J. Agric. Sci. Mansoura Univ., 29 (1):145 - 173, 2004

EFFECTS OF VARIOUS BACTERIAL INOCULANTS ON GROWTH AND YIELD OF WHEAT PLANTS (*Triticum aestivum*) GROWN UNDER DIFFERENT SALINITY LEVELS

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ABSTRACT

There is increasing attention to accommodate wheat cultivars to grow in salinities outside the natural range of tolerance to obtain an appropriate economic yield. Accordingly, a gnotobiotic experiment was conducted to study the effect of using different bacterial strains, isolated from saline soils, i.e. plant growth promoting rhizobacteria (*Klebsiella pneumoniae*) either alone or in combination with N_2-fixing (*Azospirillum brasilense* + *Azotobacter chroococcum*) or phosphate dissolving (*Bacillus megatherium*) or with both (composite), as inoculation treatments for one week-old wheat seedlings (*Triticum aestivum* L.) cv. Giza 163 growing under different levels of seawater, i.e. 0, 3, 6 and 12 dSm^{-1}. In addition, a pot experiment was carried out using the previous bacterial strains treatments for inoculation of wheat grains as well as for soil pots of wheat plants (2 and 6 weeks-old) grown under the same aforementioned levels of seawater.

In gnotobiotic experiment, the results of total bacterial counts as well as of nitrogenase activity indicated that with increasing salinity level, there were corresponding reductions in total bacterial counts as well as of nitrogenase activity were detected. Meanwhile, higher values of total bacterial counts as well as of nitrogenase activity were obtained from wheat plants inoculated with composite or *Klebsiella* + N_2-fixers inocula as compared whether with uninoculated or the other inoculation treatments under different salinity levels.

In the pot experiment, the microbiological study revealed that numbers of total bacterial counts were decreased with increasing salinity level. The highest total bacterial counts adhering to roots were recorded at nine weeks of growth period for wheat plants inoculated with composite inocula under different salinity levels. Inoculation with various bacterial strains as grain coating treatments increased the germination percentage under different salinity levels as compared each with uninoculated one.

Under different salinity levels, the mean values of germination percentage of wheat grains as well as different studied growth characters of wheat plants showed a significant progressive reduction with increasing salinity levels. Moreover, the mean values of total N, P, K^+ and Ca^{+2} concentrations decreased whereas, Mg^{+2}, Na^+, reducing and total sugar, free amino acids as well as free proline concentrations increased in roots and shoots of wheat plants. Also, the endogenous IAA and cytokinins concentrations decreased, whereas endogenous ABA increased in wheat shoots under different salinity levels. This was reflected on a significant

reduction in the different plant yield components. Such reduction was mostly resulted from reducing the number of tillers bearing spikes under salt stress.

The ability of *K. pneumoniae* to produce exopolysaccharides that binds to wheat plant roots and soil particles (Del Gallo and Fendrik, 1994) could facilitate to the other bacterial inoculants to colonize the rhizosphere of wheat roots. The exopolysaccharides might represent suitable medium for mutual utilization of substances between the plant root and the inoculated bacteria. This could increase the bacterial populations in rooting zone, allowing for more co-operations between wheat plants and inoculated bacteria. Therefore, it was found an absolute superiority of composite inocula treatment on growth of both root and shoot of wheat plants comparing with the other inoculation treatments under the different salinity levels. It appears that composite inocula treatment could partially increase the ability of wheat plants to counteract salinity by accumulating relatively higher amounts of N, K^+, Ca^{2+}, non-reducing sugar, free amino acids, free proline, endogenous auxin and cytokinins as well as lowering Na^+ and endogenous ABA concentrations in their shoots leading to better performance of wheat plants under salinity stress. This was reflected by the superiority of their grain weight/plant as compared with the other inoculation treatments under different salinity levels.

INTRODUCTION

Wheat (*Triticum aestivum)* is the most important growing cereal crop in Egypt. There is a gap between production and consumption owing to the over population in Egypt. Therefore, increasing its productivity and the cultivated areas are highly recommended. Due to restricted resources of fresh water, the use of less quality saline water, i.e. ground water or even diluted seawater became an important source of irrigation especially in the newly cultivated areas. Another problem has special relevance in Egypt, where about two million feddans of salt affected soils concentrated around the Northern lakes and the Mediterranean Sea (SediK, 1997). In addition, one of the major implications of increasing the concentration of soluble salts in the rooting zone of soil, is the reduction of rhizosphereic populations, thereby affecting the plant productivity. Therefore, it is obligatory to use some means, which enhance plant ability to tolerate salinity and minimize the plant's water requirements.

Grain germination is considered an important process in the establishment of a crop stand, and this stage is usually sensitive to salt stress condition (Naqvi, 1994). Moreover, wheat plants growing under saline conditions showed a reduction in growth and their uptake of nitrogen decreased with increasing salinity. To counter the growth depressing effects of stress, a number of investigators have reported that salinity stress could be alleviated by increasing the N supply to the soil (Pessarakli *et al.*, 1991). It minimizes salt-induced damage and apparently provides salt tolerance. In this regard, nitrogen fixation associated with roots of grasses has been recognized as a possible significant component of the nitrogen cycle in a range of ecosystems including several extreme environments (Dart, 1986). In

addition to beneficial effects of N_2-fixing bacteria associated with roots of cereal crops, these bacteria are also reported to produce growth promoting substances which could help in increasing crop yield (Lifshitz *et al.*, 1987 & De-Freitas and Germida, 1990). Moreover, the ability of *K. pneumoniae* to produce exopolysaccharides that binds to wheat plant roots and soil particles (Del Gallo and Fendrik, 1994) could facilitate to the other bacterial inoculants to colonize the rhizosphere of wheat roots.

Accordingly, this work was aimed to use bacterial inoculants containing living cells of efficient strains, isolated from saline soils, of plant growth promoting rhizobacteria, either alone or in combination with N_2-fixing or phosphate dissolving or with both, for inoculation of wheat grains and soil area with the objective of increasing the numbers of such bacteria and accelerating certain microbial processes to augment the extent of the availability of nutrients in a form which can be easily assimilated by wheat plants as well as to counter partially the detrimental effects of salinity on growth and yield. In addition, the changes in mineral nutrients, sugar, free amino acids and free proline concentrations as well as endogenous plant growth regulators of wheat plants were studied to define a possible physiological role of different bacterial inoculants under salinity stress.

MATERIALS AND METHODS

The present investigation was carried out in the Lab. of Microbiology Department as well as in the greenhouse and Lab. of the Plant Physiology Section, Faculty of Agriculture, Cairo University. The pot experiment was twice conducted under the same conditions during two successive seasons 2000/01 and 2001/02. Therefore, the tabulated and discussed data of the various determinations represent the combined analysis for the two seasons.

Bacterial strains:

A number of bacterial strains belong to the genera *Klebsiella, Azospirillum, Azotobacter* and *Bacillus* isolated from Egyptian saline sandy soils (Sedik, 1997) were purified and identified according to API microtube system, API 20B, API 20E and API 50CHE (Logan and Berkley, 1984).

Inocula preparation:

Bacterial strains, i.e. producing of exopolysaccharides and plant growth promoting substances *(Klebsiella pneumonia)*, N_2 -fixers *(Azospirillum brasilense* + *Azotobacter chroococcum)* and phosphate dissolving (*Bacillus megatherium*) were used in inoculation treatments. Each strain was precultured in nutrient broth medium at 30°C in incubator with shaking rate at 140 rpm for 3-5 days. Five combinations of bacterial strains treatments were tested for inoculation of wheat in both gnotobiotic and pot experiments as follows: 1) uninoculated, 2) 1ml of *K. pneumonia* ($7.0x10^7$ cells), 3) 1ml of *K. pneumonia* ($7.0x10^7$ cells) + 1ml of *A. brasilense* ($5.0x10^7$ cells) + 1ml of *A. chroococcum* ($3.0x10^7$ cells), 4) 1ml of *K. pneumonia* ($7.0x10^7$ cells) + 1ml of *B. megatherium* ($4.5x10^7$ cells) and 5) 1ml of *K. pneumonia* ($7.0x10^7$ cells) + 1ml of *A. brasilense* ($5.0x10^7$ cells) + 1ml of *A. chroococcum* ($3.0x10^7$ cells) + 1ml of *B. megatherium* ($4.5x10^7$ cells).

Gnotobiotic experiment:

Mature grains of wheat (*Triticum aestivum* L.) cv. Giza 163 were surface sterilized and germinated onto agar plates. After one week, seedlings were transferred to 75ml tubes (two seedlings / tube) containing 15 ml of semisolid basal medium of Reinhold *et al.* (1985) in a gnotobiotic system. Semisolid basal medium was prepared by using different dilutions of seawater to give final salt levels at electrical conductivity (E.C.) 0, 3, 6 and 12 dSm^{-1}. Cultures tubes (10-tubes/ treatment) were inoculated with different broth cultures of the inocula as previously mentioned. Culture tubes were incubated at 23-25°C in a growth chamber with a 16-h photoperiod. For each treatment, wheat plants were taken 0, 1, 2 and 3 weeks after seedling inoculation, for determination of bacterial count on plant roots and nitrogenase activity.

Pot experiment:

Mature grains of wheat were soaked for one hour in 7 days-old broth culture of the different inocula treatments, i.e. 1) uninoculated, 2) *K. pneumonia*, 3) *K. pneumonia*+ *A. brasilense*+ *A. chroococcum*, 4) *K. pneumonia*+ *B. megatherium* and 5) *K. pneumonia*+ *A. brasilense*+ *A. chroococcum*+ *B. megatherium*. After, the grains were surface dried on filter paper. The grains from each treatment were sown in plastic pots, 25 cm in diameter, filled with a mixture of 2:1 clay and fine sand, on 12th Nov, 2000 and 19th Nov, 2001, in the first and second seasons, respectively. Each pot normally fertilized with 1.4g superphosphate, 1.1g potassium sulfate and 2.5 g ammonium nitrate. Salinization treatments were immediately begun with the different levels of seawater at electrical conductivity (E.C.) 0, 3, 6 and 12 dSm^{-1}. Each pot from each treatment received 500 ml from its respective solution once every three days. Two and six weeks after sowing, the different bacterial inocula treatments were again used to inoculate wheat plants at the rate of 10 ml/pot. Each treatment was represented by 10 pots in a completely randomized design.

From each treatment, four samples were taken 3, 6, 9 and 12 weeks after sowing for determination of variable bacterial counts. Meanwhile, two samples were taken 35 and 65 days after sowing to record the growth characters including, the main stem length, root and shoot dry weights as well as number of tillers. At harvest, for each treatment grain number per spike, grain weight per spike, grain weight per plant and 1000-grain weight were recorded.

Microbiological analysis

The plate count method by using the selective medium of combined carbon medium (CCM) was carried out according to Hegazi *et al.* (1998). Technique of colony forming units (CFU) was adopted at 30°C for 2-5 days. To measure nitrogenase activity, the acetylene reduction activity (ARA) was measured by using gas-liquid chromatography (Pye Unicam PU 4550) according to Hardy *et al.* (1973). Nitrogenase activity was expressed as nmoles ethylene produced per seedling per hour. Each value represented the mean of five culture tubes (replicates).

Chemical Analysis:

Determinations of mineral nutrients, sugars, free amino acids and free

proline in roots and shoots were only carried out on the second sample. Meanwhile, determination of endogenous phytohormones was only determined in the shoots of the second sample.

Nutrient elements determination:

Determination of nutrient elements were carried out on the dry materials of roots and shoots. For total nitrogen, phosphorus, potassium, calcium, magnesium and sodium determination, the wet digestion of dry plant material was carried out as described by Piper (1947). For the determination of total N, the modified micro-Kjeldahl" apparatus of Parnas and Wagner as described by Pregl (1945) was used. Phosphorus was determined colorimetrically using the chlorostannous reduced molybdophosphoric blue color method as described by King (1951). Potassium, calcium, magnesium and sodium determinations were determined using Atomic Absorption Spectrophotometer (Unicum Sp., 1900).

Sugars and free amino acids determinations:

Extraction of sugars and free amino acids was carried out using hot ethanol (80 % v/v, 10 ml/g f.wt.). Reducing and total sugars were determined spectrophotometrically at 540 nm by phosphomolybdic acid method according to A.O.A.C. (1975). Free amino acids were determined spectrophotometrically at 570 nm by using ninhydrin reagent according to Moore and Stein (1954). Pure leucine was used as a standard.

Proline determination:

Extraction of proline was carried out by homogenizing the Freeze-dried seedlings with sulphosalicyclic acid (3% w/v, 20 ml/g f.wt.). Proline was estimated spectrophotometrically at 520 nm by using the ninhydrin method according to Bates *et al.* (1973). Pure proline was used as a standard.

Endogenous phytohormones determination:

Endogenous phytohormones, i.e. indole-3-acetic acid (IAA), cytokinins and abscisic acid (ABA) were determined in shoots according to Nesiem (1998) by using Ati-Unicum gas-liquid chromatography, 610 Series, equipped with flame ionization detector. The peaks identification and quantification of phytohormones were performed by using external authentic hormones and a Microsoft program to calculate the concentrations of the identified peaks.

Statistical analysis:

Data of growth characters and plant yield components were analyzed by using two factorial completely randomized design according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Gnotobiotic experiment:

The results of total bacterial counts adhering to roots of wheat plants grown in gnotobiotic system for different incubation periods as affected by various bacterial inoculations under different salinity levels are presented in Table (1).

Table (1): Total bacterial counts ($x10^4$ CFU. g^{-1} dry root) adhering to roots at different incubation periods of wheat plants grown in gnotobiotic system as affected by various bacterial inocula under different salinity levels.

Salinity level (dSm^{-1})	Incubation period (weeks)	uninoculated	Kleb.	Kleb. + (Azoto.+Azospi.)	Kleb. + Bacillus	Composite
0.0	0	0.3	2.0	2.7	2.4	3.8
	1	1.1	28.0	52.3	48.2	110.0
	2	3.8	152.0	330.0	202.0	569.0
	3	1.4	76.0	121.0	113.0	340.0
Mean		1.7	64.5	126.5	91.4	255.7
3.0	0	0.2	2.5	3.4	3.0	4.0
	1	0.2	40.0	62.0	52.4	127.3
	2	1.0	215.0	376.0	242.0	618.9
	3	0.4	112.0	236.0	148.0	410.0
Mean		0.5	92.4	169.4	111.4	290.1
6.0	0	0.1	1.5	2.4	2.2	3.3
	1	0.2	24.0	36.2	30.1	70.4
	2	0.7	134.3	294.6	195.0	452.0
	3	0.3	56.1	112.0	83.0	286.2
Mean		0.3	54.0	111.3	77.6	203.0
12.0	0	0.1	0.7	1.3	1.2	1.7
	1	0.1	10.2	17.8	12.1	24.4
	2	0.3	55.6	132.1	96.0	152.0
	3	0.1	22.1	57.1	39.7	90.2
Mean		0.2	22.2	52.1	37.3	67.1

Under the effect of both salinity and bacterial inoculation treatments, total bacterial counts estimated on roots of wheat plants were progressively increased with the increasing of incubation period, reaching to their maximum values after two weeks of inoculation. This was followed by a noticeable decrease in the third week. Of course, variation in bacterial population densities might be related to bacterial strain inocula as well as the nature of wheat root exudates during the different developmental stages. Fischer *et al.* (1999) demonstrated that wheat root exudates could influence the gene expression of external membrane proteins when *A. brasilense* Cd was grown under saline stress.

Meanwhile, whether in uninoculated or inoculated treatments, the total bacterial counts adhering to roots of wheat plants was proportionally decreased with increasing salinity level, except of salinity at the level of 3 dSm^{-1}, which showed a slight increase as compared to non-saline control. On the other hand, inoculation of wheat plants with either of composite (*K. pneumoniae* + *A. brasilense* + *A. chroococcum* + *B. megatherium*) or *K. pneumoniae* + N_2-fixers inocula induced higher values of total bacterial counts adhering to roots under different salinity levels as compared with other inoculation treatments at all incubation periods.

The results of nitrogenase activity expressed in terms of acetylene reducing activity (ARA) of wheat plants grown in gnotobiotic system for different incubation periods as affected by various bacterial inoculations under different salinity levels are presented in Table (2). Generally, the results of nitrogenase activity of wheat plants showed similar trends to that obtained from total bacterial counts whether under different salinity or

bacterial inoculation treatments. In addition, nitrogenase activity showed slight increase at the salinity level of 3 dSm^{-1} as compared to non-saline control. In this concern, Malik *et al.* (1991) reported that among the plants growing in saline environment, rice and wheat, which exhibited high nitrogenase activities as estimated by ARA technique. Tripathi and Mishra (1998) reported that increased NaCl inhibited growth and plant-growth-promoting activities, e.g. N_2-fixation and IAA production in *A. brasilense*. However, Mahmoud *et al.* (1973) and Malik *et al.* (1994) found that efficiency of nitrogen fixer's strains derived from saline soils were more salt tolerant than those obtained from slightly or moderately saline soils.

Table (2): Nitrogenase activity (nmol C_2H_4 . seedling^{-1} .h^{-1}) of wheat plants grown in gnotobiotic system for different incubation periods as affected by various bacterial inocula under different salinity levels.

Salinity level (dSm^{-1})	Incubation period (weeks)	uninoculated	Kleb.	Kleb. + (Azoto.+Azospi)	Kleb. + Bacillus	Composite
0.0	0	0.7	5.0	25.1	2.7	26.4
	1	1.2	13.2	96.2	10.1	127.2
	2	1.8	48.1	425.0	44.1	488.1
	3	1.1	28.0	344.0	29.7	366.0
Mean		1.2	23.6	222.6	21.7	251.9
3.0	0	0.6	5.4	26.2	3.2	30.1
	1	1.0	14.1	110.8	13.0	135.2
	2	1.7	56.2	466.1	50.2	511.1
	3	0.9	36.2	370.6	30.1	496.1
Mean		1.1	28.0	243.4	24.1	293.1
6.0	0	0.5	3.7	22.2	2.3	24.1
	1	0.8	10.1	81.0	7.8	120.2
	2	1.3	34.2	414.0	35.0	461.0
	3	0.7	23.6	305.0	22.2	340.0
Mean		0.8	17.9	205.6	16.8	236.3
12.0	0	0.2	1.1	10.1	0.9	16.6
	1	0.5	5.2	42.0	3.2	74.0
	2	0.9	17.9	204.0	14.0	256.0
	3	0.3	8.4	169.0	7.1	149.0
Mean		0.5	8.2	106.3	6.3	123.9

Under non-saline conditions, the highest values of nitrogenase activity were obtained from wheat plants inoculated with composite or *Kleb.*+ N_2-fixers inocula. Also, both inoculation treatments recorded the superiority of nitrogenase activity under the effect of different salinity levels at all incubation periods.

Pot experiment:

The capacity of wheat grains to germinate is related to the extent of imbibition from the soil culture solution and the resultant activity of the embryo. Therefore, different salinity levels reduced wheat grain germination percentage Fig (1). The reduction was proportional to the increase in the salinity level. In this respect, Raghav and Pal (1994) reported that wheat germination percentage decreased with increasing salt concentration. The reduction in germination under saline conditions could be attributed to increased osmotic pressures of the soil culture solution, which diminish the water absorption rate, leading to moisture stress in the seeds (Prisco and O'Leary, 1970) and lowering of food reserve mobilization. This could be

attributed to the negative effects of salts on enzymes responsible for hydrolysis and consequently on diminishing of hydrolysis products translocated to the embryo axis (Prisco *et al.*, 1981).

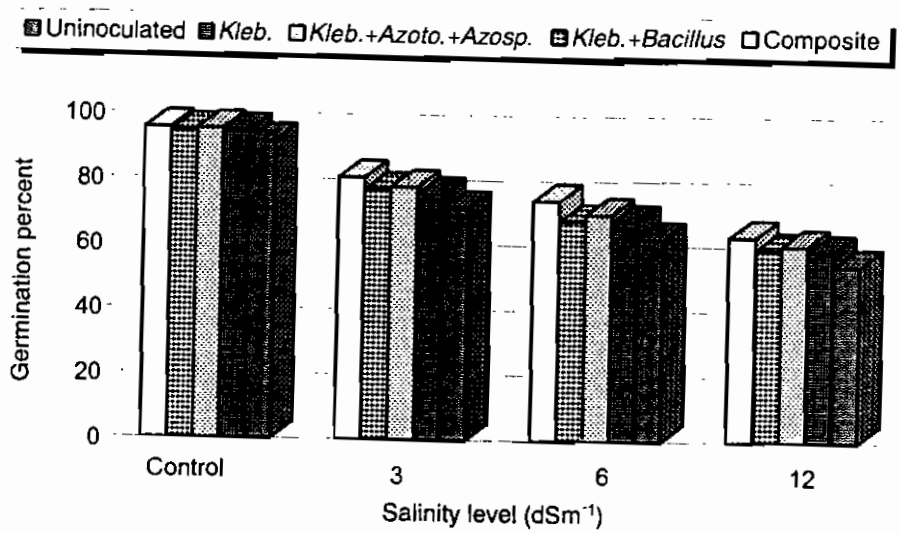

Fig.(1): Effect of various bacterial inocula treatments on germination percentage of wheat grains germinated under different salinity levels.

On the other hand, inoculation with various bacterial strains as grain coating treatments increased the germination percentage under different salinity levels as compared each with its corresponding value from uninoculated one (Fig 1). The increase was in descending order as follows: composite, *Kleb.*+ N_2-fixers, *Kleb.*+P-dissolving and finally *Kleb.*. Thus, the composite inoculation was partially able to alleviate the inhibition effect of salt stress on seed germination. In this regard, Shende *et al.* (1977) have observed an enhancement of wheat grain germinatcn by *Azotobacter* inoculation. This could be partly due to the ability of *Azotobacter* to produce growth substances and anti-fungal antibiotics. Also, the exopolysaccharides released by *Klebsiella* could protect grains against the osmotic stress induced by salt. Similar conclusion was found by Roberson and Firestone (1992) who found that bacterial exopolysaccharides could protect bacteria from various stresses.

The results of bacterial population densities adhering to roots at different growth periods of wheat plants as affected by various bacterial inocula under different salinity levels are presented in Table (3).

The results of microbiological studies revealed that the most important factors controlling the bacterial counts under soil conditions in all studied treatments were correlated with the age of wheat plant, bacterial strains inocula as well as salinity level. The results revealed that numbers of total

bacterial counts were decreased with increasing salinity level. In addition, the highest total bacterial counts adhering to roots were recorded at nine weeks of growth period for wheat plants inoculated with composite inocula under different salinity levels. However, wheat plants inoculated with *Klebsiella* alone or mixed with *Bacillus* harbored the lowest bacterial counts under different salinity levels. Certainly, the counts were still high comparing with the uninoculated one. In this regard, studying of histological assay of the colonized rice roots revealed that *Alcaligenes faeculis*, *A. brasilense* and *Azotobacter vinalandii* could be well expressed on the surface or in the endorhizosphere of rice roots under salt stress (Tripathi and Mishra, 1998). Substantial areas of agricultural potential in the tropics and subtropics are affected by salinity, which is known to have adverse effects on plant productivity and activities of plant-associated bacteria. Malik and Bilal (1988) mentioned that saline habitat soils represented a low fertility environment. So, it provides an ideal ecological niche for nitrogen fixation.

Table (3): Total bacterial counts ($x10^4$ CFU. g^{-1} dry root) adhering to roots at different growth periods of wheat plants as affected by various bacterial inocula under different salinity levels.

Salinity level (dSm^{-1})	Plant age (wks)	uninoculated	Kleb.	Kleb. + (Azoto.+Azospi)	Kleb. + Bacillus	Composite
0.0	3	22.0	52.0	74.0	58.0	100.0
	6	81.0	149.0	329.0	281.0	500.0
	9	112.0	201.0	462.0	330.0	662.0
	12	54.0	132.0	326.0	248.0	456.0
Mean		62.3	133.5	297.8	229.3	434.5
3.0	3	19.0	60.0	80.0	72.0	110.0
	6	46.0	184.0	360.0	312.0	522.0
	9	66.0	214.0	496.0	366.0	730.0
	12	39.0	152.0	360.0	270.0	486.0
Mean		48.0	152.5	324.0	255.0	462.0
6.0	3	14.0	48.0	60.0	44.0	81.0
	6	26.0	114.0	276.0	223.0	449.0
	9	60.0	162.0	376.0	290.0	633.0
	12	19.0	100.0	284.0	209.0	377.0
Mean		30.3	106.0	249.5	191.5	385.0
12.0	3	6.0	26.0	41.0	23.0	63.0
	6	11.0	51.0	180.0	98.0	206.0
	9	34.0	71.0	271.0	139.0	352.0
	12	6.0	46.0	115.0	84.0	152.0
Mean		14.3	48.5	146.8	86.0	193.8

The results of growth characters, i.e. main stem length, number of tillers as well as dry weight of both roots and shoots in the first and second samples of wheat plants as affected by various bacterial inoculation treatments under different salinity levels are presented in Tables (4 & 5). In both the first and the second samples, the mean values of main stem length, number of tillers as well as shoot dry weight showed a significant progressive reduction with increasing salinity levels, while the mean values of root dry weights showed only a significant reduction at salinity level 12 dSm^{-1} as compared each to the non-salinized control.

In this concern, Khan *et al.* (1992) stated that wheat seedling height and dry weight were negatively correlated with increasing electrical conductivity. It was believed that Na^+ toxicity might be considered the main cause of plant

growth depression under salinity. Growth reduction and salt damage appeared to be associated with ion toxicity (Heyser and Nabors, 1981), disturbance of cellular and tissue water status (Weimberg *et al.*, 1984), or perhaps increased ATP demand for osmotic adjustment (Cushman *et al.* 1990). Greenway and Munns (1980) suggested that growth reduction would presumably occur if a large proportion of photosynthates were used as osmotic solutes.

Table (4): Effect of various bacterial inoculant treatments on main stem length and number of tillers produced in the first and second samples of wheat plants grown under different salinity levels.

[a] Salinity level (dSm^{-1}) / [b] Inoculation	0.0	3	6	12	Mean	0.0	3	6	12	Mean
	First sample					Second sample				
	Main stem length									
Uninoculated	35.5	31.4	30.6	25.2	30.7	53.8	48.2	42.9	35.9	45.2
Kleb.	36.9	33.5	32.8	27.8	32.8	56.2	51.3	45.7	39.4	48..2
Kleb.+N_2-fixers	37.6	34.1	33.4	28.7	33.7	57.3	52.2	46.8	44.1	50.1
Kleb.+P-dissol.	37.3	33.8	33.1	28.4	33.2	56.8	51.8	46.4	40.2	48.8
Composite	38.4	34.7	34.0	30.8	34.5	58.4	53.2	47.6	48.2	51.9
Mean	37.1	33.5	32.8	28.4		56.5	51.3	45.9	41.6	
$LSD_{0.05}$	a = 2.4	b = 2.6	a x b = 4.5			a = 2.8	b = 3.1	a x b = 5.3		
	No. of tillers									
Uninoculated	2.1	1.5	0.9	0.6	1.3	2.5	2.0	1.3	0.9	1.7
Kleb.	2.2	1.8	1.4	1.0	1.6	2.6	2.2	1.6	1.3	1.9
Kleb.+N_2-fixers	2.3	1.9	1.5	1.1	1.7	2.7	2.3	1.9	1.6	2.1
Kleb.+P-dissol.	2.2	1.7	1.4	1.0	1.6	2.6	2.2	1.7	1.4	2.0
Composite	2.3	2.0	1.7	1.4	1.9	2.8	2.4	2.0	1.8	2.3
Mean	2.2	1.8	1.4	1.0		2.6	2.2	1.7	1.4	
$LSD_{0.05}$	a = 0.3	b = 0.3	a x b = 0.5			a = 0.4	b = 0.5	a x b = 0.8		

Table (5): Effect of various bacterial inoculant treatments on root and shoot dry weights (g) in the first and second samples of wheat plants grown under different salinity levels.

Salinity level (dSm^{-1})		0.0	3	6	12	Mean	0.0	3	6	12	Mean
noculation	Plant part	Root					Shoot				
Un-inoculated	Root	0.04	0.04	0.04	0.03	0.04	0.12	0.11	0.08	0.07	0.09
	Shoot	0.30	0.22	0.19	0.17	0.22	0.85	0.69	0.54	0.42	0.63
	Whole plant	0.34	0.26	0.23	0.20	0.26	0.97	0.80	0.62	0.49	0.72
Kleb.	Root	0.05	0.05	0.04	0.04	0.05	0.14	0.13	0.10	0.09	0.12
	Shoot	0.32	0.24	0.22	0.20	0.25	0.92	0.79	0.69	0.56	0.74
	Whole plant	0.37	0.29	0.26	0.24	0.30	1.06	0.92	0.79	0.65	0.86
Kleb. + N_2-fixers	Root	0.06	0.06	0.05	0.04	0.05	0.15	0.14	0.11	0.11	0.13
	Shoot	0.34	0.26	0.23	0.23	0.27	0.94	0.85	0.72	0.63	0.79
	Whole plant	0.40	0.32	0.28	0.27	0.32	1.09	0.99	0.83	0.74	0.92
Kleb. + P-dissol.	Root	0.05	0.05	0.04	0.04	0.05	0.14	0.13	0.10	0.09	0.12
	Shoot	0.33	0.24	0.22	0.21	0.25	0.91	0.77	0.68	0.58	0.74
	Whole plant	0.38	0.29	0.26	0.25	0.30	1.05	0.90	0.79	0.67	0.86
Composite	Root	0.06	0.06	0.05	0.05	0.06	0.16	0.14	0.12	0.11	0.13
	Shoot	0.36	0.28	0.25	0.24	0.28	0.98	0.89	0.75	0.70	0.83
	Whole plant	0.42	0.34	0.30	0.29	0.34	1.14	1.03	0.87	0.81	0.96
Mean	Root	0.05	0.05	0.04	0.04		0.14	0.13	0.10	0.09	
	Shoot	0.33	0.25	0.22	0.21		0.92	0.80	0.68	0.58	
	Whole plant	0.38	0.30	0.26	0.25		1.06	0.93	0.78	0.67	
$LSD_{0.05}$	Root	a = NS	b = 0.01	a x b = 0.01			a = 0.02	b = 0.03	a x b = 0.04		
	Shoot	a = 0.03	b = 0.03	a x b = 0.05			a = 0.11	b = 0.12	a x b = 0.20		
	Whole plant	a = 0.04	b = 0.05	a x b = 0.07			a = 0.12	b = 0.14	a x b = 0.22		

Also, it was found that shoot growth was retarded to a great extent than root growth under each salinity level, which was almost due to the reduction in the number of tillers (Table 4). In this regard, Maas *et al.* (1994) found that salinity (12-18 dSm^{-1}) significantly decreased the number of primary and secondary tillers in wheat. Leidi *et al.* (1991) mentioned that shoot growth was much more retarded than root growth in salinity-stressed wheat plants. The resultant decrease in shoot/root ratio presumably improves water balance by maintaining the potential for water absorption while reducing transpiration (Iyengar and Reddy, 1994).

Under the effect of different bacterial inoculants, the mean values of main stem length, number of tillers as well as root and shoot dry weight showed higher values in both the first and second samples as compared each with their corresponding mean value of uninoculated one. In addition, composite inoculant treatment containing plant growth promoting rhizobacteria (*K. pneumoniae*), N_2 -fixing bacteria (*A. brasilense* + *A. chroococcum*) and P-dissolving bacteria (*B. megatherium*) significantly increased all the studied growth characters as compared each whether with the other inoculant treatments or with the uninoculated one. Moreover, the increase in the mean value of shoot dry weight of composite inoculant treatment over their mean value from uninoculated treatment reached three- and five-folds from its corresponding increase in the mean value of root dry weight in the first and second samples, respectively. Such increment in the shoot dry weight was apparently due to increase in the number of tillers. Srinivason *et al.* (1997) indicated that combination of biofertilizers such as *Azotobacter* or *Rhizobium* with cellulose decomposers or *B. megatherium* produced a positive effect on wheat growth, which might be due to secretion of growth promoting substances. Abd El-Rahman (2000) reported that application of P-biofertilizer at the rate of 3kg/fed significantly increased the stem length, number of branches and leaves as well as fresh and dry weight of cantaloupe plants as compared with control. Meshref *et al.* (2000) stated that biofertilization of wheat grains by nitrobien combined with organic manure (FYM or town refuse manure) significantly increased the mean values of wheat plant dry weight.

The complex and dynamic interactions among microorganisms, roots, soil and water in the rhizosphere induce changes in soil physicochemical and structural properties (Chenu and Roberson, 1996). The capacity of plant growth-promoting rhizobacteria, i.e. *Klebsiella pneumoniae* to colonize the rhizosphere is not fully understood (El-Khawas, 2002). It is presumed that it depends on several factors, like root exudates, chemotaxis, antagonism for microorganisms, cyst formation allowing survival under adverse conditions and most importantly, the ability to produce exopolysaccharides that binds to plant roots and soil particles (Del Gallo and Fendrik, 1994). Under the effect of bacterial secretion of an exopolysaccharides on rhizosphere soil physical properties, Amellal *et al.* (1998) found that *Pantoea aglomerans* colonization of wheat root was increased at the rhizoplane and in root-adhering soil as well as improved water stability of adhering soil aggregates. The production of exopolysaccharides possibly enhances water retention in the microbial

environment (Chenu and Roberson, 1996). Aggregate stability of rye grass and alfalfa rhizosphere soil has been shown to increase with root growth and the stabilizing effect apparently originated from polysaccharide production in the rhizosphere (Reid and Goss, 1981). In addition, Ping *et al.* (1998) stated that inoculation of rice plants with *Alcaligenes faeculis* could enhance salt tolerance, promote growth as well as stimulate the formation of lateral roots and root hairs of host rice under salt stress. Several investigators reported that addition of biofertilizers such as *Azospirillum*, *Azotobacter*, *Bacillus*, *Klebsiella* and *Pseudomonas* not only fixed nitrogen but also release certain phytohormones such as GAs, cytokinins and IAA. These phytohormones could play an important role in plant growth and development, such as stimulating their nutrients uptake and photosynthetic activity (Tien *et al.*, 1979; Fayez *et al.*, 1985).

Data of plant yield components, i.e. grain number/spike, grain weight/spike, grain weight/plant and 1000-grain weight are presented in Table (6). The results obtained revealed that with increasing salinity level, there was a significant reduction in the mean values of grain number/spike, grain weight/spike, grain weight/plant and 1000-grain weight as compared each with the corresponding mean values of non-salinized control. Such reduction in grain yield/plant was mostly resulted from reducing the number of tillers bearing spikes under salt stress.

Table (6): Effect of various bacterial inoculant treatments on yield components of wheat plants grown under different salinity levels.

[a] salinity level (dSm^{-1}) / [b] Inoculation	0.0	3	6	12	Mean	0.0	3	6	12	Mean
	Grains number / spike					Grains weight (g) / spike				
Uninoculated	38.1	36.3	33.3	29.9	34.4	1.23	1.05	0.97	0.86	1.03
Kleb.	39.5	38.6	34.6	31.2	36.0	1.28	1.17	1.06	0.94	1.11
Kleb.+N_2-fixers	40.1	38.5	35.3	32.6	36.6	1.32	1.24	1.09	1.01	1.17
Kleb.+P-dissol.	40.0	38.1	35.0	31.7	36.2	1.30	1.19	1.07	0.96	1.13
Composite	40.4	39.2	36.0	34.3	37.5	1.34	1.30	1.13	1.07	1.21
Mean	39.6	38.1	34.8	31.9		1.29	1.19	1.06	0.97	
$LSD_{0.05}$	a = 2.3		b = 2.6		a x b = 4.3	= 0.10		b = 0.12		a x b = 0.19
	Grains weight (g) / plant					1000 grains weight (g)				
Uninoculated	4.31	3.03	2.16	1.58	2.77	32.22	28.90	29.19	28.88	29.80
Kleb.	4.61	3.62	2.68	2.09	3.25	32.51	30.32	30.71	30.11	30.91
Kleb.+N_2-fixers	4.88	3.96	3.07	2.55	3.62	32.85	32.28	30.97	30.95	31.76
Kleb.+P-dissol.	4.68	3.68	2.81	2.23	3.35	32.43	31.30	30.60	30.27	31.15
Composite	5.09	4.28	3.30	2.88	3.89	33.25	33.13	31.45	31.11	32.24
Mean	4.71	3.71	2.80	2.27		32.65	31.19	30.58	30.26	
$LSD_{0.05}$	0.66		b = 0.75		a x b = 1.21	= 1.15		b = 1.36		a x b = 2.10

In this regard, Hanks *et al.* (1989) reported that wheat grain yield was drastically reduced by 30 to 100% due to saline irrigation water compared with fresh water. Naresh *et al.* (1993) found that wheat irrigated with saline water (12 dSm^{-1}) produced 40% lower grain yields than control. Grieve *et al.* (1992) found that number of wheat grains per spike were decreased in response to salinity 14.3-18.1dSm^{-1}. Ahmed *et al.* (1993) indicated that saline irrigation (5000 ppm NaCl) reduced 1000-grain weight and grain yield per

plant in barley. Holloway and Alston (1992) reported that salt stress decreased tillering, dry matter production and grain yield of the stressed wheat plants. Maas *et al.* (1996) and Zeng *et al.* (2000) stated that salinity stress significantly decreased yield components of wheat and rice, respectively.

Regarding the effect of different bacterial inoculant treatments, it was found that the mean values of various plant yield components were increased as compared each with the corresponding mean value of uninoculated one. In addition, *Kleb.*+ N_2-fixers and composite inoculant treatments significantly increased the mean values of grain weight/spike, grain weight/plant and 1000-grain weight as compared each with the uninoculated one. The increase of mean values of grain weight/plant over the uninoculated control reached 30.7 and 40.4% for *Kleb.*+N_2-fixers and composite inoculants, respectively. The corresponding increase for grain weight/spike reached 13.6 and 17.5%, respectively. Meanwhile, The corresponding increase for 1000-grain weight reached 6.6 and 8.2%, respectively. However, only the composite inoculant treatment significantly increased the mean value of grain number /spike, reaching about 9.0% over the respective value of uninoculated treatment. Thus, it appears that such increment in the grain weight/plant might be mostly referred to increase in the number of tillers bearing spikes and partially to the increase in grain number/spike and 1000-grain weight.

In this concern, Ping *et al.* (1998) found under salinity stress that biofertilizers containing salts-tolerant nitrogen fixing bacteria could promote growth of rice plants and increases their yield. Subba Rao (1984) reported that inoculation with *Azospirillum brasilense* significantly increased wheat grain yield at the high level of nitrogen (120 Kg N /ha). Meanwhile, Sundara Rao (1968) found that phosphobacterin inoculation of wheat cultivated in salinized alluvial impregnated soil, resulted in an increase in grain yield to about 16-37% over control (uninoculated). Guar *et al.* (1980) demonstrated that significant increase in wheat grain yield was possible when wheat was inoculated with *Bacillus megatherium* in the presence of rock phosphate at 100 Kg P_2O_5 /ha. Hussien and Radwan (2001) found that application of dual biofertilization (phosphate dissolving bacteria and *Azospirillum* spp.) increased wheat grain yield by 6.49% over the non-biofertilized treatment.

Inoculation is successful when root colonization is achieved and followed by interaction between bacteria and the root system. However, plant response to different rhizobacteria strains inoculation depends on various factors, including mode of inoculation, chances of survival and motility (Okon, 1985), adsorption by soil particles, competition with indigenous populations of rhizospheric bacteria (Bashan and Levanony, 1990), soil fertility (Nuti and Basaglia, 1993) and consequently they are capable of having a direct effects on plant growth. Thus, the increase of wheat yield obtained by bacterial inoculation could be attributed to IAA, GAs and cytokinin-like substances produced by the bacterium. Similar conclusion was suggested by Tien *et al.* (1979) on pearl millet. The beneficial effects of N_2-fixing bacteria associated with roots of cereal crops are reported to produce growth promoting substances which helps in increasing crop yield (De-Freitas and Germida,

1990). Vande Broek and Vanderleyden (1995) attributed yield increases to mechanisms such as nitrogen fixation, phytohormones and nitrate reduction.

The results of total N, P, K^+, Ca^{2+}, Mg^{2+} and Na^+ concentrations in both roots and shoots of the second sample are presented in Table (7). Under the effect of the different salinity levels, the mean values of total N, P, K^+ and Ca^{2+} concentrations in roots and shoots were decreased as compared to the mean values of non-salinized treatment. The decrease was progressively with increasing salinity level. However, the reduction was much higher in roots than shoots.

Reduced growth in terms of dry matter production and decreased of nitrogen uptake have been reported by several investigators for wheat with different degrees of salt stress (Broadbent *et al.*, 1988 and Botella *et al.*, 1997). Zarate (1990) found that excess salts in the root medium impaired N uptake, through the competition relationship between NO_3^- and Cl^-. Meanwhile, the decreased P concentration associated with salinity could be ascribed to the higher pH value of sodium affected soil, which might hinder P availability to plants (Jeffrey, 1987).

On the other hand, potassium ion which is apart from its role as an osmotic component, it is essential for starch formation, protein synthesis, photosynthate partitioning, normal stomatal functioning as well as an activator for many enzymes (Marschner, 1995). Leidi *et al.* (1991) pointed out that K^+ concentration in shoots of wheat plants was inversely correlated with Na^+ under NaCl stress, but directly correlated with shoot dry weight. The decrease in K^+ might be attributed to the antagonism between K^+ and Na^+ cations, which increased considerably as salinity increased (Mozafer and Oertli, 1990). Meanwhile, Sharma and Gupta (1986) found that increase level of Na^+ in the plant tissue caused nutrients imbalance and displace Ca^{2+} from the exchange sites on the membranes and cell walls. Davenport *et al.* (1997) reported that translocation of Ca^{2+} to the shoots was more inhibited under saline condition in wheat.

Under the effect of different salinity levels, the mean values of Mg^{2+} and Na^+ concentrations increased in roots and shoots as compared to the mean values of the non-salinized controls (Table 7). The increase of Na^+ concentration was proportional to the increase in salinity level and this was more pronounced in roots than shoots.

Of course, the increase in Na^+ under salinity was expected since it was predominant in the root medium. Hamdy (1988) found a significant negative relationship between Na^+ concentration and the uptake of other nutrients (N, P and K^+) which clearly confirmed that high Na^+ concentration not only reduced the dry matter production, but also decreased the concentration and uptake of the major nutrients, thus exercising a double negative influence. In addition, Zhang and Liu (1993) noticed that H^+-ATPase activity of plasmamembrane and tonoplast vesicles in barley and wheat roots significantly decreased after 9 days of salt stress. This might lead to suppose that active nutrients uptake was down regulated. Moreover, Rathert (1983) reported that Na^+ has direct toxic effects on structure and function of enzymes and has a deleterious effect on integrity of cell membranes.

However, the effects of salt stress on membrane permeability and membrane lipid super-oxidation were detected earlier in roots than in shoots (Zhao and Harris, 1990). Zhao *et al.* (1993) found in wheat that salt injury induced by NaCl was positively related to the extent of superoxidation of membrane lipids and inversely to membrane Ca^{+2} concentration.

Table (7): Effect of various bacterial inoculant treatments on total N, K^{+}, P, Ca^{+2}, Mg^{+2} and Na^{+} concentrations as mg /g D.W. in roots and shoots of wheat plants grown under different salinity levels.

Salinity level (dSm^{-1})	0.0	3	6	12	Mean	0.0	3	6	12	Mean
Inoculation	Root					Shoot				
N										
Uninoculated	17.21	15.26	13.60	12.11	14.55	31.41	29.44	24.89	24.35	27.52
Kleb.	18.10	16.74	14.80	13.04	15.67	32.02	32.63	27.45	26.75	29.71
Kleb.+N_2-fixers	20.17	18.57	16.43	14.51	17.42	33.78	33.34	29.73	28.06	31.23
Kleb.+P-dissol.	18.71	17.27	15.37	13.49	16.21	32.29	33.02	27.76	27.13	30.05
Composite	21.15	20.33	18.01	15.89	19.10	34.07	33.62	30.49	28.77	31.74
Mean	19.27	17.63	15.64	13.81		32.71	32.41	28.06	27.01	
P										
Uninoculated	2.31	2.39	2.14	2.21	2.26	3.34	3.03	3.01	2.89	3.07
Kleb.	2.49	2.51	2.39	2.31	2.43	3.43	3.34	3.22	3.04	3.26
Kleb.+N_2-fixers	2.51	2.60	2.32	2.36	2.45	3.49	3.38	3.25	3.12	3.31
Kleb.+P-dissol.	2.66	2.65	2.47	2.45	2.56	3.75	3.49	3.37	3.27	3.47
Composite	2.73	2.63	2.53	2.42	2.58	3.85	3.53	3.46	3.32	3.54
Mean	2.54	2.56	2.37	2.35		3.57	3.35	3.26	3.13	
K+										
Uninoculated	17.73	15.17	11.85	11.28	14.01	31.83	29.98	27.47	19.40	27.17
Kleb.	18.05	16.58	14.95	13.23	15.70	32.38	30.48	28.67	23.95	28.87
Kleb.+N_2-fixers	19.89	17.18	15.45	14.86	16.85	33.72	32.65	30.73	24.87	30.49
Kleb.+P-dissol.	18.72	16.87	14.28	14.55	16.11	33.20	31.34	30.55	22.58	29.42
Composite	20.49	18.39	15.36	15.67	17.48	34.58	33.34	31.29	24.51	30.93
Mean	18.98	16.84	14.38	13.92		33.14	31.56	29.74	23.06	
Ca2+										
Uninoculated	4.77	4.32	3.65	3.06	3.95	9.51	9.03	5.49	4.56	7.15
Kleb.	4.96	4.93	4.11	3.38	4.35	9.88	9.67	7.58	6.88	8.50
Kleb.+N_2-fixers	5.19	5.01	4.25	3.55	4.50	10.26	10.06	8.09	7.07	8.87
Kleb.+P-dissol.	5.10	4.91	4.18	3.34	4.38	9.87	9.85	7.98	6.97	8.67
Composite	5.39	5.14	4.35	3.95	4.71	10.34	10.26	8.54	7.44	9.15
Mean	5.08	4.86	4.11	3.46		9.97	9.77	7.54	6.58	
Mg2+										
Uninoculated	2.03	2.19	2.49	2.59	2.33	2.68	3.01	3.02	2.92	2.91
Kleb.	2.19	2.27	2.58	2.57	2.40	2.77	3.11	3.22	3.02	3.03
Kleb.+N_2-fixers	2.24	2.31	2.32	2.43	2.33	2.83	3.17	3.08	3.18	3.07
Kleb.+P-dissol.	2.10	2.26	2.47	2.67	2.38	2.77	3.20	3.32	3.22	3.13
Composite	2.16	2.23	2.35	2.56	2.33	2.86	3.32	3.35	3.51	3.26
Mean	2.14	2.25	2.44	2.56		2.78	3.16	3.20	3.17	
Na+										
Uninoculated	2.30	4.52	6.03	7.17	5.01	3.29	4.90	6.75	7.52	5.62
Kleb.	2.22	4.09	5.58	5.91	4.45	3.09	4.25	5.94	6.74	5.01
Kleb.+N_2-fixers	2.24	3.86	4.46	5.57	4.03	2.96	3.96	5.53	6.24	4.67
Kleb.+P-dissol.	2.16	4.05	5.01	5.73	4.24	3.05	4.31	5.72	6.47	4.89
Composite	2.07	3.55	4.28	4.85	3.69	2.88	3.77	4.47	5.09	4.05
Mean	2.20	4.01	5.07	5.85		3.05	4.24	5.68	6.41	

Under the effect of composite inoculant treatment, wheat plants showed some sort of adaptation through improvement of their uptake for some nutrient elements, i.e. N, P, K^+ and Ca^{2+} which in turn, might competitively increase the exclusion or/and decrease the uptake potential of Na^+ ion and therefore reducing Na^+ concentration to a lower level in their shoot, despite the pre-dominance of Na^+ and Cl^- ions in the external medium (Table 7). Such enhancement might be related to the improvement effects of different bacterial inoculants on the physical, chemical and biological properties of the soil which could promote the release of some nutrients in a readily available form as well as secreting of some growth substances in rooting zone which consequently rnight increase their uptake by plants. Nitrogen fixation associated with roots of grasses has been recognized as a possible significant component of the nitrogen cycle in a range of ecosystems including several extreme environments (Dart, 1986). In this concern, Dobereiner and Day (1975) ascribed the nitrogen fixation potential of some tropical crops such maize, sorghum, wheat and rye to the activity of *Azospirillum spp.* in their roots. Rashid *et al.* (1996) found that inoculation of wheat with mixture of diazotrophic microorganisms (*Azotobacter chroococcum* + *Azospirillum lipoferum*) gave an increase in total N and P uptake of inoculated plants..

On the other hand, Saber and Kabesh (1990) found that application of some biofertilizers such as phosphate dissolving bacteria resulted in a reduction of soil pH which increased the solubility of some nutrients such as P, Fe, Zn, Mn and Cu. Guar and Ostwal (1972) mentioned that inoculation with *Bacillus megatherium* in the presence of rock phosphate significantly increased grain and straw yield as well as P uptake of wheat. They added that soil bacteria *B. megatherium* has the ability to bring insoluble phosphates in soil into soluble forms by secreting organic acids such as formic, acetic, propionic, lactic, glycolic, fumaric and succinic acids. These acids might lower the pH and bring about the dissolution of bound forms of phosphate. Also, some of the hydroxy acids may chelate with calcium and iron resulting in effective solublization and utilization of phosphates. Therefore, increase of Ca^{2+} in the saline medium possibly decreased Na^+ uptake and also reduced membrane disruption in saline solutions, leading to increased NO_3^- uptake through increase the activity of the NO_3^- transporter located in the plasmalemma of roots (Ward *et al.*, 1986). Thus, the low concentration of Na^+ as well as the increase of Ca^{+2} in wheat shoots resulting from bacterial inoculant application might induce better performance of plants under salinity stress. Zsoldos *et al.* (1990) suggested that salt tolerance of wheat was related to the restriction of Na^+ translocation from the root to the shoot. Also, Gorham *et al.* (1993) stated that salt tolerance in crops such as wheat and barley was largely determined by their ability to exclude Na^+ and Cl^- from their shoot, and their ability to maintain high shoot K^+ concentrations.

The results of reducing, non-reducing and total sugar, free amino acids as well as free proline concentrations in both roots and shoots of the second sample are presented in Table (8). Under the effect of different salinity levels, the mean values of reducing and total sugar concentrations were significantly

increased in roots and shoots as compared to their non-salinized control. The increase was proportional to increase in salinity level and was more pronounced in roots than in shoots. Meanwhile, non-reducing sugar concentrations showed a slight increase in roots and shoots under different salinity levels.

Table (8): Effect of various bacterial inoculant treatments on reducing, non-reducing and total sugar concentrations (mg/g F.W.) as well as free proline (μmol/g F.W.) and free amino acids concentrations (μmol leucine/g F.W.) in roots and shoots of wheat plants grown under different salinity levels.

Salinity level (dSm^{-1})	0.0	3	6	12	Mean	0.0	3	6	12	Mean
Inoculation	Root					Shoot				
Reducing sugars										
Uninoculated	2.88	4.09	4.53	5.80	4.33	3.13	4.25	4.79	6.09	4.57
Kleb.	2.90	3.65	4.00	5.19	3.94	3.18	3.81	4.26	5.48	4.18
Kleb.+N_2-fixers	2.95	3.49	3.82	4.96	3.81	3.21	3.65	4.07	5.24	4.04
Kleb.+P-dissol.	2.97	3.51	3.84	4.99	3.83	3.25	3.87	4.20	5.57	4.22
Composite	3.04	3.43	3.76	4.88	3.78	3.26	3.59	4.01	5.16	4.01
Mean	2.95	3.63	3.99	5.16		3.21	3.83	4.27	5.51	
Non-reducing sugars										
Uninoculated	2.80	3.23	3.14	3.71	3.10	3.17	3.66	4.46	4.04	3.83
Kleb.	2.84	3.81	4.89	5.56	4.28	3.36	4.32	5.28	5.81	4.69
Kleb.+N_2-fixers	3.80	4.40	5.28	5.79	4.55	3.55	4.58	5.68	6.03	4.96
Kleb.+P-dissol.	2.80	3.95	5.06	5.66	4.37	3.30	4.47	5.55	5.70	4.76
Ccmposite	3.17	4.16	5.44	6.17	4.74	3.67	4.81	5.95	6.50	5.23
Mean	2.94	3.84	4.76	5.28		3.41	4.37	5.38	5.62	
Total sugars										
Uninoculated	5.68	7.32	7.67	9.01	7.42	6.30	7.91	9.25	10.13	8.40
Kleb.	5.76	7.46	8.89	10.75	8.22	6.54	8.13	9.54	11.29	8.88
Kleb.+N_2-fixers	6.03	7.53	9.10	10.75	8.35	6.76	8.23	9.75	11.27	9.00
Kleb.+P-dissol.	5.78	7.46	8.90	10.65	8.20	6.55	8.34	9.75	11.27	8.98
Composite	6.17	7.59	9.20	11.05	8.50	6.93	8.40	9.96	11.66	9.24
Mean	5.88	7.47	8.75	10.44		6.62	8.20	9.65	11.12	
Amino acids										
Uninoculated	8.20	10.91	12.67	17.75	12.38	12.10	15.84	19.20	21.13	17.07
Kleb.	8.63	12.13	14.04	19.64	13.61	12.79	16.77	21.13	23.32	18.50
Kleb.+N_2-fixers	8.90	12.25	14.69	20.84	14.17	13.11	17.95	22.34	25.57	19.74
Kleb.+P-dissol.	8.78	12.20	14.42	20.74	14.04	12.98	17.36	22.03	24.45	19.21
Composite	8.97	12.40	15.62	21.04	14.51	13.27	18.22	22.56	26.04	20.02
Mean	8.70	11.98	14.29	20.00		12.85	17.23	21.45	24.10	
Proline										
Uninoculated	1.11	1.63	2.27	3.29	2.08	1.74	3.06	3.56	4.57	3.23
Kleb.	1.20	1.96	2.62	3.41	2.37	1.89	3.55	4.07	5.79	3.83
Kleb.+N_2-fixers	1.23	2.08	2.85	4.34	2.63	1.95	3.78	4.60	6.35	4.17
Kleb.+P-dissol.	1.22	2.00	2.66	4.02	2.48	1.91	3.66	4.29	6.22	4.02
Composite	1.24	2.18	2.98	4.87	2.82	1.95	3.92	4.96	6.72	4.39
Mean	1.20	1.97	2.68	4.05		1.89	3.59	4.30	5.93	

Of the various mechanisms enabling plants to cope with salinity stress, the most common is the accumulation of intracellular solutes, such as sugar and free amino acids (Heuer, 1994). In this regard, Yang *et al.* (1990) suggested that the accumulation of sugars in salt stressed leaves might be related to its reduced growth as a result of NaCl inhibition of carbohydrate translocation, or may be related to the greater energetical cost of osmotic adjustment with sugars as opposed to Na^+ or Cl^- ions. Crowe *et al.* (1993) have established that sugars (especially of sucrose) stabilize membranes and proteins in the presence of low water potential and could play a crucial role in plant survival during decreased water status.

However, the increase in reducing sugar might indicate for more stimulation of sugar hydrolysis enzymes activity. Kolupaev and Trunova (1994) reported that in wheat coleoptile, salt stress (0.5% NaCl) caused a sharp increase in invertase activity, and an accumulation of reducing sugar, most likely due to enhanced oligosaccharide hydrolysis. It was suggested that a disruption is caused in the equilibrium of synthesis-degradation system toward hydrolytic reactions in wheat under saline conditions (Chudinova, 1989).

On the other hand, different bacterial inoculants increased the mean values of total sugar concentrations in both roots and shoots as compared each with the mean value of the uninoculated one. Although total sugar concentrations increased, the reducing sugar concentrations were decreased in both roots and shoots, indicating that such increases were mainly due to the increase in non-reducing sugar concentrations. This might be interpreted as a sort of metabolism change toward the reduction in the activity of sugar hydrolysis enzymes, especially of oligosaccharide hydrolysis. Again, this could mean that an alteration toward a conserving of energetical cost from osmotic adjustment to carbohydrate exploitation through their translocation (mainly sucrose) to the newly growing points which was reflected on increasing of plant growth as a result of different bacterial inoculants.

Under the effect of different salinity levels, free amino acids and free proline concentrations increased over their non-salinized control in roots and shoots. The increase was progressively with increasing salinity level and was much higher in roots than in shoots. In this regard, Zhao *et al.* (1992) found that all NaCl treatments (50, 100 and 200 mM) reduced transpiration and increased root amino acids contents. Zidan and Malibari (1993) found in wheat that salinization with NaCl decreased growth of roots and shoots, whereas the contents of Na^+ and free amino acids including proline were increased. Also, Botella *et al.* (1994) reported that wheat plants subjected to salinity stress show increased levels of total free amino acids.

Rapid accumulation of proline, is one of the most remarkable metabolic consequences of salt stress in higher plants (Chu *et al.*, 1976). Naqvi *et al.* (1994) suggested that alteration of mitochondria ultrastructure, decrease in proline oxidase activity or an impaired incorporation of proline into protein might be responsible for free proline accumulation in salt-stressed plants. In addition, Ford and Wilson (1981) proposed that proline plays an indirect role in osmoregulation by increasing the water-binding capacity of plant cell walls to maintain the hydration of protoplasm. It was reported that high salt

concentrations inhibited most enzymes because they perturb the hydrophobic-electrostatic balance needed to maintain protein structure (Wyn Jones and Pollard, 1983). Eberhardt and Wegmann (1989) mentioned that proline may act as non-toxic osmotic solute, preferentially located in the cytoplasm and as enzyme protectant; stabilizing the structures of macromolecules and organelles. It seems that osmoregulating substances, i.e. proline could stabilize the active conformation of enzymes, thereby protect enzymes against conformational perturbations caused by salinity (Lerner, 1985). Kirst (1990) mentioned that proline is an organic solute with an amphipathic property that could protect the hydrophobic sites of proteins (enzymes) which was firstly suffer when water potential was lowered, by forming association with it, converting them into hydrophilic sites. Nomura *et al.* (1998) hypothesized that the dipole character of proline neutralize Na^+ and Cl^- during salt stress, and the hydrophobic group stabilize the hydrophobic domains of proteins.

On the other hand, different bacterial inoculants increased the mean values of free amino acids and free proline concentrations in both roots and shoots. In roots, the increase in the mean values of free amino acids concentrations over the uninoculated treatment reached 10, 14, 13 and 17%, while for shoots were 8, 16, 13 and 17% with inoculation by *Kleb.*, *Kleb.*+N_2-fixers, *Kleb.*+P-dissolving and composite, respectively. The corresponding increase in the mean values of free proline concentrations over the uninoculated control treatment reached 14, 26, 19 and 36% in roots, whereas for shoots were 19, 29, 24 and 36%, respectively. Thus, the increase percent of proline was much higher than that for free amino acids, which might be a sign of an increase of proline synthesis than those resulting from protein degradation. In this concern, Tripathi and Mishra (1998) found that IAA production by *Alcaligenes faeculis*, *Azospirillum brasilense* and *Azotobacter vinalandii* was found to be stimulated under low salinity level. This type of response may result in the proliferation of roots, resulting in enhanced exudation of osmoprotectants such as proline and betains by the host plant. This might induce proline to be more synthesized in the host plant.

Comparing the interaction effect of different bacterial inoculants and salinity treatments, it was observed that composite inoculant treatment responded with more accumulation of proline and free amino acids as compared to its control or to the other bacterial inoculant treatments. Therefore, composite treatment may partially increase the ability of wheat plants to counteract salinity by accumulating relatively higher amounts of free amino acids and free proline. Several reports correlate this phenomenon with stress resistance, indicating that a better performance and survival can be expected in species that accumulate proline (Heuer, 1994). Artlip and Wisniewski (2002) noticed that proline concentration was directly proportional to Na^+ concentration under adverse environmental conditions.

Under the effect of different salinity levels, the endogenous IAA and cytokinins concentrations decreased, whereas endogenous ABA concentrations increased in wheat shoots of the second sample when compared with their non-salinized control (Table 9). The increment or the

reduction of endogenous hormones was positively correlated with increasing of salinity level. The state of hormonal balance in plants is suggested to play a considerable role in the mediation of plant responses under salt stress (Boggess and Stewart,1980). Moreover, Naqvi *et al.* (1986) found that the free or diffusible auxin content of etiolated *Zea mays* coleoptiles decreased under salinity stress. Stefl (1988) working on wheat, found that with Increasing Na^+ concentration, the tryptophan synthase α-monomers were gradually dissociated from the oligomers producing less active isoenzyme. This would reduce the biosynthesis of L-tryptophan and consequently of IAA, so that plant growth was retarded or even stopped.

Table (9):Effect of various bacterial inoculant treatments on endogenous IAA, cytokinins (equivalent 2-isopentenyladenine) and ABA concentrations as µmol/100g F.W. In the second sample of wheat shoots grown under different salinity levels.

Salinity level (dSm^{-1}) / Inoculation	0.0	3	6	12	Mean
IAA					
Uninoculated	15.49	8.96	8.10	6.24	9.70
Kleb.	16.28	11.77	10.53	8.30	11.72
Kleb.+N_2-fixers	17.06	12.21	11.93	9.61	12.70
Kleb.+P-dissolving	16.59	11.95	10.79	8.43	11.94
Composite	17.52	13.48	12.28	10.80	13.52
Mean	16.59	11.67	10.73	8.68	
Cytokinins					
Uninoculated	17.93	14.07	12.65	10.16	13.70
Kleb.	18.49	15.85	14.32	11.38	15.01
Kleb.+N_2-fixers	19.38	16.56	14.89	12.79	15.91
Kleb.+P-dissolving	18.84	16.14	14.51	11.89	15.35
Composite	19.92	16.98	15.27	13.22	16.35
Mean	18.91	15.92	14.33	11.89	
ABA					
Uninoculated	8.93	20.22	49.33	58.78	34.32
Kleb.	9.21	14.60	40.38	48.08	28.07
Kleb.+N_2-fixers	9.37	11.77	33.48	39.85	23.62
Kleb.+P-dissolving	9.02	13.39	37.42	44.55	26.10
Composite	9.71	10.94	26.57	31.62	19.71
Mean	9.25	14.18	37.44	44.58	

On the other hand, the reduction of cytokinins in plants grown under salt stress was reported by different investigators. Roots are the first tissues exposed to salt, therefore, cytokinins level may be directly affected by salt stress (Thomas *et al.*, 1992). Meiri and Shalhevet (1973) reported that saline conditions restrict the synthesis of cytokinins in the root and their translocation to upper plant parts can also be inhibited. Kuiper *et al.* (1990) reported that growth rates of roots and shoots of wheat and barley plants were decreased after an exposure to 65 mM NaCl, and this was associated by rapid decrease in cytokinin concentration in both roots and shoots. However, Nilson and Orcutt (1996) indicated that under salinity stress, the endogenous levels of cytokinins in roots and leaves did not change or may have slightly decreased.

Concerning ABA, Gong *et al.* (1990) found that during NaCl stress, ABA content in wheat and barley leaves increased with duration and intensity of stress. Also, Maslenkova *et al.* (1993) found that endogenous level of ABA increased with salinity stress, and that level correlates with plant resistance to stress. Nilson and Orcutt (1996) attributed this increase of ABA in the tissue as a result of release from bound forms, an increase in the rate of synthesis or a decrease in the rate of destruction. Kutschera and Schopfer (1986) indicated that ABA reduced the elongation rate and also inhibited auxin-induced growth by affecting cell wall loosening in maize coleoptile. However, Pekic *et al.* (1993) mentioned that during high salinity elevated ABA levels appear to provoke discrete changes in gene expression, which could be a way of optimizing beneficial effects of ABA and thereby improving crop tolerance under salinity stress conditions.

As regards bacterial inoculation, the results of table (9) revealed that different bacterial inoculants increased the endogenous IAA and cytokinins concentrations whereas, endogenous ABA concentration decreased in shoots as compared each with the corresponding uninoculated control under different salinity levels. The increase in the mean values of endogenous IAA concentrations over the uninoculated control reached 20.8, 30.9, 22.8 and 39.4% with inoculation by *Kleb.*, *Kleb.*+N_2 -fixers, *Kleb.*+P-dissolving and composite, respectively. The corresponding increase in the mean values of endogenous cytokinins concentrations over the uninoculated control reached 9.6, 16.1, 12.0 and 19.3%, respectively. Meanwhile, the respective reduction in the mean values of endogenous ABA concentrations from the uninoculated control reached 18, 31, 24 and 43%, respectively. So, the composite inoculant treatment induced a much higher levels of both IAA and cytokinins in wheat shoots and this was accompanied by the lower levels of ABA under different salt concentrations.

Bacteria of the genera *Azospirillum* and *Azotobacter* could colonize the roots of several cereal and grasses. They are regarded as plant growth-promoting rhizobacteria due to their ability to produce phytohormones (IAA, GAs, cytokinins, etc.) and to fix atmospheric nitrogen (Bashan and Lavanony, 1990; Okon and Labandera-Gonzalez, 1994). Tripathi and Mishra (1998) found that IAA production by *Alcaligenes faeculis*, *A. brasilense* and *Azotobacter vinalandii* was found to be stimulated under low salinity level. This type of response could enhance salt tolerance, promote growth as well as stimulate the formation of lateral roots and root hairs of host rice under salt stress (Ping *et al.*, 1998). Stimulation of root proliferation could be responsible for such hormonal balance in wheat plants resulting from the composite inoculant treatment under different salinity stress. Moreover, the activities of plant-associated bacteria might have a positive effects on soil properties which could contribute to the enhancement of plant-microbe interaction and the plant ability to be tolerate more salt stress, which might stimulate plant growth and its productivity in salinity-affected soils.

In addition, the ability of *K. pneumoniae* to produce exopolysaccharides that binds to wheat plant roots and soil particles (Del Gallo and Fendrik, 1994), could facilitate to the other bacterial inoculants to colonize the rhizosphere of wheat roots. The exopolysaccharides might

represent suitable medium for mutual utilization of substances between the plant root and the inoculated bacteria. This could increase the bacterial populations in rooting z one, allowing for more co-operation between w heat plants and inoculated bacteria. Therefore, it was found an absolute superiority of composite inocula treatment on growth of both root and shoot of wheat plants comparing with the other inoculation treatments under the different salinity levels. It appears that composite inocula treatment could partially increase the ability of wheat plants to counteract salinity by accumulating relatively higher amounts of N, K^+, Ca^{2+}, non-reducing sugars, free amino acids, free proline, endogenous auxin and cytokinins as well as lowering Na^+ and endogenous ABA concentrations in their shoots leading to better performance of wheat plants under salinity stress. This was reflected by the superiority of their grain weight/plant as compared with the other inoculation treatments under different salinity levels.

However, further studies might be required to test the effectivity of composite inoculant treatment on other cereal crops as well as different soil types aiming to maximize their yields under different stress conditions.

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تأثير التلقيح بأنواع مختلفة من اللقاحات البكتيرية على نمو وإنتاجية نباتات القمح النامية تحت مستويات مختلفة من الملوحة.

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**فرع فسيولوجيا النبات-قسم النبات الزراعى-كلية الزراعة-جامعة القاهرة

هناك اهتمام متزايد نحو زيادة درجة تحمل أصناف القمح للنمو في أوساط ملحية مرتفعة وما يتبعه ذلك من زيادة العائد الاقتصادي. ولذلك تم إجراء تجربة تحت ظروف معقمة لدراسة تأثير استخدام بعض أنواع اللقاحات البكتيرية (المعزولة من بعض الأراضي الملحية) والتى تشمل *Klebsiella pneumonia* منفردا أو بالإشتراك مع *Azospirillum brasilense* +*Azotobacter chroococcum* أو بالإشتراك مع *Bacillus megatherium* أو مركبة (تحوى جميع أنواع البكتريا السابق ذكرها) لتلقيح بادرات قمح صنف جيزة ١٦٣ (عمر أسبوع) والنامية تحت مستويات مختلفة من ملوحة ماء البحر صفر،٣ ، ٦ ، ١٢ ديسيسيمنز. كذلك أجريت تجربة أصص تم فيها استخدام نفس معاملات التلقيح البكتيرى السابق ذكرها وذلك لتلقيح حبوب القمح بالإضافة إلى تلقيح النباتات (عمر ٢ ، ٦ أسابيع)والنامية تحت نفس مستويات الملوحة المذكورة سابقا.

وقد أوضحت الدراسة البكتريولوجية سواء تحت الظروف المعقمة أو خلال تجربة الأصص أن هناك تناقص لكل من أعداد الميكروبات ولدرجة نشاط إنزيم النيتروجينيز بزيادة مستويات الملوحة ، كذلك أظهرت النتائج أنه تحت تأثير جميع مستويات الملوحة فإن معاملة التلقيح المركبة أو المعاملة المشتملة على (*K.* + *A. brasilense* + *A. chroococcum pneumonia*) قد أعطت أعلى أعداد من الميكروبات وبالتبعية درجة نشاط مرتفعة لإنزيم النيتروجينيز وذلك مقارنة بالنباتات الغير ملقحة أو معاملات التلقيح الأخرى.

كذلك أظهرت الدراسة أن صفات النمو لنباتات القمح قد انخفضت معنويا مع زيادة تركيز مستويات ماء البحر ويتناسب هذا الانخفاض مع زيادة تركيز الملوحة ، كذلك وجد أنه بزيادة تركيز مستويات ماء البحر يحدث انخفاض فى تركيز كل من النيتروجين الكلى ، الفوسفور ، البوتاسيوم ، الكالسيوم بالإضافة إلى انخفاض التركيز الداخلى لكل من اندول حمض الخليك والسيتوكينينات ، بينما يحدث تراكم للمغنسيوم ، الصوديوم ، السكريات المختزلة والكلية ، الأحماض الأمينية الحرة ، البرولين وكذلك يزداد تركيز حمض الابسيسيك الداخلى . هذا وقد ظهر تأثير ذلك على الإنخفاض المعنوى لمكونات المحصول المختلفة والذى قد يرجع إلى قلة أعداد الأشطاء الحاملة للسنابل تحت تأثير الملوحة.

وعلى مايبدو أن قدرة بكتريا ال *K. pneumonia* على إنتاج السكريات العديدة الخارجية والتى قد ترتبط بكل من جذور القمح وحبيبات التربة قد تسهل من إلتصاق الملقاحات البكتيرية الأخرى على جذور القمح وإستعمارها. هذا وقد تمثل السكريات العديدة المفرزة خارجيا وسطا ملائما يسمح بزيادة تبادل المواد الكيميائية بين جذور النبات والملقحات البكتيرية الأخرى. وعلى ذلك فان المعاملة بالتلقيح البكتيرى المركب أظهرت تفوقا واضحا فى قدرتها على زيادة نمو كل من الجذر والمجموع الخضرى تحت جميع مستويات الملوحة مقارنة بالنباتات الغير ملقحة أو معاملات التلقيح الأخرى. ويبدو أن المعاملة بالتلقيح البكتيرى المركب قد تزيد من قدرة النبات على مواجهة الملوحة وذلك بتراكم كميات محسوسة من النيتروجين الكلى والبوتاسيوم والكالسيوم والسكريات الغير مختزلة والأحماض الأمينية الحرة والبرولين بالإضافة إلى زيادة التركيز الداخلى لكل من اندول حمض الخليك والسيتوكينينات ، وقد صاحب ذلك انخفاض كل من الصوديوم وحمض الابسيسيك الداخلى فى أنسجة المجموع الخضرى ، والذى قد يؤدى إلى زيادة كفاءة الأداء لنباتات القمح تحت تأثير الملوحة – والذى قد انعكس فعليا على زيادة وزن الحبوب/النبات مقارنة بالنباتات الغير ملقحة أو بمعاملات التلقيح الأخرى تحت جميع مستويات الملوحة المختبرة.