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Research article

Chloride stress triggers maturation and negatively affects the postharvest quality of persimmon fruit. Involvement of calyx ethylene production

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ABSTRACT

In recent years many hectares planted with persimmon trees in E Spain have been diagnosed with chloride toxicity. An effect of this abiotic stress on fruit quality has been reported in different crops. However, the impact of chloride stress on persimmon fruit quality is unknown. The harvest and postharvest quality of persimmons harvested from trees that manifest different intensities of chloride toxicity foliar symptoms was evaluated herein. Our results revealed that fruits from trees under chloride stress conditions underwent chloride accumulation in the calyx, which was more marked the greater the salt stress intensity trees were exposed to. Increased chloride concentrations in the calyx stimulated ethylene production in this tissue. In the fruits affected by slight and moderate chloride stress, calyx ethylene production accelerated the maturity process, as reflected by increased fruit colour and diminished fruit firmness. In the fruits under severe chloride stress, the high ethylene levels in the calyx triggered autocatalytic ethylene production in other fruit tissues, which led fruit maturity to drastically advance. In these fruits effectiveness of CO_2 deastringency treatment was not complete and fruit softening enhanced during the postharvest period. Moreover, chloride stress conditions had a marked effect on reducing fruit weight, even in slightly stressed trees.

1. Introduction

In the last few years, many hectares planted in E Spain with persimmon trees cv. Rojo Brillante have been diagnosed with chloride toxicity in areas where no soil salinity problems have been previously reported for other crops (Visconti et al., 2015). Persimmon is very sensitive to chloride salts (Mowat et al., 1995) and trees manifest toxicity symptoms as chloride accumulation in leaves. This results in leaf necrosis, and may lead to leaf abscission if symptoms are severe (Larcher, 2003).

It is known that rootstocks greatly affect tolerance to salts in fruit trees sensitive to stress given rootstocks' different abilities to absorb toxic ions, Cl^-, Na^+, and B, and to translocate ions to the canopy (Levy and Syvertsen, 2004). Worldwide, the commonest rootstocks for persimmon are *Dyospiros kaki* L.f., *Dyospiros lotus* L. and *Dyospiros virginiana* L. In Spain, the main rootstock used in recent decades has been *D. lotus* because of handling facilities in nurseries, and because it imparts elevated vigor and uniformity of development to the scion (Bellini, 2002). However, at the same time a few orchards have also been planted onto *D. virginiana*. A recent study conducted in Spain (unpublished results) has revealed clear differences between both rootstocks: chloride toxicity symptoms in leaves are manifested mainly in those trees grafted onto *D. lotus*, while they are significantly slighter, or even absent, in the persimmon trees grafted onto *D. virginiana*. This is in accordance with previous studies, which have reported that *D. virginiana* confers high salt tolerance (Incesu et al., 2014).

In other fruits, an effect of salts stress on fruit quality has been claimed. Most studies have focused on tomato because of its economical importance (Botella et al., 2000; Saied et al., 2005; Borghesi et al., 2011), but they have also examined the effect of salt stress on the quality of peppers (Chartzoulakis and Klapaki,

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2000; Azuma et al., 2010) and strawberries (Keutgen and Pawelzik, 2007, 2008). In persimmon, one work has reported that crop yield may diminish when trees are exposed to excessive chloride salts concentration (George et al., 2003). However, no studies have approached the effect of this abiotic stress on fruit quality at either harvest or during the postharvest period.

In this context, this study aimed to evaluate the effect of chloride stress on the harvest and postharvest quality of persimmon fruit cv. Rojo Brillante. As this cultivar is astringent at harvest and deastringency treatments are required for fruit commercialisation purposes, we evaluated the influence of chloride stress on the effectiveness of CO_2 deastringency treatment. We also investigated the involvement of calyx in the effect of chloride stress on fruits as we detected in a preliminary study that fruits from trees severely affected by chloride toxicity presented deteriorated calyxes that no longer looked green, but brown (unpublished results).

2. Material and methods

2.1. Vegetal material and experimental design

The study was conducted in 2014 in an orchard planted in alternate rows with two types of 'Rojo Brillante' trees: trees grafted onto the *D. lotus* rootstock and trees grafted onto the *D. virginiana* rootstock. The orchard was located at Alfarp (Valencia, Spain) (39° 16′ N, 0° 32′ W, 50 m asl). It was selected because it showed clear chloride toxicity symptoms in previous seasons. All the trees were 12 years old, and their fertilization, pest management, thinning and pruning were in accordance with conventional practices.

At mid-season (30 October), chloride toxicity foliar symptoms (FS) were evaluated on 40 trees of each rootstock. To this end, two principal branches located on the south face of each tree canopy were evaluated according to a visual scale developed by Servicio de Tecnología del Riego (STR, IVIA, Valencia) (Visconti et al., 2015). On this scale, leaves are scored between 0 and 5, depending on the extension of leaf necrosis: sound leaf, no necrosis symptoms (FS = 0); necrosis in the tip only (FS = 1); necrosis on the tip and in margins (FS = 2); necrosis extends into the blade from margins (FS = 3); necrosis reaches the centre of the blade (FS = 4); necrosis affects more than 75% of the leaf area (FS = 5).

The trees grafted onto *D. lotus* were classified as FS_L-0, FS_L-1, FS_L-2, FS_L-3, FS_L-4 and FS_L-5 trees according to manifested foliar symptoms. Those trees grafted onto the *D. virginiana* rootstock did not manifest chloride toxicity symptoms, so they were all grouped as FS*v*-0.

After classifying trees, 90 fruits were harvested from the evaluated branches of three trees randomly selected from each foliar symptom group. Besides fruits, leaves were picked from the same trees and branches to determine the chloride stress level by evaluating their chloride concentration. Ethylene production in leaves was also evaluated (six leaves per symptom; two leaves per tree).

At the arrival to the laboratory, the fruits that corresponded to each foliar symptom were divided into three homogeneous lots. One lot of fruit was directly analysed, while the other two lots were submitted to the deastringency treatment under standard conditions (95% CO_2 for 24 h at 20 °C, and 90% RH) by passing an air stream that contained 95% CO_2 through closed containers. After treatment, both lots of fruit were transferred to 15 °C. One lot was evaluated after 1 day to determine deastringency treatment efficacy. The remaining lot was evaluated after 10 days at 15 °C to know the evolution of fruits during the postharvest period.

At harvest, fruits were evaluated by taking the following measurements: external colour, fruit firmness, soluble tannins content, total soluble solids, acetaldehyde concentration and sensory evaluation of astringency. Ethylene production was analysed in whole fruits, and also individually in the pulp and calyx tissue. Chloride accumulation in both pulp and calyx was also determined.

Deastringency treatment efficacy was evaluated by all the parameters related with the astringency level: soluble tannins, acetaldehyde and sensory evaluation of astringency. After 10 days at 15 °C, external colour, fruit firmness and ethylene production were evaluated. Ethylene was determined in whole fruits, and also individually in pulp and calyx tissue.

2.2. Analytical methods

Fruit skin colour was evaluated in a Minolta Colorimeter (Model CR-300, Ramsey, N.Y., USA) on samples of 20 fruits. The 'L', 'a', 'b' Hunter parameters were measured and the results were expressed according to the skin colour index ((1000a)/(Lb)). Fruit firmness was determined with 20 fruits in a Texturometer Instron Universal Machine, model 4301 (Instron Corp., Canton, Mass., U.S.A.), using an 8-mm plunger after epicarp removal at two equidistant locations in the equatorial region of each fruit. The crosshead speed during firmness testing was set at 10 mm min^{-1}. Data were expressed as the maximum force in Newtons (N) required to break flesh.

Soluble tannins content (ST) was evaluated by the Folin-Denis method, as described by Arnal and Del Río (2004), and was expressed as mg $ST.g^{-1}$ of fresh weight (fw). As many as 15 fruits per lot were divided into three samples and were cut into four longitudinal parts. Two of the opposite parts were sliced and frozen (−20 °C) to determine ST. One fruit part not used to measure ST was placed in an electric juice extractor (Moulinex model 753, Spain) and was filtered through cheesecloth. The obtained juice was used to determine total soluble solids, acetaldehyde and ethanol production (three juices of five fruits each per lot). Total soluble solids were evaluated with a digital refractometer (Atago mod. PR1) and the results were expressed in ºBrix. Acetaldehyde production was analysed by headspace gas chromatography. Five millilitres of filtered juice were transferred to 10-mL vials with crimp-top caps, sealed with TFE/silicone septa, and frozen (−20 °C) until analysed. For the analysis, samples were put into a water bath at 20 °C for 1 h, followed by heating at 60 °C for 10 min. A 1-mL sample of the headspace was withdrawn from vials and injected into the gas chromatograph (Perkin–Elmer, Model 2000, Norwalk, Conn., USA), equipped with a flame ionisation detector (FID) and a 0.32 cm × 1.2 m Poropak QS 80/100 column. The injector was set at 175 °C, the column at 150 °C, the detector at 200 °C, and carrier gas at 12.3 psi. Acetaldehyde was identified by comparing the retention times with those of a standard solution. The results were expressed as mg AcH. L^{-1} of juice.

One fruit part not used to measure ST was destined to sensory analysis. Sensory evaluation was performed at the Sensory Laboratory of the Postharvest Department (IVIA) in composite samples of five fruits per replicate, which had been previously peeled and sliced. Eight to 10 semi-trained panellists were asked to evaluate astringency and presence of off-flavours. The panellists were familiar with the 'Rojo Brillante' cultivar as they had been tasting fruits with different levels of astringency for several years. A 4-point scale was used for astringency, where 3 = very high astringency and 0 = no astringency. Samples were presented to the panellists on trays labelled with 3-digit random codes and were served at room temperature. The panellists had to taste several segments of each sample in order to compensate, as far as possible, the biological variation of the material. Milk was provided for palate rinsing between samples.

Ethylene production of whole fruits was determined from three individual fruits of each lot. Three other individual fruits were transversally cut 3 cm below the calyx. Then the calyx was carefully removed from the upper part of the pulp (the pulp from this upper