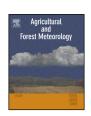
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Rainfall intensifies fruit peel cracking in water stressed pomegranate trees



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ABSTRACT

The purpose of the present study was to study the relationship between the leaf and fruit water relations of pomegranate plants at different water deficit levels during the end of the fruit growth and ripening phases. Moreover, special attention was paid to analysing whether the effect that rainfall has on peel cracking of pomegranate fruits growing in water deficit conditions is associated with changes in turgor within the fruit. For this, field-grown adult pomegranate trees (Punica granatum L.) cv. Mollar de Elche were subjected to five irrigation treatments. During the growing season, control plants (treatment T0) were irrigated above crop water requirements. During the experimental period (DOY 247-283), T1, T2, T3, and T4 treatments were irrigated as T0 except for 6 (DOY 277-283), 15 (DOY268-283), 25 (DOY258-283) and 36 (DOY247-283) days before harvest (DOY 283), respectively, when irrigation was withheld. Rain fell on DOY 270 (3.5 mm), DOY 271 (84 mm) and DOY 272 (0.9 mm), totalling 88.4 mm. During the experiment, total ETo was 162 mm, and the total amount of water received was 128, 110, 86, 49 and 0 mm for TO, T1, T2, T3 and T4, respectively, without considering precipitation. The results indicated that during the end of fruit growth and ripening phases pomegranate fruit was clearly sensitive to water deficit. During these phenological periods water could enter the fruits via the phloem rather than via the xylem. Despite this, T3 and T4 plants reached much more severe water stress levels than those reported in the literature, although leaf turgor was maintained. However, in all treatments fruit turgor was lost as a consequence of water stress, which induced a reduced expansion of fruits. When rainfall affected previously water stressed pomegranate plants an asymmetric increase in fruit turgor pressure took place, because aril turgor increased to a much greater extent than peel turgor, the pressure of the arils on the peel favouring cracking

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1. Introduction

Pomegranate (*Punica granatum* L.) and *P. protopunica* are the two species that make up the Punicaceae family. *P. protopunica*, which is considered as the ancestrer of the genus (Shilikina, 1973), is generally accepted as being endemic of the Socotra Island (Yemen) and *P. granatum*, one of the earliest domesticated plant species, is believed to be a native to the southern Caspian belt (Iran) and northern Turkey (Janick, 2007).

P. granatum has been regarded as a minor crop for a long time. However, the way pomegranate fruit is regarded has begun to change due to the crop's adaptation to a wide range of climates and soil conditions and increasing interest in of its fruits due to their organoleptic characteristics and perceived health benefits (Michel et al., 2005; Ephraim and Robert, 2007; Lansky and Newman, 2007).

Pomegranate fruits are complex in structure, showing a persistent calyx at the top of fruit, which is maintained until maturity and acts as a distinctive feature of these fruits. The fruit is technically a leathery-skinned and fleshy berry. The multi-ovule chambers (locules) are separated by membranous walls (septum) and fleshy mesocarp (Holland et al., 2009). The locules are filled with many seeds (arils) which comprise the edible portion of the fruit. The arils contain a juicy edible layer that develops entirely from the outer

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epidermal cells of the seed, which elongate to a very large extent in a radial direction (Fahan, 1976). The sap of these cells develops a turgor pressure that preserves the characteristic external shape of these cells (Holland et al., 2009). The husk comprises of two parts: the pericarp, which provides a cuticle layer and fibrous mat; and the mesocarp (also known as the albedo), which is the spongy tissue and inner fruit wall to which the arils are attached (Stover and Mercure, 2007).

Pomegranates are grown in very different climatic areas, but are frequent in areas predisposed to water scarcity (Holland et al., 2009). Studies on the water relations of pomegranate plants have mostly focused on the vegetative part of the plant, which possesses the drought tolerance characteristics that are common in xeromorphic plants, such as high leaf relative apoplastic water content and the ability to confront water stress by developing complementary stress avoidance and stress tolerance mechanisms (Rodríguez et al., 2012). For these reasons, this drought-hardy crop supports heat and thrives well in arid and semiarid areas, and even under desert conditions (Sarkhosh et al., 2006; Aseri et al., 2008). However, to the best of our knowledge, no information exists on pomegranate fruit water relations. In this sense, it is of paramount importance that one of the foremost physiological disorders, which has a severe economic impact on pomegranate fruit value, is the cracking of ripe fruit (Blumenfeld et al., 2000; Holland et al., 2009). Such defects seem to be directly related to fruit water status, because regular irrigation can decrease the damage (Prasad et al., 2003). Moreover, the conviction exists among growers that rain falling on mature pomegranate fruits following the end of the dry season can induce rapid fruit cracking (Holland et al., 2009), although no scientific evidence has been proposed for the mechanisms developed. For these reasons, the aim of the experiments described was to increase our understanding of the relationship between leaf and fruit water relations in pomegranate plants under different water deficit levels during the end of fruit growth and ripening phases. Moreover, special attention was paid to analysing whether the peel cracking caused by rainfall under water deficit conditions is associated with changes in turgor within the fruit.

2. Materials and methods

2.1. Plant material, experimental conditions and treatments

The experiment was carried out in 2012 in a farm located near the city of Alhama de Murcia (Spain) (37°47′N, 1°25′W). The soil of the orchard is a moderately saline (5.9 dS m $^{-1}$) Hyposalic Calciorthid, with a silt loam texture, moderate lime content (20% calcium carbonate), very low organic matter content (1.1%), low cationic exchange capacity (9.32 mequiv 100 g $^{-1}$), and low available potassium and high available phosphorus levels.

The plant material consisted of own rooted adult pomegranate trees (P. granatum L.) cv. Mollar de Elche. Tree spacing followed a $3 \,\mathrm{m} \times 5 \,\mathrm{m}$ pattern. The irrigation water used had an electrical conductivity of between 0.9 and $1.3 \,\mathrm{dS} \,\mathrm{m}^{-1}$. The Cl⁻ concentration in the irrigation water ranged from 67 to $78 \,\mathrm{mg} \,\mathrm{l}^{-1}$ during the experimental period. Pest control and fertilization practices were those usually used by local growers, and no weeds were allowed to develop within the orchard.

During the growing season, control plants (treatment T0) were irrigated above crop water requirements in order to ensure non-limiting soil water conditions. Irrigation was performed daily during the night using a drip-irrigation system with a lateral pipe parallel to each tree row and 3 emitters per tree, each delivering $41h^{-1}$. In-line water meters were used to measure the water supplied to each experimental unit. T1, T2, T3, and T4 treatments were irrigated as T0 except for 6 (DOY 277–283), 15 (DOY 268–283),

25 (DOY 258–283) and 36 (DOY 247–283) days before harvest (DOY 283), respectively, when irrigation was withheld. During the experimental period (DOY 247–283), total ETo (crop reference evapotranspiration) was 162 mm, and the total amount of water received by each treatment was 128, 110, 86, 49 and 0 mm for T0, T1, T2, T3 and T4 treatments, respectively, without considering precipitation (basically the 84 mm that fell on DOY 271).

2.2. Measurements

Micrometeorological data, namely air relative humidity, air temperature, solar radiation, rainfall and wind speed 2 m above the soil surface, were collected by an automatic weather station located near the experimental site. Mean daily air vapour pressure deficit (VPD_m) was calculated according to Allen et al. (1998), and daily crop reference evapotranspiration (ETo) was calculated using the Penman–Monteith equation (Allen et al., 1998).

The water relations of the leaves and fruits were measured at midday (12 h solar time). Fruits and fully expanded leaves from the south-facing side and middle third of the tree of four trees per treatment were selected for measurements. Midday leaf conductance ($g_{\rm leaf}$) was measured with a porometer (Delta T AP4, Delta-T Devices, Cambridge, UK) on the abaxial surface of two leaves per tree. Midday fruit water potential ($\Psi_{\rm fruit}$), midday leaf water potential ($\Psi_{\rm leaf}$), and midday stem water potential ($\Psi_{\rm stem}$) were measured in two fruits or two leaves similar to those used for $g_{\rm leaf}$ using a pressure chamber (PMS 600-EXP, PMS Instruments Company, Albany, USA) (McFadyen et al., 1996; Gelly et al., 2004; Dell'Amico et al., 2012). Leaves for $\Psi_{\rm stem}$ measurements were enclosed in a small black plastic bag covered with aluminium foil for at least 2 h before the measurements were made.

Midday leaf ($\Psi_{\pi \, leaf}$), fruit juice ($\Psi_{\pi \, arils}$) and fruit peel ($\Psi_{\pi \, peel}$) osmotic potentials were determined in the same leaves and fruits as used for Ψ_{leaf} and Ψ_{fruit} measurements. Leaves and fruits (peel and aril juice) were covered with aluminium foil and immediately frozen in liquid nitrogen and stored at $-80\,^{\circ}$ C. The osmotic potential was measured after thawing the samples and expressing the sap, using a vapour pressure osmometer (Wescor 5600, Logan, USA). Estimated midday leaf ($\Psi_{p \, leaf}$), fruit juice ($\Psi_{p \, arils}$) and fruit peel ($\Psi_{p \, peel}$) turgor potentials were derived as the difference between osmotic and water potentials (Kaufmann, 1970; Milad and Shackel, 1992; Mills et al., 1997; Yamada et al., 2004).

Pomegranate fruits were harvested on 10 October (DOY 283). The mean weight of marketable and peel-cracked fruit was determined according to the weight and number of fruits per box in randomly selected boxes per replicate (2–4 boxes).

2.3. Statistical design and analysis

The design of the experiments was completely randomized with four replications, each replication consisting of three adjacent rows, each with thirteen trees. Measurements were taken on the innermost tree of the central row of each replicate, which were very similar in appearance (leaf area, trunk cross sectional area, height, ground shaded area, etc.), while the other trees served as border trees. Data were analysed using SPSS software (SPPS, 2002). Analyses of variance were performed and mean values were compared by an LSD_{0.05} test. Values for each replicate were averaged before the mean and the standard error of each treatment were calculated.

3. Results

During the experimental period, average daily maximum and minimum air temperatures were 28.0 and $14.8\,^{\circ}$ C, respectively. VPD_m ranged from 0.33 to 1.87 kPa, and accumulated ETo was

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