Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2017 14(3): 525-531. **OPEN ACCESS**

Chelated Fe and Zn foliar spray improve the tolerance of kidney bean (*var. nebraska*) plants in salinized media

El-Zanaty Abd El-Motaleb Aly Abou-El-Nour[1], Mohamed Mostafa El-Fouly[1] and Zeinab Abd El-Rahman Salama[2]

[1]Fertilization Technology Department National Research Centre, 33 El-Bohouth St.,Dokki, Giza, P.O. 12622 - **Egypt**
[2]Plant Biochemistry Department National Research Centre, 33 El-Bohouth St.,Dokki, Giza, P.O. 12622 - **Egypt**

.

.***Correspondence:** elzanatyabouelnour@gmail.com **Accepted:** 05 June. 2017 **Published online:** 24 Sep. 2017

A pot experiment was conducted at the green house of the Fertilization Technology Department to explore the effect of foliar spraying of Fe chelate 0.13% and zinc chelate 12% with two levels of NaCl (0.00 - 1500 ppm) on kidney bean growth, Macronutrient and micronutrient concentrations, photosynthesis and carbonic anhydrase activity (CAA) were evaluated in the leaves of seedlings. Results showed that growth and nutrients concentration were reduced with NaCl .Potassium, Ca, P, N , Fe, Mn, Zn and Cu. Additionally, Chlorophyll a, Chlorophyll .b ,Total .Chlorophyll (a+b) and CAA were decreased with 1500 ppm NaCl. Foliar spraying with Fe chelate and Zn chelate affected stimulatory impacts on dry weight nutrient concentrations, and CAA after the salinization treatments. Therefore, It could be concluded that foliar spraying with Fe or Zn Chelate may increase kidney bean tolerance to salt stress, during early growth stages. Future studies are still needed to study the effect on late growth stages and yield.

Keywords: Zn chelate, Fe chelate, Kidney bean, Salt stress, seedlings

INTRODUCTION

Salinity is one of the key factors causing decreases in growth and productivity of almost all the crops (Szabolcs, 1994; Ashraf and Harris 2004; El-Nasharty et al. 2017). Rehm and Albert Sims (2006) reported that, micronutrients spraying led to increasing macro and micronutrients uptake as a result of improving root growth which consequently led to greater absorbing surface and nutrients uptake (Abdalla et al. 1992 and El Fouly et al. 2011).

Foliar application of nutrients can partially overcome the negative effect of stress conditions (Alpaslan et al.1999; El-Fouly and Salama, 1999; El-Fouly et al. 1997, 2002, 2004, 2010,2011&Salama et al. 2014, 2015).

Therefore, this study aimed to exploring the effects of Fe and Zn chelates foliar spray on kidney bean seedlings (Nebraska) grown in salinized media

MATERIALS AND METHODS

The present study was conducted under greenhouse conditions at the Fertilization Technology Department in National Research Centre, Cairo Egypt. Seeds of kidney bean (*var. Nebraska*) were obtained from Agricultural Research Center, were sown in Mitcherlich pots filled with 7 Kg soil. Soil analysis was done according to Ankerman and Large (1974) before fertilization (Table 1). Each pot received 0.57 g triple phosphate (37%), 1.3 g ammonium nitrate

Table 1. Soil analyses before sowing (0-30cm depth)

Characters		Macronutrient concentration (mgl100g)	
Sand %	88.8	Available – P	0.26
Silt %	4.00	Available - K	21.2
Clay %	7.2	Available - Mg	23.6
Soil Texture	Sandy	Available - Ca	143
pH	9.06	Available - Na	31.0
E.C dS/m	0.31	Micronutrient concentration (mg/kg)	
CaCO3 %	2.8	Available - Fe	3.1
O.M %	0.68	Available - Mn	2.9
		Available - Zn	0.8
		Available - Cu	0.6

(33% P_2O_5 N) and 0.7 g potassium sulfate (48 to 52% K_2O) before sowing to provide the recommended NPK for kidney bean (60-30-48). Twenty days after sowing, Plants were irrigated alternatively with saline and tap water. Level of NaCl used was1500 ppm and Fe chelated 13% and Zn chelated 12% were obtained from the pilot unit of Fertilization Technology Department, NRC. They were used to correct the nutrient imbalance caused by salt stress conditions. Micronutrient treatments were applied two times. First before salinity treatments (15 days after sowing) and the second one after salinity treatments (25 days after sowing). At flowering stage samples were taken for plant growth measurements and determination of nutrient contents according to (Chapman and Pratt 1978).

Chemicals:

Solvent and Kits were purchased from Sigma-Aldrich and Bio-diagnostic Company, Egypt were of analytical grade.

Parameters used for the investigation were:
Dry weight as indicator for early growth.
Macro and micronutrients content as indicator for nutrients status.
Chlorophyll as indicator for plant health
Carbonic anhydrase (CA) activity as biochemical indicator for diagnosis the ability of plants for withstanding salinity stress.

Nutrients content:
Nutrient contents were analyzed after digestion of plant samples with acid mixture (Nitric, Sulphoric and Perchloric). Macro and micronutrient concentrations K, Ca, Mg, Fe, Zn and Mn were analyzed in plant digests using Flame Photometer and atomic- absorption Spectrophotometry Perkin-Elmer. All macronutrients were expressed in g/1oo g DW and micronutrient concentrations were expressed in mg/ kg DW according to Chapman and Pratt, 1978.

Chlorophyll measurement:
Samples of 100 mg leaves were homogenized in chilled 80% (v/v) acetone and centrifuged at 1000 rpm for 10 min. at $4^{o}C$. Absorbance of the acetone extracts was measured at 664 and 648 according to Lichtenthaler, 1987.

Carbonic anhydrase activity assay (E. C. 4.2.1.1):
Leaves tissue (100 mg FW) were placed under ice cold conditions and homogenized with a buffered solution (pH 8.3) that contained 50 mm Veronal-H_2SO_4 and 0.2% (w/v) PVP under ice cold conditions. The homogenate was centrifuged at 12000 g for 2 min. and the supernatant was used for determination of CA activity according to Ohki, 1978.

Statistical analysis:
Data were statistically analyzed using Costat statistical package (Anonymous, 1989)

RESULTS AND DISCUSION

Soil analysis:
As shown in Table (1), the evaluation of the cultivated soil according to Ankerman and Large, 1974. The soil is sandy in texture with high pH and low salinity and organic matter. Calcium carbonate is medium. The soil is low in P and Mg while both K and Na were moderate. All micronutrients were low in their concentrations.

Effect of Fe and Zn- chelate foliar spray on plant dry weight of bean seedlings grown under salinity conditions:

Data presented in Table (3) show that plant dry weight did not significantly increase by spraying the plants with either Fe or Zn as compared with control plants. However, drastic depression in dry weight was noticed (5.51 g) due to salinity as compared with control treatment (12.59 g). Under salinity conditions, spraying plants with Fe recovered the drastic depression and the dry weight reached to about nearly double the weight of the stressed plants. The same trend was noticed due to spraying the stressed plants with Zn. Foliar spraying with Fe and Zn could partially counteract the negative effect of NaCl on nutrients may be through improving root growth and prevented the nutritional disorders and consequently, caused increases for the uptake of nutrients by the roots (El-Fouly et al. 2002). These findings are in agreement with those obtained by (Din and Flowers, 2002; Al-Ansari, 2003; Morant et al., 2004; Salama et al., 2014 &2015). Salinity increases osmotic pressure of soil solution of the root zone. This leads to reduction in growth and productivity (Abou- Hadid, 2003).The stimulation effects of applying zinc and iron on vegetative growth may be attributed to their functions. Zinc is a component of carbonic anhydrase, as well as several dehydrogenises, besides the function of zinc in CO_2 assimilation (Curie and Briat, 2003). Iron is important for leaf growth, cell number, size and cell division, as well as chlorophyll, protein, starch and sugar biosynthesis (Marschner, 1995)**.**

Effect of Fe and Zn- chelate foliar spray on micronutrients content in leaves of bean seedlings grown under salinity conditions

Data presented in Table (2) show that under normal soil condition, spraying bean plants with Fe showed marked increments in Fe concentration. On the other hand, did not significantly affect the other micronutrients i.e. Mn, Zn and Cu. While, spraying the plants with Zn significantly increased both Zn and Cu concentrations. However, under salinity condition, plants showed increments in Fe, Mn, and Zn concentrations as compared with control plants. These increments may be attributed to that salinity induce decreases in soil pH which increase the availability of micronutrients uptake, (El-Fouly et al. 2002and 2010). Spraying plants grown under salinity with Fe resulted in significant increments in both Fe and Zn concentrations as compared with control plants. Moreover, spraying the aforementioned plants with Zn showed the same trend as in case of spraying plants with Fe and resulted in increasing Cu concentration. In other words, foliar spraying with Fe and Zn could partially counteract the negative effect of NaCl on nutrients through improving root growth and prevent the nutritional disorders and consequently, caused increases in the uptake of nutrients by the roots (El-Fouly et al. 2002).

Effect of Fe and Zn- chelate foliar spray on macronutrients content in leaves of bean seedlings grown under salinity conditions:

From data presented in table (2) it is clear that spraying plants grown under normal conditions with Fe, increased N, P, Mg and Na. These increments were significant only with Mg. However, both K and Ca were significantly decreased. Moreover, spraying plants grown under normal conditions with Zn resulted in marked increases in both N and P. This treatment also resulted in marked decrement in Ca concentration. Under salinity stress condition, all macronutrients showed decrements in their concentrations except Na which showed significant increment. The decrements were significant in case of N, K, and Ca and Mg concentrations. Under salinity conditions, nutrient imbalance within plant organs appears i.e. increase in the content of Na and decrease in the essential nutrient contents, like K, Mg and Ca (Chen et al., 2007 and El-Fouly et al., 2011). Another reason for the reduction in uptake of nutrients under saline condition may be due to the Na induced blockage or reduced activity of the transporters, resulting in ionic imbalance of K , Ca and Mg as compared to Na . Several researchers (Alam, 1999; Ashraf and Harris, 2004;; Chow et al. 1990 and Botella et al. 1997) who report that imbalances may result from the effect of salinity on nutrient availability, competitive uptake, nutrient transport. Spraying plants grown under salinity stress with Fe resulted in a significant increase in N concentration and also significant decrease in Na concentration. Moreover, spraying the aforementioned plants with Zn showed significant increments in N, Ca and significant decrement in Na concentration. The positive effect of micronutrient treatments on plants grown under salinity could be explained by the decreases in uptake of Na and its translocations from old leaves to young leaves as a result of increasing membrane integrity by Zn treatment (Alpaslan et al. 1999).

Table 2. Effect of Fe and Zn- chelate foliar spray on dry weigh and micronutrients content in leaves of bean seedlings grown under salinity conditions

Treatment	Micronutrient (ppm)				Dry Weight
	Fe	Mn	Zn	Cu	(g/pot)
Control	243	58	96	14	12.59
Control + Fe	327	56	94	13	12.38
Control + Zn	286	51	99	15	12.33
Salinity	369	65	102	13	5.51
Salinity + Fe	263	47	99	19	10.47
Salinity + Zn	362	46	109	23	6.60
LSD 5%	17	7	7	2	0.22

Table 3. Effect of Fe and Zn- chelate foliar spray on macronutrients content in leaves of bean seedlings grown under salinity conditions

Treatment	Macronutrient (%)					
	N	P	K	Ca	Mg	Na
Control	3.28	0.26	3.66	3.42	0.94	0.11
Control + Fe	3.47	0.27	3.54	2.93	1.14	0.12
Control + Zn	3.67	0.33	3.50	2.39	1.02	0.17
Salinity	2.55	0.24	3.56	2.86	1.07	0.37
Salinity + Fe	3.34	0.22	3.54	2.53	1.08	0.25
Salinity + Zn	3.16	0.28	3.53	2.86	1.02	0.18
LSD 5%	0.22	0.05	0.08	0.06	0.09	0.04

Effect of Fe and Zn-chelate foliar spray on chlorophyll content in leaves of bean seedlings grown under salinity conditions

Data presented in Table (4) show that under normal condition, spraying either Fe or Zn increased significantly Chl.a. However, spraying plants with Fe or Zn did not significantly affect Chl. B. Concerning total Chl., only iron foliar spray markedly increased total chlorophyll. On the other hand, under salinity stress, Chl. a, Chl.b and Total Chl. showed significant decrease as compared with those grown under normal condition. These decreases could be recovered by spraying plants with either Fe or Zn, except in case of Chl. B, where the increment recorded was insignificant. These results are in a agreement with earlier observations in wheat and /or faba bean seedlings grown under salinity stress conditions (El-Fouly et al., 2002 & 2004)

Effect of Fe and Zn-chelate foliar spray on carbonic anhydrase activity in leaves of bean seedlings grown under salinity conditions:

Table (4) shows that under normal growth condition, spraying bean plants with Fe significantly increased carbonic anhydrase activity. However, spraying Zn did not affect the activity of the enzyme. Under salinity stress the enzyme activity showed dramatic reduction as

Table 4. Effect of Fe and Zn- chelate foliar spray on chlorophyll content and carbonic anhydrase activity in leaves of bean seedlings grown under salinity conditions

Treatment	Chl. a mg/g (fw)	Ch. B mg/g (fw)	Total Chl mg/g (fw)	CAA EU/g (fw)
Control	2.19	0.99	3.18	204
Control + Fe	2.73	1.02	3.75	216
Control + Zn	2.22	0.99	3.21	207
Salinity	1.75	0.78	2.53	82
Salinity + Fe	2.29	1.26	3.55	89
Salinity + Zn	2.22	0.82	3.04	215
LSD 5%	0.02	0.17	0.17	9

compared to control plants. Spraying the salinity stressed plants with Fe did not significantly increase the enzyme activity. However, spraying the stressed plants with Zn showed marked increase in enzyme activity as compared with either control or stress conditions. Salt stress reported to damage the photosynthetic machinery at multiple levels such as pigments, stomatal functioning and gaseous exchange, structure and function of thylakoid membrane, electron transport and enzymes (Sudhir and Murthy, 2004). Excess salts cause the closure of stomata, thereby decreasing the partial CO_2 pressure (Bethkey and Drew, 1992) as well as internal CO_2 concentration and consequently, the activity of carbonic anhydrase because its activity is regulated by to large extent CO_2 concentration (Tiwari et al , 2005). Sudhir and Murthy, 2004 also reported that the photosynthetic carbon absorption prose in bean was observed to be fundamentally discouraged by NaCl salt stress. This might be due to the diminishing the inner CO_2 fixation and hence catalyst the activity of carbonic anhydrase (Bethkey and Drew, 1992). Khan et al. 2003 was in agreement with the obtained results.

CONCLUSION

The presented results indicate that, foliar application of chelated micronutrients could be useful for improving the nutrient status, and physiological performance of bean plant during early growth stages under salinity stress conditions

.

CONFLICT OF INTEREST

The authors declared that there was no conflict of interest.

ACKNOWLEGEMENT

This study was done in the frame of the cooperation between National Research Centre (NRC) and Popov Institute for Plant Physiology Sofia-Bulgaria (Egyptian Academy of Science and Technology-Bulgarian Academy of Science). It was supported by the Egypto-German Project “Micronutrient and other Plant Nutrition Problems” implemented by the National Research Centre (NRC) The project was supported by the Egyptian Academy of Science and Technology and the German Federal Ministry for Technical Cooperation (BMZ) through the German Agency For Technical Cooperation (GTZ).

AUTHOR CONTRIBUTIONS

All authors contributed to the design of the experiment, EAAE proformed the experiment work, ZAS carried out laboratory and statistical analysis, MME wrote the manuscript. All authors approved the final version.

REFERENCES

Abdalla FE, Mobarak, ZM and El-Sayed AA, 1992.Effect of micronutrients foliar application on uptake of macronutrients by wheat and faba bean. Afr. J. Agric. Sci., 19 (1) : 181-192

Abou- Hadid AF, 2003. The use of saline water in agriculture in the Near East and North Africa Region: present and future. J. Crop Prod., 7 (1/2) : 299-323.

Alam, SM. 1999. Nutrient uptake by plants under stress conditions.In :Pssarakli M (Ed.).

Handbook of Plant and Crop Stress. Marcel Dekker, New York; Basel, pp. 285-313.

Al-Ansari, FM., 2003.Salinity tolerance during germination in two arid land varieties of wheat (*Triticum aestivum* L.). Seed Sci. Technol., 31: 597-603.

Alpaslan, M. Inal A. Gunes, A., Cikili, V and Ozcan, H 1999.Effect of zinc treatment on the alleviation of sodium and chloride injury in tomato Lycopersicum esculentum L. Mill. cv. Lale grown under salinity. Turkish Journal of Botany, 23(1): 1-6.

Ankerman, D and Large, R 1974. Soil and plant analysis. Tech. Bull. A & L. Agricultural laboratories. Inc., New York, USA, pp. 42-44 and 74-76.

Anonymous, A, 1989. Cohort Soft Ware Corp. Co-stat user manual version 3.03, Barkley Ca, USA,pp 305.

Ashraf M and Harris PJC, 2004.Potential biochemical indicators of salinity tolerance in plants. Plant Sci., 166: 3-16.

Bethkey, PC and Drew, MC. (1992): Stomata and non-stomata components to inhibition of photosynthesis in leaves of Capsicum annum during progressive exposure to NaCl salinity. Plant physiology, 99: 219-226.

Botella, M.A., Martinez, V and Pardines, JC, 1997. Salinity induced potassium deficiency in maize plants. J. Plant Physiol., 150: 200-205.

Chapman, HD. and Pratt, PF, 1978. Methods of Analysis for Soils, Plants and Waters. 1st Edn., Division Agric. Sci., Univ. California, Berkeley, U.S.A. pp: 309.

Chow W S, Ball MC and Anderson JM, 1990. Growth and photosynthetic responses of spinach to salinity .Implications of K+ nutrition for salt tolerance. Australian J. Plant Physiol., 17: 563-578.

Curie C and Briat JF, 2003. Iron transport and signaling in plants. Ann. Rev. Plant Biol., 54: 183-206.

Din J and Flowers TJ, 2002. Effect of ABA seed pre- treatment on the response of wheat (*Triticum aestivum* L.) to salinity, with special reference in plant growth, ion relations and protein patterns.Prospects for Saline Agriculture.pp. 154- 153.

El-Fouly MM, Abou El-Nour EAA and Abdel-Maguid AA, 2004.Counteracting effect of foliar application of macronutrients on spinach beet (*Beta vulgaris* var. cycla) grown under NaCl-salinity stress. Bull. Fac. Agric. Cairo Univ., 55: 587-602.

El-Fouly MM, ZM and Salama ZA, 1997. Comparative study on the effect of chelated multi micronutrient compounds on the growth and nutrient uptake in some plants. Egypt J. Physiol. Sci., 21: 447-458.

El-Fouly, M.M., Mobarak, Z.M. and .Salama, Z.A, 2002. Micronutrient foliar application control salination for horticultural productivity. Acta Hort., 573: 377-385.

El-Fouly, MM. and Salama, ZA, 1999. Can foliar fertilization increase plant tolerance to salinity? Proc. Dahlia Greidinger International . Symposium, Nutrient management under salinity and water stress, 3(1-4):113-125.

El-Fouly, MM., Mobarak, ZM. and Salama, ZA., 2010.Improving tolerance of faba bean during early growth stages to salinity through micronutrients foliar spray. Not. Sci. Biol., 2: 98-102.

El-Fouly, MM.,Mobarak, ZM.and Salama, Z.A, 2011. Micronutrients (Fe, Mn, Zn) foliar spray for increasing salinity tolerance in wheat *Triticum aestivu*m L. African Journal of Plant Science, 5: 314-322

El-Nasharty A.B. Sona Salem El-Nwehy, AbdElHalim Ibrahim Rezk and El-Zanaty Abd El-Motaleb Aly Abou El-Nour. 2017. Role of kinetin in improving salt tolerance of two wheat cultivars. Bioscience Research, 14(2): 193-200.

Khan, N. Prithviraj, B and Smith, D.L, 2003.Photosynthetic responses of corn and soybean to foliar application of salicylates. Journal of Plant Physiology,160: 485-492.

Lichtenthaler, HK, 1987. Chlorophyll and carotenoids: Pigments of photosynthetic biomembranes. Methods in Enzymology, DOI 10.1019/0076-8679 (87) 48036-1,148:350-382.

Marschner, H, 1995. Mineral Nutrition of Higher Plants, 889 pp. 2nd Ed. .Academic Press, San Diego, CA.

Morant-Manceau A, Pradier E, Tremblin G, 2004. Osmotic adjustment, gas exchanges, and chlorophyll fluorescence of hexaploidtritical and its parental species under salt stress. J. Plant Physiol., 13: 143-160.

Rehm G and Albert S, 2006. Micronutrients and Production of Hard Red Spring Wheat.Minnesota Crop News. March 7, 2006 p: 1-3.Morant-Manceau, A., E. Pradier and G.

Salama, Zeinab A., Abou El-Nour E A.A. El- Fouly MM and Gaafar A, 2014. Ascorbic acid foliar spray counteracting effect of salinity on growth, nutrients concentrations, photosynthesis, antioxidant activities and lipid peroxidation of bean (*Phaseulus vulgaris* L.) cultivars. American Journal of Agriculture and Biological Science, 9 (3): 384-393.

Salama, Zeinab A., El Fouly MM and Gaafar, AA, 2015. Role of chelated Zinc (Zn-HEDTA) foliar spray in regulation salt stress in maize plants. Analele Universităţii din Oradea, Fascicula Biologie Tom. XXII, Issue: 1, 2015, pp. 7-12

Sudhir, P and Murthy, SDS, 2004. Effects of salt stress on basic process of photosynthesis.Photosynthetica, 42:481-486.

Szabolcs I, 1994.Soils and salinization.In Handbook of Plant and Crop Stress.Ed. M Pessarakali. pp. 3–11. Marcel Dekker, New York.