Turkish Journal of FIELD CROPS

Turk J Field Crops 2023, 28(2), 279-290 DOI: 10.17557/tjfc.1262098

INTERACTION OF NITROGEN AND PLANT DENSITY ON GROWTH AND YIELD OF TWO QUINOAS (*Chenopodium quinoa* Willd.) CULTIVARS IN FARS PROVINCE, IRAN

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Received: 08.03.2023

ABSTRACT

As a field crop, quinoa (*Chenopodium quinoa* Willd.) has great potential in improving food for humans and animals even under marginal lands. Experiments were carried out at Marvdasht, Iran, in 2017 and 2018 main crop growing season to evaluate the effect of 4 levels of nitrogen (0, 75, 150, and 225 kg. ha^{-1}), three levels of density (20, 30, and 40 plants.m^{-2}), and two cultivars of quinoa (Titicaca and Sajema). The factorial experiment was performed based on a randomized complete block design with three replications. The results showed that nitrogen fertilizer significantly affected all measured traits. By increasing the nitrogen and plant density, the plant height, grain yield, and biological yield increased. The highest harvest index and nitrogen use efficiency was obtained from the highest density level. In all studied traits, Titicaca cultivar had greater than Sajema. Due to the superiority of 225 kg. ha^{-1} of nitrogen and the density of 40 plants.m^{-2} in terms of grain yield, it seems that this amount of nitrogen fertilizer and planting density to achieve good yields in the test and similar areas is also recommended. Also, the higher seed yield in Titicaca cultivar than Sajema is recommended for planting in the studied areas.

Keywords: Density, Harvest index, Nitrogen, Quinoa, Yield

INTRODUCTION

Chenopodium quinoa Willd (quinoa) is a highly nutritious pseudocereal traditionally grown for food in some parts of the world (Elizabeth and Da, 2016). The quinoa originated from the Andes in South America and is currently grown in Bolivia, Peru, the United States, Ecuador, and Canada as a food crop (Navruz-Varli and Sanlier, 2016). Its seed is reported to contain a well-balanced and significant amount of the nine essential amino acids required to fulfill human daily protein requirements (Miranda et al., 2012). The seed is also an important source of vitamins, unsaturated fatty acids, and carotenoids (Hinojosa et al., 2019). The quinoa grain is highly demanded in the USA, Europe, and Asia (Hinojosa et al., 2019).

The quinoa is sensitive to different levels of planting densities and fertilizer types, and application rates (Siavoshi et al., 2010). The planting space directly affects plant population per unit area hence the grain yield of the quinoa (Eisa et al., 2018). Low planting densities than plant densities of >150,000 plants. ha^{-1} are directly correlated to lower grain yields (Siavoshi et al., 2010). Currently, there are no specific recommended planting densities for quinoa. The variations in the planting densities are due to differences in soil fertility and general soil properties in an area (Maliro et al., 2017); hence difficult to recommend an ideal planting density for an area if the crop is newly introduced. Sief et al. (2015) found an ideal 40–80 cm spacing on planting rows with nitrogen application rates of 120 kg. ha^{-1} of urea in non-leaching soils. Nitrogen sources affect quinoa growth and development. Applying 40-160 kg/ha of inorganic nitrogen fertilizers yields high productivity (Earley et al., 2005).

The grain yield of quinoa was reported to vary from region to region, with the highest (7.50 t ha−1) recorded in Lebanon, followed by Egypt (3.87 t ha−1), and the lowest (0.23 t ha−1) recorded in Mauritania (Papastylianou et al., 2014). Bertero et al. (1998) noted that the difference in altitudes and temperature variations had a major influence on the grain yielding of quinoa. Low (<22°C) temperature encouraged high grain filling, whereas extremely high (>30°C) temperature resulted in poor grain filling (Erley et al., 2005). In areas with relatively low >22°C temperatures, the crop took a short time to reach the flowering stage and about 40–200 days to reach the physiological maturity depending on the cultivar (Martinez et al., 2009). Nevertheless, excessively high temperature during the growth phase is one of the most important abiotic stresses causing yield reduction (Hinojosa et al., 2019). In Africa, the temperature had a major influence on the adaptation of quinoa to areas of introduction (Maliro et al., 2017)

The grain yield of quinoa was positively correlated to agronomic management and fertilizer type (Maliro et al., 2017). Many workers have been proved the beneficial effects of nitrogen on quinoa yields, such as Fawy et al. (2017), Kansomjet et al. (2017), Mahmoud and Sallam (2017), Kakabouki et al. (2018), and Wang et al. (2020). The grain yield of quinoa was positively correlated to agronomic management and fertilizer type (Maliro et al., 2017). An average yield of 3.87 t ha−1 was recorded under good crop management in cool areas (Papastylianou et al., 2014).

The high costs of inputs and recurrent droughts in Iran are countering the production of Main crops, thereby intensifying food insecurity. Introducing hardy and widely adaptable crops like quinoa can be a panacea to food insecurity in the FARS region. Quinoa is a potential drought-tolerant and nutritious alternative to maize. However, the crop is new in Iran, so little agronomic information, like planting density and fertilizer requirements, is known. Therefore, the objectives of this study were to evaluate the quinoa growth performance and grain yield under two growing years (2017 and 2018) and determine the effects of different nitrogen levels, planting densities, and two quinoa varieties on the crop productivity. We hypothesized that the quinoa yield and its component vary under the two Quinoa varieties (Titicaca and Sajema) in two study regions.

MATERIALS AND METHODS

The Study Area

This research was conducted in 2017 and 2018 in Marvdasht counties in Fars province - Iran. in Marvdasht (30° 5′ 0″ N 52° 40′ 0″ E), the summers are sweltering, arid, and clear, and the winters are cold and mostly clear. Over the years, the temperature typically varies from -0°C to 37°C and is rarely below -4°C or above 40°C. The rainy period of the year lasts for 5.5 months, from November 4 to April 18, with a sliding 31-day rainfall of at least 13 millimeters. The rainless period of the year lasts for 6.5 months, from April 18 to November 4. Figure 1 shows the average minimum and maximum temperature and rainfall during the growing season in the two years of the experiment.

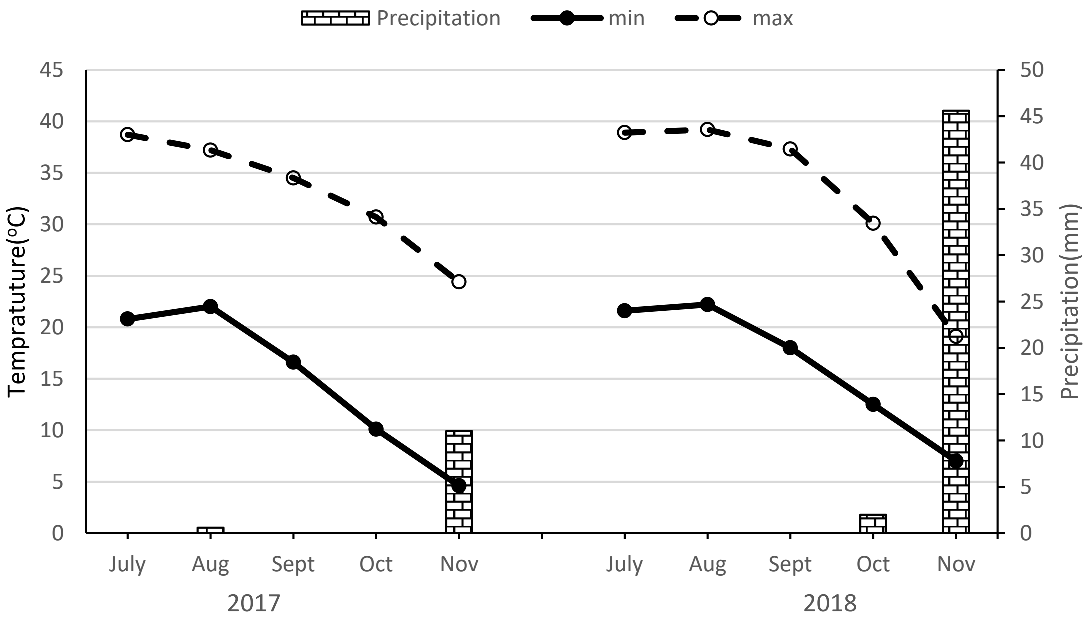

Figure 1. Average minimum and maximum temperature and rainfall during the growing season

Agricultural operations

Quinoa was planted manually on the 10th of August in the middle of the planting rows at a depth of two to three centimeters. In this experiment, quinoa was planted as a wet planting Each experimental plot was considered to have 6 rows of 4 meters (dimensions 4 x 3 meters). The distance between planting rows was 50 cm and the distance on the row was 5, 6.8 or 10 cm depending on the desired density. The irrigation system was furrow irrigation. The first irrigation was done immediately after planting. Irrigation intervals varied depending on the evaporation and transpiration conditions during the growing season. During the growth period of the plant, if necessary, pest and disease control was carried out. The weed population of the field was also controlled by hand weeding during the plant growth period.

Nitrogen from the source of urea fertilizer according to the experimental treatments, one third was given to the soil in the basal form, one third in the longitudinal growth stage of the stem (4 to 6 leaves) and one third in the budding stage.

The results of physical and chemical analysis of the studied farm soil in the experimental years are shown in Table Based on the obtained results, the studied farm soil had Medium salinity, alkaline acidity, low organic carbon, moderate to good phosphorus and potassium, and low consumption elements of iron, zinc, manganese and suitable copper and loam soil texture. Due to the high tolerance of quinoa to soil salinity, there was no need to wash the soil to reduce salinity. Also, based on these results, potassium and phosphorus fertilizers and foliar application of low-consumption elements were not used due to the appropriate level of these elements (Table 1).

Table 1. Results of physical and chemical analysis of experimental farm soil before testing.

	CLAY	SILT	SAND	Cu	Mn	Zn	Fe	K	P	N	OC	pH	Ec
Year		%				PPM					%		$dS.m^{-1}$
2017	23.3	45.1	31.6	1.52	19.85	1.53	7.4	420	13.4	0.03	0.48	7.3	3.6
2018	25.1	39.7	35.2	1.7	17.2	1.2	8.1	371	12	0.03	0.52	7.38	2.8

EC, electrical conductivity; OC, organic carbon

Experimental Design

The study was carried out using a factorial experiment in a randomized complete blocks design with three replications. The factors were two crop varieties (VAR: V1: Titicaca and V2: Sajama), Four levels of nitrogen: (NIT: N0: no nitrogen (control), N75: 75 kg. ha^{-1}, N150: 150 kg. ha^{-1}, N225: 225 kg. ha^{-1}) (Dehghani and Rezaei, 2019), and three levels of plant densities (DEN: D20: 20, D30: 30 and D40: 40 plants .m^{-2}) which were compared in two years (Year: year1: 2017 and year2: 2018). A plot was 2 × 4 m with a 1 m space between the plots. Planting was done in 2018 on July 25 and in 2017 on August 10. Irrigation was done regularly to maintain the soil moisture at field capacity in the summer.

Data Collection

Before carrying out the experiment by taking samples from a depth of 0-30 cm to determine the physical and chemical properties of the soil, including soil texture, P-Hash, electrical conductivity, the amount of organic matter as well as the amount of nutrients were measured for this purpose with an atomic absorption device. Iron, copper, manganese and zinc were also measured with a spectrophotometer. The concentration of phosphorus was determined using the yellow brush colorimetric method (Sabramanian and Charest, 1997) and the concentration of potassium was also measured after diluting the extract with a flame photometer (Ali Ahyaei and Behbahanizadeh, 2012) (Table1)

All the measured response variables are shown in Table 1. In each treatment plot, the plant height (PH) and dry biomass yield (DBW) were measured at the physiological maturity stage (when the plant leaves started to dry and fall off). The number of branches per plant (BN) was counted physically at the physiological maturity stage. The grain yield (kg. ha^{-1}) and Thousand-grain Weight (TGW) were also measured. The TGW was obtained by weighing 1000 grains of quinoa. The Harvest Index (HI) was defined as the ratio of grain yield and the above-ground biomass at maturity and Nitrogen use efficiency (NUE) was obtained by dividing grain yield by available plant nitrogen (Shajaripour, and Mojaddam, 2022). To measure the grain yield and biological yield, after removing the margin, one square meter of each plot was harvested manually, and after determining the moisture content, the grain yield was calculated based on the moisture content of 14%.

Statistical Analysis

The observations were independent of each other; data followed a normal distribution, and thus, a three-factor analysis of variance (ANOVA) combined at two years was run to compare the growth parameter of the *C. quinoa* Willd under different variety, different nitrogen rate, planting density, and growing year and the means obtained by Duncan's multiple range test were compared. All data were analyzed using minitab18 and MStatC v.4.3 statistical software (Steel and Torrie, 1980).

RESULTS

The results (Table 1) indicated that the simple effects of year, variety, nitrogen, and crop density on the studied traits were significant ($p \leq 0.01$). The interaction effect of var.× year on plant height, BN, and TB; the interaction effect of Nit× Year on all of the traits except TGW and Yield; interaction effect of Var. × Nit., Den. × Year and Den.× Nit. on all traits was significant ($p \leq 0.01$). Triple effects of Var. × Nit. × Year was significant on all treatments except TGW and YIELD. Other triple interactions significantly affected all the studied traits ($p \leq 0.01$). The results showed a significant effect of the triple interaction of the studied factors with Year on all studied factors except HI (Table 2).

Table 2. Mean Comparisons of Simple Effect of Year, Variety, Nitrogen, and Crop Density in terms of the Studied Traits in Quinoa.

Treatments	PH	BN	TB	T.G.W	YIELD	HI	NUE
Year							
2017	70.21a	6.28a	12200.75a	2.56a	2337.34a	18.52a	11.984a
2018	55.82b	5.85b	9068.16b	2.17b	1447.88b	16.08b	6.778b
Var.							
Titicaca	63.23a	6.65a	11053.84a	2.75a	2325.99a	20.94a	11.616a
Sajema	62.80 a	5.49b	10215.08b	1.97b	1459.23b	13.66b	7.146b
Nit.							
N0	59.94d	5.17d	8407.63d	1.91d	1362.78d	16.24b	-
N75	61.44c	5.74c	10059.84c	2.24c	1701.57c	16.49b	9.032b
N150	64.05b	6.19b	11172.43b	2.55b	2102.18b	18.28a	9.86a
N225	66.64a	7.18a	12897.93a	2.74a	2403.91a	18.18a	9.254b
Den							
D20	60.72c	6.42a	9675.82c	2.67a	1425.99c	14.66c	7.072c
D30	62.87b	6.05b	10727.13b	2.29b	1924.98b	17.60b	7.85b
D40	65.46a	5.74c	11500.42a	2.13c	2326.87a	19.64a	13.224a

In each column, the presence of at least one similar letter indicates that there is no significant difference based on the Duncan test.

Plant Height (PH)

Comparison of means showed lower quinoa plant height in 2017 than 2018 (20.4%). According to the results, the height of the Titicaca cultivar was higher than Sajema. The mean comparison showed that plant height increases significantly with increasing nitrogen. The highest plant height was obtained from 225 kg. ha^{-1} nitrogen, 11.7% more than the control (Table 3). Increasing the density caused a significant increase in plant height. Plant height at D30 and D40 increased by 3.5 and 7.8 percent, respectively, compared to D20.

Table 3. Mean Comparisons of Year Interaction with Variety, Nitrogen and Crop Density.

Treatments		PH	BN	TB	T.G.W	YIELD	HI	NUE
Year	Var.							
2017	Titicaca	64.50b	6.86a	13062.61a	3.05a	2762.80a	20.86a	14.28a
	Sajema	75.93a	5.70c	11338.90b	2.06c	1911.88b	16.19b	9.69b
2018	Titicaca	61.97c	6.43b	9045.06c	2.45b	1889.19b	21.02a	8.95c
	Sajema	49.68d	5.28d	9091.26c	1.88d	1006.57c	11.14c	4.61d
Year	Nit.							
2017	N0	65.71d	5.36f	10450.08d	2.09f	1660.1e	15.75e	-
	N75	68.40c	5.94d	11798.29c	2.44d	2092.3c	17.33c	11.52b
	N150	71.73b	6.44c	12741.87b	2.75b	2603.4b	19.89b	12.58a
	N225	75.01a	7.39a	13812.78a	2.95a	2993.6a	21.11a	11.85b
2018	N0	54.16g	4.98g	6365.19g	1.73g	1065.5g	16.74cd	-
	N75	54.49g	5.53e	8321.39f	2.05f	1310.8f	15.66e	6.5d
	N150	56.38f	5.93d	9602.99e	2.34e	1601.0e	16.67d	7.1c
	N225	58.26e	6.97b	11983.0c	2.54c	1814.2d	15.25e	6.65 cd
Year	Den.							
2017	D20	69.17c	6.67a	10875.85c	2.66a	1539.1cd	13.88f	5.154e
	D30	70.25b	6.26b	12339.87b	2.54b	2396.4b	19.12b	9.782b
	D40	71.22a	5.92d	13386.54a	2.46c	3076.6a	22.57a	12.02a
2018	D20	52.28f	6.16c	8475.79f	2.68a	1312.9e	15.44e	8.99c
	D30	55.48e	5.84e	9114.39e	2.03d	1453.6d	16.08d	5.916d
	D40	59.71d	5.56f	9614.30d	1.79e	1577.2c	16.71c	5.43de

In each column, the presence of at least one similar letter indicates that there is no significant difference based on the Duncan test.

Plant height in the Sajema cultivar showed an environmental response than Titicaca. The decrease in plant height in 2017 compared to 2018 was 3.9% and 34.5% for Titicaca and Sajema cultivars, respectively (Table 3).

The highest plant height was obtained in 2017 with 225 kg. ha^{-1} of nitrogen (75.01 cm). At each nitrogen level, plant height in 2018 decreased compared to 2017. This reduction was 17.58, 20.34, 21.40, 22.33% for N0, N75, N150, and N225 treatments, respectively. The height of the quinoa plant in 2017 in the D40 treatment was 71.22 cm, which was in the highest statistical group compared to other treatments. The height of the quinoa plant in 2018 compared to 2017 in D20, D30, and D40 treatments decreased by 24.42, 21.02, and 16.16%, respectively (Table 4).

Table 4. Mean Comparisons of Variety Interaction with Nitrogen and Crop Density.

Treatments		PH	BN	TB	T.G.W	YIELD	HI	NUE
Var.	Nit							
Titicaca	N0	60.37de	5.38e	8419.33f	2.22d	1671.73e	20.29b	-
	N75	62.05c	6.21c	10702.97d	2.60c	2094.49c	19.64c	11.272b
	N150	64.06b	6.84b	11793.49c	2.96b	2587.81b	21.91a	12.218a
	N225	66.46a	8.17a	13299.55a	3.22a	2949.96a	21.90a	11.362b
Sajema	N0	59.51e	4.97g	8395.93f	1.60g	1053.83g	12.20f	-
	N75	60.84d	5.27f	9416.71e	1.89f	1308.64f	13.34e	6.794d
	N150	64.05b	5.53d	10551.36d	2.13e	1616.56e	14.65d	7.502c
	N225	66.82a	6.19c	12496.31b	2.26d	1857.87d	14.46d	7.146cd
Var.	Den.							
Titicaca	D20	61.14d	7.21a	10378.09d	3.09a	1856.6c	18.29c	9.38c
	D30	63.25b	6.59b	11147.24c	2.67b	2368.3b	21.19b	9.49c
	D40	65.32a	6.15c	11636.18a	2.49c	2753.1a	23.34a	15.98a
Sajema	D20	60.31d	5.63d	8973.56e	2.25d	995.3e	11.04f	4.77e
	D30	62.49c	5.50e	10307.02d	1.90e	1481.7d	14.00e	6.21d
	D40	65.61a	5.34f	11364.66b	1.77f	1900.6c	15.94d	10.47b

In each column, the presence of at least one similar letter indicates that there is no significant difference based on the Duncan test.

There was no statistically significant difference in the height of the quinoa plant in each of the studied cultivars at each nitrogen level. The highest plant height was obtained from Sajema and Titicaca cultivars at 225 kg. ha^{-1} nitrogen (66.82 and 66.45 cm, respectively). Based on these results, the highest rate of plant height changes at 225 kg. ha^{-1} nitrogen compared to the control was related to the Sajema cultivar (12. 8%). Plant height in both cultivars increased with increasing plant density. Plant height in D40 treatment compared to D20 in Titicaca and Sajema cultivars increased by 6.8% and 8.7%, respectively. At each density level, the plant height increased with increasing the amount of nitrogen. The highest plant height was obtained from the interaction of D40 × N225 treatments (70.64 cm) (Table 3).

Due to the triple interaction of Year, cultivar, and nitrogen, the highest plant height was obtained from Sajema X N 225 treatment in 2017 (82.3 cm). The results showed a decrease in plant height in 2018 compared to 2017 in all similar treatments interaction between the three effects. By moving from 2017 to 2018, plant height in each cultivar and at each nitrogen level decreased. Based on these results, the percentage of height reduction at each nitrogen level in the Sajema cultivar was higher than Titicaca.

The plant height of the Sajema cultivar in 2017 was higher than 2018, which increased with increasing density in each cultivar. The highest plant height was obtained from Sajema X D40 interaction in 2017 (77.43 cm) (Table 4).

Branches Number (BN)

The highest number of branches per plant was obtained from quinoa plants planted in 2017 (6.28). The average number of branches per plant of Titicaca cultivar was 21.1% higher than Sajema. The results showed that the number of branches increases with increasing nitrogen levels. This increase in N225 treatment compared to N0 was 38.8%. The number of branches in N225 treatment with an average of 7.18 branches per plant was in the highest statistical group between different nitrogen levels. Increasing plant density decreased the number of branches per plant. The number of branches at D30 and D40 density levels decreased, respectively, compared to D20, 5.8, and 10.6%.

The highest number of branches in Titicaca cultivar was obtained in each experimental site. Titicaca cultivar planted in 2017 had the highest number of branches among Year and cultivar interaction treatments (6.86 branches per plant).

With increasing the amount of nitrogen in each Year, the number of branches and per plant increased. The number of branches in 2017 for levels N0, N75, N150, and N225, compared to the same levels in 2018, showed an increase of 17.6, 20.3, 21.4 22.3%, respectively. The highest number of branches was obtained from the interaction of D40 treatment in 2017 region (6.67 branches per plant) (Table 3).

The number of branches in each cultivar increased with increasing nitrogen levels. The rate of increase in N225 treatment compared to control without nitrogen (N0) in Titicaca and Sajema cultivars was 51.8% and 24.5%, respectively (Table 4).

With increasing density in each cultivar, the number of branches decreased. The decrease in the number of branches in D40 treatment compared to D20 was 14.7% in Titicaca cultivar and 5.15% in Sajema (Table 4).

The highest number of branches of Titicaca cultivar was obtained in 2017 with a nitrogen level of 225 kg. ha^{-1} (8.4 branches per plant). In 2018, the same treatment had the highest number of branches (6.6 branches per plant) (Table 4).

In the interaction of each cultivar and density, the highest number of branches was obtained from the D20 treatment. The number of branches in 2017 in similar treatments was more than 2018. Accordingly, the highest number of branches was obtained from the triple interaction of Titicaca × D20 treatment in 2017 (7.5 per plant) (Table 4).

Total Biomass t (TB)

The highest TB (12200.75 kg. ha^{-1}) with a 34.5% increase was obtained in 2017 compared to 2018 (Table 2). With increasing nitrogen levels, the TB of the plant increased. TB at 75,150 and 225 kg. ha $^{-1}$ N level increased by 19.7,32.9 and 53.4%, respectively, compared to control. According to the results compared to the control, increasing plant density increased plant dry weight per unit area (Table 5).

Table 5. Summary of treatments, Acronyms used and measurement units.

Treatments	Acronyms used	Units used
Year	Year	
Nitrogen	Nit.	
Density	Den.	
Variety	Var.	
Response variables		
Branch number per plant	BN	
Total biomass	TB	kg. ha^{-1}
Grain yield	Yield	kg. ha^{-1}
Thousand-grain weight	TGW	G
Harvest index	HI	-
Nitrogen use efficiency	NUE	$Kg.Kg^{-1}$

TB in each cultivar increased with increasing nitrogen levels. The highest amount of TB was obtained from the interaction of Titicaca × N 225 (13299.5 kb). The increase in TDB in N225 treatment compared to control (N0) was 57.5% for Titicaca cultivar and 48.8% for Sajema (Table 2). The results showed an increase in TB due to increased plant density in each cultivar.

The highest TB was obtained from the triple interaction treatment of 225 N × Titicaca in 2017 (14818.5 kg. ha^{-1}), significantly higher than other treatments. The results showed that TB changes between the 225 kg. ha^{-1} nitrogen and control treatment in each cultivar in 2017 were less than 2018. In Titicaca, the cultivar was more than Sajema (Table 4).

In 2017, the highest TB was obtained from Titicaca × D40 interaction treatment (13647.6 kg. ha^{-1}), while this value was 9624.8 kg. ha^{-1} in 2018. In all interaction treatments, density and average TB cultivar in 2018 were lower than the same treatment in 2017 (Table 4).

Grain Weight (TGW)

The average TGW in 2017 was higher than 2018 (17. 9%). The results showed that TGW in Titicaca cultivar was 39.5% higher than Sajema. In this study, increasing the amount of applied nitrogen increased TGW. The highest TGW belonged to 225 kg. ha^{-1} nitrogen (2.74 g) treatment. Increasing plant density decreased TGW (14.2% and 20.2% in D30 and D40 treatments compared to D20, respectively). Evaluation of the interaction between place and cultivar showed that the highest amount of TGW belonged to Titicaca cultivar in 2017 (3.05 g). In both places, the TGW of Titicaca cultivar was more than Sajema. The TGW loss in Titicaca and Sajema cultivars in 2018 was 19.6% and 8.7%, respectively lower than 2017. The highest TGW was obtained in 2017 using 225 kg. ha^{-1} nitrogen (2.95 g), 41.6% more than the control treatment without nitrogen. This increase was 46.8% in 2018. TGW reduction due to an increase in density in 2017 in D40 treatment compared to D20 was 7.5%, while this reduction in 2018 was 33.2%.

TGW was increased by increasing nitrogen levels in each cultivar. The highest amount of TGW was obtained from the interaction of Titicaca cultivar and N 225 treatment (3.22 g). With increasing density in each cultivar, TGW decreased. This decrease in D40 treatment compared to D20 was 19.4% for Titicaca cultivar and 21.3% for Sajema cultivar (Table 4).

The highest TGW was obtained from the interaction of 225 × Titicaca in 2017 (3.55 g), which was significantly highest. Similar treatment of nitrogen × cultivar interaction in 2018 decreased by 18.3% compared to this treatment.

Yield

The decrease in grain yield in 2018 was 38.05% lower than 2017. Also, the average yield was 2326 and 1459.3 kg. ha $^{-1}$in Titicaca and Sajema cultivars, respectively, which were divided into two statistically different groups.

An increase of nitrogen in N75, N150, and N225 treatments compared to control (N0) increased grain yield by 23.8, 54.2, and 76.4%, respectively. The results also showed an increase in grain yield due to increased plant density. The highest grain yield in the present study was obtained from D40 treatment with an average of 2326.85 kg. ha^{-1}. Changing the planting environment from 2017 to 2018 reduced grain yield in Titicaca and Sajema cultivars by 31.6% and 47.3%, respectively. Sajema grain yield was 30.7% lower than Titicaca in 2017 and 46.7% in 2018. The results showed that the increase in yield due to an increased nitrogen level of 225 kg. ha^{-1} compared to the control was 41.1% in 2017 and 46.8% in 2018. The results showed that the increase in yield due to increased nitrogen level was 225 kg. ha^{-1} compared to the control in 2017 80.3% and 2018 70.2%. The increase in yield in N0, N75, N150, and N225 treatments in 2017 compared to 2018 was 55.8, 59.6, 62.6%, and 65%, respectively.

The highest grain yield was obtained from the D40 treatment in 2017 (3076.6 kg. ha^{-1}). Grain yield increased with increasing plant density in each experimental year (Table 6). It also increased with increasing nitrogen levels in each cultivar. The highest grain yield was obtained from Titicaca cultivar in N 225 treatment (2949.9 kg. ha^{-1}). The increase in grain yield in this treatment was 76.4% compared to the same cultivar without nitrogen (control) (Table 2).

Table 6. Combined Analysis of Variance (Mean Squares) for Year, Variety, Nitrogen, Density, and their Interaction Effects in terms of Studied Traits in Quinoa.

SOV	DF	Means of Squares						
		PH	BN	TB	T.G.W	YIELD	HI	NUE
Rep.	2	1906.23^{ns}	17.68^{ns}	54284015.24^{ns}	2.61 ns	1719347.54^{ns}	143.6 ns	7.92^{ns}
Year	1	7454.22**	6.63**	353273160.81*	5.51**	28480520.05**	215.4	182.94**
Rep(year)	2	24.85	0.02	1177577.20	0.02	94935.07	0.70	0.61
Variety	1	6.65 **	48.44**	25326388.69**	21.88**	27046360.99**	1905.52**	134.76**
Var. × year	1	5060.37**	0.01**	28193615.65**	1.68**	9044.67^{ns}	243.93 ns	0.11^{ns}
Nitrogen	3	313.58**	25.93**	128419765.3**	4.78**	7470849.28**	42.05**	1.64**
Nit. × year	3	44.42**	0.03**	8168890.13**	0.01 ns	586237.51^{ns}	74.20**	0.12**
Var. × Nit.	3	4.84**	3.92**	3087113.52**	0.20**	390500.34**	5.04**	0.14**
Var. × Nit. × year	3	25.09**	0.09**	1341072.85**	0.01 ns	680.13^{ns}	0.51**	0.001**
Density	2	270.45**	5.44**	40259025.24**	3.74**	9776636.74**	300.03**	101.00**
Den. × year	2	88.43**	0.06**	5726291.99**	1.56**	4888639.36**	168.22**	215.26**
Den. × Var.	2	4.74**	1.82	3851225.04**	0.05**	3762.74^{ns}	0.10 ns	2.83**
Den. × Nit.	6	80.45**	1.22**	419335.61**	0.26**	318425.71**	11.93**	6.27**
Den. × year × Var.	2	0.74*	0.15**	6053355.20**	0.01**	126260.72**	1.74**	8.26**
Den. × year × Nit.	6	123.22**	0.06**	202580.61**	0.59**	654249.62**	44.82**	0.32*
Den. × Var. × Nit.	6	8.27**	0.45**	991124.63**	0.01**	13857.32**	2.43**	1.39**
Den.×year Var.× Nit.	6	5.74**	0.06**	1582126.06**	0.06**	35563.84**	10.61**	0.08^{ns}
Error	64	0.21	0.01	13640.71	0.01	3721.16	0.10	0.10
C.V. (%)		12.3	11.8	13.3	12.9	17.4	13.1	15.6

ns: non-significant **significant at the $p \leq 0.01$ level *significant at the $p \leq 0.05$ level

Increasing density in Titicaca cultivar from 20 to 40 plants .m^{-2}increased grain yield by 48.28%. This increase was 90.9% in Sajema cultivar. The highest grain yield was obtained from Titicaca X D40 interaction (2753.1 kg. ha^{-1}) (Table 2).

The results showed that grain yield increased with increasing density at each nitrogen level. The highest grain yield was obtained from the interaction of D40 × N 225 treatments (3044.6 kg. ha^{-1}). Based on these results, the yield of D40 treatment in interaction with N0, N75, N150, and N225 treatments;

53, 58.9, 63.4, and 72.1% increase compared to D20 treatment, respectively (Table 3).

The highest grain yield was obtained from the interaction of Titicaca ×N 22 treatments in 2017 (3537.8 kg. ha^{-1}). The lowest was obtained from the interaction of N0 × Sajema treatments in 2018 (744.7 kg. ha^{-1}). The yield increase in Titicaca cultivar in response to increased nitrogen levels in each of the studied year was greater than Sajema cultivar. With increasing nitrogen levels from 0 to 225 kg. ha^{-1} in Titicaca cultivar in 2017 and 2018, the yield increased by 80.7% and 70.04%, respectively. This increase for the Sajema cultivar was 79.7% and 79.3%, respectively (Table 4).

Grain yield in all similar treatments was higher in plant cultivar in 2017 than in 2018. D20 × Sajema in 2018 (925 kg. ha^{-1}) was obtained in the highest and lowest statistical groups (Table 7).

Table 7. Mean Comparisons of Variety Interaction with Nitrogen and Crop Density in terms.

Treatments		PH	BN	TB	T.G.W	YIELD	HI	NUE
Den.	Nit							
	D20	57.45h	13.37f	7364.69j	2.03h	1039.1i	14.23h	-
N0	D30	58.91g	12.88g	8448.49i	1.82i	1457.8g	17.23d	-
	D40	63.45e	11.76h	9409.71h	1.88i	1591.4f	17.27d	-
	D20	60.95f	14.76cd	9327.32h	2.56d	1326.8h	14.01h	7.67e
N75	D30	58.95g	14.29e	10112.22g	2.15g	1668.4f	16.19e	5.62h
	D40	64.43d	13.52f	10739.99f	2.01h	2109.5d	19.28c	13.81a
	D20	61.05f	15.12c	10139.20g	2.88b	1568.2f	15.52f	7.06f
N150	D30	67.78b	14.82cd	11172.91e	2.54d	2176.4d	19.09c	9.58c
	D40	63.33e	14.67d	12205.17c	2.22f	2562.0b	20.23b	12.94b
	D20	63.44e	16.30a	11872.08d	3.20a	1769.8e	14.90g	6.49g
N225	D30	65.83c	15.70b	13174.90b	2.65c	2397.3c	17.87d	8.35d
	D40	70.64a	15.05cd	7364.69j	2.39e	3044.6a	21.77a	12.92b

In each column, the presence of at least one similar letter indicates that there is no significant difference based on the Duncan test

Harvest Index (HI)

The results showed that the calculated HI in 2017 was 15.17% higher than 2018. Also, the harvest index in Titicaca cultivar was significantly higher than Sajema (20.94%). With increasing nitrogen levels, the harvest index increased. The harvest index in the N 150 and N 225 treatments was statistically similar. Based on the results, the harvest index increased with increasing plant density (Table 6).

The harvest index of Titicaca cultivar in 2017 and 2018 was not significantly different, but the Sajema cultivar harvest index in 2018 decreased significantly compared to 2017. In each year, the harvest index in the Sajema cultivar was lower than Titicaca. The highest harvest index in 2017 was obtained from the N 225 treatment (21.11%). In 2018, the highest average harvest index was 150 kg. ha^{-1} N (16.67%). Harvest index in each year increased with increasing density. The highest harvest index in the interaction of year and density was obtained from the D40 treatment in 2017 (22.57%) (Table 2).

The highest harvest index in Titicaca cultivar was obtained from the interaction with N 150 and N 225 treatments. These two treatments were in the highest statistical group without significant differences. In the Sajema cultivar, 150 and 225 kg. ha^{-1} N application in Sajema produced the highest HI (Table 3).

Also, the interaction of different plant density levels with the cultivar showed the highest harvest index in Titicaca X D40 treatment (23.34) (Table 3).

The highest harvest index was obtained from the D40 treatment at each nitrogen level. The highest harvest index was obtained from the D40 X N 225 treatment (21.77) (Table 4).

In 2017, the harvest index increased with increasing nitrogen level from zero to 225 kg.ha^{-1}. This increase was 28.2% and 44.8% for Titicaca and Sajma cultivars. The rate of decrease in Titicaca and Sajma cultivars was 9.1% and 8.6%, respectively (Table 7). The increase in harvest index by increasing the density from 20 to 40 plants.m^{-2} in 2017 was higher than 2018. This increase in 2017 was higher in Titicaca than in Sajma, but in 2018, the increase in harvest index in Sajema was greater than Titicaca (Table 8).

Nitrogen Use Efficiency (NUE)

The results showed Quinoa NUE in 2017 was higher than 2018 (76.8%) also greater NUE in the Titicaca cultivar (11.6 kg.kg^{-1}) compared to Sajema (7.14 kg.kg^{-1}). The NUE percentage increased to 150 kg. ha^{-1} with increasing nitrogen levels and then decreased. The highest Quinoa NUE was obtained from the D40 treatment, which increased by 86.9% and 68.4%, respectively, compared to D20 and D30 treatments.

In each studied year, NUE in Titicaca cultivar was higher than Sajema. Also, each cultivar in 2017 had more NUE than 2018. The highest calculated NUE was obtained from the Titicaca cultivar in 2017 (14.28 kg.kg^{-1}).

The results showed that the increase in yield due to increased nitrogen level was 225 kg. ha^{-1} compared to the control in 2017, 80.3% and in 2018, 70.2%. The increase in NUE in N0, N75, N150, and N225 treatments in 2017 compared to 2018 was 55.8%, 59.6, 62.6%, and 65%, respectively.

NUE in 2017 increased with increasing plant density, but this trend was declining in 2018. The highest amount of NUE was obtained from the D40 treatment in 2017 (Table 6).

Nitrogen use efficiency in each cultivar increased with increasing nitrogen till 150 kg. ha^{-1}and then decreased. In Titicaca × 150 kg. ha^{-1} N treatment, cultivar interaction at nitrogen levels was significantly higher than other interaction treatments (Table 3).

Nitrogen use efficiency in each cultivar increased with increasing plant density. The highest calculated NUE was obtained from D40 treatment in interaction with Titicaca and Sajema cultivars. The results also showed the highest NUE in interaction treatment of N 75 X D40 (13.81 kg.kg^{-1}) (Table 4).

With the increase of nitrogen level in each NUE cultivar, it increased to the level of 150 kg. ha^{-1} of nitrogen and then decreased. The highest calculated NUE belonged to the interaction of N50 in Titicaca cultivar in 2017 (14.98 kg. kg^{-1}), which was in the highest statistical group with a significant difference.

Table 8. Mean Comparisons of Year Interaction with Variety× Nitrogen and Variety × Crop Density.

Treatments			PH	BN	TB	T.G.W	YIELD	HI	NUE
Year	Var.	Nit.							
2017	Titicaca	N0	60.67i	5.6h	10626.0f	2.48e	1957.2f	18.49d	
		N75	64.00g	6.4e	12915.7c	2.90c	2475.3c	19.11d	13.82b
		N150	65.33f	7.0c	13890.3b	3.29b	3080.9b	22.13b	14.98a
		N225	68.00e	8.4a	14818.5a	3.55a	3537.8a	23.71a	14.05b
	Sajema	N0	70.76d	5.1k	10274.2g	1.69k	1363.0hi	13.02g	
		N75	72.80c	5.5i	10680.9f	1.98hi	1709.3g	15.55f	9.23d
		N150	78.12b	5.8g	11593.4e	2.22g	2125.8e	17.66e	10.17c
		N225	82.03a	6.3e	12807.1c	2.35f	2449.4cd	18.52d	9.66d
2018	Titicaca	N0	60.07i	5.2jk	6212.7l	1.95i	1386.3h	22.10b	
		N75	60.11i	6.0f	8490.2i	2.30f	1713.7g	20.18c	8.73e
		N150	62.78h	6.6d	9696.7h	2.64d	2094.7e	21.69b	9.45d
		N225	64.91f	7.9b	11780.6e	2.90c	2362.1d	20.09c	8.67e
	Sajema	N0	48.26l	4.8m	6517.7k	1.52l	744.7l	11.38h	
		N75	48.88l	5.0l	8152.5j	1.80j	908.0k	11.14hi	4.35f
		N150	60.67i	5.6h	9509.3h	2.04h	1107.3j	11.64h	4.83f
		N225	64.00g	6.4e	12185.5d	2.18g	1266.3i	10.40i	4.64f
Year	Var.	Den.							
2017	Titicaca	D20	63.75f	7.5a	12404.3c	3.18a	2012.5d	16.40e	6.64g
		D30	64.75e	6.8c	13136.0b	3.05b	2832.8b	21.40bc	11.36d
		D40	65.00de	6.3e	13647.6a	2.94c	3443.0a	24.78a	24.85a
	Sajema	D20	74.59c	5.8g	9347.4f	2.15e	1065.6g	11.37fg	3.67i
		D30	75.75b	5.7h	11543.8d	2.04f	1959.9de	16.83e	8.21e
		D40	77.43a	5.6i	13125.5b	1.99f	2710.1c	20.35d	17.18b
2018	Titicaca	D20	58.53h	6.9b	8351.9i	3.01b	1700.8f	20.18d	12.12c
		D30	61.74g	6.4d	9158.5fg	2.30d	1903.7e	20.98c	7.63f
		D40	65.63d	6.0f	9624.8e	2.03f	2063.2d	21.89b	7.10g
	Sajema	D20	46.03k	5.4j	8599.7h	2.34d	925.0h	10.71g	5.86h
		D30	49.23j	5.3k	9070.3g	1.76g	1003.5gh	11.17fg	4.20i
		D40	63.75i	5.1l	9603.8e	1.54h	1091.2g	11.53f	3.76i

In each column, the presence of at least one similar letter indicates that there is no significant difference based on the Duncan test

DISCUSSION

The results showed that Titicaca cultivar had more branches, total biomass, and thousand-grain weight than Sajema, resulting in increased crop yield. Titicaca cultivar had a higher harvest index and higher NUE. The increase in dry matter in 2017 can be considered due to the favorable temperature and length of the day during the growing season. Because dry matter production and accumulation are closely related to grain yield, a proper planting date can guarantee increased grain yield because more photosynthetic material is transferred to the grain.

This study showed that increasing nitrogen levels increased all the measured indices. Increasing plant density increased plant height, dry matter weight, grain yield, harvest index, and nitrogen use efficiency. The number of branches and seed weight decreased significantly by increasing plant density. The measured response variables in the two year were statistically significantly different. This difference in the two years may be due to differences in climatic conditions and physical and chemical properties of the soil in the experimental farms of both years. According to research, quinoa reacts strongly to nitrogen fertilizer application (Rehman et al., 2022).

The results showed that the plant height increased with increasing nitrogen application and planting density. Consumption of high amounts of nitrogen fertilizer by expanding the root surface and increasing the soil's absorption of water and elements stimulates plant growth. It also increases leaf area and photosynthetic capacity of the plant, increases the amount of assimilates; therefore, cell division and elongation increase plant height. The results showed that nitrogen and plant density on the number of branches of quinoa was significant. The number of branches decreased with increasing density. This is due to the competition at higher densities, reducing space allocated to each plant (Gimplinger et al., 2008).

The results showed at high levels of nitrogen, the investment of photosynthetic materials in the leaf and stem sections increases and increase plant biomass, followed by the leaf area index and thus better ground coverage of the field area, increases light use efficiency, which in itself leads to increases the biological yield of plants. Also, in high densities, biomass increases due to better plant use of soil and water. Geren (2015) showed that biomass accumulation is linearly and positively affected by nitrogen supply, and the maximum biomass accumulation was achieved at the highest level of nitrogen (200 kg. ha^{-1}).

Based on the results, nitrogen, plant density, and nitrogen× density interaction were significant for grain yield. An increase in yield with increasing nitrogen application is related to numerous functions of this element in essential plant processes include promoting vegetative growth, increasing photosynthetic activity, increasing the number of lateral branches, number of flowers per plant, increasing the dry matter production, and finally increasing grain yield (Sosa-Zuniga, 2017). The thousand-grain weight changes trend showed that thousand-grain weight increased in quinoa with increasing nitrogen. It seems that increasing nitrogen application prolongs the effective grain-filling period. As a result, the grains have more opportunities to fill and become heavier (Afzal et al., 2022). The results of Shoman (2018) showed that the highest thousand-grain weight in quinoa (4.75 g) was obtained from the application of 150 kg. ha^{-1} nitrogen. According to the results obtained in this study, the thousand-grain weight decreased with increasing plant density. Increasing plant density increases the number of seeds per unit area, resulting in less access to photosynthetic material per seed, reducing seed weight (Yang et al., 2020).

Harvest index, which is the ratio of grain yield to biological yield, indicates the efficiency of distribution of photosynthetic material among vegetative and production parts of the plant. The harvest index shows the amount of plant biomass allocated to the grain and indicates the plant's ability to allocate resources between vegetative and reproductive structures. In the present experiment, the effect of density and nitrogen fertilizer treatments on the harvesting index was significant at the probability level of one percent. The results showed that at each level of nitrogen, with increasing plant density, the harvest index increased due to increased biological yield compared to grain yield in this process. In this regard, Wang et al. (2020) and Shahmansouri (2015) also reported that the quinoa harvest index increased by increasing nitrogen consumption.

Also, the study of different densities on rapeseed cultivars showed that with increasing density, grain harvest index also increases, which is probably due to the decrease in the weight of vegetative components and increase in the number of seeds per unit area (Angadi et al., 2003)

The analysis of variance showed that the effect of plant density and nitrogen levels and their interaction with NUE was significant with increasing nitrogen level, NUE first increased and then decreased. The highest amount of NUE calculated was related to 150 kg. ha^{-1} nitrogen level. It has been shown that increasing the amount of nitrogen reduces the efficiency of nitrogen consumption (Modhej and Fathi, 2008). According to the law of diminishing returns, the primary fertilizer units used to have a greater effect on growth and performance improvement. As the number of used nitrogen increases, its efficiency will decrease. Increasing plant density increased nitrogen use efficiency. In investigating the effect of plant density on crop yield of two Quinoa cultivars, Asher et al. (2022) reported that the highest crop yield at the highest density.

One way to measure the productivity of fertilizers, especially nitrogen, is to evaluate the NUE. This index indicates an increase in yield per input unit (Delbert and Ulter, 1989). How to uptake, utilization efficiency, and nitrogen allocation in plants can be affected by moisture, soil fertility, and competition. Nitrogen uptake and use in grain production require effective nitrogen uptake, transfer, assimilation, and redistribution processes. The relationship between these processes and genotypic differences in nitrogen use efficiency between genetic communities and different climates and nitrogen storage (Sandhu et al., 2021). The results nitrogen use efficiency showed a statistically significant difference between the studied cultivars. This seems to be due to the genetic structure of these cultivars and their ability to absorb nitrogen. Cárdenas-Castillo et al. (2021) also reported genetic differences in nitrogen use efficiency in quinoa cultivars. Taghizadeh and Syed Sharifi (2011) also reported a significant difference between the cultivars of corn in terms of nitrogen fertilizer efficiency. The highest efficiency belonged to single cross hybrid 404 and the lowest to hybrid 301.

CONCLUSIONS

The present experiment results showed that the yield potential of quinoa seeds is affected by different years and the amount of nitrogen fertilizer used, variety, and density. In 2017, with suitable climatic conditions for growth and a longer grain filling period, a better growth environment was provided for photosynthetic materials and, finally, quinoa grain yield than 2018. The results showed that improving quinoa grain yield improves characteristics such as thousand-grain weight, harvest index, and biological yield. The results showed that increasing nitrogen significantly affects total biomass, which results in increased grain yield and biological yield. In this study, Titicaca cultivar in both years was significantly superior to Sajema cultivar, and increasing the nitrogen level to 225 kg. ha $^{-1}$ increased all the studied response variables. Accordingly, the best-proposed density in the present experimental conditions to achieve maximum grain yield in quinoa is 40 plants per square meter.

ACKNOWLEDGEMENTS

The authors are grateful to the technical and research personnel of Islamic Azad University, Arsanjan branch, for their cooperation in the implementation of this research.

This article was derived from a PhD thesis at the Islamic Azad University-Arsanjan branch.

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