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Iron biofortification and its impact on antioxidant system, yield and biomass in common bean

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ABSTRACT

The effect of application of two iron (Fe) compounds ($FeSO_4$ and Fe-EDDHA) on the activity of antioxidant enzymes, production of H_2O_2, Fe accumulation in green bean seeds and crop yield of bean plants (*Phaseolus vulgaris* L.), under greenhouse conditions was studied. This experiment was conducted in Delicias, Chihuahua, Mexico. The results indicate that the accumulation of Fe in bean seeds enhanced with the application of Fe-EDDHA, at the dose of 25 µmol. This demonstrated that low Fe application dose was enough to increase Fe levels in seeds of common bean. In addition, Fe-EDDHA application form at 50 µmol was the best treatment to improve crop yield. With respect to antioxidant system, chelated form of Fe (Fe-EDDHA) was more effective in the activation of antioxidant enzymes (catalase, superoxide dismutase and glutathione peroxidase), and a lower content of H_2O_2 in green bean seeds.

Keywords: toxic metal; enzymatic activity; fruit; oxidative stress; hydrogen peroxide

Iron (Fe) is present in the active site of enzymes on the antioxidant pathway involved in the scavenging of reactive oxygen species (ROS) (Elstner and Osswald 1994). The generation of ROS such as superoxide anion radical (O_2^-), singlet oxygen (O_2), hydrogen peroxide (H_2O_2) and hydroxide radical (••OH) can damage many cellular components, including protein, membrane lipids and nucleic acids.

To face the increased levels of H_2O_2, plants have evolved different enzymatic and non-enzymatic mechanisms (Alscher et al. 1997). These include free radical scavengers, such as superoxide dismutase (SOD), catalase (CAT), and peroxidases (GPX), and the enzymes involved in the ascorbate-glutathione cycle (Noctor and Foyer 1998). Thus, the aim of the present study was to analyse the effect of Fe biofortification, with different application rates and forms of Fe, on enzymatic activities of SOD, CAT and glutathione peroxidase (GSH-Px) as bioindicators of the antioxidant system efficiency and improve fruit yield of bean plants (*Phaseolus vulgaris* L.).

MATERIAL AND METHODS

Plants of bean (*Phaseolus vulgaris* L., cv. Strike) were grown under greenhouse conditions in Delicias, Chihuahua, Mexico. The plants were grown in individual pots of 8 L volume. Throughout the growing cycle, the bean plants received a nutrient solution (Hoagland and Arnon 1950). Fe treatments were applied in combination with the

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nutrient solution for 40 days (beginning at 20 days after seed germination).

In this study, a completely randomized experimental design was used, with different forms of Fe at different concentrations. There were two treatments of Fe chelate, at four doses and four replicates per treatment: Fe-EDDHA and $FeSO_4$ at doses of 0, 25, 50 and 100 µmol, respectively.

Plant material was sampled at 60 days when the plants had a phenological phase of complete development and fruit maturity. The organs of the plants were separated and one part of plant material (plant fresh matter) was frozen using liquid nitrogen and stored at –30°C. This material was used for antioxidant enzyme assays (SOD, CAT, APX and GSH-Px) and H_2O_2 quantification. The rest of the plant material (plant dry matter) was dried at 65°C and was used to determine the biomass, crop yield and Fe concentration in bean seed. Plant biomass was determined as the average dry weight of the entire plant and expressed as mg/dry weight (DW). Yield was expressed as the mean weight of fruits per plant in grams of dry weight. Bean fruit was weighed in the sampling of each plant.

Fe concentration was determined by an induced plasma optical emission spectrometer (Agilent Technologies 700 Series ICP-OES, California, USA) according to the method described by Karacan and Aslantaş (2008). SOD (EC 1.15.1.1) activity was determined by its ability to inhibit the formation of nitroblueformazan from NBT (nitroblue tetrazolium) according to two methods (Giannopolitis and Ries 1977), with some modifications. CAT (EC 1.11.1.6) activity was determined by spectrophotometrically following H_2O_2 consumption at 240 nm (Rao et al. 1997). GSH-Px (EC 1.11.1.9) activity was measured using H_2O_2 as substrate and following the methodology of Flohé and Günzler (1984). Concentrations of total soluble proteins extracted of bean tissue, which were used for the 3-enzyme assays (CAT, SOD and GSH-Px), were determined according to the manufacturer's protocol of quick start Bradford protein assay kit (BioRad, California, USA). The H_2O_2 content of seed samples was measured colorimetrically using the methodology of Brennan and Frenkel (1977). Data were subjected to a simple ANOVA at 95% confidence, using SAS (SAS Institute Inc., Cary, USA). Means were compared by the Tukey's test ($P \leq 0.05$). The data shown are mean values ± standard error (SE).

RESULTS AND DISCUSSION

It was found that when Fe is applied in the form of Fe-EDDHA it is more effective in increasing biomass than when this is applied in the form of $FeSO_4$ in these plants (Ortega-Blu and Molina-Roco 2007). Our results coincide with those studies, showing an improvement in the biomass, according to which Fe levels were increased (Figure 1) and crop yield increased when Fe-EDDHA form was applied (Figure 2). In this study, there were significant differences in the Fe application form. Fe-EDDHA form, at 50 µmol and 100 µmol doses, was significantly different with respect to the control (Figure 2), obtaining a 1.5 fold increase in crop yield. The results indicate that the plants tolerated and responded positively to Fe-EDDHA; even with enhanced growth of 25–50 µmol. As to the Fe accumulation in seed, Fe biofortification induced the accumulation of this mineral in bean seed with the two Fe forms applied (Figure 3). The dose of Fe applied at the lowest concentration (25 µmol) produced a highly significant increase in the accumulation of Fe in bean seed, increasing the seed levels of Fe by 29%, compared to control (Figure 3). In previous studies of Se biofortification, it was found that the content of H_2O_2 in seed can increase as the doses of Se increase, which can be toxic levels and produce oxidative stress (Ríos et al. 2009). Our results show a similar behaviour with an increase in the H_2O_2 concentration as the Fe application rate increased (25, 50 and 100 µmol Fe). In addition, the H_2O_2 concentration detected was higher when the form of iron used was $FeSO_4$. For these reasons, it was assumed that $FeSO_4$ is more toxic than Fe-EDDHA in the studied bean crop, overall at a rate of 100 µmol. In Figure 4, it can be observed that the increase of H_2O_2 levels was notably high at 50 µmol dose of Fe-EDDHA and 100 µmol dose of $FeSO_4$, compared to the control. Fe-EDDHA at 50 µmol and 100 µmol doses had significant values of H_2O_2 in bean seeds. With respect to $FeSO_4$ form, an increase of H_2O_2 concentration was more than two fold, compared to the control (Figure 4).

The effects of Fe application on CAT activity reported in this study are in agreement with studies carried out in gramineuos plants. It was observed that doses of 50 µmol and 100 µmol of Fe applied to maize plants increased CAT activity in their seeds (Sun et al. 2007). Our results are in line with

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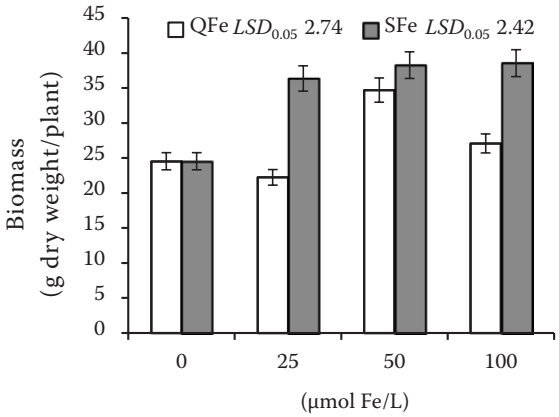

Figure 1. Biomass in plants of *Phaseolus vulgaris* L. cv. Strike subjected to different iron doses and forms. Data are means ± standard error (n = 4) ($P \leq 0.05$). QFe – iron chelate; SFe – sulfate

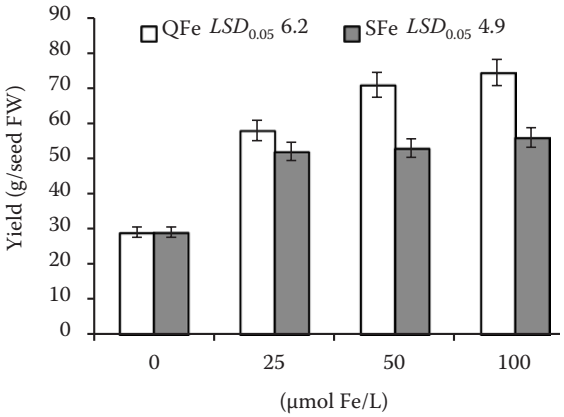

Figure 2. Yield in plants of *Phaseolus vulgaris* L. cv. Strike subjected to different iron doses and forms. Data are means ± standard error (n = 4) ($P \leq 0.05$). QFe – iron chelate; SFe – sulfate

this behaviour; CAT activity showed an increase in bean seeds as the Fe doses were elevated, with both application forms of Fe. However, $FeSO_4$ form produced a higher increase in the CAT activity of bean seed than Fe-EDDHA form at three studied doses (Table 1). Similar to CAT, it was found that SOD activity was increased in bean seeds as intermediate Fe dose were used (25 µmol and 50 µmol) with $FeSO_4$. This increase resulted as highly significant with respect to controls. However, no increase of SOD activity was detected at the highest dose of Fe used (100 µmol), for both compounds of Fe studied (Table 1).

Compared to the increase in enzymatic activity detected for CAT and SOD, such behaviour was not detected with the activity of GSH-Px in bean seeds, cultivated through biofortification with two forms of Fe. Regarding the form of Fe that was applied as $FeSO_4$, it was not possible to detect statistically significant differences when the dose of Fe was increased at concentrations of 25, 50 and 100 µmol (Table 1). In the case of Fe-EDDHA form, a small decrease in GPX activity was registered at doses of 50 µmol and 100 µmol (Table 1). In future projects, it would be necessary to confirm these results that focus on the study of another class of plant peroxidases, involved in this antioxidant system.

In conclusion, increased SOD, CAT and GSH-Px activities in the bean seeds indicate that this species has the capacity to adapt to Fe toxicity by developing an antioxidant defense system. In this

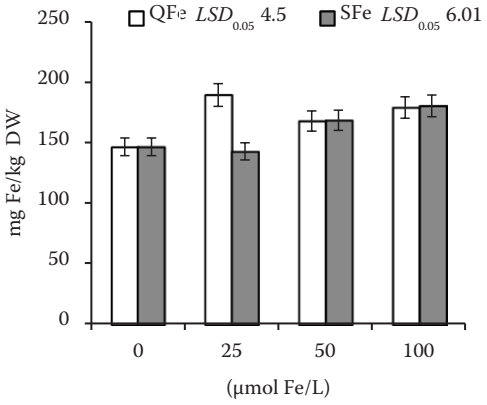

Figure 3. Iron (Fe) concentration in bean seeds subjected to different concentrations and forms of Fe. Data are means ± standard error (n = 4) ($P \leq 0.05$). DW – dry weight; QFe – iron chelate; SFe – sulfate

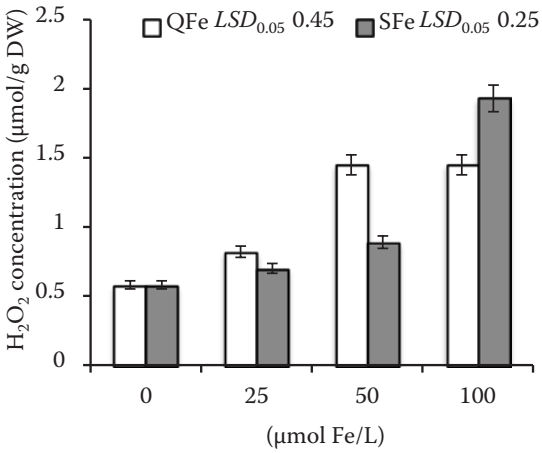

Figure 4. Hydrogen peroxide concentration in seeds of *Phaseolus vulgaris* L. cv. Strike subjected to different doses and forms of iron (Fe). Data are means ± standard error (n = 4) ($P \leq 0.05$). DW – dry weight; QFe – iron chelate; SFe – sulfate

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Table 1. Catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in bean seeds at different concentrations and forms of iron (Fe)

Dose of Fe (μmol)	CAT (μmol H_2O_2/min/g FW)		SOD (U/min/g FW)		GSH-Px (nmol GSH/min/g FW)	
	Fe-EDDHA	$FeSO_4$	Fe-EDDHA	$FeSO_4$	Fe-EDDHA	$FeSO_4$
0	1.20 ± 0.02^a	1.20 ± 0.02^d	1.72 ± 0.03^b	1.72 ± 0.03^b	1.97 ± 0.19^a	1.97 ± 0.19^a
25	1.39 ± 0.19^a	3.32 ± 0.19^c	2.56 ± 0.07^a	2.28 ± 0.10^a	1.88 ± 0.26^a	1.93 ± 0.17^a
50	1.76 ± 0.32^a	5.25 ± 0.17^b	2.81 ± 0.13^a	2.07 ± 0.05^a	1.40 ± 0.08^b	1.53 ± 0.44^a
100	2.02 ± 0.49^a	6.26 ± 0.10^a	1.40 ± 0.07^b	1.15 ± 0.01^c	1.69 ± 0.12^{ab}	1.41 ± 0.23^a

FW – fresh weight. Treatments with the same letter are not significantly different in columns ($P \leq 0.05$)

study, the antioxidant system significantly improved with respect to control, which could explain the beneficial effect of Fe found in plants subjected to diverse abiotic stress. In addition, it was indicated that the effect of Fe on this system depends largely on the form in which this trace element is applied. In bean seeds, the application of $FeSO_4$ produced a higher increase in the activity of the antioxidant enzymes than Fe-EDDHA form at the doses studied. The biofortification with Fe increased significantly the accumulation of Fe in bean seeds; it is considered that the best treatment is the application of Fe-EDDHA at 25 μmol dose, which increased in high percentage of the Fe levels in bean seeds and crop yield of this cultivar. In general, these results will contribute to defining the utility and application of Fe biofortification, which promote the application of micronutrients at low rates. This technology has a great potential to control the induction of the antioxidant system in bean plants, thereby improving crop yield, stress resistance and accumulation of antioxidant compounds in bean seeds.

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