Theor Appl Genet (2008) 116:589–602
DOI 10.1007/s00122-007-0694-y

ORIGINAL PAPER

Candidate genes and QTLs for fruit ripening and softening in melon

Eduard Moreno · Javier M. Obando · Noelia Dos-Santos · J. Pablo Fernández-Trujillo · Antonio J. Monforte · Jordi Garcia-Mas

Received: 9 July 2007 / Accepted: 28 November 2007 / Published online: 20 December 2007

Abstract Different factors affect the quality of melon fruit and among them long shelf life is critical from the consumer's point of view. In melon, cultivars showing both climacteric and non-climacteric ripening types are found. In this study we have investigated climacteric ripening and fruit softening using a collection of near-isogenic lines (NILs) derived from the non-climacteric melon parental lines PI 161375 (SC) and "Piel de Sapo" (PS). Surprisingly, we found that QTL *eth3.5* in NIL SC3-5b induced a climacteric-ripening phenotype with increased respiration and ethylene levels. Data suggest that the non-climacteric phenotypes from PI 161375 and "Piel de Sapo" may be the result of mutations in different genes. Several QTLs for fruit flesh firmness were also detected. Candidate genes putatively involved in ethylene regulation, biosynthesis and perception and cell wall degradation were mapped and some colocations with QTLs were observed. These results may provide additional data towards understanding of non-climacteric ripening in melon.

Introduction

Fruits are the essential components of human and animal diets, and for this reason the improvement of their quality is one of the main objectives of breeding programs in many crops. Different factors affect fruit quality and probably the most important ones are ripening-induced changes such as texture, aroma, flavour and pigmentation. Two types of fruit-ripening mechanisms have been described in the fully developed fruit, (1) climacteric ripening, which is characterized by a rise in respiration rate and is initiated by an autocatalytic ethylene burst, and (2) non-climacteric ripening, represented by the continuous decrease of respiration rate and ethylene production with absence of an ethylene peak (Moore et al. 2002). Fruit texture and softening are also important factors for fruit shelf life and post-harvest quality, due to fruit cell wall disassembly during ripening (Brummell and Harpster 2001). Understanding fruit ripening from a molecular point of view may have important commercial applications for fruit storage and distribution to the consumer. Advances have been made during the last years in the knowledge of the molecular mechanisms that control fruit ripening in climacteric tomato fruit, where ethylene biosynthetic and perception genes are transcriptionally regulated (Giovannoni 2007). Some important regulatory elements of ripening are the *rin* (Le-MADS-RIN) and *Cnr* genes, both encoding transcription factors (Vrebalov et al. 2002; Manning et al. 2006) or the *Gr* mutant (Green-ripe) that contains a mutation in a gene encoding a conserved protein of unknown function that disrupts ethylene signalling (Barry and Giovannoni 2006). Strawberry, a non-climacteric fruit, also contains a fruit-specific Le-MADS-RIN orthologue, suggesting the presence of common ethylene-independent regulatory networks involving MADS-box genes in both types of fruit ripening (Giovanonni 2007). The key elements that trigger ripening in a climacteric manner are not fully understood, and much less is known about the ripening mechanism in non-climacteric fruits.

Communicated by I. Paran.

E. Moreno · A. J. Monforte · J. Garcia-Mas (✉)
IRTA, Centre de Recerca en Agrigenòmica CSIC-IRTA-UAB,
Carretera de Cabrils Km2, 08348 Cabrils, Barcelona, Spain
e-mail: jordi.garcia@irta.es

J. M. Obando · N. Dos-Santos · J. P. Fernández-Trujillo
Department of Agricultural and Food Engineering,
Technical University of Cartagena (UPCT),
ETSIA and Institute of Plant Biotechnology,
30203 Cartagena, Murcia, Spain

On the other hand, fruit softening is probably the result of the controlled reorganization of cell wall structures (Brummell and Harpster 2001) together with a cell turgor pressure component (Lin and Pitt 1986; Shackel et al. 1991; Harker et al. 1997), but which enzymes are responsible for the modifications that lead to fruit softening is not completely understood (Brummell and Harpster 2001; Vicente et al. 2007).

Melon (*Cucumis melo* L.) has also been used as a model species to study climacteric ripening (Ayub et al. 1996). However, climacteric and non-climacteric varieties exist within this species. For example, Cantalupensis melon types are climacteric, whereas Inodorus melon types are non-climacteric. The coexistence of both types of ripening genotypes makes melon a suitable system to study the genetic control of climacteric ripening. Cantaloupes (Cantalupensis) are the melon type in which ripening has been studied in more detail (Ayub et al. 1996; Périn et al. 2002; Nishiyama et al. 2007). Ethylene production is drastically reduced in antisense 1-aminocyclopropane-1-carboxylate oxidase (*ACO1*) cantaloupe plants (Ayub et al. 1996). Both exogenous ethylene treatment and application of 1-methylcyclopropene (1-MCP) in *ACO1* antisense melons confirmed that fruit softening of "Charentais" melon types is completely ethylene-dependent (Nishiyama et al. 2007). The expression of some cell wall degrading enzymes is also dependent on ethylene expression, suggesting that these genes may be good candidates for pectin disassembly in climacteric melons, although other cell wall-modifying genes from the same gene families are not regulated by ethylene (Nishiyama et al. 2007).

A segregating population from a cross between the non-climacteric PI 161375 accession and the climacteric "Védrantais" line (Cantalupensis) revealed the presence of two genes (*Al-3* and *Al-4*) involved in ethylene-dependent fruit abscission and ethylene production, and four additional quantitative trait loci (QTLs) controlling the level of ethylene production (Périn et al. 2002), but the molecular nature of these genes is still unknown. Exogenous propylene treatments did not induce endogenous ethylene production in PI 161375 fruits, although it displayed the characteristic triple response induced by ethylene in seedlings, suggesting that ethylene perception is inhibited in the fruit tissue in PI 161375 (Périn et al. 2002).

In this paper we describe the identification of a region in melon linkage group III carrying a QTL that induces climacteric ripening, using a collection of near-isogenic lines (NILs) derived from two non-climacteric parental lines PI 161375 and "Piel de Sapo" (Eduardo et al. 2005). We also report the discovery of several QTLs that control the ripening-related trait fruit flesh firmness. Additionally, candidate genes for ethylene biosynthesis, perception and signalling and cell wall degradation have been placed in the PI 161375 × "Piel de Sapo" genetic map, and the possible co-segregations between QTLs and candidate genes are discussed.

Materials and methods

Plant material and DNA extraction

DNA sequence polymorphism within the candidate genes was investigated between the Spanish melon cultivar "Piel de Sapo" (PS) and the Korean accession PI 161375 "Songwhan Charmi" (SC). A double haploid line (DHL) population, developed previously from the F_1 hybrid of PS and SC to construct the melon genetic map (Gonzalo et al. 2005), was used for mapping the candidate genes. Genomic DNA from parents and DHLs was isolated from young leaves according to Doyle and Doyle (1990), with some modifications (Garcia-Mas et al. 2000).

Climacteric ripening and cell wall related traits were studied with a set of 27 near-isogenic lines (NILs) derived from the previous cross between PS and SC (Eduardo et al. 2005). The NILs were developed by marker-assisted selection after several generations of backcrossing and selfing, most of them containing a single homozygote introgression from SC, and only three of them containing a single heterozygote introgression (Eduardo et al. 2007). The introgressions represented in this set of 27 NILs together covered most of the SC genome.

Phenotyping of ethylene production and fruit softening traits

The 27 NILs and both parents PS and SC were cultivated in 2004 in Cartagena (Murcia, Spain), following soil preparation, fertigation, plant protection and other growing practices commonly used for melon cultivation in Mediterranean conditions. The field design was completely randomised with ten replications for each NIL, 50 replications for PS and five plants for SC. Further details about this experiment have been reported previously (Eduardo et al. 2007; Obando et al. 2007). In 2006 and 2007, selected NILs, PS and "Védrantais" (as a climacteric control) were cultivated with the same conditions as above in Cartagena and Cabrils (Barcelona, Spain).

Fruits were harvested in the morning based on the maturity indexes and the handling practices previously reported (Obando et al. 2007). Seven selected fruits were harvested per line and subjected to analysis. Respiration rate and ethylene production activities were measured at 20°C and 70% relative humidity after equilibrating the fruit with the environment (4 h minimum). Fruits were analysed by the static method (Kader 2000) as previously reported in

Fernández-Trujillo et al. (2005). Ethylene (C_2H_4) and carbon dioxide (CO_2) were sampled after fruits were placed in hermetic containers for 30 min and 1 h, respectively. Two samples of 0.5 mL were removed from the headspace and analysed by gas chromatography (Thermo Finnigan Trace GC 2000, Milan, Italy).

Flesh firmness was evaluated after fruit harvest using flesh cylinders (20-mm-long and 15-mm-diameter) that were trimmed from the middle flesh part of the longitudinal fruit section with an apple cork borer. This tissue corresponds to hypodermal mesocarp. In Cartagena, flesh firmness was evaluated measuring the breaking force of the above-mentioned flesh cylinders using a 4.6-mm-diameter probe (the numbers of independent measures were 50 for PS and 7 for NILs, two fruits per replicate) (Fernández-Trujillo et al. 2005). In Cabrils, flesh firmness for selected lines (four independent measures per line) was measured by using a penetrometer with an 8.0-mm-diameter probe tip.

Candidate gene selection and polymorphism discovery

Candidate genes involved in ethylene biosynthesis and perception and cell-wall degradation were selected. The sequences of *Arabidopsis thaliana* related with the traits of interest were used to perform BlastX (Altschul et al. 1990) in the MELOGEN database (http://www.melogen.upv.es, Gonzalez-Ibeas et al. 2007) and in GeneBank. Candidate genes were named with the prefix Cm (for *Cucumis melo*) followed by the abbreviated gene name and a number indicating the different genes that have been found for each candidate gene. For previously described candidate genes the same gene numbering was used.

For each of the selected ESTs, specific primers were designed using the Primer3 software (Rozen and Skaletsky 2000) with an average length of 20 nucleotides, a melting temperature around 60°C and an expected PCR product of 500–700 bp. In order to increase the probability of polymorphism discovery, we designed primers in regions near the 3′UTR or flanking positions where introns were located in *Arabidopsis*.

Genomic DNA from the parental lines of the mapping population SC and PS was amplified following the conditions described by Morales et al. (2004) after modifying the length of the PCR extension step, which varied between 30 and 90 s according to the fragment length. Amplified fragments were purified with sepharose columns and sequenced using the ABI Prism BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) in an ABI Prism 3130 sequencer (Applied Biosystems, Foster City, CA, USA).

Sequences were aligned and screened for polymorphism with the Bioedit software (Hall 1999). Sequences were also compared with EST sequences to verify the intron positions that had previously been predicted.

Bioedit software was used to generate restriction maps from sequences obtained from SC and PS. SNPs or indels producing differential restriction maps between PS and SC were used to develop cleaved amplified polymorphic sequence (CAPS) markers when possible. PCR products were digested with the corresponding enzyme and buffer.

For cases where the SNP could not be genotyped by a CAPS marker, the ABI Prism SNaPshot ddNTP Primer Extension Kit (Applied Biosystems) was used (Morales et al. 2004). Microsatellites found within candidate genes were detected by PCR amplification with primers flanking the repetitive sequence. One of the primers was labelled with IRD-800 (MWG Biotech AG, Ebersberg, Germany) and visualized with a LICOR IR^2 sequencer (Li-cor Inc, Lincoln, NE, USA) as described in Gonzalo et al. (2005).

For mapping some important candidate genes that did not show any SNP between SC and PS, we screened a melon bacterial artificial chromosome (BAC) library (van Leeuwen et al. 2003) in order to identify flanking sequences near the gene of interest. The BAC library was screened by PCR according to van Leeuwen et al. (2003) in order to isolate single positive BAC clones. Positive clones were grown in 2× YT medium (16 g of tryptone, 10 g of yeast extract and 5 g of NaCl/L) and BAC DNA was purified. After obtaining BAC-end sequences by direct sequencing, specific primers were designed and SC and PS DNA were amplified in search for polymorphisms.

In those cases in which BAC-ends were monomorphic between the parental lines, BAC DNA was digested with *Eco*RI and *Hin*dIII and the resulting fragments were subcloned in pUC19. Random clones were isolated and sequenced, specific primers were designed and SC and PS DNA were amplified in search for polymorphisms.

SNP mapping

SNPs and indels were mapped by selective genotyping using the bin-mapping strategy (Howad et al. 2006), adapted for the melon mapping population (Fernández-Silva et al. 2006). Briefly, 14 DHLs from the melon mapping population were selected to obtain the maximum resolution with a minimum number of genotypes. This reduced number of individuals defined 102 bins with an average of 18 cM per bin. SNPs and indels were placed in the bin map by visual inspection of the genotypes presumed by the markers and genotypes in the bin set.

Statistical analysis

All statistical analyses were performed with JMP v5.1.2 for Windows (SAS Institute, 2004). To study the effect of the SC introgressions on ethylene production and fruit softening,

NIL mean values were compared with the control genotype PS using the Dunnett contrast (Dunnett 1955) with type-I error $\alpha \leq 0.05$. When the mean of a NIL was significantly different from PS, it was inferred that there was a QTL for the trait within the SC introgression carried by the NIL (Eshed and Zamir 1995). The total number of QTLs controlling a trait was estimated assuming that there was only one QTL per introgression, and that when two NILs with overlapping introgressions showed significant effects the QTL was located in the overlapping region (Eduardo et al. 2007). NILs with less than four replicates were excluded from the analysis.

Results

Respiration rate and ethylene production at harvest and during postharvest ripening

During the 2004 field experiments, PS and SC had respiration rates and ethylene production values during postharvest storage typical of non-climacteric melon varieties (data not shown). No changes in skin colour, softening and aroma were observed, especially for the PS genotype. No significant differences were detected at harvest in both activities between PS and any of the 24 out of the 27 available NILs that were successfully grown (data not shown). Three additional NILs were not included in the statistical analysis because we did not obtain enough fruits. Visual inspection of the fruits of one of these NILs, SC3-5b, showed the most different ripening phenotype compared with PS or SC, the skin turned from green to yellow, the flesh developed evident softening, the fruit abscised and a strong differential aroma could be appreciated, indicating a possible climacteric ripening for this near-isogenic line. To confirm that possibility, respiration and ripening were studied more carefully during 2006 experiments for SC3-5b. PS and SC fruits behaved again as non-climacteric with ethylene values around 5 and 10 pmol kg^{-1} s^{-1}, respectively (Fig. 1). SC3-5b fruits were climacteric, showing the characteristic ethylene peak observed in typical climacteric fruits around 4 days postharvest with a maximum peak of ethylene production of 77.8 pmol kg^{-1} s^{-1} (Fig. 1). "Védrantais" fruits were also tested and an ethylene peak value of 207 pmol kg^{-1} s^{-1} was observed (data not shown). Respiration rates were also consistently higher for SC3-5b with a peak of CO_2 production of 694 nmol kg^{-1} s^{-1} around 6 days after harvest.

A QTL involved in ethylene production and climacteric response, which was named *eth3.5*, was defined on LG III (Fig. 2) inside the introgression in SC3-5b covering approximately 50 cM (Eduardo et al. 2005).

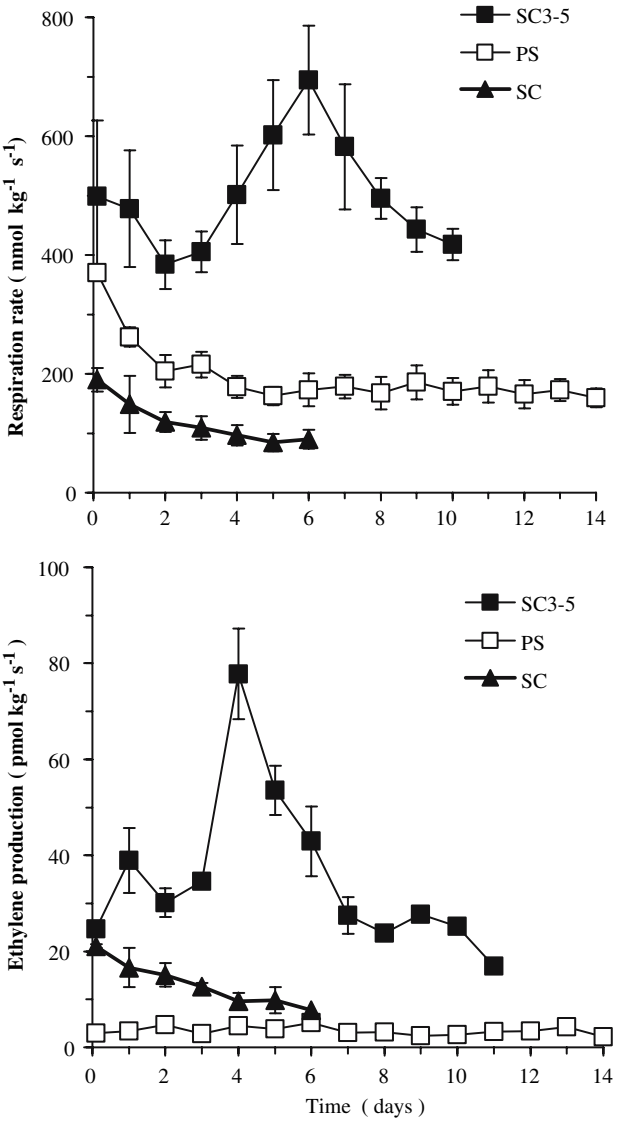


Fig. 1 Respiration rate (nmol.kg^{-1}.s^{-1}) (**a**) and endogenous ethylene (pmol.kg^{-1}.s^{-1}) (**b**) measured at 21°C in the non-climacteric parental lines *PS*, *SC* and the NIL *SC3-5b* (*SC3-5*)

Fruit firmness

Flesh firmness at harvest for PS and 24 out of the 27 available NILs were measured during 2004 in Cartagena (Table 1). NILs SC2-2a, SC8-2 and SC8-4 showed significant lower flesh firmness rates than PS, whereas SC10-2 showed a higher flesh firmness rate, defining four QTLs for this trait *ff2.2*, *ff8.2*, *ff8.4* and *ff10.2*. For the climacteric line SC3-5b, flesh firmness measured during 2006 in Cabrils was significantly lower than PS, defining another QTL (*ff3.5*) for flesh firmness (Table 1). Thus, a total of five QTLs involved in fruit firmness were detected (Fig. 2, white and lined bars). We focused on the QTLs for fruit flesh firmness in NILs SC3-5b, SC8-2 and SC10-2, which

Table 1 Flesh firmness means with their standard deviations for 25 NILs and "Piel de Sapo" (PS) control genotype in the 2004 Cartagena trial (CT 2004)

	Flesh firmness (*N*)			
	CT 2004	CB 2006	CT 2006	CB 2007
PS	4.8 ± 0.1	5.3 ± 0.4	6.9 ± 0.2	6.9 ± 0.3
SC1-3d	4.7 ± 0.4			
SC1-4a	4.8 ± 0.4			
SC1-4d	4.5 ± 0.3			
SC2-2a	3.5 ± 0.4*	n.a.	n.a.	n.a.
SC2-3d	4.0 ± 0.3			
SC3-3	5.5 ± 0.5			
SC3-5b	n.a.	3.7 ± 0.4*	4.2 ± 0.4*	4.5 ± 0.4*
SC4-1hb	4.5 ± 0.4			
SC4-4	4.4 ± 0.3			
SC5-2	4.3 ± 0.4			
SC5-3	4.0 ± 0.3			
SC6-4	4.3 ± 0.4			
SC7-2	5.5 ± 0.6			
SC7-4a	6.1 ± 0.4			
SC8-1	4.1 ± 0.3			
SC8-2	2.9 ± 0.3*	n.a.	n.a.	3.2 ± 0.6*
SC8-3	3.5 ± 0.4			
SC8-4	3.6 ± 0.3*	n.a.	n.a.	n.a.
SC9-1a	5.9 ± 0.4			
SC9-2a	4.9 ± 0.3			
SC9-3	4.3 ± 0.3			
SC10-2	6.6 ± 0.4*	n.a.	11.3 ± 0.6*	n.a.
SC11-2ahb	5.3 ± 0.4			
SC12-1ab	5.2 ± 0.4			
SC12-4hb	5.0 ± 0.3			

Some significant NILs were measured again in 2006 in Cartagena (CT 2006), Cabrils (CB 2006) and in 2007 in Cabrils (CB 2007)

n.a. Data not available, *CT* Cartagena assay, *CB* Cabrils assay

* Mean for a NIL is significantly different ($P < 0.05$) from PS

were re-evaluated in 2006 in Cartagena (SC3-5b and SC10-2) and in 2007 in Cabrils (SC3-5b and SC8-2). In all cases fruit flesh firmness was significantly different than PS, confirming QTLs *ff3.5*, *ff8.2* and *ff10.2* (Fig.2, lined bars).

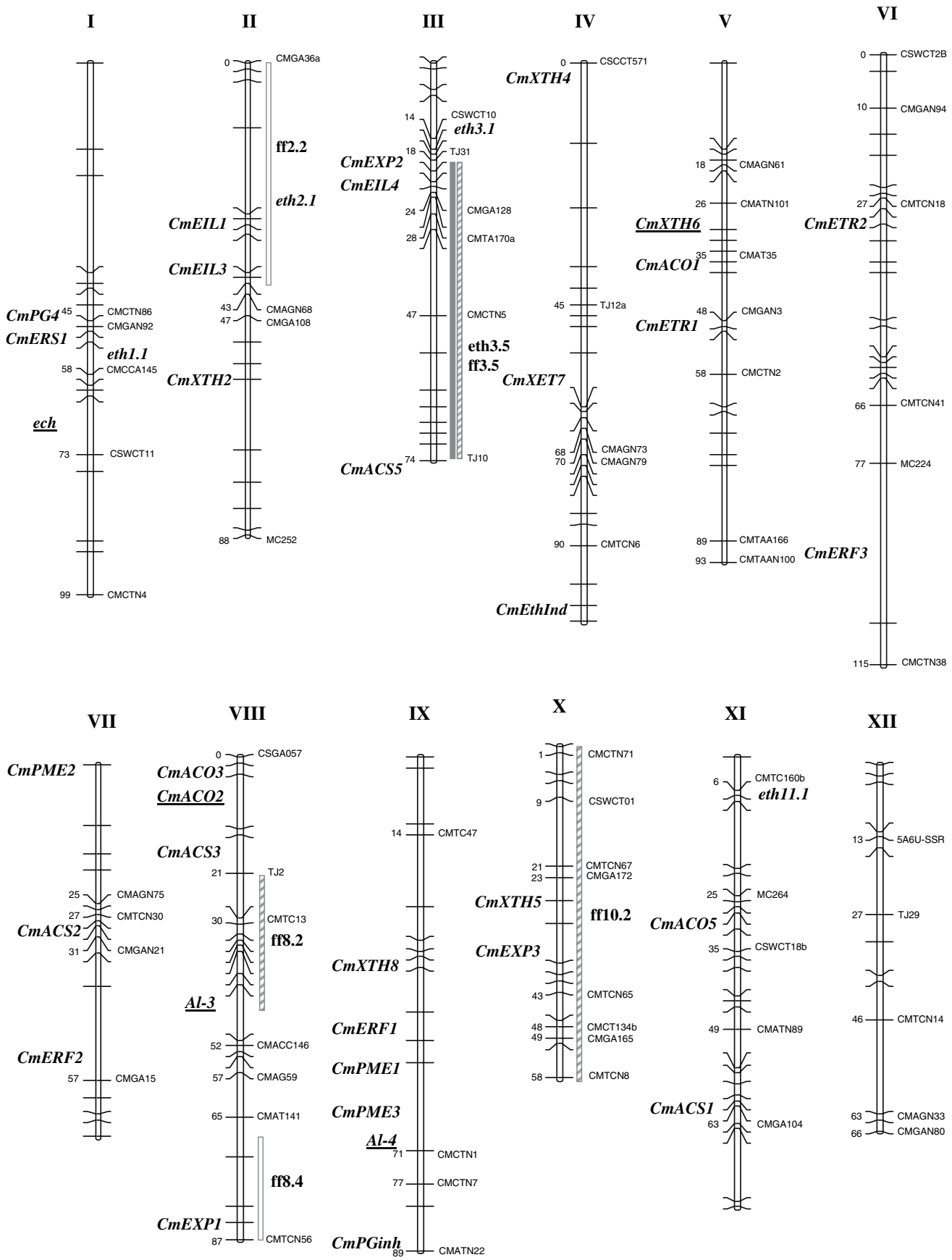
Identification of candidate genes involved in ethylene biosynthesis, ethylene signal transduction and cell wall degradation

Genes involved in ethylene biosynthesis, ethylene perception and signal transduction pathways were selected (Table 2). Candidate gene sequences were obtained from the MELOGEN database (http://www.melogen.upv.es) and from GeneBank. Four different ESTs encoding 1-aminocyclopropane-1-carboxylate synthase (ACS) were identified. PSI_39-H08 and A_02-A09 were identical to melon *CmACS2* and *CmACS3* genes, respectively (Ishiki et al. 2000) and 15d_41-D01 and DV633629 represented new melon *ACS* genes (*CmACS4* and *CmACS5*). *CmACS4* and *CmACS5* were both recovered from fruit cDNA libraries and showed the best homologies to tomato *ACS2* and *ACS5* genes, respectively. Three ESTs encoding 1-aminocyclopropane-1-carboxylate oxidase (*ACO*) were also identified. X95553 corresponded to melon *CmACO3* (Lasserre et al. 1996) and CI_07-F08 and HS_35-E12 represented new melon *ACO* genes (*CmACO4* and *CmACO5*, respectively), both obtained from a cotyledon cDNA library. *CmACO4* and *CmACO5* showed the best homologies to tomato *ACO4* and *ACO1* genes, respectively.

Three ethylene receptors putatively involved in ethylene perception were also selected as candidate genes. CI_54-H09 that was identical to melon *CmETR1* gene (Sato-Nara et al. 1999), the *CmERS1* gene (Sato-Nara et al. 1999; orthologous to tomato *Nr* gene) and AI_29-A04 (*CmETR2*) that showed the highest homology with the *ETR2* gene from cucumber (Yamasaki et al. 2000).

For ethylene signalling, several candidate genes were identified. CI_19-F07 (*CmCTR1*) showed high homology with the negative regulator kinase CTR1. PS_03-F02 (*CmEIN2*) was homologous to the positive regulator of the ethylene pathway EIN2. Three members of the EIN3-like family of transcription factors were also identified: AB063191 (*CmEIL1*), DV633356 (*CmEIL3*) and 46d_19-G04 (*CmEIL4*) that showed homology with *EIN3*-like genes. Three genes with high homology to ethylene responsive factors were also identified: melon *CmERF1* and *CmERF2* (Mizuno et al. 2006), and DV631911 (*CmERF3*) with the highest homology to an ERF from *B. oleracea*. We also identified PSI_37-A02 (*CmGRL*) as homologue to the tomato Green-ripe like (GRL) and PSI_36-G11 (*CmEthInd*) with homology to an ethylene-inducible protein from *Hevea brasiliensis*.

Enzymes involved in cell wall degradation such as polygalacturonases, expansins, xyloglucan endotransglycolase/hydrolases, pectinesterases, pectate lyases and xylose isomerases were also selected for the candidate gene approach. Three genes encoding polygalacturonases were selected: *CmPG1* (Hadfield et al. 1998), DV633624 (*CmPG4*) similar to *CmPG2* (Hadfield et al. 1998) and PSI_36-E05 (*CmPG5*). Six homologues to the xyloglucan endotransglycolase/hydrolase (*XTH*) gene family were found. 15d_18-A03 was identical to melon *CmXTH2* (ABI94062). A_15-E06 (*CmXTH4*), AI_20-E02 (*CmXTH5*), and 15d_30-C10 (*CmXTH9*) showed homologies to several XTHs from other plant species. CI_39-G03 (*CmXTHinh*) showed homology to a xyloglucan endotransglycolase inhibitor from *S. tuberosum*. DV632961 (*CmPME3*) and HS_39-B01 (*CmPME4*) were homologous to pectinesterases from *A. thaliana* and *N. tabacum*, respectively. Three


I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
CmPG4
CmERS1
eth1.1
ech
CmXTH2
CmEIL1
CmEIL3
ff2.2
eth2.1
CmEXP2
CmEIL4
eth3.1
eth3.5
ff3.5
CmACS5
CmXTH4
CmXET7
CmEthInd
CmXTH6
CmACO1
CmETR1
CmETR2
CmERF3
CmPME2
CmACS2
CmERF2
CmACO3
CmACO2
CmACS3
Al-3
ff8.2
ff8.4
CmEXP1
CmXTH8
CmERF1
CmPME1
CmPME3
Al-4
CmPGinh
CmXTH5
CmEXP3
ff10.2
CmACO5
CmACS1
eth11.1
5A6U-SSR
CMGA36a
CMCTN86
CMGAN92
CMCCA145
CSWCT11
CMCTN4
CMAGN68
CMGA108
MC252
CSWCT10
TJ31
CMGA128
CMTA170a
CMCTN5
TJ10
CSCCT571
TJ12a
CMAGN73
CMAGN79
CMCTN6
CMAGN61
CMATN101
CMAT35
CMGAN3
CMCTN2
CMTAA166
CMTAAN100
CSWCT2B
CMGAN94
CMTCN18
CMTCN41
MC224
CMTCN38
CMAGN75
CMTCN30
CMGAN21
CMGA15
CSGA057
TJ2
CMTC13
CMACC146
CMAG59
CMAT141
CMTCN56
CMTC47
CMCTN1
CMCTN7
CMATN22
CMCTN71
CSWCT01
CMCTN67
CMGA172
CMCTN65
CMCT134b
CMGA165
CMCTN8
CMTC160b
MC264
CSWCT18b
CMATN89
CMGA104
TJ29
CMTCN14
CMAGN33
CMGAN80

◀ **Fig. 2** Melon framework genetic map (SC × PS, Gonzalo et al. 2005) containing candidate genes for ethylene synthesis, signal transduction and cell wall degrading enzymes. Candidate genes are in *italics* on the left side of each linkage group. *Underlined* are candidate genes reported only in Périn et al. (2002). *Vertical bars* indicate bins where fruit QTLs for climacteric ripening (*filled bar*) and flesh firmness (*lined bars*) are located. Flesh firmness QTLs that have not been verified are represented in *white bars*. QTLs for ethylene synthesis from Périn et al. (2002) are in *italics* on the right side of each linkage group

ESTs showed homologies to members of the expansin family: 46d_01-B10 (*CmEXP1*) (Nishiyama et al. 2007), FR13C9 (*CmEXP2*) and P11.25 (*CmEXP3*). Finally, P3.84 (*CmPL*) showed homology to a pectate lyase from *Fragaria x ananassa* and P3.08 (*CmXyIso*) to a xylose isomerase from *A. thaliana*. In total, 35 melon ESTs with homology to genes involved in ethylene biosynthesis, ethylene response and cell wall degradation were selected as candidate genes for mapping (Table 2).

SNP discovery and mapping

Primers were designed for all the ESTs (Table 2). A single clear amplification product was obtained for 30 candidate genes. The size of the amplified fragment was bigger than originally predicted from the EST sequence for ten candidate genes, confirming the presence of introns in the genomic sequence. For most of the sequences the intron location was conserved with the corresponding gene in *Arabidopsis*, except for the polygalacturonase PSI_36-E05 (*CmPG5*) (data not shown). We were unable to obtain any amplification product from five ESTs (*CmACO4*, *CmCTR1*, *CmGRL*, *CmPG1* and *CmXyIso*).

For the 30 candidate genes successfully amplified, approximately 11.4 kb of genomic sequence were analysed in each of the two parental melon lines SC and PS. In total, 53 polymorphisms were identified (45 SNPs, 7 indels and 1 microsatellite) in 19 candidate genes (Table 2). The frequency of polymorphisms observed was 3.9 SNP and 0.6 indels per kilobase. Twenty-four SNPs (53.3%) were found in introns, 18 SNPs (40%) in exons and 3 SNPs (6.7%) in the 3′UTR. All indels were located in non-coding regions (two in introns and four in 3′UTR). The microsatellite in *CmACO3* was located in an intron.

All candidate genes containing at least one polymorphism between SC and PS were mapped in the SC × PS melon genetic map. The candidate genes were mapped using cleaved amplified polymorphic sequence (CAPS) markers (nine candidate genes), single primer extension with SNaPshot (eight candidate genes), one sequence characterized amplified region (SCAR) marker and one microsatellite (Table 2).

Additionally, four candidate genes (*CmACO2*, *CmETR1*, *CmERS1* and *CmETR2*) that were monomorphic between SC and PS were successfully mapped indirectly using flanking sequences obtained after screening a melon BAC library. Positive BAC ends (*CmERS1*) or internal BAC regions (*CmACO5*, *CmETR1* and *CmETR2*) were sequenced and markers were developed and successfully located in the melon genetic map (Table 3). In total, 23 out of the 30 successfully amplified candidate genes were placed in the SC × PS genetic map (Fig. 1). Additionally, the genetic map also contained the position of markers *CmACS1* (Miki et al. 1995), *CmACO1* (Lasserre et al. 1996), *CmXTH7*, *CmXTH8*, *CmPME1*, *CmPME2* and *CmPGinh* that were previously mapped in our laboratory as RFLPs or SSRs (Oliver et al. 2001) (Table 4).

Previously mapped QTLs and candidate genes

The approximate positions of several candidate genes reported in the melon genetic map from Périn et al. (2002) are also represented in Fig. 1. For *CmERS1*, *CmACS5*, *CmACS1* and *CmACO1*, the map position fitted with the position that we found for the same gene (Fig. 2). Two additional candidate genes that we did not map were also included: *CmACO2* and *CmXTH7* (Table 4). The map position of four QTLs for ethylene production (*eth1.1*, *eth2.1*, *eth3.1* and *eth11.1*), hook curvature (*ech*) and two genes involved in fruit abscission and autocatalytic ethylene production (*Al-3* and *Al-4*) (Périn et al. 2002) is also represented in Fig. 2. The approximate position of these genes in the SC × PS genetic map was determined by using common markers between both genetic maps.

Colocation of candidate genes and QTLs

Seven possible colocations between candidate genes and QTLs were detected. For fruit ripening, *eth3.5* with *CmACS5*; *eth1.1* with *ERS1*; *eth2.1* with *CmEIL1* and *CmEIL3*; *eth3.1* with *CmEIL4*. For fruit firmness, *ff10.2* with *CmEXP3* and *CmXTH5*. Additionally, *CmEXP1* colocated with the unverified QTL *ff8.4*. It should be pointed out that due to the low resolution mapping of the QTLs, we are still far from being able to correlate between QTLs and candidate genes, and further fine mapping of the QTLs is still needed.

Discussion

A novel QTL for climacteric fruit ripening derived from two non-climacteric melon types

We report the identification of *eth3.5*, a QTL involved in climacteric ripening, derived from the cross between the non-climacteric melons SC and PS. The *eth3.5* allele from

Table 2 Melon candidate genes for ethylene synthesis, ethylene signal transduction and cell wall enzymes

Sequence	GenBank accession	Gene name	Best Hit (BlastX)	*E* value	Primer sequences (forward and reverse, 5′→3′)	SNP/indel position and location	SNP detection method	Linkage group
PSI_39-H08[a]	AM721298	*CmACS2*	Aminocyclopropane-carboxylate synthase (*C. melo*) BAB18464	0.0	TCATGGATTGCCAGAGTTCA GTTCCGAGTGGATTTGAAGG	A→T 80 (intron)	CAPS (*Bcl*I)	VII
A_02-A09[a]	AM729722	*CmACS3*	Aminocyclopropane-carboxylate synthase (*C. melo*) BAB18463	0.0	TCCAACCAATACATCCACCTC GTTTAAAGCTGGCTGCGTCT CTTAAGTGAATGTAAAGGAATTAAT[c,e]	A→T 338 (intron)	SNaPshot	VIII
15d_41-D01[a]	AM739261	*CmACS4*	Aminocyclopropane-carboxylate synthase (*R.. hybrid*) AAQ88100	1E-64	GAATATCCTGGCTGCTGCTC CGAATTCGGTCCATGACAAT	Monomorphic	–	–
SSH1N10[b]	DV631608	*CmACS5*	Aminocyclopropane-carboxylate synthase (*M. charantia*) AAQ14267	2E-27	CCAAATTGGGAATTTTTGTAGG AAATAATTCCAATCCATTGTTCG	T→C 132 (exon) C→T 264 (exon)	CAPS (*Alu*I)	III
X95553[b]	X95553	*CmACO3*	Aminocyclopropane-carboxylate oxydase (*C. melo*) CAA64799	0.0	AAGAGAGGGCCCACTTTGAT TTGATGACAGGGAAATCCATC CAACGACGCCTGCGAAAACT[f] CATCAGCTCATGTGATATCCC[f]	1 SSR	Licor	VIII
CI_07-F08[a]	AM734757	*CmACO4*	Aminocyclopropane-carboxylate oxydase (*P. tremula x P. tremuloides*) AAN87846	6E-68	ATTGAGGTCCTGAGCAATGG GGTGCTGGATTGTGAAGGTT	Not amplified	–	–
HS_35-E12[a]	AM726256	*CmACO5*	Aminocyclopropane-carboxylate oxydase (*C. sativus*) AAC67232	2E-102	TCAACTTGGAGAAGCTTAATGG AGGCACGTCCACCCACTC	Monomorphic	–	XI[d]
CI_54-H09[a]	AM733709	*CmETR1*	Ethylene receptor (*C. melo*) AAC99645	0.0	AGTCGGTCAGTGACGAAAGG TTTCAAGGAACTCATCAAAACG	Monomorphic	–	V[d]
AB049128[b]	AB049128	*CmERS1*	Ethylene receptor (*C. melo* AAC99477	0.0	CAAACAAGTTGCACCCATTG ACATCGATAAGGCGCTCTGT	Monomorphic	–	I[d]
AI_29-A04[a]	AM716691	*CmETR2*	Ethylene receptor (*C. sativus*) BAA85819	2E-81	CCGATGCAGATGACATGAAC TTTTTCGAGTTCGTAACATTCC	Monomorphic	–	VI[d]
CI_19-F07[a]	AM735786	*CmCTR1*	Serine/threonine-protein kinase (*M. truncatula*) ABE89440	7E-138	GGTGTGGCCAGAGTAAAAGC TGAAGGGGTAAAAAGGTTAAAAA	Not amplified	–	–
PS_03-F02[a]	AM717510	*CmEIN2*	Ethylene signalling (*S. lycopersicum*) AAZ95507	8E-90	CAGGAAACCTTCTTCCGAATC CCCTTGCCTCAGCTTCATAA	Monomorphic	–	–
AB063191[b]	AB063191	*CmEIL1*	Ethylene insensitive (EIN3-like) (*C. melo*) BAB64344	0.0	CATCAAGCATATGTCCCCTGA AATCACCAACAGGGCAGAGT GAGCTTTATCCTGATTCTTGCCC[c]	T→A 95 (exon) A→G 160 (exon) T→C 487 (exon)	SNaPshot	II
FR13J11[b]	DV633356	*CmEIL3*	Ethylene insensitive (EIN3-like) (*F. sylvatica*) CAC09582	3E-17	GCATCTCTCTTTTTCATTCGTTT TCCTTCCACATACGCCTCTC CACTAGAACTATTACAAAGAGGG [c,e]	C→T 440 (intron) C→T 442 (intron)	SNaPshot	II
46d_19-G04[a]	AM741269	*CmEIL4*	Ethylene insensitive (*C. melo*) BAB64345	3E-13	ATAACATCTCCGACTTCAGAT GTATACAACTAGGCATTTAGATATT	T→C 153 (3′UTR)	CAPS (*Fok*I)	III

Table 2 continued

Sequence	GenBank accession	Gene name	Best Hit (BlastX)	*E* value	Primer sequences (forward and reverse, 5′→3′)	SNP/indel position and location	SNP detection method	Linkage group
AB125975[b]	AB125975	*CmERF1*	Ethylene responsive factor (*C. melo*) BAD01555	3E-93	AACGACGACGAGTTCTCACC TCCAACTTATGACCAGAAAATCA	T→C 102 (exon) G→A 268 (exon)	CAPS (*Eco*rI)	IX
AB125976[b]	AB125976	*CmERF2*	Ethylene responsive factor (*C. melo*) BAD01556	4E-150	TTCAGACGACGAAACAGAGC GTTACCGCTTCCGACATTTG	T→G 592 (exon)	CAPS (*Nco*I)	VII
SSH6B7[b]	DV631911	*CmERF3*	Ethylene responsive factor (*B. oleracea*) ABD65036	2E-20	GGCCGTGGGGTAAGTTTG ATTTTGCCACATCAGCTTCC CTACTTCGTCTTTGTCGTCGGT[c]	T→A 254 (exon)	SNaPshot	VI
PSI_36-G11[a]	AM720840	*CmEthInd*	Ethylene-inducible (*H. brasiliensis*) AAA91063	4E-63	GCCGCCATATTCTTCACTTC ATGACCGGAATCGTAACAGC GCGTTGACGACGTCCATAATTAC[c]	A→T 142 (exon)	SNaPshot	IV
PSI_37-A02[a]	AM722865	*CmGRL*	Green-ripe like (*S. lycopersicum*) ABD34616	2E-87	ACCATCAACTCGACCGAGAA ACAACCACCCAACAAAGG	Not amplified	–	–
AF062465[b]	AF062465	*CmPG1*	Polygalacturonase precursor (*C. melo*) AAC26510	0.0	AAAGAAGCACGCCTTGACTG CCTCAAACTAACGAAGGCTCA	Not amplified	–	–
FR14F9[b]	DV633624	*CmPG4*	Polygalacturonase precursor (*C. melo*) AAC26511	2E-90	GGCCTATGGAAATGCAGTCA GAAATTGCAATGGCTCTTCA	1 indel	SCAR	I
PSI_36-E05[a]	AM721008	*CmPG5*	Polygalacturonase precursor (*A. thaliana*) AAL08244	1E-58	TCTCAATGTTCCTGCAGTCG GGGACATTGACGGATTGTTC	Monomorphic	–	–
15d_18-A03[a]	AM736657	*CmXTH2*	Xyloglucan endotransglycosylase (*C. melo*) ABI94062	3E-19	AATCTGGACATTACTCGACAACAA GGATGAAAAGGGCATCTCAC CTTGAGGGAATCTCTTGGCGTC[c]	T→C 82 (exon) -→G 358 (3′UTR)	SNaPshot	II
A_15-E06[a]	AM729321	*CmXTH4*	Xyloglucan endotransglycosylase (*Arabidopsis thaliana*) AAL32776	5E-45	TAGCAATTGGGCAACATCAG CACAAAGTTCACAAACGAAATAAA	T→C 289 (exon) T→C 313 (exon)	CAPS (*Mse*I)	IV
AI_20-E02[a]	AM715612	*CmXTH5*	Xyloglucan endotransglycosylase (*P. tremula x P. tremuloides*) ABM91064	9E-85	ATGCCAATGGAAGCGTTAAG CCTTCGCTTTTCATCCATGT	C→T 113 (intron) C→T 283 (exon) G→A 498 (exon) C→T 622 (3′UTR)	CAPS (*Taq*I)	X
15d_30-C10[a]	AM737687	*CmXTH9*	Xyloglucan endotransglycosylase (*D. carota*) AAK30204	2E-90	TTCTTCCCCATTCAGTTTCAA CTTGTGCTTCCATTCCCGTA	Monomorphic	–	–
CI_39-G03[a]	AM735409	*CmXETinh*	Xyloglucan endotransglycosylase inhibitor (*S. tuberosum*) AAP84703	1E-64	GGAATATTTCATCGGCGTTA TCATCCGACCCAGTAAAGTTG	Monomorphic	–	–

Table 2 continued

Sequence	GenBank accession	Gene name	Best Hit (BlastX)	*E* value	Primer sequences (forward and reverse, 5′→3′)	SNP/indel position and location	SNP detection method	Linkage group
FR12E16[b]	DV632961	*CmPME3*	Pectinesterase (*A. thaliana*) AAN18134	2E-43	TGGAGTACAAATTGAACGAATATAA GGGCAGTACTCGAGAATCGT	T→A 73 (exon) G→T 202 (exon) C→T 311 (intron) G→A 321(intron) A→G 514 (intron) A→G 639 (intron) C→A 666 (intron) A→G 673 (intron) C→T 699 (intron) G→T 705 (intron)	CAPS (*Hin*fI)	IX
HS_39-B01[a]	AM725492	*CmPME4*	Pectinesterase (*N. tabacum*) CAB95025	6E-78	CCAAATTCCGGTCAGAAAAA GAAAGGAAAGCCCGTAGAGC	Monomorphic	–	–
46d_01-B10[a]	AM740810	*CmEXP1*	Expansin *(C. melo*) ABI94060	0.0	CTTCTCTCACGCCAGAATCC CCCCAGTTACGACTCATGCT	A→C 142 (intron) A→G 151 (intron) G→A 161 (intron) C→T 176 (intron) C→T 190 (intron) C→A 235 (intron) C→T 255 (intron) G→- 258 (intron) T→A 280 (intron) (AAATT)3→ (AAATT)4 283 (intron) A→T 391 (intron) A→C 426 (intron)	CAPS (*Dra*I)	VIII
FR13C9[b]	DV633239	*CmEXP2*	Expansin (*G. hirsutum*) ABB59694	4E-100	TGGCTCCTTCACCACTCTCT AGAGGCTCTGTCCGTTCAAA ATTCGTCATTAGGACAGAAGTCC[c,e]	C→T 372 (intron) T→−409 (intron)	SNaPshot	III
P11.25[a]	*	*CmEXP3*	Expansin precursor (*C. sativus*) AAB37746	2E-11	TGATGGTCACACTCTCACTGC CCTCTCTACCCTCTCCCAAGA GAAACGATTGACAAGCCCCAACT[c]	T→C 64 (exon) −→A 123 (3′ UTR) C→G 192 (3′ UTR) T→−238 (3’ UTR) −→T 276 (3′UTR)	SNaPshot	X

Table 2 continued

Sequence	GenBank accession	Gene name	Best Hit (BlastX)	*E* value	Primer sequences (forward and reverse, 5′→3′)	SNP/indel position and location	SNP detection method	Linkage group
P3.84[a]	*	*CmPL*	Pectate lyase (*Fragaria x ananassa*) AAK66161	1E-126	AACCGGCAATCCTATTGATG CTTACCATGGCGTTTCCAGT	Monomorphic	–	–
P3.08[a]	*	*CmXylIso*	Xylose ismoerase (*A. thaliana*) Q9FKK7	3E-87	TGAACACTGACATGGGGAGA AATCCGCCATTTCTCACAAC	Not amplified	–	–

The best BlastX hit for each sequence is indicated. Primer sequences, SNPs identified, SNP detection method and melon linkage group are indicated. For CAPS markers the restriction enzyme is also shown

* Sequences available in MELOGEN only (http://www.melogen.upv.es)

[a] ESTs from MELOGEN database. Some of them are singletons. For ESTs contained in contigs, a representative EST from the contig is given

[b] Sequences retrieved from GeneBank

[c] Primers for SNaPshot polymorphism detection

[d] Sequences mapped through BAC-end sequencing or BAC subcloning

[e] SNaPshot primers designed from intron sequences

[f] Primers for Licor polymorphism detection

SC, when introgressed into the PS genetic background, is able to induce climacteric ripening, suggesting that the non-climacteric phenotypes from PS and SC are probably produced by different genes. In fact it has been suggested that SC has impaired ethylene perception in the fruit tissue, as ethylene does not induce endogenous ethylene production or expression of known target genes (Périn et al. 2002). In climacteric melon types the treatment with exogenous ethylene can induce abscission, endogenous ethylene production and ripening. The non-climacteric "Earl's Favourite" melon (Cantalupensis) did not induce endogenous ethylene synthesis after treatment with exogenous ethylene, but *ACO1* expression was induced (Shiomi et al. 1999). Thus, the non-climacteric phenotype found in "Earl's Favourite" suggests a different type of mechanism than the one found in SC, maybe related to the impaired regulation in early ethylene biosynthesis steps, as it has been reported for the tomato *rin* and *nor* mutants (Giovannoni 2007). The same non-climacteric phenotype may also be attributed to PS, but exogenous ethylene treatments have to be performed in PS in order to make further conclusions. It should be noted that values of ethylene production for SC3-5b (77.8 pmol kg^{-1} s^{-1}) were not as high as the ones usually found in typical climacteric "Védrantais" melons (207 pmol kg^{-1} s^{-1}), suggesting that additional loci may control the levels of ethylene.

Interestingly, Monforte et al. (2004) reported an association of the genomic region containing *eth3.5* with orange flesh colour found in fruits of some individuals belonging to F_2 and DHL populations derived from the SC × PS cross, even though neither PS nor SC show orange flesh. Thus, *eth3.5*, through ethylene production, could induce the formation of orange flesh colour in combination with other loci as *ofc3.1* and *ofc12.1* (Monforte et al. 2004), in contrast with the previously reported ethylene-independent pathway of flesh colour in cantaloupe melon (Guis et al. 1997).

Several QTLs for fruit flesh softening have been detected

The genetic control of fruit firmness and related traits has been studied in a limited number of works. Four reproducible flesh firmness QTLs have been identified in apple (Liebhard et al. 2003). In tomato, however, two major QTLs for fruit firmness identified in chromosomes 4 and 9 using an RIL mapping population (Saliba-Colombani et al. 2001; Causse et al. 2002) did not have consistent effects on different years and genetic backgrounds, except the QTL on chromosome 4 in a specific genetic background (Chaïb et al. 2007). In the current report we described five QTLs related to fruit flesh firmness, three of them tested and verified in different years or locations. The SC allele of *ff10.2* increased flesh firmness, a desirable trait for "Piel de Sapo" cultivars. *ff3.5* is also interesting as it maps in the same

Table 3 Candidate genes mapped after the identification of a melon BAC clone containing them

Gene name	Positive BAC	BAC polymorphic region	SNP detection method	Primer sequences for amplification and polymorphism detection[a] (5′→3′)
CmACO5	23-4A	internal sequence	SNaPshot	GGCTTTAGGTGGGCTTGAG AATGATTATTGAGAAACGACGTAAA CTATGTTGGGTTTGGGCTTGAGT[a]
CmETR1	12-17P	internal sequence	SCAR	GGAGCAACGGATGGTCTTTA CAAAATTGATTTGTCATCCTAACC
CmERS1	41-17L	BAC-end	SNaPshot	TTTTCACCCTAACTTTGAAGTGTAAT ACTCGAAGAATATAACAAACCAAACA GAGTAATGTAATTTTTACAAATA[a]
CmETR2	20-6B	internal sequence	SNaPshot	CCTCCTTGGAAGATAAGTTGG TGGTGTGATGTCAATGTGGA GTTGATACAAAGGTAGCTTATTCT[a]

SNP detection method and primers for amplification and SNP detection are indicated

[a] Primers for SNaPshot polymorphism detection

Table 4 Candidate genes previously mapped in the PI 161375 × "Piel de Sapo" and "Védrantais" × PI 161375 genetic maps

Gene name	Marker type	Linkage group
CmACS1	RFLP	XI[a]
CmACO1	SSR (CMAT35)	V[a]
CmACO2	RFLP	VIII[b]
CmXTH6	RFLP	V[b]
CmXTH7	RFLP (MC284)	IV[a]
CmXTH8	RFLP (MC102)	IX[a]
CmPME1	RFLP (MC325)	IX[a]
CmPME2	RFLP (MC373)	VII[a]
CmPGinh	RFLP (MC348)	IX[a]
ech	Phenotipic	I[b]
Al-3	Phenotipic	VIII[b]
Al-4	Phenotipic	IX[b]

The marker name in the SC × PS map in brackets

[a] Mapped in the PI 161375 × "Piel de Sapo" framework map (Oliver et al. 2001)

[b] Mapped in the "Védrantais" × PI 161375 framework map (Périn et al. 2002)

introgression than the climacteric QTL *eth3.5*, suggesting that the decrease in flesh firmness in SC3-5b may be a consequence of the climacteric ripening phenotype due to *eth3.5*. Thus, this is the first example where QTLs for fruit firmness with consistent effects in independent experiments have been "mendelized" using a set of NILs. Previous studies have shown that cell wall changes leading to fruit softening and textural changes are complex, and involve the coordinated and interdependent activities of a range of cell wall-modifying proteins (Brummell and Harpster 2001; Rose et al. 2003). The reproducibility of the reported firmness QTLs makes NILs SC3-5b, SC8-2 and SC-10-2 an excellent genetic material to study the molecular basis of this trait. These QTLs are also useful for introducing new genetic variabilities in melon breeding programs.

Several candidate genes colocate with QTLs for fruit softening and climacteric ripening

The recent availability of gene sequences in melon allowed mining the databases for candidate genes involved in ethylene metabolism and cell wall degradation (http://www.melogen.upv.es). We have been able to map 18 genes involved in ethylene metabolism and 14 genes encoding cell wall-degrading enzymes. Many candidate genes are members of multigenic gene families from which only a few genes have been retrieved from MELOGEN (Gonzalez-Ibeas et al. 2007). When more melon sequences are available, additional candidate genes will be identified in order to further saturate the genetic map.

Transcription factors from the MADS-box, NAC-domain or SBP-box families, which are regulators of ripening in tomato as *rin*, *nor* and *Cnr*, respectively (Vrebalov et al. 2002; unpublished; Manning et al. 2006), were not included for analysis. Additional MADS-box genes that are expressed in the tomato fruit could also have a role in the control of fruit ripening (Giovannoni 2007). Several ESTs with homology to MADS-box, NAC-domain and SBP-box genes have been identified in MELOGEN. However, these genes were not considered for mapping, because it is difficult to infer only from sequence homologies which are the melon orthologues of *rin*, *nor* or *Cnr*.

Several possible colocations between candidate genes and QTLs for fruit firmness and climacteric ripening were identified, although it should be considered that the mapping resolution is still low and they could reflect simply random coincidences. Further experiments are needed in order to validate the hypothesis that the candidate genes are responsible for the QTLs.

In linkage group 10, *ff10.2* was found accounting for an increase in fruit firmness when compared with PS. The SC introgression spans all linkage group X (58 cM), and in this interval we have mapped one expansin gene *CmEXP3* and one xyloglucan endotransglycosilase/hidrolase *CmXTH5*. Further mapping resolution is needed in this region in order to locate *ff10.2* in a smaller interval.

The positions of QTLs *eth2.1* and *eth3.1* for ethylene content in linkage groups II and III (Périn et al. 2002) colocate with the position of three ethylene-insensitive gene homologues *CmEIL1*, *CmEIL3* and *CmEIL4*, respectively. *EIN3*/*EIL* encode nuclear-localized proteins that are required for responding to ethylene by binding to promoter regions of the *ERF1* gene in *Arabidopsis* (Solano et al. 2006). These genes are located downstream the ethylene receptor in the ethylene-mediated signal transduction pathway. In linkage group I, the position of the ethylene receptor *ERS1* colocated with *eth1.1* as it was previously reported by Périn et al. (2002). Further experiments are required to confirm these genes as candidates for QTLs controlling the levels of ethylene in the progeny of Védrantais × SC.

In the introgression of 50 cM containing *eth3.5* for climacteric ripening in linkage group III, we have mapped the *CmACS5* gene. The ACC synthase (ACS) multigenic family is involved in the conversion of S-adenosylmethionine to 1-aminocyclopropane-1-carboxylic acid (ACC), which is converted to ethylene by members of the ACC oxidase family. In *Arabidopsis* 12 members of the *ACS* gene family have been identified (Yamagami et al. 2003). Three *ACS* genes have been described in melon (Miki et al. 1995; Ishiki et al. 2000), and two new ESTs (*CmACS4* and *CmACS5*) have been retrieved from MELOGEN. *CmACS5* may correspond to *ACS5* in Périn et al (2002) for map position, but the *ACS5* sequence is not available. Again, a possible role of *CmACS5* in the regulation of climacteric ripening cannot be discarded, but further mapping resolution in this interval and expression studies for *CmACS5* are needed.

Genetic analysis of QTLs can be performed efficiently using NIL collections (Eshed and Zamir 1995). The results presented in this report demonstrate that the melon genomic library of near-isogenic lines (Eduardo et al. 2005) is a powerful tool to dissect complex fruit traits. These complex traits have been "mendelized", which would allow a better characterization of the underlying genes. Furthermore, unexpected allele effects have been detected, as the climacteric behaviour due to *eth3.5* or the increase of flesh firmness due to *ff10.2*. This data widens the spectrum of future research and practical applications in genetics and postharvest.

Several interesting associations between QTLs and candidate genes have been defined. Further research on these associations will help to understand the ripening process in melon. Moreover, the mapped ESTs that are not associated with any QTL can be a source of candidate genes in other works that include different melon germplasm.

Acknowledgments We thank Montserrat Saladié for critically reading the manuscript. This work was funded by grants AGL2003-09175-C02-01 and AGL2003-09175-C02-02 from the Spanish "Ministerio de Educación y Ciencia" (MEC). EM was supported by an AGAUR fellowship (Generalitat de Catalunya). JO was supported by a MAE-AECI fellowship from the Spanish Ministry of Foreigner Affairs. NDS was supported by two consecutive fellowships from FPI (Fundación Séneca de la Región de Murcia) and FPU (MEC AP2006-01565). We are indebted to Michel Pitrat (INRA, Montfavet, France) for the "Védrantais" seeds; to Fuensanta García, Claudia Miranda and María José Ferrer for technical assistance; to Plácido Varó and his team (CIFEA-Torre Pacheco, Consejería de Agricultura de la Región de Murcia) and Antoni Ortigosa for crop management.

References

Altschul SF, Gish SF, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Ayub R, Guis M, BenAmor M, Gillot L, Roustan JP, Latché A, Bouzayen M, Pech JC (1996) Expression of ACC oxidase antisense gene inhibits ripening of cantaloupe melon fruits. Nat Biotechnol 14:862–866

Barry C, Giovanonni J (2006) Ripening inhibition in the tomato *Green-ripe* mutant results from ectopic expression of a novel protein which disrupts ethylene signal transduction. Proc Natl Acad Sci USA 103:7923–7928

Brummell DA, Harpster MH (2001) Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. Plant Mol Biol 47:311–340

Causse M, Saliba-Colombani V, Lecomte L, Duffé P, Rousselle P, Buret M (2002) QTL analysis of fruit quality in fresh market tomato: a few chromosome regions control the variation of sensory and instrumental traits. J Exp Bot 53:2089–2098

Chaïb J, Devaux M-F, Grotte M-G, Robini K, Causse M, Lahaye M, Marty I (2007) Physiological relationships among physical, sensory, and morphological attributes of texture in tomato fruits. J Exp Bot 58:1915–1925

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Dunnet CW (1955) A multiple comparison procedure for comparing several treatments with a control. J Am Stat Assn 50:1096–1121

Eduardo I, Arús P, Monforte AJ (2005) Development of a genomic library of near isogenic lines (NILs) in melon (*Cucumis melo* L.) from the exotic accession PI 161375. Theor Appl Genet 6:1–10

Eduardo I, Obando J, Martínez JA, Alarcón AL, Arús P, Álvarez JM, van der Knaap E, Fernández-Trujillo JP, Monforte AJ (2007) Estimating the genetic architecture of fruit quality traits in melon (*Cucumis melo* L.) using a genomic library of near-isogenic lines. J Am Soc Hortic Sci 132:80–89

Eshed Y, Zamir D (1995) An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. Genetics 141:1147–1162

Fernández-Trujillo JP, Obando J, Martínez JA, Alarcón A, Eduardo I, Arús P, Monforte AJ (2005) Quality management of experiments with a collection of near-isogenic lines of melon. In: Atienza J, Rabasseda J (eds). Proceedings of third virtual Iberoamerican Congress of laboratory quality management. III IBEROLAB, Madrid, pp 149–158

Fernández-Silva I, Blanca J, Picó MB, Nuez F, Arús P, Monforte AJ (2006) Mapeo de marcadores microsatélites a partir de librerías de

ESTs de melón (*Cucumis melo*) mediante genotipado selectivo. Actas de Horticultura 45:109–110

Garcia-Mas J, Oliver M, Gómez H, de Vicente MC (2000) AFLP, RAPD and RFLP markers to measure genetic diversity in melon. Theor Appl Genet 101:860–864

Giovannoni JJ (2007) Fruit ripening mutants yield insights into ripening control. Curr Opin Plant Biol 10:1–7

Gonzalez-Ibeas D, Blanca J, Roig C, González-To M, Picó B, Truniger V, Gómez P, Deleu W, Caño-Delgado A, Arús P, Nuez F, Garcia-Mas J, Puigdomènech P, Aranda MA (2007) MELOGEN: an EST database for melon functional genomics. BMC Genomics 8:306

Gonzalo MJ, Oliver M, Garcia-Mas J, Monfort A, Dolcet-Sanjuan R, Katzir N, Arús P, Monforte AJ (2005) Simple-sequence repeat (SSR) markers used in merging linkage maps of melon (*Cucumis melo* L.). Theor Appl Genet 110:802–811

Guis M, Botondi R, Ben Amor M, Ayub R, Bouzayen M, Pech JC, Latche A (1997) Ripening-associated biochemical traits of cantaloupe Charentais melons expressing antisense ACC oxidase transgene. J Am Soc Hortic Sci 122:748–751

Hadfield KA, Rose JK, Yaver DS, Berka RM, Bennett AB (1998) Polygalacturonase gene expression in ripe melon fruit supports a role for polygalacturonase in ripening-associated pectin disassembly. Plant Physiol 117:363–373

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Harker FR, Redgwell RJ, Hallett IC, Murray SH, Carter G (1997) Texture of fresh fruit. Hortic Rev 20:121–224

Howad W, Yamamoto T, Dirlewanger E, Testolin R, Cosson P, Cipriani G, Monforte AJ, Georgi L, Abbott AG, Arus P (2006) Mapping with a few plants: using selective mapping for microsatellite saturation of the *Prunus* reference map. Genetics 171:1305–1309

Ishiki Y, Oda A, Yaegashi Y, Orihara Y, Arai T, Hirabayashi T, Nakagawa H, Sato T (2000) Cloning of an auxin-responsive 1-aminocyclopropane-1-carboxylate synthase gene (*CMe-ACS2*) from melon and the expression of *ACS* genes in etiolated melon seedlings and melon fruits. Asian J Plant Sci 159:173–181

Kader AA (2000) Methods of gas mixing, sampling and analysis. In: Kader AA (ed) Postharvest technology of horticultural crops. Pub no 3311. Division of agriculture and natural resources, 3a edn. University of California, pp 145–148

Lasserre E, Bouquin T, Hernández JA, Bull J, Pech JC, Balague C (1996) Structure and expression of three genes encoding ACC oxidase homologs from melon (*Cucumis melo* L.). Mol Gen Genet 251:81–90

Liebhard R, Kellerhals M, Pfammatter W, Jertmini M, Gessler C (2003) Mapping quantitative physiological traits in apple (*Malus x domestica* Borkh.). Plant Mol Biol 52:511–526

Lin YT, Pitt RE (1986) Rheology of apple and potato tissue as affected by cell turgor pressure. J Text Stud 17:291–313

Manning K, Tor M, Poole M, Hong Y, Thompson A, King G, Giovannoni J, Seymour G (2006) A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. Nat Genet 38:949–952

Miki T, Yamamoto M, Nakagawa H, Ogura N, Mori H, Imaseki H, Sato T (1995) Nucleotide sequence of a cDNA for 1-aminocyclopropane-1-carboxylate synthase from melon fruits. Plant Physiol 107:297–298

Mizuno S, Hirasawa Y, Sonoda M, Nakagawa H, Sato T (2006) Isolation and characterization of three DREB/ERF-type transcription factors from melon (*Cucumis melo*). Plant Sci 170:1156–1163

Monforte AJ, Oliver M, Gonzalo MJ, Álvarez JM, Dolcet-Sanjuan R, Arús P (2004) Identification of quantitative trait loci involved in fruit quality traits in melon (*Cucumis melo* L.). Theor Appl Genet 108:750–758

Moore S, Vrebalob J, Giovannoni J (2002) Use of genomic tools to isolate key ripening genes and analyse fruit maturation in tomato. J Exp Bot 53:2023–2030

Morales M, Roig E, Monforte AJ, Arus P, Garcia-Mas J (2004) Single-nucleotide polymorphisms detected in expressed sequence tags of melon (*Cucumis melo* L.). Genome 47:352–360

Nishiyama K, Guis M, Rose JKC, Kubo Y, Bennett KA, Wangjin L, Kato K, Ushijima K, Inaba A, Bouzayen M, Latche A, Pech JC, Bennett AB (2007) Ethylene regulation of fruit softening and cell wall disassembly in Charentais melon. J Exp Bot 58:1281–1290

Obando J, Fernández-Trujillo JP, Martínez JA, Alarcón AL, Eduardo I, Arús P, Monforte AJ (2007) Identification of melon fruit quality quantitative trait loci using near-isogenic lines. J Am Soc Hortic Sci (in press)

Oliver M, Garcia-Mas J, Cardus M, Pueyo N, Lopez-Sese AL, Arroyo M, Gomez-Paniagua H, Arus P, de Vicente MC (2001) Construction of a reference linkage map for melon. Genome 44:836–845

Périn C, Gómez-Jiménez M, Hagen L, Dogimont C, Pech JC, Latché A, Pitrat M, Leliévre JM (2002) Molecular and genetic characterization of a non-climacteric phenotype in melon reveals two loci conferring altered ethylene response in fruit. Plant Physiol 129:300–309

Rose JKC, Catalá C, Gonzalez-Carranza ZH, Roberts J (2003) Plant cell wall disassembly. In: Rose JKC (ed). The plant cell wall: annual plant reviews series, vol 8. Blackwell Publishing Ltd, Oxford, pp 264–324

Rozen S, Skaletsky H (2000) Primer3 on the WWW for general users and for biologist programmers. Meth Mol Biol 132:365–386

Saliba-Colombani V, Causse M, Langlois D, Philouze J, Buret M (2001) Genetic analysis of organoleptic quality in fresh market tomato. 1. Mapping QTLs for physical and chemical traits. Theor Appl Genet 102:259–272

Sato-Nara K, Yuhashi KI, 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 120:321–330

Shackel KA, Greve C, Labavitch JM, Ahmadi H (1991) Cell turgor changes associated with ripening in tomato pericarp tissue. Plant Physiol 97:814–816

Shiomi S, Yamamoto M, Nakamura R, Inaba A (1999) Expression of ACC synthase and ACC oxidase genes in melons harvested at different stages of maturity. J Japan Soc Hortic Sci 68:10–17

Solano R, Stepanova A, Chao Q, Ecker JR (2006) Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. Genes Dev 12:3703–3714

van Leeuwen H, Monfort A, Zhang HB, Puigdomenech P (2003) Identification and characterisation of a melon genomic region containing a resistance gene cluster from a constructed BAC library. Microcolinearity between *Cucumis melo* and *Arabidopsis thaliana*. Plant Mol Biol 51:703–718

Vicente AR, Saladié M, Rose JKC, Labavitch JM (2007) The linkage between cell wall metabolism and fruit softening: looking to the future. J Sci Food Agric 87:1435–1448

Vrebalov J, Ruezinsky D, Padmanabhan V, White R, Medrano D, Drake R, Schuch W, Giovannoni J (2002) A MADS-box gene necessary for fruit ripening at the tomato *ripening-inhibitor* (*rin*) locus. Science 296:343–346

Yamagami T, Tsuchisaka A, Yamada K, Haddon WF, Harden LA, Theologis AJ (2003) Biochemichal diversity among the 1-aminocyclopropane-1-carboxylate synthase isozymes encoded by the *Arabidopsis* gene family. Biol Chem 278:49102–49112

Yamasaki S, Fujii 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