The Journal of Medicinal Plants and By-Products

Original Article

Assessing the Interactive Effects of Priming and Drought Stress on Yield and Selected Growth Characteristics of Three Quinoa (*Chenopodium quinoa* Willd) Cultivars

Ahmad Shadmehri and Hamid Abbas dokht*

Department of Agronomy and Plant Breeding, Faculty of Agriculture, Shahrood University of Technology, Shahrood, Iran

*Corresponding author: Email: habbasdokht@shahroodut.ac.ir

Article History: Received: 09 September 2024/Accepted in revised form: 27 November 2024

ABSTRACT

Zinc and iron are vital elements for plant growth and development. This study aimed to evaluate the impact of seed priming with zinc and iron sulfate on enhancing yield and yield components in three quinoa cultivars—Q12, Giza, and Q29—under varying levels of drought stress during the 2020 and 2021 crop seasons. The experiment was conducted as a split plot design, included three levels of drought stress (100%, 75%, and 50% of field capacity) as the main plot, with the subfactors being the quinoa cultivar and two priming treatments (no priming and priming). The results revealed a significant influence of priming and drought stress on all traits across both seasons. The plant growth parameters, seed yield, seed protein content, and oil content notably decreased under drought stress in both 2020 and 2021. The greatest improvements were observed in the 100% field capacity treatment, in which the grain weight (274.2 and 298.6 gr m^{-2}), protein concentration (15.20 and 17.10%), and percentage of oil (3.33 and 3.54) increased in the seeds during both seasons. The proline (56 and 60%), superoxide dismutase (SOD) (52 and 26%), ascorbate peroxidase (APX) (70 and 67%), and catalase (CAT) (38 and 28%) activities significantly increased in 2020 and 2021, respectively. However, priming treatment effectively enhanced yield and growth attributes by mitigating oxidative damage in both seasons. The study showed that the Q12 cultivar displayed superior trait values, and priming with zinc sulfate + iron sulfate successfully sustained quinoa growth and seed yield under drought stress, even at 75% of field capacity.

Keywords: Drought stress; Priming; Quinoa; Seed coating

INTRODUCTION

The impact of climate change will undoubtedly be felt globally, with increasing temperatures and changing precipitation patterns expected to exacerbate water-related issues [1]. Among these challenges, drought stress is acknowledged as one of the most harmful abiotic stresses worldwide, resulting from fluctuations in temperature, light intensity, and decreased rainfall. It significantly impacts crop production, influencing the morphological, physiological, biochemical, and molecular traits of plants [2]. Plants have evolved an enzymatic antioxidant system, including total superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APD), to eliminate ROS and sustain individual growth and grain production, which is a common mechanism for plants to manage various abiotic stresses [3].

Quinoa (*Chenopodium quinoa Willd*) belongs to the Chenopodiaceae family and is categorized as a pseudocereal. Quinoa grains are rich in high-quality protein and all essential amino acids, along with vitamins, minerals, and antioxidants (such as flavonoids and polyphenols) that contribute to the health benefits of this crop [4]. Additionally, quinoa seeds have a high content of unsaturated fatty acids (such as oleic, linoleic, and linolenic acids) and exhibit an optimal omega-6/omega-3 fatty acid ratio, supporting the oil quality of this crop [5].

Water shortages primarily affect the initial growth stages of perennial species, as water acts as the primary trigger for germination, marking the beginning of this process [6]. Improving germination, plant growth, and yield under drought stress has become a sought-after goal in plant breeding [7], and seed priming treatments offer a partial solution in this regard [8]. Seed priming is a widely used technique aimed at enhancing seed germination and subsequent plant growth and development [9]. Improving and accelerating seed germination is a cost-effective and feasible approach for enhancing drought stress tolerance [2]. Several studies have shown that priming has a positive impact on the seed germination rate, uniformity, seedling emergence, and physiological traits of crops. In particular, priming with nutrient-enriched water, such as zinc and iron, has emerged as a promising and evolving approach [10]. Although plants require these elements in small amounts, they play a crucial role in plant growth and development [11].

In plants, Fe and Zn are essential for various biological processes because they are needed for key metabolic reactions and biological functions [11]. Fe aids in chlorophyll formation, acts as an oxygen transporter, is essential for cell division and growth [12], participates in enzyme formation (*catalase, peroxidase, cytochrome oxidase*, and *xanthine oxidase*), and is crucial for respiration, photosynthesis, nitrogen (N2) fixation, and electron transfer through cycling between Fe^{2+} and Fe^{3+} [13]. Zn is a component of more than 300 plant enzymes and vital proteins, such as Zn-finger DNA binding proteins [14]. In plant cells, it is involved in important biochemical functions, such as protein folding, catalytic activities, and regulatory functions [15].

Plants require low amounts of Fe and Zn for their physiological and metabolic processes. An excess or deficiency of these micronutrients can have negative effects on leaves, root systems, plant weight, overall biomass, photosynthesis, and DNA damage and can directly impact the cell cycle and chromosomes [16]. Therefore, one of the most cost-effective methods for enhancing micronutrient levels in crops is agronomic biofortification through foliar spraying, soil application, and/or seed priming [17].

Seed priming is a presowing technique that can be carried out using water (hydropriming) [18], aqueous solutions [19], solid matrices [20], nanoparticles [17, 21], or osmotic solutions (osmopriming) [22]. This method regulates the moisture level within the seeds and activates enzymatic and metabolic processes that enhance germination, seedling emergence and vigor, abiotic stress tolerance, initial plant growth, shoot weight and height, root length, and grain yield [20, 23].

Our current study aimed to enhance quinoa yield and yield components under drought stress conditions by priming three quinoa cultivars with the micronutrients Fe and Zn and to offer recommendations on the most suitable quinoa cultivars for cultivation in drought-affected regions.

MATERIALS AND METHODS

Site Trial Management

The experiment was conducted at the Kashmar Agricultural and Natural Resources Research Station, located in Razavi Khorasan Province, Iran (10° 10' 35" N, 50° 23' 58" E). The meteorological data for Kashmar in 2020/2021 crop seasons can be found in Table S1. The soils in Kashmar are predominantly silty. A composite soil sample was collected from the site at a depth of 0-30 cm, and standard laboratory procedures [24] were used to analyze the physical and chemical properties of the soil (Kavendish soil laboratory, Neyshaboor, Iran). The results of the soil and water analyses conducted in Kashmar are presented in Table S2.

Experimental Treatments

The experiment was conducted in a split plot design during the 2020 and 2021 crop seasons. The main plot included three levels of drought: I1 (100% of field capacity), I2 (75% of field capacity), and I3 (50% of field capacity). Field capacity and net irrigation water requirement was estimated using CROPWAT 8.0 software [25]. The subplot treatment consisted of three quinoa cultivars, Q12, Giza, and Q29, sourced from the Karaj Seed and Plant Breeding Research Institute (Alborz Province, Iran). These cultivars were subjected to two priming treatments: no priming and priming (1 hour) with a solution of zinc sulfate (ZnSO4.7H2O @ 0.03%) + iron sulfate (FeSO4.7H2O @ 0.04%) [26]. The experiment was set up in a split plot factorial design within a completely randomized block design (RCBD) with three replications, resulting in 18 treatment combinations and 54 experimental units.

Crop Management

Tillage and seedbed preparation operations, including plowing, disking, and levelling, were carried out in late February and early March in both seasons. Recommended rates of N, P, and K fertilizers (120, 90, and 30 kg ha^{-1}, respectively) were applied to each plot, following Razzaghi *et al*. [27]. The quinoa cultivar seeds were sown on March 7, 2020, in the first season, and on March 5, 2021, in the second season. The seeds were sown in subplots measuring 8 m × 3 m, with a sowing depth of 2 cm, row spacing of 45 cm, and on-row spacing of 8 cm to achieve a planting density of 280,000 plants/hectare. A nonplanted plot was included between treatments, maintaining a three-meter distance between replications.

For all treatments, irrigation was conducted every five days using specific volumetric flow meters. The amount of irrigation water applied was based on the water requirements determined for each treatment.

Initial irrigation was performed after seed planting, ensuring that the plants received full irrigation until they reached the 5-leaf stage. From that stage onward until the end of the growth period, irrigation treatments were applied according to their respective levels. The plants and seeds were harvested on July 27, 2020, in the first season and on July 29, 2021, in the second season.

Observations

The plants were collected at seed physiological rippening stage, and the plants of the three middle rows with an area of 12 square meters were harvested after removing the plot margins. The following data were recorded:

Growth Variables

Measurements included plant height, number of panicles, grain weight, and shoot weight.

Plant Physiological Measurements

The relative water content (RWC) was calculated using the formula developed by Smart and Bingham [28], which takes into account the fresh weight, turgid weight, and dry weight of the leaves:

$$RWC(\%) = (\frac{FW-DW}{TW-DW}) * 100 \quad (1)$$

FW: Fresh weight; DW: dry weight; TW turgid weight.

To assess cell membrane stability (CMS), leaf samples were collected from fully developed leaves and immediately transported to the laboratory on ice. A 0.3 g leaf sample was taken and washed three times with distilled water. Subsequently, the leaf pieces were placed in test tubes containing 25 ml of distilled water (control) and 25 ml of polyethylene glycol 6000 (PEG6000). These tubes were then incubated at a temperature of 10 °C for 24 hours. After the incubation period, the contents of the tubes were removed, and the samples were washed. Next, both the PEG-treated and control samples were placed in 25 ml of distilled water for another 24 hours. At the end of this period, the electrical conductivity was measured, and the samples were autoclaved at a pressure of one

atmosphere for 15 minutes. After autoclaving, the electrical conductivity was measured again. The following equation was used to calculate the CMS [8]:

$$Damage\ \ percentage = 1 - \frac{1-\frac{T1}{T2}}{1-\frac{C1}{C2}} * 100 \quad (2)$$

$$CMS = 100 - (Damage\ \ percentage) \quad (3)$$

Here, CMS represents cell membrane stability, C and T denote the electrical conductivity of the control and polyethylene glycol treatment, respectively, and indices 1 and 2 refer to the initial and final electrical conductivity values, respectively (5).

Biological Yield and Yield Components

The harvest index, biological yield, and seed yield were recorded at the time of harvest.

Enzyme Extractions and Assays

Fully expanded young leaves (0.5 g) from quinoa plants were sampled and immediately frozen in liquid nitrogen. The frozen samples were ground in 5 mL of Tris buffer solution containing 0.25 M sucrose, 10 mM Tris, and 1 mM EDTA at pH 7.4. The homogenate was then subjected to centrifugation at 4800 rpm for 15 minutes at 4 °C. The resulting supernatant was collected for enzyme assays.

The activity of superoxide dismutase (SOD) was determined using the SOD Assay Kit-WST following the method described in [29]. The reaction plate was incubated in a microplate reader at 37 °C for 20 minutes, and the absorbance of each reaction mixture was measured at 450 nm.

For the ascorbate peroxide activity (APX) assay, leaf tissues (0.5 g) were ground in liquid nitrogen and homogenized in 5 mL of Tris extraction buffer. The homogenate was then centrifuged at 10,000× g for 10 minutes at 4 °C. The APX activity was measured at 290 nm for 15 seconds (A1), followed by incubation of the reaction solution at 37 °C and measurement for 135 seconds (A2) using a spectrophotometer [30].

CAT activity assay: The supernatant was mixed with sodium phosphate buffer (100 mM, pH 7.0) and H_2O_2 (1 M), and the CAT activity was measured at 240 nm. One unit of CAT activity was defined as the amount of CAT required to decompose 1 mole of H_2O_2 per minute [30].

Analysis of Proline and Protein Content

Proline assay: The sample was ground in 5 mL of sulfosalicylic acid (3%, w/v), followed by centrifugation at 5000 g at room temperature for 20 minutes. One milliliter of the supernatant was mixed with 1 mL of ninhydrin and acetic acid. The mixture was then incubated in a water bath at 100 °C for 60 minutes. Afterward, 4 mL of toluene was added, and the mixture was shaken for 15 seconds. The final mixture was allowed to stand for 10 minutes, after which the absorbance was measured at a wavelength of 520 nm [31].

The protein content of the grains was determined using the Kjeldahl method. This internationally recognized method is widely used for measuring protein and nitrogen derivatives due to its high accuracy. The Kjeldahl method involves three steps: distillation, titration, and digestion. The oil content was also measured using a suction device [32].

Statistical Analysis

The data were analyzed using SAS 9.1 (SAS Inc., Cary, NC, USA) and Xlstat 2018 software to perform analysis of variance (ANOVA) to evaluate the effects of the factors and their interactions. Treatment means were compared using Duncan's new multiple-range test at a significance level of 5%.

RESULTS

Plant Growth

The impact of drought stress and priming on all traits was deemed statistically significant ($p \leq 0.01$), while the interaction between the treatments was not significant for some traits in either season. Quinoa plants irrigated at field capacity showed notably greater heights ($p \leq 0.01$) than those grown at 75% and 50% of field capacity in the 2020 and 2021 seasons, respectively (Table 1). Compared with no priming, applying priming to plants led to

significantly greater heights ($p \leq 0.01$) in both seasons. Among the cultivars, Q29 exhibited significantly greater heights ($p \leq 0.01$) in both seasons, while the heights of the Q12 and Giza cultivars did not significantly differ. The interaction effects of priming and drought stress suggested that there was no significant difference between I1 + no priming (114.7 and 124.4 cm in the 2020 and 2021 seasons, respectively) and I2 + priming (111.2 and 119.1 cm in the 2020 and 2021 seasons, respectively) in both seasons or between I3 + priming (81.6 cm) and I2 + no priming (85.1 cm) in the first season, indicating that priming countered the negative impact of mild drought stress (Table 5).

Table 1. Main and interaction effects of drought stress, cultivar and priming on plant height, peduncle number (PN), grain (SHW), biological yield, harvest index and relative water content CMS, Proline, SOD, CAT, APX, seed protein and oil weight (GW), shoot weight of quinoa.

SOV	df	H	PN	GW	SHW	BY	HI	RWC
Block	2	1052 ^{ns}	20.67 *	12007 **	250.9 **	15437 **	169.4 **	13.16 ^{ns}
Drought stress	2	16806 **	981.5 **	168970 **	278949 **	881352 **	597.2 **	4298.64 **
Error A	4	590.2	8.22	3021.8	59.11	2498	22.55	99.00
Cultivar	2	2282 *	71.05 **	2578 *	5978 **	7751 **	314.5 **	95.42 ^{ns}
Priming	1	9801 **	308.17 **	46875 **	49081 **	191888 **	37.5 ^{ns}	541.94 **
DS * C	4	198.6 ^{ns}	14.19 *	7363 **	369.2 **	9008 **	112.3 **	180.50 *
DS * P	2	1057.6 ^{ns}	38.22 **	7662 **	5992.5 **	27048 **	98.17 **	268.94 *
C * P	2	250.4 ^{ns}	7.39 ^{ns}	899.9 ^{ns}	190.3 **	1328 ^{ns}	18.67 ^{ns}	637.20 **
DS * C * P	4	743.6 ^{ns}	14.53 *	1815 *	551.9 **	2830 **	82.92 **	51.78 ^{ns}
Error B	30	514.7	4.84	515.4	26.15	505.3	17.72	60.16
CV%		11.97	10.02	13.12	6.97	6.52	8.09	11.95

SOV	df	CMS	Proline	SOD	CAT	APX	Seed Protein	Oil
Block	2	6.17 ^{ns}	1.78 ^{ns}	19.15 ^{ns}	0.00006 ^{ns}	0.005 ^{ns}	1.185 ^{ns}	0.089 ^{ns}
Drought stress	2	10229.28 **	67.33 **	5227 **	0.239 **	14.01 **	102.1 **	1.56 **
Error A	4	79.40	0.39	0.47	0.00	0.015	0.217	0.05
Cultivar	2	239.95 *	1.36 ^{ns}	129.9 **	0.003 *	0.085 **	34.65 **	0.03 ^{ns}
Priming	1	1599.44 **	1.57 ^{ns}	571.4 **	0.033 **	1.069 **	8.43 **	2.56 **
DS * C	4	231.17 **	0.92 ^{ns}	3.22 ^{ns}	0.00001 ^{ns}	0.281 **	0.097 ^{ns}	0.069 ^{ns}
DS * P	2	378.97 **	1.08 ^{ns}	14.4 ^{ns}	0.00001 ^{ns}	0.984 **	0.162 ^{ns}	0.1 ^{ns}
C * P	2	113.98 ^{ns}	0.38 ^{ns}	0.357 ^{ns}	0.00001 ^{ns}	0.003 ^{ns}	0.097 ^{ns}	1.092 **
DS * C * P	4	82.70 ^{ns}	0.49 ^{ns}	0.004 ^{ns}	0.00001 ^{ns}	0.027 *	0.097 ^{ns}	0.032 ^{ns}
Error B	30	54.98	0.65	20.11	0.0007	0.008	0.452	0.059
CV%		12.36	16.73	9.85	8.94	5.19	5.34	7.99

*, ** and ns are significant at 5%, 1% probability and not significant, respectively. H: plant height, PN: peduncle number, GW: grain weight, SHW: shoot weight, BY: biological yield, HI: harvest index, RWC: relative water content, CMS cell membrane stability, SOD: superoxide dismutase, CAT: catalyze, APX: ascorbate peroxidase.

Panicle Length (PL), grain Weight (GW), Shoot Weight (SHW), Biological Yield (BY), Harvest Index (HI), Protein Content, and Percentage of Oil

Elevated levels of drought stress led to significant ($p \leq 0.01$) decreases in PL, GW, SHW, BY, and HI during both seasons (Table 1). The intensity of drought stress notably affected BY and HI, with more severe drought stress causing considerable decreases. The most substantial enhancements in GW were observed in the I1 treatment (274.2 and 298.6 gr m^{-2} in the 2020 and 2021 seasons, respectively), which also displayed the highest protein concentrations (15.20 and 17.10%, respectively) and oil percentages (3.33 and 3.54%, respectively) in the seeds in the 2020 and 2021 seasons (Figure 1). Among the different cultivar treatments, the Q12 cultivar exhibited significantly ($p \leq 0.01$) greater values for PL, GW, SHW, BY, and seed protein in the first season, while the Giza cultivar showed greater values for PL, GW, and HI in the second season. The priming treatment led to increased values for all yield components in both seasons, except for seed protein in the second season, where the increase was not significant (Figure 1).

A significant interaction effect between priming and cultivar was observed for all yield components. Priming had greater effects on all cultivars in both seasons (Table 2, 3). Considering the interaction effect of cultivar and priming, the Q12 treatment combined with priming had the greatest effect on PL (27.1 cm), GW (217.3 g m^{-2}), SHW (217.3 cm), BY (419.9 g m^{-2}), and seed protein (14.5%) in the first season. In the first season, the Q29 treatment combined with priming had the highest HI value (58.8%), while in the second season, the Giza cultivar had higher HI values in the PL (34.7 cm), GW (190.8 g m^{-2}), SHW (278.2 cm), BY (469.0 g m^{-2}), HI (59.6%), SP (17.1%), and oil (3.35) treatments (Table 3, 4). A significant interaction effect was observed between drought stress and cultivar for all traits, with the I1 treatment having greater effects in the 2020 and 2021 seasons (Figure 2).

Table 2. Response of plant height, panicle length, grain weight and shoot weight of quinoa to the interaction of cultivar and priming in the 2020 and 2021 seasons.

Treatments		H (cm)		PL (cm)		GW (g m^{-2})		SHW (g m^{-2})	
		2020	2021	2020	2021	2020	2021	2020	2021
G	P	111.4 ab	106.4 bc	23.4 b	34.7 a	197.0 a	190.8 a	201.1 b	278.2 a
	No-P	77.52 c	102.8 c	19.9 de	24.9 c	121.8 c	118.3 c	139.1 e	206.7 b
Q12	P	108.9 ab	110.2 b	27.1 a	22.4 d	202.6 a	151.1 b	217.3 a	274.5 a
	No-P	89.82 bc	101.7 c	21.0 cd	29.6 b	152.7 b	105.1 cd	164.2 d	218.4 b
Q29	P	129.9 a	135.1 a	22.4 bc	25.6 c	207.8 a	158.4 b	187.3 c	258.0 a
	No-P	101.9 b	112.8 b	17.8 e	22.3 d	156.1 b	99.9 d	121.6 f	200.1 b

Mean pairs within a column with different letters are significantly different at the 5% probability level according to Duncan's new multiple-range test. G: Giza cultivar, H: plant height, PL: panicle length, GW: grain weight, SHW: shoot weight.

Table 3. Response of the biological yield, harvest index, seed protein content, seed oil content and relative water content of quinoa to interactions between cultivar and priming in the 2020 and 2021 seasons.

Treatments		BY (g m^{-2})		HI (%)		SP (%)		Oil (%)		RWC (%)	
		2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
G	P	398.1 b	469.0 a	50.9 bc	59.6 a	12.7 c	17.1 a	3.19 b	3.35 a	66.20 a	64.6 a
	No-P	260.9 d	325.1 c	49.7 c	61.2 a	12.07 cd	15.1 b	2.96 b	3.08 b	67.46 a	63.4 a
Q12	P	419.9 a	625.5 b	49.0 c	56.4 b	14.5 a	16.4 a	3.04 b	3.35 a	65.57 a	66.4 a
	No-P	316.9 c	323.5 c	49.1 c	59.3 a	13.6 b	16.3 a	2.97 b	3.34 a	65.35 a	63.2 a
Q29	P	395.1 b	416.4 b	58.8 a	59.6 a	11.76 d	14.6 bc	3.50 a	3.17 ab	72.36 a	69.4 a
	No-P	277.7 d	300.0 c	54.9 ab	60.0 a	10.87 e	13.9 c	2.51 c	2.79 c	52.31 b	50.8 b

Mean pairs within a column with different letters are significantly different at the 5% probability level according to Duncan's new multiple-range test. G: Giza cultivar, BY: biological yield, HI: harvest index, SP: seed protein, Oil: seed oil and RWC: relative water content.

Table 4 CMS, proline, SOD, CAT and APX of quinoa in response to interactions between cultivar and priming during the 2020 and 2021 seasons.

Treatments		CMS (%)		Proline (µg g^{-1})		SOD (U mg^{-1} protein)		CAT (U mg^{-1} protein)		APX (mg^{-1} protein)	
		2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
G	P	64.67 ab	59.2 b	5.03 a	4.58 a	41.76 cd	32.7 b	0.53 a	0.38 a	1.85 b	2.51 a
	No-P	52.94 c	49.7 d	5.20 a	4.69 a	48.22 ab	34.8 b	0.48 b	0.37 a	1.57 cd	1.18 b
Q12	P	71.80 a	66.4 a	4.52 a	5.47 a	45.02 bc	33.4 b	0.55 a	0.39 a	1.95 a	1.57 b
	No-P	56.35c	54.3 c	4.70 a	5.22 a	51.83 a	34.3 b	0.50 b	0.36 a	1.64 c	1.45 b
Q29	P	59.80 bc	52.8 cd	4.33 a	4.73 a	40.00 d	31.7 b	0.55 a	0.41 a	1.79 b	1.80 b
	No-P	54.32 c	49.5 d	5.01 a	5.21 a	46.25 bc	43.6 a	0.50 b	0.39 a	1.53 d	1.59 b

Mean pairs within a column with different letters are significantly different at the 5% probability level according to Duncan's new multiple-range test. G: Giza cultivar, CMS: cell membrane stability, SOD: superoxide dismutase, CAT: catalyst, APX: ascorbate peroxidase.

Table 5 Response of plant height, panicle length, grain weight and shoot weight of quinoa to the interaction of priming and drought stress in the 2020 and 2021 seasons.

Treatments		H (cm)		PL (cm)		GW (g m^{-2})		SHW (g m^{-2})	
		2020	2021	2020	2021	2020	2021	2020	2021
I1	P	157.4 a	145.3 a	34.0 a	33.6 a	324.8 a	353.3 a	355.3 a	368.2 a
	No-P	114.7 b	124.4 b	26.1 b	31.0 b	223.5 b	243.8 c	255.1 b	271.8 b
I2	P	111.2 b	119.1 b	21.2 c	31.1 b	192.1 b	265.6 b	177.3 c	280.5 b
	No-P	85.1 c	105.3 c	19.1 cd	24.3 c	135.3 c	207.4 d	125.3 d	189.1 c
I3	P	81.6 c	87.33 d	17.8 d	20.6 d	90.4 d	281.3 e	73.1 e	162.0 d
	No-P	69.5 c	87.44 d	13.4 e	18.9 d	71.7 d	272.1 e	44.4 f	164.3 d

Mean pairs within a column with different letters are significantly different at the 5% probability level according to Duncan's new multiple-range test. H: plant height, PL: panicle length, GW: grain weight and SHW: shoot weight.

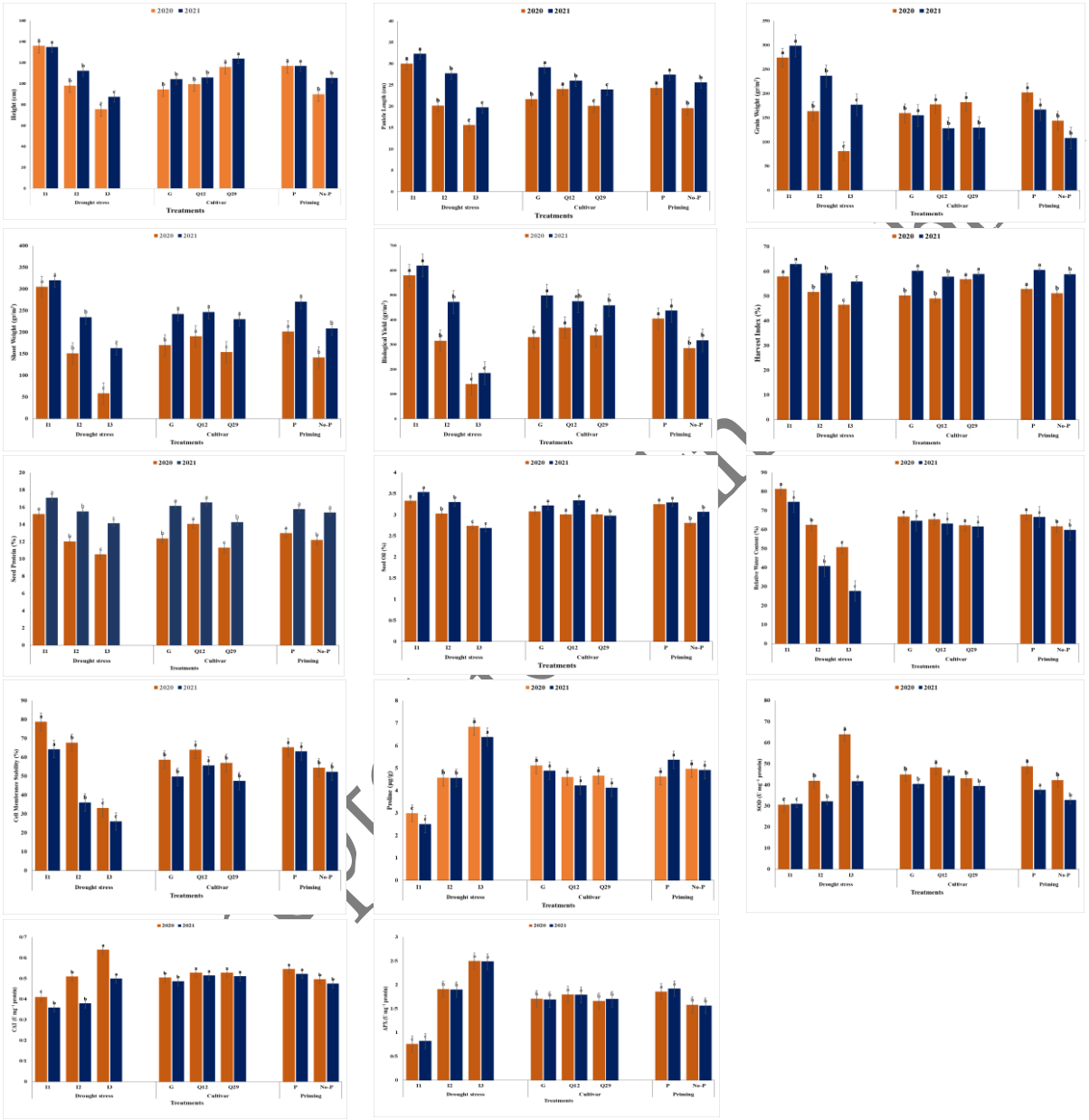

Fig. 1 Plant height, panicle length, grain weight, shoot weight, biological yield, harvest index, seed protein, seed oil, relative water content, CMS, Proline, SOD, CAT and APX of quinoa response to drought stress, cultivar and priming in 2020 and 2021 seasons. Mean pairs with different letters are significantly different at the 5% probability level according to Duncan's new multiple-range test. G: Giza cultivar, H: plant height, PL: panicle length, GW: grain weight, SHW: shoot weight, BY: biological yield, HI: harvest index, SP: seed protein, RWC: relative water content, CMS: cell membrane stability, SOD: superoxide dismutase, CAT: catalyze, APX: ascorbate peroxidase.

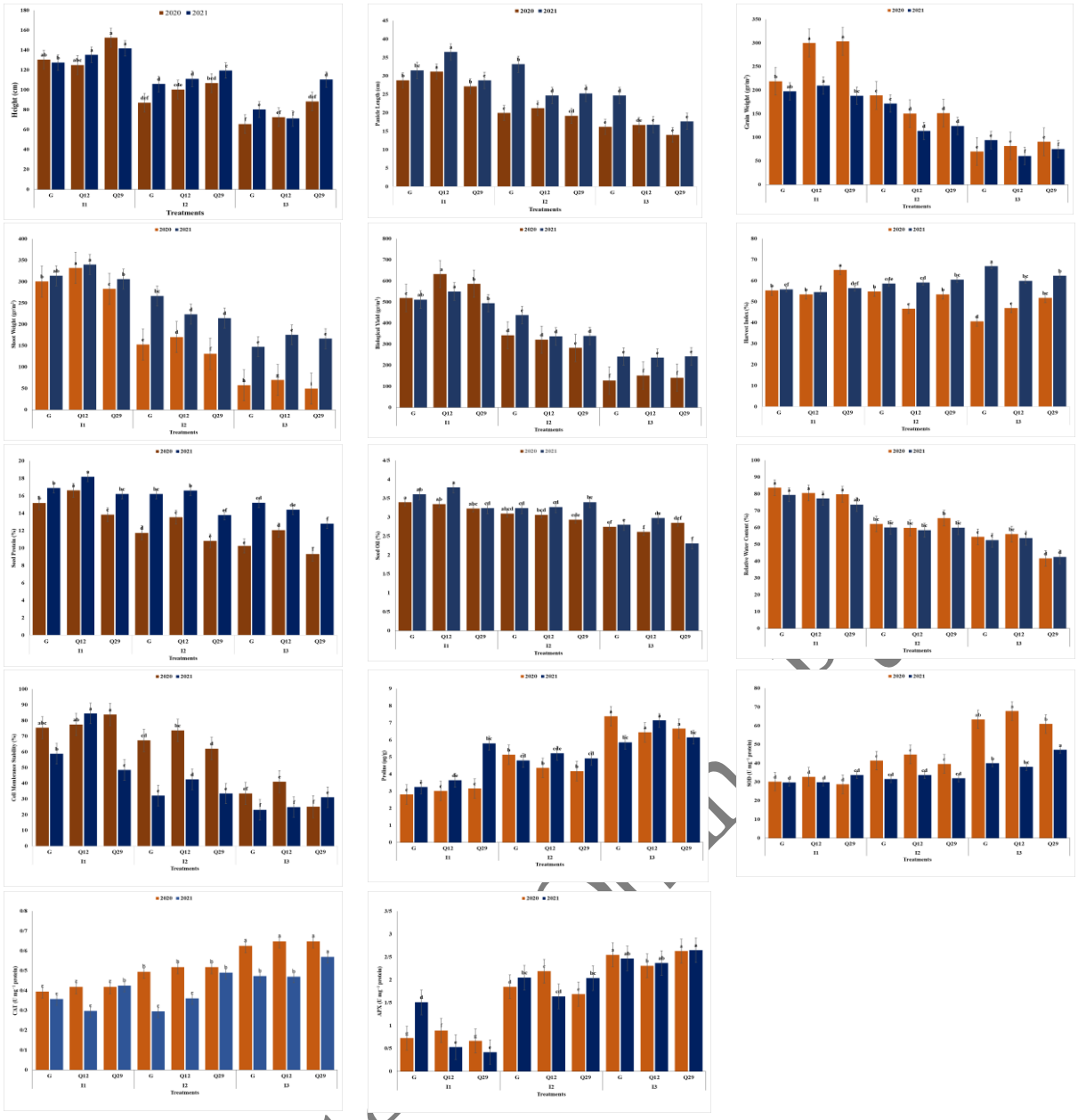

Fig. 2 Plant height, panicle length, grain weight, shoot weight biological yield, harvest index, seed protein, seed oil, relative water content CMS, Proline, SOD, CAT and APX of quinoa response to interaction of cultivar and drought stress in 2020 and 2021 seasons. Mean pairs with different letters are significantly different at the 5% probability level according to Duncan's new multiple range test. G: Giza cultivar, H: plant height, PL: panicle length, GW: grain weight, SHW: shoot weight, BY: biological yield, HI: harvest index, SP: seed protein, Oil: seed oil and RWC: relative water content, CMS cell membrane stability, SOD: superoxide dismutase, CAT: catalyze, APX: ascorbate peroxidase.

The Q12 cultivar under the I1 level of drought stress exhibited the highest values among all variables in both seasons. In terms of the interaction effect of drought stress and priming, the treatment with priming under the I1 level of drought stress exhibited the highest values for all variables in both seasons. For GW, HI, and seed oil content, no significant difference was detected between treatments without drought stress and those with no priming or between treatments with priming and those with mild stress (Tables 5, 6). In the three-way interaction, the Q12 cultivar displayed the highest values when subjected to priming and the I1 level of drought stress, showing a significant difference from the other treatments (Table S3, S4).

Relative Water Content and CMS

Table 1 shows the primary impacts of drought stress ($p \leq 0.01$), cultivar (not significant), and priming ($p \leq 0.01$) on the relative water content (RWC) in both seasons, with I1 and priming displaying higher values. Significant effects ($p \leq 0.05$) were noted in the interactions between drought stress and priming on RWC and I1, with priming showing higher values in both seasons (88.56 and 82.5%, respectively). While priming had a minimal impact on RWC at the I1 drought stress level, as drought stress levels increased, RWC notably decreased (Table 6).

The primary and interaction effects of drought stress, cultivar, and priming on CMS are illustrated in Figure 1. The greatest CMS value was recorded at the I1 drought stress level (78.95 and 64.35%), with the Q12 cultivar demonstrating the highest CMS value (64.07 and 55.70%, respectively) in both seasons. In the first season, the Q12 cultivar did not display a significant difference between the I1 and I2 drought stress levels, while Q29 exhibited the greatest difference (Figure 2).

Antioxidant Enzymes

Table 1 shows the primary impacts of drought stress, cultivar, and priming on antioxidant enzyme production. Exposure to drought stress led to a notable ($p \leq 0.01$) increase in proline (56 and 60%), superoxide dismutase (SOD) (52 and 26%), ascorbate peroxidase (APX) (70 and 67%), and catalase (CAT) (38 and 28%) activity during the 2020 and 2021 seasons, respectively. The priming treatment resulted in increased SOD, CAT, and APX activity, while proline activity remained unaffected in both seasons. Among the cultivars, Q12 displayed the highest ($p \leq 0.01$) levels of SOD, CAT, and APX activity in the 2020 and 2021 seasons (Figure 1).

Table 6. Response of the biological yield, harvest index, seed protein content, seed oil content and relative water content of quinoa to interactions between priming and drought stress in the 2020 and 2021 seasons.

Treatments		BY (g m^{-2})		HI (%)		SP (%)		Oil (%)		RWC (%)	
		2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
I1	P	680.1 a	621.5 a	61.4 a	62.0 a	15.49 a	17.2 a	3.50 a	3.58 a	88.56 a	82.5 a
	No-P	478.7 b	415.6 b	54.5 b	63.8 a	14.92 a	17.0 a	3.15 b	3.51 a	74.20 b	72.3 b
I2	P	369.4 c	446.0 b	51.8 bc	62.4 a	12.49 b	15.5 b	3.20 b	3.35 ab	61.97 c	62.4 c
	No-P	260.7 d	296.5 c	51.5 bc	55.2 b	11.59 c	15.5 b	2.86 c	3.25 b	63.04 c	61.2 c
I3	P	163.6 e	243.3 d	45.4 d	55.1 b	10.99 c	14.6 c	3.04 bc	2.93 c	53.60 d	55.6 d
	No-P	116.1 f	236.4 d	47.5 cd	56.7 b	10.09 d	13.7 d	2.43 d	2.46 d	47.89 d	50.3 d

Mean pairs within a column with different letters are significantly different at the 5% probability level according to Duncan's new multiple-range test. BY: biological yield, HI: harvest index, SP: seed protein, Oil, seed oil and RWC: relative water content.

Table 7 CMS, proline, SOD, CAT, and APX of quinoa in response to the interaction of priming and drought stress in the 2020 and 2021 seasons.

Treatments		CMS (%)		Proline (μg g^{-1})		SOD (U mg^{-1} protein)		CAT (U mg^{-1} protein)		APX (mg^{-1} protein)	
		2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
I1	P	78.55 a	72.1 a	2.91 d	3.62 d	28.12 f	27.9 d	0.435 e	0.33 d	1.12 e	1.10 c
	No-P	79.37 a	56.0 b	3.08 d	5.52 bc	33.02 e	34.2 bc	0.385 f	0.39 bc	0.41 f	0.54 d
I2	P	74.29 a	39.7 c	4.58 c	4.74 cd	38.8 d	31.1 cd	0.535 c	0.35 cd	2.09 c	2.18 a
	No-P	61.23 b	32.5 d	4.54 c	5.23 bc	44.99 c	33.8 bc	0.485 d	0.41 b	1.74 d	1.64 b
I3	P	23.83 d	28.7 d	7.29 a	6.32 a	68.29 a	44.7 a	0.615 b	0.50 a	2.39 b	2.49 a
	No-P	42.61 c	24.0 e	6.39 b	6.36 a	59.8 b	38.8 b	0.665 a	0.41 b	2.61 a	2.50 a

Mean pairs within a column with different letters are significantly different at the 5% probability level according to Duncan's new multiple-range test. CMS, cell membrane stability; SOD, superoxide dismutase; CAT, catalysis; APX, ascorbate peroxidase.

Multivariate Analysis

Pearson's correlation and principal component analysis (PCA) were performed to investigate the associations between response variables and treatments during drought stress (Figure 3, Table S7). PCA identified 13 factors (F1 to F13), with three components explaining 55.71%, 11.68%, and 8.48% of the total variance, respectively. The variables were categorized into three clusters: (1) CAT, proline, SOD, and CMS; (2) APX and Hi; and (3) other growth and physiological traits (Figure 3).

The Pearson's correlation matrix illustrated the connections among all response variables (Table S7). The plant growth and physiological traits were negatively correlated with CAT, proline, SOD, and CMS.

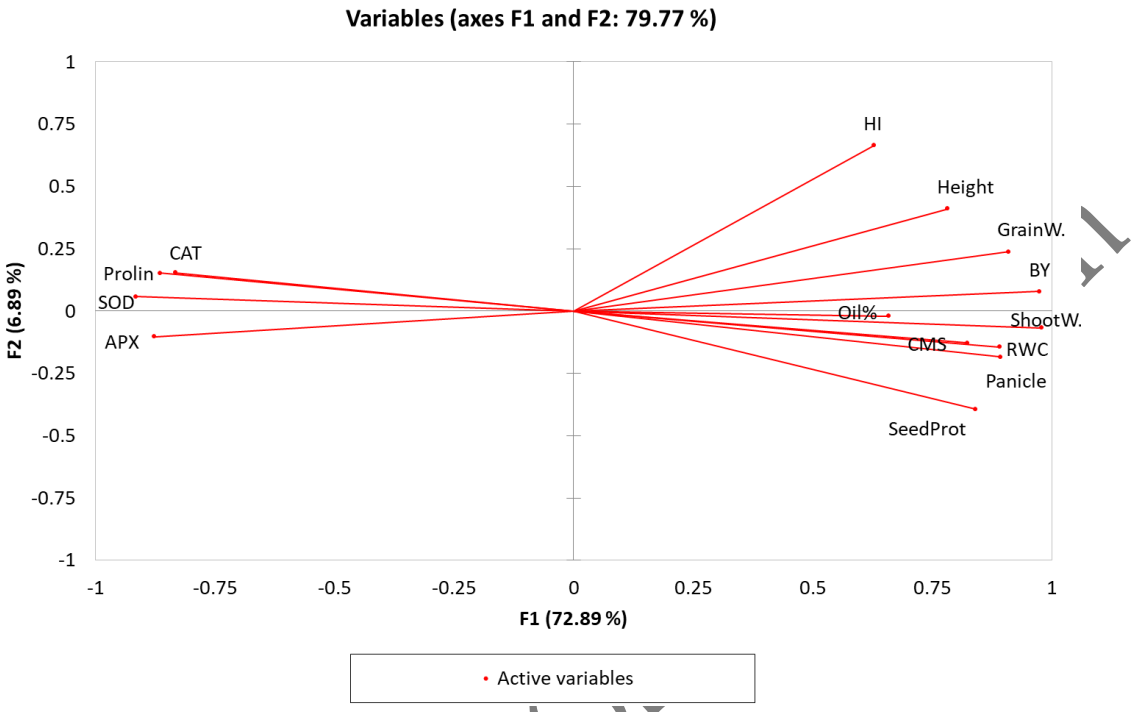

Fig. 3 Principal component analysis of different response variables of quinoa grown under drought stress and priming.

DISCUSSION

Despite similar studies in crop species such as maize, rice, wheat, pearl millet, and quinoa [17, 22, 26, among others], this study focused on the impact of seed priming with Fe and Zn and drought stress on the grain yield and yield components of three quinoa cultivars. Seed priming, an economical method to increase Zn and Fe levels in seeds before sowing, enhances seedling growth. Plants exhibit increased biomass and grain yield [33].

Seed priming, an affordable agronomic biofortification method, offers various benefits to plants by hastening germination and improving germination rate and uniformity [17]. While some argue that the efficacy of seed priming depends on the agents used and varies among crops [20], others note its role in reducing the time to seedling emergence, enhancing initial plant growth, uniformity, vigor, accelerating flowering, and improving crop yield [21]. Previous studies in quinoa and other species used methods such as hydropriming, potassium nitrate, ascorbic acid, calcium chloride, and PEG, among others, to enhance resistance to abiotic stresses and induce antioxidative defense [17, 21, 34]. The authors highlighted the benefits of seed priming for germination, seedling emergence, plant establishment, grain yield, and stress resistance.

Drought stress is a significant challenge for quinoa growth in arid regions. The implementation of drought resistance strategies such as priming at various growth stages could increase yields under stress [34]. The resilience of quinoa to abiotic stresses, including drought, salinity, low soil fertility, and frost, positions it as a promising crop for future food security amidst climate change [35, 36]. Studies have shown yield reductions under conditions such as low soil water availability, high vapor pressure deficit, elevated temperatures, and nitrogen deficiency [27, 37]. The inability of quinoa to reach full yield potential is linked to imitations in sink capacity, suggesting that enhancing reproductive partitioning could increase yields [38].

The plant height, panicle length, grain weight, shoot weight, biological yield, and harvest index were negatively affected by drought stress, especially under severe conditions. The I1 treatment had the greatest improvement in

grain weight (274.2 and 298.6 gr m-2) and the greatest increase in protein concentration (15.20 and 17.10%) and percentage of oil (3.33 and 3.54%) in the seeds in the 2020 and 2021 seasons, respectively. Among the cultivars, Q12 had significantly greater values for panicle length, grain weight, shoot weight, biological yield, and seed protein in the first season, while the Giza cultivar exhibited greater values for panicle length, grain weight, and harvest index in the second season. The priming treatment increased all yield components in both seasons except for seed protein in the second season, which was not significant. The inhibition of physiological and biochemical processes due to restricted cell elongation and division in plants has adverse effects on growth [39]. The harvest index and biological yield decreased significantly with increasing drought stress levels, leading to a decline in economic yield due to the negative impact on yield components such as the number of pods and seeds per plant. Similar reductions in the harvest index due to drought stress have been previously reported in leguminous plants [40]. Garrido *et al*. [41] observed a significant interaction between quinoa genotypes and the environment (drought stress) in terms of grain yield and harvest index. Drought stress has a negative impact on total grain yield and water use efficiency [42].

Pearson's correlation matrix revealed positive relationships between plant growth, physiological characteristics, grain weight, and quinoa yield. Selecting for traits such as panicle number and branching characteristics could result in more productive genotypes. A study by Spehar and Santos [43] revealed a significant positive correlation between panicle number and grain yield, which aligns with the findings of this study (Table S6). This suggests that selecting for these traits could result in more productive genotypes [44]. Quinoa plants with robust branching characteristics tend to develop larger inflorescences. Additionally, inflorescence length showed a positive association with plant height, indicating that lines with taller plants exhibited longer panicles [45]. Compared with those under the control conditions, plant height and shoot weight under drought stress were significantly lower [45, 46].

Drought stress and priming interactions significantly affected the relative water content, with priming showing higher values in both seasons. Priming had a minimal effect on RWC at the I1 drought stress level. However, as the drought stress level increased, the RWC significantly decreased. Similar findings have been reported in previous studies [47], where drought-induced osmotic stress resulted in reduced RWC and increased proline content in tomato plants. Quinoa possesses a distinctive ability to mitigate water uptake deficits by enhancing membrane stability and activating physiological mechanisms that enable plants to endure drought-induced stress [48].

Drought stress led to increased activities of antioxidant enzymes such as proline, superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT). The ability of quinoa to mitigate water uptake deficits by enhancing membrane stability and activating physiological mechanisms helps the plants endure drought-induced stress. This study highlighted the importance of antioxidant enzymes in reducing oxidative stress under stress conditions. Proline accumulation plays a crucial role in protecting proteins and stabilizing the cellular redox status under drought stress. The findings suggest that quinoa cultivars exhibited increased activities of CAT, SOD, and APX under drought conditions, aiding in stress mitigation. Stress conditions trigger the generation of reactive oxygen species (ROS) in plants. Various antioxidant nonenzymes and enzymes, including SOD, CAT, and POD [49], can reduce ROS-induced oxidative stress [50]. ROS detoxification under different environmental stresses occurs in a synchronized manner [2, 31]. According to Iftikhar et al. [51], SOD is critical for detoxifying superoxide (O2) radicals to H_2O_2 and O_2. In all the genotypes, drought stress increased SOD activity. Drought stress increases SOD activity in various plant species, including quinoa [50, 51]. In the case of the *C. quinoa* variety Real Blanca, there was no significant difference in CAT activity between the drought treatment and control groups, while APX activity increased significantly under drought treatment [52]. Another study also demonstrated that under drought conditions, *C. quinoa* exhibited a significant increase in SOD and APX activities compared to those in the control group [51]. In the present study, the quinoa cultivars displayed significantly increased activities of CAT, SOD, and APX, consistent with the findings mentioned earlier. Antioxidant metabolism, solute accumulation, and osmotic adjustment for sustained photosynthesis are key contributing factors to the tolerance mechanism. Drought stress induces structural changes in the photosynthetic machinery and causes a decreased concentration of photosynthetic pigments, as observed in the present study, which ultimately results in reduced photosynthesis. Previously, several studies have reported decreased concentrations of photosynthetic pigments due to overproduction of ROS under drought stress in different crops, including quinoa [53].

Proline plays a vital role in protecting proteins from dehydration-induced denaturation by binding to proteins under drought stress while also contributing to the stabilization of the cellular redox status [54]. Consequently, proline accumulates rapidly under stress and serves as an important osmoregulatory substance in plants. González et al. (2009) [55] demonstrated that the proline content of the *C. quinoa* variety Sajama increased by 21% when exposed to a soil water potential of 0.20 MPa compared to that of the control group (soil water potential of 0.05 MPa). Moreover, Sadak et al. (2019) [56] reported a significant increase in proline content in *C. quinoa* under insufficient irrigation. In the present study, the proline content of quinoa increased by 1.28-fold under the 50% water content treatment (Figure 2), indicating a more pronounced increase in proline content under drought conditions, thus mitigating the damage caused by stress [20].

Adequate nitrogen (N) uptake is crucial for plant mobilization and growth, particularly in the context of the significance of protein in quinoa seeds. However, this study revealed that as drought levels increase, the protein content in quinoa seeds decreases. This consistent decrease in seed protein concentration under drought stress is likely due to reduced nitrate absorption [57]. Insufficient nitrogen availability may also stem from disruptions in the intracellular ion balance, hindering the plant's ability to absorb nitrogen ions for transport to the leaves, as well as disturbances in carbon metabolism due to protein breakdown [58].

In summary, drought stress has a negative impact on plant growth, panicle length, seed and shoot weight, biological yield, the harvest index, and other physiological traits and on the seed yield of quinoa. Nevertheless, the introduction of exogenous Fe and Zn priming has demonstrated encouraging results in mitigating these adverse effects by preserving a favorable ionic equilibrium, boosting antioxidative enzyme functions, and enhancing seed yield. Therefore, seed priming could serve as a viable strategy to alleviate the harmful consequences of drought on quinoa production. Moreover, priming treatments also elevated the activities of antioxidant enzymes, thereby reducing the accumulation of reactive oxygen species (ROS) and malondialdehyde (MDA) levels in three quinoa varieties exposed to drought stress. Nonetheless, further investigation is necessary to confirm the efficacy of optimal priming methods under real field conditions.

Funding

This research received no external funding.

Compliance with Ethical Standards

This article does not contain any studies with human participants performed by any of the authors.

Conflict of Interest

The authors declare that they have no conflicts of interest.

REFERENCES

1. Yigit N., Sevik H., Cetin M., Kaya N. Determination of the effect of drought stress on the seed germination in some plant species. In Water Stress in Plants. Rahman I.M.M., Ed. IntechOpen: London, UK. 2016; 43–62. DOI: 10.5772/63197
2. Marthandan V., Geetha R., Kumutha K., Renganathan V.G., Karthikeyan A., Ramalingam J. Seed priming: A feasible strategy to enhance drought tolerance in crop plants. Int. J Mol Sci. 2020; 21:(21) 8258. doi: 10.3390/ijms21218258
3. Iwaniuk P., Borusiewicz A., Łozowicka B. Fluazinam and its mixtures induce diversified changes of crucial biochemical and antioxidant profile in leafy vegetable. Sci. Hortic. 2022; 298: 110988. doi.org/10.1016/j.scienta.2022.110988.
4. Gordillo-Bastidas E., Díaz-Rizzolo D.A., Roura E., Massanés T., Gomis R. Quinoa (*Chenopodium quinoa* Willd), from nutritional value to potential health benefits: An integrative review. J Nutr Food Sci. 2016; 6: 497. doi:10.4172/2155-9600.1000497.
5. Navruz-Varli S., Sanlier N. Nutritional and health benefits of quinoa (*Chenopodium quinoa* Willd.). J Cereal Sci. 2016; (69): 371–376. Doi: 10.1016/j.jcs.2016.05.004
6. Lechowska K., Kubala S., Wojtyla Ł., Nowaczyk G., Quinet M., Lutts S., Garnczarska M. New insight on water status in germinating Brassica napus seeds in relation to priming-improved germination. Int J Mol Sci. 2019; 20: 540. doi: 10.3390/ijms20030540.
7. Aydin M., Hossein Pour A., Haliloglu K., Tosun M. Effect of putrescine application and drought stress on germination of wheat (*Triticum aestivum* L.). Atatürk Univ J Agric Fac. 2015; 46: 1300–9036.
8. Nimac A., Lazarevi´c B., Petek M., Vidak M., Šatovi´c Z., Carovi´c-Stanko K. Effects of salinity and seed priming on germination of sea fennel (*Crithmum maritimum* L.). Agric Conspec Sci. 2018; 83: 81–185.

9. Lutts S., Benincasa P., Wojtyla L., Kubala S., Pace R., Lechowska K., Quinet M., Garnczarska M. Seed priming: New comprehensive approaches for an old empirical technique. In New Challenges in Seed Biology-Basic and Translational Research. Driving Seed Technology; Araújo, S., Balestrazzi, A., Eds., IntechOpen: London, UK. 2016; 1–46. DOI: 10.5772/64420
10. Reis S., Pavia I., Carvalho A., Moutinho-Pereira J., Correia C., Lima-Brito J. Seed priming with iron and zinc in bread wheat: effects in germination, mitosis and grain yield. Protoplasma. 2018; 255(4): 1179-1194. Doi: 10.1007/s00709-018-1222-4.
11. Singh S.P., Keller B., Gruissemm W., Bhullarm N.K. Rice NICOTIANAMINE SYNTHASE 2 expression improves dietary iron and zinc levels in wheat. Theor Appl Genet. 2017; 130: 283–292. DOI: 10.1007/s00122-016-2808-x
12. Morrissey J., Guerinotm M.L. Iron uptake and transport in plants: the good, the bad, and the ionome. Chem Rev. 2009; 109(10): 4553–4567. doi: 10.1021/cr900112r
13. Marenco R.A., Lopes, N.F. Fisiologia Vegetal: Fotossíntese, respiração, relações hídricas e nutrição mineral, 3rd edn. Marenco RA, Lopes NF (eds), Publisher: Editora Universidade Federal de Viçosa. 2009; 267–297
14. Laity J.H., Lee B.M., Wrightm P.E. Zinc finger proteins: new insights into structural and functional diversity. Curr Opin Struct Biol. 2001; 11: 39– 46. DOI: 10.1016/s0959-440x(00)00167-6
15. Hafeez B., Khanif Y.M., Saleem M. Role of zinc in plant nutrition – a review. Am. J. Exp Agric. 2013; 3: 374–391. DOI: 10.9734/AJEA/2013/2746
16. Ma T., Duan X.H., Yang Y.Y., Yao J., Gao T.P. Zinc-alleviating effects on iron-induced phytotoxicity in roots of *Triticum aestivum*. Biol Plant. 2017; 61(4): 733–740. Doi:10.1007/s10535-017-0720-0
17. Sheteiwy M.S., Guan Y., Cao D., Li J., Nawaz A., Hu Q., Hu W., Ning M., Hu J. Seed priming with polyethylene glycol regulating the physiological and molecular mechanism in rice (*Oryza sativa* L.) under nano-ZnO stress. Sci Rep. 2015; 5: 14278. Doi: 10.1038/srep14278
18. Afzal M., Afzal A., Jones A., Armstrong D. A rapid method for the quantification of GSH and GSSG in biological samples. Methods Mol Biol. 2002; 186: 177–122. Doi:10.1385/1-59259-173-6:117
19. Nawaz F., Ahmad R., Waraich E.A., Naeem M.S., Shabbir R.N. Nutrient uptake, physiological responses, and yield attributes of wheat (*Triticum aestivum* L.) exposed to early and late drought stress. J Plant Nutr. 2012; 35: 961–974. Doi: 10.1080/01904167.2012.663637
20. Sharma A.D., Rathore S.V.S., Srinivasan K., Tyagi R.K. Comparison of various seed priming methods for seed germination, seedling vigour and fruit yield in okra (*Abelmoschus esculentus* L. Moench). Sci Hortic. 2014; 165: 75–81. Doi: 10.1016/j.scienta.2013.10.044
21. Sheteiwy M.S., Fu Y., Hu Q., Nawaz A., Guan Y., Li Z., Huang Y., Hu J. Seed priming with polyethylene glycol induces antioxidative defense and metabolic regulation of rice under nano-ZnO stress. Environ Sci Pollut Res. 2016; 23: 19989–20002. Doi: 10.1007/s11356-016-7170-7
22. Abid M., Hakeem A., Shao Y., Liu Y., Zahoor R., Fan Y., Suyu J., Ata-Ul-Karim S.T., Tian Z., Jiang D., Snider J.L., Dai T. Seed osmopriming invokes stress memory against post-germinative drought stress in wheat (*Triticum aestivum* L.) Environ Exp Bot. 2018; 145: 12–20. DOI:10.1016/J.ENVEXPBOT.2017.10.002
23. Sarlach R.S., Sharma A., Bains N.S. Seed priming in wheat: effect on seed germination, yield parameters and grain yield. Progr Res. 2013; 8(1): 109–112.
24. FAO. Soil testing methods – Global Soil Doctors Programme - A farmer-to-farmer training programme. 2020; Rome. Doi: 10.4060/ca2796en
25. Telahigue D. C., Yahia L. B., Aljane F., Belhouchett K., Toumi L. Grain yield, biomass productivity and water use efficiency in quinoa (*Chenopodium quinoa* Willd.) under drought stress. Journal of Scientific Agriculture. 2017; 1: 222–232. Doi: 10.25081/jsa.2017.v1.67.
26. Bourhim M.R., Cheto S., Qaddoury A., Hirich A., Ghoulam C. Chemical seed priming with zinc sulfate improves quinoa tolerance to salinity at germination stage. Environmental Sciences Proceedings. 2022; 16-23. Doi: 10.3390/environsciproc2022016023
27. Razzaghi F., Ahmadi S.H., Jacobsen S.-E., Jensen C.R., Andersen M.N. Effects of salinity and soil–drying on radiation use efficiency, water productivity and yield of quinoa (*Chenopodium quinoa* Willd.). J. Agron. Crop Sci. 2012; 198: 173–184. Doi: 10.1111/j.1439-037X.2011.00496.x
28. Smart, R.E. and Bingham, G.E. Rapid Estimates of Relative Water Content. Plant Physiology, 1974, 53, 258-260. Doi: 10.1104/pp.53.2.258 .
29. Gómez M.B., Castro P.A., Mignone C., Bertero H.D., Gómez M.B., Castro P.A., Mignone C., Bertero H.D. Can yield potential be increased by manipulation of reproductive partitioning in quinoa (*Chenopodium quinoa*)? Evidence from gibberellic acid synthesis inhibition using paclobutrazol. Funct. Plant Biol. 2011; 38: 420–430. Doi: 10.1071/FP10168

30. Wattanakulpakin P., Photchanachai S., Ratanakhanokchai K., Kyu K.L., Ritthichai P., Miyagawa S. Hydropriming effects on carbohydrate metabolism, antioxidant enzyme activity and seed vigor of maize (*Zea mays* L.) Afr J Biotechnol. 2012; 11: 3537–3547. Doi: 10.5897/AJB11.3020
31. Bates L.S., Waldren R.P., Teare I. Rapid determination of free proline for water-stress studies. Plant Soil. 1973; 39: 205–207. Doi: 10.1007/BF00018060
32. Tavano OL., Miguel Amistá M., Del Ciello G., Martini Rodrigues M., Bono Nishida A., Alves Valadares L., Moreira Siqueira B., Aparecida da Silva Gomes R., Túlio Parolini M. da Silva Junior M. Isolation and evaluation of quinoa (*Chenopodium quinoa* Willd.) protein fractions. A nutritional and bio-functional approach to the globulin fraction, Current Research in Food Science. 2022; 5: 1028-1037. Doi: 10.1016/j.crfs.2022.06.006.
33. Mohsin A.U., Ahmad A.U.H., Farooq M., Ullah S. Influence of zinc application through seed treatment and foliar spray on growth, productivity and grain quality of hybrid maize. J Anim Plant Sci. 2014; 24(5): 1494–1503
34. Fallah S., Malekzadeh S., Pessarakli M. Seed priming improves seedling emergence and reduces oxidative stress in *Nigella sativa* under soil moisture stress. J Plant Nutr. 2018; 41(1): 29–40. Doi: 10.1080/01904167.2017.1381719
35. Hinojosa L., González J.A., Barrios-Masias F.H., Fuentes F., Murphy K.M. Quinoa abiotic stress responses: A review. Plants. 2018; 7: 106-112. Doi: 10.3390/plants7040106
36. Razzaghi F., Ahmadi S.H., Adolf V.I., Jensen C.R., Jacobsen S.-E., Andersen M.N. Water relations and transpiration of quinoa (*Chenopodium quinoa* Willd.) under salinity and soil drying. J. Agron. Crop Sci. 2011; 197: 348–360. Doi: 10.1111/j.1439-037X.2011.00473.x
37. Hinojosa L., Sanad M.N.M.E., Jarvis D.E., Steel P., Murphy K., Smertenko A. Impact of heat and drought stress on peroxisome proliferation in quinoa. Plant J. 2019; 99: 1144–1158. DOI: 10.1111/tpj.14411
38. Gómez M.B., Castro P.A., Mignone C., Bertero H.D., Gómez M.B., Castro P.A., Mignone C., Bertero H.D. Can yield potential be increased by manipulation of reproductive partitioning in quinoa (*Chenopodium quinoa*)? Evidence from gibberellic acid synthesis inhibition using paclobutrazol. Funct. Plant Biol. 2011; 38: 420–430. Doi: 10.1071/FP10168
39. Kumari A., Parida A.K. Metabolomics and network analysis reveal the potential metabolites and biological pathways involved in salinity tolerance of the halophyte *Salvadora persica*. Environ. Exp. Bot. 2018; 148: 85–99. Doi: 10.1016/j.envexpbot.2017.12.021
40. Kobraei S., Etminan A., Mohammadi R., Kobraee S. Effects of drought stress on yield and yield components of soybean. Anals Biol. Res. 2011; 2: 504–509.
41. Garrido M., Silva P., Silva H., Muñoz R., Baginsky C., Acevedo E. Evaluación del rendimiento de nueve genotipos de quinua (*Chenopodium quinoa* Willd.) j bajo diferentes disponibilidades hídricas en ambiente mediterráneo. Idesia. 2013; 31: 69–76. Doi: 10.4067/S0718-34292013000200010
42. Geerts S., Raes D., Garcia M., Mendoza J., Huanca R. Crop water use indicators to quantify the flexible phenology of quinoa (*Chenopodium quinoa* Willd.) in response to drought stress. Field Crop. Res. 2008; 108: 150–156. Doi: 10.1016/j.fcr.2008.04.008
43. Spehar C.R., Santos R.L.d.B. Agronomic performance of quinoa selected in the Brazilian Savannah. Pesqui. Agropecu. Bras. 2005; 40: 609–612. Doi: 10.1590/S0100-204X2005000600012
44. Mignone C., Bertero H. Identificación del período crítico de determinación del rendimiento en quínoas de nivel del mar. In Proceedings of the Congreso Internacional de la Quinua, Iquique, Chile, 2007; 23–26.
45. Yang A., Akhtar S., Amjad M., Iqbal S., Jacobsen S.E. Growth and physiological responses of quinoa to drought and temperature stress. J. Agron. Crop Sci. 2016; 202: 445–453. Doi: 10.1111/jac.12167
46. Raza A., Charagh S., Sadaqat N., Jin W. *Arabidopsis thaliana*: Model plant for the study of abiotic stress responses. In The Plant Family Brassicaceae; Springer: Singapore. 2020; 129–180. Doi: 10.1007/978-981-15-6345-4_3
47. Ahmad P., Ahanger M.A., Alyemeni M.N., Wijaya L., Alam P., Ashraf M. Mitigation of sodium chloride toxicity in *Solanum lycopersicum* L. by supplementation of jasmonic acid and nitric oxide. J. Plant. Interact. 2018; 13: 64–72. Doi: 10.1080/17429145.2017.1420830
48. Liu J., Gao H., Zheng Q., Wang C., Wang X., Wang Q. Effects of 24-epibrassinolide on plant growth, osmotic regulation and ion homeostasis of salt-stressed canola. Plant. Biol. 2013; 16: 440–450. Doi: 10.1111/plb.12052
49. Abbas G., Amjad M., Saqib M., Murtaza B., Asif N.M., Shabbir A. Soil sodicity is more detrimental than salinity for quinoa (*Chenopodium quinoa* Willd.): A multivariate comparison of physiological, biochemical and nutritional quality attributes. J. Agron. Crop Sci. 2021; 207: 59–73. Doi: 10.1111/jac.12451
50. Abbas G., Abrar M.M., Naeem M.A., Siddiqui M.H., Ali H.M., Li Y. Biochar increases salt tolerance and grain yield of quinoa on saline-sodic soil: Multivariate comparison of physiological and oxidative stress attributes. J. Soils Sediments. 2022; 22: 1446–1459. Doi: 10.1007/s11368-022-03159-2
51. Iftikhar A., Abbas G., Saqib M., Shabbir A., Amjad M., Shahid M., Qaisrani S.A. Salinity modulates lead (Pb) tolerance and phytoremediation potential of quinoa: A multivariate comparison of physiological and biochemical attributes. Environ. Geochem. Health. 2022; 44: 257–272. Doi: 10.1007/s10653-021-00937-8

52. Miranda-Apodaca J., Yoldi-Achalandabaso A., Aguirresarobe A., del Canto A., Pérez-López U. Similarities and differences between the responses to osmotic and ionic stress in quinoa from a water use perspective. Agric. Water Manag. 2018; 203: 344–352. Doi: 10.1016/j.agwat.2018.03.026
53. Iqbal H., Yaning C. Redox priming could be an appropriate technique to minimize droughtinduced adversities in quinoa. Front. Plant Sci. 2024; 15: 1253677. doi: 10.3389/fpls.2024.1253677
54. Fang Y., Xiong L. General mechanisms of drought response and their application in drought resistance improvement in plants. Cell. Mol. Life Sci. 2015; 72: 673–689. DOI: 10.1007/s00018-014-1767-0
55. González J.A., Gallardo M., Hilal M.B., Rosa M.D., Prado F.E. Physiological responses of quinoa (*Chenopodium quinoa*) to drought and waterlogging stresses: Dry matter partitioning. Bot. Stud. 2009; 50: 35–42.
56. Sadak M.S., El-Bassiouny H.M.S., Dawood M.G. Role of trehalose on antioxidant defense system and some osmolytes of quinoa plants under water deficit. Bull. Natl. Res. Cent. 2019; 43: 5-14. Doi: 10.1186/s42269-018-0039-9
57. Elzeiny H.A., Abou L.B., Gaballah M.S., Khalil S. Anti-transpirant application to sesame plant for salinity stress augmentation. Res. J. Agric. Biol. Sci. 2007; 3: 950–959.
58. Farooq M., Hussain M., Wakeel A., Siddique K.H.M. Salt stress in maize: Effects, resistance mechanisms, and management. A review. Agron. Sustain. Dev. 2015; 35: 461–481. Doi: 10.1007/s13593-015-0287-0

APPENDIX

Table S1 Meteorological data of Kashmar in in 2019 and 2020 seasons

Month	Average monthly temperature (ºC)		Average monthly maximum temperature (ºC)		Average monthly minimum temperature (ºC)		Maximum monthly wind speed (Km h^{-1})		Total monthly rainfall (mm)	
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
March	10.9	7.1	16.5	14.3	5.3	-0.1	15	17	15.7	49.1
April	18.6	15.1	25.0	23.2	12.2	7.1	15	21	1	5.1
May	23.5	20.1	29.9	28.2	17.2	12.1	10	20	56.8	16.4
June	30.4	26.6	37.2	35.5	23.6	17.8	12	17	0	0.1

Table S2 The results of the water and soil analysis of Kashmar

Soil

Parameter	Sand (%)	Silt (%)	Clay (%)	N (%)	Fe (ppm)	Zn (ppm)	Organic carbon (%)	Salinity (dS m^{-1})	pH
Value	30	52	18	0.07	1.13	0.3	0.74	1.603	7.58

Water

Parameter	Suspended solutes in water (mg l^{-1})	Sodium absorption ratio	Cl^- (mEq l^{-1})	$SO4^-$ (mEq l^{-1})	Salinity (Sμ)	pH
Value	1035.12	2.46	7.2	4.7	1625	7.29

Table S3 Response of plant height, panicle length, grain weight and shoot weight to the interaction of cultivar, priming and drought stress in the 2020 and 2021 seasons.

Treatments			H (cm)		PL (cm)		GW (g m^{-2})		SHW (g m^{-2})	
			2020	2021	2020	2021	2020	2021	2020	2021
I1	G	P	162.6 ab	130.0 bc	31.33 cde	37.0 b	292.3 b	362.0 a	356.7 b	362.3 ab
		No-P	98.4 cde	125.0 b-e	26.33 b	26.0 cde	145.3 de	233.7 d	244.0 e	265.3 cd
	Q12	P	129.0 bc	152.7 a	40.67 a	46.0 a	338.3 a	347.0 ab	366.7 a	355.4 ab
		No-P	121.3 bc	118.0 c-f	27.67 bcd	27.0 cd	262.0 bc	272.7 c	298.0 d	325.1 b
	Q29	P	180.6 a	153.3 a	30.00 bc	29.0 c	343.7 a	351.0 ab	342.7 c	386.8 a
		No-P	124.4 bc	130.3 b	24.33 def	28.7 cd	263.3 bc	225.0 de	223.3 f	225.1 def
I2	G	P	100.0 cde	116.7 def	21.00 fgh	42.3 a	233.7 c	327.7 b	176.0 h	322.4 b
		No-P	74.4 de	95.33 gh	19.00 ghi	24.0 de	144.3 de	215.7 def	129.3 k	210.1 efg
	Q12	P	111.6 cd	116.3 def	22.67 efg	24.7 cde	170.7 d	236.0 d	202.0 g	276.8 c
		No-P	89.0 cde	106.0 fg	20.00 gh	24.7 cde	130.7 def	191.3 fgh	138.7	170.2 gh
	Q29	P	121.9 bc	124.3 b-e	20.00 gh	26.3 cd	172.0 d	233.0 d	154.0 i	242.3 cde
		No-P	91.9 cde	114.7 ef	18.33 hij	24.3 cde	131.0 def	215.3 def	108.0 l	187.0 fgh
I3	G	P	71.6 de	72.67 j	18.00 hij	24.7 cde	65.0 h	182.7 ghi	70.7 n	149.9 h

		No-P	59.8 e	88.0 hi	14.33 jk	24.7 cde	75.7 gh	205.7 efg	44.0 p	144.9 h
	Q12	P	86.0 cde	61.67 k	18.00 hij	15.7 g	98.7 fgh	170.0 hij	83.3 m	191.2 fgh
		No-P	59.2 e	81.00 ij	15.33 ij	18.0 fg	65.3 h	151.3 j	56.0 o	159.8 h
	Q29	P	87.2 cde	127.7 bcd	17.33 hij	21.3 ef	107.7 efg	191.3 fgh	65.3 n	144.9 h
		No-P	89.6 cde	93.33 h	10.67 k	14.0 g	74.0 gh	159.3 ij	33.3 q	188.2 fgh

Mean pairs within a column with different letters are significantly different at the 5% probability level according to Duncan's new multiple-range test. G: Giza cultivar, H: plant height, PN: panicle number, GW: grain weight, SHW: shoot weight, BY: biological yield, HI: harvest index, RWC: relative water content.

Table S4 Plant height, panicle length, grain weight and shoot weight quinoa response to interaction of cultivar, priming and drought stress in 2020 and 2021 seasons.

Treatments			BY (g m^{-2})		HI (%)		SP (%)		Oil (%)		RWC (%)	
			2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
	G	P	649.0 b	624.3 a	63.33 ab	56.1 f-i	15.20 bc	18.1 ab	3.51 ab	3.51 bcd	89.58 ab	79.8 b
		No-P	389.3 e	398.9 d	47.33 d-g	55.7 f-i	15.13 bc	15.6 d-h	3.28 a-d	3.71 ab	78.06 a-d	57.7 d
I1	Q12	P	705.0 a	602.4 ab	53.00 c-f	55.7 f-i	17.00 a	19.0 a	3.34 abc	3.99 a	84.06 abc	81.7 ab
		No-P	560.0 c	497.7 c	54.00 cde	53.4 i	16.23 ab	17.5 bc	3.36 abc	3.59 abc	77.03 bcd	60.2 c
	Q29	P	686.3 ab	637.8 a	68.00 a	53.7 hi	14.27 cd	16.1 c-f	3.65 a	3.65 abc	92.04 a	87.9 ab
		No-P	486.7 d	350.1 def	62.33 ab	59.1 c-g	13.39 de	16.3 c-f	2.82 d-g	2.83 gh	67.51 d-g	57.9 d
	G	P	409.7 e	550.1 bc	52.67 c-g	57.0 e-i	12.20 ef	17.2 bcd	3.21 a-d	3.28 b-f	54.05 gh	89.8 a
		No-P	273.7 g	325.7 ef	57.00 bc	60.1 c-f	11.30 fg	15.2 fgh	2.98 c-f	3.21 c-g	70.24 c-f	62.5 cd
I2	Q12	P	372.7 e	412.8 d	48.00 d-g	54.9 ghi	14.00 cd	16.1 c-f	3.05 b-e	3.39 b-e	57.30 fgh	61.6 cd
		No-P	269.3 g	261.5 gh	45.33 fg	63.2 bc	13.10 de	17.0 b-e	3.07 b-e	3.14 d-g	62.23 e-h	55.3 de
	Q29	P	326.0 f	375.3 de	54.67 cd	58.2 e-h	11.27 fg	13.3 ij	3.35 abc	3.40 b-e	74.57 cde	60.6 cde
		No-P	239.0 g	302.4 fg	52.33 c-g	62.8 bcd	10.37 gh	14.3 hi	2.52 fgh	3.40 b-e	56.65 fgh	53.6 def
	G	P	135.7 ij	232.5 h	36.67 h	65.9 ab	10.70 gh	16.0 c-g	2.86 d-g	3.26 c-g	54.98 gh	51.2 ef
		No-P	119.7 j	250.6 gh	44.67 g	67.9 a	9.80 hi	14.5 ghi	2.63 efg	2.34 i	54.08 gh	45.6 fgh
I3	Q12	P	182.0 h	261.2 gh	46.00 efg	58.5 d-g	12.50 ef	15.4 e-h	2.74 efg	3.06 efg	55.34 gh	48.6 fg
		No-P	121.3 j	211.1 h	48.00 d-g	61.2 cde	11.60 fg	13.3 ij	2.49 gh	2.89 fg	56.79 fgh	43.6 ghi
	Q29	P	173.0 hi	236.2 gh	53.67 cde	66.9 ab	9.77 hi	12.4 j	3.52 ab	2.48 hi	50.48 h	44.3 ghi
		No-P	107.3 j	247.5 gh	50.00 c-g	58.0 e-i	8.87i	13.3 ij	2.18 h	2.13 i	32.79 i	35.2 i

Mean pairs within a column with different letters are significantly different at the 5% probability level according to Duncan's new multiple- range test. G: Giza cultivar, H: plant height, PN: panicle number, GW: grain weight, SHW: shoot weight, BY: biological yield, HI: harvest index, RWC: relative water content.

Table S5 Mean comparison the interaction effects of drought stress, cultivar and priming on CMS, Proline, SOD, CAT, APX, seed protein and oil.

Treatments			CMS (%)		Proline (µg g^{-1})		SOD (U mg^{-1} protein)		CAT (U mg^{-1} protein)		APX (mg^{-1} protein)	
			2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
	G	P	79.49 abc	49.3 e	2.68 i	3.20 e	27.74 ij	27.8 fg	0.42 hi	0.34 g-j	1.06 i	0.68 d
		No-P	71.39 b-e	68.6 c	2.93 hi	3.30 e	32.60 hij	31.6 b-g	0.37 j	0.37 f-i	0.40 j	0.76 d
I1	Q12	P	81.75 abc	88.6 a	3.05 ghi	3.80 de	30.19 hij	29.0 efg	0.44 gh	0.30 ij	1.35 h	0.58 d
		No-P	73.20 b-e	80.6 b	2.98 ghi	3.50 de	35.35 ghi	30.7 c-g	0.39 ij	0.29 ij	0.44 j	0.49 d
	Q29	P	91.05 a	38.0 f	2.99 ghi	3.80 de	26.42 j	27.0 g	0.44 fg	0.34 g-j	0.95 i	0.45 d
		No-P	76.86 bcd	59.2 d	3.33 f-i	8.70 a	31.12 hij	40.4 bc	0.39 gh	0.51 bcd	0.38 j	0.39 d
	G	P	62.00 efg	27.3 ghi	5.61 cde	4.32 cde	38.34 fgh	29.6 d-g	0.52 ef	0.27 j	2.03d	1.58 c
		No-P	72.78 b-e	37.2 f	4.67 def	5.28 b-e	44.46 ef	33.6 b-g	0.47 de	0.32 hij	1.67 f	1.51 c
I2	Q12	P	65.19 def	37.4 f	4.22 e-i	5.27 b-e	41.43 efg	32.7 b-g	0.54 d	0.38 f-i	2.36 c	1.57 c
		No-P	82.28 ab	47.5 e	4.52 efg	5.18 b-e	47.90 e	34.7 b-g	0.49 ef	0.34 g-j	2.03 d	1.71 bc
	Q29	P	56.51 fg	32.8 fgh	3.93 f-i	4.63 b-e	36.67 fgh	31.0 b-g	0.54 d	0.40 e-h	1.87 e	2.38 a
		No-P	67.81 c-f	34.2 fg	4.44 e-h	5.23 b-e	42.60 efg	33.0 b-g	0.49 ef	0.58 ab	1.52 g	1.70 bc
	G	P	25.43 jk	25.4 hi	7.99 a	5.50 b-e	67.59 ab	39.2 bcd	0.60 c	0.42 d-g	2.66 a	2.68 a
I3		No-P	41.74 hi	20.8 i	6.80 abc	6.23 bcd	59.21 cd	40.8 b	0.65 ab	0.53 bc	2.45 bc	2.26 ab

Q12	P	30.67 ij	26.9 ghi	6.61 abc	7.33 b	72.24 a	38.7 b-e	0.67 a	0.45 c-f	2.46 bc	2.20 ab
	No-P	51.36 gh	22.9 i	6.29 bc	6.97 bc	63.44 bcd	37.6 b-f	0.62 bc	0.49 b-e	2.15 d	2.54 a
Q29	P	15.40 k	33.9 fg	6.09 bcd	6.62 bc	65.05 abc	57.1 a	0.67 a	0.65 a	2.70 a	2.60 a
	No-P	34.74 ij	28.3 ghi	7.27 ab	5.70 b-e	56.91 d	37.0 b-f	0.62 bc	0.48 cde	2.56 ab	2.70 a

Mean pairs within a column with different letters are significantly different at the 5% probability level according to Duncan's new multiple- range test. G: Giza cultivar, CMS cell membrane stability, SOD: superoxide dismutase, CAT: catalyze, APX: ascorbate peroxidase.

Table S6 Pearson's correlation matrix of response variables of quinoa genotypes grown under drought stress and priming. Values in bold indicate a significant correlation at alpha = 0.05.

Variables	H	PL	GW	SHW	BY	HI	SP	Oil	RWC	CMS	Pro-line	SOD	CAT	APX
H	1													
PL	0.46	1												
GW	0.64	0.88	1											
SHW	0.71	0.75	0.83	1										
BY	0.70	0.86	0.96	0.95	1									
HI	0.35	0.40	0.54	0.27	0.43	1								
SP	0.43	0.84	0.79	0.79	0.83	0.30	1							
Oil	0.46	0.51	0.48	0.62	0.57	0.08	0.53	1						
RWC	0.47	0.15	0.11	0.48	0.30	-0.17	0.23	0.40	1					
CMS	-0.72	-0.73	-0.76	-0.79	-0.81	-0.37	-0.66	-0.52	-0.39	1				
Proline	-0.23	-0.14	-0.15	-0.29	-0.23	-0.03	-0.15	-0.26	-0.16	0.18	1			
SOD	-0.63	-0.71	-0.78	-0.81	-0.83	-0.48	-0.71	-0.59	-0.28	0.72	0.29	1		
CAT	-0.48	-0.73	-0.72	-0.73	-0.76	-0.32	-0.71	-0.49	-0.16	0.64	0.28	0.78	1	
APX	0.12	0.11	0.17	0.22	0.20	0.39	0.16	-0.03	-0.13	-0.11	-0.24	-0.39	-0.17	1

Values in bold are different from 0 with a significance level alpha=0.95. H: plant height, PL: panicle length, GW: grain weight, SHW: shoot weight, BY: biological yield, HI: harvest index, SP: seed protein, RWC: relative water content, CMS cell membrane stability, SOD: superoxide dismutase, CAT: catalyze, APX: ascorbate peroxidase.

Table S7 Eigen value in factor analysis by principal component

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
Eigenvalue	7.80	1.64	1.19	0.9	0.61	0.51	0.35	0.34	0.24	0.15	0.13	0.12	0.04
Variability (%)	55.7	11.7	8.5	6.4	4.34	3.60	2.53	2.39	1.71	1.10	0.93	0.85	0.27
Cumulative %	55.7	67.4	75.9	82.3	86.6	90.2	92.7	95.1	96.9	97.9	98.9	99.7	100