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Isolation and expression analysis of ethylene receptor genes from melon (*Cucumis melo*) fruit

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Abstract: Ethylene receptors are involved in ethylene sensing and signal transduction. This study was conducted to isolate genes encoding ethylene receptors from melon fruit and to determine their expression during postharvest fruit ripening. The amplification of melon fruit cDNA with degenerate primers revealed two partial cDNAs. Upon 3' and 5' RACE analysis of the cDNAs, two full length genes designated *Cm-ETR1* and *Cm-ETR2* were obtained. Cloning and sequencing of the genes showed that *Cm-ETR1* and *Cm-ETR2* genes were 2320 and 2537 bp in length, respectively. *Cm-ETR1* had a predicted open reading frame (ORF) of 2223 nucleotides encoding a protein of 740 amino acids. *Cm-ETR2* contained a predicted ORF of 2301 nucleotides encoding a protein of 766 amino acids. Structure and sequence analyses demonstrated that both genes had conserved domains found in ethylene receptor genes from other species, and were highly similar in terms of both nucleotide and amino acid sequences to the ethylene receptor sequences isolated from other species. The expression analyses of the genes revealed that the transcript levels of both receptor genes significantly increased during postharvest storage. The isolated receptor genes could be used to manipulate ethylene effects to delay the development of ethylene related physiological disorders and enhance the shelf life of melon fruit.

Key words: Ethylene, gene cloning, melon, receptor

1. Introduction

The plant hormone ethylene is sensed by receptors (Klee and Tieman, 2002). The expression of ethylene-induced genes is induced through the transduction of the ethylene signal from receptors to the corresponding transcription factors (Giovannoni, 2004). Ethylene signaling is a multistep pathway in which ethylene receptors function at the first step and play a crucial role in the regulation of ethylene responses (Giovannoni, 2004).

Ethylene receptors have been isolated from different plant species such as melon (Sato-Nara et al., 1999; Takahashi et al., 2002), tomato (Tieman and Klee, 1999), peach (Bassett et al., 2002), and pear (El-Sharkawy et al., 2003). The receptor proteins share a similar structural organization consisting of an N-terminal hydrophobic domain that contains three or four transmembrane segments and the ethylene binding sites, a GAF domain, and a histidine kinase domain. Moreover, the ETR-type receptors also have a receiver domain at the C-terminus involved in transmitting the ethylene signal.

Melon is a fruit showing climacteric ripening behavior. In climacteric fruit species, ethylene is effective in many

physiological events during postharvest ripening. It directly affects factors influencing the shelf life of a fruit including softening, wooliness, watersoaking, and related deteriorations (Karakurt and Huber, 2002; Karakurt and Huber, 2004; Mao et al., 2004; Lima et al., 2005). Ethylene influences fruit ripening through involvement in chlorophyll degradation, increasing respiration rate, carotenoid synthesis, autocatalytic ethylene production, and the expression of genes that leads to the enhancement in the activities of enzymes responsible for starch to sugar conversion and cell wall degradation (Theologis, 1993; Wilkinson et al., 1997). This has been demonstrated through the use of ethylene antagonists and the inhibitors of ethylene synthesis, which have significantly enhanced the shelf life of fruit (Ergun et al., 2005). Thus, the manipulation of receptor genes could be a good alternative to control ethylene synthesis and response, delay ripening, and prevent ethylene related physiological disorders in fruits.

This study was performed to isolate and characterize ethylene receptor genes from melon fruit and determine their expression during postharvest ripening.

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2. Materials and methods

2.1. Plant material and RNA isolation

The melons (*Cucumis melo* L. cv ananas) used in the study were purchased from a local grower (Isparta, Turkey). Fruits were harvested at the commercial ripening stage (Ergun et al., 2005) and immediately transported to the laboratory at the College of Agriculture, Süleyman Demirel University, Isparta, Turkey. The fruits were washed, surface sterilized with 200 µL/L sodium hypochlorite solution, and stored at 20 °C for 10 days. Samples of fruit were taken from storage after 0, 1, 2, 4, 6, 8, and 10 days, frozen in liquid nitrogen, and used for Northern blot analysis (Karakurt and Huber, 2004). Fifteen melons were treated with 50 µL/L ethylene at 20 °C for 12 h to induce the synthesis of ethylene receptor genes. Samples were then taken from the placental tissue and used for RNA extraction. Total RNA was isolated as previously described (Strommer et al., 1993) with modifications (Karakurt and Huber, 2004). The concentration and quality of RNA were determined with a spectrophotometer (PG instruments) and agarose gel electrophoresis (Karakurt and Huber, 2004).

2.2. cDNA synthesis

The total RNA was treated with DNase in order to eliminate any residual DNA contamination using a commercial kit (Ambion). Then the DNase-treated RNA samples were converted to cDNA with an 'Advantage RT-PCR' kit (Clontech).

2.3. Isolation of receptor gene fragments

Degenerate primers (24–27 bp) were designed with the primer BLAST program using ethylene receptor gene sequences of *Arabidopsis thaliana*, *Cucumis melo*, *Solanum esculentum*, and *Citrullus vulgaris* (Accession numbers: AF1379, AF16250, U21952, U38666, U63291, L24119, U41103) and were synthesized (Metis Biyoteknoloji, Ankara, Turkey). Primers used in PCR reactions to isolate ethylene receptor genes were given in the supplemental file (Supplemental 1; on the journal's website).

PCR amplifications were performed with the degenerate primers and melon cDNA (El-Sharkawy et al., 2003). PCR samples consisted of 5 µL of cDNA, 2 µL of degenerate primers, 5 µL of 10X PCR buffer, 1 µL of dNTPmix (10 µM), 1 µL of Taq polymerase, and 34 µL of distilled water. PCR conditions were: denaturation at 95 °C for 1 min 30 s, annealing at 58 °C for 2 min, and extension at 72 °C for 1 min for 5 cycles; denaturation at 95 °C for 30 s, annealing at 58 °C for 1 min, and extension at 72 °C for 30 s for 15 cycles; denaturation at 95 °C for 30 s, annealing at 58 °C for 1 min, and extension at 72 °C for 2 min for 20 cycles; and then final extension at 72 °C for 7 min.

PCR amplification products were run on 1.2% agarose gel and two amplification products with molecular sizes of 1250 and 950 bp were obtained. These cDNA fragments

were cut from the gel with a sterile razor blade and extracted from the gel using a NucleoTrap gel extraction kit (Clontech). The cDNAs were then cloned with a pGEMT-Easy cloning system (Promega). The recombinant colonies were grown overnight at 37 °C and their plasmids were isolated with a Wizard Plus SV Minipreps DNA Purification System (Promega) and sequenced (İontek, İstanbul, Turkey). The sequences were compared to those deposited in the NCBI GenBank with the BLAST program, and were confirmed to be ethylene receptor sequences (Altschul et al., 1997). Gene-specific primers were designed from these sequences and synthesized (Metis Biyoteknoloji). These primers were used in RACE analysis in order to obtain the full length sequences of the genes. RACE analysis was conducted using a SMART RACE cDNA amplification kit following the manufacturer's instructions (Clontech). Finally, complete full-length cDNAs were obtained using gene-specific primers prepared from both ends of the corresponding cDNA sequences determined above.

Multiple sequence alignment was performed using the Clustal W program and the phylogenetic tree was constructed using the neighbor-joining (NJ) method. The programs ExPasy (<http://www.expasy.org/tools>), PSORT (<http://psort.hgc.jp/form.html>), and SignalP (<http://www.cbs.dtu.dk/services/SignalP>) were used for the prediction of isoelectric point (pI), molecular weight, and signal peptides of the deduced proteins. The Conserved Domain Search (www.ncbi.nlm.nih.gov) program was used to determine the conserved motifs.

2.4. The expression analysis of receptor genes

Twenty micrograms of total RNA extracted as described above was used in Northern blot analysis to determine the expression of receptor genes during ripening at 20 °C (Karakurt and Huber, 2004). The receptor gene probes were labeled with DIG using a DIG High Prime labeling kit following the manufacturer's instructions (Roche). The results were analyzed with a gel imaging system (Kodak) and converted to numerical values. The expression levels of each transcript in the control fruit (day 0) were set to an arbitrary value of 1.0, to which all other time points were normalized to obtain an induction value (fold induction) (Karakurt and Huber, 2004). Quantitative gene expression data were analyzed according to a completely randomized design with three replications using the GLM program of the Statistical Analysis System (SAS, 1997).

3. Results

PCR of melon cDNA with degenerate primers resulted in the amplification of two cDNA fragments 1250 and 950 bp in size. The amplified fragments were cloned into pGEM-T easy cloning vectors and sequenced. The nucleotide and amino acid sequences of the two cDNA fragments demonstrated significant homologies to those

of ethylene receptor sequences isolated from other species. Primers were designed from these fragments and used in RACE analysis to obtain their full length sequences. The fragments amplified with 3' and 5' RACE analysis were cloned and sequenced. After their confirmation to be receptor sequences by sequence comparisons with BLAST, primers were designed from both ends of the sequences and used in a PCR to amplify the full length gene sequences. Then the full length sequences of these two genes were again cloned and sequenced. After the sequence analysis of the genes, they were designated *Cm-ETR1* and *Cm-ETR2*. The full length nucleotide sequences of these genes were submitted to the NCBI GenBank and given the accession numbers KJ150694 and KJ150695.

The sequence analysis showed that *Cm-ETR1* and *Cm-ETR2* consisted of 2320 and 2537 bp, respectively (Table 1). *Cm-ETR1* contained an open reading frame (ORF) of 2223 nucleotides encoding a predicted protein of 740 amino acids (Table 1). The gene also had 5' and 3' noncoding and a poly (A) tail consisting of 40, 49, and 8 nucleotides, respectively (Table 1). The gene called *Cm-ETR2* was composed of an ORF of 2301 nucleotides encoding a protein of 766 amino acids (Table 1). The 5'

and 3' noncoding and poly (A) tail of the gene contained 18, 209, and 9 nucleotides, respectively (Table 1). At the nucleotide level, *Cm-ETR1* demonstrated 99%, 98%, and 97% homology to *Cucumis melo* var. *Cantalupensis Cm-ETR1*, *Cucumis melo* var. *reticulatus Cm-ETR1*, and *Cucumis sativus Cs-ETR1* genes, respectively (Table 2). The gene also showed 99%, 98%, and 86% homology to *Cucumis melo* var. *cantalupensis*, *Cucumis sativus*, and *Prunus persica* ETR1 amino acid sequences in terms of predicted ORF amino acid sequence, respectively (Table 3). Nucleotide-based homology search results showed that the *Cm-ETR2* gene showed 99%, 98%, and 98% homology at the nucleotide level to the ETR2 nucleotide sequences of *Cucumis melo* var. *cantalupensis CM-ETR2*, *Citrullus lanatus Cl-ETR2*, and *Cucumis sativus CS-ETR2* (Table 2). Likewise, the predicted amino acid sequence of CL-ETR2 demonstrated 99%, 99%, and 98% similarity to the ETR2-like protein sequences of *Cucumis melo* var. *cantalupensis*, *Cucumis sativus*, and *Citrullus lanatus* (Table 3).

Multiple alignments of full-length predicted Cm-ETR proteins with other reported ETR sequences confirmed the presence of conserved motifs and structural similarities that are commonly associated with ethylene

Table 1. The nucleotide and amino acid compositions of the ethylene receptor genes isolated from melon fruit.

Gene	Nucleotide (bp)	Amino acid	5' UTR (bp)	Coding region (bp)	3' UTR (bp)	Poli (A) tail (bp)
<i>Cm-ETR1</i>	2320	740	40	2223	49	8
<i>Cm-ETR2</i>	2537	766	18	2301	209	9

Table 2. Nucleotide-based homology search results of melon ETR genes.

Gene	Species	Homology (%)	E value
<i>Cm-ETR1</i>			
<i>Cucumis melo</i> var. <i>cantalupensis Cm-ETR1</i>	<i>Cucumis melo</i>	99	0.0
<i>Cucumis melo</i> var. <i>reticulatus Cm-ETR1</i>	<i>Cucumis melo</i>	98	0.0
<i>Cucumis sativus Cs-ETR1</i>	<i>Cucumis sativus</i>	97	0.0
<i>Coffea canephora Cc-ETR1</i>	<i>Coffea canephora</i>	82	0.0
<i>Prunus persica Pp-ETR1</i>	<i>Prunus persica</i>	80	0.0
<i>Cm-ETR2</i>			
<i>Cucumis melo</i> var. <i>Cantalupensis Cm-ETR2</i>	<i>Cucumis melo</i>	99	0.0
<i>Citrullus lanatus Cl-ETR2</i>	<i>Citrullus lanatus</i>	98	0.0
<i>Cucumis sativus Cs-ETR2</i>	<i>Cucumis sativus</i>	98	0.0
<i>Pyrus pyrifolia Pp-ETR2</i>	<i>Pyrus pyrifolia</i>	78	0.0
<i>Pyrus comminus</i> putative ethylene receptor	<i>Pyrus comminus</i>	77	0.0

Table 3. Homology search results of melon receptor proteins.

Protein	Species	Homology (%)	E value
<i>Cm-ETR1</i>			
<i>Cucumis melo</i> var. <i>cantalupensis</i> Cm-ETR1	<i>Cucumis melo</i>	99	0.0
<i>Cucumis sativus</i> Cs-ETR1	<i>Cucumis sativus</i>	98	0.0
<i>Prunus persica</i> Pp-ETR1	<i>Prunus persica</i>	86	0.0
<i>Arabidopsis thaliana</i> At-ETR1	<i>Arabidopsis thaliana</i>	82	0.0
<i>Brassica oleracea</i> Bo-ETR1	<i>Brassica oleracea</i>	81	0.0
<i>Cm-ETR2</i>			
<i>Cucumis melo</i> var. <i>Cantalupensis</i> Cm-ETR2	<i>Cucumis melo</i>	99	0.0
<i>Cucumis sativus</i> Cs-ETR2	<i>Cucumis sativus</i>	99	0.0
<i>Citrullus lanatus</i> Cl-ETR2	<i>Citrullus lanatus</i>	98	0.0
<i>Prunus persica</i> Pp-ETR2	<i>Prunus persica</i>	79	0.0
<i>Pyrus comminus</i> putative ethylene receptor	<i>Pyrus comminus</i>	78	0.0

receptors (Supplementals 2 and 3; on the journal's website; Figures 1 and 2). *Cm-ETR1* predicted protein contained GAF, histidine kinase, HATPase C superfamily (histidine kinase-like ATPases), and signal receiving domains (Figure 1). Moreover, the gene had in its histidine kinase domain an ATP binding site, a Mg binding site, and a

phosphorylation site; and in its signal receiving domain phosphorylation, dimerization, and intermolecular recognition sites. Transmembrane domain analysis of the gene showed that it contained three transmembrane helices (Figure 1). Additionally, the amino end of the protein had highly conserved amino acid sequences. The protein

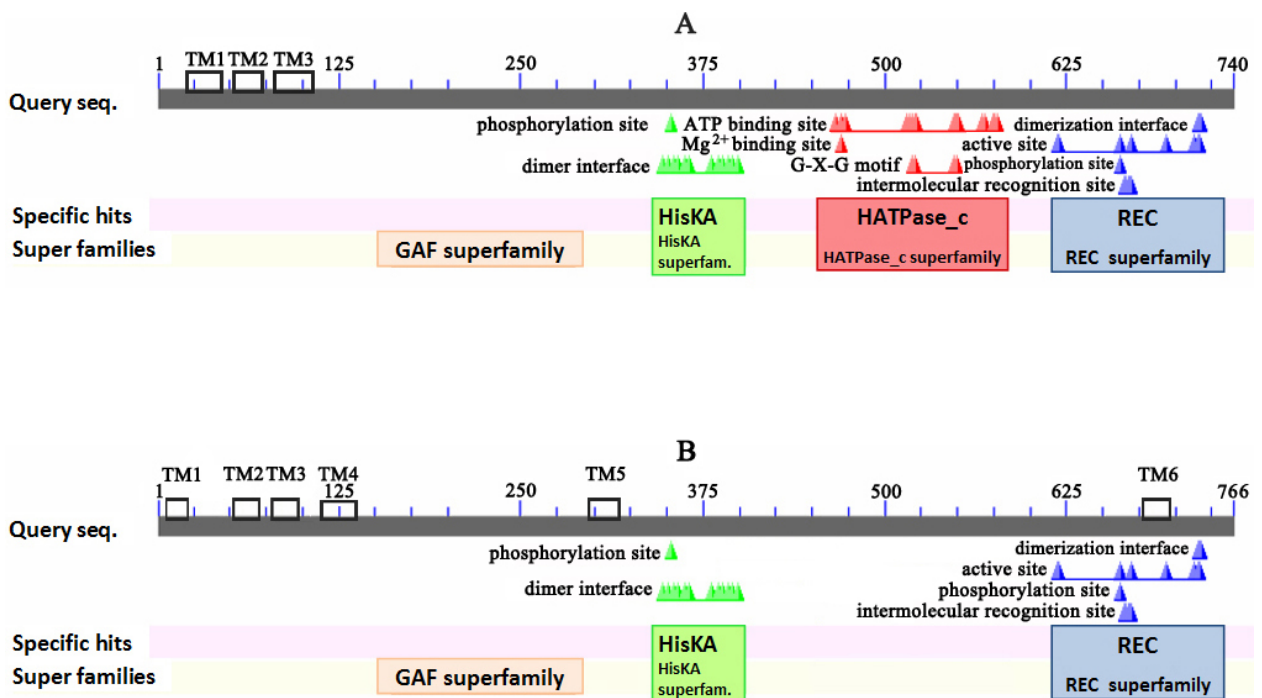


Figure 1. Domain organizations of *Cm-ETR1* (A) and *Cm-ETR2* (B) receptor proteins. TM: Transmembrane domain

encoded by the *Cm-ETR2* gene had a GAF domain, a histidine kinase domain, and a receiver domain. While the histidine kinase domain possessed a phosphorylation site, the receiver domain had phosphorylation, dimerization, and intermolecular recognition sites (Figure 1). Moreover, there were six transmembrane helices in *CL-ETR2* (Figure 1). It is noted that Mg and ATP binding sites and the HTPase-C-like domain found in ETR1-like genes were not available in the *CL-ETR2* gene. Therefore, the isolated genes structurally resemble their *Arabidopsis* homologs. Moreover, cluster analysis demonstrated that *Cucumis melo* Cm-ETR1 and *Cucumis sativus* ETR1 proteins showed more similarity to each other and were in the same group, while *Cucumis melo* ETR2 protein showed high homology to *Cucumis sativus* ETR2 proteins (Figure 2).

PSORT and SignalP analyses showed that Cm-ETR1 did not contain a signal peptide and were localized in plasma membrane, but Cm-ETR2 contained a signal sequence in its amino terminus and were localized with its carboxyl end outside and amino end inside the cytosol. Cm-ETR1 predicted hydrophobic protein had a molecular weight of approximately 83 kDa and a pI of 7.64, and contained 80 negatively and 81 positively charged amino acids. On the other hand, Cm-ETR2 predicted hydrophobic protein had a molecular weight of approximately 85 kDa and a pI of 7.86, and possessed 79 negatively and 81 positively charged amino acids.

3.1. The expression of receptor genes

To determine the expression levels of receptor genes during postharvest storage, melons were stored at 20 °C for 10 days, and fruit samples were taken at different stages of the storage and used for Northern blot analysis. The results showed a significant variation among various storage periods in the accumulation of transcripts (Figure 3; Supplemental 4; on the journal's website). The mRNA levels of both receptor genes started to increase from the

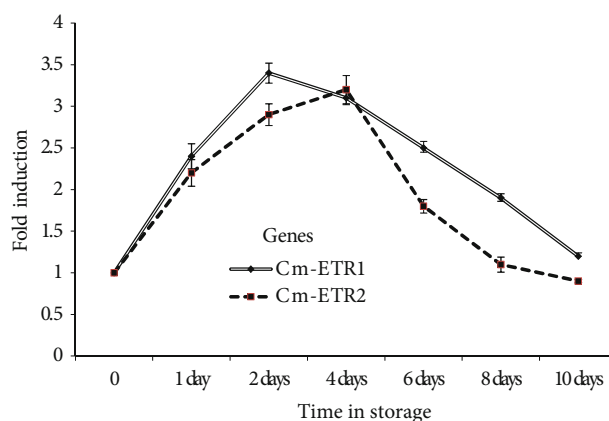


Figure 3. The changes (fold induction as compared to the control) in the mRNA transcript levels of two isolated ethylene receptor genes during storage at 20 °C. Data show the average of three replications and standard deviation.

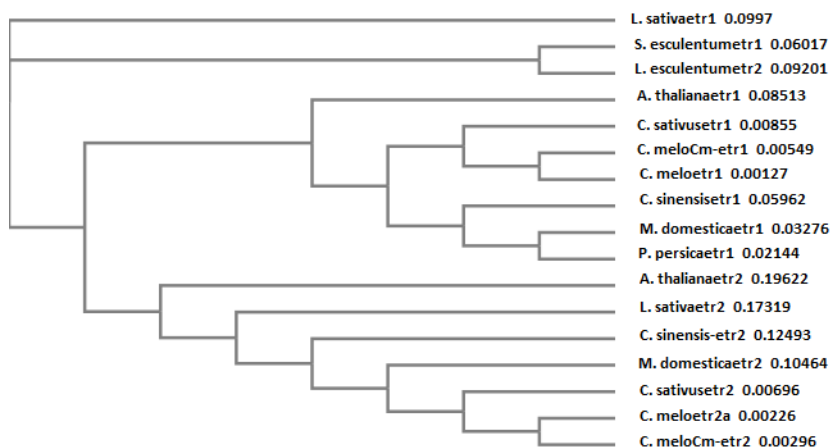


Figure 2. Phylogenetic tree of ETR1-like and ETR2-like ethylene receptors isolated from different plant species. The amino acid sequences were compared using the CLUSTAL Omega multiple alignment program, and the phylogenetic tree was generated using the neighbor-joining (NJ) method. The lengths of the branches are indicated on the right. Accession numbers in GenBank sequence databases are as follows: *AtETR1* (NM_105305) and *AtETR2* (NM_113216) in *Arabidopsis thaliana*; *Ls-ETR1* (AF350321) and *Ls-ETR2* (AF350322) in *Lactuca sativa*; *CsETR1* (NM_001280633) and *CsETR2* (AB026500) in *Cucumis sativus*; *CMeETR1* (AF054806), *CmETR1* (KJ150694), *CMeETR2a* (AB294513), and *CmETR2* (KJ150695) in *Cucumis melo*; *LeETR1* (AY600436) and *SeETR1* (AF043084) in *Lycopersicon esculentum*; *CsETR1* (GQ339592) and *CsETR2* (GQ339593) in *Citrus sinensis*; *MdETR1* (AF032448) and *MdETR2* (DQ847145) in *Malus domestica*; and *PpETR1* (AF124527) in *Prunus persica*.

early hours of storage and these increases continued up to the second day of storage in *Cm-ETR1* and up to day four of storage in *Cm-ETR2*. In general, the increase in *Cm-ETR1* transcripts was much higher than that of *Cm-ETR2*. For example, on the second day of storage, the transcript level of *Cm-ETR1* increased 3.4-fold as compared to the control (day 0). However, the increase in the mRNA level of *Cm-ETR2* was 2.9-fold and on the fourth day of storage 3.2-fold. The mRNA levels of *Cm-ETR1* and *Cm-ETR2* started to decrease on the second and fourth days of storage, respectively.

4. Discussion

Ethylene, a phytohormone, coordinates the plant's physiological responses to physiological events such as senescence, fruit ripening, and abscission, and environmental stresses including water deficit, mechanical wounding, and pathogen infection (Lelievre et al., 1997). Ethylene is received by proteins called ethylene receptors. Ethylene receptors form the first step of the ethylene signal response pathway and are the negative regulators of the pathway (Kieber et al., 1993). This study was conducted to isolate and characterize ethylene receptor genes from melon fruit, and determine their expression during postharvest ripening. At the end of the study, the full length nucleotide sequences of two ethylene receptor genes designated as *Cm-ETR1* and *Cm-ETR2* were isolated and characterized from melon fruit. The isolated genes showed significant homologies to the ethylene receptor genes previously isolated from various plant species such as watermelon, peach, tomato, and *Arabidopsis*. The genes showed 70%–99% homology both at the nucleotide and amino acid levels to the receptor genes isolated from *Cucumis melo*, *Cucumis sativus*, *Pyrus communis*, *Prunus persica*, *Prunus salicina*, *Prunus domestica*, *Zizyphus jujuba*, *Lycopersicon esculentum*, and *Arabidopsis thaliana* (Yamasaki et al., 2000; Wang et al., 2006; El-Sharkawy et al., 2007).

The two genes possessed an ethylene binding domain that uses Cu as cofactor and required for high affinity ethylene binding (Rodriguez et al., 1999) a conserved histidine kinase domain and a GAF domain (Wang et al., 2006). Only *Cm-ETR1* had the ethylene response regulator domain. A similar finding was reported from ETR1 genes previously characterized from species such as watermelon, cucumber, pear, peach, and plum (Guo and Ecker, 2004; El-Sharkawy et al., 2007). Moreover, three membrane binding sites reported for ETR1 genes isolated from other species were determined in the *Cm-ETR1* gene of melon. However, unlike ETR2 of *Arabidopsis*, which had four membrane binding sites, six potential membrane-

binding sites were observed in melon *Cm-ETR2*. *Cm-ETR1* and *Cm-ETR2* genes also had a conserved aspartate amino acid found in the autophosphorylated receiver domain of bacterial two component receptors and a GAF domain. The aspartic amino acid in the receiver domain of the protein functions as the phosphorus binding site to conserved histidine in the ETR1 receiver sequences (Gamble et al., 1998). The sequence analysis of predicted proteins of the isolated genes (*Cm-ETR1* and *Cm-ETR2*) showed that they possessed all amino acids required for receptor function, suggesting their participation in ethylene response and signal transduction (Rodriguez et al., 1999; Wang et al., 2006). Moreover, the comparison of the sequences of isolated genes with those of ETR1 and ETR2-like sequences showed that the amino acids at the N-terminus were conserved.

The isolated genes also shared similar expression trends during postharvest storage. The mRNA transcript levels of both genes showed significant increases during ripening in storage, possibly resulting from the increase in ethylene production of melon fruit. Although we did not measure the ethylene production of melon fruit during storage, increases in ethylene production during ripening have been reported for different melon cultivars (Ergun et al., 2005; Pech et al., 2008; Li et al., 2011). Moreover, it has been reported that there was a correlation between the increase in ethylene production and the increase in the transcript levels of *ETR1* and *ETR2* genes in cucumber and melon (Sato-Nara et al., 1999; Yamasaki et al., 2000; Cin et al., 2006). The increase in mRNA levels of ethylene receptors in ripening melon fruit is a natural response against increased ethylene biosynthesis to maintain the homeostatic balance and a mechanism to regulate ethylene responses (Klee et al., 2003). The expressions of ethylene receptors associate with the function and thus the capacity of the plant tissue to respond to ethylene. The fruit of the *cucurbitaceae* family such as melon and watermelon also develop watersoaking, a physiological disorder upon ethylene exposure (Karakurt and Huber, 2002; Ergun et al., 2005). The application of ethylene to melon fruit cv ananas resulted in the development of the watersoaking disorder after 9 days of storage. The ethylene treated fruit also showed 5- and 6-fold increases in mRNA transcript levels of *Cm-ETR1* and *Cm-ETR2*, respectively, after 24 h of storage (data not shown), suggesting that ethylene receptors contribute significantly to the development of the disorder. Although the functions of *Cm-ETR1* and *Cm-ETR2* genes have not been determined, their homology to the ethylene receptor genes from other species suggests that they serve similar functions. Further studies are required in order to fully determine the roles of isolated genes in fruit ripening and the development of physiological disorders.

References

- Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25: 3389–3402.
- Bassett CL, Artlip TS, Callahan AM (2002). Characterization of the peach homologue of the ethylene receptor, PpETR1, reveals some unusual features regarding transcript processing. *Planta* 215: 679–688.
- Cin VD, Rizzini FM, Botton A, Tonutti P (2006). The ethylene biosynthetic and signal transduction pathways are differentially affected by 1-MCP in apple and peach fruit. *Postharvest Biol Technol* 42: 125–133.
- El-Sharkawy I, Jones B, Li ZG, Lelièvre JM, Pech JC, Latché A (2003). Isolation and characterization of four ethylene perception elements and their expression during ripening in pears (*Pyrus communis* L.) with/without cold requirement. *J Exp Bot* 54: 1615–1625.
- El-Sharkawy I, Kim WS, El-Kereamy A, Jayasankar S, Svircev AM, Brown DCW (2007). Isolation and characterization of four ethylene signal transduction elements in plums (*Prunus salicina* L.). *J Exp Bot* 58: 3631–3643.
- Ergun M, Sargent SA, Fox AJ, Crane JH, Huber DJ (2005). Ripening and quality responses of mamey sapote fruit to postharvest wax and 1-methylcyclopropene treatments. *Postharvest Biol Tech* 36: 127–134.
- Gamble RL, Coonfield ML, Schaller GE (1998). Histidine kinase activity of the ETR1 ethylene receptor from *Arabidopsis*. *P Nat Acad Sci USA* 95: 7825–7829.
- Giovannoni JJ (2004). Genetic regulation of fruit development and ripening. *Plant Cell* 16: 170–180.
- Guo H, Ecker JR (2004). The ethylene signaling pathway: new insights. *Cur Op Plant Biol* 7: 40–49.
- Karakurt Y, Huber DJ (2002). Cell wall-degrading enzymes and pectin solubility and depolymerization in immature and ripe watermelon (*Citrullus lanatus*) fruit in response to exogenous ethylene. *Physiol Plantarum* 116: 398–405.
- Karakurt Y, Huber DJ (2004). Ethylene-induced changes in gene expression, enzyme activities and water-soaking in immature and ripe watermelon (*Citrullus lanatus*) fruit. *J Plant Physiol* 16: 381–388.
- Kieber JJ, Rothenburg M, Roman G, Feldmann KA, Ecker JR (1993). CTR1, a negative regulator of the ethylene response pathway in *Arabidopsis*, encodes a member of the Raf family of protein kinases. *Cell* 72: 427–441.
- Klee H, Tieman D (2002). The tomato ethylene receptor gene family: form and function. *Physiol Plantarum* 115: 336–341.
- Klee H, Tieman D, Ciardi J, Taylor M (2003). Regulation of ethylene responses by control of receptor levels. In: Vendrell M, Klee H, Pech JC, Romojaro F (Eds.), *Biology and Biotechnology of the Plant Hormone Ethylene III*. Amsterdam, Netherlands: IOS Press, pp. 193–197.
- Lelièvre JM, Latché A, Jones B, Bouzayen M, Pech JC (1997). Ethylene and fruit ripening. *Physiol Plantarum* 101: 727–739.
- Li XW, Jin P, Wang J, Zhu X, Yang HY, Zheng YH (2011). 1-Methylcyclopropene delays postharvest ripening and reduces decay in Hami melon. *J Food Qual* 34: 119–125.
- Lima LCO, Hurr BM, Huber DJ (2005). Deterioration of beet alpha and slicing cucumbers (*Cucumis sativus* L.) during storage in ethylene or air: responses to suppression of ethylene perception and parallels to natural senescence. *Postharvest Biol Technol* 37: 265–276.
- Mao LC, Karakurt Y, Huber DJ (2004). Incidence of water-soaking and phospholipid catabolism in ripe watermelon (*Citrullus lanatus*) fruit: induction by ethylene and prophylactic effects of 1-methylcyclopropene. *Postharvest Biol Technol* 33: 1–9.
- Pech JC, Bouzayen M, Latché A (2008). Climacteric fruit ripening: ethylene-dependent and independent regulation of ripening pathways in melon fruit. *Plant Sci* 175: 114–120.
- Rodriguez FI, Esch JJ, Hall AE, Binder BM, Schaller GE, Bleecker AB (1999). A copper cofactor for the ethylene receptor ETR1 from *Arabidopsis*. *Science* 283: 996–998.
- SAS (1997). *SAS/STAT Guide for Personal Computers, Version 6*. SAS Institute, Cary, NC.
- Sato-Nara K, Ken-Ichi Y, Higashi K, Hosoya K, Kubota M, Ezura H (1999). Stage- and tissue-specific expression of ethylene receptor homolog genes during fruit development in muskmelon. *Plant Physiol* 119: 321–329.
- Strommer J, Gregerson R, Vayda M (1993). Isolation and characterization of plant mRNA. In: Glick BR, Thompson JE (eds), *Methods in Plant Molecular Biology and Biotechnology*. Boca Raton, FL, USA: CRC Press, pp 49–65.
- Takahashi H, Kobayashi T, Sato-Nara K, Tomita K, Ezura H (2002). Detection of ethylene receptor protein Cm-ERS1 during fruit development in melon (*Cucumis melo* L.). *J Exp Bot* 53: 415–422.
- Theologis A (1993). One rotten apple spoils the whole bushel: the role of ethylene in fruit ripening. *Cell* 70: 181–184.
- Tieman DM, Klee HJ (1999). Differential expression of two novel members of the tomato ethylene-receptor family. *Plant Physiol* 120: 165–172.
- Wang W, Esch JJ, Shiu SH, Agula H, Binder BM, Chang C, Patterson SE, Bleecker AB (2006). Identification of important regions for ethylene binding and signaling in the transmembrane domain of the ETR1 ethylene receptor of *Arabidopsis*. *Plant Cell* 18: 3429–3442.
- Wilkinson JQ, Lanahan MB, Clark DG, Bleecker AB, Chang C, Meyerowitz EM, Klee HJ (1997). A dominant mutant receptor from *Arabidopsis* confers ethylene insensitivity in heterologous plants. *Nature Biotech* 15: 444–447.
- Yamasaki S, Fuji N, Takahashi H (2000). The ethylene-regulated expression of *CS-ETR2* and *CS-ERS* genes in cucumber plants and their possible involvement with sex expression in flowers. *Plant Cell Physiol* 41: 608–616.

Supplemental 1. Primers used in PCR reactions.

Primer	Usage	Sequence
ETR1-F	PCR for gene fragments	5'-gagacggg[atc]ag[ag]catgt[agct]ag[ag]atg-3'
ETR1-R	PCR for gene fragments	5'-catggg[ac]gttctattcatg[ag]ttcat-3'
ETR2-F	PCR for gene fragments	5'-cag aat tgt gcg gtt tgg atg ccg-3'
ETR2-R	PCR for gene fragments	5'-cac aac ttt aac aat ctc aat ctc ctg-3'
ETR1-F	PCR for full-length gene	5'-ctattgtccattaacatccgct-3'
ETR1-R	PCR for full-length gene	5'-agattgtccctcaacgcacatga-3'
ETR2-F	PCR for full-length gene	5'-ggtcggctccccggatacaat-3'
ETR2-R	PCR for full-length gene	5'-taccatagtattcaacagggttc-3'

Supplemental 2. Multiple amino acid sequence alignment of Cm-ETR1 and other receptor proteins.

Cmeloetr1	-----MENCYCIEPQWPADELLMKYQYISDFFIALAYFSIPELELIYFVKK
Csativusetr1	-----METCYCIEPQWPADELLMKYQYISDFFIALAYFSIPELELIYFVKK
Mdomesticaetr1	-----MLACNCIEPQWPADELLMKYQYISDFFIALAYFSIPELELIYFVKK
Ppersicaetr1	-----MEACNCIEPQWPADELLMKYQYISDFFIALAYFSIPELELIYFVKK
Athalianaetr1	-----MEVCNCIEPQWPADELLMKYQYISDFFIALAYFSIPELELIYFVKK
Csinensiset1	-----MESCNCIEPQWPADELLMKYQYISDFFIALAYFSIPELELIYFVKK
Sesculentumetr1	MGSLLRMNRLLSSIVESNCIIDPQLPADLLMKYQYISDFFIALAYFSIPELELIYFVKK
Lsativaetr1	-----MDSCNCIEPQWPADELLMKYQYISDFFIALAYFSIPELELIYFVKK
	: ** :*****:*****:*****
Cmeloetr1	SAVFPYRWVLVQFGAFIVLCGATHLINLWTFMHSRTVAVVMTTAKVLTAVVSCATALML
Csativusetr1	SAVFPYRWVLVQFGAFIVLCGATHLINLWTFMHSRTVAVVMTTAKVLTAVVSCATALML
Mdomesticaetr1	SAVFPYRWVLVQFGAFIVLCGATHLINLWTFSIHSRTVAVVMTTAKVLTAVVSCATALML
Ppersicaetr1	SAVFPYRWVLVQFGAFIVLCGATHLINLWTFMHSRTVAIVMTTAKVLTAVVSCATALML
Athalianaetr1	SAVFPYRWVLVQFGAFIVLCGATHLINLWTFTHSRTVALVMTTAKVLTAVVSCATALML
Csinensiset1	SAVFPYRWVLVQFGAFIVLCGATHLINLWTFMHSRTVAIVMTTAKVLTAVVSCATALML
Sesculentumetr1	SAVFPYRWVLVQFGAFIVLCGATHLINLWTFNMHTRNVAIVMTTAKALTALVSCITALML
Lsativaetr1	SAVFPYRWVLVQFGAFIVLCGATHLINLWTFNAHTRTVAIVMTTAKVLTAAVSCATALML
	*****:*.**:******:*** ** *****
Cmeloetr1	VHIIPDLLSVKTRFLKNAKAELDREMGLIRTQEETGRHVRLTHEIRSTLDRHTILKT
Csativusetr1	VHIIPDLLSVKTRFLKNAKAELDREMGLIRTQEETGRHVRLTHEIRSTLDRHTILKT
Mdomesticaetr1	VHIIPDLLSVKTRFLKNAKAELDREMGLIRTQEETGRHVRLTHEIRSTLDRHTILKT
Ppersicaetr1	VHIIPDLLSVKTRFLKNAKAELDREMGLIRTQEETGRHVRLTHEIRSTLDRHTILKT
Athalianaetr1	VHIIPDLLSVKTRFLKNAKAELDREMGLIRTQEETGRHVRLTHEIRSTLDRHTILKT
Csinensiset1	VHIIPDLLSVKTRFLKNAKAELDREMGLIRTQEETGRHVRLTHEIRSTLDRHTILKT
Sesculentumetr1	VHIIPDLLSVKTRFLKNAKAQLDREMGIIRTQEETGRHVRLTHEIRSTLDRHTILKT
Lsativaetr1	VHIIPDLLSVKTRFLKNAKAQLDREMGIIRTQEETGRHVRLTHEIRSTLNRHTILKT
	*****:***:*****:*****:*****:*****
Cmeloetr1	TLVEMGRTLALEECALWMPTRTGLELQLSYTLRQQNPVGYTVPINLPVISQVFNRAVK
Csativusetr1	TLVELGRTLALAECCALWMPTRTGLELQLSYTLRQQNPVGYTVPINLPVISQVFNRAVK
Mdomesticaetr1	TLVELGRTLALAECCALWMPTRTGLELQLSYTLRQQNPVGYTVPINLPVISQVFNRAVK
Ppersicaetr1	TLVELGRTLALAECCALWMPTRTGLELQLSYTLRQQNPVGYTVPINLPVISQVFNRAVK
Athalianaetr1	TLVELGRTLALAECCALWMPTRTGLELQLSYTLRQHHPVEYTVPIQLPVINQVFGTSRAVK
Csinensiset1	TLVELGRTLALAECCALWMPTRTGLELQLSYTLRQQNPVGYTVPINLPVISQVFNRAVK
Sesculentumetr1	TLVELGRTLALAECCALWMPTRTGLELQLSYTLRQHNPVGLTVPIQLPVINQVFGTNRHVK
Lsativaetr1	TLVELGRTLGLAECCALWMPTRSGLELQLSYTLRQHNPVGLTVPIQSPAINQVFNRAVK
	::*****:*****:*.** *****:*.**.*.....:..*
Cmeloetr1	ISPNSPVASLRPRAGRYVAGEVVAVRVP LLHLSNFQINDWPELSTKRYALMVMLPDS
Csativusetr1	ISPNSPVASLRPRAGRYVAGEVVAVRVP LLHLSNFQINDWPELSTKRYALMVMLPDS
Mdomesticaetr1	ISANSPVAKLRQLAGRHIPGEVVAVRVP LLHLSNFQINDWPELSTKRYALMVMLPDS
Ppersicaetr1	ISPNSPVARMRPLAGKHMPGEVVAVRVP LLHLSNFQINDWPELSTKRYALMVMLPDS
Athalianaetr1	ISPNSPVARLRPVSGKYM LGEVVAVRVP LLHLSNFQINDWPELSTKRYALMVMLPDS
Csinensiset1	ISPNCVVARLRPLAGKYM LGEVVAVRVP LLHLSNFQINDWPELSTKRYALMVMLPDS
Sesculentumetr1	ISPNSPVARLRPAG-KYMPGEVVAVRVP LLHLSNFQINDWPELSTKRYALMVMLPDS
Lsativaetr1	ISPTSPVARLRPASGKYM LGEVVAVRVP LLHLSNFQINDWPELSTKRYALMVMLPDS
	...*:* .:: *****:*** *****:*****
Cmeloetr1	RQWRVHELELVEVVADQVAVALSHAAI LEESMRARDRLMEQNVALDLARREADTAIRARN
Csativusetr1	RQWRVHELELVEVVADQVAVALSHAAI LEESMRARDPLMEQNVALDLARREAETANHARN
Mdomesticaetr1	RQWHVHELELVEVVADQVAVALSHAAI LEESMRARDLLMEQNIALDLARREAETAIRARN
Ppersicaetr1	RQWHVHELELVEVVADQVAVALSHAAI LEESMRARDLLMEQNIALDLARREAETAIRARN
Athalianaetr1	RQWHVHELELVEVVADQVAVALSHAAI LEESMRARDLLMEQNVALDLARREAETAIRARN
Csinensiset1	RQWHVHELELVEVVADQVAVALSHAAI LEESMRARDLLMQQNIALDSARREAETAIRARN
Sesculentumetr1	RQWHVHELELVEVVADQVAVALSHAAI LEESMRARDLLMEQNVALDLARREAEAVRARN
Lsativaetr1	RQWHVHELELVEVVADQVAVALSHAAI LEESMRARDLLMEQNVALDLARREAETAVRARN
	:**:***:*** *****: * :***

*Conserved amino acids

.:Similar amino acids

Supplemental 2. (Continued).

Cmeloetr1	DFLAVMNHMERTPMHAI IALSSLLQETELTPEQRLMVETILKSSNLLATLINDVLDLSRL
Csativusetr1	DFLAVMNHMERTPMHAI IALSSLLQETELTPEQRLMVETILKSSNLLATLINDVLDLSRL
Mdomesticaetr1	DFLAVMNHMERTPMHAI IALSSLLQETELTAEQRLMVETILRSSNLLATLINDVLDLSRL
Ppersicaetr1	DFLAVMNHMERTPMHAI IALSSLLQETELTPEQRLMVETILKSSHLLATLINDVLDLSRL
Athalianaetr1	DFLAVMNHMERTPMHAI IALSSLLQETELTPEQRLMVETILKSSNLLATLINDVLDLSRL
Csinensisetrl	DFLAVMNHMERTPMHAI IALSSLLQETELTPEQRLMVETILKSSNLLATLINDVLDLSRL
Sesculentumetr1	DFLAVMNHMERTPMHAI IALSSLLQETDLTPEQRLMVETILKSSNLLATLINDVLDLSRL
Lsativaetr1	DFLAVMNHMERTPMHAI IALSSLLQETDLTPEQRLMVETILKSSNLLATLINDVLDLSRL *****:*. :*****:*. :*****:*****
Cmeloetr1	EDGSLQLDIGTFNLHAVFKEVNLNIKPVTLVKKLSLTLHLGPDLPVFAVGDEKRLMQAIL
Csativusetr1	EDGSLQLDIGTFNLHAVFKEVNLNIKPVTLVKKLSLTLHLGLDLPVFAVGDEKRLMQAIL
Mdomesticaetr1	EDGSLQLEIATFNLHSVFREVHNMIKPVASIKRLSVTLNIAADLPMYAI GDEKRLMQITIL
Ppersicaetr1	EDGSLQLEIATFNLHSVFREVHNLIKPVASVKKLSVSLNLAADLPVQAVGDEKRLMQIVL
Athalianaetr1	EDGSLQLELGTFNLHTLFREVNLNIKPIAVVKKLPI TLNLAPDLPEFVVGDEKRLMQI IIL
Csinensisetrl	EDGSLQLQIGTFNLHAVFREVNLNIKPIASVKKLLVALNLAPDLPEYAVGDEKRLMQITLL
Sesculentumetr1	EDGSLQLDIGTFNLHALFREVHSLIKPIASVKKLFVTLSSLSDLPYVIGDEKRLMQI ILL
Lsativaetr1	EDGSLELDTTTTFNLHALFKEVNLNIRPVASVKRLFVTLSSLSDLPYAVGDEKRLMQI ILL *****:*. :*****:*. :*****:*. :*****:***** :*
Cmeloetr1	NVVGNAVKFSKEGSGISISAIIVAKSETFREIRVPDFHVPVSDRHFYLRVQVKDTGSGIS PQ
Csativusetr1	NVVGNAVKFSKEGSGISISAIIVAKAETFREIRVPDFHVPVSDSHFYLRVQVKDTGSGIS PQ
Mdomesticaetr1	NVVGNAVKFSKEGSGISITAFVAKSESLRDFRAPDFFPVQSDNHFYLRVQVKDSGSGINPQ
Ppersicaetr1	NVVGNAVKFSKEGSGISITAFVAKSESLRDFRAPEFFPAQSDNHFYLRVQVKDSGSGINPQ
Athalianaetr1	NIVGNAVKFSKQGSISVTLVTKS---DTRAADFFVVP TGS HFYLRVQVKDSGAGINPQ
Csinensisetrl	NVVGNAVKFTKEGNISITGFVAKSESLRDSRAPEFFVPIENHFYLRVQVKDSGSGIS PQ
Sesculentumetr1	NVVGNAVKFSKEGNVISAFVAKSDSLRDPRAPEFFAVPSENHFYLRVQIKDTGIGITPQ
Lsativaetr1	NIIGNAVKFSKEGSGISISAIMAKPDSLRDPRAPEFFPLLSDNFFLYRVQVKDTGMGISQQ *:*****:*. :*****:*. :*****:*. :*****:***** ** *
Cmeloetr1	DIPKLFTKFAQT TVGPR-NSGSGGLGLAICKRFVNLMEGHIWLESEGLGKGTATFIVKL
Csativusetr1	DIPKLFTKFAQT TVGPR-NSCGSGLGLAICKRFVNLMEGHIWLESEGLGKGTATFIVKL
Mdomesticaetr1	DIPKLFTKFAQTQALATRNSSGSGGLGLAICKRFVNLMEGHIWIESEGLGKGTATFIVKL
Ppersicaetr1	DIPKLFTKFAQTQSLATRNSSGSGGLGLAICKRFVNLMEGHIWIESEGPQKGTATFIVKL
Athalianaetr1	DIPKIFTKFAQTQSLATRNSSGSGGLGLAISKRFVNLMEGNIWIESDGLGKGTATFIVKL
Csinensisetrl	DIPNLFTKFAQQAIALRNSSGSGGLGLAICKRFVNLMEGHIWIESEGLGKGTATFIVKL
Sesculentumetr1	DIPNLFKSKFTQSALATRNSSGTGLGLAICKRFVNLMEGHIWIESEGLGKGTATFIVKL
Lsativaetr1	DMPKLFTKFAESQSPATRNPGSGGLGLAICKRFVNLMEGNIWIESEGLGKGTATFIVKL *:*****:*. :*****:*****:*****:*****:***** ** *
Cmeloetr1	GIADQSNESKLPYTSKIHENS IHTSFPGLKVLVMDDNGVSRSVTKGLLVHLGCEVTTAGS
Csativusetr1	GIAEQSNESKLPFTSKIHENS IHTSFPGLKVLVMDDNGVSRSVTKGLLVHLGCEVTTAGS
Mdomesticaetr1	GFPERSNESKLPFAPKIQANHVQTNFPGLKVLVMDDNGVSRSVTKGLLHLGCDVTVAVSL
Ppersicaetr1	GFAERSNESKLPFLTKVQANHVQTNFPGLKVLVMDDN--GSVTKGLLVHLGCDVTVVSS
Athalianaetr1	GISERSNESKQSGIPKVP AIPRHSNFTGLKVLVMDENGVSRMVTKGLLVHLGCEVTVVSS
Csinensisetrl	GIPHSNDSNLSFIPKMPVHGQTN-FPGLKVLVMDENGVSRSVTKGLLLHLGCDVMTVSS
Sesculentumetr1	GIPGRANESKLPFVTKLPANHTQMSFQGLKVLVMDENGVSRMVTKGLLTHLGCDDVTVVGS
Lsativaetr1	GFP SRLNGSRLP-HMRVPAKLQTKFPGLKVVVDDNGVSRATKGLLVHLGCDVTVVSS *:. : * * . . : : * *****:*****:***** ***** ** *
Cmeloetr1	IEEFLLRVVVSQEHKVVFM DICTPGVDGYELAIRIREKFAK--HERPFMVVLTGNSDKVTK
Csativusetr1	IEEFLLRVVVSQEHKVVFM DICTPGVDGYELAIRIREKFAK--HERPFMVVLTGNSDKVTK
Mdomesticaetr1	IDELLVHVISQEHKVVFM DVSM PGIDGYELAVRIHEKFTKR--HERPVLVALTGNSIDKITK
Ppersicaetr1	IDEFLLHVISQEHKVVFM DVCM PGIDGYELAVRIHEKFTKR--HERPVLVALTGNI DKMTK
Athalianaetr1	NEECLRVVVSHEHKVVFM DVCM PGVENYQIALRIHEKFTKQR-HQRPLLVALSGNTDKSTK
Csinensisetrl	VEECFQVVSHEHQVVFMDVCPGIDGYEVAVHIHDKFTRR--HERPLIVALTGSTDNLTK
Sesculentumetr1	RDECLRVTTHEHKVVIMDVSMQGDICYEVAVVIHERFGR--HGRPLIVALTGNTDRVTK
Lsativaetr1	GEECLQAITKDSYKVVFDVSLSSDAYNVARLVHEKLPKRHEKPPPLIVGLTGNTDKAMK :* :*****:*****:*****:*****:*****:***** ** *

*Conserved amino acids

.:Similar amino acids

Supplemental 2. (Continued).

Cmeloetr1	ESCLRAGMDGLILKPVSIDKMRSVLSELIERRVLFETS
Csativusetr1	ESCLRAGMDGLILKPVSIDKMRSVLSELIERRVLFETS
Mdomesticaetr1	ENCMRVGVDGVILKPVSVDKMRSVLSLLEHRVLF EAM
Ppersicaetr1	ENCMRVGMDGVILKPVSVDKMRSVLSLLEHRVLF EAM
Athalianaetr1	EKCMSFGLDGVLKPVSLDNIRDVLSDLLEPRVLYEGM
Csinensisetr1	DNCMRVGMGVLKPVSEKMRVSLSDLEHRVLFESM
Sesculentumetr1	ENCMRVGMDGVILKPVSVKMRVLSLLEHGVVLES-
Lsativaetr1	ESLLRAGMDGLVLPVSEKMRLLALSLEHNT-----
	.. : *:*:*:*:*:*:*: ::* .**:*:* .

*Conserved amino acids

.:Similar amino acids

Supplemental 3. Multiple amino acid sequence alignment of Cm-ETR2 and other receptor proteins.

Cmeloetr2	MLKALPSGFLILLLLASVSAAD-----NGFPRCNCDDDEGS-LWSIDSILECQRVSDFLIA
Csativusetr2	MLKALPSGFLILLLLASVSAAD-----NGFPRCNCDDDEGS-LWSIDSILECQRVSDFLIA
Mdomesticaetr2	MLKALASSLSISLLLCVVSAD-----NGFPRCNCDDDGs-WWSIESILECQRVSDFLIA
Csinensiset2	MLRALAVGLVVSLLISVSVVDGD---NNFPRCNCDDDAS-SWSIESILETQKVSDFLIA
Lsativaetr2	MSKTLASLGLISLLLVGLAVDDDGSENGFGGCNCEVEG--FFGYRNIMETQRVSDFLIA
Athalianaetr2	MVKEIASWLLILSMVVFVSPVLAING-GGYPRCNCDEGNSFWSTENILETQRVSDFLIA
Lesculentumetr2	-----MDCNCFDPLL--PADELLMKYQYISDFLIA *** . : : * : * * : * *
Cmeloetr2	VAYFSIPIELLYFVSCSNVP-FKWVLFQFI AFIVLCGLTHLLNGWTYGPHSFQLMLALTV
Csativusetr2	VAYFSIPIELLYFVSCSNVP-FKWVLFQFI AFIVLCGLTHLLNGWTYGPHSFQLMLALTV
Mdomesticaetr2	VAYFSIPIELLYFVSCSNVP-FKWVLFQFI AFIVLCGLTHLLNGWTYGPHPFQLMLALTV
Csinensiset2	VAYFSIPIELLYFVSCSNVP-FKWVLIQFI AFIVLCGLTHLLNGWTYGPHSFQLMLSLTV
Lsativaetr2	VAYFSIPIELLYFVSCSNVP-FKWVLFQFI AFIVLCGMTHLLNGWTYEPHPFQLMLALTI
Athalianaetr2	VAYFSIPIELLYFVSCSNVP-FKWVLFEFIAFI AFIVLCGMTHLLHGWTYSAHPFRLMMAFTV
Lesculentumetr2	VAYFSIPIELVYFVQSAVFPYRWVLFQFAGFI AFIVLCGATHLINLWTSTPHTRTAVMVMTT * * * * * : * * * * * . * * * * * : * * * * * : * * * * * : * * * * *
Cmeloetr2	FKILTALVSCATAITLITLIPLLLKVKVREFMLKKTWDL GREVGMILKQKEAGLHVRML
Csativusetr2	FKILTALVSCATAITLITLIPLLLKVKVREFMLKEKTWDL GREVGMILKQKEAGLHVRML
Mdomesticaetr2	FKILTALVSCATAITLITLIPLLLKVKVREFMLKKTWDL GREVGIIMRQKEAGMHVRML
Csinensiset2	FKILTALVSCATSITLITLIPLLLKVKVREFMLKKKAWDL GREVGIIMKQKEAGVHVRML
Lsativaetr2	FKFLTALVSFATAITLVTLIPLLLKVKVREFMLRKKTWDL GVEGMILKQKEAGWHVRML
Athalianaetr2	FKMLTALVSCATAITLITLIPLLLKVKVREFMLKKKAHEL GREVGLILIKKETGFHVRML
Lesculentumetr2	AKFSTAAVSCATAVMLVHIIPDLLSVKTRELF LKNKAELDREMGLIRTQETGRYVRML * : * * * * * : * : * * * * * . * * * * * : * * * * * : * * * * *
Cmeloetr2	TQEIRKSLDRHTILYTTMFELSETLGLHYCAVWMPNESKTL MNLTHELKDRSFSNGYNVS
Csativusetr2	TQEIRKSLDRHTILYTTMFELSETLGLHYCAVWMPNESKTL MNLTHELKDRSFSNGYNVFS
Mdomesticaetr2	TQEIRKSLDRHTILSTTLFELSETLGLHYCAVWMPNEIKTEMLTHELKGRNYSHAYNFS
Csinensiset2	TQEIRKSLDRHTILYTTLVELSNTLGLQNCVWMPNEIKTEMLTHQLNGRNYSDMCSS
Lsativaetr2	TQEIRKSLDRHTILYTTLDKLEILDQLNCAIWPDPNTKTVMNLTHQLKG---GQSSTI
Athalianaetr2	TQEIRKSLDRHTILYTTLVELSKTLGLQNCVWMPNDGGTEMDLTHELRGRGGYGGCSVS
Lesculentumetr2	THEIRSTLDRHTILKTTLVELGRALQLEECALWMPTRTGVELQLSYTLHHQN---PVGFT * : * * * * * : * * * * * . * * * * * : * * * * * : * * * * *
Cmeloetr2	IPISDSVVIKIKGSDGVNVLGPNLSALVVANCGESDERGPA AAI RMPMLRVSNFKGG-TPE
Csativusetr2	IPISDSVVIKIKGSDGVNVLGPNLSALVVANCGESDERGPA AAI RMPMLRVSNFKGG-TPE
Mdomesticaetr2	IPISDPDVAHIKIGSDGVSILRPDSALVHASG-DSGEPGPVAAI RMPMLRVSNFKGG-TPE
Csinensiset2	IPITDQDVVRIKGSDGVNVLGPDSELAASSGESVSESGPVAAI RMPMLRVSNFKGG-TPE
Lsativaetr2	IPIQNPVEVQEI KQSEVVKLLDHESELGTLSSGGSEPPGAVAAI RMPMLRVSNFKGG-TPE
Athalianaetr2	MEDLDVVRIRSEDEVNVLSDVSSIARASGGGGDVSEIGAVAAI RMPMLRVSNDFNG-----
Lesculentumetr2	VPIQLPVINQVFSANCAVKISPNASAVARLRPTRKYI PGEVVAVRVPVLLHLSNFQTNWDPE : * . . * * * * * : * * * * * : * * * * *
Cmeloetr2	IVPTYAAILVVLVLPGGQPRSWNKQELEI IKVVADQVAVALSHAALLEESQLMRDKLAEQN
Csativusetr2	IVPTYAAILVVLVLPGGQPRSWNNQELEI IKVVADQVAVALSHAALLEESQLMRDKLAEQN
Mdomesticaetr2	VIQACYAAILVVLVLPGGQPRCWSQDLEI IKVVADQVAVALSHAAVLEESQLMREKLAEQN
Csinensiset2	LVSACYAAILVVLVLPNEQFRFTWSNQELEI IKVVADQVAVALSHAAVLEESQHMREKLEEQN
Lsativaetr2	MIQACYAAILVVLVLPGGQIRSWTGPELEI IKVVADQVAVALSHAAVLEESQLMRDKLAEQN
Athalianaetr2	--ELSYAILVLCVLPGGTPRDWTYQEI EIKVVADQVTVALDHAAVLEESQLMREKLAEQN
Lesculentumetr2	LSPKSYALMVLMLPNSARQWHVHELELDVVDVADQVAVALSHAAIL EESMRARDLLIEQN * * : * * : * * . * * : * * : * * * * * : * * * * * : * * * * *
Cmeloetr2	RDLQQA KENALMASQARNSFQKVMSDGMRRPMHSIMGLLSMLQENNMNDDQRIILDAMVR
Csativusetr2	RDLQQA KENALMASQARNSFQKVMSDGMRRPMHSIMGLLSMLQENNMNDDQRIILDAMVR
Mdomesticaetr2	RALQQA KMKAMMASHARNAFQKVMSDGMRRPMHSILGLLSLMQDNTLDNDQRVIVDAMVR
Csinensiset2	RALQQA QKDALMASQARNAFQKVMSSGMRRPMHSILGLLSIMQDNLNSDQRMIVETMMK
Lsativaetr2	RALQQA KH DAMRASQARNLFQTVMSKSLRKPMSHIVGLLSLIQDNLNMNQKVLIDSMVK
Athalianaetr2	RALQMAKR DALRASQARNAFQKTMSEGMRPMHSILGLLSMIQDEKLSDPEQKMIVDTMVK
Lesculentumetr2	VALDLARREAE TAVRARNDFLGVMNHMERTPMHAVVALSSLLQESELIPEQRLMVETILK * : * : * * * * : * * * * * . * . . * * * * * : * * * * * : * * * * * : * * * * *

*Conserved amino acids
 .:Similar amino acids

Supplemental 3. (Continued).

Cmeloetr2	TGNVVSTLIDDVMEHPKIDSARFPLELEMRSFRLH-SMKEAACLAKCLCAYKGFQFAFEV
Csativusetr2	TGNVVSTQIDDVMEHPKIDSARFPLELEMRSFRLHSMIKEAACLAKCLCAYKGFQFAFEV
Mdomesticaetr2	TSNVLSTLINDVMDNSAKESGRFPLEMRS--FGLHATIKEAACLAKCLCVFRGFDFDAIDV
Csinensiset2	SSNVLSTLISDVMDNSPKDSGRFPLEIRS--FRLHAMIKEAACLARCLSIYRGFGFSIEV
Lsativaetr2	TSNVLSMLIDDVMDSSKE--RFPLEMRS--FRLHSLIKEAAHLAKCLCVYKGYEFVMDV
Athalianaetr2	TGNVMSNLVGDMSD---VPDGRFGTEMKP--FSLHRTIHEAACMARCLCLCNGIRFLVDA
Lesculentumetr2	SSNLLATLINDVLDLSRLEDGSLQLDVGTF--FNLHALFREVLNLIKVAAVKKLFLVTLSL .:*::: :.* : : : * ** :*: : : . . .
Cmeloetr2	QRSLPDHVMGDERRVFQVLLHVMGSLND-INQGGGYALFRVVAESGSQGRNDQRWGNWR
Csativusetr2	QRSLPDHVMGDERRVFQVLLHVMGSLND-INQGGGYALFRVVAESGSQGRNDQRWGNWR
Mdomesticaetr2	DKSLPDHVMGDERRVFQVILHVMGSLNNG-NGVGG-LVMFRVASEKGSQGRSDQRWAAWR
Csinensiset2	QRSLPDHVMGDERRVFQVILHVMGSLLN--CNSRRGTVLFVFRVVESSQGRNDQRWAWR
Lsativaetr2	DKSLPDNVMGDERRVFQVILHVMGYLLNR-GNGGGGMVLR-ILKESGSYGRNDQRWASWR
Athalianaetr2	EKSLPDNVVGDERRVFQVILHIVGSLVKPRKQEGSSLMFKVLKERGSLDRSDHRWAAWR
Lesculentumetr2	SSDFPEVAIGDEKRLMQILLNVVGNVAVKFSKEGSVSVAVNAKSESLIDPRAPEFFPVQS . . :*: :*:*:*:*:*:*:*: : : * * . :
Cmeloetr2	Q--SSSDGDAFIRFEVGINKSNSQSEGSIPNVVSGDRR-----YASDGAEEERLSFTICKK
Csativusetr2	Q--NSSDGDADFIRFEVGINKSNSQSEGSIPNMVSGDRR-----YASDGAEEERLSFTICKK
Mdomesticaetr2	H--SSSDGDCVRFEIGISNNGSQSEVTPAVQLVGRR-----YAGEVDEGLSFTICKK
Csinensiset2	Q--SSVDGDVHIRFEILLNEVGSQPEVSTSVAQLGIRR-----IVNEGIEDRMSFSVCKK
Lsativaetr2	S--NSGDGYVSVKFEIGINDHDTKLER-----SFADER-----IRSGVEQSLSFQMCRK
Athalianaetr2	SPASSADGDVYIRFEMNVENDSSSSQSFASVSSRDQEVGDVRFSGGYGLQDLSFGVCKK
Lesculentumetr2	E-----NHFYLRVQVQKDTGSGINPQDFPKLFCCKFAQNQE--PATKNSAGTGLGLAICKR . : : : : : : : *
Cmeloetr2	LVKLMQGNIIWVIPNPQGFTRSMALVLRFLRPSIAVAMPEPAGESSEH---PHSNSIFRGL
Csativusetr2	LVKLMQGNIIWVIPNPQGFTRSMALVLRFLRPSIAVAMPEPAGESSEH---PHSNSIFRGL
Mdomesticaetr2	LVQMMQGNIIWVNPQGFQSMALVLRFPRLSIAIAISEPAGESSEH---PHSNSLFRGL
Csinensiset2	LVQMLQGNIIWVPSHGFQSMGLVLRFLRPSIAIPISEGESSEY---SHSNSLLRGL
Lsativaetr2	LVEMMQGKIWVVPNPVGFQAMSLILRFQLRPSIVIGISEAGESSDHN--PLSNSIFRNL
Athalianaetr2	VVQLIHGNISVVPDGSPESTMSLLLRFRRRPSISVHGSSSESPAPDHHAHPHSNSLLRGL
Lesculentumetr2	FVNLMEGHIWIESEGVGKSTAFIVKLGIPGRLNESKLPFTAGLPAN---HMQMTFQGL .*:*. : * : : : : : : : : : : : *
Cmeloetr2	QVILADADDMNRVAVTRKMLEKLGCVNTAVSSGYECLTVMA---PAG---SSIQVVLDDLH
Csativusetr2	QVILADADDMNRVAVTRKMLEKLGCVNTAVSSGFECTVMA---PAG---SSIQVVLDDLH
Mdomesticaetr2	QVLLTDDDDVNRVVTRKMLEKLCIVTAVSSGFECTSTIG---TIGPAGSSFFQVFLDLH
Csinensiset2	QVLLADGDDMNRVVTRKLEKLGCSVSAVSTGYECLSAMGPTA-----VFQLVILDLQ
Lsativaetr2	QVLLADEDDMNRVAVTRKLEKLCIVSTVASGSDCIMALN-----QPVSYSQIILLDLH
Athalianaetr2	QVLLVDTNDNSRAVTRKLEKLGCDVTAVSSGFDCLTAIAP--GSSPSTSFQVVVLDLQ
Lesculentumetr2	KVLVMDNNGFSRMVTKSLLVHLGCDVTTIGSGDECLRILTR-----EHKVLIMDAS :*: * . . * ** . * :*** * : : : * : * : : : : *
Cmeloetr2	MPELDGFEVTTIRIKFRSQNYR--PVI IALTASAGEDWERCQVIGMNGVIRKPVQLQGIA
Csativusetr2	MPELDGFEVTTIRIKFRSQNYR--PVI IALTASAGEDWERCQVIGMNGVIRKPVQLQGIA
Mdomesticaetr2	MPELDGFEVAIRIKFRSRTWP--LIIGVTASADEVWDRCMQTGINGVIRKPVLLQGIA
Csinensiset2	MPELDGFEVALKIRKFRSRWP--LIVALTASGDEDVWEKCMQVGMNGVIRKPVLLQGIA
Lsativaetr2	MSDVDGFEVAARIRKSRSNWP--LIVALTASGDADVWERCLQMGINGVIQKPVVLLQGIS
Athalianaetr2	MAEMDGYEVAMRIR--SRSWP--LIVATTVSLDEEMWDKCAQIGINGVVRKPVVLRAME
Lesculentumetr2	ITGMNCYDVAVSVHEKFGKRLERPLI VALTGN TDQVTKENCLRVMGDGVIKPVSIDKMR : . : : : * : : : : : : : : : : : * : * : * : * : *
Cmeloetr2	HELRRALLQASKVV-
Csativusetr2	HELRRALLQASKVV-
Mdomesticaetr2	NELRRVLLQANKGMT
Csinensiset2	NELRRVLLHANKVV-
Lsativaetr2	DELRRVMVHTNKVH-
Athalianaetr2	SELRRVLLQADQLL-
Lesculentumetr2	SVLSGLEHGTVL-- * : :

*Conserved amino acids

.:Similar amino acids

Supplemental 4. mRNA transcripts of *Cm-ETR1* (A) and *Cm-ETR2* (B) genes in melon fruit during storage at 20 °C. Twenty micrograms of total RNA from fruit stored for 0 (0), 1 (1), 2 (2), 4 (4), 6 (6), 8 (8), and 10 days (10) were used for Northern blot analysis.

