# Cloning and analysis of the phytoene synthase (*BnPSY*) gene in *Brassica napus* L.

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Phytoene is an important secondary metabolite in the isoprenoid metabolic pathway in higher plants. It is one of the precursors for the biosynthesis of carotenoids, a group of accessory pigments in the photosynthesis in higher plants. Carotenoids may also protect chlorophyll from damages by high temperatures and strong light. Phytoene synthase (PSY) is a primary rate-limiting enzyme in the biosynthesis of carotenoids (Welsch R et al. 2000). Hence, the activity of phytoene synthase (PSY) affects the synthesis of carotenoids. In order to understand the functions of this enzyme in *Brassica napus* L., the full sequence of the *BnPSY* gene was cloned by RT-PCR and the bioinformatics' characteristics were analyzed in present study.

## 1. Materials and Methods

**1.1 Plant Material** A rapeseed cultivar ZHONGYOU 821 (*Brassica napus* L) was grown on the experimental farm of Sichuan Agricultural University, Ya'an, Sichuan, China and used as the experimental material.

**1.2 Isolation of Total RNA and Total DNA** Total RNA was isolated from the petals of the cultivar ZHONGYOU 821 and purified following the manufacturer's instructions. The total genomic DNA was extracted from young leaves and purified as described by Doyle, et al.(1990)

**1.3 Synthesis of cDNA** Reversed transcription PCR (RT-PCR**)** was performed to synthesize the cDNA fragments from the total RNA. The PCR was conducted with 2 ug total RNA using the TaKaRa RT M-MLV Kit following the manufacturer's instructions. The cDNA products were recovered, purified and stored at -20℃ for use.

**1.4 Amplification of Conserved Sequence of *BnPSY*** A pair of degenerate primers (forward primer: 5'-TGGGGAACTCTGCTGATGACNCCNGA-3', reverse primer: 5'- TGCTAACGTAAGCTCTCTTA GTGAAGTTRTTRTARTC -3') were designed based on the amino acid sequences of phytoene synthase reported in other related species with the software CODEHOP. The conserved sequence of *BnPSY* gene was amplified with 1 μl of the above synthesized cDNA in 50 ul reaction volume using the degenerate primers with the PrimeSTAR® HS DNA Polymerase (TaKaRa, Dalian, China) by a PTC1000 PCR Thermal Cycler (Bio-RAD, USA). The PCR program was 30 cycles of 98℃ for 10 sec, 68℃ for 1 min. The amplified products were fractionated on a 2% agarose gel and stained with Glod-View (a substitute of ethidium bromide). The band with the expected size was excised out and DNA fragments were recovered and purified using the AxyPrep DNA Gel Extraction Kit.

**1.5 Cloning of the Full Length Sequence of *BnPSY* and Analysis of Sequence** The amplified conserved sequence of *BnPSY* was cloned into the pMD®19-T Vector and then transformed into the *Escherichia coli* DH5α competent cells. The DNA insert in the recombinant clones was amplified by PCR with the designed degenerate primers used above, and then were sequenced in both directions by the Invitrogen Life Technologies Co. LTD (Shanghai, China).

The 3' and 5' RACE amplifications were performed using the GeneRacer™ Kit and the prducts were sequenced by the Invitrogen Life Technologies Co. LTD (Shanghai, China). The full length cDNA sequence of *BnPSY* gene was obtained by the electronic assembly of the 3' RACE, 5' RACE and the amplified conserved sequence of *BnPSY* using the DNAStar software. The open read frame (ORF) was predicted by the ORFFinder program (http://www.ncbi.nlm.gov/gorf/gorf.html)( Henikoff S et al. 1994). Based on the assembled full length sequence of the *BnPSY* gene, a new pair of primers (PSYF: 5'-CCGGATCCATGTCTTCTGTAGCAGTGTTATG-3' and PSYR: 5'- GCGAATTCAGTT GTTCCTCTTGAACTTGGAG-3') were designed. The pair of new primers was used to amplify the full length sequence of *BnPSY* from both the synthesized cDNA and the isolated genomic DNA fragments. The PCR amplifications were performed using an initial denaturation at 98℃ for 10 sec, followed by 30 cycles of 98℃for 10 sec, 55℃for 5 sec and 72℃ for 3 min, and with a final extension at 72℃ for 10 min. The amplified cDNA and DNA fragments were sequenced by the Invitrogen Life Technologies Co. Ltd. (Shanghai, China), and their biological information was analyzed. The homologous amino acid sequences in other related species were searched by BlastP from the non-redundant protein sequences (nr) database on the NCBI (http: // www. ncbi. nlm. nih. gov)( Shen B et al. 2002). A phylogenetic tree was constructed using the MEGA version 3.1 by the Neighbor-Joining method ( Kumar S et al. 2004) .

## 2. Results and Discussion

### 2.1 Full Length cDNA and DNA Sequences of *BnPSY* Gene

A 388 base pair (bp) fragment of the conserved sequence was obtained with the degenerate primers (Figure 1, lane 1). A fragment of 1496 bp (Figure 1, lane 2) was obtained by the 3' RACE, and a fragment of 1220 bp (Figure 1, lane 3) by the 5' RACE. The full length cDNA sequence of *BnPSY* gene obtained by the electronic assembly of the 3' RACE, the 5' RACE, and the degenerate primer amplified sequences was 1838 bp (GenBank accession number, HQ260432). The full length cDNA sequence of *BnPSY* obtained from the cDNA sample by the primer pair PSYF and PSYR was same in length (Fig. 1, lane 4). The full length genomic DNA sequence of *BnPSY* (GenBank accession number, HQ260433) was 2085 bp (Fig. 1, lane 5).

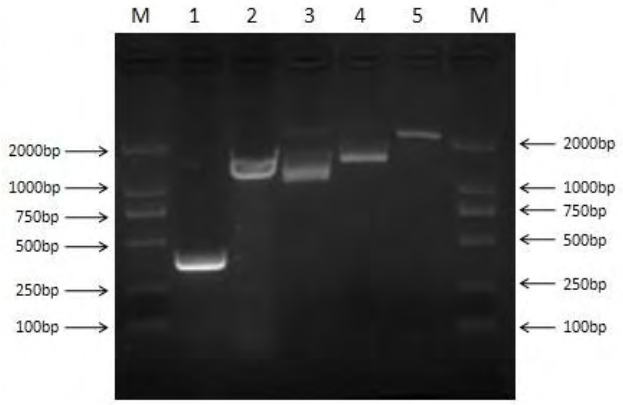


Figure 1 The electrophoresis of the 3' and 5' RACE's and the full length PCR products on 2% agarose gel. Lane 1: 3' RACE fragment; lane 2: 5' RACE fragment; lane 3: the full length cDNA sequence of *BnPSY*; Lane 4: the full length DNA sequence of *BnPSY*; lane M: DNA marker DL2000.

### 2.2 Sequence Analysis of *BnPSY*

The full length cDNA sequence of phytoene synthase in *Brassica napus L.*, denoted as *BnPSY* (GenBank access number HQ260432), was 1838 bp in length, including a 1275bp ORF (open reading frame), a 355 bp 5'UTR (untranslated region) and a 208 bp 3'UTR. The ORF encodes a peptide of 424 amino acid residues (Figure 2). The full length DNA sequence of phytoene synthase (GenBank access number HQ260433) was 2085 bp in length, containing 6 exons and 5 introns (Figure 3).

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1       aaaactaaaacttctggagaattttcggacactaacgggtcaaaccttttacttgtcgtttgattaaattaattgtt
79      tgaaaatctccttcttcctcctcctcatcagtctcaccgtgtctgttcttagatagagagatagagcgagcgaaaggt
157     tagctatttcactgttctgctactgtcgtgctctttttggggtgttcatttctatacattcagatgataaccacgac
235     gaaacgaataagcattactgtttaaccgcaaagttgggattttgattcgtcttattgaggaaaaaaacagagtaaaca
313     aaaagttgagatttttccacctctcatctcaagaagctcgagtATGTCTTCTGTAGCAGTGTTATGGGTTGCTTCCTC
1                                                   M  S  S  V  A  V  L  W  V  A  S  S
391     TTCTCCTAATCCAGACCAAATGACCACTTGTGGGTTGGTAAGGGCTGTAGAATCTTCTAGAGTGCTCGCTCGTTGTCA
13        S  P  N  P  D  Q  M  T  T  C  G  L  V  R  A  V  E  S  S  R  V  L  A  R  C  Q
469     GAATCAGAGAATGGACAATGGTAGGAGGAAGCAAACAACAAAAACGTGGACCTCTTCTTCTTCTTCCTCTCTAATGAG
39        N  Q  R  M  D  N  G  R  R  K  Q  T  T  K  T  W  T  S  S  S  S  S  S  S  L  M  S
547     CTACAGGAGGAGTGTTGTGTCTTCGAGCGTAGTAGCAAGTCATGCAGGAGAGATTGCCCTCTCATCCGAAGAGAAGGT
65        Y  R  R  S  V  V  S  S  S  V  V  A  S  H  A  G  E  I  A  L  S  S  E  E  K  V
625     TTACAACGTTGTGCTTAGACAAGCGGCTTTGGTGAACAAACAGCTCAGGTCTACTTCTCCTGAACTTGATGATGTGAA
91        Y  N  V  V  L  R  Q  A  A  L  V  N  K  Q  L  R  S  T  S  P  E  L  D  D  V  K
703     GAAACCACAGGATATTGTTCTCCCTGGGAGTTTGAGTTTGTTGGGTGAAGCTTATGATCGTTGCGGCGAAGTTTGCGC
117       K  P  Q  D  I  V  L  P  G  S  L  S  L  L  G  E  A  Y  D  R  C  G  E  V  C  A
781     TGAATATGCTAAAACCTTTTATCTCGGAACTTTGCTTATGACACCTGAGAGGCGAAAGGCCATTTGGGCTATCTACGT
143       E  Y  A  K  T  F  Y  L  G  T  L  L  M  T  P  E  R  R  K  A  I  W  A  I  Y  V
859     TTGGTGCAGAAGAACTGATGAGCTCGTAGATGGGCCCAATGCATCACACATAACACCCATGGCGTTAGATAGATGGGA
169       W  C  R  R  T  D  E  L  V  D  G  P  N  A  S  H  I  T  P  M  A  L  D  R  W  E
937     AGCAAGGTTAGAAGATCTATTCCGTGGCCGTCCTTTCGATATGCTTGATGCTGCTCTCGCTGATACCGTTGCTAGATA
195       A  R  L  E  D  L  F  R  G  R  P  F  D  M  L  D  A  A  L  A  D  T  V  A  R  Y
1015    CCCTGTTGATATTCAGCCATTTAGAGACATGATCGAAGGAATGAGAATGGATTTGAGGAAGTCCAGATACAAGAACTT
221       P  V  D  I  Q  P  F  R  D  M  I  E  G  M  R  M  D  L  R  K  S  R  Y  K  N  F
1093    TGATGATCTCTACCTTTACTGCTACTATGTAGCCGGAACCGTCGGTTTGATGAGCGTTCCGGTTATGGGAATCGATCC
247       D  D  L  Y  L  Y  C  Y  Y  V  A  G  T  V  G  L  M  S  V  P  V  M  G  I  D  P
1171    TAAGTCCAAAGCAACGACAGAGAGTGTTTACAACGCTGCCTTGGCTCTCGGTATAGCTAATCAGCTTACCAACATACT
273       K  S  K  A  T  T  E  S  V  Y  N  A  A  L  A  L  G  I  A  N  Q  L  T  N  I  L
1249    CAGAGACGTTGGCGAAGACGCGAGAAGAGGAAGAGTTTATCTGCCCCAAGATGAGTTAGCTCAAGCTGGTCTCTCAGA
299       R  D  V  G  E  D  A  R  R  G  R  V  Y  L  P  Q  D  E  L  A  Q  A  G  L  S  D
1327    TGAAGACATATTCGCGGGAAAAGTCACTGACAAATGGAGAAACTTCATGAAAATGCAGCTTAAGCGAGCAAGAATGTT
325       E  D  I  F  A  G  K  V  T  D  K  W  R  N  F  M  K  M  Q  L  K  R  A  R  M  F
1405    CTTCGACGAAGCTGAGAAAGGCGTTACCGAGCTCGACGCTGCTAGTAGATGGCCGGTATGGGCGTCGCTCCTATTGTA
351       F  D  E  A  E  K  G  V  T  E  L  D  A  A  S  R  W  P  V  W  A  S  L  L  L  Y
1483    CAGGAGAATATTGGACGAGATTGAAGCGAATGATTACAACAACTTTACAAAGAGAGCTTATGTTGGGAAAGCCAAGAA
377       R  R  I  L  D  E  I  E  A  N  D  Y  N  N  F  T  K  R  A  Y  V  G  K  A  K  K
1561    AATTGCAGCTCTGCCATTGGCTTATGCTAAATCAGTACTAAAGACTCCAAGTTCAAGAGGAACAACTTAAgaggcttt
403       I  A  A  L  P  L  A  Y  A  K  S  V  L  K  T  P  S  S  R  G  T  T  *
1639    tgtgattaaaggaaaagcttagggtcgaatttatgatgttaattaatatatacatataaacggccaaatgaaattc
1717    ttatttctctatgtaaaaagtaaatttttttattttatttctcttggctactgaagaaataggaatattttggttggag
1795    agctagttttgtgatttataaaaagaagttactgatttcaaaaa
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Figure 2 Nucleotide and deduced amino acid sequence of B*nPSY*.

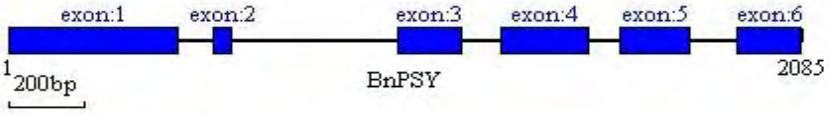


Figure 3 the composition of exons and introns of *BnPSY*

### 2.3 The Primary and Secondary Structures of Phytoene Synthase

The amino acid sequence was analyzed with the software DNAstar. It was predicted that the primary structure of PSY protein was composed of all the 20 kinds of amino acids, including 59 strong alkaline amino acids (K+R), 51 strong acidic amino acids (D+E), 150 hydrophobic amino acids (A+I+L+F+W+V) and 109 polar amino acids (N+C+Q+S+T+Y). with a molecular weight of 47.63 k Dalton and an isoelectric point of 8.905. It was indicated that the protein of phytoene synthase was a hydrophobic peptide with more hydrophobic amino acids than polar amino acids.

The predicted secondary structure of PSY protein contained 61.79% alpha helix (262 AA), 5.66% outspread (24 AA), 3.30% beta corner (14 AA) and 29.25% random coil (124 AA). Alpha helix and

random coil had the largest quantities in the structural components. The outspread chains and beta corners were scattered along the whole protein molecule.

### 2.4 Analysis of the Homology of *BnPSY* gene

An alignment of the deduced amino acids sequence of *BnPSY* was analyzed with the BLAST procedure. (Liyuhua et al. 2006; Sunxiao et al. 2005; Zhongyang et al. 2003) It was shown that the deduced amino acid sequence of Phytoene synthase from *Brassica napus* L. was 94% homologous with that from *Brassica rapa* (FJ227935), 84% from *A. thaliana* (L25812), 65% from *Narcissus tazetta* (DQ984674), and 62% from *Oryza sativa* (AY445521) (Fig.4). It was suggested that the cloned sequence was the full length sequence of *PSY* gene in *Brassica napus* L.. The successful cloning of this gene may provide a basis for further studies on the metabolic activities and functions of Phytoene synthase (PSY) in *Brassica napus* L..

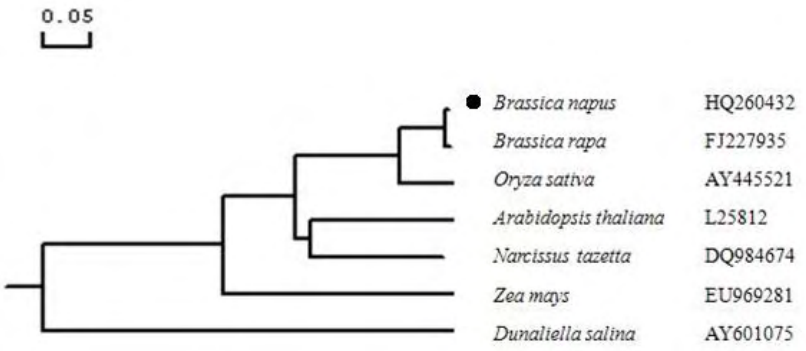


Figure 4 Phylogenetic relationship based on the amino acid sequence comparisons of *BnPSY*. GenBank accession numbers are shown followed the species.

**Acknowledgments** This work was financially supported by the China National High-tech “863” Program（2009AA101105）and the Chongqing City Natural Science Fund Program（CSTC2009BA1088）