

SALINITY RESILIENCE IN QUINOA (*CHENOPODIUM QUINOA*); INVESTIGATING ADAPTIVE MECHANISMS ACROSS DIFFERENT VARIETIES TO COMBAT SALT STRESS FOR SUSTAINABLE AGRICULTURE

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Abstract

One of the main abiotic factors influencing crop production and yield is salt stress. Quinoa (*Chenopodium quinoa* Willd.) serves as a valuable model crop for developing salt-resistant cultivated varieties through targeted breeding strategies. The purpose of this study was to evaluate how three quinoa cultivars-'UAF-Q7' (Q-1), 'White Quinoa' (Q-2), and 'Hybrid Quinoa' (Q-3)-reacted biochemically and morpho-physiologically to different salt stress levels. NaCl solutions at concentrations of 0, 100, 200, 300, 400, and 500 mmol/L were used to pretreat the seeds. To learn more about the mechanisms underlying quinoa's resistance to salt, we assessed several physiological and biochemical characteristics as well as seed germination, growth, and biomass production. The findings showed that the seed germination index, germination potential, and germination percentage first increased and then significantly decreased as the NaCl content rose, due to genetic variability among different species. In a similar vein, increased salinity was followed by a decrease in plant biomass, chlorophyll content, relative water content, soluble proteins, and antioxidant enzymatic activity including ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD). Interestingly, at 200 mmol/L NaCl, the antioxidant enzyme activities of Q-1 and Q-2 leaves were much higher than those of Q-3 71.8%, 55.5%, and 38.9% respectively. Additionally, the aerial leaves of all cultivars showed a considerable rise in soluble sugars, proline, and malondialdehyde content as the concentration of NaCl increased due to high metabolic seed activity. These results show that Q-1 and Q-2 have better physiological responses, stronger enzymatic activity, and greater salt tolerance than Q-3. This study emphasizes how quinoa may be used as a model for breeding salt-resistant cultivars and how useful it is for creating salt-tolerant crops for use in saline-affected areas and improving agricultural practices.

Key words: Salinity tolerance; Agriculture; Halophyte; Sustainability; Quinoa; Climate smart crop

Introduction

The seed crop quinoa (*Chenopodium quinoa* Willd.) belongs to the family Chenopodiaceae (now subfamily Chenopodoideae of Amaranthaceae). It has many genera (Flowers & Colmer, 2015). It is classified as a facultative halophyte, thriving well in salty and water-stressed environments, and showing amazing adaptability to different salinity levels (Cueva-Flores *et al.*, 2024). Renowned for its long history of cultivation and outstanding nutritional value. (Bazile *et al.*, 2016), quinoa was first cultivated in the Andean highlands of Bolivia and Peru approximately 5,000 to 7,000 years ago (Rashid *et al.*, 2021) as a sacred grain, "chisya mama" (or "mother grain") is esteemed by the Incas."; (Walters *et al.*, 2016; Jacobsen, 2017), quinoa has gained global importance over the past fifty years due to its outstanding nutritional profile and resilience to harsh environmental conditions. The year 2013 was declared as "International Year of Quinoa" by the United Nations Food and Agriculture Organization (FAO). The FAO also acknowledged Quinoa as a "superfood" and a climate-smart crop that can improve nutritional security and food sustainability (Vilcacundo and Hernández-

Ledesma, 2017; Bazile, 2021) also identified it as a "21st-century unique grain".

According to estimations, the global quinoa market is expected to rise at a Compound Annual Growth Rate (CAGR) of 11.1%, from \$112.72 billion in 2024 to \$125.21 billion in 2025. Increasing knowledge of quinoa's health benefits and its use in a variety of culinary applications are credited with this growth (Cruces *et al.*, 2024). Many Andean farmers have seen a development in their standard of living as a result of the economic opportunities brought about by the worldwide demand for quinoa, which has given them new sources of income and ways to escape poverty. But there are drawbacks to this demand, as well as, like shifting market conditions and the possibility of unsustainable farming methods could harm nearby ecosystems and communities (Scanlin *et al.*, 2024).

Quinoa's leaves and seeds have higher protein content (Wu *et al.*, 2016; Vilcacundo & Hernández-Ledesma, 2017), and a nutrient-dense profile than cereal grains, including barley, rice, maize, and oats (Bastidas *et al.*, 2016; Boas *et al.*, 2016; Filho *et al.*, 2017; Naz *et al.*, 2022). Bioactive compounds such as flavonoids, phenolic acids, phytosterols, bioactive peptides, and saponins are also

abundant in quinoa grains (Justino & Espindola, 2018; Olivera *et al.*, 2022). Quinoa's remarkable climate adaptability is highlighted by its resilience to several abiotic stimuli, including drought, cold, high temperatures, and salinity (Ramzani *et al.*, 2017; Piñuel *et al.*, 2019; Langyan *et al.*, 2024). However, regardless of its potential for abiotic stress studies, this plant receives limited attention in agricultural research and practices (Liu *et al.*, 2020; Patiranage *et al.*, 2022).

In agriculture one of the biggest problems is soil salinity, which drastically lowers the production of agricultural lands worldwide (Ibrahimova *et al.*, 2021; Gul *et al.*, 2022; Zhang *et al.*, 2024). Factors that negatively affect crop productivity, quality, and quantity, such as habitat loss, ecosystem degradation, and desertification, make this problem worse (García-Caparrós & Lao, 2018; Yaqoob *et al.*, 2019; Ihsanullah *et al.*, 2024). About 23% (340 million hectares) and 37% (560 million hectares) of farmed lands are affected by salinity and sodicity, respectively (Yang *et al.*, 2016; Stoleru *et al.*, 2019). Pakistan ranks eighth in terms of the areas impacted by salinity. Salt affects six million hectares of Pakistani soil, of which 2.7 million are in Punjab (Moreno *et al.*, 2018; Wang *et al.*, 2018; Stoleru *et al.*, 2019; Nazih *et al.*, 2024). Because it hinders plant growth and production, a high salt content in the soil lowers fertility and yield. Salt ions also interfere with osmotic functions, restricting water absorption and affecting seed germination. Additionally, salt ions disrupt osmotic processes, which limits water absorption and impacts seed germination (Iqbal *et al.*, 2020; Zhao *et al.*, 2020). Halophytic crops offer sustainable substitutes as they provide insights into mechanisms of salinity tolerance (Lombardi *et al.*, 2022), with the Chenopodiaceae family particularly noteworthy (Bazos *et al.*, 2021; Tipirdamaz *et al.*, 2021). Halophytes, including quinoa, can thrive in conditions with salt concentrations of 50 mM for monocots and 100–200 mM NaCl for dicot plants. (Ahmed *et al.*, 2021; Naz *et al.*, 2022). Documented for its resilience, quinoa can withstand salinity levels comparable to seawater (Hinojosa *et al.*, 2018; Causin *et al.*, 2020). Some quinoa varieties maintain nutritional value at salinity levels of 750 mM NaCl and can complete their life cycle at 500 mM NaCl (Kaur *et al.*, 2022).

Quinoa survival techniques include ion buildup in tissues to control leaf water potential, avoid dehydration, boost biomass production, and boost seed output (Jaikishun *et al.*, 2019; Sindhu & Khatkar, 2019). At the seedling/embryonic stage, its susceptibility to salinity is highest, drastically affecting the growth progress, with the lowest sensitivity at the flowering stage/ maturity stage. Salinity threshold values at different growth stages are 20, 15, and 8 dS/m for seedling emergence, blossoming, and cotyledon filling in sandy loam soil, respectively (Maleki *et al.*, 2018). Most genotypes tolerate 100 to 250 mM NaCl, with optimal growth at 100 to 200 mM NaCl (Shah & Khan, 2022; Guo *et al.*, 2023). Quinoa germination is sensitive to salinity and maintaining ionic balance (Nazih *et al.*, 2024). While moderate salinity (100 to 200 mmol/L NaCl) has little effect, higher levels (300 to 400 mmol/L NaCl) significantly hinder germination, with 500 mmol/L NaCl

being particularly inhibitory (Hussin *et al.*, 2023). A salinity level of 400 mmol/L NaCl reduces stomatal area, whereas higher levels can increase stomatal density while decreasing stomatal size in the 'Achachino' variety (Yang *et al.*, 2016; Becker *et al.*, 2017).

The compliance of quinoa to marginal soils in Pakistan is highlighted by successful cultivation and foundational production techniques. Different saline conditions result in significant physiological and agronomic variations in quinoa varieties (Afzal *et al.*, 2023). Given its nutritional value and resistance to abiotic stress, quinoa holds substantial promise as a future crop. This study evaluates the salt tolerance of three quinoa varieties-'UAF-Q7' (Q-1), 'White Quinoa' (Q-2), and 'Hybrid Quinoa' (Q-3)-to identify genotypes suitable for cultivation in salt-affected soils. This research underscores the potential of quinoa to thrive in challenging environments involving complex physiological, morphological, and biochemical mechanisms. Specifically, we seek to determine how increasing salinity influences key parameters such as germination, growth dynamics, morphophysiological and biochemical characteristics, antioxidant enzyme activity, and osmoprotectant accumulation in these quinoa accessions.

Material and Methods

Seed materials and experimental setup: The experimental plant consisted of three distinct genotypes of quinoa cultivars from Pakistan: Q-1, Q-2, and Q-3 (Table 1). These varieties were sourced from Andean Naturals (<https://www.andeannaturals.com/>), Quinoa Real (<https://www.quinuareal.bio/en-US/>), Caveman Organics (<https://www.cavemanorganics.pk/>), and the Seed Breeding and Seedling Institute at the University of Agriculture, Faisalabad's crop physiology department. The experiment was conducted in Mianwali, Punjab, Pakistan, in collaboration with Northeast Forestry University, Harbin, China. All seeds were stored at temperatures between 5 and 10 °C before experimental use. Healthy, uniformly sized, and disease-free seeds of Q-1, Q-2, and Q-3 were chosen and sterilized for 3-5 minutes with a 0.2% HgCl₂ solution, soaked for 24 hours in distilled water, and then rinsed with double-distilled water. Filter paper-lined Petri dishes containing 30 seeds each were immersed in distilled water and incubated at 25°C (Hajihashemi *et al.*, 2020), maintaining and 70% relative humidity (Panuccio *et al.*, 2014), for 12 hours in the light and 12 hours in the dark. After ten days, germination was noted. (Experimental route map and methodology are given in Fig. 1).

Pot experiment and treatments: The experiment's soil was taken from the backyard of a greenhouse in Mianwali, Punjab, Pakistan. It was then dried for a week before being ground up and sieved through a 2 mm screen. Soil characterization was carried out (Table 2). Pots (20 cm top diameter, 10 cm bottom diameter, and 15 cm height) filled with 1 kg of wet sandy loam soil were used to transplant 10-day-old quinoa seedlings. Three replicates of each treatment were used in the randomized complete block design (RCBD) experiment. Conditions in the greenhouse were maintained at 25/21 °C (day/night), 7.5 hours of natural light, 62–70% relative humidity, and frequent watering (Klute & Topp, 1994).

Table 1. Properties of three Quinoa (*Chenopodium quinoa*) varieties with different genotypes.

Quinoa cultivars/genotypes	Cultivar representation in the article	Colour of grain	Abiotic stress tolerance	Properties	References
UAF-Q7	Q-1	Pale yellow/white creamy/beige colour	Tolerant to drought, heat, and salinity	High yield potential and disease resistance. Maintains the high protein, fiber, and essential mineral content found in quinoa. Gluten-free and suitable for individuals with gluten intolerance.	(Liu <i>et al.</i> , 2020; Rashid <i>et al.</i> , 2021; Rehman <i>et al.</i> , 2022)
White Quinoa	Q-2	White colour	Tolerant to heat, drought, and salinity	Fluffy texture and neutral flavour. High in protein, fiber, iron, and essential nutrients. Provides all nine necessary amino acids, making it a complete protein source.	(Yaqoob <i>et al.</i> , 2019; Haseeb <i>et al.</i> , 2023)
Hybrid Quinoa	Q-3	Black colour	Tolerant to high temperature, salinity, and drought	Slightly earthy and nutty, with a firmer texture than white quinoa. Rich in protein, fibre, iron, magnesium, and antioxidants. May offer higher levels of certain antioxidants compared to other quinoa varieties.	(Pereira <i>et al.</i> , 2019; Piñuel <i>et al.</i> , 2019).

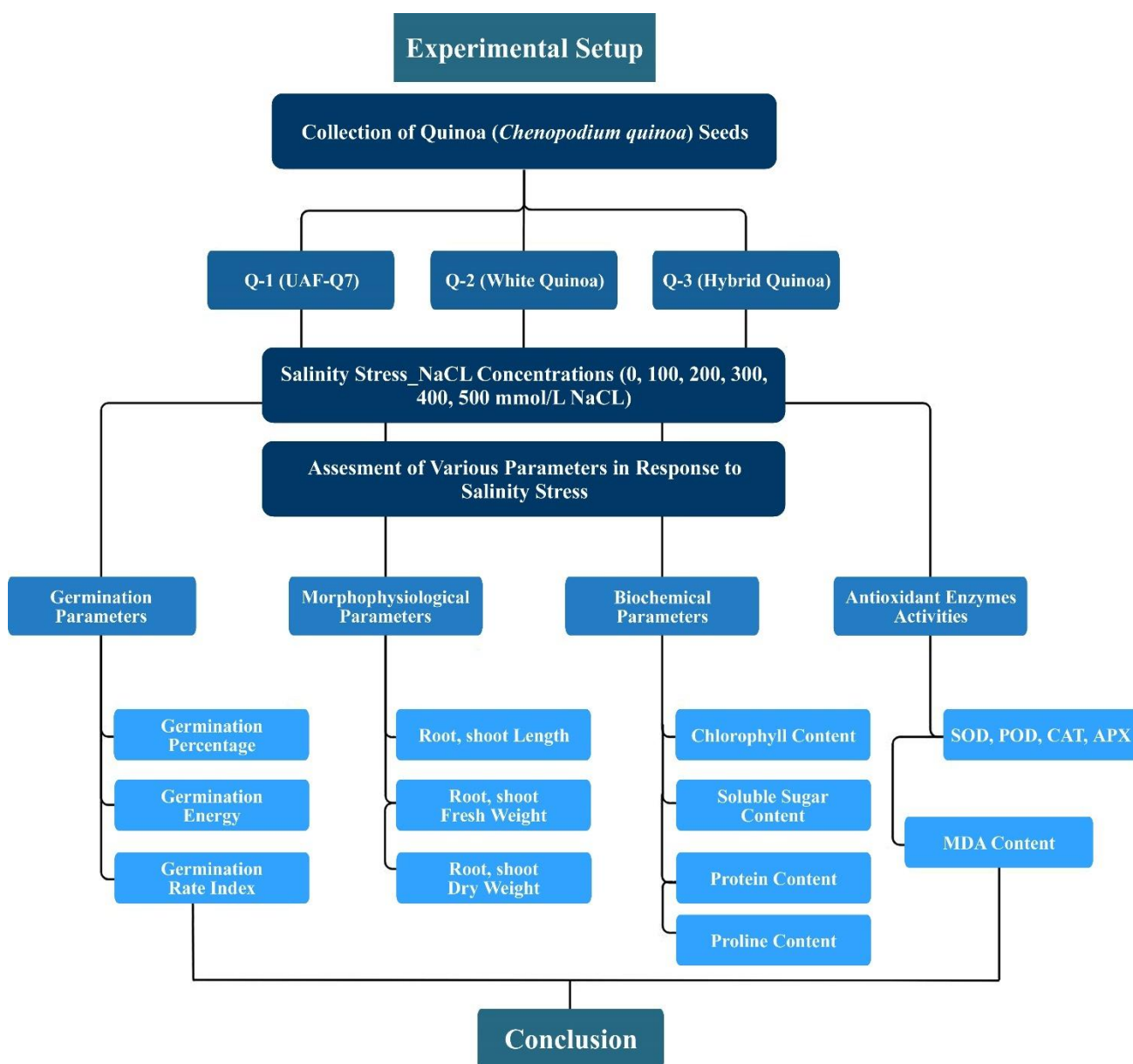


Fig. 1. Schematic representation of experimental setup and research route map.

Table 2. Physicochemical characteristics of experimental soil.

Soil Physical and Chemical Properties	Composition
*Soil Texture Classification	Sandy Loam Soil
*Soil Mapping Unit (SMU)	Alluvial/Chernozem
Sand	70.8%
Silt	40.4%
Clay	10.2%
Soil pH	7.0
Electrical Conductivity (EC)	2.5dS/m
Bulk Density	1.29kg/m ³
Humus Content	2.95%
Organic Matter (OM)	13.61g/kg
Available Nitrates (NO ₃ -N)	69.61mg/kg
Available Phosphorus (P)	59.63mg/kg
Available Potassium (K)	73.96mg/kg
Cadmium (Cd)	2.0mg/kg
Chromium (Cr)	0.50mg/kg

Six groups of three different types of quinoa (Q-1, Q-2, and Q-3) were created based on the salt treatments (0, 100, 200, 300, 400, and 500 mmol/L NaCl). While salt solutions were given to other groups, the control group was irrigated with distilled water. The distilled water used for the irrigation of quinoa seedlings has the following characteristics: EC 1.2 ds/m, K⁺ 243 mg/L, Na⁺ 139 mg/L, Cl⁻ 219 mg/L, and Mg²⁺ 54 mg/L. Pots were irrigated every day with the designated salt solution, and each treatment was repeated three times. Seedlings were trimmed to ten per pot when they reached the 7-8 leaf stage, with seed germination being tracked daily (Farooq *et al.*, 2006). After a week of NaCl treatment, the plants' morphology, physiology, and biochemistry were assessed, and their roots and leaves were frozen in liquid nitrogen for further analysis.

Determination of germination parameters: Seed germination was monitored every 24 hours following treatment until the experiment concluded. Using the procedure outlined by (Martínez-Peralta *et al.*, 2024) germination percentage, germination energy (Shah *et al.*, 2021), and germination index (Talská *et al.*, 2020) were determined using the following formulae.

1. Germination Percentage (GP)

$$GP = (N_g/N_t) \times 100\%$$

Where;

N_g = Number of germinated seeds

N_t = Total number of seeds

2. Germination Energy (GE)

$$GE = (N_{g4}/N_t) \times 100\%$$

Where;

N_{g4} = Number of germinated seeds within four days

N_t = Total number of seeds

3. Germination Rate Index (GRI)

$$GRI = n \sum_{t=1}^n (G_t/D_t)$$

Where;

G_t = Number of germinated seeds at time *t* (day)

D_t = Number of days to germination.

n = Total number of days observed

Determination of morphophysiological parameters:

After six weeks of salt stress, the plants were carefully uprooted, with soil washed. The pots were placed in a water tub for approximately one hour to ensure the safe removal of plants without damaging the roots. The roots and aerial parts of the plants were thoroughly washed multiple times with deionized water to remove any adherent soil particles. The remaining moisture on the roots and leaves was absorbed by using filter paper. The plants were then divided into aerial and subterranean parts to measure, fresh and dry weight as well as shoot and root length. The fresh weights of roots and shoots were measured using a digital electric balance. Certain plant samples were dried for 72 hours at 80°C in an oven for their dry mass.

Ten seedlings were randomly chosen from each replication to measure fresh weight and plant height.

Fresh Weight and Plant Height Measurement: The formula for Fresh Weight and Plant Height measurement is expressed as:

W_f = Fresh weight of seedling

H_p = Height of seedlings

If ten seedlings are chosen at random from each replication, the average fresh weight (*W_{avg}*) and average plant height (*H_{avg}*) was calculated then the formula will be expressed as:

$$W_{avg} = \sum_{i=1}^{10} W_{f,i} / 10$$

$$H_{avg} = \sum_{i=1}^{10} H_{p,i} / 10$$

Where;

W_{f,i} = Fresh weight of *i*th seedling

H_{p,i} = Height of seedling *i*th seedling

Determination of biochemical parameters

Chlorophyll content: To measure the amount of chlorophyll method outlined by (Agrawal & Rathore, 2007) and (Naz *et al.*, 2022) was followed, 0.2 grams of fresh leaves were thoroughly crushed with 4 mL of 80% acetone solution. After an hour of shaking in a water bath, the mixture was centrifuged for 15 minutes at 10,000 rpm. Using a UV-1800 UV spectrophotometer, the absorbance of the supernatant was measured at 470, 645, and 663 nm. One leaf was analyzed per plant, with three replications per treatment.

Soluble sugar content: The amount of soluble sugar in the leaf material was determined. 0.5 g of fresh leaves were mashed using a pestle and mortar, with 5 mL of 80%. For one hour, samples were shaken at 60°C. Then, 1 mL of the supernatant was mixed with 3 mL of anthrone reagent, which was made by dissolving 150 mg of anthrone in 72% freshly made H₂SO₄. A spectrophotometer was used to measure absorbance at 625 nm after the mixture was heated for 10 minutes and allowed to cool for 20 minutes following the procedure (Yaqoob *et al.*, 2019). One leaf was analyzed per plant, with three replications per treatment.

Soluble protein content: Fresh samples (100 mg) were homogenized in an ice-cold sodium phosphate buffer (50 mM, pH 7.2) containing 1 mM EDTA, Na₂, and 2% (w/v) PVPP to assess the protein content. For 40 minutes at 4°C, the homogenate was centrifuged at 13,000 × g. The supernatant was gathered and kept at -80°C in tiny aliquots. The supernatant was combined with Bradford reagent (B6916) and left in the dark for five minutes to determine the protein content. At 595 nm, absorbance was measured with a UV/VIS spectrophotometer. According to (Bettaieb *et al.*, 2011) the reference for calculating the concentration of soluble proteins was bovine serum albumin (BSA). One leaf was analyzed per plant, with three replications per treatment.

Proline content: Five milliliters of 3% aqueous sulfosalicylic acid were used to homogenize 0.5 grams of plant material, and the resulting homogenate was centrifuged for 10 minutes at 5,000 rpm. Two ml of the supernatant were then heated to 100°C for an hour in a test tube together with two ml of glacial acetic acid and two ml of ninhydrin reagent. Submersion in ice stopped the process. Four ml of toluene were used to extract the mixture after it had been vigorously mixed for 15-20 seconds. The absorbance of the colored toluene layer at 520 nm, using toluene as a blank was measured using a spectrophotometer (Bates *et al.*, 1973). Utilizing the following formula, the proline content was calculated as nmol.mg⁻¹ FW:

$$\text{Proline } (\mu\text{g/g FW}) = (C \times V) / (W \times 115.13)$$

Where:

C = Proline concentration (μg/mL) obtained from the standard curve

V = Volume of extract (mL)

W = Fresh weight of the sample (g)

115.13 = Molecular weight of proline (g/mol)

This formula expresses proline content in micrograms per gram of fresh weight (μg/g FW).

Malondialdehyde content: To measure the amount of malondialdehyde (MDA), a modified thiobarbituric acid (TBA) method was used. Using an extraction solution that contained 2.0 mM MgSO₄, 1.0 mM EDTA, 1.0 mM ascorbic acid, 1.0 mM phenylmethanesulfonyl fluoride (PMSF), 0.4% Triton X-100, and 2.0 mg polyvinyl polypyrrolidone (PVPP), fresh leaves (about 0.1 g) were crushed in a mortar on ice. After straining the homogenate through filter paper or muslin fabric, it was centrifuged for 15 minutes at 4 °C at 15,000 rpm. Following centrifugation, 1 ml of the supernatant with 4 ml of thiobarbituric acid (TBA) was mixed. For half an hour, this combination was kept at 95 degrees Celsius in a water bath. A spectrophotometer was used to detect absorbance at 532 and 600 nm after the reaction was stopped by submerging it in an ice tub (Vavilin *et al.*, 1998).

Antioxidant enzymatic activity: 250 mg of fresh leaf samples were crushed in 0.1 M phosphate buffer (pH 7.0) and centrifuged for 30 minutes at 15,000 rpm to evaluate the activity of antioxidant enzymes. The supernatant was

used in a UV-visible spectrophotometer at 25°C to measure the activities of ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD). The methodologies used for determining these activities were adapted from (Huang *et al.*, 2010) for SOD, (Shi *et al.*, 2010) for POD, (Aebi, 1984) for CAT, and (Prochazkova *et al.*, 2001) for APX.

Statistical Analysis: A two-way analysis of variance (ANOVA) was performed to examine the results, accounting for genotypes and treatments. The least significant difference test (LSD) at the 5% probability level was used to compare genotypes and treatments. The experiment was conducted under an RCBD, considering cultivars, salinity stress, and exogenous NaCl application (ranging from 0 to 500 mmol/L), with each treatment was replicated three times (mean ± S.E.). ANOVA was performed for each parameter, and the LSD test was used to compare the mean values at the p<0.05 and p<0.01 probability levels. Significant differences among treatments were shown by different letters (a-c). Biochemical parameters (chlorophyll content, soluble protein content, soluble proline content, enzymatic activities like SOD, POD, CAT, APX, and MDA content) and morpho-physiological parameters (germination rate, germination index, plant biomass, shoot length, and root length) were evaluated using a correlation coefficient matrix for three *Chenopodium quinoa* varieties (Q-1, Q-2, and Q-3). This approach evaluated the statistical significance of variables for different quinoa varieties at varying salt concentrations. The experimental analysis and graphical representation were executed using the 2023 version of OriginPro software.

Results

Effects of NaCl stress on seed germination of three *Chenopodium quinoa* (Q-1, Q-2, and Q-3) varieties:

Varying impacts of NaCl concentrations on the germination of different quinoa seed varieties are shown in Fig. 2. With increasing NaCl concentration, the germination rates for the three quinoa varieties initially rose but subsequently declined. Both the germination percentage and the germination rate index consistently showed a downward trend. Notably, the germination rates of the cultivars under various concentration treatments varied significantly (p<0.05). The germination rates of seeds from the different quinoa varieties peaked at 100 mmol/L NaCl, with increase of 62.50%, 40.10%, and 40.0%, respectively. However, at 400 mmol/L, there was a marked decline in the germination rates of varieties Q-1, Q-2, and Q-3. Further increasing the NaCl concentration to 500 mmol/L resulted in the lowest germination of each variety. This pattern suggests that while low salt concentrations may enhance the germination of quinoa seeds by enhancing internal defense antioxidant enzymatic system and seed metabolic rates, high salt concentrations have an inhibitory effect on their growth.

Effects of NaCl stress on shoot and root length of three *Chenopodium quinoa* (Q-1, Q-2, and Q-3) varieties:

The root length initially increased of quinoa seedlings

responded to increasing salt concentrations, followed by a reduction across different quinoa varieties. Notably, at NaCl concentration of 100–200 mmol/L, both shoot and root lengths of the quinoa varieties reached their peak, with relative increases of 18.51%, 16.25%, and 16.8% compared to the control. However, variations in root length among the varieties were not statistically significant ($p > 0.05$). A progressive decrease in root length was observed as NaCl concentrations increased further. At 500 mmol/L NaCl, the shoot lengths of the three quinoa varieties decreased by 12.1%, 10.33%, and 7.89%, respectively, relative to the control (Fig. 3a). Additionally, Fig. 3b indicates a consistent downward trend in shoot length among quinoa varieties with rising NaCl concentrations. At the highest tested concentration, the root lengths of the three quinoa types showed substantial reductions of 52.55%, 41.7%, and 37.6% compared to the control.

Effects of NaCl stress on shoot and root fresh and dry weights of three *Chenopodium quinoa* (Q-1, Q-2, and Q-3) varieties: The fresh and dried weights of the aerial sections (shoots) of different quinoa seedling varieties

show a discernible pattern as the concentration of NaCl rises, characterized by a gradual decline. This change was significantly different across treatments ($p < 0.05$). Compared to the control, at NaCl concentration of 500 mmol/L, the fresh and dry weights of the aerial parts of the three quinoa varieties were decreased by 86.1%, 71.5%, 66.8%, and 79.6%, respectively (Fig. 4). Conversely, the fresh and dry weights of the subterranean parts (roots) of quinoa seedlings were initially increased and then decreased with rising salt concentrations, displaying significant variability among different varieties and treatments ($P < 0.05$). At 200 mmol/L NaCl, the fresh and dry weights of the subterranean parts of the three quinoa varieties were increased by 51.1%, 76.7%, 89.2%, 44.3%, 55.9%, and 69.45%, respectively, compared to the control (Fig. 4).

Shoot length, root length, and both aerial and subterranean biomass were all significantly impacted by the quinoa varieties' in response to varying salt concentrations (100 mmol/L, 200 mmol/L, 300 mmol/L, 400 mmol/L, and 500 mmol/L); ($p < 0.05$). The var. Q-1 exhibited the strongest tolerance to salt concentration, followed by Q-2 and Q-3.

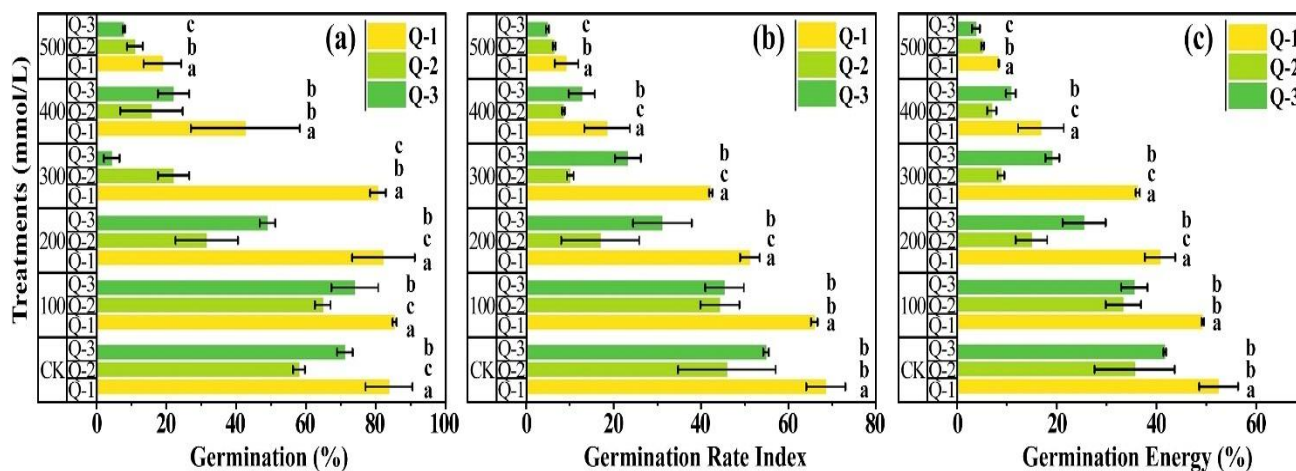


Fig. 2. Seed germination [germination (%), germination rate index, germination energy (%): a–c, index of three *Chenopodium quinoa* cultivars (Q-1, Q-2, Q-3) with or without NaCl treatments (CK, 100, 200, 300, 400, 500 mmol/L). Values are represented as means $\pm$ SD based on three biological replicates. The significance of differences ($p < 0.05$) between the various treatment groups is shown by different lowercase letters (a–c).

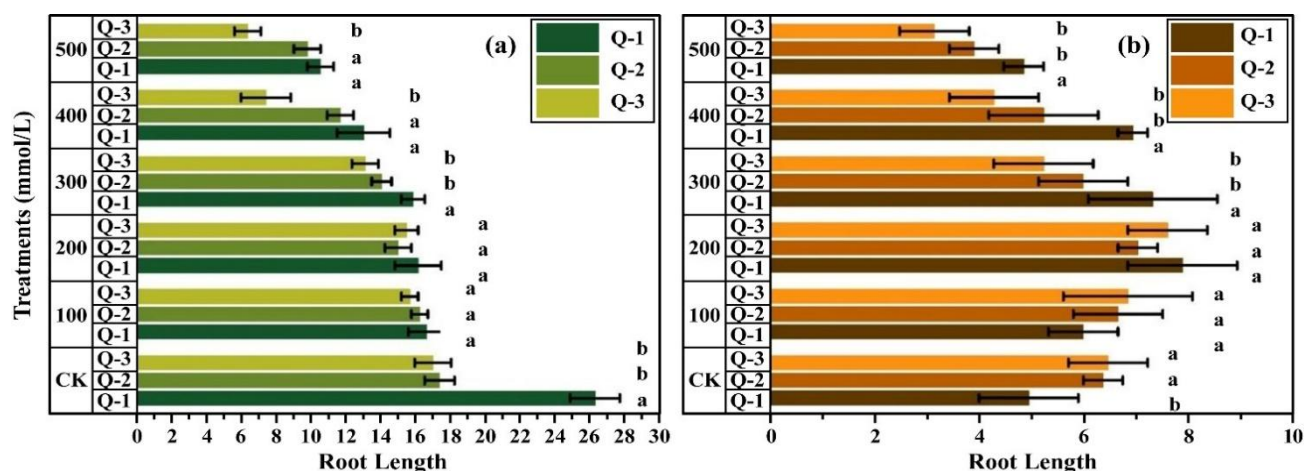


Fig. 3. a, Shoot; b, root length of three *Chenopodium quinoa* varieties (Q-1, Q-2, and Q-3) with or without NaCl treatments (CK, 100, 200, 300, 400, and 500 mmol/L). Values are represented as means $\pm$ SD based on three biological replicates. The significance of differences ($p < 0.05$) between the various treatment groups is shown by different lowercase letters (a–c).

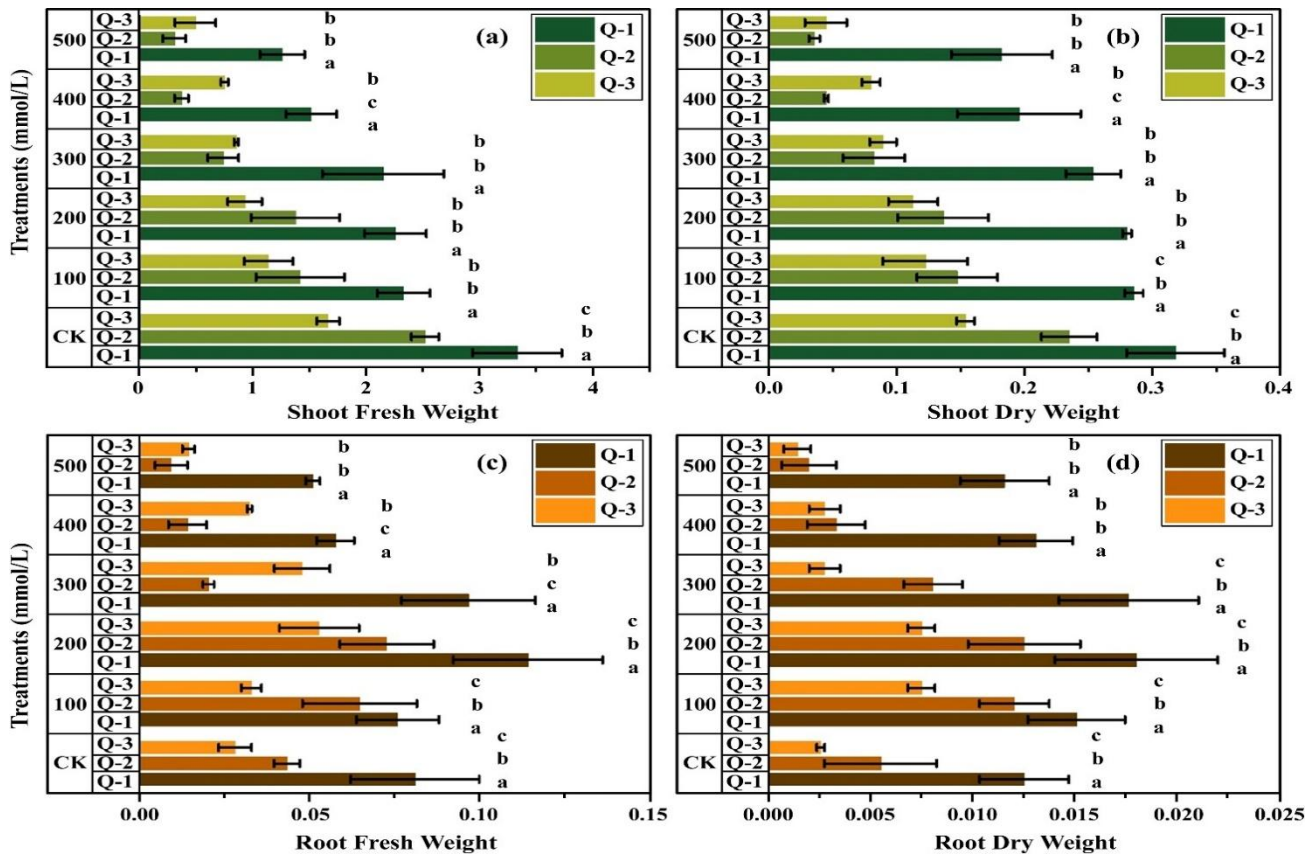


Fig. 4. a, Shoot fresh weight; b, shoot dry weight; c, root fresh weight; d, root dry weight of three *Chenopodium quinoa* varieties (Q-1, Q-2, Q-3) with or without NaCl treatments (CK, 100, 200, 300, 400, 500 mmol/L). Values are represented as means $\pm$ SD based on three biological replicates. The significance of differences ($p < 0.05$) between the various treatment groups is shown by different lowercase letters (a-c).

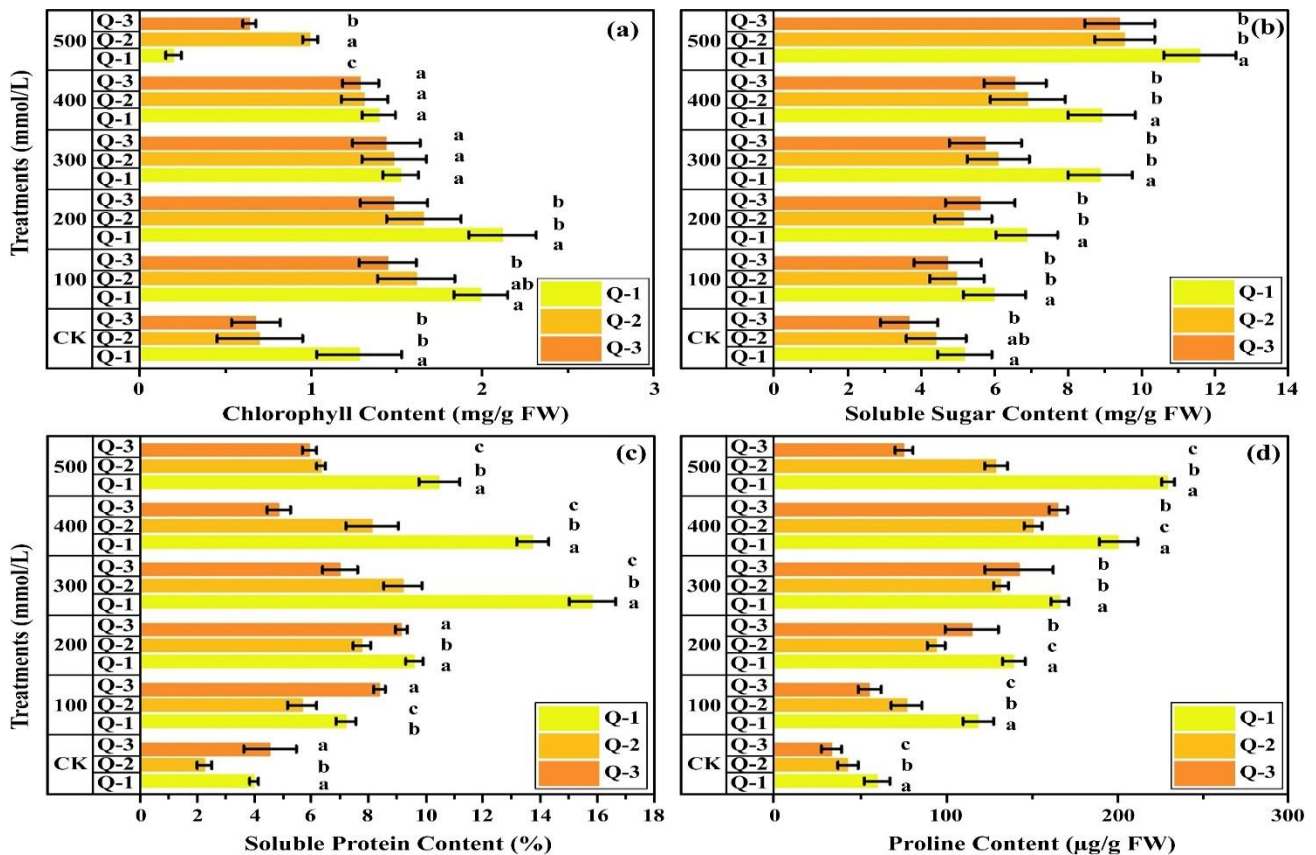


Fig. 5. a, Chlorophyll content; b, soluble sugar content; c, soluble protein content; d, proline content of three *Chenopodium quinoa* varieties (Q-1, Q-2, Q-3) with or without NaCl treatments (CK, 100, 200, 300, 400, 500 mmol/L). Values are represented as means $\pm$ SD based on three biological replicates. The significance of differences ($p < 0.05$) between the various treatment groups is shown by different lowercase letters (a-c).

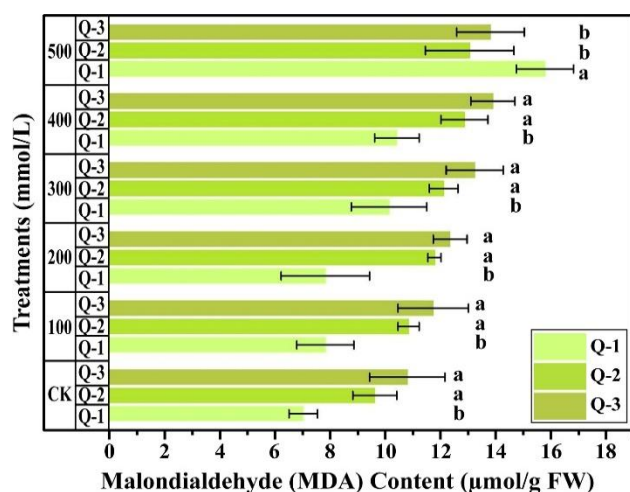


Fig. 6. Malondialdehyde content of three *Chenopodium quinoa* varieties (Q-1, Q-2, and Q-3) with or without NaCl treatments (CK, 100, 200, 300, 400, and 500 mmol/L). Values are represented as means $\pm$ SD based on three biological replicates. The significance of differences ($p < 0.05$) between the various treatment groups is shown by different lowercase letters (a-c).

Effects of NaCl stress on chlorophyll content, soluble protein, soluble sugar, and proline content of three *Chenopodium quinoa* (Q-1, Q-2, and Q-3) varieties: An intriguing trend in chlorophyll content across various quinoa seedling varieties in response to increasing NaCl concentrations is illustrated in Fig. 5a. Initially, chlorophyll content was increased afterward subsequently declined. Significant differences in chlorophyll content were observed among the varieties under control, 100, 200, and 500 mmol/L NaCl treatments ($p < 0.05$). Specifically, at 200 mmol/L NaCl, chlorophyll content peaked, showing increases of 78.8%, 68.4%, and 54.89% for the respective varieties compared to the control. However, as NaCl concentration continued to rise, a gradual decrease in chlorophyll content was noted. At 500 mmol/L, the lowest chlorophyll levels were recorded, with reductions of 7.9% and 6.4% in the Q-1 and Q-2 varieties, respectively. This pattern suggested that while low salt concentrations might promote chlorophyll accumulation, higher concentrations led to significant reductions. Additionally, sugars provide energy for metabolic functions, which can be essential for stress tolerance. Across the various treatments ($p < 0.05$), these changes were statistically significant. At 500 mmol/L NaCl, the sugar content in the leaves of the three quinoa varieties was increased by 127.1%, 119.6%, and 160.5% compared to the control. Notably, the soluble sugar content in the seedlings of the Q-1 and Q-2 varieties was higher than that in Q-3, although the difference was not obvious (Fig. 5b).

Under high salinity stress, quinoa plants demonstrated a marked decrease in soluble protein content. The most significant enhancement occurred at 300 mmol/L NaCl, where the soluble protein content in the seedlings of all three quinoa varieties was increased by 101.1%, 58.4%, and 64.8%, respectively, compared to the control. Even at 500 mmol/L NaCl, although the soluble protein content was decreased, it remained higher than the control, with significant changes among the varieties ($p < 0.05$). Among them, the protein content in the Q-1 variety was significantly greater than that in the Q-2 and Q-3 varieties (Fig. 5c).

Furthermore, the study revealed a considerable increase in proline content at varying salt concentrations. At 500 mmol/L NaCl, the proline content in the seedlings of Q-1, Q-2, and Q-3 varieties increased by 201.1%, 102.2%, and 80.95%, respectively. The proline content in seedlings of the Q-1 and Q-2 varieties was notably higher than that in Q-3 at 100 and 200 mmol/L NaCl, a statistically significant difference ($p < 0.05$) (Fig. 5d).

The effects of NaCl concentrations on malondialdehyde content in the leaves of three *Chenopodium quinoa* (Q-1, Q-2, and Q-3) varieties: Figure 6 illustrates that as NaCl concentrations rise to 400 mmol/L, and a significant surge in MDA levels becomes evident, signaling the onset of pronounced oxidative stress. The MDA content in the leaves of the three quinoa was kinds increased by 29.3%, 36.3%, and 65.9%, respectively, in comparison to the control at a concentration of 500 mmol/L NaCl. At NaCl concentrations ranging from 200 to 400 mmol/L, in the leaves of the Q-1 variety MDA levels were considerably higher than those in the other two varieties ($p < 0.05$), although MDA levels gradually increased at 500 mmol/L NaCl. This reflected an exceptional upward trend in MDA content in the Q-1 variety, indicating a heightened response to oxidative stress. While at 500mmol/L NaCl concentration; the MDA content in Q-2 seedlings was lower than in both Q-1 and Q-3, among these two types, there was no statistically significant difference.

The effects of NaCl stress on the enzymatic antioxidants' peroxidase, superoxide dismutase, catalase, and ascorbate peroxidase in the leaves of three *Chenopodium quinoa* (Q-1, Q-2, and Q-3) varieties: In response to elevated NaCl concentrations (200 mmol/L), the activities of the antioxidant enzymes-POD, SOD, CAT, and APX were increased markedly across the three quinoa varieties. Notably, SOD activity in the leaves peaked significantly at 71.8%, 55.5%, and 38.9% for the Q-1, Q-2, and Q-3 varieties, respectively (Fig. 7a). This variation in SOD activity under different salt concentrations was statistically significant ($P < 0.05$), displaying a descending order of activity from Q-1 to Q-3. As illustrated in Fig. 7b, at a higher NaCl concentration of 300 mmol/L, POD activity in the leaves of Q-1, Q-2, and Q-3 seedlings reached their peaks at 65.5%, 41.3%, and 29.3%, respectively. This represented a threefold increase over the control. The Q-1 and Q-2 varieties exhibited notably higher POD activity compared to Q-3 ($p < 0.05$). Similarly, at 200 mmol/L NaCl, CAT activity in the leaves of the three quinoa types reached its highest values-95.7%, 86.6%, and 68.3% compared to the control, with the Q-1 variety showing a significantly greater increase than the others ($p < 0.05$) (Fig. 7c). In terms of APX activity, substantial enhancement was observed across quinoa varieties. This increased by 80.1%, 70.4%, and 61.3% when the salt concentration was raised from 0 to 200 mmol/L. This upward trend was consistently seen in the Q-1 and Q-2 varieties for all enzymes-SOD, POD, CAT, and APX-following the order Q-1 > Q-2 > Q-3 (Fig. 7d). In short, the production of antioxidant enzymes like SOD, POD, CAT, and APX typically rose when quinoa was under salt stress. To protect the plant from oxidative damage caused by reactive oxygen species (ROS), which are generated when the plant is under stress, these enzymes are crucial.

Table 3. Pearson's correlation coefficient matrix shows the indexes and significance of variation between 15 indicators (I1–I15) of three *Chenopodium quinoa* (Q-1, Q-2, Q-3) varieties.

Indexes	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I14	I15
I1	0.530*	0.339	0.547*	0.431	0.595**	−0.154	0.526*	0.132	−0.245	0.551*	0.504*	0.506*	0.472*	0.402
I2		0.734**	0.537*	0.671*	0.485*	−0.596**	−0.059	−0.432	−0.738**	0.453*	−0.044	0.478*	0.394	0.299
I3			0.684**	0.896**	0.603**	−0.293	−0.065	−0.259	−0.783**	0.533*	0.114	0.409	0.351	0.199
I4				0.760**	0.838**	−0.040	0.394	0.131	−0.578**	0.744**	0.484*	0.729**	0.660**	0.579**
I5					0.730**	−0.135	0.126	−0.060	−0.751**	0.655**	0.307	0.485*	0.478*	0.592
I6						0.095	0.516*	0.250	−0.549**	0.862**	0.600**	0.732**	0.740**	0.671**
I7							0.502*	0.688**	0.428	0.112	0.502*	−0.210	−0.087	−0.087
I8								0.631**	0.053	0.443*	0.806**	0.299	0.401	0.411
I9									0.366	0.259	0.688**	0.057	0.152	0.296
I10										−0.521*	0.009	−0.509*	−0.518*	−0.365
I11											0.512*	0.766**	0.771**	0.642**
I12												0.324	0.395	0.404
I13													0.828**	0.781**
I14														0.799**

* Represents a significant correlation at $p < 0.05$, and ** Represents an extremely significant correlation at $p < 0.01$. I1–I15 represent various growth, biochemical, and enzymatic activity indices (i.e., I1: root length, I2: shoot length, I3: shoot fresh weight, I4: root fresh weight, I5: shoot dry weight, I6: root dry weight, I7: soluble sugar content; I8: proline content; I9: soluble protein contents; I10: MDA contents; I11: SOD activities; I12: POD activities; I13: CAT activities; I14: APX activities; and I15: chlorophyll contents)

Comprehensive evaluation of salt tolerance and growth indicators of three *Chenopodium quinoa* (Q-1, Q-2, and Q-3) varieties: Significant correlations ($p < 0.05$ and $p < 0.01$) between seedling development and physiological parameters across different quinoa varieties under variable salt concentration treatments are highlighted by the correlation analysis shown in Table 3. Proline concentration, soluble sugar, soluble protein, fresh and dry weight, chlorophyll content, shoot and root length, and antioxidant enzymatic activity (SOD, POD, CAT, and APX) are some of these indicators (Figs. 7, 8). Collectively, these indicators served as reliable measures for assessing the salinity tolerance of *Chenopodium quinoa* varieties. We established linear relationships between morpho-physiological and biochemical parameters across quinoa varieties using correlation coefficients, showing a significant correlation at $p < 0.05$, while * denotes an extremely significant correlation at $p < 0.01$. These parameters are labeled I1 to I15 and encompass various growth, biochemical, and enzymatic activity indices: I1: root length; I2: shoot length; I3: shoot fresh weight; I4: root fresh weight; I5: shoot dry weight; I6: root dry weight; I7: soluble sugar content; I8: proline content; I9: soluble protein content; I10: MDA content; I11: SOD activity; I12: POD activity; I13: CAT activity; I14: APX activity; and I15: chlorophyll content. The correlation coefficients varied from -1 to 1, indicating a range from low to high correlation strengths.

In comparative terms, the Q-1 and Q-2 varieties exhibited superior tolerance to moderate salinity stress (over 100–200 mmol NaCl/L), while the Q-3 variety displayed relatively low tolerance. Notably, Table 3 reveals significant positive correlations in the parameters root fresh weight and root dry weight (I4 and I6), along with MDA and soluble sugar contents (I10 and I7), which exhibited a relatively negative correlation when compared to other parameters. This analysis underscores how different quinoa varieties react differently to varied salt concentrations in terms of morpho-physiology and biochemistry. Focusing on salt tolerance among the three quinoa varieties (Q-1, Q-2, and Q-3), we compared two key indicators: germination rate and seedling vigor. The 'Comprehensive Evaluation' (CE) value, plotted on the vertical axis, indicates the overall performance of each variety; higher values signify better performance. The horizontal axis, labeled 'Range Index,' ranks the varieties from highest performing (Q-1 and Q-2) to lowest performing (Q-3) (Fig. 8).

The subordinate function and comprehensive evaluation reveal variations in CE scores for each variety at specific range indices. For instance, at range indices I1 and I15, Q-1 scored 0.37 and 0.58 in CE, Q-2 obtained 0.29 and 0.55, and Q-3 scored 0.34 and 0.49. This pattern suggested that all three varieties experienced reduced germination rates and seedling vigor under extreme saline conditions (> 300 mmol NaCl/L) compared to non-saline environments. However, Q-1 emerged as the most salt-tolerant, followed closely by Q-2. Q-1 consistently outperformed the other varieties in CE scores across nearly all range indices, except for index I3. The overall decline in CE scores with increasing range indices indicates reduced tolerance to higher salinity levels. The closer CE scores of Q-1 and Q-2 (0.58 and 0.55, respectively) compared to Q-3's lower score of 0.49 (Fig. 8) suggested a similar salt tolerance capacity in Q-1 and Q-2 compared to Q-3.

Discussion

In this study, we investigated three quinoa varieties subjected to varying salt stress treatments. In comparison to the control, salt stress conditions significantly reduced plant growth, morpho-physiology, and biomass; however, the Q-1 and Q-2 varieties demonstrated greater resistance to salinity than Q-3. The superior growth of these varieties under salt stress may be linked to chlorophyll levels and net photosynthetic rate (Wang *et al.*, 2013). Additionally, plants often accumulate compatible metabolites in response to diverse abiotic stresses (Jiang *et al.*, 2023). This protective mechanism, supported by biochemical analyses, shields plants under stress conditions (Akram *et al.*, 2017; Kahlaoui *et al.*, 2018). Notably, the robust resilience of quinoa to salinity stress makes it a promising crop for salt-affected soils (Yang FaRong *et al.*, 2017; Cai & Gao, 2020; Iqbal *et al.*, 2020). Despite its marked tolerance, *Chenopodium quinoa* is not considered a real or obligatory halophyte, because of its variable growth and resilience in different saline environments (Ruiz *et al.*, 2016; Causin *et al.*, 2020). Among the three genotypes studied, Q-1 and Q-2 displayed the highest resistance to NaCl, surpassing the resilience of Q-3. While quinoa exhibits considerable tolerance to 200 mmol/L NaCl, our findings indicate that lower salt concentrations minimally affect its germination and growth. These results align with (Panuccio *et al.*, 2014).

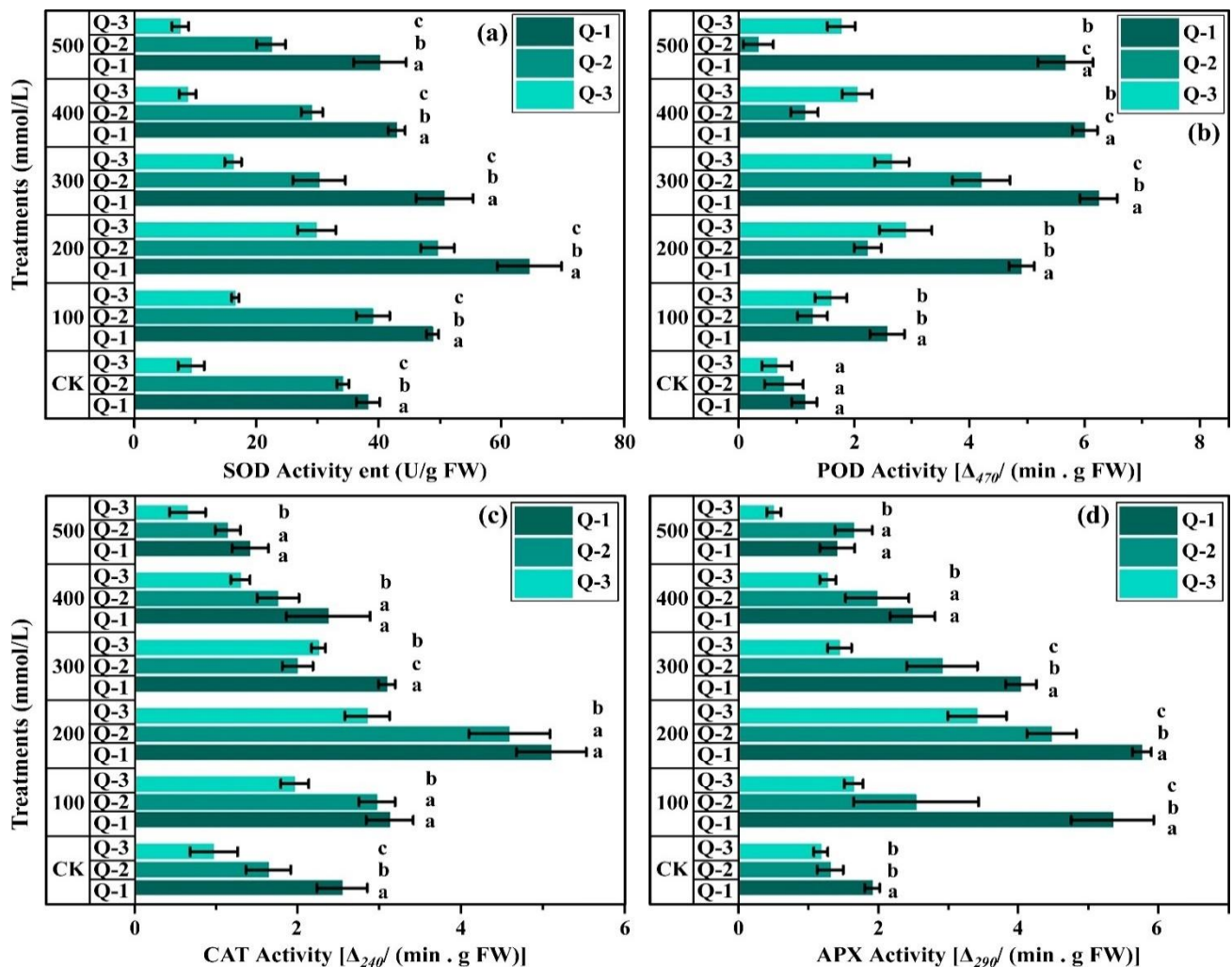


Fig. 7. a, Superoxide dismutase; b, peroxidase; c, catalase; d, ascorbate peroxidase activities in the leaves of three *Chenopodium quinoa* varieties (Q-1, Q-2, Q-3) with or without NaCl treatments (CK, 100, 200, 300, 400, 500 mmol/L). Values are represented as means $\pm$ SD based on three biological replicates. The significance of differences ($p < 0.05$) between the various treatment groups is shown by different lowercase letters (a-c).

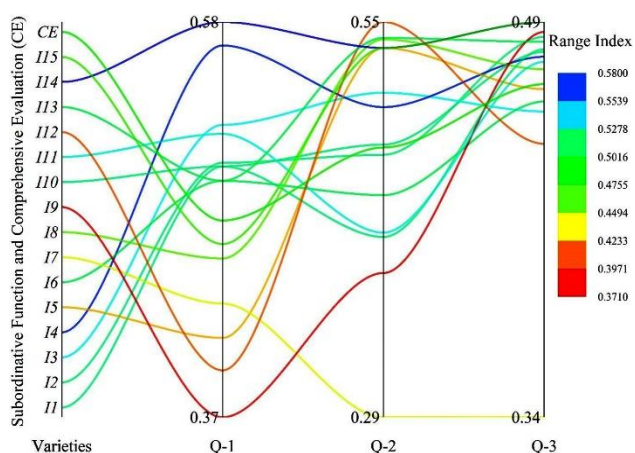


Fig. 8. Correlation coefficient matrix of indexes and significance of validation among three *Chenopodium quinoa* varieties (Q-1, Q-2, and Q-3) with or without NaCl treatments (CK, 100, 200, 300, 400, and 500 mmol/L). I1-I15 represent various growth, biochemical, and enzymatic activity indices (i.e., I1: root length, I2: shoot length, I3: shoot fresh weight, I4: root fresh weight, I5: shoot dry weight, I6: root dry weight, I7: soluble sugar content, I8: proline content, I9: soluble protein contents, I10: MDA contents, I11: SOD activities, I12: POD activities, I13: CAT activities, I14: APX activities; and I15: chlorophyll contents).

Interestingly, the lower Na^+ and Cl^- concentrations in the roots, compared to the soil medium, suggest active removal of these ions by the plants, a mechanism noted by (Jiang *et al.*, 2023). However, exposure to high NaCl concentrations (≥ 300 mM) significantly hampers germination and seedling growth of quinoa, as evidenced by a reduced germination rate, energy, and index. Similar findings have been reported in other studies; for instance, it is found that NaCl concentrations of 100 mmol/L facilitated germination in the Yanli 47 and 48 quinoa cultivars. In contrast, Kasala cultivar seedling growth was hindered at 300 mM NaCl concentrations (Yang FaRong *et al.*, 2017).

In contrast to other quinoa varieties such as Q29, which demonstrated the least tolerance to salt stress, GIZA 02 demonstrated the highest resilience, retaining superior germination rates and growth metrics even at elevated NaCl concentrations (up to 300 mM). The genetic diversity in salt tolerance among quinoa cultivars is highlighted in this study, highlighting the possibility of breeding initiatives targeted at improving crop resilience in saline conditions (Hichem *et al.*, 2024). Despite quinoa, the possibility of transgenic breeding to increase salt tolerance in crops like rice and cotton by adding genes from halophytes was examined in a study conducted by Zhou *et*

al., (2024). According to this study, creating more resilient crop types may result from a better understanding of the genetic underpinnings of salt tolerance (Zhou *et al.*, 2024)

Under salinity stress, quinoa's physiological characteristics, such as water content, protein levels, soluble sugar, proline, and chlorophyll, gradually decreased. Additionally, our investigation revealed that the chlorophyll content of all three quinoa varieties dropped as NaCl concentration increased. However, under salt stress, the total chlorophyll and soluble sugar content of the Q-1 and Q-2 varieties remained relatively higher than that of Q-3. (Gururaja, 1981) reported that chlorophyllase's breakdown of chlorophyll is the primary cause of reduced chlorophyll concentration during salt stress. Consequently, the notable shift in chlorophyll levels among the varieties indicates that Q-3 is more susceptible to salt stress than Q-1 and Q-2. Furthermore, under extreme saline stress conditions (300–500 mmol/L NaCl), these physiological characteristics of quinoa plants further declined. Other studies have also shown reductions in similar characteristics under salt stress (Abbas *et al.*, 2021; Iftikhar *et al.*, 2022). Plants combat stress-induced adversities by accumulating compatible Osmoprotectants, such as soluble proteins and proline, are crucial for regulating the osmotic balance (Szabados & Saviouré, 2010). In the three quinoa cultivars tested in this study, proline and soluble protein concentrations are effectively increased by applying salt stress as a seed treatment. Higher proline levels are associated with improved growth in tolerant quinoa types, such as Puno and Vikinga. Proline function as an Osmoprotectant, stabilizing proteins and improving salt tolerance in quinoa. Proline and soluble sugars are essential for osmotic adjustment, which helps quinoa sustain cell turgor and metabolic activity under salt stress (Jaramillo Roman, 2021). Interestingly, moderate concentrations of saline solutions positively influenced the accumulation of these Osmoprotectants in the plants. Based on these findings, proline serves as a potent protective agent that enables plants to withstand harsh abiotic conditions. Adolf *et al.*, (2013) emphasized a significant correlation between salt tolerance in *Chenopodium quinoa* embryos and the enhanced profile of compatible osmolytes accumulation such as proline, betaine, mannitol, and myo-inositol. However, Ashraf & Foolad, (2007) showed marginal variations in antioxidant capacities across different plant genotypes, suggesting that optimal quinoa germination and growth occur in low-salinity environments. MDA levels in plant cell membranes reflect the extent of oxidative stress and osmolyte imbalance. Our research indicates a rapid increase in MDA levels in response to higher salt concentrations, correlating with studies suggesting that NaCl signals the onset of oxidative stress in quinoa (Ashraf, 2009; Garg & Manchanda, 2009; Morales & Munné-Bosch, 2019). Notably, at higher salt concentrations, quinoa seeds from all varieties (Q-1, Q-2, and Q-3) maintained elevated average MDA concentrations, indicating vulnerability to oxidative damage Cai and Gao, (2020) observed that, antioxidant enzymes such as SOD, POD, CAT, and APX showed higher activity in all quinoa varieties compared to the control. However, at 100 mmol/L NaCl, MDA accumulation was not significant. At higher salt concentrations (300–500 mmol/L NaCl), marked increase in MDA concentration indicated enhanced membrane damage owing to higher reactive oxygen species (ROS) production (Iqbal *et al.*, 2023). Our findings align with

(Derbali *et al.*, 2020), demonstrating that MDA concentrations accumulate under severe salinity stress. Concurrently, SOD and POD activities declined, adversely affected by high salt levels.

According to a study, quinoa under salt stress has higher levels of antioxidant enzymes (SOD, CAT, and POD), which helps prevent oxidative damage from ROS. These enzymes are further enhanced by the application of potassium and salicylic acid, which increases salt tolerance (Alghamdi *et al.*, 2023; Iqbal *et al.*, 2023) Enhancing antioxidant capacity may help breed more salt-resilient quinoa types, as various quinoa varieties exhibit diverse antioxidant responses. ROS production to plant responses to salt stress; thus, plants require an efficient antioxidant defense system to eliminate excessive ROS. This antioxidant defense system often consists of both enzymatic and non-enzymatic elements (Asada, 2006; Lei *et al.*, 2021). The high production of antioxidant enzymes is crucial in mitigating damage induced by reactive oxygen species (Osman, 2015; Yaqoob *et al.*, 2019; Rajput *et al.*, 2021). SOD, APX, and POD are significant defensive enzymes in the enzymatic system of plants (Orendi *et al.*, 2001).

Following NaCl administration, the activities of POD, SOD, CAT, and APX in Q-1 and Q-2 varieties were substantially higher than those in control and Q-3. These findings suggest that these enzymes play a beneficial role in scavenging reactive oxygen species in salt-tolerant quinoa varieties (Q-1 and Q-2). It is also noted that proline can scavenge ROS produced under salt stress, functioning as a compatible solute that protects against oxidative damage (Ruijter *et al.*, 2003). During germination, a moderate salt concentration of 200 mmol/L NaCl markedly increased the enzyme activities of SOD, POD, CAT, and APX in all three quinoa varieties. Furthermore, at lower salt concentrations (100 mmol/L NaCl), fresh weight and seed vigor index in quinoa increased substantially, coinciding with higher antioxidant enzymatic activities of CAT, SOD, POD, and APX. This finding aligns with research demonstrating that quinoa seedlings exposed to 100–250 mmol/L NaCl significantly upregulate antioxidant enzymes (Panuccio *et al.*, 2014; Boas *et al.*, 2016).

The impact of salt stress leading to osmotic stress (e.g., salinity, drought, heat stress) may be exacerbated by mannitol. In the case of quinoa, there are conflicting reports regarding the effects of osmotic and ionic factors on germination under salinity, with variations potentially depending on genotype (Moreno *et al.*, 2018). Mannitol and proline are essential Osmoprotectants that regulate the water balance inside cells and protect proteins from denaturation in proline. Proline also has antioxidant properties that could help shield the plant from oxidative damage brought on by salt stress. plays a crucial role in protecting cells from the ionic, osmotic, and oxidative aspects of salt stress by acting as an Osmoprotectant, scavenging ROS, stabilizing proteins and membranes, and providing reducing equivalents (Tonon *et al.*, 2004; Ghimire *et al.*, 2018). Our results suggest that ionic imbalances significantly influence the varying germination responses to NaCl among genotypes, and these differences are not solely due to variations in seed imbibition rates. The biomechanical properties and composition of cell walls play a pivotal role in how plant tissues weaken and rupture

(Santos and Fernandes, 2018; Steinbrecher and Leubner-Metzger, 2018). These differences are evident in water absorption and the structural organization of seed coat layers, as corroborated by studies on *Chenopodium quinoa* seeds under varying salinities. Researchers have indicated that, under conditions of salt stress, mannitol and proline accumulation function as Osmoprotectants, aiding in osmotic balance (Shabala, 2000; Conde *et al.*, 2011; Soheilikhah *et al.*, 2013; Causin *et al.*, 2020).

According to these findings, quinoa's ability to withstand salt stress is primarily linked to modifications in antioxidative enzyme activities. Additionally, previous research has demonstrated that stress-tolerant cultivars exhibit higher antioxidant enzyme activity under various abiotic stress conditions (Mittler, 2002). Proline and soluble sugars for Osmoprotectants are two of quinoa's salt tolerance tactics, in addition to other halophytes' utilization of ion exclusion and osmotic adjustment (Kahlaoui *et al.*, 2018; Wani *et al.*, 2019) Although quinoa and other plant species like *Salicornia* and *Atriplex* share characteristics, *Suaeda salsa* has succulent leaves and exhibits high salt tolerance. Halophytes like *Aeluropus litoralis*, *Mesembryanthemum crystallinum*, *Suaeda salsa*, *Atriplex halimus*, *Thellungiella halophila*, *Cakile maritima*, *Limonium bicolor* and *Salicornia europaea* are model plants for identifying salt-responsive genes and promoters. Quinoa is unique in that it has higher antioxidant enzyme activity, which enables it to flourish in extremely salinized environments (Meng *et al.*, 2018; Ain *et al.*, 2023; Olmos *et al.*, 2024).

Our study shows that the Q-1 and Q-2 quinoa varieties are more salt-tolerant than the Q-3 variety, with this difference directly correlated to higher antioxidant enzymatic activity, elevated proline, MDA, and soluble sugar content. The knowledge of quinoa's salt tolerance for its whole growth cycle was limited as this study's primary focus was on germination and early seedling growth, morphophysiological and biochemical activities. Future studies should examine the effects of salt stress on seed growth and blooming, as well as look into molecular pathways through the use of transcriptome and proteomic techniques. Furthermore, researching combined salinity-alkalinity stress would offer a more thorough understanding of quinoa's adaptive mechanisms and more accurately reflect natural growing conditions.

Conclusion

Our results show that the three quinoa types (Q-1, Q-2, and Q-3) differ significantly in their ability to withstand salt stress during germination and the early stages of seedling growth. Q-1 and Q-2 outperformed Q-3 in terms of germination and biomass growth, especially at higher salinity levels, even though all cultivars showed some resistance to NaCl concentrations up to 200 mmol/L. These findings imply that improved germination and seedling growth are positively correlated with salt tolerance in Q-1 and Q-2, making them more robust in saline environments. This is further supported by the physiological responses, revealing that Q-1 and Q-2 had lower levels of reactive oxygen species (ROS) buildup, higher levels of antioxidant and enzyme activity (SOD, POD, CAT, and APX), and lower levels of malondialdehyde than Q-3. These characteristics highlight how well these two types regulate oxidative

damage. These results offer a solid basis for breeding initiatives aimed at creating salt-resistant quinoa types and crucial insights into the adaptive mechanisms of quinoa to deal with salt stress. Our study lays the groundwork for the production of quinoa in saline-alkaline soils by identifying the physiological indicators associated with salt tolerance, hence promoting food security and land rehabilitation. However, more investigations into the underlying genetic and biochemical processes promoting salt tolerance in these types are required to completely comprehend the molecular basis of quinoa's stress responses.

Authors contribution: Conceptualization, Z.G.; data curation, Z.G.; formal analysis, Z.G.; investigation, Z.G. and L.Q.Q.; methodology, Z.G. and M.H.; project administration, Z.G.; resources, Z.G., and L.Q.Q.; software, Z.G. and M.H.; supervision, M.A.; validation, M.A. and Z.G.; writing—original draft, Z.G. and M.A.; writing—review and editing, Z.G., M.A., and L.Q.Q. All authors have read and agreed to the published version of the manuscript.

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