



## Research Article

## ***In-silico* identification of *cis*- acting regulatory elements in 5' regulatory region of Betaine aldehyde dehydrogenase isoenzymes of selected monocot and dicot**

Hemani Sharma\*, Sumit Govil and Divya Shrivastava

School of Life sciences, Jaipur National University, Jagatpura, Jaipur, India

Received: 1/9/2018; Accepted: 1/20/2018

**Abstract:** It's challenging to understand the multifarious gene regulatory networks. Diverse biological processes, are mostly conferred by nature of *cis*-acting regulatory elements (CARE) located within the regulatory region which include abiotic stress responses, hormone responses and developmental processes. Thus, the identification of CARE and their organization is a crucial step for better understanding of spatiotemporal expression of the gene. The putative *cis*-acting element that are located within the 5' regulatory region of BADH isozymes of *Hordeum vulgare*, *Oryza sativa*, *Sorghum bicolor*, *Leymus chinensis*, *Zoysia tenuifolia*, *Arabidopsis thaliana*, *Amaranthus hypochondriacus*, *Chrysanthemum lavandulifolium*, *Populus euphratica* and *Atriplex prostrata* were identified using bioinformatics tools. Several probable *cis*-acting regulatory elements that are coupled with cellular development, hormonal and stress response were identified as: Me-JA, GARE, GT-1, MYB, W-box, I-box, Pyrimidine-box, ANAERO, ARF, CARE, ASF1, SORLIP, SURE, TAAAGSTKST1, GCC Box, CICADIAN, Sp1 and CGCG Box. These results shed light on probable *cis*-acting regulatory elements these BADH isoenzyme that confer their expression and regulation during cellular, hormonal and stress response.

**Keywords:** Biotic and abiotic stress, Betaine aldehyde dehydrogenase (BADH), *Cis*-acting regulatory elements, Cellular development, Hormonal regulation, Spatiotemporal

### Introduction

Gene expression is a completely controlled process that exists at various levels and transcription is one of the most essential step (Wyeth and Albin, 2004). In plants more than 1500 transcription factors are involved to control the expression of target gene in complex signaling network and transcription rate of gene located in the promoter region (Doelling and Pikaard, 1995). In plants the regulatory/promoter region of gene is located primarily in the untranslated region of DNA sequence (about 1000 - 1500 base pairs upstream) accountable for its activity, modulation of the neighboring regions of translated DNA and coordinate expressions (Chabouté *et al.*, 2002).

The promoter region consists of peculiar DNA sequences and responsive elements that act in the calling of regulatory proteins that aid in transcription of the protein-coding region of the gene. Each gene contains a specific combination of *cis*-acting regulatory sequence elements in their 5' regulatory region. *Cis*-acting regulatory elements are vital transcriptional gene regulatory units; that establish distinct spatiotemporal transcriptional activity (Qui, 2003). Hence identification and understanding of the *cis*-acting regulatory region bound by TFs that control gene expression will put forward the crucial information to elaborate the mechanism that how and what external stimulus may be involved in their expression. Thus, a full

understanding of the transcriptional gene regulation system will depend on successful functional analyses of *cis*-acting elements.

As well acknowledged, various environmental stresses, such as the drought, salinity and high/low temperature have hostile result on plant expansion, development and productivity (Shinozaki and Yamaguchi, 2003). By the nature, plants have developed highly intricate mechanisms and numerous response to different environmental stresses that may entail the renovation of cellular homeostasis, stress induced protein expression, synthesis of anti-microbial products; for resistant, and addition of compatible solutes such as glycine betaine (Cherian and Reddy, 2003). Glycine betaine key role in cellular osmotic adjustment, elevating drought resistance, plasma membrane integrity, also acts as a molecular chaperone by helping to stabilize protein structure and function in plants under environmental stress (Bartels and Sunar, 2005; Allakhverdiev *et al.*, 2008). In plants synthesis of glycine betaine involves two enzymes; first step oxidation catalyzed by choline monoxygenase (CMO) and second by betaine aldehyde dehydrogenase (BADH) (Rhodes and Hanson, 1993). BADH is an enzyme implicated in the biosynthesis of glycine betaine, the amount of glycine betaine exhibit in a specific plant tissue which is greatly associated and mostly controlled by

### \*Corresponding Author:

**Hemani Sharma,**

PhD student, School of Life Sciences,

Jaipur National University,

Jagatpura, Jaipur, India.

E-mail: [hemani6606@gmail.com](mailto:hemani6606@gmail.com)



statuses of environmental that can permit particular transcriptional-translational expressions of BADH genes. Various paralogous genes, which encode several isozymes of BADH were acknowledge and characteristically present in few plants. The studies also revealed and proposed that incidence of evolutionary divergence between monocot and dicot BADH isoenzymes (Munoz-Clares *et al.*, 2014) was due to several gene duplication which result in creation of many isozymes (Julian-Sanchez *et al.*, 2007). However, till now, only narrow information is present regarding BADH genes. Transcriptional phenomenon is generally regulated by binding of transcriptional elements to their related *cis*-acting elements positioned at affected gene promoter (Lee *et al.*, 2002). In plants, various stresses coupled *cis*-acting regulatory elements that trigger transcript in repose to abiotic factors, wound and pathogen response had been predicted (Ibraheem *et al.*, 2010). At present, work done on the *cis*-acting regulatory elements that modulate expression of BADH isozymes reported in abiotic and/or biotic stress is very scarce. Thus, the identification of CARE and their organization is a key step to fructify understanding expression/regulation of genes. Hence, our objective was to use accessible bioinformatics tools to reveal broad explanation and rate of occurrence of vital *cis*-regulatory elements positioned in the 5' untranslated (5'UTR) DNA (promoter region, Folorunsho *et al.*, 2014) of BADH isozymes of selected monocot and dicot plant. This analysis reveals transcription regulatory interactions of these isozymes during development or under environmental stress conditions. Hence, *in-silico* identification of *cis*-acting regulatory elements will render additional perceptivity of gene expression and regulation. In addition, this information may assist in development of higher yields tolerant varieties in plant breeding programmes.

## Materials and Methods

### Data source:

The complete nucleotide sequence, 5'UTR and the CDS of BADH isoenzymes of (*Hordeum vulgare*, *Oryza sativa*, *Sorghum bicolor*, *Leymus chinensis*, *Zoysia tenuifolia*: Monocot) (*Arabidopsis thaliana*, *Amaranthus hypochondriacus*, *Chrysanthemum lavandulifolium*, *Populus euphratica*, *Atriplex prostrata*: Dicot) registered in GenBank was obtained from National center for Biotechnology information (NCBI)

(<http://www.ncbi.nlm.nih.gov>) (Supplementary data).

### Identification and analysis of Cis-acting regulatory elements:

Detection of the CARE by scanning 5'UTR of BADH isozymes for the occurrence of probable CRE exactly alike or related motifs recorded in Genomatix Matinspector (<http://www.genomatix.de/cgi-bin/matinspectorprof/matfam.p1>; Cartharius *et al.*, 2005), PlantPan (<http://PlantPan.mbc.nctu.edu.tw>; Chang *et al.*, 2008) and PlantCARE(<http://bioinformatics.psb.ugent.be/webtools/plantcare/cgi-bin/CallMatIE5.html>; Lescot *et al.*, 2002).

### Prediction of Similarities/Divergence:

Identity/Dissimilarity among the 5' UTR, coding and protein sequences of BADH isozymes of selected monocots and dicots were also identified using Clustal Omega: EMBL-EBI, (<http://www.ebi.ac.uk/Tools/clustal/>; Mc Williams *et al.*, 2013)

## Results and Discussion

There are numerous bioinformatics tools on the World Wide Web that makes analysis on the gene expression modulation of interest more accessible. Gene transcript initiation and inactivation in the biological pathway include binding of *cis*-element to transcription factors in the affected gene promoter (Lindolf *et al.*, 2009). In the selected monocot and dicot plant species, spatial location of the putative *cis*-acting regulatory elements indicates that; HvBBD1, OsBADH1, AtBADH1 at the distal region expression of genes might be well regulated, while HvBBD2, SbBADH2, AtBADH2, ZtBADH1, AhBADH1, CibADH1, CibADH2 have the *cis*-acting regulatory elements evenly spread across 5' region. However LsBADH2, PeBADH1, PeBADH2 expression of genes is at proximal region. The alignment results of protein, coding and non-coding sequence of monocot and dicot is as presented in Table 1, Table 2 and Table 3. The analysis of protein sequence in selected monocot and dicot also revealed high conservation in these sequences (Table 1). The coding region of BADH isoenzyme of monocot and dicot plant species seems to be more conserved (Table 2) where as high divergence appeared in 5' regulatory region of selected monocot and dicot (Table 3).

**Table 1:** Range of coding sequence homology expressed in % between selected monocot and dicot BADH isoenzyme

Isozyme (A)	Isozyme (B)	Range of homology (%)	Isozyme (A)	Isozyme (B)	Range of homology (%)
Barley	Barley	66%	Rice	Rice	62.80%
	Rice	60.40-78.10%		Sorghum	54.70-78.10%
	Sorghum	53.10-73.50%		Arabidopsis	62.60-64.70%
	Leymus	61.50-85.30%		Zoysia	62.50-79.40%
	Zoysia	65.30-77.50%		Amaranthus	14.60-70.70%
	Amaranthus	17.00-56.70%		Chrysanthum	58.60-61.80%
	Chrysanthum	61.80-63.50%		Atriplex	50.30-64.10%

	Atriplex	48.50-65.30%		Populus	60.50-71.10%
	Populus	58.10-58.60%		Arabidopsis	71.0%
	Arabidopsis	55.10-64.90%		Leymus	52.90-67.50%
	Leymus	47.10-66.40%	Arabidopsis	Amaranthus	16.60-69.70%
	Zoysia	56.10-79.80%		Chrysanthmum	67.30-70.30%
	Amaranthus	13.90-59.40%		Atriplex	57.20-69.40%
	Chrysanthmum	54.30-64.40%		Populus	70.50-72.50%
	Atriplex	53.00-64.40%		Leymus	56.50%
	Populus	54.70-60.00%	Leymus	Zoysia	59.90-77.40%
	Amaranthus	18.80%		Atriplex	51.10-65.00%
Amaranthus	Chrysanthmum	17.10-66.10%		Populus	59.90-62.00%
	Atriplex	14.90-74.20%		Chrysanthmum	92.90%
	Populus	15.70-75.30%	Chrysanthmum	Atriplex	54.60-70.20%
Atriplex	Atriplex	61.90%		Populus	64.50-69.10%
	Populus	63.40-66.20%	Populus	Populus	92.60%

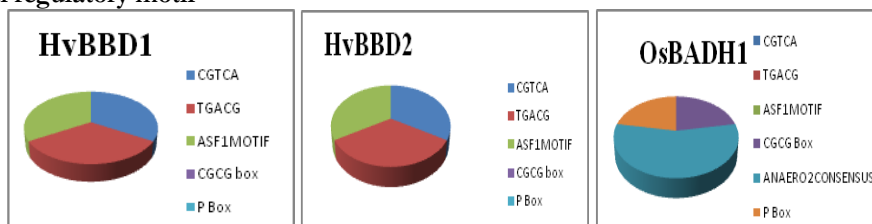
**Table 2.** Range of non-coding sequence homology expressed in % between selected monocot and dicot BADH isoenzyme

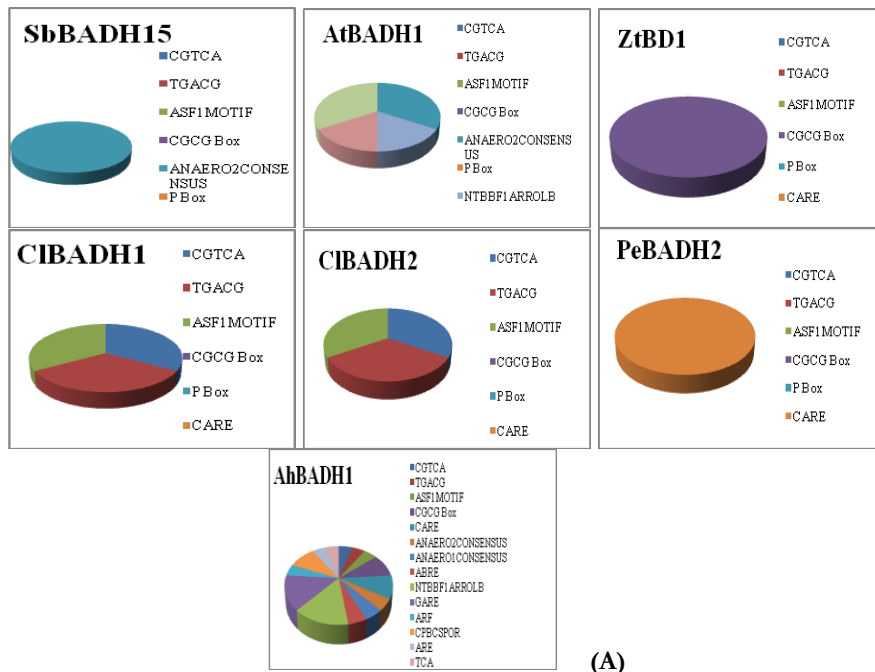
Isozyme (A)	Isozyme (B)	Range of homology (%)	Isozyme (A)	Isozyme (B)	Range of homology (%)
Barley	Barley	41.90%	Rice	Sorghum	1.50%
	Rice	27.40-32.60%		Arabidopsis	7.70-38.00%
	Sorghum	33.60-34.40%		Leymus	20.40-29.30%
	Arabidopsis	17.00-36.50%		Zoysia	10.90-26.0%
	Leymus	30.40-66.70%		Amaranthus	4.20%
	Zoysia	12.30-36.00%		Chrysanthmum	16.60-21.0%
	Amaranthus	2.20-3.60%		Atriplex	2.70%
	Chrysanthmum	2.40-37.50%		Populus	9.20-35.00%
	Atriplex	4.30-4.40%		Arabidopsis	20.90-28.90%
	Populus	16.40-24.50%		Leymus	11.50-34.70%
Arabidopsis	Arabidopsis	24.50%	Sorghum	Zoysia	17.70-41.30%
	Leymus	18.50-28.80%		Amaranthus	4.50%
	Zoysia	15.50-28.80%		Chrysanthmum	28.30-31.10%
	Amaranthus	2.40-6.00%		Atriplex	2.20%
	Chrysanthmum	19.80-40.20%		Populus	20.00-30.20%
	Atriplex	2.30-5.40%		Leymus	22.0-3.70%
	Populus	12.0-32.10%		Zoysia	18.50-34.70%
	Zoysia	14.50%		Amaranthus	1.4-1.9%
	Amaranthus	1.3-4.90%		Chrysanthmum	14.70-27.70%
	Zoysia	Chrysanthmum		13.10-31.40%	Atriplex
Chrysanthmum	Atriplex	2.50-8.30%	Amaranthus	Populus	16.20-31.60%
	Populus	4.4-29.40%		Chrysanthmum	5.60-6.40%
	Chrysanthmum	81.9%		Atriplex	0.20%
	Atriplex	1.8-2.4%		Populus	1.20-4.30%
	Populus	14.30-44.60%		Populus	3.20-14.30%
Populus	Populus	14.90%	Atriplex		

**Table 3.** Protein identity matrix in % between selected monocot and dicot BADH isoenzyme

Isozyme (A)	Isozyme (B)	Identity
HvBBD1	HvBBD2	71.33%
OsBADH1	OsBADH2	77.2%
SbBADH1	SbBADH2	66.57
AtBADH1	AtBADH2	79.44
LsBADH1	LsBADH2	71.95
AhBADH1	AhBADH2	97.73
ZtBADH1	ZtBADH2	74.55
CIBADH1	CIBADH2	98.65
ApBADH1	ApBADH2	83.52
PuBADH1	PuBADH2	92.55

**Hormonal regulatory motif**

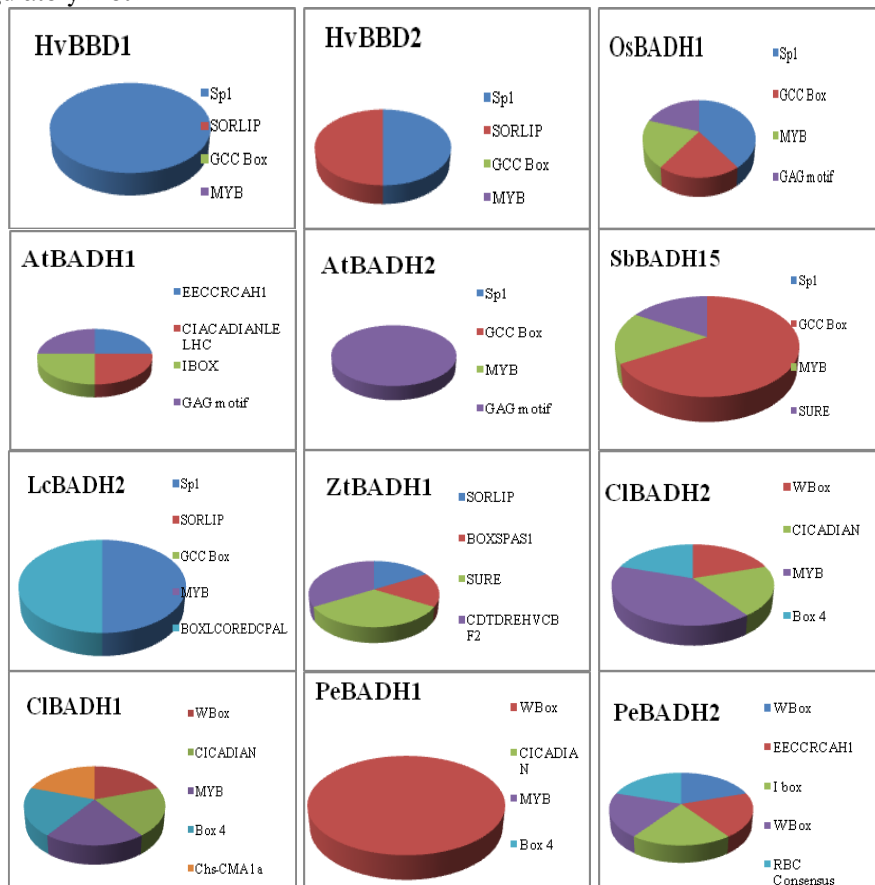


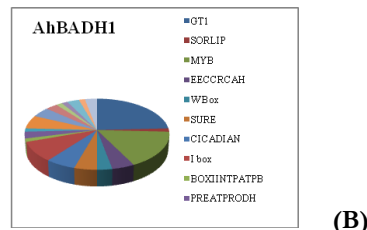


(A)

Fig. 1. Illustration of the frequencies of the cis-acting regulatory elements (hormonal regulatory motif) identified within the regulatory regions of BADH isoenzyme of selected monocot and dicot

**Stress regulatory motif**

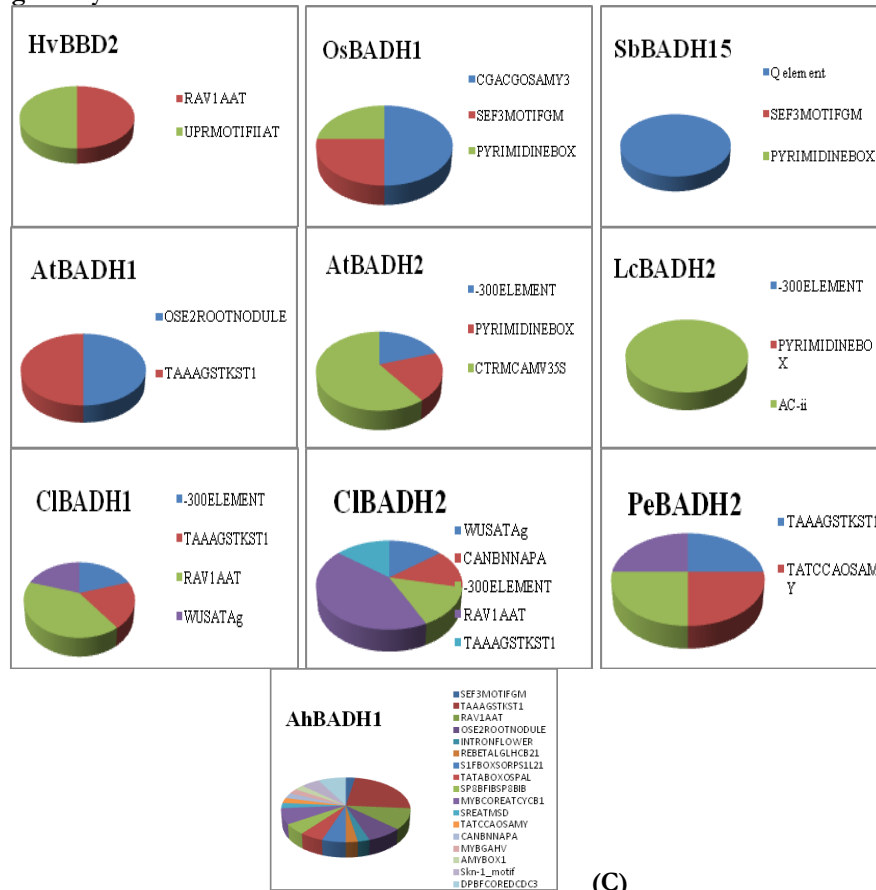




(B)

Fig. 2. Illustration of the frequencies of the cis-acting regulatory elements (stress regulatory motif) identified within the regulatory regions of BADH isoenzyme of selected monocot and dicot

Cellular regulatory motif



(C)

Fig. 3. Illustration of the frequencies of the cis-acting regulatory elements (cellular regulatory motif) identified within the regulatory regions of BADH isoenzyme of selected monocot and dicot

Sp1, GAG, Box-4, Box-I, G box, I box, L box, LAMP element, Circadian, chs-Unit 1 m1 and chs-CMA1a motif were found with variable frequency in the regulatory regions that are associated with the light response (Lam and Chau, 1989). Light is an important factor which checks the circadian rhythm of diverse processes, for example growth, development, nitrate uptake, flowering, seed development and stress responses in plants (Kumar *et al.*, 2009; Natr and Lawlor, 2005). Sp1 motif was found only in regulatory regions of HvBBD1, HvBBD2, OsBADH1 and LcBADH2 whereas GAG motifs were found only in OsBADH1 and AtBADH1. Of worth noting Box-4, G-Box, L Box, LAMP, chs-Unit 1 m1 and chs-CMA1a motif was only found in AhBADH1. I box motif found only in AtBADH2, AhBADH1, PeBADH2; a conserved

sequence in both monocots and dicots promoters of genes regulated by light (Terzaghi and Cashmore, 1995). In addition, CIACADIANLELHC essential for expression of circadian identified in the promoter region of (Lhc) genes in tomato (Piechulla *et al.*, 1998) was only found in AtBADH2 and AhBADH1 and SORLIP a key component in the light-inducible activity were found in HvBBD1, ZtBADH1, AhBADH1. It thus reflects BADH isoenzyme of selected monocot and dicot significantly influenced by light or through the involvement of particular specific receptor riposte to light. Although various binding factors and several light-responsive *cis*-elements (LREs) were discovered, none solo element has been acknowledged to only confabulate light sensitivity to minimal heterologous promoters (Lopez-Ochoa

*et al.*, 2007). Hence, it will be combinations of different *CARE* relevant for (LREs), instead of individual elements, are needed to confabulate light responsiveness alone (Chattopadhyay *et al.*, 1998). The W boxes are main *CARE* accountable for the pathogen was detected in the case of all BADH isoenzymes of Amaranthus, Chrysanthemum and Populus. Their presence merely indicates that BADH are wound responsive proteins and will be greatly modulated by wounding. This still ought to be elucidated in so as to establish response regulation of BADH isoenzyme. In previously studies, BOXLCOREDPCAL found in promoter region of DcMYB1 which activate transcription of the DcPAL2 gene in reponse to elicitor treatment (Maeda *et al.*, 2005). This would imply a MYB directed signaling pathway is triggered synthesize metabolites that facilitate defense responses. Another *cis*-element PREATPRODHD found in Amaranthus known for Pro- or hypoosmolarity activate transcription of proline dehydrogenase gene in riposte to L-Proline and hypoosmolarity (Satoh *et al.*, 2004). It is important to refer the presence of the GCC-box which was present in OsBADH1 and SbBADH2, has been notifiable as numerous PR (Pathogenesis-related) genes. Furthermore, GT1-element (for pathogen attack, light and salicylic acid) solely present in AhBADH1. GT-1 element found in the regulatory region of SCaM-4 from soybean (Glycine max) plays an important role in expression of SCaM-4 gene in riposte to pathogen and salt (Park *et al.*, 2004). EECCRCAH1 (for CO<sub>2</sub>) present in AtBADH1, AhBADH1, PeBADH2 and CIBADH2. Hbox consensus necessary for both light and elicitor stimulation restricts to AhBADH1. The presence of these elements may further be evidence of their rapid induction in stressed condition. The Heat Stress Element (HSE) was identified in regulatory region of AhBADH1. This *cis*-acting element helps to maintain a cell turgor for plant survival during high temperature stress (Larkindale and Vierling, 2008). The DRE element was only identified in regulatory region of ZtBADH1. This *cis*-acting element has been reported in modulating expression profiles of gene in abiotic stress conditions in plant (Dubouzet *et al.*, 2003). The occurrence of these motifs may connote the ability of gene to survive/acclimatize or the participation or need for glycine betaine during high/low temperature stress conditions. Li *et al.*, (2015); Tian *et al.*, (2017) also reported the function of glycine betaine in maintaining photosynthetic activity under stress conditions. Sulphur is a fundamental nutrient that is necessary for ontogenesis and plant yield (Hopkins, 1999) found in SbBADH2, ZtBADH1, and AhBADH1. P-box, *CARE* and GARE-motif detected in the regulatory regions of Rice, Arabidopsis, Populus and Amaranthus respectively that are associated to the gibberellin responsive element. In some promoters a single GARE motif is proficient to direct transcription at a high level which is hormonally

regulated as it collaborate with different *CARE* (Rogers *et al.*, 1994). CPBCSPOR a *cis*-acting element specific for cytokinin induction restricts to AhBADH1 (Fusada *et al.*, 2005). TC-rich repeats implicate with stress/ defense related responses and are uniquely found in AhBADH1. TCA, implicated to salicylic acid sensitivity reported in non-coding regions of several genes of monocot as well as dicot plant which are stimulated by single or more than one form of stress (Goldsbrough *et al.*, 1993). Salicylic acid (SA) induces the synthesis of pathogenesis associated protein and serves as a signaling molecule in plants detected in regulatory region of AhBADH1. SA facilitates plants to survive in stress conditions by stimulating biochemical and physiological mechanism (Khodary, 2004). Hence, occurrence of these motifs might indicate again the cooperative interaction between BADH isoenzyme and stress response proteins. Similarly, ASF1MOTIFCAMV found in Barley, Zoysia, and Chrysanthemum involved in stimulation of transcription of numerous genes by auxin and/or salicylic acid. With the exception of Rice, Arabidopsis, Leymus, Zoysia and Populus all other BADH isoenzymes in Barley, Amaranthus and Chrysanthemum may be regulated by MEJA and it has been reported in many wound/pathogen-sensitive genes in monocot as well as dicotyledonous plants in 5' regions (Rushton and Somssich, 1998). JA is reported to be involved in the drought-evoked betaine accretion in pear leaves (Gao *et al.*, 2004). The occurrence of the MeJA in the 5' regions of some of BADH isoenzyme infers a possible function in wound and stress signaling. LECPLEACS2 a *cis*-element of an elicitor-induced expression found in Arabidopsis; reported in solanum lycopersicum Acs2 gene for ethylene biosynthesis (Matarasso *et al.*, 2005). A process might be linked to synthesis of glycine betaine grounded on *in-silico* analysis is their involvement in reposit to flooding for the reason of occurrence of ANAERO1-3 consensus found to be spread within the regulatory region of Rice, Arabidopsis and Amaranthus. These consensus were reported in the promoter of anaerobically induced genes implicated in fermentation reaction (Mohanty *et al.*, 2005). Therefore, we may speculate that flood-response provide protective covering to cell against the acidification of cytosol (Zang *et al.*, 2000) plant growth may be modulated by BADH isoenzyme. ABRE *cis*-acting element has been well-known to aid drought tolerance in seeds, physiological changes such as: seed (growth and ontogeny) (Finkelstein and Gibson, 2002). ARF: Auxin response factor functionally identified in primary/early response genes where they adhere to auxin responsive element. Meanwhile, another *CARE* NTBBF1ARROLB implicated in tissue-specific expression and auxin keyed out in Arabidopsis and Amaranthus (Baumann *et al.*, 1999). Although ARF, ABRE found only in Amaranthus at low frequencies, so it effects on

genes may perhaps increase or suppress the transcription of genes relative to the kind of plant tissue wherein they're expressed. The *cis*-element CGCGBOX reported in promoters region of genes involved in ethylene, abscisic acid and light perception (Yang and Poovaiah, 2002) was found only in Rice, Zoysia and Amaranthus. In addition, a very interesting element WUSATAg has been solely identified at very low frequency in CIBADH1 and CIBADH2 reported as a target sequence of WUS in *AGAMOUS* gene in Arabidopsis (Lohmann *et al.*, 2003). Motifs coupled with sugar responsiveness as well as repression in expression of gene such as pyrimidine-box was located in OsBADH1 and AtBADH2. Similarly another *cis*-acting element implicated in sugar signaling response SREATMSD (Tatematsu *et al.*, 2005) detected in regulatory region of PeBADH2 and AhBADH1. On the contrary, the sugar responsiveness (Hwang *et al.*, 1998) CGACGOSAMY3 box is carried only by ZtBADH1, OsBADH1 and not by the other BADH isoenzymes. Another sugar related sequence TATCCAOSAMY found in promoter of alpha amylase mediate sugar and seed expression during germination (Chen *et al.*, 2006) located in regulatory region of AhBADH1 and PeBADH2. MYBGAHV is a *cis*-acting regulatory element partially implicated in sugar repression and reported in alpha amylase gene for GA response (Gubler *et al.*, 1995) was lower in AhBADH1 and absent in other BADH isoenzymes. This would entail that the expression profile of those genes could be modulated by levels of sugar, yet their sensitivity and physiological significance of identified *cis*-elements in 5' region will require to be identified by reporter gene experiments. Similarly, another explicit *cis*-element strongly restricts to Zoysia and Amaranthus the motif MYBCOREATCYCB1 require for activation of transcription of cyclin B1 at two different phase of cell cycle (Irehin *et al.*, 1997). Different TATA boxes such as TATAAAT, TATATAA and TATTAAT were manifest only in regulatory region of AtBADH2, AhBADH1, CIBADH1 and CIBADH2. TATA boxes are implicated in the expression of beta phaseolin gene of *Phaseolus vulgaris* (bean), responsible for encoding a seed storage protein in bean (Grace *et al.*, 2004). Another *cis*-regulatory element, CAATBOX1 reported in promoter for particular tissue activity of a pea legumin gene in tobacco (Shirsat *et al.*, 1989) has been found in AtBADH2, AhBADH1, CIBADH1, CIBADH2 and PeBADH2. It is important to mention 5' UTR Py-rich stretch which is known to confer elevate transcription doesn't require any other upstream *CARE* except for a TATA-box was detected in AtBADH2 (Daraselia *et al.*, 1996). There were some particular tissue elements such as, TAAAGSTKST1 for guard cell expression (Plesch *et al.*, 2001) found in AtBADH1, AhBADH1, CIBADH1, CIBADH2 and PeBADH2; CANBNNAPA for embryo- and endosperm-specific expression (Ellerstrom *et al.*, 1996) located

in CIBADH2 and AhBADH1; Skn-1 motif for endosperm expression (Washida *et al.*, 1999) restricts to AhBADH1; Q Element for regulating pollen expression (Hamilton *et al.*, 1998) restricts to SbBADH2; -300 element for seed storage protein (Forde *et al.*, 1985) found in AtBADH2, CIBADH1 and CIBADH2; CTRMCAMV35S serve as an inverted GAGA element which enhance gene expression (Pauli *et al.*, 2004) identified in AtBADH2; OSE2ROOTNODULE for nodule expression (Fehlberg *et al.*, 2005) found in AtBADH1 and AhBADH1; RAV1AAT and RAV1BAT key regulator of various developmental process in plant and relatively high rosette leaf and root expression (Kagaya *et al.*, 1999) detected in HvBBD2, AhBADH1, CIBADH1 and CIBADH2; REBETALGLHCB21 required for phytochrome regulation (Degenhardt and Tobin, 1996) found in AhBADH1; SEF3MOTIFGM for seed or embryo expression (Allen *et al.*, 1989) found in OsBADH1, AhBADH1 and PeBADH2. Another, embryo specific element DPBFCOREDCDC3 reported in promoter of Dc3 gene of carrot (Kim *et al.*, 1997) found in AhBADH1. CACT motif is a key element of Mem1 (mesophyll expression module1) (Gowik *et al.*, 2004) found in regulatory region of Zoysia. Motif of AC II is essential for vascular specific expression (Lui *et al.*, 2003) only found in *Leymus*. Furthermore, an AMY Box1 reported in alpha amylase promoter of rice, wheat and barley is responsible for regulating expression specific in seeds sprouting and callus (Huang *et al.*, 1990) found in AhBADH1. In addition, MYB *cis*-elements were keyed out in regulatory region of LcBADH2, OsBADH1, SbBADH2 and AhBADH1. These *cis*-elements had been accounted for drought inducible gene expression. Thus, it is tempting to cogitate that the function of betaine aldehyde dehydrogenase is restricted tissue specific because of the occurrence of particular tissue *cis*-acting elements and may be modulated by various environmental stresses and hormone signals. Numerous putative *cis*-elements identified in non-coding regulatory region that may be involved in the regulatory process while some elements implicate in cell/tissue specific expression (Supplementary data). It is assumed that expression of gene is modulated by amalgamation of various regulatory factors conferring different effects in different cell/tissue (Qui, 2003). Hence genes that have related expression may have common *cis*-acting element in 5' regulatory regions (Vilo *et al.*, 2000) which are key signature for a class of co-regulated genes. However, common CARE is one of the primary features of co-regulated genes and is frequently in regulatory region where they interact with complex protein (Wang *et al.*, 2004).

## Conclusion

Significant variations in nature and frequency observed in isoenzyme of selected monocot and dicot. Similar studies corroborate that isoenzyme

usually loose common *cis*-acting element and as a result of evolution or gene duplication of parent gene the regulatory region may be well diverse (Folorunsho *et al.*, 2014; Ibraheem *et al.*, 2010; Lynch and Force, 2000). This computational analysis is based on bioinformatics approach and mainly focus on the relative study of putative *cis*-elements keyed out in untranslated region of selected monocot and dicot. The analysis of these BADH isozymes gave an enhanced perceptiveness on how, when and type of external stimulus could be implicated in their expressions. However, transcription is littered with the conjunctive association of the several regulators that impacts the binding and property of RNA polymerase II, in riposte to numerous environs stress (Lloyd *et al.*, 2001; Zhu, 1996). This could be an excellent platform for restructuring regulatory networks of gene, that assist to amend biological research and future expressional in-vitro analysis. Hence, plant productivity and development will pivot on well regulated BADH isoenzymes expression; though *in-silico* analysis is a harbinger that assists in substantiating future expressional and functional in-vitro analysis.

### Acknowledgement

Authors are grateful to School of Life Sciences, Jaipur National University, Jaipur for providing necessary infrastructure and support for this work.

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#### Cite this article as:

Hemani Sharma, Sumit Govil and Divya Shrivastava. *In-silico* identification of cis- acting regulatory elements in 5' regulatory region of Betaine aldehyde dehydrogenase isoenzymes of selected monocot and dicot. *Annals of Plant Sciences* 7.2 (2018) pp. 2026-2036.



<http://dx.doi.org/10.21746/aps.2018.7.2.8>

**Source of support:** School of Life Sciences, Jaipur National University, Jaipur

**Conflict of interest:** Nil