved in the *de novo* fatty acid synthesis pathway of oilseed rape.

## MATERIALS AND METHODS

### Plant Materials

The rapeseed cultivar, *B. napus cv.* Ningyou12, was grown and harvested in a greenhouse. Fresh leaves were collected for DNA extraction. The roots, stems, leaves, flowers, and seeds (35 days after pollination) were harvested for RNA extraction at various development stages. *Nicotiana benthamiana* plants were grown under a 16 hour day/8 hour night photoperiod regime at a constant temperature of 25°C. The leaves of *N. benthamiana* at the four-week stage were used for *Agrobacterium* infection of the transient expression of exogenous gene.

### RNA Extraction and cDNA Synthesis

In order to analyze the tissue expression pattern, rape roots, stems, leaves, flowers, and seeds (35 days after pollination) were collected for RNA extraction. Total RNA was isolated with an RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The RNA was treated with DNaseI and the first strand cDNA was synthesized from 3 μg RNA using a PrimeScript Reverse Transcriptase kit (Takara, Shiga, Japan), according to the manufacturer's instructions.

### Gene Expression Analysis

Quantitative real-time PCR (qRT-PCR) was performed in a fluorescence thermal cycler (CFX96 Real-Time PCR Detection System, Bio-Rad, Hercules, CA, USA). The qRT-PCR conditions were as follows: 94°C for 2 minutes, followed by 45 cycles of 94°C for 15 seconds, 56°C for 30 seconds and 72°C for 90 seconds, with a final extension at 72°C for 10 minutes. The results were analyzed using CFX Manager software. The expression level of *BnActin* was used as the internal control in order to standardize the samples. The gene specific primer, *BnFatB-RT-F/R*, and the internal control primer, BnActin-F/R, are shown in Table 1. Each qRT-PCR assay was replicated three times.

### Cloning and Sequence Analysis of *BnFatB*

In order to screen the *BnFat* gene from *Brassica napus*, the cDNA sequence of the FatB family conserved region was used as a query to blast against the *Brassica napus* database and a putative EST (Expressed Sequence Tag sequences) was obtained. Using the homology based candidate gene method, a primer *BnFatB-F/R*, based on the

**Table 1.** Sequences of the primers used in this study.

| Primers | Sequences (5'-3') |
|---|---|
| BnFatB-RTF | *TTACAGGAAACGGCACTCAA* |
| BnFatB-RTR | *AACTAGCCAATCACGACGC* |
| BnActin-F | *ATGGCCGATGGTGAGGACATTC* |
| BnActin-R | *GGT GCGACCACCTTGATCTTC* |
| ScActin-F | *CTCTTTCTCCACCACTGCTGA* |
| ScActin-R | *GGCAGATTCCAAACCCAAA* |
| BnFatB-F | *GGTACCATGGTGGCCACCTCTGCT* |
| BnFatB-R | *GAATTCTTACGATGTAGTGTCCCAAGTT* |
| BnFatB-SLF | GGTGGCCACCTCTGCTACA |
| BnFatB-SLR | TTACGATGTAGTGTCCCAAGTTG |

EST sequence and the *Arabidopsis* AtFatB sequence (NM100724.2), was designed (Table 1). The cDNA sample from the seeds of Ningyou12 was PCR-amplified using *BnFatB-F/R* primers (Table1), which were designed according to the above sequence. The primer sequences were located near the start and stop codon of the CDS. A 1,350 bp length product was amplified. The PCR conditions were as follows: 94°C for 4 minutes, 35 cycles of 94°C for 40 seconds, 56°C for 40 seconds and 72°C for 90 seconds, with a final extension at 72°C for 10 minutes. The amplified fragments were purified and cloned into the pMD18-T vector (Takara, Dalian, China). Several randomly selected clones were sequenced. One clone was obtained and was named *BnFatB* (KC445243), based on the sequence alignment of the FatB protein. The Expasy database (http://www.expasy.org/) was used to analyze the characteristics of the *BnFatB* protein. Species whose *FatB* gene shared close relationship with *BnFatB* were found in ThYme (http://www.enzyme.cbirc.iastate.edu). The sequence alignment was performed using the Multalin program and the default parameters (http://www.ebi.ac.uk/Tools/msa/clustalw2/) and GeneDoc was used to edit the sequence alignment. A phylogenetic tree was constructed by the neighbor-joining method using molecular evolutionary genetics analysis (MEGA) (version 4.0). TreeView was used to construct the phylogenetic tree. Subcellular localization prediction was performed using the TargetP 1.1 Server (http://www.cbs.dtu.dk/services/TargetP/).

## Heterologous Expression of the *BnFatB* Gene in Yeast

The sequenced *BnFatB* gene was subcloned from the pMD18-T vector into the pYES2/NTC vector (Invitrogen, Carlsbad, CA, USA) *via* a multiple-cloning site. The pYES2 constructs, containing the *BnFatB* gene, were transformed into the yeast strain, InvSc1 (Invitrogen), using a lithium acetate procedure according to the manufacturer's instructions. The pYES2/NTC provided in the kit was transformed and used as the control. The transformant and the transformed yeast cell growth conditions followed Wu *et al.* (2008). Each yeast cell was prepared for *BnFatB* gene expression and lipid analysis. RNA extraction and cDNA synthesis was undertaken according to the manufacturer's instructions. *BnFatB* gene expression was detected by the primer, *BnFatB-RTF/R* and the *ScActin* gene was used as an internal control in the experiments. The ScActin-F/R and *BnFatB-RTF/R* sequences are shown in Table 1.

## Analysis of Yeast Fatty Acids

Yeast cell fatty acid methyl esters (FAMEs) were prepared according to the method described by Katavic *et al.* (2002,

2004). The cells were harvested from overnight cultures by centrifugation and washed with distilled water. Then, the cells were dried at 60°C and weighed. The cell pellets were saponified in methanolic-KOH [10% (w/v) KOH, 5% (v/v) $H_2O$ in methanol] for 2 hours at 80°C. After saponification, the samples were cooled on ice. Around 10–25 mg of the samples were weighed in a vial and then mashed with glass rods. Exactly 2 mL of 5% $H_2SO_4$ methanol solution, 25 µL 0.2% butylated hydroxytoluene (BHT) methanol solution and 300 µL toluene were added to the samples, which were then placed in a water bath at 90–95°C for 1.5 hours. After cooling to room temperature, 2 mL 0.9% NaCl, 3 mL n-hexane and 200 µL 5 mg $mL^{-1}$ C17:0 fatty acid solution were added to the vial for extraction. The supernatant was then poured into 10 mL centrifuge tubes and the supernatant was blown dry with nitrogen for about 10 minutes. The samples were dissolved in 500 µL of n-hexane, scrolled for 2–3 seconds and then centrifuged for gas chromatography (GC) analysis. GC analysis was performed on a gas chromatograph (Agilent 7890N, Santa Clara, CA, USA) fitted with a 30 m FFAP column, with an ID 0.25 mm narrowbore and a film thickness of 0.5 µm. The GC conditions followed Wu *et al*. (2008).

### *Agrobacterium*-Mediated Transient Expression and Subcellular Localization

In order to study subcellular localization, the *BnFatB* ORF, without stop coding (TAG), was amplified using primer, *BnFatB-SLF/R* (Table 1). The PCR conditions were as follows: 94°C for 4 minutes, 94°C for 40 seconds, 56°C for 40 seconds and 72°C for 90 seconds, with a final extension at 72°C for 10 minutes. The complete *BnFatB* ORF was fused to the GFP frame and was driven by the cauliflower mosaic virus 35S promoter in the pK7FWG2.0 vector (Karimi *et al*., 2002) *via* the gateway recombination system (Invitrogen). *Agrobacterium* (GV3101)-mediated transient expression assays were carried out using the methods outlined in Jo *et al*. (2011). Three days after transformation, a small part of the transient expressed *N. benthamiana* leaves were cut off and examined by laser-scanning confocal microscopy using an Olympus (Tokyo, Japan) confocal laser scanning microscope (model FV1000). The excitation and emission wavelengths for GFP and chloroplast autofluorescences were λ488/510–540 and λ633/661 nm, respectively.

### Estimation of Intracellular Neutral Lipids

Sudan black B was used as a marker to determine the neutral lipid contents in *S. cerevisiae*. Cell quantification was performed at $\lambda_{580}$ nm. The induced yeast cells were stained with a 0.1% solution of Sudan black B, prepared in 70% ethanol for 10 minutes and then rinsed in 70% ethanol six times. The measurement was performed at $\lambda_{580}$ nm (Evansa *et al*., 1985).

## RESULTS

### Cloning and Structural Analysis of *BnFatB*

In order to screen the *BnFat* gene from *Brassica napus*, the cDNA sequence of the FatB family conserved region was used as a query to blast against the *Brassica napus* database and a putative EST was obtained. Using the homology based candidate gene method, two primers, namely, *BnFatB F/R*, according to EST sequence, and the *Arabidopsis* AtFatB sequence (NM100724.2) (Table 1) were created. A 1,350 bp product was amplified by PCR using the primer *BnFatB F/R* from the *B. napus cv.* Ningyou12. The putative ORF length was 1,245 bp, as predicted by genscan. We found that *BnFatB* gene

belonged to TE14 family in ThYme database. The *BnFatB* shared a high homology with *BjFatB* (ACR56792.1) from *Brassica juncea* and *AtFatB* (NP 172327.1) from *Arabidopsis* after searching ThYme database and phylogenetic analysis (Figure 1). Therefore, this gene was named *BnFatB*. *BnFatB* encodes a polypeptide of 414 amino acids with a predicted molecular weight of 47.6 kDa and a calculated isoelectric point of 11.6 (Protparam: http://www.expasy.cn).

The deduced amino acid sequence of *BnFatB* shared about 90% identity and 93% positives with that of AtFatB. Two crucial residues (H319 and C354) for thioesterase catalytic activity were observed in the deduced peptide sequence and a putative chloroplast transit peptide (80 amino acids) was found at the N-terminal (Figure 1-A). Phylogenetic analysis was conducted according to the similarity of the FatB conserved domain sequence from different species. This analysis indicated that *BnFatB* was highly similar to BjFatB and AtFaTB, using 16:0-ACP as the preference substrate (Figure 1-B).

## Analysis of the *BnFatB* Expression Pattern

In order to investigate the *BnFatB* expression pattern in different tissues, total RNA was isolated from root, stem, leaf, flower, and seed tissues from *B. napus cv.*

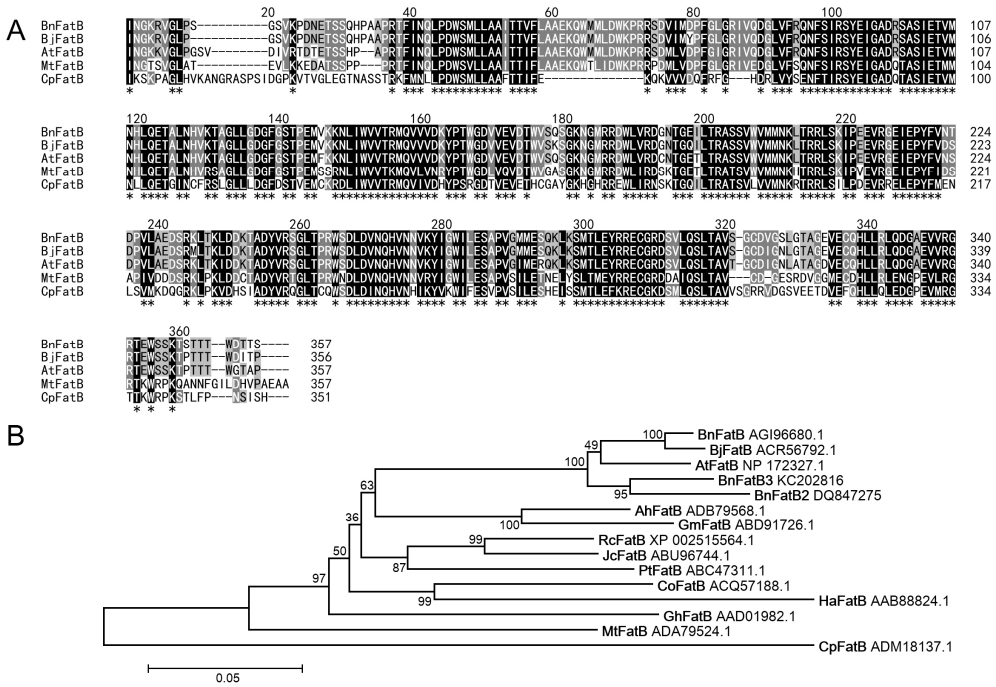


**Figure 1.** Sequence analysis of BnFatB: (**A**) Alignment of FatB sequences. The BnFatB sequence was aligned against four FatB protein sequences from different species including BjFatB, AtFatB, MtFatB and CpFatB (GenBank Acc. No. ACR56792.1, Np_172327.1, ADA79524.1 and ADM18137.1), respectively. (**B**) Protein alignments were based on Clustal W, and phylogenetic tree using NJ algorithm was generated by MEGA 4.1. The sequences of BnFatB (this study), BnFatB2 and BnFatB3 (Jin *et al.,* 2014*)*, BjFatB from *Brassica juncea*, AtFatB from *Arabidopsis thaliana*, AhFatB from *Arachis hypogaea*, GmFatB from *Glycine max*, JcFatB from *Jatropha curcas*, RcFatB from *Ricinus communis*, PtFatB from *Populus tomentosa*, CoFatB from *Camellia oleifera*, GhFatB from *Gossypium hirsutum*, MtFatB from *Macadamia tetraphylla*, HaFatB from *Helianthus annuus* and CpFatB from *Chimonanthus praecox.*

Ningyou12. The cDNA samples were used as templates in order to detect the transcription of *BnFatB* by quantitative real-time PCR (qRT-PCR). The *BnActin* gene was used as the internal control. The qRT-PCR results indicated that *BnFatB* was expressed in all the tissues tested, but was most highly expressed in seeds, similar to the *AtFatB* expression pattern (Figure 2).

### Subcellular Localization of *BnFatB* in *N. benthamiana* Leaves

In order to experimentally verify the predicted subcellular location of *BnFatB*, the *green fluorescent protein* (GFP) gene was fused to the 3'-terminal of the *BnFatB* gene *via* the gateway recombination system so that the plant expressing vector could be generated under the control of the CaMV35S promoter. The fusing protein (*BnFatB-eGFP*), was transiently expressed in *N. benthamiana* leaves using the infiltration method. The subcellular localization of *BnFatB-eGFP* was examined in crude *N. benthamiana* leaves by confocal laser-scanning microscopy. The *GFP* in the *BnFatB-eGFP* fusion protein was observed in *N. benthamiana* leaf chloroplasts at λ488/510–540 nm (Figure 3-A). Chloroplast fluorescence was detected by auto red fluorescence at λ633/661 nm (Figure 3-B). The yellow fluorescence spots were generated by the double overlapping of red fluorescence and green fluorescence. The fluorescence for *BnFatB* and the auto red fluorescence generated by the chloroplasts were overlapped and further merged (Figure 3-C). This result demonstrated that *BnFatB* was located in the chloroplasts.

### Heterogeneous Expression of the *BnFatB* Gene in *S. cerevisiae*

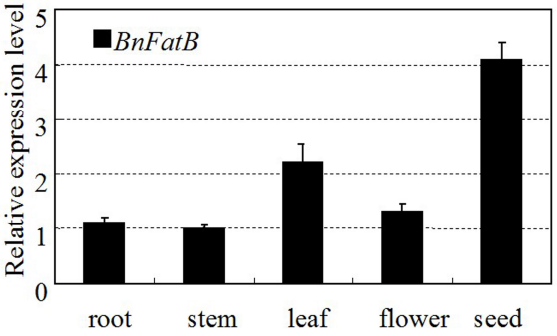


**Figure 2.** Expression pattern analysis of *BnFatB* genes in root, stem, flower, leaf, and seeds in the *Brassica napus* by qRT-PCR. The transcript level of stem was used as the calibrator whose BnFatB

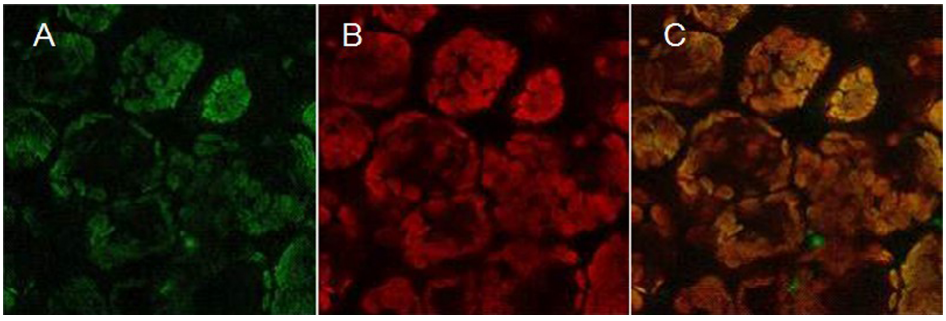


**Figure 3.** BnFatB-eGFP locates at the chloroplasts in *N.benthamiana* leaves. (A) The GFP of BnFatB-eGFP fusion protein; (B) Chloroplast was shown in red autofluorescence, and (C) The merged picture of A and B.

Previous results have shown that the lipid composition of leaf chloroplasts is similar to that of *S. cerevisiae*, which mainly comprises C16:0, C16:1, C18:0 and C18:1 (Redon *et al*. 2009). In order to test whether *BnFatB* enzyme activity affects fatty acid composition, the full length sequence *BnFatB* gene was heterogeneously expressed in *S. cerevisiae.* The semi-quantitative RT-PCR results indicated that *BnFatB* gene expression levels significantly increased compared to the control (Figure 4). In yeast cells expressing the *BnFatB* gene, the C16:0 and C18:0 saturated fatty acid contents increased by 45.7 and 21.7%, respectively, compared to the control, while C16:1 and C18:1 unsaturated fatty acid contents decreased by 15.3 and 30.6%, respectively (Table 2). The total amount of fatty acids in *S. cerevisiae* expressing the *BnFatB* gene increased by 21.4%. This result showed that the *BnFatB* gene enhanced the content of saturated C16:0 and C18:0 fatty acids. Therefore, this gene must be involved in the reaction that releases saturated fatty acids from ACP protein.

## *BnFatB* Involvement in the Synthesis of Neutral Lipids

Sudan black B was used as a marker in order to determine whether *BnFatB* was involved in the synthesis of neutral lipids and to determine the content of neutral lipids in *S. cerevisiae* (Tan *et al*., 2011). Lipid concentration quantification in the samples was performed at λ580 nm. The amount of intracellular neutral lipids increased very significantly in the yeast strain containing the pYES2_*BnFatB* vector compared to the yeast containing the empty vector (Figure 5). This result suggested that the *BnFatB* gene was involved in the synthesis of neutral

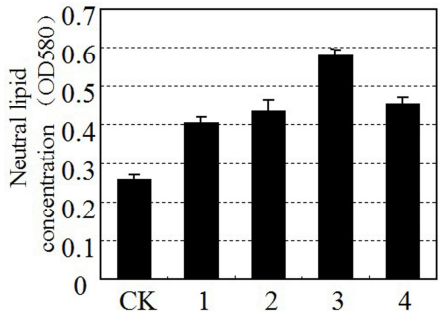


**Figure 5.** Neutral lipid detection of *S. cerevisiae* by Sudan black B staining. The induced yeast cells were examined under immersion objective. The measurement was performed at λ580 nm. (1-4) Samples from heterogeneously expressed *BnFatB* in *S. cerevisiae*, and (CK) The control (empty vector transformation).

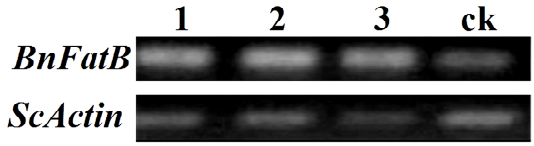


**Figure 4.** *BnFatB* gene expression in *S.cerevisiae* was detected by semiquantitative RT-PCR using the primer BnFatB-RTF/R. *ScActin* gene was used as an internal control in the experiments. (1-3) Corresponding to three different samples expressing *BnFatB* in *S. cerevisiae,* and (ck) Indicating control.

**Table 2.** The fatty acid composition in *S. cerevisiae* heterogeneously expressed *BnFatB*.

| FA[a] | pYES2[b] (mg $g^{-1}$) | pYES2-BnFat[c] (mg $g^{-1}$) |
|---|---|---|
| C16:0 | 19.8±4.0 | 65.5±6.7 |
| C16:1 | 41.8±5.5 | 26.5±3.8 |
| C18:0 | 9.42±0.94 | 31.1±2.8 |
| C18:1 | 82.8±10.5 | 52.2±5.1 |
| Total | 154±21 | 175±18 |

[a] Fatty Acid; [b] Fatty acid extracted from yeast control, [c] Fatty acid extracted from pYES2-BnFatB.

lipids.

## DISCUSSION

The plant acyl-ACP thioesterases are nuclear-encoded proteins and can be divided into two classes: FatA and FatB, according to their amino acid contents and substrate preference (Voelker *et al*., 1992; Jones *et al*., 1995; Mekhedov *et al*., 2000). FatA and FatB have different substrate specificities. The FatA class has a high specificity for 18:1-ACP and a lower specificity for 18:0- and 16:0-ACP (Knutzon *et al*., 1992; Hawkins and Kridl, 1998; Serrano-Vega *et al*., 2005). The FatB class has a greater specificity for saturated fatty acid with 8–18 carbons (Voelker *et al*., 1992; Salas and Ohlrogge, 2002). FatA protein is a polypeptide of 38 kDa and has been purified from *B. napus* and *B. campestris* (Hellyer *et al*., 1992; Pathak *et al*., 2004). FatB protein has been successfully isolated from *B. napus* (Jin-Yue Sun *et al*., 2014) as well as from several other species, such as *Arabidopsis*, sunflower, *Cuphea*, *Madhuca longifolia* and *Jatropha curcas*. (McKeon *et al*., 1982; Hellyer *et al*., 1992; Jones *et al*., 1995; Mekhedov *et al*., 2000; Beisson *et al*., 2003). This study cloned one *FatB* gene from *B. napus* cv. Ningyou12, which displayed a high homology with the *BjFatB*, *AtFatB* and *MtFatB* (from *Macadamia tetraphylla*) genes.

The *BnFatB* gene was expressed in all tissues and was most highly expressed in the seeds, consistent with a previous result reported for *Arabidopsis*. A study of ChFatB1 (from *Cuphea hookeriana*) and AtFatB1 revealed that there was a chloroplast transit peptide at the N-terminal, which contained a cleavage site before the LPDW sequence of the FatB peptide (Jones *et al.,* 1995; Dörmann *et al.,* 2000). In this study, an apparent chloroplast transit peptide (90 amino acids) was detected at the N-terminal (Figure 1-A). Subcellular localization demonstrated that the *BnFatB* was located in the chloroplasts. Plant Fat thioesterases are generally located in the plastid and terminate *de novo* fatty acid elongation (Harwood, 2005). This result suggested that *BnFatB* may carry out its functions in the chloroplast.

FatB thioesterases play an important role in the control of the fatty acid profile and in the carbon chain length of storage lipids. The *in vivo* role of *AtFatB*, as a major determinant of saturated fatty acid synthesis, has been demonstrated in *Arabidopsis* (Bonaventure *et al.*, 2003). The heterologous expression of *BnFatB* in yeast cells resulted in increased levels of saturated C16:0 and C18:0 fatty acids and in reduced levels of unsaturated C16:1 and C18:1 fatty acids. The results indicated that the cloned *BnFatB* gene was involved in a reaction with ACPs carrying saturated fatty acyl chains. Using a transgenic approach, some of the *FatB* genes have been introduced into the cultivated crop with low or no expression of *FatB* (Evansa *et al*., 1985; Porebski *et al*., 1997). The potential advantages of modifying fatty acid composition have been demonstrated by expressing *FatBs* in *Arabidopsis*. Seed-specific overexpression of the *AtFatB* gene or JcFatB1 cDNA from *Jatropha curcas* in *Arabidopsis* led to an increase in saturated fatty acids, especially palmitate, and to a reduction in unsaturated fatty acids (Dörmann *et al*., 2000; Wu *et al*., 2009). Notably, the expression of CwKAS A1 and CwFAB enzymes derived from *Cuphea* in *Arabidopsis* and oilseed rape seeds changed the long chain fatty acids (C16:0, C18:0) into shorter chain fatty acids (C8:0, C10:0, C12:0) (Dehesh *et al*,, 1996; Leonard *et al*, 1998). In this study, the newly identified *BnFatB* gene from *B. napus* functioned as a saturated acyl-ACP thioesterase, which is potentially applied for increasing the levels of saturated fatty acids in the seed oil of oilseed crops.

The *BnFatB*（KC445243）gene with a full length of 1,245 bp open reading frame (ORF) located at the chloroplast and strongly expressed in seeds. Functional expression of *BnFatB* gene in yeast cells indicated that FatB enzyme preferentially

release saturated fatty acid from the acyl-carrier protein, which can be used as candidate gene for fatty acid improvement of oilseed rape.

## ACKNOWLEDGEMENTS

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# همسانه سازی و تعیین کارکرد پروتئین حامل اسیل اسیل چرب ژن یتواستراز (*BnFatB*) در کلزا (*Brassica napus* L.)

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## چکیده

در گیاهان، پروتئین حامل اسیل اسیل چرب ژن یتواستراز (FAT)، آنزیم اصلی در تنظیم مقدار و ترکیب شیمیایی اسید ها ی چرب در چربی ها است. در این پژوهش، یک نوع cDNA متناظر پروتئین حامل اسیل اسیل چرب(acyl-ACP) آنزیم یتواستراز از بذر های کولتیوار Ningyou12 کلزا جدا سازی شد. نتایج آزمون BLASTنشان داد که cDNA تشابه زیادی با نوع*FatB* از یتواستراز های گیاه داشت. cDNA مزبور ORF دارای 1245 جفت باز بود که پروتئینی با ۴۱۴آمینو اسید را شامل می شد. نتایج مکان شناسی نشان داد که پروتئین *BnFatB* در کلروپلاست قرارداشت. ژن ( *BnFatB* KC445243)) در بسیاری از بافت ها بیان می شد و در بذر ها بیان آن شدید بود. به منظور تعیین نقش ژن *BnFatB* ، بیان هترولوگوس این ژن در سلول مخمر اجرا شد. نتایج آزمون نیمه–کمی RT-PCRگواه آن بود که سطح بیان ژن *BnFatB*در مخمر تراریخت شده در مقایسه با شاهد به طور معنی داری افزایش یافته بود. تجزیه اسیدهای چرب نشان داد که در مقایسه با شاهد، مقدار C16:0 و C18:0 در سلول مخمر با بیان *BnFatB*به ترتیب به میزان ۴۵/۷٪ و۲۱/۷٪ افزایش داشت در حالیکه C16:1 و C18:1 به ترتیب به میزان ۱۵/۳٪ و۳۰/۶٪ کاهش داشتند. این پژوهش نشان داد که ژن *BnFatB*نقش عملیاتی مشابهی مانند آنزیم *FatB*داشت و ترجیجا اسیدهای چرب اشباع شده را از پروتئین حامل اسیل آزاد میکرد و بنا بر این می تواند به عنوان کازوید هدف برای بهبود اسید های چرب در کلزای روغنی استفاده شود.