Analyses of open-access multi-omics data sets reveal genetic and expression characteristics of maize ZmCCT family genes

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Abstract

Flowering in maize (Zea mays) is influenced by photoperiod. The CO, CO-like/COL and TOC1 (CCT) domain protein-encoding genes in maize, ZmCCTs, are particularly important for photoperiod sensitivity. However, little is known about CCT protein-encoding gene number across plant species or among maize inbred lines. Therefore, we analysed CCT protein-encoding gene number across plant species, and characterized ZmCCTs in different inbred lines, including structural variations (SVs), copy number variations (CNVs), expression under stresses, dark-dark (DD) and dark-light (DL) cycles, interaction network and associations with maize quantitative trait loci (QTLs) by referring to the latest v4 genome data of B73. Gene number varied greatly across plant species, more in polyploids than in diploids. The numbers of ZmCCTs identified were 58 in B73, 59 in W22, 48 in Mo17, and 57 in Huangzao4 for temperate maize inbred lines, and 68 in tropical maize inbred line SK. Some ZmCCTs underwent duplications and presented chromosome collinearity. Structural variations and CNVs were found but they had no germplasm specificity. Forty-two ZmCCTs responded to stresses. Expression of 37 ZmCCTs in embryonic leaves during seed germination of maize under DD and DL cycles was roughly divided into five patterns of uphill pattern, downhill-pattern, zigzag-pattern, l-pattern and j-pattern, indicating some of them have a potential to perceive dark and/or dark-light transition. Thirty-three ZmCCTs were co-expressed with 218 other maize genes; and 24 ZmCCTs were associated with known QTLs. The data presented in this study will help inform further functions of ZmCCTs.

Keywords: Circadian clock rhythm; flowering; maize; photoperiod response.

Introduction

Photoperiod affects flowering in plants including maize (Zea mays) (Jackson 2009; Song et al. 2015). Modern maize gradually evolved two major types of germplasm after domestication of a tropical teosinte from Mexico and Central America: (i) tropically/subtropically adapted and photoperiod-sensitive, and (ii) temperate-adapted and less photoperiod-sensitive (Doebley 2004; Hung et al. 2012). Temperate maize is an autonomous day-neutral plant, and teosinte is an obligate short-day plant that requires uninterrupted long nights to induce flowering (Minow et al. 2018). The differences in photoperiod sensitivity are observed in and between maize populations (Jiang et al. 1999; Huang et al. 2018). Photoperiod sensitivity hinders the improvement of temperate maize through the utilization of subtropical/tropical maize germplasm (Lewis and Goodman 2003; Yamasaki et al. 2007; Wang et al. 2008).

Growing evidence indicate that many key genes in photoperiodic flowering-time regulatory pathways are conserved across diverse plant species, but unique regulatory
pathways are also present in some phylogenetic groups (Coles et al. 2010). Maize response to photoperiod is affected only by few of the flowering-time quantitative trait loci (QTLs) (Yang et al. 2013). The photoperiod-sensing pathway consists of the conserved upstream genes of con2 as the closest homolog of CONSTANS (CO) in Arabidopsis, gig21A, gig21B, id1, and the differential downstream FLOWERING LOCUS T (FT)-like genes such as ZCN8, of which the upstream gene components are conserved in maize (Miller et al. 2008).

The proteins containing the CO, CO-like/COL and TOC1 (CCT) domain in the C terminus are transcription factors involved in photoperiod sensitivity of plants (Liu et al. 2020). The CCT domain-containing proteins were usually divided into three clades including COL, PSEUDO RESPONSE REGULATOR (PRR)-like and CCT MOTIF FAMILY (CMF)-like proteins on the basis of the domains in the N terminus (Liu et al. 2020). Many transcription factors related to photoperiod sensitivity in flowering plants contain a CCT domain in their predicted sequence (Liu et al. 2020).

The maize CCT domain-containing protein (ZmCCT) genes, ZmCCTs, appear to be particularly important for the photoperiod sensitivity of maize (Ducrocq et al. 2009; Yang et al. 2013) because their consistent and high expression results in the delay in flowering of maize under long day (Hung et al. 2012). It was reported that the ZmCCT family in maize inbred line B73 contains 53 ZmCCT genes based on the version two (v2) B73 genome, with four clades, including COL, PRR-like, CMF-like and TIFY-like proteins (Jin et al. 2018). ZmCCTs are the upstream genes of ZCN8 in the photoperiod pathway, and they repress ZCN8 expression (Dong et al. 2012; Huang et al. 2018). A few ZmCCTs were functionally identified, of which ZmCCT9 and ZmCCT10, and ZmCOL3 contribute to flowering-time adaptation of maize from tropical to temperate regions (Yang et al. 2013; Huang et al. 2018; Jin et al. 2018). In addition, ZmCCTs may also be involved in growth, development, and stress response (Ku et al. 2016; Li et al. 2016, 2017; Wang et al. 2017; Xu et al. 2017; Zhang et al. 2018). There is a report in which the CCT protein number of numerous genomes was previously informed, but little is yet known about CCT protein-encoding gene number across plant species or the genetic characteristics of ZmCCTs among maize inbred lines. We speculate that the CCT protein number varies with plant species, and that the expression patterns of ZmCCTs in maize are different under continuous dark-dark (DD) versus dark-light (DL) cycles. In this study, we analyse the number of CCT protein-encoding genes of plants and disclose characteristics of ZmCCTs including structural variations (SVs); copy number variations (CNVs); expression under stresses, DD and DL cycles; interaction network; and associations with maize QTLs by referring to the latest v4 genome data of B73.

Materials and Methods

Analysis of CCT domain-containing proteins and CCT protein-encoding genes

The identification of CCT domain-containing proteins was based on the search of protein data sets using the PF06203CCT hidden Markov model (HMM) (http://pfam.xfam.org/). The protein data sets were those from temperate maize inbred lines B73, Mo17 and W22 (https://maizegdb.org/), temperate maize inbred line Huangzao4 under accession number PRJCA001247 and the tropical small-kernel maize inbred line 5K under accession number PRJNA531547 (http://bigd.big.ac.cn/gsa), and phytozome database (https://phytozome.jgi.doe.gov/). The search was conducted using the HMMER3 tool (Finn et al. 2011; http://www.ebi.ac.uk/Tools/hmmer/) according to 1 expect threshold (E) < 10^-10.

Second, to prevent from losing potential CCT proteins in the above HMM-based search, Arabidopsis CCT proteins from the Arabidopsis Information Resource database (http://www.arabidopsis.org/), and rice CCT proteins from the Rice Genome Annotation database (http://rice.plantbiology.msu.edu/) were used to search the above proteomes from maize and proteomes from other plants through the basic local alignment search tool for proteins (BLASTp) according to 1E < 10^-10. After removing redundant sequences, CCT proteins were further confirmed by searching the conserved domain database with a threshold of 0.01 and a maximum hit of 500 (Marchler-Bauer et al. 2017) and the Pfam database under 1E < 0.05 (Finn et al. 2016).

Third, the candidate protein identification (ID) number of maize were used to search and obtain the corresponding gene ID number in the maizegdb database (https://maizegdb.org/) for maize ZmCCTs and in the phytozome database (https://phytozome.jgi.doe.gov/pz/portal.html) for CCT protein-encoding genes of other plants. When there were multiple candidate proteins produced by different transcripts of the same gene, the protein with the longest amino acid sequence was selected as the representative to search the corresponding gene ID number.

Construction of phylogenetic trees of plant species

With the above information, the v0.66839 Toolkit for Biologists integrating various biological data-handling tools (TBtools) was used to generate the Newick (nwk) files with Latin names of species as input files according to the previous methods under the default parameters (Chen et al. 2018). The nwk files were employed to construct phylogenetic trees using the v6 Molecular Evolutionary Genetics Analysis software under the default parameters (Tamura et al. 2013).

The polyploidy events, such as whole-genome triplication (WGT), whole-genome duplication (WGD) and whole-genome sextuplication (WGS), of plant species came from research in Qu et al. (2018).

Analysis of chromosomal localization, collinearity and duplication time of ZmCCTs

The multiple sequence alignment was performed with the target proteins against the v4 B73 proteome data set (https://maizegdb.org/) by using the BLASTp to generate an m8-format file under 1E < 10^-10. The chromosome collinearity of the genes was analysed based on both the m8-format file and the General Feature Format (GFF) file of the v4 B73 genome by using the toolkit for collinearity detection based on an adjusted MCScan (Wang et al. 2012). Duplicate gene pairs were extracted from the collinear genes with gene’s ID number. The collinearity and chromosome localization were plotted using the Amazing Simple Circos tool (Chen et al. 2018).

The non-synonymous substitution (Ks) and synonymous substitution (Ks) rates of the duplicate gene pairs were calculated using the Parallel Alignment and back-Translation tool (Zhang et al. 2012) and Ks_Calculator 2.0 (Wang et al. 2010). The gene duplication time was estimated according to the formula: $t = \frac{2}{\lambda}$, where $\lambda = 6.5 \times 10^{-8}$ (Koch et al. 2000).
Analysis of characteristics and domains/motifs of ZmCCT-encoded proteins ZmCCTs, as well as introns and exons of ZmCCTs

The molecular weight and isoelectric point of each protein were analysed according to Artimo et al. (2012), and subcellular localization was predicted as Yu et al. (2006). The amino acid motifs were predicted (Bailey et al. 2015) and annotated through the InterProScan database (Mitchell et al. 2019). The protein domains were predicted according to Letunic and Bork (2017). The introns, exons and the domains/motifs in the genes were mapped based on the GFF files of the genes following the methods in Chen et al. (2018).

Analysis of SVs and CNVs of ZmCCTs

Structural variation analysis of the ZmCCTs was based on the maize inbred line B73 v4 genome SV loci data, which were generated by Yang et al. (2019) against the SV data set of the maize inbred line SK genome. In brief, with the SV loci of ZmCCTs in B73 as reference, the SVs of the ZmCCTs in the genomes of other 521 maize inbred lines (http://maizegdb.org/Resources.html) were scanned according to the methods described by Yang et al. (2019).

For CNV analysis, the maize genome re-sequencing data sets with a depth of > 30x, including 17 tropical/subtropical maize inbred lines and 24 temperate maize inbred lines (see Supporting Information—Table S1; https://www.ncbi.nlm.nih.gov/sra/), were first subjected to quality control (Bolger et al. 2014). Afterwards, the genome sequences were aligned to the v4 B73 genome by using the V0.7.15 Burrows-Wheeler Alignment software (Li and Durbin 2009), where the parameters used were under threads of 4, a minSeedLen of 32 and mark shorter split hits as secondary (Li and Durbin 2009). The sequences were converted into Binary Alignment/Map (BAM) format files using the Sequence Alignment/Map tools (Li et al. 2009). The BAM-format files were used to identify CNVs in other maize lines by using the Picard 1.129 software (http://sourceforge.net/projects/picard/) and the cnvnator 0.3.2 software under 1E < 0.01 (Abyzov et al. 2011).

Expression analysis of ZmCCTs

The raw data sets were used as the transcriptomes of embryonic leaves during seed germination of Z. mays cv. White Crystal under DL [dark (11 h)/light (13 h)] cycles (Liu et al. 2013) and DD (Chang et al. 2019), and the transcriptomes of abiotic (Makarevitch et al. 2015; Zenda et al. 2019; http://childslab.plantbiology.msu.edu) and biotic stress-treated maize leaves (Swart et al. 2017). These raw data had three biological replicates.

The gene expression in the embryonic leaves was presented as Fragments Per Kilobase of transcript per Million-fragments mapped (Trapnell et al. 2010). For analysis of gene expression in the tissues under stresses, the reads of the ZmCCT sequences in the Sequence Read Archive (SRA) file (https://www.ncbi.nlm.nih.gov/sra/) were transformed into FASTQ format by using the SRA toolkit 2.9.2 tool, subjected to quality control by the FastQC 0.11.8 and Trimmomatic 0.38 tools, and then aligned to the bowtie 2-indexed v4 B73 genome (https://maizegdb.org/) by using the TopHat 2.1.1 software (Trapnell et al. 2012) to generate the BAM-format files. The reads of ZmCCTs were counted by using the featureCounts tool (Liao et al. 2014) and then used for differential expression analysis by the OmicShare tools (www.omicshare.com/tools) under the default parameters (Robinson et al. 2010). Differentially expressed genes were defined according to a log2 fold change of read counts ≥ 1 at P < 0.05.

The gene expression heat maps were plotted by using the Amazing Simple HeatMap tool in TBtools (Chen et al. 2018).

Analysis of association of ZmCCTs with QTLs

The QTLs associated with ZmCCTs were identified through searching the B73 data set (https://bigd.big.ac.cn/gwas/) in the genome-wide association study (GWAS) Atlas (Tian et al. 2020) by ID number of ZmCCTs.

Analysis of gene co-expression and gene ontology enrichment

The genes co-expressed with ZmCCTs were achieved by searching the maize co-expression data set in the Arabidopsis thaliana trans-factor and cis-element prediction database-II (ATTED-II) (Obayashi et al. 2018; http://atted.jp/) with ID number of ZmCCTs. The co-expression networks of the genes were constructed by using the Cytoscape software (Shannon et al. 2003). Gene ontology (GO) enrichment was performed using the OmicShare tools under default parameters with a false discovery rate (Q) < 0.05 (www.omicshare.com/tools).

Results

CCT protein-encoding genes and CCT proteins in plants

Based on the CCT HMM model, the number of CCT protein-encoding genes was found to differ among 68 plant species, with up to 114 genes in Gossypium hirsutum and only 17 genes in Amborella trichopoda (Fig. 1A). The ID number of CCT protein-encoding genes is listed in Supporting Information—Table S2.

The temperate maize lines of B73, Huangzao 4, W22 and Mo17 had 58, 37, 59 and 48 ZmCCTs (see Supporting Information—Table S3), respectively. The tropical maize line SK (Yang et al. 2019) possessed 68 ZmCCTs. The ID number of the ZmCCTs is shown in Supporting Information—Table S3. The ZmCCTs had high hydrophilicity because of the negative overall average of hydropathicity, and they differed in amino acid sequence length, molecular weight, isoelectric point, and in subcellular locations (see Supporting Information—Table S4).

Chromosome distribution and duplication, collinearity and genomic structure of ZmCCTs in B73

Except for ZmCCT58, which was located on the unassembled genome scaffold, the remaining 57 ZmCCTs were mapped to 10 chromosomes of B73 (Fig. 1B). A total of 26 pairs of the genes with segment duplication (Table 1) showed collinear relationships (Fig. 1B). Duplication happened between 1.1 MYA (million years ago) for the ZmCCTs 28 and 37 pair and 38.947 MYA for both ZmCCT9 and ZmCCT20 (Table 1). ZmCCTs showed easily noticeable differences in genomic DNA structure in B73 genome (Fig. 2A). The number of introns in ZmCCTs of B73 inbred line varied greatly, with no introns in ZmCCT9 and 17 genes encoding genes was found to differ among 68 plant species, with up to 114 genes in Gossypium hirsutum and only 17 genes in Amborella trichopoda (Fig. 1A). The ID number of CCT protein-encoding genes is listed in Supporting Information—Table S2.

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Phylogenetic relationships and conserved domains/motifs of ZmCCTs

As previously reported (Jin et al. 2018), ZmCCTs were divided into four clades including COL, PRR-like, TIFY-like and CMF-like proteins. In addition to the CCT domain, there were the ZF-B (zinc finger B-box) domain in the COL clade, the pseudoresponse domain in the PRR-like clade, and the methyl CpG binding domain (MBD) and the GATA domain
in the TIFY-like clade (Fig. 2B; see Supporting Information—Table S5). In addition to the above domains, ZmCCTs had many conserved amino acid motifs (Fig. 2B; see Supporting Information—Table S5). The motif 3 existed in 57 ZmCCTs but not in ZmCCT5 (Fig. 2B).

CNVs of ZmCCTs

With the v4 B73 genome as reference, CNVs of ZmCCTs in 41 maize lines (17 tropical/subtropical and 24 temperate lines) were analysed (Fig. 3). The copy number of ZmCCTs 8, 9, 11, 15, 28, 43, 45, 53, 56 and 58 (accounting for 17.2 % of ZmCCTs in maize inbred line B73) increased, involving more than 25 (60.9 %) maize lines. The copy number of ZmCCTs 10, 20 and 44 (accounting for 5.2 % of ZmCCTs) decreased, involving more than 10 (24.4 %) maize lines.

The copy number of ZmCCT36 increased in 7 (41.2 %) of 17 tropical/subtropical maize lines and in 15 (62.5 %) of 24 temperate maize lines, respectively. The copy number of ZmCCT51 increased in 3 (17.7 %) of 17 tropical/subtropical maize lines and in 10 (41.7 %) of 24 temperate maize lines, respectively. The copy number of both ZmCCT42 and ZmCCT55 had no changes in tropical/subtropical maize lines but increased in 11 (45.8 %) and 7 (29.2 %) of the 24 temperate maize lines, respectively (Fig. 3).

SVs of ZmCCTs in different maize inbred lines

With the SK genome as reference, ZmCCTs 8, 29, 30, 41 and 43 were found to have SVs in the v4 B73 genome, showing deletions and insertions of DNA segments (Table 2). These SVs were divided into three types: AA, for SVs of the same structure as B73 but different structure from SK; TT, for SVs of the same structure as SK but different structure from B73; and NN, for SVs unable to evaluate (see Supporting Information—Table S6).

The genomes of 521 maize inbred lines (http://maizego.org/Resources.html) were scanned by reference to the SVs of ZmCCTs in the B73 genome according to the methods described by Yang et al. (2019). Consequently, one TT-type SV, SV7, of ZmCCT41 was found in 287 maize lines. Five TT-type SVs, SVs 9–13, of ZmCCT43 were found in 375 to 472 maize lines depending on the SV. One AA-type SV, SV2 of ZmCCT29 was found in 85 maize lines. One AA-type SV, SV4, of ZmCCT30 was found in 152 lines. One AA-type SV, SV8, of ZmCCT43 was found in 46 maize lines (see Supporting Information—Table S6).

Expression of ZmCCTs under biotic and abiotic stresses

Expression levels of 58 ZmCCTs from B73 were analysed in maize lines under biotic and abiotic stresses (Table 3). Forty-two (72.4 %) of 58 ZmCCTs responded to the stresses, and they together made a total of 84 differential expression events when compared to those in corresponding unstressed tissues of the same maize lines. The responses to drought, cold and heat accounted for 73 (86.9 %) of 84 differential expression events (Table 3), of which 53 (63.1 %) resulted from ZmCCTs in the COL clade, 17 (20.2 %) from
Table 1. Duplications of ZmCCTs in maize inbred line B73 according to v4 of the B73 genome. $K_a$, non-synonymous substitution rate; $K_s$, synonymous substitution rate; MYA, million years ago; v4, version 4; ZmCCT, ZmCCT gene.

| Duplicate pair          | $K_a$ | $K_s$ | $K_a/K_s$ | Estimated time (MYA) | Duplication type |
|-------------------------|-------|-------|-----------|----------------------|-----------------|
| ZmCCT2 ZmCCT13          | 0.406 | 2.917 | 0.139     | 22.441               | Segmental       |
| ZmCCT6 ZmCCT27          | 0.108 | 0.788 | 0.137     | 6.063                | Segmental       |
| ZmCCT1 ZmCCT27          | 0.406 | 3.176 | 0.128     | 24.429               | Segmental       |
| ZmCCT8 ZmCCT26          | 0.067 | 0.427 | 0.156     | 3.284                | Segmental       |
| ZmCCT2 ZmCCT42          | 0.483 | 2.852 | 0.169     | 21.937               | Segmental       |
| ZmCCT1 ZmCCT54          | 0.086 | 0.238 | 0.362     | 1.833                | Segmental       |
| ZmCCT57 ZmCCT9           | 0.030 | 0.242 | 0.126     | 1.864                | Segmental       |
| ZmCCT57 ZmCCT20          | 0.189 | 4.627 | 0.041     | 35.591               | Segmental       |
| ZmCCT57 ZmCCT28          | 0.463 | 2.123 | 0.218     | 18.385               | Segmental       |
| ZmCCT20 ZmCCT32          | 0.173 | 2.067 | 0.083     | 15.897               | Segmental       |
| ZmCCT9 ZmCCT20          | 0.176 | 5.063 | 0.035     | 38.947               | Segmental       |
| ZmCCT12 ZmCCT41          | 0.071 | 0.146 | 0.486     | 1.124                | Segmental       |
| ZmCCT13 ZmCCT42          | 0.107 | 0.330 | 0.323     | 2.542                | Segmental       |
| ZmCCT15 ZmCCT46          | 0.081 | 0.445 | 0.183     | 3.420                | Segmental       |
| ZmCCT20 ZmCCT32          | 0.064 | 0.515 | 0.125     | 3.959                | Segmental       |
| ZmCCT21 ZmCCT33          | 0.061 | 0.224 | 0.271     | 1.726                | Segmental       |
| ZmCCT22 ZmCCT34          | 0.095 | 0.288 | 0.332     | 2.212                | Segmental       |
| ZmCCT24 ZmCCT30          | 0.086 | 0.402 | 0.215     | 3.089                | Segmental       |
| ZmCCT19 ZmCCT36          | 0.164 | 0.592 | 0.277     | 4.555                | Segmental       |
| ZmCCT23 ZmCCT41          | 0.640 | 2.468 | 0.259     | 18.985               | Segmental       |
| ZmCCT35 ZmCCT38          | 0.269 | 2.412 | 0.112     | 18.553               | Segmental       |
| ZmCCT28 ZmCCT37          | 0.047 | 0.143 | 0.332     | 1.100                | Segmental       |
| ZmCCT31 ZmCCT52          | 0.203 | 2.627 | 0.077     | 20.210               | Segmental       |
| ZmCCT27 ZmCCT54          | 0.403 | 2.887 | 0.139     | 22.209               | Segmental       |
| ZmCCT34 ZmCCT51          | 0.247 | 2.233 | 0.111     | 17.179               | Segmental       |
| ZmCCT35 ZmCCT49          | 0.292 | 2.275 | 0.129     | 17.500               | Segmental       |
| ZmCCT38 ZmCCT49          | 0.088 | 0.447 | 0.197     | 3.435                | Segmental       |
Figure 2. The clades of ZmCCTs (A), and the conserved domains/motifs (A) of the predicated ZmCCTs in B73. COL, PRR-like, TIFY-like and CMF-like were four clades of ZmCCTs. The figures in the structures in panel (B) referred to motif numbers. ZmCCT, Maize CCT domain-containing protein; ZmCCT, ZmCCT gene.

Figure 3. The CNVs of ZmCCTs in maize inbred lines with the v4 genome of the inbred line B73 as reference. The CNVs of ZmCCTs were analysed based on the genome data set in the literature (Yang et al. 2019). CNV, copy number variation; v4, version 4; ZmCCT, ZmCCT gene.
Table 2. Structural variations (SVs) of ZmCCTs in the v4 B73 genome against the SK genome. The analyses were based on the previous open genome sequence data set in the literature (Yang et al. 2019; The data sets were from Yan's lab of the National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, China. http://maizego.org/Resources.html); SV, structural variation; UTR, untranslated region; v4, version 4; ZmCCT, ZmCCT gene.

| ZmCCT | SV | Number | Start | Stop | Type | Start | Stop | Length of insertion/deletion (bp) | Region | Distance away from start codon ATG (bp) |
|-------|----|--------|-------|------|------|-------|------|-----------------------------------|--------|----------------------------------------|
| ZmCCT8 | SV1 Insertion | 272190299 | 272190300 | 15 | Upstream | |
| ZmCCT29 | SV2 Insertion | 70352067 | 70352068 | 13 | 5’ UTR | 4141 upstream |
|         | SV3       | 70353576 | 70353577 | 2302 | 5’ UTR | 2632 upstream |
| ZmCCT30 | SV4 Insertion | 82140693 | 82140694 | 21 | Exon | 1064 downstream |
|         | SV5 Deletion | 82141884 | 82141898 | 14 | Intron | 1043–2255 downstream |
| ZmCCT41 | SV6 Deletion | 148067829 | 148068185 | 356 | Intron | 2570–3326 upstream |
|         | SV7 Deletion | 148070516 | 148070532 | 16 | Intron | 623–629 upstream |
| ZmCCT43 | SV8 Insertion | 180520885 | 180520886 | 23 | Intron | 4771 upstream |
|         | SV9 Deletion | 180520895 | 180520909 | 14 | Intron | 4747 upstream |
|         | SV10 Deletion | 180520929 | 180520950 | 21 | Intron | 4706 upstream |
|         | SV11 Deletion | 180520961 | 180521014 | 53 | Intron | 4642 upstream |
|         | SV12 Deletion | 180521021 | 180521037 | 16 | Intron | 4619 upstream |
|         | SV13 Deletion | 180521056 | 180521069 | 13 | Intron | 4587 upstream |
|         | SV14 Deletion | 180521075 | 180521121 | 46 | Intron | 4535 upstream |
|         | SV15 Deletion | 180521125 | 180521175 | 50 | Intron | 4481 upstream |
Table 3. Expression of 58 ZmCCTs from B73 line in leaves of maize inbred lines under abiotic and biotic stresses. The raw open data sets under drought, cold, heat and salt were from Makarevitch et al. (2015); Zenda et al. (2019), and the raw data set on infection with C. zeina was from Swart et al. (2017), which had three biological repeats. Black bold and negative black bold values indicate up-regulation and down-regulation of gene expression at $p < 0.05$, respectively. *The EF was calculated based on the sequence read counts of the target genes.

| ZmCCT   | Drought-stressed YE8112 leaf Log2 EF* | P-value | Drought-stressed Mo17 leaf Log2 EF* | P-value | Cold-stressed B73 leaf Log2 EF* | P-value | Heat-stressed B73 leaf Log2 EF* | P-value | Salt-stressed B73 leaf Log2 EF* | P-value | B73 leaf infected by C. zeina Log2 EF* | P-value |
|---------|-------------------------------------|---------|-------------------------------------|---------|---------------------------------|---------|---------------------------------|---------|----------------------------------|---------|-----------------------------------|---------|
| ZmCCT1  | -0.08                               | 0.95    | -0.67                               | 0.43    | -1.02                           | 0.00    | 1.48                            | 0.00    | -1.30                           | 0.05    | -1.82                            | 0.00    |
| ZmCCT2  | 0.28                                | 0.23    | 0.19                                | 0.55    | 2.76                            | 0.00    | -1.10                           | 0.00    | -0.48                           | 0.09    | -0.37                            | 0.04    |
| ZmCCT3  | 0.45                                | 0.09    | -0.89                               | 0.06    | -0.45                           | 0.00    | -0.52                           | 0.00    | -0.89                           | 0.00    | -0.41                            | 0.05    |
| ZmCCT4  | 0.00                                | 1.00    | 0.00                                | 1.00    | 3.91                            | 1.00    | 3.32                            | 1.00    | 0.00                            | 1.00    | 0.00                             | 1.00    |
| ZmCCT5  | -0.26                               | 0.51    | -1.25                               | 0.05    | 1.86                            | 0.00    | -1.17                           | 0.00    | -0.20                           | 0.63    | -0.38                            | 0.21    |
| ZmCCT6  | 4.91                                | 0.60    | 0.26                                | 0.92    | -0.06                           | 1.00    | 1.97                            | 0.00    | 0.00                            | 1.00    | 0.00                             | 1.00    |
| ZmCCT7  | 0.26                                | 0.17    | -0.24                               | 0.48    | -0.69                           | 0.00    | -0.99                           | 0.00    | -1.30                           | 0.00    | 0.16                             | 0.39    |
| ZmCCT8  | 0.10                                | 0.75    | -3.65                               | 0.00    | -3.70                           | 0.00    | -1.66                           | 0.00    | 0.39                            | 0.47    | -0.20                            | 0.40    |
| ZmCCT9  | 1.01                                | 0.00    | -0.86                               | 0.01    | -1.16                           | 0.00    | -0.77                           | 0.00    | -0.86                           | 0.00    | 0.14                             | 0.46    |
| ZmCCT10 | -4.74                               | 0.57    | 4.32                                | 1.00    | -1.00                           | 0.09    | -0.44                           | 0.50    | 0.63                            | 0.61    | -5.06                            | 1.00    |
| ZmCCT11 | -0.04                               | 0.83    | -0.09                               | 0.79    | 3.33                            | 0.00    | -1.71                           | 0.00    | -0.53                           | 0.04    | 0.03                             | 0.90    |
| ZmCCT12 | -0.12                               | 0.53    | 0.41                                | 0.26    | 5.30                            | 0.00    | -1.19                           | 0.00    | 0.67                            | 0.01    | -0.36                            | 0.05    |
| ZmCCT13 | 0.13                                | 0.93    | 0.27                                | 0.78    | -0.38                           | 0.04    | -0.46                           | 0.01    | -0.99                           | 0.42    | -0.30                            | 0.59    |
| ZmCCT14 | 0.21                                | 0.40    | -1.02                               | 0.01    | -1.15                           | 0.00    | -2.58                           | 0.00    | -0.15                           | 0.59    | -0.38                            | 0.12    |
| ZmCCT15 | 0.38                                | 0.36    | -0.34                               | 0.91    | 0.33                            | 0.35    | -1.52                           | 0.00    | -3.73                           | 0.00    | 0.43                             | 0.52    |
| ZmCCT16 | 0.00                                | 1.00    | 6.91                                | 0.14    | -0.89                           | 0.64    | 3.64                            | 0.00    | 0.00                            | 1.00    | 0.00                             | 1.00    |
| ZmCCT17 | 0.00                                | 1.00    | 4.91                                | 1.00    | 0.00                            | 1.00    | 1.00                            | 1.00    | 0.00                            | 1.00    | 0.00                             | 1.00    |
| ZmCCT18 | 0.24                                | 0.61    | -0.93                               | 0.13    | -1.95                           | 0.10    | 0.10                            | 1.00    | -0.93                           | 0.12    | -0.57                            | 0.16    |
| ZmCCT19 | 0.56                                | 0.17    | -2.01                               | 0.00    | -1.01                           | 0.00    | 0.96                            | 0.00    | -0.67                           | 0.32    | 0.27                             | 0.68    |
| ZmCCT20 | 0.40                                | 0.27    | -0.26                               | 0.53    | -1.36                           | 0.00    | -0.73                           | 0.00    | 0.79                            | 0.22    | -0.04                            | 1.00    |
| ZmCCT21 | -0.97                               | 0.00    | 0.42                                | 0.45    | 3.51                            | 0.00    | -0.14                           | 0.59    | 0.47                            | 0.17    | 0.17                             | 0.32    |
| ZmCCT22 | -0.60                               | 0.01    | -0.27                               | 0.66    | 2.55                            | 0.00    | -0.83                           | 0.00    | -0.26                           | 0.49    | -0.37                            | 0.12    |
| ZmCCT23 | -0.30                               | 0.14    | -0.01                               | 0.98    | 2.90                            | 0.00    | -1.04                           | 0.00    | 0.12                            | 0.61    | -0.06                            | 0.75    |
| ZmCCT24 | -0.61                               | 0.03    | -3.86                               | 0.00    | 0.54                            | 0.00    | -3.02                           | 0.00    | 1.98                            | 0.00    | -0.52                            | 0.02    |
| ZmCCT25 | 0.42                                | 0.09    | -0.22                               | 0.59    | -1.28                           | 0.00    | -0.35                           | 0.00    | -0.65                           | 0.04    | -0.20                            | 0.38    |
| ZmCCT26 | 0.61                                | 0.01    | -1.62                               | 0.00    | -2.45                           | 0.00    | -1.12                           | 0.00    | -0.08                           | 0.79    | 0.22                             | 0.29    |
| ZmCCT27 | -0.30                               | 0.71    | 0.09                                | 0.85    | -0.14                           | 0.82    | -0.48                           | 0.35    | -1.02                           | 0.20    | 1.89                             | 0.05    |
| ZmCCT | Log2 EF | Log2 EF | Log2 EF | Log2 EF | Log2 EF | Log2 EF | Log2 EF |
|-------|---------|---------|---------|---------|---------|---------|---------|
| ZmCCT28 | -0.15 | 0.45 | -0.34 | 0.28 | -0.34 | 0.28 | -0.05 | 0.24 |
| ZmCCT29 | 0.00 | 1.00 | 0.00 | 1.00 | 0.00 | 1.00 | 0.00 | 1.00 |
| ZmCCT30 | 0.62 | 0.24 | -0.62 | 0.07 | -0.83 | 0.00 | -0.36 | 0.14 |
| ZmCCT31 | 0.64 | 0.88 | 0.00 | 1.00 | -0.65 | 0.00 | -0.72 | 0.00 |
| ZmCCT32 | 0.00 | 0.96 | -0.11 | 0.36 | -0.93 | 0.00 | -0.26 | 0.00 |
| ZmCCT33 | 0.00 | 0.97 | -0.93 | 0.00 | -0.57 | 0.00 | -0.22 | 0.00 |
| ZmCCT34 | 0.00 | 1.00 | -0.26 | 0.24 | -2.13 | 0.00 | -0.33 | 0.00 |
| ZmCCT35 | 0.00 | 0.97 | -0.36 | 0.16 | -1.11 | 0.00 | -0.46 | 0.00 |
| ZmCCT36 | 0.00 | 0.98 | -1.44 | 0.00 | -1.25 | 0.00 | -0.91 | 0.00 |
| ZmCCT37 | 0.00 | 0.97 | -1.14 | 0.00 | -1.05 | 0.00 | -0.82 | 0.00 |
| ZmCCT38 | 0.00 | 1.00 | -1.07 | 0.00 | -1.15 | 0.00 | -0.66 | 0.00 |
| ZmCCT39 | 0.00 | 1.00 | -1.07 | 0.00 | -1.07 | 0.00 | -0.83 | 0.00 |
| ZmCCT40 | 0.00 | 0.97 | -1.44 | 0.00 | -1.05 | 0.00 | -0.82 | 0.00 |
| ZmCCT41 | 0.00 | 0.97 | -1.14 | 0.00 | -1.15 | 0.00 | -0.83 | 0.00 |
| ZmCCT42 | 0.00 | 0.97 | -1.14 | 0.00 | -1.15 | 0.00 | -0.83 | 0.00 |
| ZmCCT43 | 0.00 | 0.97 | -1.14 | 0.00 | -1.15 | 0.00 | -0.83 | 0.00 |
| ZmCCT44 | 0.00 | 0.97 | -1.14 | 0.00 | -1.15 | 0.00 | -0.83 | 0.00 |
| ZmCCT45 | 0.00 | 0.97 | -1.14 | 0.00 | -1.15 | 0.00 | -0.83 | 0.00 |
| ZmCCT46 | 0.00 | 0.97 | -1.14 | 0.00 | -1.15 | 0.00 | -0.83 | 0.00 |
| ZmCCT47 | 0.00 | 0.97 | -1.14 | 0.00 | -1.15 | 0.00 | -0.83 | 0.00 |
| ZmCCT48 | 0.00 | 0.97 | -1.14 | 0.00 | -1.15 | 0.00 | -0.83 | 0.00 |
| ZmCCT49 | 0.00 | 0.97 | -1.14 | 0.00 | -1.15 | 0.00 | -0.83 | 0.00 |
| ZmCCT50 | 0.00 | 0.97 | -1.14 | 0.00 | -1.15 | 0.00 | -0.83 | 0.00 |
| ZmCCT51 | 0.00 | 0.97 | -1.14 | 0.00 | -1.15 | 0.00 | -0.83 | 0.00 |
| ZmCCT52 | 0.00 | 0.97 | -1.14 | 0.00 | -1.15 | 0.00 | -0.83 | 0.00 |
| ZmCCT53 | 0.00 | 0.97 | -1.14 | 0.00 | -1.15 | 0.00 | -0.83 | 0.00 |
| ZmCCT54 | 0.00 | 0.97 | -1.14 | 0.00 | -1.15 | 0.00 | -0.83 | 0.00 |
| ZmCCT55 | 0.00 | 0.97 | -1.14 | 0.00 | -1.15 | 0.00 | -0.83 | 0.00 |
| ZmCCT56 | 0.00 | 0.97 | -1.14 | 0.00 | -1.15 | 0.00 | -0.83 | 0.00 |
| ZmCCT57 | 0.00 | 0.97 | -1.14 | 0.00 | -1.15 | 0.00 | -0.83 | 0.00 |
| ZmCCT58 | 0.00 | 0.97 | -1.14 | 0.00 | -1.15 | 0.00 | -0.83 | 0.00 |

Table 3. Continued
ZmCCTs in the FRR clade (12 (14.3 %) from ZmCCTs in the CMF clade and 2 (2.4 %) from a TIFY clade (Table 3).

Of the 42 stress-induced ZmCCTs, 28 (66.7 %) responded to heat in B73 leaves, of which 22 (78.6 %) were downregulated and 6 (21.4 %) were upregulated. Twenty-seven (64.3 %) responded to cold in B73 leaves, of which 15 (55.6 %) were downregulated and 12 (44.4 %) were upregulated. Fourteen (33.3 %) responded to drought in leaves of the drought-sensitive line Mo17 (Zenda et al. 2019), of which one (7.4 %) was downregulated and three (9.2 %) were upregulated. Only four ZmCCTs responded to drought in leaves of drought-tolerant YB8112 (Zenda et al. 2019), of which one (25 %) was downregulated and three (75 %) were upregulated. Very few ZmCCTs responded to salt and infection with Cercospora zeina (Table 3).

**Expression rhythm of ZmCCTs**

Based on the transcriptomes of embryonic leaves during seed germination under DL cycles and DD, there were 37 (63.8 %) of 58 ZmCCTs in inbred line B73 that showed differential expression (Fig. 4). From the beginning to the end of the treatments, ZmCCT expression could be roughly divided into five patterns: uphill pattern (i.e. the expression level tended to increase gradually); downhill-pattern (i.e. the expression level tended to decrease gradually); zigzag-pattern (i.e. the expression level had single or multiple obvious peaks and valleys); 1-pattern (i.e. the expression level was high initially, and then it decreased suddenly and remained flat till the end); and J-pattern (the expression level remained low until suddenly increased sharply at the last time point) (Fig. 4).

There were four ZmCCTs (i.e. 43, 46, 48 and 48) under DD, and eight ZmCCTs (i.e. 8, 14, 18–20, 34, 37 and 53) under DL that presented the 1-pattern. Meanwhile, there were seven ZmCCTs (i.e. 1, 21, 23, 38, 45, 52 and 57) under DD, and one ZmCCT (i.e. 21) under DL that presented the J-pattern expression (Fig. 4).

Under the two treatments, in terms of overall expression, the genes with similar expression patterns included ZmCCTs 6, 11, 14, 18, 20, 21, 23, 32, 34, 36–38, 45 and 47. At the initial time point of the two treatments, the genes with a completely opposite expression pattern included ZmCCTs 1, 3, 5, 18, 19, 22, 23, 28, 36, 43, 46, 48, 49 and 52–56. At the last time point of the two treatments, the genes with a completely opposite expression pattern involved ZmCCTs 1, 3, 8, 22, 24, 25, 47, 49 and 56 (Fig. 4).

**Association of ZmCCTs with QTLs**

Analysis of the relevant data sets in the GWAS Atlas database revealed that 24 (41.4 %) of 58 ZmCCTs were associated with QTLs in the v4 B73 genome (Table 4) in inbred line B73. ZmCCTs 11, 34, 43, 57 and 58 were linked to four QTLs. ZmCCT23 was associated with three QTLs. ZmCCTs 1, 2 and 31 were related to two QTLs. Other 17 ZmCCTs were associated with one QTL (Table 4), respectively.

**Co-expression of ZmCCTs with other maize genes**

Analysis of the transcriptome data sets of tissue samples of B73 under both control and stresses in the ATTED-II version 9.2 database showed that 33 (56.9 %) of 58 ZmCCTs in inbred line B73 were co-expressed with 218 other maize genes, including 16 transcription factors [see Supporting Information—Table S7]. ZmCCTs 6, 30, 37 and 54 were in isolated co-expression networks containing a few genes each (Fig. 5). In order to visualize the co-expression networks, the jre_8u_windows_64.exe or the jre_8u_windows_32.exe file was first downloaded and installed followed by installing the Cytoscape_3_3_0_windows_64bit or Cytoscape_3_3_0_windows_32bit file depending on the local Windows system. After installing this software, the reader can double-click and then watch the file ‘Genes co-expressed with ZmCCTs in maize inbred line B73’ through the button ‘Zoom In’ on the screen. These files are provided for readers

![Figure 4](image-url). Expression of the ZmCCTs at 13 time points in embryonic leaves during seed germination of Z. mays cv. White Crystal under DL cycles and DD. The results were based on the transcriptome data sets of seeds of Z. mays cv. White Crystal under the two conditions of DL cycles (Liu et al. 2013) and DD (Chang et al. 2019). T00 represents dry seed; T06, 12, ... represent time points post imbibition of seeds under DL cycles and DD. DD, dark-dark; DL, dark (11 h)/light (13 h); ZmCCT, ZmCCT gene.
as a compressed supplemental file package in the Supporting Information.

The functional categorization by GO analysis indicated that the co-expressed genes were significantly associated with cellular component (Fig. 6A) and biological process (Fig. 6B) rather than molecular function (Fig. 6C).

Discussion

In this study, it was found that the number of CCT domain-containing genes varies across plant species (Fig. 1A) and among maize inbred lines [see Supporting Information—Table S3]. The number of ZmCCTs identified in maize inbred line B73 based on the v4 B73 genome [see Supporting Information—Table S3] differed from the 53 ZmCCTs that were reported in B73 based on the v2 B73 genome (Jin et al. 2018) likely because of the upgrade of gene annotation.

The CNV is one of the most common and most studied forms of SVs (Swanson-Wagner et al. 2010). They are most likely caused by non-allelic homologous recombination in plants (Gabur et al. 2019). CNVs are closely associated with the chromosome ploidy (Tang and Amon 2013). In this study, plants with higher ploidy had more CCT domain-containing genes compared to diploid species, such as allotetraploid G. hirsutum (Hu et al. 2019), allotetraploid Glycine max (Kyriakidou et al. 2018), allotetraploid Triticum aestivum (Kyriakidou et al. 2018), autotetraploid Solanum tuberosum (Kyriakidou et al. 2018) and tetraploid to octoploid Panicum virgatum (Grant 2017) (Fig. 1A). This phenomenon also occurred in different species of the same genus, for example, G. hirsutum had 1.9 times as many genes as diploid G. raimondii (Hu et al. 2019), P. virgatum had 1.86 times as many genes as diploid P. hallii (Grant 2017) and S. tuberosum had 1.96 times as many genes as diploid S. lycopersicum (Al Shaye et al. 2018) (Fig. 1B).

The ancestor of maize was an ancient tetraploid (White and Doebley 1998). However, over time, its genome has reverted to functional diploid (White and Doebley 1998). With diploidization, separation of chromosome segments will lead to the change of maize inbred line-specific CNVs (Eichten et al. 2011) which have been found in maize populations (Swanson-Wagner et al. 2010). This may partly explain why the genome sizes of B73, Mo17, W22 and SK are marginally different (Springer et al. 2018; Yang et al. 2019), but the ZmCCT number differed among maize inbred lines [see Supporting Information—Table S3].

The gene SVs are important clues to domestication and/or breeding (Swanson-Wagner et al. 2010) and directly affect trait variations in maize (Gabur et al. 2019). More than 3000 SVs have been found in maize. The average length of an individual SV event is about 20 kb but ranges from 1 kb to over 1 Mb in length (Gabur et al. 2019). Expression of ZmCCTs delays flowering of maize under long day (Hung et al. 2012). Therefore, it is reasonably to believe that SVs of 5 ZmCCTs 8, 29, 30, 41 and 43 in populations of tropical/subtropical and temperate maize lines (Table 2; see Supporting Information—Table S6) were likely specific changes exerted by domestication and/or artificial selection objectives.

Several ZmCCTs are associated with flowering-time QTLs (Hung et al. 2012), drought and heat tolerance (Ku et al. 2016) and stalk rot resistance (Li et al. 2017; Wang et al. 2017). Quantitative trait loci associated with ZmCCTs are important nodes linking the photoperiod to stress tolerance responses under long day, and to how photoperiod affects the adaptability of plants to stresses (Ku et al. 2016) and vice versa (Hill and Li 2016). Association of 24 ZmCCTs with QTLs suggests that they can be considered important candidate genes of the QTLs, particularly those associated with flowering-related QTLs (Table 4). Responses to...
Table 4. Association between ZmCCTs and QTLs in the v4 B73 genome. The information was extracted from the public data set in the GWAS Atlas (Tian et al. 2020). GWAS, genome-wide association study; ID, identification; QTL, quantitative trait locus; SNP, single nucleotide polymorphism.

| ZmCCT | SNP ID number | SNP position | QTL/trait | P-values | Other gene(s) covered within the QTL | PubMed ID in NCBI database |
|-------|---------------|--------------|-----------|----------|-------------------------------------|-----------------------------|
| ZmCCT1 | zma191016     | chr1:8675749 | ear infructescence position | 3.80E-04 | Zm00001d027598 Zm00001d027596 Zm00001d027597 | 24514905 |
|       | zma43162386   | chr1:8674425 | leaf length | 6.20E-07 | Zm00001d027598 Zm00001d027596 Zm00001d027597 | 21217756 |
| ZmCCT2 | zma43149907   | chr1:60530979 | southern leaf blight resistance | 1.34E-09 | Zm00001d029149 | 25475173 |
|       | zma43156454   | chr1:60537720 | southern leaf blight resistance | 9.37E-10 | Zm00001d029149 | 25475173 |
| ZmCCT3 | zma43161909   | chr1:92222374 | ear infructescence position | 1.85E-04 | Zm00001d029886 Zm00001d029885 | 24514905 |
| ZmCCT11 | zma43153974  | chr2:145691242 | shoot biomass | 1.50E-05 | Zm00001d004875 | 27768702 |
|       | zma10098914   | chr2:145691595 | southern leaf blight resistance | 8.35E-09 | Zm00001d004875 | 25475173 |
|       | zma43157232   | chr2:145684456 | tassel length | 8.47E-11 | Zm00001d004875 Zm00001d004874 Zm00001d004875 | 26801971 |
| ZmCCT11 | zma43153974  | chr2:145691242 | transpiration efficiency | 1.50E-05 | Zm00001d004875 | 27768702 |
| ZmCCT12 | zma43145709   | chr2:201957407 | above ear plant height | 2.07E-08 | Zm00001d006212 | 29150689 |
| ZmCCT14 | zma11374170   | chr2:225517150 | days to flowering trait | 7.58E-09 | Zm00001d007240 | 23840585 |
| ZmCCT15 | zma15275529   | chr3:184827163 | plant height | 3.46E-07 | Zm00001d042958 | 24514905 |
| ZmCCT18 | zma43154150   | chr4:27316553 | ear infructescence position | 2.90E-04 | Zm00001d049347 | 24514905 |
| ZmCCT23 | zma19806124   | chr4:200744498 | days to silk | 2.92E-08 | Zm00001d052781 Zm00001d052784 Zm00001d052783 | 23144785 |
|       | zma43155891   | chr4:200740173 | ear infructescence position | 8.34E-05 | Zm00001d052782 Zm00001d052781 Zm00001d052783 | 24514905 |
|       | zma43145909   | chr4:200738304 | tassel branch zone length | 1.17E-15 | Zm00001d052782 Zm00001d052781 Zm00001d052783 | 22125498 |
| ZmCCT27 | zma21185023   | chr5:31566925 | transpiration rate | 4.17E-06 | Zm00001d014073 Zm00001d014074 | 29044609 |
| ZmCCT29 | zma21907252   | chr5:70357243 | plant height | 1.37E-07 | Zm00001d014963 | 24514905 |
| ZmCCT30 | zma43149250   | chr5:82139979 | water use efficiency | 6.92E-06 | Zm00001d015268 Zm00001d015269 | 29044609 |
| ZmCCT | SNP ID number | SNP position | QTL/trait | P-values | Other gene(s) covered within the QTL | PubMed ID in NCBI database |
|-------|---------------|--------------|-----------|----------|-----------------------------------|---------------------------|
| ZmCCT31 | zma43153506 | chr5:91969815 | plant biomass | 2.19E-06 | Zm00001d015469, Zm00001d015468 | 29044609 |
| ZmCCT34 | zma43153430 | chr5:209622204 | ear infructescence position | 3.42E-13 | Zm00001d017885, Zm00001d017886 | 24514905 |
| ZmCCT35 | zma43154216 | chr5:210408594 | leaf area trait | 2.40E-07 | Zm00001d017939 | 29044609 |
| ZmCCT38 | zma43154936 | chr6:121365959 | days to flowering trait | 4.92E-07 | Zm00001d037327 | 24514905 |
| ZmCCT41 | zma43157943 | chr7:148063144 | leaf length | 2.30E-07 | Zm00001d021291, Zm00001d021290 | 21217756 |
| ZmCCT43 | zma43155834 | chr7:180510629 | days to flowering trait | 9.63E-12 | Zm00001d022590, Zm00001d022590 | 23840585 |
| ZmCCT47 | ma31615680 | chr8:174635607 | plant height | 3.51E-10 | Zm00001d012445 | 24514905 |
| ZmCCT50 | zma43143021 | chr9:36013691 | shoot apical meristem volume | 4.37E-13 | Zm00001d045735 | 26584889 |
| ZmCCT52 | zma43151844 | chr9:111028354 | ear infructescence position | 1.85E-12 | Zm00001d046925 | 24514905 |
| ZmCCT54 | zma43154034 | chr9:155206589 | number of seminal root | 1.16E-05 | Zm00001d048369 | 30472798 |
| ZmCCT56 | zma43150378 | chr10:94434907 | photoperiod-sensitive flowering-time trait | 8.10E-09 | Zm00001d024910, Zm00001d024909 | 24089449 |
| ZmCCT56 | zma43147102 | chr10:94435667 | plant height | 8.17E-05 | Zm00001d024910, Zm00001d024909 | 24514905 |
abhonic stresses (Table 3) suggest that downregulated expression of ZmCCTs would weaken the tolerance of maize to abiotic stress. The co-expression (Fig. 5; see Supporting Information—Table S7) indicated that some ZmCCTs were involved with abiotic stimulus. Again, these results together indicate that ZmCCTs may play important roles in photoperiod sensitivity and stress adaptability in maize.

During the evenings of long days, CO proteins activate FLOWERING LOCUS T and remain stable through interactions of the LOV domain with FKF1. Meanwhile, FKF1 simultaneously removes CYCLING DOF FACTOR, which is a repressor of CO and FLOWERING LOCUS T (Song et al. 2012). The COL proteins show a range of sequence identity with CO proteins (Valverde 2011). The genes with COL, CMF and PRR domains promote flowering under short day or delay flowering under long day in cereal crops (Liu et al. 2020). In maize, ZmCOL3 represses circadian clock but enhances ZmCCT expression and therefore delays flowering (Jin et al. 2018).

The TIFY family, previously known as ZIM, is characterized by a TIFY/F/Y/XG sequence motif (Vanholme et al. 2007), which has been associated with abiotic and biotic stresses in plants such as maize (Zhang et al. 2015). Zf-B/B-box domain-containing proteins from the COL clade are a class of zinc-finger transcription factors with multiple functions (Gangappa and Botto 2014) that act as bridges between light and hormones in plants (Vaishak et al. 2019). MBDA domain-containing proteins from the TIFY-like clade can enhance transcriptional repression of CCT domain-containing genes and are involved in DNA demethylation and abiotic stress responses. Also, mutation in AtMBD8 results in a late-flowering phenotype in the C24 ecotype of Arabidopsis (Parida et al. 2018). GATA domain-containing proteins from the TIFY-like clade have been implicated in light-dependent and nitrate-dependent control of transcription (Reyes et al. 2004). The ZmCCTs were divided into four distinct clades (Fig. 2A) but the degrees of responses of the clades to the stresses differed in terms of the number of stress-responsive genes (Table 3). Taken all together, in addition to the response to photoperiod, the functions of four clades in ZmCCT family are diversified, and even ZmCCTs in the same clade appear to have also different functions. Motif 1, identified as a CCT domain motif of ZmCCTs, was only 29-amino acid residue long because these amino acid residues identified were only the most conserved core motifs in the CCT domain. Unknown motif 3 was 21-amino acid residue long [see Supporting Information—Table S5], shorter than the CCT domain FF6203 which is defined as a 43- to 45-amino acid residue domain in the database (http://pfam.xfam.org/). This is because these amino acid residues identified were only the most conserved core motifs in the CCT domain. Unknown motif 3 was 21-amino acid residue long [see Supporting Information—Table S5], existed in 57 ZmCCTs not in ZmCCT, and was closely adjacent to CCT domain (Fig. 2B), suggesting that motif 3 is likely a motif that loses region of the CCT domain.

Photoperiod refers to light time in a rhythmic day-night cycle of 24 h (Velez-Ramirez et al. 2011). The critical ‘windows’ of gene expression in the perception of photoperiod are at the transition periods of both dark-light and light-dark (Imaizumi and Kay 2006; Song et al. 2015; Shim et al. 2017). Therefore, it could be inferred that the ZmCCTs 1- or J-pattern expression under DD (i.e. ZmCCTs 43, 46, 48 and 48 for 1-pattern and ZmCCTs 1, 21, 23, 38, 45, 52 and 57 for J-pattern) and under DL cycle (i.e. ZmCCTs 8, 14, 18-20, 34, 37 and 53 for 1-pattern and ZmCCT 21 for J-pattern) would be involved in perception of light-dark and/or dark-light transitions (Fig. 4).

### Conclusions

The CCT protein gene number varies greatly across plant species. ZmCCT number also varies with maize inbred lines. The
Figure 5. Co-expression of the ZmCCTs with other genes in B73. The analysis was based on a data set in the literature (Obayashi et al. 2018). ZmCCT, ZmCCT gene.

Figure 6. GO enrichment, cellular component (A), biological process (B) and molecular function (C), of the genes co-expressed with ZmCCTs in B73. GO, gene ontology; ZmCCT, ZmCCT gene. GO, gene ontology; Q, false discovery rate.
data analysed suggest that ZmCCTs are involved in photoperiod response and stress adaptability. Based on their expression, it appears that some ZmCCTs are induced during dark-light or light-dark transitions. The data presented in this study are informative to further investigating the functions of the ZmCCTs.

Supporting Information
The following additional information is available in the online version of this article—

Table S1. Re-sequencing data sets of maize inbred lines in the Sequence Read Archive (SRA) database (https://www.ncbi.nlm.nih.gov/sra/).

Table S2. Accession no. of the CCT protein-encoding genes of other plant species other than maize in the database (https://phytozome.jgi.doe.gov/pz/portal.html).

Table S3. Accession no. of the ZmCCTs in maize inbred lines of B73, Huangzao4, W22, Mo17 and SK.

Table S4. ZmCCTs and ZmCCTs in maize inbred line B73.

Table S5. Conserved domains/motifs of maize ZmCCTs.

Table S6. Distribution of structural variations (SVs) of ZmCCTs in genomes of 521 maize inbred lines.

Table S7. Genes co-expressed with ZmCCTs in maize inbred line B73.

Network files. In order to visualize the co-expression networks, the jre_8u_windows_64.exe or the jre_8u_windows_32bit file depending on the local Windows system. After installing this software, the reader can follow by installing the Cytoscape_3_3_0_windows_32bit file or the jre_8u_windows_64.exe or the jre_8u_windows_32bit or Network files.

Supporting Information
The following additional information is available in the online version of this article—

Table S1. Re-sequencing data sets of maize inbred lines in the Sequence Read Archive (SRA) database (https://www.ncbi.nlm.nih.gov/sra/).

Table S2. Accession no. of the CCT protein-encoding genes of other plant species other than maize in the database (https://phytozome.jgi.doe.gov/pz/portal.html).

Table S3. Accession no. of the ZmCCTs in maize inbred lines of B73, Huangzao4, W22, Mo17 and SK.

Table S4. ZmCCTs and ZmCCTs in maize inbred line B73.

Table S5. Conserved domains/motifs of maize ZmCCTs.

Table S6. Distribution of structural variations (SVs) of ZmCCTs in genomes of 521 maize inbred lines.

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Conflict of Interest
None declared.

Contributions by the Authors
M.-Y.D. conducted analyses of all the data and written the first draft. L.L. helped with data analysis. X.-W.F. helped collect the literature and participated in project management. Y.-Z.L. conceived the research project, was responsible for project funds and revised the manuscript as a supervisor.

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