BMC Plant Biology

RESEARCH Open Access

Comprehensive identification and expression analysis of the *CPP* gene family in maize (*Zea mays* L.)

Lei Gu[1,2†], Tianyu Kang[1†], Tuo Zeng[1,2], Hongcheng Wang[1], Bin Zhu[1], Xuye Du[1] and Yinglang Liu[1,2*]

Abstract

Background Maize is an important crop. The cysteine-rich polycomb-like protein (CPP) is crucial for plant development and abiotic stress response. However, few reports have been reported on the function of ZmCPPs. This study conducted bioinformatics and expression analyses of the *ZmCPP* gene family.

Results Based on the homologous comparison methods, 12 *ZmCPPs* were identified in the last assembly of maize B73 genome (V5). They were unevenly distributed across six maize chromosomes. Six *ZmCPPs* formed 3 groups due to segmental but no tandem duplication, indicating segmental duplication as the key driving force of the *ZmCPPs* family expansion. Homologous evolutionary analysis classified 12 ZmCPPs into three groups, with each containing four members. *ZmCPP* gene structure and protein motif in the same group were highly conserved. The promoter regions of 12 *ZmCPP* genes containing plant growth, hormone, and abiotic stress-responsive elements. RNA-seq data indicated that the expression pattern of *ZmCPPs* exhibited organizational specificity and the *ZmCPPs* transcript levels could be influenced by abiotic/biotic stresses. RT-qPCR analysis of six *ZmCPPs* (*ZmCPP2/4/8/9/11/12*) showed that the expression of *ZmCPPs* were induced or reduced by short-term heat, cold, dehydration, and waterlogging stresses. Furthermore, ZmCPP2/9 was localized in the cytoplasm and nucleus, without transactivation activity in yeast.

Conclusion Taken together, the comprehensive analysis of *ZmCPPs* in the whole genome provides a novel perspective on the evolutionary relationship among *ZmCPP* genes and lays a foundation for further study of the biological functions of *ZmCPPs*.

Keywords Maize, *CPP* gene family, Genome-wide analysis, Bioinformatics analysis, Abiotic stress

†Lei Gu and Tianyu Kang contributed equally to this work.

*Correspondence:
Yinglang Liu
liuyl-23@126.com
[1]School of Life Sciences, Guizhou Normal University, Guiyang 550025, China
[2]Guizhou Key Laboratory of Forest Cultivation in Plateau Mountain, Guiyang 550025, China

Background

Maize is an important crop worldwide. Severe abiotic stresses (e.g., extreme temperature, drought, and salinity) affect maize growth and development, posing a serious threat to important traits and resulting in notable decreases in yield [1]. After plants perceive stress signals, transcription factors (TFs) are responsible for transducing and amplifying the signals, thereby inducing the expression of related genes in cells to cope with the damage caused by abiotic stress [2]. Compare to the large plant TF families, such as MYC, MYB, NAC, WRKY, AP2/ERF; The cysteine-rich polycomb-like protein (CPP) transcription factor family with relatively few members, but it plays an important regulatory role in plant growth, development, and responses to abiotic stress [3]. The distinctive feature of CPP proteins is that their sequences contain two conserved CXC domains (motif: CXCX-4CX3YCXCX6CX3CXCX2C, CXC1 and CXC2), as well as a short conserved sequence (RNPXAFXPK) for separated between these two CXC domains [4, 5]; These conserved region (CXCs and short linker) in CPP proteins is also known as the CRC/TCR /CHC domain, which an important characteristic of its family, responsible for binding to the promoter of the target genes and regulating its transcription [6, 7].

Based on the development of sequencing technology, the *CPP* gene family has been identified through bioinformatics analysis in many plants, such as Arabidopsis (8 genes) [8], rice (11 family members) [8], tomato (6 members) [4], Moso bamboo (*Phyllostachys edulis*) (17 genes) [5], soybean (20 family members) [9], wheat (37 genes) [10], and cucumber (*Cucumis sativus*) (5 members) [3]. The reported functions of the CPPs are primarily focused on their involvement in plant floral organ development and responses to abiotic environmental conditions; The *TSO1/AtCPP5* gene was the first identified and characterized *CPP* member in plants through map-based cloning analysis [11]. The TSO1 act as an important regulatory protein in the mitosis of flower cells in Arabidopsis, however, this specific function seems to be not present in other tissues [12]. Knockout of the *TSO1* gene in Arabidopsis disrupted the mitosis of floral organ cells, leading to a significant increase in DNA content within the nucleus; Additionally, the cell walls were incomplete at the end of mitosis, resulting in abnormal phloem development in the mutant lines, ultimately, these changes caused abnormal development of petals, stamens, and carpels [7, 13]. Further analysis indicated that TSO1 may through transcriptionally repress the expression of *AtMYB3R1* gene to positively modulating the balance of shoot or root cell proliferation and differentiation [14]. In soybean, GmCPP1 may be repressing the expression of *Gmlbc3* (leghemoglobin) to modulate the development of symbiotic root nodules [15].

Besides their function in tissue development, CPPs also respond to many environmental treatments (e.g., heat, drought, and salt). Soybean contain 20 *CPPs*, and most of them could induced by heat shock, implying its function in plant temperature stress response [9]. Tomato included 7 *CPP* genes, except for *SlCPP5*, the remaining six genes were all induced by drought treatment [4]; Further analysis revealed that tomato lines with gene silencing of *SlCPP3* significantly reduced drought tolerance [4]. A recent study indicated ZmSK1 could negatively regulate drought tolerance [16]; Further analysis revealed ZmSK1 could phosphorylate ZmCPP2, resulting in ZmCPP2 losing the ability to directly upregulating the expression of *ZmSOD4*, causing a drought-sensitive phenotype in maize [16]. While the function of other *ZmCPPs* in maize nutritional growth and the abiotic stress response is largely unknown.

Previous reports revealed maize contains 13 *ZmCPP* genes based on B73 V3 genome sequence data [17]. In this study, 12 *ZmCPP* family members were identified in the latest assembled maize genome (V5, Zm-B73-REFERENCE-NAM-5.0) and characterized. We also explored the expression patterns of *ZmCPPs* under normal and abiotic (heat, cold, dehydration, and waterlogging) conditions. The subcellular localization and transcriptional activation activity of ZmCPP2 and ZmCPP9 were further analyzed. This work provides a comprehensive perspective on the *ZmCPP* gene members in maize and may be helpful for future research on understand the functions of ZmCPPs.

Results

Identification and characterization of *ZmCPP* genes

In this study, we used AtCPPs [8], OsCPP protein sequence [8] and the hidden Markov model (HMM) of the core CXC motifs (pfam03638) as detectors to homology comparison of the maize latest assembled genome (V5); After combining the blast results, eliminating the redundant transcripts and confirming the presence of complete CXC domain, 12 putative *ZmCPPs* were identified (Fig. 1); These 12 members were unevenly distributed across six chromosomes (the other four chromosomes not contained *ZmCPP* genes) (Fig. 1). Chromosome 1 and 3 both included the most *ZmCPP*s (three genes), followed by chromosomes 6 and 8 each contains two members, chromosomes 5 and 7 each only included one *ZmCPP* (Fig. 1). We named the 12 *ZmCPP*s as *ZmCPP1* to *ZmCPP12* basing on their relative positions in the chromosome (Fig. 1). The Open Reading Frame (ORF), molecular weights and amino acids (aa) of *ZmCPPs* ranged from 1071 bp/39.67 KDa/356aa (*ZmCPP11*) to 2403 bp/84.98 kDa/800aa (*ZmCPP10*) (Table 1). The isoelectric points (PI) of all ZmCPPs were greater than 6, ranging from 6.08 to 8.81 (Table 1). Based

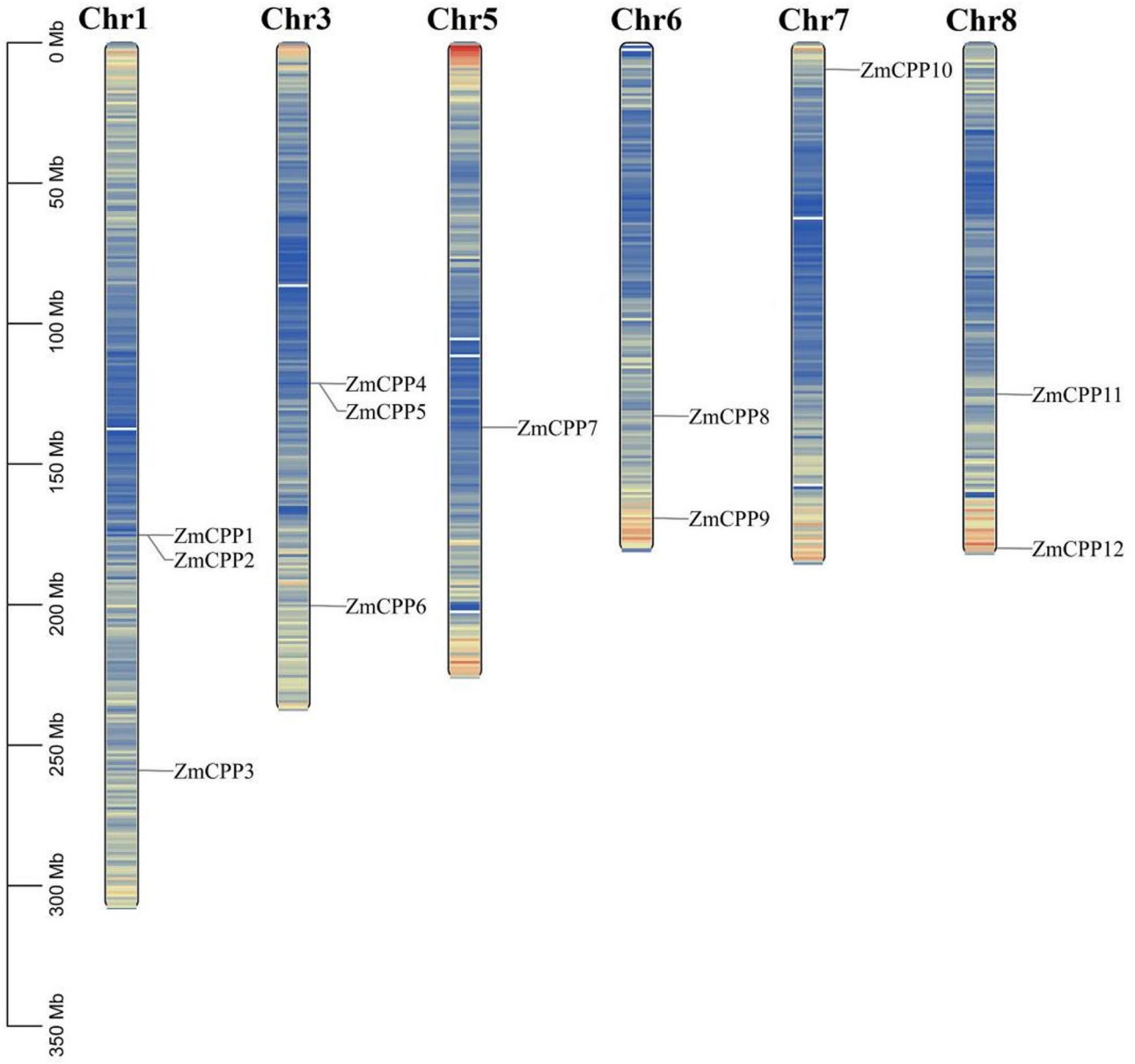

Fig. 1 Distribution of *ZmCPP* genes on maize chromosomes

on the predicted results, all ZmCPPs were localized in the nucleus (Table 1), consistent with the characteristics of a transcription factor, and may play a regulatory role in the cell.

Phylogenetic analysis of *ZmCPPs*

To better understand the evolutionary relationship among ZmCPPs, a Maximum Likelihood (ML) phylogenetic tree was created based on the full-length protein sequences of 12 ZmCPPs, 8 AtCPPs [8], 8 SbCPPs (Table S1), 11 OsCPPs [8], 37 TaCPPs [10], and 9 SiCPPs (Table S1). The phylogenetic tree indicated that the 85 CPPs were divided into four groups (Group I, II, III, and IV) (Fig. 2). Except Group IV, the other three groups both contained 4 *ZmCPPs*, indicating ZmCPPs could be divided into three subgroups (Fig. 2). Except AtCPPs, the other CPPs in group I, II, III were all from five species (maize, rice, wheat, sorghum, and foxtail millet), revealing that these CPPs may experience similar evolutionary process (Fig. 2). ZmCPPs were more closely related to the CPPs of monocotyledonous plants, such as sorghum and foxtail millet than to Arabidopsis (Fig. 2). In each cluster, the phylogenetic branches of most ZmCPPs exhibited have high bootstrap values with homologous genes in sorghum (Fig. 2), indicating a high sequence homology and a strong ancestral identity of these two species.

Table 1 Characteristics of *ZmCPP* genes

Mazie GDB ID	Gene Name	ORF (bp)	Protein Size (aa)	MW (kDa)	pI	Subcellular Location
Zm00001eb031300_T001	*ZmCPP1*	1185	394	42.99	7.76	Nucleus
Zm00001eb031310_T001	*ZmCPP2*	2313	770	83.05	6.58	Nucleus
Zm00001eb050860_T002	*ZmCPP3*	2205	734	78.95	6.18	Nucleus
Zm00001eb135740_T002	*ZmCPP4*	1185	394	42.46	6.61	Nucleus
Zm00001eb135750_T001	*ZmCPP5*	2319	772	83.07	7.19	Nucleus
Zm00001eb152420_T001	*ZmCPP6*	1824	607	66.59	8.45	Nucleus
Zm00001eb237340_T001	*ZmCPP7*	1593	530	57.88	6.08	Nucleus
Zm00001eb281570_T003	*ZmCPP8*	1500	499	55.12	7.45	Nucleus
Zm00001eb292380_T001	*ZmCPP9*	1080	359	39.94	8.29	Nucleus
Zm00001eb301170_T002	*ZmCPP10*	2403	800	84.98	6.09	Nucleus
Zm00001eb352860_T002	*ZmCPP11*	1071	356	39.67	8.41	Nucleus
Zm00001eb370470_T002	*ZmCPP12*	1845	614	67.19	8.81	Nucleus

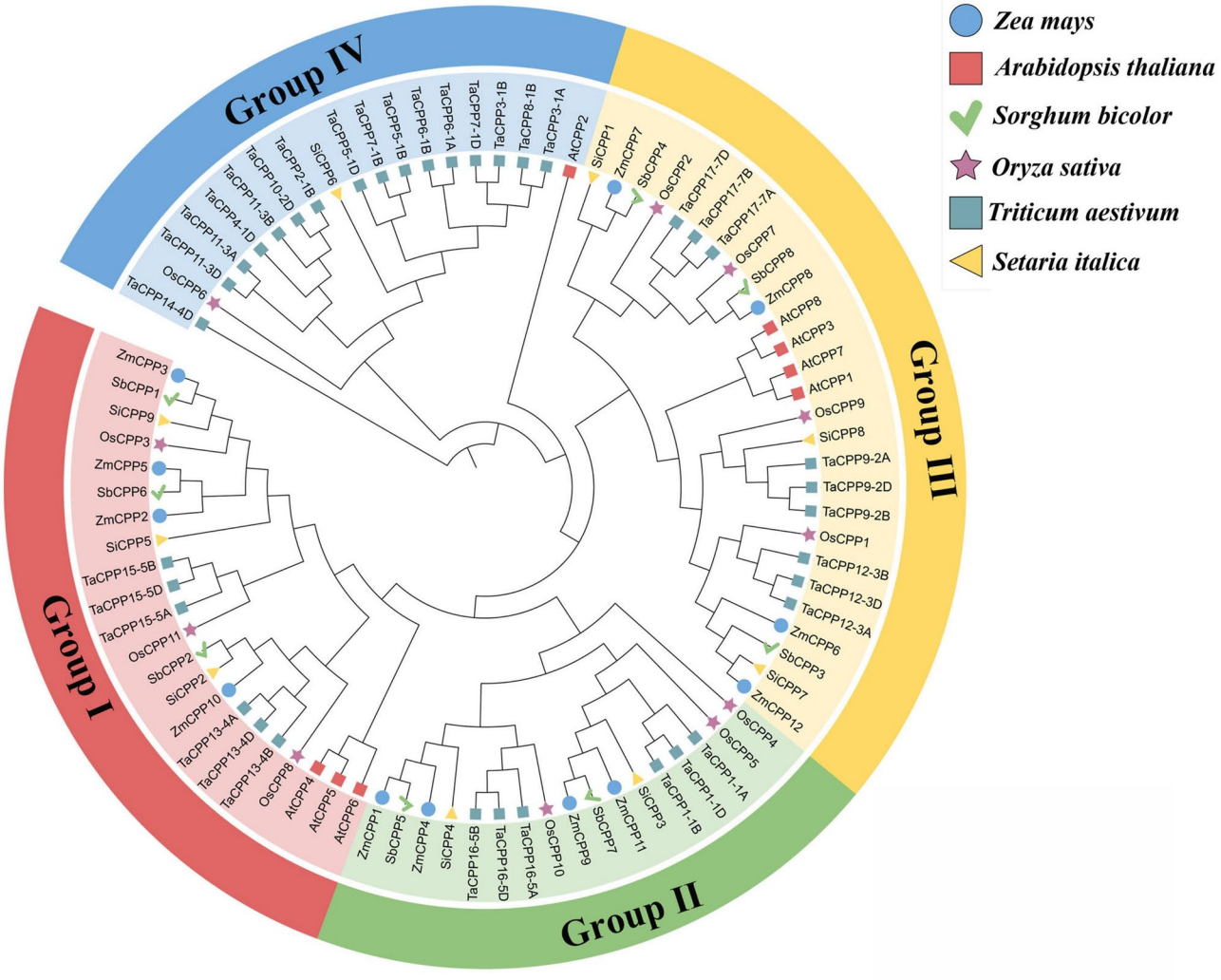

Fig. 2 Phylogenetic analysis of CPP homologs in maize (Zm), sorghum (Sb), rice (Os), wheat (Ta), foxtail millet (Si) and Arabidopsis (At). The rootless ML phylogenetic tree building by MEGA 7.0 (10,000 bootstrap replicates) was divided into four groups. Each branch is represented by specific colors

Structural analysis of *ZmCPP* genes

There were ten conserved motifs (motifs 1–10) in the 12 ZmCPPs (Fig. 3A). The TCR motif was the most conserved one, being present in all ZmCPP proteins (Fig. 3B). Except TCR domain, ZmCPPs in the same branch exhibited similar conserved domains, and there were significant differences in the number and composition of the conserved domains across different branches, which might be attributed to the varying functions of genes across different branches.

To evaluate the diversity among *ZmCPPs*, the gene structure of each *ZmCPP* was also analyzed. Each *ZmCPP* contained many exons and introns, and there were significant differences in the introns and exons of *ZmCPPs* (Fig. 3C). Like the protein domain, the gene structures within the same subfamily were similar (Fig. 3C). Combined, these findings indicated that *ZmCPPs* from the same branch might exhibit similar functions.

Collinearity among *ZmCPPs*

The 12 *ZmCPPs* are unequally distributed on 6 maize chromosomes (Figs. 1 and 4). The occurrence of gene replication events is an important inducer resulting in the expansion of gene family members. We found that the 12 *ZmCPPs* formed 3 pairs (*ZmCPP1* and *4*, *ZmCPP2* and *5*, *ZmCPP6* and *12*) as a result of gene fragment duplication events (Fig. 4). The remaining *ZmCPPs* were not identified as fragment or tandem replication pairs (Fig. 4). The relatively low frequency of gene segment duplication events may be the reason for the smaller number of *ZmCPP* members. No tandem events might also explain the distant distribution of *ZmCPPs* on maize chromosomes.

Additionally, the synteny among the *CPP* genes of maize, sorghum, rice, *Hordeum vulgare* and Arabidopsis was also examined. 12 pairs of orthologous *CPP* genes in maize and sorghum, 11 pairs in maize and rice, 7 pairs in maize and *Hordeum vulgare*, and 2 *CPP* pairs were detected in maize and Arabidopsis (Fig. 5). This finding indicated that *CPP* genes of maize and sorghum exhibited a relatively close evolutionary relationship.

Cis-acting elements of *ZmCPPs*

The *cis*-elements located in promoter regions of *ZmCPPs* were identified using TBtools. We identified 25 homeotic-acting elements in the promoter regions of *ZmCPPs* (Fig. 6). Among them, 2 elements (G-box and MRE) were related to light (Fig. 6). Five elements (CAT-box, circadian, MBSI, GCN4-motif, and O_2-site) were related to plant growth and development. Five elements (ARE, GC-motif, LTR, MBS, and TC-rich repeats) were involved in plant stress response (Fig. 6). Eight elements (ABA-responsive element (ABRE), AuxRR-core, CGTCA-motif, GARE-motif, P-box, TCA-element, TGACG-motif, and CCAAT-box) were related to the signal pathways of plant and hormone (gibberellic acid, GA and methyl jasmonate, MeJ) interaction. (Fig. 6). These results indicated that *ZmCPPs* may play significant roles in both plant

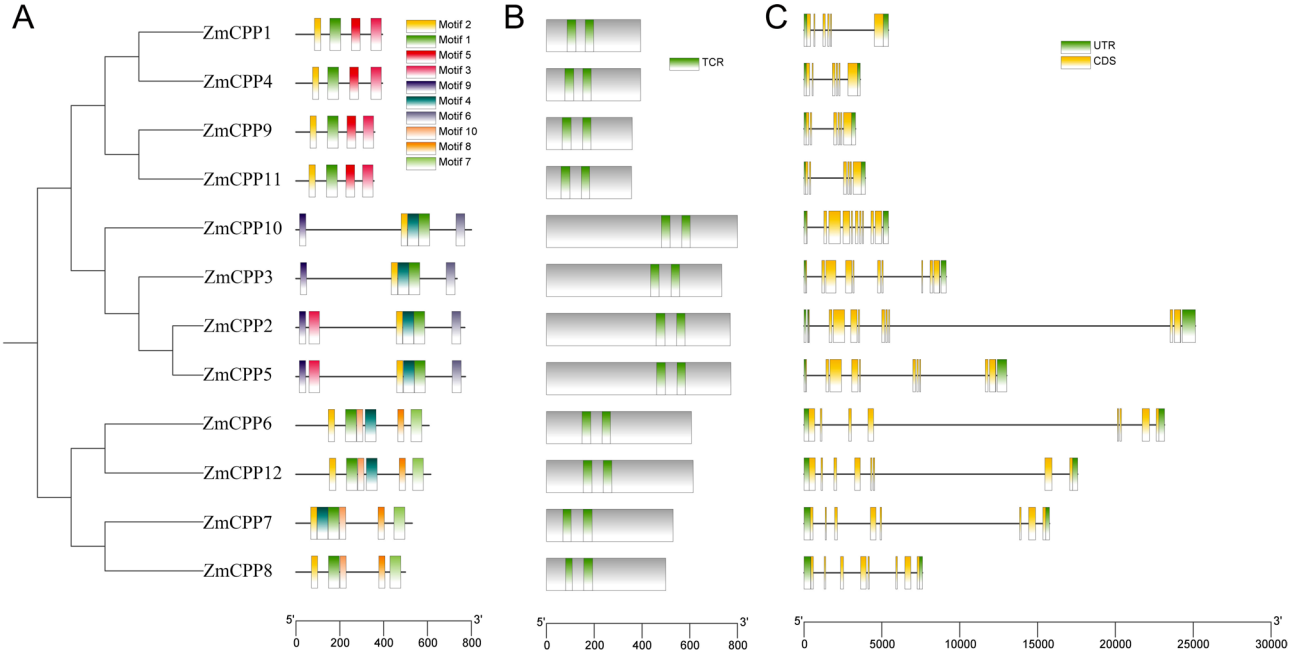

Fig. 3 The gene structures and conserved protein motifs of ZmCPPs. The clustering of *ZmCPPs* is based on the phylogenetic tree shown in Fig. 2. The distribution of the ten conserved motifs (boxes of different colors) in ZmCPPs. (**A**) The common sequences of motifs 1–10. (**B**) The location of the TCR motif. (**C**) The exon-intron structures of *ZmCPPs*

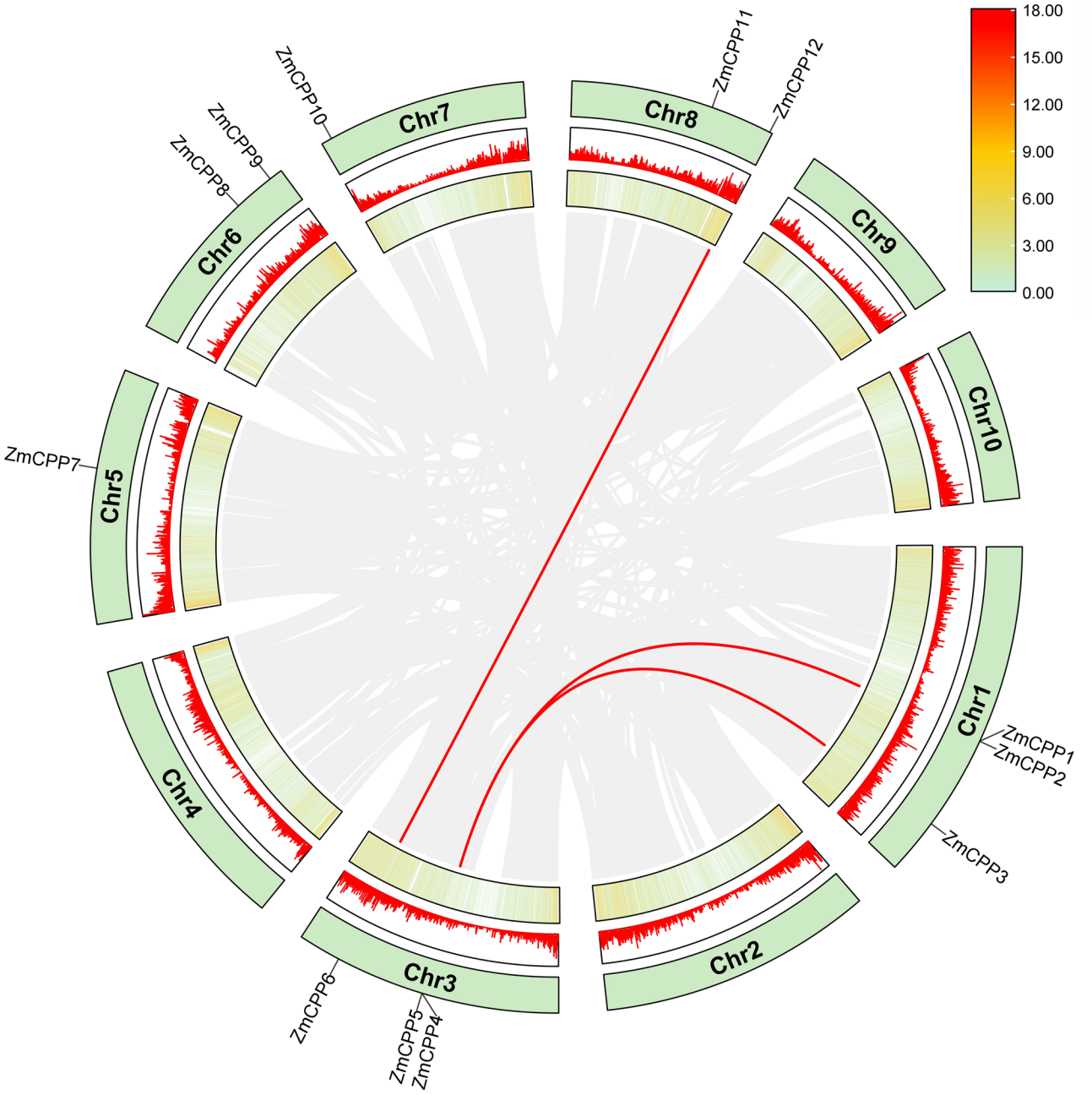

Fig. 4 Genome duplication events of *ZmCPPs*. Schematic diagram of the relationship of *ZmCPPs* on chromosomes. The heat map in the outer circle represents the gene density of chromosomes. The collinear *ZmCPPs* are connected by colored lines

development as well as in response to environmental stress.

RNA-seq profiles of *ZmCPPs*

To understand the expression profiles of *ZmCPPs*, we investigated the tissue-specific expression levels of *ZmCPPs* in different maize tissues (anthers, root, endosperm, pollen, leaf, internode, meristematic and seed) based on reported RNA-seq data [18, 19]. As shown in Fig. 7, *ZmCPP1*, ZmCPP2 and *ZmCPP5* exhibited higher expression levels in almost all explored tissues, suggesting their special role in maize development. Compared to other tissues, *ZmCPPs* exhibited lower expression levels in mature pollen, indicating that ZmCPP might be not regulate pollen development (Fig. 7). Furthermore, *ZmCPP9*, *ZmCPP10*, and *ZmCPP11* were highly expressed in the internode (Fig. 7); Compare to mature leaf, *ZmCPP10* showed higher expression level in growing leaf, suggesting it may regulate leaf development (Fig. 7). In addition, like *ZmCPP10*, *ZmCPP1/2/5* also exhibited

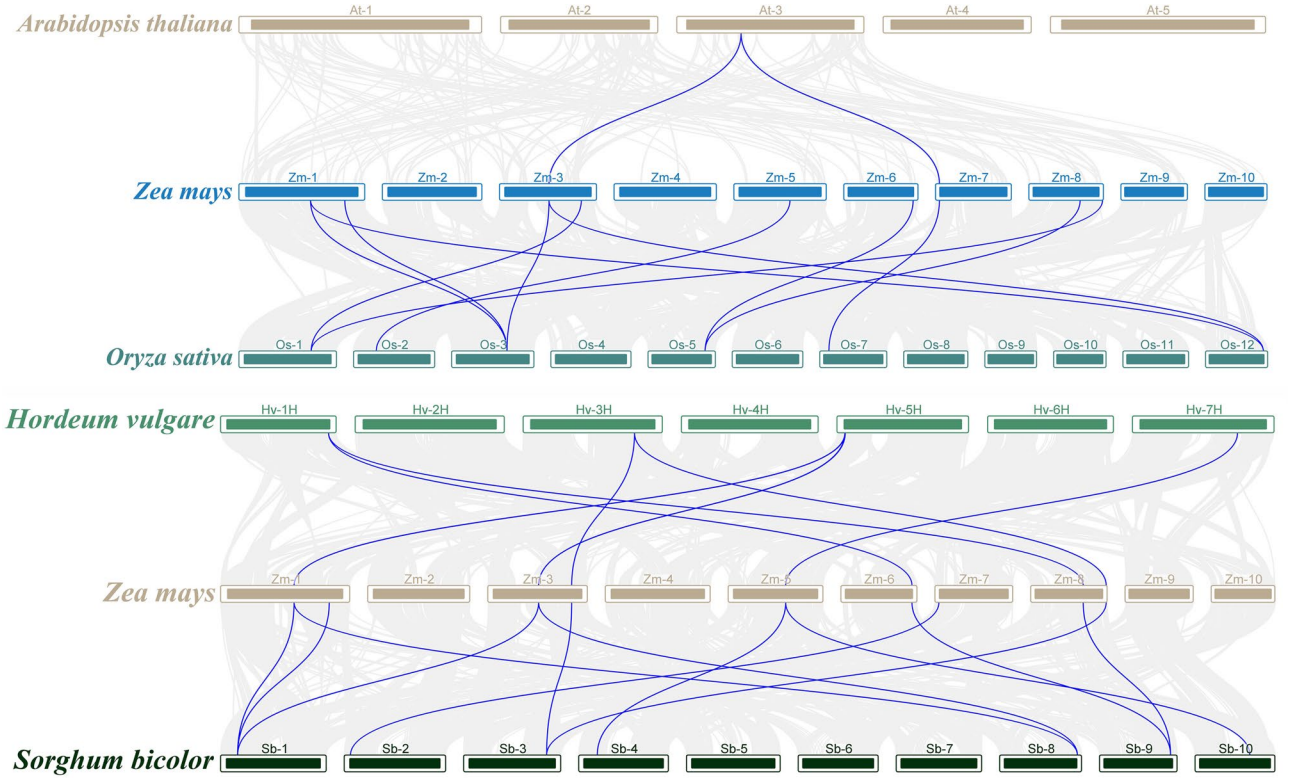

Fig. 5 The collinearity of *ZmCPPs* with the *CPP* genes of sorghum, rice, *Hordeum vulgare*, and Arabidopsis. The chromosome number is indicated below each chromosome. The gray lines in the background represent the collinear gene pairs between two species, and the *CPP* collinear genes are connected by blue lines

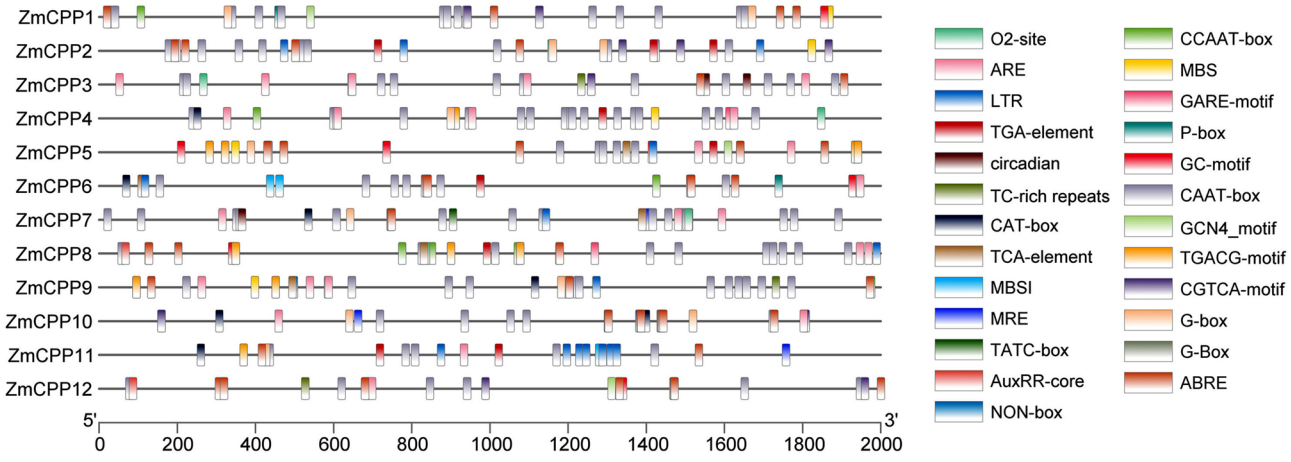

Fig. 6 Analysis of *cis*-elements in the promoter regions of *ZmCPPs*. Approximately 2 kb of the upstream promoter region of the *ZmCPPs* was downloaded from the maize B73 V5 genome

higher transcript levels in root meristem (Fig. 7), revealed these three genes may modulate root early development. ZmCPP6/7/8/12 from group III (Fig. 1) exhibited similar expression patterns, both showed low or no expression levels in selected tissues (Fig. 7), indicating that these genes were potentially not functional during maize development. Overall, these results indicated that *ZmCPPs* exhibited functional differentiation during maize growth and development, and might display regulating role in maize vegetative growth and reproductive period.

To explore the expressions of *ZmCPPs* in response to stress, we downloaded published RNA-seq data of maize B73 in response to abiotic (heat [20], cold [20], salt [20], waterlogging [21], cadmium [22], and UV [20]), biotic (Sugarcane *mosaic rirus*, SCMV [23]), and nutrition (nitrate) stress [24]. As shown in Fig. 8 and Fig. S1,

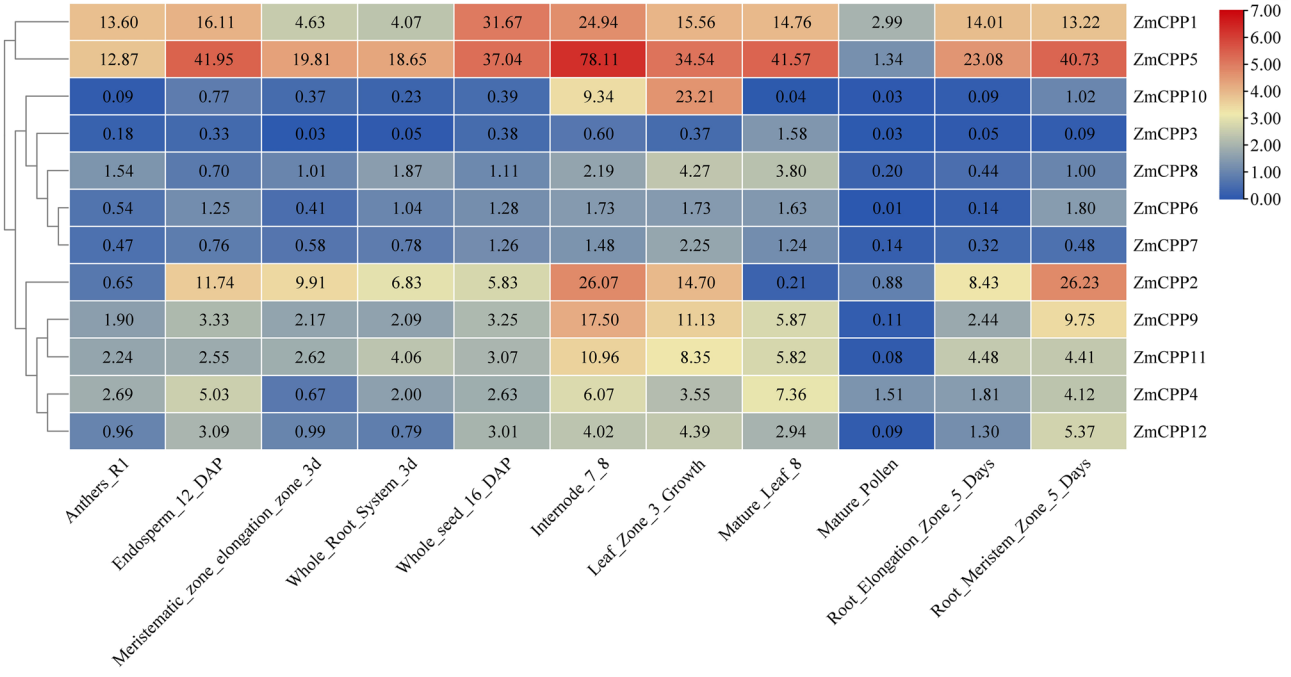

Fig. 7 Heat map of the expression levels of *ZmCPPs* in different maize tissues. Tissue name is labeled in figure. Heatmap construction was based on the fragments per kilobase per million mapped reads (FPKM) values. FPKMs data is listed in figure

almost all expression levels of *ZmCPPs* were downregulated under cold, heat, salt, and root cadmium stresses, suggesting that ZmCPPs might negatively modulate maize response to these stresses. On the contrary, the 12 *ZmCPPs* were induced by SCMV and waterlogging treatment, indicating ZmCPPs may positively modulate maize response to waterlogging stress and SCMV infection (Fig. 8 and Fig. S1). In addition, *ZmCPPs* showed different expression pattern under UV stress, and excessive nitrate treatment seemed not obviously change the expression level of *ZmCPPs* (Fig. S1). Altogether, RNA-Seq analysis indicated a significant role of ZmCPPs in the responses to various environmental stresses, with each *ZmCPP* having a specific response mechanism.

RT-qPCR validation of the expression levels of *ZmCPPs* under abiotic stress

Based on RNA-Seq analysis, six differentially expressed *ZmCPP* genes (*ZmCPP2*/*4*/*8*/*9*/*11*/*12*) were further analyzed using RT-qPCR under short-term heat, cold, dehydration, and waterlogging stress. As shown in Fig. 9A and B, the transcript of *ZmCPP2/4/9* was induced by heat and drought, while *ZmCPP12* was reduced under heat. Like heat shock, *ZmCPP2/9* upregulated by cold, and *ZmCPP8* showed conversely expression pattern (Fig. 9C). Under waterlogging stress, the expression patterns of both six genes were similar between the RT-qPCR and RNA-Seq data (Figs. 8 and 9D); All genes were upregulated by waterlogging treatment, indicating *ZmCPP* genes might positively modulating maize waterlogging stress response. Overall, these results indicated that *ZmCPPs* might be involved in the responses to abiotic stresses (heat, cold, drought, and waterlogging) in maize. However, the precise roles of these genes under abiotic stress need further exploration.

Subcellular localization and transactivation activity assays of ZmCPP2 and ZmCPP9

ZmCPP2 and ZmCPP9, which positive induced by all four abiotic stresses under RT-qPCR analysis (Fig. 9), were chosen to further explore subcellular localization and transactivation activity. The *ZmCPP2/9-GFP* or *GFP* expression vector was transformed into maize mesophyll protoplasts through the PEG-Ca method. Like the GFP control, the ZmCPP2/9-GFP signal was distributed in the cytoplasm and nucleus, indicating that ZmCPP2/9 are widely distributed in cells under normal conditions (Fig. 10A). The transactivation activities of ZmCPP2 and ZmCPP9 were analysis through yeast Y2H system. Like the negative control, yeast cells carrying BD-CPP2/9 and pGADT7 empty vectors did not survive on SD medium without tryptophan, leucine, histidine, and adenine (SD/-LWHA) (Fig. 10B). In contrast, the positive control group could grow well on SD/- LWHA, and could show a blue color after adding X-α-gal (Fig. 10B). This result revealed that ZmCPP2 and ZmCPP9 both had no transcriptional activity in yeast.

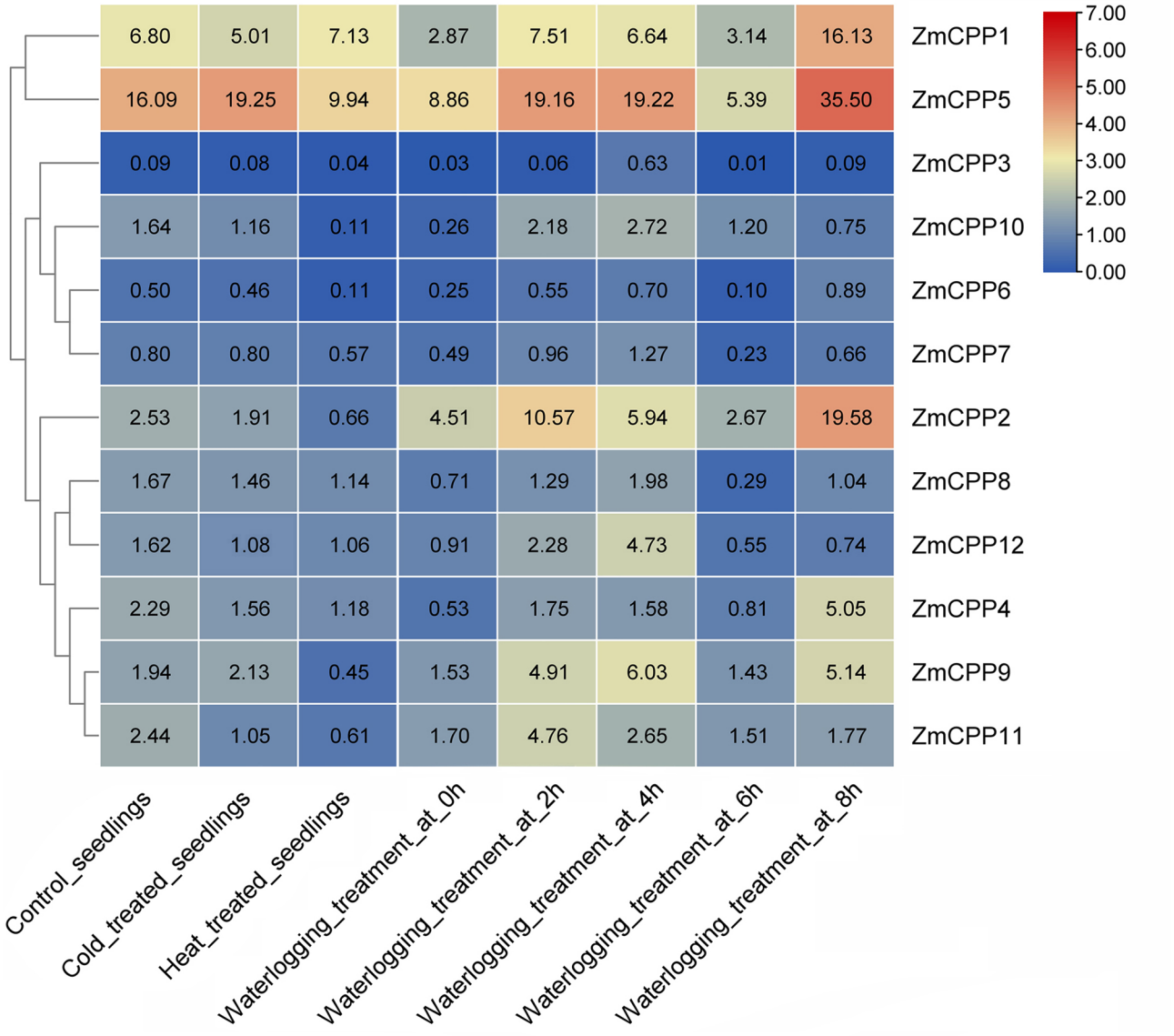

Fig. 8 Heat map of the expression levels of *ZmCPPs* in different environmental stresses. Specific stress names are marked in the figure. Heatmap construction was based on the fragments per kilobase per million mapped reads (FPKM) values. Detailed FPKMs are listed in figure

Discussion

CPP proteins play an important role in plant growth, development and stress response [12]. However, the roles of *ZmCPPs* in maize have not been reported in detail. Previous report revealed maize contain 13 *ZmCPP* genes based on B73 V3 genome sequence data, named *ZmCPP1-13* [17]. The present study used bioinformatics to identify 12 *ZmCPPs* in maize last assembled V5 genome (Fig. 1; Table 1). After comparing with our characterization results, we found *ZmCPP11* and *ZmCPP12* in previous work were located in the same gene locus in our study, and renamed with *ZmCPP12* in our work. Due to the differences in the chromosomal position annotation information, *ZmCPP1/2/3/13* in the previous studies [17] were named *ZmCPP2/3/1/12* in this study, respectively.

The phylogenetic analysis using 85 CPP protein sequences from maize, Arabidopsis, wheat, rice, sorghum, and foxtail millet in the current study showed that these CPPs group into four branches (groupI, II, III, IV) (Fig. 2); Except group IV, ZmCPPs could be divided into other three subfamily with same members in each group (Fig. 2). ZmCPPs have high bootstrap values with SbCPPs (Fig. 2), indicating a strong ancestral identity of these two species; Furthermore, group IV containing AtCPP/OsAPP/TaCPP/SiCPP and lacking ZmCPP/SbCPP; suggesting maize/sorghum, and these other species may have diverged during the evolutionary process, which could have led to CPP protein functional differences. In

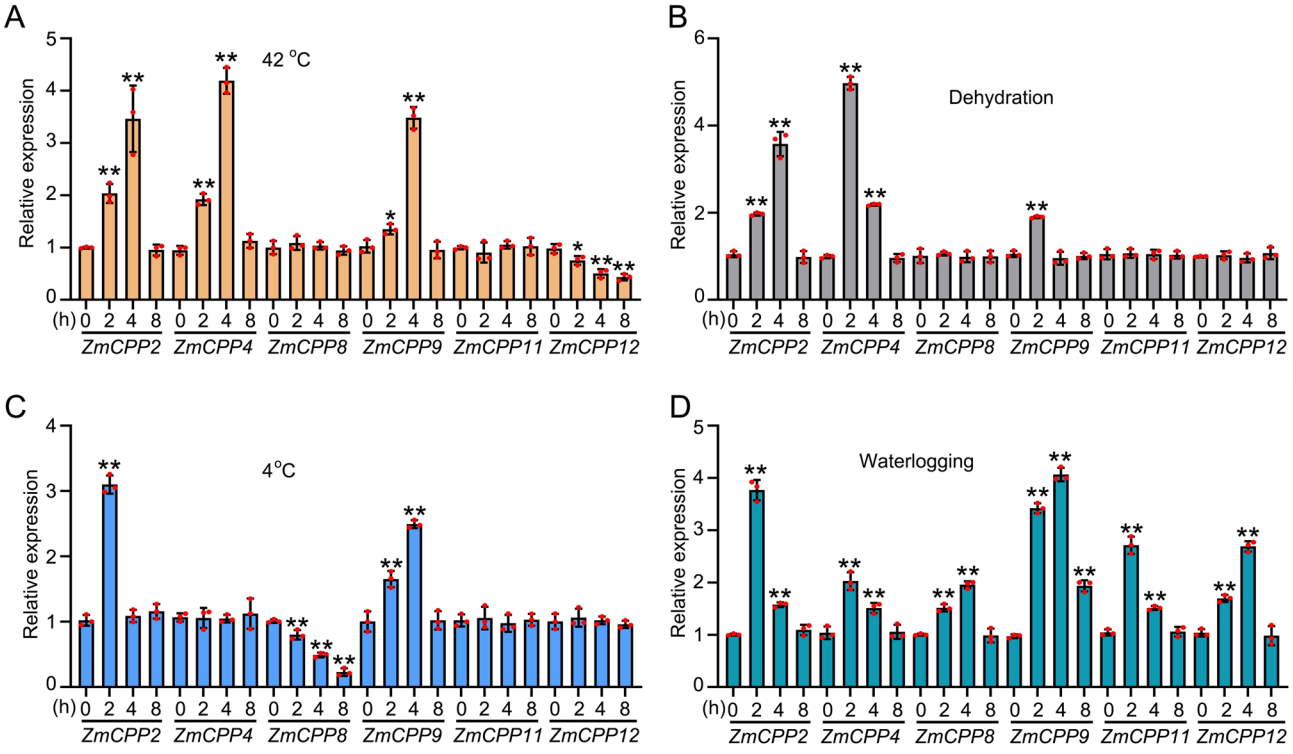

Fig. 9 RT-qPCR analysis of the expressions of *ZmCPP2/4/8/9/11/12* in maize B73 seedlings under heat (**A**), dehydration (**B**), cold (**C**), and waterlogging (**D**) treatments. The treatment times and conditions are labeled within the figure. Values represent means ± SD; $n = 3$. ** $p < 0.01$, * $p < 0.05$ compared with 0 h (Student's *t* test)

each subfamily, the number of ZmCPPs is four (Fig. 2). Moreover, the gene and protein structures are highly similar within the same group (Fig. 3), implying that homologous proteins might display similar functions. Previous studies have indicated that AtCPP4 (SOL1)/AtCPP5 (TSO1) (belong to group I in our study) play the same role in controlling floral tissue development [7, 12, 25]; ZmCPP5 showed relative high expression in anthers (Fig. 7) and homologous with AtCPP1/5 (Fig. 2), suggesting ZmCPP5 might also modulating maize inflorescence differentiation.

Gene duplication events drive the expansion of gene families within species; During the evolutionary process, gene duplication enables the genes to display new functions or differentiate from existing functions, thus enhancing plant's adaptability [26]. Tandem and segmental replications have played important roles in expanding gene families. The present study, through the analysis of gene replication events in the *ZmCPP* family, revealed that fragment replication plays a crucial role in the evolutionary expansion of *ZmCPPs* (Fig. 4). The relatively low frequency of duplication events in the *ZmCPPs* family may be the primary reason why maize has four chromosomes without *ZmCPP* gene loci and a smaller number of *ZmCPP* family. *CPP* gene family were first identified in Arabidopsis [7]. Subsequently, an increasing number of *CPPs* have been identified and functionally analyzed in multiple species, including rice and soybean [8, 9]. In Arabidopsis, eight *CPPs* have been identified [8]. In rice, 11 family members have been found [8]. In soybean, the number of *CPPs* has expanded to 20 [9]. In this study, *12 ZmCPPs* were identified (Fig. 1; Table 1), indicating that *CPP* genes have evolved in different species. Gene duplication is crucial in plant evolution and is also important in driving genome evolution [27, 28]. Homologous genes usually exhibit different functions, but they may also retain the same functions [29, 30]. We identified 12/11/7/2 pairs of orthologous *CPPs* in maize and sorghum, rice, *Hordeum vulgare*, and Arabidopsis, respectively (Fig. 5). These results indicate that these gene families have expanded in a species-specific manner. This phenomenon has been widely confirmed in other plant gene families [31–33].

The promoters of *ZmCPPs* included many *cis*-elements involve in both plant development as well as in response to environmental stress (Fig. 6). Report indicate some members of plant CPPs play important regulatory roles in the vegetative growth and development of plants. For example, the transcript of *CPPs* can be specifically expressed/detected in the juvenile leaf stage [9], pollen development stage [7], young leaves, and shoot apices. In Arabidopsis, the mutation of *TSO1* could result in changes the structure of flowers and a reduction in pollen levels, and further affects the growth of ovules and pollen

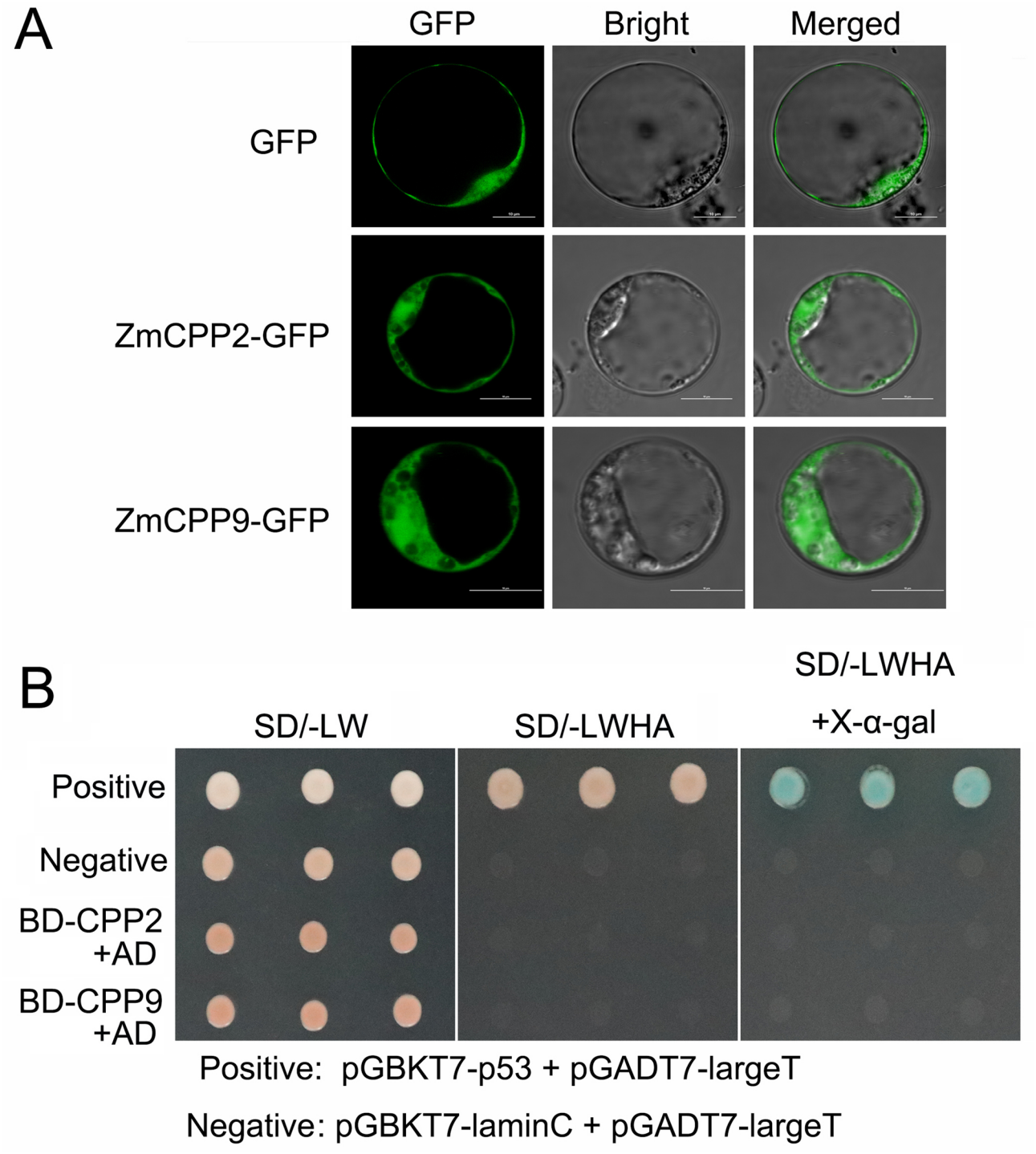

Fig. 10 Subcellular localization and transactivation activity assays of ZmCPP2 and ZmCPP9. (**A**) Subcellular localization of ZmCPP2 and ZmCPP9. The GFP (control) or ZmCPP2/9-GFP fusion expression vectors was transformed into maize mesophyll protoplasts using PEG-Ca method. The GFP signals are labeled green. Bars = 10 μm. (**B**) Transcription activation analysis of ZmCPP2 and ZmCPP9 in yeast. Full-length ZmCPP2 or ZmCPP9 was fused with the GAL4-DNA BD in the pGBKT7 vector (BD-CPP2 or BD-CPP9). BD-CPP2/9 and pGADT7(AD) were cotransformed into yeast Y2H gold. The yeast cells were cultured on SD lacking tryptophan and leucine (SD-LW), then transferred to an SD medium without tryptophan, leucine, histidine, and adenine (SD-LWHA, containing or not containing X-α-gal) to detect interactions. The positive or negative controls are labeled in the figure, respectively

[14]. *GmCPP16* is specifically expressed in young leaves and root nodules [9]. Transcriptomics analysis showed that *ZmCPPs* are expressed in various tissues, including roots, seeds, flowers, leaves, and internodes (Fig. 7). Most of these genes exhibit tissue-specific expression; For example, *ZmCPP5* has relatively high expression levels in stem internodes and endosperm, while *ZmCPP1* is highly expressed in mature leaves. These results revealed that ZmCPPs may be involved in the process of regulating maize growth and development. The expression of plant *CPPs* are also regulated by various abiotic stresses [34]. Basing on the maize heat treatment (50 ℃ for 4 h) RNA-Seq data [20], we found almost all *ZmCPPs* were downregulated (Fig. 8); while under 42 ℃, the expression of *ZmCPP2/4/9* was induced (Fig. 9). Similar to heat stress, under the treatment condition of 5 °C for 16 h, the expression of almost all *ZmCPPs* was also downregulated [20]; However, short-term treatment at 4 °C significantly induced the expression of *ZmCPP2/9* (Fig. 9). Under heat shock, two *AtCPPs* (*TCX6* and *TCX3*) are significantly downregulated in *atcngc16* knockout mutant [35]. Other published report indicated the transcript of *CPPs* are modulated by cold stress [36–38]. These results indicated *CPPs* responds to temperature stress, but the specific response mechanisms still need further research. Recent study reveal ZmCPP2 (in our work named ZmCPP1) positively regulating maize drought tolerance [16]. RT-qPCR result indicated *ZmCPP2/4/9* could induced by drought (Fig. 9), moreover *ZmCPP1* and *ZmCPP4* showed fragment duplication relationship (Fig. 4), implying ZmCPP4 may also involve in maize drought response. All *ZmCPPs* respond to root waterlogging stress (Figs. 8 and 9), indicating that ZmCPPs might play important role in the oxygen deficiency signaling transduction process in roots compared to other abiotic stresses.

ZmCPP2 and *ZmCPP9* which positively induced by short-term heat, cold, drought, and waterlogging stress (Fig. 9), were further chosen to explored the localization and transactivation activity. ZmCPP2/9 both present in the nucleus and cytoplasm under normal conditions (Fig. 10A) and these two proteins seemed unable to activate downstream genes in yeast (Fig. 10B). Although *ZmCPP2/9* is induced by stress signals, ZmCPP2/9 may negatively modulating maize stress responses. However, its needs to be further analysis.

Conclusions

In this study, 12 *ZmCPPs* were identified and characterized. Phylogenetic analysis indicated that ZmCPPs belonged to three subfamilies. Furthermore, *ZmCPPs* in the same evolutionary group shared their gene and protein structures. The analysis of *cis*-acting elements in the promoter combined with the expression heatmap based on RNA-seq data indicates *ZmCPPs* are involved in the regulation of plant growth/development and respond to various biotic/abiotic stresses. RT-qPCR assay further showed *ZmCPPs* could respond to heat, cold, drought, and waterlogging treatments. Meanwhile, ZmCPP2/9 might play negative regulatory roles in maize stress response. This study provided a theoretical foundation for further exploring the role of ZmCPPs in maize growth regulation and stress response processes.

Methods

Plant material and growth conditions

The Maize (*Zea mays* L.) inbred line B73 was used in this work. Maize plants were growing in the Crop Resource Repository of Guizhou Normal University (26°22′51.06″N, 106°38′11.80″E, 1100.5 m above sea level) to collect seeds. B73 seeds were grown under greenhouse conditions in the soil at 26 °C/22°C (day/night) and in Hoagland nutrient solution, with a photoperiod of 16 h/8 h (day/night) and continuous cultivation for 2 weeks. During the V3 stage, the seedlings were placed in a 42 ℃ or 4 ℃ growth chamber for either heat or cold stress for 0, 2, 4, or 8 h [39]. The roots were collected for waterlogging by maintaining a 2 cm water layer above the first leaf for 0, 2, 4, or 8 h [39]. For dehydration stress, the seedlings were removed from the soil for 0, 2, 4, or 8 h and harvested the same position of leaves. Three biological replicates were used for downstream analysis at each time point.

Identification of ZmCPP proteins

To identify the *ZmCPPs*, the maize B73 reference genome (Zm-B73-REFERENCE-NAM-5.0), the sequences of AtCPPs and OsCPPs were downloaded from Maize GDB (https://maizegdb.org, accessed on 18 February 2025), TAIR (https://www.arabidopsis.org/servlets/Search, visited on 18 February 2025) and rice genome annotation venture release 7 (MSU 7.0, http://rice.plantbiology.msu.edu, visited on 18 February 2025), respectively. The hidden Markov model (HMM) files of the core CXC motifs (pfam03638) were acquired from the Pfam database [40] (http://pfam-legacy.xfam.org/, visited on 19 February 2025). The AtCPP/OsCPP sequences as queries to search for *ZmCPPs* using TBtools (v2.069) (E value: 1e-5) [41]. The sequences containing CXC domain were screened using HMMER 3.0 software [42]. The ZmCPPs identified by the above two methods were combined and removed the repeat sequence, and the complete CXC domain was verified using the Pfam database (http://pfam-legacy.xfam.org/, visited on 20 February 2025) and SMART database [43] (http://smart.embl-heidelberg.de, visited on 20 February 2025). Finally, the members of *ZmCPP* gene family were obtained and named. Chromosomal localization of the *ZmCPPs* was obtained from Maize GDB and visualized using TBtools (v2.069).

Phylogenetic analysis and classification of the *ZmCPP* gene family

Multiple sequence comparisons of full-length plant CPP protein sequences (Table S1) (sorghum, rice, wheat, foxtail millet, Arabidopsis, and maize) (these species represent different plant groups, encompassing the diversity from monocots to dicots) were performed using ClustalW (v1.81) with the default parameters, and the phylogenetic tree was constructed using the MEGA11 software (v11.0.13) [44], in which the Maximum Likelihood (ML) was used. Bootstrap was set to 1000, and other options were set to the default values. The phylogenetic tree was visualized using iTOL (https://itol.embl.de/, accessed on 22 February 2025).

***Cis*-acting elements analysis and physicochemical properties**.

The properties of the ZmCPPs were analyzed using the ExPASy tool (https://web.expasy.org/cgi-bin/protparam/, accessed on 22 February 2025). The subcellular localization prediction website Plant mPloc (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/, accessed on 22 February 2025) was used to predict the subcellular localization of ZmCPPs. To determine the *cis*-elements in the promoters of *ZmCPPs*, the 2000-bp promoter sequences of *ZmCPPs* were acquired from the Maize GDB and analyzed using the Plant CARE [45] (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/, accessed on 23 February 2025). Furthermore, TBtools was employed to visualize the composition of *cis*-elements in the promoters.

Conserved motif and domain analysis

The GFF3 annotation file was downloaded from the Maize GDB database and utilized to display the structures of *ZmCPPs* in TBtools (v2.069). MEME [46] (http://alternate.meme-suite.org/tools/meme, accessed on 3 March 2025) was used to conduct a conserved motif analysis of ZmCPPs, identifying up to ten conserved motifs. MEME-derived motif sequence logos were presented using TBtools (v2.069).

Gene duplication and synteny analysis of *ZmCPPs*

Gene duplications of the *ZmCPPs* were identified and examined by TBtools. The interspecific collinearity of *ZmCPPs* with the other four species (sorghum, rice, *Hordeum vulgare*, and Arabidopsis) (these plants are relatively close in evolution, and their genome sizes and structures are relatively moderate, which facilitates detailed collinearity analysis) was used to analyze and visualize using MCScanX [47] and Circos within TBtools, respectively.

RNA-Seq data analysis

To examine the specific expression patterns of *ZmCPPs* in maize, transcriptome data for different tissues of maize and maize under various stresses were obtained from Maize GDB (https://www.maizegdb.org/, accessed on 19 March 2025). Then, the expressions of *ZmCPPs* were analyzed and used to create a heatmap using TBtools.

Real-time quantitative polymerase chain reaction (RT-qPCR) analysis

Three biological duplicate samples were collected for RT-qPCR validation analysis. Total RNA was extracted using Trizol reagent (CWBIO, Beijing, China) and reverse transcribed 2 μg of total RNA sample using a first strand synthesis kit (Thermo Fisher Scientific, Waltham, MA, USA) to generate the cDNA. The Primer-BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi) was used to design gene-specific primers. The 0 h leaf or root samples served as the control, and the relative expression of the gene in each period was calculated using the $2^{-\triangle\triangle CT}$ method [48]. The *ZmActin1* (Zm00001eb348450) was used as the internal control. The significance of the data was confirmed by the student's *t*-test ($p < 0.05$ or $p < 0.01$) using SPSS v19.0 (SPSS Inc., Chicago, IL, USA). All primers are listed in Table S2.

Subcellular localization and transactivation activity assays

The ORF of *ZmCPP2/9* was amplified using primers ZmCPP2/9-Y-F/R (Table S2) and ligated into the pROKII vector [49]. *GFP* (Empty pROKII vector) control or *ZmCPP2/9-GFP* vectors was transformed into maize B73 mesophyll protoplasts following a published report [50]. After culturing at 25 °C for 17 h, GFP signal was observed with a scanning confocal microscope (Andor Revolution WD, Belfast, Northern Ireland, UK).

The ORF of *ZmCPP2/9* was amplified using primers (ZmCPP2/9-F/R, Table S2) and ligated into pGBKT7, fused with the Gal4-DNA-binding domain (BD-CPP2/9). The pGADT7 and BD-CPP2/9 vectors were cotransformed into yeast strain Y2HGold. pGBKT7-p53 / pGADT7-largeT and pGBKT7-laminC/pGADT7-largeT were also cotransformed into yeast and used as positive and negative controls, respectively. The transformed yeast was successively cultured in SD/-Trp/-Leu and SD-Trp/-Leu/-His/-Ade/medium with or without X-α-Gal for 4 days.

Abbreviations

CPP	Cysteine-rich polycomb-like protein
TFs	Transcription factors
CXC	Cys-rich domains
ML	Maximum Likelihood
ORF	Open reading frame
HMM	The hidden Markov model
ABRE	ABA-responsive element
GA	Gibberellic acid
MeJ	Methyl jasmonate
RNA-seq	RNA-sequencing
UV	Ultraviolet
RT-qPCR	Quantitative real-time polymerase chain reaction

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06783-5.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Acknowledgements
Not applicable.

Author contributions
LG and YL conceived research plans and designed experiments. LG and TK conducted bioinformatics analysis and experiments. TZ, HW, BZ, and XD analyzed the data. LG and TK wrote the draft. LG and YL reviewed and edited this article. All authors read and approved the final manuscript.

Funding
This work was supported by the National Natural Science Foundation of China [grant numbers 32460461]. Guizhou Provincial Basic Research Program (Natural Science) [grant number QKHJCZD (2025) 047]. Guizhou Science and Technology Innovation Team Project [grant number QKHRCCXTD (2025) 055]. Guizhou Province Science and Technology Innovation Platform Construction Project [grant number QKHPTZSYS (2025) 025].

Data availability
All data generated or analyzed during this study were included in this published article and the additional files.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 22 April 2025 / Accepted: 26 May 2025
Published online: 30 May 2025

References

1. Shao J, Huang K, Batool M, Idrees F, Afzal R, Haroon M, Noushahi HA, Wu W, Hu Q, Lu X, Huang G, Aamer M, Hassan MU, El Sabagh A. Versatile roles of polyamines in improving abiotic stress tolerance of plants. Front Plant Sci. 2022;13:1003155.
2. Yang Z, Cao Y, Shi Y, Qin F, Jiang C, Yang S. Genetic and molecular exploration of maize environmental stress resilience: towards sustainable agriculture. Mol Plant. 2023;16(10):1496–517.
3. Zhou Y, Hu LF, Ye SF, Jiang LW, Liu SQ. Genome-wide identification and characterization of cysteine-rich polycomb-like protein (CPP) family genes in cucumber (*Cucumis sativus*) and their roles in stress responses. Biologia. 2018;73(4):425–35.
4. Sun Y, Jia X, Chen D, Fu Q, Chen J, Yang W, Yang H, Xu X. Genome-wide identification and expression analysis of cysteine-rich polycomb-like protein (CPP) gene family in tomato. Int J Mol Sci. 2023;24(6):5762.
5. Tan J, Xuan X, Su S, Jiao Y, Guo H, Zhang Z. Comprehensive analysis of the CPP gene family in *Moso bamboo*: insights into their role in rapid shoot growth. BMC Genomics. 2024;25(1):1173.
6. Brzeska K, Brzeski J, Smith J, Chandler VL. Transgenic expression of CBBP, a CXC domain protein, establishes paramutation in maize. Proc Natl Acad Sci U S A. 2010;107(12):5516–21.
7. Sijacic P, Wang W, Liu Z. Recessive antimorphic alleles overcome functionally redundant loci to reveal TSO1 function in Arabidopsis flowers and meristems. PLoS Genet. 2011;7(11):e1002352.
8. Yang Z, Gu S, Wang X, Li W, Tang Z, Xu C. Molecular evolution of the CPP-like gene family in plants: insights from comparative genomics of Arabidopsis and rice. J Mol Evol. 2008;67(3):266–77.
9. Zhang L, Zhao HK, Wang YM, Yuan CP, Zhang YY, Li HY, Yan XF, Li QY, Dong YS. Genome-wide identification and expression analysis of the CPP-like gene family in soybean. Genet Mol Res. 2015;14(1):1260–8.
10. Ullah U, Buttar ZA, Shalmani A, Muhammad I, Ud-Din A, Ali H. Genome-wide identification and expression analysis of CPP-like gene family in *Triticum aestivum* L. under different hormone and stress conditions. Open Life Sci. 2022;17(1):544–62.
11. Hauser BA, Villanueva JM, Gasser CS. Arabidopsis TSO1 regulates directional processes in cells during floral organogenesis. Genetics. 1998;150(1):411–23.
12. Hauser BA, He JQ, Park SO, Gasser CS. TSO1 is a novel protein that modulates cytokinesis and cell expansion in Arabidopsis. Development. 2000;127(10):2219–26.
13. Andersen SU, Algreen-Petersen RG, Hoedl M, Jurkiewicz A, Cvitanich C, Braunschweig U, Schauser L, Oh SA, Twell D, Jensen EO. The conserved cysteine-rich domain of a tesmin/TSO1-like protein binds zinc in vitro and TSO1 is required for both male and female fertility in *Arabidopsis thaliana*. J Exp Bot. 2007;58(13):3657–70.
14. Wang W, Sijacic P, Xu P, Lian H, Liu Z. Arabidopsis TSO1 and MYB3R1 form a regulatory module to coordinate cell proliferation with differentiation in shoot and root. Proc Natl Acad Sci U S A. 2018;115(13):E3045–54.
15. Cvitanich C, Pallisgaard N, Nielsen KA, Hansen AC, Larsen K, Pihakaski-Maunsbach K, Marcker KA, Jensen EO. CPP1, a DNA-binding protein involved in the expression of a soybean leghemoglobin c3 gene. Proc Natl Acad Sci U S A. 2000;97(14):8163–8.
16. Xiang Y, Liu W, Niu Y, Li Q, Zhao C, Pan Y, Li G, Bian X, Miao Y, Zhang A. The maize GSK3-like kinase ZmSK1 negatively regulates drought tolerance by phosphorylating the transcription factor ZmCPP2. Plant Cell. 2025;37(2):koaf032.
17. Song XY, Zhang YY, Wu FC, Zhang L. Genome-wide analysis of the maize (*Zea May* L.) CPP-like gene family and expression profiling under abiotic stress. Genet Mol Res. 2016;15(3):15038023.
18. Stelpflug SC, Sekhon RS, Vaillancourt B, Hirsch CN, Buell CR, de Leon N, Kaeppler SM. An expanded maize gene expression atlas based on RNA sequencing and its use to explore root development. Plant Genome. 2016;9(1):1–16.
19. Walley JW, Sartor RC, Shen Z, Schmitz RJ, Wu KJ, Urich MA, Nery JR, Smith LG, Schnable JC, Ecker JR, Briggs SP. Integration of omic networks in a developmental atlas of maize. Science. 2016;353(6301):814–8.
20. Makarevitch I, Waters AJ, West PT, Stitzer M, Hirsch CN, Ross-Ibarra J, Springer NM. Transposable elements contribute to activation of maize genes in response to abiotic stress. PLoS Genet. 2015;11(1):e1004915.
21. Yu F, Tan Z, Fang T, Tang K, Liang K, Qiu F. A comprehensive transcriptomics analysis reveals long non-coding RNA to be involved in the key metabolic pathway in response to waterlogging stress in maize. Genes (Basel). 2020;11(3):267.
22. Cheng D, Tan M, Yu H, Li L, Zhu D, Chen Y, Jiang M. Comparative analysis of cd-responsive maize and rice transcriptomes highlights cd co-modulated orthologs. BMC Genomics. 2018;19(1):709.
23. Rodriguez-Gomez G, Vargas-Mejia P, Silva-Rosales L. Differential expression of genes between a tolerant and a susceptible maize line in response to a *sugarcane mosaic virus* infection. Viruses. 2022;14(8):1803.
24. Ravazzolo L, Trevisan S, Forestan C, Varotto S, Sut S, Dall'Acqua S, Malagoli M, Quaggiotti S. Nitrate and ammonium affect the overall maize response to nitrogen availability by triggering specific and common transcriptional signatures in roots. Int J Mol Sci. 2020;21(2):686.
25. Song JY, Leung T, Ehler LK, Wang C, Liu Z. Regulation of meristem organization and cell division by TSO1, an Arabidopsis gene with cysteine-rich repeats. Development. 2000;127(10):2207–17.
26. Xu J, Zhao X, Bao J, Shan Y, Zhang M, Shen Y, Abubakar YS, Lu G, Wang Z, Wang A. Genome-wide identification and expression analysis of SNARE genes in *Brassica napus*. Plants (Basel). 2022;11(5):711.
27. Kent WJ, Baertsch R, Hinrichs A, Miller W, Haussler D. Evolution's cauldron: duplication, deletion, and rearrangement in the mouse and human genomes. Proc Natl Acad Sci U S A. 2003;100(20):11484–9.
28. Lan T, Xiong W, Chen X, Mo B, Tang G. Plant cytoplasmic ribosomal proteins: an update on classification, nomenclature, evolution and resources. Plant J. 2022;110(1):292–318.

29. Conant GC, Wagner GP, Stadler PF. Modeling amino acid substitution patterns in orthologous and paralogous genes. Mol Phylogenet Evol. 2007;42(2):298–307.
30. Ahn S, Tanksley SD. Comparative linkage maps of the rice and maize genomes. Proc Natl Acad Sci U S A. 1993;90(17):7980–4.
31. Bai J, Pennill LA, Ning J, Lee SW, Ramalingam J, Webb CA, Zhao B, Sun Q, Nelson JC, Leach JE, Hulbert SH. Diversity in nucleotide binding site-leucine-rich repeat genes in cereals. Genome Res. 2002;12(12):1871–84.
32. Zhang S, Chen C, Li L, Meng L, Singh J, Jiang N, Deng XW, He ZH, Lemaux PG. Evolutionary expansion, gene structure, and expression of the rice wall-associated kinase gene family. Plant Physiol. 2005;139(3):1107–24.
33. Jain M, Tyagi AK, Khurana JP. Genome-wide analysis, evolutionary expansion, and expression of early auxin-responsive SAUR gene family in rice (*Oryza sativa*). Genomics. 2006;88(3):360–71.
34. Kang WH, Park B, Lee J, Yeom SI. Tissue-specific RNA-Seq analysis and identification of receptor-like proteins related to plant growth in *Capsicum annuum*. Plants (Basel). 2021;10(5):972.
35. Charpentier M, Sun J, Vaz Martins T, Radhakrishnan GV, Findlay K, Soumpourou E, Thouin J, Very AA, Sanders D, Morris RJ, Oldroyd GE. Nuclear-localized Cyclic nucleotide-gated channels mediate symbiotic calcium oscillations. Science. 2016;352(6289):1102–5.
36. Yuan HM, Sheng Y, Chen WJ, Lu YQ, Tang X, Ou-Yang M, Huang X. Overexpression of *Hevea brasiliensis* HblCE1 enhances cold tolerance in Arabidopsis. Front Plant Sci. 2017;8:1462.
37. Barah P, Jayavelu ND, Rasmussen S, Nielsen HB, Mundy J, Bones AM. Genome-scale cold stress response regulatory networks in ten *Arabidopsis thaliana* ecotypes. BMC Genomics. 2013;14(1):722.
38. Jung HJ, Dong X, Park JI, Thamilarasan SK, Lee SS, Kim YK, Lim YP, Nou IS, Hur Y. Genome-wide transcriptome analysis of two contrasting *Brassica rapa* doubled haploid lines under cold-stresses using Br135K oligomeric chip. PLoS ONE. 2014;9(8):e106069.
39. Chen X, Hou Y, Cao Y, Wei B, Gu L. A comprehensive identification and expression analysis of the WUSCHEL Homeobox-containing protein family reveals their special role in development and abiotic stress response in *Zea mays* L. Int J Mol Sci. 2023;25(1):441.
40. Finn RD, Mistry J, Schuster-Bockler B, Griffiths-Jones S, Hollich V, Lassmann T, Moxon S, Marshall M, Khanna A, Durbin R, Eddy SR, Sonnhammer EL, Bateman A. Pfam: clans, web tools and services. Nucleic Acids Res. 2006;34:D247–51.
41. Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y, Xia R. TBtools: an integrative toolkit developed for interactive analyses of big biological data. Mol Plant. 2020;13(8):1194–202.
42. Prakash A, Jeffryes M, Bateman A, Finn RD. The HMMER web server for protein sequence similarity search. Curr Protoc Bioinformatics. 2017;60(1):3.15.1–3.15.23.
43. Letunic I, Doerks T, Bork P. SMART 7: recent updates to the protein domain annotation resource. Nucleic Acids Res. 2012;40(D1):D302–5.
44. Tamura K, Stecher G, Kumar S. MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol. 2021;38(7):3022–7.
45. Lescot M, Dehais P, Thijs G, Marchal K, Moreau Y, Van de Peer Y, Rouze P, Rombauts S. PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in Silico analysis of promoter sequences. Nucleic Acids Res. 2002;30(1):325–7.
46. Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, Ren J, Li WW, Noble WS. MEME SUITE: tools for motif discovery and searching. Nucleic Acids Res. 2009;37:W202–8.
47. Wang Y, Tang H, Debarry JD, Tan X, Li J, Wang X, Lee TH, Jin H, Marler B, Guo H, Kissinger JC, Paterson AH. MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. Nucleic Acids Res. 2012;40(7):e49.
48. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 2001;25(4):402–8.
49. Gu L, Hou Y, Sun Y, Chen X, Wang G, Wang H, Zhu B, Du X. The maize WRKY transcription factor ZmWRKY64 confers cadmium tolerance in Arabidopsis and maize (*Zea mays* L). Plant Cell Rep. 2024;43(2):44.
50. Gu L, Jiang T, Zhang C, Li X, Wang C, Zhang Y, Li T, Dirk LMA, Downie AB, Zhao T. Maize HSFA2 and HSBP2 antagonistically modulate raffinose biosynthesis and heat tolerance in Arabidopsis. Plant J. 2019;100(1):128–42.

Publisher's note