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IN-SILICO AND PHYLOGENETIC ANALYSIS OF DREB TRANSCRIPTION FACTOR IN *SOLANUM MELONGENA* L.

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Abstract

Solanum melongena L. belongs to family Solanaceae is an agronomically, economically and medicinally important crop plant. The crop is sensitive to severe environmental conditions thus open the door to explore its genome for possible genetic improvement. Abiotic stresses are well known to adversely affect crop productivity and yield. Dehydration responsive element binding (DREB) proteins are reported to play a significant role in abiotic stress tolerance. *Solanum melongena* genome was searched for the presence of DREB1 gene and structural-functional analyses were carried out by using different *in silico*, bioinformatics and phylogenetics tools. It was observed that SmDREB1 showed 92% and 81% sequence similarity at nucleotide and protein level respectively with *Solanum tuberosum* DREB1. Most of the short linear motifs harbored by SmDREB1 were also seen conserved in StDREB1 however, six motifs were found only in SmDREB1 which make this protein a good candidate to be used in crop engineering approaches. The translated protein has three intrinsically disordered regions, which include 89.5% of total amino acids. This feature categorizes SmDREB1 under unstructured protein that is an attribute of transcription factors. The 3D structure prediction presented nine residues mostly tyrosine and arginine involved in binding with nucleic acid. Bayesian based phylogenetic analysis inferred that DREB1 from *S. melongena* is a close homologue of DREB1 from other *Solanum* species although it may perform some additional biological functions. The results of this study highlighted the importance of SmDREB1 that could be a good candidate gene for the incorporation of abiotic stress tolerance in crop plants.

Key words: *Solanum melongena*, DREB1, Abiotic stress, Protein secondary structure, Structure/function analysis, Phylogenetics.

Introduction

Solanum melongena L., also known as Brinjal, Eggplant or Aubergine belongs to the plant family Solanaceae. It is widely cultivated as a vegetable crop in both tropical and temperate zones (Biswas *et al*., 2009; Knapp *et al*., 2019). Economically, eggplant is an important crop in Asia, Central America and Africa. Its fruits composed of beneficial minerals for human health and also low in calories. Furthermore, nutritionally important elements such as iron, potassium, calcium and magnesium are also abundantly found in eggplant fruit (Zenia & Halina, 2008).

Solanum melongena, being sessile like other plants, is exposed to various environmental stresses. These external factors ultimately decreased the crop productivity (Nawaz *et al*., 2014). Salinity and drought, two major abiotic factors, caused more than 50% yield losses in major crops the world over (Bray, 2004). These stresses are developing serious threats for major agricultural production. Eggplant often has insufficient tolerance to biotic and abiotic stresses that cause serious crop losses (Collonnier *et al*., 2001). Traditional approaches have been taken to improve its germplasm by hybridizing with wild resistant *Solanum* species, which lead to broaden genetic diversity and incorporate useful agronomic characters. However, these practices are limited by difficulties in obtaining fertile seeds and sexual incompatibilities (Collonnier *et al*., 2001). The application of *in vitro* techniques such as somatic hybridization, *in vitro* selection, plant tissue culture and genetic transformation, on the other hand resulted in considerable success in eggplant (Magioli & Mansur, 2005).

Plants respond to abiotic stresses in a highly complex manner by integrating all physiological, biochemical and molecular changes. At molecular level, the gene products that help plant to survive against stress, are usually divided into two categories: first group protect cells from the adverse effects of water stress, and second modulates signal transduction by regulating abiotic stress tolerant gene expression (Seki *et al*., 2002). The second group includes many transcription factors (TFs) and genes that interact with TFs such as dehydration responsive element binding (DREB) protein and radical-induces cell death1 gene (Yamaguchi-Shinozaki and Shinozaki, 2009; Anjum *et al*., 2015; Sadia *et al*., 2020). DREB protein binds with the cis-elements of stress-related gene promoters and up-regulate many downstream genes thus helping plant acquiring stress tolerance (Agarwal & Jha, 2010; Qamarunnisa *et al*., 2012). DREB genes are among the most studied groups of TFs related to biotic and abiotic stress tolerance in plants. They are involved in expression of many stress-inducible genes, which result in abiotic stress tolerance (Hussain *et al*., 2011; Qamarunnisa *et al*., 2015).

The studies on structural and functional relation of transcription factors specifically DREB are important to understand the molecular mechanism for recognition and expression of target genes at the genome level (Garg *et al*., 2008). *In silico* techniques can significantly reduced problems like time limitations, high costs and more labour. In the present study, *in silico* analysis, protein homology modeling and phylogenetics of the DREB1A gene was carried out. It is anticipated that the outcomes of current study will offer more insights into the structural and

functional roles of the DREB1A protein involved in the abiotic stress tolerance mechanism in *Solanum melongena*.

Materials and Methods

Homology searching: The sequence of DREB gene was retrieved and assembled from Eggplant Genome Database (Hirakawa *et al*., 2014) by using *Solanum tuberosum* DREB1 gene (Genbank accession no. HM641796) as template. The retrieved sequence was used to search homology sequences with the help of BLASTn and BLASTx online software.

Identification of DREB factor: The mRNA sequence of *Solanum melongena* dreb was used to predict conserved domains and virtually translated into amino acid sequence with ExPASy translate tool; afterwards SmDREB1 sequence was used for structural and functional annotations with the help of different bioinformatics tools.

***In-silico* analysis of SmDREB1:** ExPASy proteomics server of the Swiss Institute of Bioinformatics (SIB) was used to predict physicochemical and secondary structure characterization of *Solanum melongena* DREB protein. Conserved domain, secondary structure, protein localization and motifs were predicted by Conserved Domain Database (Marchler-Bauer *et al*., 2011), Predict Protein (Yachdav *et al*., 2014) WoLF PSORT (Horton *et al*., 2007) and Eukaryotic Linear Motif Server (Dinkel *et al*., 2013) respectively. Protein folding pattern was determined by FoldIndex© (Prilusky *et al*., 2005) and protein recruitment sites were predicted using ANCHOR© (Dosztányi *et al*., 2009). The level of conservation for SmDREB1 residues was calculated by Predict Protein (Yachdav *et al*., 2014)). SmDREB1 sequence was submitted to the Swiss Model, I-Tasser server (Roy *et al*., 2010) and Geno3D (Combet *et al*., 2002) for *in-silico* modeling. The closest template for modeling SmDREB1 structure in all software was chosen having PDB ID 1GCC.1. All software and analyses were simultaneously applied to *Solanum tuberosum* sequence HM641796 in NCBI database to obtain a comparative data set wherever required (Data not shown).

Phylogenetic analysis: A systematic phylogenetic analysis was carried out based on the similarities of AP2 domains in the proteins isolated from other plants. The list of included species with their accession numbers is given in Table 1. Bayesian Inference (BI), a character based method was applied to reconstruct the phylogeny of protein data by using BEAST (v1.8.0) software. The alignment file was converted into nexus file and BEAUti GUI application was used to create a BEAST XML file for analyzing data. Phylogenetic analysis was performed by employing the Blosum62 model. Length of chain was set as 800,000 MCMC generations. The burn-in value was set as 40 to discard the initial portion of a Markove chain sample to minimize the effect of initial value on posterior inference. The resulting tree was analyzed on Fig tree software version 1.4 (Rambaut, 2012).

Results

The homologues of DREB1 in *Solanum melongena* were searched in several databases however, only Eggplant Genome Database contains contig of DREB1 transcript. When the assembled sequence was blast against already available genomes, it was found that SmDREB1 is closest to *Solanum tuberosum* dehydration responsive element binding protein 1 (DREB1) with 92% sequence similarity at nucleotide level and 81% at protein level. The length of SmDREB1 protein is 317 amino acids having 35097.8 Dalton molecular weight. The theoretical isoelectric point (pI) was found to be acidic (4.67) with more numbers of negatively charged residues (56) than positively charged (41). The conserved domain search results in an APETALA2 (AP2) superfamily domain (Fig. 1). The secondary structure of SmDREB1 was found to be comprised of 10.41% helix, 5.68% extended strand in beta-sheet and 83.91% loops. The WoLF PSORT subcellular prediction server predicts more signals (10) to locate this protein in nucleus. The linear sequence of protein contains an eight residues long nuclear localization signal (NLS) "RKSRSRRD" at 21 position. Several short linear motifs describing specific sites and functions have been detected in SmDREB1 by ELM server. The presence of SmDREB1 motifs have also been checked in *Solanum tuberosum* DREB1 protein and the list is presented in Table 2. It was found that six motifs of SmDREB1 were absent in StDREB1.

Table 1. List of species with their protein sequence accession numbers used in phylogenetic analysis.

S. #	Species name	Family	Accession No.
1.	*Solanum pimpinellifolium*	Solanaceae	AKC42092
2.	*Solanum lycopersicum*	Solanaceae	NP001234689
3.	*Solanum tuberosum*	Solanaceae	AEI98833
4.	*Ammopiptanthus mongolicus*	Fabaceae	AHI45171
5.	*Jatropha curcas*	Euphorbiaceae	XP012086678
6.	*Erythranthe guttatus*	Phrymaceae	XP012858842
7.	*Catharanthus roseus*	Apocynaceae	CAB93939
8.	*Camellia sinensis*	Theaceae	AGG39692
9.	*Coffea canephora*	Rubiaceae	CDP13966
10.	*Nicotiana tomentosiformis*	Solanaceae	XP009612863
11.	*Nicotiana sylvestris*	Solanaceae	XP009764492
12.	*Ricinus communis*	Euphorbiaceae	XP002520794

Table 2. Several Short Linear Motifs (SLiMs) detected in SmDREB1 by ELM.

ELM name	Instances (matched sequence)	Status in StDREB1	Functional site class
DEG_SCF_FBW7_1	LDSTPVST	P	SCF ubiquitin ligase binding Phosphodegrons
DOC_MAPK_1	KPGRQEDLNF	P	MAPK docking motif
DOC_PIKK_1	DEMFDVDDL	P	HEAT Domain Ligand
DOC_PP2B_LxvP_1	LNLP	A	Calcineurin (PP2B)-docking motif LxvP
DOC_USP7_1	AKGSK, PENSQ, ATTSA, PLSSV	P	USP7 binding motif
DOC_WW_Pin1_4	AAGTPL, LDSTPV, LSNSPC	P	WW domain ligands
LIG_14-3-3_3	KSRSRR, KLDSVD	P	14-3-3 ligand
LIG_FAT_LD_1	VDDLLAMLD	P	Paxillin LD motifs
LIG_FHA_1	DGTKNVE, DETAGLP	P	FHA phosphopeptide ligands
LIG_FHA_2	EPTSIDQ	P	FHA phosphopeptide ligands
LIG_LIR_Gen_1	ECNYRGV, SGWDCL, DYGFDFL, DLAFIDL	P	Atg8 protein family ligands
LIG_PDZ_Class_1	DLDSEL	A	PDZ ligands
MOD_CK1_1	SWATTSA, SDCTVAS	P	CK1 Phosphorylation site
MOD_CK2_1	TVASGGE	P	CK2 Phosphorylation site
MOD_GSK3_1	KYPSRDDS, RDDSDSDS, DSDSDSAS, DSASWATT, SWATTSAS, GATTAAGT, AAGTPLSS, MLDSTPVS, LDSTPVST	P	GSK3 phosphorylation site
MOD_N-GLC_1	LNFSLD	A	N-glycosylation site
MOD_NEK2_1	MLDSTP	P	NEK2 phosphorylation site
MOD_NEK2_2	PLSSVK	P	NEK2 phosphorylation site
MOD_PIKK_1	PENSQCN	A	PIKK phosphorylation site
MOD_PKA_1	KRKSRSR	P	PKA Phosphorylation site
MOD_PKA_2	KRKSRSR	P	PKA Phosphorylation site
MOD_PLK	VEETLAK, SDCTVAS, KDETAGL	P	Plk phosphorylation site
MOD_ProDKin_1	AAGTPLS, LDSTPVS, LSNSPCQ	P	MAPK Phosphorylation Site
MOD_SUMO_for_1	VKNE	P	Sumoylation site
MOD_SUMO_rev_2	DSVDDEGKRM, SVDDEGKRM, DDEGKRM, DEGKRM	P	Sumoylation site
TRG_ENDOCYTIC_2	YRGV, YRGV	A	Y-based sorting signal
TRG_ER_diArg_1	YRR, RRKR, RSRR, VRQR, VRQR	P	di Arginine retention/retrieving signal
TRG_NES_CRM1_1	DEMFDVDDLLAMLD, EMFDVDDLLAMLD	P	NES Nuclear Export Signal
TRG_NLS_MonoCore_2	RRKRKS	A	NLS classical Nuclear Localization Signals
TRG_NLS_MonoExtC_3	RKRKSR, GKRMRK	P	NLS classical Nuclear Localization Signals
TRG_NLS_MonoExtN_4	RRKRKSRS, RKRKSRS, KRKSRS	P	NLS classical Nuclear Localization Signals

"P" = Present; "A" = Absent

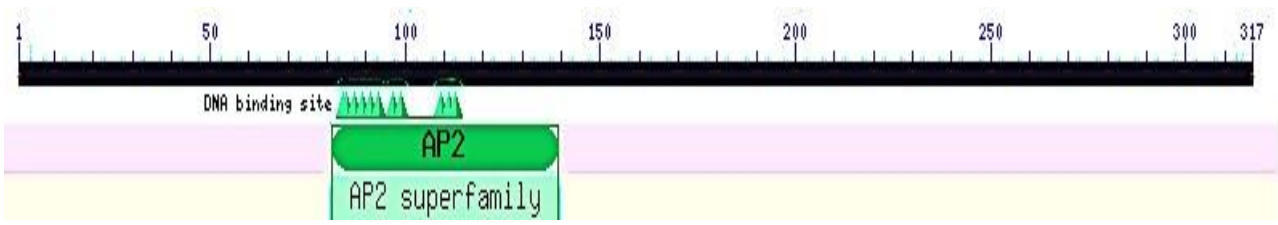

Fig. 1. Conserved domain of APETALA2 found in SmDREB1 protein.

FoldIndex© determined SmDREB1 a highly intrinsically disordered protein with 3 disordered regions (Fig. 2) containing a total number of 284 residues out of 317. The longest disordered region consists of 144 residues (Fig. 3). The same analysis conducted on StDREB1 results in 4 disordered regions including 252 residues out of 319. There were 11 binding regions in SmDREB1 as detected by ANCHOR© (Fig. 4) while in *Solanum tuberosum* protein, 9 such regions were anticipated. The level of conservation for all residues of investigated SmDREB1 was predicted and it was observed that the N terminal residues of protein including NLS and DNA binding domain (from 82 to 138 amino acids) were mostly conserved while as the sequence reaching to its C terminal the probability of variation increased (Fig. 5).

The conceptually translated protein sequence was submitted to I-Tasser server for 3D structure prediction and it demonstrated five structures. The model 1 was selected according to TM and C-score, which was 0.35±0.12 and -3.25 respectively (Fig. 6). The template 1GCC.1 that correspond to an Ethylene Responsive Element Binding Factor1 peptide of *Arabidopsis thaliana* was used to determine the structure of DNA binding domain of SmDREB1. This template showed 50% homology to investigated protein (Fig. 7). The 3D structure for DNA binding domain of SmDREB1 with estimated TM-score 0.78±0.10 and C-score 0.5 was superimposed with *Arabidopsis thaliana* DNA binding domain with a TM-score of 0.838. The ligand binding sites predicted by COACH supported by a C-score of 0.33 showed that residues at position 7, 8, 9, 11, 13, 21, 23, 25 and 46 that correspond to amino acids YRGRRERCY respectively, are used to bind with nucleic acid (Fig. 8).

The molecular systematic analysis using phylogenetic approach suggested that DREB protein in *Solanum melongena* is closely related to other species of genus *Solanum* as the clade including Solanaceae species supported by strong posterior probability value. The phylogenetic tree is divided into two major clades; the basel nodal branch consists of members from family Euphorbiaceae and Fabaceae while the upper larger clade displays different branches that represent relationship among members of different families (including *Solanum melongena*) based on their similar DREB protein (Fig. 9).

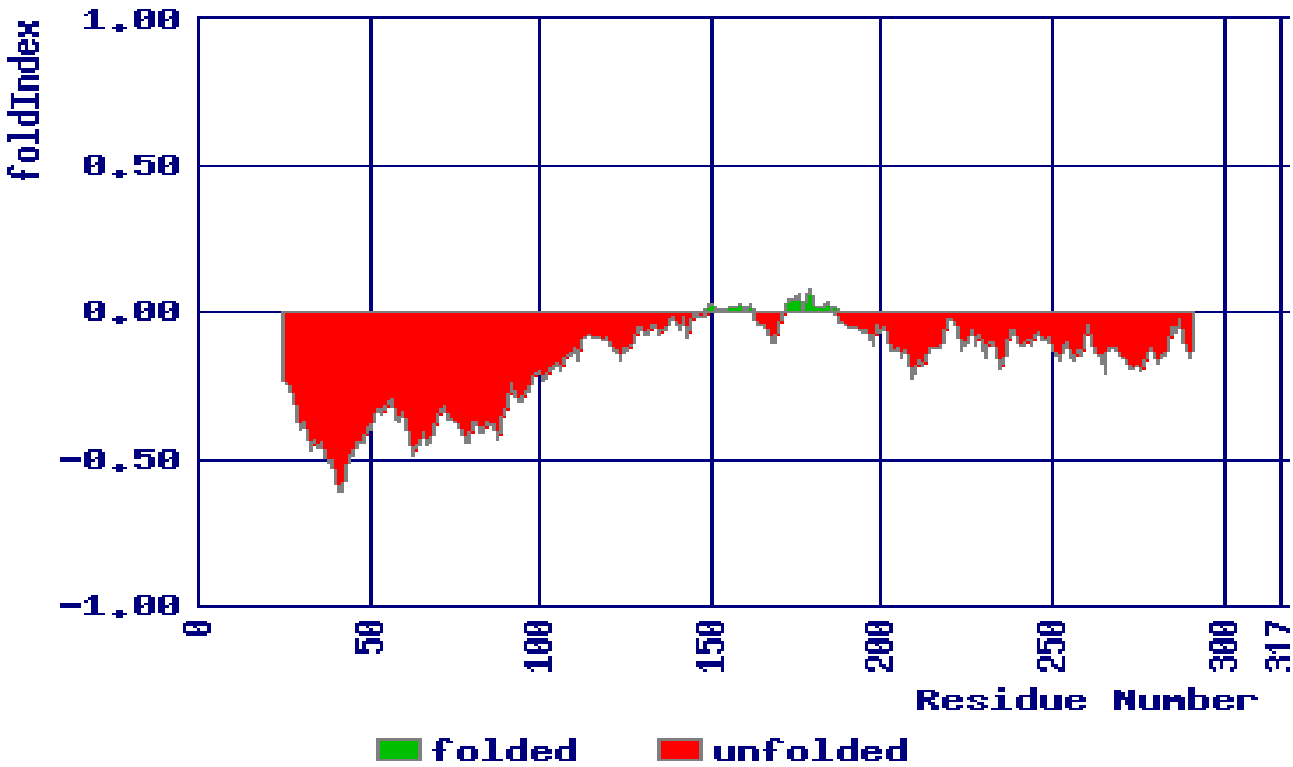

Fig. 2. Folded and unfolded regions in *Solanum melongena* DREB1 protein as determined by FoldIndex©.

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  1 MAIVDQGANM DSLPLDYRRK RKSRSRRDGT KNVEETLAKW KEYNQKLDSV
 51 DDEGKRMRKA PAKGSKKGCM KGKGGPENSQ CNYRGVRQRT WGKWVAEIRE
101 CNYRGVRQRT WGKWVAEIRE AYDEAARAMY GPCARLNLPK YPSRDDSDSD
151 SASWATTSAS DCTVASGGEV CPGEGATTAA GTPLSSVKNE GKDETAGLPG
201 EMEIVEPTSI DQDTLKSGWD CLDNLNLDEM FDVDDLLAML DSTPVSTNDF
251 GSDGKQYAYD NNLLSNSPCQ PLGDPQEMGQ EAPMTVDYGF DFLKPGRQED
301 LNFSLDDLAF IDLDSEL
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Fig. 3. Predicted disordered residues in SmDREB1 (shown in red).

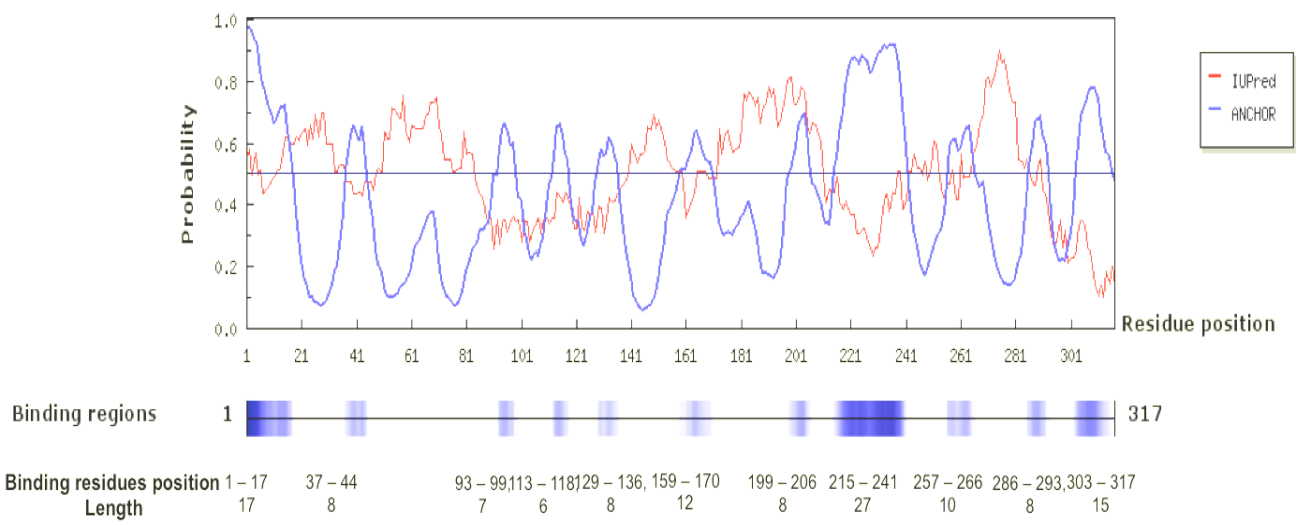

Fig. 4. Protein binding site prediction for SmDREB1 by ANCHOR©. Blue rectangular areas showing binding sites.

Fig. 5. The conserved and variable residues in SmDREB1.

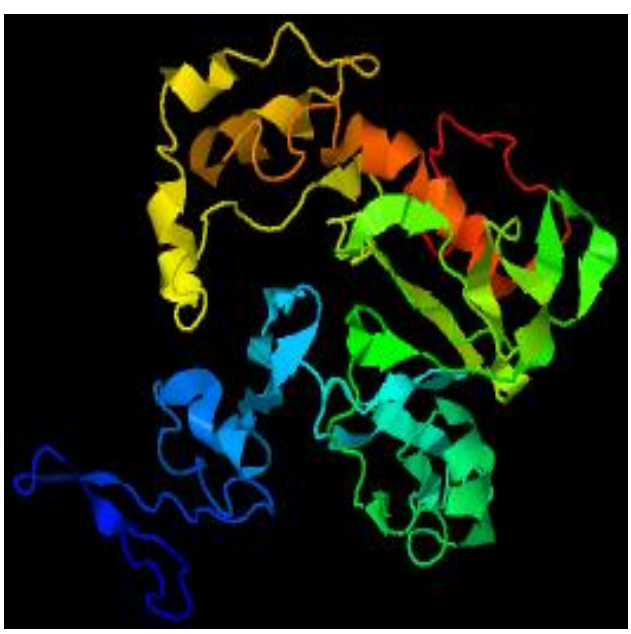

Fig. 6. Complete 3D structure of SmDREB1 as predicted by I-Tasser server.

Discussion

Solanum melongena being an important crop plant worldwide need more attention for abiotic stress tolerance as it is moderately sensitive to salinity, heat and drought (Ünlükara *et al*., 2010; Zhang *et al*., 2020). Several genes and their products have been suggested to play a significant role under water stress. DREB is one of such gene and there is a need to characterize this gene in important crop plant to make them survive under severe conditions. In the present study, detail structural and functional analysis of SmDREB1 was carried out.

Nucleotide alignment of SmDREB1 transcript indicates that it is a homologue of DREB1 family found in *Solanum* species. However, base variations were noticed in aligned sequences, which might be critical at protein level. A single nucleotide change can brought a change in amino acid sequence that can have a significant function when exposed to various stresses (Egawa *et al*., 2006).

Computational analysis with the help of softwares revealed physical and chemical properties such as number and compositions of amino acids, molecular weight (Mw) and theoretical isoelectric point (pI) of the eggplant

DREB1 protein. Structural and biophysical parameters are a prerequisite for defining protein function (Pandey *et al*., 2014). The Mw and pI of SmDREB1 and StDREB1 were almost similar suggesting they may share a common biological function. The isoelectric point of SmDREB1 was observed to be at acidic range. The pI value of a molecule can affect its solubility at a given pH. Such molecules are often precipitate out of a solution at the pH that corresponds to their pI (Friedman *et al*., 2009). This observation suggests that SmDREB1 is insoluble in a solution having pH around 4.6.

The alignment of SmDREB1 protein showed a conserved N-terminal nuclear localization signal (NLS) comprised of eight amino acid residues (RKSRSRRD) and a APETALA2 (AP2) DNA binding domain located in the range of 82 to 138 amino acid positions. The predicted NLS in SmDREB1 is also been observed in DREB2-like gene from *Dendranthema vestitum* (Liu *et al*., 2008), *Brassica napus* (TF ID; Bna005584), *Nicotiana tabacum* (TF ID; Nta007127), *Carica papaya* (TF ID; evm.model.supercontig_19.121) and *Cucumis sativus* (TF ID; Csg010412) (Zhang *et al*., 2011). Since, transcription factors only function in the nucleus, therefore NLS regulates their transportation into the nucleus (Akhtar *et al*., 2012). It was also noted that SmDREB1 protein seems to be highly conserved at N-terminal, which retains NLS and DNA binding domain residues necessary to carry out the key function of protein. This functional conservation makes SmDREB1 an important target for crop improvement for abiotic stress tolerance through plant breeding and genetic engineering approaches.

Short linear motifs (SLiMs) were also predicted in SmDREB1. These are common constituent in regulatory proteins and take part in many biological processes such as cell cycle regulation, post-translational modifications, protein-protein interactions, DNA repair and sub-cellular localization of proteins (Dinkel *et al*., 2013). SmDREB1 has several motifs that suggest its active involvements in regulatory pathways. It showed different sites phosphorylated by AGC protein kinases. This annotation can suggests its participation in hormone regulated signaling mechanisms and growth regulatory pathways. ELM found a binding site for 14-3-3 proteins in SmDREB1, these proteins are well known for providing a support to assemble signaling complexes and also help in protein shuttling (Roberts, 2003). Upon comparison, six SLiMs were found to be absent in DREB1 protein of potato. A unique Calcineurin (PP2B)-docking motif was observed in SmDREB1. The Ca^{2+}-dependent phosphatase calcineurin also known as protein phosphatase 2B (PP2B), is a highly conserved eukaryotic calcium-calmodulin-activated protein phosphatase that involve in a number of diverse signaling pathways mainly facilitates calcium-mobilizing signals to cell responses (Rusnak and Mertz, 2000). Second ligand that is only found in SmDREB1 is PDZ domain. The key role of this domain is to recognize specific five residue long motifs that usually reside at C-terminus of target protein thus regulate various biological activities (Harris and Lim, 2001). Another domain that was found absent in potato DREB1 protein is N-glycosylation site. In plants, glycosylation by N-linked oligosaccharides has a great impact in numerous biological functions of proteins such as induction of the correct folding or prevention from the proteolytic degradation (Rayon *et al*., 1998). Another signal that is an attribute of SmDREB1 is the presence of serine/threonine protein kinases, which belongs to the family of phosphatidylinositol 3-kinase-related kinases (PIKKs). They are exclusively found in eukaryotes and taking part in DNA damage and DNA repair mechanisms (Block *et al*., 2004). The tyrosine-based sorting signal is also found only in eggplant when compared with potato DREB1 protein. They interact with the mu subunit of adaptor protein complex used in vesicular traffic pathway thus determine the final destination of a particular molecule. These sites that are found only in SmDREB1 protein making it a distinctive protein that actively contribute in different regulatory pathways.

The analysis of folding pattern of SmDREB1 categorized this protein into intrinsically disorder proteins. These proteins are different from structured proteins in term of their sequence, evolution, structure, function, interactions and regulations (van der Lee *et al*., 2014). The intrinsically disorder regions (IDRs) in the proteins facilitate in acquiring different conformational states which are necessary for binding with other proteins to regulate their functions (Anjum *et al*., 2015).

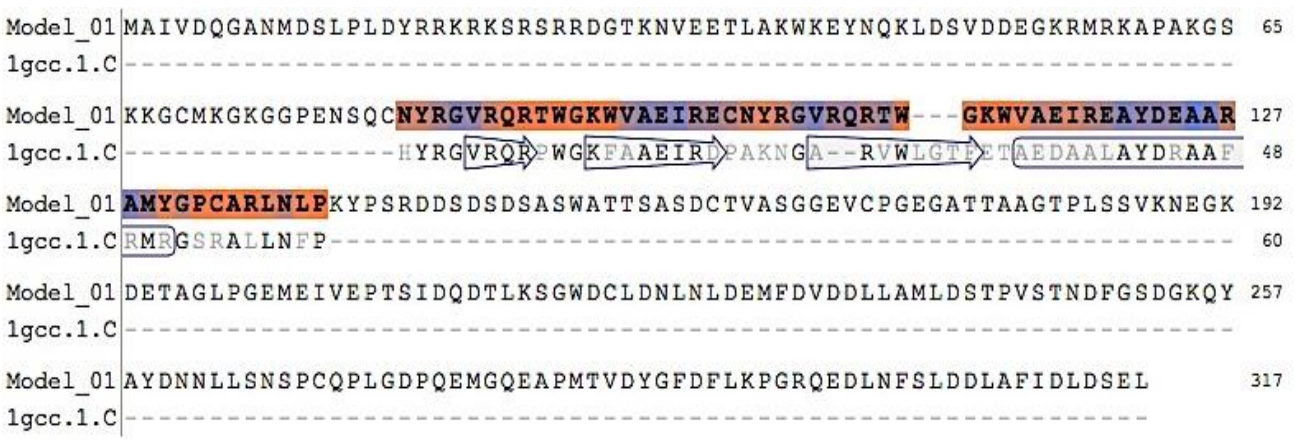

Fig. 7. Alignment of SmDREB1 and 1GCC.1 (*Arabidopsis thaliana*) template indicating sequence of DNA binding domain in highlighted area.

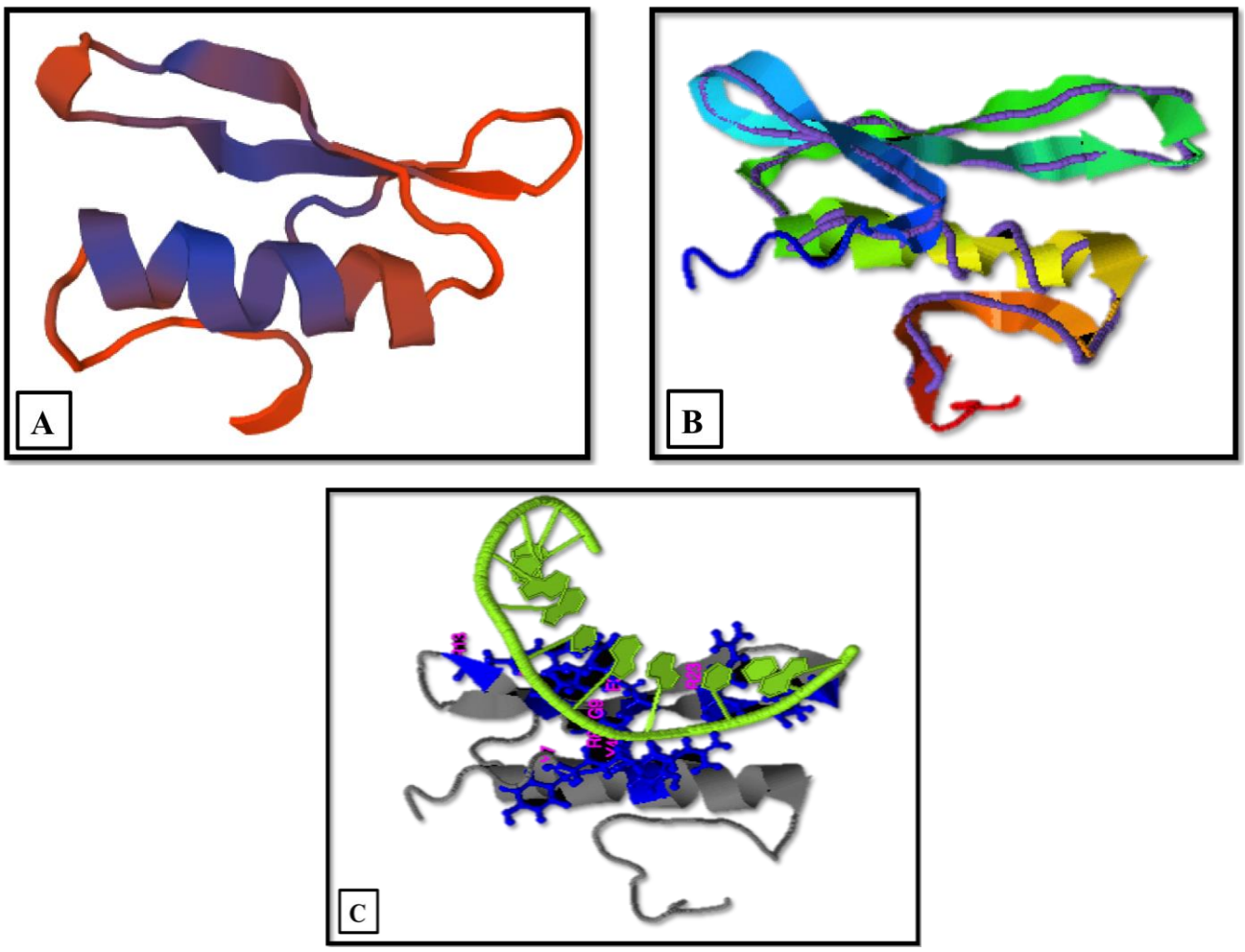

Fig. 8. 3D model structures of SmDREB1 DNA binding domain as predicted by Swiss Model and I-Tasser. A) DNA binding domain of SmDREB1; B) Superimposed structures of SmDREB1 DNA binding domain in rainbow ribbons and template (1GCC.1) in purple strand; C) Ligand binding sites in SmDREB1; grey ribbons showed SmDREB1 protein, blue side chain amino acids are ligand binding residues and green molecule is nucleic acid.

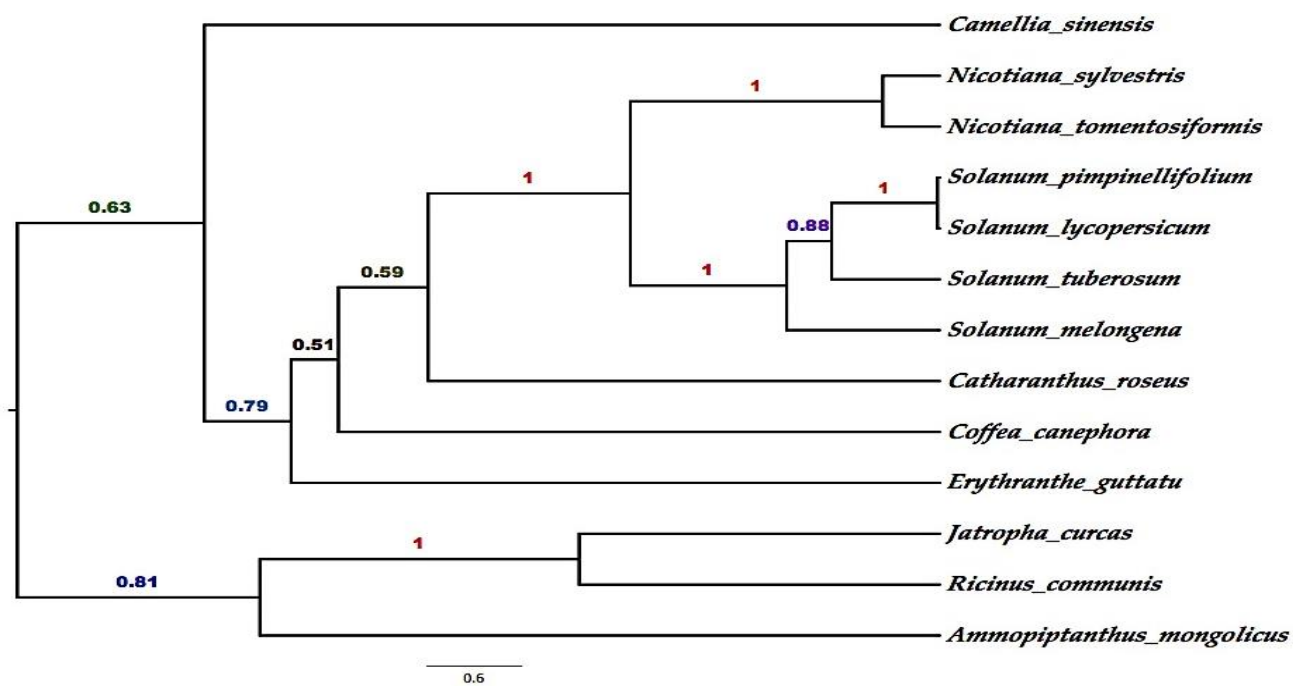

Fig. 9. Phylogenetic tree with mean branch lengths from a representative Bayesian analysis of DREB gene data. Numbers above branches represent their respective posterior probabilities.

The three dimensional structure of protein is very important to understand its function and interactions. The most common method for structure prediction is homology modeling. I-Tasser server was used to predict the 3D structure of SmDREB1, which is based on C-score and TM value. C-score is the confidence score typically in the range of -5–2. Higher C-score value implies a model of correct topology whereas template modeling (TM) value used to measure the similarity between two protein structure. A TM score greater than 0.5 signifies the correct topology of model while if this value is less than 0.17, it indicates a random similarity (Yang *et al*., 2015). In the current study, the C-score and TM value of SmDREB1 was observed as -3.25 and 0.35 respectively. The disordered and unfolded regions of SmDREB1 protein explain these lower values, as the protein does not acquire a stable structure. The basic step in homology modeling is to find out a best matching template. The template for SmDREB1 was selected based on the sequence similarity with publically available sequences. *Arabidopsis thaliana* Ethylene Responsive Element Binding Factor1 peptide having a PDB ID 1GCC.1 was selected by MODELLER as a template for structure prediction of DNA binding domain. The TM and C-score for DNA binding domain of SmDREB1 was noted as 0.78 and 0.5 respectively that correspond to a correct modeled structure. There were nine ligand binding residues found in binding domain of SmDREB1. These residues are believed to be conserved in nature when aligned with other plant DREB1 proteins. Arginine and tyrosine were the most abundant amino acids thought to be involve in DNA binding process.

Phylogenetic analysis inferred a close association between DREB1 protein from *Solanum melongena* and other *Solanum* species, suggesting the common origin of structure and function. However, *S. melongena* was observed to be in a separate branch of a clade comprised of *Solanum* species, which reveals that SmDREB1 may have some distinct features in term of its structure and biological activities; some of which have been discussed earlier. The bayesian analysis also demonstrates that DREB1 protein is conserved at family level in plant kingdom as the taxa belongs to different families appeared in separate lineages in phylogenetic tree.

The outcomes of this study provided valuable information for understanding of DREB1 protein in *Solanum melongena*, which can further be used to improve abiotic stress tolerance of the germplasm.

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