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Effect of Biofertilizers, Mycorrhiza and Foliar Spraying of some Micronutrients (Fe+ Mn+ Zn) and Potassium Silicate on Enhancing Salt Tolerance of Wheat Plant

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ABSTRACT

Two field experiments were conducted during 2009/2010 and 2010/2011 winter seasons to study the effect of biofertilizers (*Azotobacter sp, Bacillus megatherium*, *Bacillus circulans* "10^8 cfu./ml", mix of the three bacteria (1:1:1), mycorrhiza "10^4 spore/ml), micronutrients (Fe+ Mn+ Zn "25 & 50 ppm) and potassium silicate (200& 400 ppm) on growth and yield parameters and some biochemical constituents of two wheat (*Triticum aestivum* L.) cultivars Sakha 93 "Salt tolerance" and Giza 168 "salt sensitive" under salt stress. Two samples were taken at 90 and 105 days after sowing. At the 1st sample date leaves number per plant, leaf area, shoot fresh weight, shoot dry weight, total soluble carbohydrates, total sugars, Mg, Ca, Fe, Mn, Zn, Na concentrations as well as Mg:Na ratio and Ca:Na ratio (in wheat leaves) were determined. At the 2nd sample date (harvesting stage), spikes number per plant, main spike length, main spike weight and grains number per spike were recorded. Mycorrhiza treatment was strongly increased flag leaf area, shoots fresh and dry weights. Combined of bacteria with potassium silicate mitigated the adverse effect of salinity. In general, a significant increase in biochemical constituents (total soluble carbohydrates and total soluble sugars) and mineral nutrients (Ca, Mg, Zn, Mn and Fe) as well as Ca:Na and Mg:Na ratios could be considered as indicator for enhancing in salt tolerant in plant. Sakha 93 cv. has shown higher response than Giza 168 cv. under different treatments.

Key words: Wheat, *Triticum aestivum* L., Biofertilizers, Mycorrhiza, Micronutrients, Potassium silicate, Salt tolerance.

Introduction

Wheat (*Triticum aestivum* L.) is the most important cereal crops in Egypt and increasing wheat production is an essential national target to fill the gap between production and consumption. There is insufficient fresh water to develop all potential arable land. So, the use of saline water in agriculture is a subject of vital importance to cultivate arid and semi-arid lands (Tawfik *et al.,* 2006).

Salinity affects many morphological, physiological and biochemical processes, including plant growth and nutrient uptake (Willenborg *et al*., 2004). The most efficient strategy to solve the problem of salinity in developing countries is to improve the salt tolerance of crop plants because increasing the salt tolerance is much less expensive for poor farmers than using other management practices (Qureshi and Barrett, 1998). In recent years, great attention was paid for using biological application (mycorrhizal symbiosis) to alleviate soil stresses including salinity (Ibrahim *et al*., 2011; Abdel-Fattah and Asrar, 2012).

To minimize the harmful effect of salinity, using the foliar feeding of nutrients for increasing plant salinity tolerance by alleviating Na^+ and Cl^- injury to plants (El-Fouly *et al.,* 2002).

Foliar application of micronutrients generally is more effective, less costly and accepted practice for many crops. In this respect, spraying of micronutrients to plants grown on some soil of Egypt, gave better growth and more yield (El-Desuki *et al*., 2010).

The use of biofertilizers has mainly benefits such as nitrogen fixation, mobilizing phosphate and micronutrients through the production of organic acids and lowering soil pH (Saber, 1993). Moreover, microorganisms such as *Pseudomonus, Azotobacter, Azospirillium* and mycorrhiza are known as plant growth promoting rhizobacteria (PGPR) and produce gibberellins, cytokinins like substances and auxins (Hartmann *et al.,* 1983). Arbuscular mycorrhizal fungi (AMF) plays vital role to alleviating salt stress of host plants (Evelin *et al*. 2009).

AMF is known to exist in saline soil, and participates in the plant growth and development, and also improves the plant tolerance against biotic and abiotic stress (Abdel-Fattah *et al*., 2010) by regulating the physiological and biochemical process of plants (Fernanda *et al*., 2012).

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Silicon (Si) is beneficial mineral nutrients and it have been studied to counteract the adverse effects of salt stress (Epstein, 1999). On the other hand wheat is also classified as Si accumulator. It is evident that Si is beneficial for the growth of many plants under various abiotic (salt, drought and metal toxicity) or biotic (plant diseases and pests) stresses (Liang *et al.,* 2003a, b; and Ma, 2004).

This study aimed to investigate the effect of inoculation with some biofertilizers (*Azotobacter chroococcum, Bacillus megatherium* and *Bacillus circulans*) individually or in combination, arbuscular mycorrhizal fungi, foliar spraying of micronutrients (Fe + Mn + Zn) and potassium silicate on wheat plant growth, yield and chemical constituents under irrigation of saline water.

Materials and Methods

Two field experiments (November 2009/2010 & 2010/2011) were performed in new reclaimed sandy soil at 64 Km from Cairo–Alexandria desert road to study the effect of inoculation of some biofertilizers, arbuscular mycorrhizal fungi, foliar spraying of micronutrients (Fe, Mn, Zn) and potassium silicate on growth, yield and chemical constituents of wheat plants under salt stress conditions (Saline ground water 4000 ppm + 1300 ppm in soil) . Wheat cultivars kindly obtained from Agricultural Research Center, Ministry of Agric., Dokki, Cairo, Egypt.

Plant material and treatments

The experiment was arranged in complete randomized block design with three replicates. In both seasons, grains of wheat cultivars (Sakha 93 and Giza 168) were sown on 15th November in sandy soil. The plot area was 2 x 2 m. The grains were sown at one side of the rows with 13 cm between hills. Plants were thinned at 3 weeks after sowing to 4 plants / pit. Saline ground water was used for irrigation.

Treatments were as follows:

1. *Azotobacter chroococcum.* (10^8 cfu./ml)
2. *Bacillus megatherium.* (10^8 cfu./ml)
3. *Bacillus circulans.* (10^8 cfu./ml)
4. *Azotobacter chroococcum* + *Bacillus megatherium* + *Bacillus circulans* by ratio (1:1:1). (10^8 cfu./ml)
5. Mycorrhiza *(Glomus sp).* (10^4 spores/ml)
6. Fe + Mn + Zn 25 ppm (1:1:1).
7. Fe + Mn + Zn 50 ppm (1:1:1).
8. Potassium silicate 200 ppm.
9. Potassium silicate 400 ppm.
10. Saline ground water (control).

Wheat grains were mixed with individual bacteria suspension (*Azotobacter chroococcum, Bacillus megatherium, Bacillus circulans* individual or combination) and mycorrhizal fungi before sowing. The number of cells in bacterial suspension was 10^8 cfu./ ml but reach to 10^4 spores per ml for mycorrhizal fungi (Swedrzynska, 2000). The first application was presowing grains treatment and the second one was at 60 days from sowing as soil application.

Micronutrients mixture, Fe + Mn + Zn (1:1:1) at 25 & 50 ppm and potassium silicate at 200 & 400 ppm were applied as foliar spraying each two weeks intervals.

Growth and yield parameters

Plant samples were taken after 90 days from planting. The recorded growth parameters of wheat included, leaves number per plant, leaf area (cm^2), shoot fresh weight, shoot dry weight. Moreover, spikes number per plant, spike length and spike weight in both seasons.

Chemical analyses.

At 90 days after sowing, leaf samples of wheat plants were collected to determine total soluble sugars and total soluble carbohydrates concentrations and mineral nutrients (Mg, Ca, Fe, Mn, Zn and Na) concentrations in addition to Ca:Na and Mg:Na ratios.

Extraction and determination of total soluble sugars and carbohydrates

Total soluble sugars and carbohydrates were extracted according to the method described by (A.O.A.C., 1990).

Total soluble sugars and carbohydrates were estimated according to Ackerson, (1981); Chow and Landhausser (2004).

Nutritional elements

Leaf samples were taken for nutritional studies 0.1 gram samples of ground plant materials were wet digested using (H_2SO_4/H_2O_2) mixture as described by Cottenie (1980).

- The concentrations of sodium was determined using flame photometer as described by Eppendorf and Hing (1970).
- The concentrations of calcium, magnesium, iron, manganese and zinc were determined using atomic absorption (Chapman and Pratt, 1960).

Experimental design

Experiments were complete randomized block design with three replicates. The statistical analysis of data was done by SAS (1996). for separation between means, tukey test with the following model was applied

$$Yijk = \mu + Ti + Si + (T*S)\ ij + eijk$$

Results and Discussion

Growth parameters and yield

Data presented in Table (1) shows the effect of biofertilizers, micronutrients and potassium silicate treatments on leaves number/ plant and flag leaf area of wheat cultivars in both seasons. Regarding the leaves number per plant, data showed stimulated effect with all treatments and cv. Sakha 93 comparing with the control and cv. Giza 168 in the first season. The superiority was due to the potassium silicate treatment at 400 ppm. On the other hand, no significant differences were noticed in leaves number per plant by individual bacteria treatments, mix of micronutrients (Fe, Mn, Zn) and cultivars as compared to the control at 90 days after sowing in the second season.

Table 1: Effect of biofertilizers, micronutrients and potassium silicate treatments on leaves number per plant and flag leaf area of wheat plants at 90 days after sowing in both seasons under salt stress.

Treatments	1 St		2 nd		1 St		2 nd	
	Leaves no./plant				Flag leaf area			
	S 93	G 168	S 93	G 168	S 93	G 168	S 93	G 168
Control	5.00 ^{bc}	4.67 ^{c}	4.67 ^{ab}	4.00 ^{b}	33.55 ^{ab}	28.17 ^{b}	37.75 ^{bc}	22.03 ^{c}
Azotobacter sp (10^8 cfu./ml)	6.67 ^{a}	6.33 ^{a}	5.00 ^{ab}	4.67 ^{ab}	44.08 ^{ab}	46.01 ^{ab}	46.57 ^{ab}	43.15 ^{ab}
Bacillus megatherium (10^8 cfu /ml)	6.67 ^{a}	6.00 ^{ab}	5.00 ^{ab}	5.00 ^{ab}	44.37 ^{ab}	42.97 ^{ab}	46.20 ^{ab}	46.62 ^{ab}
Bacillus circulans (10^8 cfu./ml)	7.00 ^{a}	6.33 ^{a}	5.00 ^{ab}	5.00 ^{ab}	52.35 ^{a}	51.27 ^{a}	55.85 ^{a}	40.20 ^{ab}
Mix of three bacteria (1 : 1 : 1)	7.00 ^{a}	6.67 ^{a}	5.67 ^{ab}	5.33 ^{ab}	45.17 ^{ab}	44.65 ^{ab}	49.60 ^{ab}	56.75 ^{a}
Mycorrhiza (10^4 spore/ml)	6.67 ^{a}	6.00 ^{ab}	6.00 ^{a}	5.00 ^{ab}	43.25 ^{ab}	44.34 ^{ab}	54.00 ^{ab}	52.17 ^{ab}
Fe+ Mn+Zn (25 ppm)	6.00 ^{ab}	6.33 ^{a}	5.00 ^{ab}	5.00 ^{ab}	39.42 ^{ab}	44.25 ^{ab}	44.87 ^{ab}	38.27 ^{bc}
Fe+ Mn+Zn (50 ppm)	6.67 ^{a}	6.00 ^{ab}	5.67 ^{ab}	5.00 ^{ab}	44.55 ^{ab}	51.50 ^{a}	46.78 ^{ab}	47.07 ^{ab}
K_2O. $4SiO_2$ (200 ppm)	6.00 ^{ab}	6.67 ^{a}	5.33 ^{ab}	5.67 ^{ab}	44.10 ^{ab}	46.00 ^{ab}	47.15 ^{ab}	44.85 ^{ab}
K_2O. $4SiO_2$ (400 ppm)	7.00 ^{a}	7.00 ^{a}	6.00 ^{a}	6.00 ^{a}	46.67 ^{ab}	47.69 ^{ab}	50.83 ^{ab}	52.83 ^{ab}
MSD T at 5%	0.79		1.09		12.97		10.37	
MSD V at 5%	0.21		NS		NS		2.80	
MSD T x V at 5%	1.26		1.71		20.75		16.59	

S = Sakha G= Giza NS= Non significant

Regarding the effect of biofertilizers, micronutrients and potassium silicate treatments on flag leaf area of wheat plants, an increase in flag leaf area was obtained *via* both seasons at 90 days after sowing. All treatments had a significant increase in mean values of flag leaf area when compared with control in both seasons except *Bacillus megatherium*; mycorrhiza and Fe + Mn + Zn at 25 ppm which had insignificant values in the first season. Generally, the data showed that cultivar Sakha 93 produced the highest significant value of flag leaf area as compared to cv. Giza 168 in the second season.

Biofertilizers, micronutrients and potassium silicate treatments gave significant increase in shoot fresh and dry weights as compared to the control in both seasons. The highest values of shoot fresh weight were observed with mix of bacteria, mycorrhiza and potassium silicate at 400 ppm as compared to the control and rest treatments in both seasons. Concerning shoot dry weight, the superiority was due to the *Azotobacter* and mycorrhiza treatments as compared to control and other treatments in both seasons (table 2).

Generally, Sakha 93 cv. had the highest significant value of shoot fresh and dry weights as compared to Giza 168 cv. in both seasons.

Concerning yield

All treatments significantly increased spikes number per plant except *Bacillus* species; mycorrhiza and Fe + Mn + Zn at 25 ppm treatments gave insignificant values as compared to the control in the second season (Table 3). In general, the highest significant values of spikes number per plant were observed by potassium

silicate at 400 ppm in both seasons. Cv. Sakha 93 showed a significant increase in this regard as compared to cv. Giza 168 in both seasons.

Table 2: Effect of biofertilizers, micronutrients and potassium silicate treatments on shoot fresh and dry weight of wheat plants at 90 days after sowing in both seasons under salt stress.

Treatments	1 St		2 nd		1 St		2 nd	
	Shoot f.w.				Shoot d.w.			
	S 93	G 168	S 93	G 168	S 93	G 168	S 93	G 168
Control	37.9 gf	33.62 g	40.25 I	37.00 I	8.65 hi	7.77 I	9.47 hi	8.70 i
Azotobacter sp (10^8 cfu./ml)	57.69 b-d	53.26 d-f	62.28 ef	57.78 G-f	23.15 a	19.97 c-e	25.50 a-d	23.86 c-e
Bacillus megatherium (10^8 cfu./ml)	55.15 b-d	51.03 d-f	68.4 de	60.2 e-f	16.65 e-f	14.57 e-f	20.96 a-f	18.17 d-f
Bacillus circulans (10^8 cfu./ml)	52.01 d-f	49.54 d-f	70.75 cd	61.67 ef	22.30 ab	20.12 b-d	26.20 a-c	22.32 c-e
Mix of three bacteria (1 : 1 : 1)	88.64 a	80 23 a	98.17 a	90.16 a	22.32 ab	20.03 b-d	25.20 a-d	22.86 c-e
Mycorrhiza (10^4 spore/ml)	89.71 a	76.33 ab	101.03 a	85.99 b	23.58 a	19.55 c-e	28.66 a	22.50 c-e
Fe+ Mn+Zn (25 ppm)	51.17 d-f	46. 05 d-f	53.25 gh	51.03 gh	14.77 e-f	13.58 ef	16.30 e-f	15.42 e-f
Fe+ Mn+Zn (50 ppm)	59.75 b-c	53.74 d-f	67.00 de	61.29 ef	19.48 bc	17.04 e-f	22.45 a-e	18.47 e-f
$K_2O.\ 4SiO_2$ (200 ppm)	49.62 d-f	46.81 d-f	60.2 g-f	54.55 gh	13.13 e-f	11.83 f-i	14.83 e-f	13.40 f-h
$K_2O.\ 4SiO_2$ (400 ppm)	72.37 ab	64.15 bc	73.95 b-d	70.16 cd	22.35 ab	18.52 c-e	27.20 ab	20.27a-f
MSD T at 5%	10.84		9.77		4.55		5.29	
MSD V at 5%	2.93		2.64		1.23		1.43	
MSD T x V at 5%	17.35		15.64		7.28		8.46	

S = Sakha G= Giza

The effect of biofertilizers, micronutrients and potassium silicate treatments on spike length, revealed that all treatments produced a significant increase in this respect comparing with control at 105 days after sowing in the first season (Table 3). Similar trend in spike length was observed with all treatment in the second season except Fe + Mn + Zn at 25 ppm induced insignificant value as compared to the control. In general, *Bacillus circulans* produced the highest significant value comparing with the rest biofertilizers treatments. No significant differences in spike length were noticed between cultivars in both seasons.

Soil salinity significantly reduces the absorption of mineral nutrients, especially phosphorus (P), because phosphate ions precipitate with Ca^+, Mg^+ and Zn^+ ions in salt stressed soils and become unavailable to plants (Azco´n-Aguilar *et al*., 1979). Salinity stress is usually causes a decrease in crop production. It inhibits the photosynthesis of plants, causes changes of chlorophyll contents and components and damage of photosynthetic apparatus (Iyengar and Reddy, 1996). It also inhibits the photochemical activities and decreases the activities of enzymes in the Calvincycle (Sairam and Tyagi, 2004). The decreasing in FW or DW levels was also reported in salt-treated plants of wheat and barley (Sairam and Srivastava, 2002). Francois *et al.* (1984) reported that irrigation of wheat plants with 10 or 25% sea water generally decreased grain yield and their reduction was attributed to a reduction of grain weight per (spike and individual weight). The irrigation with sea water caused significant reduction of plant height, shoot fresh, spike length and spikelet's/ spike (data not showed). The whole plant dry weigh and grain yield of wheat plants were significantly reduced to 58 - 66% and 50 - 61%, respectively of that of plants irrigated tap water Abd El-Baky *et al*, (2008). D, Amico *et al.* (2004) reported that 20% (v/v) sea water inhibited growth of wheat plants causing a significantly decrease in biomass production caused by Na^+. Mineral uptake by roots is affected as a result of imbalance in the availability of different ions. In wheat the cause of reduced growth was attributed more to the reduced rate of transport of essential nutrients to the shoot (Hu and Schmidhalter, 2005).

All treatments had significant and positive effect to increase spike weight at 105 days from sowing in both seasons as compared to the control except *Bacillus circulans* and micronutrients at 25 ppm treatments in the second season gave insignificant value. The highest significant values of main spike weight were recorded by potassium silicate at 400 ppm in both seasons. These results indicated that Sakha 93 cv. had higher spike weight than the Giza 168 cv. in both seasons.

The stimulating effect observed in this study due to inoculation with selected effective microorganisms on the wheat plants crop and microbial communities and activities in the rhizosphere can be explained by the capability of such microorganisms to produce growth promoting substances and nitrogen fixation which improve the plant growth and grain yield (Ishac *et al*., 1989).

Egamberdiyeva *et al*. (2004) suggested that phosphate solubilising bacteria are able to mobilize more P to the plants and improve plant growth. Zarabi *et al.* (2011) Proved that different biofertilizers can positively affect on the growth increase of maize plant and phosphorus absorption.

Today, emphasis is put on plant growth regulating bacteria, microorganisms that are capable of increasing the rate of plant growth, by direct or indirect mechanisms, secretion of vitamins and amino acids, auxin and fixing atmospheric nitrogen by *Bacillus*, *Azotobacter* and *Azosprillum* are among the direct mechanisms of increasing root development and plant growth (Akbari *et al*., 2007).

Vesicular-arbuscular mycorrhizal (VAM) fungi are associated with improved growth of many plant species due to increased nutrients uptake, production of growth promoting substances, tolerance to drought,

salinity and transplant shock and synergistic interaction with other beneficial soil microorganisms such as N-fixers and P-solubilizer (Sreenivasa and Bagyaraj, 1989). Enhanced growth of AM plants has been partly attributed to mycorrhizically mediate enhanced nutrient acquisition, especially better P nutrition (Sharifi *et al*., 2007). AMF plays a key role in the regulation of ionome and membrane transport proteins that control the ion homeostasis of the host plants (Ramos *et al*., 2011; Song and Kong, 2012).

El-Fouly *et al*. (2002) found that all micro- and macro-nutrients uptake were negatively affected with increasing NaCl concentration. However, foliar feeding with micronutrients could partially counteract the negative effect of NaCl on nutrients uptake through improving root growth.

Used biofertilizers and foliar spraying with micronutrients significantly affected all the studied parameters of wheat plants, the highest were obtained by inoculating wheat grains with *A. brasilense* and spraying the plants with (Mn+Fe+Zn) treatment, while the lowest values were attained by un-inoculated grains (control) and spraying the wheat plants with tap water Eleiwa *et al.,* (2012). Mahmed *et al*. (2010) found that foliar spraying of wheat plants with Mn and Fe can alleviated the harmful effect of salinity on growth. The efficacy of foliar fertilization is higher than that of soil fertilizer application in these situations (Hu *et al.,* 2008).

Kassab *et al*. (2004) concluded that the foliar application with a 2% solution from each of Fe, Mn, Zn and Mg significantly increased wheat yield components including grain and straw yields as well as carbohydrates yield.fed^{-1}.

Table 3: Effect of biofertilizers, micronutrients and potassium silicate treatments on spikes number per plant, spike length and spike weight of wheat plants at 105 days after sowing in both seasons under salt stress.

Treatments	1 St		2 nd		1 St		2 nd		1 St		2 nd	
	Spikes no./plant				Spike length				Spike weight			
	S 93	G 168	S 93	G 168	S 93	G 168	S 93	G 168	S 93	G 168	S 93	G 168
Control	3.00 cd	2.33 d	4.67 ab	4.00 b	10.17 de	9.57 e	11.33 bc	10.00 c	1.55 bc	1.07 c	1.93 cd	1.59 d
Azotobacter sp (10^8 cfu./ml)	5.67 ab	5.67 ab	8.67 a	5.67 ab	12.67 a-c	12.83 a-c	11.67 a-c	13.00 ab	2.16 ab	2.10 ab	2.75 b-d	2.49 b-d
Bacillus megatherium (10^8 cfu./ml)	6.00 ab	5.00 a-c	7.00 ab	6.00 ab	13.17 a-c	14.17 ab	12.50 a-c	13.17 ab	2.12 ab	1.92 a-c	2.60 b-d	2.63 b-d
Bacillus circulans (10^8 cfu./ml)	7.33 a	4.33 b-d	7.33 ab	6.33 ab	14.00 a-c	14.17 ab	13.83 ab	14.00 a	2.27 ab	2.03 ab	2.48 b-d	2.24 b-d
Mix of three bacteria (1 : 1 : 1)	7.33 a	5.00 a-c	8.00 ab	6.67 ab	13.67 a-c	13.33 a-c	13.67 ab	13.00 ab	2.61 a	2.20 ab	3.39 b	2.83 c-d
Mycorrhiza (10^4 spore/ml)	6.33 ab	5.00 a-c	7.33 ab	5.67 ab	14.00 a-c	14.00 a-c	13.00 ab	13.00 ab	2.42 ab	2.34 ab	3.03 bc	2.73 c-d
Fe+ Mn+Zn (25 ppm)	5.00 a-c	4.67 b-d	6.00 ab	5.33 ab	12.33 bc	12.00 cd	11.67 a-c	11.83 a-c	1.97 a-c	1.93 a-c	2.22 d-f	2.48 b-d
Fe+ Mn+Zn (50 ppm)	5.67 ab	5.67 ab	7.33 ab	7.33 ab	14.67 a	13.67 a-c	12.67 ab	13.33 ab	2.29 ab	2.23 ab	3.08 bc	3.07 bc
K_2O. $4SiO_2$ (200 ppm)	6.00 ab	5.67 ab	7.67 ab	7.33 ab	13.33 a-c	12.00 cd	12.33 a-c	12.33 a-c	1.97 a-c	2.14 ab	2.88 c-d	2.58 b-d
K_2O. $4SiO_2$ (400 ppm)	7.33 a	6.67 ab	8.33 a	7.67 ab	14.33 ab	13.50 a-c	14.00 a	13.50 ab	2.66 a	2.34 ab	4.72 a	3.40 b
MSD T at 5%	1.64		2.59		1.29		1.65		0.60		0.84	
MSD V at 5%	0.44		0.70		NS		NS		0.14		0.23	
MSD T x V at 5%	2.62		4.15		2.06		2.65		0.97		1.30	

S = Sakha G= Giza NS= Non significant

The more pronounced effect on increasing grain yield was recorded by wheat plants foliar sprayed with either Fe or Mn and their mixture. The superiority of Fe on grain yield may be attributed to the indirect role of Fe in chlorophyll synthesis. In addition Iron enters in many plant enzymes that play dominant roles in redox reactions of photosynthesis and respiration (Curie and Briat, 2003). The superiority of Mn treatment resulted from the fact that manganese (Mn) is regarded as an activator of many different enzymatic reactions and takes part in photosynthesis. Manganese activates decarboxylase and dehydrogenase and is a constituent of complex PSII-protein, SOD (Super oxide dismutase) and phosphatase (Sajedi *et al*., 2009).

Ali *et al*. (2009) showed that the enhancement in shoot growth was more pronounced showing that Si application ameliorated the adverse effects of salinity by increasing root and shoot lengths and fresh and dry weights in Si-containing pots in comparison to pots where Si was not supplemented. Wheat growth is significantly and linearly correlated with Si application rate which indicates the concomitant increase in biomass with increasing levels of Si

Chemical constituents

Total soluble sugars and carbohydrates

Regarding the effect of biofertilizers, micronutrients and potassium silicate treatments on sugars and carbohydrates, (Table 4) reveal that all treatments showed a significant increase as compared to the control in both seasons. Sakha 93 cv. recorded the highest significant values of total soluble carbohydrates and total sugars were found by mix of bacteria treatment in both seasons. The highest significant values of total soluble carbohydrates and total sugars were found in the second season at 90 days from planting.

In this regard, Schwarz and Gale (1981) revealed that salinity may also increase the respiration rate of the roots, which have a higher carbohydrate requirement for maintenance respiration in saline substrates.

Tawfik *et al*. (2006) showed that raising irrigation salinity levels significantly increased the content of carbohydrate and proline. However, suggested that both proline and soluble carbohydrates act as compatible solutes under high salinity levels Murphy *et al*. (2003). Kusaka *et al.* (2005) added that, the observed increase in

the osmotic potential might be due to the accumulation of inorganic solutes, several organic components such as sucrose, glucose and amino acids including proline.

Sugars and carbohydrates were increased by biofertilizers treatments (bacteria and microrrhiza) as compared to untreated plants, these results were found tope in agreement with those of Al-Garni (2006); Hasaneen *et al.* (2009).

On the other hand, AMF has a regulatory and stimulatory influ-ence on protein, sucrose, glucose, proline and glycine-betaine (GB) synthesis; hence, these solutes may play a role in osmotic adjustment that helps plant to perform normally under salinity. The increase in total soluble carbohydrates (SC) is found to be positively correlated with mycorrhization of the host plant as reported by Thomson *et al*. (1990).

The obtained results in this study are in agreement with those obtained by Aly *et al*. (2009), Zaki *et al*. (2009) and Bashan *et al*.(2006), they found a significant increases in photosynthetic pigments (chl. a, b and carotenoids), crude protein, soluble sugar, polysaccharide, total soluble solids (T.S.S.), and sucrose in sugar beet, broccoli and wheat plants respectively inoculated with biofertilizers presowing, such as *B. polymyxa*, *A. chroococcum* and *Azospirillum* as compared with un-inoculated treatment.

Eleiwa *et al.,* (2012) revealed that all the mentioned physiological parameters were significantly increased by spraying the plants with micronutrients as compared with the control plants. The highest values obtained by spraying plants with (Mn+Fe+Zn) treatment followed by Zinc, Fe and Mn treatment in decreasing order.

Table 4: Effect of biofertilizers, micronutrients and potassium silicate treatments on total soluble sugars and carbohydrates of wheat plants at 90 days after sowing in both seasons under salt stress.

Treatments	1 St		2 nd		1 St		2 nd	
	Total soluble sugars (mg/ g f.w.)				Total soluble carbohydrates (mg/ g f.w.)			
	S 93	G 168	S 93	G 168	S 93	G 168	S 93	G 168
Control	135.33 JK	130.00 K	140.33 LM	138.33 m	211.33 i	195.00 i	215.00 h	201.00 h
Azotobacter sp (10^8 cfu./ml)	191.67 gf	150.67 Ji	194.33 e-g	156.33 KL	338.67 d	325.67 de	345.00 d	337.33 d
Bacillus megatherium (10^8 cfu./ml)	200.00 d-e	170.33 h	203.00 fe	175.00 h-gi	482.67 a	331.00 de	488.00 a	336.67 d
Bacillus circulans (10^8 cfu./ml)	216.67 b-e	214.67 e-e	224.33 b-d	223.33 cd	465.00 a	445.00 b	471.67 a	452.33 b
Mix of three bacteria (1 : 1 : 1)	284.33 a	233.33 b	290.33 a	242.00 b	481.33 a	441.33 bc	485.00 a	447.00 bc
Mycorrhiza (10^4 spore/ml)	166.33 hi	151.00 Ji	171.33 jki	161.67 KJ	469.33 a	425.00 c	475.33 a	430.00 c
Fe+ Mn+Zn (25 ppm)	204.33 d-e	190.00 fg	211.67 de	190.67 h-g	286.33 f	270.67 f-e	290.67 e	278.00 f-g
Fe+ Mn+Zn (50 ppm)	223.00 bc	217.33 b-d	231.67 bc	202.00 fe	320.00 e	276.00 fg	330.33 d	281.00 ef
K_2O. $4SiO_2$ (200 ppm)	181.00 gh	171.33 h	179.66 h-g	171.67 J-K	258.33 gh	254.00 h	268.00 fg	262.00 g
K_2O. $4SiO_2$ (400 ppm)	200.00 fe	199.33 fe	209.33 de	208.33 d-e	287.67 f	275.00 fg	290.00 e	285.67 ef
MSD T at 5%	10.88		11.12		11.07		11.12	
MSD V at 5%	NS		3.00		2.99		3.00	
MSD T x V at 5%	17.41		17.80		17.72		17.70	

S = Sakha G= Giza NS= Non significant

Minerals concentration

Calcium and magnesium

Biofertilizers, micronutrients and potassium silicate treatments showed insignificant effect on calcium and magnesium concentrations of wheat plants. As for cultivars, the superiority was due to Sakha 93 cv which showed the highest value of calcium concentration when compared to Giza 168 cv. (Table 5).

In this regard, Crowley *et al*. (1999) suggested that maintenance of high potassium and calcium in the root zone might help to offset the effect of salinity.

Iron, manganese and Zinc

It could be noticed from Table (5) that all treatments showed increments in iron concentration reaching significant value with mix of bacteria, mycorrhiza and (Fe +Mn + Zn) at 25 and 50 ppm when comparing with control. On the other hand, all treatments led to a significant increase in manganese concentration when compared to control (Table 5). The highest significant values of manganese concentration were recorded by (Fe + Mn + Zn) at 25 and 50 ppm. On the contrary, Sakha 93 cv. showed reduction in manganese concentration when compared to Giza 168 cv.

Data in Table (5) indicate that (Fe + Mn + Zn) treatments significantly increased zinc concentration as compared to the control. As for cultivars, cv. Sakha 93 showed a significant increase in zinc concentration as compared to cv. Giza 168. On the other hand, all treatments showed reduction in sodium concentration but the reduction was significant by potassium silicate treatment at 400 ppm as compared to the control. Also, Sakha 93 cv. had a significant decrease in sodium concentration as compared to Giza 168 cv.

The use of biofertilizers and foliar spraying of micronutrients can alleviate the harmful effect of salinity during growth of wheat plants. These treatments might also increase nutrients uptake from soils and prevent Na^+ translocation to shoot tissues. Mahmoud *et al*., (2008); Zuccarini and Okurowska (2008); Aly *et al.* (2009) and

Zaki *et al.* (2009) Stated that inoculating the grains before plantation with different biofertilizers significantly increased the concentration of different nutrients (N, P, K, Fe, Mn, Zn and Cu) in different plant organs.

Mycorrhizal treated plants showed reduced accumulation of Na and enhanced content of N, P, K, Ca and Mg than non-mycorrhizal treated plants. The parallel increase in the content of nutrients, photosynthetic pigments, reducing sugars, TSC, proline and protein in inoculated plants might be responsible for plants counteracting oxi-dative damage generated by salinity El-Amri *et al.,* (2013).

The favorable effect of inoculating wheat grains before planting on nutrients concentration may be due to one or more of the following reasons:

1. Increasing water and mineral uptake from the soil (Sarig *et al*., 1984).
2. Increasing of root surface area, root hairs and root elongation as affected by *Azotobacter* (Sunjaravelu and Muthukrishinan, 1993).
3. Enhancing the production of biological active fungicidal substance, which may change the microflora in the rhizosphere and affect the balance between harmful and beneficial organisms (Apte and Shendi, 1981). Moreover, Montemurro *et al*. (2008) confirmed the important role of biofertilizers in reducing soil pH value, by secreting acid such as propionic, fumaric and succinic which brought about the dissolution of nutrients bound to organic materials and render them available for growing plants.

Nassar (1997) found that addition of micronutrients (Fe+Mn+Zn) to wheat plants, simultaneously give an additional enhancing effect of N, P and K content as compared with the individual application. Moreover, the superior effect of the triple treatment may be due to the suitable balance between the aforementioned micronutrients (Fe, Zn, and Mn) which enable plants to grow well and absorb more quantities of N, P and K. Results in this study are in good agreement with those obtained by Hanafy *et al*. (2008) and Mahmoud *et al*. (2008) who stated that spraying micronutrients on plants significantly increased plant content of N, P, K, Fe, Mn, Zn and Cu as well as chlorophyll content a, b, a+b and carotenoids.

Sodium

Data in Table (5) indicate that all treatments showed reduction in sodium concentration. The highest significant reduction was recorded by potassium silicate application at 400 ppm. Also, Sakha 93 cv. a significant decrease in sodium concentration as compared to Giza 168 cv. The use of biofertilizers and foliar spraying of micronutrients can alleviate the harmful effect of salinity on growth of wheat plants. These treatments might also increase nutrients uptake from soils and prevent Na^+ translocation to shoot tissues. Higher nutrient levels due to biofertilizers and micronutrients treatments has been reported by Mahmoud *et al.,* (2008); Evelin *et al.* (2009); Mahmed *et al*. (2010).

Mycorrhizal treated plants showed reduced accumulation of Na^+ in wheat genotypes (El-Amri *et al.,* 2013).

The role of silicon to decrease sodium concentrations could be explained by several finding silicon was deposited within the roots (Gong *et al*., 2003). It inhibited the Na+ transportation to aerial parts of plants by its effect on transpiration movement (Yeo *et al*., 1999) or by making a complex with Na+ (Ahmad *et al*., 1992). Moreover, Liang (1999) found that the salt tolerance due to Si application is attributed to selective uptake and transport of K+ and Na+ by plants. Si negatively correlated with Na+, thus it reduced the concentration of Na+ in wheat leaves. Lower Na+ is a good indicator of salt tolerance in plants. Si uptake is positively correlated with K+ and negatively with Na+ uptake (Ali *et al*., 2009).

Ca:Na and Mg:Na ratios

The results concerning the effect of biotic and abiotic on K:Na, Ca:Na and Mg:Na ratios of wheat plants are presented in Table (5). Also, significant increase was obtained by mix of bacteria in Ca:Na ratio as compared to the control. On the other hand, all treatments led to insignificant values in Mg:Na ratio. Cv. Sakha 93 recorded a significant increase in all ratios when compared to cv. Giza 168.

Nutrient concentration ratios in plant tissues were found to also affected by foliar spray. The K/Na and Mg/Na ratios showed high values El-Fouly *et al*. (2011). This may be attributed to that foliar spray of micronutrients under NaCl stress could increase the capability of root system for selectivity K^+ and Mg^+ ions at high concentration of NaCl, which allows the maintenance of the transport of both ions and the limitation of Na ion uptake in the shoots (Tattini *et al*.,1993; Carvajal *et al*., 1999). Ashraf and Harris (2004) and Raza *et al*. (2007) postulated that Ca^{++}/ Na^+ ratio might valid selection criteria for assessing salinity tolerance of different crop species. The present study suggested that inoculation of fungi was effective in improving the tolerance of wheat genotypes by improving the accumulation of nutrients and soluble solutes that might be responsible for osmotic adjustment of plant to counteract oxidative damage generated by salinity El-Amri *et al. (*2013).

In addition, Si may act to alleviate salt stress in maize by decreasing the permeability of plasma membranes and maintenance of cell form and structure due to the increase of antioxidative enzymes SOD and CAT (Catalase). Si partially offset the negative impacts of NaCl stress due to increased the tolerance of maize leaves to NaCl salinity by enhancement of chlorophyll content and photosynthetic activity (Moussa, 2006).

Table 5: Effect of biofertilizers, micronutrients and potassium silicate treatments on Magnesium, Calcium, Iron, Manganese, Zinc, Sodium concentrations, Mg;Na and Ca:Na ratios of wheat plants at 90 days after sowing in the second season under salt stress.

Treatments	Magnesium (%)		Calcium (%)		Iron (mg/kg d.wt.)		Manganese (mg/kg d.wt.)		Zinc (mg/kg d.wt.)		Sodium (mg/kg d.wt.)		Ca:Na ratio		Mg:Na ratio	
	S 93	G 168	S 93	G 168	S 93	G 168	S 93	G 168	S 93	G 168	S 93	G 168	S 93	G 168	S 93	G 168
Control	0.031	0.033	0.093	0.047	188.3	216.7	5.00 ^{b}	13.33 ^{ab}	18.33 ^{cd}	3.31 ^{d}	0.039^{bc}	0.069^{a}	3.56 $^{a-e}$	1.08 ^{e}	1.06 ^{ab}	0.64 ^{b}
Azotobacter sp (10^8 cfu./ml)	0.047	0.042	0.13	0.113	303.3	348.3	16.70 ^{ab}	31.66 ^{ab}	20.00 $^{b-d}$	28.33 $^{b-d}$	0.029^{c}	0.041^{a-c}	4.84 $^{a-e}$	2.44 $^{a-e}$	1.50 ^{ab}	1.06 ^{ab}
Bacillus megatherium (10^8 cfu./ml)	0.046	0.037	0.147	0.117	290	358.3	20.00 ^{ab}	30.00 ^{ab}	25.00 $^{b-d}$	21.66 $^{b-d}$	0.028^{c}	0.040^{a-c}	4.50 $^{a-e}$	1.60 $^{c-e}$	1.413 ^{ab}	0.77 ^{ab}
Bacillus circulans (10^8 cfu./ml)	0.047	0.042	0.11	0.09	251.7	265	11.70 ^{ab}	20.00 ^{ab}	25.00 $^{b-d}$	18.33 ^{cd}	0.029^{c}	0.048^{a-c}	4.74 $^{a-e}$	2.22 $^{a-e}$	1.39 ^{ab}	0.77 ^{ab}
Mix of three bacteria (1 : 1 : 1)	0.054	0.054	0.147	0.126	371.7	431.7	25.00 ^{ab}	33.33 ^{ab}	28.33 $^{b-d}$	28.00 $^{b-d}$	0.025^{c}	0.040^{a-c}	5.83 ^{a}	3.50 $^{a-e}$	1.57 ^{ab}	1.11 ^{ab}
Mycorrhiza (10^4 spore/ml)	0.053	0.046	0.167	0.13	413.3.0	451.7	23.00 ^{ab}	31.70 ^{ab}	46.67 $^{a-c}$	28.33 $^{b-d}$	0.034^{bc}	0.047^{a-c}	5.26 ^{ab}	2.66 $^{a-e}$	1.64 ^{a}	1.16 ^{ab}
Fe+ Mn+Zn (25 ppm)	0.037	0.034	0.13	0.09	461.7.0	480	25.00 ^{ab}	35.00 ^{a}	53.33 $^{a-c}$	51.67 $^{a-c}$	0.037^{bc}	0.060^{ab}	3.63 $^{a-e}$	1.22 ^{de}	1.29 ^{ab}	0.70 ^{b}
Fe+ Mn+Zn (50 ppm)	0.043	0.036	0.133	0.11	595	581.7	30.00 ^{ab}	36.70 ^{a}	81.67 ^{a}	60.00 ^{ab}	0.031^{bc}	0.052^{a-c}	3.70 $^{a-e}$	1.50 $^{c-e}$	1.423 ^{ab}	0.91 ^{ab}
K_2O. $4SiO_2$ (200 ppm)	0.041	0.037	0.117	0.093	221.7	245	13.33 ^{ab}	23.33 ^{ab}	31.67 $^{b-d}$	25.00 $^{b-d}$	0.028^{c}	0.036^{bc}	4.43 $^{a-e}$	2.38 $^{a-e}$	1.293 ^{ab}	0.72 ^{ab}
K_2O. $4SiO_2$ (400 ppm)	0.048	0.042	0.147	0.12	251.7	388.3	23.33 ^{ab}	25.00 ^{ab}	38.33 $^{b-d}$	28.33 $^{b-d}$	0.024^{c}	0.034^{bc}	5.23 ^{ab}	2.64 $^{a-e}$	1.56 ^{ab}	1.10 ^{ab}
MSD T at 5%	NS		NS		266-84		18.67		25.39		0.018		10.10		NS	
MSD V at 5%	NS		0.02		NS		5.04		6.86		0.01		2.73		0.16	
MSD T x V at 5%	NS		NS		NS		29.88		40.63		0.03		16.16		0.93	

S = Sakha G= Giza NS= Non significant

Finally, it could be concluded that the most of treatments whether biofertilizers, micronutrients and potassium silicate led to enhance salt tolerance in wheat cultivars. As was expected, Sakha 93 cv. "salinity tolerant" has shown high response to treatments compared to Giza 168 cv. "salt sensitive", although the latter cultivar has shown reasonable response, though less. So, we recommend using the applied treatments in all wheat cultivars subjected to salinity, both in irrigation water and/or in the soil.

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