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Characterization of a Desiccation Stress Induced Lipase Gene from *Brassica napus* L.

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ABSTRACT

Lipases are known to have important functions in many physiological processes in plants. Here, we cloned a lipase gene via Rapid Amplification of cDNA Ends (RACE) technique from *Brassica napus* L., designated as *BnDIL1* (*B. napus* Desiccation-Induced Lipase 1). The lipase enzyme activity was confirmed by estimating the lipase activity and reduced lipids content in *Saccharomyces cerevisiae* (pep4) transformant. Two *B. napus* lines with different oil contents were employed to examine the transcription profiles of *BnDIL1* during the processes of seed morphogenesis, maturation, dormancy, pregermination and germination. The transcription level of lipid degradation pathway was enhanced during the processes of seed maturation, dormancy, pregermination and germination, and was higher in seeds of low oil-contents line than that of high oil-contents line. However, *BnDIL1* was significantly activated when seed desiccation started. Both "slow desiccation" and "fast desiccation" treatments on seedlings dramatically activated the transcription of *BnDIL1*, while only "slow desiccation" stress, which would induce the cell apoptosis, significantly activated the transcription of lipid degradation gene. This result demonstrated that *BnDIL1* in *B. napus* was desiccation stress dependent gene rather than fatty acids degradation gene.

Keywords: Enzyme activity, Oil-content, Rapid amplification, Seed, Transcription profiles of *BnDIL1*.

INTRODUCTION

As a member of the super family of hydrolytic/lipolytic enzymes, lipase contains a highly conserved catalytic triad ''S-D-H'' which is formed by three amino acid residues including serine, aspartic acid, and histidine (Ollis *et al*., 1992). In this catalytic triad, the Ser residue is the most important element for the fatty acid-deesterifying activity (Brick *et al*., 1995).

In plants, TriAcylGlycerols (TAGs), as the main storage oil, are aggregated into the form of oil bodies in seeds (Austin *et al*., 2006) and leaf mesophyll tissue (Sorokin, 1955). Lipid bodies with TAGs in leaves were found mainly in chloroplast, and the fatty acid composition of the chloroplast TAGs was very similar to the TAGs found in seeds (Austin *et al*., 2006; Lin and Oliver, 2008). Degradation of TAGs by various lipases are thought to be important to supply carbon source and energy for the morphogenesis, development, and defense response (Durrett *et al*., 2008; Hong *et al*., 2008; Hong *et al*., 2000; Li *et al*., 2009; Matsui *et al*., 2004). During the oilseed

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germination, massive TriAcylGlycerols (TAGs) are hydrolyzed by lipases to provide the carbon skeletons and energy that drive post germination growth.. Besides the role in carbon source supply, lipases are also known to have other important physiological functions such as converting phosphatidylcholine to substrate for galactolipid synthesis in chloroplast envelope (Andersson *et al*., 2004), maintaining the structural integrity of chloroplast (Padham *et al*., 2007), involvement in chloroplast development (Tan *et al*., 2011), mediating the onset of senescence (Hong *et al*., 2000), and affecting flowering through the regulation of gibberellin metabolism (Lin *et al*., 2011).

Lipases are generally divided into two types, the "*GXSXG*" type (Horrevoets *et al*., 1991) and "*GDSL*" type (Upton and Buckley, 1995), depending on the consensus sequence motifs "*GXSXG*" and "*GDSL*" containing active site Ser residue. Both the "*GXSXG*" and "*GDSL*" type lipases are found to be widely involved in signaling and stress responses. In the case of the "GXSXG" type lipases, they were reported to be involved in ultraviolet B stress (Lo *et al*., 2004), salt and osmotic stress (Ellinger and Kubigsteltig, 2010), antibiosis against the green peach aphid and the pathogens *Pseudomonas syringae* and *Hyaloperonospora arabidopsidis* (Louis *et al*., 2010; Louis *et al*., 2012).

Here, we aimed to study a *GXSXG* type lipase of *Brassica napus*, which was designated as *BnDIL1* (*B. napus* Desiccation-Induced Lipase 1) and was involved in responding to fast and slow desiccation stress.

MATERIALS AND METHODS

Plant Materials

Oil seeds of *Brassica napus cv.* "Ningyou16" were sterilized by bleach for 6 min. The seeds were softly washed with sterile distilled water for 6 times or more. To achieve uniform germination, seeds were soaked in sterile distilled water for 2 d at 4℃ and sowed on MS basal medium solidified with 8 g L^{-1} Agar, and then grew in growth chamber operating at 16 hours light/8 hours dark cycles, 22|±2|, and 150 μmol m^{-2} s^{-1} photosynthetically active radiation. Green seedlings were harvested at the 6th day after germination and roots were isolated from these seedlings for RNA extraction immediately. Roots, stems, leaves, and flowers were isolated from the six-month-old plants, *B. napus* lines with different oil contents EM 91(36.02% oil content), and EM 102 (50.59% oil content) were employed for gene expression analysis. Samples were collected from different stages of seed morphogenesis [from 25 Days After Pollination (DAP) to 40 DAP], maturation (form 40 to 50 DAP), dormancy stage (storage), pregermination (imbibition), and germination.

RNA Extraction, Reverse Transcription, in Silicon Cloning, 5' RACE and 3' RACE

Total RNAs of different samples including roots, stems, leaves, flowers, and seeds from *B. napus* were extracted using Plant RNA Reagent (Invitrogen, CA, USA). cDNAs synthesis was followed by the manual of Takara. Synthesized cDNAs were then diluted 10 times for the real-time RT-PCR assay.

Arabidopsis lipase gene NM_102182 was used as the query to blast *Brassica* ESTs (Expression Sequence Tags) database (TAIR, http://www.arabidopsis.org/). Six ESTs (GeneBank Acc. No. ES991495、EV023742、CD813762、EV159683、ES989073、EV060641) with high nucleotide sequence similarities were obtained. The longest EST CD813762 with the highest homology was chosen for 3' and 5' RACE. The 3' RACE was carried out according to the 3' RACE kit (Takara, Japan). Specific primers of 3' RACE for Nest PCR were *BnDIL1* 3-1 (5'-CGTTCACATGATGTATGGGC-3') and *BnDIL1* 3-2 (5'-GTGGGCATTCTCAGTTCGG-3'),

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respectively. The 5' RACE was performed by using the 5' RACE system of Invitrogen (Catalog no. 18374-058). The reverse transcription of the first strand cDNA was performed with gene-specific primer *BnDIL1*-5-RT R (5'-CTCTTGCCCTATCGTG-3'). Specific primers of 5' RACE for Nest PCR were *BnDIL1*-5-1 R (5'-GACTTCGCCGGTGGTTTTGGACT-3') and *BnDIL1*-5-2 R (5'-CGGAGCTCACTGACAGCTCC-3'), respectively. PCR products were purified and sub-cloned into pMD18-T vector followed by sequencing.

Sequence and Phylogenetic Analysis

Sequence alignments were carried out with MEGA and edited with GeneDoc. Pattern search was done with Pattern Search program in PIR (http://pir.georgetown.edu/pirwww/index.shtml) network. Molecular weight and pI of the deduced protein were detected with DNAStar. Subcellular localization prediction was performed with SoftBerry (http://linux1.softberry.com/berry.phtml) and ChloroP Server (http://www.cbs.dtu.dk/services/ChloroP-1.1/). Protein sequence analysis was performed using ExPASy Proteomics (http://au.expasy.org/) and Motif-Scan (http://myhits.isb-sib.ch/cgi-bin/motif_scan).

Crude Enzyme Activity Assay

The predicted signal peptide sequence containing 35 aa was deleted by PCR using the primers designed for the expression of *BnDIL1*. Restriction endonuclease sites *Eco*RI and *Not*I were introduced respectively into the sense primers *BnDIL1*-F (5'-TgaattcATGATTCAACGGTTGGTTGT) and *BnDIL1'*-F (5'-TgaattcATGGGAGATCTCACGCAG-3') with an extra "ATG", and antisense primer *BnDIL1*-R (5'-AATgcggccgcTTCTTCTTGGTCTCCTCC TA-3'). The intact and truncated *BnDIL1* were constructed in the yeast expression vector *pYES2*, respectively. To enhance the stability of foreign protein expressed in yeast, the protease-A-deficient (pep4) strain of *Saccharomyces cerevisia* was chosen as the expression host. Yeast transformation was carried out as described previously(Gietz and Schiestl, 2007). Empty vector *pYES2* transformant was used as the negative control. The yeast culture and protein expression conditions were performed as described in our previous work (Tan *et al*., 2011). Protein concentration was determined using the Bradford method (Bradford, 1976). For enzyme activity assay, a modified method from a previous report was used. The 600mL reaction system containing 10% non-colored substrate p-NPL, 0.5mg total protein and the other was 50 mM sodium phosphate buffer (pH 7.0), which was carried out at 22 ℃ for 0.5, 1.0, 1.5, 2.0, and 2.5 hours, respectively. The absorbance at λ= 405 nm ($A_{405\ nm}$) of each supernatant was measured immediately by spectrophotometer.

Estimation of Intracellular Lipids of *Saccharomyces cerevisia* (pep4)

Sudan black B was applied to determine the neutral lipids content of *S. cerevisiae*. Equal amount of yeast cells was stained with Sudan black B between control and samples. Cell quantification was performed at λ_{600} nm. The induced yeast cells were stained with prepared Sudan black B and measured with spectrophotometer at λ_{580} nm according to a previous report (Evansa and Gilbert, 1985). The change in total polar lipids of *S. cerevisiae* was examined by two-dimensional Thin Layer Chromatography (2D-TLC) as described before (Tan *et al*., 2011;Vyssotski *et al*., 2009). The total fatty acid composition of yeast cells was estimated by Gas Chromatograph (GC) as

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described in our previous work (Tan *et al*., 2011).

Quantitative PCR (qPCR) Analysis

Gene specific primers *BnDIL1*-RT-F (5'-ATGATTCAACGGTTGGT-3') and *BnDIL1*-RT–R (5'-TCCTTCTTCTCAGCTCCAGC-3') of *BnDIL1*, *ICL*-RT-F (5'-AAGAGGAAGGGAGATTTGAGG-3') and *ICL*-RT–R (5'-TGTTAGGAACGGTATCGTATGG-3') of *ICL*, and *BnActin*-RT-F (5'-GTTGCTATCCAGGCTGTTCT-3'), and *BnActin*-RT-R (5'-ACTGCTCTTAGCCGTCTCC-3') of *BnActin* used as an internal control were designed and used for qPCR analysis, which was carried out with the kit of SYBR® *Premix Ex Taq*™ II (Takara, Japan) and detected by Mx3000P (Stratagene, USA). The cDNAs prepared for each gene were subjected to 45 cycles of amplification under the following conditions: 95°C denaturing for 1 minute, 57°C annealing for 30 seconds, and 72°C extension for 15 seconds. In order to clearly show the relative transcription levels of each gene, the transcription level of *BnDIL1* in pre-imbibition treated seeds was normalized into 1.

Southern Blot Analysis

Genomic DNA was extracted from rapeseed of *Brassica napus cv* "Ningyou16". The DNA probe of *BnDIL1* was amplified with primers GM-F (5'-ATGATTCAACGGTTGGT-3') and GM-R (5'-CTATTCTTCTTGGTCTCCT-3'). During PCR, dNTP containing [a-^{32}P] dCTP was used for probe labeling. Every 15 ug of rapeseed genomic DNA was digested with *Bam*H I, *Eco*R I, *Eco*R V, *Hin*d III, *Xba* I, respectively, and loaded into each lane of 0.6% agarose gel for electrophoresis. After sufficient DNA migration, the digested DNA was transferred to N+ Hybond nylon membranes and hybridized with a [a-^{32}P]-labeled PCR fragment of *BnDIL1*. Southern blotting was carried out as described by Yamaguchi-Shinozaki and Shinozaki (Yamaguchi-Shinozaki and Shinozaki, 1994).

Desiccation Treatment

Seeds of *B. napus cv* "Ningyou16" were grown in sterilized water for 6 days after germination. Seedlings were washed with sterilized water carefully for desiccation treatments. Seedlings on the surface of filter papers for 2 hours at room temperature were set as the "slow desiccation" group, while seedlings embedded in high concentration of 40% (w/v) PolyEthylene Glycol (PEG) 8000 for 2 hours at room temperature were set as the "fast desiccation" group. And seedlings soaked in sterilized water for 2 hours at room temperature were used as control. RNAs were isolated from these samples immediately for qPCR analysis of each interested gene.

RESULTS

Gene Cloning and Analysis

A putative *Arabidopsis* TAG lipase gene NM_102182 was used to probe the ESTs database of *Brassica napus*. Six ESTs (GeneBank Acc. No. ES991495, EV023742, CD813762, EV159683, ES989073, EV060641) with high nucleotide sequence similarities were found. The longest EST CD813762 with the highest homology was chosen for 3' and 5' RACE as described in the section on "Materials and Methods". A cDNA sequence with 1.804 kb was obtained containing a 5' UTR (Untranslated Region) with 198 bp, a 3'UTR with 187 bp, a poly-A sequence with 12 bp, and an

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Open Reading Frame (ORF) sequence with 1.407 kb (Figure 1). The nucleotide sequence was confirmed by PCR and sequencing. The nucleotide sequence and deduced amino acid sequence of *BnDIL1* are shown in Figure 1. Calculated molecular mass of this deduced protein was 52.9 KD, and the predicted Isoelectric point (pI) was 6.39. The target P program predicted that this deduced protein is a protein that existed in the secretory pathway. Homologue alpha/beta-hydrolase family proteins as with *BnDIL1* are found to be widely distributed in plants, fungi, and bacteria via querying *BnDIL1* in GenBank database. Multi-sequence alignment of *BnDIL1* with homologue proteins from plants, fungi, and bacteria demonstrated that *BnDIL1* contains a conserved *GXSXG* motif in the typical 10-residue consensus sequence near N terminal, [LIV]-X-[LIVAFY]-[LIAMVST]-G-[HYWV]-S-X-G-[GSTAC]. The N terminal

GXSXG motif is different from the known center-located *GXSXG* type lipases, such as in *BnDIL1* (Tan *et al*., 2011), *DAD1* (Ishiguro *et al*., 2001), *PAD4* (Louis *et al*., 2012), etc. (Figure 1). The result of multi-sequence alignment also indicated a putative catalytic triad ''S-D-H'' consisting of conserved Ser^{304}, Asp^{290}, and His^{416} (Figure 1). All these results indicated that *BnDIL1* might belong to a new group of the *GXSXG* type lipase, in which *GXSXG* motif is front-located. This gene encoding a putative lipase was designated as *BnDIL1* for its desiccation inducible properties described below, and the gene sequence was submitted to GenBank (GenBank Acc. No. JX446400).

The genomic DNA sequence of *BnDIL1* was amplified with the primers used for its cDNA ORF amplification and sequencing. Through comparative analysis with cDNA ORF of *BnDIL1*, the genomic DNA sequence of *BnDIL1* ORF region was demonstrated to contain 1.878 k nucleotide base-pairs consisting of 7 exons and 6 introns (Figure 2-a). The copy number of genomic *BnDIL1*was investigated via southern blot analysis. The results showed that there were at least two clear bands detected in the lanes of genomic DNA digested with *Xba* I、*Bam*H I、*Eco*R I and *Eco*R V, respectively, while there was one clear band observed in the lane of genomic DNA digested with *Hin*d III (Figure 2b). All these results presumed that there were at least two copies of *BnDIL1* in *B. napus* genome.

Lipase Activity Analysis of *BnDIL1*

Although different sub-localization prediction results of this protein were obtained from ChloroP and Softberry, a putative signal peptide of about 35 amino acids at the N-terminus with unclear function was co-assumed, cleavage of which would produce a mature protein of approximately 49 KD. We truncated *BnDIL1* into "*BnDIL1*'" by removal of the first N-terminal 35 amino acids. Both *BnDIL1* and *BnDIL1'* were constructed into yeast expression vector *pYES2* forming *pYES2_BnDIL1* and *pYES2_BnDIL1'*, respectively, and transformed into *S. cerevisiae* (pep4) for enzyme activity analysis. Empty vector *pYES2* was transformed into *S. cerevisiae* (pep4) as the control. Total proteins of galactose induced *S. cerevisiae* (pep4) transformants were extracted for enzyme activity analysis using p-nitrophenyl laurate (C12) as the substrate. In this assay, both the intact and truncated forms of *BnDIL1* demonstrated higher lipase activity than the *pYES2* control ($P< 0.05$). Only negligible increase of lipase activity of *BnDIL1'* over *BnDIL1* was observed, which indicated that a signal peptide was probably not included in *BnDIL1*. The *in vivo* lipase activity of *BnDIL1'* on neutral lipids was further confirmed by estimation of the total intracellular neutral lipids content of *S. cerevisiae*, in which sudan black B was applied according to Evansa's method (Evansa and Gilbert, 1985). After 12 hours

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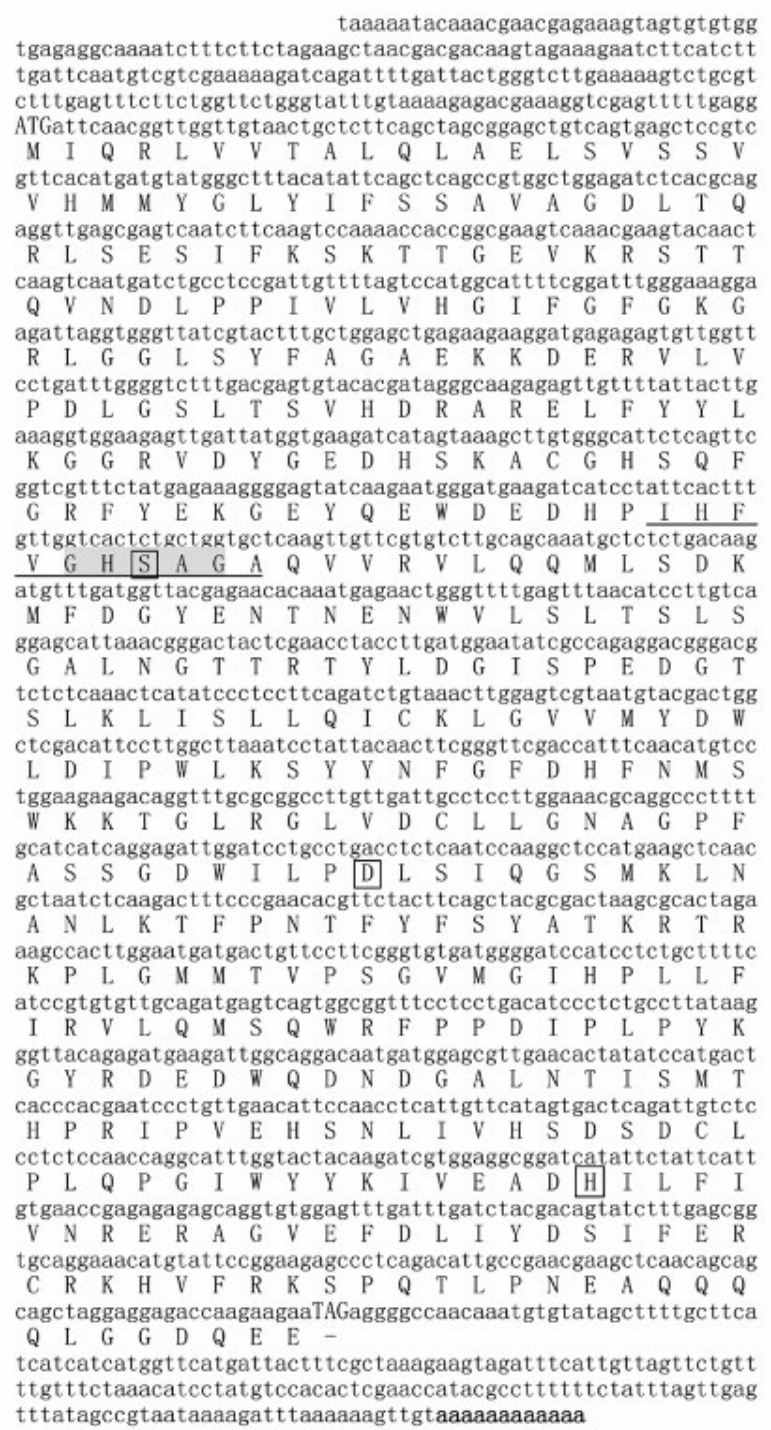

Figure 1. The nucleotide sequence and deduced protein sequence of *BnDIL1*. The nucleotide sequences of the 5' UTR region and the 3' UTR region of *BnDIL1* are shown in front of the uppercased "ATG" and behind of the uppercased "TAG", respectively. The Open Reading Frame (ORF) sequence is from the uppercased start codon "ATG" until the uppercased stop codon "TAG". A poly A sequence is bolded. The typical 10-residue consensus sequence of *GXSXG* type lipase is underlined and the *GXSXG* motif is highlighted with grey shade. The three conserved amino acids Ser^{304}, Asp^{290}, and His^{416} forming the putative catalytic triad "S-D-H" are marked with box.

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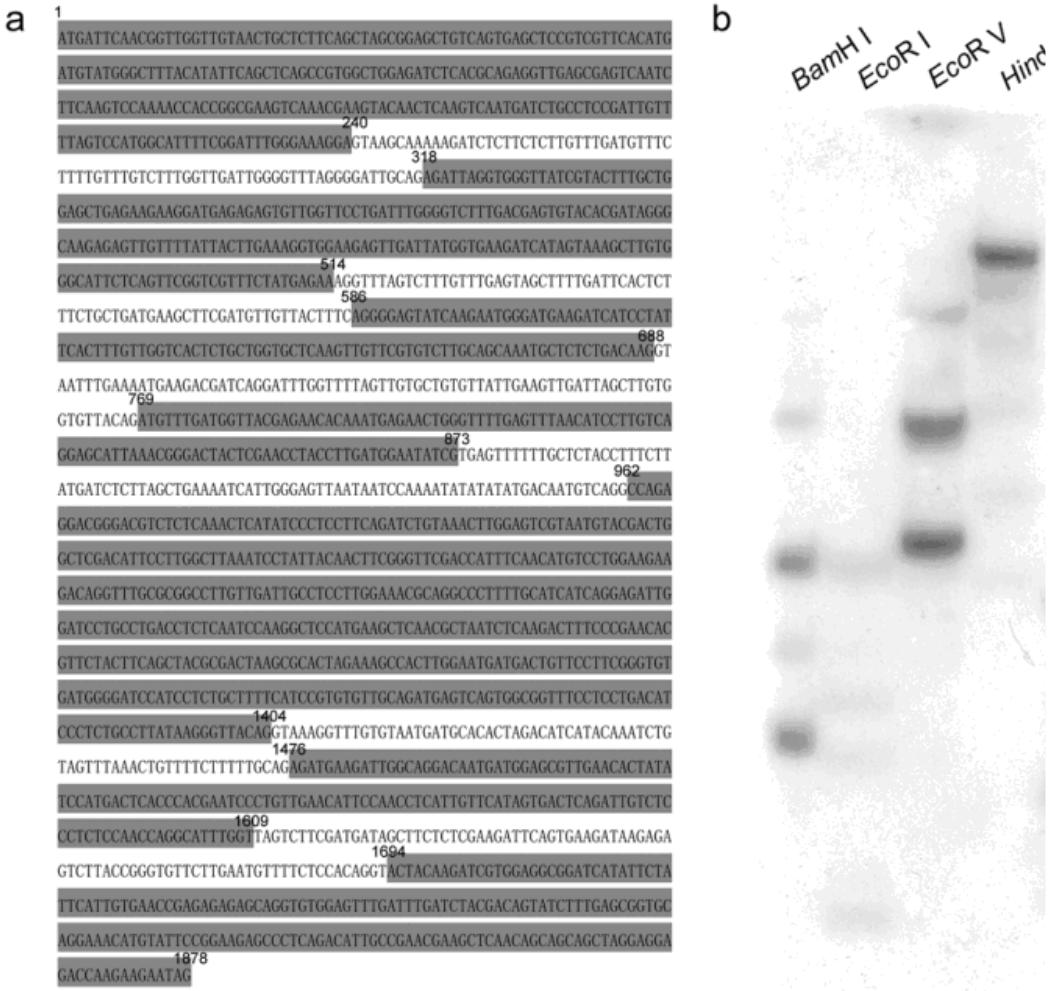

Figure 2. The genomic DNA sequence corresponded to the ORF sequence of *BnDIL1* and southern blot analysis of *BnDIL1*. (**a**) The genomic DNA sequence corresponded to the ORF sequence of *BnDIL1*. The exons are highlighted with grey shade and the introns are shown without grey shade. The numbers above the nucleotides represent the positions of the initial nucleotide and the end nucleotide of each exon. (**b**) Southern blot analysis of *BnDIL1*. Every 15 ug of rapeseed genomic DNA was digested with *Bam*H I, *Eco*R I, *Eco*R V, *Hin*d III, *Xba* I, respectively, and loaded into each lane of 0.6% agarose gel for electrophoresis. After sufficient DNA migration, the digested DNA was transferred to N+ Hybond nylon membranes and hybridized with a [a-^{32}P]-labeled PCR fragment of *BnDIL1*.

induction with galactose, the total intracellular neutral lipid content decreased by more than 14% in *BnDIL1'* transformants compared with the control (Figure 2-b). The *in vivo* lipase activity of *BnDIL1*' on polar lipids was confirmed by 2-D TLC assay. The results showed a significant decrease in polar lipids content in the *BnDIL1'* transformants, which indicated a strong lipase activity of *BnDIL1*' on polar lipids (Figure 3).

The *BnDIL1'* yeast transformants was subjected to Gas Chromatography (GC) for fatty acid composition analysis. We analyzed the contents of 4 main fatty acids of the yeast including C16:0, C16:1, C18:0, and C18:1 (Redon *et al*., 2009), and the results showed that, compared with the control, the amount of fatty acids C16:0 was

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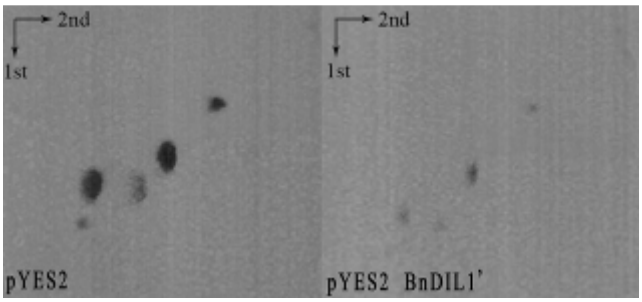

Figure 3. The effect of *BnDIL1* on phospholipids of *S. cerevisiae* (pep4). The change of total phospholipids of *S. cerevisiae* (pep4) was examined by 2D-TLC. *pYES2* represents the control *S. cerevisiae* harboring empty vector *pYES2*; while *pYES2_BnDIL1'* represents the *BnDIL1'* overexpressed *S. cerevisiae*.

reduced by 22.9%, C16:1 by 24.1%, C18:0 by 27.1%, and C18:1 by 29.2%, while the total amount of fatty acids decreased by 25.8%. All the results from the *in vitro* and *in vivo* experiments indicated that *BnDIL1* was a nonspecific lipase as other liplytic acyl hydrolases (Galliard, 1971).

Organ-Specific Expression Analysis of *BnDIL1*

Lipases and their genes can be detected in almost all the tissues, such as root, inflorescence stem, flower, silique, and leaf. To investigate the expression profiles of *BnDIL1* in different plant organs, quantitative real-time PCR analysis was performed with total RNAs isolated from mature leaf, root, flower, and stem of a single six-month-old plant. The organ-specific expression analysis of *BnDIL1* showed that *BnDIL1* expressed in all tissues, and was significantly expressed in mature leaf, flower, and root, but slightly expressed in stem (Figure 4).

Expression Profiles of *BnDIL1* and *ICL* during Seed Morphogenesis, Maturation, Dormancy, Pregermination and Germination

Fatty acids metabolism is quite important for organisms to live normally due to its roles in carbon source and energy supply, membrane repair, oil storage. *ICL* was known as the glyoxysomal marker enzyme, of which the gene expression patterns could reflect the fatty acid metabolic situation. We investigated the fatty acid metabolic situation during seed morphogenesis, maturation, dormancy, pregermination and germination, via examining the transcript levels of *BnDIL1* and *ICL* by quantitative real-time PCR analysis. Two *B. napus* lines EM91 (with low oil content: 29.62%) and EM102 (with high oil content: 50.59%) were used as materials. The expression profiles of *ICL* in both *B. napus* lines showed increasing trend from seed morphogenesis to dormancy, and staying at a high and relatively stable level from seed dormancy

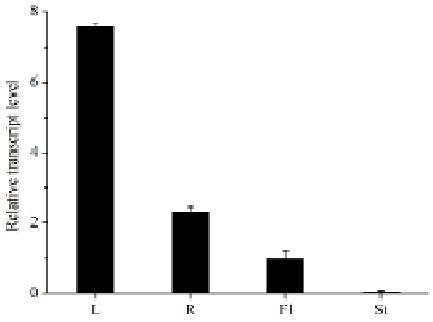

Figure 4. Organ-specific expressions of *BnDIL1* in the Leaf (L), Root (R), Stem (St), and Flower (Fl). The expression profiles of *BnDIL1* in different plant organs were investigated *via* quantitative real-time PCR using total RNAs isolated from mature leaf, root, flower, and stem of a single six-month-old plant as the template. *BnActin* was used as an internal control.

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to germination (Figure 5-a). This result indicated that the degradation of fatty acids was not only essential for seed germination, seed morphogenesis, and maturation, but even for dormancy. Most of the metabolisms are inactive in dormant seeds, but at the dormant stage, the seeds also have basic metabolic activity to prepare the next life cycle, in which the gene *BnDIL1* may be involved. The transcription of *ICL* in seeds of low oil-content line EM91 was observed to be more active than that in high oil-content line EM102 during the reserve accumulation stages from 45 to 55 Days After Pollination (DAP) (Figure 5-a), which suggested faster fatty acids degradation in low oil-content line seeds during seed maturation.

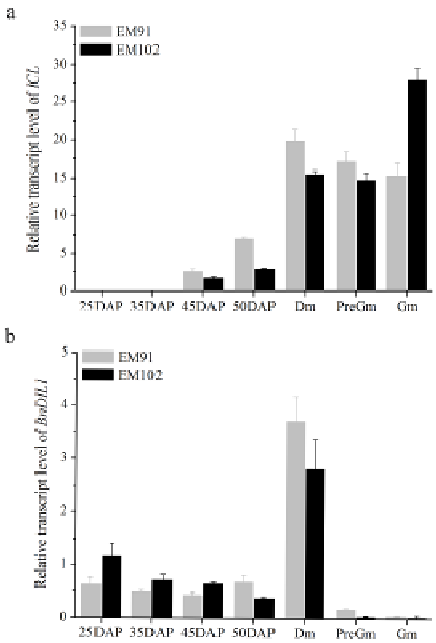

Figure 5. The transcript profiles of *ICL* and *BnDIL1* in seed lines with distinct oil content during the processes of seed morphogenesis, maturation, dormancy, pregermination, and germination. (**a**) The transcript profiles of *ICL* in seed lines with distinct oil content during the processes of seed morphogenesis, maturation, dormancy, pregermination, and germination. (**b**) The transcript profiles of *BnDIL1* in seed lines with distinct oil content during the processes of seed morphogenesis, maturation, dormancy, pregermination and germination. EM91: A *B. napus* line with low oil content (29.62%); EM102: A *B. napus* line with high oil content (50.59%); DAP: Day After Pollination; Dm: Dormancy; PreGm: PreGermination, and Gm: Germination. *BnActin* was used as an internal control.

Seeds maturation of line EM91, with low oil-content, was earlier than that in line EM102 with higher oil content (Tan *et al*., 2011). In both *B. napus* lines, the expression level of *BnDIL1* kept decreasing during seed development until the desiccation started in the maturation stage. Then, seed entered into the dormancy stage in which *BnDIL1* was significantly induced, but when the seeds imbibed water and reached the germination stages latter, the transcription of *BnDIL1* was then dramatically suppressed (Figure 5-b). This result indicated that *BnDIL1* is a desiccation stress inducible gene rather than oil breakdown gene.

The asynchronous transcription profiles of *ICL* and *BnDIL1* during seed morphogenesis, maturation, dormancy, pregermination, and germination suggested that the main role of *BnDIL1* in *B. napus* seed was for desiccation stress response instead of storage oil degradation.

Transcript Profiles of *BnDIL1* in Seedlings under Desiccation Stress

As *BnDIL1* could be detected in all the studied tissues (Figure 4), and expressed higher in desiccation seeds, understanding the transcription response of *BnDIL* of *BnDIL1* towards desiccation stress in other tissues became interesting and necessary. *B. napus* seedlings were chosen as the material for desiccation response test of *BnDIL1* due to the dramatically suppressed transcription of *BnDIL1* in germinated seeds (Figure 5-b). To verify the transcript response of *BnDIL1* towards desiccation stress, desiccation stress treatments including "Slow Desiccation" (SD) and "Fast Desiccation" (FD) were carried out on *B. napus* seedlings. The transcript profiles of *ICL* were quite different between seedlings treated with SD

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stress and FD stress. Compared with the control group that was soaked in water, there was a strong increase in the expression of *ICL* by almost 17-fold under the SD stress, while only a slight decrease in expression of *ICL* under the FD stress (Figure 6-a). Fast desiccation was known as an efficient method and widely used for long-term preservation of food, e.g. fast desiccation was commonly applied in vegetable preservation that desiccated vegetable could be recovered close to the fresh vegetable after having been immersed in water for a few hours (Hawk, 1919). The increased expression of *ICL* in SD group and decreased expression of *ICL* in FD group indicated that the fatty acids degradation was strongly activated in SD, whereas lipid degradation was inhibited under FD. Unlike *ICL*, the expressions of *BnDIL1* in both desiccation stressed groups were induced by 10-fold (Figure 6-b), which indicated that the expression of *BnDIL1* was not related to fatty acids degradation, but depending on the desiccation stress rather than the stress from cell apoptosis. The fast and dramatic transcription response of *BnDIL1* towards the desiccation stress in both seed and seedling suggested the potential role of *BnDIL1* in desiccation stress tolerance of *B. napus*, such as for the life sustaining of dominant seeds under the continuing dry conditions. Desiccation, as well as freezing or high salt, is a kind of osmotic stress inducer, which would affect membrane integrity and functionality. Remodeling of lipid composition and activation of a variety of phospholipid based signaling pathways is known as a common method for plants to survive and adapt to osmotic stress (Munnik and Meijer, 2001). Considering the irrelevant transcription patterns between *ICL* and *BnDIL1*, enzyme activity of *BnDIL1* towards polar lipids, and its desiccation stress dependent manner, *BnDIL1* was assumed to be involved in responding to desiccation stress via a phospholipid based signaling pathway. In addition, ABA was reported to be required for desiccation tolerance of plant (Angelovici *et al*., 2010; Bewley, 1997; Khandelwal *et al*., 2010), and was also involved in the phospholipid based signaling pathway (Meijer and Munnik 2003), which makes it to be very interesting to investigate the relationship between *BnDIL1* and the ABA related phospholipid based signaling pathway, in the future.

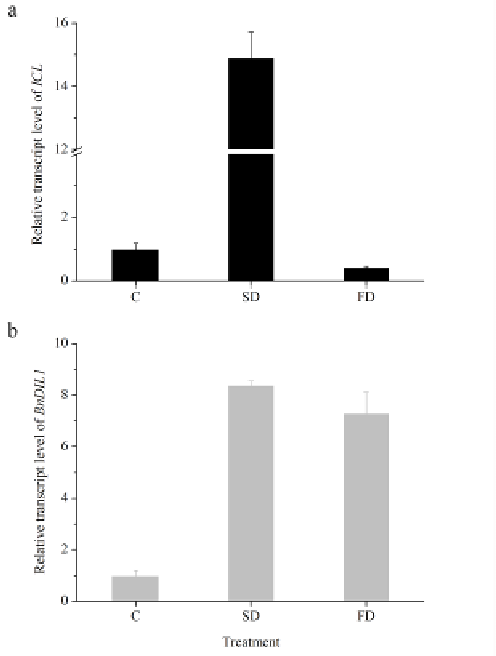

Figure 6. The transcript profiles of *ICL* and *BnDIL1* in seedlings under desiccation stress. (**a**) The transcript profiles of *ICL* in seedlings under both fast and slow desiccation stresses. (**b**) The transcript profiles of *BnDIL1* in seedlings under both fast and slow desiccation stresses. C: The control group in which seedlings were soaked in sterilized water for 2 hours at room temperature; SD: The "Slow Desiccation" stress group, where seedlings were put on the surface of filter papers for 2 hours at room temperature, and FD: The "Fast Desiccation" stress group, where seedlings were embedded into high a concentration of 40% (w/v) PolyEthylene Glycol (PEG) 8000 for 2 hours at room temperature. *BnActin* was used as an internal control.

CONCLUSIONS

The sequence of *BnDIL1* contained the lipase motif, and the *in vitro* and *in vivo* assays confirmed that the *BnDIL1* encoded a

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lipase gene. *BnDIL1* was widely expressed in all the organ, especially in desiccation seeds, and was strongly responsive to the desiccation stress in seedling stage.

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تعیین ویژگی های یک ژن لیپاز القا شده با تنش خشک کردن در کلزا *Brassica napus* L.

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چکیده

لیپازها به عنوان موادی که نقش های مهمی در فرایندهای فیزیولوژیکی گیاهان بازی می کنند شناخته شده اند. در پژوهش حاضر، یک ژن کلزا به صورت *BnDIL1* (به جای *B. napus* Desiccation-Induced Lipase 1) نشان دار شد و با روش (RACE) یا تکثیر سریع (Rapid Amplification) انتهای cDNA همسانه سازی (clone) شد. فعالیت آنزیم لیپاز با تخمین فعالیت لیپاز و کاهش مقدار چربی ها در *Saccharomyces cerevisiae (pep4)* تراریخته شده مورد تایید قرار گرفت. سپس ، برای بررسی رونویسی های *BnDIL1* در طی فرایندهای مورفوژنز بذر، بلوغ و رسیدگی، خفتگی، پیش جوانه زنی، و جوانه زنی بذر، دو لاین *B. napus* که مقادیر روغن آن ها متفاوت بود استفاده شدند. در طی فرایند های بلوغ بذر، خفتگی، پیش جوانه زنی، و جوانه زنی، سطح رونویسی مسیر فساد و تجزیه چربی ها افزایش یافت و در بذر های لاینی که روغن کمتری داشت بیش از لاینی بود که دارای روغن بیشتری بود. با این همه، هنگامی که خشک کردن بذر ها شروع شد، *BnDIL1* به طور معنی داری فعال شد. هر دو تیمار "خشک کردن آرام" و "خشک کردن سریع" گیاهچه ها، به طور بارزی رونویس *BnDIL1* را فعال کردند در حالی که فقط تنش "خشک کردن آرام" که خودکشی یاخته ای (apoptosis) را القا می کند به طور معنی داری رونویسی ژن تجزیه چربی را فعال کرد. این نتیجه نشان داد که در کلزا(*B. napus*)، *BnDIL1* ژن وابسته به تنش خشک کردن بود نه اینکه ژن فساد و تجزیه آمینو اسید ها.

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