BILINGUAL PUBLISHING CO.
Pioneer of Global Academics Since 1984

Journal of Botanical Research
https://ojs.bilpublishing.com/index.php/jbr

ARTICLE

Identification, Structure Analyses and Expression Pattern of the ERF Transcription Factor Family in *Coffea arabica*

Silvia Graciele Hülse de Souza[1] Tiago B. dos Santos[2] Douglas S. Domingues[3] Anne Bernadac[4] Mondher Bouzayen[4] Luiz F. P. Pereira[5] Giuliano Degrassi[6] Valéria Carpentieri-Pípolo[7*]

1.Laboratory of Molecular Biology, Universidade Paranaense PO box 87502-210, Umuarama, PR, Brazil
2.Instituto Agronômico do Paraná, Laboratório de Biotecnologia Vegetal, CP 481, PO box 86001-970, Londrina, PR, Brazil
3.Department of Botany, Instituto de Biociências, Universidade Estadual Paulista, UNESP, Rio Claro, SP, Brazil
4.Laboratoire de Genomique et Biotechnologie des Fruits, UMR 990 INRA/INP-ENSAT Chemin de Borderouge, PO box 107, 31326 Castanet Tolosan Cedex, France
5.Empresa Brasileira de Pesquisa Agropecuária, Embrapa Café, Parque Estação Biológica, Brasília, DF, PO box 70770-901, Brazil
6.International Centre for Genetic Engineering and Biotechnology (ICGEB), 1Industrial Biotechnology Group, Buenos Aires, República Argentina
7.Empresa Brasileira de Pesquisa Agropecuária, Embrapa Trigo, PO box 99001-970, Passo Fundo, RS, Brazil

ARTICLE INFO

Article history
Received: 19 January 2021
Accepted: 12 March 2021
Published Online: 30 March 2021

Keywords:
AP2/ERF
Coffee
Ethylene
Transcription factor

ABSTRACT

Members of the ERF Family of Transcription Factors play an important role in plant development and gene expression that regulates responses to biotic and abiotic stress. This work identified 36 ERF family genes in *Coffea arabica* within the AP2/ERF full domain, using the EST-based genomic resource of the Brazilian Coffee Genome Project. The ERF family genes were classified into nine of the ten existing groups through phylogenetic analysis of the deduced amino acid sequences and comparison with the sequences of the ERF family genes in Arabidopsis. In addition to the AP2 domain, other conserved domains were identified, typical of members of each group. The *in silico* analysis and expression profiling showed high levels of expression for libraries derived from tissues of fruits, leaves and flowers as well as for libraries subjected to water stress. These results suggest the participation of the ERF family genes of *C. arabica* in distinct biological functions, such as control of development, maturation, and responses to water stress. The results of this work imply in the selection of promising genes for further functional characterizations that will provide a better understanding of the complex regulatory networks related to plant development and responses to stress, opening up opportunities for coffee breeding programs.

**Corresponding Author:*
Valéria Carpentieri-Pípolo,
Empresa Brasileira de Pesquisa Agropecuária, Embrapa Trigo, PO box 99001-970, Passo Fundo, RS, Brazil;
Email: valeria.carpentieri-pipolo@embrapa.br

DOI: https://doi.org/10.30564/jrb.v3i1.2819

1. Introduction

With an annual worldwide production of 168.5 million of 60 kilograms bags of grains in 2019 [1], coffee is an important agricultural commodity cultivated in more than 80 countries, which represents a significant source of income mainly for developing tropical countries[2]. Brazil is the largest world producer and, together with Vietnam and Colombia, accounts for more than 50% of the world production [1]. Among the 124 identified species, only two are economically important: *Coffea arabica* and *Coffea canephora* [3]. The world market shares for these two species are 70% and 30%, respectively.

During their life cycle, crops are exposed to various biotic and abiotic stresses that limit their growth, development, and production [4,5]. To survive in stress conditions, plants have developed a complex molecular signaling network [6,7]. Gene regulation by transcription is one of the main control points of biological processes in which Transcription Factors (TFs) play a central role [8,9].

AP2/ERF superfamily is composed of ERF (Ethylene Responsive Factor), AP2, and RAV families, which consists of about 60-70 amino acids involved in DNA binding [10]. The ERF family proteins contain a single AP2 domain and the AP2 family proteins contain two repeated AP2 domains [11]. In addition to the single AP2 domain, the RAV family proteins contain a B3 domain that is a DNA-binding domain [12, 13]. The ERF family is further divided into two subfamilies: the ERF and the CBF/DREB [11, 13, 14].

Generally, the ERF family genes are partially involved in responses to biotic stress by recognizing the cis-acting sequence AGCCGCC, known as GCC-*box* [15]. The CBF/DREB subfamily genes play a crucial role on the plant responses to abiotic stress by recognizing the dehydration responsive element (DRE) with a central motif A/GTCGAC[16, 17]. The roles of the ERF and CBF/DREB proteins on the development and response to biotic and abiotic stresses in different plant species have been widely studied. Combining molecular genetic approaches, a series of ERF family regulatory genes involved in different metabolic pathways have been examined, including those related to drought [5, 18, 19, 20, 21], salinity [18, 22], low temperatures [23, 24, 25], and diseases [26, 27]. In addition to responses to diverse stress types, the ERF family genes are also involved in the development of roots [28], germination [29, 30], and development and maturation of fruits [31, 32].

Transcription Factors of the ERF subfamily and the CBF/DREB subfamily were identified in diverse species: Arabidopsis [14, 13, 33], rice [13, 34], cotton [35, 36], soybean [37], poplar [38], grape [15, 39], corn [40], tomato [8], apple [41], citrus [42] and banana [7]. Few studies with ERF family members in *Coffea* ssp were published [43]. Bustamante-Porras et al. [44] isolated the first ERF family member in *C. canephora*, whose expression is involved in processes of cell differentiation and fruit maturation. In *C. arabica*, no member of the ERF family was described until this moment.

Research on genomics and transcriptomics in coffee has gained more prominence. The Brazilian Coffee Genome Project [45, 46] has been developed to investigate the coffee characteristics through complementary DNA sequencing (cDNA). This database has a set of 265,889 expressed sequence tags (ESTs) from different tissues for *C. arabica, C. canephora* and *C. racemosa*. Therefore, the aim of this work was to identify and characterize possible ERF transcription factors in *C. arabica* from the ESTs database of the Brazilian Coffee Genome Project.

2. Material and Methods

2.1 Identification and Classification of ERF Family Genes in *Coffea arabica*

The ERF family genes in *C. arabica,* searches on the Brazilian Coffee Genome Database (http://bioinfo03.ibi.unicamp.br/cafe/) were performed using the AP2 domain of the *Solanum lycopersicon* ERF4 protein (GENBANK: AAO34706), with the BlastP software [47]. More than 265,889 ESTs sequences are available in this database, which were obtained from forty-three cDNA libraries, most of them of *C. arabica.* The cDNA was obtained from different tissues of the coffee plant (leaves, roots, flowers, seeds, fruits, among others) in different stages of development and subjected to various stress conditions [45, 46]. In order to increase the chances to identify new ESTs, searches were also performed using the following keywords: ERF, Ethylene Response Factor and EREBP. To verify the specificity of the annotated sequences, comparisons were confronted using BlastP tool with other sequences deposited in the NCBI database (http://www.ncbi.nlm.nih.gov/protein/). The deduced sequence of amino acids of each *contig* was obtained by the ORF Finder *software* (Open Reading Frame Finder - NCBI -https://www.ncbi.nlm.nih.gov/orffinder/). The sequences that presented incomplete AP2 domain or incorrect ORFs were excluded from analysis.

2.2 Phylogenetic Analysis

The protein sequences of the AP2 domain were aligned by the Clustal Omega algorithm version 2.0.3 [48]. The phylogenetic tree was constructed using the the MEGA *software* version 7.0 [49] based on neighbor-joining (NJ) method with *pair-wise* deletion, and the reliability was tested with 1,000 iterations of the *bootstrap.*

DOI: https://doi.org/10.30564/jrb.v3i1.2819

Table 1. *Coffea arabica* sequences with identity to ERF gene family in *Arabidopsis thaliana*

Arabidopsis thaliana		*Coffea arabica*			
Gene	Gene	ERF	Coverage (%)	e-value	Identity (%)
AT1G78080	RAP2.4	CaERF01	99	1,00E-68	81
AT1G78080	RAP2.4	CaERF02	97	2,00E-63	81
AT1G78080	RAP2.4	CaERF03	100	2,00E-58	80
AT1G78080	RAP2.4	CaERF04	100	5,00E-62	80
AT1G78080	RAP2.4	CaERF05	100	2,00E-34	81
AT5G67190	AtERF10	CaERF06	100	3,00E-39	74
AT5G52020	AtERF25	CaERF07	63	8,00E-30	71
AT5G52020	AtERF25	CaERF08	85	6,00E-45	65
AT244940	AtERF34	CaERF09	65	6,00E-48	77
AT240340	DREB2C	CaERF10	84	9,00E-49	68
AT240340	DREB2C	CaERF11	78	8,00E-38	70
AT1G75490	DREB2D	CaERF12	87	3,00E-37	69
AT4G27950	CRF4	CaERF13	71	3,00E-30	63
AT3G16770	ATEBP/RAP2.3	CaERF14	96	8,00E-34	72
AT3G16770	ATEBP/RAP2.3	CaERF15	93	2,00E-29	73
AT3G16770	ATEBP/RAP2.3	CaERF16	96	2,00E-29	68
AT3G16770	ATEBP/RAP2.3	CaERF17	62	1,00E-28	81
AT3G16770	ATEBP/RAP2.3	CaERF18	90	6,00E-27	81
AT3G14230	RAP2.2	CaERF19	99	8,00E-33	77
AT3G16770	ATEBP/RAP2.3	CaERF20	85	8,00E-28	77
AT3G16770	ATEBP/RAP2.3	CaERF21	96	1,00E-27	84
AT3G15210	AtERF4/RAP2.5	CaERF22	42	3,00E-30	74
AT3G15210	AtERF4/RAP2.5	CaERF23	41	5,00E-30	74
AT1G50640	AtERF3	CaERF24	82	2,00E-42	76
AT5G44210	ATERF9	CaERF25	97	4,00E-30	76
AT1G28360	ATERF12	CaERF26	68	1,00E-26	85
AT4G17500	AtERF1	CaERF27	75	9,00E-53	75
AT4G17500	AtERF1	CaERF28	86	1,00E-44	73
AT4G17500	AtERF1	CaERF29	79	5,00E-52	74
AT3G23240	ERF1	CaERF30	82	4,00E-31	73
AT4G17490	AtERF-6	CaERF31	97	5,00E-42	77
AT4G17490	AtERF-6	CaERF32	84	4,00E-31	78
AT4G17490	AtERF-6	CaERF33	93	7,00E-34	74
AT3G23240	ERF1	CaERF34	54	2,00E-15	73
AT5G61890	ABR1	CaERF35	94	2,00E-33	90
At2G33710	AtERF112	CaERF36	49	4,00E-28	75

DOI: https://doi.org/10.30564/jrb.v3i1.2819

2.3 Determination of Conserved Motifs

The identification of conserved motifs in protein sequences of *C. arabica* was analyzed using the Meme Suite version 4.11.2 (http://meme-suite.org/tools/meme), with the following parameters: ideal size: 6-80 amino acids; any number of repetitions for motifs and maximum number of motifs = 25. The resulting motifs were verified in the databases of NCBI (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) and PROSITE (http: //www.expasy.org) to verify their significance.

2.4 Gene Expression Profiling by Electronic Northern Blot

The gene expression profiles were performed by the Northern Blot technique. The specific tissue libraries investigated in this study were from Brazilian Coffee Genome Database [45]. The frequency of *reads* in each library was normalized according to Lima et al. [43]. The e-Northern Blot was performed using Genesis *software,* version 1.7.5.

3. Results

3.1 Identification and Phylogenetic Relationships of ERF Family in *C. arabica*

The analysis comprised a data mining process within the Coffee Genome Project Database to identify the ERF family members in *C. arabica*. For this, the *reads* that possibly encode the AP2 domain in *C. arabica* were selected. The search by keywords and through the AP2 domain of ESTs enabled the identification of 38 Transcription Factors encoding ERF proteins. Among these 38 possible ERFs, it was observed that only 36 ERF proteins contained a full AP2 domain, while the other 2 ERFs contained only a part of the AP2 domain and, therefore, were excluded from analysis. The identity of the ERF proteins in *C. arabica*, with their homologs in Arabidopsis, varied from 63-90% (Table 1). The conservation of the sequence in comparison with arabidopsis was higher in the Group X members, varying from 75-90%. Lower values were observed in the Groups III (65%) and VI (63%).

A phylogenetic reconstruction was obtained from the identification of 122 ERFs in Arabidopsis, previously described by Sakuma et al. [14]. The ERF sequences are highly conserved between species. It favored the distinction of the 10 main groups, named as Groups I-X by Nakano et al. [13]. According to Figure 1, the comparative analysis of the phylogenetic tree for Arabidopsis and *C. arabica* grouped a high number of identified sequences in *C. arabica* (22.22%, 8 sequences of 36) together with the arabidopsis sequences belonging to the Groups VII and IX. In *C. arabica,* a lower number of proteins was grouped into the ERFs of the Arabidopsis belonging to the Groups I and VIII (13.89%, 5 sequences), followed by the Groups III and IV (8.33%, 3 sequences). The ERF groups with fewer members in *C. arabica* were X, II and VI (5.56%, 2.78% and 2.78%; related to the sequences 2, 1 and 1, respectively). No member of *C. arabica* was found in the Group V and in the Sub-Groups VI-L and Xb-L. These two subgroups are characterized in Arabidopsis by a low homology in the C-terminal region of the AP2/ERF domain [36].

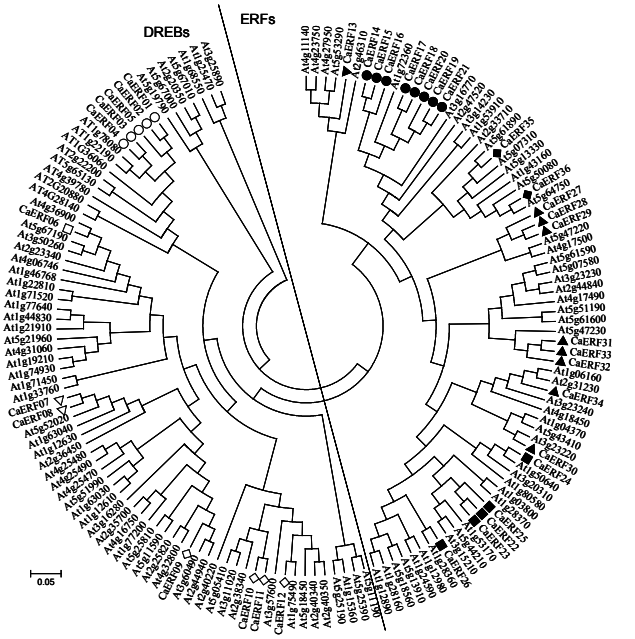

Figure 1. Phylogenetic tree of the ERFs in *C. arabica* and Arabidopsis . The phylogenetic tree was constructed with MEGA 7.0 using the NJ method. (DREB subfamily: ○ - Group I, □ - Group II, ▽ - Group III, ◇ - Group IV; ERF subfamily: ▼- Group VI, ● – Group VII, ■ - Group VIII, ▲ – Group IX, ◆ - Group X).

The ERFs were divided into two subfamilies. It was identified 12 putative ERFs as members of the DREB subfamily (Groups I, II, III and IV), in comparison with 57, 40, 75 and 58 in Arabidopsis, grape, poplar and rice, respectively. It was identified 24 encoding genes within the ERF subfamily (Groups VI, VII, VIII, IX, X) in comparison with 65, 82, 134 and 87 in Arabidopsis, grape, poplar and rice, respectively. The organization of the ERF family genes in *C. arabica* is showed in Table 2 along with the comparative distribution of Arabidopsis, grape, poplar and rice.

To study the phylogenetic relationship between the ERF family genes of *C. arabica* and Arabidopsis*,* multiple analyses were realized with the alignment of the deduced sequences of amino acids. The alignment of the AP2 do-

Table 2. Number of genes in each Group of the ERF Family in *C. arabica* and in species whose genome was completely sequenced and size of the respective genomes.

Family Subfamily	Group	Arabidopsis[a]	Vitis[b]	Poplar[c]	Rice[a]	Coffe[a]
ERF	I	10	5	8	9	5
	II	15	8	51	16	1
DREB	III	23	22	6	27	3
	IV	9	5	10	6	3
	V	5	11	42	8	0
	VI	8	2	20	6	1
	VI-L	4	5	-	10	0
ERF	VII	5	3	12	15	8
	VIII	15	11	39	15	5
	IX	17	40	19	18	8
	X	8	10	2	12	2
	Xb-L	3	0	-	3	0
Total ERFs		122	122	209	145	36
Genome Size (Mb)		125	487	465	430	1.300

[a]Nakano et al. [13], [b]Licausi et al. [15] and [c]Wang et al. [38].

main indicated that the residues Gly-4, Arg-6, Glu-16, Trp-28 and Gly-30 are completely conserved among all proteins within the ERF family in *C. arabica* and Arabidopsis. Furthermore, more than 95% of the ERF family members contain the conserved residues Arg-8, Gly-11, Ile-17, Arg-18, Arg-26, Leu-29, Ala-38, Ala-39, Asp-43 and Asn-56. As previously demonstrated by Sakuma et al. [14] the ERF gene subfamily includes two main residues of amino acids, the alanine (A) at position 14 and the aspartate (D) at position 19, which possibly contribute to a functional activity of binding to GCC-box in many ERFs. The CBF/DREB family contains a valine (V) and a glutamic acid (E) at positions 14 and 19, respectively. In the DREB subfamily, all genes present the conserved residue Valine14 and, at position 19, the genes *CaERF01-05* contain 1 Leucine (L) and the genes *CaERF06-12* contain 1 Glutamic Acid (E). The C-terminal region of the AP2/ERF domain of the *CaERF34* protein showed low homology with the region of consensus with other genes (Figure 2). This region corresponds to the half of terminal *α-helix* [50], which includes the highly conserved residues Asp-43 and Asn-57. In general, the ERF family showed significant similarity to the remaining domain.

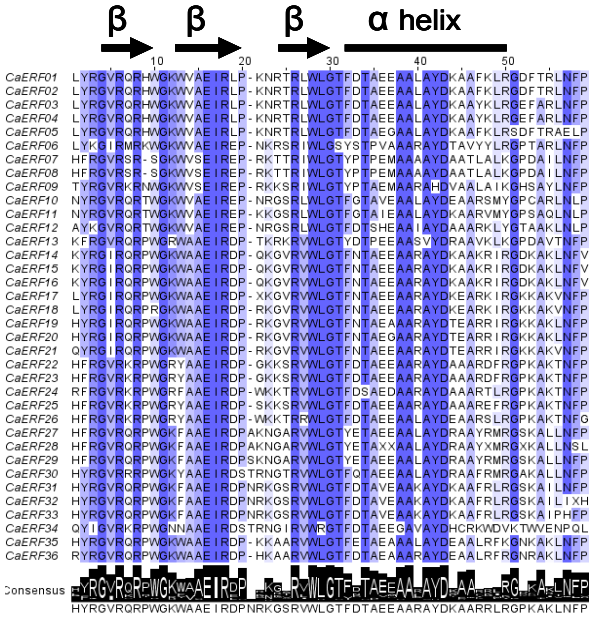

Figure 2 – Multiple sequence alignment of *C. arabica* AP2 domains from the ERF proteins. Identical and conserved amino acid residues are represented by dark and light blue shading, respectively. Black bar and arrows represent the predicted *α-helix* and *β-sheets* regions, respectively. *CaERF*01-12 belongs to the DREB subfamily; *CaERF*13-36 belongs to the ERF subfamily.

DOI: https://doi.org/10.30564/jrb.v3i1.2819

3.2 Distribution of Conserved Motifs

In general, the regions in Transcription Factors outside the DNA-binding domain contain important domains that are involved in transcription activities as the protein-protein interactions, which may be involved in nuclear localization [33]. Such functional domains are often conserved among members of a subgroup within families of transcription factors in plants. Probably these motifs are sharing the same functions [13, 51, 52].

In order to relate the putative ERFs in *C. arabica* to biological functions, other conserved motifs (CM) outside the AP2/ERF region were investigated on the deduced sequences of amino acids. Most members of the same group shared one or more motifs outside the AP2 domain (Figure 3). For instance, the Group I comprise 5 ERFs (*CaERF*01-05) and contain 5 conserved motifs (Figure 3). Except *CaERF*05, the members of this group contain the CMI-1 and CMI-2 motifs in the C-terminal region. The *CaERF*06 gene belonging to the Group II, unique member identified in this work, contains the CMII-1motif in the C-terminal region, adjacent to the AP2 domain. Belonging to the Group III are the *CaERF*07-09 genes, which contain the CMIII-1 motif in the C-terminal region. In addition to the CMIII-1 motif, the *CaERF*08 gene contains CMIII-2 and CMIII-4, and the last is identified as the LWSY conserved motif in the *OsDREB1A/B/C* and *AtCBF3/DREB1A* [18]. The *CaERF*09 represents the CMIII-6 and the CMIII-7 motifs. In the Group IV (*CaERF*10-12) only the CMIV-2 motif was found in the *CaERF*10 and *CaERF*11

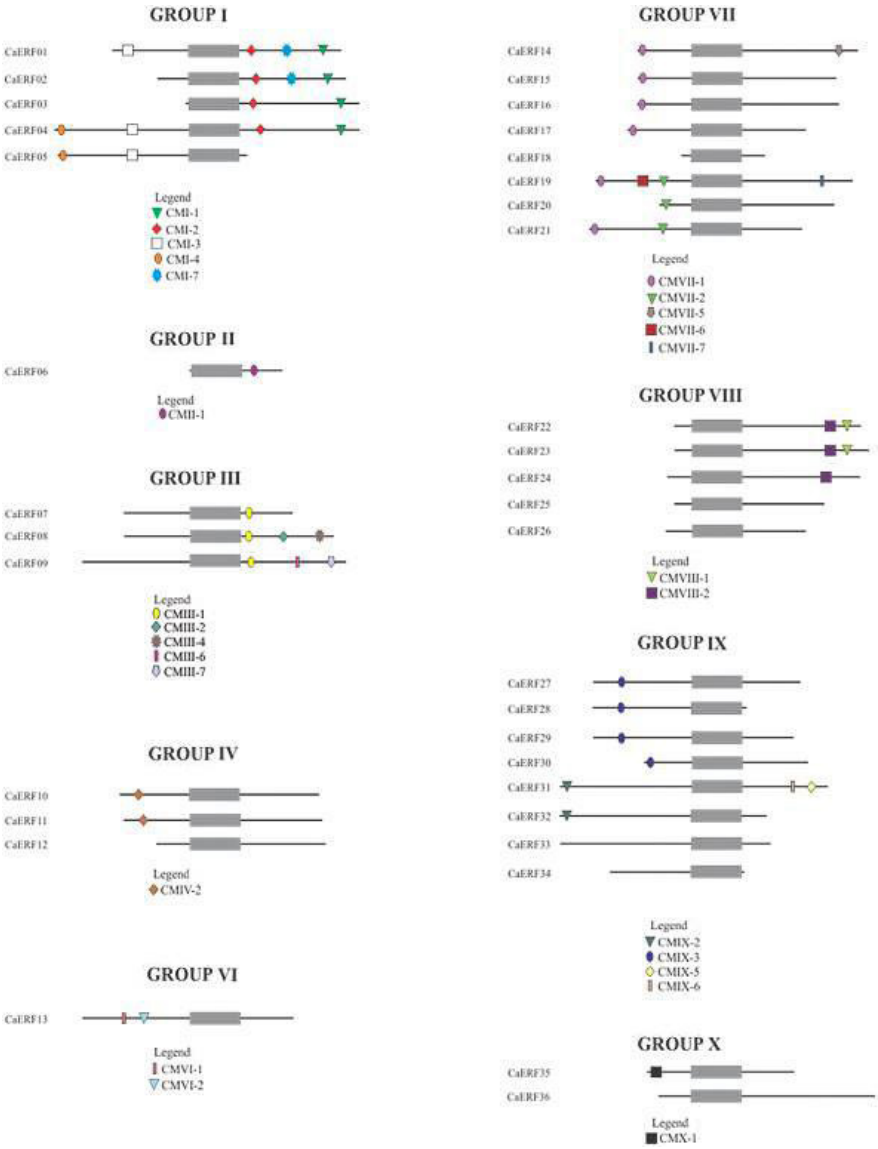

Figure 3. Conserved motifs in the ERF family in *Coffea arabica.* The motifs were identified in *C. arabica* and classified according to the classification proposed by Nakano et al. [13].

 DOI: https://doi.org/10.30564/jrb.v3i1.2819

genes. The CMIV-2 motif includes a putative nuclear localization signal [33].

The *CaERF*13 gene, unique of the Group VI, has two proteins that share the CMVI-1 and CMVI-2 conserved motif on the N-terminal region. The Group VII was firstly described by Tournier et al. [53] and is characterized by the presence of one highly conserved motif in the N-terminal region (MCGGAII/L). Within this group, 8 members were found in *C. arabica*. The EAR motif (CMVIII-1) was found in members of the Group VIII, in the ERFs *CaERF*22 and *CaERF*23, which also contain the CMVIII-2 motif. The Group IX is composed by 8 genes (*CaERF*27-34). The *CaERF*27-30 genes contain only the CMIX-3 motif while the *CaERF*32 gene contains only the CMIX-2 motif. The CMIX-3 motif corresponds to a conserved sequence that was referred previously to a DMLV motif [26]. In addition to the CMIX-3, the *CaERF*32 gene contains the CMIX-5 and CMIX-6 motifs that are probably a MAP kinase phosphorylation site, located at the C-terminal region of the protein [54]. The Group X is represented by the *CaERF*35 and *CaERF*36 genes. The group X members contain one CMX-1 conserved motif in the N-terminal region, such as the *CaERF*35, whereas the *CaERF*36 presents no conserved motif.

3.3 In Silico Gene Expression Profiling of the ERF Family in *Coffea arabica*

In order to assess the differences among transcripts of different tissues or organs, the ERF expression profiling was obtained in silico by e-Northen in the *C. arabica* libraries. High levels of expression were observed in libraries derived from tissues of fruits, leaves and flowers (Figure 4). The ERFs of the cDNA libraries from the tissues subjected to different types of stresses were also detected, however, with fewer transcripts than those from the tissues of diverse parts of the plant and different stages of development. Transcripts were detected in the majority of the evaluated libraries for the *ERFs CaERF20, CaERF21* and CaERF23. However, the majority of the 36 transcription factors of the ERF family were detected in specific libraries, showing that they are tissue specific. This is the case of *CaERF03, CaERF08, CaERF12, CaERF18, CaERF22, CaERF26, CaERF31* and *CaERF36,* which are expressed only in fruits. On the other hand, the *CaERF03, CaERF08, CaERF12, CaERF18, CaERF22, CaERF26, CaERF31* and CaERF36 genes are expressed only in flowers, leaves and roots. Expression profiling in libraries subjected to water stress were observed for *CaERF06, CaERF07, CaERF11, CaERF21* and *CaERF23*; the first three ERFs showed a higher expression in this library.

4. Discussion

Transcription factors are the principal regulators of biological factors and emerged as a powerful tool to manipulate complex metabolic pathways [55,56]. Using these proteins on plant breeding programs involves knowledge

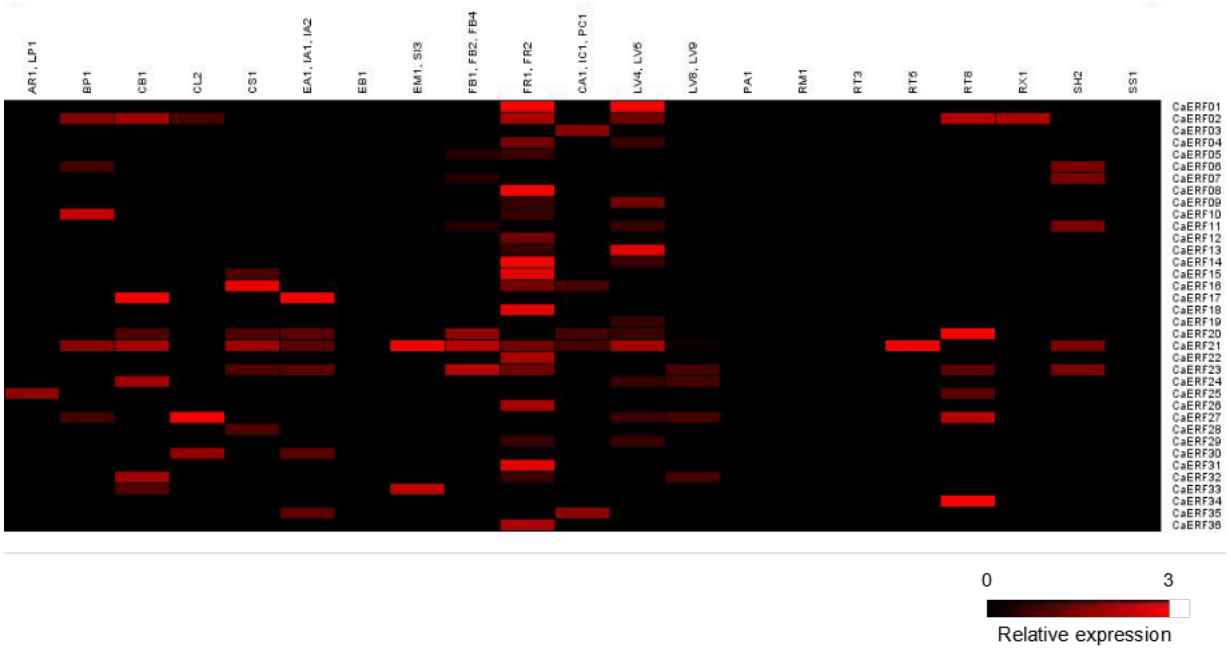

Figure 4. In-silico expression profiling of the ERFs in *Coffea arabica*. The number of reads was normalized in each library and values were represented by the relative expression scale.

DOI: https://doi.org/10.30564/jrb.v3i1.2819

on their role in gene regulatory networks. The ERF family of transcription factors presents a highly conserved element including the AP2/ERF domain, responsible for the DNA binding activity and important to plant development[14, 57, 58]. Nakano et al. [13] have systematically studied the phylogeny, the structures, and the conserved motifs of the ERF family in Arabidopsis and rice. In order to obtain more information on this family in *C. arabica*, this present work identified and analyzed 36 possible ERF proteins from the EST database in *C. arabica*, which is available at the Brazilian Coffee Genome Project [45, 46].

The AP2 conserved domains of Arabidopsis were used to group their homologs in *C. arabica*. The majority of the sequences in *C. arabica* are grouped into Groups VII and IX followed by the Groups I and VIII. Only few sequences were grouped into Groups III, VI, X, II and VI. However, the Group VII represents about 4.1% of the family in Arabidopsis [13], 2.46% in grape[15], 9.56% in poplar[38] and 10.34% in rice [13]. This group represents more than 22% of the proteins containing the single AP2 domain, found in the Coffee Genome database. In this work, from the ten main groups identified in the ERF family in Arabidopsis, nine occurred in *C. arabica*. Therefore, the methodology used by Nakano et al. [13] is applicable in this species. The presence of the majority of groups and subgroups in the two dicot species, as well as in monocot species suggests that many of the genes predate the divergence between monocotyledonous and dicotyledonous [8]. On the same way, some groups and subgroups are present in only one specie, for instance, the Groups XI-XIV occur only in the ERF family in rice, excluding Arabidopsis and other dicot species. It suggests that these groups have evolved or were lost in a certain species after divergence [37].

The structural analysis revealed that all EFR proteins contain conserved Ala-14 and Asp-19, whereas the DREB proteins contain Val-14 and Glu/Leu-19. The comparative analysis of the amino acids residues of the ERF/AP2 domain in *C. arabica* with the ERF family proteins in *Arabidopsis* showed that the AP2/ERF domains were well conserved between the two species. These conserved amino acids probably play an important role in the ERF gene family, where they can be involved with different ways of contact with DNA. According to Allen et al. [50] the AP2/ERF domain recognizes the DNA by the conserved residues arginine and tryptophan, located into *β-sheets*. The Ala-37 in the ERF domain plays an important role in the stability of the ERF domain or DNA binding to the DRE element or GCC-*box* [59].

The transcription factors generally contain conserved domains that are outside the DNA binding domain, but functionally important [60,61]. The distribution of the specific motifs into proteins belonging to the specific groups of the phylogenic tree was also observed for the ERF proteins in *C. arabica*, which demonstrated structural similarities within the same subgroup. The majority of the ERF sequences identified in *C. arabica* share one or more motifs outside the AP2 domain with their homologs in Arabidopsis, such as in rice and soybean [13, 37]. For instance, Ohta et al. [57] identified an EAR motif (ERF related to amphiphilic repression), which works as a repression domain. The EAR motif of conserved sequence, (L/F) DLN(L/F)xP, identified in this present work as CMVIII-1, is found in the C-terminal regions of the Group VIII. This motif was already identified in various repressors, including ZAT7, 10, 12, ERF3, AUX/IAA, NIMIN1, HSI2, SUPERMAN (Arabidopsis), NRR (rice), ZFT1 (tobacco) and ZPT2-3 (petunia), which play different roles - from the plant development to stress tolerance[62,63,64]. Currently the DEAR1, a DREB protein containing the EAR motif, appeared as a protein repressor of binding to dehydration responsive element, which mediates responses to biotic and abiotic stresses[65]. The CMIV-2 motif in the N-terminal region could work as a nuclear localization signal (NLS) [66]. Recently, it has been considered essential in Arabidopsis that CBF1 bind to DNA, since it is indispensable for transcriptional activity [67]. A putative nuclear localization signal (KRKRK) was identified in ERF proteins [31, 68]. The comparative analysis of the conserved motifs in *C. arabica* and Arabidopsis suggests that the protein functions were conserved and diverged during the evolution of the ERF gene family. Sharma et al. [8] showed that some motifs are specific in spermatophytes whereas many motifs have been identified in non-vascular plants, bacteria, fungi and animals. The presence of these conserved motifs in evolutionarily different organisms indicates that they play an important functional role, while specific motifs in spermatophytes may have later evolved to fulfill specific functions.

In this present work, 8 ERFs belonging to the Groups I, III, VI, VII, VIII, IX and X were expressed only in fruit libraries. Although the ERF transcription factors are regulated by a series of physical and chemical stimuli, many ERFs are responsive to ethylene, and therefore they may be involved in the maturation process of climacteric fruits. Tournier et al. [53] demonstrated that the *SlERF2*, an ERF that binds to the GCC-*box*, plays an important role during the tomato maturation process. The same was observed by Yin et al. [69] for different ERFs expressed during the kiwi maturation process. Pereira et al. [70] showed that the autocatalytic production of ethylene in fruits of green *C. arabica* is very low; however, it increases considerably during the initial stage of ripening. Such observations

 DOI: https://doi.org/10.30564/jrb.v3i1.2819

demonstrate the climacteric nature of the maturation of *C. arabica* fruits and the importance of ethylene in this process. Bustamante-Porras et al. [44] isolated an ERF gene (*CoERF*) in *C. canephora*, with expression during fruit differentiation and maturation. Comparing this *CoERF* (GENBANK: AY522505) with the *CaERF*17 in *C. arabica*, it shared 97% of identity and 98% of similarity. Given that *C. canephora* is one of the ancestors of *C. arabica*, the *CaERF*17 was expected to be expressed in fruits. However, *reads* were not found in fruits libraries for *CaERF*17. In allotetraploids, genes are expected to be present in two homologous forms, highly similar, but not identical [71]. The redundancy of genes can lead to gene silencing or functional divergence of duplicated genes[72]. Vidal et al.[73] found that, in some cases, apparently a homolog of C. canephora is recruited to be expressed in certain tissues, while *C. eugenioides* homologs are silenced. On this way, differences in expressions in *C. arabica* can be attributed to different sub-genomes of the ancestors of *C. canephora* or *C. eugenioides*. These genes may be good candidates for future characterizations that would help to understand regulation processes during development and maturation of fruits in Coffea ssp.

The majority of the ERFs have demonstrated an increase in plant tolerance to biotic and abiotic stresses [33, 75, 76]. In this work, the ERFs *CaERF*06, *CaERF*07, *CaERF*11 presented high expression in libraries subjected to water stress. These genes belong to the DREB subfamily, which play an important role in plant tolerance to abiotic stress, recognizing the Dehydration Responsive Element (DRE), the *core motif* A/GCCGAC [33]. Studies have showed that the overexpression of DREB genes in Arabidopsis activate the expression of several genes related to stress, thus improving the tolerance to drought, salinity and low temperature [33, 77, 78]. For example, the overexpression of the *AoDREB* gene of *Asparagus officinalis* L in transgenic Arabidopsis induced the expression of genes rd29A and COR15A, resulting in higher tolerance of transgenic plants to drought and salinity [79]. On this way, these genes are promising for further studies that will help to understand the regulation mechanisms of the ERF family related to responses of *C. arabica* to different stresses.

Previous work suggests the hypothesis that a group-specific expression profile is occurring. For example, from 8 genes belonging to the Group VII, 5 are expressed in fruits, where the ERFs *CaERF*14, *CaERF*15 and *CaERF*18 present a high relative expression. This group has been associated with fruit maturation. *LeERF*2 in tomato [53], *MdERF*1 in apple [87], *PsERF2a* and *PsERF2b* in plum [31] and *AdERF10* and *AdERF14* in kiwi [69] are proteins that are expressed during the maturation of fruits belonging to Group VII. The ripening induction was also related in the Group VIII in plum [80] and grape [15,39]. Other works have demonstrated that members of the Group IX present induction of expression when subjected to diverse pathogen attacks. Constitutive overexpression of the *AtERF*2 of the IXa subgroup probably induced the gene expression of the PDF1.2 gene [81]. On the same way, the overexpression of the *AtERF1* gene in Arabidopsis, a homolog next to *AtERF2*, gives resistance to *Botrytis cinerea, Sclerotinia sclerotiorum* and *Erysiphe orontii* in Arabidopsis [26]. Anderson et al. [82] demonstrated that the overexpression of the *MtERF*1-1 gene in roots of *Medicago truncatula* increased the resistance to *Rhizoctonia solani*, as well as to *Phytophthora medicaginis*. Thus, the genetic profile expression suggests a functional level of specialization for the investigated ERF Groups, although it is expected a high degree of overlapping functions in large plant genes families [83, 84, 85]. On this way, the presence of distinct expression profiles of the ERFs observed in *C. arabica* by *in silico* analysis may be associated to the phylogenetic distance among sequences, that is, the ERF phylogenetically related proteins have more similar patterns of expression than the divergent sequences.

The ERF gene family plays a crucial role in the development regulation, as well as in the responses to abiotic and biotic stresses. With the sequenced transcriptome of *C. arabica* by a Brazilian consortium (Brazilian Coffee Genome Project), 36 ERFs were identified in *C. arabica* in this work, where 12 ERFs belong to the DREB subfamily and 24 to the ERF subfamily. The gene expression profiling showed high levels of expression in libraries derived from tissues of fruits, leaves, and flowers and libraries subjected to water stress. From the comparison of the homologs with other species, whose genome was sequenced together with expression profiles, it is suggested that the ERFs of *C. arabica* are involved in different biological functions mediated by ethylene as control of development, maturation, and responses to water stress. *C. arabica* is a perennial species whose fruits have commercial value. Knowledge on the role of the ERF transcription factors in processes of development and maturation of this species opens opportunities for investments in plant breeding programs to increase the production and the coffee bean quality.

Acknowledgements

We thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior- Brazil) and CNPq (Conselho Nacional de Desenvolvimento Cientifico- Brazil) for the PhD Fellowship to Silvia G. H. de Souza.

DOI: https://doi.org/10.30564/jrb.v3i1.2819

References

[1] ICO. International Coffee Organization. Available< http://www.ico.org/documents/cy2020-21/cmr-1220-e.pdf.> Accessed 22 Jan 2021.

[2] Pay, E. The market for organic and fair-trade coffee. FAO Rome 2009. Available in http://<www.fao.org/.../organicexports/.../Market_Organic_FT_Coffee.pdf>accessed 31 October 2016.

[3] Hamon, P., Hamon, S., Razafinarivo, N.J., Guyot, R., Sonja Siljak-Yakovlev, S., Couturon, E., Crouzillat, D., Rigoreau, M., Akaffou, S., Rakotomalala, J.J., Kochko A. (2015), "Coffea Genome Organization and Evolution", Coffee in Health and Disease Prevention, edited by Victor R. Preedy, Academic Press, San Diego, 29-37.

[4] Wang, W., Vinocur, B., Altman, A. (2003), "Plant Responses to Drought, Salinity and Extreme Temperatures: Towards Genetic Engineering For Stress Tolerance", Planta, 218(1), 1-14.

[5] Yu, Y., Yang, D., Zhou, S., Gu, J., Wang, F., Dong, J., Huang, R. (2017), "The Ethylene Response Factor Oserf109 Negatively Affects Ethylene Biosynthesis and Drought Tolerance in Rice", Protoplasma, 254(1), 401-408.

[6] Fujita, M., Fujita, Y., Noutoshi, Y., Takahashi, F., Narusaka, Y., Yamaguchi-Shinozaki, K., Shinozaki, K. (2006), "Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks", Curr. Opin. Plant. Biol., 9(4), 436-42.

[7] Lakhwani, D., Pandey, A., Dhar, Y.V., Bag, S.K., Trivedi, P.K., Asif, M.H. (2016), "Genome-wide analysis of the AP2/ERF family in Musa species reveals divergence and neofunctionalisation during evolution", Sci. Rep., 6, 18878.

[8] Sharma, M. K., Kumar, R., Solanke, A. U., Sharma, R., Tyagi, A. K., Sharma A. K. (2010), "Identification, phylogeny, and transcript profiling of ERF family genes during development and abiotic stress treatments in tomato", Mol. Genet. Genomics, 284(6), 455-75.

[9] Yamasaki, K., Kigawa, T., Seki, M., Shinozaki, K., Yokoyama, S. (2013), "DNA-Binding Domains Of Plant-Specific Transcription Factors: Structure, Function, And Evolution", Trends Plant Sci., 18(5), 267-76.

[10] Riechmann, J.L., Heard, J., Martin, G., Reuber, L., Jiang, C., Keddie, J., Adam, L., Pineda, O., Ratcliffe, O.J., Samaha, R.R., Creelman, R., Pilgrim, M., Broun, P., Zhang, J.Z., Ghandehari, D., Sherman, B.K., Yu, G. (2010), "Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes", Sci., 290(5499),2105-10.

[11] Jofuku, K. D., Den Boer, B. G., Van Montagu, M., Okamuro, J. K. (1994), "Control of Arabidopsis flower and seed development by the homeotic gene APETALA2", Plant Cell, 6(9), 1211-25.

[12] Kagaya, Y., Ohmiya, K., Hattori, T. (1999), "RAV1, a novel DNA-binding protein, binds to bipartite recognition sequence through two distinct DNA-binding domains uniquely found in higher plants", Nucleic Acids Res., 27(2), 470-478.

[13] Nakano, T., Suzuki, K., Fujimura, T., Shinshi, H. (2006), "Genome wide analysis of the ERF gene family in Arabidopsis and rice", Plant Physiol., 140(2), 411-432.

[14] Sakuma Y., Liu Q., Dubouzet J. G., Abe H., Shinozaki, K. (2002), "DNA binding specificity of the ERF/AP2 domain of Arabidopsis DREBs, transcription factors involved in dehydration and cold inducible gene expression", Biochem. Biophys. Res. Commun., 290(3), 998-1009.

[15] Licausi, F., Giorgi, F. M., Zenon, S., Osti, F., Pezzotti, M., Perata, P. (2010), "Genomic and transcriptomic analysis of the AP2/ERF superfamily in Vitis vinifera", BMC Genomics, 11, 719.

[16] Thomashow, M. F. (1999), "Plant Cold Acclimation: freezing tolerance genes and regulatory mechanism", Annu. Rev. Plant Physiol., 50, 571-599.

[17] Shinozaki, K. and Yamaguchi-Shinozaki K. (2007), "Gene networks involved in drought stress response and tolerance", J. Exp. Bot., 58(2), 221-227.

[18] Dubouzet, J. G., Sakuma, Y., Ito, Y., Kasuga, M., Dubouzet, E. G., Miura, S., Seki, M., Shinozaki, K., Yamaguchi, S. K. (2003), "OsDREB genes in rice, Oryza sativa L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression" Plant J., 33(4), 751-63.

[19] Yamaguchi, S. K. and Shinozaki, K. (2006), "Transcriptional Regulatory Networks in Cellular Responses And Tolerance To Dehydration And Cold Stresses", Annu. Rev. Plant Biol., 57, 781-803.

[20] Mawlong, I., Ali, K., Kurup, D.,Yadav, S., Aruna, T. (2014), "Isolation and characterization of an AP2/ERF-type drought stress inducible transcription factor encoding gene from rice", J. Plant Biochem. Biotechnol., 23, 42-51.

[21] Gao, Y., Han, D., Jia, W., Ma, X., Yang, Y., Xu, Z. (2020), "Molecular characterization and systematic analysis of NtAP2/ERF in tobacco and functional determination of NtRAV-4 under drought stress" Plant Physiol. Biochem., 156, 420-435.

[22] Faraji, S., Filiz, E., Kazemitabar, S.K., Vannozzi,

 DOI: https://doi.org/10.30564/jrb.v3i1.2819

A., Palumbo, F., Barcaccia, G., Heidari, P. (2020), "The AP2/ERF Gene Family in Triticum durum: Genome-Wide Identification and Expression Analysis under Drought and Salinity Stresses", Genes, 11(12), 1464.

[23] Yang, T.W., Zhang, L.J., Zhang, T.G., Zhang, H., Xu, S.J., An, L.Z. (2005), "Transcriptional Regulation Network of Cold-Responsive Genes in Higher Plants", Plant Sci., 169(6), 987-995.

[24] Qin, Q.L., Liu, J.G., Zhang, Z., Peng, R.H., Xiong, A.S., Yao, Q.H., Chen, J.M. (2007), "Isolation, optimization, and functional analysis of the cDNA encoding transcription factor RdreB1 in Oryza Sativa L", Mol. Breeding, 19, 329-340.

[25] Du, C., Hu, K., Xian, S., Liu, C., Fan, J., Tu, J. (2016), "Dynamic transcriptome analysis reveals AP2/ERF transcription factors responsible for cold stress in rapeseed (Brassica napus L.)" Mol. Genet. Genomics, 291(3), 1053-1067.

[26] Gutterson, N. and Reuber, T. L. (2004), "Regulation of disease resistance pathways by AP2/ERF transcription factors", Curr. Opin. Plant Biol., 7(4), 465-471.

[27] Charfeddine, M., Bouaziz, D., Charfeddine, S., Hammami, A., Nouri-Ellouz, O., Bouzid G.R. (2015), "Overexpression of dehydration responsive element binding 1 protein (DREB1) in transgenic Solanum tuberosum enhances tolerance to biotic stress", Plant Biotechnol Rep., 9, 79-88.

[28] Banno, H., Ikeda, Y., Niu, Q. W., Chua, N. H. (2001), "Overexpression of arabidopsis ESR1 induces initiation of shoot regeneration", Plant Cell., 13(12), 2609-2618.

[29] Pirrello, J., Jaimes-Miranda, F., Sanchez-Ballesta, M. T., Tournier, B., Khalil-Ahmad, Q., Regad, F., Latche, A., Pech, J. C., Bouzayen, M. (2006), "Sl-ERF2, a tomato ethylene response factor involved in ethylene response and seed germination", Plant Cell Physiol., 9(47):1195-1205.

[30] Yoong, F.Y., O'brien, L.K., Truco, M.J., Huo, H., Sideman, R., Hayes, R., Michelmore, R.W., Bradford, K.J. (2016), "Genetic Variation for Thermotolerance in Lettuce Seed Germination is Associated with Temperature-Sensitive Regulation of Ethylene Response Factor1 (ERF1)", Plant Physiol., 170(1), 472-488.

[31] El-Sharkawy, I., Sherif, S., Mila, I., Bouzayen, M., Jayasankar, S. (2009), "Molecular characterization of seven genes encoding ethylene-responsive transcriptional factors during plum fruit development and ripening", J. Exp. Bot., 60(3), 907-922.

[32] Zhang, A.D., Hu, X., Kuanga, S., Ge, H., Yin, X.R., Chen, K.S. (2016), "Isolation, Classification and Transcription Profiles of the Ethylene Response Factors (ERFs) in Ripening Kiwifruit", Sci. Hort., 199, 209-215.

[33] Liu Q., Kasuga, M., Sakuma, Y., Abe H., Miura, S., Yamaguchi-Shinozaki, K.,Shinozaki, K. (1998), "Two transcription factors, DREB1 and DREB2, withan EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in Arabidopsis", Plant Cell., 10(8), 1391-1406.

[34] Sharoni, A. M., Nuruzzaman, M., Satoh, K., Shimizu, T., Kondoh, H, Sasaya, T., Choi, Ir., Omura, T., Kikuchi, S. (2011), "Gene Structures, Classification and Expression Models of the AP2/EREBP Transcription Factor Family in Rice", Plant Cell Physiol., 52(2), 344-360.

[35] Jin, L. G. and Liu, J. Y. (2008), "Molecular cloning, expression profile and promoter analysis of a novel ethylene responsive transcription factor gene GhERF4 from cotton", Plant Physiol. Biochem., 46(1), 46-53.

[36] Champion, A., Hebrard, E., Parra, B., Bournaud, C., Marmey, P., Tranchant, C., Nicole, M. (2009), "Molecular diversity and gene expression of cotton ERF transcription factors reveal that group IXa members are responsive to jasmonate, ethylene and Xanthomonas", Mol. Plant Pathol., 10(4), 471-485.

[37] Zhang, G., Chen, M., Chen, X., Xu, Z., Guan, S, Li Lc, Li A, Guo J, Mao L, Ma Y. (2008), "Phylogeny, Gene Structures, And Expression Patterns of the ERF Gene Family in Soybean (Glycine Max L)", J. Exp. Bot., 59(15), 4095-4107.

[38] Wang, S.,Yao, W.,Zhou, B., Jiang, T. (2016), "Structure Analysis And Expression Pattern of the ERF Transcription Factor Family in Poplar", Acta Physiol. Plant, 38(10), 239.

[39] Zhuang, J., Peng, R-H., Cheng, Z-M., Zhang, J., Cai, B., Zhang, Z., Gao, F., Zhu, B., Fu, X-Y., Jin, X-F., Chen, J-M, Qiao, Y-S., Xiong, A-S., Yao, Q-H. (2009), "Genome-Wide Analysis of the Putative AP2/ERF Family Genes in Vitis Vinifera", Sci Hortic., 1(123), 73-81.

[40] Zhuang, J., Deng, De-X., Yao., Q-H., Zhang, J., Xiong, F., Chen, J-M., Xiong, Ai- S. (2010), "Discovery, Phylogeny and Expression Patterns of AP2-Like Genes in Maize", Plant Growth Regul., 1(62), 51-58.

[41] Zhuang, J., Yao, Q-H., Xiong, A. S., Zhang, J. (2011), "Isolation, Phylogeny and Expression Patterns of AP2-Like Genes in Apple (Malus × Domestica

  DOI: https://doi.org/10.30564/jrb.v3i1.2819

Borkh)", Plant Mol. Biol. Rep., 1(29), 209-216.

[42] Ito, T.M., Polido, P.B., Rampim, M.C., Kaschuk, G., Souza, S.G.H. (2014), "Genome-wide identification and phylogenetic analysis of the AP2/ERF gene superfamily in sweet orange (Citrus sinensis)", Genet. Mol. Res., 13(3), 7839-7851.

[43] Lima, A.A., Ságio, S.A., Chalfun-Júnior, A., Paiva, L.V. (2011), "In silico characterization of putative members of the coffee (Coffea arabica) ethylene signaling pathway", Genet. Mol. Res., 10(2), 1277-1289.

[44] Bustamante-Porras, J., Noirot, M., Campa, C., Hamon, S., Kochko. A. (2005), "Isolation and characterization of a Coffea canephora ERF-like cDNA", Afr. J. Biotechnol., 2(4), 157-159.

[45] Vieira, L. G. E., Andrade, A. C., Colombo, C. A., Moraes, A. H. A., Metha, A., Oliveira A. C., Labate, C. A., Marino, C. L., Monteiro-Vitorello, C. B, Monte, D. C. et al. (2006), "Brazilian Coffee Genome Project: An Est-Based Genomic Resource", Brazil. J. Plant Physiol., 18(1), 95-108.

[46] Mondego, J. M. C., Vidal, R. O., Carazzolle, M. F., Tokuda, E. K., Parizzi, L. P., Costa, G. G. L., Pereira, L. F. P., Andrade, A. C., Colombo, C. A.., Vieira, L. G. E., Pereira, G. A. G. (2011), "Brazilian Coffee Genome Project Consortium. An EST-based analysis identifies new genes and reveals distinctive gene expression features of Coffea arabica and Coffea canephora", BMC Plant Biol., 11, 30.

[47] Altschul, S. F., Madden, T. L., Scha¨Ffer, A. A., Zhang, J., Zhang, Z., Miller, W., Lipman, D. J. (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res., 25(17), 3389-3402.

[48] Sievers, F., Wilm, A., Dineen, D., Gibson, T.J., Karplus, K., Li, W., Lopez, R., Mcwilliam, H., Remmert, M., Söding, J., Thompson, J.D., Higgins, D.G. (2011), "Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega", Mol. Syst. Biol., 7, 539.

[49] Kumar, S., Stecher, G., Tamura, K. (2016), "MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets", Mol. Biol. Evol., 33(7),1870-1874.

[50] Allen, M. D., Yamasaki, K., Ohme-Takagi, M., Tateno, M., Suzuki, M. (1998), "A novel mode of DNA recognition by a beta-sheet revealed by the solution structure of the GCC-box binding domain in complex with DNA", EMBO J., 17(18), 5484-5496.

[51] Kranz, H. D., Denekamp, M., Greco, R., Jin, H., Leyva, A., Meissner, R. C, Petroni, K., Urzainqui, A., Bevan, M., Martin, C. et al. (1998), "Towards functional characterisation of the members of the R2R3-MYB gene family from Arabidopsis thaliana", Plant J., 16(2), 263-276.

[52] Reyes, J.C., Muro-Pastor M.I., Florencio, F.J. (2004)," The GATA family of transcription factors in Arabidopsis and rice", Plant Physiol., 134(4), 1718-32.

[53] Tournier, B., Sanchez-Ballesta, M. T., Jones, B., Pesquet, E., Regad, F., Latche, A., Pech, J. C., Bouzayen, M. (2003), "New Members of the Tomato ERF Family Show Specific Expression Pattern and Diverse DNA-Binding Capacity to the GCC Box Element", Febs Lett., 550(1-3), 149-54.

[54] Fujimoto S. Y., Ohta, M., Usui, A., Shinshi, H., Ohme-Takagi, M. (2000), "Arabidopsis ethylene-responsive element binding factors act as transcriptional activators or repressors of GCC box-mediated gene expression", Plant Cell., 12(3), 393–404.

[55] Grotewolda, E. (2008), "Transcription factors for predictive plant metabolic engineering: are we there yet?", Curr Opin Biotechnol., 19(2),138-44.

[56] Zhang, Q., Zhou, W., Li, B., Li, L., Fu, M., Zhou, L., Yu, X., Wang, D., Wang, Z. (2021), "Genome-Wide Analysis and the Expression Pattern of the ERF Gene Family in Hypericum perforatum", Plants, 10(1), 133.

[57] Ohta, M., Matsui, K., Hiratsu, K., Shinshi, H., Ohme-Takagi, M. (2001), "Repression domains of class II ERF transcriptional repressors share an essential motif for active repression", Plant Cell., 13(8), 1959-1968.

[58] Cao, Y., Song, F., Goodman, R. M., Zheng, Z. (2006), "Molecular characterization of four rice genes encoding ethylene-responsive transcriptional factors and their expressions in response to biotic and abiotic stress", J. Plant Physiol., 11(163), 1167-1178.

[59] Liu, Y., Zhao, T. J., Liu, J. M., Liu, W. Q., Liu, Q., Yan, Y. B., Zhou, H. M. (2006), "The conserved Ala37 in the ERF/AP2 domain is essential for binding with the DRE element and the GCC box", FEBS Lett., 580(5), 1303-1308.

[60] De Bodt, S., Raes, J., Florquin, K., Rombauts, S., Rouze, P., Theissen, G., Van De Peer, Y. (2003), "Genome-wide structural annotation and evolutionary analysis of the type I MADS-box genes in plants" J. Mol. Evol., 56(5):573-86.

[61] Arora, R., Agarwal, P., Ray, S., Singh, A. K., Singh, V. P., Tyagi, A. K., Kapoor, S. (2007), "MADS-box gene family in rice: genome-wide identification, organization and expression profiling during reproductive development and stress", BMC Genomics., 8, 242.

 DOI: https://doi.org/10.30564/jrb.v3i1.2819

[62] Tiwari, S. B., Hagen, G., Guilfoyle, T. J. (2004), "AUX/IAA Proteins Contain a Potent Transcriptional Repression Domain", Plant Cell, 16(2), 533-543.

[63] Hiratsu, K., Mitsuda, N., Matsui, K., Ohme-Takagi, M. (2004), "Identification of the minimal repression domain of SUPERMAN shows that the DLELRL hexapeptide is both necessary and sufficient for repression of transcription in Arabidopsis", Biochem. Biophys. Res. Commun., 321(1), 172-178.

[64] Kazan, K. (2006), "Negative regulation of defense and stress genes by EAR-motif-containing repressors", Trends Plant Sci., 11(3), 109-112.

[65] Tsutsui, T., Kato, W., Asada, Y., Sako, K., Sato, T., Sonoda, Y., Kidokoro, S., Yamaguchi-Shinozaki, K., Tamaoki, M., Arakawa, K., Ichikawa, T., Nakazawa, M., Seki, M., Shinozaki, K., Matsui, M., Ikeda, A., Yamaguchi, J. (2009), "DEAR1, a Transcriptional Repressor of Dreb Protein that Mediates Plant Defense and Freezing Stress Responses in Arabidopsis", J. Plant. Res., 122(6), 633-43.

[66] EL Kayal, W., Navarro, M., Marque, G., Keller, G., Marque, C. Teulieres, C. (2006), "Expression profile of CBF-like transcriptional factor genes from Eucalyptus in response to cold", J. Exp. Bot., 57(10), 2455-2469.

[67] Canella, D., Gilmour, S. J., Kuhn, L. A., Thomashow, M. F. (2010), "DNA binding by the Arabidopsis CBF1 transcription factor requires the PKKP/RAGRxKFxETRHP signature sequence", Biochim. Biophys. Acta., 1799(5-6), 454-462.

[68] Van Raemdonck, D., Pesquet, E., Cloquet, S., Beeckman, H., Boerjan, W., Goffner, D., El, Jaziri, M., Baucher, M. (2005), "Molecular Changes Associated with the Setting up of Secondary Growth in Aspen", J. Exp. Bot., 56(418), 2211-2227.

[69] Yin, X-R., Allan, A. C., Chen, K-S., Ferguson, I. B. (2010), "Kiwifruit EIL and ERF Genes Involved in Regulating Fruit Ripening", Plant Physiol., 3(153), 1280-1292.

[70] Pereira, L. F. P., Galvao, R. M., Kobayashi, A. K., Cação, S. M. B., Esteves, V. L. G. (2005), "Ethylene production and acc oxidase gene expression during fruit ripening of Coffea arabica L", Braz. J. Plant Physiol., 17(3), 283-289.

[71] Petitot, A. S., Lecouls, A. C., Fernandez, D. (2008), "Sub-genomic origin and regulation patterns of a duplicated WRKY gene in the allotetraploid species Coffea arabica", Tree Genet. Genomes., 3(4), 379-390.

[72] Chen, Z. J. and Ni, Z. (2006), "Mechanisms of genomic rearrangements and gene expression changes in plant polyploids", BioEssays, 28(3), 240-252.

[73] Vidal, R. O., Mondego, J. M., Pot, D., Ambrosio, A. B., Andrade, A. C., Pereira, L. F., Colombo, C. A., Vieira, L. G., Carazzolle, M. F., Pereira, G. A. (2010), "A High-Throughput Data Mining of SNPS in Coffea Spp ESTs Suggests Differential Homeologous Gene Expression in the Allotetraploid Coffea arabica" Plant Physiol., 154(3), 1053-1066.

[74] Guo, Z. J., Chen, X. J., Wu, X. L., Ling, J. Q., Xu, P. (2004), "Overexpression of the AP2/EREBP transcription factor OPBP1 enhances disease resistance and salt tolerance in tobacco", Plant Mol. Biol., 55, 607-618.

[75] Zhang, G., Chen, M., Li, L., Xu, Z., Chen, X., Guo, J., Ma, Y. (2009), "Overexpression of the Soybean GMERF3 Gene, an AP2/ERF Type Transcription Factor for Increased Tolerances to Salt, Drought, and Diseases in Transgenic Tobacco", J. Exp. Bot., 13 (60), 3781-3796.

[76] Erpen, L., Devi, H.S., Grosser, J.W. et al. (2018), "Potential use of the DREB/ERF, MYB, NAC and WRKY transcription factors to improve abiotic and biotic stress in transgenic plants", Plant Cell Tiss. Organ Cult., 132, 1-25.

[77] Sakuma, Y., Maruyama, K., Osakabe, Y., Qin, F., Seki, M., Shinozaki, K., Yamaguchi-Shinozaki, K. (2006), "Functional analysis of an Arabidopsis transcription factor, DREB2A, involved in drought-responsive gene expression", Plant Cell., 18(5), 1292-309.

[78] Bouaziz, D., Jbir, R., Charfeddine, S., Saidi, M.N., Gargouri-Bouzid, R. (2015), "The StDREB1 transcription factor is involved in oxidative stress response and enhances tolerance to salt stress", Plant Cell Tiss. Organ Cult., 121(1):237-248.

[79] Liu, Y., Chen, H., Zhuang, D., Jiang, D., Liu, J., Wu, G., Yang, M., Shen, S. (2010), "Characterization of a DRE-binding transcription factor from asparagus (Asparagus officinalis L.) and its overexpression in Arabidopsis resulting in salt- and drought-resistant transgenic plants", J. Plant Sci., 171(1), 12-23.

[80] El-Sharkawy, I., Kim, W. S., El-Kereamy, A., Jayasankar, S., Svircev Amd., Brown, C. W. (2007), "Isolation and characterization of four ethylene signal transduction elements in plums (Prunus salicina L.)", J. Exp. Bot., 58(13), 3631-3643.

[81] Brown, R. L., Kazan, K., Mcgrath, K. C., Maclean, D. J., Manners, J. M. (2003), "A role for the GCC-box in jasmonate-mediated activation of the PDF1.2 gene of Arabidopsis", Plant Physiol., 132(2),1020-1032.

[82] Anderson, J. P., Lichtenzveig, J., Gleason, C., Oliver, R. P., Singh, K. B. (2010), "The B-3 Ethylene Response Factor MtERF1-1 Mediates Resistance to

  DOI: https://doi.org/10.30564/jrb.v3i1.2819

a Subset of Root Pathogens in Medicago truncatula without Adversely Affecting Symbiosis with Rhizobia", Plant Physiol., 154(2), 861-73.

[83] Soltis, D. E., Bell, C. D., Kim, S., Soltis, P. S. (2008), "Origin and early evolution of angiosperms", Ann. N. Y. Acad. Sci. 1133, 3-25.

[84] Semon M. and Wolfe, K. H. (2007), "Consequences of genome duplication", Curr. Opin. Gen. Dev., 17(6), 505-512.

[85] Wang, A., Tan D. M., Takahashi, A., Li, T. Z., Harada, T. (2007), "MdERFs, two Ethylene-Response Factors Involved in Apple Fruit Ripening", J. Exp. Bot., 58(13), 3743-3748.

 DOI: https://doi.org/10.30564/jrb.v3i1.2819