




Screening of quinoa (*Chenopodium quinoa* Willd.) germplasms under high-SAR saline water on the basis of growth, yield, and multivariate analysis

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Increasing soil and underground water salinization with decreasing availability of fresh water has become a potential threat to sustainable crop production in arid and semi-arid areas globally. Introduction and evaluation of salt-tolerant halophytic crops is one of the sustainable ways to preserve productivity in saline ecosystems. This study was aimed to screen quinoa germplasms under high-sodium adsorption ratio (SAR) saline stress. Thirteen quinoa germplasms were evaluated under four levels [best available water (BAW), 8, 16, and 24 dSm^{-1}] of high-SAR saline water irrigation. The evaluation was carried out based on growth, yield, and ionic content parameters along with statistical tools such as multivariate analysis, salt tolerance indices, and correlation. The results showed that the salinity levels of 16 and 24 dSm^{-1} resulted in increase of chlorophyll content relative to BAW and 8 dSm^{-1} . The germplasm CSQ2 recorded the highest proline content (163.7 mg g^{-1} FW) at 24 dSm^{-1} . Increasing levels of salinity reduced relative water content in plant leaves, and the germplasm CSQ2 showed minimal reduction of 4% at 24 dSm^{-1} . Na^+ and K^+ contents in the plants increased with increasing salinity levels, while the K^+/Na^+ ratio decreased. The grain yield of quinoa germplasms ranged between 3.5 and 14.1 g plant^{-1} . The germplasm EC507740 recorded the highest grain yield (7.0 g plant^{-1}) followed by CSQ1 and CSQ2 at a maximum stress of 24 dSm^{-1} . Principal component analysis (PCA) and correlation elucidated that Na^+ content in plants was negatively correlated with all the studied traits except SPAD, proline content, and K^+ content. The different salt tolerance indices indicated that the germplasms EC507740, CSQ1, CSQ2, EC507738, and IC411825 were more stable at high-SAR salinity, while PCA showed the germplasms EC507740 and CSQ2 as the most salt-tolerant germplasms.

Keywords. Growth; ionic content; principal component analysis; quinoa germplasm; salt tolerance indices; sodium adsorption ratio; yield

1. Introduction

Increasing salinization and declining availability of good-quality irrigation water for agriculture have threatened the productivity of about 960 million ha of farmlands globally and the livelihood of millions of farmers (Wicke *et al.* 2011). Nearly 2000 ha of productive land worldwide are becoming unproductive each day as a consequence of continuous spread of salinity (Shahid *et al.* 2018). Continuous use of saline underground water for irrigation purposes and faulty

irrigation management practices have affected 20% of irrigated land globally (Negacz *et al.* 2022). In India, 6.73 million ha of arable land are affected by soil salinization (2.94 million ha saline soils and 3.79 million ha sodic soils) (Sharma and Singh 2015; Sheoran *et al.* 2021). Moreover, in a survey, 32% to 84% of underground water used for irrigation purposes was declared of poor quality (Minhas 1999). Due to salinity hazards, the country is losing 16.84 million tons of food grains annually, the value of which has been estimated as INR 230.20 billion (Mandal *et al.* 2018).

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With the paucity of good-quality irrigation water in arid and semi-arid regions, farmers are compelled to use underground saline water to irrigate crops. In many parts of arid and semi-arid regions, declining groundwater levels and consequent exploitation of water from lower strata of saline aquifers having high electrolyte concentrations is increasing salt loads in the soil over time. It has been reported that such underground saline water is not only rich in electrolytes but also has excess Na^+ content relative to Ca^{++} and Mg^{++} with a resultant higher sodium adsorption ratio (SAR). Such ionic imbalance of saline water having higher SAR ($>10 \text{ mmol L}^{-1}$) is termed high-SAR saline water, which has been found to limit crop productivity when used for irrigation purposes (Minhas et al. 2004). Continuous irrigation with high-SAR saline water adversely affects both crop yield and soil properties through salinity and sodicity hazards. In such situations, the choice of crops is limited to some tolerant species and adoption of alternate irrigation strategies or bio-saline agriculture; e.g., agroforestry-based systems and growing of halophyte and semi-halophyte crops are the only feasible options to sustain productivity (Dagar 2018).

Quinoa (*Chenopodium quinoa* Willd.) is a promising facultative halophyte that has recently been recognized for its endurance in multiple abiotic stresses (drought, salinity, frost, etc.), and has a high potential for cultivation in saline and drought-prone areas of the world (Bhargava et al. 2007; Bazile et al. 2016; Hariadi et al. 2011; Hirich et al. 2013; Hirich et al. 2014; Iqbal et al. 2019; Shabala et al. 2013; Salehi 2020). Quinoa grains possess excellent nutritional quality, surpassing the values prescribed by the FAO (Bhargava et al. 2007; Hirose et al. 2010; Bazile et al. 2015). Grains are rich sources of high-quality proteins with all essential amino acids (Dini et al. 2005), vitamins, and minerals (iron, zinc, magnesium, and calcium) (Repo-Carrasco et al. 2003; Stikic et al. 2012). Quinoa grains are also free from gluten content, making them highly suitable for people suffering from celiac disease (Yazar and Incekaya 2014; Salehi et al. 2021). Further, the glycemic index of quinoa grains makes them a good food for diabetics. The outstanding nutritional properties and variety of health benefits of quinoa make it a prospective crop to fight world hunger (Pulvento and Bazile 2023).

Several studies have proven quinoa's salt-tolerant ability and showed that it can be grown productively under a wide range of salinity stresses (Jacobsen et al. 2003; Koyro et al. 2008; Eisa et al. 2012; Razzaghi et al. 2015; Goehring et al. 2019). Quinoa

exhibited no adverse effects and even showed a small increase in grain yield under moderate salinity of $8\text{--}10 \text{ dSm}^{-1}$ (Hirich et al. 2014; Geissler et al. 2015). Quinoa performed optimally at $10\text{--}20 \text{ dSm}^{-1}$ salinity and can tolerate salinity stress of up to 40 dSm^{-1} , representing seawater salinity (Jacobsen et al., 2003; Hariadi et al. 2011; Adolf et al. 2013). It has been reported that quinoa could grow up to high salinity of $50\text{--}75 \text{ dSm}^{-1}$ and complete its life cycle (Orsini et al. 2011; Eisa et al. 2017). In another study, 50% reduction in grain yield was observed at 25 dSm^{-1} salt concentration, but quinoa was able to grow and produce yield up to a 51.5 dSm^{-1} level of salinity (Razzaghi et al. 2015). Yazar et al. (2015) observed that grain yield of quinoa cv. Titicaca decreased with increase in salinity levels up to 30 dSm^{-1} . From the above findings it can be inferred that quinoa crop possesses a high degree of salt tolerance and can be grown successfully with good yield in saline environments.

Various physiological and biochemical pathways have been identified for the salinity tolerance ability and the halophytic nature of quinoa. Among them, quick Na^+ removal from the cytosol, high K^+ accumulation in the shoot and root to maintain a favorable K^+/Na^+ ratio (Sun et al. 2017), Na^+ omission and compartmentation (Hariadi et al. 2011), reduced stomatal densities (Shabala et al. 2013), accumulation of salt in the form of salt nodules in the leaves (Agarie et al. 2007), and a protected seed interior (Koyro and Eisa 2007), etc., are some of the foremost mechanisms described for salt tolerance in quinoa.

Quinoa is a new crop in Southeast Asia and its potential and suitability for saline agro-ecosystems of India have not been studied so far. Although its tolerance to salinity stress is well established through several available studies, there are few studies on its performance under high-SAR saline irrigation water or soil. Recently, it has been observed that sodicity is more detrimental for quinoa growth and yield performance than salinity stress (Abbas et al. 2021; Iqbal et al. 2019). So, it is necessary to screen suitable quinoa germplasms under sodicity stress as well. With the above facts, the current investigation attempted (i) to study the impact of high-SAR saline water on the physiological, growth, and yield parameters of quinoa germplasms, (ii) to determine the most sensitive characteristics under high-SAR saline water, and (iii) to find promising quinoa germplasms based on multivariate analysis, correlation, and salt tolerance indices.

2. Materials and methods

2.1 Experimental site

The experiment was conducted for two consecutive winter seasons (2017–18 and 2018–19) at the ICAR-Central Soil Salinity Research Institute (CSSRI), Karnal, Haryana, India. The experimental site is located at 28°717'N, 76°967'E and 244 m AMSL, and has a semi-arid sub-tropical type climate characterized by three distinct seasons, i.e., rainy season (July–October), cool winter season (November–March), and hot summer season (April–June). The mean annual precipitation of the region is 670 mm, 70% of which is received during the rainy season.

2.2 Experiment setup and treatments

Earthen pots of 24 cm diameter and 25 cm height were used for the experimental purpose. Pots were uniformly filled (up to 20 cm depth) with 9.0 kg surface (0–15 cm) soil of the experimental farm of the Institute. The filled soil was sandy loam (sl) in texture, which represents the typical soil of the Indo-Gangetic plains (IGP) of India. The physico-chemical properties of the soil were analysed, and the results are given in supplementary table 1. The experiment was set up in a pot house which was covered with a transparent polythene sheet in order to protect the pots from rainwater.

Out of the 13 germplasms of quinoa used in the study, 11 (G1 to G11) were obtained from ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi, and the remaining two, G12 and G13, were exotic germplasms being grown by farmers of Bulandshahr of Uttar Pradesh State and Kota of Rajasthan State of India, respectively (original source not known) (supplementary table 2). Three levels of high-SAR irrigation water salinity (EC_{iw}), viz., 8 dSm^{-1} , 16 dSm^{-1} , and 24 dSm^{-1} , along with good-quality irrigation water available at the institute [hereafter designated as best available water (BAW)] were taken as treatments over 13 quinoa germplasms. The experiment was planned in a factorial complete randomized design (FCRD) with three replications. The natural saline underground water available at the tube wells of the experimental farm located at Nain village of Panipat district of Haryana, which represents the semi-arid climate of IGP of India, was used for the salinity treatments. The chemical properties of saline water used for irrigation were analysed in the laboratory and are listed in supplementary table 3.

The climatic requirements for the growing period of quinoa crops are suitably met during the winter season at the experimental site. Sowing of all the germplasm lines was done on 15th and 19th November 2017–18 and 2018–19. Each germplasm was sown in four pots representing each treatment with three replications per treatment, making a total of 12 pots per germplasm in the experiment. All the pots were irrigated with BAW until germination. After seedling emergence, three plants were kept in each pot by thinning out the remaining plants. The salinity treatments started 14 days after sowing (DAS) up to maturity stage. As no recommended fertilizers levels have been standardized for the Indian conditions, we applied only nitrogen on soil weight basis (equivalent to 50 kg N ha^{-1}) through the application of a uniform dose of 0.5 g urea to each pot at the pre-flowering stage of the crop under all treatments.

2.3 Physiological and biochemical parameters

Observations on SPAD (Soil Plant Analysis Department) values, proline content, and relative water content (RWC) were recorded at vegetative and reproductive growth stages. Plant sampling and observations at the vegetative growth stage were recorded at the principal growth stage 4 (formation of vegetative harvestable parts; Sosa-Zuniga *et al.* 2017), which was observed 40–45 DAS in different germplasms. For the reproductive stage, plants were sampled when 50% of the plants completed anthesis on the main panicle (principal growth stage 6, 70–75 DAS). Third and fourth fully expanded leaves from the top of the plant were sampled between 10:00 h to 12:00 h for the estimation of RWC and proline content.

The relative chlorophyll content of leaves in terms of SPAD meter values was recorded at the vegetative and reproductive stages. SPAD meter readings were recorded between 09:00–11:00 h of the day using SPAD-502 (DL plus Konica Minolta, Japan) on the top three intact fully expanded leaves of each plant.

About 300 mg of sampled fresh leaves were homogenized in 5 mL of 3% sulphosalicylic acid and then centrifuged at 5000 rpm for 15 min, and the supernatant was taken for proline estimation. Proline content in the supernatant was determined using the method described by Bates *et al.* (1973) and quantified on fresh weight basis ($mg\ g^{-1}\ FW$).

After weighing the sample for proline content, the remaining portions of the leaves were used for estimation of RWC. After measuring the fresh weight, leaf

samples were cut into small pieces and submerged in water for 8 h for soaking. Then, the turgid weight was measured after blotting dry. These samples were oven-dried at 70°C for 48 h and their dry weight was recorded. RWC was estimated using the following formula given by Weatherley (1950):

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

where RWC is the relative water content, FW is the leaf fresh weight, DW is the leaf dry weight, and TW is the turgid leaf weight.

2.4 Antioxidant enzyme activities

For estimation of antioxidants [ascorbate peroxidase (APX), catalase (CAT), and guaiacol peroxidase (GPOX)], fully expanded fresh leaves from the tops of the plants were again sampled at the reproductive stage. The fresh leaf tissues (300 mg) were ground in 3.0 mL phosphate buffer (0.1 M, pH 7) and centrifuged at 10000 rpm (4°C, 15 min). The supernatant was collected as an extract for the estimation of antioxidant enzymes. APX activity with enzyme classification 1.11.1.11 was estimated as a decrease in optical density of the reaction mixture by ascorbate at 290 nm in a UV spectrophotometer (Nakano and Asada 1981). Reaction mixture (3 mL) was prepared by adding L-ascorbate (0.15 mL, 15 mM), H₂O₂ (0.15 mL, 10 mM), and potassium phosphate buffer (2.7 mL, 0.1 M, pH 7.0). Activity was initiated by adding 50 µL of extract and the reduction in optical density due to ascorbate was measured at 290 nm using a UV spectrophotometer for 3 min at 15 s interval. The activity in terms of APX needed to oxidize 1 nmol of ascorbate min⁻¹ was expressed as enzyme unit (EU) g⁻¹ fresh weight (FW) using an extinction coefficient of 2.8 mM⁻¹ cm⁻¹.

Activity of CAT (enzyme classification 1.11.1.6) was estimated from the measurement of the decomposition of H₂O₂ (Aebi 1984). The reaction mixture contained 2 mL potassium phosphate buffer (0.1 M, pH 7), 50 µL enzyme extract, and 0.95 mL hydrogen peroxide (15 mM). The decrement in absorbance of reaction mixture was recorded at 240 nm for 3 min. The activity of CAT as decomposition of 1 mM H₂O₂ mL⁻¹ min⁻¹ was expressed as EU g⁻¹ FW using an extinction coefficient of 36 M⁻¹ cm⁻¹. GPOX activity (enzyme classification 1.11.1.7) was estimated from the increase of optical density due to the formation of tetra-guaiacol at 470 nm (Castillo et al. 1984). The reaction was initiated by adding potassium phosphate buffer (2.5 mL,

100 mM, pH 7), guaiacol (0.6 mL, 1%), and enzyme extract (0.1 mL), and the increment in absorbance was measured at 470 nm for 3 min at 15 s intervals. GPOX activity, as the amount needed to oxidize 1 nmol of guaiacol min⁻¹ mL⁻¹, was expressed as EU g⁻¹ FW using the molar extinction coefficient of 26.6 mM⁻¹ cm⁻¹.

2.5 Ionic content

Na⁺ and K⁺ contents in the above-ground plant portion (excluding root and grains) were estimated at the maturity stage of each germplasm. Oven-dried plant samples were ground individually into fine powder and 0.5 g of ground sample was further digested in a di-acid mixture (HNO₃:HClO₄ in 10:3 ratio). The digested plant sample was then diluted to 100 mL with double-distilled water and Na⁺ and K⁺ concentrations were measured using a flame photometer (PFP7, Jenway, Bibby Scientific, UK).

2.6 Growth and yield

Plant height of germplasm lines was measured at the vegetative, reproductive, and maturity stages. At the vegetative stage (principal growth stage 4), plants were measured from the base of plant at soil level to the uppermost part of the plant. At maturity, the plants were measured in cm from the soil level to the tip of the primary panicle (Stanschewski et al. 2021). The number of branches on the main axis of each germplasm line were counted at the end of principal growth stage 6 (flowering). The length of the main panicle on each germplasm was measured in cm from base of the panicle to the end. The number of significant panicles on lateral branches which contributed to seed yield was counted at the maturity stage of each germplasm. The seeds from each plant were separated manually and weighed for seed weight per plant. For estimating 1000-grain weight (TW), seeds from each germplasm were counted using a seed counter (WAVER IC-VAI, AIDEX Co. Ltd., Japan) and the weight was recorded in grams.

2.7 Root and shoot dry weight

After harvesting grains from all panicles of every plant, the pots were watered and whole plants were uprooted. After uprooting, the roots were cut from the base. The

shoot and roots were then oven-dried separately for recording root and shoot dry biomass.

2.8 Salt tolerance indices

To assess the relative performance of the studied germplasms across the salinity treatments, different salt tolerance indices were calculated as follows:

Stability index (SI) = Y_s/Y_{ns} (Bousslama and Schapagha 1984)

Yield index (YI) = Y_s/\bar{Y}_s (Gavuzzi *et al.* 1997)

Stress tolerance index (STI) = $Y_{ns} \times Y_s/\bar{Y}_{ns}^2$ (Fernandez 1992)

Stress susceptibility index (SSI) = $(1 - Y_s/Y_{ns})/SI$; $SI = 1 - \bar{Y}_s/\bar{Y}_{ns}$ (Fischer and Maurer, 1978)

where Y_s and Y_{ns} are the grain yields of germplasms grown under saline and non-saline (BAW) treatments, respectively. \bar{Y}_s and \bar{Y}_{ns} are the average yields of all germplasms evaluated under saline (at 8, 16, and 24 dSm⁻¹ levels of irrigation water salinity) and non-saline (BAW) conditions, respectively. Salt tolerance indices were calculated for each salinity level and averaged at the germplasm level to delineate the index value of a germplasm across salinity treatments.

2.9 Statistical analysis

The growth, physiological, biochemical, and yield data were subjected to two-way analysis of variance (ANOVA) in factorial completely randomized design (FCRD) (Gomez and Gomez 1984). Analysis of data was performed using general linear model (GLM) procedure in the R software (R Core Team 2021) and treatment means were separated using Duncan's multiple range test ($p \leq 0.05$).

Principal component analysis (PCA) was employed using the 'factoextra' and 'FactoMineR' packages in the R software to determine the pattern of variability in the studied traits of quinoa germplasm under salinity levels and to identify contrasting germplasms using mean values of all the studied traits data at different salinity treatments. Data scaling was adopted before PCA to standardise the data to have zero mean and unit variance. Dimension reduction in data was done based on covariance-based factor loadings and eigenvalues with varimax rotation. Biplots for the first two important components were generated to determine the contrasting germplasm groups based on their projections on the biplot axes and the variables significantly

contributing to the variation. Germplasm ranking was then done by generating the factor scores of germplasms based on factor loadings of each variable. Heat map-based clustering was performed to identify diverse germplasm groups across salinity treatments using the R software. Pearson's correlation matrix was used to determine the degree of interrelationship among important traits.

3. Results

3.1 Physiological traits

It was evident that at the vegetative stage of quinoa germplasms, the relative chlorophyll content measured in terms of SPAD values did not vary significantly under all salinity levels (figure 1A), while at the reproductive stage, the SPAD values were significantly ($p < 0.05$) higher at salinity levels of EC_{iw} 16 and 24 dSm⁻¹ as compared with those under EC_{iw} 8 dSm⁻¹ and BAW treatments (figure 1A). The leaves at these salinity levels appeared dark green and fleshy as compared with BAW and EC_{iw} 8 dSm⁻¹.

Among the studied quinoa germplasms, EC507746 recorded the highest SPAD value (45.7), followed by the germplasms EC507740 (45.4) and CSQ 1 (45.3) at the vegetative stage (figure 1A). At the reproductive stage, significantly higher SPAD values were observed in germplasm EC507740 (51.5) followed by EC507746 (50.2) and EC507742 (48.4) compared with the remaining germplasms.

Proline content, an indicator of adjustment to abiotic stress, increased significantly ($p < 0.01$) with gradual increase in salinity levels up to EC_{iw} 24 dSm⁻¹ in all germplasms (table 1). The values of proline content ranged between 17.0 to 32.2 and 32.0 to 163.7 mg g⁻¹ FW at the vegetative and reproductive stages, respectively. A significant ($p < 0.05$) salinity and germplasm interaction was observed at both growth stages, which showed that germplasms EC507738 and CSQ2 recorded the highest proline content (32.2 and 163.7 mg g⁻¹ FW) with a salinity level of EC_{iw} 24 dSm⁻¹ at the vegetative and reproductive stages, respectively.

The relative water content (RWC) in leaves of quinoa germplasms showed a declining trend with increasing salinity levels in most germplasms (table 2). However, the germplasms did not exhibit a significant difference in RWC at the vegetative stage. The salinity and germplasm interaction was significant ($p < 0.05$) at the reproductive stage and the germplasm CSQ2 exhibited highest RWC (83.7%) with BAW irrigation. The data

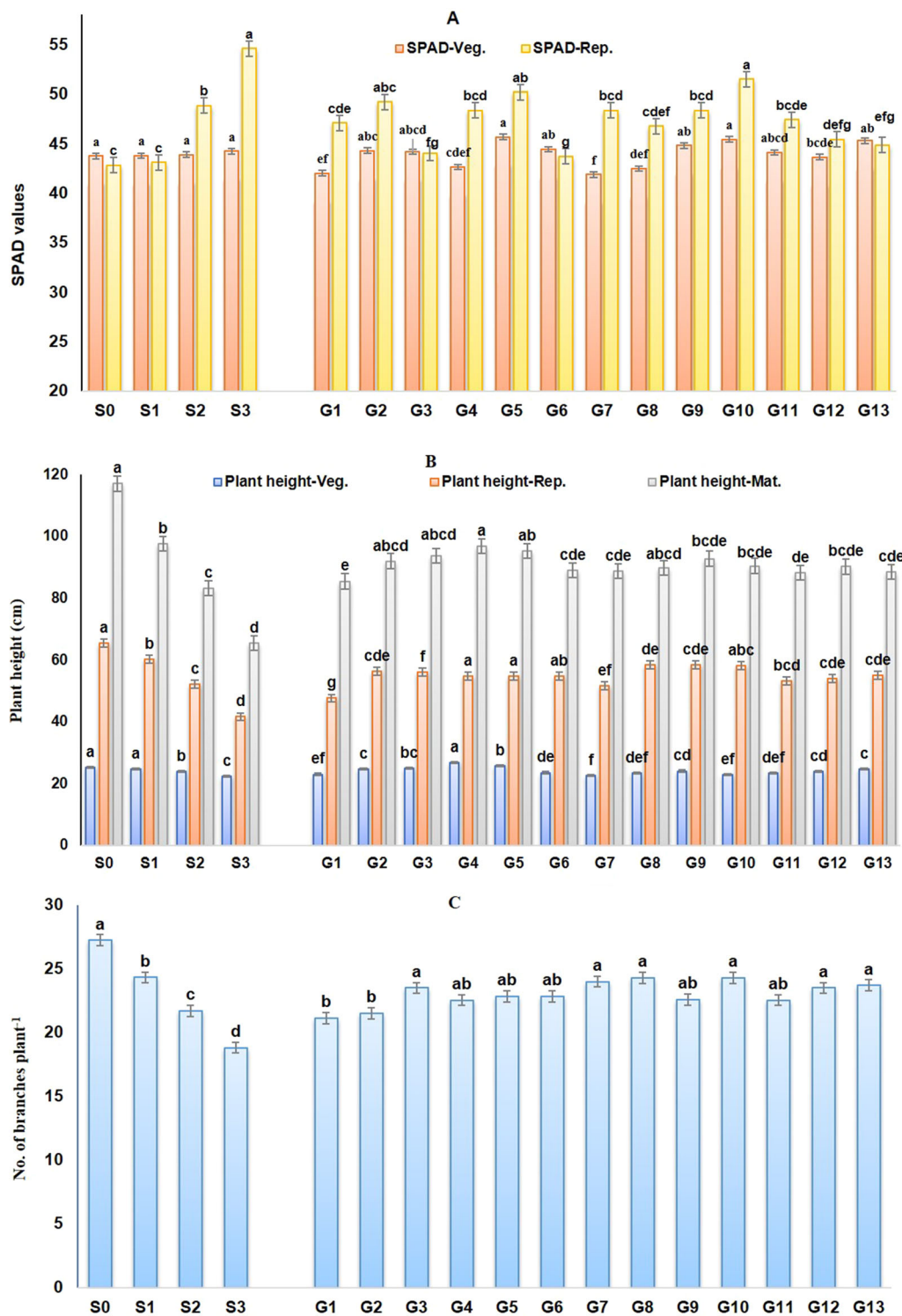


Figure 1. Effect of salinity levels on SPAD values (A), plant height (B) and number of branches per plant (C) of quinoa germplasm. Error bars indicate SEM. Bars with different lowercase letters are significantly different using DMRT ($p \leq 0.05$). S0: BAW; S1: EC_{iw} 8 dSm⁻¹; S2: EC_{iw} 16 dSm⁻¹; S3: EC_{iw} 24 dSm⁻¹; G1: EC507744; G2: EC507742; G3: IC411824; G4: EC507741; G5: EC507746; G6: EC507738; G7: EC507748; G8: EC507739; G9: IC411824-1; G10: EC507740; G11: IC411825; G12: CSQ1; G13: CSQ2.

Table 1. Effect of various salinity levels on proline content in leaves of quinoa germplasms

Salinity	Germplasm													Mean
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	
Proline content (mg g ⁻¹ FW) at vegetative stage														
S0	21.0	24.0	23.0	17.0	25.1	23.5	22.2	26.2	17.8	19.2	16.0	21.5	23.2	21.5 ^d
S1	22.0	26.0	23.3	19.2	26.8	25.1	22.4	27.0	18.0	19.4	16.2	22.0	23.5	22.4 ^c
S2	24.0	28.1	24.0	20.5	27.8	26.0	23.8	28.2	19.2	22.6	17.8	24.3	26.5	24.1 ^b
S3	26.2	23.0	26.0	24.0	30.2	26.8	24.6	32.2	23.1	26.8	20.5	27.8	28.2	26.1 ^a
Mean	23.3 ^d	25.3 ^c	24.1 ^f	20.2 ^b	27.5 ^c	25.4 ^b	23.3 ^d	28.4 ^a	19.5 ^f	22.0 ^e	17.6 ^g	23.9 ^d	25.4 ^c	
<i>p</i> -Level	Salinity (S)				Germplasm (G)				S×G					
	<0.0001				<0.0001				<0.05					
Proline content (mg g ⁻¹ FW) at reproductive stage														
S0	40.4	32.0	32.5	37.7	33.7	37.8	33.0	32.3	39.2	39.7	37.5	40.8	42.1	36.8 ^c
S1	54.1	34.9	31.2	45.2	44.8	44.8	43.4	44.6	54.1	50.5	49.5	41.2	43.5	44.8 ^c
S2	77.7	67.4	76.6	59.9	53.4	73.1	48.1	34.8	84.7	85.1	52.6	69.1	59.8	64.8 ^b
S3	135.6	79.7	67.4	110.4	97.2	145.7	58.5	64.6	110.0	91.3	61.0	147.5	163.7	102.5 ^a
Mean	77.0 ^a	53.5 ^{abcd}	52.0 ^{bcd}	63.3 ^{abcd}	57.3 ^{abcd}	75.4 ^{ab}	45.8 ^{cd}	44.1 ^d	72.0 ^{abc}	66.7 ^{abcd}	50.1 ^{cd}	74.7 ^{abc}	77.3 ^a	
<i>p</i> -Level	Salinity (S)				Germplasm (G)				S×G					
	<0.0001				<0.0001				0.001					

Means with different lower-case letters within column or within row are significantly ($p \leq 0.05$) different from each other using DMRT. S0: BAW; S1: EC_{iw} 8 dSm⁻¹; S2: EC_{iw} 16 dSm⁻¹; S3: EC_{iw} 24 dSm⁻¹; G1: EC507744; G2: EC507742; G3: IC411824; G4: EC507741; G5: EC507746; G6: EC507748; G7: EC507738; G8: EC507739; G9: IC411824-1; G10: EC507740; G11: IC411825; G12: CSQ1; G13: CSQ2.

Table 2. Effect of various salinity levels on relative water content in leaves of quinoa germplasms

Salinity	Germplasm													Mean
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	
Relative water content (%) at vegetative stage														
S0	84.2	83.0	86.0	86.1	84.2	84.2	83.5	83.6	84.6	85.8	84.5	85.4	86.1	84.7 ^a
S1	84.3	83.1	86.1	86.0	83.5	84.0	83.4	83.2	84.8	85.5	84.0	85.0	85.7	84.5 ^a
S2	80.2	83.0	84.2	85.5	83.0	83.3	81.7	82.6	82.5	84.2	82.4	83.5	84.1	83.1 ^{ab}
S3	79.0	86.0	82.0	82.2	81.5	81.6	80.1	80.4	81.2	82.9	80.1	83.1	82.5	81.7 ^b
Mean	81.9	83.8	84.6	85.0	83.1	83.3	82.2	82.5	83.3	84.6	82.8	84.3	84.6	
<i>p</i> -Level	S×G													
	0.82													
Relative water content (%) at reproductive stage														
S0	82.8	79.7	81.3	76.8	78.4	77.4	80.0	77.8	82.4	81.5	80.3	83.7	81.0	80.2 ^a
S1	75.6	80.5	79.8	69.5	74.2	76.4	75.0	84.0	74.5	81.3	79.0	74.5	80.1	77.3 ^b
S2	75.7	73.0	79.8	74.8	75.3	75.7	74.9	85.3	73.6	80.3	82.6	75.9	76.7	77.2 ^b
S3	69.7	72.3	75.0	70.9	68.7	75.0	68.7	76.8	70.7	72.7	77.2	74.5	77.7	73.1 ^c
Mean	76.0 ^{abcd}	76.4 ^{abcd}	79.0 ^{abc}	73.0 ^d	74.2 ^{cd}	76.1 ^{abcd}	74.7 ^{cd}	81.0 ^a	75.3 ^{bcd}	79.0 ^{abc}	79.8 ^{ab}	77.2 ^{abcd}	78.9 ^{abc}	
<i>p</i> -Level	S×G													
	0.005													
	Germplasm (G)													
	<0.0001													

Means with different lower-case letters within column or within row are significantly ($p \leq 0.05$) different from each other using DMRT. S0: BAW; S1: EC_{iw} 8 dSm⁻¹; S2: EC_{iw} 16 dSm⁻¹; S3: EC_{iw} 24 dSm⁻¹; G1: EC507744; G2: EC507742; G3: IC411824; G4: EC507741; G5: EC507746; G6: EC507738; G7: EC507748; G8: EC507739; G9: IC411824-1; G10: EC507740; G11: IC411825; G12: CSQ1; G13: CSQ2.

further revealed that at the highest level of salinity (EC_{iw} 24 dSm^{-1}), the germplasm CSQ2 had the highest RWC (77.7%) with only 4% decline over BAW.

3.2 Antioxidant enzyme activities

The activities of three antioxidant enzymes (APX, CAT, and GPOX) were significantly affected due to salinity and germplasm while their interaction was observed as non-significant. It was evident that while the activity of the three enzymes was unaffected due to application of EC_{iw} 8 dSm^{-1} , the salinity levels of EC_{iw} 16 and 24 dSm^{-1} significantly increased as compared with BAW and EC_{iw} 8 dSm^{-1} (figure 2A–C). The difference between EC_{iw} 16 and 24 dSm^{-1} salinity levels was also significant for APX, CAT, and GPOX values.

The studied germplasms also differed significantly in antioxidant activity (figure 2A–C). The activities of APX and CAT were estimated to be maximum in germplasm CSQ2 (40.28 and 3.34 $EU\ g^{-1}\ FW$) followed by germplasm EC507740 (39.05 and 3.19 $EU\ g^{-1}\ FW$), whereas the activity of the GPOX enzyme was higher in EC507740 (3.96 $EU\ g^{-1}\ FW$), CSQ2 (3.89 $EU\ g^{-1}\ FW$), and CSQ1 (3.89 $EU\ g^{-1}\ FW$) than the remaining germplasms.

3.3 Growth and yield

The data on plant height as affected by salinity levels at various growth stages of quinoa germplasms are depicted in figure 1B. At the vegetative stage, a non-significant difference was observed among BAW and EC_{iw} 8 dSm^{-1} treatments, whereas a significant reduction in plant height with increasing salinity levels was noticed at the reproductive and maturity stages. At the maturity stage, plant height was reduced by 16.7, 28.9, and 44.1% under 8, 16, and EC_{iw} 24 dSm^{-1} salinity levels, respectively. Among the quinoa germplasms, the tallest plants were measured in germplasm EC507741 at all growth stages. At highest salinity stress (EC_{iw} 24 dSm^{-1}), the extent of reduction in plant height was minimum in germplasm EC507740 (32.1%) over BAW.

Application of increasing salinity levels adversely affected the yield parameters of all quinoa germplasms. The number of branches $plant^{-1}$ were reduced by 10.7, 22.2, and 30.9% at EC_{iw} 8, 16, and 24 dSm^{-1} ,

respectively, as compared with that observed under BAW (figure 1C). Among the germplasms, EC507740 recorded the highest number of branches $plant^{-1}$ (24.3). All other germplasms also showed statistically similar number of branches $plant^{-1}$ to that of the aforesaid germplasm, with the exception of EC507744 (21.5) and EC507742 (21.1), which were significantly lower than EC507740.

The combined effect of salinity and germplasm was significant for number of panicles ($p<0.01$), length of panicle ($p<0.01$), and 1000-grain weight ($p<0.05$). It was evident from the interaction effect that at the highest salinity level of EC_{iw} 24 dSm^{-1} , germplasm EC507740 showed the highest number of panicles $plant^{-1}$ (19.8) and 1000-grain weight (2.1 g) (table 3) compared with those of other germplasms. The length of the main panicle was the highest in germplasm CSQ2 (8.0 cm) with BAW irrigation treatment. On the other hand, with the highest stress level of EC_{iw} 24 dSm^{-1} , germplasm EC507739 showed superiority over the others with respect to the length of the main panicle.

Root and shoot dry weights were significantly affected by salinity levels, germplasm, and their interaction. Each incremental salinity level caused marked reduction ($p<0.01$) in root and shoot dry weights of all the quinoa germplasms over BAW (table 4). Germplasms EC507741 and EC507738 recorded the highest root dry weight (1.98 g), while germplasm EC507738 recorded the highest shoot dry weight (11.47 g) with BAW irrigation. On the contrary, at the highest level of salinity (EC_{iw} 24 dSm^{-1}), germplasm EC507740 recorded maximum root dry weight $plant^{-1}$ (0.95 g), followed by germplasm CSQ2 (0.89 g). Germplasm EC507742 recorded the highest shoot dry weight (5.63 g), followed by germplasm EC507740 (5.44 g) at EC_{iw} 24 dSm^{-1} .

Variable response of quinoa germplasms was observed due to salinity levels on grain yield (table 4). There was significant effect of salinity ($p<0.001$) and germplasm ($p=0.001$) but not their interaction ($p=0.09$). A significant reduction in grain yield $plant^{-1}$ of quinoa germplasms was noted with incremental salinity levels. At BAW irrigation, germplasms EC507740 and CSQ2 produced the highest grain yield (14.1 $g\ plant^{-1}$), followed by IC411824-1 (13.6 $g\ plant^{-1}$). At the highest stress level of EC_{iw} 24 dSm^{-1} , germplasm EC507740 recorded the highest grain yield (7.0 $g\ plant^{-1}$), followed by CSQ1 (6.2 $g\ plant^{-1}$) and CSQ2 (5.7 $g\ plant^{-1}$).

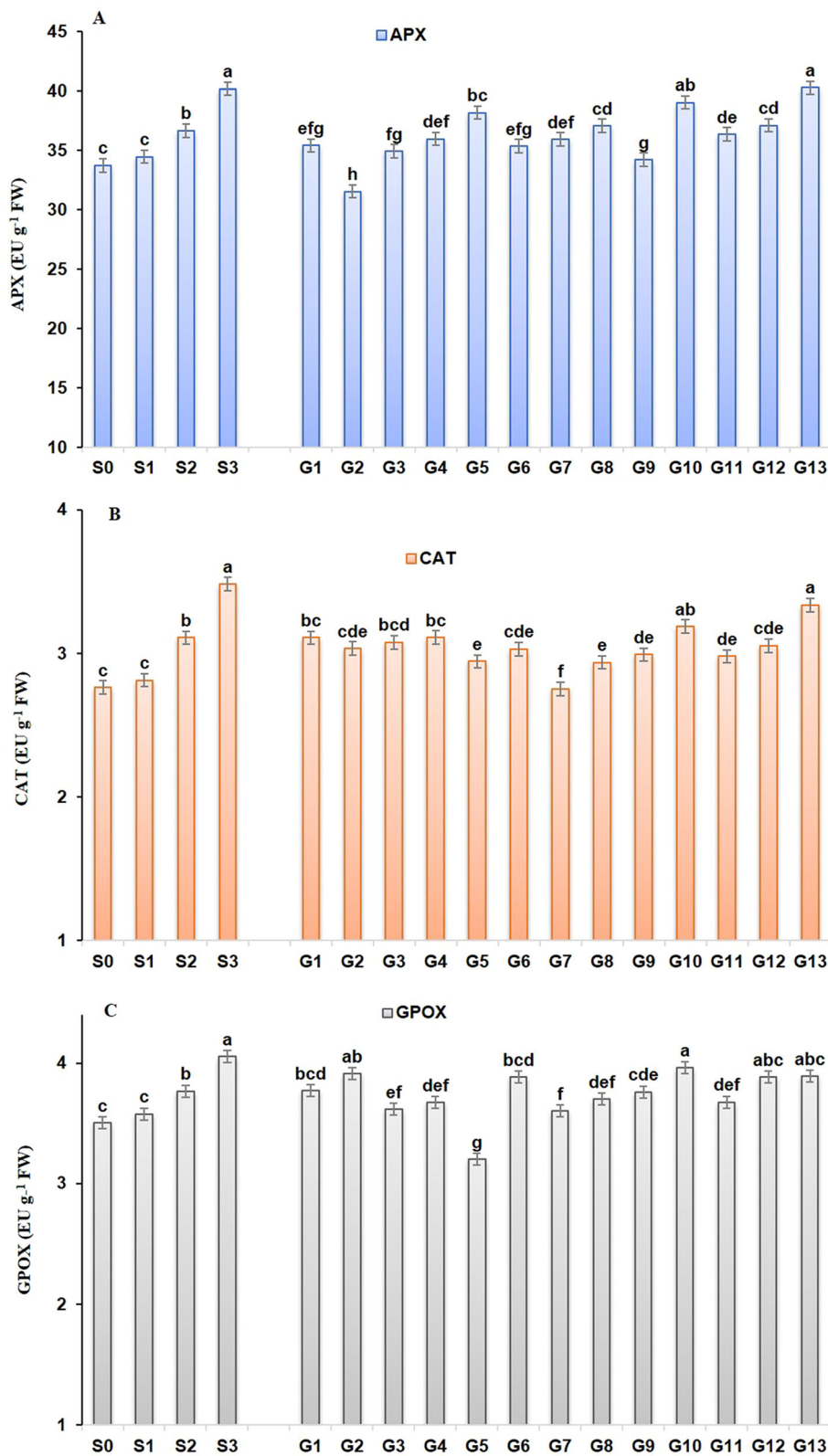


Figure 2. Effect of salinity levels on antioxidant enzyme activities of ascorbate peroxidase (A), catalase (B), and guaiacol peroxidase (C) of quinoa germplasm. Error bars indicate SEM. Bars with different lowercase letters are significantly different using DMRT ($p \leq 0.05$). S0: BAW; S1: EC_{iw} 8 dSm⁻¹; S2: EC_{iw} 16 dSm⁻¹; S3: EC_{iw} 24 dS m⁻¹; G1: EC507744; G2: EC507742; G3: IC411824; G4: EC507741; G5: EC507746; G6: EC507738; G7: EC507748; G8: EC507739; G9: IC411824-1; G10: EC507740; G11: IC411825; G12: CSQ1; G13: CSQ2.

3.4 Plant ionic content

Accumulation of Na^+ and K^+ in quinoa germplasms showed marked increase with increasing levels of salinity (table 5). The Na^+ content in plants ranged between 0.20% and 2.54% across salinity levels. Similarly, the corresponding values for K^+ content were 2.7–4.7%. At the highest level of salinity ($\text{EC}_{\text{iw}} 24 \text{ dSm}^{-1}$), germplasm EC507744 recorded the highest Na^+ content while germplasm CSQ2 accumulated the lowest Na^+ in plants as compared with the remaining germplasms.

The accumulation of K^+ also showed an increasing trend with the increasing salinity levels in most of the germplasms (table 5). The highest accumulation of K^+ (4.4%) was associated with $\text{EC}_{\text{iw}} 24 \text{ dSm}^{-1}$ in germplasm EC507738. Furthermore, the magnitude of increase in ion accumulation as a result of increasing salinity stress was relatively higher in the case of Na^+ than that of K^+ , resulting in decline of the K^+/Na^+ ratio of plants. The maximum K^+/Na^+ ratio was found in the case of the BAW treatment in all germplasms. At the highest level of salinity, germplasm EC507738 showed the highest K^+/Na^+ ratio (2.5), followed by germplasm CSQ2 (2.4).

3.5 Salt tolerance indices

To find the most tolerant germplasm across salinity levels, various salinity tolerance indices were calculated, which are depicted in figure 3. As can be seen in the figure, the stability index was maximum in germplasm EC507739 (0.68) followed by germplasms EC507740 (0.62), CSQ2 (0.60), and IC411825 (0.59). The maximum values for yield index were obtained in germplasm EC507740 (1.31) followed by CSQ1 (1.18), CSQ2 (1.17), and EC507739 (1.06). The order of germplasms with respect to stress tolerance index was $\text{EC507740} (0.76) > \text{CSQ2} (0.70) > \text{CSQ1} (0.64)$. The values of salt susceptibility index were highest in germplasms EC507742 (1.24), EC507738 (1.20), and IC411824-1, indicating the more susceptible germplasms. In agreement with the stability index data, the minimum values of the salt susceptibility index were recorded for germplasms EC507739 (0.64), IC411825 (0.80), and EC507740 (0.87).

3.6 Principal component and cluster analysis

Principal component analysis was performed for all 18 traits separately under different salinity levels. Two PCAs were performed, PCA-I with all 18 traits (figure 4)

and PCA-II with 12 traits (supplementary figure 1) after removing the 6 redundant traits. The correlation matrix (figure 6) among all the traits showed that some of the traits were highly correlated. Na, TW, PL, RDW, and PH were highly correlated with grain yield ($r > 0.8$). Similarly, CAT activity also showed high correlation with PROL ($r = 0.84$). Therefore, these 6 traits were considered redundant as these may influence the result of the PCA. The PCA biplot of the first two components showed a scattered pattern of germplasms over all the four quarters, indicating diverse germplasm groups (figure 4 and supplementary figure 1).

The PCA-I biplot of the first two components of BAW, $\text{EC}_{\text{iw}} 8 \text{ dSm}^{-1}$, 16 dSm^{-1} , and 24 dSm^{-1} salinity levels had eigenvalues greater than 1 and accounted for 47.21, 42.19, 48.08, and 44.56% cumulative variability, respectively (supplementary table 4). Similarly, a cumulative variability of 49.8, 44.3, 54.2, and 47.9 was explained by PCA-II biplots at BAW, $\text{EC}_{\text{iw}} 8 \text{ dSm}^{-1}$, $\text{EC}_{\text{iw}} 16 \text{ dSm}^{-1}$, and $\text{EC}_{\text{iw}} 24 \text{ dSm}^{-1}$ treatments, respectively (supplementary table 5).

The germplasms were ranked for their tolerance or sensitivity to salt stress based on factor scores obtained by considering the value of each trait of PCA-I (table 6). The factor scores predicted that germplasms G13 (CSQ2) and G10 (EC507740) were found to be most tolerant germplasms at all salinity levels. These two germplasms had maximum values on the x-axis of the PCA-I biplot. Germplasm G5 (EC507746) was ranked as the most sensitive germplasm at $\text{EC}_{\text{iw}} 8 \text{ dSm}^{-1}$, while germplasm G2 (EC507742) was found to be as most sensitive at $\text{EC}_{\text{iw}} 16$ and 24 dSm^{-1} salinity levels. The biplot indicated that the sensitive germplasms were projected on opposite sites of the tolerant germplasms. A similar projection of the germplasms was also obtained from PCA-II (supplementary figure 1), therefore validating the ranking obtained with PCA-I.

A heat map-based similarity matrix was generated from the quantitative traits data. The cluster analysis classified the studied germplasms into five diverse groups, clearly distinguished with a coefficient of 0.81 (figure 5). The first cluster comprised germplasms G10 (EC507740) and G13 (CSQ2). The second group constituted two germplasms, namely, G1 (EC507744) and G6 (EC507744). Germplasms G5 (EC507746), G7 (EC507748), and G9 (IC411824-1) were categorised in the third cluster. The fourth cluster included two germplasms, G8 (EC507739) and G12 (CSQ1), while the fifth cluster included four germplasms, G2 (EC507742), G4 (EC507741), G3 (IC411824), and G11 (IC411825). The most tolerant germplasms, G13 and G10, were classified in the same group.

Table 3. Effect of various salinity levels on yield parameters of quinoa germplasms

Salinity	Germplasm											Mean		
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11		G12	G13
	Number of panicles plant ⁻¹													
S0	26.5	24.7	27.8	25.3	23.5	24.8	22.8	24.2	21.0	26.3	22.0	23.8	24.3	24.4 ^a
S1	21.2	20.5	22.2	17.8	21.2	21.7	21.7	22.3	20.8	22.2	20.5	22.3	22.3	21.3 ^b
S2	20.8	18.8	19.0	16.7	20.0	18.7	20.8	21.7	19.5	21.2	19.8	21.0	21.3	19.9 ^c
S3	19.7	16.3	17.0	15.8	18.8	17.3	19.8	19.0	18.0	19.8	17.3	19.7	19.3	18.3 ^d
Mean	22.0 ^{ab}	20.0 ^d	21.5 ^{abc}	18.9 ^e	20.9 ^{cd}	20.6 ^{cd}	21.3 ^{abc}	21.8 ^{abc}	19.8 ^d	22.4 ^a	19.9 ^d	21.7 ^{abc}	21.8 ^{abc}	
<i>p</i> -Level	Salinity (S)	Germplasm (G)											S × G	
	<0.0001	<0.0001											<0.0001	
	Length of main panicle (cm)													
S0	5.8	5.7	5.5	6.1	4.5	6.4	7.9	6.2	6.3	7.0	5.6	6.8	8.0	6.3 ^a
S1	5.1	5.2	4.0	5.2	3.8	6.2	5.5	5.5	5.8	6.1	4.8	5.2	6.2	5.3 ^b
S2	4.1	4.1	3.7	4.7	3.6	5.4	3.9	4.9	5.3	5.6	4.2	4.0	5.7	4.5 ^c
S3	2.2	3.4	3.1	3.1	3.2	2.9	2.9	4.5	3.1	3.5	2.9	3.4	3.1	3.2 ^d
Mean	4.3 ^h	4.6 ^{fg}	4.1 ^{hi}	4.8 ^{ef}	3.8 ⁱ	5.2 ^{bc}	5.0 ^{cde}	5.3 ^{bc}	5.1 ^d	5.6 ^{abc}	4.4 ^{gh}	4.8 ^{def}	5.7 ^a	
<i>p</i> -Level	Salinity (S)	Germplasm (G)											S × G	
	<0.0001	<0.0001											<0.0001	
	1000-grain weight (g)													
S0	2.5	2.7	2.5	2.3	2.5	2.6	2.8	2.9	2.4	3.3	3.4	3.0	2.9	2.7 ^a
S1	2.2	2.1	2.2	2.1	2.2	2.5	2.6	2.6	2.3	3.1	3.1	2.7	2.6	2.5 ^b
S2	2.1	2.0	1.9	1.8	2.0	2.2	2.5	2.5	1.8	2.6	2.6	2.3	2.4	2.2 ^c
S3	1.8	1.7	1.7	1.3	1.5	1.8	1.8	2.0	1.3	2.1	2.0	1.9	1.9	1.8 ^d
Mean	2.2 ^{de}	2.1 ^e	2.1 ^{ef}	1.9 ^g	2.1 ^{ef}	2.3 ^{cd}	2.4 ^{bc}	2.5 ^b	2.0 ^{fg}	2.8 ^a	2.7 ^a	2.5 ^b	2.5 ^b	
<i>p</i> -Level	Salinity (S)	Germplasm (G)											S × G	
	<0.0001	<0.0001											0.03	

Means with different lower-case letters within column or within row are significantly ($p \leq 0.05$) different from each other using DMRT. S0: BAW; S1: EC_{iw} 8 dSm⁻¹; S2: EC_{iw} 16 dSm⁻¹; S3: EC_{iw} 24 dSm⁻¹; G1: EC507744; G2: EC507742; G3: IC411824; G4: EC507741; G5: EC507746; G6: EC507738; G7: EC507748; G8: EC507739; G9: IC411824-1; G10: EC507740; G11: IC411825; G12: CSQ1; G13: CSQ2.

Table 4. Effect of various salinity levels on root and shoot dry weight of quinoa germplasms

Salinity	Germplasm													Mean
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	
	Root dry weight (g plant ⁻¹)													
S0	1.75	1.76	1.62	1.98	1.66	1.96	1.98	1.67	1.75	1.95	1.61	1.53	1.78	1.77 ^a
S1	1.51	1.30	1.42	1.50	1.26	1.69	1.43	1.28	1.34	1.46	1.42	1.10	1.57	1.41 ^b
S2	0.99	1.13	1.18	1.14	0.94	1.22	1.10	0.94	1.17	1.09	1.07	0.95	1.14	1.08 ^c
S3	0.71	0.64	0.58	0.79	0.64	0.76	0.80	0.66	0.83	0.95	0.61	0.54	0.89	0.72 ^d
Mean	1.24 ^{cd}	1.21 ^{cde}	1.20 ^{cde}	1.35 ^{ab}	1.13 ^e	1.41 ^a	1.33 ^{ab}	1.14 ^e	1.27 ^{bc}	1.36 ^a	1.18 ^{de}	1.03 ^f	1.34 ^{ab}	
<i>p</i> -Level	S×G													
	<0.0001													
	Shoot dry weight (g plant ⁻¹)													
S0	7.79	8.40	7.64	6.86	8.23	11.47	7.04	5.74	6.56	9.18	7.54	6.46	7.65	7.7a
S1	6.86	6.48	6.75	6.04	5.76	9.74	6.05	5.13	5.09	7.00	7.26	5.71	7.29	6.55 ^b
S2	4.84	6.67	5.48	5.39	5.66	5.85	6.00	4.32	4.12	5.98	4.93	4.80	6.14	5.40 ^c
S3	4.33	5.63	4.62	5.07	4.98	4.45	4.62	3.24	2.91	5.44	4.98	3.02	5.25	4.50 ^d
Mean	5.95 ^d	6.79 ^b	6.12 ^{cd}	5.84 ^d	6.16 ^{cd}	7.88 ^a	5.93 ^d	4.61 ^e	4.67 ^e	6.90 ^b	6.18 ^{cd}	5.00 ^e	6.58 ^{bc}	
<i>p</i> -Level	S×G													
	<0.0001													
	Grains weight (g plant ⁻¹)													
S0	12.4	13.2	13.1	11.6	12.3	12.2	12.0	10.8	13.6	14.1	11.9	13.1	14.1	12.7 ^a
S1	8.5	8.4	9.5	8.8	8.4	7.3	9.7	9.5	8.8	10.0	10.1	8.3	10.2	9.0 ^b
S2	6.1	5.1	7.3	6.3	6.4	6.3	6.9	7.7	7.0	9.0	6.5	8.9	7.9	7.0 ^c
S3	4.3	4.2	3.8	3.9	3.7	3.5	4.1	4.7	3.5	7.0	4.4	6.2	5.7	4.6 ^d
Mean	7.8 ^{cd}	7.7 ^d	8.4 ^{bcd}	7.7 ^d	7.7 ^d	7.3 ^d	8.2 ^{bcd}	8.2 ^{bcd}	8.2 ^{BCcd}	10.0 ^a	8.2 ^{bcd}	9.1 ^{abc}	9.5 ^{ab}	
<i>p</i> -Level	S×G													
	0.09													

Means with different lower-case letters within column or within row are significantly ($p \leq 0.05$) different from each other using DMRT. S0: BAW; S1: EC_{iw} 8 dSm⁻¹; S2: EC_{iw} 16 dSm⁻¹; S3: EC_{iw} 24 dSm⁻¹; G1: EC507744; G2: EC507742; G3: IC411824; G4: EC507741; G5: EC507746; G6: EC507738; G7: EC507748; G8: EC507739; G9: IC411824-1; G10: EC507740; G11: IC411825; G12: CSQ1; G13: CSQ2.

Table 5. Effect of various salinity levels on plant ionic content of quinoa germplasms

Salinity	Germplasm													Mean
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	
S0	0.53	0.56	0.65	0.48	0.45	0.34	0.25	0.24	0.20	0.35	0.33	0.25	0.30	0.38 ^d
S1	1.35	1.08	1.05	0.96	1.14	1.09	1.01	1.03	0.83	1.02	0.99	1.04	0.99	1.05 ^c
S2	1.90	1.51	1.30	1.32	1.54	1.49	1.51	1.49	1.25	1.32	1.32	1.23	1.05	1.40 ^b
S3	2.49	2.54	2.51	2.40	2.42	1.85	1.92	2.34	1.92	2.00	2.35	2.15	1.74	2.20 ^a
Mean	1.57 ^a	1.42 ^{ab}	1.38 ^b	1.29 ^{bc}	1.39 ^{ab}	1.19 ^{cd}	1.17 ^{cd}	1.28 ^{bc}	1.05 ^d	1.17 ^{cd}	1.25 ^{bc}	1.17 ^{cd}	1.02 ^d	
<i>p</i> -Level	Salinity (S)				Germplasm (G)				S×G					
	<0.0001				<0.0001				<0.0001					
	K ⁺ content (%)													
S0	3.6	3.4	2.8	3.1	3.0	3.3	2.7	3.4	3.2	3.1	3.0	3.3	3.1	3.15 ^d
S1	3.7	3.9	3.2	3.4	3.4	3.7	3.3	3.5	3.7	3.7	3.3	3.1	3.7	3.49 ^c
S2	3.8	4.3	3.6	3.7	3.5	4.4	3.6	3.6	3.8	3.9	3.6	3.7	3.8	3.80 ^b
S3	4.2	3.5	4.0	3.7	3.9	4.7	4.3	4.3	4.1	4.2	3.7	3.9	4.3	4.05 ^a
Mean	3.81 ^{ab}	3.78 ^{ab}	3.40 ^d	3.45 ^{cd}	3.46 ^{cd}	4.02 ^a	3.47 ^{cd}	3.69 ^{bc}	3.72 ^{bc}	3.72 ^{bc}	3.40 ^d	3.47 ^{cd}	3.71 ^{bc}	
<i>p</i> -Level	Salinity (S)				Germplasm (G)				S×G					
	<0.0001				<0.0001				<0.0001					
	K ⁺ /Na ⁺ ratio													
S0	6.7	6.1	4.3	6.5	6.6	9.7	10.8	13.8	16.5	8.9	9.1	13.4	10.4	9.46 ^a
S1	2.7	3.6	3.1	3.5	3.0	3.4	3.2	3.4	4.4	3.6	3.3	2.9	3.7	3.38 ^b
S2	2.0	2.9	2.8	2.8	2.3	3.0	2.4	2.5	3.1	2.9	2.7	3.0	3.6	2.76 ^b
S3	1.7	1.4	1.6	1.5	1.6	2.5	2.2	1.8	2.2	2.1	1.6	1.8	2.4	1.88 ^c
Mean	3.28 ^{bc}	3.47 ^{bc}	2.93 ^c	3.58 ^{bc}	3.39 ^{bc}	4.65 ^{abc}	4.67 ^{abc}	5.36 ^{ab}	6.54 ^a	4.39 ^{bc}	4.18 ^{bc}	5.29 ^{ab}	5.05 ^{abc}	
<i>p</i> -Level	Salinity (S)				Germplasm (G)				S×G					
	<0.0001				<0.0001				<0.0001					

Means with different lower-case letters within column or within row are significantly ($p \leq 0.05$) different from each other using DMRT. S0: BAW; S1: EC_{iw}, 8 dSm⁻¹; S2: EC_{iw}, 16 dSm⁻¹; S3: EC_{iw}, 24 dSm⁻¹; G1: EC507744; G2: EC507742; G3: IC411824; G4: EC507741; G5: EC507746; G6: EC507738; G7: EC507748; G8: EC507739; G9: IC411824-1; G10: EC507740; G11: IC411825; G12: CSQ1; G13: CSQ2.

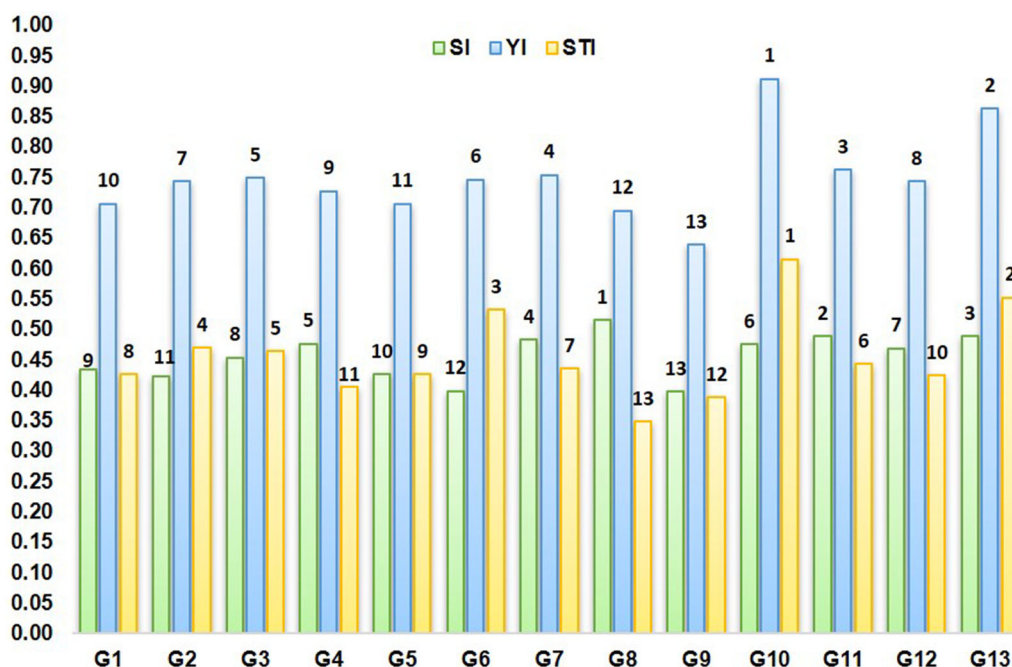


Figure 3. Salt tolerance indices (SI: stability index; YI: yield index; STI: stress tolerance index; SSI: stress susceptibility index) of quinoa germplasms across salinity levels. Numbers on the bars indicates the rank of the germplasm under respective indices. G1: EC507744; G2: EC507742; G3: IC411824; G4: EC507741; G5: EC507746; G6: EC507738; G7: EC507748; G8: EC507739; G9: IC411824-1; G10: EC507740; G11: IC411825; G12: CSQ1; G13: CSQ2.

3.7 Interrelationship among traits

To establish the degree of interrelationship among the studied traits, Pearson's correlation matrix was visualized as a heat map (figure 6). The grain yield was positive and significantly correlated with plant height ($r=0.9$), root dry weight ($r=0.88$), 1000-grain weight ($r=0.81$), number of branches plant⁻¹ ($r=0.78$), number of panicles plant⁻¹ ($r=0.78$), K⁺/Na⁺ ratio ($r=0.77$), and RWC ($r=0.67$). The plant Na⁺ content had strong negative correlation with grain yield plant⁻¹ ($r=-0.93$), plant height ($r=-0.93$), root dry weight ($r=-0.90$), number of branches ($r=-0.83$), and panicle length ($r=-0.83$), while plant Na⁺ content showed positive relationship with K⁺ content ($r=0.71$), proline content ($r=0.69$), and SPAD ($r=0.67$). Moreover, all growth traits were significantly and positively correlated with all yield traits.

4. Discussion

As a consequence of any external biotic or abiotic stress, plants tend to survive through suitable modifications of their morphological features and physiological processes. Salinity stress adversely

affects the seedling, vegetative, and reproductive stages of crop plants, thereby affecting growth and yield. The magnitude of damage due to opposing externalities mainly depends on the nature of the crop (tolerant or sensitive), the stage of the crop, and the degree of stress. Even though quinoa is a semi-halophytic crop, its growth and yield are adversely affected by high-SAR salinity stress levels of 8–24 dSm⁻¹.

Salinity stress induced proline accumulation in the leaves of quinoa germplasms, which was substantially high under EC_{iw} 24 dSm⁻¹ (table 1). Higher accumulation of proline in leaf tissues was observed in germplasm CSQ2 followed by CSQ1 at EC_{iw} 24 dSm⁻¹, which indicated higher resilience of these germplasms towards salinity stress. Proline is an important osmoprotector produced by plants in response to drought and salinity stress (Elewa *et al.* 2017) which helps in osmotic adjustment in leaves (Muscolo *et al.* 2016), thereby mitigating the adverse effect of salt stress on plant growth. Salt stress restricts water uptake, which inhibits oxidation of proline, which, in turn, results in high proline accumulation in leaf tissues (Aguilar *et al.* 2003). A high concentration of proline also helps in scavenging reactive oxygen species under high-stress conditions, which results in the formation of more

Table 6. Ranking of quinoa germplasm based on PCA factor scores under various salinity levels

Rank	EC_{iw}		
	8 dS m ⁻¹	16 dS m ⁻¹	24 dS m ⁻¹
1	3.78 (G13)	3.40 (G13)	4.71 (G13)
2	3.36 (G10)	2.61 (G10)	2.57 (G10)
3	1.49 (G8)	1.88 (G8)	2.31 (G6)
4	0.82 (G6)	1.59 (G11)	0.73 (G12)
5	0.66 (G11)	1.48 (G12)	0.07 (G7)
6	0.20 (G12)	1.05 (G3)	0.04 (G9)
7	0.16 (G9)	-0.12 (G5)	0.03 (G8)
8	-0.45 (G3)	-0.48 (G7)	-0.02 (G1)
9	-0.73 (G7)	-1.16 (G9)	-0.97 (G11)
10	-1.44 (G2)	-1.60 (G4)	-1.63 (G5)
11	-1.84 (G4)	-1.77 (G6)	-2.07 (G3)
12	-2.82 (G1)	-3.15 (G1)	-2.38 (G4)
13	-3.19 (G5)	-3.73 (G2)	-3.38 (G2)

G1: EC507744; G2: EC507742; G3: IC411824; G4: EC507741; G5: EC507746; G6: EC507738; G7: EC507748; G8: EC507739; G9: IC411824-1; G10: EC507740; G11: IC411825; G12: CSQ1; G13: CSQ2.

with SPAD readings, as observed by Marenco *et al.* (2009). They observed that reduced leaf water content and increased leaf thickness increased the SPAD

readings in leaves through interference in transmittance. Therefore, the relationship between chlorophyll content and SPAD values needs further study under salinity levels to corroborate the proposed changes in chlorophyll content under our experimental conditions.

Relative water content, an indicator of water deficit in the plant leaves, decreased to the extent of 1.29 to 15.82% under different germplasms across salinity levels. Higher salt concentration in the crop root zone under salinity stress reduces the total soil water potential, which affects plant water uptake, ultimately resulting in leaf water deficit (Razzaghi *et al.* 2015) at elevated salinity levels. However, different germplasms showed discrepancy with respect to RWC. Germplasm CSQ2 showed higher relative water content in leaves compared with the rest of the germplasms at the highest level of salinity with a reduction of only 4%. This indicated its ability to maintain favorable leaf water content required for plant metabolic processes more efficiently under salinity stress. It has been demonstrated that quinoa can regulate stomatal conductance under salt stress conditions, which helps in maintaining leaf water potential by inhibiting transpiration loss (Cocozza *et al.* 2013).

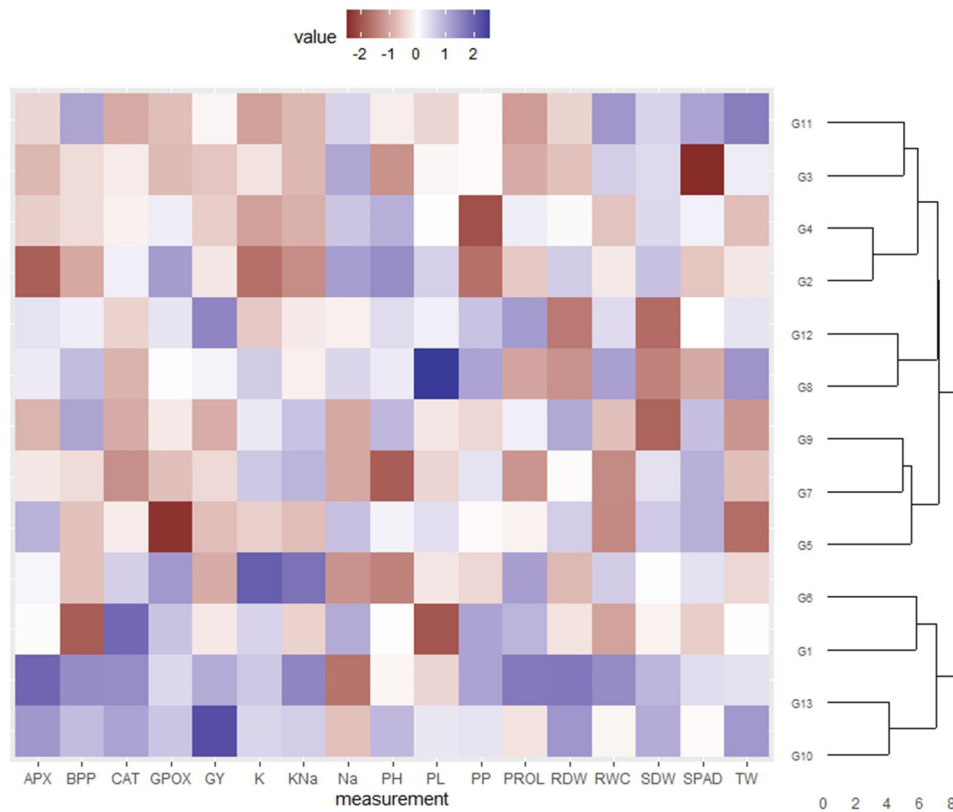


Figure 5. Heat map-based clustering of quinoa germplasms considering all studied traits. G1: EC507744; G2: EC507742; G3: IC411824; G4: EC507741; G5: EC507746; G6: EC507738; G7: EC507748; G8: EC507739; G9: IC411824-1; G10: EC507740; G11: IC411825; G12: CSQ1; G13: CSQ2.

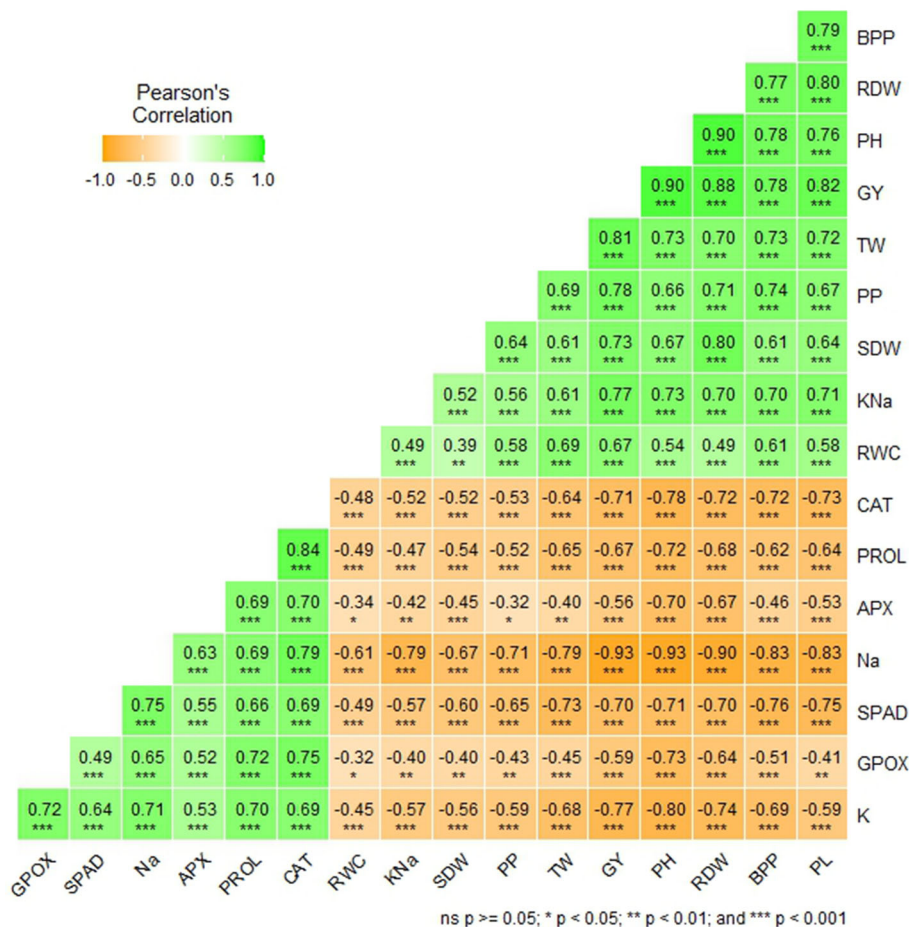


Figure 6. Pearson’s correlation matrix among studied traits. RWC: relative water content, PROL: proline content, PH: plant height, BPP: no. of branches plant⁻¹, PP: no. of panicles plant⁻¹, PL: panicle length, TW: 1000-grain weight, SDW: shoot dry weight, RDW: root dry weight, Na: Na⁺ content, K: K⁺ content, KNa: K⁺/Na⁺ ratio; APX: ascorbate peroxidase; CAT: catalase; GPOX: guaiacol peroxidase.

Irrigation with increasing high-SAR salinity levels caused a marked reduction in the number of branches per plant (figure 1C) and plant height of all germplasms (figure 1B). Under salinity stress, reduction in osmotic potential, cell wall flexibility, and turgor pressure were responsible for retardation of growth (Kumar *et al.* 2017; Pooja *et al.* 2019) due to reduced cell division and expansion. Diversion of metabolic energy towards mitigating salt-induced damage, which could otherwise be utilized for the growth and development of the plant, is also responsible for sluggish growth under stress conditions (Jaikishun *et al.* 2020).

Salinity stress starting from 8 dSm⁻¹ resulted in reduction of number of panicles per plant, length of panicles, and 1000-grain weight compared with BAW (table 3). The negative effect of salinity stress on growth parameters was also reflected in yield-attributing parameters, yield, and root and shoot biomass yield of quinoa germplasms. Under salinity stress, oxidative-

prompted closure of stomata, higher Na⁺ accumulation, reduction in RWC, reduced leaf size, etc., were presumed to lower the photosynthetic efficiency of plants (Koyro and Eisa 2007; Tavakkoli *et al.* 2010), which might have negatively impacted root and shoot biomass and grain yield. Loss of stigma receptivity along with lower pollen viability, and sluggish grain filling due to reduced starch synthetase, were also hypothesized to lower grain yield of quinoa under salt stress conditions (Koyro *et al.* 2008). Transport of plant metabolites was also affected due to reduction in water uptake under high salt concentration, resulting in lower biomass production (Hassen *et al.* 2014). The yield parameters and yields of quinoa germplasms also varied significantly due to salinity levels. Higher growth and yield-attributing characters recorded under germplasms EC507740 and CSQ2 at the highest salinity level (EC_{iw} 24 dSm⁻¹) transformed into higher biomass production and ultimately enhanced grain yield.

Germplasm variation with respect to yield performance might be due to genetic variation existing in some germplasms to withstand different stress levels as manifested through better physiological mechanisms and morphological adaptations. Earlier studies on various germplasms/cultivars of quinoa also showed genetic variation in the degree of salt tolerance (Cai and Gao 2020; Gomez-Pando *et al.* 2010; Jaikishun *et al.* 2020;).

The activities of antioxidant enzymes were increased with higher salinity stress (figure 2A–C). Experimental findings indicated that the activities of antioxidant enzymes increased in salt-tolerant genotypes compared with others. Salinity stress causes production of reactive oxygen species (ROS), which triggers the production of H_2O_2 (Maia *et al.* 2012). This induces the production of antioxidant enzymes like APX, CAT, and GPOX, which aim to neutralize H_2O_2 by conversion into water and oxygen. It has been reported that APX has greater affinity for H_2O_2 than CAT, thus playing an essential role in ROS scavenging under salt stress (Correa-Aragunde *et al.* 2013). The increased activities of antioxidants in tolerant germplasms at the reproductive stage indicate the presence of metabolic adjustment that might lower lipid peroxidation under high salinity stress and help coping under salt stress. However, Causin *et al.* (2020) did not observe a direct relationship between antioxidant enzyme activities and salt tolerance in three quinoa cultivars when examined at the establishment stage, and argued that these traits are not necessarily true indicators of salinity tolerance.

The reduction in growth and biomass yield in our experiment was evidenced even at a lower salinity level of 8 dSm⁻¹. Our results are contrary to the earlier findings of Eisa *et al.* (2012), who reported growth stimulation in quinoa with 100 mM NaCl. Similarly, a non-significant effect on dry weight of quinoa was observed by Wilson *et al.* (2002) at 11 dSm⁻¹ of mixed salt salinity. In the present study, we used high-SAR saline water which might have exerted a dual stress of soluble solutes as well as excess Na⁺, which could be responsible for declining growth and yield even at an initial salinity of 8 dSm⁻¹. It can be assumed that the extent of tolerance of quinoa under high-SAR saline water (SAR > 10 mmol L⁻¹) is lower than that of saline water with safe SAR levels. However, quinoa was able to grow and produce yield even at the highest salinity level of EC_{iw} 24 dSm⁻¹ having 41.3 mmol L⁻¹ SAR. These findings are in conformity with those of Koyro *et al.* (2008), Eisa *et al.* (2012), and Jaikishun *et al.* (2020), who opined that quinoa acts as a facultative

halophyte plant and could produce economic yield up to irrigation water salinity of 20–30 dSm⁻¹.

Application of incremental salinity levels consistently increased the Na⁺ content in plants (table 5). Quinoa is reported to act as a salt includer for osmotic adjustment in plant tissues (Ben Amor *et al.* 2005; Eisa *et al.* 2012), which is assumed to be a low energy-consuming characteristic feature described for many halophyte species (Koyro *et al.* 2011). However, excess accumulation of Na⁺ above what is required for osmotic adjustment has a deleterious effect on the growth of quinoa plants (Eisa *et al.* 2012). Further, the accumulation of K⁺ also showed an increasing trend with increasing salinity levels. This implies that quinoa plants could maintain favorable ionic balance by counteracting excess Na⁺ with K⁺ uptake. Although the increase in K⁺ concentration was not in proportion to that of Na⁺ content, it resulted in reduction of the K⁺/Na⁺ ratio with increasing salinity stress. The high-SAR irrigation water having ample amount of Na⁺ used in this study might have resulted in a higher uptake of Na⁺ and thereby accounted for higher accumulation in plants. It is also worth mentioning that all quinoa germplasms were able to maintain a K⁺/Na⁺ ratio above 1.0, which helped in mitigating any salt injury symptoms (Koyro *et al.* 2011), particularly in young leaves.

Estimation of salinity tolerance indices is helpful in delineating the groups of tolerant and stable germplasms. Selection of germplasms directly based on growth and yield traits data is difficult due to lack of germplasm consistency for all traits at the applied stress levels, and therefore, the use of different indices can help in identifying the group of best performing germplasms (Naghavi *et al.* 2013). Screening based on different tolerant indices considering all the traits values can give an account of more tolerant germplasms under a given stress (Raman *et al.* 2012; Gholinezhad *et al.* 2014; Singh *et al.* 2015). Based on yield index, germplasms EC507739, EC507740, CSQ2, and IC411825 were found to be superior in producing grain yield, while germplasms EC507740, CSQ1, CSQ2, and EC5077439 were observed to be more stable under the studied stress levels. The stress tolerance index, which gives an account of the germplasms that produce higher grain yield under stress conditions, showed that EC507740 followed by CSQ2 and CSQ1 ranked the highest among all the germplasms, showing their resilience at higher levels of salinity stress. The lowest salt susceptibility was recorded for germplasms EC507739, IC411825, and EC507740. Moreover, considering all the above

indices, germplasms EC507740, CSQ1, CSQ2, EC507738, and IC411825 may be considered more stable and consistent germplasms under high-SAR salinity stress.

Multivariate analysis through PCA has been identified as the most effective statistical method to identify the most salt-tolerant germplasm and for establishing germplasm–trait associations (Chikha *et al.* 2016; Sivakumar *et al.* 2020). PCA was performed with 13 germplasms to identify distinct salt-tolerant and salt-sensitive germplasms at different salinity levels (figure 4 and supplementary table 4). PCA biplot axes showed geometrical distances among the germplasms, which reflect the diversity among them in terms of the variables measured. The factor scores of the germplasms distinguished salt-tolerant and salt-sensitive germplasms. Germplasms G13 (CSQ2) and G10 (EC507740) were scored as tolerant germplasms while germplasms G5 (EC507746) and G2 (EC507742) were scored as salinity-sensitive germplasms. Therefore, multivariate analysis using PCA can be used as an effective tool for screening and to validate salt-tolerant germplasms. In previous studies, PCA was used to discriminate stress-tolerant wheat (Rana *et al.* 2015), barley (Allel *et al.* 2016), rice (Chunthaburee *et al.* 2016), and tomato (Sivakumar *et al.* 2023).

Dendrogram-based classification of 13 germplasms distinguished them into 5 groups (figure 5) showing diversity among the selected germplasms. The most tolerant germplasms indicated in the PCA (CSQ2 and EC507740) grouped together in the first cluster, further validating the PCA results. Interrelation among traits as Pearson's correlation metrics gives a clear picture of the association between characters which were responsible for imparting better tolerance and higher yields under salinity stress (figure 6). Na^+ content and K^+ content had strong negative association with grain yield, while K^+/Na^+ ratio had strong positive relation with growth and yield traits as well as grain yield, showing that higher K^+/Na^+ balance in plants is more effective than their individual contents in imparting salt tolerance. Wakeel *et al.* (2013) also showed that optimum K^+/Na^+ is vital to maintain the growth and development of plants by playing a crucial role in enzymatic reactions of the cytoplasm. The positive correlation of Na^+ content with K^+ content and SPAD further elucidates the unique feature of quinoa to enhance K^+ uptake and to maintain leaf greenness for counteracting salinity stress.

5. Conclusion

The quinoa germplasms evaluated in this study showed wide variation in the measured growth, physiological, biochemical, and yield traits at different high-SAR salinities. Various salinity tolerance indices, multivariate analyses, correlation, and clustering approaches were used to identify salinity-tolerant quinoa germplasms. Based on the PCA, we conclude that the proline content, K^+ , K^+/Na^+ ratio, root dry weight, and high ascorbate peroxidase activity are the adaptive traits imparting tolerance to high-SAR salinity. Overall, the results of salinity tolerance indices and PCA showed that germplasms EC507740 and CSQ2 were the most tolerant and stable germplasms which could be used for cultivation in arid and semi-arid areas affected with high-SAR salinity and for future breeding programs. Furthermore, there is need to assess the effect of salinity on nutritional properties and to identify salt-responsive markers in tolerant germplasms.

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Data availability

All the data which are used to generate results are presented in the manuscript and supplementary files and therefore available.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

References

- Abbas G, Amjad M, Saqib M, *et al.* 2021 Soil sodicity is more detrimental than salinity for quinoa (*Chenopodium quinoa* Willd.): A multivariate comparison of physiological, biochemical and nutritional quality attributes. *J. Agron. Crop Sci.* **207** 59–73
- Adolf VI, Jacobsen S-E and Shabala S 2013 Salt tolerance mechanisms in quinoa (*Chenopodium quinoa* Willd.). *Environ. Exp. Bot.* **92** 43–54
- Aebi H 1984 Catalase in vitro. *Meth. Enzymol.* **105** 121–126
- Agarie S, Shimoda T, Shimizu Y, *et al.* 2007 Salt tolerance, salt accumulation, and ionic homeostasis in an epidermal bladder-cell-less mutant of the common ice plant *Mesembryanthemum crystallinum*. *J. Exp. Bot.* **58** 1957–1967
- Aguilar PC, Cutipa Z, Machaca E, *et al.* 2003 Variation of proline content of quinoa (*Chenopodium quinoa* Willd.) in high beds (Waru Waru). *Food Rev. Int.* **19** 121–127
- Algozaibi AM, El-Garawany MM, Badran AE, *et al.* 2015 Effect of irrigation water salinity on the growth of quinoa plant seedlings. *J. Agric. Sci.* **7** 205–214
- Allel D, Ben-Amar A, Badri M, *et al.* 2016 Salt tolerance in barley originating from harsh environment of North Africa. *Aust. J. Crop Sci.* **10** 438
- Bates LS, Waldren RP and Teare ID 1973 Rapid determination of free proline for water-stress studies. *Plant Soil* **39** 205–207
- Bazile D, Bertero HD and Nieto C 2015 *State of the art report on quinoa around the world in 2013* (Rome: FAO and CIRAD)
- Bazile D, Jacobsen SE and Verniau A 2016 The global expansion of quinoa: Trends and limits. *Front. Plant Sci.* **7** 622
- Ben Amor N, Ben Hamed K, Debez A, *et al.* 2005 Physiological and antioxidant responses of the perennial halophyte *Crithmum maritimum* to salinity. *Plant Sci.* **168** 889–899
- Bhargava A, Shukla S and Ohri D 2007 Genetic variability and interrelationship among various morphological and quality traits in quinoa (*Chenopodium quinoa* Willd.). *Field Crops Res.* **101** 104–116
- Bousslama M and Schapaugh WT 1984 Stress tolerance in soybean. I. Evaluation of three screening techniques for heat and drought tolerance. *Crop Sci.* **24** 933–937
- Cai Z and Gao Q 2020 Comparative physiological and biochemical mechanisms of salt tolerance in five contrasting highland quinoa cultivars. *BMC Plant Biol.* **20** 70
- Castillo FJ, Celardin F and Greppin H 1984 Peroxidase assay in plants: Interference by ascorbic acid and endogenous inhibitors in *Sedum* and *Pelargonium* enzyme extracts. *Plant Growth Regul.* **2** 69–75
- Causin HF, Bordon DA and Burrieza H 2020 Salinity tolerance mechanisms during germination and early seedling growth in *Chenopodium quinoa* Willd. genotypes with different sensitivity to saline stress. *Environ. Exp. Bot.* **1** 172
- Chikha MB, Hessini K, Ourteni RN, *et al.* 2016 Identification of barley landrace genotypes with contrasting salinity tolerance at vegetative growth stage. *Plant Biotechnol.* **33** 287–295
- Chunthaburee S, Dongsansuk A, Sanitchon J, *et al.* 2016 Physiological and biochemical parameters for evaluation and clustering of rice cultivars differing in salt tolerance at seedling stage. *Saudi J. Biol. Sci.* **23** 467–477
- Cocozza C, Pulvento C, Lavini A, *et al.* 2013 Effects of increasing salinity stress and decreasing water availability on ecophysiological traits of quinoa (*Chenopodium quinoa* Willd.) grown in a mediterranean-type agroecosystem. *J. Agron. Crop Sci.* **199** 229–240
- Correa-Aragunde N, Foresi N, Delledonne M, *et al.* 2013 Auxin induces redox regulation of ascorbate peroxidase activity by S-nitrosylation/denitrosylation balance resulting in changes of root growth pattern in *Arabidopsis*. *J. Exp. Bot.* **64** 3339–3349
- Dagar JC 2018 Utilization of degraded saline habitats and poor-quality waters for livelihood security. *Sch. J. Food Nutr.* **1** 88–106
- Dini I, Tenore GD and Dini A 2005 Nutritional and anti-nutritional composition of Kancolla seeds: an interesting and underexploited andine food plant. *Food Chem.* **92** 125–132
- Eisa S, Hussin S, Geissler N, *et al.* 2012 Effect of NaCl salinity on water relations, photosynthesis and chemical composition of Quinoa (*Chenopodium quinoa* Willd.) as a potential cash crop halophyte. *Aust. J. Crop Sci.* **6** 357–368
- Eisa SS, Eid M, El-Samad EHA, *et al.* 2017 *Chenopodium quinoa* Willd. A new cash crop halophyte for saline regions of Egypt. *Aust. J. Crop Sci.* **11** 343–351
- Elewa T, Sadak MS and Saad AM 2017 Proline treatment improves physiological responses in quinoa plants under drought stress. *Biosci. Res.* 2017 **14** 21–33
- Fernandez GC 1992 Effective selection criteria for assessing plant stress tolerance; in *Proceeding of the International Symposium on Adaptation of Vegetables and other Food Crops in Temperature and Water Stress* (Shanhua, Taiwan) pp 257–270
- Fischer RA and Maurer R 1978 Drought resistance in spring wheat cultivars. I. Grain yield response. *Aust. J. Agric. Res.* **29** 897–907
- Gavuzzi P, Rizza F, Palumbo M, Campaline RG, Ricciardi GL and Borghi B 1997 Evaluation of field and laboratory predictors of drought and heat tolerance in winter cereals. *Canadian J. Plant Sci.* **77** 523–531
- Geissler N, Hussin S, El-Far MM, *et al.* 2015 Elevated atmospheric CO₂ concentration leads to different salt resistance mechanisms in a C3 (*Chenopodium quinoa*) and a C4 (*Atriplex nummularia*) halophyte. *Env. Exp. Bot.* **118** 67–77

- Gholinezhad E, Darvishzadeh R and Bernousi I 2014 Evaluation of drought tolerance indices for selection of confectionery sunflower (*Helianthus annuus* L.) landraces under various environmental conditions. *Not. Bot. Hort. Agrobot. Cluj-Napoca* **42** 187–201
- Goehring N, Verburg P, Saito L, et al. 2019 Improving modeling of quinoa growth under saline conditions using the enhanced agricultural policy environmental extender model. *Agron* **9** 592
- Gomez KA and Gomez AA 1984 *Procedures for agricultural research* (New York: John Wiley & Sons)
- Gomez-Pando LR, Alvarez-Castro R and Eguiluz-de la Barra A 2010 Effect of salt stress on Peruvian germplasm of *Chenopodium quinoa* Willd.: A promising crop. *J. Agron. Crop Sci.* **196** 391–396
- Hariadi Y, Marandon K, Tian Y, et al. 2011 Ionic and osmotic relations in quinoa (*Chenopodium quinoa* Willd.) plants grown at various salinity levels. *J. Exp. Bot.* **62** 185–193
- Hassen A, Souguir K and Hannachi C 2014 Effect of salt stress (NaCl) on germination and early seedling parameters of three pepper genotypes (*Capsicum annum* L.). *J. Stress Physiol. Biochem.* **10** 15–25
- Hirich A, Choukr-Allah R and Jacobsen S-E 2013 The combined effect of deficit irrigation by treated wastewater and organic amendment on quinoa (*Chenopodium quinoa* Willd.) productivity. *Desalin. Water Treat.* **52** 2208–2213
- Hirich A, Jelloul A, Choukr-Allah R, et al. 2014 Saline water irrigation of quinoa and chickpea: seedling rate, stomatal conductance and yield responses. *J. Agron. Crop Sci.* **200** 378–389
- Hirose Y, Fujita T, Ishii T, et al. 2010 Antioxidative properties and flavonoid composition of *Chenopodium quinoa* seeds cultivated in Japan. *Food Chem.* **119** 1300–1306
- Iqbal S, Basra SM, Afzal I, et al. 2019 Yield potential and salt tolerance of quinoa on salt-degraded soils of Pakistan. *J. Agron. Crop Sci.* **205** 13–21
- Jacobsen SE, Mujica A and Jensen CR 2003 The resistance of quinoa (*Chenopodium quinoa* Willd.) to adverse abiotic factors. *Food Rev. Int.* **19** 99–109
- Jaikishun S, Gang P, Song S, et al. 2020 Morpho-physiological responses of quinoa (*Chenopodium quinoa* Willd.) varieties to salinity in a hydroponic system. *Am. J. Plant Physiol.* **15** 41–51
- Koyro HW and Eisa SS 2007 Effect of salinity on composition, viability and germination of seeds of *Chenopodium quinoa* Willd. *Plant Soil* **302** 79–90
- Koyro HW, Geissler N, Seenivasan R, et al. 2011 Plant stress physiology; physiological and biochemical strategies allowing to thrive under ionic stress; in *Handbook of plant and crop stress* 3rd edition (Ed.) M Pessarakli (Florida: CRC press) pp 1051–1094
- Koyro HW, Lieth H and Eisa SS 2008 Salt tolerance of *Chenopodium quinoa* Willd., grains of the Andes: Influence of salinity on biomass production, yield, composition of reserves in the seeds, water and solute relations; in *Mangroves and halophytes: Restoration and utilisation* Vol. 43 (Eds.) H Lieth, MG Sucre and B Herzog (Netherlands: Springer) pp 133–145
- Kumar A, Lata C, Krishnamurthy SL, et al. 2017 Physiological and biochemical characterization of rice varieties under salt and drought stresses. *J. Soil Salin. Water Qual.* **9** 167–177
- Maia JM, Ferreira-Silva SL, Voigt EL, et al. 2012 Activities of antioxidant enzymes and root growth inhibition in cowpea seedlings exposed to different salt levels. *Acta Bot. Bras.* **26** 342–349
- Mandal S, Raju R, Kumar A, et al. 2018 Current status of research, technology response and policy needs of salt-affected soils in India – a review. *J. Indian Soc. Coast. Agric. Res.* **36** 40–53
- Mann A, Kaur G, Kumar A, et al. 2019 Physiological response of chickpea (*Cicer arietinum* L.) at early seedling stage under salt stress conditions. *Leg. Res.* **42** 625–632
- Marengo R, Antezana-Vera S and Nascimento H 2009 Relationship between specific leaf area, leaf thickness, leaf water content and SPAD-502 readings in six Amazonian tree species. *Photosynthetica* **47** 184–190
- Minhas PS 1999 Use of poor-quality waters; in *50 years of natural resource management research* (Eds) GB Singh and BR Sharma (Karnal: ICAR-CSSRI) pp 327–346
- Minhas PS, Sharma DR and Chauhan CPS 2004 Management of saline and alkali waters for irrigation; in *Advances in sodic land reclamation*, International Conference on Management of Sodic Lands for Sustainable Agriculture, February 9-14 (Lucknow: UPCAR) pp 9–14
- Muscolo A, Panuccio MR, Gioffre AM, et al. 2016 Drought and salinity differently affect growth and secondary metabolites of “*Chenopodium quinoa* Willd” seedlings; in *Halophytes for food security in dry lands* (Eds.) MA Khan, M Ozturk, B Gul and MZ Ahmed (San Diego: Elsevier Inc.) pp 259–275
- Naghavi MR, Aboughadareh AP and Khalili M 2013 Evaluation of drought tolerance indices for screening some of corn (*Zea mays* L.) cultivars under environmental conditions. *Not. Bot. Hort. Agrobot. Cluj Napoca* **5** 388–393
- Nakano Y and Asada K 1981 Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* **22** 867–880
- Negacz K, Malek Z, Devos A, et al. 2022 Saline soils worldwide: Identifying the most promising areas for saline agriculture. *J. Arid. Environ.* **203** 104775
- Orsini F, Accorsi M, Gianquinto G, et al. 2011 Beyond the ionic and osmotic response to salinity in *Chenopodium quinoa*: Functional elements of successful halophytism. *Funct. Plant Biol.* **38** 818–831

- Pooja Nandwal AS, Chand M, *et al.* 2019 Varietal variation in physiological and biochemical attributes of sugarcane varieties under different soil moisture regimes. *Indian J. Exp. Biol.* **57** 721–732
- Pulvento C and Bazile D 2023 Worldwide evaluations of quinoa – Biodiversity and food security under climate change pressures: advances and perspectives. *Plants* **12** 868
- R Core Team 2021 R: *A language and environment for statistical computing* (R Foundation for Statistical Computing, Vienna, Austria) <https://www.R-project.org/>.
- Raman A, Verulkar S, Mandal N, *et al.* 2012 Drought yield index to select high yielding rice lines under different drought stress severities. *Rice* **5** 31
- Rana V, Sewa R, Ramadas S, *et al.* 2015 Physiological, biochemical and morphological study in wheat (*Triticum aestivum* L.) RILs population for salinity tolerance. *J. Agric. Sci.* **7** 119–128
- Razzaghi F, Jacobsen S-E, Jensen CR, *et al.* 2015 Ionic and photosynthetic homeostasis in quinoa challenged by salinity and drought—mechanisms of tolerance. *Funct. Plant Biol.* **42** 136–148
- Repo-Carrasco R, Espinoza C and Jacobsen SE 2003 Nutritional value and use of the Andean crops quinoa (*Chenopodium quinoa*) and kañiwa (*Chenopodium pallidicaule*). *Food Rev. Int.* **19** 179–189.
- Salehi M 2020 Hypersaline water for alternative crop irrigation in Iran; in *Emerging research in alternative crops* Vol. 58 (Eds.) A Hirich, R Choukr-Allah and R Ragab (Cham: Springer) pp 55–92
- Salehi M, Dehghany F, Soltani Gerdafaramarzi V, *et al.* 2021 Identify the effective traits for the selection of quinoa (*Chenopodium quinoa* Willd.) lines in spring cultivation under saline condition. *Env. Stresses Crop Sci.* **14** 1041–1054
- Shabala S, Hariadi Y and Jacobsen S-E 2013 Genotypic difference in salinity tolerance in quinoa is determined by differential control of xylem Na⁺ loading and stomatal density. *J. Plant Physiol.* **170** 906–914
- Shahid SA, Zaman M and Heng L 2018 Introduction to soil salinity, sodicity and diagnostics techniques; in *Guideline for salinity assessment, mitigation and adaptation using nuclear and related techniques* (Eds.) Z Mohammad, SA Shabbir and H Lee (Cham: Springer) pp 1–42
- Sharma DK and Singh A 2015 Salinity research in India achievements, challenges and future prospects. *Water Energy Int.* **58** 35–45
- Sheoran P, Kumar A, Sharma R, *et al.* 2021 Managing sodic soils for better productivity and farmers' income by integrating use of salt tolerant rice varieties and matching agronomic practices. *Field Crops Res.* **270** 108192
- Singh S, Sengar RS, Kulshreshtha Neeraj, *et al.* 2015 Assessment of multiple tolerance indices for salinity stress in bread wheat (*Triticum aestivum* L.). *J. Agric. Sci.* **7** 49–57
- Sivakumar J, Prashanth JEP, Rajesh N, *et al.* 2020 Principal component analysis approach for comprehensive screening of salt stress-tolerant tomato germplasm at the seedling stage. *J. Biosci.* **45** 141
- Sivakumar J, Reddy MS, Sergeant K, *et al.* 2023 Principal component analysis-assisted screening and selection of salt-tolerant tomato genotypes. *Plant Physiol. Rep.* **28** 272–288
- Sosa-Zuniga V, Brito V, Fuentes F, *et al.* 2017 Phenological growth stages of quinoa (*Chenopodium quinoa*) based on the BBCH scale. *Ann. Appl. Biol.* **171** 117–124
- Stanschewski CS, Rey E, Fiene G, *et al.* 2021 Quinoa phenotyping methodologies: an international consensus. *Plants* **10** 1759
- Stikic R, Glamoclija D, Demin M, *et al.* 2012 Agronomical and nutritional evaluation of quinoa seeds (*Chenopodium quinoa* Willd.) as an ingredient in bread formulations. *J. Cereal Sci.* **55** 132–138.
- Sun Y, Lindberg S, Morgan S, *et al.* 2017 A comparative analysis of cytosolic Na⁺ changes under salinity between halophyte quinoa (*Chenopodium quinoa*) and glycophyte pea (*Pisum sativum*). *Environ. Exp. Bot.* **141** 154–160
- Tavakkoli E, Fatehi F, Coventry S, *et al.* 2010 Additive effects of Na⁺ and Cl⁻ ions on barley growth under salinity stress. *J. Exp. Bot.* **62** 2189–2203
- Wakeel A 2013 Potassium-sodium interaction in soil and plant under saline-sodic conditions. *J. Plant Nutr. Soil Sci.* **176** 344–354
- Weatherley PE 1950 Studies in the water relations of the cotton plant. I. The field measurement of water deficits in leaves. *New Phytol.* **49** 81–87
- Wicke B, Smeets E, Dornburg V, *et al.* 2011 The global technical and economic potential of bioenergy from salt-affected soils. *Energy Environ. Sci.* **4** 2669–2681
- Wilson C, Read JJ and Abo-Kassem E 2002 Effect of mixed salt salinity on growth and ion relations of a quinoa and a wheat variety. *J. Plant Nutr.* **25** 2689–2704
- Yazar A and Incekaya C 2014 A new crop for salt affected and dry agricultural areas of turkey: quinoa (*Chenopodium quinoa* Willd.). *Turk. J. Agric. Nat. Sci.* **2** 1440–1446
- Yazar A, Incekaya C, Sezen SM, *et al.* 2015 Saline water irrigation of quinoa (*Chenopodium quinoa*) under Mediterranean conditions. *Crop Pasture Sci.* **66** 993–1002
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