Molecular Cloning of Betaine Aldehyde Dehydrogenase (BADH) Gene from Harvested Papaya (Carica papaya) Fruit and its response to Temperature Stress

Xiaoyang Zhu1,2, Weixin Chen1 and Xueping Li1*

1. State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources/Guangdong Provincial Key Laboratory for Post harvest Science and Technology of Fruits and Vegetables, College of Horticulture, South China Agricultural University, Guangzhou 510642, CHINA
2. Université Toulouse (UPS), UMR 5546, Laboratoire de Recherche en Sciences Végétales, BP 42617, F-31326 Castanet-Tolosan Cedex, FRANCE
*lxp280477@gmail.com

Abstract
Betaine aldehyde dehydrogenase in plants can respond to drought, salt and temperature stress and can play a positive role in abiotic stress tolerance. In present study, a novel BADH gene was isolated from papaya fruit using in silico cloning and 3’- or 5’-rapid amplification cDNA ends (RACE). The full-length cDNA of CpBADH was 2052 bp, with a 1509 bp open reading frame (ORF) encoding a deduced 503 amino acid polypeptide. Sequence analysis showed that CpBADH contained some highly conserved regions of BADHs in the amino acid sequence. Subcellular localization prediction revealed that CpBADH contained a targeting signal peptide (QLFIDGE) to chloroplast and a targeting-signal (SKL) to peroxisome. Phylogenetic tree analysis revealed that CpBADH had a close genetic relationship with AtBADH1 (ALDH10A8).

Gene expression analysis showed that both temperature stresses induced CpBADH expression during storage. High temperature caused a significantly higher induction of CpBADH expression. Our results showed that CpBADH may be involved in diverse roles in temperature stress tolerance. This study has provided a foundation for further study on the CpBADH function and for improvement of papaya fruit stress tolerance.

Keywords: Papaya, BADH, stress, in silico cloning, gene expression.

Introduction
Papaya, one kind of typical tropical fruit, is widely cultivated and consumed for the favorite flavor, nutritional benefits and its pharmacological roles1. Papaya fruits suffer many problems such as susceptible to biotic or abiotic stresses and ripening rapidly due to its climacteric character usually leading to a heavy production loss2,3 and in turn causing a negatively economic impact throughout the production chain of papaya. Inappropriate post-harvested storage temperature is one of the most common problems for the loss of papaya fruit during the storage and transportation which is always accompanied by a rapid ripening, skin freckles, pulp translucency, pericarp pitting, pulp water-soaking and so on. A better understanding of the physiological, cellular and molecular mechanisms by which papaya fruit responds to the temperature stress may provide clues to select optimal temperature for storage of papaya fruit.

Betaine aldehyde dehydrogenase (BADH) belongs to the aldehyde dehydrogenase family 10 (ALDH10) of the large super family of aldehyde dehydrogenases4,5. BADHs were initially known to oxidize betaine aldehyde (BAL) to form glycine betaine (GB) which is one of the major organic osmolytes that are accumulated in various plant species in response to different ambient stresses such as salinity, drought, extreme temperatures, heavy metals and UV radiation6,7. In other cases, they can oxidize some of the metabolism-derived aminoaldehydes to the corresponding amino acids, many of which are related to osmotic regulation and stress responses8.

It has been proposed that plant BADHs virtually belong to two subfamilies: one family with high affinity specific for betaine aldehyde as substrate (true BADHs) and the other with the affinity widely for many aminoaldehydes (high BADH homology aminoaldehyde dehydrogenases, HBH-AMADHs). Some BADHs belong to such as SoBADH in spinach (Spinacia oleracea) and BBD2 in barely (Hordeum vulgare L.)4,7 belong to the true BADH. Other BADH may belong to AMADHs which do not accumulate GB such as PsAMADH and ALDH10A8 or ALDH10A9 in Arabidopsis10,11 which do not accumulate GB, may belong to AMADHs. Actually, BADH isozymes have not been classified based on the specificity of the encoded enzymes which indicated that HBH–AMADHs could not be distinguished from true BADHs just according to the amino acid sequence4.

Plant BADHs have been shown to respond to some abiotic stresses such as salt, drought and temperature stress12-14. Also, many transgenic plants expressing a foreign BADH exhibit increased tolerance to abiotic stress15,16. A recent study has identified BADH as an potential antibiotic-free marker for the screening of transgenic plants and also found a major role of BADH in 2-acetyl-1-pyrroline-based fragrance which is associated with several aromatic rice
varieties (jasmine and basmati style)\textsuperscript{3}. Over expressed BADH of transgenic alfalfa plants showed significantly higher BADH enzyme activity and betaine level when grown under higher salt condition\textsuperscript{18}. Similarly, the transgenic sweet potato over-expressing SoBADH showed the improved tolerance to various abiotic stresses, including low temperature, salt and oxidative stress\textsuperscript{19}.

BADH genes have been cloned from a vast variety of plant species such as sugar beet\textsuperscript{20,21}, spinach\textsuperscript{22}, sorghum\textsuperscript{23}, barley\textsuperscript{24}, Avicennia marina\textsuperscript{25}, Amaranthus hypochondriacus L\textsuperscript{26}, rice\textsuperscript{27}, Arabidopsis thaliana\textsuperscript{28} and Suaeda liaotungensis\textsuperscript{29}. However, limited information is available on BADH in fruit crops and very little is yet known about BADH gene in papaya.

In the present work, a full-length cDNA of CpBADH gene related to temperature response was cloned and characterized from Carica papaya. The expression profile of CpBADH was investigated in harvested papaya under both high and low temperature stresses.

Material and Methods

Plant materials and experimental conditions: Papaya fruit (Carica papaya) at color break to one-fifth yellow stage were collected from a local commercial plantation nearby Guangzhou, South China and then transported to laboratory. Fruits with the uniform size, shape and maturity and free of visual defects were randomly selected and cleaned, dipped in 2g·L\textsuperscript{-1} sodium hypochlorite solution for 10 min to astringe cut, then soaked in 500 mg·L\textsuperscript{-1} Iprodione solution (Kuaida, Jiangsu, China) and 500 mg·L\textsuperscript{-1} prochloraz solution (Huifeng, Jiangsu, China) for 1 min to eliminate potential microbes. They were allowed to be air dried at 25°C, placed into unsealed plastic bags and stored at 7°C, 25°C and 35°C respectively. For each treatment, samples were collected according to the fruit color change during ripening as described in our previous work\textsuperscript{28}. Biological triplicates were performed randomly for each temperature setting. All samples were frozen in liquid nitrogen and stored at -80°C until use.

Fruit ripening evaluation under temperature stress: Periodical measurements of ethylene production, respiration rate and visual inspection of the peel color were conducted to evaluate the fruit ripening. Fruit coloring indexes were calculated as described in our previous study\textsuperscript{28}. Ethylene production and respiration rate were measured as described previously\textsuperscript{29}.

Total RNA isolation and first strand cDNA synthesis: All frozen tissues were ground using a mortar and pestle in liquid nitrogen for RNA isolation. Total RNA extraction used the hot borate method described by Wan and Wilkins\textsuperscript{30}. Potential DNA contamination was eliminated by DNase I digestion treatment using RNAse-free kit (TaKaRa, Japan). The DNA-free total RNA with good quality was then used for first strand cDNA synthesis. For RT-qPCR experiment, cDNA was prepared using ReverTra Ace qPCR RT kit (TOYOBO, Japan) according to the product manual. 1 µg of total RNA was reverse transcribed using SMARTer\textsuperscript{TM} RACE cDNA Amplification Kit (Clontech, USA) to prepare the cDNA for RT-PCR.

Isolation of BADH full length cDNA and sequence analysis: To amplify the BADH gene from papaya, a homologous BADH gene from Arabidopsis (GenBank accession number: NM_106150.3) was used as the seed sequence for retrieval blast in papaya EST database. A highly homologous papaya BADH EST sequence (GenBank accession number: EX280809.1) was obtained and it also had a high homology with the BADHs of other plant species. A set of primers (sense: 5’TGGAAAAACCGCTTTGATGAAG3’, antisense: 5’CCCGACTGACAAATAGGACC3’) was designed based on the EST sequence. RT-PCR conditions were described as previous work\textsuperscript{28}. The PCR products with the predicted size (about 720 bp in length) were sequenced (BGI, China) used to design specific primers for obtaining the full-length cDNA of CpBADH.

Two specific primer pairs for CpBADH (3’-RACE: outer, AGCAGGGCGAGGAACCTTTCATGTG; 5’-RACE: outer, TGCTGCTGTGGCGATGCTTCCAGTGAA, inner, GGCACATCGGATGAAGCACAAGAT) were used for 3’- and 5’-RACE respectively using SMARTer\textsuperscript{TM} RACE cDNA Amplification Kit (Clontech, Mountain View, CA, USA). The full-length cDNA sequence of the CpBADH genes was obtained by assembling the sequences of the internal conserved fragment, 3’and 5’-RACE products.

Bioinformatics analysis: The NCBI Blast program (http://www.ncbi.nlm.nih.gov/BLAST) was used to identify nucleotide sequences from RT-PCR clones. Sequence alignment was conducted using DNAMAN software. NCBI ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) was used to find the open reading frame (ORF) and protein prediction. The phylogenetic tree was constructed by MEGA 5.0 software. The theoretical isoelectric point (pI) and molecular weight values were calculated using the Peptide Mass program (http://us.expasy.org/tools/peptide-mass.html). Prediction of protein subcellular localization was conducted using WoLF PSORT (http://wolfsort.org/). The three-dimensional structure of CpBADH protein domain was predicted using SWISS-MODEL workspace\textsuperscript{31}.

Real-time quantitative PCR: As the accuracy of RT-qPCR experiment is mainly due to the use of the stable expressed reference gene(s) for data normalization, the reference genes used in present study have been identified and described in our previous study which conducted systematic analysis of the stability of abundant candidate reference genes under different experimental conditions and a set of suitable reference genes for specific experimental conditions were determined\textsuperscript{32}.
information of the five reference genes (RPS, EIF, UBCE, UBQ, and SAND) used in this study were the same as those reported in our previous study\(^{28}\) and the primer pair of CpBADH for RT-qPCR was listed as:

**Forward:** 5′ TGGACGAGAACTAGGTGAATGG3′

**Reserve:** 5′ GCATCAGCAATTACAAGAATCT3′

with single product of a correct size with a high PCR efficiency (90–110%).

Specificity of the amplifications was confirmed by both melting curve analysis and agarose gel electrophoresis. RT-qPCR was carried out on Bio-Rad CFX96 Real-Time PCR System as described in the previous work as well as the reaction conditions.\(^{28}\) Each RT-qPCR analysis was performed in triplicate and the mean was used for RT-qPCR analysis. The relative expression of the BADH gene was calculated according to the method of \(\frac{\Delta\Delta Ct_{\text{target}}}{\Delta\Delta Ct_{\text{reference}}}\). The Ct values for both the target and the reference genes were the means of three independent PCR assays.

**Statistical analysis:** Experiments were arranged using a completely randomized design. Each sample time point for each treatment comprised of three independent biological replicates. Data were plotted on figures as means ± standard error (SE).

**Results**

**Fruit evaluation:** Fruit coloring index was calculated on a daily basis as our previous study.\(^{28}\) The results showed that high temperature stress accelerated the fruit color change and firmness decrease whereas low temperature completely blocked the fruit coloration.\(^{33}\) Chilling injury symptoms were also observed for fruit stored at 7°C at the 20\(^{\text{th}}\) d after storage.\(^{33}\) It was consistent with the previous study which indicated that storage below 10°C or high than 30°C would cause stresses to papaya fruit. High temperature accelerated fruit ethylene production where ethylene was detected on 4\(^{\text{th}}\) d after storage, peaked on 5\(^{\text{th}}\) d and increased to a second peak from 6\(^{\text{th}}\) d. For the control fruit, ethylene production was detected on the 6\(^{\text{th}}\) d and peaked on day 7. In contrast, low temperature completely repressed the ethylene production (Fig. 1a). As shown in fig. 1b, the respiration rate of papaya fruit stored at high temperature (35°C) was much higher than that in the control fruit stored at 25°C, indicating that high temperature promotes the respiration rate of papaya fruit. However, low temperature (7°C) repressed the respiration which was dropped to a low level and remained low throughout whole storage (Fig. 1b).

**Isolation and characterization of a full-length cDNA of CpBADH:** One fragment of a CpBADH homolog approximately 720 bp in length was initially cloned from papaya as described before. The corresponding full-length cDNA sequence, designated as CpBADH, was subsequently obtained by RACE and deposited into GenBank (accession number: JQ678786). The full-length cDNA was 2052 bp, containing an ORF of 1509-bp that encodes a predicted polypeptide of 503 amino acids (AA), with an isoelectric point (pI) of 5.25 and a predicted molecular weight of 54.84kDa.

CpBADH shared a high homology in nucleotide sequence with those BADHs in Populus euphratica (BADH2) (84%), Jatropha curcas (BADH) (83%), Coriolum heterophylla (82%), Gossypium hirsutum (82%) and Arabidopsis thaliana (80%). Although the amino acid sequences of BADH proteins appeared to be diverse in both composition and size, several major and highly conserved regions were found in the amino acid sequence including a decapetide motif ‘VTLEGKGP’, and a cysteine residue at a distance of 28 amino acid from the decapetide motif which are highly conserved among general ALDHs. The Ala-441 in SoBADH was also found in position 442 in CpBADH and other BADHs (Fig. 2) which is critical for BADH activity. The amino acid sequence had a significant homology with BADH proteins of higher plants, exhibiting higher identity with those of Jatropha curcas (84%), Chorispora bungeana (84%), Populus euphratica (83%) and Arabidopsis thaliana (84%). Sequence alignment of CpBADH protein with other plant BADHs was presented in fig. 2. We also found that CpBADH had unique residues, cysteine-lysine-leucine (SKL), at the C-terminal end which was the same as that of barley and rice BADHs\(^{25,26}\).

The signal peptide SKL is the signal of localization in the peroxisomes.\(^{26,35}\) Another series residues glutamate – leucine- phenylalanine- isoleucine- aspartate- glycine glutamate (ELFIDGE), which was quite similar to the signal peptide QLFIDGE were also found in CpBADH N-terminal end. Phylogenetic tree analysis showed that several major groups were distinguished within the phylogenetic tree (Fig. 3). CpBADH, AIALDH10A8 and AtBADH1 (ALDH10A8) belonged to the same group indicating that they may have a relatively close evolutionary relationship. However, AIALDH10A9 and AtBADH2 (ALDH10A9) were clustered in another group indicating that they have a relatively distant evolutionary relationship with CpBADH.

**Prediction of cellular localization and three-dimensional (3D) structure:** CpBADH protein was predicted to be mainly distributed within chloroplast by using WoLF PSORT program. Consistent with this prediction, several studies have also reported that BADH was predominantly localized in chloroplasts.\(^{36,37}\).

X-ray crystal analyses showed that ALDH\(^{38,39}\) and BADH\(^{40}\) enzymes in animal consist of a nucleotide-binding domain, an oligomerization domain and a catalytic domain. A predicted 3D structure of the CpBADH domain was obtained by homology modeling based on the crystal structure of Pisum sativum aminoaldehyde dehydrogenase.
Expression profiles of CpBADH in papaya fruit under temperature stresses: The product specificity was confirmed by both melting curve analysis and agarose gel electrophoresis as well as sequencing. The expression of CpBADH was determined at different time points as previously described under low and high temperature stresses (Fig 5). In the control fruit (25°C), CpBADH transcript level slightly decreased during the storage. Compared to the control, CpBADH expression at low temperature (7°C) showed a slight increase to 1.5 fold in the first 2 days but decreased to the initial level at 6 d and remained thereafter.

However, CpBADH expression was significantly up-regulated under high temperature stress (35°C). During the first 2 days, CpBADH mRNA levels sharply increased up to about 2.3 fold at 2 d decreased to 1.5 fold from 2 d to 6 d and increased again from 6 d and reached up to 2.5 fold at 10 d. Overall, both temperature stresses induced the CpBADH expression during the storage. In addition, high temperature stress caused a more remarkable induction of CpBADH expression. These results suggest that CpBADH may be a temperature-stress inducible gene involved in the regulation of GB accumulation in papaya subjected to temperature stress and that CpBADH appears to be more closely related to high temperature stress than to low temperature stress.

Discussion
Higher plants can be divided into GB accumulating and non-accumulating plants according to the functional choline monooxygenase (CMO). Plants lacking a functional CMO belong to GB non-accumulating plants such as Arabidopsis thaliana, rice and Medicago truncatula etc. while spinach (Spinacia oleracea), barley and Amaranthus are typical GB accumulating plants. BADH is the enzyme catalyzing the last step in the pathway of GB synthesis from choline and this pathway has been found in almost all GB-accumulating plant species. To date, BADH gene has been cloned from many species but few studies have focused on its roles in tree fruits. In present study, a novel BADH gene, named CpBADH, was isolated and characterized from papaya fruit. Sequence analysis revealed that CpBADH contained several conserved domains and showed high homology to BADHs of other plants; especially, it has a relatively close evolutionary relationship with A1ALDH10A8 and AtBADH1 (ALDH10A8). Many plants, such as barley, rice and Arabidopsis thaliana, possess more than one putative BADH-encoding gene homologue. Genes encoding isozymes may show different expression patterns. For instance, two BADH genes (BRD1 and BRD2) in barley showed different activities on Betaine aldehyde. Arabidopsis thaliana contains two BADH genes, ALDH10A8 and ALDH10A9. Phylogenetic tree analysis showed that CpBADH had a relatively close evolutionary relationship with A1ALDH10A8 and AtBADH1 (ALDH10A8) but showed a relatively distant evolutionary relationship with A1ALDH10A9 and AtBADH2 (ALDH10A), indicating that CpBADH may have a similar role with ALDH10A8 (Fig. 3). As shown in the phylogenetic tree, CpBADH had a relatively distant evolutionary relationship with SoBADH which has a high activity and specificity for BA as a substrate (true BADHs).

However, sequence analysis showed that CpBADH had the conserved Ala-441 in the position 442, which is the amino acid residue critical for BADH activity. It has been reported that GB accumulators can distinguish from the non GB accumulators based on these sequence characters, the former has a high-BAL-affinity isoenzyme with Ala or cysteine in this critical position while the later has low-BAL-affinity isoenzymes containing Ile in this position.

On the other hand, CMO and BADH work together in GB biosynthesis pathway in plants. It has been suggested that the lack of GB accumulation in plant is due to the lack of a functional CMO, Rice is one kind of GB non-accumulating plants and has two BADH gene homologs (OsBADH1 and OsBADH2). The lack of a functional OsCMO protein presumably results in non-GB accumulation in the tested rice plant. Therefore, it is possible that some plants show a high BADH activity but no GB accumulation as they do not have a functional CMO.

BADH proteins appear to be localized in different cellular compartments in plants including chloroplast, peroxisomes and cytosol. There are two kinds of relevant signal peptides for cellular localization. One kind of N-terminal end signal peptide, QLFIDGE, is thought to be the signal for chloroplast localization and the other is C-terminal end signal peptide, SKL which is the signal for peroxisome localization. Functional domain analysis showed that CpBADH had both signal peptides. However, the cellular localization prediction showed that the indicated CpBADH protein was mainly distributed on chloroplast. It is likely that CpBADH is localized in the chloroplast of papaya leaves.

The chloroplast localization of CpBADH has been shown in an earlier study by Weigel et al who reported that the chloroplast stromal fraction of spinach leaves contained a specific pyridine nucleotide-dependent BADH whereas the cytosol fraction contained a minor isozyme of BADH and that the specific activity of BADH increased three-fold in spinach plants grown at high salt condition. While both...
Isozymes contributed to the BADH increase, the chloroplast CpBADH is involved in oxidative and salinity responses. ALDH10A9 has been found to effectively target peroxisomes while ALDH10A8 localizes in leucoplasts in Arabidopsis thaliana.

In barley, two BADH proteins were identified and were found to be localized in peroxisomes and cytosol respectively. In rice, BADH isozyme was localized in peroxisomes. The deduced amino acid sequences of barley and rice BADHs both contain the peroxisome targeting-signal SKL, like CpBADH suggesting that CpBADH is likely localized in the peroxisomes of papaya fruit. However, the N-terminal end signal peptide, QLFIDGE, may also play a role in subcellular location in papaya. The divergence in the cellular localization of BADH isozymes indicated that they may play certain specific functions. The physiological significance of the differential sub-cellular localizations of BADHs is still unclear and further studies are needed to investigate the precise subcellular location and its roles in stresses responses.

We investigated the role of CpBADH in response to temperature stress and observed that CpBADH expressed differently in response to low and high temperature stresses. While both low and high temperature induced CpBADH expression, high temperature stress caused a more significant induction of CpBADH expression. Therefore, CpBADH may be a stress inducible gene and CpBADH appears to be more closely related to high temperature stress than to low temperature stress. Consistent with this observation, it was reported that in barely, BADH gene was induced by high salinity and other osmotic stresses. It was also reported that in leaf tissue from A. tricolor, heat stress caused increased BADH protein concentration rather than chilling stress did. The expression of a BADH homologue was induced under cold stress in Atriplex centralasiatica. In Arabidopsis, ALDH10A8 and ALDH10A9 genes were weakly induced by ABA, salt and chilling stresses.

The expression of CpBADH was increased in the first two days, then decreased from 2 d to 5 d and increased again thereafter at 35°C (Fig. 5). These changes may reflect the physiological changes within the fruits. When fruit were stored at high temperature, their metabolism and evaporation rate were stimulated leading to an increased osmotic stress and the induction of CpBADH to produce more osmotic protectants (GB or other substraces) to adjust intercellular and extracellular atmosphere. After a period of adjustment, CpBADH expression decreased but increased again when the sustaining stresses were imposed upon on fruit.

Several transgenic plants over-expressing GB-synthesizing genes exhibited an increased GB production and an enhanced tolerance to cold, salt, drought and high temperature stresses. But recent studies indicate that GB accumulation pathway may not be the only mechanism by which all putative BADHs confer abiotic stress tolerance. For example, rice is one kind of GB non-accumulating plants, it does not accumulate GB and rice BADH1 shows a much stronger affinity for c-aminobutyraldehyde (GABald) than for BA. However, rice BADH1 can respond to salt stress.

Therefore, rice BADH1 is a definitive example of HBH-AMADHs that can respond to salt stress. BADH catalyzes not only the production of GB from BA but also the production of γ-aminobutyric acid (GABA) from GABald. GABA possesses the osmotic protectant properties and plays certain roles in abiotic stress. The expression pattern of BADH in non-functional CMO transgenic tobacco with increased salt stress tolerance has also implicated that there might be GB independent mechanisms by which BADHs regulate stress tolerance. Therefore, no matter papaya is GB accumulating or non-accumulating plant, CpBADH is an important gene for improving its stress tolerance. There is an urgent need of further work to define the precise roles of BADH in stress tolerance and the underlying mechanisms.

Fig. 1: (a) The effects of temperature on fruit ethylene production and (b) fruit respiration rate during the storage. Means were derived from three independent measurements and the bars indicate standard errors
Conclusion
The papaya BADH gene, \textit{CpBADH}, was isolated from papaya fruit and characterized with bioinformatic tools. Its time-course expression profiles under low and high temperature stress conditions were examined. Our results suggest that \textit{CpBADH} may play roles in stress responses. Further experiments including transgenic attempt and small RNA interference are worthy to advance our understanding of its roles in papaya fruit stress adaption and fruit quality control.

Acknowledgement
The research work was supported by National Natural Science Foundation of China (31372112), the National Key Technology R and D Program of China (2011BAD24B02-4) and Guangdong Science Foundation (06200670).

References


Fig. 3. Phylogenetic tree showing the relationship of full-length BADHs from several plant species based on the similarity of their amino acid sequences. The tree was generated using bootstrap support based on 1000 replicates constructed using MEGA 5. Numbers at nodes indicate degree of bootstrap support. Branch distance indicates proportion of amino acid changes. NCBI accession number was shown for each BADH. The plant BADHs include Arabidopsis lyrata ALDH10A8-EFI65259.1 (Arabidopsis lyrata subsp. Lyrata), Arabidopsis thaliana BADH1 (ALDH10A8) -NP 565094.1 (Arabidopsis thaliana), Chorispora bungeana BADH-AAV67891.2 (Chorispora bungeana), Gossypium hirsutum BADH-ADW80331.1 (Gossypium hirsutum), Pyrus betulifolia BADH-AER10508.1 (Pyrus betulifolia), Corylus heterophylla BADH-ADW80331.1 (Corylus heterophylla), Glycine max BADH-ADW80331.1 (Glycine max), Populus euphratica BADH-AFA5317.1 (Populus euphratica), Colza napus BADH-AFA5317.1 (Brassica napus), Arabidopsis lyrata ALDH10A9-EFI65259.1 (Arabidopsis lyrata subsp. Lyrata), Arabidopsis thaliana BADH2 (ALDH10A9) -NP 190400.1 (Arabidopsis thaliana), Betula vulgaris BADH-EAE07176.1, Hordeum vulgare BADH-HAM62947.1, Oryza sativa Indica Group BADH-ABB83473.1, Spinacia oleracea BADH-ACG29220.1, Escherichia coli DH1 BADH-YP 006033207.1, and Staphylococcus aureus BADH-YP 001317798.1 (Staphylococcus aureus subsp. aureus JH1).

Fig. 4: Three-dimensional structures of CpBADH and AtBADH domains based on a template of Pisum sativum Aminoaldehyde dehydrogenase 2 (PSAMADH2) crystal structure. (A) CpBADH from papaya and (B) AtBADH from Arabidopsis thaliana. Estimated per-residue inaccuracy was visualized using a color gradient from blue (more reliable regions) to red (potentially unreliable regions).
Fig. 5: Relative quantification of CpBADHs using selected reference genes for normalization under different storage temperature conditions. Means are derived from three independent measurements and the bars indicate the standard errors.

10. Kopečný D., Tylicková M., Snegaroff J., Popelková H. and Šebela M., Carboxylate and aromatic active-site residues are determinants of high-affinity binding of α-aminoadaldehydes to plant aminoaldehyde dehydrogenases, FEBS J, 278(17), 3130-3139 (2011)
17. Kishitani S., Watanabe K., Yasuda S., Arakawa K. and Takabe T., Accumulation of glycinebetaine during cold acclimation and freezing tolerance in leaves of winter and spring barley plants, Plant Cell Environ, 17(1), 89-95 (1994)


(Received 30th December 2014, accepted 15th February 2015)