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Combined Application of Microbial Inoculation and Biochar to Mitigate Drought Stress in Wheat

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ABSTRACT: Drought stress spearheads the main factors threatening food security. Although many strategies might use for stress management, microbial inoculation with plant growth promoting bacterias (PGPBs) which have ACC deaminase activity and biochar amendment which is an effective way to increase soil carbon stock, improve soil physiological and biological properties are sustainable and easy-applicable methods. The experiment was laid out in a 3x10 factorial design with three replications under controlled conditions in 2020. The aim of this study was to evaluate the efficiency of microbial inoculation (MI: TV24C + TV126C + TV61C) and biochar amendments (1%BC, 2%BC and 4%BC) on growth of wheat seedlings under different irrigation levels (IL1: 80%, IL2: %50 and IL3: 25%). While biochar applications and microbial inoculation backed up to plants to alleviate drought stress, most effective results were obtained with combined applications of them. The combined application of 4% biochar+microbial inoculation increased plant height, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight up to 28.3%, 56.8%, 72.2%, 141.3% and 112.8% compared with control plants while it improved them up to 4.9%, 10.3%, 16.6%, 21.1% and 40.3% compared with optimum synthetic fertilizer under drought conditions, respectively. In conclusion, biochar applications with microbial inoculations can be considered as an effective method to cope with the destructive effects of drought.

Keywords: Bio-priming, food security, PGPB, stress tolerance index, *Triticum aestivum*, water use efficiency

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INTRODUCTION

Climate change is the most important factor threatening wheat yield as in all other agricultural products. Although all adverse conditions lead to decrease crop yield, drought stress has a vital role in agricultural production. Drought causes a reduction of up to 92% in wheat depending on the growing stage, tolerance of wheat and density of drought stress (Semenov et al., 2014). The most sensitive time to drought is the early microspore stage of pollen growth in wheat cultivation (Ji et al., 2010). Besides, if plants are exposed to drought stress during the early vegetative growth period, it negatively affects all reproductive periods from pollen formation to grain filling (Liu et al., 2016). There are different organic, synthetic and biotic applications to mitigate drought stress such as the use of the tolerant variety, in vitro selection, use of phytohormones (SA, GA, ABA, cytokinin), osmoprotectants and antioxidants as foliar treatment, utilization of seed priming techniques and microbial inoculation with plant growth promoting bacterias (PGPB) with superior strains (Ojuederie et al., 2019; Marthandan et al., 2020). Among these protective approaches, microbial inoculation with PGPBs starts up due to its beneficial complex virtues, contribution to sustainable agriculture prospect and eco-friend structure.

The PGPBs can fix free nitrogen, solubilize phosphate compounds, protect the plants from biotic and abiotic stress factors and stimulate the physiological functions of plants (Glick, 2020). Using PGPB as microbial inoculants have been gaining awareness as an eco-friendly method in sustainable agriculture compared to chemical fertilizers that damage soil structure, environment and living organisms. Although the application of PGPB inoculation dates back more than a century, it has started up for the last three decades due to commercialization in the markets of different inoculants (Santoyo et al., 2021). The basic mechanisms used by PGPBs to ameliorate drought stress are ACC deaminase activity, exopolysaccharide, siderophore and indole acetic acid (IAA) production. Out of these mechanisms, ACC deaminase activity has a critical role for plants under stress conditions due to its inhibitory impact on the ethylene hormone. Mechanism of ACC deaminase activity bases on hydrolysis of ACC (1-aminocyclopropane-1-carboxylic acid) which is the immediate precursor of ethylene hormone, thereby, reduction of ethylene level (Raghuwanshi and Prasad, 2018). Therefore, certain PGPB strains can improve stress tolerance in plants exhibiting ACC deaminase activity.

In terms of soil-based factors, carbon and nitrogen have vital importance on microorganism activities. For this reason, the amendment of organic sources into the soil is a decisive factor for soil biota. There are many organic materials used to improve soil physiological, chemical and biological properties such as farm manure, poultry manure, green manure, sewage sludge, vermicompost and biochar (Ceritoglu et al., 2018; Cheng et al., 2021). Biochar has started up recently due to its vulnerable traits such as carbon sequestration, nutrient retention via cation adsorption, rising pH in acidic soils and water holding capacity (Wang et al., 2020a). Biochar which is considered as a substantial carbon sequestration system to cope with climate change is produced by gasification or pyrolysis that led to thermal degradation of organic amendments in the lacking of oxygen (Yang et al., 2018). Besides, biochar can increase microbial activity and colonization via binding macro-nutrients and carbon sources, offset the emission of greenhouse gas, adsorb metals and purify water (Palansooriya et al., 2019). The aim of this study was to investigate the interaction of PGPB and biochar and evaluate the usage potential to mitigate drought stress in wheat during the vegetative stage.

MATERIALS AND METHODS

Location and Duration of The Study

The study was laid out at the growth chamber depends on the department of Field Crops, Siirt University, in Siirt, Turkey in 2020. The geographical position of the faculty is between 37°58’11″N and 41°50’33″E. Altitude is 606 m.

Experimental Materials

Bacterial strains used in the study were isolated from the Van Lake Basin via the TÜBİTAK project (TOVAG-108O147) in 2010. TV24C, TV61C and TV126C strains belong to *Pseudomonas agarici*, *Bacillus megaterium* and *Pseudoalteromonas tetraodonis* species, respectively. A mixture from three different strains was used for the MI because the deficit of activities in a strain can be tolerated by the other ones (Louca et al., 2018). The nitrogen fixation, phosphate solubilizing and ACC deaminase activity of strains were tested with laboratory experiments and biofertilizer potentials were investigated during the Project and field study (Erman et al., 2010). Identification of isolated strains was determined in the microbial identification systems (MIS). The Bezostaja-1 wheat variety (bread wheat) was used in the study due to its sensitivity to drought stress (Ayrancı et al., 2017). Wheat seeds were obtained from Transitional Zone Agricultural Research Institute. Biochar material was bought from a traditional company (Synpet Technology Development Corporation, İstanbul).

Experimental Design

The study was laid out as a pot experiment to eliminate ecological and biological factors. The three doses of biochar (1%BC, 2%BC and 4%BC), microbial inoculation (MI: TV24C + TV126C + TV61C), two doses of synthetic fertilizer (S1: 50% N+P, S2: 100% N+P) and control (not any treatment) was applied under three irrigation level (IL1: 80%, IL2: %50 and IL3: 25%) in the study. The urea and triple superphosphate were used as a nitrogen and phosphorus source, respectively. The experimental design was arranged in a 3x10 factorial design with three replications. The study consists of 30 treatments with the SF, MI, BC, irrigation levels and their interactions. Pots of two liters size were used in the study and plastic bags were placed in each pot. Field soil which was gathered from experimental areas of Siirt University was taken from A horizon (20 cm). Field soil was sterilized in the autoclave at 121 °C for 60 minutes. Physiochemical properties of gathered soil had neutral, no salinity and lime problem, high clay content, insufficient organic matter and available phosphorus, enough available potassium (Table 1).

Table 1. Physiochemical properties of soil gathered for the study from 0-20 cm depth from the agricultural production area of Siirt University

Soil properties	Unit	Values
Structure	%	54.3:23.0:22.7 (clay:sand:silt)
Organic matter	%	1.14
Available potassium	Kg da^{-}	81.08
Available phosphorus	Kg da^{-}	3.49
pH	-	7.30
EC	dS m^{-}	0.22
Lime	%	1.88

The 2 kg dry soil was weighed and put in control pots. Biochar applications were mixed to field soils as dry weight scale in ratios 1%, 2% and 3%. Firstly, 100% field capacity of soil in the pot was determined by weighting saturated soils 24 hours later than saturation. After determining the volume of required water for %100 field capacity, the required water amount was calculated for 25% %50 and 80% moisture levels. Protected bacterial strains in the laboratory of Siirt University were placed in a nutrient medium that was prepared with 20 g nutrient agar for each liter of distilled water for multiplication. The nutrient solution was sterilized at 121 °C for 15 minutes by autoclave. After sterilization, cooled feed-lots were transferred into Petri dishes and solidified for 24 hours at room temperature. The stock of bacterial strains was planted on agar medium by the sterile needle and incubated at 2±25°C for 24 hours. The nutrient broth (Merck-VM775843711) was used for the liquid feed-lot. The just one colony was taken from nutrient agar medium, transferred into nutrient broth liquid feed-lot and incubated at 2±26 °C for 24 h and 120 rpm in the shaker. The bacteria concentrations were turbidimetrically arranged to ~108 cfu ml- (Sonkurt and Çığ, 2019). Before bacterial inoculation, wheat seeds were subjected to surface sterilization with 70% ethyl alcohol for 1 minute and 5% sodium hypochlorite (NaOCl) for 20 minutes. Seeds were primed with bacterial strains for 3 hours. After biopriming, seeds were dried to initial moisture content for 24 hours under dark conditions. Except for seeds with bacterial treatments were subjected to the pure and sterile nutrient broth to eliminate the impacts of early water uptake on germination and seedling growth.

All pots were kept at 25%, 50% and 80% field capacity through a week before sowing. At the end of this time, ten seeds were sown to each pot. After emergence, five wheat plants were kept in each pot and out of them were excavated. The pots were irrigated once a week as appropriately to determined field capacities. Mean temperature, photoperiod and humidity were 2±25/18 °C (day/night), 14/10 (light/dark) and 50-60%, respectively. Plants were grown under an irradiance of 350 µmol (photon) m^{-2} s^{-1}. Plants were harvested at the end of 8 weeks.

Investigated traits in the study

Differences between plant height (PH), shoot fresh weight (SFW), shoot dry weight (SDW), root length (RL), root fresh weight (SFW) and shoot dry weight (RDW) depending on applications were investigated in the study. The harvested plants were carefully cut from the junction of shoot and root and then weighted separately to determine fresh weights. The PH and RL were measured by a manual meter. Plant materials were placed into the oven at 1±70 °C and dried up to there was no change among the last two dry weights. The SDW and RDW were calculated by a precision scale.

Investigated traits in the study

The results were subjected to analysis of variance using JUMP (5.0.2) according to completely randomized factorial design. The mean values were grouped by TUKEYs Multivariate test (Kalayci, 2005).

RESULTS AND DISCUSSION

This study was carried out to investigate the effects of microbial inoculation (MI), synthetic fertilizer (SF), biochar applications (BC) and co-efficient of biochar and microbial inoculation on seedling growth in wheat under different irrigation levels. In general, the results denoted that although the SF provides higher growth compared with other treatments, increasing drought stress leads to a decrease in seedling growth and efficiency of the SF. The MI and BC applications provided the mitigation of water stress. Besides, co-application of the MI with increasing BC treatments led to improve the activity of microbial strains. According to variance analysis, drought, treatments and their interactions caused significant differences (<0.01) in all traits (Table 2).

The optimum dose of synthetic nitrogen and phosphorus certainly exhibited the highest favorable impact in all traits under 80% of field capacity. However, increasing water deficiency caused decreased efficiency of the SF in all but root length. Although the effectiveness of the BC, MI and their interactions a little lower than the SF under the optimum water level, it was exactly observed that the MI and BC applications shored to plants in the struggle to drought stress. On the other hand, drought stress led to a decrease in aboveground and below-ground biomass while it promoted root elongation. The SF ensured more increase of root elongation while the MI and BC treatments restricted it under drought stress, however, they stimulated biomass growth and dry matter accumulation in both root and shoot. The combined application of 4%BC+MI increased the PH, SFW, SDW, RFW and RDW up to 28.3%, 56.8%, 72.2%, 141.3% and 112.8% compared with control while it improved them up to 4.9%, 10.3%, 16.6%, 21.1% and 40.3% compared with optimum synthetic fertilizer under 25% of field capacity, respectively (Table 3).

Table 2. Analysis of variance (ANOVA) for investigated traits grown under controlled conditions in different irrigated levels (80%, 50% and 25% of field capacity)

Source of variance	DF	Sum of Squares	Prob>F	Sum of Squares	Prob>F	Sum of Squares	Prob>F
		PH		SFW		SDW	
Irrigation level (IL)	2	449.0	**	198.7	**	3.64	**
Treatments (T)	9	601.0	**	106.0	**	2.44	**
Replication	2	6.5	ns	0.4	ns	0.01	ns
IL x T	18	77.3	**	27.1	**	0.29	**
Error	58	6.0	-	8.2	-	0.23	-
Total	89	1139.9	**	340.4	**	6.60	**
		RL		RFW		RDW	
Irrigation level (IL)	2	47.5	**	119.4	**	8.26	**
Treatments (T)	9	199.9	**	42.4	**	2.14	**
Replication	2	0.5	ns	0.6	*	0.10	ns
IL x T	18	123.3	**	22.3	**	0.99	**
Error	58	20.2	-	0.9	-	0.32	-
Total	89	391.5	**	185.6	**	11.80	**

(ns: no significant difference, **: <0.01)

Table 3. Means of investigated traits depending on synthetic fertilizer, microbial inoculation and biochar applications under different irrigation levels

	Plant Height (cm)			
	IL1	IL2	IL3	Mean±SE
Control	33.1±0.2^{jk}	30.4±0.4^{m}	27.9±0.2^{n}	30.5±2.3^{G}
S1	38.8±0.7^{e}	34.4±0.3^{i}	32.4±0.4^{kl}	34.8±2.4^{D}
S2	43.3±0.4^{a}	38.2±0.9^{e}	34.1±0.2^{ij}	38.6±4.0^{A}
MI	36.6±0.2^{fg}	35.1±0.5^{hi}	34.8±0.6^{hi}	35.5±1.0^{C}
1%BC	35.1±0.1^{hi}	31.9±0.5^{l}	28.4±0.3^{n}	31.8±2.9^{F}
2%BC	36.2±0.2^{g}	32.4±0.3^{kl}	30.2±0.3^{m}	32.2±2.6^{E}
4%BC	36.8±0.8^{fg}	34.1±0.1^{ij}	30.4±0.5^{m}	34.4±3.7^{D}
1%BC+MI	37.3±0.1^{ef}	35.8±0.3^{gh}	34.7±0.3^{hi}	35.9±1.2^{C}
2%BC+MI	39.8±0.7^{bc}	37.7±0.2^{e}	34.9±0.5^{hi}	37.5±2.1^{B}
4%BC+MI	40.4±0.5^{b}	39.1±0.4^{cd}	35.8±0.8^{gh}	38.4±2.1^{A}
Mean	37.6±2.8^{A}	34.9±2.8^{B}	32.4±2.8^{C}	
TUKEY	IL: 0.19**, T: 0.50**, ILxT: 1.03**			
CV%	0.92			
	Root Length (cm)			
	IL1	IL2	IL3	Mean±SE
Control	12.9±0.6^{no}	17.6±0.5^{b-e}	18.6±0.6^{bc}	16.4±2.7^{C}
S1	14.3±0.7^{i-o}	18.4±0.7^{b-d}	19.3±0.8^{b}	17.3±2.4^{B}
S2	16.5±0.8^{e-h}	18.8±0.4^{bc}	21.4±0.8^{a}	18.9±2.2^{A}
MI	15.6±0.8^{f-k}	16.5±0.6^{e-h}	17.2±0.9^{c-f}	16.4±1.0^{BC}
1%BC	15.2±0.4^{g-l}	15.9±0.2^{e-j}	16.7±0.3^{d-g}	15.9±0.7^{C}
2%BC	16.0±0.7^{e-i}	14.8±0.4^{h-m}	16.0±0.2^{e-i}	15.6±0.7^{C}
4%BC	14.6±0.4^{i-n}	14.1±0.4^{j-o}	15.1±0.9^{g-l}	14.6±0.7^{D}
1%BC+MI	15.4±0.7^{f-l}	13.6±0.7^{l-o}	15.1±0.7^{g-l}	14.7±1.0^{D}
2%BC+MI	15.9±0.5^{e-j}	14.6±0.2^{i-n}	14.6±0.7^{i-n}	14.5±1.4^{D}
4%BC+MI	14.3±0.4^{i-o}	13.0±0.6^{m-o}	13.9±0.1^{k-o}	13.6±0.9^{E}
Mean	15.1±2.4^{B}	12.5±2.2^{C}	16.8±1.1^{A}	
TUKEY	IL: 0.37**, T: 0.91**, ILxT: 1.90**			
CV%	3.74			

Continuation of Table 3

Root Fresh Weight (g)				
	IL1	IL2	IL3	Mean
Control	3.79±0.2^{hi}	2.06±0.2^{q}	1.43±0.1^{r}	2.43±1.1^{F}
S1	6.13±0.2^{b}	2.45±0.1^{o-q}	2.89±0.2^{pq}	3.62±1.9^{E}
S2	7.27±1.1^{a}	2.60±0.3^{n-p}	2.85±0.1^{m-o}	4.24±2.3^{CD}
MI	4.57±0.1^{ef}	3.21±0.2^{j-m}	2.96±0.1^{l-n}	3.58±1.0^{E}
1%BC	4.86±0.2^{de}	3.46±0.2^{ij}	3.01±0.1^{k-n}	3.78±0.7^{E}
2%BC	5.02±0.1^{d}	3.89±0.1^{gh}	3.21±0.1^{j-m}	4.04±0.7^{D}
4%BC	5.45±0.2^{c}	4.15±0.2^{gh}	3.34±0.1^{j-l}	4.31±0.7^{C}
1%BC+MI	5.65±0.4^{c}	4.12±0.2^{gh}	3.39±0.1^{i-k}	4.38±1.0^{C}
2%BC+MI	6.31±0.1^{b}	4.28±0.0^{fg}	3.43±0.1^{ij}	4.67±1.4^{B}
4%BC+MI	7.03±0.2^{a}	4.67±0.2^{d-f}	3.45±0.2^{ij}	5.05±0.9^{A}
Mean	5.61±0.6^{A}	3.49±0.9^{B}	2.94±1.1^{C}	
TUKEY	IL: 1.33**, T: 0.20**, ILxT: 0.42**			
CV%	3.24			

Shoot Fresh Weight (g)				
	IL1	IL2	IL3	Mean
Control	5.87±0.1e	4.26±0.1^{gh}	3.82±0.1h	4.65±0.9^{F}
S1	10.26±0.9b	5.40±0.3^{eg}	4.84±0.1eh	6.83±1.5^{BC}
S2	11.90±1.1a	7.50±0.3^{d}	5.43±0.2eg	8.29±2.9^{A}
MI	8.02±0.2cd	5.37±0.1^{eg}	5.21±0.2gh	6.20±1.2^{CD}
1%BC	7.11±0.6d	4.61±0.1^{fh}	3.89±0.3h	5.20±1.7^{E}
2%BC	7.41±0.1d	4.86±0.1^{eh}	4.24±0.1gh	5.50±1.6^{DE}
4%BC	7.69±0.6d	5.35±0.4^{eg}	4.40±0.1gh	5.81±1.8^{D}
1%BC+MI	7.80±0.1d	5.67±0.2^{ef}	5.56±0.1ef	6.45±1.0^{C}
2%BC+MI	9.18±0.6c	6.05±0.2^{e}	5.99±0.1e	7.08±1.6^{B}
4%BC+MI	10.38±0.5b	7.42±0.4^{d}	6.05±0.1e	7.98±1.9^{A}
Mean	8.67±0.9^{A}	5.65±1.0^{B}	4.94±1.7^{C}	
TUKEY	IL: 0.23**, T: 0.58**, ILxT: 1.21**			
CV%	5.88			

Shoot Dry Weight (g)				
	IL1	IL2	IL3	Mean
Control	0.907±0.02^{h-j}	0.691±0.02^{km}	0.596±0.01^{m}	0.731±0.14^{F}
S1	1.235±0.07^{c-f}	0.920±0.01^{h-j}	0.825±0.08^{j-l}	0.993±0.19^{CD}
S2	1.646±0.06^{a}	1.164±0.14^{d-g}	0.880±0.04^{g-j}	1.228±0.31^{A}
MI	1.255±0.05^{c-e}	1.034±0.09^{f-i}	0.904±0.01^{h-j}	1.064±0.18^{BC}
1%BC	1.063±0.11^{e-h}	0.819±0.02^{j-l}	0.626±0.02^{lm}	0.836±0.20^{E}
2%BC	1.263±0.04^{c-e}	0.860±0.03^{ij}	0.65±0.03^{lm}	0.924±0.27^{DE}
4%BC	1.367±0.06^{bc}	0.897±0.04^{h-j}	0.679±0.03^{lm}	0.981±0.31^{CD}
1%BC+MI	1.267±0.05^{cd}	0.988±0.06^{g-j}	0.917±0.03^{h-j}	1.057±0.17^{BC}
2%BC+MI	1.434±0.04^{bc}	1.088±0.06^{d-h}	0.920±0.03^{h-j}	1.148±0.23^{B}
4%BC+MI	1.524±0.07^{ab}	1.267±0.05^{cd}	1.026±0.06^{f-i}	1.275±0.22^{A}
Mean	1.296±0.16^{A}	0.973±0.18^{B}	0.812±0.21^{C}	
TUKEY	IL: 0.04**, T: 0.09**, ILxT: 0.20**			
CV%	6.09			

Root Dry Weight (g)				
	IL1	IL2	IL3	Mean
Control	0.556±0.04^{o-p}	0.358±0.01^{pq}	0.257±0.01^{q}	0.390±0.13^{E}
S1	1.149±0.11^{c-f}	0.622±0.07^{j-o}	0.387±0.04^{o-q}	0.700±0.36^{CD}
S2	1.535±0.10^{a}	0.853±0.04^{g-j}	0.390±0.01^{o-q}	0.926±0.50^{A}
MI	1.241±0.04^{cd}	0.572±0.03^{l-p}	0.399±0.15^{o-q}	0.737±0.37^{CD}
1%BC	0.934±0.04^{e-h}	0.612±0.01^{k-o}	0.440±0.03^{n-q}	0.662±0.22^{D}
2%BC	1.038±0.05^{d-g}	0.665±0.03^{i-n}	0.472±0.02^{m-q}	0.725±0.25^{CD}
4%BC	1.116±0.03^{c-f}	0.686±0.04^{i-m}	0.473±0.03^{m-q}	0.758±0.29^{B-D}
1%BC+MI	1.264±0.01^{b-d}	0.784±0.02^{h-l}	0.506±0.01^{m-p}	0.852±0.33^{AB}
2%BC+MI	1.356±0.02^{a-c}	0.817±0.01^{g-k}	0.541±0.02^{m-p}	0.904±0.36^{A}
4%BC+MI	1.485±0.06^{ab}	0.904±0.04^{f-i}	0.547±0.30^{l-p}	0.979±0.49^{A}
Mean	1.167±0.14^{A}	0.687±0.16^{B}	0.441±0.28^{C}	
TUKEY	IL: 0.05**, T: 0.12**, ILxT: 0.24**			
CV%	9.77			

(IL: Irrigation level, IL1: 80% of field capacity, IL2: 50% of field capacity, IL3: 25% of field capacity, S1: Half of the optimum synthetic fertilizer, S2: Optimum synthetic fertilizer, MI: Microbial fertilizer, BC: Biochar, CV%: Coefficient of variation, SE: Standart error)

Formed diagrams showed that fluctuations between irrigation levels increased in control plants and the SF treatments while it decreased with microbial inoculation. This is a significant indicator for increasing stress tolerance by the MI. The high performance of the SF sharply declined with water deficiency whereas applications with the MI exhibited more stable performance. Although the BC applications had positive impacts compared with control plants under water deficiency, it was not enough alone to struggle with drought stress. However, the supportive effects of the BC once used with microbial inoculation were noteworthy (Figure 1).

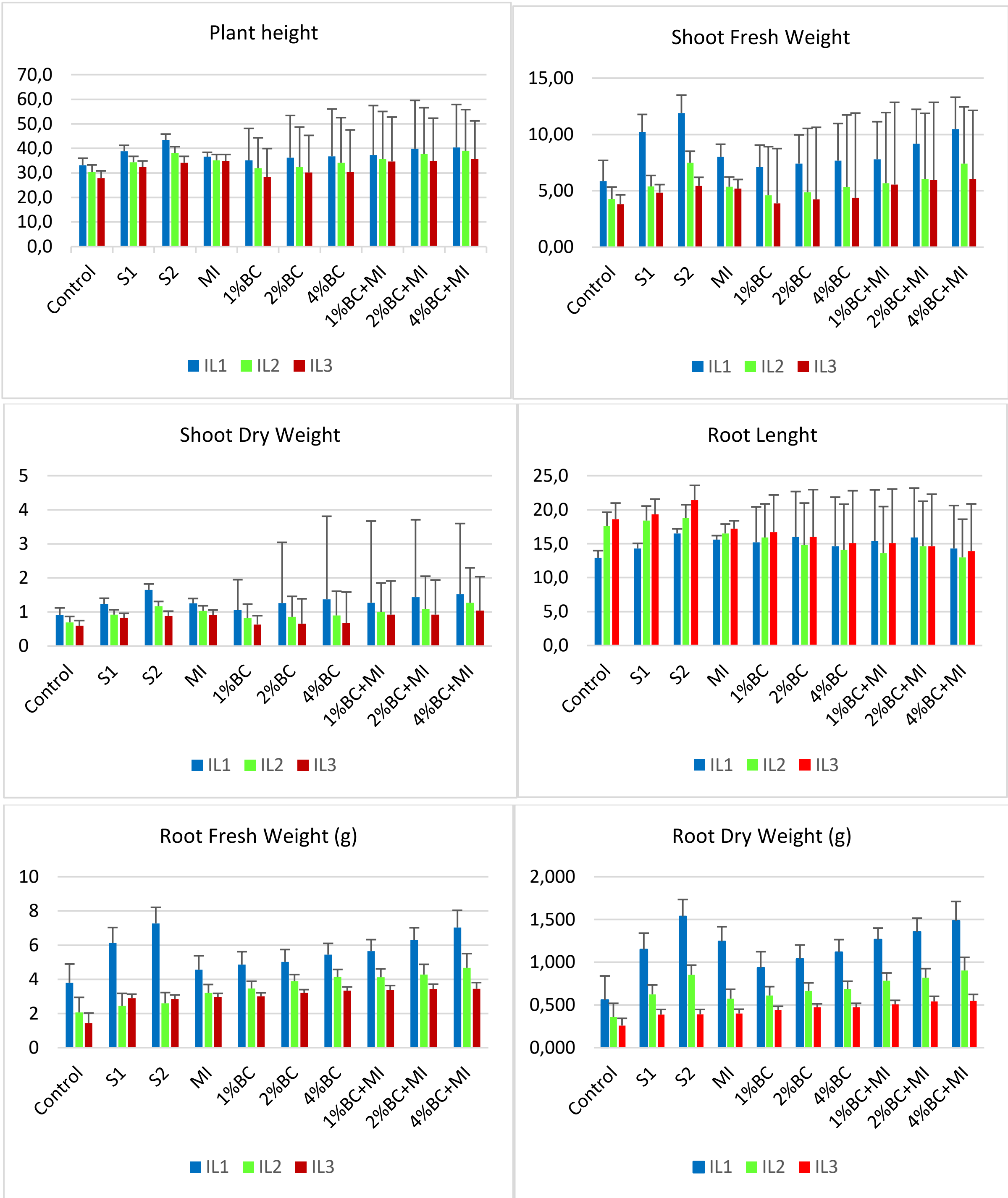

Figure 1. Distribution diagram of investigated traits depending on drought stress and treatments (IL: Irrigation level, IL1: 80% of field capacity, IL2: 50% of field capacity, IL3: 25% of field capacity, S1: Half of the optimum synthetic fertilizer, S2: Optimum synthetic fertilizer, MF: Microbial fertilizer, BC: Biochar, CV%: Coefficient of variation). Standart errors for all traits were given in figures depending on applications under different irrigation levels

The importance of using organic amendments and PGPB in agricultural production has gradually been increasing due to their contributions to the sustainable agriculture phenomenon. In a nutshell, this study showed that synthetic fertilizers present a rapid solution for plant requirement at the seedling stage under optimum water conditions, however, their impacts linearly decrease depending on rising water deficiency. The MI helps plants with water stress, especially with the BC applications.

Plant growth achieved top performance with synthetic fertilizer in 80% of field capacity. On the other hand, although organic amendments have diverse chemical and biological contents, they slowly dissolve and the in-unit volume contains a low concentration of nutrition compared with synthetic materials. Microbial inoculation that can fix free nitrogen and solve phosphate compounds provides these vital nutrients to plant step by step through the growth period depending on the ability of strains, environmental conditions and bacterial interaction. Moreover, microbial inoculation and its interaction with biochar exhibited helpful results under both optimum water conditions and also increasing water stress. As a result, it is a predictable result to obtain the best development parameters under optimum conditions with synthetic fertilizer applications. Wang et al. (2020b) stated that although PGPB cannot be an alternative to synthetic fertilizer, it helps to reduce the applicated dose.

However, a different scenario was observed with an increasing water deficit. Gargallo-Garriga et al. (2014) stated that drought stress restricts shoot metabolic activity to save water and food which would have facilitated roots growth. The MI alone supported plants to a certain degree to the nutrient deficit and water stress. Various factors such as nitrogen-fixing, phosphate solubilizing, IAA production and ACC deaminase activity play a significant role in this process. Plant growth is restricted under drought by many biochemical, physiological and molecular systems stress that led to reduce photosynthesis, impair cell division and elongation, loss of cell turgor, inhibit nutrient uptake, ethylene synthesis, affect gene expression, thereby, cause yield and quality losses (Guo et al., 2020). The two main hormones, IAA and ethylene, were secreted by plants as a response to cope with drought stress. The IAA leads to increase root depth to reach the water source while ethylene can either trigger the symptoms of stress or lead to responses that improve plant survival under stress conditions. Differences among ethylene effects depend on plant species, age, stress factor and severity, amount of secreted ethylene (Riyazuddin et al., 2020). A low peak of ethylene was secreted at the beginning of stress. This beneficial reaction initiates a protective response by plants, however, ethylene exhibited a destroyer role if the stress factor continues inveterately or more intense. Also, 1-aminocyclopropane-1-carboxylic acid (ACC) is the precursor of ethylene synthesis. The vital role of PGPBs that has ACC deaminase activity step exactly in this process due to converting ACC to α-ketobutyrate and ammonia, therefore, increasing of ethylene and stress intensity are reduced in plant cells (Glick, 2020). So, microbial inoculation with PGPB strains with ACC deaminase activity reduced plant stress and lead to improving seedling growth under water stress in the present study.

Increasing BC concentrations provided positive impacts both alone applications and co-application with the MI under optimum irrigation level and especially water deficit. It was observed that the application of increasing BC doses alone supported the plants under low irrigation levels due to their large surface area and abundant surface functional groups. These morphological properties ensure high water holding capacity, therefore, it forms a water source for plants to survive. More importantly, biochar procures a great source for microorganisms with nutrient binding and its carbon stock. For this reason, the co-application of MI and BC exhibited a higher tolerance. This interaction is an important indicator that biochar is a trigger application for not only improving soil quality and also a stimulative substrate material in terms of PGPB effectiveness. The results support the data of different studies conducted in the same subject (IJaz et al., 2019; Danish and Zafar-ul-Hye, 2019).

Synthetic fertilizer provided maximum root depth for wheat seedling following biochar amendments. It was already known that phosphorus stimulated to growth root system architecture by increasing root length, branching and promoting dry matter accumulation (Shafi et al., 2020). Moreover, all treatments stimulated root elongation by various traits such as providing carbon, nitrogen and phosphorus sources, improving soil physiology and microbiological interaction compared with control plants. However, increasing drought changed the scenario on taproot elongation and effects of applications. Water deficit sharply reduced phosphorus efficiency since it is an immobile and waterborne nutrient. Although phosphorus applications led to rising the root length dry matter accumulation decreased up to 74.6% with increasing water stress. This is an important indicator that roots could use phosphorus for root elongation to achieve water and nutrient sources but could not use for metabolic activities. Increasing root length and decreasing total root biomass agree with various studies under low P concentration (Soumya et al., 2021). Biochar amendments exhibited a balanced growth in root length and dry matter accumulation compared with control and synthetic fertilizer under water stress due to their high water holding capacity and nutrient contents. Similarly, microbial inoculation with superior strains helped seedlings to mitigate water stress. Plant roots did not need to taproot elongation in like control and synthetic fertilized plants since both nitrogen fixation and phosphate solubilizing traits and also ACC deaminase activity of strains. It is thought that these properties provided to decrease ethylene synthesis and water stress in seedlings, thereby fluctuation in root growth was restricted (Saikia et al., 2018). The MI provided to efficiently use water and nutrient sources and the BC applications formed an important C source for inoculated strains, therefore tolerance of seedlings to drought was improved.

CONCLUSION

Drought stress sharply reduced wheat seedling. Although synthetic nitrogen and phosphorus fertilizer have an effective stimulative effect on the shoot and root growth, increasing water deficit reduced phosphorus using efficiency. Biochar applications and microbial inoculation backed up to plants in mitigation of drought's inhibitory effects. Moreover, the combined application of them exhibited better performance compared with other treatments under drought stress. Most results were obtained with microbial inoculation + 4% biochar amendments under drought conditions. In conclusion, biochar applications provide to increase promoting effects of microbial inoculations and can be considered as an effective method to cope with the destructive effects of drought.

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Conflict of Interest

The authors declare that there is no conflict of interest related to the article.

Author's Contributions

FÇ, ME and MC designed and run the experiment and collected data. FÇ made statistical analysis and contributed writing, MC wrote and edited the manuscript. ME gave feedbacks. All authors read and approved the final manuscript.

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