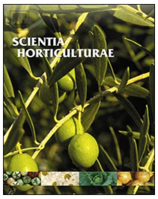
Scientia Horticulturae 224 (2017) 171–179

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti



Physiological responses of three pomegranate cultivars under flooded conditions

Antonio Olmo-Vega[a], Francisco García-Sánchez[b,*], Silvia Simón-Grao[b], Inmaculada Simón[a], Vicente Lidón[a], Manuel Nieves[a], Juan J. Martínez-Nicolás[a,*]

[a] *Escuela Politécnica Superior de Orihuela, Universidad Miguel Hernández, Ctra. de Beniel, km 3.2, Orihuela, Alicante, Spain*
[b] *Centro de Edafología y Biología Aplicada del Segura, CSIC, Campus Universitario de Espinardo, Espinardo 30100, Murcia, Spain*

ARTICLE INFO

Keywords:
Chlorophyll fluorescence
Flooding stress
Punica granatum
Stomatal conductance
Water relations

ABSTRACT

Pomegranate (*Punica granatum* L.) is known for its great resistance to abiotic stresses such as salinity or drought. However, its behavior under flooding − which is very common in the southeast of Spain, due mainly to the presence of heavy soils − has not yet been studied. This experiment was carried out in a greenhouse, with three of the pomegranate varieties most used in southeastern Spain: 'Mollar de Elche', 'Valenciana', and 'Wonderful'. The plants were immersed in containers of 20 liters capacity, leaving the water level three centimeters above the root zone for six days. Measurements of growth parameters, water relations, gaseous exchange parameters, chlorophyll fluorescence, organic solutes, oxidative stress, and the hormonal response were performed. The first variety that showed symptoms of stress was 'Valenciana', the one whose water relations, gaseous exchange parameters, total dry biomass, and chlorophyll fluorescence were affected most. It was also the one with the worst hormonal response. The flooding also negatively affected the growth parameters of 'Wonderful' and 'Mollar de Elche', but much less than in 'Valenciana'. From this study we can conclude that flooding tolerance of pomegranate crops depend on the cultivar. So, 'Valenciana' cultivar was the most sensitive and 'Mollar de Elche' the most tolerant.

1. Introduction

Pomegranate (*Punica granatum* L.) is one of the most important emerging fruit crops worldwide. The total area devoted to pomegranate cultivation in the world is over 302,000 ha, with more than 76% in five countries (India, Iran, China, Turkey, and the United States). However, other countries, such as Spain, Egypt, and Israel, with areas of between 2400 and 16,000 ha, have developed much more with regard to exports, research, market development, and new varieties (Quiroz, 2009; Melgarejo et al., 2012).

Pomegranate is known for its extreme resistance to abiotic stresses, such as salinity or drought (Parvizi et al., 2014; Mastrogiannidou et al., 2016), so its cultivation has displaced other, traditional Mediterranean fruit trees, such as *citrus*, in areas where the latter are not so profitable. Pomegranate is a crop with great adaptation to a wide range of climates and soil conditions (Rodriguez et al., 2012; Sharma et al., 2015; Hmid et al., 2016). There are several regions of pomegranate production in the world, being able to develop the crop in soils with different texture including clay soils, clay loam, chestnut, loamy, loamy-pebble soils, sandy loam soils rich with humus, black earth (Chernozem), light humus soils with pebble inclusions, Yellow yellow soils (Zheltozen), on podzolclay, alluvial soils, on seaside sands, gravel talus dry rocky hills, alkali soils, lime-rich soils as well as on limestone-rich lands of arid hills (Teixeira da Silva et al., 2013). This crop can tolerate well wet soils (Badizadegan, 2015), although high soil moisture may lead to wilt disease (Sharma et al., 2006); however, it has not been reported how flooding condition can affect to this crop.

Southeastern Spain is characterized by a semi-arid climate with an average annual precipitation that does not exceed 300 mm. However, the precipitation is concentrated in late summer, in the form of torrential rains. This is a meteorological phenomenon known as "cold drop", a relatively frequent and intense rainy phenomenon on the Iberian Peninsula, particularly on the Spanish East to South-East inlands and coasts (Díez et al., 2013). These flash flood events in

---

Abbreviations: Ψ_P, turgor potential; Φ_{PSII}, photochemical efficiency of PSII; Ψ_w, leaf water potential; Ψ_π, osmotic potential; ABA, abscisic acid; A_{CO2}, net assimilation of CO_2; Ci, intercellular CO_2 concentration; Fv'/Fm', antennas efficiency in the PSII reaction centers; gs, stomatal conductance; LDW, leaf dry weight; MDA, malondialdehyde; PAR, photosynthetically active radiation; PQ, photochemical quenching; PSII, photosystem II; QAC, quaternary ammonium compounds; RDW, root dry weight; ROS, reactive oxygen species; RS, reducing sugars; RWC, relative water content; SDW, stem dry weight; TDW, total dry weight; TNC, total nonstructural carbohydrates; TSS, total soluble sugars

[*] Corresponding authors.

E-mail addresses: fgs@cebas.csic.es (F. García-Sánchez), juanjose.martinez@umh.es (J.J. Martínez-Nicolás).

http://dx.doi.org/10.1016/j.scienta.2017.06.013
Received 20 February 2017; Received in revised form 17 May 2017; Accepted 10 June 2017

Mediterranean area are widely recorded because of their economic impact caused by the high volume of discharge, even in some years it have been reported precipitation of 200 mm/day (Ibarra, 2012). In addition, many soils of these areas, has a significant clay content and low permeability due to high sodium concentration, it causes dispersion of colloids and blockage of pores in the soil, which further facilitates their waterlogging. So, it is common for crops in these areas to suffer flood problems. Under flood conditions, water occupies the soil air pores, saturating them, reducing the gas diffusion capacity, and lowering the concentration of oxygen in the soil. The resulting conditions of hypoxia and anoxia produce anaerobic decomposition of organic matter − leading to the formation of toxic compounds, which seriously affect the uptake of water and nutrients by the roots, and increasing the concentrations of soluble Fe and Mn in the soil (Pucciarello and Perata, 2012; Syvertsen and García-Sánchez, 2014; Bhatt et al., 2015). These changes produce in the plant a decrease in the net assimilation rate of CO_2, alterations of the water status, nutritional imbalances, and intensification of the oxidative stress. This leads to epinasty, foliar chlorosis, necrosis, and foliar abscission, resulting in reduced vegetative growth and crop yield (Jackson et al., 2009). Plants, in order to alleviate the negative effects of oxygen deficiency in the soil, activate a series of morphological, physiological, and biochemical mechanisms. The typical morphological mechanisms are found mainly in forest plants: the appearance of adventitious roots (Kissmann et al., 2014), hypertrophied lenticels (Du et al., 2012), or aerenchyma (Wang and Cao, 2012). In species not adapted to this stress, morphological changes are related more to an increase in the root/shoot ratio (Gimeno et al., 2012). In addition, in all plants, the first response is usually stomatal closure to avoid water loss through transpiration (Arbona et al., 2008). In these conditions, antioxidant systems are also activated to reduce the concentration of reactive oxygen species (ROS) and thereby prevent lipid peroxidation of cell membranes (Lin et al., 2013). It has also been shown that anoxia triggers a series of chemical signals in the root that are transmitted to the aerial parts of the plant with the aim of inducing physiological responses (Dat et al., 2004). In many plants, an increase in abscisic acid (ABA) has been observed that promotes stomatal closure in flood situations, thus improving tolerance of this stress (Wu et al., 1997).

Since the responses of pomegranate plants to flood conditions are not known, the main objective of this work was to study the relative tolerance to flooding of the three pomegranate cultivars currently grown the most in the southeast of Spain (‘Mollar de Elche’, ‘Valenciana’, and ‘Wonderful’), and to establish the morphological, physiological, and/or biochemical mechanisms related to their differing tolerance. Our results could be of interest to scientists who study how plants behave under adverse conditions; to plant breeders attempting to enhance flooding tolerance; to farmers, to provide them with varieties they can plant on flood-prone soils; and to different agricultural sectors involved with pomegranates that believe that the use of rootstocks could be a good strategy to improve this crop.

2. Materials and methods

2.1. Plant material and experimental conditions

Pomegranate seedlings were obtained from woody cuttings in a commercial nursery (Caliplant, Murcia, Spain) nine months before the plants were taken to the test greenhouse. Here, the plants were cultivated before the treatments started. The climatic characteristics of the greenhouse were: maximum photosynthetically active radiation (PAR) of 1000 μmol m^{-2} s^{-1}, day/night temperature of 35/18 ± 3 °C, day/night relative humidity of 55/75 ± 5%, and a natural photoperiod of 16 h. Thirty-six plants (30–40 cm in height) were transplanted in individual 4 l pots, using 12 plants of each variety ‘Mollar de Elche’ (M), ‘Valenciana’ (V), and ‘Wonderful’ (W). The plants were watered three times per week, by a localized irrigation system with self-compensating and anti-draining drippers (4 l h^{-1}), with a complete Hoagland nutrient solution of 7.75 mM NO_3^-, 0.7 mM $H_2PO_4^-$, 4.05 mM K^+, 2.20 mM Ca^{2+} 0.5 mM Mg^{2+}, 0.5 mM SO_4^{2-}, and 0.6 mM Fe. Pots containing a universal substrate (Projar S.L. Spain) of 50% fine blond peat moss and 45% black peat moss blended with 5% perlite; with particle size ranged in 0–10 mm and density of 280 g/l. After three months of plant acclimation, the flood treatment was imposed by submerging the plants, six of each variety in 20 l containers (one container per plant), to 3 cm above the base of the stem with tap water of (mM) 8.86 SO_4^{2-}, 1.16 HCO^{3-}, 3 Cl^-, 3.5 K^+, 1.2 Ca^{2+}, 2.22 Mg^{2+}, 4.13 Na^+; and electrical conductivity EC = 0.8 dS m^{-1}. Seedlings remained submerged in these containers fill out with tap water during only once period of six days. After this period the plants were harvested. During flooding period, every day tap water without oxygen was added to replenish the water lost by evapotranspiration. Six non-flooded plants of each variety were taken as controls, maintaining the same irrigation regime as mentioned previously.

2.2. Concentration of O_2

During the six days of the trial, $[O_2]$ readings of the water that was flooding the plants were taken with a portable oximeter (Crison 330i). There was a progressive decrease in the $[O_2]$ during the six days of testing, with a rapid reduction on the second day of almost 45% and a final concentration of 2.7 mg l^{-1}, without significant differences among the varieties tested (Fig. 1).

2.3. Measurements of water relations

To determine the water status of the plants the water potential (Ψ_w), osmotic potential (Ψ_π), and turgor potential (Ψ_p) were measured in the leaves. The Ψ_w of the leaf was measured at midday (12:00–14:00) on days 0, 2, 5, and 6 of the assay. A Scholander type pressure chamber (PMS Instruments, Corvallis, OR; Scholander et al., 1965) was used. After the Ψ_w was measured, the leaves were immediately wrapped tightly in aluminum foil, frozen in liquid nitrogen, and stored in airtight plastic bags at −18 °C. After thawing, the Ψ_π of the extracted sap was measured at 25 ± 1 °C, with an osmometer (Digital Osmometer, Wescor, Logan, UT). The Ψ_p was calculated as the difference between Ψ_w and Ψ_π. The relative water content (RWC) was measured in leaves similar to those used to measure Ψ_w. At midday, leaves were picked and immediately weighed to obtain the fresh weight. The leaves were placed in vials with their petioles immersed in deionized water and kept

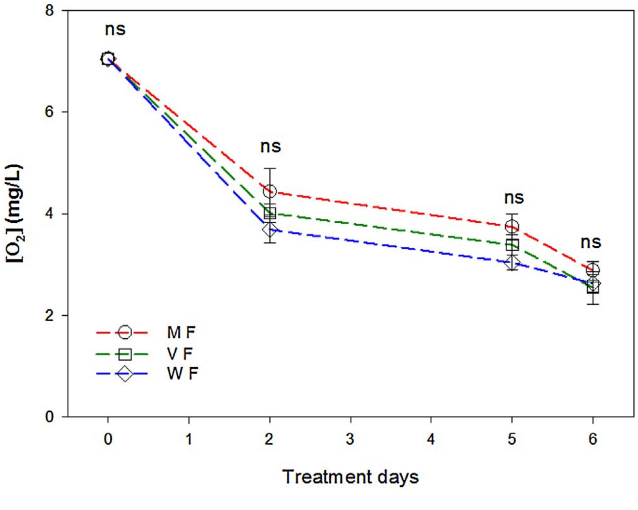


Fig. 1. Oxygen (O_2) concentration of the water used for the flooding of the pomegranate plants, on the different days of treatment (6 days): MI = ‘Mollar de Elche’ Flood; VI = ‘Valenciana’ Flood; WI = ‘Wonderful’ Flood. Ns indicates non-significant differences for $P < 0.05$. Vertical bars represent the standard error (n = 6).