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Potential effect of plant growth-promoting rhizobacteria (PGPR) on wheat (*Triticum aestivum* L.) under salinity stress

Eman.M.M.Al-Bardini[1] , Alaa.E.Omara[2], Mohamed. H.H. Abbas[1], Ibrahim. Ali[1]
[1] Soils and Water Department, Agriculture Faculty, Benha University, Egypt
[2]Agricultural Research Center, Department of Microbiology, Soils, Water and Environment Research Institute, Giza 12112, Egypt
E-Mail: eman2024@gmail.com

Abstract

The aim of this paper to investigate the influence of *Azospirillum brasilense*, *Bacillus cereus*, *Pseudomonas fluorescens*, *Bacillus subtilis, Azospirillum lipoferum,* and *Enterobacter cloacae*) on the yield and growth of two wheat varieties, namely Misr 1and Sakha 95, which were cultivated on saline clayey soil. The experiment contained four treatments for each variety (T1(control), T2(*Azospirillum brasilense* and *Bacillus cereus)*, T3(*Pseudomonas fluorescens* and *Bacillus subtilis*), and T4(*Azospirillum lipoferum*, and *Enterobacter cloacae*). The results show that Sakha 95 had higher growth and yield than Misr 1. Moreover, the used groups of PGPR significantly increased the yield and growth parameters of wheat plants. The highest plant length, spike length, spike number, the weight of 1000 grains, straw yield, and grain yield (77.95 cm, 9.67 cm, 16.14 spike, 43.5 g, 27.28 g/pot, and 19.18 g/pot, respectively) were recorded with T3 (*Pseudomonas fluorescens* and *Bacillus subtilis*) application. The interaction effects of wheat varieties and PGPR on the above-mentioned parameters, indicated that the highest plant length, spike length, spike number, weight of 1000 grains, straw yield, and grain yield were observed in Sakha 95 with application of T3 (*Pseudomonas fluorescens* and *Bacillus subtilis)* compared with Misr 1 and the control. Salt-affected soils inoculated by *Pseudomonas fluorescens* and *Bacillus subtilis* enhance the growth and reduce the cost of wheat production.

Keywords: Wheat, PGPR, Growth parameters, Yield, Saline soil

1. Introduction

Wheat (*Triticum aestivum* L.) is highly valued as one of the primary edible grains and crucial crops for human nutrition. It is the second most widely grown cereal worldwide, resulting in a significant yearly harvest of 713 million tons [1]. Zhara [2] showed that increasing wheat yield could positively contribute to food security in the coming decades. The wheat cultivation area in Egypt in 2020 covered around 1.37 million hectares, producing 9 million tons. This production accounted for a significant portion, around 42.85%, of the overall wheat consumption in the region. Adequate water and soil management is vital for enhancing wheat yield, particularly in salty soils (covering approximately 8.1 x 105 hectares), which comprise 35% of Egypt's total cultivated area [3].

Soil salinity is a major agricultural issue and one of the most significant environmental stresses worldwide. It causes agriculturally productive lands to become unproductive at around 1-2% per year, especially in dry and semi-arid regions. In addition, soil salinization has made over 7% of the soil on earth and 20% of the total arable area unfit for farming [4]. Moreover, the increase in soil salinization is a worldwide phenomenon, and forecasts suggest that almost 50% of farmland will be at risk of salinization by 2050. Elevated salt levels, a notable abiotic component, have a substantial damaging impact on the growth and development of most plants, resulting in a decline in overall crop yield. Furthermore, salinity causes significant modifications in plant growth and metabolism, including physiological, morphological, and biochemical adjustments [5]. Salt stress induces increased movement of ions into cells, disruption of oxidant balance, inhibition of cell division, and degradation of cellular membranes [6,7]. Ion toxicity and osmotic stress are the primary contributors to the salt-induced reduction in crop yield [8].

Improving the ability of plants to tolerate salt is vital for the sustainable progress of agriculture. Microorganisms are crucial in enhancing plant tolerance to biotic and abiotic stressors. Numerous investigations have evaluated the capacity of microorganisms to stimulate host plant growth under salinity stress conditions. Many studies indicated that good microorganisms for plants have intricate regulatory mechanisms that facilitate their growth and lessen the negative impacts of salt stress on their hosts [9,10] .

Plant Growth-Promoting Rhizobacteria (PGPR), also known as rhizobacteria, are a group of bacteria that live in the rhizosphere and help plants grow by directly and indirectly affecting their growth. Direct mechanisms include the synthesis of phytohormones and enhanced nutrient accessibility. Indirect processes involve inhibiting infections by antibiosis, producing lytic enzymes, and stimulating systemic resistance (ISR) [8]. Previous studies have shown that using PGPR can effectively improve crop production in harsh salinity circumstances [11-15] . In addition, rhizosphere bacteria improve plant resistance to salt stress by regulating multiple physiological factors. These factors include the control of photosynthetic efficiency, balance of ion levels, accumulation of secondary metabolites, osmotic regulators, and adjustment of gene expression through the signaling pathways of plant hormones [15-17]. Several genera of PGPR, including *Pseudomonas, Bacillus, Azospirillum, Azotobacter,* and others, have been recognized for their effectiveness in maintaining the productivity of different crops grown in saline soils [18,19] .

This study aims to examine the effectiveness of three different combinations of PGPRs in enhancing the growth of two wheat cultivars, Misr 1 and Sakha 95. This work highlights the exceptional ability of these PGPR genera to thrive in high salt concentrations, revealing their potential to enhance plant growth consistently.

2. Materials and Methods

2.1. Soil samples and analysis

A salt-rich farm in Kafr El Sheikh City, Kafr El Sheikh Governorate, provided the soil used in the experiment. It was shipped in sterile polyethylene bags with great care. The soil was dried by air, sieved through a 2-mm screen for further investigation, and was found to have inadequacies in accessible nitrogen (N), phosphorous (P), and potassium (K), as well as elevated electrical conductivity (EC) and pH levels, low organic matter content, and low quantities of these elements. The hydrometer method of Bouyoucos [20] was employed to determine the texture of the soil. A digital pH and EC meter was used to measure the EC of the soil in a soil paste, and the pH of the soil was measured in a 1:2.5 ratio solution (soil:water, w:v). Total organic matter in the soil was determined through the titration process using ferrous ammonium sulfate [21]. The method of chlorostannus-reduced molybdophosphoric blue color was used to quantify the amount of accessible phosphorous after soil was extracted using 0.5 M sodium bicarbonate [22]. Table 1 lists all the soil's specific chemical and physical characteristics.

Table (1) some physical and chemical properties of the used soil.

Properties	Values
Physical properties	
Sand (%)	14.71
Loam (%)	31.43
Clay (%)	53.86
Texture	Clay
Bulk density (g cm^{-1})	1.41
Particle density (g cm^{-1})	2.59
Chemical properties	
pH^*	8.01
EC^{**} (dS m^{-1})	7.64
CEC ($mmol_c$ kg^{-1})	35.19
OM (g kg^{-1})	1.75
$CaCO_3$ (g kg^{-1})	2.64
Soluble cations ($mmol_c$ l^{-1})	
Ca^{2+}	21.71
Mg^{2+}	9.45
Na^+	31.56
K^+	13.68
Soluble anions ($mmol_c$ l^{-1})	
CO_3^{2-}	0
HCO_3^-	21.52
Cl^-	39.18
$SO4^{2-}$	15.70
Available nutrients (mg kg^{-1})	
Available N (KCl extract)	30.74
Available P ($NaHCO_3$ extract)	5.56
Available K (CH3COO-NH_4 extract)	181.8

*** in a suspension of 1:2.5 soil:water**
**** in a soil paste extract**

2.2. Pot experiment

A pot experiment was performed in a net greenhouse in Arab Elhaswa village, Toukh City, Qalyubia Governorate, Egypt, under natural conditions in the winter season of 2022 (November 15, 2022) . Grains of two wheat cultivars (Misr 1 and Sakha 95) were attained from the Agricultural Research Center, Field Crops Research Institute, Wheat Research Department (Giza, Egypt). Three groups of PGPRs (1: *Azospirillum brasilense* and *Bacillus cereus*, 2: *Pseudomonas fluorescens* and *Bacillus subtilis* and 3: *Azospirillum lipoferum* and *Enterobacter cloacae*) were acquired from Ain Shams University, Faculty of Agriculture, Agricultural Microbiology Department. The experimental pots were collected and filled with the salt affected soil (5 kg soil per pot). The experiment was factorial with two factors (the first one was wheat varieties and the second one was the PGPRs) and had 8 treatments (T1= control, T2= *Azospirillum brasilense,* and *Bacillus cereus*, T3= *Pseudomonas fluorescens* and *Bacillus subtilis* and T4= *Azospirillum lipoferum* and *Enterobacter cloacae* for Misr 1, and Sakha 95. The grains of wheat (Misr 1 and Sakha 95) were sterilized by sodium hypochlorite (4%, w/v) for 15 min., and were then gently rinsed with distilled water to remove residual chloride. The wheat grains were microbiologically inoculated through by soaking them for 30 min in a 10 % Arabic gum solution containing the PGPR mixture. Fifteen inoculated grains (5 L/fed) from each wheat variety were sown in the soil, and then the experimental pots were irrigated with tap water till they reached the field capacity level for the soil. After 10 days from the germination, wheat seedlings were thinned to 10 plants per pot. The experimental design utilized a Randomized Complete Block configuration (RCBD). Each treatment was replicated four times. Wheat plants were fertilized and managed according to the Ministry of Agriculture and Land Reclamation recommendations. Nitrogen (N) fertilizer was applied as urea (46.5% N) at a rate of 75kg N fed^{-1} in two equal doses at 30 and 45 days after sowing. The phosphorus fertilizer was added one month before the sowing of wheat grains as calcium superphosphate (15.5% P_2O_5) at a rate of 150 kg fed^{-1}, while potassium fertilizer was added at the same time of nitrogen supplying as potassium sulfate (48% K_2O) at a rate of 50 kg fed^{-1}, respectively.

2.3. Estimation of plant growth and yield

The plants were harvested on April 25, 2023, and then separated into grains and straw for oven-drying at 70 °C for 48 h. Growth parameters and dry weights of straw and grains were measured and averaged per pot.

2.4. Statistical analysis

The study's data are the means that were determined from four replicates (n = 4). The SPSS program (version 25) was utilized to carry out a one-way ANOVA statistical analysis of the data. The statistical significance of mean differences was calculated using Duncan's multiple-range test, with a significance level of $P < 0.05$.

3. Results

Data in Table 2 showed the mean effect of wheat varieties on some growth parameters of wheat plants such as plant length, spike length, spike number and weight of 1000 grains. Sakha was higher than Misr 1 in plant length, spike length, spike number and weight of 1000 grains.

In Table 3, the mean effect of plant growth promoting rhizobacteria treatments on the above-mentioned items was shown. T3 was responsible for the highest values of these items followed by T2 and the lowest values were recorded due T4 and T1, respectively and all treatments were better than the Control (T1). Only in plant length, and spike number, the effect of T2 was similar to that of T4.

Table (2) Mean effect of wheat varieties on some growth parameters of wheat plants

	Plant length (cm)	Spike length (cm)	Spike number	W-1000 grain (g)
Misr 1	70.75 b	8.40 b	13.35 b	35.88 b
Sakha 95	74.10 a	9.03 a	13.83 a	38.68 a

Different letters within the same column indicated significant differences.

Table (3) Mean effect of plant growth-promoting rhizobacteria treatments on some growth parameters

	Plant length (cm)	Spike length (cm)	Spike number	W-1000 grain (g)
T1	66.85 c	7.67 d	11.07 c	31.5 d
T2	73.15 b	9.07 a	13.99 b	38.4 b
T3	77.95 a	9.67 b	16.14 a	43.5 a
T4	71.75 b	8.45 c	13.12 b	35.8 c

T1: Control, T2: *Azospirillum brasilense* and *Bacillus cereus,* T3: *Pseudomonas fluorescens* and *Bacillus subtilis,* T4: *Azospirillum lipoferum* and *Enterobacter cloacae*

Different letters within the same column indicated significant differences.

The interaction effect of wheat varieties and PGPR treatments was presented in Table 4. The heights plant length, spike length, spike number and weight of 1000 grains (80.5 cm, 10.13 cm ,16.39 and 45.4g) were found in T3 for sakha 95, whereas the lowest values these parameters wear recoded in the control treatment T1 (65.3cm, 7.38 am, 10.7 and 30.9g) for Misr 1.

Different letters within the same column indicated significant differences.

The Mean effect of wheat varieties on straw and grain yields was shown in Table 5. The results indicated that there was significant difference between the used varies (Misr 1, and Sakha 95) and both straw and grain yields of Sakha 95 were higher than those for Misr 1.

Different letters within the same column indicated significant differences.

The used PGPR treatments showed different pattern for straw yield and grain yields (Table 6). The largest straw and grain yields (27.4 and 19.18 g/pot) were found due to T3, followed by T 2 and T4 and all treatments showed marked effect in compassion to the control (T)

The interaction effect of PGPR and varieties showed that the use of T3 led to highest straw yield (28.65 and 26.91 g/pot) and grain yield (19.95 and 18.41g/pot) Sakha 95 and Misr 1, respectively (Table 7). However, the lowest straw and grain yields (22.46 and 11.59 g/pot) were noted in T1 for Misr 1.

Table (4) Interaction effect of plant growth-promoting rhizobacteria treatments and wheat varieties on some growth parameters of wheat plants

		Plant length (cm)	Spike length (cm)	Spike number	W-1000 grain (g)
Misr 1	1	65.3 e	7.38 e	10.78 d	30.9 e
	2	71.7 c	8.85 c	13.93 b	37.5 c
	3	75.4 b	9.20 b	15.89 a	41.6 b
	4	70.6 c	8.15 d	12.71 c	34.3 d
Sakha 95	1	68.4 d	7.95 d	11.36 d	32.8 e
	2	74.6 b	9.28 b	14.05 b	39.3 bc
	3	80.5 a	10.13 a	16.39 a	45.4 a
	4	72.9 bc	8.75 c	13.53 b	37.2 c

T1: Control, T2: *Azospirillum brasilense* and *Bacillus cereus,* T3: *Pseudomonas fluorescens* and *Bacillus subtilis,* T4: *Azospirillum lipoferum* and *Enterobacter cloacae*

Table (5) Mean effect of wheat varieties on straw and grain yields of wheat plants

	Straw yield (g/pot)	Grain yield (g/pot)
Misr 1	24.78 b	15.12 b
Sakha 95	25.69 a	16.77 a

Table 6. Mean effect of plant growth-promoting rhizobacteria treatments straw and grain yields of wheat plants

	Straw yield (g/pot)	Grain yield (g/pot)
T1	23.29 c	12.84 c
T2	25.79 b	16.49 b
T3	27.28 a	19.18 a
T4	24.59 bc	15.27 b

T1: Control T2: *Azospirillum brasilense* and *Bacillus cereus*
T3: *Pseudomonas fluorescens* and *Bacillus subtilis* T4: *Azospirillum lipoferum* and *Enterobacter cloacae*

Table (7) Interaction effect of plant growth-promoting rhizobacteria treatments and wheat varieties on straw and grain yields of wheat plants

		Straw yield (g/pot)	Grain yield (g/pot)
Misr 1	T1	22.46 d	11.59 f
	T2	25.70 b	15.86 d
	T3	26.91 b	18.41 b
	T4	24.05 c	14.63 e
Sakha 95	T1	24.12 c	14.08 e
	T2	25.87 b	17.13 c
	T3	28.65 a	19.95 a
	T4	25.13 b	15.92 d

T1: Control, T2: *Azospirillum brasilense* and *Bacillus cereus,* T3: *Pseudomonas fluorescens* and *Bacillus subtilis,* T4: *Azospirillum lipoferum* and *Enterobacter cloacae*

4. Discussion

Salinity significantly impacts the balance and characteristics of the soil in a particular area, reducing crop yields and contributing to lower economic returns. Numerous studies have repeatedly demonstrated that salinity negatively affects plants, including reduced growth and development, hampered vegetative growth, restricted germination, and impaired reproductive development. Furthermore, salinity lowers overall fertility, spikelet fertility per spike, delayed spike development, and delayed spike development, reducing grain yield [23,24]. Plant growth and metabolism are adversely affected by salty environments, mainly due to the increased build-up of sodium ions (Na) in plant tissues [25]. Reactive oxygen species (ROS) are produced more often, and the accumulation of these Na ions hinders photosynthesis. The plant is then subjected to several negative consequences from these ROS, including the acceleration of harmful processes such as membrane damage, protein deterioration, and DNA mutation [26]

PGPR can significantly facilitate plant growth of many cereal and other essential agricultural crops [27]. Rhizospheric or endophytic bacteria attached to the outside or inside of plant roots are called PGPR. Recent investigations have demonstrated that bacteria from some genera, including *Microbacterium, Pantoea, Achromobacter, Rhizobium, Pseudomonas, Bacillus, Paenibacillus, Enterobacter, Burkholderia, Methylobacterium, Azospirillum, and Variovorax*, among others, have been identified for having the capacity to provide host plants with tolerance against a variety of abiotic stressors [27-29] . Based on studies conducted by Banaei-Asl [30] and Wang [31], these microorganisms have been found to be useful in agricultural contexts as well as beneficial to mitigate a variety of abiotic stresses. Research has repeatedly shown that these bacteria improve plants' ability to withstand stress by producing gibberellins, indole acetic acid. According to a number of studies, these bacteria increase plants' ability to withstand stress through a variety of mechanisms, including the production of gibberellins, indole acetic acid, and other elements that are yet to be identified. These mechanisms increase the root surface area, length, and tips as well as, most importantly, the nutrient content, which benefits the plant's health when exposed to salt stress [28,29].

5. Conclusion

The results of this study indicate that the negative effects of salt stress on wheat plant growth could be mitigated by root inoculations with PGPB. This was shown to occur with increasing straw and grain yield and growth parameters such as plant length, spike length, spike number, and weight of 1000 grains. Furthermore, plant response to inoculation suggests that the bacterial strains *Pseudomonas fluorescens* and *Bacillus subtilis* have the most significant potential to be used as an environmentally friendly approach to combat salt stressors and then increase the productivity of wheat plants under saline conditions.

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