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Evaluation of salt tolerance in quinoa (*Chenopodium quinoa* Willd.) germplasm cultivated in Algeria through seed germination, growth performance, chlorophyll content, and non-enzymatic antioxidant levels

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ABSTRACT

This study aimed to evaluate the salt stress response of four quinoa (*Chenopodium quinoa* Willd.) varieties (Q102, Giza 02, Q101, and Black) cultivated in Algeria by assessing a range of physiological and biochemical traits. Specifically, four seed germination traits, seven plant growth parameters, three chlorophyll fractions (total, chlorophyll a, and chlorophyll b, and four non-enzymatic antioxidants (proline, soluble sugars, total phenolics, and saponins) were measured. Salt stress was simulated using seven NaCl concentrations: 0 mM (control), 50 mM, 100 mM, 150 mM, 200 mM, 250 mM, and 300 mM. During the germination phase, the absolute decrease (AD), salt tolerance index (STI), and inhibition index (II) were identified as the most informative indicators for discriminating varietal salt tolerance across treatments. Cluster analysis grouped the varieties into two distinct categories, with Black and Q101 exhibiting the highest germination-stage salt tolerance. At the maturity stage, ANOVA results indicated that root length stress tolerance index (RLSTI), seed polyphenol content, and seed saponin content were the most significant traits associated with both non-enzymatic antioxidant responses and plant growth performance under salt stress. Furthermore, biplot analysis revealed Black as the most salt-tolerant variety under 250 mM NaCl, whereas Giza 02 and Q102 exhibited reduced tolerance. These findings suggest that indices such as germination stress index, RLSTI, and the contents of seed polyphenols and saponins can serve as reliable markers for the selection of salt-tolerant quinoa genotypes.

Keywords: Quinoa, salt stress, germination traits, growth traits, chlorophyll, non-enzymatic antioxidants

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is a nutrient-dense pseudo-cereal recognized for its high agronomic value and potential contribution to global food security. Its seeds are rich in essential macronutrients and micronutrients, including high-quality proteins, essential fatty acids, lipids, phenolic compounds, minerals, dietary fiber, and vitamins (Olmos et al., 2022). Quinoa's increasing agricultural importance is largely attributed to its exceptional adaptability to harsh environmental conditions, particularly saline soils characterized by high alkalinity and limited water availability. It is classified among the most halophytic crop species (Flowers & Colmer, 2015), exhibiting a notable capacity to tolerate a wide range of abiotic stresses, including drought and salinity.

These traits position quinoa as a strategic crop for cultivation in marginal and stress-prone agroecosystems (Lallouche and Hadjkouider, 2024).

Soil salinity remains a formidable barrier to agricultural productivity worldwide and is anticipated to worsen in the future. The impact of salinity on crop performance, particularly in quinoa, has been shown to vary considerably across genotypes (Ruiz-Carrasco et al., 2011; Hariadi et al., 2011; Lallouche and Hadjkouider, 2024). This genotypic variation has driven extensive research into quinoa's potential as a salt-tolerant crop. Quinoa's capacity to maintain growth and yield under saline conditions is largely attributed to its broad genetic diversity, making it an attractive candidate for cultivation in arid and semi-arid environments (Ruiz et al., 2016; Lallouche and Hadjkouider, 2024). Kaur et al. (2022) reported that quinoa can tolerate salinity levels ranging from 100 to 750 mM NaCl, emphasizing that salinity tolerance in this species is a complex trait governed by multiple physiological and biochemical mechanisms.

A key adaptation observed in quinoa is its efficient salt-exclusion mechanism, which reduces the ionic impact of salinity and facilitates osmotic adjustment during sudden increases in NaCl concentration (Terletskaya et al., 2023). However, certain quinoa germplasm exhibit reductions in morphological and physiological traits under saline conditions ranging from 50 mM to 400 mM NaCl, including decreases in root length, leaf area, plant height, shoot biomass, and dry root as well as overall plant vigor (Cueva-Flores et al., 2024; Türkoğlu et al., 2024). Adverse effects are also evident in seed germination rates and plant survival rates under similar stress conditions (Shi and Gu, 2020; Lallouche and Hadjkouider, 2024). Furthermore, salt stress frequently triggers an increase in secondary metabolites, which play a critical role in plant adaptation and stress response (Ruiz et al., 2016; Aina et al., 2022; Huang et al., 2024).

Proline, a prominent secondary metabolite, is widely recognized as a biochemical marker of salinity adaptation in plants (Lallouche et al., 2017). Several studies have also demonstrated that the accumulation of secondary metabolites is influenced by varying environmental conditions (Ksouri et al., 2007; Huang et al., 2024). Quinoa's ability to grow under adverse environmental conditions is mediated by multiple physiological and biochemical mechanisms, including alterations in chlorophyll content (total, a, and b), non-enzymatic antioxidants, and key morphological traits. An effective approach to counteract the detrimental effects of salt stress, particularly in sensitive crops, involves the identification of new genetic sources of tolerance and the application of targeted morphological selection strategies to develop salt-tolerant cultivars (Munns, 2005; Lallouche et al., 2017). Furthermore, a crop's overall salt tolerance is strongly influenced by its performance during seed germination and early developmental stages (Jahan et al., 2019; Tarchoun et al., 2022).

To effectively identify salt-tolerant germplasm, it is essential to evaluate plant responses across the entire life cycle, from germination to the reproductive stage. Such comprehensive assessments are critical for selecting genotypes that exhibit stable and consistent tolerance throughout all developmental phases. Despite the recognized importance of this approach, global quinoa production remains significantly limited by the crop's inherent sensitivity to salt stress. In response, several countries have initiated research programs aimed at enhancing quinoa cultivation under adverse conditions. Algeria is among the nations that have adopted quinoa as a strategic crop, supported by the Food and Agriculture Organization (FAO), which provided scientific and technical assistance. This collaboration facilitated an initial evaluation of quinoa's adaptability following its introduction to Algeria during the 2013–2014 growing season. Eight experimental sites, representing diverse agro-ecological zones, were selected for this purpose: Baïnem (Algiers), Guelma, Sétif, Adrar, Relizane, Biskra, Tiaret, and El Oued. Under this FAO-led international initiative, 16 quinoa varieties including Amarilla Marangani, Q21, Santamaria, Q12, Kancolla, Q29, Giza1, Q18, Blanca de Junin, Q26, Giza2, Q22, Q27, Salcedo Inea, Amarilla Sacaca, and Sajama, were evaluated for their performance in arid and semi-arid climatic conditions.

The primary objective of this study was to evaluate the phenological development of various quinoa varieties, assess key yield-related traits across different genotypes, and test locations. The first phase of the trials commenced in the autumn of 2014, conducted at seven sites: Baïnem (Algiers), Sétif, Biskra, Tiaret, Relizane, Adrar, and El Oued. Additional trials were carried out in Guelma and Relizane during the spring of 2015. The introduction of quinoa to Algeria aims to identify alternative crops that can be cultivated

on marginal lands affected by extreme temperatures, salinity, and drought. The central focus is to determine whether quinoa possesses the resilience necessary to thrive under the current and projected environmental challenges of the Saharan agricultural landscape, particularly as desertification intensifies. However, quinoa cultivation in Algeria is still in its early stages and has not yet reached the scale or national recognition needed for broad adoption.

To enhance the understanding and optimization of quinoa cultivation techniques in Algeria, several studies have been conducted across diverse agroecological regions (Oustani et al., 2023; mri et al., 2022). A study by Lallouche and Hadj Kouider (2024) explores the effects of halopriming, hormopriming, and hydropriming on seed performance in quinoa and their influence on salt stress tolerance under Algerian conditions. Additionally, Lallouche and HadjKouider (2025) provide the first assessment of phenotypic diversity in four quinoa populations cultivated in Algeria, focusing on morphological traits. However, despite these efforts, there remains a significant gap in scientific research concerning the genetic tolerance of quinoa to salt stress. Moreover, the limited availability of genetic material presents a substantial challenge for plant breeders aiming to enhance salt tolerance (NaCl) in quinoa.

The objective of this study was to evaluate the response of different quinoa germplasm to salt stress, identify salt-tolerant genetic resources with potential applications in breeding programs, and establish reliable markers for classifying salt tolerance during the germination and seedling stages. This research advances our understanding of plant responses to salt stress, contributing to the development of more sustainable and resilient agricultural systems in the context of escalating environmental challenges.

MATERIALS AND METHODS

Plant material

The study material included four quinoa varieties cultivated in Algeria: Giza 02, Q101, Q102, and Black. The seeds, provided by the Technical Institute for the Development of Saharan Agronomy (ITDAS) in Algeria, were originally sourced from the United States Department of Agriculture (USDA).

Germination process and growing conditions

Twenty-five seeds from each variety were sterilized in a 1% sodium hypochlorite solution for 5 minutes, followed by three rinses with distilled water. The seeds were then sown in 1L plastic pots filled with a mixture of sand, agricultural soil, and potting soil in a 1:1:1 (v/v/v) ratio. The pots were placed in a greenhouse under controlled conditions of 22°C/15°C (day/night), with a photoperiod of 16/8 hours and relative humidity set at 50%. Following sowing, the pots were manually irrigated with either tap water (control; "EC = 1.55 mS/cm", "pH = 7.58") or saline solutions containing NaCl concentrations of 50 mM, 100 mM, 150 mM, 200 mM, 250 mM, and 300 mM (diluted in tap water). The experimental design was a completely randomized trial, with five replications for each variety and NaCl concentration. At the six-leaf stage, seedlings were thinned to three plants per pot. Irrigation was performed every two days during the first 25 days to maintain field capacity, after which the irrigation frequency was increased to every other day for the remaining 150 days, corresponding to the maturity stage.

Traits recorded

Germination and plant growth parameters

After seven days of germination, salt tolerance was assessed based on four parameters: germination percentage (GP), salt tolerance index (STI), inhibition index (II), and absolute decrease (AD), as outlined in Table 1. At maturity, plant growth parameters were measured, including plant fresh weight (PFW), plant height (PH), root length (RL), plant height/root length ratio (PHR), root fresh weight (RFW), and plant height stress tolerance index (PHSTI), root length stress tolerance index (RLSTI) as detailed in Table 1. Five plants were randomly selected from each treatment for measurement.

Leaf chlorophyll content

At the vegetative stage, the total chlorophyll (LChT), chlorophyll a (LCha), and chlorophyll b (LChb) contents were measured following the method of Lichtenthaler and Buschmann (2001). Briefly, fresh leaf tissues were ground in a mortar and pestle using 80% acetone. The optical density (OD) of the resulting solution was recorded for chlorophyll a and b at wavelengths of 662 nm and 645 nm, respectively, using a Shimadzu UV-1700 spectrophotometer.

Table 1. Description of traits used for evaluating quinoa varieties against salt stress during seed germination and plant growth stages

Trait	Code	Units	Formula
Germination percentage	GP	%	GP = (GS/TS) x 100
Salt tolerance index	STI	%	STI = (GPS/GPC) × 100
Inhibition index	II	%	II = [(GPC – GPS)/GPC] x 100
Absolute decrease	AD		AD = GPC – GPS
Plant height	PH	cm	after 150 days of germination
Root length	RL	cm	after 150 days of germination
Plant fresh weight	PFW	g	recorded by using a sensitive balance (Sartorius AC 1215, Germany
Root fresh weight	RFW	g	recorded by using a sensitive balance (Sartorius AC 1215, Germany)
Plant height /root length ratio	PRR		ratio of PH to RL
Plant height stress tolerance index	PHSTI	%	PHSTI = (PHS/PHC) x 100
Root length stress tolerance index	RLSTI	%	RLSTI = (RLS/RLC) x 100

Note: GS – total germinated seeds after seven days from swing, TS – total seeds, GPC – germination percentage without NaCl stress (control), GPS – germination percentage under salt stress, PHS – plant height under salt stress, PHC – plant height without NaCl stress (control), RLS – root length under salt stress, RLC – root length without NaCl stress (control).

Seeds non-enzymatic antioxidants content

Seeds collected at maturity from five plants of each treatment were analyzed for non-enzymatic antioxidants, including soluble sugars (SSS), proline (SP), saponin (SS), and polyphenols (SPh). SSS and SP contents were determined using the methods described by DuBois et al. (1956), Monneveux and Nemmar (1986), respectively. Saponin content was evaluated according to the protocol of Galindo et al. (1989), while seed polyphenol (SPh) content was measured following Romani et al. (2006).

Statistical analysis

Statistical analyses were performed on both control and stressed samples to determine significant differences across groups using a two-tailed test and ANOVA, with all computations carried out using StatBox Pro software. Mean comparisons were made using the Newman-Keuls test at a 5% significance level. Multivariate analyses were conducted using R software version 4.4.0, available from the Comprehensive R Archive Network (CRAN) at http://CRAN.R-project.org. Principal component analysis (PCA) was conducted using the FactoMineR package, and heatmap and dendrogram constructions were performed with the heatmap R library.

RESULTS

Seven NaCl concentrations (0, 50, 100, 150, 200, 250, and 300 mM) were applied to assess the direct effects of salinity on seed germination, growth, chlorophyll content, and non-enzymatic antioxidant levels of four quinoa varieties. Two-way ANOVA analyses (Tables 2, 3, and 4) indicated that salt stress significantly affected all recorded traits. These effects are detailed below.

Effect of salinity on seed germination

Figure 1 illustrates that at 0 mM NaCl, germination percentages ranged from 97% (for the Giza 02 variety) to 100% (for the Q102 variety). At low NaCl concentrations (50 mM), all varieties exhibited 100% germination, which was superior to the control. As NaCl concentrations increased from 100 to 250 mM, the germination percentage decreased, with the lowest values observed at 250 mM NaCl for all varieties. At 300 mM NaCl, germination was completely inhibited (Fig. 1). The most discriminative parameters were absolute decrease (AD) (F = 8.256), inhibition index (II) (F = 8.026), and salt tolerance index (STI) (F = 8.005), followed by germination percentage (GP) (F = 7.74). At 250 mM NaCl, the Q102 and Giza 02 varieties exhibited the highest AD and II values, indicating lower salt tolerance, while Q101 and Black demonstrated the lowest AD and II,

Table 2. Results of variance analysis based on mean square of examined seed germination

Source of variance	ddl	Mean Squares			
		GP%	AD	II	STI
Variety	3	107.86***	123.57***	124.62***	126.63***
NaCl	6	18242.04 ***	16571.25 ***	17183.76 ***	17944.75***
Variety x NaCl	18	14.02 ***	15.13 ***	14.21 ***	13.97 ***
CV %		2.31	2.85	2.76	2.53
Test F		7.749	8.256	8.026	8.005

Note: Degree of freedom (ddl); Coefficient of variance (CV %); germination percentage (GP), absolute decrease (AD), inhibition index (II), salt tolerance index (STI).

Table 3. Results of variance analysis based on mean square of examined plant growth potential traits

Source of variance	ddl	Mean Squares						
		PH	PFW	RFW	RL	PRR	RLSTI	PHSTI
Variety	3	362.48 ***	918.47 ***	0.43 ***	40.29 ***	1.18 ***	50.84 ***	156.30 ***
NaCl	6	18663.64 ***	3105.44 ***	5.12 ***	1361.218 ***	23.53 ***	11419.22 ***	11517.5 ***
Variety x NaCl	18	49.87 ***	32.79 ***	0.04 ***	10.12 ***	0.27 ***	183.91 ***	37.82 ***
CV %		1.20	2.30	4.6	3.80	2.98	1.80	4.80
Test F		74.811	61.952	14.9	17.82	36.17	155.79	4.05

Note: Plant height (PH), plant fresh weight (PFW), root fresh weight (RFW), root length (RL), plant height/root length Ratio (PRR), root length stress tolerance index (RLSTI), plant height stress tolerance index (PHSTI). *** $P < 0.001$ (highly significant).

Table 4. Results of variance analysis based on mean square of examined chlorophyll content in leaves, and non-enzymatic antioxidant levels in seeds

Source of variance	ddl	Mean Squares						
		LChT	LCha	LChb	SP	SS	SPh	SSS
Variety	3	0.37 ***	0.01 ***	0.25 ***	0.01 ***	6.95 ***	8813.76 ***	41.55 ***
NaCl	6	40.66 ***	5.47 ***	16.32 ***	11.432 ***	23.68 ***	46514.31 ***	14130.54 ***
Variety x NaCl	18	0.014 ***	0.002 ***	0.008 ***	0.001 ***	0.34 ***	255.56 ***	5.75 ***
CV %		0.39	0.81	0.51	2.97	0.86	0.42	0.95
Test F		51.76	10.65	43.14	7.89	560.53	706.74	10.95

Note: Chlorophylls (LChTT, LCha, LChb) content in leaves, proline content in seeds (SP), saponin content in seeds (SS), polyphenol content in seeds (SPh) and soluble sugar content in seeds (SSS).

indicating higher salt tolerance during germination (Fig. 1). STI was another key discriminative trait, with Black showing the highest tolerance at the 250 mM NaCl concentration, followed by Q101, which also displayed superior tolerance compared to the other varieties under this level of salt stress (Fig. 1).

Effect of salt stress on plant growth potential

The analysis revealed that salinity significantly influenced all observed growth parameters, including plant height (PH), plant fresh weight (PFW), plant height/root length ratio (PRR), and root length stress tolerance index (RLSTI). Of these, RLSTI (F-value = 155.793) was the most discriminative trait, followed by PH (F-value = 74.811), PFW (F-value = 61.95), and other traits such as PHSTI, root fresh weight (RFW), and root length (RL)which also exhibited significant genotype × NaCl interactions but with lower F-values (Fig. 2). At low NaCl concentrations (50 mM), plant growth was enhanced in all quinoa varieties compared to the

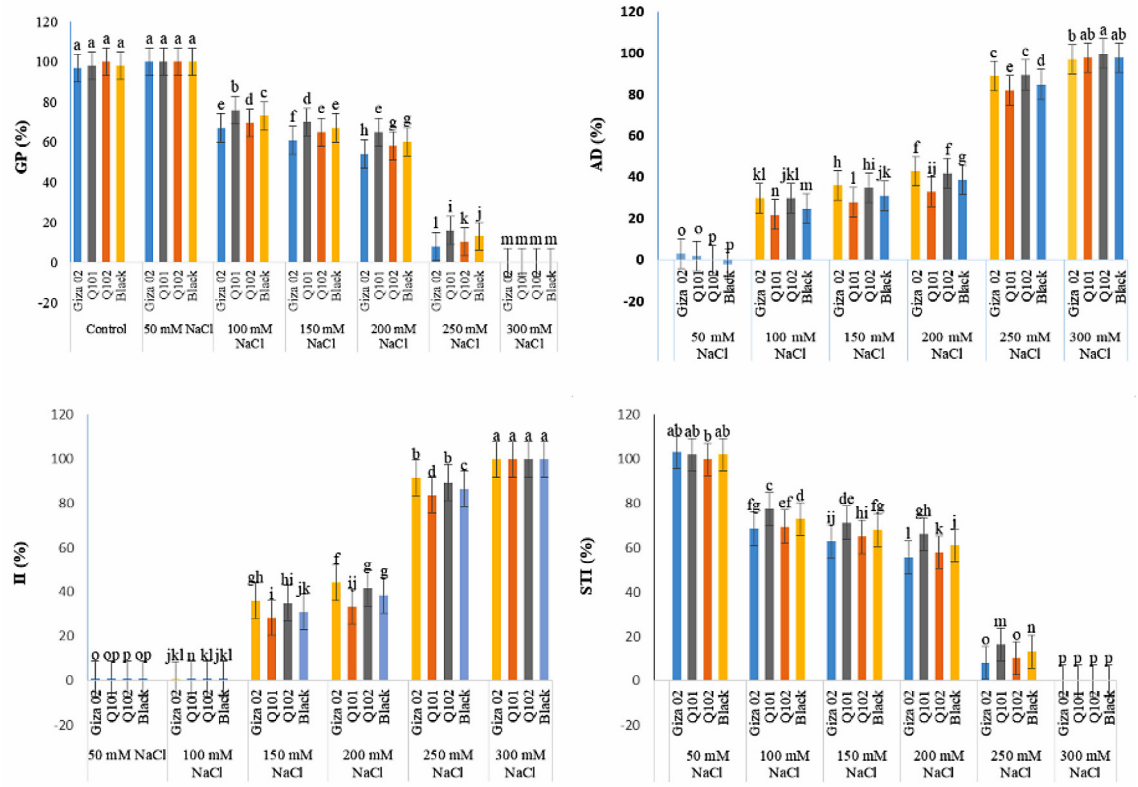

Figure 1. Changes in the rate of five measured variables related to the germination of four quinoa varieties subjected to seven levels of salt stress (NaCl): control, 50 mM, 100 mM, 150 mM, 200 mM, 250 mM, and 300 mM. The measured traits include germination percentage (GP), salt tolerance index (STI), absolute decrease (AD), and inhibition index (II). For each variable, means followed by the same letter are not significantly different at $P < 0.05$, as determined by the Newman-Keuls test. * indicates significance at $P < 0.05$, and *** indicates high significance at $P < 0.0001$.

control (0 mM). However, as NaCl concentrations increased from 100 to 250 mM, a negative impact on growth was observed across all varieties. The most discriminative parameter, RLSTI, exhibited a marked decrease under higher salinity stress (100, 150, 200, and 250 mM NaCl). Varieties Q102 and Giza 02 displayed the lowest RLSTI values (46.66% to 48.83%), indicating reduced salt tolerance, while Q101 and Black, the most salt-tolerant varieties, showed the highest RLSTI values (50.34% to 66.80%) (Fig. 2). Plant height (PH) was positively influenced by the low NaCl concentration (50 mM), with values ranging from 93.66 to 112 cm, compared to the control. However, moderate to high salinity levels (100, 150, 200, and 250 mM NaCl) resulted in a progressive reduction in plant height, with the most pronounced declines observed in Q102 and Giza 02 under 250 mM NaCl, indicating heightened sensitivity to salt stress at this concentration (Fig. 2).

Leaf chlorophyll and seed non-enzymatic antioxidant contents

ANOVA results revealed statistically significant differences in leaf chlorophyll content (total chlorophyll, LChT, chlorophyll a, LCha, and chlorophyll b, LChb) and seed non-enzymatic antioxidant contents (proline, soluble sugars, saponins, and polyphenols) across the four quinoa varieties exposed to different NaCl concentrations (0 to 250 mM) over a 150-day period. Salinity exerted a significant influence on all measured parameters, with polyphenol and saponin contents emerging as the most discriminating traits, as indicated by their high F-values (706.746 and 560.531, respectively). Although proline, soluble sugar, and chlorophyll contents were also significantly affected by the variety × NaCl interaction, they displayed comparatively lower F-values (Fig. 3). Polyphenol and saponin concentrations in seeds increased markedly with rising NaCl levels (100 to 250 mM) in all varieties. At 250

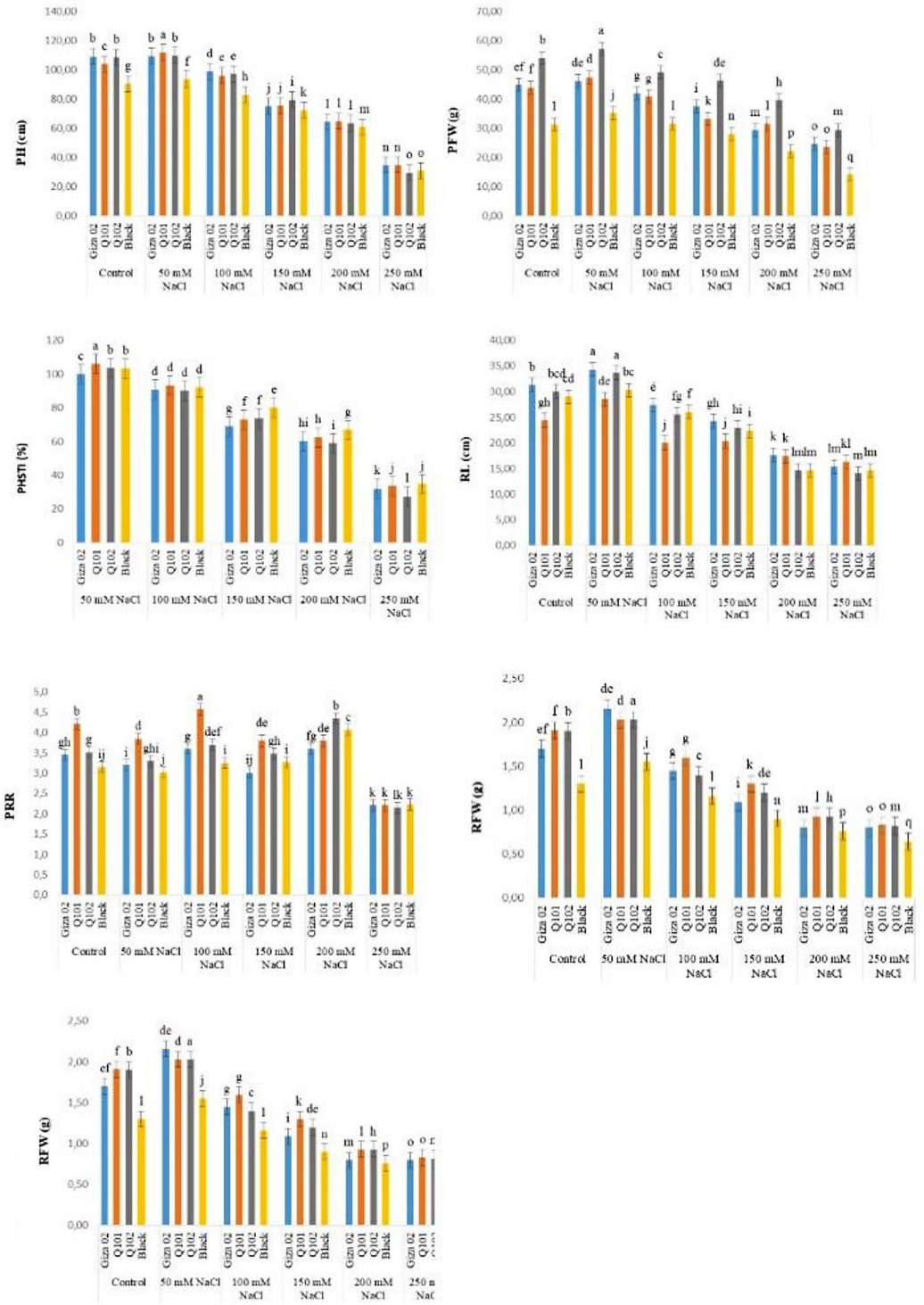

Figure 2 Variation in seven growth-related parameters among four quinoa varieties exposed to six salinity levels (NaCl): 0 (control), 50 mM, 100 mM, 150 mM, 200 mM, and 250 mM, assessed under pot conditions during the germination stage. The evaluated traits include plant height (PH), plant fresh weight (PFW), root length (RL), plant height/root length ratio (PRR), root fresh weight (RFW), root length stress tolerance index (RLSTI), and plant height stress tolerance index (PHSTI). Mean comparisons were conducted using the Newman-Keuls test; means followed by different letters are significantly different at $P < 0.05$. *** denotes highly significant differences at $P < 0.001$.

mM NaCl, polyphenol content rose by 1.09 to 1.16 times, while saponin content increased by 1.47 to 2.05 times relative to the control. Varieties Q102 and Giza 02, which exhibited the lowest polyphenol accumulation, were identified as less salt-tolerant. Conversely, Q101 and Black, the more salt-tolerant genotypes, recorded the highest polyphenol levels (Fig. 3). Proline and soluble sugar contents followed similar trends, displaying elevated concentrations in response to salt stress. In contrast, total chlorophyll (LChT) and its components (LCha and LChb) decreased

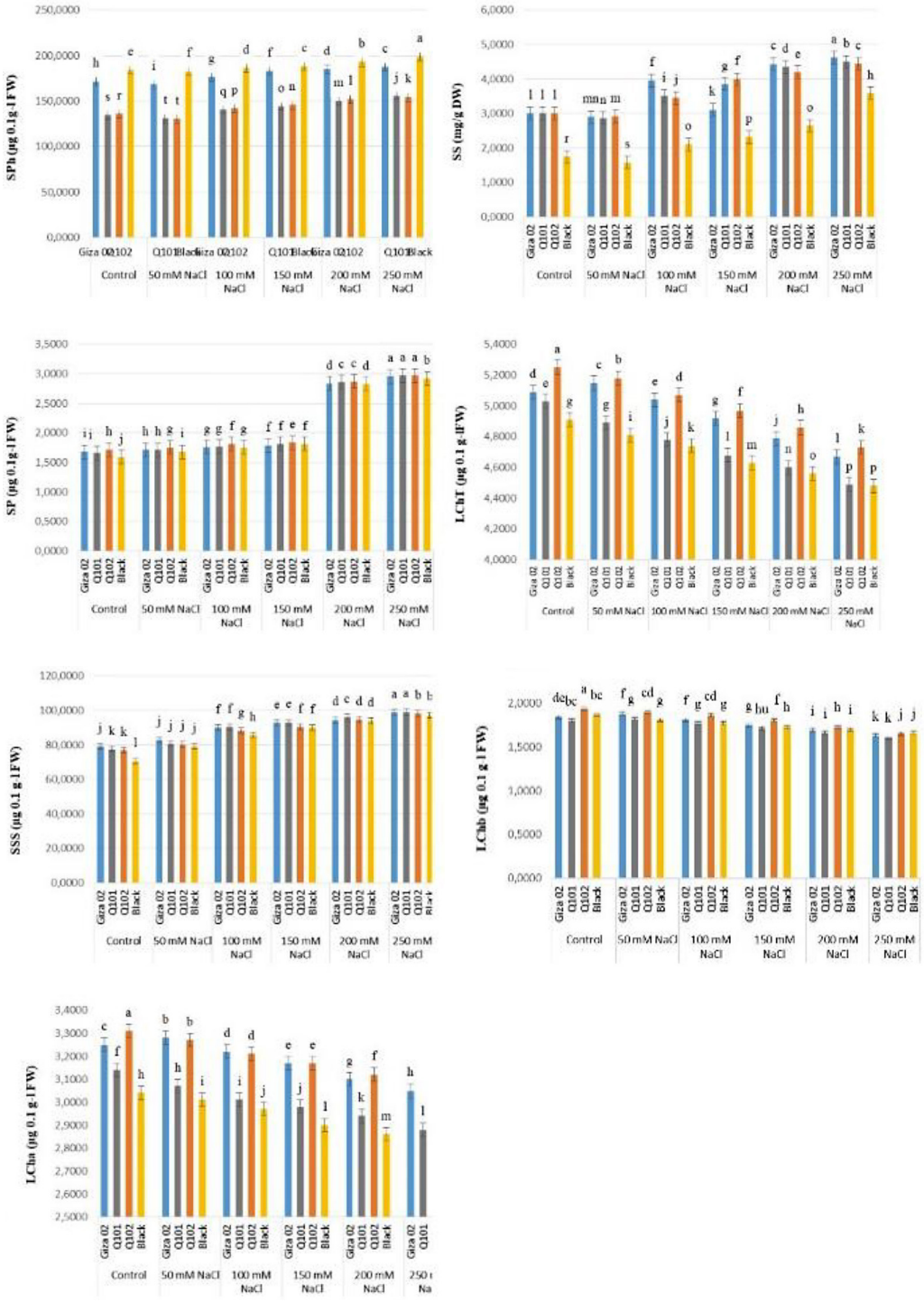

Figure 3. Variation in seven biochemical traits in seeds and leaves of four quinoa varieties subjected to six salinity levels (NaCl concentrations: 0, 50, 100, 150, 200, and 250 mM). The measured parameters include seed polyphenol content (SPh), saponin content (SS), soluble sugar content (SSS), proline content (SP), and chlorophyll contents in leaves: (T, a, b).

progressively with increasing salinity levels, particularly from 100 to 250 mM, indicating that salt stress adversely affected photosynthetic pigment accumulation and, by extension, plant physiological performance (Fig. 3).

Principal component analysis (PCA)

PCA was performed to investigate the relationships among all measured traits and to differentiate the responses of the four quinoa varieties under varying levels of salt stress. The analysis accounted for 95.1% of the total cumulative variance across germination parameters, growth traits, chlorophyll content, and non-enzymatic antioxidant levels (Fig. 4). The PCA grouped the varieties into five distinct clusters based on their overall tolerance to salinity stress.

The **first group** included Black and Q101 under 100 and 150 mM NaCl, and Giza 02 and Q102 under 100 mM. Within this group, Q101 and Black demonstrated superior salt tolerance, reflected in their higher values for RLSTI, leaf fresh weight (LFW), root length (RL), and plant height/root length ratio (PRR). In contrast, Giza 02 and Q102 exhibited lower values for these traits, indicating greater sensitivity to salt stress.

The **second group**, in direct contrast to the first, represented the threshold at which germination was severely inhibited across all varieties, marking a critical level of salt stress with a strong negative impact on seed performance.

The **final group** comprised Giza 02 and Q102 at 50 mM NaCl, which showed the most favorable response in terms of enhanced germination, seedling growth, and increased accumulation of non-enzymatic antioxidants, suggesting that mild salinity may have a stimulatory effect on these varieties.

Varieties clustering by heatmap of quinoa under salt stress conditions

Hierarchical clustering was conducted to provide an overview of the measured traits and to identify major clusters among the four quinoa varieties under varying salt stress concentrations. The clustering, based on Euclidean distance, revealed two primary clusters (A and B) for the studied varieties (Fig. 5).

Cluster A. Highly salt-tolerant varieties, characterized by their response to low salt stress (50 mM NaCl), which proved to be the most effective treatment for enhancing germination, seedling growth, and the accumulation of non-enzymatic antioxidants.

Hierarchical clustering further divided this cluster into two categories: Category 1, which included 100 mM, 150 mM, and 200 mM NaCl, represented varieties that exhibited salt tolerance but

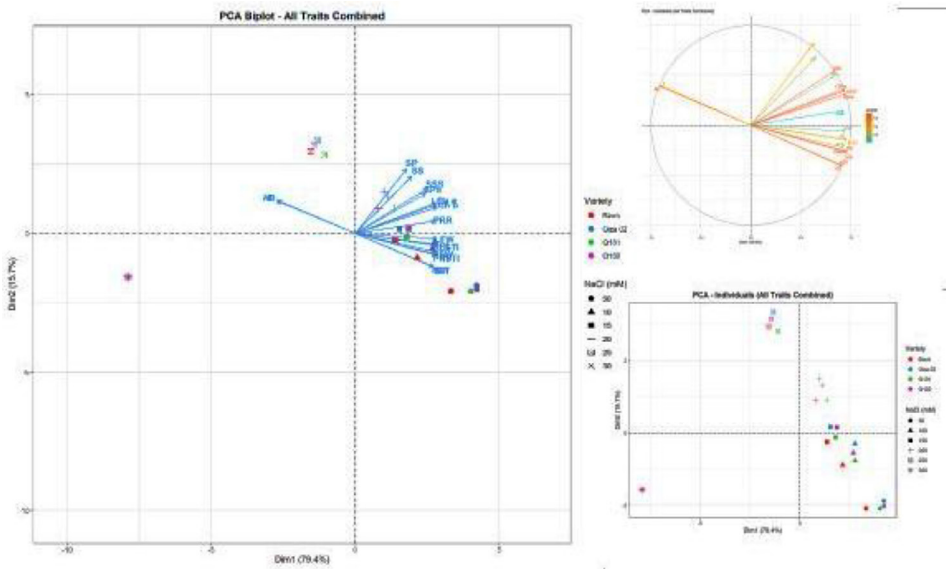

Figure 4. Principal Component Analysis (PCA) of all measured traits across four quinoa varieties subjected to varying levels of salt stress. **(A1)**: PCA biplot showing the distribution of quinoa genotypes under different salinity treatments based on their stress responses. **(A2)**: Graphical representation of each variable's contribution to the total variance in the PCA model, including the direction and magnitude of their influence on principal components.

with reduced resistance; and Category 2, which included 250 mM NaCl, where salt stress negatively impacted germination, seedling growth, and antioxidant accumulation in seeds.

Category 1. Salt-tolerant varieties, which included those exposed to salt stress at 100 mM, 150 mM, and 200 mM NaCl, were further divided into three subcategories.

Subcategory 1. Comprised of 100 mM (Giza, Q101, and Q102) and 150 mM (Q101 and Q102). This subcategory showed a decrease in germination percentage, plant growth, and chlorophyll (a and b) content in leaves, but higher levels of proline, soluble sugar, saponin, and polyphenol content in seeds.

Subcategory 2. Associated with 100 mM (Black) and 150 mM (Giza 02 and Black).

Subcategory 3. Comprised of 200 mM NaCl. This subgroup exhibited reduced germination percentage, chlorophyll (a and b) content in leaves, and plant growth, but higher levels of proline, soluble sugar, saponin, and polyphenol content in seeds. These varieties showed some tolerance but were less resistant to salt stress.

Category 2. Low salt-tolerant varieties. This category included all varieties exposed to 250 mM NaCl. It was associated with severe salt stress, which negatively affected germination, seedling growth, and antioxidant accumulation in seeds (Fig. 5). The Giza and Q102 varieties, which are relatively sensitive to salt stress, showed significant inhibition in germination and growth, along with lower non-enzymatic antioxidant accumulation compared to Q101. In contrast, Q101 exhibited potential tolerance to salt stress. Therefore, the most salt-tolerant varieties were ranked as follows: Black being the most tolerant, followed by Q101.

Cluster B. This cluster is characterized by the highest NaCl concentration (300 mM NaCl), identified as the threshold for germination inhibition, which significantly affected quinoa seed germination across all tested varieties (Fig. 5).

The analysis highlights the varying levels of salt tolerance among quinoa varieties, based on their responses in terms of germination, growth, chlorophyll content, and antioxidant accumulation under different salt concentrations. The results from the cluster analysis offer a more comprehensive understanding of how different NaCl concentrations influence quinoa seedling growth and seed antioxidant content.

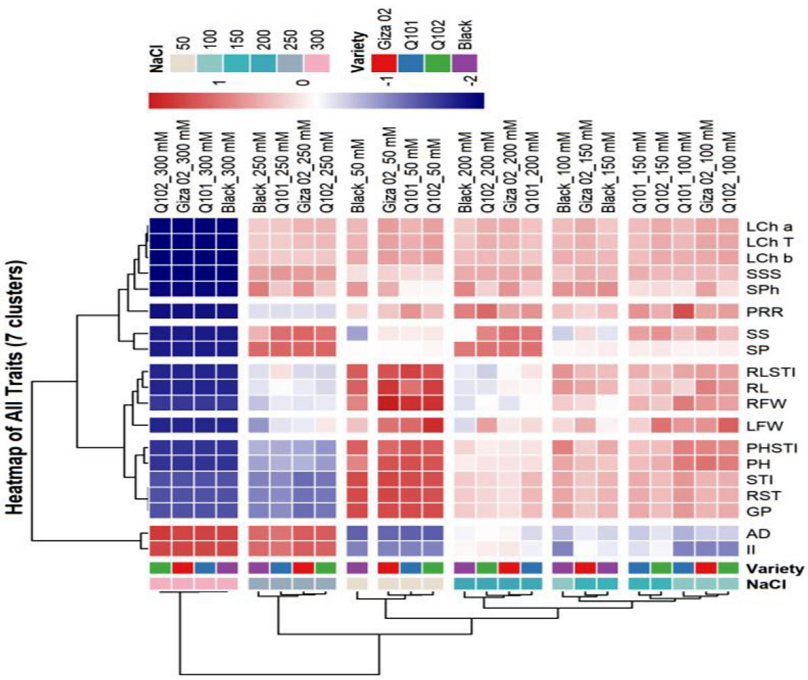

Figure 5. Heatmap illustrating hierarchical clustering of all measured traits across four quinoa varieties under varying salt stress levels (50 mM, 100 mM, 150 mM, 200 mM, 250 mM, and 300 mM NaCl). Clusters A and B represent groupings based on the varieties exposed to these salt stress concentrations. Clusters "G, H, I, J, K, L, and M" represent groupings based on the assayed traits. The color bar indicates the gradient of rate change values in response to salt stress conditions.

DISCUSSION

This study investigated the salinity tolerance of four *Chenopodium quinoa* varieties cultivated in Algeria by assessing the effects of salt stress on germination characteristics, growth parameters, and the accumulation of chlorophylls, proline, saponins, soluble sugars, and polyphenols in the seeds.

Analysis of variance (ANOVA; Tables 2, 3; Fig. 1, 3) for the evaluated parameters indicated that the inhibition index (II%), absolute decrease (AD%), and salt tolerance index (STI) during the germination stage, as well as the root length stress tolerance index (RLSTI), seed polyphenol content, and seed saponin concentration at the maturation stage, were the most discriminative and reliable indicators of salinity tolerance.

Sodium chloride (NaCl) is recognized for its phytotoxic effects when accumulated in plant root tissues. Previous studies (Epron et al., 1999; Lallouche et al., 2017; Lallouche and Hadjkouider, 2024) have extensively documented plant responses to NaCl-induced stress. In the present study, salt stress resulted in a marked reduction in germination traits, vegetative growth, and the levels of non-enzymatic antioxidants, particularly at higher NaCl concentrations (250–300 mM). Notably, at 300 mM NaCl, seed germination was completely inhibited in all tested varieties (Fig. 1, 3).

Interestingly, exposure to a low concentration of sodium chloride (50 mM NaCl) exhibited a stimulatory effect on seed germination and early plant growth (Fig. 1, 3). This concentration significantly increased germination rates, enhanced seedling development, and elevated chlorophyll content in the leaves. These observations suggest that low levels of NaCl may act as a mild abiotic stressor, eliciting adaptive metabolic responses that enhance plant vigor without inducing toxicity.

The application of 50 mM NaCl appears to trigger moderate stress that promotes beneficial physiological and biochemical processes, including improved germination, enhanced seedling growth, and increased accumulation of non-enzymatic antioxidants. In contrast, progressively higher concentrations of NaCl (100–250 mM) led to a gradual decline in these parameters, while 300 mM NaCl exerted severe inhibitory effects on both germination and plant development (Fig. 1, 3). These findings highlight the critical importance of identifying optimal salt concentrations to enhance quinoa's salinity tolerance (Fig. 4 and 5).

This study provides valuable insights into the salinity responses of quinoa, emphasizing the potential of mild salt stress to enhance seedling growth and the accumulation of non-enzymatic antioxidants across varieties with varying salt tolerance levels (Fig. 1, 3).

The beneficial effects observed at low NaCl concentrations, particularly at 50 mM, may be attributed to the synergistic roles of Na^+ and Cl^- ions in stimulating germination and early growth. These ions likely contribute to osmotic adjustment, maintenance of ion homeostasis, and the activation of stress-responsive metabolic pathways. In contrast, exposure to higher concentrations, such as 300 mM NaCl, led to complete inhibition of seed germination, consistent with the phytotoxic effects of excessive salt – especially Na^+ accumulation in the root zone. These findings suggest that the positive physiological responses to 50 mM NaCl result from the coordinated actions of Na^+ and Cl^-, but only within a defined threshold. Beyond this threshold, salt stress imposes inhibitory effects on plant development.

The biphasic response, characterized by stimulatory effects at low to moderate salinity levels followed by growth inhibition at higher concentrations, has been documented in several studies on halophytic species (Ghanem et al., 2021; Pungin et al., 2023). These findings support the notion that low salinity can function as a mild abiotic stressor, activating adaptive physiological and biochemical mechanisms, whereas elevated salinity levels disrupt cellular homeostasis and impair plant growth. Understanding this dual response is essential for elucidating the strategies by which quinoa and other halophytes tolerate and adapt to variable saline environments.

The salt tolerance index (STI), inhibition index (II), and absolute decrease (AD) are key indicators commonly employed to assess plant responses to salinity stress (Fig. 1). These metrics are widely recognized for their effectiveness in comparing the performance of different accessions under saline conditions (Alatawi et al., 2025). In the present study, AD, II, and STI proved to be critical parameters for discriminating among quinoa varieties with differing salt tolerance levels, particularly at the germination stage under 250 mM NaCl (Fig. 1, 4, and 5).

The analysis identified two distinct varietal groups based on their salinity tolerance profiles. Group 1 (G1), comprising the Black and Q101 varieties, exhibited high salt tolerance, as evidenced

by low absolute decrease (AD: 82–85), low inhibition index (II: 83.6–86.73), and high salt tolerance index (STI: 13.34–16.33) values. These metrics reflect the ability of these varieties to maintain germination and early growth under high salt concentrations. In contrast, Group 2 (G2), which included Giza 02 and Q102, was classified as low salt-tolerant. These varieties displayed higher AD (89–89.66), higher II (89.66–91.7), and lower STI (8.23–10.33) values, indicating greater sensitivity to salinity stress (Fig. 1, 4, and 5). Phenolic compounds, particularly polyphenols, play a crucial role in mitigating oxidative stress, a common consequence of salt-induced cellular damage (Ksouri et al., 2007; Aina et al., 2022; Huang et al., 2024). In the present study, a progressive increase in polyphenol content was observed in quinoa seeds with rising salinity levels up to 250 mM NaCl (Fig. 3).

This trend suggests that polyphenols may function as key antioxidant molecules, contributing to the plant's defense mechanisms under salt stress. Similar responses have been reported in other halophytic species, such as Cakile maritima (Ksouri et al., 2007), and in crops like broccoli exposed to moderate salinity (López-Berenguer et al., 2009), where elevated polyphenol accumulation was associated with enhanced stress tolerance.

Furthermore, this study observed an increase in saponin accumulation, particularly at higher NaCl concentrations (250 mM) (Fig. 3). This finding is consistent with previous research, which has demonstrated that salt stress can stimulate saponin production in species such as Acalypha wilkesiana (Odjegba and Alokolaro, 2013) and quinoa (Gómez-Caravaca et al., 2012). Like polyphenols, saponins are believed to play a role in the plant's defense mechanisms against oxidative stress, potentially enhancing the plant's resistance to salinity-induced damage.

This study underscores the differential responses of quinoa varieties to salt stress, with low to moderate NaCl concentrations promoting germination and growth, while higher concentrations inhibit these processes (Fig. 1, 3). These findings highlight the importance of elucidating the underlying mechanisms of salt tolerance in quinoa and other halophytes. The utilization of phenolic compounds and saponins as antioxidants under salt stress emerges as a potential strategy to enhance salt tolerance in quinoa. Additionally, the study emphasizes the value of employing indices such as salt tolerance index (STI), inhibition index (II), and absolute decrease (AD) for effectively classifying quinoa varieties based on their salinity tolerance. This provides critical insights for future breeding programs aimed at improving quinoa's resilience to salinity.

In the high salt-tolerant varieties, Black and Q101, a significant increase in polyphenol content and saponin accumulation was observed in seeds subjected to salt stress (250 mM NaCl) (Fig. 4, 5). This observation is consistent with previous studies that emphasize the role of secondary metabolites, such as polyphenols and saponins, in enhancing plant tolerance to salt stress. Polyphenols are well-known for their antioxidant properties, which help mitigate the oxidative stress induced by excessive salinity in plants (Wahid and Ghazanfar, 2006). Similarly, saponins act as part of the plant's defense system, classified as phytoanticipins or phytoprotectants – protective compounds synthesized in response to abiotic stresses like salinity (Lacaille-Dubois and Wagner, 2000). In contrast, low salt-tolerant varieties, such as Giza 02 and Q102, exhibited a decrease in the accumulation of these metabolites, suggesting a limited capacity to synthesize protective compounds under salt stress.

The role of secondary metabolites, including polyphenols, saponins, soluble sugars, and proline, in enhancing plant salt tolerance has been well-documented across numerous plant species exposed to salinity stress (Bistgani et al., 2019; Ghanem et al., 2021; Birhanie et al., 2022). These metabolites contribute to osmotic regulation, antioxidative defense, and membrane stabilization, all of which are essential for maintaining cellular integrity under salt stress. However, the specific effects of these metabolic changes (whether beneficial or detrimental) can vary based on factors such as plant species, developmental stage, and the intensity of salt stress. The results of the present study suggest that all quinoa varieties examined adopt a similar strategy in response to salt stress, potentially involving the coordinated accumulation of these metabolites to mitigate the adverse effects of salinity.

This strategy involves a reduction in growth parameters and chlorophyll content in the leaves, coupled with the activation of the non-enzymatic defense system, which leads to the increased biosynthesis of antioxidant compounds. These compounds, polyphenols, saponins, soluble sugars, and proline, aid in mitigating the detrimental effects of salt stress while requiring less energy compared to enzymatic defense systems (Ghanem et al., 2021). This response pattern suggests that the salt tolerance mechanisms in the quinoa

varieties studied in Algeria may be similar, with a primary focus on the accumulation of these secondary metabolites as a key adaptive strategy.

However, further in-depth research is necessary to confirm these findings and to better understand the metabolic pathways involved in the synthesis of these compounds under salt stress. Additionally, investigating the molecular mechanisms underlying salt tolerance in quinoa could provide valuable insights for developing more resilient quinoa varieties, specifically tailored for cultivation in saline environments.

CONCLUSIONS

The findings of this study suggest that quinoa varieties can achieve optimal germination (100%) under 50 mM NaCl conditions. Mild salt stress (50 mM NaCl) appears to induce salt tolerance in quinoa through several mechanisms, including enhanced seedling vigor, improved seed germination rates, increased chlorophyll synthesis, and the accumulation of secondary metabolites such as proline, soluble sugars, saponins, and polyphenols. These metabolic changes collectively contribute to enhanced salt tolerance in quinoa seedlings. At higher salt stress levels (250 mM NaCl), the Black variety demonstrated high salt tolerance, while Q101 exhibited moderate tolerance compared to the other quinoa varieties. Based on these results, specific traits, including the absolute decrease (AD), salt tolerance index (STI), inhibition index (II), root length stress tolerance index (RLSTI), and seed content of saponins and polyphenols, can serve as reliable parameters for assessing salt tolerance in quinoa varieties. Further research is necessary to confirm these mechanisms and explore the genetic and molecular foundations of salt tolerance in quinoa. Ultimately, this knowledge could facilitate the development of quinoa varieties better suited for cultivation in saline-affected regions.

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