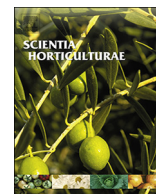




Contents lists available at ScienceDirect

Scientia Horticulturae

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

Foliar nutrient applications to 'Wonderful' pomegranate (*Punica granatum* L.). I. Effects on fruit mineral nutrient concentrations and internal quality

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ARTICLE INFO

Keywords:

Antioxidant
DPPH
Foliar nutrition
Fruit nutrient concentration
Juice
Total soluble solids

ABSTRACT

Investigations into putative health benefits of pomegranate fruit have increased interest in its consumption and production but limited evidence exists regarding fruit mineral nutrient concentrations and other internal fruit quality characteristics, especially for Wonderful, an industry standard cultivar, and even less is known about the effects of agricultural practices such as foliar fertilizer applications on these variables. Mature 'Wonderful' pomegranate trees were treated during fruit set with zinc sulfate (ZnSO_4), magnesium sulfate (MgSO_4), potassium nitrate (KNO_3), and with deionized water. Mature fruit were analyzed for mineral nutrient concentrations, antioxidant activity (AA), total phenolics (TP), total soluble solids (TSS), and titratable acidity (TA). Fruit mineral nutrient concentrations were characterized. Zinc-treated trees had greater fruit Zn concentrations. No other significant differences in fruit mineral nutrient concentrations were detected. Antioxidant activity ranged from 67.1 to 87.1 percent inhibition of 2,2'-diphenyl-1-picrylhydrazyl with no treatments resulting in AA that was significantly different from the control. Total phenolics ranged from 2091 to 3735 $\text{mg}\cdot\text{L}^{-1}$ gallic acid equivalents, with 2% KNO_3 resulting in a statistically significant increase in TP at one site. Fruit TSS and TA were not affected significantly by the treatments. The results suggest that foliar ZnSO_4 , MgSO_4 , and KNO_3 could be used as part of an efficient, sustainable fertilizer program for 'Wonderful' pomegranate while maintaining or improving fruit mineral nutrient concentrations, bioactivity, and internal quality.

1. Introduction

Pomegranate (*Punica granatum*) is a subtropical fruit tree crop cultivated in numerous subtemperate, temperate, tropical, and subtropical regions throughout the world (Verma et al., 2010). The primary uses for pomegranate are as fresh market fruit and valued-added products, including fresh and concentrated fruit juice (Melgarejo et al., 2012) and current investigations regarding the putative health benefits of pomegranate, including anti-inflammatory, anticancer, and antioxidant properties (reviewed in Lansky and Newman, 2007), have led to increased worldwide demand for pomegranate fruit and juice. Knowing the mineral nutrient content and chemical composition of commonly consumed foods is important to governmental regulatory agencies, consumers, the food and beverage industries, breeders, and growers.

There is limited peer-reviewed literature regarding the mineral nutrient concentrations of mature pomegranate fruit. In a study of 'Malas Yazdi' pomegranates in Iran, Mirdehghan and Rahemi (2007) reported that nutrient concentrations varied with the part of the fruit tested and the stage of development of the fruit. Concentrations of calcium (Ca) and sodium (Na) in the peel were greater than that in the

arils, but concentrations of nitrogen (N), phosphorus (P), potassium (K), and magnesium (Mg) were greater in the arils than in the peel. Fawole and Opara (2013) reported that for 'Ruby' pomegranates, N and K were in the greatest concentration in the arils. In a study of six Turkish pomegranate cultivars, Hepaksoy et al. (2000) reported that aril pulp N and K concentrations and peel Ca concentrations were cultivar-dependent, but no other fruit macronutrient concentrations in the aril pulp or peel were reported to be cultivar-dependent. Cultivar-specific studies of pomegranate fruit mineral nutrient concentrations are needed in order to more fully characterize the potential nutritional value of pomegranate fruit.

Though numerous studies have described the chemical characteristics of fruit of various pomegranate cultivars (Akbarpour et al., 2009; Tehranifar and Tabar, 2009; El-Rhman, 2010; Tehranifar et al., 2010; Mena et al., 2011; Khayyat et al., 2012; Fawole and Opara, 2013; Davarpanah et al., 2016; Marathe et al., 2017), few have been conducted on Wonderful (Kader et al., 1984; Gil et al., 2000; Mena et al., 2011; Beaulieu et al., 2015), the industry standard cultivar in the United States and Israel (Holland and Bar-Ya'akov, 2008). Research suggests that pomegranate juice has greater levels of antioxidant

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activity (AA) than many other beverages known to be high in AA, including red wine and green tea (Gil et al., 2000). The antioxidants identified in pomegranates include ascorbic acid and three known groups of polyphenolic compounds: anthocyanins (Tehraniifar et al., 2010), ellagic acid and its derivatives, and hydrolyzable tannins (Gil et al., 2000). Ellagitannins make up the majority of the hydrolyzable tannins found in ‘Wonderful’ pomegranate fruit and give the fruit 90% of its antioxidant capacity (Gil et al., 2000). Previous research indicates that hydrolyzable tannins are 15–30 times more effective than simple phenols at quenching peroxy, a free-radical oxidizing molecule (Hagerman et al., 1998) that is the most commonplace free radical in humans (Wang, 2006). The combined AA of juice can be measured by its ability to quench (or inhibit) an oxidizing chemical compound such as 2,2'-diphenyl-1-picrylhydrazyl (DPPH) and the polyphenolic content of pomegranate fruit can be collectively quantified as total phenolics (TP) (Gil et al., 2000). Previous studies have demonstrated that AA and TP of pomegranate fruit are cultivar-dependent (Akbarpour et al., 2009; Tehraniifar et al., 2010). Fawole and Opara (2013) found that TP of ‘Ruby’ pomegranate increased during the final stages of fruit development, thus suggesting that harvest date can also affect pomegranate fruit AA and TP. Little else is known about factors affecting AA and TP of mature pomegranate fruit.

With increased interest in the potential benefits of pomegranates to human health, demand for and production of pomegranate are likely to increase and growers will need scientifically-based recommendations for agricultural practices such as foliar fertilizer applications, which are not currently used widely in the commercial pomegranate industry, in order to more efficiently and sustainably improve plant nutrient status and increase yield. However, limited peer-reviewed evidence exists regarding the effects of such agricultural practices on mineral nutrient concentrations, AA, and TP of mature pomegranate fruit. Additionally, pomegranate fruit are harvested and evaluated based upon the maturity indices of total soluble solids (TSS) (Fawole and Opara, 2013) and titratable acidity (TA) (Kader et al., 1984). Minimum maturity guidelines established in California recommend that total TA be less than 1.85% w-vol⁻¹ citric acid (Kader, 2006). In other countries, maturity indices are based on the sugar to acid ratio of the juice (Fawole and Opara, 2013). Before recommending any new agricultural practice to growers, cultivar-specific effects of such practices on TSS and TA will be critical to ensure that fruit quality is not negatively impacted, especially with respect to proper determination of harvest maturity. Increases in pomegranate fruit TSS have been reported in response to foliar fertilizers, including zinc sulfate (ZnSO₄) (Hasani et al., 2012), chelated nano-Zn applied with chelated nano-boron (B) (Davarpanah et al., 2016), manganese sulfate (MnSO₄) (Hasani et al., 2012), potassium silicate (K₂O₃Si) (Wassel et al., 2015), and K applied as soluble potash (Tehraniifar and Tabar, 2009). Foliar applications of K as soluble potash also result in increased pomegranate juice TA (Tehraniifar and Tabar, 2009), whereas chelated nano-Zn applied foliarly with or without chelated nano-B chelate resulted in decreased TA (Davarpanah et al., 2016). Khayyat et al. (2012) reported that foliar applications of potassium nitrate (KNO₃) resulted in increased concentrations of ascorbic acid in pomegranate fruit. Of these studies, only Wassel et al. (2015) used ‘Wonderful’ pomegranate. Little else is known about the effects of foliar fertilizer applications on the pomegranate fruit characteristics that contribute to its nutritional value, putative health benefits, and internal quality. Therefore, the objectives of this study were to quantify mineral nutrient concentrations, AA, TP, TSS, and TA of ‘Wonderful’ pomegranate fruit, and to determine the effects of three foliar fertilizers, ZnSO₄, magnesium sulfate (MgSO₄), and KNO₃, on these key internal fruit quality variables.

2. Materials and methods

2.1. Plant material and experimental design

This study was conducted using 9-year-old bearing ‘Wonderful’ pomegranate trees at 2 commercial orchards in Kern County, CA, USA (site 1: latitude: 35°04'09.20"N, longitude: 119°18'47.74"W; site 2: latitude: 35°40'33.55"N, longitude: 119°55'16.72"W). Both orchards had deep, well-drained loam soils (Cerini loam and Kimberlina fine sandy loam at site 1 and site 2, respectively). The experiment was conducted using whole-tree experimental units and a randomized complete block design with 7 blocks at each site for AA, TP and TA analyses, 6 blocks at each site for TSS analyses, and 5 blocks at site 2 for fruit mineral nutrient concentration analyses. Data trees were selected at each site for uniform health, size, and vigor.

Treatments consisted of foliar applications of ZnSO₄ (0.3%, 0.4%, and 0.5%), MgSO₄ (1%, 2%, and 3%), KNO₃ (1%, 2%, and 3%), or deionized water (control), for a total of 140 data trees for AA, TP and TA analyses, 120 data trees for TSS analyses, and 50 data trees for fruit mineral nutrient concentration analyses. All solutions were formulated in deionized water with 0.50% adjuvant. Treatments were applied to runoff with a professional backpack sprayer (SP1, SP Systems International, Incorporated, Santa Monica, CA, USA) at early fruit set (July, when fruit were green to breaker with an equatorial diameter of approximately 50 mm) and late fruit set (August, when fruit were red with an equatorial diameter of approximately 70 mm), except for ZnSO₄ treatments, which were applied only at early fruit set.

2.2. Fruit collection and aril and juice extraction

Data trees at sites 1 and 2 were harvested on 22 October and 4 November, respectively. To determine fruit mineral nutrient concentrations, 10 mature unsplit fruit were collected at harvest from each data tree at site 2. An additional random sample of up to 5 unsplit fruit per data tree was collected at harvest from both sites for AA, TP, and TA analyses and stored at 5–8 °C for up to 19 d until arils could be extracted. Arils were pooled per data tree and stored for approximately 1–1.25 yr at –80 °C until conducting AA, TP, and TA analyses. Just prior to these analyses, arils were defrosted in a 0–2 °C cold water ice bath in a controlled atmosphere refrigeration unit maintained at 4.4 °C with 99% humidity. Arils thawed to 0.8–4.5 °C were pressed using a heavy-duty hand operated juice extractor (Strite-Anderson Manufacturing Company, Minneapolis, MN, USA). The juice was filtered through a 2.7 µm silica mesh syringe filter (Whatman PLC; Pittsburg, PA, USA). Immediately after filtration, each aliquot was analyzed at room temperature for AA, TP and TA. A separate random sample of up to 5 unsplit fruit per data tree was collected at harvest from both sites for TSS determination and stored at 5–8 °C for up to 19 d until arils could be manually extracted and pressed immediately using a heavy-duty hand operated juice extractor to quantify TSS of unfiltered juice.

2.3. Determination of mineral nutrient concentrations

Fruit were sent to Fruit Growers Lab, Inc. (Santa Paula, CA) for whole-fruit analysis of nutrients, including N, P, K, Ca, Mg, Na, B, copper (Cu), iron (Fe), Mn, and Zn using the Leggingwell Nutrient Analysis System (NAS) and software, procedure S-2001. Total N concentration was determined based on AOAC Combustion Method 993.13 using a Leco Analyzer Nitrogen Determinator (PP428, Leco Corporation, St. Joseph, MI, USA). Fruit were washed, cut, blended for 2 min, and dried in a forced-air drying oven at 110 °C for 12 h. Samples were then placed in glass jars, transferred to a 50 °C vacuum oven for 1 h and then run in the analyzer. The dry ash method was used to determine the concentration of all other elements. Dried, ground samples (2–3 g) were placed in a muffle furnace and the temperature was

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