Scientia Horticulturae 230 (2018) 86–91

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Research paper

Foliar calcium fertilization reduces fruit cracking in pomegranate (*Punica granatum* cv. Ardestani)

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ARTICLE INFO

Keywords:
Punica granatum
Calcium spray
Fruit cracking
Total phenolic compounds

ABSTRACT

An experiment was conducted to assess the effects of foliar sprays of a calcium fertilizer containing nanoparticles (nano-Ca) and calcium chloride ($CaCl_2.2H_2O$) on the yield and quality of pomegranate fruits cv. Ardestani, during two consecutive years, 2014 and 2015. The nano-Ca fertilizer was sprayed at concentrations of 0.25 and 0.50 g Ca L^{-1}, and $CaCl_2.2H_2O$ was used at concentrations of 1 and 2% (2.73 and 5.45 g Ca L^{-1}), with treatments being applied twice, first at full blooming and then one month later. Calcium foliar fertilization did not have significant effects on yield, number of fruits per tree and average fruit weight, whereas it increased fruit length only in the case of the $CaCl_2$ 1% treatment in the first season. The untreated trees in the orchard were moderately affected by fruit cracking, with 6–7% of the fruits being affected. Calcium foliar treatment with the nano-Ca fertilizer at 0.50 g Ca L^{-1} and 1% $CaCl_2$ (in the both seasons) and also 2% $CaCl_2$ (only in the second season) decreased significantly fruit cracking when compared with the control treatment, resulting in increases in marketable fruit yield. Foliar sprays with $CaCl_2$ 1% increased TSS by 7.6% only in the second season. Moreover, foliar nano-Ca fertilization at 0.50 g Ca L^{-1} led to minor decreases (approximately 1%) in total phenolics only in the first season. Other chemical properties, including titratable acidity, fruit maturity, total sugar, antioxidant activity and total anthocyanin contents were not affected by Ca foliar fertilization. Leaf analyses show that Ca foliar treatments increased leaf Ca concentrations in the first season, with the exception of the low dose of nano-fertilizer, whereas the leaf concentrations of N, P, K, Fe, Zn and Mn were unaffected. In summary, fertilization with a low (0.50 g Ca L^{-1}) Ca concentration in the form of a nano-Ca formulation resulted in similar decreases in pomegranate fruit cracking than those obtained with higher doses of $CaCl_2$ (2.73 and 5.45 g Ca L^{-1}).

1. Introduction

Pomegranate (*Punica granatum* L.) is one of the oldest known edible fruits, which is native of Iran and is currently cultivated in many countries, including Spain, Morocco, Egypt, Afghanistan, Burma, China, Japan, USA, Russia, Bulgaria and Italy. Pomegranate is mainly consumed as a fresh fruit and also used in form of jams, juices, wines, vinegars and jellies (Heber et al., 2006; Kingsly and Singh, 2007; Sheikh and Manjula, 2012; Gumienna et al., 2016).

It is well known that the growth and yield of fruit trees are affected by many factors, including climate and soil conditions, irrigation, cultivars, pruning, insects and plagues, as well as the tree nutritional status. Since several essential elements are directly involved in the plant growth and reproduction, fertilization with these nutrients can affect fruit yield and quality (Barker and Pilbeam, 2007; Dhillon et al., 2011; Obaid and Al-Hadethi, 2013). As an important macroelement, calcium (Ca) plays several roles in plants, including structural functions in cell walls, stabilization of cell membranes, maintenance of cell turgor pressure, as well as acting as a counter-ion for inorganic and organic anions in vacuoles and as a cytoplasmic second messenger (Picchioni et al., 1995; Sugimura et al., 1999; Mastrangelo et al., 2000; White, 2001). In the case of fruits, quality is mainly affected by Ca via Ca pectate formation, which is associated to increases in the strength of the cell wall and middle lamella (Faust, 1989; Carpita and McCann, 2000).

Calcium cannot be transported through the basic pH phloem pathway from the older tissues to other parts of the plant, and Ca xylem

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https://doi.org/10.1016/j.scienta.2017.11.023
Received 12 February 2017; Received in revised form 14 July 2017; Accepted 10 November 2017
Available online 22 November 2017

translocation depends on unidirectional transpiration stream (White and Broadley, 2003). Although the foliar application of Ca may be potentially effective for increasing the fruit Ca concentration, Ca spraying has been shown to have a low efficiency in many cases. This has been attributed to limitations in Ca uptake, penetration to fruits, epidermal characteristics, cuticle presence and composition, and may be also related to the low translocation rates of Ca in the phloem (Wojcik, 2001; Conway et al., 2002; Mengel, 2002; Danner et al., 2015). Some nutritional disorders attributed to Ca deficiency, including bitter pit in apple, cork spot in pear, blossom end rot in tomato and fruit cracking in cherry and pomegranate, can be alleviated by foliar Ca application (Kader, 2002; Erogul, 2014; Hegazi et al., 2014).

Fruit cracking is one of the serious physiological disorders in many fruit species, including pomegranate, apple, sweet cherry, grape, plum, persimmon, litchi, avocado, pistachio, citrus and banana, and leads to decreases in fruit yield and quality (Blumenfeld et al., 2000; Khadivi-Khub, 2015). Pomegranate fruit cracking can occur as a result of the pressure of quickly expanding arils on the stretched peel (Yilmaz and Ozguven, 2006). Cultivar sensitivity, day and night temperatures, soil moisture variation, relative humidity, irrigation, peel pliability and Ca and boron (B) deficiency are some of the major factors that contribute to pomegranate fruit cracking (Mir et al., 2012; Khadivi-Khub, 2015). Concerning cultivar sensitivity, fruit cracking damage was significantly different among Chinese pomegranate cultivars; the fruit cracking rate were lower than 3% in 'Gangliu', 'Zhuyeqing', 'Houpitian' and 'Qingpidazi', and above 27% in 'Daqingpitian', 'Dahongpitian', 'Sanbaitian' and 'Xiehuatian' (Hou et al., 2010). Regarding the effects of Ca fertilization on pomegranate fruit cracking, foliar sprays of $CaCl_2$ at concentrations of 2 and 4% has been shown to decrease significantly fruit cracking in the cultivars 'Manfaloty' and 'Wonderfull' (Hegazi et al., 2014).

The aim of the present study was to evaluate the effects of foliar sprays with two Ca formulations, a nano-Ca commercial product and $CaCl_2$, on pomegranate fruit yield and quality. Pomegranate is widely cultivated as commercial orchards in Iran, where the cultivation area and total production in 2015 were 81700 ha and *ca.* 990000 t, respectively, and fruit cracking is one of the major problems for pomegranate fruit production in many Iranian agricultural areas, resulting in financial damage to growers ever year.

2. Materials and methods

2.1. Experimental site, plant materials and treatments

The experiment was carried out in two seasons, 2014 and 2015, in a commercial pomegranate orchard. The orchard was located in the central part of the Razavi Khorasan province in North Eastern Iran (Tous Dasht; lat. 35° 1′ 24.33" N, long. 58° 50′ 19.61" E, altitude 967 m), and the soil was a coarse-loamy over fragmental, mixed, thermic xeric Torriorthents (64% sand, 12% clay and 24% silt), with a pH of 8.08 in water and an EC of 9.4 dS m^{-1}. The region is arid, with 248 mm of annual mean precipitation and a mean annual temperature of 14.8 °C. Trees were eight-year-old with three trunks, approximately 2.5–3 m in height. Trees were planted in regular rows and spaced at 3 × 5 m (667 trees ha^{-1}) and irrigated by a drip irrigation system. In the orchard studied, fruit cracking is known to be present even though a drip irrigation system is used and harvest is carried out before the weather gets cold at the end of the season.

Two sources of Ca were used, a "*Nano-chelated fertilizer Ca*" (250 g Ca L^{-1}; thereafter called nano-Ca) and $CaCl_2.2H_2O$. An experiment was carried out based on completely randomized block design with five treatments (Control −no Ca-, nano-Ca1, nano-Ca2, $CaCl_2$ 1%, $CaCl_2$ 2%) and four replications per treatment. Four rows of pomegranate trees were selected, and in each row one tree was sprayed with each of the five treatments. The nano-Ca fertilizer contains nanoparticles (composition patent-protected, average size 50 nm, range of 23–80 nm), and was used in spray applications at concentrations 0.25 and 0.50 g Ca L^{-1} (nano-Ca1 and nano-Ca2, respectively; 0.50 g Ca L^{-1} is the dose recommended by the manufacturer). On the other hand, $CaCl_2$ was used at concentrations of 2.73 and 5.45 g Ca L^{-1} (1 and 2% $CaCl_2{\cdot}2H_2O$, respectively), doses comparable to those used in previous foliar fertilization studies in pomegranate (2 and 4%; Ramezanian et al., 2009). The fertilizer solution was prepared by diluting the nano-Ca commercial liquid product or commercial $CaCl_2{\cdot}2H_2O$ with underground water available in wells in the orchard. Trees were sprayed twice per season, the first at full bloom, on May 12th 2014, and April 26th 2015, and the second one month after the first one, in all cases with 5.3 L per tree (until full foliage wetting; the total doses of Ca applied with nano-Ca and $CaCl_2$ fertilizers were approximately 1.3 or 2.7 g Ca $tree^{-1}$ and 14.5 or 28.9 g Ca $tree^{-1}$, respectively). Leaves were sampled from the middle part of fruiting shoots (100 leaves from the three trunks all around the canopy in each tree) on August 11th in the first season and on August 6th in the second season. Fruits were harvested on October 22nd in the first season and on October 14th in the second season, with the harvest date being based on general fruit appearance and fruit chemical properties (see below).

2.2. Plant measurements

The concentrations of macro and micronutrients in pomegranate leaves were measured in the Iranian laboratory in the first season and in the Spanish laboratory in the second season. In Iran, samples were prepared as in Chapman and Pratt (1961), and total N, P, K and Ca were determined using Kjeldhal method, spectrophotometry, flame photometry and complexometry, respectively; Fe, Zn and Mn contents were measured using flame atomic absorption spectrophotometry. In the Spanish laboratory samples were digested using a microwave device and analysed for Fe, Mn, Zn and Ca using flame atomic absorption spectrophotometry and for K using flame emission spectrometry (Carrasco-Gil et al., 2016). Three replications per treatment and year were carried out.

2.3. Fruit physical properties

In order to determine fruit physical properties, four fruits were randomly selected from each tree replication, and fruit weight was measured using an electronic balance. Fruit diameter and length, fruit calyx diameter and peel thickness were measured by using a digital Vernier gauge. In order to determine peel weight and aril percentage of each fruit, fruits were manually peeled and the weight of total arils and peel were measured. The weight of 100 arils was measured and the juice volume of 100 g arils, extracted by a manual extractor, was expressed in mL per 100 g arils. The number of fruits affected by cracking on each tree was counted and the results were expressed as percentage of fruits affected by cracking. For all physical parameters four replications per treatment and year were carried out.

2.4. Fruit chemical properties

Titratable acidity (TA) was determined by the titration method (to pH 8.2 with 0.1 N NaOH), and results expressed as percentage of citric acid. Total soluble solids (TSS) and juice pH were measured at room temperature using a digital refractometer and a digital pH meter, respectively. The TSS/TA ratio was expressed as maturity index. Four replications per treatment and year were carried out.

To determine total phenolic compounds in juice, the Folin–Ciocalteu reagent method was used (Singleton and Rossi, 1965). Anti-oxidant activity was determined using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method (Brand-Williams et al., 1995). Total anthocyanins were estimated by the pH differential method using two buffer systems; 25 mM K chloride buffer, pH 1.0, and 0.4 M Na acetate buffer, pH 4.5 (Giusti and Wrolstad, 2001). Samples were diluted with K