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Enhancement of Phytoestrogen Content of Pomegranate Seeds by Zinc Fertilization

FARHAD KHORSANDI[1] AND FATEMEH ALAEI YAZDI†
Department of Soil Science, Islamic Azad University–Darab Branch, Darab, Fars Province, Islamic Republic of Iran
†*Yazd Agriculture and Natural Resources Research Center, Yazd, Islamic Republic of Iran*
[1]Corresponding author's e-mail: fkhorsandi@gmail.com

ABSTRACT

Pomegranate (*Punica granatum*), a rich source of phytoestrogens, is grown extensively in arid regions of the world, where zinc (Zn) deficiency is common. In this study, the response of four commercial pomegranate cultivars to foliar Zn fertilization at a rate of 0.4% was evaluated in a two-year field experiment. Seed phytoestrogen contents were significantly different among cultivars. Zn application increased phytoestrogen content of the seeds in all four cultivars, which was significant in three of them. There was a significant and positive correlation between seed phytoestrogen content and leaf Zn content. The correlation between phytoestrogen content and seed Zn content was highly significant and positive in one of the cultivars. It is concluded that Zn fertilization of pomegranates cultivated in calcareous soils of arid regions has enhanced phytoestrogen content in fruits.

Key Words: Isoflavonoids; Polyphenols; *Punica granatum*; Zinc sulfate

INTRODUCTION

The interest in phytoestrogens has increased dramatically due to the ineffectiveness and unsafety of hormone replacement therapy (Cornwell *et al.*, 2004) and emergence of several lines of evidence about their possible role in preventing a range of diseases, including hormonally dependent cancers (Van Elswijk *et al.*, 2004; Albrecht *et al.*, 2004) and cardiovascular diseases (Larkin *et al.*, 2000). Phytoestrogens are a group of naturally occurring phenolic compounds present in legumes, whole grains, fruits and vegetables (Rohrdanz *et al.*, 2002). Currently four different families of plant derived phenolic compounds are considered phytoestrogens: isoflavonoids, stilbenes, lignans and coumestans (Cornwell *et al.*, 2004). Isoflavonoids and coumestans have been identified as the most common estrogenic compounds in plants (Price & Fenwick, 1985). Leguminous plants, such as soybean, chickpeas and beans are the most abundant in isoflavonoid phytoestrogen (Mazur, 2000). Recently Onyilagha and Islam (2009) studied flavanoid profiles of five cultivated *Phaseolus* species and found them to be rich sources of this compound. Grains, fiber-rich fruits and vegetables and tea are rich in lignans and pomegranate (*Punica granatum*) is one of the rich sources of phytoestrogen compounds among horticultural and fruit crops (Mazur, 2000; Cornwell *et al.*, 2004).

Pomegranate is grown extensively in many arid regions of the world. The biological uniqueness of pomegranate is that it has no close botanical relatives and consequently is a potential source for several physiological factors, which could have significant effect on human health and diseases (Lansky *et al.*, 2000). Pomegranate tree has been used extensively as indigenous medicine in many cultures, at least as far back as 1550 B.C. (Wren, 1988). The oil from the seeds contains about 80% of a rare 18-carbon fatty acid, or punicic acid (Longtin, 2003). Also present in the oil are isoflavonic phytoestrogens (genistein & daidzein) and a phytoestrogenic coumestan called coumestrol (Moneam *et al.*, 1988). Pomegranate is one of the only plants in nature known to contain the sex steroid estrone and has the highest botanical concentration of the steroid estrone at 14 mg/kg dried seed (Heftmann *et al.*, 1966). Pomegranate juice and seed oil contain phytoestrogenic compounds that has been shown to exhibit antioxidant activity (Schubert *et al.*, 1999), exert anti-proliferative effects on human breast cancer cells (Kim *et al.*, 2002), significant anti-tumor activity against human prostate cancer (Albrecht *et al.*, 2004) and heart disease prevention (Aviram *et al.*, 2004). Also, pomegranate has been recommended as medicinal food for treatment of Acquired Immune Deficiency System (AIDS) patients (Lee & Watson, 1998).

Zinc (Zn) deficiency is common in calcareous soils of arid regions (Alloway, 2004), where pomegranate production is extensive. Zn is essential for the healthy growth of plants, animals and humans. It plays an essential role in several key plant physiological pathways that are concerned with photosynthesis and sugar formation, protein and hormone synthesis, growth regulation, seed production

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and disease resistance (Alloway, 2004). It may also have an important role in the synthesis of phytoestrogenic compounds in plants.

Several studies have shown positive antagonistic relationship between Zn and phytoestrogens. Isoflavonoid effects on bone loss prevention were enhanced by Zn in studies with elderly and young rats (Gao & Yamaguchi, 1998; Yamaguchi *et al.*, 2000; Ma *et al.*, 2000). In a glasshouse experiment, foliar application of Zn increased isoflavones levels in 47 days old clover (*Trifolium subterraneum*) seedlings (Rossiter, 1967). Venkatesan *et al.* (2005) reported positive and highly significant correlation between Zn and polyphenols contents of mature leaves of tea.

It is speculated that Zn fertilization of agronomic and horticultural crops may increase their phytoestrogen content. Both Zn and phytoestrogen play an important role in human health. Pomegranate, an important source of phytoestrogen, is grown extensively as cash crop in many Zn deficient, calcareous soils of arid regions. Therefore, the main objective of this study was to evaluate the effects of Zn fertilization on the phytoestrogen contents of four commercial cultivars of pomegranate.

MATERIALS AND METHODS

Field experiments were carried out in 2002 and 2003 on a sandy loam soil at the Yazd Agricultural and Natural Resources Research Center (YANRC), Yazd Province, Iran. The experiment was factorial with randomized complete blocks design and four replications. The pomegranate cultivars were Togh Gardan (TG), Shahvar Dane Ghermez (SDG), Malas Yazdi (MY) and Zagh Yazdi (ZY). Full description of the experiment including the description of the experimental field, fertilizer applications and soil analysis of the experiment site is given as (Khorsandi *et al.*, 2009).

The fruits were harvested on 7^{th}-10^{th} of September each year. Before harvest, eight fruits from the four sides of each tree were randomly picked and were used for determination of seed Zn and phytoestrogen contents in the laboratory. To determine seed dry weights, 100 g of fresh seed was oven dried at 80^{o}C for 72 h. Afterwards, the percent dry weight was calculated. Zn content of the leaves, fruit juice and seeds were determined by dry ash method as described by Saini *et al.* (2001), using Atomic Absorption Spectrometer (5100ZL, Perkin Elmer, USA). The total content of the major phytoestrogens (daidzin, daidzein, genistin, genistein & coumestrol) in pomegranate seeds were determined by the method described by Moneam *et al.* (1988). The seeds from all four cultivars and both Zn treatments were dried and grinded. Then 10 g of grinded seeds were placed in HPLC-grade methanol and filtered. The total phytoestrogens were determined by reversed-phase HPLC apparatus (FP-920, Jasco, USA), using a non-linear methanol-deionized water gradient on a C-18 column (250 mm x 4.6 mm), at a flow rate of 0.3 mL/min for a total of 17 min per sample. The detection was analyzed by spectrophotometer at 254 nm. The data were evaluated by Analysis of Variance procedures. Duncan Multiple Range (DMR) Test at 0.05 level of probability was used for separation of mean differences.

RESULTS AND DISCUSSION

The effect of year on phytoestrogen content of seed was not significant, but it was highly significant on seed Zn content (Table I). This indicates the importance of environmental condition on this component. Although Zn fertilization did not have significant effect on the total fruit yield (Khorsandi *et al.*, 2009), its effect on seed dry weight was highly significant (Table I) and increasing it by 27-42% in all cultivars (Fig. 1). There was no significant difference in Zn content of the seeds among cultivars with or without Zn fertilization (Table I). In addition, Zn fertilization did not have any significant effect on the Zn content of the seeds (Table I).

Phytoestrogen content of pomegranate seeds was significantly different among cultivars (Table II). In control treatment, TG cultivar significantly had the lowest seed content of phytoestrogen, while it was not significantly different among other three cultivars (Table II). Zn fertilization effect on seed phytoestrogen was highly significant (Table I). Seed phytoestrogen increased in all cultivars between 3.6-14.8%, but it was significant only in TG and MY cultivars (Table II). TG cultivar also had the highest increase in seed phytoestrogen content due to Zn fertilization (Table II).

Phytoestrogen content of seeds had a significant and positive correlation with leaf Zn content at 0.05 probability level, in the pooled two years data (Table III). Within cultivars seed phytoestrogen content had positive and highly significant correlation with Zn contents of seed and leaf only in cv. TG (Table III). As was mentioned before, TG and MY cultivars had the highest increase in seed Zn content due to Zn fertilization (18 & 20.3%, respectively) (Fig. 1). The highest increase in seed phytoestrogen content was also observed in these two cultivars (14.8 & 8.1%, respectively) (Table II). Zn is not a part of phytoestrogen structures, however it is reported that the DNA-binding domain of oestrogen receptors and other steroid hormone receptors contain two Zn fingers (WGPH, 2003). Zn was used as an effective catalytic agent for synthesis of genistein (Whalley *et al.*, 2000). Therefore it seems that Zn acts as a catalytic substance for biosynthesis of phytoestrogen in pomegranate seed.

CONCLUSION

In this two years field study, foliar Zn fertilization significantly increased seed dry weights and phytoestrogen contentsin the pomegranate cultivars. Cultivar MY showed the highest phytoestrogen content either with Zn fertilized or

Table I. The *F* values for seed characteristics of pomegranate seed for the two years pooled data

Treatment	Seed Characteristics		
	Dry Weight	Phytoestrogen	Zn
	%	mg kg^{-1}	
Year (Y)	NS	NS	22.05**
Cultivar (C)	NS	15.35**	NS
Y x C	6.08**	NS	3.30*
Fertilizer (Zn)	31.26**	19.38**	NS
Zn x C	NS	NS	NS
Zn x Y	NS	NS	NS
Zn x C x Y	NS	NS	NS

*, ** significant at 5 and 1 percent probability levels, respectively
NS not significant at 5% probability level

Table II. Effect of Zn fertilization on phytoestrogen content of pomegranate seeds (two years pooled data)

Cultivar	Seed phytoestrogen content (mg kg^{-1})§	
	Control	0.4% $ZnSO_4$
MY	29.35 bc	31.72 a
ZY	29.32 bc	30.42 ab
SDG	28.48 bc	29.62 bc
TG	24.56 d	28.19 c
CV = 6.44		

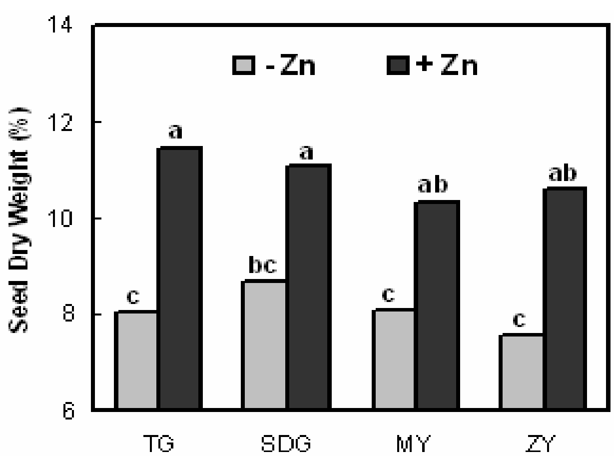
§ Means with the same letters are not significantly different ($P<0.05$) according to DMR Test.

Table III. Correlation coefficient of seed phytoestrogen content with Zn contents of leaf, juice and seed

Data Set	Leaf Zn	Juice Zinc	Seed Zn
Pooled	0.248*	0.173	0.011
TG cv.	0.790**	0.367	0.624**

*, ** significant at 5 and 1 percent probability levels, respectively.

Fig. I. Seed dry weights of pomegranate cultivars as affected by Zn treatment (0 & 0.4%) for the pooled data of two study years, Bars with the same letters are not significantly different (P<0.05) according to DMR Test



not. Cultivar TG showed a better response to Zn fertilization, which produced a greatest increase in phytoestrogen content of the seeds. The significant increase in these two components greatly increased the extractable phytoestrogen from the seed and also enhanced the value of pomegranate as a rich source of phytoestrogen. It is likely that Zn acted as catalytic agent in the biosynthesis of phytoestrogen in seeds. Zn fertilization of pomegranates in calcareous soils of arid regions is strongly recommended for correction of Zn deficiency and enhancement of fruit quality in terms of phytoestrogen content.

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