BMC Genomics

RESEARCH Open Access

Identification and expression analysis of CCCH gene family and screening of key low temperature stress response gene *CbuC3H24* and *CbuC3H58* in *Catalpa bungei*

Pingan Bao[1,2†], Jingshuang Sun[1†], Guanzheng Qu[2], Maolin Yan[3], Shiping Cheng[4], Wenjun Ma[5], Junhui Wang[5] and Ruiyang Hu[1*]

Abstract

Catalpa bungei, a tree indigenous to China, is renowned for its superior timber quality and as an ornamental in horticulture. To promote the cultivation of *C. bungei* in cold regions and expand its distribution, enhancing its cold tolerance is essential. The CCCH gene family is widely involved in plant growth, development, and expression under stress conditions, including low-temperature stress. However, a comprehensive identification and analysis of these genes have not yet been conducted. This study aims to identify key cold-tolerance-related genes within the CCCH gene family of *C. bungei*, providing the necessary theoretical support for its expansion in cold regions. In this study, 61 CCCH genes within *C. bungei* were identified and characterized. Phylogenetic assessment divided these genes into 9 subfamilies, with 55 members mapped across 16 chromosomes. The analysis of gene structures and protein motifs indicated that members within the same subfamily shared similar exon/intron distribution and motif patterns, supporting the phylogenetic classification. Collinearity analysis suggested that segmental duplications have played a significant role in the expansion of the *C. bungei* CCCH gene family. Notably, RNA sequencing analysis under 4 °C cold stress conditions identified *CbuC3H24* and *CbuC3H58* as exhibiting the most significant responses, highlighting their importance within the CCCH zinc finger family in response to cold stress. The findings of this study lay a theoretical foundation for further exploring the mechanisms of cold tolerance in *C. bungei*, providing crucial insights for its cultivation in cold regions.

Keywords *Catalpa bungei*, CCCH zinc finger gene, Gene family, Low temperature stress, Gene expression

†Pingan Bao and Jingshuang Sun contributed equally to this work.

*Correspondence:
Ruiyang Hu
hury@caf.ac.cn
[1]State Key Laboratory of Tree Genetics and Breeding, Experimental Center of Forestry in North China, Chinese Academy of Forestry, National Permanent Scientific Research Base for Warm Temperate Zone Forestry of Jiulong Mountain in Beijing, Beijing 102300, China
[2]State Key Laboratory of Tree Genetics and Breeding, Northeast Forestry University, Harbin 150040, China
[3]Inner Mongolia Academy of Forestry, Hohhot 010010, China
[4]Henan Key Laboratory of Germplasm Innovation and Utilization of Eco-economic Woody Plant, Pingdingshan University, Henan 467000, China
[5]State Key Laboratory of Tree Genetics and Breeding, Research Institute of Forestry, Chinese Academy of Forestry, Beijing 100091, China

Introduction

Catalpa bungei, belonging to the Bignonia family, is a notable species within the Catalpa genus. It holds significant importance in China, primarily due to its exceptional timber quality and remarkable ornamental characteristics, making it highly sought after for garden usage. It is widely recognized for its excellent material properties and versatility, earning it the esteemed designation of the "King of Woods" [1]. *C. bungei*, which is extensively distributed in the Yangtze River Basin and the Yellow River Basin, displays a high growth rate, superior wood quality, and remarkable adaptability [2].

Zinc finger proteins (ZFPs) are a group of transcription factors known for their characteristic zinc-binding domains, comprised of amino acid residues that specifically bind to Zn^{2+}. These proteins serve vital roles in processes such as gene transcription and translation, exerting essential functions in regulating gene expression [3]. CCCH-type zinc finger proteins (Cys-CysCys-His, with Cys referring to cysteine and His referring to histidine) consist of 1 to 6 CCCH-type zinc finger motifs. Cysteine and histidine are utilized to chelate zinc ions, forming the zinc finger structure. These proteins have the ability to recognize and bind DNA and are associated with various RNA metabolism processes in organisms [4]. The amino acid sequence of CCCH-type zinc finger motifs was initially defined as C-X6-14-C-X4-5-C-X3-H, where X represents any other amino acid. However, it is currently defined as C-X4-15-C-X4-6-C-X3-H, with the most common variations being C-X7-C-X5-C-X3-H and C-X8-C-X5-C-X3-H [5]. In recent years, there has been an increasing amount of research focused on identifying transcription factor proteins (TFPs) in plants and understanding their response to different stressors. This has led to a growing interest in TFs in plant studies. Examples of TFs include CCCH genes [6], bZIP genes [7], PLATZ [8], C2H2-ZFPs [9], BBX (B-box) [10], LIM [11], among others. However, the specific role of CCCH genes in *C. bungei* has not been thoroughly investigated, indicating the need for further examination.

CCCH zinc finger proteins play a vital role in regulating plant development, adaptation, hormone signaling, and responses to various environmental stresses, including both biotic and abiotic stress [12]. Another study on *Arabidopsis thaliana* indicates that a CCCH-type zinc finger protein, known as SOMNUS, exerts negative regulation downstream of *PIL5* and affects light-dependent seed germination [13]. In *Oryza sativa*, *OsTZF1* enhances rice stress tolerance by regulating the RNA metabolism of stress-responsive genes and also alleviates leaf senescence [14]. The expression of the *OsCCCH-Zn-1* gene plays a crucial role in low oxygen stress in rice [15]. In *Medicago sativa*, the CCCH zinc finger protein gene *MsZFN* can regulate flowering time by suppressing the expression of flowering genes under long-day conditions [16]. CCCH zinc finger proteins are crucial regulators in plant growth, development, and hormone signaling, influencing key processes such as seed germination, stress tolerance, and flowering time. Overexpression of *PdC3H17* in *Populus deltoides* enhances tolerance to drought stress [17]. *CaC3H14* enhances plant resistance to damping-off disease by regulating the antagonistic effect between salicylic acid (SA) and jasmonic acid (JA)/ethylene (ET) signaling pathways [18]. In genetically modified *Glycine max*, overexpression of *GmZF351* improves soybean's tolerance to salt and drought stress [19]. CCCH zinc finger proteins enhance plant tolerance to drought stress by influencing hormone signaling pathways and stress-response gene regulation, thereby improving plant resilience under water-deficit conditions. The zinc finger gene *AtZFP1* in *A. thaliana* plays a significant role in enhancing the plant's capacity to adapt to salt stress through the regulation of ion balance, osmotic balance, and ROS homeostasis mechanisms [20]. In *Gossypium hirsutum*, NaCl treatment induces the expression of *GhZFP1*, thereby enhancing cotton's tolerance to salt stress [21]. Transferring the *HuTZF3* gene from *Hylocereus polyrhizus* into *A. thaliana* using Agrobacterium-mediated transformation, it was observed that overexpression of *HuTZF3* enhanced the salt and heat stress tolerance in *A. thaliana* [22]. In *Phyllostachys edulis*, the zinc finger gene *PeC3H74* enhances plant drought tolerance through an ABA-dependent signaling pathway [23]. CCCH zinc finger proteins play a significant role in enhancing plant salt tolerance by regulating ion and osmotic balance, ROS homeostasis, and stress-responsive signaling pathways. In *Capsicum annuum*, CCCH genes are significantly upregulated during abiotic stress responses, particularly in cold and heat stress responses [24]. *PvC3H72* in switchgrass (*Panicum virgatum*) is the first discovered CCCH family gene involved in plant cold and freeze stress, potentially through an ABA-mediated ICE-CBF-COR pathway [25]. CCCH zinc finger proteins are pivotal in cold stress response, often acting through ABA-mediated pathways to enhance plant cold and freeze tolerance.

Overall, CCCH zinc finger proteins are integral in regulating various aspects of plant development and stress responses. They are involved in essential processes such as seed germination, flowering, and senescence. Additionally, they play crucial roles in enhancing plant tolerance to drought, salt, and cold stress by modulating hormone signaling pathways, maintaining ionic and osmotic balance, and improving ROS homeostasis. Understanding their functions can provide valuable insights into improving crop resilience under adverse environmental conditions.

In this study, leveraging bioinformatics tools and whole-genome sequence data of *C. bungei*, we identified members within the CCCH zinc finger protein family. These genes were subject to a comprehensive suite of analyses including evolutionary relationships, motif composition, gene structure characterization, and collinearity assessments, allowing for a robust genomic perspective of the CCCH family in *C. bungei*. Moreover, we explored the expression profiles of these identified CCCH genes under low-temperature stress conditions, aiming to decipher their potential roles during the cold response. Among the gene family members, *CbuC3H24* and *CbuC3H58* emerged as notably responsive gene to low-temperature stress. The systematic analysis and identification of CCCH family members in *C. bungei* provide essential insights into the zinc finger-mediated regulatory networks crucial for cold tolerance. This research yields valuable genetic resources that can significantly support breeding programs aimed at developing *C. bungei* varieties with superior cold resistance. This work underscores the mechanistic complexities of cold tolerance in *C. bungei* and sets the stage for further functional validation of candidate genes for crop improvement strategies.

Methods

Identification of the CCCH-type zinc finger family members in *C. Bungei*

To identify members of the CCCH gene family in *C. bungei*, we initiated our investigation by acquiring the seed data for the PF00642 domain associated with the CCCH gene from the PFAM database (http://pfam.xfam.org/). Utilizing the HMMER search tool, we performed a preliminary screen against the complete protein sequences derived from *C. bungei*. In this screening, genes exhibiting an E-value less than 1×10^{-5} were provisionally classified as belonging to the CCCH gene family. For further validation of these candidate genes, we employed two additional resources: the SMART database (http://smart.embl-heidelberg.de/) and the Batch search functionality available on the Pfam database (http://pfam.xfam.org/search#searchBatchBlock).

Physicochemical properties, conserved domain and gene structure of CCCH gene

To further characterize the identified members of the CCCH gene family in *C. bungei*, we leveraged TBtools-II software (version 2.007) for the analysis of amino acid sequence length, relative molecular weight (Da), and isoelectric points (pI). This facilitated a detailed understanding of the physicochemical properties of the proteins within the CCCH gene family. In addition, to elucidate the conserved motifs present in these proteins, we employed the MEME suite (http://meme-suite.org/tools/meme), a powerful tool for motif discovery. The exploration of gene structure, including the arrangement of exons and introns within the *CbuC3Hs* genes, was also conducted using TBtools-II.

Construction and subfamily classification of CCCH family phylogenetic tree

To facilitate a comparative phylogenetic analysis of the CCCH zinc finger protein gene family across different species, we acquired the respective protein sequences from *Populus trichocarpa*, *O.* and *A. thaliana*. The sequences for *P. trichocarpa* and *O. sativa* were downloaded from the Plant Transcription Factor Database (Plant TFDB, http://planttfdb.gao-lab.org/), while the sequences for *A. thaliana* were retrieved from The Arabidopsis Information Resource (TAIR, https://www.arabidopsis.org/index.jsp). Subsequently, we utilized the MUSCLE tool within the MEGA11 software for comprehensive multiple sequence alignment, ensuring the sequences were optimally prepared for phylogenetic analysis. Building upon this, we constructed a phylogenetic tree using the Maximum Likelihood method, specifically utilizing the WAG+G model, and augmented this with 1000 bootstrap replicates to ensure the reliability of the inferred phylogenetic relationships. This tree facilitated a clear classification of the subfamilies within the CCCH zinc finger protein gene family in *C. bungei*, allowing for a deeper understanding of their evolutionary relationships and functional diversification.

Cis-acting elements

To investigate the regulatory mechanisms governing the expression of the CCCH gene family, we focused on the identification of cis-acting elements within their promoter regions. Utilizing the TBtools-II software (version 2.007), we extracted sequences extending 2000 base pairs upstream of the translation start sites, which are presumed to encompass key promoter regions. The analysis for cis-acting regulatory elements within these promoter sequences was conducted through the Plant CARE database (http://bioinformatics.psb), a dedicated online platform for the prediction of such elements. The visualization and further examination of these predicted cis-acting elements were facilitated using the TBtools software. This approach enabled us to delineate the potential regulatory motifs that could play crucial roles in the transcriptional regulation of the CCCH gene family, thereby providing insights into their functional regulation and expression patterns.

Chromosome localization and collinearity analysis

To elucidate the genomic organization and evolutionary dynamics of the CCCH gene family in *C. bungei*, we undertook an analysis of their chromosomal distribution and collinearity relationships. By employing TBtools-II

software (version 2.007), we conducted a visual exploration of the CCCH genes' localization on *C. bungei* chromosomes, providing insight into their physical distribution and potential clustering patterns. Additionally, the MCScanX tool was utilized to assess the synteny and collinearity among the CCCH genes within *C. bungei* and between *C. bungei* and other species. This analysis revealed insights into the evolutionary history and functional conservation of these genes. To further understand the selective pressures acting on the CCCH gene family, we calculated the nonsynonymous (Ka) to synonymous (Ks) substitution ratio (Ka/Ks values), offering evidence on the direction and magnitude of genetic selection pressures these genes have undergone. This comprehensive genetic and evolutionary analysis contributes to our understanding of the structural and functional diversification of the CCCH gene family in *C. bungei* and their evolutionary conservation across different species.

Low temperature stress RNA-seq analysis of CCCH gene family

To decipher the response patterns of the CCCH gene family in *C. bungei* under low-temperature conditions, this study exposed *C. bungei* (9−1 type) tissue culture seedlings to a 4 °C low-temperature environment for varying durations: 0 h, 0.5 h, 1 h, 3 h, 6 h, 12 h, 24 h, 48 h, and 72 h. The material collected for analysis was specifically the leaf tissue. Post-treatment, transcriptomic analyses were conducted to investigate the expression dynamics of the CCCH gene family during these stress periods. Utilizing TBtools software for this analysis allowed for the identification of specific CCCH gene family members that are significantly responsive to low-temperature stress. The FPKM values were normalized using a Log_2 (value+1) transformation. This approach enabled us to elucidate the temporal expression patterns of these genes, shedding light on their potential roles in the physiological and molecular mechanisms of cold stress adaptation in *C. bungei*.

Co-expression network analysis of CCCH gene expression under low temperature stress

In this study, we utilized the Weighted Gene Co-expression Network Analysis (WGCNA) method, implemented through the R package with the recommended default parameters, to explore the co-expression networks associated with the *CbuC3H24* and *CbuC3H58* genes within *C. bungei*. After identifying these essential genes, we constructed the co-expression network and analyzed it using Cytoscape software. This approach allowed us to map complex gene expression patterns and identify key genes likely involved in regulating significant biological processes in *C. bungei*, providing a comprehensive view of gene interactions and functional associations within this species.

qPCR validation of CCCH gene family in *C. Bungei* in response to low temperature stress

In our qPCR experiment, we utilized precise instrumentation and specialized materials to ensure robust and accurate outcomes. The equipment included the Eppendorf Centrifuge 5810R, ABI 7500 Real-Time Quantitative PCR System, BaiJing Bio BG-Qspin™ Mini Centrifuge, and Qilinbeier VORTEX-5 Vortex Mixer. The reagents comprised SYBR Green PCR Master Mix from DBI and PCR plates from AXYGEN. Our experimental procedure involved converting total RNA into complementary DNA (cDNA) through reverse transcription and synthesizing specific primers for the target genes. The cDNA samples were then subjected to real-time quantitative PCR (RT-qPCR) using SYBR Green chemistry, allowing for the amplification and quantification of the target genes. We prepared the qPCR reaction mixture with 12.5 µl of Bestar SybrGreen qPCR Mastermix, 0.25 µl of PCR Forward Primer (10 µM), 0.25 µl of PCR Reverse Primer (10 µM), 1 µl of cDNA template, and 11 µl of sterile distilled water (ddH2O), in a total volume of 25 µl. The qPCR amplification was carried out with an initial denaturation at 95 °C for 2 min, followed by 45 cycles of 95 °C for 10 s and 60 °C for 1 min. Relative gene expression was determined using the $2^{-\Delta\Delta Ct}$ method, referencing expression levels at 0 h in the control group. The expression data are presented as mean ± standard error of the mean (SEM) from three biological replicates ($n=3$). For statistical analysis, each time point was compared to the control group using one-way ANOVA, with multiple comparisons performed using the Bonferroni test. Statistical significance was denoted by p-values: >0.1234 (ns), <0.0332 (*), <0.0021 (**), <0.0002 (***) and <0.0001 (****).

Results

Whole genome identification and physicochemical property analysis of CCCH gene family members in *C. Bungei*

A comprehensive identification of the CCCH gene family members within the *C. bungei* genome is summarized in Table 1. A total of 61 CCCH gene family members were identified and systematically named *CbuC3H01* to *CbuC3H61* based on their gene IDs. These CCCH proteins exhibit substantial variability in length, ranging from 99 amino acids (*CbuC3H08*) to 2007 amino acids (*CbuC3H61*), with corresponding molecular weights ranging from 10.48 kDa to 224.53 kDa. The theoretical isoelectric points (pI) of these proteins vary from 4.90 to 9.93. *CbuC3H14* displayed the lowest pI value of 4.90, while *CbuC3H51* exhibited the highest at 9.93. The instability indices of these proteins ranged from 32.22

Table 1 CCCH gene family members and physicochemical properties of ***C. Bungei***

Gene name[1]	Gene ID[2]	AA[3]	DNA MW[4]	pI[5]	Instability Index[6]	GRAVY[7]
CbuC3H01	evm.model.group0.1101	709	78450.44	6.07	53.78	-1.016
CbuC3H02	evm.model.group0.2096.1	307	32,590	9.36	37.19	-0.483
CbuC3H03	evm.model.group0.2154	636	69754.9	6.05	57.23	-0.455
CbuC3H04	evm.model.group0.901	108	12438.06	5.07	48.1	-0.59
CbuC3H05	evm.model.group1.1254	429	48528.75	6.56	41.69	-1.152
CbuC3H06	evm.model.group1.1409	562	62835.3	5.75	49.99	-0.396
CbuC3H07	evm.model.group1.1478	376	42285.09	7.55	57.84	-0.743
CbuC3H08	evm.model.group1.167	99	10475.96	8.65	35.76	-0.335
CbuC3H09	evm.model.group1.168	302	33930.18	7.52	54.65	-0.258
CbuC3H10	evm.model.group1.677	639	71101.84	5.29	50.43	-0.721
CbuC3H11	evm.model.group1.996	494	54571.58	6.1	55.43	-0.799
CbuC3H12	evm.model.group11.104	929	101736.4	9.27	50.06	-0.788
CbuC3H13	evm.model.group11.1360	370	41176.4	6.5	73.81	-0.647
CbuC3H14	evm.model.group13.155	510	56294.82	4.9	57.38	-0.854
CbuC3H15	evm.model.group14.221	481	50727.97	8.08	57.48	-0.383
CbuC3H16	evm.model.group15.191	347	37166.29	5.7	55.53	-0.401
CbuC3H17	evm.model.group15.193.2	687	75983.26	5.51	61.58	-0.439
CbuC3H18	evm.model.group15.402	301	31760.02	9.39	37.24	-0.505
CbuC3H19	evm.model.group15.875	420	45825.48	9.04	57.67	-0.599
CbuC3H20	evm.model.group15.929	1022	116236.1	6.29	47.44	-0.191
CbuC3H21	evm.model.group16.113	732	79125.74	6.1	63.01	-0.394
CbuC3H22	evm.model.group16.174	673	73837.43	5.56	53.66	-0.515
CbuC3H23	evm.model.group16.542	442	50064.83	8.35	67.12	-0.775
CbuC3H24	evm.model.group18.360	646	70528.4	6.84	52.33	-0.472
CbuC3H25	evm.model.group18.83	433	47300.45	8.32	32.22	-0.253
CbuC3H26	evm.model.group2.1070	706	77194.6	5.69	63.44	-0.476
CbuC3H27	evm.model.group2.1597	338	38982.28	4.91	44.87	-1.174
CbuC3H28	evm.model.group2.359	786	86424.89	6.27	51.09	-0.725
CbuC3H29	evm.model.group2.415	339	38132.78	7.23	61.25	-0.67
CbuC3H30	evm.model.group3.1204	910	99556.85	6.46	46.5	-0.652
CbuC3H31	evm.model.group3.832	316	35695.09	8.06	58.7	-0.628
CbuC3H32	evm.model.group4.1044	488	53671.35	7.12	32.52	-0.193
CbuC3H33	evm.model.group4.1108	296	33062.05	6.66	50.27	-0.778
CbuC3H34	evm.model.group4.1117	347	38234.11	6.03	46.77	-0.867
CbuC3H35	evm.model.group4.1232	332	37229.41	7.12	42.93	-1.023
CbuC3H36	evm.model.group4.196	300	32425.69	8.46	54.12	-0.372
CbuC3H37	evm.model.group4.351	157	17280.46	8.93	63.37	-0.655
CbuC3H38	evm.model.group4.659	733	79481.2	6.2	63.33	-0.422
CbuC3H39	evm.model.group4.685	345	38505.06	8.48	54.01	-0.284
CbuC3H40	evm.model.group5.1325	483	53008.44	8.7	59	-0.593
CbuC3H41	evm.model.group5.1451	597	67286.97	9.19	63.82	-1.308
CbuC3H42	evm.model.group5.707	435	47711.07	8.31	32.61	-0.25
CbuC3H43	evm.model.group6.126	521	57781.54	5.12	60.74	-0.917
CbuC3H44	evm.model.group7.1778	335	36682.43	9.05	60.54	-0.423
CbuC3H45	evm.model.group7.2227	348	37978.79	6.51	46.15	-0.829
CbuC3H46	evm.model.group7.251	380	41165.72	7.93	46.01	-0.415
CbuC3H47	evm.model.group7.3112	324	36756.23	7.58	45.93	-0.956
CbuC3H48	evm.model.group7.3194	204	21872.83	9.38	49.35	-0.658
CbuC3H49	evm.model.group7.3471	375	43143.3	5.29	40.24	-1.153
CbuC3H50	evm.model.group7.3974	632	69377.37	6.41	46.55	-0.515
CbuC3H51	evm.model.group7.492	261	28527.56	9.93	79.52	-0.47
CbuC3H52	evm.model.group8.298	458	49388.08	7.11	55.26	-0.524
CbuC3H53	evm.model.group8.710	462	51443.89	5.42	55.39	-0.824

Table 1 (continued)

Gene name[1]	Gene ID[2]	AA[3]	DNA MW[4]	pI[5]	Instability Index[6]	GRAVY[7]
CbuC3H54	evm.model.group8.859	428	46453.09	8.74	56.77	-0.666
CbuC3H55	evm.model.group9.296	352	39485.34	6.42	64.49	-0.68
CbuC3H56	evm.model.scaffold205.58	314	36496.28	9.67	80.59	-1.271
CbuC3H57	evm.model.scaffold210.62	425	46769.57	8.9	57.61	-0.543
CbuC3H58	evm.model.scaffold443.4	634	69165.1	7.05	52.69	-0.418
CbuC3H59	evm.model.scaffold453.99	694	77319.83	6.37	39.27	-0.556
CbuC3H60	evm.model.scaffold72.14	893	96967.74	7.29	46.63	-0.728
CbuC3H61	evm.model.scaffold81.28	2007	224531.7	8.34	52.91	-0.81

[1]: Refers to the gene name that the postal gene ID is uniformly converted into

[2]: Refers to the gene ID numbers in the *C. bungei* genome data

[3]: Refers to the number of amino acids

[4]: Denotes the relative molecular weight (MW) of DNA.

[5]: Refers to the theoretical isoelectric point (pI) of a protein

[6]: Refers to the instability index

[7]: Refers to the average hydrophilicity coefficient

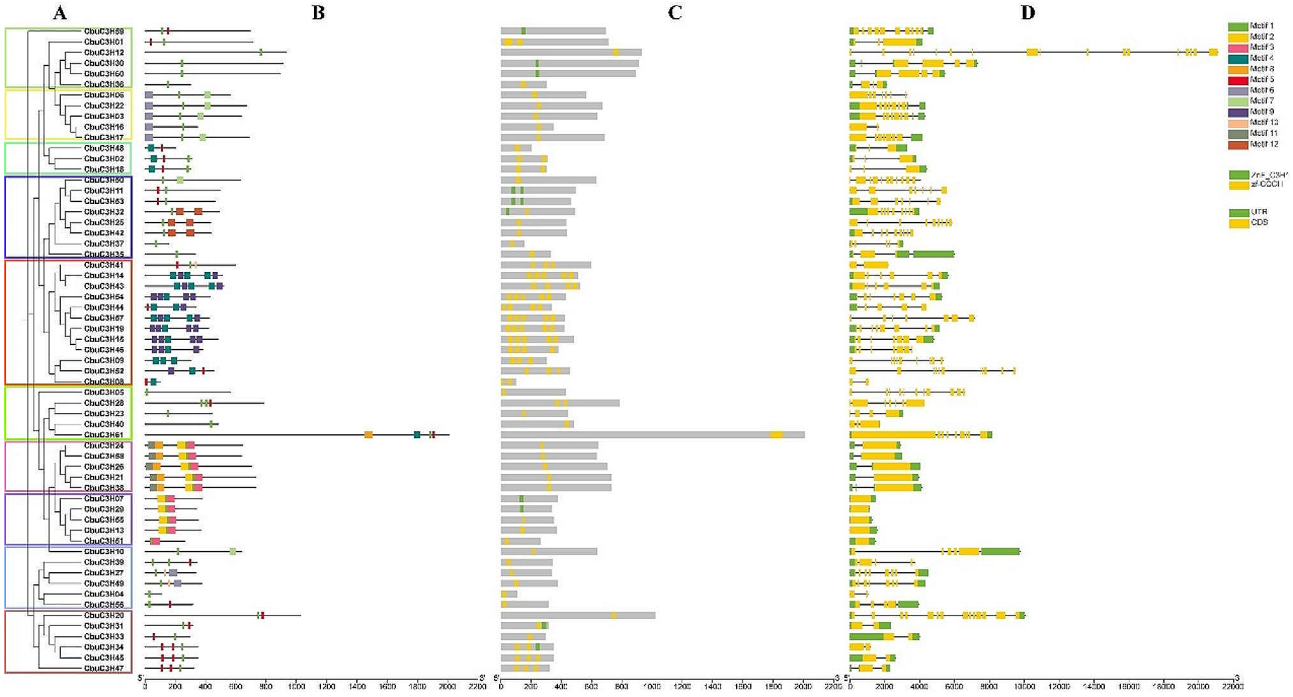

Fig. 1 Phylogenetic relationships, motif composition, domain and gene structure of *C. bungei*. A.The phylogenetic tree was constructed using the Maximum Likelihood (ML) method based on the amino acid sequences of the 61 *C. bungei* CCCH proteins to facilitate subfamily recognition; B. Schematic representation of the motifs in *C. bungei* CCCH genes; C. Schematic representation of the conserved domain structures in *C. bungei* CCCH proteins; D. Schematic representation of the exon-intron structure of *C. bungei* CCCH genes

(*CbuC3H25*) to 80.59 (*CbuC3H56*), where values below 40 indicate stability and values above 40 suggest instability. Additionally, the hydrophobicity scores of these CCCH genes varied from −1.308 (*CbuC3H41*) to -0.191 (*CbuC3H20*), emphasizing that members of the CCCH gene family are predominantly hydrophilic proteins.

Motif prediction and gene structure analysis

The structural analysis of the 61 CCCH zinc finger genes is depicted in Fig. 1. The results indicate that the majority of these genes encompass both introns and exons, with few exceptions being intronless. There is considerable variation in gene size and the number of exons across these genes, with a range from 1 to 17 exons. However, the majority of the genes have fewer than 10 exons, with an average of approximately 5.2 exons per gene. Panels A and D in Fig. 1 corroborate the intron-exon architecture, while panels A and C highlight the conserved structural domains zf-CCCH and ZnF_C3H1 present across all 61 genes. Further analysis conducted using the MEME Suite

online tool has identified 12 conserved motifs within these genes, as demonstrated in panels A and B of Fig. 1. Genes belonging to the same phylogenetic subgroups tend to exhibit similar gene structures and conserved motifs, suggesting functional homology among these evolutionary clades.

Analysis of cis-acting elements of promoters

To explore the potential transcriptional regulatory mechanisms of CCCH genes, the 2000 bp sequences of the CCCH promoter region were analyzed using PlantCare software, and the identified cis-elements were visualized using TBtools. Various cis-elements were found within the CCCH family, including those related to environmental stress, hormone signaling, and factors associated with plant growth and development (Fig. 2). These elements encompass environmental stress response, such as defense, low temperature (LTR), drought inducible (MBS), anaerobic induction (ARE), and light responsiveness. Additionally, cis-acting elements responsive to hormones such as MeJA, IAA, ABA, and GA were observed. Elements related to plant growth and development were also identified, including leaf mesophyll cell differentiation, endosperm expression, maize alcohol-soluble protein metabolism, and physiologically related cis-regulatory elements. These findings suggest that CCCH family genes may have a broader involvement in plant responses to environmental stress and in their growth and development.

Phylogenetic tree analysis of CCCH family

To investigate the evolutionary relationships of the CCCH zinc finger proteins among *C. bungei* (*CbuC3H*), *P. trichocarpa* (*PtC3H*), *O. sativa* (*LOCC3H*), and *A.*

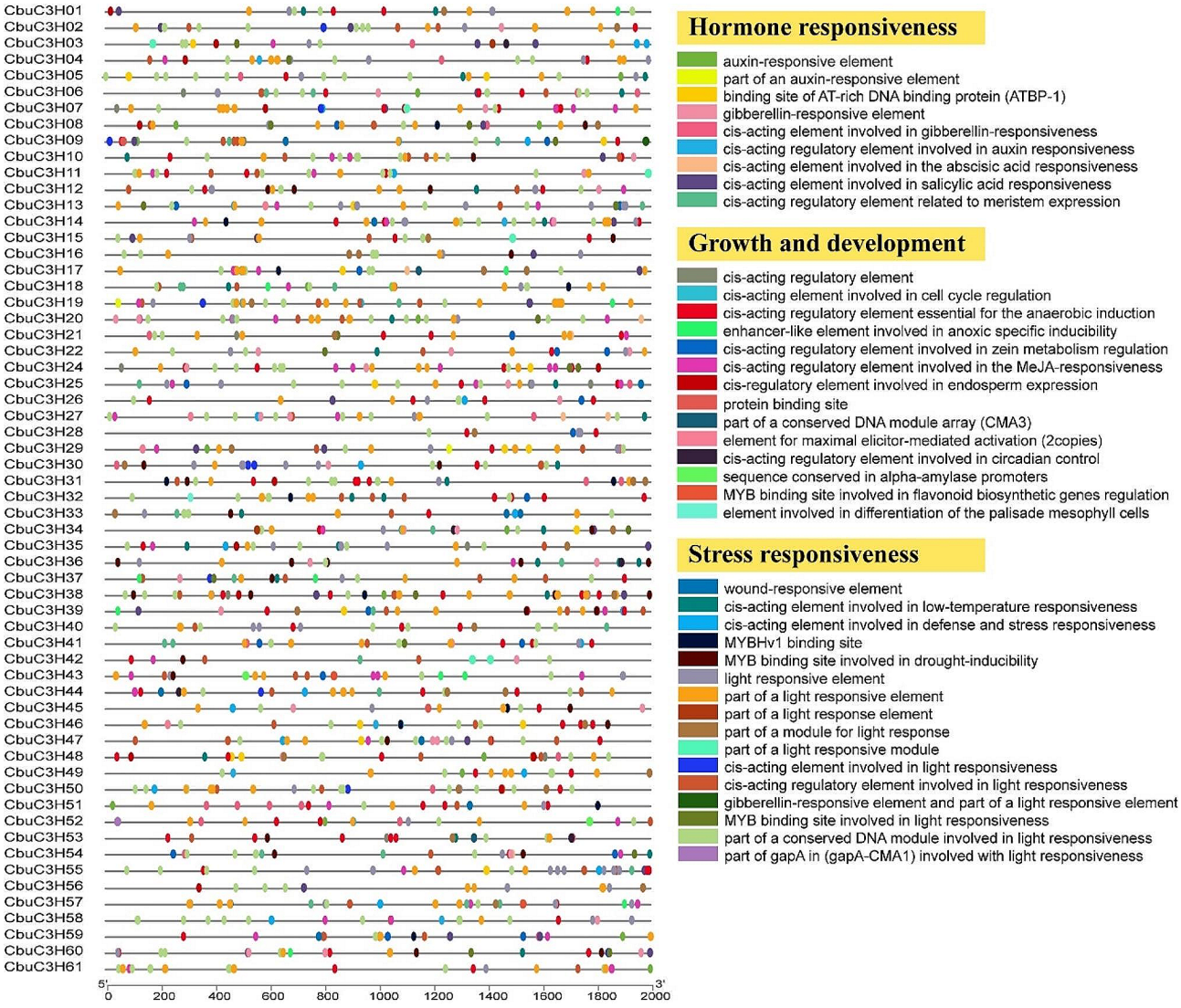

Fig. 2 Cis-element analysis of the promoter of CCCH gene in *C. bungei*

thaliana (*AtC3H*), a phylogenetic analysis was conducted as presented in Fig. 3. A total of 239 CCCH zinc finger proteins were analyzed, encompassing 61 from *C. bungei*, 61 from *P. trichocarpa*, 49 from *O. sativa*, and 68 from *A. thaliana*. These proteins were categorized into nine distinct groups, denominated as I through IX. The distribution of *CbuC3H* proteins spanned all identified groups. Variability in the abundance of CCCH zinc finger genes was observed across the assorted clades, with each exhibiting a range of 1 to 14 genes. Group IV harbored the greatest number of zinc finger protein family members, whereas Group V contained the fewest, highlighting the diversity and evolutionary divergence of the CCCH zinc finger protein family within these plant species.

Chromosome localization and collinearity analysis

Chromosomal localization analysis of the 61 CCCH genes was performed, with the results illustrated in Fig. 4. The distribution of the CCCH genes across the chromosomes was found to be uneven, genes such as

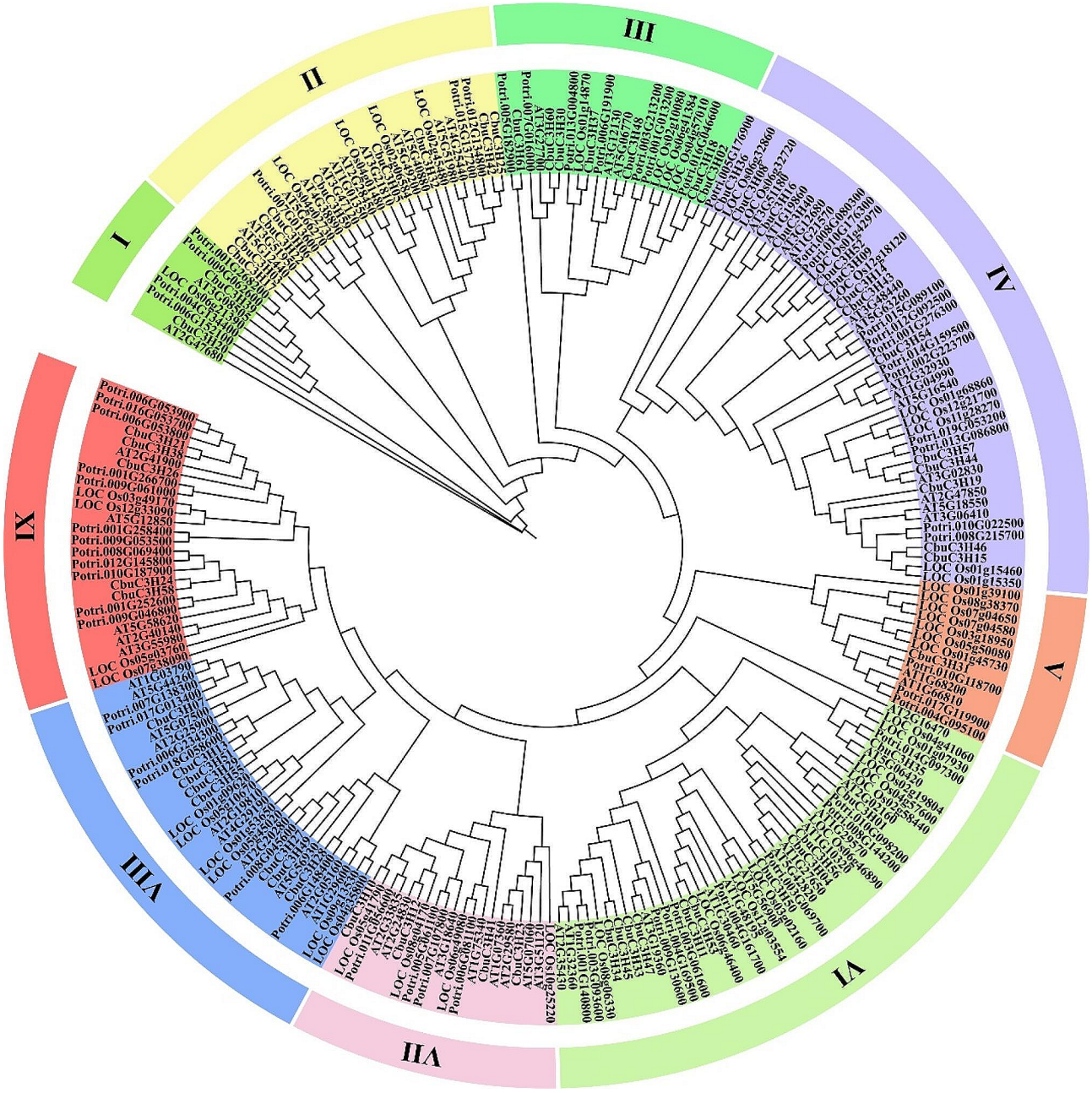

Fig. 3 Phylogenetic tree of CCCH family of *C. bungei*, *P. trichocarpa*, *O. sativa* and *A. thaliana*. The figure distinguishes subfamilies using different colors. The CCCH family members of *C. bungei* are labeled as CbuC3H01-61, those of *P. trichocarpa* begin with Potri, those of *O. sativa* begin with LOC and those of *A. thaliana* begin with AT

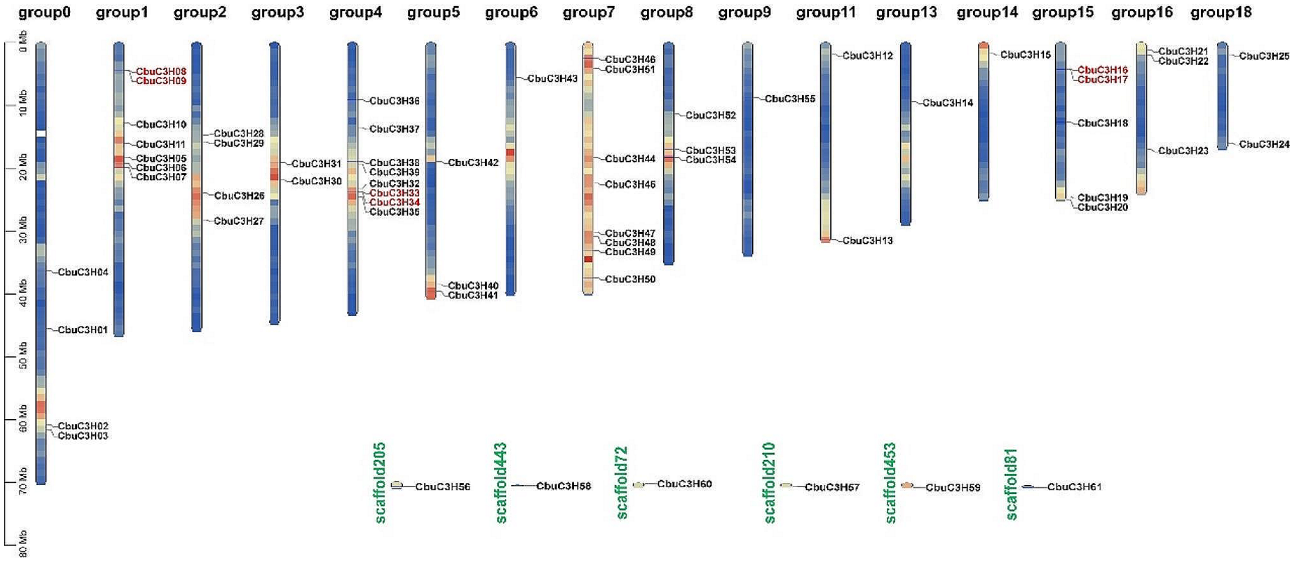

Fig. 4 Distribution of CCCH gene on chromosome of *C. bungei*. The chromosomal position was mapped according to the *C. bungei* genome. Only 55 CCCH genes (not including *CbuC3H56*, *CbuC3H57*, *CbuC3H58*, *CbuC3H59*, *CbuC3H60*, *CbuC3H61*) were mapped to the 16 chromosomes of *C. bungei*. Three pairs of tandem repeat genes are marked in red in the figure

CbuC3H56 to *CbuC3H61* were located on scaffolds rather than on specific chromosomes. Groups 4 and 7 contained the highest number of CCCH genes, with each group comprising 8 genes. In contrast, Groups 6, 9, 13, 14, and various scaffolds possessed the least number of CCCH genes, each with a single gene. Such an uneven gene distribution reflects the genetic diversity within *C. bungei* that has arisen throughout its evolutionary history. Subsequent analysis revealed collinearity among members of the CCCH gene family and identified three tandem duplication events within the *C. bungei* CCCH gene family. These events involve gene pairs from Group 1 (*CbuC3H08* and *CbuC3H09*), Group 4 (*CbuC3H33* and *CbuC3H34*), and Group 15 (*CbuC3H16* and *CbuC3H17*).

Additionally, as shown in Fig. 5, segmental duplication events were observed within the CCCH gene family, with 19 instances occurring across 30 gene members. Notably, genes *CbuC3H29* and *CbuC3H51*, located on chromosome groups 2 and 7 respectively, exhibited clear evidence of segmental duplications. An intriguing pattern of duplication involved genes *CbuC3H21*, *CbuC3H26*, and *CbuC3H38*, which are interlinked and situated on different chromosome groups (16, 2, and 4, respectively). This suggests that gene duplication might propagate across chromosomes through individual genes, underscoring the significance of such duplications in the evolution of CCCH-type transcription factors.

In a synteny analysis of the CCCH zinc finger gene family among three species—*C. bungei*, *P. trichocarpa*, and *A. thaliana*—as shown in Fig. 6, the data revealed a significantly higher degree of synteny between *C. bungei* and *P. trichocarpa* compared to the synteny observed between *C. bungei* and *A. thaliana*. This finding suggests a closer evolutionary relationship between *C. bungei* and *P. trichocarpa*, potentially attributed to both species being woody plants. The shared characteristics of the woody growth form likely contribute to the elevated homology observed within their CCCH zinc finger gene families.

To investigate the evolutionary pressures on the CCCH gene family, we utilized TBtools-II to analyze the ratio of non-synonymous to synonymous substitutions (Ka/Ks) among gene pairs with segmental duplications and collinearity, as shown in Table 2. All examined gene pairs exhibited a Ka/Ks ratio below 1, suggesting that the CCCH gene family may have been subject to significant purifying selection throughout its evolution.

Analysis of CCCH gene expression under low temperature stress

Based on the expression patterns of the CCCH gene family in *C. bungei* under varying durations of low-temperature stress (0 h, 0.5 h, 1 h, 3 h, 6 h, 12 h, 24 h, 48 h, and 72 h), the behavior of 61 CCCH genes was systematically classified into four distinct groups, as depicted in Fig. 7. Class I, consisting of genes like *CbuC3H03*, *33*, *07*, *31*, *36*, *47*, *06*, and *29*, exhibited stable expression levels, remaining largely unaffected by the low-temperature conditions. Class II, featuring 30 genes such as *CbuC3H55*, *26*, *43*, *61*, *12*, *41*, *52*, and *34*, initially showed lower expression levels under normal conditions, yet were moderately higher than Class I genes, with minimal alterations following the stress. Class III, which includes 19 genes like *CbuC3H13*, *51*, *23*, *38*, *16*, *08*, *09*, *39*, *44*, and more, had slightly higher baseline expressions than Class II. These genes

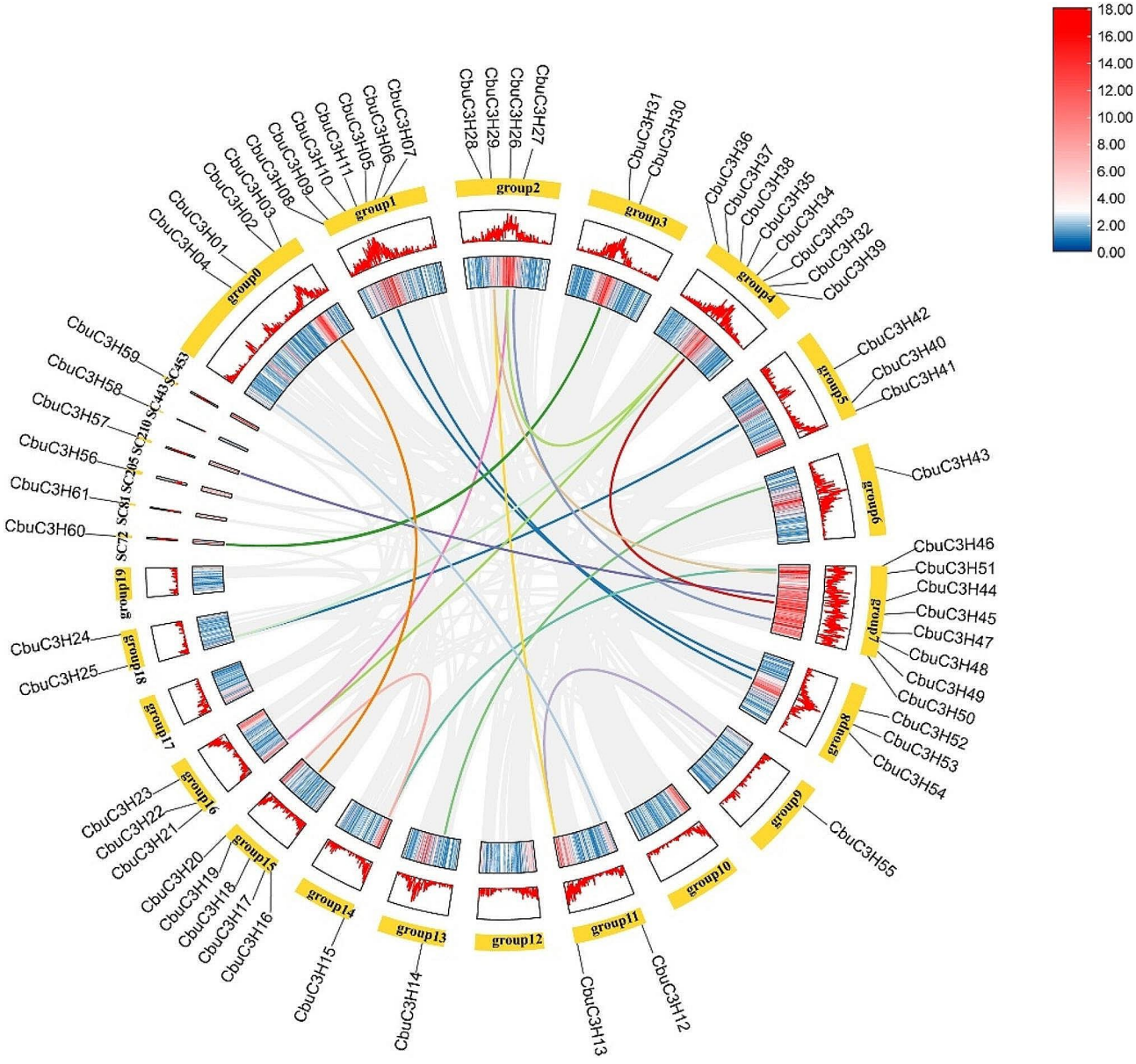

Fig. 5 Collinearity analysis of CCCH gene in *C. bungei*. Chromosomes are represented by yellow rectangles. The gray lines indicate syntenic blocks in *C. bungei* genome, and the colored lines between chromosomes delineate segmentally duplicated gene pairs. The outermost heatmap and lines represent gene density on the chromosomes

experienced some fluctuations in expression post-stress, but these were not markedly significant. Lastly, Class IV, represented by genes including *CbuC3H24*, *58*, *04*, and *57*, were the most expressive within the CCCH gene family. Particularly, *CbuC3H24* and *CbuC3H58* were significantly up-regulated, highlighting their crucial role in the response of the *C. bungei* CCCH zinc finger gene family to low-temperature stress.

To validate the accuracy of our transcriptome data, we conducted a quantitative real-time PCR (qPCR) analysis on 10 CCCH genes that showed significant expression changes under 4 ℃ cold stress conditions (Fig. 8). The qPCR results indicated that the expression levels of *CbuC3H24* and *CbuC3H58* significantly increased after 0.5 h of cold treatment, followed by a decrease at 1 h, 3 h, 6 h and 12 h. Subsequently, their expression levels rose again at 24 h, peaking at 48 h. These changes were significant under cold stress. The expression levels of *CbuC3H04*, *CbuC3H51*, and *CbuC3H13* displayed a pattern of initial decrease followed by an increase. In contrast, *CbuC3H57* showed an initial increase followed by a decrease, with the highest expression level observed at 6 h. However, the expression differences of *CbuC3H04*, *CbuC3H51*, *CbuC3H13*, and *CbuC3H57* were not as

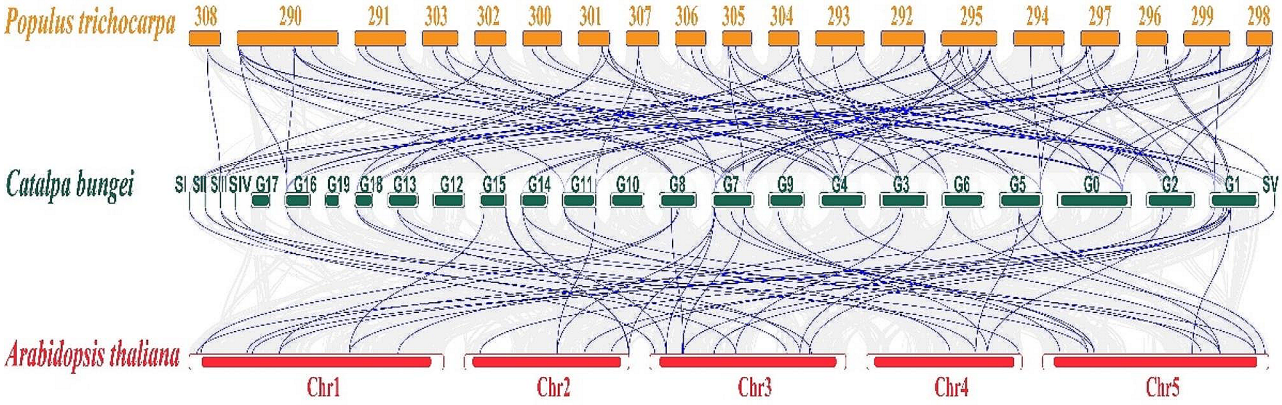

Fig. 6 Homology analysis of CCCH gene among *C. bungei*, *P. trichocarpa* and *A. thaliana*. Gray lines in the background indicate the collinear blocks within *C. bungei* and different plant genomes, whereas blue lines highlight syntenic CCCH gene pairs. In *P. trichocarpa*, the notation 290–399 corresponds to chromosome numbers, which should be prefixed with "CM009" to indicate their full names. For example, the complete chromosome notation would be CM009290 through CM009399. In *C. bungei*, the designations are as follows: "SI" refers to scaffold205, "SII" refers to scaffold453, "SIII" refers to scaffold210, "SIV" refers to scaffold72, and "SV" refers to scaffold81. The letter "G" stands for "group," with group0-19 representing chromosomes 1–19. In *A. thaliana*, the notations Chr1 through Chr5 denote chromosomes 1 through 5

Table 2 Ka/Ks values were analyzed collinearly

Seq_1	Seq_2	Ka	Ks	Ka_Ks
CbuC3H30	*CbuC3H60*	0.254947951	0.571137695	0.446386139
CbuC3H08	*CbuC3H52*	0.15123404	0.412065764	0.367014331
CbuC3H44	*CbuC3H57*	0.117877478	0.410064374	0.28746091
CbuC3H14	*CbuC3H43*	0.182241581	0.647114015	0.281622059
CbuC3H29	*CbuC3H51*	0.355985221	1.448721969	0.24572363
CbuC3H15	*CbuC3H46*	0.114888191	0.548940425	0.209290818
CbuC3H15	*CbuC3H19*	0.339987131	1.63895824	0.207440997
CbuC3H29	*CbuC3H55*	0.147904685	0.801867858	0.184450198
CbuC3H13	*CbuC3H29*	0.353577854	1.932156606	0.182996478
CbuC3H13	*CbuC3H51*	0.183647095	1.0196523	0.180107567
CbuC3H27	*CbuC3H49*	0.077406834	0.51727257	0.149644189
CbuC3H34	*CbuC3H45*	0.09630027	0.692422042	0.139077419
CbuC3H21	*CbuC3H38*	0.076769859	0.565834877	0.135675375
CbuC3H11	*CbuC3H53*	0.067909769	0.531289307	0.127820695
CbuC3H25	*CbuC3H42*	0.05936973	0.589533201	0.100706338
CbuC3H26	*CbuC3H38*	0.227410194	2.295995049	0.099046466
CbuC3H02	*CbuC3H18*	0.079074536	0.805149453	0.098211004
CbuC3H13	*CbuC3H55*	0.338821385	3.752618394	0.090289326
CbuC3H21	*CbuC3H26*	0.237402511	3.076524788	0.077165805

pronounced as those of *CbuC3H24* and *CbuC3H58*. The expression patterns of *CbuC3H21*, *CbuC3H38*, *CbuC3H49*, and *CbuC3H23* did not show clear trends, and the differences in their expression levels were not significant under cold stress at different time points.

Overall, the expression trends of these 10 genes in the qPCR results were consistent with the transcriptome data, confirming the reliability of our previous findings. This study identified *CbuC3H24* and *CbuC3H58* as key genes responding to low-temperature stress within the CCCH gene family in *C. bungei*.

Co-expression network analysis of CCCH gene expression under low temperature stress

Utilizing Weighted Gene Co-Expression Network Analysis (WGCNA), our research successfully constructed a co-expression network centered on the *CbuC3H24* and *CbuC3H58* genes (Fig. 9). This network includes a range of genes involved in crucial biological processes such as adaptation to cold and salt stress, modulation of light response, regulation of glucose metabolism, and the complex control of growth and development. Notably, the network features a set of genes responsive to cold stress, including *CbuC3H24*, *CbuC3H58*, *CBF2*, *CBF3*, *DREB2A*, *MPK3*, *MPK6*, *AZF3*, *CML24*, and *ERF6*. Among these, *CbuC3H24* and *CbuC3H58* are distinguished by their significant connectivity and occupy a central role in the network's structure. Our findings implicate the *CbuC3H24* and *CbuC3H58* genes as potentially playing a key role in the adaptive response of plants to low-temperature stress, highlighting their importance in the molecular framework governing plant cold tolerance. These insights are of considerable significance in advancing our understanding of the genetic mechanisms related to cold resistance in *C. bungei*.

Discussion

In plants, CCCH-type zinc finger proteins play crucial roles in biological processes such as RNA stability [26], transcriptional regulation [27], and signal transduction [28, 29]. CCCH-type zinc finger proteins play pivotal roles in regulating gene expression and are involved in essential physiological processes such as plant growth, development, stress responses, and hormone signal transduction. The multifaceted functions of CCCH proteins have garnered increasing significance in the realms

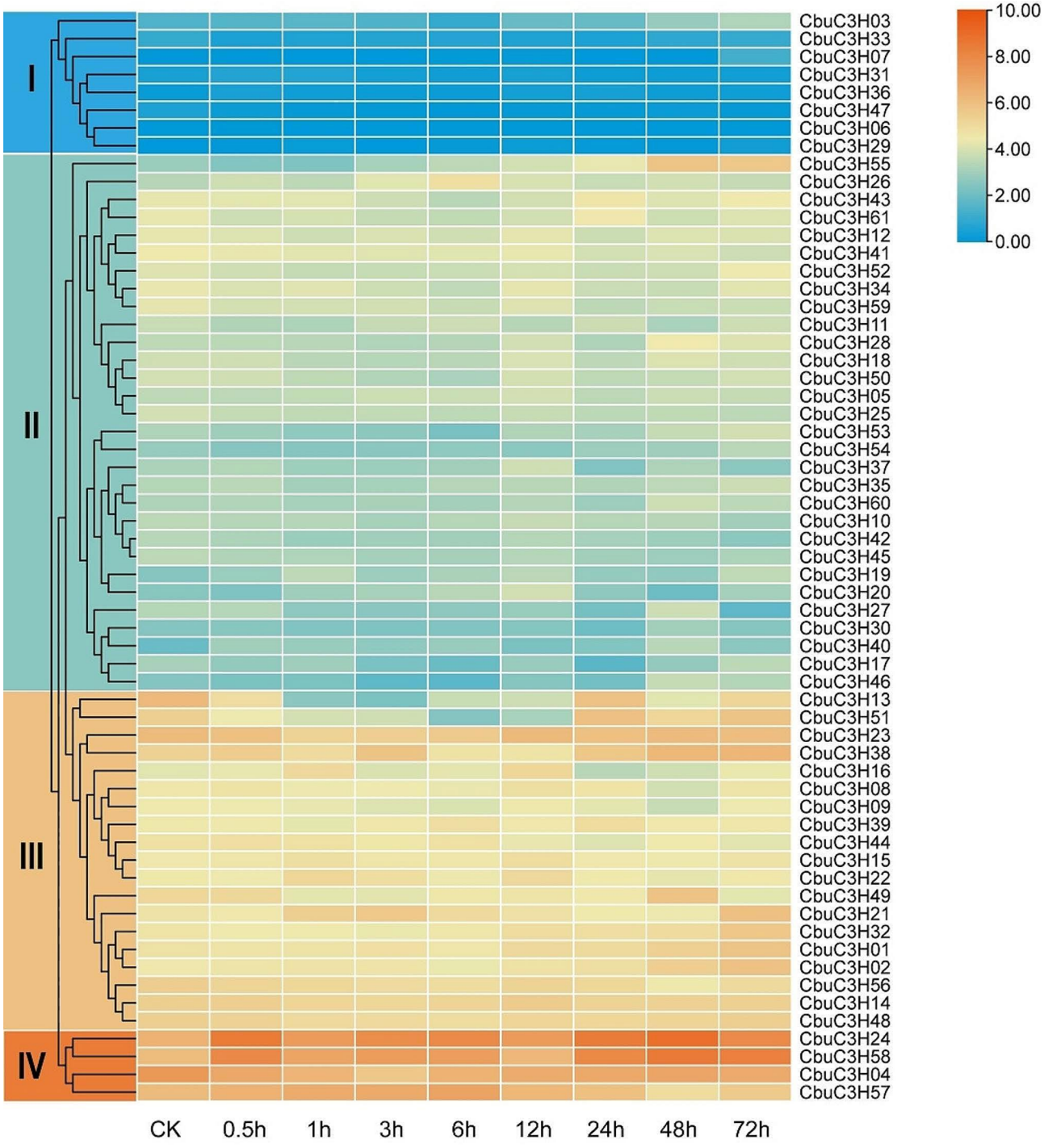

Fig. 7 Heat map of CCCH gene expression in *C. bungei* under low temperature stress. CK refers to 0 h of treatment under 4 degrees low temperature stress, 0.5 h refers to 0.5 h of treatment under 4 degrees low temperature stress, and so on. The more a color approaches red, the higher the expression level; conversely, the more it leans toward blue, the lower the expression level. The FPKM values were normalized using a Log2 (value + 1) transformation

of plant molecular biology and biotechnological research. Identified across numerous plant species, CCCH proteins not only interact with DNA but also modulate gene expression by engaging with mRNA, showcasing their versatility in cellular functions. Interestingly, the number of CCCH gene family members varied among different plant species, with 91, 68, 67, 116, 53, 68, 57, 46, 62 and 119 CCCH genes identified in *P. trichocarpa* [30], *A. thaliana* [5], *O. sativa* [5], *G. max* [31], *Hordeum vulgare* [32], *Zea mays* [33], *C. annuum* [24], *Pinus massoniana*

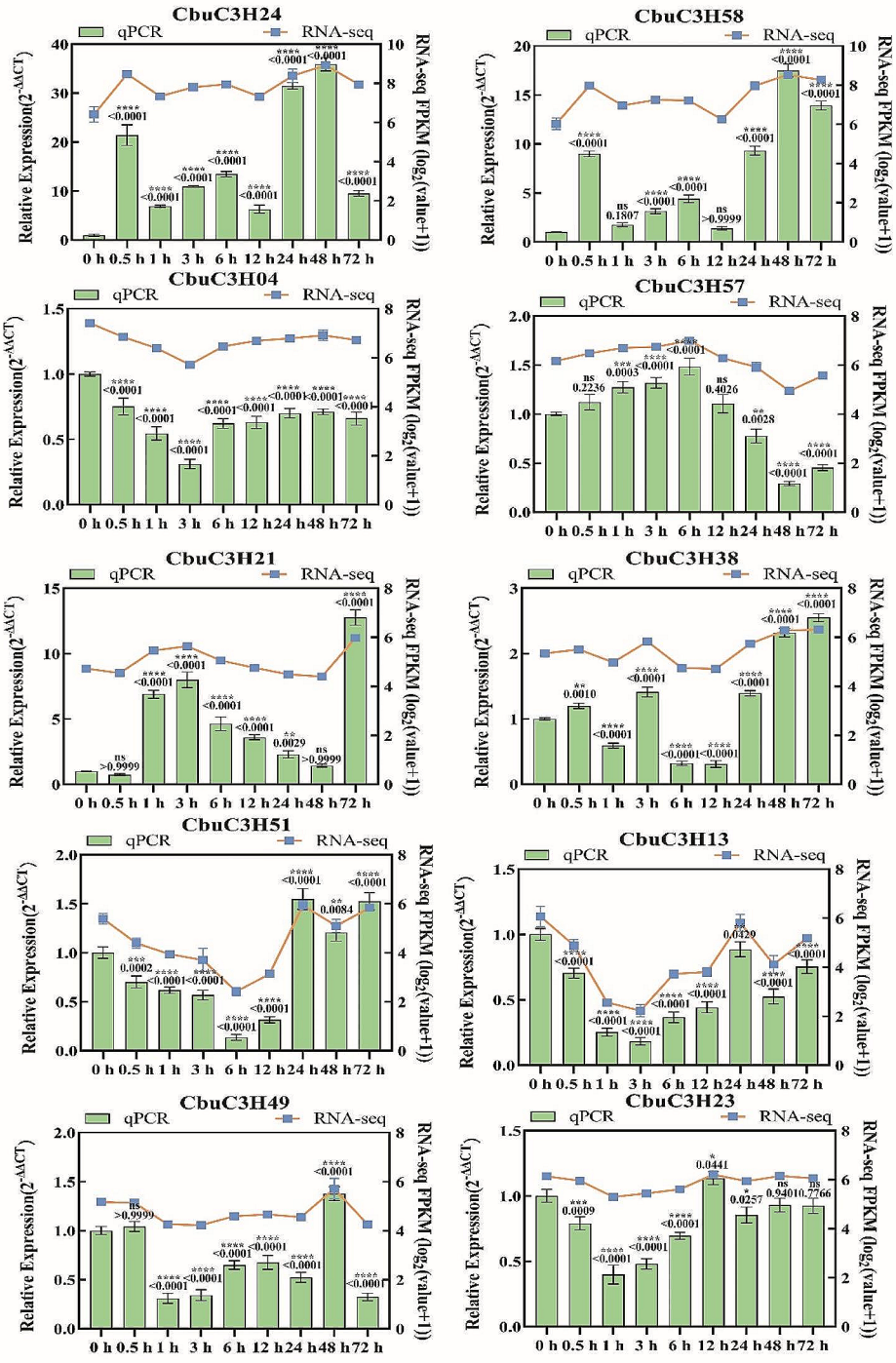

Fig. 8 Validation of RNA-seq data by qPCR for *C. bungei* CCCH genes under low temperature stress. Green bar charts represent the relative expression levels of the genes ($2^{-\Delta\Delta CT}$), while the lines indicate the expression trends of the genes as observed in the RNA-seq data (normalized FPKM values). Values are presented as the mean ± standard error of the mean (SEM) from three biological replicates ($n = 3$). Each time point was compared to the control group, and significance was calculated using one-way ANOVA. Multiple comparisons were performed using the Bonferroni test. *p*-values were denoted as follows: >0.1234 (ns), < 0.0332 (*), < 0.0021 (**), < 0.0002 (***), < 0.0001 (****)

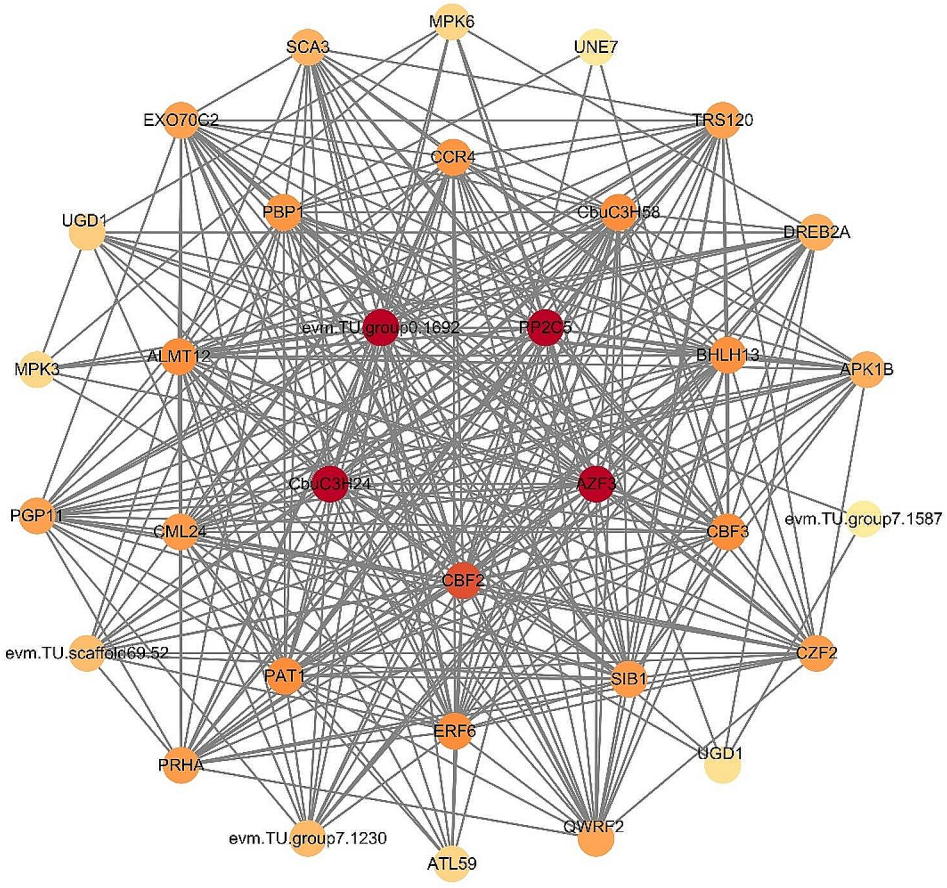

Fig. 9 The co-expression network diagram of core genes *CbuC3H24* and *CbuC3H58*. Circles represent genes and lines depict the regulatory relationships among them. The depth of a circle's color signifies the extent of its centrality within the network, where darker hues denote a greater level of interaction with other genes, thus indicating a more central position in the network

[6], *Clementine mandarin* [34], and *P. edulis* [23], respectively. These findings highlight the species-specific variations in the CCCH gene family composition.

A phylogenetic analysis of CCCH proteins in *C. bungei*, *P. trichocarpa*, *O. sativa* and *A. thaliana* revealed that the cold-responsive genes *CbuC3H24* and *CbuC3H58* are positioned in Group IX and shows high homology with *At5G58620*, *At2G40140*, *At3G55980* and *LOC Os07g38090*. These findings are consistent with previous research on *PvC3H72* in *P. virgatum*, a cold-tolerant gene belonging to the CCCH zinc finger protein family, where *PvC3H72*, *CbuC3H24* and *CbuC3H58* similarly exhibit sequence homology with *At5G58620*, *At2G40140*, *At3G55980* and *LOC Os07g38090* [25]. Additionally, existing studies indicate that *At2G40140* independently regulates cold stress responses, unrelated to the ICE-CBF-COR pathway [35]. Furthermore, no binding sites were identified for either the *CbuC3H24 or CbuC3H58* genes among the upstream and downstream regulatory elements of the *CBF* gene. Notably, a linkage was observed between these two genes in the co-expression network analysis, suggesting the possibility of an indirect regulatory interaction. However, this proposed relationship between *CbuC3H24* and *CbuC3H58* with *CBF* requires further experimental validation. These findings contribute important insights into the molecular mechanisms underlying the cold stress response in *C. bungei*.

Low temperature is one of the most common abiotic stresses encountered by plants throughout their lifecycle, greatly limiting the geographical distribution, growth and development, yield and quality of crops, and post-harvest vitality [36–38]. Understanding the effects of low temperatures on the physiological and biochemical processes in plants, as well as the molecular mechanisms underlying their responses to cold stress, is essential for developing cold-resistant crop varieties. Studies have shown that the CCCH gene family in *C. bungei* is abundant in cis-acting elements related to plant hormones and external stimuli, playing a key role in plant growth, development, and responses to environmental stresses, especially in modulating cold resistance [39]. This research analyzed the cis-acting elements of the CCCH gene family in *C. bungei* tissue culture seedlings (9−1) and investigated their differential expression following cold stress treatment,

identifying four CCCH members potentially involved in the cold stress response.

In the WGCNA co-expression network analysis, *CbuC3H24* and *CbuC3H58* were found to be part of a network that includes numerous cold-responsive genes. Notably, *CBF2* and *CBF3* occupy significant positions within this network. *CBF2* and *CBF3* are significantly induced under low-temperature conditions and activate the expression of cold and dehydration-responsive genes through binding to the CRT/DRE cis-acting elements, thereby enhancing plant stress resistance [40, 41]. Studies have shown that *CBF2* negatively regulates the expression of *CBF1* and *CBF3* in *A. thaliana* [42]. However, whether the regulatory relationship between *CbuC3H24* and *CbuC3H58* and the *CBF* genes is positive or negative remains unclear and requires further experimental validation.

Moreover, *ERF*, as one of the largest plant-specific transcription factors, plays a crucial role in plant development and stress responses. In Chrysanthemum, overexpression of the *ERF* gene can significantly enhance plant cold tolerance [43]. The *MPK6* gene encodes a protein with mitogen-activated protein kinase (*MPK*) phosphorylation residues, which is closely associated with cold resistance. It acts as a negative regulator of cold tolerance and can enhance plant cold tolerance by suppressing its expression [44]. *CML24* is expressed in all major organs, and its transcription levels increase two- to fifteen-fold in response to stimuli such as touch, darkness, heat, cold, hydrogen peroxide, abscisic acid (ABA), and indole-3-acetic acid [45]. These genes are all involved in responding to cold stress and show high connectivity with *CbuC3H24* and *CbuC3H58* in the co-expression network, further corroborating the important roles of *CbuC3H24* and *CbuC3H58* in the cold stress response. However, the regulatory networks and mechanisms among these genes remain unclear and require further experimental investigation.

Other genes in the co-expression network also exhibit significant biological functions. For instance, the *PAT1* protein regulates stress responses by mediating the degradation of ABA-responsive genes, and is associated with drought stress [46]. *PP2C5* has been identified as a negative regulator of PAMP-triggered immunity (*PTI*) and studies suggest that specific elimination of *PP2C* can enhance plant resistance without affecting growth and yield [47]. The *AZF3* gene is expressed in plant roots and its expression levels significantly increase under salt stress, participating in drought and salt stress responses via the ABA pathway [48]. *bHLH13* has been identified as a potential novel regulator of JA-mediated petal senescence, functioning independently of stamen development and leaf senescence, highlighting the unique mechanisms of petal senescence [49]. *ALMT12* is involved in stomatal closure by altering Ca^{2+} permeability. Pbp1 mediates cell growth regulation through interaction with ribosomal proteins [50]. Although these genes mainly relate to plant stress responses and growth regulation, their diverse functional attributes suggest that *CbuC3H24* and *CbuC3H58* may also play roles in various aspects of plant growth regulation. Therefore, *CbuC3H24* and *CbuC3H58* are not only associated with cold tolerance but may also be involved in multiple regulatory processes in plant growth and development, which require further experimental validation.

The expression patterns of these genes were subsequently validated under cold stress at 4 °C using qPCR, confirming their role in the cold stress response of *C. bungei*, with *CbuC3H24* and *CbuC3H58* exhibiting the most significant reaction to cold stress. Moreover, existing studies suggest that CCCH-type zinc finger protein genes can respond to cold stress; for example, the expression of the CCCH-type zinc finger protein gene *OsTZF5* in rice and *PhTZF1* in petunia is induced by cold stress [51, 52]. These findings provide valuable insights for further exploration into the regulation of the CCCH gene in *C. bungei* and its mechanism of cold resistance.

Conclusions

In this study, we comprehensively characterized 61 CCCH zinc finger genes in *C. bungei*, elucidating their evolutionary relationships, structural conservation, motif composition, and chromosomal distribution. We identified nine subfamilies, each exhibiting conserved structural features, suggesting functional similarities. Gene duplication was predominantly attributed to fragment repetition events, indicating a potential mechanism for the expansion of the CCCH gene family. Notably, *CbuC3H24* and *CbuC3H58* were identified as key genes responsive to cold stress. qPCR quantification confirmed their significant reaction under cold stress conditions and revealed an extensive co-expression network of genes implicated in low temperature adaptation. This study not only enhances our understanding of the CCCH gene family's role in plant stress responses but also provides a foundation for further investigation into the evolutionary dynamics and functional mechanisms of CCCH zinc finger genes in *C. bungei* and other plants.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-024-10690-8.

Additional file 1: Table S1: The coding sequences (CDS) of the CCCH gene family members in *C. bungei*

Additional file 2: Table S2: The amino acid sequences of the CCCH gene family members in *C. bungei*

Additional file 3: Table S3: Transcriptome data of *C. bungei* subjected to 4 °C cold stress treatment

Additional file 4: Table S4: The primers for Realtime fluorescence quantitative PCR

Additional file 5: Table S5: Raw data for Co-Expression Network Analysis

Acknowledgements
We express our gratitude to the anonymous reviewers for their critical comments on the manuscript.

Author contributions
PB conducted all the analyses and interpreted the results. JS participated in data mining. MY and SC assisted with the collection of C. bungei tissue culture seedlings and their exposure to low-temperature stress. GQ, WM and JW supervised the analysis. Ruiyang Hu conceived the project and critically revised the manuscript. All authors have read and approved the final manuscript.

Funding
This research was funded by National Key R&D Program of China (2021YFD2200301) and Henan Key Laboratory of Germplasm Innovation and Utilization of Eco-economic Woody Plant.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 28 April 2024 / Accepted: 5 August 2024
Published online: 11 August 2024

References

1. Olsen RT, Kirkbride JH. Taxonomic revision of the genus *Catalpa*. (Bignoniaceae) BRITTONIA. 2017;69(3):387–421.
2. Jian S, Zhu T, Wang J, Yan D. The current and future potential geographical distribution and evolution process of *Catalpa bungei* in China. FORESTS. 2022;13(1):96.
3. Wang Q, Song S, Lu X, Wang Y, Chen Y, Wu X, Tan L, Chai G. Hormone regulation of CCCH Zinc Finger proteins in plants. INT J MOL SCI 2022, 23(22).
4. Han G, Qiao Z, Li Y, Wang C, Wang B. The roles of CCCH Zinc-Finger proteins in Plant Abiotic stress tolerance. INT J MOL SCI 2021, 22(15).
5. Wang D, Guo Y, Wu C, Yang G, Li Y, Zheng C. Genome-wide analysis of CCCH zinc finger family in *Arabidopsis* and rice. BMC Genomics. 2008;9:44.
6. Wang D, Yao S, Agassin RH, Zhang M, Lou X, Huang Z, Zhang J, Ji K. Transcriptome-Wide Identification of CCCH-Type Zinc Finger Proteins Family in *Pinus massoniana* and RR-TZF Proteins in Stress Response. GENES-BASEL. 2022, 13(9).
7. Dong W, Xie Q, Liu Z, Han Y, Wang X, Xu R, Gao C. Genome-wide identification and expression profiling of the *bZIP* gene family in *Betula platyphylla* and the functional characterization of *BpChr04G00610* under low-temperature stress. PLANT PHYSIOL BIOCH. 2023;198:107676.
8. Zhang X, Lan Y, Wang L, Liu H, Jiang N, He W, Yan H, Wu M, Xiang Y. Whole-genome identification and multiple abiotic stresses expression pattern profiling analysis of PLATZ transcription factor family members in Pecan (*Carya Illinoensis*). INT J BIOL MACROMOL. 2023;248:125959.
9. Shao L, Li L, Huang X, Fu Y, Yang D, Li C, Yang J. Identification of C2H2 zinc finger genes through genome-wide association study and functional analyses of *LkZFPs* in response to stresses in *Larix kaempferi*. BMC PLANT BIOL 2023, 23(1).
10. Yang N, Cong Q, Wang X, Ni X, Cheng L. Identification of EgrBBX Gene Family and its expression analysis under abiotic stress in *Eucalyptus grandis*. Journol Agricultural Biotechnol. 2020;28(4):658–71.
11. Sarkar MAR, Sarkar S, Islam MS, Zohra FT, Rahman SM. A genome–wide approach to the systematic and comprehensive analysis of LIM gene family in sorghum (*Sorghum bicolor* L). Genomics Inf. 2023;21(3):e36.
12. Bogamuwa SP, Jang J. Tandem CCCH zinc finger proteins in plant growth, development and stress response. PLANT CELL PHYSIOL. 2014;55(8):1367–75.
13. Kim DH, Yamaguchi S, Lim S, Oh E, Park J, Hanada A, Kamiya Y, Choi G. SOMNUS, a CCCH-Type Zinc Finger Protein in *Arabidopsis*, Negatively Regulates Light-Dependent Seed Germination Downstream of *PIL5*. The Plant cell. 2008, 20(5):1260–1277.
14. Ilyas M, Hussain Shah S, Fujita Y, Maruyama K, Nakashima K, Yamaguchi-Shinozaki K, Jan A. *OsTZF1*, a CCCH-tandem zinc finger protein gene, driven under own promoter produces no pleiotropic effects and confers salt and drought tolerance in rice. PLANT SIGNAL BEHAV 2022, 17(1).
15. Pandey DM, Kim S. Identification and expression analysis of hypoxia stress inducible CCCH-type zinc finger protein genes in rice. J PLANT BIOL. 2012;55(6):489–97.
16. Chao Y, Zhang T, Yang Q, Kang J, Sun Y, Gruber MY, Qin Z. Expression of the alfalfa CCCH-type zinc finger protein gene *MsZFN* delays flowering time in transgenic *Arabidopsis thaliana*. Plant Sci (Limerick) 2014, 215–6:92–99.
17. Zhuang Y, Wang C, Zhang Y, Chen S, Wang D, Liu Q, Zhou G, Chai G. Overexpression of *PdC3H17* confers tolerance to Drought stress depending on its CCCH Domain in *Populus*. FRONT PLANT SCI; 2020. p. 10.
18. Qiu A, Lei Y, Yang S, Wu J, Li J, Bao B, Cai Y, Wang S, Lin J, Wang Y, et al. *CaC3H14* encoding a tandem CCCH zinc finger protein is directly targeted by *CaWRKY40* and positively regulates the response of pepper to inoculation by *Ralstonia solanacearum*. MOL PLANT PATHOL. 2018;19(10):2221–35.
19. Wei W, Lu L, Bian XH, Li QT, Han JQ, Tao JJ, Yin CC, Lai YC, Li W, Bi YD, et al. Zinc-finger protein GmZF351 improves both salt and drought stress tolerance in soybean. J INTEGR PLANT BIOL. 2023;65(7):1636–50.
20. Han G, Wang M, Yuan F, Sui N, Song J, Wang B. The CCCH zinc finger protein gene *AtZFP1* improves salt resistance in *Arabidopsis thaliana*. PLANT MOL BIOL. 2014;86(3):237–53.
21. Guo Y, Yu Y, Wang D, Wu C, Yang G, Huang J, Zheng C. GhZFP1, a novel CCCH-type zinc finger protein from cotton, enhances salt stress tolerance and fungal disease resistance in transgenic tobacco by interacting with GZIRD21A and GZIPR5. NEW PHYTOL. 2009;183(1):62–75.
22. Xu W, Jian S, Li J, Wang Y, Zhang M, Xia K. Genomic identification of CCCH-Type zinc finger protein genes reveals the role of *HuTZF3* in Tolerance of Heat and Salt stress of Pitaya (*Hylocereus polyrhizus*). Int J Mol Sci. 2023;24(7):6359.
23. Chen F, Liu H, Wang K, Gao Y, Wu M, Xiang Y. Identification of CCCH Zinc Finger proteins Family in Moso Bamboo (*Phyllostachys edulis*), and *PeC3H74* confers Drought Tolerance to transgenic plants. FRONT PLANT SCI. 2020;11:579255.
24. Tang W, Hao Y, Ma X, Shi Y, Dang Y, Dong Z, Zhao Y, Zhao T, Zhu S, Zhang Z, et al. Genome-wide analysis and identification of stress-responsive genes of the CCCH zinc finger family in *Capsicum annuum* L. FRONT PLANT SCI. 2023;14:1189038.
25. Xie Z, Lin W, Yu G, Cheng Q, Xu B, Huang B. Improved cold tolerance in switchgrass by a novel CCCH-type zinc finger transcription factor gene, *PvC3H72*, associated with ICE1-CBF-COR regulon and ABA-responsive genes. BIOTECHNOL BIOFUELS. 2019;12(1):224.
26. Xu L, Liu T, Xiong X, Liu W, Yu Y, Cao J. *AtC3H18L* is a stop-codon read-through gene and encodes a novel non-tandem CCCH zinc-finger protein that can form cytoplasmic foci similar to mRNP granules. BIOCHEM BIOPH RES CO. 2020;528(1):140–5.
27. Pi B, He X, Ruan Y, Jang JC, Huang Y. Genome-wide analysis and stress-responsive expression of CCCH zinc finger family genes in *Brassica rapa*. BMC PLANT BIOL. 2018;18(1):373.
28. Jan A, Maruyama K, Todaka D, Kidokoro S, Abo M, Yoshimura E, Shinozaki K, Nakashima K, Yamaguchi-Shinozaki K. OsTZF1, a CCCH-Tandem Zinc Finger protein, confers delayed senescence and stress tolerance in Rice by regulating stress-related genes. PLANT PHYSIOL. 2013;161(3):1202–16.
29. Verma V, Ravindran P, Kumar PP. Plant hormone-mediated regulation of stress responses. BMC PLANT BIOL. 2016;16(1):86.
30. Chai G, Hu R, Zhang D, Qi G, Zuo R, Cao Y, Chen P, Kong Y, Zhou G. Comprehensive analysis of CCCH zinc finger family in poplar (*Populus trichocarpa*). BMC Genomics 2012, 13(1).

31. Hu X, Zuo J. The CCCH zinc finger family of soybean (*Glycine max* L.): genome-wide identification, expression, domestication, GWAS and haplotype analysis. BMC Genomics 2021, 22(1).
32. Ai Q, Pan W, Zeng Y, Li Y, Cui L. Correction to: CCCH Zinc finger genes in Barley: genome-wide identification, evolution, expression and haplotype analysis. BMC PLANT BIOL. 2022;22(1):189.
33. Peng X, Zhao Y, Cao J, Zhang W, Jiang H, Li X, Ma Q, Zhu S, Cheng B. CCCH-type zinc finger family in maize: genome-wide identification, classification and expression profiling under abscisic acid and drought treatments. PLoS ONE. 2012;7(7):e40120.
34. Liu S, Khan MR, Li Y, Zhang J, Hu C. Comprehensive analysis of CCCH-type zinc finger gene family in citrus (*Clementine mandarin*) by genome-wide characterization. MOL GENET GENOMICS. 2014;289(5):855–72.
35. Wang S, Shen Y, Deng D, Guo L, Zhang Y, Nie Y, Du Y, Zhao X, Ye X, Huang J, et al. Orthogroup and phylotranscriptomic analyses identify transcription factors involved in the plant cold response: a case study of Arabidopsis BBX29. PLANT COMMUN. 2023;4(6):100684.
36. Xu G, Li L, Zhou J, Lyu D, Zhao D, Qin S. Comparison of transcriptome and metabolome analysis revealed differences in cold resistant metabolic pathways in different apple cultivars under low temperature stress. HORTIC PLANT J. 2023;9(2):183–98.
37. Aslam M, Fakher B, Ashraf MA, Cheng Y, Wang B, Qin Y. Plant low-temperature stress: signaling and response. Agronomy. 2022;12(3):702.
38. Guan Y, Hwarari D, Korboe HM, Ahmad B, Cao Y, Movahedi A, Yang L. Low temperature stress-induced perception and molecular signaling pathways in plants. ENVIRON EXP BOT. 2023;207:105190.
39. Bai H, Lin P, Li X, Liao X, Wan L, Yang X, Luo Y, Zhang L, Zhang F, Liu S, et al. *DgC3H1*, a CCCH zinc finger protein gene, confers cold tolerance in transgenic chrysanthemum. SCI HORTIC-AMSTERDAM. 2021;281:109901.
40. Guo J, Ren Y, Tang Z, Shi W, Zhou M. Characterization and expression profiling of the *ICE-CBF-COR* genes in wheat. PEERJ. 2019;7:e8190.
41. Park S, Lee CM, Doherty CJ, Gilmour SJ, Kim Y, Thomashow MF. Regulation of the *Arabidopsis CBF* regulon by a complex low-temperature regulatory network. PLANT J. 2015;82(2):193–207.
42. Kim Y, Kim S, Lim J, Kim S. ATBS1-INTERACTING FACTOR 2 positively regulates freezing Tolerance via INDUCER OF CBF EXPRESSION 1/C-REPEAT BINDING FACTOR-Induced Cold Acclimation Pathway. PLANT CELL PHYSIOL 2024:pcae072.
43. Ren M, Yang W, Zhang J, Zhao L, Quan Y, He Z, Xu Y, Zhang F, Yin M, Wang Y et al. Overexpression of *CIRAP2.4* in *Chrysanthemum* enhances tolerance to cold stress. FUNCT PLANT BIOL. 2023, 50(6):470–481.
44. Zeng R, Li Z, Shi Y, Fu D, Yin P, Cheng J, Jiang C, Yang S. Natural variation in a type-A response regulator confers maize chilling tolerance. NAT COMMUN. 2021;12(1):4713.
45. Delk NA, Johnson KA, Chowdhury NI, Braam J. *CML24*, regulated in expression by diverse stimuli, encodes a potential Ca^{2+} sensor that functions in responses to abscisic acid, daylength, and ion stress. PLANT PHYSIOL. 2005;139(1):240–53.
46. Zuo Z, Roux ME, Saemundsson HP, Muller M, Munne BS, Petersen M. The *Arabidopsis thaliana* mRNA decay factor *PAT1* functions in osmotic stress responses and decaps ABA-responsive genes. FEBS LETT. 2021;595(2):253–63.
47. Diao Z, Yang R, Wang Y, Cui J, Li J, Wu Q, Zhang Y, Yu X, Gong B, Huang Y, et al. Functional screening of the *Arabidopsis* 2 C protein phosphatases family identifies PP2C15 as a negative regulator of plant immunity by targeting BRI1-associated receptor kinase 1. MOL PLANT PATHOL. 2024;25(4):e13447.
48. Sakamoto H, Maruyama K, Sakuma Y, Meshi T, Iwabuchi M, Shinozaki K, Yamaguchi-Shinozaki K. Arabidopsis Cys2/His2-Type Zinc-Finger Proteins Function as Transcription Repressors under Drought, Cold, and high-salinity stress conditions. PLANT PHYSIOL. 2004;136(1):2734–46.
49. Wu L, Wang K, Chen M, Su W, Liu Z, Guo X, Ma M, Qian S, Deng Y, Wang H, et al. *ALLENE OXIDE SYNTHASE* (AOS) induces petal senescence through a novel JA-associated regulatory pathway in *Arabidopsis*. PHYSIOL MOL BIOL PLA. 2024;30(2):199–212.
50. Jaslan J, Marten I, Jakobson L, Arjus T, Deeken R, Sarmiento C, De Angeli A, Brosche M, Kollist H, Hedrich R. ALMT-independent guard cell R-type anion currents. NEW PHYTOL. 2023;239(6):2225–34.
51. Ning L, Li B, Bao M, Zhang W. Cloning and expression analysis of a cold responsive zinc-finger protein gene *PhTZF1* in *Petunia hybrida*. J Huazhong Agricultural Univ. 2015;34(2):24–30.
52. Selvaraj MG, Jan A, Ishizaki T, Valencia M, Dedicova B, Maruyama K, Ogata T, Todaka D, Yamaguchi Shinozaki K, Nakashima K, et al. Expression of the CCCH-tandem zinc finger protein gene *OsTZF5* under a stress-inducible promoter mitigates the effect of drought stress on rice grain yield under field conditions. PLANT BIOTECHNOL J. 2020;18(8):1711–21.

Publisher's Note