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*Short Communication*

# Isolation and characterization of a *Coffea canephora* ERF-like cDNA

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**ERFs (Ethylene-Responsive Element Binding Factors) are members of a transcription factors family unique to plants. They contain a well-conserved segment, which interacts specifically with sequences containing AGCCGCC motifs (GCC box) located in the promoter region of genes activated during biotic or environmental stress in plants. A cDNA corresponding to an ERF gene has been isolated from a *Coffea canephora* fruit cDNA library. The cDNA was 1,317 nucleotides long and has an open reading frame of 987 bp. The predicted polypeptide showed a great similitude with equivalent proteins from others plant species. The binding domain shows 98.3% identity in amino acids sequence with *Lycopersicon esculentum* ERF. This transcription factor may possibly be involved in differential cell growth or in fruit ripening process. Although it was not possible to isolate it from a leaf cDNA library, its presence in such library was confirmed.**

**Key words:** Ethylene, coffee, *Coffea canephora*, transcription factor, ERF.

## INTRODUCTION

Many plant genes are transcriptionally regulated to respond to pathogen attacks or environmental stresses. Several signaling molecules, such as salicylic acid (SA), ethylene (ET), and jasmonic acid (JA) have been shown to be important components of defense response pathways (Glazebrook, 2001). These regulated pathways require the coordination of highly specific DNA-protein and protein-protein interactions.

A family of five membrane-localized receptors (ETR1, ERS1, ETR2, ERS2 and EIN4) recognizes ethylene, which is synthesized from S-adenosyl-L-Methionine and 1-aminocyclopropane- 1-carboxylic acid (ACC). Other receptors include the CTR1, EIN2, EIN3 and finally the ERFs (Chang and Shockey, 1999; Stepanova and Ecker, 2000; Wang et al*.,* 2002).

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ERF proteins are members of the AP2/ERF family of transcription factor. They have been divided in two subfamilies based on the number of AP2/ERF domains (Riechmann and Meyerowitz, 1998): The AP2 type like APETALA2 (AP2) (Jofuku et al., 1994) and the ERF type (Ohme-Tagaki and Shinski, 1995). The first one has two DNA-binding domains whereas the ERF type contains only one. This DNA-binding domain, highly conserved, interacts specifically with sequences containing AGCCGCC motifs (GCC box) (Hao et al., 1998). ERF proteins are mainly expressed during biotic or abiotic stresses (reviewed in Wang et al., 2002). The ERF1 expression is synergistically activated by ethylene and jasmonate (Lorenzo et al., 2003). The isolation of a cDNA corresponding to a gene encoding an ERF-like transcription factor from *Coffea canephora* is described here.

Degenerated primers (forward 5'–GGGTXCTYRTXCA RTTYGGYGC-3' and reverse 5'– GGCATCCAHARXGC RCAYTC-3') were deduced from conserved regions

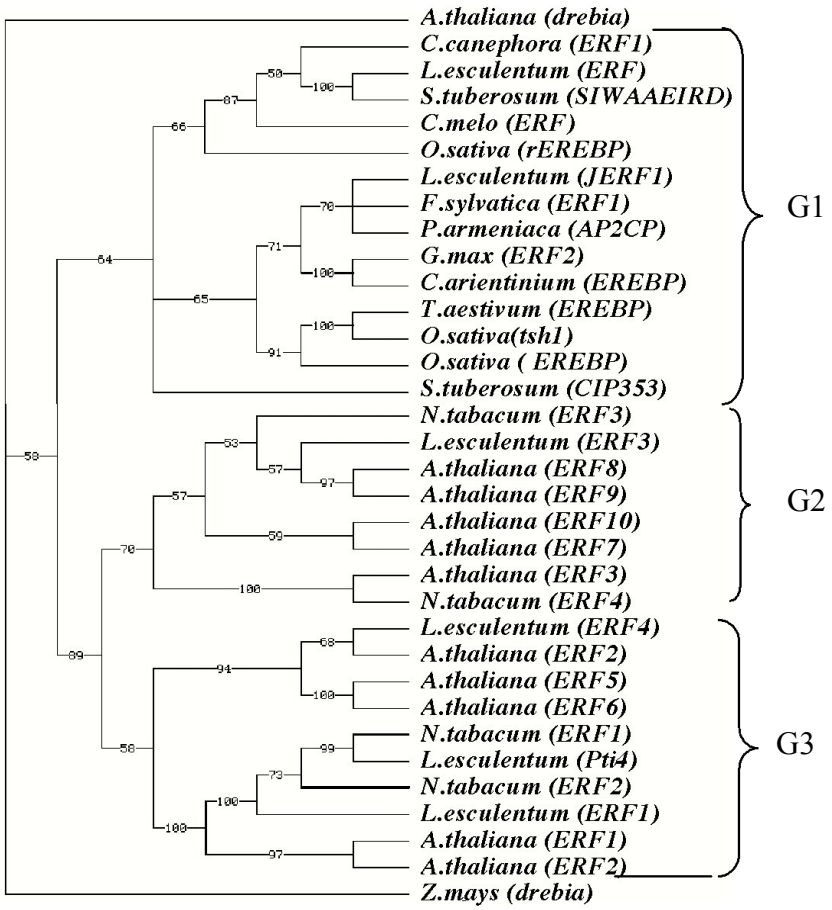


**Figure 1.** Phylogenetic relationship of the *Coffea canephora* ERF to various others plants ERF. The phylogenetic tree was constructed from an optimal alignment of proteins using Clustal W. GeneBank accession number were: *A. thaliana* (DREB1: BAA33791), At ERF1, 2, 3, 4, 5, 6, 7, 8, 9, 10 (O80337, O80338, O80339, O80340, O80341, NP_567529, BAA96653, BAB16084, BAB18560, BAB18561); *N. tabacum*: NtERF1, 2, 3, 4 (BAA07321, BAA07324, BAA07322, BAA07323); *L. esculentum*: LeERF, 1, 3, 4 (AAO34704, AAO34703, AAO34705, AAO34706), JERF1 (AAK95687), Pti4 (AAC50047); *S. tuberosum*: STWAAEIRD (AAC29516), CIP353 (BAC56862); *O. sativa*: EREBP (AAK92632), rEREBP (AAP56251), TSH1 (AAF05606); *C. arientinum* EREBP (CAD56217); *F. silvatica* EREBP1 (CAD21849); *C. melo* CmERF2 (BAD01556); *P. armeniaca* AP2DCP (AAC24587); *G. max* EREBP (AAQ10777); *T. aestivum* TaERF2 (AAP32468). *A thaliana* (dreb1a) and *Z. mays* (dreb) are ERF/AP2 related proteins, but from the DREB subfamily. They are in the cluster for showing the diversity of these family genes.

previously identified in ethylene receptors of different plant species. Using these primers, a segment of 400 bp was amplified from *C. pseudozanguebariae* nuclear DNA. This amplified fragment after random-prime labelling (Prime-a-Gene® Labeling System kit (Promega)) with $\alpha^{32}$P[dCTP], was directly used as a probe for screening a *C. canephora* cDNA library obtained from fruits harvested at different ripening stages (ZAP Express® -cDNA GigapackII gold cloning kit (Stratagene)).

## RESULTS AND DISCUSSION

A full-length CoERF cDNA (Gene Bank Accession No AY522505) was isolated. It is 1,317 nucleotides long and contains an open reading frame of 987 bp. The predicted polypeptide has 329 amino acids (39 Strongly Basic (+), 42 Strongly Acidic (-), 93 Hydrophobic and 99 Polar Amino Acids. It has a molecular mass of 36.34 kDa and an isoelectric point of 6.5. It shows very significant similitude with other equivalent genes previously isolated. The protein has a conserved DNA *G-Binding Domain* or GBD of 59 amino acids. This GBD domain which is 99.39% identical to a *Lycopersicon esculentum* ERF, has conserved secondary structure elements consisting of three -sheet strands, responsible for binding DNA (Allen et al., 1998) and one -helix.

The phylogenetic relationship of *Coffea* ERF to a panel of others plants ERF showed three major groups (Figure 1). G1 group includes the *Coffea* cDNA described here.

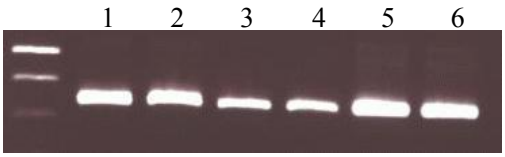


**Figure 2.** PCR with specific primers in *C. canephora* cDNA fruits libraries (1, 2), cDNA leaves library (3, 4) and *C. pseudozanguebariae* genomic DNA (5, 6).

ERF genes are often expressed during biotic or environmental stresses, nevertheless some cDNAs including those from G1 were isolated in cell suspensions (*S. tuberosum*: AAC29516), seeds (*F. sylvatica*: CAD21849) and fruits (*L. esculentum*: AAO34703, *P. armeniaca*: AAC24587 and *C. canephora*) (reported here)). In tomato, LeERF transcripts were most abundant in ripening fruit (Tournier et al., 2003). In rice, the *OsEBP-89* (Sequence CAC83122) was expressed at high levels in the developing endosperm (Yang et al., 2002). Such homologies suggest that the *Coffea* ERF may possibly be involved in differential cell growth or in the ripening process. Moreover, these genes, clustered in G1, are involved in one of the two processes described above, but not in plant defense.

To determine the expression of the *Coffea* ERF, a PCR with specific primers (Forward: GGGGGATCAGGCAGC GACC, and Reverse: CATGAAGGACTCATAGGCCAGC) were prepared. A fragment was amplified in two *C. canephora* libraries (fruits and leaves) and *C. pseudozanguebariae* nuclear DNA. Figure 2 shows that this ERF is possibly more expressed in fruits than in leaves. In addition, the PCR product obtained from *C. pseudozanguebariae* nuclear DNA has the same size, suggesting the lack of introns in this gene segment (bases 312-904). This *Coffea* ERF sequence has a highly conserved N-terminal motif (MCGGAII/L); identical to all genes clustered in the same group.

Transcription of corresponding genes LeERF (AAO34704) and STWAAEIRD (AAC29516) from tomato and potato respectively, were not affected by ethylene (Tournier et al., 2003; Campbell et al., 1998). These two sequences clustered in the G1 group (Figure 1) . Despite its close relationship and phylogenetic link, the possible role of *C. canephora* ERF gene in fruit ripening and/or cell growth needs to be confirmed.

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## REFERENCES

Allen MD, 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: 5484–5496.

Campbell MA, Woodring A, Stidd J (1998). Isolation of a cDNA from potato with structural similarity to the AP2 gene superfamily. Plant Physiol. 117: 1127.

Chang C, Shockey JA (1999). The ethylene-response pathway: signal perception to gene regulation. Curr. Opin. Plant. Biol. 2: 352-358.

Glazebrook J (2001). Genes controlling expression of defense responses in Arabidopsis-2001 status. Curr. Opin. Plant. Biol. 4: 301-308.

Hao D, Ohme-Takagi M, Sarai A (1998). Unique mode of GCC box recognition by the DNA-binding domain of ethylene-responsive element-binding factor (ERF domain) in plant. J. Biol. Chem. 273: 26857-26861.

Jofuku KD, Den Boer BG, Van Montagu M., Okamuro, JK (1994). Control of Arabidopsis flower and seed development by the homeotic gene *APETALA2*. Plant Cell 6: 1211–1225.

Lorenzo O, Piqueras R, Sánchez-Serrano JJ, Solano R (2003). ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. Plant Cell 15: 165–178.

Ohme-Takagi M, Shinshi H (1995). Ethylene-inducible DNA binding proteins that interact with an ethylene-responsive element. Plant Cell 7: 173–182.

Riechmann JL, Meyerowitz EM (1998). The AP2/EREBP family of plant transcription factors. Biol. Chem. 379: 633–646.

Stepanova AN, Ecker JR (2000). Ethylene signalling: From mutants to molecules. Curr. Opin. Plant. Biol. 3: 353–360.

Tournier B, Sanchez-Ballesta MT, Jones B, Pesquet E, Regad F, Latché A, Pech JC, 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 Letters 550: 149-154.

Wang K, Li H, Ecker JR (2002). Ethylene biosynthesis and signaling networks. Plant Cell 14: S131-S151.

Yang HJ, Shen H, Chen L, Xing YY, Wang ZY, Zhang JL, Hong MM (2002). The OsEBP89 gene of rice encodes a putative transcription factor and is temporally expressed in developing endosperm and intercalary meristem. Plant Mol. Biol. 50: 379-391.