# Identification of ABF/AREB gene family in tomato (*Solanum lycopersicum*) and functional analysis of *ABF/AREB* in response to ABA and abiotic stresses (#80477)



First submission

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# Identification of ABF/AREB gene family in tomato (*Solanum lycopersicum*) and functional analysis of *ABF/AREB* in response to ABA and abiotic stresses

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Abscisic acid (ABA) is a plant hormone that plays an important regulatory role in plant growth and stress response. The *AREB* (ABA-responsive element binding protein) /*ABF* (ABRE-binding factor) gene family is a kind of crucial transcription factors acting as ABA responsor and taking part in ABA-dependent stress responses in plants. This study was conducted to identify and analyze the response of tomato *ABF/AREB* family members. The results show that a total of 10 *ABF/AREB* members were identified in tomato, which are randomly distributed on 5 chromosomes. Domain analysis showed that these members exhibit high protein similarity, especially in the basic leucine zipper (bZIP) domain region. Subcellular localization analysis indicated that all 10 *ABF/AREB* members are localized in the nucleus. Phylogenetic tree analysis showed that tomato *ABF/ AREB* genes are divided into two groups, and they are similar with the orthologs of other plants. The analysis of *cis*-acting elements showed that most tomato *ABF/AREB* genes contain a variety of hormones and stress-related elements. Expression profiles of different tissues indicated that *SlABF2* and *SlABF10* play an important role in fruit ripening. Finally, qRT-PCR analysis revealed that 10 tomato *ABF/AREB* genes respond to ABA, with *SlABF3* being the most sensitive. *SlABF3*, *SlABF5* and *SlABF10* positively respond to salt and cold stresses. *SlABF1*, *SlABF3* and *SlABF10* are significantly induced under UV radiation treatment. *SlABF3* and *SlABF5* are significantly induced in osmotic stress. Overall, this study may provide insight into the roles of tomatoes *ABF/ AREB* homologues in plant response to abiotic stresses.

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## Abstract

Abscisic acid (ABA) is a plant hormone that plays an important regulatory role in plant growth and stress response. The *AREB* (ABA-responsive element binding protein) /*ABF* (ABRE-binding factor) gene family is a kind of crucial transcription factors acting as ABA responsor and taking part in ABA-dependent stress responses in plants. This study was conducted to identify and analyze the response of tomato *ABF/AREB* family members. The results show that a total of 10 *ABF/AREB* members were identified in tomato, which are randomly distributed on 5 chromosomes. Domain analysis showed that these members exhibit high protein similarity, especially in the basic leucine zipper (bZIP) domain region. Subcellular localization analysis indicated that all 10 *ABF/AREB* members are localized in the nucleus. Phylogenetic tree analysis showed that tomato *ABF/ AREB* genes are divided into two groups, and they are similar with the orthologs of other plants. The analysis of *cis*-acting elements showed that most tomato *ABF/AREB* genes contain a variety of hormones and stress-related elements. Expression profiles of different tissues indicated that *SlABF2* and *SlABF10* play an important role in fruit ripening. Finally, qRT-PCR analysis revealed that 10 tomato *ABF/AREB* genes respond to ABA, with *SlABF3* being the most sensitive. *SlABF3*, *SlABF5* and *SlABF10* positively respond to salt and cold stresses. *SlABF1*, *SlABF3* and *SlABF10* are significantly induced under UV radiation treatment. *SlABF3* and *SlABF5* are significantly induced in osmotic stress. Overall, this study may provide insight into the roles of tomatoes *ABF/ AREB* homologues in plant response to abiotic stresses.

**Keywords** Tomato; *ABF/AREB*; Gene family analysis; Transcription factor; Abscisic acid; Abiotic stress

## Introduction

During the whole process of plant growth, there are many abiotic stress factors that hinder plant growth and reduce yield (*Zhu 2016*). In different environments, plants produce different stress responses through perception and adaptation. When plants lack water resources, their growth, survival, distribution and productivity will be seriously affected *(Fleta-Soriano & Munne-Bosch 2016*). Soil salinization and drought are also important unfavorable factors affecting agricultural development. drought can cause damage to the above-ground and underground tissues and organs of plants, as well as change the normal relationship between plants and soil *(Qian et al. 2019*).

*ABF* (AREB binding factors) /*AREBs* (ABA response element binding protein) belong to the A subfamily of basic leucine zipper *bZIP* (Basic leucine zipper) transcription factors. They are key regulatory molecules downstream of the ABA signaling pathway regulating plant response to hormones and stresses, initiating the expression of downstream genes *(Chang et al. 2019*). *ABFs* activates the expression of ABA-regulated genes by binding to ABRE homeopathic elements. In the signal transduction of ABA, ABA binds to Pyrabactin resistance 1 (PYR1) /PYR1-like (PYL), leading to the inactivation of protein phosphatase 2C (PP2C), destroying the interaction between PP2C and Snf1-related protein kinase2 (SnRK2), and stimulating the activity of SnRk2 to activate *AREB (Fujii et al. 2009; Fujita et al. 2013; Kim et al. 2004*). In yeast one-hybrid screening, *ABF/AREB* is established using ABRE as a bait (*Zhao et al. 2016*). So far, 7 *ABF/AREB* homologues, *StAREB1-StAREB4*, *StABI5*, *StABL1* and *StABL2*, have been identified in potato. Among them, the promoter regions of *StAREB1*, *StAREB2/StABF1*, *StAREB3* and *StAREB4* have ABREs, which means that their rapid induction under exogenous ABA treatment may also be self-mediated. ABA and osmotic stress could strongly induce the transcription of *StAREB1*, *StAREB2* and *StAREB4*, indicating that these three genes may have significant roles in ABA signaling pathway (*Liu et al. 2019*).

Tomato (*Solanum lycopersicum*) has long been used as a model plant for fruit ripening, disease response, genetics and whole genome sequence studies (*Hsieh et al. 2010*). Tomato is the third most important commercial crop family in *Solanaceae* and is also the most valuable horticultural crop in the world (*Bastias et al. 2011*). In China, tomato production accounts for a quarter of world tomato production, but the growth and development of tomato is easily affected by unfavorable environmental conditions. Therefore, exploring the potential functions of genes will provide important theoretical support for improving tomato yield, quality and resistance to stress.

In order to better understand the key role of the *ABF/AREB* subfamily in plants, the coding genes of the *ABF/AREB* members in tomato were identified and analyzed in this study. For tomato *ABF/AREB* members, the secondary structure, chromosome position of the gene, gene structure, conservative motif analysis, homeopathic promoter analysis, phylogenetic relationship and subcellular location analysis were conducted. At the same time, the expression patterns of these *ABF/AREBs* in different tissue-specific processes and gene transcription analysis under different abiotic stresses and hormones were also investigated. Our study here aims to help

broaden the molecular biological functions of *ABF/AREBs* in plants.

## Materials & Methods

### Identification of the *ABF /AREB* family members in tomato

Firstly, 9 known amino acid protein conserved sequences of *Arabidopsis thaliana* were used *(Li et al. 2020*). The members of *ABF/AREB* gene family were screened as candidate genes by homology comparison in the database of tomato gene testing (https://solgenomics.net/organism/Solanum lycopersicum/genome). Secondly, the E value of 1E-20 was used to reduce the false positive, and the PFAM database (http://pfam.xfam.org/) and SMART database (http://smart.embl-heidelberg.de/) were used to further verify the *ABF/AREB* protein domain of tomato. Candidate genes that did not contain a specific domain of the *ABF/AREB* gene (accession number PF00170) were manually eliminated. The *ABF/AREB* gene family was represented by the remaining genes.

Tomato genome sequence and annotation information were downloaded by Ensemble Plants-Tomato Genome Database (http://plants.ensembl.org/index.html). The whole genome information (GFF3, FASTA, PEP, CDS) of tomato was sorted out by using TBtools software, and finally the whole genome information file of tomato *ABF/AREB* was screened out for mapping according to the gene ID of the identified members of tomato *ABF/AREB* gene family.

### Characterization of ABF/AREB transcription factor in tomato

The chromosome position, amino acid length, molecular weight, isoelectric point, molecular formula, and other physical and chemical characteristics data were used to examine the tomato ABF/AREB protein sequence (https://web.expasy.org/protparam/). Online analysis of the tomato ABF/AREB transcription factor subcellular location prediction was done using WoLFPSORT (https://wolfpsort.hgc.jp/). The secondary structure of tomato ABF/AREB family proteins were examined using the online tool (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_auto1mat.pl?page=/NPSA/npsa_sopma.html).

### Conserved motifs and protein conserved domain analysis

MEME (http://meme-suite.org/tools/meme) was used to input all protein sequences of tomato ABF/AREB online program to analyze tomato ABF/AREB transcription factor family conserved motifs. The number of predicted motifs was set to 10, while the other parameters were set as default. The multiple sequence alignment of tomato family was done through ClustalX and GeneDox software.

### Phylogenetic tree and cis-acting elements analysis

*ABF/AREB* protein sequences of *Arabidopsis thaliana*, *Solanum tuberosum*, and *Populus orientalis* were obtained from TAIR (https：//www.arabidopsis.org/). Plant Gene Database (https：//phytozome.jgi.doe.gov) (*Liu et al. 2019*) and article (*Yong et al. 2021*). Phylogenetic trees were constructed using Mega 7.0 software. A phylogenetic tree of 33 *ABF/AREB* protein

sequences was constructed by neighbor-joining method (Bootstrap parameter was set to 1000). In addition, the evolutionary tree was beautified using EvolView (https：//evolgenius.info/ /evolview-v2/#login) website. A DNA sequence of 2000bp upstream of the tomato *ABF/AREB* gene was obtained from the genome-wide information of tomato *ABF/AREB* and submitted to PlantCare online database (http：//bioinformatics.psb.ugent.be/) for analysis. After deletion and integration, TBtools software was used for analysis.

**Tissue expression analysis of ABF/AREB gene in tomato**

The IDs of the *SlABF* genes were searched in the eFT (http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi) database. Then, the data were sorted out and the expression patterns of *SlABF* in different tissues were drawn by TBtools.

**Gene location and gene structure analysis**

The GFF3 file in the whole genome information of tomato *ABF/AREB* was visualized and analyzed by TBtools software. *ABF/AREB* gene members were renamed according to their chromosomal distribution. The exon-intron structure distribution of *ABF/AREB* gene in tomato was analyzed by using the GFF3 file of *ABF/AREB* genome-wide information with TBtools software.

**Transcriptional analysis of ABF/AREB gene in tomato under different abiotic stresses and hormone treatments**

**Plant materials and treatment**

Tomato (*L. esculentum* L. 'Micro-Tom') seeds were provided by the Institute of Vegetable Science, College of Horticulture, Gansu Agricultural University. The seeds were put in a 250 mL Erlenmeyer flask with 100 mL of sterile water (45 °C), soaked for 10 minutes, and then put into a high-temperature shake flask at a speed of 180 r $min^{-1}$ (25 °C). The sterile water was changed one time every day. After germination, the tomato seeds were transferred to a plug tray containing the substrate. After the cotyledons were fully unfolded, the nutrient solution was irrigated every two days. The control growth chamber environment had a photoperiod of 16/8 h (light/dark), an air temperature of 26/20 °C (day/night), and a light intensity of 250 μ mol $m^{-2}$ $S^{-1}$. Stress treatments were carried out at the two-leaf stage of seedlings. For salt, ABA, fluridone (FLD) and PEG treatments, seedlings were grown in 1/2 nutrient solution containing NaCl (200 mM), ABA (100 mM), fluridone (an inhibitor of ABA biosynthesis) (100 mM) and PEG 6000 (20 %) without other reagents. For cold treatment, the seedlings were placed in 1/2 nutrient solution at 4 °C with no other reagents added. Some of the selected seedlings were transferred to a growth chamber with 253.7 nm Uv treatment and other growth conditions were the same as those of the control. Leaf samples were collected for qRT-PCR experiments after 0, 12, and 24 h of treatment (*Liu et al. 2022*). The collected samples were immediately frozen in liquid nitrogen and stored at -80 °C. Each treatment contained three biological replicates.

**RNA extraction and quantitative qRT-PCR**

Total RNA was extracted from the samples using TRIzol reagent (Invitrogen, Carlsbad, CA,

USA) Take advantage of FastQuant First Strand cDNA Synthesis Kit (tianen, Beijing, China) to synthesize cDNA. These reactions were executed under the following conditions: 37 °C for 15 min, 85 °C for 5 seconds, and finally ended at 4°C. LightCycler 480 Real-Time PCR System (Roche Applied Science) and SYBR Green Premix Pro Taq HS Premix kit was used for qRT-PCR. The reaction system was 2×SYBR Green Pro Taq HS Premix 10 μL, primer F 0.4 μL, primer R 0.4 μL, cDNA 2 μL, dd$H_2O$ 7.2 μL. The primers used in qRT-PCR were designed with Primer 5.0, and the internal reference was Actin (NC 015447.3) as shown in Table 1.

**Data statistics and analysis**

The data were analyzed using the 2-ΔΔCt calculation method. GraphPad Prism software was used for statistical analysis. ANOVA was used to detect the significant level of difference between different times or different treatments.

## Results

### Identifiification of ABF/AREB genes in tomato

10 tomato *ABF/AREB* genes were obtained by homologous alignment, which were named *SlABF1-SlABF10* according to the location of the genes on the different chromosomes (Table 2). The tomato *ABF/AREB* transcription factor family is unevenly distributed on 5 chromosomes of tomato. Among them, *SlABF1*, *SlABF2* and *SlABF3* are located on Chr-01, *SlABF4* and *SlABF5* are located on Chr-04, *SlABF6* is located on Chr-09, *SlABF7*, *SlABF8* and *SlABF9* are distributed on Chr-10 and *SlABF10* is located on Chr-11 (Fig. 1). The amino acid size of tomato ABF/AREB transcription factor family is between 146 aa (SlABF1) and 447 aa (SlABF5). The molecular weight is between 16689.92 and 47977.73 kDa. The isoelectric point (pI) is between 6.41 (SlABF5) and 9.72 (SlABF4). In the ABF/AREB family, only SlABF4 and SlABF8 are acidic proteins (pI < 7), and the rest are unique (pI > 7) (Table 2). The instability index is greater than 40 for all 10 tomato genes, showing that the ABF/AREB genes are unstable proteins. From the perspective of subcellular location analysis, SlABF1-S1ABF10 are all expressed in the nucleus, which speculate that the gene is related to the storage and replication of genetic material. SlABF2 is expressed in chloroplasts, suggesting that *SlABF2* may be involved in photosynthesis.

### Conserved domain and conserved motifs of tomato ABF/AREB family

ABF/AREB has a highly conserved protein structure including four conserved phosphorylation sites, three conserved domains of C1, C2 and C3 at the N-terminus, and a highly conserved domain of C4 at the C-terminus (*Fujita et al. 2005*). The tomato ABF/AREB protein has four conserved phosphorylation sites. The N-terminal is made up of C1, C2, and C3, whereas the C-terminal is made up of C4 and the bZIP region (basic region and leucine zipper). The C-terminus of tomato ABF/AREB proteins has the unique BRLZ domain of bZIP transcription factor, which has the function of recognizing and binding specific DNA sequence (Fig. 2).

In this study, 10 conserved motifs are found in the tomato ABF/AREB proteins (Fig. 3). Sequence information for the identified conserved motifs is presented in Table 3. The length of each motif is between 10 and 50 amino acids. The results show that the 10 identified tomato

ABF/AREB motifs are quite similar. Motif1 is the basal core of the bZIP domain. Motif5 and Motif4 constitute the C1 conserved phosphate site. Motif3, Motif2, and Motif6 constitute the C2, C3, and C4 conserved phosphate sites, respectively. Both Motif1 and Motif2 are presented in all tomato ABF/AREB proteins. Both Motif3 and Motif4 are presented in 9 tomato ABF/AREB proteins except for SlABF1. Except for SlABF1 and SlABF10, the other eight tomato ABF/AREB proteins contain Motif6. Motif7 and Motif9 are in four tomato ABF/AREB proteins (SlABF3, SlABF5, SlABF7 and SlABF10). Motif10 occurs in SlABF3, SlABF5 and SlABF7. Motif8 occurs in SlABF8 and SlABF9. It can be inferred that the tomato ABF/AREB members are highly conservative and may have similar functions.

**Phylogenetic analyses of the tomato ABF/AREB families**

The 33 ABF/AREB (10 SlABFs, 9 AtABFs, 7 StABFs and 7 PdABFs) proteins are divided into 2 subfamilies (Group A and Group B) (Fig. 4). Among them, SlABF3, SlABF5, SlABF7 and SlABF10 belong to Group A. They have the highest homology with StAREB1, StAREB2, StAREB3 and StAREB4, respectively. SlABF1, SlABF2, SlABF4, SlABF6, SlABF8 and SlABF9 belong to Group B. SlABF6 is more closely related to StABI5. SlABF8 is more closely related to StABL2, and SlABF9 is more closely related to StABL1. SlABF2 and SlABF4 are closely related to AtDPBF2. It can be concluded from the entire evolutionary tree that SlABFs have the highest homology with StABFs, relatively low homology with AtABFs, and the lowest homology with PdABFs.

**Analysis of the gene structure of tomato ABF/AREB family**

By analyzing the phylogenetic tree and gene structure of the tomato *ABF/AREB* gene family, the overall differences between introns and exons of the 10 tomato *ABF/AREB* genes are found to be insignificant. Tomato *ABF/AREB* genes are divided into two groups. *SlABF3*, *SlABF5*, *SlABF6*, *SlABF7*, and *SlABF10* are divided into ClASS I，and *SlABF1*, *SlABF2, SlABF4, SlABF8,* and *SlABF9* are divided into ClASS II (Fig. 5). We found that the number of exons in tomato *ABF/AREB* is between 2 and 4. The number of introns is between 1 and 4. Specifically, in ClASS I, *SlABF5*, *SlABF6*, and *SlABF10* contain 4 introns and 4 exons, *SlABF7* possesses 3 introns and 3 exons, *SlABF3* contains 1 intron and 2 exons. Both *SlABF2* and *SlABF4* in ClASS II have 3 introns and 4 exons. There are 3 exons in *SlABF1*, *SlABF8* and *SlABF9*, and 2 introns in *SlABF1* and *SlABF9*. *SlABF8* has 4 introns. In general, the genetic structures of different tomato *ABF/AREB* members are relatively similar. Interestingly, except for *SlABF1* and *SlABF3*, the other 8 genes have similar exon lengths. Thus, the function of the tomato *ABF/AREB* genes may also be relatively similar.

**Analysis of protein secondary structure of tomato ABF/AREB family genes**

The most abundant protein secondary structures within tomato ABF/AREB members are mainly alpha helix and random coil (Table 4). The 10 ABF/AREB encoded proteins have alpha helix

(29.31 %-50.68 %), extended strand (4.79 %-13.49 %), beta turn (1.01 %-3.14 %), and random coil (43.15 %-59.26 %) as their secondary protein structures.

**Analysis of cis-acting elements of tomato ABF/AREB family genes**

The tomato *ABF/AREB* genes include a total of 18 homeopathic components (Fig. 6). Among them, 3 elements (AE-box, GATA-motif, MRE) are related to light response, 7 elements (ABRE, CGTCA-motif, GARE-motif, P-box, TGACG-motif, TCA-element, TATC-box) are related to hormone response, and 4 elements (ARE, LTR, MBS, TC-rich repeats) are related to stress response. In order to further study *cis*-elements in the *ABF/AREB* promoter sequences, three main types of *cis*-acting elements are identified, including light, hormones, and stress response elements (Fig. 7). AE-box element is mainly distributed in *SlABF9*. ARE element is mainly distributed in *SlABF8*. ABRE is all tomato ABF/AREBgenes, with the exception of SlABF7 and SlABF8, and was prevalent in SlABF10. Both CGTCA-motif and TGACG-motif elements are mainly distributed in *SlABF3* and *SlABF7*. Generally speaking, the *cis*-elements correlated to hormone is relatively more abundant, which manifesting that the tomato *ABF/AREBs* gene plays a vital role in regulating hormone response.

**Tissue-specific expression pattern of tomato ABF/AREB genes**

In order to investigate the expression of the *ABF/AREB* gene in various tomato tissues during various growth stages. the expression of *ABF/AREB* genes in 14 tomato tissues is analyzed, including unopened flower bud, fully opened flower, leaf, root, 1 cm fruit, 2 cm fruit, 3 cm fruit, mature green fruit, breaker fruit, breaker fruit + 10, pimpinellifolium immature, green fruit, pimpinellifolium breaker fruit, pimpinellifolium breaker + 5 fruit and pimpinellifolium leaf (Fig. 8). Some *SlABFs*, including *SlABF2*, *SlABF3*, and *SlABF10*, are highly expressed in all tissues. In contrast, *SlABF6* and *SlABF7* are expressed at low levels in all tissues. The expression level of *SlABF5* in roots is much higher than that in other tissues. The expression of *SlABF*9 is higher in pimpinellifolium leaf, but lower in other tissues. In addition, *SlABF1, SlABF4* and *SlABF8* also show similar expression patterns.

**Expression profles analysis ABF/AREB genes in tomato under ABA and FLD treatment**

The relative expression of *SlABF1*, *SlABF2*, *SlABF3*, *SlABF4*, *SlABF5*, *SlABF8*, *SlABF9* and *SlABF10* is significantly up-regulated under ABA and FLD treatments (Fig. 9). *SlABF6* expression decreases after 12 h of ABA treatment, and then increases gradually. There is a downward trend under the treatment of FLD. *SlABF7* is up-regulated by ABA treatment, but increases first and then decreases under FLD treatment. 7 genes (*SlABF1*, *SlABF2*, *SlABF5*, *SlABF6*, *SlABF7*, *SlABF9,* and *SlABF10*) have higher relative expression levels under ABA than under FLD treatment. When compared to FLD treatment, the relative expression of *SlABF3* and *SlABF8* under ABA treatment at 12 h is marginally greater, whereas at 24 h, it was marginally

lower. At 12 h, the relative expression of *SlABF4* is higher in FLD treatment than in ABA treatment, while, at 24 h, it is lower in FLD treatment than in ABA treatment.

**Expression profles analysis of ABF/AREB genes in tomato under NaCl, UV, cold and PEG treatments**

In order to clarify the role of *ABF/AREB* in tomato under abiotic stress, the expression levels of 10 *ABF/AREB* genes in tomato under NaCl, UV, cold and PEG treatments were studied. As shown in Figure 10a, the relative expression levels of 10 *ABF/AREB* genes in tomato are different under NaCl treatment. *SlABF6* and *SlABF7* expression levels is decreased by NaCl and cold treatments (Fig. 10a and Fig. 10c). In contrast, the expression of *SlABF3*, *SlABF5*, and *SlABF10* is upregulated by NaCl and cold treatments. Moreover, *SlABF8* is also significantly upregulated by cold stress. Under NaCl treatment, 4 genes (*SlABF1*, *SlABF2*, *SlABF3*, and *SlABF4*) reach the highest levels at 12 h, with *SlABF3* showing the greatest change and increasing approximately 9.06-fold compared to 0 h. The expression levels of *SlABF6*, *SlABF7* and *SlABF9* decrease gradually with the increase of treatment time. The expression levels of the remaining 3 genes (*SlABF5*, *SlABF8*, and *SlABF10*) are highest at 24 h with NaCl treatment. Under cold treatment, the expression of the 4 genes (*SlABF1*, *SlABF3*, *SlABF4* and *SlABF5*) gradually increases and reaches the highest level at 24 h. Compared to 0 h, it is increased by 2.26, 16.80, 3.59 and 10.56-folds, respectively.

The relative expression levels of *SlABF6*, *SlABF7* and *SlABF9* are significantly inhibited by UV treatment (Fig. 10b). After 12 h, however, the relative expression levels of *SlABF2* and *SlABF8* remain essentially unaltered. As the amount of time spent receiving UV treatment is extended, the relative expression levels of *SlABF3*, *SlABF4*, and *SlABF5* steadily increase. *SlABF3* has the largest change trend, increasing by over 38.50 times at 24 h compared to 0 h. After 12 h, *SlABF1* and *SlABF10* expression levels dramatically increase, and at 24 h, they marginally reduce.

Under PEG treatment, the relative expression levels of 10 tomato *ABF/AREB* genes have a similar trend: the expression levels all reach the highest at 24 h (Fig. 10d). The biggest changes are seen in the expression levels of *SlABF1*, *SlABF3*, and *SlABF5*, which are increased by 14.98, 110.16 and 11.13 times compared with 0h, respectively). The expression levels of *SlABF1* and *SlABF4* decrease at first and then increase with the extension of treatment time.

## Discussion

ABA is an important plant hormone. Members of the *ABF/AREB* family are key transcription factors for ABA-dependent genes and they play important roles in plant hormone and abiotic stress responses. However, the *ABF/AREB* gene family in tomato has not been studied in detail. In this study, we identified 10 *ABF/AREB* genes in the tomato genome. The 10 genes of *ABF/AREB* gene family were distributed on 5 chromosomes of tomato (Fig. 1). While 7 *ABF/AREB* members were identified on 5 chromosomes in *Solanum tuberosum* (*Liu et al. 2019*), 10 *ABF/AREB* members were identified on 8 chromosomes in *Oryza sativa* (*Lu et al. 2009*), and

14 *ABF/AREB* members on 9 chromosomes in *Populus trichocarpa* (*Ji et al. 2013*). It can be seen that the more the number of chromosomes, the more the corresponding number of *ABF/AREB* genes. The possible reasons for its occurrence are the whole genome duplication event (WGD), tandem gene duplication and chromosome recombination. In *Actinidia chinensis*, *AchnABF2* is localized in the nucleus (*Wei et al. 2020*). *Arabidopsis thaliana* AREB/ABFs have been reported to localize in the nucleus and form heterodimers (*Yoshida et al. 2010*). In this study, the ABF/AREB family in tomato is mainly expressed in the nucleus, so another way to regulate the activity of tomato ABF/AREB proteins is dimerization. The secondary structures of AREB1 protein in *Vignaum bellata* and *Phaseolus vulgari* are alpha helix and random coil (*Hai-long et al. 2018*). Similarly, the secondary structure of ABF/AREB family in tomato in this study are mainly alpha helix and random coil. Therefore, irregular coil accounts for more, which may be because irregular coil connects more secondary structural elements.

ABF/AREBs structurally has 5 conserved domains (Fig. 2) , 3 N-termini (C1, C2 and C3), 1 C-terminal DNA-binding bZIP region, and 1 terminal C4 conserved domain *(Hong et al. 2013; Kim 2005*). ABA-dependent AREB1 is involved in gene regulation through multisite phosphorylation. For example, the phosphorylated active form of AREB1 can induce ectopic genes in vegetative tissues (*Bastias et al. 2011*). However, the hypothesis of the ABF/AREB phosphate site in tomato has not yet been established. This study shows that all the tomato ABF/AREB genes have a unique BRLZ domain at the C-terminus of the bZIP region, which functions to recognize and bind specific DNA sequences. The results indicate that ABF/AREB proteins are highly conserved in plant evolution. We analyzed the genetic structure of the tomato ABF/AREB and found that the intron numbers of the tomato ABF/AREB ranged from 1 to 4, this is similar to the results of genetic structure of potato and *Populus trichocarpa (Ji et al. 2013; Liu et al. 2019*). proving that the genetic makeup of the ABF/AREB family members is conserved (Fig. 5).

ABA activation is required in Arabidopsis AREB1/ABF2, AREB2/ABF4, and ABF3 to regulate ABRE-dependent signaling involved in drought stress tolerance (*Yoshida et al. 2010*). In this study, SlABF3, SlABF5, SlABF10 and AREB1/ABF2, AREB2/ABF4, ABF3 belong to the same grouping of the evolutionary tree (Group A). *SlABF3*, *SlABF5* and *SlABF10* are significantly induced by ABA, NaCl, Uv, cold and PEG treatments. In addition, the three tomato ABF/AREB transcription factors showed similar expression patterns in cellular localization, genetic structure, and tissues. The results suggest that *SlABF3*, *SlABF5* and *SlABF10* play a redundant role in ABRE-dependent ABA signaling pathway under osmotic stress. However, another member of this subgroup, SlABF7, is expressed at a lower level than SlABF3, SlABF5, and SlABF10. The expression level of ABF1 in *Arabidopsis* is lower compared to *AREB1/ABF2*, *AREB2/ABF4*, and *ABF3*, but *ABF1* is a functional homologue of *AREB1/ABF2*, *AREB2/ABF4*, and *ABF3* dependent gene expression. The cellular localization of SlABF7 in genetic structure is similar to that of SlABF3, SlABF5 and SlABF10. Therefore, *SlABF7* may be a functional homologue of *SlABF3*, *SlABF5* and *SlABF10*.

It has been observed that promoter homeopathic elements are crucial for controlling gene expression, notably when biotic and abiotic stressors are present (*Gao et al. 2021*). We determined that the promoter region of the tomato *ABF/AREB* gene has a range of *cis*-acting components related to hormone response and abiotic stress (Fig. 7). This demonstrated that the *ABF/AREB* gene may be crucial for adapting to abiotic stressors and hormonal stimulation in tomato. Our functional verification research of the *ABF/AREB* gene revealed that ABA and PEG can activate several *ABF/AREB* genes *(Vysotskii et al. 2013; Zandkarimi et al. 2015*). Some *ABF/AREB* genes that are hormone-induced have corresponding hormone-related *cis*-elements in their promoters. For example, the relative expression levels of *SlABF1*, *SlABF2*, *SlABF8* and *SlABF10* are upregulated under osmotic treatment (Fig. 10), which is consistent with the distribution of osmotic response elements (MBS) in *SlABF1*, *SlABF2*, *SlABF8* and *SlABF10*, implying that they might control gene transcription by combining active transcription factors with *cis*-acting components to produce the desired effects. Interestingly, there are also conflicting results in our analysis. *SlABF3* and *SlABF5* do not participate in the cryoresponsive element and responded significantly to osmotic stress. It might be because the transcription of the regulated genes is not influenced directly by the presence or absence of the appropriate *cis*-acting elements. In addition to the well-known ABA-induced phosphorylation by SnRK2 protein kinases, it has been demonstrated that Arabidopsis *ABFs* themselves are implicated in the induction of exogenous ABA treatment (*Wang et al. 2019*). This adds another layer of ABA control towards ABF proteins. It was found through the analysis of tomato *ABF/AREB* homeopathic elements that *SlABF1*-*SlABF6*, *SlABF9* and *SlABF10* possessed ABRE in their promoter regions (Fig. 7), implying that the rapid induction of their expression on exogenous ABA treatment might also be mediated by themselves.

ABF/AREB transcription factors participate in not only stress response, but also in hormone response. The role of *ABF/AREBs* in stress response, growth and development has been extensively studied and characterized in *Arabidopsis thaliana* and *Solanum tuberosum (Li et al. 2013; Liu et al. 2019; Vishwakarma et al. 2017*). *ABF/AREB* can bind to ABRE and activate the expression of ABA-dependent genes under drought stress (*Fujita et al. 2011*). It has been shown that the *ABF/AREB* family is sensitive to ABA response *(Liu et al. 2019; Lu et al. 2009; Zandkarimi et al. 2015*). The overexpression of *TaAREB3* in *Arabidopsis* improved osmotic and freezing tolerance and enhanced ABA sensitivity (*Wang et al. 2016*). StCDPK2, a calcium-dependent protein kinase that phosphorylates *StABF1* in vitro, is found to respond to ABA and NaCl (*Muniz Garcia et al. 2012*). However, we found that *SlABF3* is significantly induced by ABA treatment. FLD is known as an inhibitor of ABA biosynthesis and FLD affects plant growth and development and stress response by reducing ABA levels *(Ondzighi-Assoume et al. 2016; Zou et al. 2018*). The expression level of *AchABF1-1* is induced by ABA but inhibited by FLD in *Actinidia chinensis* (*Wei et al. 2022*). In the present study, exogenous ABA promoted the expression of ABF/AREB in tomato. In contrast, FLD inhibited this promotion effect. These findings implied that the *ABF/AREB* genes are crucial for response of ABA in the tomato. We also discovered that tomato *ABF/AREB* genes respond significantly to drought stress. For

instance, during osmotic stress, *SlABF3* was considerably induced up to 110.16-folds, and *SlABF5* was significantly induced up to 11.13-folds. It has been demonstrated that the *ABF/AREB* genes are crucial for response of osmotic stress in the tomato. This concurs with earlier research on *Oryza sativa* and *Nicotiana tabacum (Hossain et al. 2010; Maruyama et al. 2012*). Additionally, research revealed that excessive salt and osmotic pressure may also activate the majority of the ABA-induced genes (*Seki et al. 2003*). This suggested that there is an interaction between plant responses to hormones and abiotic stresses. Therefore the specific functions of tomato *ABF/AREB* genes need to be further investigated in depth.

## Conclusions

In summary, 10 members of the *ABF/AREB* genes family in tomato are identified and their genetic structure, conserved domains, and phylogenetic relationships are analyzed in this study. The ABF/AREB gene family is highly conserved during evolution. *Cis*-acting analysis reveals the genetic basis of *ABF/AREB* response to multiple hormones and stresses in tomato. *ABF/AREBs* are expressed in many tissues of tomato. *ABF/AREB* genes plays an important role in response to phyhormone and abiotic stresses in tomato. Appears obvious to ABA is seen in *SlABF3*. Significant induction of *SlABF1*, *SlABF3* and *SlABF10* occurred during UV stress. Osmotic stress greatly increases the expression of *SlABF3* and *SlABF5*. Salt and cold stresses significantly induce *SlABF3*, *SlABF5* and *SlABF10*. Our findings lay a theoretical foundation for further exploring the function of *ABF/AREBs* in plants.

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**Author Contributions:**

Xuejuan Pan conceived and designed the experiments, performed the experiments,prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
Chunlei Wang performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
Zesheng Liu conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
Rong Gao performed the experiments, prepared figures and/or tables, and approved the final draft.
Li Feng analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.

Ailing Li and Kangding Yao analyzed the data, prepared figures and tables, and approved the final draft.
Weibiao Liao conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

**Data availability:**

The following information was supplied regarding data availability:

Original data including tomato, potato, Arabidopsis and poplar ABF/AREB protein sequences are available in the supplementary file.

## References


**Bastias A, Lopez-Climent M, Valcarcel M, Rosello S, Gomez-Cadenas A, and Casaretto JA. 2011.** Modulation of organic acids and sugar content in tomato fruits by an abscisic acid-regulated transcription factor. *Physiol Plant* **141:**215-226 DOI 10.1111/j.1399-3054.2010.01435.x

**Chang HC, Tsai MC, Wu SS, and Chang IF. 2019.** Regulation of *ABI5* expression by *ABF3* during salt stress responses in *Arabidopsis thaliana*. *Bot Stud* **60:**16 DOI 10.1186/s40529-019-0264-z

**Fleta-Soriano E, and Munne-Bosch S. 2016.** Stress Memory and the Inevitable Effects of Drought: A Physiological Perspective. *Front Plant Sci* **7:**143 DOI 10.3389/fpls.2016.00143

**Fujii H, Chinnusamy V, Rodrigues A, Rubio S, Antoni R, Park SY, Cutler SR, Sheen J, Rodriguez PL, and Zhu JK. 2009.** In vitro reconstitution of an abscisic acid signalling pathway. *Nature* **462:**660-664 DOI 10.1038/nature08599

**Fujita Y, Fujita M, Satoh R, Maruyama K, Parvez MM, Seki M, Hiratsu K, Ohme-Takagi M, Shinozaki K, and Yamaguchi-Shinozaki K. 2005.** *AREB1* is a transcription activator of novel ABRE-dependent ABA signaling that enhances drought stress tolerance in Arabidopsis. *Plant Cell* **17:**3470-3488 DOI 10.1105/tpc.105.035659

**Fujita Y, Fujita M, Shinozaki K, and Yamaguchi-Shinozaki K. 2011.** ABA-mediated transcriptional regulation in response to osmotic stress in plants. *J Plant Res* **124:**509-525. 10.1007/s10265-011-0412-3

**Fujita Y, Yoshida T, and Yamaguchi-Shinozaki K. 2013.** Pivotal role of the *AREB/ABF*-SnRK2 pathway in ABRE-mediated transcription in response to osmotic stress in plants. *Physiol Plant* **147:**15-27 DOI 10.1111/j.1399-3054.2012.01635.x

**Gao R, Luo Y, Yun F, Wu X, Wang P, and Liao W. 2021.** Genome-Wide Identification, Expression Profile, and Alternative Splicing Analysis of *CAMTA* Family Genes in Cucumber (*Cucumis sativus L.*). *Agronomy* **11** DOI 10.3390/agronomy11091827

**Hai-long L, Li-hua D, Shu-mei F, Jian-ying L, Wen-kai M, and Xi-long L. 2018.** Bioinformatics Analysis of *AREB1* from Red Bean and Kidney Bean. 31 DOI 10. 16213 /j. cnki. scjas. 2018. 8. 002

**Hong L, Hu B, Liu X, He CY, Yao Y, Li XL, and Li L. 2013.** Molecular cloning and expression analysis of a new stress-related *AREB* gene from *Arachis hypogaea*. *Biologia plantarum* **57:**56-62 DOI 10.1007/s10535-012-0236-6

**Hossain MA, Cho JI, Han M, Ahn CH, Jeon JS, An G, and Park PB. 2010.** The ABRE-binding bZIP transcription factor *OsABF2* is a positive regulator of abiotic stress and ABA signaling in rice. *J Plant Physiol* **167:**1512-1520 DOI 10.1016/j.jplph.2010.05.008

**Hsieh TH, Li CW, Su RC, Cheng CP, Sanjaya, Tsai YC, and Chan MT. 2010.** A tomato bZIP transcription factor, *SlAREB*, is involved in water deficit and salt stress response. *Planta* **231:**1459-1473 DOI 10.1007/s00425-010-1147-4

**Ji L, Wang J, Ye M, Li Y, Guo B, Chen Z, Li H, and An X. 2013.** Identification and characterization of the *Populus AREB/ABF* subfamily. *J Integr Plant Biol* **55:**177-186 DOI 10.1111/j.1744-7909.2012.01183.x

**Kim S, Kang JY, Cho DI, Park JH, and Kim SY. 2004.** *ABF2*, an ABRE-binding bZIP factor, is an essential component of glucose signaling and its overexpression affects multiple stress tolerance. *Plant J* **40:**75-87 DOI 10.1111/j.1365-313X.2004.02192.x

**Kim SY. 2005.** The role of *ABF* family bZIP class transcription factors in stress response. *Physiologia plantarum* **126:**519-527 DOI 10.1111/j.1399-3054.2005.00601.x

**Li F, Mei F, Zhang Y, Li S, Kang Z, and Mao H. 2020.** Genome-wide analysis of the *AREB/ABF* gene lineage in land plants and functional analysis of *TaABF3* in *Arabidopsis*. *BMC Plant Biol* **20:**558 DOI 10.1186/s12870-020-02783-9

**Li XY, Liu X, Yao Y, Li YH, Liu S, He CY, Li JM, Lin YY, and Li L. 2013.** Overexpression of Arachis hypogaea *AREB1* gene enhances drought tolerance by modulating ROS scavenging and maintaining endogenous ABA content. *Int J Mol Sci* **14:**12827-12842 DOI 10.3390/ijms140612827

**Liu T, Zhou T, Lian M, Liu T, Hou J, Ijaz R, and Song B. 2019.** Genome-Wide Identification and Characterization of the *AREB/ABF/ABI5* Subfamily Members from *Solanum tuberosum*. *Int J Mol Sci* 20 DOI 10.3390/ijms20020311

**Liu Z, Pan X, Wang C, Yun F, Huang D, Yao Y, Gao R, Ye F, Liu X, and Liao W. 2022.** Genome-wide identification and expression analysis of serine hydroxymethyltransferase (*SHMT*) gene family in tomato (*Solanum lycopersicum*). *PeerJ* **10:** 12943 DOI 10.7717/peerj.12943

**Lu G, Gao C, Zheng X, and Han B. 2009.** Identification of *OsbZIP72* as a positive regulator of ABA response and drought tolerance in rice. *Planta* **229**:605-615 DOI 10.1007/s00425-008-0857-3

**Maruyama K, Todaka D, Mizoi J, Yoshida T, Kidokoro S, Matsukura S, Takasaki H, Sakurai T, Yamamoto YY, Yoshiwara K, Kojima M, Sakakibara H, Shinozaki K, and Yamaguchi-Shinozaki K. 2012.** Identification of cis-acting promoter elements in cold- and dehydration-induced transcriptional pathways in Arabidopsis, rice, and soybean. *DNA Res* **19**:37-49 DOI 10.1093/dnares/dsr040

**Muniz Garcia MN, Giammaria V, Grandellis C, Tellez-Inon MT, Ulloa RM, and Capiati DA. 2012.** Characterization of *StABF1*, a stress-responsive bZIP transcription factor from *Solanum tuberosum L.* that is phosphorylated by StCDPK2 in vitro. *Planta* **235:**761-778 DOI 10.1007/s00425-011-1540-7

**Ondzighi-Assoume CA, Chakraborty S, and Harris JM. 2016.** Environmental Nitrate Stimulates Abscisic Acid Accumulation in Arabidopsis Root Tips by Releasing It from Inactive Stores. *Plant Cell* **28:**729-745 DOI 10.1105/tpc.15.00946

**Qian D, Zhang Z, He J, Zhang P, Ou X, Li T, Niu L, Nan Q, Niu Y, He W, An L, Jiang K, and Xiang Y. 2019.** Arabidopsis ADF5 promotes stomatal closure by regulating actin cytoskeleton remodeling in response to ABA and drought stress. *J Exp Bot* **70:**435-446 DOI 10.1093/jxb/ery385

**Seki M, Kamei A, Yamaguchi-Shinozaki K, and Shinozaki K. 2003.** Molecular responses to drought, salinity and frost: common and different paths for plant protection. *Current Opinion in Biotechnology* **14:**194-199 DOI 10.1016/s0958-1669(03)00030-2

**Vishwakarma K, Upadhyay N, Kumar N, Yadav G, Singh J, Mishra RK, Kumar V, Verma R, Upadhyay RG, Pandey M, and Sharma S. 2017.** Abscisic Acid Signaling and Abiotic Stress Tolerance in Plants: A Review on Current Knowledge and Future Prospects. *Front Plant Sci* **8:**161 DOI 10.3389/fpls.2017.00161

**Vysotskii DA, de Vries-van Leeuwen IJ, Souer E, Babakov AV, and de Boer AH. 2013.** ABF transcription factors of Thellungiella salsuginea: Structure, expression profiles and interaction with 14-3-3 regulatory proteins. *Plant Signal Behav* **8:**e22672 DOI 10.4161/psb.22672

**Wang J, Li Q, Mao X, Li A, and Jing R. 2016.** Wheat Transcription Factor TaAREB3 Participates in Drought and Freezing Tolerances in Arabidopsis. *Int J Biol Sci* **12:**257-269 DOI 10.7150/ijbs.13538

**Wang X, Guo C, Peng J, Li C, Wan F, Zhang S, Zhou Y, Yan Y, Qi L, Sun K, Yang S, Gong Z, and Li J. 2019.** ABRE-BINDING FACTORS play a role in the feedback regulation of ABA signaling

by mediating rapid ABA induction of ABA co-receptor genes. *New Phytol* **221:**341-355 DOI 10.1111/nph.15345

**Wei X, Lu W, Mao L, Han X, Wei X, Zhao X, Xia M, and Xu C. 2020.** ABF2 and MYB transcription factors regulate feruloyl transferase FHT involved in ABA-mediated wound suberization of kiwifruit. *J Exp Bot* **71:**305-317 DOI 10.1093/jxb/erz430

**Wei X, Wei X, Guan W, Nong W, Chen R, Tao X, and Mao L. 2022.** ABA-responsive transcription factor *ABF1-1* promotes JA biosynthesis to accelerate suberin polyphenolic formation in wounded kiwifruit (*Actinidia chinensis*). *Postharvest Biology and Technology* 187 DOI 10.1016/j.postharvbio.2022.111850

**Yong X, Zheng T, Zhuo X, Ahmad S, Li L, Li P, Yu J, Wang J, Cheng T, and Zhang Q. 2021.** Genome-wide identification, characterisation, and evolution of *ABF/AREB* subfamily in nine Rosaceae species and expression analysis in mei (*Prunus mume*). *PeerJ* **9:**e10785 DOI 10.7717/peerj.10785

**Yoshida T, Fujita Y, Sayama H, Kidokoro S, Maruyama K, Mizoi J, Shinozaki K, and Yamaguchi-Shinozaki K. 2010.** *AREB1*, *AREB2*, and *ABF3* are master transcription factors that cooperatively regulate ABRE-dependent ABA signaling involved in drought stress tolerance and require ABA for full activation. *Plant J* **61:**672-685. 10.1111/j.1365-313X.2009.04092.x

**Zandkarimi H, Ebadi A, Salami SA, Alizade H, and Baisakh N. 2015.** Analyzing the Expression Profile of *AREB/ABF* and *DREB/CBF* Genes under Drought and Salinity Stresses in Grape (*Vitis vinifera L.*). *PLoS One* **10:**e0134288 DOI 10.1371/journal.pone.0134288

**Zhao BY, Hu YF, Li JJ, Yao X, and Liu KD. 2016.** *BnaABF2*, a bZIP transcription factor from rapeseed (*Brassica napus L.*), enhances drought and salt tolerance in transgenic Arabidopsis. *Bot Stud* **57:**12 DOI 10.1186/s40529-016-0127-9

**Zhu JK. 2016.** Abiotic Stress Signaling and Responses in Plants. *Cell* **167:**313-324 DOI 10.1016/j.cell.2016.08.029

**Zou Z, Zou X, Zhao S, Xia C, Qian K, Wang P, and Yin C. 2018.** Fluridone Induces Leaf Bleaching by Inhibiting Pigment Biosynthesis via Downregulated Transcription Levels of Pigment Biosynthetic Genes in Rice (*Oryza Sativa L.*). *Journal of Plant Growth Regulation* **37:**1385-1395 DOI 10.1007/s00344-018-9781-3

## Table 1(on next page)

Table 1

**qRT-PCR primers for expression analysis of *ABF/AREB* gene family in tomato**

1 **Table 1** qRT-PCR primers for expression analysis of *ABF/AREB* gene family in tomato

| Gene | Prime sequence | | Size /bp |
|---|---|---|---|
| *SlABF1* | F: ACTACTTGGTGAAAGCCGGG | R: CGATGTCCATAGCACCCCTC | 171 |
| *SlABF2* | F: GCTACACAGCAGAAACAGCG | R: CCATGATCTGCTTAAGTCTCTCCT | 181 |
| *SlABF3* | F: CACATTGACATGTCGTGCGAA | R: GTTGCCTTGCAGCTCTGATG | 171 |
| *SlABF4* | F: TTGGAGGCGACTTCCATGAC | R: ATCCACCGTCCTCCTAACCA | 189 |
| *SlABF5* | F: GTTTAGGAGCCAGTGGGGTC | R: CTGCCTCCTTTCAACGACCT | 176 |
| *SlABF6* | F: CAGCAACAGAACAACGGGTG | R:TGATTGCTGCTGAGGAGGTG | 162 |
| *SlABF7* | F: CAGCAACCAACTCAAAGCCC | R: GCCAGTTGGCAATTGTTCCC | 176 |
| *SlABF8* | F: GAAAGGAGGCAGAAGCGGAT | R: GCTCTGGAGGTGGAACACTC | 178 |
| *SlABF9* | F: TGTTGGGCACATTATCGGACA | R: CGAGGCGTGAAACCTTGTTC | 183 |
| *SlABF10* | F: GCGTTGTCATCTTCTGCTGC | R: CTCCCAAGGTAGATTCCCGC | 187 |
| *Actin* | F: AATGAACTTCGTGTGGCTCCAGAG | R: ATGGCAGGGGTGTTGAAGGTTTC | |

2

## Table 2(on next page)

Table 2

**Information of the ABF/AREB transcription factors in tomato**

1 **Table 2** Information of the ABF/AREB transcription factors in tomato

| Gene | Gene ID | Gene locus | ORF (bp) | Amino acid | Instability index | Molecular weight/kDa | pI | Subcellular Localization |
|---|---|---|---|---|---|---|---|---|
| SlABF1 | Solyc01g008980.3.1.ITAG3.2 | Chr01 | 441 | 146 | 55.95 | 16689.92 | 9.22 | Nucleus |
| SlABF2 | Solyc01g104650.3.1.ITAG3.2 | Chr01 | 894 | 297 | 66.49 | 32080.06 | 7.81 | Nucleus Chloroplast |
| SlABF3 | Solyc01g108080.3.1.ITAG3.2 | Chr01 | 1245 | 414 | 58.79 | 45028.31 | 9.64 | Nucleus |
| SlABF4 | Solyc04g071510.3.1.ITAG3.2 | Chr04 | 927 | 308 | 56.19 | 33874.15 | 6.71 | Nucleus |
| SlABF5 | Solyc04g078840.3.1.ITAG3.2 | Chr04 | 1344 | 447 | 50.23 | 47977.73 | 9.42 | Nucleus |
| SlABF6 | Solyc09g009490.3.1.ITAG3.2 | Chr09 | 1281 | 426 | 54.70 | 46072.80 | 8.79 | Nucleus |
| SlABF7 | Solyc10g050210.2.1.ITAG3.2 | Chr10 | 1137 | 378 | 52.09 | 41170.31 | 9.72 | Nucleus |
| SlABF8 | Solyc10g076920.2.1.ITAG3.2 | Chr10 | 975 | 324 | 62.54 | 36282.85 | 6.41 | Nucleus |
| SlABF9 | Solyc10g081350.2.1.ITAG3.2 | Chr10 | 1053 | 350 | 55.95 | 38396.10 | 8.63 | Nucleus |
| SlABF10 | Solyc11g044560.2.1.ITAG3.2 | Chr11 | 1098 | 365 | 61.45 | 40007.81 | 8.51 | Nucleus |

2

## Table 3(on next page)

Table 3

**Details of the 10 conserved motifs of tomato ABF/AREB proteins**

1 **Table 3** Details of the 10 conserved motifs of tomato ABF/AREB proteins

| Motif | Width (aa) | Motif Sequence |
|---|---|---|
| Motif 1 | 50 | EKVVERRQRRMIKNRESAARSRARKQAYTVELEAEVAKLEEENERLKKKK |
| Motif 2 | 26 | GZRQSTLGEMTLEDFLVKAGVVREDA |
| Motif 3 | 29 | SLQRQGSLTLPRTLSQKTVDEVWRDIQKE |
| Motif 4 | 21 | GGLGKDFGSMNMDELLKNIWT |
| Motif 5 | 18 | LARQSSIYSLTFDELQNT |
| Motif 6 | 21 | LPNVPKREPLRCLRRTLSGPW |
| Motif 7 | 25 | NLDTSSLSPSPYAFNEGGRGRKSCS |
| Motif 8 | 50 | WSQYQIPAMQPLPPQQHQQQQQNIPPVFMPGHPIQQPLPIVANPIIDAAY |
| Motif 9 | 33 | QQQPLFPKQTTVEFASPMQLGNNGQLASPRTRA |
| Motif 10 | 10 | MGSYLNFKNF |

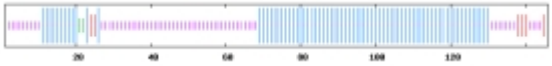
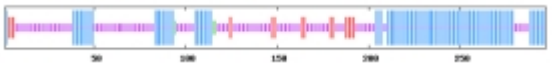
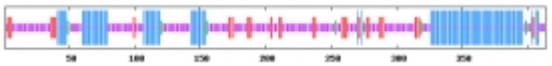
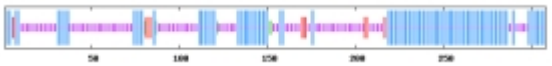
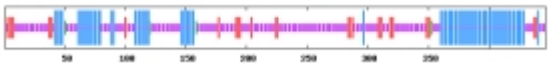
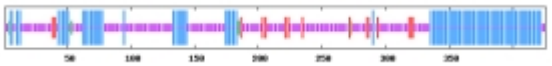
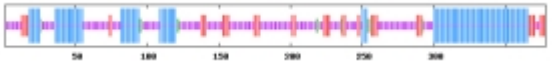
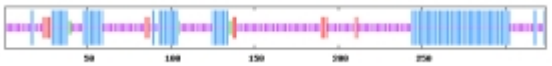
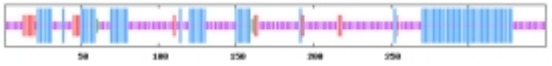
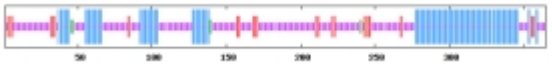
2

## Table 4(on next page)

Table 4

**The secondary structure of ABF/AREB gene family protein sequence in tomato.** Blue indicates alpha helix; Green indicates beta turn; Red indicates extended strand; Purple indicates random coil

**Table 4** The secondary structure of ABF/AREB gene family protein sequence in tomato. Blue indicates alpha helix; Green indicates beta turn; Red indicates extended strand; Purple indicates random coil

| Protein | Alpha helix (%) | Extended strand (%) | Beta turn (%) | Random coil (%) | Distributionofsecondarystructureelements |
|---|---|---|---|---|---|
| SlABF1 | 50.68 | 4.79 | 1.37 | 43.15 |  |
| SlABF2 | 40.07 | 7.07 | 1.01 | 51.85 |  |
| SlABF3 | 31.40 | 11.59 | 3.14 | 53.86 |  |
| SlABF4 | 44.48 | 4.87 | 1.62 | 49.03 |  |
| SlABF5 | 29.31 | 10.07 | 1.57 | 59.06 |  |
| SlABF6 | 34.51 | 6.81 | 1.41 | 57.28 |  |
| SlABF7 | 32.54 | 13.49 | 2.91 | 51.06 |  |
| SlABF8 | 33.95 | 5.25 | 1.54 | 59.26 |  |
| SlABF9 | 34.29 | 7.14 | 1.43 | 57.14 |  |
| SlABF10 | 32.88 | 9.04 | 1.92 | 56.16 |  |

## Table 5(on next page)

Table 5

**Each of the tomato *ABF/ABRE* gene family has the original function of homeopathy**

1 **Table 5** Each of the tomato *ABF/ABRE* gene family has the original function of homeopathy

| Cis-Element | Number of Genes | Sequence of Cis-Element | Functions of Cis-Elements |
|---|---|---|---|
| ABRE | 18 | TACGTGTC | cis-acting element involved in the abscisic acid responsiveness |
| ACE | 4 | CTAACGTATT | cis-acting element involved in light responsiveness |
| AE-box | 5 | AGAAACAA | part of a module for light response |
| ARE | 15 | AAACCA | cis-acting regulatory element essential for the anaerobic induction |
| Box 4 | 32 | ATTAAT | part of a conserved DNA module involved in light responsiveness |
| CGTCA-motif | 7 | CGTCA | cis-acting regulatory element involved in the MeJA-responsiveness |
| GARE-motif | 6 | TCTGTTG | gibberellin-responsive element |
| GATA-motif | 6 | GATAGGA | part of a light responsive element |
| G-Box | 17 | TACGTG | cis-acting regulatory element involved in light responsiveness |
| LTR | 5 | CCGAAA | cis-acting element involved in low-temperature responsiveness |
| MBS | 6 | CAACTG | MYB binding site involved in drought-inducibility |
| MRE | 3 | AACCTAA | MYB binding site involved in light responsiveness |
| P-box | 4 | CCTTTTG | gibberellin-responsive element |
| TATC-box | 5 | TATCCCA | cis-acting element involved in gibberellin-responsiveness |
| TCA-element | 5 | CCATCTTTTT | cis-acting element involved in salicylic acid responsiveness |
| TC-rich repeats | 3 | ATTCTCTAAC | cis-acting element involved in defense and stress responsiveness |
| TGACG-motif | 7 | TGACG | cis-acting regulatory element involved in the MeJA-responsiveness |
| circadian | 5 | CAAAGATATC | cis-acting regulatory element involved in circadian control |

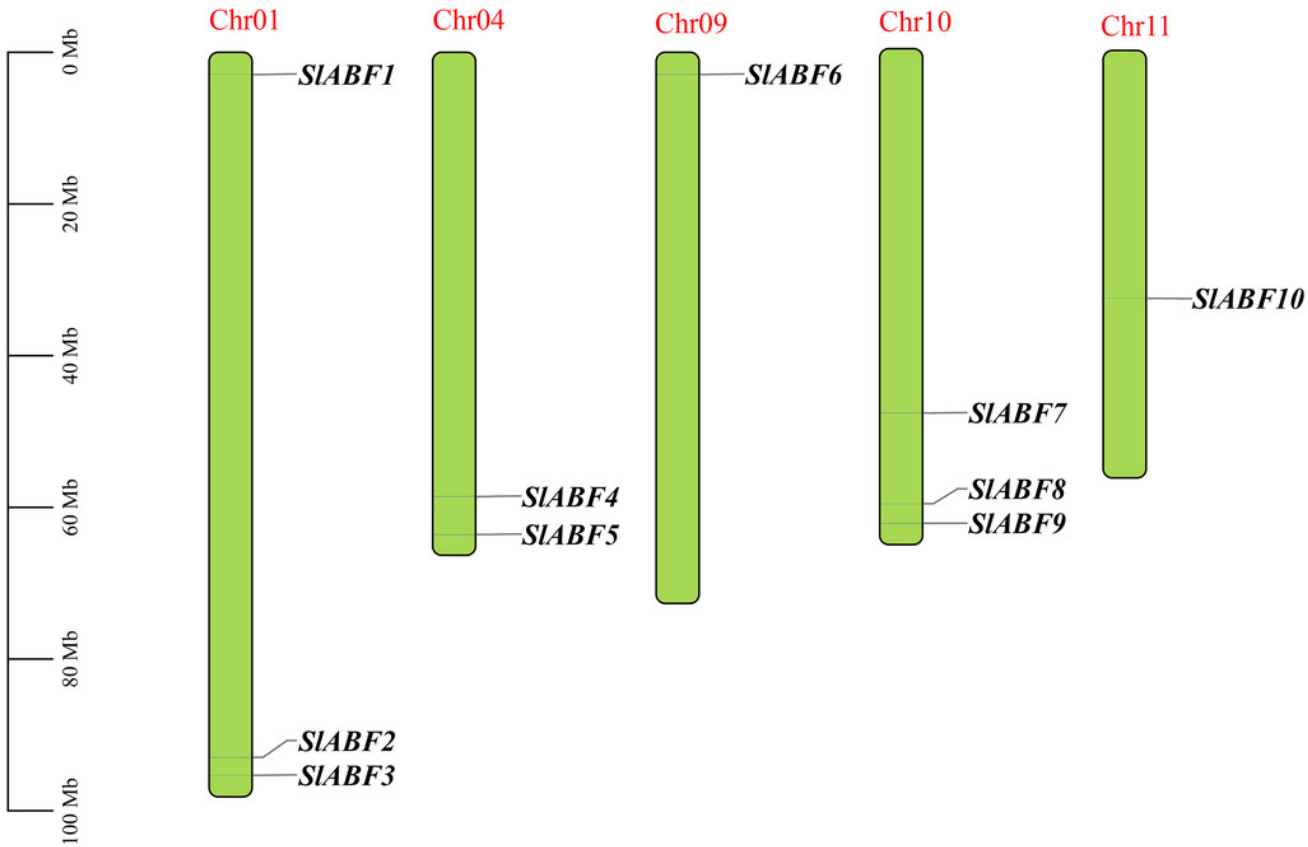
2

# Figure 1

Figure 1

**The distribution of *ABF/AREB* gene family members of chromosomes in tomato.**

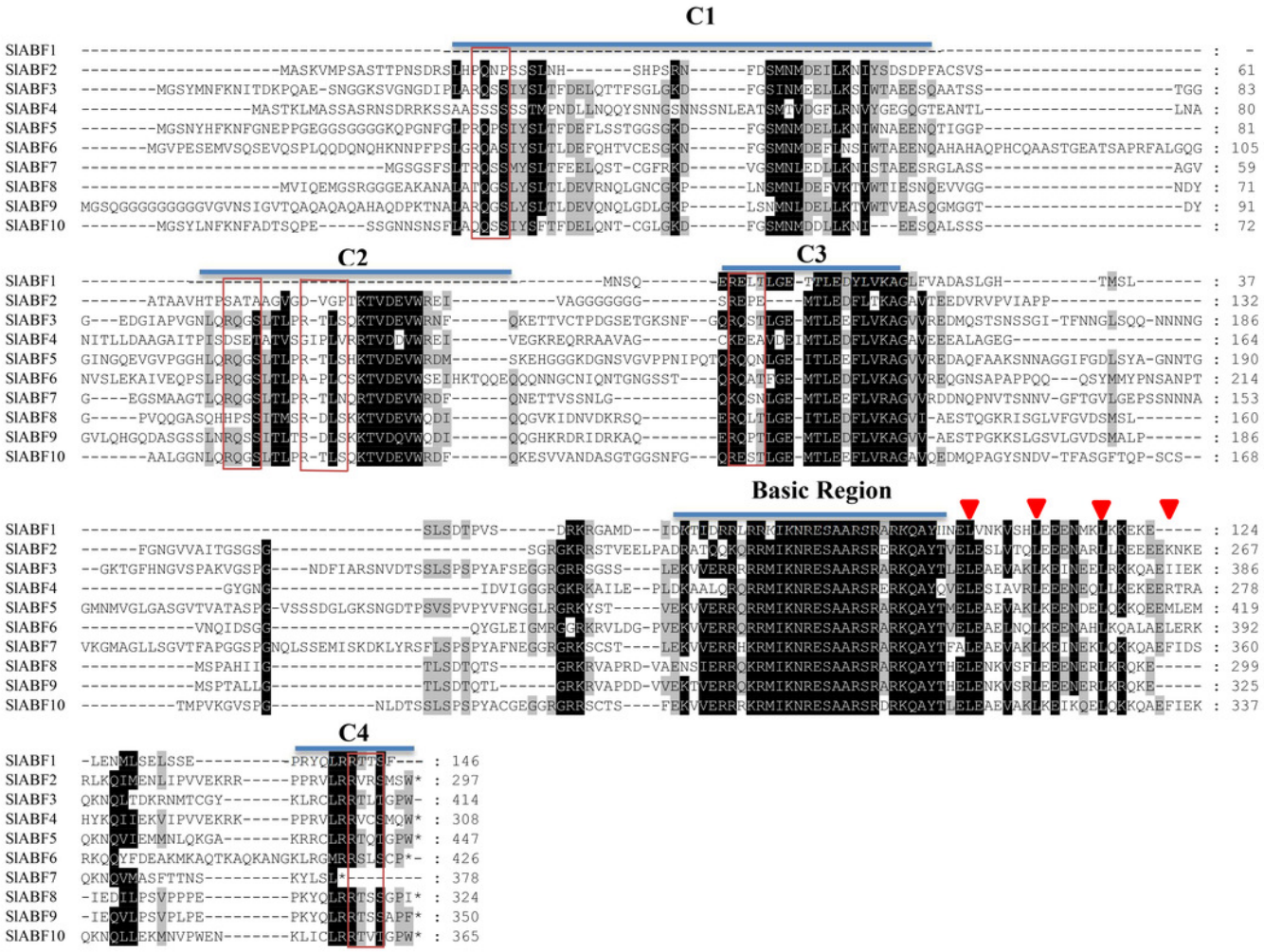
Chromosome positioning was based on the physical location of the 10 tomato *ABF/AREBs*. Chromosome numbers are shown at the top of each bar chart. Gene names are indicated in black. The scale bar is on the left.



# Figure 2

Figure 2

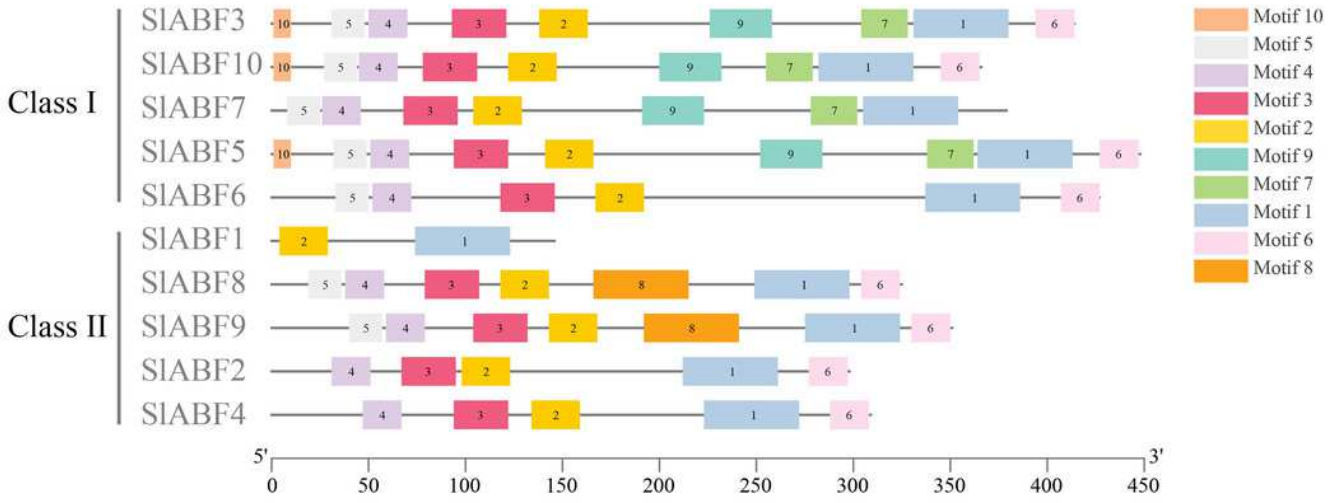
**Multiple sequence alignment of tomato ABF/ AREB members.** Residues are shaded in black and light black, respectively. The positions of C1 to C4 are conserved Domains and basic regions are represented by lines above the protein sequence. Potential phosphorylated residues (R-S-S-X/T) of the characteristic phosphorylation sites are indicated by red boxes. Positions of conserved Leu residues in Leu zippers Domains are represented by red triangle



# Figure 3

Figure 3

**Sequence analysis of ABF/AREB gene family in tomato.** The different colored rectangles are different motifs

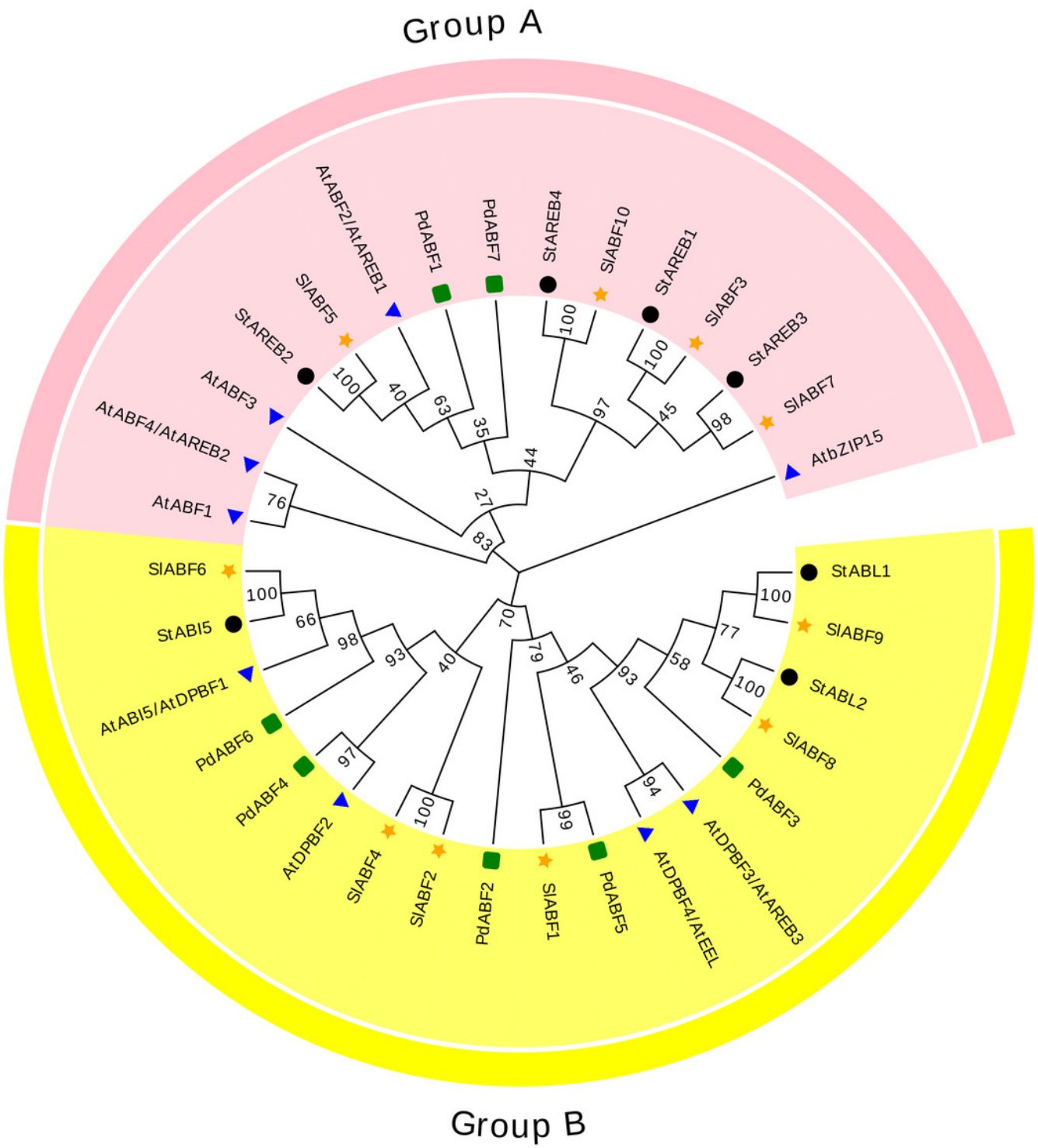


# Figure 4

Figure 4

**Phylogenetic relationship of tomato ABF/ AREB homologs in different species.**

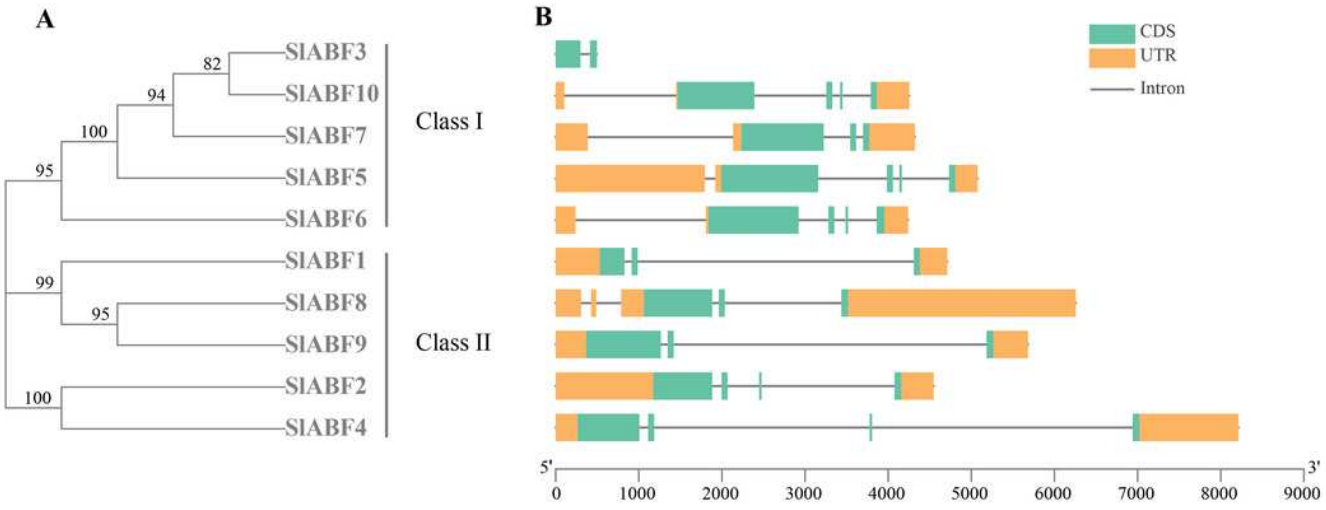
Tomatoes are marked as a yellow five-pointed star


Group A
AtABF2/AtAREB1
PdABF1
PdABF7
StAREB4
SlABF10
StAREB1
SlABF3
StAREB3
SlABF7
AtbZIP15
SlABF5
StAREB2
AtABF3
AtABF4/AtAREB2
AtABF1
100
40
63
35
44
97
100
45
98
27
76
83
SlABF6
StABI5
AtABI5/AtDPBF1
PdABF6
PdABF4
AtDPBF2
SlABF4
SlABF2
PdABF2
SlABF1
PdABF5
AtDPBF4/AtEEL
AtDPBF3/AtAREB3
StABL1
SlABF9
StABL2
SlABF8
PdABF3
100
66
98
93
40
70
79
46
93
58
77
100
100
97
100
99
94
Group B

# Figure 5

Figure 5

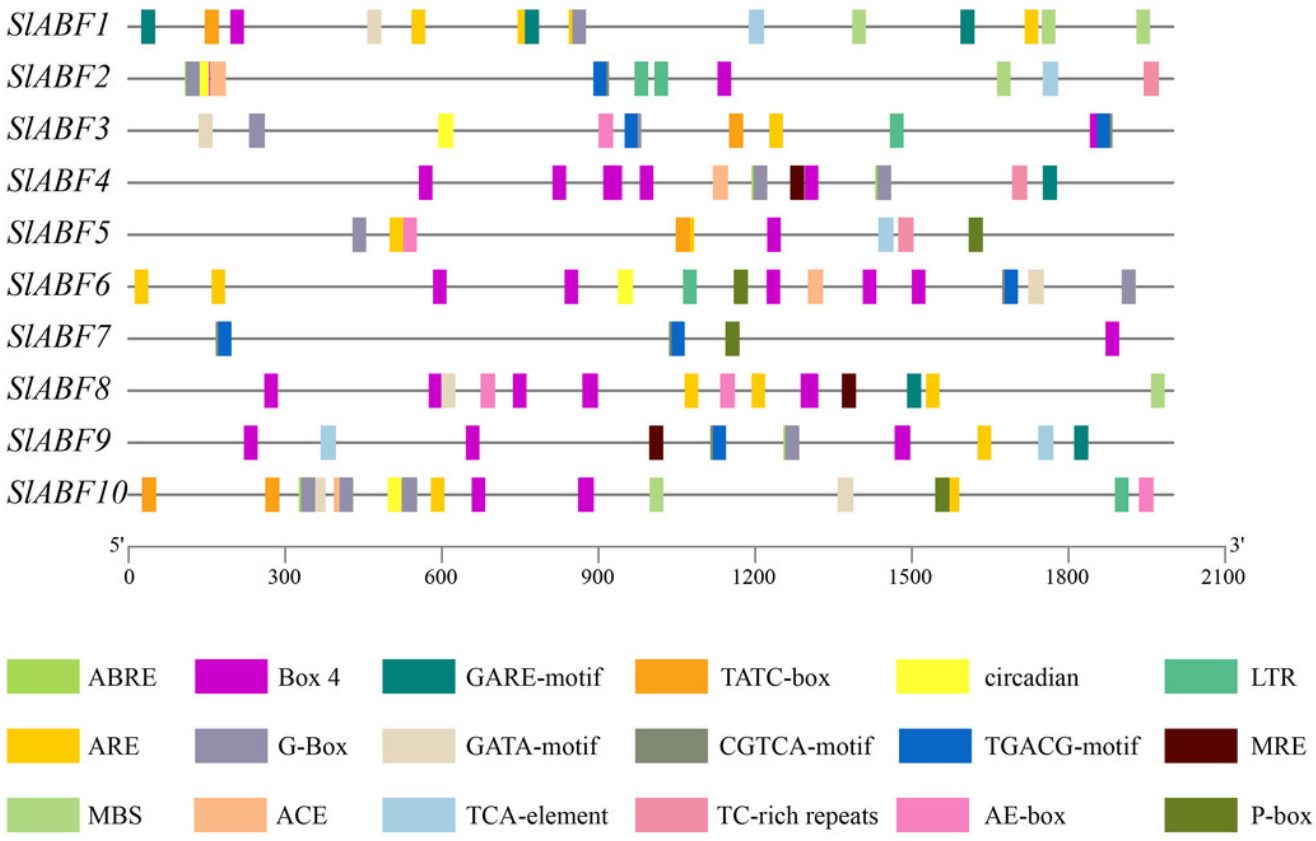
**Exon-intron structure of *ABF/AREB* gene family in tomato.** (A) A phylogenetic tree was constructed based on the full-length tomato ABF/AREB protein sequence using MEGA7.0 software. (B) The exon-intron map of the tomato *ABF/AREB* gene was drawn using TBtools. Green rectangles represent exons, and orange rectangles represent upstream and downstream noncoding regions of genes. Solid black lines represent introns. The scale bar represents the length of the DNA sequence



# Figure 6

Figure 6

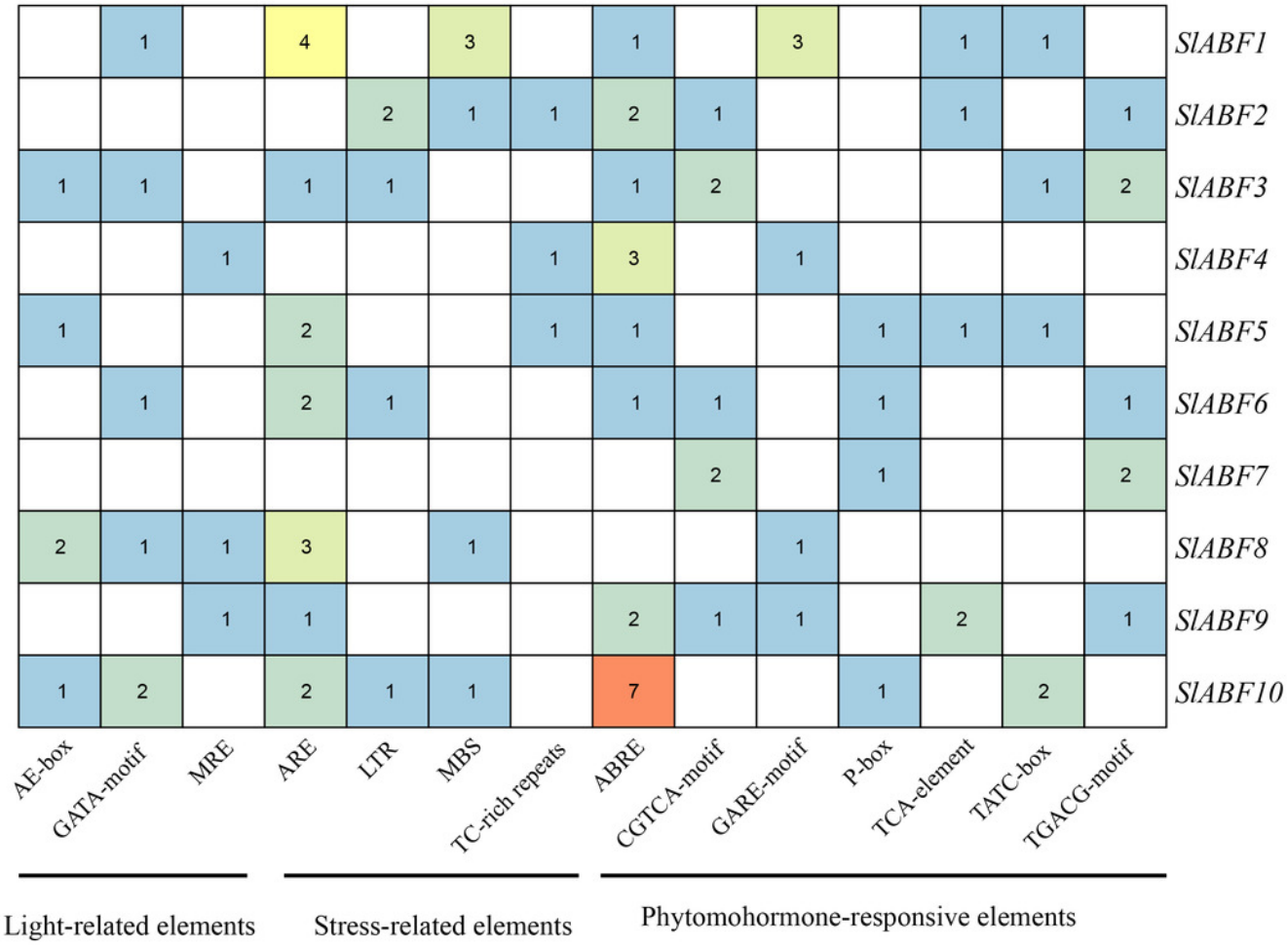
**The distribution of *cis*-acting elements in tomato ABF/AREB genes.** Different colored wedges represent different cis elements. The length and position of each *SlABF* genes were mapped to scale. The scale bar represents the length of the DNA sequence



# Figure 7

Figure 7

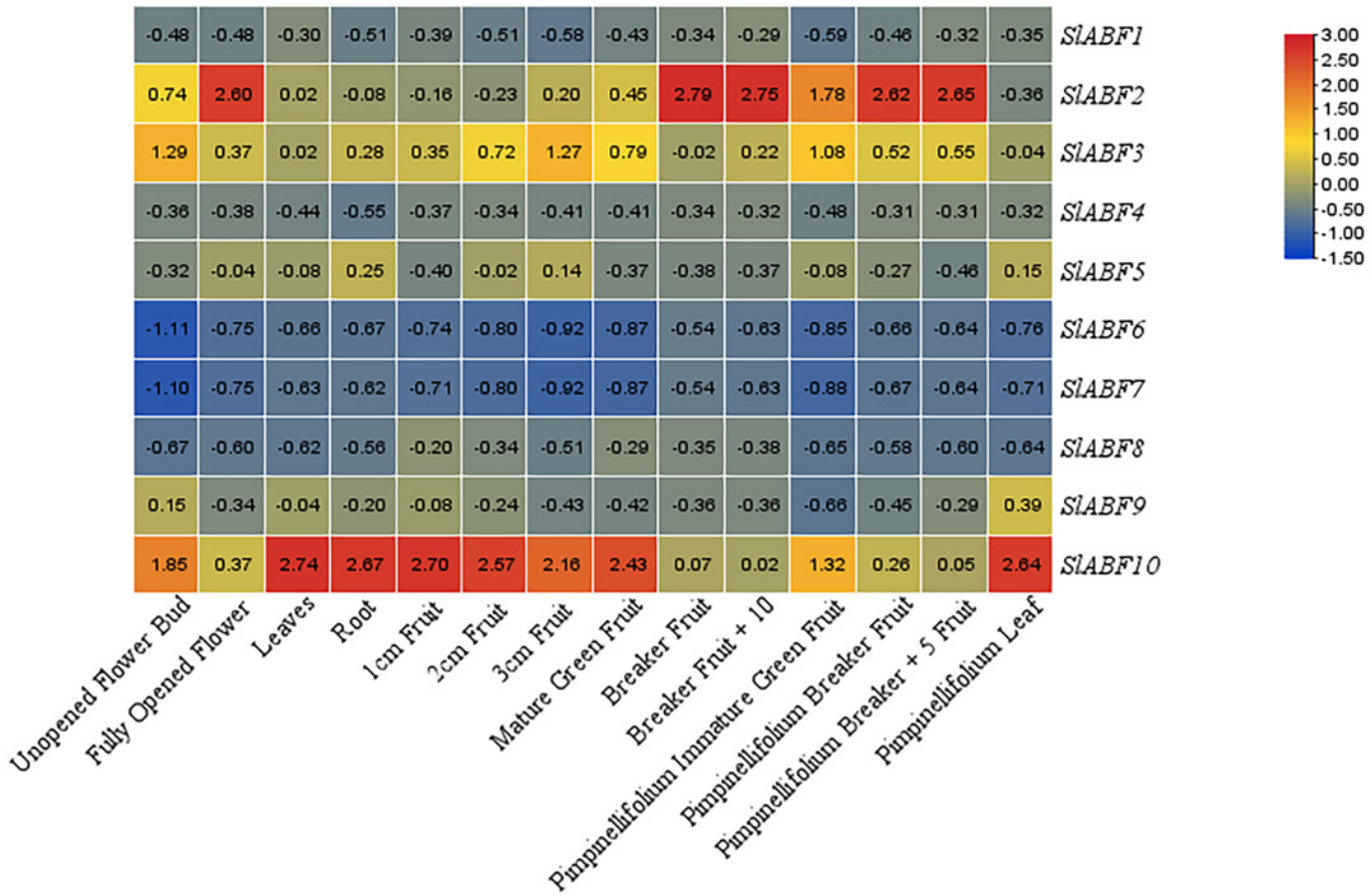
**The number of *cis*-acting elements in tomato *ABF/AREB* genes**



# Figure 8

Figure 8

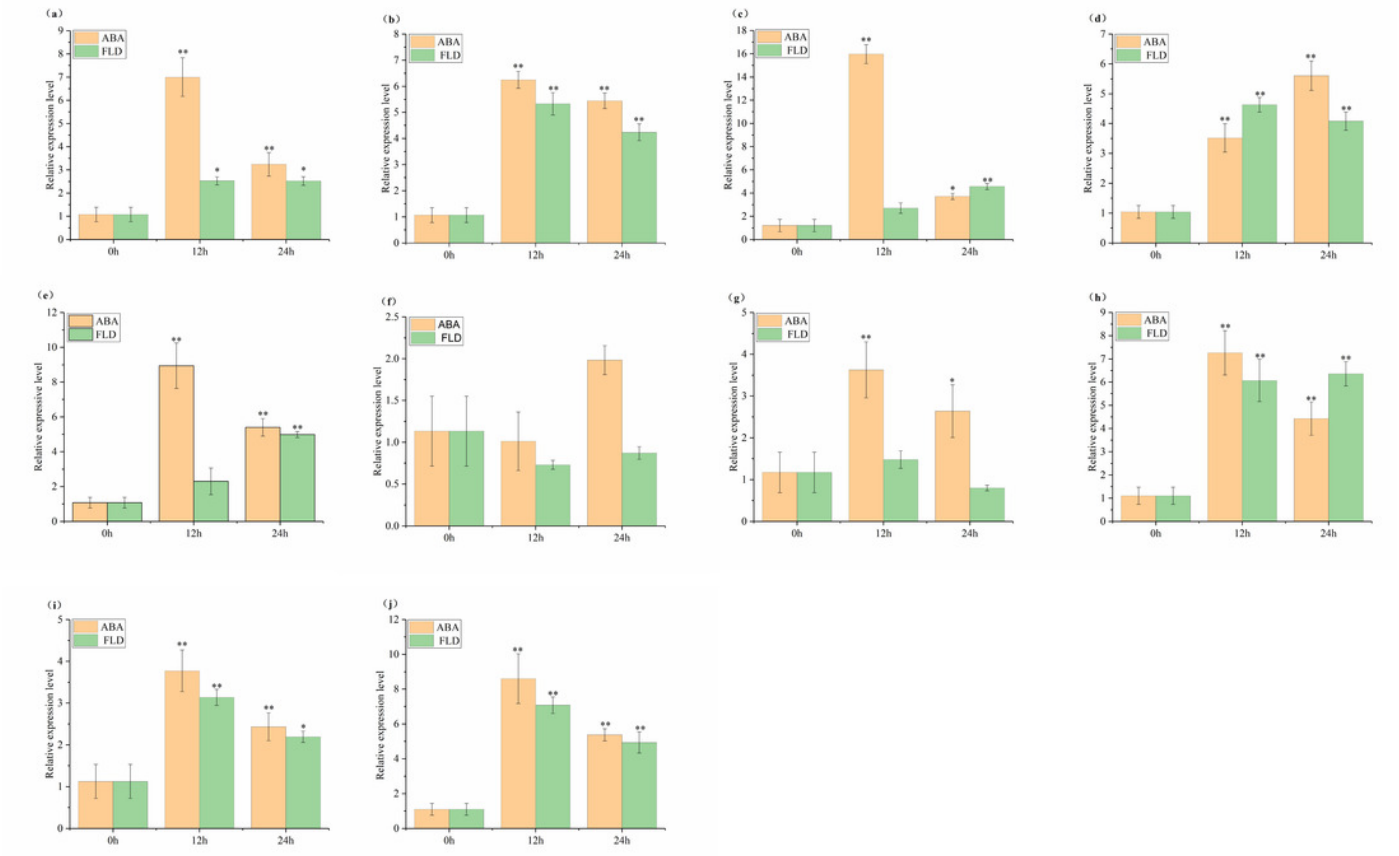
**Expression patterns of tomato *ABF/AREB* in different tissues.** Color scale represents fold change normalized by log2 transformed data. Heatmaps are shown in blue/yellow/red for low/medium/high expression respectively



# Figure 9

Figure 9

**Relative expression analysis of *SlABF* gene under ABA and FLD treatments.** (a) *SlABF1*; (b) *SlABF2*; (c) *SlABF3*; (d) *SlABF4*; (e) *SlABF5*; (f) *SlABF6*; (g) *SlABF7*; (h) *SlABF8*; (i) *SlABF9*; (j) *SlABF10*. The asterisk (*) indicates that the expression level of the stress group is significantly different from that of the control group (* $p < 0.05$, ** $p < 0.01$, one-way ANOVA, Tukey test)



# Figure 10

Figure 10

**Analysis of relative expression of *ABF/AREB* genes in tomato under abiotic stresses including NaCl (a), Uv (b), Cold (c) and PEG (d).** The asterisk (*) indicates that the expression level of the stress group is significantly different from that of the control group (* $p < 0.05$, ** $p < 0.01$, one-way ANOVA, Tukey test)