Gene expression and volatile production during melon ripening

Expresión génica y producción de volátiles durante la maduración del melón

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Abstract

Transcriptome sequencing was performed in order to analyze the genes associated to volatile synthesis expressed during ripening and to understand the molecular mechanisms that differentiate a melon Near-isogenic Line (NIL) SC10-2 and its parental Piel de Sapo (PS). CmLOX18 gene (Similar to Lipoxygenase 18) was differentially expressed in the NIL SC10-2 compared with PS associated with the aroma volatile compound hexanal as a target compound of the non-climacteric ripening. The expression of CmACO1 (1-aminocyclopropane-1-carboxylate oxidase 1) gene associated with ethylene biosynthesis did not change during ripening. The introgression in LG X was associated with the differential hexanal production of the NIL and PS. An eQTL located in LG X is probably controlling the production of aroma volatiles due to CmLOX18 in LG I.

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

Resumen

Se realizó una secuenciación de transcriptoma para analizar los genes implicados en la formación de aromas expresados durante la maduración y para comprender los mecanismos moleculares que diferencian una línea casi isogénica (NIL) SC10-2 de melón y su parental Piel de Sapo (PS). El gen CmLOX18 (similar a la lipoxigenasa 4) se expresó diferencialmente comparando la NIL SC10-2 y PS y se asoció a la producción de hexanal, un compuesto diana e indicador del proceso de maduración no climatérica. La expresión del gen de la CmACO1 (1-aminocyclopropane-1-carboxylate oxidase 1) implicado en la biosíntesis de etileno no manifestó diferencias durante la maduración. La introgresión en LG X estuvo asociada a la diferente producción de hexanal entre la NIL y PS. Se propone un eQTL en el LG X que controla la producción de aromas del gen CmLOX18 localizado en LG I.

Palabras clave: Calidad de fruto; ARN-seq; Líneas casi isogénicas; Loci de Caracteres Cuantitativos (QTLs).

1. INTRODUCTION

Melon is one of the most economically important fruit crops worldwide. LOX is an essential enzyme involved in the volatile biosynthetic pathways, and it is particularly regulated by ethylene during fruit ripening [1]. The near-isogenic line (NIL) SC10-2 has been studied previously due to its interest for improving melon flesh firmness associated with cell wall modifications compared with its ''Piel de Sapo'' (PS) parental [2]. Also, the NIL showed a late ripening compared with other non-climacteric melons producing differential aroma volatile profile and at harvest [3; 4]. The goal of this paper was to identify genes associated with aroma formation and delayed fruit ripening during postharvest ripening that may be involved in the differential behavior of SC10-2 compare with the recurrent PS.

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]. 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 -25°C. 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 plus a 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 had more than 9850 sequences in our experience, and 1434 showing differential expression. Twenty one genes were identified for fruit ripening of which nine genes were expressed in SC10-2 and twelve in PS (only nine in common). Because of the differences in harvest time between lines were evident (data not shown), a gene associated with ripening and ethylene biosynthetic process was investigated known MELO3C014437 (*Cm*ACO1), LG V (Fig. 1B). However, no significant effects were found but one fruit of SC10-2 with showed a remarkably high ACO1 expression that is usually concomitant with high ester levels [4], ACO1 expression does not follow a normal distribution and mean and interaction were significant at $P<0.1$, which could be of interest. ACO1 gene, which regulated the last step of the biosynthetic pathway, can contribute to non-climacteric melon senescence or the biosynthesis of ethylene-dependent aroma volatiles [4]. Hexanal levels were consistently higher in SC10-2 over ripened fruits compared with the control PS (Figure 1A). Therefore levels of genes of the lipoxygenase (LOX) pathway were investigated. We only found the expression of the gene MELO3C024348 (*Cm*LOX18), linkage group I (Fig. 1C). None of the other *Cm*LOX genes reported by Zhang et al. (2014), particularly

*Cm*LOX1 and *Cm*LOX3 involved in the last stages of development and ripening, showed expression in our data. Other CmLOx are located in other LG´s (one in LG X) though at least two of them associated with *Cm*LOX1 were not positioned any LG because of the lack of information in MELOGEN. Expression levels of *Cm*LOX18 showed no significant interaction but significantly higher levels in SC10-2 than in PS and convex quadratic changes over time were also significant (P=0.01) with a similar trend in both lines (minimum after 8d of ripening). Among the roles associated with fruit ripening, LOX is involved in the generation of C6 alcohols and aldehydes such as hexanal, which constitute major volatile flavor components in ripening particularly of non-climacteric fruits [10]. In this experiment, *Cm*LOX18 expression preceded the upsurge of hexanal. For the same reason, when the level of volatile was very high the expression of the gene was diminishing (Figs. 1A and 1C). Environmental conditions and preharvest history of each fruit can affect the gene expression and production of volatile organic compounds [11]. Remarkably, *Cm*LOX18 expression markedly increased here as occurred with other *Cm*LOX genes in strawberry [12], but also in climacteric fruit [13; 1], and in melon ripening [14]. The introgression of SC10-2 is located in homozygosis in whole LG X [4], but *Cm*LOX18 is only in LG I and its expression might be controlled by an expression QTL (eQTL) located in LG X that would have a contribution in differential hexanal production between SC10-2 and PS during ripening. Further research to verify the association between this e-QTL and ACO1 gene is required.

4. CONCLUSIONS

The expression of *Cm*LOX18 gene located in LG I was associated with differential hexanal production linked to the introgression in LG X. An e-QTL located in LG X is proposed to partly modify the expression of *Cm*LOX18.

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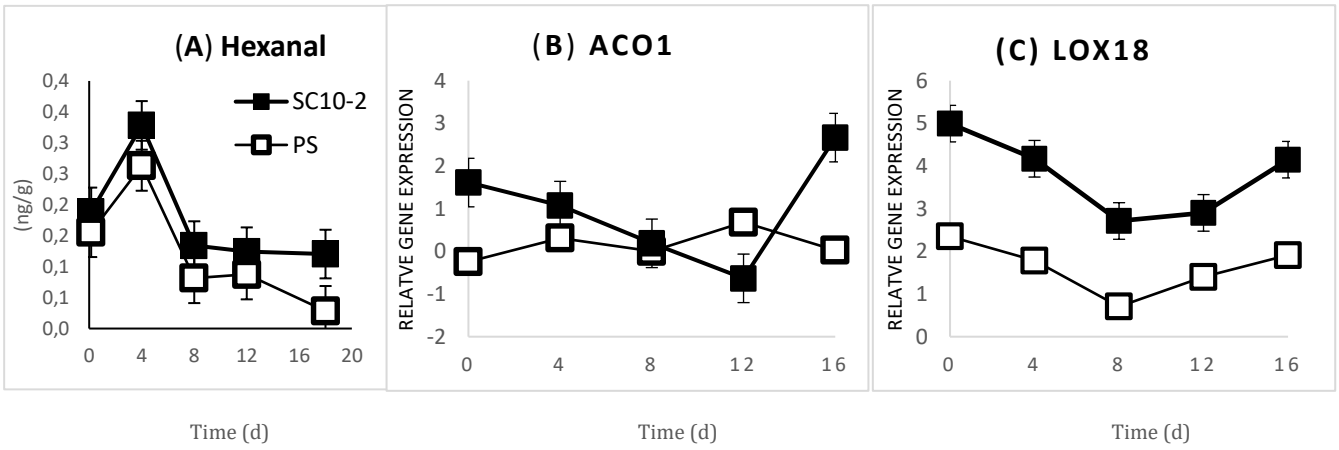

Figure 1. (**A**) Changes of hexanal present in melon aroma of the flesh during ripening at 20.5ºC and 88% RH (mean ±SE, n=3). (**B**) Expression of the melon *Cm*ACO1 gene of SC10-2 and its parental control PS. (**C**) *Cm*LOX18 gene expression over ripening of NIL SC10-2 and its parental control PS. (mean ± SE, n=3).