# Characterization of three ethylene receptor genes in *Coffea canephora* Pierre

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## 1. Introduction

The phytohormone ethylene plays a central role in physiological and developmental processes, such as germination, growth, flower initiation, senescence of leaves and flowers, organ abscission, and fruit ripening (Abeles *et al.*, 1992). It is also a major signal, mediating responses to a range of biotic and abiotic stresses. At the level of gene expression, ethylene has been shown to induce transcription of a wide range of genes involved in wound signalling and defense against pathogens. A family of five receptors mediates ethylene perception in Arabidopsis: ETR1, ERS1, ETR2, ERS2, and EIN4 (Hua and Meyerowitz, 1998; Sakai *et al.*, 1998). The ETR1 receptor is a homodimer localized in the endoplasmic reticulum membrane (Chen *et al.*, 2002).

Coffee plants are of the climacteric type, thus events during fruit maturation are tightly linked to ethylene perception, but there is little information on the response of coffee fruits to ethylene. Coffee quality depends on the stage of fruit maturation when harvested. Studies on possible relationships between ethylene receptor gene expression and fruit development and maturation should give new insights into a possible role of these receptors on coffee cup quality. Here we present results on the isolation and characterization of three genes encoding ethylene receptors in coffee (*CcETR1*, *CcETR2*, and *CcEIN4*).



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## 2. Results and Discussion

Table 1 gives a general description of three ethylene receptor genes from *Coffea canephora* (CAN). The *CcETR1* gene is present as a unique copy in the CAN genome. Aspects of the gene structure might indicate strong regulation at different levels of expression:

**Table 1.** General characterization of three ethylene receptors genes in *C. canephora*.

| | ***CcETR1* cDNA** | ***CcEIN4* cDNA** | ***CcETR2* cDNA** |
|---|---|---|---|
| **Length** | 2,649 bp<br>2,683 bp<br>3,162 bp | 2,906 bp | 2,985 bp |
| **ORF** | 2,223 bp | 2,298 bp | 2,289 bp |
| **Putative protein** | 740 aa, 82.48 kDa | 765 aa, 85.63 kDa | 762 aa, 85.46 kDa |
| **Identity** | 87.1% to *ETR1* of *Petunia × hybrida* | 74.4% to *LeETR5* of *Solanum lycopersicum*<br>35.3% to *CcETR1* | 71.5% to *LeETR4* of *Solanum lycopersicum*<br>37.7% to *CcETR1*<br>59.7% to *CcEIN4* |
| | **GENOMIC SEQUENCE** | | |
| **Intron in coding region** | 5 | 1 | 1 |
| **Intron size** | 1,240; 125; 95; 163; 1,148 bp. | 2,045 bp | 652 bp |
| **Upstream Open Reading Frame (uORFs)** | Yes, 35 aa | Not | Not |
| **Intron in 5' UTR** | Yes, 978 bp | Not | Not |

Three independently isolated full-length cDNA clones had the same coding sequence and an identical 5'UTR, but differed in 3'UTR length, suggesting that these three clones only differed by alternative polyadenylation sites. Indeed, some AATAAA-like motifs were found upstream of the poly (A) tail. The three RNA forms may have a different turnover depending on the length of their 3'UTR (Meyer *et al.*, 2004).

The 5'UTR comprised a short putative open reading frame (uORF). This uORF is interrupted by one intron, which is conserved only in some *Coffea* species. In others, under the same conditions it was not possible to identify the intron by PCR amplification, indicating the absence of that intron or a strong divergence in the sequences corresponding to the primers.

RT-PCR analysis made from RNA isolated at different fruit development stages in CAN and *Coffea pseudozanguebariae* (PSE), a wild East African *Coffea* species, showed that the *ETR1* primary transcript had

different alternative splice sites that take various forms (Fig. 1). They are related with retained introns at the transcript terminus or internal sections of the transcript. They include: exon skipping, unspliced introns, alternative 5′ splice donor site, and alternative 3′ splice acceptor site. In addition to the 3′ UTR, two polyadenylation sites that might be responsible for incomplete splicing at the 3′ terminus are present within the fifth intron. In some cases, these intron retentions and alternative splicing may lead to truncated proteins that interfere with the most abundant functional receptor. In other cases, the resulting protein deduced from the aberrant RNAs might be nonfunctional, but the aberrant RNAs themselves could intervene in a negative regulation of gene expression, post-transcriptional gene silencing or nonsense-mediated decay (Isshiki *et al.*, 2001).

Overexpression of *CcETR1* or *CcEIN4* in etiolated transgenic Arabidopsis plants grown on a medium without ethylene precursor (ACC) or inhibitor of ethylene synthesis (AVG) yielded a loss of gravitropic regulation of hypocotyl growth (Fig. 2), indicating a possible interference between the introduced ethylene receptors and auxin distribution (Philosoph-Hadas *et al.*, 1996).

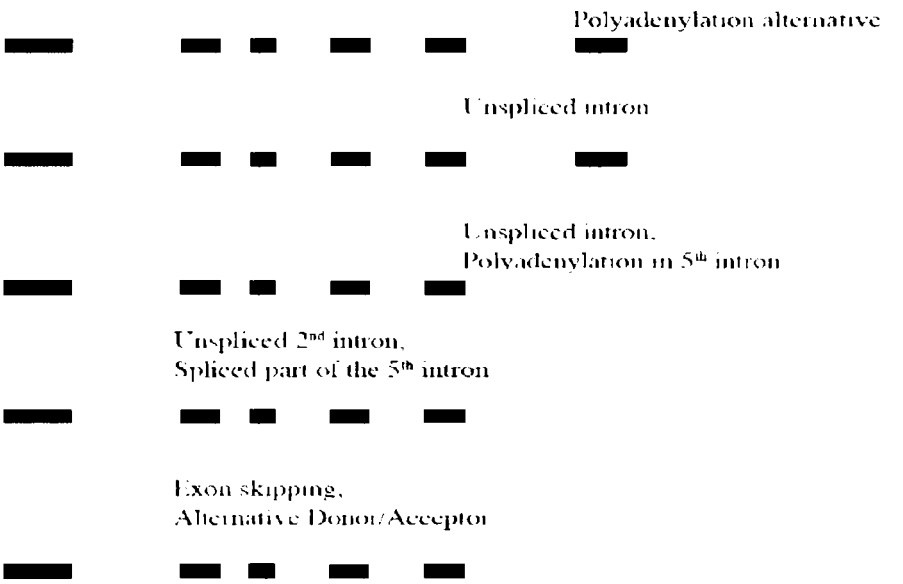


**Fig. 1.** Mature transcript types and alternative splicing observed in *Coffea pseudozanguebariae*.

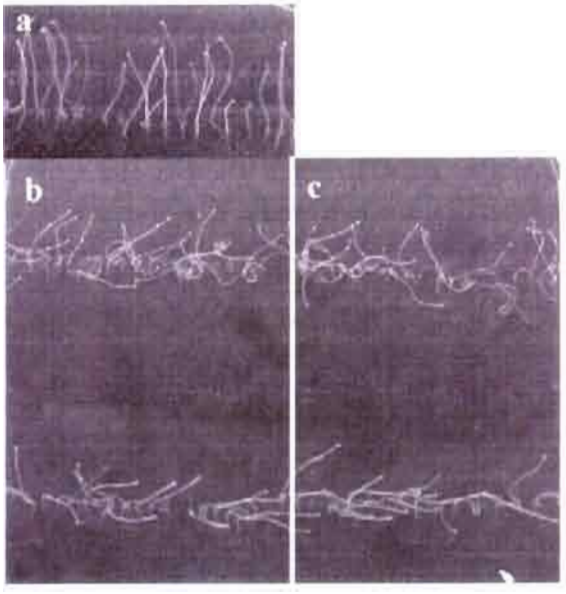


**Fig. 2.** (Color figure in the Annex, p.455) Effect of *CcETR1* or *CcEIN4* over-expression in *Arabidopsis* wild-type (Col-0) dark-grown seedlings, untransformed Col-0 **(a)** and two lines of transformed Col-0 with *CcETR1* **(b)** and *CcEIN4* **(c)**.

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