Research Article

Bacteria Polysaccharides Elicit Resistance of Wheat Against Some Biotic and Abiotic Stress

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ABSTRACT

Plant growth-promoting rhizobacterium (PGPR) have a well-established obtaining system and use in plant protection by induction of resistant. The role of exopolysaccharides (EPSs) from a plant growth-promoting rhizobacterium, *Pseudomonas fluorescens*, *Bacillus amyloliquefaciens* and *Bacillus polymyxa* on elicitation of wheat resistance toward some biotic and a biotic stress was investigated. Purified EPSs of three PGPR showed ability to activate multiple plant defense mechanisms against wheat powdery mildew caused by *Blumeriagraminis* and leaf rust caused by *Puccinia recondite*, under controlling conditions. At the same time, it increasing ACC deaminase, Indol acetic acid (IAA), proline, peroxidase, chitinase and soluble protein contents under saline and normal soil. The maximum level of diseases protection and plant growth was noted when seeds and plant sprayed with 200 ppm of the EPS of test bacteria. Under saline soil at Sinai and under natural infection conditions, application of bacteria polysaccharide (slime type) elicits induced resistance of wheat against some biotic and abiotic stress under. In addition, plants treated with bacteria EPS increased growth parameters and yield. This study indicates that they could be used as a promising plant growth enhancer for biotic and a biotic stress. Our results indicate that EPS from specific rhizobacteria can elicit induced resistance and suggest that bacterial EPS might be a useful elicitor of resistance.

Keywords: Bacteria polysaccharides, Biotic and a biotic Stress, *Bacillus amyloliquefaciens* and *Bacillus polymyxa, Pseudomonas fluorescens,* Wheat.

INTRODUCTION

Wheat (*Triticumaestivum*L.) is the most important grain crop, a staple food for more than one third of the world population and in Egypt [13]. Knowing diseases that may cause injuries and are likely to affect plant health and quality is critical to minimizing the gap between attainable yield and actual yield. On wheat, the most prevalent diseases were Yellow or stripe rust (*Pucciniastriiformis,* f.sp. *tritici*); Leaf or brown rust (*Pucciniarecondita,* f.sp. *tritici*);Stem or black rust (*Pucciniagraminis,* f.sp.*tritici*); Loose smut (*Ustiligotritici*); Tan spot (Yellow leaf spot) (*Drechesleratritici-repentis* (syn. *Helminthosporiumtritici-repentis)* as in not final stage; *Pyrenophoratritici-repentis*as a complete stage);- Septoria leaf blotch (*Septoriatritici*as in not complete stage, *Mycosphaerellagraminis*as a complete stage); Septoria leaf and glume blotch (*Septorianodorum*as in not complete stage; *Stagonosporanodorum*as a complete stage); Helimintosporium leaf blotch (*Bipolarissorokiniana*, syn. *Helminthosporium sativum*) and Powdery mildew (*Blumeriagraminis*f. sp. *Tritici.* [14,15]

Concerns have been raised about both the environmental impact and the potential health risk related to the use of chemical fungicides compounds. At any improvement in agricultural system that results in higher production should reduce the negative environmental impact of agriculture and enhance the sustainability of the system. In recent years, the induction of the plant defense response by natural products as well as microorganisms as fungi and bacteria that normally colonize living plants without causing visual damage, has received ample attention. Rhizobacteria that are associated with plant roots, are known to promote plant growth, as well as to induce systemic resistance to diseases in plants.[25]

Bacterial polysaccharides represent a diverse range of macromolecules that include peptidoglycan, lipopolysaccharides, capsules and exopolysaccharides; compounds whose functions range from structural cell-wall components (eg peptidoglycan), and important virulence factors, to permitting the bacterium to survive in harsh environments.[4,11] Polysaccharide biosynthesis is a tightly regulated, energy intensive process and understanding the subtle interplay between the regulation and energy conservation, polymer modification and synthesis, and the external ecological functions is a huge area of research. Several poly and oligosaccharides exhibit different kind of biological activities.[10-12,20] Some bacteria, developed capsular adaptations by secreting polysaccharides that help their adhesion to surfaces and prevent the desiccation. These capsular polysaccharides, including peptidoglycans, lipopolysaccharides and exopolysaccharides, are water soluble, acids and participate in the host-pathogen interaction.[21] The production of biologically active substances which promotes the growth of bacteria and other plant organisms has been reported.[12] The bacteria cells can release extra-cellular polysaccharides (EPS) into the

environment; these EPS are ecologically important through their influence on carbon cycle and microbial diversity.[7] Plant growth promoting rhizobacteria (PGPR) are a group of free-living saprophytic bacteria that can be found in the rhizosphere in association with root system and enhance the growth and development of plant either directly or indirectly. Interestingly these PGPR strains also possess the enzyme ACC deaminase and this enzyme can cleave the plant ethylene precursor ACC to ammonia and a-ketobutyrate thereby lowers the level of ethylene under various biotic and abiotic stresses such as salt stress[27], drought stress[16]and pathogen attack.[26] ACC deaminase-containing plant growth promoting rhizobacteria lowers the level of ACC in the stressed plants, thereby limiting the amount of stress ethylene synthesis and hence the damage to the plant. These bacteria are beneficial to plant growth as plants are often subjected to ethylene producing stresses. Soil borne fluorescent pseudomonads have excellent root colonizing ability, catabolic versatility and produce a wide range of enzymes and metabolites that favor the plant weight and under varied biotic and abiotic stress conditions.[16]

The objective of this study was to determine whether EPSs isolated from PGPR strain B *Pseudomonas fluorescens*, *Bacillus amyloliquefaciens* and *Bacillus polymyxa* could elicit ISR in wheat against biotic (diseases as powdery mildew and leaf rust) and abiotic (salinity stress).

MATERIALS AND METHODS

Test microorganisms

Three bacteria species were selected for polysaccharide production i.e., *Pseudomonas fluorescens*, *Bacillus amyloliquefaciens* and *Bacillus polymyxa*. The tested bacteria species were isolated from rhizosphere of wheat grown in a salt-affected soil and identified in Plant Pathology Department, National Research Centre, Egypt. *Pseudomonas fluorescens* was serially diluted and appropriate dilutions were spread plated on solid King's B medium. The plates were incubated at 28±2°C and fluorescent colonies were selected for exopolysaccharide production The cultured media were incubated at 25 ± 2 °C and shaken to prevent bacteria cell clumping and adherence to the containers. *Bacillus amyloliquefaciens* and *Bacilluspolymyxa* were spread onto nutrient agar plates from stocks. These plates were incubated overnight at 25^{0}C and used to inoculate 100 ml nutrient broth (Oxoid) in 250 ml conical flasks. The cells were diluted to 2 x 10^5 cells/ml with sterile water and then resuspended in 0.01 M potassium phosphate buffer (pH 7.0) plus 0.01% Tween-80 as surfactant. After incubation at 28°C under shaking conditions (120 rpm) for 24 h, growth was estimated by measuring the optical density at 600 nm using a spectrophotometer. The growth of the isolates at various stress levels was recorded.

Measurement of extra-cellular polysaccharides (IPS)

The extra-cellular polysaccharides were estimated according to (Berge*t al.,*[4] To precipitate proteins from the bacteria, trichloroacetic acid (TCA) was added in a final concentration of 4% and the filtrate was stirred for 2 h. Precipitated proteins were removed by centrifugation. The clear supernatant was collected which contains EPS. Extra-cellular polysaccharides was precipitated by ethanol and determined.

Pot trials

Pot experiments were conducted by employing Completely Randomized Design. Seeds of highly susceptible wheat cultivar to diseases and stress (cv. Giza 160) were surface sterilized with 0.1% $NaCl_2$ for 2 min and washed with sterilized water. For treatment of wheat seeds with EPS, seeds were treated with 100 µl of 200 ppm EPS. For spray application of EPS, 30-day-old wheat plants were sprayed with each treatment. The sand was sterilized by autoclaving thrice before the experiment while natural soil was used without sterilization. Hoagland solution was provided whenever required. The moisture content of the soil was maintained at 60% WHC (water holding capacity) by loss in weight method throughout the experiment.

Trial 1 This experiment was conducted in in (8 cm diameter) plastic pots containing sand salinized with 6 days intervals (300 mM). First salinization was done before seed sowing while 2nd was done at 6-days old seedling stage and 3rd was done at 12days old seedlings. Hoagland solution and saline water were alternatively provided to plant whenever required. The plants grown without sand salinization were used as positive control.

Trial 2 This experiment was conducted in (8 cm diameter) plastic pots containing normal sand soil. Artificial infestation of pathogens (10^4 spores/ ml) Leaf or brown rust (*Pucciniarecondita,* f.sp. *tritici* and Powdery mildew *Blumeriagraminis*f. sp. *tritici*).[15] Inoculation at the third leaf stage was preferred by spraying the seedling with 5 x 10^5 spores per ml of suspension. Leaf rust inoculations were preformed on seedlings at 2-4 leaf stage. The inoculation was carried out by dusting fresh urediospores of *Pucciniahordei* diluted 10 times with talcum powder, over the seedlings when the second leaf of the seedlings had emerged. After inoculation, seedlings were incubated overnight in complete darkness and at a relative humidity of 100%.

- After inoculation, seedlings were incubated overnight in complete darkness and at a relative humidity of 100%. Seedlings were then transferred to a growth chamber at 18–22C and white fluorescent light (12 h light/12 h dark). Inoculated seedlings and plants 18-21h at greenhouse temperatures in a dark room. Each treatment was replicated three times. Seedling and plants were moved to natural light greenhouse chambers at 22- 24 2^{o}C, and disease responses were assessed 15 days after inoculation. The severity of

rust disease was assessed as the percentage area of leaves infected during growth periods.

Chemical analysis

Ten days after foliar sprays, three leaves per plant were separately collected, frozen for 36 h, dried and powdered. Generally, 100 mg dried sample were used for analysis.

Screening of stress tolerant bacteria

ACC deaminase positive stress tolerant isolates were tested *in vitro* for their multiple PGP traits. The method of Gordon and Weber [9] was followed for the estimation of indole acetic acid (IAA). Luria Bertani broth (LB) amended with 5-mmol tryptophan was inoculated with overnight raised bacterial cultures (0.5 OD at 600 nm) and incubated at 28o C for 48 h. Absorbance was read at 530 nm. Concentration of the proteins in the pellet was determined[6] and the amount of IAA produced was expressed µg/mg.

Peroxidase and chitinase assays

Total protein was extracted from wheat leaves and the supernatant prepared according to Tuzun*et al*.[24] Peroxidase activity was measured according to the methods described by Allam.[1] The chitinase activity was determined by the colorimetric method of Boller and Mauch.[4.]

Field experiment

Two field experiments were designed in a randomized complete block design with three replications in field at in Sinai (Elqantara) and under natural infection conditions. Soil and water characteristics are mentioned in Table 1. Seeds were soaked for 2 h in a 48 h old bacterial broth (non slime type, experiment 1) or in four or five days old bacterial broth (slime type, experiment 2) and sown. Seeds were soaked in sterile distilled water as a control. The weather is very hot and dry from May to October where temperatures can reach up to 40 °C. On the other hand, the weather is usually warm during winter months and rainfall is rare. 1% of carboxy methyl cellulose (CMC) was added to the culture to increase its viscosity to gel form to act as adhesive biostabilizer, the addition of CMC was made just before using. The experiment was carried out in a randomized block design with three replications. Experimental unit measured 3.0 m in width and 4 m in length. Wheat (Giza 160 cv.) was shown on the 10th of November in each season. The other cultural practices were carried out as recommended for the crop.

Data recorded

Fresh samples of ten guarded plants were taken from each plot, where the different irrigation treatments coincided with each other for recording a set of traits that seemed to be related to drought tolerance assessments. The data will record for:

1. Diseases development was measured during growth periods.
2. Growth parameters and yield /plant. At harvest time, ten fertile stems were taken at random from each plot for measuring plant height, spike length, 1000-kerenl weight was estimated for each plot. Meanwhile, grain and yields were estimated at plot basis.

Statistical analysis

Results are presented as mean ±SD (standard deviation) for three replicates. Data obtained were analyzed statistically to determine the degree of significance between treatments using one, two and three way analysis of variance (ANOVA) at $P \leq 0.001$.

Table 1: Physical and chemical properties of the experimental soil and water irrigation analysis

Locations	Physical properties			Chemical properties										
	Sand	Silt and clay	Soil texture	EC dS/m	ppm	pH	Cations (meq/L)				Anions (meq/L)			
							Ca++	Mg++	Na+	K+	CO3=	HCO3-	Cl-	SO4=
	a) Physical and chemical analysis of the soil													
Elqantara	96.50	3.41	Sandy	1.14	703	7.6	3.50	0.83	2.36	0.14	---	1.4	3.35	1.90
				b) Water irrigation analysis										
Elqantara				5.02	3200	7.5	13.85	4.26	28.31	0.85	---	5.70	36.90	4.66

RESULTS AND DISCUSSION

Data in Figure (1) show the various concentrations of extra-cellular polysaccharides (EPS) production by *Pseudomonas fluorescens*, *Bacillus amyloliquefaciens* and *Bacillus polymyxa*. Maximum production was recorded in *Bacillus polymyxa* forward by *Bacillus amyloliquefaciens*.

Induced Systemic Resistance on Wheat by Bacterial EPS

To evaluate the elicitation of Plant Growth promoting Rhizobacteria by EPS, we conducted experiments with wheat as a model plant against the wheat diseases (Leaf rust caused *by Pucciniarecondita,* f.sp. *tritici* and Powdery mildew caused by *Blumeriagraminis*f. sp. *tritici)*. Pretreatment of three different concentrations of EPS (50, 100 and 200 ppm) as seeds and foliar spray significantly reduced the number of both powdery mildew and rust spots numbers in compared with untreated control. Since, Wheat treated with water control developed spots lesions. Our results showed that even 200 ppm EPS elicited plant resistance against wheat

diseases (Table 2). Among EPS, EPS from *Bacillus polymyxa* was the highest effect forward by *Bacillus amyloliquefaciens* and *Pseudomonas fluorescens*. *Bacillus polymyxa* was effective against *Pucciniarecondite*, mean while, *Bacillus amyloliquefaciens* was effect against *Blumeriagraminis*.

Results showed that strongly increased growth rate of wheat plants as well as resistance to pathogenic organisms (Table 3). Under saline soil, wheat growth was greatly reduced. The same trend was also found that pretreatment of three different concentrations of EPS (50, 100 and 200 ppm) as seeds and foliar spray significantly increased root length, shoot length and plant dry matter. EPS at 200 ppm gave the highest growth under saline soil. EPS from *Bacillus polymyxa* was effective in increased wheat growth.

Chemical analysis

The results of enzymes activities (ACC µM/mg protein/h, IAA, Proline, Peroxidase and Chitinase) and soluble protein in leaves of wheat plants treated with exopolysaccharide (EPS)-producing bacterial strains under greenhouse conditions are summarized in (Table 4). Under saline condition, all enzymes activities were decreased. Also production of proline increased with increase in salt concentration. All EPS were found positive for all enzymes, proline production and soluble protein under both normal and saline soil. Treated wheat with EPS of *Bacilluspolymyxa* was found the most effective to increase all enzymes and soluble protein. The most effective concentration screened by a previous study was determined to be 200 ppm, and we used this concentration for further tests.

Effect of EPS on Wheat diseases control and growth under natural saline soil

We investigated the appropriate method to elicit resistant with promoting plant growth. All treatments exhibit effects on diseases control and plant growth in two experiments (Tables 5 and 6). Pretreatment of slime type of bacteria as seeds and foliar spray significantly reduced the number of both powdery mildew and rust incidence in compared with untreated control (Table 5). *Bacillus polymyxa*was the highest effect forward by *Bacillus amyloliquefaciens* and *Pseudomonas fluorescens*. *Bacillus polymyxa* was effective against *Pucciniarecondite*, meanwhile, *Bacillus amyloliquefaciens* was effect against *Blumeriagraminis* in two experiments. Also, the same trend was also found that pretreatment of three different of slime type of bacteria as seeds and foliar spray significantly increased plant height, spike length, 1000-kerenl weight, grain and yields . *Bacillus polymyxa* gave the highest growth under saline soil in two experiments.

Development of economically feasible and environmentally friendly agricultural technologies able to provide stability of agricultural ecosystems, to promote wide use of biocontrol, and to guarantee improvement of quality, is one of the challenges of modern agriculture. Salinity is one of the most common environmental stress factors that adversely plant growth and crop production in cultivated areas worldwide. Plant productivity in saline soils is considerably reduced dueto nutrient imbalance[19], osmotic stress, and partial closure of stomata. Soil salinity has been reported to reduce plant growth, photo-synthetic capacity, protein synthesis, energy and lipid metabolism, and the total nitrogen contents.[19] Therefore, to improve plant growth under stress conditions, and for sustainable crop production, it is necessary to improve salt stress tolerance in crops. PGPR are able to increase plant tolerance via different mechanisms such as lowering ethylene concentration in plants, producing phytohormones, regulating nutrient uptake, inducing and augmenting stress-response gene expression or the production of antioxidants. Beneficial effects of drought tolerant *Pseudomonas* strains on drought-stressed maize plants were observed at the morphological and physiological level. Interestingly, antioxidant enzyme activities were lower in bacteria-inoculated plants compared to control plants. This shows that the biochemical response of inoculated plants correspond to a less stressed plant[21]. PGPR containing ACC-deaminase can improve maize plant growth under salt stress conditions through better nutrient uptake[18] and exopolysaccharides produced by PGPR allow maize plants to tolerate salt stress by binding Na^+, resulting in a reduced salt uptake by the plant.[3] The bacteria belonging to *Bacillus* spp. are ubiquitous microorganisms, present in the soil and in the phylloplane, and they are also able to live as endophytes. They have been studied for their antagonistic activity and induction of plant resistance against stresses. In the last years, endophyte isolates of *B. subtilis*, able to control several diseases caused by leaf and soil pathogens, have been identified.[2] Many *Bacillus* isolates can promote plant vegetative development by producing several extracellular substances, so acting as PGPMs[2].*Paenibacilluspolymyxa*, a common soil bacterium, belongs to this group. A range of activities has been found to be associated with *P. polymyxa* treatment, some of which might be involved in plant growth promotion.[10,23,12,22] Inoculation by the PGPR *P. polymyxa* can protect *A. thaliana* against a bacterial pathogen and drought stress in a gnotobiotic system. This effect correlates with an increase in the expression not only of genes associated with biotic stress (*PR-1, HEL,ATVSP*) but also of those associated with drought stress (*ERD15, RAB18*).

In conclusion, EPS purified from a rhizobacterium strains, efficiently elicited resistant against wheat diseases at 200 ppm. Seed treatment and foliar sprays would be practical application methods to use in field trials.

The presented results showed the release of bacteria polysaccharides into the culture medium and in the natural environment, is of ecologically importance because they may increase the polysaccharide contents of the water column, growth, reduce their toxicity to aquatic organisms, protect from environment and

improve the significance of the results with the aim of using them as models of the real environment.

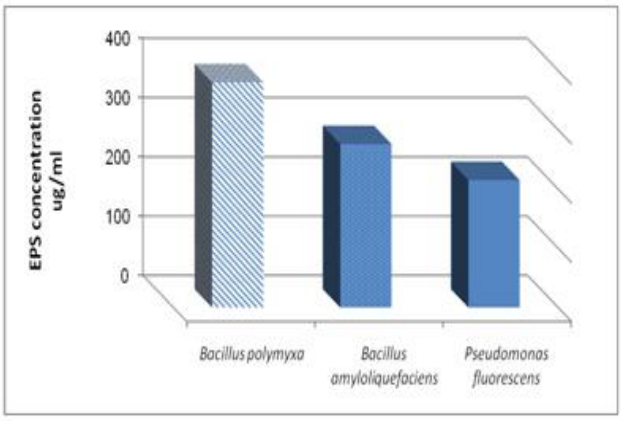

Figure 1: Ex polysaccharide production by Plant Growth promoting Rhizobacteria.

Table 2: Effect of different concentration of Ex polysaccharide production by Plant Growth promoting Rhizobacteria on induced systemic resistance against powdery and leaf rust of wheat under greenhouse conditions.

Treatments	Concentration ppm	Number of lesions per plant	
		Powdery mildew	Leaf rust
Pseudomonas fluorescens	50	20.3	13.5
	100	13.4	7.3
	200	4.5	3.5
Bacillus amyloliquefaciens	50	19.1	12.4
	100	11.3	4.5
	200	3.1	2.1
Bacillus polymyxa	50	17.6	8.5
	100	9.4	6.6
	200	2.2	2.5
Water	0	65.5	43.4
LSD	-	2.1	2.3

Table 3: Effect of different concentration of ex polysaccharide production by Plant Growth promoting Rhizobacteria on wheat growth under greenhouse conditions.

Treatments	Concentration ppm	Wheat growth parameters					
		Root length		Shoot length		Plant dry matter	
		Normal soil	Saline soil	Normal soil	Saline soil	Normal soil	Saline soil
Pseudomonas fluorescens	50	13.4	12.7	47.7	44.7	49.3	48.7
	100	17.5	15.7	49.8	45.7	51.3	50.5
	200	19.4	18.8	51.9	49.7	55.6	52.4
Bacillus amyloliquefaciens	50	13.5	11.9	47.7	44.6	50.6	48.5
	100	14.2	12.8	48.6	47.8	53.5	40.7
	200	14.9	13.8	50.7	50.0	54.6	51.4
Bacillus polymyxa	50	13.5	12.8	48.7	44.6	50.5	43.4
	100	14.6	13.3	50.3	47.6	56.4	51.4
	200	19.6	17.5	55.6	51.8	65.8	60.5
Water	0	9.7	5.4	36.3	20.5	37.8	19.8
LSD		1.6	2.5	3.2	3.6	2.5	3.4

Table 4: Enzymes activities and Soluble protein in leaves of wheat plants treated with exopolysaccharide (EPS)-producing Plant Growth

Treatments	Concentration ppm	Enzymes activities										Soluble Protein	
		ACC µM/mg protein/h		IAA		Proline µg mg¡1 protein		Peroxidase (unit)		Chitinase (unit)			
		Non	Saline	Non	Saline	Non	Saline	Non	Saline	Non	Saline	Non	Saline
Pseudomonas fluorescens	50	4.5	3.7	109.8	98.7	5.8	8.6	12.8	11.6	5.1	5.0	27.8	24.6
	100	4.6	4.0	116.6	108.8	7.6	9.7	14.8	12.6	6.4	6.0	31.9	27.6
	200	5.2	4.6	124.5	117.7	8.5	10.8	16.8	15.7	8.1	7.2	33.8	29.7
Bacillus amyloliquefaciens	50	4.3	3.5	106.8	96.7	7.3	8.0	13.7	12.4	5.7	5.0	21.9	19.7
	100	4.6	4.1	107.4	101.5	8.6	9.0	14.8	12.5	6.3	5.5	26.9	24.7
	200	4.4	4.2	121.4	116.8	9.4	10.1	18.3	17.5	7.5	6.3	30.8	27.6
Bacillus polymyxa	50	4.6	4.0	108.8	98.7	7.4	8.6	11.7	10.7	7.1	6.0	27.5	25.6
	100	4.5	4.2	111.3	106.7	8.5	9.7	15.8	14.1	7.3	6.4	31.5	27.5
	200	5.1	4.6	139.7	129.7	9.4	11.4	19.7	17.6	8.7	7.5	35.4	30.1
Water	0	4.0	-	99.6	-	3.1	-	11.7	-	3.2	-	12.4	-
Saline Water		-	1.5	-	44.6	-	4.3	-	4.8	-	1.4	-	7.5

Promoting Rhizobacteria under greenhouse conditions.

Table 5: Efficiency of bacteria EPS against the leaf rust and powdery mildew of wheat grown under natural saline soil

Treatments	% Diseases control											
	Leaf rust				Powdery mildew				Complex of leaf spots			
	Slime type		Non slime type		Slime type		Non slime type		Slime type		Non slime type	
	Exp. I	Exp.II	Exp. I	Exp.II	Exp. I	Exp.II	Exp. I	Exp.II	Exp. I	Exp.II	Exp. I	Exp.II
Pseudomonas fluorescens	2.4	3.3	6.5	6.8	3.3	3.1	7.5	6.4	3.3	2.4	6.7	6.4
Bacillus amyloliquefaciens	1.1	2.0	7.5	8.1	2.4	2.5	5.4	6.3	2.3	2.6	6.7	5.3
Bacillus polymyxa	2.2	1.6	6.4	7.3	2.3	2.4	5.7	5.7	2.3	1.6	5.4	4.7
Water	32.4	35.3	32.4	36.4	44.9	6.44	44.9	43.5	21.3	20.8	21.3	23.5
LSD	1.3	1.4	1.4	1.3	1.4	1.3	1.5	1.5	1.4	1.3	1.5	1.3

Table 6: Efficiency of bacteria EPS on wheat growth and yield under saline soil

Treatments	Plant growth															
	Plant height (cm)				Spike length (cm)				1000- kernel weight (g)				Grain yield (t/ha)			
	Slime type		Non slime type		Slime type		Non slime type		Slime type		Non slime type		Slime type		Non slime type	
	Exp. I	Exp.II	Exp. I	Exp.II	Exp. I	Exp.II	Exp. I	Exp.II	Exp. I	Exp.II	Exp. I	Exp.II	Exp. I	Exp.II	Exp. I	Exp.II
Pseudomonas fluorescens	105.4	112.1	98.6	99.4	12.5	13.4	10.2	10.6	41.3	43.2	36.2	37.6	3.978	4.24	3.01	3.12
Bacillus amyloliquefaciens	106.4	108.4	97.4	101.4	13.4	14.2	9.6	9.7	45.3	41.8	36.7	38.4	4.043	3.98	3.04	3.23
Bacillus polymyxa	110.5	121.2	102.3	103.4	14.5	16.4	11.3	10.8	46.5	47.3	40.4	41.3	4.542	4.34	3.13	3.54
Water	90.5	93.4	90.5	93.4	8.65	7.87	8.65	7.87	25.5	22.4	25.5	22.4	2.545	2.43	2.54	2.43
LSD	3.4	3.5	2.6	2.8	1.4	1.7	1.3	1.5	2.3	2.4	2.1	2.0	0.5	1.0	0.6	0.7

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