Effect of nutri-priming on germination indices and photosynthetic pigments of quinoa (*Chenopodium quinoa*) seedling under drought stress

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Abstract

Recently the use of plant products instead of chemical is one of the most important needs of the modern civilization in terms of human safety. On the other, drought is one of the abiotic environmental stresses that reduce the performance of the plant products. The objective in this study was to assess the effect of nutri-priming with (control, Fe, Zn, Fe + Zn, nano- Fe, nano- Zn, and nano- Fe + nano- Zn) on quinoa seed germination indices and physiological characteristics in PEG 6000 (polyethylene glycol), drought condition (0, -3, -6, -9 and -12 atm). Results indicated that the effect of drought in nutri-priming were significant on germination percentage, germination rate, mean of germination time, germination uniformity, seedling length, seedling length index, seedling weight, seedling weight index and photosynthetic pigments. Drought stress decreased the percentage and rate of germination and seedling indexes, however, nutri-priming reduces the adverse effects of drought stress conditions. Drough stress also reduced the level of photosynthetic pigments, in spite of all that this decrease was very low in terms of nutri-priming with Fe, Zn, Fe + Zn, nano- Fe, nano- Zn, and nano- Fe + nano- Zn) in high drought level (-12 atm). drought stress increased, mean of germination time, germination uniformity, that nutri-priming especially with the control more increased. The finding of this study leads to the conclusion that nutri-priming with nutrients, especially nano- Fe + nano- Zn increased the germination characteristics of the plant to improve germination and seedlings growth of quinoa under stress conditions.

Keywords: nutri-priming, drought stress, quinoa, photosynthetic pigments, germination indices.

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Introduction

Medicinal plants are a precious heritage and of global importance, which have provided great wealth to the world. During the past decades, there has been a wide expansion in the range of herbal treatments, which has led to a rapid growth in the demand for herbal medicines and course, medicinal plants in the world (Omidi et al., 2015). Quinoa (*Chenopodium quinoa* Willd) is a dicotyledonous plant, allotetraploid (2n=4x=36), from the Amaranthaceae family, C_3 and optional halophyte (Yasui et al., 2016). Quinoa with about 95% self-fertilization originates from the Andes

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mountains on the west coast of Latin America (South). It is one of the plants under the spinach and sugar beet family, and despite its high nutritional value, it can be cultivated well in conditions of low or limited fertility and produces a suitable crop (Chitambo, 2019).

Quinoa seed contains 14-20% protein and is rich in essential amino acids such as lysine and methionine, which are found in very small amounts in most cereal plants (Ferreira et al., 2015). The very high nutritional value of quinoa seed has caused it to be compared to powdered milk in the World Food Organization (FAO), and it has been called multivitamin or herbal caviar (Vahidi et al., 2021). The absence of gluten in this grain has made it possible for celiac patients and those who are allergic to wheat gluten to consume it, because the rate of hydrolysis of quinoa proteins by bacterial lactic acid is higher than the hydrolysis of wheat protein (Caeiro et al., 2022).

Plants are constantly exposed to various biotic and abiotic stresses. Among these stresses, drought stress is one of the most important factors affecting the growth and fertility of plants, and as the most important abiotic stress, it plays an important role in reducing the yield of medicinal plants (Seleiman et al., 2021).

Seed priming is one of the methods used to develop drought tolerance in plants (Seleiman et al., 2021). Seed priming is a technique by which seeds are physiologically and biochemically ready for germination before being placed in their substrate and facing the ecological conditions of the environment. This can cause many biological and physiological manifestations in primed seeds and the resulting plant. So that these cases can be observed in the way of germination, early plant establishment, utilization of environmental inputs, early maturity, quantitative and qualitative increase of the crop (Tabassum et al., 2018).

In seed priming with micronutrient elements (Nutripriming), micronutrient elements are used as an osmotic material (Bhatia and Gupta, 2022). Primed seeds usually have better and more uniform germination (Waqas et al., 2019)due to less drinking time (Bourioug et al., 2020)and the production of metabolites that increase germination ((Pawar and Laware, 2018).

The positive effect of iron and zinc micronutrients on dry matter performance may be due to increasing auxin biosynthesis, increasing chlorophyll concentration, increasing the activity of phosphoenol pyruvate carboxylase (PEPC) and ribulose biphosphate carboxylase/oxygenase (Rubisco), reducing sodium ion accumulation in plant tissues and increasing the efficiency of nitrogen and phosphorus absorption in the presence of It is the elements of micronutrients. Micronutrients are vital for human health and plant growth(Mbarki et al., 2018).

Iran has a variety of climates, and the cultivation of quinoa in terms of production, especially in the southern regions, will create diversity in crops, sustainable production and food security (Fathi and Kardoni, 2020).

Due to lack of micronutrients in soils of Iran and on the other hand the importance of nutrients, especially Fe and Zn salient role in the vital processes and metabolism of plants under drought stress condition, the objective in this study was to evaluate nutrient seed priming with Fe, and Zn (nano and non-nano forms) on germination indices and biochemistry characteristic under drought stress in herb quinoa.

Materials and Methods

Seed collection

Freshly matured seeds (Giza1 Cultivar) of quinoa (Chenopodium quinoa) were obtained in April 2018 from Karaj Seed and Plant Breeding Research Institute.

Nutri-priming and drought stress treatments

To assess the effect of seed priming with Fe (source was $FeSO_4.7H_2O$, 4 g/L), Zn ($ZnSO_4.7H_2O$, 3 g/L), Nanoforms of iron (FeO oxide) and zinc (ZnO oxide) were also used in the 1 g/L. on germination indices and photosynthetic pigments of seedling

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 Rate	GR = Σ Ni / Ti	(Mangure, 1962)
Mean of Germination Time	MGT= (Σ Ni. Ti) / Σ Ti	(Matthews and Khajeh-Hosseini, 2007)
Germination Uniformity	GU= D90- D10	(Soltani et al., 2001)
Seedling Length vigour Index	SLVI=GP×Mean (SL)	(Abdul-Baki and Anderson. 1973)
Seedling weight vigour index	SWVI= GP×Mean (SDW)	(Abdul-Baki and Anderson. 1973)

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 time germination, Ni*: seed number at germination peak, SL= Seedling Length, SDW= Seedling Dry Weight,

under drought stress, was conducted a factorial experiment based on completely randomized design (CRD) with Four replications at the Laboratory of Seed Science and Technology of Shahed University of Tehran in 2018. Experiment factors were drought (PEG 6000) stress (0, -3, -6, -9 and -12 atm) and nutri-priming in seven combinations, control (without priming), Fe, Zn, Fe + Zn, nano-Fe, nano -Zn, nano-Fe + nano-Zn.

Procedure and seed germination test

The germination test was conducted in February 2017 in the Faculty of Agriculture of Shahid University of Tehran in the Laboratory of Seed Science and Technology. To perform this test, the seeds were first disinfected with ethanol (70%) for one minute and sodium hypochlorite solution (10%) for 3 minutes, and then they were washed 3 times with sterile distilled water (Hajihashemi and Ehsanpour, 2013).

In order to apply priming, the seeds were placed in the desired solutions (Fe, Zn, Fe + Zn, nano- Fe, nano- Zn, nano- Fe + nano- Zn) and after 6 hours, removed from the solution, on filter paper They were dried for 24 hours at room temperature (Farooq et al., 2011). The duration of priming is also based on the review of sources (Ashrafi and Razmjoo, 2010). After drying, 100 treated seeds and control (without prime) and 7 ml of PEG 6000 solutions with concentrations of 0, -3, -6, -9 and -12 atm was added. were wrapped with impermeable Parafilm to avoid the loss of moisture and evaporation, and the standard germination test performed in 4 repetitions at a temperature of 23 ± 1 °C, relative humidity of 75% and a light period of 16 h and 8 h of darkness for 14 days. At the end of the germination period, the characteristics of germination speed, germination percentage, average germination duration and seedling vigour indices were calculated based on the equations presented in Table 1.

Chlorophyll and Carotenoid Contents

0.5 g of fresh plant material was crushed in a mortar using liquid nitrogen. Then, 10 ml of 80% acetone was added to it in the dark and centrifuged for 10 minutes at 4°C with 6000 revolutions per minute (rpm). The upper part of the extract was transferred to a new test tube and covered with foil. Readings were then taken using a spectrophotometer at wavelengths of 663 nm, 645 nm, and 470 nm to measure chlorophyll a, chlorophyll b, and carotenoids, respectively (Arnon, 1967)

$$\text{Chlorophyll a} = (12.25 \times OD_{663.2}\ \text{nm} - 2.79 \times OD_{646.8}\ \text{nm})$$

$$\text{Chlorophyll b} = (21.21 \times OD_{646.8}\ \text{nm} - 5.1 \times OD_{663.2}\ \text{nm})$$

$$\text{Total Chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

$$\text{Carotenoid} = [(1000 \times OD_{470}\ \text{nm} - 1.8 \times \text{Chlorophyll a} - 85.02 \times \text{Chlorophyll b})/198]$$

Statistical Analysis

The data obtained from the experiment were analyzed using software of SAS (Statistical Analysis System software, Version 9.4) and the mean data were compared using the least significant

Table 2
Analysis of variance for the effect of drought stress and nutri-priming on quinoa seed germination indices

S. O. V	Df	(MS)					
		GP (%)	GR (Hourse)	MGT	GU	SLVI	SWVI
Nutri -priming (NP)	6	4722.57**	3.23**	1.02**	0.00**	11593.23**	966.23**
Drought (D)	4	18089/10**	21.27**	4.02**	0.04**	89733.05**	6984.34**
NP× D	24	177.05**	0.18**	0.03**	0.01**	1702.70**	84.66**
Experimental error	105	3.73	0.00	0.00	0.00	154.43	3.25
Coefficient of Variation (%)	-	2.92	1.60	3.33	1.03	23.21	10.28

ns, *, and **: non-significant, significant at 5%, and 1%, respectively; df: degrees of freedom; GP: Germination Percentage, GR: Germination Rate, MGT Mean of Germination Time, GU: Germination Uniformity, SLVI: Seedling Length Vigour Index, SWVI: Seedling weight vigour index

Table 3
Mean comparison of the effects of drought stress and nutri-priming on seed germination indices of quinoa

Treatment	GP (%)	GR (hour)	MGT (hour)	GU	SLVI	SWVI
Nutri -priming						
Control	88.95e	0.95g	1.25a	1.35a	13.77d	5.38e
Fe	93.20d	1.53e	0.84b	1.34b	43.30c	14.88d
Zn	92.55d	1.50f	0.85b	1.34b	41.43c	14.40d
Fe + Zn	97.40b	1.90b	0.66d	1.32d	65.44b	21.29b
Nano-Fe	96.00c	1.80c	0.70c	1.33c	59.70b	19.61c
Nano-Zn	95.60c	1.77d	0.71c	1.33c	59.90b	19.51c
Nano-Fe+Nano-Zn	99.65a	2.24a	0.54e	1.31e	91.28a	27.68a
Drought						
0	99.68a	2.94a	0.36e	1.29e	136.64a	40.11a
-3	83.89b	2.12b	0.53d	1.32d	82.34b	26.09b
-6	70.89c	1.49c	0.73c	1.33c	40.81c	14.91c
-9	47.68d	0.99d	1.07b	1.36b	5.87d	4.37d
-12	33.11e	0.81e	1.28a	1.38a	2.07d	2.21e

In each column, means having at least one same letter are not significantly different

difference test (LSD) at 0.05 statistical probability levels.

Results

Germination percentage (GP) and Rate (GR)

Effect of drought, nutri-priming, and the interaction of these two was significant (P≤0.01) on germination percentage (GP) and rate (GR) (Table 2). Analysis of the main effects showed that the maximum GP and GR were related to drought condition of control (0 bar) and nutri-priming (nano-Zn + nano-Fe) (Table 3). Increasing drought levels caused significantly decreased of GP and GR. The highest GP and GR in nutri-priming (nano-Zn + nano-Fe) in the drougt of cntrol (0 bar) (Fig.I and II).

Mean of germination time (MGT) and germination uniformity (GU)

Effect of drought, nutri-priming, and the interaction of these two was significant (P≤0.01) on mean of germination time (MGT) and germination uniformity (GU) (Table 2). Analysis of the main effects showed that the maximum MGT and GU were related to drought condition of

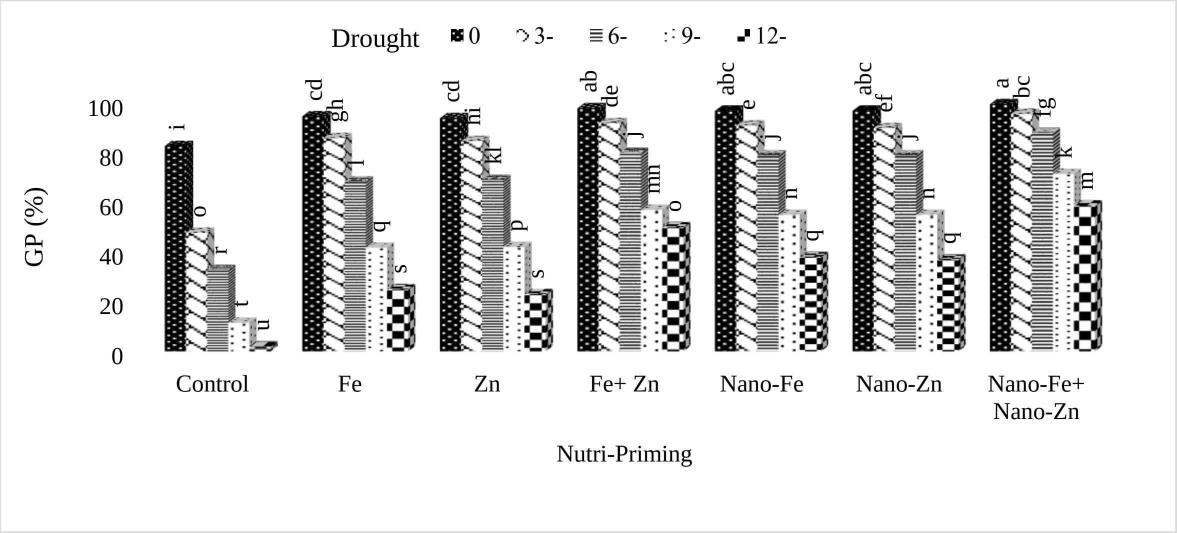

Fig. I The effect of nutria-priming on seed germination Percentage (GP%) of quinoa under drought stress conditions (LSD 5%= 1.29); Bars represent ± SE.

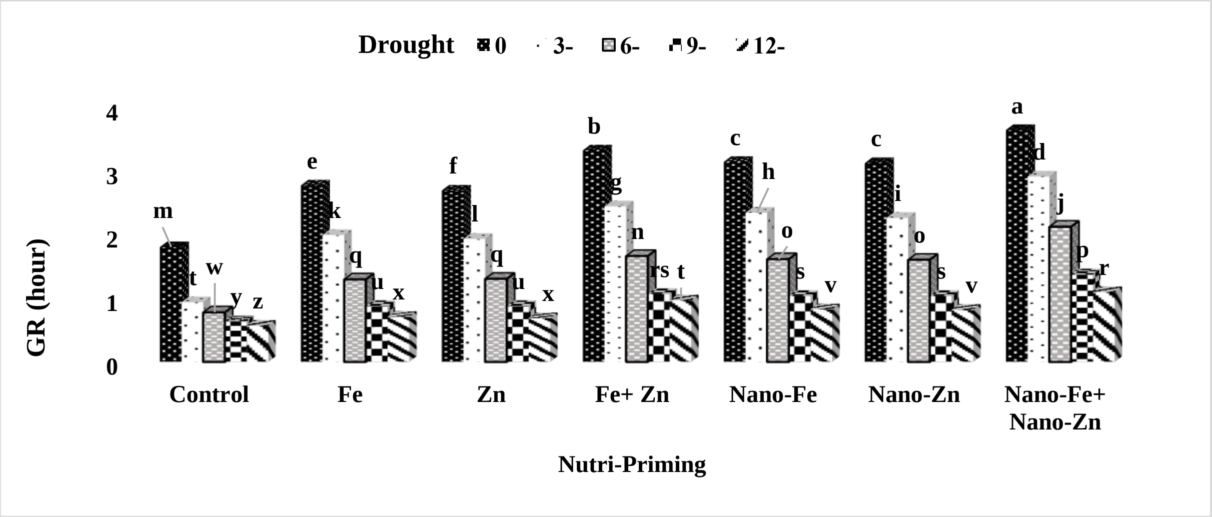

Fig. II. The effect of nutria-priming on seed germination rate (GRh) of quinoa under drought stress conditions (LSD 5%= 1.29); Bars represent ± SE.

control (12 bar) and nutri-priming (control) (Table 3). Increasing drought levels caused significantly increased of MGT and GU. The highest MGT and GU in nutri-priming (control) in the drougt of (12 bar) (Fig. III and IV).

Seedling lengh vigor index (SLVI) and Seedling weight vigor index (SWVI)

Effect of drought, nutri-priming, and the interaction of these two was significant (P≤0.01) on Seedling length vigor index (SLVI) and Seedling weight vigor index (SWVI) (Table 2). Analysis of the main effects showed that the maximum SLVI and SWVI were related to drought condition of control (0 bar) and nutri-priming (nano-Zn + nano-Fe) (Table 3). Increasing drought levels caused significantly decreased of SLVI and SWVI. The highest PLVI and PWVI in nutri-priming (nano-Zn + nano-Fe) in the drougt of control (0 bar) (Fig. V and VI).

Chlorophylls and carotenoid contents

According to the results (Table 4), the effect of drought stress and nutri-priming, as well as the interaction of drought × nutri-priming on

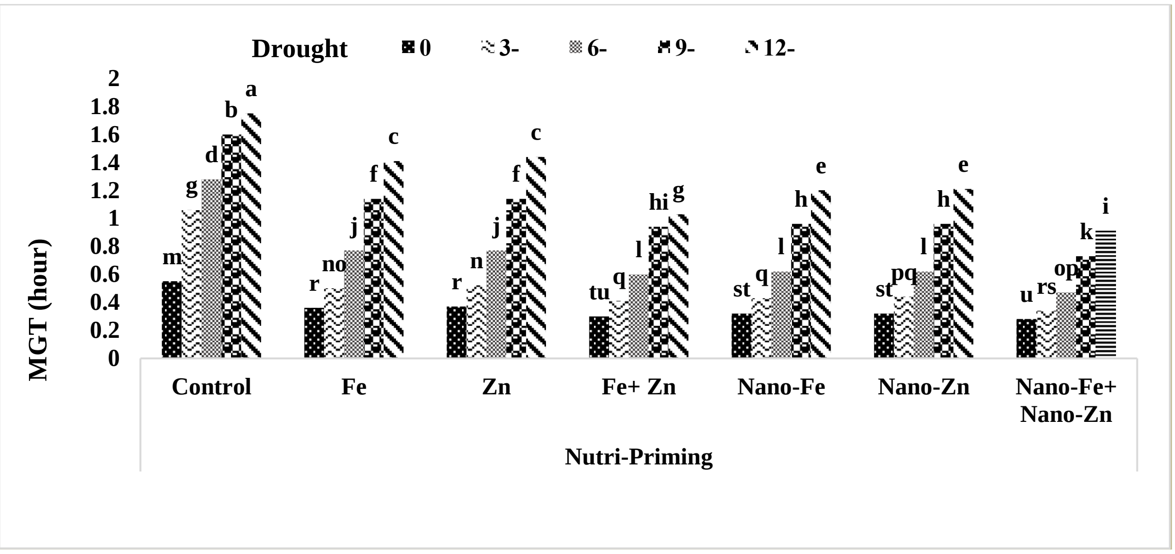

Fig. III. The effect of nutria-priming on mean of germination time (MGT) of quinoa under drought stress conditions (LSD 5%= 1.29); Bars represent ± SE.

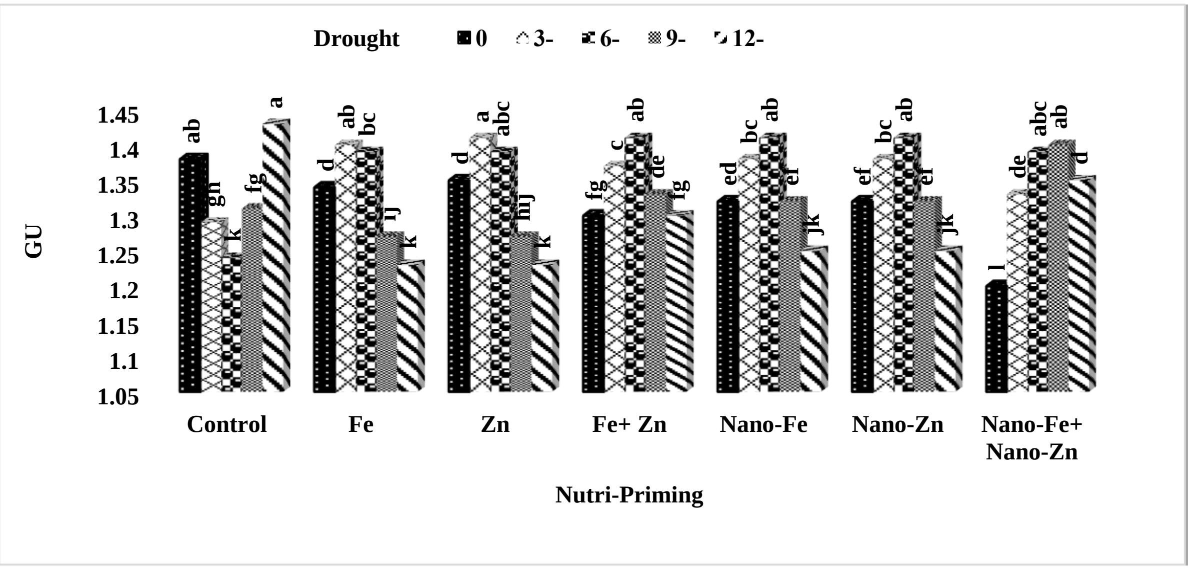

Fig. IV. The effect of nutria-priming on germination uniformity (GU) of quinoa under drought stress conditions (LSD 5%= 1.29); Bars represent ± SE.

chlorophyll a, b, and total chlorophyll and also carotenoid contents were significant (p≤0.01).

As shown in Table 5, the maximum content of chlorophyll a, b chlorophyll, total and carotenoids contents were related to no drought stress (0 bar) and nutri-priming (nano-Zn + nano-Fe). Also interaction of the treatments showed the highest chlorophyll a, b, total chlorophyll and carotenoid contents were obtained in Nano-Zn + Nano-Fe nutrient treatment in without drought stress. Also, the lowest chlorophyll a, b chlorophyll, total chlorophyll, and also carotenoids content were obtained in nutrient control treatment under drought stress condition (Fig VII-X).

Discussion

The purpose of this research was to investigate the effect of nutri-priming on germination and physiological indicators of quinoa seedlings in drought conditions. According to the results, drought stress had adverse effects on germination indicators, which decreased the rate and percentage of germination, seedling length, and seedling vigor indices.

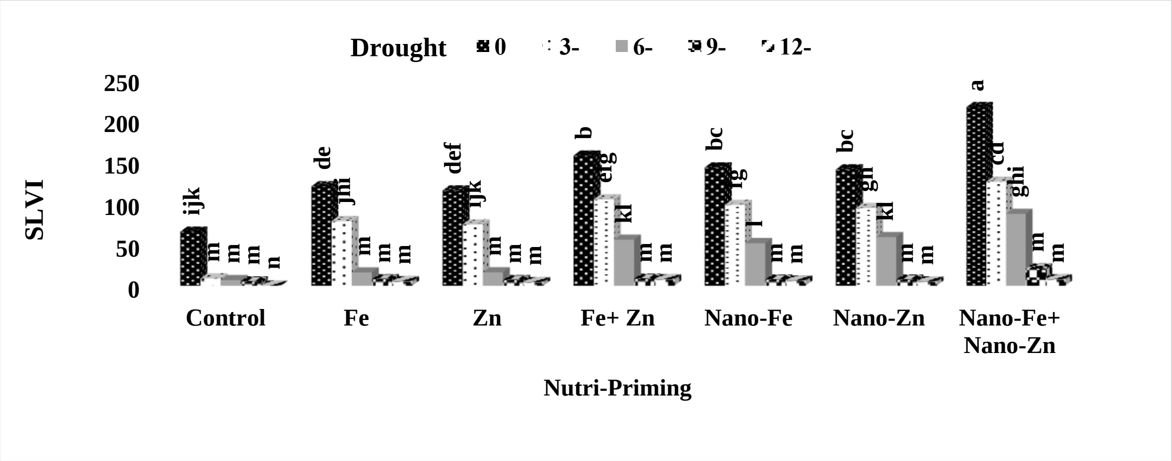

Fig. V. The effect of nutria-priming on seedling lengh vigor index (SLVI) of quinoa under drought stress conditions (LSD 5%= 1.29); Bars represent ± SE.

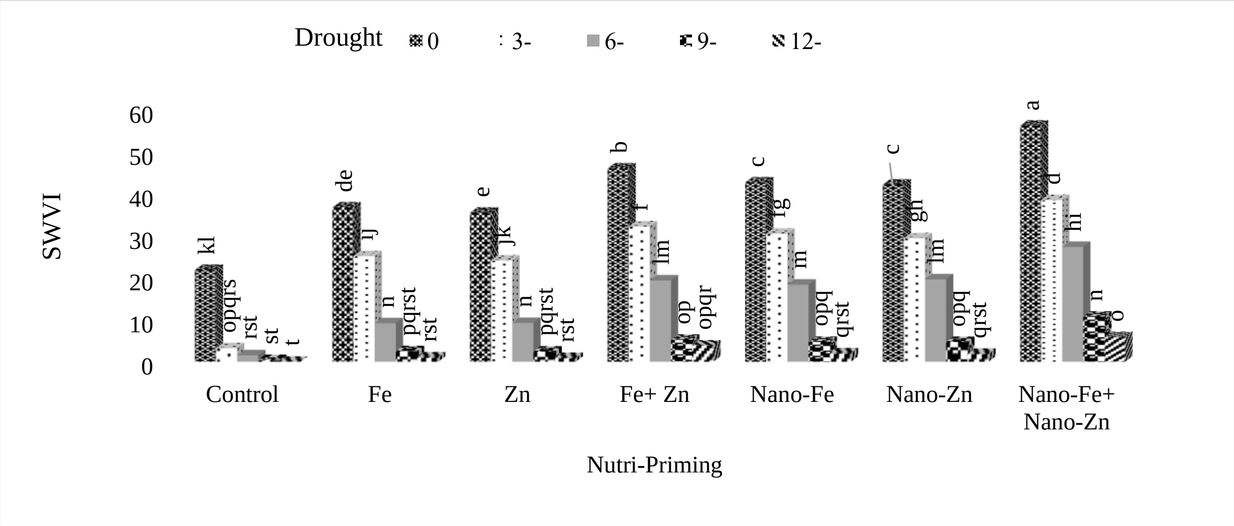

Fig. VI. The effect of nutria-priming on seedling weight vigor index (SWVI)of quinoa under drought stress conditions (LSD 5%= 1.29); Bars represent ± SE.

Among the drought stress levels, the increase in drought levels compared to the control (without drought stress), caused a decrease in seed germination percentage. In recent years, drought stress has been recognized as an important environmental factor that seriously affects productivity (Seleiman et al., 2021).

A decrease in water absorption by seeds and an increase in the level of ROS in water shortage conditions lead to poor and irregular germination (Zhang et al., 2021), which naturally leads to a disturbance in the transfer of nutrients to the seeds, resulting in less food storage and a decrease in uniformity and early ripening(Gough, 2020). According to reports, under stress conditions, changes in some enzymes and seed hormones can lead to a decrease in germination (Ali and Elozeiri, 2017).

Seed priming with nutrients (nutri-priming) caused beneficial effects such as increasing germination components and reducing the harmful effects of drought stress(Hussain et al., 2022). Among the nutritional treatments, the combination of nano-iron and nano-zinc had more positive effects on germination indices (Dhaliwal et al., 2021).

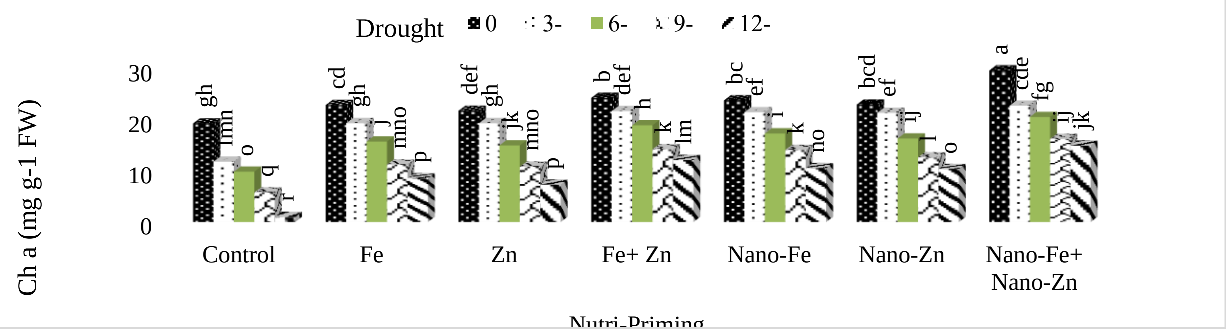

Fig. VII. The effect of nutria-priming on Chlorophyll a of quinoa under drought stress conditions (LSD 5%= 1.29); Bars represent ± SE.

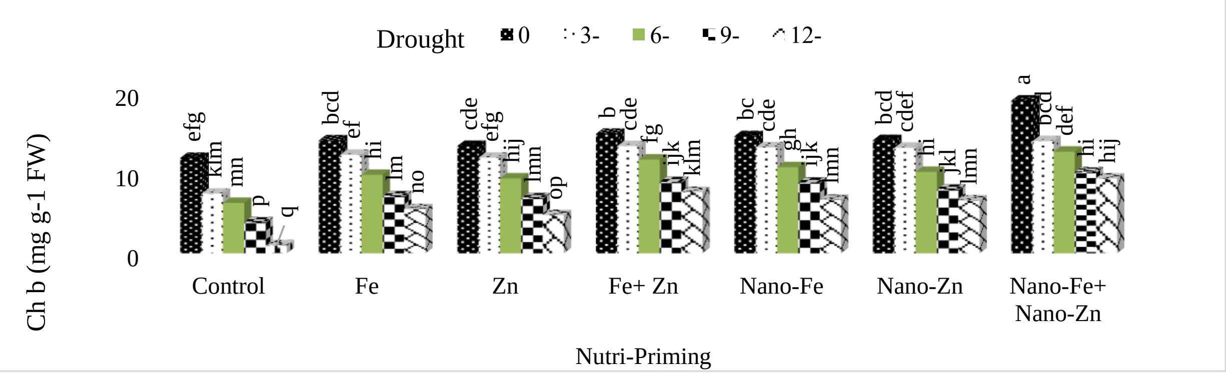

Fig. VIII. The effect of nutria-priming Chlorophyll b of quinoa under drought stress conditions (LSD 5%= 1.29); Bars represent ± SE.

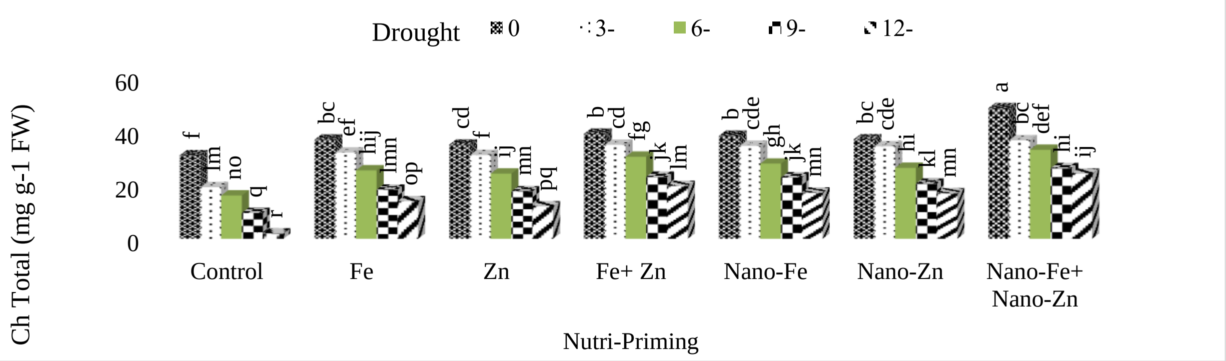

Fig. IX. The effect of nutria-priming Chlorophyll total of quinoa under drought stress conditions (LSD 5%= 1.29); Bars represent ± SE.

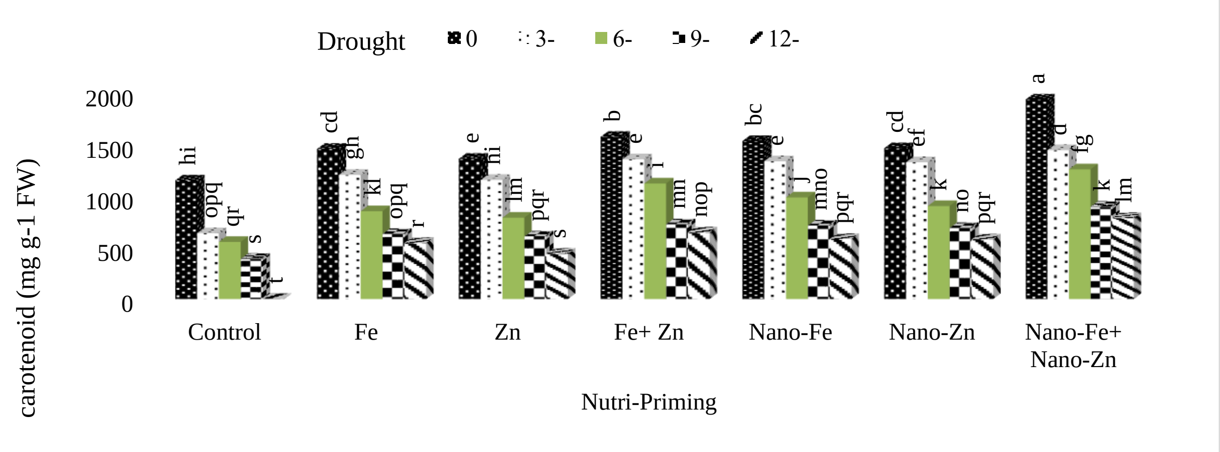

Fig. X. The effect of nutria-priming on Carotenoids of quinoa under drought stress conditions (LSD 5%= 1.29); Bars represent ± SE.

Table 4
Analysis of variance for the effect of drought stress and nutri-priming on chlorophyll and carotenoids of quinoa

		(MS)			
S. O. V	Df	Chlorophyll a	Chlorophyll b	Chlorophyll Total	Carotenoids
Nutri -priming (NP)	6	256.60**	97.08**	668.92**	982732.40**
Drought (D)	4	933.33**	343.59**	2408.11**	4505657.63**
NP × D	24	5.01**	2.36**	14.10**	22813.58**
Experimental error	105	0.81	0.87	3.34	2818.90
Coefficient of Variation (%)	-	5.55	9.26	6.96	5.51

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

Table 5
Mean comparison of the effects of drought stress and nutri-priming on chlorophyll and carotenoid contents of quinoa

Treatment	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Chlorophyll Total (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
Nutri -priming				
Control	9.36g	5.92e	15.27f	548.29g
Fe	15.69e	9.78d	25.47d	940.10e
Zn	14.87f	9.29d	21.15e	872.42f
Fe+ Zn	18.29b	11.33b	29.61b	1087.50b
Nano-Fe	17.48c	10.85bc	28.32c	1032.98c
Nano-Zn	16.76d	10.42c	27.18c	994.70d
Nano-Fe+Nano-Zn	20.87a	13.05a	33.92a	1267.47a
Drought				
0	23.56a	14.61a	38.16a	1500.32a
-3	19.80b	12.23b	32.02b	1212.30b
-6	16.28c	10.13c	26.40c	925.92c
-9	12.17d	7.68d	19.85d	666.04d
-12	9.14e	5.81e	14.94a	512.18e

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

synthesis, protein synthesis, membrane repair, and also improves the antioxidant defense system (Khan et al., 2019). The beneficial effects of priming with nutrients have been successfully reported by different scientists in different crops (Zulfiqar, 2021). Seedling establishment index is important for the rapid establishment of mass and early growth of plants, as reported by Mao and coworkers (Mao et al., 2019).

In the findings of different researchers, favorable effects of nutria- priming with nano-iron and nano-zinc nutritional compounds have been observed on various indicators of plant seed germination (Naghdi Badi et al., 2017). The improvement of seed germination indices in the effect of priming with nutrients can lead to a significant increase in seedling length and weight, as well as seed germination speed, which may be due to the effect of these elements in increasing cell division and meristem growth (Younis et al., 2019). Seed Priming with micronutrients allows to seeds that absorb water quickly and expand metabolism and germination, resulting in higher germination percentage, improved seedling growth, and increased tolerance to drought stress (Waqas et al., 2019).

Conclusions

The results of this study showed that priming with nutrients under drought stress improves the germination characteristics of quinoa seeds. The process of adaptation to water stress, started by nutritional treatment, can be attributed to pre-priming with nutrients before applying water stress. Among the nutritional treatments, the combination treatment of nano-iron and nano-zinc showed better results at all levels of drought stress. Among the drought stress levels, a significant difference was seen in most of the traits, which showed better results in the treatment of drought stress. In general, it can be stated that due to the better and more uniform germination of primed seeds with micronutrient elements, it will improve the initial establishment, utilization of environmental inputs, early arrival, increase in quantity and quality of the product.

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