Foliar application of Iron and Zinc on quinoa under drought stress affects its seed germination and biochemical properties

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Abstract

Recently the use of plant products instead of chemical materials is one of the most important needs of the modern civilization in terms of human health. Drought stress during growth and seed maturation is one of the major environmental constraints, which influences seed grain filling and consequential germination and early growth stages of plants. In this study, the impact of foliar application of micronutrients (control, Zn, Fe, Zn + Fe, nano-Zn, nano-Fe, and nano-Zn + nano-Fe) were evaluated on quinoa at 2 reproductive stages (50% and 100% of flowering stage) under 2 levels of drought stress (50% and 85% of depletion of soil moisture content). Results revealed that germination traits including germination percentage, and seed technological parameters such as germination value and germination energy, antioxidant enzymes activities, and chlorophyll (Chl) content were significantly affected by foliar application of micronutrients, time of micronutrient spraying, and drought stress level. The correlation results showed that 1000- grain weight had a positive and significant relationship with grain filling period, physiological maturity, germination percentage (GP), and germination speed (GS). Drought stress also increased the antioxidant enzymes activities of catalase (CAT) and peroxidase (POD) in all nutrient treatments. Drought stress at seed formation stage had a significant effect on seed filling and its weight and could decrease seed germination rate. It was concluded that application of nano-Zn + nano-Fe in 50% flowering stage could promote the germination performance, seedling growth, and the antioxidant capacity under drought stress conditions.

Keywords: *quinoa*, antioxidant enzymes activity, chlorophyll, micronutrients, seed technological parameter

Pakbaz, N., H. Omidi, H. A. Naghdi Badi, A. Bostani. 2022. 'Foliar application of Iron and Zinc on quinoa under drought stress affects its seed germination and biochemical properties'. *Iranian Journal of Plant Physiology* 12 (2), 4153- 4167.

Introduction

Chenopodium species have been used by humans for centuries and contain high protein and balanced amino acid spectrum with high lysine (5.1-6.4) and methionine (0.4-1.0%). *Chenopodium album* as a leafy vegetable and *Chenopodium quinoa* and *C. album* are of great interest for human consumption or animal feed (Prakash and Pal, 1998; Bhargava et al., 2003). *Chenopodium quinoa* is a native of the Andean region and is nowadays known as a pseudo cereal

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Received: August, 2021
Accepted: November, 2021

(Cusack, 1984; Koziol, 1993; Repo-Carrasco et al., 2003; Jacobsen, 2003).

Food production is decreasing due to the detrimental effects of various biotic and abiotic stresses; therefore, minimizing these losses is a major concern to ensure food security under the changing climate. Drought, being the most important environmental stress, severely impairs plant growth and development and limits the crop performance more than any other environmental factor (Shao et al., 2009). Its negative effects have been addressed on growth, yield, membrane integrity, pigment content, osmotic adjustment water relations, and photosynthetic activity (Benjamin and Nielsen, 2006; Praba et al., 2009).

Drought stress is affected by climatic, edaphic, and agronomic factors. The susceptibility of plants to drought stress varies depending on the degree of stress, different accompanying stress factors, plant species, and their developmental stages (Demirevska et al., 2009). Acclimation of plants to water deficit is the result of different events, which leads to adaptive changes in plant growth and physio-biochemical processes, such as changes in plant structure, growth rate, tissue osmotic potential, and plants' antioxidant defense (Duan et al., 2007). It has become imperative to elucidate the responses and adaptation of crops to water deficit, and take actions to improve the drought resistance ability of crop plants and to ensure higher crop yields against unfavorable environmental stresses.

Nutrient deficiency and diet-related issues are the main cause of death on the earth while this can be prevented by sustainably supplying nutrients and finding solutions to malnutrition (Graham et al., 2007). Human body needs 51 essential nutrients, and a short supply or lack of even one of them can cause metabolic problems resulting in poor health, sickness, and economic and social costs to the community (Graham et al., 2007; Singh et al., 2018; Branca and Ferrari, 2002; Golden, 1991).

Zinc and iron microelements play a salient role in the vital processes of plants such as metabolism, transpiration rate, photosynthesis, dysfunction of relevant enzymes, reducing nutrient absorption, plant wilting, imbalanced water relations, and crop quality and quantity (Afsahi et al., 2020; Prasad, 2004; Dhir et al., 2011; Fageria, 2016). Foliar application of micronutrients on numerous occasions, while eliminating their deficiencies, also enhances the quantitative and qualitative yield of the plant (Whitty and Chambliss, 2005). Besides, the policy of various governments world-wide, including those of the European Union (EC, 2007) had deeply prohibited the overuse of agrochemical inputs, pushing to assume alternative and sustainable strategies. One of most innovative and sustainable strategies to overcome the problem of micronutrient deficiency in alkaline soil is converting the required salts into nano-forms and spraying them as foliar fertilizers on plants or coating the nanofertilizers with nano-materials to control nutrient release (Rashid and Ryan, 2004). Green synthesized nanofertilizers can be defined as nano-nutrients synthesized from plant and/or microorganism materials, which are believed to be an improvement over applying chemical materials that leave hazardous compounds in the environment (Thakur et al., 2018). In the agriculture arena, nanotechnology can provide environmental friendly strategy to remediate water and soils, thus promoting world food production and quality (Prasad et al., 2014; Sekhon, 2014). In this regard, foliar application of nano formulation micronutrient is also considered more proper than the land application, because of its potential to quickly overcome the deficiency, ease of applying, minimization of the toxicity resulting from accumulation of micronutrient, and avoiding the immobilization of trace elements in the soil (Dhir et al., 2011; Elanchezhian, 2017).

Quinoa is resistant to drought and salinity and is a valuable dietary food as is gluten free, and as a medicinal plant, it also contributes to the health of the community. Given the high nutritional value of its grain, World Food Organization compares it to milk powder (Schoenlechner et al., 2010).

Generally, because seeds from different flowers are produced under different environmental conditions and/or at different positions on the mother plant, it is reasonable to expect them to differ in their germination responses. Micronutrient supplementation in quinoa production is of growing interest to producers and

agronomists as a means to further increase yield. Despite many studies carried out on mineral nutrient absorption during grain filling period, many aspects of foliar fertilization on seedling performance are still unknown. This study was an attempt to investigate foliar application, spraying time, and drought stress during seed maturation on germinability, stand establishments, and early seedling growth of quinoa.

Materials and Methods

Field practice, spraying and treatments

This study was conducted in the Research Farm of Shahed University, Iran in 2018 (34° 35'_N, 51° 8'_E, 1050 m ASL). In the experiment, Fe and Zn were supplied from sources of iron (II) sulfate heptahydrate ($FeSO4.7H_2O$, 4 g/L) and zinc sulfate heptahydrate ($ZnSO4.7H_2O$, 3 g/L), respectively. Nanoforms of iron (FeO) and zinc (zink oxide) were also used in the 1 g/L. The treatments were 7 levels of foliar application (control, Zn, Fe, Zn + Fe, nano-Zn, nano-Fe, and nano-Zn + nano-Fe) at 2 time (50% and 100% flowering stage) and 2 levels of drought stress, 50% (control) and 85% (stress) moisture discharge. The foliar application was done at 50% flowering and full flowering stages. These micronutrients were sprayed on the plants in pots by a manual pump sprayer until run-off. Tween 20 (0.01 %, v/v) was added to all spray solutions as the surfactant to improve the foliar uptake. Plants treated with distilled water were utilized as controls. For morphophysiological evaluations, healthy, impurity-free seeds harvested from the mother plant were stored in paper bags in the dark at 4 ℃.

Phenological stages and weight of 1000- grains weight

Table 1
Equations used in the study to calculate germination parameters in the experiment

Parameters	Formula	Reference
Germination Percentage	GP = (N × 100)/M	Liopa-Tsakalidi *et al*., 2012
Germination Speed	GS = Σ Ni / Ti	Mangure, 1962
Germination value	GV = GP × MDG	Czabator, 1962
Germination Energy	GE=(Ni*/N)×100	Mangure, 1962

N: the sum of germinated seeds at the end of the experiment, M: the total number of planted seeds, n: the number of germinated seeds at time D, T: throughout the germination period, Ti: number of days after germination, MDG: mean daily germination, Ni*: seed number at germination peak.

During the growing season, phenological stages such as grain filling period and physiological maturity were recorded according to the Zadoks scale (Zadoks et al., 1974). The 1000-grain weight of quinoa seeds was also measured using a digital scale with an accuracy of one thousandth of a gram.

Procedure and seed germination test

The seeds' germination assay was carried out at the Seed Science and Technology Laboratory of Agricultural College, Shahed University of Tehran, Iran in 2018. The germination test was performed based on a completely randomized design (CRD) with four replications. To conduct the germination, quinoa seeds were sterilized in 70% (v/v) ethanol for 1 min and 20% (v/v) sodium hypochlorite solution for 15 min before they were rinsed three times with sterile distilled water (Hajihashemi and Ehsanpour, 2013). Then, 50 seeds were placed in Petri dishes with 5 ml of distilled water and were wrapped with impermeable Parafilm to avoid the loss of moisture and evaporation. Subsequently, all dishes were transferred to a programmed germination chamber at 23 ± 2 ℃, 16/8 h light/darkness and 75% relative humidity (Liopa-Tsakalidi et al., 2012). The germinated seeds were daily counted. The seeds whose radicles were 2 mm long or more were considered as germinated seeds (ISTA, 2010). Eventually, at the end of the germination period (14 days), germination percentage, germination rate, mean germination time, seedling vigor index, and germination value were calculated based on the equations presented in Table 1. After two weeks of growth, the

seedlings from each replicate were collected, immediately frozen in liquid nitrogen, and stored in an ultra-low freezer at -80 ℃ for subsequent physiological studies.

Chlorophyll and carotenoid contents

Half a gram (0.5 g) fresh leaf tissues were ground in 5 ml of 80% (v/v) acetone with the help of a mortar and pestle. The suspension was centrifuged at 6,000 g for 10 min at 4 ℃. Absorbance of the solution was then measured at 645 and 663 for chlorophylls and at 470 for carotenoid. The chlorophyll and carotenoid contents were determined using the formula given (Arnon, 1967) and the following formula:

Chlorophyll a (mg/g fresh weight) = [19. 3 (A_{663}) - 0. 86 (A_{645})] v/100w

Chlorophyll b (mg/g fresh weight) = [19. 3 (A_{645}) - 3. 6 (A_{663})] v/100w

Total chlorophyll (mg/g fresh weight) = [20. 8 (A_{645}) + 8. 02 (A_{663})] v/100w

Carotenoids = [(100 (A_{470}) - 3. 27 (mg chl a) - 104 (mg chl b)]/227

A = absorption of light at wavelengths of 663, 645, and 470 nm

V = the volume of the upper solution in centrifuges

W = weight of the sample in grams

Antioxidant enzymes assay

To evaluate the activity of antioxidant enzymes, 0.5 g of frozen samples were homogenized in 5 mL of cool extraction buffer, 50 mM potassium phosphate buffer (pH = 7.5), containing 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM dithiothreitol (DTT) and 2% (w/v) polyvinyl pyrrolidine (PVP)). The homogenate was centrifuged at 15,000 × g for 25 min and the obtained supernatant was used as the enzyme source for CAT and POD assays. The whole extraction process was done at ℃.

The CAT activity (EC.1.11.1.6) was assayed according to the method of Chance and Maehly (1955), which is briefly described here. The reaction mixture consisted of 50 mM sodium phosphate buffer (pH = 7.0), 15 mM H_2O_2, and 25 µL of the enzyme extract in a total volume of 3 mL. The absorbance at 240 nm for 1 min at 25 ℃ was recorded spectrophotometrically. One unit of CAT was defined as the amount of enzyme that decomposes 1 µM of H_2O_2 per min.

The POD activity (EC 1.11.1.7) was determined following the method of MacAdam et al. (1992) with some modification. In this method, the enzymatic activity was assayed by adding 50 µL of enzyme extract to the reaction mixture (3 mL), containing 0.1 M potassium phosphate buffer (pH 6.0), 50 µL guaiacol, and 50 µL H_2O2 (3%), and absorption alterations were immediately recorded at 436 nm for 3 min per 15 s by a spectrophotometer. The control contained 3 mL of 0.1 M potassium phosphate buffer, 50 µL guaiacol and 50 µL H2O2.

Statistical Analysis

After checking the data distribution normality (Kolmogorov-Smirnov and Shapiro-Wilk test) assumption, the data were statistically analyzed by the Statistical Analysis System software (SAS Institute, Cary, NC, USA, Version 9.4). The differences among means were separated using the least significant difference test (LSD) at 0.05 statistical probability levels. To determine the correlation coefficients and regression analysis, statistical package for social science software (SPSS, Version 25) was used.

Results

Germination percentage (GP), speed (GS), value (GV), and energy (GE)

Drought stress, nutrient application, and time of foliar spraying had significant effects (P≤0.01) on germination percentage (GP), speed (GS), value, and energy (Table 2). Analysis of the main effects showed that the maximum GP, GS, GV, and GE were related to control condition (no drought stress), spaying at 50% flowering, and treatment with nano-Zn + nano-Fe nutrients (Table 3). As shown in Table 2, the germination energy (GE) was significantly (P≤0.01) affected by the interaction of drought × foliar spraying time, foliar spraying time × nutrients, and drought × foliar spraying time ×

nutrients. Furthermore, the interaction of drought × foliar spraying time had significant effect on all seed germination indices of quinoa.

According to the results (Table 4), the effect of drought stress, foliar application time, and nutrient treatments, as well as the interaction of drought × foliar application time × nutrients on

Table 2
Analysis of variance for the effect of drought stress, micro-nutrients, and foliar spraying time on quinoa seed germination indices

		Mean square			
S.O.V	Df	GP	GS	GV	GE
Drought	1	165.14**	41.29**	590635.89**	0.00**
Foliar spraying time	1	302.29**	75.57**	1072510.37**	0.00**
Nutrients	6	214.62**	53.65**	749692.06**	0.00**
Drought × Foliar spraying time	1	5.14*	1.29*	22402.85*	0.00**
Drought × Nutrients	6	0.14^{ns}	0.04^{ns}	587.71^{ns}	0.00^{ns}
Foliar spraying time × Nutrients	6	1.29^{ns}	0.32^{ns}	4583.80^{ns}	0.00**
Drought × Foliar spraying time × Nutrients	6	0.14^{ns}	0.036^{ns}	570.79^{ns}	0.00**
Experimental error	84	1.05	0.261	3726.00	0.00
Coefficient of variation (%)	-	1.14	1.15	2.29	1.62

ns, *, and **: non-significant, significant at 5%, and 1%, respectively; df: degrees of freedom; GP: Germination Percentage, GS: Germination Speed, GV: Germination Value, GE: Germination Energy

Table 3
Mean comparison of the effects of foliar application of micronutrients at different times on seed germination indices of quinoa under drought stress conditions

Characteristics	GP (%)	GS	GV	GE
		(seed/day)		
Drought stress				
Control	90.57^{a}	45.29^{a}	2739.71^{a}	0.018^{a}
Stress	88.14^{b}	44.07^{b}	2594.47^{b}	0.018^{b}
Foliar spraying time				
50% flowering	91.00^{a}	45.50^{a}	2764.95^{a}	0.018^{a}
100% flowering	87.71^{b}	43.86^{b}	2569.24^{b}	0.017^{b}
Micro-nutrients				
Control	83.75^{e}	41.88^{e}	2339.83^{f}	0.016^{e}
Zn	85.75^{d}	42.88^{d}	2452.83^{e}	0.017^{d}
Fe	87.75c	43.87^{c}	2568.50^{d}	0.017^{c}
Zn + Fe	91.75^{b}	45.88^{b}	2807.83^{b}	0.018^{b}
Nano-Zn	91^{b}	45.50^{b}	2761.33^{c}	0.018^{b}
Nano-Fe	91.75^{b}	45.88^{b}	2807.83^{b}	0.018^{b}
Nano- Zn + Nano- Fe	93.75^{a}	46.88^{a}	2931.50^{a}	0.019^{a}

In each column, means having at least one same letter are not significantly different according to Duncan's multiple range test (p≤0.05). GP: Germination Percent, GS: Germination Speed, GV: Germination Value, GE: Germination Energy

The main effects of the treatments (Table 3) showed that the maximum GE was achieved in without drought stress treatment (0.018), 50% flowering (0.018), and nano-Zn + nano-Fe nutrient (0.019). The interaction of the treatments showed that the highest GE was related to nano-Zn + nano-Fe nutrient at 50% flowering stage without drought stress conditions (Fig. I).

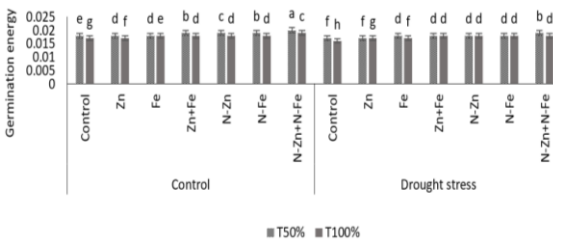

Fig. I. The effect of foliar application of micronutrients at different times on seed germination energy of quinoa under drought stress conditions (LSD 5%= 1.29); Bars represent ± SE.

Chlorophylls and carotenoid contents

chlorophyll a, b, and total chlorophyll and also

Table 4
Analysis of variance for the effect of foliar application of micronutrients at different times on chlorophyll and carotenoids content and antioxidant enzyme activities of quinoa under drought stress conditions

S.O.V	df	Mean square					
		Ch a	Ch b	Ch T	Carotenoids	CAT	POD
Drought	1	192.76**	462.51**	1837.73**	1297583.84**	31.84**	18.01**
Foliar spraying time	1	101.81**	34.18**	71.25**	425393.43**	68.51**	12.98**
Nutrients	6	165.17**	516.33**	1478.66**	2686612.56**	4.90**	2.41**
Drought × Foliar spraying time	1	4.26**	108.34**	0.72^{ns}	124824.11**	8.25**	0.36^{ns}
Drought × Nutrients	6	1.71**	66.58**	13.22^{ns}	43324.92**	2.84*	0.23^{ns}
Foliar spraying time × Nutrients	6	0.74^{ns}	51.92**	55.52**	12567.99*	1.33^{ns}	0.10^{ns}
Drought × Foliar spraying time × Nutrients	6	2.51**	26.87**	37.03**	24553.01**	0.31^{ns}	0.04^{ns}
Experimental error	84	0.48	5.52	6.49	4900.32	1.03	0.51
Coefficient of variation (%)	-	2.43	10.72	5.13	3.00	12.79	8.84

ns, * and, **: non-significant, significant at 5%, and significant at 1%, respectively; df: degrees of freedom; Cha: chlorophyll a, Ch b: chlorophyll b, Ch T: Total chlorophyll, CAT: Catalase, POD: Peroxidase (POD)

Table 5
Mean comparison of the effects of foliar application of micronutrients at different times on chlorophyll and carotenoid contents and also antioxidant enzyme activities of quinoa under drought stress conditions

Characteristics	Ch a	Ch b	Ch T	Carotenoids	CAT	POD
	(mg/g Fw)				min-1 mg-1 Fw	
Drought stress						
Control	29.81^{a}	23.95^{a}	53.75^{a}	2442.96^{a}	1.83^{b}	1.44^{b}
Stress	27.19^{b}	19.89^{b}	45.65^{b}	2227.69^{b}	2.90^{a}	2.24^{a}
Foliar spraying time						
50% flowering	29.46^{a}	22.47^{a}	50.50^{a}	2396.96^{a}	3.15^{a}	2.18^{a}
100% flowering	27.55^{b}	21.37^{b}	48.90^{b}	2273.70^{b}	1.58^{b}	1.50^{b}
Micro-nutrients						
Control	24.02^{e}	16.37^{e}	35.43^{g}	1811.44^{g}	1.60^{d}	1.28^{c}
Zn	24.74^{d}	17.17^{de}	41.90^{f}	1986.22^{f}	1.73^{cd}	1.43^{c}
Fe	26.93^{c}	18.75^{d}	45.66^{e}	2155.86^{e}	2.26^{bcd}	1.66^{bc}
Zn + Fe	30.96^{b}	28.03^{b}	58.97^{b}	2564.68^{b}	3.15^{a}	2.19^{ab}
Nano-Zn	30.45^{b}	18.46^{d}	48.90^{d}	2328.20^{d}	2.46^{abc}	2.01^{ab}
Nano-Fe	30.57^{b}	23.95^{c}	54.51^{c}	2449.06^{c}	2.71^{ab}	2.01^{ab}
Nano- Zn + Nano- Fe	31.84^{a}	30.69^{a}	62.51^{a}	3051.81^{a}	2.66^{ab}	2.29^{a}

In each column, means having at least one same letter are not significantly different according to Duncan's multiple range test (p≤0.05). Chlorophyll a (Ch a), chlorophyll b (Ch b), Total chlorophyll (Ch T), Catalase (CAT), Peroxidase (POD)

carotenoid contents were significant (p≤0.01). As shown in Table 5, the maximum content of chlorophyll a (29.81), b (23.95), total (53.75) and carotenoids (2442.96) were related to no drought stress, 50% flowering, and treatment with nano-Zn + nano-Fe spraying. Also interaction of the treatments (Table 6) showed the highest chlorophyll a, b, total chlorophyll and carotenoid contents were obtained in N-Zn + N-Fe nutrient treatment at 50% flowering stage without drought stress. Also, the lowest chlorophyll a, b, total chlorophyll, and carotenoid contents were observed in nutrient control treatment at 100% flowering stage under drought stress condition (Table 6).

Antioxidant enzymes activities

The effects of drought stress, foliar application time, and nutrient treatments were significant (p≤0.1) on the activity of CAT and POD enzymes (Table 4). The main effects showed that the highest activity of CAT and POD were observed in drought stress condition, at 50% flowering stage, and application of Zn + Fe and nano-Zn + nano-Fe (Table 5).

Phenological stages

The effect of drought stress, foliar spraying time, nutrient treatments, and their interactions on the physiological maturity and grain filling period were significant (p≤0.01) as shown in Table 7. The highest period of physiological maturity (107.1 day) and grain filling (52.11 day) were related to

Table 6
Effect of foliar application of micronutrients at different times on chlorophyll and carotenoid contents and also antioxidant enzyme activities of quinoa under drought stress conditions

Characteristics			Ch a	Ch b	Ch T	Carotenoids
Drought stress	Foliar spraying time	Nutrients	(mg/g.fw)			
Control	Flowering 50%	Control	26.1^{g}	13.90^{k}	40.08^{qrs}	1972.99^{mn}
		Zn	27.6^{f}	16.31^{ijk}	43.97^{nopq}	2112.05^{jkl}
		Fe	29.3^{de}	16.92^{ijk}	46.21^{mno}	2281.44^{hi}
		Zn + Fe	32.83^{ab}	33.91^{ab}	66.72^{b}	2797.10^{d}
		N-Zn	33.62^{a}	18.94^{ij}	52.55^{ghij}	2671.39^{ef}
		N- Fe	33.41^{a}	27.45^{def}	60.84^{cd}	2748.18^{de}
		N- Zn + N-Fe	33.72^{a}	37.20^{a}	70.90^{a}	3182.69^{a}
	Flowering 100%	Control	23.49^{hi}	14.68^{k}	38.16^{rst}	1921.47^{n}
		Zn	24.50^{h}	24.16^{fg}	48.65^{jklm}	2053.82^{klm}
		Fe	26.85^{fg}	24.83^{efg}	51.67^{hijk}	2190.82^{i}
		Zn + Fe	31.66^{c}	29.41^{cd}	61.05^{cd}	2598.00^{f}
		N-Zn	30.33^{d}	20.04^{hi}	50.36^{ijkl}	2301.64^{h}
		N-Fe	31.47^{c}	25.87^{defg}	57.32^{def}	2435.25^{g}
		N- Zn + N-Fe	32.67^{bc}	31.67^{bc}	64.02^{bc}	2934.65^{c}
Drought Stress	Flowering 50%	Control	24.02^{hi}	23.25^{gh}	27.43^{u}	1750.80^{o}
		Zn	23.78^{hi}	14.24^{k}	38.01^{rst}	1910.71^{n}
		Fe	27.10^{fg}	16.52^{ijk}	43.6^{opq}	2108.82^{jkl}
		Zn + Fe	30.38^{d}	25.83^{defg}	56.19^{efg}	2428.83^{g}
		N-Zn	28.87^{e}	19.05^{ij}	47.90^{klmn}	2181.88^{ij}
		N-Fe	29.92^{de}	23.04^{gh}	52.95^{ghi}	2336.62^{gh}
		N- Zn + N-Fe	31.58^{c}	28.02^{de}	59.58^{de}	3073.90^{b}
	Flowering 100%	Control	22.39^{j}	13.66^{k}	36.04^{t}	1600.52^{p}
		Zn	23.02^{ij}	13.97^{k}	36.99^{st}	1868.31^{n}
		Fe	24.46^{h}	16.73^{ijk}	41.18^{pqr}	2042.35^{lm}
		Zn + Fe	28.95^{e}	22.96^{gh}	51.90^{hij}	2434.80^{g}
		N-Zn	29.00^{e}	15.82jk	44.81^{mnop}	2157.91^{jk}
		N-Fe	27.48^{f}	19.45^{ij}	46.92^{lmno}	2276.22^{hi}
		N- Zn + N-Fe	29.71^{de}	25.86^{defg}	55.55^{fgh}	3016.01^{bc}

In each column, means having at least one same letter, are not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

Table 7
Analysis of variance for the effect of drought stress, foliar spraying time, nutrient treatments, and their interactions on the physiological maturity, grain filling period, and weight of 1000- grains weight of quinoa

S.O.V	Df	Mean square		
		Physiological maturity (day)	Grain filling period (day)	1000- grain weight (g)
Replication	2	1.75**	1.75^{**}	0.01^{**}
Drought	1	5376.00**	5376.00^{**}	13.05^{**}
Replication × Drought	2	0.00	0.00	0.00
Foliar time	1	1647.73^{**}	1647.43^{**}	0.84^{**}
Nutrients	6	76.96^{**}	76.96^{**}	2.74^{**}
Drought × Foliar time	1	15.43^{**}	15.43^{**}	0.00^{ns}
Drought × Nutrients	6	0.25^{**}	0.25^{**}	0.08^{**}
Foliar time × Nutrients	6	1.18^{**}	1.18^{**}	0.00^{**}
Drought × Foliar time × Nutrients	6	1.18^{**}	1.18^{**}	0.00^{**}
Coefficient of variation (%)	-	10.01	10.00	10.32

ns, * and **: non-significant, significant at 5% and 1%, respectively. df: degrees of freedom

control treatment (without drought stress), 50% flowering stage, and nano-Zn + nano-Fe treatment (Table 8). The interaction effects of treatment showed that the highest physiological maturity and grain filling period were recorded in nano-Zn + nano-Fe nutrients at 50% flowering stage, and under control treatment (without drought stress) while the lowest maturity was observed in

nutrient control group at 100% flowering stage under drought stress conditions (Table 9).

stage, and nano-Fe + nano-Zn nutrient application (Table 8). The infraction effect of treatments

Table 8
Mean comparison of the effects of drought stress, micronutrients, and foliar spraying time on quinoa characteristics

Characteristics	Physiological maturity (day)	Grain filling period (day)	1000- grain weight (g)
Drought stress			
Control	107.1[a]	52.11[a]	3.10[a]
Stress	91.1[b]	36.11[b]	2.32[b]
Foliar time			
Flowering 50%	103.5[a]	48.54[a]	2.81[a]
Flowering100%	94.7[b]	39.68[b]	2.61[b]
Nutrients			
Control	95.0[g]	40.00[g]	1.77[g]
Zn	97.3[f]	42.25[f]	2.52[f]
Fe	98.3[e]	43.25[e]	2.66[e]
Zn + Fe	101.3[b]	46.25[b]	3.02[b]
N- Zn	99.3[d]	44.25[d]	2.81[d]
N- Fe	100.3[c]	45.25[c]	2.92[c]
N- Zn +N- Fe	102.5[a]	47.50[a]	3.27[a]

In each column, means having at least one same letter are not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

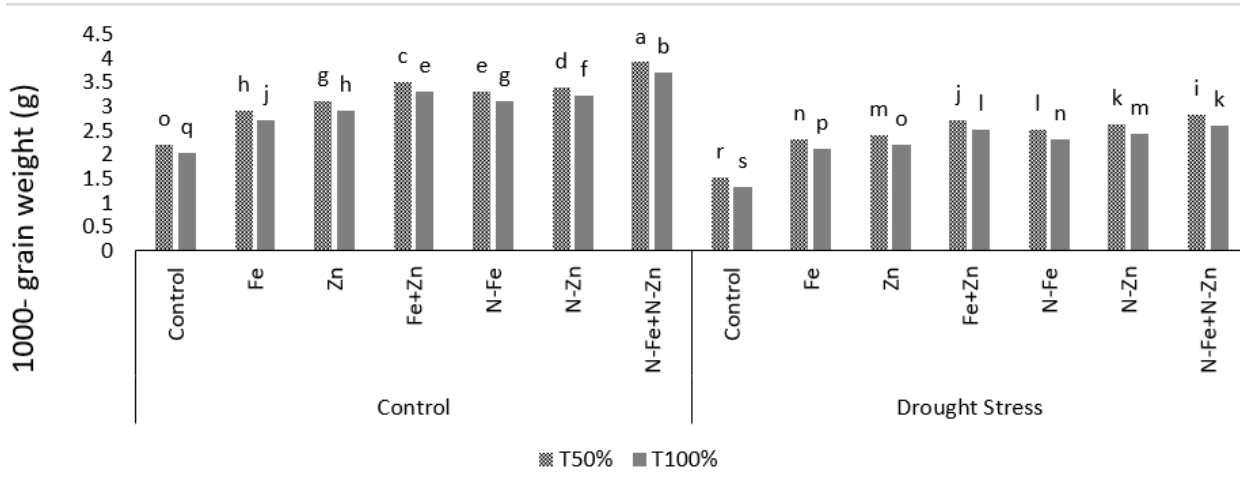

Fig. II. The 1000- grain weight in micronutrients and foliar application time under drought stress conditions of quinoa (LSD 5%= 1.29); bars represents ± SE.

1000- grain weight

Drought stress, nutrient spraying, and spraying time had significant effects ($p \leq 0.01$) on 1000-grain weight (Table 7). As shown in Table 7, the interaction effects of drought stress × spraying time, drought stress × nutrient spraying, and spraying time × nutrient spraying, and drought stress × nutrients spraying × spraying time had significant effects on 1000- grain weight trait ($p \leq 0.01$). Maximum 1000- grain weight was observed in no drought stress (control), 50% flowering indicated that the highest 1000- grain weight was related to nano-Zn + nano-Fe nutrient application at spraying time of 50% flowering stage under no drought stress conditions. Also, the lowest 1000-grain weight was observed in no nutrient application at 100% flowering stage under drought stress conditions (Fig. II).

Correlation

Correlation results showed that 1000-grain weight had a positive and significant relationship with grain filling period, physiological maturity period,

Table 9
Effect of different micronutrients and foliar time under drought stress conditions of quinoa on physiological maturity and grain filling period

Characteristics			Physiological maturity	Grain filling period
Drought stress	Foliar time	Nutrients	(day)	
Control	Flowering 50%	Control	106.3^{g}	51.25^{g}
		Zn	110.3^{f}	55.25^{f}
		Fe	111.3^{e}	56.25^{e}
		Zn + Fe	114.3^{b}	59.25^{b}
		N- Zn	112.3^{d}	57.25^{d}
		N- Fe	113.3^{c}	58.25^{c}
		N- Zn + N- Fe	116.3^{a}	61.25^{a}
	Flowering 100%	Control	99.3^{n}	44.25^{n}
		Zn	100.3^{m}	45.25^{m}
		Fe	101.3^{l}	46.25^{l}
		Zn + Fe	104.3^{t}	49.25^{t}
		N- Zn	102.3^{k}	47.25^{k}
		N- Fe	103.3^{j}	48.25^{j}
		N- Zn + N- Fe	105.3^{h}	50.25^{h}
Stress	Flowering 50%	Control	91.3^{u}	36.25^{u}
		Zn	93.3^{t}	38.25^{t}
		Fe	94.3^{s}	.39.25^{s}
		Zn + Fe	97.3^{p}	42.25 ^{p}
		N- Zn	95.3^{r}	40.25^{r}
		N- Fe	96.3^{q}	41.25^{q}
		N- Zn + N- Fe	98.3^{o}	43.25^{o}
	Flowering 100%	Control	83.3^{b}	28.25^{b}
		Zn	85.3^{a}	30.25^{a}
		Fe	86.3^{z}	31.25^{z}
		Zn + Fe	89.3^{w}	34.25^{w}
		N- Zn	87.3^{y}	32.25^{y}
		N- Fe	88.3^{x}	33.25^{x}
		N- Zn + N- Fe	90.3^{v}	35.25^{v}

In each column, means having at least one same letter are not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

Table 10
Correlations between 1000-grain weight grain filling period, physiological maturity period, CAT, POD, GP, and GS of quinoa plants under study

	1000-grain weight (g)	Grain filling period (day)	Physiological maturity (day)	CAT (mg/g.Fw)	POD (mg/g.Fw)	GP (%)	GS (seed/day)
1000-grain weight (g)	1						
Grain filling period (day)	0.80**	1					
Physiological maturity (day)	0.80**	1.00**	1				
CAT	-0.12^{ns}	-0.31**	-0.31**	1			
POD	-0.19	-0.50**	-0.50**	0.82**	1		
GP (%)	0.84**	0.65**	0.65**	0.22*	0.20	1	
GS	0.84**	0.65**	0.65**	0.22*	0.20	1.00**	1

** and *: significant correlations at 0.01 and 0.05; ^{ns}: no significant correlation

GP, and GS. Also, grain filling and physiological maturity period had a positive and significant relationship with GP and GS while having a negative and significant relationship with CAT and POD (Table 10).

Discussion

The purpose of this study was to investigate the effect of drought stress on some seed germination and physiological characteristics of quinoa seeds produced from their mother plant under drought stress as well as their seedlings. In recent years, drought stress is known as an important environmental factor which seriously impacts the productivity of many crops and can retard their growth and development (Lipiec et al., 2013). The results of our study showed that water restriction imposed by drought stress on mother plant had significant effects on seed germination rate. Also, the seed germination indices of quinoa were affected by the deleterious effects of drought, so that GP, GS, GV, and GE significantly decreased under drought stress (Table 3).

The poor and erratic germination might be attributed to the lower water uptake by seeds and the elevation of ROS levels under water deficiency (Kaya et al., 2006); this leads to impaired transfer of nutrients to the seeds and consequently less food storage in them. It has been reported that under stress conditions, the alteration of some enzymes and hormones found in the seed could lead to the reduction in final germination (Botía et al., 1998). These results are consistent with the findings presented in previous research in other plants (Patane et al., 2013; Zaher- Ara et al., 2016).

In our study, total Chl content significantly diminished under drought stress as compared to the control conditions or without drought stress. The reduction of Chl content has been considered as a usual symptom of oxidative stress due to water stress (Fathi and Barari, 2016) and could be due to damage to the chloroplast membrane (Madany and Khalil, 2017). It has already been reported that drought stress reduced chlorophyll content, whereas nutrients treatments minimized the deleterious effects of stress conditions (Hussain et al., 2017; Madany and Khalil, 2017). Zn is an essential element for Chl synthesis, pollen function, and germination (Cakmak, 2008). It has been reported that Zn element by the protection of the sulfhydryl group keeps the Chl content (Latef et al., 2017). Fe is an essential element required for the maintenance of chloroplast structure and is involved in Chl synthesis (Rout and Sahoo, 2015; Naghdi-Badi, 2018). The limitation of this element has a remarkable effect on the productivity of photosynthetic organisms (Tewari et al., 2013).

This study showed that due to water restriction in the quinoa mother plant, the activity of antioxidant enzymes such as CAT and POD in quinoa seedlings increased. CAT and POD are most important antioxidant enzymes that protect plants against cell oxidative damages caused by drought and other environmental stresses (Shams Peykani and Farzami Sepehr, 2018). Plants have enzymatic and nonenzymatic antioxidant mechanisms to deal with oxidative stress caused by reactive oxygen species. Superoxide dismutase radically converts superoxide to hydrogen peroxide and is converted into water by ascorbate peroxidase in chloroplasts (Omidi, 2010; Dashab and Omidi, 2021).

Increased enzymatic activity, as seen in our study, has been considered as a part of seed strategy to scavange free radicals (Chiu et al., 2005). In this study, nano-Zn + nano-Fe had a positive effect on increasing the activity of these enzymes (Table 5). Also, ROS production has been reported under both normal and stress conditions, and the increase in ROS production in response to drought stress is due to oxidative stress (Sharma et al., 2012). Plants usually have several defensive mechanisms to overcome the oxidative stress (Lipiec et al., 2013), which include enzymatic and non-enzymatic antioxidants as well as reparation systems that orchestrate stress signaling and block the adverse effects of ROS (Demidchik, 2015). CAT and POD are described to be two of the most important antioxidant enzymes that protect plants against oxidative damages in cell caused by drought and other environmental stresses (Huang et al., 2016). These enzymes play an important role in scavenging H_2O_2 (Hussain et al., 2016).

Grain filling period is a determining factor in grain yield and performance. Increasing the duration of this period makes it possible to transfer more assimilates from the source (photosynthetic organ) to the sink (grain) and thus increase grain yield. Asseng et al., (2003) stated that the relative contribution of remobilization to the yield in grains under different environmental conditions

depends largely on source/sink interactions during grain filling. In favorable environmental conditions where photosynthesis is sufficient and as a result, access to resources is enough, there is a necessary coordination between the source and the sink and the source material is stored in the sink in an appropriate amount. But in conditions of environmental stress, lack of access to nutrients may upset the balance of the source and the sink, and in such cases, the sink capacity is greater than that of the source (Seyed-Sharifi and Haydari Siahkhalaki, 2016). Also, regarding the effect of foliar application of Fe on grain yield, it is noted that Fe in the photosynthesis process plays an important role in the oxidation and reduction processes due to the presence of proteins such as cytochromes and ferredoxins (grain., 2020). In our study, drought stress and spraying micronutrients on mother plant were effective in verifying differential responses for attributes related to germination. Nanomaterial recently has been used widely in various scientific areas as medicinal, pharmaceutical, physical, and agricultural science (Duhan et al., 2017). These materials have been employed in several agriculture practices due to their efficiency in plant protection and nutrition (Iavicoli et al., 2017). Zinc plays a key role in many physiological and biochemical processes in plant cells, including protein synthesis, membrane function, and the expression of plant genes. This metal component acts as a regulatory element for many enzymes, and plays a major role in plant defense systems against stress conditions (Cakmak, 2000; Reis et al., 2018), For example, Aboutalebian and Nazari (2017) mentioned that application of ZnSO4 increased the activity of SOD, POD, and CAT enzymes in canola under chilling stress. Fe also is well known as an important co-factor for many antioxidant enzymes such as CAT and POD (Kusvuran et al., 2016). Ruiz et al. (2000) reported that the activities of the CAT and POD enzymes were correlated with the Fe content of leaves. In the present study, drought stress during the grain filling stage not only reduced the germination percentage, but also slowed down the speed of the process. According to Table 10, restrictions on water access due to drought stress have reduced grain filling time (Seyed-Sharifi, 2018) and certainly have a significant and often reducing effect on most morphological and physiological traits (Amiri et al., 2013; Abdoli and Saeedi, 2013). The damage caused by drought stress during the reproductive stage is evident in the form of reducing the number of seeds and 1000-seed weight (Rahman et al., 2009). Photosynthesis and plant growth are affected by drought stress, but lant growth is even more affected by drought stress and increases with the stop of photosynthetic production (Maali-Amiri et al., 2007; Chen et al., 2011).

Conclusions

In general, this study indicated that drought stress condition at grain filling period had inhibitory effects on all germination indices of the produced quinoa seeds. Foliar application of zinc and iron on the mother plant under drought stress conditions significantly increased germination indices, chlorophyll content, and antioxidant activities of the seedlings. Foliar application of zinc and iron at 50% flowering stage of the mother plant improved the drought tolerance of quinoa seedlings by increasing the activity of CAT and POD enzymes. Overall, the results showed that foliar application of zinc and iron at 50% flowering stage on quinoa plants could improve drought tolerance of seedlings. However, confirmatory trials under field conditions are required over several years to ensure that the studied treatments improve seedling emergence and vegetative growth of quinoa as well as its resistance to drought stress in the field.

Acknowledgements

The authors are thankful to colleagues in the Central Laboratory of the Shahed University, for their help with field management and chemical analysis.

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