African Journal of Biotechnology Vol. 7 (20), pp. 3577-3581, 20 October, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Cloning and characterization of a cDNA encoding phytoene synthase (*PSY*) in tea

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Accepted 9 September, 2008

Carotenoids are an important group of precursors of volatile flavour compounds in tea and phytoene synthase (PSY) is a key enzyme during the biosynthesis pathway of carotenoids. A cDNA encoding PSY in tea shoot [*Camellia sinensis* (L.) O. Kuntze] was cloned and sequenced in the present study. The obtained tea PSY cDNA was 1296 bp with an open reading frame of 987 bp. The predicted protein displayed a sequence of 329 amino acids. The phylogenetic tree showed that the tea *PSY* was clustered closely to the *PSY* of *Adonis palaestina*, with an overall amino acid identity of 90%.

Key words: *Camellia sinensis*, carotenoids, phytoene synthase, gene cloning, sequencing, phylogenetic tree.

INTRODUCTION

The expression of phytoene synthase (PSY) gene was reported to have high correlation with the carotenoids accumulation in tomato fruit (Giuliano et. al., 1993), citrus (Ikoma et al., 2001) and pepper fruit (Romer et al., 1993). Seed from PSY transgenic *Brassica napus* contained up to a 50-fold increase in carotenoids (Shewmaker et al., 1999). In tea, a close correlation of the expression pattern of PSY gene with the carotenoids accumulation has been observed (Borthakur et al., 2008). Carotenoids are an important group of precursors of volatile flavour compounds in tea (Ravichandran, 2002). Thus the study of PSY gene including its sequencing and expression patterns should be helpful to understand the regulation mechanism of carotenoids biosynthesis. In addition, complete sequencing of the PSY gene is required for designing a STS (sequence tagged sites) marker that can be used to screen germplasm with regard to high carotenoid content since the level has a high correlation to the tea flavour attributes.

A PCR based method was adopted to clone a cDNA encoding PSY from the actively growing tea shoot.

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MATERIALS AND METHODS

Cloning of cDNA encoding PSY

Actively growing shoots with two leaves and a bud from *Camellia sinensis cv.* Longjing-43 were harvested from the Experimental Tea Farm of Zhejiang University (Hangzhou, China) in the early summer in 2007 and kept at -80℃ for total RNA extraction. Total RNA was extracted using TRIZOL reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) as described by Mamati et al. (2005). RACE primers (Table 1) for cloning *PSY* cDNA were designed using the Lasergene Primer Select software (DNAStar, Madison WI, USA) based on the published PSY sequence (GanBank accession No. EF545005). The 5'-RACE and 3'-RACE were performed using 5'-Full RACE Core Set Ver. 2.0 and 3'-Full RACE Core Set Ver. 2.0 (TAKARA Biotechnology (Dalian) Co., Ltd.) according to manufacturer's instruction, respectively. The PCR products of the "outer PCR" were then pooled and 2 µL was used for the 'inner PCR'. The obtained sequences were then combined to construct the full length cDNA using the SeqMan software (DNAStar, Madison WI, USA).

Construction of phylogenetic tree

The amino acid sequences of the *PSY* proteins reported from other plant species were obtained from the public database NCBI and multiple alignments was performed by clustalW (Thompson et al., 1994). The out put of the multiple alignment was then used to construct the phylogenetic tree. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The evolutionary distances were computed using the Kimura 2-

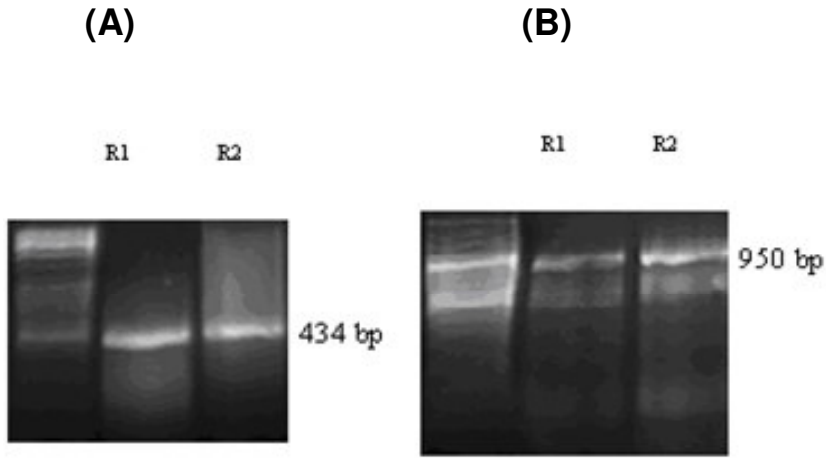

Figure 1. Result of normal PCR (A) using PSY gene specific primers and RACE (B) of the amplification of a cDNA encoding phytoene synthase (*PSY*) in tea.

Table 1. RACE primer pairs for the amplification of a cDNA encoding phytoene synthase (*PSY*) of tea.

Primer	Oligonucleotide sequence
3'-Outer primer	TCAGGGATGTTGGAGAAGAT
3'-Inner primer	AGGGCTTTCAGATGAGGACAT
5'-Outer primer	GGCTGAATATCAACGGGAA
5'-Inner primer	AGCAGCATCGAGCATATCAA

parameter method (Kimura, 1980). Phylogenetic analyses were conducted using MEGA4 (Tamura et al., 2007). The presence of the chloroplast transit peptide sequence was analyzed by chloroplast targeting signal recognition program ChloroP (Emanuelsson et al., 1999)

RESULTS AND DISCUSSION

A PCR fragment of 434 bp was obtained by using PSY gene specific primers as described in our previous paper (Borthakur et al., 2008) and is presented in Figure 1A. 5' RACE was performed and a *PCR* amplicon of 950 bp was obtained (Figure 1B). The overlapping sequence was removed and a sequence of 1296 bp was finally recorded. The *PSY* nucleotide sequence was compared with the *PSY* sequences reported from other species using BLASTn program. A high degree of similarity was found with *PSY* genes from *Carica papaya* (84%, DQ 666828), *Citrus sinensis* (84%, AY 669084), *Prunus mumu* (82%, AB 253628), *Coffea* (81%, DQ157164) and *Adonis (*79%, AY 61705).

The obtained length of the cloned PSY cDNA from tea plant was 1296 with an open reading frame of 987 bp and a 5'-UTR of 307 bp. The predicted protein displayed a sequence of 329 amino acids (Figure 2) with a calculated molecular mass of 37.5 kDa. However, the poly A tail or 3'-UTR that may present in the predicted *PSY* sequence was not cloned. The 3'-UTR or the poly A tail may be very short in this gene or the PCR fragments used to design the 3' RACE primers may represent almost the whole of the 3' end of the gene.

Table 2. ChloroP (V1.1) prediction results of the conceptual translation of the obtained cDNA encoding phytoene synthase (*PSY*) of tea.

Name	Length	Score	cTP	CS-Score	cTP length
PSY (Tea)	428	0.545	Y	1.832	56

Analysis of the conceptual translation of the obtained cDNA with the ChloroP indicated the presence of chloroplast transit peptide (cTP) (Table 2) of 56 amino acid. Since the product of this gene is expected to be chloroplast targeted, the plastid targeting of this enzyme will have to be experimentally confirmed.

The phylogenetic tree showing the evolutionary relation of tea *PSY* with the *PSY* genes from the other plant species was presented in Figure 3. It demonstrated that the tea *PSY* was closely related to the *PSY* of *Adonis palaestina*. However, this PSY cDNA was found to distantly related with the *PSY* from *Dunaliella* (73%) and *Chlamydomonus* (66%).

The degradation products of carotenoids in tea were β-Ionone, α-ionone, β-damascone theaspirone, 4-oxo-beta-ionone, dihydroactinodiolide, 4-oxoisophorone, safranal, β-cyclocitral etc. External addition of natural carotenoid to the cut *dhool* during tea manufacturing would increase the flavour compounds of carotenoids origin, resulting in improvement of quality parameters of tea (Ravichandran, 2002; Sanderson and Graham, 1973). Flavoursome black tea was reported to be produced from green leaf with high carotenoids (Ravichandran, 2002). Moreover, carotenoids in photosynthetic tissue have functions both in the acquisition of light energy and in the

GGA AAG ATC CCT TTC TTT GAG TCT TCT ACT TGT ATA CAT GCG ACT ATA CAA
AAT TTT GCT TTT AGG GGT TTT GGT TTT TTC TCC AGA AAA CAG AGT TTG TTT
TCC TGT TCA AGA CCT CAA TAC CTC TTC TTC AAC TGT AGT ATT TTG ATT TGG
TTT TTG GGA TTT ACT AAA CTT TCC CTC AAA AAG GAA CCT GGG TTT TGC TTG
AAA GTG GAA AAA AAC CAG TGG GTT GTT TGT AAT TTA ACC ATT TAC CGC AAG
GGA AAA GGA GTA GAT AAT TTC AAA AGT GCT TTC GTT TTC AAG TTC ATC
ATG TCT GCA GCT CTG TTA TGG GTT GTT TCG CCC AAT TCT GAG GTC TCT AGT
M S A A L L W V V S P N S E V S S
GGG TTC GGA TTC TTA GAA TCT GTC CGA GAA GGA AAC AGT CTC TTA GAT TCA
G F G F L E S V R E G N S L L D S
TCC AAA TTC AGC CCT AGA GAG AGG ACT TTG ATT TGC CAT GGC AGA TTC AAA
S K F S P R E R T L I C H G R F K
AAG TCA AGA AAC AAA GCT ACA AGA TAT AGA AGA AAG GCA ATT TTC CTG TAC
K S R N K A T R Y R R K A I F L Y
TTT CAA GCA TGG TTG CAA ACC CTG CTG GAG AAT TGG CAA TCA CAT CTG AAC
F Q A W L Q T L L E N W Q S H L N
AAA AGG TTT ACG ATG TTG TTT TTG AAA CAA GCT GCG TTA GTT AAG AAA CAC
K R F T M L F L K Q A A L V K K H
CTC AAA TCT GAA GAG GAG TAT GAT GTG AAA CCT GAT ATT GTT CTT CCG GGG
L K S E E E Y D V K P D I V L P G
ACT TCG AGC TTG TTG AGC GAG GCC TAT GAC CGG TGT GGC GAA GTT TGT GCA
T S S L L S E A Y D R C G E V C A
GAG TAT GCA AAG ACA TTT TAC TTG GGA ACG CTG CTA ATG ACG CCC GAG AGG
E Y A K T F Y L G T L L M T P E R
CGA AGA GCT ATC TGG GCA ATA TAT GTG TGG TGT AGG AGG ACA GAT GAG CTC
R R A I W A I Y V W C R R T D E L
GTT GAT GGG CCT AAT GCG TCA CAC ATA ACT CCT ACA GCT TTA GAC CGA TGG
V D G P N A S H I T P T A L D R W
GAA TCT AGA CTT GAA GAT CTT TTT CGA GGA AGG CCA TTT GAT ATG CTC GAT
E S R L E D L F R G R P F D M L D
GCT GCT TTG TCA GAT ACG GTT ACA AAG TTT CCC GTT GAT ATT CAG CCA TTT
A A L S D T V T K F P V D I Q P F
AAA GAT ATG ATA GAA GGA ATG AGA TTG GAC CTG AAG AAG TCT AGA TAC AAG
K D M I E G M R L D L K K S R Y K
AAC TTT GAT GAA TTA TAT CTC TAC TGT TAC TAT GTG GCC GGG ACT GTC GGA
N F D E L Y L Y C Y Y V A G T V G
TTG ATG AGT GTT CCG GTT ATG GGA ATT GCG CCT GAA TCT CAG GCG ACA ACA
L M S V P V M G I A P E S Q A T T
GAG AGC GTC TAT AAT GCG GCC TTG GCT TTA GGG ATT GCG AAT CAG CTG ACC
E S V Y N A A L A L G I A N Q L T
AAC ATT CTC AGG GAT GTT GGA GAA GAT GCC AGA AGA GGA AGG GTA TAC CTA
N I L R D V G E D A R R G R V Y L
CCA CAA GAT GAA TTG GCA CAG GCA GGG CTT TCA GAT GAG GAC ATA TTT GCA
P Q D E L A Q A G L S D E D I F A
GGA AAA GTA ACA GAG AAA
G K V T E K

Figure 2. Nucleotide sequence and deduced amino acid sequence of the putative *PSY* gene from tea (*Camellia sinensis*). The 5' UTR underlined.

protection of photosynthetic apparatus against excessive light damage (Demmig-Adams et al., 1996) and albino tea shoots induced by strong light illumination might be connected with its lacking of the photo-protection pigments carotenoids (Du et al., 2006; Du et al., 2008). The present paper revealed the sequence of tea PSY gene, a key gene during carotenoids biosynthesis pathway. It will be of significance for the further studies of these subjects.

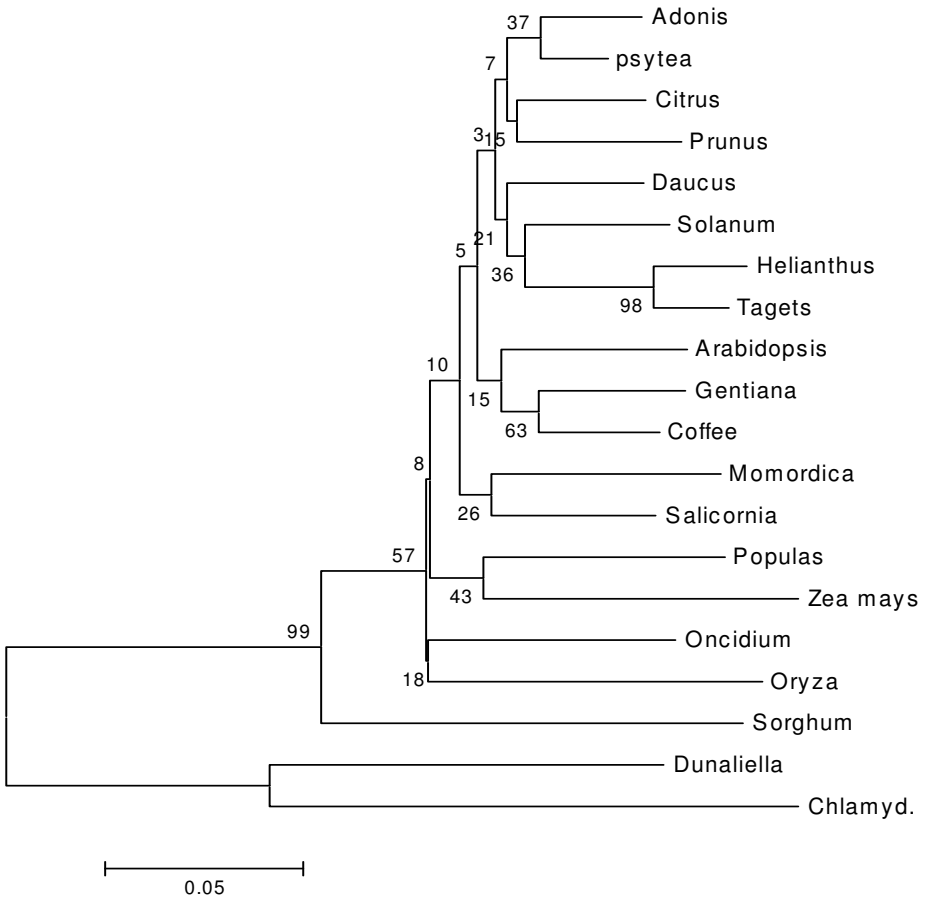

Figure 3. Phylogenetic relationship of the phytoene synthase gene from tea (*Camellia sinensis*; psytea) with those from the other species. The protein sequences used to construct the tree and accession number are: *Adonis* (AAV74394.1), *Arabidopsis* (AAM62787.1), *Chlamydomonas* (XP_001701192.1), *Citrus* (AAF33237.1), *Coffee* (ABA43898.1), *Daucus* (ABB52068.1), *Dunaliella* (ABY50091.1), *Gentiana* (BAE45299.1), *Helianthus* (CAC19567.1), *Momordica* (AAR86104.1), *Oncidium* (AAX84686.1), 1*Oryza* (AAS18307.1), *Populas* (CAI63877.1), *Prunus* (BAF49052.1), *Salicornia* (AAX19898.1), *Solanum* (ABU40771.1), *Sorghum* (AAW28997.1), *Tagets* (AAM45379.1) and *Zea mays* (AAS02284.1).

ACKNOWLEDGEMENT

The present study was financially supported by the National Science Foundation of China (project No.30771374).

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