

Identification and bioinformatics analysis of MADS-box family genes containing K-box domain in maize

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Abstract

The MADS-box family genes are involved in the development of plant roots, leaves, flowers, and fruits, and play a crucial role in plant growth and development. Studying MADS-box genes with K-box domain is crucial to distinguish different types of MADS-box genes. This study systematically analysed the genomic structural information of maize MADS-box family members containing the K-box Domain at the genome-wide level using the maize (*Zea mays*) B73 genome as the reference sequence, and provided insight into the biological functions of the maize MADS-box family containing the K-box domain. According to the findings, 52 MADS-box family genes with K-box domain were identified and divided into 4 subgroups. The distribution of motif in the same subgroup was found to be relatively conservative, and all of them had MADS-box conserved domain and K-box domain. Gene structure analysis showed that the introns and exons of the same subgroup genes have similar gene structure, and different types of genes containing the K-box domain showed different exon/intron structure characteristics. Chromosome mapping showed that 52 genes containing the K-box domain were unevenly distributed on the 10 chromosomes of maize, most of which were distributed at both ends of the chromosome and a small number of genes were distributed near the centromere. Based on the analysis of cis-acting elements of its up-stream promoter, it was found that MADS-box family genes may be involved in light response, IAA, GA, ABA, and LTR signal pathways, indicating that they play a certain role in stress response and hormone signal transduction. The expression analysis of genes with the K-box domain in maize leaves treated with auxin and gibberellin revealed that MADS-box genes may have a regulatory effect on certain plant hormones. Through the identification and bioinformatics analysis of MADS-box family genes containing the K-box domain, it is helpful to further study the function and pathway of MADS-box family genes, and provide a theoretical basis for further re-search for the molecular mechanism of maize growth and development.

Keywords: bioinformatics analysis; gene expression analysis; K-box domain; MADS-box gene family; maize

Received: 18 Jun 2023. Received in revised form: 29 Sep 2023. Accepted: 31 Oct 2023. Published online: 16 Nov 2023.

From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Introduction

Transcription factor (TF) occupies a certain proportion in plant genomes (Du, 2021), has the potential to improve crop and fine-tune traits, and can play an important role in plant development and response to external environment by activating or inhibiting gene transcription (Wei *et al.*, 2022), which is beneficial to crop evolution and domestication (Martínez-Ainsworth and Tenailon, 2016). As one of the largest plant transcription factor families, MADS-box gene family is involved in different stages of plant growth and development. The name of MADS-box is composed of the initials of four protein factors: MCM1 (*Saccharomyces cerevisiae*), AGAMOUS (*Arabidopsis thaliana*), DEFICIENS (*goldfish grass*) and SRF (*human*) (Ma *et al.*, 2006). All four proteins have a highly conserved MADS domain, which consists of 56-58 amino acids (Schwarz-Sommer *et al.*, 1990). According to the different conserved domains, MADS-box genes in the plant kingdom can be divided into type I and type II (Zhang *et al.*, 2022). Type I MADS-box genes generally contain only 1-2 exons, and the encoded protein does not contain K (Keratin-like)-box domain (Huang *et al.*, 2012). Type II MADS-box gene generally consists of 7 exons and 6 introns. The protein encoded is mainly composed of the most conserved MEF2-like MADS (M) domain, semiconserved Keratin-like domain (K) domain, Intervening domain (I) domain and the least conserved C-terminal domain (C) domain (Huang *et al.*, 2012). Therefore, it is also called MIKC type. In type II MADS-box genes, the semi-conserved K-box domain exists in most genes, so type II can be divided into K-box-containing and K-box-free types. Many MADS-box genes have been found in existing plants, animals and fungi, and expressed in different growth and development stages and different parts of different organisms (Wang *et al.*, 2009). In plants, MADS-box gene can not only regulate flowering time and determine the characteristics of floral meristem and floral organ, but also play a regulatory role in the development of root, stem, leaf, ovule and fruit (Cui *et al.*, 2003; Lv and Meng, 2007; Wang *et al.*, 2021). MIKC type MADS-box genes are involved in almost all aspects of plant development, such as root, flower, seed and embryo development (Gramzow and Theissen, 2010), and they are also involved in different stress responses (Arora *et al.*, 2007; Jia *et al.*, 2018; Wei *et al.*, 2018). Therefore, the study of MADS-box gene is very important for understanding crop development, crop breeding and crop improvement (Boden and Østergaard, 2019).

At present, studies have been carried out to identify the MADS-box gene family in wheat (Jiao *et al.*, 2017) (*Triticum aestivum*), cotton (Wang, 2010) (*Gossypium* spp.), soybean (Martínez-Ainsworth and Tenailon, 2016) (*Glycine max*), rape (Gao *et al.*, 2017) (*Brassica napus*), pineapple (Hu *et al.*, 2017) (*Ananas comosus*), dragon fruit (Wei and Zhang, 2018) (*Hylocereus undatus*), radish (Li, 2016) (*Raphanus sativus*) and marijuana (Wan *et al.*, 2021) (*Cannabis sativa*). For example, the expression in stem has been frequently reported (Ma *et al.*, 1991; Mandel and Yanofsky, 1995). Barley (*Hordeum vulgare*) MADS-box gene *BMI* (Barley MADS-box1) is expressed in the meristematic cell layer of stem node and vascular system (Schmitz *et al.*, 2000). The transcription of *OsMDP* (Oryza Sativa Mads Domain Containing Protein) gene in rice (*Oryza sativa*) was inhibited dark induction and brassinolide (BR) treatment, resulting in shorter primary roots, longer coleoptile and larger leaf inclination. Previous studies have shown that *ZmMADS3* gene in maize is expressed in endothelial cells, fertilized eggs in vivo and in vitro, immature pistil and unpollinated and pollinated mature pistil (2 and 5 days after pollination), but not in isolated immature and mature embryos (Heuer *et al.*, 2001).

There are differences in the expression patterns of MADS-box genes in different plants. Maize, as an important grain and feed crop and indispensable industrial raw material in China, plays an irreplaceable role in ensuring food security and promoting industrial development, but the systematic research report of maize MADS-box gene family is limited. Semi-conserved K-box domain is an important basis for distinguishing type I and II (MIKC) MDAS-box genes, which not only promotes the dimerization of two or more MADS domain proteins, but also facilitates the formation of advanced complexes, so it is of great significance to study MADS-box genes containing this domain (Melzer *et al.*, 2009; Theißen *et al.*, 2018; Schilling *et al.*, 2020). In this study,

all members of MIKC type MADS-box family containing the K-box domain in maize were identified by bioinformatics method. The evolutionary relationship, structure, distribution position on the chromosome, cis-acting element and expression under auxin and gibberellin treatment of 52 maize genes containing the K-box domain were systematically analysed at the whole genome level. It laid a foundation for understanding the MIKC type MADS-box family genes containing the K-box domain in maize, and also provided a reference for further determining the function of MIKC type MADS-box genes containing the K-box domain.

Materials and Methods

Identification and sequence Analysis of MADS-box family genes containing K-box domain in maize

Firstly, the proteome and genome sequence of B73 V4 version are downloaded from maize genome database Ensemblplants (http://plants.ensembl.org/Zea_mays/Info/Index). Secondly, the corn proteome and genome database are constructed by Blast localize software, and then the hidden Markov model (Hidden Markov Model) of the K-box conserved domain in Pfam (Finn *et al.*, 2016) database (<http://pfam.xfam.org>) is used. HMM file (sequence number PF01486) is the search sequence. The hidden Markov model file of MIKC type MADS-box gene family is found and downloaded. The hidden Markov model file and the transcribed protein sequence file of all genes in maize are searched by HMMER (<http://hmmer.org>) software. The sequence containing this conservative domain is extracted, and the screening threshold (E-value) parameter is set to 1.2×10^{-16} . Get the candidate members of MIKC type MADS-box containing the K-box domain in corn. In order to ensure that the candidate members contain K-box domain, three major database websites (SMART, CDD, Pfam) were used to identify the MIKC type MADS-box candidate members containing the K-box domain in maize. The uncertain gene ID was removed and a reliable gene list of gene family was obtained. Finally, the protein and gene coding sequences (coding sequence, CDS) of the identified maize MADS-box family members were extracted from the local corn proteome and genome database as the basis for later analysis. The on-line software WOLFPSORT (<https://www.genscript.com/wolf-psort.html>) and Primer3web (<https://primer3.ut.ee>) were used to predict and analyse the physicochemical characteristics such as chromosome position, the number of amino acids, molecular weight, hydrophilicity and isoelectric point.

Gene structure analysis

The visualization map of gene structure was drawn by using online software GSDS 2.0 (<http://gsds.gao-lab.org/>) and the genome sequence information of 52 maize MADS-box family genes containing the K-box domain and the corresponding cDNA sequence information, and beautified by Adobe Illustrator software.

Chromosome mapping analysis

The starting and ending positions of 52 maize MADS-box family genes containing K-box domain were extracted from maize B73 genomic Gff3 files by TBtools software, and then located on chromosomes, and the distribution of these genes on 10 maize chromosomes was analysed. Pairwise comparison with BLAST was used to find replicative genes, and the screening conditions were as follows: the alignment rate of 12 genes was more than 70% (compared with the longer genes), and the similarity of genes was more than 70%. When the two MADS-box genes meet the above conditions at the same time, it indicates that there is gene replication.

Evolutionary tree and conservative motif analysis of protein sequences of MADS-box family containing K-box domain in maize

The corn protein and coding sequence were downloaded from NCBI database, and the phylogenetic tree was constructed by MEGA 7.0 the amino acid sequences were compared by Clustal W method, and the phylogenetic tree of MADS-box family was constructed by adjacency methods (neighbor-joining, NJ). The

Bootstrap Replications value was set to 1000, and the other parameters were kept by default, and the evolutionary tree was beautified by Evolview software. The conservative motif of MADS-box family gene protein containing the K-box domain in maize was identified by MEME software, except that the cardinality parameter was set to 10, the other parameters were default parameters, and then visual analysis was carried out by TBtools (Guo *et al.*, 2016) software. The vector map of the position information of motif on the gene was obtained in order to better understand the domain characteristics of maize MADS-box family genes containing the K-box domain.

Analysis of cis-acting elements upstream of promoter

The promoter sequence of 1500 bp upstream of maize MADS-box family genes containing K-box domain initiation codon was analysed by online software PlantCARE (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>), and those elements related to abiotic stress response were analysed. Such as auxin response element (AuxRE, TGA-element), gibberellin response element (GARE-motif), abscisic acid response element (ABRE), salicylic acid response element (TCA-element), ethylene response element (ERE), low temperature stress element (LTR), drought stress element (MBS), hypoxia specific inducer element (GC-motif) for further analysis (Shinwari *et al.*, 1998; Narusaka *et al.*, 2003; Gou *et al.*, 2010; Yun *et al.*, 2010).

Analysis of the expression of MADS-box family genes containing K-box domain in maize under plant hormone treatment

According to the analysis of cis-acting elements upstream of the promoter, it was found that there were a large number of auxin and gibberellin response elements in the promoter region of this family, and it was speculated that these genes might be more sensitive to these two kinds of hormones, so 10 genes with the largest number of auxin and gibberellin response elements were screened, and distilled water treatment was used as control to set different concentration gradients of IAA and GA. The expression of MADS-box gene containing the K-box domain in maize leaves under different concentrations of auxin and gibberellin was studied. The concentrations of IAA treatment were 1 $\mu\text{mol}\cdot\text{L}^{-1}$, 10 $\mu\text{mol}\cdot\text{L}^{-1}$, 100 $\mu\text{mol}\cdot\text{L}^{-1}$, 200 $\mu\text{mol}\cdot\text{L}^{-1}$ and 500 $\mu\text{mol}\cdot\text{L}^{-1}$, respectively. The concentrations of GA were 1 $\mu\text{mol}\cdot\text{L}^{-1}$, 10 $\mu\text{mol}\cdot\text{L}^{-1}$, 100 $\mu\text{mol}\cdot\text{L}^{-1}$, 200 $\mu\text{mol}\cdot\text{L}^{-1}$ and 500 $\mu\text{mol}\cdot\text{L}^{-1}$, respectively. The seedlings of B73 were treated with 50 ml of different concentrations of hormone every 3 days. RNA was extracted from the leaves of B73 seedlings when the seedlings grew to the stage of three leaves and one heart (about 3 times of hormone treatment), the RNA extraction kits and reverse transcription kits of B73 seedlings in the extraction clover stage were provided by Ackeri Biological Co., Ltd., according to the operation instructions of SteadyPure plant RNA extraction kit and Evo M-MLV reverse transcription reagent premixed solution. The extracted RNA was stored at $-80\text{ }^{\circ}\text{C}$, reverse transcription was carried out by PCR instrument (37 $^{\circ}\text{C}$ 15 min, 85 $^{\circ}\text{C}$ 5sec, 4 $^{\circ}\text{C}$ preservation), and cDNA was stored in $-20\text{ }^{\circ}\text{C}$ refrigerator. The reaction conditions of real-time quantitative PCR were as follows: Pre-denaturation at 95 $^{\circ}\text{C}$ for 30 s, denaturation at 95 $^{\circ}\text{C}$ for 5 s, annealing at 60 $^{\circ}\text{C}$ for 30 s for 40 loops, 95 $^{\circ}\text{C}$ for 15 s, 60 $^{\circ}\text{C}$ for 60 s, 95 $^{\circ}\text{C}$ for 15 s. The relative expression level of MADS-box gene was calculated by $2^{-\Delta\Delta\text{CT}}$ method, and then converted into \log_2 value. According to the results of cis-acting elements, 10 genes with the most obvious response to auxin and gibberellin were screened, and primers were designed by using the online website of Sheng gong Bioengineering (Shanghai) Co., Ltd. 18S is used as the internal reference gene, and the sequence of the primers used in the experiment is shown in Table 1.

Table 1. Primer design sequence

Gene	Forward primer	Reverse primer
<i>Zm00001d007949</i>	GGGAAATTGGTGCCAGAAT	CCTTCTGCAGAGCCTTGTC
<i>Zm00001d018142</i>	GGGAGAGGCGGGAGTAAAG	GCTGTTCTGTTTTCCCAGG
<i>Zm00001d027425</i>	CGGAAGTGCCCAGAAAACAA	CCCTGATGCAATGAAGGCTC
<i>Zm00001d018587</i>	TTGTGCCTCATGAAACAGCC	GGACAACAACGCGAACATA
<i>Zm00001d027957</i>	CCGGCAAACCTTCCACTTC	CCTCTCCTCTCATCTGCCTG
<i>Zm00001d035053</i>	CTGCGGCGACAATAACAGAA	CTTTGCAGAGCGATGTTGT
<i>Zm00001d048082</i>	GCAAGAAACAAGTCCGCAGA	TGTAGGCCTGCTGATGTAC
<i>Zm00001d048474</i>	AGTGCGCAGAAAAGATTGA	GGAGCAGTCTTCCAACCTCT
<i>Zm00001d050897</i>	GGGGAGGGGAAGATTGTGA	TTTGTGACGACTTGCTGCTC
<i>Zm00001d052180</i>	GCGACGGGAGATAAGAGGA	TTCATACTGAGCTGGCGAA

Results

Identification and Analysis of MADS-box Family genes containing K-box Domain in Maize

After removing the redundancy, the candidate genes were uploaded to three major database websites (SMART, CDD, Pfam) for identification, 58 MADS-box family members were obtained, the conservative domains in the other sequences were examined, and 6 uncertain genes ID were removed. 52 maize MADS-box family candidate genes containing K-box domain were identified by whole genome identification. As shown in Table 2, the analysis of physical and chemical properties showed that the amino acid content of 52 maize genes was between 30,286 aa and the average number of amino acids was 211 aa, in which *Zm00001d037737_P001* encoded the largest protein length and *Zm00001d030375_P002* the smallest, and the molecular weight range was 3465.85-32175.03 Da, the highest molecular weight gene was *Zm00001d037737_P001*, and the smallest gene was *Zm00001d030375_P002*. The hydrophilic range of 52 protein sequences is -0.078 copyright 0.996, and the GRAVY values are all negative, which indicates that these proteins are all hydrophilic proteins with different hydrophilicity, among which the weakest hydrophilic protein is *Zm00001d002332_P005* and the strongest protein is *Zm00001d050388_P001*. The range of isoelectric point (PI) was 4.36 to 9.81. The Pi values of 40 proteins were between 7 and 10, and the Pi values of 12 proteins were between 4 and 7, of which *Zm00001d027425_P001* was the most alkaline and *Zm00001d030375_P002* was the most acidic. Most of the genes (42) were located in the nucleus, 9 genes in chloroplast and *Zm00001d027955_P001* in the intercellular space. Therefore, generally speaking, maize MADS-box family is rich in acidic amino acids. 52 maize MADS-box family genes containing K-box domain have the characteristics of diversity of amino acids, isoelectric point and molecular weight, which are distributed on 10 maize chromosomes, most of them are located in the nucleus and the proteins have certain hydrophilicity.

Gene structure analysis

GSDS 2.0 software and the genomic sequences and corresponding cDNA sequences of 52 maize MADS-box genes were used to analyse the gene structure. As shown in Figure 1, the gene structure analysis of 52 maize MADS-box family genes belonging to different subgroups showed that the intron and exon contents of 52 MADS-box genes were quite different. The number of introns varied from 1 to 7, including 3 (5.7%) intron deletions, 14 (26.9%) genes with 1 intron, 27 (51.9%) genes with 2 introns, 5 (9.6%) genes with 3 introns, *Zm00001d022088_P001* with 4 introns, and *Zm00001d002332_P005* with the most complex structure with 8 introns. In general, genes with few or no introns usually show a low level of expression in plants, which is different from that in animals (Ren *et al.*, 2006). The results showed that on the whole, the exon-intron distribution of MADS-box gene was conservative, the intron number and exon length of the same

subgroup had similar gene structure, while different subgroups of MADS-box gene showed different exon / intron structure.

Table 2. The characteristics of identified MADS-box family genes containing K-box domain in maize

Gene ID	Chromosome	Number of amino acids	MW	Grand average of hydropathicity	PI	Subcellular Localization
Zm00001d002332_P005	2	285	30577.22	-0.078	8.92	nucleus
Zm00001d003168_P001	2	255	29348.66	-0.557	9.07	nucleus
Zm00001d003408_P001	2	151	16878.09	-0.674	5.07	nucleus
Zm00001d007949_P001	2	264	30279.50	-0.863	9.16	nucleus
Zm00001d008882_P001	8	259	29267.22	-0.708	9.22	nucleus
Zm00001d010232_P001	8	212	24667.06	-0.903	9.16	nucleus
Zm00001d010233_P001	8	212	24891.41	-0.902	9.39	chloroplast
Zm00001d011748_P001	8	197	22508.57	-0.712	7.72	nucleus
Zm00001d013258_P001	5	241	27340.18	-0.582	7.06	nucleus
Zm00001d013259_P001	5	245	28050.23	-0.665	9.41	nucleus
Zm00001d015381_P001	5	259	29000.85	-0.528	6.08	nucleus
Zm00001d016957_P001	5	240	27414.99	-0.683	8.54	chloroplast
Zm00001d017614_P001	5	255	29133.19	-0.691	8.85	nucleus
Zm00001d017932_P002	5	184	20735.59	-0.662	9.30	nucleus
Zm00001d018142_P001	5	228	25526.59	-0.676	5.41	nucleus
Zm00001d018587_P001	5	232	26971.49	-0.370	9.13	nucleus
Zm00001d018667_P002	7	271	31094.38	-0.863	9.17	nucleus
Zm00001d018767_P001	7	255	29337.59	-0.563	8.50	nucleus
Zm00001d019189_P001	7	146	17195.91	-0.534	9.44	nucleus
Zm00001d019289_P001	7	116	13385.35	-0.786	8.88	nucleus
Zm00001d022088_P001	7	251	28372.32	-0.731	9.45	chloroplast
Zm00001d023736_P001	10	245	27460.04	-0.585	8.42	nucleus
Zm00001d025479_P001	10	199	22894.39	-0.429	8.46	chloroplast
Zm00001d027425_P001	1	204	23537.18	-0.823	9.81	nucleus
Zm00001d027955_P001	1	180	19979.75	-0.252	4.92	extracellular space
Zm00001d027957_P001	1	233	26365.80	-0.695	7.70	nucleus
Zm00001d028217_P001	1	241	28007.66	-0.754	6.12	nucleus
Zm00001d030375_P002	1	30	3465.85	-0.683	4.36	nucleus
Zm00001d031399_P001	1	128	14449.57	-0.356	8.96	nucleus
Zm00001d031591_P001	1	144	16939.66	-0.533	9.55	nucleus
Zm00001d032219_P001	1	121	14070.92	-0.723	7.07	chloroplast
Zm00001d033701_P001	1	242	27348.35	-0.411	8.69	nucleus
Zm00001d034045_P003	1	245	28131.28	-0.692	9.34	nucleus
Zm00001d035053_P001	6	221	25142.91	-0.335	6.97	chloroplast
Zm00001d036210_P001	6	113	13382.59	-0.546	9.39	nucleus
Zm00001d036279_P001	6	67	8095.36	-0.940	6.82	nucleus
Zm00001d036425_P001	6	227	25841.32	-0.638	8.95	nucleus
Zm00001d037737_P001	6	286	32175.03	-0.830	9.31	nucleus
Zm00001d039434_P001	3	262	29487.25	-0.777	9.25	nucleus
Zm00001d041781_P001	3	269	30229.47	-0.519	9.11	nucleus
Zm00001d042618_P001	3	209	24062.57	-0.711	8.43	chloroplast
Zm00001d043589_P004	3	269	30115.94	-0.714	8.43	nucleus
Zm00001d045231_P001	9	225	25725.00	-0.625	5.54	nucleus
Zm00001d047356_P001	9	119	13747.63	-0.963	5.75	nucleus
Zm00001d047742_P001	9	146	17190.93	-0.528	9.41	nucleus
Zm00001d048082_P001	9	240	27831.42	-0.795	6.67	nucleus
Zm00001d048474_P001	9	232	26404.25	-0.841	9.38	nucleus
Zm00001d049692_P001	4	227	25473.23	-0.341	8.30	nucleus
Zm00001d050388_P001	4	166	19398.71	-0.996	7.11	chloroplast
Zm00001d050897_P001	4	226	25669.10	-0.666	9.25	chloroplast
Zm00001d051465_P001	4	255	28913.84	-0.639	8.43	nucleus
Zm00001d052180_P001	4	228	25480.54	-0.644	5.52	nucleus

The results of the gene length analysis showed that most of the genes in this family were between 91 and 15000bp, a few genes were in 15000~25000bp, and the length of *ZMM4* (*Zm00001d034045_P003*) gene was

the largest, exceeding that of 25000bp. The expression of *ZMM4* starts from the leaf primordium of the vegetative stem tip and then increases in the elongated meristem with inflorescence characteristics.

Studies have shown that *ZMM4* may play a role in flower induction and inflorescence development (Wei *et al.*, 2018). The above results showed that the MADS-box family genes of maize were highly conservative.

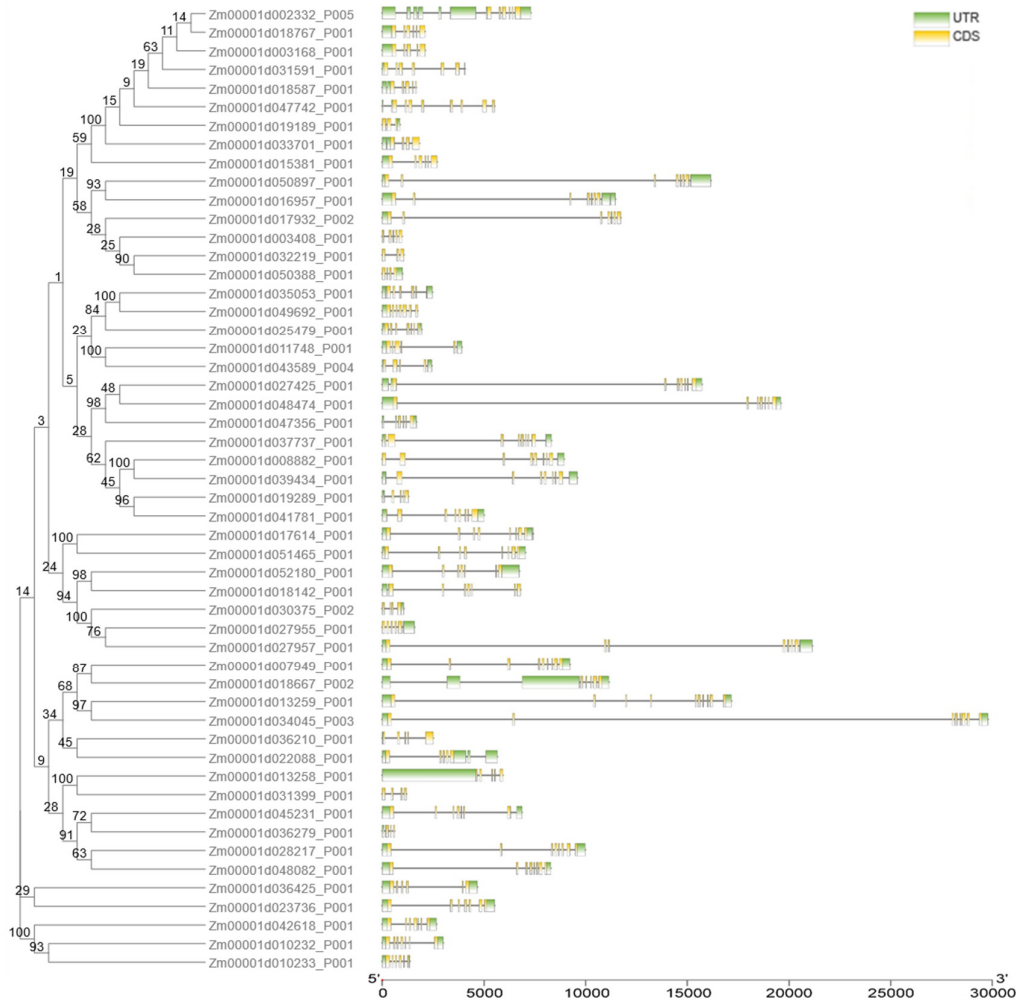


Figure 1. Gene structural analysis of MADS-box family genes containing K-box domain in maize

Chromosome mapping analysis

There are 10 chromosomes in maize. The length of chromosomes from 1 to 10 is 307041717 bp, 244442276 bp, 235667834 bp, 246994605 bp, 223902240 bp, 174033170 bp, 182381542 bp, 181122637 bp, 159769782 bp and 150982314 bp. As shown in Figure 2, 52 MADS-box genes are unevenly distributed on all 10 chromosomes of maize, most of them are evenly distributed at both ends of the chromosome, and a small number of genes are distributed near the centromere of the chromosome. Among them, there are 10 genes (19.23%) on chromosome 1 and 2 genes (3.85%) on chromosome 10, and the length of these two genes is less than 5000 bp.

After BLAST comparison, seven pairs of tandem repeat genes were obtained: *Zm00001d006094_P001* and *Zm00001d021057_P001*, *Zm00001d017614_P001* and *Zm00001d051465_P001*, *Zm00001d027425_P001* and *Zm00001d048474_P001*, *Zm00001d050897_P001* and

Zm00001d016957_P001, *Zm00001d052180_P001* and *Zm00001d018142_P001*, *Zm00001d002332_P005* and *Zm00001d018767_P001*, *Zm00001d010232_P001* and *Zm00001d010233_P001*. Therefore, the expansion of MADS-box family genes in maize may be closely related to tandem repeats.

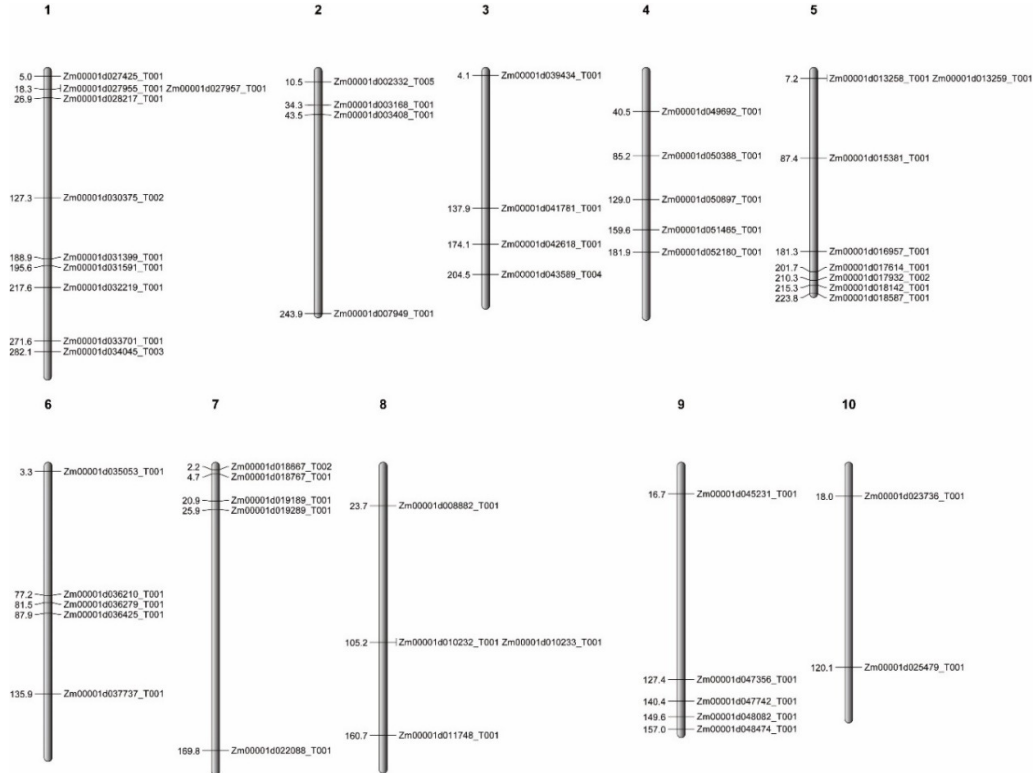


Figure 2. Chromosomal locations of MADS-box family genes containing K-box domain in maize

Evolutionary tree and conservative motif analysis of MADS-box family gene protein sequences containing K-box domain in maize

In order to better understand the evolution of protein sequences and the number and species of conserved motifs of MADS-box family members, MEGA 7.0 software was used to analyse the evolutionary tree of maize MADS-box family, MEME software was used to study the conserved motifs of maize MADS-box family proteins, and TBtools software was used for visual analysis. Results as shown in Figure 3, the phylogenetic tree of protein sequence showed that the MADS-box family of maize could be divided into 4 subfamilies, with the largest number of members of subgroup I (35), followed by members of subgroup II (12), and less members of subgroup III and IV, with 2 and 3, respectively. Protein conserved motif analysis showed that there were 10 conserved motifs in MADS-box protein: motif 1~motif 10, and all the conserved motifs were arranged in a certain manner, which was motif 3-1-5-4-2-7-9-8-10-6. Each conserved motif contains 857 amino acids, of which motif 1, 6, 8 and 10 contain the most amino acids, with an average of 50, and motif 9 contains the least, which is 8.

As shown in Figure 3, most of the MADS-box proteins in each subgroup have a highly conserved motif, and different subgroups have different conserved motif. For example, members of subgroup IV contain motif 1, 2, 3, 4, 5, and their number and location distribution of different protein sequences are similar. Motif 1, 2, 3, 4 and 5 were found in most proteins, of which motif 2 was found in all proteins, while motif 6, 7, 8, 9 and 10 were relatively few.

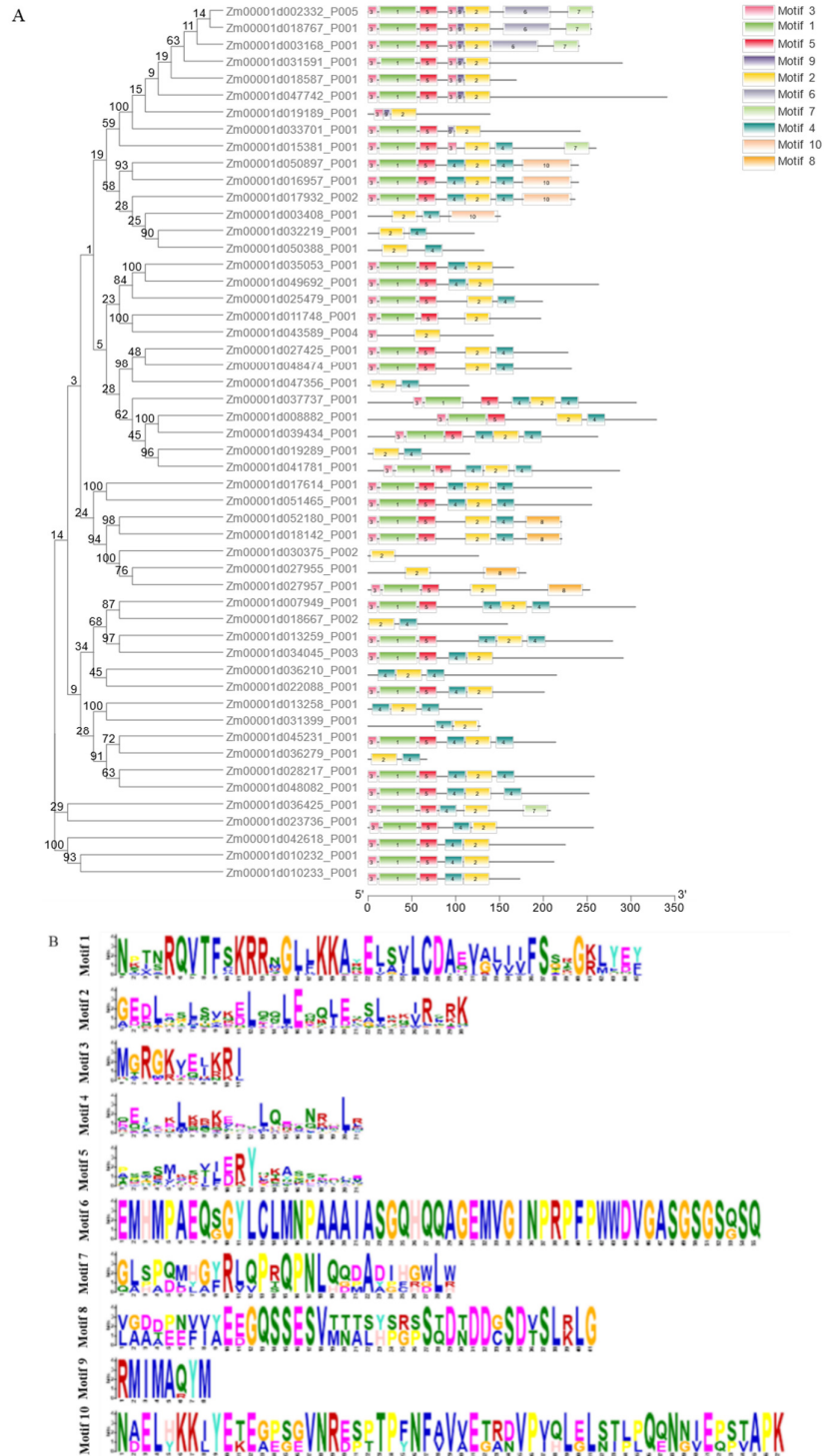


Figure 3. Conserved motif analysis and typical motif sequence logos of MADS-box family members containing K-box domain in maize (A) Motif analysis of maize MADS-box family genes containing K-box domain; (B) Typical motif sequence of MADS-box

Note: The size of each base corresponding figure character is proportional to the frequency of the base at that position, which can reflect the sequence preference.

The motif distribution of the whole MADS-box family proteins is relatively consistent, in which motif 1, 3 and 5 are regularly concentrated in the conserved N-terminal region of the protein sequence, indicating that they are closely related to the conserved domain of MADS-box protein. Motif 7, 8, 10 are regularly concentrated in the less conservative C-terminal region, and the length change of MADS-box gene is also caused by the change of C-terminal. The prediction results of MADS-box family proteins show that each protein contains a different number of conserved motifs, ranging from 1 to 7, and 52 proteins contain conserved MADS-box domains. In summary, there are some differences in the number, type and location distribution of conserved motifs in different proteins, which reveals the differences in gene structure and the functional similarity of genes with similar structure.

Analysis of cis-acting elements upstream of promoter of MADS-box family genes containing K-box domain in maize

Cis-acting elements are the binding sites of transcription factors and participate in the regulation of gene expression, so the analysis of the type and distribution of cis-acting elements in gene promoter sequence is helpful to study the function of genes. Figure 4 shows the distribution of cis-acting elements in the 1500 bp promoter region upstream of maize MADS-box gene. Different colors and shapes represent auxin (IAA) response elements, gibberellin (GA) response elements, abscisic acid (ABA) response elements, salicylic acid (SA) response elements, light response elements and defense and stress response elements, respectively. These cis-acting elements can be divided into three types: light response elements (G-Box, I-box), hormone response-related cis-acting elements (AuxRE, TGA-element, GA-motif, GARE-motif, ABRE, TCA-element, ERE) and stress-related cis-acting elements (LTR, MBS, GC-motif). Each gene contains multiple light, hormone and stress response cis elements, and the type and number of cis elements are different. Among them, the number of light response elements (G-box) and auxin response elements (AuxRE, TGA-element) are more and widely distributed, G-box is distributed in 46 genes, and the largest number in *Zm00001d019289_T001*; AuxRE and TGA-element are distributed in 34 genes, and the largest number in *Zm00001d048474_T001*. Gibberellin response elements (GA-motif, GARE-motif) were most distributed on *Zm00001d007949_T001* and *Zm00001d018587_T001*, drought stress response elements (MBS) were the largest on *Zm00001d036279_T001*, hypoxia specific induction elements (GC-motif) were the largest on *Zm00001d027425_T001*, and other elements (I-box, ABRE, TCA-element, ERE, LTR) were also distributed but less in some members. It is suggested that maize MADS-box family genes may respond to plant hormones and stress and other signals, including auxin, gibberellin, abscisic acid, salicylic acid, ethylene and other hormone small molecular signals, as well as hypoxia, drought, low temperature and other abiotic stress.

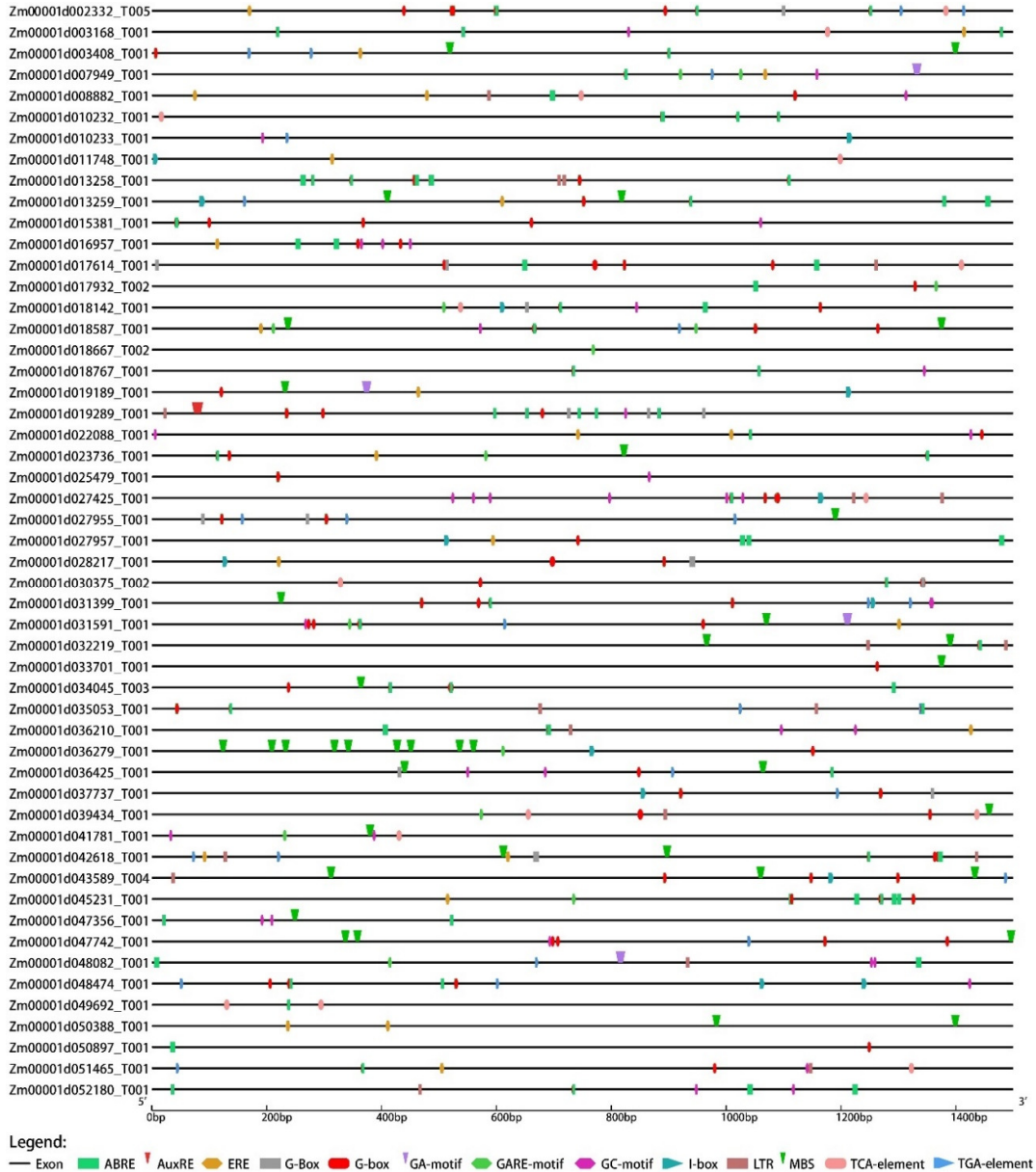


Figure 4. Homeopathic element analysis of MADS-box family genes containing K-box domain in maize

Expression analysis of members of MADS-box gene family containing K-box domain under exogenous auxin treatment

Based on the analysis of the cis-acting elements upstream of the promoter of maize MADS-box family genes containing K-box domain, it was found that the proportion of auxin and gibberellin response elements in this family gene was relatively large, and some gene promoters contained both auxin and gibberellin response elements, which indicated that the transcriptional expression of MADS-box genes containing K-box domain was likely to be affected by plant hormones. In order to explore the expression of MADS-box family genes containing the K-box domain in maize leaves treated with different concentrations of auxin, 10 genes with the largest number of auxin response elements were screened, and the expression of these 10 genes under different concentrations of auxin treatment was analysed. The results are shown in Figure 5. Compared with other genes,

the expression levels of *Zm00001d018142*, *Zm00001d048474*, *Zm00001d050897* and *Zm00001d052180* were higher than other genes under different concentrations of auxin, while the expression levels of *Zm00001d007949*, *Zm00001d027425*, *Zm00001d018587*, *Zm00001d027957*, *Zm00001d035053* and *Zm00001d048082* were lower as a whole. With the increase of auxin concentration, the gene expression level increased at first and then decreased. The expression level of most genes was the highest under 10 $\mu\text{mol}\cdot\text{L}^{-1}$ and 100 $\mu\text{mol}\cdot\text{L}^{-1}$ auxin treatment, and the expression level was lower under 1 $\mu\text{mol}\cdot\text{L}^{-1}$ and 500 $\mu\text{mol}\cdot\text{L}^{-1}$ auxin treatment. Compared with the control treatment, *Zm00001d007949*, *Zm00001d018142*, *Zm00001d027425*, *Zm00001d018587*, *Zm00001d027957*, *Zm00001d048474* and *Zm00001d050897* were up-regulated significantly under 100 $\mu\text{mol}\cdot\text{L}^{-1}$ auxin treatment, *Zm00001d035053* was up-regulated most significantly under 200 $\mu\text{mol}\cdot\text{L}^{-1}$ auxin treatment, *Zm00001d052180* was up-regulated most significantly under 10 $\mu\text{mol}\cdot\text{L}^{-1}$ treatment, *Zm00001d027425* was down-regulated most significantly under 500 $\mu\text{mol}\cdot\text{L}^{-1}$ treatment, and *Zm00001d035053* was down-regulated most significantly at 10 $\mu\text{mol}\cdot\text{L}^{-1}$ treatment. The above results suggest that MADS-box gene has a certain regulatory effect on auxin.

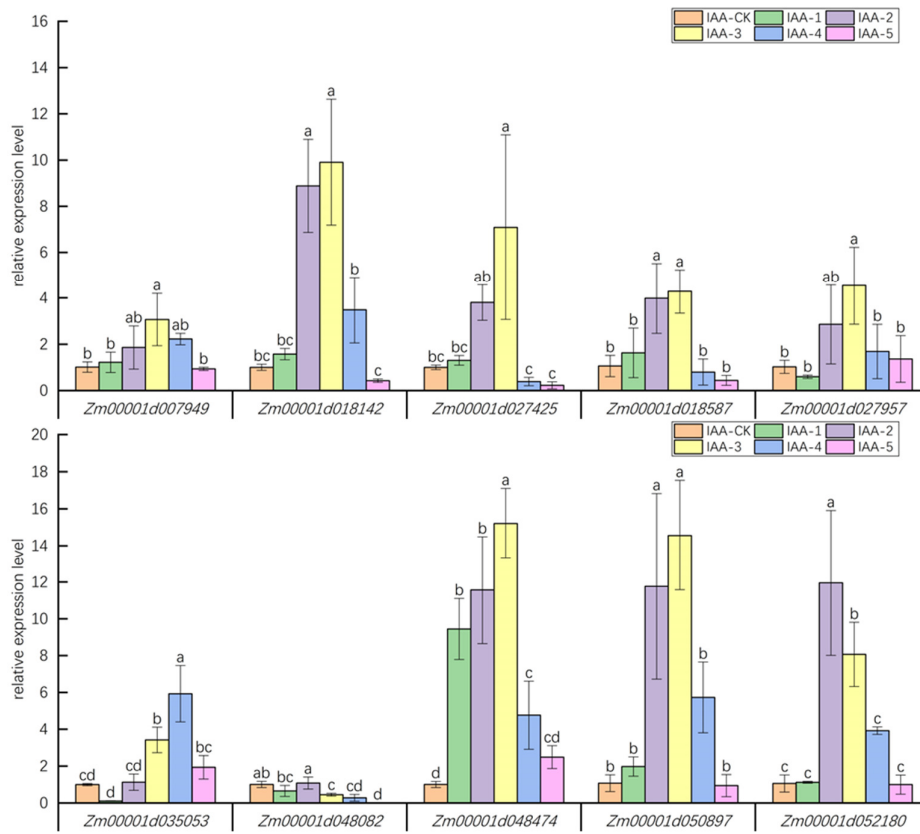


Figure 5. Expression characteristics of MADS-box family genes containing K-box domain treated with auxin

Note: The O-X axis represents different genes, The O-Y axis represents the expression level of the same gene under different concentrations of IAA. In the figure, IAA-CK, IAA-1, IAA-2, IAA-3, IAA-4 and IAA-5 represent auxin concentrations of 0 $\mu\text{mol}\cdot\text{L}^{-1}$, 1 $\mu\text{mol}\cdot\text{L}^{-1}$, 10 $\mu\text{mol}\cdot\text{L}^{-1}$, 100 $\mu\text{mol}\cdot\text{L}^{-1}$, 200 $\mu\text{mol}\cdot\text{L}^{-1}$ and 500 $\mu\text{mol}\cdot\text{L}^{-1}$; Different lowercase letters indicate significant expression differences of genes at the 0.05 level.

Expression analysis of members of MADS-box gene family containing K-box domain under exogenous gibberellin treatment

In order to explore the expression of MADS-box family genes containing the K-box domain in maize leaves under different concentrations of gibberellin treatment, 10 genes with the largest number of gibberellin response elements were screened, and the expression of these 10 genes under gibberellin treatment was analysed. The results are shown in Figure 6. Compared with other genes, the expression levels of *Zm00001d027425*, *Zm00001d048082* and *Zm00001d048474* were lower under different concentrations of gibberellin. Compared with the control treatment, the expression levels of *Zm00001d007949*, *Zm00001d027425*, *Zm00001d035053*, *Zm00001d048082* and *Zm00001d050897* were the highest under 10 $\mu\text{mol}\cdot\text{L}^{-1}$ gibberellin treatment, *Zm00001d018142* and *Zm00001d018587* were the highest under 100 $\mu\text{mol}\cdot\text{L}^{-1}$ treatment, and *Zm00001d027957*, *Zm00001d048474* and *Zm00001d052180* were up-regulated most significantly under 200 $\mu\text{mol}\cdot\text{L}^{-1}$ treatment. The above results suggest that MADS-box gene has a certain regulatory effect of gibberellin.

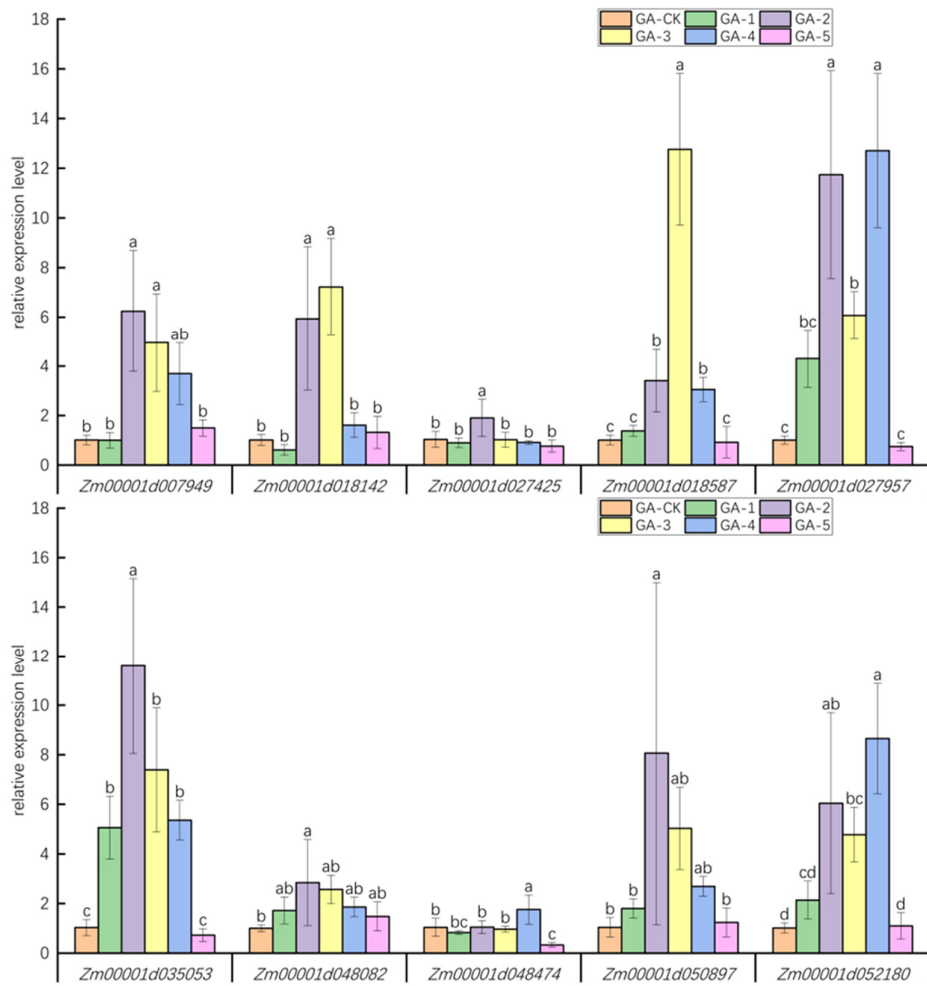


Figure 6. Expression characteristics of MADS-box family genes containing K-box domain treated with gibberellin

Note: The O-X axis represents different genes, The O-Y axis represents the expression level of the same gene under different concentrations of GA. In the figure, GA-CK, GA-1, GA-2, GA-3, GA-4 and GA-5 represent gibberellin concentrations of 0 $\mu\text{mol}\cdot\text{L}^{-1}$, 1 $\mu\text{mol}\cdot\text{L}^{-1}$, 10 $\mu\text{mol}\cdot\text{L}^{-1}$, 100 $\mu\text{mol}\cdot\text{L}^{-1}$, 200 $\mu\text{mol}\cdot\text{L}^{-1}$ and 500 $\mu\text{mol}\cdot\text{L}^{-1}$; Different lowercase letters indicate significant expression differences of genes at the 0.05 level.

Discussion

MADS-box gene family is one of the largest transcription factor families in eukaryotes, encoding transcription factors that play a key role in plant growth and development (Ma *et al.*, 2018). This family of genes are involved in almost all aspects of plant growth and development, not only in the development and formation of floral organs in higher plants, but also in the biological processes of plant organ shedding, fruit ripening, transformation from vegetative growth to reproductive growth and stress resistance (Zhao *et al.*, 2011; Schilling *et al.*, 2018; Zhao *et al.*, 2021). In this study, it was found that 52 MADS-box genes were unevenly distributed on the 10 chromosomes of maize. The number of amino acids varied greatly and most of them were acidic. Most of the proteins were located in the nucleus and the family proteins had certain hydrophilicity. The motif distribution and gene structure of the same subgroup were relatively conservative, and there were some differences in the number, type and distribution of conservative motifs contained in each protein. This shows that the MADS-box family proteins containing K-box domains are evolutionarily conservative, and the diversity of motifs also indicates that these genes may have more potential functions, which is consistent with previous studies (Shu *et al.*, 2013; Schilling *et al.*, 2018; Liu *et al.*, 2019; Zhao *et al.*, 2021).

Auxin is a small molecular organic acid that affects the final shape and function of plant cells and tissues by affecting cell division, elongation and differentiation, and coordinates plant responses to the environment (Teale *et al.*, 2006; Ljung, 2013; Brumos *et al.*, 2018). Gibberellin is a diterpenoid composed of four isoprene, which is involved in regulating physiological processes such as seed germination, Hypocotyl elongation, leaf elongation, flower, fruit and seed development (Ogawa *et al.*, 2003; Cheng *et al.*, 2004). Analysis of cis-acting elements of upstream promoter of MADS-box family gene containing the K-box domain showed that this family group contains not only a large number of light response elements I-box (Terzaghi and Cashmore, 1995) composed of GATAA, but also a variety of plant hormone response elements and stress response elements, among which auxin response elements (AuxRE, TGA-element) and gibberellin response elements (GA-motif, GARE-motif) are numerous and widely distributed. It is suggested that MADS-box family genes containing the K-box domain in maize play a certain role in response to plant hormones and environmental stress, which is helpful to the growth and development of maize and its adaptation to various environments. The expression analysis of MADS-box family genes containing K-box domain in maize leaves under different concentrations of auxin and gibberellin showed that compared with distilled water treatment, the expression level increased at first and then decreased with the increase of auxin and gibberellin concentration, and most genes were highly expressed under $10 \mu\text{mol}\cdot\text{L}^{-1}$, $100 \mu\text{mol}\cdot\text{L}^{-1}$ and $200 \mu\text{mol}\cdot\text{L}^{-1}$ auxin and gibberellin treatments. It is suggested that the MADS-box gene containing K-box domain has a certain regulatory effect on auxin and gibberellin, which affects the growth and development of maize. Studies have found that MADS-box gene is involved in a variety of hormone processes, indicating that plant hormones regulate MADS-box gene in a variety of ways, and there may be synergism or antagonism among different plant hormones (Zhao *et al.*, 2021; Song *et al.*, 2023). At present, the regulatory role of MADS-box in some plant hormones (such as ABA, BR) and drought stress signal transduction has been proved (Duan *et al.*, 2006; Shao *et al.*, 2010; Ma, 2022), but its role in other hormones and stress signal transduction has not been reported. At the same time, the presence of a response element in the MADS-box gene containing the K-box domain is not necessarily induced by the corresponding hormone or environment, on the contrary, some response elements lacking in the MADS-box gene containing the K-box domain can also be induced by the corresponding hormone or environment.

Although the study of MADS-box gene family containing the K-box domain has been quite extensive and in-depth, the research is mainly focused on its regulation of plant reproductive growth, such as the regulation of floral organ development, flowering time, ovule and fruit development, and only reasonable and clear explanation of the action mechanism and signal pathway of some genes (Gramzow and Theissen, 2010; Harris *et al.*, 2017; Yang *et al.*, 2017; Li *et al.*, 2019; Schilling *et al.*, 2020). The functions and pathways of many

genes need to be further explored, for example, there are few studies on the regulation of leaf and leaf pillow development of crop vegetative growth, but leaves and leaf pillows can affect plant type and crop yield. Therefore, through the bioinformatics analysis of maize MADS-box gene family containing the K-box domain, we can not only understand the basic characteristics of maize MADS-box genes containing the K-box domain, provide the basis for the study of maize stress resistance and improve maize yield, but also provide a basis for the future study of the function of this gene.

Conclusions

In this study, 52 MADS-box family genes containing the K-box domain were identified in maize genome. These genes were unevenly distributed on 10 maize chromosomes. There were diversity characteristics of amino acid, isoelectric point and molecular weight among the genes. Most of them were located in the nucleus and the proteins had certain hydrophilicity. Cis element analysis showed that the upstream promoter region of MADS-box gene containing the K-box domain contained different acting elements in response to hormone and stress, and the type and number of these elements were also different in different genes, indicating that maize MADS-box gene containing the K-box domain may have different functions under different hormones and environments. Generally speaking, the exon-intron distribution of maize MADS-box gene containing the K-box domain is relatively conservative, and 52 MADS-box proteins containing the K-box domain have conservative MADS-box domain, indicating that maize MADS-box family genes are highly conserved. There are some differences in the number, type and distribution of conserved motifs in different proteins, which further reveals the different functions of MADS-box genes containing the K-box domains in maize. The analysis of the expression of MADS-box family genes containing the K-box domain in maize leaves auxin and gibberellin treatment showed that MADS-box genes containing the K-box domain could regulate some plant hormones to some extent. To sum up, this study systematically analysed the MADS-box gene containing the K-box domain in maize by bioinformatics method, identified the structure of MADS-box family gene containing the K-box domain, and speculated the gene function, which laid a foundation for further study on the functional characteristics and action pathway of maize MADS-box gene, and also provided an important basis for the study of MADS-box family genes involved in maize growth and development.

Authors' Contributions

YW conceptualized and prepared the draft of the manuscript. XJ reviewed and improved the manuscript. ZZ and YZ provided technical assistance. YP supervised the study. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This work was supported by the Major Special Projects in Gansu Province (No. 22ZD6NA009) and the National Key Research and Development Project (No.2022YFD1201804).

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

- Arora R, Agarwal P, Ray S, Singh A, Singh V, ... Kapoor S (2007). MADS-box gene family in rice: genome-wide identification, organization and expression profiling during reproductive development and stress. *BMC Genomics* 8(1):242-252. <https://doi.org/10.1186/1471-2164-8-242>
- Boden S A, Østergaard L (2019). How can developmental biology help feed a growing population. *Development* 146(3):dev172965. <https://doi.org/10.1242/dev.172965>
- Brumos J, Robles LM, Yun J, Vu T C, Jackson S, Alonso JM, ... Stepanova AN (2018). Local auxin biosynthesis is a key regulator of plant development. *Developmental Cell* 47(3):306-318. <https://doi.org/10.1016/j.devcel.2018.09.022>
- Cheng H, Qin L, Lee S, Fu X, Richards DE, Cao D, ... Peng J (2004). Gibberellin regulates Arabidopsis floral development via suppression of DELLA protein function. *Development* 131(5):1055-1064. <https://doi.org/10.1242/dev.00992>
- Cui YL, Zhang L, Huang MR (2003). Research progress of MADS box genes in plants. *Chinese Journal of Biological Engineering* 23(9):50-54. <https://doi.org/10.3969/j.issn.1671-8135.2003.09.012>
- Du C (2021). Function and application of the WRKY transcription factor superfamily in plant response to stresses. *Pratacultural Science* 38(7):14-21. <https://doi.org/10.11829/j.issn.1001-0629.2020-0662>
- Duan K, Li L, Hu P, Xu SP, Xu ZH, Xue HW (2006). A brassinolide-suppressed rice MADS-box transcription factor, *OsMDP1*, has a negative regulatory role in BR signaling. *The Plant Journal for Cell and Molecular Biology* 47(4):519-531. <https://doi.org/10.1111/j.1365-313X.2006.02804.x>
- Finn RD, Coghill P, Eberhardt RY, Eddy SR, Mistry J, Mitchell AL, ... Bateman A (2016). The Pfam protein families database: towards a more sustainable future. *Nucleic Acids Research* 44(D1):D279-D285. <https://doi.org/10.1093/nar/gkv1344>
- Gao HH, Zhang YX, Hu SW, Guo Y (2017). Genome-wide survey and phylogenetic analysis of MADS-box gene family in *Brassica napus*. *Journal of Integrative Plant Biology* 52(006):699-712. <https://doi.org/10.11983/CBB16244>
- Gou J, Strauss SH, Tsai CJ, Fang K, Chen YR, Jiang XG, ... Busov VB (2010). Gibberellins regulate lateral root formation in *Populus* through interactions with auxin and other hormones. *Plant Cell* 22(3):623-639. <https://doi.org/10.1105/tpc.109.073239>
- Gramzow L, Theissen G (2010). A hitchhiker's guide to the MADS world of plants. *Genome Biology* 11(6):214. <https://doi.org/10.1186/gb-2010-11-6-214>
- Guo R, Tu M, Wang X, Zhao J, Wan R, Li Z, ... Wang XP (2016). Ectopic expression of a grape aspartic protease gene, *API3*, in *Arabidopsis thaliana* improves resistance to powdery mildew but increases susceptibility to *Botrytis cinerea*. *Plant Science* 248:17-27. <https://doi.org/10.1016/j.plantsci.2016.04.006>
- Harris F, Eagles HA, Virgona JM, Martin P J, Condon JR, Angus J F (2017). Effect of *VRN1* and *PPD1* genes on anthesis date and wheat growth. *Crop and Pasture Science* 68:195-201. <https://doi.org/10.1071/CP16420>
- Heuer S, Hansen S, Bantin J, Bretschneider R, Kranz E, Lörz H, ... Dresselhaus T (2001). The maize MADS box gene *ZmMADS3* affects node number and spikelet development and is co-expressed with *ZmMADS1* during flower development, in egg cells, and early embryogenesis. *Plant Physiology* 127(1):33-45. <https://doi.org/10.1104/pp.127.1.33>
- Hu JY, Chen Z, Hu FC, Luo ZW, Nian YW, He F, ... Zhang ZL (2017). Bioinformatics analysis of MADS-box gene family in pineapples. *Genomics and Applied Biology* 36(8):3042-3052. <https://doi.org/10.13417/j.gab.036.003042>
- Huang F, Chi YJ, Yu DY (2012). Research progress of MADS-box gene in plants. *Journal of Nanjing Agricultural University* 35(5):9-18. <https://doi.org/10.7685/j.issn.1000-2030.2012.05.002>
- Jia JT, Zhao PC, Cheng LQ, Yuan GX, Yang WG, Liu S, ... Li XX (2018). MADS-box family genes in sheepgrass and their involvement in abiotic stress responses. *BMC Plant Biology* 18(1):42-51. <https://doi.org/10.1186/s12870-018-1259-8>

- Jiao ZX, Li JC, Niu JS (2017). Analysis of MIKC- type MADS- box gene family in Wheat (*Triticum aestivum*). Journal of Agricultural Biotechnology 025(011):1756-1769. <https://doi.org/10.3969/j.issn.1674-7968.2017.11.003>
- Li C (2016). Characterization of MADS-box transcription factor gene family and biological functional validation of RsFLC and miR172 in radish (*Raphanus sativus L.*). MSc Dissertation, Nanjing Agricultural University, Nanjing.
- Li C, Lin H, Chen A, Lau M, Jernstedt J, Dubcovsky J (2019). Wheat *VRN1*, *FUL2* and *FUL3* play critical and redundant roles in spikelet development and spike determinacy. Development 146(14): dev175398. <https://doi.org/10.1242/dev.175398>
- Liu M, Fu Q, Ma Z, Sun W, Huang L, Wu Q, ... Chen H (2019). Genome-wide investigation of the MADS gene family and dehulling genes in tartary buckwheat (*Fagopyrum tataricum*). Planta 249(5):1301-1318. <https://doi.org/10.1007/s00425-019-03089-3>
- Ljung K (2013). Auxin metabolism and homeostasis during plant development. Development 140(5):943-950. <https://doi.org/10.1242/dev.086363>
- Lv SH, Meng Z (2007). Gene duplication and functional diversity of MADS-box gene family. Bulletin of Botany 24(1):60-70. <https://doi.org/10.3969/j.issn.1674-3466.2007.01.005>
- Ma H, Yanofsky MF, Meyerowitz EM (1991). *AGL1-AGL6*, an Arabidopsis gene family with similarity to floral homeotic and transcription factor genes. Genes & Development 5(3):484-495. <https://doi.org/10.1101/gad.5.3.484>
- Ma H, Zhang ZJ, Luo SP (2006). Research progress of MADS-box gene in plants. Bulletin of Biotechnology 2006(06):14-18. <https://doi.org/10.3969/j.issn.1002-5464.2006.06.004>
- Ma J, Yang Y, Luo W, Yang C, Ding P Y, Liu YX, ... Lan XJ (2017). Genome-wide identification and analysis of the MADS-box gene family in bread wheat (*Triticum aestivum L.*). PLoS One 12(7):181-189. <https://doi.org/10.1371/journal.pone.0181443>
- Ma XL (2022). Bioinformatics analysis and expression analysis of MADS-box and type III PRX gene family in Millet under drought stress. MSc Dissertation, Hebei normal University of Science and Technology, Hebei.
- Mandel MA, Yanofsky MF (1995). The Arabidopsis *AGL8* MADS box gene is expressed in inflorescence meristems and is negatively regulated by APETALA1. Plant Cell 7(11):1763-1771. <https://doi.org/10.1105/tpc.7.11.1763>
- Martínez-Ainsworth NE, Tenaillon MI (2016). Superheroes and masterminds of plant domestication. Comptes Rendus Biologies 339(7-8):268-273. <https://doi.org/10.1016/j.crv.2016.05.005>
- Melzer R, Verelst W, Theißen G (2009). The class E floral homeotic protein SEPALLATA3 is sufficient to loop DNA in 'floral quartet'-like complexes in vitro. Nucleic Acids Research 37(1):144-157. <https://doi.org/10.1093/nar/gkn900>
- Narusaka Y, Nakashima K, Shinwari ZK, Sakuma Y, Furihata T, Abe H (2003). Interaction between two cis-acting elements, ABRE and DRE, in ABA-dependent expression of Arabidopsis rd29A gene in response to dehydration and high-salinity stresses. Plant Journal 34(2):137-148. <https://doi.org/10.1046/j.1365-313x.2003.01708.x>
- Ogawa M, Hanada A, Yamauchi Y, Kuwahara A, Kamiya Y, Yamaguchi S (2003). Gibberellin Biosynthesis and Response during Arabidopsis Seed Germination. Plant Cell 15(7):1591-1604. <https://doi.org/10.1105/tpc.011650>
- Ren XY, Vorst O, Fiers MW, Stiekema WJ, Nap JP (2006). In plants, highly expressed genes are the least compact. Trends in Genetics 22(10):528-532. <https://doi.org/10.1016/j.tig.2006.08.008>
- Schilling S, Kennedy A, Pan S, Jermini LS, Melzer R, Jermini Lars S, ... Melzer Rainer (2020). Genome-wide analysis of MIKC-type MADS-box genes in wheat: pervasive duplications, functional conservation and putative neofunctionalization. New Phytologist 225(1):511-529. <https://doi.org/10.1111/nph.16122>
- Schilling S, Pan S, Kennedy A, Melzer R (2018). MADS-box genes and crop domestication: the jack of all traits. Journal of Experimental Botany 69(7):1447-1469. <https://doi.org/10.1093/jxb/erx479>
- Schmitz J, Franzen R, Ngyuen TH, Garcia-Maroto F, Pozzi C, Salamini F, ... Rohde W (2000). Cloning, mapping and expression analysis of barley MADS-box genes. Plant molecular biology 42(6):899-913. <https://doi.org/10.1023/a:1006425619953>
- Schwarz-Sommer Z, Huijser P, Nacken W, Saedler H, Sommer H (1990). Genetic control of flower development by homeotic genes in antirrhinum majus. Science (New York, N.Y.) 250(4983):931-936. <https://doi.org/10.1126/science.250.4983.931>
- Shao SQ, Li BY, Zhang ZT, Zhou Y, Jiang J, Li XB (2010). Expression of a cotton MADS-box gene is regulated in anther development and in response to phytohormone signaling. Journal of Genetics and Genomics 37(12):805-816. [https://doi.org/10.1016/S1673-8527\(09\)60098-9](https://doi.org/10.1016/S1673-8527(09)60098-9)

- Shinwari ZK, Nakashima K, Miura S, Kasuga M, Seki M, YamaguchiShinozaki K, ... Shinozaki K (1998). An arabidopsis gene family encoding DRE/CRT binding proteins involved in low-temperature-responsive gene expression. *Biochemical and Biophysical Research Communications* 250(1):161-170. <https://doi.org/10.1006/bbrc.1998.9267>
- Shu Y, Yu D, Wang D, Guo D, Guo C (2013). Genome-wide survey and expression analysis of the MADS-box gene family in soybean. *Molecular Biology Reports* 40(6):3901-3911. <https://doi.org/10.1007/s11033-012-2438-6>
- Song M, Zhang Y, Jia Q, Huang S, An R, Chen N, ... Hu S (2023). Systematic analysis of MADS-box gene family in the U's triangle species and targeted mutagenesis of BnaAG homologs to explore its role in floral organ identity in Brassica napus. *Front Plant Science* 13:1115513. <https://doi.org/10.3389/fpls.2022.1115513>
- Teale WD, Paponov IA, Palme K (2006). Auxin in action: signalling, transport and the control of plant growth and development. *Nature Reviews Molecular Cell Biology* 7(11):847-859. <https://doi.org/10.1038/nrm2020>
- Terzaghi W B, Cashmore A R (1995). Light-Regulated Transcription. *Annual review of plant biology* 46(1):445-474. <https://doi.org/10.1146/annurev.pp.46.060195.002305>
- Theißen G, Rümpler F, Gramzow L (2018). Array of MADS-box genes: facilitator for rapid adaptation. *Trends in Plant Science* 23(7):563-576. <https://doi.org/10.1016/j.tplants.2018.04.008>
- Wan ZT, Lu M, Wu SS, Mi YL, Zhai JW (2021). Identification and expression analysis of the MIKC-type MADS-box gene family in Cannabis sativa L. *Acta Pharmaceutica Sinica* 56(11):3173-3183. <https://doi.org/10.16438/j.0513-4870.2021-0892>
- Wang LN (2010). Molecular cloning, expression profile and function analysis of MADS-box genes in cotton. MSc Dissertation, Chinese Academy of Agricultural Sciences, Beijing.
- Wang X, Guo XB, Wang CY, Hou YJ, Yi J (2009). Advances of MADS-box genes in plant. *Anhui Agricultural Sciences* 37(35):17372-17375. <https://doi.org/10.13989/j.cnki.0517-6611.2009.35.027>
- Wang Y, Mu YX, Wang J (2021). Advances in the regulation of plant floral organ development by MADS-box gene family. *Zhejiang Journal of Agricultural Sciences* 33(06):1149-1158. <https://doi.org/10.3969/j.issn.1004-1524.2021.06.21>
- Wei KF, Zhang W (2018). Transcriptome-wide identification and expression profiling of the MADS-box gene family in *Hylocereus Undatus*. *Journal of Minnan Normal University (Nat. Sci.)* 004:31-38. <https://doi.org/10.16007/j.cnki.issn2095-7122.2018.04.008>
- Wei M, Wang Y, Pan R, Li W (2018). Genome-Wide identification and characterization of MADS-box family genes related to floral organ development and stress resistance in hevea brasiliensis Müll. *Arg. Forests* 9(6):304-315. <https://doi.org/10.3390/f9060304>
- Wei N, Li YP, Ma YT, Liu WX (2022). Genome-wide identification of TCP gene families in Alfalfa and analysis of their expression patterns under drought stress. *Prataculturae Science* 31(01):118-130. <https://doi.org/10.11686/cyxb2021189>
- Yang W, Lou X, Li J, Pu M, Mirbahar A A, Liu D, ... Zhang A (2017). Cloning and functional analysis of MADS-box genes, *TaAG-A* and *TaAG-B*, from a Wheat K-type cytoplasmic male sterile line. *Front Plant Science* 8:1081. <https://doi.org/10.3389/fpls.2017.01081>
- Yun KY, Park MR, Mohanty B, Herath V, Xu FY, Mauleon R, ... Bruskiwich R (2010). Transcriptional regulatory network triggered by oxidative signals configures the early response mechanisms of japonica rice to chilling stress. *BMC Plant Biology* 10(1):1-29. <https://doi.org/10.1186/1471-2229-10-16>
- Zhang Y, Wang J Q, Yu ZJ, Xu Q, Zhang L, Pan YX (2022). Bioinformatics analysis of MIKC-type MADS-box gene family in legumes. *Chinese Journal of Oil Crop Sciences* 44(04):798-809. <https://doi.org/10.19802/j.issn.1007-9084.2021175>
- Zhao D, Chen Z, Xu L, Zhang L, Zou Q (2021). Genome-Wide analysis of the MADS-Box gene family in Maize: gene structure, evolution, and relationships. *Genes* 12(12):1956. <https://doi.org/10.3390/genes12121956>
- Zhao W, Zhang LL, Xu ZS, Fu L, Pang HX, Ma YZ, ... Min DH (2021). Genome-Wide analysis of MADS-Box genes in Foxtail Millet (*Setaria italica* L.) and functional assessment of the role of *SiMADS51* in the drought stress response. *Front Plant Science* 12:659474. <https://doi.org/10.3389/fpls.2021.659474>
- Zhao Y, Li X, Chen W, Peng X, Cheng X, Zhu S, ... Cheng B (2011). Whole-genome survey and characterization of MADS-box gene family in maize and sorghum. *Plant Cell, Tissue and Organ Culture (PCTOC)* 105(2):159-173. <https://doi.org/10.1007/s11240-010-9848-8>



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