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Identification and expression analysis of two small heat shock protein cDNAs from developing seeds of biodiesel feedstock plant *Jatropha curcas*

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ABSTRACT

Plant small heat shock proteins (sHSPs) are known to be important for environmental stress tolerance and involved in various developmental processes. In this study, two full-length cDNAs encoding sHSPs, designated *JcHSP-1* and *JcHSP-2*, were identified and characterized from developing seeds of a promising biodiesel feedstock plant *Jatropha curcas* by expressed sequence tag (EST) sequencing of embryo cDNA libraries and rapid amplification of cDNA ends (RACE). *JcHSP-1* and *JcHSP-2* contained open-reading frames encoding sHSPs of 219 and 157 amino acids, with predicted molecular weights of 24.42 kDa and 18.02 kDa, respectively. Sequence alignment indicated that both *JcHSP-1* and *JcHSP-2* shared high similarity with other plant sHSPs. Real-time quantitative RT-PCR analysis showed that the transcriptional level of both *JcHSP-1* and *JcHSP-2* increased along with natural dehydration process during seed development. A sharp increase of *JcHSP-2* transcripts occurred in response to water content dropping from 42% in mature seeds to 12% in dry seeds. Western blot analysis revealed that the accumulation profile of two cross-reacting proteins, whose molecular weight corresponding to the calculated size of *JcHSP-1* and *JcHSP-2*, respectively, was well consistent with the mRNA expression pattern of *JcHSP-1* and *JcHSP-2* in *Jatropha* seeds during maturation and natural dehydration. These results indicated that both *JcHSPs* might play an important role in cell protection and seed development during maturation of *J. curcas* seeds.

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1. Introduction

Small heat shock proteins (sHSPs) are a family of low molecular mass proteins (15–42 kDa) [1], which play an important role in the defense of organisms against a variety of environmental stresses and in various developmental processes [2]. sHSPs are the most abundant heat stress-induced proteins in higher plants where they were induced as molecular chaperones that play a protective role against various stresses such as cold, salt, drought and oxidants besides high temperature [3–7]. Moreover, many sHSPs appear to be also involved in various plant developmental processes, such as pollen development [8], embryo development [9,10], seed maturation [11,12], as well as cell proliferation and

differentiation [13]. Three *Arabidopsis* sHSPs (*AtHsp17.4*, *AtHsp17.6* and *AtHsp17.7*) accumulated during the middle stage of seed maturation, and their concentration remained high during the late stage and in mature dry seeds [12]. Similarly, rice *Oshsp16.9A* abundantly accumulated in mature dry seeds [14]. *Arabidopsis* mutant plants sensitive to desiccation contained lower amounts of sHSPs during seed maturation [15]. The synthesis of sHSPs during seed maturation suggests their probable role involved in the desiccation tolerance. As well as late embryogenesis abundant proteins (LEA) accumulated during seed maturation and acquisition of desiccation tolerance [16].

Plant sHSPs are all encoded by nuclear multigene families and classified into six subfamilies. Three subfamilies of sHSPs are localized to cytosol, and the other three organelle sHSP subfamilies localized to the chloroplast (CP), the endoplasmic reticulum (ER) and the mitochondria (MT), respectively [17,18]. The common feature of most sHSPs contains a conserved C-terminal alpha-crystallin domain (ACD) of approximately 90 amino acid residues, which is homologous to alpha-crystallin proteins of the vertebrate eye lens [19]. The structural organization of sHSPs is evolutionarily con-

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served, and the proteins of the same class from different plant species show a high sequence similarity [20].

Jatropha curcas L. (hereafter refer as *jatropha*) belongs to the genus *Jatropha* of the family *Euphorbiaceae* and mainly distributes in tropical and subtropical regions of South America and South Asia. It can be used as a biodiesel feedstock plant, a source of medicine products and biological pesticides, and for soil and water conservation and land reclamation [21]. For its high content of seed oil (up to 40%) [22] that has the similar characteristics to those of fossil diesel [23] and its strong adaptability to the drought and impoverished soil, *jatropha* is considered a promising biodiesel feedstock plant [24,25]. Some genes encoding fatty acid biosynthesis enzymes and affecting seed oil contents in *jatropha* have been cloned [26,27]. However, the cloning and characterization of sHSPs during *jatropha* seed maturation has not yet been reported.

In this study, we cloned two full-length cDNAs encoding sHSPs from developing *jatropha* seeds, designated *JcHSP-1* and *JcHSP-2*, by the approaches of expressed sequence tag (EST) sequencing of embryo cDNA libraries and rapid amplification of cDNA ends (RACE). Sequence analysis revealed that *JcHSP-1* and *JcHSP-2* shared high similarity with sHSPs from other plants. Their expression patterns during maturation and natural desiccation processes of *jatropha* seeds were investigated by real-time quantitative RT-PCR (qRT-PCR) analysis and western blot analysis.

2. Materials and methods

2.1. Plant materials

J. curcas L. seeds were collected from a local population in the Xishuangbanna Tropical Botanical Garden (XTBG), Chinese Academy of Sciences, Yunnan Province, PR China. When the plants came into blossom, the fruits development was recorded throughout the fruiting period. The whole seeds at different developmental stages (50, 55, 60, 65, 70, 90 and 100 days after flowering (DAF)) were harvested, flash frozen in liquid nitrogen and stored at -80°C for molecular analysis.

2.2. Fresh and dry weight and water content analysis

A random sampling of fifteen seeds at different developmental stages was weighed to determine the fresh weight (FW). The dry weight (DW) of seeds was determined by reweighing after oven drying for 17 h at 103°C . Water content (WC, %) was calculated based on FW and DW. Three independent replicates of fifteen seeds were used for individual stages.

2.3. Heat shock protein preparation and western blot analysis

Seeds at different developmental stages were ground to fine powder in liquid nitrogen. The powder was transferred immediately to a 10-ml tube containing extraction buffer [50 mM phosphate, pH 7.0, 0.2% (v/v) Triton-X-100, 7 mM β -mercaptoethanol and 5 mM ascorbic acid]. The homogenate was centrifuged at 12,000 rpm for 15 min at 4°C . The supernatant was transferred to fresh tube and incubated at 100°C for 10 min, then the tube was transferred to ice for 5 min. The heat-treated supernatant was centrifuged at 12,000 rpm for 15 min, and 50–100 μl of supernatant was transferred to new tubes for direct use or storage at -80°C . Concentrations of heat-stable proteins were determined according to the Bradford protocol [28]. SDS-PAGE was performed with a discontinuous buffer system, as described by Laemmli [29]. Protein samples in 1 \times SDS gel-loading buffer were denatured by heated at 95°C for 5 min before loaded into the gel. Fifteen micrograms of protein samples were separated electrophoretically on low range SDS-PAGE and transferred onto polyvinylidene fluoride

(PVDF) microporous membranes (Millipore, Cat. No. IPVH00010) using the Trans-Blot[®] cell (Bio-Rad). The western blotting procedures were carried out according to Mazhar and Basha [30] using anti-sHSPs antibody (anti-AtHsp17.6 class I, kindly supplied by Dr. E. Vierling, University of Tucson, Arizona, USA) [12] and goat anti-rabbit IgG antibody conjugated with alkaline phosphatase (Vector Laboratories, CA 94010, USA). Fresh-developing buffer (100 μl of NBT solution and 100 μl of BCIP solution in 10 ml of alkaline phosphatase buffer) [31] was used for membrane staining, and the reaction was stopped by washing the membrane in ddH_2O .

2.4. Cloning of *jatropha* sHSP cDNAs

Two sHSP unigene sequences [GenBank FM894116 for *JcHSP-1* and GenBank FM891678 for *JcHSP-2*] were obtained from the EST database of *jatropha* embryo cDNA libraries (GenBank FM887038-FM896881). Total RNA was extracted from *jatropha* seeds at different developmental stages using silica particle protocol [32]. Contaminated DNA was removed by incubating at 37°C for 30 min with DNase I. The concentration and purity of RNA was determined by measuring the absorbance at 260 and 280 nm. Reverse transcription-polymerase chain reaction (RT-PCR) was used to clone sHSP cDNAs with primers derived from sHSP EST sequences. The 3' ends of sHSP cDNA were obtained by using 3' Rapid Amplification of cDNA Ends (3'-RACE) according to the protocol of Rapid Amplification of cDNA Ends (Clontech). The 3'-RACE products were purified and sequenced.

2.5. qRT-PCR analysis

First-strand cDNA was obtained from 2 μg total RNA, M-MLV reverse transcriptase (Promega), dNTP minixture, and Oligo-d(T)18 primer (TaKaRa) incubating at 42°C for 60 min in a 10 μl reaction volume. Then 2 μl cDNA sample diluted at 1:5 (v/v) was used as template for quantitative RT-PCR of 20 μl reaction volume. Real time quantitative RT-PCR (qRT-PCR) were performed with LightCycler[®] 480 Instrument (Roche) and LightCycler[®] 480 Relative Quantification Software. *JcHSP-1* specific primers (XT68, 5'-ATGGCGTACTCGGTGCTCTTAA-3'; XT69, 5'-GT-GTTCTGTTCCA-CGGAGACCTT-3') and *JcHSP-2* specific primers (XT5, 5'-TTCCCTTCATCTTCTCCCTCGTC-3'; XT6, 5'-TGAAGTGGCACTGCTAGCTCC-3') were designed based on our *jatropha* EST sequences of *JcHSP-1* (GenBank FM894116) and *JcHSP-2* (GenBank FM891678) using Primer Premier v5.0 (Premier Biosoft International). *Jatropha* actin gene (GenBank FM894455) was used as an internal control for the relative amount of RNA, which was amplified with the gene specific primers XT24 (5'-TGACAGACCGTATGAGCAAGGAGATC-3') and XT25 (5'-CCA-GAGGGACCATTACAGTTGAGCC-3'). Only a single peak presented in each of the melting curves for amplification of *JcHSP-1*, *JcHSP-2* and *actin*, respectively, demonstrating that all the primer pairs used were specific.

Total 20 μl of reaction mixture containing the following components: 1 \times TransStart Green qPCR SuperMix, primers (0.25 mM each of them), 1 \times Passive Reference Dye/PCR Enhancer (50 \times) and 2 μl of the first-strand cDNAs diluted at 1:5 (v/v). Amplifications were performed under the following thermal cycling parameters: pre-denaturation at 94°C for 3 min, 40 cycles of 94°C for 30 s, 54 – 59°C (a specific annealing temperature for each gene) for 50 s and 72°C for 50 s, and followed by 1 cycle of final elongation at 72°C for 7 min. Amplification-product specificity was checked with a heat-dissociated protocol (melting curves in 56 – 90°C) following the final step of PCR. The efficiency-corrected quantification was performed automatically by the LightCycler[®] 480 Relative Quantification Software based on relative standard curves, which described

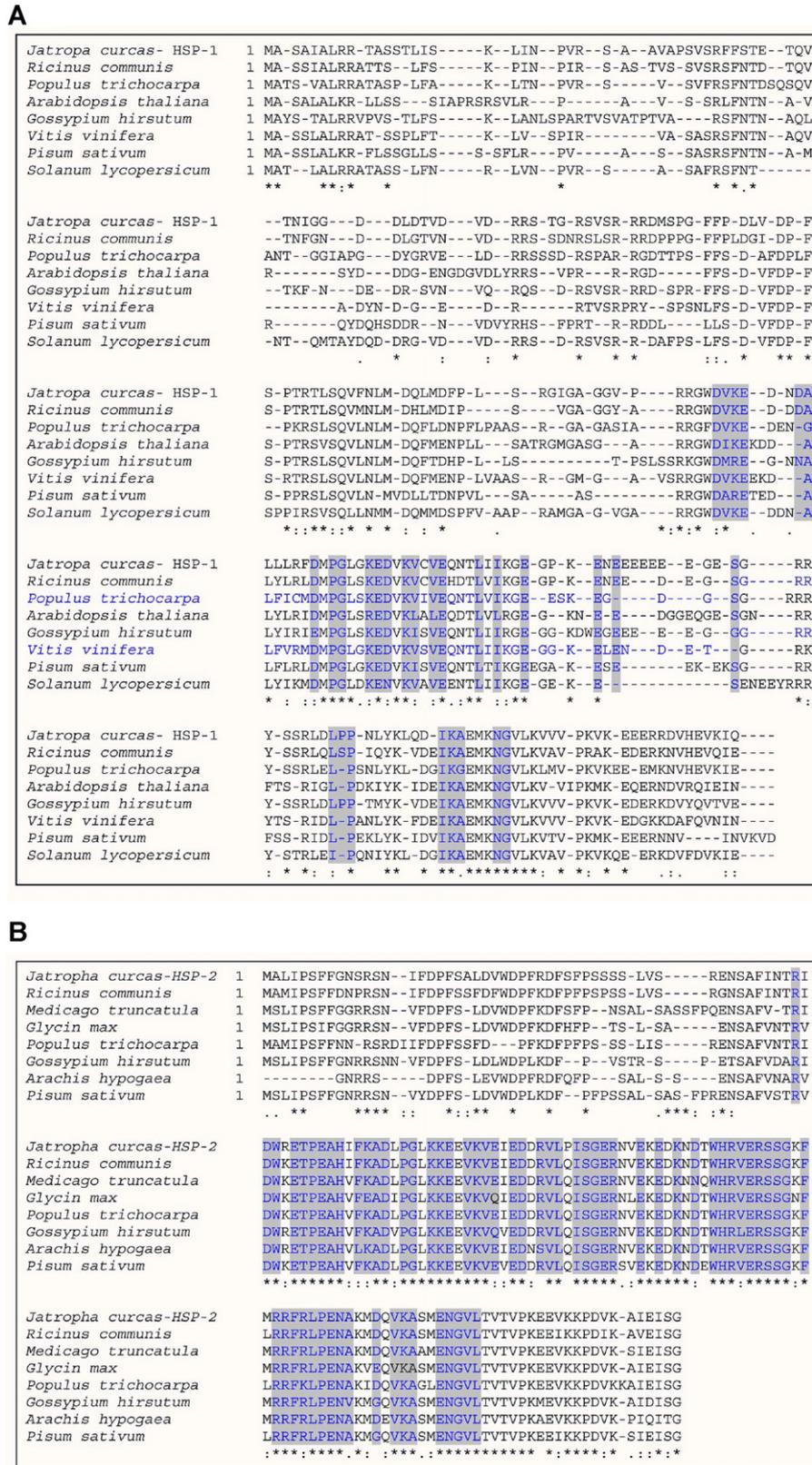


Fig. 1. The optimized alignment of JcHSP-1 and JcHSP-2 with other closely related plant sHSPs. (A) The alignment of JcHSP-1 with sHSPs from castor bean (*Ricinus communis*, EEF33459), poplar (*Populus trichocarpa*, XP_002304270), Arabidopsis (*Arabidopsis thaliana*, CAA67022), upland cotton (*Gossypium hirsutum*, ABW89471), grape (*Vitis vinifera*, XP_002270632), pea (*Pisum sativum*, P46254) and tomato (*Solanum lycopersicum*, BAA32547). (B) The alignment of JcHSP-2 with sHSPs from castor bean (*Ricinus communis*, EEF41877), barrel medic (*Medicago truncatula*, ABD32352), soybean (*Glycin max*, P04793), poplar (*Populus trichocarpa*, XP_002313360), upland cotton (*Gossypium hirsutum*, ABW89468), peanut (*Arachis hypogaea*, ABC46712) and pea (*Pisum sativum*, AAN74634). Gaps are marked with dashes. Shaded areas indicated the conserved residues of alpha-crystallin domain. (*) Indicates to the identical residue; (:) indicates to strong positive residue; (.) indicates to weaker positive residue.

the PCR efficiencies of the target and the reference genes. Three replicates were tested for each sample.

2.6. Sequence and bioinformatic analysis

The conserved sequence of plant sHSP cDNAs was identified by multiple sequence alignment analysis with the Clustal W Program (<http://www.ch.embnet.org/software/ClustalW.html>), and used as the query sequence in a blast search against our *Jatropha* embryo EST database (DDBJ/EMBL/GenBank FM887038–FM896881). The ORF Finder (Open Reading Frame Finder) program (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>) on the website of National Center for Biotechnology Information (NCBI) was used to analyze the ORF of the full length cDNAs of the two HSPs obtained by RACE. Sequence homology of the two JcHSPs with other plant HSPs were made via the BLAST program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Protein calculator v.3.3 online software (<http://www.scripps.edu/cgi-bin/cdputnam/protcalc3>) was used to determine the molecular weights and isoelectric points of the two JcHSPs.

3. Results

3.1. Isolation and characterization of *Jatropha* cDNAs encoding sHSP

To obtain the full-length cDNAs encoding sHSP in *Jatropha*, we did a blast search against our *Jatropha* EST database, which was established from the embryo cDNA libraries of *Jatropha* developing seeds (DDBJ/EMBL/GenBank FM887038–FM896881), using conserved sequences of plant sHSP cDNAs as the search query. Two unigenes (GenBank FM894116 and FM891678) were found that showed significant similarity to the known plant sHSP sequences in GenBank database. By alignment with the protein sequence of other plant sHSPs, these two EST sequences were predicted to contain the entire N-terminal sequences of sHSP but lacking the C-terminal sequence. Gene specific primers were then designed according to the sequences of these two sHSP ESTs, and used for PCR amplification of the 3' ends of the cDNAs by 3'-RACE. The full-length cDNA sequences, *JcHSP-1* and *JcHSP-2*, were obtained by assembling the sequences of the 3'-RACE fragment with the corresponding EST fragment.

The full-length *JcHSP-1* cDNA sequence (GenBank GU325780) was 842 bp in length, and contained a 660-bp open reading frame (ORF) encoding a protein of 219 amino acids, as shown in Fig. 1A. The calculated molecular mass of JcHSP-1 is 24.43 kDa, and the deduced isoelectric point was 5.44. Conserved alpha-crystallin domain (ACD) was identified in JcHSP-1 precursor protein (Fig. 1A), which is consistent with the ACD of other plant sHSPs [33,34]. JcHSP-1 shared 53–60% identity and 68–74% similarity to sHSPs from other plants in GenBank (Fig. 1A). The highest sequence homology (70% identity and 81% similarity) was found to a sHSP (GenBank EEF33459) from castor bean (*Ricinus communis*).

The full-length *JcHSP-2* cDNA sequence (GenBank GU325781) was 956 bp in length, and contained a 474-bp ORF encoding a protein of 157 amino acids, as shown in Fig. 1B. The deduced molecular weight of JcHSP-2 is 18.02 kDa with a predicted isoelectric point of 5.71. JcHSP-2 also contained ACD. Homology analysis revealed that JcHSP-2 shared 79–85% identity and 87–93% similarity to sHSPs from other plants in GenBank (Fig. 1B), with the highest sequence homology to another castor bean sHSP (GenBank EEF41877). JcHSP-1 and JcHSP-2 shared 50% identity and 54% similarity in protein sequence, suggesting a certain phylogenetic divergence and functional diversity between the two proteins.

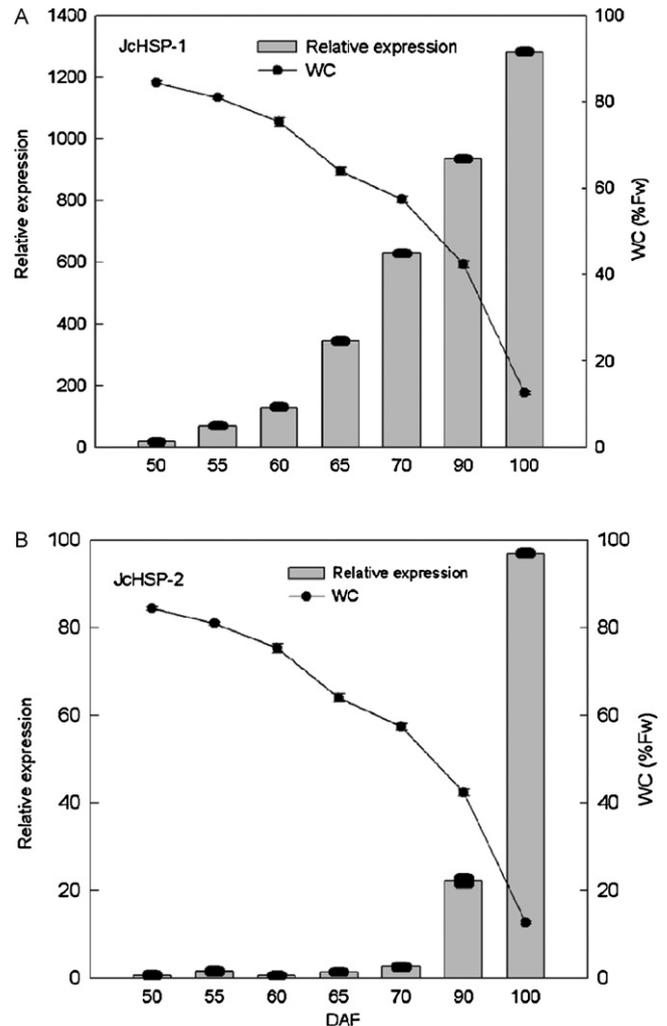


Fig. 2. qRT-PCR analysis of *JcHSP-1* (A) and *JcHSP-2* (B) mRNA expression. qRT-PCR was carried out with first-strand cDNAs obtained from the seeds at different developmental stages. Quantification is based on C_p values that were normalized using the C_p value corresponding to an internal control gene *actin* (GenBank FM894455). Expression of the *Jatropha actin* was used as the reference point (=1) for calculations of the relative expression level. Water content (WC) of seeds was expressed on a fresh weight (FW) basis. Three replicates for each sample examined. Each value is the mean \pm SD.

3.2. Expression pattern of *JcHSP-1* and *JcHSP-2* in *Jatropha* seeds at different developmental stages

In order to investigate the gene expression patterns of *JcHSP-1* and *JcHSP-2* in *Jatropha* seeds, real time quantitative RT-PCR analysis was carried out with total RNAs extracted from *Jatropha* seeds at different developmental stages. Although the highest expression level of both of *JcHSP-1* and *JcHSP-2* was in dry seeds (100 DAF), the two sHSP cDNAs exhibited different expression patterns in developing *Jatropha* seeds. *JcHSP-1* transcripts increased during seed development from 17-fold (comparing to the control gene, *actin*) to about 1200-fold of the control from 55 to 100 DAF (Fig. 2A). On the other hand, *JcHSP-2* transcripts showed a low expression during the early stages, where it increased from 0.6-fold to 22-fold of the control from 50 to 90 DAF (Fig. 2B). After that, a sharp increase of *JcHSP-2* transcripts from 22-fold in mature seeds (90 DAF) to 96-fold in dry seeds (100 DAF) occurred in response to water content dropping from 42% in mature seeds to 12% in dry seeds (Fig. 2B).

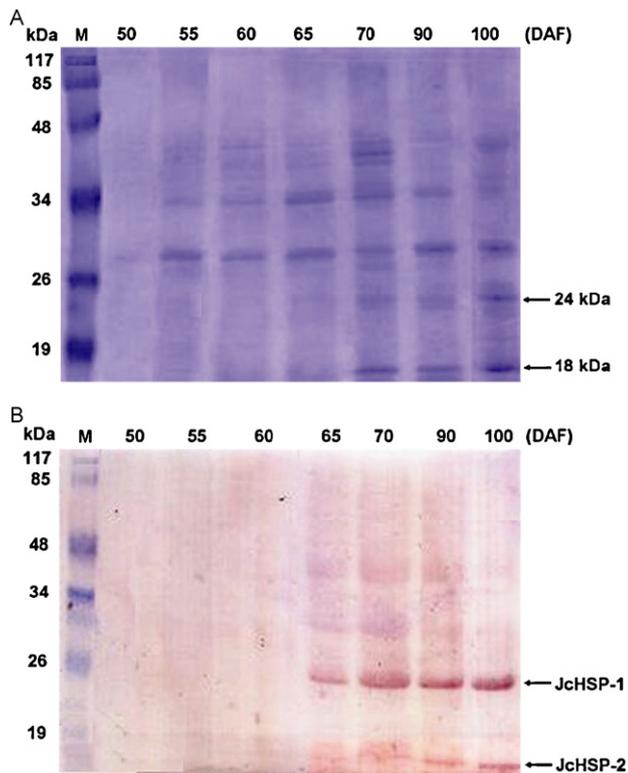


Fig. 3. Heat-stable proteins from jatropha seeds at different developmental stages. (A) SDS-PAGE of heat-stable protein fractions extracted from jatropha seeds at different developmental stages; (B) Western blot analysis of the corresponding gel probed with anti-sHSP [AtHsp 17.6 (class I)] antibody. M, molecular weight marker; DAF, days after flowering.

3.3. Detection of jatropha sHSPs during seed maturation and natural dehydration processes

The profile of sHSPs in jatropha seeds at different developmental stages was analyzed by SDS-PAGE and western blot analysis at protein level (Fig. 3). The number of heat-stable polypeptides increased from none in the seeds at 50 days after flowering (DAF) to about 7 in the seeds at 100 DAF (Fig. 3A). The immunoblot of the corresponding gel was probed with anti-AtHsp17.6 class I, which was generated against the ACD domain of carboxyl terminus of sHSPs [12]. Two predominant cross-reacting bands were detected in jatropha seeds during maturation and natural dehydration (Fig. 3B). The molecular weight of the two cross-reacting proteins was about 24 kDa and 18 kDa, respectively, which was corresponding to the calculated size of JcHSP-1 and JcHSP-2. And furthermore, the accumulation profile of each of the two cross-reacting proteins (Fig. 3B) was well consistent with the mRNA expression pattern of *JcHSP-1* and *JcHSP-2*, respectively (Fig. 2). In addition, some faint bands above JcHSP-1 were found (Fig. 3B), which could be some other HSPs containing the ACD domain.

4. Discussion

This study showed that two different sHSPs (JcHSP-1 and JcHSP-2), which belong to the cytosolic class I sHSPs and contain the alpha-crystallin domain (ACD) [35], were synthesized during jatropha seed development and maturation. And the differential expression of JcHSP-1 and JcHSP-2 was observed at both mRNA and protein level.

It is well established that sHSPs express at specific developmental stages of many plants, in addition to being synthesized in response to stresses [8,18]. *Arabidopsis* cytosolic class I sHSPs begin

to accumulate during embryo mid-maturation and are abundant throughout the late maturation program [12]. In pea, class I and class II sHSPs appear in embryos during reserve synthesis at mid-maturation and are most abundant in dry seeds [36]. Here, we show that *JcHSP-1* transcripts increased gradually along with seed development processes from 55 to 100 DAF, whereas *JcHSP-2* expression was very low during the early stages of seed development and significantly increased during natural dehydration processes from 90 to 100 DAF. Rapid accumulation of *JcHSP-2* expression appeared to be in response to dehydration stress, which was caused by water content decreasing from 42% FW to 12% FW in jatropha seeds. The different expression patterns of *JcHSP-1* and *JcHSP-2* might indicate they play the different roles and were regulated by different mechanisms during seeds maturation and desiccation [37]. Considering JcHSP-1 and JcHSP-2 accumulation in the late stages of seed maturation, the two sHSPs might function as molecular chaperones that suppress protein aggregation and protect cells against dehydration stress [38]. The presented data also suggest that the sHSP expression during seed maturation is important in acquisition of desiccation tolerance of jatropha seeds.

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References

- [1] E. Vierling, The small heat shock proteins in plants are members of an ancient family of heat induced proteins, *Acta Physiologiae Plantarum* 19 (1997) 539–547.
- [2] F. Narberhaus, Alpha-crystallin-type heat shock proteins: socializing minichaperones in the context of a multichaperone network, *Microbiology and Molecular Biology Reviews* 66 (2002) 64–93.
- [3] N. Banzet, C. Richaud, Y. Deveaux, M. Kazmaier, J. Gagnon, C. Triantaphylides, Accumulation of small heat shock proteins, including mitochondrial HSP22, induced by oxidative stress and adaptive response in tomato cells, *Plant Journal* 13 (1998) 519–527.
- [4] E.W. Hamilton, S.A. Heckathorn, Mitochondrial adaptations to NaCl. Complex I is protected by anti-oxidants and small heat shock proteins, whereas complex II is protected by proline and betaine, *Plant Physiology* 126 (2001) 1266–1274.
- [5] A. Sabehat, S. Lurie, D. Weiss, Expression of small heat-shock proteins at low temperatures – a possible role in protecting against chilling injuries, *Plant Physiology* 117 (1998) 651–658.
- [6] Y. Sato, S. Yokoya, Enhanced tolerance to drought stress in transgenic rice plants overexpressing a small heat-shock protein, sHSP17.7, *Plant Cell Reports* 27 (2008) 329–334.
- [7] A. Soto, I. Allona, C. Collada, M.A. Guevara, R. Casado, E. Rodriguez-Cerezo, C. Aragoncillo, L. Gomez, Heterologous expression of a plant small heat-shock protein enhances *Escherichia coli* viability under heat and cold stress, *Plant Physiology* 120 (1999) 521–528.
- [8] V. Zarsky, D. Garrido, N. Eller, J. Tupy, O. Vicente, F. Schoffl, E. Heberlebers, The expression of a small heat-shock gene is activated during induction of tobacco pollen embryogenesis by starvation, *Plant Cell and Environment* 18 (1995) 139–147.
- [9] M.A. Coca, C. Almoguera, J. Jordano, Expression of sunflower low-molecular-weight heat-shock proteins during embryogenesis and persistence after germination – localization and possible functional implications, *Plant Molecular Biology* 25 (1994) 479–492.
- [10] W.N. Sun, C. Bernard, B. van de Cotte, M. Van Montagu, N. Verbruggen, At-HSP17.6A, encoding a small heat-shock protein in *Arabidopsis*, can enhance osmotolerance upon overexpression, *Plant Journal* 27 (2001) 407–415.
- [11] D. Löw, K. Brändle, L. Nover, C. Forreiter, Cytosolic heat-stress proteins Hsp17.7 class I and Hsp17.3 class II of tomato act as molecular chaperones in vivo, *Planta* 211 (2000) 575–582.
- [12] N. Wehmeyer, L.D. Hernandez, R.R. Finkelstein, E. Vierling, Synthesis of small heat-shock proteins is part of the developmental program of late seed maturation, *Plant Physiology* 112 (1996) 747–757.
- [13] A.P. Arrigo, In search of the molecular mechanism by which small stress proteins counteract apoptosis during cellular differentiation, *Journal of Cellular Biochemistry* 94 (2005) 241–246.

- [14] J.C. Guan, T.L. Jinn, C.H. Yeh, S.P. Feng, Y.M. Chen, C.Y. Lin, Characterization of the genomic structures and selective expression profiles of nine class I small heat shock protein genes clustered on two chromosomes in rice (*Oryza sativa* L.), *Plant Molecular Biology* 56 (2004) 795–809.
- [15] N. Wehmeyer, E. Vierling, The expression of small heat shock proteins in seeds responds to discrete developmental signals and suggests a general protective role in desiccation tolerance, *Plant Physiology* 122 (2000) 1099–1108.
- [16] K. Goyal, L.J. Walton, A. Tunnacliffe, LEA proteins prevent protein aggregation due to water stress, *Biochemical Journal* 388 (2005) 151–157.
- [17] K.D. Scharf, M. Siddique, E. Vierling, The expanding family of *Arabidopsis thaliana* small heat stress proteins and a new family of proteins containing alpha-crystallin domains (Acid proteins), *Cell Stress and Chaperones* 6 (2001) 225–237.
- [18] E.R. Waters, G.J. Lee, E. Vierling, Evolution, structure and function of the small heat shock proteins in plants, *Journal of Experimental Botany* 47 (1996) 325–338.
- [19] W.W. de Jong, G.J. Caspers, J.A.M. Leunissen, Genealogy of the alpha-crystallin – small heat-shock protein superfamily, *International Journal of Biological Macromolecules* 22 (1998) 151–162.
- [20] E.M. Kalemba, S. Pukacka, Possible roles of LEA proteins and sHSPs in seed protection: a short review, *Biological Letters* 44 (2007) 3–16.
- [21] K. Openshaw, A review of *Jatropha curcas*: an oil plant of unfulfilled promise, *Biomass & Bioenergy* 19 (2000) 1–15.
- [22] J. Kandpal, M. Madan, *Jatropha curcas*: a renewable source of energy for meeting future energy needs, *Renewable Energy* 6 (1995) 159–160.
- [23] H.R.S. Makkar, K. Becker, *Jatropha curcas*, a promising crop for the generation of biodiesel and value-added coproducts, *European Journal of Lipid Science and Technology* 111 (2009) 773–787.
- [24] D. Fairless, Biofuel: the little shrub that could – maybe, *Nature* 449 (2007) 652–655.
- [25] K. Sanderson, Wonder weed plans fail to flourish, *Nature* 461 (2009) 328–329.
- [26] L. Tong, P. Shu-Ming, D. Wu-Yuan, M. Dan-Wei, X. Ying, X. Meng, C. Fang, Characterization of a new stearyl-acyl carrier protein desaturase gene from *Jatropha curcas*, *Biotechnology Letters* 28 (2006) 657–662.
- [27] P.Z. Wu, J. Li, Q. Wei, L. Zeng, Y.P. Chen, M.R. Li, H.W. Jiang, G.J. Wu, Cloning and functional characterization of an acyl-acyl carrier protein thioesterase (JcFATB1) from *Jatropha curcas*, *Tree Physiology* 29 (2009) 1299–1305.
- [28] M.M. Bradford, Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein-dye binding, *Analytical Biochemistry* 72 (1976) 248–254.
- [29] U.K. Laemmli, Cleavage of structural proteins during assembly of head of bacteriophage-T4, *Nature* 227 (1970) 680–685.
- [30] H. Mazhar, S.M. Basha, Effects of desiccation on peanut (*Arachis hypogaea* L.) seed protein composition, *Environmental and Experimental Botany* 47 (2002) 67–75.
- [31] A.A.P. Ahumada, Antioxidant defence and the role of the peroxiredoxins during silique development and seedling establishment in oil seed plants, Bielefeld, 2006.
- [32] L.W. Ding, Q.Y. Sun, Z.Y. Wang, Y.B. Sun, Z.F. Xu, Using silica particles to isolate total RNA from plant tissues recalcitrant to extraction in guanidine thiocyanate, *Analytical Biochemistry* 374 (2008) 426–428.
- [33] G.J. Caspers, J.A.M. Leunissen, W.W. Dejong, The expanding small heat-shock protein family, and structure predictions of the conserved alpha-crystallin domain, *Journal of Molecular Evolution* 40 (1995) 238–248.
- [34] K. Scharf, M. Siddique, E. Vierling, The expanding family of *Arabidopsis thaliana* small heat stress proteins and a new family of proteins containing α -crystallin domains (Acid proteins), *Cell Stress and Chaperones* 6 (2001) 225–237.
- [35] E.R. Waters, G. Lee, E. Vierling, Evolution, structure and function of the small heat shock proteins in plants, *Journal of Experimental Botany* 47 (1996) 325–338.
- [36] A.E. Derocher, E. Vierling, Developmental control of small heat-shock protein expression during pea seed maturation, *Plant Journal* 5 (1994) 93–102.
- [37] S. Kotak, E. Vierling, H. Baumlein, P. von Koskull-Doring, A novel transcriptional cascade regulating expression of heat stress proteins during seed development of *Arabidopsis*, *Plant Cell* 19 (2007) 182–195.
- [38] M.E. Feder, G.E. Hofmann, Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology, *Annual Review of Physiology* 61 (1999) 243–282.