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Exogenous Application of Proline and Salicylic Acid can Mitigate the Injurious Impacts of Drought Stress on Barley Plants Associated with Physiological and Histological Characters

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Abstract: Barley is a very important crop worldwide and has good impact in preserving food security. The impacts of 10 mM proline and 0.5 mM salicylic acid were evaluated on water stressed barley plants (*Hordeum vulgare* L. Giza126). Salicylic acid and proline treatments led to increased stem length, plant dry weights, chlorophyll concentration, relative water content, activity of antioxidant enzymes, and grain yield under drought stress. Nevertheless, lipid peroxidation, electrolyte leakage (EL), superoxide ($O_2^{\cdot-}$), and hydrogen peroxide (H_2O_2) significantly decreased in treated barley plants with proline and salicylic acid in both growing seasons as compared with drought treatment only, which caused significant decrease in stem length, plant dry weights, chlorophyll concentration, activity of antioxidant enzymes, as well as biological and grain yield. These results demonstrated the importance of salicylic acid and proline as tolerance inducers of drought stress in barley plants.

Keywords: Barley; drought; enzymes activity; electrolyte leakage; scanning electron microscope

1. Introduction

The barley (*Hordeum vulgare* L.) plant belongs to the family *Poaceae*, playing a main role in human food and animal feeding [1]. The production of barley grains was around 142.37 million tons in 2017/2018 (www.statista.com/statistics/271973/world-barley-production). However, it was influenced by environmental factors, particularly drought. Drought is a very damaging factor which causes decreases in morphological and physiological parameters and reduces plant growth and

production [2]. By 2050, the population of the world will reach more than 9 billion people and the adverse climate changes will extremely threaten food security [3]. The plants under deficit water stress have distinctive morphological symptoms such as wilting, etiolation, yellowing, and leaf downfall [4,5]. Stomatal closure and reduction in CO_2 concentration are the first responses to drought stress in leaves, consequently reducing photosynthetic activity. The stomatal density too is associated with tolerance of drought stress [6]. Drought stress led to significant reductions in chlorophyll concentrations and yield characters of flax plants [7].

Generally, biotic and abiotic stress cause oxidative damage, resulting in increase of electrolyte leakage and respiration rate, which lead to increased reactive oxygen species production (superoxide and hydroxyl radicals as well as hydrogen peroxide); however, stem length and root dry weights were reduced in many plants [8–11]. One of the main reasons of crop loss during the growth and flowering stages is drought, which causes decrease in the yield of most crops [12]. Morphological characters and grains yield significantly decreased under water-deficit stress conditions [13]. High concentration of reactive oxygen species (ROS) induces lipid peroxidation; causes injury to proteins; can damage to photosynthetic processes, thylakoid membranes, and pigments; and finally causes programmed cell death [14]. Accordingly, the plants can avoid ROS injury with upregulation of enzyme activity like catalase and polyphenol oxidase, which play vital roles in ROS scavenging [15–17]. Salicylic acid (SA) plays a main role under stress conditions in the regulation of photosynthesis and many physiological processes [13,18], proline metabolisms, and antioxidant defense systems as well as provides protection under abiotic stresses [19] and improves the growth characters and yield parameters of canola plant [4]. Use of SA significantly alleviated the injurious impacts of drought and decreased lipid peroxidation in wheat plants [20], increased growth characters and yield of faba bean plants, as well as improved activity of antioxidant enzymes [13]. Proline accumulation is induced in plant tissues under drought conditions, and it is considered as osmoregulators, which helps in osmoregulation and protects the structure of organs and membranes of plant cells [21]. Furthermore, proline plays a pivotal role in alleviating the injurious effects of drought and salinity stress in addition to protecting chloroplasts, mitochondria, and DNA from oxidative damage [22]; improving photosynthetic processes; and increasing soluble sugar accumulation. Thus, our study aimed to assess the impact of salicylic acid and proline on morphophysiological, biochemical characters and yield as well as scanning electron microscope investigation of barley plants under drought conditions.

2. Materials and Methods

2.1. Plant Materials

The research experiments were conducted on the farm of the Faculty of Agriculture, Plant Pathology, and Biotechnology Laboratory (PPBL) and Egyptian Phytomicrobial Collection for Research and Sustainability Excellence Center (EPCRS) Excellence Center, Kafrelsheikh University, Egypt. The meteorological data of this location is presented in Table 1 according to NASA POWER Data Access Viewer-Prediction of Worldwide Resource (https://power.larc.nasa.gov/data-access-viewer).

Table 1. The meteorological data of the experimental location from sowing to harvesting date.

Meteorological Data	Season	December	January	February	March	April	May
Precipitation (mm day^{-1})	2017	8.36	7.78	7.85	0.58	47.42	0.03
	2018	8.13	32.80	10.80	1.61	3.09	0.01
Relative Humidity at 2 Meters (%)	2017	67.35	68.84	67.23	63.81	59.35	53.37
	2018	67.82	68.49	67.61	55.02	54.00	54.25
Maximum Temperature at 2 Meters (°C)	2017	21.88	17.16	18.86	21.86	25.25	29.92
	2018	19.65	18.69	20.99	25.41	27.58	31.35
Minimum Temperature at 2 Meters (°C)	2017	14.21	9.70	10.37	12.50	14.21	18.37
	2018	13.66	11.64	12.15	13.66	15.76	19.90

Some of the physiological studies were conducted in the center lab of excellence center in biotechnology research, king Saud university during two growing seasons of 2017/2018 and 2018/2019. The research study was designed to study the influence of proline and salicylic acid in mitigating the harmful impacts of drought on morphophysiological, biochemical, and yield characteristics of *Hordeum vulgare* L. (Giza 126). The experiment was laid out as a randomized complete block design with three replicates; the plot size was 2.5 × 2.5 m^2. The previous crop was maize (*Zea mays* L.). The recommended doses of chemical fertilizers were nitrogen at 110 kg N ha^{-1} ammonium sulfate and phosphorus at 125 kg P_2O_5 ha^{-1} superphosphate. Calcium superphosphate was added according to the recommendation of the Ministry of Agriculture during seedbed preparation. The amount of N fertilizer was added to each plot at two doses: the first dose was at sowing date, while the second one was at booting stage. Barley grains (120 kg ha^{-1}) were sown on 4 December 2017 and on 8 December 2018 in typical clay soil. The characters of the experimental soil were recorded according to Association of Officinal Analytical Chemists (AOAC; 2005) [23] as follows: pH 8.2; electrical conductivity 1.8 dS m^{-1}; available phosphorus 10.5 ppm; available nitrogen 32.4 ppm; available potassium 289 ppm; silt 35.5%; sand 17.3%; clay 47.2%; and texture clay. The treatments were four as follows: 1) control (well water plants) plants receive five irrigations during growing season; 2) drought treatment (D) plants receive one irrigation only after germination; 3) drought treatment + 0.5 mM SA; and 4) drought treatment + 10 mM proline. Foliar application of SA and proline was done one time after 21 days from sowing date. The harvested dates were on 2 May 2018 and 7 May 2019, respectively.

2.2. Morphophysiological and Biochemical Studies

Ten plants were randomly taken at anthesis stage to determine length of stem, number of leaves, fresh and dry weights, chlorophyll concentrations as well as proline, electrolyte leakage (EL), Lipid peroxidation (MDA), and relative water content. According to Lichtenthaler (1987) [24], concentrations of chlorophyll a and chlorophyll b were determined as mg g^{-1} fresh leaves and the absorbance was determined at 663 and 648 nm. Electrolyte leakage was measured according to Szalai et al (1996) [25]. As described by Simaei et al. (2011) [26], proline content was determined in fresh leaves as mM g^{-1} FW (fresh weight). Lipid peroxidation was determined according to Davenport et al. (2003) [27] by calculating the amount of malondialdehyde. Lipid peroxidation (MDA) was recorded as follow: (nmol g^{-1}fw) = (6.45 × (A532 − A600) − (0.56 × A450)) × V-1W, where V = volume (ml) and W = weight (g).

Hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\cdot-}$) were assayed as described by Ádám et al. (1989) [28].

Catalase activity (CAT) was measured according to Aebi et al. (1983) [29]. Peroxidase (POX) activity was measured as described with Hammerschmidt et al. (1982) [30]. Polyphenol oxidase (PPO) activity was measured as described with Malik and Singh (1980) [31].

2.3. Scanning Electron Microscope (SEM) Investigation and Yield Characters

Histological structure of barley flag leaf epidermal tissue was investigated with scanning electron microscope (SEM). Samples of flag leaf at 50 days from sowing (4 mm^2) were taken and prepared for scanning electron microscope investigation according to Harley and Ferguson (1990) [32]. At harvest date (2 May 2018 and 7 May 2019), the yield components; (length of spike, no. of grains $spike^{-1}$, 1000 grains weight, and biological and grain yield ton ha^{-1}) were determined.

2.4. Statistical Analysis

One- and two-way analysis of variances (ANOVAs) were carried out using IBM-SPSS Statistics for Mac OS, version 23.0. (IBM SPSS, Armonk, NY: IBM Corp), and the means were compared using Tukey's honestly significant difference (HSD) post hoc test at $p \leq 0.05$ when the differences were significant ($p \leq 0.05$). Data were represented as mean ± standard error (SE) (Gomez and Gomez 1984, Duncan 1955, O'Mahony 1986) [33–35].

3. Results

3.1. Effect of Proline and SA on Morphophysiological Characters and Biochemical Analysis of Barley Plants under Drought Stress

The stem lengths (cm) of barley plants in the untreated control group in the first and second season were 59.25 ± 1.58 cm and 60.25 ± 2.79 cm, which significantly ($p < 0.05$) decreased to 51.25 ± 1.45 cm and 54.00 ± 2.29 cm in the drought stressed plants, respectively (Table 2). According to the results during the two growing seasons 2017/2018 and 2018/2019 presented in Table 2, drought stress significantly ($p < 0.05$) decreased fresh and dry weight of barley plants compared to the control plants group (well water) and other treatments. The highest values of stem length recorded were 66.0 ± 1.30 cm and 67.5 ± 2.42 cm in the barley plants under drought stress and treated with SA in both growing seasons. However, the highest fresh weights of 8.01 ± 0.26 g and 9.44 ± 0.35 g in the drought + SA group and that for dry weight at 3.61 ± 0.15 and 3.6 ± 0.15 g were obtained with drought stressed plants treated with salicylic acid (D + salicylic acid), which were significantly ($p < 0.05$) different from drought stressed group. The lowest values of stem length (51.25 ± 1.45 and 54.0 ± 2.29 cm), fresh weight (5.45 ± 0.14g and 5.71 ± 0.12 g), and dry weight (2.06 ± 0.06 g and 2.52 ± 0.09 g) in the two growing seasons respectively were recorded in drought stressed plants (Table 2). Contrariwise, there was no significant difference ($p > 0.05$) among the treatments in leaves number/plant (Table 2). Under drought stress, chlorophyll a and b concentrations were considerably reduced in stressed plants compared to the control plants. Proline and SA treatments led to increased concentrations of chlorophyll a and b in the stressed plants compared with control and untreated stressed plants in both seasons. The obtained data pointed out that the maximum levels of chlorophyll a and b were recorded in water stressed plants treated with proline (1.74 ± 0.17 and 1.83 ± 0.24 mg g^{-1} FW and 1.13 ± 0.03 and 1.2 ± 0.30 mg g^{-1} FW) and with SA (1.66 ± 0.24 and 1.50 ± 0.15 mg g^{-1} FW and 1.07 ± 0.03 and 0.86 ± 0.09 mg g^{-1} FW) respectively, in the two growing seasons (Figure 1A,B). Moreover, the highest leaf total chlorophyll contents of 2.87 ± 0.19 mg g^{-1} FW were recorded in proline-treated barley under drought stress. Generally, treatments of barley plants with either proline or SA significantly ($p < 0.05$) improved chlorophyll b and total chlorophyll contents. Differences were assessed by one- and two-way ANOVAs followed by Tukey's HSD post hoc tests at $p < 0.05$.

Table 2. Stem length, number of leaves, and fresh and dry weights of barley plants treated with salicylic acid and proline under drought stress during two growing seasons (2017/2018–2018/2019). Data are represented as mean (±standard error (SE)); differences between treatment groups were assessed by ANOVA. Means in the same column followed by the different letter are significantly different according Tukey's HSD (honestly significant difference) test at $p < 0.05$.

Treatments	Stem Length (cm)		Number of Leaves		Fresh Weight (FW, g plant^{-1})		Dry Weight (DW, g plant^{-1})	
	First	Second	First	Second	First	Second	First	Second
	Season		Season		Season		Season	
Control	59.25b ± 1.58	60.25ab ± 2.79	5.75a ± 0.53	6.50a ± 0.46	7.36a ± 0.35	8.91a ± 0.35	2.99a ± 0.32	3.52a ± 0.14
Drought (D)	51.25c ± 1.45	54.0 b ± 2.29	5.00a ± 0.73	4.75a ± 0.14	5.45b ± 0.14	5.71b ± 0.12	2.06b ± 0.06	2.52b ± 0.09
(D) + Salicylic acid	66.00a ± 1.30	67.50a ± 2.42	5.50a ± 1.00	5.75a ± 0.26	8.01a ± 0.26	9.44a ± 0.35	3.61a ± 0.15	3.60a ± 0.15
(D) + Proline	62.25ab ± 0.87	62.13ab ± 2.21	5.25a ± 0.29	5.50a ± 0.47	7.66a ± 0.47	9.02a ± 0.26	3.06a ± 0.16	3.47a ± 0.10
ANOVA: F-ratio	24.48	4.82	0.197	3.28	9.53	37.29	11.17	18.86
p-value	<0.001 ***	0.033 *	>0.05 ns	>0.05 ns	0.005 **	<0.001 ***	0.003 **	<0.001 ***

* Significant at $p < 0.05$; ** highly significant at $p < 0.01$; *** very highly significant at $p < 0.001$; NS, nonsignificant at $p > 0.05$.

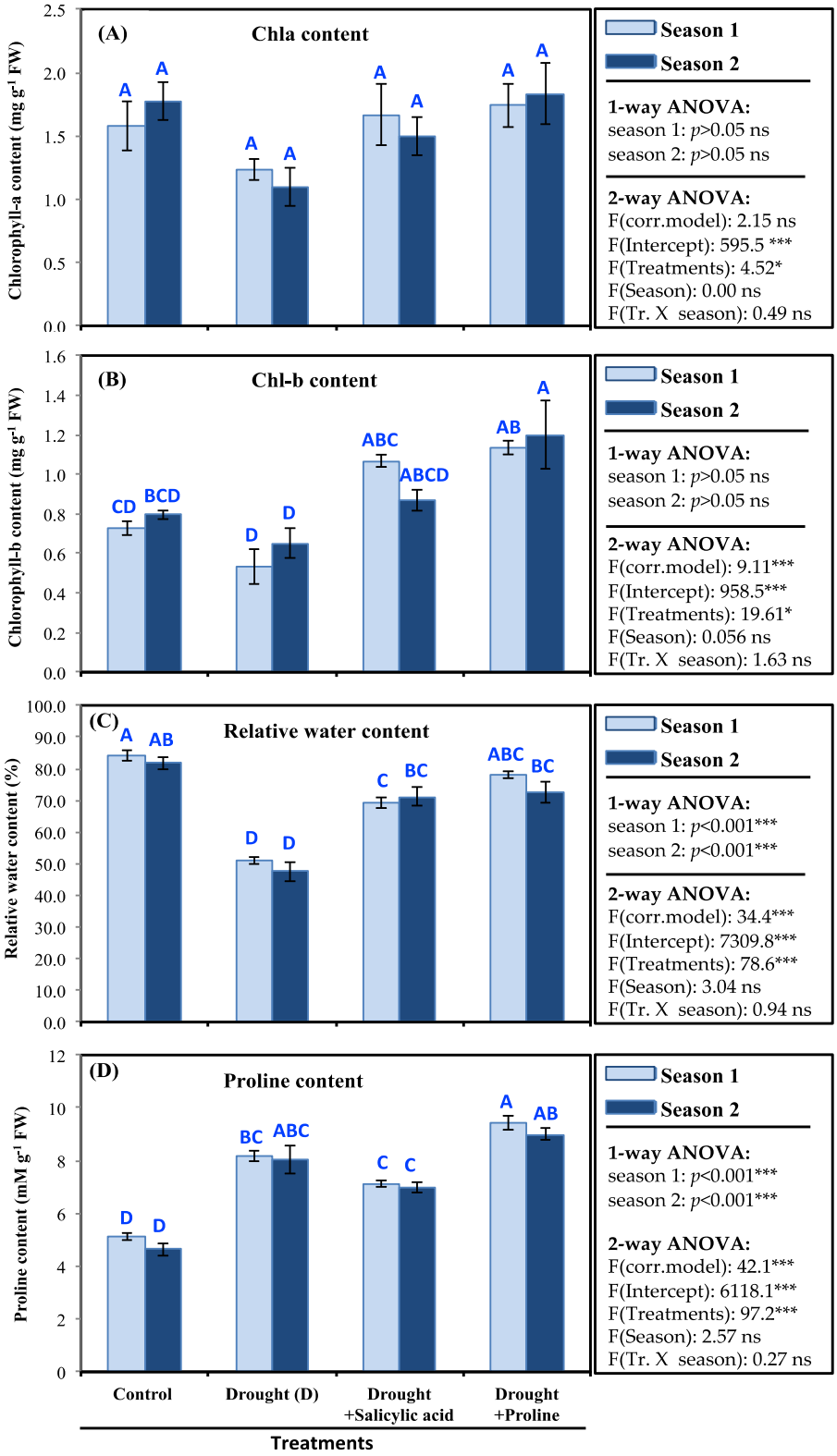

Figure 1. Effect of salicylic acid and proline on chlorophyll a and b concentrations (**A**,**B**), relative water content (**C**), and proline content (**D**) in barley plants under drought stress during two growing seasons: season 1 (2017/2018) and season 2 (2018/2019). Data represented as mean ± SE); differences between treatment groups were assessed by one- and two-way ANOVAs. Bars with different letters are significantly different according to Tukey's HSD at $p < 0.05$. * Significant at $p < 0.05$; ** highly significant at $p < 0.01$; *** very highly significant at $p < 0.001$; NS, nonsignificant at $p > 0.05$.

The effects of either proline or SA treatments on relative water contents and proline content in barley are presented in Figure 1C,D. Drought caused significant reduction ($p < 0.05$) in relative water content (58.3% ± 1.35 % and 53% ± 3.11%) in both seasons, respectively; nevertheless, the maximum values (81.5% and 84%) were recorded in control plants. Additionally, proline and SA treatments led to significant increase in relative water content (RWC; 72.5% and 74.6%, and 76% and 78.2%, respectively) in both seasons compared with stressed untreated plants (Figure 1C). From Figure 1D, it could be observed that water stressed-plants showed significant increase in proline contents (7.4 and 7.9 mM g^{-1} FW) compared with control plants (5.2 and 4.5 mM g^{-1} FW) in the two seasons, respectively. Interestingly enough, proline contents were enhanced with application of proline and SA; the maximum values (9.6 and 9.1 mM g^{-1} FW) were obtained with proline treatment in both seasons. Exposing plants to drought stress led to increased electrolyte leakage (43% and 41.5%) in the two seasons compared with control and the treated stressed plants with proline and SA (Figure 2A). Our results indicated that application of proline and SA led to decreased electrolyte leakage (EL) in stressed treated plants (21% and 24%, and 25% and 23.5%) in both seasons, respectively; the best result was obtained with stressed treated plants with SA. As a response of drought, data presented in Figure 2B indicated that drought stress significantly increased lipid peroxidation (MDA) (5 and 5.2 μmol g^{-1} FW) compared to control plants (3.9 and 4.2 μmol g^{-1} FW) in both seasons, respectively. However, application of proline and SA treatments significantly decreased lipid peroxidation (3.5% and 3.6%, and 3.6 and 4.7 μmol g^{-1} FW) compared to stressed untreated plants in the two seasons.

The presented data in Figure 3 revealed that accumulation of hydrogen peroxide and superoxide in stressed plants was higher than control plants and other treatments (Figure 3A,B). Likewise, concentrations of $O_2^{\cdot-}$ and H_2O_2 were increased significantly in water-stressed plants (39 and 43, and 34 and 35) compared with control plants (28 and 30, and 17 and 18) in the two seasons, respectively. Conversely, foliar application of proline and SA led to decreased concentration of $O_2^{\cdot-}$ (27 and 30, and 28 and 33) in both seasons and concentration of H_2O_2 (21.5 and 23, and 25 and 27) in the two seasons, respectively (Figure 3C,D).

In the current study, the results showed that CAT, POX, and PPO enzyme activities were increased significantly by exposure to drought stress (Figure 4A,C). CAT activity was increased significantly (176.4 and 181 mM H_2O_2 g^{-1} FW min^{-1}) in stressed barley plants compared with control (149.6 and 142.8 mM H_2O_2 g^{-1} FW min^{-1}) in both seasons (Figure 4A). However, application of SA led to improved CAT activity (139 and 146.5 mM H_2O_2 g^{-1} FW min^{-1}) in both seasons; likewise, proline led to improved CAT activity (142.6 and 127.8 mM H_2O_2 g^{-1} FW min^{-1}) in compared to control in the two seasons. Correspondingly, proline and SA treatments led to improve POX and PPO activities in barley plants under drought conditions in both seasons (Figure 4B,C).

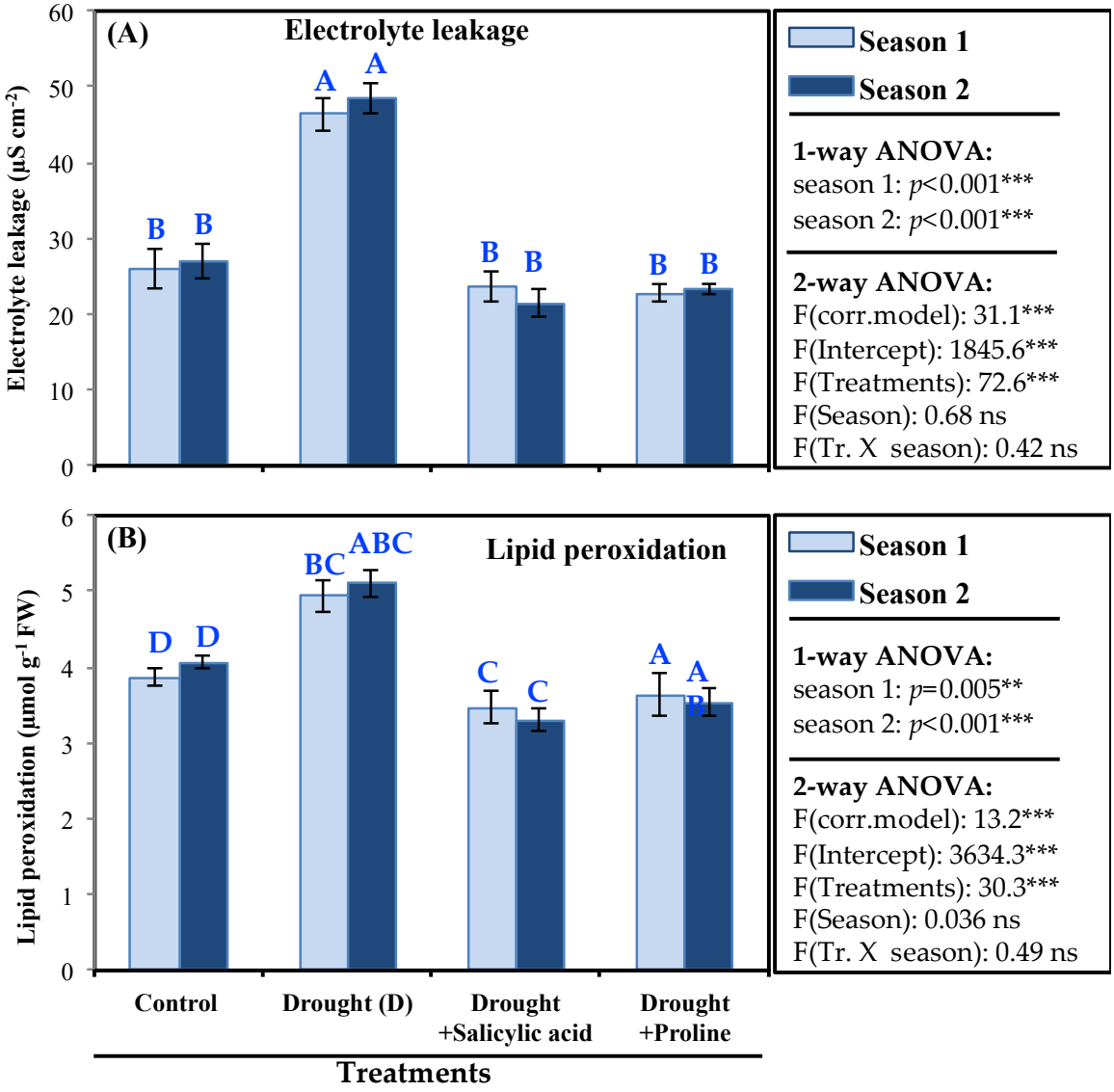

Figure 2. Effect of salicylic acid and proline on electrolyte leakage (**A**) and lipid peroxidation (**B**) in barley plants under drought stress during two growing seasons: season 1 (2017/2018) and season 2 (2018/2019). Data are represented as mean (±SE); differences between treatment groups were assessed by one- and two-way ANOVAs. Bars with different letters are significantly different according to Tukey's HSD at $p < 0.05$. * Significant at $p < 0.05$; ** highly significant at $p < 0.01$; *** very highly significant at $p < 0.001$; NS, nonsignificant at $p > 0.05$.

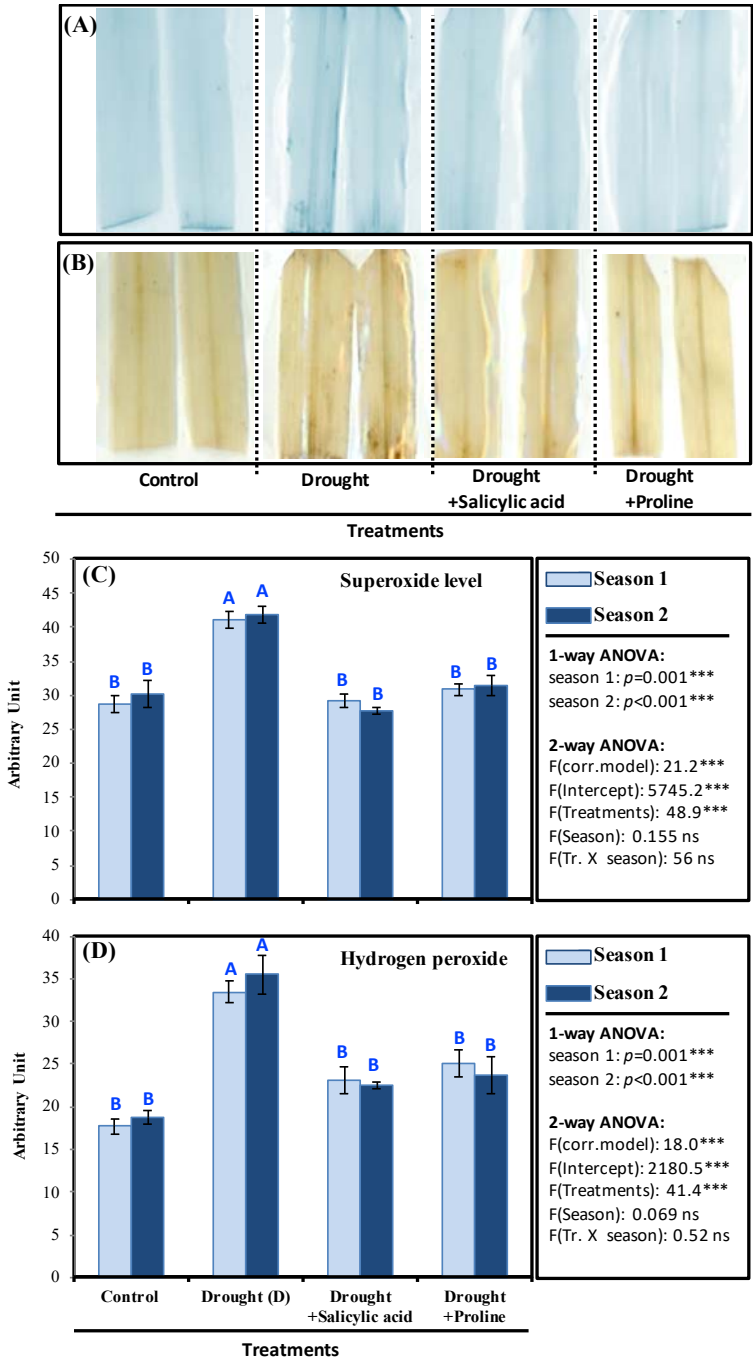

Figure 3. Effect of salicylic acid and proline on accumulation of superoxide, $O_2^{\cdot-}$ (**A**), and hydrogen peroxide, H_2O_2 (**B**), during second season (2018/2019) and concentrations of superoxide (**C**) and hydrogen peroxide (**D**) in barley plants under water deficit stress during two seasons: season 1 (2017/2018) and season 2 (2018/2019). Data are represented as mean (±SE); differences between treatment groups were assessed by one- and two-way ANOVAs. Bars with different letters are significantly different according to Tukey's HSD at $p < 0.05$. * Significant at $p < 0.05$; ** highly significant at $p < 0.01$; *** very highly significant at $p < 0.001$; NS, nonsignificant at $p > 0.05$.

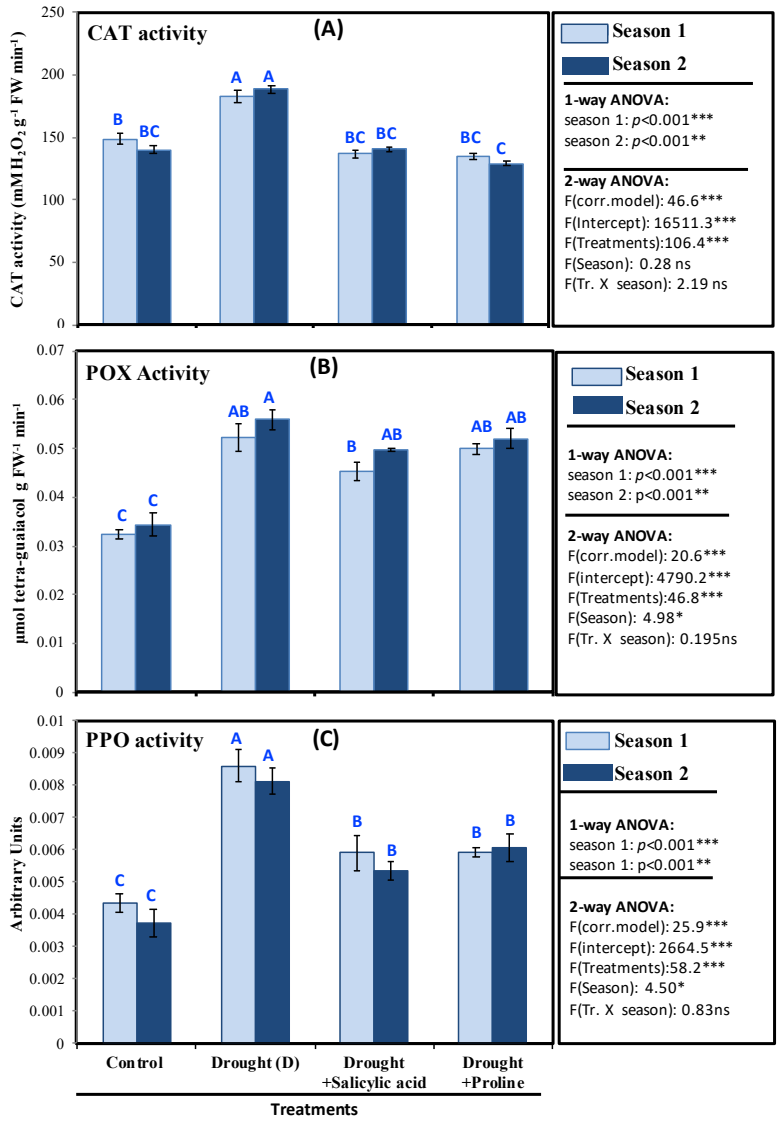

Figure 4. Effect of salicylic acid and proline on activities of catalase, CAT (**A**); peroxidase, POX (**B**); and polyphenol oxidase, PPO (**C**), enzymes in barley plants under drought stress during two growing seasons: season 1 (2017/2018) and season 2 (2018/2019). Data are represented as mean (±SE); differences between treatment groups were assessed by one- and two-way ANOVAs. Bars with different letters are significantly different according to Tukey's HSD at $p < 0.05$. * Significant at $p < 0.05$; ** highly significant at $p < 0.01$; *** very highly significant at $p < 0.001$; NS, nonsignificant at $p > 0.05$.

3.2. Effect of Proline and SA on Yield and Scanning Electron Microscope (SEM) Investigation of Barley Plants under Drought Stress Conditions

The decrease in spike length (cm), grain $spike^{-1}$, grain yield, and biological yield (ton ha^{-1}) as well as 1000 grains weight (g) under drought conditions are presented in Table 3. Drought stress led to significant decreases in spike length (6.25 and 6.65 cm) compared with control plants (8 and 7.75 cm) in both seasons; however, application of proline and SA significantly increased spike length (8 and 8.20, and 8.6 and 8.5 cm) under drought stress in both seasons, respectively. Furthermore, grains per spike were significantly ($p < 0.05$) decreased according to water-deficit stress (48 and 46.5) compared with control plants (60 and 57). Foliar treatment with SA led to increases in grains per spike, but the increase was not significant ($p > 0.05$) (54 ± 2.05 and 51.5 ± 0.68) compared to the control in both seasons, while proline application led to significant increases in grains per spike (58.5 and 60.5) in both seasons. The weight of 1000 grains and biological and grain yield were considerably decreased in both seasons

compared with control plants. Proline and SA treatments led to improve yield characters; the highest values of biological yield (5.46 ± 0.07 and 5.77 ± 0.11 ton ha^{-1}) and grain yield (2.98 ± 0.12 and 2.98 ± 0.19 ton ha^{-1}) as well as weight of 1000 grains (66.22 ± 1.27 g and 67.18 ± 0.92 g) were obtained with SA treatment in both seasons, respectively. The scanning electron microscope (SEM) investigation presented in Figure 5 shows that the epidermal tissue consists of small cells in water-stressed barley plants as compared with control plants. Application of proline and SA gave small sunken guard cells compared with guard cells in control plants; these normal guard cells were arranged in parallel rows. Also, these treatments led to improved epidermis cell structure in the stressed plants.

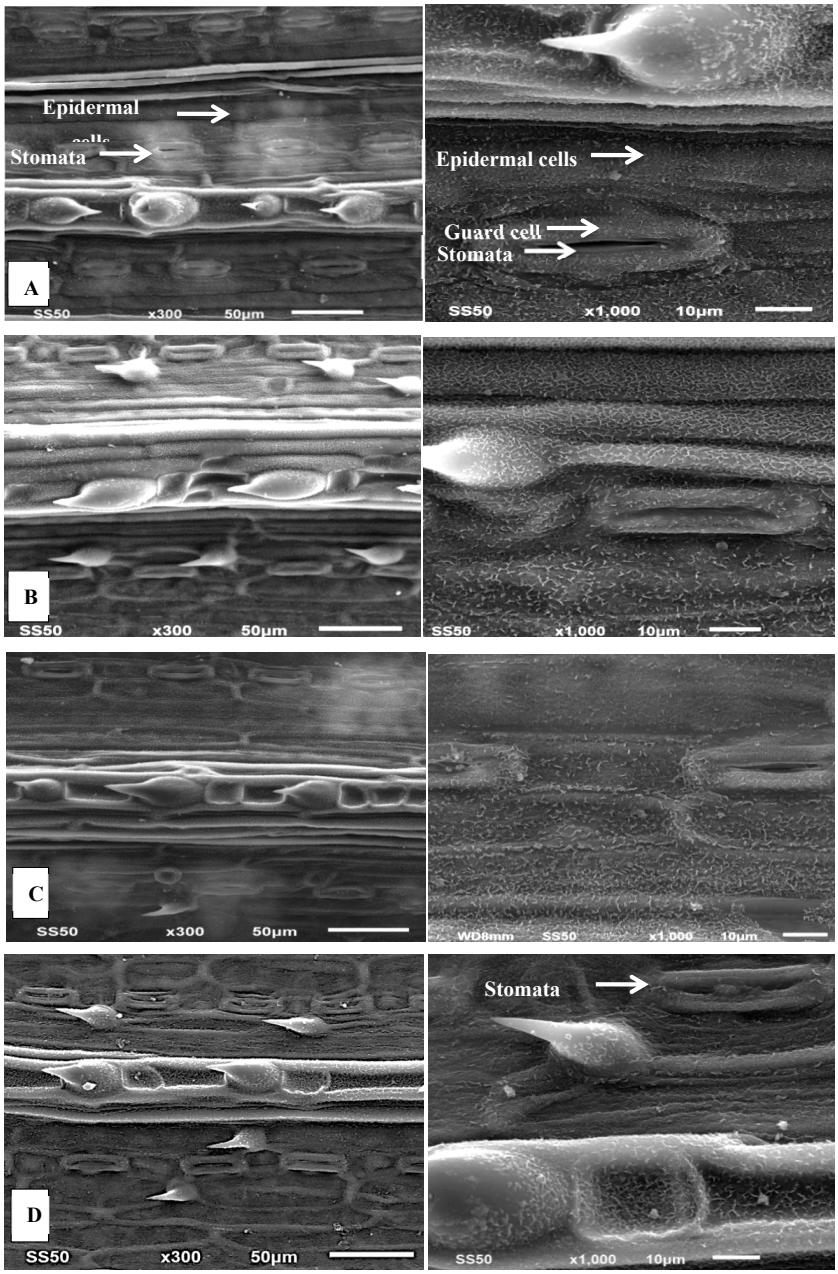

Figure 5. Effect of salicylic acid and proline on barley leaves by Scanning Electron Microscope (SEM) during growing season (2018/2019): (**A**) control, (**B**) water-deficit stress (**D**), (**C**) D + salicylic acid, and (**D**) D + proline. Bar = 10 µm and bar = 50 µm.

Table 3. Spike length, grains per spike, biological yield, grain yield, and weight of 1000 grains of barley plants treated with salicylic acid and proline under drought stress during two growing seasons (2017/2018–2018/2019): Data are represented as mean (±SE); differences between treatment groups were assessed by ANOVA. Means in the same column followed by the different letter are significantly different according Tukey's HSD at $p < 0.05$.

Treatments	Spike Length (cm)		Grains per Spike		Biological Yield (ton ha^{-1})		Grain Yield (ton ha^{-1})		Weight of 1000 Grains (g)	
	First Season	Second Season	First Season	Second Season	First Season	Second Season	First Season	Second Season	First Season	Second Season
Control	8.00a ± 0.32	7.75b ± 0.23	60.0a ± 2.00	57.0a ± 0.88	5.47a ± 0.12	5.35a ± 0.18	2.20b ± 0.07	2.24b ± 0.05	61.53b ± 0.86	57.99a ± 1.98
Drought (D)	6.25b ± 0.06	6.65c ± 0.29	48.0b ± 0.52	46.5c ± 2.52	3.07b ± 0.14	3.01b ± 0.10	1.42c ± 0.06	1.43c ± 0.07	49.30c ± 0.87	53.07b ± 1.56
(D) + Salicylic acid	8.00a ± 0.15	8.60a ± 0.24	54.0a ± 2.05	51.5b ± 0.68	5.46a ± 0.07	5.77a ± 0.11	2.98a ± 0.12	2.98a ± 0.19	66.22a ± 1.27	67.18a ± 0.92
(D) + Proline	8.20a ± 0.16	8.50a ± 0.11	58.5a ± 1.06	60.5a ± 0.29	5.56a ± 0.14	5.63a ± 0.17	2.70a ± 0.13	3.02a ± 0.15	61.33b ± 2.72	65.04a ± 0.98
ANOVA: F-ratio	7.7	8.8	12.3	19.8	98.8	77.4	45.4	31.04	21.5	21.5
***p*-value**	0.010 **	0.006 **	0.002 **	<0.001 ***	<0.001 ***	0.001 ***	<0.001 ***	0.001 ***	<0.001 ***	<0.001 ***

* Significant at $p < 0.05$; ** highly significant at $p < 0.01$; *** very highly significant at $p < 0.001$; NS, nonsignificant at $p > 0.05$.

4. Discussion

The significant ($p < 0.05$) decrease in leaves number, stem lengths, and dry weights of barley plants (Table 2) under drought stress conditions could be due to the reduction of water absorption from the soil and consequently to decreased cell division and elongation as well as plant growth [36,37]. The impact of drought on leaves number can result from the adverse effect on cell elongation and division as well as nucleic acid synthesis [13]. The reduction in growth characters of barley plants under drought stress conditions agrees with the results of [4,13] in various plants. Likewise, drought stress significantly decreased photosynthetic rate, which resulted in reduction in the yield production. The negative effect of drought on the growth characters might be due to the key role of water in physiological processes, for example, stimulating the photosynthetic rate, which affects plant dry weight [38]. In the current research, the injurious impact of drought conditions on relative water content and chlorophyll concentrations in barley could be due to the reduction in water flow, to decline of chlorophyll, to disorder of thylakoid, to dehydration of protoplasm, and to photo-assimilation level, as well as to oxidative damage to the chloroplasts; induces stomatal closure; and decreases Co_2 concentration in the mesophyll cells [39]. This adverse effect could be due to the chlorophyll oxidation. The effect of drought stress in increasing Lipid peroxidation (MDA) and electrolyte leakage (EL) may be a result of protoplasm dehydration [39], consequently causing oxidative stress to cell membranes. The same trend was recorded under salinity stress in many plants [9,40,41]. Relative water content (RWC) significantly decreased under drought stress conditions according to the increase in plasma membrane permeability and the reduction in water supply. Moreover, proline accumulation was higher in stressed plants, which may be due to the key role of proline in osmotic regulation under drought stress. Likewise, $O_2^{\cdot-}$ and H_2O_2 significantly increased in mesophyll cells of water-stressed plants because of the drought role in cellular dehydration of plants, causing protein degradation and thus maximizing oxidative stress [29]. Contrariwise, the activity of antioxidant enzymes (CAT, PPO, and POX) increased due to drought stress; the plants depend on these enzymes as a defense system to scavenge the ROS [35,36]. The injurious impact of drought stress on yield components may be related to the decrease in leaves numbers, RWC, cell division, and photosynthetic process [39,40]. Under drought conditions, the significant decrease in barley grain yield was also reported mostly because of decreased grains number as well as 1000 grain weight. SA treatment increased morphological characters of barley plants under drought conditions. The effect of SA may be related to increased element uptake as well as photosynthetic rate. Additionally, SA can improve enzymatic (CAT, POX, and PPO) and nonenzymatic antioxidant activity and plays a main role in the enhancement of plant stress tolerance as well as decreased oxidative stress [42], consequently improving plant growth of drought-stressed plants. SA significantly increased yield characters because of its role in improving flower formation and grain yield. In agreement with our findings, application of SA enhanced number of pods and 100-seed weights of faba beans under drought stress [13]. These impacts of SA could be due to the greater role of SA to reserve water in plant cells and to enhance the enzyme activities under stress conditions [43], consequently increasing yield characters. Under drought stress, proline treatment led to adjustments in osmotic pressure and improvements in the root system to get water under low water potentials [44]. Moreover, proline accumulation significantly increased in drought-stressed plants and in tolerant rather than sensitive cultivars [32]. The valuable impact of proline might be due to its role as a scavenger and osmolyte, consequently improving growth characters and yield production under drought conditions. Some researchers reported that osmoregulators enhance growth and yield characters in some plants under drought, salinity and biotic stress conditions [45–48]. Stomatal pores are a very important part of leaves, considered the main way through which upper and lower epidermises exchange gas and supply water. Under drought conditions, the reduction in stomatal aperture in stressed barley plants (Figure 5) may lead to conserved water in the leaf, to reduced transpiration levels, and to saved soil moisture, consequently avoiding the injurious effect of drought stress conditions on barley plants [6].

5. Conclusions

To conclude, this research was planned to examine the impact of proline and salicylic acid on barley plants under drought stress. The growth characters and biochemical and yield characters of barley were considerably decreased in stressed plants compared with control (well-watered) plants. Nevertheless, foliar application of proline and SA led to improvements in the plant growth characters, such as stem length, number of leaves, fresh and dry weight of plants, as well as relative water content; however, electrolyte leakage and lipid peroxidation were decreased. Furthermore, foliar application of proline and SA led to improved yield characters in stressed plants compared with untreated stressed barley plants.

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