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Nitrogen Nutrition and Adaptation of Halophyte *Chenopodium quinoa* to Salt Stress

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Abstract

There is little evidence that the nitrogen nutrition supply at rates above or less than what is considered optimal in non-saline conditions improves growth and yield of halophyte crop cultivated under salt stress. Therefore, hypothesize of the present work was to find out the magnitude to which N could restore the harmful effects of salt stress on quinoa plants. A pot experiment was performed in greenhouse conditions to evaluate quinoa's response grown under water salinity treatments (0.0 & 200 mM NaCl) when nitrogen nutrition rates were limiting (50ppm), adequate (250 ppm), and excess (450 ppm) to guide proper application rate of nitrogen fertilizer under salinity stress. The results indicated that, salinity caused a significant decrease in the vegetative growth of the plant. Consequently, all vegetative measurements were negatively affected. As a result, the seed yield decreased to more than 50%. The application of a moderate level of nitrogen (250 ppm) caused a significant ameliorative effect on seed yield by 126% under non saline conditions and 34.5 % under saline conditions compared to the low nitrogen level. The results did not improve any further with the application of a higher level of nitrogen. These results indicate that applying (N) in adequate may improve most traits and prove to be a physiological treatment to increase resistance against the negative effects of salt stress in quinoa.

Keywords: Salt Stress, Nitrogen Nutrition, Nitrogen Use e Efficiency, Seed Yield, *Chenopodium quinoa*

1 Introduction

Salinity is one of the main environmental stressors, as it causes growth and crop productivity to be reduced in many regions of the world (Liang et al 2018). Salinity affects plant growth by two major threats: osmotic and ionic stresses (Flower and Colmer 2008). Moreover, it causes oxidative stress (Rafiq et al 2017), gas exchange reduction (Hu et al 2017), lessening leaf water content and photosynthetic pigments (Abbas et al 2017), which leads to inhibition of plant growth and decrease plant biomass production (Abbas et al 2015, Negrão et al 2017). To make matters worse, most traditional crops do not tolerate salinity. Several researches have focused on finding solutions to this problem. One of these solutions is to utilization of halophyte plants, which can resist high levels of salt stress (Adolf et al 2012). Quinoa is an annual herbaceous crop that belongs to *Amaranthaceous* family, it originates from the Andean region. This region is characterized by its harsh nature, and quinoa has shown a high resist for many environmental factors such as drought, frost, wind, hail and salt stress (Hariadi et al 2011). It is a facultative halophytic plant and can resistance elevated levels of salinity of up to more than 400

mM sodium chloride (Koyro and Eisa 2008, Hariadi et al 2011). The salinity tolerance limit for both biomass and quinoa seed yield was noticed at 200 mM NaCl (Eisa et al 2017). Proper nutrition under salinity conditions is one of the most essential agricultural practices to deal with the adverse effects of salinity stress. Of all the essential elements, nitrogen is required in greater quantities and has a major role in the growth and productivity of most crops, whether under non-saline (Hou et al 2007) or saline conditions (Chen et al 2010). Optimization of nitrogen nutrition could be a convenient strategy to alleviate the harmful effects of salinity on plants by ameliorating nutrients imbalances and/or ion toxicity through its impacts on uptake and transport of ions in plant. In addition to its vital role in osmotic adjustment by inducing synthesis of osmoprotectants like proline and glycine betaine (Siddiqui et al 2010, Rais et al 2013, Ashraf et al 2018). Although, numerous researches have been performed to study the individual effect of salt stress or nitrogen nutrition on plant development and productivity, there are few studies on their interactive effects, and most of these studies have been conducted on conventional crops (Esmaili et al 2008, Chen et al 2010, Zhang et al 2012, Ibrahim et al 2018). And very few studies have been conducted on cash crops for halophytes (Hessini et al 2011, Hessini et al 2013, Amal Mahmoud et al 2019). Therefore, the aim of the present study was to compare the response of halophyte quinoa to salinity stress when nitrogen nutrition rates were limited, adequate, or in excess to guide proper nitrogen fertilizer rate that could restore yield losses induced by salinity.

2 Materials and Methods

2.1 Plant growth conditions and treatments

Seeds of *Chenopodium quinoa* willd cv. *Hualhuas*, (origin: International Potato Center, Lima, Peru) were surface sterilized before sowing by soaking for 10 seconds with 70% ethanol and then were washed for several times with distilled water. Five seeds were then transferred onto a plastic pot (25 cm inner diameter and 30 cm height, with 5 drain holes at the bottom). The pots were filled with washed sandy soil "7.5 kg per pot" under controlled conditions in the greenhouse of Agricultural Botany Dept., Faculty of Agriculture, Ain Shams University (latitude 30° 06' 48" and longitude 31° 14' 52").

Plants were regularly watered with a modified nutrient solution (Arnon and Hoagland 1940). The nutrient solution was modified by replacing all sources of nitrogen by ammonium nitrate salt, with maintaining other nutrient concentrations. All pots were exposed to photoperiod of (14h light/ 10h dark) with a day/night temperature of (28/15 ± 3°C) and relative humidity ranged from 40 to 60%. Daylight radiation type metal halide lamps (Osram, 200 watt, light intensity 300 µmol m^{-1} s^{-1}) were used for lighting.

Plants thinned out to maintain one plant per pot. After a further one week the treatment began. Treatments have been organized in totally randomized design with 12 replicates. Each replicate was included in a combination of two levels of salinity [control and 200 mM NaCl]. "Some studies mention that optimal growth and productivity of quinoa can be carried out between 10 and 20 dS/m **(**Hariadi et al 2011, Adolf et al 2013)" therefore, we tried to increase the resistance of quinoa to raise the tolerance limit to above 200 mM for NaCl salinity. With three levels of nitrogen (50, 250 and 450 ppm).

2.2 Samples and harvest

Two samples were taken to assess the impact of nitrogen nutrition rates on growth and productivity of *C. quinoa* willd. cv. *Hualhuas* grown under salinity treatments.

The first sample (8 weeks after sowing) to estimate some growth and some physiological parameters, by randomly selecting for six replicates of each treatment.

2.3 Growth parameters

The plants were divided into leaves (L), stem(S), and root (R) after determine plant height (Ph). To calculate the dry weight for all plant organs, samples of about 10 g were dried at 70° C until constant weight was reached.

2.4 Physiological parameters

2.4.1 Determination of osmotic potential

The freeze-point depression method was used to measure the osmotic potential of the press sap of roots and leaves using a cryo osmometer (Osmomat 030, Genotec GMBH, Berlin 2006).

2.4.2 Determination of mineral elements

About 0.2 g of powdered dried plant samples from each plant organ was put in glass flask and added 10 ml of concentrated sulfuric acid to start the digestion of samples and kept overnight at room temperature. The next day, 1 ml of hydrogen peroxide was added to each flask, they were placed in a digestion block whose temperature was set to 350°C for 45 minutes. The extracts were cooled, then 2 ml of hydrogen peroxide was added, then they were brought back to the digestive block, this step was repeated several times until the extracts became colorless. After that, the extracts were filtered and supplemented to 50 ml with distilled water. The concentration of total nitrogen (N) in these digests was analyzed using the modified micro Kjeldahl method in a distilling unit VELP UDK-127 according to the procedure described by Cottenie et al (1982). The amount of Na^+ and K^+ contents was estimated using a Flame photometer (Jenway PFP7, ELE Instrument Co. Ltd., UK).

2.4.3 Nitrogen Use Efficiency (NUE)

Total Nitrogen uptake was calculated as the product of (N) concentration of whole plant components in corresponding to its dry mass (Chen et al 2010). Two indices of nitrogen use efficiency, including agronomic nitrogen use efficiency (aNUE) and physiological nitrogen use efficiency (pNUE) were calculated after the following equations used by Song et al (2019).

$$aNUE = (YN - YC)/FN \ldots\ldots\ldots (1)$$

Where aNUE = Agronomic efficiency of applied N (g yield increase per g N applied)

YN refer to grain yield [g pot^{-1}] in the N-applied treatments (N2 and N3)

YC refer to grain yield [g pot^{-1}] in the low N applied (N1)

FN = rate of N applied [g pot^{-1}]

$$pNUE = (YN - YC)/(UN - UC) \ldots\ldots\ldots (2)$$

Where pNUE = Physiological efficiency of applied N (g yield increase per g increase in N uptake).

UN and UC = is total nitrogen uptake in the nitrogen applied treatment and its control.

The second sample (yield) was taken after ripening (18 weeks after sowing) by randomly selecting for six replicates of each treatment. The Inflorescences of six replicates of each treatment were separated from the stems, the seeds were separated from inflorescences by hand. The seeds weight of each plant was recorded, as well as the weight of 1000 seeds per plant.

2.4.4 Statistical analysis

All data were statistically analyzed of variance procedure using SAS software Version 9 (SAS, 2006).

3 Results and Discussion

The results of two-way ANOVA analysis for different morphological, physiological, and seed yield traits are presented in **Table 1**. The fresh weight of shoot, plant height, shoot dry weight, root fresh weight, root dry weight and stem diameter were significantly affected by water salinity, nitrogen treatments, and their interactions. The seed yield of quinoa

responded significantly to the treatments of salinity, nitrogen and their interaction. Conversely, there were no statistically significant differences in the weight of 1000 grains per plant under all treatments **Table 1**. The correlation coefficients for all measured morphological traits showed significant positive correlation with seed yield **Table 2**. The highest correlation value was recorded for shoot fresh weight (0.905) followed by stem diameter (0.893), plant height (0.883), shoot dry weight (0.882), root dry weight (0.767), and root fresh weight (0.704), respectively.

The effects of saline water and N addition rate on seed yield of quinoa are illustrated in **Fig. 1** Under the treatments of non-saline conditions (S0), increasing the nitrogen application rate from low (N1) to moderate (N2) significantly increased seed yield by 126%, but further rising into the highest level (N3) resulted in a significant decrease in the seed yield by 8.3% less than (N2) rate. Application of salinity in irrigation water (200 mM NaCl) significantly decreased the seed yield of quinoa by 50.9% less than the non-saline low nitrogen treatment. Elevation of nitrogen application rate under the saline condition from (N1) to (N2) significantly increased the seed yield of quinoa by 34.5%. In contrast to the non-saline condition, raising the level of nitrogen application from (N2) to (N3) led to insignificant increase ($P \leq 0.05$) of seed yield by 8.8% higher than the (N2) rate.

As for the physiological traits, a significant effect of saline water, nitrogen rates and the interaction between them on the osmotic potential of root were observed. But, the osmotic potential of leaves was significantly affected only by salinity meanwhile, nitrogen levels or the interactive between salinity and nitrogen treatments had no effects **Tables 1 & 3**. The correlation coefficient between osmotic potential and seed yield is presented in **Table 2**. Either osmotic potentials of root or leaves showed positive association with seed yield, the leaves osmotic potential recorded a higher correlation value (0.776) than that of root osmotic potential (-0.370).

The statistical analysis presented in **Table 1.** Showed that the nitrogen concentration, either in root or leaves, was significantly affected by nitrogen application rates ($P< 0.001$), while the effect of salinity was non-significant on nitrogen concentration in root and was relatively limited in leaves ($P< 0.05$). The highest significant concentration of nitrogen in root tissues was obtained at a moderate level of nitrogen (N2) in both non-saline (S0) and saline (S1) treatments **Table 3.** and the nitrogen concentration in the root displayed a positive association with seed yield **Table 2**. Meanwhile, the highest significant value of nitrogen concentration in leaves was observed at the highest nitrogen application rate (N3) to record 5.07% under non- saline treatment and 4.03 % under salinity treatment, on dry weight basis **Table 3**. However, the nitrogen content in leaves had no significant correlation with seed yield **Table 2**. Therefore, the results of the present work clearly indicated that increases of nitrogen concentration in plant leaves might not be translated into increases in seed yield for halophytic species like quinoa.

The potassium concentration, whether in roots or in leaves, was significantly affected by salinity and nitrogen treatments, while the interaction effect showed a significant effect on the potassium concentration in the root, but in the leaves was insignificant **Table 1**. Data presented in (**Table 3)** clearly showed an opposite trend between the effects of salinity treatment and nitrogen fertilizer on potassium concentration in different plant parts. In general, Salinity treatment led to decrease potassium concentration in roots, which was about 15% less than that of non-saline treatment. Meanwhile, potassium concentration in the roots was significantly increased by increasing nitrogen application rates up to (N2) to record 14.4% greater than (N1) treatment. Moreover, the results of the interaction effects showed that the salinity treatment caused a linear decrease of the potassium concentration in the root tissues under the application rates of N1, N2, and N3 to being 6.7%, 4.5% and 30.8% less than non-saline, respectively. However, the increase in nitrogen

Table 1. Two-way ANOVA analysis of plant traits by salinity (S), nitrogen (N), and their interaction (S*N)

Parameters	Salinity (S)	Nitrogen (N)	Interaction (S*N)
Plant height [cm]	2454***	55.47***	20.68***
Stem diameter [cm]	68.64***	26.91***	15.5***
Root fresh weight [g plant $^{-1}$]	796.83***	79.91***	74.94***
Shoot fresh weight [g plant $^{-1}$]	3631.68***	960.08***	728.50***
Root dry weight [g plant $^{-1}$]	672.68***	151.66***	138.34***
Shoot dry weight [g plant $^{-1}$]	880.56***	232.65***	170.19***
Seed yield [g plant $^{-1}$]	1836.03***	323.58***	160.83***
Weight of 100 seed [g]	.02 n.s	2.17 n.s	.74 n.s
Osmotic potential in root [MPa]	74.62***	8.25**	8.09**
Osmotic potential in leaves [MPa]	353.0***	1.95 n.s	2.26 n.s
Nitrogen concentration in root [%]	3.91 n.s	26.32***	2.81 n.s
Nitrogen concentration in leaves [%]	5.00*	195.00***	33.80***
Potassium concentration in root [%]	20.02***	8.00**	9.52**
Potassium concentration in leaves [%]	24.00***	59.04***	0.12 n.s
Sodium concentration in root [%]	512.00***	22.62***	.87 n.s
Sodium concentration in leaves [%]	976.07***	2.47 n.s	4.07*

Number represent F-values *P< 0.05, **P< .01, ***P< 0.001, n.s., non-significant

application rates from N1 to N3 led to a significant increase in the potassium content in the roots by about 34.7% under non-saline treatment, but it had no effect under saline treatment **Table 3**. In contrast to the roots, the potassium concentration in leaves sharply decreased as affected by increasing nitrogen levels. The highest potassium concentration value was recorded at saline treatment (S1) under low nitrogen nutrition level (N1). However, increasing nitrogen application rates led to significant decrease of potassium content in leaves under both of non-saline and saline treatments **Table 3**. Potassium concentration in the root displayed a significant positive association with seed yield, while the potassium content in leaves presented a significant negative association with seed yield **Table 3**.

The two-way ANOVA results presented in **Tables 1** and **3** showed that the sodium concentration in the roots and leaves was significantly increased under salinity treatment to record 87.5% and 300% higher than the non-saline treatment in the roots and leaves, respectively. Sodium concentration in roots was significantly increased by N treatments. While, N treatments had no significant effect on the sodium content in leaves **Table 1**. The correlation coefficient between sodium content in roots or leaves with seed yield is presented in **Table 2**. Sodium concentration in roots and leaves exhibited negative significant correlation with seed yield. Water salinity treatment significantly increased sodium concentrations in both of roots and leaves. This led to a gradual reduction in K+/ Na+ ratio in both organs **Fig. 2** However K+/Na+ ratio in the leaves were progressively lower than those of the roots.

3.1 Nitrogen use efficiency

Nitrogen use efficiency as indicated by agronomic nitrogen use efficiency (aNUE) and physiological nitrogen use efficiency (pNUE) are presented in **Table 4**. Salinity treatment significantly decreased both of aNUF and pNUF to recored 82.8% and 18.8% less than non-saline treatments.

As for interaction between salinity and nitrogen treatments, the highest level of nitrogen application (N3) significantly reduced both of aNUF and pNUF under non-saline treatment, while it had no significant effect under saline treatments.

Table 2. Correlation coefficient among 17 traits in *Chenopodium quinoa*

Parameters	Seed yield	Plant length	Stem diameter	Root fresh weight	Root dry weight	Stem fresh weight	Stem dry weight	O.P Root	O.P Leaves	Root (K) conc. (%)	Leaves (K) conc. (%)	Root (Na) conc. (%)	Leaves (Na) conc. (%)	Root (N) conc. (%)	Leaves (N) conc. (%)	Root K/Na ratio	Leaves K/Na ratio
Seed yield		0.88346 <.0001	0.89355 <.0001	0.70485 0.0011	0.76713 0.0002	0.9056 <.0001	0.88279 <.0001	-0.37013 0.1306	-0.77696 0.0001	0.7101 0.001	-0.60835 0.0074	-0.66603 0.0025	-0.79776 <.0001	0.63457 0.0047	0.41336 0.0882	0.75475 0.0003	0.55401 0.0171
Plant length			0.79385 <.0001	0.90331 <.0001	0.84881 <.0001	0.85595 <.0001	0.85814 <.0001	-0.71636 0.0008	-0.93899 <.0001	0.54538 0.0192	-0.40539 0.0951	-0.85297 <.00019	-0.96475 <.0001	0.32871 0.1829	0.09096 0.7196	0.78419 0.0001	0.87079 <.0001
Stem diameter				0.74735 0.0004	0.85922 <.0001	0.94476 <.0001	0.94344 <.0001	-0.32684 0.1856	-0.60517 0.0078	0.48 0.0438	-0.4162 0.0858	-0.46369 0.0526	-0.65603 0.0031	0.53593 0.0219	0.21459 0.3925	0.48907 0.0394	0.45532 0.0576
Root fresh weight					0.94598 <.0001	0.84354 <.0001	0.86493 <.0001	-0.78343 0.0001	-0.82715 <.0001	0.23339 0.3513	-0.10596 0.6756	-0.71728 0.0008	-0.83414 <.0001	0.06869 0.7865	-0.28915 0.2445	0.50559 0.0323	0.85716 <.0001
Root dry weight						0.94564 <.0001	0.96117 <.0001	-0.57461 0.0126	-0.73481 0.0005	0.28975 0.2435	-0.11189 0.6585	-0.55751 0.0162	-0.71237 0.0009	0.20042 0.4252	-0.22923 0.3602	0.42087 0.082	0.68473 0.0017
Stem fresh weight							0.99638 <.0001	-0.39925 0.1007	-0.70989 0.001	0.48686 0.0405	-0.34061 0.1666	-0.52041 0.0268	-0.70926 0.001	0.46638 0.0511	0.0645 0.7993	0.50615 0.0321	0.56087 0.0155
Stem dry weight								-0.42827 0.0762	-0.70792 0.001	0.46441 0.0522	-0.31493 0.203	-0.52146 0.0265	-0.71151 0.0009	0.42951 0.0753	0.02132 0.9331	0.49214 0.038	0.58332 0.0111
O.P Root									0.76738 0.0002	-0.02869 0.91	0.02545 0.9202	0.79062 <.0001	0.77333 0.0002	0.26056 0.2963	0.32408 0.1895	-0.50586 0.0322	-0.91203 <.0001
O.P Leaves										0.54156 0.0203	0.29303 0.238	0.93653 <.0001	0.94306 <.0001	-0.15781 0.5317	-0.04089 0.872	-0.84053 <.0001	-0.89464 <.0001
Root (K) conc. (%)											-0.64269 0.004	-0.47837 0.0446	-0.55119 0.0177	0.7057 0.0011	0.62773 0.0053	0.80002 <.0001	0.2561 0.305
Leaves (K) conc. (%)												0.33598 0.1728	0.39152 0.1081	-0.71013 0.001	-0.81964 <.0001	-0.57961 0.0117	-0.12373 0.6247
Root (Na) conc. (%)													0.91544 <.0001	-0.04633 0.8551	-0.10934 0.6658	-0.87645 <.0001	-0.86658 <.0001
Leaves (Na) conc. (%)														-0.28274 0.2556	-0.12111 0.6321	-0.84467 <.0001	-0.91019 <.0001
Root (N) conc. (%)															0.72235 0.0007	0.4093 0.0917	-0.05605 0.8252
Leaves (N) conc. (%)																0.4515 0.06	-0.21496 0.3917
Root K/Na ratio																	0.66279 0.0027
Leaves K/Na ratio																	

Table 3. Effect of various levels of nitrogen adding and two levels of water salinity on Root O.P, Leaves O.P, Root (N) content (DW %), Leaves (N) content (DW %), Root (K) content (DW %), Leaves (K) content (DW %), Root (Na) content (DW %), Leaves (Na) content (DW %) of quinoa plants

Treatment	Root O.P (MPa)	Leaves O.P (MPa)	Root (N) content (DW %)	Leaves (N) content (DW %)	Root (K) content (DW %)	Leaves (K) content (DW %)	Root (Na) content (DW %)	Leaves (Na) content (DW %)
S0	-0.55^{b} ±0.062	-1.22^{b} ±0.027	2.0^{a}±0.159	3.6^{a}±0.367	2.24^{a}±0.110	2.86^{b}±0.109	0.80^{b}±0.047	0.78^{b}±0.011
S1	-0.97^{a} ±0.035	-2.14^{a} ±0.047	1.8^{a}±0.120	3.4^{b} ±0.168	1.90^{b}±0.054	3.12^{a}±0.111	1.50^{a}±0.037	2.1^{a}±0.052
L.S.D	0.0939	0.1055	0.2083	0.1624	0.1569	0.1186	0.0685	0.0938
N1	-0.62^{b} ±0.135	-1.62^{a}±0.174	1.42^{a}±0.087	2.8^{c}±0.089	1.88^{b}±0.040	3.32^{a}±0.083	1.10^{b}±0.157	1.52^{a}±0.340
N2	-0.78^{a} ±0.088	-1.68^{a}±0.219	2.12^{a}±0.060	3.3^{b}±0.093	2.15^{a}±0.056	3.10^{b}±0.067	1.30^{a}±0.152	1.47^{a}±0.268
N3	-0.83^{a}±0.047	-1.73^{a}±0.227	2.20^{a}±0.135	4.6^{a}±0.232	2.2^{a}±0.186	2.60^{c}±0.068	1.15^{b}±0.172	1.40^{a}±0.298
L.S.D	0.115	0.1292	0.2551	0.1989	0.1922	0.1453	0.0839	0.1148
S0 N1	-0.32^{a}±0.0300	-1.23^{c}±0.033	1.40^{c}±0.057	2.67^{e}±0.088	1.93^{bc}±0.033	3.2^{b}±0.066	0.70^{d}±0.033	0.77^{c}±0.033
S0 N2	- 0.60^{b}±0.008	-1.20^{c}±0.057	2.17^{ab}±0.088	3.17^{cd}±0.066	2.20^{b}±0.100	2.93^{c}±0.088	1.0^{c}±0.057	0.80^{c} 0
S0 N3	-0.74^{b}±0.038	-1.23^{c}±0.066	2.43^{a}±0.033	5.07^{a}±0.088	2.6^{a}±0.152	2.5^{d}±0.066	0.8^{d}±0.033	0.80^{c} 0
S1 N1	-0.91^{a}±0.059	-2.0^{b}±0.057	1.40^{c}±0.185	2.93^{de}±0.120	1.8^{c}±0.066	3.5^{a}±0.088	1.4^{b}±0.033	2.30^{a}±0.120
S1 N2	-0.95^{a}±0.089	-2.2a^{b}±0.066	2.1^{b}±0.088	3.40^{c}±0.145	2.1^{c}±0.057	3.2^{b}±0.033	1.7^{a}±0.033	2.0^{b} 0
S1 N3	-0.91^{a}±0.052	-2.3^{a}±0.066	1.9^{b}±0.145	4.03^{b}±0.033	1.8^{c}±0.066	2.7^{c}±0.033	1.5^{b}±0.033	2.1^{ab} ±0.033
L.S.D	0.1626	0.1828	0.3607	0.2813	0.2717	0.2054	0.1186	0.1624

Values are presented as the mean of six replicates ± standard error (SE). Values with the different letters represent a significant difference at ($P < 0.05$) within each treatment.(L.S.D) represent the Least Significant Difference within each treatment

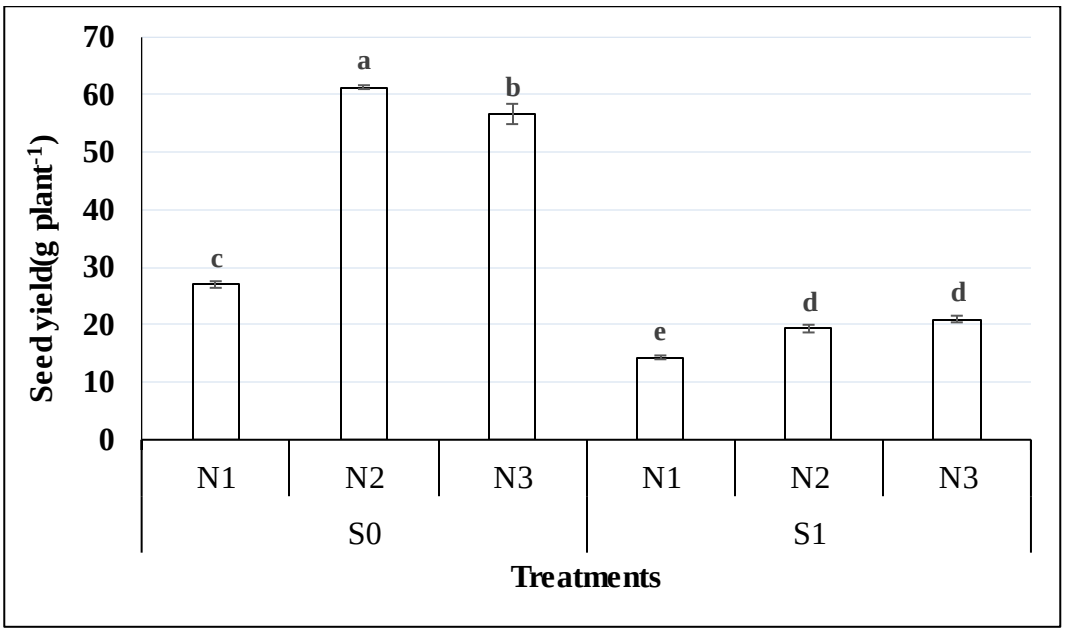

Fig 1. Impact of various rates of nitrogen adding and two levels of water salinity on seed yield. Each column shows the mean values of six replications and the bars represent (S.E) standard errors. The same letters are not significantly different at ($P \leq 0.05$)

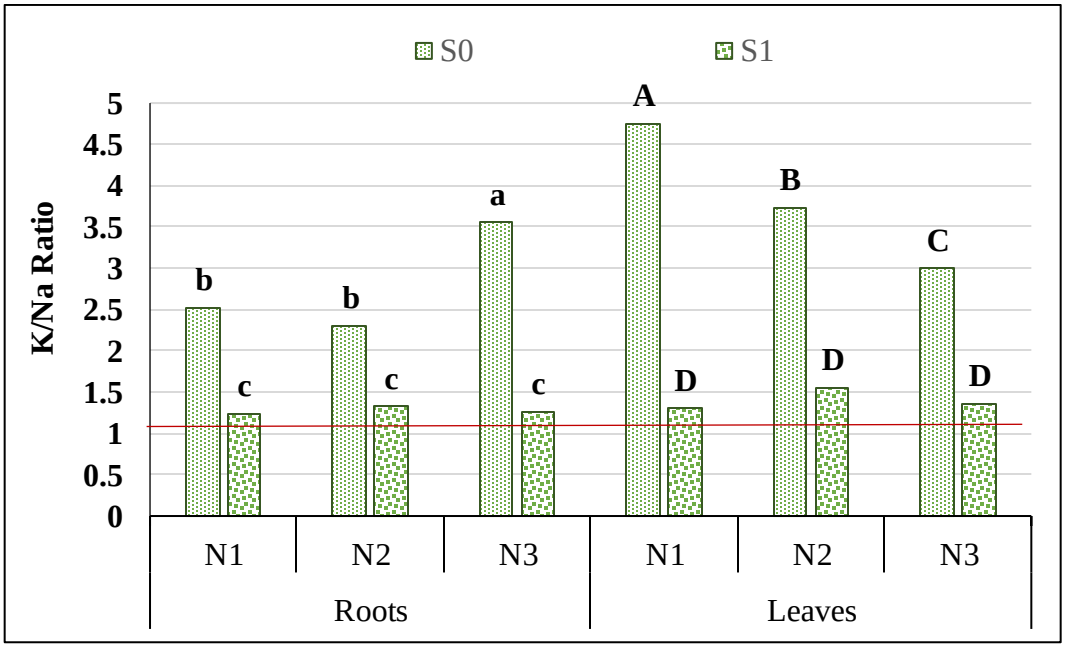

Fig 2. Impact of various rates of nitrogen adding and two levels of water salinity on K/Na ratio in both leaves and roots. Each column shows the mean values of six replications and the bars represent (S.E) standard errors. The same letters are not significantly different at ($P \leq 0.05$) within each organ

Table 4. Effect of adding various levels of nitrogen adding and two levels of water salinity on agronomic nitrogen use efficiency (aNUE) and physiological nitrogen use efficiency (pNUE)

Treatment	aNUE	pNUE
S0	123^{a}±21.8	69^{a}±2.9
S1	21^{b}±2.3	56^{b}±1.8
L.S.D	6.6235	4.449
N2	98^{a}±32.6	65^{a}±4.9
N3	45^{b}±12.8	61^{a}±1.6
L.S.D	6.6235	4.449
S0 N2	171^{a}±4.1	75^{a}±1.4
S0 N3	74^{b}±2.7	63^{b}±1.5
S1 N2	25^{c}±2.8	54^{c}±1.8
S1 N3	16^{c}±0.6	59^{bc}±2.6
L.S.D	9.367	6.2918

Values are presented as the mean of six replications ± standard error (SE). Values with the different letters represent a significant difference at ($P < 0.05$) within each treatment. (L.S.D) represent the Least Significant Difference within each treatment

The individual effects of water salinity or nitrogen fertilizers on the development and yield of crops have been extensively studied, but the effect of their interaction has not yet been fully understood. Nevertheless, the growth and productivity attributes of plants are a direct result of various biochemical and physiological mechanisms, and their diminishing due to salinity is highly dependent on the plant species. For example, halophyte species are considered to be highly tolerant to salinity compared to glycophytes. Moreover, halophytes differ in their resistance to salinity and this is one of the bases used to assess their potential of utilization (Koyro et al 2008). The salt concentration leading to the yield depression of 50% relative to its yield under non-saline conditions is defined as the limit of salinity tolerance (Kinzel and Bhattacharjee 1982). Quinoa was classified as a facultative halophyte with an ability to grow under salinity levels similar to those found in seawater, and the limit of salinity resistance (C50 value) for biomass production and seed yield was estimated to be approximately at 20 dSm^{-1}. Whereas, the optimum growth and productivity of different quinoa species was at salt concentration of less than 10-20 dSm^{-1} (Jacobsen et al 2003, Koyro and Eisa 2008, Hariadi et al 2011, Adolf et al 2013, Eisa et al 2017). Our present study showed a similar result in terms of a significant reduction in seed yield by 50.9% under salt stress (20 dSm^{-1}) less than the non-saline treatment. Likewise, the individual effects of nitrogen fertilizers on the growth and production of quinoa seeds have been studied, and the optimal levels of nitrogen nutrition for the quinoa crop have been estimated to be ranged between 75 to 160 kg N ha^{-1} (Jacobsen et al 1994, Erley et al 2005, Basra et al 2014, Geren 2015). The proper use of nitrogen nutrition to mitigate the harmful effects of salt stress has been discussed on several glycophyte crops (Chen et al 2010, Zhang et al 2012, Ashraf et al 2018, Ibrahim et al 2018, Hessini et al 2019, Song et al 2019, Qin et al 2020, Zhu et al 2020). But so far, not much attention has been paid to cash halophyte crop (Pessarakli et al 2012). It is well known that, the salinity stress causes adverse effects on the uptake, transport and assimilation of the nutrients within plant tissues (Ashraf et al 2018). In accordance with our results, the growth and productivity of the quinoa plants were significantly reduced in response to water salinity treatment (200 mM NaCl). In this regards, there are a number of factors that may limit plant growth and productivity under salt stress conditions. The initial deleterious constraint of salt stress on plant is due to an osmotic stress (Munns 2005). As shown in **(Table 2)**, osmotic potential of root or leaves significantly decreased and became lower values than non-saline treatments. However, the results

clearly showed that quinoa plants could efficiently decrease leaves osmotic potential to be lower than those of roots. This clearly implies that quinoa plants, grown under salinity stress, are able to adjust their osmotic potential to maintain a positive water uptake, and this has been previously reported by many researchers (Hariadi et al 2011, Eisa et al 2012). On the other hand, the dropping of osmotic potential of plant organs to avoid salt stress was concomitant with progressive sodium accumulation, particularly in the leaves **Table 2**. This led consequently to reduce in K^+/Na^+ ratio for both of root and leaves **Fig 2** This may be an explanation for the reduction of growth and seed yield under salinity treatments due to ion imbalance. However, quinoa is known to be considered as a salt- includer halophyte, tending to immediately translocate sodium and potassium from roots into leaves and utilize them to adjust osmotic potential. This may explain why the potassium accumulation is higher in leaves under the saline treatment than the non-saline treatment **Table 2**. In this context, Huchzermeyer and Koyro (2005) reported that potassium accumulation is much more preferred under salt stress. Moreover, (Shabala et al 2010, Hariadi et al 2011) reported that the resistance of salinity in quinoa is attributed to its highly efficient potassium retention.

The proper of nitrogen application rate under salinity conditions could be an effective physiological remedy to increase the plant tolerance against the harmful effects of salt stress (Siddiqui et al 2010). The results from the present study clearly showed that the increase of nitrogen application rate, under salinity treatment, from low nitrogen rate (N1) up to moderate nitrogen rate (N2) significantly increased nitrogen and potassium concentration in root, but increasing nitrogen application up to highest rate (N3) decreased both of nitrogen and potassium concentration in root. This ill effect of high nitrogen application rate could be attributed to reduce water uptake as a result of decreasing the soil water potential, and/or by competitive processes occurring on membranes of root cells, at the sites of ion transport between Cl^- and $NO3^-$ on one hand, and/ or between Na^+ and other captions of the essential element such as K^+ and $NH4^+$ on other hand (Ashraf and Harris 2004, Shawer 2014, Song et al 2019). The results also clearly showed that the increase in nitrogen application rates led to a significant decrease in the osmotic potential of roots (**Tables 1 & 3)**. This phenomenon could be explained by the application of large amount of nitrogen greater than the ability of the plant to absorb, then additional nitrogen residual in the soil solution leading to a secondary salinization (Song et al 2019).

Concerning the nitrogen use efficiency, the present study used aNUE and pNUE as the two-most prevalent measures. Agronomic nitrogen use efficiency is an important economic indicator leading to an estimate the maximum value of the crop: cost ratio, evaluating the benefits of investment since both parameters are closely related for input and output prices (Vanlauwe et al 2011). In the present study either under salinity treatment or non-saline ones, moderate N rate gave higher aNUE and pNUE than high N rate. This result suggested that the NUE reduced with increasing N rates higher than adequate rate (N2).

4 Conclusion

In summary, decreasing N rate to 250 ppm either under salinity stress or non- saline treatments would reduce the cost related to N fertilizer use and improved NUE by proper management of nitrogen fertilizer especially under salinity stress.

Abbreviations: O.P, osmotic potential

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