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ORIGINAL PAPER

Interaction between QTLs induces an advance in ethylene biosynthesis during melon fruit ripening

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Abstract The coexistence of both climacteric and non-climacteric genotypes and the availability of a set of genetic and genomic resources make melon a suitable model for genetic studies of fruit ripening. We have previously described a QTL, *ETHQB3.5*, which induces climacteric fruit ripening in the near-isogenic line (NIL) SC3-5 that harbors an introgression on linkage group (LG) III from the non-climacteric melon accession PI 161375 in the, also non-climacteric cultivar, "Piel de Sapo" genetic background. In the current study, a new major QTL, *ETHQV6.3*, on LG VI was detected on an additional introgression in the same NIL. These QTLs are capable, individually, of inducing climacteric ripening in the non-climacteric background, the effects of *ETHQV6.*3 being greater than that of *ETHQB3.5*. The QTLs interact epistatically, advancing the timing of ethylene biosynthesis during ripening and, therefore, the climacteric responses. *ETHQV6.3* was fine-mapped to a 4.5 Mb physical region of the melon genome, probably in the centromeric region of LG VI. The results presented will be of value in the molecular identification of the gene underlying *ETHQV6.3*

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Introduction

Fruit development is one of the most important processes for a plant and requires a large amount of energy. The compensation is the dispersion of the seeds, in some cases over long distances, for species survival and colonization. Plant species originally domesticated for human consumption have been further selected to produce fruit which is more appealing to human preferences, by traditional farmers and, more recently, by modern breeding techniques. Dramatic biochemical changes occurred to convert the immature fruit into attractive ripe fruit, for both animal and human consumption. A number of ripening-associated changes are common in most species, including the conversion of starch to sugars, fruit softening, accumulation of pigments such as carotenoids, and synthesis of aroma volatiles (Seymour et al. 1993). These features, related to organoleptic components, have been frequent targets of physiological and molecular studies over the last few years; knowledge of the control of the mechanisms underlying their production may have important commercial applications.

The general fruit development model (Gillaspy et al. 1993) distinguishes three major stages: cell division, cell expansion, and, finally, ripening. Fruit ripening is achieved through two main mechanisms based on the role of the ethylene hormone in the process: (1) climacteric ripening, characterized by an increase in respiration and concomitant autocatalytic ethylene synthesis upon initiation of ripening, and (2) non-climacteric ripening, characterized by a continuous decrease in respiration rate and ethylene production

in the absence of the autocatalytic response (McMurchie et al. 1972). However, despite these two clearly different types of hormonal controls, there are several common features that accompany the onset of fruit ripening, suggesting common pathways that might be shared by the two models.

The study of the regulation and molecular basis of ripening has been a major focus in plant research, especially in fleshy fruits, with the climacteric fruit of tomato being the most studied model. Important advances have been made over the years to decipher the biosynthetic pathway (Alexander and Grierson 2002) and the components of the perception (Klee 2004) and transduction (Adams-Phillips et al. 2004) systems of the hormone ethylene during tomato fruit ripening. The availability of tomato mutants with impaired fruit ripening has greatly helped to unravel the mechanisms that control ripening. In the *ripening inhibitor* (*rin*), *non-ripening* (*nor*) and *colorless non-ripening* (*cnr*) mutants, inhibition of fruit ripening is severe. They also share some physiological features: (a) complete development of a mature green fruit with mature seeds, (b) inability to undergo the climacteric rise in respiration or ripening-associated ethylene production, and (c) insensitivity to exogenous ethylene (Eriksson et al. 2004; Giovannoni 2007; Manning et al. 2006). The cloning of *Rin* (Vrebalov et al. 2002), *Cnr* (Manning et al. 2006) and *Nor* (patent US 6762347B1) revealed that all of them are transcription factors. Additional transcription factors have been identified demonstrating the role of TAGL1 (Itkin et al. 2009) and LeHB-1 (Lin et al. 2008) in controlling the fruit ripening. While these transcription regulators are all positive effectors, the first negative regulator has recently been identified, an APETALA2 gene family member (Chung et al. 2011; Karlova et al. 2011). Currently the main research efforts are dedicated to find new candidate genes regulating fruit ripening as well as understanding the interactions among them (Klee and Giovannoni 2011; Fujisawa et al. 2012; Kumar et al. 2012; Bemer et al. 2012). In spite of these advances, the full complexity of the climacteric ripening regulatory system and the interplay between ethylene-dependent and -independent mechanisms still remain to be unraveled.

Even though climacteric ripening is a well-conserved mechanism, there is a variation in climacteric ripening responses in fleshy fruit species such as apple, peach, banana and melon, which gives a better understanding of the physiological and biochemical processes that account for climacteric fruit ripening. This is the case of carotenoid content, which has been reported to be partially ethylene-dependent in tomato (Lee et al. 2012) and papaya (Barreto et al. 2011), but is ethylene-independent in melon, as silencing *Aco1* in melon transgenic plants does not produce a reduction of carotenoids (Silva et al. 2004).

In recent years, melon (*Cucumis melo L.*) has emerged as an alternative model system for fruit ripening studies due to the coexistence of genotypes that follow either climacteric or non-climacteric fruit ripening, which can provide new insights into the complexity of cross talk between the two types of ripening. Furthermore, the genetic and genomic tools developed in recent years make possible the comprehensive study of this process. The availability of genetic populations such as double haploid lines (DHLs) (Gonzalo et al. 2011) and near-isogenic lines (NILs) (Eduardo et al. 2005), saturated genetic maps (Diaz et al. 2011), microarrays (Mascarell-Creus et al. 2009), reverse genetic platforms (Gonzalez et al. 2011) and the genome sequence (Garcia-Mas et al. 2012), provides us with a complete system to find new elements of this complex developmental regulatory network.

Cantalupensis melon varieties have typical climacteric behavior and, consequently, these varieties have often been used as a model to study fruit ripening (Ezura and Owino 2008). In a pioneer study, ethylene production was drastically reduced in antisense *Aco1* cantaloupe transgenic plants (Ayub et al. 1996). Both exogenous ethylene treatment and application of 1-methyl-cyclopropene (1-MCP) in ACO1 antisense melon transgenic plants confirmed that fruit softening is an ethylene-dependent process (Nishiyama et al. 2007). Fruit ripening processes such as the increase in fruit softening, the aroma profile, fruit abscission and rind color are ethylene-dependent, whereas flesh color, accumulation of sugars and carotenoids, and loss of acidity are ethylene-independent processes (Pech et al. 2008). In contrast, the ripening processes in non-climacteric melon varieties have not been studied in depth.

Périn et al. (2002) analyzed a population of recombinant inbred lines (RILs) derived from the climacteric variety "Charentais" and the Korean non-climacteric accession PI 161375. They studied the segregation for fruit abscission and ethylene production and found that both characters are controlled by two major independent loci, abscission layer *Al-3* and *AI-4*, as well as several QTLs involved in differences in ethylene accumulation among climacteric RILs. A collection of NILs developed from the Spanish cultivar "Piel de Sapo" and PI 161375 (both of them non-climacteric) has been extensively studied for fruit quality traits (Eduardo et al. 2007; Moreno et al. 2008; Fernandez-Silva et al. 2010; Obando-Ulloa et al. 2010). Unexpectedly, fruits from the introgression line SC3-5 showed climacteric ripening (Moreno et al. 2008; Obando et al. 2008), indicating the presence of a gene for climacteric ripening in the introgression from PI 161375 on LG III. Further molecular characterization of SC3-5 showed that an additional, previously undetected, introgression from PI 161375 was also present on LG VI (Vegas et al. 2010), spanning 32 cM, making impossible to discern whether the climacteric

ripening was actually induced by genes on LG III, LG VI or genes from both LG would be necessary to induce climacteric ripening. Therefore, the objectives of the current research were to verify the effects of the introgression on LG III, determine whether the additional introgression on LG VI also has effects on ripening, and study possible interactions between the introgressions, and to increase the mapping resolution of the genes involved in melon fruit ripening behavior.

Materials and methods

Plant material and phenotyping

Two *Cucumis melo* non-climacteric varieties, "Piel de Sapo" (PS) and the Korean accession PI 161375 (SC) were the parents of the genetic stocks used in this study. The climacteric near-isogenic line (NIL) SC3-5, harboring an introgression from SC on LG III in the PS genetic background (Eduardo et al. 2005), also carried a second, previously undetected, introgression on LG VI (Vegas et al. 2010). This NIL with two introgressions was renamed SC3-5-1, whereas a new NIL, carrying only the LG III introgression, was developed by marker assisted selection and named SC3-5 (Online Resource 1). Additional populations were derived from the cross SC3-5-1 × PS (Fig. 1). All the plants were grown in a greenhouse in peat bags and drip irrigated as described previously (Moreno et al. 2008). Fruits were hand pollinated and the pollination day for each fruit was recorded.

The two approaches used to determine climacteric behavior were visual inspection and ethylene production (μL kg^{-1} h^{-1}), measured by gas chromatography, according to Chiriboga et al. (2011). Briefly, melon fruits were placed in 1.5 L flasks in an acclimatized chamber at 20 °C, continuously ventilated with humidified air at a flow rate of 1.5 L h^{-1}. Gas samples (1 ml) were taken of effluent air from respiration jars, using a 1 ml syringe, and injected into a gas chromatograph (Agilent Technologies 6890, Wilmington, Germany) fitted with an FID detector and an alumina column F1 80/100 (2 m × 1/8 × 2.1, Teknokroma, Barcelona, Spain). The injector was kept at 120 °C and the detector at 180 °C.

Climacteric melon fruit usually has a number of phenotypic characteristics that contrast with non-climacteric melon fruit, such as the formation of an abscission layer in the peduncle, fruit detachment (Abeles 1992), degreening of the rind, shelf-life, chilling injury and aroma production (Guis et al. 1997; Ben-Amor et al. 1999). Therefore, we also used fruit phenotypic traits to classify them as climacteric or non-climacteric (see Result section for further explanation). To ensure an adequate classification, fruit was left on the plant up to 60 days after anthesis (when the first symptoms of putrefaction appear in non-climacteric fruits) to ensure the formation of the abscission layer (climacteric fruits usually detach before 40 DAP). Total soluble solids content was estimated in flesh juice by measuring the Brix index with a digital refractometer (PAL-1; Atago, Tokyo, Japan). Flesh firmness was measured using a hand penetrometer fitted with an 8 mm cylindrical probe (fruit pressure tester, model FT-011; Italy).

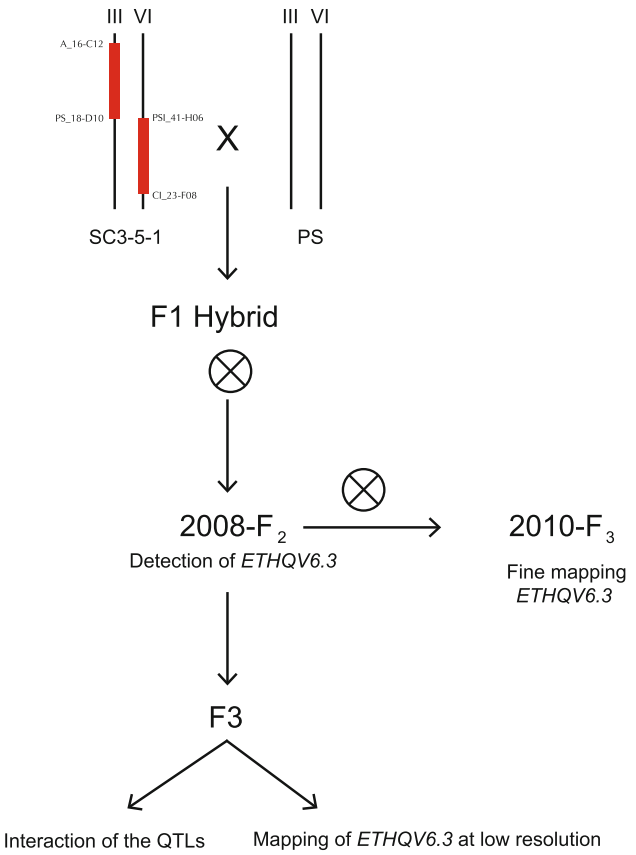

Fig. 1 Populations used in this study were from the cross between the climacteric line SC3-5-1 and "Piel de Sapo" (PS). *Solid bars* represent the introgressions from Korean accession PI 161375 (SC) into the Spanish cultivar PS genetic background, indicating the markers flanking the introgressions. F_2 and F_3 populations used for the detection and mapping of *ETHQV6.3* and the study of the interaction between both QTLs are also shown

DNA extraction and genotyping

Genomic DNA from all the plants was isolated from young leaves according to Doyle and Doyle (1990) with some modifications (Garcia-Mas et al. 2000).

Twenty-two molecular markers were used in this study; most of them already described in the literature and databases (Diaz et al. 2011, www.icugi.org). Additional new markers were designed from the melon genome sequence (Garcia-Mas et al. 2012). Simple sequence repeat markers

(SSRs) were developed by searching microsatellite motifs in the region on LG VI covered by the introgression of SC in SC3-5-1 using Tandem Repeat Finder (http://tandem.bu.edu/trf/trf.html) (Benson 1999). The primers flanking the repeats were designed with Primer 3 (http://frodo.wi.mit.edu/primer3/) (Rozen and Skaletsky 2000). PCR reactions were in a final volume of 15 µl with 1 × Taq buffer [10 mM Tris–HCl, 50 mM KCl, 0.001 % gelatin (pH 8.3)], 1.5–3.5 mM $MgCl_2$, 166 µM dNTPs, 5 pmol of each primer, 2 units of Taq DNA polymerase and 20 ng of DNA. Amplification was in a PTC-200 Thermocycler (MJ Research, Waltham, MA, USA) as follows: an initial cycle at 94 °C for 1 min, followed by 35 cycles at 94 °C, 30 s, 40–60 °C, 30 s and 72 °C, 1 min, and a final cycle at 72 °C for 5 min. To label the PCR products, a 20-nucleotide sequence from the M13 cloning vector (5′ CACGACGTTGTAAAACGACC 3′) was attached to the 5-end of the forward primers. PCR was as above, with the addition of 2 pmol of each primer and 0.66 pmol of IRD700- or IRD800-labeled oligonucleotide complementary to the 20-mer M13 sequence. Amplified fragment bands were separated and visualized using a LI-COR IR2 sequencer (Li-Cor Inc, Lincoln, NE, USA). After the addition of 5 µl of loading buffer (95 % formamide, 20 mM EDTA, 0.05 % bromophenol blue, 0.05 % xylene cyanol), the amplified products were electrophoresed in denaturing conditions at 50 °C in TBE (90 mM Tris–borate, 2 mM EDTA pH 8.0 and 7.5 M urea) using 6 % polyacrylamide gels (AA:BIS = 19:1).

Additional SNP markers between PS and SC were searched within the sequence of candidate genes using the melon genome sequence (Garcia-Mas et al. 2012). When SNPs were located within a restriction enzyme target, a cleaved amplified polymorphic sequence (CAPS) assay was designed. The marker names, amplification conditions and references are listed in Online Resource 2.

Statistical analysis

Analysis of variance, mean comparisons, mean distributions and χ^2 contingency tests were performed with JMP v5.12 for Windows (SAS Institute Inc., NC).

Experimental design and data analysis

Characterization of ripening behavior in SC3-5-1

Three fruits from three independent plants of each genotype, PS and SC, SC3-5-1 and the hybrid SC3-5-1 × PS, were harvested at 30 days after pollination (DAP). Ethylene synthesis was recorded every day until either the climacteric peak was detected or fruit showed the first symptoms of putrefaction.

Verification of effects of introgressions on LG III and VI on ripening behavior

From the cross between SC3-5-1 × PS (named 2008-F2), 152 F2 plants were grown in a completely randomized design in a greenhouse during the summer of 2008 in Cabrils (Barcelona). Fruits were harvested at 35 DAP and ripening behavior was classified visually as described above, recording "1" as climacteric and "0" as non-climacteric. All plants were genotyped with markers included in the introgressed regions on LG III (A_16-C12 and PS_18-D10) and LG VI (PSI_41-H06, CMN61_14, ECM14 and CI_23-F08) and a genetic map was constructed using MAPMAKER 3.0. The association between ripening behavior and the introgressions was investigated using a contingency test ($p < 0.05$) and composite interval mapping (CIM) (Zeng 1994) with qGENE (Nelson 1997), calculating the LOD score threshold for $\alpha < 0.05$ by a permutation test with 1,000 resampling.

Fine mapping

Nine 2008-F2 recombinants between markers PSI_41-H06 and CI_23-F08 were selected to fine map the QTL within the introgression in LG VI. Twenty seeds for each of the F3 families were sown for progeny testing. All individuals were genotyped using additional molecular markers in the interval (MU10920, AI_19-F11, CMBR002, AI_03-B03, FR14-P22, CMTCN41, CMN61_14 and TJ 14). Ripening was scored in the F3 recombinants, as previously described, to confirm the nine 2008-F2 recombinants phenotypes and, thus, map the QTL.

For the second high-resolution map, a new population of 967 individuals (named 2010-F3), obtained after selfing a 2008-F2 plant homozygous for the SC allele at the LG III introgression and heterozygous for the LG VI introgression, was screened. DNA was extracted from each individual and screened with the flanking markers, PSI_41-H06 and CMTCN41. Nine additional markers were genotyped in the recombinant plants to determine the recombination break point. The recombinants were cultivated in a greenhouse, selfed by hand pollination, and the fruit climacteric behavior was evaluated, measuring the days to fruit dehiscence from the pollination date (DAP). The mean distribution of dehiscence measured as DAP was checked for normality. The position of the QTL was estimated by CIM as explained above. Furthermore, progeny testing of five selected recombinants was carried out to verify the estimated position. Twenty F4 plants for each selected recombinant were grown and phenotyped to infer the ripening behavior of the parental line, and genotyped with nine tightly linked markers to refine the position of the locus. One-way ANOVA was used to test the markers significantly

associated with climacteric ripening, and differences in days to ripening between progeny test families and the control line SC3-5-1 were analyzed using Dunnett's two-tailed test with a minimum significance level of 0.01. Additionally, because the distribution of dehiscence mean departed from normality, a Kruskal–Wallis test was also used to confirm the Dunnett test for QTL fine mapping.

Interaction between LG III and LG VI

Four plants from the 2008-F2 population representing the four possible allele combinations for both introgressions, (1) PS alleles for both introgressions, (2) SC allele for LG VI and PS allele for LG III, (3) SC allele for LG III and PS for LG VI and (4) SC alleles for both introgressions, were selected and selfed. Ten replicates of each progeny were grown in a completely randomized design during 2008–2009 in greenhouses in Cabrils. In both years, ripening behavior was determined by visual inspection and determination of ethylene production by gas chromatography.

Results

Genetic control of fruit ripening in SC3-5-1

The climacteric behavior of melon fruits was assayed in the parental lines (PS and SC), SC3-5, SC3-5-1, and the hybrid SC3-5-1 × PS by measuring the evolution of ethylene production at 30 DAP (Fig. 2). PS and SC fruits displayed low ethylene production and the absence of an ethylene peak, typical of non-climacteric ripening, although ethylene production was slightly higher in SC than in PS fruits, confirming previous observations (Moreno et al. 2008). In contrast, ethylene biosynthesis and a clear ethylene peak were observed in SC3-5 and SC3-5-1 fruit, behaving as climacteric. SC3-5-1 fruits produced more ethylene (3.63 μL kg^{-1} h) and the peak was detected earlier (40 DAP) than in SC3-5 fruits, which had an ethylene production of 2.17 μL kg^{-1} h and a peak at 43 DAP. The hybrid SC3-5-1 × PS did not show climacteric behavior, indicating that the gene action of climacteric ripening may be recessive in this genotype. The increase in the ethylene levels in climacteric fruits was associated with three typical climacteric responses: change of external fruit color from green to yellow, fruit abscission, and production of typical aromas. Climacteric fruits usually detached before 40 DAP, whereas non-climacteric fruits did not detach from the plant and showed the first symptoms of putrefaction after 60 DAP. Previously, Obando-Ulloa et al. (2010) studied the aroma profile of several NILs from the same collection (including SC3-5-1), finding that climacteric NILs had a volatile profile similar to the standard climacteric cultivars, composed mainly of esters, especially acetate derivates, being responsible for the characteristic sweet aroma of cantaloup melons (Beaulieu and Lea 2003). In contrast, non-climacteric NILs, PS and other inodorous varieties produce lower levels of volatiles and an abundance of volatile aldehydes and alcohols rather than esters (Shalit et al. 2001), compounds characterized by the lack of aroma. These differences in aroma and external fruit characteristics make it possible to distinguish climacteric and non-climacteric melon fruit based on visual and olfactory parameters. (Fig. 3).

Fruits from 152 individuals of the 2008-F2 population, both parental lines and the hybrid were visually phenotyped and classified into climacteric or non-climacteric groups according to the criteria described in the Materials and methods section. To verify whether one or both introgressions on LG III and LG VI affected climacteric ripening, we investigated the association between it and markers from both introgressions using a contingency test. SC alleles at both markers, A_16-C12 on LG III and CMN61_14 on LG VI, were significantly associated ($\chi^2 = 14.345$, $p = 0.0002$ and $\chi^2 = 44.076$, $p < 0.0001$, respectively), demonstrating that both introgressions harbor the genes involved in climacteric ripening.

Composite interval mapping was also performed on LG VI, confirming the presence of a QTL (LOD = 14.2) for fruit ripening, *ETHQV6.3* (according to Diaz et al. 2011 nomenclature), where climacteric ripening was induced by the SC alleles. A QTL for flesh firmness (*FFQV6.3*, LOD = 2.7) was also detected in the same region as the *ETHQV6.3*. Flesh firmness was lower in climacteric fruits than in non-climacteric fruits ($p < 0.0001$) in the 2008-F2 population (Fig. 4a); therefore, the QTL for flesh firmness probably is due to a pleiotropic effect of *ETHQV6.3*. On the other hand, no QTL was detected for soluble solid concentration and, concomitantly, no differences in SSC were observed between climacteric and non-climacteric fruits (Fig. 4b).

Given that both QTLs were involved in climacteric ripening, a two-QTL model was adopted, taking PSI_18-D10 and CMN61_14 as the closest markers to the QTLs on LGs III and VI, respectively. Two-way ANOVA with these markers indicated significant interaction ($p < 0.048$) between PSI_18-D10 and CMN61_14 (Table 1). The interaction is shown graphically in Fig. 5. The maximum proportion of climacteric fruits was observed when both loci were homozygous for SC alleles (Fig. 5a). Differences in ripening behavior could be distinguished among the three genotypes at *ETHQV6.3* when *ETHQB3.5* was either heterozygous or homozygous for SC. When *ETHQB3.5* was homozygous for PS, it was not possible to distinguish

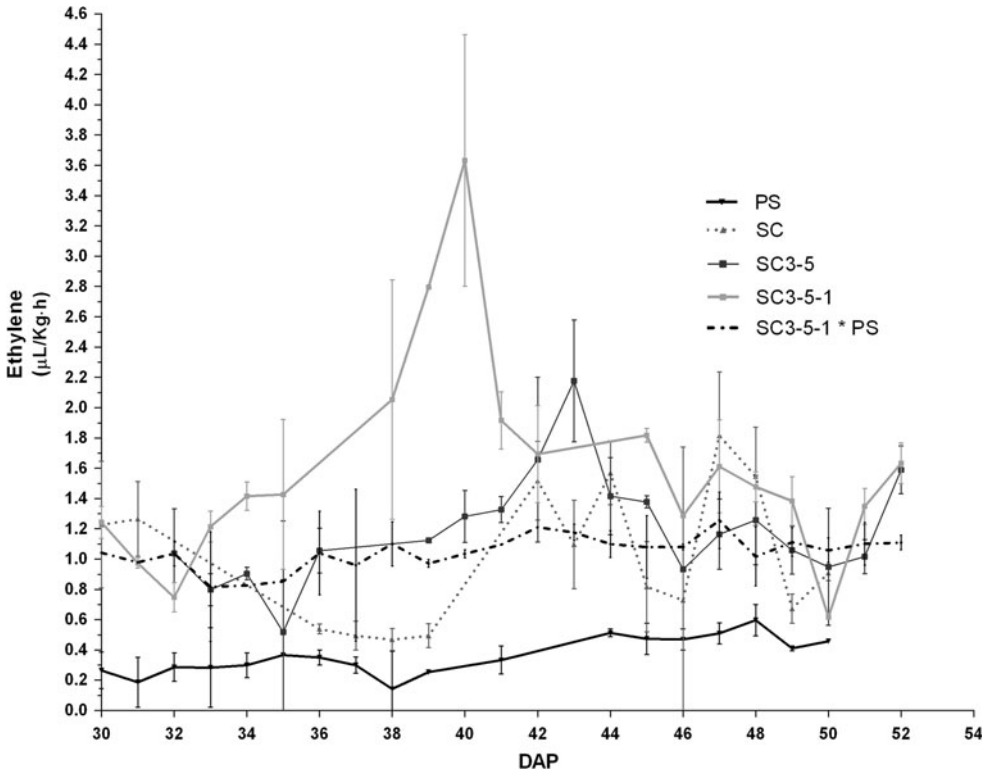

Fig. 2 Ethylene production (mean ± SD, $n = 3$) during fruit ripening (days after pollination, DAP) in the parental lines "Piel de Sapo" (PS) and the Korean accession PI 161375 (SC), the near-isogenic lines SC3-5 and SC3-5-1 and the hybrid SC3-5-1xPS

the heterozygote at *ETHQV6.3*, nor the homozygous PS. Furthermore, the effects of *ETHQB3.5* were evident only when *ETHQV6.3* was heterozygous or homozygous for the SC allele. These results suggest that *ETHQV6.3* effects were easier to distinguish when *ETHQB3.5* was homozygous for SC.

Fine mapping of *ETHQV6.3*

To increase the mapping resolution of *ETHQV6.3* we selected nine 2008-F2 individuals with recombination events in the LG VI interval, between markers PSI_41-H06 and CI_23-F08 (Fig. 6a), and SC alleles fixed in LG III. Twenty F3 individuals from each recombinant family were genotyped with markers PSI_41-H06, MU10920, AI_19-F11, CMBR002, AI_03-B03, FR14-P22, CMTCN41, CMN61_14, TJ14 and CI_23-F08, to construct a medium genetic resolution map. Fruits from these progenies were visually phenotyped and classified into climacteric and non-climacteric to confirm the ripening behavior of each F_2 recombinant. Climacteric ripening segregated in six of the nine families (Table 2), suggesting that *ETHQV6.3* is located between markers CMBR002 and CMN61_14. Analysis of individual recombinants from the progeny test families indicated that *ETHQV6.3* is most likely located between markers FR14-P22 and CMCTN41 (Online Resource 3).

A 2010-F3 population was screened with flanking markers CMTCN41 and PSI_41-H06 to construct a higher resolution genetic map for *ETHQV6.3*. Forty-three recombinant plants were obtained, which were genotyped with additional markers developed from the melon genome sequence (Garcia-Mas et al. 2012) and a high-resolution map was constructed (Fig. 6b). Segregation of early-dehiscent fruits, similar to SC3-5-1, and non-dehiscent fruits was observed among the 44 recombinants, with a range from 31 to 67 days, an average of 44.2 days, to reach fruit dehiscence, following a non-normal distribution (Shapiro–Wilk, $p = 0.2119$) (Fig. 7). QTL analysis gave a maximum LOD score (15.20) close to marker 28.37, explaining 48 % of the phenotypic variation of fruit dehiscence. To further resolve the position of *ETHQV6.3*, a progeny test of six informative 2010-F3 recombinants was carried out. Fruits from families 10M80-25, 10M80-26 and 10M80-90 were significantly less dehiscent than SC3-5-1 ($p < 0.01$), according to both the Dunnett and non-parametric Wilcoxon/Kruskal–Wallis tests (Fig. 8; Table 3). By substitution mapping, we conclude that *ETHQV6.3* is located between markers AI_03-B03 and FR14-P22 in a region of 4.5 Mbp (Fig. 6c). One-way ANOVA showed the highest significance linkage between marker 28.1723 and the climacteric behavior ($p < 0.0001$; $R^2 = 65.26$ %) in the new progeny test recombinants, although a further progeny test should be carried out to confirm this.

Fig. 3 Fruit from different genotypes showing the climacteric and non-climacteric phenotypes: the two non-climacteric PS (**a**) and the hybrid PS × SC3-5-1 (**b**), and the climacteric SC3-5-1 (**c**) and SC3-5 (**d**) showing the characteristic degreening of the rind and formation of the abcission layer

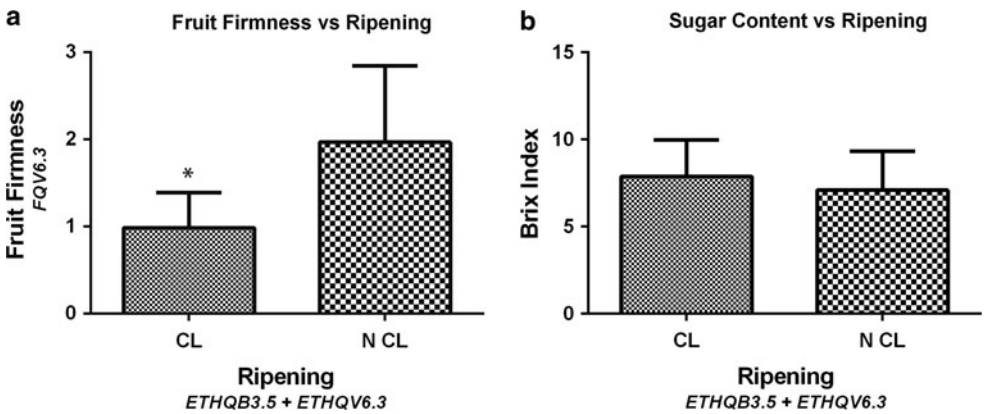

Fig. 4 Differences in fruit firmness (A, measured in N^{-1} cm^2) and sugar content (B, measured as Brix) between climacteric (CL) and non-climacteric (NCL) fruits in the 2008-F2 populations. An *asterisk* indicates statistically significant differences at $p < 0.005$

Interaction between *ETHQB3.5* and *ETHQV6.3*

To study more thoroughly the effects of both loci and their interaction, the climacteric behavior of the progeny of selected 2008-F2 plants representing the four possible genetic combinations of both introgressions in homozygosis was evaluated in two trials: (1) PS homozygous for both loci (8M29), (2) SC homozygous only for *ETHQB3.5* (8M35), (3) SC homozygous only for *ETHQV6.3* (8M40) and (4) SC homozygous for both loci (8M31).

As expected, 8M29, with no SC introgressions, had non-climacteric behavior (Table 4). Both lines carrying a single introgression (8M35 and 8M40) had a typical climacteric phenotype, although the effects were less pronounced in 8M35 (*ETHQB3.5*). Although all the fruits formed the abscission layer, most were dehiscent at 45 DAP, whereas

Table 1 Two-way ANOVA for PSI_18-D10 (*ETHQB3.5*) and CMN61_14 (*ETHQV6.3*) markers and their interaction for fruit ripening behavior in the 2008-F2 segregating population

Source	*DF*	Sum of squares	Mean square	*F* ratio	R^2
Analysis of variance					
Model	8	11.14	1.39	14.04	0.44
Error	142	14.08	0.10	Prob > F	
C. Total	150	25.22		<0.0001*	

Source	*DF*	Sum of squares	F ratio	Prob > F
Effect test				
PSI_18-D10	2	1.06	5.37	0.0057*
CMN61_14	2	7.10	35.8	<0.0001*
PSI_18-D10 × CMN61_14	4	0.98	2.46	0.048*

Asterisk highlights statistically significant effects

all 8M40 (*ETHQV6.3*) fruits were dehiscent at 47 DAP or earlier. These results confirm that these loci, independently, may induce climacteric ripening in melon, although complete fruit detachment in 8M35 (*ETHQB3.5*) was only observed in 2010, suggesting an interaction between this QTL and the environment. On the other hand, fruits from 8M31 (*ETHQB3.5* + *ETHQV6.3*) had early climacteric behavior; with an abscission layer and dehiscence at 35 DAP, approximately 10 days before the single QTL lines.

For better characterization of the climacteric ripening, ethylene was measured in these lines during 2010. Fruits were harvested at different developmental stages due to the different ripening patterns observed previously in the four lines. For 8M31, carrying both loci, fruits were harvested at 30 DAP, whereas the rest of the lines were harvested at 35 DAP. As expected, there was no peak in the production of ethylene in 8M29 fruits (Online Resource 4). The 8M35 (*ETHQB3.5*) and 8M40 (*ETHQV6.3*) fruits also behaved as climacteric fruits, with a peak of 2.55 and 2.01 μL kg^{-1} h at 40–43 DAP, respectively. This confirms that both loci are capable of inducing climacteric ripening independently, although it is induced earlier in *ETHQV6.3* than in *ETHQB3.5*. The 8M31 fruits gave the characteristic ethylene peak at 35 DAP with a maximum ethylene value of 4.36 μL kg^{-1} h. The amount of synthesized ethylene is additive compared with the single QTL lines. On the other hand, QTLs do interact epistatically by inducing earlier ethylene biosynthesis and, concomitantly, changes in fruit traits (dehiscence, aromas, softening, rind color), reducing the time from pollination to fully ripe fruit by 9 days compared with the introgression line carrying only *ETHQV6.3*.

Discussion

In the current report, we show that the genetic control of the climacteric ripening previously observed in the introgression line SC 3-5, derived from the cross between the non-climacteric genotypes PS and SC (Eduardo et al. 2005; Moreno et al. 2008), is more complex than we had hypothesized. Two loci are actually responsible for the climacteric ripening in this introgression line, the previously reported *ETHQB3.5* and a new locus, *ETHQV6.3* on LG VI. Both loci may induce climacteric ripening independently but, when they are combined, the climacteric ripening starts earlier in fruit development. The combination also has stronger pleiotropic effects on other fruit traits typical of the climacteric ripening observed in *cantalupensis* varieties (Pech et al. 2008), such as fruit softening, changes in rind color and aroma profile

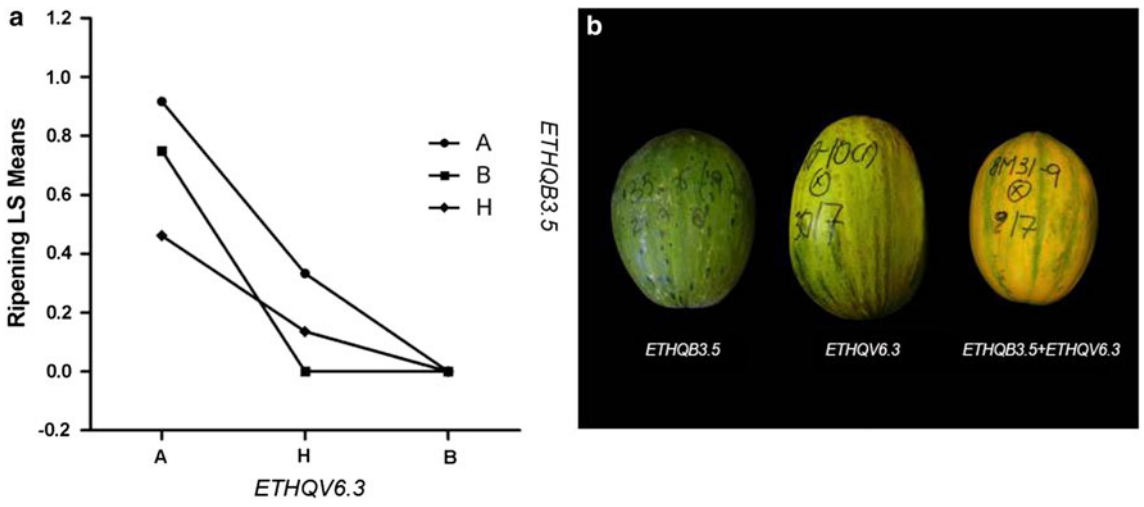

Fig. 5 **a** Allele interaction between markers significantly linked with both QTLs, *ETHQB3.5* and *ETHQB6.3*. *A* homozygous SC; *B* homozygous PS and *H* heterozygous. The type of ripening is shown on the *y* axis: 1 climacteric, 0 non-climacteric. **b** Representative fruits of plants with SC alleles homozygous for *ETHQB3.5*, for *ETHQV6.3* and for both of them

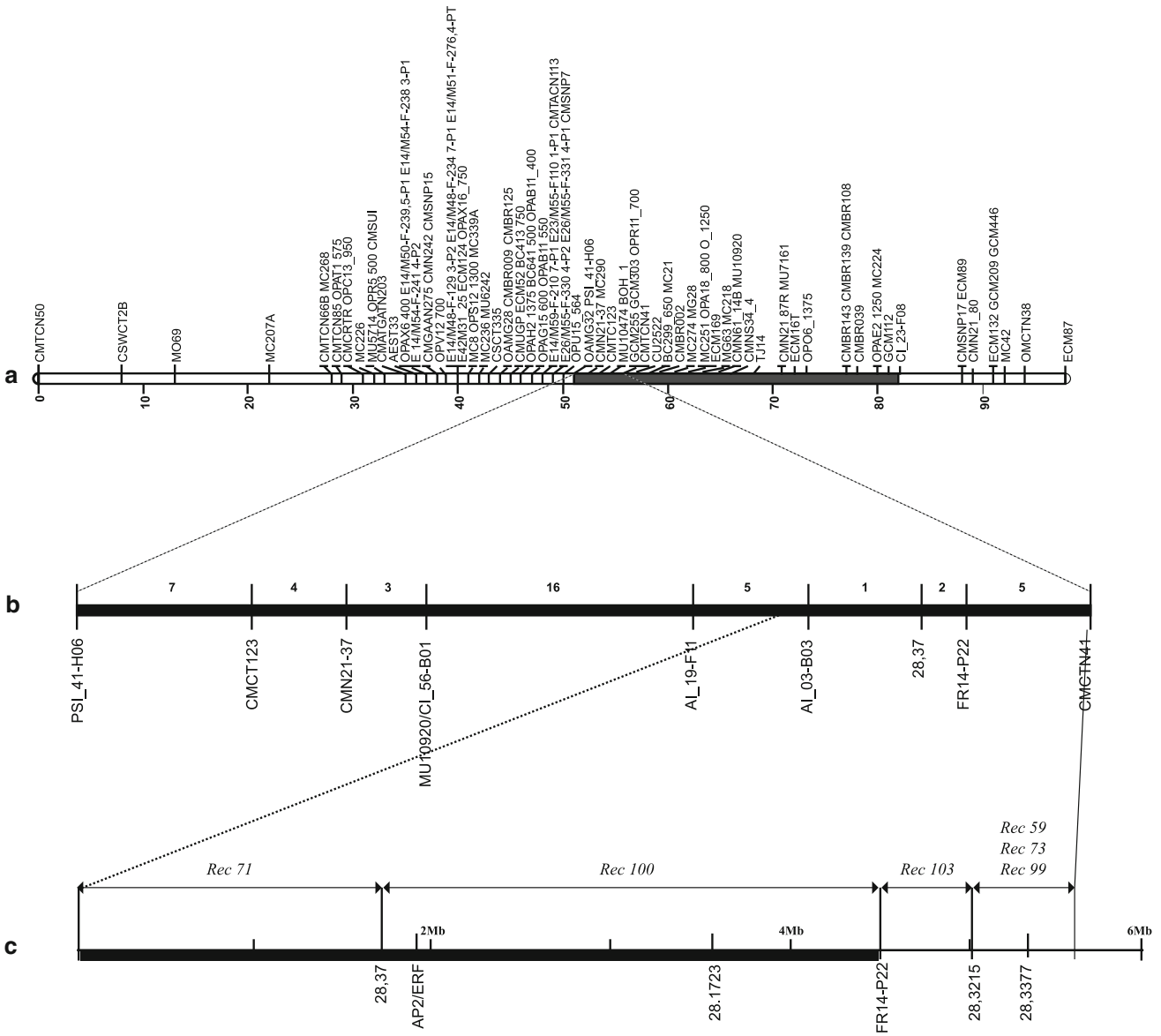

Fig. 6 **a** Consensus genetic map of melon LG VI (Diaz et al. 2011). Introgression from SC in the genetic background of PS in the climacteric SC3-5-1 is represented on the map as a *gray bar*. **b** High-resolution genetic map of the *ETHQV6.3* region using the 2010-F3 population. The number of recombinants in each interval is indicated between markers. **c** Physical map of the region of *ETHQV6.3* (in Mb). 2010-F3 recombinants selected for progeny test are indicated in *italics*. The interval of the QTL position after progeny testing is indicated by a *dark gray bar*

(Obando et al. 2008), and no differential effects on sugar accumulation.

Périn et al. (2002) studied the genetic control of climacteric ripening in a collection of recombinant inbred lines, derived from the same parental "SC" and the climacteric variety "Vedrantais", and found that two major genes on LGs VIII and IX, *Al-3* and *Al-4*, respectively, controlled ethylene production and fruit senescence. They also found additional QTLs controlling the amount of ethylene production. However, neither the alleles *Al-3* and *Al-4* nor the QTLs controlling the amount of ethylene mapped in the same regions as *ETHQB3.5* or *ETHQV6.3*. These contrasting results are attributable to the differences in allelic composition of the segregating populations in the studies. As "Vedrantais" is a standard climacteric variety it carries the major genes responsible for climacteric ripening, with the effects being detectable in a segregating population with a non-climacteric variety such as SC. On the other hand, PS is a standard non-climacteric variety. The fact that two loci involved in climacteric ripening have been detected in segregating populations with SC indicates that SC also carries genes involved in climacteric ripening.

Table 2 Study of the ripening phenotype of the F3 families from 2008-F2 recombinants in progeny tests and mapping of *ETHQV6.3*

2008-F_2 Recombinants	Phenotype	PSI_41-H06	MU10920	AI_19-F11	CMBR 002	AI_03-B03	FR14-P22	CMTCN 41	CMN 61_14	TJ 14	CI_23-F08	Progeny Test Phenotype CL	NCL
8M38-3	NCL	A	A	A	H	H	H	H	H	H	H	5	11
8M40-2	NCL	B	H	H	H	H	H	H	H	H	H	6	8
8M38-4	NCL	A	A	A	A	B	B	B	B	H	H	0	16
8M71-11	NCL	H	H	H	H	H	H	H	A	A	A	6	8
8M71-6	NCL	H	H	H	H	H	H	H	A	A	A	9	4
8M39-3	CL	A	A	A	A	A	A	A	A	H	H	16	0
8M39-2	CL	A	A	A	A	A	A	A	A	H	H	12	0
8M71-19	NCL	H	H	H	H	H	H	H	A	A	H	4	10
8M71-13	NCL	H	H	H	H	H	H	H	A	A	A	4	8
						*	*	*					

The 2008-F2 recombinant plants and their phenotype for ripening behavior (*CL* climacteric; *NCL* non-climacteric) are indicated in the two first columns. The following columns indicate the marker genotypes in the F3 progeny test families. *A* homozygous SC, *B* homozygous PS and *H* heterozygous. The last two columns indicate the number of plants with CL and NCL fruits. The interval position of *ETHQV6.3* is indicated with asterisks below the corresponding markers

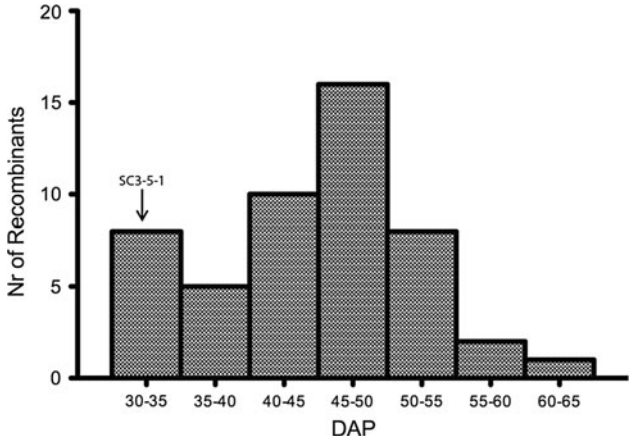

Fig. 7 Frequency distribution of days to fruit dehiscence after pollination (DAP) in the recombinant 2010-F3 population. SC3-5-1 (mean = 34.4, SD = 1.7) is represented by an *arrow*, PS is not shown because it is non-dehiscent

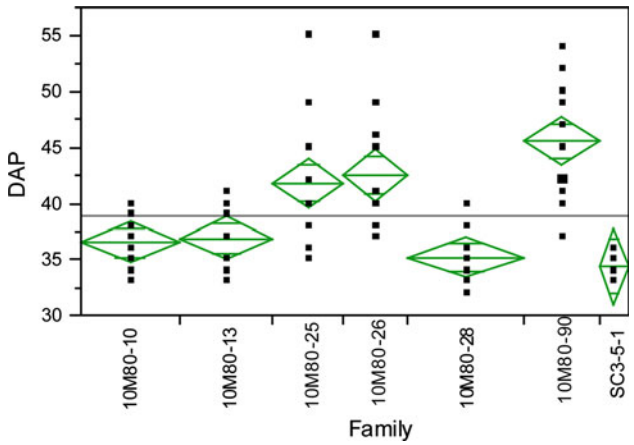

Fig. 8 Analysis of the differences among progeny test families from 2010-F3 recombinants in days to fruit dehiscence after pollination (DAP), and comparison with the control climacteric line SC3-5-1 using Dunnett's test ($\alpha = 0.05$). Families indicated in *gray* are not statistically different compared with SC3-5-1, whereas families in *black* are statistically different from SC3-5-1

The effects of the SC loci can only be detected in a non-climacteric background, such as PS. Consequently, SC does not carry the climacteric-inducing alleles of *Al-3* and *Al-4*, but other loci involved in climacteric ripening that were not detected previously in the climacteric genetic background. These results also suggest that SC cannot be considered as a standard non-climacteric line. *ETHQB3.5* and *ETHQV6.3* must interact in a different way when present in an SC or PS genetic background, preventing ripening in the SC background or triggering climacteric ripening in the PS background. New loci involved in climacteric ripening should be detected if the genetic control was studied in crosses involving cultivars with different climacteric behavior, for example, in a "Vedrantais" × PS cross.

Even though loci involved in ethylene production have not been previously detected in LG VI, other ripening-related traits have been detected in this genomic region. Monforte et al. (2004) found QTLs on LG VI involved in early fruit maturity in DHL (Gonzalo et al. 2011), and F2 populations originating from the same PS × SC cross, which can probably be attributed to the pleiotropic effects of *ETHQV6.3*. Cuevas et al. (2009), studying a cross between the early fruit maturity Chinese line "Q 3-2-2-2" and the late maturity cultivar "Top Mark", also detected a

Table 3 Fine mapping of *ETHQV6.3* in the recombinants selected from 2010-F3, considering the QTL as a molecular marker whose genotype is inferred after progeny tests using Dunnet's test

F_2 Recombinants	PSI_41-H06	AI_03-B03	28.37	AP2/ERF	28.1723	FR14-P22	28.3215	28.3377	CMCTN41	Dehiscence of fruit (DAP)	Phenotype after progeny test	*ETHQV6.3*
10M80-13 *(rec 73)*	A	A	A	A	A	A	A	A	H	33	CL	A
10M80-10 *(rec 59)*	A	A	A	A	A	A	A	H	H	32	CL	A
10M80-28 *(rec 103)*	A	A	A	A	A	A	H	H	H	33	CL	A
10M80-90 *(rec 71)*	A	A	H	H	H	H	H	H	H	51	N CL	H
10M80-25 *(rec 99)*	H	H	H	H	H	H	H	H	A	40	N CL	H
10M80-26 *(rec 100)*	H	H	H	H	H	A	A	A	A	37	N CL	H
			*	*	*							

The markers are indicated at the top of the columns. *A* homozygous for SC; *B* homozygous for PS and *H* heterozygous. The original name of the recombinants is shown in italics. The interval position of *ETHQV6.3* is indicated with asterisks below the corresponding markers, and the inferred genotype for *ETHQV6.3* is shown in the last column

Table 4 Ripening-associated phenotypes for the fixed lines during the 2009–2010 seasons

Trial	Lines	Genotype	Dehiscence (DAP)		Degreening of rind	Aromas
			Mean	SD		
2009	8M29	–	–	–	No	No
	8M35	*ETHQB3.5*	–	–	Yes	Yes
	8M40	*ETHQV6.3*	44.88	1.16	Yes	Yes
	8M31	*ETHQB3.5* + *ETHQV6.3*	35.00	1.62	Yes	Yes
2010	8M29	–	–	–	No	No
	8M35	*ETHQB3.5*	45.37	3.92	Yes	Yes
	8M40	*ETHQV6.3*	47.44	3.32	Yes	Yes
	8M31	*ETHQB3.5* + *ETHQV6.3*	36.3	2.31	Yes	Yes

Mean and standard deviation (SD) of fruit dehiscence in days after pollination (DAP), color change of the rind and presence of aroma are shown. Genotypes for each line are indicated, with *ETHQB3.5* SC alleles for the QTL in LG III; and *ETHQV6.3* SC alleles for the QTL in LG VI

QTL associated with fruit maturity (*fm6.1*) in the same region near CMTCN41.

A high-resolution genetic map of the region has been constructed (Fig. 6) locating *ETHQV6.3* in an interval of 4.5 Mbp, flanked by the markers AI_03-B03 and FR14-P22. The physical/genetic distance ratio (0.34 Mbp^{-1} 1 cM) calculated in the 2010-F3 population is almost twice the average of the total melon genome sequence (0.18 Mbp^{-1} 1 cM). Indeed, the low number of recombinants in the 2010-F3 between markers AI_03-B03 and FR14-P22 suggests a suppression of recombination in this region. The increment in the genetic/physical distance ratio, and therefore the low recombination rate observed, might be due to the centromeric localization of this interval, as observed in this region of LG VI in the melon genome sequence (Garcia-Mas et al. 2012). A larger segregating population is necessary to identify a candidate gene for *ETHQV6.3*, although the use of transcriptomic data and the availability of genome sequence information in public databases could greatly accelerate this process.

The interaction between *ETHQB3.5* and *ETHQV6.3* induces an advance in triggering of the ethylene peak, therefore, inducing early fruit ripening. To the best of our knowledge, there have been no previous reports of this phenomenon, despite fruit earliness being an economically important trait. Earliness can be defined in different ways, but basically represents the time that the plant takes from sowing to produce a fruit. The variation is due to two main processes: an earlier switch from vegetative to reproductive growth or faster ripening of the fruit (Doganlar et al. 2000; Tanksley 2004). In a collection of a *Solanum pennelli* introgression line population, the QTL *hi2-1* has been described as having an effect on fruit earliness, measured from sowing to the appearance of the first ripe fruit, and

harvest index. In the same study, an analysis of the flowering pattern of the introgression line that carries the QTL suggests that *hi2-1* may affect these traits, changing the plant architecture and the flowering rate (Gur et al. 2010). In contrast, in our study earliness is essentially due to faster ripening of the fruit due to an advance in an ethylene control mechanism.

To date, several tomato mutants with an impaired ethylene production (*rin*, *nor*, *cnr* and *Nr*) have been described, but none of them overproduces ethylene. In *Arabidopsis thaliana*, the *eto* mutants (*ethylene overproducers*) show an ethylene constitutive response in etiolated seedlings (Guzmán and Ecker 1990). One of these mutants encodes ETO1, a negative regulator of ACS5 activity through enzyme degradation via proteasome, producing elevated levels of ethylene (Wang et al. 2004). This means the plant may reach a certain level of the hormone earlier, accelerating the ripening process. It is possible that a similar regulatory mechanism of ethylene synthesis is involved in the earlier production of ethylene in the climacteric SC3-5-1; therefore, the level of the hormone needed to induce climacteric ripening would be reached earlier because two genes control synthesis of the hormone, compared with the NILs with only one of the loci.

On the other hand, the expression of antisense LeETR4 in tomato plants results in increased ethylene sensitivity, showing significant earlier fruit ripening (Tieman et al. 2000). This mutant phenotype could be restored with overexpression of the NR receptor. These authors also observed that lines with reduced expression of other ethylene receptors, such as LeETR1, LeETR2, NR and LeETR5, did not affect ethylene-associated developmental processes. In another study (Kevany et al. 2007), the same authors provide evidences of the role of the LeETR4 and LeETR6 receptors in modulating ripening time. These ethylene receptors act as negative regulators of ethylene signaling, therefore, it seems paradoxical that mRNA levels rise upon ripening, repressing the ethylene signal. It has been demonstrated that LeETR4 and LeETR6 levels drop during the maturation process; therefore, they may be reduced by either antisense RNA or protein degradation via 26S proteasome-mediated levels. In our case, the early ripening phenotype cannot be attributed only to a mutation in one of these receptors due to the absence of ethylene receptor genes annotated in the region of both QTLs. It must be noted that other elements, independent of the ethylene pathway, may be influencing the initiation of ripening.

How the fruit measures ethylene content and triggers the process, and how this complex regulatory system is connected, remains unknown. The identification of the genes behind these two QTLs (*ETHQB3.5* and *ETHQV6.3*) would help to understand the interaction between them, to provide early melon fruit maturation and an alternative model for ripening studies. The results presented here are a good basis for further investigation and future cloning of these QTLs, especially with the release of the melon genome sequence, which will help in the identification of the candidate genes. The cloning of these genes could help in analyzing the differences between the two types of ripening observed in melon and will bring new opportunities for breeders to control fruit maturation.

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References

Abeles (1992) Ethylene in plant biology, 2nd edn, Academic Press, San Diego

Adams-Phillips L, Barry C, Giovannoni J (2004) Signal transduction systems regulating fruit ripening. Trends Plant Sci 9:331–338

Alexander L, Grierson D (2002) Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. J Exp Bot 53: 2039–2055

Ayub R, Guis M, Ben Amor 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

Barreto GPM, Barreto GP, Fabi J, De Rosso V, Cordenunsi BR, Lajolo FM (2011) Influence of ethylene on carotenoid biosynthesis during papaya postharvesting ripening. J Food Compos Anal 24:620–624

Beaulieu JC, Lea JM (2003) Aroma volatile differences in commercial orange-fleshed cantaloupes, the inbred parental lines and stored fresh-cuts. Proceeding XXVI IHC—Issues and Advances in Postharvest Hort, Prange RK (ed), Acta Hort (ISHS) 628: 809–815

Bemer M, Karlova R, Ballester AR, Tikunov YM, Bovy AG, Wolters-Arts M, Rossetto PB, Angenent GC, de Maagd RA (2012) The tomato FRUITFULL homologs TDR4/FUL1 and MBP7/FUL2 regulate ethylene-Independent aspects of fruit ripening. Plant Cell 24:4437–4451

Ben-Amor M, Flores B, Latché A, Bouzayen M, Pech JC, Latché A (1999) Inhibition of ethylene biosynthesis by antisense ACC oxidase RNA prevents chilling injury in Charentais cantaloupe melons. Plant, Cell Environ 22:1579–1586

Benson G (1999) Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res 27:573–580

Chiriboga M-A, Schotsmans WC, Larrigaudière C, Dupille L, Recassens I (2011) How to prevent ripening blockage in 1-MCP-treated "Conference" pears. J Sci Food Agric 91: 1781–1788

Chung M-Y, Vrebalov J, Alba R, Lee JM, McQuinn R, Chung J-D, Klein P, Giovannoni J (2011) A tomato (*Solanum lycopersicum*) APETALA2/ERF gene, SlAP2a, is a negative regulator of fruit ripening. Plant J 64:936–947

Cuevas HE, Staub JE, Simon PW, Zalapa JE (2009) A consensus linkage map identifies genomic regions controlling fruit maturity

and beta-carotene-associated flesh color in melon (*Cucumis melo* L.). Theor Appl Genet 119:741–756

Diaz A, Fergany M, Formisano G, Ziarsolo P, Blanca J, Zhanjung F, Staub J, Zalapa J, Cuevas H, Dace G, Oliver M, Boissot N, Dogimont C, Pitrat M, Hofstede R, van Koert P, Harel-Beja R, Tzuri G, Portnoy V, Cohen S, Schaffer A, Xu Y, Fukino N, Matsumo S, Garcia-Mas J, MOnforte AJ (2011) A consensus linkage map for molecular markers and quantitative trait loci associated with economically important traits in melon (*Cucumis melo* L.). BMC Plant Biol 11:111

Doganlar S, Tanksley SD, Mutschler MA (2000) Identification and molecular mapping of loci controlling fruit ripening time in tomato. Theor Appl Genet 100:249–255

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

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 PI161375. Theor Appl Genet 112:139–148

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

Eriksson EM, Bovy A, Manning K, Harrison L, Andrews J, De Silva J, Tucker G, Seymour G (2004) Effect of the colorless non-ripening mutation on cell wall biochemistry and gene expression during tomato fruit development and ripening. Plant Physiol 136:4184–4197

Ezura H, Owino WO (2008) Melon ripening and ethylene. Plant Sci 175: 121–129

Fernandez-Silva I, Moreno E, Essafi A, Fernandez-Silva I, Moreno E, Essafi A, Fergany M, Garcia-Mas J, Martín-Hernandez AM, Alvarez JM, Monforte AJ (2010) Shaping melons: agronomic and genetic characterization of QTLs that modify melon fruit morphology. Theor Appl Genet 121:931–940

Fujisawa M, Shima Y, Higuchi N, Nakano T, Koyama Y, Kasumi T, Ito Y (2012) Direct targets of the tomato-ripening regulator RIN identified by transcriptome and chromatin immunoprecipitation analyses. Planta 235:1107–1122

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

Garcia-Mas J, Benjak A, Sanseverino W, Bourgeois M, Mir G, Gonzalez VM, Henaff E, Camara F, Cozzuto L, Lowy E, Alioto T, Capella-Gutierrez S, Blanca J, Canizares J, Ziarsolo P, Gonzalez-Ibeas D, Rodriguez-Moreno L, Droege M, Du L, Alvarez-Tejado M, Lorente-Galdos B, Mele M, Yang L, Weng Y, Navarro A, Marques-Bonet T, Aranda MA, Nuez F, Pico B, Gabaldon T, Roma G, Guigo R, Casacuberta JM, Arus P, Puigdomenech P (2012) The genome of melon (*Cucumis melo* L.). Proc Natl Acad Sci USA 109:11872–11877

Gillaspy G, Ben-David H, Gruissem W (1993) Fruits: a developmental perspective. Plant Cell 5:1439–1451

Giovannoni JJ (2007) Fruit ripening mutants yield insights into ripening control. Curr Opin Plant Biol 10:283–289

Gonzalez M, Xu M, Esteras C, Roig C, Monforte AJ, Troadec C, Pujol M, Nuez F, Bendahmane A, Garcia-Mas J, Picó B (2011) Towards a TILLING platform for functional genomics in Piel de sapo melons. BMC Res Notes 4:289

Gonzalo MJ, Claveria E, Monforte AJ, Dolcet-Sanjuan R (2011) Parthenogenic haploids in melon: generation and molecular characterization of a doubled haploid line population. J Am Soc Hortic Sci 136:145–154

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

Gur A, Osorio S, Fridman E, Fernie AR, Zamir D (2010) *hi2-1,* a QTL which improves harvest index, earliness and alters metabolite accumulation of processing tomatoes. Theor Appl Genet 121:1587–1599

Guzmán P, Ecker JR (1990) Exploiting the triple response of *Arabidopsis* to identify ethylene-related mutants. Plant Cell 2:513–523

Itkin M, Seybold H, Breitel D, Rogachev I, Meir S, Aharoni A (2009) TOMATO AGAMOUS-LIKE 1 is a component of the fruit ripening regulatory network. Plant J 60:1081–1095

Karlova R, Rosin FM, Busscher-Lange J, Parapunova V, Do PT, Fernie AR, Fraser PD, Baxter C, Angenent GC, de Maagd RA (2011) Transcriptome and metabolite profiling show that APETALA2a is a major regulator of tomato fruit ripening. Plant Cell 23:923–941

Kevany BM, Tieman DM, Taylor MG et al (2007) Ethylene receptor degradation controls the timing of ripening in tomato fruit. Plant J 51:458–467

Klee HJ (2004) Ethylene signal transduction. Moving beyond Arabidopsis. Plant Physiol 135:660–667

Klee HJ, Giovannoni JJ (2011) Genetics and control of tomato fruit ripening and quality attributes. Annu Rev Genet 45:41–59

Kumar R, Sharma MK, Kapoor S, Tyagi AK, Sharma A (2012) Transcriptome analysis of *rin* mutant fruit and in silico analysis of promoters of differentially regulated genes provides insight into LeMADS-RIN-regulated ethylene-dependent as well as ethylene-independent aspects of ripening in tomato. Mol Genet Genomics 287:189–203

Lee JM, Joung J-G, McQuinn R, Chung M-Y, Fei Z, Tieman D, Klee H, Giovannoni J (2012) Combined transcriptome, genetic diversity and metabolite profiling in tomato fruit reveals the ethylene response factor SlERF6 to play an important role in ripening and carotenoid accumulation. Plant J 70:191–204

Lin Z, Hong Y, Yin M, Li C, Zhang K, Grierson D (2008) A tomato HD-Zip homeobox protein, LeHB-1, plays an important role in floral organogenesis and ripening. Plant J 55:301–310

Manning K, Tor M, Poole M, Hong Y, Thompson AJ, King GJ, Giovannoni J, Seymour GB (2006) A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. Nat Genet 38:948–952

Mascarell-Creus A, Cañizares J, Vilarrasa-Blasi J, Mora-García S, Blanca J, Gonzalez-Ibeas D, Saladié M, Roig C, Deleu W, Picó B, López-Bigas N, Aranda MA, Garcia-Mas J, Nuez F, Puigdomènech P, Caño-Delgado A (2009) An oligo-based microarray offers novel transcriptomic approaches for the analysis of pathogen resistance and fruit quality traits in melon (*Cucumis melo* L.). BMC Genomics 10:467

McMurchie EJ, McGlasson WB, Eaks IL (1972) Treatment of fruit with propylene gives information about the biogenesis of ethylene. Nature 237:235–236

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

Moreno E, Obando JM, Dos-Santos N, Fernandez-Trujillo JP, Monforte AJ, Garcia-Mas J (2008) Candidate genes and QTLs for fruit ripening and softening in melon. Theor Appl Genet 116:589–602

Nelson JC (1997) QGENE: software for marker-based genomic analysis and breeding. Mol Breeding 3:239–245

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

Obando J, Fernandez-Trujillo JP, Martinez JA, Alarcon AL, Eduardo I, Arus P, Monforte AJ (2008) Identification of melon fruit quality quantitative trait loci using near-isogenic lines. J Am Soc Hortic Sci 133:139–151

Obando-Ulloa JM, Ruiz J, Monforte AJ, Fernández-Trujillo JP (2010) Aroma profile of a collection of near-isogenic lines of melon (*Cucumis melo* L.). Food Chem 118:815–822

Pech JC, Bouzayen M, Latché A (2008) Climacteric fruit ripening: ethylene-dependent and independent regulation of ripening pathways in melon fruit. Plant Sci 175:114–120

Périn C, Gomez-Jimenez M, Hagen L, Périn C, Gomez-Jimenez MC, Hagen L, Dogimont C, Pech J-C, Latché A, Pitrat M, Lelièvre 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

Rozen S, Skaletsky H (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics methods and protocols. Humana Press, Totowa

Seymour GB, Taylor JE, Tucker GA (1993) Biochemistry of fruit ripening. Chapman & Hall, London

Shalit M, Katzir N, Tadmor Y, Larkov O, Burger Y, Shalekhet F, Lastochkin E, Ravid U, Amar O, Edelstein M, Karchi Z, Lewinsohn E (2001) Acetyl-coa: alcohol acetyltransferase activity and aroma formation in ripening melon fruits. J Agric Food Chem 49:794–799

Silva JA, da Costa TS, Lucchetta L, Marini LJ, Zanuzo MR, Nora L, Nora FR, Twyman RM, Rombaldi CV (2004) Characterization of ripening behavior in transgenic melons expressing an antisense 1-aminocyclopropane-1-carboxylate (ACC) oxidase gene from apple. Postharvest Biol Technol 32:263–268

Tanksley SD (2004) The genetic, developmental, and molecular bases of fruit size and shape variation in tomato. Plant Cell 16(Suppl):S181–S189

Tieman DM, Taylor MG, Ciardi JA, Klee HJ (2000) The tomato ethylene receptors NR and LeETR4 are negative regulators of ethylene response and exhibit functional compensation within a multigene family. In: Proceedings of the National Academy of Sciences of the United States of America, vol 97, pp 5663–5668

Vegas J, Garcia-Mas J, Monforte AJ (2010) Characterization of fruit ripening in the melon near isogenic line SC3-5-1. Proc Cucurbitaceae 2010. Section Breeding and Genetics. ASHA, Alexandria, p 3–7

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

Wang KL-C, Yoshida H, Lurin C, Ecker JR (2004) Regulation of ethylene gas biosynthesis by the *Arabidopsis* ETO1 protein. Nature 428:945–950

Zeng ZB (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468