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Chen, Yi and Grimplet, Jérôme and David, Karine and Castellarin, Simone and Terol, Javier and Wong, Darren C.J and Luo, Zhiwei and Schaffer, Robert and Celton, Jean-Marc and Talon, Manuel and Gambetta, Gregory Alan and Chervin, Christian *Ethylene receptors and related proteins in climacteric and non-climacteric fruits.* (2018) Plant Science, 276. 63-72. ISSN 0168-9452

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Ethylene receptors and related proteins in climacteric and non-climacteric fruits

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ARTICLE INFO

Keywords:
Ethylene
Perception
Plant hormone signaling
Ripening
Climacteric fruit
Non-climacteric fruit
Phylogenetic analysis
RNAseq

ABSTRACT

Fruits have been traditionally classified into two categories based on their capacity to produce and respond to ethylene during ripening. Fruits whose ripening is associated to a peak of ethylene production and a respiration burst are referred to as climacteric, while those that are not are referred to as non-climacteric. However, an increasing body of literature supports an important role for ethylene in the ripening of both climacteric and non-climacteric fruits. Genome and transcriptomic data have become available across a variety of fruits and we leverage these data to compare the structure and transcriptional regulation of the ethylene receptors and related proteins. Through the analysis of four economically important fruits, two climacteric (tomato and apple), and two non-climacteric (grape and citrus), this review compares the structure and transcriptional regulation of the ethylene receptors and related proteins in both types of fruit, establishing a basis for the annotation of ethylene-related genes. This analysis reveals two interesting differences between climacteric and non-climacteric fruit: i) a higher number of ETR genes are found in climacteric fruits, and ii) non-climacteric fruits are characterized by an earlier ETR expression peak relative to sugar accumulation.

1. Introduction

The plant hormone ethylene was discovered in the early 20th century by observing that gas containing ethylene affected plant growth [1]. Ethylene is involved in most aspects of plant development including seed germination, root elongation, flower development, fruit ripening, and organ senescence and abscission [2–4]. It also has a role in response to many biotic and abiotic stresses such as pathogen, heavy metal toxicity, and wounding among others [5].

Fleshy fruit are essential in human nutrition and health, and ethylene is critical for the proper ripening of many of them [6]. It regulates several processes associated with fruit ripening including softening, color change, sugar accumulation, organic acid production, as well as the accumulation of secondary metabolites [7]. Based on the

Abbreviations: CTR, constitutive triple response; Cyb5, cytochrome b5; ein2, ethylene insensitive 2; ETR, ethylene receptor; GRL, green-ripe like; RAN1, response to ANtagonist 1; RTE, reversion to ethylene sensitivity; SMART, simple modular architecture research tool; TMpred, transmembrane prediction; TPR1, tetratrico peptide repeat 1

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https://doi.org/10.1016/j.plantsci.2018.07.012

respiration profile and ethylene production during ripening, fruits can be divided into two classes: climacteric and non-climacteric. Climacteric fruits include tomato, banana, apple, mango, and pear, while grape, citrus, and watermelon belong to the non-climacteric class [6].

However, more contemporary research looking at a larger range of fruits and including changes of ethylene-related genes (synthesis and signaling) suggests that the classification of fruits as either climacteric or non-climacteric is not obvious. Some fruits, like melons, can display both climacteric and non-climacteric behaviors [8], while kiwifruit displays a more complicated regulation where the first stage of ripening is not dependent on ethylene while the second stage is [9]. Furthermore, there are climacteric and suppressed-climacteric plum varieties, and it has been suggested that the latter have impaired ethylene sensing capacities [10]. Even in fruits typically classified as non-climacteric current molecular studies suggest a role of ethylene in ripening. For example, in strawberry, transcript levels of ethylene receptors increase at the onset of ripening like in climacteric fruit [11], and in grape, traditionally classified as non-climacteric, ethylene sensing seems necessary for fruit ripening [7,12]. Additionally, exogenous ethylene was found to stimulate sweet cherry respiration, a fruit classified as non-climacteric [13]. It is now proven that differences in behaviour are not as clear cut as previously thought.

The genes/proteins involved in ethylene production and signaling are generally well characterized. Ethylene biosynthesis has been well described and reviewed [14]. In the cytoplasm, it starts with S-adenosyl-L-methionine (SAM) production in the Yang's cycle. This substrate is then converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthases. ACC is further converted to ethylene by ACC oxidases [15].

Ethylene is perceived by transmembrane-receptor proteins localized in the endoplasmic reticulum (ER) belonging to the EThylene Receptor (ETR) family [2] (Fig. 1). The signal transduction pathway involves other proteins localized in, or close to, the ER, such as CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1) and ETHYLENE INSENSITIVE 2 (EIN2). Ultimately, EIN2's C-terminal end is cleaved and moves to the nucleus, leading to the stabilization of a specific transcription factor, EIN3, which promotes the expression of ethylene-dependent genes. The ethylene receptor itself is regulated by a range of proteins localized at the endoplasmic reticulum such as RTE/GTL, RAN1, TPR1, and Cyb5. This review will present current knowledge regarding ethylene perception and signaling in fleshy fruits, and with the use of additional data described below, compare the transcriptional regulation of the key perception and signaling proteins among two climacteric and two non-climacteric fruits.

We explored additional data from four different fruits, all details are given in Supp. Table 9. Three criteria were used to choose the fruit species: (i) fruit with an important role in agriculture, (ii) fruit with access to genomic (gene sequences) and transcriptomic data (RNAseq) over the ripening period, and (iii) an equal number of climacteric and non-climacteric fruit. Using those criteria we selected tomato (*Solanum lycopersicum* cv Heinz), apple (*Malus domestica* cv 'Royal Gala'), grape (*Vitis vinifera* cv Merlot), and clementine mandarin, a popular citrus species (*Citrus clementina* cv Hernandina). All of these fruits are of global economic importance having annual production according to the FAO of: tomato 170, oranges and mandarins 127, apples 125, and grape 87, in millions of tons in 2014 (http://www.fao.org/faostat/en/#home). Access to high quality RNAseq data and the availability of a well-annotated, up-to-date reference genome were critical requirements in providing a reliable correspondence between orthologous genes.

The four fruit species selected have different ripening characteristics. Table 1 shows the roles of ethylene production and perception, according to previous studies. Although these four fruits can all produce ethylene, the non-climacteric fruits, grape and citrus, show a much lower level of ethylene production, especially citrus, in comparison with the climacteric fruits, apple and tomato. Exogenous applications of ethylene or its precursor ACC can accelerate fruit ripening in all four species, while applications of the ethylene receptor inhibitor, 1-methylcyclopropene (1-MCP), or the ethylene production inhibitor, aminoethoxyvinylglycine (AVG), can delay fruit color changes and ripening-related processes (see references in Table 1).

These data indicate that both climacteric and non-climacteric fruit share some identical responses to ethylene that need to be clarified. With this objective, we detail in the following the differences and similarities in ethylene receptors, and the specific role of ethylene receptor partner proteins between climacteric and non-climacteric fruits.

2. Are there differences at the ethylene receptor level among climacteric and non-climacteric fruits?

2.1. There are more ETR genes in climacteric fruits than in non-climacteric fruits

The ETRs, the first elements of the ethylene signaling cascade, are transmembrane proteins located in the endoplasmic reticulum that bind ethylene, forming a stabilized dimer with two disulfide bonds at the N-terminus [16]. ETRs are part of a multigene family. The Arabidopsis ETR family contains 5 members and were used as anchors to establish the phylogenetic relationships between ETRs across the four fruits (Fig. 2). The location of ETRs in the membrane facilitates interaction with ethylene as it is more soluble in lipid environments [16]. ETRs are negative regulators of the signalling cascade, meaning that in the absence of ethylene, ETRs block downstream signal transduction. For example, in tomato, transgenic plants down-regulated for*SlETR4* produce early ripening fruits [17]. Additionally, point mutations in the ethylene binding pocket of ETRs confer ethylene insensitivity. In Arabidopsis, this is the case for the *etr1-1* mutant, which doesn't display the characteristic triple response in presence of ethylene, and for tomato the *nr* mutant (for *never ripe,* a mutation of the *SlETR3* gene) which produces fruits with delayed ripening [16,18].

Across the fruit species examined here, previous studies reported different number of ETR genes in the climacteric and non-climacteric fruits (Fig. 2); 7 ETRs in tomato [18], 9 in apple [19], 4 in clementine, and 4 in grapevine [20]. Considering that all receptors may have similar ethylene binding on a per unit protein basis, as described previously for Arabidopsis and tomato [21], this higher number of receptors in climacteric fruits may contribute to the need for a greater level of ethylene in triggering a response in climacteric species. This is worthy of further research at the protein level to validate a quantitative relationship between ethylene and receptors within species.

Members of the two ETR subfamilies 1 and 2, are found in all four fruit species. For each fruit, there is an even distribution of ETRs within the two subfamilies (Fig. 2). In apple, there is a higher number of homologs resulting from a genome-wide duplication [22]. It is possible that errors in the editing of the old apple genome led to *in silico* mis-splicing of some proteins, like the unusual long N-terminus in MdETR1b (Fig. 3). The recent publication of a new version of the apple genome [23] allowed us to check the correspondence between the old and new annotations of the *MdETRs* (Sup. Table 8). According to a previous study, there were 9 *ETRs* in apple genome, reduced from 15 genome accession numbers after comparing the genome data with EST data [19]. We kept these 9 annotations in the phylogenetic tree (Fig. 2). After comparing old and new apple genome version (Supp. Table 8), we found that both *MdETR1b* (MDP0000267951) and *MdETR101* (MDP0000300556) correspond to MD02G1161700 in the new genome. And indeed both *MdETR1b* and *MdETR101* are very closely related on the phylogenetic tree (Fig. 2). However, as the only set of apple RNAseq data during fruit development was performed using the old annotation [19,24] we have left both annotations in our text and figures.

Fig. 3 also shows the number of transmembrane domains that were estimated using the TMpred tool (https://embnet.vital-it.ch/software/TMPRED_form.html) (Sup. Table 1). The newly described SlETR7, by Liu and collaborators [18], contains only three transmembrane

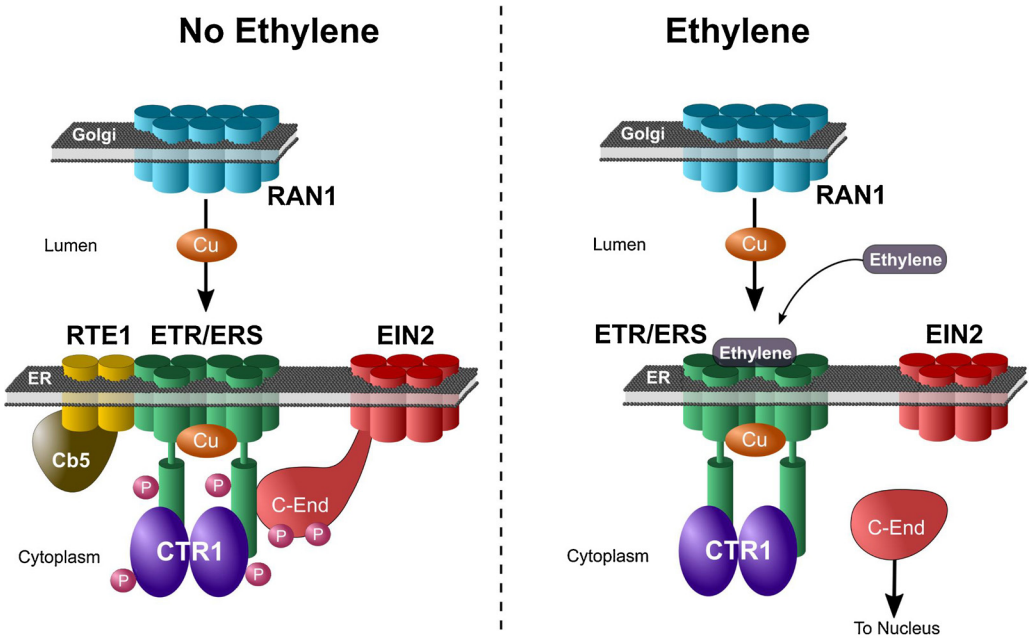

Fig. 1. Ethylene perception mechanisms, at the endoplasmic reticulum (ER) membrane level, adapted from Ju and Chang [2]. In presence of ethylene, the decrease of phosphorylated receptors and receptor-partners leads to the cleavage of EIN2 C terminal, which goes to nucleus to carry on further signal. ETR stands for EThylene Receptor, ERS stands for Ethylene Response Sensor, EIN2 stands for Ethylene INsensitive 2, CTR1 stands for Constitutive Triple Response 1, RTE1 stands for Reversion To Ethylene sensitivity 1, RAN1 stands for Response to Antagonist 1, Cb5 stands for Cytochrome b5, Cu is copper. RTE1 and Cb5 are not represented in the right panel to save space, but may still be important when ethylene is present.

Table 1
Climacteric and non-climacteric fruit responses to ethylene. Note that all timings of inception of ripening, expressed in time units after full bloom, depend on the growth conditions, particularly light and temperature. ACC stands for aminocyclopropane carboxylic acid, which a precursor of ethylene in the plant biosynthesis pathway; 1-MCP stands for 1-methylcyclopropene, which is an inhibitor of ethylene perception; AVG stands for aminoethoxyvinylglycine, which an inhibitor of ethylene synthesis.

	Species	Ethylene production during fruit development	Effect of ethylene or precursor	Ethylene signal inhibitors
Climacteric	Tomato	Ethylene peaks near breaker stage (around 42 days after full bloom) (inception of ripening) [18]	ACC accelerated the transition from green to orange/red [56] The ripening process was induced by exogenous ethylene treatment [57]	1-MCP treatment delayed softening, total soluble solids accumulation, and titratable acidity decrease, inhibited the increase of weight loss, and suppressed the rise in respiration rate and ethylene production. Moreover, 1-MCP treatment also inhibited the lycopene accumulation and chlorophyll degradation [58]. AVG delayed tomato fruit ripening [33]
	Apple	Ethylene production during fruit development peaks at 105 days after full bloom (inception of ripening) [59]	Exogenous ethylene treatment accelerated the onset of ethylene production and the climacteric peak [60]	1-MCP treatment drastically reduced the ethylene production, impaired skin yellowing and fruit softening in cold-stored fruit at post-harvest [61] 1-MCP treatment resulted in delayed respiration and ethylene climacteric peaks [60]. AVG delayed colour development at harvest [62]
Non- Climacteric	Grape	A small ethylene production peak occurs at 7 weeks after flower full bloom (inception of ripening) [12]	Exogenous ethylene enhanced anthocyanin accumulation [63] and reduced titratable acidity [64]	1-MCP inhibited anthocyanin accumulation [12]
	Clementine	Fruitlets exhibited a rise in ethylene production, but not the mature fruits [65]	Ethylene accelerated chlorophyll degradation [66]	1-MCP delayed chlorophyll degradation and can prolong storage time [67]

domains although its strong phylogenetic relationship with SlETR5 and the absence of a conserved HATPase_c (Fig. 3) shows that it is clearly a member of subfamily 2. SlETR6 and MdETR102 (sub-family 2) have only three transmembrane domains (Fig. 3 and Sup. Table 1). There are occasionally variations in the estimation of transmembrane domains between TMpred (Supp. Table 1, and black figures at the N term on Fig. 3) and SMART images (blue rectangles in Fig. 3), however the TMpred tool provides more robust predictions as it is specialized in analysis of transmembrane helices. In the ETR sub-family 1, most ETRs have three transmembrane domains (Fig. 3), but MdETR1 and CclERS1 have four. Regarding the SMART analyses (http://smart.embl-heidelberg.de/), particular attention should be paid to the various domains located in the C terminal portion of the ETR involved in the phosphorelay [16]. The predicted functionality of the HATPase_c domains (histidine kinase-like ATPase) is a strong indicator of the ETR sub-family. All sub-family 1 ETRs have a HATPase_c domain with strong likelihood of being functional, i.e. very low E-value (Fig. 3), and all sub-family 2 ETRs have a HATPase_c domain with weak likelihood of being functional, i.e. rather high E-value.

The *ETR* expression data were obtained from RNAseq datasets that were produced and validated in previous studies (see data origin in Supp. Table 9), and these data were plotted across fruit development in

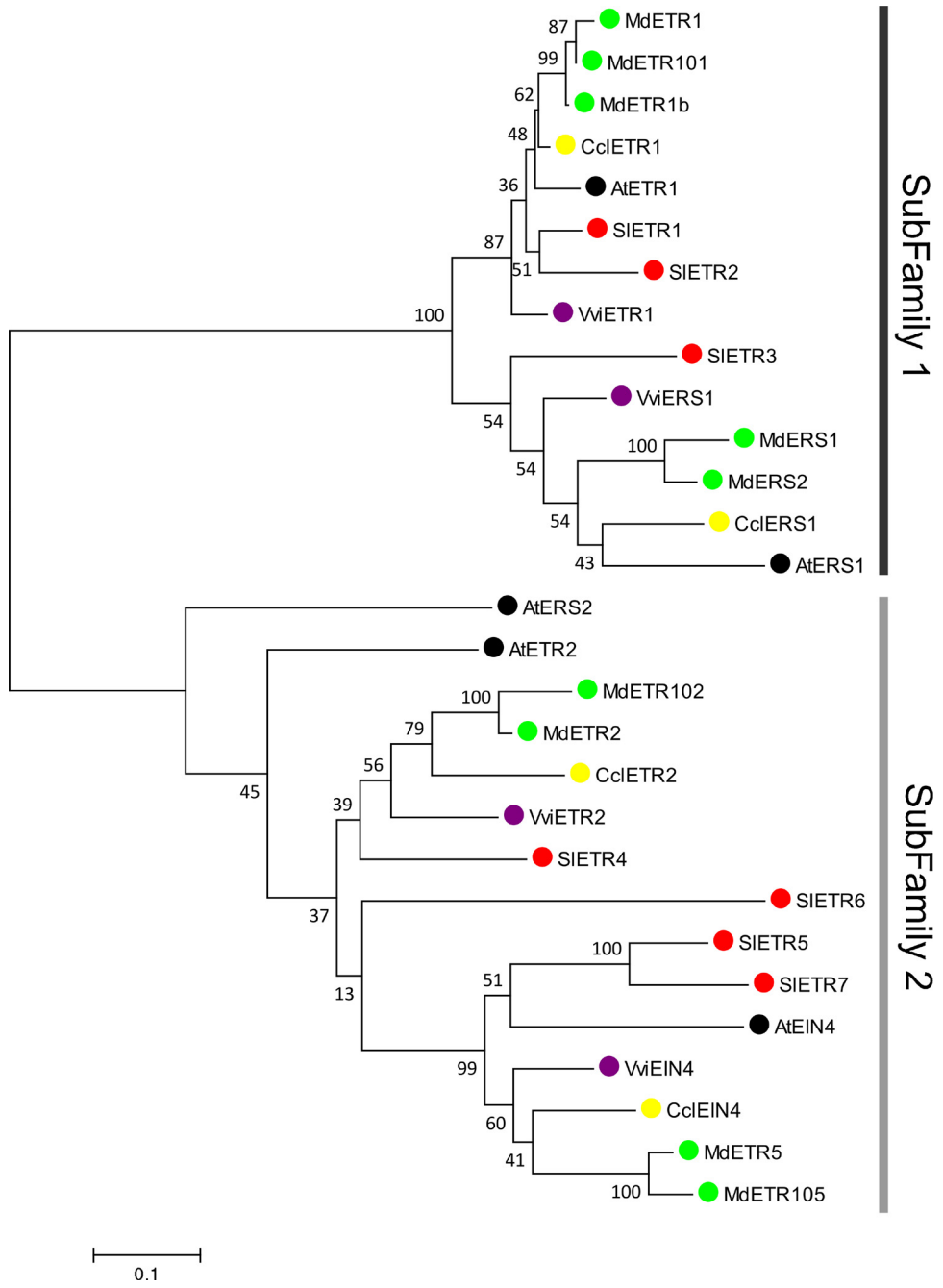

Fig. 2. Phylogenetic tree of ETRs, constructed from a MUSCLE alignment by the maximum likelihood method. The numbers at the branches are confidence values based on bootstrap method ($B = 500$ replications). Sl stands for *Solanum lycopersicum*, Md stands for *Malus domestica*, Vvi stands for *Vitis vinifera*, Ccl stands for *Citrus clementina* and At stands for *Arabidopsis thaliana*.

the four species (Fig. 4). In all four fruit, specific *ETRs* have a high expression at the onset of fruit ripening. This coincided with the start of sugar accumulation and to a lesser extent to color change (Fig. 5). In apple, the last two sampling dates were chosen slightly late in the ripening process, but that is the only set of RNAseq data currently available. In non-climacteric fruits, peak *ETR* expression occurred earlier than in climacteric fruits.

Whether sugars regulate *ETR* expression remains to be confirmed, although previous studies showed that sucrose can act as a signal accelerating ripening in non-climacteric fruits such as strawberry [25], grape [26], and citrus [27], but also in a climacteric fruit such as tomato [25]. In tomato and citrus, two *ETRs*, one from subfamily 1 and one from subfamily 2, had a high level of expression at the onset of ripening, while in apple and grape, the *ETRs* that displayed a peak belong to subfamily 2 (Fig. 4). Regarding the apple data, we assessed a set of microarray data (Sup. Fig. 11) confirming that *MdETR2* is the ETR with the highest expression during ripening. Moreover looking at the RNAseq data (Fig. 3), *MdETR101* and *MdETR1b* show same expression trend during the apple ripening, confirming that they are the same gene, as discussed in the phylogenetic analysis above. Thus it seems that there are only 8 ETR genes in apple. In this review, we focused our analysis on ETRs at the mRNA level. However, there is clearly a need for more data on protein levels across fruit development. A study in 2007 revealed a negative correlation between *ETR* mRNA and protein levels in tomato [28], but this was not confirmed in a more recent study by the same team [29]. Another nuance of ETR function, suggested by

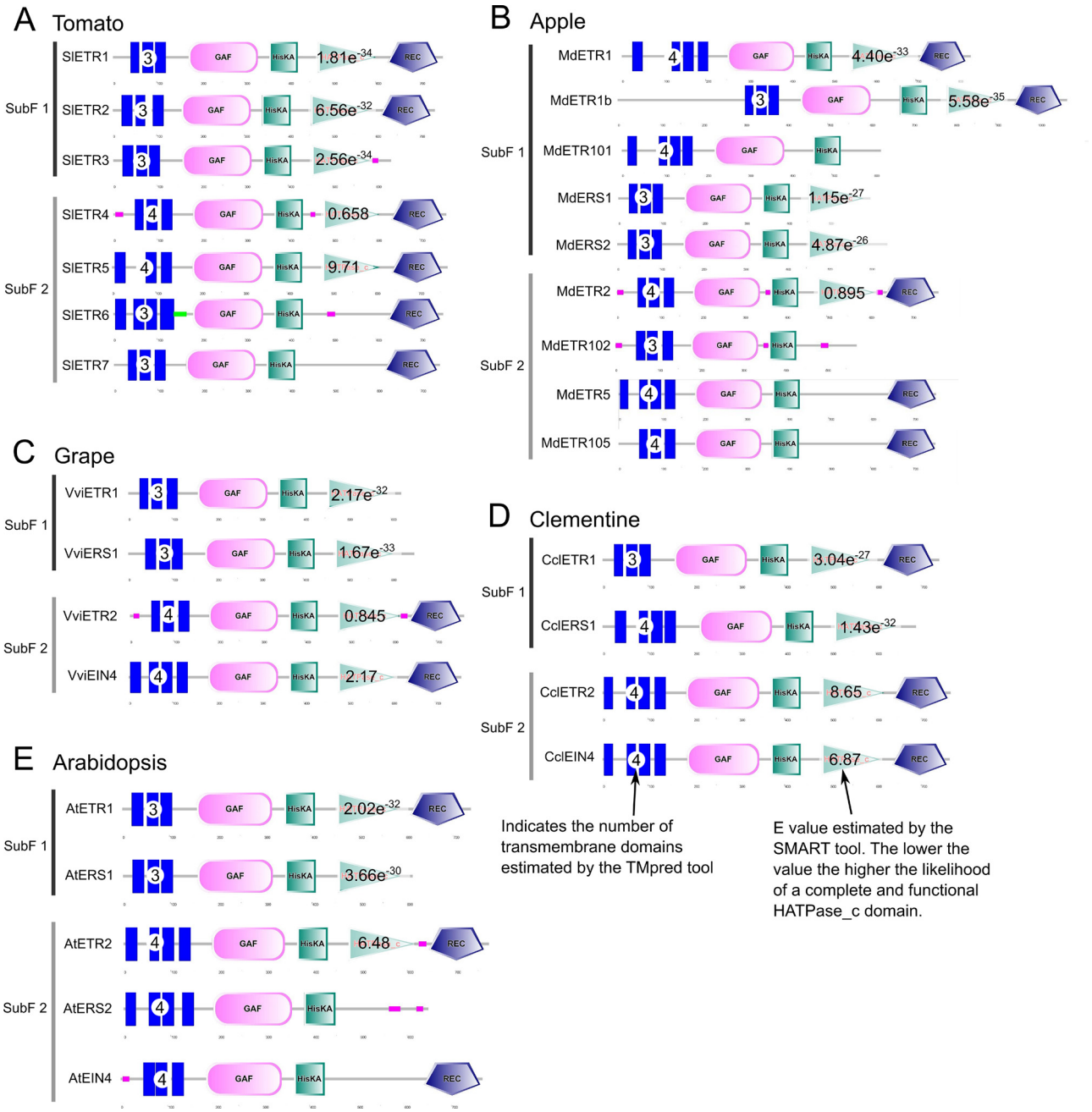

Fig. 3. SMART images of the various ETRs (http://smart.embl-heidelberg.de/), and the number of transmembrane domains (black figures in white circles) was estimated by the TMpred tool (https://embnet.vital-it.ch/software/TMPRED_form.html). In SMART images, a HATPase_c domain with a very low E value has a strong likelihood of being functional.

Arabidopsis studies, is the sub-functionalization of the individual ETR homologs [16]. Individual ETRs might have limited specific roles in specific plant responses, such as salt stress [30].

2.2. *CTRs may not be essential for ethylene signaling in some fruit*

CTRs (CONSTITUTIVE TRIPLE RESPONSE) are the second component in ethylene signal transduction at the ER membrane level, acting as a mediator between ETRs and EIN2s [2]. It is worth noting that SlETR4, a key regulator of fruit ripening [31], does not bind to any CTR [32]. This suggests that ETR-CTR interaction may not be essential for tomato fruit ripening. Furthermore, the direct link between SlETR1 and EIN2 seems essential to tomato ripening [33], and this interaction does not involve CTRs. CTRs are Raf-like protein kinases that act as a negative regulator of the ethylene response. In Arabidopsis, *ctr* mutant seedlings show a typical triple response in the absence of ethylene, i.e. a constitutive response [16]. Studies have shown that CTR1 can phosphorylate EIN2 in Arabidopsis [34]. CTRs are most likely cytosolic proteins, which are sometimes mobilized to the ER when co-expressed with ETRs [32].

The Raf-like kinase family is large, and thus we chose to construct a tree with the three gene families forming the CTR1/EDR1 clade (Supp. Fig. 1) according to recent work on plant CTRs [35]. All the CTRs could be subdivided into the CTR1, CTR1-related, and EDR1 sub-families (Supp. Fig. 1). Each of the fruit species considered in this study had at least two CTRs phylogenetically related to AtCTR1. The CTR1 Related Group (also named CRG) was kept in the tree as there are proteins representative of the four fruits, one of them being annotated

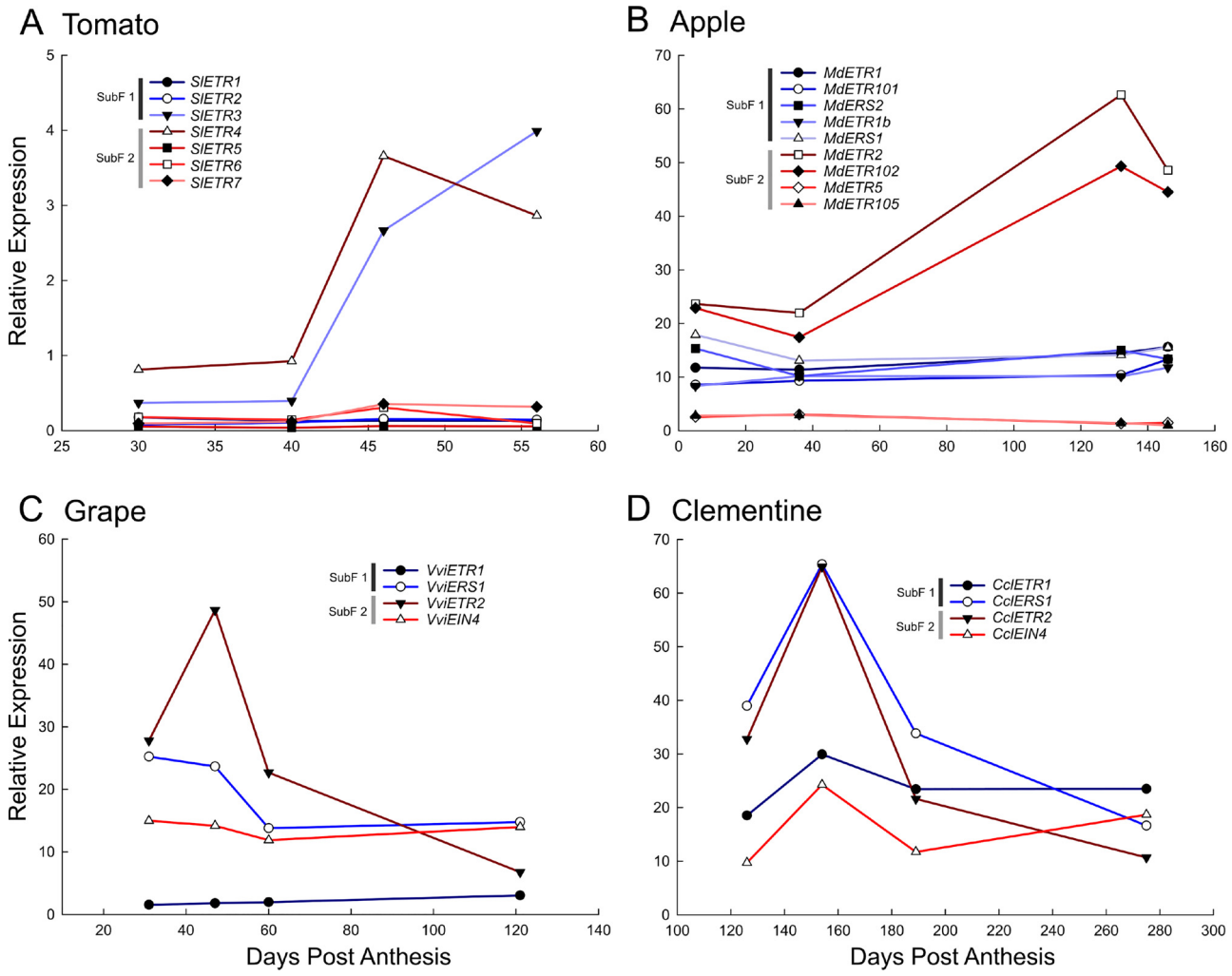

Fig. 4. mRNA accumulation of ETRs over fruit development (for details on RNAseq data, see Supp. Table 9). Data were collected from different published RNAseq works. The relative abundance of gene expression was calculated differently. Tomato data are mean counts per base pair, apple and clementine data are in RPKM: Reads Per Kilobase per Million mapped reads, and grape data are in FPKM: Fragments Per Kilobase of exon per Million mapped reads. These different units still allow comparisons of relative expression within one species.

MdCTRL3, for CTR-like. The EDR1 sub-family (Enhanced Disease Resistance) was kept in the tree as SlCTR2 belongs to this sub-family, which function has been described previously [35,36]. However, SlCTR2 has been shown to interact with SlETR1 and SlETR2, similar to SlCTR1, SlCTR3 and SlCTR4 [32]. SlCTR2 does not cluster with the three other SlCTRs (Supp. Fig. 1), as previously shown [37]. Moreover, SlCTR2 has three transmembrane domains (Supp. Table 2), like SlEDRL3, VviEDRL2 and CclEDRL3, while SlCTR1, SlCTR3, SlCTR4, VviCTR1, and CclCTRL1 have one or two transmembrane domains, although with a weak score. This indicates that SlCTR2 belongs to the EDRL family. The SMART images of the CTRs (Supp. Fig. 3) show that most apple and clementine CTRs harbor a serine/threonine kinase domain, while this is not the case in tomato and grape. This serine/threonine kinase domain may not be critical for signaling in the ethylene pathway, as underlined by studies in Arabidopsis [16]. This is also supported by the fact that SlCTR1, which lacks an active kinase domain (Sup. Fig. 3), is functional in ethylene signaling restoring ethylene signaling in the Arabidopsis mutant *ctr1-1* [38].

Changes in *CTR* expression (Supp. Fig. 2) do not exhibit any clear trends between the climacteric and non-climacteric fruits. Tomato *SlCTR1* expression peaks at onset of ripening, but in apple (e.g. *MdCTR1*) it remains unclear due to the lack of sampling between 36 and 130 days. In a previous study [39], *MdCTR1* expression was peaking in one apple cultivar, around 100 dpa (qPCR data). A RNAseq series over apple fruit development with a more comprehensive sampling is a suggestion for further studies. In all four fruit, the expression levels of *CRG* (CTR1 Related Group) orthologs were very low. If protein accumulation kinetics correlate to RNA expression levels, CRGs are unlikely to play an important role in fruit ripening. In non-climacteric fruits, the *EDRs* have the highest expression profile over all sampling dates (Supp. Fig. 2), but functional analyses are necessary to prove that they may play a critical role in the onset if ripening. The role of CTRs in the ethylene signal will also be discussed in the following paragraph, when evoking the link between EIN2s and ETRs.

2.3. *The crucial EIN2 is a single copy gene*

EIN2 (ETHYLENE INSENSITIVE 2) is another protein localized in the endoplasmic reticulum membrane and has similarities to NRAMP proteins (Natural Resistance Associated Macrophage Protein). Originally described in the 90's [40,41], it is the "last protein" in the ethylene signaling pathway located in the endoplasmic reticulum membrane. Ethylene binding to the ETR receptor leads to the cleavage of EIN2 C-terminus which moves to the nucleus where the ethylene signaling cascade continues [34,42]. More recently two studies provided new information about the role of EIN2 showing that: 1) EIN2 represses EBF1/2 translation, and therefore the degradation of ethylene response transcription factor EIN3/EIL1 [43], which are the first step of

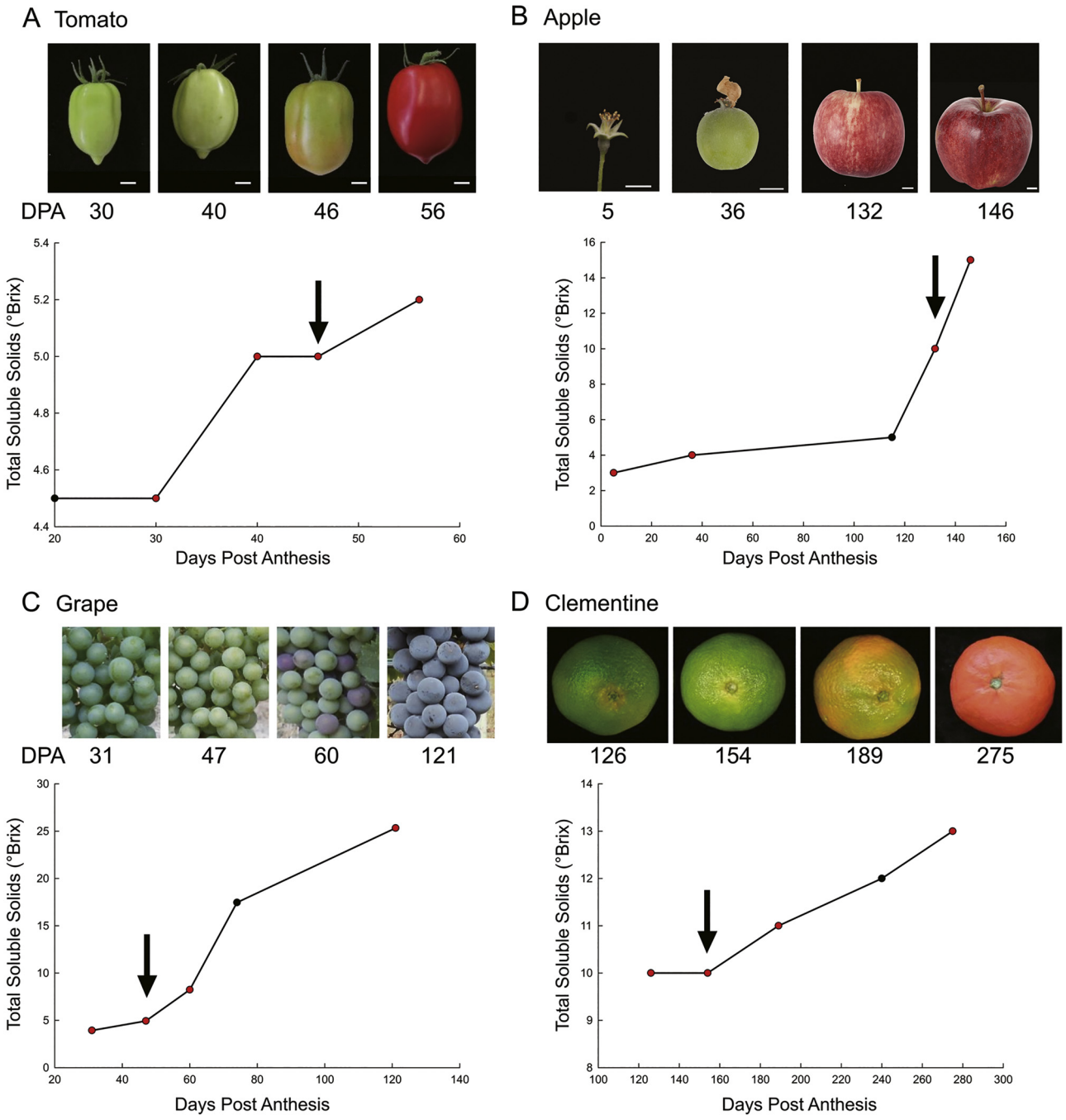

Fig. 5. Pictures of fruit sampled for RNAseq analyses, and Brix changes over these fruit development, red dots correspond to sampling dates for the RNAseq. The arrow shows the time at which the ETRs peak (according to Fig. 4 data). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ethylene signaling in the nucleus, and 2) there may be a direct interaction between ETRs and EIN2s, suggesting that CTRs may be by-passed in some cases [33,44].

There is only one copy of EIN2 in each fruit genome (Fig. 6A). Only apple has two *EIN2* genes, however, this is consistent with the ancestral apple whole genome duplication [22]. The fact that they are single copy genes could indicate that they are a critical point in the ethylene signaling pathway since mutations would potentially lead to the complete impairment of the signaling pathway. One can speculate that these mutations would be so deleterious that they could not be perpetuated. Further genetic studies would be interesting to understand how such a crucial element in ethylene signaling has been maintained as a single copy gene in these fruit species. EIN2s have 11–12 transmembrane domains within the first 500 amino acids of the N-terminal end (Supp. Table 3, and Supp. Fig. 5).

EIN2 expression increased over all fruit development (Supp. Fig. 4A). In tomato and clementine, there was a slight peak at the onset of fruit ripening, but not for apple and grape. In a previous study (qPCR data), two out of three apple cultivars exhibit an EIN2 peak around 100 dpa [39], thus the lack of sampling at this time in the RNAseq data we used may be a problem, as outlined above in the CTR paragraph. Nevertheless if EIN2 protein accumulation follows mRNA accumulation, the EIN2 increase over the fruit ripening period may compensate a decreasing sensitivity to ethylene. Once again, there was no clear distinction between climacteric and non-climacteric fruit regarding the EIN2 mRNA expression patterns.

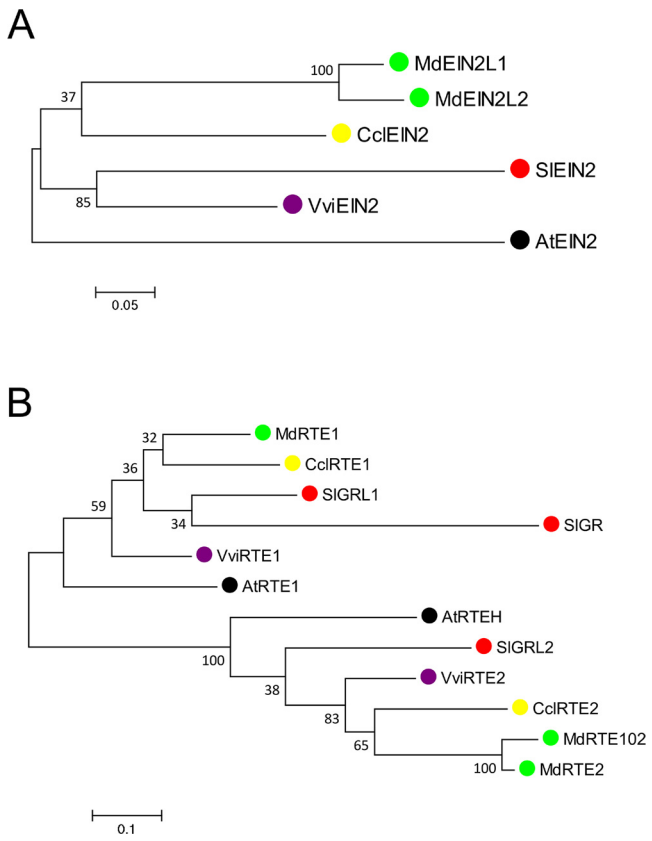

Fig. 6. Phylogenetic tree of A] EIN2s and B] GRLs/RTEs, constructed from a MUSCLE alignment by the maximum likelihood method.

3. What are the ETR-protein partners in climacteric and non-climacteric fruits?

3.1. ETRs' helpers: GRLs/RTEs

Green-Ripe Like (GRLs) or Reversion To Ethylene sensitivity (RTEs) are proteins mediating the ethylene receptor signal output, but their mode of action is still unknown. It was suggested that RTE1 might be involved in a conformational change of ETR1 important for its activity [45]. The *Green-Ripe* mutation in tomato, on *SlGR* gene, has been studied initially [46], while in the same year the role of a similar protein, RTE1, was elucidated in Arabidopsis [45]. Chang suggests that RTE1 may perform the oxidative folding of ETR1 [47]. Overexpression of Arabidopsis *RTE1* in tomato leads to a reduced ethylene sensitivity in some tissues, but ripening was not affected [48]. These authors studied the three homologs, SlGR, SlGRL1, and SlGRL2 in tomato. Their study suggests that each protein has a specific role in various ethylene responses in different tissues and development stages. For example, *SlGR* is highly expressed in seeds, *SlGRL1* is highly expressed in ripening fruit and in response to ethylene in leaves, and *SlGRL2* is moderately expressed in ripening fruit.

The phylogenetic tree (Fig. 6B) confirms previous observations for eudicot species [48]. Indeed, SlGRL1 is closely related to AtRTE1, VviRTE1, CclRTE1 and MdRTE1. These different orthologous proteins may have different roles. Sub-functionalization has been suggested previously [48]. SlGR is slightly different from AtRTE1 and SlGRL1, and has no strong ortholog in other fruit, whereas SlGRL2 is closely related to AtRTEH, VviRTE2, CclRTE2, MdRTE2 and MdRTE201. This second group may not be related to ethylene signaling [47,48]. This study showed that the *SlGR* gene is not highly expressed in tomato fruit, even though the original mutation in the 5'-flanking region leading to ectopic expression of the protein was associated to the non-ripening phenotype [46]. All proteins of the GRL/RTE family have either two or three transmembrane domains according to the predictions (Supp. Table 4). MdRTE1 harbors an 'excess' of 100 aa at the N-terminus before the consensus RTE sequence and its homolog, MdRTE101, harbors an excess of 1000 aa in N-terminus and thus was not considered for the TMpred analysis.

SlGRL1 peaks at the same time as *SlETR3* and *SlETR4* (Supp. Fig. 4), and it is noteworthy that all orthologs *SlGRL1, MdRTE1*, *MdRTE101*, *VviRTE2*, and *CclRTE1* showed an increase in mRNA levels (Supp. Fig. 4B) at the onset of ripening, concomitantly with the increased expression of *ETRs* (Fig. 4). This matches the potential role of RTEs/GRLs in complementing ETRs' function and provides insights into which ortholog(s) may be active in the different fruits studied here, climacteric or not. Notably, the expression data for *SlGR*, *SlGRL1*, and *SlGRL2* in the 'Heinz' tomato cultivar reported here is similar to the one reported in a qRT-PCR study that considered a different tomato cultivar, 'Ailsa Craig' [48].

3.2. Copper delivery to ETRs by RAN1

RAN1 (Response to ANtagonist 1) is a P-type ATPase localized in the Golgi membrane [2] and pumps cytosolic Cu and delivers it to membrane proteins such as ETR1 [49]. The copper ion is essential for the ethylene binding activity of the ethylene receptors [50]. These authors showed that RAN1 complements yeast mutants lacking a RAN1 ortholog (Δccc2). The absence of copper in the N-terminus of ETRs induces constitutive ethylene responses in Arabidopsis, as demonstrated by co-suppression of RAN1 [49] or in a *ran1* loss-of-function mutant [50]. RAN1 is also called HMA7 (Heavy Metal ATPase 7) [51], and is supposed to receive its copper from a metal chaperone, AtCCH1 [52].

To our knowledge, the RAN1 orthologs have not been studied in the four fruit examined here. The protein sequences found in the various fruit databases are all around 1000 amino acids. As in the case of *EIN2s*, the *RAN1s* are mostly single copy genes (Supp. Fig. 6A), except for apple, in which there are MdRAN1 and MdRAN2 due to a genome duplication as described above. All the RAN1 proteins harbor 8–11 putative transmembrane spanning domains (Supp. Table 5), which is consistent with their potential function as ATPase Cu-transporters. The expression profiles between climacteric and non-climacteric fruits (Supp. Fig. 7A) revealed no difference between these two classes of fruit. The Cu transport function is critical for many physiological processes over plant and fruit development [53] and that may be one reason for their constitutive expression.

3.3. The TPR1 may be an antagonist to ETRs

TPR1 (TetratricoPeptide Repeat 1) is a protein that is suggested to have an antagonist role to ETRs, either by competing with CTRs or by facilitating ETRs' degradation [14]. SlTPR1 is the ortholog of AtTRP1. Initial studies were in tomato and the gene was named *SlTPR1*, but the Arabidopsis ortholog [54,55] was named *AtTRP1*, as *AtTPR1* already existed. Both SlTPR1 and AtTRP1 were shown to interact with ETRs by yeast two-hybrid assays.

TPR1s are short proteins of 270 amino acids without detectable transmembrane domains. This suggests that the localization in membranes observed for SlTPR1 [54] is due to interaction with ETRs. As for with EIN2 and RAN1, TPR1s are also single copy genes (Supp. Fig. 6B), except in clementine, where two close homologs were found. CclTPR1 and CclTPR1L are 274 and 277 amino acids long respectively, and they share a consensus sequence of 198 amino acids (more than 70%). Contrary to all proteins described previously and involved in the ethylene perception complex, no transmembrane domain was found by TMpred for all five protein sequences except CclTPR1L (data not shown). CclTPR1L harbors a transmembrane domain, but in a region that is absent from CclTPR1. The analysis of protein sequences with the SMART tool revealed three tetratricopeptides motifs in all TPR1s from the four species (Supp. Fig. 8).

Regarding the mRNA transcription levels (Supp. Fig. 7A) all fruit TPR1s showed an increase during ripening, except in apple. This difference in *TPR1* expression could be a noticeable difference between apple and the other fruits. This data needs to be confirmed by more thorough expression studies.

3.4. Cytochromes b5, the potential ETR partners

Cyb5s are cytochromes that have recently been identified as RTE-1 binding proteins regulating ETRs [47], in particular the isoform D on which we will focus. Cyb5s seem to act upstream of RTEs and perform electron transfer in oxidation/reduction reactions. The C-terminal end of Cyb5 could interact with RTE1 leading to oxidative folding of ETR1. Alternatively, Cy5b may alter the composition of the membrane and thus the activity of ETR1.

To increase the probability of sorting cytochromes b5 among the 5 classes: A, B, C, D and E, a phylogenetic tree was constructed with the five Cyb5 classes found in Arabidopsis and the orthologs found in the four fruits studied here (Supp. Fig. 9). The D class is shown at the top of the tree. In the four fruit, there are orthologs of AtCyb5D, with the clementine ortholog being slightly more distant from the others (Supp. Fig. 9). However, they all harbor the typical heme/steroid binding domain of cytochromes (Supp. Fig. 6) and they all have a unique transmembrane domain confirmed by the TMpred analysis (Supp. Table 6). The expression data in tomato, apple, and clementine showed that particular Cyb5s (Supp. Fig. 7B) are roughly correlated with the increase in ETR at the onset of ripening (Fig. 4), suggesting that they may act in ethylene signaling. In apple, all three orthologs showed a similar pattern of expression so it is not possible to suggest that a specific Cyb5 ortholog is required for ethylene signaling. In clementine, the CclCyb5-D expression peaks over fruit ripening, but for grape there was no obvious correlation between VviCyb5-D and the ETR expression (Supp. Fig. 7B). More research is necessary in fruit regarding these Cyb5-Ds to validate their involvement in ripening.

4. Conclusions and perspectives

The phylogenetic analysis performed on the ethylene receptors and membrane localized proteins, as well as the determination of their mRNA accumulation levels on two climacteric (tomato and apple) and two non-climacteric fruits (grape and citrus), indicated that both climacteric and non-climacteric fruits share many aspects of ethylene perception and signaling during fleshy fruit ripening. Despite these similarities, we identified two remarkable differences: climacteric fruit possesses a higher number of ETR genes (7 in tomato, 8 in apple) than non-climacteric fruit (4 in grape, 4 in clementine), while this last class of fruit exhibits an earlier ETR expression peak coincident with the onset of sugar accumulation. This relation between *ETR* expression and sugar accumulation in climacteric and non-climacteric fruits needs further investigation. In all fruit, the ETR classification in subfamily 1 or subfamily 2 relies on the non-functional HATPase_c domain present in the subfamily 2 members. Both subfamilies are present in climacteric and non-climacteric fruits and subfamily does not seem to be a discriminant factor when considering the expression across ripening.

Regarding CTRs, the subfamily CRG (CTR1-Related Group) shows very low transcript accumulation during fruit development in the four species studied here. Until functional data is obtained the gene transcription data do not support a role for this gene family in ethylene signaling. In non-climacteric fruits, the EDRs show a higher accumulation over the course of fruit development than in climacteric fruits. Functional studies are necessary to determine the roles of these proteins in regulating fruit ripening.

EIN2 represents a key step in ethylene signaling and all species contain a single gene copy, except apple due to the ancestral whole genome duplication. The fact that such a critical gene is single copy in most fruit genomes deserves further study. *EIN2* expression tends to increase during ripening, suggesting an increasing ethylene signal during ripening that could be independent of the actual ethylene signal.

Regarding the protein partners of the ETR-CTR-EIN2 core, it is noticeable that GRLs-RTE1s follow an accumulation pattern somewhat similar to ETRs over fruit development, consolidating their expected role as ETR helpers. Finally, RAN1s, TRP1s, and Cyt5b-Ds are found in all fruits considered, but their transcript accumulation patterns do not reveal any major difference between climacteric and non-climacteric fruit.

Additionally, sampling on whole fruit tissues may mask differences between climacteric and non-climacteric fruit that exist at the level of specific cells and/or tissues. This would therefore necessitate further studies.

This review sheds light on the first steps of ethylene perception at the membrane level in four global fruit crops and establishes a basis for the annotation of ethylene-related genes. In addition to the differences between climacteric and non-climacteric fruits that have been highlighted here, there may be differences at the protein level that need further studies.

Availability of data and materials

All datasets used in this review paper are available to readers either in supplemental material or in publicly available repositories listed in the manuscript.

Conflict of interest

The research was conducted in the absence of any potential conflict of interest.

Acknowledgments

The authors thank the China Scholarship Council for the PhD fellowship to Yi Chen. We also thank Anis Djari, Elie Maza, Pierre Frasse, Mohamed Zouine and Mondher Bouzayen (GBF, ENSAT) for the development of the TomExpress data basis. Finally, thanks to the editor and two anonymous reviewers for their time and comments.

Appendix A. Supplementary data

References

[1] R.F. Lacey, B.M. Binder, How plants sense ethylene gas—the ethylene receptors, J. Inorg. Biochem. 133 (2014) 58–62.
[2] C. Ju, C. Chang, Mechanistic insights in ethylene perception and signal transduction, Plant Physiol. 169 (2015) 85–95.
[3] H. El-Maarouf-Bouteau, et al., Reactive oxygen species, abscisic acid and ethylene interact to regulate sunflower seed germination, Plant Cell Environ. 38 (2015) 364–374.
[4] I.H. Street, et al., Ethylene inhibits cell proliferation of the Arabidopsis root meristem, Plant Physiol. 169 (2015) 338–350.
[5] F. Wang, X. Cui, Y. Sun, C.H. Dong, Ethylene signaling and regulation in plant growth and stress responses, Plant Cell Rep. 32 (2013) 1099–1109.
[6] V.A. Bapat, P.K. Trivedi, A. Ghosh, V.A. Sane, T.R. Ganapathi, P. Nath, Ripening of fleshy fruit: molecular insight and the role of ethylene, Biotechnol. Adv. 28 (2015) 94–107.
[7] S. Cherian, C.R. Figueroa, H. Nair, "Movers and shakers" in the regulation of fruit ripening: a cross-dissection of climacteric versus non-climacteric fruit, J. Exp. Bot. 65 (2014) 4705–4722.
[8] J.P. Fernández-Trujillo, J.M. Obando-Ulloa, J.A. Martínez, E. Moreno, J. García-Mas, A.J. Monforte, Climacteric and non-climacteric behavior in melon fruit. 2. Linking climacteric pattern and main postharvest disorders and decay in a set of near-isogenic lines, Postharv. Biol. Technol. 50 (2008) 125–134.
[9] P.A. McAtee, et al., The hybrid non-ethylene and ethylene ripening response in kiwifruit (Actinidia chinensis) is associated with differential regulation of MADS-box transcription factors, BMC Plant Biol. 15 (2015) 304.
[10] N. Abdi, W.B. McGlasson, P. Holford, M. Williams, Y. Mizrahi, Responses of climacteric and suppressed-climacteric plums to treatment with propylene and 1-methylcyclopropene, Postharv. Biol. Technol. 14 (1998) 29–39.

[11] L. Trainotti, A. Pavanello, G. Casadoro, Different ethylene receptors show an increased expression during the ripening of strawberries: does such an increment imply a role for ethylene in the ripening of these non-climacteric fruits? J. Exp. Bot. 56 (2005) 2037–2046.
[12] C. Chervin, A. El-Kereamy, J.P. Roustan, A. Latché, J. Lamon, M. Bouzayen, Ethylene seems required for the berry development and ripening in grape, a non-climacteric fruit, Plant Sci. 167 (2004) 1301–1305.
[13] Y.P. Gong, X.T. Fan, J.P. Mattheis, Responses of' Bing' and' Rainier' sweet cherries to ethylene and 1-methylcyclopropene, J. Am. Soc. Hortic. Sci. 127 (2002) 831–835.
[14] Z. Lin, S. Zhong, D. Grierson, Recent advances in ethylene research, J. Exp. Bot. 60 (2009) 3311–3336.
[15] A. Bakshi, J.M. Shemansky, C. Chang, B.M. Binder, History of research on the plant hormone ethylene, J. Plant Growth Regul. 34 (2015) 809–827.
[16] S.N. Shakeel, X. Wang, B.M. Binder, G.E. Schaller, Mechanisms of signal transduction by ethylene: overlapping and non-overlapping signalling roles in a receptor family, AoB Plants 5 (2013) plt010.
[17] B.M. Kevany, M.G. Taylor, H.J. Klee, Fruit-specific suppression of the ethylene receptor LeETR4 results in early-ripening tomato fruit, Plant Biotechnol. J. 6 (2008) 295–300.
[18] M. Liu, J. Pirrello, C. Chervin, J.P. Roustan, M. Bouzayen, Ethylene control of fruit ripening: revisiting the complex network of transcriptional regulation, Plant Physiol. 169 (2015) 2380–2390.
[19] H.S. Ireland, et al., Mining the apple genome reveals a family of nine ethylene receptor genes, Postharv. Biol. Technol. 72 (2012) 42–46.
[20] C. Chervin, L. Deluc, Ethylene signalling receptors and transcription factors over the grape berry development: gene expression profiling, Vitis 49 (2010) 129–136.
[21] R.C. O'Malley, F.I. Rodriguez, J.J. Esch, B.M. Binder, P. O'Donnell, H.J. Klee, A.B. Bleecker, Ethylene-binding activity, gene expression levels, and receptor system output for ethylene receptor family members from Arabidopsis and tomato, Plant J. 41 (2005) 651–659.
[22] R. Velasco, et al., The genome of the domesticated apple (Malus × domestica Borkh.), Nat. Genet. 42 (2010) 833–839.
[23] N. Daccord, et al., High-quality de novo assembly of the apple genome and methylome dynamics of early fruit development, Nat. Genet. 49 (2017) 1099–1106.
[24] R.J. Schaffer, H.S. Ireland, J.J. Ross, T.J. Ling, K.M. David, SEPALLATA1/2-suppressed mature apples have low ethylene, high auxin and reduced transcription of ripening-related genes, AoB Plants 5 (2013) pls047.
[25] J. Haifeng, et al., Abscisic acid and sucrose regulate tomato and strawberry fruit ripening through the abscisic acid-stress-ripening transcription factor, Plant Biotech. J. 14 (2016) 2045–2065.
[26] G.A. Gambetta, M.A. Matthews, T.H. Shaghasi, A.J. McElrone, S.D. Castellarin, Sugar andabscisic acid signaling orthologs are activated at the onset of ripening in grape, Planta 232 (2010) 219–234.
[27] D.J. Iglesias, F.R. Tadeo, F. Legaz, E. Primo-Millo, M. Talon, In vivo sucrose stimulation of colour change in citrus fruit epicarps: Interactions between nutritional and hormonal signals, Physiol. Plant. 112 (2001) 244–250.
[28] B.M. Kevany, D.M. Tieman, M.G. Taylor, V.D. Cin, H.J. Klee, Ethylene receptor degradation controls the timing of ripening in tomato fruit, Plant J. 51 (2007) 458–467.
[29] Y. Kamiyoshihara, D.M. Tieman, D.J. Huber, H.J. Klee, Ligand-induced alterations in the phosphorylation state of ethylene receptors in tomato fruit, Plant Physiol. 160 (2012) 488–497.
[30] R.L. Wilson, H. Kim, A. Bakshi, B.M. Binder, The ethylene receptors ETHYLENE RESPONSE1 and ETHYLENE RESPONSE2 have contrasting roles in seed germination of Arabidopsis during salt stress, Plant Physiol. 165 (2014) 1353–1366.
[31] D.M. Tieman, M.G. Taylor, J.A. Ciardi, H.J. Klee, The tomato ethylene receptors NR and LeETR4 are negative regulators of ethylene response and exhibit functional compensation within a multigene family, Proc. Natl. Acad. Sci. 97 (2000) 5663–5668.
[32] S. Zhong, Z. Lin, D. Grierson, Tomato ethylene receptor–CTR interactions: visualization of NEVER-RIPE interactions with multiple CTRs at the endoplasmic reticulum, J. Exp. Bot. 59 (2008) 965–972.
[33] M.M.A. Bisson, et al., Peptides interfering with protein-protein interactions in the ethylene signaling pathway delay tomato fruit ripening, Sci. Rep. 6 (2016) 30634.
[34] C. Ju, et al., CTR1 phosphorylates the central regulator EIN2 to control ethylene hormone signaling from the ER membrane to the nucleus in Arabidopsis, Proc. Natl. Acad. Sci. 109 (2012) 19486–19491.
[35] Y. Yasumura, R. Pierik, S. Kelly, M. Sakuta, L.A. Voesenek, N.P. Harberd, An ancestral role for CONSTITUTIVE TRIPLE RESPONSE1 proteins in both ethylene and abscisic acid signaling, Plant Physiol. 169 (2015) 283–298.
[36] X. Shen, H. Liu, B. Yuan, X. Li, C. Xu, S. Wang, OsEDR1 negatively regulates rice bacterial resistance via activation of ethylene biosynthesis, Plant Cell Environ. 34 (2011) 179–191.
[37] L. Adams-Phillips, C. Barry, J.J. Giovannoni, Signal transduction systems regulating fruit ripening, Trends Plant Sci. 9 (2004) 331–338.
[38] J. Leclercq, et al., LeCTR1, a tomato CTR1-like gene, demonstrates ethylene signaling ability in Arabidopsis and novel expression patterns in tomato, Plant Physiol. 130 (2002) 1132–1142.
[39] V. Singh, A. Weksler, H. Friedman, Different preclimacteric events in apple cultivars with modified ripening physiology, Front. Plant Sci. 8 (2017) 1502.
[40] J.M. Alonso, T. Hirayama, G. Roman, S. Nourizadeh, J.R. Ecker, EIN2, a bifunctional transducer of ethylene and stress responses in Arabidopsis, Science 284 (1999) 2148–2152.
[41] P. Guzmán, J.R. Ecker, Exploiting the triple response of Arabidopsis to identify ethylene-related mutants, Plant Cell 2 (1990) 513–523.
[42] H. Qiao, et al., Processing and subcellular trafficking of ER-Tethered EIN2 control response to ethylene gas, Science 338 (2012) 390–393.
[43] W. Li, et al., EIN2-directed translational regulation of ethylene signaling in Arabidopsis, Cell 163 (2015) 670–683.
[44] M.M.A. Bisson, G. Groth, Targeting plant ethylene responses by controlling essential protein-protein interactions in the ethylene pathway, Mol. Plant 8 (2015) 1165–1174.
[45] J.S. Resnick, C.K. Wen, J.A. Shockey, C. Chang, REVERSION-TO-ETHYLENE SENSITIVITY1,a conserved gene that regulates ethylene receptor function in Arabidopsis, Proc. Natl. Acad. Sci. 103 (2006) 7917–7922.
[46] C.S. Barry, J.J. Giovannoni, Ripening in the tomato green-ripe mutant is inhibited by ectopic expression of a protein that disrupts ethylene signaling, Proc. Natl. Acad. Sci. 103 (2006) 7923–7928.
[47] J. Chang, J.M. Clay, C. Chang, Association of cytochrome b5 with ETR1 ethylene receptor signaling through RTE1 in Arabidopsis, Plant J. 77 (2014) 558–567.
[48] Q. Ma, W. Du, F. Brandizzi, J.J. Giovannoni, C.S. Barry, Differential control of ethylene responses by GREEN-RIPE and GREEN-RIPE LIKE1 provides evidence for distinct ethylene signaling modules in tomato, Plant Physiol. 160 (2012) 1968–1984.
[49] T. Hirayama, et al., RESPONSIVE-TO-ANTAGONIST1, a Menkes/Wilson disease-related copper transporter, is required for ethylene signaling in Arabidopsis, Cell 97 (1999) 383–393.
[50] B.M. Binder, F.I. Rodríguez, A.B. Bleecker, The copper transporter RAN1 is essential for biogenesis of ethylene receptors in Arabidopsis, J. Biol. Chem. 285 (2010) 37263–37270.
[51] M. Zimmermann, et al., Metal binding affinities of Arabidopsis zinc and copper transporters: selectivities match the relative, but not the absolute, affinities of their amino-terminal domains, Biochemistry 48 (2009) 11640–11654.
[52] K.E. Woeste, J.J. Kieber, A strong loss-of-function mutation in RAN1 results in constitutive activation of the ethylene response pathway as well as a rosette-lethal phenotype, Plant Cell 12 (2000) 443–455.
[53] M. Pilon, Moving copper in plants, New Phytol. 192 (2011) 305–307.
[54] Z. Lin, et al., SlTPR1, a tomato tetratricopeptide repeat protein, interacts with the ethylene receptors NR and LeETR1, modulating ethylene and auxin responses and development, J. Exp. Bot. 59 (2008) 4271–4287.
[55] Z. Lin, C.W. Ho, D. Grierson, AtTRP1 encodes a novel TPR protein that interacts with the ethylene receptor ERS1 and modulates development in Arabidopsis, J. Exp. Bot. 60 (2009) 3697–3714.
[56] L. Su, et al., Carotenoid accumulation during tomato fruit ripening is modulated by the auxin-ethylene balance, BMC Plant Biol. 15 (2015) 114.
[57] H. Zegzouti, et al., Ethylene-regulated gene expression in tomato fruit: characterization of novel ethylene-responsive and ripening-related genes isolated by differential display, Plant J. 18 (1999) 589–600.
[58] M. Wang, J. Cao, L. Lin, J. Sun, W. Jiang, Effect of 1-methylcyclopropene on nutritional quality and antioxidant activity of tomato fruit (Solanum Lycopersicon L.) during storage, J. Food Qual. 33 (2010) 150–164.
[59] T. Li, et al., Apple MdACS6 regulates ethylene biosynthesis during fruit development involving ethylene-responsive factor, Plant Cell Physiol. 56 (2015) 909–1917.
[60] X. Yang, J. Song, L. Campbell-Palmer, S. Fillmore, Z. Zhang, Effect of ethylene and 1-MCP on expression of genes involved in ethylene biosynthesis and perception during ripening of apple fruit, Postharv. Biol. Technol. 78 (2013) 55–66.
[61] A. Rizzolo, M. Grassi, M. Vanoli, Influence of storage (time, temperature, atmosphere) on ripening, ethylene production and texture of 1-MCP treated "Abbé Fétel" pears, Postharv. Biol. Technol. 109 (2015) 20–29.
[62] S.K. Whale, Z. Singh, M.H. Behboudian, J. Janes, S.S. Dhaliwal, Fruit quality in "Cripp"s Pink' apple, especially colour, as affected by preharvest sprays of aminoethoxyvinylglycine and ethephon, Sci. Hortic. 115 (2008) 342–351.
[63] L. Li, T. Kaplunov, Y. Zutahy, A. Daus, R. Porat, A. Lichter, The effects of 1- methylcyclopropene and ethylene on postharvest rachis browning in table grapes, Postharv. Biol. Technol. 107 (2015) 16–22.
[64] R.J. Weaver, R. Montgomery, Effect of Ethephon on Coloration and Maturation of Wine Grapes, Am. J. Enol. Vitic. 25 (1974) 39–41.
[65] E. Katz, P.M. Lagunes, J. Riov, D. Weiss, E.E. Goldschmidt, Molecular and physiological evidence suggests the existence of a system II-like pathway of ethylene production in non-climacteric citrus fruit, Planta 219 (2004) 243–252.
[66] E. Alós, G. Distefano, M.J. Rodrigo, A. Gentile, L. Zacarías, Altered sensitivity to ethylene in "Tardivo", a late-ripening mutant of Clementine mandarin, Plant Physiol. 151 (2014) 507–521.
[67] R. Porat, B. Weiss, L. Cohen, A. Daus, R. Goren, S. Droby, Effects of ethylene and 1-methylcyclopropene on the postharvest qualities of 'Shamouti' oranges, Postharv. Biol. Technol. 15 (1999) 155–163.