ISOLATION, CHARACTERIZATION AND EXPRESSION ANALYSIS OF *HORDEUM VULGARE 22* (*HVA 22*) GENE FROM A DROUGHT TOLERANT SUGARCANE VARIETY

T.S. Sarath Padmanabhan, S. Dharshini, V.M. Manoj, J. Ashwin Narayan, C. Appunu and G. Hemaprabha*

Abstract

Sugarcane is an important commercial crop in India. Water deficit stress is the major limitation for sustainable sugarcane production across the country. Owing to its socio-economic importance, predicted climate changes and increase in the possibility of water shortage, a drought responsive gene *Hordeum vulgare* 22 (*HVA 22*) was cloned and characterized from drought tolerant sugarcane variety Co 740. The open reading frame of this gene is 945 bp that encodes for a single polypeptide of 314 amino acids. *In silico* analysis of *HVA 22* using bioinformatics tools revealed the ~ 35.56 kDA size protein with theoretical pI 9.17, instability index 38.70, aliphatic index 66.53 and GRAVY of -0.742. Subcellular localization by WOLFPSORT server suggested that *HVA 22* expression is localized in the nucleus, mitochondria and chloroplast. TMHMM analysis suggests that *HVA 22* protein contains three transmembrane domains. SignalP showed no signal peptide and the phosphorylation sites *viz*. 30 serine, 8 threonine and 3 tyrosine residues were identified using Netphos server. The phylogenetic tree exhibited that *HVA 22* from sugarcane variety Co 740 is closely associated with that of HVA 22 of *Zea mays* and *Oryza sativa.* qRT-PCR gene expression analysis showed that *HVA 22* was differentially upregulated in drought tolerant and susceptible genotypes under water deficit stress conditions.

Key words: Sugarcane, drought, *HVA* gene

Introduction

Drought is the single most important abiotic stress responsible for reduced agricultural production. Improved productivity under periodic drought stress is a major challenge for global agriculture. It has been estimated that crops attain only about 25 % of their potential yield because of the detrimental effects of environmental stress (Boyer 1982). Therefore, increasing the yield of agricultural crops grown under drought conditions is challenging because of the low heritability of the trait, the unpredictable nature of most periods of drought stress encountered in growing areas, and gaps in our understanding of drought biology (Bruce et al. 2002; Tuberosa et al. 2002). As a consequence, in addition to conventional breeding, biotechnological approaches were also used for improving the performance of crops grown under periodic drought conditions. During periods of decreased water availability, plants often exhibit stress symptoms, including increased leaf rolling and drying of leaf and cellular damage due to photooxidative stress, as well as reduced leaf expansion and normal photosynthetic activity (Nelson et al. 2007). Sugarcane (*Saccharum* spp. hybrids) is one of the vital agricultural crops cultivated in both tropical and sub-tropical countries which produce nearly 80% of the white sugar worldwide. India ranks second in sugarcane area and production, which contributes approximately 15% of global sugar requirement. Sugarcane, an important source of sugar and ethanol, is a relatively high water-demanding crop

T.S. Sarath Padmanabhan, S. Dharshini, V.M. Manoj, J. Ashwin Narayan, C. Appunu and G. Hemaprabha
ICAR-Sugarcane Breeding Institute, Coimbatore-641 007, Tamil Nadu, India
*Corresponding author: ghemaprabha1@gmail.com

and its growth is highly sensitive to water deficit conditions (Lakshmanan and Robinson 2014). Its production and productivity are impacted by drought throughout the life cycle, with the greatest losses being observed when stress occurs in the formative phase.

Hordeum vulgare 22 (*HVA 22*) gene belonging to LEA proteins (group III) was shown to confer increased tolerance to salt and drought stress when introduced to monocots (Xu et al. 1996; Sivamani et al. 2000). It is an ABA-inducible gene in barley (*H. vulgare* L.) which shares little homology with other ABA-responsive genes such as LEA (late embryogenesis abundant) and RAB (responsive to ABA) genes (Shen et al. 2001). *HVA 22* is known to be highly induced in leaves by ABA and drought (Guo et al. 2008). The abscisic acid induced *HVA 22*-like protein regulating vesicular trafficking, enhances the nutrient mobilization and delays the coalescence of vacuolar protein (Chen et al. 2002; Guo and Ho, 2008). Overexpression of *HVA 22* proteins inhibited gibberellin (GA) induced formation of large digestive vacuoles, which is an important aspect of GA-induced programmed cell death in aleurone cells (Guo and Ho 2008). In both barley and Arabidopsis (*Arabidopsis thaliana*), transcripts of *HVA 22* homologs in leaves were highly induced by ABA, drought, cold, and salt stresses (Shen et al. 1993, 2001; Chen et al. 2002). So far, *HVA 22* has not been characterized in sugarcane. In this study, *HVA 22* gene was cloned, characterized and its expression response to drought treatment was determined.

Materials and methods

Identification of *Hordeum vulgare 22 (HVA 22)*

Drought responsive *H. vulgare* 22 (*HVA 22*) gene was identified from previous reports (Shen et al. 2001; Chen et al. 2002). All monocot *HVA 22* sequences were retrieved from NCBI (https://www.ncbi.nlm.nih.gov/nucleotide/) and gene specific primers were designed by analyzing the conserved regions. Primer pair F- ATGGGCTCCGGATCTTTGCTCAAGGTC and R- TCAGCAGTCTGGGTGACCTCGGTTCCA was used for cloning of *HVA 22* gene from drought tolerant sugarcane variety Co 740.

cDNA preparation and isolation of *HVA 22*

Total RNA was extracted from frozen leaf tissues collected from drought tolerant sugarcane variety using Trizol (Invitrogen, CA) and DNA contamination was removed using RNase free DNase I (Thermo Scientific, USA). The quality and quantity of total RNA were analyzed by agarose gel and NanoDrop Spectrophotometer 2000 (Thermo Scientifc, USA). First-strand cDNA was synthesized from total RNA using the Fermentas first-strand cDNA synthesis kit (Fermentas International Inc., Ontario, Canada) following the manufacture's instruction. PCR amplification was carried out as follows: Initial denaturation of 4 min at 94 °C, 35 cycles (94°C for 45s, 62°C for 45s, 72°C for 45s) and final extension of 10 min at 72°C. The PCR product was analyzed on 1 % agarose gel and desired DNA fragment was purified using GeneJET Gel Extraction Kit (Thermo Scientific, USA).

Cloning of *HVA 22* gene

Eluted fragment of 945 bp was ligated into pTZ57R/T vector provided by InsTAclone PCR Cloning Kit (Thermo Scientific, USA) and the ligated product was transformed in *E. coli* DH5α cells. Recombinant colonies were selected in media containing Ampicillin (*AmpR*). Positive colonies were confirmed using M13 and *HVA 22* gene specific primers. Recombinant plasmid was isolated using Plasmid isolation kit (Qiagen, Hilden) and sequenced using Sanger method.

Bioinformatics analysis

Physio-chemical characterization of *HVA 22 viz.*, theoretical molecular weight (MW), isoelectric

point (pI), instability index (II) (Guruprasad *et al*., 1990), aliphatic index (AI) (Ikai AJ, 1980) and grand average hydropathy (GRAVY) (Kyte and Doolottle 1982) were computed using the Expasy's ProtParam server (Gasteiger et al. 2005).

Conserved domains of *HVA 22* gene were predicted using simple modular architecture research tool (SMART) (http://smart.embl-heidelberg.de/) (Schultz et al. 1998; Letunic et al. 2014). Subcellular location of *HVA 22* protein was predicted using WoLFPSORT (http://wolfpsort.org) (Nakai and Kanehisa 1992; Bannai et al. 2002). Netphos server was used in the prediction of phosphorylation sites. SignalP v4.1 was used for detection of signal peptides (Nielsen and Krogh, 1998). TMHMM server v2.0 (http://www.cbs.dtu.dk/services/TMHMM/) was used for transmembrane domains (TMD) prediction (Korgh et al. 2001).

Secondary structure of *HVA 22* was predicted using programmes PSIPRED (McGuffin et al. 2000) (http://bioinf.cs.ucl.ac.uk/psipred/), GOR (https://npsa-prabi.ibcp.fr/cgi bin/npsa_automat.pl?page=/NPSA/npsa_gor4.html), HNN (Hierarchical neural network) (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_hnn.html) and SOPMA (Self-Optimized Prediction Method with Alignment) (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html). RaptorX server was used for protein structure, function and 3D model construction (http://raptorx.uchicago.edu/BindingSite/). RaptorX server uses an in-house free modelling programme (Rumpi *et al*., 2015). The structure was minimized using chiron energy minimization server (http://troll.med.unc.edu/chiron/processManager.php) and the quality of the refined model was evaluated using PROCHECK, ERRAT and VERIFY_3D of SAVES server (http://nihserver.mbi.ucla.edu/SAVES/). Final refined protein structure obtained was submitted in Protein Model database (PMDB).

Phylogenetic analysis

Gene sequences of *HVA 22* from different monocot plants were retrieved from the NCBI database. Phylogenetic tree was constructed in MEGA 7 software using Neighbor joining method (Kumar et al. 2016). Average pathway methods (Nei and Kumar 2000) were used to calculate the branch lengths and *HVA 22* been represented as the units of number of changes over the whole sequence. The resulting percentages of replicate (1000 replicates) trees where the associated taxa cluster together are indicated next to the branches (Felsenstein 1985).

Relative expression analysis

A total of four clones of *Saccharum* complexes: IND 00-1044 (*S. spontaneum*), Co 740 (Drought tolerant commercial variety), Co 775 (Drought susceptible commercial variety) and NG 77-232 (*S. officinarum*) were used for *HVA 22* gene expression studies. All these plants were vegetatively propagated in pots for 90 days and drought stress was imposed by withholding irrigation for 10 days. Leaf samples were collected and snap frozen in liquid nitrogen and stored in -80°C until further use. Total RNA was isolated using Trizol (Invitrogen) and DNAse (Fermentas International Inc., Ontario, Canada) treatments was carried out to remove the DNA contamination. First-strand cDNA was synthesized as detailed above. Specific qRT-PCR primers were designed using IDT-primer quest tool. The primer pair, F- CGAGTGCTTCAAGACGGTGG and R-ATGGGGCGAAGAAAGGTGTC was used to perform qRT-PCR using Quantinova q-PCR kit (Qiagen) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as an internal control (Dharshini et al., 2016). The CT values for both the target and internal control genes

Table 1. Physio-chemical properties

	Number of amino acids	Molecular weight (kilo dalton)	Theoretical pI	Instability index	Aliphatic index	Grand average of hydropathicity (GRAVY)
HVA 22	314	35.56	9.17	38.70	66.53	-0.742

were used for the quantification of transcripts by comparative CT method normalization. Later, the products were analyzed through a melt-curve analysis to check the specificity of PCR amplification. Three biological and three technical replicates were used to minimize the error (Livak and Schmittgen 2001).

Results and discussion

Cloning of *Hordeum vulgare HVA 22* gene

HVA 22 gene was amplified with gene specific primers designed using conserved sequences of monocots such as *Sorghum bicolor* and *Zea mays*. *HVA 22* gene of 945 bp was sequenced which showed 89.7 % and 94.2% similarity with *HVA 22* of *Z. mays* and *S. bicolor*, respectively. Also, it showed 88% and 82% identity at the nucleotide level with *HVA 22* of *Setalia italica* and *Oryza sativa,* respectively. A complete coding region of *HVA 22* gene sequence was submitted to NCBI GenBank database under the accession number KY595440.1.

Bioinformatics and phylogenetic analysis

Physico-chemical characterization such as theoretical isoelectric point (pI), instability index (II), molecular weight (MW), aliphatic index (AI) and grand average hydropathy (GRAVY) of various proteins in different crops was studied using the Expasy's ProtParam server (Sahay et al. 2010; Filiz et al. 2013; Han et al. 2015; Goyal et al. 2017) and results are given in Table 1. The molecular weight of *HVA 22* protein was found to be ~ 35.56 kDa, it is alkaline in nature (pI 9.17) and exhibited moderate stability with instability index of 38.70. A protein whose instability index is lesser than 40 is predicted as stable (Guruprasad et al. 1990). This protein scored an aliphatic index of 66.53, a moderate level of aliphatic side chains and thus expected moderate stability over the temperature fluctuations. GRAVY value of *HVA 22* is -0.742 which showed a good interaction of protein with water (Kyte and Doolittle 1982).

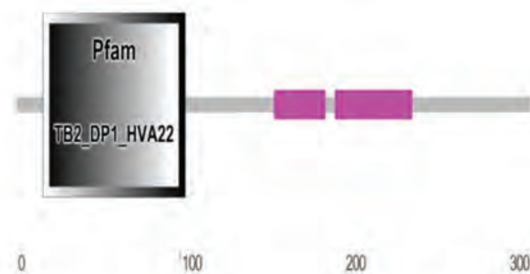

Fig. 1. Domain prediction stage

Domain prediction using SMART server showed that *HVA 22* has one TP2_DP1_*HVA 22* domain in 16-103 amino acid region with E-value 3.8e-28 and two low complexity regions (158-187 and 195-240 amino acid) (Table 2; Fig. 1). *HVA 22* protein sequence was predicted in WOLFPSORT

Table 2: Domain analysis

Server	Domain name	Position	E-Value
SMART	Pfam TP2_DP1_*HVA 22*	16-103	3.8e-28
	Low complexity region	158-187	N/A
	Low complexity region	195-240	N/A

id	site	distance	identity	comments
At1g16060.1	nucl	193.221	13.8728%	[Arath]
At1g64380.1	nucl	195.298	10.7463%	[Arath]
At1g22190.1	nucl	196.62	17.5159%	[Arath]
E13K_TOBAC	extr	230.048	10.8761%	[Uniprot] SWISS-PROT45:Extracellular.
At1g72360.1	nucl	231.514	14.0127%	[Arath]
At5g47230.1	nucl	243.242	15.3605%	[Arath]
At2g41710.1	nucl	254.637	14.1844%	[Arath]
At1g25540.1	nucl	255.213	8.37321%	[Arath]
WR29_ARATH	nucl	258.204	12.381%	[Uniprot] SWISS-PROT45:Nuclear.
At1g22300.1	plas	258.265	12.4204%	[Arath]
ADT1_SOLTU	mito	270.446	13.9896%	[Uniprot] SWISS-PROT45:Integral membrane protein. Mitochondrial inner membrane.
CB28_PEA	chlo	273.063	11.465%	[Uniprot] SWISS-PROT45:Chloroplast thylakoid membrane.
At1g78080.1	nucl	273.31	17.0659%	[Arath]
At2g28550.1	nucl	276.255	11.1359%	[Arath]

Fig. 2. Subcellular localization of *HVA 22*

(Bannai et al. 2002; Horton et al. 2007) to identify the subcellular localization. The analysis revealed its localization expression in the nucleus, mitochondria (Grelet et al. 2005) and chloroplast region (N'Dong 2002). Different subcellular localization of a protein suggests that they could play different roles in cell physiological processes under abiotic stress conditions (Cao et al. 2017). The screenshot of result is given in Fig. 2. SignalP server (Nielsen and Krogh 1998) inferred that there is no signal peptide in the protein. NetPhos phosphorylation site prediction tool revealed the presence of phosphorylation site (*viz)* 30 serine, 8 threonine and 3 tyrosine residues (Fig. 3).

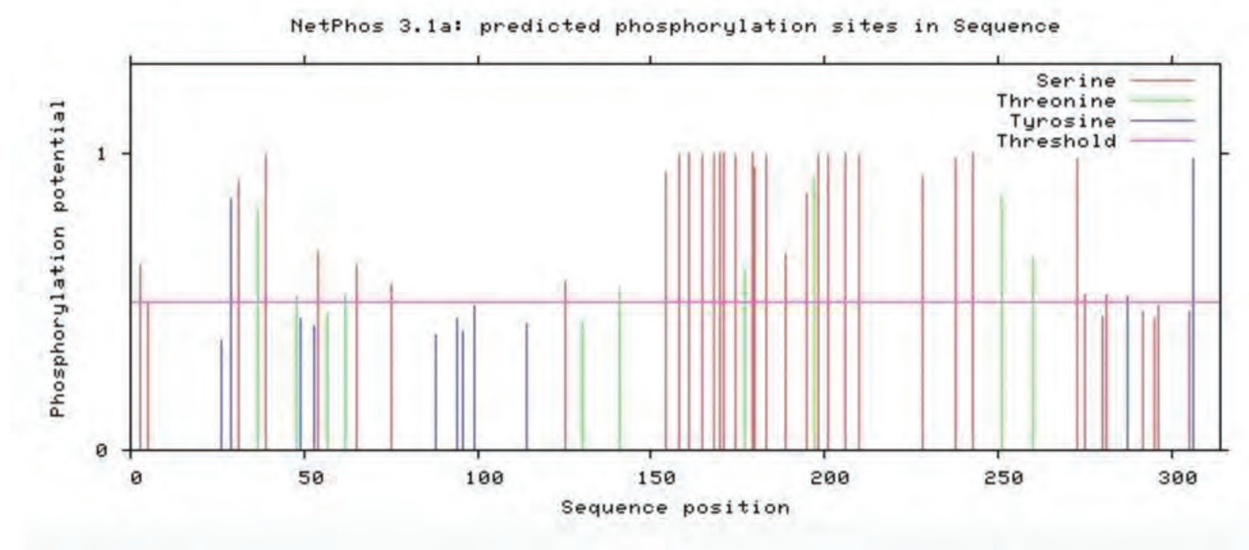

Fig. 3. Phosphorylation site prediction of *HVA 22*

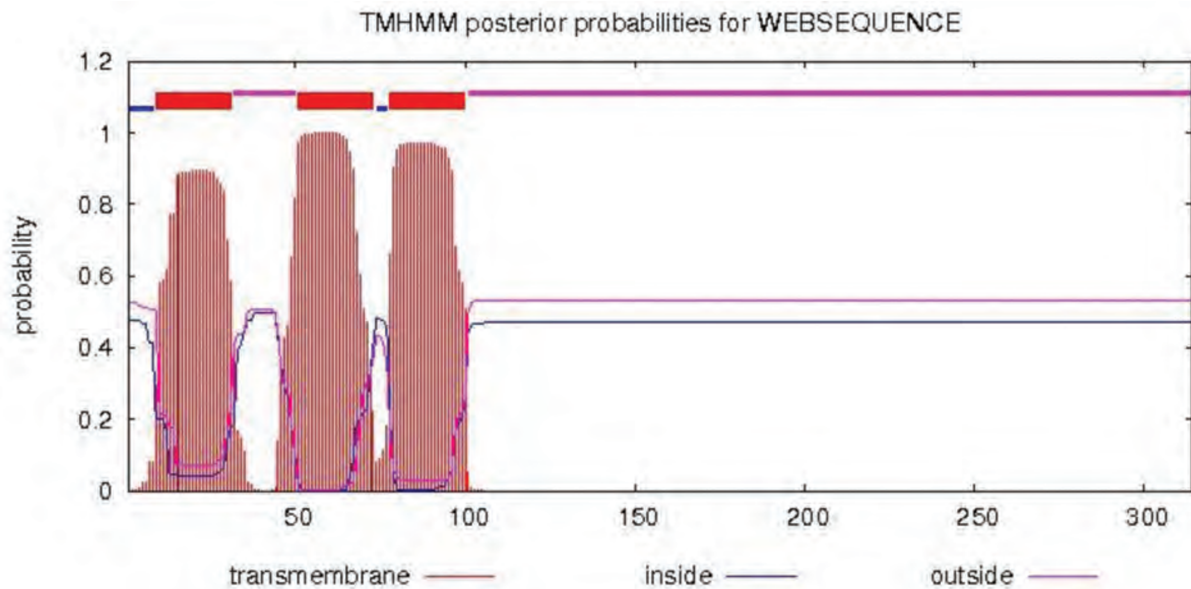

Fig. 4. Transmembrane prediction of *HVA 22*

Table 3. Analysis of secondary structures

Server name	α-Helix		Extended-strand		Random coil	
	No. of residues	Percentage (%)	No. of residues	Percentage (%)	No. of residues	Percentage (%)
GOR	104	33.12	51	16.24	159	50.64
HNN	118	37.58	25	7.96	171	54.46
SOPMA	106	33.76	43	13.69	129	42.08
PSIPRED	111	35.3	15	4.77	194	59.87

TMHMM server (Korgh et al. 2001) confirmed the presence of three transmembrane sites in *HVA 22* protein (Fig. 4).

Secondary structure of *HVA 22* predicted using PSIPRED, HNN, GOR and SOPMA servers showed dominance of random coil (42.08-59.87%), followed by α-helix (33.12-37.58%) and extended strand (4.77-16.24%). Random coils *HVA* `important functions in proteins for flexibility and conformational changes (Buxbaum 2007). Detailed secondary structure prediction is given in Fig. 5. All these servers use different algorithms and approaches to predict the secondary structure pattern from primary amino acid sequences, among all the presence of random coils in secondary structure was found to be dominant (Yaqoob et al. 2016; Chandra et al. 2015). Analysis of secondary structure details are given in Table 3.

The 3D structure of *HVA 22* protein was modelled using RaptorX server. The obtained 3D modelled

Table 4. Stereo-chemical analysis

	Ramachandran Plot			ERRAT	ProSA
	Favoured region (%)	Allowed region (%)	Outlier region (%)		
HVA 22	83.6	12.3	1.4	82.76	-2.9

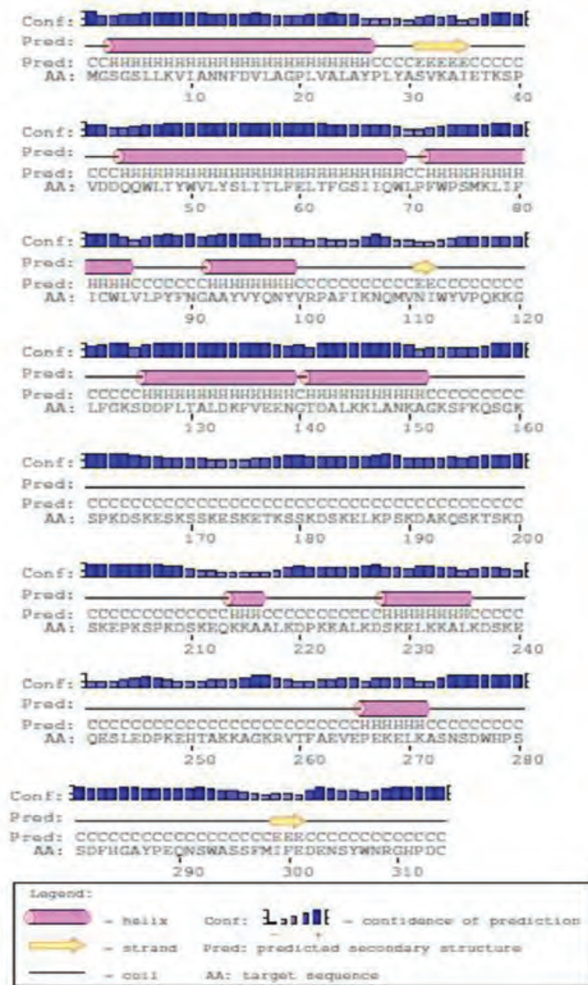

Fig. 5. Secondary structure prediction of *HVA 22*

structure from RaptorX was then optimized in Chiron energy minimization server. The optimized 3D modelled structure was validated in SAVES server and ProSA (Nagaraju et al. 2018; Chandra et al. 2015). The overall quality showed for *HVA 22* protein was 81.752%, Ramachandran plot showed 83.6% of the amino acid residue are in favored region (Fig. 6) and ProSA server showed Z score of -2.9 (Table 4). The final 3D structure is shown in Fig 7. The final refined model was deposited to the PMDB (http://bioinformatics.cineca.it/PMDB/) and can be accessed using PMDB ID: **PM0081235**.

Protein sequences of *HVA 22* from 15 different monocot plants were retrieved from NCBI database, and multiple sequence alignment was performed using ClustalX with default parameters (Thompson et al. 1997) and it revealed the presence of conserved domains (Fig. 8). Based on sequence similarity three major clades were formed in the phylogenetic tree. *HVA 22* gene from Sugarcane commercial variety Co 740 showed its close association with HVA 22 of *Zea mays* and *Oryza sativa* (Fig. 9). Grouping of various *HVA 22* gene into clusters indicate the presence of conserved domains in *HVA 22* protein gene family during evolution.

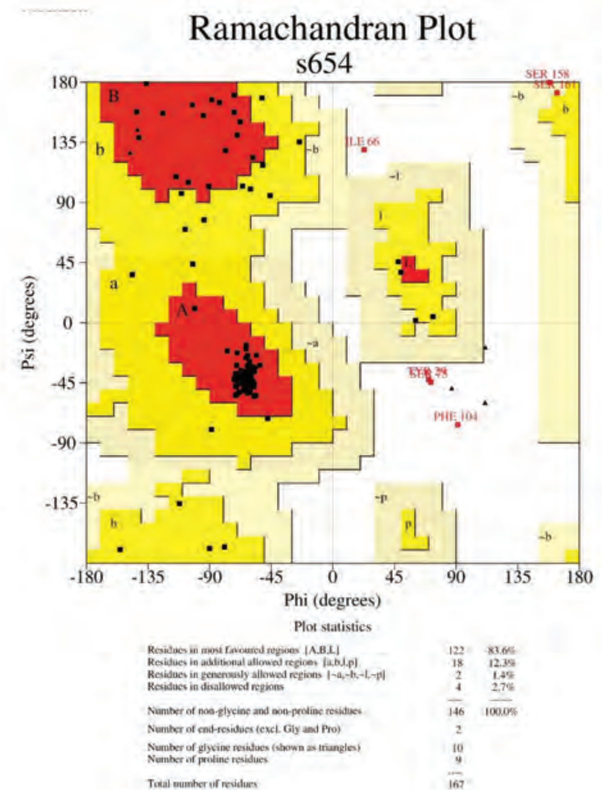

Fig. 6. Ramachandran plot

Relative expression analysis

Relative expression of *HVA 22* gene during drought stress was studied using qRT-PCR. This gene was highly upregulated in drought tolerant clones *viz., S. spontaneum* IND 00-1044 (6.06

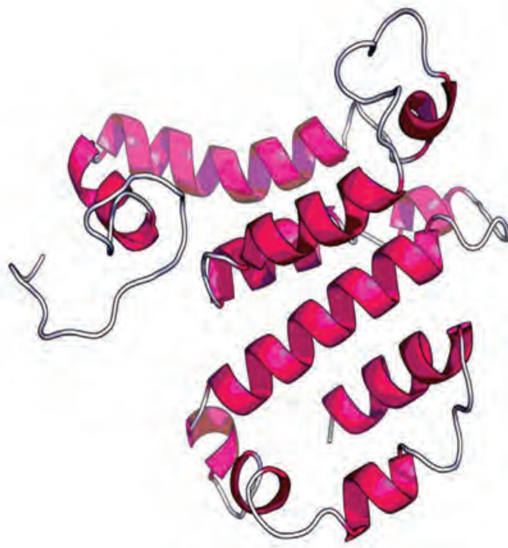

Fig. 7. Tertiary structure if *HVA 22*

fold) and commercial variety Co 740 (3.01 fold) compared to drought susceptible *S. officinarum* NG 77-232 (0.46 fold) and commercial variety Co 775 (0.96 fold) and to its respective control plants. *HVA 22* gene expression level under drought condition is shown in Fig. 10. *S. spontaneum* IND 00-1044 (which is highly drought tolerant) recorded higher expression followed by drought tolerant commercial hybrid Co 740 upon drought stress. *S. officinarum* NG 77-232 and commercial variety Co 775 are known drought sensitive genotypes exhibited low level of expression in comparison with tolerant genotypes. Many studies suggesting that different group of LEA proteins are upregulated during abiotic stresses like drought, cold and salinity stress (Nagaraju et al. 2018; Yang et al. 2012; Cao et al. 2018). Overexpressing

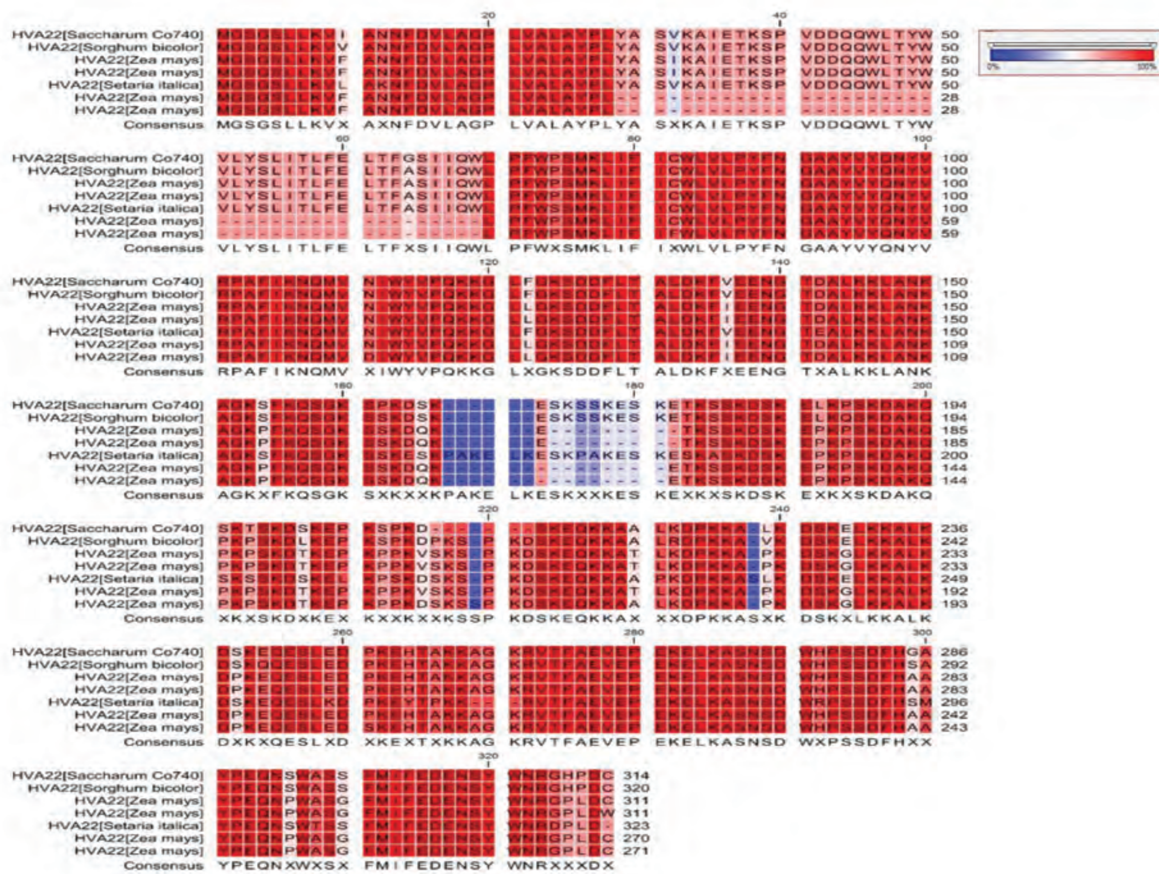

Fig. 8. Multiple sequence alignment of *HVA 22*

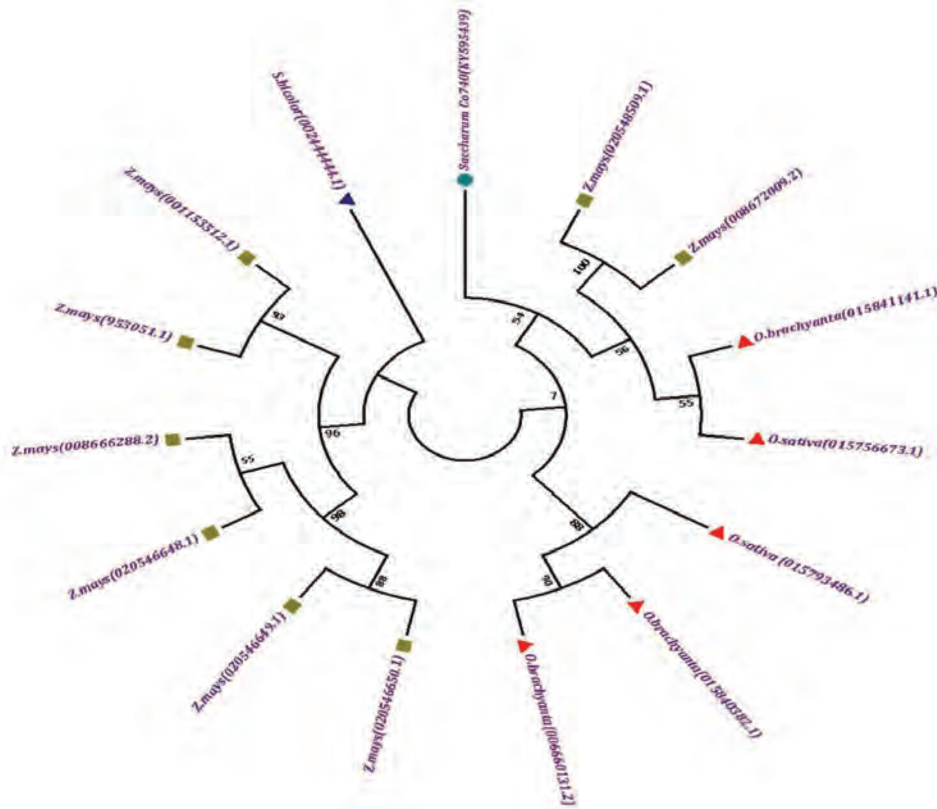

Fig. 9. Phylogenetic tree of *HVA 22*

HVA 22, a group 3 LEA protein could serve as a good candidate gene to improve drought tolerance (Leprince and Butink 2010; Amara 2012; Sharon and Suvarna 2017).

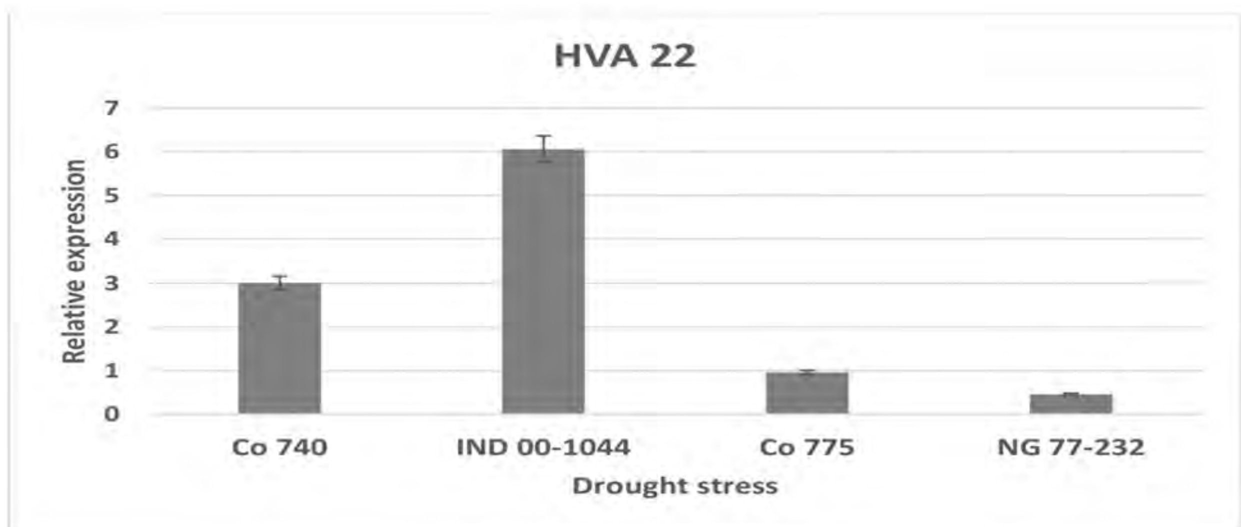

Fig. 10. Relative expression of *HVA 22* during drought stress

Conclusion

In this study, a drought responsive gene *HVA 22* was isolated from *a* drought tolerant commercial sugarcane variety Co 740. Using bioinformatics approach the primary, secondary, tertiary structure, transmembrane and domain of *HVA 22* were predicted. The physiochemical characterization showed that the protein is stable and contains three transmembrane sites. The subcellular localization prediction showed its function in nucleus, mitochondria and cytoplasm. Phosphorylation site prediction showed *HVA 22* to contain 30 serine, 8 threonine and 3 tyrosine residues. Expression analysis revealed that this gene is highly upregulated during drought conditions in drought tolerant genotypes and thus could be a good candidate gene for improving drought tolerance in sugarcane.

Acknowledgements

Authors thank to Indian Council of Agricultural Research (ICAR), New Delhi for financial support. We also wish to thank the Director, ICAR-Sugarcane Breeding Institute, Coimbatore for providing sugarcane material.

References

Amara I (2012) Abiotic stress in plants: Late Embryogenesis Abundant proteins. Barcelona. Spain

Bannai H, Tamada Y, Maruyama O, Nakai K, Miyano S (2002) Extensive feature detection of N-terminal protein sorting signals. Bioinformatics 18(2): 298-305

Buxbaum E (2007) Fundamentals of Protein Structure and Function. Springer Science and Business Media, LLC, New York.

Cao Y, Xiang X, Geng M, You Q, Huang X (2017) Effect of *HbDHN1* and *HbDHN2* genes on abiotic stress responses in *Arabidopsis*. Frontiers in Plant Science 8: 470

Chandra S, Dutta AK, Chandrashekara KN, Acharya K (2017) In silico characterization, homology modeling of *Camellia sinensis* chitinase and its evolutionary analyses with other plant chitinases. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 87(3): 685-695

Chen CN, Chu CC, Zentella R, Pan SM, Ho TH (2002) *AtHVA 22* gene family in Arabidopsis: phylogenetic relationship, ABA and stress regulation, and tissue-specific expression. Plant Molecular Biology 49(6): 631-642

Dharshini S, Chakravarthi M, Manoj VM, Naveenarani M, Kumar R, Meena M, Ram B, Appunu C (2016) *De novo* sequencing and transcriptome analysis of a low temperature tolerant *Saccharum spontaneum clone* IND 00-1037. Journal of Biotechnology 231:280-294

Doerks T, Copley RR, Schultz J, Ponting CP, Bork P (2002) Systematic identification of novel protein domain families associated with nuclear functions. Genome Research 12(1): 47-56

Felsenstein J (1985). Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39(4): 783-791

Filiz E, Ozyigit II, Tombuloglu H, Koc I (2013) In silico comparative analysis of LEA (Late Embryogenesis Abundant) proteins in *Brachypodium distachyon* L. Plant Omics 6(6): 433

Gasteiger E, Hoogland C, Gattiker A, Wilkins MR, Appel RD, Bairoch A (2005) Protein identification and analysis tools on the

ExPASy server. In The proteomics protocols handbook. Humana press 571-607

Goyal M, Chauhan S, Kumar P (2017) In silico analysis, structural modeling and phylogenetic analysis of EPSP synthase of Phaseolus vulgaris. Agricultural Science Digest 37(3): 185-190

Grelet J, Benamar A, Teyssier E, Avelange-Macherel MH, Grunwald D, Macherel D (2005). Identification in pea seed mitochondria of a late-embryogenesis abundant protein able to protect enzymes from drying. Plant Physiology 137(1): 157-167.

Guo WJ, Ho TH (2008). An abscisic acid-induced protein, *HVA 22*, inhibits gibberellin-mediated programmed cell death in cereal aleurone cells. Plant Physiology 147(4): 1710-1722

Guruprasad K, Reddy BB, Pandit MW (1990) Correlation between stability of a protein and its dipeptide composition: a novel approach for predicting in vivo stability of a protein from its primary sequence. Protein Engineering, Design and Selection 4(2):155-161.

Han, Y, Zheng QS, Wei YP, Chen J, Liu R, Wan HJ (2015) In silico identification and analysis of phytoene synthase genes in plants. Genetics and Molecular Research 14(3): 9412-9422.

Horton P, Keun-Joon P, Takeshi O, Naoya F, Hajime Harada CJ, Adams C, Kenta N (2007) WoLF PSORT: protein localization predictor. Nucleic Acids Research 35: W585–W587 Web Server issue.

Ikai A (1980) Thermostability and aliphatic index of globular proteins. The Journal of Biochemistry 8(6): 1895-1898

Krogh A, Larsson B, Von Heijne G, Sonnhammer EL (2001) Predicting transmembrane protein topology with a hidden markov model: application to complete genomes1. Journal of Molecular Biology 305(3): 567-580

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7): 1870-1874

Kyte J, Doolittle RF (1982). A simple method for displaying the hydropathic character of a protein. Journal of Molecular Biology, 157(1): 105-132.

Lakshmanan P, Robinson N (2014). Stress physiology: Abiotic stresses. Sugarcane: Physiology, Biochemistry and Functional Biology 411-434.

Leprince O, Buitink J (2010) Desiccation tolerance: from genomics to the field. Plant Science 179(6): 554-564

Letunic I, Doerks T, Bork P (2014) SMART: recent updates, new developments and status in 2015. Nucleic Acids Research 43(1): 257-260

Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25(4): 402-408.

Lobell DB, Schlenker W, Costa-Roberts J (2011) Climate trends and global crop production since 1980. Science 333(6042): 616-620

Mahajan S, Tuteja N (2005) Cold, salinity and drought stresses: an overview. Archives of Biochemistry and Biophysics 444(2): 139-158

McGuffin LJ, Bryson K, Jones DT (2000) The PSIPRED protein structure prediction server. Bioinformatics 16(4): 404-405

Nagaraju M, Reddy PS, Kumar SA, Kumar A, Suravajhala P, Ali A, Srivastava RK, Kishor PK, Rao DM (2018) Genome-wide in silico analysis of dehydrins *in Sorghum bicolor*, *Setaria italica* and *Zea may*s and quantitative analysis of dehydrin gene expressions under abiotic stresses in *Sorghum bicolor*. Plant Gene 13: 64-75

Nakai K, Kanehisa M (1992) A knowledge base for predicting protein localization sites in eukaryotic cells. Genomics 14(4): 897-911.

N'Dong C, Danyluk J, Wilson KE, Pocock T, Huner NP, Sarhan F (2002) Cold-regulated cereal chloroplast late embryogenesis abundant-like proteins. Molecular characterization and functional analyses. Plant Physiology 129(3): 1368-1381.

Nei M, Kumar S (2000) Molecular evolution and phylogenetics. Oxford university press.

Nielsen H, Krogh A (1998) Prediction of signal peptides and signal anchors by a hidden Markov model (1998) Proceedings of International Conference Intelligent Systems for Molecular Biology 6: 122-130

Rampino P, Pataleo S, Gerardi C, Mita G, Perrotta C (2006) Drought stress response in wheat: physiological and molecular analysis of resistant and sensitive genotypes. Plant Cell and Environment 29(12): 2143-2152

Rumpi G, Upadhyay AD, Roy AK, Samik A (2017) Structural analysis of cytochrome C Genes of Major Carp and utility of Forensic Investigation. Journal of Forensic and Crime Investigation 1(1): 1-8

Saha D, Rana RS, Arya L, Mondal TK (2017) Genomic organization and structural diversity of germin-like protein coding genes in foxtail millet (*Setaria italica* L.). Agri Gene 3:87-98

Sahay A, Shakya M (2010) In silico Analysis and Homology Modelling of Antioxidant Proteins of Spinach. Journal of Proteomics and Bioinformatics 3: 148-154

Schultz J, Milpetz F, Bork P, Ponting CP (1998) SMART, a simple modular architecture research tool: identification of signaling domains. Proceedings of the National Academy of Sciences 95(11): 5857-5864

Sharon K, Suvarna S (2017) Cloning of *HVA 22* Homolog from *Aloe vera* and preliminary study of transgenic plant development. International Journal of Pure and Applied Biosciences 5(6): 1113-1121

Shen Q, Chen CN, Brands A, Pan SM, Tuan-Hua DH (2001) The stress-and abscisic acid-induced barley gene *HVA 22*: developmental regulation and homologues in diverse organisms. Plant Molecular Biology 45(3): 327-340

Shen Q, Uknes SJ, Ho TH (1993) Hormone response complex in a novel abscisic acid and cycloheximide-inducible barley gene. Journal of Biological Chemistry 268(31): 23652-60.

Sivamani E, Bahieldin A, Wraith JM, Al-Niemi T, Dyer WE, Ho TH, Qu R (2000) Improved biomass productivity and water use efficiency under water deficit conditions in transgenic wheat constitutively expressing the barley *HVA*1 gene. Plant Science 155(1): 1-9

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25(24): 4876-4882

Wang W, Vinocur B, Altman A (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218(1): 1-4

Xu D, Duan X, Wang B, Hong B, Ho TH, Wu R (1996) Expression of a late embryogenesis abundant protein gene, *HVA1*, from barley confers tolerance to water deficit and salt stress in transgenic rice. Plant Physiology 110(1): 249-257

Yadav DK, Shukla D, Tuteja N (2014) Isolation, in silico characterization, localization and expression analysis of abiotic stress-responsive rice G-protein β subunit (RGB1). Plant Signaling and Behvaior 9(5): e28890

Yang Y, He M, Zhu Z, Li S, Xu Y, Zhang C, Singer SD, Wang Y (2012) Identification of the dehydrin gene family from grapevine species and analysis of their responsiveness to various forms of abiotic and biotic stress. BMC Plant Biology 12(1): 140.

Yaqoob U, Kaul T, Nawchoo IA (2016) In-silico analysis, structural modelling and phylogenetic analysis of *Acetohydroxy acid* synthase gene of *Oryza sativa*. Medicinal and Aromatic Plants (Los Angel) 5(272): 2167-0412

Received: January, 2019; Revised & Accepted: March, 2019