RESEARCH ARTICLE

Impact of Global Warming and Environmental Stress Conditions on Proteins in *Triticum aestivum* L.: A Computational Analysis

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ABSTRACT

Crop yield of *Triticum aestivum* L. (wheat), the leading cereal crop of India, deteriorates under environmental stress conditions of heat, drought, and associated stresses due to the expression of specific proteins. In the current study, we explored the structural and functional aspects of these stress proteins by establishing the 3D structure through homology modeling for the first time. Our results revealed the specific proteins get expressed under each stress, and most of their activity was regulated by their interactions, catalyzing their structural modification and complex formation. When analyzed with respect to each other, all these proteins provided common features which affect them at a particular time under a specific stress. The phylogenetic relationships offer insight into the differentiation process of proteins from each other during evolutionary processes and their respective structures during evolution. The present findings help in developing stress-tolerant varieties for crop improvement and increased production.

Keywords: Abiotic stress, Computational biology, Drought stress, Global warming, Heat stress, Protein.

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INTRODUCTION

Environmental pressures create various abiotic stress conditions in cultivated and wild plants. Climate change results in drastic impacts of stresses such as drought, salinity, toxicity and mineral deficiency which are encountered recurrently on crops under the influence these stress conditions. In most cases, the operative simultaneous stresses adversely influence crop production and diminish their yield. The deterioration in crop yield of the main cereal crop of India, *Triticum aestivum* L. (wheat), is found to occur under abiotic stress conditions due to high temperature (heat stress) and reduced water availability (drought stress), leading to associated stresses of high salinity, toxicity and mineral deficiency (Brini *et al*., 2007; Alessio *et al.,* 2009). Rise in temperature is, thus, the trigger point of all the successive stresses as heat stress results in loss of water uptake leading to drought stress, which in turn, lowers cellular osmotic levels creating osmotic stress. Hence, if a crop experiences one kind of stress, it automatically gives way to another and the cumulative impact results in the expression of such specific proteins which otherwise remain suppressed under normal conditions and cause reduction of crop yield (Campbell *et al*., 2001).

Abiotic stress due to climate change is, therefore, estimated as the most plausible cause of diminished biomass productivity of *Triticum aestivum* L. (wheat) in India as the chain of events that begin under heat stress conditions suppress the photosynthetic activity of chloroplasts, the most sensitive function of all cells, lowering water potential and loss of turgor pressure in the guard cells, with enhanced abcissic acid production and increased leaf fall. These drought affected crops also encounter soil resistance during root penetration following prolonged exposure of leaves to excessive excitation energy (Ding *et al*., 2009), photo reduction of oxygen and release of highly toxic reactive oxygen species (ROS) compounds such as super oxides and peroxides. Which cause chemical damage of the DNA and proteins which are lethal for cellular metabolism (Gutha and Reddy, 2008) resulting in reduced growth, reproduction and ultimate loss of overall biomass productivity (Alessio *et al*., 2009).

The present analysis was therefore made to study the impact of global warming and environmental stress conditions on major cereal crop of India, *Triticum aestivum* L. and provide information for developing genetically modified crop varieties resistant to differential stress conditions (Nieva *et al.,* 2005) through modification in corresponding protein expression.

MATERIALS AND METHODS

The biochemical pathways and complex response of plants exposed to global warming cause environmental stress conditions is analyzed through proteomic and genomic tools. In order to study the impact of abiotic stress conditions on the quantitative expression and activity status of proteins, the *in-silico* approach was adopted because the activity of many proteins is regulated by their interactions, which lead to the formation of complexes or catalyze structural modifications in them. Hence all proteins were analyzed with respect to each other to assess the common stress conditions at a particular time under a particular stress. For this, similarity and/or differences in protein structures were assessed and their evolutionary relationships were established to get an insight on their structural similarities/differences as well as the basis of differentiation from each other during evolution and, finally, the

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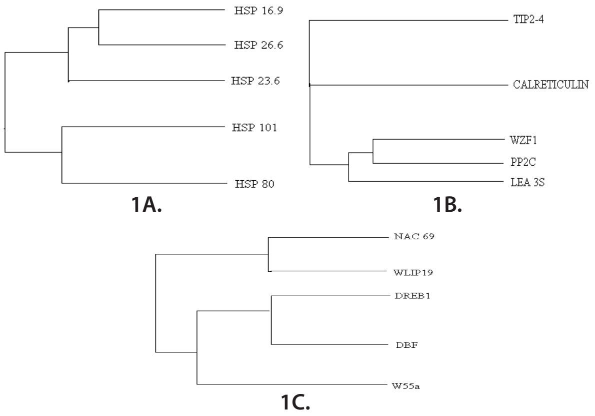
Table 1: Triticum aestivum L. Proteins expressed under different stress conditions.

S.No.	*Protein*	*Plant*	*Reference*
	HEAT STRESS PROTEIN		
1.	HSP16	Wheat	Krishan et al.(1989)
2.	HSP23	Wheat, maize	Yildiz and Terz. (2006)
3.	HSP26	Wheat	Krishan et al (1989)
4.	HSP80	Wheat	Guo et al.(2008)
5.	HSP101	Rice, Wheat	Campbell et al.(2001)
Drought stress protein			
6.	protein phosphatase 2C catalytic subunit gene (PP2C)	Rice, Wheat	Liu et al.(2008)
7.	aquaporin genes (TIP)	Rice, Wheat	Forrest and Bhave.(2008)
8.	Zinc Finger protein (WZF1)	Rice, Wheat	Huang et al.(2009)
9.	late embryogenesis abundant (LEA3S)	Wheat	Wang et al.(2009)
10.	CRT (calreticulin)	Wheat	Jia et al.(2008)
Successive stress protein			
11.	Serine-threonine protein kinase (W55a)	Wheat	Xu et al (2009)
12.	basic region/leucine zipper protein (LIP19)	Wheat	Kobayashi et al.(2008)
13.	NAC69	Wheat, Rice, Arabidopsis, Maize	Zheng et al.(2009)
14.	DRE binding factor (DBF)	Rice, Wheat, Arabidopsis	Saleh et al.(2006)
15.	DREB1B	Rice, Wheat	Gutha and Reddy.(2008)

Table 2: Secondary structure of proteins

Protein	*Alpha helix(%)*	*Beta sheet(%)*	*Extended coil(%)*
Heat stress			
HSP 16.9	28	21	49
HSP 23.6	27	22	50
HSP 26.6	42	11	46
HSP 80	51	14	35
HSP 101	63	9	29
Drought stress			
PP2C	31	20	47
CALRETICULIN	23	20	55
LEA 3S	73	6	20
TIP2-4	9	36	55
WZF1	37	10	52
Successive stress			
DBF	33	13	53
DREB1	37	13	49
NAC 69	13	21	66
W55a	26	24	48
WLIP19	52	6	42

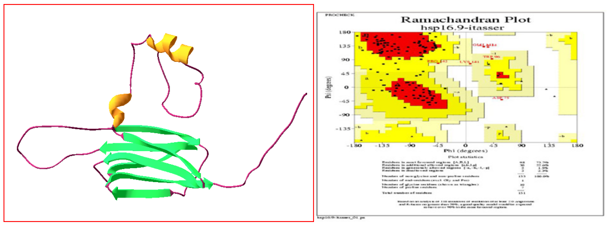
functional sites present in the sequence were located. During this analysis, the proteins affected by abiotic stress conditions were selected, analyzed in terms of their structure, functional site and evolutionary relationships and their 3D structure was established through homology modeling. The functional site of proteins was located through functional analysis using various servers and their structural variation was established through structural analysis.



Figs. 1A-C: Phylogenetic relationship of. A- heat stress proteins. B- drought stress proteins. C- successive stress proteins.

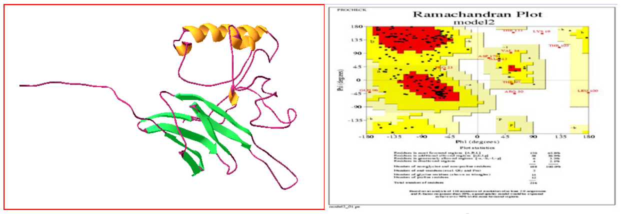
Nomenclature of proteins was based on the kind of stress reported earlier (Table 1) and their sequences were retrieved by Uniport (www.uniprot.org/) and NCBI (www.ncbi.nlm.nih.gov/). The dataset was divided in three subheads - heat stress (Fig. 2A-E), drought stress (Fig. 3A-E) and successive stress (Fig. 4A-E), which were aligned through Successive Sequence Alignment Tool-Clustal W2. Phylogenetic analysis was carried out through MEGA v.11 and Phylip v. 3.695 softwares and structure analysis was carried out by studying their secondary structure through GOR IV and Hierarchical Neural Network method (HNN) of which predicts the tertiary protein structure using the tools of homology modeling, including 1-metaserver I-Tasser (www.zhanggroup.org/I-TASSER/). Protein structures were visualized in the visualization tool and energy minimization was done by GROMOS96, in swiss pdb viewer (spdbv.unil.ch/content.html) followed by validation of structures by procheck (www.ebi.ac.uk/thornton-srv/software/PROCHECK/) (Table 3). For functional analysis, motif search, domain search, of cleavage

STRUCTURE OF HEAT STRESS PROTEINS (Figs. 2A-E)



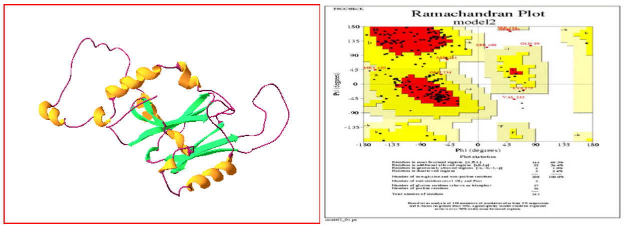
(a) (b)

Fig. 2A: HSP 16.9 a.Tertiary structure as seen in biodesigner. b. Ramachandran plot generated by Procheck.



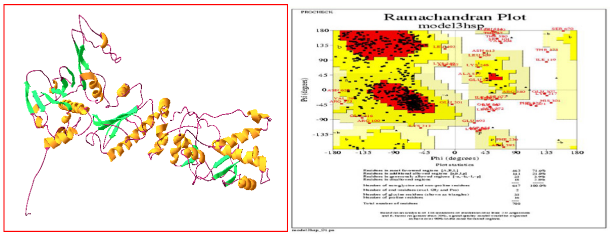
(a) (b)

Fig. 2B: HSP 23.6 a. Tertiary structure as seen in biodesigner. b. Ramachandran plot generated by Procheck.



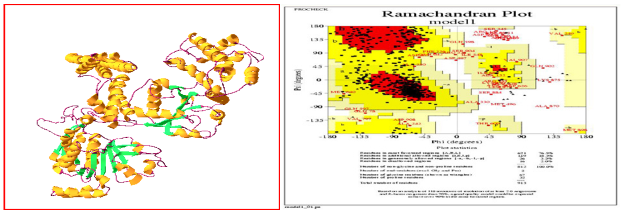
(a) (b)

Fig. 2C: HSP 26.6 a. Tertiary structure as seen in biodesigner. b. Ramachandran plot generated by Procheck.



(a) (b)

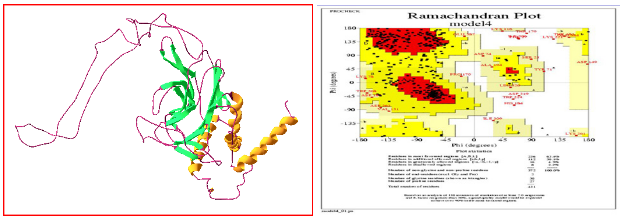
Fig. 2D: Hsp 80 a. Tertiary structure as seen in biodesigner. b. Ramachandran plot generated by Procheck.



(a) (b)

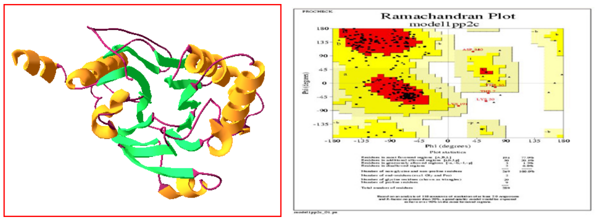
Fig. 2E: Hsp 101 a. Tertiary structure as seen in biodesigner. b. Ramachandran plot generated by Procheck.

STRUCTURE OF DROUGHT STRESS PROTEIN (Figs. 3A-E)



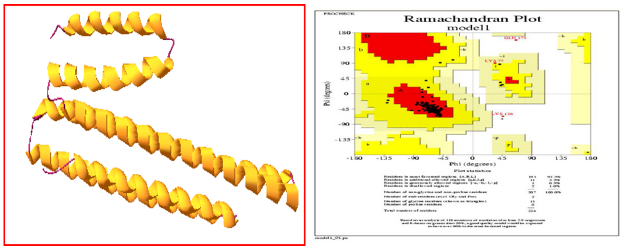
(a) (b)

Fig. 3A: CALRETICULIN a. Tertiary structure as seen in biodesigner. b. Ramachandran plot generated by Procheck.



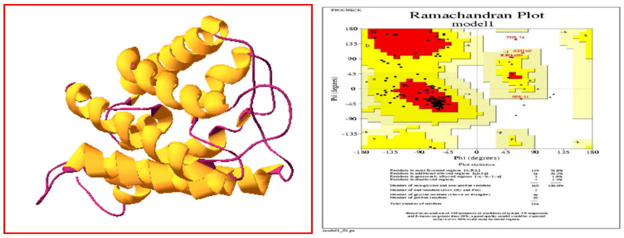
(a) (b)

Fig. 3B: PP2C a. Tertiary structure as seen in biodesigner. b. Ramachandran plot generated by Procheck.



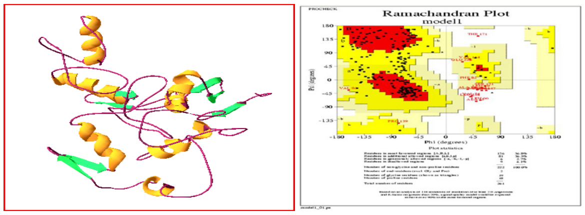
(a) (b)

Fig. 3C: LEA 3S a. Tertiary structure as seen in biodesigner. b. Ramachandran plot generated by Procheck.



(a) (b)

Fig. 3D: TIP2-4 a. Tertiary structure as seen in biodesigner. b. Ramachandran plot generated by Procheck.



(a) (b)

Fig. 3E: WZF1 a. Tertiary structure as seen in biodesigner. b. Ramachandran plot generated by Procheck.

Table 3: Energy value and Procheck result of proteins

PROTEIN	*ENERGY*	*RESIDUE IN MOST FAVOURED REGION(%)*	*RESIDUE IN ADDITIONAL ALLOWED REGION(%)*	*RESIDUE IN GENEROUSLY ALLOWED REGION(%)*	*RESIDUE IN DISALLOWED REGION(%)*
HEAT STRESS PROTEIN					
HSP 16.9	-4844.456	73.3	22.6	1.9	2.3
HSP 23.6	-4216.976	63.8	30.9	3.2	2.1
HSP 26.6	-11711.768	69.2	26.4	1.9	2.4
HSP 80	-27152.479	71.6	21.8	3.9	2.8
HSP 101	-47082.273	76.9	18.3	3.2	2
DROUGHT STRESS					
PP2C	-11616.073	77.9	22.1	1.2	0.8
CALRETICULIN	-18605.557	63.4	30.1	4.3	2.1
LEA 3S	-10951.218	93.2	5.3	0.9	1
TIP2-4	-4911.335	76.8	20.2	1.9	1.2
WZF1	-8056.391	56.8	36.9	2.7	4.1
SUCCESSIVE STRESS					
DBF	-7715.699	56.8	34.3	5.9	3
DREB1	-7973.487	50.8	38.2	7.1	3.8
NAC 69	-12202.6	55.4	39	3.3	2.3
W55a	-15589.788	71.6	22.4	2.3	3.6
WLIP19	-8467.758	89.9	8.9	0.8	0.8

Table 4: Location of the functional sites in proteins.

PROTEIN	*SERINE SITE*	*THEORINE*	*TYROSINE*	*ARG/LYS*
HEAT STRESS				
HSP 16.9	32, 36, 82, 103	23, 51, 86	--	101
HSP 23.6	31, 49, 51, 88, 95, 177	43	51, 58, 124, 181	113
HSP 26.6	15, 34, 43, 55, 95, 123, 154, 168, 213	119, 233	--	--
HSP 80	27, 51, 56, 101, 117, 140, 157, 220, 257, 303, 426, 440, 443, 449, 471, 516, 527, 568, 596, 602, 631, 650, 670, 694	139, 164, 165, 214, 215, 278, 327, 441, 595	26, 407, 465, 489, 493, 600	--
HSP 101	63, 77, 79, 100, 104, 108, 156, 158, 345, 410, 440, 477, 644, 674, 735, 769, 853, 904	30, 84, 89, 318, 546, 702	325, 360, 689, 655, 814, 847, 859	--
DROUGHT STRESS				
PP2C	5, 8, 9, 54, 97, 177, 183,	57, 305, 308	84, 90, 252, 269	--
CALRETICULIN	5, 57, 72, 92, 99, 207, 340	47, 82, 140, 155	123, 130, 211, 238, 250, 286, 300, 321, 353, 412, 416, 427	--
LEA 3S	9, 68, 102, 152	30, 37, 48, 59, 117, 132, 164, 204, 205, 213, 214, 220	97	--
TIP2-4	23, 110, 176	--	--	--
WZF1	32, 36, 49, 54, 118, 146, 151, 152, 163, 192, 193, 204, 208, 249	31, 110	102	51, 53
SUCCESSIVE STRESS				
DBF	9, 163, 192, 202, 218, 229, 231, 233, 234, 235, 236, 243	4, 144, 207, 211, 215	--	--
DREB1	6, 25, 30, 51, 133, 162, 168, 170, 173, 192, 212, 223	26, 34, 108, 213, 219	116, 238, 240, 266	--
NAC 69	37, 73, 153, 154, 176, 178, 195, 203, 206, 230, 232, 258, 259, 328, 329, 331	22, 141, 148, 190, 191, 299	51, 70, 79, 242, 276, 287, 294, 313, 320, 344	--
W55a	81, 96, 154, 158, 172, 218, 222, 230, 287, 334	--	4, 146, 165, 226, 272	--
WLIP19	5, 8, 9, 10, 12, 21, 23, 35, 39, 43	80	--	7

Table 5: Proteins with their functions.

Protein	Function
Heat stress	
HSP 16.9	alpha crystalline domain
HSP 23.6	alpha crystalline domain
HSP 26.6	alpha crystalline domain
HSP 80	activated and phosphorylated under thermal stress
HSP 101	show response in thermal stress
Drought stress	
PP2C	mutation in it causes growth defects
CALRETICULIN	storage of calcium in ER and SR
LEA 3S	no domain region found
TIP2-4	responsible for diffusion of water, amino acids and/or peptides from the tonoplast interior to the cytoplasm.
WZF1	zinc finger protein
Successive stress	
DBF	ethelene regulation
DREB1	ethelene regulation
NAC 69	involve in developmental processes and plant hormonal control and defence
W55a	ATP binding and contain aspartic acid residue responsible in catalytic activity of enzyme.
WLIP19	required for dimerization

sites prediction, transmembrane helix and prediction sub cellular location of protein was used. Functional sites were predicted by functional site prediction servers like Net Phos k (Xu *et al.,* 2007).

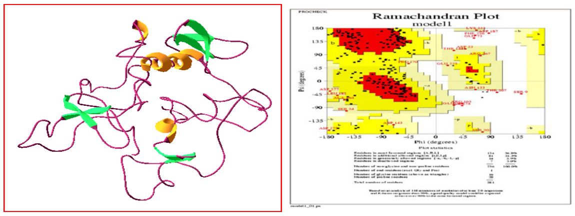
RESULT AND DISCUSSION

Computational analysis of proteins affected under environmental abiotic stress conditions in the major cereal crop of India, *Triticum aesticum* L., through homology modeling revealed that specific proteins get expressed under these stresses and majority of their activity were regulated by their interactions with each other, which catalyzes their structural modification and complex formation. When analyzed with respect to each other, all these proteins provided common features which affect them at a particular time under a particular stress (Torres, 2007). The detailed expression of their structure, including similarity and/or differences, functional sites and phylogenetic relationships (Kobayashi *et al.,* 2008), and their 3D structures (predicted for the first time) are provided for evolving methods of crop improvement and development of stress-tolerant varieties. The Secondary and tertiary structure of the proteins (Table 2 and 3; Figs. 2A-E) analyzed on the basis of all the tools and then refined by energy minimization with GROMOS96 in Swiss pdb viewer predicted the energy of various structures (Table 3) which provide a hypothetical structure of proteins and their percentage of sequence validation for each protein. The Functional sites of all proteins (Table 4) indicated that in heat stress protein, all small HSPs contain alpha crystalline domain, which is activated by high temperature, a factor that is responsible for heat stress tolerance. Similarly, the big HSPs contain a particular protein which gets activated under heat stress and show a response when the plant is under stress (Figs 2A-E). Further, in drought stress conditions, PP2C protein (Fig. 3B) helps in the growth of the plant and calreticulin (Fig. 3A) stores calcium in the smooth and rough endoplasmic reticulum, but no domain region was found in LEA 3S protein (Fig. 3C). TIP2-4 protein (Fig. 3D) was responsible for diffusion of water and amino acid in the cytoplasm while WZF1 (Fig. 3E) was a zinc finger protein (Jia *et al.,* 2008). Under the successive stress both DBF and DREB1 proteins (Figs. 4A-B) help in ethylene regulation (Matsukura, 2010) the NAC69 (Fig. 4C) helps in development processes of plant, W55a (Fig. 4D) catalyses activity of many enzymes as it contains aspartic acid residue and WLIP19 (Fig. 4E) is required for dimerization process (Table 5).

The predicted functional site of the proteins (Table 4) and their phylogenetic relationships provide insight on the differentiation process of proteins from each other during evolutionary processes and their respective structures during evolution. The predicted structure of the protein thus portrayed under differential stress conditions, is useful in analyzing the variations present in their respective structure and functional sites. The small heat shock proteins hsp 16.9 and hsp 26.6 were found to be interrelated and correlated with hsp 23.6. The other group of larger HSPs, hsp 101 was interrelated with hsp 80, forming a group of big heat shock proteins and the two groups. The small and big heat shock proteins were in turn phylogenetically interrelated (Fig. 1A). Similarly, under drought stress, the WZF1 proteins were interconnected with PP2C protein which in turn, were also correlated with LEA 3S protein. However, the TIP2-4 and Calreticulin protein were phylogenetically different from all other proteins but were interrelated (Fig. 1B) with each other. Furthermore, under successive stress, NAC 69 and WLIP protein were closely interrelated with each other, but DREB1 and DBF proteins which were interrelated with one another, were also related with W55a protein, and these two groups finally interrelated (Fig. 1C) with each other.

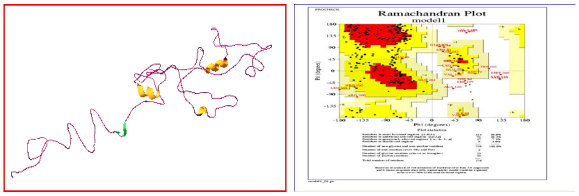
The computational analysis of proteins affected under environmental abiotic stress conditions in the major cereal crop of India, *Triticum aesticum* L., through homology modeling of thus, revealed that the environmental stress conditions, which are abiotic in nature, get triggered by elevated temperature levels resulting from global warming under climate change, leading to successive stresses causing drought conditions, loss of turgor pressure and leaf abscission which are responsible

STRUCTURE OF SUCESSIVE STRESS PROTEIN (Figs. 4A-E)



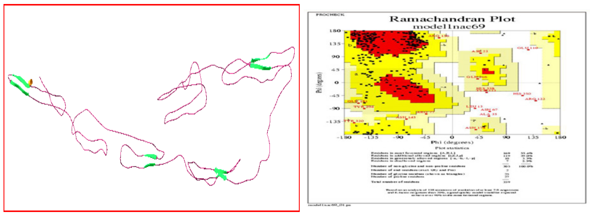
(a) (b)

Fig. 4A: DBF a. Tertiary structure as seen in biodesigner. b. Ramachandran plot generated by Procheck.



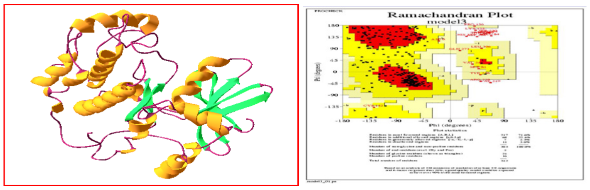
(a) (b)

Fig. 4B: DREB1 a. Tertiary structure as seen in biodesigner. b. Ramachandran plot generated by Procheck.



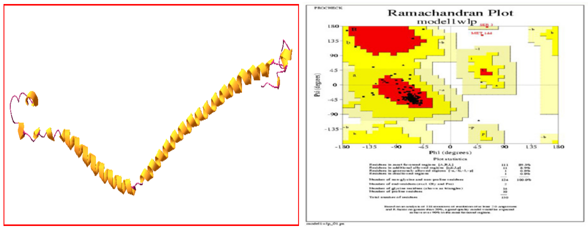
(a) (b)

Fig. 4C: NAC69 a. Tertiary structure as seen in biodesigner. b. Ramachandran plot generated by Procheck.



(a) (b)

Fig. 4D: W55a a. Tertiary structure as seen in biodesigner. b. Ramachandran plot generated by Procheck.



(a) (b)

Fig. 4E: WLIP19 a. Tertiary structure as seen in biodesigner. b. Ramachandran plot generated by Procheck.

for anomalies in plant growth responses, due to expression of specific proteins which otherwise remain dormant and thus, pose major threat to the crop due to diminished biomass production and crop yield.

CONCLUSION

Climate change with elevated temperature and humidity is responsible for operational stresses of drought, salinity, toxicity and mineral deficiency in plants, most deleterious for crop yield. Diminished crop yield of *Triticum aestivum* L. (wheat), the main cereal crop of India, under elevated temperature and associated stress conditions was found to be responsible for expression of such suppressed proteins, which get triggered under heat stress, resulting in loss of photosynthesis, water potential, turgor pressure and increased abcissic acid formation causing senescence, as well as soil resistance to root penetration. While elevated temperature activates HSPs, the diminished humidity activates PP2C protein (Fig. 3B). Calreticulin stores calcium in the smooth and rough endoplasmic reticulum, but no domain region occurs for LEA 3S protein (Fig. 3C). The TIP2-4 protein (Fig. 3D) was responsible for water and amino acid diffusion in cytoplasm while WZF1 (Fig. 3E) was a zinc finger protein. Under successive stresses both DBF and DREB1 proteins (Fig.4A-B) help in ethylene regulation, NAC69 (Fig. 4C) helps in development, W55a (Fig. 4D) catalyzes enzymatic activity and WLIP19 (Fig. 4E) is required for dimerization process (Table 5). The detailed structure, similarity and/or differences, functional sites, phylogenetic relationships and 3D structures (predicted for the first time) of specific proteins also revealed interrelationships among the small hsp 16.9, hsp 26.6 and hsp 23.6 and the bigger hsp 101, hsp 80 heat stress proteins (Fig.1A), between WZF1, PP2C and LEA 3S drought stress proteins and TIP2-4, Calreticulin, NAC 69, WLIP, the DREB1, DBF and W55a protein for successive stress proteins (Fig.1C). Expression of the specific proteins which otherwise remain dormant, pose threat to the crop, leading to diminished crop yield and simultaneous oxidative photo reduction, release of highly toxic reactive oxygen species (ROS) compounds which are lethal for cellular metabolism, result in loss of overall biomass productivity. The present findings are of wide scale applications in crop improvement through genetic engineering for development of resistant, stress tolerant and genetically modified crop varieties of cereal crops in India.

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