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Influence of seed priming and water stress on selected physiological traits of borage

Soheila Dastborhan, Kazem Ghassemi-Golezani*

Department of Plant Ecophysiology
Faculty of Agriculture, University of Tabriz
Tabriz, Iran

ABSTRACT

Borage is a valuable medicinal plant with various constituents in leaves, flowers and seeds. Hence, it is important to improve the performance of this medicinal plant under different environmental conditions. Thus, two field experiments were arranged as split-plots based on a RCB design with three replications in 2012 and 2013, to evaluate the effects of seed priming and different irrigation intervals on selected physiological properties of borage leaves. Irrigation intervals (irrigation after 60, 90, 120, 150 mm evaporation from Class A pans, respectively) and priming treatments (control, water, KNO_3 and KH_2PO_4) were allocated to the main and sub plots, respectively. The chlorophyll content index was enhanced under limited irrigation treatments, mainly due to a decrease in leaf area index and intercepting more radiation. However, the membrane stability index was stable under different irrigation intervals. Decreased relative water content and leaf area index and increased leaf temperature under lower water availability led to some reductions in the grain yield of borage. All of the priming techniques, particularly hydro-priming, enhanced the seedling emergence rate, leaf area index and consequently grain yield per unit area. Therefore, seed hydro-priming can be used to improve the field performance of borage, particularly when sufficient water is available.

Key words: borage, chlorophyll content, seed priming, water stress

INTRODUCTION

Medicinal plants have gained a considerable importance in agricultural production, pharmacy and exportation because of their use as a raw material for the pharmaceutical industry (Abou-Arab and Abou-Donia 2000). Borage (*Borago officinalis* L.) is an annual medicinal plant belonging to the Boraginaceae family that is called gavzaban in Persian. The stem and leaves are covered with coarse, prickly hairs, and the flowers are large, star-shaped and bright blue with contrasting black anthers. Borage leaves and flowers contain mucilage, tannin, saponins, essential oil, alkaloid (pyrrolizidine), vitamin C, calcium and potassium (Gupta and Singh 2010) and are traditionally used as diuretic, diaphoretic, expectorant, anti-inflammatory and as a mild sedative and anti-depressant (Van-Wyk and Wink 2004). Borage seeds contain 30-40% oil, which is a rich source of gamma-linolenic acid.

Drought is known as a major abiotic factor that limits plant growth and production in arid and semiarid regions more than other environmental stresses and is the most significant factor in world security and sustainability in agricultural production. Morphological and physiological changes in response to drought stress can be used

*Corresponding author.
Tel.: +98 41 33392028; fax: +98 41 33356003;
e-mail: golezani@gmail.com (K. Ghassemi-Golezani).

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to help identify resistant plant species for better productivity under drought stress (Nam et al. 2001).

Relative water content (RWC) is a good sign for water status in plants and is a better indicator than water potential (Sinclair and Ludlow 1985). Leaf relative water content reflects the metabolic activity of tissues and is used as a meaningful index for dehydration tolerance (Anjum et al. 2011). Change in leaf temperature may be an important factor in controlling leaf water status under drought stress. Leaf pubescence is a xeromorphic trait that helps protect the leaves from excessive heat load. Hairy leaves have reduced leaf temperatures and transpiration (Sandquist and Ehleringer 2003).

Biological membranes are the first targets of many abiotic stresses. It is generally accepted that the maintenance of the integrity and stability of membranes under water stress is a major component of drought tolerance in plants (Bajji et al. 2002). The membrane stability index (MSI) is another physiological index that has been widely used to evaluate drought and heat tolerance (Blum and Ebercon 1980). Desiccation of plant cells causes cell membrane leakage of ions and electrolytes (Bandurska 2001). Pessarakli (1999) stated that photosynthesis persistence and leaf chlorophyll conservation under stress conditions are physiological indices of resistance to stress. Chlorophyll plays a key role in trapping sunlight and converting it into chemical energy, so any disturbance in chlorophyll content may result in a reduction in photosynthesis (Azhar et al. 2011). Water stress also limits the size of individual leaves and leaf number.

One of the most effective and simple techniques for reducing the adverse effects of water stress on plants is seed priming. In priming, seeds are partially hydrated to a point where germination related metabolic processes begin, but radicle emergence does not occur (Farooq et al. 2006). Different seed priming techniques lower the time for seedling emergence compared with the control treatment. Faster emergence, better and more uniform stands, more vigorous plants, better drought tolerance, earlier flowering and higher grain yield in many crops are reported as the beneficial effects of priming (Harris et al. 1999). Since seed priming can improve the physiological performance and yield of medicinal plants under stressful conditions (Ghassemi-Golezani et al. 2012a,b, Gholami et al. 2013), this research was carried out to evaluate the effects of several priming techniques on the physiology and yield of borage under well watering and limited irrigation conditions.

MATERIAL AND METHODS

Seeds of borage were obtained from the Pakan Bazr Company of Isfahan, Iran. These seeds were divided into four sub-samples. A sub-sample was kept as a control (unprimed) and the three other sub-samples were primed with water, 1% KNO_3 and 1% KH_2PO_4 solutions for eight hours. Priming treatments were performed in an incubator adjusted to 15 ± 1°C under dark conditions. After priming, seeds were washed with tap water and then dried for about three hours at room temperature (20-25°C).

Two experiments were conducted in 2012 and 2013 at the Research Farm of the Faculty of Agriculture (latitude 38°5′ N, longitude 46°17′ E, altitude 1360 m above sea level), at the University of Tabriz, Iran. Average maximum and minimum temperatures, rainfall and relative humidity during the experiment in 2012 and 2013 are shown in Table 1.

The experiments were arranged as a split plot based on a randomised complete block design in three replications, with irrigation treatments (I_1, I_2, I_3, I_4: irrigation after 60, 90, 120 and 150 mm evaporation from class A pans, respectively) in the main plots and priming techniques (P_1, P_2, P_3, P_4: Control (unprimed) and priming with water, 1% KNO_3 and 1% KH_2PO_4 solutions, respectively) in subplots. Each plot consisted of eight rows with three meters length and 25 cm apart. Before

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

sowing, 50 kg ha^{-1} potassium sulphate, 50 kg ha^{-1} triple super phosphate and 60 kg ha^{-1} urea (46% N) were applied to the plots. Borage seeds were treated with 2 g kg^{-1} Benomyl and then were sown with a density of 80 seeds m^{-2} on 11 May 2012 and 30 April 2013. All plots were irrigated immediately after sowing and irrigation treatments were applied after seedling establishment. Hand weeding of the experimental area was carried out during crop growth and development.

The number of emerged seedlings in an area of 1 m^2 within each plot was counted in daily intervals until seedling establishment became stable. The seedling emergence rate was calculated according to Ellis and Roberts (1980). Select physiological properties of borage leaves including relative water content (RWC), leaf temperature, membrane stability index (MCI), leaf area index (LAI) and chlorophyll content index (CCI) were measured just before irrigation at the flowering stage, using the following procedures

Relative Water Content (RWC)

Five young fully expanded leaves were selected from each sub-plot at 11-12 AM local time and placed in plastic bags within an ice tank and immediately transferred to the laboratory. 10 leaf disks of uniform size were provided and the fresh weight of the disks was recorded (F_w). Then, leaf disks were put in distilled water for 24 h at 4°C in the darkness. After soaking, water on the leaf surface was quickly and carefully dehumidified with tissue paper prior to the determination of turgid weight (T_w). Finally, dry weight was obtained after drying the leaf disks for 24 h at 80°C (D_w). The value of RWC was determined using the following equation:

$$RWC = [(F_w - D_w)/(T_w - D_w)] \times 100$$

where, F_w, D_w and T_w are fresh weight, dry weight and turgid weight, respectively.

Leaf temperature

Leaf temperature was measured remotely at midday (12-13 PM) using a hand-held infrared thermometer with a laser beam (Tes1327-K). On the days when the measurements were taken, the weather was completely cloud-free and sunny. For this purpose, three plants were marked in each plot and measurements were made on three fully expanded leaves of the main stem of each plant.

Membrane Stability Index (MSI)

The membrane stability index was determined by recording the electrical conductivity of leaf leakages in double distilled water at 40^{o}C and 100^{o}C (Deshmukh et al. 1991). After a quick washing of the leaves with distilled water to remove dust and dirt, leaf disks (200 mg) were taken in test tubes containing 20 ml of double-distilled water in two sets. One set was kept at 40°C for 30 min. Initial conductivity (C_1) was recorded using a conductivity meter after bringing the sample to 25°C. Another set was placed in a boiling water bath (100°C) for 15 min and then cooled to 25°C and final conductivity (C_2) was recorded. The membrane stability index (MSI) was calculated as:

$$MSI = [1 - (C_1/C_2)] \times 100$$

Leaf Area Index (LAI)

Five plants were randomly selected from the central rows of each plot and then all of the leaves were counted and detached from the shoots. Leaf area was measured using a leaf area meter (LI-COR, Model Li-3100C Area Meter, USA). The leaf area index was calculated as:

$$LAI = \text{Leaf area} / \text{Ground area}$$

Chlorophyll Content Index (CCI)

Leaf chlorophyll content index (CCI) was measured using a portable chlorophyll meter (CCM-200, Opti-Sciences, USA). For this purpose, three plants were marked in each plot and the CCI of the upper, middle and lower leaves of each plant was measured at the flowering stage. Subsequently, the mean CCI for each treatment and replicate was calculated.

Grain yield

In both years, the grains of plants in 1 m^2 of the middle part of each plot were harvested at maturity and grain yield per unit area was determined. In the second year, nets with small meshes were spread above the ground and just below the inflorescence to prevent ants from removing shed grains.

Statistical analysis

The data were tested for homogeneity and normality of residuals using the Kolmogorov-Smirnov and Bartlett tests, respectively. The data were then analysed by SPSS 16 software, considering year as a random factor. The means were compared using the Duncan multiple range test at $p \leq 0.05$. Excel software was used to draw figures.

RESULTS

Seedling emergence

The rate of seedling emergence was significantly ($p \leq 0.01$) affected by year and seed priming.

Table 2. Means of rate and percentage of borage seedling emergence affected by year and seed priming

Treatment		Emergence (%)	Emergence rate (per day)
Year	2012	86.26 [a*]	0.0859 [a]
	2013	70.60 [b]	0.0587 [b]
Seed priming	Control	78.17 [a]	0.0697 [b]
	Water	77.97 [a]	0.0731 [a]
	KNO_3	77.59 [a]	0.0730 [a]
	KH_2PO_4	80.00 [a]	0.0737 [a]

*Different letters in each column for each treatment indicate a significant difference at $p \leq 0.05$

Table 3. Effects of year and irrigation intervals on select physiological traits of borage

Treatment		Relative water content (%)	Leaf temperature (°C)	Chlorophyll content index (CCI)
Year	2012	67.16 [b*]	27.65 [a]	37.17 [a]
	2013	74.26 [a]	27.09 [b]	39.96 [a]
Irrigation intervals	60 mm (I_1)	72.66 [ab]	25.80 [b]	33.99 [b]
	90 mm (I_2)	73.77 [a]	25.44 [b]	35.61 [b]
	120 mm (I_3)	70.18 [b]	28.91 [a]	41.06 [a]
	150 mm (I_4)	66.23 [c]	29.33 [a]	43.61 [a]

*Explanations: see Table 2

The effect of year on emergence percentage was also significant ($p \leq 0.01$), but seed priming had no significant effect on this trait ($p > 0.05$). The percentage and rate of emergence in 2012 were higher than those in 2013. Borage seed priming significantly increased seedling emergence rate, so that the emergence rate of seedlings from primed seeds was 4.8-5.8% more than that from unprimed seeds. However, there were no significant differences in the emergence rate among the priming techniques (Tab. 2).

Physiological traits

Relative water content was significantly influenced by year and irrigation treatments ($p \leq 0.01$), but seed priming had no significant effect on this trait ($p > 0.05$). RWC in the second year was significantly higher than that in the first year. Water deficit significantly reduced the relative water content of borage leaves. Moderate (I_3) and severe water deficit (I_4) led to a 3.4 and 8.8% reduction in the relative water content of borage, respectively. However, RWC under I_1 and I_2 was statistically similar (Tab. 3).

Leaf temperature was significantly affected by year and water supply ($p \leq 0.05$), but it was not significantly influenced by seed priming ($p > 0.05$). The interaction of year × irrigation was also significant for the leaf temperature of borage ($p \leq 0.05$). The leaf temperature of borage in the first year was higher than that in the second year. Moderate (I_3) and severe water deficit (I_4) increased leaf temperature by about 3 and 4°C, respectively (Tab. 3). In both years, the leaf temperature of borage under I_3 and I_4 was higher than that under I_2 and I_1.

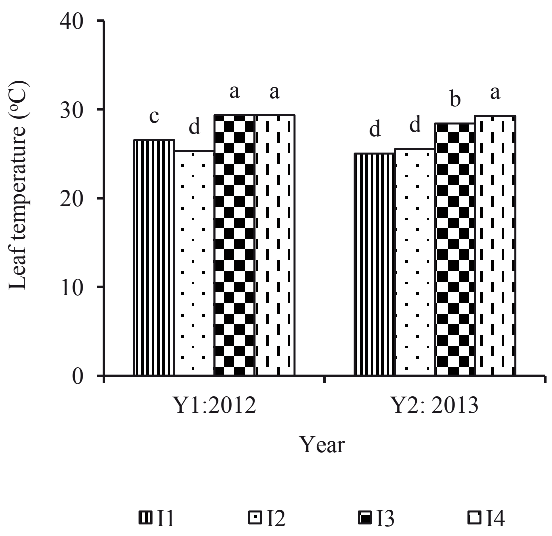

Figure 1. Means of borage leaf temperature for irrigation × year interaction

I_1, I_2, I_3, I_4: irrigation after 60, 90, 120 and 150 mm evaporation, respectively
Different letters indicate a significant difference at $p \leq 0.05$

In the second year, borage leaf temperature under I_4 was significantly higher than that under I_3, while in the first year, it was statistically similar under I_3 and I_4 (Fig. 1). In contrast, the effects of year, irrigation intervals and seed priming on membrane stability index were not significant ($p > 0.05$).

The leaf area index was significantly affected by year and irrigation intervals ($p \leq 0.01$), but the effect of seed priming on this trait was not significant ($p > 0.05$). Interactions of year × seed priming ($p \leq 0.05$) and irrigation × seed priming ($p \leq 0.01$) were also significant for LAI. Borage LAI in 2012 for different priming treatments was similar, while in 2013, the LAI of plants from primed seeds was significantly improved compared to the control. In general, the mean leaf area index in 2013 was lower than that in 2012 (Fig. 2a). LAI under better watering (I_1) was enhanced by priming treatments, particularly by priming with water and KNO_3. The priming of seeds with water and KH_2PO_4 was the superior treatment under moderate water stress (I_3), while only priming with KH_2PO_4 was the best treatment under severe water stress (I_4) for improving borage

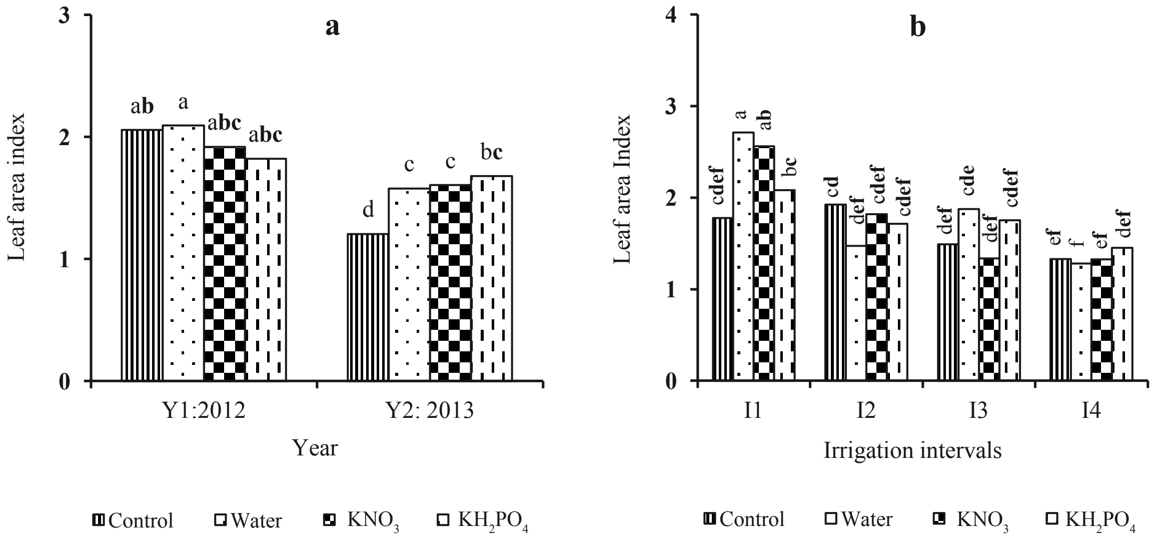

Figure 2. Means of borage leaf area index for year × priming (a) and irrigation × priming (b) interactions

I_1, I_2, I_3, I_4: irrigation after 60, 90, 120 and 150 mm evaporation, respectively
Different letters indicate a significant difference at $p \leq 0.05$

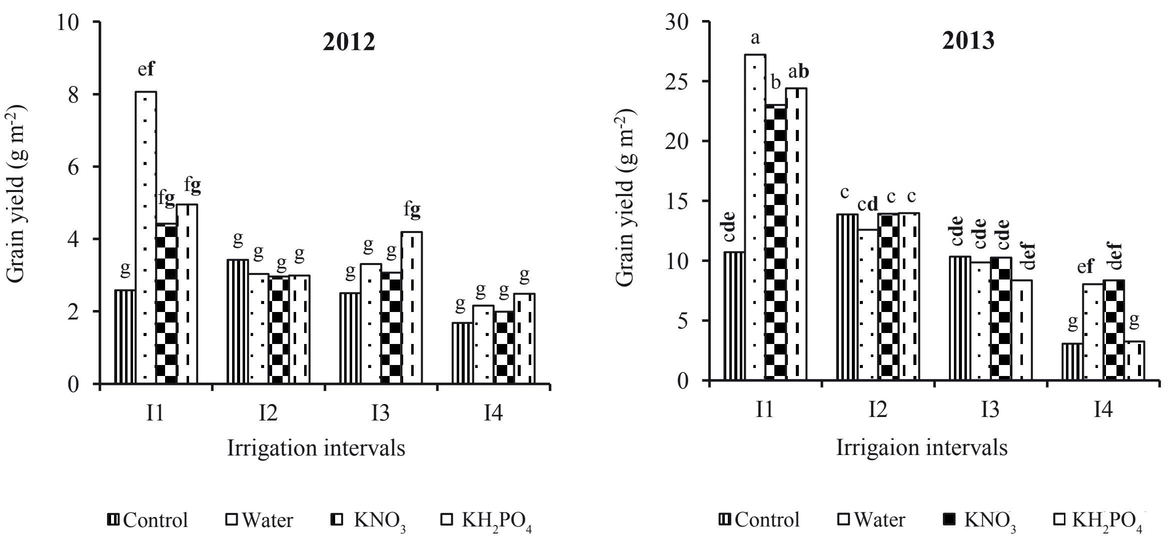

Figure 3. Mean grain yield of borage for year × irrigation × priming

I_1, I_2, I_3, I_4: irrigation after 60, 90, 120 and 150 mm evaporation, respectively
Different letters indicate a significant difference at $p \leq 0.05$

LAI. The mean leaf area index generally decreased with increasing irrigation intervals (Fig. 2b).

The chlorophyll content index was only affected by irrigation intervals ($p \leq 0.01$). The effects of year and seed priming on CCI were not significant ($p > 0.05$). The leaf chlorophyll content under good irrigation (I_1) was minimal and increased with increasing water deficit. However, there was no significant difference between I_1 and I_2 and also between I_2 and I_3 treatments (Tab. 3).

Grain yield

The grain yield of borage was significantly influenced by year and the interaction of year × irrigation ($p \leq 0.01$). The effect of irrigation treatments and seed priming on this trait were not significant ($p > 0.05$), while the interaction of year × priming and year × irrigation × priming were significant for grain yield per unit area ($p \leq 0.05$). In both years, plants from primed seeds significantly improved the grain yield of borage under better watering (I_1), which was more pronounced in the second year. Seed priming with water and KNO_3 also led to a significant increase in the grain yield under the lowest water supply (I_4) in the second year. In other irrigation treatments, the grain yield of plants from primed and unprimed seeds was statistically similar in each year (Fig. 3).

DISCUSSION

The low percentage and rate of borage emergence in 2013 compared with 2012 (Tab. 2) was due to low temperatures during seedling emergence (Tab. 1). The high emergence rate of borage seedlings from primed seeds (Tab. 2) can be attributed to faster water uptake in primed seeds (Ghassemi-Golezani et al. 2008), mediation of cell division in germinating seeds (Sivritepe et al. 2003), increasing the activity of enzymes such as amylase, protease and lipase, which play a large role in the breakdown of macromolecules for growth and embryo development (Dell-Aquila and Tritto 1990) and metabolic repair during imbibition. Iqbal and Ashraf (2005) reported that although priming improves the rate of germination, the effectiveness of different priming agents varies with different concentrations of priming solution and crop species. Ample reports are documented in the literature that different priming agents lower the mean emergence time due to shortening the lag phase (Neamatollahi et al. 2009, Ghassemi-Golezani et al. 2011).

A decrease in the relative water content of borage leaves due to water stress (Tab. 3) indicates a loss of turgor that resulted in limited water availability for the cell extension process (Katerji et al. 1997). Under water deficit, cell membranes are subject to changes such as penetrability and a decrease in sustainability (Blokhina et al. 2003). A decrease in RWC in medicinal plants under drought stress has also been observed in *Melissa officinalis* L. (Munné-Bosch and Alegre 1999), *Rosmarinus officinalis* L. (Munné-Bosch et al. 1999) and *Hibiscus rosa-sinensis* (Egilla et al. 2005).

The increase in leaf temperature under moderate and severe water deficit (Fig. 1) was closely related with a reduction of relative water content of borage leaves under limited irrigation conditions (Tab. 3). When a plant is under water deficit conditions, it tends to close the stomata to decrease transpiration and then it cannot cool efficiently, which may lead to increased leaf temperature and reduced photosynthetic production (Duffková 2006). Borage leaves are covered with coarse hairs that can reflect some of the sunlight and energy. Under high temperature and radiation stresses, hairiness increases the light reflectance and minimizes water loss by increasing the boundary layer resistance to water vapour movement away from the leaf surface (Farooq et al. 2009).

A stable cell membrane that can function well under water deficit conditions plays a pivotal role in adaptation to high temperature and resistance to drought (Chaves and Oliveira 2004). Maintenance of membrane integrity and functions under a given level of dehydration stress has been used as a measure of drought tolerance (Deshmukh et al. 1991). The membrane stability index of borage leaves was not affected by water deficit ($p > 0.05$), which is an indication of plant resistance to drought. Since the membrane is the first line of defence, as it has many heat responsive sensors that help the plant to activate its defence mechanism well in advance against heat shock, the integrity of the membrane is one of the most important parameters for plant resistance under stress.

The leaf area index of borage decreased under limited irrigation conditions (Fig. 2b). Drought decreased the leaf area, owing to a loss of turgor and reduced leaf numbers. A reduction in leaf turgor and photosynthesis under water stress condition suppresses cell expansion and growth, leading to the diminution of leaf area (Anjum et al. 2011). The reduction in leaf area is a mechanism adapted to avoid a higher rate of transpiration and to reduce surfaces for radiation due to water deficit (Hayatu et al. 2014). Seed priming had the most beneficial effect on leaf area index under well watering (Fig. 2b), indicating that the earlier advantage in rapid seedling emergence due to seed priming was more pronounced under favourable conditions.

An increase in the chlorophyll content index of borage under water stress (Tab. 3) may be related to the decrease in leaf area index (Fig. 2). Low leaf area enables leaves to intercept sufficient sunlight for chlorophyll synthesis. The ability to synthesize more chlorophyll under water stress is a good criterion for a species tolerant to drought (Poljakoff-Mayber and Gale 1975). Increment in leaf chlorophyll as a result of water deficit has also been observed in shrub species (Yanqiong et al. 2007), potato (Teixeira and Pereira 2007) and plantago (Rahimi et al. 2010).

The higher grain yield of borage in the second year (Fig. 3) was closely associated with the reduction of ant damage in that year. The possible decrease in the grain yield of borage under water stress is probably influenced by low LAI (Fig. 2) and RWC and high temperatures rather than by chlorophyll content variation (Tab. 3). The increased grain yield of plants from primed seeds, particularly those from hydro-primed seeds, in both years under well watering (I_1) (Fig. 3) can be attributed to improvements in the leaf area index of these plants (Fig. 2b). Indeed, the advantage of seed priming in improving borage grain yield per unit area was closely related with rapid seedling emergence and optimal stand establishment (Tab. 2). The early emergence of seedlings from primed seeds results in the more efficient and longer use of light and soil resources during the growth and development of plants. Consequently, the grain yield of plants from primed seeds are comparatively higher than that of plants from unprimed seeds (Ghassemi-Golezani et al. 2012a), particularly under better watering.

CONCLUSIONS

1. Borage seedlings from primed seeds emerged earlier than those from unprimed seeds, probably because of faster water uptake and the promotion of germination processes.
2. Increased leaf temperature under moderate and severe water deficit was closely related with a reduction of the relative water content of the leaves under these conditions.
3. The membrane stability index of borage was almost stable under different irrigation intervals, from 60 to 150 mm evaporation, indicating the resistance of this plant to drought.
4. The chlorophyll content index increased under water stress, mainly due to a decrease of the leaf area index and intercepting more sunlight.
5. Decreased relative water content and leaf area index and increased leaf temperature under limited irrigation led to a reduction in grain yield per unit area.
6. Priming techniques, particularly hydro-priming, had positive effects on the emergence rate, leaf area index and consequently grain yield of borage, especially when the water supply was sufficient.

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AUTHOR CONTRIBUTIONS

S.D. performed analytical measurements and statistical analysis, K.G.G. designed the experiments and wrote the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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