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Identification of DREB family genes in banana and the functional under drought and cold stress

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Abstract: Bananas are one of the most important cash crops in the tropics and subtropics. The industry is affected by drought and low temperature stress.~~Abiotic stress such as drought and low temperature affect the normal growth and development of bananas and have serious implications for the development of the banana industry. . As one of the major transcription factors, the~~ The DREB(dehydration responsive element binding protein) gene family, as one of the major transcription factors, plays crucial~~an important~~ role in defence against abiotic stress. Currently,~~At present~~, systematic analyses of the banana DREB (*MaDREB*) gene family have not yet been reported. In this study, 103 members of the *MaDREB* gene were identified in the banana genome. In addition, transcriptomic analysis~~transcriptome sequencing~~ results revealed~~indicated~~ that *MaDREB*s responded to drought and cold stress. The expression of *MaDREB14/22/51* was induced by drought and cold stress, which were further selected for analysis~~The expression of three members, *MaDREB14/22/51*, which was highly induced by drought and cold stress and was therefore selected for further study. .~~ The qRT-PCR validation results confirmed these transcriptome results.~~were consistent with those obtained using the transcriptome~~. Additionally, transgenic Arabidopsis plants overexpressing *MaDREB14/22/51* exhibited enhanced resistance to drought and cold stress~~Further, the transgenic Arabidopsis with *MaDREB14/22/51* overexpression showed enhanced drought and cold resistance by reducing MDA content and increasing PRO and soluble sugar content~~. This study enhances~~deepens~~ the understanding of the function~~characterisation~~ and evolution of the *MaDREB* gene family, provides ~~a~~ new insights into their regulatory role under abiotic stress, and lays a good foundation for improving drought and cold stress-tolerance banana verities.~~basis for further understanding of the regulatory role of *MaDREB* genes under abiotic stress, and lays a good foundation for providing candidate members for stress tolerance breeding in banana.~~

Keywords: Genome-wide, DREB, Climate Change, Drought, Cold.~~DREB; banana; gene family; abiotic stress~~

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1. Introduction

Abiotic stresses such as drought and cold temperature limits plant growth and development under climate change circumstances.~~Abiotic stress such as drought, salinity and temperature changes have a negative impact on plant growth and development, resulting~~

~~in reduced agricultural production and severe economic losses, especially in a changing climate~~[1]. As a result, plants have evolved a variety of regulatory system~~in order~~ to protect themselves from the damage caused by harsh environmental conditions.~~, plants have developed a variety of regulatory systems.~~ Banana (*Musa acuminata* L.) is the fourth largest food crop in the world and its widely grown in tropical and subtropical regions. It is a majestic annual monocotyledonous herbaceous plant, holds a cherished place in the hearts and diets of people worldwide. Its delectable fresh fruits are not only a delight to consume but also contribute significantly to global food security and —
nutrition. The banana plant's unique combination of growth habits, nutritional value, and economic importance makes it a cherished and indispensable crop in many tropical and subtropical regions. Banana cultivation requires sufficient water, drought stress has a severe impact on banana plant growth and fruit development. ~~at~~ At the same time, banana is also highly sensitive to cold temperatures~~typically a cold-sensitive crop~~. The critical temperature for growth inhibition is 10°C. A decrease to 5°C will result in the leaves turning yellow due to cold damage, while a further drop to 2.5°C will cause chilling injury to the banana plant, leading to the rotting and death of the pseudostem centre-~~When bananas are grown at low temperatures (< 12°C), browning and pockmarking of the fruit can occur~~. Therefore, abiotic stresses such as drought and temperature adversely affects both growth and productivity.~~can affect its growth and industrial development~~.

Transcription factors, which are proteins that regulate gene expression by binding to specific cis-acting elements in gene promoters, and play a crucial role in mediating plant responses to abiotic stress ~~Transcription factors, otherwise known as executing factors, which control pressure responsive qualities by specifically restricting cis-acting sites in their regulators and activating or repressing records~~[2-3]. Several types of stress~~A number of stress~~ response transcription factors, including MYB[4],WRKY[5], bZIP[3,6] and AP2/ERF[7], are activated in response to abiotic stimuli. The AP2/ERF (APETALA2/ethylene-responsive factor) family consists of~~was isolated into~~ four major subfamilies: AP2, ERF, DREB and RAV[8,9]. Among these~~A key subgroup of the AP2/ERF gene family~~, the DREBs subgroups is characterized by a conserved AP2 domain that interacts with the DRE (Dehydration-Response Element) element ~~, includes a single conserved AP2 domain that contains the DRE element~~[10-12]. The sequence~~element,~~ A/GCCGAC ~~sequence were~~ found in genes responsive to drought and cold~~in the drought and cold sensitive genes~~ [13-16]. Based on the sequence similarities within their DNA-binding domains, DREB proteins are further categorized into six subgroups (A1- A6). Members of these subgroups serve distinct functions in plants. Notably, the A-1 subgroup, comprising DREB1/CBF (C-repeat Binding Factor)-like genes, is primarily activated by low temperatures and orchestrates the expression of numerous cold stress-responsive genes. In contrast, the A-2 subgroup, consisting of DREB2 genes, primarily functions in response to osmotic stress [8]. In mung bean (Vigna radiata L.), VrDREB2A is induced by drought and salt stresses, and its heterologous expression enhances tolerance to both drought and high salt stress without compromising growth [17]. Similarly, in Arabidopsis, DREB2A and

DREB2B have been identified and shown to actively respond to dehydration and high salinity stress [18].Additionally, BjDREB2 plays a pivotal role in ABA-independent gene expression during drought stress [19].

~~According to reports, DREB family members can be divided into six groups[16].~~

In recent years, DREB has been implicated in the response of plants to a wide range of abiotic stress. For example, 41 DREB genes have been identified~~found~~ in barley with most responding to drought and salt stress ~~. Most of them can respond to drought and salt stress described, five of which are altogether upregulated with drought conditions[17,20]~~. Overexpressing of *OsDREB1A* ~~makes~~ in transgenic plants has been shown to confer resistance~~resistant~~ to salt, drought and low temperature stress[18,21]. Similarly, ~~The~~ the expression of *PpDBF1* ~~had been~~is up-regulated during salt and drought stress[19,22], while the ~~expression of~~ *SbDREB2A* ~~had been~~is induced under drought, NaCl and high temperature stress[23,30]. Additionally, ~~Under the salt stress, the expression of~~ *AhDREB1* expression is induced under salt stress and Arabidopsis DREB1/CBF regulate the expression of many genes in response to drought and cold stress ~~was induced[21]. Arabidopsis DREB1/CBF can regulate the expression of many genes in response to drought and cold stress[24-25,52]~~.

Several DREB transcription factor genes have been identified in various plants, including maize , Arabidopsis, soybean, and sesame ~~A number of genes from maize[23], Arabidopsis, soybean and sesame have so far been identified that encode the DREB transcription factor[26-27,4]~~. ~~Nevertheless~~However, there are fewer reports on this family in bananas. The sequencing of the entire banana ~~genome of the banana~~ has enabled~~allowed us to carry out~~ a comprehensive examination of the banana's genetic traits~~qualities~~. As DREBs play a critical role in the response to various abiotic stress, we selected this family for extensive study in banana. In this research, the DREB gene of banana (*MaDREB*) have been identified, also the phylogenetic relationship, gene structure, the expression patterns have been analysed. Specifically, ~~Three~~ three of the *MaDREB*s named *MaDREB14*, *MaDREB22*, and *MaDREB51* have been choosed and transform to Arabidopsis. The result provides a foundation for understanding the regulatory roles of MaDREBs and offer significant insights for banana bio-breeding and stress management.~~a good starting point for focusing on the management tool of *MaDREB*s and also gives significant qualities to banana bio-breeding~~.

2. Results

2.1. *Phylogenetic analysis*~~*The evolutionary tree construction*~~

A total of 103 banana *MaDREB*s were identified,with in length ranging ~~in length~~ from 447 bp to 1314 bp (Table S2). The DREB family sequences of Arabidopsis were obtained from the TAIR website, and a phylogenetic tree was constructed to examine the evolutionary relationship between the banana and Arabidopsis DREB family members. This tree was constructed using the neighbor-joining method in MEGA-X~~the phylogenetic relationship tree between the banana DREB family members and the Arabidopsis DREB family members was constructed by the neighbour-joining method using MEGA-X~~ (Fig.1). Based on the classification of Arabidopsis DREB families and the closeness of their phylogenetic relationships, the banana DREB family was clustered into six subgroups: A1, A2, A3, A4, A5, and A6. These subgroups contained 8, 13, 2, 43, 25, and 12 genes, respectively.

~~The banana DREB family was clustered into six subgroups with Arabidopsis related families based on the classification of Arabidopsis DREB families and the closeness of their phylogenetic relationships. Among the six banana subgroups, the A1, A2, A3, A4, A5 and A6 subgroups contained 8, 13, 2, 43, 25 and 12 genes, respectively.~~ Among these genes, some showed one-to-one correspondence with Arabidopsis DREB family genes. For

example, *MaDREB12* in the A4 subgroup is homologous to ~~the~~ AT4G16750. Additionally,~~And~~ some of the genes have ~~a~~ duplicated counterpart. For instance, in t~~T~~he A2 subgroup, AT1G75490 and AT3G57600 of ~~A2 subgroup~~ correspond to *MaDREB17/98* and *MaDREB24/59*, respectively~~,~~. In A3 subgroup, the AT2G40220 ~~of A3 subgroup~~ corresponds to *MaDREB20/73*, and the AT2G35700 gene of A4 subgroup corresponds to *MaDREB12*.

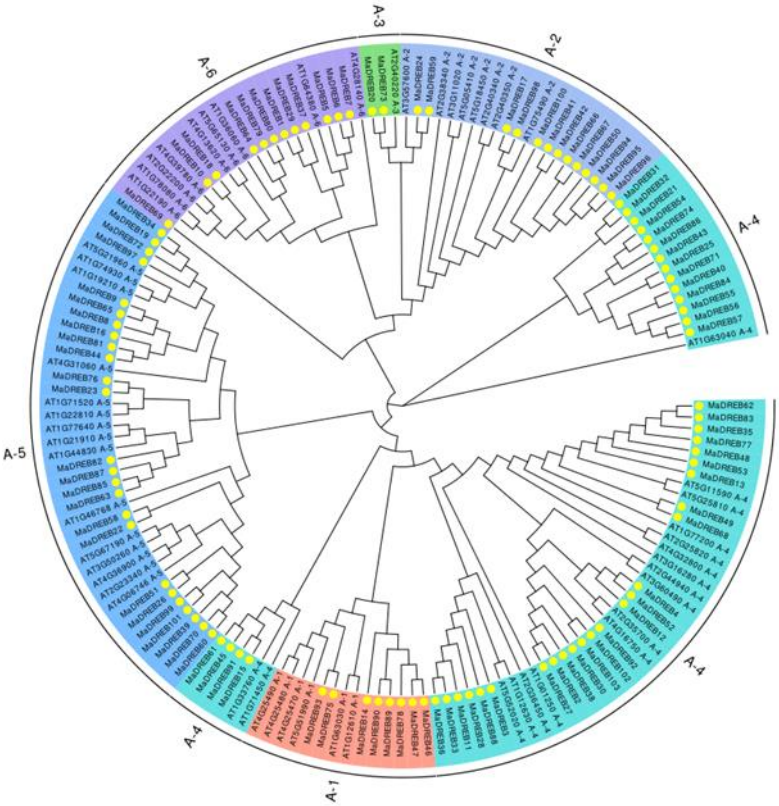

Figure 1. Construction of phylogenetic tree of banana DREB gene family. The phylogenetic tree illustrates the relationships within the DREB family across banana and Arabidopsis thaliana.

2.2. *Analysis of structural domain*

~~Prediction of t~~The conserved motifs of the 103 members of the banana DREB family were predicted using the~~via the~~ MEME website (Fig.2). The results showed that all identified members of the banana DREB family were structurally conserved, containing motif1, motif2 and motif3~~,. and it was hypothesised that this class of~~These motifs are hypothesized to be major conserved motifs of the DREB family~~the major conserved motifs of the DREB family, as speculated above~~. Gene structures of the identified banana DREB family members were visualized using TBtools, with the GFF3 annotation file as a reference~~T-Btools was used to visualise the gene structures of the identified banana DREB family members using the obtained GFF3 annotation file as a reference~~. The results indicated~~showed~~ that most ~~of the genes of the~~ banana DREB family members do not contain introns, ~~and~~ only ~~some~~ a few of the members contain 1-3 introns within their structures~~,~~. These introns appear only as coding sequences (CDSs)~~which appear only as CDSs~~. Additionally~~In addition~~, most members contain 5' and 3' untranslated regions (UTRs)~~UTRs~~.

Figure 2. Analysis of DREB gene family domain in banana. The motifs are depicted through the use of three distinct color-coded boxes. A schematic representation of the UTR, exon, and intron regions, where green frames denote the UTR, exons are symbolized by yellow boxes, introns are signified by black lines, providing a clear visualization of the gene structures.

2.3. Analysis ~~the structures~~ of MaDREB promoters structures

Promoter analysis of the 103 MaDREB genes (-2000 bp) revealed the presence of TATA and CATA motifs in all promoters.~~TATA and CATA motifs were present in all 103 *MaDREBs* promoters (2000 bp),~~ Additionally, a large number of cis-acting elements associated with abiotic stress responses were identified, including elements linked to salicylic acid, low temperature, abscisic acid, and light response.~~along with a large number of cis-acting elements linked to abiotic stress, including salicylic acid, low temperature, abscisic acid, and light response~~. The promoters also contained hormonal response elements such as those responsive to auxin, MeJA (methyl jasmonate), and gibberellin~~There are also hormonal response elements such as auxin, MeJA, gibberellin and so on~~ (Fig. 3).

Figure 3. The promoter element analysis. The distribution and density of cis-acting elements within the upstream promoter region of *MaDREB*s are shown.

2.4. Collinearity analysis of MaDREBs

Gene duplication events are critical for the evolution of plant genomes. To determine the duplication events of the *MaDREB* genes, we used TBtools to perform intraspecific covariance of banana DREB genes. Using Gene Density Profile and Gene Location Visualize from the GTF/GFF programs, the 103 identified DREB family genes were located on the chromosomes of the banana genome based on the genome annotation information~~, and the results are shown in~~ (Fig. 3). The DREB family genes are distributed on across 10 chromosomes of the banana genome., Chromosome 10 has the highest number of genes (16), followed by chromosomes 2, 4, 5, 6, 9, and 11, each with 9 genes. Chromosomes 4, 7, and 8 have 14, 12, and 7 genes, respectively.~~It can be seen that the genes of the DREB family are distributed on 10 chromosomes of the banana, with chromosome 10 having the highest number of genes (16), followed by chromosomes 2, 4, 5, 6, 9 and 11 (9), there are 14,12,7 members in chromosomes 4,7 and 8, respectively~~.

All genes were displayed on ~~ten chromosomes~~a circle representation of the ten chromosomes, and 42 collinearity pairs were identified~~found~~ (Fig. 4A). ~~According~~ In ~~to~~ the interspecific covariance analysis (Fig. 4B), 1744 and 10371 genes in Arabidopsis and rice, respectively, were found to collinear with genes in banana. Most of the DREB members in banana shared 1 or 2 homologous gene s with Arabidopsis or rice.

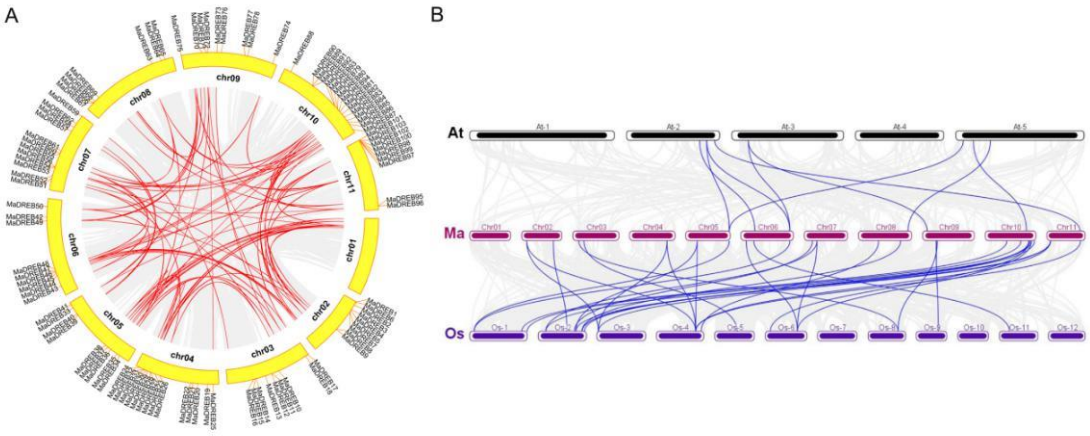

Figure 4. The collinearity distribution of *MaDREB*s (A) and synteny collinearity of banana, Arabidopsis and rice genomes (B). The blue line represented the associated the gene pairs, in this map, blue lines denote homologous associations between the two species. ~~.~~

2.5. Protein interaction analysis

An analysis of protein interactions revealed that the majority of the proteins interacting with *MaDREB*s were mainly AP2 (APETALA 2), ABF2 (ABRE binding factor 2), RHL41 (zinc finger protein 41), PFT1 (pore-forming toxin-like), HSFA3/A2 (heat shock transcription factor A3/A2), EXO (exocyst gene), and others.~~etc.~~ These proteins are are largely involved in~~What's more, their functions were largely concerned with~~ plant resistance and development, providing important insights for further practical research into their cooperative roles and guiding principles~~the guiding principles of cooperation~~ (Fig.5).

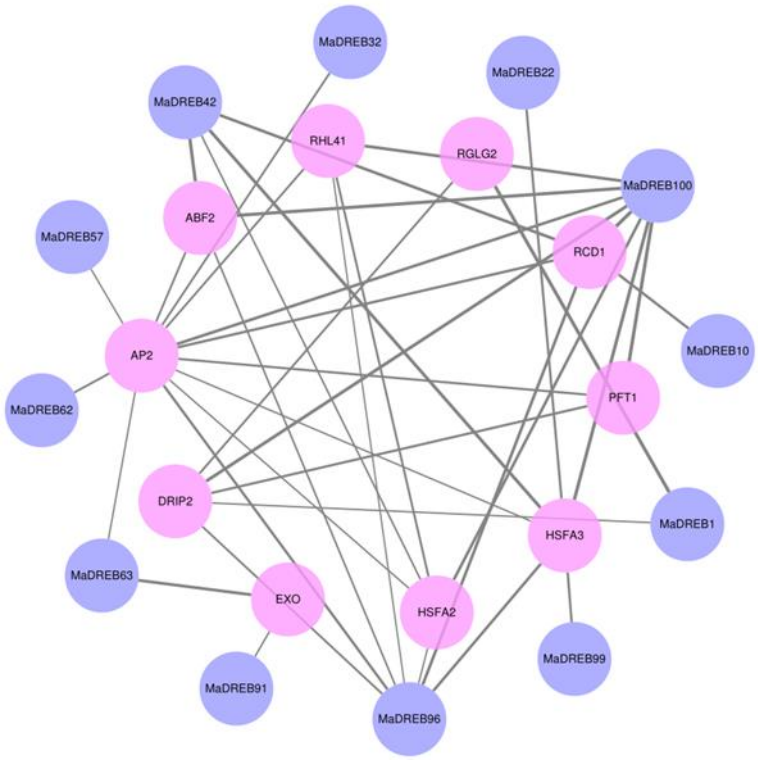

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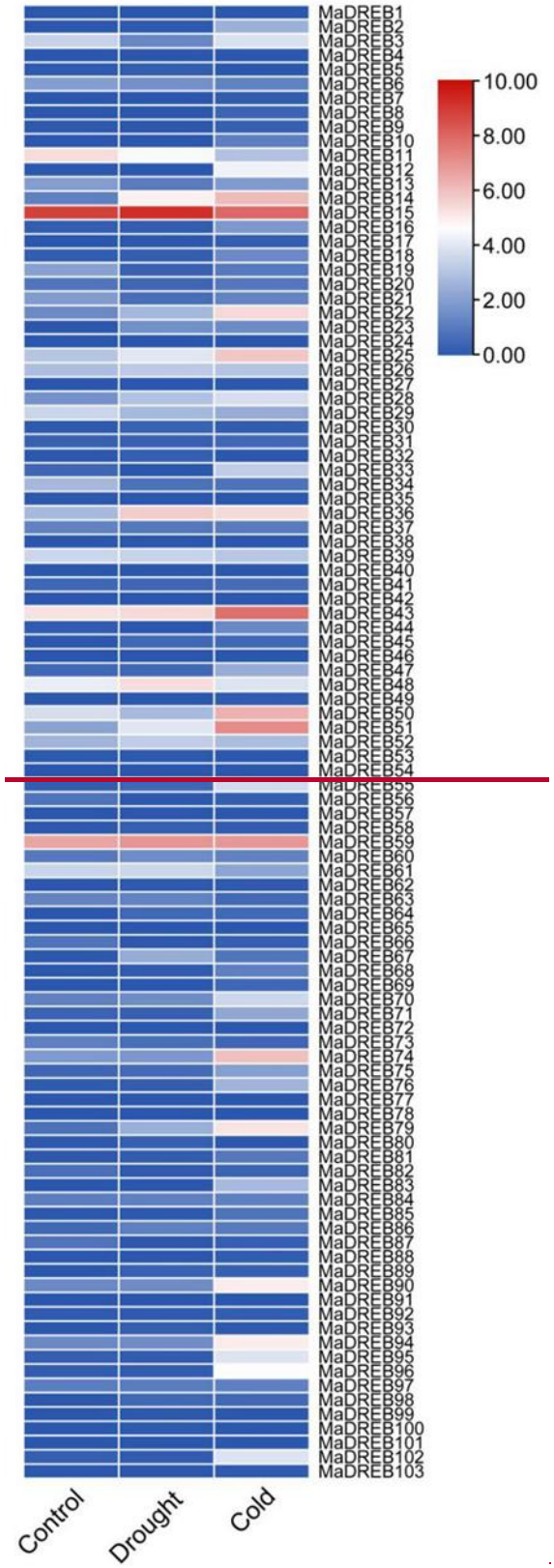
Figure 5. The predicted interaction networks of *MaDREB* proteins. This visualization provides insights into the potential functional associations and networks that ***MaDREB*** genes may be a part of within the cellular context.

2.6. Three MaDREBs were selected to respond to drought and cold stress

Based on the transcriptome data, there are many members that respond to drought and cold stress (Fig.6A). Notably~~Of these~~, more members were found to respond to cold stress compared to drought stress~~responded to cold stress than to drought stress~~. The members that responded to both stress conditions include~~can respond to both stress are~~ *MaDREB14/22/25/36/51/67/79/80/81*. Among these,~~Three members with relatively high expression,~~ *MaDREB14*, *MaDREB22* and *MaDREB51* exhibited relatively high expression levels and were selected for further investigation.~~, were selected for further study~~. Further quantitative real-time PCR (qRT-PCR) analysis was subsequently conducted on these three genes~~qRT-PCR was performed on these three genes~~ (Fig.~~7~~6B). The qRT-PCR results demonstrated that *MaDREB14*, *MaDREB22*, and *MaDREB51* were indeed responsive to both drought and cold stress, showing expression patterns consistent with the transcriptome findings.~~The results showed that the three genes were able to respond to drought and cold stress, and the expression trends were consistent with the transcriptome results~~.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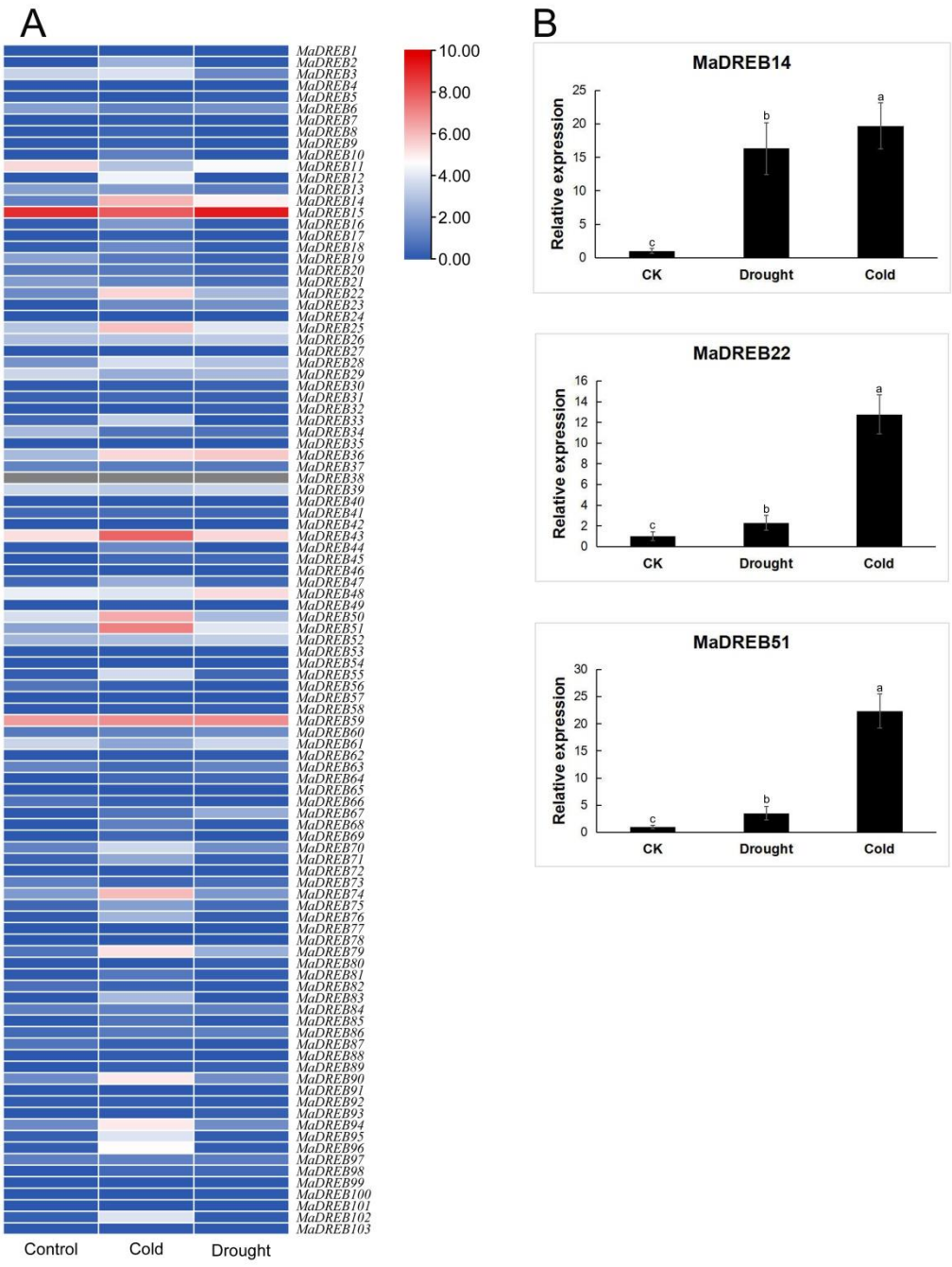


Figure 6. Transcriptome *MaDREB*s responding to drought and cold stress(A) and qRT-PCR of *MaDREB*s responding to drought and cold stress(B). Red and blue indicated high and low expression levels, respectively. The a-c was marked on top of the blank bar, which represented differential significance.

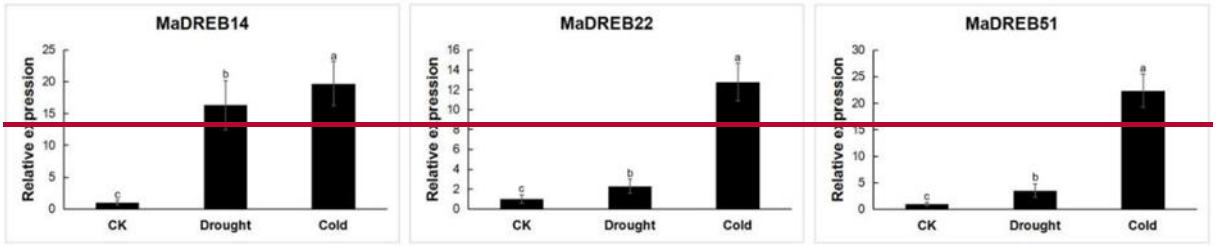


Figure 7. qRT-PCR of *MaDREBs* responding to drought and cold stress. The a–c was marked on top of the blank bar, which represented differential significance.

2.7. Overexpression of MaDREB enhances transgenic Arabidopsis tolerance to drought and cold stress

Currently, tThe ability of DREBs in plants to respond to abiotic stress in plants has been extensively studieddiscussed. In this research, we focused on *MaDREB14/22/51*, which is are induced under drought and cold stress (Fig.8 7).

The three genes were transformed into Arabidopsis thaliana, resulting in three independent transgenic lines for each gene. These transgenic lines were subjected to both drought and cold stress respectivelyand three independent transgenic plants were obtained from each, which were subjected to drought and cold stress, respectively. The results showed that under drought stress, all three genes enhanced the drought tolerance of the transgenic Arabidopsis plants, with *MaDREB14* showing the greatest improvement in drought resistancewhen the transgenic Arabidopsis were subjected to drought stress, all three genes improved the plants' drought tolerance. Among them, MaDREB14 showed the strongest drought resistance. In contrastFor cold stress, the phenotypic differences between transgenic plants and wild-type (WT) plants under cold stress were not significant. Nevertheless,uUpon returning to normal temperature, some of the phenotypic traitsphenotypes of the transgenic plants were restored.

2.8. Chlorophyll fluorescence measurements under drought and cold stress

The plant cChlorophyll fluorescence measurement were used to evaluate plant damage inducedsystem was used to further detect the damage caused to plants by drought and cold stress. Before stress treatments, there were minimal differences in chlorophyll fluorescence color between WT and transgenic plantsAs shown in the figure, there was little difference in chlorophyll fluorescence colour between wild type and transgenic plants before the stress treatments, However, underwhereas when subjected to drought and cold stress, all plants were damaged to varying degrees, with transgenic plants being less damaged than WT (the false colour of normal plant leaves was blue, whereas the false colour of leaves changes from green to yellow or even black, indicating more damage to the plant). Among the transgenic lines, *MaDREB14* showed the least damage, as indicated by the fluorescence images*MaDREB14* was the least damaged, as shown by the chlorophyll fluorescence colours (Fig.8 7).

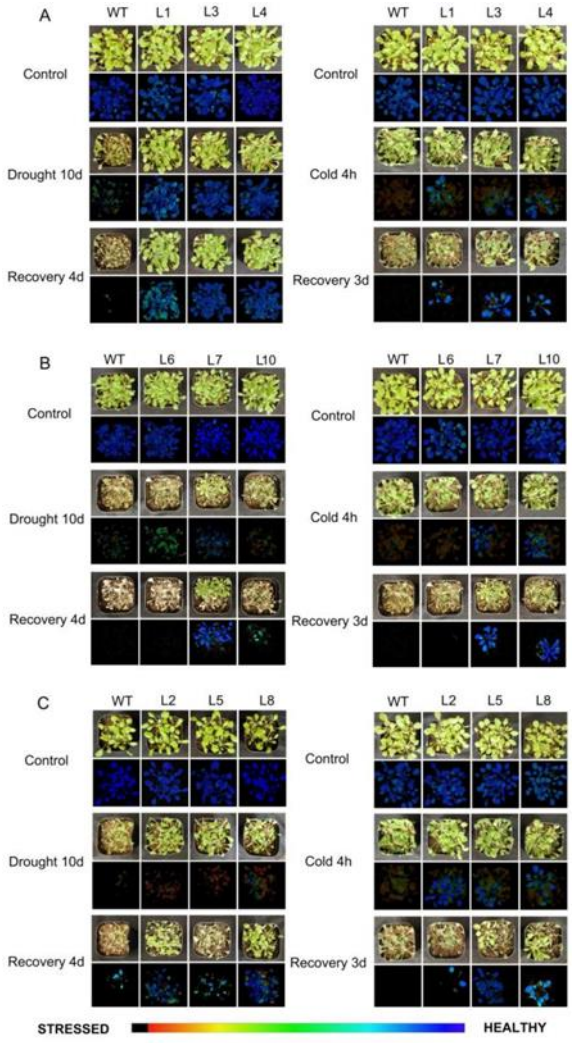
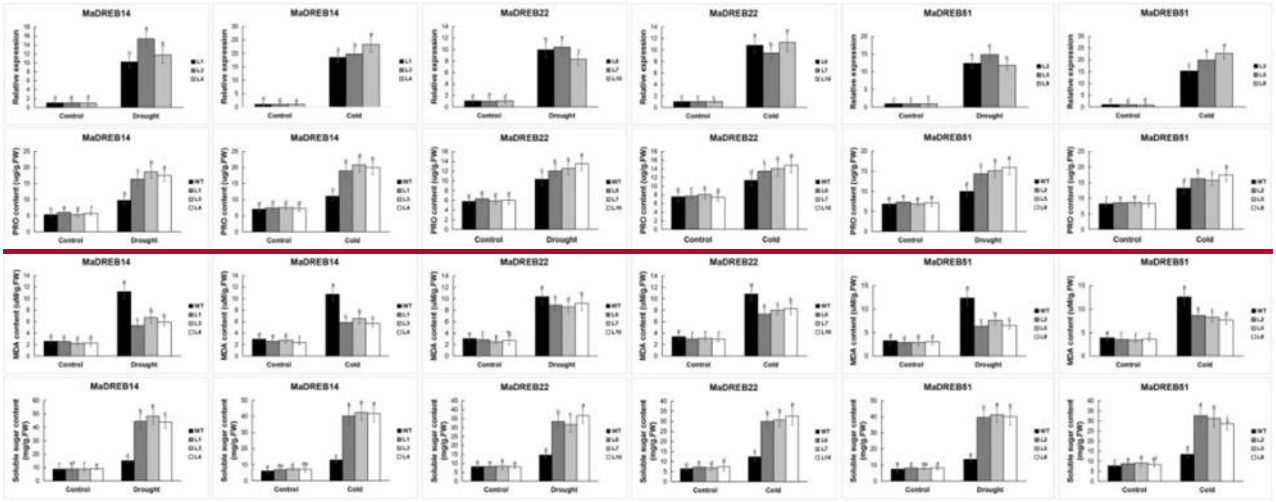


Figure ~~8~~7. Phenotypes of transgenic Arabidopsis under drought and cold stress treatments and chlorophyll fluorescence measurements. A *MaDREB14* B.*MaDREB22* C.*MaDREB51.*

2.9. Measurement of Physiological indicators ~~measurement of~~ in transgenic Arabidopsis

The expression levels of different transgenic Arabidopsis thaliana under drought and low temperature stress were significantly higher than those under normal conditions(Fig.8).Physiological indicators, including malondialdehyde (MDA), proline (PRO), and soluble sugars, were measured in WT and transgenic Arabidopsis plants. Under normal conditions, there were no significant differences in MDA, PRO, and soluble sugar levels between WT and transgenic plants. However, after exposure to drought and cold stress, transgenic plants showed significantly lower MDA levels and higher PRO and soluble sugar levels compared to WT plants. These results indicate that *MaDREB14/22/51* enhance drought and cold tolerance in transgenic Arabidopsis ~~Physiological indicators of WT and transgenic plants were analysed and there were no massive differences in MDA, PRO and soluble sugar levels between WT and transgenic plants under normal conditions. After drought and cold stress, the transgenic plants had lower MDA and higher PRO and soluble sugar levels than the WT. *MaDREB14/22/51* appeared to enhance the drought and cold tolerance of transgenic Arabidopsis~~ (Fig.~~9~~9).



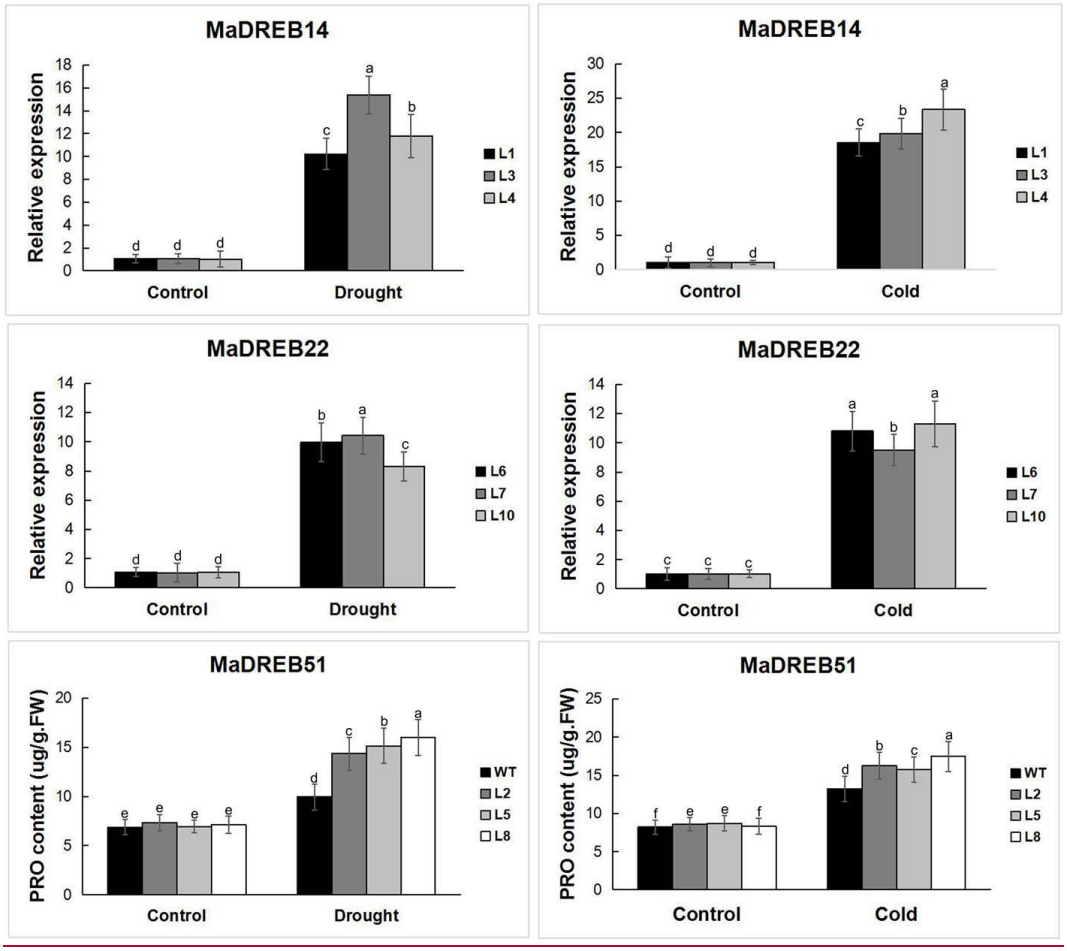


Figure 98. Detection of relative expression ~~and physiological indices~~ of *MaDREB14/22/51* in transgenic Arabidopsis thaliana under drought and cold stress.

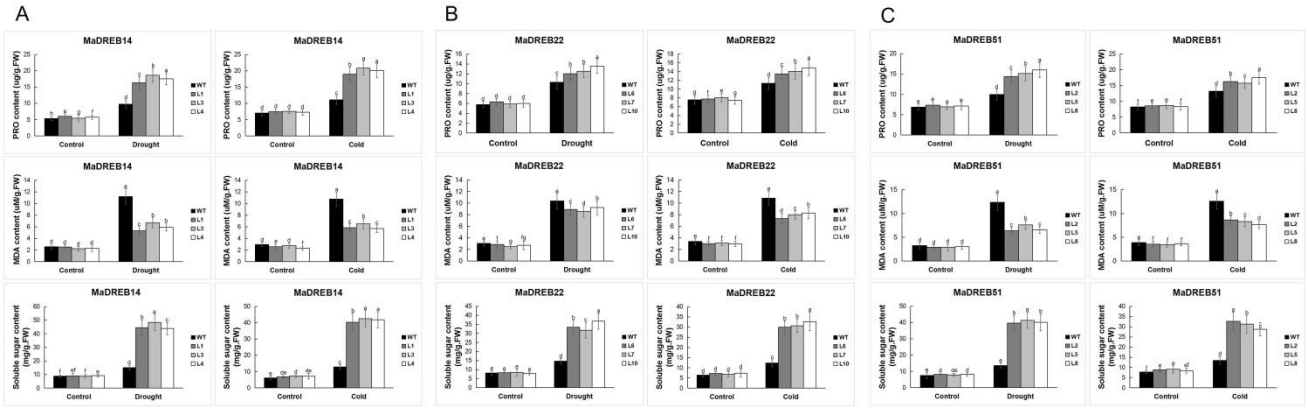


Figure 9.Measurement of physiological indicators in transgenic Arabidopsis thaliana(A: *MaDREB14; B:MaDREB22;C:MaDREB51*) under drought and cold stress.

3. Materials and methods

3.1. Identification and gene family Analysis of DREBs in banana

The protein sequence of the *M. acuminata* genome (DH-Phang-V4) was obtained from the Banana Genome Database (https://banana-genome-hub.southgreen.fr/). Evolutionary tree construction, analyses of gene conserved structural domain analysis, promoter element, collinearity and protein interaction prediction were performed on the *MaDREB* gene family[28,31]. The relevant software and websites are listed in Table S1. ~~The information~~ Information ~~of~~ on the CDS length, the protein length, the molecular formula, the molecular weight (MW) and the protein iso-electric point (pI) was listed in Table S2.

3.2. Plant materials, transcriptome, and qRT-PCR analysis

~~Screening for d~~Differentially expressed genes were screened using banana drought and cold stress transcriptome data[32]. The raw data was deposited in NCBI-SRA database (accession number: PRJNA343716). The transcriptome data of *MaDREB*s under the drought and cold stress treatment are shown in Table S3. Plants of the banana (*Musa acuminate* L. AAA group cv. Cavendish, BX) were used in this study. The seedlings were planted in an incubator with 28 ℃, 16 h light/8 h dark cycle and 70% relative humidity. The pressure drugs were applied to banana plants at the five-leaf stage. For the cold stress, plants were treated for 22 hours at 4°C. For the drought treatment, plants were treated with 200 mM mannitol for 7 days at 28 ℃. These samples were used for the qRT-PCR analysis, which were using the instruments[31]. Relative expression was determined by 2-ΔΔCt and normalised to *MaDREB*s.

3.3. Cloning and vector construction of MaDREB14/22/51

The cDNA of *MaDREB14/22/51* and its promoter region were amplified by PCR from the banana (*Musa acuminate* L. AAA group cv. Cavendish, BX) and then cloned separately into the pCAMBIA1304 vector, named pCAMBIA1304-*MaDREB14/22/51*. The oligo primers of *MaDREB*s used for qRT-PCR are shown in Table S4.

3.4. Plant transformation and drought and cold treatment

Agrobacterium transformed with pCAMBIA1304-*MaDREB14/22/51* was cultured ~~shaken~~ overnight with shaking. Gene transformation experiments were conducted using flowering Arabidopsis thaliana via the~~by~~ pollen tube passage method[30]. The transgenic Arabidopsis (30 d) of the~~with~~ T3 generation were~~was~~ subjected to drought and cold treatments. For the drought and cold stress, the plants (30 d) were treated ~~simulated~~ in the

incubator, respectively[31,34]. Three independent lines were taken from each transgenic plant for the experiment.

3.5. Analyses of physiological indices

The content of Malondialdehyde (MDA), proline and soluble sugar were determined using the detection kits, separately (SH113W-100, Jinkelong, Beijing, China; SH140K-50, Jinkelong, Beijing, China; A145-1-1, Jiancheng, Nanjing, China). Chlorophyll fluorescence images were taken with a camera mounted above the plant pot. The background was removed from the image and only the plant was photographed. The camera has a 10x13 cm field of view and a resolution of 640 x 480 pixels. Light-emitting diodes (LEDs) were have been placed around the lens of the camera. The fluorescence intensity is displayed in false colours. A 450 nm blue LED provides pulse modulated excitation light for both light and saturation pulses, and a red long pass filter in front of the CCD chip limits the wavelength to 620 nm. Wild type and 4 four plants of from each transgenic line were tested separately.

4. Discussion

Many DREB genes have been cloned as more and more species gene families from various species are mined and analyzed analysed. Moreover, some research has shown that DREBs play a role crucial role in regulating gene expression in response to abiotic stressors such as drought, cold, and high-intensity stresses. What's more, some research has shown that DREBs play a role in controlling quality articulation due to abiotic stress, such as drought, cold and high intensity stress. HoweverNevertheless, the characteristics and functionsthe personality and ability of banana DREBs remained unclear. In this studyresearch, DREB family membersrelatives were identifiedrecognised in the banana genome and the potential roles ofexpected jobs for *MaDREB*s in responses to abiotic stress were elucidatedillustrated.

Recently, several DREB homologues have been identifieddistinguished in various plants, such as soybean[32], Arabidopsis[33,36], wheat[34,37], sorghum[35,38], rice[36,39], tomato[37,40], tobacco[41,38], barley[42,39], grape, maize[43,0] and Chinese cabbage[44,1]. The number of DREB gene members varies amongfrom some species. This study identified 103 DREB genes in the banana, a number similar to the number of172 DREB genes found in alfalfa (172)[45,2]. In comparison, And the number of the DREB genes maize[46,3], the grape, the Arabidopsis, the rice, the Chinese cabbage is are 18, 38, 56, 56, 65, respectively separately[44,1]. In addition, cotton contains a significantly higher the number of DREBs totalingthe number of DREBs in cotton is 535.

In this research, the *MaDREB*s were subdivided into six groups based on the classificationgrouping of Arabidopsis thaliana, a result consistent with that of Brassica rapa[44,1] and cotton[47,4], In contrastwhereas in Medicago sativa there arehas only five taxagroups[45,2]. The *MaDREB* members are distributed on chromosomes 2 to 11 a totalwith 103 members, exhibiting collinearity among themwith covariance between members. This result is similar to finding inthat obtained in *B. oleracea*[48,5]., This suggests suggesting that *BrDREB* genes may have been generated by chromosome duplication and gene duplication events[44,1]. Elemental analysis allows further elucidate thedetermination of the structural unity of gene family members. In Fig.2, there are three elements in the sequence of the banana DREB member contain three conserved elements that , which are very neatly arranged. However, and the number of elements in the gene sequences is not consistent across species, where there are 10 elements in the sequence of the *MsDREB* members[45,2].

Introns are sequences of nucleotides in the coding region of a gene that do not code for a protein and are an important part of the structure of the gene. In contrast, While exons are nucleotide sequences in the coding region of a gene that are responsible for translating proteins, its and the most criticalimportant part of the gene. The Exonexon-intron structure can reveal evolutionary links within gene families[49,6]. In banana, most of the genes of *MaDREB* family members do not contain introns, only 34 out of 103 DREB

members have introns, and most genes contains only one exon~~while the number of exons is mostly only 1~~. In *MsDREB*, the number of exons varies from one to four. For example, ~~The~~ the 'gene62759' contains one intron and four exons. This indicates~~In conclusion~~, a large variation in the number and content of motifs ~~was revealed~~ between ~~the~~ different subfamilies of *MsDREB*s, which ~~differed from~~with their less variable gene structures, where~~for which~~ 137 members ~~had~~ have only one exon or no intron. Gene structure analysis showed that introns are absent in most *FvDREB*s[20~~17~~,50~~47~~]. Meanwhile, all *FvDREB* protein sequences ~~had~~ contain elements associated with the AP2 domain, indicating that the AP2 domain ~~was~~ is highly conserved in *FvDREB*s. Similarly, ~~Most~~ most of the DREB member genes ~~of~~ in cotton do not contain introns ~~in their structures~~. The proportions of UTRs and CDSs also differed among the four cotton species.The differences in the structure of the DREB genes indicate a diversity of functions that may be related to the evolution of plant genomes[51~~48~~].

It is well known that cis-acting elements are specific DNA sequences with transcriptional regulatory functions, which play an important role in gene transcription and expression[52~~49~~]. The finding in this study are consistent with previous research on~~Similar to the findings of~~ *BrDREB* in Brassica and *MnDREB* in mulberry[53~~0~~], ~~the~~ our results suggest that the cis-acting elements of the *MaDREB* gene include elements that respond to abiotic stresses and hormones[4~~11~~]. Furthermore, it has been demonstrated~~was shown~~ that DREB transcription factors are involved in the ~~expressing~~ expression resistance genes ~~in~~ through both ABA-dependent and ABA-independent pathways[54~~1~~]. An ABA-related element was identified~~found~~ in the *MaDREB* sequence~~element~~, suggesting~~and it was hypothesised~~ that this gene may ~~be able~~ regulate downstream gene expression through an ABA-dependent signal~~l~~ing pathway. For instance, ~~Overexpression~~ overexpression of *SlDREB3* in tomato has been shown to~~can~~ reduce ABA levels, thereby enhancing~~to increase~~ root growth and ~~increase~~ increasing photosynthetic rate ~~in plants~~[55~~2~~]. Furthermore~~In addition~~, ~~it has been found that~~ the promoter region of the DREB gene contains a high number of light-responsive elements (Fig.5), indicating a potential~~suggesting that phase may be intrinsically~~ link~~ed~~ to circadian regulation. These results suggest that, in addition to the basic TATA-box and CAAT-box elements, the cis-acting elements in the *MaDREB* promoter can be categorized~~divided~~ into three groups: stress-responsive, phytohormone-responsive, and growth-developmental elements. Abiotic stresses such as drought and cold temperatures adversely affect the ~~normal~~ plant growth and development ~~of plants~~[56~~3~~]. It can lead to dehydration of plant cells and trigger a series of tolerance responses in plants[57~~4~~]. As an important branch of AP2/ERF, many studies have been reported showing that DREB family members are valuable for~~used~~ ~~as genes to improve~~ improving crop stress tolerance for the past few years[58~~5~~].

In this research, the results indicated that some *MaDREB* members responded to drought and cold stress, ~~among which~~with *MaDREB1* and *MaDREB5* notably~~were able to improve~~ improving drought and cold tolerance in Arabidopsis. Similar finding have been reported in other studies~~Several studies have reported similar results.~~ The *FvDREB* gene was shown to~~can~~ enhance drought tolerance in plants was demonstrated by transcriptome sequencing and qRT-PCR assays[59~~6~~]. *SsDREB2D/2F* improves drought and cold tolerance in sugarcane[60~~57~~]. Beyond~~In addition to~~ drought and cold stress, there are several reports showing that DREB genes respond to other abiotic stress. *BrDREB2B* was able to improve the survival of transgenic plants, which grew better under high salt and high temperature stress. Overexpression of *OsDREB2A*[61~~58~~], *TaDREB3*[12] and *GmDREB2A*[62~~59~~] in Arabidopsis has enhanced tolerance to various abiotic stresses[4~~11~~]. Similarly, *ZmERF135* was significantly up-regulated after heat treatment, and the expression levels of its~~two~~ homologous genes, *OsDREB2A* and *AtDREB2A*, were previously reported to~~shown to have~~ similar ~~expression levels~~[63~~0~~]. Overexpression of *TaDREB3* improved salt tolerance in wheat[12], a result similar to the expression of DREB in barley[42~~39~~], *GmDREB2* in soybean, and *DvDREB2A* in chrysanthemum[6~~11~~].

5. Conclusion

The DREB gene family plays a crucial role in ~~enhances~~ enhancing plant stress tolerance. In this study, 103 DREB members were identified in banana ~~had been identified~~. *MaDREB*s could respond to drought and cold stress. Among these, ~~Three members named~~ *MaDREB14*, *MaDREB22*, and *MaDREB51*, ~~which~~ were highly responsive to drought and cold stress, and their expression levels were confirmed ~~was also verified~~ by qRT-PCR. The transgenic Arabidopsis with three genes showed drought and low temperature tolerance. This study provided a new basis for understanding the regulatory functions of *MaDREB*s during abiotic stress and screened three stress-resistant genes as candidate members, laying a good foundation for further creation of stress-resistant germplasm. In the next work, we will conduct more in-depth studies to resolve the mechanism of action of *MaDREB*s to enhance drought and cold tolerance in plants.

Supplementary Materials: Table S1. The software and website for *MaDREB* gene family analysis. Table S2. MaDREBs genes physicochemical property analysis. Table S3. The transcriptome data of MaDREBs under the drought and cold stress treatment. Table S4. qRT-PCR primers for genes.

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