Paper S6 Components of qRT-PCR reaction

Reagent	Volume (μL)
ddH_2O_2	5
2X SYBR Green Fast qPCR Mix	7.5
Forward primer（10μM）	0.4
Reverse primer（10μM）	0.4
cDNA	1
Total	10

Paper S7 Reaction Step

Step	Temperature	Time
1	95 ℃	3 min
2	95 ℃	5s
3	60 ℃	30 s
4	+ H10Plate Read，Go to step 2	40-45 cycles
5	Melt Curve 65-95℃	
6	+ Plate Read，increment 0.5℃	0.5 s
7	end	

Paper S8 Primers used in RT-PCR

Gene	Forward primer	Reverse primer
Zm00001d012321	TTATCGTTCTCGAGGCCAGC	GGCTCCGGTTGAACTCTCTC
Zm00001d037590	TTCTGCAGCCAAGCATGGAA	CCGTAAAGTGGTGGTTCGCT

Paper 9

Subcellular localization prediction

The subcellular localization prediction was performed using the WoLF PSORTT (https://wolfpsort.hgc.jp/) website. After selecting plants for classification, upload the protein sequences of the hub gene family and submit them. Predict the location of each protein in the cell, where nuclear represents localization in the nucleus, cytoplasmic means localization in the cytoplasm, and similarly chloroplast, mitochondrial, plasma membrane, and extracellular represent localization in the chloroplast, mitochondria, plasma membrane, and extracellular, respectively. After obtaining the data, the positioning results were visualized using the Pathway Builder Tool 2.0 drawing software.

Gene Structure and Cis-control element analysis

Analysis of Gene Structural Characteristics

The plant database (Ensembl Plants: http://plants.ensembl.org/) was used to search the candidate gene ID, and download the structural annotation information of the gene, followed by performing the statistical analysis, including gene name, gene length, Exons (start, stop), and introns.

Cis-control element analysis

For promoter analysis of candidate genes, each gene's upstream 2kb sequence was downloaded, and the Cis-acting regulatory elements were obtained by submitting all sequences to the Plant CARE database (http://bioinformatics.psb.ugeniant.Be/webtools/plantcare/html/). After sorting the data of the obtained cis-acting regulatory elements, the elements with functional annotation or with more attention were retained, the core promoters with no actual function were eliminated, and the CAAT-box and TATA-box were also eliminated. This study only retained the four categories, including hormones, light response, environmental stress, and cis-regulatory elements related to growth and development as research objects.

Gene family identification among different maize varieties

In this study, the identification, phylogenetic analysis and selection pressure analysis of the homologous gene families of *Zm00001d012321* and *Zm00001d037590* in 15 different maize inbred lines were carried out. Among them, the different maize inbred lines of maize species include B97, CML52, CML247, DK105, Ki3, Ki11, Ky21, Mo17, Mo18, Ms71, NC350, Oh43, P39, W22, and Mexican ruminant grass (Zx-PI566673), all data Both are downloaded from the Maize GDB database.

The homologous genes of *Zm00001d012321* and *Zm00001d037590* were identified by the similarity between the target sequence and the aligned sequence, and the genes whose % identity between the aligned sequence and the target sequence was higher than 60% were classified as paralogous genes. Blast-2.9.0 was used to construct a BLAST database using the protein sequences corresponding to the longest transcripts extracted from the genomes of inbred lines of each maize species, and then compared with the protein sequences of the longest transcripts of *Zm00001d012321* and *Zm00001d037590*, and the comparison parameters: Score value$\geqslant$100, E <1e−5, and the output format is '6' to identify *Zm00001d012321* and *Zm00001d037590*

homologous gene sequences.

MEGA_X_10.0.5_win64 was used to construct a phylogenetic tree. First, the protein sequences of *Zm00001d012321* and *Zm00001d037590* were aligned with ClustalW, and the alignment parameters were set to default parameters. After obtaining the comparison results, the ML maximum likelihood method based on the Poisson correction model was used to construct the phylogenetic tree, and Bootstrap was used for detection, and the parameter was set to 1000 times.

The CDS sequences of the above-mentioned gene family members were aligned respectively. The sequence alignment was performed by the ClustalW method of the MEGA-X software, and the selection pressure analysis was performed on the alignment results by the KaKs_Calculator 2.0 software, and YN was selected as the model parameter. According to the size of Ka/Ks, the genes are divided into three categories and counted. The division principles are as follows:

Ka>Ks->Ka/Ks > 1, positive selection

Ka＝Ks->Ka/Ks=1, neutral evolution

KaKa/Ks < 1, purify selection

Paper 10

Gene Structure and Cis-control element analysis

Analysis of Gene Structural Characteristics

The gene annotation files of the three hub genes were extracted, and the length and related information of the hub genes were counted. The results showed that the Zm00001d012321 gene was 1425 bp in length and contained a CDS region; the Zm00001d037590 gene was 4643 bp in length, including 2 UTR regions and 10 CDS regions. The results of hub gene sequence statistics are presented in Table S15. Zm00001d012321 gene contains 1 exon,and no intron; the Zm00001d037590 gene contains 12 exons and 11 introns. The statistics of the exon distribution of each hub gene are shown in Table S16.

Cis-control element analysis

The extracted cis-acting elements in the upstream 2kb sequence of the hub gene

are shown in Table S17. In the Zm00001d012321 gene, there was 1 cis-acting element, each containing MYB transcription factor binding and recognition sites, and there were 7 hormone-responsive cis-acting elements. There were 5 cis-acting elements involved in adversity defense response, 3 cis-acting elements involved in light response, and 1 cis-acting regulatory element related to meristem expression. It is speculated that the hub gene Zm00001d012321 may be mediated by the regulation of hormonal signaling systems in maize introgression lines for regulating stress defense under adversity. In the Zm00001d037590 gene, 10 cis-acting elements were found to respond to hormones, 7 cis-acting elements involved in the abscisic acid response, 9 cis-acting elements involved in adversity defense response, and cis-acting elements involved in the light response. It is inferred that the hub gene Zm00001d037590 may be related to the abscisic acid-mediated hormone signal transduction system in regulating the response to external stress.

Gene family identification among different maize varieties

In this study, the protein sequences corresponding to the longest transcripts of maize inbred lines B97, CML52, CML247, DK105, Ki3, Ki11, Ky21, Mo17, Mo18, Ms71, NC350, Oh43, P39, W22 and ZX-PI566673 were compared, the results showed that 31 paralogous genes with Zm00001d012321 were identified from the above 15 maize inbred lines, In addition to the identification of 1 paralogous gene in ZX-PI566673, 2 paralogous genes were identified in the remaining 14 maize inbred lines. The Zm00001d012321 homologous genes identified were highly similar in terms of gene length, gene structure, DNA, CDS, and protein sequences (Table S18), it is inferred that this gene is highly conserved in maize.

There are 109 paralogous genes with Zm00001d037590 identified from the above-mentioned 15 maize inbred lines, Among them, 7 paralogous genes were identified in maize inbred line B73; 11 paralogous genes were identified in maize inbred line B97; 5 paralogous genes were identified in maize inbred line DK105; Seven paralogous genes were identified in the maize inbred line Ki3; 1 paralogous gene was identified in the maize inbred line Ky21; 4 paralogous genes were identified in the maize inbred line Mo18; Seven paralogous genes were identified in the inbred line Ms71; seven

paralogous genes were identified in the maize inbred line NC350; seven paralogous genes were identified in the maize inbred line Oh43; the maize inbred line 4 paralogous genes were identified in P39; 7 paralogous genes were identified in the maize inbred line NC350; 4 paralogous genes were identified in ZX-PI566673; 11 paralogous genes were identified in inbred line CML52; 13 paralogous genes were identified in maize inbred line Ki11; 8 paralogous genes were identified in maize inbred line CML247; maize inbred line Mo17 2 paralogous genes were identified (Table S19).

Phylogenetic analysis was performed on the DNA sequences of the target gene Zm00001d012321 and 31 HSP genes in inbred lines of other maize species (Figure S10-a), the results of phylogenetic analysis showed that the above 31 HSP orthologous genes were divided into 2 Subfamily, HSP1 and HSP2 respectively. The results showed that in the same homology group, the genes from different maize inbred lines were highly similar or even identical in gene length, gene structure, base sequence, CDS sequence and protein sequence. However, there were significant differences in gene length, gene structure, base sequence, CDS sequence and protein sequence among different groups (namely HSP1 and HSP2). In addition, this study also showed that Zm00001d012321 was closely related to the maize inbred lines oh43 and ky21.

Phylogenetic analysis of the protein sequences encoded by the target gene Zm00001d037590 and 109 genes in other maize species (Figure S10-c) showed that the above 109 PDIL gene family member genes were divided into 3 subfamilies, namely PDIL1, PDIL2 and PDIL3. In addition, the study also showed that the same within the family, maize from different kinds of genes in the gene, gene structure, the base sequence length, height of CDS sequences and protein sequences are similar, but different length of the gene, gene structure, between the family base sequences, CDS, protein sequences, such as obvious differences. The PDIL1 subgene family has the most family members identified, and the PDIL3 subgene family has the least family members. It is speculated that the gene family members have different degrees of differentiation in the evolutionary process.

In this study, the selection pressure analysis of Zm00001d012321 and Zm00001d037590 paralog genes in 16 maize inbred lines (Figure S10-b, d) showed

that in 16 maize inbred lines Zm00001d012321 and Zm00001d037590 paralogs The mutations of the homologous genes affected by the natural environment are significantly lower than those caused by the non-natural environment. The Ka/Ks values in each maize inbred line showed a trend of <1, and peaked at 0.5 and 0.7. It shows that Zm00001d012321 and Zm00001d037590 and their homologous genes in the inbred line of maize species have the effect of purification and selection, and are relatively conservative in natural evolution.