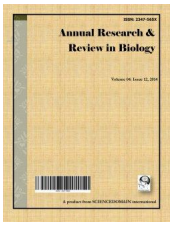

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Enhancement of Plant Growth and Yield of Wheat (*Triticum aestivum* L.) under Drought Conditions Using Plant-growth-promoting Bacteria

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

To reduce negative effects of drought on plants, the use of plant growth- promoting rhizobacteria (PGPR) is an effective way to investigate that. The aim of the present study was to assess the bacterial characteristics *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas koreensis*, *Pseudomonas fluroscence*, and *Enterobacter cloacae* as growth, IAA production, phosphate solubilization, seed germination under different concentrations of polyethylene glycol (PEG 6000), and their efficacy of single or dual inoculation with two superior strains in lyzimeter experiment for improving growth and yield of sensitive variety of wheat (*Triticum aestivum* L.) cv. Sids 1 under different stress irrigation water 100, 70 and 35% of field capacity. Among the tested strains only 2 strains *B. subtilis* and *P. koreensis* showed a stable growth even in the maximum 40% PEG concentration. Also, *P. koreensis* produced the highest amount of IAA (1.84 µg ml^{-1}), and solubilise maximum amount of P (1.59 µg ml^{-1}), and improved seed germination at 30% PEG concentration. On the other hand, in gnotobiotic sand system experiment, PGPR increase growth dynamics as well as proline content and root colonisation of wheat plants over uninoculated control under drought-stressed conditions.

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In lyzimeter experiment, single and dual inoculation treatments showed a significant increase of physiological and biochemical parameters of the plant under different drought stress treatments. Also, maximum increase 29.08 % in ascorbate peroxidase and 27.38% in catalase activities due to dual inoculation treatments T_{12} (Inoculation with *B. subtilis* + *P. koreensis* and irrigated at field capacity 35%), with respect to the corresponding unstressed control T_{10} (Inoculation with *B. subtilis* + *P. koreensis* and irrigated at field capacity 100%). Also, significant increase in grain yield, straw yield, biological yield and harvest index were observed under different drought stress. These results may be related to increase uptake of water and nutrients in wheat plant and reflected in better plant growth and yield.

Keywords: Plant growth-promoting rhizobacteria; polyethylene glycol; field capacity; wheat; yield.

1. INTRODUCTION

Nowadays, the agricultural sector is severely affected by abiotic and biotic stresses. The abiotic stresses consist of drought, salinity, temperature, flood, air pollution and heavy metals. Additionally, a decrease in rainfall and increase in CO_2 as well as 4.5°C increase in temperature are expected in the future. These factors lead to yield reduction of agriculture crops individually or in combination and these reductions reach up to 82% upon the plant types and duration of stress [1,2].

Drought stress is one of the major factors that influence crop production in arid and semi-arid regions around the world. This stress induces various physiological changes in plants causing a reduction in growth and yield [3]. Also, oxidative stress was increased under drought stress conditions lead to an increase of reactive oxygen species (ROS) levels as a result of the imbalance in the electron transport rates [4]. So, plants have developed advanced antioxidant defence systems in both enzymatic and non-enzymatic forms to overcome drought stress [5].

To cope drought stress, various biotechnological approaches can be used such as gene manipulation to the production of drought-tolerant plants or using of plant growth promoting rhizobacteria (PGPR) to mitigate this stress [6,7,8]. Several PGPRs genera including *Pseudomonas*, *Bacillus*, *Azospirillum, Bradyrhizobium, Aeromonas, Enterobacter, Acetobacter, Sinorhizobium, Flavobacterium* etc. have been famous for improving the growth and yield of different crops cultivated in drought-affected soils. Plant growth promoting rhizobacteria can associate with plant roots as endophytes or on the surface of plant roots as the rhizoplane or within the zone of the soil as the rhizosphere [6,9]. These bacteria can offer many benefits to plants such as the production of phytohormones, increased plant root system and water uptake, production of ACC deaminase, increased availability of plant nutrients, osmolyte accumulation in plants, induced systemic tolerance, induced plant synthesis of antioxidative enzymes and production of extracellular polymeric substances [10,11,12,13, 14].

In all main crop systems such as wheat, maize and rice, a positive effect from application of PGPR is well documented. [8], showed that application of the drought-tolerant rhizobacteria *Bacillus amyloliquefaciens*, *Bacillus thuringiensis* and *Enterobacter aerogenes* can help to ameliorate negative effects of the wheat plant grown in drylands. Application of bacterial inoculation with *Burkholderia phytofirmans* on wheat growth under drought stress at different growth stages in the field conditions improved growth, relative water content and biomass of wheat. Also, grain yield was reduced when plants were exposed to drought stress at the tillering and flowering stage, but inoculation showed in better grain yield up to 21 and 18% higher, respectively, compared to control [15].

According to Armada et al. [16], the water limitation and osmotic stress showed negatively impact on plant growth of *Lavandula dentate* and *Salvia officialis* but with bacterial inoculation by *B. megaterium*, *Enterobacter* sp., *B. thuringiensis* and *Bacillus* sp. were able to mitigate drought effects by improving nutritional, physiological and metabolic plant activities.

The aims of this study were the following: (1) to determine the bacterial characteristics *Bacillus subtilis* SARS 11, *Bacillus cereus* SARS 101, *Pseudomonas koreensis* MG209738, *Pseudomonas fluroscence* SARS 5, and *Enterobacter cloacae* KX034162 as growth, IAA production and phosphate solubilization under different concentrations of drought stress; (2) to

investigate the ability of bacterial strains under study to ameliorate the seed germination and growth of wheat (*Triticum aestivum* L.) under stressed and non-stressed conditions; and (3) to assess the efficacy of single or dual inoculation with two superior strains for improving growth and yield of sensitive variety of wheat (Sids 1) under different stress irrigation water 100, 70 and 35% of field capacity.

2. MATERIALS AND METHODS

2.1 Microorganisms and Growth Conditions

Five bacterial strains, *Bacillus subtilis* SARS 11, *Bacillus cereus* SARS 101, *Pseudomonas koreensis* MG209738, *Pseudomonas fluroscence* SARS 5, and *Enterobacter cloacae* KX034162 were used in the present study which provided from Bacteriology Laboratory, Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt. To examine tolerance level of different strains to drought stress, 1 mL of the bacterial culture ($1x10^8$ CFU ml^{-1}) were inoculated to 200 mL of Nutrient Broth (NB) medium in a 500-mL Erlenmeyer flask amended with different concentration (0, 5, 10, 20, 30 and 40 %) of polyethylene glycol (PEG 6000). Each treatment was performed in triplicate. After incubation at 30 °C under shaking conditions (200 rpm) for 24, 48, 72 and 96 h, growth was counted by using standard serial dilution and plated by spread plate count method and expressed in terms of log_{10} [17].

2.2 Plant Growth Promotion Traits

In drought stress conditions at 0, 5, 10, 20, 30 and 40 % of polyethylene glycol (PEG 6000), two plant growth promotion traits were analysed: IAA production and inorganic phosphate solubilization. Each experiment was performed in triplicate and repeated at least thrice.

2.2.1 Assay of IAA production

Using the method described by Ivanova et al. [18] for assay the indolic compounds were determined. Briefly, 1 mL of the bacterial suspensions ($1x10^8$ CFU ml^{-1}) were inoculated into Nutrient Broth medium supplemented with 1 g L^{-1} filter sterilised L-tryptophan as IAA precursor in the presence of drought stress conditions and incubated at 30°C on a shaker at 200 rpm for 72 h. After the incubation period, bacterial cells were centrifuged at 8000 rpm for 10 min and take supernatant for IAA assay (0.5 mL of the supernatant was supplemented with 2ml of the Salkowski Reagent) and read the pink auxin complex at 540 nm using UV/ Visible Spectrophotometer (model 6705). The concentration of IAA produced by the cultures was measured from a calibration curve using a standard IAA and expressed as µg ml^{-1}.

2.2.2 Assay of phosphate solubilization

Solubilization of tribasic calcium phosphate was quantitatively measured in Pikovskaya (PVK) liquid medium inoculated with 1 mL of bacterial suspensions ($1x10^8$ CFU ml^{-1}) in the presence of drought stress conditions and left on shaker incubator at 200 rpm and 30°C for 5 days. The concentration of soluble phosphate was determined colourimetrically after centrifugation of liquid cultures at 5,000 rpm for 15 min [19]. The absorbance of generated blue colour was measured at 610 nm, and soluble phosphorus is detected from a standard curve of K_2HPO_4 and expressed as µg P_2O_5 ml^{-1}.

2.3 Germination of Seeds

This experiment aims to investigate the ability of bacterial strains to ameliorate the seed germination and growth of Wheat (*Triticum aestivum* L.) under elevated drought stress. For this purpose, the experiment was conducted in complete randomised blocks with four replicates using a sensitive variety of wheat cv. Sids 1 [20], which provided by the Field Crop Institute, Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt.

Ten seeds of each treatment were first rinsed with 70% (v/v) ethanol and surface sterilised with diluted sodium hypochlroide (3% v/v) for 3 min and then washed five times with sterile distilled water. Seeds were soaked in bacterial suspensions ($1x10^8$ CFU ml^{-1}) overnight before germinated in a sterile 15-cm Petri plates lined with sterile double filter paper saturated with 15 mL of sterile water supplemented with different concentrations 0, 5, 10, 20, 30 and 40% of polyethylene glycol (PEG 6000) then incubated at 20°C in a dark place. For control, seeds were soaked in autoclaved inocula. All Petri plates were sealed well to prevent from drying across the experiment period of 10 days. A daily count of germinant was done and at the end of the experiment, the dry mass was taken for each replicate. Germination indicators were calculated as follows:

a. Final germination percentage (FGP, %) was calculated according to Ranal and Santana [21] using the formula:

$$FGP, \% = \left[\frac{TNG}{TNP}\right] \times 100$$

Where, FGP, % is the final germination percentage, TNG is the total number of germinated seeds, and TNP is the total number of planted seeds.

b. Mean germination time (MGT) was used to evaluate seedling emergence and computed by the formula cited by Mauromicale and Licandro [22].

$$MGT = \sum \left(\frac{ni \times ti}{ni}\right)$$

Where, MGT is the mean germination time, ni is the number of germinated seeds on germination days, and ti is the number of days during the germination period (between 0 and 10 days).

c. Vigor index (VI) was calculated using the formula of Kharb et al. [23], as follows:

$$VI = \left[\frac{SDM(g) \times GP}{100}\right]$$

Where, VI is the vigor index, SDM is the seedling dry mass (g), and GP is the germination, %.

2.4 Plant Growth in a Gnotobiotic Sand System

The effect of inoculation with *B. subtilis*, *B. cereus*, *P. koreensis*, *P. fluroscence*, and *E. cloacae* on the growth and root colonisation of a sensitive variety of wheat cv. Sids 1 exposed to drought stress in glass tubes (2.5 cm in diameter, 20 cm in length) as described by Simons et al. [24], with 10 replicates (N=10). Sixty grams from sterilised mixture of washed sand and vermiculite (1:1) was added to each tube and soaked with 6 ml of the nutrient solution [25]. Drought treatments were established by watering every 5 days but unstressed treatments were watering every 2 days.

Sterilized seeds were planted into sterile tubes, one seed per tube and inoculated with 0.5 mL from the bacterial suspensions ($1x10^9$ CFU ml^{-1}), whereas control seeds were inoculated with autoclaved inocula. All tubes were grown in a growth cabinet with a photoperiod of 16:8 h at 20°C. After four weeks, the fresh and dry weight of plants, as well as length of shoots and roots, were measured. Also, proline content and root colonisation were determined.

2.4.1 Proline content

According to Bates et al. [26], proline content was measured with colourimetric assay at 520 nm. Briefly, 0.01 g fresh plant were ground with 0.4 mL sulfosalicylic acid (3%) and placed overnight at 5°C. The suspension was centrifuged at room temperature at 3000 rpm for 5 min. The supernatant was mixed with 0.4 mL of acidic ninhydrin reagent then heated in a boiling water bath for 60 min. Thereafter, the content in the tubes was cooled and the mixture was extracted with 0.4 mL of toluene. The absorbance of the formed complex was determined using UV/Visible Spectrophotometer (model 6705), and the proline concentrations were measured using a standard curve and calculated as mg g^{-1} fresh weight.

2.4.2 Colonisation efficiency

The ability of studied microorganisms to colonise wheat roots were tested after 30 days of germination of seeds in glass tubes. Plants were obtained from the experimental set-up as described above. From each treatment, 3 plants were taken randomly, and roots were washed thoroughly to remove soil then weighted and kept in test tube containing 9 mL sterile saline solution (0.85% NaCl) then shaking on a vortex every 10 min for 1 h. Serial dilutions were done and 0.1 mL from each diluent was spread on nutrient agar plates and incubated at 30°C for 48 h. The experiment was replicated three times, and the number of viable cells was counted and calculated as CFU g^{-1} root [27].

2.5 Lyzimeter Trial under Drought Stress

Lyzimeter trial was conducted at the Experimental Farm, Sakha Agricultural Research Station, Kafr El-Sheikh to assess the efficacy of single or dual inoculation with *B. subtilis* SARS 11 and *P. koreensis* MG209738 strains for improving growth and yield of sensitive variety of wheat cv. Sids 1 under drought stress conditions during Nov. to Apr. 2017–2018. The soil was clayey soil having pH, 7.24; EC, 3.07 dS m^{-1}; organic matter (%), 1.73; soluble cations Ca^{+2}, Mg^{+2}, Na^{+} and K^{+} (meq L^{-1}), 4.28, 8.25, 9.30 and 0.23, respectively; soluble anions CO_3^{--}, HCO_3, Cl^{-} and SO_4^{--} (meq L^{-1}), 0.0, 3.65, 6.82 and 11.59, respectively; available N (mg Kg^{-1}), 38.0; available P (mg Kg^{-1}), 7.88; available K (mg Kg^{-1}), 234.2. Physical and chemical properties

were measured according to Black, Jackson [28,29], respectively.

The experiment in lyzimeter was carried out in randomised complete block design which composed of 12 treatments with single or dual bioinoculants, and different drought stresses conditions (100, 70 and 35% field capacity), with 3 replicates (Table 1).

Wheat seeds were inoculated (coated) before sowing with 30 g of a sterilised carrier containing 15 ml (1×10^8 CFU ml^{-1}) from each strain using a sticking material then sown by broadcasting method at the rate of 7.2 g for each lyzimeter unit (recommended for wheat cultivation per hectare). Fertilizer program was applied according to the recommendations of Ministry of Agriculture and Land Reclamation.

2.6 Measurements

After 70 days from sowing, random selection of five plants were made from each replication to measure the growth dynamics, i.e. plant height (cm $plant^{-1}$), dry weight (g $plant^{-1}$), chlorophyll content, total carotenoids, relative water content, total soluble sugars and antioxidant enzymes. At the end of the experiment, biological yield, grain yield and straw yield were determined as ton ha^{-1} as well as harvest index as %.

Harvest Index (H.I) = Grain yield / Biological yield × 100

2.6.1 Chlorophyll and carotenoid

According to Mousa et al. [30]. 0.1 g of fresh leaf tissue was grinded with 5 ml acetone 80% then centrifugation at 13,000 rpm for 10 min. The absorbance of the supernatant was read at 645, 663 and 470 nm. The amount of chlorophyll and carotenoid (mg g^{-1} FW) in the extract was calculated as below:

Chl a = 12.7 (A_{663}) – 2.69 (A_{645})
Chl b = 25.8 (A_{645}) – 4.68 (A_{663})
Total Chl = 20.21 (A_{645}) + 8.02 (A_{663})
Carotenoids = (1000 (A_{470}) – 2.27 (Chl a) – 81.4 (Chl b)) / 227

2.6.2 Total soluble sugar

Total soluble sugar was estimated using anthrone reagent according to Ibragimova et al. [31]. For this purpose, 0.1 mL of alcoholic leaf extract was added to 3 mL freshly prepared anthrone reagent and mixed then boiling in a water bath for 10 min and measured at 620 nm. A calibration curve prepared from glucose was used to quantify soluble sugar in plant samples.

2.6.3 Relative water content

For relative water content (RWC) measurement. Fresh weight (FW) of five leaves from each sample was recorded using a digital electrical balance then dipped in test tubes containing distilled water. After 24 h, the leaves were wiped with a tissue paper and the turgid weight (TW) was recorded. The samples were dried at 65°C for three days and dry weight (DW) was determined. Relative water contents were calculated according to Barrs et al. [32].

$$\mathrm{RWC} = (\mathrm{FW} - \mathrm{DW})/(\mathrm{TW} - \mathrm{DW}) \times 100$$

2.6.4 Enzymes activity

In order to estimate the ascorbate peroxidase (APX) and catalase (CAT) enzyme activities, flag leaves of wheat were collected 70 days after sowing and homogenized in a cooled 0.1 mol L^{-1} Tris-HCl buffer at pH 7.8 containing 1 mmol L^{-1} EDTA, 1 mmol L^{-1} dithiothreitol and 5 mL of 4% polyvinyl pyrrolidone per one gram of fresh weight. Two mL reaction mixture consisting of 20 µL crude leaf extract, 660 µL potassium phosphate buffer (pH 7.0), 660 µL ascorbic acid solution, and 660 µL H_2O_2 was used to measure ascorbate peroxidase (APX) activity. Enzyme activity was tested by observing the ascorbate reduction through H_2O_2 at 290 nm for 3 min [33]. On the other hand, catalase (CAT) activity was extracted by grinding 1 g of leaf tissues in 0.1 M sodium phosphate buffer at pH 7.1 in a porcelain mortar. Reaction mixture contained 25 mM L^{-1} Tris-acetate buffer (pH 7.0), 0.8 mM L^{-1} EDTA-Na, and 20 mM L^{-1} H_2O_2 at 25°C. Enzyme activity was tested by observing H_2O_2 consumption at 240 nm for 3 min [34]. Enzymes activities were calculated in the form of µM H_2O_2 min^{-1} g^{-1} FW.

2.7 Statistical Analysis

Using one-way and two-way analysis variances (ANOVA), data were analysed by software SPSS 14.0 for windows and Duncan's multiple range test was used for comparison among the treatment means [35].

Table 1. Treatment used for lyzimeter experiment

Symbol	Treatment	Symbol	Treatment
T_1	Control (100% FC)	T_7	Inoculation with PS (100% FC)
T_2	Control (70% FC)	T_8	Inoculation with PS (70% FC)
T_3	Control (35% FC)	T_9	Inoculation with PS (35% FC)
T_4	Inoculation with BS (100% FC)	T_{10}	Inoculation with BS+PS (100% FC)
T_5	Inoculation with BS (70% FC)	T_{11}	Inoculation with BS+PS (70% FC)
T_6	Inoculation with BS (35% FC)	T_{12}	Inoculation with BS+PS (35% FC)

BS: *B. subtilis SARS 11;* ***PS:*** *P. koreensis MG209738;* ***FC:*** *Field Capacity*

3. RESULTS

3.1 Screening of Bacterial Strains to Drought Stress

To determine tolerance level of different plant growth promoting strains *B. subtilis*, *B. cereus*, *P. koreensis*, *P. fluroscence* and *E. cloacae* to drought stress conditions, cells were cultured in NB medium containing variable concentrations of polyethylene glycol. After different incubation periods for 24, 48, 72 and 96 h in liquid medium containing 0, 5, 10, 20, 30 and 40% PEG, viable cell numbers were determined (Fig. 1).

Generally, viable cell numbers of tested strains showed survived poorly in the medium with increasing PEG concentrations. On the other hand, after incubation time with 72 h, viable cell numbers showed greater growth as compared to other different incubation times in presence of different treatments of drought stress. Also, all strains were able to grow in all tested PEG concentrations at up to 30%, only 2 strains (*B. subtilis* SARS 11 and *P. koreensis* MG209738) showed a stable growth even in the maximum 40% PEG concentration tested.

3.2 Plant Growth Promotion Traits

Plant growth promoting properties of these strains, *B. subtilis*, *B. cereus*, *P. koreensis*, *P. fluroscence* and *E. cloacae* under different stressed conditions are presented in Table 2. All these strains were found positive for the tested PGP traits (IAA and P_2O_5) up to 30% PEG with a few exceptions (*B. cereus* and *P. fluroscence* could not produce IAA). Among these strains, *P. koreensis* produced highest amount of IAA (1.84 and 19.41 µg ml^{-1} under stressed and non-stressed conditions, respectively) and solubilised maximum amount of P under non-stressed (7.70 µg ml^{-1}) and stressed (1.59 µg ml^{-1}) conditions when compared with other strains under respective conditions.

3.3 Germination of Seeds

Results of wheat seeds germinated under different concentrations of PEG with plant growth promoting strains are depicted in Fig. 2. Increasing drought stress drastically affected the final germination percentage of wheat seeds Fig. 2a. Also, the FGP decreased to 10% at 40% PEG when treated with *B. subtilis*, *B. cereus*, *P. fluroscence* and *E. cloacae* and 20% when treated with *P. koreensis* compared to 100% for control (zero PEG). Among all strains, *P. koreensis* improved FGP which recorded higher values followed by *B. subtilis* at 30% PEG than other strains.

On the other hand, all strains led to a decrease of mean germination time at low PEG concentrations up to 10% (Fig. 2b). Among all treatments, the shortest MGT was found when wheat seeds treated with *P. koreensis* under 0, 10, 20, 30 and 40% PEG recording 2, 2.5, 2.5, 3.1, 3.5 and 4 days, respectively.

A negative impact was observed on the vigour index at high PEG concentrations (Fig. 2c). The VI values were dramatically reduced with increasing PEG percentages, particularly when the germination medium was supplemented with 40% PEG. The application of 10% PEG maximised the VI by 0.014 and 0.012 for *P. koreensis* and *B. subtilis* compared to control (no PEG).

3.4 A Gnotobiotic Sand System

3.4.1 Growth parameters

Response of drought-affected wheat plants to the inoculation with plant growth promoting strains under a gnotobiotic sand system is showed in Table 3. Generally, the strains increased the fresh weight, dry weight, root and shoot length of wheat plants over uninoculated control both

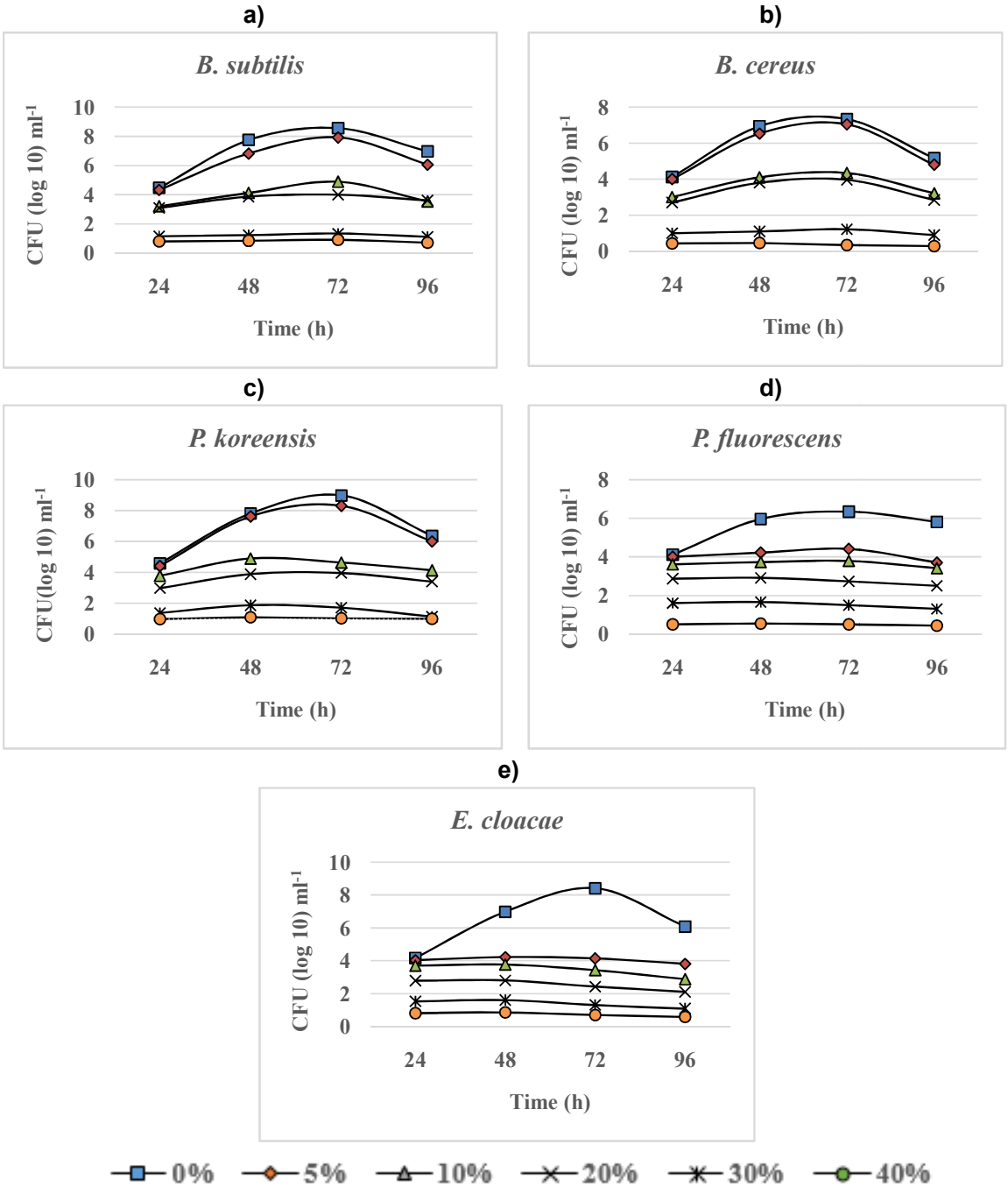


Fig. 1. Viable cells numbers of plant growth promoting strains growing in nutrient broth medium supplemented with increasing concentrations of PEG at different incubation times (24 to 96 h)

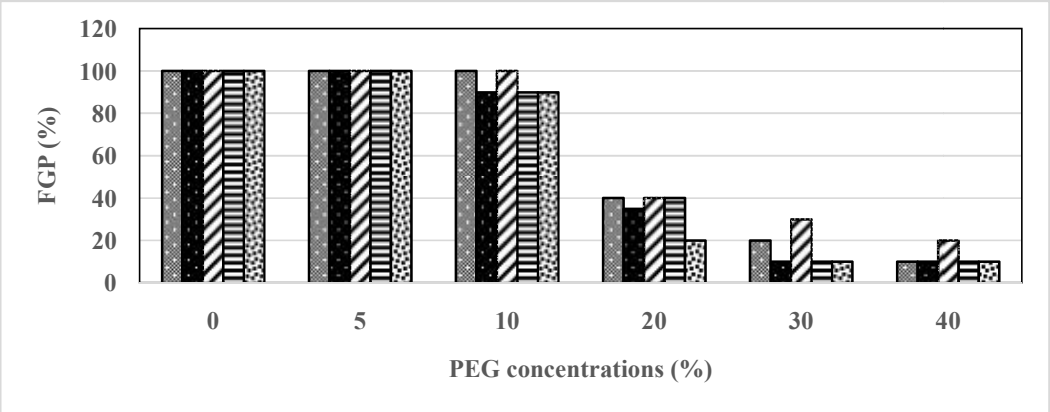
under unstressed and drought-stressed conditions. A significant improvement of fresh weight was observed with *P. koreensis* (55 and 53.12%) followed by *B. subtilis* (52.5 and 34.37%) over control under unstressed and drought-stressed conditions, respectively. Among all strains, *P. koreensis* improved dry weight, root and shoot length recording 0.013 g plant^{-1}, 9.66 cm plant^{-1} and 15.66 cm plant^{-1}, respectively under stressed-drought.

Table 2. Effect of different drought-stress conditions on production of IAA and phosphate solubilization by different plant growth promoting strains

Strains	PEG concentrations (%)					
	0	5	10	20	30	40
	IAA µg ml^{-1}					
B. subtilis	18.73±0.15	15.28±0.15	11.27±0.05	6.20±0.07	1.41±0.03	-
B. cereus	18.18±0.07	14.64±0.10	9.66±0.20	3.31±0.10	-	-
P. koreensis	19.41±0.10	16.27±0.15	13.14±0.11	8.22±0.12	1.84±0.05	-
P. fluorescence	16.75±0.11	13.22±0.11	8.23±0.12	2.50±0.04	-	-
E. cloacae	17.64±0.10	14.76±0.10	10.19±0.09	5.18±0.07	0.79±0.02	-
	P_2O_5 µg ml^{-1}					
B. subtilis	7.17±0.04	5.50±0.09	4.23±0.03	2.57±0.07	1.00±0.01	-
B. cereus	6.98±0.03	5.21±0.10	3.96±0.05	1.99±0.06	0.50±0.05	-
P. koreensis	7.70±0.07	6.30±0.08	4.88±0.08	3.06±0.05	1.59±0.07	-
P. fluorescence	6.93±0.06	4.87±0.05	3.44±0.05	2.11±0.05	0.59±0.05	-
E. cloacae	7.14±0.11	6.26±0.06	4.11±0.05	2.07±0.02	0.70±0.04	-

IAA: *Indole Acetic Acid; Data are presented as the mean ± SD with n = 3*

a



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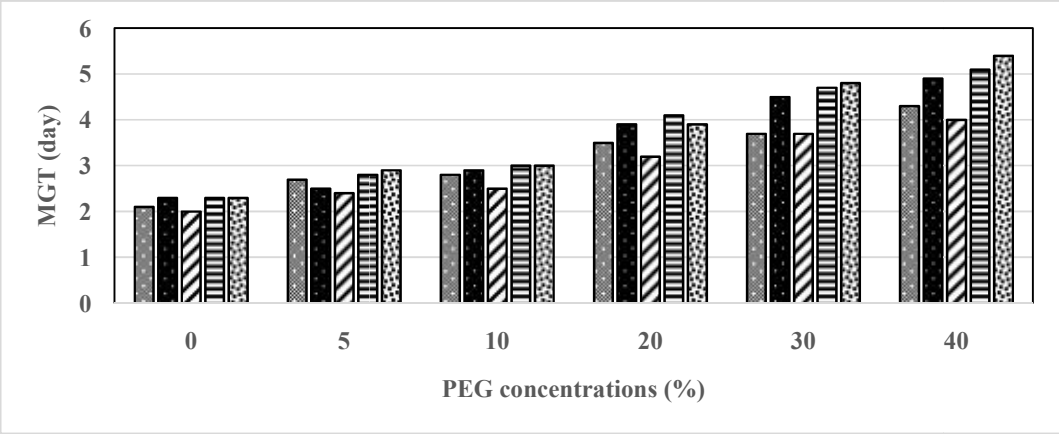


c

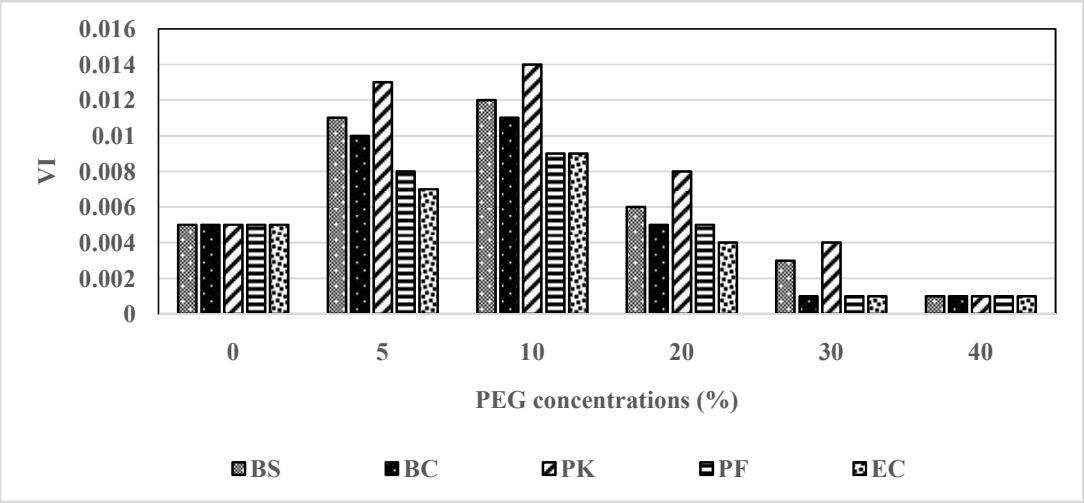


Fig. 2. Effect of plant growth promoting strains *B. subtilis* (BS), *B. cereus* (BC), *P. koreensis* (PK), *P. fluroscence* (PF) and *E. cloacae* (EC) on germination parameters of wheat seeds (Sids 1) under different concentrations of PEG (0, 5, 10, 20, 30 and 40%). a) Final germination percentage. b) Mean germination time. c) Vigour index

Table 3. Inoculation with plant growth promoting strains and its effect on some growth parameters of wheat plant under drought stress-conditions

Treatment		Fresh weight (g)	Dry weight (g)	Root length (cm)	Shoot length (cm)
Unstressed	Control	0.040 ^{de}	0.009	11.00	17.00
	B. subtilis	0.061 ^{a}	0.016	12.33	17.33
	B. cereus	0.047 ^{bc}	0.013	10.66	16.33
	P. koreensis	0.062 ^{a}	0.016	12.00	17.33
	P. fluorescence	0.044 ^{bcd}	0.011	11.33	15.33
	E. cloacae	0.048 ^{bc}	0.012	11.33	17.00
Stressed	Control	0.032 ^{f}	0.005	7.33	13.66
	B. subtilis	0.043 ^{cde}	0.011	9.66	15.00
	B. cereus	0.041 ^{de}	0.010	8.66	14.00
	P. koreensis	0.049 ^{b}	0.013	9.66	15.66
	P. fluorescence	0.038 ^{e}	0.007	9.33	14.33
	E. cloacae	0.044 ^{bcd}	0.010	9.66	15.33
LSD 0.01		0.004 **	NS	NS	NS
Significance due to					
Main (LSD 0.01)		0.002 **	NS	NS	NS
Sub main (LSD 0.01)		0.002 **	NS	NS	NS

Data were analysed by two-way ANOVA analysis followed by Tukey's multiple comparisons

3.4.2 Proline and root colonisation

Applied of inoculation with PGPR strains showed decreased the proline content of wheat plant significantly over the control under unstressed conditions but an increased amount of proline was observed under drought stress (Table 4). Under unstressed condition, *P. koreensis* was more efficient in reducing the proline content of wheat (4.48 mg g^{-1} FW) over the uninoculated control (5.03 mg g^{-1} FW) as well as over that of other strains. In contrast, a significant increase in proline content was higher 72.11 and 55.33% for *P. koreensis* and *B. subtilis* over control, respectively under drought-stressed conditions.

Regarding root colonisation, data showed that inoculation with PGPR strains increased the

number of bacteria on the root surface of wheat plants under unstressed compared to drought-stressed conditions and control (Table 4). The highest bacterial CFU g^{-1} root was observed with *B. subtilis* (18.66 × 10^5 g^{-1} root) followed by *P. koreensis* (18 × 10^5 g^{-1} root) compared to control under unstressed conditions. Also, under drought-stressed conditions, *B. subtilis* was the most tolerant as maximum bacterial (16.33 × 10^5 g^{-1} root) was observed over the other strains. So, *P. koreensis* and *B. subtilis* showed better results in case of root colonisation under drought-stressed conditions.

Table 4. Inoculation with plant growth promoting strains and its effect on proline content and root colonisation of wheat plant under drought-stress conditions

Treatment		Proline (mg g^{-1} FW)	Root colonisation (CFU×10^5 g^{-1} root)
Unstressed	Control	5.03 ^{ef}	0.00 ^{e}
	B. subtilis	5.23 ^{e}	18.66 ^{a}
	B. cereus	4.56 ^{f}	15.00 ^{c}
	P. koreensis	4.48 ^{f}	18.00 ^{ab}
	P. fluorescence	4.53 ^{f}	14.00 ^{c}
	E. cloacae	4.53 ^{f}	14.00 ^{c}
Stressed	Control	4.59 ^{f}	0.00 ^{e}
	B. subtilis	7.13 ^{b}	16.33 ^{bc}
	B. cereus	6.30 ^{c}	14.00 ^{c}
	P. koreensis	7.90 ^{a}	15.66 ^{c}
	P. fluorescence	5.36 ^{de}	10.33 ^{d}
	E. cloacae	5.76 ^{d}	9.33 ^{d}
LSD 0.01		0.443**	1.94**
Significance due to			
Main (LSD 0.01)		0.181**	0.795**
Sub main (LSD 0.01)		0.313**	1.378**

Data were analysed by two-way ANOVA analysis followed by Tukey's multiple comparison test.

Table 5. Effect of different inoculation treatments on height, dry weight and chlorophyll content of wheat plant under grown drought-stress conditions

Treatment	Height (cm $plant^{-1}$)	Dry weight (g $plant^{-1}$)	Chl a	Chl b	Chl t
T_1	82.33 ^{ab}	6.46 ^{d}	1.88 ^{c}	0.95 ^{b}	2.84 ^{c}
T_2	52.36 ^{e}	4.38 ^{h}	1.31 ^{f}	0.67 ^{e}	1.98 ^{g}
T_3	27.75 ^{g}	2.52 ^{j}	0.94 ^{h}	0.42 ^{h}	1.36 ^{j}
T_4	88.66 ^{a}	7.16 ^{c}	2.08 ^{b}	1.07 ^{a}	3.15 ^{b}
T_5	58.00 ^{de}	4.76 ^{g}	1.44 ^{e}	0.77 ^{d}	2.21 ^{f}
T_6	31.66 ^{fg}	2.67 ^{j}	0.99 ^{h}	0.44 ^{h}	1.43 ^{j}
T_7	91.00 ^{a}	7.52 ^{b}	2.19 ^{a}	1.12 ^{a}	3.31 ^{a}
T_8	65.33 ^{cd}	5.26 ^{f}	1.69 ^{d}	0.87 ^{c}	2.57 ^{e}
T_9	38.33 ^{fg}	3.25 ^{i}	1.07 ^{g}	0.49 ^{g}	1.57 ^{i}
T_{10}	83.00 ^{ab}	7.96 ^{a}	2.24 ^{a}	1.09 ^{a}	3.33 ^{a}
T_{11}	71.66 ^{bc}	6.16 ^{e}	1.76 ^{d}	0.90 ^{c}	2.67 ^{d}
T_{12}	39.33 ^{f}	3.43 ^{i}	1.13 ^{g}	0.56 ^{f}	1.69 ^{h}
LSD 0.05	11.55**	0.219**	0.067**	0.047**	0.096**

In a column means followed by a common letter are not significantly different at 5% level by DMRT; T_1: Control without inoculation and irrigated at felid capacity 100%; T_2: Control without inoculation and irrigated at field capacity 70%;T_3: Control without inoculation and irrigated at field capacity 35%; T_4: Inoculation with B. subtilis and irrigated at field capacity 100%; T_5: Inoculation with B. subtilis and irrigated at field capacity 70%; T_6: Inoculation with B. subtilis and irrigated at field capacity 35%; T_7: Inoculation with P. koreensis and irrigated at field capacity 100%; T_8: Inoculation with P. koreensis and irrigated at field capacity 70%; T_9: Inoculation with P. koreensis and irrigated at field capacity 35%; T_{10}: Inoculation with B. subtilis + P. koreensis and irrigated at field capacity 100%; T_{11}: Inoculation with B. subtilis + P. koreensis and irrigated at field capacity 70%; T_{12}: Inoculation with B. subtilis + P. koreensis and irrigated at field capacity 35%. Chl a: Chlorophyll a; Chl b: Chlorophyll b; Chl t: Total chlorophyll.

3.5 Lyzimeter Experiment

3.5.1 Height plant, dry weight and chlorophyll content

The two bacterial strains namely *Bacillus subtilis* SARS 11 and *Pseudomonas koreensis* MG209738 were selected on the basis of tolerance drought conditions and their efficiency as plant growth promoting. The influence of single and dual inoculation on plant growth promotion of wheat (Sids 1) was evaluated under different treatments of stress water 100, 70 and 35% of field capacity.

After 70 days from sowing, results showed that all plant growth parameters were significantly increased in the case of single or dual inoculation under water stress (Table 5). Positive effect was observed in height plant which attained 65.33 for T_8 treatment (inoculation with *P. koreensis*) and 58.00 cm plant^{-1} for T_5 treatment (inoculation with *B. subtilis*) compared to 52.36 cm plant^{-1} for T_2 treatment (uninoculated control), at stress water 70% of field capacity. Similar trend was also exhibited in dry weight parameter.

Under dual inoculation, chlorophyll a, chlorophyll b and total chlorophyll were 34.35, 34.32 and 34.84% more for T_{11} treatment (inoculation with *B. subtilis* + *P. koreensis* and irrigated at felid capacity 70%), and 20.21, 33.33 and 24.26% more for T_{12} treatment (inoculation with *B. subtilis* + *P. koreensis* and irrigated at felid capacity 35%), respectively, in comparison to control under the same stress conditions.

3.5.2 Carotenoids, total soluble sugars and relative water content

As shown in Table 6, carotenoids, total soluble sugar contents and relative water content (%) were decreased significantly ($P \leq 0.05$) under low and high drought conditions (70 and 35% of field capacity).

Carotenoids content in PGPR-inoculated treatments showed significant variation in comparison to control plants under stress conditions. Dual inoculation showed a significant increase in carotenoid content recording 0.69 for T_{11} treatment (inoculation with *B. subtilis* + *P. koreensis* and irrigated at felid capacity 70%), compared to 0.46 for T_2 treatment (uninoculation control and irrigated at felid capacity 70%). Moreover, total soluble sugar was found significantly enhanced in single bacterial inoculated under water stress 35% of field capacity which recorded 3.91 µg g^{-1} FW for *P. koreensis* and 3.74 µg g^{-1} FW for *B. subtilis* than 2.65 µg g^{-1} FW for uninoculated control. Similarly, relative water content unaffected at water stress 70% of field capacity in all treatments. However, a significant increase of 75.33% was observed with dual inoculation treatment, in comparison to control (71.50%) under drought stress 35% of field capacity. Overall, physiological parameters were significantly increased when wheat seeds were inoculated with PGPR compared with control in the presence of different water stress conditions.

3.5.3 Antioxidant enzymes activity

At 70 days from sowing, single and dual bacterial inoculation decreased antioxidant enzymes APX and CAT activity in leaves of wheat significantly over the control under non-stressed conditions, but an increased amount of these enzymes was observed under drought stress (Fig. 3). Under drought-stressed conditions, *B. subtilis* and *P. koreensis* efficiently increased the APX content by 6.53% and 16.98% at 70% of field capacity, but at 35% of field capacity the increased rate reached to 11.25% and 16.74%, respectively, over the uninoculated control.

Regarding CAT activity, the highest values was observed with *P. koreensis* (21.43 µM H_2O_2 min^{-1} g^{-1} FW) followed by *B. subtilis* (20.32 µM H_2O_2 min^{-1} g^{-1} FW) compared to control (19.60 µM H_2O_2 min^{-1} g^{-1} FW), under water stress conditions (35% of field capacity). On the other hand, stress at 35% of field capacity, maximum increase 29.08 % in ascorbate peroxidase and 27.38% in catalase activities due to dual inoculation treatments (T_{12}) with respect to the corresponding unstressed control (T_{10}).

3.5.4 Yield

Table 7, indicates that the differences among different inoculation and water stress treatments for grain yield, straw yield, biological yield and harvest index. Under stressed conditions (70% of field capacity), treatment T_8 showed a significant increase in grain yield (6.33 ton ha^{-1}), followed by (6.23 ton ha^{-1}), for treatment T_5 in comparison with the control treatment T_2 (5.82 ton ha^{-1}) in case of the single inoculation treatments.

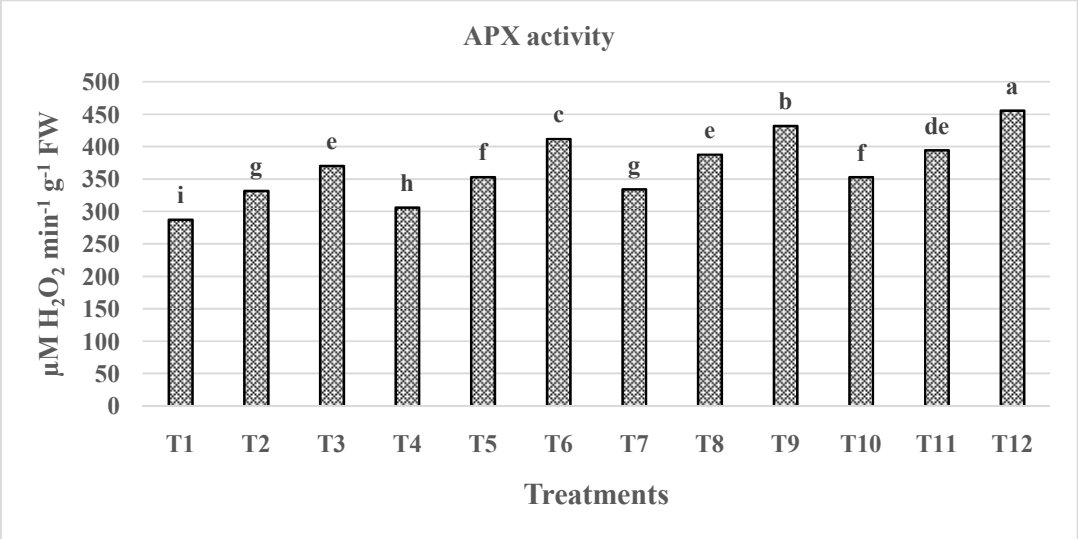
Under dual inoculation, straw yield and biological yield were 26.16 and 18.96% more for T_{11} treatment (inoculation with *B. subtilis* + *P.*

koreensis and irrigated at felid capacity 70%), and 4.80 and 7.25% more for T_{12} treatment (inoculation with *B. subtilis* + *P. koreensis* and irrigated at felid capacity 35%), respectively, in comparison to control under the same stress conditions. Also, harvest index was highly significantly affected by different treatments which T_9 treatment gave the highest harvest index 45.81% as a single inoculation, whereas T_{12} treatment gave 45.32% as a dual inoculation treatment as compared to control T_3 treatment 44.08% under drought stress 35% of field capacity.

Table 6. Effect of different inoculation treatments on carotenoids, total soluble sugars and relative water contents of wheat plant grown under drought-stress conditions

Treatment	Carotenoids	TSS µg g^{-1} FW	RWC (%)
T_1	0.73 ^{c}	3.97 ^{fg}	82.30 ^{bcd}
T_2	0.46 ^{g}	3.86 ^{h}	81.93 ^{cd}
T_3	0.31 ^{i}	2.65 ^{j}	71.50 ^{f}
T_4	0.83 ^{b}	4.55 ^{b}	84.03 ^{ab}
T_5	0.57 ^{f}	4.10 ^{e}	85.33 ^{a}
T_6	0.37 ^{h}	3.74 ^{i}	71.30 ^{f}
T_7	0.90 ^{a}	4.79 ^{a}	83.33 ^{bc}
T_8	0.65 ^{e}	4.33 ^{d}	80.83 ^{d}
T_9	0.46 ^{g}	3.91 ^{gh}	75.33 ^{e}
T_{10}	0.89 ^{a}	4.82 ^{a}	85.36 ^{a}
T_{11}	0.69 ^{d}	4.45 ^{c}	80.83 ^{d}
T_{12}	0.48 ^{g}	3.98 ^{f}	75.33 ^{e}
LSD 0.05	0.036**	0.060**	1.769**

In a column means followed by a common letter are not significantly different at 5% level by DMRT; T_1: Control without inoculation and irrigated at felid capacity 100%; T_2: Control without inoculation and irrigated at field capacity 70%;T_3: Control without inoculation and irrigated at field capacity 35%; T_4: Inoculation with B. subtilis and irrigated at field capacity 100%; T_5: Inoculation with B. subtilis and irrigated at field capacity 70%; T_6: Inoculation with B. subtilis and irrigated at field capacity 35%; T_7: Inoculation with P. koreensis and irrigated at field capacity 100%; T_8: Inoculation with P. koreensis and irrigated at field capacity 70%; T_9: Inoculation with P. koreensis and irrigated at field capacity 35%; T_{10}: Inoculation with B. subtilis + P. koreensis and irrigated at field capacity 100%; T_{11}: Inoculation with B. subtilis + P. koreensis and irrigated at field capacity 70%; T_{12}: Inoculation with B. subtilis + P. koreensis and irrigated at field capacity 35%. RWC: Relative water content; TSS: Total soluble sugars.



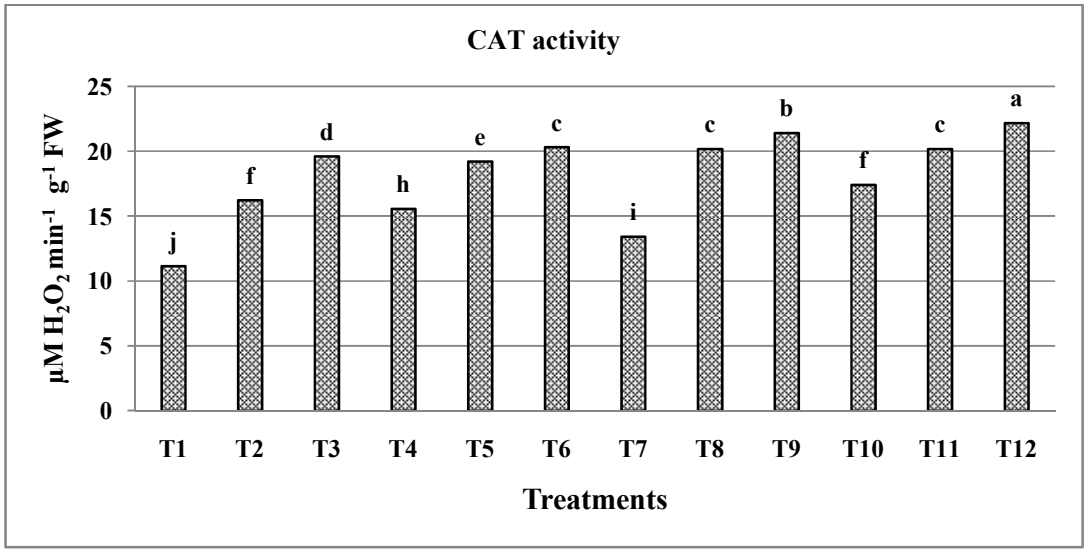


Fig. 3. Effect of different inoculation treatments on ascorbate peroxidase (APX) and catalase (CAT) contents of leaves wheat plant grown under drought-stress conditions T_1 *Control without inoculation and irrigated at field capacity 100%; **T_2:** Control without inoculation and irrigated at field capacity 70%;**T_3:** Control without inoculation and irrigated at field capacity 35%; **T_4:** Inoculation with B. subtilis and irrigated at field capacity 100%; **T_5:** Inoculation with B. subtilis and irrigated at field capacity 70%; **T_6:** Inoculation with B. subtilis and irrigated at field capacity 35%; **T_7:** Inoculation with P. koreensis and irrigated at field capacity 100%; **T_8:** Inoculation with P. koreensis and irrigated at field capacity 70%; **T_9:** Inoculation with P. koreensis and irrigated at field capacity 35%; **T_{10}:** Inoculation with B. subtilis + P. koreensis and irrigated at field capacity 100%; **T_{11}:** Inoculation with B. subtilis + P. koreensis and irrigated at field capacity 70%; **T_{12}:** Inoculation with B. subtilis + P. koreensis and irrigated at field capacity 35%. Bars with different letters are significantly different at P < 0:05 using Duncan's test*

Table 7. Effect of different inoculation treatments on grain yield, straw yield, biological yield and harvest index of wheat plant grown under drought-stress conditions

Treatment	Grain yield (ton ha^{-1})	Straw yield (ton ha^{-1})	Biological yield (ton ha^{-1})	Harvest index (%)
T_1	8.24 ^{c}	11.90 ^{c}	20.14 ^{d}	40.90 ^{d}
T_2	5.82 ^{f}	7.30 ^{g}	13.13 ^{h}	44.36 ^{b}
T_3	3.28 ^{h}	4.16 ^{i}	7.44 ^{k}	44.08 ^{b}
T_4	8.39 ^{bc}	12.14 ^{b}	20.53 ^{c}	40.86 ^{d}
T_5	6.23 ^{e}	8.45 ^{f}	14.68 ^{g}	42.44 ^{c}
T_6	3.56 ^{g}	4.22 ^{i}	7.79 ^{j}	45.78 ^{a}
T_7	8.61 ^{a}	12.17 ^{b}	20.78 ^{a}	41.42 ^{d}
T_8	6.33 ^{de}	8.61^{e}	14.94 ^{f}	42.35 ^{c}
T_9	3.57 ^{g}	4.22 ^{i}	7.80 ^{j}	45.81 ^{a}
T_{10}	8.52 ^{ab}	13.10 ^{a}	21.62 ^{a}	39.41 ^{e}
T_{11}	6.40 ^{d}	9.21 ^{d}	15.62 ^{e}	40.99 ^{d}
T_{12}	3.61 ^{g}	4.36 ^{h}	7.98 ^{i}	45.32 ^{a}
LSD 0.05	0.155**	0.065**	0.162**	0.691**

In a column means followed by a common letter are not significantly different at 5% level by DMRT; T_1: Control without inoculation and irrigated at felid capacity 100%; T_2: Control without inoculation and irrigated at field capacity 70%;T_3: Control without inoculation and irrigated at field capacity 35%; T_4: Inoculation with B. subtilis and irrigated at field capacity 100%; T_5: Inoculation with B. subtilis and irrigated at field capacity 70%; T_6: Inoculation with B. subtilis and irrigated at field capacity 35%; T_7: Inoculation with P. koreensis and irrigated at field capacity 100%; T_8: Inoculation with P. koreensis and irrigated at field capacity 70%; T_9: Inoculation with P. koreensis and irrigated at field capacity 35%; T_{10}: Inoculation with B. subtilis + P. koreensis and irrigated at field capacity 100%; T_{11}: Inoculation with B. subtilis + P. koreensis and irrigated at field capacity 70%; T_{12}: Inoculation with B. subtilis + P. koreensis and irrigated at field capacity 35%.

4. DISCUSSION

In arid and semi-arid regions, drought stress is considered a recurrent climatic factor faced by plants which lead to incidence changes physiological and biochemical processes that affect growth and yield. To allow plant growth in such stressful conditions, inoculation of plants with plant growth promoting rhizobacteria (PGPR) can increase the productivity of crops. Previous studies showed that applications of the plant with PGPR have the potential to ameliorate a drought stress [8,12,14,36,37]. Therefore, in the present study, we investigated the drought stress mitigation of wheat by inoculation with drought-tolerant PGPRs.

Screening of rhizobacteria was accomplished on the basis of their viable cell numbers as well as auxin production and phosphate solubilisation. Viable cell numbers of the tested strains showed survived poorly in the medium with increasing PEG concentrations. Also, all strains were able to grow in all tested PEG concentrations at up to 30%, only 2 strains (*B. subtilis* and *P. koreensis*) showed a stable growth even in the maximum 40% PEG concentration. These results may be due to an alteration of synthesis patterns of lipopolysaccharides and protein in bacteria subjected to adaptation of drought stress [16,38]. Among the tested strains, *P. koreensis* produced highest amount of IAA (1.84 µg ml^{-1}), and solubilize maximum amount of P (1.59 µg ml^{-1}) under stressed conditions (30%) when compared with other strains. This observation indicates superior performance of *B. subtilis* and *P. koreensis* in IAA production and solubilizing phosphorous and may also contribute to improved nutrition of plants growing under drought conditions [8,12,39,40,41,42].

In our study, *P. koreensis* improved FGP which recorded higher values followed by *B. subtilis* at 30% PEG and all strains led to a decrease of mean germination time at low PEG concentrations up to 10%. Also, the application of 10% PEG maximised the VI by 0.014 and 0.012 for *P. koreensis* and *B. subtilis* compared to control (no PEG). This result indicates that increasing PEG decreased relative water content in seedlings and inhibit of water absorption by seeds causing an inequity in the uptake of water and nutrients. So PGPR can improve the uptake of water and mitigate the poisonous effects [43,44].

On the other hand, in a gnotobiotic sand system experiment, the strains increased the fresh weight, dry weight, root and shoot length of wheat plants over uninoculated control both under unstressed and drought-stressed conditions. Among all strains, *P. koreensis* improved fresh weight, dry weight, root and shoot length recording 0.049 g $plant^{-1}$, 0.013 g $plant^{-1}$, 9.66 cm $plant^{-1}$ and 15.66 cm $plant^{-1}$, respectively under stressed-drought conditions. This finding was supported by Singh et al. and Egamberdieva et al. [45,46], who showed that inoculation of wheat and rice plants with PGPR under stress conditions results in increased root length, shoot length, fresh and dry weight. Also, a significant increase in proline content was higher 72.11% for *P. koreensis* over control under drought-stressed conditions. For root colonisation, *P. koreensis* and *B. subtilis* showed better results in case of root colonisation under drought-stressed conditions [8,12,46,47,48].

The potential of using PGPR *B. subtilis* and *P. koreensis* for improving growth, physiology, antioxidant activity and yield of wheat was evaluated under different drought stress applied in lyzimeter experiment conditions.

In general, our results revealed that drought stress applied had strong negative effects on the growth of uninoculated wheat plants, especially on height, dry weight of plant and chlorophyll content. Similarly, changes in different physiological and biochemical processes of the plant were observed due to drought stress. In contrast, a positive effect was observed in the growth of inoculated wheat plants. For example, height plant attained 65.33 for T_8 treatment (inoculation with *P. koreensis*) and 58.00 cm $plant^{-1}$ for T_5 treatment (inoculation with *B. subtilis*) compared to 52.36 cm $plant^{-1}$ for T_2 treatment (uninoculated control), at stress water 70% of field capacity. Similar trend was also exhibited in dry weight and chlorophyll content. This results are confirmed in wheat [8,15,49]; mung bean [42]; finger millet [36]; tomato [14].

In agreement with some of the earlier studies [37,48], our results demonstrated that two strains used in this study can protect the wheat plant from the deleterious effects caused by increased levels of drought stress. The carotenoids, total soluble sugar and relative water content of PGPR inoculated plants were observed to be

higher compared to that of uninoculated plants during different drought stress.

Among the antioxidant enzymes, the highest values were observed with *P. koreensis* (21.43 μM H_2O_2 min^{-1} g^{-1} FW) followed by *B. subtilis* (20.32 μM H_2O_2 min^{-1} g^{-1} FW) compared to control (19.60 μM H_2O_2 min^{-1} g^{-1} FW), under water stress conditions (35% of field capacity) for catalase activity. On the other hand, stress at 35% of field capacity, maximum increase 29.08% in APX and 27.38% in catalase activities due to dual inoculation treatments (T_{12}) with respect to the corresponding unstressed control (T_{10}). This finding may be due to drought pretreatment which it can improve the wheat plant to scavenge active oxygen and reduce adverse effects. Also, alleviation of water stress seems to bacterial inoculation [14,41].

In lyzimeter experiment, single and dual inoculation treatments showed a significant increase in grain yield, straw yield, biological yield and harvest index under different drought stress. These results may be related to the increased uptake of water and nutrients in plant tissues and led to improved nitrogen metabolism in different parts of the plant and reflected in better plant growth and yield. Indeed, these results corroborate those of Omara et al. Karimi et al. [38,50].

5. CONCLUSIONS

The present work aims to the utilisation of two superior strains *B. subtilis* and *P. koreensis* for plant growth promotion of wheat plant and alleviation of water stress. Positive changes in different physiological and biochemical processes of the plant were observed due to single or dual inoculation under different irrigation water stresses and these results are reflected in better plant growth and yield.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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