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ALLEVIATION OF DROUGHT STRESS IN HIGH YIELDING MODERN BARLEY CULTIVAR WITH THE HELP OF COMBINATIONS OF ENDOPHYTIC BACTERIA AND VITAMIN B

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Abstract

Barley (*Hordeum vulgare* L.) is amongst the most extensively cultivated food crops around the globe. Increasing pollution and environmental constraints negatively affect overall climatic features. The effects of these agents on agricultural dynamics have motivated various biotic and abiotic stresses that have significantly decreased global rainfall patterns causing drought conditions and affecting sustainable agriculture directly. Endophytes have recently gained heedfulness for their usage in the alleviation of several types of stresses. Similarly, the use of certain growth regulators like thiamine (vitamin B) is also proved to be a promising approach. A pot investigation was done on barley to determine the impact of seed coating using thiamine (Vitamin B) and soil inoculation of *Pseudomonas putida* and *Bacillus subtilis* bacterial strains on drought stress mitigation at two irrigation regimes. The results showed that under drought stress, drought-tolerant endophytic bacteria improved barley seedling development and biochemical characteristics. The bacterial strain *Pseudomonas putida* and thiamine significantly decreased the severe impacts of drought stress, according to morphological, biochemical, and molecular characterization of barley. Combined bacterial strains and vitamin B enhanced plant vigour under drought stress. Our data validate the use of bacterial root endophytes as a potential tool for diminishing the impacts of drought on the early growth stages of barley seedlings.

Key words: *Pseudomonas*, *Bacillus*, Barley, Abiotic stresses, Endosymbionts, Vitamin-B, Chlorophyll.

Introduction

Barley (*Hordeum vulgare* L.) is native to the Poaceae group. It is a broadly raised cereal, the fourth most significant in the world, following rice, wheat and maize. It is a major cereal of various arid regions around the globe and an essential source of livelihood for many farmers. It is crucial for the human food supply and is used as livestock forage. Barley is competing with different cereals with its caloric worth and valuable protein content. It possesses extended levels of convincing nutritional significance, including vitamin E and water-soluble fibre β-glucans (Aman & Graham, 1987; Oscarsson *et al*., 1996). The current world is facing many stresses to agricultural commodities, and so is barley, where abiotic stresses (Cattivelli *et al*., 2010; Ansari *et al*., 2012), heavy metals (Stiborova *et al*., 1987), nutrient deficiency (McCauley *et al*., 2009; Postma *et al*., 2013), salinity (Soltani *et al*., 2006; Akbarimoghaddam *et al*., 2011) and drought (Mokhberdoran *et al*., 2009) are most devastating stresses which lower crop production (Ashraf *et al*., 1992; Jorenush & Mohsen, 2015; Lalarukh *et al*., 2022).

An extreme reduction of more than 50% in final crop yield occurs annually because of soil water deficiency (Stiborova *et al*., 2002). Changing weather conditions, including less humidity, increasing earth temperature, and low soil water, are causes behind water scarcity in many regions. Such factors result in extensive evapotranspiration leading to the less water-holding capacity of the rhizosphere and uneven rainfall patterns (Gargallo-Garriga *et al*., 2014; Fischer *et al*., 2019). Minimum fertilization can lower production costs and minimize the emission rate of Carbon and nitrogen oxides into the atmosphere, which can increase global warming (Guardia *et al*., 2019; Hidangmayum *et al*., 2019). The time needs to concern water relations in cereal crops and inoculation with rhizospheric bacteria in areas facing water scarcity. Frequently used seeds for agricultural practices based on their tolerance to physical agents, storage capacity and less effort to manageability (Gadalla, 2013; Kuźniar *et al*., 2019; Hussain, 2021) and this is too a fact that different microorganisms and fluctuations in the rhizosphere keep challenging the plants. Several rhizobia establish beneficial associations with plant roots and make endosymbiotic relations (Glick *et al*., 1998; Santi *et al*., 2013). Rhizospheric bacteria are being used to diminish the consequences of biotic stress (Vacheron *et al*., 2015; Aguilar-Marcelino *et al*., 2020; Sharma *et al*., 2020) by inhibiting different plant pathogens (Mohammed *et al*., 2011, 2013, 2014; Al-Ani *et al*., 2020; Singh *et al*., 2021), inducing systemic resilience (Al-Ani, 2006; Al-Ani & Al-Ani, 2011), and enhancing plant growth (Al-Ani, 2017; Soumare *et al*., 2021). The rhizosphere bacteria are effective alternatives to chemical nitrogen fertilizers and combat environmental contamination caused by these fertilizers (Founoune *et al*., 2002; Baris *et al*., 2014).

These endosymbionts (bacteria in membrane-enclosed vesicles present in host cells, lower ambient nitrogen to stimulate plant growth by disseminating ammonia in exchange for carbon resources and energy) increase height, plant biomass, dry weight, root length, yield and evocation of resilience to biotic/abiotic stress (Kondorosi *et al.*, 2013; Diagne *et al.*, 2020). Bacterial-inoculated cereal plants exhibit a pervasive root system. The benefit of these bacterial associations is greatly imparted to roots, where they affect the root physiological states and stimulate other stress-tolerating factors in plants (Dimkpa *et al.*, 2009; Kaymak *et al.*, 2009). These inoculations also stimulate acclimatizing comebacks of plants contrary to biotic/abiotic stresses and improve water use efficiency and nutrient uptake (Azcon & El-Atrash, 1997; Grover *et al.*, 2011). However, an imperative participant can be given to the plant to assist this inoculation in diminishing the detrimental outcomes of drought stress, i.e., externally applied vitamins and vitamin B (thiamine) can be proved to be very important in this regard. Typically, yellowing of leaves, stunted plant growth, leaf curling and plant wilting are significant consequences of drought stress, including reduced phenolics, ascorbic acid and plant biomass in cereal (Ashraf *et al.*, 1992). Seeds soaked in vitamin B are most favorable to ion accumulation and proved inhibitory for abiotic stress. Plant, though, has extensive root system to cope with drought stress, but osmotic adjustments need to be enhanced significantly. These include solute accumulation, stimulated antioxidant defence system, stomatal dynamics, stress-related plant contents like hydrogen peroxide (H_2O_2), malondialdehyde (MDA), glycine betaine (GB), proline, superoxide dismutase (SOD), catalases (CAT) and peroxidases (POD) (Ahmed *et al.*, 2020; Zia *et al.*, 2021). Hence, the Exogenous application of thiamin becomes essential to improve all these stress-mitigating characteristics improving plant growth attributes, osmoprotectants in leaves and photosynthetic pigments biosynthesis and membrane integrity. Vitamin B application is also reported to reduce potassium leakage (Murphy *et al.*, 2015). This treatment can significantly increase plant proline contents and decrease hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) even at 50% field capacity. Vitamin B and rhizobacterial association contribute notably to inducing drought countering in plants and minimizing the devastating activity of reactive oxygen species (ROS) (Murphy *et al.*, 2015). Barley is the most cultivated cereal among food crops in Pakistan and is one of the ten main contributors to edibles (Martinez *et al.*, 1992; Naheed *et al.*, 2015). As a country's staple food crop, barley faces yield losses due to drought stress (Wahid, 2006). The current investigation aims to assess endophytic bacteria and vitamin B combinations to minimize the aftereffects of drought stress in high-yielding modern barley cultivars. The pre-sowing administration of thiamine and the inoculation of endophytic bacteria were considered to assist barley more effectively withstanding water deficit conditions.

Material and Methods

Experimental location and design: A pot trial was conducted in the Government College University Faisalabad experimental location (at 30° to 31.5° N; 73° to 74° E and 184.4 m above sea level). The experimental design was tri-replicated and used a randomized complete block design (RCBD).

Soil characteristics: A textured clay loam soil (collected at 7-10 inches depth) was obtained from a Faisalabad village agricultural site. This soil was desiccated before being sieved with a 2 mm sieve. Some soil parameters and chemical elements are; organic matter=0.38%, pH=8.3, EC=7.79 dS m^{-1}, total available N=0.0031%, available P=5.93 mg kg^{-1} and potassium (K) 132.3 mg kg^{-1}.

Seed collecting and sterilizing: A well-suited barley cultivar (Frontier-87) was selected for this study (Sadiq *et al.*, 2013). Seeds were disinfected completely with 95% ethanol, then cleaned in 20% sodium hypochlorite mixture and again cleansed thrice with distilled water.

Bacterial isolation, characterization and identification: *Bacillus subtilis* (CC-pg104) strain was isolated from the compost sample, and *Pseudomonas putida* (CC-FR2-4) was obtained from root surroundings of *Ficus religiosa* (Rekha *et al.*, 2007) on nutrient agar along with a serially diluted (up to 10^{-9}) soil sample. A standard procedure was used to prepare LB agar plates (10 g tryptone, 5 g yeast extract, and 15 g of agar l^{-1}). A colony of both bacteria was injected in 5 ml of medium and cultured at 37°C overnight to develop the inoculum (Mokkapati *et al.*, 2018). The emergence of colonies was tested for their ACC utilization capabilities by culturing bacteria on growth media nourished with three mM ACC (Sigma-Aldrich, USA) and gestated for 48 to 72 hours at 30°C. The nitrogen-fixing activity was quantified on the nitrogen-free solid plate (Rennie, 1981). The isolate was chosen based on its subsequent growth.

The 16S rRNA gene sequence was amplified and scrutinized to identify the chosen strains. For this, genomic DNA was derived, and 16S rRNA gene was elaborated with the help of universal primers [1494 Rc (5′-TACGGCTACCTTTACGAC-3′) and 27 F1 (5′-AGAGTTTGATCMTGGCTCAG-3′)]. The PCR amplicon was sequenced at the Department of Microbiology, University of Agriculture Faisalabad. Nucleotide sequences were matched against the GenBank database. The isolate was identified as *Bacillus subtilis* (CC-pg104) strain and *Pseudomonas putida* (CC-FR2-4).

Application of Treatments and seed sowing: The treatments having two sets of pots [viz; drought stress=40 FC (40% field capacity) and well-watered=75FC (75% field capacity)] were: **1** control [untreated], **2** EB1 [*Pseudomonas putida*], **3** EB2 [*Bacillus subtilis*], **4** Vit B [thiamine supplementation], **5** EB1+VitB [a combined application of EB1 and Vit B], **6** EB2+VitB [a combined application of EB2 and Vit B].

The pots were placed in canopies after 20 days of sowing to monitor irrigation regimes and inflict drought stress closely. Drought stress was kept at 40 FC, whereas well-irrigated conditions were kept at 75 FC. Drought stress levels were vindicated based on soil water holding capacity. Twenty (20) days following the last normal watering to the soil, the plants were subjected to drought by deficit irrigation, whereas soil moisture content gradually decreased until it reached 40% field capacity (FC) as a moderate drought treatment and 75% FC as a normal well-irrigated condition. Until harvesting, the plants were kept at these limited water regimes. Drought stress was applied at the seedling stage because we wanted to observe how well barley plants performed with given amendments under drought stress at such an early growth stage.

Ten seeds were distributed in two Petri plates on Whatman filter paper no. 1, moistened with 10 ml 100 ppm of thiamine-supplemented solution. Plants were covered with parafilm for 5 days and incubated to maintain moisture levels. Emerging seedlings were kept in petri plates for a total of ten days. Afterwards, seedling roots were inoculated with *Pseudomonas putida* and *Bacillus subtilis* bacterial strains. The seedlings were then transplanted into soil-filled containers. There was a pre-sowing treatment of thiamine. Barley seeds were soaked in 100 ppm of thiamine-supplemented solution for 24 h before sowing. Barley was sown in pots at a depth of 2 cm. Ten seeds were initially propagated in each pot (23 × 27 cm diameter height) with 10 kg soil. Following the final emergence, saplings were shrinked to 6 plants per pot.

Harvesting and measurement of growth parameters: Plants were reaped 60 days after germination, and the lengths and fresh weights of roots, along with shoots, were instantly measured on the site. For further fresh analysis, samples were stored at -30°C in a biomedical freezer. Three samples out of each treatment were oven desiccated for three days at 65°C to assess their ionic analyses and dry weights. All chemicals utilized were of pure analytical grades.

Photosynthetic pigments: A method for assessing the chlorophyll content was proposed by Dere *et al.* (1998). 0.2 g of leaves were standardized in 10 ml of 96% methanol for 60 seconds, filtered and centrifuged at 2500 g for 10 minutes. The supernatant was gathered and measured chlorophyll concentration at wavelengths of 666 nm (chlorophyll a), 653 nm (chlorophyll b), and 470 nm (total carotenoids) with the help of a spectrophotometer (Model SM1200; Randolph, NJ, USA). Fresh leaf samples were used to compute carotenoids using the Arnon protocol (Arnon, 1949). Net photosynthesis was measured for three days on three randomly chosen plants from each treatment on the third or fourth fully developed leaves throughout the treatment period. This was done with the IRGA apparatus (CIRAS-2, PP-systems, MA, USA).

Leaf biochemical analysis: Healthy and properly developed leaves had been taken, wrapped in aluminium foil, and sealed in plastic zipper envelopes. Fresh samples were kept at -80°C for subsequent investigation. The biochemical examination was carried out as follows: 50 mg of desiccated leaves were extracted in 10 mL of 80% ethanol and filtered out in order to quantify biochemical assay such as total soluble proteins (Bradford, 1976), total soluble sugars (Dubois *et al*., 1956), ascorbic acid (Azuma *et al*., 1999), proline (Bates *et al*., 1973) and phenolics (Bray & Thorpe, 1954).

Measurement of malondialdehyde and H_2O_2 contents: The Heath & Packer (1968) method was used to assess lipid peroxidation in chloroplasts, which included approximating malondialdehyde (MDA) concentrations with a thiobarbituric acid reaction. Mukherjee & Choudhari (1983) method calculated the H_2O_2 content. 0.1 g of leaf sample was introduced into 10 mL cold acetone and then centrifuged at 10,000 rpm. The solution was mixed with 5 mL concentrated ammonium and 4 mL titanium reagent. After centrifugation at 10,000 rpm for 5 minutes, the solution was poured into 10 mL of 2N H_2SO_4. The obtained solution was centrifuged once more to eliminate any suspended fragments. The optical density at 415 nm was noted using blank with the help of a spectrophotometer (UV-2550; Shimadzu, Kyoto, Japan).

Determination of mineral ions: The cations (Na^+) in the extracts of acid-digested root and shoot plant materials were evaluated using flame emission spectrophotometry according to the Havre (1961) methodology.

Statistical analysis

OriginPro 2021 software was used to perform statistical analysis using two-way ANOVA under a completely randomized block design with the split-plot arrangement. At a 5% significant level ($p<0.05$), LSD test was done to differentiate the means of all the treatments. Transforming logarithmic data into a near-normal distribution was done before analyzing, where necessary. To determine the relationship between variables, Pearson's correlation analysis was employed.

Results

Vegetative growth characters and photosynthetic pigment contents: Effects of treatments were significant for root length (RL), shoot length (SL), root fresh weight (RFW), shoot fresh weight (SFW), root dry weight (RDW), and shoot dry weight (SDW) of barley. EB2+VitB treatment was found to be effective in increasing RL of barley when compared with control-treated plants. Application of EB1, EB2 and Vit B alone also performed significantly better for RFW than control, but EB1+VitB was even better than them. In the case of barley, EB1+VitB and VitB alone were statistically identical but differed significantly from the control. Treatment EB1 remained significantly better over control for RL of barley. On average, VitB caused a maximum increase (54%) in RL over control. However, barley showed 24% significant increase RL over untreated plants (Fig. 1). For SL, EB2+VitB remained significantly better than control in drought-stressed barley. Application of EB1 and EB2 also significantly increased SL, and VitB differed significantly, but EB1 did not remain significant for SL over control. On average, EB2 caused a maximum increase (85%) in SL and an average increase of 60% SL in barley was observed compared to untreated drought-stressed plants.

The addition of EB2+VitB significantly enhanced RDW in barley more than in control. Treatments EB1 and EB2 also significantly improved RFW-treated plants over control. On average, EB2+VitB caused a maximum increase (69%) in RFW over control. However, barley showed 41% significantly higher RFW over control. No significant variations were observed in SDW where EB2+VitB and EB1+VitB were given. Yet, both EB2+VitB and EB1+VitB differed significantly over control for SDW. Treatments EB1 and EB2 were statistically similar to each other for SDW. For barley, a significant increase in RDW, Chl a, but a decline in root length was found in EB2+VitB, EB1+VitB, and VitB compared to the control. On average, control caused a minimum Chl a, Chl b, T Chl and carotenoids over EB2+VitB. For SDW, EB2+VitB application caused 67% significant increase over control. However, plants showed 50% and 51% significantly higher vegetative growth compared to treatments (Fig. 2). However, the difference among varieties for all vegetative traits was constant for FC 40 and FC 75 plants.

A significant change was noted in chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (T Chl), and carotenoids by application of treatments. The addition of EB2+VitB was significantly different for enhancement in Chl. an over control. Application of EB1, EB2 and VitB alone also differed significantly for Chl a compared to untreated plants. On average, EB2+VitB caused a maximum increase (65%) in Chl an over control. For Chl b, EB2+VitB remained significantly better than control in drought-stressed plants. Application of EB1+VitB, EB1, EB2 and VitB were less significant for Chl b. All the treatments remained to differentiate for Chl b over control (Fig. 2). On average, EB2+VitB caused a maximum increase (82%) in Chl b against control. However, varieties showed no significant difference when average values for chlorophyll contents were observed. The addition of EB2+VitB significantly enhanced T Chl and carotenoids in treated plants over control. Treatments EB1+VitB, EB1, EB2 and VitB were significantly different for TChl and carotenoids (Fig. 2). On average, EB2+VitB caused a maximum increase (56%) in TChl over control.

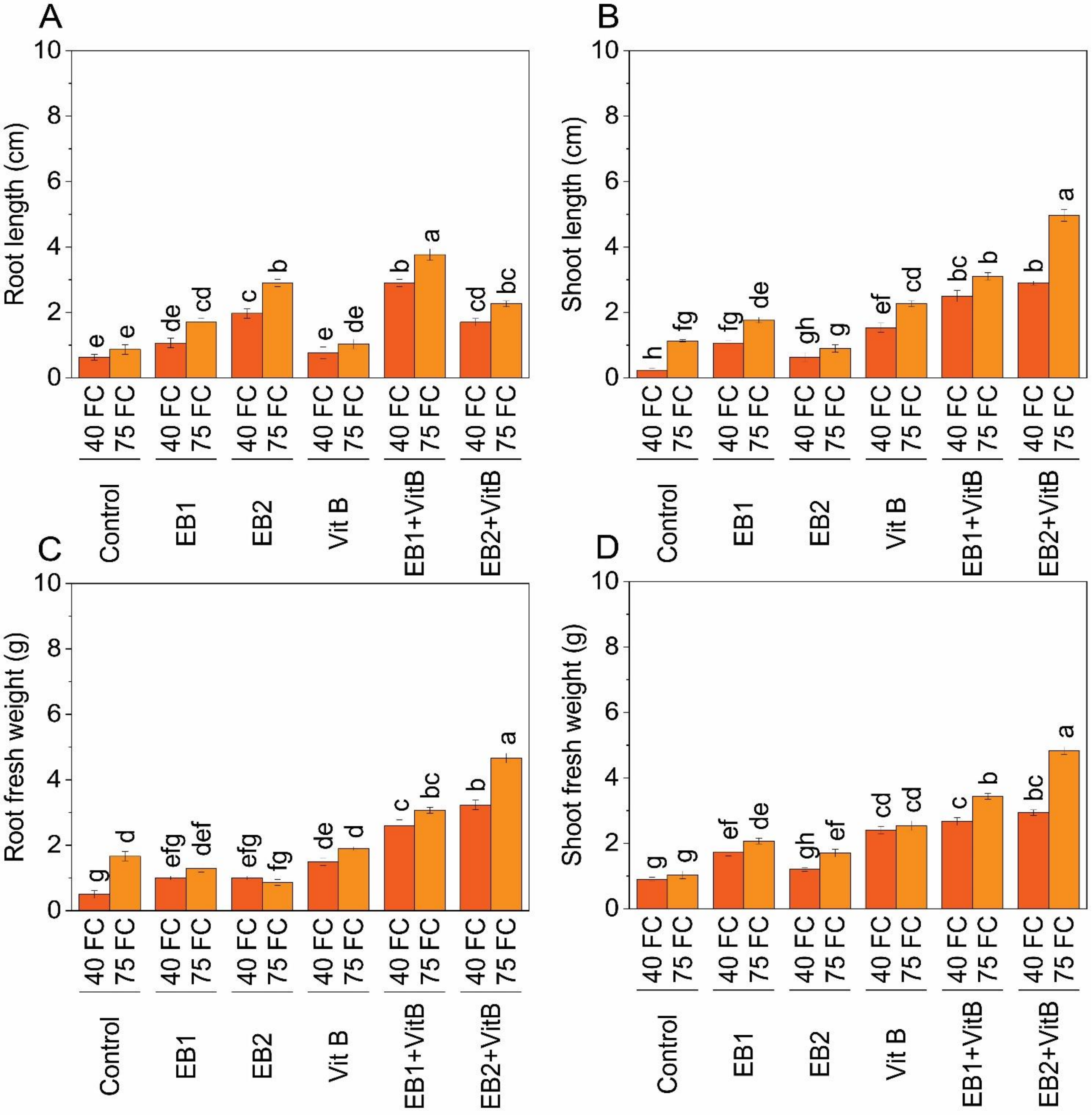


Fig. 1. Influence of different amendments on barley growth parameters grown in well-irrigated and drought-stressed soils. All figures are means of three replicates ± SD. Various labels represent significantly different alphabets by LSD test. Control [untreated], EB1 [*Pseudomonas putida*], EB2 [*Bacillus subtilis*], Vit B [thiamine supplementation], EB1+VitB [a combined application of EB1 and Vit B], EB2+VitB [a combined application of EB2 and Vit B].

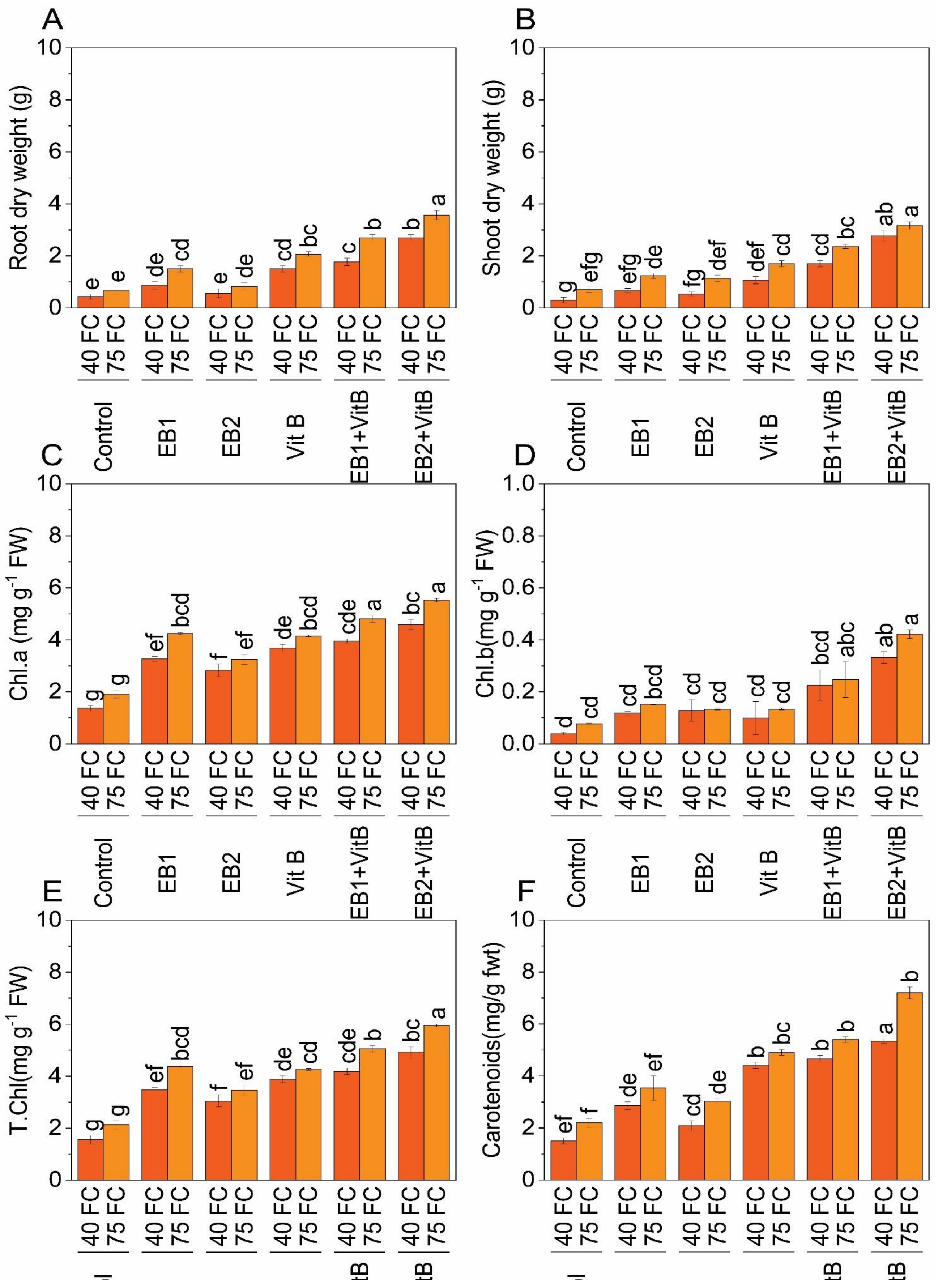


Fig. 2. Influence of different amendments on barley root dry weight, shoot dry weight and photosynthetic pigments grown in well-irrigated and drought-stressed soils. All figures are means of three replicates ± SD. Various labels represent significantly different alphabets by LSD test. Control [untreated], EB1 [*Pseudomonas putida*], EB2 [*Bacillus subtilis*], Vit B [thiamine supplementation], EB1+VitB [a combined application of EB1 and Vit B], EB2+VitB [a combined application of EB2 and Vit B].

Osmolytes, net photosynthesis oxidative stress indicators and Na ions: The effect of amendments was considered significant for anthocyanin in the root total soluble proteins (TSP), total soluble sugars (TSS), malondialdehyde (MDA), hydrogen peroxide (H_2O_2), proline contents, ascorbic acid (ASA), shoot Na, root Na, phenols and net photosynthesis in barley gave variability under given treatments where the addition of EB2+VitB was significantly different for the barley was found significant (Figs. 3 and 4). For TSP and TSS treatment EB2+VitB showed increment. In contrast, MDA, H_2O_2, Proline and ASA were decreased under treatments EB2+VitB and were observed to be highest in untreated plants under drought stress. However, net photosynthesis was highest in plants under treatment EB2+VitB, and plants observed the least phenols with EB1+VitB, and EB2+VitB treated plants gave higher phenols over EB1+VitB, but the phenols in untreated plants were maximum of all.

Statistical data showed a significant difference among all treated plants over control in barley. Application of EB1 and EB2 was even better than untreated plants. On average, EB2+VitB caused a maximum change in TSP (81%), TSS (114%), MDA (83%), H_2O_2 (89%), proline (82%) and phenols (149%) over EB1+VitB and control. However, the difference among varieties was not considerable (Figs. 3 and 4).

Pearson correlation: According to the Pearson correlation, the outcomes of drought stress on plants were significantly adverse regarding root and shoot fresh weights, dry weights, lengths, photosynthetic pigments, net photosynthesis, total soluble proteins and sugar contents. Ascorbic acid, proline, phenolics, malondialdehyde, hydrogen peroxide, and sodium levels had a significant positive relationship with drought stress (Fig. 5). Drought stress increased antioxidants while decreasing wheat growth characteristics, photosynthetic pigments, and osmolytes content.

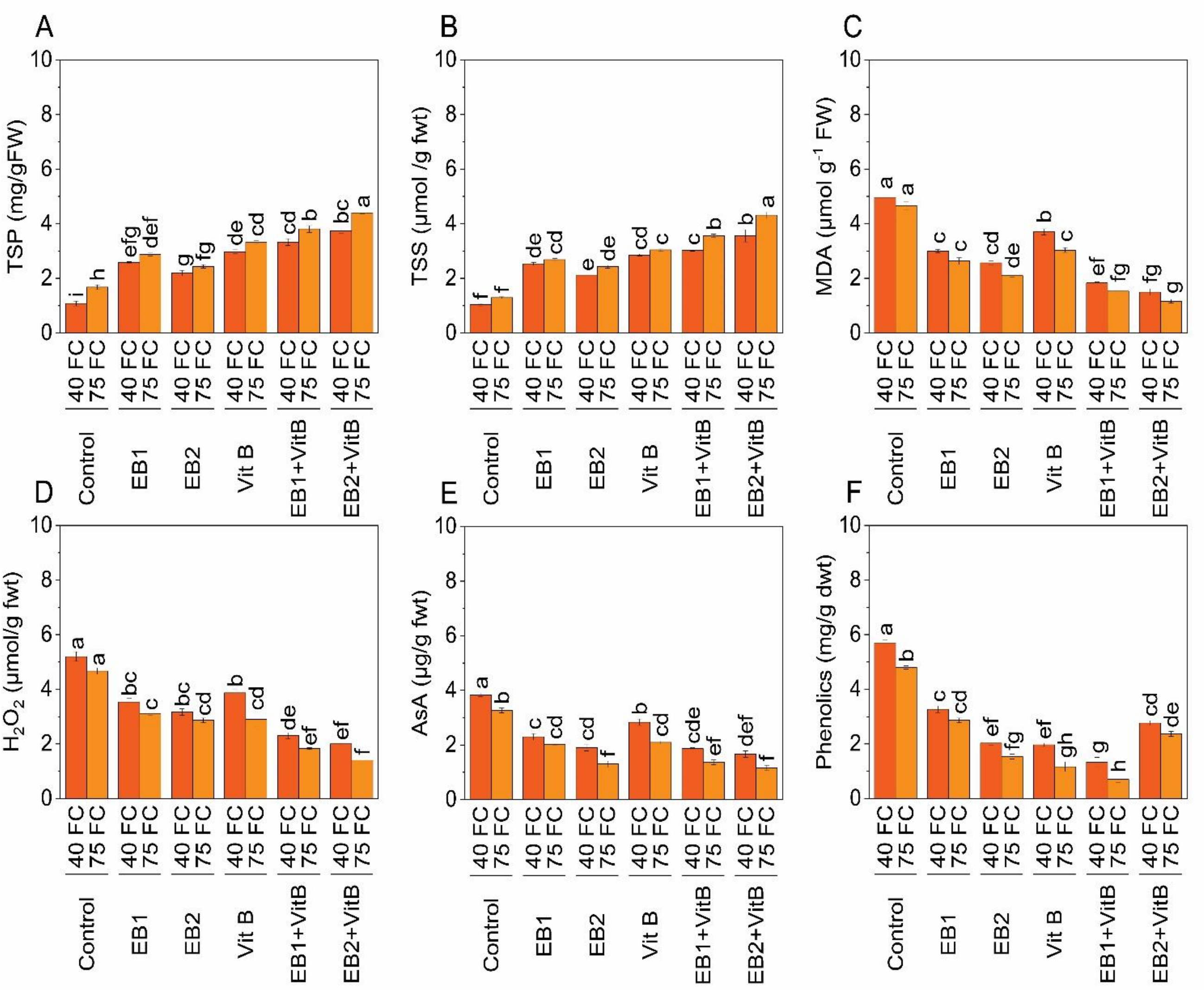


Fig. 3. Influence of solitary and integrated amendments of *Pseudomonas putida*, *Bacillus subtilis* and thiamine supplementation on total soluble proteins, total soluble sugars, malondialdehyde, hydrogen peroxide, ascorbic acid and proline of barley under well-watered and drought stressed soils. Bars indicate means of three replicates. Error bars depict standard errors. Various letters illustrate significant differences at $p \leq 0.05$; Tukey Test. EB1=Pseudomonas putida, EB2=Bacillus subtilis, Vit B=thiamine supplementation, EB1+VitB=a combined application of EB1 and Vit B, EB2+VitB=a combined application of EB2 and Vit B, TSP=total soluble proteins, TSS=total soluble sugars, AsA=ascorbic acid, MDA=malondialdehyde, H_2O_2=hydrogen peroxide, Na=sodium ions.

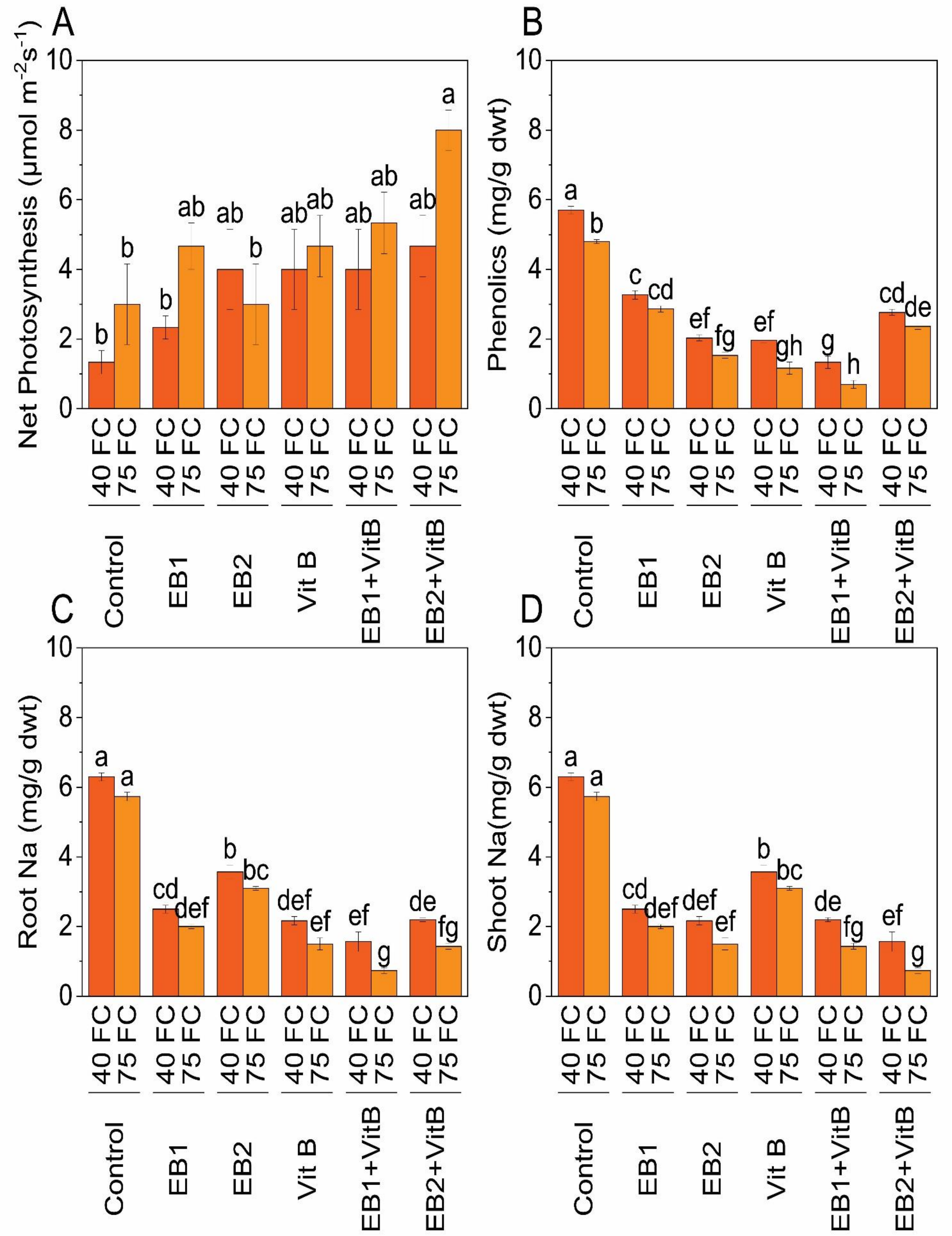
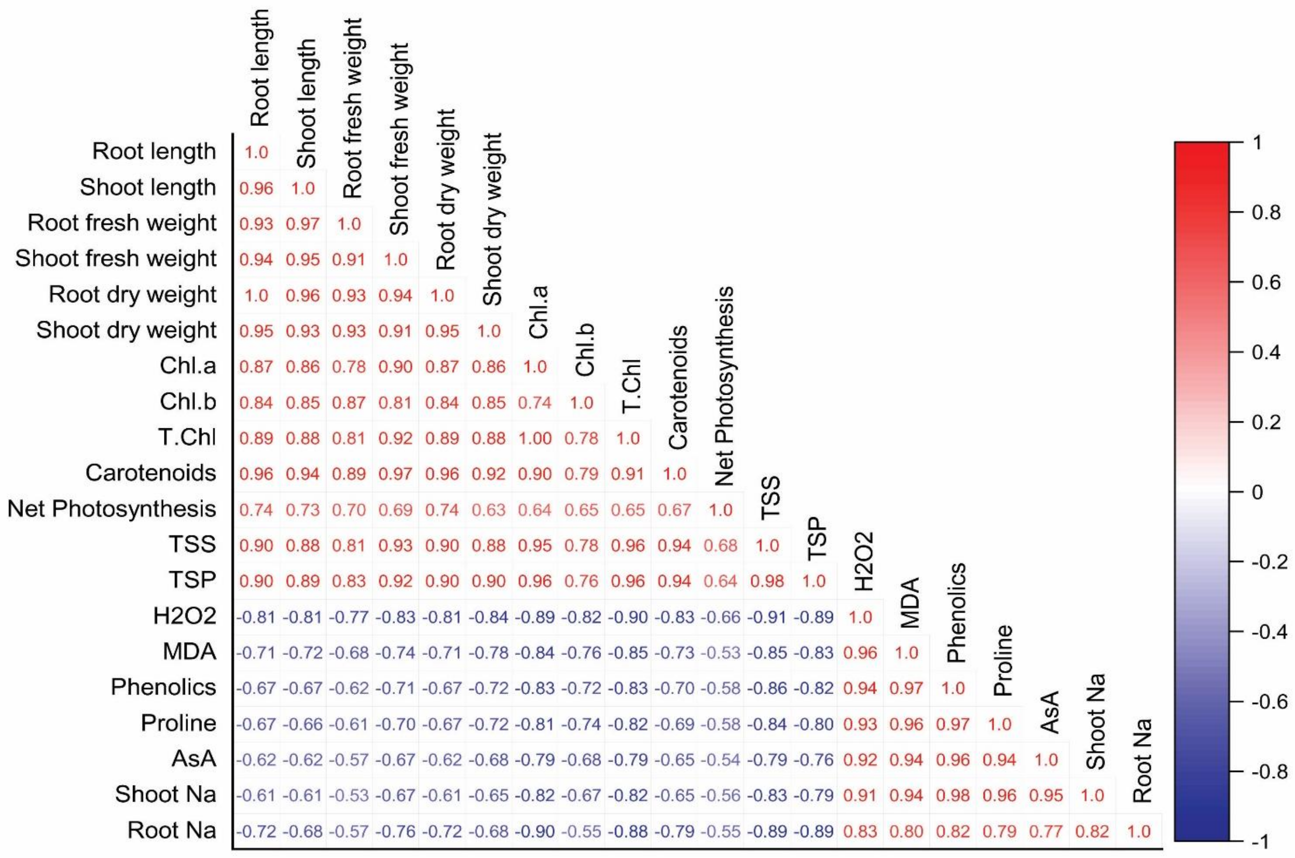


Fig. 4. Influence of solitary and integrated amendments of *Pseudomonas putida*, *Bacillus subtilis* and thiamine supplementation on net photosynthesis, phenolics, root and shoot sodium contents of barley under well-watered and drought stressed soils. Bars indicate means of three replicates. Error bars depict standard errors. Various letters illustrate significant differences at $p \leq 0.05$; Tukey Test. EB1=Pseudomonas putida, EB2=Bacillus subtilis, Vit B=thiamine supplementation, EB1+VitB=a combined application of EB1 and Vit B, EB2+VitB=a combined application of EB2 and Vit B, TSP=total soluble proteins, TSS=total soluble sugars, AsA=ascorbic acid, MDA=malondialdehyde, H_2O_2=hydrogen peroxide, Na=sodium ions.



	Root length	Shoot length	Root fresh weight	Shoot fresh weight	Root dry weight	Shoot dry weight	Chl.a	Chl.b	T.Chl	Carotenoids	Net Photosynthesis	TSS	TSP	H2O2	MDA	Phenolics	Proline	AsA	Shoot Na	Root Na
Root length	1.0																			
Shoot length	0.96	1.0																		
Root fresh weight	0.93	0.97	1.0																	
Shoot fresh weight	0.94	0.95	0.91	1.0																
Root dry weight	1.0	0.96	0.93	0.94	1.0															
Shoot dry weight	0.95	0.93	0.93	0.91	0.95	1.0														
Chl.a	0.87	0.86	0.78	0.90	0.87	0.86	1.0													
Chl.b	0.84	0.85	0.87	0.81	0.84	0.85	0.74	1.0												
T.Chl	0.89	0.88	0.81	0.92	0.89	0.88	1.00	0.78	1.0											
Carotenoids	0.96	0.94	0.89	0.97	0.96	0.92	0.90	0.79	0.91	1.0										
Net Photosynthesis	0.74	0.73	0.70	0.69	0.74	0.63	0.64	0.65	0.65	0.67	1.0									
TSS	0.90	0.88	0.81	0.93	0.90	0.88	0.95	0.78	0.96	0.94	0.68	1.0								
TSP	0.90	0.89	0.83	0.92	0.90	0.90	0.96	0.76	0.96	0.94	0.64	0.98	1.0							
H2O2	-0.81	-0.81	-0.77	-0.83	-0.81	-0.84	-0.89	-0.82	-0.90	-0.83	-0.66	-0.91	-0.89	1.0						
MDA	-0.71	-0.72	-0.68	-0.74	-0.71	-0.78	-0.84	-0.76	-0.85	-0.73	-0.53	-0.85	-0.83	0.96	1.0					
Phenolics	-0.67	-0.67	-0.62	-0.71	-0.67	-0.72	-0.83	-0.72	-0.83	-0.70	-0.58	-0.86	-0.82	0.94	0.97	1.0				
Proline	-0.67	-0.66	-0.61	-0.70	-0.67	-0.72	-0.81	-0.74	-0.82	-0.69	-0.58	-0.84	-0.80	0.93	0.96	0.97	1.0			
AsA	-0.62	-0.62	-0.57	-0.67	-0.62	-0.68	-0.79	-0.68	-0.79	-0.65	-0.54	-0.79	-0.76	0.92	0.94	0.96	0.94	1.0		
Shoot Na	-0.61	-0.61	-0.53	-0.67	-0.61	-0.65	-0.82	-0.67	-0.82	-0.65	-0.56	-0.83	-0.79	0.91	0.94	0.98	0.96	0.95	1.0	
Root Na	-0.72	-0.68	-0.57	-0.76	-0.72	-0.68	-0.90	-0.55	-0.88	-0.79	-0.55	-0.89	-0.89	0.83	0.80	0.82	0.79	0.77	0.82	1.0

Fig. 5. Pearson correlation showing the effect of different amendments on barley attributes grown in well-watered and drought-stressed soils.

Discussion

In the current study, untreated (control) plants showed significantly less growth and productivity in barley. The decline in production was associated with adverse drought stress. Munns (2002) argued that water scarcity restricts root and shoot metabolic activity. Low metabolism facilitates water conservation and transfers the stored food from the shoot towards the root in the plant body. This transfer of food and conservation of water help in plant root elongation for survival under drought (Zeiger & Taiz, 2010; Elkelish *et al*., 2021). Findings of current research work also justify these facts. Root length (cm) was small in untreated plants and in plants where EB1, EB2 and vit B were solely applied. However, RL was high in EB2+VitB-treated plants. It also signified that vitamin B2, along with higher inoculation of endophytic bacteria, played an imperative role in alleviating drought stress in barley as compared to EB1+VitB, and similar results were observed for shoot length, root and shoot fresh and dry weight, chlorophyll contents and carotenoids (Paul *et al*., 2018; Amjad *et al*., 2021b). Accomplishments of variety were found to be negligible in this regard. However, net photosynthesis was a key component in boosting plant growth (Matile *et al*., 1997; Sánchez-Díaz *et al*., 2002). Drought stress changes pigment activity in plants leading to poor photosynthesis caused by less water availability and high temperature in untreated plants (Kishor *et al*., 2014). Less photosynthesis induced a considerable decline in root and shoots dry weight, while poor TSS and TSP due to changes in plant metabolic rates decreased the fresh weight of root and shoot after fewer photoassimilates (Seleiman *et al*., 2021). Increased drought stress imparted an imperative change in the enhancement of stress-generating hormones (Kaya *et al*., 2006). Stimulating these stress-induced hormones, especially ethylene, led to less activity of photosynthetic pigments during these abiotic stress conditions (Basu *et al*., 2010). Moisture-deficient conditions denatured proteins and enzymes (Basu *et al*., 2010). For the protection of these proteins, the plant used several osmoprotectants. It permitted the osmotic adjustment where proline, phenolic, MDA and H_2O_2 were observed to highly synthesize in untreated plants, being the most important antioxidants that validated the adverse effects of drought to be tolerated by barley (Doblas-Miranda *et al*., 2017). Osmoregulation maintained the turgor potential in cells and improved physiological attributes to endure desiccation (Farooq *et al*., 2006). H_2O_2 has many growth-related and physiological roles in plants to regulate drought tolerance in cereals (Basu *et al*., 2010).

Nutrients in root and shoots, especially sodium ions, were significantly affected by these treatments where root and shoot Na was negatively associated with shoot fresh and dry weight and with barley chlorophyll constituents. Correlation analysis indicated that root/shoot length, root dry and fresh weight, shoot dry and fresh weight, net photosynthesis, chlorophyll contents, carotenoids, TSS and TSP were all positively correlated, and these observations were similar to Lebeis *et al.*, (2015). In contrast, proline, phenolics, MDA and H_2O_2 were observed to be negatively associated with all other traits that show the treatments' validity and coordinate with the findings of Kaya *et al.*, (2006). Generally, the findings were that the prescribed vitamin B could invigorate barley development by mitigating drought stress. Accordingly, Amjad *et al.*, (2021) analyzed that marinating wheat seeds in thiamin boosted shoot and root fresh along with dry weights compared to the control. Thiamin (Vitamin B) is expected to play a decisive role in synchronizing plant metabolism. It was mentioned that endophytic bacteria mitigate the drought-stimulated ROS to influence plant growth and metabolism antagonistically; hence, a decrease in antioxidants by combined application of Vitamin B and endophytic bacteria signified their imperative effectiveness towards alleviating drought stress in barley.

The plant's relationship with bacteria facilitates plant growth growing in drought-adapted soil. Water deficiency significantly lowers nutrient absorption due to less available soil solutions, mainly NPK, in non-inoculated plants. EB in plants gave significantly higher concentrations of P (Sahin *et al.*, 2004), N (Cakmakci *et al.*, 2001) and K (Hasan *et al.*, 2019) as compared to the control. These findings allowed us to reduce the elevated water scarcity tolerance in plants grounded on endophytic bacteria association. As per findings of current studies, it was observed that vitamin B had been a potential inducer to resistance against water stress along with endophytic bacteria by increasing the total phenol contents and these vitamins are essential for photosynthesis, redox metabolism and enzymatic activity of energy production in plants (Abdelaal *et al.*, 2021).

Furthermore, treating Vitamin B improves plant vulnerability to biotic and abiotic stress and diminishes bacterial, fungal and pathogenic attacks on plants (Capozzi *et al.*, 2012). However, for endophytic bacterial inoculation, it was observed that the process in each signaling system could probably be with a different mechanism of action (Ahn *et al.*, 2007; Kuzniar *et al.*, 2019; Amjad *et al.*, 2021a). Experimental analysis of both factors has given a tool for identifying plant tolerance to water scarcity (Amjad *et al.*, 2021b).

The other researchers also have found some varying biochemical changes in barley when grown in water-deficient soils with endophytic bacteria and vitamin B application, and these biochemical changes included less levels of H_2O_2, ROSs, SODs etc., due to induction of increased flavonoids compared to untreated plants indicating production of inducer compounds evaluating a biochemical effect in plant response towards given treatments (Mansoora *et al.*, 2021; Rehman *et al.*, 2022). It is probably due to conditions that induced carnation in plants by advantages during responses to endophytic bacteria since reported enhanced antioxidant capacity (Boubakri, 2020). However, understanding molecular mechanisms involve an unknown fact from being completely understood and studies on Constitutive and inducible biochemical levels at the root level need to be evaluated (Amjad *et al.*, 2021b; Hussain, 2021; Yaseen *et al.*, 2021).

Conclusions

Overall, during drought, combining *Pseudomonas putida* or *Bacillus subtilis* bacterial strains and thiamine improves barley plants' development and physiological and ionic traits. A considerable improvement in fresh root weight, shoot dry weight, and root and shoot of barley substantiated the efficacy of endophytic bacteria and thiamine solution in reducing drought stress. The treatment EB1+VitB proved more efficient in tolerating drought stress than other applied amendments at both irrigation levels. More field research is needed under various climatic circumstances before EB1+VitB can be declared the optimal combination of *Pseudomonas putida* and thiamine as the most effective drought amendment.

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