The synergistic effects of hydro and hormone priming on seed germination, antioxidant activity and cadmium tolerance in borage

Parisa Sheikhzadeh*, Nasser Zare, Fatemeh Mahmoudi

University of Mohaghegh Ardabili, Faculty of Agriculture Natural Resources, Department of Agronomy and Plant Breeding, Ardabil, Iran

Abstract – This research investigated the effects of priming with water for 24 hours and hormone-priming with different concentrations of gibberellic acid (GA$_3$) (50, 100, 150 mg L^{-1}) for 24 hours and hydro and hormone priming techniques either alone or in combination on the germination, growth and biochemical properties of borage seedlings under cadmium stress conditions. The results showed that cadmium stress reduces seed germination and seedling growth indices. Seed priming led to a significant increase in the germination percentage and rate, seedling dry weight and length, seedling vigor indices, as well as the catalase and peroxidase activity of borage seedlings under both cadmium stress and non-stress conditions. Among the priming treatments, the combination of hydro and hormone priming showed the greatest effects on the improvement of germination and seedling growth under cadmium conditions, significantly greater than those achieved with the individual uses of hydro or hormone priming. At all levels of cadmium stress, utilization of combined hydro and hormone priming led to a 0.9 to 11.53-fold, and 0.95 to 2.63-fold increase in seedling dry weight as compared with the control treatment and individual use of hydro or hormone priming, respectively. The highest activity of catalase and peroxidase enzymes in borage seedlings was obtained from seeds primed with the combination of hydro priming with 150 mg L^{-1} GA$_3$, which was significantly higher than those of the other treatments. Generally, at all levels of cadmium stress, combined hydro and hormone (specially 150 mg L^{-1} GA$_3$) priming had the most positive effects on seed germination, growth and biochemical properties of borage seedlings.

Keywords: *Borago officinalis*, gibberellic acid, heavy metal, seed priming technique, seedling growth

Introduction

Heavy metals are the most hazardous environmental pollutants and their toxicity is a significant problem, for ecological, evolutionary, nutritional and environmental reasons. Today, soil and water pollution with heavy metals, and their accumulation in agricultural products are one of the most critical environmental issues that threaten human and plant life due to the adverse effects on crop growth and food safety (Hasan et al. 2009, Jaishankar et al. 2014). Cadmium (Cd) due to its significant solubility in water and high toxicity is one of the most important heavy metal pollutants of the environment and agricultural products. Cadmium gets easily and rapidly absorbed by roots and quickly translocates to the aerial parts of plants (Hasan et al. 2009). Altho-

ugh other heavy metals are also released to the environment from various other industrial and agricultural sources, including sewage treatment, plating, mining and the production of plastic as well as pest control (Jaishankar et al. 2014), it is cadmium that has the most adverse effects on many physiological, biochemical and metabolic processes such as seed germination and seedling growth, photosynthesis, respiration, plant water relations and function of stomata in plant cells (Hasan et al. 2009). It has been shown that cadmium decreases CO$_2$ fixation and consequently, photosynthesis in the plants by reducing chlorophyll synthesis and the inhibition of rubisco. In addition, cadmium stress induces the production of various reactive oxygen species

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* Corresponding author e-mail: sheikhzadehmp@gmail.com

(ROS) and oxidative stress, which cause peroxidation of the lipids, cell membrane damage and disruptions to cell growth and function (Mittler 2002, Tiryakioglu et al. 2006). It has been shown that in the germination and emergence stage, the presence of cadmium reduces the percentage and rate of germination and seedling growth (Munzuroglu and Zengin 2006, Mahmoudi et al. 2017).

Borage (*Borago officinalis* L.) is one of the most important medicinal plants, having many applications in the pharmaceutical industries and traditional medicine. The flowers of borages are usually prescribed as an anti-inflammatory, and were once widely used for the prevention of colds, bronchitis and respiratory infections as well as digestive and cardiovascular disorders. Furthermore, oleic acid and palmitic acid from borage flowers have on the effect of lowering blood cholesterol (Gupta and Singh 2010).

Seeds of most medicinal plant species have uneven and weak germination, which limits the cultivation and production of these plants. Several methods and approaches have been suggested for the improvement of seed germination and seedling growth, as well as for decreasing the adverse effects of environmental stresses. Seed priming is a simple, efficient, and environmental-safe method that improves seed vigor and germination under favorable and unfavorable conditions (McDonald 2000, Ghassemi Golezani et al. 2008). In this technique, the seeds are treated with distilled water (hydro-priming), hormones or other chemical materials for a specified period, so that the germination processes are started, without the radicle emerging from the seed. Among the priming methods, hydro-priming is a very simple and economical as well as the most commonly used method. Seed priming was suggested as an approach for improving seed vigor, seed germination, seedling emergence and establishment, and resistance to environmental and biotic stress, and consequently improving product quantity and quality (McDonald 2000, Ghassemi Golezani et al. 2008). Also, seed priming, by increasing the activity of antioxidant enzymes such as catalase, peroxidase, superoxide dismutase, glutathione reductase, and other enzymes, leads to the elimination of reactive oxygen species (ROS) (Gill and Tuteja 2010). In general, the beneficial effects of seed priming on germination and seedling establishment and growth of barley seeds (Munzuroglu and Zengin 2006), pigeon pea (Sneideris et al. 2015) and chickpea (Ghassemi Golezani et al. 2008) subjected to heavy metals stress have been reported.

Plant hormones include various groups of regulatory and signaling molecules which are synthesized in small quantity in different plant cells, and work in a complex signaling network to regulate various aspects of plant growth, development, fruit ripening, reproduction and response to environmental stresses. Gibberellic acid (GA_3) is one of the growth-promoting hormones that play a crucial role in the seed germination process through stimulating the degradation of seed storage materials into usable products for the embryo and consequently triggering the germination process (Kumar et al. 2014). Gibberellic acid is one of the growth-promoting hormones that influence the seed ger-

mination process in two ways. First, GA_3 increases embryo growth through stimulating expression of the genes involved in cell expansion, which causes the weakness of seed cover. Second, endogenous increase of GA_3 level or exogenous application of GA_3 stimulate the expression, activation and secretion of hydrolytic enzymes including α-amylase which degrade the seed food reserves (proteins, carbohydrates and lipids) into the soluble amino acids, sugars and other products that are necessary for embryo growth (Gupta and Chakrabarty 2013, Kumar et al. 2014).

The accumulation of toxic heavy metals in agricultural soils can affect the production of plants, especially medicinal plants through the reduction of seed germination, seedling growth, yield, and quality of these plants (Munzuroglu and Zengin 2006, Mahmoudi et al. 2017). Therefore, recognizing the effects of cadmium stress on germination and growth of borage seedlings and the development of suitable treatments to improve borage seed germination and seedling growth under cadmium stress conditions is highly valuable. Regarding the medical importance of the borage plant and weak seed germination this plant, this study aimed to investigate the effects of hydro and hormone priming techniques, either alone or in combination, on the germination and growth characteristics, and biochemical properties of borage seedlings under cadmium-free and cadmium stress conditions.

Materials and methods

Plant material and seed priming

Matured borage seeds were obtained from the Medicinal plants research centre of The academic center for education, culture & research. In order to apply hydro and hormone priming, first the borage seed lots were washed with distilled water, then the seeds were soaked in distilled water (hydro-priming) or solutions containing 50, 100 or 150 mg L^{-1} of GA_3 (hormone priming) for 24 hours at 10 °C. For sequential application of hydro and hormone priming of borage seeds, firstly, the seeds were treated for 24 hours in distilled water at 10 °C, and then, these seeds were treated with different concentrations of GA_3 (50, 100, 150 mg L^{-1}) at 10 °C for 24 hours. The primed seed lots were rinsed with distilled water, and then dried at room temperature (20 to 22 °C) until they reached the initial moisture content (McDonald, 2000). Unprimed borage seed lots were used as the control.

Germination test

In order to perform the germination test, borage seeds (primed and non-primed) were placed on filter paper (Whatman No. 1) in 10 cm diameter Petri plates. Three replicates with 25 seeds per replicate were used for each treatment. The filter paper was moistened with 4 mL of water solution containing different concentrations of cadmium (0, 10, 50 and 100 mg L^{-1} CdCl$_2$); this was repeated every 2 days. The CdCl$_2$ (0, 10, 50 and 100 mg L^{-1} CdCl$_2$) solutions were prepared by dissolving appropriate amounts of CdCl$_2$ in 100 mL distilled

water. The experiment was carried out in the dark in an incubator at 20 °C. The number of germinated seeds was counted daily for 10 days, and the emergence of a 2 mm rootlet was considered as the criterion for seed germination (ISTA, 2017). The seed germination rate was calculated using the following equation (Ellis and Roberts 1981).

$$\bar{R} = \frac{\sum n}{\sum Dn}$$

In this formula, $\bar{R}$ is the average seed germination rate, D the number of days from the beginning of the experiment, and n is the number of seeds germinated during the day (D).

Seedling dry weight and vigor index

At the end of the germination test (after 10 days), the percentage of seed germination) was calculated, and the length of seedlings was measured. Dry weight of seedlings was measured after drying at 80 °C in an oven until a constant weight was recorded.

Seed vigor is the sum of those seed attributes that characterize the level of activity and performance of the seed lot during germination and seedling emergence. Seed vigor is an important quality parameter, which supplements germination and viability tests to achieve insights into the seed lot performance in the field or in experimental condition. It can be evaluated by various methods, such as seedling vigor indices. Seedling vigor index I and II were calculated as follows (Vashisth and Nagarajan 2010):

Seedling vigor index I = Germination (%) × mean seedling length (cm)

Seedling vigor index II = Germination (%) × mean seedling dry weight (mg)

Enzyme activity assay

Crude protein extract from seedlings (10 days old) was prepared using the Chang and Koa (1988) method. Briefly, 0.8 g of seedlings were thoroughly ground into fine powder in liquid nitrogen using a mortar and pestle and then homogenized in 6 mL of 0.2 M potassium phosphate buffer, (pH 7.0 and containing 0.1 mM EDTA). The homogenate was filtered and centrifuged at $15000 \times g$ for 20 min at 4 °C, and the supernatant fraction was used as a crude extract for protein and antioxidant enzyme activity assays. Total protein content was estimated according to the Bradford (1976) method using bovine serum albumin as the standard.

Catalase (EC 1.11.1.6) activity was determined based on the consumption of H_2O_2 and the decrease in absorbance at 240 nm for 1 min (Aebi 1984). Briefly, one mL of reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0) and 10 mM H_2O_2 and 50 µL of enzyme extract. The activity of catalase was calculated using the extinction coefficient of H_2O_2 (40 mM^{-1} cm^{-1} at 240 nm). Enzyme activity was expressed in terms of mmol of H_2O_2 min^{-1} g^{-1} fw.

Peroxidase activity was determined based on the conversion of guaiacol to tetraguaiacol in the presence of H_2O_2 (Fielding and Hall 1978). Briefly, 1 mL of reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 7.2 mM guaiacol, 11.8 mM H_2O_2, and 50 µL of enzyme extract. The reaction was initiated by adding enzyme extract, and the increase in absorbance at 470 nm was measured for 1 min. The activity of peroxidase was calculated using the extinction coefficient of tetraguaiacol (22.6 mM^{-1} cm^{-1} at 470 nm) and expressed as micromole of guaiacol oxidized per min.

The proline content of the seedlings was estimated according to the method of Bates et al. (1973). Seedling samples (0.5 g) were ground in liquid nitrogen and extracted with 3% sulfosalicylic acid. After centrifugation (10,000 rpm, 10 minutes), 2 mL of the supernatant was taken and 2 mL of glacial acetic acid and 2 mL of ninhydrin reagent (2.5% of ninhydrin in glacial acetic acid and 6 M orthophosphoric acid mixture) was added and incubated in boiling water for 60 min. The reaction was terminated by placing the tubes on ice. Then, 4 mL of toluene was added to the tubes, vigorously mixed for 20 s and incubated at room temperature for 5 min. The absorbance of the upper layer was read at 520 nm, and the proline content was determined using the L-proline standard curve and expressed as µmol g^{-1} fresh weight.

Statistical analysis

In order to investigate the effect of hydro-priming and hormone priming on the germination, growth and biochemical activities of borage (*Borago officinalis* L.) seedlings under cadmium stress, a factorial experiment based on a completely random design (CRD) was carried out with three independent replications and 25 seeds per replication. The experimental factors include cadmium stress [0 (control), 10, 50 and 100 mg L^{-1}] and different seed priming (hydro-priming for 24 hours, priming with different concentration of gibberellic acid (GA$_3$) (50, 100, 150 mg L^{-1}) for 24 hours, sequential hydro-priming and hormone priming and control seeds (non-primed).

Distribution of data sets for all traits was analyzed using different normality tests including Kolmogorov-Smirnov, skewness and kurtosis, and the results showed that the data sets follow a normal distribution. All data were subjected to ANOVA followed by Duncan's multiple range test (parametric methods) and a nonparametric Kruskal-Wallis test for group comparison (P < 0.05). Given that the Kruskal-Wallis test for group comparison showed a pattern of significance similar to those of ANOVA and Duncan's Multiple Range test, with very little and negligible changes, here we report the results of the parametric analysis method. Analyses were performed using SPSS ver. 16 (SPSS Inc, Chicago, IL.) and SAS Ver. 9 (SAS Institute, Cary, NC, USA) software and the graphs were produced using Microsoft Office Excel 2010. All values are presented as mean ± SE (Standard Error) and with significance at P ≤ 0.05.

Results

Seed germination

Data analysis indicated that seed germination and rate were significantly influenced by cadmium stress and seed priming and their interaction. Both the percentage and rate of borage seed germination were decreased with an increase in the intensity of cadmium stress. Therefore, the highest and lowest seed germination percentage and rate were observed under non-stress (without cadmium stress) and 100

mg L^{-1} cadmium treatments, respectively (Fig. 1 and 2a). As shown in Fig. 1, seed priming significantly improved the percentage of seed germination in both non-stress and stress conditions. Furthermore, the seed germination rate of primed seeds was significantly higher than that of non-primed seeds (Fig. 2b). These results indicate that the sequential application of hydro and hormone priming of seed led to an increase in seed germination rate (Fig. 2b). Under cadmium stress, the highest percentage of germination percentage was obtained from seeds primed with distilled water
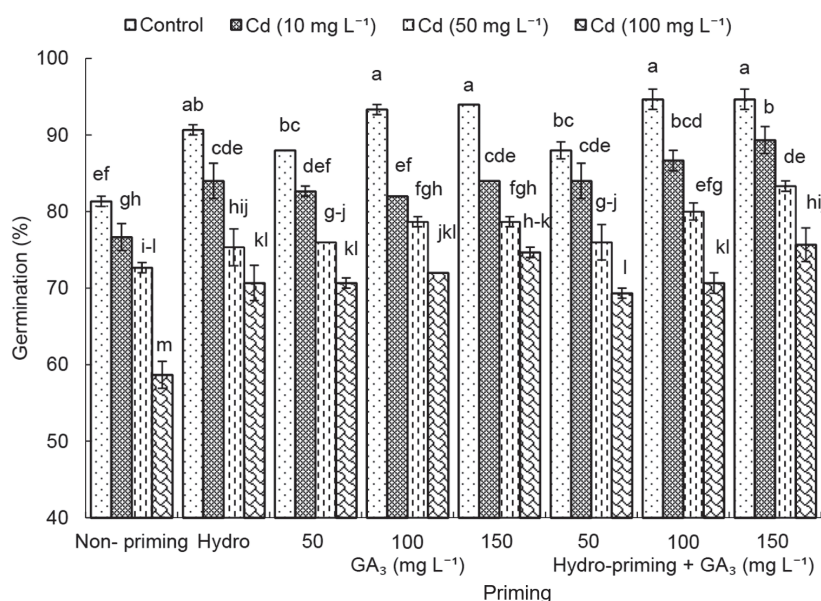

Fig. 1. The effects of cadmium (Cd) stress (10, 50, 100 mg L^{-1}) and seed priming (non-priming, hydro-priming, hormone priming (50, 100, 150 mg L^{-1} GA$_3$) and sequential application of hydro and hormone priming (hydro-priming + GA$_3$) (50, 100, 150 mg L^{-1}) for 24 hours) on the germination percentage of borage (*Borago officinalis* L.) seeds. The values present mean of data from three technical replicates (n=3) and 25 seeds per replicate. The error bars show standard error (SE). Values followed by different letters are statistically different (Duncan's Multiple Range test, P <0.05).
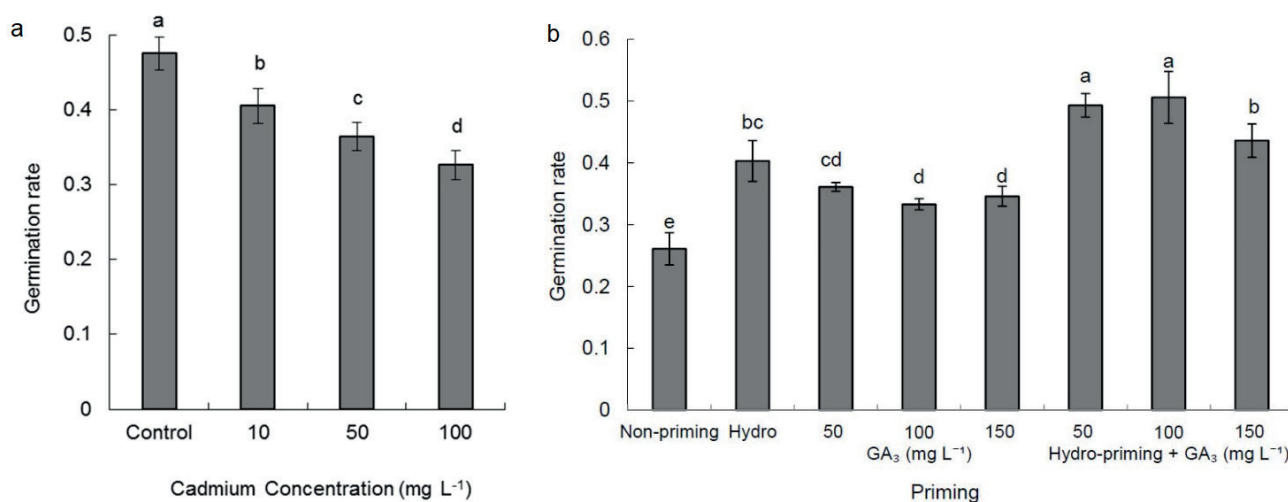

Fig. 2. The effects of cadmium stress (10, 50, 100 mg L^{-1}) (a) and seed priming (Non-priming, Hydro-priming, hormone priming (50, 100, 150 mg L^{-1} GA$_3$) and sequential application of hydro and hormone priming (hydro-priming + GA$_3$) (50, 100, 150 mg L^{-1}) for 24 hours) (b) on the germination rate of borage (*Borago officinalis* L.) seeds. The values represent mean data from three technical replicates (n=3) and 25 seeds per replicate. The error bars show standard error (SE). Values followed by different letters are statistically different (Duncan's Multiple Range test, P <0.05).

(hydro-priming) + 150 mg L^{-1} GA$_3$ and with distilled water (hydro-priming) + 100 mg L^{-1} GA$_3$, which was significantly higher than that of the non-primed seeds and other treatments. On the other hand, as shown in Fig. 1, in non-stress conditions there were no considerable differences among the different priming methods (hydro-priming, hormone priming and sequential hydro and hormone priming), but at the 10 mg L^{-1} cadmium stress condition, the percentage of seed germination observed for the seeds primed with the combination of hydro-priming with 100 or 150 mg L^{-1} GA$_3$ (distilled water + 100 or 150 mg L^{-1} Gibberellic acid) was significantly higher than that of either hydro or hormone-priming treatments, as well as that of non-primed seeds.

Seedling growth characteristics

Seedling growth characteristics, including seedling dry weight and length, and seedling vigor indices (vigor index I and II), were significantly influenced by cadmium stress, seed priming, and their interaction. As shown in Figs. 3, 4 and 5a,b, with increasing cadmium concentration, seedling growth characteristics were significantly decreased, so that the lowest and the highest growth of borage seedlings were related to the treatment with 100 mg L^{-1} cadmium and the control (cadmium-free), respectively (Figs. 3, 4, 5a,b). At non-stress and lower (10 mg L^{-1}) cadmium stress conditions, the highest seedling dry weight and vigor index II were obtained from hydro-priming and its combination with 50, 100 or 150 mg L^{-1} gibberellin (distilled water + (50, 100 or 150 mg L^{-1} GA$_3$)), which were significantly higher than those of the other treatments (Figs. 3 and 5b). However, at higher concentrations of cadmium (50 and 100 mg L^{-1}),

seedling dry weight and vigor index II obtained from sequential hydro and hormone priming treatments were higher than those of either hydro or hormone priming and as well as non-primed seeds (Figs. 3 and 5b).

In non-stress conditions, the longest seedlings and highest vigor index I were obtained from seeds primed with distilled water + 100 or 150 mg L^{-1} GA$_3$, which were significantly higher than those from other priming treatments and non-primed seeds. At lower level (10 mg L^{-1}) of cadmium stress, seedling length and vigor index I of seedlings obtained from hormone priming and its combination with hydro-priming were significantly higher than those of hydro-primed and non-primed seeds, but there were no significant differences between GA$_3$ alone and GA$_3$ in combination with hydro-priming treatments (Figs. 4, 5a). However, at higher concentrations (50 and 100 mg L^{-1}) of cadmium, sequential application of hydro and hormone priming resulted in a statistically significant increase in seedling length and in vigor index I as compared with only hormone or hydro-priming treatments as well as with non-primed seeds (Figs. 4, 5a). These results indicate the synergistic effects of hormone and hydro-priming techniques on the improvement of seedling growth and performance, especially under cadmium stress conditions.

Antioxidant enzymes activity and proline content

According to the results of variance analysis, the activity of catalase and peroxidase enzymes and proline content in borage seedlings were significantly influenced by cadmium stress, seed priming, and their interaction. Cadmium stress caused a significant decrease in the activity of catalase
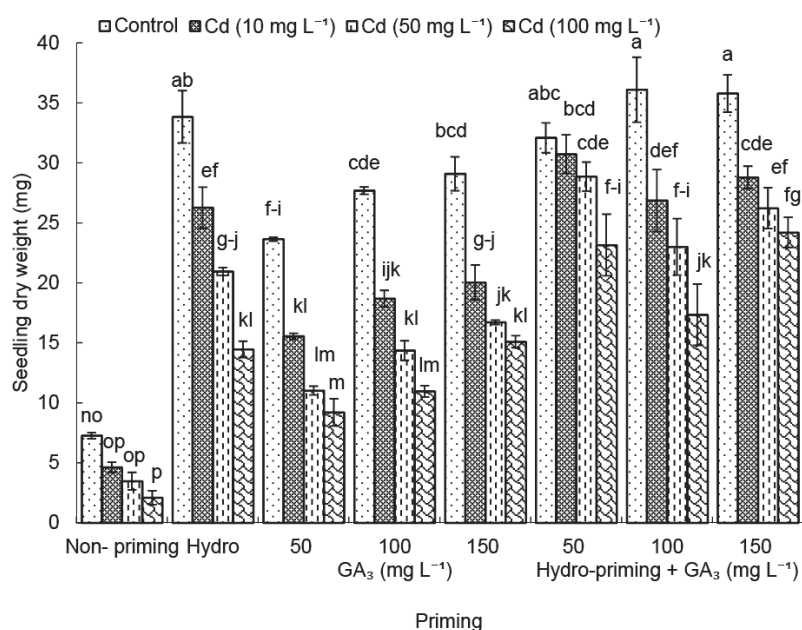

Fig. 3. The effects of cadmium stress (10, 50, 100 mg L^{-1}) and seed priming (non-priming, hydro-priming, hormone priming (50, 100, 150 mg L^{-1} GA$_3$) and sequential application of hydro and hormone priming (hydro-priming + GA$_3$) (50, 100, 150 mg L^{-1}) for 24 hours)) on the seedling dry weight of borage (*Borago officinalis* L.). The values represent mean data from three technical replicates (n=3) and 25 seeds per replicate. The error bars show standard error (SE). Values followed by different letters are statistically different (Duncan's Multiple Range test, P <0.05).
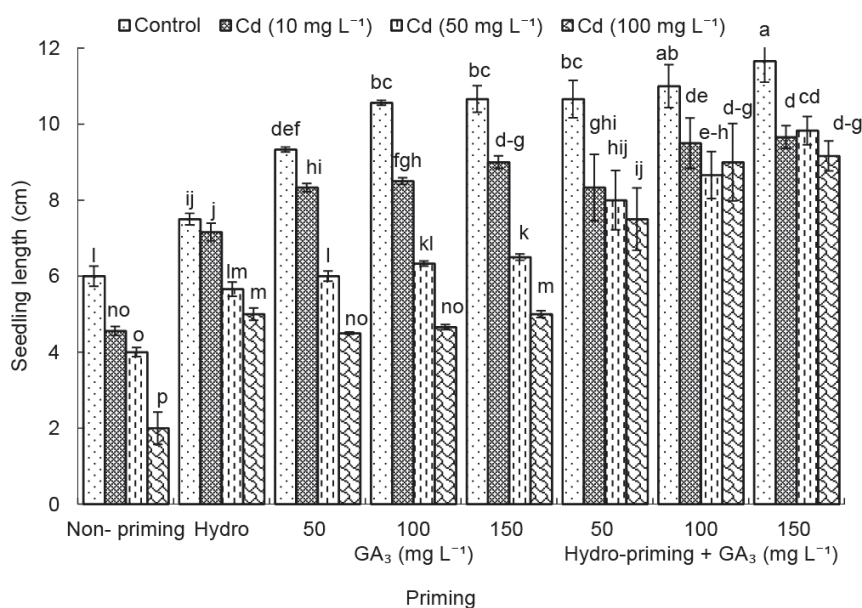
Fig. 4. The effects of cadmium (Cd) stress (10, 50, 100 mg L^{-1}) and seed priming (non-priming, hydro-priming, hormone priming (50, 100, 150 mg L^{-1} GA$_3$) and sequential application of hydro and hormone priming (hydro-priming + GA$_3$) (50, 100, 150 mg L^{-1}) for 24 hours)) on the seedling length of borage (*Borago officinalis* L.). The values represent mean data from three technical replicates (n=3) and 25 seeds per replicate. The error bars show standard error (S.E.). Values followed by different letters are statistically different (Duncan's Multiple Range test, P <0.05).

and peroxidase enzymes, but seed priming treatments significantly increased the activity of these enzymes in both non-stress and cadmium stress environments. On the other hand, under both non-stress and cadmium stress conditions, the activity of catalase and peroxidase enzymes in seedlings obtained from primed seeds was significantly higher than that of seedlings from non-primed seeds (Fig. 6a, b). Under both non-stress and cadmium stress conditions, the highest activity of catalase was observed in the seedlings obtained from the seeds primed with the combination of hydro and hormone, which was significantly higher than that of seedlings obtained from non-primed seeds and other seed priming treatments (Fig. 6a). However, the highest activity of peroxidase enzyme was observed in seedlings obtained from seeds primed only with distilled water (hydro-priming) and 150 mg L^{-1} GA$_3$ under both non-stress and cadmium stress conditions, which was significantly higher than its activity in seedlings obtained from other treatments, especially from seedlings obtained from sequential hydro and hormone priming treatments (Fig. 6b).

The proline content of borage seedlings significantly increased after applying cadmium stress and seed priming, and the amount of enhancement was varied depending on seed priming treatments and cadmium stress level (Fig. 7). As shown in Fig. 7, the proline content of seedlings significantly increased with an increase in the severity of cadmium stress, so that, at all seed priming treatments the highest amount of proline in borage seedlings was related to the 100 mg L^{-1} cadmium stress, which was significantly higher than the other levels of cadmium stress. The highest amount of proline was recorded under severe cadmium (100 mg L^{-1}) stress in seedlings obtained from seeds primed with dis-

tilled water and distilled water (hydro priming) +150 mg L^{-1} GA$_3$ treatments, which was significantly higher than that of the other treatments.

Discussion

Seed germination

Generally, cadmium stress negatively affected the germination of borage seeds (Fig. 1). These adverse effects could be attributed to the accumulation of cadmium in the cell and its inhibition effects on nutrient uptake and translocation as well as cellular metabolism and photosynthesis (Gill et al. 2013). Furthermore, it has been shown that cadmium uptake and accumulation in the cells lead to the destruction of protein and enzyme structures or inhibit their function and activity (Hasan et al. 2009, Hussain et al. 2016) as well as their synthesis. These enzymes and structural proteins are needed in cell growth and division, and germination processes (Tiryakioglu et al. 2006, Dar et al. 2016). Nevertheless, seed priming significantly reduces these adverse effects of cadmium; and leads to an increase in the percentage of borage seed germination under cadmium stress conditions (Fig. 1). The improved and accelerated germination of borage seeds under both non-stress and cadmium stress conditions could be attributed to the improving effects of seed priming, especially of combined hydro and hormone priming, on biochemical processes in the plant cell. It has been shown that seed priming induces the cytoplasmic membrane and DNA damage repairing systems, DNA, RNA and protein synthesis and activity of starch-degrading enzymes such as alpha-amylase and reduces the leakage of metabolites. These
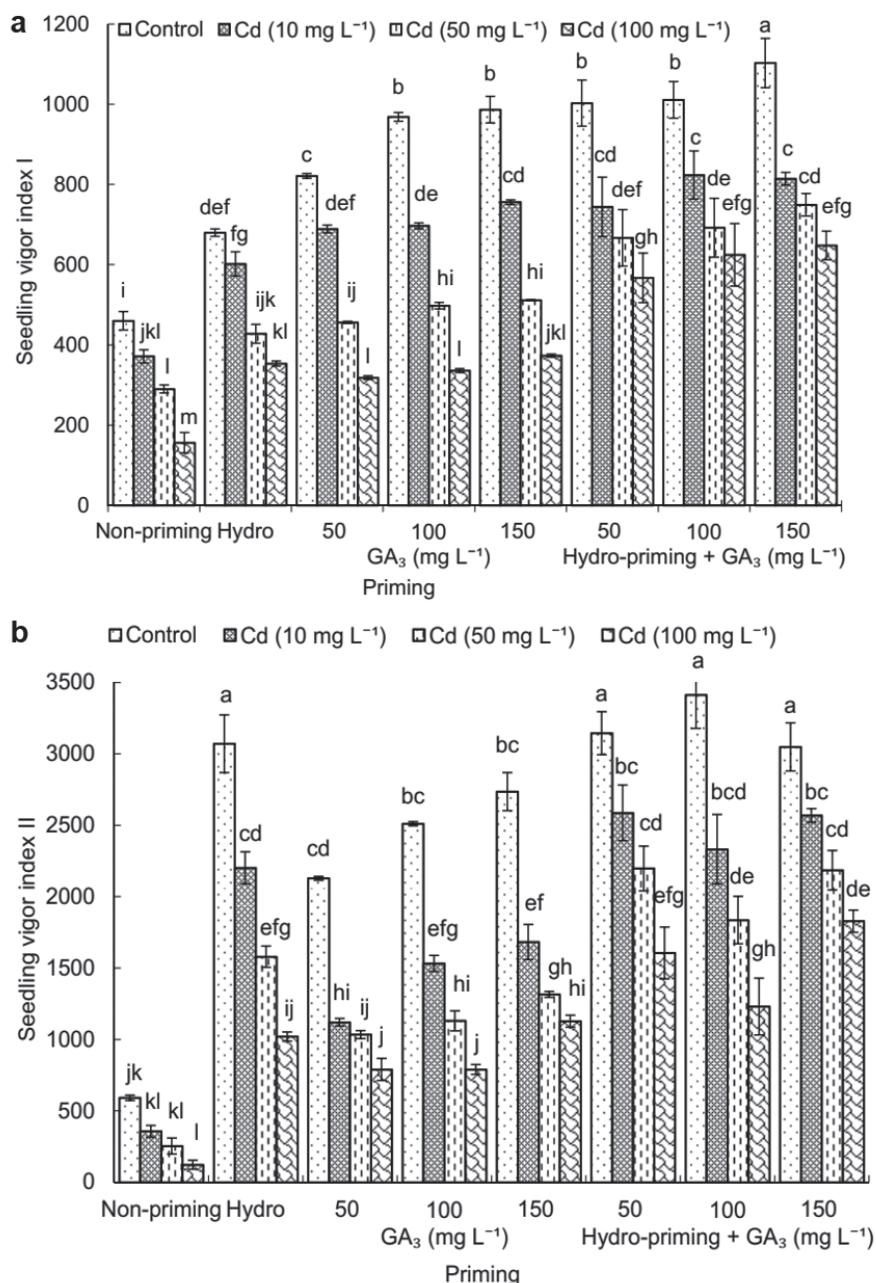
Fig. 5. The effects of cadmium (Cd) stress (10, 50, 100 mg L^{-1}) and seed priming (non-priming, hydro-priming, hormone priming (50, 100, 150 mg L^{-1} GA$_3$) and sequential application of hydro and hormone priming (hydro-priming + GA$_3$) (50, 100, 150 mg L^{-1}) for 24 hours)) on the seedling vigor index I (a) and seedling vigor index II (b) of borage (*Borago officinalis* L.). The values represent mean data from three technical replicates (n=3) and 25 seeds per replicate. The error bars show standard error (SE). Values followed by different letters are statistically different (Duncan's Multiple Range test, P <0.05).

improved cellular and molecular processes lead to an increase in the cell division and embryo growth, and finally improved seed germination and vigor (McDonald 2000, Hussain et al. 2016). The improved percentage and rate of seed germination through hydro or hormone priming have also been reported in chickpea (Ghassemi Golezani et al. 2008), pigeon pea (Sneideris et al, 2015), in borage (Mahmoudi et al. 2017), and maize (Li et al. 2017). In borage, the seed priming increases varied from 1.1-1.2-fold under control (without cadmium) condition and 1.03-1.3-fold under cadmium stress conditions, as compared with non-primed seeds (Fig. 1).

Seedling growth characteristics

The superiority of primed seeds in terms of the enhanced borage seedling growth under non-stress and cadmium stress conditions can be attributed to the higher germination rate (Fig. 2b) of these seeds. Seed priming treatments improved the seedling growth characteristics in both non-stress and cadmium stress conditions, so at all levels of cadmium stress, dry weight and length, and seedling vigor indices of seedlings obtained from primed seeds were significantly higher than those obtained from non-primed seeds (Figs. 3, 4, 5a, b). The fact that primed seeds had a higher germination rate than non-primed seeds
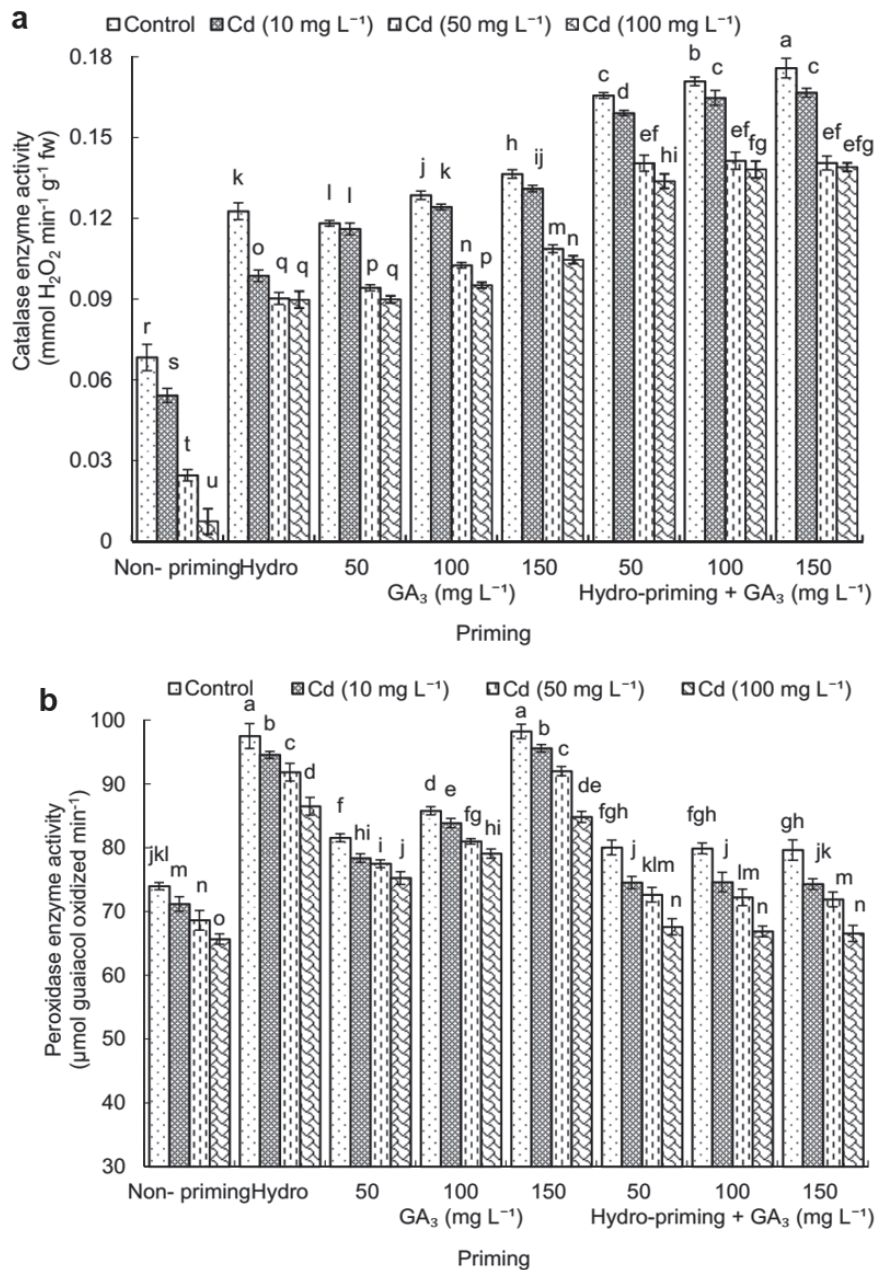
Fig. 6. The effects of cadmium stress (Cd) (10, 50, 100 mg L^{-1}) and seed priming (non-priming, Hydro-priming, hormone priming (50, 100, 150 mg L^{-1} GA$_3$) and sequential application of hydro and hormone priming (hydro-priming + GA$_3$) (50, 100, 150 mg L^{-1}) for 24 hours)) on the catalase (a) and peroxidase (b) enzymes activity of borage (*Borago officinalis* L.) seedlings. The values represent mean data from three technical replicates (n=3) and 25 seeds per replicate. The error bars show standard error (SE). Values followed by different letters are statistically different (Duncan's Multiple Range test, P <0.05).

means a quicker seed germination and the production of higher biomass than in non-primed seeds. It has been shown that seed priming increases the activity of α-amylase in maize (Li et al. 2017) and rice (Hussain et al. 2016) seeds, which could increase the germination rate and finally growth characteristics of the seedlings. Therefore, seed priming through the reduction of embryonic growth barriers and increasing seed vigor improves seed germination and growth of seedlings, which leads to the production of more vigorous seedlings. However, there were significant differences among the various seed priming treatments in terms of the abovementioned growth characteristics. Our

results indicated that in the non-stress condition, combined hydro and hormone priming led to about 0.9 to 4.41-fold increases in the borage seedling weight as compared with seedlings obtained from the non-primed seeds. Under cadmium stress conditions, the combined hydro and hormone priming caused a 5.80–11.53 and a 1.02-2.63-fold increase in the seedling dry weight in comparison with the seedlings obtained from non-primed seeds and seeds primed using either hydro or hormone, respectively (Fig. 3). As shown in Fig. 4, under severe cadmium stress (100 mg L^{-1}), the combination of hydro and hormone priming increased the borage seedling length by 3.75–4.58 and 1.5–2.03 times in compar-
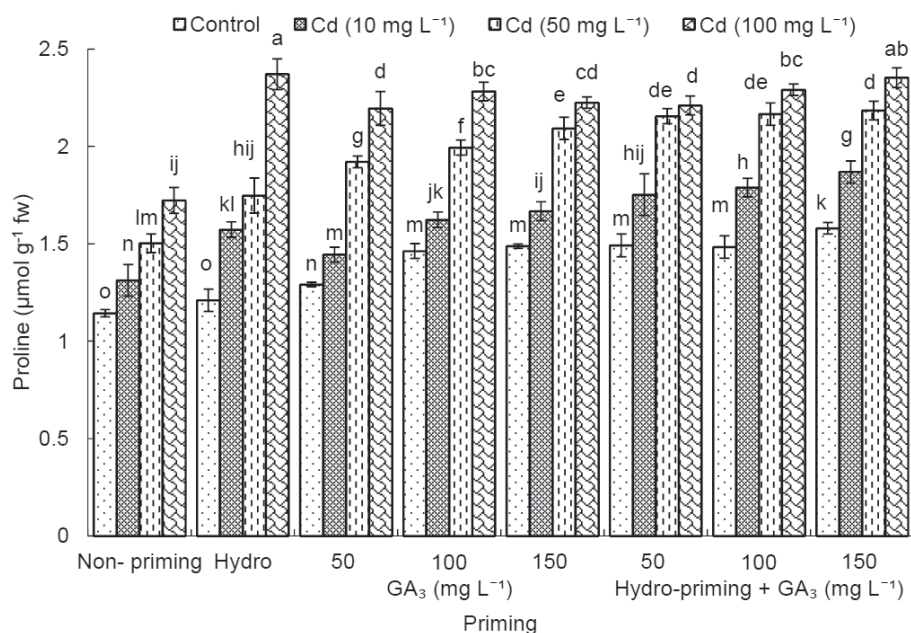
Fig. 7. The effects of cadmium stress (Cd) (10, 50, 100 mg L⁻¹) and seed priming (Non-priming, Hydro-priming, hormone priming (50, 100, 150 mg L⁻¹ GA₃) and sequential application of hydro and hormone priming (hydro-priming + GA₃) (50, 100, 150 mg L⁻¹) for 24 hours) on proline content of borage (*Borago officinalis* L.) seedlings. The values represent mean data from three technical replicates (n=3) and 25 seeds per replicate. The error bars show standard error (S.E.). Values followed by different letters are statistically different (Duncan's Multiple Range test, $P < 0.05$).

ison with that of the seedlings obtained from non-primed seeds and either hydro or hormone primed seeds, respectively. In other words, the sequential application of hydro and hormone priming on borage seeds enhances the positive effects of each of the techniques on the seedling growth characteristics such as seedling length (Fig. 4) and seedling vigor index I (Fig. 5a). These results are consistent with the findings of Tiryakioglu et al. (2006) in barley, Mahmoudi et al. (2017) in borage.

Antioxidant enzymes activity and proline content

As shown in Fig. 6a, the activity of catalase in borage seedlings from the seeds primed with the combination of distilled water and GA₃ were significantly higher than those of the seeds primed with alone distilled water or GA₃, as well as non-primed seeds. These results emphasize the synergistic effects of hydro and hormone priming techniques, which lead to improvement of the biochemical and antioxidant potential of borage seedlings. It has been shown that environmental stresses such as cadmium stress induces the production of ROS in plant cells, which attack biological molecules, including proteins and enzymes at the cellular and subcellular levels, and finally reduce or stop cell growth and the proliferation process (Mittler 2002, Tiryakioglu et al. 2006). The plant cells adapted various enzymatic and non-enzymatic mechanisms that scavenge ROS molecules and protect the biological molecules and cells from the damaging effects of environmental stress. Catalase and peroxidase, the most important antioxidant enzymes of plant cells, play a crucial role in the ROS scavenging process. These enzymes catalyze the conversion of H_2O_2 (hydrogen peroxide) to H_2O and O_2

in plant cells (Gill and Tuteja 2010). On the other hand, the balance between ROS production and activity of antioxidant systems may determine the plant's responses to stressful environments, such as cadmium stress, and their damaging effects. It could be concluded that hydro and hormone priming of borage seeds enhanced the activity of catalase and peroxidase enzymes in the borage seedlings, which in turn enhance the antioxidant capacity of the cells and their ability for germination (Fig. 1) and growth (Figs. 3–5b) under cadmium stress. For example, the sequential application of hydro and hormone priming led to 2.42–2.57 and 2.93–18.72 fold increase in the activity of catalase enzyme under non-stress and cadmium stress conditions, respectively, as compared with those of non-primed seeds (Fig. 6a).

The relationship between reduced damaging effects of environmental stresses and enhanced activity of antioxidant enzymes have been reported in several plants, including rice (Guo et al. 2006, Hussain et al. 2016) response to drought and chilling, sugar beet (Bor et al. 2003) response to salinity, and mung bean (Tiryakioglu et al. 2006) response to heavy metal. On the other hand, it could be concluded that these seed priming techniques mitigate the damaging effects of cadmium in borage seedlings through enhancing the activity of antioxidant systems and reducing adverse effects of cadmium-induced oxidative stress in the cells (Fig. 6a,b).

Among amino acids, proline biosynthesis and catabolism in plant cells play vital roles in physiological responses and plant adaptation to environmental and biotic stresses (Dinakar et al. 2008). It has been shown that this amino acid acts as a free radical scavenger and chemical chaperone that stabilizes proteins in their native conformation, cellu-

lar and subcellular membranes, especially under stressful conditions (Hossain et al. 2014). In borage, the proline content of seedlings from primed seeds was significantly higher than that of seedlings obtained from non-primed seeds, but the amount of increase in proline content varied from a 1.06 to a 1.45-fold change depending on cadmium stress level and seed priming treatments (Fig. 7). This elevated level of proline improves the cellular and physiological processes in borage seedlings and facilitates the activation of detoxification pathways (Dar et al. 2016), cytoplasmic osmotic adjustment and the balancing of cell redox reactions (Hossain et al. 2014). On the other hand, the content of proline in borage seedlings was increased 1.45 and 1.37 times through combined hydro and 150 mg L^{-1} GA$_3$ priming treatment under 10 mg L^{-1} and 100 mg L^{-1} cadmium stress, respectively, as compared to the non-primed seeds (Fig. 7). This regulation of proline concentration in the plant cells is achieved through its biosynthesis, catabolism, and transport between cells and subcellular organelles (Man et al. 2011, Hossain et al. 2014), and these processes are strongly influenced by various factors, especially plant genotype, environmental conditions and hormone balance in the plant cells (Dar et al. 2016). Accumulation of free proline in plant cells and tissues in response to the heavy metal and other abiotic and biotic stresses has been reported in a wide range of plant species. For example, increased levels of proline content was found to occur in *Arachis hypogaea* L. seedlings (Dinakar et al., 2008) and *Ocimum basilicum* L. leaves (Georgiadou et al., 2018) in response to heavy metal stress, and in tall fescue (Man et al. 2011) in response to drought stress.

Conclusions

There is little information in the literature concerning the relationship between seed priming techniques and cadmium toxicity and tolerance in plants. In the present study, for the first time, we demonstrated that the hydro and hormone seed priming techniques effectively mitigated the damaging effects of cadmium stress and improved seed germination and seedling growth under both non-stress and cadmium stress conditions. Among the seed priming treatments, combined hydro and hormone seed priming improved borage seed germination and seedling growth under stressful environments. This improved germination and seedling growth of primed borage seeds was associated with an increased activity of antioxidant enzymes (catalase and peroxidase) and an elevated proline content in borage seedlings.

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