Full Length Research Paper

Cloning and characterization of ATP synthase CF1 α gene from sweet potato

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ATP synthase CF1 α subunit protein is a key enzyme for energy metabolism in plant kingdom, and plays an important role in multiple cell processes. In this study, the complete *atpA* gene (accession no. JN247444) was cloned from sweet potato (*Ipomoea batatas* L. Lam) by reverse transcriptase-polymerase chain reaction (RT-PCR). This *atpA* gene contains an open reading frame (ORF) of 1524 bp coding for a peptide of 507 amino acids with a molecular mass of 55.36 kD. Sequence analysis showed that *atpA* gene from sweet potato has high homology with the other plant chloroplast *atpA*. The transcript levels of the *atpA* gene in young leaves, mature leaves, stems and tuberous roots were examined by the digital gene expression profiling (DGE), and then confirmed by semi-quantitative RT-PCR. The results demonstrate that the highest transcription of *atpA* gene was found in young leaves, but it was relatively lower in other three tissues. In addition, the *atpA* gene was successfully expressed in *Escherichia coli*.

Key words: Sweet potato, *atpA* gene, gene expression, digital gene expression profiling, quantitative analysis.

INTRODUCTION

Sweet potato (*Ipomoea batatas* L. Lam) is the seventh important crop in the world and has been planted widely due to its high and stable yield, strong adaptability, rich nutrient content, high photosynthetic capacity, being easy to manage and multiple uses.

ATPase is the key enzyme for energy metabolism in the vegetation and occurs from eukaryotic chloroplast, mitochondria to bacteria, and widely takes part in oxidative phosphorylation and photosynthetic phosphorylation. ATPase consists of two rotary motors named as Fo and F1. F1 consists of 5 subunits named as α, β, γ, δ and ε (Hudson et al., 1987; Zurawski et al., 1982; Boekema and Bjttcher., 1992; Unji et al., 1988).

The α subunit of ATPase is a key chloroplast envelope membrane protein encoded by *atpA* gene of chloroplast genome, which has extra conservation and low mutation rate in plant kingdom and therefore used as the object of the evolution and classification (Palmer., 1987; Gao et al., 2009; Xu et al., 2001). Previous studies showed that the expression of *atpA* gene associated to cold-resistance because under low temperature the change at the transcriptional level was positively related to ATPase activity (He et al., 2005; Mao et al., 2003; Ganim et al., 1998). The activity of ATPase played an important role in promoting plant resistance to cold weather. ATPase activity has been found to be associated with plant male sterility since the plant with ATPase mutation would suffer from shortage of energy supply during flower development, thus resulting in abnormal microspore development (Ducos et al., 2001; Ellora et al., 1987; Hernould and Suharto., 1993). The sequence differences of *atpA* genes in the maintainer and cytoplasmic male sterility (CMS) have also been shown to have an important influence on the male sterility (Siculella et al., 1990; Zhang et al., 2003).

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Abbreviations: ATP, Adenosine triphosphate; **RT-PCR,** reverse transcriptase-polymerase chain reaction; **ORF,** open reading frame; **DGE,** gene expression profiling.

Digital gene expression profile (DGE) is a novel approach to genome-wide expression profiling using next generation sequencing rather than hybridization methodologies (Peter et al., 2008). This approach allows researchers to identify, quantify and annotate the expression of any transcript from any organism, and is even more accurate and repeatable than fluorescent quantitation PCR. The Ib*atpA* probably plays important roles in metabolic pathways in sweet potato. However, Ib*atpA* gene in this important crop has not been cloned and studied. For this purpose, we describe the isolation, characterization and the issue specific expression pattern of Ib*atpA* gene in sweet potato.

MATERIALS AND METHODS

Strains and materials

The sweet potato cultivar Xushu18 was obtained from Sichuan Academy of Agricultural Sciences, and planted in the experimental farm of Sichuan University with exposure to natural sunlight from June to November of 2009. Samples for experiments grew normally as wild. Various tissues/organs including young leaves, mature leaves and stems were sampled at 90 days after planting. Harvested tuberous roots were gained at fifth month after planting. Tissues of sweet potato were harvested, washed and frozen immediately in liquid nitrogen for short store. pET32a(+) was used for constructing prokaryotic expression vector. *Escherichia coli* strains of JM109 and BL21(DE3) were used for routine cloning and recombinant expression, respectively.

Isolation of total RNA and cloning of *atpA* gene

Total RNA was extracted using TRIZOL (Invitrogen) following the instructions of the manufacturer. First-strand cDNA was synthesized from total RNA of sweet potato Xushu 18 by M-MLV reverse transcriptase (Invitrogen). Reverse transcription (RT) was performed using the primer oligo(dT)$_{15}$. The following conditions were used: incubation at 37°C for 2 min, then addition of 1 µL (200 U) M-MLV reverse transcriptase, incubation at 37°C for 50 min, heating at 70°C to terminate the reaction. The resulting RT-products were used to amplify *atpA* gene by PCR with the forward primer atpA-F (5'-GAAAACCTGTACTTCCAGGGTATGGTAACCATTCAAGCCG-3') and the reverse primer atpA-R (5'-GTTTAGAGGCCCCAAGGGGTTATACTTGTTGCTGAAGTAGAAAAC-3'). The nucleotide sequences of the above primers were designed based on the corresponding nucleotide sequences of *atpA* from other plants and sweet potato transcriptome database (our laboratory database). The underlined nucleotide sequences are identical to the vector pET32a(+), which was used to clone *atpA* gene into the vector via sequence and ligation independent cloning (SLIC) method (Cheo et al., 2004; Wang et al., 2010).

The PCR reaction was performed using DNA polymerase KOD-Plus-Neo (TOYOBO) under the condition including a pre-denaturation at 94°C for 2 min, and following 30 cycles of denaturation at 98°C for 10 s, annealing at 54°C for 30 s, extension at 68°C for 90 s, and final extension at 68°C for 10 min. The PCR product was analyzed and recovered from 1.5% (w/v) agarose gel.

For preparing the vector DNA fragment, a pair of primers were designed based on the pET32a(+) sequence. The primers contained the following sequences: pET-F (5'-TTTTCTACTTCAGCAACAAGTATAACCCCTTGGGGCCTCTAAAC-3'),pET-R(5'-ACCCTGGAAGTACAGGTTTTCACCAGAAGAATGATGATGATGATGG-3'). The underlined sequences are complemented to the *atpA* gene. The PCR reaction was performed under the condition at 94°C for 2 min, 30 cycles with 98°C for 10 s, 54°C for 30 s and 68°C for 3 min. The amplified vector sequence was recovered from the agarose gel. The purified *atpA* and vector DNA fragments were treated by T4 DNA pol (TAKARA) for 30 s at 37°C, respectively. Afterward, these two PCR products were mixed and annealed under the condition containing an initial denaturation at 75°C for 10 min and cooling down to the room-temperature naturally. Finally, the annealed DNA mixture was directly transformed into *E. coli* competent cells. The resulting recombinants were verified by PCR and *Xba* I digestion, and then nucleotide sequencing. The confirmed recombinant plasmid was named as pE32-IPatpA.

Isolation of genomic sequence

Total DNA of flesh tissues was extracted as template for PCR to obtain genomic sequence of *atpA*. One pairs of PCR primers were used same with the cDNAs of *atpA*. The purified *atpA* fragment was also ligated to the amplified pET32a(+) vector DNA fragment as mentioned above by SLIC. Recombinants were verified by PCR and *Xba*I digestion, and the nucleotide sequencing was carried out soon after.

Bioinformatics analysis of sequence data

Database searches and the protein analysis (including molecular weight) were performed with the NCBI server (http://www.ncbi.nlm.nih.gov) and the ProtParam tool (http://www.expasy.ch/tools/protparam.html). Multiple protein sequence alignments were analyzed using the software DNAMAN. Phylogenetic analysis and tree construction were completed using MEGA4.0 (Tamura et al. 2007). The nucleotide-binding domain and nucleotide-binding active site prediction of the deduced amino acid sequence were performed using InterProScan Sequence Search of EMBL-EBI (http://www.ebi.ac.uk/Tools/pfa/iprscan/). The SignalP 3.0 server (http://www.cbs.dtu.dk/services/SignalP/) and TMHMM Server ver. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/) were used to predict the AtpA signal peptide and transmembrane regions. The NetPhosK 1.0 (http://www.cbs.dtu.dk/services/NetPhosK/) was used to predict kinase-specific phosphorylation sites.

Digital gene expression profiling

The raw 21 bp DGE tags coming from four different tissues of young leaves, stems, mature leaves and harvested tuberous roots were generated according to the Illumina pipeline and the clean tags were mapped to *atpA* complete CDS using Bowtie (Langmead et al., 2009a,b; Trapnell et al., 2009) at Galaxy's web platform allowing only one base mismatch. All clean tags corresponding to *atpA* gene gave a raw expression level in different tissues, and then an empirical approach described by edgeR package (Robinson et al., 2010) was used to normalize the expression levels by estimating the bias introduced by RNA composition (Robinson and Oshlack, 2010) and different sequencing library size. After the normalizing analysis, a more accurate and scientific measurement of gene expression model in different tissues was obtained ('t Hoen et al., 2008; Morrissy et al., 2009).

Semi-quantitative RT-PCR

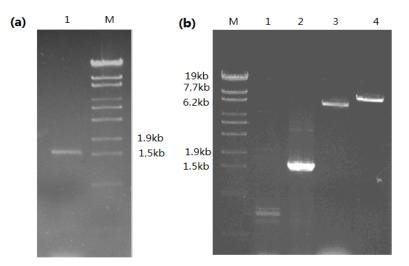Semi-quantitative RT-PCR was performed to validate the results off

Figure 1. (a) The PCR result of the cDNA. M, DNA marker; lane 1, full length cDNA of *atpA* gene by RT-PCR. (b) Identification of the recombinant clone by PCR and restriction enzyme digestion. M: DNA marker; lane 1, the PCR product of empty pET-32a (+); lane 2, the PCR product of pE32-IPatpA; lane 3, the digested product of empty pET-32a (+) by *Xba* I; lane 4, the digested product of pE32-IPatpA.

DGE-tag profiling. The housekeeping gene of glyceraldehyde-3-phosphate dehydrogenase gene (*GAPDH*, SpEST212, CB330166.1) was used as an internal control to normalize the PCR efficiency. According to *GAPDH* and *atpA* gene of sweet potato, two pairs of primers were designed as follows: GAPDHsq-F (5'-GAACTGAGAAAAAGGCTACC), GAPDHsq-R (5'-CCCATTCGTTGTCATACC), atpAsq-F (5'-TAATGTGGGTATCTCTGTTTCC) and atpAsq-R (5'-CTTCTGCTTCCTCGGTA). The semi-quantitative RT-PCR data were calibrated relative to the corresponding gene expression level in four different tissues from sweet potato.

Recombinant expression of atpA gene

The recombinant plasmid pE32-IPatpA was transformed into *E. coli* BL21(DE3) competent cells and selected on LB plates amended with 50 μg/ml ampicillin according to the molecular cloning (Sambrook et al., 1989). A single colony of the transformed *E. coli* was inoculated into 3 ml of LB medium (containing 50 μg/ml ampicillin) and was grown at 37°C overnight. Then, each 10 ml of LB medium plus 50 μg/ml ampicillin was inoculated with 0.5 ml of the above culture and grown at 37°C. When cell density of the cultures reached an A_{600} up to about 0.5, various concentrations of IPTG were separately added in each 10 ml of cultures to induce gene expression. The cultures were subsequently incubated at 18°C for 16 h. The cells from 1 ml of each culture were precipitated by centrifugation and suspended with 0.1 ml SDS-PAGE loading buffer. Finally, 30 μL sample was separated on 10% SDS-PAGE.

RESULTS

Cloning and analysis of *atpA* gene

The *atpA* gene encoding ATP synthase CF1 α subunit from sweet potato was successfully amplified by RT-PCR

from total RNA using the specific primers. The recombinant clones were initially confirmed by *Xba* I digestion and PCR (Figure 1). DNA sequencing revealed that the cDNA was 1524 bp in length, coding for an open reading frame of 507 amino acids with the predicted molecular weight of 55.36 kD and pI of 5.25, respectively (Figure 2), and this gene was designated as *IbatpA*. The sequence of *IbatpA* has been deposited in GenBank with the accession number JN247444. The prediction results of the ProtParam tool showed that this polypeptide with an aliphatic index of 106.43 and the grand average of hydropathicity (GRAVY) of 0.010, suggesting that this polypeptide was a hydrophobin. In the high-level structure assay, AtpA peptide contained deduced α helixes (221 amino acids, 43.59%) and extended strands (115 amino acids, 22.68%) that can be separated into 3 groups, and some random coils (171 amino acids, 33.73%), indicating a rather compacted structure. By InterProScan sequence search, nucleotide-binding domain (149 to 365) and nucleotide-binding active sites (356-365) were found in AtpA peptide (Figure 2). Moreover, NetPhosK analysis indicated that the AtpA protein may possess multiple phosphorylation sites, such as PKA, PKB, PKC, PKG, CKII, et al. Therefore, this hints that the AtpA may be served as substrates of some kinases involved in energy metabolism, signal transduction, even low-temperature reaction.

In addition, standard nucleotide-nucleotide and protein-protein Basic Local Alignment Search Tool (BLAST) were performed. The results revealed that the *atpA* gene of sweet potato shared a high identity in nucleotide and amino acid sequence with the chloroplast *atpA* from other plants. Multiple amino acid sequences alignment of the

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1     ATGGTAACCATTCAAGCCGACGAAATTAGTAATCTTATCCGTGAACGTATTGAACAATATAATAGAGAAGTCAATATTGTAAATACCGGT
1      M  V  T  I  Q  A  D  E  I  S  N  L  I  R  E  R  I  E  Q  Y  N  R  E  V  N  I  V  N  T  G

91    ACCGTACTTCAAGTAGGCGATGGCATTGCTCGTATTTACGGTCTTGATGAAGTAATGGCAGGGGAATTGGTAGAATTTGAAGAGGGTACA
31     T  V  L  Q  V  G  D  G  I  A  R  I  Y  G  L  D  E  V  M  A  G  E  L  V  E  F  E  E  G  T

181   ATCGGCATTGCTCTCAATTTGGAATCAAAAAATGTTGGTGTTGTATTAATGGGCGATGGGTTGATGATACAAGAAGGAAGTTCTGTAAAA
61     I  G  I  A  L  N  L  E  S  K  N  V  G  V  V  L  M  G  D  G  L  M  I  Q  E  G  S  S  V  K

271   GCAACGGCAAGAATTGCTCAGATACCAGTGGGTGACGCCTATTTGGGTCGTGTTATAAATGCCCTGGCTAACCCTATTGATGGTAGGGGC
91     A  T  A  R  I  A  Q  I  P  V  G  D  A  Y  L  G  R  V  I  N  A  L  A  N  P  I  D  G  R  G

361   GAAATTTCAGCTTCTGGATTTCGATTAATTGAATCCCCTGCTCCGGGGATTATTTCGCGTCGTTCCGTATATGAGCCTCTTCAAACCGGG
121    E  I  S  A  S  G  F  R  L  I  E  S  P  A  P  G  I  I  S  R  R  S  V  Y  E  P  L  Q  T  G

451   CTTATTGCTATCGATTCGATGATCCCTATAGGACGTGGTCAGCGAGAATTAATTATTGGGGACAGACAAACCGGTAAAACAGCAGTAGCC
151    L  I  A  I  D  S  M  I  P  I  G  R  G  Q  R  E  L  I  I  G  D  R  Q  T  G  K  T  A  V  A

541   ACGGATACGATTCTCAATCAACAAGGTCAAAATGTAATATGTGTTTATGTAGCTATTGGGCAAAAGCATCTTCTGTGGCTCAGGTAGTT
181    T  D  T  I  L  N  Q  Q  G  Q  N  V  I  C  V  Y  V  A  I  G  Q  K  A  S  S  V  A  Q  V  V

631   ACTACCTTACAGGAAAGGGGGGCGATGGAATACACTATTGTGGTAGCCGAAACGGCGGATTCCCCTGCTACATTACAATACCTCGCTCCT
211    T  T  L  Q  E  R  G  A  M  E  Y  T  I  V  V  A  E  T  A  D  S  P  A  T  L  Q  Y  L  A  P

721   TATACAGGAGCAGCTCTGGCTGAATATTTTATGTATCGTGAAAGACACACTTTAATCATTTATGATGATCTCTCCAAACAAGCCCAAGCT
241    Y  T  G  A  A  L  A  E  Y  F  M  Y  R  E  R  H  T  L  I  I  Y  D  D  L  S  K  Q  A  Q  A

811   TATCGCCAAATGTCTCTTCTATTACGCAGACCGCCCGGTCGCGAAGCTTATCCAGGAGATGTTTTTTATTTGCACTCACGCCTTTTGGAA
271    Y  R  Q  M  S  L  L  L  R  R  P  P  G  R  E  A  Y  P  G  D  V  F  Y  L  H  S  R  L  L  E

901   AGAGCTGCTAAATTAAGTTCTAAGTTAGGTGAGGGGAGTATGACCGCCTTACCTATAGTTGAAACGCAATCGGGAGATGTTTCGGCTTAT
301    R  A  A  K  L  S  S  K  L  G  E  G  S  M  T  A  L  P  I  V  E  T  Q  S  G  D  V  S  A  Y

991   ATTCCTACTAATGTAATTTCCATTACTGATGGACAAATATTCTTATCCGCCGATCTATTCAATGCTGGAATCAGACCTGCTATTAATGTG
331    I  P  T  N  V  I  S  I  T  D  G  Q  I  F  L  S  A  D  L  F  N  A  G  I  R  P  A  I  N  V

1081  GGTATCTCTGTTTCCCGAGTGGGGTCTGCAGCTCAAATTAAAGCCATGAAACAGGTAGCTGGTAAATTAAAATTGGAACTGGCGCAATTC
361    G  I  S  V  S  R  V  G  S  A  A  Q  I  K  A  M  K  Q  V  A  G  K  L  K  L  E  L  A  Q  F

1171  GCAGAATTAGAAGCCTTTGCACAATTTGCTTCTGATCTCGATAAAGCTAGTCAAAATCAATTGGCAAGAGGTCAACGATTACGTGAATTG
391    A  E  L  E  A  F  A  Q  F  A  S  D  L  D  K  A  S  Q  N  Q  L  A  R  G  Q  R  L  R  E  L

1261  CTTAAACAATCCCAATGCGCTCCTCTCTCGGTGGCAGAGCAGATAATAACTATTTTTACTGGAATAAATGGTTATCTTGATTCATTAGAA
421    L  K  Q  S  Q  C  A  P  L  S  V  A  E  Q  I  I  T  I  F  T  G  I  N  G  Y  L  D  S  L  E

1351  CTTGGACAGGTAAAGAAATTTATTGTTGAGTTACGTACTTACTTAAAAACTAATAAACCTCGGTTCGAAGAAATCATATCTTCTACTAAG
451    L  G  Q  V  K  K  F  I  V  E  L  R  T  Y  L  K  T  N  K  P  R  F  E  E  I  I  S  S  T  K

1441  ATATTTACCGAGGAAGCAGAAGCCCTTTTGAAAGAAGCTATTCGGGACCAAATGGATCGTTTTCTACTTCAGCAACAAGTATAA
481    I  F  T  E  E  A  E  A  L  L  K  E  A  I  R  D  Q  M  D  R  F  L  L  Q  Q  Q  V
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Figure 2. The nucleotide sequence and the deduced amino acid sequence of the full-length cDNA of *atpA*. The nucleotide-binding domain is underlined, and the nucleotide-binding active sites are boxed.

AtpA proteins were derived from sweet potato (accession no.JN247444) and other plants (Figure 3). The amino acid sequence of AtpA from sweet potato has an overall identity from 91.5 to 99.0% with the selected 22 species of plant, respectively. Phylogenetic analysis of *IbAtpA* and other plant chloroplast AtpA proteins indicated that *IbAtpA* protein exhibited some similarities with that of *Ipomoea purpurea* (99.0%) (Figure 3). According to sequenced result and phylogenetic analysis, *IbatpA* was proved to be a chloroplast gene.

Genomic DNA amplification and comparison for *IbatpA*

The primers of *atpA*-F and *atpA*-R were also applied to amplify the genomic fragment corresponding to the cDNA of *atpA*. A 1524-bp DNA fragment was obtained from genomic DNA, which was completely identical to the cDNA sequence (data not shown) and no intron was present in *IbatpA* genomic DNA. The Blast result in NCBI demonstrated that *IbatpA* gene was most likely encoded by the chloroplast genome. Hence, sweet potato

contains only a single copy of *IbatpA* gene.

Digital gene expression profiling

On the basis of the results of the digital gene expression profiling, the transcripts of *IbatpA* in four different tissues were counted as: 26.84 TPM (transcript per million) for young leaf; 1.16 TPM for stem; 3.21 TPM for mature leaf; 3.11 for harvested tuberous root (Figure 4). The results show that the highest transcription level of *IbatpA* was in young leaf, but lowest in stem, without obvious different between mature leaf and harvested tuberous root.

Semi-quantitative RT-PCR

Subsequently, semi-quantitative analysis was performed to confirm the difference of transcription of *IbatpA* in the four various tissues. The results are shown in Figure 5. The transcription of *IbatpA* in young leaf was higher than other three tissues, while there were no obvious differences amongst the mature leaf, stem and harvested
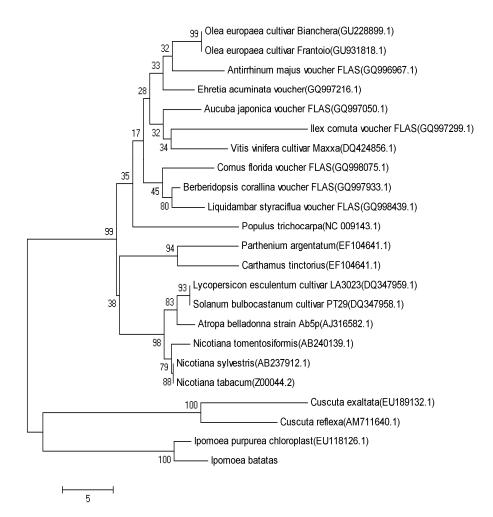
Figure 3. Amino acid sequence phylogenetic tree (Neighbor-joining) of AtpA polypeptide from 23 plant species.
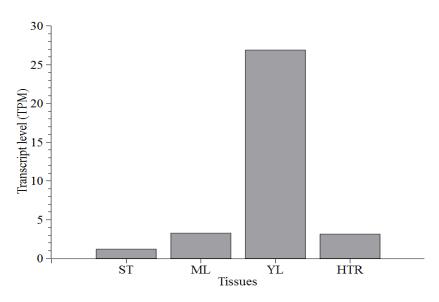

Figure 4. The result of digital gene expression profiling of *atpA* gene in various tissues of sweet potato. TPM means the transcript per million. ST, stem; ML, mature leaf; YL, young leaf; HTR, harvested tuberous root.
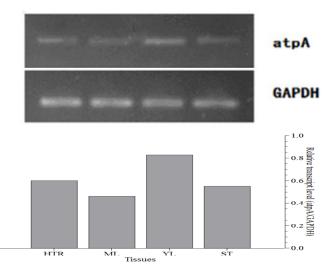
Figure 5. The semi-quantitative PCR analysis of *atpA* gene in various tissues of sweet potato. ST, Stem; ML, mature leaf; YL, young leaf; HTR, harvested tuberous root.
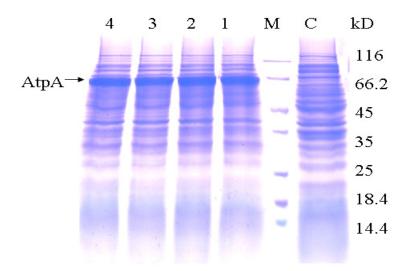

Figure 6. SDS-PAGE analysis of the recombinant expression of sweet potato *atp*A. M, Protein marker; C, empty pET-32a(+) induced by IPTG; lanes 1 to 4, pE32-IPatpA induced by 0.1 , 0.5 , 1 and 2 mM of IPTG, respectively.

tuberous root. Accordingly, the semi-quantitative RT-PCR results were similar to the preliminary data from the digital gene expression profiling.

Recombinant expression

The *IbatpA* gene was expressed in *E. coli* BL(DE3) as a fusion protein with *trx*A. And an expected protein band with a molecular weight of about 66 kD on the SDS-

PAGE was obviously induced (Figure 6). However, there was no distinct difference in protein expression level when different concentrations of IPTG (0.1, 0.5, 1 and 2. mM) were used for induction. More also, the recombinant

DISCUSSION

In this study, *IbatpA* gene which contained 1524 bp nucleotide and encoded a peptide with 507 amino acids

from sweet potato cDNA pool was isolated. ATP-binding sites were separately distributed in α and beta subunits; those sites on the α subunits are regulatory and the sites on the beta subunits are catalytic. The conformation changes of α and beta subunits lead to the formation or hydrolysis of ATP (Leyva et al., 2003). Protein sequence analysis showed that the protein of *atpA* may possess multiple protein kinase receptor sites which occur widely in eukaryotic proteins. Therefore, this protein may be likely involved in various regulatory reactions, especially via phosphorylation and dephosphorylation.

ATPases are membrane-bound enzyme complexes which synthesize and/or hydrolyze ATP by the transport of protons across mitochondrial or chloroplast membrane (Cross and Muller., 2004; Rappas et al., 2004). ATP synthase CF1 α and beta subunits constitute the cylinder of rotary motors, while the gamma subunit forms the rotor (Yasuda et al., 2001). *AtpA* peptide of sweet potato is predicated as a compact hydrophobic protein, which contains larger part of α helixes, but less extended strands and some random coils. No strong signal peptide sequence was found, which was thought as an important part to make up the cylinder of rotary motors, suggesting that the AtpA could not be transported to cytosol from mitochondria or chloroplast.

Also, by digital gene expression profiling and semi-quantitative RT-PCR analysis, the transcript level of *atpA* gene in different tissues was elaborated, and the highest transcription of *atpA* was found in young leaf since photosynthetic phosphorylation and energy metabolism was expected at a higher level in the faster-growing tissues or organs. Besides, *atpA* gene is usually considered as a chloroplast gene. Moreover, the activity of ATPase declined quickly after cold incubation and the decline was reversed completely upon warming of cells (Yoshida et al., 1993). A close correlation between the activity of the enzyme and chilling treat seemed to exist (Kasamo et al., 1988; Zhou et al., 1989; Jian et al., 1983). These results therefore provided a new clue for probing the role of ATPase CF1 α-subunit in plant defence against chilling stress (He et al., 2005). Additionally, cytoplasmic male sterility (CMS) has been demonstrated relative to *atpA* (Yang et al., 2009; Senda et al., 1993; Ji et al., 1998; Kohler et al., 1991). However, most sweet potato cultivars do not bloom under normal growing conditions (Lu et al., 1998). It would be interesting to probe if the flowerless in sweet potato is or not relevant to the energy metabolism.

To our knowledge, there has been no report about an array of ATP associated synthesis or hydrolysis genes in sweet potato, ATP synthesis and related metabolic pathways research in sweet potato is also very limited. Therefore, isolation and characterization of the *atpA* could be the initial step for further studies on the energy metabolism or stress tolerance in sweet potato. Our findings have important implications for studying the conservation of the genetic diversity and origins of sweet

potato.

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