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Improving Productivity of Some Bread Wheat Cultivars under Water Deficit Stress Using Endophytic *Bacillus* sp. NGB-WhE3

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PLANT growth-promoting endophytic bacteria (PGPEB) are beneficial microbes that can be applied to improve plant responses to drought stress. In this study, seven bacterial isolates were purified from the root-endosphere of field-grown wheat (*Triticum aestivum* L.) in Egypt. Bacterial isolates were identified and *in-vitro* screened for plant growth-promoting (PGP) traits and water deficit stress alleviation. Then, the effect of bacterial inoculation on growth promotion and performance of wheat was investigated under full irrigation and water deficit stress in greenhouse and field experiments. Based on 16S rRNA gene sequences, three isolates were classified as *Bacillus* sp., whereas, four isolates were identified as *Enterobacter*, *Paenibacillus*, *Pseudomonas*, and *Sphingomonas* sp. All isolates produced indole acetic acid, solubilized inorganic phosphate, secreted siderophore, and exhibited tolerance to osmotic stress. Under greenhouse conditions, growth, shoot-N content and leaf proline content of wheat plants inoculated with most bacterial isolates were significantly ($P< 0.05$) increased under both normal irrigation and water deficit stress (50% water holding capacity). Based on greenhouse results, *Bacillus* sp. strain NGB-WhE3 was evaluated to alleviate water deficit stress on five wheat cultivars (Giza 171, Misr 1, Misr 3, Shandaweel 1, and Sids 14) in two-year field trials. Bacterial inoculation significantly ($P< 0.05$) improved the agronomic traits and some physiological characteristics of wheat plants under water deficit stress than the uninoculated control. Grain yield of inoculated wheat plants showed significant ($P< 0.05$) increases from 5.6 to 14.6% under normal irrigation and from 3.2 to 12.5% under water deficit stress.

Keywords: Endophytes, Inoculation, Plant growth promotion, Water deficit, Wheat cultivars.

Introduction

Wheat (*Triticum aestivum* L.) is the world's most important grain crop and is a stable source of food for ~ 40% of the world's population (Giraldo et al., 2019). It contains 80-85% carbohydrates, 10-15% protein, fiber, vitamins, minerals, and phytochemicals (Shewry & Hey, 2015). Currently, wheat is the most widely grown crop worldwide, cultivated on > 216 million ha, producing 766 million tons per year (FAO, 2021). In Egypt, the total area cultivated with wheat is 1.4 million ha, with a yield of 9 million tons (FAO, 2021). However, there is still a big gap, (~50%) between production and consumption (Abdelmageed et al., 2019). Egyptian land available for new agricultural expansion projects is generally categorized as semiarid land. Under these semiarid conditions, the main factor limiting wheat production and its nutritive value is water deficit "commonly known as drought" (Eissa et al., 2018). Globally, drought reduces cereal production by 9- 10%, via deleterious effects on plant growth, nutrition, physiology, and yield (Fahad et al., 2017; Zhang et al., 2018). The adverse effects of drought are expected to increase due to the warming climate (Haile et al., 2020).

Worldwide extensive research is being conducted to reduce drought effects on plants,

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including the development of drought-tolerant varieties, resource management practices, or genetic engineering approaches, but most of these technologies are cost-intensive. Recent studies indicate that beneficial microorganisms, particularly plant growth-promoting (PGP) endophytic bacteria (PGPEB), can also help plants cope with drought stress (Ullah et al., 2019). Generally, endophytic microbes are microorganisms inhabiting plants without causing any apparent harm to the host plant (Lata et al., 2018). PGPEB receive considerable attention and may have a preference advantage over rhizospheric bacteria in supporting plant health and growth (Oukala et al., 2021) because they have more intimate contact with their host plants (Santoyo et al., 2016). They can increase tolerance to abiotic stresses by providing the plant with several direct and indirect PGP benefits (Fadiji & Babalola, 2020; Farahat et al., 2020). Events include enhancing root growth, antioxidants, and relative water content (Vurukonda et al., 2016; Ullah et al., 2019). In addition, endophytic bacteria also release several plant growth regulating and drought-resistant substances such as phytohormones, abscisic acid, gibberellins, exopolysaccharides, and 1-aminocyclopropane-1-carboxylate deaminase (Egamberdieva et al., 2017). Numerous studies have demonstrated the positive effects of PGPEB inoculation in alleviating deleterious effects of drought stress on the growth and yield of different crops, including wheat (Naveed et al., 2014; Chen et al., 2017), soybean (Dubey et al., 2021), rice (Saddique et al., 2018), maize (Sandhya et al., 2017) and others (Ullah et al., 2019). However, the efficiency of bacterial inoculation is highly dependent on plant genotypes of the same species. For example, the same bacterial strain can have different growth effects on the performance and vigor of a plant, depending on the plant's genotype (Kazi et al., 2016; Schlemper et al., 2018).

Considering the importance of endophytic bacteria in improving wheat tolerance to water deficit stress, this study aimed to isolate endophytic bacteria associated with the roots of wheat grown under water deficit conditions. The bacterial isolates were genetically identified using 16S rDNA sequencing and their capacities to promote plant growth were tested *in vitro*. Bacterial isolates were also screened for their growth-promoting activity in wheat plants grown under drought stress in the greenhouse. This study hypothesized that PGPEB would trigger different growth plant responses in different wheat cultivars. To test this hypothesis, two-year field experiments were established to evaluate the potential effect of the highest efficient PGP bacterium in enhancing the growth and yield of five wheat bread cultivars under sandy soil conditions.

Materials and Methods

Bacterial isolation

Bacterial endophytes were isolated from fresh roots of wheat plants (cv. Misr 1) grown in Mallawi, Minya Governorate (27°43'12.0"N 30°43'12.0"E) as described by Youseif (2018). Briefly, root samples were washed with running tap, then were surface-sterilized using 70% ethanol for 1min, and finally rinsed 6 times with sterile distilled water. The roots were then immersed for 10min in 3% sodium hypochlorite solution (NaClO), then washed 6 times with sterile distilled water. To confirm the efficiency of sterilization protocol, an aliquot (100µL) from the sixth wash solution was streaked on a nutrient agar medium composed of (g/L): peptone, 5; beef extract, 3; sodium chloride, 5 and agar, 18 (pH 7). The surface-sterilized roots were aseptically ground in a mortar with a pestle, and 100µL aliquots were plated on a nutrient agar medium and incubated at 28°C for 5 days.

DNA isolation and molecular characterization

Total genomic DNA of bacterial cells was extracted using GeneJet™ Genomic DNA Purification Kit (Thermo Scientific®, Massachusetts, USA). The procedures were done as described in the manufacturer's instructions. Bacterial 16S rDNA was amplified using 27F/1492R primers (Lane, 1991; Turner et al., 1999). Polymerase chain reaction (PCR) was carried out in T100 Thermal Cycler (Bio-Rad, California, USA) using the standard reaction (25µL) containing: 1× PCR buffer, 200mM of each dNTPs, 15 pmol of each primer, 1 unit Taq polymerase enzyme (Promega® Corporation, Madison, USA), 1.5mM $MgCl_2$ and 50 ng DNA template. Thermal cycling conditions were as follows: initial denaturation at 94°C for 5min; 30 cycles of 94°C for 1min, 55°C for 1min, and 72°C for 1min; and final elongation at 72°C for 10min. 16S rRNA PCR products were purified using the QIAquick PCR purification kit (Qiagen, Germany) and sequenced at Macrogen Inc., South Korea.

Phylogenetic analysis

Sequence reads were assembled using

DNASTAR software (Lasergene, Madison, USA). The taxonomical identification of bacterial isolates was made to the genus level by blasting partial 16S rRNA gene sequences at EzBioCloud (http://eztaxon-e.ezbiocloud.net) databases. The obtained sequences were aligned using Clustal W version 1.8 (Altschul et al., 1997). 16S rRNA phylogenetic tree was generated using the Maximum likelihood (ML) algorithm in the MEGA X software (Kumar et al., 2018) using the Tamura-Nei model (Tamura & Nei, 1993). Bootstrap (BT) support for each node was evaluated with 1000 replicates. The percentage of average nucleotide identity (ANI) between tested isolated and closely related reference strains was calculated using the MEGA X software.

In-vitro screening of plant growth-promoting activities

A quantitative analysis of P-solubilization activity was performed using the molybdate blue color method (Watanabe & Olsen, 1965). Briefly, bacterial isolates were inoculated in a 25mL Pikovskaya broth medium (Pikovskaya, 1948) then incubated for 7 days at 28°C. Bacterial cultures were centrifuged and 1 ml supernatant was mixed with 10mL chloromolibidic acid then the volume was made up to 45mL with distilled water. A 0.25mL of cholorostannous acid was added, and the volume was brought up to 50mL with sterilized distilled water. The absorbance of the resulting blue color was measured at 600nm by a spectrophotometer (Evolution 100, Thermo Scientific®, Massachusetts, USA). The amount of solubilized phosphate was detected using a standard curve produced with dilutions of a KH_2PO_4 solution (Sigma-Aldrich®, St. Louis, USA).

Indole acetic acid (IAA) production by bacterial isolates was determined as described by Rahman et al. (2010). Bacterial isolates were inoculated into LB medium composed of (g/L): tryptone, 10; yeast extract, 5 and sodium chloride, 5 (pH 7). L-tryptophan was added at the rate of 0.5mg/mL. Bacterial cultures were incubated at 28 °C with continuous shaking at 150rpm for 48h. Bacterial cultures (2mL) was centrifuged at 15,000 rpm for 1min, then the supernatant (1mL) was mixed with 2mL Salkowski's reagent (150mL concentrated perchloric acid, 7.5mL of 0.5M iron (III) chloride hexahydrate, and 250mL distilled water) and incubated for 20min at room temperature in the dark. IAA production was determined by the development of a pink-red color, and absorbance was detected at 530nm using a spectrophotometer. The IAA concentration was calculated using a standard curve prepared from pure IAA solutions (Sigma-Aldrich®, St. Louis, USA).

Bacterial isolates were quantitatively assayed for siderophore production using the modified microplate method as described by Arora & Verma (2017) by a microplate reader (Infinite 200 Pro, Life Sciences/Tecan, Mannedorf, Switzerland), in which a 0.1mL culture supernatant was mixed with 0.1mL Chrome Azurol S (CAS) reagent (Acros Organics®, Belgium). Absorbance was detected at 630nm against a reference consisting of 0.1mL uninoculated broth and 0.1mL CAS reagent. Siderophore production was measured as the percent siderophore unit (psu) according to the following equation (Payne, 1994):

Siderophore production unit % (psu)= Ar - As/Ar × 100

where, Ar= Absorbance of the reference (CAS solution and uninoculated broth) and As= Absorbance of the sample (CAS solution and cell-free supernatant of the sample).

Bacterial growth under drought stress

For evaluation of the growth of isolated endophytic bacteria under drought stress, nutrient broth medium of different osmotic potentials (−0.05, −0.30, −0.73, -1.03, and −1.37 MPa) was prepared by adding polyethylene glycol 6000 (PEG-6000) according to the equation supplied by Mcclendon (1981). Bacterial cultures were inoculated into nutrient broth media with different osmotic potentials. Each treatment was done in six replicates. After incubation at 28°C for 24h with shaking at 150rpm, growth was determined by measuring the optical density at 600nm using spectrophotometry.

Greenhouse experiment

A pot experiment was carried out in the controlled greenhouse of the National Gene Bank, Agricultural Research Center (Giza, Egypt) to study the effect of bacterial inoculations on different growth parameters of wheat plants under water deficit stress. Seeds of wheat (cv. Misr 1) were surface sterilized according to (Turan et al., 2012). Each pot (13cm diameter) was filled with 2.5kg sandy soil collected from the Ismailia Agricultural Research Station. The physicochemical characteristics of the soil were analyzed as described in (Page & Keeney, 1982) and are presented in Table 1. Ten wheat seeds were cultivated in each pot and inoculated with 5mL bacterial culture (10^9 Colony-forming units/mL) at planting. After 10 days, the plants were thinned to six plants per pot. The greenhouse experiment was carried out in a complete randomized block design of eight treatments (seven bacterial isolates and uninoculated control) and two irrigation regimes: well-watered and severe water deficit, corresponding to 100 and 50% of water holding capacity (WHC), with four replicates. Water deficit stress was applied after seedling establishment (i.e. 15 days after seedling emergence), before that time, all pots were maintained uniformly at 100% WHC. All treatments received the recommended dose of N, P, and K-fertilizers as the follows superphosphate (12.5% P_2O_5) and potassium sulfate (48.5% K_2O) at a rate of 0.5 g/pot (480kg/ha) and 0.25 g/pot (240kg/ha), respectively (Abd El-Megeed & Youseif 2018). All treatments received the recommended N dose of ammonium sulfate (20.5% N) at a rate of 0.73g/pot (144 kgN/ha). Plants were grown in a controlled greenhouse at 12°C/24°C (night/day), with a relative humidity of 50–60%, and a 10/24h photoperiod. After 50 days of cultivation, plants were uprooted, and the dry weight of shoots and roots was measured. The N-uptake in shoots was determined using Kjeldahl methodology (Kirk, 1950), whereas the proline content in the leaf was assayed as described previously (Bates et al., 1973).

Field experiment

Field experiments were conducted in the new reclaimed sandy soil at Al Gharirah, Esna, Luxor Governorate (lat. 25.482485, long. 32.448397) for two successive winter growing seasons, 2019–2020 and 2020–2021. Physicochemical characteristics of the soil were analyzed as described in Page & Keeney (1982) and are presented in Table 1. Five cultivars of bread wheat (Giza 171, Misr 1, Misr 3, Shandaweel 1, and Sids 14) were used in this study due to their tolerance to abiotic stresses (heat and drought). The seeds were sown using the drill method at a seeding rate of 240 kg seeds/ha. A total of 30 same size plots (3 x 3.5m= 10.5m^2) were prepared and randomly divided into five triplicate treatments (T) (5 × 3= 15), applied at two levels of irrigation (Irr), following a split-plot arrangement in randomized complete block design, where the main plot was allocated to cultivars and inoculations were represented in the split plots.

TABLE 1. Physical and chemical properties of different soils used in this study

Property	Value		
	Greenhouse experiment	Field experiments	
		2019/2020	2020/2021
pH	7.66	7.97	8.00
EC (dS m^{-1} at 25ºC)	0.54	0.34	0.31
Texture grade	Sandy	Sandy	Sandy
$CaCo_3$ (%)	2.20	6.20	7.80
Saturation percent (%)	18.60	21.80	23.0
Total N (%)	0.016	0.014	0.017
Total Soluble-N (ppm)	15.60	12.50	11.30
Available-P (ppm)	3.72	3.20	2.50
Available-K (ppm)	144.5	102.4	65.0
Organic matter (%)	0.30	0.21	0.11
Soluble cations (meq/L)			
Ca^{+2}	1.73	0.87	0.65
Mg^{+2}	1.10	0.56	0.45
Na^+	1.95	1.45	1.61
K^+	0.67	0.49	0.41
Soluble anions (meq/L)			
CO_3^{-2}	0.00	0.00	0.00
HCO_3^-	1.64	0.71	0.70
Cl^-	2.32	1.89	1.65
SO_4^{-2}	1.49	0.77	0.77
DTPA extractable (ppm)			
Fe	1.14	1.10	1.00
Mn	0.72	0.56	0.51
Zn	0.88	0.29	0.21
Cu	0.06	0.03	0.02

Treatments were irrigated every 6 days (full irrigation) or 12 days (water deficit stress). Based on the greenhouse experiment, *Bacillus* sp. strain NGB-WhE3 was prepared as an inoculant for use in the field trial as described previously (Youseif et al., 2014). At the time of sowing, wheat seeds were inoculated at a rate of 10 g vermiculite/peat inoculant/kg seeds, using a gum Arabic solution (16%) as an adhesive agent to coat the seeds

(Youseif et al., 2021). All treatments received the recommended dose of chemical fertilizers as mentioned previously in the greenhouse experiment. At the tillering stage, proline content in the leaf was measured. At the end of the growing season, plants were harvested to estimate the overall yield and yield components of wheat plants.

Statistical analysis

Data were statistically analyzed using MSTAT analysis software (Snedecor & Cochran, 1980). Data means were analyzed by analysis of variance (ANOVA). The least significant difference (LSD) values were used to compare treatment means ($P< 0.05$).

Results

Bacterial isolation and in-vitro *characterization for PGP traits*

Seven bacterial isolates were purified and obtained from surface-sterilized roots of wheat plants collected from Mallawi, Minya Governorate (Table 2). All seven isolates were characterized for their activity to produce PGP traits (Table 2). Bacterial isolates were able to solubilize phosphate with the range of 47.0 to 108.5 µg/ml. Isolates NGB-WhE4 and NGB-WhE5 showed the highest phosphate solubilization activities with 108.5 ± 8.96µg/mL and 103.1 ± 8 .87µg/mL, respectively. All bacterial isolates produced IAA in the presence or absence of L-tryptophan induction. However, in the absence of L-tryptophan precursor, the detected amount of IAA was low. In the presence of tryptophan, bacterial isolates produced IAA ranging between 72.2 and 140.3µg/mL. In the absence of tryptophan, the isolated bacteria could produce IAA from 13.7 to 37.9µg/mL. The highest amount of IAA under both conditions was recorded by the root endophytic bacterium NGB-WhE3. Based on the CAS-blue assay, all tested isolates produced siderophores ranging from 18.63 to 58.30% of siderophore units. The maximum amount of siderophore units was produced by isolate NGB-WhE7 (58.3 ± 5.57%) followed by isolate NGB-WhE4 (51.4 ± 5.37%).

Molecular characterization of endophytic bacteria

Nearly full-length 16S rRNA gene products (1500bp) were amplified and sequenced from endophytic bacterial isolates under this study. The sequences of the 16S rRNA gene were blasted to the EZBioCloud database (Table 2). The sequences of 16S rRNA gene from isolates NGB-WhE1 and NGB-WhE3 shared 99.34 and 99.17% similarity, respectively, with *Bacillus halotolerans* ATCC 25096^T. Isolate NGB-WhE2 showed 98.56% 16S rRNA sequence similarity with *Sphingomonas trueperi* LMG 2142^T. Isolate NGB-WhE4 had 99.52% similarity with *B. tequilensis* KCTC 13622^T. Isolate NGB-WhE5 exhibited 99.62% 16S rRNA sequence identity with *Enterobacter cloacae* ATCC 13047^T. Isolates NGB-WhE6 and NGB-WhE7 shared 99.59 and 99.77% 16S rRNA sequence similarity with *Paenibacillus graminis* DSM 15220^T and *Pseudomonas rhodesiae* CIP 104664^T, respectively.

According to the ML-phylogenetic tree based on 16S rRNA sequences, bacterial isolates were closely affiliated to five genera corresponding to two phyla; Proteobacteria (Alpha- and Gammaproteobacteria classes) and Firmicutes and were grouped in five distinct clusters (Fig. 1). Due to the low phylogenetic power at the species level, the newly isolated bacteria in this study were assigned only to the genus level. Isolate NGB-WhE7 was identified as *Pseudomonas* sp. and tightly grouped with type strains of *P. fluorescence*, *P. grimontii*, and *P. rhodesiae s*upported by 100% BT value and 99.7% ANI. Isolate NGB-WhE5 was assigned to *Enterobacter* sp. and grouped with the two subspecies of *E. cloacae*; sub. *dissolvens* and sub. *cloacae* (100% BT, 99.6% ANI). Isolate NGB-WhE2 was assigned as *Sphingomonas* sp. and closely related to *S. azotifigens* NBRC 15497^T and *S. trueperi* LMG 2142^T (94.0% BT, 98.6% ANI). Isolates NGB-WhE1, WhE3 and WhE5 were classified as *Bacillus* sp. and grouped with the type strains of *B. halotolerance*, *B. rugosus* and *B. tequilensis* (99.4% ANI). Isolate NGB-WhE6 was identified as *Paenibacillus* sp. and showed a high resemblance to DSM 15220^T, the type strain of *P. graminis* supported by 99.0% BT.

Tolerance of bacterial growth to drought stress

The isolated endophytic bacteria were screened for drought tolerance by analyzing their ability to grow in varying levels of PEG 6000 as a dehydrating agent. All strains were sensitive to the matric stress caused by PEG 6000 in the medium (- 0.05 to -1.37 MPa) as the optical density strongly declined as stress was increased (Fig. 2). *Bacillus* sp. NGB-WhE3 and NGB-WhE4 exhibited the maximum tolerance to the high level of osmotic stress (-1.37 MPa) as the growth was more vigorous and reached higher cell density compared to other tested strains.

TABLE 2. Taxonomic affiliation of root-endophytic bacteria isolated from wheat plants and their plant growth-promoting activities

Bacterial strain	Identity based on 16S rRNA gene sequence using EZTaxon blast					IAA production (µg/mL)		Phosphate solubilization (µg/mL)	Siderophores production (psu%)
	Length (bp)	NCBI Access.	Closest species	Accession No.	Identity (%)	With tryptophan	Without tryptophane		
NGB-WhE1	1221	LC639743	*Bacillus halotolerans* ATCC 25096^T	LPVF01000003	99.34	72.2±2.74	26.1±3.61	52.9±4.38	23.9±2.20
NGB-WhE2	1254	LC639744	*Sphingomonas trueperi* LMG 2142^T	X97776	98.56	109.4±7.61	32.9±2.86	47.0±3.26	18.63±1.94
NGB-WhE3	1331	LC639745	*Bacillus halotolerans* ATCC 25096^T	LPVF01000003	99.17	140.3±6.56	37.9±4.17	73.4±4.12	34.4±4.58
NGB-WhE4	1265	LC639746	*Bacillus tequilensis* KCTC 13622^T	AYTO01000043	99.52	88.8±6.64	18.9±2.90	108.5±8.96	51.4±5.37
NGB-WhE5	1328	LC639747	*Enterobacter cloacae* ATCC 13047^T	CP001918	99.62	106.1±4.5	30.9±5.44	103.1±8.87	34.9±6.48
NGB-WhE6	1213	LC639748	*Paenibacillus graminis* DSM 15220^T	CP009287	99.59	92.3±4.69	24.7±1.77	80.4±5.72	22.1±3.41
NGB-WhE7	1306	LC639749	*Pseudomonas rhodesiae* CIP 104664^T	AF064459	99.77	117.1±9.15	13.7±3.46	98.1±6.48	58.3±5.57

(IAA) indole acetic acid. IAA was tested with (500µg/mL) and without the addition of precursor. Data are average values of three replicates

Pot experiment under water deficit stress

A significant difference (P< 0.05) was detected in root and shoot dry weight of wheat plants inoculated with bacterial strains compared to control in both fully-irrigated and drought-affected plants (Fig. 3). During full irrigation, bacterial inoculation gave significant increases (P< 0.05) in root (14.7 - 28.1%) and shoot) 15.6 - 50.0%) dry weight compared to the uninoculated control. Under water deficit stress, bacterial inoculation significantly (P< 0.05) increased root (16.5 - 48.5%) and shoot (20.6 - 56.7%) dry weight compared to the uninoculated control. Similarly, the shoot N-content was significantly (P< 0.05) improved due to inoculation in both irrigation (2.5-21.6%) and water deficit-stressed conditions (6.1-25.4%) compared to the uninoculated control (Fig. 4). Under full irrigation, there was no significant difference in the leaf proline content in most inoculated treatments compared to the uninoculated plants (Fig. 4). However, under water deficit stress, all inoculated plants accumulated leaf proline content (11.5 - 30.6%) significantly (P< 0.05) greater than the uninoculated plants (Fig. 4). It is noteworthy to mention that *Bacillus* sp. NGB-WhE3 recorded the highest shoot dry weight, shoot N-content, and leaf proline content under both irrigated and water deficit-stressed conditions. Consequently, it was selected for further evaluation with various wheat cultivars under field conditions.

Field experiments under water deficit stress

ANOVA showed that inoculation, irrigation, and the cultivar significantly (P< 0.05) affected all studied parameters (Table 3). The effect of bacterial inoculation on grain yield and yield components varied due to different wheat cultivars and irrigation regimes (Tables 4 and 5).

The number of spikes per square meter was significantly (P< 0.05) improved under well-irrigation in all inoculated cultivars (9.4 - 17.3%) than uninoculated plants (Table 4). Similarly, under water deficit-stressed conditions, there were significant (P< 0.05) increases (10.9-13.0%) in the number of spikes per square meter compared to uninoculated plants. The number of kernels per spike was also significantly enhanced in inoculated plants (7.0-10.0%) compared to uninoculated control under well irrigation conditions (Table 4). However, this effect was greatly observed (11.8-28.6%) under water deficit stress. A similar trend was detected for the 1000-kernel weight parameter, in which significant (P< 0.05) increases were recorded in inoculated plants under both normal (7.6 - 12.6%) and stress (6.8 - 17.2%) conditions higher than the uninoculated plants (Table 4).

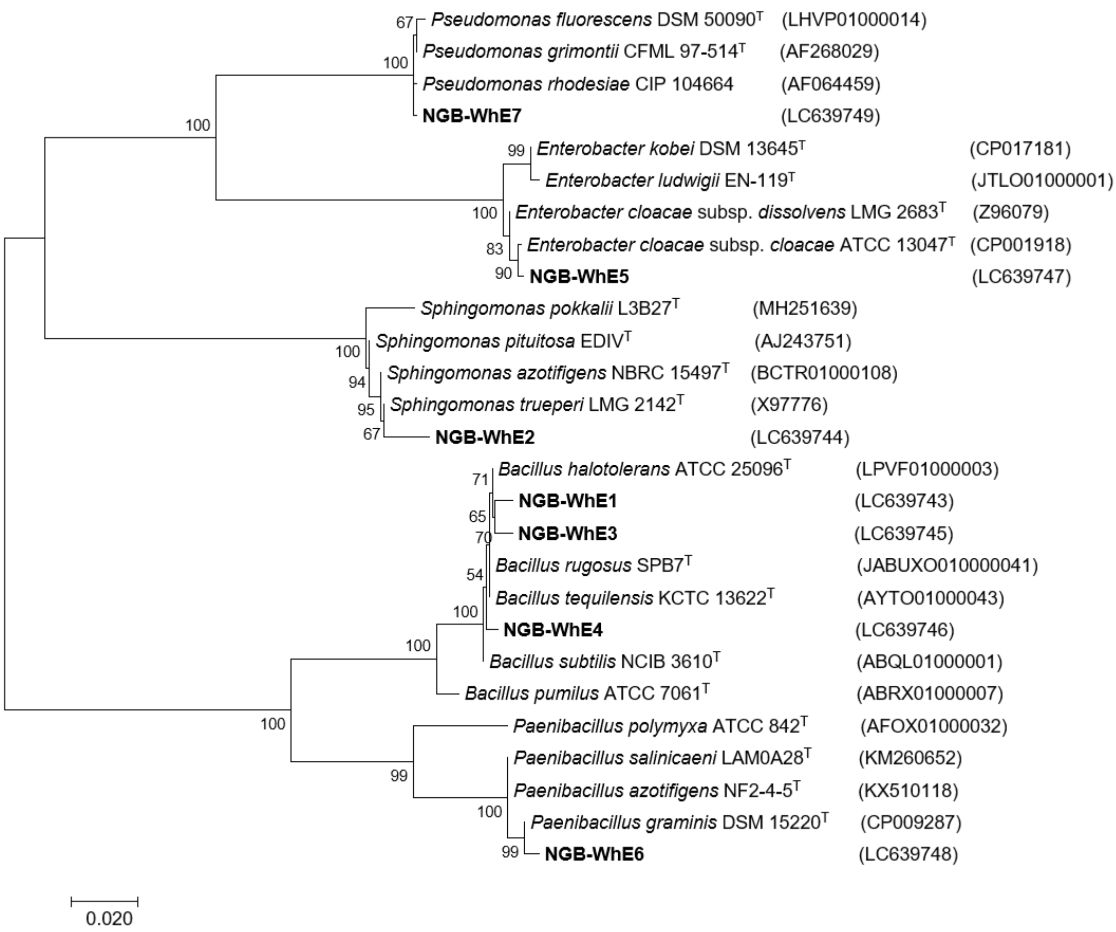

Fig. 1. The phylogenetic relationships between PGPEB isolated in this study (in bold) and closely related reference strains based on 16S rRNA gene sequences. NCBI accession numbers are in parentheses [Bootstrap values are indicated for each node (1000 replicates)]

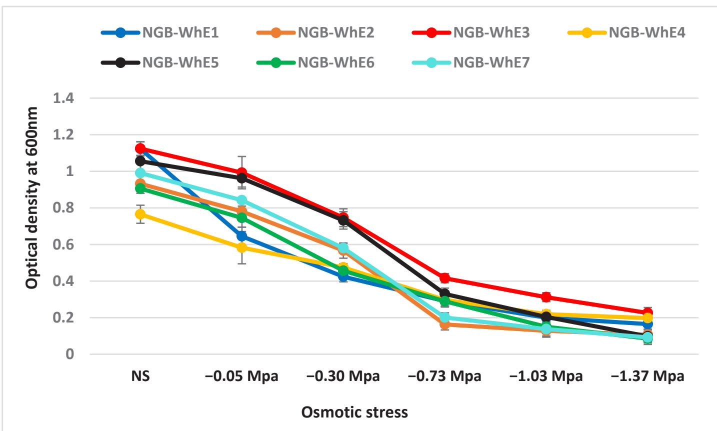

Fig. 2. Growth patterns of the isolated endophytic bacteria under non-stressed (NS) and water deficit-stressed conditions of different osmotic potential

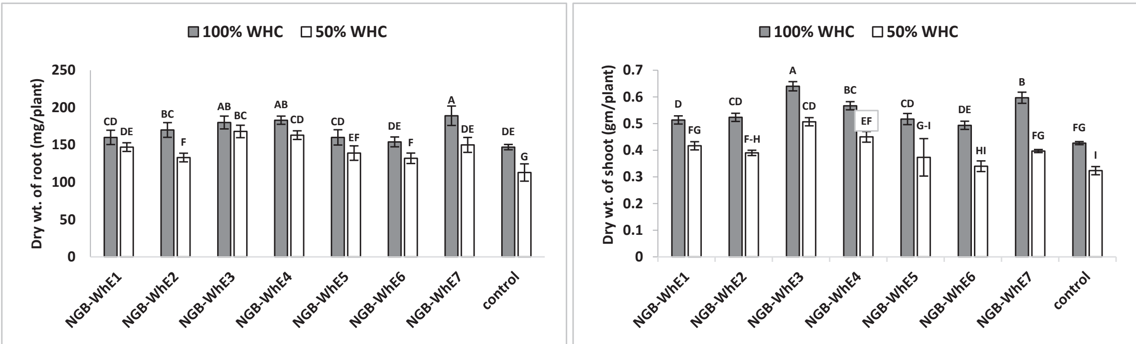

Fig. 3. Effect of PGPEB strains on root and shoot dry weight of wheat plants (Misr 1) cultivated under full irrigation (100% WHC) and water deficit stressed-conditions (50% WHC)

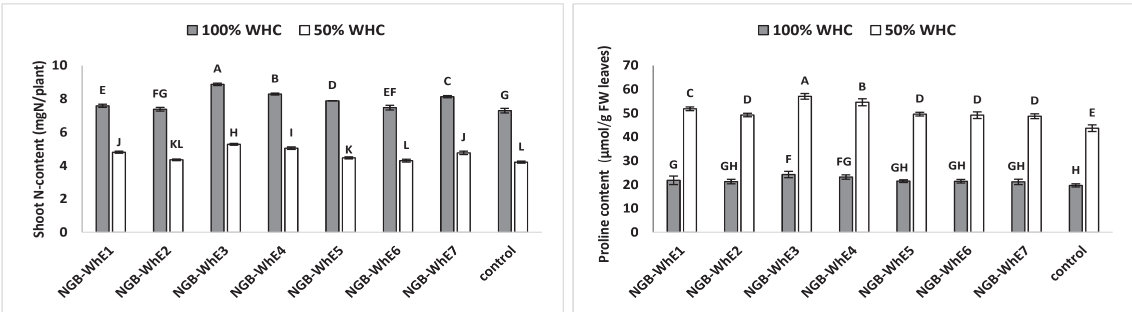

Fig. 4. Effect of PGPEB strains on shoot N-content and leaf proline content of wheat plants (Misr 1) cultivated under full irrigation (100% WHC) and water deficit stressed-conditions (50% WHC)

TABLE 3. Mean squares of the combined analysis of variance for the studied characters as affected by irrigation, bacterial inoculation and wheat cultivars of the two growing seasons (2019/2020 and 2020/2021)

S.O.V	d.f	No. Spikes/m^2	No. Kernels/ spike	1000- kernel weight	Grain yield	Proline content
Seasons (S)	1	30083**	525**	183**	8.103**	1481**
Error	4	2492	18.9	6.99	0.273	6.10
Irrigation (Irr)	1	23631**	3910**	1125**	17.13**	10137**
(S x I)	1	294	57.4	12.9	0.277	190**
Error	4	1269	20.5	1.75	0.357	5.01
Inoculation (I)	1	37171**	468**	372**	8.67**	1502**
(S x I)	1	213	10.2	5.20	0.012	20.5*
(Irr x I)	1	76.8	1.878	2.95	0.001	826**
(S x Irr x I)	1	43.2	5.20	5.42	0.003	6.15
Error	8	2110	15.3	11.0	0.122	3.42
Cultivars (C)	4	7718**	113**	62.0**	1.11**	447**
(S x C)	4	122	12.6	3.92	0.050	8.54
(Irr x C)	4	43.3	44.9*	3.40	0.287	34.3**
(S x Irr x C)	4	113	5.30	0.742	0.018	3.76
(I x C)	4	201	3.01	2.585	0.017	21.4**
(S x I x C)	4	262	2.85	0.715	0.028	2.01
(Irr x I x C)	4	90	0.271	0.957	0.044	4.97
(S x Irr x I x C)	4	216	1.73	0.536	0.003	0.627
Error	64	579	14.6	11.13	0.270	5.53

TABLE 4. Average of grain yield components as affected by irrigation, bacterial inoculation, and wheat cultivars over the two growing seasons (2019/2020 and 2020/2021)

Cultivar	Full irrigation			50% irrigation		
	Number of spikes/m²					
	Inoculated	Non-inoculated	Increase (%)	Inoculated	Non-inoculated	Increase (%)
Giza 171	363	327	11.0	335	298	12.4
Misr 1	353	301	17.3	322	285	13.0
Misr 3	313	286	9.4	285	254	12.2
Shandaweel 1	329	293	12.3	295	266	10.9
Sids 14	342	309	10.7	315	280	12.5
Mean	340	303	12.2	310	277	11.9
L.S.D 0.05	25	27	---	23	30	---
	Number of kernels/spike					
	Inoculated	Non-inoculated	Increase (%)	Inoculated	Non-inoculated	Increase (%)
Giza 171	52	48	8.3	42	35	20.0
Misr 1	49	45	8.9	38	34	11.8
Misr 3	44	40	10.0	36	28	28.6
Shandaweel 1	45	41	9.8	32	27	18.5
Sids 14	46	43	7.0	36	32	12.5
Mean	47	43	9.3	37	31	19.4
L.S.D 0.05	4.3	5.8	---	4.7	5.3	---
	1000-kernel weight					
	Inoculated	Non-inoculated	Increase (%)	Inoculated	Non-inoculated	Increase (%)
Giza 171	44.50	41.12	8.2	37.79	33.08	14.2
Misr 1	41.92	37.73	11.1	35.62	31.49	13.1
Misr 3	39.10	35.39	10.5	32.46	30.40	6.8
Shandaweel 1	36.03	32.00	12.6	30.47	26.00	17.2
Sids 14	39.14	36.39	7.6	33.28	29.48	12.9
Mean	40.14	36.53	9.9	33.92	30.09	12.7
L.S.D 0.05	4.46	4.83	---	4.08	4.74	---

Grain yield, the most important parameter for plant health, was negatively influenced by water deficit stress (Table 5). In uninoculated plants, a significant ($P< 0.05$) reduction (6.8-19.9%) in grain yield was recorded for all wheat cultivars when plants were subjected to 50% irrigation. Cultivar Misr 3 was highly sensitive to water deficit stress conditions, whereas cultivar Sids 14 was drought-tolerant. However, there was a significant decrease in grain yield (9.0 - 16.5%) in the inoculated treatments due to water deficit stress. Remarkably, plants with bacterial inoculation showed a significant ($P< 0.05$) increase in grain yield under both well-irrigation (5.6- 14.6%) and water deficit stress (3.2- 12.5%) than plants without bacterial application. Under normal conditions, the maximum positive effect of inoculation in grain yield (14.6%) was observed in Sids 14 cultivar. Under water deficit stress, the highest increase due to inoculation in grain yield (12.5%) was recorded for Misr 1 and Misr 3, followed by (12.3%) Shandweel 1 cultivar.

TABLE 5. Average of grain yield and proline content as affected by irrigation, bacterial inoculation, and wheat cultivars over the two growing seasons (2019/2020 and 2020/2021)

Cultivar	Grain yield (ton/ha)					
	Full irrigation			50% irrigation		
	Inoculated	Non-inoculated	Increase (%)	Inoculated	Non-inoculated	Increase (%)
Giza 171	6.46	6.12	5.6	5.62	5.27	6.6
Misr 1	5.98	5.43	10.1	5.39	4.79	12.5
Misr 3	5.81	5.38	8.0	4.85	4.31	12.5
Shandaweel 1	6.02	5.69	5.8	5.48	4.88	12.3
Sids 14	5.72	4.99	14.6	4.80	4.65	3.2
Mean	6.0	5.52	8.٧	5.23	4.78	9.4
L.S.D 0.05	0.609	0.572		0.550	0.594	
	Leaf proline content (µmol/g FW)					
	Full irrigation			**50% irrigation**		
	Inoculated	**Non-inoculated**	**Increase (%)**	**Inoculated**	**Non-inoculated**	**Increase (%)**
Giza 171	36.5	34.2	6.7	60.9	47.3	28.8
Misr 1	34.5	32.4	6.5	61.6	47.2	30.5
Misr 3	29.4	27.9	5.4	48.8	38.5	26.8
Shandaweel 1	32.2	29.1	10.7	58.1	43.5	33.6
Sids 14	26.9	26.7	0.7	48.2	39.7	21.4
Mean	31.9	30.1	٥,٩	55.5	43.2	28.٥
L.S.D 0.05	2.39	2.36	---	3.57	2.79	---

Drought stress also increased the production of proline in plant leaves (Table 5). Under water deficit stress, plants with bacterial treatments showed higher production of proline (21.4-33.6%) compared to the uninoculated plants. Under the water deficit stress, plants of Misr 1 and Giza 171 with inoculation produced the highest amount of proline (61.6 and 60.9 µmol/g FW); without inoculation, their proline content was found to be 47.2 and 47.3µmol/g FW, respectively.

There were significant effects of irrigation, cultivars, and bacterial inoculation on grain yield traits and leaf proline content. Data of grain yield traits and leaf proline content of wheat as affected by seasons, irrigation, bacterial inoculation, and cultivars (combined analysis of 2019/2020 and 2020/2021 seasons) are presented in Table 6. Irrigation significantly affected yield and its components and leaf proline content; however, the effect differed between cultivars. Most cultivars performed significantly better at full irrigation; this demonstrated the importance of water quantity when evaluating the performance of different wheat cultivars. The results showed a highly significant effect between irrigation in all studied characters. Significant highest values of the total number of spikes/meter^2 (322), number of kernels/spike (45), 1000-kernel weight (38.33 gm), and grain yield (5.830 ton/ha) were found for full irrigation. Inoculation also had a significant effect on all studied traits. Results indicated significant differences between inoculated and uninoculated grains. The increase between inoculated and uninoculated grains was 12.07, 10.81, 10.51, 10.38, and 19.30% for the number of spikes/meter^2, number of kernels/spike, 1000-kernel weight, grain yield, and leaf proline content, respectively. Cultivars had a significant effect on all studied characters. The superiority for Giza 171 was shown in the number of spikes per square meter (331), number of kernels per spike (41), 1000-kernel weight (37.37 gm), grain yield (5.731 ton/ha), and leaf proline content (44.73 µmol/g^{-}FW). Conversely, Misr 3 gave the lowest values for the number of spikes per square meter (285) and grain yield (5.133 ton/ha). Finally, the interactions of (irrigation X inoculation), (irrigation X cultivars), and (inoculation X cultivars) were also statistically significant for leaf proline content. For grain yield traits, the interaction (irrigation X cultivars) was significant only for the number of kernels per spike.

TABLE 6. The combined average of yield and yield components and proline content as affected by irrigation, bacterial inoculation, and cultivars of the two growing seasons (2019/2020 and 2020/2021)

Factor	No. spikes/m^2	No. kernels/ spike	1000-kernel weight (gm)	Grain yield (ton/ha)	Leaf proline content (µmol/g FW)
Irrigation (Irr)					
Full Irrigation	322	45	38.33	5.830	30.97
50% Irrigation	294	33	32.21	5.074	49.36
Reduction (%)	8.70	26.67	15.97	12.97	-59.38
F. test	**	**	**	**	**
Inoculation (I)					
Inoculated	325	41	37.03	5.721	43.70
Non-inoculated	290	37	33.51	5.183	36.63
Increased (%)	12.07	10.81	10.50	10.38	19.30
F. test	**	**	**	**	**
Cultivars (C)					
Giza 171	331	41	37.37	5.731	44.73
Misr 1	315	40	36.44	5.463	43.90
Misr 3	285	37	34.58	5.133	36.15
Shandaweel 1	296	36	33.37	5.518	40.71
Sids 14	311	39	34.57	5.415	35.34
L.S.D 0.05	13.88	2.21	1.92	0.300	1.36
Interaction					
Irr x I	ns	ns	ns	Ns	**
Irr x C	ns	**	ns	Ns	**
I x C	ns	ns	ns	Ns	**
Irr x I x C	ns	ns	ns	Ns	ns

Discussion

Wheat is the most important food crop worldwide and is grown across millions of hectares. It supplies ~ 20% of the calories and proteins for human diets (Giraldo et al., 2019). Water deficit stress has negative effects on all agronomic traits of the wheat crop. An average of 25.0 and 27.5% of biomass and yield, respectively of the wheat crop are decreased due to water deficiency stress (Zhang et al., 2018). PGPEB have been found effective in increasing the drought tolerance of plants that can be used for sustainable agriculture practices (Ullah et al., 2019). This can be achieved by inducing many mechanisms, including alteration of root architecture, osmoregulation, production of phytohormones and extracellular polysaccharides, and regulation of antioxidants (Vurukonda et al., 2016).

In this study, seven bacterial endophytes were obtained from surface-sterilized roots of wheat plants. These bacteria are related to two major phyla, Firmicutes (four isolates) and Proteobacteria (three isolates). Of the seven isolates, three were identified as *Bacillus* sp., whereas the other isolates were classified as *Enterobacter*, *Paenibacillus*, *Pseudomonas,* and *Sphingomonas* sp.. All isolated strains had PGP traits, as supported by previous studies that demonstrated the PGP capacity of endosphere bacteria associated with wheat plants (Rana et al., 2020). In the same regard, Albdaiwi et al. (2020) reported that a large group of bacterial strains (62) isolated from both rhizosphere and endosphere of durum wheat were dominantly classified into Firmicutes (61.3%) and Proteobacteria (29.0%) phyla. In agreement with this study, Majeed et al. (2015) also isolated 12 strains from the rhizosphere and endosphere of wheat plants that belonged to the genera *Staphylococcus*, *Pantoea*, *Sphingobium*, *Bacillus*, *Kosakonia*, and *Micrococcus*. The PGP potential including N_2-fixation, P-solubilization, and IAA production of these strains was confirmed (Majeed et al., 2015).

The endophytic bacteria isolated in this study were found to tolerate a water potential up to −1.37 MPa, indicating their drought tolerance. Similar to this study, PGPEB corresponding to *Klebsiella*, *Enterobacter*, and *Flavobacterium* sp. isolated from roots of wheat plants were able to grow up to 25% PEG (−0.73MPa) (Gontia-Mishra et al., 2016). Bacterial cells have the ability to accumulate osmolytes, proline, and exopolysaccharides to promote cell growth under water deficit stress conditions (Ilyas et al., 2020).

This study elucidates the role of PGPEB in improving the performance of wheat plant cv. Misr 1 under both normal irrigation and drought stress. This was observed in the case of inoculated plants in terms of the better dry weight of roots and shoots compared to the uninoculated plants (Fig. 3). The positive impact detected in inoculated plants under normal irrigation compared to control treatment could result from the beneficial functions of the applied PGPEB isolates, such as IAA production and P-solubilization. These findings are in line with previous results by other researchers, who reported the potential of PGPEB to enhance the growth of wheat plants (Majeed et al., 2015; Boleta et al., 2020). The improvement in inoculated plants under water deficit stress may be related to the bacterial ability to increase root growth, biomass, and proline accumulation in leaves. Proline is an important biochemical indicator of stress tolerance in plants by maintaining osmotic balance (Gontia-Mishra et al., 2016). This study follows previous studies that reported that PGPEB mitigate water deficit stress in wheat plants by increasing the proline content and changing the root architecture (Ullah et al., 2019). For example, inoculation of wheat plants with *B. subtilis*, *Azospirillum brasilense*, and their coinoculation increased leaf proline content by 14, 28.12, and 30% respectively under water deficit stress (Ilyas et al., 2020).

Wheat is known to be susceptible to even mild or moderate drought. Therefore, it is important to select cultivars that achieve high yields under both stressed and normal conditions (Mwadzingeni et al., 2016). Based on greenhouse data, *Bacillus* sp. NGB-WhE3 was evaluated to alleviate drought stress in five wheat cultivars under field conditions. Expectedly, drought stress had strong negative effects on the growth and yield of uninoculated wheat plants. However, the field study demonstrated an improvement in the growth and performance of all wheat cultivars under both well-irrigation and water deficit-stressed conditions. The beneficial impact of *Bacillus* sp. NGB-WhE3 on wheat cultivars was evident due to the observed significant increases in the number of spikes per square meter, the number of kernels per spike, weight of 1000 kernels, and grain yields compared to the uninoculated plants. However, this improvement was significantly varied according to the wheat cultivar and the water regime. For example, the highest increase in grain yield due to inoculation was recorded for Sids 14 (14.6%) under normal irrigation and Misr 1 and Misr 3 (12.5%) under water deficit stress. These data supported the finding of Furlan et al. (2017), who found that wheat genotypes showed different performances under standard water and water shortage regimes when inoculated by *A. brasilense* and *Herbaspirillum seropedicae*. This study is in line with previous studies that confirmed the role of bacterial inoculation in improving grain yield and other agronomic traits of wheat plants under water deficit stress (Camaille et al., 2021). For example, wheat plants inoculated with *Burkholderia phytofirmans* demonstrated higher grain yield (18% - 21%) under water deficit stress compared to uninoculated plants (Naveed et al., 2014). Also, inoculation with *Agrobacterium fabrum* or *B. amyloliquefaciens* under three levels of irrigations (4, 3, and 2 irrigations) exhibited significant increases in grain yield and yield parameters of wheat plants compared to the uninoculated control (Zafar-Ul-Hye et al., 2019). In conclusion, this study reported the isolation, identification, and utilization of newly isolated endophytic bacteria from wheat roots for promoting plant growth and mitigating drought stress of wheat plants. Among isolated PGPEB, inoculation with *Bacillus* sp. NGB-WhE3 exhibited significant increases in yield and yield components of various wheat cultivars under field normal irrigation and drought stress.

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تحسين إنتاجية بعض أصناف قمح الخبز تحت ظروف نقص المياه باستخدام بكتيريا الباسيلليس المتعايشة داخل الجذور الـNGB-WhE3

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تعتبر البكتريا المتعايشة داخل النبات والمحفزة لنمو النبات (PGPEs) هى كائنات دقيقة نافعة يمكن إستخدامها لتحسين استجابة النباتات للإجهاد المائي. أجريت هذه الدراسة بغرض عزل وتنقية سبع عزلات بكتيرية من داخل جذور نباتات القمح (*Triticum aestivum* L.) والمنزرعة تحت الظروف المصرية. تم تعريف هذه العزلات البكتيرية ودراسة مدي قدرتها علي تحسين نمو النبات وتخفيف الإجهاد المائي تحت ظروف المعمل. ثم تم دراسة تأثيرها كلقاح بكتيري على تحفيز نمو نباتات القمح تحت ظروف الري الطبيعي (100%ري) وظروف الإجهاد المائي (50% ري) في تجارب الصوب الزراعية والتجارب الحقلية. وبناءً على تحليل التتابع النيكليوتيدي لجين الـ 16S rRNA,تم تصنيف ثلاث عزلات على أنهم *Bacillus* sp. بينما تم تعريف الأربع عزلات الأخرى على أنها *Enterobacter*،*Paenibacillus* ، *Pseudomonas* و*Sphingomonas* sp. وكانت جميع العزلات لديها القدرة على إنتاج حامض اندول الخليك (Indole acetic acid, IAA)واذابة الفوسفات غير العضوي وانتاج مخلبيات الحديد (siderophore) وتحمل الإجهاد الاسموزي. وتحت ظروف الصوب الزراعية، أدي التلقيح بمعظم العزلات البكتيرية الي زيادة النمو ومحتوي النيتروجين ومستوي البرولين في الاوراق لنباتات القمح بشكل معنوي ($P<0.05$) وذلك تحت ظروف الري الطبيعي والإجهاد المائي. وبناءً على النتائج تحت ظروف الصوب الزراعية، تم اختيار العزلة البكتيرية *Bacillus* sp. NGB-WhE3 لاختبار قدرتها على تحمل الإجهاد المائي في خمسة أصناف من قمح الخبز (جيزة 171، مصر 1، مصر3، شندويل1، سدس14) في تجارب حقلية أجريت لموسمين زراعيين (2019/2020 و2020/2021) في منطقة الظهير الصحراوي الغربي بمحافظة الأقصر. وأدى التلقيح البكتيري إلى تحسين معنوي ($P<0.05$) في الصفات المحصولية وبعض الصفات الفسيولوجية لنباتات القمح تحت ظروف الإجهاد المائي مقارنة بالمعاملات الغير ملقحة. وأظهر محصول الحبوب لمعاملات القمح الملقحة زيادة معنوية ($p<0.05$) من 5.6 الى 14.6% ومن 3.2 الى12.5% تحت ظروف الري الطبيعي وتحت ظروف الاجهاد المائي، على الترتيب وذلك مقارنة بالمعاملات الغير ملقحة.