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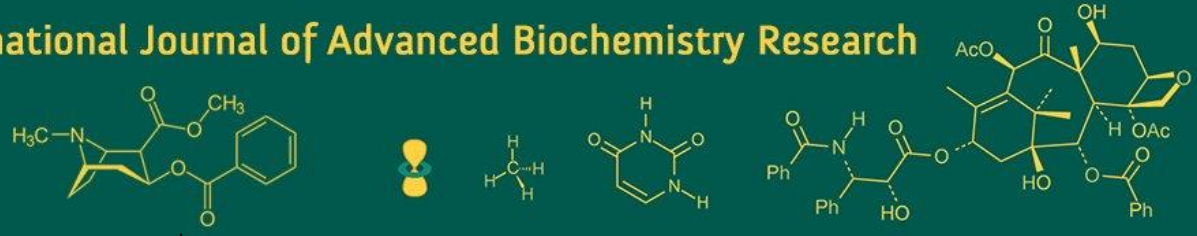

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In-silico analysis of AREB1 gene in tomato for draught resistance

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Abstract
Abiotic stresses have a negative impact on the production, productivity, and quality of the tomato (*Solanum lycopersicum.* L), one of the most significant vegetable crops. Almost every phase of the tomato life cycle is impacted by abiotic factors such drought, excessive heat, and high salinity. Abiotic stress causes a yield loss of around 70%, depending on the stage of the plant and the length of the stress. Several wild tomato species possess the genes for stress resistance, but due to considerable genetic distance and other restrictions, it is highly challenging to introduce these genes into cultivars. One of the most environmentally friendly strategies for the successful production of tomatoes are the development of cultivars with improved abiotic stress tolerance. Attempts are being made in this area to comprehend the mechanism of stress tolerance, the finding of genes, and the interaction of genetic and environmental factors. For growing tomatoes, a number of omics strategies, instruments, and resources have previously been created. Studies on tomato genomes and transcriptomics have advanced significantly thanks to modern sequencing technology. Abiotic stress responses in plants have been linked to transcription factors from the abscisic acid-responsive element binding protein (AREB) family. Abscisic acid (ABA), a plant hormone that is vital in regulating stress-responsive gene expression under osmotic stress conditions including drought and excessive salinity, primarily functions through three bZIP transcription factors called AREB1/ABF2, AREB2/ABF4, and ABF3. Although their induction methods differ, the AREB members have been observed to be receptive to ABA and a variety of environmental stimuli. The AREB/ABF genes function in two ways: first, by producing functional proteins that stabilise plant cells and interact with other regulatory proteins in response to stress; second, by directly or indirectly influencing the expression of downstream genes through ABA.

Keywords: AREB1, abiotic stress, *in-silico*, omics, transcriptomics, bioinformatics

Introduction
Tomato (*Solanum lycopersicum* L.) is the most common and considerable crop grown worldwide, including in India. It has a diploid genome with 12 chromosome pairs and a genome size of 950 Mb (Barone *et al.*, 2008) [4]. This crop is widely known for its origin in Western South America and was later domesticated throughout the world (Kimura and Sinha 2008) [9].

Tomato production is known to be drastically reduced as a result of various biotic and abiotic factors. Nematodes, fungi, bacteria, and viruses are examples of living agents, and they were thought to be the main causes of significant damage in the tomato industry (Kaur and Asthir *et al.*, 2017) [8]. It is very problematic because tomatoes are extremely sensitive to drought stress and can experience yield losses of up to 79%. (Aliche *et al.*, 2018) [1].

During the process of plant response to drought stress, a large number of genes were induced. These genes were classified into two major groups according to their putative functional modes (Laila *et al.*, 2019) [10]. The first group comprises the genes encoding structural proteins, which were downstream effectors in the stress response pathway including osmoregulatory genes, antioxidant proteins, and late embryogenesis abundant (LEA) proteins, whereas, the second group consists of the genes encoding regulatory proteins, which were early response transcriptional activators including transcription factors and protein kinesis including gbZIP, WRKY, MYB, and AP2/EREBP proteins have been proved to play important roles in the regulation of drought tolerance (Wang *et al.*, 2006) [15].

Several water deficit (drought) stress-inducible genes have been uncovered and found to be activated by abscisic acid ABA. AREB-I gene mutant families are more tolerant to ABA. There are other single and double mutants with respect to for primary root growth, and it exhibits low drought tolerance (Takuya *et al*., 2010) [14].

AREB/ABF transcription factors induced as a consequence of environmental stresses, only AREB1 are reported to be regulated by ABA-dependent phosphorylation (Fujita *et al*., 2005) [6]. ARBB1 is required for ABA to be fully activated. Abscisic acid is a plant hormone that regulates many important processes in plant metabolism, such as seed germination and dormancy, stomatal opening and closing, and absence and adaptation to water stress (Redenbaugh *et al*., 1992) [13].

Gene-expression profiling is a powerful technique for studying biological processes at the molecular level. Gene activity or gene expression can be assessed by protein identification but gene expression is usually investigated by examining the RNA-transcript and the application of *in-silico* tools for significantly improving the detection of genes and creating variations for a beneficial purpose (Orellana *et al.,* 2010) [12]. Further, the *in-silico* analysis will help to find the drought-associated genes in tomatoes and their associations with other co-expressed genes. In this study, we aim to contribute to a better understanding of genes and their *in-silico* characterization for drought-associated genes in tomato genotypes.

Materials and Methods

The study was conducted at College of Agriculture, department of biotechnology bioinformatic cell, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, India (2021-2022).

For achiving the goal of the present study NCBI (https://www.ncbi.nlm.nih.gov), a public library or database is use. This contains information about proteins, genes, SNPs, Domains, and Primers, among other molecular entities was one of the three primary databases that exist and was redundant. The NCBI's Gene and Protein Databases were utilized to retrieve the sequences, as well as some key information such as accession number, functional areas, active sites etc.

Basic Local Alignment Search Tool analysis

Extracted FASTA (Fast Adaptive Shrinkage Threshold Algorithm) sequences of drought-responsive genes from NCBI were subjected to the Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov) (Yu *et al.*, 2006) [16] for Solanum tax Id with default parameter settings. Out of 100, a total of 5 stress-responsive sequences which were showing nearly 80-100 percent identity were selected from solanum tax id. Out of these 100 genes which were selected our gene of interest AREB1 was taken into consideration and nearly about 5 sequences were identified for 90-100% similarity between the sequences.

Conservation and phylogenetic study using CLUSTAL W of EMBL

The CLUSTAL W (https://www.genome.jp›tools-bin›clustalw) tool from EMBL (https://www.embl.org) was used to detect sequence conservation at both the genomic and proteomic levels of the AREB1gene. The evolutionary tree development followed similarity, gaps, conservation, and most importantly relationship between the 5 sequences. The phylogenetic tree can be conserved based on the results of the multiple sequence alignment.

Structural studies of the protein

Primary Structure Analysis using PHYRE

Protparam an Expasy tool (https://www.expasy.org) was used for completing the physio-chemical characterization of a protein. This tool falls under the category of primary structure prediction tools. The tool can predict protein sequence-based parameters such as length, molecular weight, iso-electric point, GRAVY etc.

Secondary Structure prediction using SWISS Model

The secondary structure must be studied in order to identify internal structural confirmations such as loops, turns, and Homology (or comparative) modeling methods to generate models for evolutionary-related proteins using experimental protein architectures ("templates") ("targets").

Tertiary Structure prediction using PHYRE

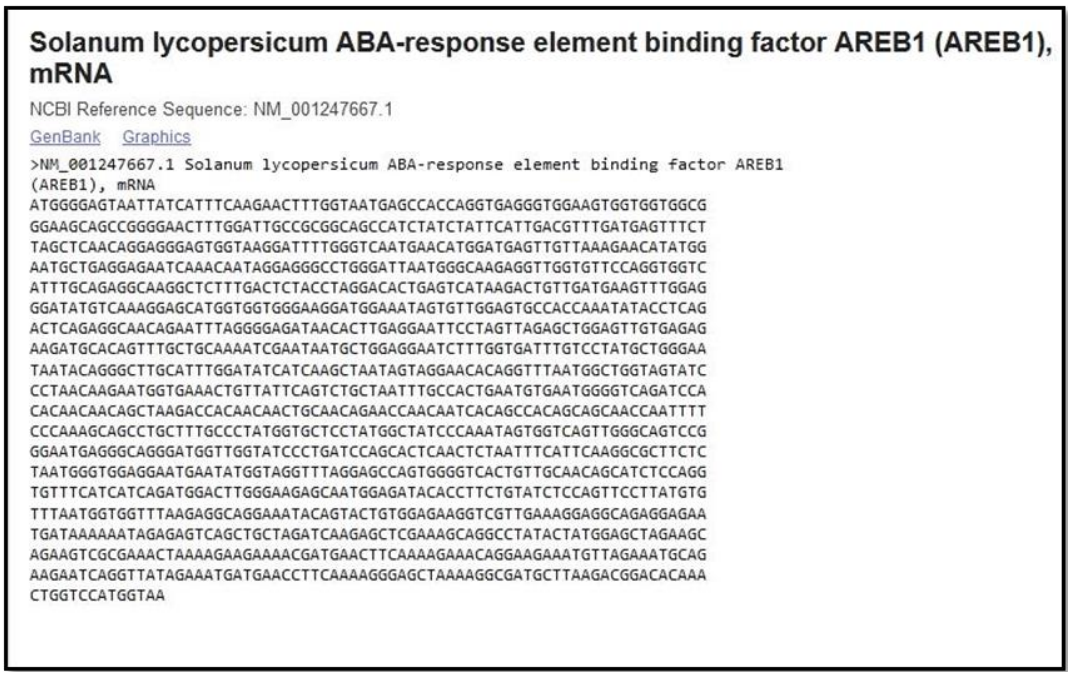
Phyre, a 3D structure prediction tool that uses a BLAST-like algorithm to compare the user-entered sequence to a list of protein sequences contained in the PDB database, with the result displayed as the best PDB that shares the greatest sequence similarity was used to predict the 3D structure of the AREB1 gene. The PDB Ids of the structure that were already saved in the Protein Data Bank were retrieved here. The structure can be seen by obtaining this PDB from the Protein Data Bank. SWISS-MODEL (https://swissmodel.expasy.org) used was a structural bioinformatics web-server 3D protein homology modelling. Homology modelling was currently the most accurate method for generating credible three-dimensional protein structure models, and it was employed in a wide specialized range of practical applications.

Results and Discussion

In-silico Screening of Tomato for Drought Related Gene

Retrieval of protein FASTA sequence of (AREB1) gene from NCBI

The sequence for the selected gene was retrieved, and the protein and nucleotide sequences were stored in FASTA format in text file. The sequences were obtained in query search from which only drought proteins belonging to tomato were screened out for more specificity. This FASTA format was further used for similarity searching and domain analysis. Among all proteins, AREB1 protein was selected and studied as it was responsible for major drought stress in tomato.



Solanum lycopersicum ABA-response element binding factor AREB1 (AREB1), mRNA

NCBI Reference Sequence: NM_001247667.1

GenBank Graphics

>NM_001247667.1 Solanum lycopersicum ABA-response element binding factor AREB1 (AREB1), mRNA

ATGGGGAGTAATTATCATTTCAAGAACTTTGGTAATGAGCCACCAGGTGAGGGTGGAAGTGGTGGTGGCG
GGAAGCAGCCGGGGAACTTTGGATTGCCGCGGCAGCCATCTATCTATTCATTGACGTTTGATGAGTTTCT
TAGCTCAACAGGAGGGAGTGGTAAGGATTTTGGGTCAATGAACATGGATGAGTTGTTAAAGAACATATGG
AATGCTGAGGAGAATCAAACAATAGGAGGGCCTGGGATTAATGGGCAAGAGGTTGGTGTTCCAGGTGGTC
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ACTCAGAGGCAACAGAATTTAGGGGAGATAACACTTGAGGAATTCCTAGTTAGAGCTGGAGTTGTGAGAG
AAGATGCACAGTTTGCTGCAAAATCGAATAATGCTGGAGGAATCTTTGGTGATTTGTCCTATGCTGGGAA
TAATACAGGGCTTGCATTTGGATATCATCAAGCTAATAGTAGGAACACAGGTTTAATGGCTGGTAGTATC
CCTAACAAGAATGGTGAAACTGTTATTCAGTCTGCTAATTTGCCACTGAATGTGAATGGGGTCAGATCCA
CACAACAACAGCTAAGACCACAACAACTGCAACAGAACCAACAATCACAGCCACAGCAGCAACCAATTTT
CCCAAAGCAGCCTGCTTTGCCCTATGGTGCTCCTATGGCTATCCCAAATAGTGGTCAGTTGGGCAGTCCG
GGAATGAGGGCAGGGATGGTTGGTATCCCTGATCCAGCACTCAACTCTAATTTCATTCAAGGCGCTTCTC
TAATGGGTGGAGGAATGAATATGGTAGGTTTAGGAGCCAGTGGGGTCACTGTTGCAACAGCATCTCCAGG
TGTTTCATCATCAGATGGACTTGGGAAGAGCAATGGAGATACACCTTCTGTATCTCCAGTTCCTTATGTG
TTTAATGGTGGTTTAAGAGGCAGGAAATACAGTACTGTGGAGAAGGTCGTTGAAAGGAGGCAGAGGAGAA
TGATAAAAAATAGAGAGTCAGCTGCTAGATCAAGAGCTCGAAAGCAGGCCTATACTATGGAGCTAGAAGC
AGAAGTCGCGAAACTAAAAGAAGAAAACGATGAACTTCAAAAGAAACAGGAAGAAATGTTAGAAATGCAG
AAGAATCAGGTTATAGAAATGATGAACCTTCAAAAGGGAGCTAAAAGGCGATGCTTAAGACGGACACAAA
CTGGTCCATGGTAA

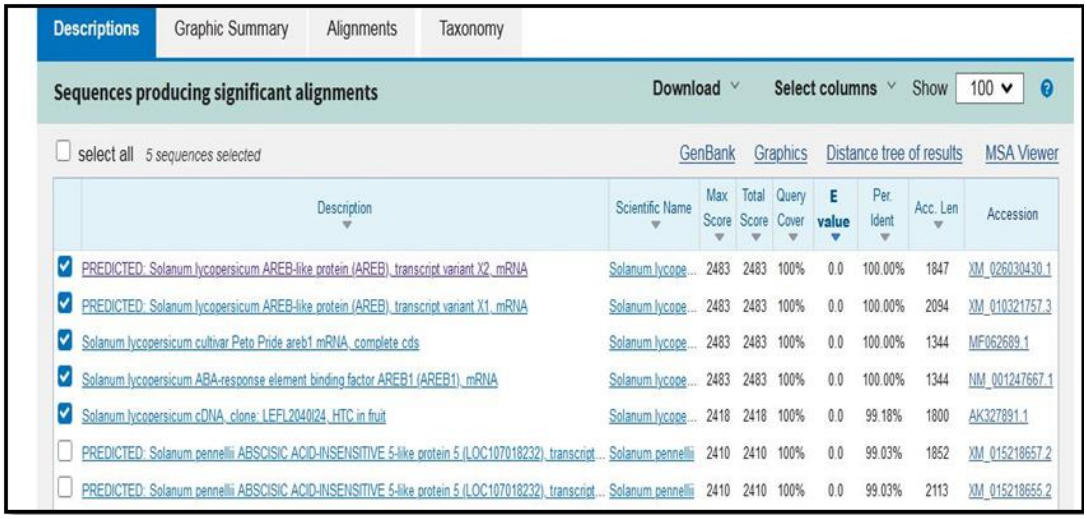
Fig 1: FASTA sequence of (AREB1) gene

BLAST

This tool helps in identifying the similar sequences between the nucleotide and protein sequences or nucleotide-nucleotide sequences or protein-protein sequences. Therefore, results obtained from the similarity search using BLAST tool. BLAST results showed the similar sequences within the percent identity if 90-100% and their query cover, gaps loci and their respective accession ID's which were the basis for a similarity search using BLAST tool.

Multiple sequence alignments (MSA)

For the analysis of protein primary, secondary and tertiary structures, phylogenetic reconstruction, and protein function prediction, BLAST was needed from which multiple sequence alignment was used to compare homologous sequences and the similarities between them. A protein family's conserved areas may be seen in biologically high-quality and accurate alignments, which can reveal connections and the homology between various sequences and provide important structural and functional information that can be utilized to further discover new members of the family. For the multiple sequence alignment, the sequence of AREB1 (NM_001247667.1) retrieved from the NCBI website. The sequence of (AREB1) was obtained from NCBI and put in BLAST tool in order to align various sequences. Numerous sequences that demonstrate sequence similarity and were subjected to considerable alignments were revealed as the findings of nucleotide and protein analysis.



Descriptions | Graphic Summary | Alignments | Taxonomy

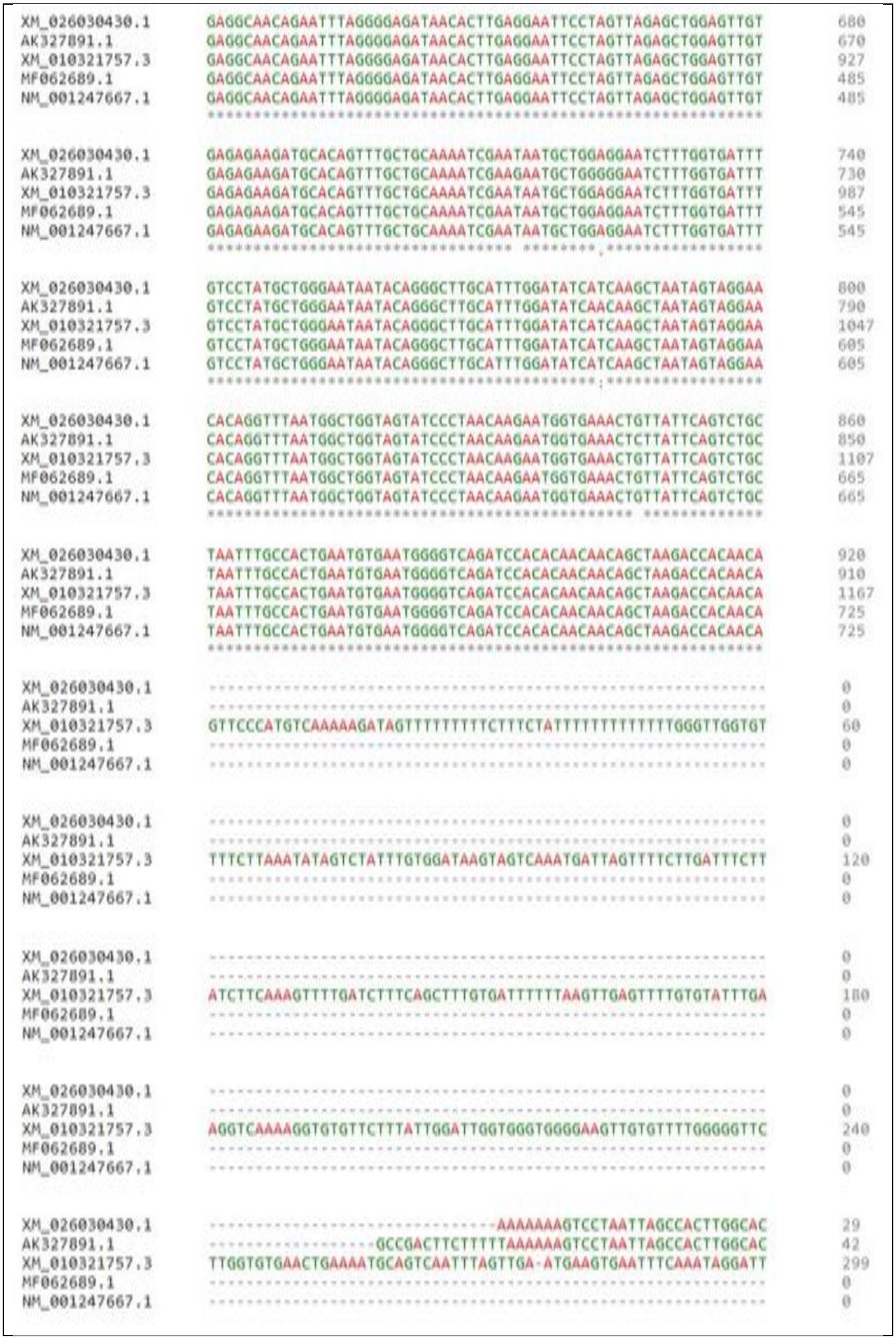
Sequences producing significant alignments — Download | Select columns | Show 100

select all 5 sequences selected — GenBank | Graphics | Distance tree of results | MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
PREDICTED: Solanum lycopersicum AREB-like protein (AREB), transcript variant X2, mRNA	Solanum lycope...	2483	2483	100%	0.0	100.00%	1847	XM_026030430.1
PREDICTED: Solanum lycopersicum AREB-like protein (AREB), transcript variant X1, mRNA	Solanum lycope...	2483	2483	100%	0.0	100.00%	2094	XM_010321757.3
Solanum lycopersicum cultivar Peto Pride areb1 mRNA, complete cds	Solanum lycope...	2483	2483	100%	0.0	100.00%	1344	MF062689.1
Solanum lycopersicum ABA-response element binding factor AREB1 (AREB1), mRNA	Solanum lycope...	2483	2483	100%	0.0	100.00%	1344	NM_001247667.1
Solanum lycopersicum cDNA, clone: LEFL2040I24, HTC in fruit	Solanum lycope...	2418	2418	100%	0.0	99.18%	1800	AK327891.1
PREDICTED: Solanum pennellii ABSCISIC ACID-INSENSITIVE 5-like protein 5 (LOC107018232), transcript...	Solanum pennellii	2410	2410	100%	0.0	99.03%	1852	XM_015218657.2
PREDICTED: Solanum pennellii ABSCISIC ACID-INSENSITIVE 5-like protein 5 (LOC107018232), transcript...	Solanum pennellii	2410	2410	100%	0.0	99.03%	2113	XM_015218655.2

Fig 2: Top five sequences selected for sequence for alignment

Sequences with differences in the similarity percent values more than 100% and strong query coverage were chosen from among them, and both the nucleotide and protein versions of those sequences were downloaded. These nucleotide and protein sequences were put through Multiple Sequence Alignment using tool Clustal W programme, which was readily available online. The multiple sequence alignment of protein template sequences shows similar alignment sequences (XM_026030430.1, MF062689.1, NM_001247667.1, AK327891.1, XM_026030430.1 and X8M_010321757.3).


XM_026030430.1 GAGGCAACAGAATTTAGGGGAGATAACACTTGAGGAATTCCTAGTTAGAGCTGGAGTTGT 680
AK327891.1 GAGGCAACAGAATTTAGGGGAGATAACACTTGAGGAATTCCTAGTTAGAGCTGGAGTTGT 670
XM_010321757.3 GAGGCAACAGAATTTAGGGGAGATAACACTTGAGGAATTCCTAGTTAGAGCTGGAGTTGT 927
MF062689.1 GAGGCAACAGAATTTAGGGGAGATAACACTTGAGGAATTCCTAGTTAGAGCTGGAGTTGT 485
NM_001247667.1 GAGGCAACAGAATTTAGGGGAGATAACACTTGAGGAATTCCTAGTTAGAGCTGGAGTTGT 485
XM_026030430.1 GAGAGAAGATGCACAGTTTGCTGCAAAATCGAATAATGCTGGAGGAATCTTTGGTGATTT 740
AK327891.1 GAGAGAAGATGCACAGTTTGCTGCAAAATCGAAGAATGCTGGGGGAATCTTTGGTGATTT 730
XM_010321757.3 GAGAGAAGATGCACAGTTTGCTGCAAAATCGAATAATGCTGGAGGAATCTTTGGTGATTT 987
MF062689.1 GAGAGAAGATGCACAGTTTGCTGCAAAATCGAATAATGCTGGAGGAATCTTTGGTGATTT 545
NM_001247667.1 GAGAGAAGATGCACAGTTTGCTGCAAAATCGAATAATGCTGGAGGAATCTTTGGTGATTT 545
XM_026030430.1 GTCCTATGCTGGGAATAATACAGGGCTTGCATTTGGATATCATCAAGCTAATAGTAGGAA 800
AK327891.1 GTCCTATGCTGGGAATAATACAGGGCTTGCATTTGGATATCAACAAGCTAATAGTAGGAA 790
XM_010321757.3 GTCCTATGCTGGGAATAATACAGGGCTTGCATTTGGATATCATCAAGCTAATAGTAGGAA 1047
MF062689.1 GTCCTATGCTGGGAATAATACAGGGCTTGCATTTGGATATCATCAAGCTAATAGTAGGAA 605
NM_001247667.1 GTCCTATGCTGGGAATAATACAGGGCTTGCATTTGGATATCATCAAGCTAATAGTAGGAA 605
XM_026030430.1 CACAGGTTTAATGGCTGGTAGTATCCCTAACAAGAATGGTGAAACTGTTATTCAGTCTGC 860
AK327891.1 CACAGGTTTAATGGCTGGTAGTATCCCTAACAAGAATGGTGAAACTCTTATTCAGTCTGC 850
XM_010321757.3 CACAGGTTTAATGGCTGGTAGTATCCCTAACAAGAATGGTGAAACTGTTATTCAGTCTGC 1107
MF062689.1 CACAGGTTTAATGGCTGGTAGTATCCCTAACAAGAATGGTGAAACTGTTATTCAGTCTGC 665
NM_001247667.1 CACAGGTTTAATGGCTGGTAGTATCCCTAACAAGAATGGTGAAACTGTTATTCAGTCTGC 665
XM_026030430.1 TAATTTGCCACTGAATGTGAATGGGGTCAGATCCACACAACAACAGCTAAGACCACAACA 920
AK327891.1 TAATTTGCCACTGAATGTGAATGGGGTCAGATCCACACAACAACAGCTAAGACCACAACA 910
XM_010321757.3 TAATTTGCCACTGAATGTGAATGGGGTCAGATCCACACAACAACAGCTAAGACCACAACA 1167
MF062689.1 TAATTTGCCACTGAATGTGAATGGGGTCAGATCCACACAACAACAGCTAAGACCACAACA 725
NM_001247667.1 TAATTTGCCACTGAATGTGAATGGGGTCAGATCCACACAACAACAGCTAAGACCACAACA 725
XM_026030430.1 0
AK327891.1 0
XM_010321757.3 GTTCCCATGTCAAAAAGATAGTTTTTTTTTCTTTCTATTTTTTTTTTTTTTGGGTTGGTGT 60
MF062689.1 0
NM_001247667.1 0
XM_026030430.1 0
AK327891.1 0
XM_010321757.3 TTTCTTAAATATAGTCTATTTGTGGATAAGTAGTCAAATGATTAGTTTTCTTGATTTCTT 120
MF062689.1 0
NM_001247667.1 0
XM_026030430.1 0
AK327891.1 0
XM_010321757.3 ATCTTCAAAGTTTTGATCTTTCAGCTTTGTGATTTTTTAAGTTGAGTTTTGTGTATTTGA 180
MF062689.1 0
NM_001247667.1 0
XM_026030430.1 0
AK327891.1 0
XM_010321757.3 AGGTCAAAAGGTGTGTTCTTTATTGGATTGGTGGGTGGGGAAGTTGTGTTTTGGGGGTTC 240
MF062689.1 0
NM_001247667.1 0
XM_026030430.1 -------------------------------AAAAAAAGTCCTAATTAGCCACTTGGCAC 29
AK327891.1 ------------------GCCGACTTCTTTTTAAAAAAGTCCTAATTAGCCACTTGGCAC 42
XM_010321757.3 TTGGTGTGAACTGAAAATGCAGTCAATTTAGTTGA-ATGAAGTGAATTTCAAATAGGATT 299
MF062689.1 0
NM_001247667.1 0

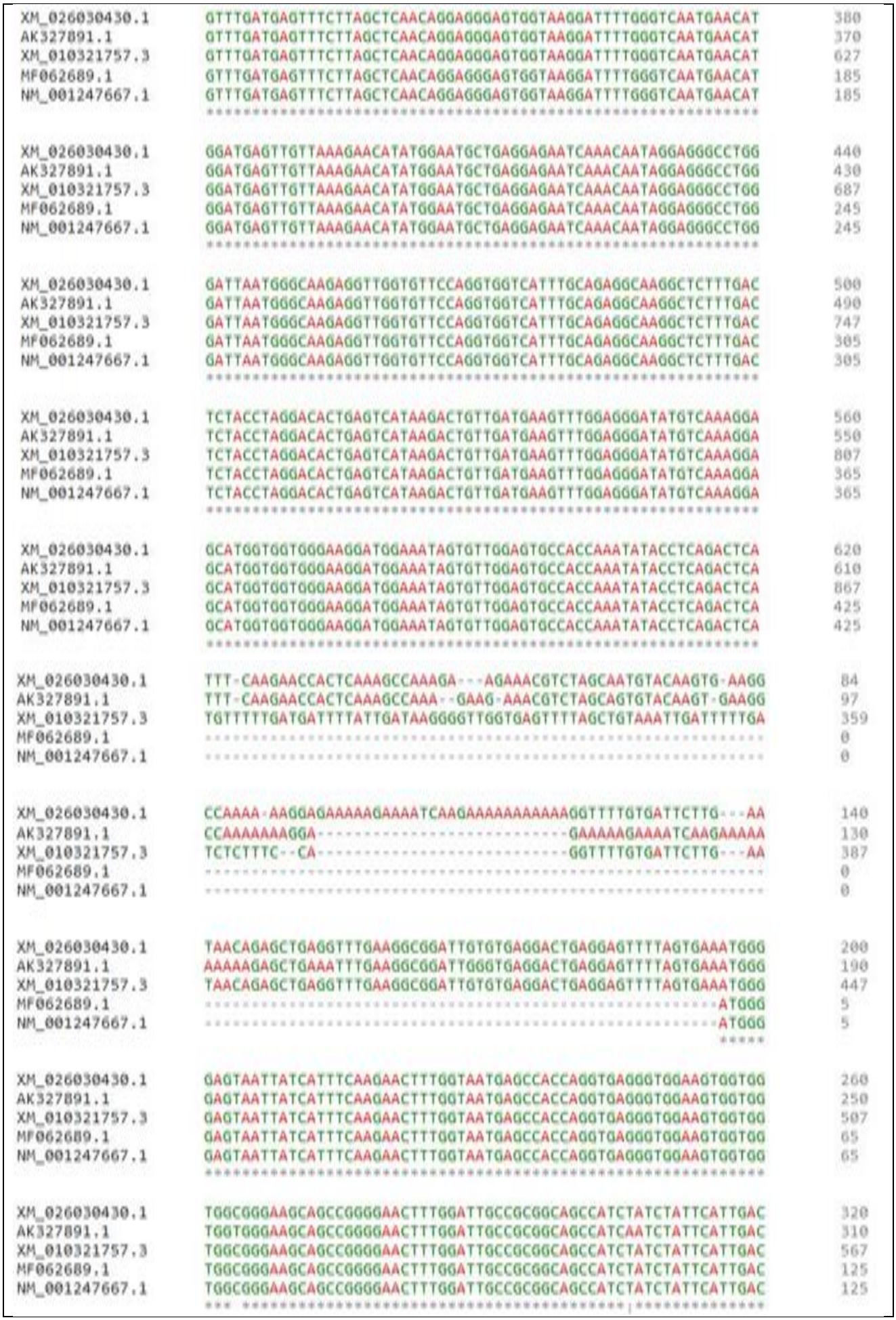


Fig 3: Multiple sequence alignment of AREB1

Structure Model of Protein

The Swiss-model website assists in developing and creating the protein secondary structures from which each and every detail of the proteins can be obtained along with its query cover, sequence identity, structural assessment etc.

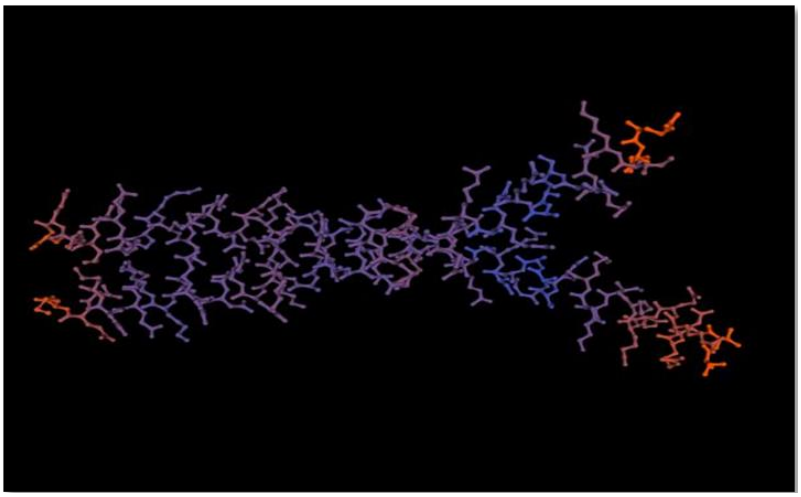


Fig 4: Secondary structure model of the AREB1 protein

Ramachandran plot of AREB1 gene

The Ramachandran plot was a plot of the torsional angles – phi and psi of the amino acid's residue in a peptide and alpha helices, beta strands and turns were the most likely confirmations for a polypeptide chain to adopt in this plot.

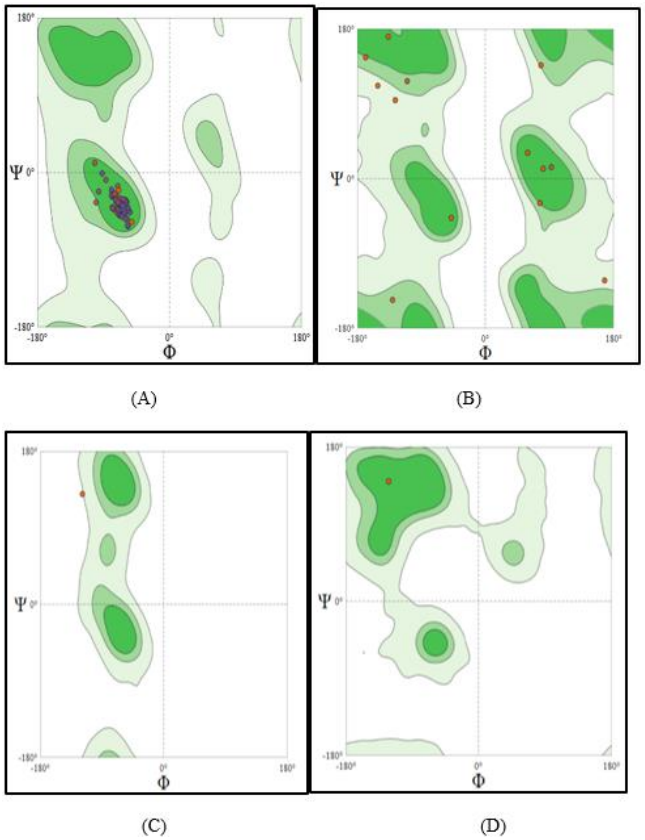


Fig 5: The sterochemical validation of hypothetical model of AREB 1 using Ramachandran plot (A) General proportion, (B) Glycine favored, (C) Pre-polinefavoured, (D) Proline favored

Phylogenetic analysis

The phylogenetic tree was obtained by using Clustal omega tool, in which the sequences of protein of different organisms were uploaded in FASTA format and then submitted to search the multiple sequence alignment. After that the result page shows the various options like cladogram in order to obtain detailed tree structure.

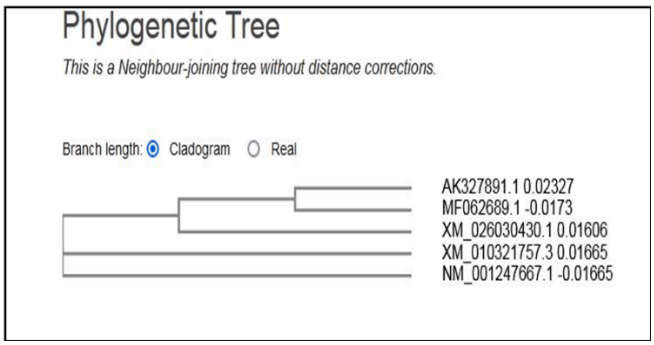


Fig 6: Phylogenetic tree of AREB1 gene

Construction of Phylogenetic Tree

The phylogenetic tree showing the similarity between multiple sequences of similar AREB1 proteins among different organisms. The branches shown in this tree was showing the phylogenetic similarity between the sequences. The short branch length was showing high phylogenetic similarity whereas; longer branch length was showing less similarity.

Based on the percentage identity matrix of the nucleotide of 5 template sequences, a phylogenetic tree was constructed using Clustal omega online available tool. The phylogenetic tree showed that all sequences were interlinked with each other. The cluster grouped all the nucleotide template sequences in two distinct cluster.

Discussion

There were many genes for drought stress in tomatoes which includes DREB, APETALA and AREB etc. and many more genes were also reported previously.

Accordingly, AREB gene was selected for our work, in which the gene similarity sequences were selected and many bioinformatics programs were also conducted. To identify similar genes with respect to AREB gene this gene was checked for its FASTA sequences which was downloaded from NCBI website. The BLAST of this FASTA sequence resulted in several genes similar to the selected gene. Out of

all the genes, the similar sequences were selected within 50-100% sequences similarity and which were expressed only in tomato germplasms. Later, the Phyre2 software along with the Swiss-Model was used in identifying the protein primary and secondary structures along with their domain analysis. In this software, the protein structures of the related genes were also observed and recorded.

The similar work is performed by (Zhou *et al*., 2016) [17], reported that the gene ABF1 was markedly induced by drought, high salinity and ABA treatments, although its expression level was lower even under stress conditions than AREB1, AREB2 and ABF3. However the ARBB1 gene was identified as a robust gene along with the Dreb gene in drought responsive pathways in Arabidopsis, tomato, rice and other members of Solanaceae (Alves and Setter., 2004) [3], (Ja'afar *et al*., 2018) [7] (Aliche *et al*., 2021) [1]. The ARBB1 gene confers a very low sensitivity to abscisic acid; and, therefore, suggested that it may be involved in an ABA independent pathway (Barry., 2001) [5].

Also, the phylogenetic tree along with the multiple sequence alignment was drawn using the Bio-edit software where the similarity between the sequences was understood and the phylogenetic relatedness between different genes was also studied. Due to these advantages, of understanding a particular gene, its similar sequences, and their protein structures along with phylogenetic tree analysis provides maximum data with resected concerning gene AREB (Abscisic acid- Responsive Element Binding protein) gene.

The same cluster is found to be closely related and share a common evolutionary trend. A similar trend was reported by Molla *et al*. (2015) [11] for Oryza sativa and *Oryza glaberrima* with some closely related species in the same cluster.

Conclusion

In conclusion, the in-silico screening of the tomato for drought-related genes, particularly focusing on the AREB1 gene, involved several bioinformatics tools and analyses. The retrieval and screening of protein FASTA sequences from NCBI, followed by BLAST similarity searches, facilitated the selection of relevant sequences specific to tomato drought proteins. Multiple sequence alignments allowed for comparison and identification of conserved regions among homologous sequences. Structural modeling using tools like Swiss-model provided insights into the secondary structure of the AREB1 protein. Additionally, phylogenetic analysis revealed evolutionary relationships between AREB1 sequences from different organisms. The discussion highlighted the significance of the AREB gene in drought response pathways, corroborated by previous research findings. Overall, the comprehensive analysis of AREB1 gene and its related sequences enhances our understanding of drought tolerance mechanisms in tomatoes and provides valuable insights for further research in this area.

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