DESERT

Desert
Online at http://desert.ut.ac.ir

Desert 22-2 (2017) 145-155

The effect of bicarbonate on iron (Fe) and zinc (Zn) uptakes by soybean varieties

B. Motesharezadeh[a*], A. Hesam-Arefi[a], Gh.R. Savaghebi[a]

[a] *Department of Soil Science Engineering, University College of Agriculture & Natural Resources, University of Tehran, Karaj, Iran*

Received: 8 June 2016; Received in revised form: 28 June 2017; Accepted: 7 October 2017

Abstract

Bicarbonate anion, has a very high concentration in the calcareous soil, and in fact, is the main cause of chlorosis in calcareous soils. This study was conducted to evaluate eight soybean cultivars with iron deficiency and bicarbonate presence. The present research was carried out with the aim of assessing the effect of irrigation with water containing bicarbonate on enzyme activities and Fe and Zn uptakes in soybean. We also compared Fe-efficient and non-Fe-efficient varieties regarding their tolerance against bicarbonate stress. This experiment was done in two parts; the first part was performed in order to identify Fe-efficient and non-Fe-efficient varieties (8 soybean varieties: 'Perishing', 'Zaan', 'Clark', 'Williams', 'M7', 'M9', 'B.P', and 'L17'). 'Perishing', with an Fe efficiency index of 0.98, was considered Fe-efficient, and 'Clark', with an Fe efficiency index of 0.43, was identified as non-Fe-efficient. In the second part, the treatments included two levels of Fe, four levels of bicarbonate (0, 10, 20, and 40 mM), and two varieties different in terms of Fe uptake (Fe-efficient ['Perishing'] and non-Fe-efficient ['Clark']) were selected. The results showed that under conditions of bicarbonate stress caused by the irrigation water, the Fe-efficient variety ('Perishing') had more resistance than the non-Fe-efficient variety ('Clark'). Zinc uptake by 'Perishing' was greater than that by 'Clark'. The activity of catalase enzyme decreased with increasing the bicarbonate concentration, while that of super oxide dismutase increased in both varieties, but more strongly in 'Perishing'.

***Keywords*:** Fe-efficiency; Enzyme activity; Chlorosis; Crops; Micronutrients

1. Introduction

Bicarbonate is an anion, which abounds in calcareous soils in arid and semi-arid areas. It is often created by dissolving carbonate minerals, which are present in the parent material and also by combining water and CO_2 (Colla *et al*., 2010; Zhao and Wu, 2017). Bicarbonate is known as the main factor to outbreak Fe-deficiency in plants (Mengel *et al*., 1994). Different studies have shown that the presence of bicarbonate in the irrigation water and soil solution has a negative effect on nutrient uptake and hence on plant nutrition (Cuenca *et al*., 2013; Mengel *et al*., 1983). Karst regions are characterized by calcareous soils with a low bioavailability of plant nutrients (e.g., P, Zn, and Fe), high calcium content (in the form of calcium carbonate), and high alkalinity (pH 7.5 to 8.5) (Zhao and Wo., 2017). A high concentration of bicarbonate buffers the soil pH. As a result, mineral Fe concentration reduces by reducing insoluble sediments. H+, produced by the activity of proton pomp, is neutralized and inactivated in the presence of bicarbonate. Consequently, secreting phenolic compounds, which are responsible to complex Fe, reduce, and the reduction rate of Fe^{3+} in plasma membrane decreases. Thus, the plant faces Fe-deficiency (Marschner, 1995). Bicarbonate also affects the balance between Fe - reductase and ATPase activities in roots of citrus seedlings (Cuenca *et al*., 2013). In an experiment, it was observed that bicarbonate decreased shoot Zn content in rice varieties by decreasing organic acid synthesis and decreasing the root volume (Hajiboland *et al*., 2005). Bicarbonate buffers plant apoplast pH, so Fe is accumulated in the

* Corresponding author. Tel.: +98 912 2022388
Fax: +98 26 32231787
Email address: moteshare@ut.ac.ir

plant in an inactive form. The majority of scientists believe that the presented Fe in leaves is distributed with apoplast pH, and bicarbonate prevents plasma membrane from acidification and neutralizes the H^+ produced by ionic pomp activity (Mengel *et al*., 1994; Ishwar *et al*., 2016). Therefore, the metabolic activities of active Fe (Fe^{2+}) in the plant decrease, and many of the electron transmission processes in plant metabolic reactions is disturbed. Fe plays an important role in plant nutrition, whose deficiency causes oxidative stress, thus disturbing enzyme activities in plants (Tewari *et al*., 2013). Fe is an essential element for chemical and bio-chemical activities in plants, whose limitation has a clear effect on the plant production and photosynthesis (Frey and Reed, 2012). Investigations indicate that the total absorbed Fe by plants is not used, and part of that is spent for the formation of insoluble compounds. Therefore, in some cases, the total Fe of a plant is not a suitable criterion to monitor the nutritional status of the plant. Usually, Fe^{3+} is immobile in plants, and the active form is Fe^{2+} (Rashid *et al*., 1997; Nikolic and Romheld, 1999).

Fe-deficiency in high sensitive plants creates oxidative damages. In a study, Reactive Oxygen Species (ROS) accumulation was observed in Fe-deficient sunflower plants (Rodriguez *et al*., 2001). For many antioxidant enzymes, Fe is important for electron transmission in reactions. A potential targeted comparison of oxygen active types such as hydrogen peroxide (H_2O_2), hydroxyl (OH), reduction of H_2O and the tendency of oxidation like O_2 (radical of superoxide anion), is along with reduction of Fe^{3+} to Fe^{2+}. In some plants, when there is Fe-deficiency, the level of lipid peroxidation decreases. Reactive oxygen species, including peroxide (O_2^-), singlet oxygen (O_2), hydrogen peroxide (H_2O_2), and hydrogen radicals (OH), are produced during environmental stresses. A defensive mechanism in plants against these compounds is the production of antioxidant enzymes, such as peroxidase (APX), superoxide dismutase (SOD), and catalase (CAT), and non-antioxidant enzymes, such as glutathione-ascorbate and carotenoid (Tewari *et al*., 2005; Molassiotis, 2006). Molassiotis (2006) reported that under conditions of Fe-deficiency, catalase activity in the leaves and roots of peach decreased, while that of superoxide dismutase increased. The ability of root to create suitable conditions for Fe absorption is different among various plants. The plants that are resistant to Fe-deficiency have an efficient root system to absorb Fe. The root system of sensitive plants does not have enough efficiency to absorb Fe (Marschner, 1995). Plants adopt two types of mechanisms (Strategy I and II) for iron acquisition from the soils. Strategy I is found among dicots and monocots, except graminaceous species which adopt Strategy II. The Strategy I mechanism involves proton release at the rhizosphere that lowers the pH of soil solution and increasing solubility of Fe^{3+}, Fe(III) chelate reductase activity that reduces Fe^{3+} to more soluble Fe^{2+}, and transportation of Fe^{2+} into the root by metal transporters (Ishwar *et al*., 2016). Strategy II plants acquire Fe through naturally synthesized mugineic acid (MA) family phytosiderophores, which dissolve insoluble Fe^{3+} in the rhizosphere and acquire Fe(III)-MA complexes. Soybean is a strategy I plant, whose yield in calcareous soils decreases because of the chlorosis induced by Fe-deficiency. A method to investigate the effects of the genetics on plant nutrition is using different varieties of a given plant with different abilities to absorb a certain element (Marcchner, 1995). The efficiency of Fe absorption by genotypes is the ability of them to better grow and produce more yields under conditions of Fe-deficiency in comparison with other genotypes (Barton and Abed, 2006; Behl *et al*., 2003; Lamont *et al*., 2002).

Fe is essential for physicochemical and methalo-protein activities. Fe is also necessary for the activities of many enzymes (Marschner, 1995). An optimum concentration of Fe is essential for the functionality of antioxidant enzymes. Fe-deficiency reduced the activities of catalase, peroxidase, and superoxide dismutase, while the production of free radicals increases simultaneously, which influences electron chain, damages chloroplast, and reduces carotenoid synthesis (Mathey *et al*., 1996). The aims of this research was to investigate the effects of bicarbonate on Fe concentration, active Fe in the shoot, and the activities of catalase and super oxide dismutase enzymes in two varieties of soybean different in Fe-efficiency, and also to compare the effect of this ion on each variety.

2. Materials and Methods

2.1. Soil selection and analysis

This experiment, arranged in a completely randomized block design with three replications in greenhouse conditions, was conducted in two parts. The primary part was performed in order to identify Fe-efficient and non-Fe-efficient varieties of soybean. For this purpose, seeds of eight commercial and common varieties of soybean in Iran were provided and cultivated in a soil with less than 2 mg/kg available Fe (less than the critical level). Some physicochemical properties of the soil such as texture, saturation moisture percent, pH, electrical conductivity (EC), calcium carbonate value, organic matter, available potassium, available phosphorous, soil nitrogen, and cation exchange capacity are shown in table 1 (Ali-ehyae and Behbahanizadeh, 2002).

2.2. Plant cultivation in pre-treatment

In order to evaluate the eight varieties of soybean grown under a soil with Fe deficiency (to identify Fe-efficient and non-Fe-efficient varieties), a greenhouse factorial trial was carried out, being arranged in a completely randomized block design. The first factor was Fe at two levels: no Fe fertilization and optimized Fe fertilization at planting time (5 mg/kg; Sequestrin), and the second factor was 8 soybean varieties: 'Perishing', 'Zaan', 'Clark', 'Williams', 'M7', 'M9', 'B.P', and 'L17'.

In the first part, the seeds were disinfected and then sown in three-kg pots. In this part, one treatment of Fe adequacy and one treatment of Fe deficiency were applied (Fe fertilizer was sourced from Fe EDDHA), and then the pots were transferred to a growth chamber with an appropriate temperature for the soybean growth. The other required nutrients were applied optimally. Irrigation was done by hand till 0.7 of field capacity (FC), and after eight weeks, the plants were harvested. Plant samples were kept at a temperature of 70˚C for 72 hours. The dry matter weights were measured, and the concentration of Fe in the plant tissue was extracted with hydrochloric acid 1 N and read by atomic absorption spectrometer. There were significant differences regarding Fe uptake and transmission among the different varieties tested, so they were classified under two groups of resistant (Fe-efficient) and sensitive (non-Fe-efficient) (Mahmoudi *et al*., 2005).

Fe-efficient and non-Fe-efficient varieties were identified by using element-efficiency equation (Sadrarhami *et al*. 2010):

$$Fe-efficient = \frac{Ys}{Yp} \quad (1)$$

Y_s: yield under conditions of Fe-deficiency
Y_p: yield under conditions of Fe-adequacy

Various varieties were compared with each other, and two varieties of Fe-efficient and non-Fe-efficient were selected by calculating Fe efficiency index and indices of tolerancence and sensitivity against stress.

2.3. The cultivation of Fe-efficient

The second part of the present experiment was performed in order to compare the two varieties (Fe-efficient and non-Fe-efficient) of soybean with two soil bicarbonate content. Through this part, the seeds of both varieties (Fe-efficient and non-Fe-efficient) were disinfected and cultivated in three-kg pots, and two treatments of fertilization (Fe-adequacy) and non-fertilization (Fe-deficiency) were administered. The other nutrients required for the plant growth were applied optimally. They were then transferred to a germinator with a day/night temperature of 28/22˚C, a relative day/night humidity of 74/54%, and a photoperiod of 16/8 hours (dark/light). According to research report (Malakouti and Shahabi, 2002) Bicarbonate was added to the irrigation water by dissolving ammonium bicarbonate salt therein at four concentrations: 0, 10, 20, and 40 mM and added to soil. In fact, the Fe-efficient variety ('Perishing') and the non-Fe-efficient one ('Clark'), which were selected in the first part of the experiment, were subjected to bicarbonate stress at four levels. This was also a factorial trial arranged in a completely randomized block design with three replications. The first factor was Fe fertilization (with and without Sequestrin, according to the critical level of Fe in the soil for both varieties). The second factor was bicarbonate at four levels. Irrigation was done by hand till 0.7 FC, and after eight weeks, the plants were harvested. In this part of the study, Fe, active Fe, Zn, and some of enzymes were measured.

2.4. Measuring active Fe

The active Fe is an important part of plant tissue and is considered the available part of Fe for metabolic reactions. To measure the active Fe, some opened and matured leaves were provided from each pot and washed with distilled water. After that, the active Fe of green leaves was measured according to Katyal and Sharma's method. To measure the active Fe in the plants, the solution of ortho phenanthroline 1.5% was used and then the amount of active Fe was calculated by using the below equation (Katyal and Sharma, 1980):

Fe^{++} (mg/kg) = Fe^{++} (mg/kg) (from calibration curve) A/Wt; Wt: the dry weight of 2 g fresh plant sample that was oven-dried

A: the volume of extractant.

Measuring Fe and Zn: Fe and Zn in the plant samples were measured by the dry ashing method and by extracting with hydrochloric acid 1N. Fe concentration in filtered samples was read by atomic absorption spectrometer (Oserkowsky, 1933).

2.5. Measuring antioxidant enzymes activity

To evaluate the activities of the enzymes, a spectrophotometer device (UV_160A_ SHIMADZU) with a quartz cuvette was used and reported in the unit of u min^{-1} mg^{-1} protein.

2.6. Data analysis

The data were analyzed by using SAS software, and the graphs were drawn by using Excel. The means were compared according to Duncan's multiple-range test (P<0.05).

3. Results

3.1. The selection of Fe-efficient and non-Fe-efficient varieties

The comparison of Fe efficiency index in the eight varieties of soybean in the preliminary study showed that the variety had a considerable effect on Fe uptake. 'Perishing', with an Fe efficiency index of 0.98, had the highest Fe-efficiency, while 'Clark', with an Fe efficiency index of 0.43, had the lowest Fe-efficiency. The difference between 'Williams' and 'M7' was insignificant statistically (P<0.05). Similarly, there were no significant differences between 'Perishing', 'M9', 'B.P', 'Zan' and 'L17', but 'Perishing' and 'Clark' showed the highest difference (Fig. 1).

Means within the same columns followed by the same letter(s) have no significant differences (P<0.05). Based on table 2, Fe fertilization and the variety had significant effects on the concentrations of Fe, Zn, and active Fe in the shoot, whereas the effect of bicarbonate was only significant on the concentrations of Fe and active Fe in the shoot. Additionally, the interactive effects of Fe and bicarbonate on the concentrations of Fe, Zn, and active Fe of shoot were significant. The interactive effects of bicarbonate and variety were significant only on the concentration of Fe in the shoot. The interactive effects of Fe fertilization, bicarbonate, and variety were significant on the concentrations of Fe and active Fe in the shoot.

Table1.The studied soil properties

Characteristics	Unit	Value
Soil Texture Class		Loam
EC	dS/m	0.80
FC	%	19.00
pH	-	7.80
CEC	$cmolc^{+}/kg$	11.07
$CaCO_3$	%	6.15
N	%	0.03
OC	%	0.08
$NaHCO_3$-Pe	mg/kg	3.90
NH_4Ac-K	mg/kg	530.00
DTPA-Zn	mg/kg	0.86
DTPA-Fe	mg/kg	1.25

Table 2. Analysis of variance of the concentrations of Fe, active Fe, and Zn in the shoot of soybean

		Mean Square		
Source of variations	Degree of freedom	Fe in the shoot	Active Fe	Zn
Iron	1	30402.3**	516**	5598.72**
Variety	1	123340.9**	364**	369.63ns
Bicarbonate	3	28127**	507**	10348.72ns
Fe*Var	1	621.936**	60.60**	7.05ns
Fe*Bic	3	889.90**	4.8*	1414.94*
Var*Bic	3	6031.74**	1.7ns	7.74556ns
Var*Bic*Fe	3	534.98**	8.34**	48.11ns
Error	32	38.52**	1.18	158.60

Note: ns: non-significant; * and **: significant at 5% and 1% probability levels, respectively; CV: coefficient of variation

3.2. Fe concentration in the shoot and root

The concentration of Fe in the shoot was much more under conditions of Fe-availability (Fe fertilization) than under conditions with no Fe fertilization. At all concentrations of bicarbonate (0, 10, 20, and 40 mM), Fe fertilization had an incremental effect on the concentration of Fe in the shoot, but with increasing bicarbonate concentration, the concentration of Fe in the shoot decreased. Moreover, the concentration of Fe under conditions with no Fe fertilization decreased with increasing bicarbonate concentration (Table 3).

In both varieties, 'Clark' (non-Fe-efficient) and 'Perishing' (Fe-efficient), with increasing bicarbonate concentration in the irrigation water, shoot Fe concentration decreased, but at all levels of bicarbonate, shoot Fe concentration in 'Perishing' (Fe-efficient) was much more than that in 'Clark' (non-Fe-efficient) (Table 3).

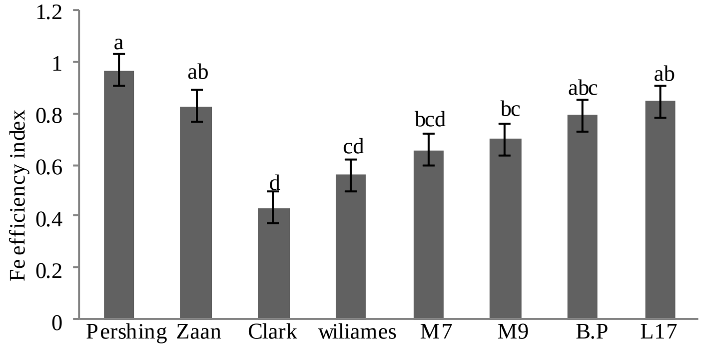

Fig. 1. Fe efficiency of eight varieties of soybean

Table 3. Fe and Zinc concentration in conditions of fertilization and non-fertilization at different concentrations of bicarbonate.

	Varieties								Fe levels							
	Pershing				Clark				+Fe				-Fe			
Bicarbonate levels (mg/kg)	0	10	20	40	0	10	20	40	0	10	20	40	0	10	20	40
Fe in shoot (mg/kg)	210.6a	166.5b	115.2c	80.9e	138.9d	110.6e	73.8f	48.5g	210.6a	166.5b	115.2d	80.93e	138.9c	110.6d	73.8e	48.5f
Fe in root (mg/kg)	224.6e	257.0d	341.5c	361.7b	213.8e	269.7d	359.3b	424.1a	248.9e	383.9d	406.0b	455.6a	189.5f	243.3e	294.8d	330.2c
Zn in shoot (mg/kg)	105.6a	91.5ab	56.6c	44.0cd	102.46a	91.56ab	50.4cd	38.1d	128.8a	101.3b	59.1d	40.6e	79.2c	75.0c	47.0ed	41.4e
Active iron in shoot (mg/kg)	28.9a	25.3b	18.8c	15.05d	24.3b	19.5c	13.0e	9.0f	30.5a	25.7b	19.4d	14.4e	22.7c	19.1d	12.4e	9.6g

Based on the data in table 3, shoot Fe concentration under conditions with Fe fertilization and with no Fe fertilization and at levels of 0 (control), 10, 20, and 40 mM bicarbonate were 71.1, 55.37, 41.1, and 32.38 mg/kg, respectively. These results indicated that with increasing bicarbonate concentration, Fe fertilization efficiency (Fe fertilizer recovery) decreased substantially, and these variations were statistically significant ($P<0.05$) at all bicarbonate concentrations. With increasing bicarbonate concentration, Fe concentration in the root increased strongly under conditions of Fe adequacy (Fe fertilization). In fact, with increasing bicarbonate concentration, root Fe concentration increased. At all levels of bicarbonate, root Fe concentration under conditions with Fe fertilization was more than that under conditions with no Fe fertilization (Table 3).

Results showed that with increasing bicarbonate concentration, root Fe concentration increased in both varieties. Root Fe concentration in 'Clark' (non-Fe-efficient) at all concentrations of bicarbonate was much more than that in 'Perishing' (Fe-efficient) and increased with increasing bicarbonate level (Table 3).

The results showed that root Fe concentration in 'Pershing' and 'Clark' under 0 and 10 mM of bicarbonate was not significant, while it was significant ($P<0.05$) at levels of 20 and 40 mM of bicarbonate. Fe concentration in 'Clark' (non-Fe-efficient) was much more than that in 'Pershing' (Fe-efficient).

3.3. Active Fe

Research shows that there is not much correlation between total Fe and Fe deficiency-mediated chlorosis, and in most cases, total Fe in chlorotic leaves are more than in normal leaves, which is known as the iron contradiction. However, these chlorotic leaves had lower active Fe (Abadia, 1992; Therios *et al*., 2005). Various methods of Fe extraction from avocado tress showed that the correlation between Fe(II) extracted with phenanthroline and the degree of chlorosis was more than that obtained by other methods. Therefore, extraction with phenanthroline can be a suitable method for the recognition of Fe deficiency in these trees (Neaman and Aguirre, 2007). Ortho-phenanthroline forms a stable complex with Fe(II), which produces a specific orange color, whose intensity is read at 510 nm and then calculated by using a standard curve (absorption against Fe(III) concentration) (Katayal and Sharma, 1980). Some earlier studies demonstrate that there is good correlation between active Fe and the degree of chlorosis, which is accordingly an appropriate method for the recognition of Fe deficiency (Tagliavini *et al*., 2000; Romheld, 2000).

At all bicarbonate concentrations (0, 10, 20, and 40 mM), the active Fe concentration in the shoot under conditions of soil Fe-adequacy was much more (Fe fertilizing) than that under Fe-deficiency conditions, and this variation was statistically significant at all levels of bicarbonate ($P<0.05$) (Table 3). The active Fe concentration in both varieties decreased with increasing the bicarbonate level, but at all levels of bicarbonate; the active shoot Fe concentration in 'Pershing' (Fe-efficient) was much more than that in 'Clark' (non-Fe-efficient) (Table 3).

The active shoot Fe concentration under Fe fertilization and non-Fe fertilization at levels of 0, 20, and 40 mM of bicarbonate were 7.79, 6.59, and 4.73 mg/kg, respectively. These variations indicated that with increasing bicarbonate concentration, the differences among the active shoot Fe concentration in conditions with Fe fertilization and non-Fe-fertilization reduced. However, at 0 and 10 mM of bicarbonate, Fe fertilization was more effective on increasing active Fe concentration in the shoot.

The results showed that bicarbonate decreased shoot Fe concentration in both varieties, and with increasing Fe concentration, a stronger reduction was observed, which was due to a bicarbonate-mediated disruption in the process of Fe uptake (Table 3). In addition, active shoot Fe concentration in both varieties decreased in the presence of bicarbonate (Table 3).

3.4. Shoot Zn concentration

The results showed that shoot Zn concentration reduced with increasing bicarbonate concentration. In both varieties, with increasing the bicarbonate concentration, shoot Zn concentration reduced. The difference between the two varieties regarding Zn concentration was not significant at 0 and 10 mM of bicarbonate, but it was statistically significant at 10 and 20 mM. However, between 20 and 40 mM, the difference between the two varieties was not significant statistically. In general, shoot Zn concentration reduced with increasing bicarbonate concentration, and at all levels of bicarbonate concentration; this reduction in 'Clark' (non-Fe-efficient) was more than that in 'Pershing' (Fe-efficient) (Table 3).

At concentrations of 0 and 10 mM of bicarbonate, shoot Zn concentration was not significantly different in 'Pershing' (Fe-efficient) and 'Clark' (non-Fe-efficient). Additionally, shoot Zn concentration in 'Pershing' was not significantly different at 20 and 40 mM of bicarbonate, whereas the difference was significant between bicarbonate 0 and 10 mM and 20 and 40 mM in variety of 'Pershing' (Fe-efficient), and between 10 and 20 mM was a considerable difference (Figure 8). The presence of bicarbonate in the irrigation water not only prevents the uptake of Fe, but also disrupts that of Zn. In both varieties, shoot Zn concentration reduced, but this reduction was stronger in the non-Fe-efficient variety (Table 3).

3.5. Catalase Activity (CAT)

As shown in figure 9, with increasing bicarbonate concentration, the catalase activity in both varieties decreased. The catalase activity in 'Pershing' (Fe-efficient) was more than in 'Clark' (non-Fe-efficient) at all levels of bicarbonate. The difference between the two varieties in the case of catalase activity was statistically significant ($P<0.05$) at all levels of bicarbonate. In fact, there was a decreasing trend. The highest CAT activity was observed in the 'Pershing' plants irrigated with water containing bicarbonate 0 mM, while the lowest in 'Clark' plants irrigated with water containing bicarbonate 40 mM (Figs. 2 and 3).

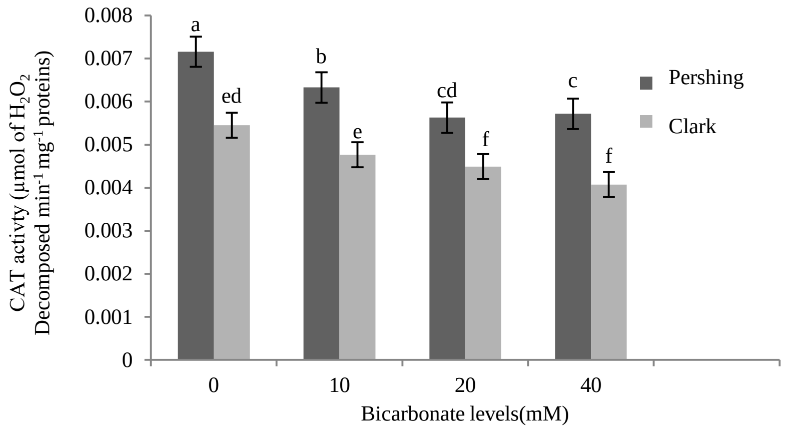

Fig. 2. The activity of catalase in the 'Perishing' and 'Clark' plants irrigated with water containing different concentrations of bicarbonate

3.6. Superoxide dismutase (SOD) activity

In both 'Pershing' and 'Clark', the activity of superoxide dismutase enhanced with increasing the bicarbonate concentration, which was significant statistically ($P<0.05$) at all levels of bicarbonate. At all levels of bicarbonate, the activity of superoxide dismutase in 'Clark' (non-Fe-efficient) was more than in 'Pershing'. The highest activity was observed in 'Clark' irrigated with water containing bicarbonate 40 mM, and the lowest in 'Pershing' (Fig. 3).

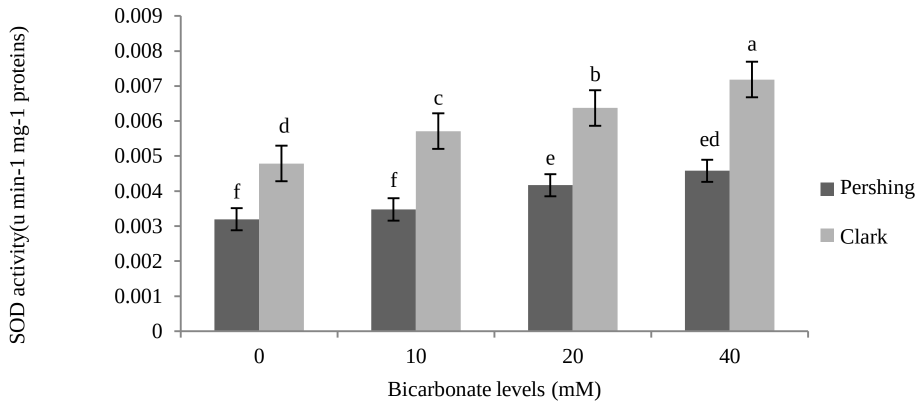

Fig. 3. The activity of SOD in the 'Perishing' and 'Clark' plants irrigated with water containing different concentrations of bicarbonate

4. Discussion

The results of the present study showed the differences between various Glycine soja cultivars regarding "iron efficiency" (Fig. 1). In order to understand the possible reasons and the mechanisms behind iron efficiency, it is important to recognize the differences between 'Pershing' and 'Clark', iron efficient and non-efficient, respectively. This Fe difference could be attributed to better root growth and biomass production (formulas used to calculate iron efficiency index: data of Fig. 1). Furthermore, some of mechanisms of iron efficiency for increasing active iron in the plant and the ability to transfer the Fe from root to shoot are attributed to the plant's aerial organs (data in Table 3).

A high Fe concentration in the leaves at the time of chlorosis induced by Ca can be because of its restrictive effect on the other factors required for leaf growth and chloroplast growth and organization (Ksouri *et al*., 2005). Fe-deficiency is common among plants, e.g.

almonds, rice, beans, soybean, and sorghum (Fageria, 2008). A plant's Fe requirement changes proportionally with its ability to utilize Fe from the environment. For example, differences were observed among plant different varieties of a species such as sunflower, barley, corn, oat, wheat, rice, corn, tomato, cucumber, and soybean (Tinker and Launchi, 1984; Marschner, 1995). As shown in figure 1, the different varieties of soybean showed varied yields under conditions of Fe deficiency, which was because of variation in their genetic properties and their specific mechanism against Fe deficiency. Severe Fe deficiency in plants increases the root secretion compounds such as phenolic materials, riboflavin, and organic acids. In addition to their indirect effects on the soil microbial population, the secreted compounds affect micronutrient availability by increasing the acidity of root zone, forming chelates and reducing Fe^{3+} to Fe^{2+} (Ksouri *et al*., 2005). Various studies have shown that under conditions of Fe deficiency, yields and growth of different plants reduce significantly, but with less severity in resistant varieties (Norvell and Adams, 2006; Yousfi *et al*., 2009). Plants that are Fe deficient do not perform photosynthetic processes completely because of inadequate chlorophyll content, thereby diminishing the growth and yields. Generally, the plants that are capable of converting Fe to available forms are considered Fe-efficient under conditions of Fe-deficiency. The utilization of Fe-efficient varieties increases Fe uptake due to morphological and/or physiological mechanisms like secreting organic acids (citric acid) and increasing reductive capacity of the root (Marcshner, 1986). Bertamini and Nadunchezhian (2005) declared that Fe-deficiency reduced plant fresh and dry weights and leaf area in grapes. Pestana *et al*., (2012) showed that adding Fe to nutrient solution of sugarcane increased shoot and root Fe concentration and had a substantial effect. The results of this experiment showed that under conditions of Fe sufficiency, Fe-reductase activity decreased.

Bicarbonate is an important factor in the occurrence of chlorosis induced by Fe-deficiency. A high concentration of bicarbonate in the root may indirectly affect leaf Fe utilization value. In the majority of varieties sensitive to chlorosis, a high concentration of bicarbonate stops important processes including: root growth, root respiration, and root pressure power to transfer solution through xylem and to send out cytokinin to branches.

The findings of the present study showed that with increasing bicarbonate in various treatments, Fe and Zn concentrations decreased in all treatments (table 3), however at different rates. Iron efficient plants such as 'Pershing' resisted the increased bicarbonate very well, with a slight decreasing gradient. However, Iron non-efficient plants lost more Fe and Zn. Ours results are in consistency with those of Kosegarten *et al*. (2001). Kosegarten *et al*., (2001) indicated that the presence of bicarbonate led to the accumulation of Fe in apoplsat of root epidermic cells, the increase of apoplast pH, and the restriction of Fe transfer from the root to the shoot. The increased apoplast pH leads to the reduction of Fe in the leaves, hampers Fe uptake and transmission to the shoot, and reduces the capacity of the reduction of Fe^{3+} and inactivates active Fe (Mengel *et al*., 1994). Yang *et al* (2003, 1994) observed that bicarbonate decreased Zn and Fe concentrations in non-Zn-efficient varieties but not in Zn-efficient varieties. These genotypes grow in calcareous soils easily. Moreover, bicarbonate can neutralize Zn, Fe, and other micronutrients in plants. Bicarbonate makes Fe to sediment in leaf apoplast, and Fe concentration decreases subsequently (Nicolik *et al*., 1998).

Bicarbonate in the rhizosphere is considered an important factor to cause plant Zn-deficiency in calcareous soils. In a Hajiboland *et al.,* (2005) study, the effect of bicarbonate on rooting, distribution, and secretion of organic acids in Zn-efficient and non-Zn-efficient genotypes was investigated, and the results indicated that bicarbonate considerably increased the number of fine roots in the Zn-efficient variety. In the non-Zn-efficient variety, the growth decreased and the plants secreted more citrate and malate. Bicarbonate increased malate secretion in the fine roots in 1-2 cm depth of the soil, and this secretion was stronger in the Zn-efficient variety than in the non-Zn-efficient one. Furthermore, bicarbonate decreased rooting in the non-Zn-efficient variety and led to a more accumulation of organic acids in the root. In the non-Zn-efficient variety, the accumulations of malate and citrate in fine roots reduced the secretion of these compounds in the rhizosphere and diminished their transfer to the shoot (Hajiboland *et al*., 2005). It has been stated in numerous reports that bicarbonate ions decrease root volume, prevent Zn uptake, and decrease Fe mobility in the root, thus reducing its transfer to the shoot (Ferno *et al*., 1975).

Fe mobility in plants is affected by bicarbonate ions more than Fe uptake value. It has been shown that with the application of bicarbonate in the nutrient solution, Fe content increased in the stem and petioles but decreased in the leaves. Additionally, the pH of cell extract is related to the total Fe and soluble Fe therein. When the pH of a cell increases, the total Fe content increases, whereas the soluble Fe decreases. When there is a high concentration of bicarbonate or CO_2 (which produce bicarbonate under poor soil aeration) in the irrigation water, the absorption of nutrients, especially micronutrients, is disturbed, which is due to the elevated soil pH (Koasch and Hofner, 1984).

The results revealed that the plants grown under stress of bicarbonate experienced changes in the activities of the enzymes. The activity of catalase enzyme (CAT) showed a decreasing trend with increasing bicarbonate (Fig. 1), while that of superoxide dismutase (SOD) displayed an increasing trend (Fig. 2). As bicarbonate increased, the activities of catalase decreased in both cultivars, but at a lower rate in ‘Pershing’ (Fig. 2) but these changes were stronger at higher concentrations of bicarbonate. The comparison between SOD activities revealed that ‘Pershing’, Fe efficient, has a higher SOD activity. In other words, Fe efficient cultivars resist bicarbonate stress with the help of SOD antioxidant activity, which is a defense mechanism. Our results are in agreement with those of Cuenca *et al*. (2013) and Rivero *et al*. (2003). Researchers reported that after 21 days, citrus seedlings were irrigated with bicarbonate 10mM, and then Fe concentrations in the different parts were measured. Additionally, the effect of bicarbonate on the enzyme activity and Fe availability were examined. The seedlings that were grown under Fe deficiency and in the presence of bicarbonate had less shoot Fe concentration than those grown only under conditions of Fe-deficiency. Bicarbonate affects the balance of Fe-reductase and ATPase activities in the roots of citrus seedlings. The activities of these enzymes in the presence of bicarbonate and lack of Fe increase (Cuenca *et al*., 2013). Biochemical and ultra-structural changes are the primary indications of Fe-deficiency in calcareous soils. In a study, the effects of different temperatures on Fe uptake and the activities of APX, GPX, CAT, and SOD were investigated (Rivero *et al*., 2003). The lack of Fe increased the production of active oxygen types, thus provoking oxidative stress and lipid peroxidation. Therefore, the activity of increased (Donnini *et al*., 2011; Tewari *et al*., 2005). Fe-deficiency in sunflower caused oxidative stress and decreased the activities of POD and APX. Decreased Fe concentration in plant tissue reduced Fe-SOD activity in citrus, peas, and tobacco. Moreover, decreased Fe concentration in the leaves of strawberry, maize, and cauliflower changed the activities of catalase and peroxidase. It also increases active types of oxygen and SOD (Tewari *et al*., 2005). Therefore, bicarbonate leads to oxidative stress in two ways: disrupting oxidation-reduction reactions (Klimov *et al*. 1995) and provoking Fe-deficiency in plants and thereupon oxidative stress.

The severe deficiency of Fe is not due to OH production; it usually provokes lipid peroxidation, though the concentrations of H_2O_2 and O_2 and the activity of SOD increase. Iturbe-Ormaexte *et al*. (1995) stated that H_2O_2 is accumulated in plant leaves treated with bicarbonate. In an experiment on peach seedlings, Fe in the leaves and roots as well as the activity of Fe-reductase in the root reduced strongly under the lack of Fe and the presence of bicarbonate. Fe deficiency increased superoxide-dismutase activity, whereas the activity of catalase reduced in the roots.

5. Conclusion

Oure results showed that Fe-efficient and non-Fe-efficient varieties have different reactions to soil bicarbonate. In both varieties, with increasing the bicarbonate concentration, the concentrations of Fe and active Fe in the shoot decreased, but Fe-efficient variety was more tolerant against this stress. Furthermore, bicarbonate affected shoot Zn concentration. Shoot Zn concentration decreased with increasing the bicarbonate concentration, but the Fe-efficient variety had a more Zn concentration in its shoot than the non-Fe-efficient variety. Fe fertilization increased its absorption by both varieties. Therefore, it is strongly recommended that Fe efficient cultivars be recognized at first, since the administration of Fe fertilizers is not a sustainable method. Recognition and utilization of these cultivars can be both eco-friendly and economical. Accordingly, to deal with soils with a high lime content and water sources with a high bicarbonate level, it is essential to utilize Fe efficient cultivars. The other important note is to consider ‘Active Iron Index’. This index, which evaluates Fe (II) in the plant, can very well display Fe deficiency. Therefore, farmers should put more importance on it.

References

Abadia, J., 1992. Leaf responses to Fe deficiency: a review. Journal of Plant Nutrition, 15; 1699-1713.

Abdelmajid, K., B.H. Karim, A. Chedly, 2008. Symbiotic response of common bean (*Phaseolus vulgaris* L.) to iron deficiency. J. Acta Physiol Plant. 30; 27–34.

Aebi, H., 1984. Catalase in vitro. Methods Enzymol., 105; 121–6.

Ali-ehyae, M., A.A. Behbahanizadeh, 2002. Method of soil Analysis, TAAT Publication, Bulletin No. 893, Tehran, Iran.

Barton, L.L., J. Abad, 2006. Iron nutrition in plants and rhizospheric microorganisms. Published by Springer 8; 23-59

Bertamini, M., N. Nadunchezhian, 2005. Grapevine Growth and Physiological Response to Iron Deficiency. J. Plant Nutr., 28; 737-749

Bradford, M.M, 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. J. Anal Biochem., 72; 248–54.

Bremner, J.M., 1996. Nitrogen-total. In: Methods of soil analysis part 3. (Ed.). D.L. Sparks. Am. Soc. Agron., 1085-1122.

Chapman, HD., 1965. Cation-exchange capacity. In: C.A. Black (ed.). Methods of soil analysis - Chemical and microbiological properties. Agronomy, 9; 891-901.

Colla, G., Y. Rouphael, M. Cardareill, A. Salerno, E. Rea, 2010. The effectiveness of grafting to improve alkalinity tolerance in watermelon. J. Environ Exp Bot., 68; 283-291.

Cuenca, M.C., F. Legaz, M.A. Forner-Giner, E. Primo-Millo, J. Iglesias, 2013. Bicarbonate blocks iron translocation from cotyledons inducing iron stress responses in Citrus roots. J. Plant Physiol., 170; 899-905.

Donnini, S., A. Castagna, A. Ranieri, G. Zocchi, 2009. Differential responses in pear and quince genotypes induced by Fe deficiency and bicarbonate. J. Plant Physiol., 166; 1181–1193.

Fageria, N.K. 2008. The use of nutrients in crop plants, CRC Press, 448 p.

Forno, DA., S. Youshida, C. J. Asher, 1975. Zinc deficiency in rice. II. Studies on two varieties differing in susceptibility to zinc deficiency. J. Plant Soil. 42; 551–563.

Frey, PA., G.H. Reed, 2012. The ubiquity of iron. ACS Chemical Biol., 7; 1477–1481.

Gee, G.W., J.M. Bauder, 1986. Partical-size analysis. p. 383-411.In Methods of Soil Analysis, Part 1, Physical and Mineralogical Methods. Agronomy Monogroph No. 9 (2nd edition), American Society of Agronomy, Madison, WI.

Hajiboland, R., X.E. Yang, V. Romheldand, G. Neumannc, 2005. Effect of bicarbonate on elongation and distribution of organic acids in root and root zone of Zn-efficient and Zn-inefficient rice (*Oryza sativa L.*) genotypes. J. Environ Exp Bot., 54; 163-173.

Ishwar, H., K. Boodi-Santosh, D. Basavaraj, K. Gopalakrishna, P. Virupakshi-Chimmad, A. Kanatti, V. Kumar, M.K. Debnath, 2016. Morpho-Physiological parameters associated with iron deficiency chlorosis resistance and their effect on yield and its related traits in groundnut, J. Crop Sci. Biotech., 19 (2); 177-187. DOI NO. 10.1007/s12892-016-0005-8

Iturbe-Ormaetxe, I., JFC. Moran, Y. Arrese-Igor, R.V. Gogorcena, M. Becana, 1995. Activated oxygen and antioxidant defences in iron-deficient pea plants. J. Plant Cell Environ., 18; 421–429.

Katyal, JC., B.D. Sharma, 1980. A new technique of plant analysis to resolve iron chlorosis. J. Plant Soil., 55; 103-119.

Klimov, V.V., S.I. Allakhverdiev, Y.M. Feyziev, S.V. Baranow, 1995. Bicarbonate requirement for the donor side of photosystem II. FEBS15399, 363; 251-255.

Koasch, H., M. Oktey, W. Hofner, 1984. Effect of iron chlorosis-inducing factors on the pH of the cytoplasm of sunflower. J. Plant Soil. 82; 215-221.

Kosegarten, U., B. Hoffmand, K. Mengel, 1999. Apoplasic PH, and reduction in intaac sunflower leaves. J. Plant Physiol., 121; 1069-1079.

Ksouri, R., M. Gharsalli, M. Lachaal, 2005. Physiological responses of Tunisian grapevine varieties to bicarbonate-induced iron deficiency. J. Plant Physiol., 162; 335-341.

Lamont, BB., 2003. Structure, ecology and physiology of root cluster-a review. J. Plant Soil. 248; 1-9.

Loeppert, RH., D.L. Suarez, 1996. Carbonate and gypsum. In: D.L. Sparks (Editor), Methods of Soil Analysis: Chemical Methods, Soil Science Society of America, Inc. and American Society of Agronomy, Inc., Madison, Wisconsin, USA, pp. 437–474.

Lombardi, L., L. Sebastianin, 2003. Physiological, biochemical, and molecular effects of in vitro induced iron deficiency in peach rootstock. J. Plant Nutr., 26; 2149-2163.

Mahmoudi, H., R. Ksouri, M. Gharsalli, K. Lachaal, 2005. Differences in responses to iron deficiency between two legumes: lentil (*Lens culinaris*) and chickpea (*Cicer arietinum*). J. Plant Physiol., 162; 1237-1245.

Manthey, J.A., D.L. Coy, D.E. Cloley, 1996. Root response of sterile grown onion plants to iron efficiency. J. Plant Nutr., 19; 145-141.

Malakouti, M.J., A.A. Shahabi, 2002. The role of bicarbonate on nutritional disorders of fruit trees, Sana Publication, Tehran, Iran.

Marschner, H., 1986. Adaptation of plants to adverse chemical soil conditions, In Mineral nutrition of higher plants, Academic Press, New York, NY, USA. 477-542.

Marschner, H., 1995. Mineral nutrition of higher plants, 2nd ed, Academic Press. New York, NY, USA.

Mengel, K., 1994. Iron availability in plant tissues – iron chlorosis on calcareous soils. J Plant Soil., 165; 275–283.

Mengel, K., R. Planker, B. Hoffmann, 1994. Relationship between leaf apoplast pH and iron chlorosis of sunflower (*Helianthus annuus L.*). J. Plant Nutr., 17; 1053–65.

Mengel, K., S. Schybert, 1985. Active extrusion of protons into deionized water by roots of intact maize plants. J. Plant Physiol., 79; 344-348

Miller, GW., A. Shigematsu, G.W. Welkie, N. Motoji, M. Szlek, 1990. Potassium effect on iron stress in tomato. II. The effects on root CO_2-fixation and organic acid formation. J. Plant Nutr., 13; 1355-1370.

Molassiotis, A., G. Tanou, G. Diamantidis, A. Patakas, I. Therios, 2006. Effects of 4-month Fe deficiency exposure on Fe reduction mechanism, photosynthetic gas exchange, chlorophyll fluorescence and antioxidant defense in two peach rootstocks differing in Fe deficiency tolerance. J. Plant Physiol., 163; 176–185.

Neaman, A., L. Aguirre, 2007. Comparison of different methods for diagnosis of iron deficiency in avocado. Journal of Plant Nutrition., 30; 1098 – 1108.

Nelson, EW., L.E. Sommers, 1996. Total Carbon, Organic Carbon, and Organic Matter. In Methods of Soil Analysis, Chemical Methods, Part 3,D L Sparks, editor. Soil Sci. Soc. of Am., Madison WI.

Nikolic, M., V. Romheld, 1999. Mechanism of Fe uptake by the leaf symplast: Is Fe inactivation in leaf a course of fe deficiency chlorosis*?*. J. Plant Soil. 215; 229-237.

Norvell, WA., M.L. Adams, 2006. Screening soybean cultivars for resistance to iron-deficiency chlorosis in culture solutions containing magnesium or sodium bicarbonate. J. Plant Nutr., 29; 1855–1867.

Olsen, SRC., V. Cole, F.S. Watanabe, L.A. Dean, 1954. Estimation of available phosphorus in soil by extraction with sodium bicarbonate, USDA. Cir. 939. Us Govern. Printing Office, Washington.

Oserkowsky, J., 1933. Quantitative relation between chlorophyll and iron in green and chlorotic leaves. J. Plant Physiol., 8; 449–68.

Panjtandoust, M., Sorooshzadeh, A., Ghanati, F., 2013. Influence of Iron Application Methods on Seasonal Variations in Antioxidant Activity of Peanut. Soil Sci Plant Anal., 44; 2118-2126.

Pestana, M., P. Correia, T. Saavedra, F. Gama, A. Abadia, A. Varennes, 2012. Development and recovery of iron deficiency by iron resupply to roots or leaves of strawberry plants. J. Plant Physiol Biochem., 53; 1-5.

Ramirez-Rodriguez, V., M.F. Nieto-Jacobo, J. Lopez-Bucio, L. Herrera- Estrella, 2001. Effect of citrate overproduction on iron nutrition in plants. J. Plant Nutr., 92(1); 44–45

Rao, K.V.M., T.V.S. Sresty, 2000. Antioxidant parameters in the seedlings of pigeon pea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. J. Plant Sci., 157; 113–128.

Rashid, A., J. Rafique, J. Din, S.N. Malik, M.Y. Arain, 1997. Micronutrient deficiencies in rain fed calcareous soil of Pakistan. I. Iron chlorosis in peanut plant. J. Plant Nutr., 28; 135-148.

Rhodes, JD., 1996. Salinity: Electrical Conductivity and Total Dissolved Solids .p. 417-435. In 138-Methods of Soil Analysis, Part 3, Chemical Methods. Soil Science Society of America, Inc., Madison, USA ,WI.

Rivero, R., J. Ruiz, L. Romero, 2003. Influence of temperature on biomass, iron metabolism and some related bioindicators in tomato and watermelon plants. J. Plant Physiol., 160; 1065–1071.

Romheld, V., 2000. The chlorosis paradox: Fe inactivation as a secondary event in chlorotic leaves of grape vine. Journal of Plant Nutrition, 13; 1629-1643.

Sadrarhami, A., A.H. Khoshgoftarmanesh, H.R. Sharifi, 2010. Using stress tolerance indicator (STI) to select high grain yield iron-deficiency tolerant wheat genotypes in calcareous soils. Field Crops Research, 1; 12-19

Sumner, ME., W.P. Miller, 1996. Cation exchange capacity, and exchange coefficients. In: D. L. Sparks (ed.) Methods of soil analysis. Part 2: Chemical properties, (3rd ed.) ASA, SSSA, CSSA, Madison, WI.

Sun, BT., Y. Jing, K.M. Chen, L.L. Song, F.J. Chen, L.X. Zhang, 2007. Protective effect of nitric oxide on iron deficiency-induced oxidative stress in maize (*Zea mays*). J. Plant Physiol., 164; 536–543.

Tagliavini, M., Abadia, J., Rombola, A.D., Abadia, A., Tsipouridis, C., B. Marangoni, 2000. Agronomic means for the control of iron deficiency chlorosis in deciduous fruit trees. Journal of Plant Nutrtion, 23; 2007-2022.

Tagliavini, M., A.D. Rombola, 2001. Iron deficiency and chlorosis in orchard and vineyard ecosystems. European Journal of Agronomy, 15; 71– 92. 55.

Tewari, R.K., P. Kumar, P.N. Sharma, 2005. Signs of oxidative stress in the chlorotic leaves of iron starved plants. J. Plant Sci., 16; 1037–1045.

Therios, I., V. Chouliaras, D. Bizas, S. Boukouvalas, E. Palioura, E. Hatzidimitriou, D. Basioukas, V. Gioldasis, N. Stavrou, 2005. Changes in leaf biochemical and physiological indices due to iron deficiency in citrus. AgroThesis., 1; 18 – 25.

Thomas, GW., 1996. Soil pH and soil acidity. In: D.L. Sparks (Editor), Methods of Soil Analysis: Chemical Methods. Soil Science Society of America, Inc. and American Society of Agronomy, Inc., Madison, Wisconsin, USA, pp. 475–490.

Tinker, P.B., A. Lauchli, 1984. Advances in plant nutrition; Academic Publisher: Dordrecht, The Netherlands.

Wilcox, JR., 1987. Soybean improvement, production and uses– 2d Ed. Agronomy monograph. No. 16. Wisconsin. USA.

Yang, XE., V. Romheld, H. Marschner, 1994. Effect of bicarbonate on root growth and accumulation of organic acids in Zinc efficient and Zn-efficient rice cultivars (*Oryza sativa* L.). J. Plant Soil. 164; 1–7.

Yang, XE., V. Romheld, H. Marschner, 1994. Micronutrient uptake by hybrid and conventional rice cultivars from different types of soils. J. Plant Nutr., 17; 319–332.

Yousfi, S., M. Rabhi, C. Abdelly, M. Gharsalli, 2009. Iron deficiency tolerance traits in wild (*Hordeum aritimum*) and cultivated barley (*Hordeum vulgare*). C. R. Biol., 332; 523–533.

Zhao, K., Y. Wu, 2017. Effects of Zn deficiency and bicarbonate on the growth and photosynthetic characteristics of four plant species. Plos One, 11; 1-14. DOI:10.1371/journal.pone.0169812.