



# Effects of seed priming with boron on the chemicals and antioxidants of quinoa

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## Abstract

A factorial experiment was conducted in a completely randomized design with four replications to investigate the effect of boron priming on the chemical and antioxidant content of seedlings of different quinoa genotypes. Experimental factors included seed priming (at six levels of control, hydropriming, and boron concentrations of 1 g/L, 2 g/L, 3 g/L, and 4 g/L) and 3 quinoa genotypes (Titicaca, Giza 1, and Sajama). The traits in this study included chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, anthocyanins, saponins, total phenols, peroxidase, and superoxide dismutase. Results showed that boron priming has a significant effect on all traits. The effect of genotype on all traits except anthocyanin content was significant. The highest levels of chlorophyll and carotenoids in Titicaca and Sajama genotypes were obtained by applying 2 g/L boron concentrations while the highest level of these traits was obtained by applying 1 g/L boron in Giza 1 genotype. In the studied genotypes, by increasing the concentration of boron solution, the saponin content increased by 33.3% and the highest level of saponin (1.13 mg/g DW) was observed in the Titicaca genotype. The highest total phenol contents in Titicaca and Sajama genotypes were obtained by applying 1 g/L boron concentration while boron priming reduced the total phenol content in Giza 1, compared to hydroprimed seeds. In addition, the response of anthocyanin content to different levels of priming was variable in different genotypes. The highest anthocyanin contents in Titicaca, Giza 1, and Sajama genotypes were obtained under 2 g/L, 4 g/L, and 0 g/L (control) boron concentrations, respectively. At a boron concentration of 2 g/L, Titicaca and Sajama genotypes had the highest superoxide dismutase enzyme contents while Giza 1 had the highest level of this enzyme at a 1 g/L concentration of boron. The highest peroxidase activity was observed in 4 g/L boron concentration.

**Keywords:** boron, *Chenopodium quinoa* willd, peroxidase, saponin, superoxide dismutase

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## Introduction

Quinoa, scientifically known as *Chenopodium quinoa* willd, is a dicotyledonous plant which belongs to the Chenopodiaceae family. It has a strong root system and can withstand a variety of environmental stresses, including salinity and

drought. Moreover, it can grow in marginal soils (Jacobsen et al., 2009).

The protein content of quinoa seeds is high and they are rich in lysine and methionine (Maradini-Filho et al., 2017; James, 2009). They also contains other amino acids including proline, aspartic acid, glutamic acid, cysteine, serine, and tyrosine which are not found in wheat (Bhargava et al., 2006). Quinoa seeds are high in carbohydrates (mostly starch) and low in sugar. Further, they contain

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fiber, vitamins B, E, and C, and minerals such as magnesium, iron, potassium, calcium, phosphorus, manganese, zinc, copper, and sodium. The composition of quinoa fats is similar to that of soybean oil fatty acids (James, 2009).

Germination is considered as one of the most crucial stages in a plant growth. Seed priming is a highly successful method of increasing the physiological and biochemical preparation of seeds for germination. In this technique, the seed absorbs some water so that the physiological steps take place before germination, but the root does not come out of the seed (Schwember and Bradford et al., 2010). Advantages of seed priming include dormancy failure, more and faster germination, uniformity in germination, and increased seedling growth in a wide variety of environmental conditions (Harris et al., 2007).

Seed priming with microelements is a priming method which involves enriching seeds with a microelement, e.g. boron, solution before sowing (Rehman et al., 2012). Boron positively affects many plant functions include cell elongation, nucleic acid production, hormonal responses, membrane function, cell cycle regulation, covalent bonding in the cell wall (Taiz and Zeeiger, 2006), water availability, sugar transport, cation and anion adsorption, and metabolism of nitrogen, phosphorus, carbohydrates, and fats (Oyinola, 2007), phenol metabolism (Goldbach et al., 2001), salt uptake, flowering, and fruiting process and pollen germination (Rafeii and Pakkish, 2014). On the other hand, the effect of boron on the plant is still unknown. Taiz et al. (2015) reported that the boron content in the dry matter of most plants is about 20 ppm, and the soil should be supplemented with this microelement if its content is less. Boron content in the dry matter of quinoa is about 8.58 ppm (Mhada et al., 2020), which is far from the normal. The difference could be attributed to quinoa's inability to absorb boron from the soil. Priming was found to be more effective than soil and foliar application methods (Rehman et al., 2014). The positive effect of boron priming has been reported on many crops including rice (Rahman et al., 2012; Johnson et al., 2005), wheat (Farooq et al., 2012), corn (Harris et al., 2007), chickpeas (Johnson et al., 2005), and dill (Mirshekari et al., 2012).

This study aimed to examine how seed priming with boron affects the chemical content of seedlings from three different quinoa genotypes.

## Materials and Methods

### *Seedling preparation*

The present study was performed in the Seed Processing Laboratory of the Faculty of Agriculture, Shahed University of Tehran. The experiment was performed as a factorial experiment in a completely randomized design with 4 replications. Experimental factors included seed priming (at six levels of control, hydropriming, and boron concentrations of 1 g/L, 2 g/L, 3 g/L, and 4 g/L) and also 3 quinoa genotypes (Titicaca, Giza 1, and Sajama). The seeds were disinfected by soaking in a 70% ethanol solution for one minute; then, they were soaked in a 10% sodium hypochlorite solution for three minutes, before being washed three times with distilled water (Hajhashemi et al., 2013). The seeds were disinfected before being put in boron solutions and immersed in darkness at a temperature of 24 °C for 12 hours. Sterilized Whatman paper No. 1 was used as the culture medium in Petri dishes. On hundred (100) seeds were placed in each Petri and the dishes were transferred to a germinator with the temperature of 20 ±1°C, 70% humidity, 16 hours of light, and 8 hours of darkness (ISTA, 2013). The germination period was completed in 6 days. Then, at 20 °C, all normal seedlings were transferred to a Hoagland solution (hydroponic culture conditions) and were kept there until they reached the 6-leaf stage for biochemical measurements.

### *Chlorophyll and carotenoid assays*

To measure chlorophyll a, b, and carotenoid contents of the seedlings, 0.2 g of fresh leaf tissue was extracted completely with 20 ml of 90% methanol as an extraction medium. To separate the leaf tissue, the resulting mixture was centrifuged at 10,000 rpm for 10 minutes. The upper solution was then isolated and the amount of light absorption at wavelengths of 652.4, 665.2, and 470 nm was measured by ELISA. The contents of leaf chlorophyll and carotenoids were

calculated by the following equations in mg/g FW (Hartmut et al., 2001).

$$\text{Chl a} = (16.72 B) - (9.16 A)$$

$$\text{Chl b} = (34.09 A) - (15.28 B)$$

$$\text{Carotenoid} = (1000 C - 1.63 Ca - 104.96 Cb) / 221$$

where A, B, and C are the light absorption at 652.4, 665.2, and 470 nm, respectively.

### **Saponins assay**

One hundred (100) mg dried seedling tissue was powdered and dissolved in 10 ml boiling water, then heated for 30 minutes in a boiling water bath. The resulting solution was filtered and vortexed for 15 seconds after cooling. Finally, the height of the produced foam was measured, and the content of seedling saponins was determined using Merck's pure saponins as a standard (World Health Organization, 1998).

### **Total phenol content assay**

To measure the total phenol content, the Folin–Ciocalteu method (Ordoñez et al., 2006) was used with a slight modification. For this purpose, 0.5 g of plant tissue in 10 ml of 80% ethanol was completely ground. The mixture was centrifuged at 10,000 rpm for 20 minutes. Then, 0.5 ml of the obtained extract was mixed with 7 ml of distilled water and 5 ml of 10% Folin–Ciocalteu reagent. After 5 minutes, 4 ml of 1 M sodium carbonate was added. The amount of light absorption was measured at 760 nm after 2 hours of incubation at room temperature.

### **Anthocyanin assay**

Tasgin et al. (2003) method was used to measure the amount of leaf anthocyanin. 0.1 g of fresh plant tissue was thoroughly ground in a Chinese mortar with 10 ml of acidic methanol (99 cc of pure methanol and 1 cc of pure hydrochloric acid), and the extract was poured into a helical test tube and kept in the dark for 24 hours and centrifuged at 25 °C. Then, it was centrifuged for 10 minutes at 4000 rpm and the adsorption of the upper solution was measured at a wavelength of 550 nm. The concentration was 3300 cm/M by using the

following equation and considering the extinction coefficient ( $\epsilon$ ).

$$A = \epsilon b c$$

where A indicates the adsorption number, b shows the cuvette width, and c is the concentration of the desired solution.

### **Antioxidant Enzymes Assay**

For antioxidant enzymes assays, 200 mg of fresh leaf tissue in 10 ml of phosphate buffer (pH = 6.8, 0.1 M) was completely ground in ice, and then centrifuged at 2 °C for 15 minutes at 17000 rpm. The upper phase was isolated and stored at -80 °C until various enzymes were measured (Kar and Mishra, 1976).

**Superoxide Dismutase (SOD):** At 560 nm, the adsorption rate of 1 mM EDTA, 50 mM phosphate buffer (pH = 7.8), 12 mM L-methionine, 75% NBT, 50 mM sodium carbonate, 1  $\mu$ M riboflavin, and 300  $\mu$ L enzyme extract was measured so that the final volume of the solution became 3 ml. The superoxide dismutase activity was then calculated as the amount of enzyme required to inhibit 50% of NBT reduction (Giannopolitis and Reis, 1977).

**Peroxidase Activity Assay:** 100  $\mu$ l of enzyme extract, 500  $\mu$ l of 28 mM guaiacol, and 2 ml of potassium phosphate buffer were mixed. Then, 500  $\mu$ l of 5 mM hydrogen peroxide was added to the solution. The activity of peroxidase was measured in terms of mM of hydrogen peroxide per gram fresh leaf weight per minute (mM/g FW/min) through a rapid measurement of light absorption at 470 nm (once every 10 seconds for 3 minutes) (Ghanati et al., 2002).

### **Statistical Analysis**

The data were analyzed with SAS 9.1 software, and their means were compared using Duncan's multiple range test at the  $p \leq 0.05$ .

## **Results**

### **Chlorophyll content**

The effect of boron priming, genotype, and the interaction of boron priming and genotype on the content of chlorophyll a, chlorophyll b, and total

chlorophyll was significant at  $p \leq 0.01$  (Table 1). The response of different genotypes to different levels of boron priming was different. In Titicaca genotype, the results indicated the highest amount of chlorophyll a (1.91 mg/g FW) with 2 g/L boron treatment, chlorophyll b (0.93 mg/g FW) with 3 g/L treatment, and total chlorophyll (2.84 mg/g FW) with applications of 2 and 3 g/L boron. In Gaza 1 genotype, the highest levels of chlorophyll a (0.79 mg/g FW), chlorophyll b (0.37 mg/g FW), and total chlorophyll (1.16 mg/g FW) were obtained by 1 g/L boron treatment (Table 2). Boron priming treatment reduced the chlorophyll a, b, and total in Sajama genotype, and hydropriming treatment performed better in this genotype. Chlorophyll content decreased in all genotypes as the boron solution concentration

increased above 3 g/L. The highest chlorophyll content was observed in Titicaca genotype.

### Carotenoid content

The simple and interaction effects of boron and genotype on carotenoid contents were significant at  $p \leq 0.01$  (Table 1). Genotypes responded differentially to different levels of boron priming regarding carotenoid contents. Titicaca genotype showed the most positive response to boron treatment. The highest amount of carotenoid content compared to the control was obtained as follows: in Titicaca with the application of 2 g/L (170% increase), in Giza 1 with the application of 1 g/L (29.6% increase), and in Sajama with the application of hydropriming (38.6% increase) (Table 2). Increased boron concentration

Table 1

Analysis of variance for effect of different level of Boron priming and Genotype q

Source of variation	df	Mean of Squares			
		Chl a	Chl b	Total Chl	Carotenoids
Boron (B)	5	4.47**	0.33**	5.50**	0.04**
Genotype (G)	2	0.71**	0.57**	2.52**	0.04**
B×G	10	0.21**	0.06**	0.46**	0.002 <sup>ns</sup>
Error	54	0.018	0.003	0.023	0.001
C.V.%		20.27	15.04	14.53	27.41

ns, \*, and \*\*: non-significant and significant at the 0.05 and 0.01 levels of probability, respectively

Table 2

Comparison of the interaction between different priming treatments and genotype

Genotype	Priming	Chl a (mg/g FW)	Chl b (mg/g FW)	Total Chl (mg/g FW)	Carotenoids (mg/g FW)
Titicaca	Control	1.01 <sup>b</sup>	0.27 <sup>gh</sup>	1.28 <sup>cd</sup>	0.12 <sup>eh</sup>
	Hydroprime	1.19 <sup>b</sup>	0.49 <sup>d</sup>	1.69 <sup>b</sup>	0.17 <sup>ce</sup>
	Boron 1 (g/L)	1.16 <sup>b</sup>	0.5 <sup>d</sup>	1.67 <sup>b</sup>	0.27 <sup>ab</sup>
	Boron 2 (g/L)	1.91 <sup>a</sup>	0.93 <sup>a</sup>	2.84 <sup>a</sup>	0.29 <sup>a</sup>
	Boron 3 (g/L)	1.19 <sup>b</sup>	0.21 <sup>hi</sup>	1.41 <sup>c</sup>	0.17 <sup>df</sup>
Giza 1	Boron 4 (g/L)	0.56 <sup>df</sup>	0.06 <sup>j</sup>	0.63 <sup>hi</sup>	0.12 <sup>fh</sup>
	Control	0.48 <sup>df</sup>	0.18 <sup>i</sup>	0.67 <sup>h</sup>	0.07 <sup>hi</sup>
	Hydroprime	0.54 <sup>de</sup>	0.34 <sup>eg</sup>	0.89 <sup>fg</sup>	0.12 <sup>eh</sup>
	Boron 1 (g/L)	0.79 <sup>c</sup>	0.37 <sup>ef</sup>	1.16 <sup>de</sup>	0.22 <sup>bc</sup>
	Boron 2 (g/L)	0.66 <sup>cd</sup>	0.31 <sup>fg</sup>	0.72 <sup>gh</sup>	0.12 <sup>eh</sup>
Sajama	Boron 3 (g/L)	0.42 <sup>eg</sup>	0.16 <sup>i</sup>	0.45 <sup>ij</sup>	0.08 <sup>hi</sup>
	Boron 4 (g/L)	0.12 <sup>h</sup>	0.05 <sup>j</sup>	0.17 <sup>k</sup>	0.07 <sup>i</sup>
	Control	0.12 <sup>h</sup>	0.18 <sup>i</sup>	0.31 <sup>jk</sup>	0.1 <sup>gi</sup>
	Hydroprime	0.43 <sup>eg</sup>	0.61 <sup>c</sup>	1.05 <sup>ef</sup>	0.12 <sup>eh</sup>
	Boron 1 (g/L)	0.6 <sup>ce</sup>	0.69 <sup>bc</sup>	1.3 <sup>cd</sup>	0.19 <sup>dc</sup>
Sajama	Boron 2 (g/L)	0.66 <sup>cd</sup>	0.73 <sup>b</sup>	1.39 <sup>c</sup>	0.15 <sup>dg</sup>
	Boron 3 (g/L)	0.42 <sup>eg</sup>	0.39 <sup>e</sup>	0.82 <sup>gh</sup>	0.11 <sup>fh</sup>
	Boron 4 (g/L)	0.2 <sup>h</sup>	0.14 <sup>i</sup>	0.35 <sup>jk</sup>	0.04 <sup>i</sup>

Similar letters at each column indicate the non-significant difference at the 0.05 level of probability.

exhibited a negative effect on the carotenoid contents in all genotypes. In this study, the highest carotenoid content was measured in the Titicaca genotype.

**Saponin content**

Based on the results of ANOVA, the effect of boron priming, genotype, and their interaction on seedling saponin contents was significant ( $P \leq 0.01$ ) (Table 3). The highest and lowest amounts of saponin were measured in Titicaca genotype (1.13 mg/g DW) and Giza 1 genotype (0.42 mg/g DW), respectively. Boron priming increased saponin contents of Titicaca and Sajama genotype seedlings compared to control and hydropriming treatment while the highest saponin content was obtained by hydropriming treatment in Giza 1 (0.61 mg/g DW) (Table 4). Saponin contents

increased when the concentration of boron solution increased in all genotypes. Compared to the control, the amount of saponin increased by 57.9% in Titicaca, 29% in Giza, and 58.1 % in Sajama genotypes after the 4 g/L boron treatment (Table 4).

**Polyphenol content**

The effects of genotype, boron priming, and their interaction on seedling polyphenol contents were significant ( $P \geq 0.01$ ) (Table 3). Findings revealed that the polyphenol content of Titicaca and Sajama seedlings increased by applying priming treatment to the control while the polyphenol content of Giza 1 seedlings decreased. The polyphenol content of seedlings in various genotypes decreased when the concentration of boron solutions increased. The highest polyphenol

Table 3  
Analysis of variance for the effects of priming and genotypes

Source of variation	df	Mean of Squares				
		Saponin	Polyphenols	Anthocyanin	SOD	Peroxidase
Boron (B)	5	3.09**	0.15**	4.16**	107.44**	3.23**
Genotype (G)	2	0.70**	1.28**	0.35 <sup>ns</sup>	424.10**	5.94**
B×G	10	0.16**	0.34**	1.43**	16.70**	2.28**
Error	54	0.008	0.016	0.229	2.537	0.238
C.V.%		11.42	11.13	21.16	10.11	20.28

ns, \*, and \*\*: non-significant and significant at the 0.05 and 0.01 levels of probability, respectively

Table 4  
Comparison of the interaction between different levels of Boron priming and Genotype

Genotype	Priming	Saponin (mg/g DW)	Polyphenols (µg/ml)	Anthocyanin (mg/g FW)	SOD (U/mg FW)	Peroxidase (mM/g FW/min)
Titicaca	Control	0.71 <sup>f</sup>	1.19 <sup>eg</sup>	2.27 <sup>dg</sup>	9.17 <sup>hi</sup>	2.13 <sup>cf</sup>
	Hydroprime	0.87 <sup>e</sup>	1.33 <sup>de</sup>	2.5 <sup>be</sup>	10.75 <sup>gh</sup>	5.64 <sup>a</sup>
	Boron 1 (g/L)	0.96 <sup>e</sup>	1.67 <sup>ab</sup>	2.42 <sup>be</sup>	18.28 <sup>c</sup>	1.88 <sup>df</sup>
	Boron 2 (g/L)	1.1 <sup>e</sup>	1.43 <sup>cd</sup>	2.82 <sup>ad</sup>	22.22 <sup>b</sup>	2.19 <sup>cf</sup>
	Boron 3 (g/L)	1.47 <sup>b</sup>	0.92 <sup>h</sup>	3.32 <sup>a</sup>	19.79 <sup>c</sup>	2.55 <sup>bd</sup>
Giza 1	Boron 4 (g/L)	1.69 <sup>a</sup>	0.46 <sup>k</sup>	3.07 <sup>ab</sup>	6.13 <sup>j</sup>	2.6 <sup>bc</sup>
	Control	0.39 <sup>h</sup>	1.41 <sup>cd</sup>	1.7 <sup>fh</sup>	10.79 <sup>gh</sup>	1.93 <sup>cf</sup>
	Hydroprime	0.61 <sup>fg</sup>	1.49 <sup>bd</sup>	2.37 <sup>cf</sup>	12.63 <sup>fg</sup>	3.1 <sup>b</sup>
	Boron 1 (g/L)	0.22 <sup>i</sup>	1.15 <sup>fg</sup>	2.47 <sup>be</sup>	22.77 <sup>b</sup>	1.5 <sup>f</sup>
	Boron 2 (g/L)	0.37 <sup>h</sup>	0.88 <sup>hi</sup>	1.22 <sup>h</sup>	18.08 <sup>cd</sup>	1.74 <sup>ef</sup>
Sajama	Boron 3 (g/L)	0.36 <sup>h</sup>	0.72 <sup>ij</sup>	2 <sup>eg</sup>	15.83 <sup>de</sup>	2.19 <sup>cf</sup>
	Boron 4 (g/L)	0.55 <sup>g</sup>	0.62 <sup>jk</sup>	2.7 <sup>ad</sup>	7.94 <sup>ij</sup>	2.54 <sup>bd</sup>
	Control	0.54 <sup>g</sup>	0.79 <sup>hj</sup>	2.97 <sup>ac</sup>	12.21 <sup>fg</sup>	1.7 <sup>ef</sup>
	Hydroprime	0.57 <sup>g</sup>	1.11 <sup>g</sup>	2.22 <sup>dg</sup>	13.73 <sup>ef</sup>	2.09 <sup>cf</sup>
	Boron 1 (g/L)	0.64 <sup>fg</sup>	1.78 <sup>a</sup>	1.87 <sup>eh</sup>	22.76 <sup>b</sup>	1.57 <sup>f</sup>
Sajama	Boron 2 (g/L)	0.71 <sup>f</sup>	1.53 <sup>bc</sup>	1.82 <sup>eh</sup>	26.67 <sup>a</sup>	2.31 <sup>ce</sup>
	Boron 3 (g/L)	1.11 <sup>d</sup>	1.32 <sup>df</sup>	1.3 <sup>h</sup>	22.46 <sup>b</sup>	2.61 <sup>bc</sup>
	Boron 4 (g/L)	1.29 <sup>c</sup>	0.67 <sup>j</sup>	1.6 <sup>gh</sup>	11.3 <sup>gh</sup>	3.1 <sup>b</sup>

Similar letters at each column indicate the non-significant difference at the 0.05 level of probability.

contents in untreated seedlings were observed in Giza 1 (1.41  $\mu\text{g/ml}$ ), Titicaca (1.19  $\mu\text{g/ml}$ ), and Sajama (0.79  $\mu\text{g/ml}$ ), respectively (Table 4). The highest polyphenol content was obtained by applying boron 1 g/L treatment while the lowest polyphenol content was obtained by applying boron 4 g/L priming treatment. Hydropriming treatment increased the content of seedling polyphenols compared to the control (Table 4).

#### ***Anthocyanin content***

Boron priming as well as the interaction of priming and genotype had a significant effect on saponin contents of the seedlings ( $P \geq 0.01$ ) while the effect of genotype was not significant (Table 3). Hydropriming and boron priming increased anthocyanin contents of the seedlings. The anthocyanin content of seedlings increased with the increase in concentration of boron. The highest anthocyanin level was obtained by applying 3 g/L boron to Titicaca genotype seeds while the lowest anthocyanin content was recorded in 2 g/L boron priming treatment of Giza 1 seeds (Table 4).

#### ***Superoxide dismutase activity***

Based on the results of ANOVA, the effect of boron priming, genotype, and their interaction on SOD activity was significant ( $P \leq 0.01$ ) (Table 3). The highest and lowest activities of superoxide dismutase were observed in Sajama (18.19 U/mg FW) and Titicaca genotypes (14.39 U/mg FW), respectively. When the priming solution concentration increased to 2 g/L, the enzyme activity initially increased while decreasing until priming with 4 g/L boron solution and the enzyme activity reduced by 21% compared to the control treatment (Table 4).

#### ***Peroxidase activity***

The effect of genotype, priming boron, and their interaction on peroxidase activity was significant ( $P \leq 0.01$ ) (Table 3). The highest peroxidase activity was observed in Titicaca genotype (2.83 mM/gFW/min) and the lowest activity was observed in Giza 1 (2.16 mM/gFW/min). At low concentrations (1 g/L), boron priming treatment reduced peroxidase activity by 14% compared to the control while peroxidase activity increased

with an increase in the boron concentration. Treatment with 4 g/L boron solution increased peroxidase activity by 29.9% compared to the control. Hydropriming increased peroxidase activity more than the boron treatment in this study (Table 4).

#### **Discussion**

The results showed that seed priming of different quinoa genotypes with low boron concentrations increased the chlorophyll content in seedlings. However, the content of chlorophyll decreased when the concentration of boron solution increased. Changes in seedling carotenoid content were similar to chlorophyll. Rehman et al. (2012) reported similar results. Inbaraj and Muthuchelian (2011) reported that boron deficiency in the plant reduces chlorophyll a and b and carotenoids. Furthermore, Mouhtaridou et al. (2004) found that low boron concentrations increased leaf chlorophyll content while high boron concentrations had the opposite effect. Numerous studies confirmed the involvement of boron in chlorophyll synthesis (Sheng et al., 2009; Rehman et al., 2012). Boron is an important micronutrient for plant growth while excess amount of Boron has toxic effects in plants (Ghanati et al., 2002). Boron affects hill reactions and photosynthesis by acting on chlorophyll and photosynthetic enzymes (Prabhu Inbaraj and muthuchelian, 2011). In addition, boron deficiency alters the transfer of  $\text{CO}_2$  to primary and secondary metabolites (Dixit et al., 2002). In an anatomical study of kiwifruit leaves, boron deficiency reduced the volume of mesophilic cells and increased the volume of intercellular space leading to damage to photosynthetic cells (Sotiropoulos et al., 2002).

Saponins are glycoside triterpene compounds or steroids which have foaming properties (Gurfinkel and Rao, 2003). They are molecules with a high molecular weight which are formed by binding a sugar part to a terpenoid or steroid aglycone. The non-aglycone part of the molecule is called saponin, genein, or sapogenin, which binds to the sugar part through glycosid transplantation. The structure of saponins includes arabinose, galactose, glucose, ramosis, xylose, galacturonic acid, and glucuronic acid (Sun and Pan, 2006). These biochemicals are highly considered for their

antioxidant properties (Hu et al., 2002) and protection against pests, fungi, and viruses (Oleszek and Bialy, 2006). In the present study, boron priming and hydropriming could increase the saponin content of plants. In a study aimed at investigating the effect of saponin priming on quinoa resistance under salinity stress conditions, the seeds primed with saponins showed more resistance to stress (Gheysari et al., 2019). Some studies have shown that boron priming can increase plant resistance to stress by increasing saponin levels.

Phenols are released in response to a variety of stresses in the plant. Based on the results of the present study, the phenol content of the plants first increased and then decreased when the concentration of boron solution increased. In another study aimed at evaluating the effect of boron on photosynthetic pigments of phenol and protein compounds of *Vigna radiata* L., the amount of boron in the environment increased to the level of toxic concentrations of phenol (Seth and Aery, 2014). Chlorophyll degradation is a regulated process and is catalyzed by enzymes. Chlorophyllase, chlorophyll oxidase, and peroxidase are three of the identified chlorophyll-degrading enzymes. The peroxidase system requires phenolic compounds in the degradation of chlorophyll (Yamauchi and Watada, 1994). The enzymatic degradation of chlorophyll is probably facilitated by increasing phenol content under high levels of boron via the hydrogen peroxidase system.

There is always oxygen in the plant, even in the absence of environmental stresses of radical oxygen species (Ananeiva et al., 2002). Reactive oxygen species (ROS) including hydrogen peroxide ( $H_2O_2$ ), hydroxyl (HO), superoxide ( $O^{-2}$ ), and single oxygen cause oxidative stress in the plant. These molecules inflict a lot of damage to giant molecules and the overall structure of cell (Ariano et al., 2005). Antioxidants, both enzymatic and non-enzymatic, help plants tolerate oxidative stress. The plant defense against oxidative stress is made up of enzymes including peroxidase, superoxide dismutase, catalase, glutathione reductase, ascorbate peroxidase, and pigments like anthocyanins and carotenoids (Ariano et al.,

2005; Gill and Tuteja, 2010). Superoxide dismutase is the first line of defense in the plant against reactive oxygen species and converts superoxide radicals to hydrogen peroxide and oxygen (Bailly, 2004). The peroxidase enzyme is found in the cell wall, endoplasmic reticulum, vacuole, and Golgi apparatus and is responsible for hydrogen peroxide radical decomposition (Ahmadpour Dehkordi and Balouchi, 2013). Anthocyanins have a number of critical roles in the plant including antioxidant properties and light protection.

The amount of anthocyanin in plant vacuoles increases in response to oxidative stress or freezing (Gould, 2004), and anthocyanin has been found to play a key function in flower color and pollination (Akhbari et al., 2015). Anthocyanins along with flavonoids prevent pests from invading the plant and inhibit the growth of insect larvae in the plant (Tiwari et al., 2009). The results of different studies showed that different priming methods increase the antioxidant content of plants (Cruzde Carvalho, 2008; Moosavi et al., 2009; Ahmadpour Dehkordi and Balouchi, 2013). These findings are consistent with those of the present study. Currently, it is unclear how priming can increase the antioxidant content of plants. In addition, priming quinoa seeds with boron increased the antioxidant content of the plant more than hydropriming did, which may be related to the increased metabolism, especially that of nitrogen, in seedlings primed with boron.

## Conclusion

Results revealed the positive effects of boron priming on photosynthetic pigments. Boron priming increased the content of photosynthetic pigments, i.e. chlorophylls and carotenoids. It was also found that boron priming can increase the enzymatic and non-enzymatic antioxidant contents such as anthocyanins, superoxide dismutase, and peroxidase. Furthermore, an increase occurred in seedling saponin levels under the priming treatment, especially with boron. The effects of different concentrations of boron on quinoa seedling traits were different. It is difficult to comment on the best concentration of boron solution for priming quinoa seeds and further research is needed. Finally, Titicaca genotype in

this study had more antioxidant, photosynthetic pigment, and saponin content than other genotypes primed with boron.

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