EFFECTS OF ROW SPACING ADVANCES AND CROP ROTATION WITH SORGHUM ON ROOT PERFORMANCE, YIELD AND QUINOA PRODUCTIVITY

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(Received 26th Jul 2025; accepted 2nd Oct 2025)

Abstract. The quinoa experimental base was established to investigate the effects of different row spacing's on the agronomic traits and yield formation of different quinoa varieties, and to clarify whether. The main results were as follows: Compared with the row spacing of 20 cm and 60 cm, the quinoa-sorghum row spacing of 40 cm significantly increased the root length, projected area, root surface area, root diameter, root volume, and root tip number. The number of root branches and root crosses significantly improved agronomic characteristics in each growth period sorghum-quinoa line with a row spacing of 40 cm and quinoa-sorghum with a row spacing of 20 cm, which was conducive to the accumulation of dry matter in the shoots. Quinoa-sorghum with a row spacing of 40 cm increased the SPAD value of leaves, and the effect was better in the middle and late stages, which was beneficial to plant photosynthesis. It improved the nitrogen content of plants at various growth stages, and the difference was significant at the panicle, flowering and grain filling stages, and increases the grain albumin in the mature stage. Plant height, primary branch, secondary branch, stem diameter under panicle, stem base diameter and main ear length were positively correlated with yield. Nitrogen fertilizer utilization efficiency, increasing grain protein content, improving grain quality, and achieving the goal of high yield, and quality.
Keywords: *quinoa, row spacing, fertility characteristics, soluble sugar, yield, quality*

Introduction

The cropping system, or cropping sequence, is a simple practice with a significant impacts agroecological conditions (Sun et al., 2016). In recent years, a combination of environmental and anthropogenic factors have exacerbated global warming and water scarcity, resulting in elevated plant transpiration rates and soil moisture depletion. Among the abiotic factors, drought is a significant environmental stress that profoundly affects crop growth (Riedesel et al., 2024). Water deficiency severely curtails plant growth, leading to diminished growth rates, dehydration, wilting, and even crop mortality, ultimately causing a substantial decline in crop yields (Raja et al., 2020). Furthermore, plants subjected to drought stress experience a reduction in leaf relative water content, which adversely affects chlorophyll synthesis (Bouchemal et al., 2017). Therefore, to further develop the quinoa industry in Shanxi, it is necessary to breed varieties that are adapted to the ecological environment of various regions. In the breeding work of quinoa-sorghum, domestic researchers only introduced some excellent varieties from abroad to conduct product ratio tests, and then obtained high-yield, high-efficiency, and high-quality quinoa-sorghum (Alotaibi et al., 2023; Müjgan et al., 2024).

Temperature is another significant form of abiotic stress compounded by climate change (Shehab et al., 2020). Although the quinoa industry has developed to a certain extent in China, there are widespread problems in quinoa production. It is easy to lodge, has low yield, poor disease resistance, and few excellent varieties, which have seriously affected the further expansion of the quinoa industry (Barman et al., 2024). Despite its importance, research on the impact of temperature changes on the physiological parameters of quinoa-sorghum remains limited (Falcioni et al., 2024). Some studies have suggested that temperature fluctuations affect leaf stomatal size, photosynthesis, and chlorophyll content and can lead to a reduction in leaf relative water content (Shuyskaya et al., 2023). The effects of temperature and drought on plants increase their osmotic pressure, with the tolerance threshold of plants in arid regions during their lifecycle largely dependent on environmental temperatures. Studies have demonstrated that high temperatures and drought often coincide, with one factor exacerbating the impact of the other, thereby imposing greater stress on the plants (Elakhdar et al., 2023).

Quinoa is an annual dicotyledonous plant of the amaranth family and a non-cereal grain crop (Sadak et al., 2019). Renowned for its nutritional richness, including proteins, amino acids, minerals, and other nutrients, quinoa-sorghum has earned the reputation of a 'complete nutritional food (Rakhmankulova et al., 2023). Indigenous to high-altitude, cool regions of South America, quinoa exhibits excellent biological characteristics such as cold and drought tolerance, making it highly recommended by the Food and Agriculture Organization of the United Nations for cultivation in arid and saline-alkaline regions (Jiang et al., 2024). Although recent studies on quinoa have primarily focused on variety selection, cultivation techniques, and molecular biology, there are limited reports on the effects of abiotic environmental stresses, particularly temperature and drought stress (Song et al., 2023). Therefore, it is important to study and explore the disease resistance characteristics of quinoa-sorghum and improve the yield and quality per unit area. Before the 21st century, there were few studies on quinoa in China (Oustani et al., 2023; Jiang et al., 2023). This experiment utilized the effects of temperature, drought, and their interaction on (1) the growth physiology performance of sorghum-quinoa, (2) to provide a theoretical basis and technical methods for high-yield cultivation of quinoa-sorghum, and (3) the chemical evaluation method was used to explore the quality of sorghum-quinoa, and the quality of different row spacings was further explored.

Materials and methods

The experiment was conducted in a field (37°42 'N,112°53' E) at the Experimental Base of the Sorghum Research Institute, Shanxi Agricultural University, China 2022-2024 (*Figure 1*). Located in the east of the Loess Plateau, Jinzhong Basin northeast, has a warm temperate continental climate, arid and semi-arid areas, with an altitude of 791 m (*Figure 2*). On April 20, soil fertility of 0-20 cm was measured, organic matter 12.65 $g·kg^{-1}$, alkaline nitrogen 42.56 $mg·kg^{-1}$, Available phosphorus 15.65 $mg·kg^{-1}$, available potassium 210.66 $mg·kg^{-1}$. The experiment consisted of two cropping methods: sorghum–fallow–sorghum continuous cropping, and quinoa–sorghum rotation. The sorghum seeds (Jinza 22) used in this study were sourced from the Sorghum Research Institute, Academy of Agriculture Sciences, Taiyuan, Shanxi Province, China. The seeds of the ‘Jiaqi 1’ variety of quinoa were procured from Shanxi Jiaqi Quinoa Development Co., Ltd. (Shuozhou, China).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2306_1123311255

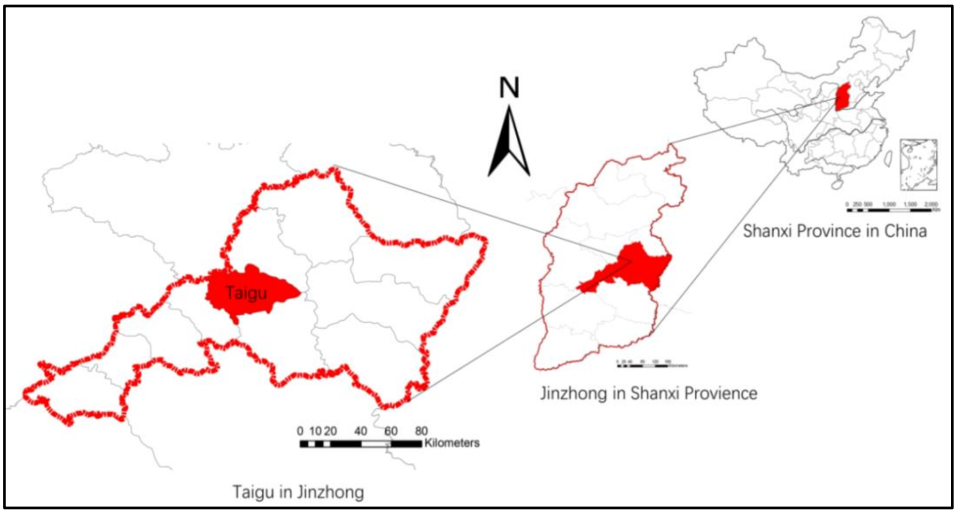

Figure 1. *Location and general situation of the test site at the Base of the Sorghum Research Institute, Shanxi Agricultural University, China*

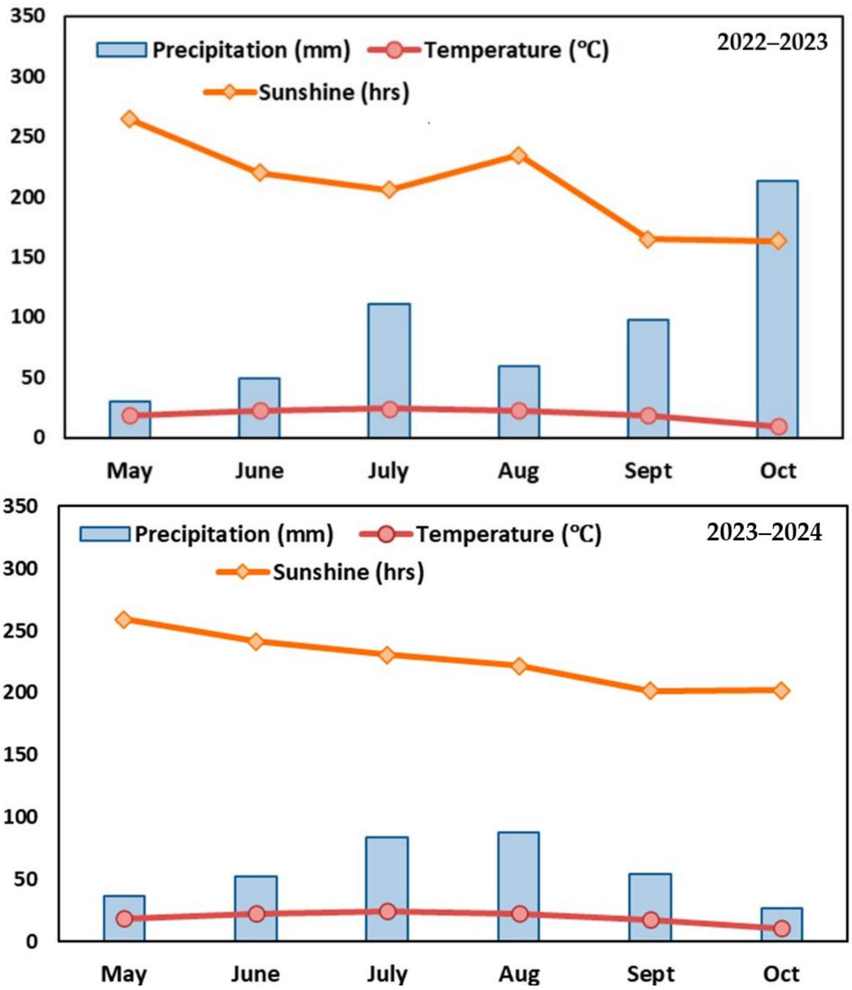

Figure 2. *The average monthly temperature, total monthly precipitation, and accumulated sunlight hours during the sorghum and quinoa crop growth period in (A) 2022–2023 and (B) 2023–2024*

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Experimental materials and design

The field experiment employed A two-factor split-plot design was conducted from May to October 2022–2024 at the Wheat Research Base of the Shanxi Agricultural University. Uniform-sized quinoa seeds were selected and disinfected by soaking in 75% alcohol for 2 min, followed by three rinses with sterile water, and drying with sterile paper. Ten seeds were evenly planted in each pot filled with a mixture of 2.5 kg soil and vermiculite (3:1). The seeds were placed in a constant-temperature chamber with a light intensity of 30000 lx for 14 h per day. The seeds of both crops were sown with a drill on May 5, 2022, and May 2, 2023. The area under each cropping system was 100 m2 (5 × 20 m), and each treatment was repeated three times. Sorghum was planted at a planting density of 117,000 plants ha^{-1}, row spacing of 50 cm, and plant spacing of 17 cm; the quinoa crop was planted at a planting density of 135,000 plants ha^{-1}, row spacing of 50 cm, and plant spacing of 15 cm. The crops were harvested on October 12, 2023, and October 9, 2024. Sorghum was sown in both years (2022 and 2023), whereas under the sorghum–quinoa rotation, quinoa was sown in 2022 and sorghum was sown in 2023. In the first year (2022), the soil properties were estimated at two different depths under the two cropping systems. In the second year (2023), soil properties were determined at different growth stages of sorghum, and sorghum yield was determined. The treatment design for both years was a randomized complete block design with three blocks. The amount of nitrogen, phosphorus, and potassium fertilizer applied was 180 kg ha^{-1} during the growth period, timely artificial weeding, and pest control.

Morphological index measurement and root length decay rate

The heading, anthesis, and filling stages of mature quinoa plants were uprooted from each treatment. After carefully washing the roots to remove soil, the seedlings were blotted dry using filter paper. Plant height and root length were measured using a ruler and fresh weights were recorded. Roots from the same layer across all three monoliths were pooled in a single string-mesh bag. After gently washing away the soil and removing organic debris, root length was quantified using a flat-bed scanner (Epson V600 Photo, Los Alamitos, CA, USA). Root dry weight was determined after oven-drying at 60 °C to constant mass (Noor et al., 2024).

Root weight density (RWD, g m^{-3})=root dry weight/excavated soil volume (Eq.1)

Root length density (RLD)=root length/excavated soil volume (Eq.2)

Leaf area index and relative chlorophyll content

At the anthesis, heading, anthesis, and filling stages, all green leaves were separated and their areas were measured using a leaf area meter (LI–3100C, LI–COR, Lincoln, NE, USA), and these measurements were then used to calculate the leaf area index. The relative chlorophyll content, expressed as SPAD values, was measured using a chlorophyll meter (SPAD-502 Plus, Japan). Five sampling points were selected for each experimental plot using the five-point sampling method. SPAD readings were obtained from the flag leaves at each sampling point, and the average of these measurements was recorded as the representative SPAD value for the leaf.

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Yield and its constituent factors

At the maturity stage of sorghum, two rows were harvested from each subplot (block) and the panicle fresh weight was recorded. The panicles were dried in an oven and the dry weight was measured. The grains were then removed, and the weight of grains per spike was measured. The spike fertility index (spike harvest index) was calculated as the ratio of the grain weight of the spike to the spike dry weight. The remaining panicles were harvested from the field and sun-dried until the moisture content reached 14% or less, and the grain yield (kg m^{-2}) was calculated and converted to tons per hectare.

Protein component content in grain

The protein and albumin, globulin, gliadin, and gluten contents in the grain were determined by continuous extraction.

Sucrose, soluble sugar and total starch content in the grain

The soluble sugar content in the grain was determined by anthrone colorimetry, and the sucrose content was determined by resorcinol colorimetry. Total starch content was measured using the anthrone colorimetric method.

Statistical analysis

The soil and yield data recorded during the two years were analyzed using ANOVA, and differences among treatments were determined using a post hoc test (Tukey test at $p < 0.05$). The correlation graph of soil traits at the maturity stage in 2022 and yield traits was drawn using the Corrplot package in RStudio (version 2023.06.1+524).

Results

Soil depths under continuous cropping and crop rotation

Sorghum–quinoa rotation (S-Q) likely improves soil fertility compared to continuous sorghum (S), as indicated. Higher total N, available N, and organic matter were observed (due to diversified crop residues and root exudates) (*Figure 3*). Possibly higher available K if quinoa contributes to K cycling. pH moderation (quinoa may influence soil acidity/alkalinity differently than sorghum). Total Nitrogen (TN) showed no significant differences between depths (0–20 cm vs. 20–40 cm) or cropping systems (continuous sorghum vs. rotation). This suggests that N accumulation is slow, and short-term rotation (1 year) may not noticeably alter the TN. Alkaline Hydrolyzable N (AN), Available K (AK), and Organic Matter (SOM) were higher in the topsoil (0–20 cm) than in the subsoil (20–40 cm), which is consistent with typical nutrient stratification. Rotation (S-Q) showed numerically higher values (especially in the topsoil), but the effects were non-significant in the first year. This implies that soil fertility improvements from rotation may require long-term implementation to become statistically evident. The initial pH was alkaline (8.98), but declined under both systems, likely due to root exudates, nitrification, or organic acid release. Depth effects dominated AN, AK, and SOM, highlighting the importance of topsoil management.

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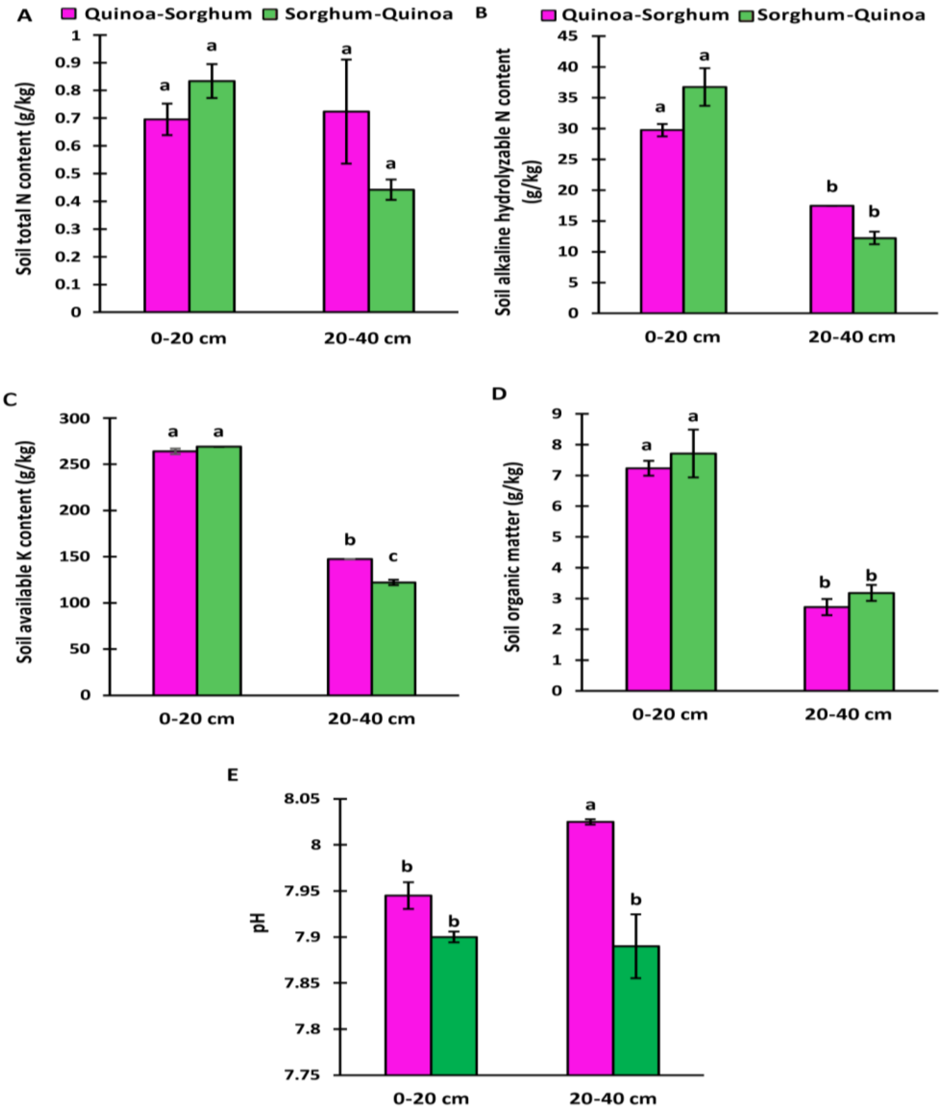

Figure 3. *Soil physio-chemical properties from 0 to 20 and 20 to 40 cm soil depth under sorghum–quinoa continuous cropping and sorghum–quinoa crop rotation. (A) Soil total nitrogen (TN) content, (B) Soil alkaline hydrolysable nitrogen (AN) content (a measure of readily available N), (C) Soil available potassium (AK) content, (D) Soil organic matter (SOM) content, (E) Soil pH. Different letters on bars indicate significant differences according to the Tukey test (p < 0.05). Vertical bars correspond to the standard error*

Soil properties under sorghum continuous cropping vs. sorghum-quinoa rotation

Soil total nitrogen content did not differ significantly between cropping systems (continuous sorghum vs. rotation) or soil depths (0–20 cm vs. 20–40 cm) (*Figure 4*). TN was less responsive to short-term (1-year) crop rotation, as N build-up depends on long-term organic matter dynamics. The Soil Alkaline Hydrolyzable Nitrogen (AN) Content Higher in the topsoil (0–20 cm) than in the subsoil (20–40 cm), reflecting greater microbial activity and organic N mineralization near the surface. Rotation (S-Q) may show a numeric increase (likely due to quinoa’s N cycling effects), but the differences were not statistically significant in the first year. Soil Available Phosphorus (AP) Content

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Crop rotation might enhance P availability if quinoa mobilizes P (e.g., through root exudates or mycorrhizal associations). Higher in topsoil, with possible (but non-significant) increase under rotation due to quinoa K uptake/release dynamics. The Soil Available Potassium (AK) content was higher at 0–20 cm than at 20–40 cm, as K was less mobile and concentrated near organic residues. Rotation may improve K availability (if quinoa is a strong K recycler), but the effects may need a longer time to become significant. Significantly higher in the topsoil (0–20 cm) due to crop residue and root biomass accumulation. Rotation (S-Q) showed a numeric increase (likely from diversified crop inputs) but was not statistically significant at 1-year.

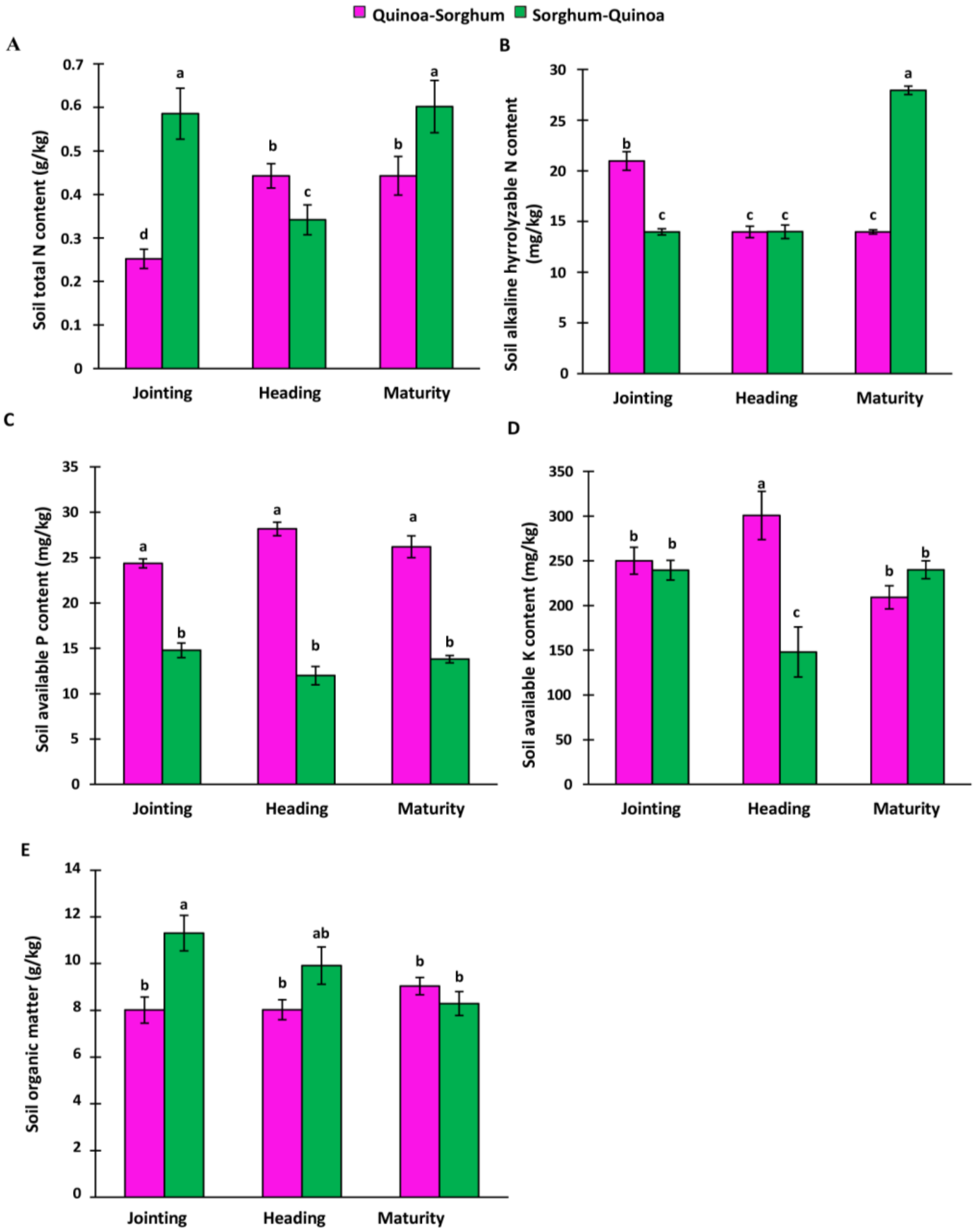

Figure 4. *Soil properties under sorghum continuous cropping vs. sorghum-quinoa rotation. (A) Soil Total Nitrogen (TN) Content, (B) Soil Alkaline Hydrolyzable Nitrogen (AN) Content, (C) Soil Available Phosphorus (AP) Content, (D) Soil Available Potassium (AK) Content, (E) Soil Organic Matter (SOM) Content. Different letters on bars indicate significant differences according to the Tukey test ($p < 0.05$). Vertical bars correspond to the standard error*

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Cropping and crop rotation row spacing on root characteristics of quinoa at 0–20 cm at each growth stage

Compared with quinoa-sorghum, the root length of quinoa in the 0–20 cm soil layer in each growth stage was significantly increased (*Table 1*). Compared with 20 cm and 60 cm, the 40 cm row spacing significantly increased the root length of the 0–20 cm soil layer in the heading, filling, and maturity stages of quinoa-sorghum, and significantly reduced the root length of the 0–20 cm soil layer in the heading and flowering stages of quinoa-sorghum significantly increased the root length of the 0–20 cm soil layer in the filling stage. A row spacing of 40 cm was conducive to root elongation in the cultivated layer of the quinoa-sorghum line.

Table 1. *The sorghum continuous cropping sorghum–quinoa crop rotation in row space on the 0–20 cm root length of quinoa at different growing stages*

Year	Crop Rotation	Row space (cm)	Heading stage	Anthesis stage	Filling stage	Mature stage
2022-2023	Quinoa-Sorghum	20	380.23 c	839.60 a	471.67 c	373.09 d
		40	580.41 a	718.53 b	568.48 a	914.09 a
		60	415.67 b	392.33 e	327.56 e	619.51 b
2023-2024	Sorghum-Quinoa	20	225.36 d	735.70 b	427.23 d	615.28 b
		40	185.75 e	494.39 d	510.35 b	448.09 c
		60	353.86 c	526.86 c	491.55 bc	435.49 c
Probability level of ANOVA		Y	**	**	*	*
		C	**	**	**	**
		Y × C	**	ns	**	**

Note: * and **, significant at 0.01 and 0.05 probability levels, respectively; ns, indicates not significant at 0.05 probability level

Cropping and crop rotation row spacing on projection area of sorghum–quinoa

The strain had a significant effect on the projected area of quinoa roots in the 0–20 cm soil layer at each growth stage, and row spacing had a significant effect on the projected area of quinoa roots in the 0–20 cm soil layer at each growth stage (*Table 2*). Compared with the control group, the projection area of roots in the soil layer of 0–20 cm in the development stage was significantly increased in the quinoa-sorghum management and control groups, and the projection area of roots in the development stage of 0-20 cm in the development stage was significantly increased in the quinoa-sorghum management. The root projection area of the 0–20 cm soil layer in the treatment with 40 cm row spacing significantly increased the root projection area of the 0-20 cm soil layer in the imputation stage of the quinoa-sorghum filling stage, and the root projection area of the 60 cm row spacing significantly increased the root projection area of the 0-20 cm soil layer in the imputation stage of the quinoa-sorghum flowering stage, but reduced the root projection area of the 0-20 cm soil layer in the imputation stage of the quinoa-sorghum flowering stage. In conclusion, when the row spacing was 40 cm, it was beneficial to increase the root projection area of the cultivated layer in the quinoa-sorghum line.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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***Table 2.** The quinoa continuous cropping and sorghum–quinoa crop rotation in row spacing on 0–20 cm root projection area of quinoa at different growing stages*

Year	Crop Rotation	Row space (cm)	Heading stage	Anthesis stage	Filling stage	Mature stage
2022-2023	Quinoa-Sorghum	20	44.16 a	31.62 f	37.48 e	903.59 a
		40	34.38 b	36.88 e	95.86 a	363.47 f
		60	41.83 a	48.11 d	28.76 f	614.01 b
2023-2024	Sorghum-Quinoa	20	22.55 d	49.91 c	48.98 b	609.78 c
		40	22.42 d	61.48 b	41.12 d	442.59 d
		60	26.46 c	65.25a	47.20 c	429.99 e
Probability level of ANOVA		Y	**	**	*	*
		C	**	**	**	**
		Y × C	**	ns	**	ns

Note: * and **, significant at 0.01 and 0.05 probability levels, respectively; ns, indicates not significant at 0.05 probability level

Cropping and crop rotation row spacing on root surface area

Strain had a significant effect on the root surface area of quinoa in the 0–20 cm soil layer at each growth stage, and row spacing had a significant effect on the root surface area of quinoa in the 0–20 cm soil layer at each growth stage (*Table 3*). The root surface area of the soil layer of 0–20 cm in the developmental stage was significantly increased in the management of quinoa compared with sorghum, and the root surface area of the soil layer of 0–20 cm in the developmental stage was significantly increased in the management of sorghum compared with quinoa. The root surface area of the 0–20 cm soil layer in the treatment with 40 cm row spacing significantly increased the root surface area of the 0–20 cm soil layer in the imputation stage of the quinoa-sorghum cultivation stage, and the root surface area of the 0–20 cm soil layer in the treatment with 20 cm row spacing significantly increased the root surface area of the quinoa-sorghum cultivation stage and reduced the root surface area of the 0–20 cm soil layer in the imputation stage of the quinoa-sorghum flowering stage. A row spacing of 40 cm was conducive to the improvement of the root surface area of the arable layer in the quinoa-sorghum line.

***Table 3.** The quinoa continuous cropping and sorghum–quinoa crop row spacing on the 0–20 cm root surface area of quinoa at different growing stages*

Year	Crop Rotation	Row space (cm)	Heading stage	Anthesis stage	Filling stage	Mature stage
2022-2023	Quinoa-Sorghum	20	134.81 a	152.22 d	118.83 e	238.11 a
		40	104.38 b	194.22 b	302.24 a	196.74 b
		60	129.35 a	157.88 c	91.41 f	171.51 d
2023-2024	Sorghum-Quinoa	20	71.90 d	206.06 a	154.95 b	178.25 c
		40	66.80 d	100.91 f	130.75 d	140.19 f
		60	85.77 c	117.92 e	149.34 c	147.89 e
Probability level of ANOVA		Y	**	**	*	*
		C	**	**	**	ns
		Y × C	**	ns	ns	**

Note: * and **, significant at 0.01 and 0.05 probability levels, respectively; ns, indicates not significant at 0.05 probability level

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Cropping and crop rotation row spacing on root diameter in quinoa-sorghum

Root diameter in 0–20 cm soil layer showed an increasing trend under different row spacing treatments at the same growth period (*Table 4*). The root diameter of the soil layer aged 0–20 cm was significantly increased in the management of quinoa compared with sorghum, and the root diameter in the treatment of quinoa-sorghum was significantly increased in the management of quinoa-sorghum and flowering stage, while it was reduced in the management of quinoa-sorghum and flowering stage. The treatment with row spacing of 40 cm significantly increased the root diameter of the soil layer of quinoa-sorghum and 0–20 cm in the maturity stage, but reduced the root diameter of the soil layer of quinoa-sorghum and 0-20 cm in the maturity stage, and the growth rate of blooming to grain filling was relatively obvious. The row spacing of 40 cm was conducive to the improvement of root diameter in the arable layer of the quinoa-sorghum line.

Table 4. *The quinoa continuous cropping and sorghum–quinoa crop row spacing on the 0–20 cm root diameter of quinoa at different growing stage*

Year	Crop Rotation	Row space (cm)	Heading stage	Anthesis stage	Filling stage	Mature stage
2022-2023	Quinoa-Sorghum	20	0.46 e	0.58 f	0.80 f	0.84 e
		40	0.87 b	0.88 c	1.28 a	1.72 a
		60	1.02 a	1.07 a	1.19 b	1.20 b
2023-2024	Sorghum-Quinoa	20	0.85 b	0.90 b	1.14 c	1.16 b
		40	0.56 d	0.66 e	0.82 e	1.01 d
		60	0.60 c	0.70 d	0.98 d	1.09 c
Probability level of ANOVA		Y	**	**	*	na
		C	**	**	**	**
		Y × C	ns	ns	ns	ns

Note: * and **, significant at 0.01 and 0.05 probability levels, respectively; ns, indicates not significant at 0.05 probability level

Cropping and crop rotation row spacing on root volume in quinoa-sorghum

The root volume in the 0-20 cm soil layer under different row spacing treatments during the same growth period showed an increasing trend (*Table 5*). The root volume of the 0–20 cm soil layer was significantly higher in quinoa than in sorghum. Treatment with 60 cm row spacing significantly increased the root volume of the soil layer of 0–20 cm at the quinoa-sorghum and flowering stages but decreased the root volume of 0–20 cm at the quinoa-sorghum and flowering stages. Treatment with row spacing of 40 cm significantly increased the root volume of the 0–20 cm soil layer in the development stage, reducing the root volume of the 0–20 cm soil layer in the development stage. A row spacing of 40 cm was conducive to the improvement of root volume in the cultivated layer of quinoa-sorghum line.

Cropping and crop rotation row spacing on dynamic changes of SPAD

Compared with quinoa-sorghum, the SPAD value of leaves at the panning stage increased, and the difference was significant when the row spacing was 60 cm. The SPAD value of the leaves at the flowering and filling stages significantly increased when the row spacing was 40 cm (*Table 6*). In the development group, the SPAD value of leaves

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DOI: http://dx.doi.org/10.15666/aeer/2306_1123311255

at different stages was increased by 40 cm row spacing compared to 20 cm and 60 cm, and the difference was significant at the flowering and filling stages. In the development group, the SPAD value of leaves at 20 cm row spacing was higher than that at 40 and 60 cm row spacing. The SPAD values of leaves increased at both the panicle and flowering stages, with significant differences at the flowering stage. In conclusion, a spacing of 40 cm of quinoa-sorghum could increase the SPAD value of leaves, and the effect was better in the middle and late stages, which was conducive to plant photosynthesis and promoted dry matter accumulation.

Table 5. *The quinoa continuous cropping and sorghum–quinoa crop row spacing on the 0–20 cm root volume of quinoa in different growing stages*

Year	Crop Rotation	Row space (cm)	Heading stage	Anthesis stage	Filling stage	Mature stage
2022-2023	Quinoa-Sorghum	20	2.65 b	1.19 f	2.37 e	5.01 d
		40	2.27 c	4.14 c	8.50 a	9.71 a
		60	3.30 a	5.02 a	4.16 b	5.11 c
2023-2024	Sorghum-Quinoa	20	1.89 d	4.64 b	4.16 b	5.44 b
		40	1.88 d	1.95 e	2.69 d	3.52 f
		60	1.72 e	2.06 d	3.67 c	4.01 d
Probability level of ANOVA		Y	ns	ns	ns	ns
		C	**	**	**	*
		Y × C	**	ns	**	**

Note: * and **, significant at 0.01 and 0.05 probability levels, respectively; ns, indicates not significant at 0.05 probability level

Table 6. *The quinoa continuous cropping and sorghum–quinoa crop row spacing on the dynamic changes of SPAD in quinoa during each growth stages*

Year	Crop Rotation	Row space (cm)	Heading stage	Anthesis stage	Filling stage
2022-2023	Quinoa-Sorghum	20	56.92 a	55.24 c	57.42 c
		40	57.24 a	64.18 a	64.92 a
		60	55.28 a	54.34 c	57.72 c
2023-2024	Sorghum-Quinoa	20	56.14 a	58.62 b	60.34 bc
		40	52.76 ab	50.26 d	58.10 bc
		60	48.96 b	55.06 c	60.96 b
Probability level of ANOVA		Y	ns	ns	ns
		C	**	**	**
		Y × C	ns	**	**

Note: * and **, significant at 0.01 and 0.05 probability levels, respectively; ns, indicates not significant at 0.05 probability level

Cropping and crop rotation row spacing on protein content and its components in grain

Grain albumin, globulin, alcohol-soluble protein, gluten, and protein contents in the maturity stage were higher in patients holding quinoa than in those holding sorghum (*Table 7*). Under quinoa-sorghum cultivation, the levels of albumin, globulin, soluble protein, gluten, and protein at maturity stage increased by 1.6%-39.5%, 4.7%–42.0%,

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2306_1123311255

2.3%–14.6%, 8.8%-49.1%, and 4.9%-20.9%, respectively, at 40 cm row spacing. The grain albumin, globulin, ethanol-soluble protein, gluten, and protein contents of patients with row spacing of 40 cm were increased significantly compared with those of patients with row spacing of 20 cm and 60 cm, and those of patients with row spacing of 20 cm and 60 cm, respectively. The grain albumin, globulin, gliadin, glutenin, and protein contents at the maturity stage were increased by 40 cm row spacing of quinoa-sorghum. The levels of albumin, globulin, gliadin, gluten, and protein in the maturity stage were the highest when the row spacing was 40 cm, which laid the foundation for the development of quinoa quality and sustainable research.

Table 7. *Effects quinoa continuous cropping and sorghum–quinoa crop row spacing on grain protein components of quinoa at mature stage*

Year	Crop Rotation	Row space (cm)	Albumin (%)	Globulin (%)	Gliadin (%)	Glutelin (%)	Protein content (%)
2022-2023	Quinoa-Sorghum	20	2.29 b	1.67 c	4.65 cd	4.55 c	13.16 d
		40	2.4 a	2.23 a	5.33 a	5.95 a	15.91 a
		60	1.72 e	2.01 b	4.78 c	5.46 b	13.97 b
2023-2024	Sorghum-Quinoa	20	1.8 d	1.57 cd	4.98 b	5.46 b	13.81 bc
		40	2.36 a	2.13 ab	5.21 ab	5.47 ab	15.17 ab
		60	2.13 c	2.12 ab	5.21 ab	3.99 d	13.45 c
Probability level of ANOVA		Y	*	ns	ns	*	ns
		C	**	**	**	**	**
		Y × C	**	ns	*	**	**

Note: * and **, significant at 0.01 and 0.05 probability levels, respectively; ns, indicates not significant at 0.05 probability level

Cropping and crop rotation row spacing on grain soluble sugar, sucrose and starch contents

The contents of soluble sugar, sucrose, and starch in grains at the maturity stage were higher in patients holding quinoa than in those holding sorghum (*Table 8*). Under the condition of quinoa-sorghum, 40 cm row spacing increased soluble sugar, sucrose, and starch contents at maturity stage by 5.2%-25.7%, 0.3%-11.3% and 2.7%-34.0%, respectively. The contents of soluble sugar, sucrose, and starch in grains at the maturity stage increased by just 40 cm spacing compared with those at 20 cm and 60 cm spacing. The contents of soluble sugar, sucrose, and starch in grains at the maturity stage increased by just 40 cm spacing compared with those at 20 cm and 60 cm spacing. The contents of soluble sugar, sucrose, and starch in grains at the maturity stage were only increased in patients with a row spacing of 40 cm, and the contents of soluble sugar, sucrose, and starch in grains at the maturity stage were the highest, but there was no significant difference compared with patients with a row spacing of quinoa-sorghum.

Cropping and crop rotation row spacing on plant height

The height of quinoa plant increased gradually with the growth process (*Table 9*). Compared with quinoa-sorghum, the plant height of patients with a row spacing of 20 and 40 cm was significantly increased at the branching stage and panning stage, and was significantly increased at the flowering, filling, and maturity stages. With the increase of

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2306_1123311255

row spacing, the branching stage and panning stage of quinoa-sorghum gradually decreased, and the plant height was the highest at 40 cm from flowering stage to maturity stage. The plant height was the lowest at 40 cm during the panning stage and maturity stage, and was the highest at 20 cm during the branching stage, panning stage, flowering stage, and maturity stage. The row spacing of 40 cm was conducive to the growth of quinoa, and the increase of plant height laid a solid foundation for grain formation and yield improvement.

Table 8. *The effects quinoa continuous cropping and sorghum–quinoa crop row spacing on the sucrose, soluble sugar and starch content of quinoa grains at maturity stage*

Year	Crop Rotation	Row space (cm)	Soluble sugar (%)	Saccharose (%)	Starch (%)
2022-2023	Quinoa-Sorghum	20	16.16 b	42.01 c	54.51 d
		40	17.83 a	45.18 a	73.06 a
		60	15.52 bc	40.61 d	61.20 c
2023-2024	Sorghum-Quinoa	20	15.25 c	44.16 b	64.97 c
		40	16.95 ab	45.03 a	71.14 a
		60	14.19 d	44.40 b	64.48 c
Probability level of ANOVA		Y	**	*	*
		C	ns	ns	ns
		Y × C	ns	**	**

Note: * and **, significant at 0.01 and 0.05 probability levels, respectively; ns, indicates not significant at 0.05 probability level

Table 9. *The effect quinoa continuous cropping and sorghum–quinoa crop of row space on plant height of quinoa in different growing stages*

Year	Crop Rotation	Row space (cm)	Branching stage	Heading stage	Anthesis stage	Filling stage	Mature stage
2022-2023	Quinoa-Sorghum	20	21.10 a	65.72 a	88.43 d	133.97 b	174.90 b
		40	18.50 b	58.76 b	121.2 a	161.17 a	178.40 a
		60	15.78 c	54.48 b	106.26 c	113.10 c	174.45 b
2023-2024	Sorghum-Quinoa	20	20.36 b	46.16 c	113.70 b	121.80 b	147.05 c
		40	17.92 c	31.92 d	86.93 d	114.10 c	136.90 d
		60	17.34 c	44.70 c	91.57 d	128.53 b	135.20 d
Probability level of ANOVA		Y	ns	ns	ns	ns	ns
		C	**	**	**	**	**
		Y × C	**	ns	**	**	**

Note: * and **, significant at 0.01 and 0.05 probability levels, respectively; ns, indicates not significant at 0.05 probability level

Cropping and crop rotation row spacing on stem branch

The growth process and number of primary and secondary branches under different row spacing treatments in the same growth period showed an increasing trend (*Table 10*). Compared with quinoa-sorghum, the primary and secondary branches of quinoa in each growth stage were significantly increased. The number of first-level branches was higher

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2306_1123311255

at a field spacing of 40 cm than at the other field spacing in all growth stages, and the difference was significant at a field spacing of 60 cm, but there was no significant difference between the field spacing of 20 cm and the spacing of 40 cm. A row spacing of 40 cm significantly increased the number of secondary branches in quinoa-sorghum growing periods but reduced the number of secondary branches in quinoa-sorghum growing periods. Quinoa-sorghum was conducive to the improvement of the number of primary and secondary branches at each growth stage, and a row spacing of 40 cm was more conducive to the improvement of the number of primary and secondary branches, which laid a foundation for grain formation and yield improvement.

Table 10. *The effect quinoa continuous cropping and sorghum–quinoa crop of row spacing on the primary and secondary branches of quinoa at each growth stage*

Year	Crop Rotation	Row space (cm)	First level branch			Secondary branch		
			Branching stage	Heading stage	Filling stage	Branching stage	Heading stage	Filling stage
2022-2023	Quinoa-Sorghum	20	18.00 a	20.00 a	26.33 ab	8.00 bc	14.00 ab	32.00 cd
		40	17.60 a	21.20 a	27.33 a	13.00 a	15.00 a	54.67 a
		60	14.80 b	18.00 b	23.00 c	8.20 bc	9.00 c	42.00 b
2023-2024	Sorghum-Quinoa	20	14.00 b	15.60 c	23.00 c	9.00 abc	12.00 b	35.33 c
		40	12.00 c	14.80 c	23.33 b	10.40 ab	12.00 b	28.00 d
		60	15.80 a	18.00 b	21.00 d	7.40 c	7.60 d	27.00 d
Probability level of ANOVA		Y	ns	ns	ns	ns	ns	ns
		C	**	**	**	**	**	**
		Y × C	*	**	**	*	*	*

Note: * and **, significant at 0.01 and 0.05 probability levels, respectively; ns, indicates not significant at 0.05 probability level

Cropping and crop rotation row spacing on canopy parameters at flowering and filling stages

In the quinoa–sorghum management group, the extinction index and leaf area index of quinoa increased, and the diffuse transmission coefficient of sorghum decreased during the flowering and filling stages (*Table 11*). The extinction coefficient at 20 cm was significantly higher than that at 40 cm and 60 cm, and the difference was significant. The extinction index at 20 cm was significantly higher than that at 40 cm and 60 cm, but there was no significant difference between 20 cm and 70 cm. Moreover, it significantly increases the diffuse transmission coefficient during the filling period. The leaf area index at the flowering and maturity stages was significantly higher at the 40 cm level than at the 20 cm and 60 cm levels. The diffused transmission coefficient at the 40 cm level was significantly higher at the flowering stage than at the 20 cm and 60 cm depths. The leaf area index at 60 cm at the flowering stage was significantly higher than that at 20 cm and 40 cm at a row spacing of 60 cm, but there was no difference between the two groups. The extinction index and leaf area index of quinoa-sorghum were relatively high, and the diffuse transmission coefficient was the lowest, which was more conducive to the absorption and utilization of light energy by leaves.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2306_1123311255

Table 11. *The effect quinoa continuous cropping and sorghum–quinoa crop of row spacing on the extinction index of quinoa during flowering and filling period*

Year	**Crop Rotation**	**Row space (cm)**	**Extinction index**		**Leaf area index**		**Diffuse transmission**	
			Filling stage	**Mature stage**	**Filling stage**	**Mature stage**	**Filling stage**	**Mature stage**
2022-2023	Quinoa-Sorghum	20	0.93 a	1.00 a	1.07 b	1.04 c	0.38 c	0.38 d
		40	0.85 bc	0.81 b	1.49 a	1.18 a	0.47 b	0.35 e
		60	0.83 bc	0.76 c	0.84 b	1.12 b	0.47 b	0.40 c
2023-2024	Sorghum-Quinoa	20	0.89 ab	0.96 a	1.12 b	0.81 d	0.36 c	0.48 a
		40	0.74 c	0.72 c	0.92 c	1.01 c	0.49 a	0.45 b
		60	0.73 c	0.66 d	1.49 a	1.11 b	0.33 d	0.44 b
Probability level of ANOVA		Y	ns	ns	ns	ns	ns	ns
		C	**	**	**	**	**	**
		Y × C	*	**	**	*	*	*

Note: * and **, significant at 0.01 and 0.05 probability levels, respectively; ns, indicates not significant at 0.05 probability level

Cropping and crop rotation row spacing on dry matter accumulation in each growth period

Dry matter accumulation at the maturity stage was higher in patients with a row gap of 40 cm than in patients with a row gap of quinoa-sorghum, but the difference was not significant (*Table 12*). The dry matter accumulation at the panning, flowering, and filling stages of 40 cm was higher than that at 20 cm and 60 cm, and the difference was significant between the panning and flowering stages and the row spacing of 20 cm. The increase in row spacing and dry matter accumulation in the branching stage of quinoa-sorghum gradually decreased, and was significantly higher at a row spacing of 20 cm, while it gradually increased in the maturity stage, and was the highest at a row spacing of 60 cm, but there was no significant difference between the row spacing of 40 cm and 60 cm. The dry matter accumulation at the flowering stage of patients with a row spacing of 40 cm was higher than that at 20 cm and 60 cm, but the difference was not significant. The dry matter accumulation of patients with row spacing of 20 cm was higher than that of 40 cm and 60 cm only at the flowering stage. The row spacing of quinoa-sorghum was 40 cm and that of quinoa-sorghum was 20 cm, which was conducive to dry matter accumulation in the ground.

Cropping and crop rotation row spacing on quinoa yield

The content of quinoa increased the 1000–grain weight, output, and economic coefficient compared with sorghum (*Table 13*). Compared with the 20 cm and 60 cm, the 1000–grain weight, yield, and economic coefficient of quinoa were significantly increased by 3.81%-4.32%, 8.37%-15.35% in the fields of just 40 cm. Compared with the row spacing of 20 cm and 60 cm, the spacing was equal to quinoa-sorghum. Under 40 cm row spacing, 1000–grain weight, yield, and economic coefficient of quinoa-sorghum were significantly increased by 7.49%-8.19%, 6.64%-7.08%, and 0.06%-9.89%, respectively. It was found that quinoa-sorghum was conducive to the improvement of 1000–grain weight, yield, and economic coefficient, and the 1000–grain weight, yield, and economic coefficient were the highest when the row spacing was 40 cm, which laid a foundation for the study of quality and sustainability of quinoa-sorghum.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2306_1123311255

***Table 12.** The effect quinoa continuous cropping and sorghum–quinoa crop of row space on the total dry matter accumulation of quinoa in different growing stages*

Year	**Crop Rotation**	**Row space (cm)**	**Branching stage ($kg\cdot ha^{-1}$)**	**Heading stage ($kg\cdot ha^{-1}$)**	**Anthesis stage ($kg\cdot ha^{-1}$)**	**Filling stage ($kg\cdot ha^{-1}$)**	**Mature stage ($kg\cdot ha^{-1}$)**
2022-2023	Quinoa-Sorghum	20	1992.90 a	6244.35 a	10789.80 c	18614.40 abc	16771.72 ab
		40	1619.10 b	6817.65 a	14486.85 a	21413.70 a	17448.31 ab
		60	710.85 d	5180.70 ab	13747.65 ab	15545.25 c	17943.38 a
2023-2024	Sorghum-Quinoa	20	2001.30 a	4384.80 b	12971.70 abc	20049.75 ab	16390.25 b
		40	1548.75 b	3747.45 b	13055.70 abc	18223.80 abc	16033.19 bc
		60	1092.00 c	4072.95 b	11788.35 bc	17234.70 bc	14822.05 c
Probability level of ANOVA		Y	**	*	*	ns	ns
		C	ns	ns	ns	ns	ns
		Y × C	ns	**	**	**	**

Note: * and **, significant at 0.01 and 0.05 probability levels, respectively; ns, indicates not significant at 0.05 probability level

***Table 13.** The effect quinoa continuous cropping and sorghum–quinoa crop of row spacing on quinoa yield*

Year	**Crop Rotation**	**Row space (cm)**	**1000–grain weight (g)**	**Yield ($kg\ ha^{-1}$)**	**Economic coefficient**
2022-2023	Quinoa-Sorghum	20	2.02 c	3287.79 b	0.2021
		40	2.33 a	3429.74 a	0.2026
		60	2.15 b	3303.95 b	0.1898
2023-2024	Sorghum-Quinoa	20	2.41 b	2570.45 b	0.1617
		40	2.57 a	2762.89 a	0.1777
		60	2.40 b	2553.63 b	0.1776
Probability level of ANOVA		Y	**	*	*
		C	ns	ns	ns
		Y × C	ns	**	**

Note: * and **, significant at 0.01 and 0.05 probability levels, respectively; ns, indicates not significant at 0.05 probability level

Correlation analysis between sorghum–quinoa on quinoa yield and grain protein

The optimized soil-regulated vegetation index was closely related to the grain protein of quinoa-sorghum, and both were in the positive phase (*Figure 5*). The green normalized vegetation index was positively correlated with the grain gliadin and total protein contents, which were normalized red edge index of chemical difference was significantly positively correlated with grain albumin, and was extremely significantly positively correlated with grain gliadin. In conclusion, the green normalized vegetation index, leaf chlorophyll content, normalized difference red edge index, normalized difference vegetation index, and optimized soil-regulated vegetation index were positively related to the grain protein of quinoa.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2306_1123311255

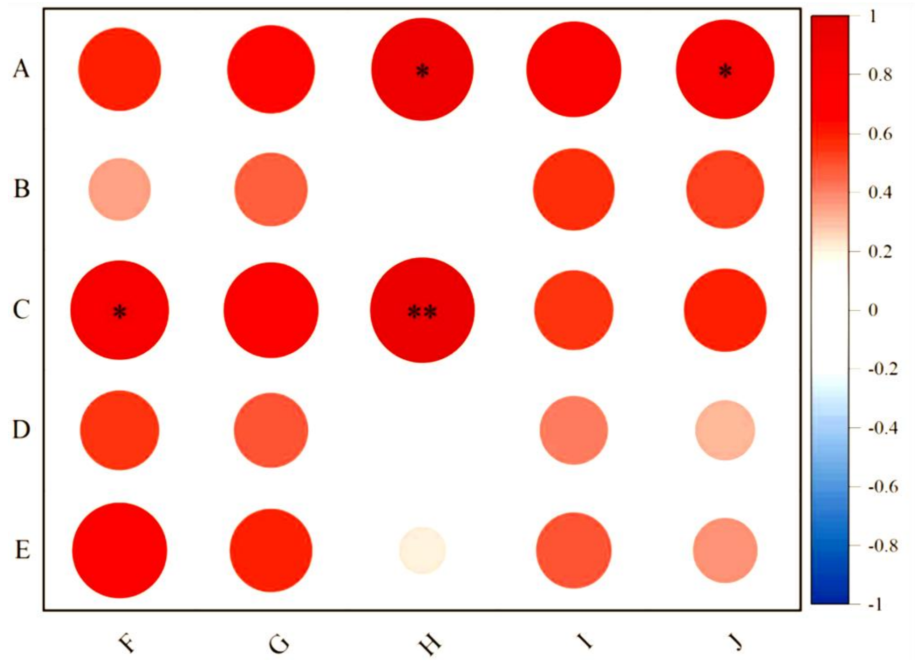

Figure 5. *Relationship between yield, economic coefficient and grain protein. Note: A; Soluble sugar, B; Saccharose, C; Starch, D; Yield, E; Economic coefficient, F; Albumin, G; Globulin, H; Gliadin, I; Glutelin, J; Protein content*

Discussion

The growth period of quinoa-sorghum was heading, anthesis, and filling stages of mature quinoa, and there were differences between the different lines. Compared with quinoa-sorghum heading, anthesis, and filling stages of mature quinoa, 3.3°C the daily average temperature, and 2 days the emergence to branch. The average daily temperature was lower by 5.04°C, and the average daily temperature was higher 1.75°C, 1 day less from panicle to flowering, 2.25°C lower daily average temperature, 5 days less from flowering to grain filling, low daily average temperature 1.46°C, the heading, anthesis, and filling stages of mature longer, the daily average temperature was 1.09°C higher, and the total number of heading, anthesis, and filling stages of mature in the growth period was 2 days less (Voronov et al., 2023; González et al., 2023). Although the total number of days of the growth period was two days less, mainly due to the number of days in each key growth period before grain filling, the duration was longer after grain filling. The root system is an important organ that fixes the plant, absorbs and transports water and nutrients from the soil. Plant density can also affect root system (Li et al., 2023). Our results showed that the total root length, projected root area, root surface area, root diameter, root volume, number of root tips, number of branching roots, and number of crossing roots per plant decreased with increasing plant density. There is a significant correlation between crop root characteristics and above-ground traits (Kausar et al., 2023; Ramakrishnan et al., 2024). The total root length, root projection area, root surface area, root diameter, root volume, number of root tips, number of root branches, and number of root crossings per plant differed in the 0–20 cm soil layer under the same planting density and row spacing. The total root length, root projection area, root surface area, root diameter, root volume, root tip number, root branching number, and root crossing number of different quinoa lines under 20 cm or 60 cm row spacing were better than those under

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2306_1123311255

40 cm row spacing in the 0–20 cm soil layer at the panicle and branching stages (Gonzalez et al., 2012; Mufari et al., 2018). However, under 40 cm row spacing in the filling and maturity stages, the total root length, root projection area, root surface area, root diameter, root volume, root tip number, root branching number, and root crossing number of different quinoa lines were better than those under 40 cm row spacing in the 0–20 cm soil layer. The total root length, root projection area, root surface area, root diameter, root volume, root tip number, root branching number, and root crossing number were higher in the 0–20 cm soil layer were better (Ruiz et al., 2014; Wefers et al., 2015). Previous studies have found that quinoa germplasma has rich genetic diversity, the coefficient of variation of yield and grain weight per plant is large, and that there are significant differences in agronomic traits among different varieties of quinoa. At the same time, abiotic factors (photoperiod, temperature, radiation, and rainfall) affect the growth of quinoa, and biological factors such as leaf spot disease and weeds also affect the yield of quinoa (Bhargava et al., 2007; Gabriel et al., 2012; Motaet al., 2016). Row spacing can build a reasonable and efficient population structure of quinoa-sorghum, effectively promote the growth and development of maize individuals, improve the ventilation environment of quinoa-sorghum, more effectively promote the growth of quinoa-sorghum grain, and promote high and stable yield of maize (Cordeiro et al., 2012). The highest yield in the wide and narrow row spacing treatment (80 cm+40 cm) was 7.96% higher than that in the equal row spacing treatment (60 cm+60 cm) (Tarnawa et al., 2023). The results showed that reasonable row spacing is an important way to improve maize yield. Since the appearance characteristics of quinoa are similar to those of corn, row spacing also has a certain impact on the growth characteristics and yield quality of quinoa (Bouchyoua et al., 2024). It was found that different sowing times affected the length of the growth period of quinoa; the earlier the sowing time, the longer the growth period, and quinoa-sorghum sown after June could not mature in the northern foot of Yinshan Mountain in Inner Mongolia (Ru et al., 2023). Quinoa-sorghum materials and found that the growth period of each quinoa-sorghum material was 91-145d, the plant height was 54.3-205 cm, the 1000–grain weight was 0.9–3.9g, and the yield was 112.8–2795.4 kg·ha^{-1}. Yield was positively correlated with 1000–grain weight and grain weight per plant. There is a significant positive correlation with the length of the main inflorescence length (Bakaeva et al., 2024). The study of plant height was 145.5-200.4 cm, 1000-grain weight was 2.0-3.6 g, and the highest yield was observed 4246.6 kg·ha^{-1} (Fakhrzad et al., 2023). This study showed that there were significant differences in agronomic traits between the two lines of quinoa, with plant height ranging from 135.20 to 178.40 cm, 1000–grain weight was 2.02–2.57 g, and the yield was 2553.63-3429.74 kg·ha^{-1}. Compared with quinoa-sorghum, plant height was significantly increased at 20 cm and 40 cm row spacing at the branching and filling stages, and significantly increased at the panning and maturity stages (Barboričová et al., 2022; Mathur et al., 2023). The increase in row spacing and the branching and panicle stages of quinoa-sorghum gradually decreased, and the plant height was highest at 40 cm from flowering to maturity. For sorghum, the plant height was lowest at 40 cm from the panning stage to the maturity stage, and the plant height was highest at 40 cm at the branching, panning, flowering, and maturity stages. A row spacing of 40 cm significantly increased the number of first and second branches in each growth stage for quinoa but decreased the number of first and second branches in each growth stage for sorghum (Fukudome et al., 2022; Narayan et al., 2024). Quinoa sorghum increased the 1000–grain weight and yield of Quinoa compared to quinoa sorghum. Compared with the 20 cm and

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2306_1123311255

60 cm row spacing, the 1000–grain weight, yield, and economic coefficient of quinoa under 40 cm row spacing were significantly increased by 3.81%–4.32% and 8.37%-15.35% under sorghum, respectively. Compared with 20 cm and 60 cm row spacing, sorghum significantly increased the 1000-grain weight and yield of quinoa with 40 cm row spacing by 7.49%-8.19% and 6.64%-7.08%, respectively (Tian et al., 2023; Sarkar et al., 2024). This study demonstrates that with the intensification of quinoa-sorghum, grain albumin, globulin, glitin, gluten, and protein content at the maturity stage compared with quinoa-sorghum. The grain albumin, globulin, glitin, gluten, and protein contents at the maturity stage were the highest when the row spacing was 40 cm. The main nutrient composition of quinoa seeds from the Taigu district of Jinzhong City in Shanxi Province was compared with those from other producing areas. The differences in the composition of quinoa seeds from different production areas were compared in terms of their basic nutrient composition. In terms of nutritional composition, the protein content of quinoa was slightly higher from 13.16% to 15.91% (Xu et al., 2022; Liu et al., 2024; Abdelrady et al., 2024). The results showed that under 40 cm row spacing in the filling and maturity stages, the total root length, root projection area, root surface area, root diameter, root volume, root tip number, root branching number, and root crossing number of different quinoa-sorghum lines were better than those under 40 cm row spacing in the 0–20 cm soil layer. The total root length, root projection area, root surface area, root diameter, root volume, root tip number, root branching number, and root crossing number in the 0–20 cm soil layer were better. The green-normalized vegetation index was significantly positively correlated with grain gliadin and total protein content. The normalized difference red edge index was significantly positively correlated with grain albumin and grain gliadin was significantly positively correlated. In summary, the green normalized vegetation index, foliar chlorophyll content, normalized differential red edge index, and normalized differential vegetation at the early stage of quinoa maturation were analyzed and found to be positively correlated with the SPAD value of quinoa-sorghum at the early maturity stage.

Conclusions

The findings demonstrate significant interactions between the suitable planting variety and suitable row spacing range of quinoa. This study provides an important scientific basis for the development of the quinoa industry in the Shanxi Province. The scientific and reasonable selection of appropriate row spacing is a necessary condition for quinoa to achieve high yield and efficiency, and unmanned aerial vehicles can monitor crop growth conveniently, quickly, and accurately. The allocation of 40 cm row spacing was conducive to the growth of the root system, which promotes the absorption and utilization of water in the root, was conducive to the better photosynthesis of the plant of each vegetative organ in each growth period, which improved the nitrogen accumulation in each growth period, and then improved the content of protein components, soluble sugar, sucrose, and starch in the grain. Quinoa-sorghum with a row spacing of 40 cm had the highest content of albumin, globulin, glitin, gluten, protein in mature grains, and its nutritional value was equivalent to that of meat. It is a high-quality plant protein, which lays a foundation for research on the quality and sustainability of quinoa. Agriculture in the province was developing towards water-saving, high–quality, high-efficiency, and multi-functional urban agriculture, and it was of great significance to introduce new high-efficiency, stress-resistant, and multi-functional crops.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2306_1123311255

Funding. The authors are thankful to Science and Technology Innovation Enhancement Project of Shanxi Agricultural University (CXGC202431); Shanxi Key R&D Program (202302140601008-03); Technical System of Current Agricultural Coarse Grain Industry in Shanxi Province 2025 (CYJSTX03-16). for financial support of this study.

Acknowledgements. The authors are thankful to Science and Technology Innovation Enhancement Project of Shanxi Agricultural University (CXGC202431); Shanxi Key R&D Program (202302140601008-03), Technical System of Current Agricultural Coarse Grain Industry in Shanxi Province 2025 (CYJSTX03-16) for financial support of this study.

Availability of data and materials. The datasets generated and/or analyzed during the current study are available in the Shanxi Jiaqi Agricultural Technology Co., Ltd repository. All data generated or analyzed during this study are included in this published article files.

Competing interests. The authors declare that they have no competing interests.

Authors' contributions. Conceptualization, H.N, G.L, L.S, J.Z, Y.Z.; formal analysis, W.B. and G.L.; investigation, H.N.; resources, G.L.; data curation, H.N.; writing—original draft preparation, H.N.; writing—review and editing, L.S.; supervision, J.Z.; Software, W.B.; funding acquisition, G.L, L.S, Y.Z. All author has read and agreed to the published version of the manuscript.

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