Full Length Research Paper

Cloning and characterization of the 14-3-3 protein gene from *Ipomoea batatas* (L.) Lam

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The 14-3-3 proteins are a group of highly conserved and widely distributed eukaryotic proteins with diverse functions. In plants, it regulates basal metabolism, ion transport, cell passage, enzyme activity and gene expression, and it is also important in disease resistance, anti-oxidation and drought resistance. Based on assembled sequences of sweet potato (*Ipomoea batatas* L. Lam) transcriptome, 14-3-3 protein cDNAs were cloned for the first time, and the sequencing analysis revealed the presence of three highly homologous isoforms of 14-3-3 protein cDNAs, designated as Ib14-3-3a, Ib14-3-3b and Ib14-3-3c. Three cDNA isoforms are all 1176 bp in length with 7 mutation sites and have a 789 bp of open reading frame encoding a polypeptide of 262 amino acids. The results from digital gene expression (DGE) profiling showed that the expression levels of 14-3-3 genes are different among tissues, predominantly in harvested tuberous roots (223.08 TPM) and very low in mature leaves (85.07 TPM) , which were also confirmed by semi-quantitative Reverse transcription polymerase chain reaction (RT-PCR) analysis.

Key words: 14-3-3 protein, *Ipomoea batatas*, gene cloning, digital gene expression profiling, RT-PCR analysis.

INTRODUCTION

14-3-3 proteins were found in the classification research on mammalian brain proteins in 1960s, and are highly conserved regulatory proteins in eukaryotic cells (Aitken et al., 1992; Ferl, 1996). They are named after the migration patterns in DEAE cellulose membrane chromatography and the position of starch gel electrophoresis (Ferl, 2004). Ordinarily, 14-3-3 proteins exist as homodimers or heterodimers, with a molecular weight of 60~70kDa. Each monomer can bind to a target peptide sequence in target proteins (Liu et al., 1995; Xiao et al., 1995; Obsil et al., 2001) and this binding to target peptide sequence could change the activity of target proteins, e.g. the subcellular location of target proteins, or mediate the formation of a protein complex (van Hemert

et al., 2001). The naming of 14-3-3 proteins in different organisms is different, e.g. GF14 for higher plants, BMH1 for yeast, and KCIP-1 for sheep. It has been known that 14-3-3 proteins of vertebrate are encoded by at least seven different genes (Aitken et al., 1995; Aitken, 2006; Xue et al., 2009).

14-3-3 proteins were found in plants in 1990s (Brandt et al., 1992) and they are involved in regulating basic metabolism, ion transport, cell channel, enzyme activity and gene expression (Ferl, 1996; Aducci et al., 2002; Sehnke et al., 2002). Previous research showed that the 14-3-3 protein regulated plant cells in the function of anabolic metabolism, vesicles shuttle, cell cycle, apoptosis and cell signaling, and is crucial in carbon metabolism, such as the synthesis of sugar, starch, amino acid, nucleic acid and protein (Bunney et al., 2002; Fulgosi et al., 2002; Huber et al., 2002; Milne et al., 2002; Roberts et al., 2002; Sehnke et al., 2002). Arabidopsis 14-3-3 protein Gf14λ was shown to interact with several

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proteins, including APX3 (an antioxidant protein that protects plants under oxidative and water-deficit conditions) and AKR2A (a protein involved in disease resistance and protein targeting) (Zhang et al., 1997; Yan et al., 2002; Zheng et al., 2003; Shen et al., 2010). Transgenic cotton expressing the GF14λ gene from *Arabidopsis* demonstrated a "stay-green" phenotype and improved drought tolerance. These lines wilted less and maintained higher photosynthesis than segregated non-transgenic control plants under water-deficit conditions (Yan et al., 2004). Therefore, 14-3-3 protein may play a key role in plant resistance and the increase of production.

Sweet potato (*Ipomoea batatas* L. Lam), a hexaploid (2n=6x = 90)dicotyledonous plant, is the seventh most important crop in terms of production (Loebenstein, 2009), and is extensively used in food, feed and industry (including bio-fuels). During energy crisis, oil price increases and resource shortage become rampant, more attention is given to finding new energy sources such as fuel ethanol that is called "21st century green power" (Ihemere et al., 2006). Development of fuel ethanol industry from non-food crops may ease the energy crisis and also not threaten world food security. The production and application of fuel ethanol with sweet potatoes as raw material may reduce the demand in corn and wheat. Sweet potato cv. Xushu 18 is widely planted in China, and is the leading variety in China both in total planting area and in total root production (Loebenstein and Thottappilly, 2009), but the starch content and stress resistance of this sweet potato variety still need to be improved. Besides, this cultivar is susceptible to some diseases. Therefore, increasing the production of sweet potato, improving disease resistance and stress resistance are significantly important.

Using 14-3-3 proteins, we may be able to improve sweet potato's stress tolerance and disease resistance, we therefore cloned 14-3-3 proteins genes from sweet potato and conducted sequence analysis, protein modeling, digital gene expression profiling, and RT-PCR analysis in different tissues and developmental stages. Our results will lay a solid foundation for the research on increasing production and improving disease-resistance and stress-resistance in sweet potato.

MATERIALS AND METHODS

Plant materials and chemicals

Sweet potato cultivar (*I. batatas* cv. Xushu 18) was planted in the experimental farm of Sichuan University under natural light and temperature from late May to late October. Samples for experiments were taken after transplanting. *Escherichia coli* DH5α, BL21 (DE3) and pET-32a(+) vector were conserved in our laboratory. RNA TRIzol reagent and reverse transcriptase M-MLV were purchased from Invitrogen (CA, USA), and KOD-Plus-Neo for PCR from TOYOBO (Japan). Restriction enzymes, T4 DNA polymerase, Taq DNA Polymerase, dNTP were obtained from Fermentas. Other reagents were commercially available in China.

Sampling and extraction of DNA and RNA

Young leaves, mature leaves, stems, and harvested tuberous roots of sweet potato (Xushu 18) were frozen in liquid nitrogen, and ground in a mortar, then RNAs were extracted using the TRIzol reagent (Invitrogen, CA, USA). Mixed RNAs from all four tissues were used in clone of sweet potato 14-3-3 protein cDNAs, and each tissue sample RNAs were used in digital gene expression profiling and RT-PCR analyses.

DNA extraction by using the CTAB method was adopted as follows, Plant tissues were frozen in liquid nitrogen, and ground in a mortar, extracts were re-suspended in pre-warmed (60°C) CTAB extraction buffer and incubated at 60°C, added into chloroform /isoamylalcohol (24:1) solution, RNase (DNase-free) and isopropanol (2/3 of the recovered volume) was added, spinned at 4°C with 13,000 g for 10 min to pellet the DNA. The pellets were washed with cold alcohol, re-suspended in TE (pH 8.0) and stored at -20°C.

Cloning of sweet potato 14-3-3 protein cDNAs

Total cDNAs were synthesized from 1 µl of mixed RNA (from young leaves, mature leaves, stems, and harvested tuberous roots of Xushu 18) with Moloney murine leukemia virus (M-MLV) reverse transcriptase (Invitrogen, CA, USA) using oligo (dT) as primer following the manufacturer's instructions. The resulting cDNA was subjected to PCR using primers Ib14-3-3F and Ib14-3-3R, which were designed according to the transcriptome data created in our laboratory. The vector primers VectorF and VectorR were designed from the pET-32a(+) (Novagen, Wisconsin, USA). Their sequences are shown below:

Ib14-3-3F: 5′-GAAAACCTGTACTTCCAGGGTATGGTGTCAACTGAGTCAACTC-3′

Ib14-3-3R: 5′-GTTTAGAGGCCCCAAGGGGTTACTGCGGCCCCTCGCCTG-3′

VectorF: 5′-AATCAGGCGAGGGGCCGCAGTAACCCCTTGGGGCCTCTAAAC-3′

VectorR: 5′-ACCCTGGAAGTACAGGTTTTCACCAGAAGAATGATGATGATGATGG-3′

In all four primers, the sequences without underline were from the 5′- and 3′-terminal sequence of 14-3-3 coding region, the underlined sequences were from the vector pET32, and the boxed sequences were designed as the TEV recognition site. The sweet potato 14-3-3 protein cDNA was amplified by primers Ib14-3-3F and Ib14-3-3R. VectorF and VectorR were used for the amplification of vector pET-32a(+). Amplification was carried out with 35 cycles of 2 min at 94°C, 10 s at 98°C, 30 s at 59°C, and 30 s at 68°C with KOD-Plus-Neo (TOYOBO, Japan). The PCR product was sub-cloned into the pET-32a(+) vector by using a modified SLIC method (Sequence and Ligation Independent Cloning: Elledge and Mamie, 2007; Bai et al., 2008; Wang et al., 2010) to generate plasmid pET-23a(+)-IB14-3-3, which was then used to transform *E. coli* strain BL21 (DE3).

Cloning of sweet potato 14-3-3 protein genes from genomic DNAs

Genomic DNA fragments of sweet potato 14-3-3 protein were amplified using KOD FX DNA polymerase (TOYOBO, Tokyo, Japan) by using Ib14-3-3F and Ib14-3-3R. The T-cloning method
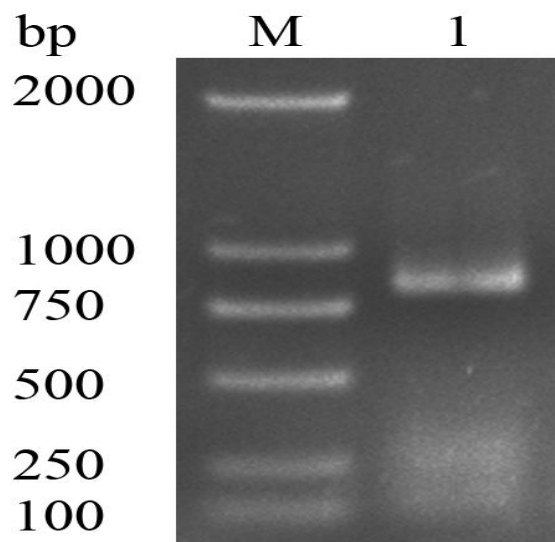
Figure 1. PCR amplification of an ORF from 14-3-3 protein gene ORF from *Ipomoea batatas.* Lane M, molecular weight marker (DL2000); lane 1, PCR product.

was employed to clone the 14-3-3 protein genes by using pGM-T vector (TIANGEN BIOTECH, Beijing, China).

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 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 14-3-3 protein signal peptide and transmembrane regions. The three-dimensional model build by SWISS-MODEL (http://swissmodel.expasy.org/workspace/index.php?func=modelling_simple1) (Guex and Peitsch, 1997; Schwede et al., 2003; Arnold et al., 2006).

Expression of recombinant sweet potato 14-3-3 protein gene

The transformant colony of recombinant sweet potato 14-3-3 protein gene was grown overnight at 37°C with constant shaking in 3 mL LB medium containing 50 g/L ampicillin. The overnight culture was diluted to 1:100 with 100 mL LB containing 50 g·L^{-1} ampicillin and grew at 37°C for about 2 h. Then IPTG was added in a final concentration of 0.1 mM to 2 mM to induce the expression of recombinant sweet potato 14-3-3 protein and the growth was continued at 18°C for an additional 16 h. The control culture was grown under the same conditions except that no IPTG was added. Cells were collected and resuspended in 5 mL of lysis buffer (50 mM Tris, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.1 mM PMSF, and 0.1% Triton X-100). The cell suspension was sonicated with a Branson ultrasonic disintegrator for 10 short bursts of 3 s followed by intervals of 2 s in a cooling bath. Cell lysates were boiled for 15

min at 100°C and then mixed with 5×SDS-PAGE loading buffer (250 mM Tris-HCl pH 6.8; 10% SDS; 0.5% Bromphenol Blue; 50% Glycerol; 5% β-Mercaptoethanol).

Digital gene expression profiling of sweet potato 14-3-3 protein gene

The raw 21bp DGE tags coming from seven different tissues: young leaves, mature leaves, stems, fibrous roots, initial tuberous roots, expanding tuberous roots and harvested tuberous roots were generated according to the Illumina pipeline, and the clean tags were mapped to 14-3-3 protein gene complete CDS using Bowtie (Langmead et al., 2009; Langmead et al., 2009; Trapnell et al., 2009) at Galaxy's web platform allowing only one base mismatch. All clean tags corresponding to 14-3-3 gene gave a raw expression level for different tissues, and then we used an empirical approach described by edgeR (Robinson et al., 2010) package 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 could be obtained.

Semi-quantitative reverse transcription-PCR (RT-PCR) analyses

Total RNAs (2.5 μg) from each sample of sweet potato were reversely transcribed, and the resulting cDNA solution was then diluted tenfold with TE (10 mM Tris–HCl, pH 8.0, 1 mM EDTA). Semi-quantitative RT-PCR reaction was performed within linear range of amplification for each targeted fragment examined at 28 cycles for stems and harvested tuberous roots with the template of 0.5 μl, and 30 cycles for young leaves and mature leaves with the template of 1 μl, and 25 cycles for the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene of sweet potato. Gene-specific primers for 14-3-3 protein were RT-5490F (TTGAAGATGAAAGGCGATTA) and RT-5490R (TCAGAAGTCCACAGCGTTA), and those for GAPDH were GAPDH-F (GAACTGAGAAAAAGGCTACC) and GAPDH-R (CCCATTCGTTGTCATACC). GAPDH gene expression levels were served as internal controls for quantification of relative amounts of cDNAs.

RESULTS

Cloning of sweet potato 14-3-3 protein cDNAs

One cDNA sequence of 1176 bp from transcriptome database of sweet potato subjected to BLAST-n analysis showed high similarity to known 14-3-3 protein genes from *Chlamydomonas reinhardtii*, Tobacco, *Pisum sativum L.*, Arabidopsis, Tomato, *Ipomoea nil* and other organisms, implying that it may be a fragment of a 14-3-3 protein gene. This 14-3-3 protein gene cDNA has an ORF of 789 bp from position 181 to 969 of the sequence, except for a 180 bp 5′-UTR and a 207 bp 3′-UTR. In order to know more information about the sweet potato 14-3-3 protein gene and express this gene in *E. coli*, its ORF was amplified using primers Ib14-3-3F and Ib14-3-3R (Figure 1) and cloned.

Sequencing of 15 clones revealed the presence of three highly homologous 14-3-3 cDNAs, which were

Table 1. Differences in nucleotides and amino acids among sweet potato 14-3-3 genes.

Sites	216	240	526	534	634	765	772
Ib14-3-3a	C	T	C	A	C	A	G
Ib14-3-3b	T	T	A	C	C	G	C
Ib14-3-3c	C	G	A	A	A	G	G
Amino acid changed	none	none	none	none	L→I	none	G→R

Figure 2. Predicted three dimensional structure of sweet potato 14-3-3 protein.

designated as Ib14-3-3a (GenBank accession number: JN564798), Ib14-3-3b (GenBank accession number: JN564799) and Ib14-3-3c (GenBank accession number: JN564800), respectively. For each gene in this study, the absence of PCR-introduced mutations was verified by the identical sequences obtained from products of different PCRs. Furthermore, Direct sequencing of the PCR product of the full-length ORFs was also carried out and the presence of three highly similar isoforms was confirmed. All ORFs encode a polypeptide of 262 amino acid residues and shared 99.7% identity of the nucleotides and 99.75% identity of the amino acids. There are 7 nucleotides differences among three 14-3-3 protein ORFs, which causes 2 amino acid replacements (Table 1).

Bioinformatics analysis of sequence data

Protein sequence prediction analysis revealed that the molecular weight of sweet potato 14-3-3 proteins is 29.5 kDa and the isoelectric point for all three proteins is 4.46. Glu and Leu are the most two abundant amino acids, accounting for 13.4 and 10.3% respectively. The result from SignalP 3.0 Serve analysis (Bendtsen et al., 2004)

showed that this protein contains a signal peptide of 29 amino acid residues at the N-terminus. The three-dimensional model build by SWISS-MODEL (Guex and Peitsch, 1997; Schwede et al., 2003; Arnold et al., 2006) demonstrated that they contain 9 alpha helix bundles and 8 loops (Figure 2).

Homology analyses of sweet potato 14-3-3 proteins

Using the BlastN program to search the NCBI database, we found that the nucleotide sequences of the sweet potato 14-3-3 protein genes are highly similar to other 14-3-3 protein genes found in other species, and so are the amino acid sequences for sweet potato 14-3-3 proteins. For example, the amino acid sequences of 14-3-3 proteins of sweet potato are almost identical to those of *I.nil*, *Manihot esculenta* and *Gossypium hirsutum*, while above 93% homology with those in other plant species. To determine the relationship among the 14-3-3 proteins of sweet potato and other plants, phylogenetic analysis was carried out. The results revealed that the genetic relationship of the three 14-3-3 proteins between sweet potato and *I. nil* are very closed (Figure 3).

Cloning of sweet potato 14-3-3 protein gene genomic DNAs

Genomic DNA fragments of 1698 bp were also successfully cloned with the PCR products amplified by primer pairs Ib14-3-3F/Ib14-3-3R, and the coding sequences was found to be identical to those of Ib14-3-3a, Ib14-3-3b and Ib14-3-3c, respectively. Comparison of the genomic DNA sequences (Ib14-3-3A, Ib14-3-3B and Ib14-3-3C) and cDNA sequences revealed that sweet potato 14-3-3 protein gene contained 5 introns with the identical positions but different sequences.

Expression analyses of sweet potato 14-3-3 protein gene

Both semi-quantitative RT-PCR and digital gene expression were performed to determine the transcript levels of sweet potato 14-3-3 protein gene in different organs, including young leaves, mature leaves, stems,
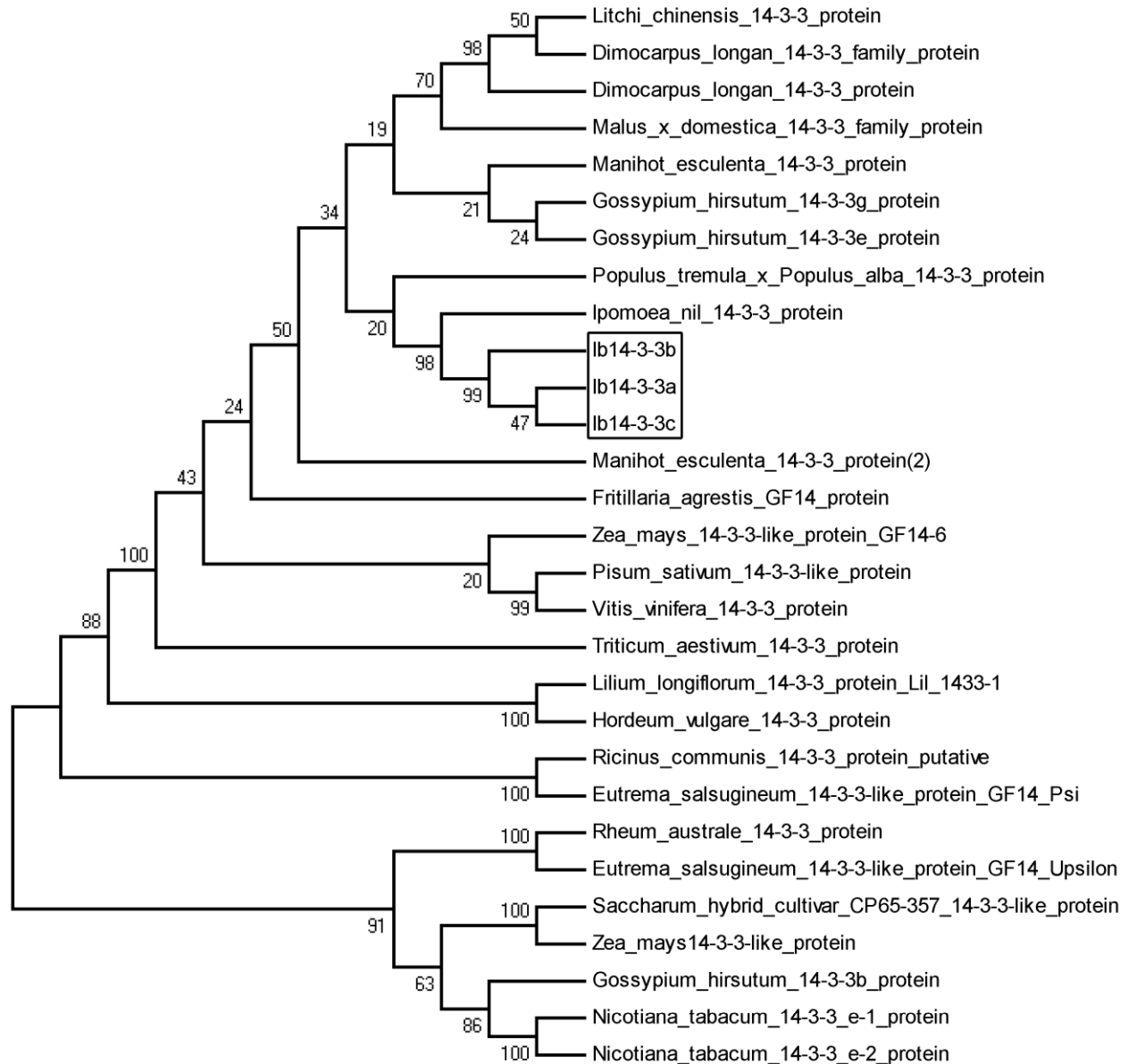
Figure 3. Phylogenetic analysis of Ib14-3-3a, Ib14-3-3b and Ib14-3-3c with other 14-3-3 proteins: *Nicotiana tabacum* gi |44917151|, *Dimocarpus longan* gi |291162643|, *Malus x domestica* gi |55375985|, *Hordeum vulgare* gi |2266662|, *Ipomoea nil* gi |124484407|, *Litchi chinensis* gi |310007371|, *Manihot esculenta* gi |67107029|, *Populus tremula x Populus alba* gi |8515888|, *Rheum australe* gi |197312897|, *Solanum lycopersicum* gi |22217852|, *Triticum aestivum* gi |40781605|, *Vitis vinifera* gi |226295434|, *Lilium longiflorum* gi |126508568|, *Ricinus communis* gi |255545792|, *Gossypium hirsutum* gi | 193290377|, *Manihot esculenta* gi |291293221|, *Pisum sativum* gi |4850247|, *Saccharum hybrid cultivar* CP65-357 gi |37903393|, *Zea mays* gi |162458469|, *Eutrema salsugineum* gi |309952059|, 14-3-3-like protein gi |12229593|, *Brassica napus* gi |224981577|, 14-3-3-like protein E gi |3912950|, *Fritillaria agrestis* gi |2921512|. The three 14-3-3 proteins identified in the present study were boxed. The bootstrap consensus tree from 1000 replicates was constructed based on neighbor-joining method, using MEGA 3.1 (http://www.megasoftware.net/) from initial multiple alignment performed by ClustalX 1.83 (http://www.clustal.org/).

and harvested tuberous roots. Due to the high similarity among the three different 14-3-3 protein genes, it was difficult to distinguish them accurately by semi-quantitative RT-PCR technique. Therefore, primers designed from conserved coding sequences of three 14-3-3 protein genes were used and a 337 bp PCR-amplified band was observed. The results displayed that 14-3-3 protein gene was expressed predominantly in harvested tuberous roots, but very low in mature leaves (Figure 4).

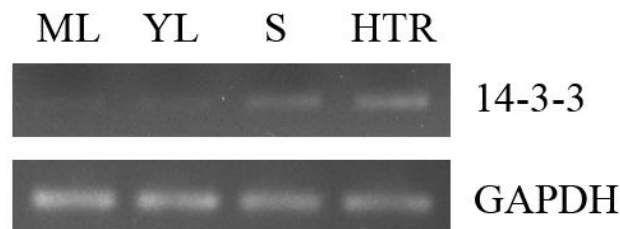A similar result was obtained from the digital gene

Figure 4. RT-PCR analyses of 14-3-3 protein gene in different tissues of sweet potato. At least two independent RT-PCR experiments were carried out in each case, and a representative data are presented here. GAPDH (glyceraldehyde-3-phosphate dehydrogenase) of sweet potato served as an internal loading control. YL, young leaves; ML, mature leaves; S, stems; HTR, harvested tuberous roots.
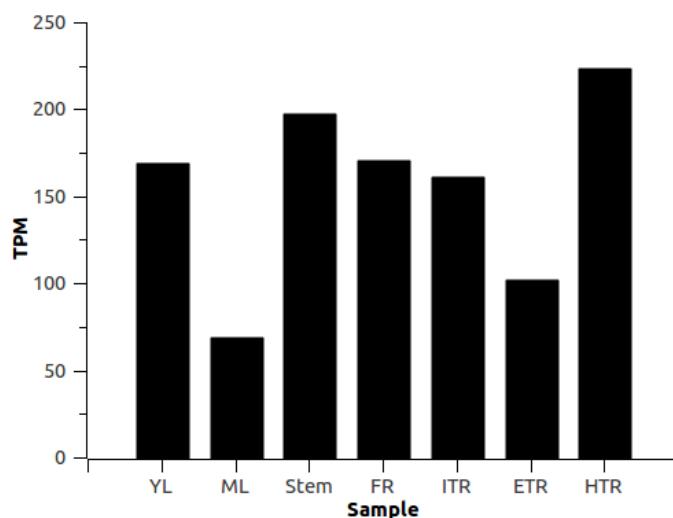

Figure 5. Digital gene expression profiling pattern of 14-3-3 proteins in different tissues or developmental stages of sweet potato. YL, young leaves; ML, mature leaves; Stem, stems; FR, fibrous roots; ITR, initial tuberous roots; ETR, expanding tuberous roots; HTR, harvested tuberous roots.

expression profiling and showed that the expression level of 14-3-3 protein gene in seven different tissues is varying (Figure 5). 14-3-3 protein genes were predominantly expressed in harvested tuberous roots (223.08 TPM), but very low in mature leaves (85.07 TPM). The differences of expression levels in young leaves, stems, fibrous roots and initial tuberous roots were not big (Figure 5).

Expression of recombinant sweet potato 14-3-3 protein gene

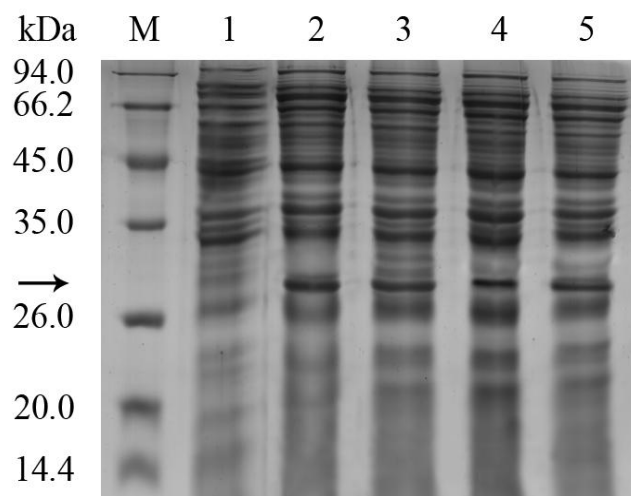14-3-3 fusion protein was expressed in *E. coli* strain BL21

Figure 6. SDS-PAGE analysis of the expressed product of a recombinant 14-3-3 protein gene. M, protein molecular weight standard; Arrows indicate the target protein bands; lane 1, engineered bacteria strain harboring pET-32a(+) after induced for 16 h under 18°C; lanes 2-5, total proteins of engineered bacteria strains after induced for 16 h under 18°C, the IPTG concentration are 0.1, 0.5, 1 and 2 mM respectively.

(DE3). After sonication, centrifugation and SDS-PAGE analysis, the fusion protein was found to be mainly expressed in the inclusion body. After coomassie blue staining, the purified protein displayed as a distinct, clear band of around 29 kD. This band was matched with the expected size of the sweet potato 14-3-3 fusion protein (Figure 6).

DISCUSSION

Since 14-3-3 proteins were found in plants in 1990s, researches are mainly focused on the regulatory roles of 14-3-3 proteins in enzyme activities such as H^+-ATP enzyme on plasma membrane (Bunney et al., 2002; Fulgosi et al., 2002; Huber et al., 2002; Milne et al., 2002; Roberts et al., 2002; Sehnke et al., 2002). In plants, 14-3-3 protein plays an very important role in the regulation of basal metabolism, ion transport, cell passage, enzyme activity and gene expression, which is important in disease resistance, anti-oxidation and drought resistance, and is the regulate center of glucose metabolism, protein metabolism and nucleic acid metabolism (Bunney et al., 2002; Fulgosi et al., 2002; Huber et al., 2002; Milne et al., 2002; Roberts et al., 2002; Sehnke et al., 2002).

Therefore, it is reasonable to link improving expression of 14-3-3 genes and improving cellular metabolism. The functions of 14-3-3 protein in drought resistance and stress resistance are widely reported in literatures. As the earth environmental deterioration, this is more and more important for sweet potato and other higher plants

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ORIGIN
ib14-3-3          ..MVSTESTREENVYMAKLAEQAERYEEMVEFMEKVAKTVDV.DELTVEERNLLSVAYKN    57
nicotiana_tabacu  ....ma-----------------------------------.e---------------    55
dimocarpus_longa  ..-sp---s----------------------------------.e--------------- 57
malus_x_domestic  ..-sp-d-s----------------------------------.e--------------- 57
nicotiana_tabacu  ....mad-s--------------------i---------g---.e--------------- 55
hordeum_vulgare   .msapg-ls----------------------------------s.e-------------- 58
ipomoea_nil       ..-------s---------------------------------.--------------- 57
litchi_chinensis  ..-sp---s------------------------d---------.e--------------- 57
manihot_esculent  ..-sps-ps---h-----------------------------.e--------------- 57
populus_tremula_  ..-sp---s-------------------------------n.e---m------------- 57
rheum_australe    ...m--ves------l---------------------v-----.e--------------- 56
solanum_lycopers  ....mad-s------l-------------i---------a--.e--------------- 55
triticum_aestivu  ..-sta-a-----------------------------a--.g--------------- 57
vitis_vinifera    ..-aaap-a---------------------saa--s.e--------------- 57
lilium_longiflor  ..-spv--s-d-s-----------------------------age-------------- 58
ricinus_communis  ......maa---------------------------sasi-n.e--------------- 53
gossypium_hirsut  maaaa-s-p------------------------sasa-n.e--------------- 59
pisum_sativum     ..-aaahtp---------------------sana-s.e--------------- 57
saccharum_hybrid  .......ms----------------------y---------.e--------------- 52
nicotiana_tabacu  ....ma-----------------------------------.e--------------- 55

ib14-3-3          VIGARRASWRIISSIEQKEESRGNEDLVKTIKEYRGRIEAELSKICDGILNLLESHLIPS   117
nicotiana_tabacu  -------------------------h-ss------k----------------------v   115
dimocarpus_longa  -----------------------h-av--d---k--s----------g---------   117
malus_x_domestic  -----------------------h-ai-----sk--t-------------------   117
nicotiana_tabacu  -----------------------h--------ak------------g---------   115
hordeum_vulgare   -----------------------r-tl------k--t----------k---t--v--   118
ipomoea_nil       ---------------------e-----------------s------v--   117
litchi_chinensis  -----------------------h-av------k--s----------g---------   117
manihot_esculent  -v---------------------h-ti------k---------g---s--d------a   117
populus_tremula_  -----------------------h-ti------k-----c--------s---t--v--   117
rheum_australe    -----------------------h-ti--d--sk--t-------------d---v--   116
solanum_lycopers  -----------------------h-n------sk------------s----n----   115
triticum_aestivu  -----------------------ay-as-----t---t--n-------k--d---v--   117
vitis_vinifera    ----------------------dhh-am-rd--sk--s---s------k--d-r----   117
lilium_longiflor  ------------------------h-vl--d---k------------k--d---v--   118
ricinus_communis  ------------------------h-sl-rd--ak--s---s------k--dtr----   113
gossypium_hirsut  ----------------------d-h-a--rd--ak--s--ts--n---k--dtr-v--   119
pisum_sativum     ------------------------h-dv-rd--sk--s---n------k--dtr----   117
saccharum_hybrid  -----------v----------k--eh-nl------k------n------k--d---v--   112
nicotiana_tabacu  -------------------------h-ss------k----------------------v   115

ib14-3-3          ASTAESKVFYLKMKGDYHRYLAEFKTGAERKEAAESTLLAYKSAQDIALGELAPTHPIRL   177
nicotiana_tabacu  -------------------------------------n------------a---------   175
dimocarpus_longa  --s------------------------------------------------a---------   177
malus_x_domestic  --s------------------------------g------------------a---------   177
nicotiana_tabacu  --a------------------------------s-------n------------t---------   175
hordeum_vulgare   stap-------------y-------s-p---d---n-mv---a------a---------   178
ipomoea_nil       --------------------------------------------------------------   177
litchi_chinensis  --s------------------------------------------------a---------   177
manihot_esculent  --s-------------y--------as------------------ia---------   177
populus_tremula_  --a-------------------------------s--------s---------   177
rheum_australe    -as-------------------t-------n-----------ad---------   176
solanum_lycopers  -----------------------t-------n------------a---------   175
triticum_aestivu  -ta--------------------a---------n--v---------ad-pt------   177
vitis_vinifera    --sgd-------------------------------t---------na---------   177
lilium_longiflor  -a--------------------v-------n------------ad---------   178
ricinus_communis  -aagd-------------------------t---------na---------   173
gossypium_hirsut  --sgd-------------------------t---------na---------   179
pisum_sativum     --sgd---------------------------a---------na--p---------   177
saccharum_hybrid  sta-------------------------s----mv---a------a---------   172
nicotiana_tabacu  ---------------------------------n-----------a---------   175
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ib14-3-3       GLALNFSVFYYEILNSPDRACNLAKQAFDEAISELDTLGEESYKDSTLIMQLLRDNLTLW   237
nicotiana_tabacu -------------------------s----------d--a-------------------   235
dimocarpus_longa ------------------------------------------------------f-------   237
malus_x_domestic --------------------------------------------------------------   237
nicotiana_tabacu --------------------------------------------------------------   235
hordeum_vulgare  -----------------------------------------s--------------------   238
ipomoea_nil      ------------------------------l-------------------------------   237
litchi_chinensis --------------------------------------------------------------   237
manihot_esculent --------------------------------------------------------------   237
populus_tremula_ --------------------s-----------------------------------------   237
rheum_australe   ------------------k-------------------------------------------   236
solanum_lycopers --------------------------------------------------------------   235
triticum_aestivu ------------------------------a---s---------------------------   237
vitis_vinifera   ------------------------------a-----------------------h---     237
lilium_longiflor --------------s-----------------------------------------------   238
ricinus_communis ------------------------------a-------------------------------   233
gossypium_hirsut ------------------------------a-------------------------------   239
pisum_sativum    ------------------------------a-------------------------------   237
saccharum_hybrid ------------------k-------------------------------------------   232
nicotiana_tabacu ------------------s-----------d--a----------------------------   235

ib14-3-3        TSDTADDAGDEI.KEASKRESGEGPQ....   262
nicotiana_tabacu ----t--------.-----------e-....   260
dimocarpus_longa ---it--------.--------v--q.....   261
malus_x_domestic ---it--------.--------s-tq-....   262
nicotiana_tabacu ---n-e-----d-.---p-h--s--q-....   260
hordeum_vulgare  ---ite-tae-eir--p-hd-s--q.....   263
ipomoea_nil      -------g----.--------------....   262
litchi_chinensis ---it--------.--------a--q.....   261
manihot_esculent ---it-e------.----------qpqq..   264
populus_tremula_ ---it--------.----------d---...   262
rheum_australe   ---ateega---.---t-g-t---q.....   260
solanum_lycopers ---n---v--d-.------p-----q-....   260
triticum_aestivu ---n-eeg----.---askpe---h.....   261
vitis_vinifera   ---mq--ga---.---p--ddeqq......   260
lilium_longiflor ---ite------keapp--d-v--q.....   263
ricinus_communis ---mq--ga---.---apkp-d-qq.....   257
gossypium_hirsut ---mq--ga-d-.---p--deq-qq-kpeq   268
pisum_sativum    ---mq--ga---.---apkadeqq......   260
saccharum_hybrid ---lte-ga--g.-----gda---q.....   256
nicotiana_tabacu ---st--------.---------d-e-....   260
```

Figure 7. Alignment of 14-3-3 protein using DNAMAN program. Multi-alignments were made by DNAMAN of the deduced Ib14-3-3 amino acid sequence with the 19 most homologous 14-3-3 proteins from *Nicotiana tabacum* gi |44917151|, *Dimocarpus longan* gi |291162643|, *Malus x domestica* gi |55375985|, *Nicotiana tabacum* gi |44917163|, *Hordeum vulgare* gi |2266662|, *Ipomoea nil* gi |124484407|, *Litchi chinensis* gi |310007371|, *Manihot esculenta* gi |67107029|, *Populus tremula x Populus alba* gi |8515888|, *Rheum australe* gi |197312897|, *Solanum lycopersicum* gi |22217852|, *Triticum aestivum* gi |40781605|, *Vitis vinifera* gi |226295434|, *Lilium longiflorum* gi |126508568|, *Ricinus communis* gi |255545792|, *Gossypium hirsutum* gi |193290377|, *Pisum sativum* gi |4850247|, *Saccharum hybrid cultivar CP65-357* gi |37903393|, *Nicotiana tabacum* gi |44917153|. The completely identical amino acids were boxed.

growing in harsh environment.

Presently, knowledge of the 14-3-3 protein is still limited, because this protein is a key regulator of many cellular signal transduction pathways and cell functions in plants, and interacts with many target proteins at same time. In arabidopsis, 14-3-3 protein gene family contains 12 genes (Rosenquist et al., 2001), and this is common in allopolyploid plants. Presence of gene families of 14-3-3 proteins in the sweet potato reflects their essential role in plant physiology.

This study fills a gap in our knowledge on 14-3-3

proteins genes in sweet potato. The sweet potato 14-3-3 protein cDNAs are all 1176 bp long, and they contain an ORF of 789 bp that encodes a polypeptide of 262 amino acids and 7 mutation sites. It is generally believed that sweet potato is allohexaploid (Gao et al., 2011), but our sequencing results on sweet potato (variety Xushu 18) 14-3-3 protein genomic genes showed that not only the coding sequences but also the positions of introns in the three genes are identical, and the same results are found in Ib-Hsp70, Ib-AGP (ADP-Glucose Pyrophosphorylase), Ib-SBE (starch branching enzyme) and several other

genes from Xushu 18 (data not shown)，implying that that Xushu 18 is homologous hexaploid. Protein sequence analyses showed that there were four highly conserved domains at positions 8–34, 42–83, 121–144 and 169–240 respectively (Figure 7), which may play a key role in 14-3-3 protein functions. In addition, digital gene expression profiling and semi-quantitative RT-PCR were performed to test the expression levels of 14-3-3 protein genes in different tissues. The results of the digital gene expression profiling demonstrated that the 14-3-3 protein genes were widely expressed among the tissues of sweet potato, which is reasonable for 14-3-3 protein's pivotal role. The expression levels of 14-3-3 protein genes are distinct (very high in harvested tuberous roots and very low in mature leaves) and these results were also confirmed by semi-quantitative RT-PCR analyses. Our results provide a solid foundation for further study on improving the sweet potato's resistance against pathogen infection and various environmental stresses.

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