Australian Journal of Basic and Applied Sciences, 2(3): 575-582, 2008
ISSN 1991-8178

The Potential Impact of Biofertilization, Antioxidant and Micronutrients Application in Reducing Salinity Stress on Two Wheat Cultivars

[1]Gomaa, A.M., [2]T. El-Mesiry and [3]M. Rady

[1]Agricultural Microbiology Department, National Research Centre, Cairo, Egypt.
[2]Water Relations and Field Irrigation Department, National Research Centre, Cairo, Egypt.
[3]Faculty of Agriculture, Fayoum University, Egypt.

Abstract: A field experiment was conducted for the two growing seasons of 2006 and 2007 at three soil sites of different salinity levels (4.72, 7.79 and 10.86 dSm^{-1}). The experiment was designed to study the influence of salt affected soil in addition to years, micronutrients, antioxidant application and their interaction on two wheat cultivars (Sakha 93 and Sakha 94). Obtained results indicated that application of biofertilization generally raised the shoot fresh weight values in comparison with non biofertilized ones, although treatments using micronutrients in addition to 100 & 200 ppm antioxidant improved plant fresh weight to reach those of the positive control (100% NPK). At high soil salinity levels a remarkable observation was found where the biofertilized treatments significantly overcame the non-biofertilized treatments and similar observation was estimated for plant shoot dry weight under the same previous mentioned conditions. Biofertilization with *Candida tropicalis* increased significantly the leaf area of tested wheat cultivars under and soil salinity levels. At high salinity level, it was found that treatments supplied by biofertilization with yeast mitigated the adverse effect of salinity. Application of yeast produced the highest spikes number m^2 irrespective of salinity level and cultivar type, that ranged from 221 – 323, in comparison with the non biofertilized ones (72 - 273). For grain yieldm the same previous trend was recorded at lower salinity levels where biofertilized wheat yield ranged from 8 to 22 ardab/fed, while non biofertilized plants ranged from 0.73 to 14.8 Ardab/fed.

Key words: Biofertilization, antioxidant, micronutrients, wheat cultivars, salinity stress

INTRODUCTION

Egypt vitally needs sustained agricultural development to cope with the social and economic obligations that are the normal consequences of the continued high rates of population growth. This urgent need requires continuous scientifically based implementation of effective agricultural practices. On the limited cultivable land area it is essential to obtain the maximum yield per unit of irrigation water used. Wheat (*Triticum aestivum* L.) is the most important cereal crop in Egypt and increasing wheat production is an essential national target to fill the gap between production and consumption. Production could be increased through cultivation of high yielding cultivars and appropriate agronomic practices, among which water management is most important. The increased emphasis on wheat-water relationships can be attributed to its role as a controlling factor in production and optimization of water use. Water and salt stress are the most important limiting factors in wheat productivity in the semi-arid regions of the world. Most crop plants suffer after exposure to saline conditions and showed decline in growth. The deleterious effect of salinity was suggested as a result of water stress, ion toxicities, ion imbalance or combination of all these factors Kurt *et al.* (1986).

Salinity problem is defined as a condition where the salts in solution within the crop root zone accumulate in high concentrations which decrease crop yield. Salts in the soil water solution can reduce evapotranspiration by making soil water less available for plant root extraction (Allen *et al.,* 1998). Using biofertilization is of great importance but largely unfulfilled aim of agricultural development (Gaballah and Gomaa, 2004). The main effects of salinity are associated with ion accumulation in the plant rather than with reduced water availability in the substrate, where there is incomplete adjustment.

The work within hand aims at studying the combined influence of biofertilization, micronutrient and foliar application of ascorbic acid, as antioxidant, on growth and yield parameters of two wheat varieties (Giza 93 and Giza 94) grown under three soil salinity levels i.e., 4.72, 7.79 and 10.86 dSm^{-1}.

Corresponding Author: Abu-Bakr Gomaa, Agricultural Microbiology Department, National Research Centre, Cairo, Egypt.
E-mail: abgomaa@yahoo.com

MATERIALS AND METHODS

A field experiment was conducted for the two growing seasons of 2006, 2007 at three different sites of soil salinity level (4.72, 7.79 and 10.86 dS/m) at Demo Experimental Farm, Faculty of Agriculture Fayoum University, Egypt. The experiment was designed in a randomized complete block design with three replications to study the influence of soil salinity in addition to antioxidant, micronutrients and biofertilizer application on two wheat cultivars (Sakha 93 and 94) in addition to study the effect of biofertilization on mitigating the adverse impacts of salinity stress on two wheat cultivars growth.

The biofertilization treatment was soil yeast (*Cabdida tropicalis*) and was applied before cultivation where wheat seeds were inoculated with biofertilizer yeast loaded on sterilized peat moss. Firstly, wheat seeds were mixed with the Arabic gum (4%) and then spread on a clean plastic sheet in a shady place for fifteen minutes. Wheat seeds were mixed carefully with tested yeast inoculum. The inoculated wheat seeds were directly in the experimental plots according to the respective treatment.

Growth parameters were evaluated at 105 days after sowing (shoot fresh and dry weight g/plant and leaf area (cm^2/plant). At harvest, yield characters (spikes number/m2, Grain number/spike, 1000 grains weight (g) and Grains yield (Ardab/fed.) were recorded. At 75-days-old of wheat plants, leaf samples were collected randomly for estimation of polyphenol oxidase activity (nM/min/g.fr.wt), L-phenylalanine ammonia-lyase (nM min/g.fr.wt), peroxidase activity (nM/min/g.fr.wt) and a-amylase activity (mg/glucose/hr/g.fr.wt).

Enzyme Extraction:

Leaf samples were homogenized with 2ml of 0.1M sodium phosphate buffer (pH 7.0) at 4°C. The homogenate was centrifuged for 20 min at 10000 rpm. Protein extract prepated from leaves was used for estimating of peroxied (PO), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL).

Spectrophotometric Assay:

Assay of peroxidase/PO. Peroxidase activity was assayed spectrophotometrically (Hartee, 1955). The reaction mixture consisted of 1.5 ml if 0.05M pyrogalld, 0.5 ml of the enzyme extract and 0.5 ml of percent H_2O_2. The reaction mixture was incubated at room temperature (28 $\pm$ 1°C). The change in absorbance was recorded at 30 sec. interval for 3 min. The boiled enzyme preparation served as blank. The enzyme activity was expressed as change in absorbance at 420n M min-1 g-1 on fresh weight basis (Hammerschmidt *et al.,* 1982).

Assay of Polyphenol Oxidase (PPO):

A sample of 1g wheat leaves was used for polyphenol oxidase estimation. The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 200 ml of the enzyme extract. To start the reaction, 200 ml of 0.01 M catechol was added and the activity was expressed as changes in absorbance at 495 n m Min^{-1} g^{-1} fresh weight of tissue.

Assay of L-phenylalanine Ammonia-lyase (PAL):

The phenylalanine ammonia lyase assay was conducted as described by Ross and Sederoff (1992). The assay mixture containing 100 ml of enzyme, 500 ml of 50 mM Tris HCl (pH 8.8) and 600 ml of mML-phenylalanine ammonia lyase was incubated for 60 min. The reaction was stopped by adding 2 N HCl. Later 1.5 ml of toluene fraction containing trans-cinnamic acid was separated. The toluene phase was measured against the blank of toluene. Standard curve was drawn with graded amounts of cinnamic acid in toluene as described before. The enzyme activity was expressed as nM of cinnamic acid at 290 nM $min^{-1}g^{-1}$ of fresh tissue.

Assay of α-amylase Activity:

BOL97\f"Symbol"\s12-amylase activity was determined according to Petrova and Bolatina (1956) where a-anylase enzyme was extracted from leaves using phosphate buffer (pH 6.0). The activity of the enzyme was determined using soluble starch as substrate and the glucose produced was estimated (as mg glucose/g fresh wt/hr).

Also, wheat leaves were collected from plants of 105 days-old for estimating proline mg/g dry wt), total chlorophyll (mg/g fr.wt) and total carotenoids mg/g fr.wt. following the method of Saric *et al.* (1967).

The Experimental Design Was as Follows:

1 Positive control (100% NPK)
2 Positive control + yeast (*Candida tropicalis*)
3 Negative control (without NPK)
4 Negative control + yeast
5 Micronutrients
6 Micronutrients + yeast
7 Antioxidant at 100 ppm level
8 Antioxidant at 100 ppm + yeast
9 Antioxidant at 200 ppm level
10 Antioxidant at 100 ppm + yeast
11 Micronutrients + antioxidant at 100 ppm
12 Micronutrients + antioxidant at 100 ppm + yeast
13 Micronutrients + antioxidant at 200 ppm level
14 Micronutrients + antioxidant at 100 ppm level + yeast

The previously mentioned treatments were applied to both wheat varieties i.e., Sakha 93 and Sakha 94.

RESULTS AND DISCUSSION

The data presented in Table (2) show that the high level of salinity negatively affected shoot dry weight and leaf area irrespective of the treatment. Salinity can damage the plant through its osmotic effect, which is equivalent to a decrease in water activity through specific toxic effects of ions and by disturbing the uptake of essential nutrients (Lauchli and Epstein, 1990; Marschner, 1995 and Dorais *et al.,* 2000).

For shoot dry weight, the various tested treatments failed to give similar results to the positive control (100% NPK) in the non-biofertilized treatments for both tested varieties (Sakha 93 and Sakha 94) in the different salinity levels. Reduction in plant growth as a result of salt stress has also been reported in several other plants species (Ashraf and O'leary 1997 and Turkman *et al.,* 2008). Moreover, in both sites of high salinity levels i.e., E.C 7.79 and E.C 10.86, wheat plants completely failed to grow in most treatments. Some nutritional disturbances are expected under saline condition. In presence of excess NaCl in medium, Na and Cl are accumulated in plant organs, and these saline ions can affect other mineral elements uptake through competitive interactions or by affecting the ion selectively if membrane, which causesnutrient deficiencies in plants (Bohro and Doffling, 1993).

Biofertilization of wheat plants mitigated the adverse effects of salinity, where the plants were able to grow in the majority of treatments in comparison with the non-biofertilized ones. The biofertilized treatments induced comparable results to the positive control without significant differences for both tested wheat varieties especially in both sites of E.C. 4.72 and E.C. 7.79 salinity levels The obtained results was confirmed by those recorded with Gaballah and Gomaa (1994). Besides, the same trend was recorded for the leaf area parameter in the site of E.C. 4.72. In the site of E.C 4.72, the shoot dry weight of Sakha 93 variety ranged from 4.50 to 11.60 g/plant and from 4.07 to 11.47 for Sakha 94 in the non-biofertilized treatments. Biofertilization enhanced the growth of wheat plants where the shoot dry weight ranged from 11.17 to 12.67 g/plant for Sakha 93 and from 11.20 to 12.30 for Sakha 94 under the same salinity level (E.C 4.72). In the site of high salinity level (E.C. 10.86). Wheat shoot dry weight ranged from 1.93 to 2.33 g/plant and from 1.17 to 1.30 g/plant respectively for Sakha 93 and Sakha 94 in the non-biofertilized treatments, meanwhile these ranges reached 2.40 to 2.83 g/plant for Sakha 93 and 1.27 to 1.87 g/plant for Sakha 94 in the biofertilized treatments. The previously mentioned results are in harmony with those obtained by Karmer (1975) and Jonas *et al.* (1992) who noted that dry matter accumulation significantly decreased with increasing salinity under stress conditions where photosynthesis was reduced by closure of the stomata, which decreased the supply of carbondioxide and thus growth.

For leaf area, it ranged from 19.93 to 35.53, 17.23 to 33.53, 32.03 to 46.23 and 28.97 to 45.73 cm^2/plant respectively for Sakha 93 and Sakha 94 barieties in the non-biofertilzed and biofertized treatments in the salinity level of E.C. 4.72. In the site of high salinity level, theses ranges reached 11.80 to 17.93, 11.10 to 13.30, 19.90 to 21.27 and 13.47 to 14.23 cm^2/plant respectively as the same previously mentioned order.

Table 1: Some physiological chemical and physical properties of studied soil

Physical and chemical properties	Site 1	Site 2	Site 3
Clay %	36.50	33.00	31.50
Silt %	21.50	20.00	23.00
Sand %	42.00	47.00	45.00
Soil texture	Sandy clay loam		
EC (dS/m)	4.72	7.79	10.86
pH	7.45	7.58	7.51
$CaCO_3$ %	8.41	7.31	6.07
Organic matter %	0.96	0.84	0.80

Table 2: Salinity & Biofertilization, Antioxidants, Micronutrients, Wheat Cultivars Minimizing the Effect of Salinity Stress on two wheat cultivars using biofertilization Antioxidants and Micronutrients.

			Shoot dry wt. (g/plant)			Leaf area (cm2/plant)		
Treatments			Site 1 (EC=4.72)	Site 2 (EC=7.79)	Site 3 (EC=10.86)	Site 1 (EC=4.72)	Site 2 (EC=7.79)	Site 3 (EC=10.86)
Without biofertilizer	Sakha-93	T_1	11.0	9.37	2.33	35.53	30.30	17.93
		T_2	4.50	0.00	0.00	19.93	0.00	0.00
		T_3	8.13	7.00	0.00	25.97	21.27	0.00
		T_4	4.93	0.00	0.00	21.73	0.00	0.00
		T_5	6.10	5.30	1.93	24.07	1.33	11.80
		T_6	10.57	8.30	0.00	25.80	21.07	0.00
		T_7	10.80	8.53	2.17	29.00	23.27	16.43
	Sakha-94	T_1	11.47	8.77	1.30	33.53	29.97	13.30
		T_2	4.07	0.00	0.00	17.23	0.00	0.00
		T_3	7.93	6.77	0.00	23.00	19.00	0.00
		T_4	4.63	0.00	0.00	19.03	0.00	0.00
		T_5	4.70	5.13	1.17	21.17	18.60	11.10
		T_6	10.33	7.13	0.00	23.20	20.90	0.00
		T_7	10.63	7.70	1.27	25.97	22.47	13.07
With biofertilizer	Sakha-93	T_1	12.67	9.73	2.60	46.10	40.23	21.27
		T_2	11.17	8.67	0.00	32.03	26.80	03.00
		T_3	11.63	9.30	2.4	38.63	33.57	19.90
		T_4	11.47	9.23	0.00	32.23	27.10	0.00
		T_5	11.90	9.47	2.47	40.63	35.23	20.90
		T_6	12.37	9.57	2.63	42.87	35.83	20.80
		T_7	12.57	9.80	2.70	46.23	40.37	21.07
	Sakha-94	T_1	12.30	9.57	1.87	45.17	39.33	14.23
		T_2	11.20	8.17	0.00	28.97	25.40	0.00
		T_3	11.43	8.90	1.27	36.53	33.23	13.47
		T_4	11.30	8.47	0.00	30.37	25.70	0.00
		T_5	11.77	9.13	1.33	38.77	35.10	13.57
		T_6	12.17	9.37	1.53	39.50	35.10	13.63
		T_7	12.20	9.60	1.63	45.73	39.07	13.90
$LSD_{5\%}$				0.47			1.12	

T1: Positive control (100% NPK), T2: Negative control (without NPK), T3: Micronutrients, T4: Antioxidant at 100 ppm level, T5: Antioxidant at 200 ppm level, T6: Micronutrients + antioxidant at 100 ppm, T7: Micronutrients + antioxidant at 200 ppm level

Under saline conditions, a non-uniform distribution of ions in the successive leaves within the shoots and between the leaf blade and sheath has been observed frequently (Yaser *et al.,* 2006). Salt treatment affects differently early growth stages of plants and has both osmotic and specific ion effects on plant growth Dionisio-Sese and Tobita (2000).

The impact of various tested treatments on wheat yield parameters was shown in Table (3). For spikes number per m^2, it was found that the positive control overcame the different tested treatments for both cultivars (Shaka 93 and Sakha 94) at E.C. 4.72 and 7.79 salinity levels. The only exception is the biofertilized treatments supplied by micronutrients and 200 ppm antioxidant where the differences were not significant in comparison with the positive control for both cultivars. The uptake of some mineral nutrients dissolved in water is also restricted in plants. Growth and development of plants are inhibited due to occurring defect in metabolism Cramer *et al.* (1985).

It is worthy to mention that at E.C. 7.79 salinity level, wheat plants were not able to grow except the positive control and micronutrients accompanied by 200 ppm antioxidant for both cultivars in the non-biofertilized treatments. Mass and Grieve (1990) mentioned that grain yield decreased as salinity increased by reducing grains number more than grain weight. Salts stress decreased the yield potential of cultivars by

Table 3: Effect of biofertilizations antioxidant and micronutrients on wheat yield parameters.

			Spikes number/m²			Grains number/spike			1000 grain weight (g)			Grains yield (Ardab/fed.)		
Treatments			Site 1 (EC=4.72)	Site 2 (EC=7.79)	Site 3 (EC=10.86)	Site 1 (EC=4.72)	Site 2 (EC=7.79)	Site 3 (EC=10.86)	Site 1 (EC=4.72)	Site 2 (EC=7.79)	Site 3 (EC=10.86)	Site 1 (EC=4.72)	Site 2 (EC=7.79)	Site 3 (EC=10.86)
Without biofertilizer	Sakha-93	T_1	273.70	226.30	0.00	46.33	42.67	22.00	43.73	41.63	19.33	14.80	10.73	0.9
		T_2	72.33	0.00	0.00	21.00	0.00	0.00	12.17	0.00	0.00	0.53	0.00	0.00
		T_3	171.70	147.070	0.00	31.00	29.00	0.00	31.77	25.20	0.00	4.50	2.87	0.00
		T_4	89.33	0.00	0.00	22.67	0.00	0.00	191.17	0.00	0.00	1.07	0.00	0.00
		T_5	117.70	74.0	0.00	28.00	21.33	0.00	31.37	25.33	0.00	2.73	1.07	0.00
		T_6	199.00	14.30	0.00	33.67	30.67	0.00	32.37	28.67	0.00	5.77	3.83	0.00
		T_7	216.30	195.70	54.00	36.33	32.00	14.33	38.17	35.60	18.53	8.00	5.97	0.40
	Sakha-94	T_1	271.30	226.00	56.33	45.33	41.67	17.00	42.33	40.50	13.00	13.87	10.27	0.33
		T_2	54.00	0.00	0.00	19.67	0.00	0.00	9.77	0.00	0.00	0.27	0.00	0.00
		T_3	167.30	140.70	0.00	28.33	26.33	0.00	27.27	21.57	0.00	3.47	2.13	0.00
		T_4	75.00	0.00	0.00	22.00	0.00	0.00	16.77	0.00	0.00	0.73	0.00	0.00
		T_5	113.0	74.67	0.00	27.33	20.33	0.00	26.27	22.97	0.00	2.17	0.93	0.00
		T_6	191.70	153.30	0.00	30.67	28.33	0.00	31.17	27.73	0.00	4.87	3.20	0.00
		T_7	215.00	194.00	43.67	33.00	29.67	11.67	37.50	34.33	13.83	7.10	5.27	0.20
With biofertilizer	Sakha-93	T_1	323.30	304.30	145.7	52.00	51.00	31.00	48.77	45.07	23.07	21.83	18.70	2.80
		T_2	222.70	189.70	0.00	38.33	33.67	0.00	37.37	35.13	0.00	8.50	5.93	0.00
		T_3	284.00	265.00	78.67	46.67	43.67	25.67	43.60	41.33	18.80	15.40	12.77	1.00
		T_4	241.70	213.70	0.00	40.67	36.33	0.00	42.07	40.73	0.00	11.03	8.40	0.00
		T_5	296.00	270.30	92.67	47.67	46.00	28.67	43.93	41.57	18.10	16.50	13.77	1.30
		T_6	291.00	270.70	100.00	47.33	45.33	28.00	46.97	44.53	21.53	17.23	14.60	1.60
		T_7	319.00	294.30	132.70	51.00	49.67	30.00	49.77	45.37	23.70	21.60	17.67	2.53
	Sakha-94	T_1	319.30	303.00	87.33	51.33	50.00	24.33	46.87	41.60	16.03	20.47	16.77	0.90
		T_2	220.70	194.70	0.00	35.33	31.67	0.00	36.20	34.57	0.00	7.53	5.70	0.00
		T_3	285.70	253.00	60.00	45.67	42.67	19.67	42.47	40.90	13.30	14.77	11.80	0.40
		T_4	239.30	214.30	0.00	37.67	34.00	0.00	41.23	39.80	0.00	9.90	7.73	0.00
		T_5	294.70	29.30	65.00	47.67	45.00	21.33	42.63	40.33	12.90	15.97	13.03	0.47
		T_6	291.00	262.70	68.33	47.00	44.00	21.67	45.97	41.40	15.63	16.77	12.77	0.63
		T_7	312.30	293.70	85.00	50.67	47.67	23.33	46.87	41.73	17.67	19.77	15.57	0.97
$LSD_{5\%}$				12.5			2.61			2.08			0.91	

T1: Positive control (100% NPK), T2: Negative control (without NPK), T3: Micronutrients, T4: Antioxidant at 100 ppm level, T5: Antioxidant at 200 ppm level, T6: Micronutrients + antioxidant at 100 ppm, T7: Micronutrients + antioxidant at 200 ppm level

Table 4: Effect of biofertilizations antioxidant and micronutrients on proline, total chlorophyll and total carotenoids.

			Proline (mg/g dry wt.)			Total chlorophyll (mg/g leaves fresh wt.)			Total carotenoids (mg/g leaves fresh wt.)		
Treatments			Site 1 (EC=4.72)	Site 2 (EC=7.79)	Site 3 (EC=10.86)	Site 1 (EC=4.72)	Site 2 (EC=7.79)	Site 3 (EC=10.86)	Site 1 (EC=4.72)	Site 2 (EC=7.79)	Site 3 (EC=10.86)
Without biofertilizer	Sakha-93	T_1	0.26	0.31	0.18	0.76	0.60	0.41	0.32	0.24	0.17
		T_2	0.18	0.00	0.00	0.65	0.43	0.00	0.28	0.20	0.00
		T_3	0.22	0.24	0.00	0.67	0.48	0.00	0.30	0.20	0.00
		T_4	0.18	0.00	0.00	0.64	0.00	0.00	0.27	0.00	0.00
		T_5	0.22	0.25	0.17	0.68	0.49	0.24	0.30	0.21	0.16
		T_6	0.23	0.27	0.00	0.68	0.48	0.25	0.30	0.21	0.16
		T_7	0.25	0.29	0.17	0.70	0.51	0.28	0.32	0.23	0.17
	Sakha-94	T_1	0.25	0.30	0.16	0.75	0.58	0.34	0.33	0.21	0.15
		T_2	0.19	0.00	0.00	0.63	0.00	0.00	0.29	0.00	0.00
		T_3	0.21	0.23	0.00	0.66	0.45	0.00	0.30	0.20	0.00
		T_4	0.17	0.00	0.00	0.62	0.00	0.00	0.26	0.00	0.00
		T_5	0.21	0.24	0.17	0.66	0.47	0.21	0.29	0.19	0.00
		T_6	0.22	0.25	0.00	0.66	0.46	0.00	0.29	0.20	0.00
		T_7	0.24	0.29	0.14	0.69	0.49	0.25	0.31	0.23	0.13
With biofertilizer	Sakha-93	T_1	0.33	0.44	0.24	0.89	0.71	0.54	0.36	0.27	0.21
		T_2	0.25	0.30	0.00	0.75	0.55	0.00	0.32	0.24	0.00
		T_3	0.29	0.35	0.21	0.79	0.63	0.43	0.33	0.24	0.18
		T_4	0.25	0.30	0.00	0.74	0.58	0.39	0.32	0.23	0.17
		T_5	0.3	0.36	0.23	0.82	0.65	0.46	0.33	0.25	0.19
		T_6	0.31	0.37	0.23	0.80	0.64	0.44	0.32	0.24	0.18
		T_7	0.36	0.43	0.25	0.86	0.68	0.50	0.34	0.26	0.20
	Sakha-94	T_1	0.32	0.41	0.22	0.88	0.69	0.46	0.34	0.26	0.19
		T_2	0.25	0.30	0.00	0.72	0.53	0.00	0.32	0.23	0.00
		T_3	0.26	0.31	0.18	0.77	0.59	0.40	0.32	0.23	0.17
		T_4	0.23	0.28	0.00	0.74	0.55	0.00	0.31	0.23	0.00
		T_5	0.29	0.36	0.20	0.80	0.62	0.41	0.32	0.24	0.19
		T_6	0.32	0.36	0.19	0.78	0.61	0.41	0.32	0.23	0.18
		T_7	0.33	0.41	0.21	0.84	1.67	0.42	0.32	0.25	0.18
$LSD_{5\%}$			0.03			0.05			0.05		

T1: Positive control (100% NPK), T2: Negative control (without NPK), T3: Micronutrients, T4: Antioxidant at 100 ppm level, T5: Antioxidant at 200 ppm level, T6: Micronutrients + antioxidant at 100 ppm, T7: Micronutrients + antioxidant at 200 ppm level

reducing the number of tillers. Abo El-Soud (1989) found that decrease in N uptake was due to the decrease in dry matter as well as the decrease in grain yield. It was declared that the micronutrients are generally less affected by salt stress compared with macronutrients (El-Fouly and Salama, 1999; Hu and Schmidhalter, 2001and Turhan and Eris, 2005).

Biofertilization of wheat plants with *Candida tropicalis,* mitigated the adverse effect of salinity where the plants of both cultivars were able to grow and survive for the majority of treatments. Moreover, the biofertilized treatment of micronutrients + 200 ppm antioxidant overcome the positive control in the produced number of spikes per m^2. The same previously mentioned trend was observed for grains number per spike and 1000 grain weight for both tested wheat cultivars. Besides, additional treatment (micronutrients + 100 antioxidant) gave comparable results to the positive control at the three tested salinity levels. For grain yield (Ardab/fed). The biofertilized treatment of micronutrients + 200 ppm antioxidant was the only treatment that

Table 5: Effect of biofertilizations antioxidant and micronutrients poly phenol oxidase activity, phenyl alanine ammonia lyase, peroxidase activity and amylase activity.

			Poly phenol oxidase activity (M/min/g leaves fresh wt.)			Phenyl alanine ammonia Lyase (M/min/g leaves fresh wt.)			Peroxidase activity (M/min/g leaves fresh wt.)			Amylase activity (mg glucose/hr/g leaves fresh wt.)		
Treatments			Site 1 (EC=4.72)	Site 2 (EC=7.79)	Site 3 (EC=10.86)	Site 1 (EC=4.72)	Site 2 (EC=7.79)	Site 3 (EC=10.86)	Site 1 (EC=4.72)	Site 2 (EC=7.79)	Site 3 (EC=10.86)	Site 1 (EC=4.72)	Site 2 (EC=7.79)	Site 3 (EC=10.86)
Without biofertilizer	Sakha-93	T_1	0.34	0.25	0.19	0.40	0.32	0.19	0.33	0.25	0.18	0.69	0.59	0.45
		T_2	0.25	0.19	0.00	0.35	0.27	0.00	0.17	0.11	0.00	0.44	0.36	0.00
		T_3	0.27	0.20	0.00	0.36	0.28	0.00	0.24	0.16	0.00	0.61	0.43	0.00
		T_4	0.23	0.00	0.00	0.34	0.00	0.00	0.19	0.00	0.00	0.47	0.00	0.00
		T_5	0.27	0.20	0.15	0.30	0.29	0.12	0.24	0.18	0.12	0.60	0.45	0.31
		T_6	0.27	0.20	0.16	0.37	0.30	0.13	0.27	0.20	0.14	0.62	0.46	0.31
		T_7	0.29	0.21	0.17	0.39	0.31	0.15	0.30	0.22	0.15	0.66	0.51	0.37
	Sakha-94	T_1	0.35	0.23	0.15	0.39	0.30	0.15	0.34	0.23	0.16	0.70	0.59	0.37
		T_2	0.23	0.00	0.00	0.32	0.00	0.00	0.16	0.00	0.00	0.40	0.00	0.00
		T_3	0.24	0.19	0.00	0.36	0.26	0.00	0.25	0.14	0.00	0.59	0.42	0.00
		T_4	0.24	0.00	0.00	0.32	0.00	0.00	0.18	0.00	0.00	0.44	0.00	0.00
		T_5	0.26	0.19	0.12	0.36	0.28	0.10	0.20	0.19	0.09	0.60	0.41	0.30
		T_6	0.25	0.19	0.00	0.37	0.27	0.00	0.25	0.21	0.00	0.60	0.46	0.00
		T_7	0.28	0.21	0.13	0.38	0.29	0.13	0.29	0.20	0.13	0.64	0.53	0.34
With biofertilizer	Sakha-93	T_1	0.38	0.28	0.22	0.43	0.35	0.24	0.39	0.32	0.24	0.80	0.68	0.53
		T_2	0.32	0.23	0.00	0.39	0.31	0.00	0.25	0.19	0.00	0.49	0.39	0.00
		T_3	0.32	0.24	0.20	0.41	0.33	0.20	0.33	0.25	0.19	0.67	0.49	0.29
		T_4	0.34	0.24	0.18	0.39	0.31	0.17	0.31	0.22	0.15	0.54	0.40	0.26
		T_5	0.32	0.26	0.21	0.41	0.33	0.21	0.34	0.27	0.20	0.64	0.49	0.38
		T_6	0.34	0.25	0.21	0.41	0.33	0.21	0.35	0.27	0.21	0.68	0.53	0.40
		T_7	0.35	0.27	0.22	0.44	0.34	0.22	0.37	0.29	0.23	0.74	0.63	0.51
	Sakha-94	T_1	0.37	0.26	0.18	0.41	0.32	0.20	0.37	0.30	0.20	0.78	0.65	0.47
		T_2	0.37	0.21	0.00	0.38	0.31	0.00	0.23	0.19	0.00	0.44	0.40	0.00
		T_3	0.32	0.22	0.16	0.39	0.31	0.16	0.33	0.24	0.16	0.65	0.47	0.31
		T_4	0.34	0.21	0.00	0.37	0.30	0.00	0.31	0.20	0.00	0.53	0.38	0.00
		T_5	0.32	0.25	0.17	0.40	0.32	0.17	0.33	0.25	0.17	0.63	0.49	0.35
		T_6	0.34	0.23	0.17	0.40	0.32	0.19	0.35	0.26	0.18	0.67	0.53	0.38
		T_7	0.37	0.25	0.18	0.42	0.32	0.20	0.35	0.27	0.19	0.74	0.61	0.42
$LSD_{5\%}$				0.04			0.03			0.05			0.05	

T1: Positive control (100% NPK), T2: Negative control (without NPK), T3: Micronutrients, T4: Antioxidant at 100 ppm level, T5: Antioxidant at 200 ppm level, T6: Micronutrients + antioxidant at 100 ppm, T7: Micronutrients + antioxidant at 200 ppm level

gave comparable grain yield to the positive control without significant variations especially at E.C. 4.72 and 10.86 salinity levels. Ascorbic acid is a small, water-soluble anti-oxidant molecule which acts as a primary substrate in the cyclic pathway for enzymatic detoxification of hydrogen peroxide. In addition, it acts directly to neutralize superoxide redicals, singlet oxygen or superoxide and as a secondary anti-oxidant during reductive recycling of the oxidized fro of a-tocopherol, another lipophilic anti-oxidant molecule Noctor and Foyer (1990).

Zhang and Kirkham (1996) proved that water stress increased (TBARS) thiobarbituric acid reactive substances accumulation, and as with salt stress, this increase was partially inhibited when plants were supplied with exogenous ascorbic acid. However, the level of osmotic stress was too low to allow a parallel investigation of the effects on seedling survival.

The addition of ascorbic acid would inhibit stress-induced increases in the leakage of essential electrolytes following peroxidative damage to plasma membranes (Lechno *et al.*, 1997 and McKersie *et al.,* 1999). However, additional ascorbic acid did not inhibit increases in leakage of electrolytes from roots of salt-stressed plants. Nor did additional ascorbic acid significantly reduce the undesirable accumulation of sodium in the stems of salt-stressed plants. Possibly, the protective effect of ascorbic acid is more related to reduced AOS damage to essential proteins and/or nucleic acids (Inze' and Van Montague 1995; Becana *et al.,* 1998 and Noctor and Fayer, 1998). Kerk *et al.* (2000) found that low ascorbic acid levels have been associated with mitotic quiescence in root meristems.

Table (4) indicates the influence of diverse tested treatments on each of proline, total chlorophyll and total carotenoids. For proline content, it was found that the highest amounts were recorded at the site of E.C. 7.79 irrespective of the treatment and variety. Furthermore, the treatment of micronutrients + 200 ppm antioxidant gave comparable results to the positive controls for both tested varieties in all salinity levels. In addition, more increases in proline content were recorded due to biofertilization with *Candida tropicalis* in both varieties for all tested salinity levels.

Regarding the total chlorophyll, the determined amounts decreased with increasing salinity level. The positive controls significantly surpassed the different non-biofertilized treatments, while the application of soil yeast *Candida tropicalis* in biofertilizing wheat plants improved chlorophyll content where the treatment of micronutrients + 200 ppm antioxidant induced results were comparable to the positive controls for both tested varieties without significant differences. El-Gabas (2006) found that ascorbic acid increased photosynthetic pigments which was attributed to simulation of biosynthesis of chlorophyll.

The same trend was recorded for total carotenoids where the determined amounts were decreased with increasing salinity level. Moreover, the majority of tested treatments produced amounts of carotenoids were comparable to positive controls without significant differences for both tested wheat varieties.

Table (5) illustrates the enzyme activity of each of polyphenol oxidase, phenylalanine ammonia lyase, peroxidase and amylase. Generally, the obtained results indicated that the enzyme activity decreased with increasing salinity level irrespective of the treatment and wheat variety. Abed And Peter (2001) concluded that the increase in plant resistance to salt-stress was associated with the anti-oxidant activity of ascorbic acid and a partial inhabitation of salt-induced increases in lipid peroxidation by active oxygen species.

Furthermore, biofertilization of both wheat varieties with the soil yeast *Candida tropicalis* augmented the enzymes activity in comparison with the non-biofertilized treatments at all salinity levels. Table (5) also shows that the highest values of enzymes activity were recorded with the positive controls at the three tested salinity levels.

REFERENCES

Abed Shalata and Peter, M. Neuman, 2001. Exogenous ascorbic acid (Vitamin C) increases resistance to salt stress and reduces lipid peroxidation. Journal of Experimental Botany., 52(346): 2207-2211.

Abou El-Soud, M., 1987. Effect of Irrigation Regime and Water Quality on Water and Salt Balance and Crop Production under Lysimeters Conditions. Ph. D. Thesis Soil Department Faculty of Agriculture, El-Mansoura University.

Ashraf, M. and J.M. O'leary, 1997. Ion distribution in leaves of salt-tolerant and salt-sensitive lines of spring wheat under salt stress. Acta. Bot. Neerl., 46(2): 207-217.

Becana, M., J.F. Moran, Iturbe and I. Ormaetxe, 1998. Iron dependent oxygen free radical generation in plants subjected to environmental stress; toxicity and antioxidant protection. Plant and Soil, 201: 137-147.

Bohra, J.S. and K. Doffling, 1993. Potassium nutrition of rice (Oryza sativa L.) varieties under NaCl salinity. Plant Soil. 152: 299-303.

Boyer, J.S., 1969. Effects of osmotic water stress n metabolic rates of cotton plants with open Stomata. Plant Physiol., 40: 229-234.

Cramer, G.R., A. Lauchli and Politovs, 1985. Displacement of Ca+2 by Na+ from the plasma lemma of root cellos. Plant Physiol., 79: 207-211.

Dionisio-Sese, M.L. and Tobita, 2000. Effects of salinity on sodium content and photosynthetic responses of rice seedlings differing in salt tolerance. J. Plant Physiol., 157: 54-58.

Dorais, M., A.P. Papadopoulos and A. Gosselin, 2001. Green house tomato fruit quality. Horticult. Rev. 26: 239-319.

El-Fouly, M.M. and Z.H. Salama, 1999. Can foliar fertilization increase plant tolerance to salinity: Dahlia Greidinger International Symposium: Nutrient Management under Salinity and Water Stress. 1-4 March. Teknion-ITT Haifa.

El-Gabas, N.M.M., 2006. Physiological studies on the effect of ascorbic acid and micronutrients on sunflower plants grown under salinity stress. H. Sc. (Botany). Fac. Sci., Al-Azhar Univ.

Gaballah, M.S. and A.M. Gomaa, 2004. Performance of faba bean varieties grown under salinity stress and biofertilized with yeast. Pakistan Journal of Applied Science, 4(1): 93-99.

Hammerschmidt, R., E.M. Nuckles and J. Kuc, 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to Colletotrichum Lagenarium. Physiol. Plant Pathol., 20: 73-82.

Hy, Y. and U. Schmidholter, 2001. Effect of salinity and macronutrient levels on micronutrients in wheat. J. Plant Nutr., 24(2): 272-281.

Inze' D. and M. Van Montague, 1995. Oxidative stress in plants. Current Opinion in Biotechnology, 6: 153-158.

Jonas, O.A., M.C. Pereyra, C. Cebeza, A.D. Golberg and J.F. Ledent, 1992. Recovery of nitrate reductase activity in wheat leaves after a period of severe water stress. Cereal Research Communications, 20: 13-18.

Karmer, P.J., 1975. Plant, Soil and Water Relationships 2nd Ed., Tata McGraw-Hill.

Kerk, N.M., K.N. Jiang and L.J. Feldman, 2000. Auxin metabolism in the root apical meristem. Plant Physiology 122: 925-932.

Kurt, E., G.R. Cramer, A. Lauchli and E. Epstein, 1986. Effects of NaCl and CaCl2 on cell enlargement and cell production in cotton roots. Plant Physiol., 82: 1102-1106.

Lauchli, A. and Epstein, 1990. Plant responses to saline and sodic conditions. In : Tanji (Ed), Agricultural Salinity Assessment and management American Society of Civil Engineers, New York, pp: 113-137.

Marschner, H., 1995. Mineral Nutrient of Higher Plant. 2nd. Ed. London Academic, pp: 889.

Mass, E.V. and J.F. Grieve, 1990. Spike and leaf development in salt-stressed wheat. Crop Science Society

of America, 30: 1309-1313.

Noctor, G. and C.H. Foyer, 1998. Ascorbate and glutathione: Keeping active oxygen under control. Annual Review of Plant Physiology and Plant Molecular Biology 49, 249-279.

Petrova, A.N. and T.T. Bolotina, 1956. Studies on the enzymes of starch metabolism in potato tubers during growth. Biochema, 21: 4-15.

Ross, W.W. and R.R. Sederoff, 1992. Phenylalanine ammonia lyase from loblolly pine: Purification of the enzyme and isolation of complementary DNA clones. Plant Physiol., 98: 380-386.

Saric, M.R., R. Kastrori, T. Curie, Cupina and I. Gric, 1967. Chlorophyll Determination Univerzitet U Noven Sodu Praktikum iz fiziologize Biljaka-Beograd, Hayena Anjiga, pp: 215.

Turhan, E. and A. Eris, 2005. Changes of Micronutrients, dry weight and chlorophyll contents in strawberry plants under salt stress conditions. Comm. Soil Sci. Plant Anal., 36: 1021-1028.

Turkmen, O., S. Sensory, S. Demir and C. Erdinc, 2008. Effects of two different AMF species on growth and nutrient content of pepper seedlings grown under moderate salt stress. Afr. J. Biotechnol., 7(4): 392-396.

Yaser, F., O. Uzal, S. Tufenkci and K. Yildiz, 2006. Ion accumulation in different organs of freen bean genotypes grown under salt stress. Plant Soil Environ., 52(10): 476-480.