

HOSTED BY



Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

The influence of different bacteria consortium and irrigation levels on yield and physiological attributes of durum wheat (*Triticum durum* Desf.) grown under organic farming conditions



Arzu Mutlu

Department of Organic Agriculture, Akcakale Vocational High School, Harran University, Akcakale District, Sanliurfa, Turkey

ARTICLE INFO

Article history:

Received 13 June 2022

Revised 2 September 2022

Accepted 5 October 2022

Available online 12 October 2022

Keywords:

Durum wheat
Bacteria consortia
Sugar content
Grain yield
Organic farming
Turkey

ABSTRACT

Background: Inorganic fertilizers help to prevent yield and quality losses in agricultural production; however, adversely affect soil quality and human health. The rising public awareness is switching the production focus from conventional to organic farming. Therefore, obtaining higher yields under organic farming conditions is inevitable. Different bacterial consortia have been found helpful in improving crop yields under stressful and benign environments. Therefore, current study tested the impact of two bacterial consortia on yield and physiological attributes of durum wheat (*Triticum durum* Desf.) grown under organic farming conditions with two different irrigation levels.

Methods: Two bacteria consortiums [(consortium 1 = *Bacillus megaterium* RCK-869 + *Pantoea agglomerans* RK-120 + *Paenibacillus polymyxa* RCK-540 + *Bacillus subtilis* RCK-561), (consortium 2 = *Azospirillum*, *Rhizobium*, *Azotobacter* and *Acetobacter*) and two irrigation levels, i.e., 50 and 100% of the required moisture were included in the study. Data on chlorophyll index, flag leaf area, soluble sugar content, plant height, protein ratio, and grain yield were recorded.

Results: The GY in bacteria-free treatment was 3512.0 kg ha⁻¹, which increased to 4868.3 kg ha⁻¹ with the application of bacteria consortium 1. The application of 50% irrigation significantly reduced the yield and related traits, whereas the application of bacteria consortium 1 significantly improved these traits under normal and 50% irrigation. Overall, higher values of the studied traits were recorded during 1st year than 2nd year. The decrease in total precipitation and relative humidity, and the increase in temperature in the second year caused a decrease in grain yield, agronomic and physiological parameters.

Conclusion: Bacteria consortium consisting of *Bacillus* bacteria significantly improved the yield and related traits of durum wheat under deficit and normal irrigation. Therefore, bacterial consortium consisting of *B. megaterium* + *P. agglomerans* + *P. polymyxa* + *B. subtilis* could be used to improve durum wheat production under organic farming conditions and deficit irrigation.

© 2022 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Wheat is one of the most widely produced cereals in the world and in Turkey. Approximately 25% of the 790 million tons of wheat produced in the world is durum wheat (FAO, 2022). In Turkey, approximately 25% of total wheat production consists of durum wheat (TÜİK, 2022)). Southeastern Anatolia region of Turkey is

E-mail address: arzu.usm@gmail.com

Peer review under responsibility of King Saud University.



the homeland of durum wheat, and rich in genetic resources. This region is a special area where quality durum wheat with high protein content is produced. High temperatures during the grain filling period increases protein ratio; thus, helps to obtain uniform and high-quality wheat grains. The world population is expected to reach approximately 9.5 billion in 2050 (Grim et al., 2015); therefore, crop production per unit area must be increased to meet the needs of the increasing population. Environmentally friendly practices should be adopted to conserve soil and ecosystem while increasing crop yields. Many toxic and hazardous chemicals are used in conventional agriculture, which contaminate food, soil, underground and surface waters. Organic agriculture, integrated management and good agricultural practices are the alternative solutions to increase the quality and yield in agricultural

<https://doi.org/10.1016/j.jksus.2022.102366>

1018-3647/© 2022 The Author(s). Published by Elsevier B.V. on behalf of King Saud University.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

production. The use of plant growth promoting rhizobacteria (PGPR) has been introduced to protect plants against from pathogen infestation and promote plant growth with lesser use of inorganic chemicals (Mehboob et al., 2022, 2021; Sagar et al., 2021). The use of bacteria has been proposed as an effective and environment-friendly alternative to chemical fertilizers in crop production (Smith et al., 2016).

The PGPRs improve biological nitrogen fixation, synthesis of plant growth hormones (IAA, gibberellic acid, cytokinin, and ethylene), production of siderophores, increase availability of nutrients such as zinc, potassium and phosphorus (Egamberdieva et al., 2015; Hu et al., 2017; Verma et al., 2015), and promote uptake of macro- and micronutrients in the soil by increasing root fringing (Emami et al., 2019, 2018). Previous studies reported that bacteria improve soil aggregation, facilitate infiltration and storage of water, increase plant resistance to low temperature, drought, and soil salinity (Mehboob et al., 2021; Ullah et al., 2020). Shah et al. (2021) reported that bacterial applications increased wheat grain yield by improving plant height by 30%, spike length by 20%, leaf area by 44% and number of grains per spike by 25% compared to the bacteria-free treatment. Similarly, Sheirdil et al. (2019) stated that bacterial applications increased tillering by 25%, and improved IAA synthesis and nitrogen and phosphorus use without any decrease in yield and yield components. In addition, free nitrogen binding bacteria caused 11% increase in wheat yield due to inoculant properties.

Soil bacteria with different genes including *Pseudomonas*, *Bacillus*, *Azospirillum*, *Azotobacter*, *Burkholderia*, *Enterobacter* and *Rhizobium* help plants to produce siderophore and absorb iron in soils, and improve uptake of micronutrients such as iron and zinc; thus, increase plant growth and yield (Gouda et al., 2018). The studies showed that bacterial applications increased the availability of phosphorus by dissolving insoluble phosphorus in soils through the production of organic acids and acid phosphates, lowering soil pH, dissolution of mineral phosphate, all of which increase the availability of phosphorus (Emami et al., 2019, 2018). Co-application of organic fertilizer and bacteria increased wheat grain yield by 20%, the number of grains per ear by 35% and the plant height by 9% (Mahato and Kafle, 2018). In addition, the bacterial applications caused 40% increase in root growth, 12% increase in stem development and 16% increase in grain yield of wheat (Naiman et al., 2009).

Over the last two decades, researchers have studied the effects of co-inoculation with two or more PGPRs on a single plant (Mpanga et al., 2019). When comparing inoculation of single bacterium, the use of bacterial consortia has increased in the production and development of different crops (Molina-Romero et al., 2021). Different abiotic stresses, i.e., moisture deficiency (Gamalero and Glick, 2022), salinity (Ahmad et al., 2013), and heavy metals have been mitigated by inoculation of PGPR consortia (Hassan et al., 2014). Plants injected with a bacterial consortium needed lesser chemical fertilizers to produce high yields (Shahzad et al., 2013) However, these consortia have been rarely tested to improve durum wheat production under moisture deficiency and in organic farming conditions.

Moisture deficiency in wheat significantly reduce yield and physiological attributes (Farooq et al., 2015, 2017). The application of bacteria in wheat reduces drought stress (Ilyas et al., 2020) due to physiological, enzymatic, and biochemical changes in the plants. The bacterial applications in water shortage cause synthesis of siderophore, phytohormones and enzymes in wheat plants, and especially bacillus bacteria increase tolerance of plants to abiotic stress by regulating the ethylene level (Lastochkina et al., 2019). Additional irrigations in wheat increase nitrogen availability in soils, improve grain yield and yield components, however, yield and yield components are decreased under limited irrigation (Liu

et al., 2022; Yu et al., 2020). Similarly, Ilyas et al. (2020) indicated that sufficient irrigation and bacteria application increase grain yield and yield components.

Although there are reports indicating the yield improvement in wheat under different abiotic stresses, the use of bacterial consortia to improve durum wheat production under organic farming is rarely tested. Furthermore, the interactive effect of deficit and sufficient irrigation and bacterial consortium on durum wheat production under organic farming conditions in southeastern Anatolia region has never been explored. Therefore, this study investigated the physiological and agronomic changes in durum wheat plants grown in a semi-arid region with different bacterial consortia and irrigation levels. It was hypothesized that deficit irrigation will reduce yield and related traits, whereas the use of bacterial consortia will reverse the impacts of deficient irrigation.

2. Materials and methods

2.1. Climate and characteristics of plant material

The 'Cesare' durum wheat variety widely cultivated in Mediterranean countries and the world, was used as plant material. Field experiment was conducted during 2018–19 and 2019–20 durum wheat growing seasons in organic farming experimental fields at Akcakale Vocational School, Harran University, Sanliurfa Turkey. The study area is situated between 36° 43' 10" N, 38 ° 56' 48" E latitudes and 362 m altitude.

The relative humidity and total precipitation in the first year were higher than second year, while the temperature values were lower (Table 1). The total precipitation and relative humidity in second year decreased and temperature increased in March, April, and May. The amount of precipitation during the experiment was lower than long-term and temperature was higher. The weather data of the study area during growing seasons are given in Table 1.

2.2. Characteristics of soil, bacteria, and farmyard manure

The physical and chemical properties of the soil in the experimental field are given in Table 2. Mean electrical conductivity (EC) value indicated no salinity problem. The soil was clayey and organic matter content was 1.24%. Soil was rich in potassium, while poor in zinc (Table 2).

The chemical properties of the farmyard manure used in the experiment are given in Table 3. Fermented manure was rich in organic matter, pH was suitable for plant growth, and macro- and micronutrient contents were sufficient. In addition, two different liquid solutions consisting of bacterial consortia were applied during the stem elongation period.

2.3. Characterization of bacteria consortium

Bacteria consortium 1 consisted of *Bacillus megaterium* RCK-869, *Pantoea agglomerans* RK-120, *Paenibacillus polymyxa* RCK-540 and *Bacillus subtilis* RCK-561. Total number of living microorganisms was 1×10^7 cfu ml⁻¹. Application dose of the consortia was 1000 ml per hectare. Bacterial isolates were mixed in 20 L water to contain 100 ml of living bacteria, sugar was added, and kept in a closed environment for 24 h. The mixture was applied with a backpack sprayer during the stem elongation period.

Bacteria consortium 2 consisted of *Azospirillum*, *Rhizobium*, *Azotobacter* and *Acetobacter*. Total number of living microorganisms was 1×10^6 cfu ml⁻¹. Application dose of the consortia was 3000 ml per hectare. Bacterial isolates were mixed in 10 L water to contain 100 ml of living bacteria, sugar was added, and kept in

Table 1
The long term and season data of the experimental site.

		November	December	January	February	March	April	May	June
Total precipitation (mm)	2018–19	177.6	125.4	75.6	79.6	115.6	104.8	10.2	0.8
	2019–20	2.6	126.2	25.2	3.0	83.6	18.4	0.2	0.0
	Long-term	49.9	76.8	78.0	67.9	63.5	44.4	26.8	5.4
The highest temperature (°C)	2018–19	28.0	18.1	17.5	17.9	21.3	26.7	40.0	44.2
	2019–20	28.1	19.6	14.2	20.6	26.9	29.2	38.8	41.6
	Long-term	29.2	26.0	20.5	25.5	29.5	36.4	40.3	44.1
The lowest temperature (°C)	2018–19	3.3	–1.2	–3.8	0.4	0.5	4.5	9.5	17.4
	2019–20	0.5	0.7	–2.0	–7.8	1.9	4.2	10.1	13.2
	Long-term	–2.7	–6.4	–6.8	–9.3	–7.3	–0.7	7.4	12.3
Mean temperature (°C)	2018–19	18.1	12.8	11.6	14.0	16.4	20.6	32.2	37.9
	2019–20	22.6	13.5	11.8	12.5	19.8	24.2	31.0	36.5
	Long-term	12.9	7.7	5.9	7.3	11.4	16.6	22.5	28.5
Mean relative humidity (%)	2018–19	81.0	89.4	79.3	79.3	75.5	73.1	42.3	34.4
	2019–20	47.2	85.1	76.8	71.5	70.5	64.1	45.9	33.9
	Long-term	61.1	70.3	70.6	66.4	60.5	61.0	56.1	46.0

Table 2
Physical and chemical characteristics of experimental soil.

Soil properties	2018/19	2019/20
EC (dS m ⁻¹)	0.73	0.98
pH	8.37	8.12
Organic matter (%)	1.14	1.34
Lime (%)	36.66	34.84
Sand (%)	13.95	13.43
Clay (%)	67.12	68.22
Silt (%)	19.57	18.35
P ₂ O ₅ (kg ha ⁻¹)	66.22	62.34
K ₂ O (kg ha ⁻¹)	1896	2235
Cu (mg kg ⁻¹)	1.52	1.41
Mn (mg kg ⁻¹)	4.35	4.65
Fe (mg kg ⁻¹)	4.73	4.87
Zn (mg kg ⁻¹)	0.85	0.97

Table 3
Chemical characteristics of farmyard manure used in the experiment.

Characteristic	Value
Organic matter content (%)	40.2
Total nitrogen (%)	2.1
Organic nitrogen (%)	2.2
pH	6.5–8.5
Humic and Fulvic Acid (%)	28.2
Potassium (%)	2.1
Phosphorus (%)	2.3
Magnesium (%)	1.1
Iron (%)	0.2
Zinc (mg kg ⁻¹)	129.5
Manganese (mg kg ⁻¹)	90.6

a closed environment for 24 h. The mixture was applied with a backpack sprayer during the stem elongation period.

2.4. Experimental setup

The experiment was laid out split-plot randomized blocks with 3 replications. The irrigation treatments (50% deficit irrigation, 100% full irrigation) were placed in the main plots, and bacteria treatments (without bacteria (control), and consortia) were randomized in the sub-plots. Before sowing, 20 t ha⁻¹ farmyard manure was applied homogeneously to the experimental field.

2.5. Cultural practices

Sowing was done on 15 November 2019 and 20 November 2020 during 1st and 2nd year, respectively. The seeds were manually

sown on the opened incisors. Each plot had 6 rows with 5 m length and 20 cm interrow spacing (5 m × 1.2 m = 6 m²). Sowing depth was 4–6 cm and the number of seed per unit area was 475 m⁻². An isolation distance of 1 m was maintained between plots and 3 m between blocks. Chemicals were not used in the experimental field. Fermented manure was applied at a rate of 20 t ha⁻¹ to the whole experimental field before sowing in both years, and the manure was distributed homogeneously with a rake and mixed into the soil. Irrigation treatments composed of full irrigation (100%) and deficit irrigation (50%). The irrigation method was drip irrigation. Plants were irrigated from sowing to the physiological maturity period (in all growth stages of the wheat plants).

2.6. Irrigation process

The irrigation water was applied to bring moisture content of soil to the field capacity. The experimental field was irrigated with the sprinkler irrigation system to ensure homogeneous germination before the drip system was installed. The irrigation was carried out at 7-day intervals considering the moisture content at 0–60 cm soil depth. In deficit irrigation, 50% of the water calculated for full irrigation was applied.

Soil samples were collected from 0 to 30 and 30–60 cm soil depths of the middle-replicated plots before each irrigation, and the soil moisture content was determined. The amount of irrigation water to bring the moisture content to the field capacity was applied using a water meter. Plant water consumption was calculated by the moisture depletion method based on the water balance equation. The water budget equation (Eq. (1)) was used to calculate the plant water consumption.

$$ET = I + P + K - D - R \pm \Delta S \quad (1)$$

In the equation, I is irrigation water (mm), P is precipitation (mm), K is capillary rise (mm), D is infiltration (mm), R is runoff (mm), ΔS is moisture loss in soil profile (mm), and ET stands for plant water consumption (mm).

The value of I was calculated using the following equation (Eq. (2)).

$$I = Qfc - Qc \quad (2)$$

In the equation, Qc is the current moisture content before irrigation (mm), Qfc stands for the field capacity moisture content (mm).

The volume of water to be given for each plot was calculated by multiplying the total water amount, the plot size, the deficit ratio (1 and 0.50) and the cover percentage (Eq. (3)).

$$V = dT \times A \times U_o \times P \quad (3)$$

In the equation, V is the volume of water to be applied for each plot (L), dT is total amount of water for effective root depth, A is plot area (m²), U_o is the deficit ratio (%) and P is the cover percentage. The coverage percentage was considered 1.0 in all treatments.

Harvest time was carefully determined to prevent grain loss. The wheat was harvested on 13th and 9th June in the first and second year of the experiment, respectively.

2.7. Data collection

The grain yield of each plot was determined at harvest. The whole plots were harvested, and plot yields were converted to kg ha⁻¹. The distance from the crown to the tip of flag leaf was measured to determine plant height before harvest. The chlorophyll index of three leaves in each plant was measured during the flowering period after bacterial consortia applications using a SPAD meter (Model 502, Spectrum Technologies, Plainfield, IL, USA).

Leaf samples were collected during the flowering period to determine the soluble sugar content. Fresh leaf samples (0.1 g) were mixed with 80% methanol (3 ml), and the solution was heated in a water bath at 70 °C for 30 min. An equal volume of 5% phenol and 0.5 ml extract was mixed with 1.5 ml of concentrated sulfuric acid and incubated in the dark for 30 min. The absorbance of solutions at 490 nm was determined using a spectrophotometer. Standard curve for glucose solution was prepared to determine the sugar content and expressed in mg g⁻¹ FW (Ilyas et al., 2020).

Flag leaf area (FLA) was determined in samples collected during flowering period. The FLA was determined as an average of ten leaves using the Eq. (4).

$$FLA = FLL \times width \ at \ center \times 0.95 \quad (4)$$

In the equation FLL is flag leaf length.

Protein ratio (PR) of grains were determined using ICC standard method (AACC Method 46–30). Two-hundred gram of grain samples were taken the harvested plots, and straw and stones were removed. The PR of samples was determined using the FOSS NIRS 6500 spectrophotometer calibrated by Celdhl NIT (near infrared transmittance) technique.

2.8. Data analysis

The effects of treatments on yield and yield components were determined using variance analysis (ANOVA). The differences among bacterial consortia and irrigation treatment were tested using a least-significance difference test (LSD) at 95% probability level, where ANOVA indicated significant differences. All statistical analysis of the data were analyzed using JUMP 13.2.0 statistical software. The differences among years were significant; therefore, data of the years were analyzed separately. Similarly, two-way interaction of irrigation levels and bacterial consortia were significant for all parameters; therefore, only interactions were presented and interpreted.

3. Results

Grain yield (GY), protein ratio (PR), leaf area (LA), chlorophyll index (CI) and soluble sugar content (SSC) significantly differed among years at $p \leq 0.01$ level, while plant height (PH) differed at $p \leq 0.05$ level. The effect of bacteria consortium and irrigation treatments on PH was significant at $p \leq 0.01$ and $p \leq 0.05$, respectively. The interaction of irrigation and bacteria consortium had a significant effect ($p \leq 0.01$) on GY, PH, PR, LA and SSC parameters,

while interactive effect on CI was significant at $p \leq 0.05$ level (Table 4).

The values of GY, PH and PR were significantly different among years, bacteria applications and irrigation treatments (Table 5). The GY in the first year of the experiment was 4401.39 kg ha⁻¹, while decreased to 4229.66 kg ha⁻¹ in the second year. The highest GY (4868.33 kg ha⁻¹) throughout the experiment was obtained with the application of consortium 1, followed by consortium 2 (4566.25 kg ha⁻¹) and bacteria free (3512.00 kg ha⁻¹) application. The grain yield loss in bacteria-free treatment at 50% water deficit was 13.13% compared to full irrigation, while the grain yield loss in consortium-1 and consortium-2 treatments was 3.70 and 4.20%, respectively. The least grain yield loss in water deficit \times consortium-1 interaction indicates that consortium-1 improved the tolerance of wheat plants to water stress.

Plant height in 2019 and 2020 was 91.55 and 88.29 cm, respectively. The plants were shorter in deficit irrigation compared to full irrigation. Deficit irrigation compared to full irrigation caused a 11.79, 0 and 0.70% decrease in plant heights in the bacteria-free, consortium-1 and consortium-2 applications. The difference in plant heights between full irrigation \times consortium-1 and deficit irrigation \times consortium-1 interactions was not statistically significant.

Abruptly suppressing temperature and drought during the grain filling period promoted protein accumulation in wheat. The highest protein content (14.70%) was obtained in the second year of the experiment with consortium 1 treatment, while the lowest protein content (11.37%) was obtained in bacteria-free treatment in the first year. The protein ratios were slightly increased in the deficit irrigation compared to the full irrigation. The bacteria consortium treatments yielded similar results that the increase in protein content under water deficit conditions.

The effect of year and irrigation and bacterial consortium treatments on FLA, CC and SSC values were statistically significant (Table 6). The highest FLA (35.42 cm²) in bacterial consortium treatments was measured in the consortium-1 application, while the FLA value decreased by 3.39 and 16.74% in the consortium-2 and bacteria-free treatments, respectively.

The CI decreased in deficit irrigation compared to full irrigation. Bacterial treatments helped wheat plants to tolerate the adverse effects of deficit irrigation, and this tolerance was at the highest level in consortium-1 treatment.

The SSC is one of the important quality parameters of wheat. The SSC values in the deficit irrigation increased by 11.24, 18.83 and 21.92% in the bacteria-free, consortium-1 and consortium-2 treatments, respectively compared to the full irrigation. The results showed that drought stress promoted SSC.

The lowest grain yield was obtained in bacteria free \times deficit irrigation and bacteria free \times full irrigation treatments, while the highest grain yield was obtained in the bacteria 1 \times full irrigation treatment (Table 4). However, grain yield in the deficit irrigation \times consortium 1 treatment was very close to the full irrigation \times consortium 1 application.

4. Discussion

A certain level of irrigation was carried out in addition to the precipitation occurred in both years of the experiment. However, the sudden suppression of temperatures and low humidity, especially in the post-spiking period, caused an increase in the protein content of the grain, a decrease in the starch ratio, and thus a decrease in grain yield. The result clearly showed that drought, which is one of the most important abiotic stress factors, reduces the plant growth and quality. The bacteria application increases the phosphorus content of wheat from 41.2 to 96.4% by increasing

Table 4

Analysis of variance (mean squares) for yield and yield components of durum wheat grown under organic farming conditions with different irrigation levels and bacteria consortium.

Source	DF	GY (kg ha ⁻¹)	PH (cm)	PR (%)	LA (cm ²)	CI (SPAD)	SSC (mg g ⁻¹ FW)
Year (Y)	1	265397**	95.09*	0.75**	37.86**	43.56**	1.32**
Irrigation levels (I)	1	761256**	116.89*	0.35**	4.97**	15.21**	106.44**
Y × I	1	2584.03 ns	0.198 ns	0.02 ns	0.060 ns	0.01 ns	0.0064 ns
Bacteria consortium (BA)	2	6084675**	798.40**	14.67**	117.93**	1149.98**	65.69**
Y × BA	2	1287 ns	1.70 ns	0.126*	0.091 ns	0.22 ns	0.01 ns
I × BA	2	92381**	95.60**	2.98**	0.37**	1.75*	4.80**
Y × I × BA	2	71.5278 ns	0.49 ns	0.007 ns	0.03 ns	0.003 ns	0.02 ns
Error	16	2501	9.66	0.031	0.03	0.40	0.04

Here, DF = degree of freedom, GY = grain yield, PH = plant height, PR = protein ratio, CI = chlorophyll index, SSC = soluble sugar content, * = significant at 95% probability, ** = significant at 99% probability, ns = non-significant, DF = degrees of freedom.

Table 5

The influence of different irrigation levels and bacteria consortium on grain yield, plant height, and protein ratio of durum wheat grown under organic farming conditions.

	2018–2019		2019–2020	
	50%	100%	50%	100%
<i>Grain yield (kg ha⁻¹)</i>				
Bacteria free	3356.66f	3835.00e	3174.00f	3682.33e
Bacteria-1	4860.00b	5030.00a	4693.33b	4890.00a
Bacteria-2	4576.66d	4750.00c	4360.00d	4578.33c
LSD 0.05	99.31		88.15	
<i>Plant height (cm)</i>				
Bacteria free	77.56c	87.96b	73.73c	83.56b
Bacteria-1	97.83 a	97.36 a	94.53a	95.10a
Bacteria-2	94.06 ab	94.50 ab	91.00a	91.86a
LSD 0.05	6.53		5.03	
<i>Protein ratio (%)</i>				
Bacteria free	11.37d	12.42c	11.83e	12.69d
Bacteria-1	14.59a	13.90b	14.70 a	13.91b
Bacteria-2	13.58b	12.78c	14.03b	13.23c
LSD 0.05	0.36		0.25	

Here, 50% = water deficit, 100% = full irrigation, the means indicated with the same letter in the same column and row are non-significant at 95% probability.

Table 6

The influence of different irrigation levels and bacteria consortium on flag leaf area, chlorophyll index and soluble sugar content of durum wheat grown under organic conditions.

	2018–2019		2019–2020	
	50%	100%	50%	100%
<i>Flag leaf Area (cm⁻²)</i>				
Bacteria free	30.50d	30.71d	28.09	28.67
Bacteria-1	36.13b	36.76 a	33.99	34.80
Bacteria-2	34.60c	35.73b	32.75	33.83
LSD 0.05	0.41		NS	
<i>Chlorophyll index (SPAD)</i>				
Bacteria free	42.43e	44.63d	40.40e	42.53d
Bacteria-1	61.10ab	61.90a	58.66ab	59.30a
Bacteria-2	59.03c	60.06bc	57.03c	58.03b
LSD 0.05	1.54		0.66	
<i>Soluble sugar content (mg g⁻¹ FW)</i>				
Bacteria free	17.36c	15.43 d	17.86c	15.83d
Bacteria-1	22.63a	18.23b	22.83a	18.66b
Bacteria-2	18.30b	14.23e	18.66b	14.63e
LSD 0.05	0.54		0.39	

Here, 50% = water deficit, 100% = full irrigation, the means indicated with the same letter in the same column and row are non-significant at 95% probability, NS = non-significant.

the available water and nutrient contents in soils (Wang et al., 2020). In addition, the application of bacteria increased the grain yield by 59% by increasing availability of phosphorus to plants (Sheirdil et al., 2019). Khan et al. (2020) reported that nitrogen is one of the most important nutrients, and the bacteria containing *Bacillus* species have a high ability to fix atmospheric nitrogen, which encourages plant growth. The studies revealed that *Bacillus* bacteria species produce high concentrations of IAA (Khan et al.,

2020). The IAA promotes plant growth by increasing tillering by about 25% and reduces fertilizer use in wheat. Therefore, several studies reported that the bacteria application increases spike length, plant height, number of tillering, thousand grain weight and grain yield (Shah et al., 2021; Sheirdil et al., 2019).

Plant height is one of the most studied characteristics that affect yield and yield elements in wheat. The bacteria contribute to plant growth by increasing availability of nitrogen, phosphorus and

potassium in soils and cause IAA synthesis. The IAA improves cell division, root growth and stem elongation by increasing the surface area of the roots so that plants can obtain water and nutrients (Wang et al., 2020). In addition, the bacteria application also promotes nitrogen content and vegetative growth in the plant, which in turn increases the plant height (Mpanga et al., 2019). The comparison of bacteria free and bacteria added treatments revealed that the use of bacteria increased plant height even in water deficit conditions (Mehboob et al., 2022, 2021).

The CI and FLA values are directly proportional to each other. The decrease in CI decreases in FLA values. The decrease in leaf area caused a decrease in the amount of photosynthesis and thus a decrease in the CC of wheat leaves (Farooq et al., 2015, 2017). The plant, exposed to water stress due to drought, closes its stomata, minimizing the level of transpiration. Therefore, photosynthesis is decreased, and carbon dioxide uptake is reduced under water stress, and plant growth is adversely affected (Onen et al., 2017). In addition, drought stress causes an increase in ethylene production, which disrupts the integrity of cell membrane by disruption of oil molecules due to the direct contact with chloroplast, which activates the chlorophyllase gene. The activation of the chlorophyllase gene causes a severe reduction of chlorophyll production in plants. The decrease in chlorophyll production constrains the root growth and stem elongation.

Wheat plants reduce chlorophyll content to tolerate drought stress. The bacteria application in arid regions improves root growth, nitrogen, and phosphorus uptake, and increases photosynthesis and chlorophyll content. In addition, the use of bacteria increases IAA production (Emami et al., 2019) and chlorophyll synthesis through increasing the water and osmotic potential in plants (Ilyas et al., 2020).

Sugar concentration in the leaves was significantly altered in bacteria-treated plants. The sugar concentration in water-deficit plants was higher than in plants grown under full irrigation. Previous studies indicated that the application of bacteria helps plants in regulating osmotic pressure by increasing the sugar concentration, especially in arid conditions (Ullah et al., 2020). Osmolyte accumulation in a plant is an indicator of drought tolerance of the plant, because osmolyte prevents water loss and regulates osmosis. Soluble sugar accumulation caused by the bacteria improves drought tolerance in plants.

The bacterial applications have been reported reducing oxidative stress by increasing the antioxidant activities. In addition, foliar application caused a significant increase in sugar concentration in the leaves. Consistent with our findings, Ilyas et al. (2020) also reported increasing soluble sugar contents under drought stress conditions.

The results obtained in this study suggested that the decrease in photosynthesis under drought stress was occurred due to the closure of the stomata and the deterioration in metabolic processes, while the closure of the stomata caused a decrease in the transpiration rate, CO₂ entry and nutrients, and deterioration of metabolic activities such as photosynthesis (Jha et al., 2014). Leaf area decreased in plants exposed to drought conditions in both experimental years, on the contrary, leaf area increased with the use of bacteria even under deficit water treatments. Leaf area is very important for in wheat plants to tolerate water stress under drought conditions. The drought tolerance of plants is closely associated with the size of leaf area (Hussain et al., 2018). The studies revealed that leaf area increased with the use of bacteria compared to the control (Shah et al., 2021).

The IAA is a hormone that increases the rooting area by increasing the root growth and root length, thus increasing the leaf area by providing more nutrients; thus, directly promote plant growth (Shah et al., 2021). The results of our study revealed that the bacteria used increased the production of IAA even in the water deficit

treatment, helped the plant to perform more photosynthesis and increased the leaf area of the plant.

The main criterion that determines the quality of durum wheat are the hardness and vitreousness of the grains. The higher the glassiness of the grain, the higher the protein ratio. The protein content of the grain was higher in the driest year of the experiment. The protein content of grains is affected by the changes in climatic conditions, especially the temperature and precipitation during the grain filling period, and the cultural practices such as top fertilizer and other cultural practices. Although drought stress significantly reduces protein production in grain, protein content of grains under bacterial application increased due to the stimulating effect of bacteria. The plants treated with bacteria consortium probably synthesized higher protein than control plants and leading to the expression of new proteins that confer drought tolerance on plants. In addition, bacteria can promote the synthesis of heat shock proteins that bind to other proteins under stress conditions. The heat shock proteins help rapid protein synthesis under drought stress conditions due to containing high ribosomes contents (Tas and Mutlu, 2021).

5. Conclusion

Water is an absolute necessary natural resource for biochemical activities. Plant growth, development and productivity depend on the availability of sufficient water in soils and the appropriate osmotic balance. The wheat plants were grown under deficit and full irrigation, and two living bacteria consortia were applied to the leaves during the stem elongation period. The results revealed that applications of bacterial consortium consisting of *Bacillus* bacteria significantly improved durum wheat yield under full and deficit irrigation conditions. The foliar application of bacteria consortium improved the nutrient use of plants even under the deficit water conditions. The results revealed that bacteria consortium consisting of *Bacillus* bacteria species could be used to alleviate the effect of drought stress. The results obtained provide valuable contributions to the literature on alleviating the effects of drought stress and adapting wheat farming to arid and semi-arid areas.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- Ahmad, M., Zahir, Z.A., Khalid, M., Nazli, F., Arshad, M., 2013. Efficacy of Rhizobium and Pseudomonas strains to improve physiology, ionic balance and quality of mung bean under salt-affected conditions on farmer's fields. *Plant Physiol. Biochem.* 63, 170–176. <https://doi.org/10.1016/j.plaphy.2012.11.024>.
- Egamberdieva, D., Wirth, S., Alqarawi, A.A., Abd Allah, E.F., 2015. Salt tolerant *Methylobacterium mesophilicum* showed viable colonization abilities in the plant rhizosphere. *Saudi J. Biol. Sci.* 22, 585–590. <https://doi.org/10.1016/j.sjbs.2015.06.029>.
- Emami, S., Alikhani, H.A., Pourbabaee, A.A., Etesami, H., Motashare Zadeh, B., Sarmadian, F., 2018. Improved growth and nutrient acquisition of wheat genotypes in phosphorus deficient soils by plant growth-promoting rhizospheric and endophytic bacteria. *Soil Sci. Plant Nutr.* 64, 719–727. <https://doi.org/10.1080/00380768.2018.1510284>.
- Emami, S., Alikhani, H.A., Pourbabaee, A.A., Etesami, H., Sarmadian, F., Motesharezadeh, B., 2019. Effect of rhizospheric and endophytic bacteria with multiple plant growth promoting traits on wheat growth. *Environ. Sci. Pollut. Res.* 26, 19804–19813. <https://doi.org/10.1007/s11356-019-05284-x>.
- FAO, 2022. FAO [Food and Agriculture Organization]. URL www.faostat.fao.org.
- Farooq, S., Shahid, M., Khan, M.B., Hussain, M., Farooq, M., 2015. Improving the productivity of bread wheat by good management practices under terminal drought. *J. Agron. Crop Sci.* 201, 173–188. <https://doi.org/10.1111/jac.12093>.

- Farooq, S., Hussain, M., Jabran, K., Hassan, W., Rizwan, M.S., Yasir, T.A., 2017. Osmopriming with CaCl₂ improves wheat (*Triticum aestivum* L.) production under water-limited environments. *Environ. Sci. Pollut. Res.* 24, 13638–13649. <https://doi.org/10.1007/s11356-017-8957-x>.
- Gamalero, E., Glick, B.R., 2022. Recent advances in bacterial amelioration of plant drought and salt stress. *Biology (Basel)* 11, 437.
- Gouda, S., Kerry, R.G., Das, G., Paramithiotis, S., Shin, H.-S., Patra, J.K., 2018. Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. *Microbiol. Res.* 206, 131–140. <https://doi.org/10.1016/j.micres.2017.08.016>.
- Grim, B., Johnson, T., Skirbekk, V., Zurlo, G., 2015. Global Population Projections by Religion: 2010–2050, in: *Yearbook of International Religious Demography 2015*. BRILL, pp. 99–116. https://doi.org/10.1163/9789004297395_004.
- Hassan, W., Bano, R., Bashir, F., David, J., 2014. Comparative effectiveness of ACC-deaminase and/or nitrogen-fixing rhizobacteria in promotion of maize (*Zea mays* L.) growth under lead pollution. *Environ. Sci. Pollut. Res.* 21, 10983–10996. <https://doi.org/10.1007/s11356-014-3083-5>.
- Hu, J., Wei, Z., Weidner, S., Friman, V.-P., Xu, Y.-C., Shen, Q.-R., Jousset, A., 2017. Probiotic *Pseudomonas* communities enhance plant growth and nutrient assimilation via diversity-mediated ecosystem functioning. *Soil Biol. Biochem.* 113, 122–129. <https://doi.org/10.1016/j.soilbio.2017.05.029>.
- Hussain, M., Farooq, S., Hasan, W., Ul-Allah, S., Tanveer, M., Farooq, M., Nawaz, A., 2018. Drought stress in sunflower: Physiological effects and its management through breeding and agronomic alternatives. *Agric. Water Manage.* <https://doi.org/10.1016/j.agwat.2018.01.028>.
- Ilyas, N., Mumtaz, K., Akhtar, N., Yasmin, H., Sayyed, R.Z., Khan, W., Enshasy, H.A.E., Dailin, D.J., Elsayed, E.A., Ali, Z., 2020. Exopolysaccharides producing bacteria for the amelioration of drought stress in wheat. *Sustainability* 12, 8876. <https://doi.org/10.3390/su12218876>.
- Jha, U.C., Chaturvedi, S.K., Bohra, A., Basu, P.S., Khan, M.S., Barh, D., 2014. Abiotic stresses, constraints and improvement strategies in chickpea. *Plant Breed.* 133, 163–178.
- Khan, M.S., Gao, J., Chen, X., Zhang, M., Yang, F., Du, Y., Moe, T.S., Munir, I., Xue, J., Zhang, X., 2020. Isolation and characterization of plant growth-promoting endophytic bacteria *Paenibacillus polymyxa* SK1 from *Lilium lancifolium*. *Biomed Res. Int.* 2020, 1–17. <https://doi.org/10.1155/2020/8650957>.
- Lastochkina, O., Aliniaiefard, S., Seifikalhor, M., Yuldashev, R., Pusenkova, L., Garipova, S., 2019. Plant Growth-Promoting Bacteria: Biotic Strategy to Cope with Abiotic Stresses in Wheat, in: *Wheat Production in Changing Environments*. Springer Singapore, Singapore, pp. 579–614. https://doi.org/10.1007/978-981-13-6883-7_23.
- Liu, Y., Han, M., Zhou, X., Li, W., Du, C., Zhang, Y., Zhang, Y., Sun, Z., Wang, Z., 2022. Optimizing nitrogen fertilizer application under reduced irrigation strategies for winter wheat of the north China plain. *Irrig. Sci.* 40, 255–265. <https://doi.org/10.1007/s00271-021-00764-w>.
- Mahato, S., Kafle, A., 2018. Comparative study of *Azotobacter* with or without other fertilizers on growth and yield of wheat in Western hills of Nepal. *Ann. Agrar. Sci.* 16, 250–256.
- Mehboob, N., Hussain, M., Minhas, W.A., Yasir, T.A., Naveed, M., Farooq, S., Alfarraj, S., Zuan, A.T.K., 2021. Soil-applied boron combined with boron-tolerant bacteria (*Bacillus* sp. mn54) improve root proliferation and nodulation, yield and agronomic grain biofortification of chickpea (*cicer arietinum* L.). *Sustain.* <https://doi.org/10.3390/su13179811>.
- Mehboob, N., Minhas, W.A., Naeem, M., Yasir, T.A., Naveed, M., Farooq, S., Hussain, M., 2022. Seed priming with boron and. *Crop Pasture Sci.* 73, 494–502. <https://doi.org/10.1071/CP21377>.
- Molina-Romero, D., Juárez-Sánchez, S., Venegas, B., Ortiz-González, C.S., Baez, A., Morales-García, Y.E., Muñoz-Rojas, J., 2021. A bacterial consortium interacts with different varieties of maize, promotes the plant growth, and reduces the application of chemical fertilizer under field conditions. *Front. Sustain. Food Syst.* 4. <https://doi.org/10.3389/fsufs.2020.616757>.
- Mpanga, I., Nkebiwe, P., Kuhlmann, M., Cozzolino, V., Piccolo, A., Geistlinger, J., Berger, N., Ludewig, U., Neumann, G., 2019. The form of N supply determines plant growth promotion by P-solubilizing microorganisms in maize. *Microorganisms* 7, 38. <https://doi.org/10.3390/microorganisms7020038>.
- Naiman, A.D., Latrónico, A., de Salamone, I.E.G., 2009. Inoculation of wheat with *Azospirillum brasilense* and *Pseudomonas fluorescens*: impact on the production and culturable rhizosphere microflora. *Eur. J. Soil Biol.* 45, 44–51.
- Onen, H., Farooq, S., Gunal, H., Ozaşlan, C., Erdem, H., Gunal, H.O.H., Ozaşlan, C., Halil Erdem, S.F., 2017. Higher tolerance to abiotic stresses and soil types may accelerate common ragweed (*Ambrosia artemisiifolia*) invasion. *Weed Sci.* 65, 115–127. <https://doi.org/10.1614/WS-D-16-00011.1>.
- Sagar, A., Rathore, P., Ramteke, P.W., Ramakrishna, W., Reddy, M.S., Pecoraro, L., 2021. Plant growth promoting rhizobacteria, arbuscular mycorrhizal fungi and their synergistic interactions to counteract the negative effects of saline soil on agriculture: key macromolecules and mechanisms. *Microorganisms* 9, 1491. <https://doi.org/10.3390/microorganisms9071491>.
- Shah, D., Khan, M.S., Aziz, S., Ali, H., Pecoraro, L., 2021. Molecular and biochemical characterization, antimicrobial activity, stress tolerance, and plant growth-promoting effect of endophytic bacteria isolated from wheat varieties. *Microorganisms* 10, 21. <https://doi.org/10.3390/microorganisms10010021>.
- Shahzad, S.M., Arif, M.S., Riaz, M., Iqbal, Z., Ashraf, M., 2013. PGPR with varied ACC-deaminase activity induced different growth and yield response in maize (*Zea mays* L.) under fertilized conditions. *Eur. J. Soil Biol.* 57, 27–34. <https://doi.org/10.1016/j.ejsobi.2013.04.002>.
- Sheirdil, R.A., Hayat, R., Zhang, X.-X., Abbasi, N.A., Ali, S., Ahmed, M., Khattak, J.Z.K., Ahmad, S., 2019. Exploring potential soil bacteria for sustainable wheat (*Triticum aestivum* L.) production. *Sustainability* 11, 3361. <https://doi.org/10.3390/su11123361>.
- Smith, P., House, J.L., Bustamante, M., Sobocká, J., Harper, R., Pan, G., West, P.C., Clark, J.M., Adhya, T., Rumpel, C., Paustian, K., Kuikman, P., Cotrufo, M.F., Elliott, J.A., McDowell, R., Griffiths, R.I., Asakawa, S., Bondeau, A., Jain, A.K., Meersmans, J., Pugh, T.A.M., 2016. Global change pressures on soils from land use and management. *Glob. Chang. Biol.* 22, 1008–1028. <https://doi.org/10.1111/gcb.13068>.
- Tas, T., Mutlu, A., 2021. Morpho-physiological effects of environmental stress on yield and quality of sweet corn varieties (*Zea mays* L.). *PeerJ* 9, e12613.
- TÜİK, 2022. Türkiye İstatistik Kurumu.
- Ullah, A., Farooq, M., Nadeem, F., Rehman, A., Hussain, M., Nawaz, A., Naveed, M., 2020. Zinc application in combination with zinc solubilizing *Enterobacter* sp. MN17 improved productivity, profitability, zinc efficiency, and quality of desi chickpea. *J. Soil Sci. Plant Nutr.* <https://doi.org/10.1007/s42729-020-00281-3>.
- Verma, J.P., Jaiswal, D.K., Meena, V.S., Meena, R.S., 2015. Current need of organic farming for enhancing sustainable agriculture. *J. Clean. Prod.* 102, 545–547. <https://doi.org/10.1016/j.jclepro.2015.04.035>.
- Wang, J., Li, R., Zhang, H., Wei, G., Li, Z., 2020. Beneficial bacteria activate nutrients and promote wheat growth under conditions of reduced fertilizer application. *BMC Microbiol.* 20, 38. <https://doi.org/10.1186/s12866-020-1708-z>.
- Yu, L., Zhao, X., Gao, X., Siddique, K.H.M., 2020. Improving/maintaining water-use efficiency and yield of wheat by deficit irrigation: A global meta-analysis. *Agric. Water Manag.* 228. <https://doi.org/10.1016/j.agwat.2019.105906>.