Scarecrow like protein 1, (Ct-SCL1) involved in drought stress tolerance by interacting with SWI3B component of Chromatin modelling complex in Cluster bean, *Cyamopsistetragonaloba*(L.) Taub.

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ABSTRACT *Drought is one of the important challenges to the agricultural produce in tropics. The cropping in this region is mainly rain fed. Clusterbean, Cyamopsistetragonaloba L. a highly valued and cultivated crops of arid and semi-arid region but resulting in low yields because of susceptibility to abiotic stresses like drought and salinity. Therefore, stress tolerant varieties of cluster bean have gained importance in agriculture of semi-arid tropics. The current study carried out at isolation, characterization of a transcription factor, Scarecrow like protein 1 (Ct-SCL-1)belonging to the class of GRAS family transcription factors from Cluster bean variety RGC-1025. The real time PCR screening of the drought stressed and control samples of the Cluster bean variety RGC-1025 revealed overexpression of the Ct-SCL1 under drought stress. Structural and phylogenetic analysis of Ct-SCL1 sequences with the other SCL proteins revealed the structurally and functionally conserved residues of the SCL proteins. All the findings of the study reveal that Ct-SCL1 is having a key role in the survival of the Cluster bean under drought stress through interaction with SWI3B protein through chromatin remodelling and stress based epigenetic memory for the further generations of the plant variety.*

Keywords:

Introduction

The tropical regions of the world are more prone to frequent droughts due to low rainfall and precipitation. AnantapuramDistrict of Andhra Pradesh is one of the more drought prone areas in India due low and erratic rainfall. Hence there is more need in evaluation and development of the plant varieties which are drought resistant. More in depth understanding physiological mechanisms, gene functions and biochemical interactions that are required to enhance plant performance under drought and the increased quality of production, environmental adaptability is needed to overcome drought stress. Among the genes that control the trait of drought resistance, transcription factors play a major role as they could alter the expression of many genes that are in down stream of the transcriptional start site. Many model systems such as *Arabidopsis thaliana*, *Oryza sativa* etc., have been extensively used to study transcription factors that study drought stress. There have been many transcription factors that regulate drought stress such as WRKY, MYB, NAC, GRAS etc., GRAS transcription factors or proteins are involved in alteration of various functions in the plants. The name GRAS was actually obtained from abbreviation of GAI, RGA and SCR proteins. Bolle, 2004; Hirsch and Oldroyd, 2009 suggested that these GRAS proteins could also function as transcription factors. The GRAS transcription factors comprises of SCR (Scarecrow)subfamily, SHR (Short Root) subfamily, Scarecrow-Like (SCL) subfamily, OG-NSP (Nodulation Signalling Pathway) subfamily, OG-LS (Lateral Suppressor), HAM (Hairy Meristem) subfamily, DELLA subfamily, PAT (Phytochrome A Signal Transduction) subfamily, DLT (Dwarf and Low-Tillering) subfamily, RAM (Reduced Arbuscular Mycorrhization) subfamily, RAD (Required for Arbuscule Development) subfamily, LISCL subfamily etc., Among these GRAS TFs, the SCL family has a considerable role in combating drought stress. The gene products of this SCL subfamily has been shown to be Gibberellin-Acid Insensitive (GAI) and the Repressor of GA1(RGA) loci (Peng et al, 1997; Silverstone et al., 1998). The SCL genes are predominantly expressed more in the root and some of them also have a tissue specific expression pattern. Besides they have a key role in physiology and development of higher plants (Pysh et al., 1999). In the current study, we aimed to screen the levels of SCL-1 gene under physiological stress. Further molecular characterization of the gene was also done.

Materials and methods

Plant material, Drought Stress Induction

Cluster bean seeds var RGC-1025 were procured from Regional Agricultural Research Station (RARS), Rekulakunta, Anantapuramu. The seeds were surface sterilized and grown in earthern pots with red soil and manure at 3:1 ratio, in two sets for 3 weeks in the green house (28°±2°C, Relative humidity 70 ± 5 % and natural photo period).One set of 3 week old plants were stressed by withholding water for 10 days. Another set of plants were with full irrigation and served as control. Leaf samples from both control and stressed plants were collected at 10 days for the further study.

Total RNA extraction& quantification

Plant leaf samples were ground to fine powder in Liquid Nitrogen andTotal RNA from the control and stress leaf samples was isolated using the Trizol Reagent according to the standard protocol. 100 mg of ground Leaf sample powder was added to 1 ml of Trizol reagent and mixed thoroughly. Then 0.2 ml of Chloroform was added and mixed vigorously, the samples were stabilised for 5 min at ambient. Then the tubes were centrifuged at 12,000g for 5 min at 4°C. The aqueous phase was collected and the lower organic and interphases were discarded. 2/3 volumes of isopropanol were added to the collected aqueous phase and the samples were centrifuged at 12,000g for 10 min at 4°C. The pellet was collectedand the supernatant was discarded. Then the pellet was washed with 70% ethanol by centrifugation at 9500g for 5 min at 4°C. The pellet was briefly air dried and the pellet was dissolved in nuclease free sterile water. The sample was then quantified in a Nanodrop spectrophotometer.

cDNA synthesis

The total RNA with 260/280 ratio ~2.0 was treated with DNAse to remove any contamination of DNA using Turbo DNAse treatment kit as per the manufacturer's instructions. Further 1µg of RNA was used to synthesize the cDNA with oligo dT priming treated RNA using Revert aid Reverse transcriptase (Thermoscientific, USA) according to the manufacturer's instructions.

qPCR analysis of the expression of SCL-1 gene under drought stress

qPCR analysis of the expression of SCL-1 gene in control plant and plants under drought stress was done using the Power green SYBR Green kit (Applied Biosystems, USA) in a Step one real time PCR machine (Applied Biosystems, USA).A primer pair used for the qPCR was designed using Primer express tool (Applied Biosystems, USA). The primers were synthesised at Bioserve Biotechnologies Pvt Ltd., Hyderabad.The master mix comprised of 1X Power SYBR Green master mix, 0.2 µM of each primer (forward and reverse), ~20 ng of cDNA and nuclease free water in a 20 µl reaction. The standard cycling conditions of the Step one real time PCR machine were applied i.e., 95°C for 20 sec followed by 40 cycles of 95°C for 1 sec and 60°C for 20 sec. The Ct values of the corresponding samples were considered for the analysis. Melt curve analysis was also performed to evaluate the presence of single species amplicon.

RT-PCR amplification and Sequencing of the SCL-1 gene

Primer pair was designed from the SCL-1 of the Glycine max available in the Genbank database. ~20 ng of cDNA was used to amplify ~ 1.8 kb fragment. PCR mix comprises 1X PCR buffer, 0.2 mM dNTPs, 0.2 µM of each primer (forward and reverse), 1.5mM MgCl2, ~20 ng of cDNA and 2.5 units of Taq DNA polymerase (Thermoscientific, USA). Primers include SCL-1F and SCL-1R.Thermal cycling conditions comprises of 94°C for 4 min followed by 35 cycles of 94°C for 45 sec; 60°C for 30 sec and 72°C for 2 min and a final extension of 72°C for 10 min. The DNA amplicon was gel purified, cloned and sequenced at Bioserve Biotechnologies Pvt Ltd., Hyderabad.

Sequence, analysis of the SCL-1 gene and 3D structure modelling of the SCL-1

The sequence reads were aligned using the CAP contig assembly program of the Bioedit V. 7.0.5.3 package. Blast analysis was done using NCBI blast tool (https://blast.ncbi.nlm.nih.gov). Phylogenetic tree construction was done using Clustal X2 V. 2.1 package. 3D structure of SCL-1 was modelled using Phyre server2 V 2.0 (http://www.sbg.bio.ic.ac.uk/~phyre2.html). Domain recognition was done using Interpro scan (https://www.ebi.ac.uk/interpro/search/sequence/). Validation of the structure was done using Procheck tool online(https://servicesn.mbi.ucla.edu/PROCHECK/). Structural overlap was done using the magic fit algorithm of Swiss PDB viewer.

Results and Discussion

qPCR analysis of the expression of SCL-1 gene under drought stress

qPCR analysis of the study showed in the upregulation of SCL-1 gene during the drought stress revealing the role of SCL-1 gene during drought stress. The results of fold change in expression of SCL-1 transcripts both in control and drought stressed cluster bean samples is represented in the Figure 1.

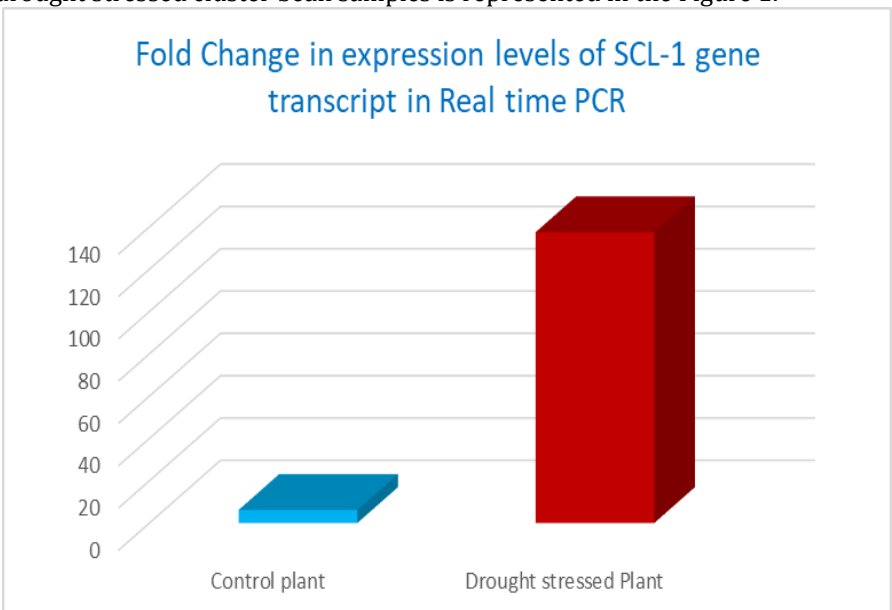

Figure 1Relative expression of SCL-1 gene transcript in Real time PCR, percent fold change is represented in control and drought stressed plants

RT-PCR amplification of the SCL-1 gene in cluster bean

RT-PCR amplification of the SCL-1 gene resulted in ~1.8Kbp amplicon in PCR (Figure 2). The product was sequenced,and the sequence was analysed for the characteristic domain of the GRAS transcription factors.

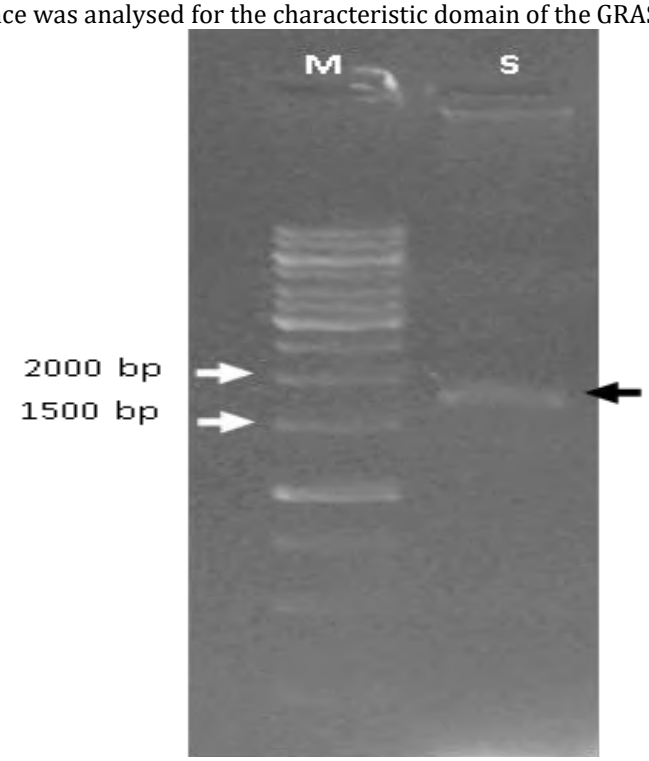

Figure 2~1.8 Kbp size amplification of the SCL-1 gene in RT-PCR

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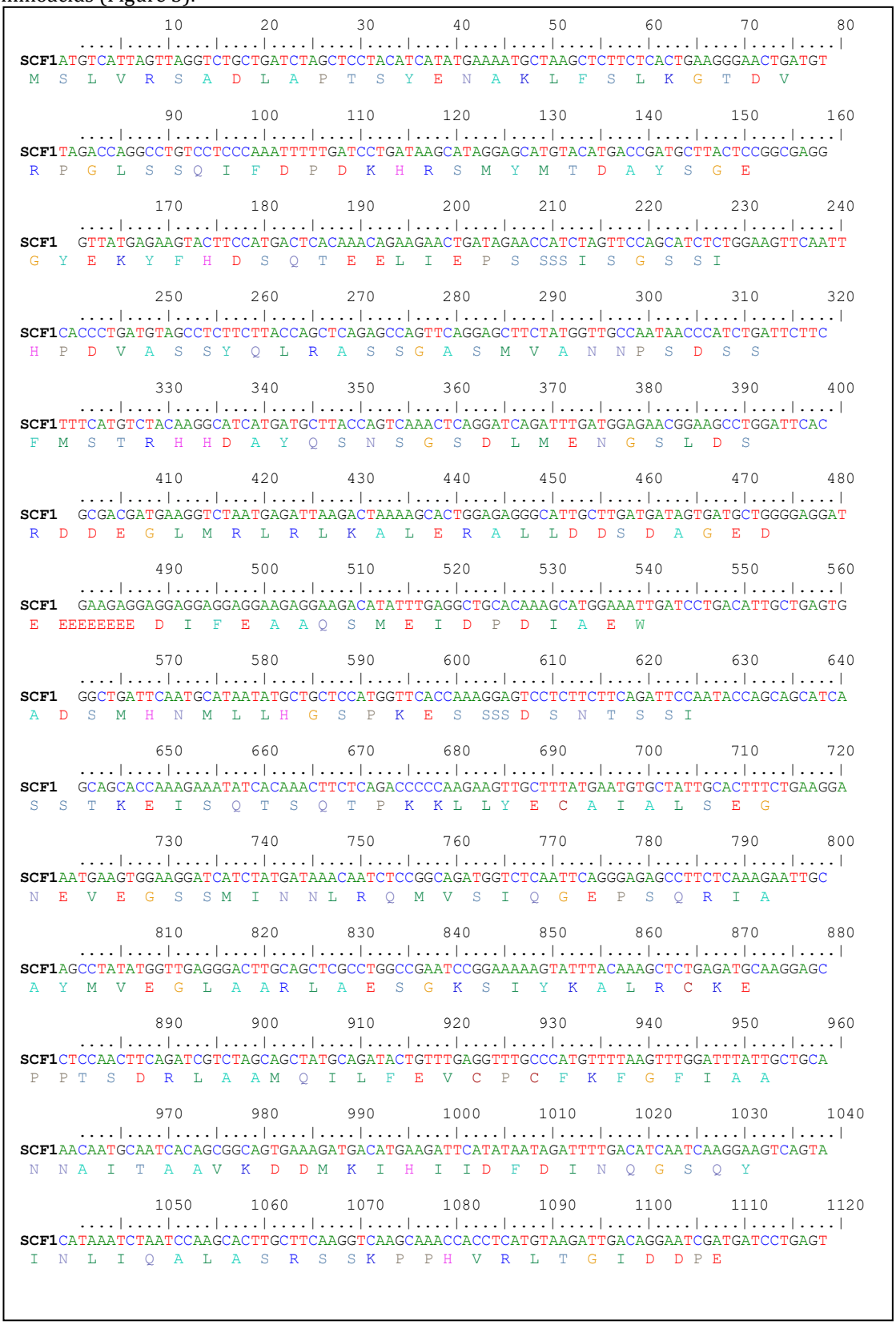
Sequence analysis of the SCL-1 gene and its product

Sequencing of the SCL-1 gene revealed 1800 bp nucleic acid coding sequence and a protein sequence of 599aminoacids (Figure 3).



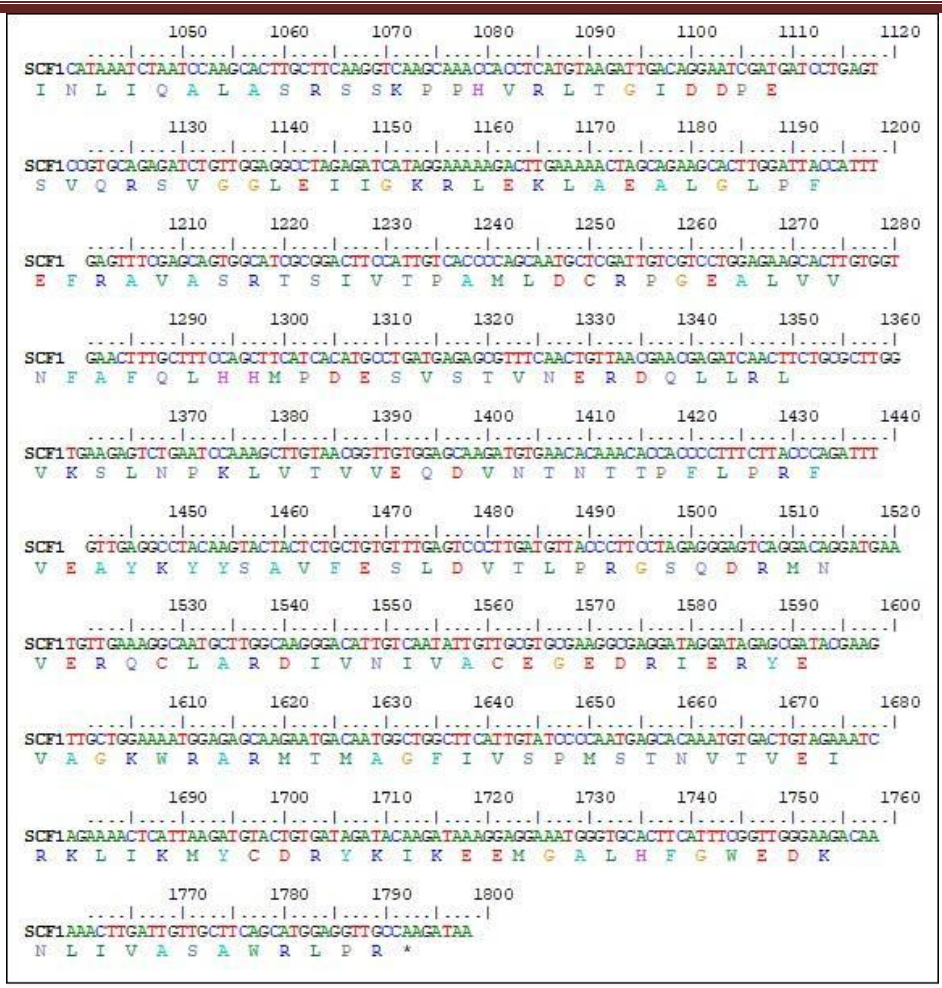


Figure 3 Sequence of SCL-1 gene and deduced Aminoacid Sequence

Database homology search using BLASTn tool (http://www.ncbi.nlm.nih.gov/BLAST) has showed that the SCL-1 gene has a high level of homology with *Glycine max* and *Glycine soja*at nucleic acid level (~95%) and amino acid level (~97%). Analysis of the conserved domains of the sequence with Interpro scan(https://www.ebi.ac.uk/interpro/search/sequence/)revealed the presence of GRAS domains (Figure 4) and Prosite scan(https://prosite.expasy.org/scanprosite/)revealed the presence of GRAS domain which is a characteristic domain of SCL-1 protein (Figure 5). Phylogenetic analysis of the SCL-1 protein with the other SCL like proteins of various plant genera (Figure 6).

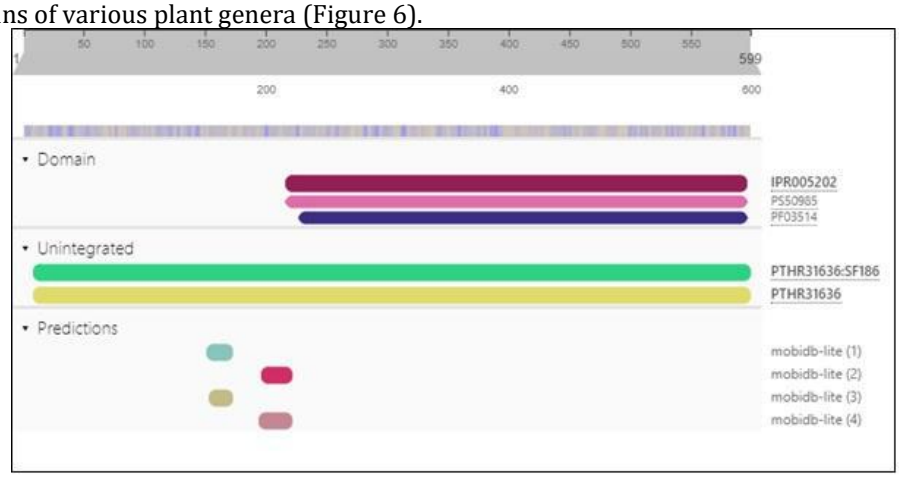


Figure 4GRAS Interpro domains identified in SCL-1 Protein Sequence

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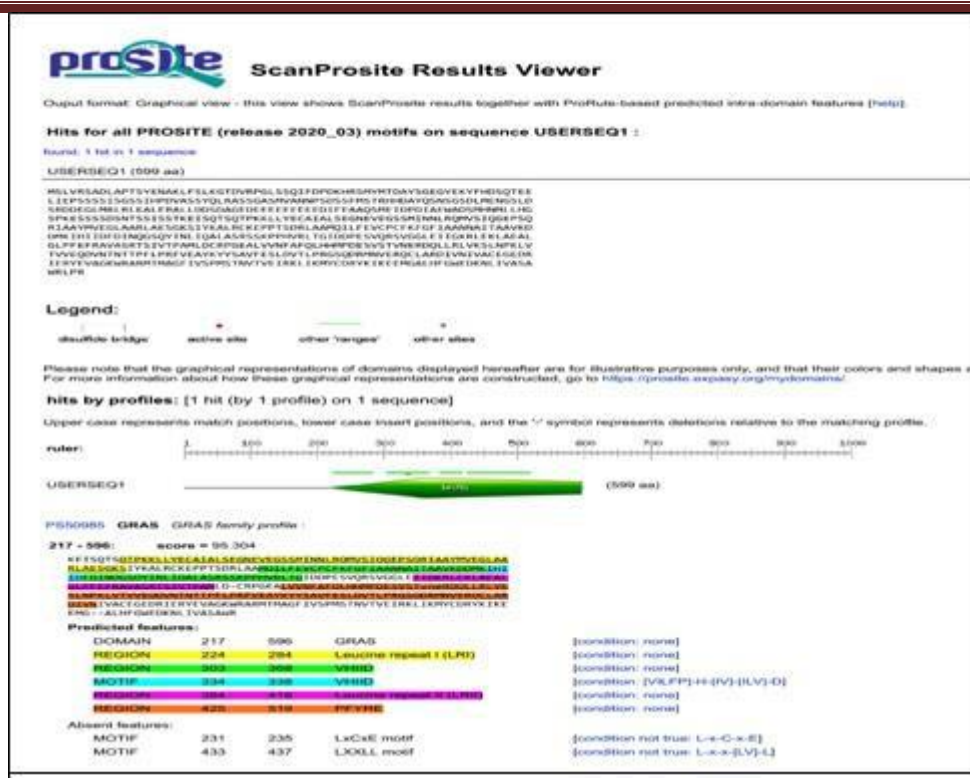

Figure 5Prosite motifs identified in SCL-1 Protein Sequence

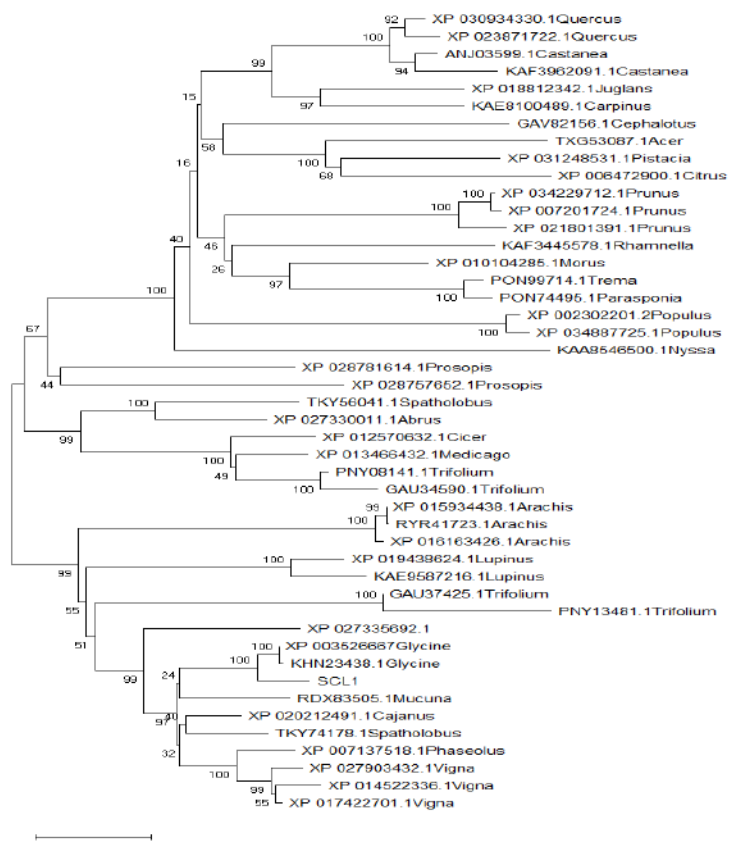

Figure 6 Phylogenetic tree showing the homology of SCL-1 Protein with SCL proteins of other plants

Modelling of the 3D structure of SCL-1 protein

Inorder to evaluate the structural conservation of SCL-1 protein, 3D structures of SCL-1 of the study and SCL-1 from Glycine max were predicted by Phyre server. The native structure of the SCL-1 protein is represented in Fig. 7. The CA trace overlap done using SPDB viewer tool magic fit algorithm is represented in Fig. 8 which shows that the SCL protein of study was structurally conserved with SCL protein from *Glycine max* with 0.04A RMSD. The integrity of the predicted structures are verified using Procheck analysis (Fig 9) and Verify 3D analysis (Fig 10). The procheck results show that 90% of the residues are in the core region and almost all the residues are above threshold line (0) of the verify 3D graph generated revealing that the predicted structure is very consistent.

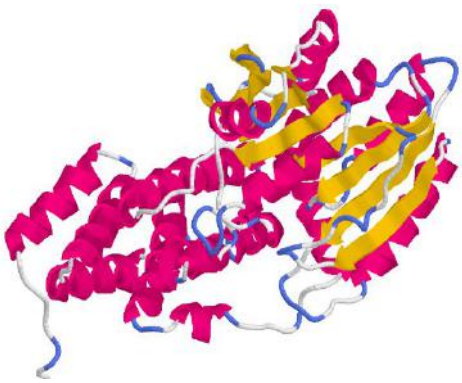

Figure 7Predicted3D structure of SCL-1 protein from Cyamopsistetragonaloba

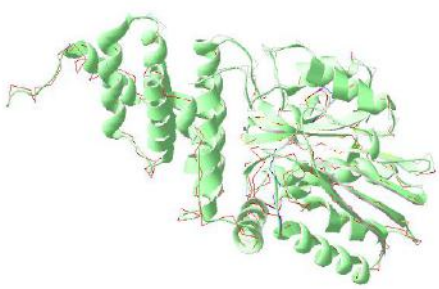

Figure 8Structure overlap of the SCL-1 protein of the study (green ribbon) and the wired frame structures of the SCL protein from Glycine max (Red)

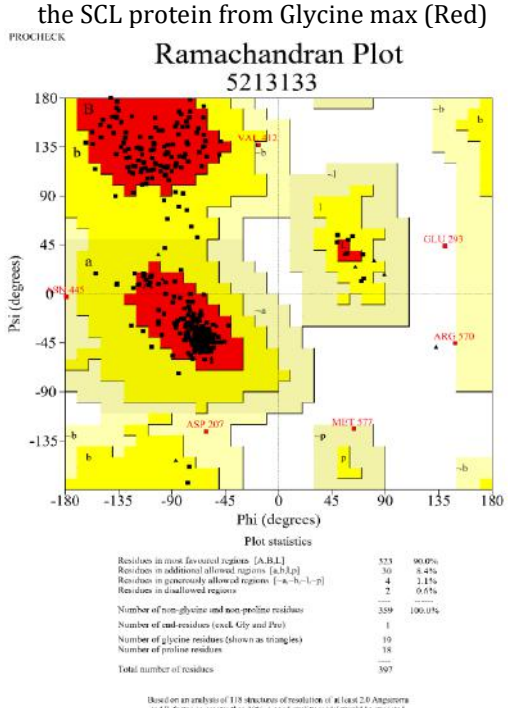

Figure 9Procheck based Ramachandran plot forPredicted3D structure of SCL-1 protein from Cluster bean

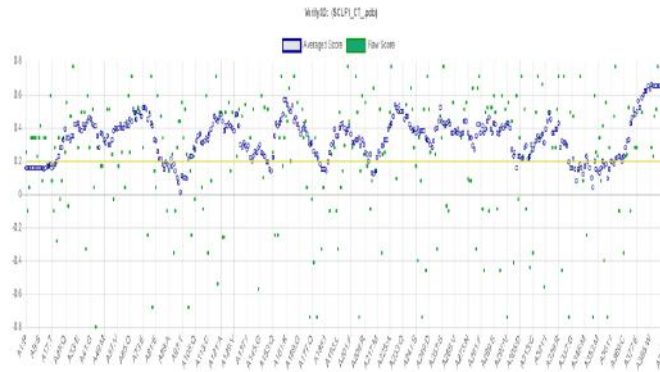

Figure 10 Verify 3D Plot forPredicted3D structure of SCL-1 protein from Cluster bean

Interaction Pathway Database Search

To identify the interactions of SCL-1 protein with other gene products at the cellular level, we used a String database search (https://string-db.org/) using Arabidopsis as a model plant for the evaluation. The search results showed that SCL-1 protein (Fig 11) interacted with 10 genes involved in cellular division and transcriptional regulation that include 1) HD1 Encodes a histone deacetylase that enhances AtERF7-mediated transcriptional repression 2) AT1G55290 which encodes 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein; 2-oxoglutarate (OG)- and Fe(II)-dependent dioxygenase (2OGD)involved in scopoletin biosynthesis 3) AT4G02725 which encodes a Spindle pole body-associated protein 4) AT5G53990 which encodesUDP-Glycosyltransferase superfamily protein with transferase activity, transferring glycosyl groups involved in metabolic process and expressed in root 5) BPC1 6) BPC3 and 7) BPC2 (Protein BASIC PENTACYSTEINE1, 2, 3; Transcriptional regulator that specifically binds to GA-rich elements (GAGA-repeats) present in regulatory sequences of genes involved in developmental processes. 8) E2F1 9) E2F3 proteins from class of E2F transcriptional factors expressed throughout the cell cycleandtheir abundance increased by auxin through stabilization of the protein and finally MYB17 transcription factor that may play a role in flower development by repressing ANT which also known to be a stress responsive transcription factor (Chen et al., 2014). These results states that SCL-1 protein of the study has regulatory role in cell division and developmental processes during the stress and could also help the plant to combat abiotic stress.

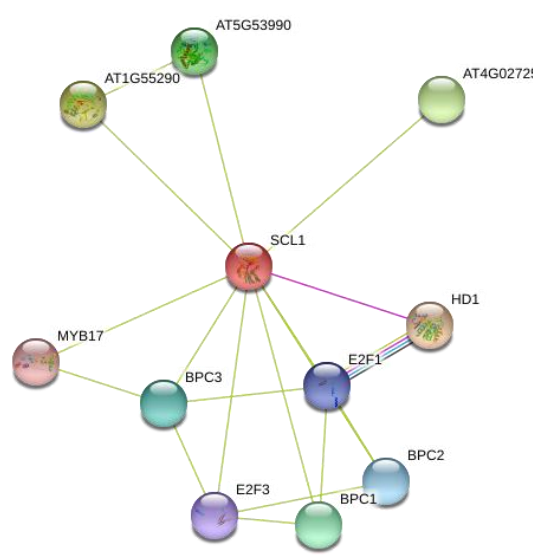

Figure 11String database search results of SCL-1 protein from Cluster bean

A similar interaction database search was done for the interactions of SCL-1 protein using BioGRID database (https://thebiogrid.org/). The search resulted in interaction of SCL-1 protein with HD1 as it was in the string database search followed by interaction with SWI3B which is CHROMATIN REMODELING

COMPLEX SUBUNIT B. ATPases alter histone-DNA interactions that remodel chromatin utilizing ATP non covalently promotes changes in nucleosome position, occupancy and composition. Among these, there are four subfamilies that are well known among which SWI/SNF chromatin remodellers are implicated in water stress responses in plants. In Arabidopsis, it has been demonstrated that SWI family of proteins has been linked to ABA and drought responses (Han et al., 2012; Umezawa et al., 2013; Wang et al., 2013).

To conclude, the Scarecrow like protein -1 isolated and characterized from *Cyamopsistetragonaloba*(L.)(Ct-SCL1) has been found to have a regulatory role in drought stress by regulating and interacting with various proteins such as SWI3B which can induce alterations in the chromatin organization and/or in the activity of chromatin remodelling and modifying enzymes may furthermore contribute to stress based epigenetic memory. This stress induced memory in the plant could make the variety to tolerate the drought stress in the further generations of the variety.

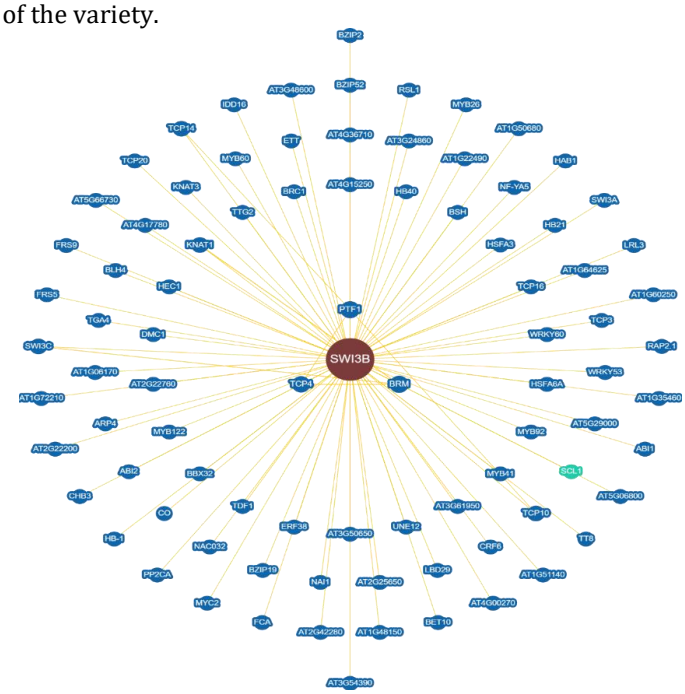

Figure 12BioGRID database search results of SCL-1 with SWI3B gene product/protein

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