Journal of Medicinal Plants Research Vol. 6(10), pp. 1997-2003, 16 March, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.1703
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

cDNA cloning, sequence characterization and expression analyses of a novel gene-*TPI* from pepper cytoplasmic male sterility (CMS)

Ming-hua DENG[1,2], Jin-fen WEN[3], Jin-long HUO[4], Hai-shan ZHU[2], Xiong-ze DAI[1], Zhu-qing ZHANG[1], Hui ZHOU[2] and Xue-xiao ZOU[1]*

[1]Institute of Vegetable Crops, Hunan Academy of Agricultural Science, Changsha 410125, China.
[2]College of Horticulture and Landscape, Yunnan Agricultural University, Kunming 650201, China.
[3]Faculty of Modern Agricultural Engineering, Kunming University of Science and Technology, Kunming 650224, China.
[4]Faculty of Animal Science and Technology, Yunnan Agricultural University, Kunming 650201, China.

Accepted 10 February, 2012

The complete coding sequence (CDS) of the gene triose phosphate isomerase (*TPI*) was amplified using the reverse transcriptase polymerase chain reaction based on the conserved sequence information of some Solanaceae plants and known highly homologous pepper ESTs. The results showed the pepper *TPI* gene encodes a protein of 254 amino acids that has high homology with the proteins of six other species, *Solanum tuberosum* (95%), *Arabidopsis thaliana* (87%), *Ricinus communis*, *Vitis vinifera* and *Oryza sativa* (87%) and *Zea mays* (85%). The pepper *TPI* gene was expressed in root, stem, leaf, flower, pericarp, placenta and seed. At the abortion stages, the ATP and NADH pool in male-sterile line were distinctly lower than those in the maintainer; The activities and expression levels of *TPI* in anthers of sterile line were obviously reduced, while levels in F_1 hybrid and maintainers kept normal, which indicated that stable transcripts of *TPI* are beneficial to keep the energy metabolism with a normal level. But when the restorer gene was transferred into CMS line, activities and expression level of *TPI* could be regulated by the restorer gene and kept stably. The restorer gene likely plays an important role in keeping the balance of the energy metabolism with a normal level during the microspore development.

Key words: Pepper (*Capsicum annuum* L.), triose phosphate isomerase, cloning, characterization, cytoplasmic male sterility.

INTRODUCTION

Cytoplasmic male sterility (CMS), a maternally inherited trait resulting in the inability to produce or release functional pollen, has been identified and characterized in many crop species (Wise and Pring, 2002). Numerous studies have shown that CMS is related to abnormal mitochondrial gene reorganization (Touzet and Budar, 2004; Linke and Börner, 2005). The reorganization of mitochondria often leads to abnormality of CMS energy metabolism (Connettm and Hanson, 1990). In aerobic tissues, mitochondria provide energy for cell activities (Balk and Leaver, 2001; Sweetlove et al., 2002). Numerous studies have shown that inadequacy of energy metabolism is related to CMS (Sun et al., 2009; Carlsson et al., 2007; Fujii et al., 2007; Teixeira et al., 2005).

Triose phosphate isomerase (TPI) (Lolis et al., 1990) is the glycolytic enzyme (EC:5.3.1.1) that catalyses the reversible interconversion of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. TPI plays an important role in several metabolic pathways and is essential for efficient energy production. It is present in eukaryotes as well as in prokaryotes. TPI is generally considered as an extremely efficient enzyme, and is even referred to as a perfect catalyst (Albery and Knowles, 1976).

There is an abundance of literature on the role, structure and regulation of animal TPIs (Oláh et al., 2002; Ahmed et al., 2003). This situation is in sharp contrast to the current research on plant TPIs. In fact, TPI catalyzes a very important step in carbohydrate metabolism, and

*Corresponding author. E-mail: zou_xuexiao@163.com.

TPI activity could play a role in the balance of metabolic fluxes in plant primary metabolism. In vascular plants, a small number of studies have been conducted on TPI expression (Xu and Hall, 1993; Zhang and Chinnappa, 1994; Dorion et al., 2005), but until today the pepper TPI gene has not been reported yet.

In the present experiment, we will isolate the complete coding sequences of the pepper (*Capsicum annuum* L.) *TPI* gene; we will also study the tissue expression distribution of the gene, and finally we will also study the activity and expression level of *TPI* gene in anthers of pepper CMS-9704A, its maintainer line 9704B and F_1, in order to establish the primary foundation of further understanding of the pepper gene *TPI* and the relationship with the CMS.

MATERIALS AND METHODS

Samples collection, RNA extraction and first-strand cDNA synthesis

A typical CMS line, 9704A, its near-isogenic fertile maintainer line, 9704B and a F_1 hybrid line were grown in experimental fields of Yunnan Agricultural University on campus in the summer of 2011. Flowers at four developmental stages were picked out from the corresponding young flowers and collected for experiments. The whole flower development was divided into four stages according to the microsporogenesis progress by checking under the microscope, that is (Stage 1) sporogenous cell division stage, (Stage 2) pollen mother cell meiosis stage, (stage 3) uninucleate microspore stage and (Stage 4) mature pollen stage. Fresh tissues (root, stem, leaf, flower, pericarp, placenta and seed) in the full bloom stage for RNA isolations were frozen in liquid nitrogen and stored at -80°C until further use.

Total RNA and first-strand cDNA synthesis for those samples were performed as the methods described by previously (Deng et al., 2011).

Isolation of the pepper TPI gene

The reverse transcription PCR (RT-PCR) was performed to isolate the pepper gene using the cDNAs of CMS line and maintainer line from different tissues aforementioned. The primers for pepper *TPI* gene isolation was designed based on the conserved coding sequences information from tomato, tobacco, potato *TPI* genes and their highly homologous pepper ESTs. The primers for pepper *TPI* were: 5'- GTTCATCTACATCATATCCAAA -3' and 5'- CTGCAAAATTACAATCGC -3'. RT-PCR was carried out as previously described (Deng et al., 2011). After the PCR, the gene product was cloned into pMD18-T vector (TaKaRa, Dalian) and sequenced bidirectionally with the commercial fluorometric method. At least five independent clones were sequenced.

Bioinformatics analysis

Sequence analysis of pepper *TPI* gene was performed using software in NCBI (http://www.ncbi.nlm.nih.gov) and ExPaSy (http://www.expasy.org). The cDNA sequence was predicted using the GenScan software (http://genes.mit.edu/GENSCAN.html). Putative protein theoretical molecular weight (Mw) and isoelectric point (pI) prediction, signal peptide prediction, subcellular localization prediction and transmembrane topology prediction were performed using the Compute pI/Mw Tool (http://us.expasy.org/tools/pi_tool.html), SignalP 3.0 server (http://www.cbs.dtu.dk/services/SignalP/), PSort II (http://psort.hgc.jp/), TMHMM-2.0 server (http://www.cbs.dtu.dk/services/TMHMM-2.0/), respectively. The Blastp program and Conserved Domain Architecture Retrieval Tool were used to search for similar proteins and conserved domain, respectively (http://www.ncbi.nlm.nih.gov/Blast). The alignment of the nucleotide sequences and deduced amino acid sequences were computed using ClusterX, and the phylogenetic trees were computed using the ClustalX and Mega 4.0 software with standard parameters. Secondary structures of deduced amino acid sequences were predicted with SOPMA (http://npsa-pbil.ibcp.fr/). The 3D structure was predicted based on the existed 3D structures by the amino acids homology modeling on Swiss server (http://swissmodel.expasy.org/).

Semi-quantitative RT-PCR

Semi-quantitative RT-PCR was performed as previously reported (Deng et al., 2011). We selected the housekeeping gene Actin as a positive control. The control primers used were: 5'-TGCAGGAATCCACGAGACTAC -3' and 5'-TACCACCACTGAGCACAATGTT -3'. The pepper primers which were used to perform the semi-quantitative RT-PCR for tissue expression profile analysis were the same as the primers for isolation RT-PCR aforementioned.

Enzyme activity determinations

Enzyme activity was measured at 20 and monitored spectrophotometrically at 334 nm in a total volume of 1 ml. One unit (U) of enzyme activity corresponds to the disappearance of 1 µmol GAP per minute. TPI activity was assayed as described in Tomlinson and Turner (1979) and Dorion et al. (2005).

Isolation of mitochondria

Mitochondria were isolated according to the methods described by Bergman et al. (1980) and Deng et al. (2010).

Measurement of NAD^+-NADH pool and ATP

The amount of ATP was measured by the luciferin–luciferase method (Wan et al., 2007). NAD^+–NADH content in mitochondria was measured as described by Wan et al. (2007).

Statistical analyses

The experiments were carried out in three independent series with three replicates. expressed the mean±SD.

RESULTS

Cloning and identification of pepper *TPI* cDNA

Through RT-PCR with different tissue cDNAs, for pepper *TPI* gene, the resulting PCR product was 818 bp (Figure 1). These cDNA nucleotide sequence analysis revealed that pepper *TPI* gene was not homologous to any of the

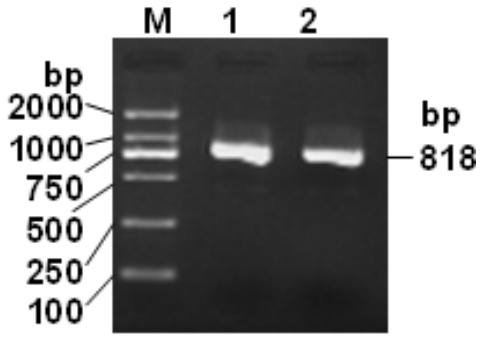

Figure 1. RT-PCR result for pepper *TPI* gene. M: DL2000 DNA marker, 1: PCR product for pepper *9704A*; 2: PCR product for pepper *9704B*.

known pepper genes and they were then deposited into the GenBank database (Accession No. JN885195). The sequence prediction showed that the 765 bp cDNA sequences represent a single gene which encoded 254 amino acids. The cDNA sequences in 9704A and 9704B are exactly the same. It indicated that the *TPI* gene CMS line was not reorganized. The complete CDS and the encoded amino acids were presented in Figure 2.

Bioinformatics analysis of pepper *TPI*

The pI and Mw of pepper *TPI* were 5.72 and 27094, respectively. The signal peptide prediction revealed that pepper *TPI* did not contain a potential signal peptide (Bendtsen et al., 2004). Transmembrane topology prediction showed that pepper *TPI* was not potential membrane protein. Subcellular localization analysis showed pepper *TPI* was probably located in the cytoplasm (Nakai and Horton, 1999).

Examined indicated that pepper *TPI* contains one separated conserved domain—TIM-phosphate_binding. The prediction of secondary structure indicates that the deduced pepper *TPI* contains 126 alpha helices, 43 extended strands, 12 beta turn and 73 random coils.

The homology modeling of *TPI* was performed to estimate their 3D structure (Benkert et al., 2011). The 3D structure of the *TPI* (3-249AA) was similar to that of the rabbit *TPI* (1r2r: C).

The deduced protein sequence of pepper *TPI* was submitted to generate BLAST reciprocal best hits, and similarity comparison revealed that pepper *TPI* protein has high homology with the *TPI* proteins of six other species, *Solanum tuberosum* (95%), *Arabidopsis thaliana* (87%), *Ricinus communis* (87%), *Vitis vinifera* (87%), *Oryza sativa* (87%) and *Zea mays* (85%) (Figure 3).

The phylogenetic tree analysis revealed that the pepper *TPI* gene has a closer genetic relationship with the *TPI* genes of *S. tuberosum* than with those of *A. thaliana*, *R. communis*, *V. vinifera*, *O. sativa* and *Z. mays* (Figure 4).

mRNA tissue-specific expression profile

To check the relative expression levels of pepper *TPI* mRNA in various pepper tissues, semi-quantitative RT-PCR was performed in seven pepper tissues aforementioned. Results revealed that pepper *TPI* gene was expressed in root, stem, leaf, flower, pericarp, placenta and seed (Figure 5).

ATP and NAD^+-NADH content in isolated mitochondria

The ATP levels in 9704A, 9704B and a F_1 hybrid line preserved a durative uptrend, but the ATP levels in 9704A were significantly lower than that in 9704B and F_1 at each of the developmental stage (Figure 6a). The NAD^+-NADH levels in the extracted mitochondria were also investigated, and a gradual rise was found in 9704B and a F_1 hybrid line. However, except a notable decline at the Stage 2, no distinct change was observed in other three stages in 9704A (Figure 6b). At the same time, the ATP and NAD+-NADH levels were no more different in 9704B and a F_1 hybrid line at every stage (Figure 6).

Activities and expression level

The activity and expression level of *TPI* was investigated in this paper (Figure 7). Results showed the activity of TPI had no significant difference in 9704B and F_1 in the all stages. But the activities of TPI were obviously reduced, relative to the anthers of 9704B and F_1 especially in the Stage 2.

Transcriptional analysis of *TPI* gene by semi-quantitative RT-PCR was used to test the gene expression difference in the process of flower development among the anthers of 9704A and 9704B. The amplification results showed that the expressions of *TPI* were very similar to the activities of TPI. As could be seen in Figure 7, the levels of *actin* mRNA were relatively constant in all of the RNA samples analyzed.

DISCUSSION

It had been reported that there are very highly energy demands during pollen development in higher plants (Tedege and Kuhlemeier, 1997). A widely accepted hypothesis on the mechanism of CMS is that the increased demand for respiratory function and cellular energy in the form of ATP during anther development may be compromised by expression of the aberrant mitochondria genes.

```
ATGGGCAGAAAATTCTTCGTCGGCGGCAACTGGAAATGCAATGGAACCAGTGACGAGATCAAGAAGATAATATCAACACTCAATGCTGGT
 M  G  R  K  F  F  V  G  G  N  W  K  C  N  G  T  S  D  E  I  K  K  I  I  S  T  L  N  A  G
CAAGTGCCATCACAAGATGTTGTTGAGGTTGTTGTGAGCCCCCCATTTGTATTTCTTCCTCTGGTCAAGAATGAGCTGCGACCTGATTTT
 Q  V  P  S  Q  D  V  V  E  V  V  V  S  P  P  F  V  F  L  P  L  V  K  N  E  L  R  P  D  F
CATGTTGCCGCCCAAAATTGTTGGGTTAAGAAAGGCGGTGCTTTCACCGGGGAAGTTAGTGCTGAGATGCTTGTTAATCTGAGCATTCCT
 H  V  A  A  Q  N  C  W  V  K  K  G  G  A  F  T  G  E  V  S  A  E  M  L  V  N  L  S  I  P
TGGGTCATTTTGGGTCACTCTGAAAGGAGAGCCCTTTTGGGTGAATCAAACGAGTTCGTTGGAGACAAGGTAGCATATGCTCTTTCTCAA
 W  V  I  L  G  H  S  E  R  R  A  L  L  G  E  S  N  E  F  V  G  D  K  V  A  Y  A  L  S  Q
GGTTTGAAGGTAATTGCATGTGTTGGGGAGACTCTTGAGCAAAGAGAATCAGGATCCACAATGGATGTTGTTGCTGCACAAACGAAGGCT
 G  L  K  V  I  A  C  V  G  E  T  L  E  Q  R  E  S  G  S  T  M  D  V  V  A  A  Q  T  K  A
ATTGCAGATCTAGTAAAAGATTGGACAAATGTTGTGATAGCTTATGAGCCTGTTTGGGCTATTGGTACTGGAAAGGTTGCAACCCCTGCA
 I  A  D  L  V  K  D  W  T  N  V  V  I  A  Y  E  P  V  W  A  I  G  T  G  K  V  A  T  P  A
CAAGCTCAGGAAGTACATGCTGAATTGAGGAAATGGCTTCAAGCAAATGTTAGCGCTGAAGTGGCTGCCTCAACCAGGATCATCTATGGA
 Q  A  Q  E  V  H  A  E  L  R  K  W  L  Q  A  N  V  S  A  E  V  A  A  S  T  R  I  I  Y  G
GGATCTGTAAGCGGTGCAAACTGCAAGGAGTTGGCTGGACAGCCAGATGTTGACGGTTTTCTTGTAGGTGGAGCTTCTTTGAAGCCGGAG
 G  S  V  S  G  A  N  C  K  E  L  A  G  Q  P  D  V  D  G  F  L  V  G  G  A  S  L  K  P  E
TTCATTGACATCATCAAGGCTGCTGAGGTGAAGAAAAGTGCCTGA
 F  I  D  I  I  K  A  A  E  V  K  K  S  A  .
```

Figure 2. The complete cDNA sequence and amino acid sequence of the protein encoded by *TPI* (GenBanaccession number: JN885195).

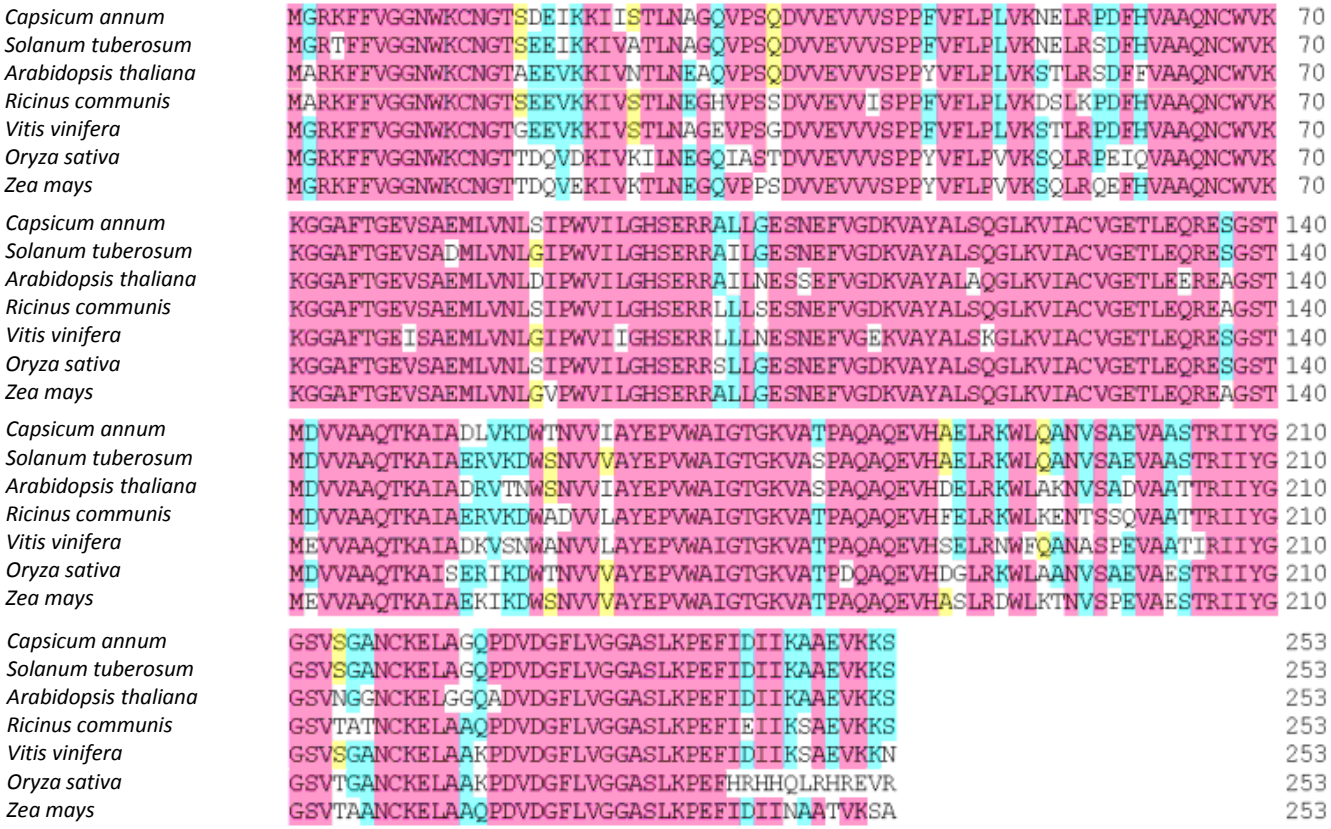

Figure 3. Alignment of the protein encoded by the pepper *TPI* and six other types of *TPI* from *Solanum tuberosum* (ABB02628), *Arabidopsis thaliana* (NP_191104), *Ricinus communis* (XP_002533147), *Vitis vinifera* (XP_002283693), *Oryza sativa* (AAB63603) and *Zea mays* (ACG24648).

In aerobic tissues, mitochondria provide energy for cell activities. Numerous studies have shown that CMS is related to abnormal mitochondrial gene reorganization (Touzet and Budar, 2004; Linke and Börner, 2005).

The citric acid cycles a series of enzyme-catalyses chemical reactions, which is of central importance in all living cells, especially those that use oxygen as part of cellular respiration. In eukaryotic cells, the citric acid

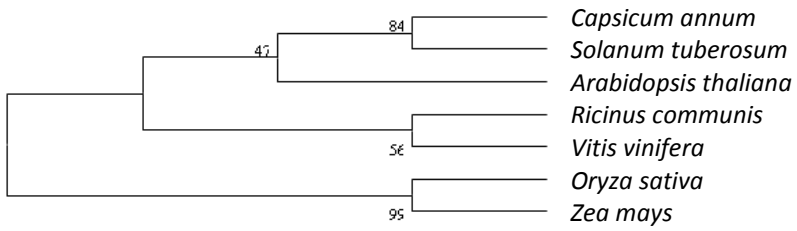

Figure 4. Phylogenetic tree for pepper *TPI* gene.

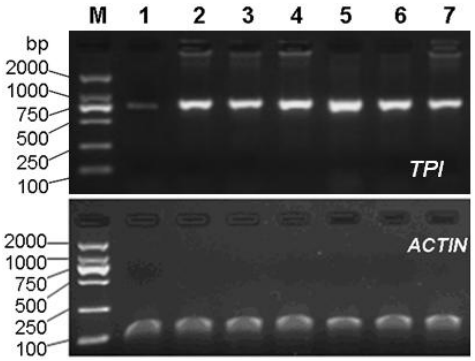

Figure 5. Tissue expression profile of pepper *TPI* gene.1, root; 2, stem; 3, leaf; 4, flower; 5, npericarp; 6, placenta; 7, seed. The *Actin* expression level is used for the internal control. M: DL2000 DNA mark.

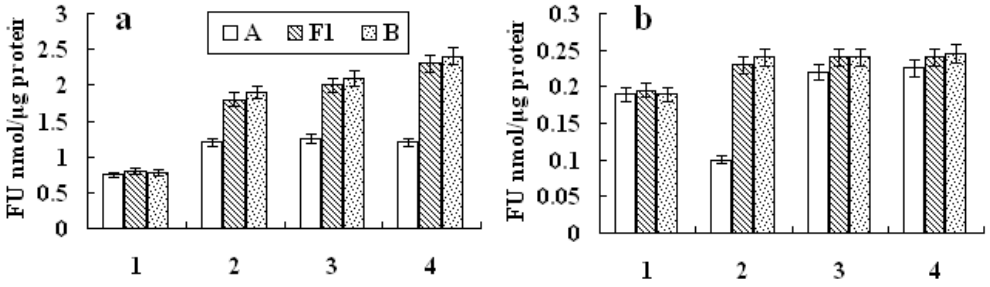

Figure 6. ATP and NAD^+-NADH content in isolated mitochondria during flowers development. A, Generation of ATP in isolated mitochondria from the anthers. B, Content of NAD^+-NADH in the mitochondria.

cycle occurs in the matrix of the mitochondrion. TPI is an enzyme in the Embden-Meyerhof pathway which catalyzes the reversible conversion of dihydroxyacetone phosphate to glyceraldehyde-3-phosphate and is essential for efficient energy production (Lolis et al., 1990). TPI has been found in nearly every organism searched for the enzyme, including animals such as mammals and insects as well as in fungi, plants, and bacteria (Dorion et al., 2005).

Our results (Figure 6) showed that the ATP and NADH pool in male-sterile line were distinctly lower than that in the male fertile (maintainer and F_1). This indicates that the CMS plants are in a sub-hungriness state and the reduced availability of energy. At the same time, the low the transcripts and protein activities of TPI were checked in the in male-sterile line (Figure 7). So we can speculate that the transcripts and protein activities of TPI were too low to keep balance of the energy metabolism in sterile line, but they were significantly higher in the anthers of maintainer and hybrid F_1, which indicated that stable

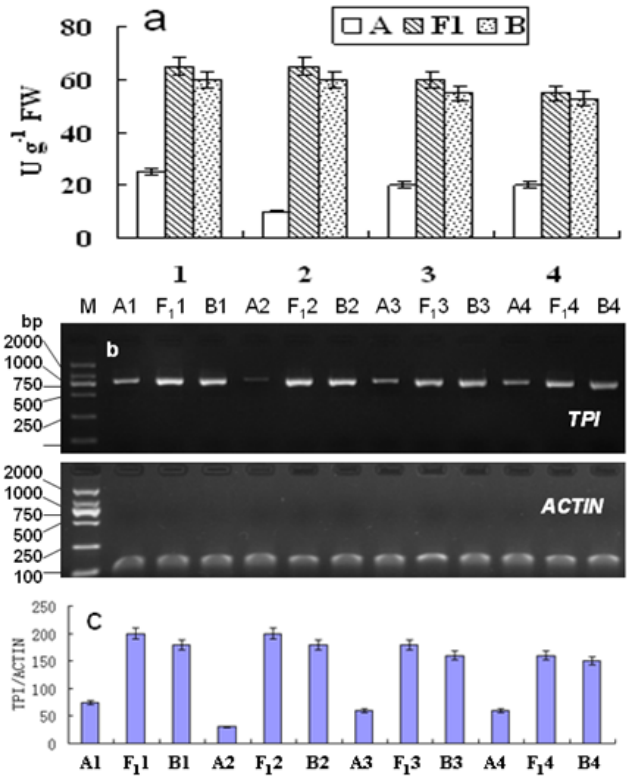

Figure 7. Activities and expression level of pepper *TPI* gene.

transcripts of TPI are beneficial to keep the energy metabolism with a normal level.

Large quantities of literature have revealed that nucleus gene expression can regulate mitochondrial gene expression through mitochondria-to-nucleus signaling (Karpova et al., 2002; Rhoads et al., 2006). In our study, there was an interesting finding that during the anther abortion, not like the sterile line, hybrid F_1 always kept stable transcripts and protein activities of *TPI* with the maintainer. There are not many reports about this phenomenon up to date. We presume that during the stage of anther abortion, the transcripts and protein activities of *TPI* in hybrid F_1 keeping consistent with the maintainer likely result from the regulation of nuclear restorer gene.

ACKNOWLEDGEMENTS

This study was supported by the Earmarked Fund for Modern Agri-industry Technology Research System, National Department Public Benefit Research Foundation (200903025), National Natural Science Foundation of China (Grant No. 31160394), the Foundation of Natural Science of Yunnan Province, China (2009CD055), and Specialized Research Fund for the Doctoral Owner of Yunnan Agricultual University.

REFERENCES

Ahmed N, Battah S, Karachalias N, Babaei-Jadidi R, Horanyi M, Baroti K, Hollan S, Thornalley PJ (2003). Increased formation of methylglyoxal and protein glycation, oxidation and nitrosation in triose phosphate isomerase deficiency. Biochem. Biophys. Acta, 1639: 121-132.

Albery WJ, Knowles NR (1976). Evolution of enzyme function and the development of catalytic efficiency. Biochemistry, 15: 5631-5640.

Balk J, Leaver CJ (2001). The PET1-CMS mitochondrial mutation sunflower is associated with premature programmed cell death and cytochrome c release. Plant Cell, 13: 1803-1818.

Bendtsen JD, Nielsen H, von Heijne G, Brunak S (2004). Improved prediction of signal peptides: SignalP 3.0. J. Mol. Biol., 340: 783-795.

Benkert P, Biasini M, Schwede T (2011). Toward the estimation of the absolute quality of individual protein structure models. Bioinformatics, 27: 343-350.

Bergman A, Gardeström P, Ericson I (1980). Method to obtain a chlorophyll-free preparation of intact mitochondria from spinach leaves. Plant Physiol., 66: 442-445.

Carlsson J, Lagercrantz U, Sundström J, Teixeira R, Wellmer F, Meyerowitz EM, Glimelius K (2007). Microarray analysis reveals altered expression of a large number of nuclear genes in developing cytoplasmic male sterile *Brassica napus* flowers. Plant J., 49(3): 452-462.

Connettm B, Hanson MR (1990). Differential mitochondrial electron transport throungh the cyanidesensitive and cyanide-insensitive pathways in isonuclear lines of cytoplasmicmale sterile, male fertile, and restored petunia. Plant Physiol., 93: 1643-1640.

Deng M-H, Wen J-F, Huo J-L, Zhu H-S, Dai X-Z, Zhang Z-Q, Zhou H, Zou X-X (2011). Relationship of metabolism of reactive oxygen species with cytoplasmic male sterility in pepper (*Capsicum annuum* L.). Sci. Hortic-Amsterdam, 134: 232-236.

Deng MH, Wen JF, Zou XX, Zhu HS, Zhang ZQ, Zhao K (2010). Analysis of mtDNA of cytoplasmic male sterility (CMS) line, its maintainer line and restorer line of pepper (*Capsicum annuum* L.) by RAPD. J. Yunnan Agric. Univ., 25(4): 541-544 (in Chinese).

Dorion S, Parveen, Jeukens J, Matton DP, Rivoal J (2005). Cloning and characterization of a cytosolic isoform of triosephosphate isomerase developmentally regulated in potato leaves. Plant Sci., 168: 183-194.

Fujii S, Komatsu S, Toriyama K (2007). Retrograde regulation of nuclear gene expression in CW-CMS of rice. Plant Mol. Biol., 63: 405-417.

Karpova OV, Kuzmin EV, Elthon TE, Newton KJ (2002). Differential expression of alternative oxidase genes in maize mitochondrial mutants. Plant Cell, 14: 3271-3284.

Linke B, Börner T (2005). Mitochondrial effects on flower and pollen development. Mitochondrion, 5: 389-402.

Lolis E, Alber T, Davenport RC, Rose D, Hartman FC, Petsko GA (1990). Structure of yeast triosephosphate isomerase at 1.9-A resolution. Biochem., 29 6609-6618.

Nakai K, Horton P (1999). PSORT: a program for detecting the sorting signals of proteins and predicting their subcellular localization. Trends Biochem. Sci., 24: 34-35.

Oláh J, Orosz F, Keserü GM, Kovári Z, Kovács J, Hollán S, Ovádi J (2002). Triosephosphate isomerase deficiency: a neurodegenerative misfolding disease. Biochem. Soc. Trans., 30: 30-38.

Rhoads DM, Umbach AL, Subbaiah CC, Siedow JN (2006). Mitochondrial reactive oxygen species. Contribution to oxidative stress and interorganellar signaling. Plant Physiol., 141: 357-366.

Sun Q, Hu C, Hu J, Li S, Zhu Y (2009). Quantitative proteomic analysis of CMS-related changes in Honglian CMS rice anther. Protein J., 28: 341-348.

Sweetlove LJ, Heazlewood JL, Herald V, Holtzapffel R, Day DA, Leaver CJ, Milla AH (2002). The impact of oxidative stress on Arabidopsis mitochondria. Plant J., 32: 891-904.

Tedege M, Kuhlemeier C (1997). Aerobic fermentation during tobacco pollen development. Plant Mol. Biol., 35: 3433-3454.

Teixeira RT, Knorpp C, Glimelius K (2005). Modified sucrose, starch, and ATP levels in two alloplasmic male-sterile lines of *B. napus*. J. Exp. Bot., 56(414): 1245-1253.

Tomlinson JD, Turner JF (1979). Pea seed triose phosphate isomerase. Phytochem., 18: 1959-1962.

Touzet P, Buda RF (2004). Unveiling the molecular arms race between two conflicting genomes in cytoplasmic male sterility. Trends Plant Sci., 9: 568-570.
Wan C, Li S, Wen L, Kong J, Wang K, Zhu Y (2007). Damage of oxidative stress on mitochondria during microspores development in Honglian CMS line of rice. Plant Cell Rep., 26: 373-382.
Wise RP, Pring DR (2002). Nuclear-mediated mitochondrial gene regulation and male fertility in higher plants: Light at the end of the tunnel. Proc. Natl. Acad. Sci. U.S.A., 99(16): 10240-10242.
Xu Y, Hall TC (1993). Cytosolic triosephosphate isomerase is a single gene in rice. Plant Physiol., 101: 683-687.
Zhang XH, Chinnappa CC (1994). Triose phosphate isomerase of Stellaria longipipes (Caryophyllaceae). Genome, 37: 148-156.