Lower relative differential expression of two genes is associated with delayed ripening in melon

Una menor expresión diferencial relativa de dos genes está relacionada con maduración más lenta en melón

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Abstract

The expression of selected genes during ripening was studied considering a melon Near-isogenic Line (NIL) SC10-2 and its parental "Piel de Sapo" (PS). The expression of *Cm*GGP (GDP-L-galactose phosphorylase 1), *Cm*AP2-like X1 (AP2-like ethylene-responsive transcription factor TOE3 isoform X1) and *Cm*RAP2-11 (ethylene-responsive transcription factor RAP2-11) were differentially expressed in the NIL SC10-2 compared with PS. Consequently, expression of genes that mapped in LG X such as one ethylene response transcription factors or ascorbic acid metabolism gene were probably associated with delayed ripening.

Keywords: fruit quality; near-isogenic lines; RNA-Seq; Quantitative Trait Loci (QTLs).

Resumen

Con el fin de comparar la expresión génica de un melón cerca de la línea isogénica (NIL) SC10-2 y su parental Piel de Sapo (PS) durante la maduración y para comprender los mecanismos de diferenciación, se realizó una secuenciación transcriptoma. Dos genes de *Cm*GGP (GDP-L-galactosa fosforilasa 1) y *Cm*RAP2-11 (factor de transcripción sensible al etileno RAP2-11) mostraron menor expresión relativa en la NIL SC10 -2 versus PS debido a la introgresión en LG X. Sin embargo, no existieron diferencias en expresión de *Cm*AP2-like X1 (factor de transcripción sensible al etileno, similar a AP2 TOE3 isoforma X1). En consecuencia, la expresión de genes que mapearon en el grupo de ligamiento X como un factor de transcripción de respuesta a etileno o del metabolismo del ácido ascórbico estuvieron probablemente asociados con el retraso de maduración.

Palabras clave: ARN-seq; calidad de fruto; líneas casi isogénicas; Quantitative Trait Loci (QTLs).

1. INTRODUCTION

Melon (*Cucumis melo* L.) is an important annual diploid plant belonging to the Cucurbitaceae family. Unfortunately, non-climacteric melon fruit ripening and quality have been little studied compared with climacteric melons. *Cm*GGP is a GDP-L-galactose phosphorylase 1, *Cm*AP2-like X1 and *Cm*RAP2-11 genes are ethylene-responsive transcription factors, which are involved

generally with the other genes in so many important developmental processes and interact with so many plant hormones [1]. The *Cm*GGP gene was involved in the ascorbate biosynthetic pathway in plants *Arabidopsis thaliana* [2] and in different fruits such as kiwifruit, strawberry, tomato [3] and probably melon. The *Cm*GGP, *Cm*AP2-like X1 and *Cm*RAP2-11 genes which involved in the GDP-D-glucose phosphorylase activity, catalytic activity and glucose metabolic process, and ethylene-responsive transcription factors [4], act directly and/or indirectly on the fruit quality traits. The goal of this paper was to compare the gene expression during melon fruit ripening using the NIL SC10-2 and its parental PS as a model system.

2. MATERIALS AND METHODS

The melon near-isogenic line (*Cucumis melo* L.) SC10-2 was obtained through marker assisted breeding from a cross between a Korean accession "Songwhan Charmi" PI 161375 (SC) and the Spanish cultivar T111 type "Piel de Sapo"(PS) [5]. SC10-2 carries an introgression on linkage group (LG) X from SC into the PS genome. Melon cultivation was under Mediterranean conditions in Torre Pacheco (Murcia, Spain) [6]. Melons were allowed to ripen at 20.5ºC and 88% relative humidity during 16 d (n=3). Flesh samples were obtained according to Dos-Santos et al. (2007) and stored at -80ºC before freeze drying [7]. Freeze-dried samples for transcriptomic analysis were stored at -25ºC. The RNA extraction was performed two times using TRI Reagent RNA isolation protocol. Highly pure total RNA was quantified with a NanoDrop ND-1000 spectrophotometers (Thermo Scientific, Germany). RNA quality was verified by calculating two absorbance ratios (260/280 nm and 260/230 nm, respectively) and by electrophoresis analysis. The library from DNA free total RNA was constructed following the TruSeq™ Stranded mRNA Sample Preparation kit protocol (Illumina Inc., Redwood. CA, USA) and was sequenced using TruSeq SBS Kit v3-HS, in paired end mode with the read length 2x101bp. The transcriptomic analysis was performed in CNAG (Barcelona) according to the gene sequence reported by Garcia-Mas et al. (2012) [8]. On the other hand, flesh juice mixed with calcium chloride served for aroma volatile extraction by solid phase microextraction and GC-MS analysis for semiquantitative quantification [9]. A two-way ANOVA and a post-hoc Tukey HSD test (p=0.01) with interaction was performed to determine the effects of the pedigree (factor P) and the ripening time (factor t) on the aroma volatiles and gene expression using JMP 5.1 (Systat) and Statgraphics Plus for Windows 2.1 (Statistical Graphics Corp., Herndon, VA, USA).

3. RESULTS AND DISCUSSION

We focus on three genes with differential expression in NIL SC10-2 versus PS (Fig. 1). *Cm*GGP gene is a GDP-L-galactose phosphorylase 1 known MELO3C013136, located in CM3.5_scaffold00019 from 883408 to 886606 [10] (Fig. 1), *Cm*AP2-like X1 gene is an ethylene-responsive transcription factor RAP2-11 known MELO3C014722, located in CM3.5_scaffold00022 from 5246528 to 5247177 [11] (Fig. 1) and *Cm*RAP2-11 gene is similar to floral homeotic protein APETALA 2 (*Arabidopsis thaliana*) known MELO3C020848, located in CM3.5_scaffold00045 from 1724235 to 1728074 [12] (Fig. 1). *Cm*GGP gene expression showed significantly higher levels in PS than in SC10-2 till 8 d during ripening (Fig. 1). GDP-L-galactose phosphorylase, involved in the ascorbate biosynthetic pathway in *Arabidopsis thaliana* [2]. Also, is the only significant source of ascorbate in *A. thaliana* seedlings, and that ascorbate is essential for seedling growth [2]. Moreover, the relative expression of the GGP gene was important for regulation of ascorbic acid (AsA) biosynthesis [13]. Overexpression of kiwifruit or *Arabidopsis* GGP in strawberry, potato and tomato have been shown to significantly increase AsA [3]. The highest increase of ascorbate in tomato fruits reported so far has been about six-fold and was achieved by ectopically expressing GGP from kiwi [13]. There can be several explanations for these differences in fruit development, first the ascorbate increase in strawberry fruit is smaller, thus not being enough to solubilize

pectins, second strawberry is a false fruit with the real fruits (the achenes) located outside the fleshy part, and third the composition of the cell wall surrounding the fruits might be different in terms of pectin composition [14]. *Cm*AP2-like X1 gene expression showed similar relative level in both lines during ripening with maximum values at harvest and following a convex trend over time (Fig. 1). Apparently, the gene was downregulated during postharvest ripening but upregulated during melon senescence. On the contrary, the expression of *Cm*RAP2-11 diminished during ripening but having higher levels in PS than in SC10-2 at harvest and after 8 d (Fig. 1). The transcription of ethylene-regulated genes is mediated by ethylene response factors (ERFs) [16]. The plant hormone ethylene is involved in a wide range of developmental processes and physiological responses such as flowering, fruit ripening, organ senescence, abscission, root nodulation, seed germination, programmed cell death, cell expansion, and responses to abiotic stresses and pathogen attacks [16]. Transcription factors (TFs) like APETALA2/ETHYLENE RESPONSE FACTORS (AP2/ERFs) are an integral component of these signalling cascades because they regulate expression of a wide variety of downstream target genes related to stress response and development through different mechanism [17]. AP2/ERF family of TFs are regulated by different plant growth regulators (PGRs) and their role in retrograde signalling. With multiple responses there comes both positive and negative regulation which needs proper concern before generation of recombinant plants [17]. The downstream regulation of transcript does not always positively or beneficially affect the plant but also, they display some developmental defects like senescence and reduced growth under normal condition or sensitivity to stress condition [17]. AP2/ERFs are one of the most important families of TF in plants which regulate various developmental and stress responsive pathways [18, 19].

The introgression of SC10-2 is located in homozygosis in whole LG X [20], and also the gene MELO3C013136 (*Cm*GGP), MELO3C014722 (*Cm*AP2-like X1) and MELO3C020848 (*Cm*RAP2-11) that surely have a contribution delaying ripening of such NIL vs PS [21]. Environmental conditions and preharvest history of each fruit can also affect the gene expression [22].

4. CONCLUSIONS

The expression of two genes were diminished due to the introgression of SC10-2 in the LG X that probably explained the delayed ripening in the NIL.

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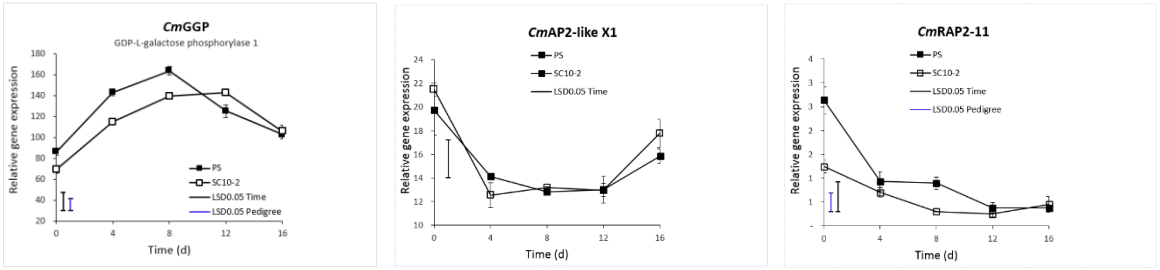

Figure 1. Relative gene expression of the NIL SC10-2 and its parental control PS during ripening at 20.5ºC and 88% relative humidity (mean ± SE, n=3). ***Cm*GGP:** GDP-L-galactose phosphorylase 1/ ***Cm*AP2-like X1:** AP2-like ethylene-responsive transcription factor TOE3 isoform X1/ ***Cm*RAP2-11:** ethylene-responsive transcription factor RAP2-11.