Genome-Wide Identification, Expression and Potential Function Analysis of the ERF and DREB Subfamily Members in Tomato

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Abstract

Background: APETALA2/ethylene responsive factors (AP2/ERFs) are unique regulators found in the plant kingdom that are involved in all life activity processes, including flowering, fruit ripening, floral meristem growth, and defense responses. In tomato (*Solanum lycopersicum*), there are 60 DREB and 80 ERF subfamily members, however, their functionality remains poorly understood.

Results: In this work, the AP2 domain conserved amino acid sequences of 68 ERF proteins from 20 plant species were compared and a Multiple Em for Motif Elicitation (MEME) analysis was conducted. Results revealed that the 9^{th} amino acid of the AP2 domain exhibited marked characteristics during the selection of DRE/CRT and/or GCC boxes as protein binding sites. Moreover, motifs near the AP2 domain may be involved in protein binding to DNA, whereas motifs far away from the AP2 domain may function as a part of the transactivation domain. Furthermore, we compared the expression levels of all ERF genes in 30 tomato organs and under biotic and abiotic stresses. Results indicated that most of 17 ERF and DREB repressor genes were highly expressed in almost all tomato organs and under some biotic and abiotic stress. The transcripts per million (TPM) value ratios of all repressor genes exceeded that of all activator genes in 16 tomato organs. Thus, it can be inferred that these repressor genes play vital roles in balancing the regulatory functions of activator genes and activator genes may also conversely compete with repressor genes to ensure normal growth, development, and defense responses in tomato.

Conclusions: This work uncovered the potential functions of all ERF and DREB genes that regulate tomato growth, development, and defense responses, and considers the binding ability of the AP2 domain unique sequences with DRE/CRT and GCC boxes, as well as the relationship of unique motifs with the transactivation domain. These findings will expand upon our understanding of the functions of ERF and DREB genes in tomato.

Background

Plant hormones are involved in vital processes of complex signal transduction pathways and affect the expression of various genes at different time periods and in different organs, which regulate plant growth, development, and defense responses. To ensure survival and reproduction, diverse hormones, such as auxin (IAA), abscisic acid (ABA), ethylene (ET), gibberellin (GA), cytokinin (CTK), and jasmonate (JA), are synthetized and regulate different life activities in their metabolic networks [1, 2]. In these complex networks, transcription factors (TFs) are critical regulators that play essential roles [3].

Among these TFs, the APETALA2/ethylene responsive factor (AP2/ERF) is widely distributed in the plant kingdom and plays important roles in regulating growth and development. With updates to the plant genome database, more AP2/ERF genes have been identified. Thus far, 147, 291, 170, 163, and 136 AP2/ERF genes have been found in *Arabidopsis thaliana* [4], Chinese cabbage [5], *Salvia miltiorrhiza* [6], rice [7] and melon [8], respectively. Currently, RNA sequencing of many species has been conducting, lying an important foundation for the study of AP2/ERF gene families during plant growth and development. *Jatropha curcas* L. *JcERF035* was identified in the roots and leaves under Pi deficiency conditions by RNA sequencing and its overexpression affected root development in *Arabidopsis thaliana* [9].

AP2/ERF family members have been divided into 5 groups according to *Arabidopsis* classifications: ERF, DREB, AP2, RAVs and soloist [4, 10]. These subfamilies exhibit different structural characteristics. Among them, differences between ERF and DREB subfamily members is that the 14th and 19th amino acids of the DREB proteins are valine (V) and glutamate (E) in the AP2 domain, but alanine (A) and aspartate (D) in ERF proteins. This difference affects the ability of proteins to interact with DRE or GCC boxes during the regulation of their downstream target genes during transcription [11]. It also suggests that ERF and DREB subfamily members may act in different regulatory pathways. For example, *PUCHI*, an ERF subfamily gene, regulates lateral root development, floral meristem identity, and organ initiation in *Arabidopsis* [12, 13]. *Arabidopsis DREB2A* overexpression enhanced drought and heat tolerance in transgenic plants [14, 15]. Additionally, DREB2A affected leaf senescence by interacting with radical-induced cell death 1 (RCD1) under heat stress [16]. Thus, an ERF protein is often involved in several regulatory networks, which cause some ERF proteins to exert the same or opposite function during different processes. AtERF1 activates the defense-related gene, *PDF1.2* [17], while AtERF4 represses *PDF1.2* in biotic stress tolerance [18]. Moreover, AtERF2 and AtERF5 are activators and AtERF3 is a repressor, which regulate downstream target genes during transcript in defense responses [19].

Although different ERFs may exhibit opposing functions in different vital processes, an ERF may function in several of these processes. For example, *AtDREB1A* in transgenic *Arabidopsis* plants resulted in the dwarfed phenotypes and freezing and dehydration tolerance, whereas *AtDREB2A* transgenic plants exhibited slight growth retardation [20]. Additionally, the wild-type *Arabidopsis* plants overexpressing *AtERF53* exhibited unstable drought tolerance, while *rglg1rglg2* double mutant plants overexpressing *AtERF53* exhibited stable drought tolerance, as RGLG1 and RGLG2 together negatively regulate *AtERF53* transcription [21]. However, *AtERF7* overexpression decreased the sensitivity of guard cells to ABA and increased water loss during transpiration, which reduced drought tolerance in transgenic plants. Contrasting results were found in *AtERF7* RNA interference plants [22]. These studies suggest that although the regulatory pathways of DREB and ERF proteins differ, they can achieve the same effects in different vital processes.

Solanum lycopersicum (tomato), as an important fruit vegetable, is widely planted in many countries. Tomato fruit is abundantly nutrition and has a unique flavor, and can be eaten raw, boiled, or processed into ketchup or juice. Thus, improving the fruit yield and quality of tomato is the primary goal of tomato production. To achieve this goal, our understanding of the underlying molecular mechanisms of different vital processes must be enhanced, including seed germination, fruit ripening and softening, flower development, and defense responses to biotic and abiotic stresses. Among these processes, ERFs as regulators or repressors play important roles that affect different gene networks. In this study, we identified, corrected, and analyzed all ERF and DREB subfamily members based on *S. lycopersicum* genome database versions 2.0, 3.2, and 4.0. To understand the potential functions of ERF and DREB subfamily members, several RNA sequencing databases were used to analyze gene expression levels during vital processes, including growth, development, and defense responses to biotic and abiotic stresses. These works will help establish the regulatory networks of ERF and DREB subfamilies and uncover effective ways to improve tomato yield and quality.

Methods

Identification of ERF and DREB subfamily members in tomato

The genome sequences of *S. lycopersicum* were downloaded from a database of gene annotations, SGN, (Release v4.0 and v3.2, http://solgenomics.net/organism/solanum_lycopersicum/genome). The hidden Markov model (HMM) profile of the AP2 domain (PF00847) was downloaded from the Pfam database (http://pfam.xfam.org/). HMMER v3.3 was used to search for candidate AP2/ERF genes from the tomato genome database. The default parameters were used and the cutoff value was set to 0.001. All of the candidate AP2/ERF proteins with only a single AP2 domain were selected as candidate ERF proteins. The Pfam, SMART (http://smart.embl-heidelberg.de/), and NCBI CDD databases (https://www.ncbi.nlm.nih.gov/cdd) were used to validate the candidate ERF proteins. Finally, the identification results of the 3 genome versions (2.0, 3.2, and 4.0) and NCBI database were compared to determine the final ERF subfamily members of *S. lycopersicum*.

Phylogenetic analysis

Multiple sequence alignments of the tomato ERF proteins were performed using CLUSTAL W based on the complete sequences. To understand the relationship among the tomato ERF proteins, a phylogenetic tree was inferred using the maximum likelihood method based on the Whelan and Goldman model [23] of MEGA v7.0 with the following parameters: JTT + G model, partial deletion with 80% site coverage cutoff, and 1000 bootstrap replications [24].

Gene structure and conserved motif analyses

According to the cluster analysis results of the tomato ERF gene subfamily, the structural domain analysis of the ERF protein sequences of different groups was conducted using Jalview software [25]. Homologous alignments were compared using T-Coffee software [26]. The protein sequences of non-conservative regions were deleted. The alignment results were preserved in EPS format. Conserved motifs of the tomato ERF subfamily proteins were identified using the Multiple Em for Motif Elicitation (MEME) online tool v5.1.1 (http://meme-suite. org/tools/meme) with the following parameters: number of occurrences of a single motif distributed among the sequences within the model, 0 or 1 per sequence; maximum number of motifs, 20; optimum width of each motif, 6–50 residues.

Transcriptome data sources and bioinformation analysis

Transcriptome sequencing data were downloaded from the NCBI SRA database (https://www.ncbi.nlm.nih.gov/sra/) using the SRA toolkit. The project number of transcriptome data used in this article is as follows: PRJNA507622 (*S. lycopersicum*, 30 tomato organs) [27], PRJNA560638 (cultivated tomato, *S. lycopersicum*, heat, leaf), PRJNA484882 (Jinlingmeiyu, *S. lycopersicum*, drought, leaf), PRJNA312788 (Moneymaker, *S. lycopersicum*, low temperature, leaf), PRJDB1863 (*S. lycopersicum*, LED red light, leaf) [28], PRJNA530354 (Jinpeng No.1, *S. lycopersicum*, strong light, leaf), PRJNA434798 (Moneymaker, *S. lycopersicum*, TYLCSV, leaf) [29], PRJNA413551 (SW-7 and Fla. 8059, *S. lycopersicum*, TSWV, leaf), PRJNA407898 (Moneymaker and Motelle, *S. lycopersicum*, *F. oxysporum*, root), PRJNA531929 (*S. lycopersicum*, tomato psyllid, leaf) [30], and PRJNA565137 (M82, *S. lycopersicum*, STV, leaf) [31].

The transcripts per million (TPM) expression values of the transcriptomes of different organs, biotic and abiotic stressors of tomato were obtained using the SRA toolkit and Salmon software [32]. Subsequently, the TPM values were processed to quantify of gene expression levels of the original data. The expression heat map of the ERF genes in different organs, and biotic and abiotic stressors of tomato were drawn using R-pheatmap based on the TPM values.

Results

Sequence correction of ERF and DREB subfamily genes

To ensure the sequence accuracy of all AP2/ERF genes, the Pfam model (pf00847) of the AP2 domain downloaded from the Pfam website was used to search the tomato v4.0 protein database. A total of 166 AP2/ERF proteins with an AP2 domain E-value < 0.001 were obtained. Among

these proteins, 20 had ≥ 2 AP2 domains, while 146 proteins had single AP2 domain. Among the latter, 3 proteins with the B3 domain were RAV-type AP2/ERF proteins. Thus, there were 143 ERF subfamily proteins with a single AP2 domain. The 143 protein sequences were submitted to the Pfam, CDD, and smart websites for conservative domain analysis. Subsequently, 140 tomato ERF subunit genes with a single AP2 domain were identified. The sequences of these genes were compared in 3 tomato genome sequencing DNA, CDS, cDNA, and protein databases (versions 2.0, 3.2, and 4.0); 26 genes were found to be different (Table S1). The CDS and protein sequences of these 26 genes were compared and confirmed according to the tomato genome and NCBI databases (Tables S2 and S3). Finally, the corrected protein sequences were used for subsequent analyses (Tables S2).

Characteristics, polarity, and chemical structure analysis of the 14th and 19th amino acids in the AP2 domain

Among the 140 ERF genes with a single AP2 domain, the 14th amino acid of the AP2 domain was V in 57 genes. Among these 57 genes, the 19th amino acid of the AP2 domain was glutamic acid (E) in 30 genes, aspartic acid (D) in 4 genes, ssparagine (N) in 1 gene, glutamine (Q) in 4 genes, histidine (H) in 6 genes, leucine (L) in 10 genes, alanine (A) in 1 gene, and V in 1 gene (Tables 1 and S4). These 57 genes were identified as DREB genes. Additionally, the 14th and 19th amino acids of the AP2 domain were isoleucine (I) and D, respectively, in SlERF2-5, SlERF10-6, and SlERF10-8. The codon of I was AUA/AUC, GUA/GUG/GUU/GUC for V, but GCA/GCG/GCU/GCC for A. The characteristics, polarity, and chemical structure of I and V were hydrophobic, nonpolar, and aliphatic, while A was neutral, nonpolar, and aliphatic (Tables 1 and S4). Thus, I can only be a V mutation. Accordingly, the 3 genes were identified as DREB genes. In the 19th amino acid of the AP2 domain, the hydrophilic amino acids included E, D, N, Q, and H, the hydrophobic amino acids included L and V, and the neutral amino acids included A. The negative charged amino acids (E and D), uncharged amino acids (N and Q), and positively charged amino acids (H) were polar; the nonpolar amino acids included L, A, and V. Additionally, H had a heterocycle chemical structure, while the others were aliphatic (Table 1). These differences may affect the functionality of DREB protein interactions with DRE and GCC boxes.

Table 1
The 14th /19th amino acid analysis of the DREB subfamily AP2 domain

Gene number	14th	19th	14th codon	19th codon	14th /19th Characters	14th polarity	14th chemical structure	19th polarity	19th chemical structure
30	V	E	GUA/GUG/ GUU/GUC	GAA/ GAG	Hydrophobic/Hydrophilic	Nonpolarity	Aliphatic	Polarity with negative charge	Aliphatic
4	V	D	GUU	GAU/ GAC	Hydrophobic/Hydrophilic	Nonpolarity	Aliphatic	Polarity with negative charge	Aliphatic
1	V	N	GUU	AAC	Hydrophobic/Hydrophilic	Nonpolarity	Aliphatic	Polarity, without charge	Aliphatic
4	V	Q	GUA/GUG/ GUU	CAA	Hydrophobic/Hydrophilic	Nonpolarity	Aliphatic	Polarity without charge	Aliphatic
6	V	H	GUU/ GUC	CAU/ CAC	Hydrophobic/Hydrophilic	Nonpolarity	Aliphatic	Polarity with positive charge	Heterocycle
10	V	L	GUA/GUG/ GUU/GUC	UUG/CUU/ CUA/UUA	Hydrophobic/Hydrophobic	Nonpolarity	Aliphatic	Nonpolarity	Aliphatic
1	V	A	GUA	GCA	Hydrophobic/Neutral	Nonpolarity	Aliphatic	Nonpolarity	Aliphatic
1	V	V	GUG	GUU	Hydrophobic/Hydrophobic	Nonpolarity	Aliphatic	Nonpolarity	Aliphatic
2	I	D	AUA	GAC	Hydrophobic/Hydrophilic	Nonpolarity	Aliphatic	Polarity with negative charge	Aliphatic
1	I	V	AUC	GUU	Hydrophobic/Hydrophobic	Nonpolarity	Aliphatic	Nonpolarity	Aliphatic

Among the 80 ERF subfamily members, the 14th and 19th amino acids of the AP2 domain were A and D in 70 genes. Additionally, there was an A and tyrosine (Y) in 1 gene, A and N in 1 gene, threonine (T) and D in 1 gene, serine (S) and D in 4 genes, E and D in 1 gene, glycine (G) and N in 2 genes, and I and V in 1 gene (Tables 2 and S5). In the 14th amino acid of the AP2 domain, the neutral amino acids included A, T, S, and G, and

the hydrophilic amino acid included E. The nonpolar amino acid was A, the polar amino acids without charges were T, S, and G, and the chemical structure of these amino acids is aliphatic. In the 19th amino acid of the AP2 domain, D, Y, and N comprised the hydrophilic amino acids, the negative charged amino acids (D), the uncharged amino acids (Y and N) were polar, and the chemical structure of these amino acids was aliphatic (Table 2). Thus, the 80 genes with a single AP2 domain were identified as ERF subfamily members. These differences may affect the functionality of ERF protein interactions with GCC boxes.

Table 2
The 14th /19th amino acid analysis of the ERF subfamily AP2 domain

Gene number	14th	19th	14th codon	19th codon	Characters	14th polarity	14th chemical structure	19th polarity	19th chemical structure
70	A	D	GCA/GCG/ GCU/GCC	GAU/GAC	Neutral/Hydrophilic	Nonpolarity	Aliphatic	Polarity with negative charge	Aliphatic
1	A	Y	GCA	UAU	Neutral/Hydrophilic	Nonpolarity	Aliphatic	Polarity without charge	Aromatic
1	A	N	GCU	AAU	Neutral/Hydrophilic	Nonpolarity	Aliphatic	Polarity without charge	Aliphatic
1	T	D	ACG	GAU	Neutral/Hydrophilic	Polarity without charge	Aliphatic	Polarity with negative charge	Aliphatic
4	S	D	UCU/ UCA	GAU/GAC	Neutral/Hydrophilic	Polarity without charge	Aliphatic	Polarity with negative charge	Aliphatic
1	E	D	GAA	GAU	Hydrophilic/Hydrophilic	Polarity with negative charge	Aliphatic	Polarity with negative charge	Aliphatic
2	G	N	GGA	AAC	Neutral/Hydrophilic	Polarity without charge	Aliphatic	Polarity without charge	Aliphatic

Phylogenetic analysis of ERF and DREB proteins

To understand their genetic relationships, the protein sequences of the 60 DREB and 80 ERF subfamily members were classified into 6 groups (Fig. 1). The I group included 51 DREB proteins. Among these proteins, 37 and 14 proteins differentiated into the I-A and I-B subgroups, respectively. The I-A subgroup included 30 proteins with V14E19, 3 proteins with V14Q19 (SlERF12-9, SlERF1-13, and SlERF7-1), 1 protein with V14A19 (SlERF11-4), 1 protein with V14V19 (SlERF1-5), and 2 proteins with V14L19 (SlERF6-5 and SlERF12-3) (Table S4). Seven CBF proteins (SlERF3-7, SlERF3-22, SlERF3-6, SlERF8-2, SlERF8-3, SlERF12-11, and SlERF1-3) clustered together and were in the I-A subgroup (Fig. 1). Additionally, the I-A subgroup included 4 repressor proteins (SlERF9-1, SlERF2-10, SlERF4-10, and SlERF4-11) with EAR motif (DLNxxP or LxLxL) (Table S4). However, the I-B subgroup only included 6 proteins with V14H19 and 8 proteins with V14L19. SlERF9-10 and SlERF8-14 were repressor proteins in the I-B subgroup (Fig. 1; Table S4).

Group II included 49 ERF subfamily proteins. Among these proteins, 14 and 35 proteins differentiated into the II-A and II-B subgroups, respectively (Fig. 1). All members of the II-A subgroup belonged to ERF proteins with A14D19. In the II-B subgroup, there were 33 proteins with A14D19, 1 protein with T14D19 (SlERF1-10), and 1 protein with S14D19 (SlERF1-11) (Table S5). In the II-B subgroup, 6 proteins with the EDLL transactivation motif (ExxxxDxxxLxxxL) clustered together (SlERF3-1, SlERF9-7, SlERF9-3, SlERF9-4, SlERF3-2, and SlERF9-8) (Fig. 1). SlERF5-8 was also a repressor protein in the II-B subgroup. Groups III and IV included 13 and 16 ERF subfamily proteins, respectively. However, group III also included a DREB protein with V14D19 (SlERF1-4) that clustered with an ERF-type protein (SlERF1-2). One protein with S14D19 (SlERF1-15) and 1 repressor protein (SlERF4-1) were clustered into group III. Among these proteins in group IV, there were 2 proteins with S14D19 (SlERF3-16 and SlERF12-1), 2 proteins with G14N19 (SlERF12-6 and SlERF12-7), and all others belonged to proteins with A14D19. Additionally, 9 repressor proteins (SlERF10-1, SlERF7-5, SlERF12-1, SlERF7-2, SlERF2-6, SlERF7-3, SlERF10-2, SlERF3-4, and SlERF3-16) were in group IV (Fig. 1; Table S5). Group V had 8 DREB proteins, including 3 proteins with V14D19, 1 protein with V14Q19, 1 protein with V14N19, 2 proteins with I14D19, and 1 protein with I14V19 (Fig. 1; Table S4). Group VI included 1 protein with E14D19 (SlERF2-1) and 1 protein with A14D19 (SlERF9-2) (Table S5).

Motif analysis of ERF and DREB protein sequences

To understand the constructional characteristics of ERF and DREB proteins, a Multiple Em for Motif Elicitation (MEME) analysis was conducted to calculate the possible motifs of the 140 proteins. β1 of the AP2 domain was located on the left of motif 2, β2 was located on the right of

motif 2 and left of motif 3, and β3 and α were located on motif 1. All 140 proteins, except SlERF9-1, SlERF10-9, SlERF6-1, SlERF8-4, SlERF8-12, SlERF3-8, SlERF2-1, and SlERF9-2, had motifs 1, 2, and 3. SlERF9-1, SlERF10-9, SlERF6-1, SlERF8-4, and SlERF8-12 had only motifs 1 and 3, as well as a same sequence to motif 25 in front of motif 3. SlERF3-8 had motifs 2, 3, and 16. Motif 16 had a similar sequence as motif 1. SlERF2-1 and SlERF9-2 did not have motif 1, 2 and 3, but had a similar sequence as motif 16 with motif 1 (Fig. 2). These results suggest that SlERF9-1, SlERF10-9, SlERF6-1, SlERF8-4, SlERF8-12, SlERF3-8, SlERF2-1, and SlERF9-2 may have the low ability to bind with GCC or DRE boxes.

In addition to motifs 1, 2, 3, 16, and 25, some motifs were located on both sides of the AP2 domain of many ERF and DREB proteins. For example, motifs 10 and 20 were near the left of the AP2 domain in 7 CBF and SlERF1-13, respectively, while motifs 4 and 5 were near the right of motif 1 and especially motif 4 (Fig. 2). These findings suggest that motifs 4, 5, 10, and 20 may be involved in the process of AP2 domain binding with GCC or DRE boxes. However, motifs 6–9, 11–15, 17–19, 21, 23, and 24 were relatively far away from the AP2 domain, may be located in the transactivation or repression domains, and may be involved in regulating the expression of their downstream target genes. However, some ERF and DREB proteins, including SlERF4-10, SlERF7-1, SlERF10-5, SlERF6-7, SlERF3-15, SlERF6-9, SlERF3-16, SlERF12-6, SlERF12-7, SlERF3-4, SlERF7-3, SlERF2-6, SlERF3-13, and SlERF2-5, did not have other motifs, except motifs 1, 2, 3, and 4 (Fig. 2). Nevertheless, a few of these proteins had a typical EAR (LxLxL or DLNxxP) repression domain, including SlERF4-10, SlERF3-16, SlERF3-4, SlERF7-3, and SlERF2-6. These proteins bound to DNA with the AP2 domain and repressed the expression of downstream target genes with the EAR domain. However, SlERF7-1, SlERF10-5, SlERF6-7, SlERF3-15, SlERF6-9, SlERF12-6, SlERF12-7, SlERF3-13, and SlERF2-5 especially protein sequences with < 100 amino acids (SlERF12-6 and SlERF12-7) may competitively inhibit other ERF and DREB proteins (Fig. 2).

Unique amino acids affected the ability of protein to bind with DRE and GCC boxes

Previous studies found that some DREB and ERF subfamily proteins only bound to DRE or GCC boxes, but most of these proteins can also interact with these boxes. However, the correlation between the characteristics and binding ability of DREB and/or ERF subfamily proteins remains unclear. To distinguish the difference between DREB and ERF proteins during binding with DRE or GCC boxes, the AP2 domain amino acid sequences of 49 *Arabidopsis* and 19 other species ERF proteins, including 8 tomato ERF proteins, were compared. The binding assays of the 68 ERF proteins with DRE and GCC boxes were completed through an electrophoretic mobility shift assay (EMSA), yeast one-hybrid, or proteome chip assays. Among these proteins, there were 42 protein AP2 domains that included P9, 5 included H9, 5 included S9, 6 included N9, 3 included Q9, 2 included K9, 2 included T9, and 1 included I9 (Fig. 3). Only 19 proteins bound with GCC box, including 17 ERF with P9, 2 DREB with 1 P9 and 1 H9. Additionally, 37 proteins bound with DRE and GCC boxes, including 23 ERF with P9, 14 DREB with 1 P9, 4 H9, 4 N9, 2 Q9, 1 T9, 1 K9, and 1 I9. Only 12 proteins bound with DRE, including 1 ERF with P9, 11 DREB with 5 S9, 2 N9, 2 K9, 1 Q9, and 1 T9 (Fig. 3). These results suggest that almost all ERFs with P9 and H9 can interact with GCC box, and most can also bind with DRE. All DREB with S9 can only interact with DRE, but other DREBs with N9, K9, Q9, T9, and I9 may only bind with DRE or with DRE and GCC boxes. The A14 and A15 amino acids of ERF AP2 domain were conserved, but the 13th amino acid may be Y, F, or W. The W13 and V14 amino acids of the DREB AP2 domain were conserved, but the 15th amino acid may be S, A, or C (Fig. 3). These characteristics of ERFs and DREBs may affect the ability of proteins to bind with DRE and GCC boxes.

In tomato DREB subfamily members, there are 10 DREBs with S9W13V14S15 (SlERF3-20, SlERF5-5, SlERF6-8, SlERF8-10, SlERF8-11, SlERF8-12, SlERF9-1, SlERF10-9, SlERF11-4, and SlERF12-9) and 1 DREB with S9W13V14C15 (SlERF8-4) (Table S1), which suggests that these 11 DREBs may only bind with DRE. There was 1 DREB with S9W13I14A15 (SlERF10-6). However, the ability of this protein to bind with DRE and GCC boxes has not been determined. There were 4 DREBs with H9W13V14S15 (SlERF12-4, SlERF6-7, SlERF3-15, and SlERF6-9) and 9 DREB with H9W13V14A15 (SlERF3-14, SlERF9-10, SlERF8-5, SlERF8-14, SlERF4-6, SlERF12-13, SlERF4-9, SlERF7-4, and SlERF12-5) (Table S1). DREB with H9W13V14A15 can interact with GCC or both DRE and GCC boxes. Seven CBF proteins exist in tomato DREB subfamily members, including 5 CBF with N9W13V14C15 (SlERF3-7, SlERF3-22, SlERF3-6, SlERF8-2, and SlERF1-3) and 2 CBF with D9W13V14C15 (SlERF8-3 and SlERF12-11). There were 3 DREB with N9W13V14S15 (SlERF6-1, SlERF8-6, and SlERF1-13), 5 DREB with K9W13V14S15 (SlERF11-5, SlERF11-6, SlERF1-5, SlERF6-5, and SlERF12-3), 4 DREB with K9W13V14A15 (SlERF2-10, SlERF4-10, SlERF7-1, and SlERF1-4), 6 DREB with T9W13V14A15 (SlERF10-4, SlERF10-5, SlERF10-7, SlERF4-4, SlERF4-11, and SlERF5-11), 3 DREB with 1 Q9W13V14S15 (SlERF6-4), 1 I9W13V14A15 (SlERF6-2), and 1 A9W13V14S15 (SlERF12-2), 5 DREB with P9W13V14A15 (SlERF2-2, SlERF1-1, SlERF3-13, SlERF1-14, and SlERF3-19), and 1 DREB with P9W13I14A15 (SlERF10-8) (Table S1). These DREB proteins may interact with DRE, some of which may also bind with GCC boxes.

In tomato ERF subfamily members, there were ERFs with 22 P9Y13A14A15, 21 P9F13A14A15, and 21 P9W13A14A15 (Table S1). There were ERFs with K9Y13A14A15 (SlERF3-18), P9Y13A14S15 (SlERF5-7), P9Y13G14A15 (SlERF12-7), P9Y13G14V15 (SlERF12-6), T9F13A14A15 (SlERF3-2), K9F13A14T15 (SlERF5-1), Q9F13S14A15 (SlERF1-11), P9F13S14A15 (SlERF12-1), Q9F13T14A15 (SlERF1-10), E9W13A14A15 (SlERF1-2), 2 with K9W13A14A15 (SlERF4-1 and SlERF5-2), and 2 with P9W13S14A15 (SlERF1-15 and SlERF3-16) (Table S1). This indicates that P9A14A15 of the ERF AP2 domain may play an important role in binding with GCC box. However, it has not been determined why most ERF proteins can also interact with DRE in comparison with these ERF proteins that only bind with GCC boxes.

Expression analysis of CBF genes in tomato

To understand the function of different ERF and DREB genes in tomato growth and development, the expression levels of ERF and DREB subfamily members were analyzed according to tomato RNA-Seq data abtained from NCBI SRA library. Seven tomato CBF proteins had the PKKPAGR motif in the N-terminal and the DSAWR motif in the C-terminal of the AP2 domain, but these highly similar proteins to CBF did not have the same motif (Fig. 4a). CBF proteins had N/D9W13V14C15 in β2, while other proteins had N/S/A9W13V14S15 (Fig. 4a). This analysis suggests that CBF and its highly similar proteins may process different abilities with GCC and/or DRE boxes, which leads them to play different roles in regulating tomato growth, development, and defense responses to abiotic stress.

In different tomato organs, *SlERF8-2* was mainly expressed in mature petals and sepals, cotyledons, hypocotyl, and leaf lamina and veins; *SlERF8-3* was only expressed in mature petals and sepals, and hardly in other organs. *SlERF12-11* and *SlERF1-3* were hardly expressed in all organs, except *SlERF1-3* in young stamen. *SlERF3-6* was mainly expressed in mature sepals, hypocotyl, leaf lamina and veins, and internodes. *SlERF3-7* was expressed in mature anthers and petals, young and mature sepals, meristems after transitioning to flowering, cotyledons, hypocotyl, leaf lamina and veins, root apexes and roots without apexes, and internodes, especially in cotyledons, hypocotyl, and leaf lamina. *SlERF3-22* was expressed in mature anthers, petals and sepals, meristems after transitioning to flowering, cotyledons, hypocotyl, leaf lamina, and internodes (Fig. 4b; Data S1). These results indicate that 6 CBF genes, except *SlERF12-11*, may be involved in regulating the development of some floral organs, meristems after transitioning to flowering, cotyledons, hypocotyl, leaves, roots, and internodes. Although other highly similar DREB proteins to CBF do not have PKKPAGR and DSAWR motifs, most of these proteins play roles in some tomato organs. For example, *SlERF6-1* was expressed in mature petals and sepals, meristems after transitioning to flowering, cotyledons, hypocotyl, and leaf veins. *SlERF8-12* and *SlERF8-11* were highly expressed in mature anthers and young sepals, respectively, but were lowly expressed or undetectable in other organs. *SlERF3-20* was highly expressed in some organs, including mature anthers, floral meristems, meristems after transitioning to flowering, vegetative meristems, hypocotyls, leaf veins, young leaves, and internodes; *SlERF6-8* was highly expressed in hypocotyls and internodes. These results indicate that CBFs and some DREB genes that are highly similar to CBF regulate the development of the same organs, such as the expression of *SlERF8-2*, *SlERF3-7*, *SlERF3-22*, *SlERF6-1*, and *SlERF3-20* in meristems after transitioning to flowering (Fig. 4b; Data S1).

Among 7 CBF genes, *SlERF12-11* and *SlERF1-3* did not respond to abiotic stress, including drought, heat, cold, strong light, and red light. *SlERF8-2* and *SlERF8-3* positively responded to cold and red light, but not to drought, heat, or strong light. *SlERF3-6* was positively induced by heat and red light, negatively regulated by drought, and did not respond to cold or strong light. *SlERF3-7* was positively induced by heat, cold, and strong and red lights, but did not change due to drought. *SlERF3-22* responded to cold, and strong and red lights, but did not change due to drought or heat (Fig. 4c; Data S2). These results suggest that different CBF genes may play different roles in response to different abiotic stressors. However, some DREB genes that are highly similar to CBF also responded to abiotic stress, including *SlERF6-1*, *SlERF1-13*, *SlERF12-9*, and *SlERF6-8*, which up-regulated by drought, and *SlERF6-1*, *SlERF8-4*, *SlERF8-12*, and *SlERF12-2*, which up-regulated by cold. *SlERF9-1* and *SlERF10-9* were not expressed or induced by abiotic stress, while *SlERF8-10* and *SlERF8-11* were lowly expressed under abiotic stress (Fig. 4c; Data S2).

Under biotic stress, among 7 CBF genes, *SlERF8-2*, *SlERF3-6*, *SlERF3-7*, and *SlERF3-22* were up-regulated by the tomato spotted wilt virus (TSWV), did not respond to the southern tomato virus (STV), tomato leaf curl *Sardinia* virus (TYLCSV), and tomato psyllid (*Bactericera cockerelli*), and were down-regulated by *Fusarium oxysporum*. *SlERF8-2* was down-regulated by *F. oxysporum* and did not respond to other biotic stressors. *SlERF12-11* and *SlERF1-3* were not expressed by any biotic stress (Fig. 4c; Data S3). Among the DREB genes that were highly similar to CBF, *SlERF1-13*, *SlERF5-5*, *SlERF12-2*, and *SlERF6-8* only responded to TSWV. *SlERF6-1* and *SlERF8-12* were only down-regulated by *F. oxysporum* and hardly responded to biotic stress. Other DREB genes were hardly regulated by any biotic stressor (Fig. 4c; Data S3).

Expression analysis of repressor genes in tomato

Among the 140 ERF proteins, 11 ERF and 6 DREB subfamily proteins, which have 1 or 2 typical EAR (LxLxL or DLNxxP) motifs, were found. *SlERF10-1*, *SlERF7-5*, *SlERF12-1*, *SlERF7-2*, *SlERF4-11*, *SlERF9-1*, *SlERF2-10*, and *SlERF4-10* had a DLNxxP motif in the C-terminal, *SlERF2-6*, *SlERF3-16*, *SlERF8-14*, and *SlERF4-1* had an LxLxL motif in the C-terminal, and *SlERF9-10* and *SlERF5-8* had an LxLxL motif in the N-terminal. *SlERF7-3*, *SlERF10-2*, and *SlERF3-4* had an independent LxLxL motif and DLNxxP motif in the C-terminal. The DLNxxP motif of *SlERF7-3* and *SlERF10-2* connected with LxLxL sequences and formed a strong repressive motif (Fig. 5a).

To understand the function of the 17 repressor genes, the expression levels of these genes were analyzed in tomato flowers, fruits, meristems, seeds, leaves, and roots. Among these genes, *SlERF10-1*, *SlERF7-5*, *SlERF12-1*, *SlERF7-2*, *SlERF7-3*, *SlERF10-2*, and *SlERF2-10* were largely expressed in almost all organs. *SlERF2-6*, *SlERF3-4*, *SlERF4-10*, and *SlERF5-8* were expressed in most organs, including flowers, seeds, and leaves. *SlERF2-6* and *SlERF3-4* were not expressed in the fruits, in contrast to *SlERF4-10* and *SlERF5-8*. *SlERF3-16* was only expressed in the floral and vegetative meristems, seeds, and young leaves. *SlERF4-11* was only expressed in the seeds. *SlERF8-14* and *SlERF4-1* were lowly expressed in almost all organs. *SlERF9-10* and *SlERF9-1* were not expressed in almost all organs (Fig. 5b; Data S1). These results indicated that *SlERF10-1*, *SlERF7-5*, *SlERF12-1*, *SlERF7-2*, *SlERF7-3*, *SlERF10-2*, and *SlERF2-10* were involved in regulating the development of almost all organs, while other repressor genes, except *SlERF9-10* and *SlERF9-1*, played roles in regulating the development of some organs.

Among the 17 repressor genes, at least 13 were expressed in every organ, especially 16 in green large seeds (Fig. S1a; Data S1). Most activator genes were expressed in every organ, including a maximum of 94 genes in mature petals and minimum of 65 in red pulp (Fig. S1a; Data S1). However, the total TPM values of 17 repressor genes had a very high ratio among all ERF genes in every organ and exceeded the activator genes in most organs, including 56.14% in mature flowers, 57.64% in floral meristems, 56.58% in vegetative meristems, and 60.05% in cotyledons (Fig. S1b; Data S1). These results suggested that the 17 repressor genes play important roles in balancing the regulatory functions of other ERF and DREB subfamily genes and their downstream target genes during tomato growth and development.

Under drought stress, the expression of *SlERF3-4* and *SlERF4-10* increased, but other repressor genes did not change compared to the control (CK). The expression of *SlERF10-1*, *SlERF2-6*, *SlERF10-2*, *SlERF5-8*, and *SlERF4-1* increased under heat stress, in contrast to *SlERF8-14* (Fig. 5c; Data S2). *SlERF7-3*, *SlERF10-2*, *SlERF8-14*, *SlERF5-8*, and *SlERF4-1* responded to cold stress, but other genes were not induced. Only *SlERF10-2* was positively regulated by strong light at forenoon and *SlERF8-14* was negatively regulated under strong light. *SlERF10-1*, *SlERF2-6*, *SlERF7-3*, *SlERF10-2*, *SlERF2-10*, and *SlERF4-10* responded to red light stress (Fig. 5c; Data S2). A handful of ERF repressor genes responded to some biotic stressors, including *SlERF10-1*, *SlERF7-5*, *SlERF7-3*, and *SlERF10-2*, which were up-regulated after induction with TSWV in tomato species SW-7 and Fla.8059. *SlERF2-6* was down-regulated by the STV, in contrast to the TYLCSV. *SlERF8-14* was down-regulated by the TSWV and *F. oxysporum*. However, most ERF repressor genes, including *SlERF7-2*, *SlERF3-16*, *SlERF9-10*, *SlERF4-11*, *SlERF9-1*, *SlERF2-10*, and *SlERF4-10*, did not respond to the STV, TYLCSV, tomato psyllid, TSWV, or *F. oxysporum* (Fig. 5c; Data S3). These results suggest that some repressor genes are involved in regulating tomato tolerance to abiotic and biotic stress.

Expression analysis of ERF genes in the II-A subgroup

Among the 14 ERF subfamily genes in the II-A subgroup, the 5th, 23th, 24th, 46th, and 48th amino acids of the AP2 domain exhibited unique characteristics. SlERF4-2, SlERF4-5, SlERF4-7, SlERF12-12, SlERF3-17, SlERF6-10, SlERF12-8, SlERF1-16, and SlERF10-3 had V5A23A24L/I46F48, while SlERF1-6, SlERF9-6, SlERF12-10, SlERF3-21, and SlERF6-6 had I5G23V/I24R/K46I48 (Fig. 6a). These differences may affect the ability of ERF proteins to bind with DRE and GCC boxes during the regulation of growth, development, and tolerance to abiotic and biotic stress.

In 30 tomato organs, *SlERF1-6*, *SlERF9-6*, *SlERF12-10*, *SlERF3-21*, and *SlERF6-6* were largely expressed in almost all organs, but *SlERF4-7*, *SlERF12-12*, *SlERF3-17*, *SlERF12-8*, *SlERF1-16*, and *SlERF10-3* were only expressed in some organs, including *SlERF4-7* in mature anthers, styles, and seeds, and *SlERF12-12* in mature anthers, mature carpels, styles, ovaries, mature petals, mature sepals, and seeds. *SlERF4-2*, *SlERF4-5*, and *SlERF6-10* exhibited low transcription levels in some organs and were not expressed in most organs (Fig. 6b; Data S1). These results suggest that most of the II-A subgroup genes play roles in regulating tomat growth and development.

All II-A subgroup genes did not respond to drought or strong light stress, but some of these genes were up-regulated by heat, cold, and red light. For example, the expression of *SlERF4-2*, *SlERF1-16*, and *SlERF6-6* increased under cold and red light stress, and *SlERF9-6*, *SlERF12-10*, and *SlERF6-6* increased under heat stress (Fig. 6c; Data S2). These results indicate that some of the II-A subgroup genes improve heat, cold, and red light tolerance. However, all II-A subgroup genes did not respond to biotic stress and some genes were even down-regulated, including *SlERF1-16* and *SlERF10-3* under *F. oxysporum* treatment (Fig. 6c; Data S3). These results suggest that the II-A subgroup genes were not involved in enhancing the tolerance to biotic stress.

Expression analysis of DREB genes in the I-B subgroup

Among the 14 DREB genes in the I-B subgroup, the 12th, 19th, 39th, 43th, 47th, 48th, and 49th amino acids of the AP2 domain exhibited unique characteristics. SlERF3-14, SlERF12-4, SlERF6-4, SlERF6-7, SlERF3-15, and SlERF6-9 had S12H19R39E43L47M48S/C49, while SlERF9-10, SlERF8-5, SlERF8-14, SlERF4-6, SlERF12-13, SlERF4-9, SlERF7-4, and SlERF12-5 had K12L19F/Y/L/M39T/R/K/V43M/K/R47L48R49 (Fig. 7a). SlERF9-10 and SlERF8-14 also show an EAR motif. These characteristics indicate that the ability of the I-B subgroup genes to bind with DRE and/or GCC boxes may be different, and these genes play different roles in regulating tomato growth and development.

Among the 14 DREB genes, *SlERF4-6* and *SlERF4-9* were highly expressed in all tomato organs, but other genes were specifically expressed in some organs, including *SlERF3-14* in young stamens, young sepals, and senescent leaves, *SlERF6-4* in young sepals and seeds, and *SlERF3-15* in red and dry seeds. *SlERF12-5* was not expressed in any tomato organs (Fig. 7b; Data S1). These results suggest that the I-B subgroup genes, except *SlERF12-5*, are involved in regulating different tomato growth and development processes.

Under abiotic stress, the expression of all I-B subgroup genes did not increase and *SlERF8-5* was down-regulated by drought stress. Under heat stress, the expression of *SlERF6-4* and *SlERF8-14* decreased, *SlERF4-9* and *SlERF7-4* increased, and other genes did not change. *SlERF8-14*, *SlERF4-9*, and *SlERF7-4* were up-regulated by cold stress, while other genes did not change. Almost all I-B subgroup genes did not respond to strong or red light stress, but *SlERF3-14* and *SlERF6-7* were positively regulated and *SlERF6-4* was negatively regulated by red light (Fig. 7c; Data S2). All I-B subgroup genes did not positively respond to biotic stress (Fig. 7c; Data S3). These results indicate that a few I-B subgroup genes play roles in abiotic stress responses and all I-B subgroup genes are not involved in biotic stress responses.

Discussion

Conserved sequence characteristics of the AP2 domain affected the ability of ERF proteins to bind with DRE and GCC boxes

DRE/CRT and GCC boxes are two *cis*-acting elements that ERF and DREB subfamily members bind to in higher plants. In previous reports, the 14th and 19th amino acids of the AP2 domain were used to distinguish DREB and ERF proteins, including V14E19, and A14D19, which were respectively named DREB and ERF proteins. Additionally, DRE/CRT and GCC boxes only interact with DREB and ERF proteins, respectively [11]. However, an increasing number of reports have proved that some DREB and ERF proteins can interact with both DRE/CRT and GCC boxes [34, 37, 38, 44, 53, 54], while a few DREB proteins can only bind with GCC box and a few ERF proteins can only bind with DRE/CRT element [34]. These findings suggest that the 14th and 19th amino acids of the AP2 domain play certain roles in interacting with DRE/CRT and GCC boxes, but some other amino acids also have important functions.

In this study, 140 AP2/ERF proteins with a single AP2 domain in tomato were classified into 6 groups (Fig. 1). Among these proteins, 60 and 80 members were distinguished as DREB- and ERF-type, respectively. Among the DREB-type proteins, there were 30 proteins with V14E19, 27 with V14 and D/N/G/H/L/A/V19, and 3 proteins with I14 and D/V19. E and D have the same characteristics, but others exhibit different characteristics (Table 1). Thus, AP2 domains with either E19 or D19 are not affected by the ability to bind with DRE/CRT or GCC boxes. For example, At5g19790 with V14D19 and At1g75490 with V14E19 can interact with DRE/CRT and GCC boxes [34]. Among the ERF-type proteins, there were 70 proteins with A14D19, 4 with A14Y19, A14N19, T14D19, and E14D19, 4 with S14D19, and 2 with G14N19 (Table 2). In tomato, SlERF3-12 and SlERF5-8 with A14D19 only bind to GCC box [33], but SlERF9-9, SlERF3-21, SlERF6-6, and SlERF9-6 with A14D19 can interact with DRE/CRT and GCC boxes [39, 41–43]. In addition, SlERF5-7 with S14D19 also bound to DRE/CRT and GCC boxes (Fig. 3) [36]. These results suggest that the 14th and 19th amino acids of the AP2 domain cannot be the only standard by which to evaluate the ability of ERF and DREB subfamily members binding with DRE/CRT and GCC boxes.

In this study, we compared the AP2 domain sequences of 68 ERF and DREB proteins from 20 species. The ability of these proteins to bind with DRE/CRT and GCC boxes were demonstrated by EMSA, yeast one-hybrid, and proteome chip assays. We found that 46 proteins with P/H9 in the AP2 domain, except At4g23750, can interact with GCC box, 28 of which can also bind with DRE/CRT box (Fig. 3). However, 5 DERB proteins with S9 in the AP2 domain only interacted with DRE/CRT box. Other site sequences of these 5 proteins did not display some typical differences compared to other DREB proteins that bind to DRE/CRT and GCC boxes (Fig. 3). These results indicate that the 9th amino acid of the AP2 domain plays an important role in protein selection at its binding sites for DRE/CRT and/or GCC boxes. In tomato, 11 DREB proteins had S9, and 70 ERF and 19 DREB had P/H9 at the 9th amino acid of the AP2 domain. These results suggest that DREB proteins with S9 may only interact with DRE/CRT box, and ERF and DREB proteins with P/H9 may bind to GCC box or to both DRE/CRT and GCC boxes. However, the ability of these proteins without S/P/H9 binding with DRE/CRT and GCC boxes is poorly understood. For example, the 9th amino acid of 6 CBF proteins in tomato is N or D, and some *Arabidopsis* DERB proteins with N9 can interact with only DRE/CRT or both DRE/CRT and GCC boxes [34, 55, 56]. The abilities of DREB proteins with D9 were not demonstrated by EMSA, yeast one-hybrid, or proteome chip assays.

Functionally, ERF and DREB subfamily proteins work as activators or repressors in plant growth, development, and defense responses. Proteins, including EAR motif, can repress the expression of downstream target genes by recruiting a histone deacetylase complex to affect chromatin structures [57–59]. However, although some reports have verified that EDLL motif is an activating domain [60–62], the activating domain of ERF and DREB subfamily proteins is complex and remains elusive. In tomato, EDLL motif is only displayed in some the II-B group members (Fig. S2), but this motif was included in motif 6. Some members of the I-A group including CBF and a few members of the II-B group also had motif 6 (Fig. 2). These findings suggest that motif 6 instead of the EDLL motif may be a more reliable transactivation domain. The MEME analysis results suggest that motifs 1, 2, 3, and 4 are located in the AP2 domain, while others are distributed outside the AP2 domain. Thus, motifs relatively distant from the AP2 domain, including motifs 7–9, 11, 12, 14, 17–19, and 22–24 may also act as a part of the transactivation domain. Motifs near the AP2 domain, including motifs 5, 10, 13, 15, 20, 24, and 25 may help the AP2 domain bind to DRE/CRT and GCC boxes (Fig. 2). For example, the PKKPAGR signature sequence of *Arabidopsis* CBF1 is located near the left of the AP2 domain and mutations within this motif reduce the ability of CBF1 to bind to DRE/CRT element and decrease the expression levels of the COR gene [63]. In this study, motif 10 included the PKKPAGR signature sequence and was located near the left of the AP2 domain in 7 tomato CBF proteins (Fig. 4a). These results indicate that these motifs near the AP2 domain may play important roles in helping the AP2 domain to bind to DRE/CRT and GCC boxes, but the functions of motifs far away from the AP2 domain remain unclear during the regulation of transcription of downstream genes.

ERF repressors balance the regulatory functions of other ERF activating genes during tomato growth, development, and defense responses

AP2/ERF TFs play important roles in regulating plant growth, development, and defense responses. For example, *AtERF115* can repress adventitious rooting in *Arabidopsis* through the JA and CTK signaling pathways [64], but *PUCHI* (At5g18560) can positively regulate floral meristems, organ initiation, and lateral root development in *Arabidopsis* [12, 13]. Rice *OsERF101* regulates leaf senescence and responses to drought stress in reproductive tissues [65, 66]. These findings suggest that some ERF proteins often regulate several plant life processes. However, an ERF protein can only act as an activator or repressor. In this study, we found that there were 11 ERF and 6 DREB proteins including

the EAR repression domain, in tomato. Among these proteins, SlERF7-3 and SlERF10-2 had 2 LxLxL and 1 DLNxxP, and SlERF3-4 had 1 LxLxL and 1 DLNxxP at the C-terminal. These results suggest that SlERF7-3, SlERF10-2, and SlERF3-4 may recruit more histone deacetylase complexes to repress the expression of their downstream target genes. The expression levels of *SlERF10-2* (*LeERF3b*) markedly increased in low ET tomato fruit containing an ACC oxidase sense-suppression transgene and in the ET insensitive mutant never ripe (Nr), in contrast to *SlERF5-10* (*Pti4*) without the EAR motif [67]. *SlERF5-10* regulated fruit ripening, seed germination, and responses to biotic and abiotic stress [68–71]. In this study, *SlERF7-3*, *SlERF10-2*, and *SlERF3-4* were expressed in several organs, especially *SlERF7-3* and *SlERF10-2*, which were highly expressed in tomato all flowers, fruits, meristems, seeds, leaves, and roots (Fig. 5b). The 3 genes also responded to some biotic and abiotic stressors (Fig. 5c). These results indicate that *SlERF7-3*, *SlERF10-2*, and *SlERF3-4* as repressors are involved in several metabolic pathways that affect tomato growth, development, and defense responses. Other genes, including *SlERF10-1*, *SlERF7-5*, *SlERF12-1*, *SlERF7-2*, *SlERF2-6*, *SlERF2-10*, *SlERF4-10*, *SlERF5-8*, and *SlERF4-1*, were also highly expressed in several tomato organs. Overexpression of *SlERF10-1* (*SlERF36*) caused early flowering and plant senescence, and affected stomatal density, photosynthesis, and plant growth [72]. These findings suggest that they may play the same or similar roles, like *SlERF7-3*, *SlERF10-2*, and *SlERF3-4*. However, *SlERF9-1* was not expressed in any tomato organ or under biotic and abiotic stress. Moreover, its EAR motif was located in the AP2 domain terminal. Thus, *SlERF9-1* may be an invalid gene. *SlERF3-16*, *SlERF9-10*, *SlERF8-14*, and *SlERF4-11* were expressed some tomato organs and under biotic and abiotic stress (Fig. 5b and c). *SlERF3-16* (*ENO*) was involved in the CLAVATA-WUSCHEL signaling pathway that regulates floral meristem development [73]. These results indicate that these genes play repression roles in some organs and under certain environmental conditions.

Although there are 60 DREB and 80 ERF subfamily members in tomato, only 11 ERF and 5 DREB genes, except *SlERF9-1*, as repressors were expressed in all or some tomato organs or under biotic and abiotic stress. In this study, we analyzed the expressed gene numbers of all repressors and activators in every tomato organ and found that at least 13 repressor genes were expressed in several floral organs, red pulp, cotyledons, and senescent leaves, and, at most, 16 repressor genes were expressed in green large seeds (Fig. S1a). However, most activator genes were expressed in every organ, including the maximum of 94 genes in mature petals and the minimum of 65 in red pulp (Fig. S1a). The TPM value ratios of all expressed repressors and activators were compared. Results indicated that the ratio of repressors exceeded that of activators in 16 tomato organs, especially in cotyledons (Fig. S1b), suggesting that the 16 repressor genes control the activation function of other ERF and DREB genes by competing with the binding sites of their target genes, and finally balancing the expression of their target genes to ensure normal growth and development.

Among the activators, the expression of most members showed tissue and spatiotemporal specificity, including *SlERF8-3*, *SlERF3-6*, *SlERF3-7*, *SlERF3-22*, *SlERF6-1*, *SlERF12-2* (*LeDREB2*), and *SlERF8-12* in the I-A subgroup (Fig. 4b and c), *SlERF4-7*, *SlERF12-12*, and *SlERF10-3* in the II-A subgroup (Fig. 6b and c), and *SlERF3-14*, *SlERF6-4*, *SlERF3-15*, and *SlERF8-14* in the I-B subgroup (Fig. 7b and c). Among these genes, only *SlERF12-2* responded to drought, cold, and salt [74], and the functions of other genes remain unclear. Some activator genes were expressed in almost all tomato organs and responded to some biotic and abiotic stressors, including *SlERF1-6*, *SlERF9-6*, *SlERF12-10*, *SlERF3-21*, and *SlERF6-6* in the II-A subgroup (Fig. 6b and c), and *SlERF4-6* and *SlERF4-9* in the I-B subgroup (Fig. 7b and c). Their expression patterns were highly similar with most repressor genes. Although *SlERF1-6* regulated the biosynthesis of carotenoids and ET during fruit ripening [75], *SlERF9-6* regulated ET biosynthesis, seed germination, and defense responses [43, 76–78], and *SlERF4-9* was involved in plant growth, root architecture, and responses to abiotic stress [79–81]. However, their functions in other tomato organs remain unclear. Moreover, these genes may conversely limit repressor activities, and ensure the normal growth and development of tomato plants is not overly inhibited.

Conclusions

This work highlights that much remains to be understood about the relationship between repressors and activators in tomato ERF and DREB subfamily members. By performing Pfam model (pf00847) search and sequences comparing in 3 tomato genome sequencing databases (versions 2.0, 3.2, and 4.0), 140 AP2/ERF genes were identified. These genes included 60 DREB and 80 ERF subfamily members and were classified into six subgroups. The expression profiles of DREB and ERF genes in 30 tomato organs as well as under biotic and abiotic stresses were investigated and compared, which could considered as the candidates for further study of their function in plant growth, development, and defense response.

Declarations

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Availability of data and material

All of the datasets supporting the results of this article are included within the article and its Additional files.

Authors' contributions

LZ, MQ, and YL conceived and designed this study. LC, SP, QZ, SQ, and YL carried out genome-wide identification and sequence analysis of the AP2/ERF genes. TX, LZ, and YL carried out expression analysis of the AP2/ERF genes. LZ, MQ, and YL drafted and revised the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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Figures

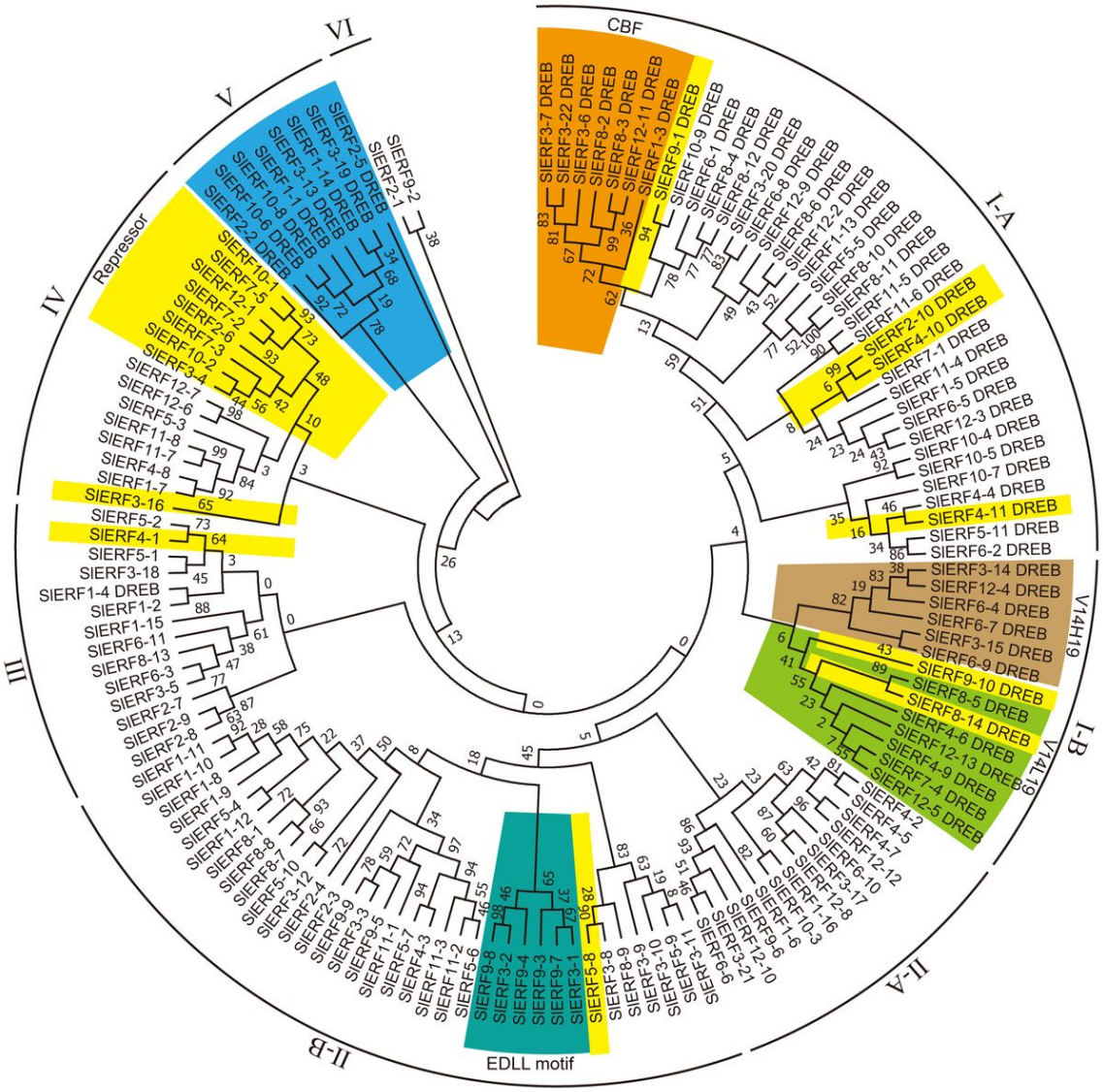
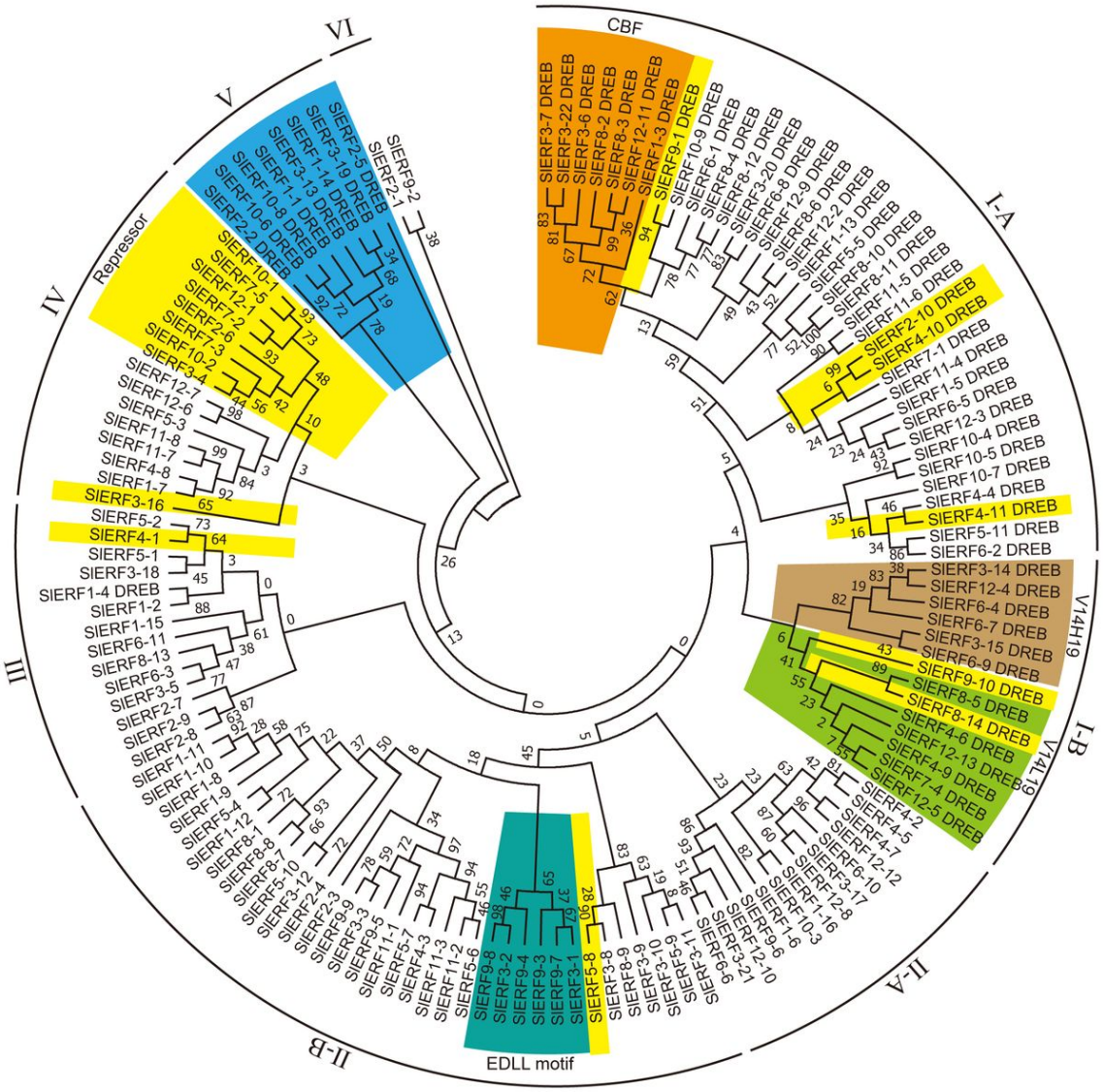


Figure 1

Phylogenetic analysis of 60 DREB and 80 ERF proteins in tomato. Blue, DREB proteins in group Ⅵ; yellow, repressors; orange, CBF proteins; emerald green, activators with EDLL motif; yellow green and latin yellow, respectively indicate DREB proteins with V14L19 and V14H19. Maximum likelihood method was used to structure phylogenetic tree based on the Whelan and Goldman model of MEGA v7.0 [23], parameters: JTT + G model, partial deletion with 80% site coverage cutoff, and 1000 bootstrap replications [24].



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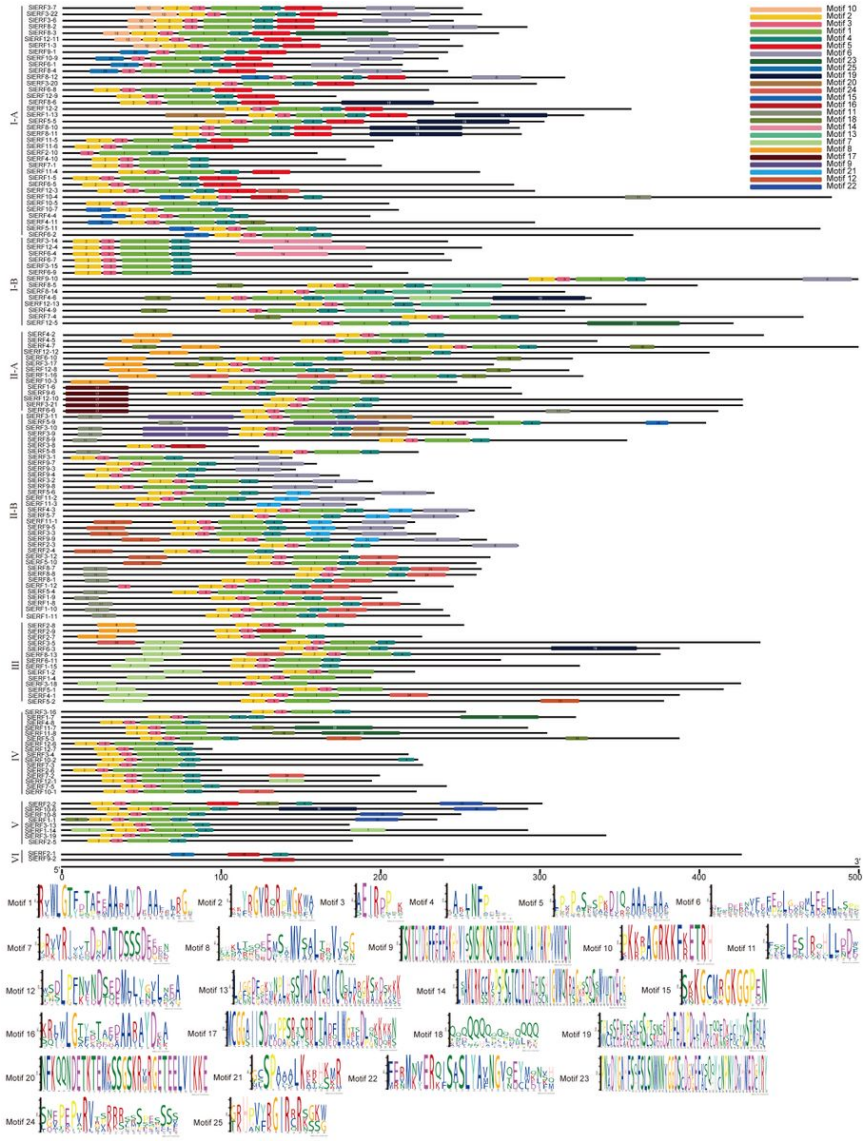
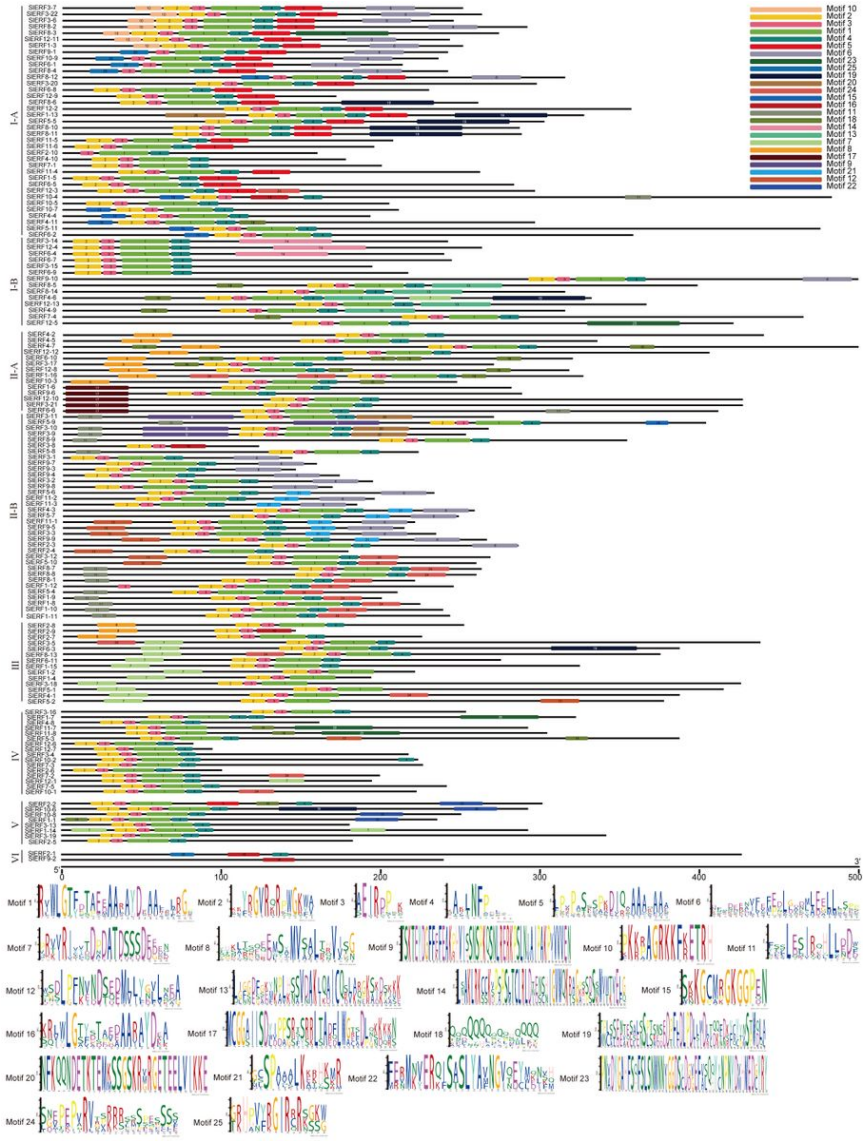


Figure 2

25 motif predictive analysis of 60 DREB and 80 ERF proteins in tomato using a Multiple Em for Motif Elicitation (MEME).



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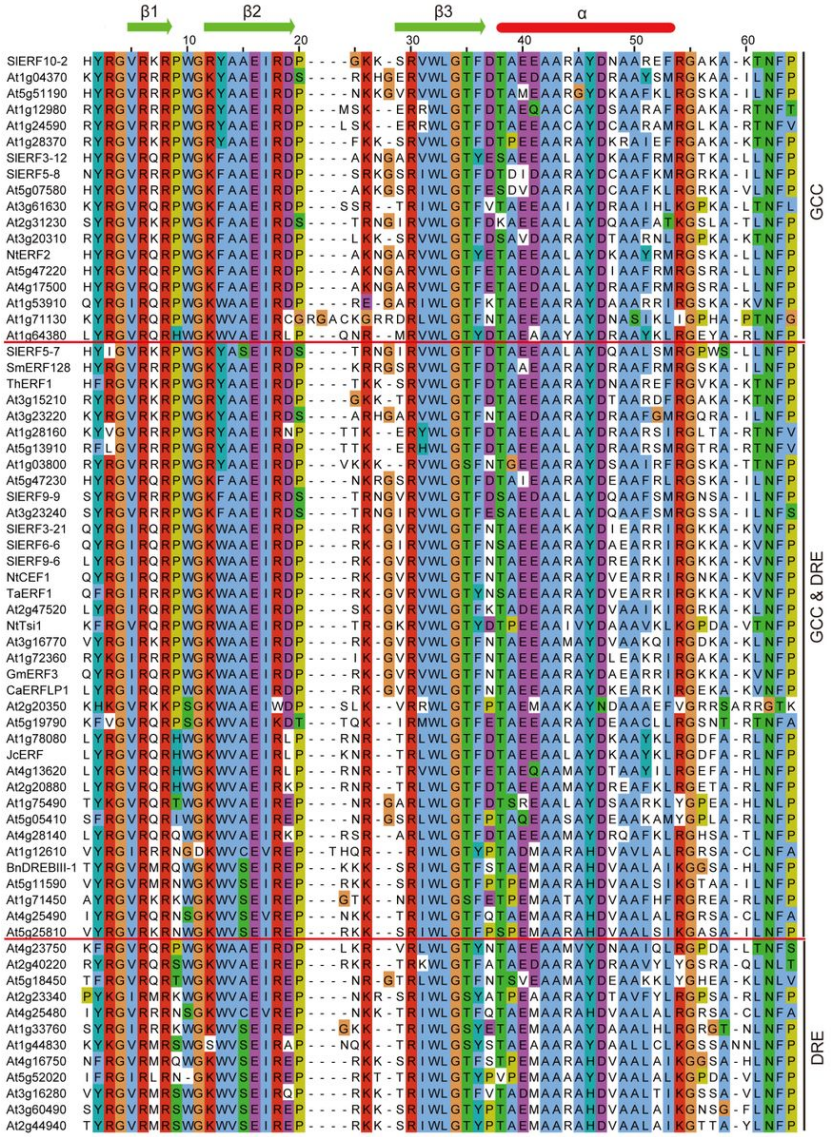
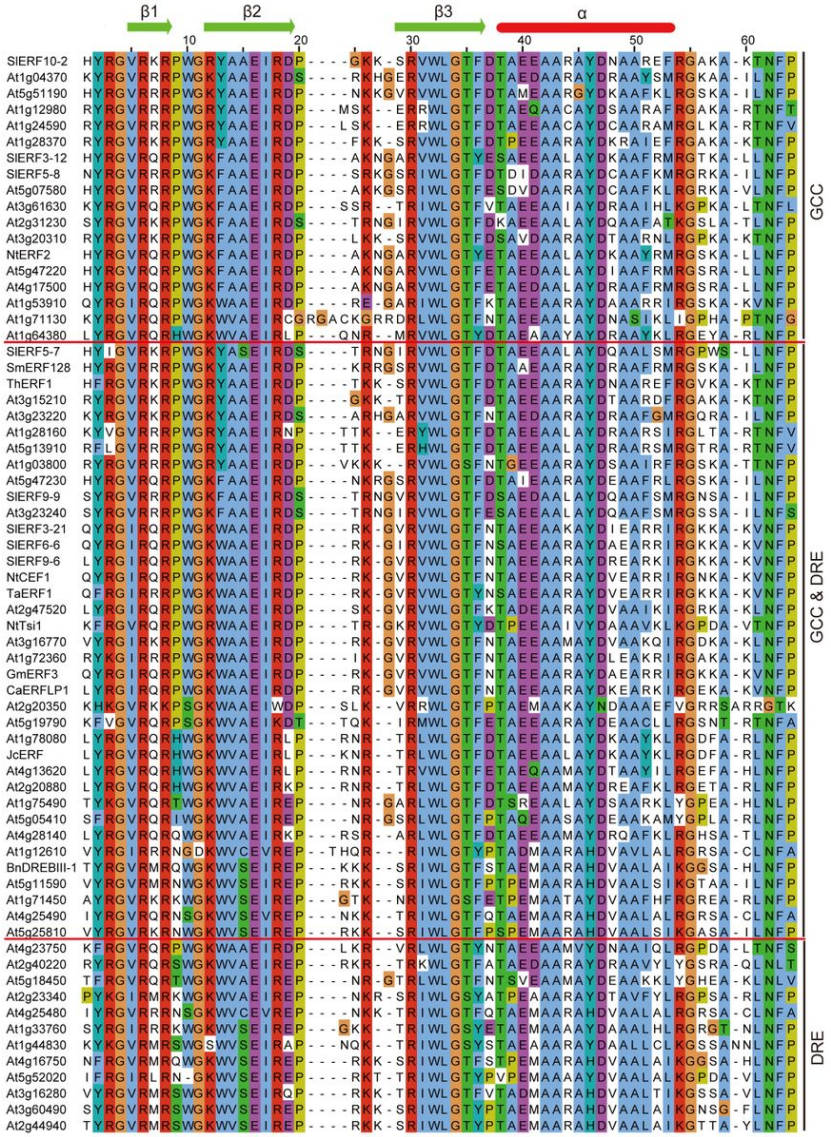


Figure 3

Comparison of the AP2 domain amino acid sequences of 49 Arabidopsis and 19 other species ERF proteins. SlERF10-2, SlERF3-12, SlERF5-8 [33]; At1g04370, At5g51190, At1g12980, At1g24590, At1g28370, At5g07580, At3g61630, At2g31230, At3g20310, At4g17500, At1g53910, At1g71130, At1g64380, At3g23220, At1g28160, At5g13910, At1g03800, At2g20350, At5g19790, At4g13620, At2g20880, At1g75490, At4g28140, At1g12610, At1g71450, At4g25490, At2g40220, At5g18450, At2g23340, At4g25480, At1g33760, At1g44830, At4g16750, At5g52020, At3g16280, At3g60490, At4g23750, and At2g44940 [34]; NtERF2 [35], At5g47220, At3g15210, At5g47230 [19], SlERF5-7 [36], SmERF128 [37], ThERF1 [38], SlERF9-9 [39], At3g23240 [40], SlERF3-21 [41], SlERF6-6 [42], SlERF9-6 [43], NtCEF1 [44], TaERF1 [45], At2g47520 [46], NtTsi1 [47], At3g16770 [48], At1g72360 [49], GmERF3 [50], CaERFLP1 [51], At1g78080 [52], JcERF [53], At5g05410 [11], BnDREBIII-1 [54], At5g11590 [55], At5g25810 [56].



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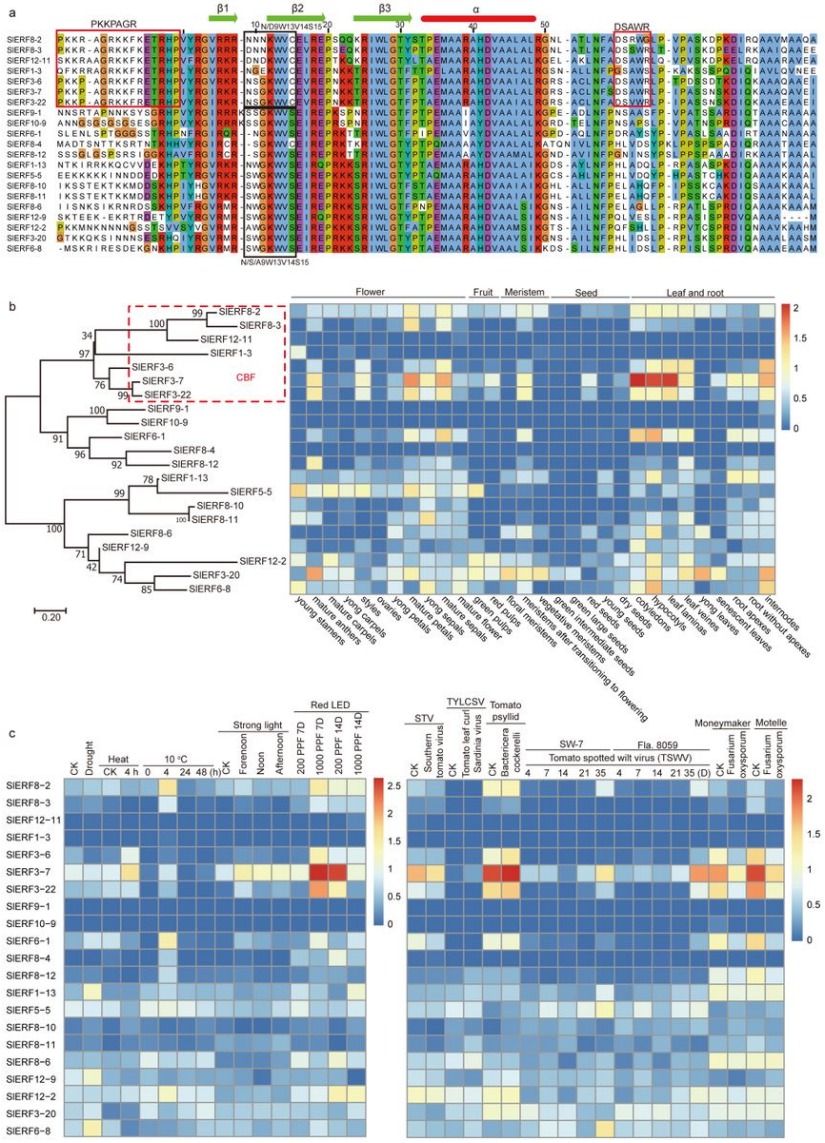
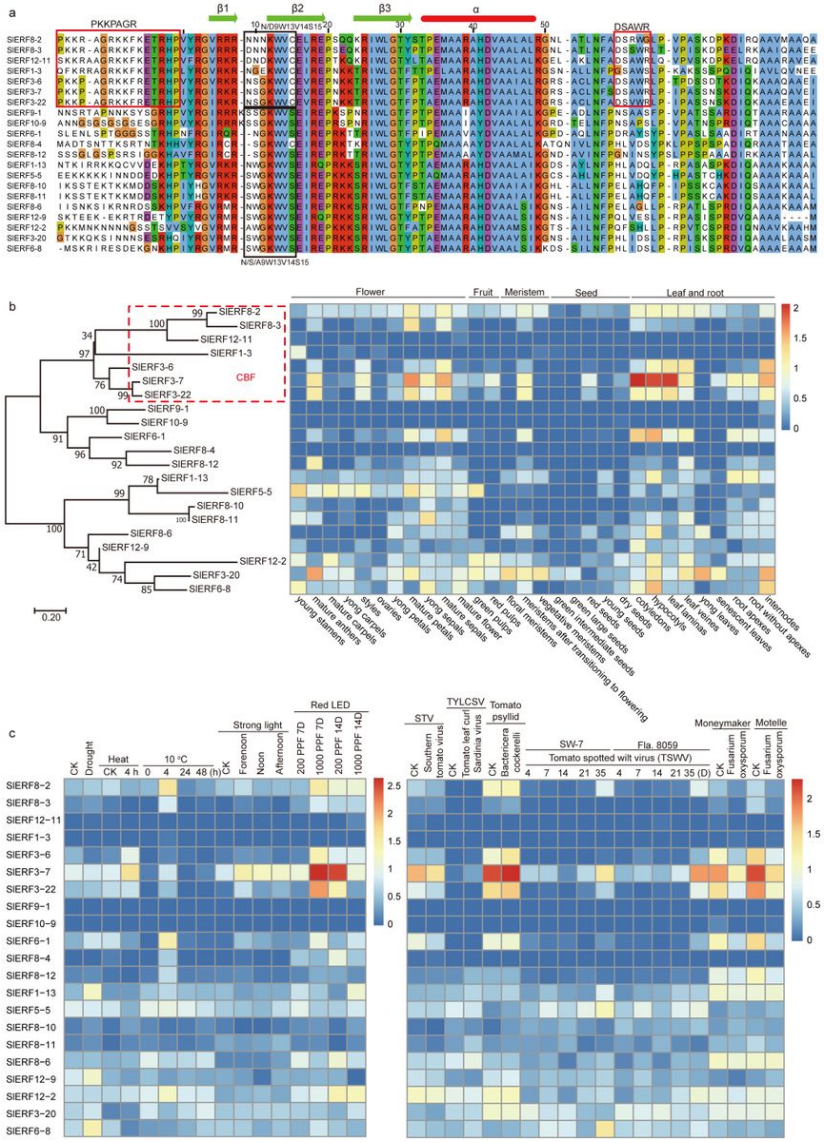


Figure 4

Expression levels of CBFs and their similar DREB genes in 30 tomato organs and under different biotic and abiotic stresses. (a), AP2 domain sequences comparison; (b and c), heatmap analysis of CBFs and their similar DREB based on TPM values in 30 tomato organs and under different biotic and abiotic stresses, respectively. TSWV, tomato spotted wilt virus; STV, southern tomato virus; TYLCSV, tomato leaf curl Sardinia virus.



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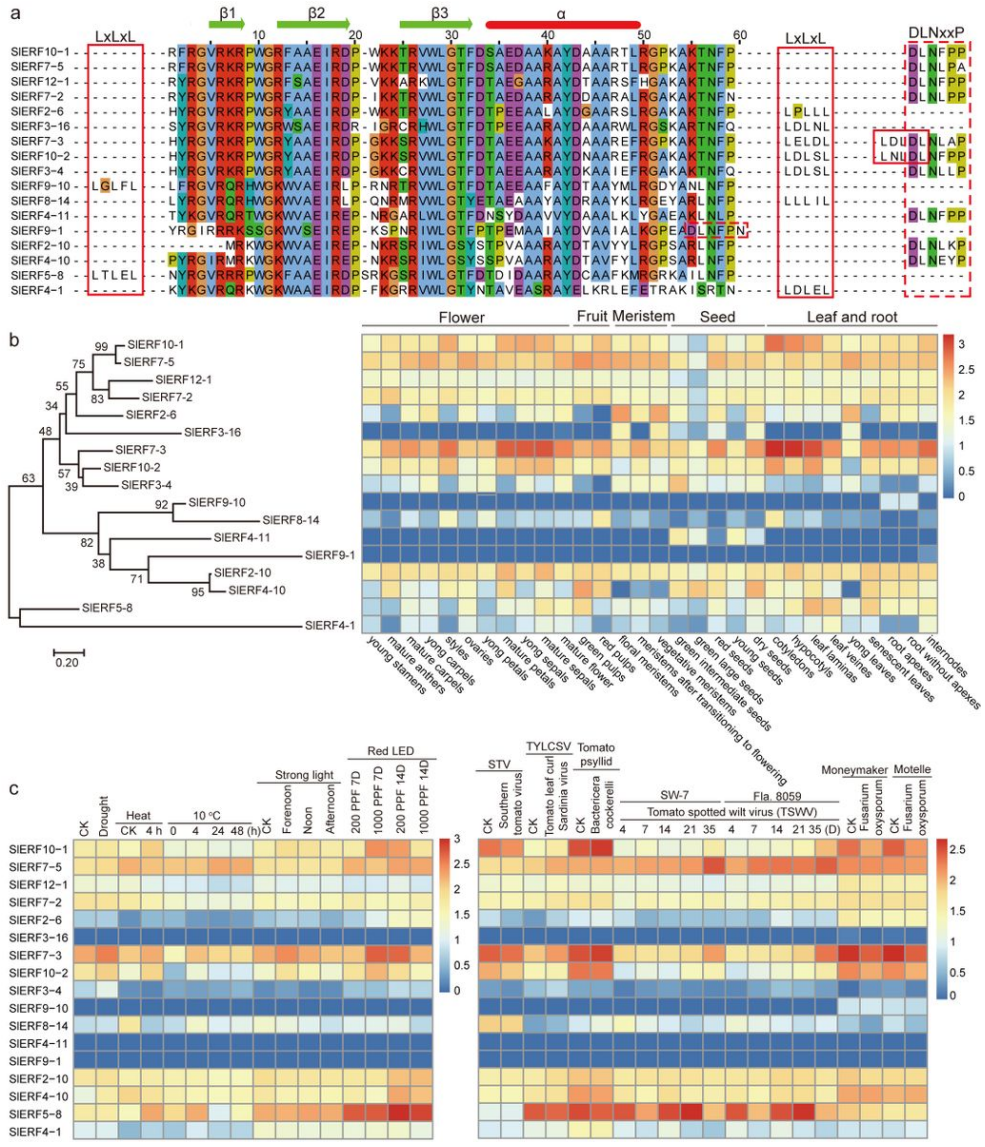

Figure 5

Expression levels of all repressor genes in 30 tomato organs and under different biotic and abiotic stresses. (a), AP2 domain sequences and EAR motifs analysis; (b and c), heatmap analysis of all repressor genes based on TPM values in 30 tomato organs and under different biotic and abiotic stresses, respectively.

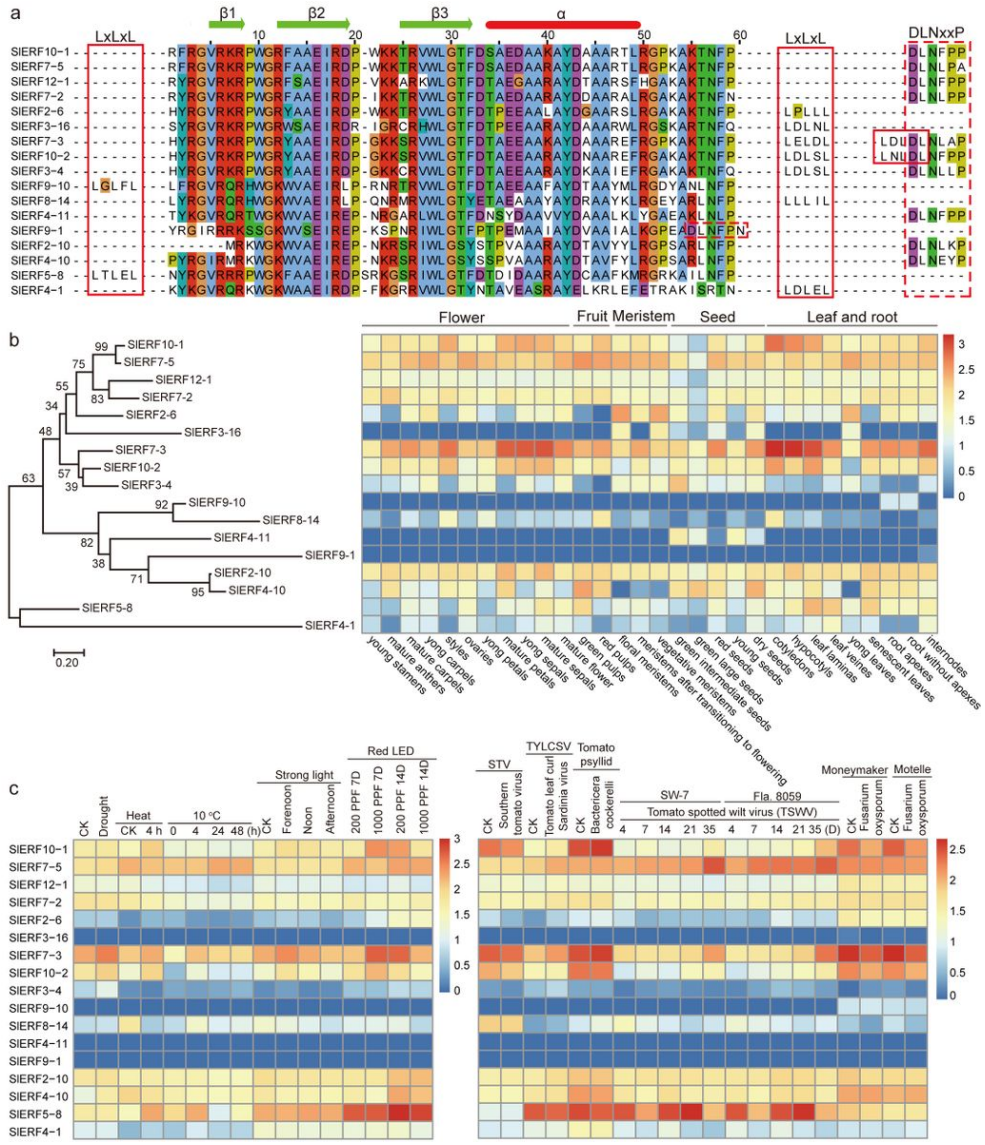

Figure 5

Expression levels of all repressor genes in 30 tomato organs and under different biotic and abiotic stresses. (a), AP2 domain sequences and EAR motifs analysis; (b and c), heatmap analysis of all repressor genes based on TPM values in 30 tomato organs and under different biotic and abiotic stresses, respectively.

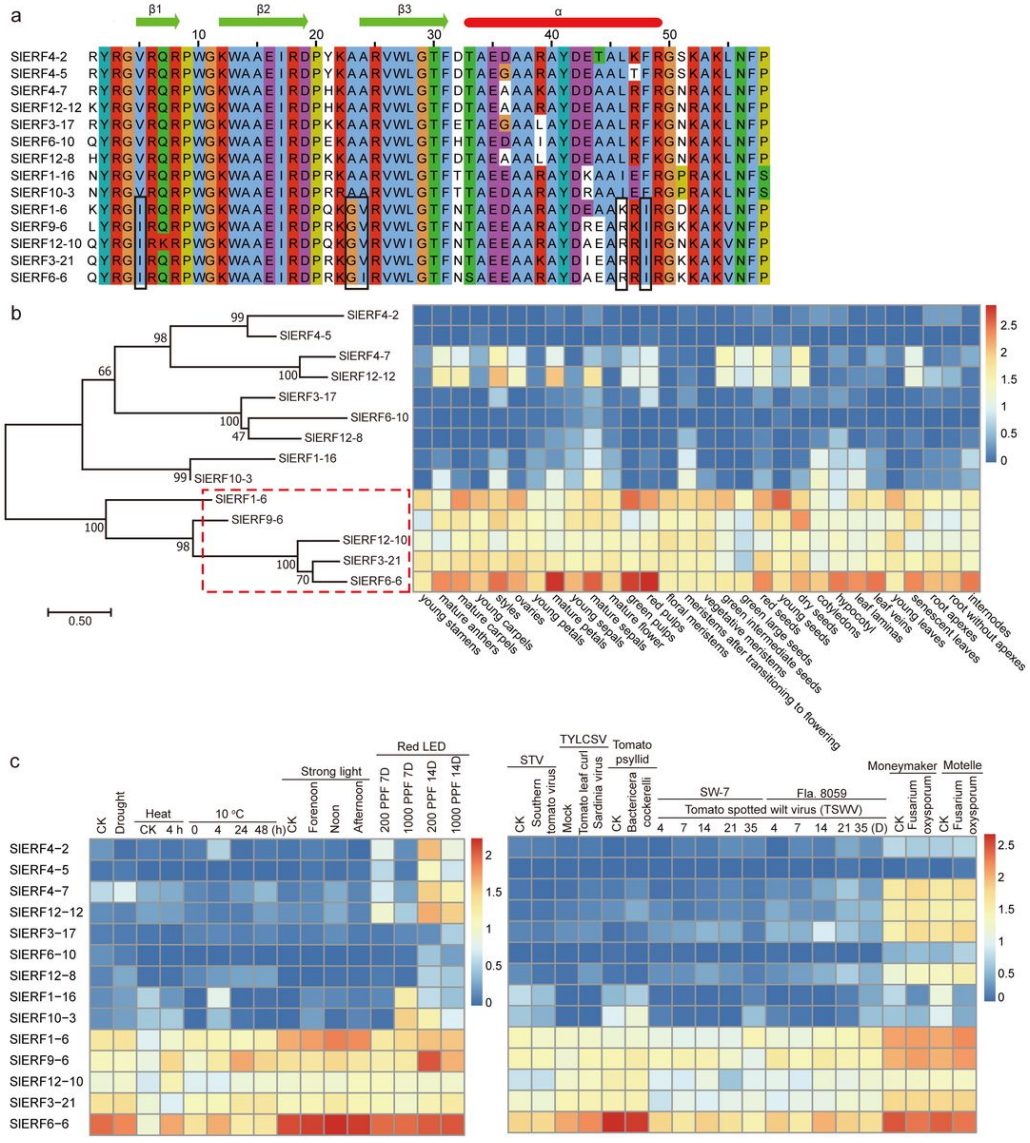

Figure 6

Expression levels of the ERF A-5 subgroup ERF genes in 30 tomato organs and under different biotic and abiotic stresses. (a), AP2 domain sequences comparison; (b and c), heatmap analysis of the ERF A-5 subgroup ERF genes based on TPM values in 30 tomato organs and under different biotic and abiotic stresses, respectively.

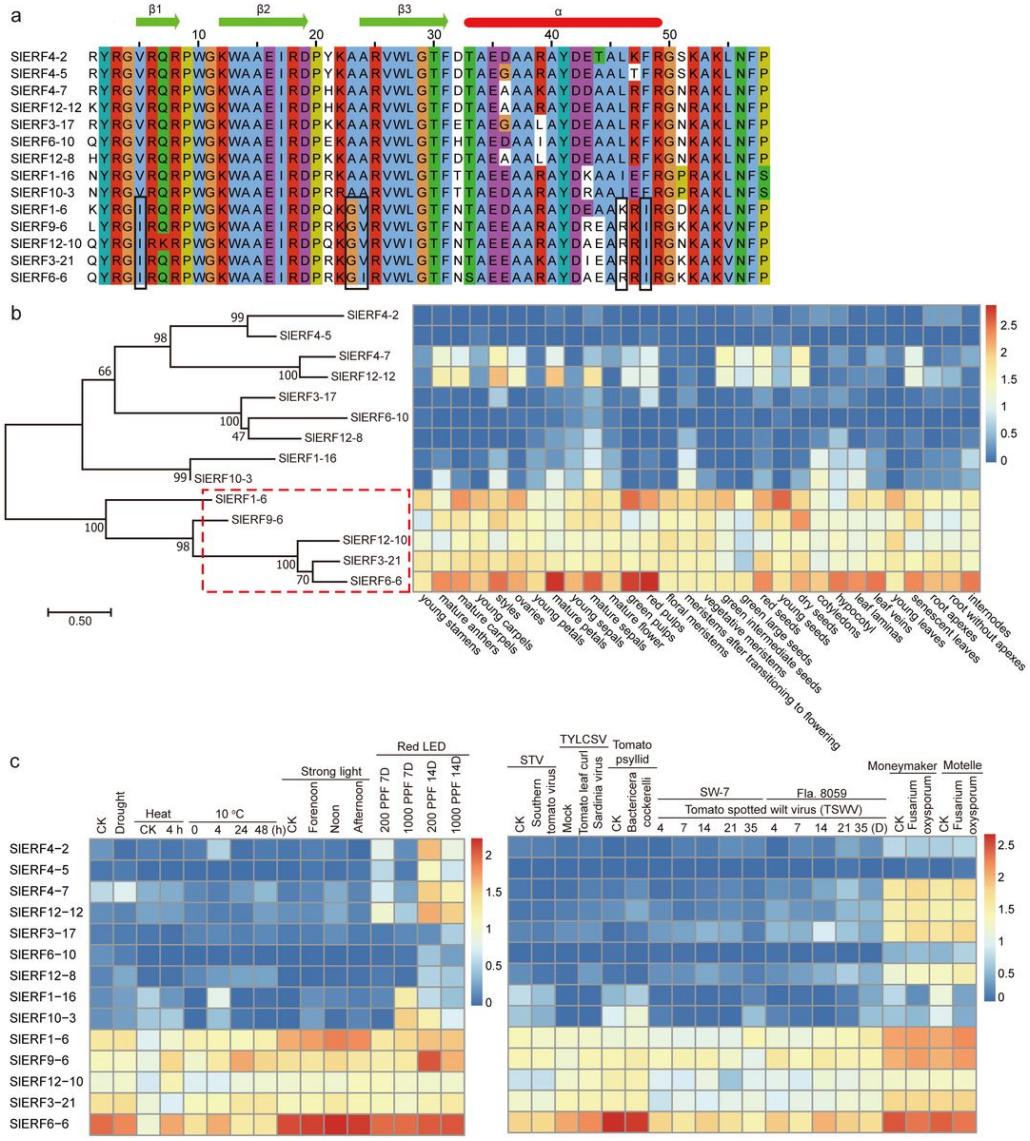

Figure 6

Expression levels of the -A subgroup ERF genes in 30 tomato organs and under different biotic and abiotic stresses. (a), AP2 domain sequences comparison; (b and c), heatmap analysis of the -A subgroup ERF genes based on TPM values in 30 tomato organs and under different biotic and abiotic stresses, respectively.

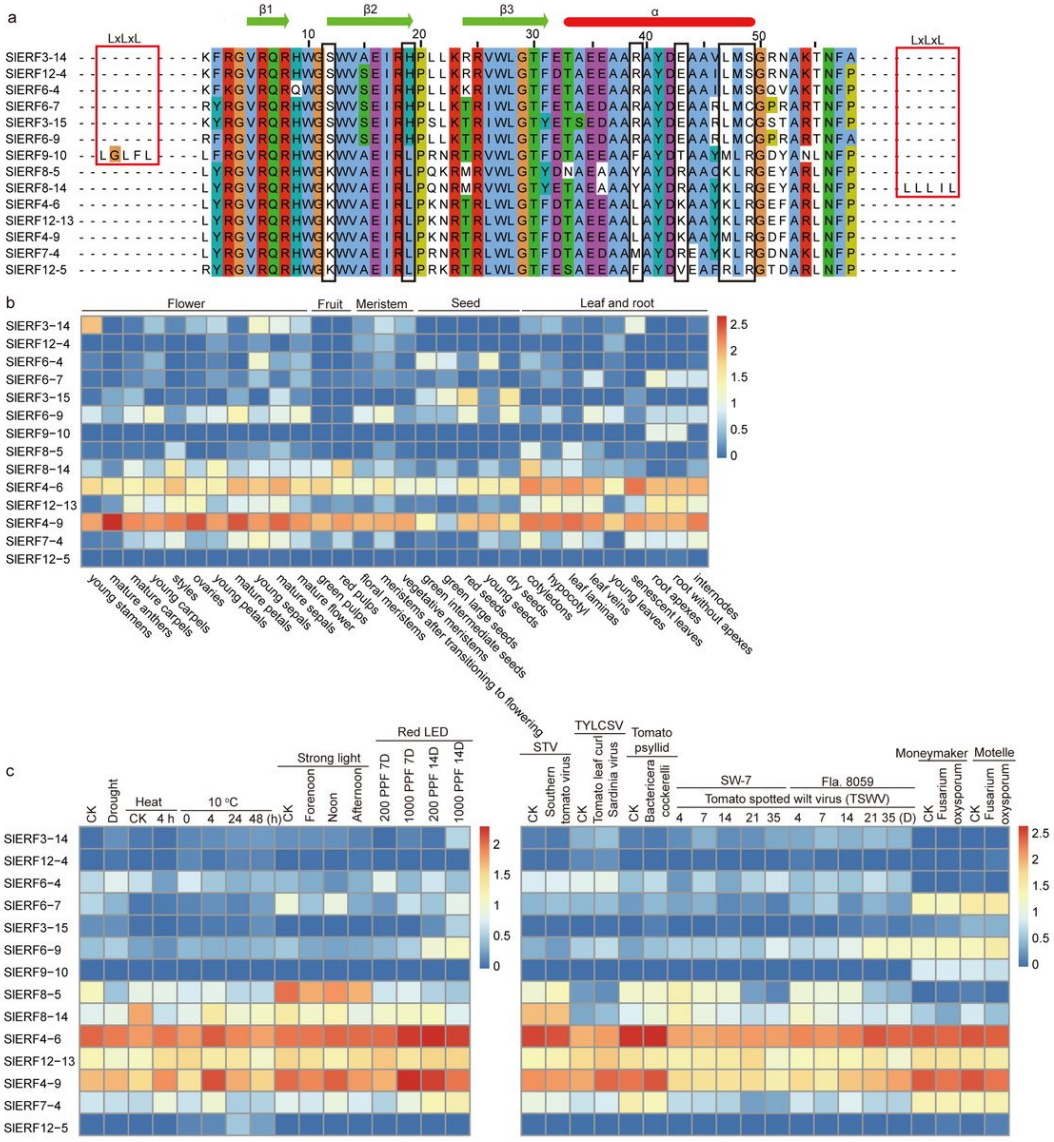

Figure 7

Expression levels of the ▯-B subgroup DREB genes in 30 tomato organs and under different biotic and abiotic stresses.(a), AP2 domain sequences and EAR motifs analysis; (b and c), heatmap analysis of the ▯-B subgroup DREB genes based on TPM values in 30 tomato organs and under different biotic and abiotic stresses, respectively.

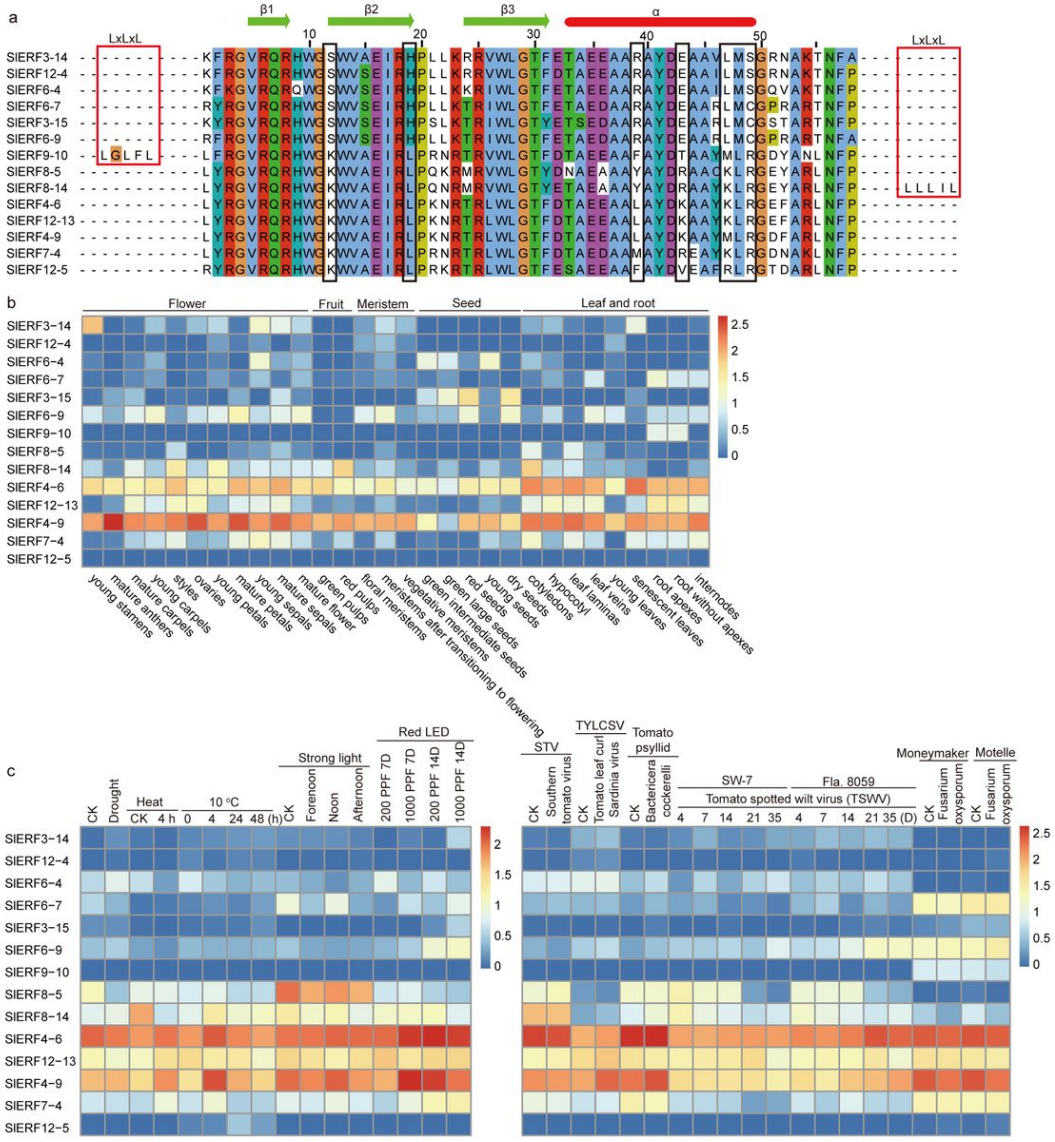

Figure 7

Expression levels of the Ⅱ-B subgroup DREB genes in 30 tomato organs and under different biotic and abiotic stresses.(a), AP2 domain sequences and EAR motifs analysis; (b and c), heatmap analysis of the Ⅱ-B subgroup DREB genes based on TPM values in 30 tomato organs and under different biotic and abiotic stresses, respectively.

Supplementary Files

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