High relative expression of two genes of a melon near-isogenic line versus its parental during ripening
Mayor expresión de dos genes en una línea de melón casi isogénica versus su parental durante la maduración

M. Zarid[1*], C. Esteras[2], A.G. Sifres[2], X. Cañizares[2], J. Esteva[3], M. Belén Picó[2], A.J. Monforte[4], J.P. Fernández-Trujillo[1, 5]

[1]Department of Agricultural & Food Engineering, Regional Campus of International Excellence "Campus Mare Nostrum" (CMN), Technical University of Cartagena (UPCT). Paseo Alfonso XIII, 48, ETSIA. E-30203 Cartagena, Murcia, Spain.

[2]Instituto de Conservación y Mejora de la Agrodiversidad, Universitat Politècnica de València (COMAV-UPV), Camino de Vera s/n, E-46022 Valencia, Spain.

[3]Department of Plant Production, ETSIA. CMN, UPCT, Cartagena, Murcia, Spain.

[4]Instituto de Biología Molecular y Celular de Plantas (IBMCP) UPV-CSIC, Ciudad Politécnica de la Innovación Edificio 8E, Ingeniero Fausto Elio s/n, E-46022 Valencia, Spain.

[5]Institute of Plant Biotechnology, CMN, UPCT, Cartagena, Murcia, Spain.

*m.zarid@gmail.com

Abstract

In order to compare the gene expression of a melon Near-isogenic Line (NIL) SC10-2 and its parental Piel de Sapo (PS) during ripening and to understand the differentiate mechanisms, a transcriptome sequencing was performed. *Cm*TCP15 (Transcription factor activity) and *Cm*GDSL (Esterase and lipase activity) genes were high differentially expressed in the NIL SC10-2 compared with PS due to the introgression in LG X. Consequently, some fruit quality traits such as aroma, sweetness and probably others can be affected by such genes.

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

Resumen

Con el fin de comparar la expresión génica de una línea casi isogénica (NIL) SC10-2 de melón y su Piel de Sapo (PS) parental durante la maduración y para comprender los mecanismos de diferenciación, se realizó una secuenciación de transcriptoma. Los genes *Cm*TCP15 (Factor de actividad de transcripción) y *Cm*GDSL (actividad de la esterasa y la lipasa) tenían una alta expresión diferencial en el NIL SC10-2 en comparación con el PS debido a la introgresión en LG X. En consecuencia, algunos atributos de calidad de fruto como el aroma, dulzura y, probablemente otros pueden estar afectados por tales genes.

Palabras clave: Calidad de fruto; ARN-seq; Líneas casi isogénicas; Loci de Caracteres Cuantitativos (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 has been little studied compared with climacteric melons. *Cm*TCP15 gene is a transcription factor activity, which is involved generally with the other TCP transcription factors in so many important

developmental processes and interact with so many plant hormones [1]. The *Cm*GDSL gene which involved in the esterase and lipase activity, also in the hydrolase activity, lipid metabolic process, alpha-L-fucosidase activity and acting on ester bonds [2], which 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) [3]. 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) [4]. Flesh samples were obtained according to Dos-Santos et al. (2007) and stored at -80ºC before freeze drying [5]. 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) [6]. 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 [7]. 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

Though in this experiment we had almost 1500 showing differential expression, we focus on two genes with higher differential expression in NIL SC10-2 versus PS that are located in LG X (Fig. 1). First, a gene associated with the transcription activity known MELO3C012086 (*Cm*TCP15), located in CM3.5_scaffold00016 from 2960759 to 2962531 [8] (Fig. 1A). On the other hand, we also studied a gene associated with the esterase and lipase activity known MELO3C011939 (*Cm*GDSL esterase/lipase), located in CM3.5_scaffold00016 from 1874087 to 1877752 [9] (Fig. 1B).

*Cm*TCP15 in addition of the transcription activity with others factors affect the synthesis of methyl jasmonate, a hormone that has multiple functions in plant development [10] does also affect the cell cycle [11]. Apart from hormonal control of growth, *Cm*TCP15 with others TCP transcription factors are also involved in other biological processes that in turn affect growth [12], and implying a role in diurnal regulation of transcripts of the mitochondrial oxidative phosphorylation machinery [13]. Also, *Cm*GDSL gene expression showed significantly higher levels in SC10-2 than in PS during ripening (Fig. 1B). *Cm*GDSL esterase/lipase with the esterase and lipase activity up-regulated and significantly correlated with ethylene production [14]. In melon as in apple, the typical aroma compounds are fruity esters that develop during ripening with the maximum endogenous ester concentration occurring at the climacteric peak [15]. These compounds can be broadly separated into straight- and branched-chain esters. Straight-chain esters are synthesized from fatty acids via the lipoxygenase (LOX) pathway, whereas branched-

chain esters are produced from the metabolism of branched-chain amino acids such as isoleucine [16]. Fatty acids are important precursors in the formation of the characteristic aroma in tomato, apple, kiwifruit pear and melon [17, 18].

The introgression of SC10-2 is located in homozygosis in whole LG X [19], also MELO3C012086 (*Cm*TCP15) and MELO3C011939 (*Cm*GDSL esterase/lipase), that surely have a contribution delaying ripening of such NIL vs PS [20]. Environmental conditions and preharvest history of each fruit can also affect the gene expression [21].

4. CONCLUSIONS

The introgression of SC10-2 in the LG X was associated with two differentially expressed genes that can be associated with noticeable differences in fruit quality and delayed ripening in the NIL with introgression in LG X.

5. ACKNOWLEDGEMENTS

Financial support: Fundación Séneca de la Región de Murcia (11784/PI/09), MINECO & UE-FEDER funds (AGL2010-20858). Thanks for the technical assistance to P. Varó and his team in CIFEA-Torre Pacheco (Consejería de Agricultura, Región de Murcia), N. Dos-Santos, E. Cuadros, M. García-Gutiérrez, A. Hakmaoui (UPCT), M.J. Roca (SAIT-UPCT), and IRTA-CRAG for the seeds of the NIL.

6. REFERENCES

[1] Danisman, S. 2016. TCP Transcription Factors at the interface between environmental challenges and the plant's growth responses. Front Plant Sci. 7: 1930. DOI: 10.3389/fpls.2016.01930

[2] www.melonomics.net

[3] Perpiñá, G., Esteras, C., Gibon, Y., Monforte, A.J., Picó, B. 2016. A new genomic library of melon introgression lines in a cantaloupe genetic background for dissecting desirable agronomical traits BMC Plant Biol. 16: 154. https://doi.org/10.1186/s12870-016-0842-0

[4] Eduardo I, Arus P., Monforte A.J., 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: 130-148. DOI: 10.1007/s00122-005-0116-y

[5] Dos-Santos, N., Obando, J., Ostos, I., Melgarejo, LM., Moreno, E., Monforte, A.J., Fernández-Trujillo, J.P. 2007. Optimization of the extraction of organic acids in lyophilized melon pulp and analysis by high-performance liquid chromatography. Proc. IV Ibero-American Virtual Congress of Quality Management in Laboratory. May 2-June 30, 2007. pp. 203-211. I.S.B.N 978-84-491-0796-2.

[6] Garcia-Mas J., Benjak A., Sanseverino W., Bourgeois M., Mir G., González V. M., Hénaff E., Câmara F., Cozzuto L., Lowy E., Alioto T., Capella-Gutiérrez S., Blanca J., Cañizares J., Ziarsolo P., Gonzalez-Ibeas D., Rodríguez-Moreno L., Droege M., Du L., Alvarez-Tejado M., Lorente-Galdos B., Melé M., Yang L., Weng Y., Navarro A., Marques-Bonet T., Aranda M. A., Nuez F., Picó B., Gabaldón T., Roma G., Guigó R., Casacuberta J. M., Arús P., Puigdomènech P. 2012. The genome of melon (*Cucumis melo* L.). Proc. Nat. Acad Sci. 109:11872-11877.

[7] Chaparro-Torres, L.A., Bueso, M.C., Fernández-Trujillo, J.P. 2016. Aroma volatiles at harvest obtained by HSSPME/GC-MS and INDEX/MS-E-nose fingerprint discriminate climacteric behavior in melon fruit. J. Sci. Food Agr. 96: 2352-2365. DOI: 10.1002/jsfa.7350

[8] https://melonomics.net/feature/MELO3C012086/

[9] https://melonomics.net/feature/MELO3C011939/

[10] Schommer C., Palatnik J.F., Aggarwal P., Chételat A., Cubas P., Farmer E.E., Nath U., Weigel D. 2008. Control of jasmonate biosynthesis and senescence by miR319 targets. PLoS Biol. 6(9): 1991-2001. https://doi.org/10.1371/journal.pbio.0060230

[11] Swiatek A., Lenjou M., Van Bockstaele D., Inzé D., Van Onckelen H. 2002. Differential effect of jasmonic acid and abscisic acid on cell cycle progression in tobacco BY-2 cells. Plant Physiol. 128(1): 201-211. DOI: 10.1104/pp.010592

[12] Welchen E., Gonzalez D.H. 2006. Overrepresentation of elements recognized by TCP-domain transcription factors in the upstream regions of nuclear genes encoding components of the mitochondrial oxidative phosphorylation Machinery. Plant Physiol. 141(2): 540-545. DOI: 10.1104/pp.105.075366

[13] Giraud E., Ng S., Carrie C., Duncan O., Low J., Lee C.P., Van Aken O., Millar A.H., Murcha M., Whelan J. 2010. TCP transcription factors link the regulation of genes encoding mitochondrial proteins with the circadian clock in Arabidopsis thaliana. Plant Cell. 22: 3921-34. DOI: 10.1105/tpc.110.074518

[14] Zhang Z., Jiang S., Wang N., Li M.,Ji X., Sun S., Liu J., Wang D., Xu H., Qi S., Wu S., Fei Z., Feng S., Chen X. 2015. Identification of differentially expressed genes associated with apple fruit ripening and softening by suppression subtractive hybridization. PLoS One. 10(12): 1-17 (e0146061). DOI: 10.1371/journal.pone.0146061

[15] Defilippi B.G., Dandekar A.M., Kader AA. 2005. Relationship of ethylene biosynthesis to volatile production, related enzymes, and precursor availability in apple peel and flesh tissues. J. Agric. Food Chem. 53: 3133-3141. DOI: 10.1021/jf047892x

[16] Souleyre E.J., Greenwood D.R., Friel E.N., Karunairetnam S., Newcomb R.D. 2005. An alcohol acyl transferase from apple (cv. Royal Gala), MpAAT1, produces esters involved in apple fruit flavor. FEBS J. Jun; 272: 3132-3144.

[17] Schwab W., Davidovich-Rikanati R., Lewinsohn E. 2008. Biosynthesis of plant-derived flavor compounds. Plant J. May. 54(4): 712-732. DOI: 10.1111/j.1365-313X.2008.03446.x

[18] Zarid M., Esteras C., Gemma Sifres A., Cañizares X., Esteva J., Picó B.M., Monforte A.J., Fernández Trujillo J.P. 2017. Gene expression and volatile production during melon ripening. In: Proc. 6th Workshop Agri-Food Res., 1st ed, F. Artés-Hernández, M. Egea-Cortines, J.A. Fernández-Hernández, J. Calatrava, E. Aguayo, J.J. Alarcón, J.E. Cos, eds. (CRAI Biblioteca. Universidad Politécnica de Cartagena, Murcia, España). Pp. 27-30. http://hdl.handle.net/10317/6706

[19] Escudero, A.A., Zarid, M., Bueso, M.C., Fernández-Trujillo, J.P. 2017. Aroma volatiles during non-climacteric melon ripening and potential association with flesh firmness. VIII International Postharvest Symposium. Postharvest 2016. Cartagena 21-24/06/2016: Enhancing Supply Chain and Consumer Benefits –Ethical and Technological Issues Eds.: F. Artés-Hernández, P., Gómez, E. Aguayo, F. Artés. Pages 363-366. https://www.ishs.org/ishs-article/1194_50

[20] Dos-Santos, N., Bueso, M., Fernández-Trujillo, J.P. 2013. Aroma volatiles as biomarkers of textural differences at harvest in non-climacteric near-isogenic lines of melon. Food Res. Int. 54: 1801-1812.

[21] Zarid, M., Fernández-Trujillo, J.P. 2016. Seasonal effect on selected quality traits of a melon near-isogenic line. In: Proc. 5th Workshop Agri-Food Res., 1st ed, F. Artés-Hernández, M. Egea-Cortines, J.A. Fernández-Hernández, J. Calatrava, E. Aguayo, J.J. Alarcón, J.E. Cos, eds. (CRAI Biblioteca. Universidad Politécnica de Cartagena, Murcia, España). Pp. 44-47.

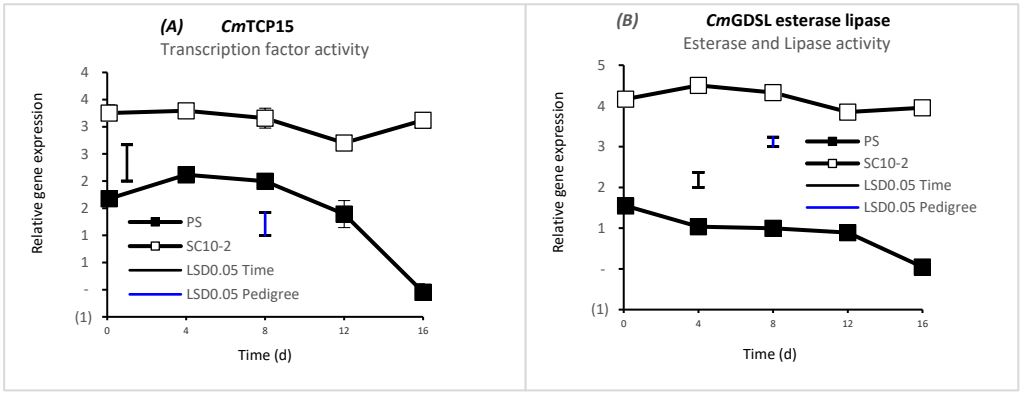

Figure 1. Relative genes expression of the NIL SC10-2 and its parental control PS, (mean ±SE, n=3). (**A**) ***Cm*TCP15:** Transcription factor TCP15, (**B**) ***Cm*GDSL**: Esterase and lipase activity.