Journal of Plant Physiology and Breeding
2023, 13(2): 197-215 ISSN: 2008-5168

Research paper

The effect of sowing date and planting density on grain yield and some nutritional quality characteristics of three seed-quinoa genotypes

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Received: August 24, 2022 Accepted: November 9, 2022
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Abstract
Quinoa (*Chenopodium quinoa* Willd) is a plant with high nutritional value and has good adaptability to different growing conditions. To investigate the effects of planting date and density on the seed quality of three quinoa genotypes (Q29, Titicaca, and Red Carina), a field experiment was conducted in 2019-2020 at the research field of Agriculture and Natural Resources Campus, Razi University, Kermanshah, Iran. The seeds were sown on three dates (15th March, 15th April, and 15th May) under two planting densities (40 and 60 plants/m^2). The experiment was conducted as a split-plot factorial design based on the randomized complete block design with three replications. The results revealed that both sowing date and plant density influenced the nutritional composition of the quinoa genotypes. The grain yield varied between 1642 and 2351 kg/ha, and the amino acids profile varied according to planting date and planting density. The amino acids histidine, glutamic acid, leucine, and lysine increased with delaying sowing date whereas threonine, tyrosine, arginine, methionine, glycine, proline, isoleucine, valine, serine, alanine, aspartic acid, asparagine, cysteine, phenylalanine, and glutamine decreased with delaying the sowing date from 15 March to 15 May. The Titicaca genotype produced maximum grain yield under the third sowing date while in the case of the first sowing date, all studied genotypes were more responsive regarding most amino acids. Also, the most suitable plant density to result in the highest grain yield was 60 plants/m^2. The mineral content of the quinoa seeds was not influenced significantly by the sowing date, plant density, genotype, and their interactions, except for calcium which was affected significantly by the genotype. The lowest saponin content was observed in Titicaca under the planting density of 40 plants/m^2. These results indicated that both sowing dates and plant densities influence the nutritional composition of quinoa genotypes.

Keywords: cultivar, grain yield, minerals, nutritional value, protein

How to cite: Rabbani B, Khoramivafa M, Saeidi M, Zarei L, Bagheri M. 2023. The effects of sowing date and planting density on grain yield and some nutritional quality characteristics of three seed-quinoa genotypes. J Plant Physiol Breed. 13(2): 197-215.

Introduction

As a pseudocereal, quinoa (*Chenopodium* quinoa Willd.) is a native plant in the Andean region (Sharma *et al*. 2015). It has a broad genetic diversity, thus considerable adaptability to challenging environments such as highlands and frosts (Nowak *et al*. 2016). Quinoa is a broadleaf plant with starchy dicotyledonous seeds. Therefore, the quinoa seeds can be used as cereals or as whole grains in salads, cooked meals, breakfast, or soups, and it can be milled into flour to produce pasta,

bread, biscuits, and pancakes (De Bock *et al*. 2021).

Interestingly, quinoa was also called the "mother grain" in the indigenous culture (Sharma *et al*. 2015). Quinoa has high protein content with high quality due to its essential amino acids. In addition, quinoa seeds contain essential unsaturated fatty acids and minerals (Präger *et al*. 2018). Protein nutritional quality is determined by the proportions of essential amino acids (Miranda *et al.* 2012). Depending on the variety, the protein content of quinoa seed is in the range of 12 to 23%. Its protein content is higher than common grains but lower than oilseeds and legumes (Dakhili *et al*. 2019). Quinoa has a gluten-free protein, and one of the few plant foods that contain all nine essential amino acids. It is also high in fiber, magnesium, B-vitamins, iron, potassium, calcium, phosphorus, vitamin E, and various beneficial antioxidants (Awadalla and Morsy 2017).

Planting date for some quinoa genotypes play a prominent role in their production. Variation exists in the quinoa germplasm for response to planting date (Hinojosa *et al*. 2018). Planting date impacts plants' phonological and growth characteristics (Jahanbkhsh *et al*. 2020). Rathore *et al*. (2019) demonstrated that protein content in quinoa seeds was significantly higher when the crop was sown on February 15. Hammad *et al*. (2021) reported that planting quinoa plants (cv. Regalona) on October 10 at 84.000 plants/acre significantly increased most of the quality traits of the quinoa crop under the climatic conditions of Aswan Governorate.

Plant density per unit area is one of the critical factors that determines the yield of quinoa. In very high densities, the intra-species competition reduces yield, and in densities of less than optimal, the environmental facilities such as light, space, and soil, are not used optimally, thus reducing yield (Xia *et al.* 2019). Eisa *et al*. (2018) reported that protein, calcium, and magnesium concentrations in quinoa seeds increased at the low planting density, whereas carbohydrate concentration decreased.

Quinoa is a newly introduced crop in the Kermanshah, Iran. Therefore, exploring its adaptability to local agro-climatic conditions under varying sowing dates is necessary. Therefore, a field study was conducted in this area to evaluate the effect of sowing date and planting density on grain yield and amino acid profile of the quinoa seed.

Materials and Methods

Plant material and field experiment

A field experiments was conducted during the 2019–2020 growing season at the experimental field of the Faculty of Agriculture, Razi University, Kermanshah, Iran (longitude of 47° 6′ E, altitude of 36° 19′ N, and altitude of 1318 m above sea level).

Plant material was kindly provided by the Seed and Plant Improvement Institute, Karaj, Iran. Long-term average temperature and precipitation during growth period of quinoa (March to August) at the experimental site were 11.6–29.5 ºC and 194.99 mm, respectively. The soil samples were taken from 0-30 cm depth before planting the quinoa genotypes and the soil properties are shown in Table 1.

Table 1. The physical and chemical characteristics of the experimental field.

pH	Texture	EC[1] ($dS.m^{-1}$)	OM[2] (%)	BD[3] (g/cm^{-3})	FC[4] (%)	N (%)	P (ppm)	K (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)	B (ppm)
7.75	Loam	0.83	1.16	1.30	35	0.116	11.4	440	1.14	1.03	4.8	3.23	0

Experimental design

The experiment was conducted as a split-plot factorial design based on a randomized complete block design with two replications. The sowing dates (March 15, April 15, and May 15) assigned to main plots, and the factorial combinations of two plant densities (40 and 60 plants/m^2) and three varieties (Titicaca, Q29, and Red Carina) were arranged in the subplot. The plot had the size of 4 m^2 (3.20 m × 1.25 m), including five rows with a between-row distance of 0.25 m. Three months before planting, 2000 kg ha^{-1} cattle manure was used, and the field was furrowed to a depth of 0.3 m with disk plow to prepare seedbeds before sowing. Seedbed up to 8 cm deep was prepared two days before sowing using rotary harrow. Planting was done mechanically on the three mentioned planting dates at 1–2 cm depth, with 40 and 60 plants/m^2.

The rain system was used to irrigate the quinoa plants whenever needed. Plots were harvested from three central rows (1.25 m^2). To avoid border effects, 0.50 m was removed at the beginning and the end of each plot. The harvested seeds were stored at room temperature in dry conditions for further analysis.

Mineral composition

The mineral content of dry grains was analyzed following Miranda *et al*. (2012). The phosphorus content was determined using a spectrophotometer (Shimadzu Instruments, Inc., Spectrophotometer UV-120-02, Kyoto, Japan) at 470 nm. Potassium content was determined through the flame atomic emission spectroscopy. Calcium, sodium, iron,

[1] Electrical conductivity
[2] Organic material
[3] Bulk density
[4] Field capacity

manganese, and zinc content were measured using a flame atomic absorption spectrophotometer (phoenix-986 England). The minerals were expressed in mg/kg.

Amino acids profile

***The extraction and hydrolysis of samples for amino acid analysis*:** For each unit weight of samples, 5 ml of 5.5 N hydrochloric acid was added and incubated at 110 °C for 18 h. The hydrolyzed liquid was neutralized with bicarbonate and centrifuged at 1300 g for 10 min. The liquid was separated on a float, and 200 µL was taken and mixed separately with 50 µL of the substance (4–(4-dimethylaminophenylazo) benzene sulfonyl chloride (mg/ml) to convert the amino acids into dansyl chloride and was incubated for 60 min at 60 °C. Then, acetone in the solution of dansyl chloride was evaporated and used for high-performance liquid chromatography (HPLC) injection (Qi *et al*. 1991).

***Evaluation of the extracted amino acids*:** To measure amino acids, 20 micro liters of the extracted sample was injected into HPLC (Unicamcrystal-200, England). The device column was a C_{18} reverse phase column with an inner diameter of 250 mm x 4.6 mm, a diameter of particles inside the column of 5 µm. The mobile phase consisted of two solutions: ultrafine acetonitrile and a solution of 25 mmol sodium acetate with pH 5.9. The mobile phase passed through the column at a flow rate of 0.1 ml/min at a temperature of 40 °C. Initially, the mobile phase was only a buffer (acetonitrile). After four minutes, the concentration ratio reached 60% to 40% (pure acetonitrile/sodium acetate) and remained with this ratio for 10 minutes. Then, washing with 100% acetonitrile continued until the ending of time. The percentage of each amino acid was determined based on the area under the peak output curve and relative to the standard peak of each amino acid (Wayne 2012).

Saponin content

To determine the saponin content, a 5 ml of deionized water was added to 0.5 g of the quinoa seeds in a tube. The tube was shaken for 40 seconds (four shakes per second), After the formation of a white foam, the tube was turned upside down and the height of the created foam on the water was measured. Then, the content of saponin was calculated by by the following formula (Koziol 1991):

$$\text{Saponin (mg)} = \frac{\{0.008 + \text{Created foam } (cm) \times (0.423)\}}{\text{Sample weight (gr)}}$$

Statistical analysis

The normality of the data was evaluated using the Lilliefors-corrected Kolmogorov-Smirnov test by SPSS IBM 23. The analysis of variance based on the split-plot factorial design was done using the PROC MIXED procedure of the SAS software (Ver. 9.1). Multiple comparisons were made using the least

significant difference (LSD) test at a probability level of 5%. The Pearson's correlation coefficients were computed by SPSS IBM 23.

Results and Discussion

Grain yield

The effect of sowing date × planting density × genotype interaction on grain yield of the quinoa was statistically significant ($p \leq 0.01$) (Table 2). The highest grain yield (2350.67 kg/ha) was observed in the Titicaca genotype on the May 15 sowing date and plant density of 60 plants/m^2, while the Q29 genotype displayed the lowest grain yield (1642.00 kg/ha) on the sowing date of March 15 and plant density of 40 plants/m^2 (Figure 1). It seems that the reason for increasing grain yield on the third planting date is the optimum temperature in May, resulting in faster plant growth, more vigorous plants, and higher grain yield as suggested by Mirzaie *et al*. (2020). Bagheri (2021) reported significant differences ($p \leq 0.01$) for the effect of sowing date on seed yield. February 5 and May 5 were the best sowing dates for the Q29 and Titicaca genotypes, respectively. Khanalizadegan *et al*. performed an investigation into the effect of planting date on yield and yield components of quinoa. The results showed that planting date has a significant effect on the grain yield, 1000-grain weight, number of panicles, and biomass. The planting date of February 19 and August 20 had the highest plant height as compared to other dates. The highest hectoliter weight was obtained on February 19. The relationship between the maximum temperature and yield at first significantly increased and then decreased (cubic model). The highest yields from February 19 to August 20 were related to the suitable planting conditions in spring and also the higher water use efficiency in this period, so the most suitable planting dates were 19 and 29 of February.

Increasing density to 60 plants/m^2 resulted in a higher grain yield than 40 plants/m^2, while Wang *et al*. (2020) reported that the grain yield of quinoa decreased with increasing plant density in China. Rodríguez Gómez *et al*. (2021) reported that grain yield in quinoa varied according to location, genotype, and agro-ecological conditions. Sayad *et al.* conducted an experiment in Egypt with one quinoa cultivar and two planting densities namely, 56.000 plant ha^{-1} (Low) and 167.000 plant ha-1 (High). Grain yield increased by 34.7% with the increase of plant density from 56.000 plant ha^{-1} to 167.000 plant ha^{-1}. The increase in plant density significantly decreased 1000-seed weight and hectoliter weight. Owji *et al.* (2021) showed that increasing the seeding rate from 6 to 10 kg ha^{-1} led to an increase of grain yield from 1716.33 to 3622.92 kg ha^{-1} in the Fars province of Iran.

Mineral elements

The entire mineral contents of quinoa seeds were not changed significantly by the sowing date, plant density genotypes, and their interactions, except for calcium that was affected significantly by the genotype (Table 2). The highest calcium content (0.308%) was recorded in Q29 and the lowest content (0.150%) was obtained in Red Carina and Titicaca (Table 3). Similar results were found by Matías *et al.* (2021). They illustrated that Ca content differed significantly among

Table 2. The effect of planting date, planting density, and genotype on grain yield, minerals, and amino acids of quinoa.

SOV	df	Mean squares								
		Grain yield	Zn	Fe	Mn	Na	Ca	K	P	Saponin
Replication	1	1283.5	20334.8	2629965.0	756.6	0.00008	0.123	0.111	0.093	0.218
Sowing date (SD)	2	1447556**	3338.2	1211282.4	0.37	0.00016	0.187	0.022	0.050	0.614
Error 1	2	539.6	2158.5	1097885.5	754.7	0.00003	0.048	0.045	0.010	0.034
Density (D)	1	4760.2*	10346.3	897.0	29.0	0.00005	0.023	0.004	0.006	0.284**
Genotype (G)	2	9586.7**	3960.0	308173.1	628.5	0.00000	0.100*	0.039	0.001	3.035**
SD × D	2	623.4	2058.8	114104.5	35.6	0.00007	0.041	0.092	0.002	0.008
SD × G	4	5127.5**	4285.1	267884.1	199.2	0.00005	0.033	0.044	0.004	0.044
D × G	2	794.4	3549.8	12677.3	22.3	0.00004	0.006	0.019	0.001	0.004
SD × D × G	4	3784.3**	4520.1	68518.85	114.7	0.00003	0.010	0.031	0.005	0.062
Error 2	15	905.4	4204.7	175957.6	232.6	0.00007	0.018	0.048	0.013	0.028
CV (%)		1.5	13.7	14.9	23.2	31.160	16.9	15.2	17.8	3.3

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

Table 2 continued

SOV	df	Mean squares									
		His	Thr	Val	Tyr	Lys	Ser	Arg	Ala	Cys	Asp
Replication	1	0.047	0.175	0.107	0.006	0.018	0.127	0.026	0.001	0.127	0.003
Sowing date (SD)	2	2.670**	4.632**	6.854**	2.587**	4.760**	4.658**	7.911**	6.776**	5.817**	6.419*
Error 1	2	0.018	0.048	0.031	0.017	0.018	0.025	0.023	0.006	0.051	0.071
Density (D)	1	0.050	0.108	0.260**	0.094	0.149*	0.227**	0.327**	0.308**	0.029	0.033
Genotype (G)	2	1.827**	2.866**	4.217**	0.960**	2.792**	2.857**	4.361**	3.788**	3.405**	3.963**
SD × D	2	0.002	0.045	0.001	0.002	0.025	0.035	0.047	0.016	0.089**	0.063
SD × G	4	0.154**	0.095*	0.076	0.102**	0.062	0.019	0.088*	0.067	0.049**	0.085
D × G	2	0.012	0.066	0.028	0.344**	0.020	0.013	0.038	0.025	0.018	0.081
SD × D × G	4	0.063	0.020	0.030	0.026	0.014	0.015	0.039	0.074	0.059**	0.045
Error 2	15	0.022	0.024	0.028	0.021	0.021	0.022	0.022	0.035	0.010	0.076
CV (%)		8.5	7.2	6.5	7.3	6.0	5.456	6.961	6.719	4.846	11.093

*, **: Significant at the 5% and 1% probability levels, respectively; Amino acids abbreviation: His: Histidine, Thr: Threonine, Val: Valine, Tyr: Tyrosine; Lys: Lysine, Ser: Serine, Arg: Arginine, Ala: Alanine, Cys: Cysteine, Asp: Aspartic acid.

Table 2 continued

SOV	df	Mean squares									
		Glu	Met	Gly	Pro	Phe	Ile	Leu	Gln	Asn	Try
Replication	1	0.360	0.072	0.072	0.105	0.414	0.099	0.031	0.016	1.613	0.013
Sowing date (SD)	2	8.129**	4.604**	2.278*	4.571**	8.263**	3.029**	5.967**	7.184**	21.064*	4.111**
Error 1	2	0.011	0.042	0.039	0.002	0.012	0.001	0.043	0.008	0.275	0.016
Density (D)	1	0.120*	0.279**	0.081	0.163**	0.165*	0.167**	0.295**	0.164	2.181*	0.045
Genotype (G)	2	5.598**	2.582**	1.266**	2.675**	4.850**	1.315**	3.599**	3.568**	9.723**	2.482**
SD × D	2	0.009	0.099**	0.222	0.008	0.030	0.075*	0.022	0.020	0.448	0.026
SD × G	4	0.079*	0.095**	0.236*	0.154**	0.185**	0.064*	0.125**	0.046	0.403	0.022
D × G	2	0.078	0.068*	0.179	0.089*	0.007	0.051	0.053*	0.101	0.061	0.053
SD × D × G	4	0.063	0.029	0.045	0.041	0.108*	0.011	0.031	0.140*	0.418	0.018
Error 2	15	0.021	0.013	0.060	0.018	0.023	0.018	0.013	0.037	0.323	0.027
CV (%)		4.743	5.632	13.399	7.485	4.123	6.869	6.021	5.571	8.332	7.366

*, **: Significant at the 5% and 1% probability levels, respectively; Glu: Glutamic acid, Met: Methionine, Gly: Glycine, Pro: Proline, Phe: Phenylalanine, Ile: Isoleucine, Leu: Leucine, Gln: Glutamine, Asn: Asparagine, Try: Tryptophan.

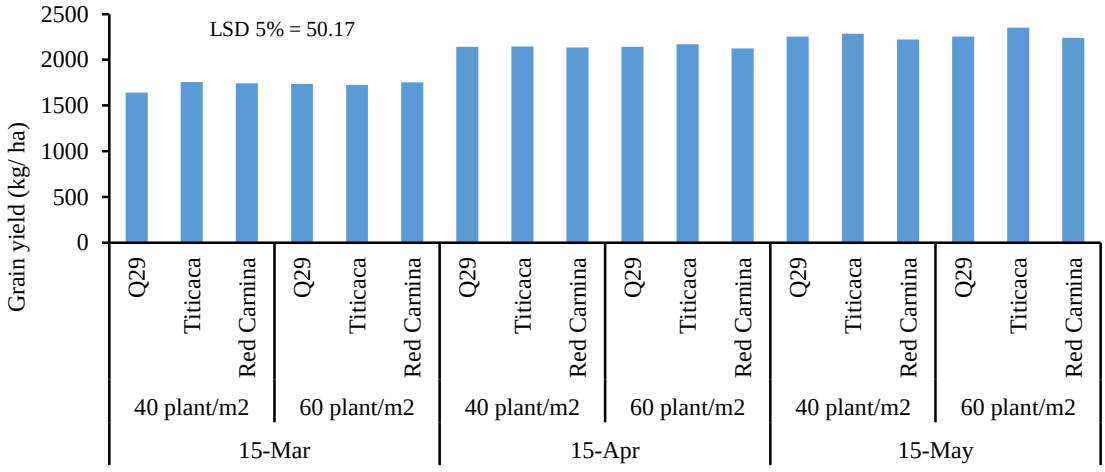

Figure 1. The combined effect of planting date, planting density, and genotype on grain yield of quinoa seeds.

the genotypes. Rodríguez Gómez *et al.* (2021) indicated no significant difference among quinoa varieties in terms of phosphorus, calcium, and iron, while potassium, magnesium, and sodium led to significant differences. Granado-Rodríguez *et al.* (2021) showed that for some minerals, such as magnesium, no significant difference was found among genotypes, while for others, such as potassium, a slight variation was observed among the quinoa genotypes. According to Prado *et al.* (2014), the mineral concentration of quinoa grains showed a high variability depending on the genetic and environmental factors. Moreover, Awadalla and Morsy (2017) found that mineral elements (P, K, Ca, and Fe) in the quinoa grain was affected significantly by sowing date and genotype and

the highest contents were recorded in the Regalona genotype and the sowing date of 1st November.

Amino acid profile

***Histidine*:** The main effects of sowing date and genotype, and their interaction were statistically significant ($p \leq 0.01$) for the histidine in the quinoa grain (Table 2). The maximum histidine content (2.770 mg/100g) was observed in the Titicaca on May 15, and the minimum histidine content (1.125 mg/100g) was recorded in Q29 on March 15 (Table 4). Quinoa contains more histidine than the wheat and rice proteins but is comparable to corn (Dakhili *et al*. 2019). Präger *et al*. (2018) also found significant differences among cultivars and years for the histidine. Präger *et al*. (2018) and Rodríguez Gómez *et al*. (2021) reported the highest content of histidine (2.48 g/100g) in the Puno variety of quinoa.

***Threonine*:** The effects of sowing date, genotype, and the interaction of sowing date × genotype on the threonine amino acid of the quinoa grains were significant (Table 2). The mean comparisons indicated that the maximum threonine content (2.755 mg/100g) was observed in the Q29 on March 15, and the minimum threonine content (1.173 mg/100g) was recorded in the Red Carina on May 15 (Table 4). Präger *et al*. (2018) reported considerable variations in the composition of the threonine across varieties. Quinoa proteins are high in threonine, which are, in general, the

Table 3. The effect of planting date, planting density, and genotype on some amino acids and Ca of quinoa grains (mg/100 g)

Planting date	Val	Lys	Ser	Ala	Asp	Asn	Tryp
15 March	3.387	1.782	3.361	3.569	3.223	8.172	2.858
15 April	2.513	2.474	2.615	2.682	2.463	6.772	2.184
15 May	1.883	3.039	2.123	2.075	1.760	5.523	1.693
LSD (5%)	0.311	0.236	0.277	0.135	0.468	0.921	0.225

Plant density	Val	Lys	Ser	Arg	Ala	Glu	Ile	Asn	Saponin
40 plant/m^2	2.679	2.367	2.779	2.236	2.868	3.015	2.033	7.067	5.005
60 plant/m^2	2.509	2.496	2.621	2.046	2.683	3.131	1.897	6.576	5.183
LSD (5%)	0.119	0.103	0.105	0.105	0.132	0.104	0.096	0.404	0.119

Genotype	Val	Lys	Ser	Ala	Asn	Ca	Asp	Tryp	Saponin
Q29	3.161	1.989	3.154	3.321	7.577	0.308	1.860	2.261	5.192
Titicaca	2.644	2.946	2.184	2.807	7.064	0.150	2.593	2.692	4.550
Red Carina	1.978	2.360	2.762	2.198	5.826	0.150	2.993	1.783	5.542
LSD (5%)	0.146	0.126	0.128	0.162	0.495	0.116	0.239	0.144	0.146

His: Val: Valine, Lys: Lysine, Ser: Serine, Ala: Alanine, Asp: Aspartic acid, Asn: Asparagine, Try: Tryptophan; Arg: Arginine, Ala: Alanine, Glu: Glutamic acid, Ile: Isoleucine.

Table 4. The combined effect of planting date and genotype on some amino acids of quinoa grains (mg/100 g)

Sowing date	Genotype	His	Thr	Tyr	Arg	Glu	Met	Gly	Pro	Ile	Leu
15 March	Q29	1.125	2.755	2.307	2.470	1.655	2.257	2.673	2.948	2.375	1.580
	Titicaca	1.455	2.257	2.260	3.053	2.122	2.975	2.248	2.480	2.875	1.143
	Red Carina	1.240	2.448	2.775	3.475	2.820	2.905	1.952	2.000	2.295	0.845
15 April	Q29	1.347	2.238	1.583	1.225	2.412	1.420	2.120	2.310	1.833	2.757
	Titicaca	2.297	2.740	2.019	2.127	3.063	1.995	1.360	1.815	2.312	1.803
	Red carina	1.550	1.447	2.197	2.762	4.088	2.510	1.898	1.010	1.407	1.353
15 May	Q29	1.780	1.563	1.140	0.815	3.270	0.935	1.742	1.588	1.463	3.188
	Titicaca	2.770	2.002	1.670	1.473	3.735	1.632	0.990	1.172	1.795	2.523
	Red Carina	2.100	1.173	1.752	1.867	4.495	1.877	1.535	1.002	1.333	2.080
LSD (5%)		0.112	0.117	0.109	0.112	0.109	0.086	0.185	0.101	0.101	0.086

His: Histidine, Thr: Threonine, Tyr: Tyrosine, Arg: Arginine, Glu: Glutamic acid, Met: Methionine, Gly: Glycine, Pro: Proline, Ile: Isoleucine, Leu: Leucine.

limiting amino acids in the important cereals like wheat and maize (Nowak *et al*. 2016).

***Valine*:** Table 2 data shows that sowing date, plant density, and genotype had significant effect ($p \leq 0.01$) on valine of the quinoa grains, but the combined impact of these factors were not significant. The maximum amount of valine (3.387 mg/100g) was recorded on March 15 (Table 3). The maximum valine content (2.679 mg/100g) for the plant density was observed under 40 plants/m2. Similarly, Craine and Murphy (2020) reported that the valine content of the quinoa grains varied by location, sowing date, and planting density. The maximum valine content was observed in the Q29 variety (3.161 mg/100g) (Table 3). According to Khaitov *et al*. (2020), the genotype significantly influenced the composition of the valine essential amino acid in the quinoa.

***Lysine*:** Table 2 shows that the effect of sowing date, plant density, and genotype on the lysine content of quinoa seeds were statistically significant ($p \leq 0.01$). However, their combined effects were not significant. The maximum content of lysine (3.039 mg/100g) was recorded on May 15. The maximum content of lysine (2.496 mg/100g) was measured at 60 plants/m^2 density. The maximum content of lysine (2.946 mg/100g) was observed in Titicaca (Table 3). Reguera *et al*., (2018) reported that Regalona seeds grown in Spain showed higher lysine and methionine contents than the Chilean Regalona seeds. It is evident that quinoa protein has high lysine content, which agrees with the findings of Nowak *et al.* (2016) and Reguera *et al*. (2018).

***Serine*:** This amino acid was affected significantly ($P \leq 0.01$) by sowing date, plant density, and genotype. The combined effects were not significant (Table 2). The maximum

serine content (3.361 mg/100g) was recorded on March 15. The maximum serine content (2.779 mg/100g) was obtained at 40 plants/m^2 plant density. The maximum serine content (3.154 mg/100g) was observed in Q29 (Table 3). Miranda *et al*. (2012) reported that the amino acid content of quinoa seeds differed by genotype aand Villarrica genotype had the highest amino acid content, including histidine, leucine, lysine, methionine, phenylalanine, tyrosine, taurine, glycine, and serine. In contrast, Cáhuil displayed the lowest content among amino acids.

***Arginine*:** The effect of sowing date, plant density, and genotype on the arginine content of quinoa seeds was statistically significant ($p \leq 0.01$). Also, the interaction of sowing date with genotype for arginine content was significant ($p \leq 0.05$) (Table 2). The maximum content of arginine (3.475 mg/100g) was obtained on March 15 in Red Carina and the minimum content (0.815 mg/100g) was recorded on May 15 with Q29 genotype (Table 4). After glutamic amino acid content, the most abundant amino acid in the quinoa seeds analyzed in the present study was arginine. The same results were reported by (Reguera *et al*. 2018).

***Alanine*:** The results of the analysis of variance showed that the effect of sowing date, density, and genotype was significant ($p \leq 0.01$) on the alanine content of the quinoa seeds (Table 2). The maximum alanine content (3.569 mg/100g) was recorded on March 15. The maximum alanine content (2.868 mg/100g) was obtained at 40 plants/m^2. Also, the maximum alanine content (3.321 mg/100g) was measured in Q29 and the lowest content of alanine (2.198 mg/100g) was obtained in Red Carina (Table 3). This result corroborated the results of Khaitov *et al*. (2020) regarding the fact that the amino acid composition of quinoa seeds varied among genotypes.

***Cysteine*:** The amino acid cysteine content was affected significantly ($p \leq 0.01$) by sowing date, genotype, and the interaction between sowing date and genotype, sowing date and density, and sowing date, density, and genotype (Table 2). The highest cysteine content (3.425 mg/100g) was recorded on March 15 at 40 plants/m^2 in Titicaca. Besides, the minimum cysteine content (0.880 mg/100g) was obtained on May 15 at 60 plants/m^2 plant density in Red Carina (Figure 3). Reguera *et al*. (2018) similarly revealed that amino acid profiles in quinoa seeds depend on the location, climate, and cultivar.

Tyrosine: Planting date, genotype, and planting date × genotype and plant density × genotype interaction for the tyrosine amino acid concentration were significant ($p \leq 0.01$) (Table 2). The highest concentration of tyrosine (2.775 mg/100 g) was on the planting

date of March 15 and the Red Carina genotype. The lowest concentration of tyrosine (1.140 mg/100 g) was obtained on the planting date of 15 May and the Q29 genotype (Table 4). The highest and lowest concentrations of tyrosine (2.283 and 1.563mg/100 g) were obtained in the Red Carina and Q29 genotypes at the density of 40 plants/m^2 respectively. (Figure 2). Präger *et al*. (2018) also reported a significant difference among cultivars and years for the quinoa essential amino acid.

***Aspartic acid*:** The results of the analysis of variance showed that only the effects of the sowing date and genotype were significant ($p \leq 0.01$) on the aspartic acid content of the

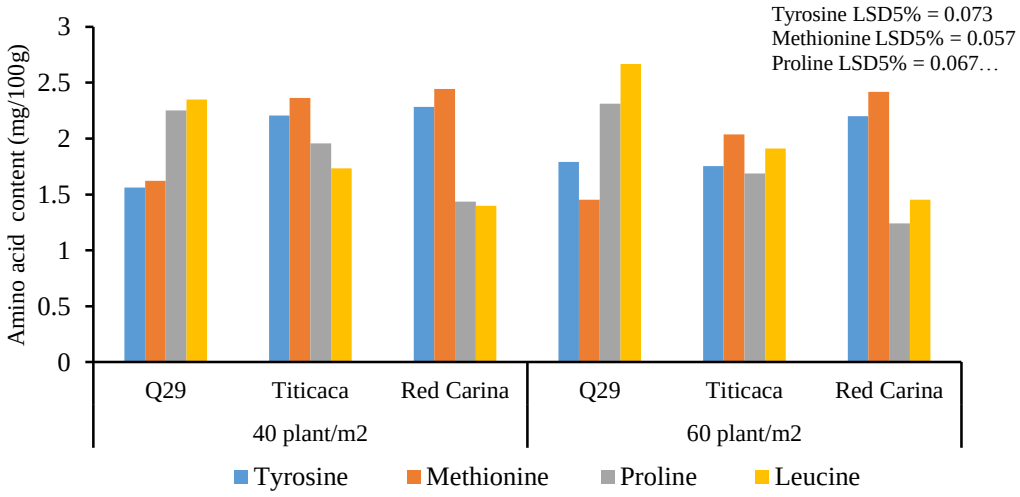

Figure 2. The combined effect of planting density and genotype on some amino acids of quinoa grains.

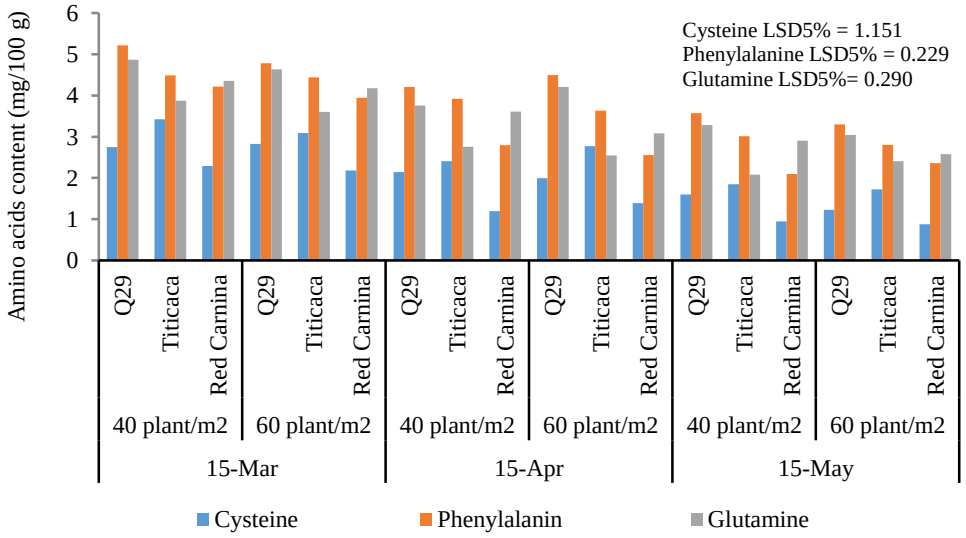

Figure 3. The combined effect of planting date, planting density, and genotype on some amino acids of quinoa grains.

quinoa seeds (Table 2). The highest content of aspartic acid (3.223 mg/100g) was measured on Mach 15. This study illustrated that the aspartic acid content decreased when the sowing date was delayed. The same result was reported by (Thanapornpoonpong *et al.* 2008), who indicated that aspartic acid content was affected by sowing date in the amaranth seeds. The maximum content of aspartic acid (2.993 mg/100g) was found in Red Carina, and the minimum aspartic acid content (1.860 was measured in Q29 genotype (Table 3). Relevant studies indicated that the amino acid content in quinoa seeds depended on the seeds' cultivation region and genetic variability (Gonzalez *et al*. 2012).

Glutamic acid: The effects of sowing date and genotype were significant ($p \leq 0.01$). Also, the interaction of sowing date with genotype was significant ($p \leq 0.05$) (Table 2). Comparing the means of the interactions between the sowing date showed that the highest glutamic content (4.445 mg/100g) was found on May 15 in Red Carina, and its lowest content (1.655 mg/100g) was measured on March 15 in Q29 genotype (Table 4). According to Rodríguez Gómez *et al*. (2021), was a significant difference in the glutamic acid concentration of quinoa varieties (11.84 - 15.21 g per 100 g), and glutamic was the predominant amino acid in all varieties.

***Methionine*:** The table of analysis of variance showed that the effects of sowing date, plant density, genotype, and their interactions (sowing date and genotype), (sowing date and density), and (density and genotype) were significant on the methionine content of quinoa seeds (Table 2). The highest content of methionine (2.975 mg/100g) was found on March 15 in Titicaca, and the lowest content of methionine (0.935 mg/100g) was measured on May 15 in Q29 (Table 4). Delaying the sowing date evidently decreased the methionine content.

The highest and lowest methionine content (2.897 and 1.405 mg/100 g) were observed for the planting date of March 15 with a density of 40 plants/m^2 and the planting date of May 15 and the density of 60 plants/m^2, respectively. Also, the highest methionine content (2.445 mg/100 g) was obtained at the density of 40 plants/m^2 for the Red Carina genotype, while the lowest methionine content (1.452 mg/100 g) was observed at the density of 60 plants/m^2 for the genotype Q29 (Figure 2). The range of contents of the amino acids described was similar to those in Gonzalez *et al*. (2012). In contrast, Granado-Rodriguez *et al*. (2021) found no significant difference among quinoa cultivars for methionine content in the seeds.

***Glycine*:** Table 2 indicates that the glycine content of the quinoa seeds was affected significantly by sowing date, genotype, as well as the interaction of the sowing date and genotype. The highest content of glycine (2.673 mg/100g) was found on March 15 in Q29, and the lowest content of glycine (0.990 mg/100g) was recorded on May 15 in Titicaca genotype (Table 4). Delaying the sowing date decreased glycine content. The same results were found by Jacobsen and Christiansen (2016). They reported that the amino acid compositions in amaranth seeds decreased significantly by delaying the sowing date.

***Proline*:** According to Table 2, the effect of sowing date, plant density, genotype, and their interactions (sowing date and genotype) and (density and genotype) were significant ($p \leq 0.01$). The highest proline content (2.948 mg/100g) was found on March 15 in the Q29 genotype (Table 4). The interaction between density and genotype indicated that the highest proline content (2.312 mg/100g) was obtained at 60 plants/m^2 density in Q29 (Figure 2). Proper planting dates can enhance biosynthesis and photosynthesis processes and complete the seed ripening process to maximize yield, thus reducing the risk of unfavorable environmental conditions and improving the quality of quinoa seeds (Ning *et al*. 2020). In line with our results, Granado-Rodriguez *et al*. (2021) reported the highest proline content in Q29. They indicated a remarkable variation in the proline content among varieties.

***Phenylalanine*:** The analysis of variance showed that sowing date, planting density, genotype, and their interactions (sowing date and genotype) and (sowing date, density, and genotype) had significant ($p \leq 0.01$) effect on phenylalanine content of the quinoa seeds (Table 2). The maximum content of phenylalanine (5.215 mg/100g) was obtained on 15 March at 40 plants/m^2 plant density in Q29, whereas the minimum phenylalanine content (2.100 mg/100g) was recorded on May 15 at 40 plants/m^2 plant density in the Red Carina genotype (Figure 3).

***Isoleucine*:** Table 2 indicates that the effect of sowing date, density, and genotype was significant ($p \leq 0.01$) on the isoleucine content of the quinoa seeds. The interaction of the sowing date and genotype was also significant ($p \leq 0.05$). The highest content of isoleucine (2.875 mg/100g) was recorded on March 15 in the Titicaca genotype. On the other hand, the lowest isoleucine content (1.333 mg/100g) was obtained on May 15 in Red Carina (Table 4). Thanapornpoonpong *et al.* (2008) reported the highest essential amino acid content in the Tango variety. The opposite results indicated no differences in the amino acid composition in the quinoa seeds based on the genotype (Miranda *et al*. 2012).

***Leucine*:** Results showed the significant ($p \leq 0.01$) effect of sowing date, density, genotype, and sowing date × genotype and density × genotype interactions on the leucine content of the quinoa seeds (Table 2). The highest leucine content (3.188 mg/100g) was obtained on May 15 in Q29, whereas the lowest (0.845 mg/100g) was recorded on March 15 in the Red Carina genotype (Table 4). De Bock *et al*. (2021) observed a lower variation for the leucine content (204–278 mg/g protein) among the tested genotypes in Germany. The highest leucine content (2.667 mg/100g) was obtained at 60 plants/m^2 plant density in the Q29 genotype, whereas its lowest content (1.400 mg/100g) was recorded at 40 plants/m^2 plant density in Red Carina (Figure 2). Remarkable variation was found in the present study among the genotypes for the leucine profile in the quinoa seeds. The same results were reported by Präger *et al*. (2018). Shams and Galal (2014) reported that quinoa seeds contained high amount of essential amino acids, except for isoleucine, leucine, and phenylalanine.

***Glutamine*:** Data presented in Table 2 show that the sowing date and genotype had significant ($p \leq 0.01$) effects on glutamine content of the quinoa seeds. Also, the sowing date × density × genotype interaction was significant ($p \leq 0.05$). The maximum glutamine content (4.875 mg/100g) was recorded on March 15 at 40 plants/m^2 plant density in Q29. The minimum glutamine content (2.080 mg/100g) was observed on March 15 at 40 plants/m^2 plant density in Titicaca (Figure 3). The glutamine content decreased by delaying the sowing date.

***Asparagine*:** The analysis of variance (Table 2) revealed that the asparagine content of the quinoa seeds was significantly affected by the sowing date and plant density ($p \leq 0.05$), and also, by the genotype ($p \leq 0.01$). The highest (8.172 mg/100g) and the lowest (5.523 mg/100g) asparagine contents were obtained at March 15 and May 15, respectively, indicating that delaying the sowing date reduced the asparagine content. The highest asparagine content (7.068 mg/100g) was obtained at the 40 plants/m^2 plant density, whereas the lowest asparagine content (6.576 mg/100g) was recorded at the 60 plants/m^2 plant density. The results indicated that increasing plant density decreased the asparagine content. Also, the highest asparagine content (7.577 mg/100g) was measured in Q29, while Red Carina displayed the lowest asparagine content (5.826 mg/100g) (Table 3). Reguera *et al*. (2018) noted that varieties grown in Chile had no difference in amino acid content, while the cultivation of different varieties of quinoa in Spain caused variation in the asparagine content.

***Tryptophan*:** The results showed that

tryptophan significantly ($p \leq 0.01$) changed under sowing date and genotype, but their interaction was not significant (Table 2). The highest tryptophan content (2.858 mg/100g) was obtained on March 15, while the lowest tryptophan content (1.693 mg/100g) was recorded on May 15 (Table 3). This indicates that delaying the sowing date declined the tryptophan content in the quinoa seeds. The highest tryptophan content (2.692 mg/100g) was observed in Titicaca, whereas its lowest content (1.783 mg/100g) was recorded in Red Carina (Table 3). De Bock *et al.* (2021) found that the essential amino acid content of the quinoa seeds in different growing seasons varied but there was no difference between cultivars. The variations in amino acid contents could be attributed to the genetic or environmental growth conditions (Gonzalez *et al*. 2012; Miranda *et al*. 2012).

Sayad *et al*. showed that two planting densities (56.000 plant ha^{-1} and 167.000 plant ha^{-1}) affected protein, carbohydrate, and ash content. However, there were no significant differences between the two planting densities for the crude fiber and total fat. The calcium and magnesium content significantly increased at low density compared with high planting density. Meanwhile, no significant effects of plant density on phosphorus, potassium, iron, and zinc content in quinoa seeds were detected. They concluded that the yield increase at the higher plant density is associated with a significant reduction in seed quality in terms of protein content. On the other hand, low plant density significantly increased 1000-seed weight and hectoliter, which was reflected in the grain size. This trait is considered a very important parameter for the quinoa global market preference. Proper planting date may enhance biosynthesis processes and photosynthesis and complete the seed ripening process and consequently lead to higher yield, lower the risk of unfavorable environmental conditions, and improve the seeds quality (Hammad *et al*. 2021).

Saponin

The saponin content of quinoa, a bitter-testing set of terpenoids located in the outer layers of the seed coat, was affected significantly ($p \leq 0.01$) by plant density and genotype. No significant differences were found concerning the sowing date and all interactions (Table 2). The highest content of saponin (5.183 mg/g) was obtained with the plant density of 60 plants/m^2 and the lowest content of saponin (5.006 mg/g) was recorded with the plant density of 40 plan/m^2 (Table 3). Increasing plant density increased the saponin content. The same result was reported by Hammad *et al*. (2021) who found that increasing concentration of saponin quinoa seed was associated with increasing plant density and the maximum saponin (3.55%) was obtained under 84000 plants/ha in two seasons.

The highest concentration of saponin (5.542 mg/g) was obtained in Red Carina, and its lowest concentration was recorded in Titicaca (Table 3). It should be noted that the variation of saponin content in the quinoa seeds depends strongly on the genotype (Rodríguez Gómez *et al*. 2021). However, Granado-Rodríguez *et al.* (2021) by comparing two varieties, reported higher saponin content in Titicaca was higher compared to the Vikinga variety.

Conclusion

Our results highlighted that different sowing dates, planting density, and genotype could significantly have varied the seed yield and amino acid composition of quinoa seed, impacting the seed quality. Although not all the amino acids evaluated varied to the same extent, one can affirm that sowing date, planting density, and genotype determined the amino acid profile of the quinoa seeds.

Quinoa has become an important product in recent years due to its nutritional benefits and adaptability to different weather conditions. Thus, considering the effect of planting date and planting density on the nutritional quality characteristics of different quinoa seed genotypes can help farmers make more informed decisions on how to optimize their yield while maximizing the nutritional value of their crop. The effect of planting date and planting density on nutrition has practical implications for farmers and consumers who want to maximize the nutritional benefits of quinoa. However, considering the importance of income for farmers, the priority is the selection of genotype, planting date and density based on grain yield. Therefore, later sowing dates (April 15 and May 15) and the higher plant density of 60 plants/m^2 resulted in higher grain yield compared to the early sowing date and lower plant density. Furthermore, the Titicaca cultivar produced the highest grain yield on May 15 sowing date and a plant density of 60 plants/m^2.

On the other hand, if grain quality becomes more important to the farmer, it is recommended to choose the first planting date, because based on the results of the present experiment, most of the amino acids had better values in the first planting date. Additionally, the quinoa seeds' minerals did not show any significant response to planting date, plant density, genotype, and their interaction, except for calcium. Several areas of future research could help further our understanding of the impact of these factors on nutritional quality.

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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تأثیر تاریخ و تراکم کاشت بر عملکرد دانه و برخی خصوصیات کیفی تغذیه‌ای سه ژنوتیپ کینوا

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چکیده

کینوا (*Chenopodium quinoa* Willd) گیاهی با ارزش غذایی بالا است که به شرایط مختلف رشد نیز سازگاری قابل توجهی دارد. به منظور بررسی اثر تاریخ کاشت و تراکم بر کیفیت دانه سه ژنوتیپ کینوا (Q29، تیتیکاکا و ردکارینا)، یک آزمایش مزرعه‌ای در زمستان ۱۳۹۸ و بهار ۱۳۹۹ در مزرعه تحقیقاتی پردیس کشاورزی و منابع طبیعی دانشگاه رازی کرمانشاه در سه تاریخ کاشت (هفته سوم اسفند، هفته سوم فروردین و هفته سوم اردیبهشت) و دو تراکم (۴۰ و ۶۰ بوته در متر مربع) به صورت طرح اسپلیت پلات فاکتوریل در قالب طرح بلوک‌های کامل تصادفی در سه تکرار انجام شد. نتایج آزمایش نشان داد ترکیب غذایی ژنوتیپ‌های کینوا تحت تاثیر تاریخ کاشت و تراکم قرار می‌گیرد. عملکرد دانه بین ۱۶۴۲ و ۲۳۵۱ کیلوگرم در هکتار نوسان داشت و مشخصات اسیدهای آمینه بر اساس تاریخ کاشت و تراکم کاشت متفاوت بود. اسیدهای آمینه هیستیدین، اسید گلوتامیک، لوسین و لیزین با تاخیر در تاریخ کاشت افزایش یافتند ولی ترئونین، تیروزین، آرژنین، متیونین، گلیسین، پرولین، ایزولوسین، والین، سرین، آلانین، اسید آسپارتیک، آسپاراژین، سیستئین، فنیل آلانین و گلوتامین با تأخیر در تاریخ کاشت از ۱۵ اسفند تا ۱۵ اردیبهشت ماه کاهش یافتند. ژنوتیپ تیتیکاکا در تاریخ کاشت سوم حداکثر عملکرد دانه را تولید کرد، در حالی که در تاریخ کاشت اول همه ژنوتیپ‌های مورد مطالعه در مورد اکثر اسیدهای آمینه پاسخ بهتری نشان دادند. همچنین مناسب‌ترین تراکم بوته برای دستیابی به بیشترین عملکرد دانه، ۶۰ بوته در متر مربع بود. مواد معدنی بذر کینوا نسبت به تاریخ کاشت، تراکم بوته، ژنوتیپ و اثر متقابل آن‌ها تغییر معنی داری نشان ندادند به جز کلسیم که به طور معنی‌داری تحت تأثیر ژنوتیپ قرار گرفت. کمترین میزان ساپونین در ژنوتیپ تیتیکاکا در تراکم کاشت ۴۰ بوته در متر مربع مشاهده شد. این نتایج نشان داد که ترکیب مواد مغذی ژنوتیپ‌های کینوا تحت تاثیر تاریخ کاشت و تراکم قرار می‌گیرد.

واژه‌های کلیدی: ارزش تغذیه‌ای، پروتئین، رقم، عملکرد دانه، مواد معدنی