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Original Research

Bio-priming with *Brevibacterium frigoritolerans* and Foliar Phytohormones Boost Plant Growth, Photosynthesis, and Antioxidant Mechanisms in Barley under Drought Stress

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Abstract

Using plant growth-promoting bacteria (PGPB) or exogenous plant growth promoters, i.e., salicylic acid (SA) and gibberellic acid (GA), is an effective strategy for stress management in plants. A few publications have reported that *Brevibacterium frigoritolerans* has some superior characteristics in the mitigation of biotic and abiotic stresses in plants. Here we report the action of *B. frigoritolerans* on plant growth, photosynthesis, drought stress, and antioxidant defense system in barley with or without SA and GA treatments. According to the results, the rate of increase was 20.2% at root dry weight, 2.8% at root length, 8.7% at plant height, 248% at SOD, 309% at APX, 234% at CAT, and 20.8% at TSP in Tarm-92 compared with Zeynel Ağa. Root fresh weight, shoot fresh weight, shoot dry weight, plant height, and total chlorophyll content decreased at 25% FC (field capacity) by 34%, 41%, 54%, 15% and 16%, respectively. In addition, high drought stress (25% FC) increased SOD, APX, CAT, MDA, and total soluble protein (TSP) by 140%, 215%, 417%, 198% and 151%, respectively. Control plants exhibited the lowest enzymatic antioxidants and TSP, while they had the highest MDA concentrations in plants. The highest SOD (2001 ng mL^{-1}), APX (8.83 ng mL^{-1}), and CAT (29.08 ng mL^{-1}) were determined with PGPB+GA, PGPB, and GA-treated plants, respectively. On the other hand, the lowest MDA (15.77 ng mL^{-1}) was observed in PGPB+SA+GA-treated plants. In conclusion, foliar application of 1.5 mM SA or 110 mg L^{-1} GA with *B. frigoritolerans* bio-priming had the most mitigating action on drought stress in barley.

Keywords: antioxidants, beneficial bacteria, plant growth promoter, stress management, water scarcity

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Introduction

Barley (*Hordeum vulgare* L.), a member of the Poaceae family, has significant potential for human nutrition and animal feed. Annual barley production worldwide exceeded 156.4 million tons in 2019-2020, while Türkiye produced 8.3 million tons in the same period [1]. However, adverse environmental factors related to climate change, especially drought stress, significantly reduce barley production. Studies show that drought stress limits morphological and physiological development in barley and reduces crop yield and quality [2]. Therefore, innovative and sustainable approaches have pivotal importance in mitigating the devastating effects of drought stress.

Innovative, easy-to-implement, low-cost, and sustainable methods for stress management and drought stress mitigation in agricultural production are gaining prominence. Commonly used methods to improve stress tolerance include developing resistant varieties, using organic materials, water and nutrient management, plant growth-promoting bacteria (PGPB) application, the use of plant growth regulators, seed priming, and biotechnological techniques [3]. Among these methods, bio-priming with PGPB and exogenous hormonal regulator treatments stand out as effective, sustainable, and easily applicable methods for stress management.

PGPB, which can colonize various tissues and the rhizosphere of the plant, fix nitrogen, mineralize phosphate compounds, and increase plant tolerance to biotic and abiotic stress factors, can be used both to promote plant growth and protect against negative effects through direct and indirect mechanisms [4]. Although PGPB are widespread in the natural flora and widely distributed in geographical areas, the primary reason for their isolation and propagation is to select strains that exhibit superior properties in terms of desired characteristics and to improve these properties. The efficiency of biological fertilizer applications with PGPBs is highly affected by environmental factors, and plant-microorganism interaction plays an important role in the character of biofertilizers [5]. Although microorganisms have a wide range of adaptability, strains adapted to harsh living conditions are more effective in terms of stress management [6].

Therefore, evaluating the strain performance obtained from the Siirt region represents one of the novelties of this experiment. In addition, the exogenous application of hormonal regulators, which is another effective method of stress management, is a widely used method that increases tolerance to stress. It will be possible to investigate the application of salicylic acid (SA) and gibberellic acid (GA), their co-application, and interaction with PGPB on barley plants under optimum and drought conditions. Various researchers have stated that PGPB applications promote phytohormone production in plants and have positive effects on stress tolerance [7]. However, there is not enough information on the co-application and interactive effects of PGPB and plant growth regulators in barley under drought stress. This experiment is important for understanding the changes in morphological, physiological, and molecular responses to drought stress in barley under the influence of PGPB and plant growth regulators. Moreover, each bacterial genus, species, and even strain has different characteristics and behavior under various growth conditions. *Brevibacterium frigoritolerans*, which was isolated from Siirt province, was subjected to barley seeds with the bio-priming technique.

According to Choi et al. [8], the *Brevibacterium* genus comprises at least 49 species that have been isolated from various sources, including foods, humans, saline and beach environments, salt lake sediments, and different field soils [9]. *B. frigoritolerans* strains that can be categorized as PGPB were reported as an effective material on some biotic and abiotic stress conditions, such as alleviation of salinity stress in *Oryza sativa* [10], drought stress in *Poa annua* [11], and suppression of *Fusarium* stalk rot of maize [12] and *Alternaria alternata* in tomato [13]. In addition, some other researchers indicated the plant growth-promoting properties of *B. frigoritolerans* in *Flaveria bidentis* [14], Aloe vera [15], lentil [16], and *Acacia cyanophylla* [17]. However, *B. frigoritolerans* is an original microbial material and was used for the first time on barley plants under drought conditions. On the other hand, the use of *B. frigoritolerans* in combination with foliar SA and GA treatments to alleviate drought stress and their effects on antioxidant enzymes and chlorophyll activity are other critical points that increase the novelty of the paper.

Materials and Methods

Experimental Area

The experiment was conducted in the growth chamber of the Field Crops Department, Faculty of Agriculture, Siirt University, Türkiye. The average temperature was set at 24±2ºC. Light and dark periods were set as 16 and 8 hours, respectively. Light intensity averaged 500-600 µmol m^{-2} $s^{-1,}$ and the distribution over the pots was homogeneous. Nevertheless, the position of the pots was changed regularly so that small differences in the amount of light falling on the pots did not affect the results.

Experimental Materials

Two barley varieties were used in the experiment, one of which was drought-tolerant and the other was sensitive. The varieties were Tarm-92 and Zeynel Ağa, which were bred by the Central Research Institute of Field Crops. Tarm-92 is a 2-row, awned, long-spiked barley with white-grained kernels, thin and long leaves, an average height of 90-100 cm, an alternate growth habit, resistance to lodging, tolerance to drought

and microelements (zinc and boron) toxicity, high tillering capacity, high stability in crop rotation, medium-early maturity, resistance to spike breakage, and easy threshability [18]. Zeynel Ağa is a 2-row barley, 80-100 cm tall, resistant to lodging, with light green, semi-erect leaves and long, dense, semi-erect spikes bearing parallel and toothed awns. In addition, it has an alternate growth habit, is winter hardy, medium-early, shows a good reaction to fertilizer, and is relatively sensitive to arid conditions [19].

SA and GA were used as plant growth regulator materials. *B. frigoritolerans,* which was coded as KF58B, was selected based on its superior characteristics from a wide collection that was isolated from the plains of Siirt province (38°05'18''N and 41°46'01''E) and the mountainous areas of Kayabağlar region (37°59'03''N and 41°42'20''E). The strain was characterized by laboratory tests for nitrogen fixation, phosphate solubilization, ACC deaminase activity, and siderophore production [20]. The effectiveness of KF58B under various stress conditions has been reported [16, 21]; therefore, its use was decided upon in this research. The strain with superior characteristics, as identified through laboratory tests, was selected using molecular methods. 16S rDNA sequence was used for the identification of bacterial strains (Accession number: KF58B-27F-1492R). For this purpose, 16S rDNA regions were first amplified using universal primers 27F and 1492 R and sent to BM Lab for sequence analysis after clean-up [22]. The superior properties of KF58B are given in Table 1.

Preparation of Bacterial Solutions and Bio-Priming Process

Firstly, the bacterial colony was transferred to a nutrient agar (NA) medium using a loop. After an incubation period of 72 h, a single colony was taken from the NA medium and transferred to the nutrient broth (NB) medium. Bacteria-inoculated NB was incubated at 30ºC overnight on a horizontal shaker at a speed of 120 rpm min^{-1}. The concentration of the suspensions was adjusted by the turbidimetric method to ~10^8 CFU mL^{-1}.

Barley seeds were weighed and kept in 5% NaOCl for 3 min for surface sterilization. After sterilization, they were rinsed three times with distilled water and dried between 2 layers of Whatman filter paper in a sterile cabinet. Bacterial suspensions were added at a seed: suspension ratio of 1:3. Petri dishes were incubated at room temperature (24ºC) for 2 h with gentle shaking at regular intervals. Primed seeds were allowed to dry on filter paper for 10-12 h. Control seeds were hydro-primed with distilled water. Hydro-priming was applied as described above for 2 h.

Experimental Design and Laying Out

Bio-priming, foliar SA and GA applications, and their different combinations were applied to 2 different barley cultivars. The study was conducted according to a completely randomized factorial design with 4 replications. Drought levels were set as 75% field capacity (FC), 50% FC, and 25% FC. The concentrations of SA and GA were set at 1.5 mM and 110 mg L^{-1}, respectively [23]. The treatments were: i) control, ii) bio-priming treatment with KF58B, iii) 1.5 mM SA, iv) 110 mg L^{-1} GA, v) SA+GA, vi) KF58B+SA, vii) KF58B+GA, and viii) KF58B+SA+GA. Foliar SA and GA were applied in two different periods; the first one was 5 mL per plant 15 days after emergence, and the second one was 10 mL per plant 30 days after emergence. Tween-20 was added to the prepared solutions at the rate of 0.01% as a surfactant for better spreading and contact with the leaf surface. The same amount of distilled water was sprayed on the leaves of SA/GA untreated plants.

A mixture of field soil and peat in a ratio of 2:1 by volume was sterilized in an autoclave at 121ºC for 60 min. The soil mixture was used as a growing medium in two-liter pots. To determine the amount of water for FC, the soil-peat mixture was weighed and watered abundantly to reach saturation. After 24 h, weights were taken again and were accepted as 100% FC. Therefore, the amount of water for 75%, 50%, and 25% FC was calculated. Basic fertilizers were applied to all pots at a rate of 200 mg kg^{-1} N, 100 mg kg^{-1} P, 2.5 mg kg^{-1} Fe (Fe-EDTA), and 1.0 mg kg^{-1} Zn ($ZnSO_4.7H_2O$) to eliminate nutrient stress. Urea and triple superphosphate were used as nitrogen and phosphorus sources, respectively. Before transplanting, all pots were adjusted to the optimum humidity level (75% FC). After thinning, irrigation continued according to drought levels. Ten homogeneously sized, intact, problem-free seeds were carefully selected and sown in each pot. Thinning was carried out 7 days after sowing, and 5 seedlings were left in each pot. The pots continued to be watered once a week at the determined amounts. The study was completed 40 days after sowing, before flowering.

Table 1. Identification and characterization of bacterial strain (KF58B).

Code	Taxonomy	N_2 fixation	Phosphate solubilization	ACC deaminase activity	Siderophore production
KF58B	*Brevibacterium frigoritolerans*	+	L	+++	++

Note: L: Low, +: Active, ++: High, +++: Very high.

Experimental Observations

A SPAD chlorophyll meter (SPAD-502 Konica Minolta Sensing, Inc., Japan) was used to determine the total chlorophyll content in the leaves on the harvest day. Readings were taken from the young leaves of the plants, and the average value was calculated to represent the pot.

At the end of 40 days, two plants from each pot were cut with scissors at the soil level for molecular analysis, quickly packaged, and placed in a -86ºC freezer. After that, the other plants in each pot were carefully removed together with their soil, and the roots were cleaned of rhizosphere soil. Root and shoot wet weights were recorded as fresh weight. Plant height and root length were determined. The weights of the measured plants were monitored periodically at 68ºC until there was no change in weight. They were then weighed to determine dry matter accumulation in roots and shoots. The methods of Çığ et al. [21] were used to calculate the stress tolerance index (STI).

Total soluble protein (TSP), superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) enzyme activities and malondialdehyde (MDA) concentrations were determined to investigate stress response in plants. Leaf samples were frozen with liquid nitrogen and crushed in a porcelain mortar. Each sample was homogenized with three times its weight of homogenization buffer (pH 7.5, 50 mM K-phosphate, 0.35 mM PMSF, and 1 mM EDTA) with a pestle and mortar. The resulting mixture was centrifuged at 15,000 rpm at 4ºC for 1 h, and the supernatant was used as a homogenate. The Bradford method was used to determine the TSP in plant tissues. CAT was determined using the method based on the hydrolysis of H_2O_2. APX activity was measured according to

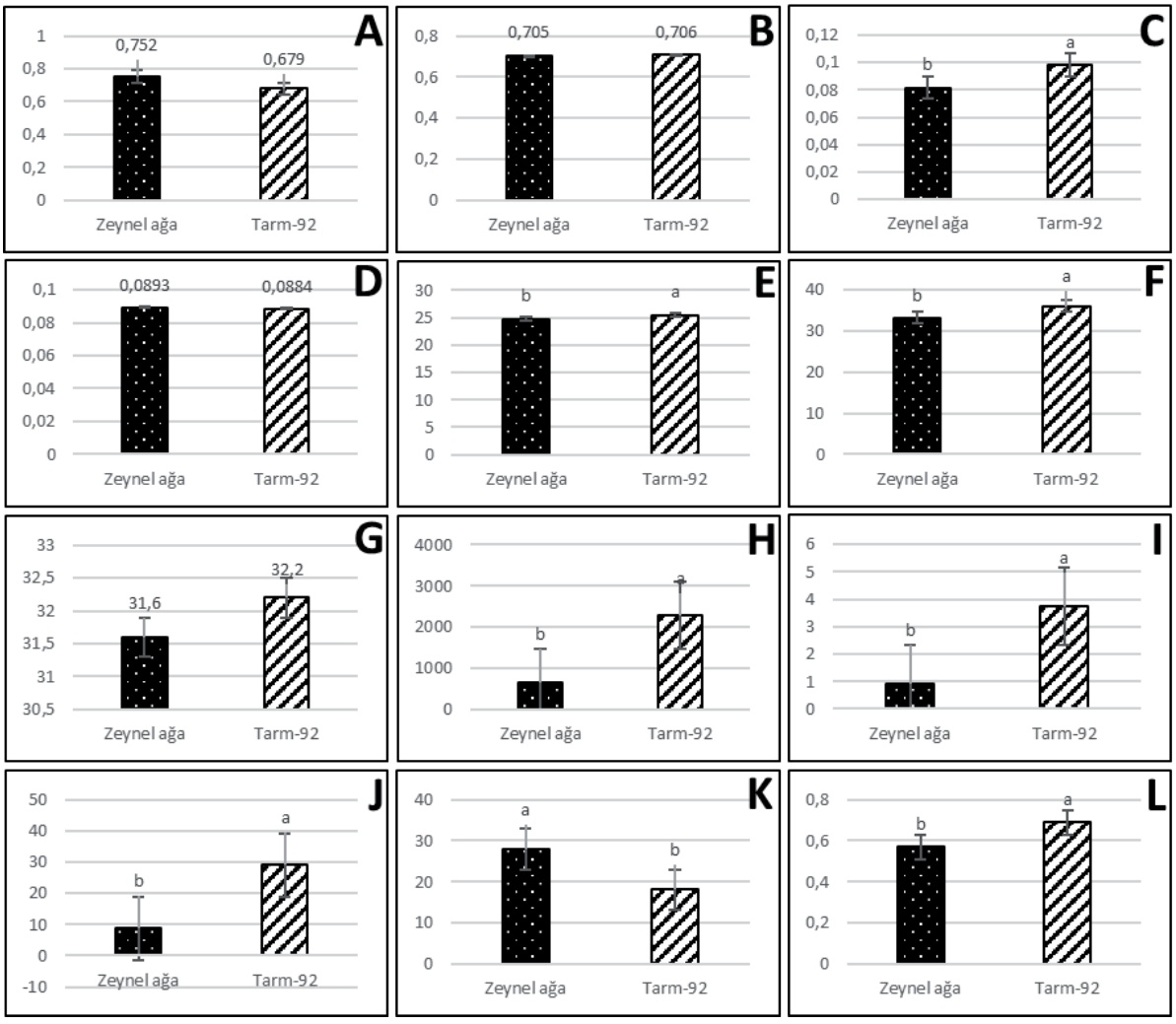

Fig. 1. Alterations of experimental characteristics between genotypes.
Statistically significant differences among columns are indicated by letters. Columns sharing different letters represent distinct statistical groups, whereas columns shown with direct values indicate no significant differences. Detailed ANOVA results are provided in the supplementary file. (A: Root fresh weight (g), B: Shoot fresh weight (g), C: Root dry weight (g), D: Shoot dry weight (g), E: Root length (cm), F: Plant height (cm), G: Total chlorophyll content (%), H: SOD (ng mL^{-1}), I: APX (ng mL^{-1}), J: CAT (ng mL^{-1}), K: MDA (ng mL^{-1}), L: Total soluble protein (mg mL^{-1})).

the decrease in ascorbate per minute. The method based on spectrophotometric measurement at 560 nm was used for SOD activity. The TBARS method was used to determine MDA concentration in the plant tissues. Laboratory analyses were laid out according to methods described by Fujita and Hasanuzzaman [24].

Statistical Analysis

Data were subjected to the Levene homogeneity test. The homogeneous data were subjected to analysis of variance according to a completely randomized factorial design. Tukey's HSD (honestly significant difference test) multiple comparison test was used to group averages. The JMP (5.0.1) software was used for statistical calculations.

Results

Genotypes, irrigation levels, and treatments caused statistically significant differences ($p<0.05$ or $p<0.01$) in the investigated characteristics. Very significant differences ($p<0.01$) were determined in plant height, SOD, APX, CAT, MDA, and TSP, while significant differences ($p<0.05$) were observed in shoot fresh weight, root dry weight, and root length among genotypes. Differences in root fresh weight, shoot dry weight, and total chlorophyll content were not significant. Irrigation levels were not effective on root dry weight and root length; however, they caused very significant differences ($p<0.01$) in other characteristics, except for SOD, as it was affected at the 5% level. According to treatments, very significant differences ($p<0.01$) were determined in root length, total chlorophyll content, SOD, APX, CAT, MDA, and TSP, whereas other characteristics were not

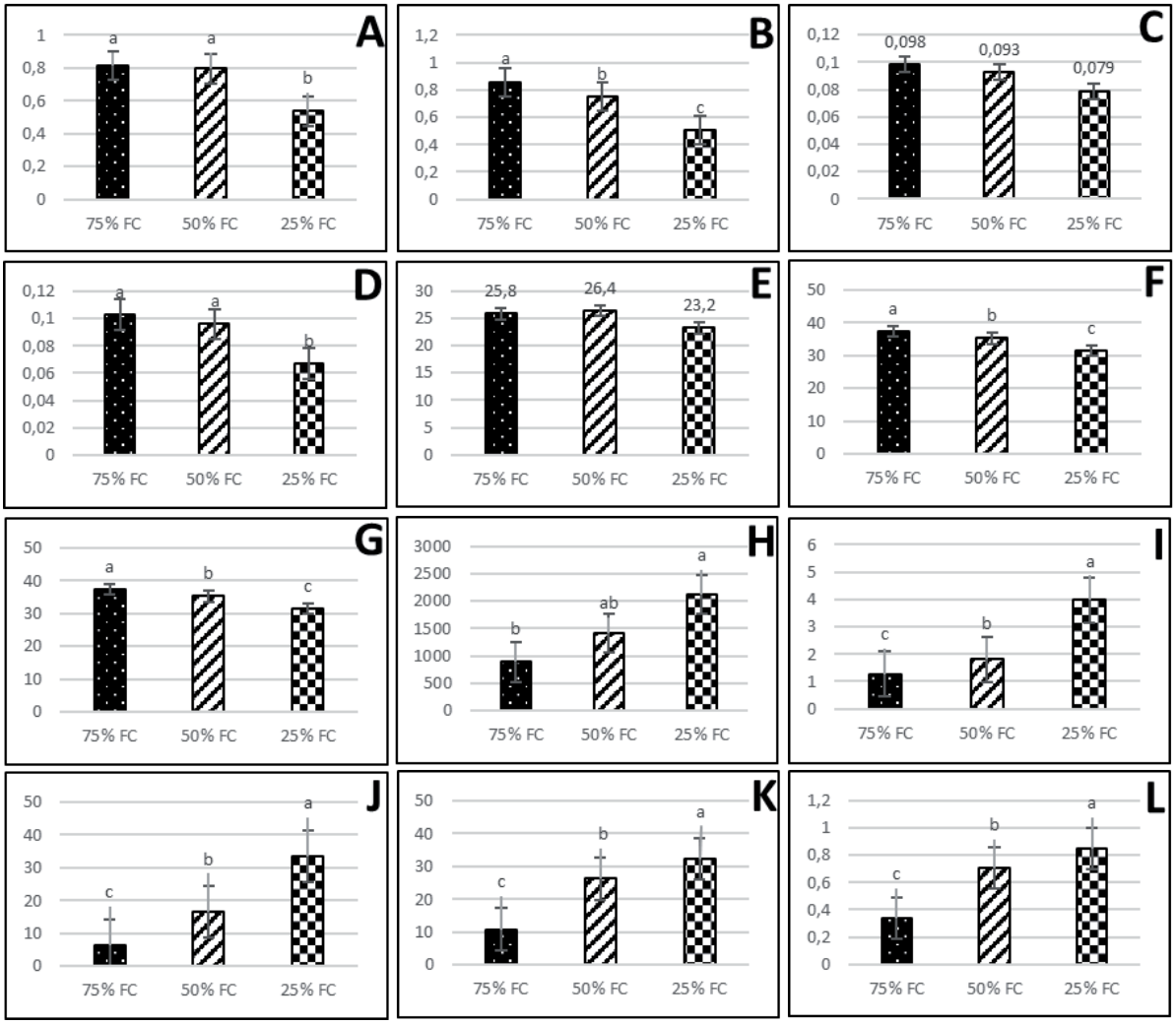

Fig. 2. Alterations of experimental characteristics depending on irrigation levels.
Statistically significant differences among columns are indicated by letters. Columns sharing different letters represent distinct statistical groups, whereas columns shown with direct values indicate no significant differences. Detailed ANOVA results are provided in the supplementary file. (A: Root fresh weight (g), B: Shoot fresh weight (g), C: Root dry weight (g), D: Shoot dry weight (g), E: Root length (cm), F: Plant height (cm), G: Total chlorophyll content (%), H: SOD (ng mL^{-1}), I: APX (ng mL^{-1}), J: CAT (ng mL^{-1}), K: MDA (ng mL^{-1}), L: Total soluble protein (mg mL^{-1})).

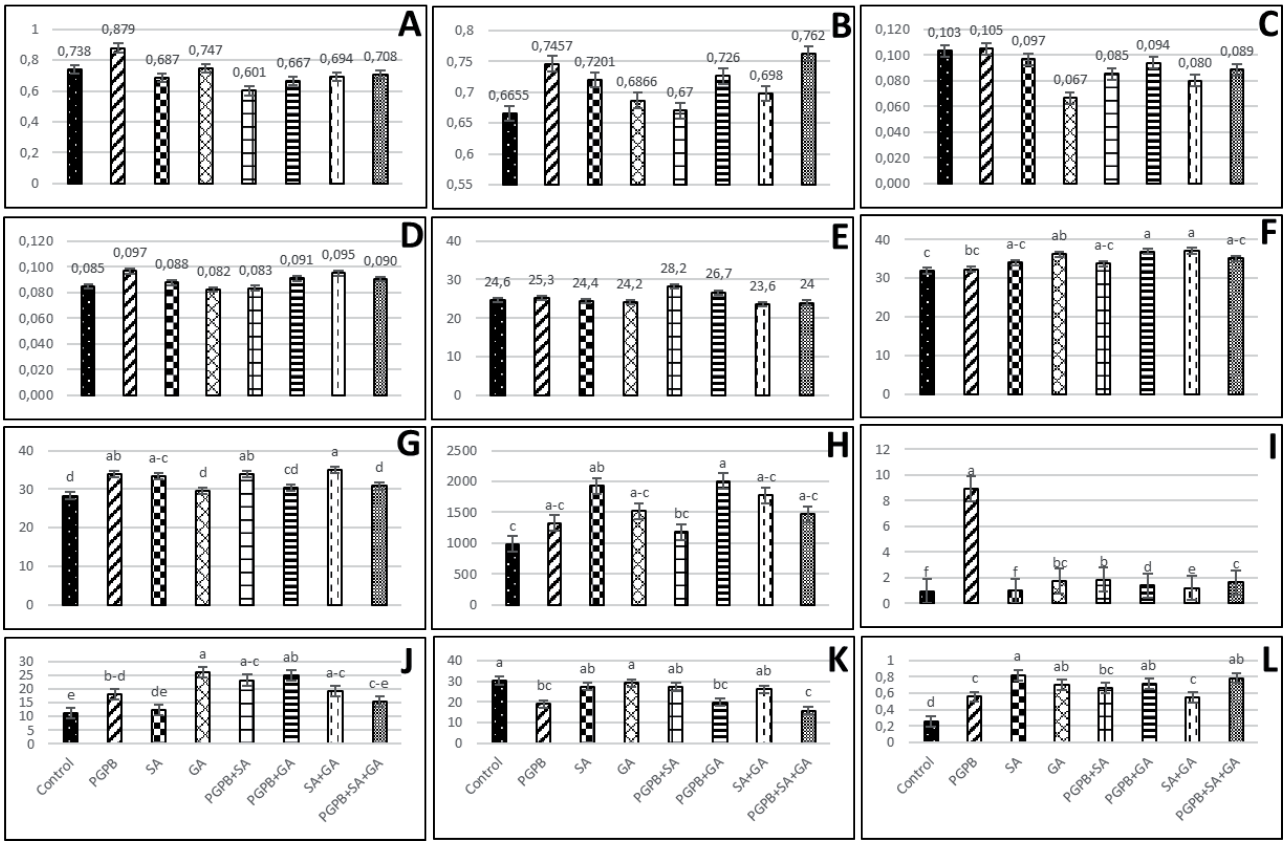

Fig. 3. Alterations of experimental characteristics depending on biological and hormonal treatments.
Statistically significant differences among columns are indicated by letters. Columns sharing different letters represent distinct statistical groups, whereas columns shown with direct values indicate no significant differences. Detailed ANOVA results are provided in the supplementary file. (A: Root fresh weight (g), B: Shoot fresh weight (g), C: Root dry weight (g), D: Shoot dry weight (g), E: Root length (cm), F: Plant height (cm), G: Total chlorophyll content (%), H: SOD (ng mL^{-1}), I: APX (ng mL^{-1}), J: CAT (ng mL^{-1}), K: MDA (ng mL^{-1}), L: Total soluble protein (mg mL^{-1})).

affected. G×I interaction caused statistically significant differences at 5% in total chlorophyll content and 1% in MDA, while it did not influence other characteristics. G×T, I×T, and G×I×T interactions caused very significant differences ($p<0.01$) in APX, MDA, and TSP; however, not in other characteristics (Supplementary file, Table S1).

Root fresh weight, shoot fresh weight, shoot dry weight, and total chlorophyll content varied between 0.679-0.752 g, 0.705-0.706 g, 0.0884-0.0893 g, and 31.6-32.2% depending on genotypes, respectively. Tarm-92 (drought-tolerant genotype) exhibited superior performance in root dry weight, root length, plant height, SOD, APX, CAT, and TSP, whereas MDA was higher in Zeynel Ağa (drought-sensitive genotype). The rate of increase was 20.2% at root dry weight, 2.8% at root length, 8.7% at plant height, 248% at SOD, 309% at APX, 234% at CAT, and 20.8% at TSP in Tarm-92 compared with Zeynel Ağa. On the other hand, MDA decreased in Zeynel Ağa by 20.8% over Tarm-92 (Fig. 1).

Root dry weight and root length varied between 0.079-0.098 g and 23.2-26.4 cm, respectively. Decreasing irrigation levels caused detrimental alterations among morphological characteristics while promoting antioxidant enzyme activity. Root fresh weight, shoot fresh weight, shoot dry weight, plant height, and total chlorophyll content decreased with 25% FC by 34%, 41%, 54%, 15%, and 16%, respectively. In addition, decreasing irrigation levels increased SOD, APX, CAT, MDA, and TSP by 140%, 215%, 417%, 198%, and 151%, respectively. Thus, drought stress was more effective on antioxidant enzyme activity compared to morphological characteristics (Fig. 2).

Root fresh weight, shoot fresh weight, root dry weight, shoot dry weight, and root length varied between 0.601-0.879 g, 0.6655-0.7620 g, 0.067-0.105 g, 0.082-0.097 g, and 24.0-28.2 cm, respectively. The lowest plant height (31.9 cm) and total chlorophyll content (28.3%) were determined in control plants, whereas the highest plant height (37.1 cm) and total chlorophyll content (34.9%) were observed with SA+GA-treated plants. Control plants exhibited the lowest enzymatic antioxidants and TSP, while they had the highest MDA concentrations in plant tissues. The highest SOD (2001 ng mL^{-1}), APX (8.83 ng mL^{-1}), and CAT (29.08 ng mL^{-1}) were determined with PGPB+GA, PGPB, and GA-treated plants, respectively. On the other hand, the lowest MDA (15.77 ng mL^{-1}) was observed

in PGPB+SA+GA-treated plants. Finally, SA treatment indicated the highest TSP content in plants (Fig. 3).

According to the G×I interaction, the lowest total chlorophyll content (29.7%) was determined with 25% FC in Zeynel Ağa, whereas the highest one (34.7%) was obtained from 75% FC in Tarm-92 (Supplementary file, Table S2). G×T interaction indicated that the lowest APX (0.54 ng mL^{-1}) was determined with control and GA treatment in Zeynel Ağa, while the highest one (17.16 ng mL^{-1}) was observed with PGPB in Tarm-92. In terms of I×T interaction, the lowest (0.55 ng mL^{-1}) and highest (22.48 ng mL^{-1}) APX were determined with SA treatment in 75% FC and PGPB treatment in 25% FC, respectively. Triple interaction (G×I×T) was effective on APX activity. Thus, the lowest APX was determined with control, PGPB, SA, and GA (no APX activity) in Zeynel Ağa under 75% FC, whereas the highest APX (44.1 ng mL^{-1}) was obtained with PGPB treatment in Tarm-92 under 25% FC (Supplementary file, Table S3). G×I, G×T, I×T, and G×I×T interactions caused very significant differences ($p<0.01$) in MDA, which is a pivotal indicator of plant stress. In terms of G×I, the lowest (7.31 ng mL^{-1}) and highest (44.43 ng mL^{-1}) MDA concentrations were obtained from Tarm-92 under 75% FC and Zeynel Ağa under 25% FC, respectively. G×T interaction denoted that the lowest MDA (9.33 ng mL^{-1}) was determined with PGPB+SA+GA treatment in Tarm-92, whereas the highest MDA (43.27 ng mL^{-1}) was observed with PGPB+SA in Zeynel Ağa. The lowest MDA according to I x T (6.10 ng mL^{-1}) was seen with SA+GA treatment under 75% FC, while the highest one (57.25 ng mL^{-1}) was determined in the control plants under 25% FC. According to the G×I×T interaction, PGPB+SA under 75% FC and PGPB+SA+GA under 50% FC in Zeynel Ağa and PGPB, SA, and PGPB+SA+GA treatment in Tarm-92 under 75% FC did not exhibit MDA activity. On the other hand, the highest MDA (79.70 ng mL^{-1}) was determined with SA+GA in Tarm-92 under 50% FC (Supplementary file, Table S4). G×T interaction for TSP indicated that the lowest value (0.242 mg mL^{-1}) was observed with control plants in Zeynel Ağa, whereas the highest one (1.016 mg mL^{-1}) was determined with GA treatment in Tarm-92. According to the I×T interaction, the lowest TSP (0.015 mg mL^{-1}) was seen with control plants under 75% FC, while the highest one (1.288 mg mL^{-1}) was determined with GA treatment in 25% FC. In the G×I×T interaction, control treatment in Zeynel Ağa under 75% and 50% FC with PGPB and SA treatment in Zeynel Ağa under 75% FC exhibited a negligible TSP. On the other hand, the highest TSP (2.364 mg mL^{-1}) was determined with GA treatment in Tarm-92 under 25% FC (Supplementary file, Table S5).

Discussion

According to the results, total biomass, growth, dry matter accumulation, and total chlorophyll content decreased, while antioxidant enzyme activity and total protein content increased with increasing drought. Irrigation levels showed statistically significant differences in shoot and root fresh weights. Optimum water content in the soil promotes greater storage in vacuoles, increases turgor pressure, and consequently results in higher total biomass. Fresh weight in plants is directly related to water content because the majority of living organisms are composed of water. Different researchers reported that fresh weight decreases in plants under dry conditions, whereas it increases in parallel with higher water content in plants [25].

Root dry weight showed significant differences among the varieties, while shoot dry weight showed more variability according to different irrigation levels. Another factor affecting early seedling development is the germination process. Several studies have reported that the germination characteristics of lentil seeds were negatively affected by increasing drought levels or decreasing ambient water levels. The germination characteristics of lentil seeds are negatively affected; radicle emergence is delayed, and seedling growth characteristics are slowed down compared to the control medium [26]. Inhibition of seed germination is directly related to reserve mobilization, energy production through respiration, enzyme and hormonal activity, and dilution of protoplasm to increase metabolism for successful embryonic growth [27]. Since insufficient water in the medium inhibits the activities of hydrolytic enzymes, the stored reserve cannot be broken down, and energy cannot be sufficiently utilized for radicle output, thus indirectly negatively affecting seedling vigor and development. Therefore, the water status of soil is one of the pivotal factors affecting dry matter accumulation in plants.

Similar results were observed in root and shoot length as well as fresh weight and dry matter accumulation of above- and below-ground organs. Shoot and root length of Tarm-92, which has higher resistance to drought conditions, increased more. This is because it provides greater root depth and lateral root formation to reach water, and shoot development is higher by providing more efficient transport of water to plant cells [28, 29]. Shoot length was also significantly affected by foliar applications. SA+GA and KF58B+GA were found to have the most positive effect on shoot growth. Studies indicate that SA and GA applications have positive results in terms of plant growth and development of stress tolerance [30]. SA functions as a defense signaling molecule for plants, promotes plant growth and development, improves stress tolerance, and stimulates antioxidant defense systems [31]. On the other hand, GA is effective in many growth stages from seed germination to generative stages and accelerates root and shoot development [32]. The increase in shoot length in plants due to SA and GA is consistent with previous studies. In addition, KF58B promoted shoot growth due to its nitrogen-fixing and phosphate-solubilizing ability and contributed to the development of stress tolerance

due to ACC deaminase activity. Different researchers reported that bacterial strains showing ACC deaminase activity contribute to the improvement of drought stress in plants and support plant growth [33, 34].

Total chlorophyll content decreased significantly with restricted irrigation, while it increased compared to the control, depending on the treatments. Photosynthetic pigments such as chlorophyll-a, chlorophyll-b, and carotenoids, and photosynthetic activity decrease in plants under water stress [35]. Photosynthesis is a process in which plants use solar energy to convert carbon dioxide and water into sugar and oxygen [36]. Chlorophyll is an important component of this process, and water is an essential component in chlorophyll production. Khaleghi et al. [37] examined the variation of total chlorophyll content in leaves of olive trees according to water availability in the soil and found that chlorophyll content decreased with lower water availability. Chegini et al. [38] reported that as the severity and duration of drought increased, the level of oxygen needed in photosystem II decreased, and the photosynthesis process was damaged. Waziri et al. [39] investigated the effects of water availability on chlorophyll content in wheat at different irrigation levels and under controlled conditions and reported that both chlorophyll-a content and total chlorophyll content in wheat leaves decreased with decreasing water availability. On the other hand, KF58B, KF58B+SA, and SA+GA treatments prevented significant photosynthetic pigment loss. Anwar et al. [40] found that priming with GA and potassium nitrate increased total chlorophyll content and photosynthetic activity in cucumber. Moradi and Mozafari [41] reported that PGPB-priming increased chlorophyll-a and chlorophyll-b concentrations in lentil. Tamindžić et al. [42] found that SA-priming application increased the chlorophyll content in pea seeds compared to the control group. The data obtained in terms of chlorophyll content in this study are consistent with previous studies.

Enzymatic antioxidant concentration was relatively higher in the resistant variety (Tarm-92) compared to the relatively drought-sensitive one (Zeynel Ağa). Previous studies have reported a positive and strong correlation between stress tolerance and antioxidant enzyme activity [43]. Various researchers reported that there is a wide variation among different bean and strawberry cultivars in terms of antioxidant capacity [44, 45]. Therefore, higher enzymatic activity in Tarm-92, especially under increased stress conditions, is an expected result. Resistant varieties were less affected by stress conditions and could show significant growth even under high drought conditions [46]. This suggests that mitochondrial respiration may play an important role in providing ATP to the chloroplasts, thus supporting chloroplast functions and ultimately plant survival [47]. Moreover, the soluble sugar and proline content in plant tissues exposed to drought stress are important indicators of STI. García-Coronado et al. [48] indicated that drought-tolerant genotypes produced more soluble sugars and proline but depleted the available starch reserve faster. Therefore, among genotypes under drought stress, those with high proline production potential gain an advantage and can develop tolerance to stress. Due to these physicochemical properties, the responses and tolerance thresholds of each genotype differ at various drought levels.

Antioxidant enzymes contribute to the reduction of osmotic stress by removing reactive oxygen species (ROS) from cells, thereby enhancing stress tolerance in plants [49]. On the other hand, MDA and TSP increase in plant tissues under stress conditions. MDA arises as a product of lipid peroxidation in plant cells and is generally used as an indicator of oxidative stress. It occurs due to the oxidation of unsaturated fatty acids in cell membranes by free radicals. High MDA levels indicate oxidative damage to cellular membranes [50]. Therefore, water scarcity leads to osmotic stress in plants, resulting in increased lipid peroxidation and consequently MDA synthesis. Another molecule that increases under stress conditions is TSP. The reason for this increase is stress proteins (LEA, heat shock proteins, etc.) that are produced in plants under stress conditions [51]. Abid et al. [52] reported an increase in TSP in plants under stress; however, photosynthesis might come to a halt under intense stress conditions. Therefore, protein production may be disrupted, leading to a tendency for total protein content to decrease. These phenomena support our results.

Seed-based and foliar applications positively affected enzymatic antioxidant activity, MDA, and TSP compared to the control plants. Mohammadi et al. [53] investigated the effects of SA and GA on the germination characteristics of lentil (*Lens culinaris* L.). According to their results, SA and GA were applied separately and in different combinations, and their effects on germination percentage, germination rate, root and shoot growth of seedlings, polyphenol oxidase (PPO), and peroxidase (POX) enzyme activities were observed. According to the results, the combination of 150 µM SA+100 µM GA showed a significant increase in germination percentage compared to the control. Enzyme activity increased with GA alone, and SA reduced antioxidant enzyme activity. Bouallegue et al. [54] observed the effect of seed priming on physiological and biochemical responses of lentils (*Lens culinaris* L.) at the germination stage. In the study, seed priming with 0.1 mM SA and 0.1 mM H_2O_2 was effective in alleviating the negative effects of salt stress on germination, seedling development, and physiological activities. Kaur et al. [55] examined the effects of SA priming on the nitrogen fixation activities, plant development, seed yield, and antioxidant defense mechanism of chickpea (*Cicer arietinum*) varieties under salinity stress. The researchers reported that SA-priming preserved biomass and photosynthetic efficiency, prevented nodule senescence, reduced oxidative stress by activating the ROS scavenging mechanism, and consequently increased the yield potential of chickpea plants depending on the genotype

under both stress and optimal conditions. SA-priming reduced oxidative stress and increased the total antioxidant content in flax plants and reduced stress effects in lentil [56]. Similarly, numerous studies indicate that PGPB treatments promote the production of enzymatic and non-enzymatic antioxidants in plants and enhance stress resistance [57].

Conclusions

Effects of *B. frigoritolerans*, SA, and GA treatments in two barley genotypes (sensitive and tolerant) under drought stress were investigated in this experiment. First of all, we should mention that, as expected, the genotype with higher tolerance was more successful in combating stress. The genotype with higher stress tolerance (Tarm-92) showed higher antioxidant enzyme activity under stress conditions and was able to maintain plant growth. In the study, the concentration of SOD, APX, CAT, MDA, and TSP increased with increasing drought stress. Moreover, the changes in antioxidant enzymes, MDA, and TSP concentrations were significantly altered by seed priming and foliar applications. *B. frigoritolerans*, SA, and GA treatments did not significantly affect other morphological indicators except plant height. However, if there had been a longer vegetation period, the results would surely have been different. On the other hand, *B. frigoritolerans*, SA, and GA treatments directly affected photosynthetic activity and antioxidant defense system components. However, since the antioxidant defense system is very complex, a single treatment did not affect all parameters similarly. The findings of the study indicated that priming with PGPB containing ACC deaminase activity or foliar application of SA or GA, alone or in combination, showed positive results on photosynthetic and antioxidant activity compared to the control. In conclusion, foliar application of 1.5 mM SA or 110 mg L^{-1} GA with *B. frigoritolerans* priming ameliorated drought stress in barley. There is a need for deeper research on specific action mechanisms of exogenous treatments on antioxidant mechanisms. Here, we report that *B. frigoritolerans* has a noteworthy potential to mitigate drought stress in barley and has a synergistic relationship with SA and GA.

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Conflict of Interest

The authors declare no conflict of interest.

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Supplementary Material

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