Effects of hydro- and bio-priming on some physiological and biochemical characteristics of quinoa (*Chenopodium quinoa*) seedlings under drought stress

Saba Dashab and Heshmat Omidi*

Department of Agronomy, Faculty of Agriculture, Shahed University, Tehran, Iran

Abstract

Quinoa (*Chenopodium quinoa* Willd.) is a grain-like crop with a high-grown potential in Iran. It has high nutritional value and therefore, is compared to milk by the FAO. This study was conducted to evaluate quinoa seedlings' physiological and biochemical properties under the effects of priming and drought stress treatments. The experiment was factorial based on a completely randomized design in a greenhouse at the Agriculture Faculty of Shahed University, Tehran, Iran. Experimental factors included priming at two levels (hydro-priming and bio-priming) and drought stress at five levels (control (0), -0.5, -1, -1.5, and -2 MPa with polyethylene glycol). Analysis of variance showed that the effects of priming and drought stress were significant on most of the studied traits. The results showed a significant decrease in quinoa seedling indices (such as shoot and root dry weight) under high levels of drought stress. However, the application of bio-priming (growth-promoting bacteria) improved physiological indices, such as proline content and antioxidant enzyme activity. Interactions of priming and drought stress had significant effects on fructose, glucose, and proline content. The highest seedling proline content was related to -2.0 MPa in bio-priming (8.67 µmol/g FW) while the lowest proline content was recorded under non-stress conditions in hydro- and bio-priming (2.95 and 3.11 µmol/g FW). This study showed that the highest mean dry weight of shoots, protein content, and enzymatic and non-enzymatic antioxidants were observed at the bio-priming level. The priming treatments (hydro and bio) led to improvements in characteristics such as root dry weight, proline content, superoxide dismutase activity, and anthocyanin content. These led to the mitigation of the adverse effects of drought stress.

Keywords: antioxidant activity, growth-promoting bacteria, polyethylene glycol, proline content, shoot and root dry weight

Dashab, S. and **H. Omidi.** 2021. 'Effects of hydro- and bio-priming on some physiological and biochemical characteristics of quinoa (*Chenopodium quinoa*) seedlings under drought stress'. *Iranian Journal of Plant Physiology ,11*(3):3659-3670.

Introduction

*Corresponding author
E-mail address: omidi@shahed.ac.ir
Received: December, 2020
Accepted: February, 2021

Quinoa (*Chenopodium quinoa* Willd) belongs to the Chenopodiaceae family. This plant is native to the Andes Mountains in Bolivia, Chile, and Peru and is widely adopted and the most important

producers of this plant are Bolivia, Peru, and Ecuador (Bazile et al., 2014). Since quinoa is rich in protein, it is an excellent substitute for rice (*Oryza sativa* L.), and the protein in quinoa is one of the few non-animal proteins that is quantitatively and qualitatively better than other grains and has twice the protein content of wheat (*Triticum aestivum* L.) (Ceccato et al., 2011). Demand for quinoa seeds is growing in the United States, Europe, and Asia, so this demand has led to quinoa cultivation as a strategic crop in most non-native areas (Talebnejad and Sepaskhah, 2015). Quinoa grain is gluten-free, rich in essential amino acids, vitamins (A, B_2, and E), minerals (K, Ca, Fe, and Mn), oils containing large amounts of linoleate, linolenate, and natural antioxidants, polyunsaturated fatty acids such as omega 3 and 6, and carbohydrates (Yang et al., 2016). The crop has begun to be known as the 'grain of the 21st century due to its high protein content with a balanced presence of essential amino acids (González et al., 2012). Quinoa has recently been received as an alternative crop in arid and semi-arid agriculture worldwide (Iqbal et al., 2019). In Iran, there is considerable attention to the quinoa plant, and recently it has been tested successfully in many different parts of the country (Talebnejad and Sepaskhah, 2016).

Abiotic stress is one of the leading causes of plant loss that reduces plant yield by more than 50% worldwide (Hinojosa et al., 2018). Among many of the most important crops, quinoa is suitable for growing in dry and saline conditions. In arid and semi-arid regions, abiotic stresses such as drought and salinity stress effectively reduce the percentage and rate of germination due to insufficient moisture required for germination in soil surface layers. Eventually, this leads to the poor seedling establishment in these areas (Rahimi, 2013). The arid and semi-arid regions in Iran, which cover more than 1.5 million square kilometers, means that drought is one of the most critical abiotic stress in this region. Modification of the crops for drought tolerance has always faced its challenges (Lawal, 2018).

Using appropriate techniques to prepare seeds against adverse conditions is considered a way to reduce the harmful effects of environmental stresses on the plant and improve yield. One of the methods that have received particular attention today is the seed priming technique (Rehman et al., 2018). Seed priming has been approved to improve germination and subsequently increase growth in plants such as wheat, coriander, and quinoa (Ceccato et al., 2011; Ibrahim, 2016; Tabassum et al., 2017). The use of priming as a solution to improve germination and seedling establishment has been investigated by researchers on plants such as corn (Jiang et al., 2016), wheat (Ulfat et al., 2017), stevia (Aghighi Shahverdi et al., 2017), and rice (Hussain et al., 2017).

The use of biological and natural methods for priming seeds has been considered in recent years, including the priming of grains with various bio-fertilizers. Bio-fertilizers contain several beneficial soil microorganisms that improve plant growth in a variety of ways, including the production of plant hormones, nitrogen fixation, facilitating the uptake of nutrients from the soil, and the output of bio-controllers for environmental pathogens (Etesami and Maheshwari, 2018; Gouda et al., 2018). Plant growth-promoting bacteria affect different growth indices through other mechanisms. These mechanisms include the production and secretion of growth regulators such as auxins, gibberellins, cytokines, and improving nitrogen fixation, insoluble phosphates' solubility, and other nutrients as well as the control of pathogens (Bakhtiari et al., 2020). Therefore, quinoa cultivation is a valuable opportunity as a potential crop given current conditions and future climate change (Mabhaudhi et al., 2019). This study aimed to investigate quinoa seedlings' physiological and biochemical properties under different osmotic potentials of polyethylene glycol. The study investigated the potential of priming by adding hydro and bio-priming levels to reduce the effects of drought stress.

Materials and Methods

This study was conducted in the research greenhouse of the Faculty of Agricultural Sciences, Shahed University during 2019 (longitude of 51° 8' North and latitude of 35° 34' East, altitude 1190 m, average rainfall 216 mm, and average temperature 17.1°C). This factorial study was

conducted based on a completely randomized design (CRD) with four replications. Experimental factors included two levels of priming (hydro-priming and bio-priming) and drought stress with five levels (0 as control, -0.5, -1, -1.5, and -2 MPa polyethylene glycol).

Quinoa seeds were obtained from Karaj Seed and Plant Breeding Research Institute. First, quinoa seeds were disinfected with 0.5% sodium hypochlorite for five minutes and then washed three times with distilled water. Priming treatments included hydro-priming (seeds for 12 hours at 4 ℃ in distilled water) and bio-priming (inoculation of seeds with *Bacillus subtillis* for 24 hours in a moist environment at 4 ℃, with concentration of 10 ml solution in one liter of distilled water).

After the seeds were dried, 50 seeds were sown in each petri dish on Whatman filter paper, and distilled water was added to each petri dish. After ten days, the seedlings were transferred to the Hoagland culture medium. Forty pots were prepared with Hoagland culture medium. In each replication of the treatment, five seedlings were placed in each container, and to each box was added the complete Hoagland nutrient solution with distilled water. Hoagland solution was prepared from hydroponic fertilizer with Hoagland and Schneider formula, including liquid stock-A and stock-B fertilizers. After the establishment and full growth of seedlings (6 to 8 leaves), five drought levels were applied. The greenhouse temperature was 22-25 ℃ and the light intensity was provided by a combination of fluorescent and tungsten lamps. Plastic containers with a volume of 500 ml were used as plant growth sites.

An aquarium pump was used to aerate the hydroponic solution. To prepare the containers as a culture medium, five holes with a diameter of 2 cm were made on the containers' lids, then sponges with the same diameter were placed on them. Slits were made in the sponges, and the seedlings were passed through the slit so that the roots were immersed in the nutrient solution. When preparing Hoagland solution based on the type of the treatment, the solution ratios and concentrations were controlled and applied. Polyethylene glycol was used to prepare drought levels. Hoagland solution is a hydroponic nutrient solution and one of the best nutrient compounds for growing plants. Hoagland solution provides all the nutrients necessary for plant growth and is suitable for developing many types of plant species. After preparing the stock solution, it was stored in sealed bottles in a cool dark place with a particular label (Arnon and Hoagland, 1944). After two months, sampling was performed and scales with an accuracy of 0.001 were used to measure the fresh and dry weights of different organs and physiological traits.

Measurement of physiological characteristics

Anthocyanin content

Wagner's (1979) method was used to measure the amount of anthocyanin in leaf tissue. First, 0.1 g fresh plant leaf tissue was thoroughly ground in a porcelain mortar with 10 ml acidic methanol (pure methanol and pure hydrochloric acid in a volume ratio of 99: 1), and the extract was poured into a twisted test tube which was then placed for 24 h in the dark conditions at 25 ℃. Then, centrifugation was carried out at 4000 rpm for 10 min and absorbance of the supernatant at 550 nm was measured. The concentration was calculated using the following formula, taking into account the extinction coefficient (ε) of 33000 cm/mol (Taşgín et al., 2003):

$$[1] \quad C = A / \varepsilon b$$

where A is the amount of light absorption, b is the cut width, and C is the concentration of the desired solution.

Measurement of leaf proline content

Bates et al. (1973) method was used to measure leaf tissue proline content. The top layer containing toluene and proline was used to measure the proline content at 520 nm against pure toluene control. Pure proline with concentrations of 0, 4, 8, 12, 16, and 20 mg per liter was used to draw the standard curve. Then, the amount of dissolved proline was obtained with the help of the obtained standard curve in gram fresh weight of the plant.

Table 1
Analysis of variance of different traits of quinoa seedling under priming and drought stress treatments

		Mean square (MS)									
S.O.V	df	Shoot dry weight	Root dry weight	Proline content	Soluble carbohydrate content	Fructose content	Glucose content	Protein content	SOD activity	Phenol content	Anthocyani content
Priming (P)	1	0.55*	0.008ns	0.18ns	5.25ns	6.45ns	10.20ns	27.50**	31.60**	0.01*	1.80ns
Drought stress (D)	4	1.77**	0.02ns	33.40**	315.2*	113.40ns	103ns	16.70**	33.70**	0.006*	32.30ns
D× P	4	0.05ns	0.02*	29.02**	0.06ns	225.02*	217.03*	0.05ns	24.63**	0.003ns	52.02**
Error	30	0.12	0.04	6.22	102.60	98.40	104.90	2.77	2.78	0.002	13.60
CV (%)	-	16.60	19.02	21.30	21.30	16.60	19.50	11.35	10.62	8.61	20.60

ns: non-significant; * and **: significantly at the probability level of 5% and 1%, respectively

Measurement of soluble carbohydrates

Measurement of soluble carbohydrates was performed by (Castonguay et al., 1995) method. To measure soluble carbohydrates, 0.1 ml alcoholic extract was mixed with 3 ml freshly prepared Antron (150 mg Antron + 100 ml of 72% sulfuric acid). The solution was then placed in a boiling water bath for 10 minutes to react and stain. To prepare the alcoholic extract, 0.5 g of dry plant powder was soaked in 5 ml of 80% ethanol for 48 h and then centrifuged for ten minutes at 3000 rpm. The absorbance of the solution was read with a spectrophotometer at 625 nm and the amount of soluble sugars was calculated. To prepare sugar standards using pure glucose and based on its molecular weight, the mother solution was prepared and from this solution, concentrations of 500, 1000, 1500, and 2000 ppm pure glucose were prepared to perform the relevant tests.

Measurement of protein content

The amount of soluble protein in seedlings was measured by the Bradford method (Bradford, 1976). To prepare Bradford solution, 25 mg of Kumasi Brilliant Blue was dissolved in 12.5 ml of 95% ethanol. Then 25 ml of 88% concentrated autophosphoric acid was added. It was then placed on a shaker for 12 hours and the final volume was reduced to 250 ml with distilled water and then passed through a piece of filter paper. Protein concentration (absorption 595 nm) in micrograms per gram of fresh tissue was calculated using a standard curve.

Measurement of superoxide dismutase activity

Superoxide dismutase activity was performed by (Muthukumarasamy et al., 2000). To ptassium phosphate at pH = 7.8 (containing 400 mM KH_2PO_4 at 50 ml and 400 mM K_2HPO_4 at 50 ml distilled water), 25 ml potassium phosphate at pH = 7.8, methionine 13 mM, NBT 75 µM, riboflavin 2 Mm, and EDTA 0.1 µM were added to obtain 250 ml solution. Adsorption was read at a wavelength of 560 nm.

Measurement of total leaf tissue phenol

In order to measure the total phenol content of leaf tissue the method of Meda et al. (2005) was used. To measure the content of total phenol, to 100 µl of plant extract, 2 ml of sodium carbonate (2%), 2.8 ml of distilled water, and 100 µl of Folin–Ciocalteu reagent (50%) were added. After half an hour, their absorption was recorded at 720 nm compared to the control. Gallic acid was used as the standard to draw the standard curve. All extracts' phenol content was reported in mg equivalent of gallic acid per gram dry plant weight.

Statistical Analysis

The obtained data were analyzed and the means were compared based on Duncan's test at the level of 5% using the SAS statistical program version 9.1. Excel software was also used to draw the charts.

Results

Shoots and roots dry weights

Table 2
Effect of priming treatment on shoot dry weight, protein content, superoxide dismutase activity, and phenol content of quinoa seedlings

Priming	Shoot dry weight (g)	Protein content (µmol/g FW)	SOD activity (U/mg protein)	Phenol content (mg/g FW)
Hydro-priming	1.96 b	13.84 b	14.82 b	51.0 b
Bio-priming	2.19 a	15.50 a	16.60 a	52.0a

Means with similar letters in each column have no statistical difference based on Duncan's multiple range test at the 5% probability level.

Table 3
Interaction effects of drought stress and seed priming on morpho-physiological characteristics of quinoa seedling

Drought stress (MPa)	Priming Treatments	Root dry weight (g)	Proline content (µmol/g FW)	Fructose content (µg/g FW)	Glucose content (µg/g FW)	SOD activity (U/mg protein)	Anthocyanin content (mg/g FW)
Control	Hydro-priming	2.95 c	2.95 f	102.75 e	116.33 e	10.42 e	6.85 ef
	Bio-priming	2.72 d	3.11 f	115.32 e	105.74 e	12.01 e	6.77 f
-0.5	Hydro-priming	3.48 ab	3.75 e	124.10 d	142.51 d	18.95 d	6.95 ef
	Bio-priming	3.40 ab	3.46 ef	133.81 d	133.74 d	17.63 d	7.32 e
-1.0	Hydro-priming	3.75 a	3.95 e	157.42 c	156.36 c	24.11 c	8.15 d
	Bio-priming	3.79 a	4.03 e	142.52 c	174.19 b	24.75 c	8.01 d
-1.5	Hydro-priming	3.01 bc	5.75 d	174.12 b	178.12 b	31.20 b	9.18 bc
	Bio-priming	2.89 cd	6.34 c	172.80 b	198.49 ab	33.72 b	8.57 cd
-2.0	Hydro-priming	2.89 cd	8.12 b	198.47 a	204.73 a	39.85 a	10.70 a
	Bio-priming	2.45 f	8.67 a	200.13 a	218.80 a	38.05 a	9.68 b

The means with common letters in each treatment column do not have any statistical significant difference at 5% level of probability based on the Duncan's multiple test.

The effects of priming and droughts stress and their interaction were significant on the shoot and dry root weights, respectively (Table 1). As shown in Table 2, the shoot dry weight increased in the bio-priming application (2.19 g) and decreased under hydro-priming (1.96 g). The trend of dropping in the first stage of stress and the relative increase at higher levels of drought stress is also evident in quinoa seedling dry weight. The highest shoot dry weight was obtained in non-stress and at -2 MPa stress which was not statistically different from that at control level. The lowest shoot dry weight was observed at 0.5 MPa (Fig. I). Results indicated that seed priming (hydro and bio) under -1.0 MPa drought stress increased root dry weight (3.75 and 3.79 g) compared to the other treatments, respectively. The lowest root dry weight (2.45 g) was achieved in bio-priming under high drought stress levels (Table 3).

Proline content

The effects of drought stress and its interaction with priming were significant ($p \leq 0.01$) on proline content (Table 1). Increasing drought stress levels increased the proline content of leaf tissue. The highest proline content (6.07 µmol/g FW) was observed in the -2 MPa level while the control level (no stress) showed the lowest amount of proline (1.12 µmol/g FW) (Fig. II). As for the interaction of drought and priming (Table 3), the highest proline content of seedlings was related to -2.0 MPa in bio-priming (8.67 µmol/g FW), and the lowest proline level was related to non-stress

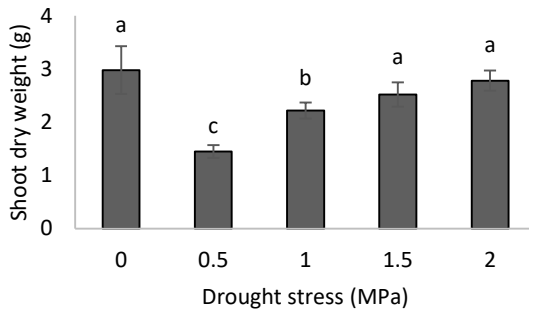

Fig. I. The effect of drought stress (0, -0.5. -1.0, -1.5, and -2 MPa) on quinoa seedling's shoot dry weight (means with similar letters in each figure are not statistically different).

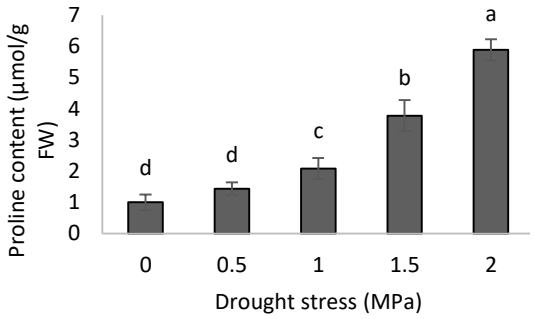

Fig. II. The effect of drought stress (0, -0.5. -1.0, -1.5, and -2 MPa) on proline content of quinoa seedlings (means with similar letters in each figure are not statistically different).

conditions in hydro- and bio-priming (2.95 and 3.11 µmol/g FW).

Soluble carbohydrate content

As shown in Table 1, the effect of drought stress was significant on the soluble carbohydrate content ($p \leq 0.05$). In general, results showed that the drought stress increased the total carbohydrate content so that the highest mean of this trait was related to -1.5 MPa (33.5 mg/g FW) while the lowest mean (16.4 mg/g FW) was observed in non-stress treatment (Fig. III).

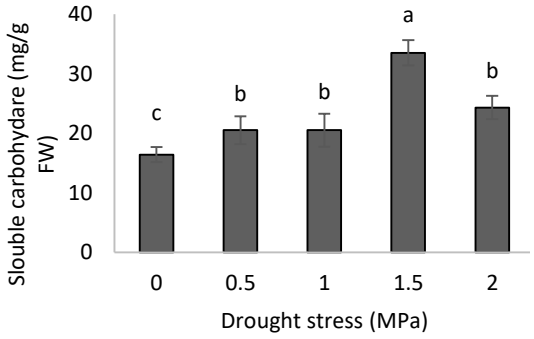

Fig. III. The effect of drought stress (0, -0.5. -1.0, -1.5, and -2 MPa) on the soluble carbohydrate content of quinoa seedlings (means with similar letters in each figure are not statistically different).

Fructose and glucose content

As shown in Table 1, results indicated that the interactions of drought and priming effects had significant effects on fructose and glucose content ($p \leq 0.05$). An increase in fructose and glucose content was observed under drought stress treatment. The lowest fructose and glucose contents were marked in the application hydro- and bio-priming under non-stress conditions.

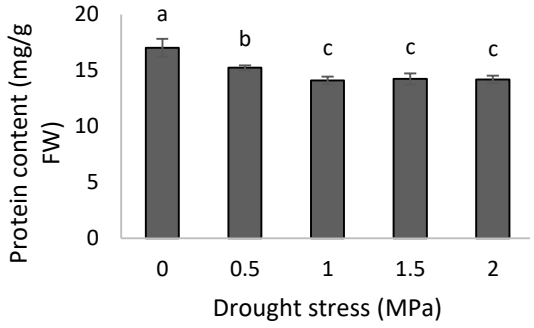

Fig. IV. The effect of drought stress (0, -0.5. -1.0, -1.5, and -2 MPa) on protein content of quinoa seedling (means with similar letters in each figure have no statistical difference).

Protein content

As shown in Table 2, analysis of the data showed that the effect of drought stress and priming was significant on protein content ($p \leq 0.01$). Comparison between the two seed priming methods showed that bio-priming compared to hydro-priming led to an increase by 10.70% in protein content (Table 2).

SOD activity

As shown in Table 1, the effects of priming, drought, and the interaction of drought and priming were significant on SOD activity ($p \leq 0.01$). As for the interaction effects, the highest SOD activity was related to hydro- and bio-priming

under -2.0 MPa (39.85 and 38.05 U/mg protein, respectively). The lowest SOD activity was observed in hydro- and bio-priming under control stress treatment (10.42 and 12.01 U/mg protein, respectively) (Table 3).

negative correlations between traits. For example, the root dry weight was significantly and positively correlated with shoot dry weight, soluble carbohydrates, fructose, glucose, and phenol content. Also, the SOD activity was significantly and negatively correlated with proline content.

Table 4
Correlation coefficients among physiological characteristics of quinoa (*Chenopodium quinoa* Willd) under salinity stress and varieties

	1	2	3	4	5	6	7	8	9	10
1	1									
2	0.37*	1								
3	-0.15ns	-0.07ns	1							
4	0.59**	0.63**	-0.15ns	1						
5	0.39*	0.50**	-0.30*	0.86**	1					
6	0.35*	0.46**	-0.27ns	0.85**	0.99**	1				
7	-0.54**	-0.33*	0.16ns	-0.41**	-0.49**	-0.44**	1			
8	0.11ns	0.10ns	0.84**	0.10ns	-0.01ns	-0.01ns	0.18ns	1		
9	0.06ns	0.11ns	-0.12ns	0.32*	0.28ns	0.28ns	-0.26ns	-0.24ns	1	
10	0.61**	0.79**	-0.19ns	0.64**	0.66**	0.63**	-0.46**	0.12ns	0.03ns	1

ns: non-significant; * and **: significantly at the probability level of 5% and 1%, respectively
1: Root dry weight; 2: Shoot dry weight; 3: Proline content; 4: Soluble carbohydrate; 5: Fructose content; 6: Glucose content; 7: Protein content; 8: SOD activity; 9: Anthocyanin content; 10: Phenol content

Phenol content

Priming and drought stress significantly affected total phenol content at a 5% probability level (Table 1). Based on the comparison of the means, bio-priming application caused a slight increase in phenol content compared to the hydro-priming (Table 2). The total phenol content decreased initially under -0.5 MPa stress and increased with increasing drought stress (Fig. V).

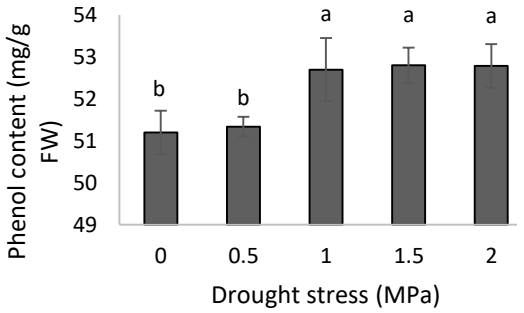

Fig. V. The effect of drought stress (0, -0.5. -1.0, -1.5, and -2 MPa) on quinoa seedling phenol content (means with similar letters in each figure are not statistically different).

Anthocyanin content

As shown in Table 1, the interaction of drought and priming had a significant effect on anthocyanin content ($p \leq 0.01$). In general, drought stress increased anthocyanin content. The highest anthocyanin content was achieved in hydro-priming under -2.0 Mpa (10.70 mg/g FW). The lowest anthocyanin content was related to the bio-priming under non-stress conditions (6.77 mg/g FW) (Table 3).

Correlation coefficients

The results of simple correlation analysis (Pearson) between morpho-physiological traits are presented in Table 4, which shows positive and

Discussion

Drought stress is one of the most critical environmental stresses in arid and semi-arid regions that affect the quantitative and qualitative traits of plants and limits the growth of plants in these areas. Researchers have found that plant growth is significantly reduced under water stress (Afshari et al., 2020; Safahani and Noora, 2020).

Research has shown that drought stress affects quinoa seedlings' morphological, physiological,

and biochemical traits (Ali et al., 2019). Inoculation of quinoa seeds with growth-promoting bacteria increases characteristics such as seedling dry weight, protein content, total phenol content, and SOD activity. Maximum shoot fresh and dry weights were obtained in inoculation of lemongrass and Licorice (*Glycyrrhiza glabra*) seeds with growth-promoting bacteria (Ghabooli et al., 2020; Mirzaei et al., 2020).

The results of the present study are also consistent with the results of Ahmadniaye Motlagh et al. (2019), who reported that inoculation of growth-promoting bacteria increased shoot and root dry weights and total dry weight compared with non-bacterial inoculation. Based on the previous research, it can be stated that *Pseudomonas* species is one of the stimulants of plant growth that has been effective in accelerating and increasing growth, root formation, root development, and expansion (Vurukonda et al., 2018; Rostami and Azhdarpoor, 2019).

The dry weight of shoots and roots initially decreased under stress, and with increasing drought stress, these traits showed an increasing trend. Growth-promoting bacteria by producing indole acetic acid, gibberellin, and some other substances, increase root length, root uptake level, number of capillary roots, increasing nutrient uptake and ultimately improving plant growth under stress (Rezazadeh et al., 2019; Mirzaei et al., 2020).

In plants, the osmotic potential depends on the number of molecules of soluble matter, and osmotic regulation is controlled by the conversion of insoluble polysaccharides such as starch and fructan to soluble sugars such as oligosaccharides, sucrose, and glucose (Choct et al., 2010; Harding et al., 2017).

Photosynthesis and plant growth are affected by drought stress, but plant growth is more affected by drought stress and increases with the stop of photosynthetic production (Maali-Amiri et al., 2007; Chen et al., 2011). During drought stress, the osmotic potential of the turgor pressure is maintained to keep photosynthesis active and to continue growing by increasing the concentration of soluble solutes in the cell. Carbohydrates and proline are the most important of these compounds, among which proline is used as an indicator of stress resistance. Free proline in many plants accumulates in response to dehydration stresses such as drought and salinity (Afshari et al., 2020; Fathi et al., 2020). Increased proline content by 3-300 times has been reported in different cultivars and treatments (Kazerani et al., 2019; Afshari et al., 2020). Accumulation of proline and some proteins in leaves to maintain the turgor pressure of plant cells is part of the mechanisms of resistance to moisture stress (Salehi-Lisar and Bakhshayeshan-Agdam, 2016). Increasing the drought level increased the activity of antioxidant enzymes and proline and sugar contents compared with the control (distilled water). Studies showed that with the increase in drought stress levels, the amount of proline and total sugar in the leaves increased (Algosaibi et al., 2017; Trivedi et al., 2020). Proline accumulation was also observed in the stressed tissues of *Tanacetum parthenium* (Esmaili et al., 2020), which is in line with the findings of the present study.

The increase in the antioxidant enzyme activity in the studied quinoa seedlings was to reduce the severity of damage to biomolecules and oxidative stress. Previous studies showed that the amount of osmotic regulators such as proline increases with any factor that reduces the water potential. Accumulation of proline under drought conditions has several biological effects. The soil solution's aqueous potential decreases and free proline production increases to eleven micromoles per day per gram of fresh weight underwater fraction, which increases the osmotic pressure of the cell (Amiri et al., 2018).

Reducing the rate of pure photosynthesis under drought stress conditions damages plant biochemical processes and non-foaming agents, including changes in protein structure. The decrease in protein content under drought stress is due to the reaction of protein with free radicals, differences in amino acids, increased protein degradation activity, decreased protein synthesis, and accumulation of free amino acids (Dubey et al., 2019). Plants have enzymatic and non-enzymatic antioxidant mechanisms to deal with oxidative stress caused by reactive oxygen species. Superoxide dismutase radically converts

superoxide to hydrogen peroxide and is converted to water by ascorbate peroxidase in chloroplasts (Omidi, 2010).

Drought stress was reported to reduce protein content and increase peroxidase and superoxide dismutase enzymes in Balango (Ahmadi and Omidi, 2017). It has been reported that with increasing drought levels, the activity of antioxidant enzymes ascorbate peroxidase, superoxide dismutase, and guaiacol peroxidase in plant leaf tissue increased significantly (Bahrampoor et al., 2019).

In this study, it was also shown that the increase in drought stress levels, antioxidant enzyme activity, and protein content in the leaf tissue of quinoa seedlings increased and decreased, respectively. In different plants such as durum wheat (Naeemi et al., 2018) and corn (Anjum et al., 2017), as in this study, with increasing drought stress, the amount of total phenol increased. Moharramnejad et al. (2016) in their analysis of the effect of drought stress on maize lines found that drought stress significantly increases total polyphenols' concentration.

Results of the study indicated that the application of bio-priming with growth-promoting bacteria (*Bacillus subtillis*) increased shoot dry weight, enzyme activity, and polyphenol content. Therefore, to accelerate seedling growth and improve quinoa's quality traits, seeds should be inoculated with *Bacillus subtilis*. Drought stress also showed a decrease in proline and sugar content traits and an increase in biochemical characteristics. The interactions of drought and priming treatments were significant in some characteristics such as root dry weight, proline content, superoxide dismutase activity, and anthocyanin content. These led to the motion of the adverse effects of drought stress. It is recommended to use different levels and treatments of seed priming in future experiments.

References

Afshari, F., Nakhaei, F., Mosavi, S., and Seghatoleslami, M. 2020. 'Evaluating the role of nutri-priming in improving PEG-induced drought stress tolerance of stevia (*Stevia rebuadiana* Bertoni)'. *Iranian Journal of Plant Physiology*, 11,3509-3522.

Aghighi Shahverdi, M., Omidi, H., and Tabatabaei, SJ. 2017. 'Effect of nutri-priming on germination indices and physiological characteristics of stevia seedling under salinity stress'. *Journal of Seed Science*, 39,353-362.

Ahmadi, K., and Omidi, H. 2017. 'Evaluate the effect of drought stress on the quantity and quality of medicinal plant populations Balangu (*Lallemantia royleana* Benth.)'. *Environmental Stresses in Crop Sciences*, 10.

Ahmadniaye Motlagh, H., Safari, O., and Paolucci, M. 2019. 'Effect of different levels of milkweed (*Calotropis persica*) seed powder on the growth parameters, immunity and gut microbiota of Oncorhynchus mykiss'. *Iranian Journal of Veterinary Science and Technology*, 11,43-50.

Algosaibi, AM., Badran, AE., Almadini, AM., and El-Garawany, MM. 2017. 'The effect of irrigation intervals on the growth and yield of quinoa crop and its components'. *Journal of Agricultural Science*, 9.

Ali, OI., Fghire, R., Anaya, F., Benlhabib, O., and Wahbi, S. 2019. 'Physiological and morphological responses of two quinoa cultivars (*Chenopodium quinoa* Willd.) to drought stress'. *Gesunde Pflanzen*, 71,123-133.

Amiri, H., Dousty, B., and Hosseinzedeh, SR. 2018. 'Water stress-induced changes of morphological, physiological and essential oil compounds in *Thymus eriocalyx* from Iran'. *Journal of Essential Oil Bearing Plants*, 21,1210-1223.

Anjum, SA., Ashraf, U., Tanveer, M., Khan, I., Hussain, S., Shahzad, B., Zohaib, A., Abbas, F., Saleem, MF., and Ali, I. 2017. 'Drought induced changes in growth, osmolyte accumulation and antioxidant metabolism of three maize hybrids'. *Frontiers in plant science*, 8.

Arnon, D., and Hoagland, D. 1944. 'The investigation of plant nutrition by artificial culture methods'. *Biological Reviews*, 19,55-67.

Bahrampoor, M., Dehestani-Ardakani, M., Shirmardi, M., and Gholamnezhad, J. 2019. 'Effect of Different Substrates and Nano Potassium Fertilizer on Morpho-Physiological Characteristics of Pot Marigold (*Calendula officinalis* L.) under Drought Stress'. *Iranian Journal of Horticultural Science and Technology*, 20,65-78.

Bakhtiari, M., Mozafari, H., Karimzadeh Asl, K., Sani, B., and Mirza, M. 2020. 'Plant Growth, Physiological, and Biochemical Responses of Medic Savory [*Satureja macrantha* (Makino) Kudô] to Bio-organic and Inorganic Fertilizers'. *Journal of Medicinal plants and By-product*, 9,9-17.

Bates, LS., Waldren, RP., and Teare, I. 1973. 'Rapid determination of free proline for water-stress studies'. *Plant and soil*, 39,205-207.

Bazile, D., Martínez, EA., and Fuentes, F. 2014. 'Diversity of quinoa in a biogeographical Island: A review of constraints and potential from arid to temperate regions of Chile'. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 42,289-298.

Bradford, MM. 1976. 'A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding'. *Analytical biochemistry*, 72,248-254.

Castonguay, Y., Nadeau, P., Lechasseur, P., and Chouinard, L. 1995. 'Differential accumulation of carbohydrates in alfalfa cultivars of contrasting winterhardiness'. *Crop Science*, 35,509-516.

Ceccato, DV., Bertero, HD., and Batlla, D. 2011. 'Environmental control of dormancy in quinoa (*Chenopodium quinoa*) seeds: two potential genetic resources for pre-harvest sprouting tolerance'. *Seed Science Research*, 21,133-141.

Chen, W., Yao, X., Cai, K., and Chen, J. 2011. 'Silicon alleviates drought stress of rice plants by improving plant water status, photosynthesis and mineral nutrient absorption'. *Biological trace element research*, 142,67-76.

Choct, M., Dersjant-Li, Y., McLeish, J., and Peisker, M. 2010. 'Soy oligosaccharides and soluble non-starch polysaccharides: a review of digestion, nutritive and anti-nutritive effects in pigs and poultry'. *Asian-Australasian journal of animal sciences*, 23,1386-1398.

Dubey, AK., Kumar, N., Kumar, A., Ansari, MA., Ranjan, R., Gautam, A., Sahu, N., Pandey, V., Behera, SK., and Mallick, S. 2019. 'Over-expression of CarMT gene modulates the physiological performance and antioxidant defense system to provide tolerance against drought stress in Arabidopsis thaliana L'. *Ecotoxicology and Environmental Safety*, 171,54-65.

Esmaili, S., Tavallali, V., and Amiri, B. 2020. 'Nano-Silicon Complexes Enhance Growth, Yield, Water Relations and Mineral Composition in *Tanacetum parthenium* under Water Deficit Stress'. *Silicon*,1-16.

Etesami, H., and Maheshwari, DK. 2018. 'Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: Action mechanisms and future prospects'. *Ecotoxicology and Environmental Safety*, 156,225-246.

Fathi, A., Oveysi, M., Nasri, M., Tohidi, H., and Kasraei, P. 2020. 'Physiological responses of peppermint (*Mentha piperita* L.) to plant growth regulators and salinity stress'. *Iranian Journal of Plant Physiology*, 11,3499-3508.

Ghabooli, M., Rezaei, E., Movahedi, Z., and Mohsenifard, E. 2020. 'Effect of Piriformospora indica inoculation on some morphophysiological parameters in licorice (*Glycyrrhiza glabra* L.) under drought stress'. *Iranian Journal of Plant Physiology*, 10,3379-3389.

González, JA., Konishi, Y., Bruno, M., Valoy, M., and Prado, FE. 2012. 'Interrelationships among seed yield, total protein and amino acid composition of ten quinoa (*Chenopodium quinoa*) cultivars from two different agroecological regions'. *Journal of the Science of Food and Agriculture*, 92,1222-1229.

Gouda, S., Kerry, RG., Das, G., Paramithiotis, S., Shin, H-S., and Patra, JK. 2018. 'Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture'. *Microbiological Research*, 206,131-140.

Harding, SE., Tombs, MP., Adams, GG., Paulsen, BS., Inngjerdingen, KT., and Barsett, H. 2017. *An introduction to polysaccharide biotechnology* (CRC Press).

Hinojosa, L., González, JA., Barrios-Masias, FH., Fuentes, F., and Murphy, KM. 2018. 'Quinoa abiotic stress responses: A review'. *Plants*, 7,106.

Hussain, M., Farooq, M., and Lee, DJ. 2017. 'Evaluating the role of seed priming in improving drought tolerance of pigmented and non-pigmented rice'. *Journal of Agronomy and Crop Science*, 203,269-276.

Ibrahim, EA. 2016. 'Seed priming to alleviate salinity stress in germinating seeds'. *Journal of plant physiology*, 192,38-46.

Iqbal, R., Raza, MAS., Saleem, MF., Khan, IH., Zaheer, MS., Ahmad, S., Haider, I., Aslam, MU., and Toleikiene, M. 2019. 'Foliar applied iron and zinc improves the growth, physiological and yield related traits of wheat under drought'. *International Journal of Biosciences*, 14,376-387.

Jiang, X., Li, H., and Song, X. 2016. 'Seed priming with melatonin effects on seed germination and seedling growth in maize under salinity stress'. *Pak J Bot*, 48,1345-1352.

Kazerani, B., Navabpour, S., Sabouri, H., Ramezanpour, SS., Zaynali Nezhad, K., and Eskandari, A. 2019. 'Evaluation of proline content and enzymatic defense mechanism in response to drought stress in rice'. *Plant Physiology*, 9,2749-2757.

Lawal, SA. 2018. 'The response of southern African vegetation to droughts in past and future climates', University of Cape Town.

Maali-Amiri, R., Goldenkova-Pavlova, I., Yur'Eva, N., Pchelkin, V., Tsydendambaev, V., Vereshchagin, A., Deryabin, A., Trunova, T., Los, D., and Nosov, A. 2007. 'Lipid fatty acid composition of potato plants transformed with the Δ12-desaturase gene from cyanobacterium'. *Russian Journal of Plant Physiology,* 54,600-606.

Mabhaudhi, T., Chimonyo, VGP., Hlahla, S., Massawe, F., Mayes, S., Nhamo, L., and Modi, AT. 2019. 'Prospects of orphan crops in climate change'. *Planta*,1-14.

Meda, A., Lamien, CE., Romito, M., Millogo, J., and Nacoulma, OG. 2005. 'Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity'. *Food chemistry*, 91,571-577.

Mirzaei, M., Ladan Moghadam, A., Hakimi, L., and Danaee, E. 2020. 'Plant growth promoting rhizobacteria (PGPR) improve plant growth, antioxidant capacity, and essential oil properties of lemongrass (*Cymbopogon citratus*) under water stress'. *Iranian Journal of Plant Physiology*, 10,3155-3166.

Moharramnejad, S., Sofalian, O., Valizadeh, M., Asgari, A., and Shiri, M. 2016. 'Response of Antioxidant Defense System to Osmotic Stress in Maize Seedling'. Fresen. *Environ. Bull*, 25,805-811.

Muthukumarasamy, M., Gupta, SD., and Panneerselvam, R. 2000. 'Enhancement of peroxidase, polyphenol oxidase and superoxide dismutase activities by triadimefon in NaCl stressed Raphanus sativus L'. *Biologia plantarum*, 43,317-320.

Naeemi, T., Fahmideh, L., and Fakheri, BA. 2018. 'The Impact of Drought Stress on Antioxidant Enzymes Activities, Containing of Proline and Carbohydrate in Some Genotypes of Durum Wheat (*Triticum turgidu* L.) at Seedling Stage'. *Journal of Crop Breeding*, 10,22-31.

Omidi, H. 2010. 'Changes of proline content and activity of antioxidative enzymes in two canola genotype under drought stress'. *American Journal of Plant Physiology*, 5,338-349.

Rahimi, A. 2013. 'Seed priming improves the germination performance of cumin (*Cuminum syminum* L.) under temperature and water stress'. *Industrial Crops and Products*, 42,454-460.

Rehman, A., Farooq, M., Naveed, M., Nawaz, A., and Shahzad, B. 2018. 'Seed priming of Zn with endophytic bacteria improves the productivity and grain biofortification of bread wheat'. *European Journal of Agronomy*, 94,98-107.

Rezazadeh, S., ilkaee, M., Aghayari, F., Paknejad, F., and Rezaee, M. 2019. 'The physiological and biochemical responses of directly seeded and transplanted maize (*Zea mays* L.) supplied with plant growth-promoting rhizobacteria (PGPR) under water stress'. *Iranian Journal of Plant Physiology*, 10,3009-3021.

Rostami, S., and Azhdarpoor, A. 2019. 'The application of plant growth regulators to improve phytoremediation of contaminated soils: A review'. *Chemosphere*, 220,818-827.

Safahani, A., and Noora, R. 2020. 'Alleviatory activities of salicylic acid and chitosan in burdock plant (*Arctium lappa* L.) under drought stress'. *Journal of Plant Environmental Physiology*, 14,39-56.

Salehi-Lisar, SY., and Bakhshayeshan-Agdam, H. 2016. 'Drought stress in plants: causes, consequences, and tolerance.' in, *Drought Stress Tolerance in Plants, Vol 1* (Springer).

Tabassum, T., Farooq, M., Ahmad, R., Zohaib, A., and Wahid, A. 2017. 'Seed priming and transgenerational drought memory improves tolerance against salt stress in bread wheat'. Plant *Physiology and Biochemistry*, 118,362-369.

Talebnejad, R., and Sepaskhah, A. 2015. 'Effect of deficit irrigation and different saline groundwater depths on yield and water productivity of quinoa'. *Agricultural Water Management,* 159,225-238.

Talebnejad, R., and Sepaskhah, A. 2016. Quinoa: a new crop for plant diversification under water and salinity stress conditions in Iran. In *International Symposium on the Role of Plant Genetic Resources in Reclaiming Lands and Environment Deteriorated by Human and 1190*, 101-106.

Taşgín, E., Atící, Ö., and Nalbantoğlu, B. 2003. 'Effects of salicylic acid and cold on freezing tolerance in winter wheat leaves'. *Plant Growth Regulation*, 41,231-236.

Trivedi, G., Patel, P., and Saraf, M. 2020. 'Synergistic effect of endophytic selenobacteria on biofortification and growth of *Glycine max* under drought stress'. South *African Journal of Botany*, 134,27-35.

Ulfat, A., Majid, SA., and Hameed, A. 2017. 'Hormonal seed priming improves wheat (*Triticum aestivum* l.) field performance under drought and non-stress conditions'. *Pak. J. Bot*, 49,1239-1253.

Vurukonda, SSKP., Giovanardi, D., and Stefani, E. 2018. 'Plant growth promoting and biocontrol activity of Streptomyces spp. as endophytes'. *International journal of molecular sciences*, 19,952.

Wagner, GJ. 1979. 'Content and vacuole/extravacuole distribution of neutral sugars, free amino acids, and anthocyanin in protoplasts'. *Plant Physiology*, 64,88-93.

Yang, A., Akhtar, SS., Iqbal, S., Amjad, M., Naveed, M., Zahir, ZA., and Jacobsen, S-E. 2016. 'Enhancing salt tolerance in quinoa by halotolerant bacterial inoculation'. *Functional Plant Biology,* 43,632-642.