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Genome-Wide Identification, Characterization, and Expression Profile of *PDCB* Gene Family in *Zea mays* L.

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Abstract: The plant kingdom harbors the Plasmodesmata Callose Binding Protein (*PDCB*) gene family, which plays essential roles in plant growth, development, environmental adaptation, and yield. *PDCB* genes are closely involved in regulating cell-to-cell communication and controlling callose deposition at plasmodesmata (PD) throughout the whole plant. Remarkably, their functions remain largely unknown in many crops, including maize. This study sought to identify the members of the *PDCB* gene family within the maize genome and analyze their physicochemical properties and expression patterns. Utilizing bioinformatics methodologies, a comprehensive genome-wide analysis of the *PDCB* gene family was performed. The findings revealed that *PDCB* genes were highly abundant in maize, with a total of 56 *PDCB* genes identified and categorized into six distinct groups. Members of the *PDCB* family were dispersed across all chromosomes. The *PDCBs* within each group exhibited significant similarity in their conserved motifs and gene structures; all members contained the X8 domain, comprising one to five exons, while displaying a straightforward genomic structure. Numerous *cis*-acting elements associated with plant growth and development, light response, stress-associated responses, and plant hormones were identified in the promoter regions of *PDCB* genes. Moreover, the *PDCBs* exhibited diverse expression patterns across various tissues. This study improves the comprehension of the *PDCB* gene family and provides a robust foundation for further research on maize.

Keywords: Plasmodesmata Callose Binding Protein (PDCB); genome-wide analysis; *cis*-acting elements; motifs; gene structure; transcriptome analyses; maize (*Zea mays*)

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1. Introduction

Plants are profoundly reliant on plasmodesmata (PD) for intercellular communication [1]. The growth and development of plants are significantly influenced by PD [2]. These tiny apertures in the plant cell wall enable adjacent cells to connect through extensions of their cell membranes into these pores. The smooth endoplasmic reticulums of the two cells are also interconnected, forming the PD. It is articulated that PD plays a pivotal role in facilitating symplasmic cell-to-cell transfer in plants [3,4]. Homeodomain transcription factors, small RNA molecules, and viral genomic material are specifically transported to neighboring cells via PD microchannels [5]. Additionally, sucrose is conveyed from cell to cell through PD into the sieve elements (SEs), which are sequentially arranged to form sieve tubes. These tubes act as a conduit for the long-distance transport of sucrose from source tissues to the sinks [6,7]. In maize xylem exudate, sucrose was found to be the sole sugar present [8]. It stands as the primary product of photosynthesis and is instrumental in the distribution of assimilated carbon within plants [9]. The process known as whole-plant carbohydrate partitioning is employed by plants to transfer carbon, produced through photosynthesis, via the phloem. Carbohydrate allocation profoundly impacts all aspects of

plant growth and crop yield; thus, PD are potential factors influencing crop development, stress tolerance and yield [10].

PD are associated with numerous proteins, including plasmodesmata callose binding proteins (PDCBs), callose synthases (CalS), remorin (REM), plasmodesmata-located proteins (PDLPs), and β-1,3-glucanase (BGs), among others [11]. The pores of PD permit the selective deposition or degradation of callose [12]. Callose, a type of β-(1,3)-D-glucan found in many higher plants, is critical for proper growth and development and plays a crucial role in plant defense mechanisms. Notably, callose accumulates between the plasma membrane and cell wall at the sites of pathogen attack, including PD, thereby impeding pathogen entry and dissemination [13]. Although in the model plant *Arabidopsis thaliana*, callose constitutes merely about 0.3% of the cell wall, it regulates various biological processes such as phloem and pollen development, cell division, organ formation, response to pathogenic invasion, and adaptation to changing soil nutrients and toxic metal levels [14]. The size exclusion limit (SEL) of PD and consequently the mobility of large macromolecules and signaling proteins and RNA molecules that mediate plant developmental and environmental responses are influenced by callose deposition [14,15].

Key contributors to the accumulation of callose at PD include AtBG_PP, PDCB, glycan synthase-like (GSL), and PDLP [16]. Despite lacking apparent enzymatic activity, the PDCB family has been shown to regulate callose deposition at the neck of PD. Thus, PDCBs have emerged as significant regulators of cell-to-cell communication [17,18]. Studies have demonstrated that overexpression of *PDCB1* results in increased callose accumulation [18], and *PDCB1*, *PDCB2*, and *PDCB3* showed overlapping tissue-specific expression suggesting the functional redundancy in this gene family. Due to possessing callose-binding domains, PDCBs have been linked to the closure of PD during plant development [19].

The potential correlation between PDCB-mediated callose deposition and intercellular communication in *Arabidopsis thaliana* has been implicated in the regulation of callose homeostasis in plasmodesmata [16,18]. The callose synthases GSL1, GSL2, GSL5, GSL8, and GSL10 are essential for callose production during pollen development, playing a vital role in ensuring the fertility and/or survivability of the pollen [19]. In maize mutants, abnormal callose and lignin accumulation has been observed in the phloem; and genetic modifications in poplar trees that induce callose deposition can alter the permeability, structure, water-holding capacity, and potentially the lignin content of the secondary cell wall, leading to an increased distance between lignin and cellulose molecules, which ultimately enhances biomass resistance to degradation [20,21].

Although callose holds great significance and the research on the *PDCB* gene family is being conducted extensively, pertinent reports are rare. It has been documented that there were 37 *AtPDCBs* in *Arabidopsis thaliana*, 25 *PbPDCBs* in pear fruit, and 45 *GhPDCBs* in cotton [18,20,21]. The *Arabidopsis* PDCB proteins belong to a larger protein superfamily, which encompasses proteins characterized by conserved X8 domains adjacent to β-1,3-glucanase catalytic domains. PDCBs interact with callose through their X8 domain, and the presence of PDCBs affects the transference of substances through PD between cells by influencing callose deposition [18]. In pear fruit, the formation of stone cells is associated with the lignification transmitted by PD. *PbPDCB16* is considered to enhance callose deposition at PD, thereby affecting intercellular connectivity mediated by PD and resulting in a reduction in lignin concentration [20]. Furthermore, *GhPDCB9* was believed to be related to fiber elongation in cotton, contributing positively to increasing cotton production [21]. Although the *PDCB* gene family in *Arabidopsis*, pear, and cotton has been studied, they remain largely unknown in most crops, such as maize. Maize is a widely cultivated cereal crop, grown globally for fuel, fiber, food, and feed. Moreover, maize serves as an important model crop for studying various biological phenomena [22], and has its genome information sufficiently available [23].

Maize is also a representative of the C4 photosynthetic pathway [24]. In contrast to C3 plants, C4 plants possess two structurally and biochemically specialized photosynthetic cell types: mesophyll (M) and bundle sheath (BS) [25]. These BS and M cells surround the leaf

veins, forming a wreath-like structure [26]. A significant number of PD exist between BS and M cells, facilitating material exchange between these cellular types and enabling their coordinated effort in achieving carbon assimilation during plant photosynthesis [25,27]. Currently, there is little research on the *PDCB* gene family. Even with regard to rice, one of the most important food crops and relevant model plants, the *PDCBs* have not been reported. Thus, investigating *PDCBs* in important crops could elucidate their role in plant growth and development, and provide new insights into the study of carbohydrate transport and potential for agronomy trait improvement. In this study, we identified members of the maize *PDCB* gene family and subsequently analyzed their gene structures, conserved domains, evolutionary relationships, *cis*-acting elements, and expression patterns across different tissues. This study could furnish valuable information for comprehending the functions of the *PDCB* gene family in maize and provide new insights for crop breeding potential including yield, quality, and so on. Additionally, it might promote further exploration of *PDCB* genes in other significant crops, such as rice, and also facilitate a more comprehensive grasp of plant growth and metabolism.

2. Materials and Methods

2.1. Detection of PDCB Gene Family Members in Maize and Rice

We downloaded the *Arabidopsis thaliana* PDCB gene family proteins from TAIR (https://www.arabidopsis.org, accessed on 29 February 2024) [28]. The maize genome (Zm-B73-REFERENCE-NAM-5.0) and annotated files were obtained from Ensembl plants (http://plants.ensembl.org/index.html, accessed on 29 February 2024) [29].

Then, the Sequence Files program of TBtools was used to search PDCBs within the maize database, with the PDCB proteins of *Arabidopsis* as the query for identification of the *PDCB* genes. Furthermore, the HMM profile of the X8 domain (Pfam: PF07983) was downloaded from the Pfam database (http://pfam.xfam.org, accessed on 1 March 2024) [30] and was effectively utilized to accurately identify the *PDCB* genes with an E-value lower than 1×10^{-5}. The proteins lacking X8 domain were eliminated. The candidate sequences were calculated using the TBtools Protein Parameter Calc (ProtParam-based) software (v2.119) for determining the molecular weights (MW) and theoretical isoelectric points (pI) and the WOLF PSORT (https://wolfpsort.hgc.jp, accessed on 2 March 2024) for subcellular localization prediction [31].

The rice genome (T2T-NIP) was downloaded from the Rice Super Pan-genome Information Resource Database (http://www.ricesuperpir.com/web/nip, accessed on 6 August 2024) [32]. The genome-wide identification of PDCB proteins in rice was conducted by using the same process mentioned above.

2.2. Developing Phylogenetic Trees, Analyzing Motifs, and Determining Gene Structures

A phylogenetic tree was constructed by following the neighbor-joining (NJ) method and employing MEGA11 with 1000 replicates [33]. TBtools was utilized to extract *PDCB* location data and view the gene structure. The conservative pattern was found using TBtools' Simple MEME Wrapper program, which was configured to yield a maximum of 20 motifs. Following this, the motif was shown using TBtools' Visualize MEME Motif Pattern application.

2.3. Chromosome Location and Synteny Analysis for PDCBs

PDCB positions on chromosomes were displayed using TBtools using genomic annotation files from the *Zea mays* species [34]. *PDCB* collinearity was examined using MCScanX, which analyzes intra- and extra-genomic collinearity based on genome annotation files. Collinear gene pairs were shown using TBtools' Advanced Circossoftware (v2.119).2.4. Examination of the Cis-Acting Element inside Promoters

The 2000 bp region chosen as the promoter was located upstream of the *PDCB* gene's translation start codon ATG. The *cis*-acting elements present in the promoter region of the *PDCBs* were estimated using the PlantCare website [35]. Then, the number of *cis*-

acting elements was counted, and the *cis*-acting elements were visualized using the TBtools HeatMap program (v2.119).

2.4. ZmPDCBs Expression Patterns under Different Tissues

Transcriptome data for expression patterns of *ZmPDCBs* in 18 different tissues were downloaded from MaizeGDB (https://www.maizegdb.org/genome/assembly/Zm-B73-REFERENCE-NAM-5.0,accessed on 5 March 2024) [36–38]. The tissues include internode, vegetative meristem, ear primordium, embryo, endosperm, endosperm crown, germination kernels, pericarp aleurone, female spikelet, silk, root cortex, root meristem zone, secondary root, root elongation zone, primary root, mature pollen, mature leaf, and leaf zone. Genes with FPKM values greater than 1 were classified as expressed genes. Using the value of $\log_2$ (FPKM+1), the HeatMap software (v2.119) of TBtools was used to visualize the expression patterns of the *ZmPDCBs*.

3. Results

3.1. Zea mays PDCB Identification and Characterization

Fifty-six members of the *PDCB* gene family were identified in *Zea mays*. The X8 domain (PF07983) was present in every protein sequence. Further analysis was conducted on the characteristics of ZmPDCBs (Table S1). The protein length ranged from 81 aa (amino acids) in ZmPDCB9 to 665 aa in ZmPDCB43 and ZmPDCB54. The molecular weight (MW) of PDCBs varied from 9.07 kDa (ZmPDCB9) to 73.12 kDa (ZmPDCB54), with an average MW of 41.19 kDa. The isoelectric point (pI) values of ZmPDCB proteins were either above or below 7, demonstrating both alkaline and acidic characteristics. The GAH ranged from −0.532 (ZmPDCB24 and ZmPDCB55) to 0.382 (ZmPDCB51), revealing their differences of hydrophilicity. The PSL (predicted subcellular location) indicated that the subcellular localization of the *ZmPDCBs* was also highly diverse. It may be located on membrane structures such as the plasma membrane and vacuolar membrane, or in organelles such as chloroplasts, nucleus, and Golgi apparatuses. These results indicate that ZmPDCB proteins present multiplicity in their physicochemical properties.

3.2. Phylogenetic Study and Classification of Maize PDCBs

To investigate the evolutionary relationships among the PDCB protein family in maize and other plants, we constructed a phylogenetic tree using the PDCB protein sequences from maize (*Zea mays*) and *Arabidopsis thaliana*. Furthermore, we identified 47 PDCB proteins from rice (*Oryza sativa*) to better characterize the evolutionary traits of the PDCB proteins in the grass family (Table S2). The phylogenetic relationships among PDCB proteins from these three species are illustrated in Figure 1. The phylogenetic tree divided the PDCB proteins into six branches. ZmPDCBs were distributed in all branches with an uneven pattern. Branches B and F contained more PDCB members, with 13 members in branch B and 27 members in branch F. In contrast, branches A, C, D, and E contained only four members, respectively. Notably, each branch contained PDCB proteins from both maize and rice, but not all branches included PDCB proteins from *Arabidopsis* (e.g., branch F), indicating an evolutionary divergence between monocots and dicots. Meanwhile, in branch F, there was a subbranch containing only ZmPDCB14, 41, 42, 43, which was significantly different from the subbranch nearby constituted by OsPDCBs (28, 29, 30, 31, 32, 38). These results indicate that the functions of the *PDCB* homolog genes in maize might be similar, and the phylogenetic relationship between maize and rice, both members of the grass family, is closer than that between maize and *Arabidopsis*, which belong to the Brassicaceae family.

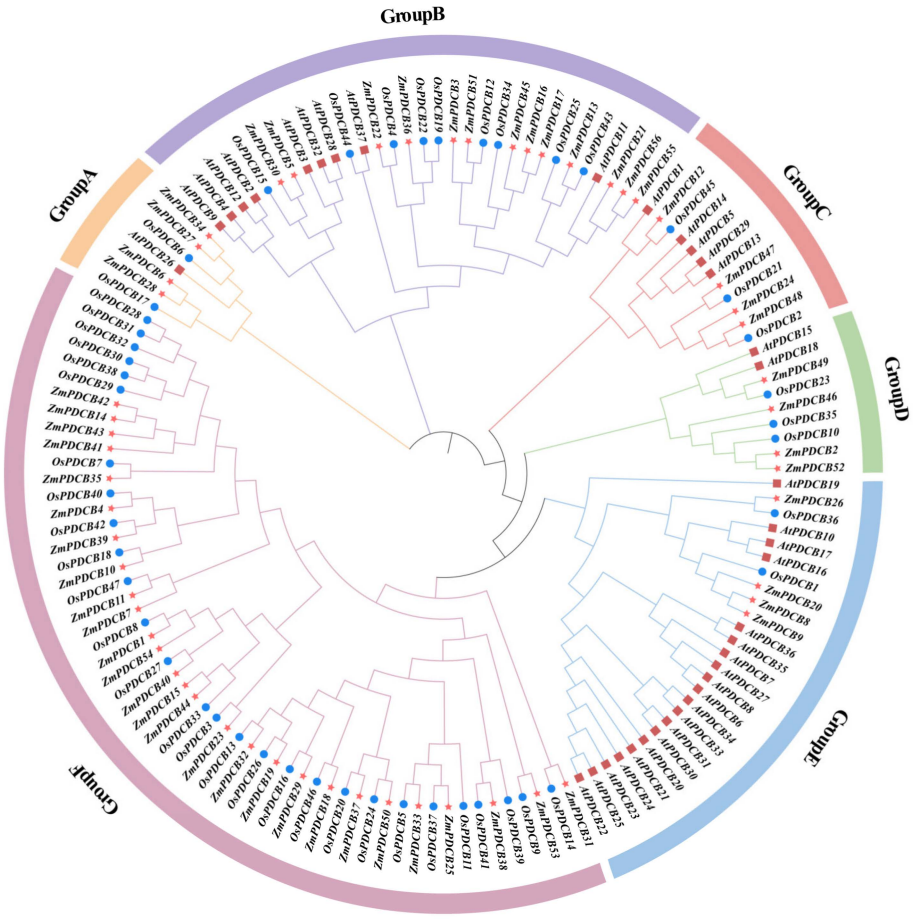

Figure 1. Plasmodesmata Callose Binding Proteins (PDCBs) from *Zea mays*, *Arabidopsis*, and rice underwent phylogenetic analysis. *Zea mays*, *Arabidopsis* and rice PDCBs are denoted by pink stars, red squares, and blue circles, respectively.

3.3. Gene Structure and Conserved Motif Analysis of ZmPDCB Genes

Variations in conserved motifs and the diversity of gene architecture serve as signs of the evolution of multigene families [39]. To comprehend the variation in gene structure, the spatial arrangement of *PDCB* exon/intron sections was investigated (Figure 2). The majority of *PDCBs* (36%) had four exons, out of a total of five exons. The exons of *PDCB* genes showed a higher degree of similarity within the same group (Figure 2C).

The fundamental framework of any feature sequence is defined by the motif, a conserved segment within the sequence. There were 20 possible motifs identified (Figure 2A). Remarkably, only motif 4 was present in every PDCB. The majority of PDCBs were ordered in the motif 4-1-2 pattern. The conserved motifs of PDCBs exhibited variations among groups, whereas the amount and configuration of motifs within a particular group of PDCBs were more similar compared to those of other groups. The remaining PDCBs, except for PDCB9, were placed in the sequence of motif 4-19-2.

The PDCB proteins were shown to have five conserved domains: PHA03247, X8, PRK14971, PLN02807, and Glyco_hydro superfamily domains (Figure 2B). The X8 domain was present in all PDCBs, and approximately half of them contained Glyco_hydro superfamily domains. The PHA03247 superfamily was present in ZmPDCB6, ZmPDCB13, and ZmPDCB28; the PRK14971 superfamily was present in ZmPDCB12; and the PLN02807 superfamily was present in ZmPDCB21. Based on these findings, we deduced that while the majority of *PDCB* members were conserved in maize, there was a minor degree of divergence in terms of gene structure and protein sequences.

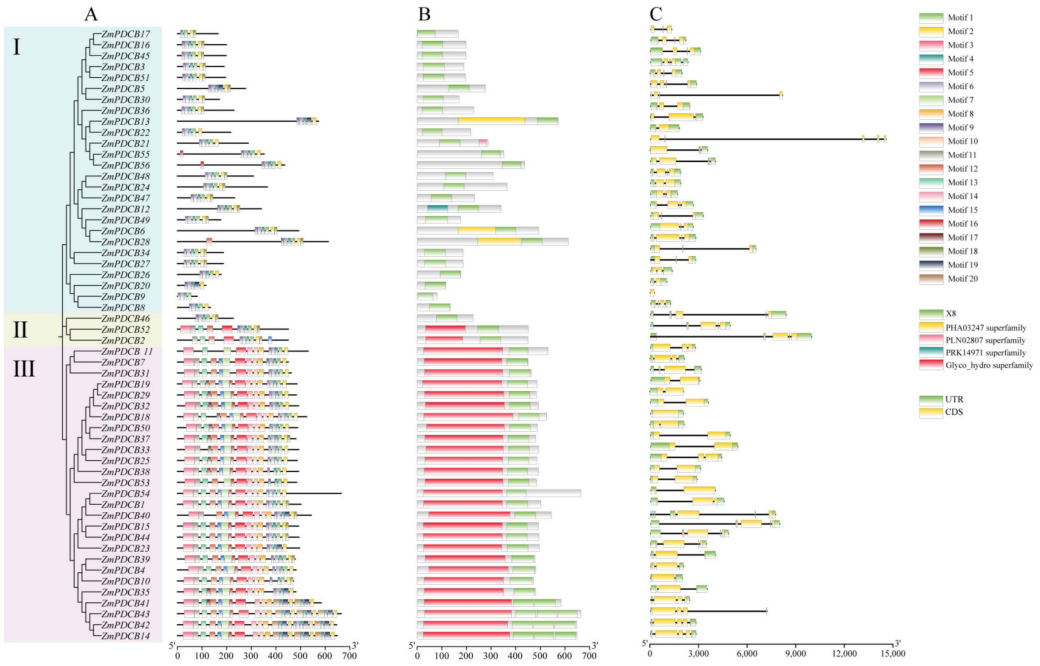

Figure 2. Conserved motif, conserved domain, and exon–intron distribution of *PDCB* genes in maize. (**A**) The conservative motif of *PDCBs* and MEGA11 were used to build the evolutionary tree of *PDCBs*. The 20 motifs are shown in various colored boxes. (**B**) Conserved domains of PDCB proteins. (**C**) Gene structures of *PDCB* genes. I, II, III represent the groups of the phylogenetic tree.

3.4. Chromosomal Location and Synteny Analysis of the PDCBs in Zea mays

Chromosome mapping showed that there were 55 *ZmPDCBs* distributed across 10 chromosomes, with 6 *ZmPDCBs* on Chr01, 13 *ZmPDCBs* on Chr02, 5 *ZmPDCBs* on Chr03, 3 *ZmPDCBs* on Chr04, 8 *ZmPDCBs* on Chr05, 2 *ZmPDCBs* on Chr06, 9 *ZmPDCBs* on Chr07, 2 *ZmPDCBs* on Chr08, 6 *ZmPDCBs* on Chr09, and 1 *ZmPDCB* on Chr10. Additionally, 1 *ZmPDCB* was located on scaffold_539. Most *PDCBs* were found near the telomeres or centromeres of chromosomes (Figure 3).

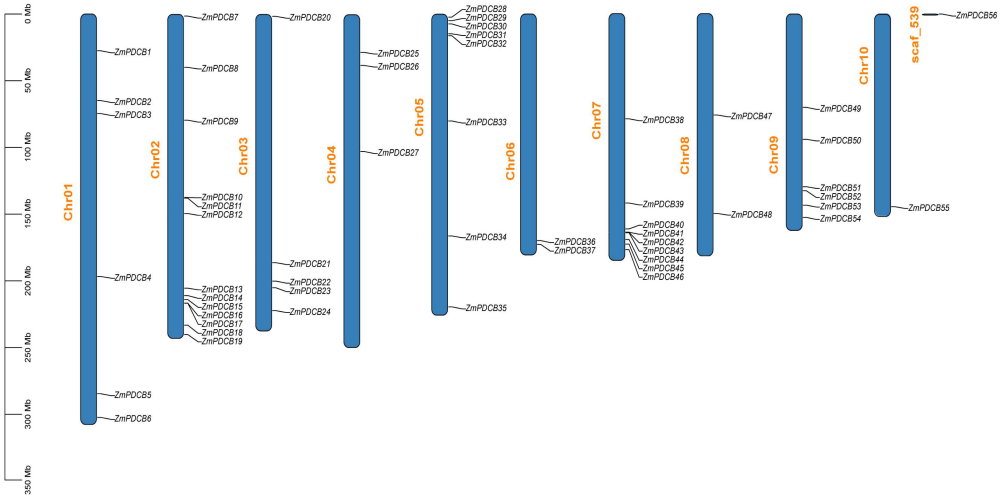

Figure 3. The distribution of 56 *PDCBs* on the chromosomes of maize. The chromosome name is located to the left of each chromosome, whereas the gene ID is situated to the right.

The distribution or arrangement of homologous genes within or between species is referred to as collinearity [40]. We examined the intra-genomic collinearities to demonstrate the collinearity of *PDCB* genes (Figure 4A). A total of 16 pairs of duplicated genes were

distributed across 7 chromosomes, and most of them spanned chromosomes, suggesting that segmental duplication was a crucial aspect in the evolution of the *PDCB* gene family in maize. Then, we conducted an analysis of the collinearity among maize, rice, and *Arabidopsis* to reveal their evolutionary relationships. The synteny relationships in *ZmPDCBs* and *OsPDCBs*, and in *ZmPDCBs* and *AtPDCBs* were denoted by red and blue lines, respectively. A total of 48 pairs of collinearity were identified, of which 47 pairs of collinearity occurred in *ZmPDCBs* and *OsPDCBs*, while *ZmPDCBs* and *AtPDCBs* had only 1 pair of collinearity. The number of homologous gene pairs between maize and rice was much higher than the number between maize and *Arabidopsis*, and which was consistent with the evolutionary relationship shown in Figure 1, further indicating that maize and rice are both grass family members with a closer relationship.

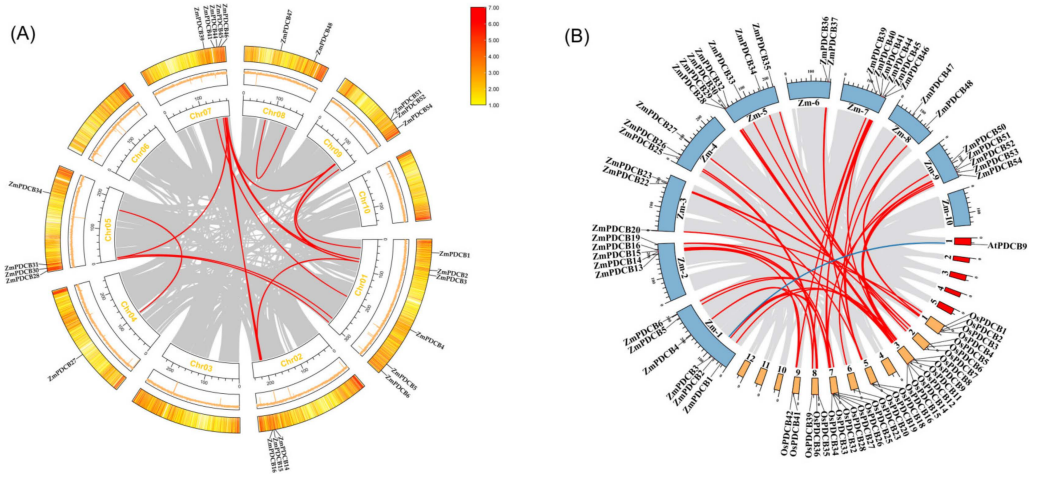

Figure 4. Collinearity analysis of *PDCB* genes. (**A**) Synteny analysis of *PDCB* genes in *Zea mays*. (**B**) Synteny analysis of *PDCB* genes among *Zea mays*, *Arabidopsis*, and rice.

3.5. Cis-Acting Elements in the Promoter of PDCB Genes

The *cis*-acting elements in the promoter region may facilitate the regulation of gene transcription. An analysis of the *cis*-acting elements upstream of *PDCB* genes can illuminate their potential functions and enhance our comprehension of their regulatory mechanisms (Figure 5). A total of 10 *cis*-acting elements linked to phytohormone responses were identified. For instance, there was 1 salicylic acid-responsive element (TCA-element), 3 auxin-responsive elements (TGA-element, TGA-box, and AuxRR-core), 3 gibberellin-responsive elements (GARE-motif, P-box, and TATC-box), 2 methyl jasmonate-responsive elements (TGACG-motif and CGTCA-motif), and 1 abscisic acid-responsive element (ABRE). All *ZmPDCB* promoters contained the ABRE, with *ZmPDCB29* having the largest number of this element. Given that maize is a C4 plant, these components, especially auxRR-core and ABRE, may play a role in regulating *ZmPDCBs*, thereby enhancing maize agronomy traits like deep rooting and stress tolerance.

Additionally, 30 light-responsive elements were identified, with G-Box and Sp1 existing in almost all *ZmPDCB* promoters. Six environmental stress-responsive *cis*-acting elements were identified, including defense and stress responsiveness (TC-rich repeats), wound-responsive elements (WUN-motif), anaerobic induction (ARE), enhancer-like elements involved in anoxia-specific inducibility (GC-motif), the MYB binding site involved in drought inducibility (MBS), and low-temperature responsiveness (LTR). Additional *cis*-acting elements associated with plant development were also found, including those regulating the cell cycle (MSA-like), zein metabolism (O2-site), and MYB binding sites, which are involved in the regulation of flavonoid biosynthesis genes (MBSI), light responsiveness (MRE), and drought inducibility (MBS). One MYBHv1 binding site, termed CCAAT-box, was also identified, with HvMYB1 known as a positive regulator of drought tolerance [41]. In conclusion, the quantity and diversity of *cis*-acting elements in the *Zm-*

PDCB promoter region were not only indicators of functional complexity, but also shown to be key regulators that contribute significantly to the unique growth characteristics and stress tolerance of maize as a C4 plant.

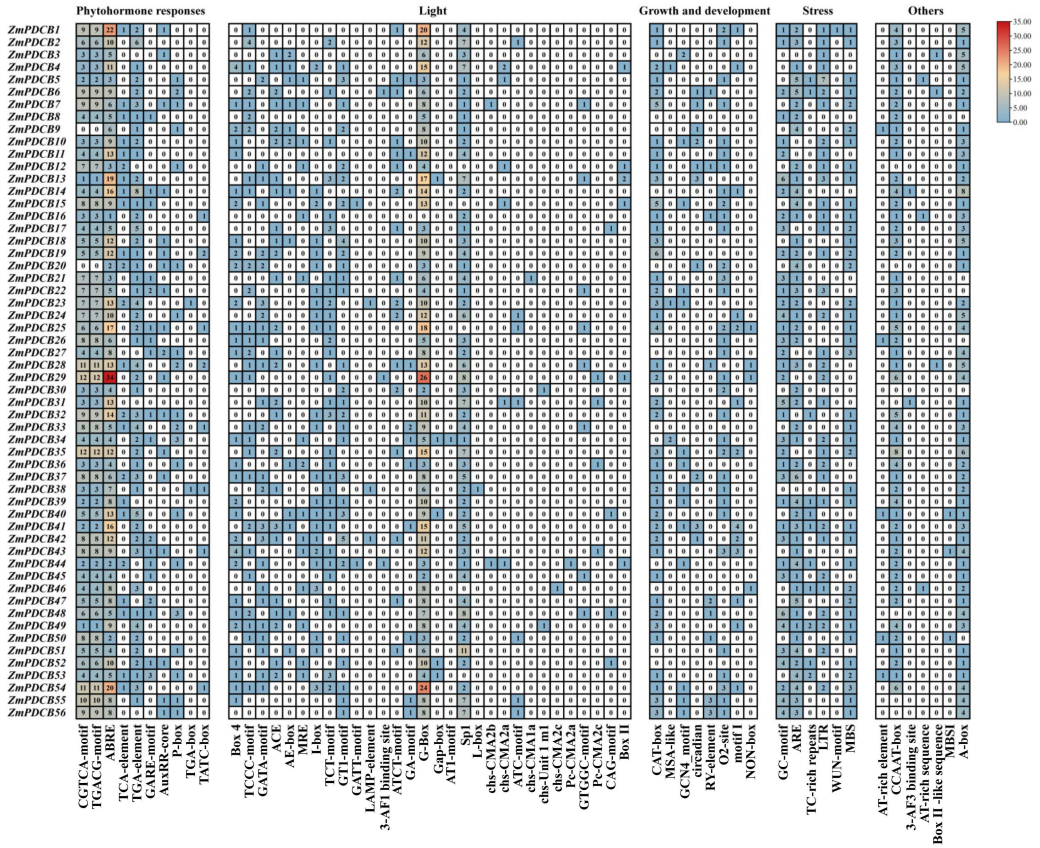

Figure 5. *Cis*-regulatory elements identified in the promoter region of *ZmPDCBs*. The different colors and numbers of the squares represent the numbers of different promoter elements in *ZmPDCB* genes.

3.6. ZmPDCBs Expression Patterns in Various Tissues

Transcriptomic data from specific tissues can be utilized to predict the functionality of a gene during particular plant developmental stages. Various tissues displayed expression of *ZmPDCBs*, with some exhibiting high expression levels. Notably, 2 out of the 56 *ZmPDCB* genes, namely *ZmPDCB11* and *ZmPDCB24*, were expressed across all 18 tissues, albeit at varying levels (Figure 6). Conversely, five genes, *ZmPDCB4*, *9*, *21*, *42*, and *43,* showed no expression in any tissue. Eighteen *PDCBs*, namely *ZmPDCB*2, 4, 9, 10, 13, 14, 18, 21, 25, 26, 27, 28, 32, 37, 42, 43, 47, and 55, exhibited extremely low expression (FPKM value < 10) across all tissues. The remaining genes were generally expressed in at least six tissues. Furthermore, *ZmPDCB5*, *8*, *23*, *35*, *36*, and *45* displayed elevated expression levels in most tissues relative to other genes. *ZmPDCB5* demonstrated significant expression (FPKM value > 90) in internode, vegetative meristem, ear primordium, endosperm, and leaf. *ZmPDCB8* and *ZmPDCB36* demonstrated significant expression (FPKM value > 80) in 10 and 15 samples in different tissues, respectively, with higher expression (FPKM value > 190) in root, indicating that these genes may play important roles in root development. *ZmPDCB23* demonstrated significant expression (FPKM value > 90) in internode, vegetative meristem, ear primordium, leaf, and embryo. *ZmPDCB35* demonstrated significant expression (FPKM value > 80) in internode, ear primordium, embryo, leaf, and root. *ZmPDCB45* demonstrated significant expression (FPKM value > 110) in internode, vegetative meristem, leaf, and female spikelet, with the highest expression patterns in female spikelet compared to other genes. Notably, one gene (*ZmPDCB14*) was expressed only in secondary root. Five genes showed significant tissue-specific expression in reproductive tissues. For instance,

ZmPDCB8, *11*, *24*, and *50* were specifically expressed in mature pollen, with *ZmPDCB50* exhibiting an extraordinarily high expression level, suggesting a potential role for the genes in pollen maturation and function. Furthermore, the expression levels of *ZmPDCB20* increased extremely in silk, indicating its significance in the development of pistils in maize. In summary, the expression profiles of *ZmPDCBs* revealed the diverse and specific expression patterns of *ZmPDCB* genes in different tissues and developmental stages of maize. The prominent expression of certain genes, such as *ZmPDCB8*, *20*, *36*, *45*, and *B50*, in vegetative tissues like roots and reproductive tissues like mature pollen, female spikelets, and silk indicates their essential regulatory roles in these processes.

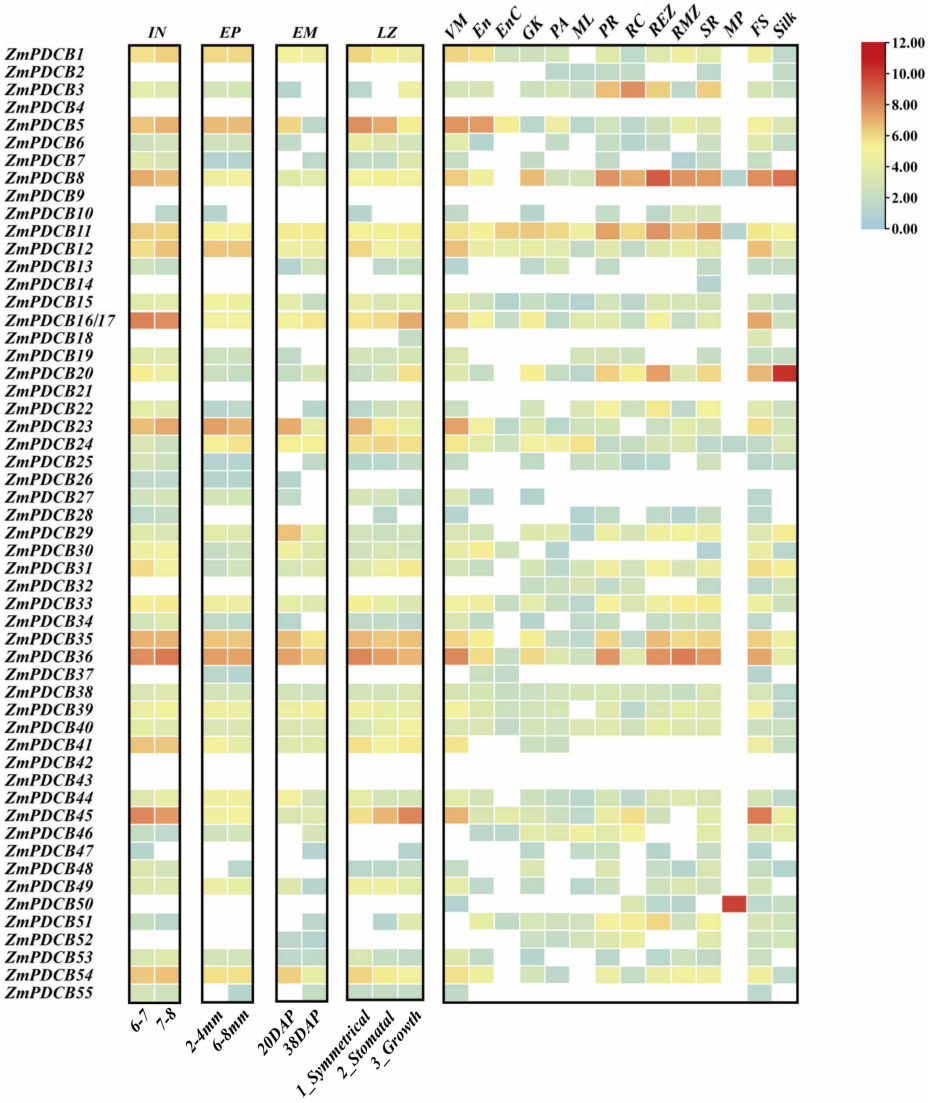

Figure 6. Transcriptome analyses of *ZmPDCB* genes in 18 tissues. The expression levels of *ZmPDCB* genes in distinct tissues can be observed by plotting the normalized Log_2 (FPKM+1) values against the relevant tissues. Internode (IN), vegetative meristem (Vm), ear primordium (EP), embryo (Em), endosperm (En), endosperm crown (EnC), germination kernels (GK), pericarp aleurone (PA), leaf zone (LZ), root meristem zone (RMZ), root cortex (RC), primary root (PR), mature leaf (ML), root elongation zone (REZ), secondary root (SR), mature pollen (MP), and female spikelets collected on the same day as silk (FS) are presented in abbreviated forms.

4. Discussion

Maize holds a pivotal position in human nutrition as a staple food, provides a valuable source of raw materials for various industries, and is highly esteemed as the primary feed

for livestock [42], but its *PDCB* coding gene family remains unknown. PDCBs are involved in regulating the function and localization of callose in the vicinity of PD [18]. Callose is crucial for numerous biological processes related to plant development and growth, as well as plant defense against adverse environmental factors [43]. In plants, the best studied *PDCB* gene families were those of *Arabidopsis thaliana* and pear fruit [20,44]. This study employs bioinformatics approaches to identify and evaluate *PDCBs* in *Zea mays*, aiming to gain insights into the involvement of the PDCB family in maize development.

A total of 56 *PDCBs* were identified in maize genome, categorized into six groups based on their evolutionary relationships (Figure 1). Not all groups contained homologous genes from *Arabidopsis*. Each group comprised between 4 to 27 *ZmPDCB* members; *ZmPDCBs* and *OsPDCBs* were predominantly found in group F, in which there were 27 *ZmPDCBs*, implying they might be specific orthologs of cereal. On the other hand, the majority of group E were 20 *AtPDCBs*, with just four *ZmPDCBs* and two *OsPDCBs* included. Group F, a subbranch containing only *ZmPDCB14*, *41*, *42*, and *43*, was significantly different from the nearby subbranch constituted by *OsPDCBs* (*28*, *29*, *30*, *31*, *32*, and *38*). Based on FPKM values obtained from RNA-sequencing data, the expression patterns of *ZmPDCBs* in 18 tissues were scrutinized to identify which *PDCB* genes might be implicated in regulating the growth of specific tissues or organs in maize (Figure 5). Interestingly, the transcription level in various tissues indicated the expression of *ZmPDCB41* was general in nine tissues investigated, but the expression of *ZmPDCB14* was only detected in secondary root, and the expressions of *ZmPDCB42*, *43* were absent. According to the relationship demonstrated by the phylogenetic tree, the *ZmPDCB41* showed a big genetic distance from *ZmPDCB14*, *42*, *43*, which might explain partially the difference in their expression profile. However, as showed in Figure 3, it is very likely that *ZmPDCB41*, *42*, *43* were in one gene cluster, thus *ZmPDCB14* possibly originated from them by chromosome duplication and merging. The expression profile of *ZmPDCB42*, *43* remained unknown. It is clear that *PDCB* genes have been linked to callose accumulation in maize [11]. The study on tissue-specific expression of this gene family provides insights into its potential roles in various developmental processes [22].

Considering the vital roles of structural diversity in the evolution of gene families, we conducted the gene structure and conserved motif analysis of *ZmPDCB* genes (Figure 2). The *ZmPDCBs* exhibited notable congruence in their conserved motifs and gene architectures within the same group. However, for group II, there was an exception. This may be because some motifs in these three *PDCBs* were not conserved. The PDCB proteins typically featured five conserved domains: X8, PLN02807, PHA03247, PRK14971, and Glyco_hydro superfamily domains. However, not all ZmPDCB proteins contained the Glyco_hydro superfamily, PLN02807, PHA03247, or PRK14971 domains. All ZmPDCB proteins contained the X8 domain, which belongs to a broader family and is essential for callose-binding activity [18,20]. Additionally, 16 pairs of homologous genes were identified within the maize genome.

Moreover, *cis*-acting elements relevant to plant growth and development, light response, stress-associated responses, and plant hormones were analyzed within the promoter sequences of the *ZmPDCB* genes (as showed in Figure 5). These components may play a role in regulating *ZmPDCBs*. C4 plants are well-known for their superior agronomy traits, like deep rooting and stress tolerance, of which maize is typical and outstanding [24,45]. In plants, auxin (IAA) is a master regulator of morphogenesis, pattern formation, tropic responses, and development, including root architecture. Present research checked expression profiles of the *ZmPDCBs* in root meristem zone (RMZ), root cortex (RC), primary root (PR), root elongation zone (REZ), and secondary root (SR). More than half of the *ZmPDCBs* showed expression in these root tissues (Figure 6). Among these genes, *ZmPDCB3*, *8*, *11*, *20*, *35*, and *36* had relatively higher expression levels than the others. The presentation of *cis*-regulatory element auxRR-core was identified in the promoter regions of *ZmPDCB3*, *20*, and *35*, which hinted at the involvement of an auxin signal regulating the expression of *ZmPDCBs* in the process of root development. There is also research reporting that the

quantity of callose at the plasmodesmal region is controlled by the auxin-GSL8 feedback circuit [46]. The results also showed that all the promoter regions of these *ZmPDCBs* have *cis*-regulatory elements concerned with stress responding and tolerance, and ABRE were detected and relatively abundant. These are key factors determining abscisic-acid-mediated transcriptional regulation of stress-related genes. ABA is vital in responding to various adverse conditions and is significantly involved in callose production [47]. Furthermore, environmental stressors, such as low temperatures, reactive oxygen species, and early viral defense systems, also influence callose deposition [15]. All these results implied that *ZmPDCBs* are highly involved in the maize root development and stress response.

5. Conclusions

Within this scholarly investigation, we delineated 56 ZmPDCBs within the maize genome, categorizing them into six distinct groups according to their evolutionary profiles. The *ZmPDCBs* exhibited notable congruence in their conserved motifs and gene architectures within the same group. Numerous *cis*-acting elements associated with plant hormones, light, plant growth dynamics, and stress conditions were identified within the promotor regions of the *ZmPDCB* genes. Significantly, every *ZmPDCB* promoter encompassed the abscisic-acid-responsive element. Through transcriptomics analysis of the *ZmPDCBs* across 18 distinct tissues, diverse and specific expression patterns emerged across various developmental stages of maize. Certain genes displayed extraordinarily high expression levels in vegetative tissues, including roots and reproductive tissues such as mature pollen, female spikelets, and silk, underscoring their pivotal regulatory functions in tissue-specific gene expression. These outcomes lay the groundwork for forthcoming endeavors in elucidating the crucial roles of the PDCBs in cereal crops, especially in the study of carbohydrate transport mechanisms.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy14081858/s1, Table S1: Detailed information and analysis of the physicochemical analysis of the Plasmodesmata Callose Binding Proteins (PDCBs) in *Zea mays* L.; Table S2: Detailed information of the *PDCB* gene family in *Oryza sativa* L.

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