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Identification and Expression Analysis of DREB Transcription Factor Family in Pineapple (*Ananascomosus* (L.) Merr.)

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Abstract

The *Dehydration Responsive Element Binding* (*DREB*) gene family is one of the most im
transcription factor families in plants, which plays a crucial role in regulating plant growt
development as well as response to diverse stresses. Although *DREBs* have been thoroug
characterized in many plant species, the genome-wide identification of *DREB* gene famil
pineapple has not been reported yet. In order to analyze the gene and protein properties o
gene family members in pineapple, a comprehensive genome-wide screening was perfor
20 *AcoDREBs* were obtained. Based on phylogenetic analysis, *DREB* genes were located
pineapple chromosomes and divided into 5 subgroups. *AcoDREBs* within the same phylo
group share similar gene structure and domain composition. In addition, gene structural a
showed that most of the *DREB* genes do not contain introns. *Cis*-element analysis showe

thatpromoter regions of*AcoDREBs*consist with at least one stress response *cis*-element. Expression pattern of *AcoDREB*gene family showed that about 4 genes overexpressed an genes underexpressed in all tissues. Eight*AcoDREB* genes were induced under abiotic str Our study provides new insight for future studies to find out the functions of *DREB* genes pineapple.

Keywords: DREB transcription factors, Pineapple, Gene structure, Expression profiles

Introduction

Abiotic stresses, such as high salinity, drought, and high or low temperatures severely aff growth and development of plants. To drive the plant growth and development against ab stresses, plants evolve complex signaling transduction pathways and various mechanisms abiotic stress tolerance by inducing the expression of plant functional proteins and regula proteins. The functional proteins consist of membrane proteins (membrane transporters a water channel proteins), key enzymes (proline, betaine, and sugars, etc.), detoxification e (catalase, superoxide dismutase, ascorbate peroxidase, and glutathione *S*-transferase, etc.) other proteins for protection of macromolecules (LEA protein, osmotin, antifreeze protei mRNA binding protein, etc.). The regulatory proteins include transcription factors (bZIP, MYB and DREB, etc.), protein kinases (receptor protein kinase, MAP kinase, CDP kinas transcription-regulation protein kinase, etc.), and proteinases (phospholipase C and phosphoesterases, etc.). Among those regulatory proteins, transcription factors (TFs) play roles in response to abiotic stresses through interacting with *cis*-elements present in the p region of specific sets of stress-responsive genes to activate or repress their expression *(A et al., 2006)*.

Dehydration response element binding (DREB) proteins are essential stress-responsive regulatory factors. Members of the DREB transcription factor family can specifically bin DRE/CRT *cis*-acting elements to control down-stream gene expression, and enhance plan tolerance to abiotic stresses. DREB transcription factor family belongs to the AP2/ERF (ethylene-responsive element binding factors) superfamily of transcription factors. The A superfamily is well known for its APETALA2 (AP2) domain, which consists of about 60 amino acids. In each AP2 domain, there are two conserved sequence blocks: YRG eleme RAYD element. The YRG element consists of 19-22 amino acids, contains the conserved motif, which may be related to the DNA specific binding of AP2 protein. The RAYD ele has a conserved core region that can form an amphipathic α-helix in the AP2 domains (O et al. 1997). The AP2 domain of DREB subfamily compare with other subfamilies has sli important differences in specific amino acid sites. Valine (Val) at position-14 and Glutam (Glu) at position-19 are conserved in the DREB subfamily(Sakuma et al. 2002).

In *Arabidopsis thaliana*, the genes of DREB subfamily were divided into 6 groups, name A-6 or DREB1 to DREB6 (Sakuma et al. 2002). There are many reports about the functio A-1 and A-2 group' members. The first identified DREB gene was *AtCBF1* from A-1 sub

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which strongly induced by low-temperature. Three genes *AtCBF1*, *AtDREB1A*, and *AtDR* were suggested to have a positive function in low-temperature stress response. A typical r of the DREB1 in sweet potato (*Ipomoea batatas*) *SwDREB1*,showed involvement in plan response to low temperature (Kim et al. 2008). Overexpression of the *ZjDREB1.4* in *Arabidopsis*, showed an increase in tolerance to high and freezing temperature stresses w obvious growth inhibition (Feng et al. 2019).In rice, the interaction of *OsDREB1A*, *OsDR* and *OsDREB1C* with the GCC box increased the cold tolerance of rice plants (Donde et a 2019). As a whole, DREB1 mainly takes part in the cold stress regulation in plants. Acco the previous reported studies of DREB2, the function of the DREB2 is mainly involved ir responses to drought and salt stress (Liu et al. 1998). The first reported At*DREB2A* and At*DREB2B* were induced by dehydration and high-salt stress (Sakuma et al. 2002). Overexpression of *GmDREB2* in *Arabidopsis* resulted in enhanced tolerance to high-salt and without growth retardation (Chen et al. 2007a). In Sugar cane, overexpression of *EaI* can greatly enhance the tolerance to drought and salinity stress compared to untransforme plants (Augustine et al. 2015). Compared with DREB1 and DREB2, the genes of the DRI DREB6 subgroups have only been reported in recent years. A-4 subgroup gene *ZmDREB* cloned from maize (*Zea mays*), and it was found to play an important role in the negative regulation of plant growth and development (Li et al. 2018a). A novel A-5 type DREB ge *ScDREB8*, can improve the salt tolerance in *Arabidopsis* at the seedling stage by up-regul the expression of down-stream stress-related genes (Liang et al. 2017). *CmDREB6* was cl into the DREB6 subgroup, whose overexpression enhanced the tolerance of chrysanthem heat stress (Du et al. 2018). The DREB family have been identified in several plant speci including *Arabidopsis thaliana* (Hwang et al. 2012), *Perennial ryegrass* (Xiong and Fei 2 *Triticum L* (Mondini, Nachit and Pagnotta 2015), *Dendronthema*(Yang et al. 2009), *Zea m* (Qin et al. 2007), and *Oryza sativa L.*(Cui et al. 2011, Gumi et al. 2018, Matsukura et al. 1 However, there are no reports of *DREB* genes of pineapple, and the whole genome characteristics of DREB transcription factors in pineapple have not been studied. Therefo there is an urgent need for a thorough bioinformatic analysis and characterization of *DRE* in pineapple genome.

Pineapple, a tropical fruit, has an important economic value. Pineapple is widely grown ir tropical and subtropical regions. The cultivation of pineapple is of great significance to th development of local agriculture. With the change of global climate, various abiotic stress seriously affect the growth of pineapple. In this study, we identified 20 pineapple *DREB* g and divided into five subgroups. The gene structure, protein structure, protein motifs, gen locations on chromosomes, and expression profiles of *AcoDREB* genes were analyzed. O results provide novel insights into the stress responses of *DREB* genes and broaden our understanding of the function of *DREB* genes in pineapple.

Materials & Methods

Identification of the DREB family members in Pineapple

The *DREB* amino acid sequences in *Oryza sativa* and *Arabidopsis* were obtained from Ri
Genome Annotation Project (http://rice.plantbiology.msu.edu/index.shtml) and TAIR
(http://www.arabidopsis.org), respectively. To identify the DREB amino acid sequences,
have used the DREB genes of *Arabidopsis* to search pineapple genome with BLAST-P ar
on a Hidden Markov Model (HMM), we downloaded the AP2（PF00847）domain as qu
execute an hmmer search (https://www.ebi.ac.uk/Tools/hmmer/search/hmmsearch) with
parameters set default in pineapple genome. Subsequently, we deleted the redundant seq
and used SMART (http://smart.embl-heidelberg.de/) to verify the completeness and exist
the core domain from these sequences. Finally, the remaining sequences were used for fu
phylogenetic analysis.

Protein characteristics and chromosomal localization

The information about gene lengths, number of amino acids, CDS length and chromosom
localization of *AcoDREB* genes was collected from Phytozome. The molecular weight ar
isoelectric points of predicted *AcoDREB* were detected using the ExPASy proteomics ser
(http://expasy.org/) (Gasteiger et al. 2003). According to the genes start position and the l
of related chromosome, MapChart was used to visualize the 20 *DREB* genes mapped on 2
chromosomes and scaffolds localization.

***Cis*-acting element analysis of *AcoDREB* gene promoters**

The up-stream sequences (2.0 kb) of the *AcoDREB*-coding regions were retrieved from th
Phytozome and then submitted to PlantCARE
(http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) to identify six regulatory el
abscisic acid (ABA)-responsive elements, involved in the ABA responsiveness; dehydrat
responsive elements (DREs), involved in dehydrated, low-temp and salt stresses; low
temperature-responsive elements (LTRE), involved in low-temperature response; TC-rich
repeats, involved in defense and stress response; W-boxes, binding site of WRKY transcr
factor in defense responses; and MBS, MYB binding site involved in drought-inducibility

Sequence alignment and phylogenetic analysis

The CDS sequences of the *AcoDREB* genes were obtained from the Phytozome, importec
DNAMAN for sequence alignment. The phylogenetic tree was constructed by IQ tree wi
Maximum likelihood (ML) method (Chernomor, von Haeseler and Minh 2016, Nguyen e
2015), the parameters were set default, except for the ultrafast bootstrap option n=1000 (I
et al. 2018) after performing multiple sequence alignments using MUSCLE 3.7(Edgar 20
with default parameters. Neighbor-joining (NJ) method of MEGA7(Kumar, Stecher and
2016) was also used to construct tree and to validate the ML method results.

Gene structural analysis and conserved motif identification

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The *DREB* genes structure including the number and position of exon and intron were determined using the Gene Structure Display Server (http://gsds.cbi.pku.edu.cn/) (Guo et 2007). To analyze the amino acid sequences of the 20 *AcoDREBs*, MEME (Multiple EM Motif Elicitation) (http://meme-suite.org/tools/meme) was used, the maximum number of was set to 10, and default options were used.

Plant Material and Growth Conditions

Pineapple (*Ananascomosus*) variety MD2 was provided by Qin Lab, Haixia Institute of S and Technology, Fujian Agriculture and Forestry University, Fujian, China (www.qinlab. Plant crowns were grown on soil mixture [peat moss:perlite,2:1(v/v)] in plastic pots place greenhouse at ~30 °C with light intensity of 60–70 umol m^{-1} s^{-1} photons, under 70% hun with 16-h light/8-h dark photoperiod (Ali et al. 2017, Rahman et al. 2017).

RNA-Seq for Different Tissues in Pineapple

We used RNA extraction kit (Omega Bio-Tek, Shanghai, China) to extract total RNA fro different tissues of pineapple (MD2), including sepal, gynoecium, ovule, petal, and stame collection of different tissues was done following the previous method (Chen et al. 2017) NEBNext Ultra RNA Library Prep Kit for Illumina was used to prepare qualified librarie then sent for sequencing. The RNA-Seq data of root, leaf, leaf base and leaf tip, flower ar at different development stages were collected fromPineapple GenomicsDatabase （http://pineapple.angiosperms.org/pineapple/html/index.html）(Ming et al. 2015). Usin TopHatv2.1.1 (Trapnell et al. 2012) with default parameter settings, the trimmed pair-enc of all tissues were aligned to pineapple genome. Cufflinks v2.2.1 software and Cuffdiffv2 were used to estimate the FPKM values, then the heatmap of DREB genes expression pro was generated by pheatmap a packages of R.

Plant material and treatments

One-month-old plants in rooting medium were used as the panting material for the stress treatment. Uniform tissue cultured seedlings were obtained from Qin Lab. (Priyadarshani 2018). Seedlings were subjected to the stress treatments including low-temperature (4 ℃) temperature (45 ℃), drought (350 mM mannitol) and high salt (150 mM NaCl) conditions root and leaf of samples were collected at 6h, 12h, 24h and 48h time periods after treatme et al. 2017). Samples from seedlings those were not subjected to stress treatments were us control. Collected samples were immediately stored in liquid nitrogen prior to total RNA extraction (Rahman et al. 2017).

RNA Extraction and Quantitative real-time PCR

Total RNA was extracted using RNA plant extraction Kit (Omega Bio-Tek, Shanghai, Cl following manufacturer's protocol. According to the supplier's instructions to use AMV r transcriptase (Takara), 1μg of purified total RNA was reverse transcribed to cDNA in a 2

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μLreaction volume (Cai et al. 2019). To determine the relative transcript levels of selecte
*DREB*genes, real-time PCR was performed with gene specific primers according to the
manufacturer's instructions on the Bio-Rad Real-time PCR system (Foster City, CA, USA
specific primers used in this experiment are given in Supplemental Table S2. The PCR pr
was set: 95 °C for 30 s; 40 cycles of 95 °C for 5 s and 60 °C for 34 s; 95 °C for 15 s. Thre
technical replicates and at least three independent biological replicates were performed in
case (Cai et al. 2017, Zhang et al. 2018).

Results

Genome-Wide Identification and Chromosomal location of Pineapple *DREB* genes

To identify *AcoDREB* amino acid sequences, we searched the pineapple genome, based o
DREB family in *Arabidopsis*. A total of 20 *DREB* amino acid sequences were obtained fr
pineapple proteome. According to the gene ID, the candidate genes named *AcoDREB1* to
AcoDREB20 and the amino acid sequences of these genes are shown in Table S1. The
characteristics of the 20 *DREB* genes are listed in Table 1, including the gene name, gene
gene and amino acids length, pI (Isoelectric points), Mw (Molecular weights). The numb
amino acids in 20 *AcoDREB* varied from 149 (*AcoDREB13*) to 463 (*AcoDREB20*). The l
CDS also varied widely from 450 (*AcoDREB13*) to 1392 (*AcoDREB20*) bp. The relative
molecular weight of these DREB proteins range from 16316.44 (*AcoDREB13*) to 49311.
(*AcoDREB20*) kDa, and the predicted isoelectric points varied from 4.71 (*AcoDREB10*) t
(*AcoDREB07*). According to our mapped results, these 20 *DREB* genes were mapped on
pineapple chromosomes (Fig.1). Among them, Chr2 possesses three *AcoDREB* genes, fou
chromosomes (Chr3, Chr5, Chr6, and Chr17) contain two genes, and the anothernine
chromosomes possess one gene in each.

Multiple alignments and Phylogenetic analysis of *DREB* Gene Family

Multiple alignments with the AP2 domain of AcoDREBs indicated that 20 AcoDREBs ha
highly conserved domain and exhibited the typical characteristics of DREBs (Fig.2). Exc
the conserved YRG and RAYD motif, all of them also contained the conserved amino ac
the specific sites. The AP2 domain of DREBs contained conserved Val at position-14 and
position-19. Specifically, Val at position-14 is important in DNA-binding specificity to th
and Glu-19 not as important as Val-14 (Sakuma et al. 2002). According to the sequence
alignment results, we found that all the AcoDREBshave conserved Val at position-14, an
while Glu at position-19.

To clarify phylogenetic relationships of the *DREBs* in pineapple, the multi-species phylog
tree was constructed based on the full-length amino acid sequences of *DREBs* from pinea
Arabidopsis and rice. Specifically, *AT3G57600* and *AT2G40220* (red frame) belong to th
Arabidopsis groups A-2 and A-3, respectively. Compared with the *AtDREBs*, the pineapp
DREB genes lack the homologous gene of the A-3 group. Therefore, we divided *AcoDRE*
five subgroups, Ⅰ to Ⅴ (Fig.3). Among the groups, group Ⅳ has five members. *AcoDRE*

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AcoDREB02, and *AcoDREB03* were in the same group (group Ⅰ) and *AcoDREB04*, *05*, *0*
19 were assigned to group Ⅱ. There are four members in group Ⅲ(*AcoDREB07*, *AcoDRI*
AcoDREB09 and *AcoDREB10*) and Ⅴ(*AcoDREB16*, *AcoDREB17*, *AcoDREB18*, and
AcoDREB20）.

Stress-related *cis*-elements in *AcoDREBs* promoters

To study stress-related conservative *cis*-elements distribution pattern of *AcoDREB* genes
stress response, the 2.0-kb up-stream sequences from the translation start sites of were an
using PlantCARE. We found six abiotic stress response elements namely ABA-responsiv
elements, DRE, LTRE, TC-rich repeat, MBS and W-box, and displayed in Fig.4. *AcoDR*
genes possessed at least one kind of *cis*-acting regulatory element, which indicated that th
expressions of *AcoDREBs* were associated with these abiotic stresses. In total, 9 *AcoDRE*
one or more LRTEs, meaning a potential low-temperature response under low-temperatu
conditions. One to eight ABA-responsive elements existed in 16 *AcoDREBs*, and only
AcoDREB09, *12*, and *17* had TC-rich repeat. MBSs were located in 7 *AcoDREBs*, W-box
DREs all appeared in 10 *AcoDREBs*. The results of the *cis*-element analysis showed that
AcoDREB genes could respond to various kinds of abiotic stresses.

Gene structure and Conserved motifs in DREB proteins

Structural diversity is very common in duplicated genes and has resulted in the generatio
distinct paralogs functionally. To understand the gene structural diversity further, the nun
and positions of exons and introns were determined by comparing the full-length cDNA
sequences and the corresponding genomic DNA sequences (Fig.5). The results indicated
most *AcoDREB* genes have no introns. Among these genes, four genes (*AcoDREB18*, *04*,
13) have one intron each, and *AcoDREB05* has three introns. Interestingly, the members
Ⅱ are different in the number of exons, introns and the length of UTR, which implies that
four paralogs may play different roles in the growth and development of pineapple.
As shown in Fig.6, the distribution of the motifs in *AcoDREBs* are relatively conserved. M
2 and 3 are present in all genes and the motifs in different subgroups showed a certain de
divergences. For example, motif 1, 2, 3, 4 and 9 were harbored by three proteins all in sul
Ⅰ. Motif 7 was only present in two proteins (*AcoDREB07* and *AcoDREB08*) of subgroup
Motif 4 was only presented in *AcoDREB05* of subgroup Ⅱ. *AcoDREB* members within th
subgroups were generally found to share a common motif composition. The results sugge
that these members in the same subfamily might have similar functions (Fig.S1).

Expression profile of *DREB* genes in various tissues at different developmental stag

For understanding of the expression profiles of *AcoDREB* genes in different tissues, we u
transcriptome sequencing to discover the expressions of the 20 *AcoDREBs* in nine differe
tissues, which include root, leaf, flower, fruit, gynoecium, stamen, petal, calyx and ovule.
expression level of 20 *AcoDREBs* can be divided into four clusters based on the hierarchi

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cluster (Fig.7). In cluster i, four genes (*AcoDREB05*, *AcoDREB16*, *AcoDREB17*, and *AcoDREB20*) had high expression level in all tissues, implying that those genes play an important role in plant growth, flower and seed development, and others. The expression *AcoDREB09* in stamen is lower than in other tissues. The expression level of *AcoDREB1* is the lowest, suggesting that it may not take part in the crucial functions of the pineapple addition, the 6 genes (*AcoDREB02*, *AcoDREB04*, *AcoDREB07*, *AcoDREB08*, *AcoDREB1* *AcoDREB13*) of cluster iii were expressed in all tissues at very low levels, suggesting tha genes might be expressed under special conditions. In cluster ii and iv, most genes are specifically expressed in certain tissues or stages. Two genes (*AcoDREB01* and *AcoDRE* showed higher expression levels only in sepal when compared to other parts, suggesting t these genes may play a positive role in flower organs. *AcoDREB06* was expressed highly 6 of stamen, meaning that this gene might be related to the maturity of the stamen. *AcoD* showed high expression during stamen development. *AcoDREB11* was expressed in ovul stamen, and gynoecium, suggesting this gene may function widely during the gametophy development and *AcoDREB03* was highly expressed in 4 different tissues, including root, petal, and gynoecium. According to previous studies (Azam et al. 2018, Su et al. 2017), d stages of the pineapple reproductive organs were defined. To validate RNA-Seq data, we selected 16 genes (except *AcoDREB04*, *AcoDREB07*, *AcoDREB08* and *AcoDREB13*) to p quantitative real-time PCR (qRT-PCR) analysis. We found that the expression of selected (Fig.8) in the root, leaf and other seven tissues coincide with the RNA-Seq data.

Expression Patterns of *DREB* Genes under Abiotic Stress

To further explore the expression patterns in the*AcoDREB* genes under various abiotic str conditions including salt, drought, cold and heat, we examined the expression patterns of *AcoDREB* genes (*AcoDREB01,03, 06, 09, 11, 14, 18* and *19*) in '*MD2*' variety of pineapp qRT-PCR with 3 biological and 3 technical replications. The relative expression level of *AcoDREB*genes under all stress conditions fluctuated during the 48-h treatment. Cold stre abiotic stress that also has drastic effects on plant growth and development and causes ma to crop yield (Cai et al. 2015). In the shoot, most of the *AcoDREB* genes were more sensi cold stress than other parts of the plant. Among these genes, 3 (*AcoDREB01*, *AcoDREB0* *AcoDREB18*) of them responded rapidly under cold stress and reached to the highest leve hours. The two genes (*AcoDREB09* and *AcoDREB19*) reached to the highest expression l after 24 hours. The expression levels of *AcoDREB06*weredown regulated significantlyaft subjected to 4 abiotic stresses in the shoot of pineapple. After 48-h, the expression levels stress and drought stress were restored. Salt stress is also major stress, which adversely af plants and causes a major loss in crop yield. According to the expression analysis of eigh in roots after 12 hours of 150 mM salt treatment, we found that the expression levels of a increased rapidly and reached to a maximum level after 12 hours. According to the exper results we found that three genes (*AcoDREB03*, *AcoDREB06*, and *AcoDREB14*) were mo sensitive to cold stress than salt stress, and *AcoDREB03* and *AcoDREB14* reached to the l

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expression level after 48 hours. Clearly, the *AcoDREB11*gene was sensitive to drought sti
expression level lowered rapidly compared with the control.

Discussion

With the change of global climate, all kinds of environmental stresses have brought serio
to the growth of plants and severely inhibited the survival, development finally reducing
yield of crop plants. In the process of growth and development, plants can respond to vari
environmental stresses through changing the expression of functional genes activated due
external environment. These stress resistance genes can be roughly divided into two categ
the first group is functional genes that are directly responsible for the production of prote
stress resistance in plants, such as aquaporin, LEA protein, antioxidant enzyme, etc. The
group is anti-stress genes that encode regulatory proteins, such as transcription factors an

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protein kinases. Among them, transcription factors can specifically recognize and bind *ci*

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elements in promoters, to regulate the transcription of down-stream genes. There are hun
transcription factors found in higher plants, which play an important role in plant reprodu
development and physiological metabolism (Liu, Zhang and Chen 2001). Transcription fa
are involved in regulating plant growth and development and in response to environmenta
by control a broad range of down-stream genes. *AtMYB4* is a transcription factor in the ba
against UV (Hemm, Herrmann and Chapple 2001). Transcription factor *GmMYB22* can e
drought tolerance, salt tolerance, and ABA sensitivity in *Arabidopsis thaliana*(Shan et al.
One class of bZIP proteins, TGA/OBF family members, can interact with NPR1 to involv
the SA defense signaling pathway (Singh, Foley and Onate-Sanchez 2002). The DREB
transcription factors contain a conserved AP2/ EREBP domain, which is involved in plan
response to external environmental stress. By specific combining with DRE (Dehydration
responsive element) *cis*-acting elements, DREB regulates the expression of down-stream
related genes and improves plant resistance. It was found that the mutation of DRE bindi
could result in the inability of DREB transcription factors to bind (Dubouzet et al. 2003,
al. 1998). And *DREB1A* binding sites had a preference for two DRE sequence between
Arabidopsis and *Oryza sativa* (Dubouzet et al. 2003, Sakuma et al. 2002).
Several studies have elucidated the functions and evolutionary history of *DREB* genes in
plant species such as *Arabidopsis,* rice, and corn. Until now, there are increasing reports
the function of *DREB* family genes in the process of plant stress tolerance. The *DREB* ge
were first cloned from *Arabidopsis* in 1998 (Liu et al. 1998). It was shown that *DREB1* a
DREB2 play trans roles in two separate signal transduction pathways under low-temperat
dehydration conditions (Liu et al. 1998). In *Arabidopsis*, *VuDREB2a* was reported to enh
ability of plants to drought resistance (Sadhukhan et al. 2014). DREB also protects plants
both biotic and abiotic stresses by regulating some genes responsible for anthocyanin syn
(Song et al. 2019). In addition, the members of *MaDREB1-MaDREB4* (*Achr9G04630*,
Achr5G280, *Achr6G32780*, *Achr11G24820*), which are induced by ethylene in bananas (

acuminata), play an important role in regulating fruit ripening (Kuang et al. 2017). There
DREB plays a significant role in plant growth and development.
As a tropical fruit crop with important economic value, pineapple has important research
plant stress tolerance. However, little is known about the *DREB* gene family in pineapple
we performed a series of analyses on pineapple *DREB* genes, including their characteristi
isoelectric point (*pI*), molecular weight, chromosome location, gene structure and motif,
phylogeny relationships, domain architecture, promoter acting elements, and expression p
under abiotic stresses (Li et al. 2018b). The DREB family was identified in the whole ger
pineapple, and 20 *AcoDREB* genes were finally obtained (Table.1). This condition is simi
that reported for *Phyllostachys pubescens*,which had 27 *DREB* genes (Wu et al. 2015). H
there are significant differences with *Arabidposis* and rice; both have 57 *DREB* genes (Na
al. 2006). The reason for these differences may be due to the loss of some genes in the ev
process of these species. The number of amino acid residues of the *DREB* family ranged
149 (*AcoDREB13*) to 463 (*AcoDREB20*). The average amino acid length is 255, which is
similar to rice and Chinese jujube (Zhang and Li 2018b). The relative molecular weights
from 16316.44 to 49311.65 kDa, and the predicted pI varied from 4.71 (*AcoDREB10*) to
(*AcoDREB07*) (Table.1). The biochemical characteristics of DREB transcription factors v
stable in many species. This means that the function of DREB may also be conserved.
To clarify the phylogenetic relationships of the *AcoDREB* gene family, an unrooted phylo
tree was constructed based on the multi-alignment of the sequence of DREBs from Pinea
Arabidopsis, and rice. The results showed that *AcoDREB* genes could be grouped into fiv
subgroups, Ⅰ to Ⅴ (Fig.3) according to the cluster analysis and comparison with *Arabido*
rice in evolution. The number of genes per subgroup is 3, 4, 4, 5 and 5, respectively (Fig.
Furthermore, the DREB family was divided into 6 subcategories (A1, A2, A3, A4, A5, ar
and A3 has only one gene in *Arabidopsis*. In the current study we found that, *AcoDREB0*
AT2G40220 (A3 Group) and AT3G57600 (A2 Group) were divided into the same branch
evolutionary tree (Fig.3). According the analysis of sequence and domain, we finally clas
AcoDREB04 into A2 subgroup (Nakano et al. 2006). So, we suggest that there is no A3 s
in DREB family of pineapple. These genes were evenly distributed in the five subgroups.
Additionally, the intron-exon structure and core domain analyses of *AcoDREBs* were carr
based on the phylogenetic tree. The introns are very few in the whole DREB family. The
maximum number of introns were found in *AcoDREB05*, while the other genes didn't hav
introns, as reported previously in grapes and jujube (Zhang and Li 2018a, Zhao et al. 201
According to the results from core domain analysis, we found that all members of *AcoDR*
have three conserved domains (Fig.6). Among the three domains, two of them (YRG and
RAYD) form the AP2 structure, which is the feature of the whole DREB family (Okamur
1997). The AP2 domain of DREBs was found to contain conserved Val at position-14 an
position-19. Specifically, Val at position-14 is important in DNA-binding specificity to th
(Sakuma et al. 2002). In this study, we found that all the 20 *AcoDREBs* had conserved Va
position-14, and 11 *AcoDREB*s possessed Glu at position-19 (Fig.2). Except for the conse

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amino acids in the specific sites, all of them also contained the conserved YRG and RAY (Fig.2).

We further studied their expression levels and found that the expression of some *AcoDRE* genes were parallel whit other species. According to the results of RNA-Seq and q-PCR, *AcoDREB19* is highly expressed in anthers (Fig.7, Fig.8), which is consistent with its rice homologous gene (LOC_Os08g27220) (Davidson et al. 2012). *AcoDREB16* and homolog gene (LOC_Os10g22600) are highly expressed in root. It suggests that homologous gene different species maybe have similar expression patterns.

We subjected pineapple seedlings to different abiotic stress conditions to study the expres changes of eight *AcoDREB* genes. Under different abiotic stress conditions, DREB genes the ability to response to stress (Torres et al. 2019). However, these genes show different characteristics in shoot and root. The expression patterns of the eight genes were almost i and induced in the shoot and root under salt stress (Fig.9a). According to the earlier repor subgroup could play an important role in plant response to salt and drought stress in *Arabidopsis*(Yamaguchi-Shinozaki and Shinozaki 2006). According to our analysis *AcoL* and *AcoDREB03* classified as group I, correspond to A1 subgroup in *Arabidopsis thalian* showed induced expression level when subjected to salt and drought stress (Fig.9a). The expression of AcoDREB01 and AcoDREB03 were obviously induced in root under salt s Under the drought stress, AcoDREB01 and AcoDREB03 were induced in shoot. It indica AcoDREB01 and AcoDREB03 may play important roles in root and shoots respectively salt and drought stress. In addition, it was found that some genes, belonging to the same evolutionary branch, show similar expression patterns under abiotic stress. *AcoDREB01* a *AcoDREB03* mRNA increasingly accumulated and reached to its maximum level after 24 of treatment in the pineapple leaves (Fig.9). This expression pattern is similar to that in th though its highest point is at 12 hours after treatment. Under mannitol treatment, the expr in the leaves was increased, while the expression in roots was reduced (Fig.9b). At the tir sampling, the expression patterns of the two genes were consistent, indicating that the tw in the same subgroup maybe have similar functions in response to salt and drought stress

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the group IV members, the expression level of *AcoDREB11* and *AcoDREB14*, were signi increased under both salt and cold stresses (Fig.9), which is similar with the A5 group me *GmDREB2* (Chen et al. 2007b), indicating that the function is conserved between pineapp soybean. As the member of the V subgroup, *AcoDREB18* and *AcoDREB19* have the same response with different abiotic stresses. The results showed that the genes of the same sub were functionally conserved. Expression of *AcoDREB06* was inhibited in the leaves and increased in the roots. It shows that the enhancing of the expression of *AcoDREB06* in pla help to increase the resistance of roots to different abiotic stresses. On the contrary, *AcoD* may regulate other pathways to respond to plant stress in the case of decreased expressio shoot (Fig.9), such as HARDY (AT2g36450) can improve drought and salt tolerance by r transpiration in (Abogadallah et al. 2011). The RNA-Seq data analysis results showed tha *AcoDREB19* was almost not expressed in roots. In our study, the expression of

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*AcoDREB19*increased significantly under abiotic stresses, indicating that *AcoDREB19* ma a key role in plants response to abiotic stress and can enable plants to cope with a variety adverse environments. In addition, *DREB* genes are also involved in the process of plants responding to biological stress, which will be great significance.

Conclusions

In conclusion, we accomplished the first genome-wide analysis of the DREB TFs family pineapple and identified 20 genes encoding *DREB* family transcription factors.Further, w conducted a detailed investigation of DREB transcription factors with respect to their stru characterization, and expression profiles under various abiotic stresses. To the best of our knowledge, this report is the first genome-wide analysis of *DREB* genes in pineapple, and data provide insights into potential functions of pineapple*DREBs*. The results will provid useful basis for the further understanding of the structure-function relationship of *DREB* transcription factor family members. Further, this study will help in developing strategies future improvement of stress tolerance in pineapple.

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