Melon, an alternative model plant for elucidating fruit ripening

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Abstract

Ethylene perception has been studied using Arabidopsis and tomato as model plants during last two decades. Arabidopsis has been an ideal model system for gene identification and subsequent functional analysis of the identified gene. On the other hand, tomato is not only the model of choice to study climacteric fruit ripening but also crops of agronomic importance and hence has been at the forefront of the comparative analysis with Arabidopsis. A number of fruit development and ripening studies in melon has been conducted by many laboratories in the last decade, leading to the accumulation of a great deal of information. These include genetic transformation techniques, isolation of related genes, physiological information and genetics resources. The information accumulated has enabled melon to carve a niche for itself as an alternative model system for fruit for studies. In addition, International Cucurbit Genomics Initiative (ICuGI) was launched 2005, in which melon became a model species in Cucurbit genomics research. In next decade, genomic resources including large collection of ESTs, precise maps and so on will be gathered, indicating that melon will be an alternative model plant for studying fruit ripening in addition to ethylene perception and signaling. In this review, we will summarize the information accumulated so far and discuss the perspectives.

Keywords: Melon; Ethylene perception; Signaling; Functional genomics

1. Introduction

Plant hormones are signaling molecules that influence numerous aspects of growth and development at a tissue and organ level by communicating between cells. They function as signals that integrate internal developmental and external environmental inputs and translate them into appropriate responses. Hormone regulation can occur at the level of synthesis, transport, uptake, and turnover of the hormone. Regulation can also occur at the level of perceptions or signal transduction. Any understanding of how the growth and developmental complexities are initiated by these simple molecules requires an understanding of how they are synthesized, perceived and transduced.

The plant hormone ethylene is known to regulate multiple physiological and developmental processes in plants, such as leaf and flower senescence, fruit ripening, organ abscission and growth transition from vegetative phase to reproductive phase, and is also involved in the reactions of plants to abiotic and biotic stresses [1-4]. The mechanisms by which the ethylene signal is perceived and transduced to mediate phenotypic responses is not fully understood. However many elegant studies exploiting genetic screens in the model plant *Arabidopsis* led to the identification of critical components of this signaling pathway. Mutations were identified that increased or decreased the response of a whole plant to the hormone ethylene. These mutants include those that are insensitive to the ethylene receptors *etr1* (ethylene response) [5-7]. Cloning and characterization of genes disrupted in these mutants have defined a mostly linear pathway for ethylene signal transduction leading from initial hormone perception to transcriptional regulation. This not only allowed genetic and biochemical studies but also initiated a comprehensive comparison of how multicellular plants, which most

likely evolved independently use the same hormone to organize growth and development

Arabidopsis has been and is still the ideal model plant for many genetic and molecular analyses and it would have been impossible to assemble such a comprehensive understanding of the individual components of the ethylene perception in any other plant species. Arabidopsis has been ideal as a model system for gene identification and subsequent functional analysis of the identified gene. The Arabidopsis silique is a dehiscent fruit characteristic of the legumes and thus represents an important fruit type in terms of human food. The ripening of dehiscent fruit such as Arabidopsis silique is facilitated by separation of the valves at an abscission layer (termed the dehiscence zone) that is formed between the valve-replum boundary. Unlike Arabidopsis, fleshy fruits undergo ripening process in which the biochemistry, physiology and structure of the organ are developmentally altered to influence appearance, texture, flavour and aroma in ways designed to attract seed-dispersing organisms (8). The specific biochemical programs culminating in ripening differ from one fleshy fruit to another. The importance of ethylene in regulating traits of agronomic importance, particularly fruit ripening has driven research on the identification and functional characterization of components of the ethylene signaling pathway in crop species.

Climacteric fruits including apple, avocado, banana, passion fruit, melon, peach etc differ from non-climacteric fruits in their ability to continue to ripen after harvest [1]. The ripening of climacteric fruit features autocatalytic ethylene synthesis stage, which is preceded by a rapid increase in respiration followed by a series of developmental

processes that allow plants to produce edible fruit and disperse seeds [8]. The tomato fruit is the model of choice to study climacteric fruit ripening and has also been at the forefront of the comparative analysis with Arabidopsis. Though there are significant differences in the way ethylene signaling is regulated in Arabidopsis and tomato, the building blocks of ethylene signal transduction are very similar between the species. The Tomato is the model system of choice for studying the changes during ripening of fleshy fruit due to its commercial importance, a rich source of genetic and biochemical information, relatively small genome, relative ease of genetic transformation and availability of developmental mutants that are ripening impaired.

Some of the pleiotropic tomato mutants that have aided the elucidation of ripening control mechanisms include; *ripening-inhibitor* (*rin*), *Never-ripe (Nr), non-ripening* (*nor*), *Green-ripe* and *Colorless non-ripening* (*Cnr*). These mutants can be grouped into two classes based on their ability to synthesize ethylene during ripening and for the ability of ethylene to restore ripening-related gene expression. The first class is characterized by the *rin, nor,* and *Cnr* loci. Ethylene synthesis does not increase during ripening in fruits of these mutants but remains at a baseline level. However, treatment of mutant fruits with ethylene will restore some ethylene-regulated gene expression but not fruit ripening. For these reasons, it has been proposed that these loci regulate an ethylene-independent component of fruit ripening and may govern the competency of the fruits to ripen [9, 10]. The *rin, Cnr and nor* loci encode putative transcription factors demonstrating that other factors, act upstream of ethylene and their control of ripening process is no less important that that played by the hormone ethylene [11]. The second class of ripening mutant is characterized by *Gr* and the ethylene receptor mutant *Nr*.

These mutants produce ethylene during ripening, but the expression of ripening-related genes is greatly reduced and cannot be restored by ethylene treatment, indicating that the physiological basis of ripening inhibition is due to reduced ethylene sensitivity. It is these genomic studies in tomato that have led to the hypothesis that ripening in climacteric fruits is regulated by both ethylene dependent and independent pathways [8]. In contrast to the amount of information regarding the regulation of ripening in climacteric fruits, much less is known about non-climacteric ones. To date, no single growth regulator appears to play a positive role analogous to the role played by ethylene in the ripening of climacteric fruits. Strawberry is the most widely studied system for nonclimacteric fruit ripening and has been useful in identification and characterization of numerous ripening-related genes that affect cell wall metabolism, color, and aroma [12-14]. However the possible role of ethylene produced during the ripening phase of strawberries is yet to be elucidated despite the accumulation of ethylene biosynthesis and ethylene receptor genes during ripening of these fruits [15].

Notwithstanding, evolutionary processes have resulted in a variety of developmental manifestations of structures of fleshy fruit that differ in design and function. Because of differences in structural design, biochemistry and physiology among fleshy fruit species, it would be improper to extrapolate findings in tomato as universal in other climacteric fruit species. Therefore, the use of alternative model plant systems that differ from tomato in quantitative and/or qualitative aspects of ripening are necessary in providing novel insight into the general ripening processes and in explaining the ripening process disparity that exists among the fruit species. Melon is an ideal alternative model fruit for studies on ethylene perception and sensitivity due to its agronomic importance; its

development has distinct stages; the flesh, embryo, placenta and seeds are well ordered; the fruit development can be clearly divided into ethylene-insensitive and ethylene sensitive stage and the developing fruit has a lower sensitivity to ethylene than does the ripening fruit, availability of both climacteric and non-climacteric types, availability of genetic and genomic resources including EST collections, BAC library and cDNA arrays [16, 17, http://cucurbit.bti.cornell.edu/]. For instance, usefulness of melon as an alternative model system to study fruit ripening was demonstrated by experiments examining the temporal sequence of cell wall disassembly during ripening. Because of the distinct differences in softening of melon and tomato, Charentais melon provided information on the molecular events associated with the disassembly of each cell wall polymer [18].

2. Melon ripening and ethylene

Fruit ripening is a genetically programmed event that is characterized by a series of physiological and biochemical changes that alter fruit color, texture, firmness, aroma and flavor, making fruit scent and flavor appealing to consumers. In many fruits such as apple, tomato, avocado and melon, ripening is associated with a transient increase in respiration and autocatalytic ethylene production. It is generally thought that ethylene induces the increased respiration when fruit reaches the appropriate stage [19]. The presence or absence of the respiratory burst or climacteric has been used to classify fruits into climacteric fruits or non-climacteric fruits [1]. Melon fruit varieties exhibit different respiratory climacteric pattern. For instance the *cantaloupensis* and *reticulatus* varieties tend to have a shorter respiratory climacteric [20], whereas the respiratory climacteric of *inodorus* and *saccharinus* varieties tend to be prolonged or it may be absent [21, 22]. The respiration rate of melons appeared to depend on the amount of

time between harvest and gas sampling. When the Honeydew melons (*inodorus* variety) were harvested at full size and held in storage, they went through a characteristic increase in carbon dioxide and ethylene production [23]. However, when carbon dioxide and ethylene production were measured shortly after harvest, there was the expected rise in ethylene, but no concomitant rise in carbon dioxide production [24]. The respiratory climacteric was observed in harvested but not on the vine 'Caravelle', 'Mission' and 'Explorer' netted muskmelons, suggesting that the climacteric might be an artifact of harvest and not a natural phenomenon associated with ripening of climacteric fruit [25]. However, Charentais melons (*reticulatus* variety) attached to the plant exhibited a respiratory climacteric during ripening that is equal in magnitude to the respiratory climacteric of harvested melons. The ripening associated increase in ethylene production as wells as the respiratory climacteric occurred in the charentais cultivar while fruit were ripened attached to the plant discounting the idea that the detachment of fruit must precede the rise in carbon dioxide concentration that characterizes climacteric fruit [25].

Divergent ethylene production rates in selected melon genotypes have been documented. In the orange fleshed varieties (*cantaloupensis* group), there is a burst of ethylene production concurrent with fruit maturity and abscission [25, 26]. In contrast, green and white fleshed honeydew and other long shelf-life varieties (*inodorus* group) are generally lower ethylene producers, and generally do not form an abscission zone at marketable maturity. The *reticulatus* melon variety (netted melon) produce considerable quantities of climacteric ethylene at or to close harvest whereas in the *inodorus* variety (non netted melon) climacteric ethylene production delays and tend to occur as late as 20 days after harvest [27]. The ethylene production of melon has been suggested to

correlate with the rind type. The surface meshworks (‘net’) found in melon epidermis consist of an elaborate system of lenticels derived from the subepidermal periderm [28]. The netted rind was reported to be associated with higher ethylene production and could be as a result of enhanced gas exchange of the melon mesocarp afforded by the lenticels. The *inodorus* fruit types on the other hand may require postharvest exogenous ethylene treatment, in order to obtain a more uniform and rapid ripening, as well as better development of color, wax, and aroma [29]

Ethylene is a dominant hormonal trigger for ripening of climacteric fruit and both ethylene-dependent and ethylene independent regulatory pathways coexist to coordinate the ripening process in melon fruit. Thus there are some physiological processes during ripening which have been defined to be dependent on ethylene whereas others are either ethylene independent or extremely sensitive to low levels of ethylene. Charentais melon fruit undergo a ripening associated decrease in fruit firmness accompanied by a dramatic increase in internal ethylene [18]. Treatment of melon fruit with 1-Methylcyclopropene (1-MCP), an inhibitor of ethylene action completely halted the subsequent softening process indicating that the overall process of cell wall disassembly in ripening charentais melons is ethylene regulated [30]. However there is considerable variation between patterns of regulation of divergent families of wall-modifying proteins and between individual members of those families.

Climacteric respiration, formation of the peduncular abscission zone, yellowing and carotenoid content of the rind, in Charentais type Cantaloupe melon (cv. Védrantais) and in ‘Galia’ male parental line (cv. Krymka)are processes that are ethylene-dependent [31-33]. Membrane deterioration and volatiles synthesis have been described as partially dependent on ethylene [32, 34].Other biochemical processes

during ripening of melon such as sugar accumulation, titratable acidity, organic acid metabolism, total soluble solids (TSS), fruit weight and size, seed number, mesocarp size, and carotenoid content in the flesh of melons have been described to be ethylene-independent [31,33]

3. Isolation and characterization of genes related to ethylene biosynthesis in melon

The ethylene biosynthetic pathway is well established [35]. Ethylene biosynthesis is initiated from the amino acid, methionine, via S-adenosylmethionine (Adomet) through the intermediate 1-aminocyclopropane-1-carboxylic acid (ACC) controlled by ACC synthase (ACS) that catalyzes the conversion of Adomet to ACC, and ACC oxidase that catalyzes the conversion of ACC to ethylene (Fig. 1). The regulation of the expression of the main ethylene biosynthetic genes, ACC synthase and ACC oxidase has been described in many species and tissues [36, 37]. The onset of ethylene production in ripening climacteric fruits is known to be associated with increased activities of ACC synthase and ACC oxidase [38]. However, the regulation of the ethylene climacteric in fruit development and ripening is far more complicated. Investigations into the molecular basis of ethylene in melon were initially devoted to the isolation of genes involved in the ethylene biosynthetic pathway. Several ACC synthase and ACC oxidase isoenzymes have been identified in melon tissues and both enzymes are encoded by multigene family [39-41], as shown in Table 1.

To date five ACC synthase genes and three ACC oxidase genes have been isolated from the melon fruit [39-43]. The *CMe-ACS1* gene is wound responsive and is expressed mainly in the mesocarp tissues in the ripening fruit and most likely plays a key role in the increases production of ethylene at the ripening stage [40]. The *CMe-ACS2* gene was isolated from auxin-treated etiolated seedlings [43]. The

CMe-ACS2 gene expression was highest 3 days after pollination, being maintained for at least 12 days during the period when the melon fruit was rapidly developing, suggesting its induction by auxin during this period of rapid fruit growth [43]. The *CMe-ACS2* gene expression was transiently detected in fruit at the preclimacteric stage which declined at the time of harvest. In order to understand the mechanisms regulating *CMe-ACS2* expression, Ishiki et al, [43] investigated the *CMe-ACS2* promoter sequence and suggested that the promoter region of *CMe-ACS2* contains several putative auxin-responsive elements (AuxRDs). In another study, Mizuno et al., [44] also found a DNA-binding motif GCCGAC sequence located upstream of the transcription initiation site of *CMe-ACS2*. The GCCGAC sequence is a dehydration responsive element/C-repeat (DRE/CRT) cis-acting element, which is involved in cold, drought and high salt stress responsiveness. The DRE-binding protein1/CRT binding factor (DREB1/CBF) and DREB2 transcription factors bind to the DRE/CRT element and induce the expression of some DRE/CRT downstream genes, and then enhance cold and osmotic stress tolerance in plants. Other stress-inducible transcription factor members also include the ethylene-responsive element binding factor (ERF) family [45]. In order to isolate cDNAs encoding transcription factors that interact with the GCCGAC sequence in the *CMe-ACS2* promoter, three melon transcription factors were isolated (*CMe-DREB1*, *CMe-ERF1* and *CMe-ERF2*) using the yeast one hybrid screening method [44]. This study suggested that *CMe-DREB1* may function as a transcriptional activator of *CMe-ACS2* through the GCCGAC sequence in the presence of jasmonate, and that *CMe-ERF1* and *CMe-ERF2* are unlikely involved in the expression of *CMe-ACS2*. The *CM-ACS3* is also an auxin-responsive gene and its expression pattern was similar to that of *CMeACS2* in the melon fruit at the preclimacteric stage, although

the *CMe-ACS3* expression level was lower than that of *CMe-ACS2*. The role of *CMe-ACS4* has been reported not to be clear, whereas *CMe-ACS5* has been reported to be highly expressed in ripening fruit, but is ethylene independent [46].

The *CM-ACO1* gene expression was induced during fruit ripening, and also in response to wounding and ethylene treatment in leaves. The *CM-ACO2* gene expression was detected at low levels in etiolated hypocotyls, whereas *CM-ACO3* was expressed in flowers and was not induced by any treatment tested [40, 42, 47]. Shiomi et al [48] compared the ethylene biosynthetic activity and expression patterns of ACS and ACO genes by using two different netted melon cultivars, ‘Andes’ and ‘Earl’s Favourite’ which differ in their ethylene biosynthesis capability during ripening. ‘Andes’ melon fruit produce a considerable amount of ethylene and tend to have a shorter shelf-life, compared to ‘Earl’s Favourite’ fruit which produce a lesser amount of ethylene. The *CMe-ACS1* transcripts accumulated in the mesocarp and placenta of ‘Andes’ fruit harvested at commercial harvest maturity, but not in any of the tissues of ‘Earl’s Favourite’ at all harvest maturities. The *Cm-ACO1* transcript accumulated in the mesocarp and placenta of both cultivars, whereas *Cm-ACO2* was constitutively expressed. The *CMe-ACS1* transcript was induced by ethylene in preclimacteric ‘Andes’ but not ‘Earl’s Favourite’. 1-Methylcyclopropene inhibited the accumulation of *CMe-ACS1*, *CMe-ACO1* and *CMe-ACO2* in ‘Andes’. They suggested that the ‘Earl’s Favourite’ fruit behaved like non-climacteric fruit and that the main difference between the two cultivars in the ethylene-forming capability results from the expression of *CMe-ACS1* gene during ripening. The melon *CM-ACO1* expression is also induced by ethylene treatment or upon wounding, and functional studies of the *CM-ACO1* promoter suggested that wound and ethylene induction of this gene occurs via two direct and

independent transduction pathways [49].

Studies carried out to elucidate the molecular genetics of ethylene production in melon revealed strong evidence for linkage of RFLPs of the ACC oxidase and ACC synthase genes to ethylene production in two melon cultivars were reported by Zheng et al., [50]. The ‘TAM Uvalde’ cultivar had high ethylene production whereas ‘TAM Yellow Canary’ cultivar had low levels of ethylene production. Their single-copy-reconstruction assays suggested that the *CMACO-1* was a single copy gene, whereas the *CMACS-1* gene was a component of a multigene family in both melon cultivars. These results could be useful in mapping the quantitative trait loci (QTLs) of ethylene production and the RFLPs may be used in marker-assisted selection in developing melons with a more desirable low ethylene production rate for enhancing postharvest life.

4. Isolation and characterization of genes related to ethylene perception and signaling in melon

In order to understand the regulation of the various physiological and biochemical processes by ethylene, there is the need to study both the biosynthesis and perception of this hormone. Genetic studies on mutants exhibiting altered responses to ethylene in Arabidopsis *(Arabidopsis thaliana*) have established a linear signal transduction pathway at the early steps in transducing the ethylene signal in plants [6, 51, 52]. These studies have revealed that after ethylene is synthesized, the signal is perceived and then transduced via a transduction machinery to trigger specific biological responses [53].

In Arabidopsis, ethylene is perceived by a family of five membrane-bound receptors (ETR1,ERS1,ETR2, EIN4 and ERS2) which transmit the signal to

downstream effectors. Based on distinguishing structural features and overall sequence similarity, the members of the ethylene receptor family in Arabidopsis can be divided into two subfamilies: subfamily I and II [54]. Subfamily I ethylene receptors (ETR1 and ERS1) have three transmembrane domains and a well-conserved histidine kinase domain. On the other hand, Subfamily II (ETR2, EIN4 and ERS2) receptors contain a putative signal peptide in addition to the three conserved transmembrane domains and a histidine kinase domain that lacks one or more elements that are necessary for catalytic activity. The involvement of ethylene and its receptors in the ripening of climacteric fruits has been reaffirmed in several fruits even though it has also emerged that the role played by ethylene is not exclusive, since ethylene-independent pathways are also involved in the ripening process [8].

In muskmelon, three ethylene receptor genes have been isolated and characterized (Table 1). Cm-ERS1 and Cm-ETR1 structurally belong to Subfamily 1 whereas Cm-ETR2 belongs to Subfamily II ethylene receptors [55, 56]. Gene expression analyses showed that the *Cm-ERS1* mRNA level increased during enlargement of young fruits and decreased at the end of enlargement, whereas the level of *Cm-ETR1* mRNA was high in the seed and placenta of developing and fully enlarged fruit and increased concurrent with the beginning of ethylene production during ripening, suggesting that each of these proteins has a specific role in fruit development [55]. However, it was unclear whether the amount of ethylene receptor mRNA accurately reflects the level of the corresponding proteins. The melon subfamily II ethylene receptor *Cm-ETR2* mRNA, exhibit earlier accumulation compared to *Cm-ETR1* during ripening, and its transcript accumulation increased during melon ripening, and declined in parallel with a reduction in ethylene production. Furthermore the *Cm-ETR2* mRNA

was induced by ethylene treatment and inhibited by 1-MCP [56].The Cm-ERS1 protein was not detected at the ripening stage despite the presence of Cm-ERS1 *mRNA* [17]. The difference in the accumulation patterns of mRNA and protein for Cm-ERS1 suggests that its expression is post-transcriptionally regulated. Western tissue print analysis showed that Cm-ERS1 protein was present at high levels in the pericarp of both cultivars at the early stages of fruit development, where frequent cell division occurs suggesting a role for Cm-ERS1 protein in cell division and expansion.

Biochemical analysis has been carried out to determine the subcellular localization and membrane topology of the melon Cm-ERS1. Membrane fractionation and GFP imaging analyses revealed that the melon receptor Cm-ERS1 protein is localized at the endoplasmic reticulum [57]. An *N*-glycosylation mutagenesis strategy was then applied to determine the precise membrane orientation of the short hydrophilic N-terminal region of Cm-ERS1 protein. The topology studies indicated that Cm-ERS1 has three membrane-spanning domains, with its N-terminus facing the luminal space and the large C-terminal portion being located on the cytosolic side of the ER membrane. Biochemical analysis is in progress to determine the subcellular localization of the melon Cm-ETR2 [Owino et al, unpublished studies].

5. Elucidation of fruit ripening using transgenic melon plants

Since ethylene induces ripening of climacteric fruit, it is a potential target for control of ripening. The genetic manipulation of ethylene production is one way in which climacteric fruit ripening can be controlled in order to increase storability [38]. The transgenic melons in which the ethylene biosynthesis has been inhibited provide a useful model for elucidating the role of ethylene in triggering and regulating ripening related pathways. One approach that has been applied is the down-regulation of genes

encoding the ethylene biosynthesis enzymes. The genetic transformation processes that have been utilized to obtain melon transgenic plants include the *Agrobacterium tumefaciens* [58] and particle gun bombardment [59].

Ayub et al., [60], used an antisense construct to suppress *Cm-ACO1* gene expression via *Agrobacterium*-mediated transformation in cantaloupe 'Charentais' (cv Vedrantais') melon fruit in order to reduce ethylene production, and as a consequence improve fruit quality by delaying ripening and softening. The reduction of ethylene production in these transgenic melon fruit was 97-99% compared to the wild-type, even at very late stages of fruit development. However, this strong inhibition of ethylene production completely arrested the ripening process in these fruit. Attached and detached melon transgenic fruits showed the same results of inhibition on rind yellowing, peduncle detachment, a large proportion of flesh softening, production of volatiles and the respiratory climacteric. Therefore, continuously ethylene exposure was needed in order to restore fruit ripening, as observed by rind yellowing, fruit softening and activation of the peduncle abscission zone [60, 61]. Some ethylene independent pathways, such as coloration of the flesh, accumulation of sugars and organic acids were not affected.

In a similar approach, Peters et al.,[62] generated four transgenic Cantaloupe melon plants cv. Védrantais lines by inserting and overexpressing an ACC oxidase gene from apple in antisense orientation. Despite the antisense ACC oxidase gene being from apple, showing less than 100% identity to the orthologous gene in the melon plant, one line (AS3) showed extreme inhibition of ethylene production. In analyzing this transgenic line (AS3) so as to discriminate between ethylene-regulated and ethylene-independent pathways, Silva et al., [63] showed that the loss of chlorophyll in

the rind, softening of the flesh, reduction of acidity and maturation of the peduncular abscission zone are inhibited in the transgenic line, indicating that these processes are ethylene-dependent. However the accumulation of carotenoids and soluble solids occurs as normal in transgenic fruit indicating that these processes are not significantly influenced by ethylene.

The male parental line (cv. ‘Krimka’) of ‘Galia’ muskmelon was transformed with the ACC oxidase (CMACO-1) antisense gene in an attempt to delay fruit ripening [64, 65]. Two independent diploid ACC oxidase antisense transgenic plants of ‘Galia’ inbred parental line (TGM-AS-1 and TGM-AS-2) were obtained. Comparison of the fruit quality characteristics between transgenic ACC oxidase (TGM-AS) and wild type melon fruit were carried out. The ethylene synthesis was reduced in the transgenic melon fruit most likely due to low ACO activity. As a result if the low ethylene production in transgenic ‘Galia’ fruit, several parameters such as yellowing of the rind, ripening index, decrease in titratable acidity and fruit softening were altered indicating that these processes are ethylene dependent. Traits such as fruit size, seed development and mesocarp total soluble solids and pH were not affected by the CMACO-1 transgene indicating that the physiological processes behind these traits are ethylene-independent.

S-adenosylmethionine is the metabolic precursor of ACC, the proximal precursor of ethylene. Clendennen et al., [66] utilized the product of the S-adenosylmethionine hydrolase (SAMase or AdoMetase) gene (from T3 bacteriophage) to catalyze the conversion of SAM to methylthioadenosine. Clendennen et al., [66] used a fruit specific promoter (chimeric ethylene-responsive E8/E4 promoter) to overexpress the SAMase gene in Cantaloupe lines and evaluated several postharvest fruit quality parameters in the transgenic lines. Transgenic melon showed

significant reduction in ethylene biosynthesis (up to 75%) both as inbred homozygous and as hybrids. The concentration of soluble sugar was also found to be higher in transgenic fruit. The onset of maturity, measured on four different dates, was not significantly delayed in transgenic fruit compared to wild type. SAMase expression had minimal effect on other horticultural traits and yield.

Transgenic melon plants conferring reduced ethylene production are significant experimental materials for isolating components related to ethylene perception and signaling. Using above mentioned transgenic melon plants and other molecular techniques, ethylene responsive genes involved in aroma production have been isolated and characterized in melon [67-70].

6. Perspective

Elucidation of mechanisms accounting for diversification is a fundamental question in biology. During last two decades, ethylene perception and signaling in plants were extensively studied using Arabidopsis and tomato as model plant species, and those studies have provided important information regarding ethylene perception and signaling in plants. Though have found common features in ethylene perception and signaling between both model species and melon (Fig. 1), we also have recognized unique features, such as diversification of the components in each species. In order to dissect the molecular mechanism of diversification in the ethylene perception and signaling of plants, intensive studies using other model plant species will be highly required. Just as in most climacteric fruits, in melon several genes are up-regulated during ripening and/or by ethylene and inhibited by ethylene antagonists (Table 1). As described previously, fundamental components in ethylene biosynthesis and signaling

have been already identified and characterized in melon. Several tools for melon functional genomics such as BAC library [71, 72], EST collections [73] and EcoTILLING [74] are already available. In addition, International Cucurbit Genomics Initiative (ICuGI) has been lunched from 2005, in which melon became a model species. The object of the Initiative will build up a platform for Cucurbit genomics; 1) Functional Genomics: sequencing of 100,000 ESTs from different melon genotypes and tissues, 2) Mapping: merging the existing melon genetic maps using SSRs as anchor markers, 3) Bioinformatics: development of a webpage for the ICuGI, where certain genomic tools would be available for the cucurbit research community (http://www.icugi.org/). Furthermore, development of other tools for melon functional genomics including EMS mutant families and DNA pools for TILLING (Japan and other countries), and genetically engineered *Agrobacterium* conferring higher ability of gene transfer to melon (Ezura, submitting) are in progress. These information and tools definitely make melon to be an alternative model plant for elucidating ethylene perception and signaling.

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Table 1. Some examples of ripening related genes in melon fruit

Gene	Homology	Gene Accession	Function	Reference
CMe-ACS1	ACC synthase	AB025906	Catalyzes ACC formation during wounding and ripening	[40, 41, 43, 46]
CMe-ACS2		D86242		
CMe-ACS3		D86241		
CMe-ACS5		Unpublished in [46]		
CM-ACO1	ACC oxidase	X95551	Catalyzes ACC conversion during wounding and ripening	[40-42]
CM-ACO2		X95552		
Cm-ERS1	Ethylene receptor	AF037398	Ethylene binding	[17,55, 56, 57]
Cm-ETR1		AF054806		
Cm-ETR2		AB29513		
Cm-PG1	Polygalacturonase	AF062465	Depolymerizes pectin	[26, 30]
Cm-PG2		AF062466		
Cm-PG3		AF062467		
Cm-XTH1,	Xyloglucan	DQ914794	Depolymerizes xyloglucan by acting in a hydrolytic mode or as a transglycosylase	[30]
Cm-XTH2	endotransglycosylase	DQ914795		
Cm-XTH3	/hydrolase	DQ914796		
Cm-Exp1	Expansin	DQ914793	Disrupts hydrogen bonds in wall matrix	[30]
Cm-ADH1	Alcohol	ABC02081	Participate in the biosynthetic pathway of aroma volatiles in fruit by interconverting aldehydes to alcohols and providing substrates for the formation of esters	[67]
Cm-ADH2	dehydrogenases	ABC02082		
Cm-AAT	Alcohol	CAA94432	Participate in the biosynthetic pathway of aroma volatiles in fruit by esterification of volatile esters	[68,69]
Cm-AAT2	acyl-transferase	CAAL77060		
Cm-AAT3		AAW51125		
Cm-AAT4		AAW51126		
pmPAL1	Phenylalanine ammonia lyase	X76130	The first enzyme of phenylpropanoid biosynthesis involved in the synthesis of a multiplicity of plant natural products.	[70]

Figure Legend

Figure 1. Schematic representation of key events during ethylene biosynthesis, perception and signal transduction, finally leading to the regulation of ethylene responsive genes in melon fruit.

Ethylene is produced from methionine, which is converted to S-adenosyl-methionine (SAM). SAM is then converted to 1-aminocylopropane-1-carboxylic acid (ACC) by either of the five ACC synthases. ACC is then converted to ethylene by either of the three ACC oxidases. The ethylene is then perceived by a family of ethylene binding receptors. Upon ethylene binding, these receptors are most likely inactivated and in turn represses the yet to be identified downstream components thus activating the ethylene signaling pathway. The downstream components regulate among others the transcription of several members of the family of transcription factors which triggers a transcriptional cascade and as a result, the transcription of numerous ethylene responsive genes [60-63] is regulated.

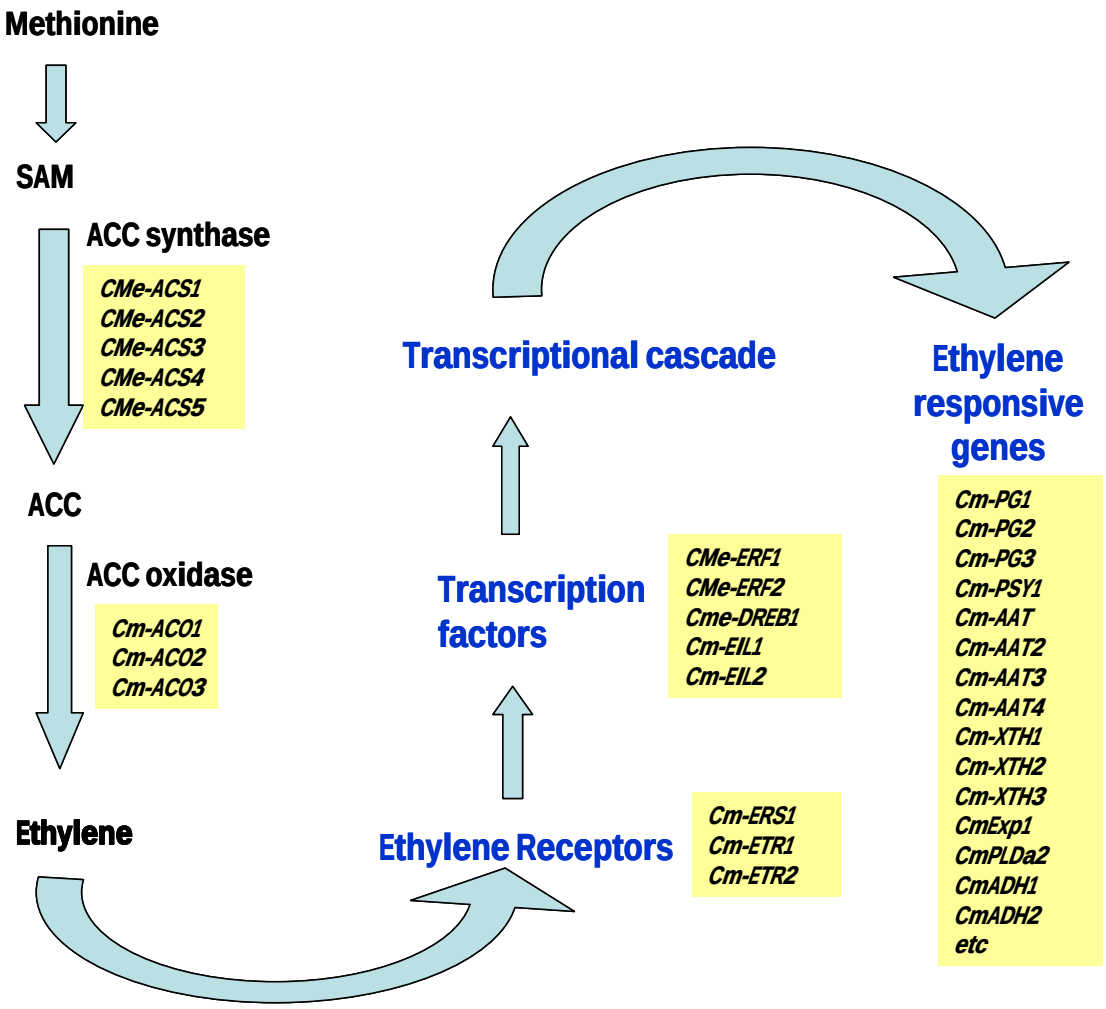
Methionine
SAM
ACC synthase
CMe-ACS1
CMe-ACS2
CMe-ACS3
CMe-ACS4
CMe-ACS5
ACC
ACC oxidase
Cm-ACO1
Cm-ACO2
Cm-ACO3
Ethylene
Ethylene Receptors
Cm-ERS1
Cm-ETR1
Cm-ETR2
Transcription factors
CMe-ERF1
CMe-ERF2
Cme-DREB1
Cm-EIL1
Cm-EIL2
Transcriptional cascade
Ethylene responsive genes
Cm-PG1
Cm-PG2
Cm-PG3
Cm-PSY1
Cm-AAT
Cm-AAT2
Cm-AAT3
Cm-AAT4
Cm-XTH1
Cm-XTH2
Cm-XTH3
CmExp1
CmPLDa2
CmADH1
CmADH2
etc

Fig. 1