New Charentais Lines with Delayed Climacteric Ripening Derived from an Introgression Lines Collection.

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Introduction

Melon (*Cucumis melo* L., 2n = 2x = 24) is one of the most variable species within the Cucurbitaceae family, with a current total world production of over 29 million tons (5). The *cantalupensis* horticultural group includes some of the most important melon varieties cultivated worldwide. 'Vedrantais' is a reference variety for the Charentais-type market class Cantaloupe. It produces medium-size, oval-to-round, sutured and orange-fleshed fruits, with a typical climacteric ripening behavior, that are aromatic and have a medium sugar content (9). One of the main problems of Charentais melons is the short posthaverst life associated to a reduction of flesh firmness and sugar content throughout the postharvest process.

The recent availability of new markers derived from massive sequencing melon projects (1, 2, 3) may be integrated in more efficient breeding programs. New mapping and pre-breeding populations are developed using these genomic tools. Introgression lines (IL) are an excellent breeding tool to incorporate exotic natural variation into recurrent genetic backgrounds. We have recently developed a new melon IL collection by introgressing the exotic genetic background of a Japanese cultivar 'Ginsen makuwa' (MAK) melon (*Cucumis melo* var. *makuwa*) into a French 'Vedrantais' (VED) cultivar (8). This collection includes some interesting ILs showing favorable traits that can be used to develop new cultivars. In this paper, we characterize one of these ILs containing a major introgression of the MAK genome in the chromosome 10 (MAK_10). This line shows a delay in the climacteric ripening behavior, providing a longer postharvest life associated with firmer fruits.

Materials and Methods

The assay was conducted in Paiporta (Valencia, Spain) (Latitude 39º 25' 2.208'' N, Longitude 0º 25' 3, 01'' W and Altitude 17 m), in the spring-summer season 2016 (from March to July), in a greenhouse with automatic control of temperature with cooler and window aperture. Plants were grown in substrate bags of 29 kg (70 % coconut fiber and 30 % coconut chips). Nutrients were provided through the irrigation system and pruning was done manually when necessary to regulate vegetative growth and flowering. Fifty plants of the VED cultivar and thirty of MAK_10 were cultivated. These plants were self-pollinated and the pollination day was recorded with the aim to harvest the fruits at different days after pollination (DAP), from 30 to 39, from 40 to 49 and >50 DAP. All the fruits were phenotyped for flesh firmness (measured as kg/cm^2 with a fruit pressure tester, FT 327, with a plunger diameter of 8 mm, Alfonsine, Italy) and for the presence of abscission layer (an external signal of climacteric ripening in melon, scored visually as 0, absent and 1, present). Also fruits of the two genotypes collected at 40-49 DAP were stored in a chamber at room temperature and phenotyped at 5 and 10 days after harvesting (DAH), with the aim to study the postharvest behavior.

The fruits collected at 40-49 DAP were additionally phenotyped for fruit weight (FW in grams, with digital scale), fruit length and diameter (FL and FD in millimeters, with graduated rule), cavity width (CW, as the ratio of the width of the seminal cavity to the fruit diameter), flesh and rind thickness (Fth and Rth in mm, with electronic digital caliper, I.C.T, S.L., La Rioja, España), occurrence of external aroma of the whole fruit and netting (AR and NET, scored visually as 0, absent and 1, present), flesh color measured with a CR-400 colorimeter (Konica Minolta, Inc., Tokyo, Japan) with Hunter Lab coordinates where L* expresses luminosity (L=0 black and L=100 white), a* expresses the color direction between red (positive) and green (negative) and b* expresses the color direction between yellow (positive) and blue (negative)) (FCHl, FCa, FCb), and soluble solids content (SSC, measured as ºBrix from drops of juice (with a hand-held "Pocket" refractometer (PAL-α), Atago CO., LTD, Tokyo, Japan).

A t-test was used to identify significant differences between the fruits of the recurrent parent and those of the IL MAK_10.

Results

Differences in the formation of the abscission layer between VED and MAK_10 were observed clearly. The abscission layer started to appear in VED fruits collected at 40-49 DAP and was fully formed in all fruits collected at >50 DAP. No fruits of MAK_10 formed the abscission layer at any of the harvesting times (Figure 1).

Apart from the abscission layer, the climacteric ripening process is associated with a loss of flesh firmness. VED fruits, as expected, suffered a progressive decrease of flesh firmness during ripening, dropping from 6.18 ± 0.46 kg/cm^2 in immature fruits (collected at 30-39 DAP) (Figure 2) to 1.38 ± 0.32 kg/cm^2 in overripe fruits (collected at >50 days DAP). MAK_10 fruits had a clearly different behavior maintaining a more constant flesh firmness throughout all the ripening process (ranging from 4.00 ± 0.49 kg/cm^2 to 3.71 ± 0.39 kg/cm^2). T-test results indicated that these differences in fruit firmness between MAK_10 and the recurrent parent VED were significant during all the ripening period (Figure 2 and Table 1). Flesh firmness differences between MAK_10 and VED are important in fruits collected at commercial maturity (40-49 DAP), the fruits of MAK 10 being firmer (4.35 ± 0.3 *versus* 3.19 ± 0.2 kg/cm^2 in MAK_10.1 and VED respectively). At this ripening time the fruits of both genotypes only differed in the external aroma (VED fruits were more aromatic than MAK_10), VED and MAK_10 were similar in fruit weight, length, diameter, cavity width, rind thickness, soluble solids content and flesh color (Table 2).

The differences in flesh firmness found between the two genotypes in fruits collected at 40-49 days were maintained at 5 and 10 days after harvesting (DAH) and conservation at room temperature. Fruits of the ILs MAK_10 conserved a higher flesh firmness (2.5 ± 1 kg/cm^2 and 2.1 ± 0.05 kg/cm^2 at 5 and 10 DAH) than VED fruits that were too soft to be marketable at this postharvest time (0.6 ± 0.05 kg/cm^2 and 1.06 ± 0.14 kg/cm^2) (Table 3).

Discussion

Fruit ripening is a complex process regulated by multiple genetic pathways. Ethylene plays a major role in the regulation of some ripening-associated processes, such as the formation of the abscission layer and the synthesis of aroma volatiles. Other processes related to ripening seem to be ethylene-independent, like sugar accumulation (7). The flesh softening process that is correlated to cell wall degrading is an ethylene-dependent and independent process (4). The suppression of ethylene maintains the flesh firmness and inhibits the development of the abscission layer (4, 6, 7).

In this study, we characterized a new line, MAK_10, derived from the introgression lines collection described by Perpiñá et al. (8), generated by introgressing the MAK genome into a VED background. Unlike VED fruits, MAK_10 fruits do not form an abscission layer during the growing cycle and the postharvest period. Likewise, MAK_10 presents a constant flesh firmness until harvested, unlike fruits of VED that lose flesh firmness throughout the ripening process. Also MAK_10 has an extended shelf-life during the post-harvest process retaining a marketable flesh firmness. This line has a major introgression of 0.9 Mb in LGX of the MAK genome not shared with any other IL of the collection, being IL lacking abscission layer at maturity. In the specific genomic region of LGX several ripening-related genes are located, annotated as NAC transcription factor (belonging to the same TF family as the ripening regulator NOR, non ripening), ERF (ethylene responsive transcription factor), and PEI (pectin esterase inhibitor).

The occurrence of MAK alleles in NAC TFs, ethylene signaling components and genes involved in cell wall metabolism of the MAK_10 introgression might alter the climacteric ripening process causing the absence of abscission layer formation, the low aroma production and the delay in flesh softening.

In conclusion, this line provides fruits with a slower ripening process and a longer postharvest life. A deeper study of the candidate genes involved in the ripening behavior of this line will serve to understand the ripening process in melon.

Acknowledgments

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