

## Full Length Research Paper

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

D. Borthakur, Y. Y. Du, H. Chen, J. L. Lu, C. Lin, J. J. Dong, J. H. Ye, X. Q. Zheng and Y. R. Liang\*

Zhejiang University Tea Research Institute, 268 Kaixuan Road, Hangzhou 310029, China.

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.

## MATERIALS AND METHODS

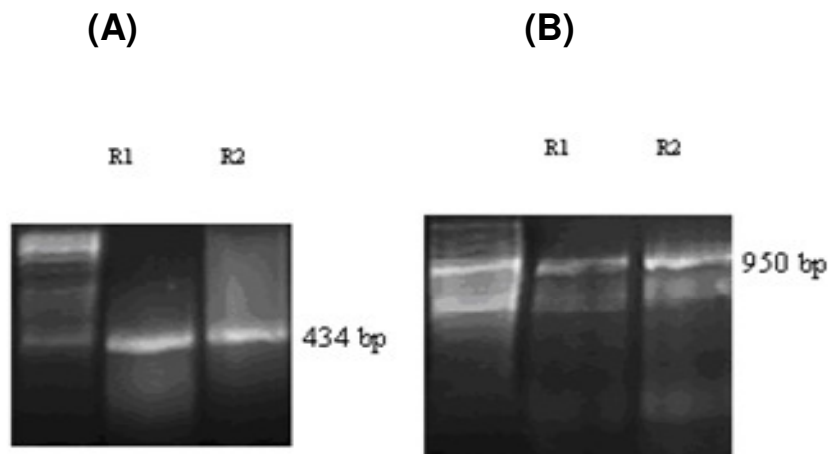
### Cloning of cDNA encoding PSY

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°C 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 (DNASar, Madison WI, USA) based on the published PSY sequence (GenBank 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 (DNASar, 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-

\*Corresponding author. E-mail: yrliang@zju.edu.cn. Fax: +86 571 86971704.



**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	AGGGCTTTTCAGATGAGGACAT
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

**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
<i>PSY</i> (Tea)	428	0.545	Y	1.832	56

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.

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 *Chlamydomonas* (66%).

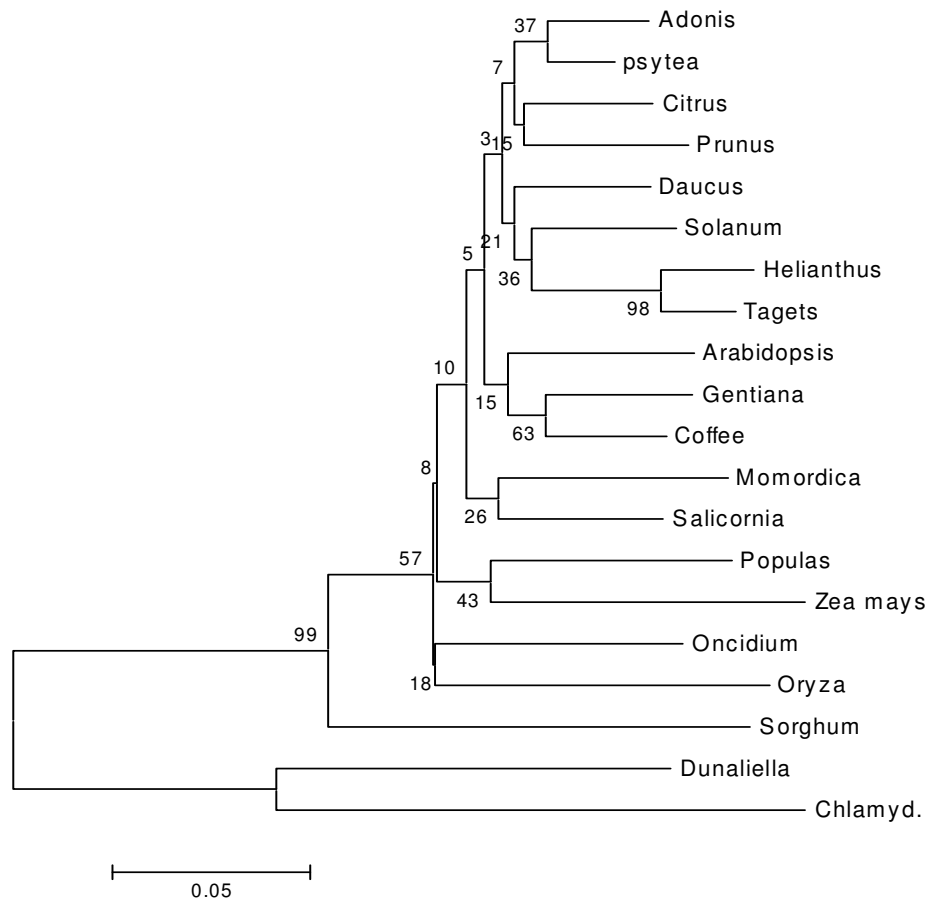
The degradation products of carotenoids in tea were  $\beta$ -ionone,  $\alpha$ -ionone,  $\beta$ -damascone theaspiron, 4-oxo-beta-ionone, dihydroactinodioid, 4-oxoisophorone, safranal,  $\beta$ -cyclocitral etc. External addition of natural carotenoid to the cut *dhool* during tea manufacturing would 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

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

**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), *Oryza* (AAS18307.1), *Populus* (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).

## REFERENCES

- Borthakur D, Lu JL, Chen H, Lin C, Du YY, Liang YR (2008). Expression of phytoene synthase (psy) gene and its relation with accumulation of carotenoids in tea [*Camellia sinensis* (L) O Kuntze]. *Afr. J. Biotechnol.* 7: 434-438.
- Demmig-Adams B, Gilmore AM, Adams WW, III (1996). *In vivo* functions of carotenoids in higher plants. *FASEB J.* 10:403-412.
- Du YY, Chen H, Zhong WL, Wu LY, Ye JH, Lin C, Zheng XQ, Lu JL, Liang YR (2008). Effect of temperature on accumulation of chlorophylls and leaf ultrastructure of low temperature induced albino tea plant. *Afr. J. Biotechnol.* 7: 1881-1885.
- Du YY, Liang YR, Wang H, Wang KR, Lu JL, Zhang GH, Lin WP, Li M, Fang QY (2006). A study on the chemical composition of albino tea cultivars. *J. Hort. Sci. Biotech.* 81: 809-812.
- Emanuelsson O, Nielsen H, Heijne GV (1999). ChloroP, a neural network-based method for predicting chloroplast transit peptides and their cleavage sites. *Prot. Sci.* 8: 978-984.
- Giuliano G, Bartley GE, Scolnik PA (1993). Regulation of carotenoid biosynthesis during tomato development. *Plant Cell.* 5: 379-387.
- Ikoma Y, Komatsu A, Kita M, Ogawa K, Omura M, Yano M, Origuchi T (2001). Expression of phytoene synthase gene and characteristic carotenoid accumulation during citrus fruit development. *Physiol. Plant.* 111: 232-238.
- Kimura M (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16: 111-120.
- Mamati GE, Liang YR, Lu JL (2005). Expression of basic genes involved in tea polyphenol synthesis in relation to accumulation of catechins and total tea polyphenols. *J. Sci. Food Agric.* 86: 459-464.
- Ravichandran R (2002). Carotenoid composition, distribution and degradation to flavour volatiles during black tea manufacture and the effect of carotenoid supplementation on tea quality and aroma. *Food Chem.* 78: 23-28.
- Romer S, Huguency P, Bouvier F, Camara B, Kuntz M (1993). Expression of genes encoding the early carotenoid biosynthetic enzyme in *Capsicum annum*. *Biochem. Biophys. Res. Commun.* 196: 1414-1421.

- Saitou N, Nei M (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4: 406-425.
- Sanderson G, Graham HN (1973). On the formation of black tea aroma. *J. Agric. Food Chem.* 21: 576-585.
- Shewmaker CK, Sheehy JA, Daley M, Colburn S, Ke DY (1999). Seed-specific overexpression of phytoene synthase: increase in carotenoids and other metabolic effects. *Plant J.* 20: 401-412.
- Tamura K, Dudley J, Nei M, Kumar S (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* 24: 1596-1599.
- Thompson JD, Higgins DG, Gibson TJ (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22: 4673-4680.