Journal of
Plant Productions

Research Article

Plant Prod., 2021, 43(4), 477-486
http://plantproduction.scu.ac.ir/

ISSN (P): 2588-543X
ISSN (E): 2588-5979

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Effect of Iron-Amino Acid Chelates on Antioxidant Capacity and Nutritional Value of Soybean

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Citation: Jalali, M. (2021). Effect of iron-amino acid chelates on antioxidant capacity and nutritional value of soybean. *Plant Productions*, 43(4), 477-486.
10.22055/ppd.2020.30728.1810

ARTICLE INFO

Received: 18.08.2019
Accepted: 27.06.2020

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Keywords:
Antioxidant activity,
Aminochelate,
Biometric traits,
Chelating agents,
Fe-EDTA

ABSTRACT

Introduction: Supplying a sufficient amount of available iron (Fe) for plant growth in hydroponic nutrient solutions is a great challenge. There are various Fe fertilizers to eliminate iron deficiency in crops. The chelators commonly used to supply Fe in nutrient solutions have several disadvantages and may negatively affect plant growth. The objective of the current paper was to evaluate the effects of some various Fe- chelates, (Fe-arginine, Fe-glycine, Fe-histidine, Fe-lysine, Fe-glutamine and Fe-EDTA) on the physiological properties and nutraceutical potential of soybean (*Glycine max* (L.) Merr.) grown in nutrient solution.

Materials and Methods: The experiments had a randomized complete block design with three replications and the treatments were arranged in factorial combination. The plants were grown in a greenhouse of Faculty of Agriculture at Lorestan University in 2018. In this study, Fe- chelates were synthesized. Then, soybean seeds were sterilized and germinated in 11 µm filter paper. Seven-day seedlings were transplanted into perlite and watered regularly with distilled water for a week. Then, Two-pair leaflet seedlings were placed in continuously aerated solution under controlled conditions of the greenhouse. Six different iron treatments (at Fe level=50 µM) were applied including: Fe-EDTA, Fe-arginine, Fe-Glycine, Fe-histidine, Fe-glutamine, and Fe-lysine. The plant leaves were collected at 10-leaves stages. The seed collection was done in the maturity of harvest (R8). Afterwards, physiological parameters, antioxidant enzymes activity and seeds quantity and quality were measured.

Results and Discussion: Fe-amino acid application significantly ($P \leq 0.05$) enhanced root and shoot dry matter yield, total chlorophyll content, 1000 seed weight, seed yield, seed protein content, seed oil content, oleic acid, and number of seeds in each pod in comparison with Fe-EDTA treatment. The maximum chlorophyll content, seed protein and seed oil were observed in the Fe-glycine treatment (increased by 66.25%, 103.48% and 85.11%, respectively compared to that of control). Concentrations of ferritin, iron, zinc, and nitrogen in soybean seeds were also higher in Fe-amino acid chelate treatments compared to Fe-EDTA. The effect of Fe-amino acid chelates on the Fe content in seeds was in the order Fe-glycine> Fe-glutamine> Fe-lysine> Fe-histidine> Fe-arginine. Catalase activity (EC 1.11.1.6) and ascorbate peroxidase (EC 1.11.1.11) significantly ($P \leq 0.05$) increased in all Fe-amino acid chelates treatments compared to Fe-EDTA treatment.

Conclusion: According to the results, Fe-amino acid chelates could provide the required amount of iron to soybean and this resulted in seed quality enhancement. Therefore, Fe-amino acids can be used as more efficient fertilizers than Fe-EDTA in nutrient solution, and they can be used as an alternative to Fe-EDTA to supply Fe in nutrient solutions.

Introduction

Iron (Fe) is the most deficient micronutrient in plants, especially in arid and semi-arid regions, and play the main role in physiological processes including respiration, photosynthesis and heme proteins'sythesis, RNA, DNA and hormones (Jashani et al., 2017).

The quality of soybean seeds, significantly depends on the quality and quantity of proteins, oil, and fatty acids (Jalili Sheshbahre et al., 2013). The quality and yield of this plant can be increased significantly following the use of biostimulants of soybean nutrient solution (Soares et al., 2016).

There are several researches confirming that Fe uptake and translocation in plants can be facilitated by the presence of chelating agents (Mengel, 1994). Nowadays, amino acids have attracted much attention due to the increasing Fe bioavailability to plants. Various amino acids like arginine, lysine, glycine, histidine and glutamine play principal roles in plant and human nutrition by ameliorating Fe nutrition in plants (Amin et al., 2011).

In nutrient solution cultures, synthetic Fe chelates are widely used to maintain a desirable concentration of a micronutrient element for the plant. The most common Fe sources used in nutrient solutions are Fe-EDTA and Fe-DTPA. Although these chelates are widely used to eliminate iron deficiency in hydroponic solutions, increasing ligand concentration in the solution after iron uptake can decrease the metal bioavailability to plants by enhancing the possibility of a complex formation between free ligands and other micronutrients (like Zn, Cu, and Mn) (Vadas et al., 2007).

Amino acids as an alternative chelator with less sensitivity to photodegradation are able to correlate metal ions (such as Fe) via their carboxyl groups (Aravind and Prasad, 2005). Therefore, biological degradation of amino acids limits accumulation of free ligands in nutrient solution culture which may result in lower effects on the micronutrients uptake.

It has been shown that the use of some amino acids in nutrient solutions improves Fe uptake by crops (Sanchez et al., 2005). Kocira et al. (2018) demonstrated a significant increase in the soybean yield and antioxidant potential. Moreover, Noroozlo et al. (2019) indicated that the application of glycine and glutamine amino acids can have beneficial effects on lettuce growth, as higher fresh yield, leaf chlorophyll content and vitamin C were obtained by low to moderate concentrations of glycine and/or glutamine amino acids.

Due to several disadvantages of Fe-EDTA (for example, toxic side effects on plants and impaired micronutrient balance), finding a suitable alternative for Fe-EDTA in hydroponic nutrient solutions is of great importance. There is limited information on the possibility of using Fe–amino acid complexes as a plant growth stimulator and Fe source in nutrient solution cultures. Therefore, the present research was performed to synthesize Fe-amino acid chelates and evaluate their efficacy as Fe sources for soybean plants grown in nutrient solution. Arginine, glycine, glutamine, histidine, glycine, and lysine were chosen as ligand amino acids. The L-enantiomers (natural forms in plants) of amino acids were used, and some factors which were considered in the selection of these amino acids were abundance in the plant rhizosphere, significance in plant and human nutrition, and stability of their Fe complexes in water.

Materials and Methods

Synthesis of Fe-Amino Acid Chelates

Iron chelates have been prepared using arginine, glutamine, histidine, glycine, and lysine amino acids as complexing agents. A solution of arginine, glutamine, histidine, glycine and lysine (2 mmol) in 5 ml distilled water was slowly added to a solution of $FeSO_4$ (1 mmol) in 2 ml distilled water. The mixture was heated at reflux temperature for 2 h while being stirred vigorously. Evaporation of solvent at room temperature yielded brown microcrystals of Fe-amino acid chelates. The products were washed with cold ethanol followed by diethyl ether and air-dried. All complexes were characterized by different analytical techniques (Ghasemi et al., 2012).

Sample Collection and Treatments

Soybean (*Glycine max* (L.) Merr.) was selected for the present study since it is one of the most protein-rich grain crops, with a seed protein content of about 40% of dry

weight, and it reacts to iron concentration changes well. Soybean seeds were sterilized and germinated in 11 µm filter paper. Seven-day seedlings were transplanted into perlite and watered regularly with distilled water for a week. Then, Two-pair leaflet seedlings were placed in continuously aerated solution under controlled conditions of greenhouse. The nutrient solution used throughout contained 68 mg·L^{-1} KH_2PO_4, 505 mg L^{-1} KNO_3, 114 mg L^{-1} K_2HPO_4 $3H_2O$, 102 mg L^{-1} Ca $(NO_3)_2$, 240 mg L^{-1} $MgSO_4$, 2.86 mg L^{-1} H_3BO_3, 0.31 mg L^{-1} $MnSO_4$ H_2O, 2.29 mg L^{-1} $ZnSO_4$ $7H_2O$, 0.71 mg L^{-1} $CuSO_4$ $5H_2O$, 0.22 mg L^{-1} $COCl_2$, and 0.023 mg L^{-1} $NaMoO_4 \cdot 2H_2O$. The pH was set to 6.0±0.2 using HNO_3 or NaOH (Qiao and Murray 1998).

Every 7 days, the water consumed by soybean was replenished and also the solution was renewed. Six different iron treatments (at Fe level=50 µM) were applied including: Fe-EDTA, Fe-arginine, Fe-Glycine, Fe-histidine, Fe-glutamine, and Fe-lysine. Fe-amino chelates were prepared based on the standard protocol (Ghasemi et al., 2012). Each treatment had three replicates in the three pots. Three plants were planted in each pot. The plant leaves were collected at 10-leaves stage. The seed collection was done in maturity of harvest (R8). This research was conducted in the greenhouse of Faculty of Agriculture at Lorestan University (33.4647° N, 48.3390° E) with average day/night temperatures of 32/20°C, average relative humidity of 60%, and 16 h photoperiod with a photosynthetic photon flux of 250 µmol m^{-2} s^{-1} (400–700 nm). It is noteworthy that pest and disease control operations were also carried out in all the treatments and no specific disease was observed in those treatments.

Physiological Parameters

To measure dry weight of the plants, shoot and root samples were separately dried in an oven for 24h at 60^0C. Leaf chlorophyll was measured in acetone leaf extracts (Arnon, 1949) at stage V5. Total content of seeds protein was measured using Bradford (1976) method and BSA as standard.

Antioxidant Enzymes Activity

Leaf samples were homogenized in 0.1 M sodium phosphate buffer (pH=7.5) containing 0.5 mM ethylenediaminetetraacetic acid (EDTA) using pre-chilled pestle and mortar. The extract was centrifuged for 15 min at 4°C and 15000× g. For the purpose of measuring the ascorbate peroxidase (APX) activity, the reaction mixture contained 50 mM of sodium phosphate buffer (pH=7.0), 0.5 mM ascorbate, 0.1 mM H_2O_2, and appropriate content of enzyme extract. The absorbance decrease was read at 290 nm (Nakano and Asada 1981). According to H_2O_2 consumption at 240 nm for 1 min, the catalase (CAT) activity was measured (Aebi, 1984).

Seeds Quantity and Quality

To evaluate seed quantity, the weight of 1000-seeds was measured. The oil concentration in seeds was determined using spectrometer (Inframatic 8620 near-infrared). The calibration was set for soybean. The content of fatty acid composition in seeds samples was measured by gas chromatography (Agilent 6890).

Fe^{2+} ion concentration in seeds was measured according to O-phenanthroline method (Katyal and Sharma 1980). The total amount of nitrogen in seeds was measured by Kjeldahl method (Saez-Plaza et al., 2013). Phosphorus and potassium concentrations in seeds were measured by colorimetric (Murphy and Riley, 1962) and flame photometric (Sparks, 1996) method, respectively. Calcium, zinc, and manganese concentrations in seeds were determined by atomic absorption spectrophotometer (Agilent 240FS AA, USA) (Basgel and Erdemoglu, 2006).

A competitive ELISA protocol was applied to measure ferritin content in seeds (Rajabbeigi et al., 2013). One g of frozen seed samples was homogenized on ice in 2 ml of extraction buffer (10 mM Na-Pi buffer containing 100 mM NaCl, 2% PVP (polyvinylpyrrolidone) and 1 mM PMSF (phenylmethanesulfonyl fluoride), pH=7.2). The slurry was centrifuged at 12,000 g for 10 min at 4.8°C, and the supernatant was used. Fifty microliters of extract or standard was added to plates whose wells were

precoated with antiferritin antibody, following the manufacturer's instructions (Pishtaz Teb Co., Tehran, Iran). Ferritin content was determined according to the results of complexes formed during the antigen–antibody reaction, using an ELISA kit (Pishtaz Teb Zaman Diagnostics, Tehran, Iran), and the absorbance was detected at 450 nm using an ELISA reader (Anthuos 2020, Australia).

Statistical Analysis

The experiments had a randomized complete block design with three replications and the treatments were arranged in a factorial combination. The significant differences of means were separated through analysis of variance (ANOVA) after least significant difference (LSD) test at $p \leq 0.05$. SPSS 15.0 statistical package (SPSS, Inc., Chicago, U.S.A.) was used to analyze the data.

Results and Discussion

Root and Shoot Dry weights and Leaf Chlorophyll

Comparison of means showed that all Fe-amino acid treatments significantly increased root and shoot dry weights compared to Fe-EDTA treatment (Figure 1). The maximum and minimum amount of dry weight of root and shoot were observed in Fe-glycine and Fe-EDTA treatments, respectively. Shoot and root dry weights decreased in treatments in the following order: Fe-arginine ≤Fe-histidine ≤ Fe-lysine ≤ Fe-glutamine ≤ Fe-glycine.

Several studies have reported the growth-synergetic effect of amino acids. For instant, the application of tryptophan and methionine improved plant growth via increasing auxin and ethylene production in soil and plant tissues (Wang et al., 2007; Amin et al., 2011). Kocira et al. (2018) investigation also showed growth stimulation of soybean treated with a biostimulant containing free amino acids. The use of amino acids also caused growth stimulation of many other plants, including legumes (Boghdady et al., 2016). In another study, it was found that replacing $N–NO_3^-$ by 20% in nutrient solution with amino acid would result in a significant increase of shoot fresh and dry weights of pak-choi (*Brassica chinensis* L.) (Wang et al., 2007).

The analysis of soybean plants indicated that all Fe-amino acid chelates applied to the nutrient solution significantly increased total chlorophyll of leaves compared to Fe-EDTA treatment. The Fe-amino acid chelate influence on chlorophyll depends on its type since Fe-glycine was the most effective treatment in chlorophyll enhancement (Figure 2).

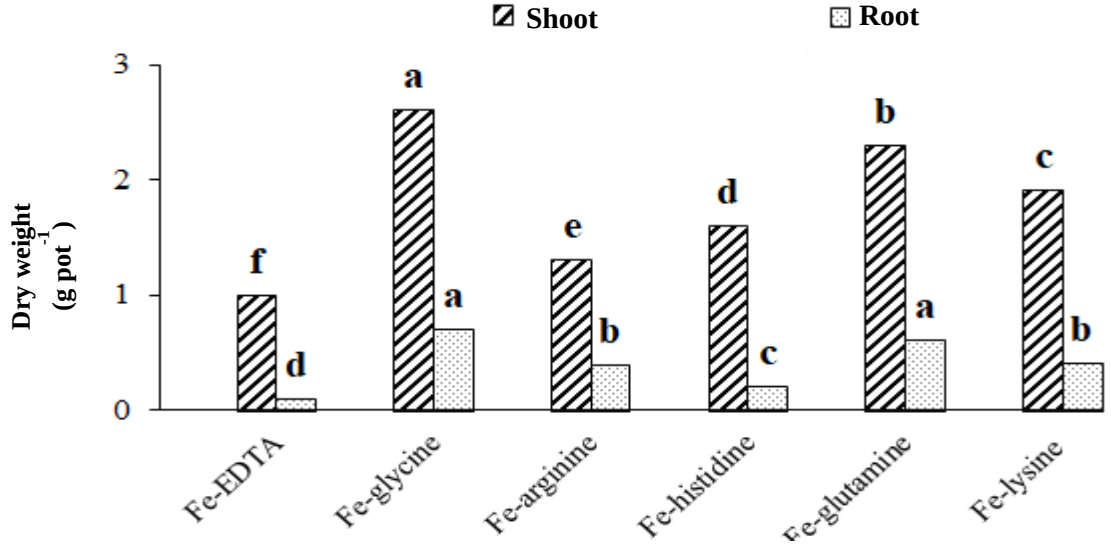

Figure 1. The effect of different Fe-chelates on shoot and root dry weights of soybean. Each value is the mean ±SD, n = 3. Columns with similar letters are not significantly different at $p \leq 0.05$

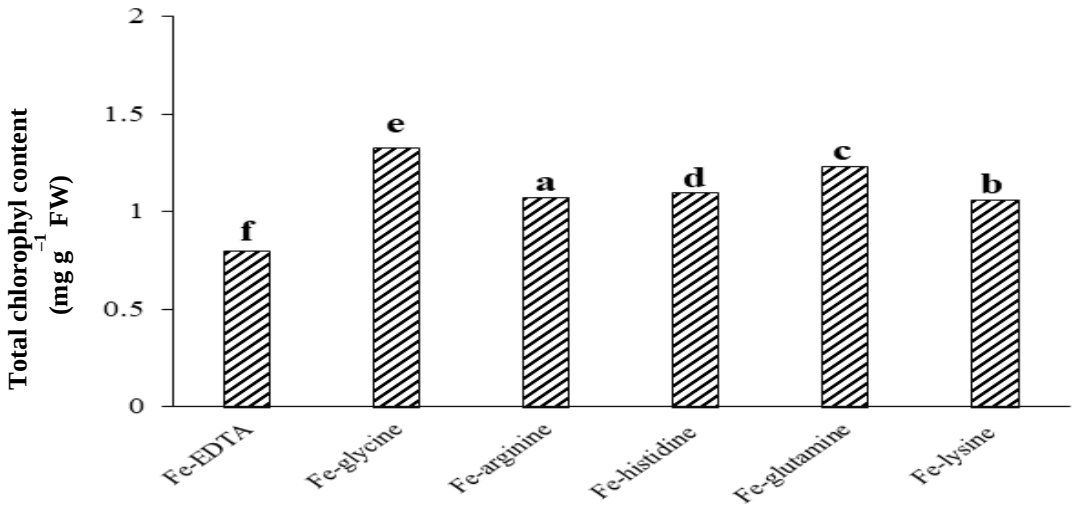

Figure 2. The effect of different Fe-chelates on total chlorophyll content of soybean. Each value is the mean ±SD, n = 3. Columns with similar letters are not significantly different at $p \leq 0.05$

An increase in chlorophyll content, due to the application of amino acids, has also been reported in other studies (Amin et al., 2011; Fahimi et al., 2016; Mohammadipour and Souri, 2019). Higher chlorophyll content of leaves could be due to the stimulating effect of amino acids on chlorophyll biosynthesis and a simultaneous decrease in chlorophyll degradation, as well (Souri et al., 2017; Fahimi et al., 2016).

Amino acids induce biosynthesis of chlorophyll and thereby improve the photosynthesis rate (Amin et al., 2011). Various components of amino chelates including nitrogen, different amino acids, and single or several micronutrients can significantly contribute to an improve of chlorophyll biosynthesis (Souri, 2016).

Amino acids are a reduced form of nitrogen and it has been shown that the application of reduced forms of nitrogen, such as ammonium (Dehnavard et al., 2017) and glycine (Fahimi et al., 2016; Souri et al., 2017), probably via restriction of leaf area expansion, can result in higher leaf chlorophyll content.

Activity of Antioxidant Enzymes in the Leaf

All Fe-amino acid chelates significantly increased CAT activity in shoot compared to Fe-EDTA (Figure 3A) as the maximum CAT activity was evaluated in Fe-glutamine and Fe-glycine treatments. In addition, Fe-amino acid chelates application increased APX activity compared to Fe-EDTA treatments. The maximum and minimum APX activity were determined in Fe-glycine and Fe-EDTA, respectively (Figure 3B). Increasing CAT and APX activities in the presence of glycine may result in an important role that glycine plays in the stress response because on one hand, it can increase or make signal of glycine betaine directly. On the other hand, glycine involves in the route of glyoxylate production, a compound that can reduce H_2O_2 content in plants under oxidative stress condition. Moreover, glyoxylate can also synthesize energy molecules of ATP and NADPH, used in various metabolic processes (Alhasawi et al., 2015). Also, glycine has the smallest molecular weight, as the most effective amino acid in plant nutrition. Therefore, no limitation seems to be on the movement of Fe (II)–glycine via cell wall pores toward the cell membrane (Ghasemi et al., 2014). Also in commercial manufacturer of chelating fertilizers, glycine is the most common amino acid to manufacture (Souri, 2016).

Moreover, glutamine becomes a precursor of other amino acids to eliminate oxidative stress indirectly. It facilitates GSH production, which is a compound that binds some free radicals to decrease plant stress, and is also used as a substrate for some enzymes such as glutathione synthetase, as well as glutathione

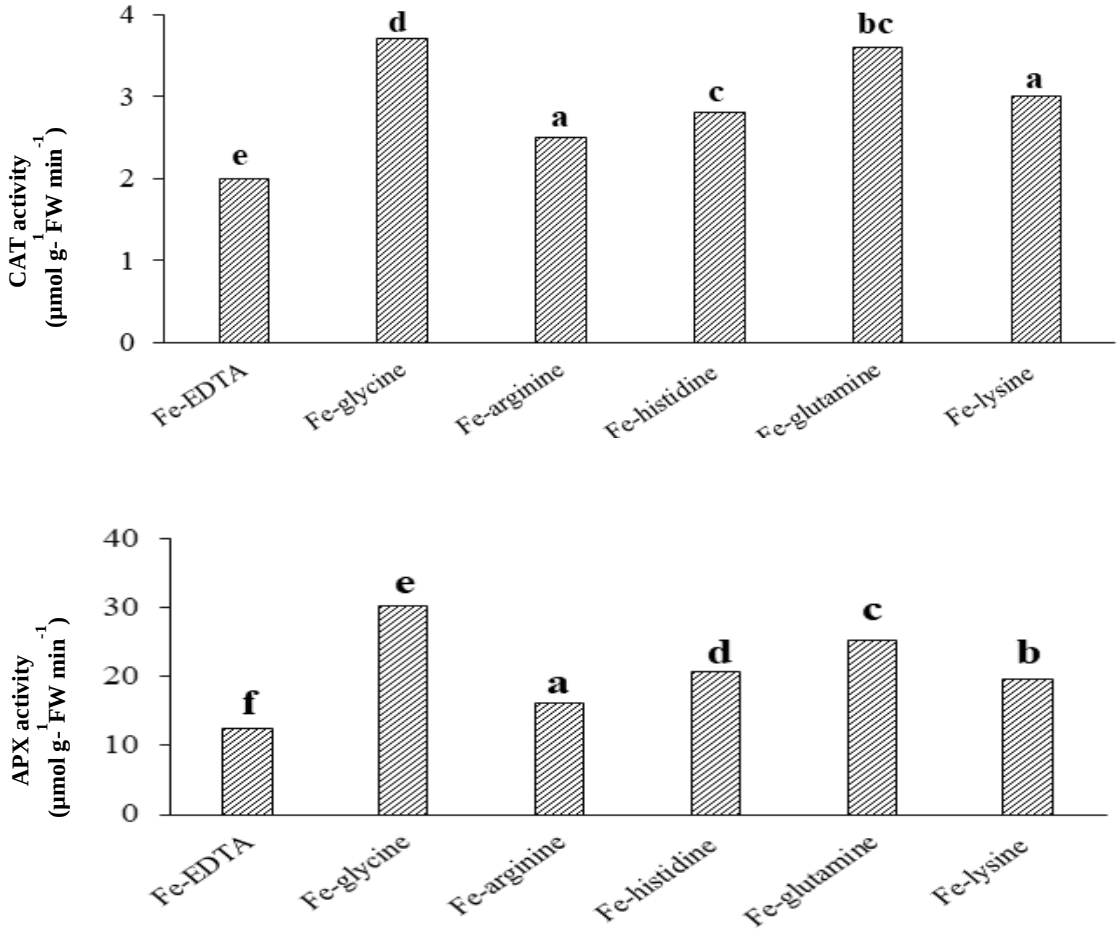

Figure 3. The effect of different Fe-chelate fertilizers on CAT (A) and APX (B) activities of soybean. Each value is the mean ±SD, n = 3. Columns with similar letters are not significantly different at $p \leq 0.05$

peroxidase, resulting in oxidative metabolisms (Gill and Tuteja, 2010). In addition, glutamine receptors can improve physiological effects such as plant stress signaling, carbon metabolism, photosynthesis, and plant immunity and stomatal movements (Weiland et al., 2015).

Also, The EDTA used in nutrient solutions may also be transported into the plant tissue (Vadas et al., 2007) probably via an undeveloped caesarian band at the root tip. High concentrations of EDTA can remove calcium (Ca^{2+}) from the cell membrane and impair root membrane integrity (Vassil et al., 1998).

Yield and Biometric Traits of Soybean

Fe-amino acid chelates significantly increased 1000-seeds weight, Seed yield and the number of seeds per pod in comparison with Fe-EDTA (Table 1). The descending order of 1000-seeds weight, seed yield and the number of seeds per pod were observed in amino acid treatments as: Fe-glutamine, Fe-glycine, Fe-lysine, Fe-histidine, and Fe-arginine. There was no significant difference in the number of pods per plant between amino acid treatments (Table 1).

Dissimilar observations were made by other authors who evaluated the effects of various amino acids on 1000-seeds weight, like an extract from seaweed in chickpea (Boghdady et al., 2016) or Terra Sorb Complex in common bean 'Toska' (Kocira et al., 2017).

Similar findings were reported after the use of amino acids in soybean cultivation, including increased numbers of pods and seeds (Kocira et al., 2018).

Table 1. Properties of soybean seeds treated with different Fe-chelate fertilizers. Each value is the mean ±SD, n = 3. Treatments with similar letters are not significantly different at $p \leq 0.05$

Treatments	1000-seed Weight (g)	Seed yield (t ha^{-1})	Number of seeds per pod	Number of pods per plant	Protein content (mg.g^{-1} FW)	Seed oil (%)	Oleic acid (%)	Linoleic acid (%)	Ferritin content (ng g^{-1} FW)
Fe-EDTA	98.36±6.7^{c}	1.19±0.094^{d}	1.75±0.13^{d}	51.32±3.6^{b}	20.11±2.8^{c}	14.24±1.28^{d}	17.76±1.25^{c}	40.04±2.2^{a}	256.1±25^{d}
Fe-glycine	128.92±5.4^{a}	2.64±0.091^{a}	3.74±0.2^{a}	62.01±4.2^{a}	40.92±3.3^{a}	26.36±2.21^{a}	26.51±1.68^{a}	39.79±2.5^{a}	600.7±38^{a}
Fe-arginine	112.54±7.4^{b}	1.81±0.086^{b}	2.28±0.21^{c}	65.61±2.1^{a}	27.23±3.5^{bc}	18.21±2.18^{c}	19.41±1.34^{c}	38.18±1.2^{a}	362.4±23^{c}
Fe-histidine	119.71±9.2^{b}	2.02±0.075^{b}	2.32±0.2^{c}	60.43±5.3^{a}	31.61±2.5^{b}	16.06±1.79^{cd}	23.11±2.18^{b}	40.79±2.7^{a}	501.4±32^{b}
Fe-glutamine	134.23±10.1^{a}	2.81±0.13^{a}	3.93±0.31^{a}	61.17±4.8^{a}	35.83±2.2^{b}	22.13±1.67^{b}	27.76±2.36^{a}	41.15±2.2^{a}	540.6±27^{b}
Fe-lysine	120.12±6.3^{b}	2.38±0.15^{ab}	2.88±0.24^{b}	64.25±4.2^{a}	34.33±2.9^{b}	17.93±2.51^{c}	22.61±1.68^{b}	42.79±3.4^{a}	520.2±21^{b}

The increased pods per plants and seeds per pod at the presence of amino acids may be attributed to nutrient rapid availability during the growth, facilitating vegetative growth, increasing photosynthetic capacity and absorption process, and allocating more photosynthetic materials to reproductive areas (Monica and Cremonini, 2009). Also, the stimulating effect of amino acids on the yield and quality of crops is due to the increased mRNA transcription, activation of sugar synthesis, and increasing protein content (Keutgen and Pawelzik, 2008).

There were significant differences between the treatments in terms of protein and oil content in seeds (Table 1). According to Fe-amino acid chelates application, regardless of the type of amino acid, it significantly increased both the protein content and the oil concentration in soybean seeds in comparison with Fe-EDTA. The amount of protein and oil increase depended on the type of chelating amino acid. The maximum level of protein and oil content was measured in Fe-glycine treatment at $p \leq 0.05$.

The positive effect of biostimulants such as amino acids on protein content in legume seeds was demonstrated by other authors in their experiments conducted on common bean (Zewail, 2014; Kocira et al., 2017). The treatment of Fabaceae plants with biostimulants based on amino acids caused an increase in protein content of seeds of common bean (Zewail, 2014). However, some studies on the chemical composition of soybean seeds demonstrated a decreased content of protein after foliar application of free amino acids and the extract from A. *nodosum* (Kocira et al., 2018).

Increasing the seed protein content in Fe-amino acid treatment specially Fe-glycine and Fe glutamine may be due to the stimulating effect of amino acids on phytohormones and peptide synthesis (Ghasemi et al., 2014).

There is some evidence that shows the five main fatty acids present in soybean seeds are palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) (Farno, 2005). Soybean plants supplied with amino chelate accumulated higher oleic acid in their seeds than EDTA. The highest oleic acid was obtained in Fe-glutamine and Fe-glycine treatments. But no significant difference was observed between Fe-amino acids and Fe-EDTA treatments regarding the Linoleic acid percentage in the seeds (Table 1).

Fe-amino acid treatments also increased the seed ferritin content compared to Fe-EDTA. Fe-glycine treatment had the maximum seed ferritin content (Table 1). So far, several transporters of amino acids in cell membranes of plants have been recognized (Haydon and Cobbett, 2007). It is assumed that the ferritin content is correlated with Fe^{2+} ion in seeds. Induction of ferritin, mediated by increasing Fe^{2+} ion, has been confirmed by Briet et al. (2010). Probably, fertilizers play the main role to control cellular Fe homeostasis, since they are members of Fe storage superfamily (Liao et al., 2012).

Nutritional elements concentrations in seeds in various treatments were shown in Table 2.

Table 2. Macro and micro nutrient concentration of soybean seeds treated with different Fe fertilizer sources. Each value is the mean ±SD, n = 3. Treatments with similar letters are not significantly different at $p \leq 0.05$

	N (%)	P (%)	K (%)	Ca (%)	Mn (mg/kg)	Fe (mg/kg)	Zn (mg/kg)
Fe-EDTA	4.67±0.31^{e}	0.66±0.01^{b}	2.16±0.31^{a}	0.36±0.09^{b}	2.14±0.01^{a}	61.86±3.51^{f}	60.16±5.31^{e}
Fe-glycine	7.61±0.42^{a}	0.76±0.08^{a}	2.22±0.2^{a}	0.45±0.06^{a}	2.16±0.07^{a}	119.2±10.4^{a}	81.21±7.12^{a}
Fe-arginine	5.42±0.5^{d}	0.80±0.07^{a}	2.17±0.2^{a}	0.46±0.03^{a}	2.10±0.04^{a}	72.17±4.32^{e}	67.47±4.15^{d}
Fe-histidine	5.38±0.24^{d}	0.74±0.05^{a}	2.20±0.1^{a}	0.43±0.09^{a}	2.09±0.08^{a}	83.37±6.1^{d}	70.37±3.05^{c}
Fe-glutamin	6.97±0.5^{b}	0.77±0.02^{a}	2.15±0.07^{a}	0.44±0.08^{a}	2.15±0.06^{a}	108.17±9.15^{b}	75.57±6.25^{b}
Fe-lysine	6.05±0.35^{c}	0.81±0.05^{a}	2.19±0.09^{a}	0.47±0.05^{a}	2.14±0.09^{a}	95.36±5.05^{c}	71.23±5.1^{c}

The results showed that using amino acids in nutritional culture solution increased Fe concentration in seeds compared with EDTA. The maximum and minimum Fe concentrations in amino acid treatments were measured in Fe-glycine and Fe-arginine, respectively. Although seeds of amino acid-treated plants had higher N and Zn concentrations compared with EDTA, type of applied amino acid did not have any significant effects on Ca, P, K and Mn concentrations in seeds (Table 2).

Application of amino acids, via the root system or foliar feeding, has been known to improve the uptake and concentrations of plant nutrients (Garcia et al., 2011; Mohammadipour and Souri, 2019). Such effects have been widely reported for micronutrients, mainly iron and zinc (Souri et al., 2018).

Amino acids have affinity with nutrient elements and some amino acids can make chelates with nutrients. This characteristic has been widely used to improve the uptake and delivery of micronutrients, mainly Fe, in humans, animals and more recently in plants. Application of glycine in nutrient solution has resulted in higher leaf concentrations of N, K, Mg and Zn compared to the control plants (Mohammadipour and Souri, 2019). Higher iron uptake by plants in the presence of amino acids rather than EDTA is probably related to the fact that permeability of cell membrane for amino acids is rather high (Haydon and Cobbett, 2007; Souri, 2016).

Increasing Fe, N, and Zn in the seeds on the one hand may result in important role of amino acids in translocation, re-translocation, and inner division of many micronutrients (Souri and Yarahmadi, 2016). On the other hand, under improved N uptake condition, Fe transporters in root cell membranes express and act better, resulting in higher Fe concentration in plant tissues (Ghasemi et al., 2014). Garcia et al. (2011) found that a mixture of various amino acids applications through nutrient solution significantly increased plant growth and mineral concentrations of calcium, potassium, iron, copper, and manganese in tomato compared to those plants without amino acid supply.

However, it seems that no significant change in manganese concentration of seeds in Fe-amino acid chelates and Fe-EDTA was the consequence of low binding constant of manganese with all the chelating agents (Eskandari et al., 2017). It is concluded that regardless of the synergetic effect of amino acids on plant growth, it is complicated to dissect whether the effect is due to better Fe uptake, more nitrogen supplied in the form of amino acids, or the hormonal effect of amino acids.

Conclusion

In general, the results showed that, the Fe-amino acid complexes such as Fe-arginine, Fe-glycine, Fe-histidine, Fe-lysine, and Fe-glutamine improved quantitative and qualitative characteristics of soybean in comparison with Fe-EDTA. More activity of CAT and APX confirmed the improvement of plant nutritional status of Fe. The application of Fe-glycine and Fe-glutamine was more effective in increasing total chlorophyll content, 1000-seed weight, total protein content, percentage of seed oil, and oleic acid. Moreover, Fe-glycine showed a significant increase in the content of N, Fe, and Zn in seeds. Conforming to the results, Fe-amino acid chelates especially Fe-glycine and Fe-glutamine can be used as alternatives to Fe-EDTA.

Acknowledgments

The author also wishes to thank the Central Laboratory of Lorestan University.

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