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Cloning and sequence characterization of a novel pepper gene – *SCS* and the relationship with cytoplasmic male sterility

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Key words: *Capsicum annuum* L.; succinyl-CoA synthetase; cloning; characterization; cytoplasmic male sterility

1 SUMMARY

Based on the information on tomatoes' (*Solanum lycopersicum*) succinyl-CoA synthetase (*SCS*) gene and some highly homologous pepper (*Capsicum annuum* L.) ESTs, the complete CDS sequence of the pepper *SCS* gene was amplified using the Reverse Transcriptase-Polymerase Chain Reaction, The results showed the pepper *SCS* gene cDNA is 981 bp long, contained a 326 amino acids polypeptide. The sequence of reduced protein was highly homologous with the protein sequences of other five plant species – *S. lycopersicum* (Tomato) (94%), *Vitis vinifera*(Grape) (87%), *Sorghum bicolor* (Broomcorn) (85%), *Zea mays (Maize)* (85%) and *Arabidopsis thaliana*(Arabidopsis) (82%). The pepper *SCS* gene expression in pericarp was over-expressed; expression in stem and flower was moderate; and expression in root, leaf, placenta and seed was weak. At the abortion stages, the ATP and NADH pools in the maintainer buds were significantly higher than those in the cytoplasmic male sterility (CMS) buds. The activities and expression levels of *SCS* in buds of the CMS line were obviously reduced, while levels in the F_1 hybrid and maintainer buds remained normal, indicating that stable transcripts of *SCS* were beneficial in maintaining energy metabolism at a normal level.

2 INTRODUCTION

Cytoplasmic male sterility (CMS) has been reported in more than 150 plant species as a genetic characteristic. CMS involves not producing or releasing viable pollen and has been widely used in hybrid seeds production (Wise and Pring 2002). Many researchers have elucidated that the mitochondrion of CMS line was reorganized and this recombination maybe disturbs the energy metabolism of the CMS (Connettm and Hanson 1990, Touzet and Budar 2004, Linke and Börner 2005).In plants, succinyl-CoA synthetase (SCS; EC6.2.1.5) catalyzes the reversible thioesterification of succinate:

$$\text{Succinate} + \text{CoA} + \text{ATP} \overset{Mg^{2+}}{\rightleftharpoons} \text{Succinyl-CoA} + \text{ADP} + P_i$$

SCS is an important mitochondrial enzyme. In the absence of oxygen, it can produce ATP

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though substrate level phosphorylation. SCS also plays a vital role in ketone metabolism and the citric acid cycle. It has the important functions of generation succinyl-CoA from succinate for anabolic purposes (Kaufman *et al.* 1953, Labbe *et al.* 1965). In aerobic organisms, energy for cell activities is provided by mitochondria. Many researchers have reported that there is relationship between CMS and inadequate energy metabolism (Balk and Leaver 2001, Sweetlove *et al.* 2002, Teixeira *et al.* 2005, Fujii *et al.* 2007, Carlsson *et al.* 2007; Sun *et al.* 2009). Due to the key role of SCS in energy metabolism, it is very important to study the SCS gene for enriching the molecular mechanism of CMS. The *SCS* genes have been cloned and characterized from a variety of species (Bush 1969, David *et al.* 1986, Studart-Guimaraes *et al.* 2005, Phillips *et al.* 2009). However, up to now the pepper (*Capsicum annuum* L.) SCS gene has not been reported. In this paper, the pepper *SCS* gene were cloned and analyzed, and the relationship of the *SCS* gene with the CMS were primary studied.

3 MATERIALS AND METHODS

3.1 Buds collection: A pepper CMS line (9704A), its near-isogenic maintainer line (9704B) and a F_1 hybrid line were used in this study. Young buds at four developmental stages were collected from each line for the experiments. Buds development stages were divided according to development process of microsporogenesis by a microscopic examination: stages 1–4 were sporogenous cell division, pollen mother cell meiosis, uninucleate microspore and mature pollen stages, respectively. Liquid nitrogen was used to preserve the fresh tissues (root, stem, leaf, bud, pericarp, placenta and seed) collected in the full bloom stage for RNA isolations.

3.2 RNA extraction and first-strand cDNA synthesis: Total RNA and first-strand cDNA synthesis for samples were carried out according to the methods described by Deng *et al.* (2012).

3.3 Isolation of the pepper *SCS* gene: Using the cDNAs of the 9704A and 9704B from the different tissues mentioned above, Reverse Transcription PCR (RT-PCR) was performed to isolate the full length cDNA of pepper *SCS* gene. The primers (5'-3') for pepper *SCS* isolation were: up primer: AAAACCTAAACGGGACG and down primer: GCATAATGAGGGTAAACTGA based on the information of *Solanum lycopersicum SCS* gene and some highly homologous pepper ESTs. RT-PCR was performed according to the methods of deng *et al.* (2012). After PCR, the products were linked into pMD18-T vector (TaKaRa). At last five independent clones were sequenced.

3.4 Bioinformatics analysis: Software from NCBI (Blast, 2013) and ExPaSy (Protscale. 2013) was used for analysis sequence of pepper *SCS* gene; GenScan software (GenScan 2013) was used for prediction the cDNA sequences. Software from expasy (ProtParam 2013) was used for Putative protein theoretical molecular weight (Mw) and isoelectric point (pI) prediction; SignalP 3.0 server (SignalP 3.0 server 2013) was used for signal peptide prediction; Software from PSort www server (PSort II 2013) was used for subcellular localization prediction; TMHMM-2.0 server (TMHMM-2.0 server 2013) was used for transmembrane topology prediction; Conserved domains and similar proteins was performed using the software from NCBI (Blast, 2013); Software from Clustal (ClustalX 2013) and from megasoftware (Mega 4.0 2013) with standard parameters was used for the alignment of the nucleotide sequences and deduced amino acid sequences; Software from NPS (sopma 2013) was used for secondary structures of deduced amino acid sequences prediction; Software from ExPaSy (swissmodel 2013) was used for the 3D structure prediction based on existing 3D structures by the amino acids homology modeling.

3.5 Semi-quantitative RT-PCR: semi-quantitative RT-PCR was performed according to the method of Deng *et al.* (2012). *Actin*, a housekeeping gene as a positive control was selected and the primers were 5'-TGC AGG AAT CCA CGA GAC TAC-3' and 5'-TAC CAC CAC TGA GCA CAA TGT T-3'. The primers of pepper *SCS* gene expression were the primers used in SCS gene isolation.

3.6 Enzyme activity determinations: SCS activity was measured using the method of Alarcon (2002) at 25°C. The content of protein was measured according to the Bradford (1976).

3.7 Mitochondria isolation: Mitochondria were isolated from pepper buds using the method described by Bergman *et al.* (1980) and in our

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previous study (Deng *et al.* 2010).

3.8 Measurement of NAD$^+$–NADH pool and ATP: The amount of ATP was determined using the method described by St. John (1970) and Wan *et al.* (2007). The content of NAD$^+$–NADH was determined using the method described by Tretter and Adam-Vizi (2000) and Wan *et al.* (2007).

3.9 Statistical analyses: The experimentations were performed in three independent series with 3 replications. Values given are the mean ± standard deviation (SD).

4 RESULTS

4.1 Cloning and identification of pepper *SCS* cDNA: Using different tissue cDNAs, the RT-PCR product for the pepper *SCS* gene was 1194 bp (Fig. 1). The cDNA sequence of the pepper *SCS* gene was analyzed. The result showed the cDNA sequence was not identical to any known pepper genes. The gene was submitted to the genebank and JN885190 is accession number. The result of sequence prediction revealed that the 981-bp long cDNA sequences represented a single gene that encoding 326 amino acids. The cDNA sequences in maintainer l and CMS lines were exactly the same, indicating that the *SCS* gene in the CMS line had not been reorganized. The complete CDS nucleotide sequence and the deduced amino acids of the pepper *SCS* gene are presented in Fig. 2.

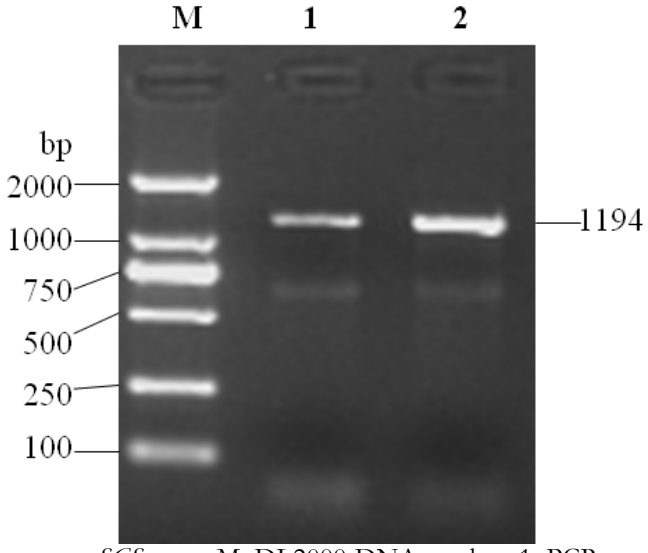

Fig. 1 RT-PCR result for pepper *SCS* gene. M: DL2000 DNA marker, 1: PCR product for pepper 9704A; 2: PCR product for pepper 9704B.

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ATGGCTCGTCAAGCGACAAGAGTGATCTCAAAGTGGAGTACAAAGCTTAACCCTAATGAGTATCGCTACTTTGGATCGTCAGCACCACCG
 M  A  R  Q  A  T  R  V  I  S  K  W  S  T  K  L  N  P  N  E  Y  R  Y  F  G  S  S  A  P  P
CCCGCTGTTTTCGTTGATAAGAACACCCGCGTCATCTGTCAAGGCATCACCGGCAAAAACGGCACTTTCCACACTGAACAAGCCATTGAA
 P  A  V  F  V  D  K  N  T  R  V  I  C  Q  G  I  T  G  K  N  G  T  F  H  T  E  Q  A  I  E
TACGGTACTAAAATGGTTGGTGGAGTGACCCCCAAGAAGGGTGGAACAGAACATTTGGGGCTTCCTGTTTTCAATACTGTTGCAGAGGCA
 Y  G  T  K  M  V  G  G  V  T  P  K  K  G  G  T  E  H  L  G  L  P  V  F  N  T  V  A  E  A
AAAGCAGAGACAAAGGCTAATGCTTCAGTGGTTTATGTCCCTCCACCATTTGCTGCAGCAGCTATTATGGAGGCCATGGAAGCTGAGTTG
 K  A  E  T  K  A  N  A  S  V  V  Y  V  P  P  P  F  A  A  A  A  I  M  E  A  M  E  A  E  L
GACTTAGTTGTCTGCATAACGGAGGGAATTCCTCAGCATGATATGGTTCGCGTAAAAGCTGCTCTTAAGAAGCAATCAAGAACTCGGCTG
 D  L  V  V  C  I  T  E  G  I  P  Q  H  D  M  V  R  V  K  A  A  L  K  K  Q  S  R  T  R  L
ATTGGGCCAAATTGTCCAGGTATTATCAAACCAGGAGAGTGCAAAATTGGAATTATGCCTGGATACATTCACAAACCTGGGCGTATTGGA
 I  G  P  N  C  P  G  I  I  K  P  G  E  C  K  I  G  I  M  P  G  Y  I  H  K  P  G  R  I  G
ATTGTTTCTCGATCTGGTACATTGACATATGAAGCAGTTTTTCAGACAACTGCTGTTGGTCTTGGACAGTCAACTTGTGTAGGGATTGGT
 I  V  S  R  S  G  T  L  T  Y  E  A  V  F  Q  T  T  A  V  G  L  G  Q  S  T  C  V  G  I  G
GGTGATCCTTTTAATGGTACAAATTTTGTCGATTGCTTAGAGAGATTTATTGCTGATCCACAGACAGAAGGTATTGTTCTTATTGGTGAG
 G  D  P  F  N  G  T  N  F  V  D  C  L  E  R  F  I  A  D  P  Q  T  E  G  I  V  L  I  G  E
ATCGGTGGTACAGCAGAAGAAGATGCTGCAGCCTTGATAAAGGAAAGTGGAACTCAAAAGCCTGTTGTTGCATTTATTGCTGGACTAACT
 I  G  G  T  A  E  E  D  A  A  A  L  I  K  E  S  G  T  Q  K  P  V  V  A  F  I  A  G  L  T
GCTCCTCCTGGACGACGAATGGGTCATGCTGGAGCCATTGTATCGGGTGGAAAGGGAACAGCACAGGACAAGATCAAGGCTCTCAAGGAA
 A  P  P  G  R  R  M  G  H  A  G  A  I  V  S  G  G  K  G  T  A  Q  D  K  I  K  A  L  K  E
GCTGGGGTTACAGTATGCGAGTCTCCTGCTAAAATTGGGATCACAATGCTTGATGTGTTCAAACAGAGGGGTCTTGCGTAA
 A  G  V  T  V  C  E  S  P  A  K  I  G  I  T  M  L  D  V  F  K  Q  R  G  L  A  .
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Fig. 2 The complete cDNA sequence and amino acid sequence of the protein encoded by *SCS* (GenBank accession number: JN885190).

4.2 Physical and chemical characteristics of pepper SCS: Some of the physical and chemical characteristics of pepper *SCS* gene were predicted. The pI was 8.99 and the Mw was 34204; Pepper *SCS* gene did not contain a potential signal peptide (Bendtsen *et al.* 2004, Comlekcioglu *et al.* 2010) and was not potential membrane protein (Moller *et al.* 2001); pepper SCS was probably located in the mitochondrial matrix space (Nakai *et al.* 1999).

4.3 Prediction and analysis of structures and conserved domains of pepper SCS: Proteins often contain several domains, each with their own evolutionary origins and functions. Examination indicated that pepper SCS protein contained one conserved domain – PLN00125 (Fig. 3). The result of secondary structure prediction showed that deduced pepper SCS protein contained 28.8% alpha helices, 20.6% extended strands, 8.3% beta turns and 42.3% random coils (Fig. 4). The 3D structure of pepper SCS protein was similar to that of pig SCS (1euc Chain: A) (Wang *et al.* 2007, Benkert *et al.* 2011).

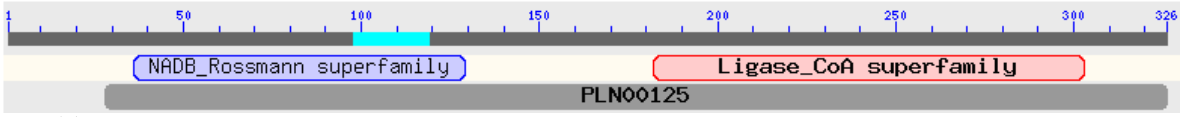

Fig. 3 The putative domains of the protein encoded by pepper *SCS*

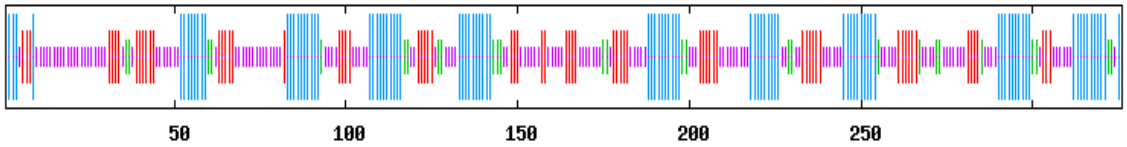

Fig. 4: The secondary structure of the pepper *SCS* protein predicted by SOPMA. Helices, strands and coils are indicated, respectively, with long, middle and short vertical lines.

4.4 Analysis of sequence identity and evolutionary relationships of pepper SCS: The homology of the pepper *SCS* gene were performed and analyzed. The result showed that the pepper SCS protein had high homology with this gene of five other plant species: *S. lycopersicum* (94%), *Vitis vinifera* (87%), *Sorghum bicolor* (85%), *Zea mays* (85%) and *Arabidopsis thaliana* (82%) (Fig. 5). Evolutionary relationships analysis revealed a closer genetic relationship between the pepper *SCS* gene and that of *S lycopersicum* than with those of *A. thaliana*, *Z. mays*, *S. bicolor* and *V. vinifera* (Fig. 6).

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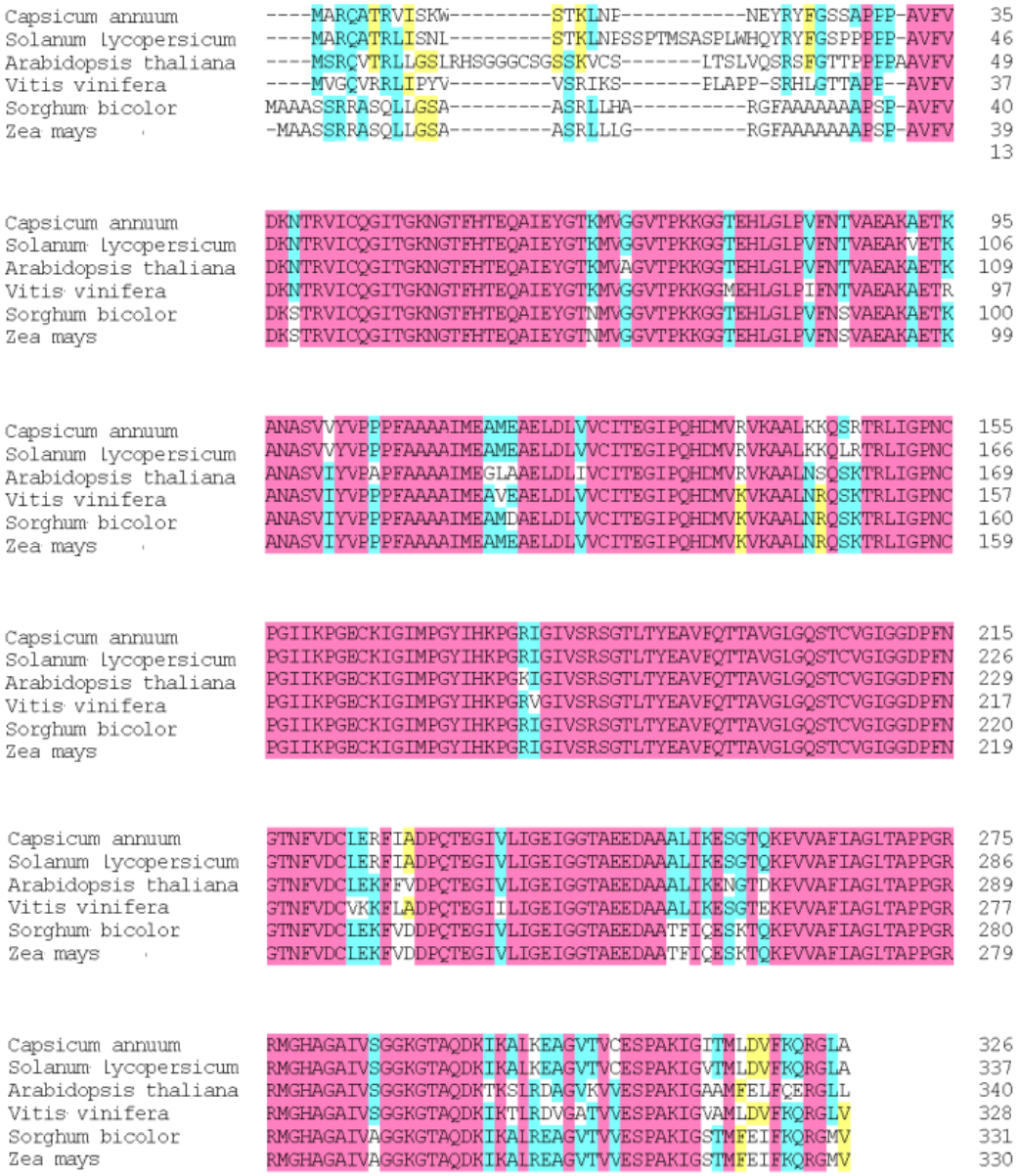

Fig. 5: lignment of the protein encoded by the pepper *SCS* and five other types of *SCS* from S*olanum lycopersicum* (AAT67464), *Arabidopsis thaliana* (AAM65450), *Zea mays* (NP_001136494) and *Sorghum bicolor* (XP_002460917), *Vitis vinifera* (XP_002271746)

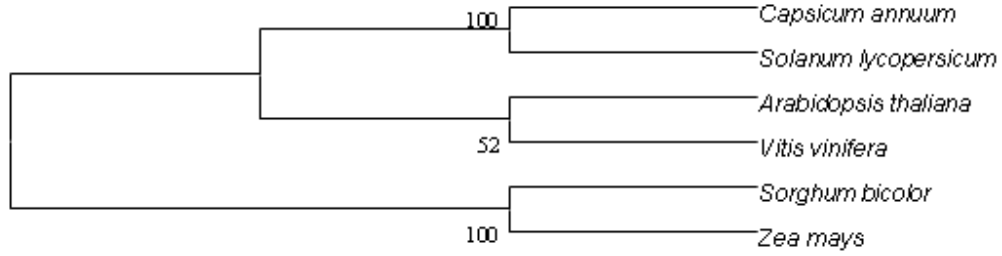

Fig. 6: Phylogenetic tree for pepper *SCS* gene.

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4.5 mRNA tissue-specific expression profile: Using semi-quantitative RT-PCR, relative expression levels of pepper *SCS* mRNA in seven pepper tissues were checked. Results revealed that pepper *SCS* gene was over-expressed in pericarp; moderately expressed in stem and flower; and weakly expressed in root leaf, placenta and seed (Fig. 7).

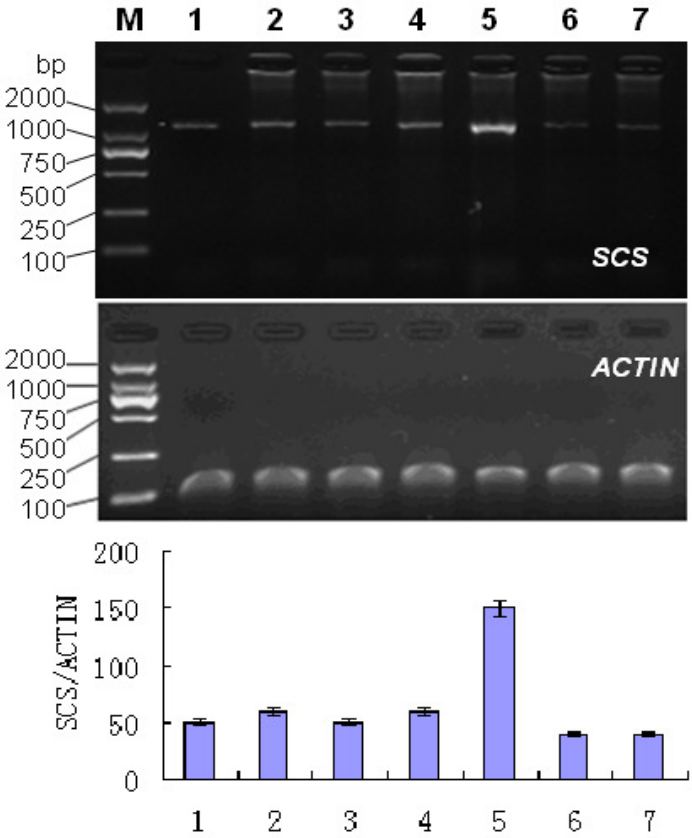

Fig. 7: Tissue expression profile of pepper *SCS* gene.
1-root; 2-stem; 3-leaf; 4-flower; 5-pericarp; 6-placenta; 7-seed. The *Actin* expression level is used for the internal control. M: DL2000 DNA mark

4.6 ATP and NAD$^+$–NADH content in isolated mitochondria: The ATP levels in CMS (9704A), its maintainer (9704B) and F_1 hybrid buds preserved a continuous uptrend, but ATP levels in CMS were remarkably lower than those in maintainer and F_1 buds in all stages (Fig. 8a). The NAD$^+$–NADH levels showed a gradual rise in maintainer and F_1 buds. However, there were no distinct changes in the other three stages for the CMS buds except for a clear decline at stage 2 (Fig. 8b). At the same time, the ATP and NAD$^+$–NADH levels were no more difference in maintainer and F_1 buds at all stages (Fig. 8).

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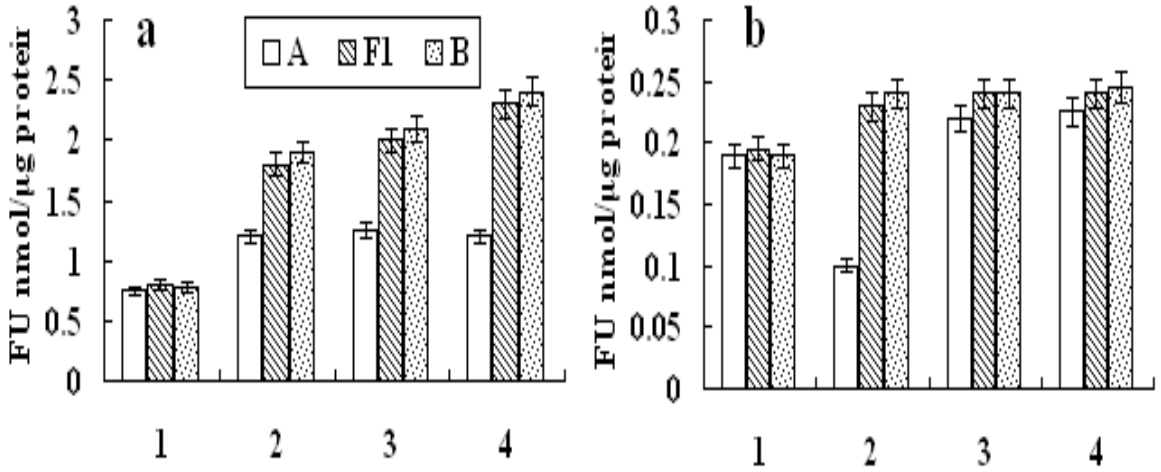

Fig. 8: ATP and NAD$^+$-NADH content in isolated mitochondria during flowers development. a Generation of ATP in isolated mitochondria from the anthers. b Content of NAD$^+$-NADH in the mitochondria.

4.7 Activities and expression level: The assay of SCS activity showed that in stage 1 there were no more changes in CMS, maintainer and F_1 buds (Fig. 9). In contrast, in stages 2–4, the SCS activity in the CMS buds was 48.7, 30.0 and 34.8% lower than in the maintainer buds, respectively, and correspondingly 49.0, 33.3 and 31.8% lower than the F_1. Transcriptional analysis of the *SCS* gene was used to test differences in gene expression between the sterile and fertile buds during buds development. During stage 1 there were no significant differences in *SCS* expression in buds of CMS, maintainer and F_1 lines. At stages 2–4, expression of *SCS* mRNA in buds of the CMS line was clearly lower than in the buds of maintainer and F_1 lines. The levels of *Actin* mRNA remained relatively constant in all RNA samples analyzed (Fig. 9).

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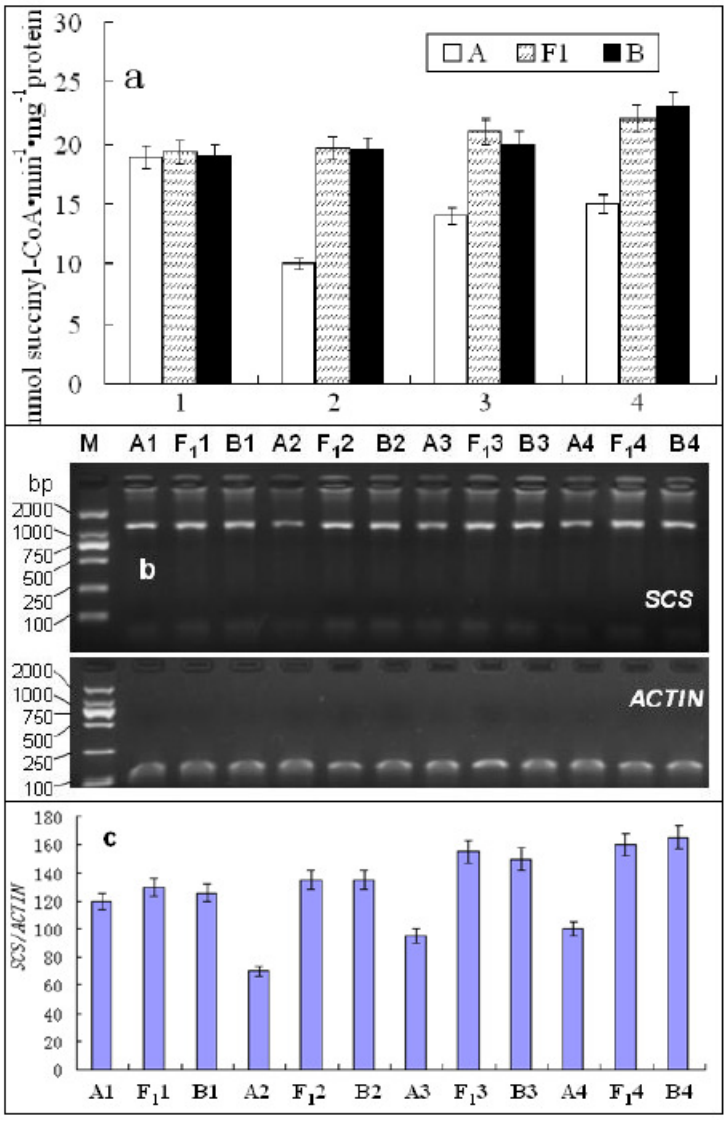

Fig. 9 Activities and expression level of pepper *SCS* gene in anthers of 9704A (CMS), 9704B (maintainer) and F_1 during different development stages. a Activities of pepper *SCS* gene. b Semi-quantitative RT-PCR of pepper *SCS* gene. c Relative transcript amount of pepper *SCS* gene.

5 DISCUSSION

Pollen will consume very high energy during development in higher plants (Tedege and Kuhlemeier 1997, Heydari *et al.* 2010). A hypothesis on the mechanism of CMS has been widely accepted. The hypothesis is that expression of the aberrant mitochondria genes will affect the energy supply and disturb the normal development of pollen.

In aerobic organisms, the energy for all activities is provided by the mitochondria. A great deal of research has revealed that abnormal mitochondrial gene reorganization is related to CMS (Touzet and Budar 2004, Linke and Börner 2005). There are a series of enzymes in the citric acid cycle, such as *SCS*, and these enzymes catalyze enzyme-catalyzed chemical reactions. The enzyme-catalyzed reactions play most important role in the living cells of aerobic respiration.

Succinyl-CoA synthetase (SCS) catalyzes the substrate-level phosphorylation, an important reaction, in aerobic metabolism (Park *et al.* 1997). The enzyme have two various subunits - α and β. The SCS consists 2 isoforms, one is the GTP-specific

(G-SCS) and the other is ATP-specific (A-SCS) (Fraser *et al.* 1999). It is well-known that the 2 SCS isoforms have the same α-subunit and different β-subunit (Labbe *et al.* 1965).

The ATP and NADH pools in the CMS buds were significantly lower than those in the male fertile buds (i.e. maintainer and F1), indicating that the CMS buds were in a insufficient energy supply state and had reduced availability of energy. At the same time, the low level of SCS transcripts and SCS protein activities were checked in the CMS buds. Due to the importance of ATP production by SCS, we speculate that the SCS transcripts and SCS protein activities were too low to maintain energy metabolism in the CMS buds; however, they were significantly higher in the buds of maintainer and F1 lines, indicating that stable transcripts of SCS were beneficial in keeping energy metabolism at a normal level.

6 ACKNOWLEDGEMENTS

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7 REFERENCE

Alarcon, C., B. Wicksteed, M. Prentki, B. E. Corkey, and C. J. Rhodes. (2002). Succinate is a preferential metabolic stimulus-coupling signal for glucose-induced proinsulin biosynthesis translation. Diabetes. 51: 2496-2504.

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

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

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

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

Blast. Accessed 2013. http://www.ncbi.nlm.nih.gov

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt. Biochem. 72: 248-254.

Bush, L. P. (1969). Influence of certain cations on activity of succinyl CoA synthetase from tobacco. Plant Physiol. 44: 347-350.

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

ClustalX . Accessed 2013. http://www.clustal.org/

Comlekcioglu, U., E. Ozkose, A. Tutus, I. Akyol and M. S. Ekinci, 2010. Cloning and characterization of cellulase and xylanase coding genes from anaerobic fungus *Neocallimastix* sp. GMLF1. Int. J. Agric. Biol. 12: 691–696

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

David, B., M. E. Spencer, and J. R. Guest. (1986). Cloning and expression of the succinyl-CoA synthetase genes of *Escherichia coli* K12. J. Gen. Microbiol. 132: 1753-1762.

Deng, M.-H., J.-F. Wen, J.-L. Huo, H.-S. Zhu, X.-Z. Dai, Z.-Q. Zhang, H .Zhou, and X.-X. Zou. (2012). Relationship of metabolism of reactive oxygen species with cytoplasmic male sterility in pepper (*Capsicum annuum* L.) Scientia Horticulturae. 134: 232-236.

Deng, M. H., J. F. Wen, X. X. Zou, H. S. Zhu, Z. Q. Zhang, and K. Zhao. (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: 541-544 (in Chinese).

Fraser, M. E., M. N. James, W. A. Bridger, and J. Wolodko. (1999). A detailed structural description of Escherichia coli succinly-CoA synthetase. J. Mol. Biol. 285: 1633-1653.

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

GenScan. Accessed 2013. http://genes.mit.edu/GENSCAN.html

Heydari, A., K. Shayesteh, N. Eghbalifam and H. Bordbar, 2010. Studies on the respiration rate of banana fruit based on enzyme kinetics. Int. J. Agric. Biol. 12: 145–149

Kaufman, S., C. Gilvarg, O. Cori, and S. Ochoa. (1953). Enzymatic oxidation of alpha-ketoglutarate and coupled phosphorylation. J. Biol. Chem. 203: 869-888.

Labbe, R. F., T. Kurumada, and J. Onisawa. (1965). The role of succinyl-CoA synthetase in the control of heme biosynthesis. Biochim. Biophys. Acta. 111: 403-415.

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

Mega 4.0. Accessed 2013. http://www.megasoftware.net /

Moller, S., M.D. Croning, and R. Apweiler (2001). Evaluation of methods for the prediction of membrane spanning regions. Bioinformatics. 17: 646-653.

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

Park, S. J., G. Chao, and R. P. Gunsalus. (1997). Aerobic regulation of the sucABCD genes of *Escherichia coli*, which encode alpha-ketoglutarate dehydrogenase and succinyl coenzyme A synthetase: roles of ArcA, Fnr, and the upstream sdhCDAB promoter. J. Bacteriol. 179: 4138-4142.

Phillips, D., A. M. Aponte, S. A. French, D. J. Chess, and R. S. Balaban. (2009). Succinyl-CoA synthetase is a phosphate target for the activation of mitochondrial metabolism. Biochemistry. 48: 7140-7149.

ProtParam. Accessed 2013. http://us.expasy.org/tools/pi_tool.html

Protscale. Accessed 2013. http://www.expasy.org

PSort II. Accessed 2013. http://psort.hgc.jp/

SignalP 3.0 server. Accessed 2013. http://www.cbs.dtu.dk/services/SignalP/

Sopma. Accessed 2013. http://npsa-pbil.ibcp.fr/

St. John, J. B. (1970). Determination of ATP in chlorella with the luciferin–luciferase enzyme system. Anal. Biochem. 37: 409-416.

Studart-Guimaraes, C., Y. Gibon, N. Frankel, C. C. Wood, M. I. Zanor, A. R. Fernie, and F. Carrari. (2005). Identification and characterisation of the alpha and beta subunits of succinyl CoA ligase of tomato. Plant Mol. Biol. 59: 781-791.

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

Sweetlove, L. J., J. L. Heazlewood, V. Herald, R. Holtzapffel, D. A. Day, C. J. Leaver, and A. H. Milla. (2002). The impact of oxidative stress on *Arabidopsis* mitochondria. Plant J. 32: 891-904.

Swissmodel. Accessed 2013. http://swissmodel.expasy.org /

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

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

TMHMM-2.0 server. Accessed 2013. http://www.cbs.dtu.dk/services/TMHMM-2.0/

Touzet, P., and R. F.Buda. (2004). Unveiling the molecular arms race between two conflicting genomes in cytoplasmic male sterility. Trends Plant. Sci. 9: 568-570.

Tretter, L., and V. Adam-Vizi. (2000). Inhibition of Krebs cycle enzymes by hydrogen peroxide: a key role of a-ketoglutarate dehydrogenase in limiting NADH production under oxidative stress. J. Neuro. Sci. 20: 8972-8979.

Wan, C., S. Li, L. Wen, J. Kong, K. Wang, and Y. Zhu (2007). Damage of oxidative stress on mitochondria during microspores development in Honglian CMS line of rice. Plant Cell Rep. 26: 373-382.

Wang, Y., K. J. Addess, J. Chen, L. Y. Geer, J .He, S.

He, S. Lu, T. Madej, A. Marchler-Bauer, P. A. Thiessen, N. Zhang, and S. H. Bryant.(2007). MMDB: annotating protein sequences with Entrez's 3D-structure database. Nucleic. Acids. Res. 35: 298-300.

Wise, R. P., and D. R. Pring. (2002). Nuclear-mediated mitochondrial gene regulation and male fertility in higher plants: Light at the end of the tunnel. Proc. Natl. Acad. Sci. USA. 99: 10240-10242.