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Genome-wide Identification, Characterization and Expression Profiling of bZIP Gene Family in Wheat using Bioinformatics Approach

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ABSTRACT

Background: The basic leucine zipper (bZIP) represents one of the largest as well as most diverse transcription factor (TFs) families, and well-studied for critical role in stress response, growth and sustainable devel opment of the plant.

Methods: In the current study a total of 266 bZIP genes were identified in wheat genome using BLAST algorithm against the fully annotated reference genome (International Wheat Genome Sequencing Consortium - IWGSC) available in Ensembl Plants 42.0 using AtbZIP11 protein from *A. thaliana* as a query. Coding sequence (CDS) analysis, physicochemical property analysis, homology model ling, structure validation, subcellular localization, phylogenetic analysis and expression profiling has been done to determine bZIP TFs in *Triticum aestivum*. Overall quality factor of protein has been studied using ERRAT, while expression profiling has been done using Wheat Expression Browser powered by expVIP.

Result: Sequence analysis revealed that the coding sequence (CDS) length of deduced TabZIP TFs genes ranged from 422 to 3669 bp and corresponding protein length ranged from 131 to 649aa. All the 266 genes were studied for the stability of their proteins. 26 out of them were considered further to study their overall quality factor using ERRAT and thereby 13 genes were found to have an ERRAT score of more than 95 for their deduced protein, further studied for their physicochemical properties revealed a molecular weight ranging from 18035.44 to 37304.66 and isoelectric point from 6.08 to 9.42. The subcellular location of all the encoding proteins is found in nucleus. RNA-Seq based expression profiling obtained through Wheat Expression Browser powered by expVIP reveals that many of the identified TabZIP were induced by heat and drought stress. Conclusively the discovered members of bZIP gene family may provide resource for genetic improvement and promote stress tolerance efficiency in wheat.

Key words: Basic leucine zipper (bZIP), Common wheat, Expression profile, *Triticum aestivum*.

INTRODUCTION

Wheat (*Triticum aestivum* L.), is one of the most important staple food crops of the world, occupying 17% (one sixth) of crop acreage worldwide, feeding about 40% (nearly half) of the world population and providing 20% (one fifth) of total food calories and protein in human nutrition (Gupta *et al.* 2008). Wheat is one of the most widely farmed crops in the world, with a yield of 7.34×108 tonnes over an area of 2.14× 106 km^2 (FAOSTAT, 2020). Increased population coupled with consumption preferences has resulted in a substantial increase in the demand for wheat in last 50 years (Kajla *et al.*, 2015). However, there is an urgent need to increase the yield and productivity of wheat to feed the burgeoning world population along with increased tolerance against various biotic and abiotic stresses limiting wheat production by significantly decreasing crop yield (Wani *et al.* 2018).

When plants encounter adverse conditions, a variety of genes regulating different functions are either upregulated or downregulated to confer stress tolerance in plants (Bhattacharjee *et al.* 2016). Regulation of various processes at transcriptional level plays crucial roles in controlling myriad of plant biological processes and signalling transduction cascades and interaction of transcription factors (TFs) with promoter binding region of target genes either repress or activate downstream genes. TFs play a key role in modulating stress-sensory pathways and precise response towards stress, TFs are involved in nearly every biological function in plants (Jakoby *et al.* 2002; Deinlein *et al.* 2014). Several families of stress responsive TFs, including ERF/AP2, bZIP, MYB, MYC, NAC and WRKY have been well characterized for their regulatory roles in eliciting stress-responses in plants (Meraj *et al.* 2020; Joshi *et al.* 2016).

The basic leucine zipper (bZIP) represents one of the largest as well as most diverse transcription factor (TFs) families. The bZIP proteins possess an eponymous bZIP domain, which is comprised of a basic region and a leucine

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zipper (Hurst, 1995). Transcription factors of the basic leucine zipper (bZIP) family control important processes in all eukaryotes. In plants, bZIPs are regulators of many central developmental and physiological processes including photomorphogenesis, leaf and seed formation, energy homeostasis and abiotic and biotic stress responses.

The basic leucine zipper (bZIP) family of proteins in plants is one of the largest and most diverse dimerizing transcription factor families. It regulates a variety of processes in plants, including stress and hormone signalling, floral induction and development, seed maturation and germination, photomorphogenesis and pathogen defence *etc* (Nijhawan *et al.* 2008). In this study, we have identified and annotated the members of bZIP family with utilizing wheat reference genome data available in public domain.

MATERIALS AND METHODS

The whole work is done in different steps sequentially as mentioned below.

Identification of bZIP TFs genes in the wheat genome

BLAST algorithm has been used to identify all the potential wheat genes containing bZIP domain. A BLASTP search was performed against the fully annotated reference genome [IWGSC] available in EnsemblPlants 42.0 (https://plants.ensembl.org/index.html) (Bolser *et al.* 2016) using the amino acid sequence of 17bZIP domain-containing protein from A. thaliana AtbZIP11 (Kumar *et al.* 2018). All sequences having an E-value less than 1.0 were considered and collected. Pfam was used to confirm the presence of other domains alongside the bZIP TFs domain in the bZIP TFs genes found through BLAST searches (Finn *et al.* 2017).

Homology modelling, structure evaluation and structure alignment

The protein's 3D structure is more conserved than amino acid sequences and it is crucial for cellular functions like differentiation and gene regulation (Kumar *et al.*, 2013). Protein data bank (PDB) repository (rcsb.org), still not have solved crystal and NMR structures of many plant bZIP TFs proteins. Furthermore, structural biologists face a difficult problem in molecular modelling of bZIP TFs proteins. Hence, homology modelling could be a viable alternative to 3D structure modelling of wheat bZIP TF proteins.

As a result, homology modelling (also known as comparative modelling) is used in the current study to estimate the 3D structure of wheat bZIP TFs proteins based on the resolved structure of homologous proteins.

Table 1: List of 26 importantbZIP TFs *genes* in wheat genome along with their length, corresponding protein length, chromosome location.

Gene	Ensembl plants ID	Chromosome	Coordinates	Length (bp)	Length (aa)	Splice variants
TabZIP2-1A	TraesCS1A02G096300	1A	91602861-91604969	1,255	335	-
TabZIP5-1A	TraesCS1A02G273000	1A	466896602-466899623	1409	253	2
TabZIP7-1A	TraesCS1A02G306300	1A	498532631-498536872	1771	324	-
TabZIP10-1A	TraesCS1A02G409800	1A	571286613-571291700	1869	283	-
TabZIP12-1B	TraesCS1B02G127400	1B	156565523-156567675	1293	340	5
TabZIP17-1B	TraesCS1B02G317100	1B	542135029-542139174	1723	322	-
TabZIP27-1D	TraesCS1D02G306000	1D	403273278-403277398	1741	327	-
TabZIP36-2A	TraesCS2A02G245700	2A	355454121-355461260	1706	378	3
TabZIP58-2D	TraesCS2D02G236200	2D	231595977-231603989	1675	379	6
TabZIP61-2D	TraesCS2D02G529000	2D	616525223-616530794	1999	425	-
TabZIP71-3A	TraesCS3A02G337200	3A	584015491-584019577	1668	338	-
TabZIP85-3B	TraesCS3B02G368300	3B	580468739-580472902	1619	343	2
TabZIP92-3B	TraesCS3B02G411300	3B	647819883-647823019	1158	250	-
TabZIP95-3D	TraesCS3D02G194800	3D	187831578-187841857	1619	332	-
TabZIP100-3D	TraesCS3D02G330300	3D	442811263-442815265	1372	337	-
TabZIP105-3D	TraesCS3D02G371900	3D	485069390-485072293	1381	228	2
TabZIP147-5A	TraesCS5A02G141500	5A	313904287-313908271	1445	313	-
TabZIP169-5B	TraesCS5B02G139800	5B	263544557-263548522	1481	313	-
TabZIP212-6B	TraesCS6B02G193200	6B	227641866-227651596	2116	458	2
TabZIP219-6D	TraesCS6D02G154400	6D	129486855-129495855	1869	466	3
TabZIP224-7A	TraesCS7A02G170600	7A	126576805-126581468	1363	343	-
TabZIP238-7A	TraesCS7A02G542700	7A	719469821-719473122	1433	164	-
TabZIP239-7B	TraesCS7B02G075600	7B	85127850-85133586	1421	340	-
TabZIP252-7B	TraesCS7B02G464700	7B	721062468-721065544	1454	165	-
TabZIP253-7D	TraesCS7D02G171300	7D	123653486-123658548	1455	345	-
TabZIP266-7D	TraesCS7D02G527500	7D	622544927-622548011	1324	164	-

(-) means no splice variants.

The best homologs template structures were identified using position-specific iterated BLAST algorithm (PSI -BLAST) against PDB to identify based on high score and lower e-value (Altschul *et al.* 1997). The automated Swiss-Model server was used to anticipate the appropriate 3D structures for wheat bZIP proteins (Arnold *et al.* 2006; Biasini *et al.* 2014).

Furthermore, Ramachandran plots of modelled wheat bZIP proteins were computed using PSVS (http://psvs-1_5-dev.nesg.org) assessing phi (Φ) and psi (Ψ) torsion angles and covalent bond quality, most favoured regions *etc*. Quality of 3D structure of proteins was analysed using ERRAT (http://saves.mbi.ucla.edu) (Colovos *et al.* 1993) a so-called "overall quality factor" for non-bonded atomic interactions. A higher score indicates higher quality.

UCSF CHIMERA 1.10 (Pettersen *et al.* 2004) was used to render projected models of wheat bZIP TFs proteins in various 3D coordinates.

Physiochemical properties and subcellular localization

The ProtParam tool embedded on the Expasy website (https://web.expasy.org/protparam/) was utilized to determine various physiochemical properties such as molecular weight (Mw), isoelectric point (pI), aliphatic index (AI) and grand average of hydropathicity (GRAVY) (Gasteiger *et al.* 2005).

For the prediction of subcellular localization of the TabZIP TFs proteins of wheat, Novel integrated web-server named asBUSCA (Savojardo *et al.*, 2018) and Plant-mPLoc (Chou and Shen, 2010) were used.

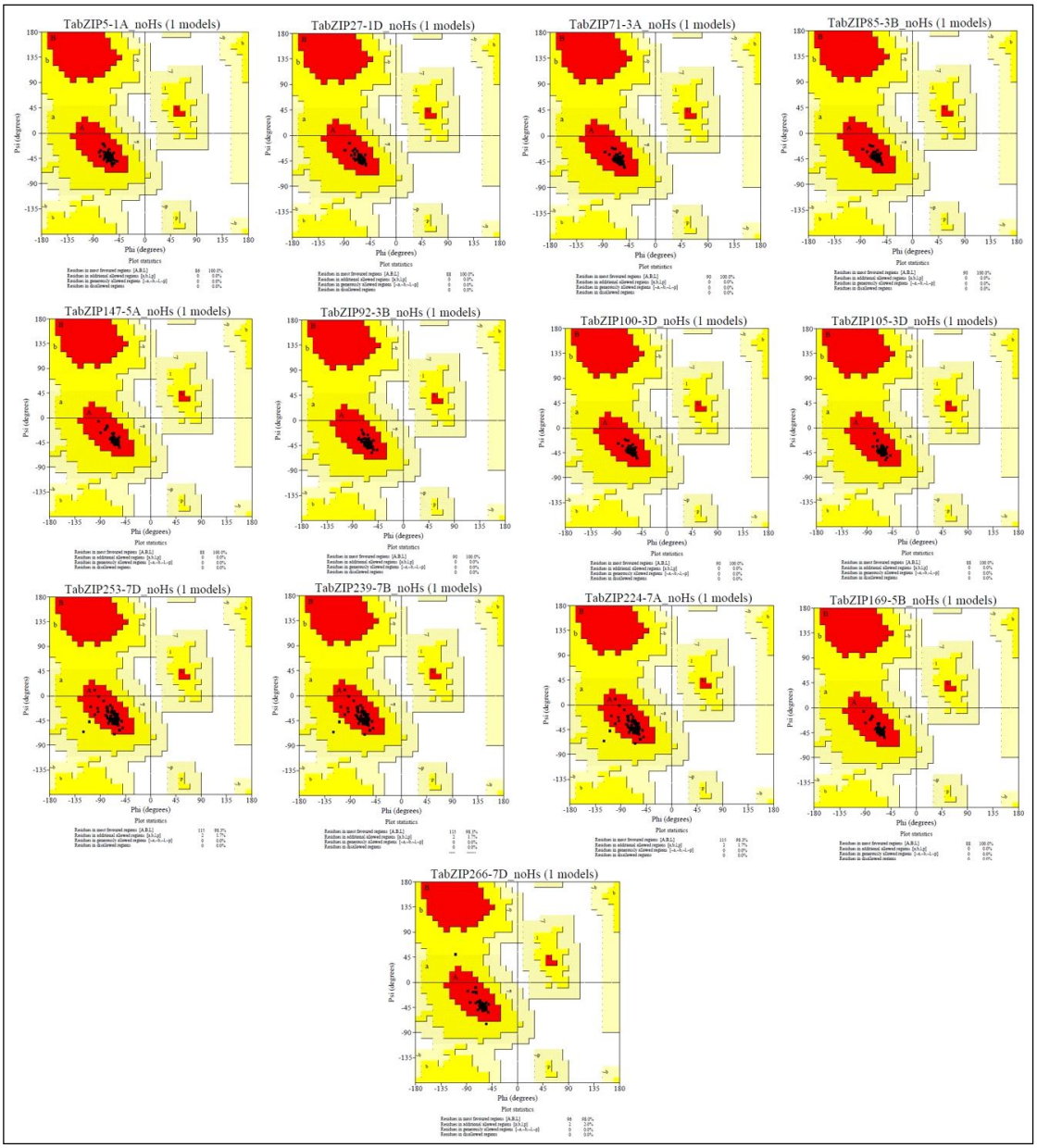

Fig 1: Ramachandran plots of modelled wheat bZIP proteins were computed using PSVS tool.

Analysis of evolutionary relation among TabZIP TFs under study

We utilized MEGA 6.0 (Tamura *et al.*, 2013) to confirm the evolutionary link among TabZIP TFs proteins in plants and ClustalW ver. 2.1 for multiple sequence alignment (MSA) of the full length deduced amino acid sequences of TabZIP TFs domain-containing proteins in *T. aestivum* (Larkin *et al.*, 2007). Subsequently, we built a phylogeny tree using the neighbor-joining method using the Poisson substitution model, uniform rates and pairwise deletion (Saitou *et al.*, 1987; Nei *et al.*, 2000), with bootstrap values determined for a percentage of 1000 iterations for the established phylogeny tree (Felsenstein, 1985).

Expression profiling

To perform the expression profile patterns of identified TabZIP genes under various stress conditions *e.g.*, drought and heat., RNA-seq expression data was obtained through Wheat Expression Browser (http://www.wheatexpression.com/) powered by expression Visualization and Integration Platform (expVIP) (Borrill *et al.* 2016).

RESULTS AND DISCUSSION

Identification of TabZIP TFs gene family in wheat

In the present study, initially a total of 266 different wheat loci encoding bZIP TFs proteins in wheat have been identified using genome-wide approach. Identified TabZIP TFs were filtered by removing redundant sequences and different transcripts of the same gene. To further verify the reliability of these TabZIP TFs genes, the amino acid sequences of all 266 proteins were searched for the presence of the conserved TabZIP TFs domain and other associated domains using by InterPro and PROSITE databases. The identified 266 TabZIP TFs genes were named TabZIP1 -1A to TabZIP266 -7D according to their chromosomal positions.

Sequence analysis revealed that the coding sequence (CDS) length of deduced TabZIP TFs genes ranged from 422 (TabZIP128 -4D) to 3669 bp (TabZIP26 -1D) and corresponding protein length ranged from 131 (TabZIP38 -2A, TabZIP59 -2D) to 649aa (TabZIP14 -1B).

Table 2: List of 26 of the total 266 identified TabZIP genes in wheat genome along with the results of other studies *e.g.*, homology modelling, % similarity, corresponding SMTL ID, properties of Ramachandran plot and ERRAT score (representing overall structure quality).

Gene	Ensembl plants ID	SMTL ID	% Similarity	Most favored regions (%)	Additional allowed regions (%)	Generously allowed regions (%)	Disallowed regions (%)	ERRAT
TabZIP2-1A	TraesCS1A02G096300	5zk1.1.B	48.84%	93.0	7.0	0.0	0.0	100
TabZIP5-1A	TraesCS1A02G273000	1dh3.1.C	40.00%	100.0	0.0	0.0	0.0	100
TabZIP7-1A	TraesCS1A02G306300	1dh3.1.C	48.94%	93.3	6.7	0.0	0.0	97.4
TabZIP10-1A	TraesCS1A02G409800	6iak.3.B	44.83%	96.3	3.7	0.0	0.0	100
TabZIP12-1B	TraesCS1B02G127400	1dh3.1.C	53.85%	94.9	3.8	1.3	0.0	85.04
TabZIP17-1B	TraesCS1B02G317100	1dh3.1.C	48.94%	93.3	6.7	0.0	0.0	97.4
TabZIP27-1D	TraesCS1D02G306000	1dh3.1.C	41.30%	100.0	0.0	0.0	0.0	100
TabZIP36-2A	TraesCS2A02G245700	1ysa.1.C	46.30%	90.2	8.0	1.8	0.0	100
TabZIP58-2D	TraesCS2D02G236200	1ysa.1.C	45.45%	91.2	7.0	1.8	0.0	100
TabZIP61-2D	TraesCS2D02G529000	1dh3.1.C	44.19%	94.3	4.5	1.1	0.0	100
TabZIP71-3A	TraesCS3A02G337200	1dh3.1.C	40.43%	100.0	0.0	0.0	0.0	100
TabZIP85-3B	TraesCS3B02G368300	1dh3.1.C	40.43%	100.0	0.0	0.0	0.0	100
TabZIP92-3B	TraesCS3B02G411300	1dh3.1.C	40.43%	100.0	0.0	0.0	0.0	100
TabZIP95-3D	TraesCS3D02G194800	1dh3.1.C	48.78%	92.9	6.0	1.2	0.0	100
TabZIP100-3D	TraesCS3D02G330300	1dh3.1.C	40.43%	100.0	0.0	0.0	0.0	100
TabZIP105-3D	TraesCS3D02G371900	1dh3.1.C	41.30%	100.0	0.0	0.0	0.0	100
TabZIP147-5A	TraesCS5A02G141500	1dh3.1.C	41.30%	100.0	0.0	0.0	0.0	98.66
TabZIP169-5B	TraesCS5B02G139800	1dh3.1.C	41.30%	100.0	0.0	0.0	0.0	98.66
TabZIP212-6B	TraesCS6B02G193200	1dh3.1.C	46.15%	92.5	7.5	0.0	0.0	100
TabZIP219-6D	TraesCS6D02G154400	1dh3.1.C	46.15%	91.2	6.2	2.5	0.0	100
TabZIP224-7A	TraesCS7A02G170600	6iak.1.A	40.91%	98.3	1.7	0.0	0.0	95.19
TabZIP238-7A	TraesCS7A02G542700	1dh3.1.C	40.38%	96.0	3.0	1.0	0.0	100
TabZIP239-7B	TraesCS7B02G075600	6iak.1.A	40.91%	98.3	1.7	0.0	0.0	95.19
TabZIP252-7B	TraesCS7B02G464700	1dh3.1.C	42.31%	96.0	3.0	1.0	0.0	91.95
TabZIP253-7D	TraesCS7D02G171300	6iak.1.A	40.91%	98.3	1.7	0.0	0.0	95.19
TabZIP266-7D	TraesCS7D02G527500	1dh3.1.C	41.18%	98.0	2.0	0.0	0.0	100

The gene ID, length, corresponding protein length, chromosome location, splice variants and coordinates of some selected TabZIP TFs, which found important in further investigations (*i.e.*,homologous modelling, structural evaluations) out of the 266 different wheat loci encoding bZIP TFs proteins are listed in Table 1.

Homology modeling, structure evaluation and structure alignment

On superposition, the modelled 3D structures of typical TabZIP proteins reveal less than 1 RMSD with respect to the homolog templates. The Ramachandran plots for the phi (Φ) and psi (Ψ) torsion angles analysis revealed that the modelled 3D structures of typical proteins have excellent geometry (Fig 1). As seen in the plots, the modelled structure revealed that up to 100% of residues were fallen in the most favoured locations (Table 2). According to the PROCHECK algorithm, a good quality model should contain above 90% residues in the most favoured regions (Laskowski *et al.*, 1993). Calculating the Ramachandran plot is a well-known stage in the verification of proteins' three-dimensional structures (Kumar *et al.*, 2016; Kumar *et al.*, 2018). At the structural level, modelled 3D structures of wheat TabZIP proteins gave a base that helped to comprehend the complex mechanism of bZIPs. Predicted 3D structures, having a similarity of 40% or more as deduced by using automated Swiss-Model server, % similarity with corresponding SMTL ID is mentioned in Table 2.

Protein structures, modelled through homology modelling using automated Swiss -Model server that have an ERRAT score more than 95, were further visualized in CPK by UCSF CHIMERA are shown in Fig 2. The structure generated using CHIMERA is comparable to the established bZIPs.

Physiochemical properties and subcellular localization

Various physicochemical properties and subcellular localization of the selected TabZIP TFs are listed in Table 3 as determined with ProtParam tool and BUSCA, respectively. As revealed from study through ProtParam as well as

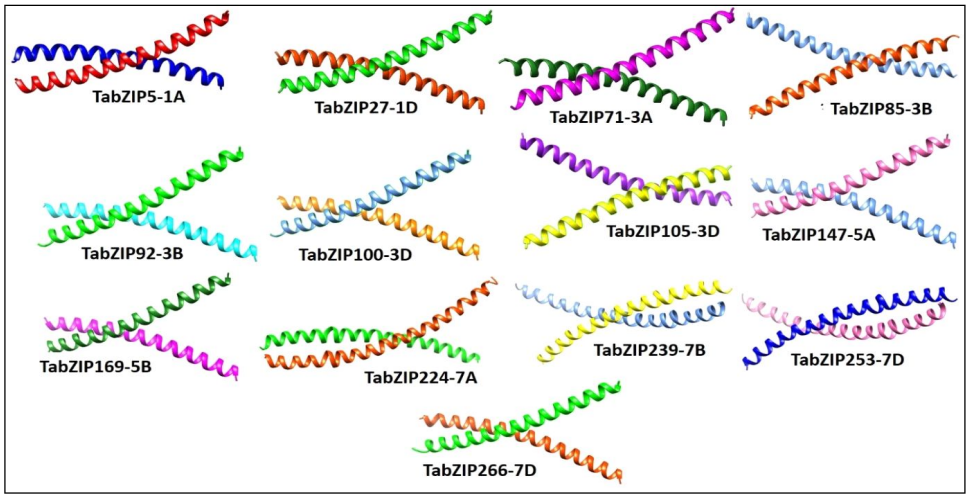

Fig 2: 3D structure images of TabZIP proteins, as were generated using the UCSF Chimera program from the resource for Biocomputing, Visualization and Informatics (http://www.cgl.ucsf.edu/chimera).

Table 3: Predicted physicochemical properties and subcellular localization of TabZIP TFs proteins.

Protein	Molecular weight	Isoelectric point	Aliphatic index	GRAVY	Subcellular localization
TabZIP5-1A	26633.69	6.08	61.98	-0.64	Nucleus
TabZIP27-1D	35401.03	6.63	72.23	-0.639	Nucleus
TabZIP71-3A	36726.31	9.26	68.99	-0.66	Nucleus
TabZIP85-3B	37353.08	9.42	70.23	-0.671	Nucleus
TabZIP92-3B	26792.04	8.01	61.48	-0.765	Nucleus
TabZIP100-3D	36528.07	9.37	69.2	-0.651	Nucleus
TabZIP105-3D	24030.02	6.02	66.97	-0.509	Nucleus
TabZIP147-5A	34883.24	6.4	76.36	-0.689	Nucleus
TabZIP169-5B	34887.28	6.4	75.11	-0.693	Nucleus
TabZIP224-7A	36979.25	8.5	74.02	-0.399	Nucleus
TabZIP239-7B	36778.07	8.84	72.91	-0.423	Nucleus
TabZIP253-7D	37304.66	8.84	74.14	-0.408	Nucleus
TabZIP266-7D	18035.44	9.42	72.07	-0.591	Nucleus

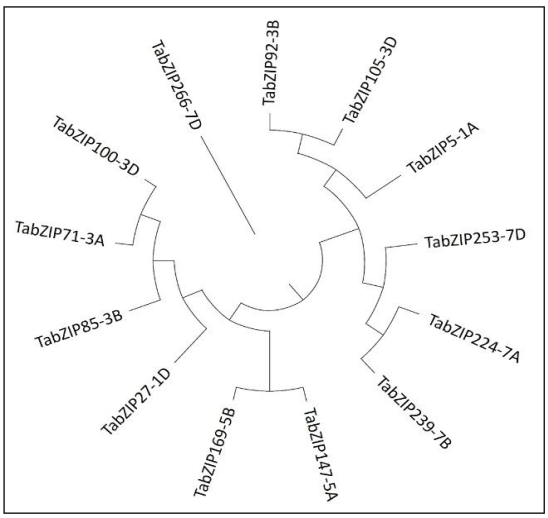

Fig 3: Evolutionary relationship among TabZIP, based on the sequence alignment of the different TabZIP proteins.

BUSCA the subcellular localization of proteins is found to be nucleus which supports their role as transcription factors (TFs).

Evolutionary relation among TabZIPs

The unrooted tree was generated using ClustalW 2.1 program and displayed by MEGA 6.0. (Fig 3) TabZIP TFs were mainly classified into 5 subgroups. TabZIP100-3D, TabZIP71-3ATabZIP85-3B and TabZIP27-1D forms one subgroup, TabZIP92-3B, TabZIP105-3D and TabZIP5-1A forms another subgroup, TabZIP253-7D, TabZIP224-7A and TabZIP239-7B forms a different subgroup, TabZIP147-5A and TabZIP169-5B forms a different subgroup while TabZIP266-7D falls in a separate subgroup.

Expression profile

The expression profile under drought and heat stress revealed the expression of three genes TabZIP5-1A, TabZIP92-3B, TabZIP147-5A, TabZIP169-5B (Fig 4). Mainly TabZIP92-3B exhibited the higher expression under stress

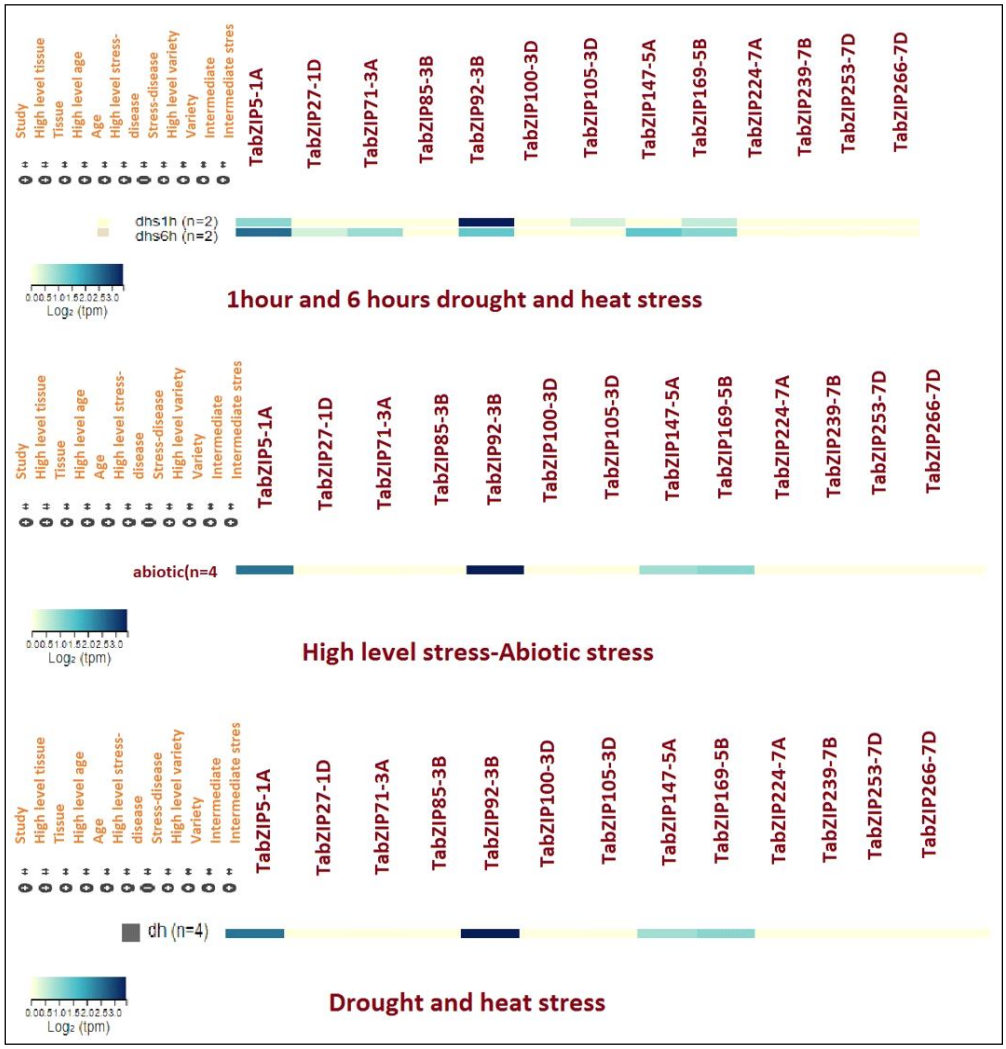

Fig 4: Expression profiles of *TabZIP* genes. The heatmap was constructed using the expVIP and the FPKM (fragments per kilobase of transcript per million fragments) value of *TabZIP* genes were transformed by log2. The yellow and blue colours represent the lower and higher relative abundance of the transcript, respectively.

condition of heat of draught, as revealed by the higher relative abundance of the transcript.

CONCLUSION

Using bioinformatics-based techniques and algorithms, this comprehensive genome-wide study provides a systematic identification and functional annotation of the wheat bZIP gene family (TabZIP). A total of 266 TabZIP genes were discovered on wheat chromosomes and they were located in an uneven distribution. Finally, 13 bZIP genes were deduced to three have a stable protein structure, out of them 6 are found to have an expression under stress conditions *i.e*., drought and heat. Overall, this study delivers the first-hand functional and structural knowledge of bZIP gene family and the results will help found a groundwork for the further functional verification of the TabZIP gene family during osmotic stress responses, leading to wheat improvement.

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