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Leaf Chlorophyll Changes and Morphological Features of Quinoa (*Chenopodium quinoa*) Cultivars by P-Zn Ratios in Greenhouse Condition

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ABSTRACT

BACKGROUND: Sufficient nutrients supplying is one of the most important factors in realizing the crops potential performance and achieving high yield, which in conventional agriculture is provided by using chemical fertilizers.

OBJECTIVES: This study aims to evaluate the root and shoot structure of quinoa cultivars in response to the phosphorus and zinc sulfate fertilizers application in the Research, Agricultural Education and Natural Resources Center of Khuzestan province.

METHODS: The statistical model was factorial based on completely randomized design (CRD) with three factors including the use of triple superphosphate fertilizer at four amounts (0, 6, 12, 18 mg. kg^{-1} soil weight), foliar application of zinc sulfate in three concentrations (water without zinc sulfate (control), foliar application at 4 and 8 g. L^{-1} concentrations) and three quinoa cultivars (Giza 1, Q26, Titicaca) in P.V.C pots and in three replications.

RESULT: The simultaneous effect of experimental factors on chlorophyll index and shoot morphological traits was significant. Root length to shoot length ratio was affected by the main effect of P_2O_5 fertilizer and the cultivars differences, and the effect of zinc sulfate was not significant on this trait. In most of the analysed traits, the 4 g. L^{-1} concentration of zinc sulfate was more effective than the 8 g. L^{-1} concentration. The total grain weight of the three cultivars was affected by the amount of fertilizer, and the best values of this attribute belonged to the 18 mg. kg^{-1} triple superphosphate and 4 g. L^{-1} concentration of zinc sulfate in Q26 cultivar. This trait (TGW) showed the most positive correlation with the plant height, number of lateral branches, stem diameter, and chlorophyll index.

CONCLUSION: Based on the results of this experiment, the optimal application of P-Zn ratios by reducing the antagonism effects led to the root and shoot structure development, and increased quinoa cultivars production.

KEYWORDS: *Correlation, Oil seed, Phosphorus, Root density, Zinc.*

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1. BACKGROUND

Quinoa is a dicotyledonous, annual, allotetraploid crop, which is classified in the pseudocereals group (Salehi and Dehghani, 2018). Introduction this crop into the country's farming pattern can make the country free from the import of rice to some extent, and it can open its place in the country's agricultural production as a supplement along with other products. The quinoa research topics are increasing and due to the high demand in the world for this valuable crop, it is possible to invest in the cultivation development of this crop in the country (Khoshkam, 2017). At present, studies on quinoa fertilization are very limited and more work has been done on the use of animal manure for the purpose of organic quinoa production (Garcia *et al.,* 2015). Phosphorus is one of the widely consumed elements that have many structural and catalytic roles in plants (Jorfi *et al.,* 2022b; Hawkesford *et al.,* 2012). Despite the need of plants for phosphorus in the range of highly consumed elements, its available amount in different soils is usually low. In many cases, there may not be a deficiency in the total amount of phosphorus in the soil in the root area, but due to the binding of an important part of this element to the organic and mineral compounds of the soil, its bioavailability for plants decreases (Vance *et al.,* 2003). Feeding the plant with sufficient phosphorus creates a nutrient environment around the germinating seeds and provides nutritional support to the plant in the early stages of growth and development (Bindraban *et al.,* 2020). It also causes the root growth and strengthening, the stems strengthening and thickening, the increase in the seeds volume, the increase in the yield and early maturity of the crops, and it is involved in the flower's pollination (Reddy *et al.,* 2020). Zinc deficiency exists in a large part of the hot and cold regions soils in the world, and it is considered one of the most important deficiencies of micronutrients in the world (Mostafavi Rad *et al.,* 2018). Calcareous soils with high pH, little organic matter and soil salinity, high bicarbonate in irrigation water and unbalanced use of fertilizers lead to a decrease in micronutrient elements. These elements improve the general conditions of the plant and participate in biochemical reactions as catalysts. To increase the yield, farmers use one of the common fertilization methods, including soil application, through irrigation, mixing with seeds and foliar spraying, which foliar spraying is one of the fast methods to meet the need for fertilizer, which in this method also saves on the use of fertilizer and as a result, in addition to the positive economic aspect, the environment is protected from chemical pollution, which is very effective in achieving sustainable agriculture. If micronutrient elements such as zinc be available through the leaves of the product, they completely compensate for the deficiency caused by soil, and are considered a suitable substitute in this field (Aytak *et al.,* 2015). Studying the interaction effect of genotype and environment is absolutely necessary and providing plans related to the cultivation development and increasing the production of new quinoa cultivars are of particular importance (Birami *et al.,* 2020). Most researchers are trying to introduce cultivars with performance stability in limited access to nutritional elements conditions (Afshari *et al.*, 2020). The absorb nutrient's ability is different in cultivars. Compared to other experimental environments, often one or more cultivars perform better than other cultivars, which indicates the high adaptability of that cultivar in that particular ecological condition (Bazile *et al.,* 2016).

2. OBJECTIVES

Considering valuable place of nutrients in crop structure development, this research was conducted with the aim of evaluating the effect of phosphorus and zinc elements on the chlorophyll index, and the morphological and functional characteristics of quinoa crop.

3. MATERIALS AND METHODS

3.1. Experimental Site and Design

The experiment was conducted during the autumn and winter of 2018-2019 agronomic year in the Ahvaz station of Khuzestan Agriculture and Natural Resources Research Center. This research site is located at 31^{o} 30′N and 48^{o} 65′E, at an elevation of 12 m above sea level. The experiment was laid out as factorial based on completely randomized design with 3 factors and 3 replicates in cylindrical pots made of polyethylene. The treatments were included: amounts of triple superphosphate fertilizer at 4 levels (0, 6, 12, 18 mg. kg^{-1} soil), foliar application of zinc sulfate in 3 concentrations (water without zinc sulfate (control)), 4 and 8 g. L^{-1} zinc sulfate) and 3 cultivars of quinoa (Giza 1, Q26, Titicaca).

Fig. 1. View of the test site and treated pots (Ahvaz research station).

3.2. Crop Management

In this experiment, 108 cylindrical pots of polyethylene with a 60 cm height and an eight-inch diameter were prepared (Fig. 1). The pots were kept in an open space so that they were exposed to the same situation as the field crops. They were placed at a certain distance from each other on the ground. For proper drainage of the pots, 2 cm of coarse sand was poured on the bottom of each pot, then the soil was added to this layer. The potting soil was prepared from a research farm. The average of some meteorological indicators of the research station is presented in table 1.

Table 1. The average of meteorological indicators of the research station in 2018-2019

Month	October	November	December	January	February
Maximum temperature (^oC)	43.1	32.3	26.5	25	26
Minimum temperature (^oC)	12.1	11.1	5.6	2.9	6.1
Average temperature (^oC)	28.3	19.4	15.3	14.2	15.5
Rainfall (mm)	37.8	159.3	20.5	45.1	13.8
Monthly evaporation (mm)	194.9	74.7	44.7	49.57	71.98

The required fertilizer, including nitrogen and potassium, was used based on soil test results. Nitrogen fertilizer (urea) was used as a base, and in 2 stages of cluster emergence, and flowering and a potassium fertilizer from potassium sulfate source was used pre-planting. Phosphorus fertilizer was supplied from a triple superphosphate source (46%) and was used pre-planting in each experimental pot according to the prescribed treatment. Triple superphosphate and potassium sulfate fertilizer were mixed with potting soil before planting, but nitrogen fertilizer was used with irrigation water. In this study, three cultivars of quinoa were evaluated. Twenty seeds were planted in each pot. The seeds were sown in a 1–2 cm depth. Extra seedlings were thinned at 2–4 leaf stage. The first irrigation was done immediately after planting. Then, until the full establishment of seedlings (4 to 5 leaves), irrigation was done routinely, providing 100% water requirements of the plant. Subsequent irrigations were carried out according to the plant's water needs so that no stress was applied to the plant. The seeds were obtained from the seed bank of the Khuzestan Agriculture and Natural Resources Research Center. Proteus pesticide with 2 g. L^{-1} concentration was used to control the leaf-eating larvae of Caradrina at 5 and 8 days after germination. Also, in the cluster emergence stage, by observing the effects of the pest, the pots were sprayed with diazinon insecticide in 2 g.L^{-1} concentrations. For zinc sulfate foliar application in the flowering stage, the desired concentration has been prepared, and according to the treatment prescribed in each pot, spraying was done in the early hours of the day. Root extraction was carried out after physiological maturity and when grain moisture reached about 14%.

3.3. Measurements

After ending the growth stages, the aerial parts of each pot were cut, and then the necessary measurements were performed. There were 5 plants per pot, and the root obtained from the entire soil in the pot was attributed to those 5 plants, and it was expressed per plant basis in the results. In this experiment, the following traits were measured: Chlorophyll index (SPAD), plant height, distance of the first lateral branch to the soil surface, stem diameter (SD), number of lateral branches (NLB), root density (RD), root length to shoot length ratio, total grain weight (TGW). To measure the chlorophyll index from five points of leaf in the flowering stage with a chlorophyll meter device (SPAD 502) the amount of chlorophyll was calculated. The average of these five numbers was considered as the desired data (Jorfi *et al.,* 2022a). To determine the plant height, from the tip of the main cluster to the end of the stem (near the soil surface) was measured in centimetres. The distance of the first lateral branch to the soil surface was measured in centimetres with a ruler (Mousavi *et al.,* 2015). The stem diameter was measured by digital calliper in mm (Sarani *et al.,* 2016). To determine the number of lateral branches, the number of branches from the main axis of the stem (below the main cluster to the end of the stem (near the soil surface)) were counted (Mousavi *et al.,* 2015). The root density was obtained using the following formula (Ganjali *et al.,* 2010):

Equ. 1. Root density ($g.cm^{-3}$) = root dry weight (g)/ root volume (cm^3)

Root length to shoot length ratio was calculated by dividing the length of the root by the length of the shoot (Armin and Kivanlou, 2014). To determine the total grain weight, 5 plants (from each pot) were sampled and harvested at maturity. The harvested plants were dried in an oven at 75°C for 48 h to record the total grain weight per plant (Mousavi *et al.,* 2015).

3.4. Statistical Analysis

Statistical analysis of data was conducted using Minitab software (version 16.2). Means were compared according to the Fisher's least significant difference (LSD) test at $P \leq 0.05$.

4. RESULT AND DISCUSSION

The results of variance analysis showed that the effect of triple superphosphate fertilizer on plant height, the distance of the first branch to the soil surface, stem diameter, number of lateral branches, root density and total grain weight was significant. The chlorophyll index differences of quinoa cultivars were significant at the 1% probability level. Root length to shoot length ratio was affected by the main effect of P_2O_5 fertilizer and cultivars differences, and the effect of zinc sulfate was not significant on this trait. Also three-fold interaction effect of factors on studied traits was significant (Table 2 and 5).

4.1. Chlorophyll index (*SPAD*)

In the examining differences of cultivars, it was observed that the Q26 cultivar with an average of 56.71 had the highest chlorophyll index, and Titicaca cultivar had the lowest value of this trait (Table 3). In this regard, Mansouri *et al.* (2021) stated that the maximum and minimum values of this index belonged to Q26 and Titicaca cultivars with values of 60.13 and 46.59, respectively. Results revealed that in all three quinoa cultivars, the chlorophyll index was enhanced by increasing amount of P_2O_5 and $ZnSO_4$ fertilizer up to a certain level. The three-fold interaction of P_2O_5 and $ZnSO_4$ and cultivars indicated that chlorophyll index ranged from 44.86 in P_0Zn_0+Titicaca treatment to 63.36 in $P_{18}Zn_4$ + Q26 treatment. No difference was found between $P_{18}Zn_4$ + Q26 cultivar treatment and Titicaca cultivar in applying 12 mg kg^{-1} P_2O_5 and 4 g. L^{-1} $ZnSO_4$ ($P_{12}Zn_4$ + Titicaca cultivar).

Table 2. Variance analysis of physiological and morphological traits of quinoa

S.O.V	df	Chlorophyll index	Plant height	Distance of the first lateral branch to the soil surface	Stem diameter	Number of lateral branches
Phosphorus (P)	3	20.55^{ns}	593.06^{**}	2.86^{**}	3.13^{**}	65.04^{**}
Zinc (Zn)	2	35.08^{ns}	400.32^{**}	3.35^{**}	2.55^{**}	2.62^{*}
Cultivar (C)	2	258.49^{**}	2347.77^{**}	4.65^{**}	19.94^{**}	61.5^{**}
P × Zn	6	61.21^{*}	447.95^{**}	3.49^{**}	1.63^{**}	1.04^{ns}
P × C	6	42.89^{ns}	300.52^{**}	2.69^{**}	3.02^{**}	12.74^{**}
Zn × C	4	5.79^{ns}	61.38^{**}	1.47^{**}	1.50^{*}	0.38^{ns}
P × Zn × C	12	46.50^{*}	401.87^{**}	1.79^{**}	4.15^{**}	2.80^{**}
Error	72	20.45	6.17	0.18	0.43	0.65
CV (%)		8.37	6.08	10.36	9.77	6.37

^{ns} = Not significant; * = $P \leq 0.05$; ** = $P \leq 0.01$.

In three cultivars, by increasing $ZnSO_4$ to 4 g. L^{-1} level, this trait was improved (Table 4). Increasing the chlorophyll index in 4 g. L^{-1} $ZnSO_4$ treatment compared to the control treatment shows the positive role of this element in improving the physiological processes of quinoa. Zn stimulates the tryptophan production, auxin growth hormone, and nitrate reductase enzymes, which increase chlorophyll in leaf area and increase photosynthesis (Liu *et al.,* 2016). In stating the reason for the superiority of the combination of chemical fertilizers treatment, it can be stated that the appropriate, sufficient and gradual supply of phosphorus and zinc elements probably caused the quinoa crop to have sufficient access to the elements required for the chlorophyll production, and other elements needed in photosynthesis and chlorophyll synthesis can also be provided through the use of these fertilizers.

4.2. Plant height

According to the main effect results, applying 18 mg. kg^{-1} triple superphosphate (P_{18}) and 4 g. L^{-1} $ZnSO_4$ (Zn_4) produced the highest plant height. There was a significant difference between the 18 mg. kg^{-1} triple superphosphate and the control mode (no fertilizer use) as well as zinc sulfate foliar application with a 4 g. L^{-1} concentration and the water without zinc sulfate treatment (Table 3). In the optimal rates of fertilizer, the root volume increases, and with the proper root expansion, the water and ions absorption level improve, and the growth of the aerial part increases. Also, with the proper production of plant growth regulating substances such as auxin, the rooting capacity of the plant increases, the macro, and micro elements absorption improves, and it will increase the vegetative characteristics of the plant (Malinowska *et al.,* 2015). The results showed that the Q26 cultivar ranked first (47.11 cm) with a little difference from the Giza 1 cultivar, and the Titicaca cultivar had the lowest plant height (31.73 cm) (Table 3).

Table 3. The main effect of treatments on physiological and morphological traits of quinoa

Treatments	Chlorophyll index	Plant height (cm)	Distance of the first lateral branch to the soil surface (cm)	Stem diameter (mm)	Number of lateral branches
Phosphorus $_0$	-	39.14	4.05	6.33	11
Phosphorus $_6$	-	35.16	3.65	6.53	11
Phosphorus $_{12}$	-	43.18	4.24	7.06	13
Phosphorus $_{18}$	-	45.87	4.41	6.93	14
LSD $_{(p\leq 0.05)}$	-	1.34	0.23	0.35	0.43
Zinc sulfate $_0$	-	39.76	3.96	6.66	13
Zinc sulfate $_4$	-	44.58	4.43	7.00	13
Zinc sulfate $_8$	-	38.62	3.77	6.49	12
LSD $_{(p\leq 0.05)}$	-	1.16	0.19	0.30	0.37
Giza 1	53.86	43.67	4.27	6.80	13
Q26	56.71	47.11	4.32	7.41	14
Titicaca	51.36	31.73	3.67	5.93	11
LSD $_{(p\leq 0.05)}$	2.12	1.16	0.19	0.30	0.37

The means were compared independently with the LSD test at the 5% probability level.

The three-fold interaction effect of the factors showed that Q26 cultivar had the highest plant height in P_{18}+Zn_4 treatment. Although this treatment did not show a statistically significant difference with the P_{12}+Zn_4/ Giza 1 treatment. The lowest plant height was attributed to the Titicaca cultivar in P_0+Zn_0 treatment (24.20 cm) (Table 4). Fluctuations in plant height are usually the most obvious characteristic of genetic conditions and changes in environmental conditions in most crops. Sometimes increasing the plant height is considered an advantage in terms of competition with other plants in a community, and one of its results is the new leaves formation on top of the vegetation. This feature puts the most useful leaves in the best position in terms of photosynthesis. Phosphorus, as the carrier of ATP energy, participates in all cellular interactions, interferes in cell division and rapid growth of meristem cells, and causes the plant to increase in height at high levels of phosphorus consumption (Yang *et al.,* 2021).

4.3. Distance of the first lateral branch to the soil surface

As for the distance of the first lateral branch to the soil surface shown in Tables 3, it is deduced that applying 18 mg. kg^{-1} triple superphosphate produced the highest distance of the first lateral branch to the soil surface (4.41 cm), and the lowest values of this trait was allocated to P_6 treatment. Foliar application of $ZnSO_4$, especially at 4 g. L^{-1} concentration, produced the maximum of this trait. High concentrations of zinc consumption by influencing and disrupting the physiological and biochemical processes causes a decrease in crop growth (Khavarinejad *et al.,* 2011). In terms of distance of the first lateral branch to the soil surface of quinoa cultivars, the Q26 cultivar was at a higher level than the Giza 1 and Titicaca cultivars. Based on three-fold interaction, under control conditions, Giza 1 and Titicaca cultivars had the same distance of the first lateral branch to the soil surface, but first lateral branch distance of the Giza 1 cultivar was more than the Titicaca cultivar.

Table 4. The interaction effect of treatments on physiological and morphological traits

Traits		Chlorophyll index			Plant height (cm)			Distance of the first lateral branch to the soil surface (cm)		
P_2O_5 (mg.kg^{-1})	$ZnSO_4$ (g.L^{-1})	Giza 1	Q26	Titicaca	Giza 1	Q26	Titicaca	Giza 1	Q26	Titicaca
0	0	48.10	53.50	44.86	32.60	36.66	24.20	3.33	3.73	3.00
	4	53.43	56.73	58.00	48.00	60.03	47.63	5.53	4.20	4.50
	8	50.23	56.20	54.43	39.36	34.56	29.16	4.30	3.40	4.50
6	0	56.16	60.20	55.46	35.80	57.76	28.23	4.86	4.66	3.50
	4	52.56	57.73	48.83	28.23	44.83	25.36	3.10	3.60	3.00
	8	54.63	55.50	46.90	33.76	36.06	26.43	3.60	3.46	3.10
12	0	50.26	54.63	51.30	50.40	35.73	33.20	3.30	4.20	3.90
	4	56.30	51.10	58.90	67.53	36.06	48.13	5.13	4.03	4.70
	8	55.36	58.93	48.73	33.66	56.86	27.10	4.20	4.50	4.20
18	0	57.76	54.06	50.13	62.73	47.86	32.00	5.36	4.80	2.96
	4	56.40	63.36	46.73	36.40	67.93	24.80	5.26	7.30	2.90
	8	55.20	58.66	52.06	55.60	51.00	34.50	3.26	4.00	3.86
LSD $_{(P\leq 0.05)}$		7.36			4.04			0.69		

Traits		Stem diameter (mm)			Number of lateral branches		
P_2O_5 (mg. Kg^{-1})	$ZnSO_4$ (g. L^{-1})	Giza 1	Q26	Titicaca	Giza 1	Q26	Titicaca
0	0	4.75	7.85	4.85	10	13	11
	4	7.18	6.86	7.29	11	13	11
	8	6.11	6.11	5.99	12	11	10
6	0	7.06	7.70	5.97	11	11	11
	4	5.52	7.24	5.95	11	12	11
	8	6.02	7.33	6.02	11	13	11
12	0	8.02	6.84	5.82	16	13	12
	4	8.97	7.80	5.85	17	14	12
	8	7.15	8.04	5.11	13	14	11
18	0	8.65	6.39	6.06	15	16	12
	4	6.59	9.56	5.26	15	18	11
	8	5.54	7.27	7.05	15	16	12
LSD $_{(P\leq 0.05)}$		1.06			1.31		

The means were compared independently with the LSD test at the 5% probability level.

The Q26 cultivar in 18 mg. kg^{-1} P_2O_5 fertilizer and 4 g. L^{-1} $ZnSO_4$ level produced the maximum average (7.30 cm) of this trait (Table 4). Creating a suitable substrate and improving the growth environment provided better use of climatic parameters and increased the distance of the first lateral branch to the soil surface. The importance of the formation of the first lateral branch is effective on how to reduce the damage of harvesting. The greater the distance of the first lateral branch from the soil surface, harvesting is easier and less damage is done. This issue is due to genetic diversity between cultivars and their maturation period. These results are consistent with the results reported by Bagheri (2018).

4.4. Stem diameter

It is noticed from results shown in Table 3, 12 mg. kg^{-1} triple superphosphate application caused an increase in stem diameter. Increasing the Zn concentration to 4 g.L^{-1} level, led to an increased in stem diameter. Regardless of P and Zn levels, the Q26 cultivar had higher stem diameter, and Giza 1 genotype ranked second. In an experiment by Goma (2013), the phosphorus fertilizer application increased the quinoa stem diameter compared to the control treatment. The quinoa is very sensitive in rainy seasons and high fertilizer use, so cultivars with a larger stem diameter will be more resistant compared to cultivars with a smaller stem diameter. The interaction effects of elements and cultivars (P×Zn×C) showed that pots were treated by 18 mg. kg^{-1} triple superphosphate and 4 g. L^{-1} $ZnSO_4$ foliar application in Q26 cultivar achieved maximum SD (9.56 mm), which showed difference compared to P_0 + Zn_0/ Giza 1, P_0 + Zn_0/ Q26, and P_0 + Zn_0/ Titicaca treatments (Table 4). Shirinnejad and Torabi Mahmoudi (2019) acknowledged that cultivars with larger stem diameter are capable of providing more reproductive units and allocate more dry matter to these units, which was consistent with results of present study.

4.5. Number of lateral branches

Consistent with main effect results, in the 18 mg. kg^{-1} P_2O_5 treatment, the highest mean of lateral branches number (NLB) was obtained with numerical values of 14 branches. The lowest mean of the mentioned trait was obtained in the non-application of P_2O_5 fertilizer. The NLB of the Zn_8 treatment was less than the Zn_0 treatment, and Zn_0 and Zn_4 treatments were at a similar statistical level. Among the tested cultivars, the Q26 cultivar was an efficient cultivar in the terms of NLB, and the Titicaca cultivar was recognized as the weakest cultivar (Table 3). The superiority of the lateral branches number of Q26 cultivar was consistent with the results of Mansouri *et al.*'s research (2021). According to these researchers, the NLB of the quinoa crop, in addition to being influenced by nutritional elements, is also affected by the genetics of the crop variety. Based on interaction of factors, Giza 1 and Titicaca cultivars obtained maximum NLB at 12 mg. kg^{-1} of triple superphosphate and 4 g. L^{-1} of $ZnSO_4$, which showed a significant difference with control treatment. The Q26 cultivar in 18 mg. kg^{-1} P_2O_5 fertilizer and 4 g. L^{-1} $ZnSO_4$ level produced the maximum average of this trait (Table 4). It seems that the use of triple superphosphate and zinc sulfate fertilizers has stimulated the vegetative growth of the plant and increased the amount of assimilates available to the lateral buds through increasing the level of photosynthesis. This factor has stimulated the growth of lateral buds and the emergence of more lateral branches.

4.6. Root density

The results of this research showed the effect of experimental factors and their interaction on root density changes (Table 5). Based on the results of Table 6, there was no obvious difference in terms of root density between the P_0 treatment and the amount of 6 mg. kg^{-1} of triple superphosphate fertilizer, and the use of 12 mg. kg^{-1} of this fertilizer improved this trait compared to the other amounts of P_2O_5 fertilizer.

Table 5. Variance analysis of quinoa quantitative traits

S.O.V	df	Root density	Root length to shoot length ratio	Total grain weight
Phosphorus (P)	3	0.23^{**}	0.08^{**}	82.04^{**}
Zinc (Zn)	2	0.07^{**}	0.001^{ns}	18.90^{**}
Cultivar (C)	2	0.003^{**}	0.21^{**}	65.74^{**}
P × Zn	6	0.04^{**}	0.07^{**}	12.75^{**}
P × C	6	0.06^{**}	0.08^{**}	49.45^{**}
Zn × C	4	0.11^{**}	0.01^{**}	7.13^{**}
P × Zn × C	12	0.08^{**}	0.03^{**}	19.98^{**}
Error	72	0.0006	0.004	0.09
CV (%)	-	4.37	9.59	7.75

^{ns} = Not significant; * = $P \leq 0.05$; ** = $P \leq 0.01$.

In the investigation of the effect of $ZnSO_4$ concentrations, it was observed that the 8 g. L^{-1} concentration of zinc sulfate with an average of 0.61 g.cm^{-3} was identified as the best treatment in terms of this trait. It seems that with the increase in chemical fertilizers in the phenological stages, the roots expansion in the soil has been done well, and the increase in the root dry weight per unit of soil volume has followed. Examining the differences between quinoa cultivars showed that Giza 1 cultivar had the highest root density with an average of 0.86 g.cm^{-3}, and Q26 and Titicaca cultivars with averages of 0.58 and 0.56 were in second and third place, respectively (Table 6). It seems that the genetic potential of the cultivars in increasing or decreasing the root dry weight per unit volume of soil is the main factor of superiority or non-superiority of quinoa root density. The highest root density with an average of 0.97 g.cm^{-3} was obtained under the conditions of 12 mg. kg^{-1} of triple super phosphate fertilizer application and in Q26 cultivar, which showed a significant difference with other amounts of fertilizer, and also with other quinoa cultivars. In Titicaca cultivar, under the conditions of the maximum application of triple super phosphate fertilizer (P_{18}) and zinc sulfate (Zn_8) recorded the highest root density with values of 0.89 g.cm^{-3}, which is compared to the P_0Zn_0 treatment, increased by 70.79% (Table 7). Foliar application of zinc sulfate becomes very important when there is an antagonistic phenomenon for the transfer of some materials through the root, or when a compound is added to the soil that destroys soil organisms. This feeding method can provide elements to the plant in the fastest time and be effective in the growth of roots and shoots of crops and vegetables (Miransari *et al.*, 2015). Based on the results of the present study, the minimum of this trait was obtained in the Giza 1 cultivar with an average of 0.78 g.cm^{-3} under the conditions of 12 mg. kg^{-1} of triple super phosphate fertilizer application with 8 g. L^{-1} concentration of zinc sulfate and the further increase of phosphorus fertilizer (P_{18}) in varying concentrations of zinc sulfate caused a decrease in root density in this cultivar (Table 7).

Table 6. The main effect of treatments on quinoa quantitative traits

Treatments	Root density ($g.cm^{-3}$)	Root length to shoot length ratio	Total grain weight ($g. plant^{-1}$)
Phosphorus $_0$	0.49	0.64	2.17
Phosphorus $_6$	0.49	0.66	2.56
Phosphorus $_{12}$	0.68	0.73	5.29
Phosphorus $_{18}$	0.60	0.60	5.45
LSD $_{(p\leq 0.05)}$	0.01	0.03	0.16
Zinc sulfate $_0$	0.55	-	3.46
Zinc sulfate $_4$	0.52	-	4.71
Zinc sulfate $_8$	0.61	-	3.51
LSD $_{(p\leq 0.05)}$	0.01	-	0.14
Giza 1	0.86	0.59	3.83
Q26	0.58	0.63	5.24
Titicaca	0.56	0.74	2.54
LSD $_{(p\leq 0.05)}$	0.01	0.02	0.14

The means were compared independently with the LSD test at the 5% probability level.

4.7. Root length to shoot length ratio

The findings of the main effect of the experimental treatments showed that the highest root length to shoot length ratio with an average of 0.73 was assigned to the 12 mg. kg^{-1} of triple super phosphate fertilizer treatment, which was significantly different from other treatments. Among the cultivars studied, the Titicaca cultivar had the highest root length to shoot length ratio with values of 0.74, and the lowest values of this trait were assigned to the Giza 1 cultivar. The Q26 cultivar with an average of 0.63 took the second place (Table 6). The results showed that Titicaca cultivar had the maximum root length to shoot length ratio with an average of 0.99 in the P_{12}+Zn_8 treatment. In this treatment, the Giza 1 cultivar also obtained the maximum of this attribute. In the control mode conditions (P_0), increasing the concentration of zinc sulfate resulted in a decrease in the root length to shoot length ratio. While in the P_{12} treatment, increasing the concentration of zinc sulfate from zero to 8 g. L^{-1} caused an increase this trait. In the maximum use of phosphorus fertilizer conditions (P_{18}), the 4 g. L^{-1} concentration of zinc sulfate compared to the maximum concentration affected this trait in all three cultivars (Table 7). Since the higher ratio of roots to aerial organs (absorbing organs compared to consuming organs) determines the crop ability to adapt the unfavorable growth conditions, therefore, most physiologists introduce this ratio as a criterion for selecting efficient cultivars (Hoseinzade *et al.,* 2013). Probably, the greater longitudinal growth of the aerial part of Q26 cultivar compared to other cultivars has reduced this ratio. The research of Slovak *et al.* (2015) showed that increasing the growth of the root system in unfavorable growth conditions in some cultivars is a mechanism to increase the resistance of these cultivars against the restriction of access to nutritional elements, which was consistent with the results of this study.

4.8. Total grain weight

Comparing the mean showed that the best value of TGW (5.45 g. plant^{-1}) was obtained in 18 mg. kg^{-1} triple superphosphate. Also, the foliar application of $ZnSO_4$ with 4 g. L^{-1} concentration increased total grain weight (4.71 g. plant^{-1}). Highly significant differences were observed among the test cultivars. Q26 cultivar with the mean of 5.24 g. plant^{-1} gained maximum total grain weight, and Giza 1 cultivar ranked second. The weakest cultivar was Titicaca (2.54 g. plant^{-1}) (Table 6). Moulai and Bagheri (2020) reported that the Q26 cultivar has the highest grain yield. The grains of this cultivar are relatively large, the length of its growth period matches with the growing season in the tested area, and its cultivation is recommended. Three-fold interactions of factors significantly improved total grain weight. Regarding Table 7, maximum TGW belonged to Q26 cultivar+ 18 mg. kg^{-1} triple superphosphate+ 4 g. L^{-1} zinc sulfate foliar application. Foliar application at the rate of 8 g. L^{-1} zinc sulfate in different levels of phosphorus fertilizer and cultivars reduced total grain weight; however, the lowest value of TGW was achieved in P_0+Zn_0+ Giza 1, P_0+Zn_0+ Q26, P_0+Zn_0+ Titicaca, and P_6+Zn_4+ Titicaca treatments.

Table 7. The interaction effect of treatments on quantitative traits

Traits		Root density (g.cm^{-3})			Root length to shoot length ratio			Total grain weight (g. plant^{-1})		
P_2O_5 (mg.kg^{-1})	**$ZnSO_4$ (g.L^{-1})**	**Giza 1**	**Q26**	**Titicaca**	**Giza 1**	**Q26**	**Titicaca**	**Giza 1**	**Q26**	**Titicaca**
0	**0**	0.42	0.55	0.26	0.56	0.75	0.73	0.73	1.20	0.95
	4	0.51	0.46	0.54	0.54	0.68	0.59	2.46	3.58	5.31
	8	0.64	0.41	0.64	0.50	0.80	0.60	2.30	1.85	1.11
6	**0**	0.41	0.44	0.89	0.66	0.59	0.94	2.56	7.60	1.76
	4	0.45	0.45	0.36	0.69	0.44	0.66	2.16	2.43	1.09
	8	0.31	0.56	0.53	0.67	0.71	0.58	1.69	2.49	1.38
12	**0**	0.64	0.97	0.44	0.54	0.66	0.73	7.80	2.18	1.64
	4	0.63	0.64	0.52	0.71	0.69	0.82	9.20	2.53	8.57
	8	0.78	0.73	0.82	0.88	0.57	0.99	5.80	8.35	1.60
18	**0**	0.63	0.66	0.37	0.44	0.49	0.75	4.75	8.67	1.69
	4	0.66	0.64	0.45	0.57	0.79	0.79	2.33	14.30	2.46
	8	0.66	0.41	0.89	0.40	0.39	0.76	4.25	7.65	2.90
LSD $_{(P\leq 0.05)}$		0.03			0.10			0.48		

The means were compared independently with the LSD test at the 5% probability level.

The results suggest the positive role of phosphorus and zinc elements on the production of acceptable yield. Apparently, foliar application of zinc sulfate and consumption of phosphorus fertilizer has caused more nitrogen uptake in the plant, and thus increased the grain weight of quinoa. Increased grain yield due to zinc and phosphorus consumption is due to root development and increased uptake of elements from the soil, increased storage materials, and reduced source constraints that cause transferring materials into the grain. Other researchers have reported a positive effect of zinc and phosphorus application on increasing grain yield, which is most likely due to the effect of these elements on indole acetic acid (IAA) (Ziaei *et al.,* 2020).

4.9. Correlation of studied traits

Based on the results of correlation analysis, leaf chlorophyll index showed a significant correlation with all traits except number of lateral branches and root traits (Table 8). This means that the increase in leaf chlorophyll improved the vegetative structure of the quinoa crop (plant height, stem diameter and distance from the first lateral branch to the soil surface) and total grain weight. In one research on quinoa cultivars, the traits identification with strong correlation by grain yield was suggested as an indicator for selecting desirable cultivars with high yield (Al-Naggar *et al.,* 2017).

Table 8. The Correlation results of studied traits

Traits	1	2	3	4	5	6	7
2	0.71^{**}						
3	0.66^{**}	0.64^{**}					
4	0.64^{**}	0.73^{**}	0.63^{**}				
5	0.44^{ns}	0.71^{**}	0.51^{*}	0.66^{**}			
6	0.17^{ns}	0.10^{ns}	0.28^{ns}	0.25^{ns}	0.31^{ns}		
7	-0.15^{ns}	-0.40^{ns}	-0.07^{ns}	-0.17^{ns}	-0.28^{ns}	0.19^{ns}	
8	0.63^{**}	0.79^{**}	0.63^{**}	0.64^{**}	0.73^{**}	0.18^{ns}	-0.11^{ns}

^{ns} = Not significant; * = P ≤ 0.05; ** = P ≤ 0.01.

1: Chlorophyll index**, 2:** Plant height, **3**: Distance of the first lateral branch to the soil surface, **4**: Stem diameter, **5**: Number of lateral branches, **6**: Root density, **7**: Root length to shoot length ratio, **8**: Total grain weight.

In another study on the quinoa crop, the stem diameter and plant dry weight were reported as two traits that are very effective in increasing grain yield, and the selection of plants with thick stems, more inflorescences and higher dry weight, was suggested to increase the quinoa yield (Bhargava *et al.,* 2003). The results showed that the total grain weight had a statistically significant correlation with all morphological and physiological traits and there was no significant correlation between this trait (TGW) and root density and the ratio of root length to shoot length. Although the ratio of root length to shoot length had a negative correlation with most of the investigated traits, but this correlation was not significant (Table 8).

5. CONCLUSION

It is concluded from the results of this research that there was a positive and direct relationship between morphological and physiological indicators of quinoa cultivars with production. Optimum ratios of P-Zn nutrients by increasing the photosynthetic system efficiency and better growth of roots and shoots improved the production of this crop. The 4 g. L^{-1} concentration of zinc sulfate improved the morphological indicators. The increase in root density by improving the root connection with soil particles, increased the quinoa production. The Q26 cultivar was recognized as the best cultivar compared to another investigated cultivars, and the Titicaca cultivar had an underdeveloped and weaker root system.

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FOOTNOTES

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