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Genome-Wide Identification, Characterization, and Expression Analysis of TUBBY Gene Family in Wheat (*Triticum aestivum* L.) under Biotic and Abiotic Stresses

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Abstract: The TUBBY gene family is a group of transcription factors found in animals and plants with many functions. *TLP* genes have a significant role in response to different abiotic stresses. However, there is limited knowledge regarding the TUBBY gene family in *T. aestivum*. Here we identified 40 *TaTLP* genes in wheat to reveal their potential function. This study found that TUBBY (*TaTLP*) genes are highly conserved in wheat. The GO analysis of *TaTLP* genes revealed their role in growth and stress responses. Promoter analysis revealed that most *TaTLPs* participate in hormone and abiotic stress responses. The heatmap analysis also showed that *TaTLP* genes showed expression under various hormonal and abiotic stress conditions. Several genes were upregulated under different hormonal and temperature stresses. The qRT-PCR analysis confirmed our hypotheses. The results clearly indicate that various *TaTLP* genes showed high expression under temperature stress conditions. Furthermore, the results showed that *TaTLP* genes are expressed in multiple tissues with different expression patterns. For the first time in wheat, we present a comprehensive *TaTLP* analysis. These findings provide valuable clues for future research about the role of *TLPs* in the abiotic stress process in plants. Overall, the research outcomes can serve as a model for improving wheat quality through genetic engineering.

Keywords: wheat; temperature stress; hormonal stress; genes; *TaTLPs*

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1. Introduction

Global warming has resulted in significant decreases in crop production over the last few decades [1]. Plants are exposed to numerous environmental stresses that disrupt biochemical and physiological processes [2]. Temperature, heat, drought, and salt stress directly reduce the quality and total yield [3,4]. To overcome annual yield losses in crops such as wheat, it is critical to identify and understand new sources of defense biomarkers. The TUBBY-like proteins are a family of bipartite transcription factors discovered in plants [5–7]. It was possible to trace the TUBBY-like gene family's phylogenetic history back to the earliest stages of eukaryotic evolution after discovering TUBBY-like genes in both single-celled and multicellular eukaryotes [7]. TUBBY-like proteins are distinguished

from other proteins by the presence of the conserved C-terminal tubby domain, which is composed of 12 antiparallels closed β-barrel strands with a central hydrophobic α–helix [5]. A conserved N-terminal F-box domain and the C terminal tubby domain are found in the *TLP* family of plants, which is much larger than the *TLP* family found in animals [8]. The function of *TLP* genes was studied in various plants such as *Arabidopsis thaliana, Oryza sativa, Populus deltoides* [9], *Malus domestica* [8], *Zea maize* [10], *Solanum lycopersicum* [11], and cotton [12]. In *A. thaliana,* 11 TUBBY family genes were identified, whereas in *Oryza sativa* 14, *Malus domestica* 15, *Zea maize* 10, *Solanum lycopersicum* 11, and cotton 105 *TLP* genes were previously identified [8–12]. *TLP* shows different expression levels in tissues in plants in response to various environmental and hormonal stresses [7,8,13]. It was found that At*TLP*3 and At*TLP*9 play an essential role in abscisic acid and osmotic stress [13], whereas At*TLP*9 plays a significant role in salt and drought stress [13,14]. Many TUBBY family genes showed up-regulation in *Malus domestica* in response to abiotic stresses, suggesting a substantial role of *TLP* genes in abiotic stresses [8]. Previous observation showed that Ca*TLP*1 in *Cicer arietinum* plays a vital role in dehydration stress resistance, and its overexpression in tobacco offers salt and drought stress resistance [15]. Thus, *TLP*s seem to have a significant role in abiotic stress tolerance in plants. However, the function of *TLP*s and their mode of action in plants is an unexplored topic [11].

Wheat is an important crop providing sustenance to 35% of the world's population. However, unpredictable climatic conditions have stagnated wheat production in the past two to three decades. Biotic and abiotic stresses affect the growth of wheat crops and have decreased the plant's output and performance [16]. Wheat crops' evolutionary diversity allows them to adapt to different environmental conditions, although the molecular basis of this adaptation is unknown. Therefore, we were interested in the evolution of the wheat TUBBY family genes and their function in response to abiotic stress. This research aimed to understand wheat TUBBY family genes to improve wheat production, plant quality, and abiotic stress response.

2. Materials and Methods

2.1. Wheat TUBBY Family Genes Identification

We retrieved the protein sequences of TUBBY genes from the Arabidopsis [17] and wheat [18] using the Hidden Markov Model (HMM). The extracted *Triticum aestivum L.* protein sequences were analyzed using CD-search NCBI (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi, http://www.ebi.ac.uk/interpro/search/sequence/) and SMART (http://smart.embl-heidelberg.de/) (accessed on 7 March 2022) databases. Proteins that do not exhibit the TUBBY domain were excluded. The chemical properties of TaTUBBY proteins were examined using the Expasy online server (http://web.expasy.org/protparam/) (accessed on 7 March 2022). The CELLO2GO [19] online server was used to predict the subcellular location of TaTUBBY genes.

2.2. Phylogenetic Tree, Digital Expression, and Motif Analysis

The Mega (version 7.0) program was used to create the maximum likelihood phylogenetic tree [19]. The conserved motif in the TUBBY gene was predicted using the online MEME server (latest Version 4.12.0) (http://meme-suite.org/tools/meme) (accessed on 8 March 2022). In response to biotic and abiotic stress, the gene expression levels were determined at various stages in all available tissue. The RNA-seq data were retrieved in transcripts per million (TPM) from the expVIP wheat Expression Browser (http://www.wheat-expression.com/) (accessed on 8 March 2022) [20,21]. The abiotic stress was comprised of temperature stress ranging from 20 to 40 °C, and biotic stresses were comprised of abscisic acid (ABA), gibberellic acid (GA), and a combination of *Fusarium graminearum* (FG), ABA, and GA. The ratio of the expression value under treatment to the control was calculated to determine the regulation patterns of a given gene subjected to stress. Ratios greater than or less than 1.0 under a given treatment indicated that the stress treatment had altered gene expression levels. In contrast, a ratio equal to 1.0 showed that

the treatment did not affect gene expression levels [20]. The heatmap was created using the Heml 1.0 software tool (http://hemi.biocuckoo.org/faq.php) (accessed on 8 March 2022).

2.3. Chromosomal Location and Protein-Protein Interaction of TUBBY Genes

The chromosomal location of the TUBBY genes was determined using plants from the Ensemble genomes (https://plants.ensembl.org/Triticum_aestivum/Info/Annotation/) (accessed on 9 March 2022) [20]. MAPDraw was also used to map the physical location of TUBBY genes, and nomenclature followed the order in which they appeared on the chromosomes. Analyses of Arabidopsis protein–protein interactions were conducted using the STRING online server (http://string.embl.de) (version. 10) (accessed on 9 March 2022).

2.4. Gene Structure and Conserved Motif Analysis

In the Gene Structure Display server program (http://gsds.cbi.pku.edu.cn/) (accessed on 10 March 2022), genomic and CDS sequences of *TaTLP*s genes were used to create an exon/intron map [22]. The conserved motifs in the TUBBY proteins were discovered using the online server MEME 4.11.3 (http://meme-suite.org/tools/meme) (accessed on 10 March 2022) [23].

2.5. Gene Ontology and Cis-Elements Analysis of TUBBY Family Genes

A 1.5 Kb genomic DNA sequence upstream of each identified *TaTLP* gene's start codon was obtained from the Ensemble Plants database (http://plants.ensemble.org/Triticum_aestivum) (accessed on 11 March 2022) using the Ensemble Plants search engine (ATG). The online Plant CARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) (accessed on 11 March 2022) database was used to identify cis-regulatory elements for all the TUBBY genes. Ontology analysis of the *TaTLP* protein sequences was performed using the Blast2GO program Ver.2.7.2 (http://www.blast2go.com) (accessed on 11 March 2022), and the groups of GO classification (molecular functions, biological process, and cellular component) were documented.

2.6. RNA Isolation and cDNA Synthesis

Total RNA was isolated from stress-exposed seedlings at selected time points, including 0 (control) and stress using the RNeasy plant mini kit (Qiagen, Redwood City, CA, USA), as per the manufacturer's instructions. The quantity and quality of isolated RNA were determined by spectrophotometry (Nanodrop 2000, Thermo Fisher Scientific, Waltham, MA, USA) and formaldehyde-based gel electrophoresis, respectively. For cDNA synthesis,1 µg of total RNA was transcribed in 20 µL using Revert Aid First Strand cDNA Synthesis Kit (Fermentas Life Sciences, Waltham, MA, USA) using oligo (dT)primers as per the manufacturer's instructions.

2.7. Expression Analysis of Different Genes

To examine the temporal expression patterns of selected genes, qRT-PCR was performed. The qRT-PCR was performed in a CFX-96 Real-time PCR Detection 4 System (Bio-Rad, Hercules, CA, USA). Reactions were conducted in a total volume of 20 pl using 50 ng of cDNA, 10 pmol of forward and reverse primers, and 10 L of 2× Sso Fast Eva GreenqPCR Supermix (Bio-Rad, Hercules, CA, USA). The cycling conditions were as per the manufacturer's protocol with a primer-specific annealing temperature. The threshold cycle (Ct) was automatically determined for each reaction using the system set with default parameters. The transcript levels were normalized to the actin transcript, and the fold differences of each amplified product in the samples were calculated using the 2-AACt method.

3. Results

3.1. Identification and Analysis of TaTLPs Genes

In the current study, 40 *TaTLP* proteins from wheat were retrieved using the Ensemble Plants (http://plants.ensemble.org/Triticum_aestivum) (accessed on 11 March 2022) database. The genes were named based on their chromosomal position from *TaTLPq1* to *TaTLP40* (Table 1). Among these, *TaTLP2, TaTLP4, TaTLP5, TALP13, TaTLP16,* and *TaTLP35* genes were located in the *Extracellular* region, *TaTLP14* and *TaTLP24* in the mitochondrial region, and *TaTLP23* was located in the chloroplast, while the remaining 31 *TaTLPs* genes were found in the nucleus (Table 1). More details regarding *TaTLPs* were also recorded, including Locus ID, Proteins, and Molecular weight.

Table 1. The gene features of the wheat TUBBY gene family.

Gene Name	Locus ID	Proteins	MW	PI	SL
TaTLP1	Traes_1AL_399C1DBF5	269	-	-	N
TaTLP2	Traes_1AL_45AB9EF34	247	27,640.24	9.22	EC
TaTLP3	Traes_1BL_BDF2FCEC7	175	19,434.98	9.08	N
TaTLP4	Traes_1DL_106460E68	50	5544.55	9.42	EC
TaTLP5	Traes_1DL_9DE76F004	251	27,986.78	9.16	EC
TaTLP6	Traes_1DL_FAB396374	204	22,671.21	9.80	N
TaTLP7	Traes_2AL_436D234EE	471	52,097.90	9.28	N
TaTLP8	Traes_2AS_11876C298	203	22,666.81	9.14	N
TaTLP9	Traes_2BL_181C4AA28	472	52,073.74	9.22	N
TaTLP10	Traes_2BS_9DCC9CC7A	203	22,657.80	9.14	N
TaTLP11	Traes_2DL_79449BF6D	347	38,850.30	9.73	N
TaTLP12	Traes_2DS_AC89AEF36	203	22,661.79	9.14	N
TaTLP13	Traes_3AL_108550E28	126	14,455.70	8.34	EC
TaTLP14	Traes_3AL_731AE8008	60	6858.86	10.08	M
TaTLP15	Traes_3AL_9B4AF9950	152	17,297.97	9.37	N
TaTLP16	Traes_3AL_C3AEDD333	82	9123.34	10.72	EC
TaTLP17	Traes_3B_021E89FE5	377	-	-	N
TaTLP18	Traes_3B_02CE045341	194	22,052.95	9.33	N
TaTLP19	Traes_3B_C967E97B9	180	20,367.29	9.30	N
TaTLP20	Traes_3DL_697C6F117	211	23,933.51	9.69	N
TaTLP21	Traes_3DL_C81B58D98	279	31,811.57	9.63	EC
TaTLP22	Traes_4AL_EDE236978	440	48,793.98	9.39	N
TaTLP23	Traes_4AS_0C8542099	402	44,497.04	9.43	C
TaTLP24	Traes_4BL_7E9BC637F	559	61,782.42	9.73	M
TaTLP25	Traes_4BS_D5B5C14F6	440	48,817.02	9.39	N
TaTLP26	Traes_4DL_7D905B6BC	404	44,726.34	9.29	C
TaTLP27	Traes_4DS_620432A0D	440	48,833.02	9.39	N
TaTLP28	Traes_5AL_83533B97D	361	40,470.04	9.54	N
TaTLP29	Traes_5AL_D38708404	64	7023.98	4.75	N
TaTLP30	Traes_5BL_ECCAFFEB4	440	49,017.86	9.34	N
TaTLP31	Traes_5DL_FA0200E13	439	48,856.72	9.25	N
TaTLP32	Traes_6AL_058D829C3	374	-	-	N
TaTLP33	Traes_6AS_FB1249AB4	321	35,747.87	9.70	N
TaTLP34	Traes_6BL_9F6ACF02D	322	35,555.86	9.39	N
TaTLP35	Traes_6BS_5C281F303	177	20,314.47	9.98	EC
TaTLP36	Traes_6DL_E7A7DAE5C	368	40,756.92	9.26	N
TaTLP37	Traes_6DS_D6AD8C3ED	177	20,355.52	9.98	N
TaTLP38	Traes_7AL_52F3AE87C	373	41,569.32	9.80	N
TaTLP39	Traes_7BL_8312EAB48	441	-	-	N
TaTLP40	Traes_7DL_037F2A4F4	238	26,443.41	9.69	N

CDS: Coding Sequence, MW: Molecular Weight, SL: Sub Cellular Location, EC: Extracellular, N: Nuclear, PM: Plasma membrane, C: Chloroplast.

3.2. Phylogenetic Analysis of TaTLPs

We used the Neighbor-Joining method to construct a phylogenetic tree that included *Triticum aestivum*, *A. thaliana*, and *O. sativa TLPs* to investigate their phylogenetic relationship (Figure 1). The results showed that 40 *TaTLPs*, 15 *OsTLPs*, and 14 *AtTLPs* were clustered and further divided into three families, namely, A, B, and C. Furthermore, Family A was divided into two subfamilies, Family AI and Family AII. Family AI was the largest family containing most *TLPs* including 18 *TaTLPs*, (*TaTLP1, TaTLP6, TaTLP7, TaTLP9, TaTLP11, TaTLP16, TaTLP17, TaTLP20, TaTLP22, TaTLP25, TaTLP27, TaTLP28, TaTLP29, TaTLP30, TaTLP31, TaTLP38, TaTLP39, TaTLP40*), six *OsTLPs*, and three *AtTLPs*. The subfamily AII contained 14 *TLPs* including nine *TaTLPs* (*TaTLP2, TaTLP3, TaTLP4, TaTLP5, TaTLP13, TaTLP14, TaTLP18, TaTLP19, TaTLP21)*, four *OsTLPs*, and one *AtTLP*. Family B was the second-largest family, containing 10 *TaTLPs* including (*TaTLP8, TaTLP10, TaTLP12, TaTLP15, TaTLP23, TaTLP24, TaTLP26, TaTLP33, TaTLP35, TaTLP37)*, four Os*TLPs*, and five At*TLPs*. Family C was the smallest, containing three *TaTLPs*, including (*TaTLP32, TaTLP34, TaTLP36,)* one *OsTLP*, and two *AtTLPs*. The results confirmed that the evolutionary relationships of *A. thaliana*, *O. sativa*, and *Triticum aestivum* are closer.

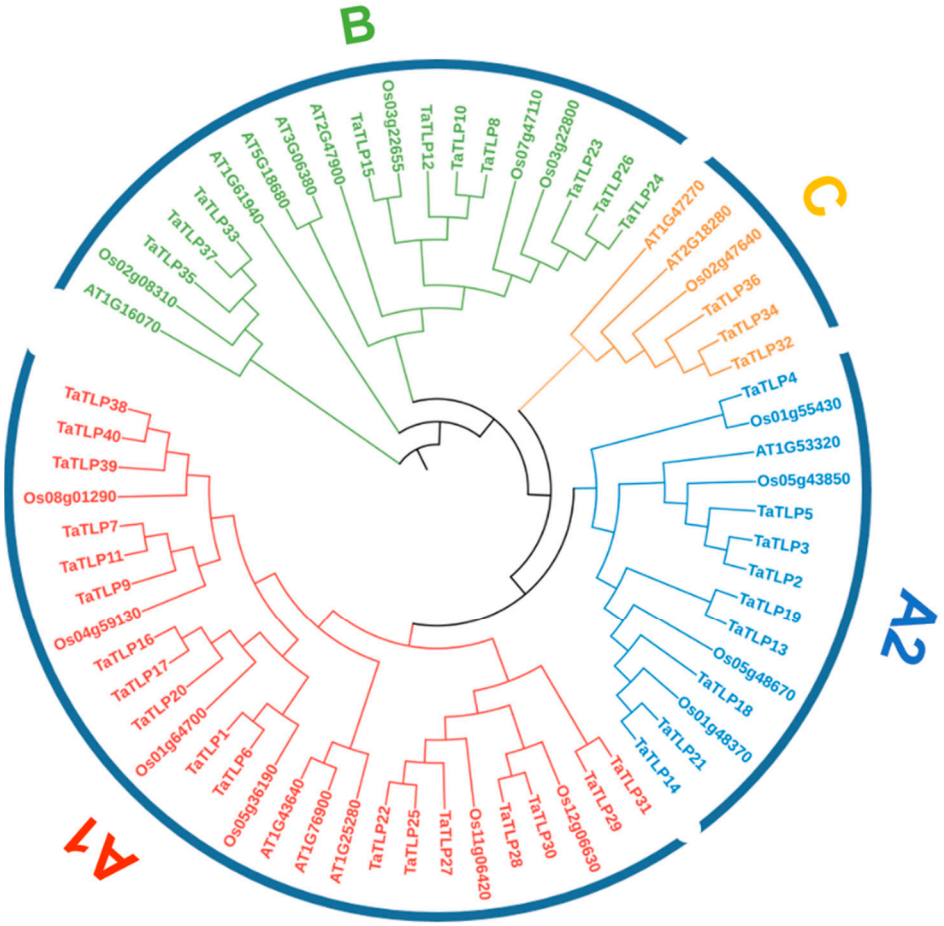

Figure 1. *TLPs* protein phylogeny in three plants: *T. aestivum*, *A. thaliana*, and *O. sativa*. MEGA 7 was used to generate the phylogenetic tree using the following parameters: Bootstrap D 1000 replicates, Neighbor-Joining method, Poisson correction. All group members are divided into four groups, each represented by an assorted color. Different labels are used to identify members of various species.

3.3. Conserved Motif Analysis of TaTLPs-Genes

A total of 10 conserved motifs were discovered using the MEME online server, and they were found to be appropriate for explaining the *TaTLPs'* structure (Figure 2). Among

the 40 *TaTLP*s genes, *TaTLP7*, *TaTLP9*, *TaTLP22*, *TaTLP24*, and *TaTLP39* contained more than seven *TLP*s motifs.

Figure 2. The *TaTLP*s genes' conserved motifs.

3.4. Gene Ontology of TaTLP Genes

For the functional prediction of *TaTLP* genes, we used gene ontology (GO) enrichment pathway analysis to identify potential pathways. Three different processes were studied and predicted in their functional outcomes: molecular, biological, and cellular (Figure 3). The molecular prediction suggested that *TaTLP* genes participate in many activities such as signal transduction activity, hydrolase activity, lipid binding, ion binding activity, and cytoskeletal protein binding activity. The biological prediction suggested that *TaTLP* genes participate in various biological processes such as cellular protein modification, signal transduction, vesicle-mediated transport, anatomical structural development, abiotic stresses, cell differentiation, lipid metabolic process, morphogenesis, and embryo development. The cellular prediction suggested that *TaTLP* genes are located in the plasma membrane, intracellular, cytoplasm, nucleus, and extracellular region. Based on the results, it is suggested that *TaTLP* genes play an essential role in plant growth regulation by modulating biological, molecular, and cellular activities.

3.5. Protein-Protein Interaction of TaTLPs

The *TaTLP* protein prediction analysis revealed various other proteins that predictably interact with TaTLP5 (Figures 2 and 4). Thus, TaTLP5 possibly interacts closely with ATG2G20050, which plays a vital role in signal transduction, ATP binding, metal ion binding, and protein serine phosphatase activity, as highlighted by the bit score. The putative bit score of 0.837 closely interacts with our reference gene *TaTLP5*, a member of the TUBBY gene family. Similarly, it interacted with ATG2G35680, Phosphotyrosine protein phosphatase superfamily protein, and Possesses phosphate activity. Furthermore, these genes interact with AT3G12370, AT3G10330, pBRP2, AT2G45910, ENDOL9, ATGRIP, AT2G04940, and MLO1. The details are given in Table 2.

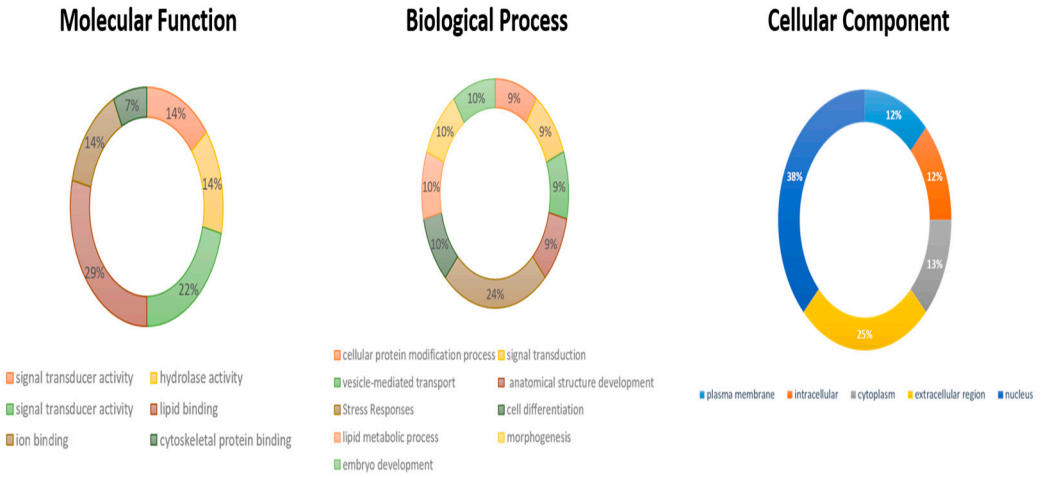

Figure 3. *TaTLP* gene GO enrichment analysis shows molecular function, biological process, and cellular component data.

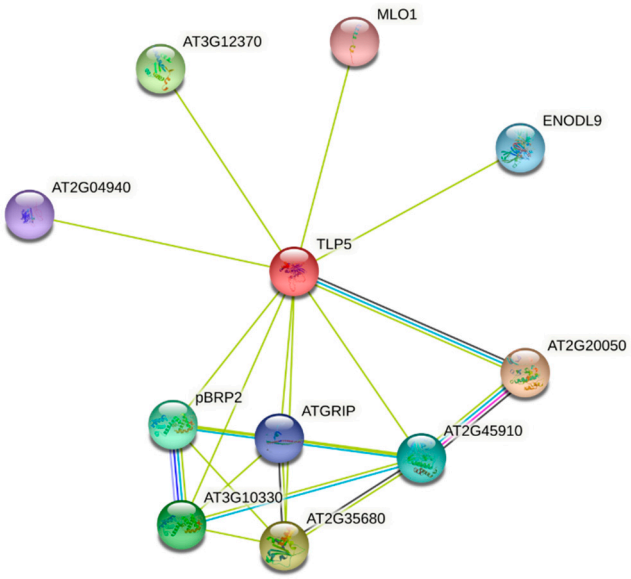

Figure 4. The predicted functional partners of *TaTLP* protein.

Table 2. Different predicted protein families interacted with *TaTLP* genes.

Gene Name	Protein Family	Putative Function	Interactive-Bit Score
ATG2G20050	Protein phosphatase 2C and cyclic nucleotide-binding	Signal transduction, ATP binding, metal ion binding, protein serine phosphatase activity	0.837
ATG2G35680	Phosphotyrosine protein phosphatase superfamily protein	Possess phosphate activity	0.698
AT3G12370	EMB3136—Ribosomal protein L10 family protein	The function is a structural protein	0.691
AT3G10330	Cyclin-like family protein	DNA-templated transcription, initiation, transcription preinitiation complex assembly	0.637
pBRP2	Plant-specific TFIIB-related protein 2	Regulation of endosperm proliferation, DNA-templated transcription, initiation	0.637
AT2G45910	U-box domain-containing protein kinase family protein	Cellular response to oxygen-containing compound, defense response to the bacterium, flower development,	0.633

Table 2. *Cont.*

Gene Name	Protein Family	Putative Function	Interactive-Bit Score
ENDOL9	Early nodulin-like protein 9	electron carrier activity	0.623
ATGRIP	Golgi-localized grip domain-containing protein	Involved in Golgi protein trafficking. AtARL1 binds directly to the GRIP domain of AtGRIP in a GTP-dependent manner.	0.616
AT2G04940	scramblase-like protein	plasma membrane phospholipid scrambling	0.603
MLO1	Transmembrane domain protein	barely mildew resistance	0.588

3.6. TaTLPs Cis-Regulatory Elements

According to the results of the in-silico analyses of the *TaTLP*-genes, the upstream region of the *TaTLP* genes contained 13 hormonal, stress, and growth responsive cis-regulatory elements, six of which were responsive to hormones. Seven of these elements were responsive to stress and growth-related changes (Table 3). The hormone-responsive cis-elements were ABRE, which participates in the abscisic acid responsiveness, TCA (cis-acting element involved in salicylic acid responsiveness), TATC-Box (gibberellin-responsive element), AuxRR-Core (auxin-responsive element), CGTCA (cis-acting regulatory element involved in the MeJA-responsiveness), and TGACG (Cis-acting regulatory element involved in the MeJA-responsiveness). The stress and growth responsive cis-elements were ARE (stimulate mRNA decay), ACE (cis-acting element involved in light responsiveness), G-Box (cis-acting element involved in light responsiveness), LTR (Long-terminal repeat), CAT-Box (Cis-acting element involved in meristem development), O2-Site (Cis-acting regulatory element involved in zein metabolism regulation), and MSA-Like (Cis-acting regulatory element involved in the cell cycle). The presence of these cis-elements in the promoter region of *TaTLP* genes indicates that they regulate gene expression in response to various environmental stimuli at various stages of development.

Table 3. Analysis of the diverse types and numbers of cis-acting regulatory elements involved in growth, development, stress, and hormonal response.

Category	Cis-Elements	Annotations
Hormone	ABRE	Cis-acting element involved in the abscisic acid responsiveness.
	TCA	Cis-acting element involved in salicylic acid responsiveness.
	TATC-Box	Gibberellin-responsive element.
	AuxRR-Core	Auxin-responsive element.
	CGTCA	Cis-acting regulatory element involved in the MeJA-responsiveness.
	TGACG	Cis-acting regulatory element involved in the MeJA-responsiveness.
Stress and Growth	ARE	Stimulate mRNA decay.
	ACE	Cis-acting element involved in light responsiveness.
	G-Box	Cis-acting element involved in light responsiveness.
	LTR	Long-terminal repeat.
	CAT-Box	Cis-acting element involved in meristem development.
	O2-Site	Cis-acting regulatory element involved in zein metabolism regulation.
	MSA-Like	Cis-acting regulatory element involved in the cell cycle.

3.7. Expression Analysis of TaTLP Genes in Different Wheat Cultivars in Response to Fusarium graminum Stress

The gene expression pattern in different wheat cultivars subjected to *Fusarium graminum* stress was drawn on the heatmap (Figure 5). The *TaTLP7, TaTLP22, TaTLP26, TaTLP27, TaTLP35,* and *TaTLP36* showed dominant expression in all cultivars compared to other *TLPs* genes, whereas *TaTLP9, TaTLP12, TaTLP20,* and *TaTLP39* showed dominant expression in annonng0771 and zhongmai66 cultivars as compared to the control and sumai3. The *TaTLP6* showed low expression levels in annonng0771 and zhongmai66 cultivars compared to the

control and sumai3. The *TaTLP*6 showed lower expression levels for cultivars annonng0771 and zhongmai66 than the control and sumai3 cultivar, which showed low expression levels. Similarly, *TaTLP15* and *TaTLP30* showed lower expression levels for cultivar zhongmai66, and in comparison, the control, sumai3, and annonng0771 showed higher expression levels. *TaTLP10, TaTLP37,* and *TaTLP24* displayed higher expression levels for annonng0771 and zhongmai66 cultivars, and lower expression levels were recorded for the control and sumai3. The *TaTLP1, and TaTLP2,* showed lower expression levels in sumai3 than the control, annonng0771, and zhongmai66 cultivars. The *TaTLP4* and *TaTLP31* showed high expression levels in the control and sumai3 compared to annonng0771 and zhongmai66 cultivars. Furthermore, *TaTLP8, TaTLP23, TaTLP29, TaTLP3, TaTLP25, TaTLP38, TaTLP13* and *TaTLP28* displayed high expression levels as compared to *TaTLP17, TaTLP18, TaTLP5, TaTLP16, TaTLP33, TaTLP32, TaTLP40, TaTLP19, TaTLP11, TaTLP21, TaTLP14*, and *TaTLP*34 which displayed low expression levels, except for *TaTLP19, TaTLP11*, and *TaTLP21* which showed higher expression levels for annong0711 cultivar under *Fusarium graminum* stress.

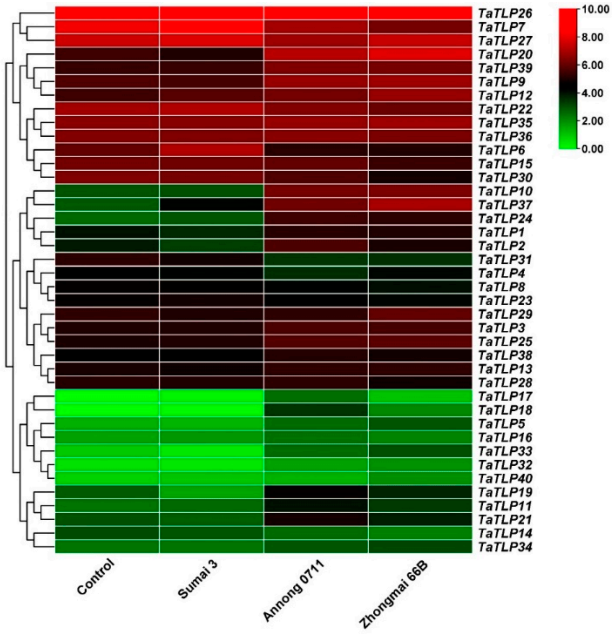

Figure 5. Heatmap representing expression analysis of *TaTLP* genes in different wheat cultivars subjected to *Fusarium graminum* stress.

3.8. Expression Analysis of TaTLP Genes in Wheat in Response to Different Temperatures

The gene expression pattern in response to temperature stress was drawn on the heatmap (Figure 6). *TaTLP*10, *TaTLP*15, *TaTLP*28, *TaTLP*2 *TaTLP*34, *TaTLP*24, *TaTLP*26, *TaTLP*16, *TaTLP*30, *TaTLP*7, *TaTLP*40, *TaTLP*20, *TaTLP*31, *TaTLP*38, *TaTLP*22, *TaTLP*14, and *TaTLP*17 displayed a high expression level under temperatures of 20 and 30 °C, whereas *TaTLP*24 and *TaTLP*22 showed the highest expression levels in comparison to other *TaTLP* genes. Similarly, *TaTLP*25 and *TaTLP*39 showed higher expression levels at 30 °C than under 20 or 40 °C.

The role of *TaTLP* proteins in response to temperature stress is of great interest. To reveal the potential role of *TaTLP* genes in response to temperature stress, we used qRT-PCR to detect *TaTLP* expression levels for 0, 3, and 6 h stress at 40 °C (Figure 7). *TaTLP* genes were found to respond positively to temperature stress. For example, *TaTLP1, TaTLP 2, TaTLP6, TaTLP8,* and *TaTLP15* expression increased at 3 and 6 h temperature stress, indicating that these genes are stress responsive in wheat. Further, *TaTLP3, TaTLP12, TaTLP16*, and *TaTLP32* expression significantly decreased under high-temperature stress. *TaTLP4, TaTLP5, TaTLP10. TaTLP17, TaTLP34,* and *TaTLP36* expression decreased under

3 h temperature stress, and, as temperature stress increased, the expression levels of these genes significantly increased.

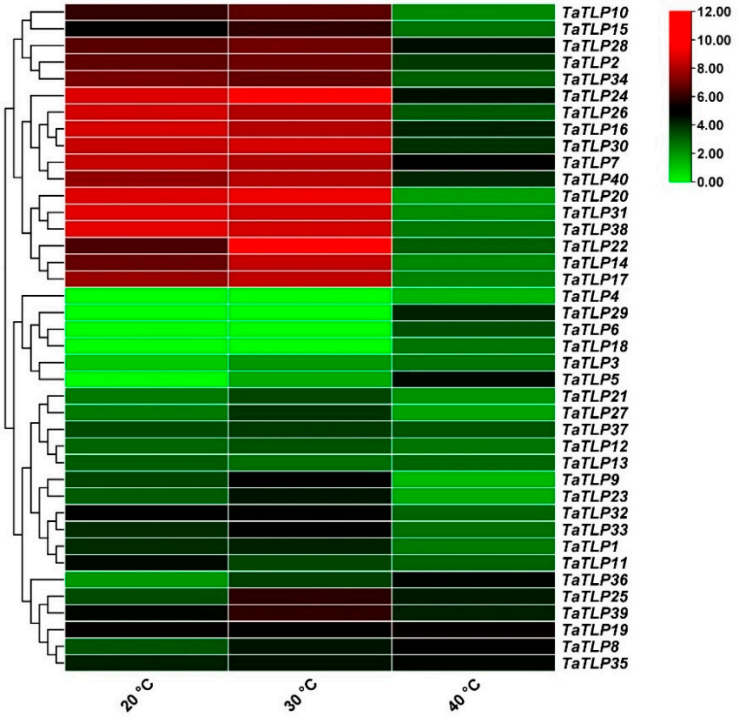

Figure 6. Heatmap representing expression analysis of *TaTLP* genes in wheat subjected to different temperatures.

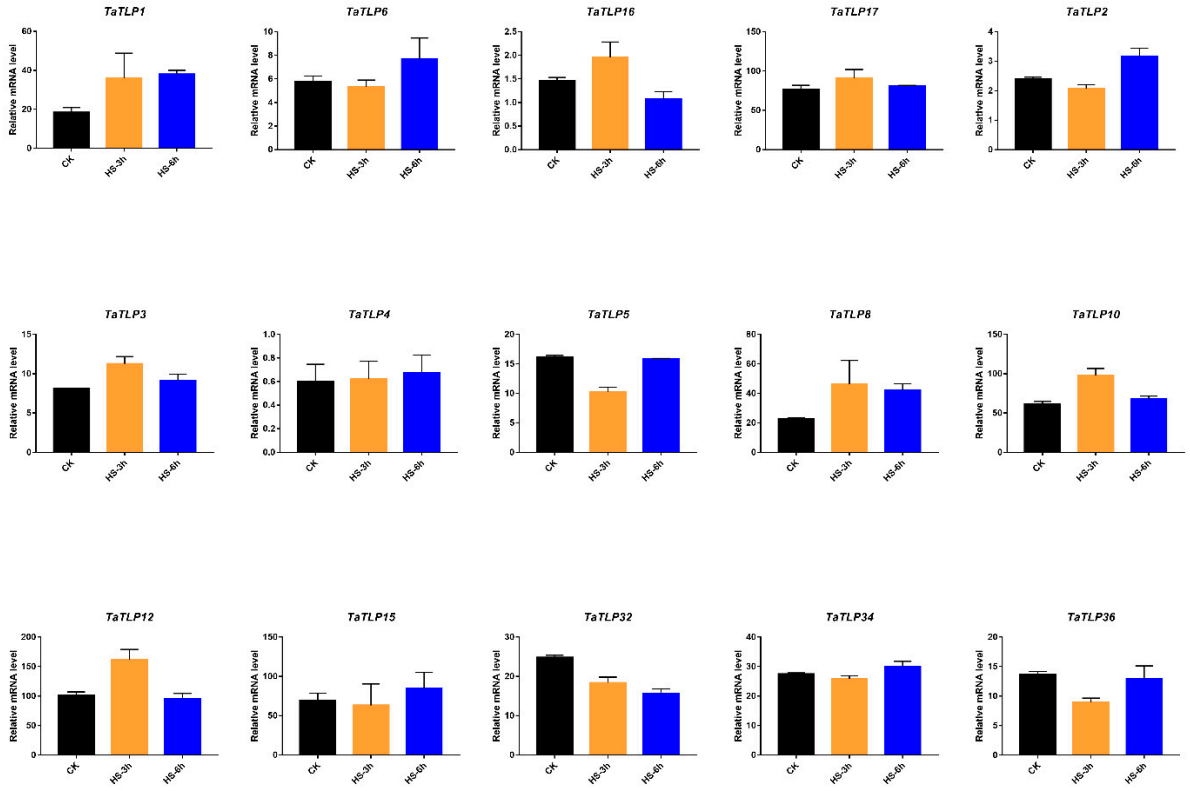

Figure 7. Expression analysis of *TaTLP* genes under high temperature at different time points.

3.9. Expression Analysis of TaTLP Genes in Wheat Subjected to Hormonal Treatment

The heatmap analysis showed different expression patterns under various hormonal treatments. *TaTLP*11, *TaTLP*2, *TaTLP*9, *TaTLP*12, *TaTLP*34, *TaTLP*13, *TaTLP*35, *TaTLP*30, *TaTLP*22, *TaTLP*23, *TaTLP*29, *TaTLP*10, *TaTLP*4, *TaTLP*37 *TaTLP*3 *TaTLP*19, *TaTLP*38, and *TaTLP*17 showed high levels of expression in comparison to other *TATLP* genes under control (CK) and a similar expression patterns were observed for ABA (abscisic acid) treatment, whereas *TaTLP*11, *TaTLP*2, *TaTLP*9, and *TaTLP*34 showed the highest expression level under CK and ABA treatments (Figure 8). Furthermore, *TaTLP*40, *TaTLP*11, *TaTLP*2, *TaTLP*9, *TaTLP*12, *TaTLP*34, *TaTLP*13, *TaTLP*35, *TaTLP*30, *TaTLP*22, *TaTLP*23, *TaTLP*3 *TaTLP*19, *TaTLP*38, *TaTLP*17, *TaTLP*1, and *TaTLP*27 showed a high expression level under GA (gibberellic acid) treatment as compared to other *TLP* genes. The expression pattern of *TaTLP* genes in response to the combination of ABA and FG (*Fusarium graminum*) treatment showed a similar expression pattern as that of GA treatment. The expression pattern of *TaTLP* genes in response to the combination of GA and FG was different; for example, *TaTLP*16, *TaTLP*25, *TaTLP*7, *TaTLP*15, *TaTLP*11, *TaTLP*12, *TaTLP*34, *TaTLP*13, *TaTLP*35, *TaTLP*22, *TaTLP*23, *TaTLP*37, *TaTLP*19, *TaTLP*38, *TaTLP*17, *TaTLP*1, and *TaTLP*27 displayed high expression levels as compared to other *TLP* genes.

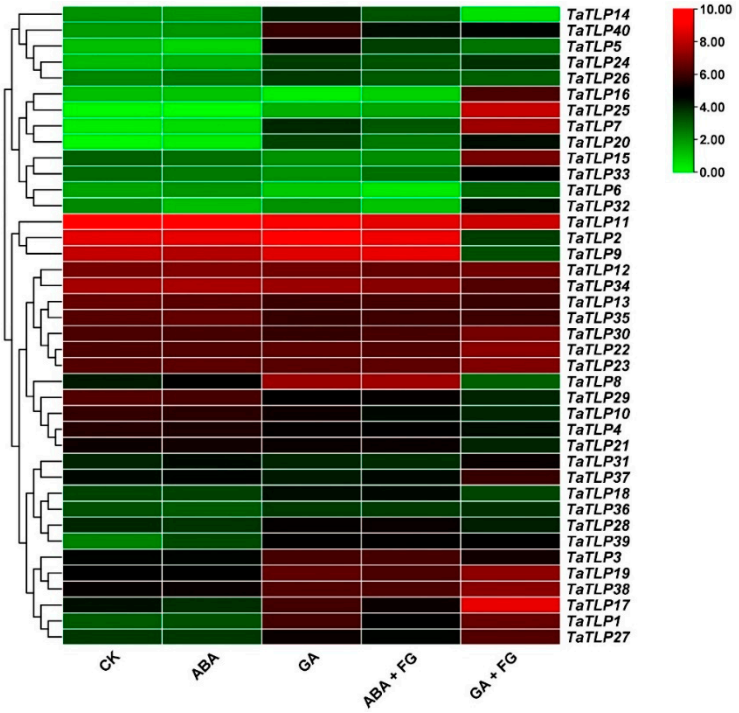

Figure 8. Heatmap represents expression analysis of *TaTLP* genes in wheat subjected to hormonal treatment and hormones + *Fusarium graminum.* ABA (abscisic acid), GA (gibberellic acid), ABA+FG (abscisic acid + *Fusarium graminum*), GA + FG (gibberellic acid + *Fusarium graminum*).

3.10. Expression Analysis of TaTLP Genes in Wheat in Response to Iron Deficiency Stress

The heatmap analysis showed varying expression patterns under iron deficiency conditions in roots and leaf tissues, as shown in Figure 9. *TaTLP7, TaTLP17, TaTLP24, TaTLP16, TaTLP22, TaTLP2, TaTLP7, TaTLP710, TaTLP7,14, TaTLP720, TaTLP32, TaTLP35,* and *TaTLP39* displayed the highest expressions levels as compared to other *TLP* genes in root-control and root low-Fe conditions, whereas *TaTLP*26 showed high expression as compared to root-control. The remaining *TLP* genes' expression levels were unaffected under root low-Fe conditions. Similarly, *TaTLP7, TaTLP17, TaTLP24, TaTLP16, TaTLP22, TaTLP10, TaTLP14,* and *TaTLP20* showed high expression levels as compared to other *TLP*Ss, but similar expression levels between leaf-control and leaf low-Fe conditions. *TaTLP*34 showed a high expression level under the leaf low-Fe condition, whereas *TaTLP*40 and

*TaTLP*1 showed a high expression level in leaf-control compared to the leaf low-Fe condition. The remaining *TLP* gene expression levels were unaffected under leaf low-Fe conditions.

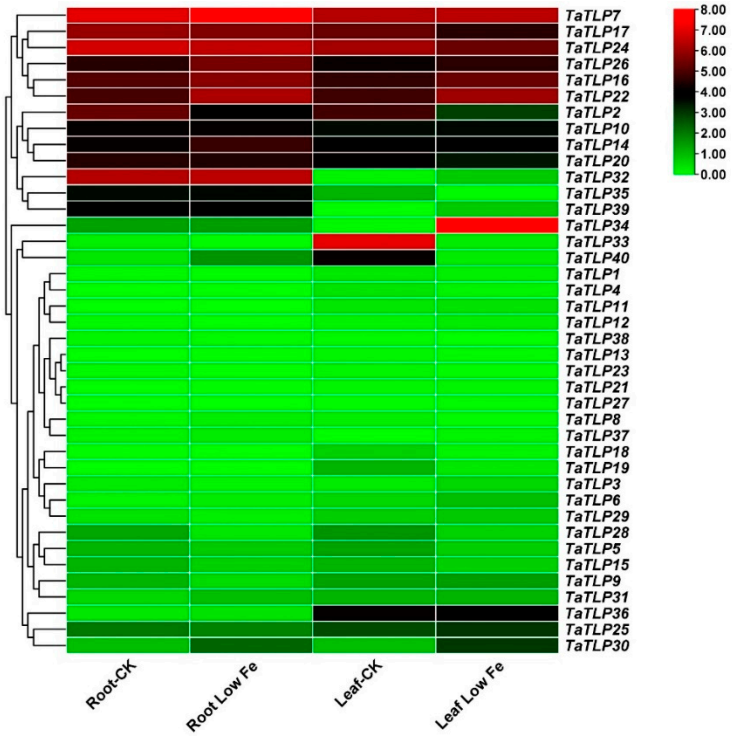

Figure 9. Heatmap representing expression analysis of *TaTLP* genes in wheat under iron deficiency stress.

3.11. Expression Analysis of TaTLP Genes in Different Wheat Tissues

To further study the responses of *TaTLP* genes against biotic and abiotic stresses, we used qRT-PCR to analyze the expression patterns in various wheat tissues (Figure 10). The results showed that *TaTLP*s were expressed in different tissues. The *TaTLP1* transcript level showed high expression in the leaf compared to other tissues. *TaTLP6* and *TaTLP16* showed significantly high expression levels in the root, leaf, and spikelet. *TaTLP17* and *TaTLP6* showed significantly high expression in the stem and leaf, respectively.

Similarly, *TaTLP3* and *TaTLP4* showed significantly high expression levels in leaf, stem, and spikelet tissues. *TaTLP5* was highly expressed in the leaf and stem compared to other tissues, whereas *TaTLP8* expression level was higher in the leaf. *TaTLP10* was expressed in almost all tissues, and the *TaTLP12* expression level was much higher in spikelets than in other tissues. *TaTLP15* and *TaTLP32* were expressed in all tissues, and the expression level was significantly high in the stem and leaf, respectively. *TaTLP34* showed significantly high expression levels in spikelets. *TaTLP36* showed a high expression level in the root, stem, and spikelet. The various *TaTLPs* were expressed in different tissues at varying levels, indicating that they may play a significant role in wheat against various biotic and abiotic stresses.

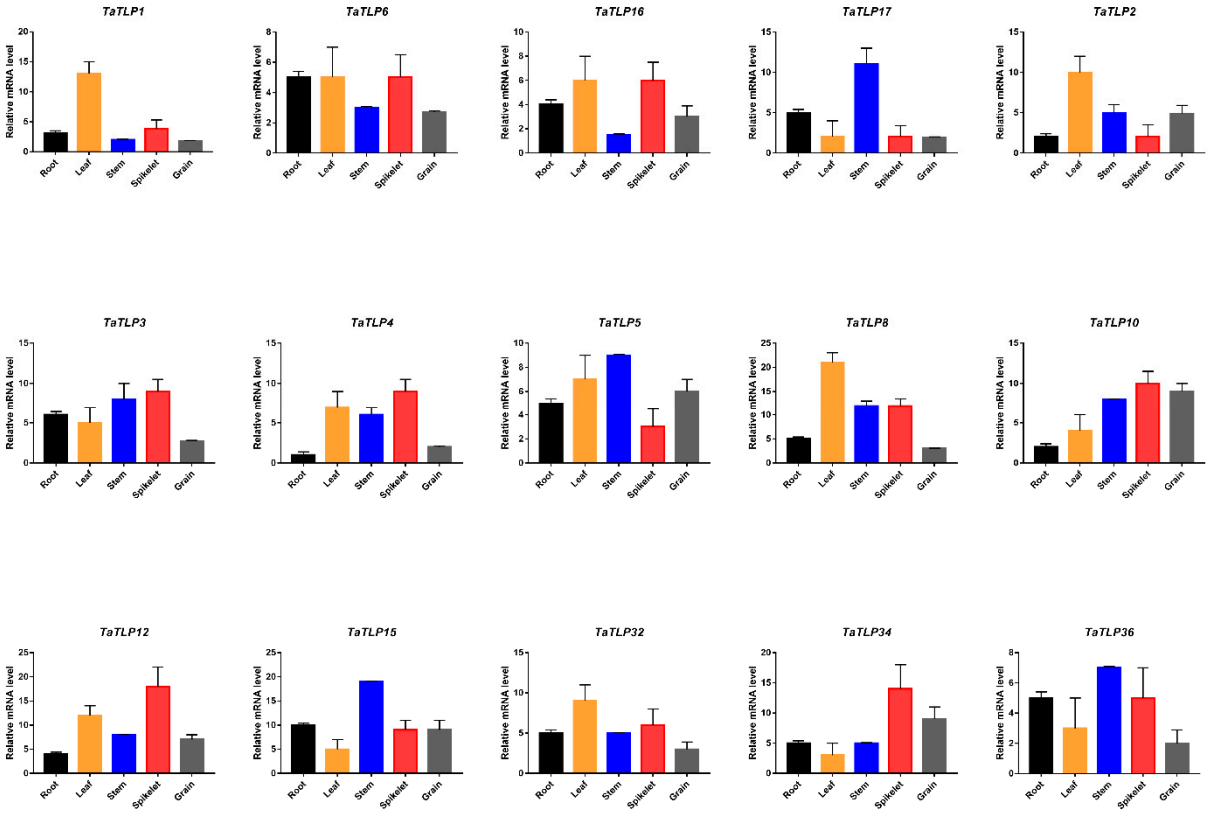

Figure 10. Expression analysis of *TaTLP* genes in different tissues of wheat.

4. Discussion

Plants contend with various environmental conditions throughout their life cycle that may interfere with their development. *TaTLP* genes are members of a gene family found in multiple animals. Plants have a smaller number of functionally studied *TLPs* than animals. *TLPs* have only been discovered in a few plant species, including Arabidopsis [13], rice [7], maize [10], *Solanum lycopersicum* [11], and cotton [12]. This study found 40 genes encoding *TLP* proteins in wheat, which is higher than the numbers found in other plants: 11 in Arabidopsis, 14 in rice, and 15 in maize. This research will advance the knowledge and understanding of their functional characteristics in the future.

4.1. TaTLP Genes Are Distributed Widely in the Wheat Genome

The hexaploid wheat, created by crossing Triticum and Aegilops, is a valuable tool for studying allopolyploidization evolution [24]. An analysis of the phylogenetic tree (Figure 1) shows that the *TaTLP*s are clustered and divided into three large subfamilies: A, B, and C. The A subfamily has two groups: AI and AII. This grouping matches previous *S. lycopersicum* reports [8,10,11]. The *TLP*s within each subfamily share a high degree of homology and have evolved close to one another [25]. Interestingly, we found that *TLP*s possess the F-box domain related to plant stress resistance [13,26,27]. This finding suggests that *TLP*s in wheat are highly conserved and may have additional functions, as demonstrated in Figure 3.

4.2. TaTLP Genes Are Thought to Be Involved in Critical Biological and Molecular Processes

The GO analysis revealed that *TaTLP* genes perform a wide range of biological, molecular, and cellular functions (Figure 3). Many genes are directly involved in cell wall biosynthesis, which is the first line of defense against abiotic and biotic factors [28]. The TUBBY gene family appears to be essential for wheat plant growth in both normal and stressful conditions. Trans-acting elements are required for any biological or molecular process in plants. Multiple signaling pathways regulate plant stress responses, and there is

much overlap between the gene expression patterns induced by different stresses [29–31]. Several transcription factors influence the expression of stress-related genes in plants. Several closely related transcription factors can frequently activate or repress genes via cis-acting sequences in response to specific stresses [14,32]. In our study, many hormones (ABRE, TCA, TATC-BOX, AUXRR-Core, CGTCA, and TGACG), stress, and growth-related (ARE, ACE, G-Box, LTR, CAT-Box, O2-Site, MSA-Like) cis-elements were identified in the promoter region of *TaTLP* genes (Table 3). These elements are primarily involved in drought, low-temperature, and hormone responses [33,34].

The CGTCA and TGACG motif were found in nearly all *TLP* promoters, indicating that they were associated with the jasmonate acid response in most cases. Also found in most *TLP* promoters where the enzymes ARE (associated with anaerobic reaction) and ABRE (associated with ABA response) [7,8,13,25]. Based on these results, we suggest that *TaTLP*s may play an essential role in stress responses, but this needs further experimental verification. The PPI analysis demonstrated that *TaTLP*s interact with other essential proteins, such as ATG2G20050, which play an indispensable role in signal transduction, ATP binding, metal ion binding, and protein serine phosphatase activity. Similarly, the *TLP* gene interacted with ATG2G35680, AT3G12370, AT3G10330, pBRP2, AT2G45910, ENDOL9, ATGRIP, AT2G04940, and MLO1 (Table 2 and Figure 4).

Plant *TLP* gene families have been previously studied, and it has been discovered that multiple *TLP* genes are involved in the responses of plants to biological and abiotic stresses [7,8,10–13]. According to this, *TLP* genes can be used as candidate genes in plant resistance breeding.

4.3. TaTLP Genes Control Plant Response to Hormones and Abiotic and Biotic Stresses

To further investigate the response of *TaTLP*s to abiotic stress, the expression patterns of 40 putative *TaTLP*s in wheat were determined using a heatmap analysis and confirmed through qRT-PCR to analyze the expression patterns in various tissues and under temperature stress (Figures 7 and 10). TaTLP genes were induced to varying degrees under multiple conditions, including high temperature, GA, exogenous ABA, and low iron deficiency stress. Due to their immobility, plants face abiotic stresses. Abiotic stresses can significantly reduce crop yields by impeding their physiological and biochemical processes [35,36]. Modern research breakthroughs have relied heavily on understanding the impact of changing climate. The underlying mechanism in systematic temporal variation is complex and challenging to comprehend. Our current results showed that many *TaTLP* genes, such as *TaTLP16, TaTLP20, TaTLP22*, and *TaTLP24*, displayed upregulated expression patterns under different degrees of temperature stress. The findings of this study are consistent with those of previous studies [11,12,25]. GA is a plant hormone involved in seed germination, phase transition, flowering, fruit, and grain development [37–39]. Here, *TaTLP40, TaTLP11, TaTLP2, TaTLP9, TaTLP12, TaTLP34, TaTLP13, TaTLP35, TaTLP30, TaTLP22, TaTLP23, TaTLP3 TaTLP19, TaTLP38, TaTLP17, TaTLP1*, and *TaTLP27* showed higher expression levels under GA treatment (Figure 8). Our findings suggest that genes may be involved in GA-mediated plant growth activities, but more research is needed. Most *TaTLP* genes showed a decrease in expression in response to ABA, FG, and a combination of these factors, in addition to low iron deficiency stress.

5. Conclusions

This study identified and analyzed 40 *TLPs* in wheat (*Triticum aestivum* L.*)*. We performed a comprehensive analysis of *TaTLPs* that included gene identification, phylogenetic analysis, chromosomal location, protein–protein interactions, cis-regulatory elements, and expression analysis. Forty *TaTLP*s were identified and classified into three subfamilies based on their domain and structural characteristics. A heatmap analysis revealed the expression of *TaTLPs* in different cultivars in response to biotic and hormonal stress. The qRT-PCR analysis showed that the expression patterns under high temperature and in various wheat tissues were significantly high, suggesting that these genes may play a

role in wheat resistance mediation. Stress regulation is also a complicated mechanism to comprehend. The in-silico analysis provided valuable information for future functional stress biology studies. More research is needed to fully understand the regulation and pathways of the mechanism of *TaTLPs* in wheat.

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