Genome-wide analysis and functional characterization of CHYR gene family associated with abiotic stress tolerance in bread wheat (Triticum aestivum L.)

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Abstract
Background: CHY zinc-finger and RING finger (CHYR) proteins have been functionally characterized in plant growth, development and various stress responses. However, the genome-wide analysis was not performed in wheat.
Results: In this study, a total of 18 TaCHYR genes were identified in wheat and classified into three groups. All TaCHYR genes contained CHY-zinc finger, C3H2C3-type RING finger and zinc ribbon domains, and group III members included 1–3 hemerythrin domains in the N terminus regions. TaCHYR genes in each group shared similar conserved domains distribution. Chromosomal location, synteny and cis-elements analysis of TaCHYRs were also analyzed. Real-time PCR results indicated that most of selected 9 TaCHYR genes exhibited higher expression levels in leaves during wheat seedling stage. All these TaCHYR genes were up-regulated after PEG treatment, and these TaCHYRs exhibited differential expression patterns in response to salt, cold and heat stress in seedling leaves. The growth of yeast cells expressing TaCHYR2.1, TaCHYR9.2 and TaCHYR11.1 were inhibited under salt and dehydration stress. Moreover, gene ontology (GO) annotation, protein interaction and miRNA regulatory network of TaCHYR genes were analyzed.
Conclusions: These results increase our understanding of CHYR genes and provide robust candidate genes for further functional investigations aimed at crop improvement.
Keywords: CHYR genes, Genome-wide identification, Gene expression, Abiotic stress, Wheat (Triticum aestivum L.)

Background
Wheat (Triticum aestivum L.) is one of the most important grain crops in the world. However, as sessile organism, wheat typically suffers from various adverse conditions including drought, salinity, cold or high temperature during the growth and development periods, which can directly affect the overall wheat production. Therefore, plants have developed complex regulatory mechanisms to avoid or defend against adverse conditions in the long-term evolution process [1]. CHYR protein (CHY zinc-finger and RING finger protein) is one of important stress-responsive protein to respond to abiotic stress in plants [2, 3].

CHYR proteins contain CHY-zinc finger and C3H2C3-type RING finger domains [2]. According to conserved motifs and phylogenetic relationships analysis, CHYR proteins are essentially classified into three groups: group I, II, and III [2]. CHY zinc-finger domain is defined based on its N-terminal conserved amino acid sequences ‘CXHY’, this domain contains many conserved cysteine and histidine residues, which can play roles in physical interaction, ubiquitination and binding to zinc ions.
[3–6]. C3H2C3-type RING finger domain, also referred to as RING-H2 finger, exists in the C-terminal region of CHYR protein, that can bind two atoms of zinc and may be involved in mediating protein–protein interactions [4, 7]. Besides, group III members, also named as BTS/BTSL (BRUTUS/BRUTUS-like) or HRZ (hemerythrin RING zinc-finger) proteins, which contain N-terminal hemerythrin (HHE) domains and play an essential role in regulating iron homeostasis [8–11].

CHYR proteins, belonging to RING-type E3 ubiquitin-protein ligase, perform vital functions in plant growth, development and various stress responses via protein ubiquitination degradation [3, 8, 12]. Arabidopsis AtCHYR1 gene can positively regulate stomatal closure and improve drought tolerance via SnRK2.6-mediated phosphorylation [3]. PeCHYR1, which was isolated from Populus euphratica, also enhances drought tolerance via ABA-induced stomatal closure caused by hydrogen peroxide (H₂O₂) production in transgenic poplar plants [13]. However, the homologous gene of AtCHYR1, rice (Oryza sativa) OsRZFP34 gene enhances stomatal opening [14]. MIEL1 can interact with transcription factor MYB30 resulting in MYB30 proteasomal degradation and the suppression of Arabidopsis defense and hypersensitive responses (HR) [12, 15]. The MIEL1 also negatively regulates cuticular wax biosynthesis in Arabidopsis stems [15]. BTS genes play crucial roles in drought stress responses by facilitating the degradation of transcription factors Vascular plant One-Zinc finger 1/2 (VOZ1/2) protein [16]. BTS also plays an essential role in regulating iron homeostasis of plants [11, 17].

CHYR genes have been identified in diverse plants, such as maize (Zea mays), Arabidopsis, rice, and soybean (Glycine max) [2, 8, 14, 18], however, a genome-wide identification of CHYR genes in wheat was not performed. In this study, a genome-wide analysis of CHYR genes was performed in wheat to characterize their sequences, evolutionary relationships, expression patterns and stress tolerance in yeast cells under various abiotic stress treatments. These results will provide a valuable foundation for further functional studies of TaCHYR genes under abiotic stress.

**Results**

**Characteristics and phylogenetic analysis of TaCHYRs in T. aestivum**

A total of 18 CHYR genes, encoding 50 transcripts, were identified from a wheat genome based on a Hidden Markov Model (HMM) search against of a CHY zinc-finger domain (PF05495) (Table S1). According to the chromosome position and phylogenetic relationship of TaCHYR genes, we named them from TaCHYR1 to TaCHYR18. These 18 predicted TaCHYR genes encoded polypeptides of 245 (TaCHYR7.2) to 1245 (TaCHYR17.8) amino acid residues with the putative molecular weights ranging from 28.03 to 140.21 kDa. The isoelectric point (pI) value varied from 5.59 (TaCHYR16.5) to 7.55 (TaCHYR9.2), and the calculated grand average of hydropathic index (GRAVY) was from -0.166 (TaCHYR6.2 and TaCHYR6.3) to -0.484 (TaCHYR11.2), suggesting that these 18 TaCHYR genes were hydrophilic proteins. In addition, the subcellular localization prediction indicated that TaCHYR proteins were all located in the nucleus.

To further investigate the phylogenetic relationship of TaCHYRs, AtCHYRs and GmCHYRs were also used to construct a phylogenetic tree (Fig. 1 and Table S2), the results indicated that TaCHYR genes were divided into 3 subfamilies: group I, II, and III (Fig. 1 and Fig. 2A), which was consistent with previous phylogenetic analyses of plant CHYR genes [2]. Group I (TaCHYR1–6), group II (TaCHYR7–12) and group III (TaCHYR13–18) subfamilies included 6 members in wheat, respectively (Fig. 1 and Table S1).

**Gene structure and conserved motifs analysis of TaCHYR genes**

To investigate the structural characteristics of TaCHYR genes, the exon–intron structures and conserved motifs of 18 TaCHYR genes were analyzed (Fig. 2). The TaCHYR genes contained 10–15 exons, the coding sequence (CDS) of group III members were longer than that of other two groups, and most genes with closer evolutionary relationships had similar exon–intron structures (Fig. 2B). Furthermore, the conserved domains of TaCHYRs were analyzed, the results showed that all TaCHYRs contained CHY-zinc finger, C3H2C3-type RING finger and zinc ribbon domains (Fig. 2C and Fig. 3). These results further verified the reliability of the identified TaCHYR gene family members. Besides, group III members contained 1–3 hemerythrin domains in the N-terminus. Meanwhile, we predicted the conserved motifs of TaCHYR gene family using MEME tools (Fig. 2C and Fig. S1). Among the detected 10 motifs, motif 2, 4 and 6 formed CHY-zinc finger domain, motif 3 and 9 made up RING finger domain. Zinc ribbon domain consisted of motif 7 and 9. Hemerythrin domain included motif 8 and 10. Additionally, motif 1, 5 and 10 were unique to group III members. These results indicated that the composition of conserved motifs were varied among different CHYR subfamilies, but TaCHYRs with closer evolutionary relationships had more similar conserved domains.

**Chromosomal location, synteny and Ka/Ks analysis of TaCHYRs**

The chromosomal location and synteny of TaCHYR gene family were analyzed according to their genomic
Fig. 1 The neighbor-joining (NJ) phylogenetic tree of CHYR proteins. The tree was constructed with protein sequences encoded by the longest transcript of each CHYR gene in *T. aestivum* (Ta), and CHYR proteins sequences in *A. thaliana* (At) and *G. max* (Gm) with bootstrap values of 1000 replicates. Different groups of CHYR proteins were distinguished by different colors.

Fig. 2 Phylogenetic classification A, exon–intron structures B and conserved domains C analysis of TaCHYR genes. A The neighbor-joining (NJ) phylogenetic tree was constructed with protein sequences encoded by the longest transcript of each CHYR gene in *T. aestivum* with bootstrap values of 1000 replicates. B Green boxes, yellow boxes and black lines indicated UTR, exons and introns, respectively. C Conserved domain compositions of TaCHYR proteins in wheat. CHY-zinc finger, C3H2C3-type RING finger, zinc ribbon and hemerythrin domains and motif 1–10 were showed by different colors.
sequences (Fig. 4 and Fig. S2). TaCHYR genes were distributed on chromosome 1, 3, 4, and 5. Eighteen TaCHYR genes were evenly distributed among A, B, and D subgenomes: TaCHYR1, 4, 7, 10, 13 and 16 were located on A subgenome; TaCHYR2, 5, 8, 11, 14 and 17 on B subgenome; TaCHYR3, 6, 9, 12, 15 and 18 on D subgenome.

Chromosome 1A, 1B, 1D, 3A, 3B and 3D contained two TaCHYR genes, respectively. Chromosome 4A, 4B, 4D, 5A, 5B and 5D contained only one TaCHYR gene, respectively (Fig. S2). A total of 22 paralogous TaCHYR gene pairs were determined in wheat genome, which all underwent WGD (whole genome duplications) or segmental duplication events (Fig. 4 and Table S3). Eighteen genes were all involved in gene duplication events, and these results indicated that TaCHYR genes might be produced by segmental duplication events and these duplication events might contribute to the evolution of TaCHYR genes. Moreover, the non-synonymous (Ka) and synonymous substitution (Ks) ratios were calculated to investigate selective pressure of these 22 paralogous gene pairs (Table S3). The Ka/Ks ratios of paralogous gene pairs were all less than 1, indicating these TaCHYR genes were under strong purifying selection to maintain the function of TaCHYR gene family.

Furthermore, we analyzed the synteny of CHYR genes between T. aestivum (AABBDD, hexaploid) with T. urartu (AA, diploid), Ae. tauschii (DD, diploid), B. distachyon (diploid) and O. sativa (diploid) (Fig. 5 and Table S4). The orthologous gene pairs of CHYR genes were identified between T. aestivum with T. urartu (15), Ae. tauschii (22), B. distachyon (22) and O. sativa (20), respectively. And 11, 17, 14 and 15 TaCHYR genes showed collinearity relationships with CHYR genes in T. urartu, Ae. tauschii, B. distachyon and O. sativa, respectively. Moreover, the synteny analysis showed 3 orthologous gene pairs which located on the same chromosomes between wheat A subgenome and T. urartu were identified with two on chromosome 1A (TaCHYR1/TuG1812G0100001751, TaCHYR13/TuG1812G0100004139), and one on chromosome 5A (TaCHYR10/TuG1812G0500001083). Similarly, 6 orthologous gene pairs between wheat D subgenome and Ae. tauschii were identified which located on the same chromosomes with two on chromosome 1D (TaCHYR15/AET1Gv20902300, TaCHYR17/AET1Gv20902300), two on chromosome 3D (TaCHYR6/AET3Gv20682500, TaCHYR18/AET3Gv20635000), one on chromosome 4D (TaCHYR9/AET4Gv20335500), and one on chromosome 5D (TaCHYR12/AET5Gv20264800) (Fig. 5 and Table S4). These TaCHYR genes might be originated from orthologous genes in T. urartu and Ae.
tauschii with the occurrence of natural hybridization events.

**Cis-elements analysis of TaCHYR genes**

The variable cis-elements in the gene promoters might indicate that these genes perform different functions in plant growth, development and various stress responses. To further investigate the functions of TaCHYR genes, 1.5 kb of the upstream region of each TaCHYR gene transcription start site (TSS) was extracted and then analyzed by using the PlantCARE database to identify and count the cis-elements (Fig. 6). These cis-elements were divided into four categories, i.e., stress responsive-, light responsive-, hormone responsive-, and growth and development related-elements. The promoter of TaCHYR genes included masses of stress and hormone responsive-elements, especially DRE (drought-responsive element), MBS (MYB binding site), MYC and STRE (stress-responsive element) among stress responsive-elements, and ABRE (ABA-responsive element) and CGTCA-motif (MeJA-responsive element) among hormone responsive-elements. Additionally, group I members had abundant ARE (anaerobic induction-responsive element). G-box abounded in TaCHYR promoters, which could interact with bZIP or bHLH transcription factors to participate in biological processes [19]. These results indicated that the TaCHYR genes might play significant roles in plant growth, development and respond to various stresses in wheat.

**GO annotation and network construction of TaCHYRs**

To further understand the function of TaCHYR from molecular levels, all TaCHYR proteins were annotated by gene ontology (GO) (Fig. 7A and Table S6). These TaCHYR proteins were assigned with 22 GO terms belonging to the cellular component, molecular function and biological process (Fig. 7A). Under the cellular component category, most TaCHYR proteins (74%) were located in the nucleus (GO:0005634). Under the molecular function category, all TaCHYRs were involved in
metal ion binding (GO:0046872). Under the biological process category, most TaCHYRs were involved in protein metabolic process (GO:0019538), protein modification process (GO:0036211), protein catabolic process (GO:0030163), and homeostatic process (GO:0042592). According to protein–protein interactions (PPIs) analysis, TaCHYR proteins could bind other TaCHYR family members or other E3 ubiquitin-protein ligases to form heterodimers (Fig. 7B and Table S7).

The putative microRNA (miRNA) targeting TaCHYR genes were analyzed using psRNATarget tools [20], the results demonstrated that 21 miRNAs were identified to target 15 TaCHYR genes encoding 43 transcripts, while 3 TaCHYR genes were not targeted by miRNAs (Fig. 7C and Table S8). The most transcripts were degraded via cleavage (75.68%), while the rest of TaCHYR transcripts were inhibited by miRNA via translation (24.32%). The tae-miR2275-3p, tae-miR5048-5p, tae-miR9657c-3p, tae-miR9661-5p, tae-miR9664-3p and tae-miR9773 targeted 3 TaCHYR genes, respectively. Besides, tae-miR2275-3p, tae-miR5048-5p, tae-miR9657c-3p, tae-miR9661-5p, tae-miR9664-3p and tae-miR9773 targeted 3 TaCHYR genes, respectively. These results provided a valuable foundation for future functional investigations of TaCHYR genes.

Expression patterns of TaCHYR Genes in different tissues
To investigate the tissue-specific expression patterns of the TaCHYR genes, the gene expression levels of 9 selected TaCHYR genes belonging to group I (TaCHYR1, 2 and 4), group II (TaCHYR8, 9, 11 and 12) and group III (TaCHYR13 and 17) members were determined by using real-time PCR in roots, stems, and leaves tissues during wheat seedling stage (Fig. 8). Most of selected 9 TaCHYR genes exhibited higher expression levels in leaves, such as TaCHYR1, 4, 8, 13, and 17 were highly expressed in leaves. Group I (TaCHYR1, 2 and 4) and group III (TaCHYR13 and 17) members were predominantly expressed in leaves and
stems, followed by roots. Group II (TaCHYR8, 9, 11 and 12) members showed differential expression profiles in roots, stems, and leaves tissues. TaCHYR8 and TaCHYR9 showed highest expression levels in leaves and roots, respectively. TaCHYR11 and TaCHYR12 exhibited the highest expression levels in stems, followed by leaves, and finally in roots. In particular, most paralogous genes showed similar expression patterns, e.g., TaCHYR1/2, TaCHYR11/12, and TaCHYR13/17. However, the paralogous genes TaCHYR8/9 had different expression profiles in various tissues, indicating TaCHYR8 and 9 might have diverse function due to ecological or environmental adaptation. These might be caused by modification in their promoters, some special regulatory elements, or functional segregation with a long-term evolutionary process.

**Expression patterns of TaCHYR genes under abiotic stress**

To insight into the function of TaCHYR genes under abiotic stress, real-time PCR was used to determine the expression patterns of these 9 TaCHYR genes under PEG, salt and heat and cold stress in leaves during the wheat seedling stage (Fig. 9). Under PEG stress, all these TaCHYR genes were up-regulated and reached the highest expression level at 36 h. TaCHYR11 and TaCHYR12 were significantly up-regulated more than 65-fold and 21-fold under PEG stress compared with the control, respectively (Fig. 9A). These TaCHYR genes exhibited differential expression patterns in seedling leaves in response...
to salt, cold and heat stress. In salt stress condition, TaCHYR2 and 4 were obviously up-regulated and reached the highest expression level at 36 h, and TaCHYR8 and 9 were significantly down-regulated (Fig. 9B). After heat stress treatment, most these TaCHYR genes were down-regulated compared with control, except TaCHYR4 was up-regulated (Fig. 9C). Under cold stress, TaCHYR2, 4, 11, 12, 13 and 17 were obviously up-regulated, and the expression of TaCHYR1, 8 and 9 were significantly suppressed compared with control (Fig. 9D). Moreover, the paralogous genes had similar expression patterns under PEG, salt, heat or cold stress. These results suggested that TaCHYR genes had different response to different stress and play various regulatory roles in abiotic stress resistance.

**Negative regulation of dehydration and salt stress tolerance in recombinant yeast cells**

According to the expression profiles, three TaCHYR genes (TaCHYR2.1, TaCHYR9.2, and TaCHYR11.1) were selected to clone into pGADT7 vector, and then transformed into the osmotic-sensitive yeast mutant BY4741 (Δhog1) to verify the ability in response to stress tolerance in yeast mutant cells (Fig. 10). The results suggested that the growth of the Δhog1 yeast cells containing these recombinant vectors (pGADT7-TaCHYR2.1, pGADT7-TaCHYR9.2 or pGADT7-TaCHYR11.1) were inhibited
in YPD medium containing NaCl (0.4 and 0.6 M) and D-Sorbitol (1.0 and 1.2 M) compared with the control (pGADT7 empty vector), and the growth of yeast cells were inhibited more obviously with the increase of NaCl and D-Sorbitol concentration (Fig. 10A and 10B). Moreover, the growth of yeast expressing \( TaCHYR2.1 \) was severely inhibited in salt and dehydration treatment conditions. The growth of yeast expressing \( TaCHYR9.2 \) was similar to \( TaCHYR11.1 \), which was slightly inhibited under salt and dehydration stress. These results revealed that \( TaCHYR2.1 \), \( TaCHYR9.2 \) and \( TaCHYR11.1 \) might participate in protein catabolic and homeostatic process under salt and dehydration stress in wheat.

**Discussion**

Previously, \( CHYR \) genes have been identified in diverse plants, such as maize, \( Arabidopsis \), rice and soybean [2, 8, 14, 18]. However, \( CHYR \) genes have not been genome-wide identified in wheat. In this study, 18 \( CHYR \) genes were identified in wheat and classified into 3 groups (Fig. 1). All \( TaCHYR \) proteins contained CHY-zinc finger, C3H2C3-type RING finger and zinc ribbon domains (Fig. 2C). Besides, there were 1–3 hemerythrin domains
Fig. 9 Expression patterns of TaCHYR genes in response to PEG (polyethylene glycol) A, salt B, heat C and cold D treatments determined by real-time PCR. Wheat seedling leaves were sampled after 0, 12, 24 and 36 h under stress conditions. The expression level of the wheat actin gene was used as the internal control to standardize the RNA samples for each reaction. The values were the mean ± SE from three samples.

Fig. 10 The ability of the tolerance in response to salt A and dehydration B stress in recombinant yeast cells. The osmotic-sensitive yeast mutants Δhog1 carrying the empty vector (pGADT7, negative control), pGADT7-TaCHYR2.1, pGADT7-TaCHYR9.2, or pGADT7-TaCHYR11.1 were spotted onto YPD medium plates containing NaCl (0.4 and 0.6 M) or D-Sorbitol (1.0 and 1.2 M), and cultured at 30°C for 3 d.
in the N-terminus of group III members, which may play vital roles in regulating iron homeostasis [8].

Previous studies showed that CHYR genes occurred during evolution of green plants, group I members were present in Bryophyta, Pteridophyta and Gymnosperms, while group II members were absent, suggesting the differentiation of CHYRs between group I and II members might occur after angiosperm differentiation [2]. Therefore, group I and II members could be found to coexist in monocotyledonous (wheat and rice) and dicotyledonous (Arabidopsis and soybean) plants (Fig. 1 and Fig. 5). Group III members were far away from the other two groups in plants evolution process, indicating that they might originated from different ancestors [2]. In this study, we also analyzed the synteny relationships of CHYR genes between the wheat and other plant species, three orthologous gene pairs that located on the same chromosomes between wheat A subgenome and T. urartu and Ae. tauschii were identified. Similarly, six orthologous gene pairs were identified on the same chromosomes between wheat D subgenome and Ae. tauschii were identified (Fig. 5 and Table S4). These TaCHYR genes might be originated from orthologous genes in T. urartu and Ae. tauschii with the occurrence of natural hybridization events. Moreover, 22 paralogous gene pairs were identified among 18 TaCHYR genes in wheat genome, which all undergone WGD or segmental duplication events and a strong purifying selection pressure (Fig. 4 and Table S3). These results indicated that WGD or segmental duplications played crucial roles in the expansion of the TaCHYR genes.

CHYR genes participated in various biological processes to play vital roles in plant growth, development and various stress responses [3]. Real-time PCR results indicated that most of selected 9 TaCHYR genes exhibited higher expression levels in leaves during wheat seedling stage (Fig. 8), suggesting these TaCHYRs might play an important function in leaves. The tissue-specific expression of TaCHYR genes mainly related with the variable cis-elements present in its promoters (Fig. 6). Under PEG stress, all TaCHYR genes were up-regulated, which might be regulated by masses of stress and hormone responsive-elements in the TaCHYR promoter regions (Fig. 6 and Fig. 9A). Moreover, the similar expression patterns were detected in the paralogous genes, e.g., TaCHYR11/12 were significantly induced more than 65-fold and 21-fold compared with the control after PEG treatment, respectively. However, less stress and hormone responsive-elements were identified in the promoter of TaCHYR11, probably due to the lack of the 425 bp genomic sequences in TaCHYR11 promoter region (Fig. 6 and Fig. S3). Therefore, we speculated that TaCHYR genes played vital roles when plants suffered dehydration stress. MiRNAs also played an important role in regulating the expression of downstream genes [21]. Here, 21 miRNAs were identified, and tae-miR9657a-3p targeted 6 TaCHYR genes, implying tae-miR9657a-3p played a key role in regulating the expression of TaCHYR genes (Fig. 7C and Table S8).

GO annotation analysis indicated TaCHYR genes were E3 ubiquitin-protein ligases (Fig. 7A and Table S6), and regulated the plant adaptive response to abiotic stress via ABA-mediated signaling, and especially through modulating the stability of bZIP and bHLH transcription factor [19, 22]. Previous studies have showed that CHYR1 promoted ABA-induced stomatal closure, reactive oxygen species production, and plant drought tolerance in Arabidopsis, the similar results were also found in poplar [3, 13]. CHYR gene CaASRF1 positively modulates ABA signaling and the drought stress response via modulation of bZIP transcription factor CaAIB1 stability [22]. In contrary, the rice E3 ubiquitin-protein ligase drought-induced SINA protein 1 (OsDIS1) negatively regulated drought stress response through transcriptional or post-translational regulation of various stress-related genes [23]. Rice OsRZFP34 gene enhances stomatal opening, leaf cooling and ABA insensitivity [14]. Arabidopsis E3 ubiquitin ligase PUB11 negatively regulated drought tolerance by degrading the receptor-like protein kinases LRR1 and KIN7 [24]. The C3HC4-type RING finger E3 ubiquitin ligase TaSADR1 negatively regulates drought resistance in transgenic Arabidopsis [25]. In our study, the growth of yeast cells expressing TaCHYR2.1, TaCHYR9.2 and TaCHYR11.1 were inhibited in salt and dehydration treatment conditions, probably caused by the ubiquitination of interacting proteins resulting in degradation of stress-related proteins. In the future, these genes would be knocked out through CRISPR technology to obtain drought-resistant wheat. These results still require to be confirmed in wheat, and E3 ubiquitin-protein ligase activity of TaCHYR genes also require further experimental verification.

Conclusions
In this study, 18 TaCHYR genes were identified, which can be divided into three groups. All TaCHYR genes contained CHY-zinc finger, C3H2C3-type RING finger and zinc ribbon domains, and group III members included 1–3 hemerythrin domains. TaCHYR genes were distributed on chromosome 1, 3, 4, and 5, and evenly distributed among A, B, and D subgenomes. Ka/Ks analysis showed that the TaCHYR genes undergone a strong purifying selection pressure during the evolution process. Twenty-two paralogous gene pairs were identified in wheat, and 15, 22, 22 and 20 orthologous gene pairs were identified between wheat with T. urartu, Ae. tauschii, B. distachyon
and *O. sativa*, respectively. The promoters of TaCHYR genes contained masses of stress and hormone responsive-elements. Real-time PCR results suggested that most of selected 9 TaCHYR genes exhibited higher expression levels in leaves during wheat seedling stage. All these TaCHYR genes were up-regulated after PEG treatment, and these TaCHYRs exhibited differential expression profiles in response to salt, cold and heat stress. The growth of yeast cells overexpressing TaCHYR2.1, TaCHYR9.2 and TaCHYR11.1 was suppressed under salt and dehydration stress. Moreover, gene ontology (GO) annotation, protein interaction and miRNA regulatory network of TaCHYR genes were analyzed. These results provide useful information for further functional studies of TaCHYR genes, and lay a foundation to improve wheat quality traits in molecular breeding under abiotic stress.

**Methods**

**Identification of the CHYR family genes**

The genome sequence of *T. aestivum* was downloaded from EnsemblPlants database ([http://plants.ensembl.org/index.html](http://plants.ensembl.org/index.html)). The Hidden Markov Model (HMM) profiles ([http://pfam.xfam.org](http://pfam.xfam.org)) of CHY zinc-finger domain (PF05495) obtained from Pfam database ([http://pfam.xfam.org](http://pfam.xfam.org)) were used to HMM search against the local genome database of *T. aestivum* using TBtools [26]. All the identified TaCHYR candidates were verified by using Pfam ([http://www.ebi.ac.uk/Tools/hmmer/](http://www.ebi.ac.uk/Tools/hmmer/)) and SMART database ([http://smart.embl.de/](http://smart.embl.de/)), then we retrieved 18 TaCHYR genes. The physiological and biochemical parameters of the TaCHYR proteins were analyzed by WheatOmics 1.0 ([http://202.194.139.32/](http://202.194.139.32/)) [27], and the subcellular localization of the TaCHYR proteins were predicted using Plant-mPLoc ([http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/](http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/)).

**Phylogenetic relationships, gene structures and domains analysis**

The phylogenetic tree was constructed by the neighbor-joining (NJ) method with 1000 bootstrap replicates using MEGA11 software and Evolv online service [28, 29]. The amino acid sequences of AtCHYRs and GmCHYRs were obtained from a previous report [28, 29]. The exon–intron structures were identified by comparing CDS and genomic DNA sequences using TBtools [26]. The conserved domains and motifs were annotated using the SMART database ([http://smart.embl.de/](http://smart.embl.de/)) and MEME online server ([http://meme-suite.org/index.html](http://meme-suite.org/index.html)).

**Chromosomal location, synteny and Ka/Ks analysis**

The position of TaCHYR genes on chromosomal were obtained according to wheat genome annotation data and then marked on the chromosomes by using the TBtools and circos [26, 30]. Multiple collinear scanning toolkits (MCScanX) were used to detect the gene replication events [31]. TBtools was used to determine the Ka (non-synonymous rate), Ks (synonymous rate), and Ka/Ks ratios of the syntenic gene pair with the Nei-Gojobori (NG) method [26].

**GO annotation, microRNA targeting and protein interactions analysis**

Gene Ontology (GO) annotation of TaCHYR proteins was analyzed using the eggNOG-mapper ([http://egg nog-mapper.embl.de/](http://eggno-g-mapper.embl.de/)) and OmicsBox ([https://www.biobam.com/](https://www.biobam.com/)), then displayed by the WEGO2.0 website ([https://wego.genomics.cn/](https://wego.genomics.cn/)). Protein–protein interactions (PPIs) were predicted using the STRING database ([https://string-db.org/](https://string-db.org/)). The combined score > 0.9 in the STRING database was used to confirm the interaction network. The microRNA (miRNA) targeting TaCHYR genes was searched using the psRNATarget tools ([http://plantgrnbb.noble.org/psRNATarget](http://plantgrnbb.noble.org/psRNATarget)) with default parameters [20]. The Cytoscape software ([https://cytoscape.org/](https://cytoscape.org/)) was used to visualize the regulatory network [32].

**cis-element analysis in the promoter**

The promoter sequences, 1.5 kb upstream sequences of the transcription start site (TSS) of the TaCHYR genes, were acquired from the wheat database, and the cis-elements in the promoters were analyzed using PlantCARE database ([http://bioinformatics.psb.ugent.be/webtools/plantcare/html/](http://bioinformatics.psb.ugent.be/webtools/plantcare/html/)) [33].

**Real-time PCR analysis**

Wheat seeds of “Chinese Spring”, which were obtained from the Northwest A&F University, were germinated on moist filter paper at 25/18 °C (day/night) with a photoperiod of 16 h light/8 h dark at Henan University of Science and Technology on 20 December 2021. For abiotic stress treatment, seedlings grown in hydroponic culture for two weeks were exposed to 20% PEG6000 (w/v), high salinity (300 mM NaCl), high temperature (42 °C), and cold (4 °C). In each treatment, the leaf tissues were collected every 12 h for 36 h, frozen in liquid nitrogen, and stored at −80 °C [34].

RNAiso Plus (Takara) was used to isolate total RNA from each frozen sample, and the first-strand cDNA was synthesized from total RNA (1 μg) by using Prescript III RT ProMix (CISTRO) according to the manufacturer’s instructions. The sequence was amplified using gene-specific primers (Table S9) with 2 × Ultra SYBR Green qPCR Mix (CISTRO), and the *actin* gene was used as an internal control. The real-time PCR cycling parameters were 95 °C for 30 s, followed by 45 cycles at 95 °C for 5 s and 60 °C for 30 s, with a melting curve analysis. All reactions were performed in triplicate to ensure the reproducibility of the results.
Stress tolerance assay in yeast cells

The coding sequence (CDS) of TaCHYR genes were cloned into a pGADT7 vector using the BM seamless cloning kit (Biomed), and then transformed into osmotically sensitive yeast mutants Δhog1 (MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, hog1::KanMX4). The primers were shown in Table S9. To analyze stress resistance, the yeast cells carrying the empty vector (pGADT7), pGADT7-TaCHYR2.1, pGADT7-TaCHYR9.2, or pGADT7-TaCHYR11.1 were cultured in YPD liquid medium (1% yeast extract, 2% peptone, 2% glucose) at 30°C until density reached an OD₆₀₀ of 1.0, then serially diluted (10⁻⁰, 10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵) with ddH₂O. The cells were spotted onto YPD medium plates (1% yeast extract, 2% peptone, 2% glucose) at 30°C until dried. The yeast cells were cultured in YPD medium (1% yeast extract, 2% peptone, 2% glucose, 2% agar) containing NaCl (0.4 and 0.6 M) or D-Sorbitol (1.0 and 1.2 M), and cultured at 30°C for 3 d.

Abbreviations

CHYR: CHY zinc-finger and RING finger protein; CDS: Coding sequence; BTS/BTSL: BRUTUS/BRUTUS-like; HRZ: Hemerythrin RING zinc-finger; HR: Hypersensitive responses; WGD: Whole genome duplications; bHLH: Basic helix-loop-helix; DRE: Drought-responsive element; MBS: MYB binding site; STR: Stress-responsive element; ABRE: ABA-responsive element; CGTCA-motif: MeJα-responsive element; GO: Gene ontology; PPI: Protein-protein interaction; ABA: Abscisic acid.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-022-03589-7.

Additional file 1: Fig. S1. Conserved motifs of TaCHYR proteins in wheat.

Additional file 2: Fig. S2. Chromosomal localizations of TaCHYR genes in wheat. Group I, II and III members were indicated by red, black and blue, respectively.

Additional file 3: Fig. S3. The promoter sequences of TaCHYR11. The transcription start site (TSS) and start codon were indicated by red and yellow, respectively.

Additional file 4: Table S1. The characteristics of CHYR genes in wheat.

Additional file 5: Table S2. CHYR genes used in the phylogenetic tree construction.

Additional file 6: Table S3. Paralogous CHYR gene pairs among T. aestivum.

Additional file 7: Table S4. Orthologous relationships between TaCHYR genes in T. aestivum with other CHYR genes in T. urartu, Ae. tauschi, B. distachyon, and O. sativa.

Additional file 8: Table S5. The cis-elements analysis in the promoter regions of TaCHYR genes.

Additional file 9: Table S6. Gene ontology (GO) annotation of TaCHYR genes in T. aestivum.

Additional file 10: Table S7. The protein-protein interaction network between TaCHYRs and other proteins in wheat.

Additional file 11: Table S8. The regulatory network between the putative miRNAs and their targeted wheat CHYR genes.

Additional file 12: Table S9. Specific primers used in the study.

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Authors’ contributions

Designed the experiment: H.L. Performed the experiments and analyzed data: H.L. Contributed reagents/materials/analysis tools: H.L., H.X., Z.Z., G.K. and N.L.. Drafted the manuscript: H.L. Revised the manuscript: H.L., H.X. and W.Y. All authors read and approved the manuscript.

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Availability of data and materials

Wheat seeds of “Chinese Spring”, which were obtained from the Northwest A&F University, were germinated on moist filter paper at 25/18 °C (day/night) with a photoperiod of 16 h light/8 h dark at Henan University of Science and Technology on 20 December 2021. All of the datasets supporting the results of this article are included within the article and its Additional files. The DNA and Protein sequences of TaCHYR are available in the EnsemblPlants database (http://plants.ensembl.org/index.html), and the gene ID provided in Additional file 3 Table S1. The amino acid sequences of ATCHYR and GMCHYR were obtained from The Arabidopsis Information Resource (https://www.arabidopsis.org/) and Phytozome database (https://phytozome-next.jgi.doe.gov/pz/portal.html), respectively, and the gene ID provided in Additional file 4 Table S2. The genomes of T. aestivum, T. urartu, Ae. tauschi, B. distachyon and O. sativa were obtained from the EnsemblPlants database (http://plants.ensembl.org/index.html).

Declarations

Ethics approval and consent to participate

The experiments did not involve endangered or protected species. The data collection of plants was carried out with permission of related institution, and complied with national or international guidelines and legislation.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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