Journal of Microbiology Research 2018, 8(2): 43-53
DOI: 10.5923/j.microbiology.20180802.03

Potential Use of Beneficial Salt Tolerant Bacteria for Improving Wheat Productivity Grown in Salinized Soil

Nanis G. Allam[1], Ramadan Kinany[2], Elham El-Refai[1], Wael Y. Ali[3,*]

[1]Microbiology Unit, Botany Department, Faculty of Science, Tanta University, Tanta, Egypt
[2]Agricultural Research Center, Research Institute of Lands, Water and Environment, Sakha, Kafr El.Sheikh, Egypt
[3]Microbiology Unit, Botany Department, Faculty of Science, Kafr El.Sheikh University, Kafr El.Sheikh, Egypt

Abstract Wheat is one of the major crops cultivated in the black clay soil, the salinized soil reduces the productivity due to the improper nutrition of plants as well as osmotic and drought stress. The current study used osmoadaptive salt tolerant *Azotobacter chroococcum* and *Azotobacter vinelandii*, isolated from salt-affected soils to tolerate salt and facilitate plant growth in saline soils. Inoculated wheat crops with bacteria gave better results than uninoculated one in growth criteria as stem high, weight, grains weight, straw weight, and 100 grains weight. The improvement ability of bacteria was correlated to the production of plant growth regulators as auxins (IAA), which indicated that using these salt tolerant bacteria with wheat cultivation, improve crop productivity in saline soil.

Keywords *Azotobacter*, Salinity, Wheat, Salt tolerant, Bacteria

1. Introduction

Salinity is considered the scourge of intensive agriculture [1]. The costs, associated with soil salinity are potentially enormous, and the effects of salinity may impact heavily on agriculture, biodiversity and the environment [2]. Salinity is one of the major factors that reduce crop productivity worldwide. High amounts of salts in soil reduced the seed germination [3, 4] and plant growth [5].

Resulting from high concentrations of sodium ions in the soil, Seed germination is decreased under saline conditions either by creating osmotic potential or by toxic effects [6]. These concentrations of soluble salts through their high osmotic pressures affect plant growth by restricting the uptake of water and balance absorption of essential nutritional ions by the roots thus, reduce the rate of germination and may retard plant development [7, 8].

Nearly 40% of world's surface, suffer from salinity problems [9]. In Egypt, two to three m3 of saline drainage wastewater are annually used for irrigating about 405.000 hectares of land [10] and the majority of salt-affected soils are located in the Northern-Center part of the Nile Delta. Soil in Delta is classified according to alluvial that varies in texture from light (30% clay content) to heavy (80% clay content) [11]. Kafr El-Sheikh governorate was chosen as the study area because of its terrain and climatic conditions.

Increase in salinity tolerance for the world's two major crops, wheat, and rice is an important goal as the world's population is increasing more rapidly than the area of agricultural land [12]. Wheat is considered a major staple food crop for more than one-third of the world population and it is the main staple food of Asia [13], originated in South Western Asia and has been a major agricultural commodity since prehistoric times. Total production area in Pakistan is 8.2 mha and the average yield is 2170 kg/hectare [14]. The average production of wheat (Tritium aestival) in Egypt 2.745 tons/feddan, Pakistan 2660 Kg ha^{-1}, China 4710 Kg ha^{-1} and India (2910 Kg ha^{-1}) [15].

There are biotechnological approaches, used to improve crop productivity in saline soil [16, 17]. Salt-tolerant plant growth promotes Rhizobacteria (ST-PGPR) as *Azotobacter*ia helps to improve soil fertility through decomposition of organic matter and nutrient cycling, by fixation of atmospheric nitrogen or production of growth hormones [18-21]. Growth of wheat plants performs better under saline environment as inoculated with different rhizobia strains due to the production of ethylene under stressed conditions; reduction in sodium uptake by the utilization of different rhizobia strains under saline environment is a positive sign to mitigate salt stress biologically [22]. *Azotobacter* is a useful organism in many ways as it can fix atmospheric nitrogen, producing plant growth hormones like gibberellins, auxins and cytokinins and can solubilize phosphate which reduces the use of fertilizers [23, 24]. *Azotobacter* can increase seed's

* Corresponding author:
waelyoussef.com@hotmail.com (Wael Y. Ali)
Published online at http://journal.sapub.org/microbiology

germinating ability by 20–30% because of the production of the plant growth-promoting compounds [25]. Inoculation by *A.chroococcum* substantially reduced the effect of salt stress on plant growth parameters such as root length, plant height, fresh shoot, root weight, dry shoot and root weight [26]. The most appropriate solution in salinization problem is to use salt-tolerant useful bacteria in developing strategies to facilitate plant growth in saline soils. Therefore, this study aimed at improving wheat yield and solving the problem of low growth, low productivity for crop plants, caused by soil salinity by using local salt tolerant bacterial inoculants with Osmoadaptive strategies to increase tolerance to high salt, capable of producing plant growth promoting substances to facilitate wheat growth in saline soils.

2. Material and Methods

2.1. Soil Sampling and Preparation

Soil samples were collected from different cultivated areas in Kafr El-Sheikh governorate, including the following towns, mentioned below:

Region (I): Desouk city, West Kafr El-Sheikh

Region (II): Sidi Salem town, North Kafr El-Sheikh

Region (III): University, South of Kafr El-Sheikh

Region (IV): Baltim and Hamul, North East of Kafr El-Sheikh

Soil samples were obtained after removal of 5 cm of surfaces and collected in clean plastic bags then, samples were air dried, ground in a mortar and sieved through 2 mm sieve. Water suspensions of soil were prepared and spread on the surface of solid nitrogen free media in Petri dishes [27].

2.2. Measuring Electrical Conductivity (EC) dS/m of Soils

Electrical conductivity (E.C dSm^{-1}) of soil extract for the collected samples was measured according to Richards [27].

2.3. Isolation and Purification of *Azotobacter* sp.

Soil suspensions were prepared to spread on the surface of solid nitrogen-free media Vancura and Macura [28] (Sucrose 30gm, K_2HPO_4 0.16gm, NaCl 0.2gm, $MgSO_4.7H_2O$ 0.2gm, $CaCO_3$ 2gm, $Fe_2(SO_4)_3$ 0.005gm, $NaMoO_4.2H_2O$ 0.005gm, $NaBO_3$ 0.005gm, Dist.H_2O 1000 ml) in Petri dishes and incubated at 30°C with inverted position for 4-6 days, the colonies which appeared on the surface of plates were transferred to new plates containing fresh media for more purification. Several subcultures were carried out in order to purify the isolated bacteria [29].

2.4. Characterization and Identification of *Azotobacter* Isolates

Isolates were characterized morphologically, examined for Gram stain to identify *Azotobacter* sp. according to Bergey's Manuals Systematic of bacteriology [30].

2.5. 16s Ribosomal RNA (rRNA) Identification for the Two Isolated Strain

Genomic DNA was extracted as described by Carozzi [31] and the 16S rRNA gene of the bacterial isolates was amplified by PCR using 16S rRNA primers. PCR was performed for 5 min by initial denaturation at 95°C, followed by 35 cycles for each denaturation process at 95°C for 30 sec, annealing at 65°C for 1 min, extending at 72°C for 1.30 min, and a final extension at 72°C for 10 min. The PCR product of 0.5 kb was extracted from the gel using Ferments gel extraction kit (Thermo), and the amplified products were electrophoresed on 1% agarose gel. Sequencing was carried out by the Genetic Analysis System model Gene JET™ PCR Coulter Inc., Fullerton, CA, USA. The 16S rRNA gene sequences were compared with others found by BLAST searching (Basic Local Alignment Search Tool) in the database of National Centre for Biotechnology Information. Then, phylogenetic analysis was performed using neighbourhood joining method [32] to identify strains selected under this study.

2.6. Osmoadaptive Strategies to Increase Salt Tolerance of Isolates

Fixed amount of selected *Azotobacter* isolates were inoculated to free nitrogen media Vancura and Macura supplemented with different concentration of NaCl range from (0.009, 5 and 20 g/100ml) and incubated at 30°C with inverted position for 4-6 days then, growth was measured in each culture by measuring Optical Density (OD) to raise salt tolerance, reaching the maximum available tolerance against salt concentration of the media.

2.7. Evaluation of Production of Indole-3Acetic Acid (IAA) by the Isolated Bacteria

One ml of inoculums were transferred to a flask containing 50 ml of (NFB) medium, supplemented with 0.3 g/L tryptophan and incubated at 28°C with agitation at 100 rpm in the dark. Clusters were centrifuged at 4000 rpm for 15 min and two millilitres of supernatants were mixed with one millilitre Pilet-Chollet reagent consisting of 12g $FeCl_3$/1 of 7.9 H_2SO_4 and the mixtures were lifted in the dark for 30 minutes at room temperature. The appearance of pink colour indicates that IAA was produced. The absorption spectra of mixtures were determined at 530 nm [33] for isolates previously exposed to different salt concentrations compared with non-salted one and IAA production was determined from the standard curve.

2.8. Studying the Effect of the Most Tolerated Bacteria on the Cultivation of the Wheat Plants

This study was based on Split Plot Design [34, 35] with two different factors in both field and pots experiments to investigate the changes in plant growth criteria under the effect of osmoadaptive bacteria and different concentration of N-fertilizer. The most tolerated bacteria were inoculated to wheat plant through soil during seeding stage to study the

effect of bacteria on crop production of wheat compared with uninoculated one grown in salinized soil in field and pots experiments. The experimental design was 3 lines of articulating squares each of which $1m^2$, one line represented inoculated plant with *A.chroococcum*, the second one represented control (uninoculated plant) and the third represented inoculated plant with *A.vinelandii* with different replicates and different fertilizer concentrations. The field experiment shown in photos 4, 5, 6, 7, and 8. The same experimental design used for pots experiment shown in photos 13, 14, 15, 16, 17, and 18.

2.9. Determination of Plant Growth Criteria

Determination of plant growth criteria (stem high, $1m^2$ weight, grains weight, straw weight and 100 grains weight) in the inoculated and uninoculated plants were done by collecting crops and measuring length and weight by sensitive balance and meter.

2.10. Statistical Analysis

Numerical variables are expressed by descriptive statistics as mean, standard deviation. Two ways" ANOVA and post hock test (tukey)" were used to compare quantitative data. Correlation analyses showed a relation between parameters. P-value <0.05(*) was considered significant difference and P-value <0.001(**) was considered highly significant difference. Statistical analyses were performed using Statistical Package for Social Sciences (SPSS version 23).

3. Results and Discussion

3.1. Soil Sampling

Eight Soil samples were collected from four regions in Kafr El-Sheikh governorate where Region (I) 3 samples, Region (II) 2 samples, Region (III) 1 sample, and Region (IV) 2 samples.

3.2. Electrical Conductivity (EC) dS/m of Soil Samples

Table (1). Electrical Conductivity of the collected soil samples

Region	No. of sample	pH	E.C* dSm^{-1}
Region (I)	1	7.24	5.95
	2	4.14	40.32
	3	7.14	4.76
Region (II)	4	7.04	4.48
	5	6.98	16.32
Region (III)	6	7.01	6.33
Region (IV)	7	7.01	28.16
	8	6.98	48

* Electrical Conductivity (EC) dS/m

According to the standard classification of soil depending on salinity from AGRIS.FAO organization [36], soil is slightly consider as a saline from (2–4) dS/m and considered as a very strongly saline when EC reach more than (16 dS/m). Result showed that sample number 2 in region (I) and sample number 8 from region (IV) showed high electrical conductivity, so they are considered as a strong saline.

3.3. Isolation and Purification of *Azotobacter* sp.

Twenty isolates were collected from soil suspension of different cultivated regions of Kafr El-Sheikh governorate:

Table (2). Number of isolates

Region	Region (I)	Region (II)	Region (III)	Region (IV)
Isolates number	11	5	1	8
	12	6	2	9
	13		3	10
	14		4	
	15		5	
	16		6	
	17		7	
	18			
	19			
	20			

3.4. Characterization of *Azotobacter* Isolates

Table (3). Characterization of *Azotobacter* isolates

Region	Isolate No.	Gram test	Shape	Color	Evaluation
Region (I)	11	(G -ve)	Coccoid	Brown	Convex
	12	(G -ve)	Coccoid	Brown	Convex
	13	(G -ve)	Coccoid	Brown	Convex
	14	(G -ve)	Coccoid	Brown	Convex
	15	(G -ve)	Coccoid	Brown	Convex
	16	(G -ve)	Coccoid	Brown	Convex
	17	(G -ve)	Coccoid	Brown	Convex
	18	(G -ve)	Coccoid	Brown	Convex
	19	(G -ve)	Coccoid	Brown	Convex
	20	(G -ve)	Coccoid	Brown	Convex
Region (II)	5	(G -ve)	Coccoid	Creamy	Convex
	6	(G -ve)	Coccoid	Brown	Convex
Region (III)	1	(G -ve)	Coccoid	Brown	Convex
	2	(G -ve)	Coccoid	Brown	Convex
	3	(G -ve)	Coccoid	Brown	Convex
	4	(G -ve)	rod	Creamy	Convex
	5	(G -ve)	Coccoid	Brown	Convex
	6	(G -ve)	rod	Creamy	Convex
	7	(G -ve)	rod	Creamy	Convex
Region (IV)	8	(G -ve)	Coccoid	Brown	Convex
	9	(G -ve)	rod	Creamy	Convex
	10	(G -ve)	rod	Creamy	Convex

According to the results of electrical conductivity which determined salinity degree of soil samples and the morphological characterizations of the bacterial isolates, two different isolates from two high saline regions were selected to be under this experiment:

Isolate (no. 17) from region (I) and soil sample (no. 2) was labelled Isolate (A)

Isolate (no. 9) from region (IV) and soil sample (no. 8) was labelled Isolate (B)

3.5. 16s rRNA Identification

16s ribosomal RNA (rRNA) sequence for the two isolated strains in the present study were used for identification of strain level. Phylogenetic analysis using neighbourhood joining method shown in figure 1 and figure 2.

Table (4). 16s rRNA Sequence of isolates

Sequence of Isolate (A)	Sequence of Isolate (B)
TAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACAC GGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAA TGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGT CTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCTGTAAG CGAATACCTTGCAGTTTTGACGTTACCGACAGAATAAGCACCGG CTAACTTTGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCG TTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTTGGT AAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTGCATCC AAAACTGCCTGACTAGAGTACGGTAGAGGGTGGTGGAATTTCCT GTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGC GAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGC GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA AACGATGTCGACTAGCCGTTGGGCTCCTTGAGAGCTTAGTGGCG CAGCTAACGCATTAAGTCGACCGCCTGGGGAGTACGGCCGCAAG GTTAAA	TACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCG AAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTC TTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCGC TCGGTGAATACCCAAGCGTCTTGACGTTACCGACAGAAT AAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATAC GAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAA GCGCGCGTAGGTGGTTCGGCAAGTTGGATGTGAAAGCCC CGGGCTCAACCTGGGAACCGCATCCAAAACTACTGGGCT AGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGG TGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAA GGCGACCACCTGGACCGATACTGACACTGAGGTGCGAA AGCGTGGGAGCAAACAGGATTAGATACCCTGGTAGTCCA CGCCGTAAACGATGTCGACTAGCCGTTGGGCTCCTTGAG AGCTTAGTGGCGCAGCTAACGCATTAAGTCGACCGCCTG GGGAGTACGGCCGCAAGGTTAAAA

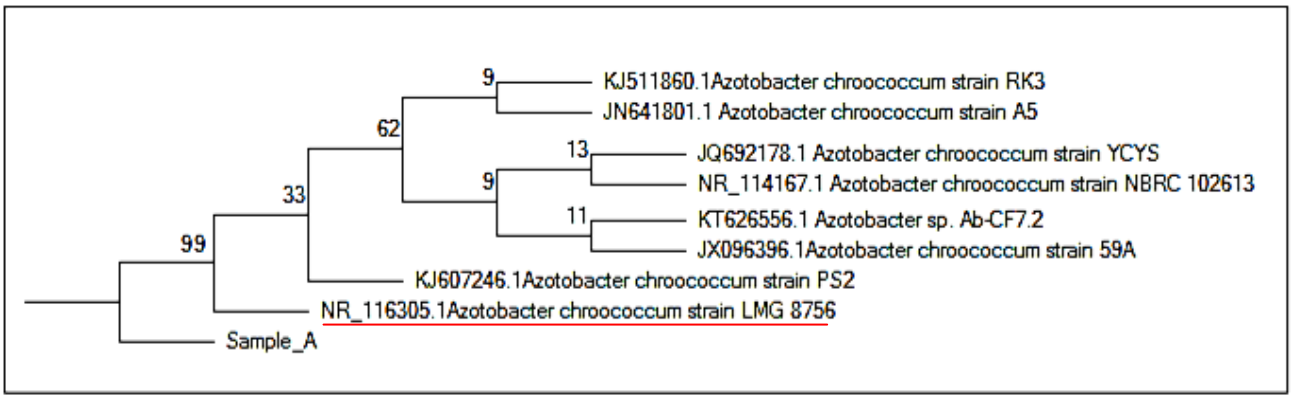


Figure 1. Phylogenetic tree constructed from the 16S rRNA gene sequence with 99% similaritie percentage to *Azotobacter chroococcum*

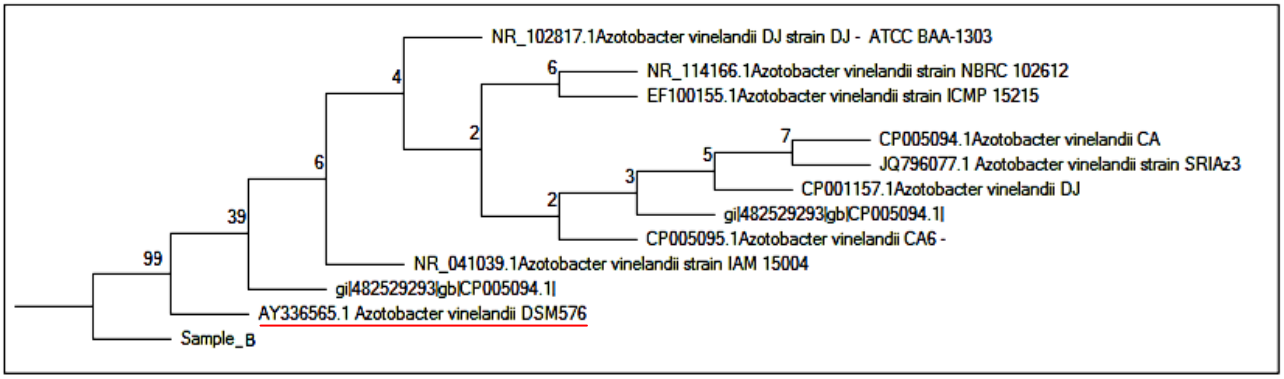


Figure 2. Phylogenetic tree constructed from the 16S rRNA gene sequence with 99% similaritie percentage to *Azotobacter vinelandii*

Results showed that the two species selected under this study were:

Isolate (no. 17) from region (I) and soil sample (no.2), Isolate (A) was *Azotobacter chroococcum.*

Isolate (no. 9) from region (IV) and soil sample (no.8), Isolate (B) was *Azotobacter vinelandii.*

3.6. Osmoadaptive Strategies to Increase Salt Tolerance of Isolates

Increasing the ability of the selected species to tolerate salt was achieved by exposing isolates to gradually increase in salt concentrations in the media and the result shown in Table (5). R is correlation coefficient in the statistical results for Table (5), when r is (-) it is mean there is negative relation between two groups (one increase while the other decrease), when r is (+) it is mean there is positive relation between two groups (the 2 groups increase together or decrease together). If r <0.5 there is a weak relation, r>0.5 there is a strong relation.

Results as illustrated in Table (5) showed that there was a negative strong relation between salt concentrations of media & *A.chroococcum* or *A.vinelandii* growth: when salt concentration increase, the growth of *A.chroococcum* and *A.vinelandii* decrease. Increase of NaCl concentraion in the media reduce the bacterial growth, where there was a gradual decrease in the bacterial growth, accompanied with as an increase of NaCl concentration until the maximum concentration (5g/100ml) of salt gets peak, after which the growth began to decrease sharply, indicating that osmoadaptive bacteria reached the maximum available tolerance against salt concentration of the media.

3.7. Evaluation of Production Indole-3Acetic Acid (IAA) by Isolates

Referring to IAA standard curve (Figure 3), result in table (6) showed that osmoadaptive species of bacteria previously exposed to different salt concentration produced more Indole-3Acetic Acid (IAA) than controlled one which were not exposed to salt previously in both species *A.chroococcum* and *A.vinelandii* which indicate that osmoadaptive isolates produce more auxins and can be used as salt-tolerant plant growth promoting rhizobacteria (ST-PGPR).

Many authors reported that IAA has a positive effect on plants and play a major role in the growth. The IAA producing bacteria significantly raaised seedling root growth up to 25% in non-salinated conditions and up to 52% at 100 mM NaCl, compared with control plants. It is concluded that growth regulators considerably alleviated salinity-induced dormancy of wheat seeds. Root colonizing bacteria produce phytohormone to alleviate salt stress of wheat grown under conditions of soil salinity [37].

Table (5). Effect of different NaCl concentrations on *Azotobacter* growth

salt g /100ml	0.009766	0.019531	0.039063	0.078125	0.15625	0.3125	0.625	1.25	2.5	5	10	20
OD** *Azotobacter chroococcum*	1.296	1.397	1.278	1.121	1.173	1.165	0.903	0.884	0.856	0.844	0.048	0.041
OD** *Azotobacter vinelandii*	0.804	0.8	0.860	0.809	0.783	0.782	0.769	0.768	0.732	0.715	0.085	0.046

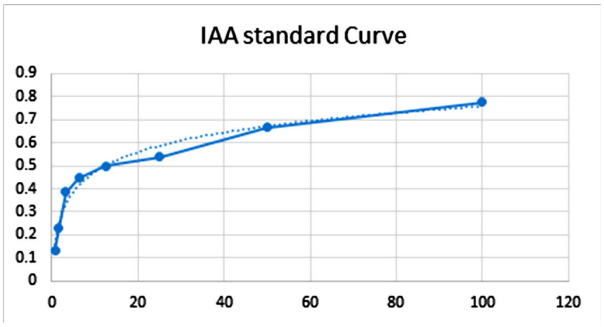
** Optical Density
OD** (control) *Azotobacter chroococcum* 1.455
OD** (control) *Azotobacter vinelandii* 0.912

Parameters	R	p-value
salt g /100ml & dsm *Azotobacter chroococcum*	-0.896	0.000**
salt g /100ml & nrrl *Azotobacter vinelandii*	-0.931	0.000**

R is correlation coefficient.

Table (6). Effect of osmoadaptation (exposure to salt) of bacteria on Indole-3Acetic Acid (IAA) producing ability

Sample	Absorbance	IAA Conc. µg/ml
A.chroococcum (Non-exposed to salt)	0.252	1.63
A.chroococcum (Exposed to salt)	0.451	8.23
A.vinelandii (Non-exposed to salt)	0.28	2.05
A.vinelandii (Exposed to salt)	0.472	9.76



Absorbance vs. Concentration µg/ml

Figure 3. IAA Standard Curve

It also reported that pre-sowing wheat seeds with plant growth regulators like IAA, gibberellins alleviated the growth inhibiting effect of salt stress [38-41]. Worth mentioning, it was observed that the plant growth stimulating substances can alleviate the effect of salinity on germination [42-45]. It is also possible that under high salt concentrations, natural present hormones may be suppressed [46, 41]. Salinity results in a progressive decline in the level of IAA in the root system of plants [47]. In this condition, seed soaking with plant growth regulators and an application of additional natural phytohormones supplied sufficient hormones for normal plant development and a growth in saline conditions [46, 41]. Inoculation of the soil with osmoadaptive isolates selected under this study during seedling stage of wheat plant raised the production of IAA and reduced the salt stress.

3.8. Effect of Using Tolerated Bacteria on the Wheat Plant Productivity

It was found that *Azotobacter* could increase seed's germinating ability and reduced the effect of salt stress on plant growth parameters such as root length, plant height, fresh shoot and root weight and dry shoot and root weight [25, 26]. In this study, both species *Azotobacter chroococcum* or *Azotobacter vinelandii* were inoculated to wheat plant, growth criteria were determined as showen in Figures (9, 10, 11, and 12) for field experiments and Tables (7, 8, 9, and 10) for pots experiments as:

Figure 4

Figure 5

Figure 6

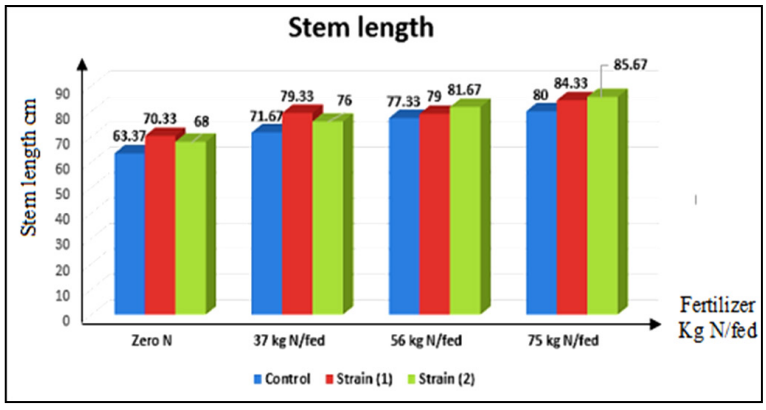
Figure 7

Figure 8

Figure 4, 5, 6, 7, and 8 represent stages of wheat planting in field experiments.

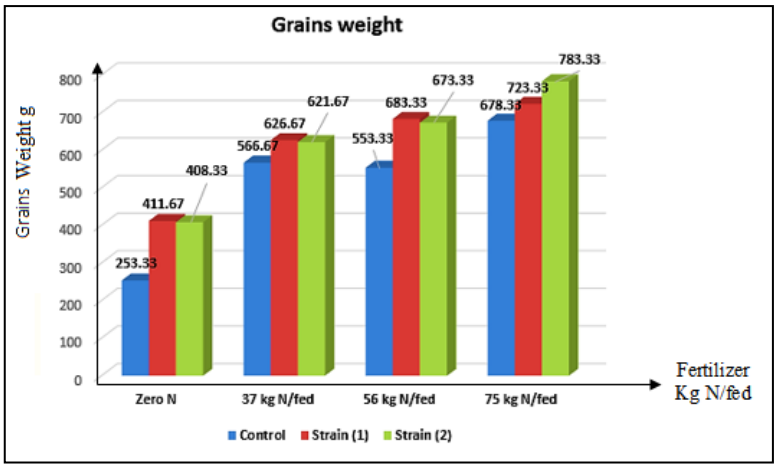
There were 3 lines of articulating squares represented *A.chroococcum*, control, and *A.vinelandii* with different replicates for different fertilizer concentrations and were based on Split Plot Design.

3.9. Results of Field Experiment



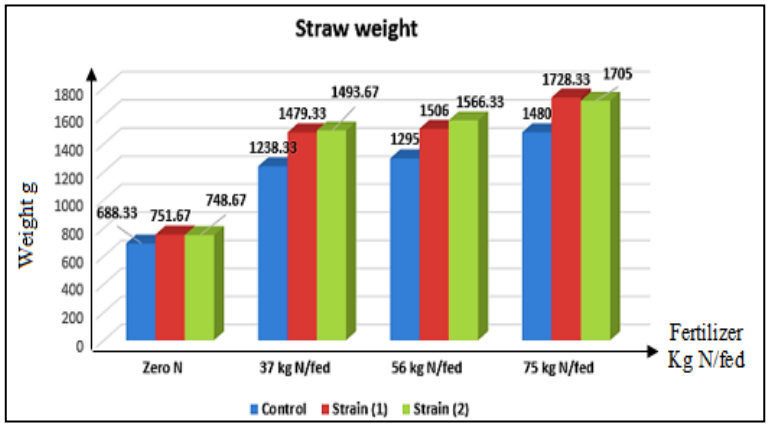
Control Uninoculated plant
Strain (1) *A.chroococcum*
Strain (2) *A.vinelandii*

Figure 9. Effect of *A.chroococcum* and *A.vinelandii* on Stem length



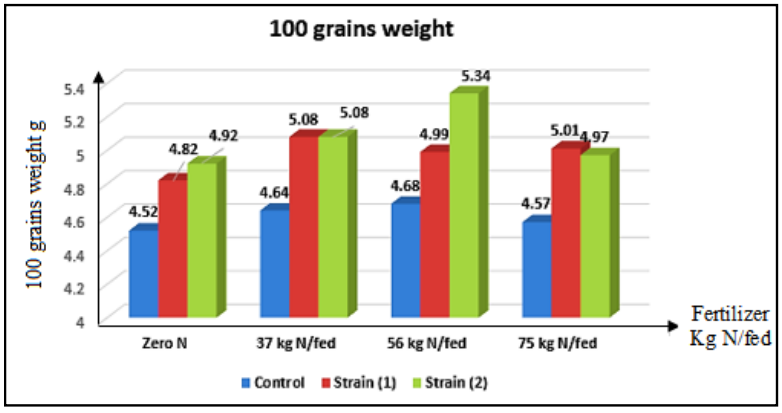
Control Uninoculated plant
Strain (1) *A.chroococcum*
Strain (2) *A.vinelandii*

Figure 10. Effect of *A.chroococcum* and *A.vinelandii* on Grains weight



Control Uninoculated plant
Strain (1) *A.chroococcum*
Strain (2) *A.vinelandii*

Figure 11. Effect of *A.chroococcum* and *A.vinelandii* on Straw weight



Control Uninoculated plant
Strain (1) *A.chroococcum*
Strain (2) *A.vinelandii*

Figure 12. Effect of *A.chroococcum* and *A.vinelandii* on 100 grains weight

The result as illustrated in Figure (9) showed that there was a significant increase in stem length of wheat grown in salinized soil inoculated with *Azotobacter* spp. than uninoculated (control) especially with using *A.chroococcum*. On other hand, the increase of N-fertilizer in combination with osmoadaptive species led to increase of stem length especially with *A.vinelandii.*

In recent study, it was reported that inoculation of crop plants with certain strains of PGPR at an early stage of development improves biomass production through direct effects on root and shoot growth [48]. Soil inoculation with PGPB had a positive impact on plant growth in combination with the organic fertilizer that was added [49]. Osmoadaptive strains selected in this study produced phytohormones as IAA which influenced the growth and length of the shoot of wheat to tolerate the effect of salt stress so, inoculated wheat with *Azotobacter* species give better results in shoot length than inoculated one.

Inoculation of crop plants with certain strains of PGPR at an early stage of development improves biomass production [48]. There was a significant increase in grains weight of wheat grown in salinized soil inoculated with *Azotobacter* spp. than uninoculated one (control) especially with using *A.vinelandii*. On other hand, the increase of N-fertilizer with using these osmoadaptive species led to increase in weight of wheat. Similar enhancement reported by inoculation of PGPB during wheat cultivation [56].

Results as illustrated in Figure (11) showed that there was a significant increase in straw weight and biomass of wheat grown in salinized soil inoculated with *Azotobacter spp*. than uninoculated (control) on both 2 species where results were better with species *A.vinelandii,* on the other hand, the increase of N-fertilizer with using these osmoadaptive species led to increase of straw weight of wheat.

The results revealed a significant increase in 100 grains weight of wheat grown in salinized soil inoculated with *Azotobacter* spp. than uninoculated (control) on both 2 species espicially with species *A.vinelandii.*

It was reported that the use of PGPB could be a frontier goal to achieve a positive effect on plants and reduce the negative impact of chemical and fertilizers on the environment [50]. Plant had a higher grain yield with the application of liquid phosphor-bacteria followed by *Azotobacter* [51]. *Azotobacter* plays different beneficial roles by producing different types of secondary metabolites in the soil such as vitamins, amino acids, plant growth hormones, antifungal substances, hydrogen cyanide and siderophores. These secondary metabolites have direct influence on growth of shoot, root and seed germination of many agriculture crops [52]. The production of auxins as IAA by selected isolates of *Azotobacter* stimulate plant growth and improve its productivity under saline soil.

3.10. Results of Pots Experiment

Figure 13

Figure 14

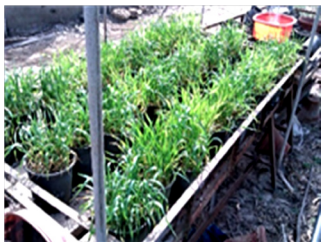


Figure 15

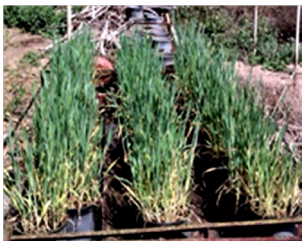


Figure 16

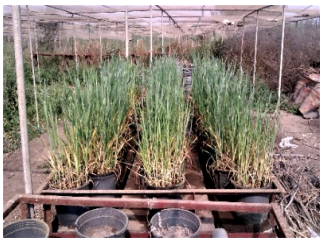


Figure 17

Figure 18

Figures 13, 14, 15, 16, 17, and 18 represent stages of wheat planting in pots experiments.

There were 3 lines of articulating squares represented *A.chroococcum*, control, and *A.vinelandii* with different replicates for different fertilizer concentrations and were based on Split Plot Design.

Table (7). Effect of *Azotobacter* species on Stem length

Parameter	Treatment	Control	*A.chroococcum*	*A.vinelandii*	p-value
Stem Length (cm)	Zero N	48 ± 2	52.67 ± 4.04	52 ± 2	0.301
	37 kg N/fed	46.33 ± 1.15	51.33 ± 1.15	50.67 ± 2.31	
	56 kg N/fed	49 ± 1.73	52.33 ± 2.89	53 ± 4.36	
	75 kg N/fed	45.67 ± 3.06	50 ± 1	53 ± 3	
	p-value	0.000**			---------

Table (8). Effect of *Azotobacter* species on Grains weight

Parameter	Treatment	Control	*A.chroococcum*	*A.vinelandii*	p-value
Grains Weight (g)	Zero N	1.19 ± 0.112	1.62 ± 0.322	1.57 ± 0.099	0.017*
	37 kg N/fed	1.48 ± 0.143	1.70 ± 0.160	1.78 ± 0.215	
	56 kg N/fed	1.82 ± 0.124	1.75 ± 0.111	1.72 ± 0.172	
	75 kg N/fed	1.04 ± 0.105	1.64 ± 0.192	1.69 ± 0.525	
	p-value	0.003*			---------

Table (9). Effect of *Azotobacter* species on Straw weight

Parameter	Treatment	Control	*A.chroococcum*	*A.vinelandii*	p-value
Straw Weight (g)	Zero N	9.55 ± 1.24	11.85 ± 0.786	10.15 ± 0.467	0.005*
	37 kg N/fed	11.52 ± 0.952	14.42 ± 1.08	14.18 ± 1.86	
	56 kg N/fed	11.27 ± 1.69	14.49 ± 0.453	13.53 ± 2.74	
	75 kg N/fed	9.67 ± 2.62	12.78 ± 1.80	13.74 ± 1.86	
	p-value	0.000**			---------

Table (10). Effect of *Azotobacter* species on 100 grains weight

Parameter	Treatment	Control	*A.chroococcum*	*A.vinelandii*	p-value
100 Grains Weight (g)	Zero N	4.08 ± 0.407	4.17 ± 0.900	4.22 ± 0.114	0.102
	37 kg N/fed	3.21 ± 0.197	4.11± 0.522	4.04 ± 0.311	
	56 kg N/fed	2.88 ± 0.442	4.09 ± 0.543	3.55 ± 0.475	
	75 kg N/fed	3.68 ± 0.739	3.80 ± 0.452	4.34 ± 0.778	
	p-value	0.019*			---------

Results in Table (7) showed that there was a significant increase in stem length of wheat grown in salinized soil inoculated with *Azotobacter* spp. than uninoculated one (control) especially with *A.vinelandii*. On other hand, increase of N-fertilizer with using these osmoadaptive species led to increase of stem length which indicated that, inoculation with *Azotobacter* spp. give better results on wheat cultivation under saline soil.

Results as illustrated in Table (8, 9, and 10) indicated that, there was a significant increase in grains weight, straw weight, and 100 grains weight of wheat grown in salinized soil inoculated with *Azotobacter* spp. than uninoculated one (control) on both *A.chroococcum* and *A. vinelandii.* On other hand, increase of N-fertilizer with using these osmoadaptive species led to increase of grains weight, straw weight, and 100 grains weight of wheat which indicated that inoculation with *Azotobacter* spp. give better results on wheat cultivation under saline soil.

Plant growth promoting bacteria PGPB are the most studied phytohormone producers [53] because the use of PGPR is an efficient and cheaper method that induces salt stress tolerance in plants [54]. The application of indole acetic acid synthesizing plant growth-promoting bacteria may represent an important alternative approach to decrease the impact of salt stress on crops [55]. PGPB have a favourable effect on plant growth, tolerance against stresses. Moreover, they are considered as a promising alternative to inorganic fertilizer for promoting plant growth, yield and quality. PGPR colonize at the plant root, raised germination rates, promote root growth, yield, leaf area, chlorophyll content, nitrogen content, protein content, tolerance to drought, shoot and root weight, and delayed leaf senescence [56].

Osmoadaptive bacteria selected under this study were exposed to gradually increasing salt concentrations in order to increase its availability of salt tolerance, when inoculated with wheat plant during seedling stage, it showed the ability to produce more growth regulating hormones as IAA than non-adaptive bacteria, led to enhance the wheat productivity in biomass and shoot length. Results of field and pots experiments showed that using of osmoadaptive salt tolerant bacteria (*A.chroococcum* or *A.vinelandii*) gave better results in growth criteria in inoculated wheat plant than uninoculated plant, some criteria were better with using *A.chroococcum* and others with using *A.vinelandii*. On other hand, increase of N-fertilizer gave better results with both species, such biofertilizers can be used as a proper tool for increasing wheat yield under salinity condition [57].

4. Conclusions

The obtained results demonstrated that, crop productivity of wheat grown in salinized soil was improved by using osmoadaptive salt tolerant *Azotobacter* spp. There was a significant increase in plant growth criteria (stem high, grains weight, straw weight and 100 grains weight) in the inoculated plants especially with osmoadaptive bacteria

(*A.chroococcum* or *A.vinelandii*) during seeding stage, the improvement ability was correlated to production of plant growth regulators as auxins (IAA) by bacteria which indicated that applying of salt-tolerant *Azotobacter* spp. was beneficial and effective in improving the productivity of wheat under salinity stress conditions and suggested the ability of using of osmoadaptive bacterial species during cultivation of wheat in salinized soils.

REFERENCES

[1] Mer, R.K., P.K. Prajith, D.H. Pandya and A.N. Pandey, 2000. Effect of salts on germination of seeds and growth of young plants of Hordeum vulgare, Triticum aestivum, Cicer arietinum and Brassica juncea. J Agronomy and Crop Sciences, 185: 209-217.

[2] Paul, D., & Nair, S. (2008). Stress adaptations in a Plant Growth Promoting Rhizobacterium (PGPR) with increasing salinity in the coastal agricultural soils. Journal of Basic Microbiology, 48(5), 378-384. doi:10.1002/jobm.200700365.

[3] Kayani, S.A. and M. Rahman, 1987. Salt tolerance in Corn (Zea mays L.) at the germination stage. Pak. J. Bot., 19: 9-15

[4] Rahman, M., S.A. Kayani and S. Gul, 2000. Combined effects of temperature and salinity stress on corn cv. Sunahry, Pak. J. Biological Sci., 3(9): 1459-1463.

[5] Pandey, A.N. and N.K. Thakrar, 1997. Effect of chloride salinity on survival and growth of Prosopis chilensis seedlings. Trop. Ecol., 38: 145-148.

[6] Hussain N, Ali A, Khan AG, Rehman OU, Tahir M (2003). Selectivity of ion absorption as nmechanism of salt tolerance I rice (variety Shaheen Basmati). Asian J plant Sci 2: 445-448.

[7] Tester M, Davenport R (2003). Na^+ tolerance and Na+ transport in higher plants. Ann Bot 91(5): 503–27.

[8] Poljakoff-Mayber A, Somers GF, Werker E, Gallagher JL. Seeds of Koteletzkya virginica (Malvaceae): their structure, germination and salt tolerance. Am J Bot. 1994;81:54–59. doi: 10.2307/2445562.

[9] Jadhav GG, Salunkhe DS, Nerkar DP, Bhadekar RK. Isolation and characterization of salt tolerant nitrogen fixing microorganisms from food. J Eur Asia Bio Sci. 2010;4:33–40. doi: 10.5053/ejobios.2010.4.0.5.

[10] Abu-Zeid, M. (1988). Egyptian policies for using low quality water for irrigation. In R. Bouchet, ed. Proc. of the Cairo/Aswan seminar "Reuse of low quality water for irrigation in Mediterranean Countries". Cairo.

[11] Amer MH, de Ridder NA (1989). Land drainage in Egypt. Drainage Research Institute for Land Reclamation and Improvement, Cairo.

[12] FAO (2010). Database Collections (Food and Agriculture Organization, Land and Plant Nutrition Management Service.) Available from: http://www.fao.org.

[13] Shirazi, M.U., S.M. Asif, B. Khanzada, M.A. Khan and A. Mohammad, (2001). Growth and ion accumulation in some wheat genotypes under NaCl stress. Pak. J. Biol. Sci., 4: 388-391.

[14] Anonymous, (1999). Agricultural statistics of Pakistan: Ministry of Food, agriculture and livestock, economics wing, Islamabad, pp: 3-4.

[15] FAO (2005). Database Collections (Food and Agriculture Organization, Fertilizer used by crop in Egypt.).

[16] Zahir ZA, Arshad M, Frankenberger WT. Plant growth promoting rhizobacteria: applications and perspectives in agriculture. Adv Agron. 2004; 81: 97–168. doi: 10.1016/S0065-2113(03)81003-9.

[17] Naz I, Bano A, Rehman B, Pervaiz S, Iqbal M, Sarwar A, Yasmin F. Potential of *Azotobacter vinelandii* Khsr1 as bio-inoculant. Afr J Biotechnol. 2012;11:10368–10372.

[18] Sturz AV, Nowak J. Endophytic communities of rhizobacteria and the strategies required to create yield enhancing associations with crops. Appl Soil Ecol. 2000; 15: 183–190. doi: 10.1016/S0929-1393(00)00094-9.

[19] Magda MA, Sabbagh SM, El-shouny WA, Ebrahim KH. Physiological response of Zea maysto NaCl stress with respect to *Azotobacter chroococcum* and Streptomyces niveus. Pakistan J Biol Sci. 2003;6:2073–2080. doi: 10.3923/pjbs.2003.2073.2080.

[20] Wu CH, Bernard SM, Anderson GL, Chen W (2009). Developing microbe interactions for applications in plant growth promotion and disease control, production of useful compounds, remediation and carbon sequestration. Microbiol Biotechnol 2: 428–440.

[21] Sindhu SS, Dua S, Verma MK, Khandelwal A (2010). Growth promotion of legumes by inoculation of rhizosphere bacteria. In: Khan MS, Zaidi A, Musarrat J (eds) Microbes for legume improvement. Springer-Wien, NewYork, pp 195–235.

[22] Muhammad Arshadullah, Syed Ishtiaq Hyder, Imdad Ali Mahmood, Tariq Sultan and Sadiq Naveed. (2017). Mitigation of salt stress in wheat plant (Triticum aestivum) by plant growth promoting rhizobacteria for ACC deaminase. Int. J. Adv. Res. Biol. Sci. 4(6): 41-46.

[23] Rahim Nosrati., Parviz Owlia., Horieh Saderi., Iraj Rasooli., Mohammad Ali Malboobi, (2014). Iran J Microbiol,6(4): 285-295.

[24] Sartaj A Wani., Subash Chand and Tahir Ali, (2013). Current Agriculture Research Journal, 1(1): 35-38.

[25] Gurikar C., Naik M.K., Sreenivasa M.Y. (2016). *Azotobacter*: PGPR Activities with Special Reference to Effect of Pesticides and Biodegradation. In: Singh D., Singh H., Prabha R. (eds) Microbial Inoculants in Sustainable Agricultural Productivity. Springer, New Delhi.

[26] Silini, A., Cherif-Silini, H., & Yahiaoui, B. (2016). Growing varieties durum wheat (Triticum durum) in response to the effect of osmolytes and inoculation by *Azotobacter chroococcum* under salt stress. African Journal of Microbiology Research, 10(12), 387-399.

[27] Richards, L. A. (ed.) (1954). "Diagnosis and improvement of saline and alkaline soils" U.S.D.A. Agric, Handbook No. 60.

[28] Vancura V and J Macura. (1960). Indole derivatives in *Azotobacter* cultures. Fol. Microbiol. 5: 293-298.

[29] Dobereiner, J. (1978). Influence environmental factors on the occurance of Spirillum lipoferum in soil and roots. In: Granhall U (Ed), Environmental role of nitrogen-fixing bleu-green algae and asymbiotic bacteria. Ecological Bulletins, 26, Stockholm: Swedish Nature, p. 343-352.

[30] Brenner, D.J., Krieg, N.R., Staley, J.T. and Garrity, G.M. (2005). Bergey's manual of Systematic Bacteriology 2nd Ed., Vol. 2, Part (A), The Proteobacteria, Introductory Essays, New York, Springer.

[31] Carozzi, N.B., Kramer, V.C., Warren, G.W., Evola, S. and Koziel, M.G. (1991). Prediction of insecticidal activity of Bacillus thuringiensis strains by polymerase chain reaction product profiles. Appl. environ. Microbiol, 57, 306-3057.

[32] Saitou N, Nei M (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406-425.

[33] Joseph G Pigeon (2012). Statistics for Experimenters: Design, Innovation.and. Discovery, Technometrics, 48: 2, 303-304, DOI: 10.1198/tech.2006.s379.

[34] Saville D.J., Wood G.R. (1991). Split Plot Design. In: Statistical Methods: The Geometric Approach. Springer Texts in Statistics. Springer, New York, NY.

[35] Pilet PE, Chollet R. (1970). Sur le dosage colorimetrique de l'acide indolylacetique. C R Acad Sci Ser D. 1970; 271: 1675–1678.

[36] Miller, R.W. and R.L. Donahue, (1995). Soils in our environment, 7th Ed. Prentice Hall Inc., Englewood Cliffs, New Jersey. 649p. Available from: agris.fao.org (http://www.fao.org/docrep/x5871e/x5871e04.htm).

[37] Egamberdieva, D. Acta Physiol Plant (2009) 31: 861. https://doi.org/10.1007/s11738-009-0297-0.

[38] Singh H, Dara BL (1971). Influence of presoaking of seeds with gibberellin and auxins on growth and yield attributes of wheat (Triticum aestivum L.) under high salinity, sodiumadsorption ratio and boron levels. Indian J Agric Sci 41: 998–1003.

[39] Datta KS, Varma SK, Angrish R, Kumar B, Kumari P (1998). Alleviation of salt stress by plant growth regulators in Triticum aestivum L. Biol Plant 40:269–275. doi: 10.1023/A:1001076805595.

[40] Sastry EVD, Shekhawa KS (2001). Alleviatory effect of GA3 on the effect of salt at seedling stage in wheat (Triticum aestivum). Indian J Agric Res 35: 226–231.

[41] Afzal I, Basra S, Iqbal A (2005). The effect of seed soaking with plant growth regulators on seedling vigor of wheat under salinity stress. J Stress Physiol Biochem 1(1): 6–14.

[42] Khan MA, Weber DJ (1986). Factors influencing seed germination in Salicornia pacifica var utahensis. Am J Bot 73:1163–1167. doi: 10.2307/2443795.

[43] Gul B, Khan MA, Weber DJ (2000). Alleviation salinity and dark-enforced dormancy in Allenrolfea occidentalis seeds under various thermoperiods. Aust J Bot 48:745–752. doi: 10.1071/BT99069.

[44] Khan MA, Gul B, Weber DJ (2001). Seed germination characteristics of Halogeton glomeratus. Can J Bot 79: 1189–1194. doi: 10.1139/cjb-79-10-1189.

[45] Khan MA, Gul B, Weber DJ (2004). Action of plant growth regulators and salinity on seed germination of Ceratoides lanata. Can J Bot 82:37–42. doi: 10.1139/b03-140.

[46] Kabar K (1987). Alleviation of salinity stress by plant growth regulators on seed germination. J Plant Physiol 128:179–183.

[47] Sakhabutdinova AR, Fatkhutdinova DR, Bezrukova MV, Shakirova FM (2003). Salicylic acid prevents the damaging action of stress factors on wheat plants. Bulg J Plant Physiol 314–31.

[48] Nagargade M., Tyagi V., Singh M.K. (2018). Plant Growth-Promoting Rhizobacteria: A Biological Approach Toward the Production of Sustainable Agriculture. In: Meena V. (eds) Role of Rhizospheric Microbes in Soil. Springer, Singapore.

[49] Saia S, Rappa V, Ruisi P, et al. (2015). Soil inoculation with symbiotic microorganisms promotes plant growth and nutrient transporter genes expression in durum wheat. Front Plant Sci 6: 815.

[50] Maria et al, (2017). The role of Plant Growth Promoting Bacteria in improving nitrogen use efficiency for sustainable crop production: a focus on wheat, AIMS Microbiology, 3(3): 413-434. DOI: 10.3934/microbiol.2017.3.413.

[51] S. K. Dubey et al, (2017). Effect of *Azotobacter* and Phosphobacteria on Yield of Wheat (Triticum aestivum), International journal of plant research, DOI: 10.5958/2229-4473.2017.00130.6.

[52] Chennappa G., Sreenivasa M.Y., Nagaraja H. (2018). *Azotobacter* salinestris: A Novel Pesticide-Degrading and Prominent Biocontrol PGPR Bacteria. In: Panpatte D., Jhala Y., Shelat H., Vyas R. (eds) Microorganisms for Green Revolution. Microorganisms for Sustainability, vol 7. Springer, Singapore.

[53] Spaepen S, Vanderleyden J, Remans R (2007). Indole 3 acetic acid in microbial and microorganism plant signaling. FEMS Microbiol Rev 31: 425–448.

[54] Kadmiri, I.M., Chaouqui, L., Azaroual, S.E. et al. Arab J Sci Eng (2018). https://doi.org/10.1007/s13369-017-3042-9.

[55] Li, H.Q. & Jiang, X.W. Russ J Plant Physiol (2017) 64: 235. https://doi.org/10.1134/S1021443717020078.

[56] Ramazan et al., (2017). The Role of Soil Beneficial Bacteria in Wheat Production: A Review http://dx.doi.org/10.5772/67274.

[57] Raouf Seyed Sharifi, Razieh Khalilzadeh & Jalal Jalilian (2016). Effects of biofertilizers and cycocel on some physiological and biochemical traits of wheat (Triticum aestivum L.) under salinity stress, Archives of Agronomy and Soil-Science, 63:3, 308-318, DOI: 10.1080/03650340.2016.1207242.