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

Silvia Graciele Hülse de Souza¹ Tiago B. dos Santos² Douglas S. Domingues³

Anne Bernadac⁴ Mondher Bouzayen⁴ Luiz F. P. Pereira⁵ Giuliano Degrassi⁶

Valéria Carpentieri-Pípolo^{7*}

1.Laboratory of Molecular Biology, Universidade Paranaense PO box 87502-210, Umuarama, PR, Brazil

2.Instituto Agrônômico 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

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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

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*.

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

<i>Arabidopsis thaliana</i>		<i>Coffea arabica</i>			
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

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].

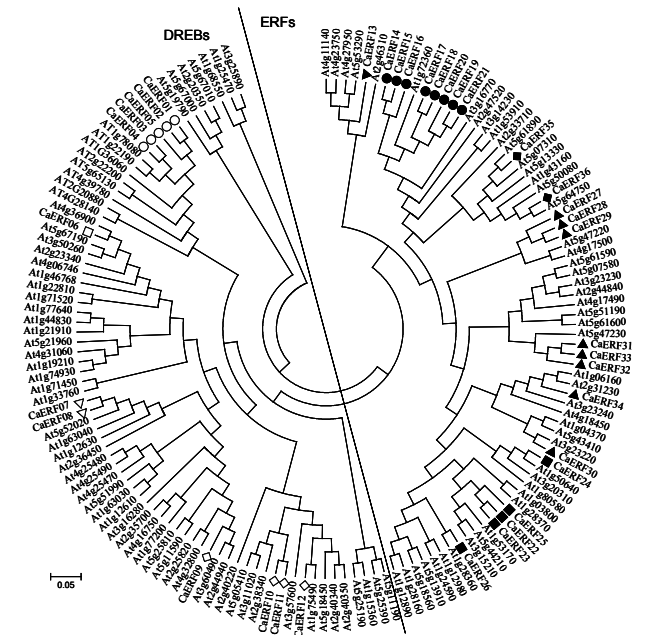


Figure 1. Phylogenetic tree of the ERFs in *C. arabica* and Arabidopsis. The phylogenetic tree was constructed with MEGA 7.0 using the NJ method. (DREB subfamily: ○ - Group I, □ - Group II, ▽ - Group III, ◇ - Group IV; ERF subfamily: ▼ - Group VI, ● - Group VII, ■ - Group VIII, ▲ - Group IX, ◆ - Group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

^aNakano et al. [13], ^bLicausi et al. [15] and ^cWang 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.

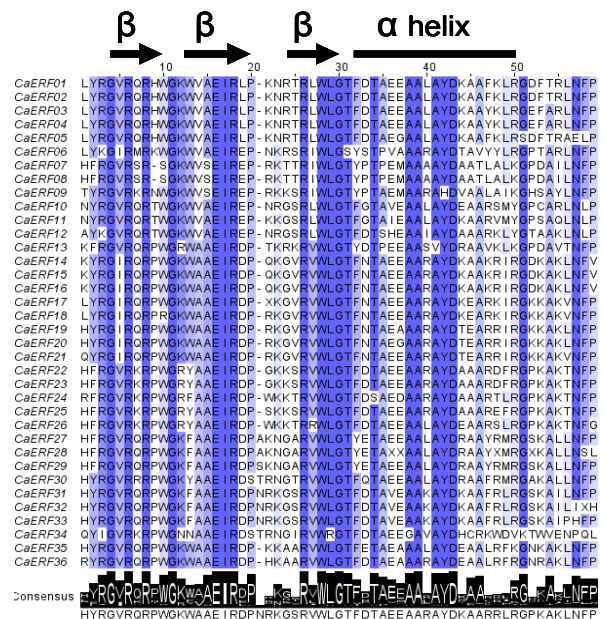


Figure 2 – Multiple sequence alignment of *C. arabica* AP2 domains from the ERF proteins. Identical and conserved amino acid residues are represented by dark and light blue shading, respectively. Black bar and arrows represent the predicted α -helix and β -sheets regions, respectively. *CaERF01-12* belongs to the DREB subfamily; *CaERF13-36* belongs to the ERF subfamily.

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 (*CaERF01-05*) and contain 5 conserved motifs (Figure 3). Except *CaERF05*, the members of this group contain the CMI-1 and CMI-2 motifs in the C-terminal region. The *CaERF06* gene belonging to the Group II, unique member identified in this work, contains the CMII-1 motif in the C-terminal region, adjacent to the AP2 domain. Belonging to the Group III are the *CaERF07-09* genes, which contain the CMIII-1 motif in the C-terminal region. In addition to the CMIII-1 motif, the *CaERF08* 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 *CaERF09* represents the CMIII-6 and the CMIII-7 motifs. In the Group IV (*CaERF10-12*) only the CMIV-2 motif was found in the *CaERF10* and *CaERF11*

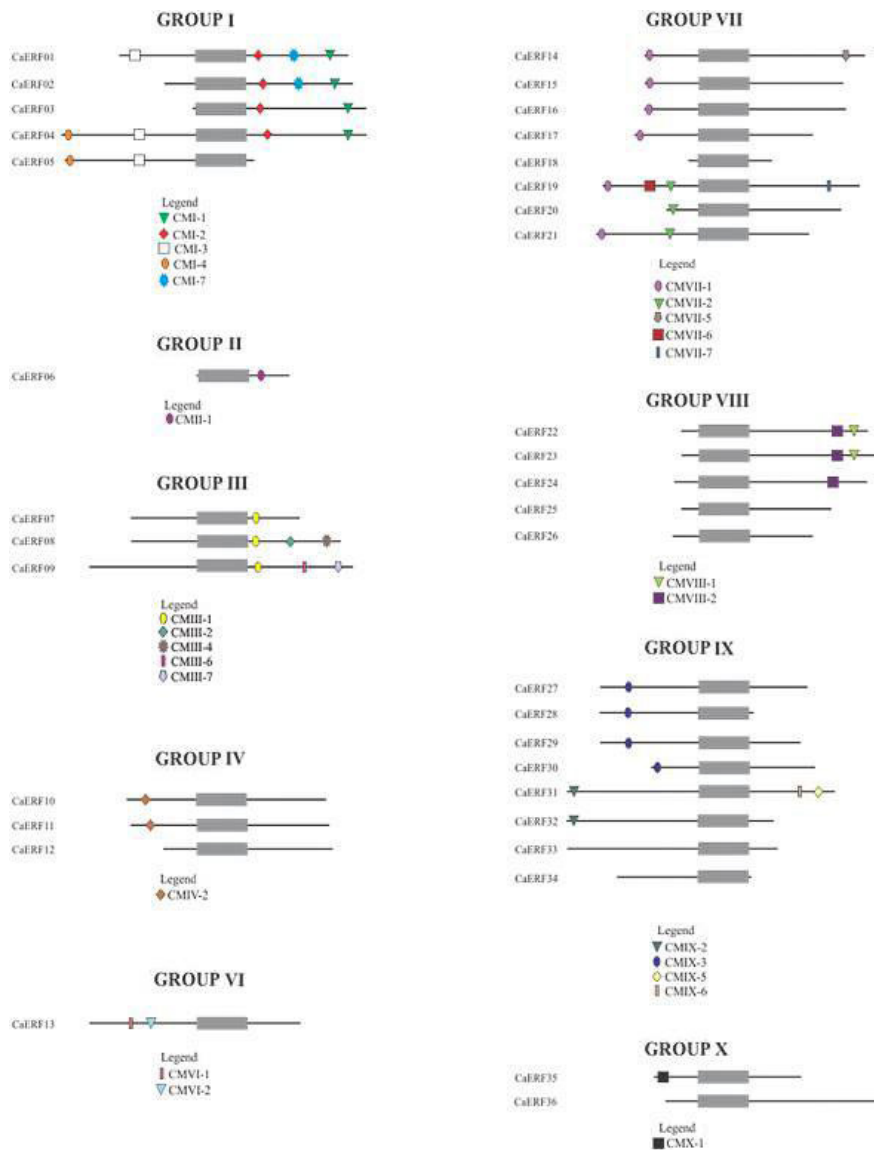


Figure 3. Conserved motifs in the ERF family in *Coffea arabica*. The motifs were identified in *C. arabica* and classified according to the classification proposed by Nakano et al.^[13].

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

The *CaERF13* 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 first described by Tournier et al.^[53] and is characterized by the presence of one highly conserved motif in the N-terminal region (MCGGAIL/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 *CaERF22* and *CaERF23*, which also contain the CMVIII-2 motif. The Group IX is composed by 8 genes (*CaERF27-34*). The *CaERF27-30* genes contain only the CMIX-3 motif while the *CaERF32* 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 *CaERF32* 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 *CaERF35* and *CaERF36* genes. The group X members contain one CMX-1 conserved motif in the N-terminal region, such as the *CaERF35*, whereas the *CaERF36* 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

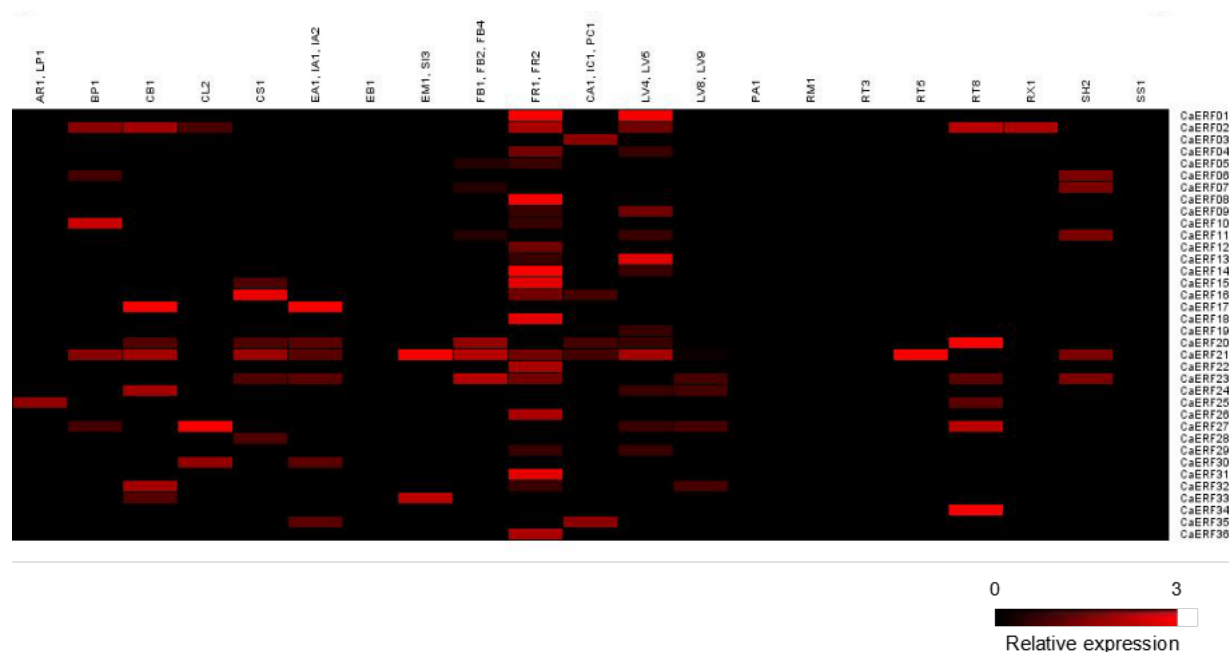


Figure 4. In-silico expression profiling of the ERFs in *Coffea arabica*. The number of reads was normalized in each library and values were represented by the relative expression scale.

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 spe-

cific motifs into proteins belonging to the specific groups of the phylogenetic 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.^[81] 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 *SIERF2*, 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

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 *CaERF17* 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 *CaERF17* was expected to be expressed in fruits. However, reads were not found in fruits libraries for *CaERF17*. 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 *CaERF06*, *CaERF07*, *CaERF11* 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 *CaERF14*, *CaERF15* and *CaERF18* present a high relative expression. This group has been associated with fruit maturation. *LeERF2* in tomato^[53], *MdERF1* 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 *AtERF2* 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 *MtERF1-1* 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.

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