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ABSTRACT: *Heat shock proteins play a significant role in response to different weather conditions. In this study, hsp70 protein sequences from cultivar c273 of Triticum aestivum were used. The main purpose for doing this research would be to execute an in-silico evaluation and estimate the 3D structure of the hsp70 protein. The aliphatic index divulge 86.33 values refers that proteins were thermal stable. The calculated grand average hydropathy value is −0.8; this value shows better interaction of the hsp70 protein with water. A thorough bioinformatics investigation of the Hsp70 gene in c273 and five different species was therefore performed in this study. A phylogenetic study separates them into six subclades. In contrast to other species, Triticum turgidum and TRINITY_DN11241_c0_g1_i4 are unique. The 3D structure of the hsp70 was done by using Swiss model. The selected model`s results were evaluated using the Saves server. This research provide insight into Hsp70's Recent research has elucidated the processes behind the actions of Hsp70s and their co-chaperones, indicating new possibilities for modifying disease-related Hsp70 roles.*

KEYWORDS*: Wheat, Protein, Secondary Structure, Triticum aestivum, Three-dimensional Structure*

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1. INTRODUCTION

Crop production is significantly influenced by a wide range of environmental parameters, especially temperature, water, and light. The continual increase in greenhouse gases over the last few years has contributed to the ongoing rise in global temperature. Heat stress has become a significant factor that has a negative impact on crop cultivation and yield because it can increase life span by shortening the plant's development lifecycle (Kim et al., 2015; Kumar et al., 2017).

Wheat, the most cultivated crop worldwide, is affected by high temperatures, notably during pollination and grain-filling stages, which also have a detrimental impact on wheat growth and yield (Abhinandan et al., 2018) High temperatures cause significant changes in the physiology of plants, which have major impacts on the yield of crops (Jung et al., 2012; Lobell et al., 2011; Spiertz et al., 2006).Every 1 degree Celsius increase in temperature reduces wheat production by 6% globally(Asseng et al., 2015). Moreover, the increasing population needs more grain, so to satisfy the growing demand for wheat; it is significant to develop heat-tolerant varieties for the future. Plants produce a variety of stress-responsive proteins in response to elevated temperatures, including a family of proteins known as heat shock proteins (HSPs). It is crucial to thoroughly study HSPs because of the numerous roles they play in plants.

Mostly all species rely on a family of cellular proteins known as heat shock (or stress) proteins (HSPs) to defend them from stress stimuli and metabolic insults. Hsps has always been primarily expressed at high temperatures and other stresses. It is necessary required for cell survival (Maio et al.,1999; Lindquist et al., 1988).

The 70 heat shock proteins (hsp70) are one of the important HSPs present in the multigene family and occur in both eukaryotes and prokaryotes. Hsp70s is one of the most prevalent and well-researched families of conserved proteins (Lin et al., 2001; Boorstein et al., 1994).

The Hsp70 is embedded with two genes: the heat shock cognate (hsc) 70 gene, which is closely correlated with physiological mechanisms, and a stress-inducible gene. Hsp70 plays a role in protein function as a molecular chaperone in numerous cellular processes, including translocation, denaturation, assembly, transport through membrane channels, and folding (Mayer et al., 2005; Marshall et al., 1990).

Hsp70s play important roles in development and respond to environmental stresses such as high temperature, dehydration, injury, cold, heavy metals, oxidative stress, and others (Heikkila et al., 1984; Dhankher et al., 1997; Chong et al.,1998; Uenishi et al.,2006). Moreover, Hsp70 is also significant during pathological processes. Furthermore, Hsp70 expression in plants is induced by pathogen infection, and plant-pathogen interactions are rare (Aranda et al.,1996). According to studies, expression of Hsp70 has been demonstrated to be positively connected with the development of heat tolerance as well as resistance to drought and salt stress. The fundamental methodologies of hsp70, which mediates many times elevated temperature tolerance, and the function of heat shock protein-70 under stressful conditions are still unknown. In order to fully comprehend the classification of the heat shock protein 70 family in a plant species, it is required to understand how hsp-70s react to cell function and retention against environmental stresses. This is caused by the variable regulation of the plant heat shock protein 70 gene family and the expected diversity of its members. There is currently no experimental structure or three-dimensional structure of the hsp70 protein in *Triticum aestivum* available. The main disadvantages of the experimental method used to characterise proteins were its high cost and time consumption. As a result, these methods were incompatible with high-throughput techniques. These issues can be solved using in-silico approaches. Researchers can use computational tools to learn about the physicochemical and structural properties of proteins. So evaluating HSP70 in wheat cultivar c273 becomes critical to analysing the putative mechanisms of its functioning in *Triticum aestivum and* its interactions with other molecules during heat stress conditions. In the present study, hsp70 protein sequences from cultivar c273 of *Triticum aestivum* were modelled using in silico analysis, which included describing the gene ontology, conserved motifs, physiochemical characteristics of crafted secondary structure, homology modelling of hsp70 proteins' 3-D structure, and validating the model's structure using computational tools.

2. METHODS

2.1 Plant Material and RNA Isolation

The seeds of Triticum aestivum cv. c273 collected from Punjab Agricultural University,Ludhiana. Isolated total RNA from wheat plant tissues using the RNeasy Plant Mini Kit (Qiagen, Germany). Assessed RNA quality and quantity using Nanodrop 1000 (Thermo Fisher Scientific). Constructed RNA libraries with the TruSeq method. Performed paired-end sequencing on the Illumina HiSeq 2000 platform. Assembled the transcript for further insights. The transcriptomic data of the cv. c273 *Triticum aestivum* were submitted in NCBI database with Accession No. SRX15502755 (https://www.ncbi.nlm.nih.gov/sra/SRX15502755).

2.2 Identification of Heat shock protein (hsp 70)

The transcripts were assembled *de novo* with Trinity (v. 2.7.0) after removing the adaptors and low-quality sequences using Trimmomatic (v. 0.39) (Bolger et al., 2014).Assembled transcripts were converted to proteins using TransDecoder (v. 5.5.0) with default settings (Brian et al., 2013). In order to predict gene ontology concepts for hsp70, the retrieved protein sequences were used as a query in a BLAST search against the NCBI NR database using Blast2Go (Conesa et al., 2008). Sequences shorter than 200 base pairs were eliminated, redundant sequences were manually removed and sorted from the HSP70 proteins, and the remaining sequences were then evaluated using the PfamScan database, and MEME (the motif-based sequence analysis program) was used to identify the motifs of the synthesised Hsp70 proteins (Bailey et al., 2009). The protein sequences were used for further analysis.

2.3 Physiochemical properties

The physical and chemical properties of the hsp70 proteins, such as theoretical pI, aliphatic index, instability index, molecular weight, and GRAVY (grand average of hydropathy), were calculated by the tool ProtParam (http://web.expasy.org/protparam/).

2.4 Sequence alignment, phylogenetic analyses

Protein sequences of cv. c273 Hsp70s and hsp70 from *Arabidopsis thaliana*, rice (*Oryza indica*), barley (*Horvu morex*),durum wheat (*Triticum turgidum*), and wheat *Triticum urartu* sequences were aligned using the MUSCLE program(Edgar, 2004) in MEGAX (Kumar et al.,2018).Aligned sequences were used to construct the phylogenetic tree using the Maximum Likelihood (ML) method whose parameters including Poisson correction, pairwise deletion, 1000 bootstrap replicates were used in the process analysis (Kumar et al.,2008).Finally, The phylogenetic tree was visualized by iTOL (http://itol.embl.de)(Letunic et al.,2007).

2.5 Secondary structure estimation

The secondary structural essential features of the chosen hsp70 protein sequences taken into consideration, such as the alpha helix, extended strand, and beta turn of selected amino acid sequences, were to be calculated using SOPMA, a self-optimized prediction technique with alignments (Geourjon et al., 1995).

2.6 Homology protein modelling and evaluation of models

The 3D protein structure was modelled using a Swiss model. Ramachandran plot analysis had been used to evaluate the protein's overall stereochemical characteristics. Structure model validation obtained from the SAVES server v. 6.0 (performed by using PROCHECK, ERRAT (Colovos et al.,1993) and VERIFY3D (Bowie et al., 1991) was used to analyse the quality of the selected models.

3. RESULTS

3.1 Identification of Heat shock protein (hsp 70)

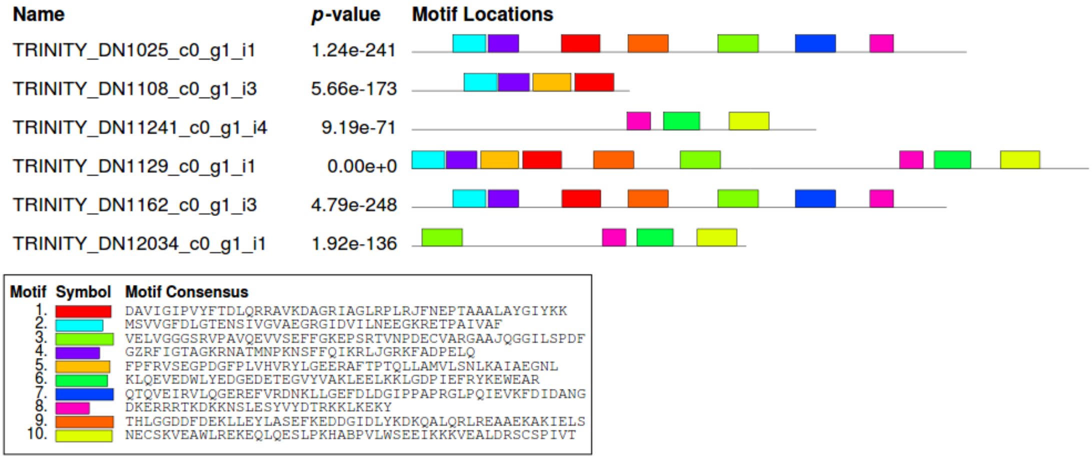
In this study, protein sequences were retrieved from Transdecoder, and the best hits and homologs were identified, in which query sequence TRINITY_DN1108_c0_g1_i3 shows a lower e-value that is more significant among other sequences in blastp and was further used for GO enrichment analysis to identify a total of 38 different heat shock proteins, like hsp90, hsp70, and hsp100. After removing the redundant sequences, sequences shorter than 200 bp yielded six distinct hsp70 protein ids. The maximum number of amino acids (842) is present in TRINITY_DN1129_c0_g1_i1. Gene ontology shows all the sequences were helpful in protein folding and all belong to the same protein family identified by PfamScan (Table 1). Using the MEME suite, the protein sequence motifs of each hsp70 were determined. Ten motifs were the maximum allowed for each protein.

Table 1 Basic information of the selected protein sequences as query with blast e-value, gene ontology and protein family.

Query Sequences	*e-value*	*No of A.A*	*Gene ontology*	*Go term*	*Pfam*
TRINITY_DN1025_c0_g1_i1	0	691	ACT65562.1	GO:0005739	Family
TRINITY_DN1108_c0_g1_i3	4.10522E-146	271	EMS61926.1	GO:0009507	Family
TRINITY_DN11241_c0_g1_i4	0	503	XP_020146994.1	GO:0005634	Family
TRINITY_DN1129_c0_g1_i1	0	842	EMS52665.1	GO:0005634	Family
TRINITY_DN1162_c0_g1_i3	0	665	XP_020168136.1	GO:0005739	Family
TRINITY_DN12034_c0_g1_i1	0	416	XP_020176226.1	GO:0005634	Family

The lack of shared motifs in all HSPs indicates that the protein sequence motifs were conserved within subfamilies but not between them. The number of conserved motifs varied among the different HSP subfamilies. For instance, of the nine motifs predicted in the TRINITY_DN1129_c0_g1_i1 sequence of Hsp70s, only three motifs were shared by TRINITY_DN11241_c0_g1_i4. In contrast, the TRINITY DN1162 c0 g1 i3 and TRINITY DN1025 c0 g1 i1 showed seven predicted motifs. Trinity DN1108 c0 g1 i3 and Trinity DN12034 c0 g1 i1, both of which had four conserved motifs, are exceptions shown in Figure1.

Figure1. Identified motifs in the selected hsp70 proteins using MEME. Name of the sequences id, p-value and motif location are shown with each motif represented with unique colour.



Name	*p*-value	Motif Locations
TRINITY_DN1025_c0_g1_i1	1.24e-241	
TRINITY_DN1108_c0_g1_i3	5.66e-173	
TRINITY_DN11241_c0_g1_i4	9.19e-71	
TRINITY_DN1129_c0_g1_i1	0.00e+0	
TRINITY_DN1162_c0_g1_i3	4.79e-248	
TRINITY_DN12034_c0_g1_i1	1.92e-136	

Motif	Symbol	Motif Consensus
1.	🟥	DAVIGIPVYFTDLQRRAVKDAGRIAGLRPLRJFNEPTAAALAYGIYKK
2.	🟦	MSVVGFDLGTENSIVGVAEGRGIDVILNEEGKRETPAIVAF
3.	🟩	VELVGGGSRVPAVQEVVSEFFGKEPSRTVNPDECVARGAAJQGGILSPDF
4.	🟪	GZRFIGTAGKRNATMNPKNSFFQIKRLJGRKFADPELQ
5.	🟨	FPFRVSEGPDGFPLVHVRYLGEERAFTPTQLLAMVLSNLKAIAEGNL
6.	🟩	KLQEVEDWLYEDGEDETEGVYVAKLEELKKLGDPIEFRYKEWEAR
7.	🟦	QTQVEIRVLQGEREFVRDNKLLGEFDLDGIPPAPRGLPQIEVKFDIDANG
8.	🟪	DKERRRTKDKKNSLESYVYDTRKKLKEKY
9.	🟧	THLGGDDFDEKLLEYLASEFKEDDGIDLYKDKQALQRLREAAEKAKIELS
10.	🟨	NECSKVEAWLREKEQLQESLPKHABPVLWSEEIKKKVEALDRSCSPIVT

3.2 Physiochemical properties

Protparam is a tool being used to compute the parameters for physicochemical analysis characteristics for the hsp70 proteins shown in Table 2. The computed isoelectric point shows the value in TRINITY_DN1025_c0_g1_i1 was 5.0 and the value obtained from the instability index was 29.71. The aliphatic index for the TRINITY_DN1162_c0_g1_i3 protein sequence value was 85.70. GRAVY (grand average hydropathy) value was -0.801 for Trinity_DN11241_c0_g1_i4.

Table 2 Physiochemical properties using Protparam shows molecular weight, theoretical pI,Instability Index,Aliphatic index and GRAVY of the selected proteins sequences.

Sequence ID	***Molecular weight***	***Theoretical pI***	***Instability index***	***Aliphatic index***	***GRAVY***
TRINITY_DN1025_c0_g1_i1	73514.14	5.01	29.71	86.33	-0.268
TRINITY_DN1108_c0_g1_i3	29504.53	9.16	44.45	82.88	-0.203
TRINITY_DN11241_c0_g1_i4	56223.72	5.21	49.14	72.37	-0.801
TRINITY_DN1129_c0_g1_i1	93315.85	5.10	43.50	76.24	-0.470
TRINITY_DN1162_c0_g1_i3	71425.01	6.01	40.83	85.70	-0.293
TRINITY_DN12034_c0_g1_i1	47193.89	5.02	52.75	80.62	-0.651

3.3 Sequence alignment, phylogenetic analyses

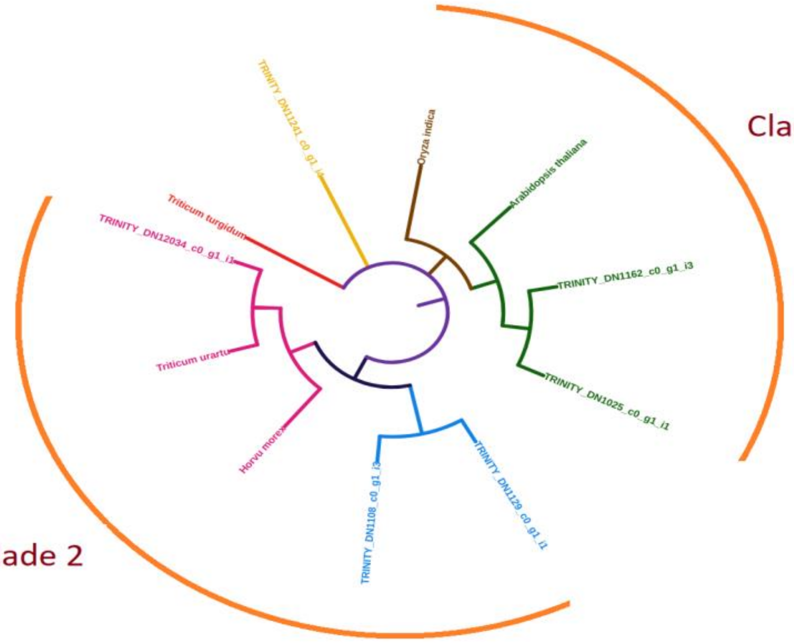
The study's findings about the Hsp70 protein sequences as well as those from *Arabidopsis thaliana*, *Oryza indica*, *Horvu morex*, *Triticum turgidum* and *Triticum urartu* sequences were utilized to create the ML phylogenetic tree: 6 from cv. C273 and 1 each from *A. thaliana*, *O. indica* , *H. morex* ,*T. turgidum* and *T. urartu* .Considering their similarity to Hsp70 proteinsin c273 and selected species the phylogenetic tree is separated into two clades as illustrated in Figure 2.

There are two subclades within Clade-I. TRINITY_DN1025_c0_g1_i1 and TRINITY_DN1162_c0_g1_i3 of the cv. c273 were displayed by Subclade-I exhibits strong similarities to Arabidopsis thaliana and also shows a connection to subclade-II, Oryza indica.

Clade II was split into two subclades. TRINITY_DN12034_c0_g1_i1 and Triticum urartu are closely related to Horvu morex in subclade-I. TRINITY_DN1108_c0_g1_i3 and TRINITY_DN1129_c0_g1_i1 are twining to one another, according to Subclade-II.

It is interesting to note that we discovered an entirely novel early independent branch of *Triticum turgidum*, and TRINITY_DN11241_c0_g1_i4 was located in the outgroup connected to the root. This branch's classification differed from that of the other subclades, indicating that it split off early in evolution.

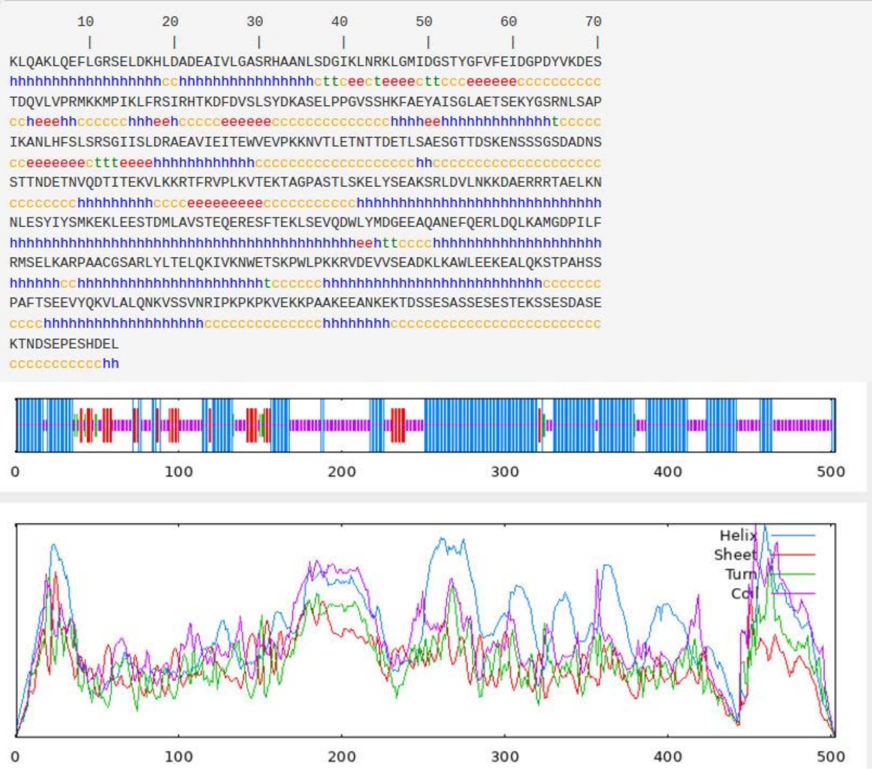
Figure2.Phylogenetic study of the crop cv.c273 and others.The hsp 70 proteins from the following species were used to build the phylogenetic tree: cv.c 273, *A. thaliana*, *O. indica*, *H. morex*, *T. turgidum*, and *T. urartu*.MEGA-X uses the maximum likelihood method to build the tree with bootstrap 1000 and visualized with ITol.



3.4 Secondary structure Estimation

The secondary structure of selected hsp70 protein sequences was evaluated using the Secondary Structure Prediction Method (SOPMA), which predicts the extended strand, alpha-helix, random coil, and beta-turn in Figure 3.

Figure 3 SOPMA analysis of TRINITY_DN1162_c0_g1_i3 (h – helix, e- sheet, t- turn, c- coil)



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        10        20        30        40        50        60        70
         |         |         |         |         |         |         |
KLQAKLQEFLGRSELDKHLDADEAIVLGASRHAANLSDGIKLNRKLGMIDGSTYGFVFEIDGPDYVKDES
hhhhhhhhhhhhhhhhhhcchhhhhhhhhhhhhhhhcttceecteeeecttccceeeeeecccccccccc
TDQVLVPRMKKMPIKLFRSIRHTKDFDVSLSYDKASELPPGVSSHKFAEYAISGLAETSEKYGSRNLSAP
ccheeehhcccccchhheehccccceeeeeeccccccccccccchhhheehhhhhhhhhhhhhtccccc
IKANLHFSLSRSGIISLDRAEAVIEITEWVEVPKKNVTLETNTTDETLSAESGTTDSKENSSSGSDADNS
cceeeeeeeclllteeeehhhhhhhhhhhhccccccccccccccccccchhcccccccccccccccccccc
STTNDETNVQDTITEKVLKKRTFRVPLKVTEKTAGPASTLSKELYSEAKSRLDVLNKKDAERRRTAELKN
cccccccchhhhhhhhhcccceeeeeeeeeccccccccccchhhhhhhhhhhhhhhhhhhhhhhhhhhhh
NLESYIYSMKEKLEESTDMLAVSTEQERESFTEKLSEVQDWLYMDGEEAQANEFQERLDQLKAMGDPILF
hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhheehttcccchhhhhhhhhhhhhhhhhhhh
RMSELKARPAACGSARLYLTELQKIVKNWETSKPWLPKKRVDEVVSEADKLKAWLEEKEALQKSTPAHSS
hhhhhhcchhhhhhhhhhhhhhhhhhhhhhtcccccchhhhhhhhhhhhhhhhhhhhhhhhhhccccccc
PAFTSEEVYQKVLALQNKVSSVNRIPKPKPKVEKKPAAKEEANKEKTDSSESASSESESTEKSSESDASE
cccchhhhhhhhhhhhhhhhhhhccccccccccccchhhhhhhhccccccccccccccccccccccccc
KTNDSEPESHDEL
ccccccccccchh
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In the predicted secondary structure of hsp70 proteins, alpha helices showed 50.7% and 48.5% in TRINITY_DN11241_c0_g1_i4 and TRINITY_DN12034_c0_g1_i1, respectively. It is followed by extended strands 19.70, 18.99, and 18.82 with 7.52% and 6.23% in the beta turn, and random coils 41.09 and 40.59 (Table 3). The hsp70 proteins divulged the pervasive existence of helix and coiling, emphasising the hsp proteins' more compressed, firmly bonded, and transmembrane position.

Table 3. Secondary structure prediction by SOPMA

Sequence id	*Alpha-helix*	*Extended strand*	*Beta-turn*	*Random coil*
TRINITY_DN1025_c0_g1_i1	41.01%	18.99%	6.23%	33.77%
TRINITY_DN1108_c0_g1_i3	36.16%	18.82%	4.43%	40.59%
TRINITY_DN11241_c0_g1_i4	50.70%	9.34%	2.39%	37.57%
TRINITY_DN1129_c0_g1_i1	42.76%	12.83%	3.33%	41.09%
TRINITY_DN1162_c0_g1_i3	43.6%	19.70%	7.52%	29.3%
TRINITY_DN12034_c0_g1_i1	48.085	14.42%	3.61%	33.89%

3.5 Model building and Structure validation

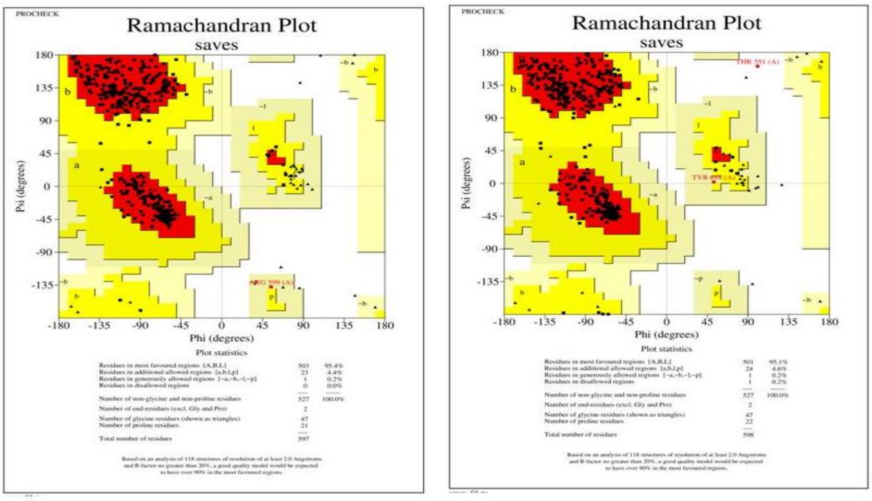
The Swiss model was used to generate six models for hsp70 sequences, with the best model in terms of stereochemical property as anticipated for sequences with greater identity to the template (Table 4).

Table 4. Swiss model analysis of all the selected proteins.

Sequence id	*Template*	*Seq Identity*	*Mol Probity Score*
TRINITY_DN1025_c0_g1_i1	5nro.1.A	54.61%	1.1
TRINITY_DN1108_c0_g1_i3	3d2e.1.A	39.81%	2.25
TRINITY_DN11241_c0_g1_i4	3d2e.1.A	20.66%	2.11
TRINITY_DN1129_c0_g1_i1	3d2e.1.A	38.41%	2.18
TRINITY_DN1162_c0_g1_i3	7ko2.3.A	60.54%	0.62
TRINITY_DN12034_c0_g1_i1	3c7n.1.A	32.93%	2.13

The TRINITY_DN1162_c0_g1_i3 model in the Ramachandran plot showed zero residues in the outlier region as well as 95.98% of residues being present in the favoured region (Figure. 4).

Figure 4. Ramachandran plot analysis of the two best models.



TRINITY_DN1162_c0_g1_i3 **TRINITY_DN1025_c0_g1_i1**

In Ramachandran's plot using PROCHECK, TRINITY_DN1025_c0_g1_i1 had 95.10% of its residues in the favourable region, but 0.20% of its residues were in the unfavourable region (Figure.3). Additional assessment through the use of VERIFY3D and ERRAT servers revealed that both. TRINITY_DN1162_c0_g1_i3 and TRINITY_DN1025_c0_g1_i1 had a high overall quality for homology modeling shown in Table 5.

Table 5. SAVES server analysis shows the protein models evaluation

Sequence id	***ERRAT***	***VERIFY3D***	***Ramachandran plot***			
			core	***allow***	***gener***	***disall***
TRINITY_DN1025_c0_g1_i1	94.9477	91.81%	95.10%	4.60%	0.20%	0.20%
TRINITY_DN1108_c0_g1_i3	88.024	83.50%	89.9%	8.9%	0.6%	0.6%
TRINITY_DN11241_c0_g1_i4	82.243	58.68%	82.9%	12.6%	3.9%	0.6%
TRINITY_DN1129_c0_g1_i1	86.2481	88.30%	88.2%	10.0%	1.6%	0.2%
TRINITY_DN1162_c0_g1_i3	96.8696	95.98%	95.4%	4.4%	0.2%	0.0%
TRINITY_DN12034_c0_g1_i1	88.1295	53.54%	87.8%	10.8%	0.3%	1.0%

4. DISCUSSIONS

Hsp70 is crucial for plant growth, acting primarily in response to stressful situations (Sunget al.,2001). In addition to rice, Arabidopsis, common beans, soybeans, and tobacco have all undergone Hsp70 gene family analysis (Jung et al., 2013; Sarkar et al., 2013; Lin et al.,2001). We thoroughly examined the Hsp70 sequences in Triticum aestivum c273, including gene ontology, conserved motifs, physiochemical properties of crafted secondary structure, homology modelling of the 3-D structure of the Hsp70 proteins, and we laid the groundwork for future screening of key genes for wheat stress response.

The 113 Hsp70 genes in c273 wheat found in this study were comparable to the findings of Lai et al. and Kumar et al., showing that the study's findings were accurate(Kumar et al., 2020; Lai et al.,2021).This may be due to the heterohexaploid nature of wheat, which was created by crossing three diploid ancestors from the A, B, and D subgroups twice recently (Glover et al.,2015).The hsp70 protein sequences identified by gene ontology were then analysed for physiochemical properties using the ProtParam tool. The isoelectric point was calculated for all the protein ids and was less than 7 (pI<7). This reveals that all the proteins were to be acidic, andto create a buffer system to do purification using the isoelectric method, the deliberate isoelectric point will be useful. The instability index calculates the stability of the proteins. Proteins with low instability index values (II <40) are predicted to be stable, whereas proteins with high values (II > 40) are predicted to be unstable (Guruprasad et al.,1990). The II (instability index) value for TRINITY_DN1025_c0_g1_i1(29.71) was found to be less than 40, indicating that it was stable, whereas TRINITY_DN12034_c0_g1_i1 (52.75) had a higher instability index, showing that it was an unstable protein (Lee et al., 2004;Kimet al., 2011).The aliphatic index, which describes the proportion of total protein volume occupied by aliphatic side chains (A, V, I, and L), is assumed to be a significant indicator for globular protein heat stress tolerance. The aliphatic index value for the TRINITY_DN1025_c0_g1_i1value was found to be 86.33. The value predicts heat-tolerant proteins. GRAVY is calculated by dividing the total hydropathy of all the amino acids by the total number of residues in the protein sequence. The GRAVY indices of the TRINITY_DN11241_c0_g1_i4 protein were discovered to be -0.801, indicating. the potential for improved water interaction (Pramanik et al., 2017; Verma et al., 2016).

To examine the evolutionary connections between genes, phylogenetic trees are used. The 11 Hsp70 proteins from c273, A. thaliana, O. indica, H. morex, T. turgidum, and T. urartu were arranged into phylogenetic trees. The Hsp70 proteins of the above species were classified into two clades and six sub-clades. According to research on tobacco Hsp70, Triticum turgidum, and TRINITY_DN11241_c0_g1_i4, these are early outcomes of plant evolution (Song et al., 2019). A new early independent two-separate branch that deviates from the classification of the other subclades was also discovered, showing that the subclade split off early.

Secondary structure was calculated using SOPMA. The findings indicate that among secondary structure components, the alpha-helix predominated, followed by random coils, beta-turns, and an extended strand. These studies concur with Dhiman et.al., findings, according to which the above-mentioned amino acids predominate in proteins with healthy alpha-helical structures (Dhimanet al., 2021)Homology modelling is used to build 3D models of the selected proteins. The Swiss model tool was an integrated web-based modelling tool. Table 4 shows the template and sequence identity among them, with 60.54% seq identity in TRINITY_DN1162_c0_g1_i3.

The most precise computational method for producing accurate structural models was homology modeling. It was used for a wide range of biological benefits. The quality of model evaluation tools was used to predict the models' stability. Following classification, Ramachandran map initial estimates generated by the PROCHECK programme were used to assess the stereochemical properties of the fitted models as well as the protein model's accuracy. Figure 3 depicts the evaluation of the selected model produced with the Swiss model. The Trinity_DN1162_c0_g1_i3 model shows 95.98% of residues in favourable regions, and the Ramachandran plot shows the best models.

5. CONCLUSIONS

Wheat is a main cereal crop that is grown globally, and its production is likely to be impacted by heat stress in the coming decades. Heat shock proteins are a category of proteins that play a role in controlling and sustaining the responsive conformation of a wide range of other proteins for the survival of the cell; nevertheless, they also undergo significant conformational changes at high temperatures in order to function. Computational conformational observations are reliable methods for perceiving protein function. In the present study, hsp70, which plays an essential role in the heat stress tolerance of proteins, was selected for wheat. The molecular weight, isoelectric point, instability index, GRAVY index, and aliphatic index were used to calculate the physicochemical properties. Secondary structure prediction tells us that alpha-helixes were dominant among other elements, i.e., random coils, extended strands, and beta-turns. The 3D modelling structure of the selected hsp70 proteins was done by Swiss modellers. The model results were validated using the Saves server. In the field of plant genetic engineering, expressing this hsp70 proteins may offer a dependable method for shielding crops against stressors.

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