

RESEARCH

Open Access



# Genome-wide analysis of *MdPLATZ* genes and their expression during axillary bud outgrowth in apple (*Malus domestica* Borkh.)

Jiuyang Li<sup>1</sup>, Yongliang Zhao<sup>1</sup>, Yaohui Zhang<sup>1</sup>, Feng Ye<sup>1</sup>, Zhengcun Hou<sup>1</sup>, Yuhang Zhang<sup>1</sup>, Longjie Hao<sup>1</sup>, Guofang Li<sup>1</sup>, Jianzhu Shao<sup>1\*</sup> and Ming Tan<sup>1\*</sup>

## Abstract

**Background** Branching is a plastic character that affects plant architecture and spatial structure. The trait is controlled by a variety of plant hormones through coordination with environmental signals. Plant AT-rich sequence and zinc-binding protein (PLATZ) is a transcription factor that plays an important role in plant growth and development. However, systematic research on the role of the PLATZ family in apple branching has not been conducted previously.

**Results** In this study, a total of 17 *PLATZ* genes were identified and characterized from the apple genome. The 83 *PLATZ* proteins from apple, tomato, *Arabidopsis*, rice, and maize were classified into three groups based on the topological structure of the phylogenetic tree. The phylogenetic relationships, conserved motifs, gene structure, regulatory *cis*-acting elements, and *microRNAs* of the *MdPLATZ* family members were predicted. Expression analysis revealed that *MdPLATZ* genes exhibited distinct expression patterns in different tissues. The expression patterns of the *MdPLATZ* genes were systematically investigated in response to treatments that impact apple branching [thidazuron (TDZ) and decapitation]. The expression of *MdPLATZ1*, 6, 7, 8, 9, 15, and 16 was regulated during axillary bud outgrowth based on RNA-sequencing data obtained from apple axillary buds treated by decapitation or exogenous TDZ application. Quantitative real-time PCR analysis showed that *MdPLATZ6* was strongly downregulated in response to the TDZ and decapitation treatments, however, *MdPLATZ15* was significantly upregulated in response to TDZ, but exhibited little response to decapitation. Furthermore, the co-expression network showed that *PLATZ* might be involved in shoot branching by regulating branching-related genes or mediating cytokinin or auxin pathway.

**Conclusion** The results provide valuable information for further functional investigation of *MdPLATZ* genes in the control of axillary bud outgrowth in apple.

**Keywords** Apple, *PLATZ* transcription factor, Gene expression, Axillary bud outgrowth, Shoot branching

## Background

Transcription factors (TFs) are proteins that specifically bind to *cis*-acting elements in promoter regions and thereby regulate gene expression [1]. They play important roles in diverse life processes, such as regulation of plant growth and development, defense against stress, and signal transduction [2]. In recent years, increased research attention has been focused on TFs.

Plant AT-rich sequence and zinc-binding protein (PLATZ) is a TF, first identified in pea, that is composed

\*Correspondence:

Jianzhu Shao

yysjz@hebau.edu.cn

Ming Tan

tanming@hebau.edu.cn

<sup>1</sup> College of Horticulture, Hebei Agricultural University, Hebei 071000, China



© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

of two zinc finger domains in pea: C-X2-H-X11-C-X2-C-X (4–5)-C-X2-C-X (3–7)-H-X2-H and C-X2-C-X (10–11)-C-X3-C (X represents any amino acid) [3]. In recent years, *PLATZ* TFs have been identified in many plant species; for example, 12 *PLATZ* family members have been identified in *Arabidopsis* [4], 17 members in maize [5], 15 members in rice [4], 62 members in wheat [6], and 86 members in *Malus* [7].

Many studies have confirmed that *PLATZ* TFs are associated with the regulation of plant growth and development as well as response to abiotic stresses [3, 8]. In maize, the *PLATZ* gene *FL3* is involved in regulating the transcription of tRNA and 5S rRNA during endosperm development and flowering [9]. In rice, *GL6* has been identified as a *PLATZ* gene and functions in regulating the length and number of rice panicles [10]. ORESARA 15 an *Arabidopsis* *PLATZ* TF, promotes the rate and duration of leaf cell proliferation and inhibits leaf senescence. In *Arabidopsis*, *PLATZ1* and *PLATZ2* play important roles in tolerance of seed dehydration [11], and *PLATZ2* can also affect plant sensitivity to salt stress [12]. Studies on sugarcane show that a *PLATZ* gene may be a transcriptional regulator of secondary cell wall synthesis [13].

Shoot branching is a quality trait important in the establishment of plant architecture. The planting of ideally branched nursery trees is crucial in modern apple orchards [14, 15]. The lateral buds on branches generally develop into flower buds in the first year, and contribute to fruit number and quality in the succeeding year [16]. The highest yield in initial and subsequent stages of orchard management can be achieved with nursery trees with an ideal branch architecture. However, different treatments are applied in nurseries to increase the number of branches, such as application of exogenous hormones [e.g., 6-benzylaminopurine (6-BA), or thidiazuron (TDZ)] or pruning methods. Therefore, understanding the molecular mechanism responsible for apple shoot branching would be greatly beneficial.

The axillary buds of apple, located in the leaf axils, are generally dormant owing to the correlative inhibition exerted by apical buds [17]. Outgrowth of axillary buds (i.e., branching) is regulated by multiple endogenous and exogenous factors [18–20]. Previous studies have demonstrated that auxin indirectly inhibits branching [21, 22]. Cytokinin (CK) stimulates the outgrowth of axillary buds directly [23, 24]. Our recent study showed that exogenous CK and decapitation can induce the activation and outgrowth of axillary buds in apple, and suppression of CK synthesis restricts the outgrowth of axillary buds induced by decapitation [25]. Spring budburst in apple is specifically triggered by CKs from the shoot [26]. Strigolactone (SL), a secondary messenger of auxin that

directly inhibits axillary bud outgrowth [27], is reported to act antagonistically with CK in regulating bud outgrowth [28]. In addition to hormone signals, the control of functional genes contributes to the regulation of bud activation. There is evidence that *PLATZ* is involved in the transition from primary growth to secondary growth during bud development in poplar [29], which provides a clue to the role of *PLATZ* genes in the control of bud growth. However, there is little corresponding research on apple.

In the present study, we identified the *MdPLATZ* family members in the entire apple genome, characterized their basic properties, and explored their phylogenetic relationships and expression patterns. To clarify the role of *MdPLATZ* genes in shoot branching in apple, we investigated their expression patterns in treated axillary buds using transcriptomic data. Selected *MdPLATZ* genes were further analyzed in response to TDZ and decapitation treatments by quantitative real-time PCR (qRT-PCR) analysis, and were also used for interaction network analysis. Changes in gene expression in response to the branching-related treatments and prediction of proteins that interact with *MdPLATZ* proteins provided evidence for their potential roles in apple bud outgrowth. The results provide a foundation for further analysis of the functional role of *MdPLATZ* genes in apple shoot branching.

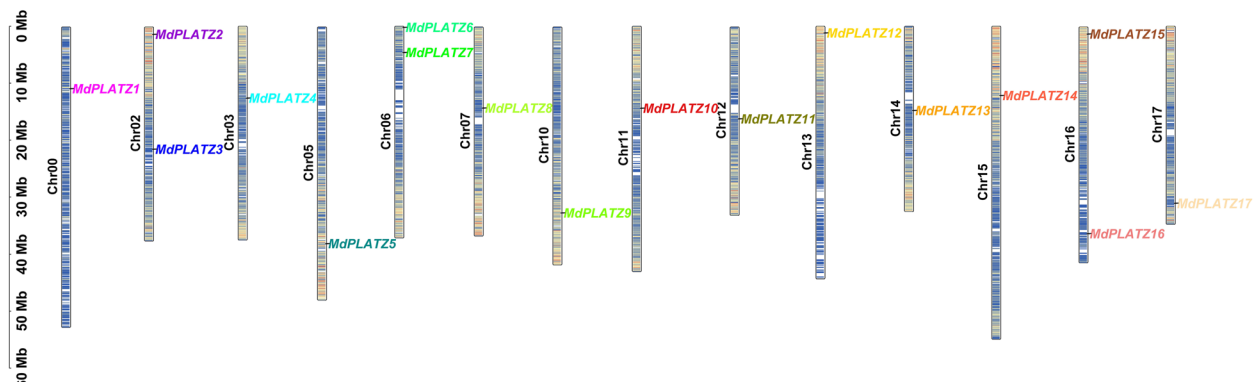
## Results

### Identification of *MdPLATZ* gene family members in apple

A total of 17 *MdPLATZ* genes were identified from the apple genome database (GDDH13.1–1). The *MdPLATZ* genes were named based on their chromosomal location (Table 1). The sequence lengths of the *MdPLATZ* proteins ranged from 147 (*MdPLATZ17*) to 257 (*MdPLATZ2*) amino acids and, accordingly, the molecular weight varied from 17.23 to 29.40 kDa. The theoretical isoelectric point (pI) of all 17 *MdPLATZ* proteins was more than 7. All proteins had a grand average hydrophobicity values of less than zero, suggesting that they were all hydrophilic proteins. The instability index of all *MdPLATZ* members was more than 40, indicating that they are unstable proteins. The *MdPLATZ* proteins were predicted to be localized in the nucleus, except for three proteins: *MdPLATZ5* (localized to chloroplasts), and *MdPLATZ6* and *MdPLATZ9* (localized to chloroplasts and the nucleus). The reliability of the tertiary structure of all *MdPLATZ* proteins was greater than 80% (Supplementary Fig. S1). Detailed information on the protein secondary structure is listed in Table S2. The 17 *MdPLATZ* genes were distributed on 14 chromosomes (Chr) (Fig. 1). Chromosomes 02, Chr06, and Chr16 contained the highest number with two *MdPLATZ* genes

**Table 1** Information on *MdPLATZ* gene family members in apple

Gene name	Gene ID	Amino acids (aa)	pI	Molecular mass (kD)	Instability index	Grand average of hydropathicity	Subcellular localization
<i>MdPLATZ1</i>	MD00G1059100	252	8.55	28.54	55.19	-0.496	Nucleus
<i>MdPLATZ2</i>	MD02G1017000	257	8.56	29.39	59.17	-0.496	Nucleus
<i>MdPLATZ3</i>	MD02G1208800	221	8.82	24.67	58.34	-0.239	Nucleus
<i>MdPLATZ4</i>	MD03G1129200	192	9.51	21.97	64.26	-0.843	Nucleus
<i>MdPLATZ5</i>	MD05G1248500	254	8.39	28.49	57.66	-0.355	Chloroplast
<i>MdPLATZ6</i>	MD06G1001300	252	8.78	28.44	51.01	-0.515	Chloroplast. Nucleus
<i>MdPLATZ7</i>	MD06G1035500	222	9.25	24.88	49.62	-0.698	Nucleus
<i>MdPLATZ8</i>	MD07G1117000	245	8.81	27.39	52.81	-0.189	Nucleus
<i>MdPLATZ9</i>	MD10G1229000	255	8.39	28.55	56.28	-0.408	Chloroplast. Nucleus
<i>MdPLATZ10</i>	MD11G1151300	212	9.3	24.31	68.23	-0.695	Nucleus
<i>MdPLATZ11</i>	MD12G1103000	197	8.88	22.04	59.31	-0.514	Nucleus
<i>MdPLATZ12</i>	MD13G1017800	214	9.5	24.71	46.55	-0.249	Nucleus
<i>MdPLATZ13</i>	MD14G1097400	197	8.52	22.00	64.2	-0.458	Nucleus
<i>MdPLATZ14</i>	MD15G1161500	255	8.75	29.10	62.78	-0.442	Nucleus
<i>MdPLATZ15</i>	MD16G1015800	227	9.02	25.76	46.5	-0.423	Nucleus
<i>MdPLATZ16</i>	MD16G1273400	226	9.33	25.26	48.58	-0.687	Nucleus
<i>MdPLATZ17</i>	MD17G1254800	147	8.24	17.23	43.3	-0.916	Nucleus



**Fig. 1** Chromosome location of apple *MdPLATZ* genes. The colors on the chromosomes represent the gene density. Blue: smaller gene density, red: greater gene density. Mb: chromosome length, 1 Mb = 1,000,000 bp. The apple chromosomes shown in Apple GDDH13\_v1.1 genome are of the 17 types chr01-17, however, there are 801 contigs assembled into one pseudomolecule that could not be assigned to a chromosome, which named Chr00

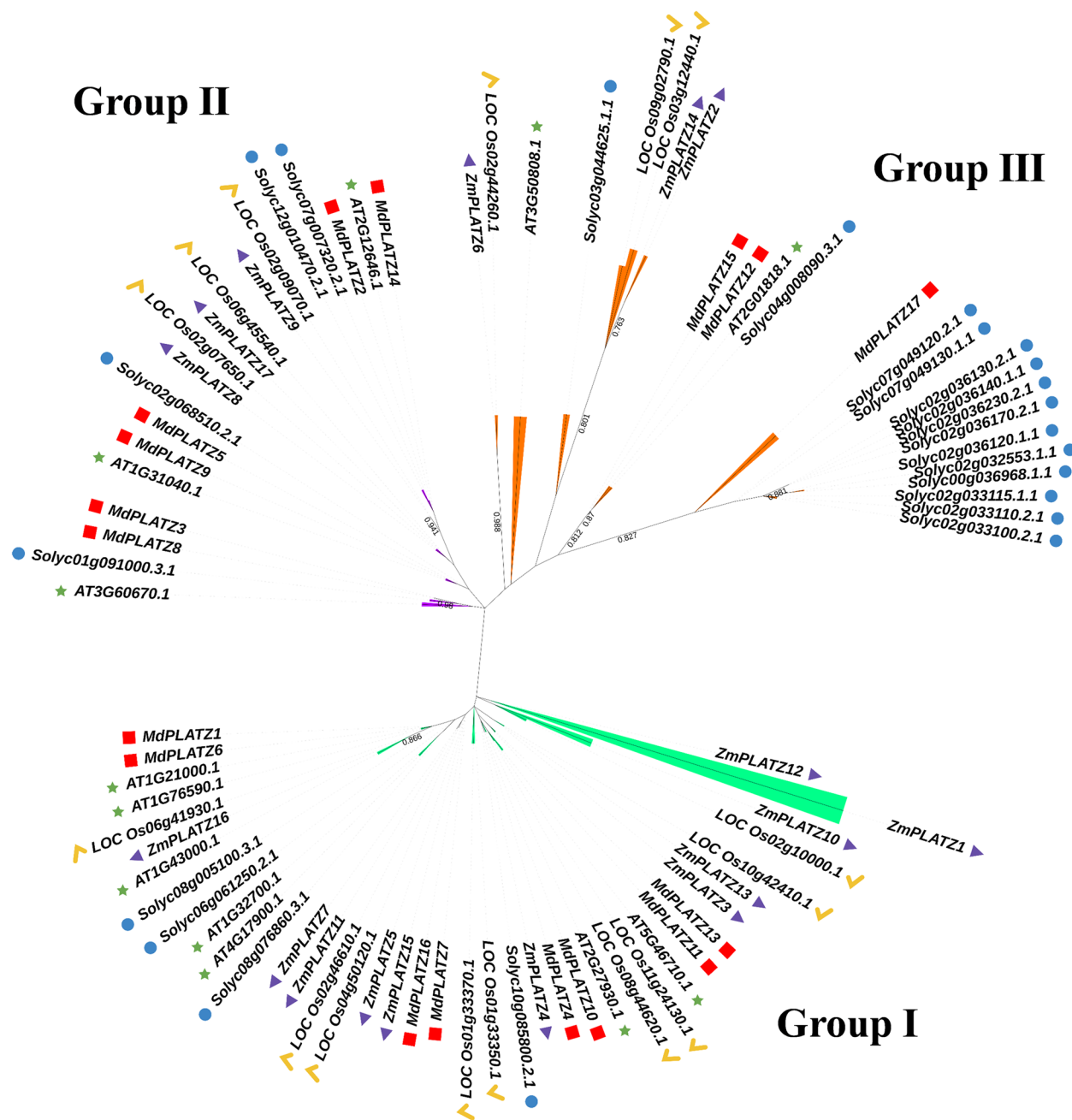
whereas Chr00, Chr03, Chr05, Chr07, Chr10, Chr11, Chr12, Chr13, Chr14, Chr15, and Chr17 each carried only one *MdPLATZ* gene.

#### Phylogenetic relationships and gene structure analysis of *MdPLATZ* family members

To explore the evolution of the *MdPLATZ* proteins, a phylogenetic tree of 83 *PLATZ* members, comprising those from apple (17 members), tomato (22 members), *Arabidopsis* (12 members), rice (15 members), and maize (17 members), was constructed (Fig. 2). The *PLATZ*s

proteins were classified into three subfamilies (Groups I to III) based on the topology of the phylogenetic tree. The *MdPLATZ* proteins were distributed in all three groups. Among the three groups, the apple *PLATZ* proteins were mainly aggregated in Groups I and II, suggesting that these *PLATZ* genes were conserved. *MdPLATZ17* was only clustered with tomato *PLATZ* proteins in Group III (Fig. 2). In addition, most apple *PLATZ*s shared sister clades with *Arabidopsis* or tomato *PLATZ*s.

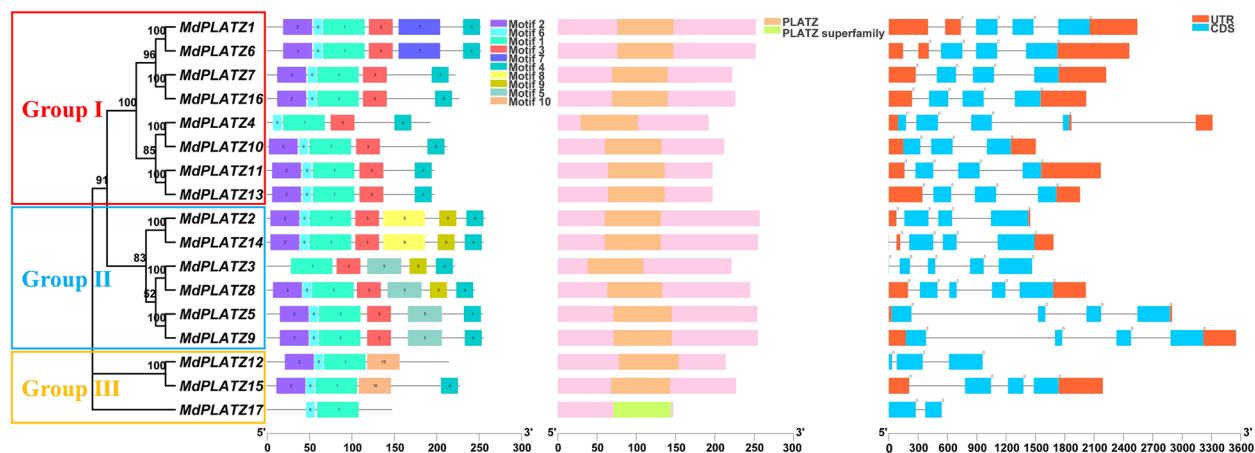
The 17 apple *MdPLATZ*s were clustered in Groups I to III (Fig. 3A). Protein structure analysis of the *MdPLATZ*



**Fig. 2** Phylogenetic tree of PLATZ genes from apple, tomato and *Arabidopsis*, rice, and maize. A red box, blue circle, green star, purple check, and brown triangle represent the PLATZ proteins from apple, tomato, *Arabidopsis*, rice, and maize, respectively. Groups I to III are indicated by different colors

family members identified ten regular motifs (Fig. 3B). All MdPLATZ proteins contained motif1, and most members also contained motif2, 4, and 6. Except for the MdPLATZ proteins of Group III, all proteins contained motif3. A total of six MdPLATZ proteins contained motif5, 8, and 9, which were only detected in the proteins of Group II. Only MdPLATZ12 and MdPLATZ15 (Group III) contained motif10. All MdPLATZ proteins contained

the PLATZ conserved domain (Fig. 3C). Two zinc finger domains, C-X2-H-X11-C-X2-C-X(4-5)-C-X2-C-X(3-7)-H-X2-H and C-X2-C-X(10-11)-C-X3-C), were present in the MdPLATZ primary amino acid sequence except in MdPLATZ3 and MdPLATZ4, which contained only the C-X2-C-X(10-11)-C-X3-C domain (Supplementary Fig. S2). The gene structures show that the *MdPLATZs* in Group I contain 4 CDS, except for *MdPLATZ10* which



**Fig. 3** Gene structure and conserved motif analyses of MdPLATZ family members. **A** Phylogenetic tree of MdPLATZ proteins. The numbers on the evolutionary tree represent the bootstrap values. The larger the value, the higher the credibility, while the smaller the value, the lower the credibility. **B** Distribution of conserved motifs in MdPLATZ proteins. Boxes of different colors represent the ten putative motifs. **C** Distribution of conserved domains in MdPLATZ proteins. **D** Exon/intron structure of MdPLATZ genes

has 3 CDS. The *MdPLATZs* of Group II mostly contain 4 CDS, and only *MdPLATZ3* and *MdPLATZ8* contain 5 CDS. The *MdPLATZs* of Group III contain more than 1 CDS. Except for *MdPLATZ17*, which contains 2 exons, all other *MdPLATZ* family members contain more than 2 exons (Fig. 3D). Only *MdPLATZ3*, *MdPLATZ12*, and *MdPLATZ17* did not contain an UTR (Fig. 3D).

#### Syntenic analysis of the *MdPLATZ* gene family

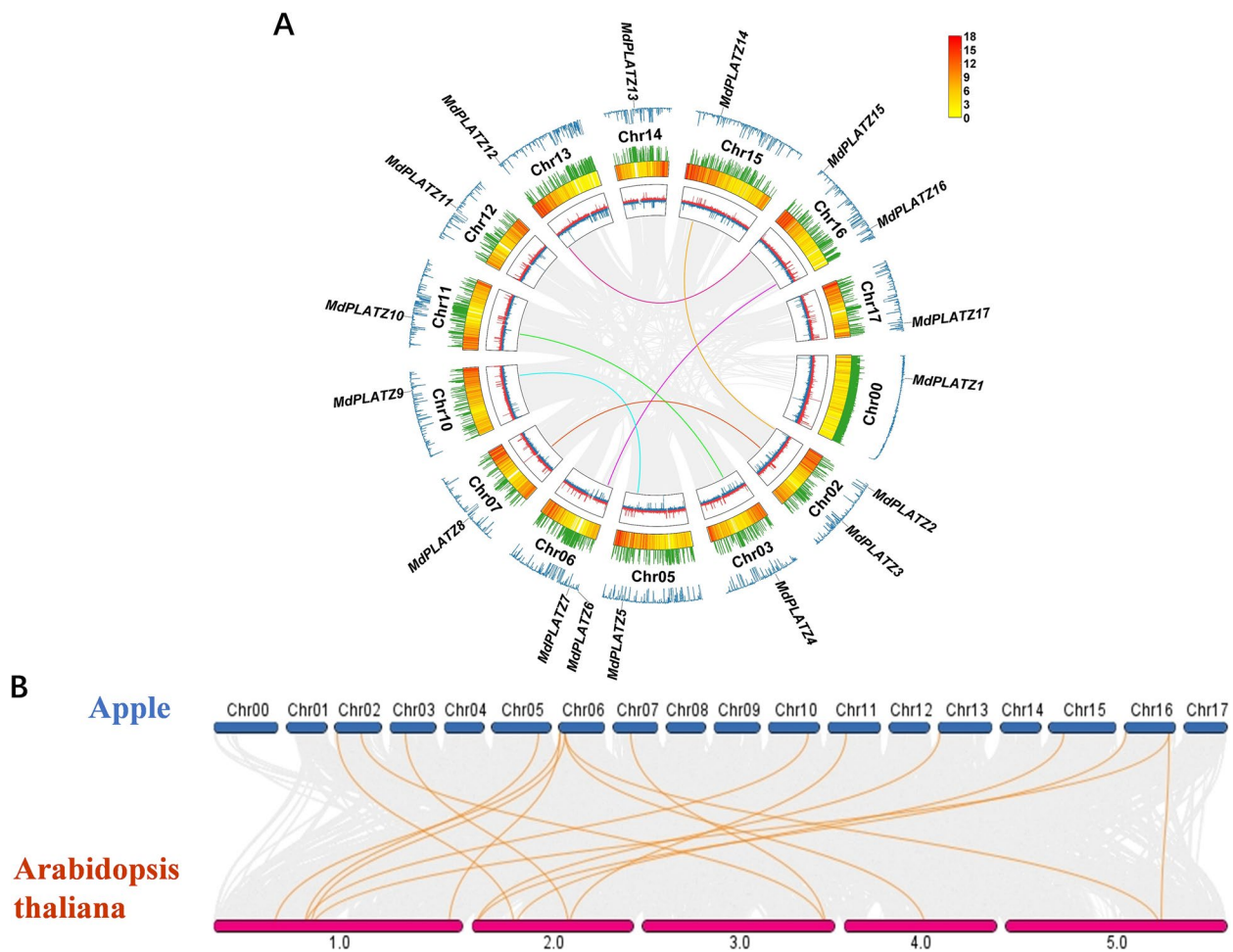
The 17 identified *MdPLATZ* genes were distributed on 14 of the 17 apple chromosomes. To analyze the evolution of the *MdPLATZ* gene family in apple, gene segmental and tandem duplication events were analyzed (Fig. 4A, Supplementary Table S3). Segmental duplications were identified as homologs on different chromosomes. Six pairs of segmental duplicates were detected among the 17 *MdPLATZ* genes: *MdPLATZ2/MdPLATZ14*, *MdPLATZ3/MdPLATZ8*, *MdPLATZ4/MdPLATZ10*, *MdPLATZ5/MdPLATZ9*, *MdPLATZ7/MdPLATZ16*, and *MdPLATZ12/MdPLATZ15*. No *MdPLATZ* genes were derived from tandem duplication events. The  $K_a/K_s$  ratio of the duplicated gene pairs ranged from 0.08 to 0.60 (less than 1) (Supplementary Table S4), suggesting that purifying selective pressure occurred during *MdPLATZ* gene family evolution and a conserved function may be shared among these genes. In addition, a comparative syntenic map between the apple and *Arabidopsis* genomes was constructed. As shown in Fig. 4B, 13 apple *MdPLATZ* genes were collinear with 10 *AtPLATZ* genes in *Arabidopsis*, suggesting that these orthologous pairs may be important for plant evolution.

#### Identification of *cis*-acting elements in the promoter of *MdPLATZ* genes

The 2 kb upstream of the genomic DNA sequence of the transcription start site (TSS) of the *MdPLATZ* genes was analyzed. As shown in Fig. 5, the number of *cis*-acting elements in the *MdPLATZ* genes ranged from 17 (*MdPLATZ5*) to 43 (*MdPLATZ11*). These included 219 photoresponsive elements, 124 hormone-responsive elements [to abscisic acid (48), auxin (12), gibberellin (12), methyl jasmonate (MeJA) (40), and salicylic acid (12)], 82 stress-responsive elements (to drought, low-temperature, and anaerobic stresses), and eight meristem expression-related elements (Fig. 5). All gene promoters contained a large number of light-responsive elements. In addition, abscisic acid-, MeJA-, and anaerobic- responsive elements were predicted to be present within the *MdPLATZ* promoter regions. In particular, several genes contained one or two hormone-related elements (auxin and gibberellin) in the promoter region. Moreover, six *MdPLATZ* gene promoters contained a meristem expression related element, suggesting that these *MdPLATZ* genes may play an important role in apple growth and development.

#### MicroRNA analysis of *MdPLATZ* genes

To investigate the post-transcriptional regulation of the *MdPLATZ* genes, the associated microRNAs of these genes were predicted and their stem-loops were visualized. As shown in Table 2, three microRNAs were predicted for each of *MdPLATZ5*, *MdPLATZ9*, *MdPLATZ12*, and *MdPLATZ15*. Two microRNAs were predicted for each of *MdPLATZ1* and *MdPLATZ10*. A single microRNA was predicted for *MdPLATZ3*.



**Fig. 4** Synteny analysis of *PLATZ* genes in apple and *Arabidopsis*. **A** Gene location, duplication, and synteny analysis of *MdPLATZ* genes. The gray curves indicate the collinear regions in the apple genome and the colored curves indicate the gene pairs that have undergone segmental duplication. From the inside out, the first circle is GC skew, the second circle is gene density, the third circle is N-ratio, and the fourth circle is GC ratio. The color scale represents the number of genes per 100,000 bin on a chromosome. Red represents a high number of genes, while yellow represents a low number of genes. **B** Synteny analysis of *PLATZ* genes between apple and *Arabidopsis*. The gray lines in the background indicate the syntenic blocks between species. The collinear gene pairs are linked with orange lines

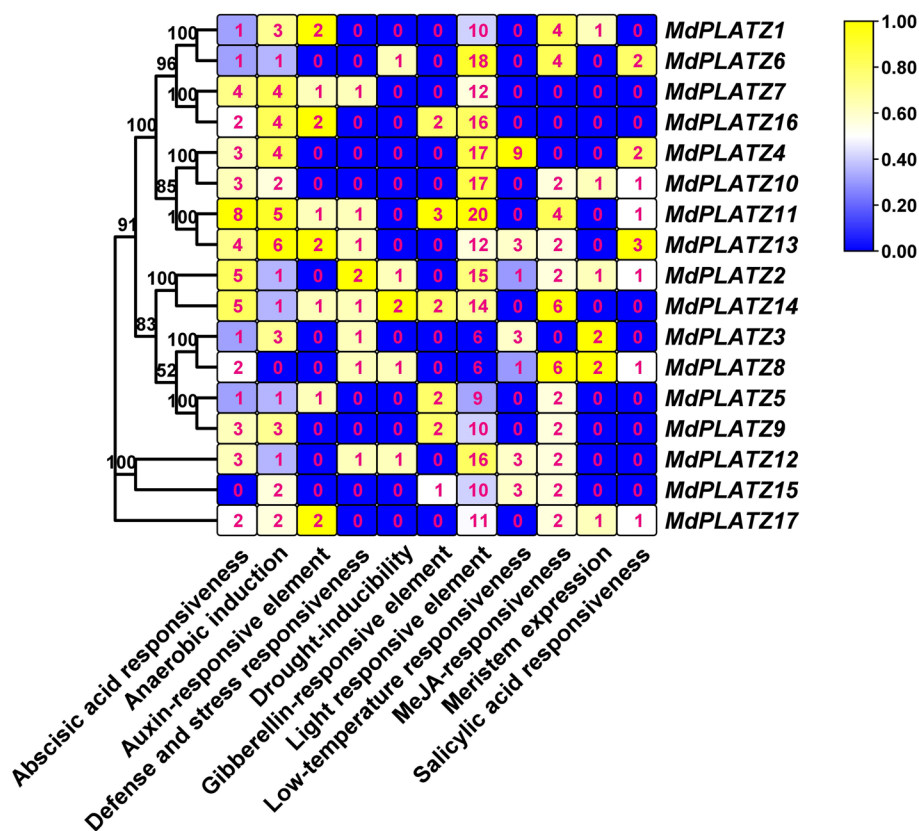
#### Expression patterns of *MdPLATZ* genes in different tissues and at different developmental stages

The expression patterns of *MdPLATZ* genes in different tissues and at different developmental stages were determined and analyzed based on the apple multidimensional omics (Apple MDO) database [30]. The *MdPLATZ* genes were considered to show no expression with FPKM value less than 1. As shown in Fig. 6, *MdPLATZ1*, *MdPLATZ6*, *MdPLATZ7*, and *MdPLATZ16* were expressed in all tissues analyzed except pollen. *MdPLATZ3*, *MdPLATZ5*, *MdPLATZ8*, and *MdPLATZ9* were highly expressed in buds. *MdPLATZ2* was barely expressed in all

tissues, *MdPLATZ12* was specifically expressed in pollen, *MdPLATZ14* was specifically expressed in fruit (1 week after flowering), *MdPLATZ17* was specifically expressed in dormant buds (1 month). The expression level of *MdPLATZ15* decreased gradually during fruit ripening.

#### Expression patterns of *MdPLATZ* genes in response to decapitation and TDZ treatment

To evaluate the putative roles of *MdPLATZ* genes in apple shoot branching, RNA-sequencing (RNA-seq) data obtained from the axillary buds after decapitation and TDZ treatment were analyzed. The expression of



**Fig. 5** Analysis of *cis*-acting elements in the promoter of *MdPLATZ* genes. The color scale represents  $\log_2$  transformed number of *cis*-acting elements. Yellow represents a high number of *cis*-acting elements. Blue represents a small number of *cis*-acting elements. The numbers in the colored boxes are the numbers of *cis*-acting elements present. The numbers on the evolutionary tree represent the bootstrap values

*MdPLATZ1*, *MdPLATZ6*, *MdPLATZ7*, and *MdPLATZ16* was downregulated in response to decapitation (Fig. 7A) and TDZ treatment (Fig. 7B), whereas *MdPLATZ8*, *MdPLATZ9*, and *MdPLATZ15* were upregulated at several times points in response to TDZ treatment (Fig. 7B). To further explore the regulation of the *MdPLATZ* genes in axillary bud outgrowth, qRT-PCR analysis was used to examine the expression of *MdPLATZ6* and *MdPLATZ15* in response to TDZ and decapitation treatments. The expression of *MdPLATZ6* was strongly downregulated in response to TDZ and decapitation at 12, 24, and 48 h, respectively (Fig. 8A). In contrast, the expression of *MdPLATZ15* was significantly upregulated in response to TDZ treatment at 4 h to 48 h; however, *MdPLATZ15* was significantly upregulated only at 4 h after decapitation (Fig. 8B).

#### Interaction network analysis of *MdPLATZ* proteins

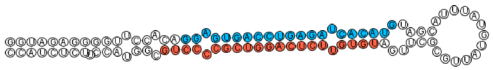
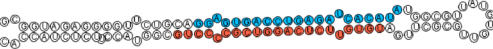
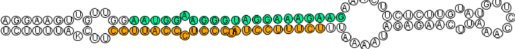
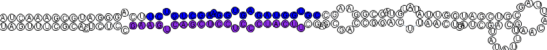
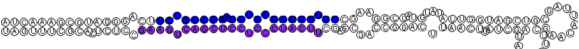
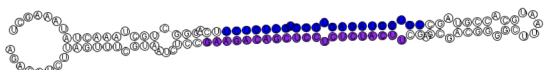

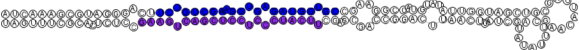
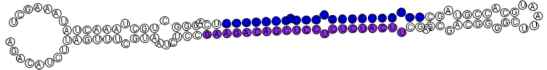


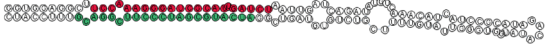




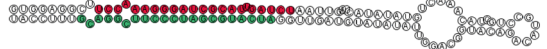
Protein–protein interaction (PPI) of seven *MdPLATZ* proteins (*MdPLATZ1*, *MdPLATZ6*, *MdPLATZ7*, *MdPLATZ8*, *MdPLATZ9*, *MdPLATZ15*, and *MdPLATZ16*) that may

be associated with bud outgrowth was predicted and analyzed. The seven *MdPLATZ*s were predicted to interact with 234 co-expressed proteins, including members of the bZIP, MYB, TCP, and NAC TF families (Fig. 9A, Supplementary Table S5). Additionally, *MdPLATZ8* and *MdPLATZ9* were significantly correlated with GRF genes (Supplementary Table S5). Gene ontology (GO) showed that the co-expressed proteins contained 20 GO categories, which belonged to cellular component, biological process and molecular function (Fig. 9B). In addition, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis indicated that many co-expressed proteins were enriched into the plant hormone signal transduction, the photosynthetic carbon metabolism, and sugar metabolism pathways (Fig. 9C).

#### Discussion

In recent years, genome-wide analysis of the *PLATZ* gene family in numerous plant species has been conducted. The genes perform different functions in plant growth and development, and response to stress. In

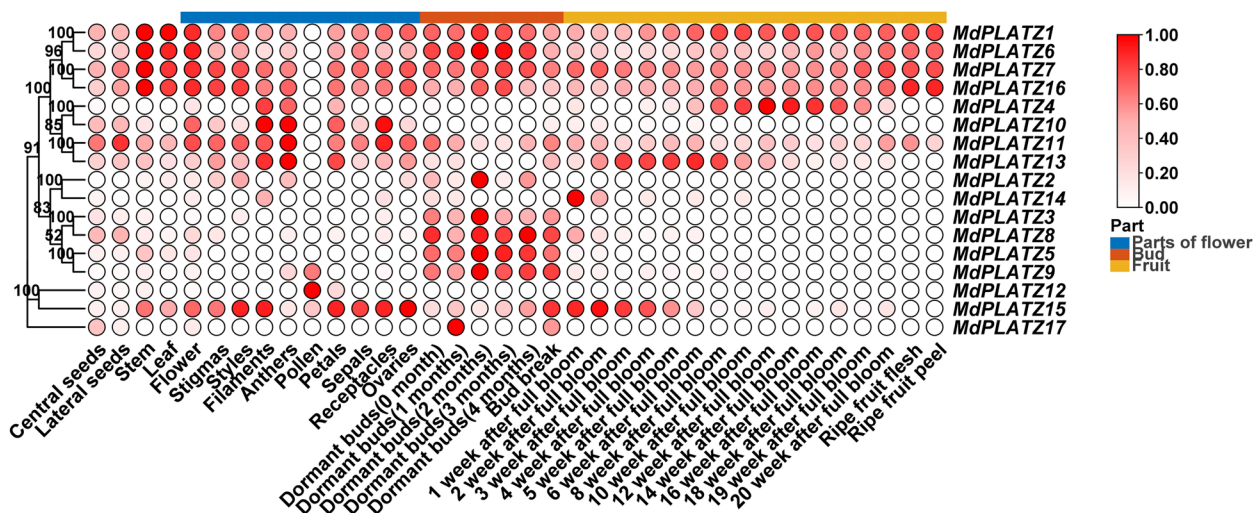
**Table 2** Information on the related microRNAs predicted for *MdPLATZ* genes

Gene name	MicroRNA name	Mature sequence	Stem-loop
<i>MdPLATZ1</i>	miR398b	UGUGUUCUCAGGUCGCCCCUG	
	miR398c	UGUGUUCUCAGGUCGCCCCUG	
<i>MdPLATZ3</i>	miR482d	AAUGGAAGGGUAGGAAAGAAG	
<i>MdPLATZ5</i>	miR5225a	UCUGUCGAAGGUGAGAUGGUGC	
	miR5225b	UCUGUCGAAGGUGAGAUGGUGC	
	miR5225c	UCUGUCGUGGGUGAGAUGGUGC	
<i>MdPLATZ9</i>	miR5225a	UCUGUCGAAGGUGAGAUGGUGC	
	miR5225b	UCUGUCGAAGGUGAGAUGGUGC	
	miR5225c	UCUGUCGUGGGUGAGAUGGUGC	
<i>MdPLATZ10</i>	miR7123a	AAGAGCGGAUGUGUAAAAGG	
	miR7123b	AAGAGCGGAUGUGUAAAAGG	
<i>MdPLATZ12</i>	miR393d	AUCAUGCGAUCCCUUCGGACG	
	miR393e	AUCAUGCGAUCCCUUCGGACG	
	miR393f	AUCAUGCGAUCCCUUCGGACG	
<i>MdPLATZ15</i>	miR393d	AUCAUGCGAUCCCUUCGGACG	
	miR393e	AUCAUGCGAUCCCUUCGGACG	
	miR393f	AUCAUGCGAUCCCUUCGGACG	

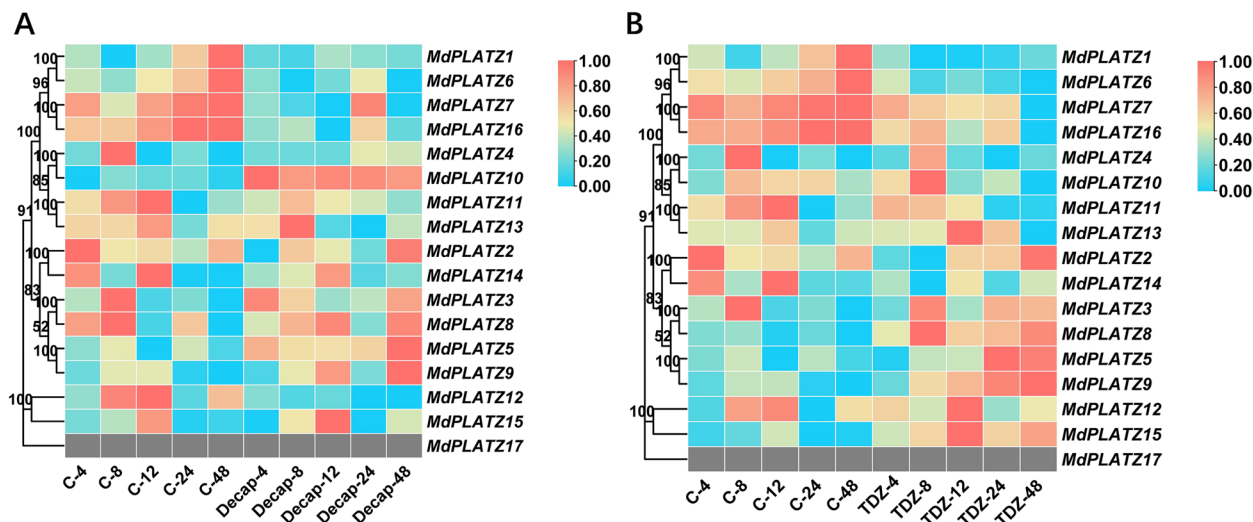
this study, 17 *MdPLATZ* family members in apple were identified, which is more than identified in watermelon (10), *Arabidopsis* (12), and rice (15). Thus, the *PLATZ* family members have expanded in the apple genome during evolution. The identification and chromosome locations of 17 *MdPLATZ* genes were consistent with Sun's research [7], but with a difference in naming methods of *MdPLATZ* genes. Generally, TFs are located in

the cytoplasm and are transported to the nucleus, where they interact with *cis*-acting elements after receiving cell membrane signal transduction signals [31]. In this study, the predicted subcellular localization of *MdPLATZ* proteins was mostly in the nucleus, except for *MdPLATZ5* (predicted to be localized to chloroplasts). The proteins that were localized in the nucleus had at least one signal peptide, except *MdPLATZ17*. This indicated that





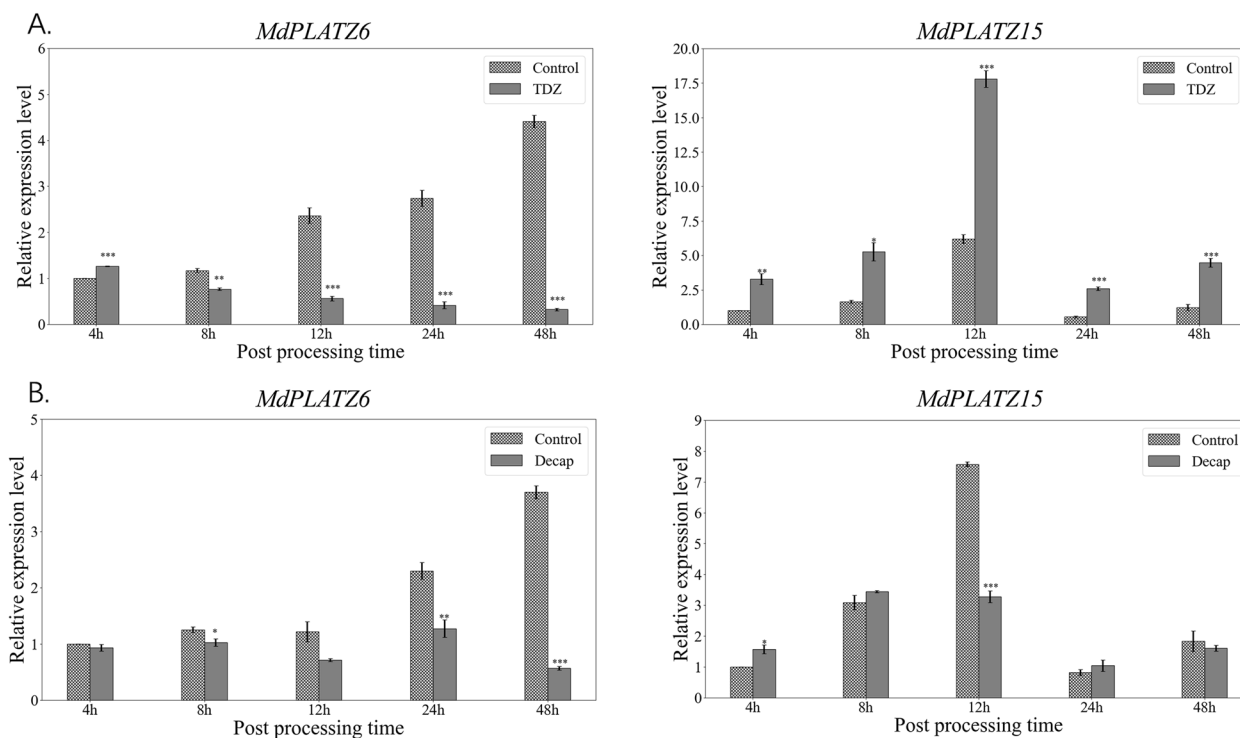
**Fig. 6** Hierarchical clustering of the expression level of apple *MdPLATZ* genes in different tissues and at different developmental stages. Transcriptome data were used to estimate the relative expression level of each gene. The heatmap shows expression patterns of *MdPLATZ* genes at different developmental stages and in different tissues. The color scale represents  $\log_2$  transformed FPKM values; FPKM: a fragments per kilobase of transcript per million mapped reads. Deep red indicates a high expression level and light red indicates a low expression level. The numbers on the evolutionary tree represent the bootstrap values



**Fig. 7** Hierarchical clustering of the expression profiles of *MdPLATZ* genes in transcriptome data for apple axillary buds after decapitation (A) and TDZ (B) treatments. “C-” represents the untreated control; “Decap-” represents the decapitation treatment. “4, 8, 12, 24, and 48” indicate the number of hours after treatment. The color scale represents  $\log_2$  transformed FPKM values. Red indicates a high expression level and blue indicates a low expression level; grey represents no expression detected. The numbers on the evolutionary tree represent the bootstrap values

*MdPLATZ17* may bind to another protein and be transported into the nucleus. To determine the relative chromosomal positions of the *MdPLATZ* genes, we mapped the distribution of the 17 *MdPLATZ* genes onto the individual apple chromosomes. The results revealed that the *MdPLATZ* genes were unevenly distributed on 14 chromosomes. According to previous studies, chromosomes 3 and 11, 5 and 10, 9 and 17, and 13 and 16

of apple are mainly derived from the common ancestor [32]. In the present study, *MdPLATZ4/MdPLATZ10*, *MdPLATZ5/MdPLATZ9*, *MdPLATZ3/MdPLATZ10*, and *MdPLATZ12/MdPLATZ15* were located on chr03/chr11, chr05/chr10, chr04/chr11, and chr13/chr16, respectively, and at almost identical positions. This finding indicates that the chromosomal distribution of the genes may reflect the evolution of the species.

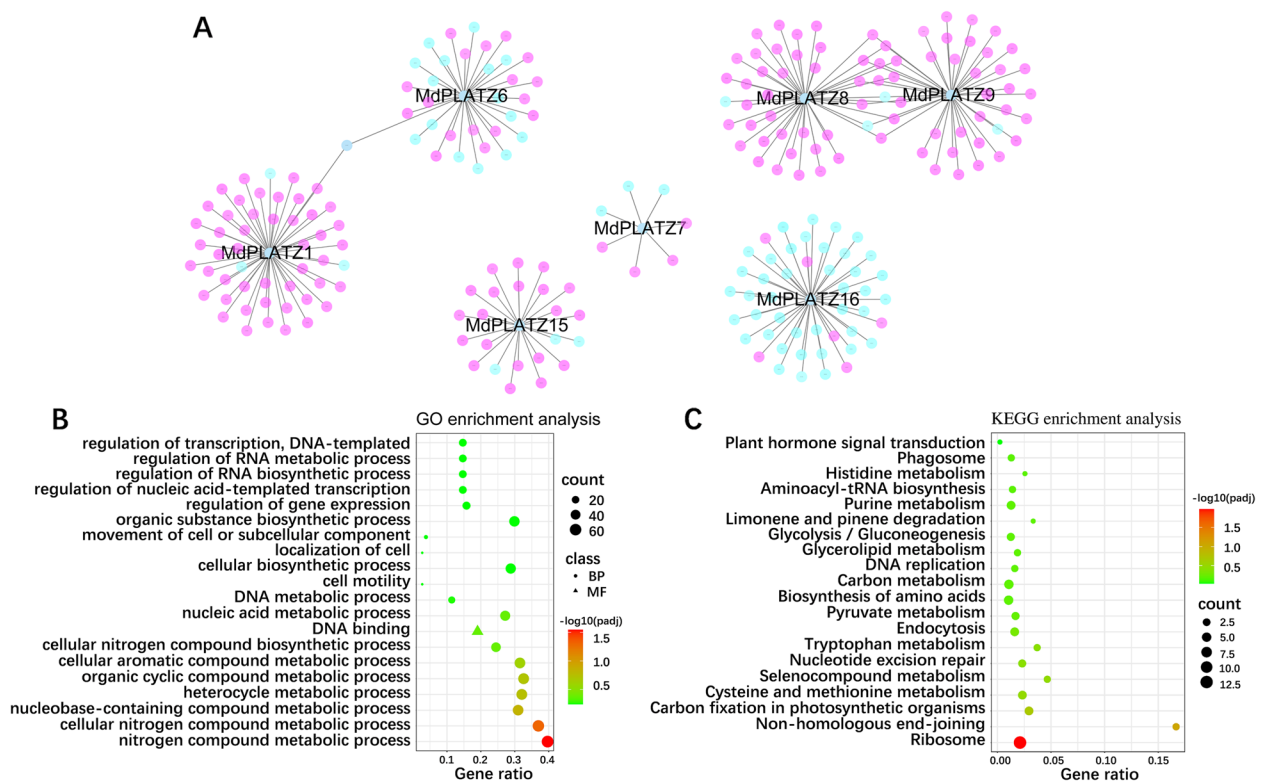


**Fig. 8** Expression patterns of *MdPLATZ6* (A) and *MdPLATZ15* (B) in axillary buds after TDZ and decapitation treatment. The relative expression levels of *MdPLATZ6* and *MdPLATZ15* were determined by qRT-PCR analysis. Error bars represent the SD of three biological replicates. Data are the mean ( $\pm$  SD),  $n=3$ . \*  $P < 0.05$ ; \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

Sequence comparison and phylogenetic analysis of the PLATZ proteins of apple, tomato, *Arabidopsis*, rice, and maize was conducted. The phylogenetic tree resolved into three groups, and apple PLATZs were mainly clustered with *Arabidopsis* or tomato PLATZs, suggesting that the PLATZ genes in dicotyledons had a closer evolutionary relationship. In the previous research, the phylogenetic tree based on the PLATZ protein sequences of *Malus* species, *Fragaria vesca* v4.0, *Prunus persica*, *Pyrus communis* L., *A. thaliana*, and *Oryza sativa* was constructed by the maximum likelihood method, and categorized into seven groups [7]. However, it implied that PLATZs in apple were more closely related to AtPLATZs rather than OsPLATZs [7], which was consistent with our study. Each group of genes shared similar motifs and gene structures, and may perform similar biological functions. Fifteen *MdPLATZ* genes contained two distant conserved zinc finger regions, which comprised cysteine and histidine residues. The N-terminal region of the genes resembled a double zinc finger region [4], such as C3H4 or C2H5 [33]. The N-terminus of the remaining two *MdPLATZ* genes may exhibit conserved regional diversity owing to nucleotide substitutions and minor deletions [34]. Fragment replication and tandem replication are the main factors that affect the expansion of gene

families and are closely associated with genetic evolution in plant genomes [35]. Therefore, we performed a synteny analysis on the *PLATZ* gene family of apple alone, and those of apple and *Arabidopsis*. This may be associated with the duplication of the apple genome, which accounts for the increased number of gene family members [36]. There are six gene pairs that exhibit fragment duplication in apple, which was consistent with previous study in apple [7]. These genes may have the same biological functions and may prevent gene loss-of-function caused by gene mutations and deletions [37]. Additionally, a comparative syntenic map between the apple and *Arabidopsis* genomes was constructed in the present study, which was not analyzed in Sun’s research [7]. Syntenic relationships were observed for the 10 *Arabidopsis* *PLATZ* genes and 13 apple *PLATZ* genes. Using this comparative genomic analysis, the function of some *PLATZ* genes may be predicted based on their homologs.

Expression of genes is regulated by *cis*-acting elements. The *cis*-acting elements of *MdPLATZ* genes related to drought stress have been analyzed in apple [7]. In the current study, the *cis*-acting elements of *MdPLATZs* containing a variety of elements responsive to plant hormones, such as auxin, abscisic acid, and gibberellin, and other hormone-responsive elements that were reported



**Fig. 9** Co-expression network of MdPLATZ genes in apple. **A** Protein-protein interaction networks of seven MdPLATZ proteins that may be associated with axillary bud growth. Pink represents positive interaction, blue represents negative interaction. **B, C** GO and KEGG pathway enrichment analysis of MdPLATZ proteins and their co-expressed proteins. “count” represents the number of genes contained in the pathway. “class” represents the classification of pathways. BP: Biological process; MF: Molecular function. Color scale represents  $-\log_{10}$  transformed corrected p-value

to associate with bud outgrowth were focused. It has been shown that PLATZ TFs play a role in regulating cell proliferation and division [38], indicating that they may participate in apple branch development. MicroRNAs are a type of endogenous non-coding small RNA molecules that exist widely among plants [39]. They inhibit the expression of target genes at the post-transcriptional level by binding to the target-gene mRNA [40]. In the present study, we observed that microRNA393, 398, 482, 5225, and 7123 target several *MdPLATZ* genes. Previous studies have reported that miR393 is involved in the regulation of plant taproot and root-cap growth [41], tillering, and response to biotic [42] and abiotic stresses [43]. The miR398 participates in plant oxidative stress response [44]. The miR482 increases the scavenging capacity of reactive oxygen species, promotes the accumulation of proline and other regulatory substances, and improves the salt and drought tolerance of plants. The expression of *PLATZ* genes is tissue-specific in plant. For example, pea *PLATZ* genes are specifically expressed in root tips and apical buds [3]. Cotton *PLATZ* transcripts are more abundant in the root, stem, and cotyledon, but lower in the leaf and seed [45]. *PLATZ* genes are preferentially

expressed in the ear of rice at the early stages of development [10]. Similarly, in the current study, it was observed that *MdPLATZ1*, *MdPLATZ6*, *MdPLATZ7*, and *MdPLATZ16* were expressed in all organs analyzed except pollen. These genes may be important for maintaining the growth and development of apple. The expression level of *MdPLATZ15* gradually decreased with fruit ripening, which may be associated with fruit hardness. The *MdPLATZ3*, *MdPLATZ5*, *MdPLATZ8*, and *MdPLATZ9* genes were highly expressed in the bud.

The *PLATZ* gene family has been identified in Rosaceae species including apple, and provided evidences that *PLATZ* genes are involved in drought stress [7], which was consistent with previous studies [46–48]. However, in this study, we focused on the functional research of *MdPLATZ* genes in the control of plant growth and development, especially for axillary bud outgrowth, which has rarely been reported. It has been reported that the removal of plant apical buds can reduce the synthesis of auxin, thus reducing the strength of apical dominance and promoting the outgrowth of lateral buds [49]. TDZ has strong CK activity and can directly promote the outgrowth of plant axillary buds [50]. In

apple, decapitation and exogenous CK treatment can significantly induce axillary bud outgrowth [51]. Hence, screening and analysis of the expression of *MdPLATZ* family members in transcriptome data for axillary buds after decapitation and TDZ treatment were performed. Seven *MdPLATZ* genes (*MdPLATZ1*, *MdPLATZ6*, *MdPLATZ7*, *MdPLATZ16*, *MdPLATZ8*, *MdPLATZ9*, and *MdPLATZ15*) were identified to associated with axillary bud outgrowth. Two genes (*MdPLATZ6* and *MdPLATZ15*) were selected for qRT-PCR analysis, and the results were similar to the transcriptome data which preliminarily verified their functions in axillary bud outgrowth, laying a foundation for further in-depth research.

The *MdPLATZ* co-expressed genes mainly involved in drought stress were analyzed in apple [7]. In this study, the protein co-expression network was predicted using the selected seven *MdPLATZ* proteins according to Fig. 7, and their co-expressing genes which were reported to associated with axillary bud outgrowth were concerned. These include bZIP, MYB, NAC, and TCP TFs. According to previous studies, *Chrysanthemum CmbZIP1* is mainly expressed in apical and axillary buds, and the number of branches in transgenic *Arabidopsis* overexpressing *CmbZIP1* is decreased compared with that of the wild type [52]. In addition, *ELONGATED HYPOCOTYL 5* of the bZIP TF family in *Arabidopsis* can significantly increase the number of branches [53]. In rice, *MYB* transcription factors are involved in the regulation of tillering [54], among which the *RAX* gene encodes R2R3-MYB TFs, which are involved in the regulation of the growth of tillering buds [55]. The tomato *Blind* gene, which is homologous to *RAX*, also affects the number of lateral buds [56]. In *Arabidopsis*, three *CUP-SHAPED COTYLEDON* genes encode NAC TFs. These three TFs affect the formation of lateral buds in *Arabidopsis* through functional redundancy [57, 58]. *BRANCHED1 (BRC1)/TEOSINTE BRANCHED 1 (TB1)*, a member of class II TB1 CYCLOIDEA PCF (TCP) type TFs is considered to be a repressor of branching [59]. *MdPLATZ1*, down-regulated in response to decapitation and TDZ treatment (Fig. 7), was predicted to positively interact with BRC1, indicating that the role of *MdPLATZ1* in axillary bud outgrowth is worth further investigation. *MdPLATZ8* and *MdPLATZ9* were significantly correlated with GRF genes, which were observed to modulates plant architecture [60]. Our previous study showed that apple bud outgrowth is correlated with the expression of cytokinin biosynthetic genes (isopentenyl transferase, IPT) [25]. *MdPLATZ6* was predicted to negatively interact with Isopentenyl Transferase 9 (IPT9), which was belong to cytokinin biosynthetic genes and was associated with apple bud outgrowth. Additionally, a number

of *MdPLATZ8* and *MdPLATZ9* co-expressed genes were involved in auxin pathway, such as PIN6, PIN1, ARF2, and YUCCA4, suggesting that *MdPLATZ8* and *MdPLATZ9* may mediate auxin to regulate axillary bud outgrowth.

## Conclusions

This study provides identification and bioinformatic analysis of the *MdPLATZ* gene family in apple. Based on the expression level in the transcriptome of axillary buds, two genes, *MdPLATZ6* and *MdPLATZ15*, were selected that may be associated with axillary bud outgrowth in apple. Through co-expression network analysis, it was demonstrated that multiple branching-related genes, including BRC1, GRF, and MYB, might be regulated by *MdPLATZ*s. Moreover, enrichment analysis showed that *MdPLATZ* genes may regulate plant axillary bud outgrowth through CK or auxin pathway. The results lay a foundation for further research on the functions and mechanisms of *MdPLATZ* family members in axillary bud outgrowth.

## Materials and methods

### Identification and characterizations of *MdPLATZ* genes in apple

All apple protein sequences were downloaded from the Genome Database for Rosaceae (GDR; [https://www.rosaceae.org/species/malus/malus\\_x\\_domestica/genome\\_GDDH13\\_v1.1](https://www.rosaceae.org/species/malus/malus_x_domestica/genome_GDDH13_v1.1)). The PLATZ domain (Pfam:PF04640.17) was downloaded from the Pfam database (<http://pfam-legacy.xfam.org/>) to filter the putative apple PLATZ protein sequences using TBtools. All of the obtained putative apple PLATZ amino acid sequences were submitted to the Conserved Domain Database of the NCBI (<http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>) to identify the presence of domain signatures. The 17 putative *MdPLATZ* genes were designated *MdPLATZ1* to *MdPLATZ17* according to their chromosomal locations.

Characterization of the encoded amino acids (aa), theoretical isoelectric point (pI), molecular weight (MW), instability index, and grand average hydropathicity of the apple *MdPLATZ* genes were calculated using ExPASy (<https://web.expasy.org/protparam/>). The subcellular localization of the proteins was predicted with Plant-mPLOC (<http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/#>). Nuclear signal peptides were predicted using INSP (<http://www.csbio.sjtu.edu.cn/bioinf/INSP/>). The secondary and tertiary structures of the PLATZ proteins were predicted using SOPMA ([https://npsa-prabi.ibcp.fr/cgi-bin/npsa\\_automat.pl?page=npsa\\_sopma.html](https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html)) and Phyre2 (<http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index>), respectively.

### Sequence alignment, phylogenetic relationships and gene structure analysis

The amino acid sequences of the MdPLATZ proteins were aligned using DNAMAN software. The sequence logos were obtained with the WebLogo tool (<https://weblogo.threeplusone.com/create.cgi>). The conserved domain sequences of PLATZ proteins of apple, *Arabidopsis*, tomato, rice, and maize were aligned using ClustalW. A phylogenetic tree was constructed in MEGA7 using the Neighbor-Joining (NJ) method with 1000 bootstrap replicates, and visualized and adjusted with ITOL (<https://itol.embl.de/>).

The conserved motifs of MdPLATZ genes were identified with the MEME suite (<http://meme-suite.org/>), and visualized with TBtools software. Gene structure (introns and exons) analysis of the MdPLATZ genes was conducted with TBtools.

### Chromosomal location and synteny analysis of the MdPLATZ genes

Information on the physical location of the MdPLATZ genes was obtained from the apple genome annotation gff3 format file and visualized with TBtools, which detected and visualized tandem duplication events.

The syntenic map was constructed using MCScanX and was visualized with TBtools. The non-synonymous ( $K_a$ ) and synonymous ( $K_s$ ) substitution rates of the collinear gene pairs were estimated using TBtools to predict the divergence time ( $t$ ) and evolutionary rate ( $K_a/K_s$  ratio).

### Analysis of cis-acting elements in the MdPLATZ genes

The 2,000 bp sequence upstream from the 5'-end of the MdPLATZ genes were extracted as the promoter region and used for cis-acting elements analysis with the PlantCare database (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>), and visualized with TBtools.

### Expression patterns of MdPLATZ genes in different tissues of apple

We obtained a total of 48 expression profiles of the 17 MdPLATZ genes from the Apple Multi-Dimensional Omics Database (<http://bioinformatics.cau.edu.cn/AppleMDO/>). These profiles included 36 tissues and different developmental stages [central seed, lateral seed, stem, leaf, flower, petal, stigma, style, ovary, anther, filament, sepal, receptacle, pollen, four dormant bud stages, break bud, 14 fruit developmental stages from 1 week after full-bloom (WAF1) to harvest (WAF20), ripe fruit skin, and fruit flesh]. In this database, all RNA-seq data had been

quality controlled and the FPKM values were extracted. Expression heatmaps were generated with TBtools.

### Prediction of microRNAs associated with MdPLATZ and interaction network analysis

The microRNAs associated with the MdPLATZ genes were predicted using psRNATarget (<https://www.zhaolab.org/psRNATarget/>). The query Stem-loop and mature sequences were identified in the miRBase database (<https://www.mirbase.org/>) and plotted with sRNAMiner software.

The interaction network for MdPLATZ family members was predicted using the Apple Multi-Dimensional Omics Database (<http://bioinformatics.cau.edu.cn/AppleMDO/>) and adjusted using Cytoscape software. The GO enrichment analysis, using the GSeq method, and KEGG pathway [61–63] enrichment analysis were conducted using KOBAS 2.0.

### Plant materials and treatments

One-year-old apple SH40 scions grafted onto *Malus robusta* Rehd. rootstocks were grown at the experimental nursery in Xiaochen village, Xiaochen Township, Li County, Hebei Province, China. The plants were cultivated in the nursery using a spacing of 50 cm × 25 cm. The following steps were used in the propagation and cultivation of the apple trees. First, seeds of *M. robusta* Rehd. were sown in spring, 2020 and then SH40 buds were grafted onto the rootstocks at approximately 20 cm above ground level in autumn, 2020. Second, the rootstock portion located above the SH40 bud was excised in spring, 2021; after pruning, only the SH40 buds were allowed to grow during the growing period. The one-year-old SH40/*M. robusta* Rehd. grafted combinations were used in the experiments in July, 2021. The plant materials were subjected to standard management practices.

The decapitation and exogenous TDZ treatments were executed on the grafted apple trees when the terminal shoot had attained 70–80 cm in length (during July, 2021), as measured from the grafting point to the tip of the main shoot. Eight full axillary buds were marked continuously in the apical sections of newly developing scion shoots. Exogenous TDZ solution (5 mmol/L) supplemented with 0.05% DIMETHYL SULFOXIDE (DMSO) was applied to the marked section of the scion (containing the eight axillary buds) as a single spray application. The solution was applied by hand using a 500 mL Solo Snazy pressurized hand sprayer. For the decapitation treatment, the approximately 2 cm apical portion of the terminal shoot was decapitated. Each treatment comprised three biological replicates, with 15 plants per replicate. Axillary buds were sampled at 4, 8, 12, 24, and 48 h

after treatment. All sampled materials were immediately frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until further use. The transcriptome was sequenced by Tianjin Nuohe Zhiyuan Bioinformation Technology Co., Ltd.

### Total RNA extraction and qRT-PCR analysis

Total RNA was extracted using the RNA Plant Plus Reagent Kit (Tiangen, Beijing, China) in accordance with the manufacturer's instructions. The cDNA was synthesized with the EasyScript<sup>®</sup> One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen, Beijing, China) following the manufacturer's instructions. The qRT-PCR assays were performed with the SYBR Green PCR Master Mix on a LightCycler<sup>®</sup> 96 system (Roche, Basel, Switzerland). All reactions included 12.5  $\mu\text{L}$  of  $2\times$  SYBR Premix Ex Taq II (Accurate Biotechnology, Hunan, China), 1.0  $\mu\text{L}$  cDNA template, 0.5  $\mu\text{L}$  forward and reverse primers, and 10.5  $\mu\text{L}$  ddH<sub>2</sub>O, made up to a 25  $\mu\text{L}$  volume. The thermal-cycling protocol consisted of  $95^{\circ}\text{C}$  for 30 s, followed by 40 cycles of  $95^{\circ}\text{C}$  for 5 s and  $60^{\circ}\text{C}$  for 30 s, followed by melting curve analysis. Quantitative estimation of gene expression was calculated using the  $2^{-\Delta\Delta\text{Ct}}$  method [64]. The *MdACTIN* gene served as a reference gene. All primers used in the qRT-PCR analyses were designed using Primer Premier 6.0 software. Primer information presented in Supplementary Table S1.

### Statistical analysis

Statistical analysis of qRT-PCR data was performed in Microsoft Excel. Significant differences in the data were analyzed using the Statistical Program for Social Science 18 (SPSS, Chicago, IL, USA). Differences in values were considered statistically significant at (\*  $P < 0.05$ ; \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ) level according to independent samples t-test. Figures were generated by Python.

### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12864-023-09399-x>.

**Additional file 1: Supplementary Table S1.** Primers used for qRT-PCR. **Supplementary Table S2.** Information of the secondary protein structures of apple MdPLATZs. **Supplementary Table S3.** Collinear gene pairs of the apple MdPLATZ gene family. **Supplementary Table S4.** Ka and Ks analysis of PLATZ genes in apple and Arabidopsis. **Supplementary Table S5.** The candidate genes co-expressed with MdPLATZs in apple. **Fig. S1.** Predicted tertiary protein structures of MdPLATZ proteins. Percentages represent credibility. **Fig. S2.** Multiple sequence alignments of MdPLATZ proteins.

### Acknowledgements

We thank Liwen Bianji (Edanz) ([www.liwenbianji.cn](http://www.liwenbianji.cn)) for editing the English text of a draft of this manuscript.

### Authors' contributions

JY L, GF L, M T and JZ S participated in the experimental design and data analysis. JY L, GF L, YH Z (Yaohui Zhang) and YL Z performed material sampling and the laboratory data measurement. JY L, M T, YH Z (Yuhang Zhang), F Y, ZC H, LJ H and JZ S participated in the paper writing and manuscript amend. All authors have read and agreed to the published version of the manuscript.

### Funding

This work was supported by the National Natural Science Foundation of China (32002068); the Natural Science Foundation of Hebei Province (C2021204172), and Hebei Agricultural University Talent Introduction Scientific Research Special Project (YJ2020034, YJ2020053).

### Availability of data and materials

All data generated during this study are included in this published article [and its supplementary information files].

### Declarations

#### Ethics approval and consent to participate

Not applicable. Experimental research and field studies on plants including the collection of plant material are comply with relevant guidelines and regulation.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare no competing interests.

Received: 29 January 2023 Accepted: 23 May 2023

Published online: 15 June 2023

### References

- Riechmann JL, Heard J, Martin G, Reuber L, Jiang CZ, Keddie J, Adam L, Pineda O, Ratcliffe OJ, Samaha RR, Creelman 8R, Pilgrim M, Broun P, Zhang JZ, Ghandehari D, Sherman BK, Yu CL. Arabidopsis transcription factors: Genome-wide comparative analysis among Eukaryotes. *Science*. 2000;290(5499):2105–10.
- Singh KB, Foley RC, Onate-Sanchez L. Transcription factors in plant defense and stress responses. *Curr Opin Plant Biol*. 2002;5(5):430–6.
- Nagano Y, Furuhashi H, Inaba T, Sasaki Y. A novel class of plant-specific zinc-dependent DNA-binding protein that binds to A/T-rich DNA sequences. *Nucleic Acids Res*. 2001;29(20):4097–105.
- Azim JB, Khan Md, Hassan L, Robin AHK. Genome-wide characterization and expression profiling of plant-specific PLATZ transcription factor family genes in Brassica rapa L. *Plant Breed Biotechnol*. 2020;8(1):28–45.
- Wang JC, Ji C, Li Q, Zhou Y, Wu YR. Genome-wide analysis of the plant-specific PLATZ proteins in maize and identification of their general role in interaction with RNA polymerase III complex. *BMC Plant Biol*. 2018;18(1):211.
- Fu YX, Cheng MP, Li ML, Guo XJ, Wu YR, Wang JR. Identification and characterization of PLATZ transcription factors in Wheat. *Int J Mol Sci*. 2020;21(23):8934.
- Sun Y, Liu Y, Liang J, Luo J, Yang F, Feng P, Wang H, Guo B, Ma F, Zhao T. Identification of PLATZ genes in Malus and expression characteristics of MdPLATZs in response to drought and ABA stresses. *Front Plant Sci*. 2023;13:1109784.
- Huang DQ, Wu WR, Abrams SR, Cutler AJ. The relationship of drought-related gene expression in Arabidopsis thaliana to hormonal and environmental factors. *J Exp Bot*. 2008;59(11):2991–3007.
- Li Q, Wang JC, Ye JW, Zheng XX, Xiang XL, Li CS, Fu MM, Wang Q, Zhang ZY, Wu YR. The maize imprinted gene Floury3 encodes a PLATZ protein required for tRNA and 5S rRNA transcription through interaction with RNA polymerase III. *Plant Cell*. 2017;29(10):2661–75.
- Wang AH, Hou QQ, Si LZ, Huang XH, Luo JH, Lu DF, Zhu JJ, Shangguan YY, Miao JS, Xie YF, Wang YC, Zhao Q, Feng Q, Zhou CC, Li Y, Fan DL, Lu YQ,

- Tian QL, Wang ZX, Han B. The PLATZ transcription factor GL6 affects grain length and number in rice. *Plant Physiol.* 2019;180(4):2077–90.
11. Gonzalez-Morales SI, Chavez-Montes RA, Hayano-Kanashiro C, Alejo-Jacuinde G, Rico-Cambron TY, de Folter S, Herrera-Estrella L. Regulatory network analysis reveals novel regulators of seed desiccation tolerance in *Arabidopsis thaliana*. *P Natl Acad Sci.* 2016;113(35):E5232–5241.
  12. Liu SS, Yang R, Liu M, Zhang SZ, Yan K, Yang GD, Huang JG, Zheng CC, Wu CA. PLATZ2 negatively regulates salt tolerance in *Arabidopsis* seedlings by directly suppressing the expression of the CBL4/SOS3 and CBL10/SCaBP8 genes. *J Exp Bot.* 2020;71(18):5589–602.
  13. Kasirajan L, Hoang NV, Furtado A, Botha FC, Henry RJ. Transcriptome analysis highlights key differentially expressed genes involved in cellulose and lignin biosynthesis of sugarcane genotypes varying in fiber content. *Sci Rep.* 2018;8(1):11612.
  14. Wertheim SJ, Webster D. Propagation and nursery tree quality. In: Ferree DC, Warrington IJ, editors. *Apples: Botany, Production and Uses*. Cambridge: CABI Publishing; 2003. p. 125–51.
  15. Wertheim SJ. Pruning. In: Tromp J, Webster AD, Wertheim SJ, editors. *Fundamentals of Temperate Zone Tree Fruit Production*. Leiden: Backhuys Publishers; 2005. p. 176–89.
  16. Costes E, Lauri PE, Regnard JL. Analyzing fruit tree architecture: implications for tree management and fruit production. *Hortic Rev.* 2006;32:1–61.
  17. Olesen T, Muldoon SJ. Branch development in custard apple (*cherimoya Annona cherimola* Miller x sugar apple *A. squamosa* L.) in relation to tip-pruning and flowering, including effects on production. *Trees Struct.* 2009;23(4):855–62.
  18. Leyser O. The control of shoot branching: an example of plant information processing. *Plant Cell Environ.* 2009;32(6):694–703.
  19. Janssen BJ, Drummond RSM, Snowden KC. Regulation of axillary shoot development. *Curr Opin Plant Biol.* 2014;17:28–35.
  20. Gonzalez GE, Pajoro A, Franco ZJM, Tarancon C, Immink RGH, Cubas P. Abscisic acid signaling is controlled by a BRANCHED1/HD-ZIP1 cascade in *Arabidopsis* axillary buds. *Proc Natl Acad Sci.* 2016;114(2):E245.
  21. Brewer PB, Dun EA, Ferguson BJ, Rameau C, Beveridge CA. Strigolactone acts downstream of auxin to regulate bud outgrowth in pea and *Arabidopsis*. *Plant Physiol.* 2009;150(1):482–93.
  22. Young NF, Ferguson BJ, Antoniadis I, Bennett MH, Beveridge CA, Turnbull CG. Conditional auxin response and differential cytokinin profiles in shoot branching mutants. *Plant Physiol.* 2014;165(4):1723–36.
  23. Shimizu-Sato S, Tanaka M, Mori H. Auxin-cytokinin interactions in the control of shoot branching. *Plant Mol Biol.* 2009;69(4):429–35.
  24. Waldie T, Leyser O. Cytokinin targets auxin transport to promote shoot branching. *Plant Physiol.* 2018;177(2):803–18.
  25. Tan M, Li G, Qi S, Liu X, Chen X, Ma J, et al. Identification and expression analysis of the IPT and CKX gene families during axillary bud outgrowth in apple (*Malus domestica* Borkh.). *Gene.* 2018;2018(651):106–77.
  26. Cook NC, Bellstedt DU, Jacobs G. Endogenous cytokinin distribution patterns at budburst in Granny Smith and Braeburn apple shoots in relation to bud growth. *Sci Hortic.* 2001;87(1–2):53–63.
  27. Hayward A, Stirnberg P, Beveridge C, Leyser O. Interactions between auxin and strigolactone in shoot branching control. *Plant Physiol.* 2009;151(1):400–12.
  28. Dun EA, Germain ADS, Rameau C, Beveridge CA. Antagonistic action of strigolactone and cytokinin in bud outgrowth control. *Plant Physiol.* 2012;158(1):487–98.
  29. Chao Q, Gao ZF, Zhang D, Zhao BG, Dong FQ, Fu CX, Liu LJ, Wang BC. The developmental dynamics of the *Populus* stem transcriptome. *Plant Biotechnol J.* 2019;17(1):206–19.
  30. Da LL, Liu Y, Yang JT, Tian T, She JJ, Ma XL, Xu WY, Su Z. Apple MDO: A multi-dimensional omics database for apple co-expression networks and chromatin states. *Front Plant Sci.* 2019;10:1333.
  31. Liu Y, Li PY, Fan L, Wu MH. The nuclear transportation routes of membrane-bound transcription factors. *Cell Commun Signal.* 2018;16(1):12.
  32. Velasco R, Zharkikh A, Affourtit J, Dhingra A, Cestaró A, Kalyanaraman A, Fontana P, Bhatnagar SK, Troglio M, Pruss D. The genome of the domesticated apple (*Malus x domestica* Borkh.). *Nat Genet.* 2010;42(10):833–9.
  33. Schwabe JWR, Klug A. Zinc mining for protein domains. *Nat Struct Biol.* 1994;1(6):345–9.
  34. Purugganan MD, Wessler SR. Molecular evolution of the plant R regulatory gene family. *Genetics.* 1994;138(3):849–54.
  35. Han XL, Liu K, Yuan GP, He SS, Cong PH, Zhang CX. Genome-wide identification and characterization of AINTEGUMENTA-LIKE (AIL) family genes in apple (*Malus domestica* Borkh.). *Genomics.* 2022;114(2):110313.
  36. Meyers BC, Kozik A, Griego A, Kuang HH, Michelmore RW. Genome-wide analysis of NBS-LRR-encoding genes in *Arabidopsis* (vol 15, pg 809, 2003). *Plant Cell.* 2003;15(4):1683.
  37. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA. The Sequence of the Human Genome. *Science.* 2001;291(5507):1304.
  38. Shi R, Zhou HW. The rice PLATZ protein SHORT GRAIN6 determines grain size by regulating spikelet hull cell division. *J Integr Plant Biol.* 2019;2:847–64.
  39. Su C, Yang XZ, Gao SQ, Tang YM, Zhao CP, Li L. Identification and characterization of a subset of microRNAs in wheat (*Triticum aestivum* L.). *Genomics.* 2014;103:298–307.
  40. Khan Z, Suthanthiran M, Muthukumar T. MicroRNAs and transplantation. *Clin Lab Med.* 2019;39:125–43.
  41. Bian HW, Xie YK, Guo F, Han N, Ma SY, Zeng ZH, Wang JH, Yang YN, Zhu MY. Distinctive expression patterns and roles of the miRNA393/TIR1 homolog module in regulating flag leaf inclination and primary and crown root growth in rice (*Oryza sativa*). *New Phytol.* 2012;196(1):149–61.
  42. Lionel N, Patrice D, Florence J, Benedict A, Nihal D, Mark E, Olivier V, Jonathan DGJ. A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. *Science.* 2006;312(5572):1286.
  43. Iglesias MJ, Terrile MC, Windels D, Lombardo MC, Bartoli CG, Vazquez F, Estelle M, Casalogue CA. MiR393 regulation of auxin signaling and redox-related components during acclimation to salinity in *Arabidopsis*. *PLoS ONE.* 2014;9(9):e107678.
  44. Mittler R. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* 2002;7(9):405–10.
  45. Zhang SC, Yang R, Huo YQ, Liu SS, Yang GD, Huang JG, Zheng CC, Wu CA. Expression of cotton PLATZ1 in transgenic *Arabidopsis* reduces sensitivity to osmotic and salt stress for outgrowth and seedling establishment associated with modification of the abscisic acid, gibberellin, and ethylene signalling pathways. *BMC Plant Biol.* 2018;18(1):218.
  46. So HA, Choi SJ, Chung E, Lee JH. Molecular characterization of stress-inducible PLATZ gene from soybean (*Glycine max* L.). *Plant Omics.* 2015;6:479–84.
  47. Zenda T, Liu S, Wang X, Liu G, Jin H, Dong A, Yang Y, Duan H. Key maize drought-responsive genes and pathways revealed by comparative transcriptome and physiological analyses of contrasting inbred lines. *Int J Mol Sci.* 2019;20(6):1268.
  48. Zhao JY, Zheng L, Wei JT, Wang YX, Chen J, Zhou YB, Chen M, Wang FZ, Ma YZ, Xu ZS. The soybean PLATZ transcription factor GmPLATZ17 suppresses drought tolerance by interfering with stress-associated gene regulation of GmDREB5. *Crop J.* 2022;10(4):1014–25.
  49. Law RD, Plaxton WC. Purification and characterization of a novel phosphoenolpyruvate carboxylase from banana fruit. *Biochem J.* 1995;307(Pt3):807–16.
  50. Wu H, Lu J, Fu ZK, Li HW, Zhou XF. Effect of TDZ on adventitious bud growth of *Rosa chinensis minima*. *Hubei Agric Sci.* 2013;52(13):3178–3179:3202 (in Chinese).
  51. Sun HY, Tao JH, Xin L, Sun QR. Effects of TDZ and BA on adventitious bud regeneration from leaf explants of pear cultivars Jinhua and yali. *Shandong Agric Sci.* 2016;48(01):26–8 39. (in Chinese).
  52. Yuan C, Shi J, Zhao L. The CmbZIP1 transcription factor of chrysanthemum negatively regulates shoot branching. *Plant Physiol Biochem.* 2020;151(100):69–77.
  53. Li T, Lian HM, Li HJ, Xu YF, Zhang HY. HY5 regulates light-responsive transcription of microRNA163 to promote primary root elongation in *Arabidopsis* seedlings. *J Integr Plant Biol.* 2021;63(8):1437–50.
  54. Wang R, Yang X, Guo S, Wang Z, Zhang Z, Fang Z. MiR319-targeted OsTCP21 and OsGAMYB regulate tillering and grain yield in rice. *J Integr Plant Biol.* 2021;63(7):1260–72.
  55. Keller T, Abbott J, Moritz T, Doerner P. *Arabidopsis* REGULATOR OF AXIL-LARY MERISTEMS1 controls a leaf axil stem cell niche and modulates vegetative development. *Plant Cell.* 2006;18(3):598–611.
  56. Schmitz G, Tillmann E, Carriero F, Fiore C, Cellini F, Theres K. The tomato Blind gene encodes a MYB transcription factor that controls the formation of lateral meristems. *Proc Natl Acad Sci USA.* 2002;99(2):1064–9.

57. Aida M, Ishida T, Fukaki H, Fujisawa H, Tasaka M. Genes involved in organ separation in Arabidopsis: an analysis of the cup-shaped cotyledon mutant. *Plant Cell*. 1997;9(6):841–57.
58. Hibara KI, Karim MR, Takada S, Taoka KI, Furutani M, Aida M, Tasaka M. Arabidopsis CUP-SHAPED COTYLEDON3 regulates postembryonic shoot meristem and organ boundary formation. *Plant Cell*. 2006;18(11):2946–57.
59. Wang M, Le Moigne MA, Bertheloot J, Crespel L, Perez-García MD, Ogé L, Demotes-Mainard S, Hamama L, Davière JM, Sakr S. BRANCHED1: A Key Hub of Shoot Branching. *Front Plant Sci*. 2019;10:76.
60. Chen Y, Dan Z, Gao F, Chen P, Fan F, Li S. Rice GROWTH-REGULATING FACTOR7 Modulates Plant Architecture through Regulating GA and Indole-3-Acetic Acid Metabolism. *Plant Physiol*. 2020;184(1):393–406.
61. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res*. 2000;28(1):27–30.
62. Kanehisa M. Toward understanding the origin and evolution of cellular organisms. *Protein Sci*. 2019;28(11):1947–51.
63. Kanehisa M, Furumichi M, Sato Y, Kawashima M, Ishiguro-Watanabe M. KEGG for taxonomy-based analysis of pathways and genomes. *Nucleic Acids Res*. 2023;51(D1):D587–92.
64. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR. *Methods*. 2001;25(4):402–8.

### Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more [biomedcentral.com/submissions](https://biomedcentral.com/submissions)

