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Effects of drought stress on yield and morphophysiological traits of quinoa (*Chenopodium quinoa* Willd.) at different levels of nitrogen

Mohammad Reza VaziriMehr[1], Alireza Sirousmehr[2*], Ahmad Ghanbari[2], and Hamid Reza Fanaei[3]

[1]PhD student, Department of Agronomy, Faculty of Agriculture, University of Zabol, Zabol, Iran
[2]Department of Agronomy, Faculty of Agriculture, University of Zabol, Zabol, Iran
[3]Seed and Plant Improvement Institute, Agricultural Research, Education, and Extension Organization (AREEO), Karaj, Iran
[*]Corresponding author; asirousmehr@uoz.ac.ir

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Abstract

Objective: This study aimed to investigate the effect of drought stress and nitrogen fertilizer on grain yield and some morphophysiological traits of quinoa.
Methods: A factorial experiment was performed for the quinoa Titikaka cultivar. The factors included three levels of water-deficit stress based on soil moisture depletion [45% (control), 65%, and 85%] and three levels of nitrogen fertilizer including 50 (control), 100, and 150 kg/ha.
Results: The highest leaf area index in the first year was obtained at 45% soil moisture depletion (without drought stress) and the highest chlorophyll index was obtained at 65% soil moisture depletion level. In the second year, the highest leaf area index (11.3) and the highest chlorophyll index (64.20) were obtained from 65% and 85% soil moisture depletion combined with 150 kg/ha of nitrogen fertilizer, respectively. In the first year of the experiment, the 45% soil moisture depletion with 150 kg/ha of nitrogen fertilizer had the highest number of panicles per plant (13.1). Also, the highest 1000-seed weight (2.47 g), grain yield (1926.63 kg/ha), and plant height (49.15 cm) were obtained from the 45% soil moisture discharge and 150 kg/ha of nitrogen fertilizer.
Conclusion: The population structure and genetic relationships of the Iranian bread wheat landraces presented here highlight their diverse genetic architecture. The results of this study provide valuable information for the utilization of landraces in the genetic improvement of bread wheat.

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Introduction

Quinoa (*Chenopodium quinoa* Willd) is a broadleaf plant of the Amaranthaceae family (Langeroodi *et al.* 2020). Recently, interest in quinoa seeds for human nutrition has increased worldwide (Goldberger and Detjens 2019; Langeroodi *et al.* 2020). From an agricultural point of view, this plant is important due to its resistance to abiotic stresses such as drought and salinity (Fathi and Kardoni 2020). Crop yield is limited by environmental stresses and therefore there is a significant difference between potential and actual crop yield. Lack of moisture at different stages of growth reduces nutrient uptake, water uptake, transfer of nutrients within the plant, and yield (Payandeh *et al.* 2018). Nitrogen is involved in forming amino acids, nucleic acids, enzymes, chlorophyll, vitamins, and hormones (Robertson and Vitousek 2009). If adequate nitrogen is available to the plant, it increases the growth of the plant and stores protein in the grain (An *et al.* 2024). It was found that N improves quinoa drought tolerance and improves seed yield and quality (Alandia *et al.* 2016). Due to the importance of using quinoa as a drought-tolerant plant, this study aimed to evaluate grain yield and some morphophysiological traits of quinoa under water-shortage stress at different levels of nitrogen fertilizer.

Materials and Methods

This study was conducted in 2017 and 2018 in the Sistan Agricultural and Natural Resources Research Center, Zahak, Iran. Zahak is located at an altitude of 481 m above sea level. According to Koppen's classification, the region's climate is classified as arid and very hot with hot and dry summers. According to the statistics of Zahak Meteorological Station, long-term (>75 years) average annual rainfall is approximately 53 mm and evaporation is 4000-4500 mm/year (Sistan and Baluchistan Provincial Portal). The meteorological information of the study area in 2017 and 2018 is given in Table 1.

Before starting the experiment, different parts of the field soil were randomly sampled from a depth of 0-30 cm. Then, a composite sample was selected, transferred to the laboratory, and subjected to chemical and physical analysis. Tables 2 and 3 show the results of the analysis of soil samples.

The experiment was performed as factorial based on a randomized complete block design with three replications. The factors were water-deficit stress (using TDR) at three levels of 45% (control), 65%, and 85% soil moisture depletion and nitrogen fertilizer (Urea) at three levels of 50 (control), 100, and 150 kg/ha.

Tillage operations included plowing and two vertical discs, followed by leveling and plotting. Each plot consisted of six planting rows at a distance of 25 cm with a length of 3 m and a plant-to-

Table 1. Meteorological information of the study area (www.timeanddate.com).

2017						2018					
Month	**Temperature (°C)**			**Precipitation**	**Wind**	**Month**	**Temperature (°C)**			**Precipitation**	**Wind**
	Max	**Min**	**Mean**	**(mm)**	**(km/h)**		**Max**	**Min**	**Mean**	**(mm)**	**(km/h)**
Oct.	30	-2	15	0	9	March	35	4	23	1.5	13
Nov.	26	-11	9	0.9	10	Apr.	36	11	26	0.2	12
Dec.	24	-4	9	4	12	May	42	17	31	0	13
Jan.	28	-7	13	2	15	June	42	18	33	0	14
Feb.	34	5	20	3.6	15	July	39	13	30	0	11

Table 2. Physical and chemical properties of the soil in the experimental site.

Texture	**Clay %**	**Sand %**	**Silt %**	**Depth Cm^3**	**EC ds/m**	**pH**	**TNV %**	**Total N %**	**P ppm**	**K ppm**	**OC %**	**Fe ppm**	**Zn ppm**	**Cu ppm**	Mn **ppm**
Sandy loam	12	64	24	0-30	2.1	7.6	12.5	0.03	12	170	0.32	4.08	0.42	0.59	3.83

Table 3. Anions and cations (meq/l) in the soil samples of the experimental site.

Cd ppm	**Bd g/cm^3**	**CEC cmol/kg**	**Plaster %**	**K**	**ESP**	**SAR**	**Na**	**Mg**	**Ca**	**pH**	**EC ds/m**
N.D	1.71	5.7	Less than 0.5	1.5	3.1	3.06	7.5	4	8	7.6	2.1

plant distance of 5 cm within a row. Before planting, furrow irrigation was conducted and according to the soil test (Table 2), 50 kg/ha of triple superphosphate was applied with the irrigation water. Sowing was done on November 10 in both years. The Titi-Kaka cultivar quinoa was obtained from Gorgan Agricultural and Natural Resources Research Center, Gorgan, Iran. The weeds were controlled by hand throughout the growing season. To control *Aphidoidea* and *Agrotis* spp., two grams per litter of diazinon were sprayed on the shoots three times in 7-day intervals. Water-deficit stress was applied at the stem elongation stage.

Irrigation regime time was determined by using the soil water curve with the application of digital hygrometer equipment of Time-Domain Reflectometry (TDR, Model Trim, FM3, Germany). Nitrogen fertilizer was applied with the irrigation water at two stages ½ of the fertilizer at 6-8 leaf stage and the remaining half before the flowering.

Plants were harvested manually on 20 June at physiological maturing when quinoa plants were completely yellow. The seeds within the inflorescence were easily separated by hand.

Measurements of the physiological traits were made at a fixed time of day between 11.00 to 13.00 h at 50% flowering stage. Three plants were randomly taken at the flowering stage and leaf area was measured using the Delta T Device. SPAD values or leaf chlorophyll index (SPAD-502, Minolta Co Ltd, Osaka, Japan), were measured as an average of 10 leaves. Also, canopy temperature (Tc), was measured by a portable infrared thermometer (USA), and air temperature (Ta) was measured by a hydrargyric thermometer. In each plot, plants of four central rows were harvested to determine seed yield.

Relative water content (RWC) was calculated according to the method of Weatherley (1950). Leaf samples were saturated in 100 ml of water for 4 h and their turgid weights were recorded. Then, they were rolled in dried butter paper and oven dried at 65 ^{o}C for 48 h, then, their dry weights were recorded. RWC was calculated as:

RWC= (fresh weight-dry weight)/(turgid weight- dry weight)

Canopy temperature was measured using a thermometer on sunny days, 11-13 hours, and 90 days after planting. At the blooming stage, three plants were randomly separated and leaf area was measured using the Delta T Device and then the leaf area index (LAI) was calculated.

Plant height was measured from the crown (ground connection) to the tip of the plant, using a calibrated wooden ruler. One thousand seeds were randomly selected from the threshed seeds and weighed using a digital scale with an accuracy of 0.01 g. A digital scale was used to quantify the biological yield per unit area after the plants were harvested and dried. The harvest index was computed by dividing the grain yield by the biological yield multiplied by 100 The number of seeds per panicle was acquired by counting the number of seeds per 10 panicles per plot. To determine the biological yield and grain yield of the crop, harvesting was done from the middle part of each plot by observing the marginal effects and removing half a meter from the sides and half a meter from the beginning and end of the plot. The experiment in the second year was performed similarly to the first year.

After collecting the data, the Bartlett test of homogeneity of variances between experimental errors of the years was performed for the studied traits. The chi-square statistic was not significant for the biological yield, the number of seeds per panicle, and relative leaf water content, so a two-year combined analysis was performed for these traits. Separate analyses for each year were performed for the other traits. After analysis of variance, the means were compared by Duncan's multiple range test at the 5% probability level. The data were analyzed using SAS v.9.4 statistical software and the diagrams were drawn using Excel software.

Results and Discussion

Leaf area index

The effect of nitrogen fertilizer in both years and water deficit stress in 2017 on the leaf area index was significant. However, the interaction of these factors for leaf area index was only significant in 2018 (Table 4). In the first year, the maximum leaf area index (4.38) was produced from 45 percent soil moisture depletion, and in the case of nitrogen fertilizer, the highest value (5) was obtained for 150 kg ha^{-1} (Table 5). In the second year, the mean comparison of treatments revealed that 65 percent soil moisture depletion with 150 kg ha^{-1} nitrogen fertilizer produced the greatest leaf area index (11.33) followed by 45 percent soil moisture depletion with 150 kg ha^{-1} nitrogen fertilizer (Table 6). The results of this study in the first year are consistent with the studies conducted by Ghassemi-Golezani and Mousavi (2022) on the reduction in leaf area index in corn in the face of high levels of drought stress. Also, the experimental results of Geren (2015) in quinoa and Bahamin *et al.* (2019) in corn showed that using nitrogen fertilizer increased the leaf area index in these plants.

Chlorophyll index (SPAD)

The first year's data analysis revealed that only the effect of drought stress on chlorophyll index was significant (Table 4). With 65 percent soil moisture depletion, the maximum chlorophyll index (54.40) was achieved but increasing the drought stress to 85 percent soil moisture depletion decreased it significantly (46.92) (Table 5). However, the analysis of variance of data in the second year showed that the effect of drought stress, nitrogen fertilizer, and their interactions was significant on the chlorophyll index (Table 4). With 45 percent soil moisture depletion and 150 kg ha^{-1} nitrogen fertilizer, the maximum chlorophyll index (64.20) was obtained. When compared to the control levels of drought stress and nitrogen fertilizer, this treatment increased by 24.29 percent. The lowest chlorophyll index (48.6) was attained by depleting 85 percent of soil moisture with 50 kg ha-*1* nitrogen fertilizer (Table 6). Fghire *et al.* (2015) stated that the application of drought stress reduced the pigmentation of quinoa leaves and, as a result, the content of chlorophyll in this plant. The reasons for reducing quinoa chlorophyll due to drought stress could be due to the degradation of chloroplast thylakoid membrane, chlorophyll optic oxidation because of increased activity of reactive oxygen species, and increased chlorophyllase activity (Hinojosa *et al*. 2018). Also, the reason for the superiority of 150 kg/ha of nitrogen fertilizer could be due to the adequate and gradual supply of nitrogen may have caused quinoa to produce chlorophyll and other elements required for photosynthesis. Others have also reported the significant effect of chemical fertilizers on increasing the chlorophyll content in quinoa (Gomaa 2013).

Table 4. Analysis of variance for physiological traits of quinoa under the influence of water-deficit stress and nitrogen fertilizer in two crop years.

Source of variation	df	Mean squares					
		Leaf area index		Chlorophyll (SPAD)		Canopy temperature	
		2017	2018	1st year	2nd year	1st year	2nd year
Replication	2	0.194	3.37	39.55	24.96*	53.72**	20.15
Water-deficit stress (S)	2	1.750**	3.70	132.11*	36.75**	1.068	2.46
Nitrogen fertilizer (N)	2	10.361**	9.93*	13.27	56.91**	1.748	39.85*
S × N	4	0.528	12.48**	30.65	90.89**	1.322	18.97
Error	16	0.278	1.954	34.35	5.790	2.416	7.21
Coefficient of variation (%)	-	12.99	15.09	11.68	4.54	10.04	9.63

* and **: Significant at the 5% and 1% probability levels, respectively.

Table 5. Means of quinoa physiological traits under drought stress and nitrogen fertilizer in two crop years.

Treatment	Leaf area index	Chlorophyll (SPAD)	Canopy temperature (ºC)	
	1st year	1st year	1st year	2nd year
Soil moisture depletion				
45%	4.38a	49.21ab	15.12a	27.30a
65%	4.22a	54.40a	15.81a	27.95a
85%	3.55b	46.92b	15.46a	28.33a
Nitrogen fertilizer				
50 kg / ha	2.88c	50.90a	15.83a	25.43b
100 kg / ha	4.27b	48.77a	14.97a	29.10a
150 kg / ha	5.00a	50.85a	15.58a	29.05a

According to Duncan's multiple range test, means with different letters for each factor in each column are significantly different at the 5% probability level.

Table 6. Means of quinoa leaf area index and chlorophyll (SPAD) for the combination of drought stress and nitrogen fertilizer factors in two crop years.

Treatment	Leaf area index	Chlorophyll (SPAD)
	2nd year	2nd year
S_1N_1	9.00^{ab}	49.2^{c}
S_1N_2	10.00^{a}	55.2^{b}
S_1N_3	11.00a	64.2^{a}
S_2N_1	9.33^{ab}	49.2^{c}
S_2N_2	6.00^{c}	51.9^{bc}
S_2N_3	11.33^{a}	54.0^{bc}
S_3N_1	$7.00b^{c}$	48.6^{c}
S_3N_2	10.67^{a}	51.3^{bc}
S_3N_3	9.00^{ab}	52.8^{bc}

According to Duncan's multiple range test, means with different letters in each column are significantly different at the 5% probability level; S1 (control), S2, and S3 are 45%, 65%, and 85% soil moisture depletion; N_1, N_2, and N_3 are 50, 100, and 150 kg/ha nitrogen fertilizer.

Canopy temperature

The analysis of variance in the first year showed no significant effects of drought stress, nitrogen fertilizer, and their interaction on canopy temperature in quinoa. But in the second year, this trait wasonly affected by nitrogen fertilizer (Table 4). According to Table 1, the maximum temperatures during the growth period of the first year were lower than the second year, and probably for this reason, no significant effect was observed in the first year.

Increasing the nitrogen fertilizer from 50 to 100 or 150 kg/ha, increased the canopy temperature significantly (Table 5). It seems that the presence of more nutrients due to nitrogen fertilizer and consequently, more plant growth has increased the plant's water requirement, however, the water absorbed and transferred by the roots did not compensate for the plant's transpiration and consequently, the canopy temperature rose. High temperatures often intensify and complicate the effects of drought and can sometimes affect plant survival under severe stress. Riahinia *et al.* (2006) reported a positive linear relationship between leaf water potential and canopy temperature in maize, sunflower, cotton, and beans.

Number of panicles per plant

Based on the analysis of variance the effect of nitrogen and its interactions with nitrogen fertilizer was significant on the number of panicles per plant in quinoa in the first year (Table 7). However, in the second year, none of the factors and their interaction had a significant effect on this trait (Table 7). According to Table 8, irrigation at 45 percent soil moisture depletion (no stress) with 100 kg/ha of nitrogen fertilizer produced the highest number of panicles (13.10), followed by 65% and 85% soil moisture depletion at this nitrogen level 12.88 and 12.42, respectively. However, the number of panicles decreased significantly with 150 kg/ha at all moisture levels. Basra *et al.* (2014) also reported the reduction of spike number in quinoa when 75 kg of nitrogen fertilizer was applied per hectare. It looks like that in our experiment the optimum nitrogen level for the number of panicles at all soil moisture levels is 100 kg/ha.

In this experiment, the soil moisture stress did not affect the number of panicles at all nitrogen levels. However, Jamali *et al*. (2018) indicated that reducing the amount of water had a negative impact on yield and yield components of quinoa.

1000-seed weight

According to the first year of the experiment, the effects of drought stress and nitrogen fertilizer and their interactions on the 1000-seed weight of quinoa were significant but in the second year, none of these effects were significant (Table 7). With 45 percent soil moisture depletion (no stress) and 150 kg ha^{-1} nitrogen fertilizer, the maximum 1000-seed weight (2.47 g) was obtained followed by 65 percent soil moisture depletion at the same nitrogen fertilizer level. Increasing the use of nitrogen in quinoa reduces the physiological removal of flowers and increases the number of sub-branches in the plant, increases the production of photosynthetic materials, flower fertility, and flowering period, and thus 1000-seed weight (Saeedi *et al.* 2019). The flow of photosynthetic material and the re-transfer of carbon and nitrogen to the grain at the grain-filling stage depend on the source-reservoir ratio.

Table 7. Analysis of variance of quinoa agronomic traits under the influence of drought stress (S) and nitrogen fertilizer (N) in two crop years.

Source of variation	df	Panicle/ plant		1000-seed weight		Plant height		Grain yield		Harvest index	
		1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year
Replication	2	0.091	1.149	0.00009	0.0209	0.58	7.09	625.4*	25998	1.28	3.8
S	2	0.030	2.301	0.2126**	0.0368	79.37**	50.61**	6064**	188677*	134.4**	120.2**
N	2	2.87**	0.515	0.017**	0.0164	20.70*	12.4*	17056**	93968	32.0**	312.2**
S × N	4	0.51**	2.011	0.4590**	0.0416	75.49**	1.73	23878**	236147	89.3**	70.6**
Error	16	0.101	0.778	0.0027	0.0184	5.37	3.54	157.1	36932	2.98	5.92
CV (%)	-	2.61	10.60	2.48	7.04	5.25	4.01	0.71	11.40	9.96	11.69

* and **: Significant at the of 5 and 1%probability levels, respectively.

Table 8. Means of quinoa agronomic traits under the influence of drought stress and nitrogen fertilizer in two crop years.

Treatment	Harvest index (%)		Grain yield (kg/ha)	Plant height (cm)	1000-seed weight (g)	Panicle/ plant
	1st year	2nd year	1st year	1st year	1st year	1st year
S_1N_1	14.08^{c}	25.97^{b}	1721.07^{de}	44.77^{bc}	2.22^{bc}	11.55^{d}
S_1N_2	12.60^{c}	15.35^{de}	1741.26 ^{cd}	47.80^{ab}	2.14^{c}	13.10^{a}
S_1N_3	11.90^{c}	22.69 ^{bc}	1926.63^{a}	49.15^{a}	2.47^{a}	11.92^{cd}
S_2N_1	19.60^{b}	18.64^{cd}	1681.05^{fg}	39.07 ^{ef}	1.71^{d}	11.73^{d}
S_2N_2	26.85^{a}	13.85^{e}	1781.41^{b}	47.63^{ab}	2.15^{c}	12.88^{ab}
S_2N_3	12.32^{c}	18.25 ^{de}	1718.72^{de}	48.40^{ab}	2.40^{a}	11.92^{cd}
S_3N_1	22.35^{b}	36.41^{a}	1670.96^{g}	36.48^{f}	1.69^{d}	12.33^{bc}
S_3N_2	14.91^{c}	16.67^{de}	1754.77^{c}	40.48^{de}	1.70^{d}	12.42^{bc}
S_3N_3	21.30^{b}	19.41^{cd}	1700.29^{ef}	43.52^{cd}	2.30^{b}	11.50^{d}

According to Duncan's multiple range test, means with different letters in each column are significantly different at the 5% probability level; S1 (control), S2, and S3 are 45%, 65%, and 85% soil moisture depletion; N1, N2, and N3 are 50, 100, and 150 kg/ha nitrogen fertilizer.

Higher drought stress (85 percent soil moisture depletion) decreased 1000-seed weight at all nitrogen levels, indicating the negative impact of severe drought stress on the seed weight of quinoa (Table 8). Drought stress reduces leaf gas exchange and thus the size of the source and reservoir in the plant. In this case, the plant's discharge and distribution of photosynthetic material are impaired (Farooq *et al.* 2009). According to Elewa *et al.* (2017), drought stress reduces the 1000-seed weight of quinoa.

Plant height

The effects of drought stress and nitrogen fertilizer and their interactions in the first year were significant on the plant height of quinoa (Table 7). With 45 percent soil moisture depletion and 150 kg ha^{-1} nitrogen fertilizer, the maximum plant height (49.15 cm) was obtained (Table 8). Increasing nitrogen fertilizer at all soil moisture depletion levels increased the plant height of quinoa. However, drought stress decreased the plant height at all nitrogen levels.

In the second year, the effects of drought stress and nitrogen fertilizer were significant on plant height, but their interaction was not significant (Table 7). The maximum plant height was attained with 45 percent soil moisture depletion (48.90 cm) and 150 kg ha^{-1} nitrogen fertilizer (47.90 cm). The lowest plant height was attained with 85 percent soil moisture depletion (44.23 cm) and 50 kg ha^{-1} nitrogen fertilizer (45.56 cm) (Table 9).

The 85% soil moisture depletion may reduce the amount of non-structural carbohydrates stored in the stems, and leaf area, thereby reducing dry matter production. The reduction in the quinoa plant height in response to drought stress may be due to reduced cell length, turgor, and volume (Elewa *et al*. 2017).

It seems that increasing the use of nitrogen fertilizer in this study increased the photosynthetic capacity of quinoa and therefore, had a positive effect on plant height. The results of a study on quinoa in Pakistan showed that nitrogen fertilizer increased all growth-related traits such as crop growth rate, leaf area index, and plant height. It was stated that this increase is because nitrogen is an integral part of the photosynthetic system (chlorophyll and chloroplasts) (Basra *et al.* 2014).

Grain yield

The effects of drought stress and nitrogen fertilizer and their interactions were significant on grain yield in the first year, but in the second year, only the effect of drought stress was significant (Table 7). In the first year, the maximum grain production (1926.63 kg ha^{-1}) was observed with 45 percent soil moisture depletion (no stress) and 150 kg ha^{-1} nitrogen fertilizer (Table 8). At this nitrogen rate, the grain yield decreased under all drought-stress conditions. The lowest grain yield (1670.96 kg ha^{-1}) was obtained from the soil moisture depletion of 85 percent with 50 kg ha^{-1} nitrogen fertilizer

Table 9. Means for quinoa plant height and grain yield under the influence of drought stress and nitrogen fertilizer in two crop years.

Treatment	Plant height (cm)	Grain yield (kg/ha)
	2nd year	2nd year
Soil moisture depletion		
45%	48.90a	1802.81a
65%	47.30a	1730.04a
85%	44.23b	1523.69b
Nitrogen fertilizer		
50 kg/ha	45.56b	-
100 kg/ha	46.96ab	-
150 kg/ha	47.90a	-

According to Duncan's multiple range test, the means with different letters for each factor in each column are significantly different at the 5% probability level.

(Table 8). The positive interaction between nitrogen and water indicates that nitrogen uptake by plants is directly related to water availability (Ercoli *et al.* 2008).

In the second year, the 45 percent soil moisture depletion showed the highest grain yield (1802.81 kg ha^{-1}) and the 85 percent soil moisture depletion resulted in the lowest grain yield (1523.69 kg/ha) (Table 9). It seems that the reduction in yield compared to the control (no stress) was due to the reduction in photosynthetic pigments, plant height, and 1000-seed weight. Gomaa (2013) reported that nitrogen fertilizer increased vegetative growth, metabolic process, and the accumulation of dry matter in quinoa. The results of an experiment performed on the effect of nitrogen fertilizer levels (0, 50, 75, 100, 125, 150, and 175 kg ha^{-1}) on quinoa grain yield in the Mediterranean climate showed that the best amount of nitrogen for the highest grain yield, harvest index, and 1000-grain weight was 150 kg ha^{-1} (Geren 2015).

Harvest index

In the first and second years of the experiment, the effects of drought stress, nitrogen fertilizer, and their interaction were significant on the harvest index (Table 7). In the first year, with 65 percent soil moisture depletion (no stress) and 100 kg ha^{-1} nitrogen fertilizer, the maximum harvest index (26.85 percent) was obtained. When compared to the control treatment (no drought stress and 50 kg ha^{-1} of nitrogen fertilizer, it exhibited a 48.35 percent increase. The lowest harvest index (11.90%) was observed with 45 percent soil moisture depletion combined with 50 kg ha^{-1} nitrogen fertilizer (Table 8). In the second year, the maximum harvest index (36.41 percent) was produced from 85 percent soil moisture depletion with 50 kg ha^{-1} nitrogen fertilizer. When compared to the control, this treatment increased the harvest index by 24.42 percent (Table 8). The opposite results from the second year compared to the first year, could be due to the more pronounced effect of the environmental conditions in the first year on grain yield than biological yield.

Biological yield

The combined analysis of variance of data showed that year × drought stress × nitrogen fertilizer interaction was significant on the biological yield of quinoa (Table 10). The highest biological yield (15319.1 kg ha^{-1}) in the first year was obtained from 45% soil moisture depletion (no stress) with 100 kg ha^{-1} of nitrogen fertilizer. When compared to the control treatment, this treatment increased by 22.41 percent. Drought stress normally reduces plant biological yield (Hirich *et al*. 2014; Elewa *et al.* 2017), attributed to reduced uptake of nutrients, stomatal conductance, and thus the photosynthetic capacity of quinoa (Yang *et al.* 2016). In a field experiment on canola with four levels of irrigation (Irrigation after 70, 100, 130, and 160 mm evaporation), it was found that water shortage increased leaf temperature and decreased leaf water content, chlorophyll content, and plant biological yield, which resulted in the reduction of the grain yield per unit area (Ghassemi-Golezani *et al.* 2023).

The lowest biological yield (3966.6 kg ha^{-1}) was observed from the soil moisture depletion of 85 percent with 50 kg ha^{-1} nitrogen fertilizer in the second year (Table 11). This could be the reason why the highest harvest index was observed for the combination of 85 percent soil moisture depletion with 50 kg ha^{-1} nitrogen fertilizer because it seems that drought stress in the second year has affected the vegetative organs more than the grain yield.

Number of seeds per panicle

The combined analysis of variance showed that year × drought stress × nitrogen fertilizer interaction was significant for the number of seeds per panicle in quinoa (Table 10). The largest number of seeds per spike (19.49) was produced in the first year from 45 percent soil moisture depletion (without stress) combined with 100 kg ha^{-1} nitrogen fertilizer. When compared to the control, there was a 32.22 percent increase in the number of seeds per panicle. The lowest number of seeds per spike (12.56) was obtained in the second year for the 85 percent soil moisture depletion combined with the 50 kg ha^{-1} nitrogen fertilizer (Table 11). In general, the number of seeds per panicle determines the capacity of plant reservoirs. The higher the number of seeds, the more reservoirs the plant has produced to store the photosynthetic material, and consequently will result in the yield increase.

The reduction in the number of seeds per panicle in the second year can be attributed to undesired temperature conditions at the flowering and pollination stages of the plant. Tavoosi and Sepahvand (2014) reported that due to the increase in mean air temperature at the flowering stage and decrease at the seed formation stage, the number of seeds per plant of quinoa decreased. Co-occurrence of flowering, pollination, and grain filling stages with heat stress at the end of the season reduces grain yield by reducing the number of seeds and 1000-seed weight.

Relative leaf water content

The results of the combined analysis of variance showed that the interaction between year, drought stress, and nitrogen fertilizer for the relative leaf water content of quinoa was significant (Table 10). The maximum relative leaf water content (95.13 percent) was recorded from the first year, when the soil moisture was depleted by 45 percent (without drought stress), and 150 kg ha^{-1} of nitrogen fertilizer was applied. When compared to the control treatment this treatment exhibited a 22.05 percent increase in the relative water content. However, the lowest relative water content (57.62 percent) was produced in the second year of the experiment from 85 percent soil moisture depletion combined with 50 kg ha^{-1} nitrogen fertilizer (Table 11). Increasing the nitrogen fertilizer application in dehydrated conditions where grain yield decreases can increase water use efficiency. The interaction between water and nitrogen causes faster leaf growth at high levels of nitrogen due to the increase in stomatal closure and minimized ammonium evaporation. In an experiment on quinoa at different levels of irrigation (based on 50, 75, and 100% water requirements) it was found that the relative water content of the leaves decreased with the increase of the water deficit stress (Mostafaee et al. 2023). Also in research on pepper, the relative water content of leaves decreased with increasing drought stress (Khazaei and Estaji 2021).

Table 10. Combined analysis of variance of quinoa traits under the influence of drought stress and nitrogen fertilizer in two crop years.

Source of variation	df	Biological yield	Number of seeds per panicle	Leaf relative water content
Replication	2	224496.4	0.485	651.59**
Year (Y)	1	61587309.5**	79.267**	10.471
Replication /Y	2	33253.6	0.123	10.431
Drought stress (S)	2	49150984.2	2.282*	128.86**
S × Y	2	37185201.6**	0.622	45.671
Nitrogen fertilizer (N)	2	50995769.0	3.011	108.57
N × Y	2	16724123.8**	1.826*	80.159*
S × N	4	5643537.3	8.206	503.80
S × N × Y	4	24200788.8**	7.462**	1053.3**
Error	32	354178.2	0.491	18.978
CV (%)	-	6.01	4.82	5.72

* and **: Significant at the 5% and 1% probability levels, respectively.

Table 11. Means of quinoa traits for the combinations of drought stresses, nitrogen fertilizers, and years.

Treatment	Biological yield (kg/ha)	Number of seeds per panicle	Leaf relative water content (%)
$Y_1S_1N_1$	11885.8c	13.21h	71.97ef
$Y_1S_1N_2$	15319.1a	19.49a	75.26de
$Y_1S_1N_3$	14517.4ab	16.15bcd	95.13a
$Y_1S_2N_1$	8803.2de	16.37bc	70.49efg
$Y_1S_2N_2$	6608.2f	15.01def	73.50def
$Y_1S_2N_3$	14133.3b	15.14cde	94.13a
$Y_1S_3N_1$	8047.1e	16.46b	74.74def
$Y_1S_3N_2$	11399.4c	14.72efg	76.48de
$Y_1S_3N_3$	7924.5e	15.13cde	85.91bc
$Y_2S_1N_1$	6588.7f	13.09h	63.21ghi
$Y_2S_1N_2$	11098.0c	13.77fgh	74.71def
$Y_2S_1N_3$	8843.4de	13.71fgh	94.17a
$Y_2S_2N_1$	8592.8de	12.86h	59.27hi
$Y_2S_2N_2$	13761.9b	13.75fgh	66.82fgh
$Y_2S_2N_3$	9235.8d	13.83fgh	92.34ab
$Y_2S_3N_1$	3966.6g	12.56h	57.62i
$Y_2S_3N_2$	8734.8de	12.66h	62.05hi
$Y_2S_3N_3$	8592.8de	13.64gh	81.33cd

According to Duncan's multiple range test, means with different letters in each column are significantly different at the 5% probability level; Y1: 1st year, Y2: 2nd year; S1 (control), S2, and S3 are 45%, 65%, and 85% soil moisture depletion; N1, N2, and N3 are 50, 100, and 150 kg/ha nitrogen fertilizer.

Conclusion

According to the results of this research, it can be said that under drought stress, the use of nitrogen fertilizer reduced the effect of drought stress on the several morphophysiological traits of quinoa. This study showed that some quinoa traits, including the grain yield, responded better to the application of 150 kg/ha of nitrogen fertilizer than other levels of nitrogen in Sistan conditions of Iran. Also, quinoa yield components were enhanced by increasing the nitrogen fertilizer, especially under water availability conditions, which consequently resulted in higher grain yield.

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Ethical considerations

The authors avoided data fabrication and falsification.

Conflict of interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

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