Research Article

Assessment of Growth and Physiological Traits of *Chenopodium quinoa* Lines Under Different Salt Levels

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Abstract | Elevating salinity level has become a worldwide threat to agricultural lands. High level concentration of salts hampers growth of plants and its yield of biomass by impacting major physiological mechanisms i.e., ionic, oxidative and osmotic stress. One possible and climate resilient strategy is to introduce new crops that can bear high level salinity and allow irrigation with saline water. Quinoa has great potential to grow under saline conditions having outstanding nutritious value. Pot based complete block design was conducted in COMSATS University Abbottabad, Pakistan during winter season. Five quinoa lines (L30, L81, L11, L9, and L24) were grown in sandy loam soil containing 15 dS m^{-1} and 30dS m^{-1} NaCl salt treatments. The results revealed that better growth was noticed in line L30 and L24 under 15 and 30 dSm^{-1} NaCl Salt treatments as compared to other three lines. Whereas, minimum plant growth was noticed in genotype L9 and L81 under 15 and 30 dSm^{-1} NaCl salt treatments. Highest leaf area, chlorophyll contents, and protein contents were noted in all condition in line L30 and L24 as compared other lines. High levels of potassium, calcium and magnesium and lowest sodium were noted in quinoa lines L30 and L24 aided in resisting salt stress and may be the cause of increased growth in both saline and non-saline soil. The present trial recognised the maximum salt-tolerant lines under severe salt stress, it might be applied to improve quinoa's tolerance to salt in a later breeding phase.

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Introduction

Chenopodium quinoa (Willd.) is cultivated mainly for its edible grains, is considered as one of the crops that may be able to maintain food security in this century due to its remarkable nutritional qualities, such as a high protein content, fiber, lipids, free of gluten, having considerable amount of fatty-acids, vitamins, minerals, and phytochemicals (Vilcacundo *et al*., 2017; Hinojosa *et al*., 2018; Akram *et al*., 2021; Hafeez *et al*., 2022). Additionally, the amounts of all essential amino acids exceed by WHO/FAO recommended in all age group categories (Joint, 2007; Filho *et al*., 2017). Moreover, its tremendous ability to withstand under abiotic stress conditions (Akram *et al*., 2021, 2023; Rivelli *et al*., 2023) attracts the world's attention towards its introduction in new areas.

Globally, in arid and semi-arid areas salinity is the main threat for production of crops (Sabagh *et al*., 2020). In arid areas, effect of salinization enhances because of low rainfall, high evapotranspiration, high temperature and inappropriate soil and water management practices (Minhas *et al*., 2020; Victoria *et al*., 2023; Waqas *et al*., 2023a). In the world more than 45 million hectares (Mha) of irrigated land is salt affected and each year 1.5 M ha land become unproductive because of high salinity (Munns and Tester, 2008). Soil salinization rate is rapidly escalating and is expected to affect 50% of arable cropland by 2050 (Shrivastava and Kumar, 2015). Among arable land of Pakistan, 6.8 M ha soil is salt affected from which 2.7 M ha soil is in Punjab province (Yaseen and Rao, 2002). There are two reasons for salt deposition and arable land degradation, natural (primary) and anthropogenic (secondary) (Sakadevan and Nguyen, 2010). Salt-affected land loses its aesthetic and economic value due to spread of salinization in continuous populated and already economically challenged countries which include Bangladesh, Pakistan and India which is causing unsustainability of agriculture.

Numerous approaches could be exploited to mitigate the adverse effects of salt stress, i.e., use of phytohormones, Osmo-protectants, crop water extracts, cultivation of salt-tolerant crops, fertilizer amendments, and different cultural practices (Zahra *et al*., 2021; Saddiq *et al*., 2019; Zahra *et al*., 2022; Waqas *et al*., 2023b). The cultivation naturally salt tolerant plants (halophytes) gaining popularity in the world (Zhang *et al*., 2018; Hafeez *et al*., 2021). Halophytes can be grown in different salty environments range from coastal areas to desert (Bueno and Cordovilla, 2020). Halophytes can tolerate higher salt concentrations with adaptations such as high uptake of potassium (K^+) as compared to sodium (Na^+), ions compartmentalization in vacuole, accumulation of organic solutes, and salt secreting bladders and glands (Yun and Shabala, 2020; Nazeer *et al*., 2022). They are capable of not only being surviving (100-200 mM NaCl) but gaining benefits from highly saline irrigation, therefore suggested for arid and semiarid agro-ecological areas, therefore in some halophyte species no significant yield reduction was found even at sea water irrigations, i.e., *Chenopodium quinoa* Willd (Hariadi *et al*., 2011). Any conventional known crop specie is not capable to tolerate such high salt concentrations.

Quinoa (pseudo-cereal) is a known as facultative halophyte whose germplasm being able to survive salinity even at sea water of 400 mM NaCl level (Eisa *et al*., 2017). They also observed that under saline condition the protein, phosphorus, potassium, sodium, iron, and copper contents were improved while decreased grain yield, weight of 1000 seeds, zinc, calcium and carbohydrate as compared to normal conditions. Iqbal *et al*. (2017) reported that increased the gaseous exchange indicators, proline, phenolics, plant height and main panicle length at salinity level 10 dS m^{-1} in all lines as compared to control. Saleem *et al*. (2017) observed that the chlorophyll index, shoot and root Na^+ and K^+ were improved but shoot fresh and dry weight were not affected at 100 mM salinity level as compared to non-saline conditions.

Quinoa as a future alternate crop has huge diversity in its germplasm for salt tolerance under different climate and salt conditions. Therefore, this trial was conducted to examine the five quinoa lines (L30, L81, L11, L9, and L24) for salt tolerance (0 dSm^{-1}, 15 dSm^{-1}, and 30 dSm^{-1}) having better growth and physiological mechanisms.

Materials and Methods

Pots experiment was conducted at department of environmental sciences, in COMSATS University Islamabad (Abbottabad Campus-Pakistan). Five lines of Quinoa (L30, L81, L11, L9 and L24) were obtained from University of Agriculture Faisalabad and seeds were sown in the nursery for homogeneous germination of Quinoa accessions in September 2019. Seeds were sown in sand and started to germinated after 4 days and then macro and micro nutrient solutions were prepared with specific concentration to obtain desired concentration of the nutrient solution KH_2PO_4 (200 mM), K_2SO_4 (500 mM), $Ca(NO_3)_2$ (500 mM), $CaCl_2$ (500 mM), $MgSO_4$ (500 mM), Fe-

EDTA (200 mM), H_3BO_3(5 mM), $MnSO_4$ (2 mM), $ZnSO_4$ (0.5 mM), $CuSO_4$ (0.3 mM), (NH_4) Mo_7O_{24} (0.01 mM). Until the 4th leaf stages seedlings were irrigated every day with nutrient solution. After that by following the required treatments T1: Control, T2:15 dSm^{-1} and T3:30 dSm^{-1} seedlings were transferred into soil. After 1 month of transplanting Quinoa was harvested for further biochemical analysis.

About 3 kg soil were collected from 0-20 cm depth and sieve by using 2 mm pore size for soil characteristics. Soil pH measured by using the pH meter (PHS-25CW Microprocessor pH/mV meter) (Makanjuola and Coker, 2019) that was 7.2. Electric conductivity of soil was measured by using the HANNA HI 98129 PH/EC/TDS tester meter, HI98129 (Talukdar *et al*., 2024) that was 2.1. Soil moisture content was measured by following the (Schulte *et al*., 2012). Organic matter content of the soil was determined by the wet oxidation method using $K_2Cr_2O_7$ (Walkley and Black, 1934) that was 0.71. Texture of soil measured by (Dewis and Freitas, 1970) method that was sandy loam. Ionic analysis of the soils (Na^+, K^+, Ca^{2+} and Mg^{2+}) were determined by following the (Goyal *et al*., 1993). The Soil absorption ratio was measured by (Richards, 1954) method that was 9. By following the (Khalil *et al*., 2015) soil saturation percentages were measured. Three different salinity levels *i.e.*, control, 15 dSm^{-1} and 30 dSm^{-1} with three replications (total 45 pots) were produced by following the outlines of U.S.D.A. Salinity Laboratory Handbook 60 (Richards, 1954) method. Every pot irrigated with tap water by maintaining the field capacity level (Algosaibi *et al*., 2015). Every pot received fertilizers (NPK) at the concentration of Urea (CH_4N_2O), 0.1224 g first half dose before sowing and 0.1224 g second half dose after 2 week of germination, DAP $(NH_4)_2HPO_4$, 0.212 g before of sowing and MOP (KCl), 0.1126 g before of sowing, respectively.

After harvesting, plant growth parameters (leaf area, leaf length, shoot height and root length) were analyzed by following the (Saiz-Fernández *et al*., 2020) method. Fresh and dry weight of the leaves and roots were measured by using the analytical balances (Qadir *et al*., 2017). Total ion concentration (Na^+, K^+, Ca^{2+} and Mg^{2+}) in leaves and roots were measured by following the (Shahzad *et al*., 2012). Relative Water Contents RWC (%) of leaves and roots were measured by using the following Equation.

$$RWC\ (\%) = FW\text{-}DW/FW \times 100$$

Protein content in the Quiona leaves were measured by the (Bradford, 1976) method (Koyro *et al*., 2008).

The design was completely randomized design with factorial arrangements. Statistical analysis was done by using the statistic 8.1 and HSD tukey test was done to compare the means value.

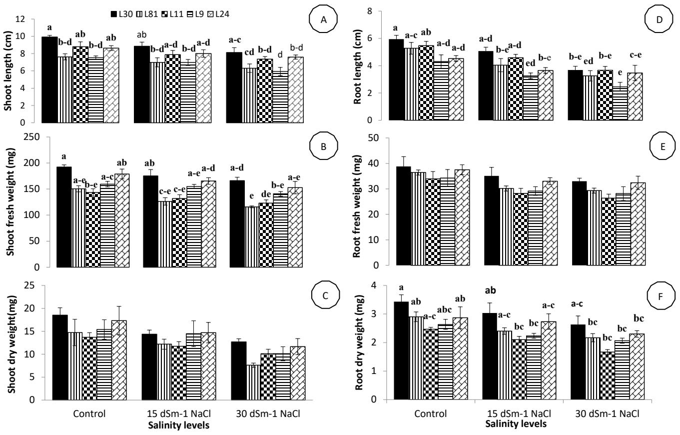

Figure 1: *Effect of salinity stress on different lines of quinoa growth related attributes.*

Results and Discussion

Shoot length, shoot fresh and dry weight significantly affected by salinity, however, maximum shoot length under all salinity levels was observed in L30 followed by L24, L81, L11 and L9 under all conditions. Similarly, highest shoot fresh and dry weight were noted in L30 and L24 as compared to other genotypes (Figure 1A-C). Growth of quinoa genotype L30 as compared to others L81, L11, L9, and L24 was not significantly affected by the salinity up to the 15 dSm^{-1} for root length that decreased at rising salt levels. Likewise, highest root fresh and dry weight were noted in L30 and L24 followed by L81, L9 and L11 (Figure 1D-F). The salinity can provoke osmotic stress by increasing extracellular solute concentration, that can lead to a decrease in water potential and loss of cellular turgor potential (Abotbatta, 2020). Thus, the reduction in plant development indices, particularly root and shoot fresh and dry weights, as salinity levels rise is connected to an increase in osmotic potential in water challenged plants (Betzen *et al*., 2019) this could be owing to elevated extracellular solute concentrations or lower cell volumes during drought. In quinoa plants, thick-walled cells adapted to water loss under osmotic stress do not lose turgor even in extreme water stress (Adolf *et al*., 2013). Quinoa plant height is one of the most salinity sensitive features (Hussain *et al*., 2020). In the current trial, the alteration in shoot length of five lines in response to salt stress was distinct. However, L30 retained shoot length up to 15 dSm^{-1} salinity after that there was a decline in shoot length, while in L81, L11, L9, L24, the shoot length dropped at both saline levels. Lines L30 and L24 maintained plant growth parameters up to 15 dSm^{-1} salinity level and then salt stress affected their growth parameters at further levels, this could be related to quinoa plants' ability to retain water status even in a salt condition while L11 displayed sensitivity even at 15 dSm^{-1} salinity level. This reduction in growth could be related to photosynthetic rate and chlorophyll contents, both of which had been exposed to decrease under severe salinity stress (Waqas *et al*., 2021).

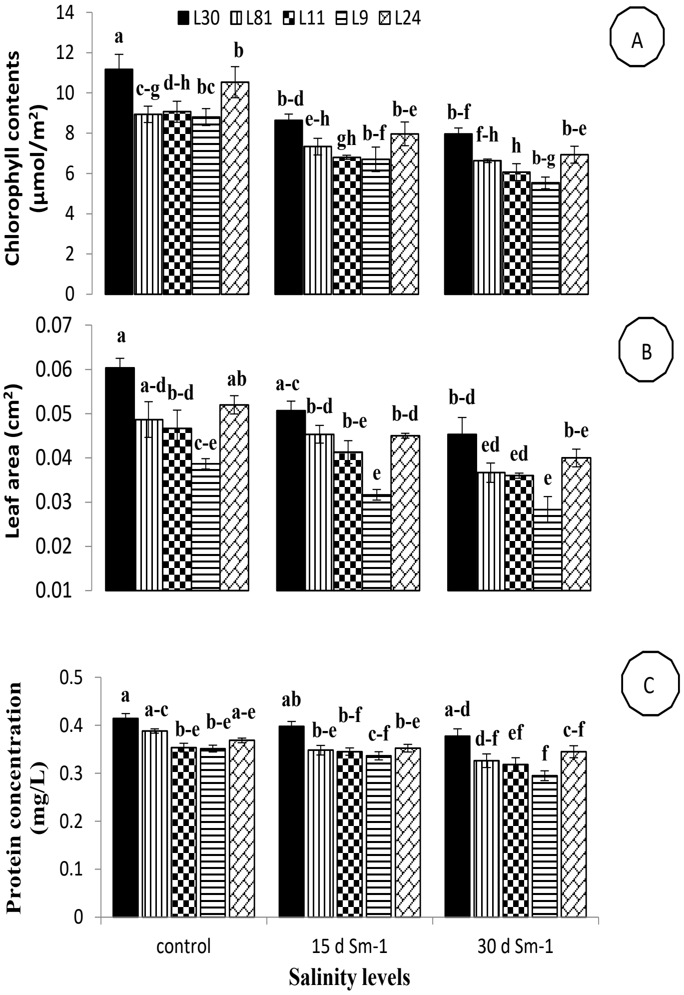

Figure 2: *Effect of salinity stress on different lines of quinoa chlorophyll contents, leaf area and protein contents.*

In current trial chlorophyll contents were also decreased by increasing level of salinity, however, highest chlorophyll content was noted in L30 followed by L24, L81, L9 and L11 (Figure 2A). A considerable decrease in photosynthesis activity and chlorophyll contents were allied with a noteworthy decrease in stomatal conductance and high levels of Na^{+} buildup in leaf tissues, both of which significantly reduce plant photosynthetic capability. The drop in photosynthetic capability could be attributed to a decrease in the activity of photosynthetic enzymes such as Rubisco, which decreases with salinity level (Hussin *et al*., 2020). The maximum protein and leaf area was significantly altered by salinity levels. The highest protein and leaf area was observed in L30 followed by L24 under 15 dSm^{-1} and 30 dSm^{-1} while minimum was noted under both salinity levels in L9 (Figure 2B, C). Turcios *et al*. (2021) noted that increase in leaf area of quinoa under salinity levels showed the salt tolerance. The decrease in chlorophyll contents in quinoa plants under salinity stress is a common indication of oxidative stress, and it is frequently associated with a lack of chlorophyll synthesis as well as the activation of its breakdown by the enzyme chlorophyllase that ultimately cause the reduction leaf area (Qureshi and Daba, 2020).

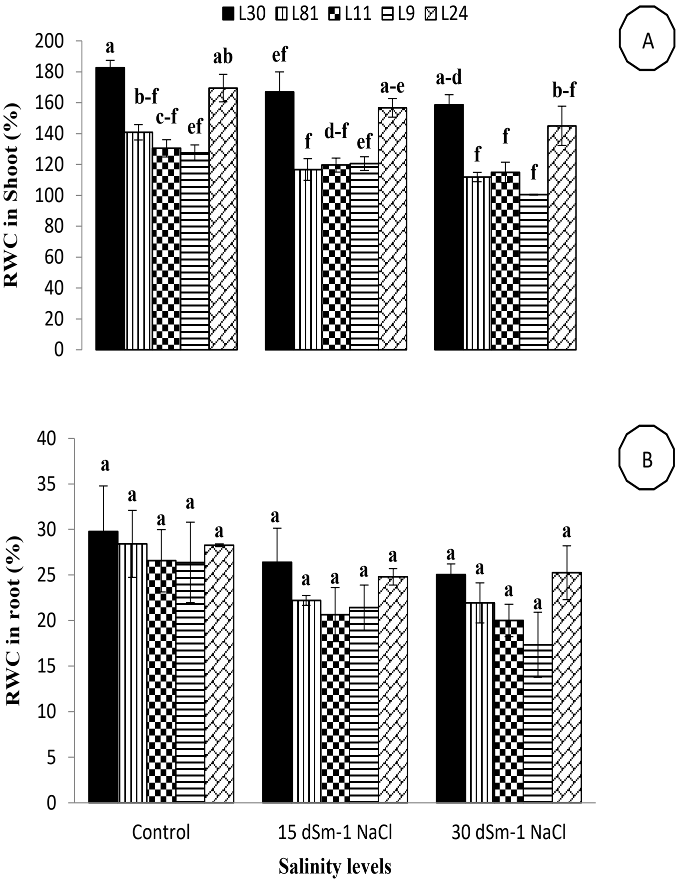

Figure 3: *Effect of salinity stress on different lines of quinoa relative water contents in shoot and root.*

RWC was not significantly declined at lower salinity level 15 dSm^{-1} in both genotypes L30 and L24, confirming the salt loving nature of both genotypes while decreased 30 dSm^{-1} while minimum RWC was observed in L11 under both salinity levels that showed this a sensitive genotype (Figure 3A-B). Similarly, Abbas *et al.* (2021) noted that under above 10 dSm^{-1} the RWC in quinoa lines was not affected, however, further increase in salinity level decreased the RWC. However, the continuation balance of water directly links with ionic interactions (Shuyskaya *et al.*, 2023).

Quinoa lines appear to have regulated water-relations by enhancing inorganic osmotica uptake accumulation potassium and sodium in root and shoot, it is termed as "hypertonic" condition. In quinoa, salinity tolerance is substantially linked with inhibition of shoot potassium deficiency and suitable leaf cytosolic potassium and sodium ratio (Cai and Gao, 2020). In the present trial, lines L30 and L24 had better leaf potassium, calcium, magnesium and sodium at all salt levels, thus representing salt tolerance (Figures 4, 5). This could be due to effective sodium dumping in the leaf vacuole or sodium translocation to older leaves. In the current trial, sodium was evaluated in young leaves, and young leaves typically had a lower degree of accumulation (Adolf *et al.*, 2013). It appears that lines L30 and L24 responded at the whole plant level by translocating sodium in older leaves, resulting in low Na$^+$ loads in young leaves, or it appears that there are sodium restrictions at the root paranchyma, as root Na$^+$ contents of L30 and L24 were higher when grown in saline solutions, particularly at 30 dSm^{-1}. This approach of minimal sodium buildup could also be linked to preferential K$^+$ uptake at the root parenchyma and translocation to the leaf, as leaf K$^+$ concentration was observed to be higher in L30 and L24. Under salt stress, quinoa plants acquire higher K$^+$ in their leaves (Mohammadi *et al.*, 2022), which was validated in this investigation. Another probable explanation is a change in Na$^+$ loading in the xylem. Nutrient intake at the root parenchyma, radial ion transport, and loading to the shoot have all been described as being mainly uncoupled (Lu and Fricke, 2023; Karahara and Horie, 2021). According to a recent halophyte review, thermodynamically, sodium loading in xylem is undoubtedly an active process assisted by *SOS1* and Na$^+$/H$^+$ exchangers (Cuin *et al.*, 2011). It also appears that xylem potassium and sodium loadings are uncoupled in quinoa, and hence tolerant lines may have higher activity of Na$^+$/H$^+$ exchangers positioned at the xylem parenchyma border. This hypothesis' confirmation could be included in future research initiatives.

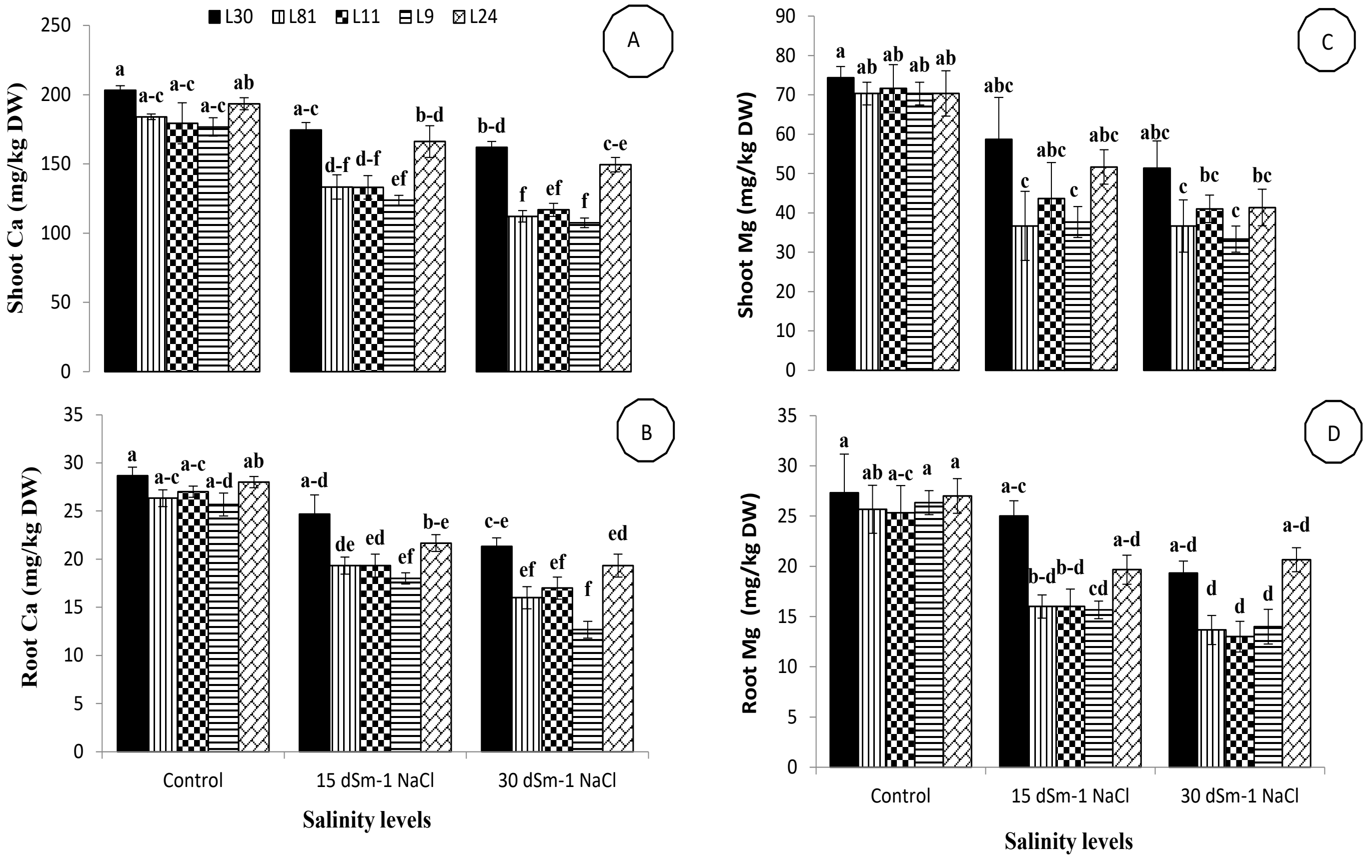

Figure 4: *Effect of salinity stress on different lines of quinoa calcium and magnesium in shoot and root.*

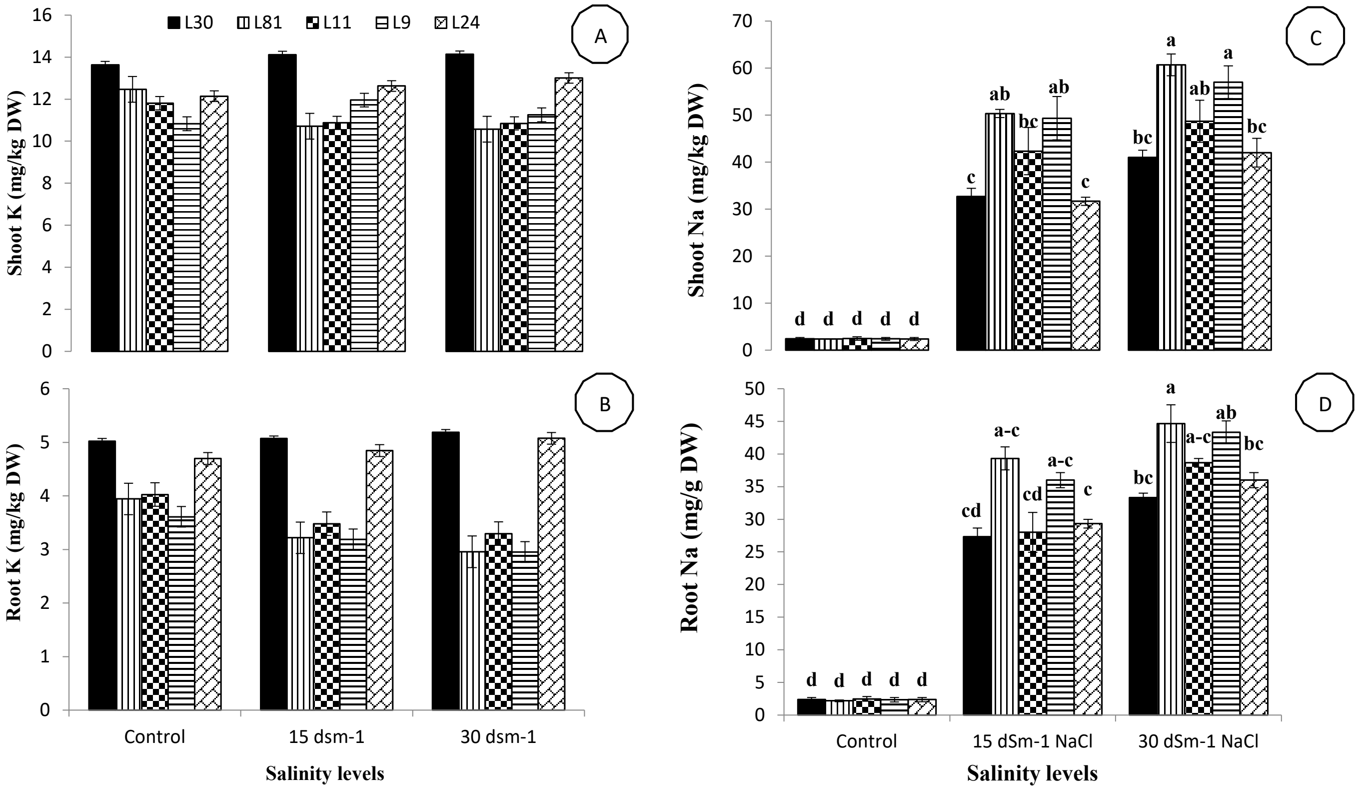

Figure 5: *Effect of salinity stress on different lines of quinoa potassium and sodium in shoot and root.*

Conclusions and Recommendations

The better salt tolerance was displayed by line L30, followed by L24. These lines have better growth attributes, chlorophyll contents, protein contents, relative water contents and ions uptake as compared to L81, L9, and L11. The present trial recognised the maximum salt-tolerant lines under severe salt stress, it might be applied to improve quinoa's tolerance to salt in a later breeding phase. Considering the increase in Quinoa tolerance to salinity after establishment of vegetative part in the soil, it is recommended to perform irrigation by water with salinity equal or less than 30 dS m^{-1}.Because quinoa has the feasibility of irrigation by high-saline water. Salinity does not have significant decreasing effect on the growth of Quinoa at vegetative growth stage in this study. But, the amount of growth and yield reduction strongly depended on the method you apply salinity. High levels of K+/Na+ ratio of Quinoa varieties V30 and V24 helped to withstand salt stress and might be the cause of high growth under both normal and salt affected soil. So, it is concluded that quinoa keeps effective homeostatic mechanisms related to K+ retention and osmotic adjustment, make it astonishing salt-tolerant plant. Quinoa plant mineral and protein concentration also did not decrease drastically under different salt stress in this study. By considering the results of this study, it is recommended to cultivate Quinoa varieties on saline affected areas of Pakistan.

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Novelty Statement

The study showed that Quinoa has the ability to withstand under higher salinity conditions by osmotic adjustment.

Author's Contribution

Mehwish Zafar: Conductance of experiment and data collection.
Muhammad Shahzad: Supervise the whole experiment as project head
Muhammad Zubair Akram: Initial draft and writing
Quratulain: Statistical analysis
Mehak Shehzad: Reviewed the manuscript
Samreen Nazeer: Review the manuscript and final drafting of manuscript.
This study is part of MS thesis of Mehwish Zafar.

Conflict of interest

The authors have declared no conflict of interest.

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