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Author contributions

JTC, KM, DG and VFZ conceived and conceptualized the research. JTC designed and performed the experiment, generated and analysed the data, and wrote the manuscript. All the authors read and approved the final manuscript.

Genotypic variation in quinoa's (*Chenopodium quinoa* Willd.) response to salinity stress: Insights from germination and seedling establishment stages.

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Abstract

Salinity negatively affects plant growth and development, particularly during the seed germination and seedling establishment stages, which are the most vulnerable due to combined osmotic stress and ionic toxicity. This study investigates the effects of salinity, simulated using NaCl, on the germination and early growth of ten quinoa genotypes (Colorado 407D, Rosa Junin, Sayana, Baer, Faro, Titicaca, Negra Collana, 11WAQ, Puno, and Vikinga). The experiment was conducted under controlled conditions with varying salinity levels (50, 100, 200, 300, and 400 mM NaCl). Results revealed that increasing salinity levels significantly reduced germination percentage, seedling shoot length, root length, fresh weight, and dry biomass, while increasing mean germination time, time to 50% germination, chlorophyll A, B, and carotenoid content, and superoxide dismutase, catalase, and peroxidase activities. Genotypes varied in their response to salinity stress, with some showing higher salinity tolerance than others. Genotypes like Colorado 407D, Sayana, Titicaca, Faro, Rosa Junin, and 11WAQ exhibited greater resilience, suggesting their potential for cultivation in salt-affected regions. This study highlights the importance of identifying and cultivating salt-tolerant quinoa genotypes to enhance food security in saline environments.

Keywords: Quinoa; salinity stress; germination; seedling establishment

Introduction

Salinity is one of the most significant abiotic stresses affecting plant growth and agricultural productivity in the 21st century (Chele et al., 2021), where poor irrigation and drainage practices have been the primary causes of increasing soil salinity in arable lands (Tarolli et al., 2024). Currently, approximately 23% of the world's 1.5 billion hectares of cultivated land is affected by salt stress, posing a serious threat to food security (Negacz et al., 2022; Stoleru et al., 2019). This problem is especially severe in arid and semi-arid regions due to high evaporation and low precipitation, which prevent natural salt leaching (Tarolli et al., 2024). High soil salinity significantly reduces soil fertility, limiting crop productivity and agricultural sustainability (Gondek et al., 2020). Salinity is most harmful during germination and seedling establishment due to ion toxicity and its negative effect on osmosis (Stoleru et al., 2019). As the global extent of saline soils continues to expand, addressing soil salinization is essential for sustainable agriculture and long-term food production. Therefore, identifying and cultivating salt-tolerant species is crucial for improving germination success and enhancing crop resilience, thereby supporting global food security in salt-affected areas.

Quinoa (*Chenopodium quinoa* Willd.) is a nutrient-dense grain crop that has gained significant global attention due to its exceptional nutritional profile and adaptability to diverse growing conditions (Angeli et al., 2020). Originating in the Andean region of South America, quinoa has been a staple food for indigenous populations for thousands of years (Hinojosa et al., 2021). It is highly valued for its complete protein content, containing all nine essential amino acids, making it a rare plant-based source of high-quality protein (Dakhili et al., 2019). Additionally, quinoa is rich in dietary fibre, vitamins, and minerals, and is naturally gluten-free (Angeli et al.,

2020; Zevallos et al., 2014; Graf et al., 2015), which has contributed to its rising popularity as a health food in recent years. As a result, quinoa is not only a subject of agricultural and nutritional interest, but also a potential contributor to food security in regions facing climatic challenges.

Understanding quinoa's abiotic stress adaptation mechanisms is essential to its global expansion and wider establishment as a nutritious and resilient crop. With this in mind, the current study aims to understand the morpho-physiological and biochemical response of ten quinoa genotypes to salinity stress during seed germination and seedling establishment stages.

Material and methods

This study was conducted at Cockle Park Farm (Northumberland, UK) in Newcastle University's Pawson Lab, with ten quinoa genotypes selected for the experiment (Table 1). These genotypes were chosen based on their origin to represent a wide range of genetic diversity, which is crucial for assessing the impact of salinity on quinoa.

Table 1 Origin of quinoa genotypes used.

Genotype	Origin
Colorado 407D	Colorado, USA
Rosa Junin	Cuzco, Peru
Sayana	La Paz, Bolivia
Baer	South of Chile
Faro	North of Chile
Titicaca	Denmark
Negra Collana	Puno region, Peru
11WAQ	North of Chile
Puno	Denmark
Vikinga	Denmark

A Petri dish experiment was conducted using a completely randomized design with a 6x10 factorial setup, involving five NaCl concentrations (50mM, 100mM, 200mM, 300mM, and 400mM) and ten quinoa genotypes (Table 1), each with five replications, in a growth chamber set at 23°C with 10 h light and 14 h dark conditions. Distilled water was used as a control treatment. Seeds were sterilized with 20% sodium hypochlorite (NaClO) (v/v) for 10 minutes, then washed with distilled water six times for 30 minutes to remove saponins and prevent potential contamination (Panuccio et al., 2014). One hundred quinoa seeds were placed in each of the 300 Petri dishes used, which were lined with two Whatman No. 2 filter papers and contained 3 ml of either NaCl solution or distilled water. The NaCl quantities corresponding to the required molar concentrations for the experiment were calculated using the Sigma molarity calculator (Table 2). This experiment was run for eight days.

Table 2 Mass of NaCl in g/L to obtain solutions with different levels of molar concentration.

Molar concentration (mM)	NaCl (g/L)
0	0
50	2.922
100	5.844
200	11.688
300	17.532
400	23.376

Germination assessment

Germination percentage

The germination percentage (GP) was calculated using the equation below (Scott et al., 1984).

$$GP = \left(\frac{\sum n_g}{n} \right) \times 100 \quad (1)$$

Where

n_g = Number of seeds germinated after eight days

n = Number of seeds sown

Mean germination time

The mean germination time (MGT) was calculated using the equation below (Kader, 2005).

$$MGT = \frac{\sum (n \cdot d)}{\sum n} \quad (2)$$

Where

n= Number of seeds germinated on day (d)

Days to 50% germination

Days to 50% germination (T_{50}) was calculated as described by Soltani et al. (2015).

$$T_{50} = t_i \frac{\left(\frac{N}{2} - n_i\right) (t_j - t_i)}{(n_j - n_i)} \quad (3)$$

Where

N= Final number of germinated seeds

n_j and n_i = Cumulative number of seeds germinated by adjacent counts at times t_j (day) and t_i (day) respectively, when $n_i < N/2 < n_j$

Seedling growth assessment

To assess seedling growth, ten seedlings were randomly selected from each Petri dish at the end of the germination period. The shoot length (SL) and root length (RL) were measured. Subsequently, the dry biomass of the seedlings (DB) was assessed by drying the samples in an oven at 70°C for 48 hours.

Seedling vigour index

Seedling vigour index (SVI) was calculated according to Orchard (1977) equation.

$$SVI = (SL + RL) * GP \quad (4)$$

Salinity tolerance indices

To assess the salinity tolerance of each genotype, the stress susceptibility index (SSI) and stress tolerance index (STI) were calculated according to equations presented by Fischer and Maurer (1978) and Fernandez (1993), respectively.

$$SSI = \left(1 - \left(\frac{Y_s}{Y_p}\right)\right) / \left(1 - \left(\frac{\bar{y}_s}{\bar{y}_p}\right)\right) \quad (5)$$

$$STI = (Y_p * Y_s) / (\bar{y}_p)^2 \quad (6)$$

In both equations, Y_p and Y_s represent the average seedling dry biomass of a given genotype under non-stress and salinity stress conditions, respectively. $\bar{y}_p$ and $\bar{y}_s$ represent the average seedling dry biomass of all genotypes under non-stress and salinity stress conditions, respectively.

Physiological and biochemical analysis

Seedling samples were randomly collected from each Petri dish 8 days after germination to determine photosynthetic pigments (chlorophyll A, chlorophyll B, and carotenoids) and the activities of antioxidant enzymes (superoxide dismutase, catalase and peroxidase) using standard procedures.

Photosynthetic pigments analysis

Approximately 1.0 g of quinoa seedling samples from each Petri dish were snap-frozen with liquid nitrogen for a few seconds to stop the metabolic processes of the tissue and then ground in 80% acetone. The ground mixture was centrifuged at 3000x g for 10 minutes. After centrifugation, the supernatant was collected, and its absorbance was measured at wavelengths of 645 nm, 663 nm, and 480 nm using a Jenway Model 7315 UV/Visible Single Beam Spectrophotometer. Chlorophyll A, chlorophyll B, and carotenoids were calculated to determine their content and expressed in mg/g fresh weight using the equations below (Lichtenthaler, 1987).

$$\text{Chlorophyll A} = [(12.25 * A_{663}) - (2.79 * A_{645})] \quad (7)$$

$$\text{Chlorophyll B} = [(21.50 * A_{645}) - (5.10 * A_{663})] \quad (8)$$

$$\text{Carotenoids} = [(A_{470} - (0.114 * A_{663}))(0.638 * A_{645})] \quad (9)$$

Antioxidant enzyme activity assessment

Approximately 0.5 g of seedling samples from each Petri dish were snap-frozen with liquid nitrogen for a few seconds to stop the metabolic processes of the tissue, then ground in 2 ml of extraction buffer (0.1 M phosphate buffer containing 100 mg soluble polyvinylpyrrolidone and 0.1 mM EDTA, pH 7.0). The homogenized mixture was then filtered through one layer of Whatman No.2 filter paper and centrifuged at 15,000 x g at 4°C for 15 minutes. The supernatant was collected and stored at -20°C for subsequent enzyme activity measurements. All enzyme activities were measured using a Jenway Model 7315 UV/Visible Single Beam Spectrophotometer.

Superoxide dismutase (SOD) activity

SOD activity was determined using the photo-reduction of nitroblue tetrazolium (NBT) method (Giannopolitis and Ries, 1977), as cited in (Iqbal et al., 2018). The reaction mixture for SOD activity comprised 1500 µl of 100 mM potassium phosphate buffer (pH 7.8), 100 µl of 200 mM methionine, 100 µl of 2.25 mM NBT, 100 µl of riboflavin, 100 µl of 3 mM EDTA, 1000 µl distilled water and 50 µl enzyme extract. The reaction mixture was illuminated for 5 minutes, and absorbance was recorded at 560 nm. SOD activity was defined as the amount of enzyme required to inhibit 50% of NBT reduction at 560 nm.

Catalase (CAT) activity

CAT activity was determined by monitoring the consumption of H₂O₂. The reaction mixture contained 200 µl of enzyme extract, 5 ml of potassium phosphate buffer (50 mM, pH 7.0), and 25 µl of H₂O₂. The decrease in absorbance due to the consumption of H₂O₂ was observed at 240 nm. One unit of CAT activity was defined as the amount of CAT required to decompose 1 µmol of H₂O₂ as per calculated from the extinction coefficient for H₂O₂ at 240 nm of 0.039 cm²µmol⁻¹ (Iqbal et al., 2018; Duman and Kaya, 2013).

Peroxidase (POD) activity

POD activity was assessed based on H₂O₂ peroxidation with guaiacol as an electron donor (Iqbal et al., 2018). The reaction mixture for POD activity contained 2.5ml of potassium phosphate buffer (0.1 M, pH 7.0), 50 µl of guaiacol, 37.5 µl of H₂O₂ and 100 µl enzyme extract. POD activity was determined by observing the absorbance at 470 nm. POD activity was quantified by the amount of guaiacol from the extinction coefficient for guaiacol at 470 nm of 26.6 mM⁻¹cm⁻¹ (Panuccio et al., 2014).

Statistical analysis

Overall effects were assessed by two-way ANOVA (NaCl concentration and genotypes as factors) and non-metric multidimensional scaling (NMDS) with Bray-Curtis similarity as the effect distribution measure. Where main effects were determined by ANOVA, post-hoc comparisons were undertaken as appropriate using the least significant difference (LSD, P<0.05) test. To demonstrate similarity between genotypes, a hierarchical agglomerative cluster analysis with percentage similarity as the similarity measure and the unweighted pair group method for classification tree construction was used. Statistical analysis packages R, SAS JMP Pro, and Origin Pro were used for calculations.

Results

A two-way ANOVA was performed to assess the overall effect of salinity stress on ten quinoa genotypes and the summary of results is presented below (Table 3). The results showed that both NaCl concentration and genotype had significant effects on all parameters at P ≤ 0.001 level. Notably, NaCl concentration had a larger F ratio than genotype in all parameters, indicating that NaCl concentration explained more variation in these parameters than genotypes. The interaction effect between NaCl concentration and genotype observed in all parameters suggested that the response to salinity stress varies among different genotypes. However, the smaller F ratio in NaCl concentration and genotype interaction than either factor alone for all parameters imply that the interaction effect explained less variation in these parameters than either factor alone.

Table 3 Combined analysis of variance showing F values of different parameters of ten quinoa genotypes subjected to five NaCl levels.

Parameters	Source of variation			
	NaCl	G	NaClxG	CV %
Germination (%)	1330.00***	84.15***	21.02***	12.2
Mean Germination Time (days)	692.02***	210.50***	5.74***	15.1
Time to 50% germination (days)	661.05***	302.63***	7.59***	21.4
Shoot length (cm)	1012.62***	35.78***	4.71***	27.1
Root length (cm)	821.16***	21.16***	2.36***	39.8
Seed Vigour Index	2868.92***	59.19***	7.09***	50.3
Fresh weight (mg)	1036.12***	688.09***	16.34***	24.4
Dry biomass (mg)	2134.74***	230.99***	13.54***	18.4
Chlorophyll A (mg/g FW)	473.99***	527.21***	20.19***	22.8
Chlorophyll B (mg/g FW)	884.35***	233.75***	30.52***	33.6
Carotenoids (mg/g FW)	703.83***	233.38***	25.76***	26.4
Superoxide dismutase (ΔA/g FW)	850.66***	281.03***	23.96***	15.9
Catalase (µmol H ₂ O ₂ /g FW)	1406.07***	149.09***	31.99***	19.7
Peroxidase (µmol guaiacol/g FW)	780.29***	227.01***	8.56***	25.1

The significant test P values= * indicates $P \leq 0.05$, ** indicates $P \leq 0.01$, *** indicates $P \leq 0.001$, highly significance; G= genotype; CV= Coefficient of variation; x= interaction effect.

Salinity reduced germination percentage (GP), seedling shoot length (SL), seedling root length (RL), seedling fresh weight (FW), seedling vigour index (SVI), seedling dry biomass (DB), chlorophyll A (Chla), chlorophyll B (Chlb) and carotenoids (Car), but increased Mean germination time (MGT), time to 50% germination (T50), and superoxidase dismutase (SOD), peroxidase (POD) and catalase (CAT) activities (Fig 1). The response to salinity varied among genotypes and the NaCl concentration, as demonstrated on the NMDS diagram (Fig 1), where the first ordination axis represents salinity gradient while the second axis represents the differences in genotype response. Samples are distributed from left (0mM NaCl) to the right side of the diagram (400 mM NaCl) and the spread is increasing along NMDS 1 axis. This distribution highlights the varied response of quinoa genotypes to salinity conditions.

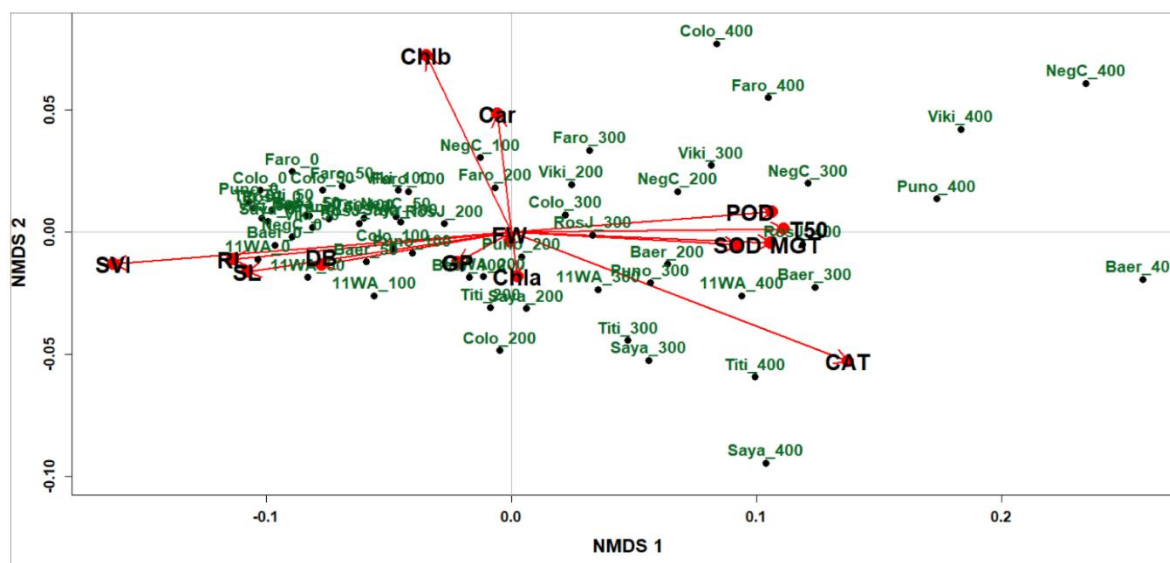


Fig 1 Non-metric multidimensional scaling ordination diagram (NMDS with Bray-curtis similarity measure) of ten quinoa genotypes in five salinity treatments.

Germination assessment

Salinity stress affected quinoa's germination parameters, with variations observed among genotypes. Significant differences in GP were observed among the ten quinoa genotypes in all NaCl levels except under control and 50 mM treatments (Fig 2a). Between control and 50 mM NaCl treatments genotypes Vikinga and Negra Collana recorded the highest reductions in GP, while Sayana, Rosa Junin, Baer, and Faro showed no reduction. Significant variations in GP were observed among the ten genotypes under 100 mM to 400 mM NaCl treatments. Sayana, Negra Collana, Vikinga, and Puno recorded the highest GP reductions under 100 mM, 200 mM, 300 mM, and 400 mM NaCl treatments, respectively. Conversely, genotype Faro showed no reduction in GP under 100 mM NaCl and the least reduction under 200 mM and 300 mM NaCl treatments, while genotype Titicaca recorded the minimum reduction under 400 mM NaCl treatment.

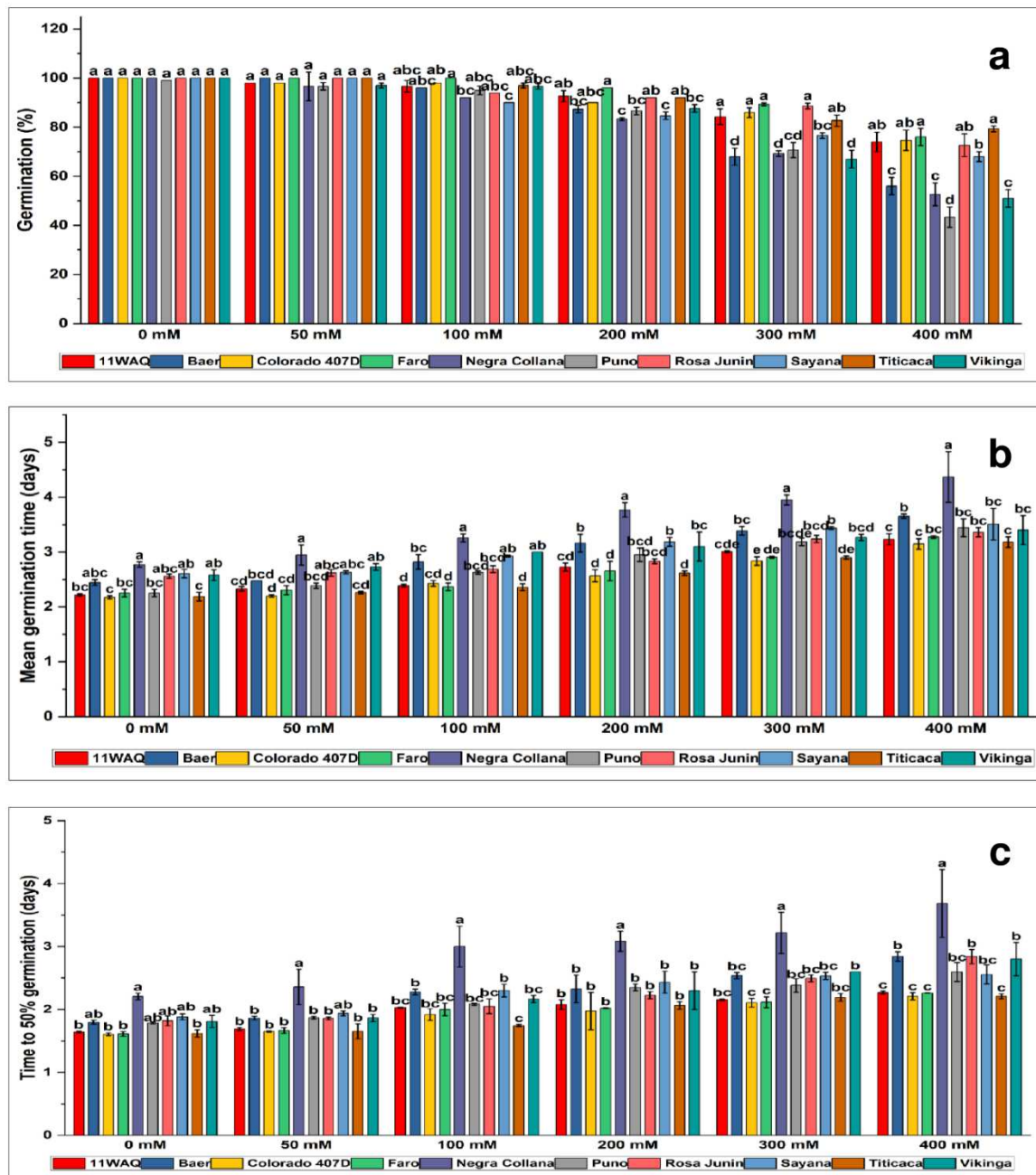


Fig 2 Germination percentage (a), mean germination time (b) and time to 50% germination (c) of ten quinoa genotypes subjected to five NaCl concentration levels. Data represent the mean values across n=100 samples, with five replicates per genotype. Error bars indicate the standard deviation of five replicates. Genotypes not connected by the same letter within a treatment level are significantly different.

MGT was also affected by salinity stress, with variations observed among the genotypes with significant differences observed across all stress levels (Fig 2b). Genotype Negra Collana recorded the highest increase in MGT across all salinity stress levels compared to the control treatment. Conversely, genotype Sayana recorded the lowest increase in MGT under 50 mM NaCl, while genotype Rosa Junin recorded the lowest increase in MGT under 100 mM, 200 mM, 300 mM, and 400 mM NaCl treatments. The highest increase in T50 was also recorded in the genotype Negra Collana at all salinity stress levels (Fig 2c). Conversely, the lowest T50 increase was observed in genotype Titicaca at 50 mM and 100 mM NaCl, Rosa Junin at 200 mM, Colorado 407D and 11WAQ at 300 mM, and Sayana at 400 mM.

Seedling establishment assessment

Salinity had a significant impact on seedling establishment parameters in all quinoa genotypes studied. Significant differences in SL were observed at all salinity levels except under control conditions. Genotype Baer showed the greatest SL reduction across all salinity stress levels (Fig 3a). In contrast, the smallest SL reduction was recorded in genotype Puno at 50 mM and 300 mM, 11WAQ at 100 mM and 200 mM, and Sayana at 400 mM. Under non-saline conditions, no significant differences in RL were observed among the genotypes. However, significant variations emerged under salinity stress (Fig 3b). Similar to SL, the greatest reduction in RL compared to the control was seen in Baer, followed by Negra Collana across all stress levels. On the other hand, the smallest reduction in RL was observed in Faro at 50 mM and 200 mM, in Puno at 100 mM, and in 11WAQ at 300 mM and 400 mM salinity levels.

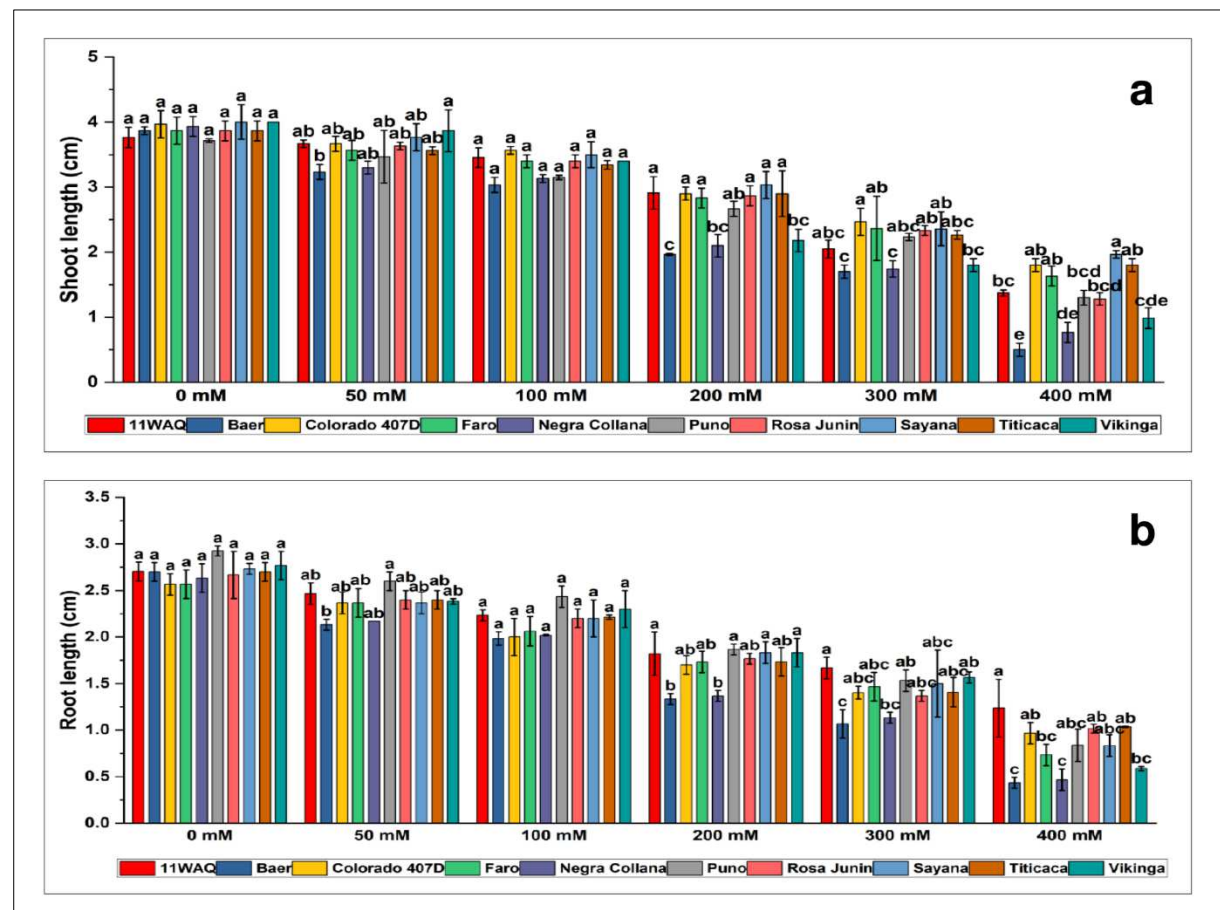


Fig 3 Shoot length (a) and root length (b) of ten quinoa genotypes subjected to five NaCl concentration levels. Data represent the mean values across n=10 samples for SL and with five replicates per genotype. Error bars indicate the standard deviation of five replicates. Genotypes not connected by the same letter within a treatment level are significantly different.

A similar trend was observed in FW, DB and SVI (Fig 4a-4c). Significant differences in FW were observed across all salinity stress levels (Fig 4a), with the greatest reduction seen in genotype Negra Collana across all salinity levels. In contrast, the smallest reduction in DB was observed in Sayana at 50 mM, Rosa Junin at 100 mM, and Colorado 407D at 200 mM, 300 mM, and 400 mM. Like FW, significant differences in DB were observed across all salinity levels (Fig 4b), with the highest decrease observed in genotype Negra Collana while the lowest decrease was shown in genotype Colorado 407D across all stress levels. Significant differences in SVI were also observed at all salinity stress levels, except under control conditions (Fig 4c). The highest decrease in SVI was found in the genotypes Negra Collana and Baer across all salinity levels, while the lowest decrease occurred in Puno at 50 mM and 100 mM, Faro at 200 mM and 300 mM, and Titicaca at 400 mM.

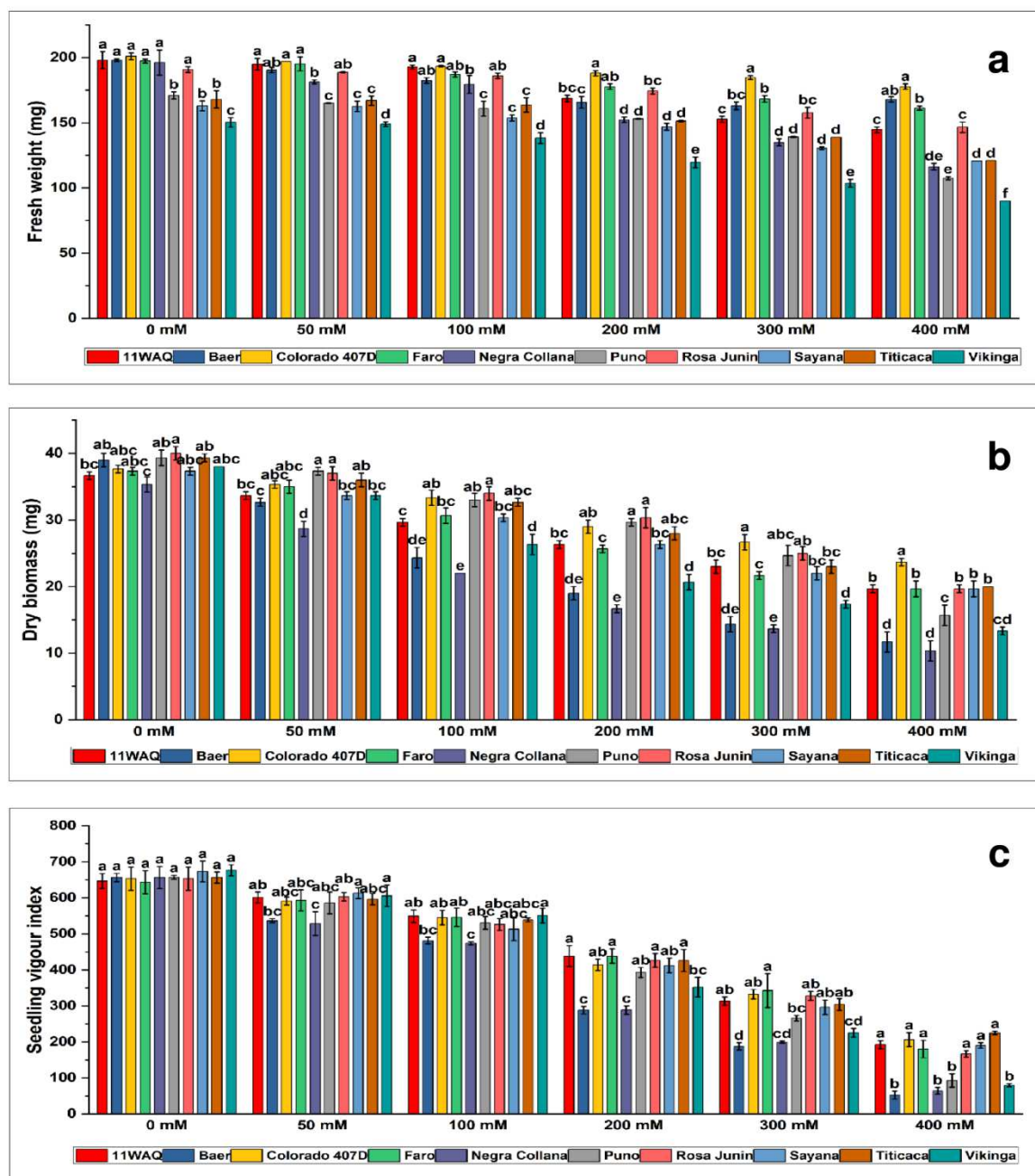


Fig 4 Fresh weight (a), dry biomass (b) and seedling vigour index (c) of ten quinoa genotypes subjected to five NaCl concentration levels. Data represent the mean values across $n=50$ samples with Five replicates per genotype. Error bars indicate the standard deviation of five replicates. Genotypes not connected by the same letter within a treatment level are significantly different.

Physiological traits assessment

Photosynthetic Pigment Content Assessment

Salinity significantly affected photosynthetic pigments content, with varying degrees of impact observed across genotypes. Significant differences in all photosynthetic pigment content were observed at all salinity levels (Fig 5a-5c). Genotype Negra Collana exhibited the highest reduction in chlorophyll A content across all salinity stress levels (Fig 5a). In contrast, the lowest reduction in chlorophyll A content was observed in Colorado 407D across all salinity stress levels, except at 200 mM, where Sayana recorded the lowest reduction.

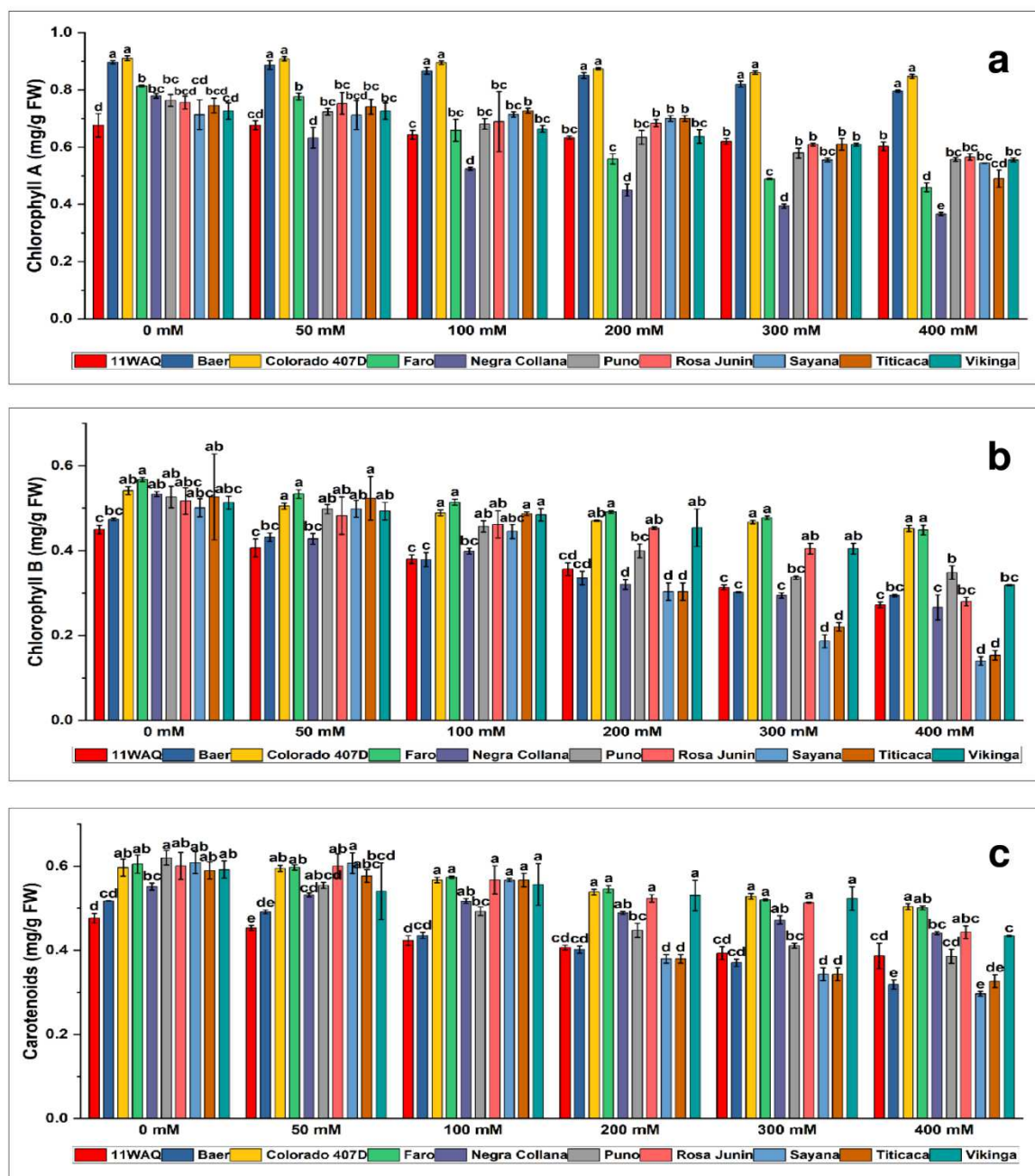


Fig 5 Chlorophyll A content (a), chlorophyll B content (b) and carotenoids content (c) of ten quinoa genotypes subjected to five NaCl concentration levels. Error bars indicate the standard deviation of five replicates. Genotypes not connected by the same letter within a treatment level are significantly different.

Similar reductions were observed in chlorophyll B and carotenoid content. Genotype Negra Collana showed the greatest reduction in chlorophyll B content under 50 mM and 100 mM, Titicaca under 200 mM, and Sayana under 300 mM and 400 mM (Fig 5b). In contrast, the smallest reduction in chlorophyll B content was observed in Sayana at 50 mM, Vikinga at 100 mM and 200 mM, and Colorado 407D at 300 mM and 400 mM treatments. The highest reduction in carotenoid content was recorded in Puno at 50 mM and 100 mM, and Sayana at 200 mM, 300 mM, and 400 mM treatments (Fig 5c). Conversely, the lowest reduction in carotenoid content was recorded in Rosa Junin at 50 mM, Titicaca at 100 mM, and Colorado 407D at 200 mM, 300 mM, and 400 mM NaCl treatments.

Antioxidant Enzyme Activity Assessment

The activities of antioxidant enzymes were also significantly influenced by salinity, with enzyme activities generally increasing as salinity stress intensified (Fig 6a-6c). However, the response varied among the ten quinoa genotypes studied. Genotype Puno exhibited the highest increase in superoxide dismutase (SOD) activity across

all salinity levels, while genotype 11WAQ showed the smallest increase in SOD activity across all stress treatments (Fig 6a). The greatest increase in catalase (CAT) activity was observed in genotype Colorado 407D across all stress levels, whereas the lowest increases were noted in Titicaca at 50 mM, Puno at 100 mM and 200 mM, Faro at 300 mM, and Negra Collana at 400 mM (Fig 6b). Genotype Puno also recorded the highest increase in peroxidase (POD) activity across all stress levels, while the lowest increases in POD activity were observed in Vikinga at 50 mM, Rosa Junin at 100 mM, and Faro at 200 mM, 300 mM, and 400 mM NaCl treatments (Fig 6c).

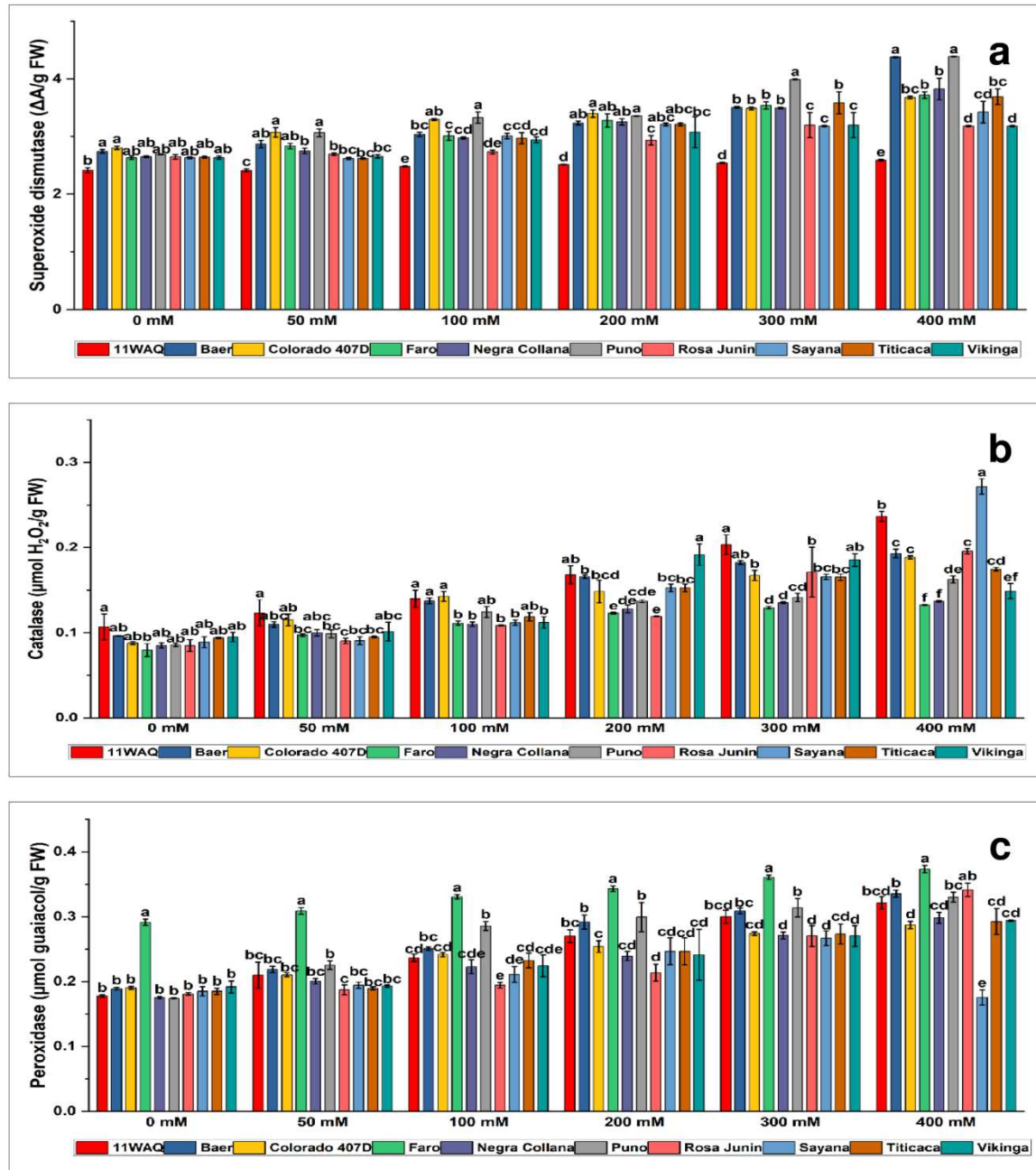


Fig 6 Superoxide dismutase activity (a), catalase activity (b), and peroxidase activity (c) of ten quinoa genotypes subjected to five NaCl concentration levels. Error bars indicate the standard deviation of five replicates. Genotypes not connected by the same letter within a treatment level are significantly different.

Salinity tolerance

Tolerance indices, SSI (Stress Susceptibility Index) and STI (Stress Tolerance Index), were used as proxies to assess the salinity tolerance of the ten quinoa genotypes under study. Under salinity stress conditions, the highest SSI values were recorded in genotype Negra Collana, followed by Baer across all NaCl stress levels. In contrast, the lowest SSI values were observed in genotype Puno at 50 mM and Colorado 407D at the remaining salinity

treatment levels. The STI values varied among the genotypes but remained above 1.0 for all genotypes and NaCl stress levels, indicating desirable salt tolerance, except for Negra Collana.

Table 4 Salinity susceptibility and tolerance indices of ten quinoa genotypes in five salinity stress treatments.

Tolerance Indices	Genotypes	50 mM	100 mM	200 mM	300 mM	400 mM
Stress susceptibility index (SSI)	Colorado 407D	0.64	0.52	0.68	0.66	0.68
	Sayana	1.01	0.85	0.87	0.93	0.87
	Rosa Junin	0.77	0.68	0.72	0.84	0.93
	Baer	1.67	1.71	1.52	1.43	1.29
	Faro	0.64	0.81	0.92	0.95	0.87
	Titicaca	0.87	0.77	0.85	0.94	0.90
	Negra Collana	1.94	1.73	1.56	1.48	1.30
	11WAQ	0.84	0.87	0.84	0.84	0.85
	Puno	0.52	0.73	0.73	0.84	1.11
	Vikinga	1.17	1.39	1.35	1.23	1.19
Stress tolerance index (STI)	Colorado 407D	1.13	1.43	1.72	2.25	2.97
	Sayana	1.07	1.29	1.55	1.84	2.44
	Rosa Junin	1.26	1.55	1.92	2.24	2.62
	Baer	1.08	1.08	1.17	1.25	1.51
	Faro	1.11	1.30	1.51	1.81	2.44
	Titicaca	1.20	1.46	1.74	2.03	2.62
	Negra Collana	0.86	0.89	0.93	0.98	0.99
	11WQA	1.05	1.24	1.52	1.89	2.40
	Puno	1.25	1.48	1.84	2.17	2.05
	Vikinga	1.09	1.14	1.24	1.47	1.69

The classification tree, which considered all germination, growth, physiological, and biochemical parameters, revealed similarities in quinoa's germination and seedling establishment under the control treatment (Fig 7). However, differences in salinity tolerance became evident when the genotypes were exposed to salinity. The classification tree identified Baer and Negra Collana as the most salt-sensitive genotypes. When these genotypes were grown in a 50 mM NaCl solution, they clustered together with the remaining genotypes grown in a 100 mM NaCl solution. Similarly, when exposed to a 100 mM NaCl solution, these genotypes grouped with others subjected to 200 mM NaCl. This trend continued when these genotypes were grown in 200 mM, 300 mM, and 400 mM NaCl solutions. Notably, genotype Vikinga exhibited salt sensitivity when grown in 200 mM, 300 mM, and 400 mM NaCl solutions, while Puno displayed sensitivity when grown in 400 mM NaCl solution. Genotypes Baer, Negra Collana, Vikinga, and Puno under 400 mM formed a separate group (cluster 7) and exhibited the lowest germination and seedling establishment parameters.

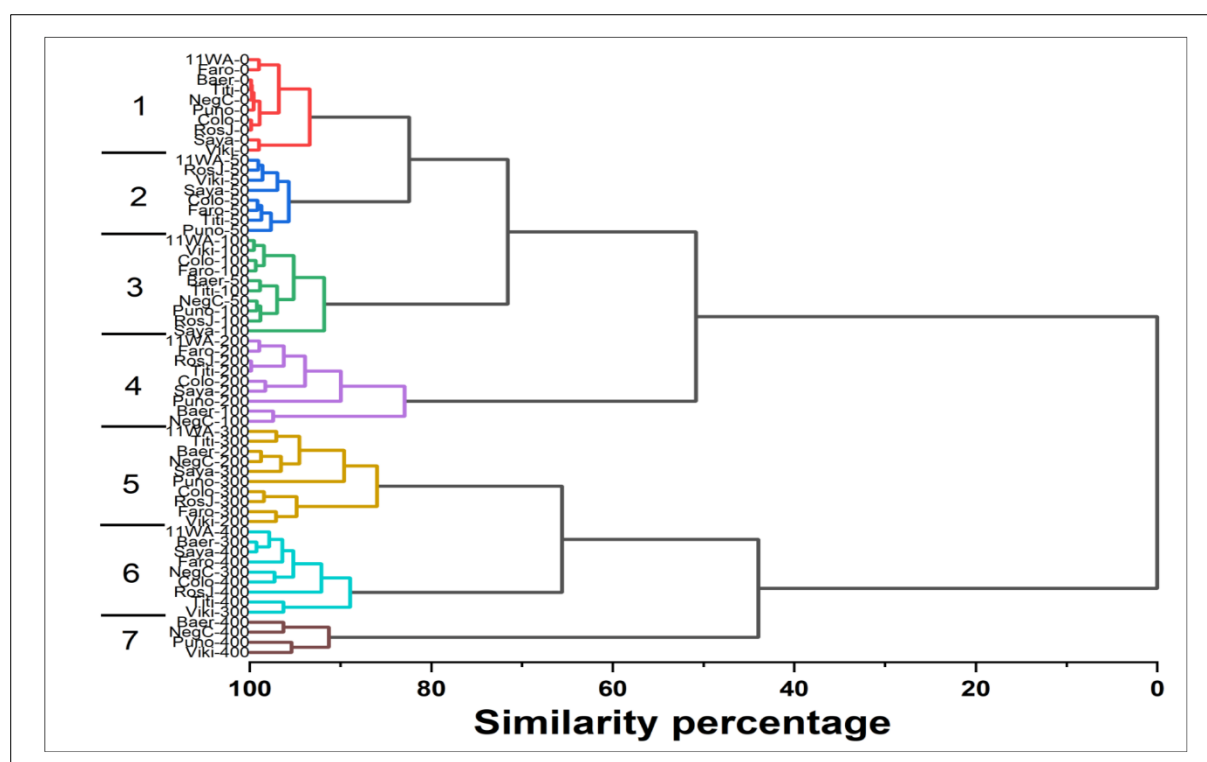


Fig 7 Hierarchical agglomerative cluster analysis based on all parameters in all Five NaCl concentration and control treatments. Clusters 1-7 according to similarity in germination, growth and physiological parameters.

Discussion

The early stages of plant development are crucial, as they heavily influence the plant's overall health, growth, and productivity (Wolny et al., 2018). During these phases, plants are particularly vulnerable to environmental stresses such as salinity. High salinity levels can cause osmotic stress, leading to reduced water uptake, and ionic toxicity, which can disrupt essential cellular processes. This can result in stunted growth, chlorosis, and even plant death. The effect of salinity on growth varies by species and genotype (Atta et al., 2023). Therefore, monitoring genetic variability is essential for improving salinity tolerance and understanding salinity tolerance mechanisms (Muhammad et al., 2024). This study aimed to explore quinoa's response to salinity and identify salinity-tolerant and sensitive genotypes during germination and seed establishment.

Quinoa genotypes showed varied responses to salinity during early plant development. The study found that as salt stress increased, GP, SL, RL, FW, DB, SVI, Chla, Chlb and Car decreased, while MGT, T50, and antioxidant enzyme activities (SOD, CAT, POD) increased. Despite these adverse effects, all ten genotypes successfully germinated at all salinity levels, indicating at least some level of resilience across all varieties studied. This suggests that these genotypes have potential for breeding salt-tolerant crops, which is vital for food security, economic stability, and environmental sustainability in salinity-affected regions.

Salinity significantly reduced seed germination percentages across all tested genotypes. This finding is consistent with previous studies demonstrating the negative impact of salinity stress on germination rates in various crops. For example, seed germination was reduced by 25% in wheat (Gholizadeh et al., 2021), 30.4% in soybeans (Zhou et al., 2023), and 57% in sorghum (Rajabi Dehnavi et al., 2020) under saline conditions. Both in these studies and the current study, the negative correlation between salinity and germination rate varied depending on the salt concentration. This correlation can be attributed to the osmotic and ionic factors associated with salinity (Panuccio et al., 2014).

At low NaCl concentrations, the reduction in seed germination is primarily due to osmotic stress, which decreases the water potential in the growth medium, making it difficult for seeds to absorb water. As a result, seeds are unable to initiate and sustain the metabolic, enzymatic, and hormonal processes important for germination. At higher salt concentrations, ionic stress becomes a significant factor in reducing seed germination. High concentrations of sodium (Na⁺) and chloride (Cl⁻) ions can be toxic to seeds, interfering with cellular processes (Alharbi et al., 2022), reducing metabolic activity (Balasubramaniam et al., 2023), disrupting enzymatic activities (de la Reguera et al., 2020), disturbing hormonal balance (Zhao et al., 2021), and reducing the use of seed reserves

(Atta et al., 2023). Among the ten quinoa varieties tested, four exhibited significant reductions in germination: Sayana at 100 mM, Negra Collana at 200 mM, Vikinga at 300 mM, and Puno at 400 mM. These differences in response to different salinity levels highlight the vast genetic and inheritance variation among quinoa genotypes.

The effects of both osmotic and ionic factors significantly extended the time required for seeds to begin and complete the germination process. This extension affected the overall mean germination time and the time to 50% germination for the tested genotypes. Among them, Negra Collana recorded the highest increase in both traits. The prolonged germination time indicates the seeds' struggle to overcome the adverse conditions caused by high salinity, further emphasizing the inhibitory effects of salt stress. Similar effects have been observed in other studies, such as the response of quinoa to various salts, which showed significant impacts on germination and seedling growth (Panuccio et al., 2014). Additionally, research has highlighted that salinity delays germination and reduces germination rates through osmotic stress, ion toxicity, and oxidative stress (Cüneyt, 2020). Furthermore, the combined osmotic and specific ion effects on seed germination have been documented, illustrating the multifaceted nature of salt stress on plants (Panuccio et al., 2014; Sosa et al., 2005).

A similar pattern to the seed germination traits was observed in seedling growth parameters, where SL, RL, FW, DB, and SVI decreased in each assessed genotype with increasing NaCl concentration. Similarly to germination parameters, the degree of reduction differed depending on the genotype. Under saline conditions, the reduction of seedling shoot and root length is common because roots and shoots are the initial points of contact with the salts (Atta et al., 2023) and osmotic and ionic effects inhibit the maintenance of necessary nutrient levels essential for plant growth, ultimately limiting root emergence and seedling growth (Panuccio et al., 2014). The decrease in SL, RL, FW, DB, and SVI of quinoa genotypes with increasing salt concentration observed in this study aligns with results reported on sorghum (Rajabi Dehnavi et al., 2020), barley (Dell'Aversana et al., 2021), and quinoa (Parvez et al., 2020). Among the ten genotypes studied, Baer and Negra Collana showed the highest reduction in SL, RL, DB, and SVI at almost all NaCl concentration levels. Linking these results to the germination parameters examined, this study suggests that Baer and Negra Collana are the most salt-sensitive among the ten genotypes tested.

Salinity stress is reported to affect photosynthetic efficiency by damaging the photosynthetic machinery (Balasubramaniam et al., 2023). This study observed a decrease in photosynthetic pigment content with increasing NaCl concentration. Among the ten genotypes, the highest reduction in chlorophyll A and chlorophyll B content was observed in Negra Collana, while Puno and Sayana recorded the highest reduction in carotenoid content. Considering that this reduction in chlorophyll content directly impacts photosynthetic efficiency, it is not surprising that Negra Collana also exhibited the greatest declines in SL, RL, FW, DB, and SVI. The subsequent diminished photosynthetic capacity likely led to reduced energy availability for growth and development, resulting in the observed reductions in these growth parameters.

In their study on sorghum, (Rajabi Dehnavi et al., 2020) reported that reduced seed establishment parameters are due to the toxic effects of Na⁺ and Cl⁻ ions accumulating in chloroplasts, disrupting the photosynthetic machinery and reducing chlorophyll content. They demonstrated that salinity reduces intracellular CO₂ concentration and photosynthesis rate by causing stomatal closure. Furthermore, under high salt levels, Na⁺ can lower the transport rate of essential ions, such as NO₃⁻, reducing N-containing compounds and ultimately inhibiting plant growth and biomass accumulation (Atta et al., 2023).

Salinity stress in plants also triggers the overproduction of reactive oxygen species (ROS), leading to oxidative damage that can impair cellular functions and overall plant health (Hasanuzzaman et al., 2021). To mitigate these effects, plants activate various antioxidant defence mechanisms, including the production of enzymes such as SOD, CAT, and POD, which help to neutralize ROS and protect cellular components (Sachdev et al., 2021). In this study, we assessed SOD, CAT, and POD activities to understand the biochemical response mechanisms employed to mitigate ROS-induced damage and maintain cellular redox status. The results showed that antioxidant enzyme activities in quinoa increased with higher NaCl concentrations. However, the response varied among genotypes, suggesting inherent genetic differences in salt stress response mechanisms. The highest SOD and POD activities were recorded in genotype Puno, while the highest CAT activities were observed in Colorado 407D, indicating a potential genetic advantage in coping with oxidative stress caused by salinity.

Two tolerance indices, SSI and STI, were used to assess the salt tolerance of the ten quinoa genotypes based on their dry biomass. The SSI index is used to determine stress-tolerant genotypes based on the rate of yield changes compared to non-stress conditions, indicating higher yield stability (Rajabi Dehnavi et al., 2020). On the other hand, STI identifies genotypes with high yield potential and stress tolerance (Mubushar et al., 2022). Based on the SSI values obtained in this study, genotype Colorado 407D, followed by Rosa Junin, Faro, Titicaca, and Sayana, showed the highest stability under salinity conditions during initial growth stages. However, this stability does not necessarily indicate better performance under saline conditions, where based on STI values all genotypes

except Negra Collana demonstrated high yield potential and salt tolerance. It should be noted, however, that this study applied these indices based on dry biomass results and not actual yield. Therefore, an assessment of these genotypes throughout their full life cycle under salinity stress is recommended for more conclusive results.

Based on the individual parameters alone, it is difficult to discuss the salinity tolerance of all ten assessed genotypes comprehensively. This difficulty arises because salinity stress impacts multiple physiological and biochemical processes in plants, including water uptake, ion balance, and metabolic functions (Shams and Khadivi, 2023). Each genotype may respond differently to these stress factors, making it challenging to draw broad conclusions from isolated parameters. Therefore, a cluster analysis was used to compare all parameters across all treatments simultaneously. This holistic approach allows for a more integrated understanding of how each genotype copes with salinity stress. The results identified genotypes Baer, Negra Collana, Vikinga, and Puno as salt sensitive. However, the salt sensitivity of Vikinga and Puno was more pronounced at higher NaCl concentrations.

Conclusion

This study demonstrated significant differences among quinoa genotypes under salinity stress during germination and seedling establishment stages, with the findings highlighting the critical impact of saline conditions on quinoa during these stages. Identifying and selecting salt-tolerant genotypes is essential for agriculture, where this study demonstrates that quinoa shows considerable genetic variability under saline stress. These genetic differences provide valuable information about quinoa genotypes that can be cultivated in salt-affected areas, potentially improving production of this important crop across wide areas of farmland. Additionally, understanding the extent of salt tolerance in different quinoa genotypes can inform breeding programs aimed at enhancing salt tolerance.

Data availability All relevant data are within the manuscript.

Statements and Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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