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Research paper

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

Sohrab Davarpanah[a], Ali Tehranifar[a,*], Javier Abadía[b], Jesús Val[b], Gholamhossein Davarynejad[a], Mehdi Aran[c], Reza Khorassani[d]

[a] *Department of Horticultural Science and Landscape, Ferdowsi University of Mashhad, Iran*
[b] *Department of Plant Nutrition, Estación Experimental de Aula Dei (CSIC), Zaragoza, Spain*
[c] *Department of Horticultural Science, College of Agriculture, University of Zabol, Zabol, Iran*
[d] *Department of Soil Science, Ferdowsi University of Mashhad, Iran*

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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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* Corresponding author.
E-mail address: tehranifar@um.ac.ir (A. Tehranifar).

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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 chloride buffer until the A_{510} was within the linear range of the spectrophotometer (Cecil Bio Quest, CE 2502). The same dilution factor was later used to dilute the sample with the Na acetate buffer. Readings were performed at 510 and 700 nm in the two buffers after 15 min of incubation, four times per sample, and total anthocyanin contents was calculated as $[(A \times MW \times DF \times 100)/MA]$, where $A = (A_{510}–A_{700})\ pH_{1.0} - (A_{510}–A_{700})\ pH_{4.5}$, where MW is the molecular weight (449.2), DF is the dilution factor, and MA the molar absorptive coefficient of cyanidin-3-glucoside (26900). Results were expressed as mg cyanidin-3-glucoside 100 g^{-1} of juice. Four replications per treatment and year were carried out.

Total soluble sugar contents in juice were determined using the anthrone reagent method (Dubois et al., 1951). A certain volume of juice was diluted with distilled water, and then 0.1 mL of the diluted juice was added to four mL of anthrone (150 mg pure anthrone in 100 mL of H_2SO_4 72%). The sample was heated for 10 min in a boiling water bath, and after cooling at room temperature A_{625} was determined using a spectrophotometer. The total sugar contents was calculated by using a glucose standard curve. Four replications per treatment and year were carried out.

2.5. Statistical analysis

The experimental design used was a randomized complete block design with four replications. Data were statistically evaluated by analysis of variance (ANOVA). Data were analyzed using SAS (Statistical Analysis System; SAS Institute Inc., Cary, NC, U.S.A.) base 9 software. Means were compared using Duncan's multiple range test at the $p < 0.05$ level.

3. Results

3.1. Changes in leaf mineral concentrations with Ca fertilization

Results obtained showed that the mean leaf concentrations of N, P, K and Ca in August in both seasons of the control trees were approximately 1.79, 0.11, 0.90, and 2.40%, whereas the mean Fe, Mn and Zn leaf concentrations were 113.16, 74.35, and 14.07 mg kg^{-1} DW, respectively (Table 1). Calcium fertilization increased the Ca leaf concentration in comparison with the control trees only in the first season (by 13–21%, depending on the treatment; Table 1), and the highest Ca leaf concentrations were obtained in both seasons with $CaCl_2$ 1%. On the other hand, the concentrations of N, P, K, Fe, Zn and Mn were never affected by any of the foliar Ca treatments. Application of the highest concentration of $CaCl_2$ (2%) led to leaf burn in both seasons.

3.2. Changes in yield, number of fruits per tree and fruit physical properties with Ca fertilization

Foliar Ca application had no significant effects on fruit yield and number of fruits per tree in both seasons (Table 2). Results obtained also showed that fertilization with $CaCl_2$ 1% increased fruit length only in the first season, whereas other fruit physical properties such as average fruit weight, fruit diameter, skin thickness, arils and skin percentages, aril/skin ratio, weight of 100 arils and fruit juice were unaffected by Ca fertilization in both seasons studied (Tables 2, 3 and 4Tables 2, 3 and 4). However, fruit lengths with the $CaCl_2$ 1 and 2% treatments were not significantly different in both seasons (Table 2).

3.3. Changes in fruit cracking percentage with Ca fertilization

Fruit cracking in the untreated trees was observed in 6.1–7.3% of the fruits, a value relatively low but still leading to a significant loss for the grower. The foliar treatments nano-Ca2 and 1% $CaCl_2$ (in both seasons) and 2% $CaCl_2$ (only in the second season) decreased significantly pomegranate fruit cracking in comparison with the control treatment (Table 3). The lowest rates of fruit cracking were observed in the trees sprayed with nano-Ca2, followed by the $CaCl_2$ 1% treatment, whereas the highest rates were observed in the nano-Ca1 and unsprayed trees in the first and second season, respectively (Table 3).

3.4. Changes in TSS, TA, maturity index and total sugars with Ca fertilization

The only treatment increasing significantly TSS was $CaCl_2$ 1% in the second season, with Ca fertilization having no significant effect in the first one. Results also show that foliar Ca sprays did not have significant effects on TA, maturity index and total sugars (Table 4 and Table 5). In the second season, the highest TSS (18.02%) was observed in the $CaCl_2$ 1% treatment, while the lowest one (16.75%) was observed in the control (Table 4).

3.5. Changes in total phenolic compounds, antioxidant activity and total anthocyanins with Ca fertilization

The only foliar treatment that decreased significantly the total phenolic compounds in pomegranate fruit juice when compared with the control treatment was the highest dose of nano-Ca fertilizer (only in the first season and by 1%), although the difference between the nano-Ca2 and $CaCl_2$ 1% treatments was not statistically significant (Table 5). The highest value for total phenolic compounds (409.13 mg 100 g^{-1} FW) was observed in the control treatment, whereas, the lowest one (404.52 mg 100 g^{-1} FW) was obtained in the treatment nano-Ca2 (Table 5). On the other hand, Ca fertilization had no significant effects on antioxidant activity and total anthocyanin contents (Table 5).

4. Discussion

Results showed that foliar Ca application led to an increase in the Ca leaf concentration when compared with the control trees. Increases in leaf Ca concentrations after foliar Ca applications have been previously reported in kiwi (Koutinas et al., 2010), blueberry (Ochmian, 2012) and apple trees (Danner et al., 2015). Regarding the effect of Ca application on the concentrations of other macro and micro elements in previous

Table 1
Effects of foliar applications of nano-Ca and calcium chloride fertilizers on pomegranate leaf mineral concentration in 2014 and 2015.

Treatment	N (%)		P (%)		K (%)		Ca (%)		Fe (mg/kg)		Mn (mg/kg)		Zn (mg/kg)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Control	1.83^{a}	1.75^{a}	0.10^{a}	0.11^{a}	0.94^{a}	0.85^{a}	2.14^{c}	2.66^{a}	108.33^{a}	117.99^{a}	75.97^{a}	72.72^{a}	15.67^{a}	12.47^{a}
Nano-Ca1	1.84^{a}	1.69^{a}	0.11^{a}	0.11^{a}	0.96^{a}	0.88^{a}	2.23^{bc}	2.59^{a}	101.92^{a}	116.01^{a}	75.9^{a}	71.77^{a}	14.84^{a}	12.57^{a}
Nano-Ca2	1.87^{a}	1.85^{a}	0.11^{a}	0.11^{a}	1.00^{a}	0.89^{a}	2.41^{ab}	2.48^{a}	107.40^{a}	106.87^{a}	73.04^{a}	69.79^{a}	14.86^{a}	13.37^{a}
$CaCl_2$ 1%	1.94^{a}	1.90^{a}	0.11^{a}	0.12^{a}	0.96^{a}	0.97^{a}	2.58^{a}	2.71^{a}	113.00^{a}	104.37^{a}	73.02^{a}	68.86^{a}	15.18^{a}	13.68^{a}
$CaCl_2$ 2%	1.86^{a}	1.79^{a}	0.10^{a}	0.11^{a}	0.92^{a}	0.93^{a}	2.48^{a}	2.47^{a}	112.13^{a}	108.46^{a}	77.61^{a}	70.68^{a}	14.96^{a}	12.38^{a}

Nano-Ca was used at rates of 0.25 (nano-Ca1) and 0.50 (nano-Ca2) g Ca L^{-1}, and $CaCl_2$ was used at rates of 2.73 ($CaCl_2$ 1%) and 5.45 ($CaCl_2$ 2%) g Ca L^{-1}, respectively. Means with the same letter in each column were not significantly different at $p < 0.05$. $n = 3$ in each of the seasons.

Table 2
Effects of foliar applications of nano-Ca and calcium chloride fertilizers on pomegranate fruit yield, number of fruits per tree, fruit weight, fruit length and fruit diameter in 2014 and 2015.

Treatment	Yield (kg tree $^{-1}$)		Number of fruits (tree^{-1})		Fruit weight (g)		Fruit length (mm)		Fruit diameter (mm)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Control	12.38^{a}	16.24^{a}	45.50^{a}	55.30^{a}	272.50 ^{a}	293.36^{a}	76.14^{b}	79.49^{a}	74.71^{a}	77.51^{a}
Nano-Ca1	12.43^{a}	16.82^{a}	50.00^{a}	53.50^{a}	249.34 ^{a}	314.31^{a}	74.44^{b}	81.05^{a}	74.25^{a}	80.78^{a}
Nano-Ca2	13.17^{a}	17.07^{a}	51.00^{a}	54.30^{a}	258.63 ^{a}	314.89^{a}	73.30^{b}	83.39^{a}	76.26^{a}	80.81^{a}
$CaCl_2$ 1%	13.47^{a}	16.54^{a}	48.00^{a}	53.80^{a}	280.15 ^{a}	309.50^{a}	83.13^{a}	84.44^{a}	77.13^{a}	80.27^{a}
$CaCl_2$ 2%	12.80^{a}	15.61^{a}	46.00^{a}	51.50^{a}	280.36^{a}	303.56^{a}	80.56^{ab}	83.51^{a}	78.94^{a}	82.80^{a}

Nano-Ca was used at rates of 0.25 (nano-Ca1) and 0.50 (nano-Ca2) g Ca L^{-1}, and $CaCl_2$ was used at rates of 2.73 ($CaCl_2$ 1%) and 5.45 ($CaCl_2$ 2%) g Ca L^{-1}, respectively. Means with the same letter in each column were not significantly different at $p < 0.05$. n = 4 in each of the seasons.

Table 3
Effects of foliar applications of nano-Ca and calcium chloride fertilizers on pomegranate fruit calyx diameter, peel thickness, fruit cracking, total aril, total peel and aril/peel ratio in 2014 and 2015.

Treatment	Fruit calyx diameter (mm)		Peel thickness (mm)		Fruit cracking (%)		Total aril (%)		Total peel (%)		Aril/peel ratio	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Control	19.77^{a}	20.58^{a}	22.30^{a}	22.27^{a}	6.10^{a}	7.26^{a}	54.07^{a}	58.27^{a}	46.03^{a}	41.73^{a}	1.17^{a}	1.40^{a}
Nano-Ca1	20.73^{a}	21.46^{a}	23.12^{a}	23.26^{a}	6.01^{a}	7.00^{a}	54.95^{a}	56.95^{a}	45.05^{a}	43.05^{a}	1.22^{a}	1.33^{a}
Nano-Ca2	20.43^{a}	21.63^{a}	23.49^{a}	23.74^{a}	2.93^{c}	3.70^{c}	53.60^{a}	57.02^{a}	46.40^{a}	42.98^{a}	1.16^{a}	1.32^{a}
$CaCl_2$ 1%	21.61^{a}	21.08^{a}	23.31^{a}	23.10^{a}	4.17^{bc}	4.23^{bc}	52.82^{a}	55.33^{a}	47.18^{a}	44.67^{a}	1.12^{a}	1.24^{a}
$CaCl_2$ 2%	21.68^{a}	21.44^{a}	23.50^{a}	24.31^{a}	4.95^{ab}	5.38^{b}	53.03^{a}	57.89^{a}	46.97^{a}	42.11	1.14^{a}	1.37^{a}

Nano-Ca was used at rates of 0.25 (nano-Ca1) and 0.50 (nano-Ca2) g Ca L^{-1}, and $CaCl_2$ was used at rates of 2.73 ($CaCl_2$ 1%) and 5.45 ($CaCl_2$ 2%) g Ca L^{-1}, respectively. Means with the same letter in each column were not significantly different at $p < 0.05$. n = 4 in each of the seasons.

Table 4
Effects of foliar applications of nano-Ca and calcium chloride fertilizers on pomegranate weight of 100 arils, juice content, Juice pH, total soluble solids (TSS) and titratable acidity (TA) in 2014 and 2015.

Treatment	Weight of 100 arils (g)		Juice content (mL 100 g $^{-1}$ arils)		Juice pH		TSS (%)		TA (%)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Control	37.05^{a}	38.02^{a}	61.25^{a}	62.50^{a}	3.51^{a}	3.68^{a}	16.32^{a}	16.75^{b}	1.82^{a}	1.74^{a}
Nano-Ca1	38.80^{a}	39.94^{a}	62.20^{a}	65.17^{a}	3.70^{a}	3.80^{a}	16.70^{a}	16.80^{b}	1.77^{a}	1.72^{a}
Nano-Ca2	38.63^{a}	39.40^{a}	60.86^{a}	61.07^{a}	3.67^{a}	3.91^{a}	17.25^{a}	17.65^{ab}	1.62^{a}	1.76^{a}
$CaCl_2$ 1%	38.68^{a}	39.53^{a}	62.10^{a}	63.37^{a}	3.75^{a}	3.68^{a}	17.27^{a}	18.02^{a}	1.74^{a}	1.77^{a}
$CaCl_2$ 2%	37.14^{a}	39.60^{a}	61.80^{a}	61.52^{a}	3.62^{a}	3.91^{a}	16.95^{a}	17.70^{ab}	1.76^{a}	1.70^{a}

Nano-Ca was used at rates of 0.25 (nano-Ca1) and 0.50 (nano-Ca2) g Ca L^{-1}, and $CaCl_2$ was used at rates of 2.73 ($CaCl_2$ 1%) and 5.45 ($CaCl_2$ 2%) g Ca L^{-1}, respectively. Means with the same letter in each column were not significantly different at $p < 0.05$. n = 4 in each of the seasons.

studies, the leaf concentration of K was decreased in apple trees (Danner et al., 2015), whereas no significant effects on the leaf concentrations of N, P, K and Mg were found in grape (Bonomelli and Ruiz, 2010), and the leaf concentrations of N, P, Mg, Fe, Mn, and Zn were unaffected in kiwi (Koutinas et al., 2010). Also in apple trees, foliar application of different rates of Ca materials increased leaf Ca and Mg concentrations, whereas the concentrations of K and N decreased (Raese and Drake, 2000). The different changes in elemental concentrations found in all these studies likely reflect the widely different systems (crop species, formulations, etc.) used.

Foliar Ca fertilization had no significant effects on fruit yield and number of fruits per tree. This is in line with previous results in which foliar and soil applications of Ca had no significant effects on the yield of kiwifruit (Antunes et al., 2004), apple (Domagała-Świątkiewicz and

Table 5
Effects of foliar applications of nano-Ca and calcium chloride fertilizers on pomegranate maturity index, total sugars, total phenolic compounds, antioxidant activity and total anthocyanins in 2014 and 2015.

Treatment	Maturity index (TSS/TA ratio)		Total sugars (g 100 g $^{-1}$ juice)		Total phenolic compounds (mg GAE 100 g $^{-1}$ juice)		Antioxidant activity (%)		Total anthocyanins (mg C3G 100 g $^{-1}$ juice)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Control	8.95^{a}	9.65^{a}	14.32^{a}	14.18^{a}	409.13^{a}	405.05^{a}	24.23^{a}	24.02^{a}	7.76^{a}	7.56^{a}
Nano-Ca1	9.40^{a}	9.80^{a}	14.34^{a}	14.28^{a}	407.77^{a}	404.26^{a}	24.11^{a}	24.35^{a}	7.94^{a}	7.54^{a}
Nano-Ca2	10.66^{a}	10.06^{a}	14.73^{a}	14.15^{a}	404.52^{b}	403.27^{a}	23.73^{a}	23.58^{a}	8.20^{a}	8.83^{a}
$CaCl_2$ 1%	9.99^{a}	10.18^{a}	14.44^{a}	14.80^{a}	406.62^{ab}	404.21^{a}	24.33^{a}	23.44^{a}	8.46^{a}	8.06^{a}
$CaCl_2$ 2%	9.65^{a}	10.54^{a}	14.33^{a}	14.34^{a}	408.02^{a}	404.20^{a}	23.81^{a}	23.15^{a}	7.54^{a}	7.70^{a}

Nano-Ca was used at rates of 0.25 (nano-Ca1) and 0.50 (nano-Ca2) g Ca L^{-1}, and $CaCl_2$ was used at rates of 2.73 ($CaCl_2$ 1%) and 5.45 ($CaCl_2$ 2%) g Ca L^{-1}, respectively. Means with the same letter in each column were not significantly different at $p < 0.05$. n = 4 in each of the seasons.

Błaszczyk, 2007), strawberry (Lanauskas et al., 2006), grape (Bonomelli and Ruiz, 2010), and cherry (Erogul, 2014). In contrast, increases in yield and number of fruits per tree with Ca fertilization have been reported in palm (El-Baz and El-Dengawy, 2003), peach (El-Shazly et al., 2013) and pomegranate (Sheikh and Manjula, 2012; Bakeer, 2016). In addition, Ca spraying in palm fruit increased the force required to removing fruits and led to decreases in fruit drop (El-Baz and El-Dengawy, 2003). It has been also reported that Ca increased tomato yield through increasing mineral contents, flower cluster, fruit set percentage and decreasing fruit physiological disorders (Abbasi et al., 2013). The contradictory results mentioned in the cases of effect of Ca fertilization on fruit yield and number of fruits per tree can be related to difference in plant species and cultivars, time and concentration of Ca sprayed, form of Ca and trees nutritional status.

Foliar fertilization with $CaCl_2$ 1% resulted in increases in fruit length only in the first season, whereas other fruit physical properties were unaffected by Ca fertilization. In previous studies, sprays with different Ca sources such as $CaCl_2$ with polyethylene wrapping increased fruit size in peach (El-Alakmy, 2012), whereas in pomegranate, application of $CaCl_2$ led to increases in fruit size only in combination with urea (Ramezanian et al., 2009). Improved fruit length as a resulted of Ca spraying can be due to the roles of Ca in cell division, elongation, permeability and functionality of cell membranes (Carpita and McCann, 2000). With regard to average fruit weight, no significant effects on average fruit were found in kiwifruit (Koutinas et al., 2010) and cherry (Erogul, 2014). In pomegranate, contrasting results on fruit weight have been reported; positive effects of foliar sprays with $CaCl_2$ was found in three studies (Ramezanian et al., 2009; Rouhi et al., 2015; Bakeer, 2016), whereas no effects on this parameter was found in a fourth one (Hasani, 2011). In this respect, the effectiveness of Ca foliar application could be influenced by environmental conditions. For instance, it has been reported that foliar sprays of Ca in areas with high temperatures and low air relative humidity are not very effective and/or may cause inconsistent results (Val et al., 2008).

Foliar Ca application led to decreases in pomegranate fruit cracking. In previous studies, Ca foliar fertilization has been shown to decrease fruit cracking. For instance, in cherry, foliar application of different Ca compounds, including caseinate, $CaCl_2$, $Ca(OH)_2$ and $Ca(NO_3)_2$ caused 38–66% decreases in fruit cracking in comparison with the control (Erogul, 2014). In pomegranate, the rate of cracking in trees sprayed with 0.5 and 1% $CaCl_2$ were 5.5 and 5.2%, respectively, down from the 28.4% rate found in the unsprayed trees (Sheikh and Manjula, 2012). In another study, foliar sprays with $CaCl_2$ 2% 'Manfalouty' pomegranate trees decreased fruit cracking by approximately 10% in comparison with the control trees (Bakeer, 2016).

Calcium may have an effect on fruit cracking due to its important roles in the cell wall, influencing the mechanical properties of plant tissues (Shear, 1975; Huang et al., 2005). Calcium and boron (B) are two elements that when present in deficient amounts can result in fruit cracking (Mir et al., 2012). Inverse correlations between fruit Ca concentration and fruit cracking have been reported in previous studies. In litchi, trees with high Ca concentrations had less cracking, whereas the concentration of Ca was lower in cracked fruits than in healthy ones (Li et al., 2001). Also in litchi, the concentration of Ca in the fruit pericarp was higher in tolerant cultivars than in sensitive ones (Huang et al., 2005). The effect of Ca application in decreasing pomegranate fruit cracking has been attributed to the role of Ca in the cohesion of cell walls, since it interacts with pectic acid (Bakeer, 2016).

Results show that TSS increased after Ca foliar fertilization with $CaCl_2$ 1% only in the second season, whereas foliar Ca treatment had no significant effects on TA, maturity index and total sugars. In previous studies, it has been reported that the application of Ca had no significant effects on TSS and TA in strawberry (Lanauskas et al., 2006), kiwifruit (Koutinas et al., 2010), cherry (Erogul, 2014) and apple (Danner et al., 2015). In pomegranate, it has been reported that TSS and TA were not affected by foliar applications of $Ca(NO_3)_2$ (Korkmaz and Aşkın, 2015), whereas foliar sprays of $CaCl_2$ increased soluble solid contents (Ramezanian et al., 2009). In other crops, Ca application led to increases in TSS in cherries and palm (El-Baz and El-Dengawy, 2003; Vangdal et al., 2008) and also increased TA in apple fruit juice (Mosa et al., 2015). Inconsistent results in different studies might be related to the different plant species and cultivars, plant nutritional status, applied concentrations and timing spray. For instance, concerning the time of fertilization, it has been reported in pomegranate that foliar applications of $CaCl_2$ at full bloom decreased TA in juice, whereas one month after full bloom they had no significant effects (Ramezanian et al., 2009). With regard to total sugars, it has been reported that foliar Ca fertilization had no significant effects on total, reducing and non-reducing sugars in apple (Mosa et al., 2015) and in total sugars in strawberry (Lanauskas et al., 2006), whereas it increased total sugars in palm (El-Baz and El-Dengawy, 2003). Among the possible reasons for the contradictory results reported after foliar Ca application, atmospheric-dependent effects on Ca absorption, uneven distribution of Ca in fruits within the canopy and the condition and management of orchard have been mentioned (Yamane, 2014).

Foliar Ca application with the highest dose of nano-Ca fertilizer in the first season led to decreases in total phenolic compounds, whereas antioxidant activity and total anthocyanin contents were unaffected after Ca fertilization. In soybean seedlings, applications of Ca led to 14 (at 1 or 2.5 mM Ca) and 27% (at 5 mM Ca) decreases in total phenolic compounds, with the decrease being attributed to a 30% decrease in the activity of phenylalanine ammonia-lyase (PAL), a key enzyme in the synthesis of phenolic compounds (Teixeira et al., 2006). Calcium has also an effect on the enzymes peroxidase and polyphenol oxidase, which are involved in the oxidation of phenolic compounds (Teixeira et al., 2006). Calcium spraying decreased the total phenolic compounds and PAL activity in sweet cherry (Thurzo et al., 2008), and foliar application of $CaCl_2$ decreased the amount of total phenolic compounds in lettuce (Perucka and Olszówka, 2011). On the other hand, foliar application of 10 μM $CaCl_2$ increased PAL activation in lemon seedlings (Castañeda and Pérez, 1996).

5. Conclusion

Results obtained from the present study show that Ca fertilization as foliar sprays at full bloom and one month later decreased significantly fruit cracking in an orchard moderately affected by this disorder (6–7% of the fruits). Results show that Ca fertilization lead to 26–52% decreases in fruit cracking in two different seasons in comparison with the control, with the best effect being found with the nano-Ca fertilizer at 0.50 g Ca L^{-1}. Foliar Ca fertilization did not have any effects on pomegranate fruit yield, number of fruits per tree, average fruit weight, TA, fruit maturity, total sugars, antioxidant activity and total anthocyanins. Fruit length increased by 9% with the $CaCl_2$ 1% treatment in the first season, and minor effects on total phenolics (by 1% with the nano-Ca2 treatment in the first season) and TSS (by 8% with the $CaCl_2$ 1% in the second season) were also observed. Results obtained indicate that foliar applications of Ca fertilizers led to decreases in fruit cracking, which is still a relevant problem in well-managed pomegranate orchards in Iran. In particular, results indicate the potential of low doses of Ca in new formulations for reducing fruit cracking in pomegranate. Additional studies would be necessary to further optimize the concentration and timing of the Ca applications with these new formulations, with the aim to reduce the incidence of this physiological disorder in crops such as pomegranate, where it limits fruit quality and yield every year.

Conflict of interest

None.

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