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Biochemical and physiological response of borage to seed priming and water deficit: antioxidant enzymes, osmolytes, photosynthetic pigments, and fluorescence parameters

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Abstract

Aim of study: To investigate the general response patterns of the borage plant to water fluctuations from a biochemical and physiological perspective.

Area of study: East Azerbaijan Province of Iran during the period 2012 and 2013.

Material and methods: The study investigated the effects of irrigation (after 60, 90, 120 and 150 mm evaporation) and priming (unprimed, and primed seeds with water, 1% KNO_3 and 1% KH_2PO_4) on the antioxidant enzymes, osmolytes, photosynthetic pigments, and fluorescence parameters of borage using a split-plot experimental design.

Main results: The statistical analyses showed no effect of seed priming on all evaluated traits other than extracellular superoxide dismutase SOD3 activity where it was significantly enhanced by seed pretreatment with 1% KNO_3 and 1% KH_2PO_4. However, irrigations after 120 and 150 mm evaporation increased Cu/Zn-superoxide dismutase (SOD1), SOD2 and SOD3, soluble sugars, and initial fluorescence (F_0). The mean contents of Ch *a*, Ch *b*, and Ch *a*+Ch *b* under mild, moderate and severe water deficit were significantly higher than those under normal irrigation. Severe drought stress gave the highest carotenoids content and quantum yield baseline parameter (F_0/F_m) of borage leaves. However, water limitation decreased Chl *a*/Chl *b* ratio, maximum primary yield of photosystem II (F_v/F_0), and maximum quantum yield of photosystem II (F_v/F_m).

Research highlights: Based on these findings, it is postulated that the increase in soluble sugars and SOD activity under stress, and the accumulation of carotenoids under severe water limitation indirectly enhance the tolerance of borage plants to drought stress.

Additional key words: *Borago officinalis* L.; chlorophyll; compatible solutes; drought stress; fluorescence; superoxide dismutase.

Abbreviations used: APX (ascorbate peroxidase); CAT (catalase); Ch (chlorophyll); F_m (maximal fluorescence); F_0 (ınitial fluorescence); F_0/F_m (quantum yield baseline); F_v (variable fluorescence); F_v/F_m (maximum quantum yield of PSII photochemistry); F_v/F_0 (maximum primary yield of PSII); FW (fresh weight); NBT (nitro blue tetrazolium); PAGE (polyacrylamide gel electrophoresis); POX (peroxidase); ROS (reactive oxygen species); SOD (superoxide dismutase).

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Introduction

Borage (*Borago officinalis* L.), from the Boraginaceae family, has industrial, pharmaceutical, and forage applications (Peiretti *et al.,* 2004). The leaves, the flowers, and the oil extracted from the seeds are used as medicine. It contains various biologically active substances that can be used in treating diseases such as arthritis, diabetes, heart diseases, multiple sclerosis, and eczema (Asadi-Samani *et al.,* 2014). Plant growth is a dynamic process that is continuously affected by environmental conditions (Khadem Moghadam *et al.,* 2020; Wang *et al.,* 2020; May *et al.,* 2021). Based on the appearance and morphological characteristics of borage, it seems that this plant can tolerate water deficit to some extent after seedling establishment.

Plants experience several biotic and abiotic stresses during the growing season (López-Serrano *et al.*, 2017; Jin *et al.,* 2021; Tiwari *et al.,* 2022). Among the different types of stresses, drought emerges as the most critical abiotic environmental stress, which causes various biochemical and physiological changes, and limits plants' growth and production (Heshmat *et al.*, 2021; Raza *et al.,* 2021; Ullah & Farooq, 2021). Some adverse effects of water deficit on plants can be diminished by seed priming. Seed priming is used to speed up and synchronize the seedling emergence of many species and to produce vigorous plants with better tolerance to adverse environmental conditions (Ashraf & Foolad, 2005; Abbasi Khalaki *et al.*, 2021). Seedling emergence and establishment may be improved by hydro- and osmo-priming treatments (Ghassemi-Golezani *et al.,* 2008). Seed pretreatment with potassium nitrate (KNO_3) and monopotassium phosphate (KH_2PO_4) has been recommended as a low-cost and efficient way of priming seeds (Abdulrahmani *et al.,* 2007; Ghassemi-Golezani *et al.,* 2008).

Plants subjected to various abiotic stresses can overproduce reactive oxygen species (ROS) (Aliyari Rad *et al.*, 2021). These species are typically the result of activating O_2 to form singlet oxygen ($^1O_2$) or reducing O_2 to superoxide radical ($O_2^{-\bullet}$) and hydroperoxyl radical ($HO_2^{\bullet}$), and other byproducts like hydrogen peroxide (H_2O_2) or hydroxyl radical ($OH^{\bullet}$) (Tuteja *et al.*, 2011; Halliwell & Gutteridge, 2015). A low level of ROS is essential for intracellular signaling and acclimation of plants to abiotic stresses (Suzuki & Mittler, 2006), but their production at high levels can seriously damage biological macromolecules and eventually leads to cell death (Farooq *et al.,* 2009). Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) have a crucial role in scavenging these harmful molecules, membrane integrity preservation, and protection of DNA and proteins (Sharma *et al.,* 2012). Changes in antioxidant activity or content reflect the environmental stress effects on plant metabolism.

Under drought stress, plants accumulate various organic and inorganic compatible solutes (osmolytes) in the cytosol to reduce the osmotic potential of the cell and maintain the cell volume and turgidity against dehydration, which is identified as osmotic adjustment (Anjum *et al.,* 2011). Osmolytes are non-toxic compounds with low molecular weight, which do not interfere with cell metabolism and protect plants from adverse effects of stress (Farooq *et al.,* 2009). Compounds such as proline and soluble sugars have a significant role in osmotic adjustment, and protection of membranes and macromolecules.

Light energy is absorbed by chlorophylls and carotenoids, and is transferred into the plant photosynthetic apparatus (Uvalle-Sauceda *et al.,* 2008). Under drought stress, the level of photosynthetic pigments changes dramatically. Chlorophyll (Ch) has a key role in light trapping and converting it into chemical energy, so any decline in chlorophyll content may decrease the photosynthesis. Chlorophylls *a* and *b* are the main photoreceptors for photosynthesis (Pareek *et al.,* 2017). Carotenoids not only contribute to light absorbance (Jaleel *et al.*, 2009), but also protect cell membranes from ROS-induced oxidative damage (Verma & Mishra, 2005). Specifically, β-carotene can directly quench triplet chlorophyll that prevents singlet oxygen generation, inhibits lipid peroxidation, and stabilizes membranes (Farooq *et al.,* 2009). Plant species with higher carotenoids content under oxidative stress show effective defense and better tolerance against water deficit (Farooq *et al.,* 2009).

The capacity of plants for photochemical activity is limited. Excess solar energy absorbed (more than photochemical consumption) is released as heat in non-photochemical processes or reflected as red light known as chlorophyll *a* fluorescence. Measuring chlorophyll *a* fluorescence including initial fluorescence (F_0), maximal fluorescence (F_m), variable fluorescence (F_v), quantum yield baseline (F_0/F_m), maximum primary yield of photosystem II (F_v/F_0), and maximum quantum yield of PSII photochemistry (F_v/F_m) provide basic information about many aspects of the photosynthetic mechanism in plants (Ranjbar-Fordoei *et al.,* 2006). The amount of F_v/F_m indicates the health of the thylakoid membrane and the relative efficiency of electron transfer from PSII to PSI under stress (Rahbarian *et al.,* 2011). Also, F_v/F_0 is an index of the number and size of active photosynthetic reaction centers (Cen *et al.,* 2017), and shows the photosynthetic capacity of leaves (Li *et al.,* 2006).

Despite the fact that borage is widely cultivated in various parts of the world, little information is available about the antioxidant enzyme, compatible solutes, photosynthetic pigments and photosynthesis mechanism under limited irrigation and severe drought conditions. Therefore, the aim of this study is to examine the general response patterns of the plant to water fluctuations from a biochemical and physiological perspective, with the main target of providing reliable physiological criteria for the screening of drought-resistant genotypes in the future.

Table 1. Average values of maximum and minimum temperatures, rainfall, and relative humidity during the experiments in 2012 and 2013.

Month	Minimum temperature (°C)		Maximum temperature (°C)		Rainfall (mm)		Minimum relative humidity (%)		Maximum relative humidity (%)	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
May	8.71	7.23	23.77	21.52	0.82	1.61	15.35	35.77	74.93	77.93
June	12.87	12.96	28.03	28.21	1.68	0.47	14.47	30.38	62.27	72.93
July	16.19	16.27	31.22	32.13	0.10	0.00	22.81	30.61	77.29	66.64
August	18.17	15.28	35.09	31.30	0.00	0.03	18.13	32.55	47.09	70.64

Material and methods

Seed sample

Seeds of borage were supplied by the Pakan Bazr Company of Isfahan, Iran. Seed priming was done using the procedures detailed in McDonald (2000) method. A sub-sample of borage seeds was kept unprimed as control and three other sub-samples were treated with water, 1% KNO_3 and 1% KH_2PO_4 solutions for eight hours under dark conditions in an incubator adjusted to 15 ± 1 °C.

Experimental design and sowing

The experiments were conducted at the Research Farm of the University of Tabriz, Iran (38°5′ N, 46°17′ E and altitude 1360 m) in 2012 and 2013. The experimental field is located in the semi-arid (steppe) climate zone (BS) according to Köppen's classification. Weather conditions during the experiments in 2012 and 2013 are shown in Table 1. Table 2 shows the physicochemical properties of the soil.

The experimental design was split-plot with Irrigation being the whole plot factor and Priming being the sub-plot factor. The levels of Irrigation were: irrigation after 60-, 90-, 120-, and 150-mm evaporation from class A pans, representing normal irrigation, and mild, moderate, and severe water limitation, respectively. The levels of Priming are unprimed, and seed priming with water, 1% KNO_3, and 1% KH_2PO_4 solutions, respectively. The 1% KNO_3 and 1% KH_2PO_4 doses, and the levels of irrigation were chosen based on preliminary studies (Ghassemi-Golezani *et al.,* 2008, 2015; Rezaei-Chiyaneh *et al.,* 2013). The experiment was conducted with 3 blocks within each year.

Each plot consisted of eight 3-m rows with a distance of 25 cm. Based on soil analysis, 60 kg ha^{-1} urea (46% N), 50 kg ha^{-1} potassium sulfate and 50 kg ha^{-1} triple superphosphate were added to each plot before planting. Seeds were sown on 11 May 2012 and 30 April 2013 in 2 cm depth of soil (80 seeds m^{-2}). All plots were irrigated by furrow irrigation method regularly up to the establishment of seedlings and after that, irrigation intervals were adjusted based on treatments. Weeds were removed from the experimental area during crop growth and development in both years. All samplings and measurements were performed at 50% flowering and just before the irrigation of every plot.

Enzymes' extraction and electrophoresis

Superoxide dismutase (SOD) and CAT activities were determined in native polyacrylamide gel electrophoresis (PAGE) in the second year of the experiment. Enzyme extraction from healthy and fresh leaves of borage was carried out according to Valizadeh *et al.* (2011). Due to the vulnerability and deformation of enzymes at high temperatures, all stages of gel extraction and loading were performed in 0-4 °C. Electrophoresis was conducted at 4 °C for 3 hours (voltage of about 180 V and constant current of 30 mA). The staining for determination of SOD and CAT was carried out according to Soltis & Soltis (1990). For the detection of SOD isoforms, the gels were incubated in 50 mL Tris-HCl buffer (pH = 8) containing 10 mg nitro blue tetrazolium (NBT), 2 mg riboflavin and 1 mg EDTA for 30 min in the dark condition and then developed for 30-45 min under moderate light intensity. For CAT staining, another layer of slab gel was soaked in a solution containing 60 mL double-distilled water (dd H_2O) and 30 µL H_2O_2

Table 2. Physicochemical properties of the research farm soil in 2012 and 2013 before planting.

Year	Sand (%)	Silt (%)	Clay (%)	pH	EC (dS m^{-1})	Organic carbon (%)	Total N (%)	Available P (ppm)	Available K (ppm)
2012	62	22	16	8.2	2.08	1.50	0.13	96.7	1540
2013	74	14	12	8.0	2.92	0.37	0.04	4.9	255

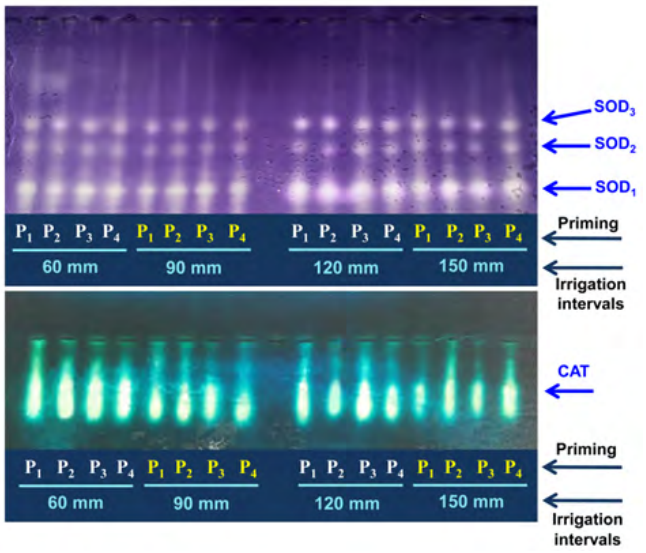

Figure 1. Examples of SOD and CAT activities in borage leave under different irrigations (after 60, 90, 120 and 150 mm evaporation) and primings (unprimed, and seed priming with water, 1% KNO_3, and 1% KH_2PO_4 represented by P_1, P_2, P_3, and P_4, respectively).

for 20 min in darkness. Then, this solution was rinsed and stained in a freshly prepared solution containing 60 mL dd H_2O, 600 mg potassium ferricyanide and 600 mg ferric chloride for 15-20 min. Three isoforms for SOD and only one isoform for CAT were detected in the leaves of the plant. Quantification of the enzymes' activity was conducted by the MCID 0.7 analysis, and the densitometric activities of SOD and CAT were determined as an extension of the study on water deficit and seed priming effects on borage.

Leaf proline content

The extraction of proline was done according to Bates *et al.* (1973) method. A 500 mg sample of fresh leaves from each plot was homogenized with 10 mL of 3% aqueous sulfosalicylic acid and centrifuged at 4000 rpm for 15 min. The supernatant was used to assay proline content; 2 mL ninhydrin acid reagent and 2 mL glacial acetic acid were added to 2 mL of the supernatant within the test tubes. The tubes were placed in a water bath for 1 hour at 90 °C and then transferred to an ice bath for termination of the reaction. After adding 4 mL toluene to the mixture to separate the proline-containing toluene from the aqueous phase, the samples were stirred vigorously for 15 sec and placed at room temperature for 20 min. After that, the pink-red upper phase was carefully collected and was poured in a cuvette. The fraction absorbance was read at 520 nm by a spectrophotometer (SPEKOL 1500, Germany). Leaf proline content was specified through the calibration curve and reported as mg g^{-1} fresh weight (FW). The L-proline was used to prepare a standard curve.

Soluble sugars content

Soluble sugars content was specified using the phenol-sulfuric acid method (Kochert, 1978). A sample of 100 mg of dry leaves powder of borage was added to glass containers and homogenized with 10 mL of 70% ethanol. The solutions were put in the refrigerator (4 °C) in darkness for a week and were stirred daily. Then solutions were centrifuged at 4000 rpm for 10 min and the insoluble residue was removed by centrifuge, and the precipitate was used for the measurement of soluble sugars content; 2 mL distilled water, 2 mL 5% phenol and 10 mL 60% sulfuric acid were added to 2 mL of supernatant and strongly vortexed for 20 sec. The glasses containing the mixture were placed in a water bath for 20 min at 70 °C and then for 30 min in cold water. Finally, the solutions absorbance was recorded at 485 nm by the spectrophotometer. Soluble sugars content was calculated by applying glucose to obtain the standard curve and was reported as mg g^{-1} of dry leaf weight.

Chlorophylls and carotenoids content

A 250 mg sample of fresh, young, and fully expanded leaves were homogenized with 10 mL of 80% acetone. Then homogenates were centrifuged at 4000 rpm for 15 min and transferred to a graduated cylinder and diluted by 80% acetone to 10 mL. The supernatant absorbance was read by the spectrophotometer (SPEKOL 1500, Germany) at 470, 646 and 663 nm wavelengths against 80% acetone as blank. The chlorophylls *a*, *b,* and the total carotenoids (carotenes + xanthophylls) contents were calculated according to the formulas proposed by Lichtenthaler & Wellburn (1983).

Table 3. ANOVA *p*-values that show the significance of the main and interaction effects of irrigation and priming, the two factors of interest, on antioxidant enzymes activity and osmolytes response variables (CAT, SOD1, SOD2, SOD3, Proline, and Soluble sugar).

Effect	CAT	SOD1	SOD2	SOD3	Proline	Soluble sugar
Irrigation	**0.004**	**0.013**	**0.054**	**0.010**	0.242	**0.002**
Priming	0.784	0.680	0.212	**0.056**	0.147	0.405
Irrigation*Priming	0.192	0.859	0.924	0.691	0.405	0.984

Significant effects that require multiple means comparison are shown in bold.

Chlorophyll *a* fluorescence measurements

Chlorophyll *a* fluorescence measurements (F_0, F_m and F_v/F_m) were determined by a portable fluorometer (OS-30, OPTI-SCIENCES, USA) at 12 AM on young and fully expanded leaves of three randomly selected plants from each plot. First, a particular plastic clip was attached to a leaf and the shutter plate was closed to start the dark adaptation. After 20 min of dark adaptation, the sensor head was carefully fitted over the location ring of the leaf clip to seal out the light. The shutter plate was then opened and the measurement started by pressing the button on the sensor head with two seconds light pulse at 1000 μE m^{-2} s^{-1} on dark-adapted leaves. A weak beam and saturated white light through fluorometer led to an estimate of the F_0 and F_m, respectively. F_0 and F_m and also F_v/F_m were read from the fluorometer.

Statistical analysis

In the split-plot design analysis, for the antioxidant enzymes activity response variables that were measured only in the second year, the three blocks within the second year were used as blocks; but for chlorophyll and fluorescence response variables measured in both years, the combinations of year and block (6 blocks) were used as blocks. The whole plot error used to test the main effect of Irrigation was the interaction between Block and Irrigation. However, for testing the main effect of Priming and the interaction between Irrigation and Priming, the pooled subplot error (Block*Priming and Block*Irrigation*Priming), which is described in Montgomery (2020) as alternative split-plot model was used. For each response variable, the validity of model assumptions was verified by examining the residuals as described in Montgomery (2020). For the responses where either the main effect or interaction effect was significant ($p< 0.05$) or marginally significant ($0.05 <p< 0.1$), multiple means comparison was completed using the lsmeans statement of SAS, and letter groupings were generated at the 5% level of significance. The analysis was completed using the Mixed Procedure of SAS 9.4 (SAS Institute Inc. 2014).

Results

Antioxidant enzymes

Fig. 1 shows patterns of SOD and CAT enzymes in plants grown from control seeds and plants obtained from seeds treated with water, KNO_3 and KH_2PO_4 under various irrigation intervals. The Analysis of Variance (ANOVA) results shown in Table 3 indicate that irrigation has a significant ($p < 0.05$) effect on CAT, SOD1, and SOD3, and a marginally significant ($0.05 < p< 0.1$) effect on SOD2. The main effect of priming was marginally significant only on SOD3. The irrigation by priming interaction effect was not significant on any of the antioxidant enzymes (Table 3), which suggests that the impact of irrigation is consistent across all priming levels.

Table 4. Mean CAT, SOD1, SOD2, SOD3, and soluble sugar (mg g^{-1}) obtained from the four irrigation levels (after 60, 90, 120 and 150 mm evaporation), and mean SOD3 obtained from the four priming methods (unprimed, and seed priming with water, 1% KNO_3, and 1% KH_2PO_4).

Irrigation	CAT	SOD1	SOD2	SOD3	Soluble sugar	Priming	SOD3
60 mm	0.221 a[1]	0.134 b	0.046 bc	0.053 b	35.41 bc	Unprimed	0.062 b
90 mm	0.165 b	0.115 b	0.042 c	0.049 b	30.66 c	Water	0.062 b
120 mm	0.177 b	0.189 a	0.070 a	0.081 a	44.62 ab	1%KNO_3	0.068 a
150 mm	0.158 b	0.173 a	0.065 ab	0.078 a	50.84 a	1%KH_2PO_4	0.068 a

[1] Within each column, means sharing the same letter are not significantly different.

Table 5. ANOVA *p*-values that show the significance of the main and interaction effects of irrigation (Irr) and priming (Pri), the two factors of interest, on the chlorophyll and fluorescence response variables.

Effect	Ch *a*	Ch *b*	Ch *a*+Ch *b*	Ch *a*/Ch *b*	Carotenoids	F_o	F_m	F_v/F_m	F_v/F_0	F_0/F_m
Irr	**0.068**	**0.004**	**0.030**	**0.001**	**0.006**	0.001	**0.051**	**0.007**	**0.013**	**0.007**
Pri	0.481	0.448	0.535	**0.051**	0.962	0.243	0.146	0.285	0.336	0.285
Irr*Pri	0.402	0.397	0.415	0.245	0.107	**0.089**	0.429	0.266	0.281	0.266

Significant effects that require multiple means comparison are shown in bold.

The multiple means comparison results shown in Table 4 indicate that significantly higher CAT activity was obtained from the normal irrigation treatment (after 60 mm evaporation), and a significant decrease in the enzyme activity occurred under water deficit conditions (after 90 mm, 120 mm and 150 mm evaporation). Water deficit had a significant effect on SOD1, SOD2, and SOD3. The means shown in Table 4 reveal no significant difference between 60 mm and 90 mm, as well as between 120 mm and 150 mm in all three activities. In all three activities, moderate and severe water deficit (120 mm and 150 mm) significantly increased their activity. Comparison of the four priming levels in terms of SOD3 shows that priming with 1% KNO_3 and 1% KH_2PO_4 significantly increases SOD3 activity (Table 4).

Osmolytes

The ANOVA results shown in Table 3 indicate that only irrigation has a significant ($p < 0.05$) effect on soluble sugar, but none of the effects is significant on proline. The overall mean proline was 8.183 mg g^{-1} FW. The leaf soluble sugar content of borage in moderate and severe water deficit was significantly higher than that of normal and mild irrigation treatment (Table 4).

Photosynthetic pigments

The ANOVA results shown in Table 5 indicate that the main effect of irrigation was either significant or marginally significant on chlorophyll *a* (Ch *a*) and Ch *b* contents, Ch *a* + Ch *b*, Ch *a*/Ch *b*, and carotenoids. The main effect of priming was significant only on Ch *a*/Ch *b*, and the interaction effect was not significant on any of these response variables. The mean contents of Ch *a*, Ch *b*, and Ch *a* + Ch *b* of mild, moderate and severe water deficit were significantly higher than that of normal irrigation (Table 6). However, the Ch *a*/Ch *b* obtained from the normal irrigation treatment was significantly higher than those of the water deficit treatments, indicating the increase in Ch *b* was higher than that in Ch *a* as the water deficit increases (Table 7). The Ch *a*/Ch *b* values resulted from unprimed, and primed with water and 1% KH_2PO_4 were not significantly different from each other, but primed with 1% KNO_3 gave the highest Ch *a*/Ch *b* value (Table 7).

The carotenoids content of borage leaves was significantly enhanced under severe water deficit, but not under moderate and mild stresses. Leaf carotenoids content under severe water deficit was 12.4 % more than that under normal irrigation (Table 6).

Chlorophyll *a* fluorescence

The interaction effect of irrigation and priming was marginally significant on initial fluorescence (F_0) (Table 5). The multiple means comparison results shown in Table 8 indicate that all four priming methods give the highest F_0 when there is severe water deficit. On the other hand, the lowest F_0 value was obtained in all priming levels when normal irrigation was applied. The next higher F_0 values were obtained from all four priming levels when moderate stress irrigation was used. Under moderate water deficit, the F_0 value was significantly lower

Table 6. Mean Ch *a*, Ch *b*, Ch *a*+Ch *b*, Ch *a*/Ch *b*, carotenoids, F_m, F_v/F_m, F_v/F_0, and F_0/F_m obtained from the four irrigation levels (after 60, 90, 120 and 150 mm evaporation).

Irrigation	Ch *a*	Ch *b*	Ch *a*+Ch *b*	Carotenoids	F_m	F_v/F_m	F_v/F_0	F_0/F_m
60 mm	0.659 b [1]	0.220 b	0.879 b	0.169 b	325 ab	0.652 a	2.40 a	0.348 b
90 mm	0.718 ab	0.254 a	0.971 a	0.165 b	337 a	0.672 a	2.28 a	0.328 b
120 mm	0.706 ab	0.255 a	0.961 ab	0.172 b	323 ab	0.646 a	2.02 a	0.354 b
150 mm	0.747 a	0.275 a	1.023 a	0.190 a	298 b	0.598 b	1.72 b	0.402 a

[1] Within each column, means sharing the same letter are not significantly different.

Table 7. Mean Ch *a*/Ch *b* obtained from the four irrigation (after 60, 90, 120, and 150 mm evaporation) levels, and the four priming methods (unprimed, and seed priming with water, 1% KNO_3, and 1% KH_2PO_4).

Irrigation	Ch *a*/Ch *b*	Priming	Ch *a*/Ch *b*
60 mm	3.00 a [1]	Unprimed	2.80 b
90 mm	2.83 b	Water	2.84 ab
120 mm	2.77 bc	1% KNO_3	2.88 a
150 mm	2.73 c	1% KH_2PO_4	2.81 b

[1] Within each column, means sharing the same letter are not significantly different.

when the seeds were primed with 1% KH_2PO_4 than unprimed seeds (Table 8).

Water stress, but not seed priming has a significant effect on F_m (Table 5). The mean F_m obtained from mild water deficit was significantly higher than that from severe water deficit (Table 6). Only the main effect of irrigation was significant on F_v/F_m, F_v/F_0, and F_0/F_m (Table 5). Among the four irrigation levels, only severe water deficit gave significantly lower F_v/F_m and F_v/F_0, but the pattern was the opposite for F_0/F_m where severe water deficit gave significantly higher F_0/F_m (Table 6).

Discussion

It is reported that seed priming increases borage field performance by improvement the emergence rate of seedling and leaf area index of this medicinal plant (Dastborhan & Ghassemi-Golezani, 2015). In the present study, the effect of seed priming was significant only on SOD3 and Ch *a*/Ch *b* ratio, probably due to the natural capability of this plant to reduce harmful effects of water limitation.

Based on our findings, the activity of SOD_1, SOD_2 and SOD_3 isoforms were considerably increased in borage under moderate and severe water deficit, indicating that borage can remove the ROS under stress by SOD increment. Water deficit disturbs the equilibrium between trapping and utilization of light, which reduces photosynthetic activity in leaves. The excess energy of light in the photosynthetic system results in excessive production of ROS (Lisar *et al.,* 2012). The capability of antioxidant enzymes to scavenge the ROS is related to plant resistance to water deficit and oxidative stress caused by it (Anjum *et al.,* 2011). SOD isoforms are strong antioxidants that act as the first line of defense against ROS (Wang *et al.,* 2018) and catalyze superoxide anion ($O_2^{-\bullet}$) dismutation to hydrogen peroxide (H_2O_2) in almost all plant cellular compartments. This relatively stable product can be converted to H_2O by peroxidase and catalase (Apel & Hirt, 2004). Therefore, the rising of SOD activity could enhance the plants' ability to scavenge $O_2^{-\bullet}$ radicals, thereby preventing membrane damage (Aydin *et al*., 2011). Excessive H_2O_2 content is toxic for cells. Therefore, rapid scavenging of H_2O_2 by the antioxidant defense system is crucial for plants (Guo *et al.,* 2006).

Catalase activity in borage was very high under normal irrigation, while it was reduced with increasing irrigation intervals. The reduction of CAT activity under stress can be attributed to synthesis inhibition of the enzyme, its degradation by peroxisomal proteases or its photo-inactivation (Abedi & Pakniyat, 2010). The balance of CAT, ascorbate peroxidase (APX) and SOD activities is crucial for suppressing toxic ROS levels in a cell. Sometimes, an increase in activity of one antioxidant enzyme may cause a decrease in the activity of others, suggesting a delicate balance related to what these enzymes deactivate (Apel & Hirt, 2004).

Osmolytes have an essential role in plant resistance to drought by preventing water loss and maintaining cell turgidity. Changes in leaf carbohydrate status in response to stresses can act as a metabolic signal. The soluble sugars accumulation in borage plants under moderate and severe water limitation increases resistance to drought stress. Under water deficit, soluble sugars can act as osmotic agents (facilitators of osmotic adjustment) or osmo-protectors (stabilizers for the structure of proteins, enzymes, and membranes) (Marcińska *et al.,* 2013). Some functions of plant solutes are associated with their highly hydrophilic property, and therefore may replace water molecules around membranes, proteins, and nucleic acids during drought stress. The accumulation of soluble sugars in leaves under water deficit may result from degrading the complex sugars into simple sugars and reducing their transport to the other parts such as grains (Jinyou *et al.,* 2004).

The ability of plants to retain the biological functions of photosynthesis under water deficit conditions is an important evidence of stress tolerance. Furthermore, chlorophyll content changes during drought stress depend on the severity and duration of water limitation (Fathi & Barari, 2016). The results of the current study showed the chlorophyll content of plant leaves under drought stress has increased, which is consistent with the findings of some researchers (Teixeira & Pereira, 2007; Azhar *et al.,* 2011). The increment in chlorophyll content under stress conditions could help the plants to cope with environmental stress and compensate for any damage that could affect the

Table 8. Mean F_0 (initial fluorescence) obtained from the 16 combinations of irrigation (after 60, 90, 120, and 150mm evaporation) and priming (unprimed, and seed priming with water, 1% KNO_3, and 1% KH_2PO_4) levels.

Irrigation	Priming	F_0
60 mm	Unprimed	97.4 e [1]
60 mm	Water	99.6 de
60 mm	1% KNO_3	96.7 e
60 mm	1% KH_2PO_4	100.5 de
90 mm	Unprimed	105.7 bc
90 mm	Water	103.4 cd
90 mm	1% KNO_3	103.5 cd
90 mm	1% KH_2PO_4	103.5 cd
120 mm	Unprimed	109.9 a
120 mm	Water	107.6 ab
120 mm	1% KNO_3	108.1 ab
120 mm	1% KH_2PO_4	105.2 b
150 mm	Unprimed	111.2 a
150 mm	Water	111.4 a
150 mm	1% KNO_3	110.5 a
150 mm	1% KH_2PO_4	108.7 ab

[1] Means sharing the same letter are not significantly different.

integrity and function of the photosynthetic system. The increase in chlorophyll content due to decreasing water supply may be associated with a reduction in leaf area and relative water content (Dastborhan & Ghassemi-Golezani, 2015), and an increase in leaf thickness and chloroplast density in stressed borage plants. This could be a defensive response to decrease the adverse effects of drought stress (Farooq *et al.,* 2009). On the other hand, chlorophyll and proline are both synthesized from the common constituent of glutamate. In most plants that are under water deficit, the synthesis of chlorophyll is limited due to increasing conversion of glutamate to proline by the enzyme of gamma-glutamyl kinase (Handa *et al.,* 1986). Since proline accumulation in borage plants under the studied irrigation treatments was not significant compared to normal irrigation, glutamate may be used in the synthesis of chlorophyll. The decrement in chlorophyll *a/b* ratio under water limitation (Table 7) is due to the larger increase of chlorophyll *b* compared to chlorophyll *a* in stressed plants. Sharifi & Mohammadkhani (2016) also found a reduction in the Chl *a*/Chl *b* ratio in wheat genotypes under water deficit conditions.

The results of our work revealed that irrigation up to 120 mm evaporation did not cause any change in leaf carotenoids content, but severe water stress acted as a stimulus to produce more carotenoids in borage leaves. Plants can protect cell structures against active radicals produced under stress through the production of antioxidant compounds such as carotenoids (Bettaieb-Rebey *et al.,* 2011). Carotenoids are isoprenoid compounds that contribute not only to light absorbance and passing of solar energy to chlorophyll but also to helping plants to withstand water limitation (Jaleel *et al.,* 2009) and to protect chlorophyll from photodamage. Carotenoids are among the most potent phytochemicals because of their properties as antioxidants and their role in the protection of reaction centers and wasting extra light energy (Shah *et al.,* 2017). Plant species with a higher content of carotenoids under oxidative stress show effective defense and better tolerance against drought stress (Farooq *et al.,* 2009).

In the current study, the enhancement of F_0 and reduction of F_m under drought stress led to an increment in F_0/F_m and a decrement in F_v/F_0 and F_v/F_m concurrently. Chlorophyll *a* fluorescence is a simple, rapid, cheap, and non-destructive tool for evaluating light-dependent photosynthetic reactions and screening genotypes for water deficit tolerance (Kalaji *et al.,* 2016). Water deficit influences F_v/F_m and reduces the rate and capacity of electron transport. Therefore, the system reaches the F_m state faster and leads to a reduction in the F_v state. In the F_0 state, plastoquinone electron acceptor pool (QA) is at its complete oxidation level (Li *et al.,* 2006). The increment in F_0 can damage

the photosynthetic system (Yamane *et al.,* 1997). The reduction of F_m due to drought stress could be related to the increment of non-photochemical dissipation (as heat) or decrement in the activity of enzyme complex that catalyzes the water-splitting (Lucena *et al.,* 2012).

In the present study, F_0/F_m significantly increased as a result of water shortage. The higher F_0/F_m under drought stress shows that the initial reduction rate of the plastoquinone A (PQA) was more than its re-oxidation rate by the plastoquinone B (PQB) and PSI activity (Lucena *et al.,* 2012) when the plants were irrigated with longer irrigation interval.

The F_v/F_0 decrease in borage under water deficit (Table 6) could be due to a disorder in the photosynthetic apparatus and a reduction in the number of reaction centers. F_v/F_0 ratio was negatively correlated with the chlorophyll content index under water deficit (Akhkha, 2009).

The F_v/F_m ratio can be used to detect damages to PSII and possible photo-inhibition. Decreasing F_v/F_m under drought stress (Table 4) revealed that the PSII might be damaged in different levels due to the water deficit effect (Li *et al.,* 2006). Drought stress through its adverse impacts on the CO_2 entry reduces the capacity of reception and electron transport. As a result, fluorescence is rapidly maximized, leading to a decrement in F_v. Indeed, limitation of CO_2 absorption due to stomatal closure under water deficit disturbs the equilibrium between the photochemical activity of PSII and the photosynthesis electron need and damages the centers of PSII (Zlatev & Lidon, 2012).

In conclusion, moderate and severe water stresses led to an increment in SOD_1, SOD_2 and SOD_3 activities, soluble sugars, and initial fluorescence. The mean contents of Ch *a*, Ch *b*, and Ch *a* + Ch *b* under mild, moderate and severe water deficit were significantly higher than that of normal irrigation. The highest carotenoids content and quantum yield baseline parameter (F_0/F_m) of borage leaves were obtained under severe drought stress. However, water limitation decreased the Chl *a*/Chl *b* ratio, F_v/F_0 and F_v/F_m in borage leaves. It seems that the improvement in SOD activity and increasing soluble sugars and carotenoids accumulation have a decisive role in enhancing drought tolerance in borage plants. Seed pretreatment had no meaningful effect on the activity of antioxidant enzymes, accumulation of compatible solutes, photosynthetic pigments content and most of the fluorescence parameters in borage leaves.

Authors' contributions

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