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Research Article

Salt Tolerance Variation among *Chenopodium quinoa* Accessions of Contrasting Geographic Origins: Germination Performance and Antioxidant Defense under NaCl Stress

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Abstract

Background and Objective: *Chenopodium quinoa* Willd. is a strategic crop for food security in salt-affected regions; however, genotype-specific physiological responses to salinity during early germination remain insufficiently characterized. Understanding variation among geographically distinct accessions is essential for identifying salt-tolerant genotypes. This study aimed to evaluate germination performance and antioxidant defense responses of three quinoa accessions of contrasting geographic origins under salinity stress. **Materials and Methods:** Three quinoa accessions 18GR (Ames 13724, USA), R-132 (PI 478418, Bolivia) and DE-1 (PI 674266, Ecuador) obtained from the USDA National Plant Germplasm System were evaluated under four NaCl concentrations (0, 100, 200 and 300 mM) under controlled laboratory conditions. Germination percentage, mean germination time (MGT), radicle length and germination kinetics were recorded. Reversibility assays were conducted to differentiate osmotic and ionic effects of salinity. Oxidative stress markers, including Hydrogen Peroxide (H₂O₂) and malondialdehyde (MDA), were quantified along with antioxidant enzyme activities such as peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX). Proline accumulation was also assessed as an indicator of osmotic adjustment. **Results:** Salinity significantly affected germination traits in an accession-dependent manner. At 300 mM NaCl, germination rates were 90, 60 and 33.33% for 18GR, R-132 and DE-1, respectively. Reversibility assays indicated osmotic stress predominance at 200 mM, while ionic toxicity dominated at higher concentrations. NaCl stress increased H₂O₂ and MDA accumulation in all accessions. POD activity increased consistently across treatments. CAT and APX activities in 18GR showed a biphasic response, being stimulated up to 200 mM and inhibited at 300 mM, indicating stress threshold regulation. Proline accumulation increased with salinity, with 18GR showing the highest osmotic adjustment capacity. **Conclusion:** Geographic origin significantly influences salt tolerance during germination in quinoa. Accession 18GR exhibited superior stress tolerance and antioxidant regulation, making it a promising candidate for saline agriculture. The contrasting biochemical responses provide a physiological basis for selecting salt-tolerant quinoa genotypes adapted to arid and semi-arid environments.

Key words: *Chenopodium quinoa* Willd., germination, oxidative stress, antioxidant enzymes, NaCl tolerance, genotype selection

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INTRODUCTION

Global water shortages and soil salinization are being made worse by climate change, which poses serious risks to agricultural production and food security worldwide. Due to the scarcity of water in arid and semi-arid areas, these interconnected problems are especially severe in those regions^{1,2}.

Salinity is a growing problem that affects both soil and water and it is the major challenge for farmers, environmentalists and communities around the world. This abiotic stress causes detrimental effects on plants. Soil salt is an abiotic obstacle that plants have to overcome, notably in dry and semi-arid areas. Maintaining and conserving food production requires the agronomic trait of tolerance to increased salt levels³. Salinity highly affects the germination process owing to ion toxicity and negative effects on osmosis⁴. In addition, these ions cause oxidative stress, which can lead to an imbalance between the antioxidant defense system and the generation of reactive oxygen species (ROS). Overproduction of ROS can harm lipids, proteins, DNA and other biological components, which will hinder germination. The processing of the nutrients that seeds retain to produce energy for germination involves several enzymes. These enzymes' activity can be inhibited by salinity, which prevents nutrients from being mobilized and energy from being produced for seed germination^{3,5,6}.

Considering climate change and food insecurity, several scientific communities worldwide have recently concentrated study on quinoa as a phytoresource with various benefits. In fact, this species has a growing significance as a resilient crop in regions with challenging environmental conditions like temperature elevation, water scarcity and salinization. It is a crop with excellent potential for the arid conditions of North Africa, where population growth and climate change make it essential to mobilize substantial resources for food production. Furthermore, quinoa has gained worldwide recognition for its nutritional quality as an important protein resource, containing all essential amino acids, mineral salts, being gluten-free and rich in phenolic compounds. For these reasons, the FAO designated 2013 as the International Year of Quinoa and highlighted the grain's potential to help reduce global poverty and hunger⁷.

Quinoa is a facultative halophyte; this plant contains physical characteristics that may help it withstand salt stress, such as high stomatal density or the existence of epidermal bladder cells, which allow salt to be temporarily stored and dispersed⁸. Additionally, quinoa offers several ways to integrate stress signaling and salinity detection with internal

developmental responses⁶, such as triggering the antioxidant system to shield cell constituents from harmful ions⁷. Regarding salt tolerance, several studies⁹⁻¹¹ noted that quinoa accessions exhibit variations in their sensitivity to salinity during germination and subsequently throughout growth. Furthermore, quinoa has a high tolerance to salt compared to other cereals like wheat, barley and corn, as well as vegetable crops including spinach, onions, carrots and asparagus⁵. The impact of NaCl on quinoa seed germination has been the subject of several research investigations on quinoa's salt tolerance^{5,12,13}.

Germination of quinoa under salt stress conditions is important to further explore plant resistance mechanisms against salinity, which is important for agricultural sustainability, food security and the development of strategies to enhance crop resilience and tolerant genotype selection. Investigating these mechanisms deepens our understanding of the enzymatic and non-enzymatic antioxidant profiles involved in the salt stress response.

Despite the growing body of literature on quinoa salt tolerance, a critical knowledge gap remains: The biochemical basis of germination-stage salt tolerance has not been systematically compared across accessions from geographically distinct origins (Andean highlands, Ecuadorian lowlands and North American germplasm collections). Most existing studies focus on a single cultivar or geographic region, preventing the identification of origin-linked adaptive strategies. Moreover, the relationship between antioxidant enzyme profiles and germination performance across a salinity gradient remains poorly characterized at the radicle level. Addressing this gap is essential to rational selection of quinoa genotypes for saline soils in North African agroecosystems.

Given these considerations, our aim is to investigate the germination of three quinoa accessions under varying NaCl treatments (0, 100, 200 and 300 mM). Furthermore, our focus is on examining the enzymatic (POD, CAT and APX) and non-enzymatic (proline) defense mechanisms of seedling roots following a 7-day germination period.

MATERIALS AND METHODS

The germination experiment was conducted in the Agronomy Laboratory at the CRRRA (Regional Center for Agronomic Research) in Sidi Bouzid during February 2022. The trial was carried out in an incubator under controlled conditions for a period of 7 days and the resulting seedlings were immediately used for the subsequent biochemical analyses. The protocols used for measuring the evaluated parameters are described in the following section.

Table 1: Name, origin and source of three studied quinoa accessions

Accession code	PI number	Origin	Source
T8GR	Ames 13724	New Mexico, USA	USDA-NPGS
R-132	PI 478418	Potosí, Bolivia	USDA-NPGS
DE-1	PI 674266	Ecuador	USDA-NPGS

USDA-NPGS: United States Department of Agriculture, North Central Regional Plant Introduction Station of the US National Plant Germplasm System

Germination experiment: In this investigation, three quinoa accessions were exposed to trials (Table 1). Seeds were obtained from the USDA National Plant Germplasm System (USDA-NPGS) through official material transfer agreements; no field collection was conducted and no additional permits were required.

A Petri dish experiment was performed as a complete randomized design in a 4×3 factorial (four salt treatments: 0, 100, 200 and 300 mM NaCl) with three quinoa genotypes and 3 replications (each replicate containing 10 seeds) in an incubator at 25°C. For each accession, quinoa seeds were placed in each of the 12 Petri dishes lined with Whatman filter paper and watered with different NaCl solutions and monitored for 7 days in. Seeds were sterilized with 5% sodium hypochlorite for 3 min followed by rinsing with distilled water before placing in Petri dishes. Filter papers were moistened with 5 mL of distilled water for the control and the same amount of saline NaCl solutions for treatments.

Germination evaluation: Seeds were considered germinated when the tip of the radicle was visible. The following parameters were assessed:

- **Germination percentage (GP):** Measured on the seventh day using the formula:

$$GP (\%) = \frac{\text{Total number of germinated seeds}}{\text{Total seeds}} \times 100$$

- **Mean germination time (MGT):** Calculated according to Benidire *et al.*¹⁴. It represents the average time for 50% of the seeds to germinate
- **Germination kinetics:** Expressed as the number of germinated seeds at a given time (7 days)¹⁴. This kinetics is established from the cumulative rates of germinated seeds, representing germination rate variation
- **Reversibility effect of NaCl:** Seeds that had not germinated in saline concentrations were transferred to a new non-saline medium. After two days, the number of

germinated seeds was counted. This parameter makes it possible to determine the osmotic and/or toxic origin of the depressive salt effect¹⁴

- **Radicle length:** This parameter corresponds to the final length reached by the radicle after 7 days of growth in different salt concentrations, measured using a caliper
- **Root growth kinetics:** Root growth kinetics represent the evolution of root growth through measurements made daily by a caliper

Radical oxidative stress parameters: The determination of all oxidative traits was taken from primary root (radicle) samples of germinated seeds collected after 7 days of different treatments. Radicles were stored before analysis in liquid nitrogen to preserve enzymes:

- **Hydrogen peroxide and malondialdehyde content:** The H₂O₂ and MDA content were determined following the protocols Slimani *et al.*¹¹
- **Enzymatic antioxidant activities:** A 0.1 M phosphate buffer (pH 7.0) was used to homogenize 250 mg of quinoa radicles. The resulting mixture was centrifuged at 15,000×g for 30 min at 4°C. Catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) activities were measured according to the protocol described by Slimani *et al.*¹¹

Radicle proline content: Proline determination was performed at the end of the experiment on whole radicles following the colorimetric method of Stoleru *et al.*¹⁵ with the following modifications: Fresh radicle tissue (0.1 g) was homogenized in 3% sulfosalicylic acid, centrifuged at 12,000×g for 10 min at 4°C and absorbance was read at 520 nm. Results were expressed as mg proline per g fresh weight (mg/g FW).

Statistical analysis: IBM SPSS 26.0 software was used. Two-way Analysis of Variance (ANOVA) was performed and differences between data means were evaluated using Duncan tests (p<0.05). Same-letter averages are not substantially different at p<0.05. Unless stated otherwise, all biochemical parameters are expressed on a fresh weight (FW) basis.

RESULTS AND DISCUSSION

In this section we present results concerning germination traits, oxidative stress markers determination and enzymatic and non-enzymatic parameters involved in defense mechanisms against salt stress in three accessions of quinoa. Understanding the variations in quinoa tolerance to chloride salinity will provide essential information for more precise usage of this crop in Tunisia's center-western areas impacted by soil salinity. Salt amounts utilized in this study (0, 100, 200 and 300 mM of NaCl) correspond to the region's soil salinity classes: Moderately saline (100 mM or 10 mS/cm) and severely saline (200 and 300 mM, respectively 20 and 30 mS/cm).

Germination traits variance analysis: Variance analyses revealed that all assessed parameters were dependent on accession, treatment and the accession-treatment interaction effect (Table 2). This analysis shows that there is a clear variability between the three accessions justified by a different response to saline stress, which could be linked to the origin of each accession having a specific genetic profile. Several authors have noted this variation in the capacity to germinate under salt stress, noting that some varieties are resistant and others susceptible¹⁵⁻¹⁷.

Salinity effect on kinetics and germination rate: The data showed a rapid germination of seeds of these accessions (Fig. 1). Just one day is enough to start the germination process. The germination of 18GR accession reached its highest rate at the second day, when 100% of the untreated (0 mM) seeds were germinated. In untreated conditions, seed germination kinetics of three quinoa accessions describe a sigmoid-shaped curve making it possible to distinguish three phases: (i) A lag phase, necessary for the appearance of the first germination, which is short (a few hours) for 18GR and DE-1 and one day for R-132; (ii) A second substantially linear phase, showing an increase in germination rate over time 18GR seeds reach their maximum germination in 2 days,

those of DE-1 in 4 days and R-132 in 6 days; (iii) The third phase, reflecting the final germination capacity of the seeds.

Evolution curves of germination as a function of time for the three studied accessions showed clearly that kinetics takes different patterns depending on accession and applied NaCl concentration (Fig. 1a-b). We did not note a slowing down of germination with salinity increase for 18GR, but germination rate was decreased by at least 30%, after 48 hrs, in 300 mM NaCl treatment. However, for the two other accessions, NaCl treatment after 24 hrs significantly affected germination at all used concentrations compared to untreated seeds. At 300 mM NaCl, the germination rate at day five was decreased by at least 10, 26.66 and 70.33% compared to untreated seeds for 18GR, R-132 and DE-1, respectively.

Regarding germination capacity, at low concentration, similar germination capacities were reported between 18GR and R-132, but at high concentration (200 and 300 mM), significant discrimination ($p \leq 0.01$) was noted between seeds of the three accessions. The final germination rate varied from 93.33 to 90%; 76.66 to 60%; and 40 to 33.33% respectively for 18GR, R-132 and DE-1. This response variability has been mentioned in the literature^{15,16,18}. The high ability to germinate in the presence of salt was explained by the fact that the pericarp covering the quinoa seed acts as a barrier to prevent toxic ions passage inside the seed. Furthermore, our results agree with many others^{17,18} indicating that seed germination of highland quinoa cultivars and lowland quinoa genotypes in Chile did not decrease at low salinity level (100 mM NaCl).

A variation according to accession was noted regarding final seed germination rate (Fig. 2). Germination decreases significantly with increasing NaCl concentration for the three quinoa accessions, by 10, 37 and 64%, respectively for 18GR, R-132 and DE-1. Results confirm the behavior of a facultative halophyte which shows a great variability of response to salt stress. The three accessions studied can germinate up to 300 mM with relatively high rates exceeding 33% after 7 days. These results agree with previous ones

Table 2: Accession, treatment and their interaction effect analysis of all measured germination traits of three quinoa accessions

Parameter	Accession (A)	Treatment (T)	A × T interaction
Germination percentage (GP)	S	S	S
Mean germination time (MGT)	S	S	S
Radicle length (RL)	S	S	S
H ₂ O ₂ content	S	S	S
MDA content	S	S	S
CAT activity	S	S	S
POD activity	S	S	S
APX activity	S	S	S
Proline content (PC)	S	S	S

Accession, Treatment and their Interaction (Treatment × Accession) effect, Fisher (F) test based on paired (independent) linear comparisons among the estimated marginal means, S: significant at $p \leq 0.001$

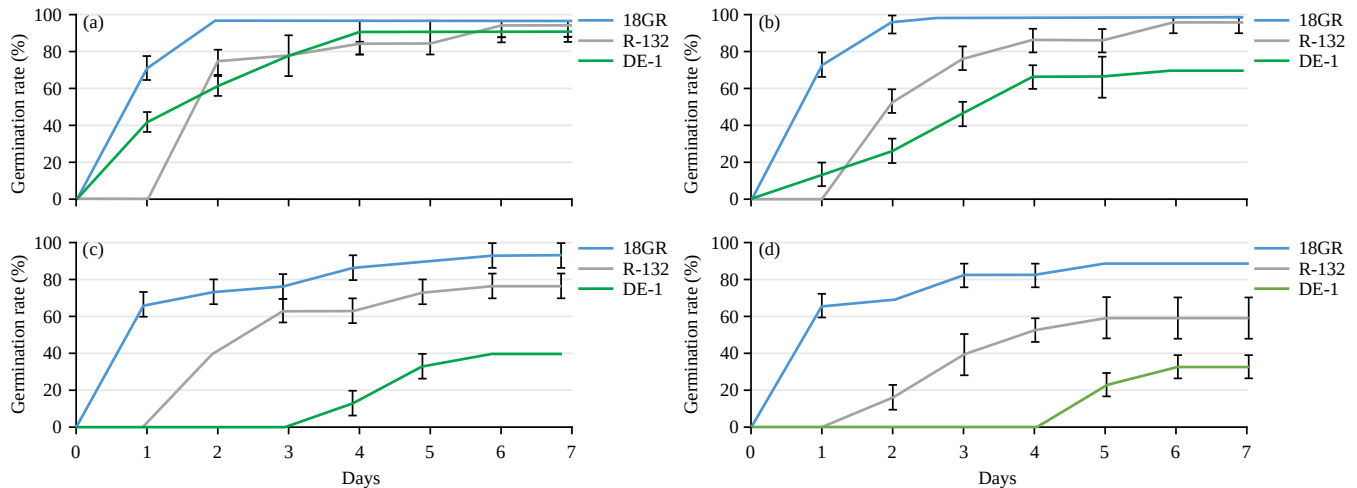


Fig. 1(a-d): Germination kinetics of the three studied quinoa accessions in the presence of different concentrations of NaCl (a) Control conditions 0 mM, (b) 100 mM, (c) 200 mM and (d) 300 mM

Each value represents the mean $\pm$ the standard deviation of three independent replicates. Means comparison of each accession and treatment within seven days is mentioned in Appendix A

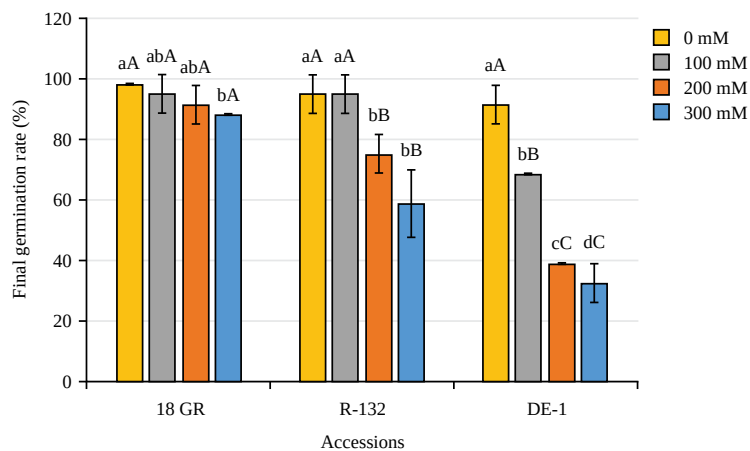


Fig. 2: Final germination rate of quinoa accession seeds to different NaCl concentrations (0, 100, 200 and 300 mM)

Each histogram represents the mean value $\pm$ the confidence interval of three independent replicates. (a, b, c, A, B, C): means followed by the same letters are not significantly different at $p \leq 0.05$ according to Duncan's test. Upper case letters compare means between accessions for each treatment; lower case letters compare means between treatments for the same accession

showing that salt concentrations between 100 and 250 mM NaCl do not affect germination rates in most quinoa genotype¹⁹. Additionally²⁰, comparing germination rates of three genotypes of different origins (CICA, Peru; Chademo and Villarrica, Chile), found rates at 300 mM NaCl of 97, 73 and 46%, respectively, confirming that differences in behavior with respect to salt stress are strongly linked to the accession effect.

NaCl effect on MGT (T50): The MGT is the point at which 50% of the seeds have germinated (Fig. 3). For all accessions, untreated seeds presented a low MGT of less than 2 days. However, treated seeds exhibited significant discrimination ($p \leq 0.05$) between accessions. Accession 18GR keeps the same

MGT (1 day) for all salt concentrations. The MGT of R-132 seeds varies from 1 to 4 days with increasing NaCl concentration. A significant increase of MGT was revealed for seeds of DE-1 accession, with the high concentration appearing inhibitory slowing the MGT to more than 10 days.

Analysis of this parameter confirms the genotype effect on salt stress response variability. "Titicaca" accession, found the MGT was around 3.7 for treatments ranging from 100 to 400 mM NaCl⁵. The MGT differed significantly ($p \leq 0.05$) between the three accessions with increasing NaCl concentration. Several studies confirmed that salt tolerance was related to the genetic variability of plant species. The study of Jacobsen *et al.*²¹ focused on Peruvian cultivars

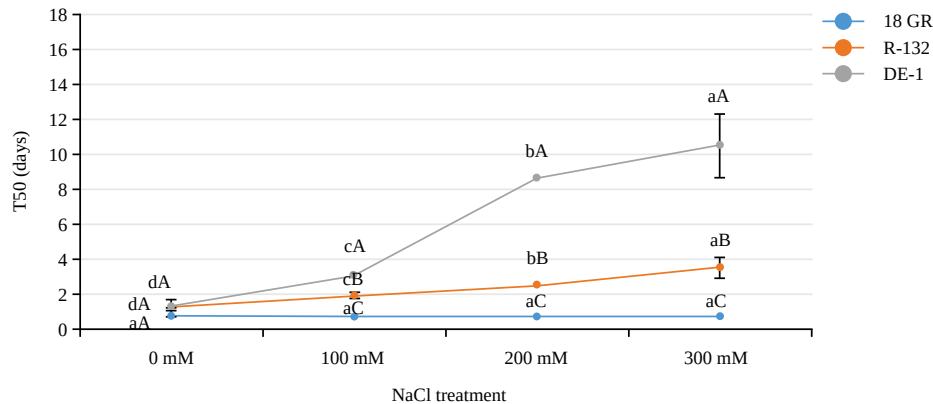


Fig. 3: Effects of different concentrations of NaCl (0, 100, 200 and 300 mM) on mean germination time (T50) variation of three studied quinoa accessions

(a, b, c, d, A, B, C): means followed by the same letters are not significantly different at $p \leq 0.05$ according to Duncan's test

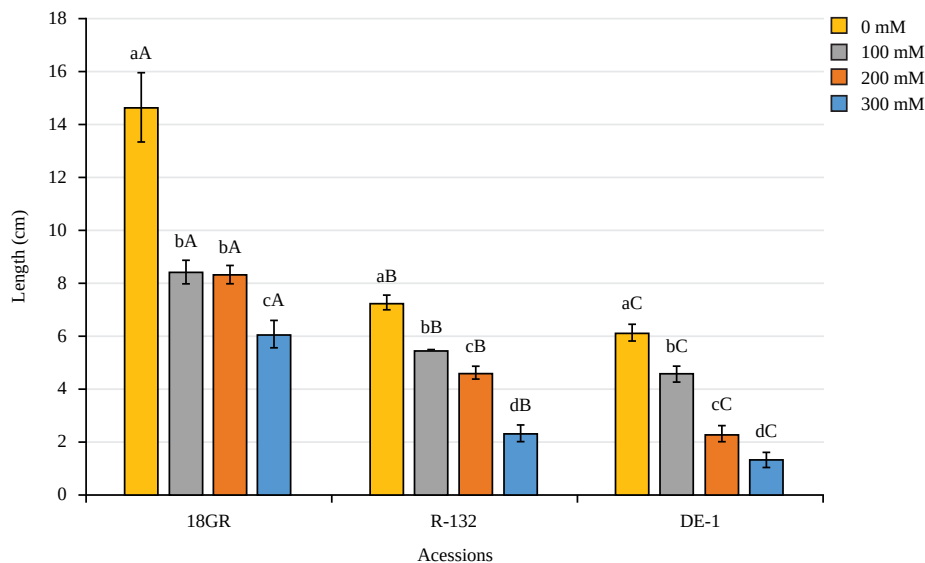


Fig. 4: NaCl effect on final radicle length of the three studied quinoa accessions

Each histogram represents the mean value $\pm$ the confidence interval of three independent replicates. (a, b, c, d, A, B, C): means followed by the same letters are not significantly different at $p \leq 0.05$ according to Duncan's test

showed these cultivars were able to germinate in salinity conditions up to 57 mS/cm. Indeed, the quinoa germination stage shows highly variable responses, with some genotypes having tolerance limit of 100 mM NaCl such as Bolivian cultivars²² Besides, Gómez-Pando *et al.*²³ indicate that of 182 Peruvian accessions, the 15 most tolerant showed germination above 60% at a salinity level of 25 dS/m.

NaCl effect on final radicle lengths and growth kinetics:

Untreated quinoa seeds showed significant differences ($p \leq 0.05$) in root length. After 7 days, length averages were 14.6 ± 1.3 ; 7.26 ± 0.2 and 6.16 ± 0.1 cm respectively for 18GR, R-132 and DE-1 (Fig. 4). With NaCl treatment, root length decreased as salt concentration increased. At 300 mM NaCl,

final root lengths were 6.1 ± 0.5 ; 2.33 ± 0.3 and 1.33 ± 0.2 cm, respectively for 18GR, R-132 and DE-1. Our results were in accordance with Souid *et al.*²⁴ who reported that seedling root length of quinoa variety Red Faro was inhibited with increasing NaCl concentration. Radicle growth involves a sequence of metabolic processes that encourage cell division; however, the presence of elevated levels of toxic ions like Na^+ and Cl^- inhibits these reactions²⁴. The resistance of this toxicity depends on the genotype.²⁰

Salt effect on root growth kinetics: Concerning root growth kinetics, a significant decrease ($p \leq 0.05$) was observed with the increase of NaCl concentrations, while maintaining the same pattern of genetic variability among the three accessions

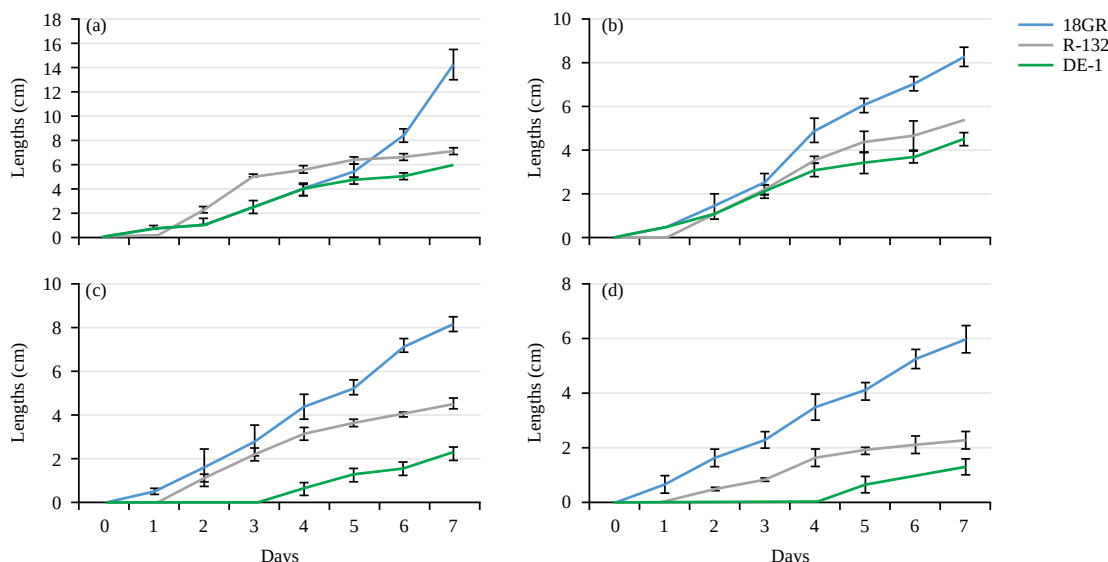


Fig. 5(a-d): NaCl effects on the root growth kinetics of studied quinoa accessions: (a) Control conditions 0 mM, (b) 100 mM, (c) 200 mM and (d) 300 mM

Each value represents the mean $\pm$ the standard deviation of three independent replicates. Means comparison of each accession, treatment within seven days is mentioned in Appendix B

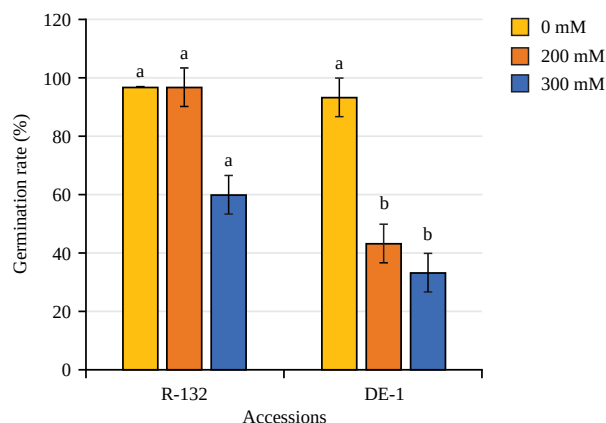


Fig. 6: Reversibility test: Seed germination rate of R-132 and DE-1 quinoa accessions

Each histogram represents the mean value $\pm$ the confidence interval of three independent replicates. (a, b): means followed by the same letters are not significantly different at $p \leq 0.05$ according to Duncan's test

(Fig. 5a-d). Varietal discrimination was more significant ($p \leq 0.05$) with increasing NaCl concentration. This inhibitory effect on radicle growth could be due to physiological disturbances exerted by salt, including osmotic and toxic effects¹⁴. Causin *et al.*²⁰ reported that high concentrations of NaCl can inhibit enzyme activity of seeds and delay radicle outflow and development.

Reversibility of NaCl effect: To understand whether NaCl inhibitory effect on germination is due to a toxic or osmotic effect, the reversibility test was used. The transfer of R-132 seeds that could not germinate at 200 mM, to distilled water,

was followed by resumption of germination of an average of 57% of seeds due to the salt osmotic effect. For 300 mM treatment, there was no resumption of germination, indicating a salt toxic effect (Fig. 6). For DE-1, no resumption of germination in distilled water was observed, indicating toxic effect as the inhibiting factor. This could be explained by the accumulation of Na^+ and Cl^- ions in the embryo, which contributes to the alteration of germination metabolic processes or embryo death²⁰.

To highlight the relationships between the different germination parameters, a Pearson correlation was carried out (Table 3). Only one significant correlation was revealed in

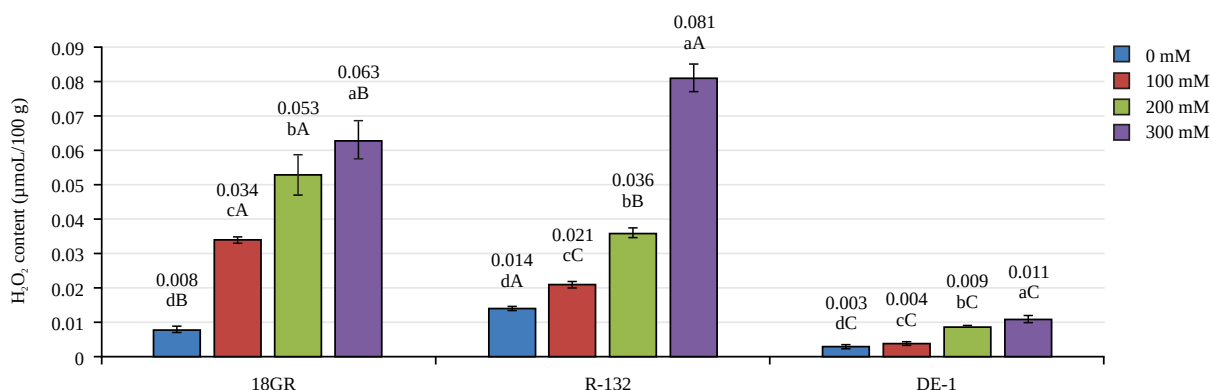


Fig. 7: NaCl effects on root H₂O₂ content of studied quinoa accessions

Each value represents the mean $\pm$ the standard deviation of three independent replicates. Means comparison based on the Duncan test was calculated between accessions (capital letters) and between treatments (lowercase letters)

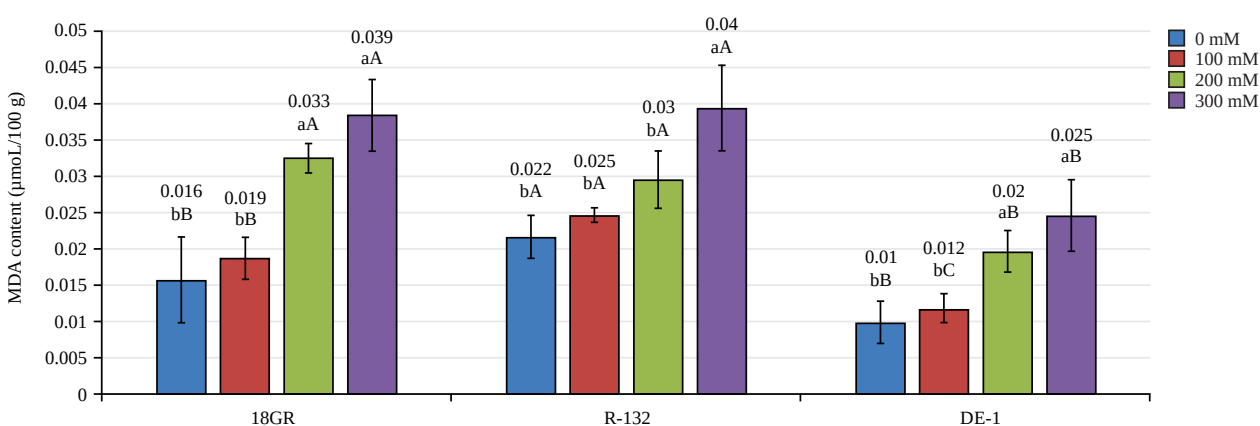


Fig. 8: NaCl effects on root MDA content of studied quinoa accessions

Each value represents the mean $\pm$ the standard deviation of three independent replicates. Means comparison based on the Duncan test was calculated between accessions (capital letters) and between treatments (lowercase letters)

Table 3: Correlation matrix between measured parameters at germination stage, in different NaCl treatments of three quinoa accessions

Parameter	GP	MGT	RL	PC
GP	1	-0.888**	0.812*	-0.654
MGT	-0.888**	1	-0.756*	0.891**
RL	0.812*	-0.756*	1	-0.772**
PC	-0.654	0.891**	-0.772**	1

*The correlation is significant at $p \leq 0.05$ level. **: The correlation is significant at $p \leq 0.01$ level. GP: Germination %, MGT: Mean germination time (T₅₀), RL: Radicle length and PC: Proline content in radicle

control treatment between MGT and RL ($r = -0.888$; $p < 0.01$) and all correlations between measured traits were significant under 200 and 300 mM. This result proves that salinity affects seed germination process through physiological stress.

Radical oxidative stress parameters

Hydrogen peroxide and malondialdehyde content:

Statistical analysis showed a significant increase of H₂O₂ content in radicles of different quinoa accessions with increasing NaCl applied dose. In the control treatment, H₂O₂ average content ranged from 0.003 to 0.1 $\mu\text{mol/g}$ FW. By increasing salt stress severity, H₂O₂ contents reached values of

0.08, 0.065 and 0.013 $\mu\text{mol/g}$ FW, respectively, for R-132, 18GR and DE-1 about 700, 622 and 333% increases compared to controls (Fig. 7).

Concerning H₂O₂, several studies showed that during imbibition and early stages of germination, seeds accumulate H₂O₂ and other ROS following their intra- and extracellular production^{25,26}. H₂O₂'s function in germination and post-germination physiology has been discussed by a number of authors^{27,28} who emphasize its dual role: It functions as a secondary messenger at low concentrations, but at high levels it reacts as a toxic compound contributing to cellular damage. One of the most well-studied harmful effects of

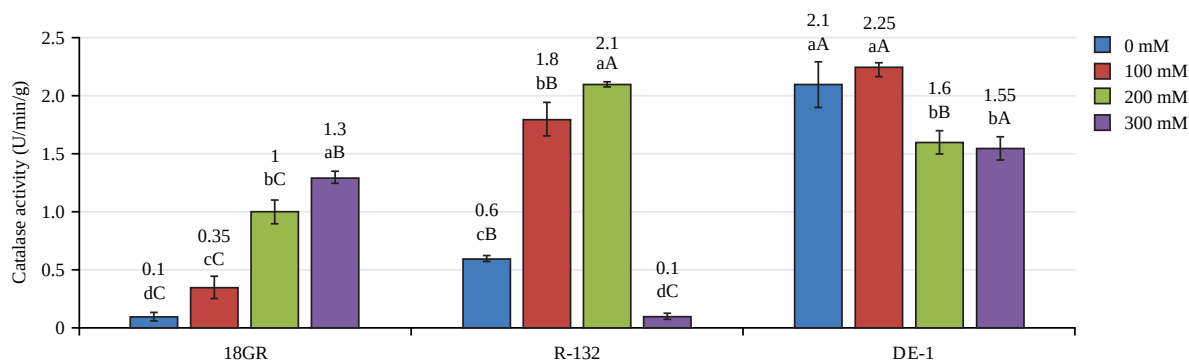


Fig. 9: NaCl effects on catalase activities of studied quinoa root accessions

Each value represents the mean $\pm$ the standard deviation of three independent replicates. Means comparison based on the Duncan test was calculated between accessions (capital letters) and between treatments (lowercase letters)

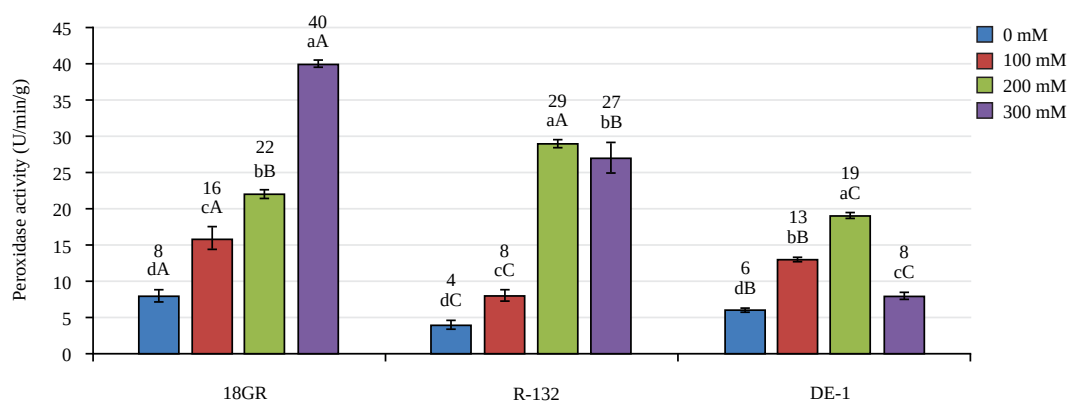


Fig. 10: NaCl effects on peroxidase activities of studied quinoa root accessions

Each value represents the mean $\pm$ the standard deviation of three independent replicates. Means comparison based on the Duncan test was calculated between accessions (capital letters) and between treatments (lowercase letters)

H₂O₂ on biological molecules is lipid peroxidation²⁸. Our results showed a proportional evolution of H₂O₂ and MDA with the increase of NaCl concentrations (Fig. 7 and 8), confirmed by significant positive correlations between these two compounds ($r = 0.871$; $p < 0.01$), ($r = 0.846$; $p < 0.01$) and ($r = 0.914$; $p < 0.01$) respectively for 18GR, DE-1 and R-132.

MDA content stimulation was significant only at 200 mM of NaCl for 18GR and DE-1 and at 300 mM for R-132. Regardless of treatment, the lowest concentration of H₂O₂ and MDA was recorded by DE-1 accession (Fig. 8). MDA was found to be elevated in a sensitive rice variety under saline stress²⁸ and in maize²⁹. Our results are in concordance with Amjad *et al.*³⁰ who found that a rise in lipid peroxidation was correlated with a drop in the membrane stability index in quinoa.

Enzymatic antioxidant defense systems: The CAT, POD and APX enzyme activities were assessed to understand how the studied quinoa accessions coped with oxidative stress during germination. These enzymatic antioxidant activities were dependent on accession, treatment intensity and their interactions.

Regarding CAT activity, at the control stage there was variability among accessions, with DE-1 showing the highest average at 2.06 U/min/g. At 200 mM, synthesis of this enzyme increased by 10 and 3.5 times compared to the control for 18GR and R-132, respectively, while a decrease of 23.8% was observed for DE-1 (Fig. 9). At 300 mM, only 18GR continues to synthesize this enzyme, showing an increase of around 13 times compared to the control; in contrast, synthesis decreases significantly by 6 times for R-132 and 1.35 times for DE-1.

Accession 18GR exhibits a significant positive correlation ($r = 0.948$; $p < 0.01$) between H₂O₂ secretion and CAT activity. This enzyme is crucial for the transformation and elimination of hydrogen peroxide into water³¹. The biphasic pattern of CAT and APX activity observed in accession 18GR stimulation at moderate salinity (100-200 mM) followed by inhibition at 300 mM NaCl likely reflects a dual mechanism: At moderate ionic concentrations, salinity-induced ROS act as secondary messengers that upregulate antioxidant gene expression, while at severe concentrations (300 mM), accumulation of Na⁺ and Cl⁻ ions likely causes enzyme denaturation and inhibits de

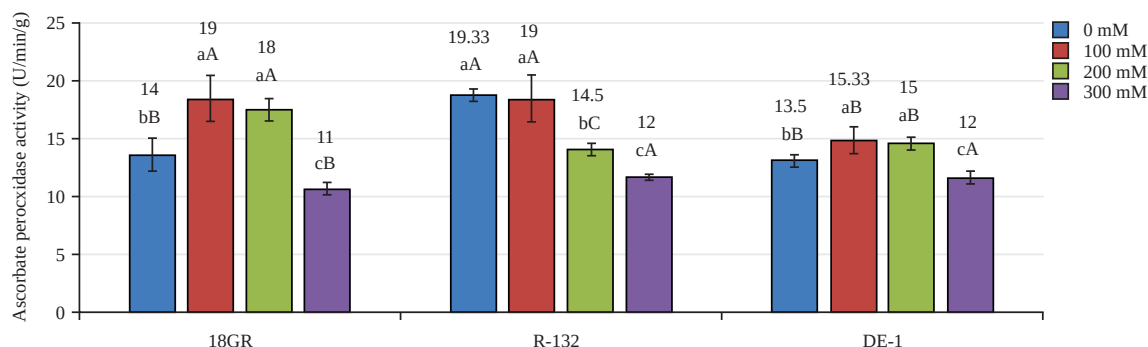


Fig. 11: NaCl effects on ascorbate peroxidase activities of studied quinoa root accessions

Each value represents the mean $\pm$ the standard deviation of three independent replicates. Means comparison based on the Duncan test was calculated between accessions (capital letters) and between treatments (lowercase letters)

novo protein synthesis³². Additionally, H_2O_2 itself can inactivate CAT at high concentrations through oxidation of its catalytic heme group. Waqas *et al.*³³ and Iqbal *et al.*³⁴ showed that under salt and drought stress in quinoa CAT and POD activities were lower, but Fghire *et al.*³⁵ observed enhanced CAT and POD activities. Haifa *et al.*³⁶ showed that CAT activity rose as the degree of salt stress increased for durum wheat. These findings are also in line with turf research by Shrivastava and Kumar³⁷ and studies in alfalfa³⁸ and *Spartina alterniflora*³⁹.

Concerning POD activity, a notable difference among the three accessions under controlled conditions was observed (Fig. 10). At 200 mM, a significant increase in this enzyme was observed, with concentrations 2.75, 7.25 and 3.1 times higher compared to the control for 18GR, R-132 and DE-1, respectively. At 300 mM, 18GR accession continues to synthesize this enzyme, reaching around 40.8 U/min/g, while for the other two accessions a significant reduction was observed compared to the 200 mM result.

POD is among essential enzymes for quinoa's reaction to salt stress; it helps detoxify hydrogen peroxide by breaking it down into oxygen and water. Positive significant correlations between this enzyme and H_2O_2 concentrations were found for all studied accessions (Appendix C). The decline in POD activity observed for R-132 and DE-1 has also been mentioned by Toderich *et al.*⁴⁰ and may point to a sophisticated regulation mechanism. The work of Souid *et al.*²⁴ showed a significant increase of POD and CAT with increasing NaCl concentrations, similar to our finding for 18GR. Results of Nader Ben Amor *et al.*⁴¹ demonstrated an increase in POD activity as a function of salt in *Crithmum maritimum* and Li⁴² showed similar stimulation in *Limonium bicolor*. On quinoa, Khalofah *et al.*⁴³ verified the rise in POD activity under metal stress.

APX activity for the R-132 accession shows a significant decrease with increasing NaCl concentration (Fig. 11). However, at moderate concentrations, 18GR and DE-1 accessions showed significant stimulation of this activity, with stimulation percentages of 36% and 19% under 100 mM treatment and 30 and 18% under 200 mM treatment. Our findings concerning R-132 accession concurred with those of Toderich *et al.*⁴⁰ and Hajhashemi *et al.*⁴⁴ and who demonstrated that, at all stress intensities, APX activity was lower than the control values. APX activity was also reported as reduced in wheat varieties under saline stress, dependent on species sensitivity and environmental stress intensity^{37,45}. In contrast, Souid *et al.*²⁴ showed that APX activity is stimulated for quinoa seedling under different salt stress conditions, as in 18GR and DE-1 at moderate concentrations.

Overall, when salt stress is moderate, we observed a significant and positive correlation between oxidative stress markers (H_2O_2 and MDA) and defense enzymes (CAT, POD and APX). However, this correlation disappeared when the stress became severe (300 mM). The absence of this correlation also depends on the accession, particularly for R-132 and DE-1.

Radicle proline content: Under control conditions, the three accessions maintained low and constant radicle proline levels (Fig. 12). A proportional increase in proline contents with increasing salt concentration was reported, except for DE-1 accession which showed similar contents for 200 and 300 mM NaCl treatments. Proline contents between control and 300 mM treatment were from 0.08 ± 0.009 to 1.2 ± 0.02 ; from 0.09 ± 0.008 to 1 ± 0.04 ; and from 0.08 ± 0.008 to 0.88 ± 0.01 mg/g FW respectively for 18GR, R-132 and DE-1. This accumulation of proline could have a protective effect against salt toxic effects by participating in osmotic adjustment which improves root growth.

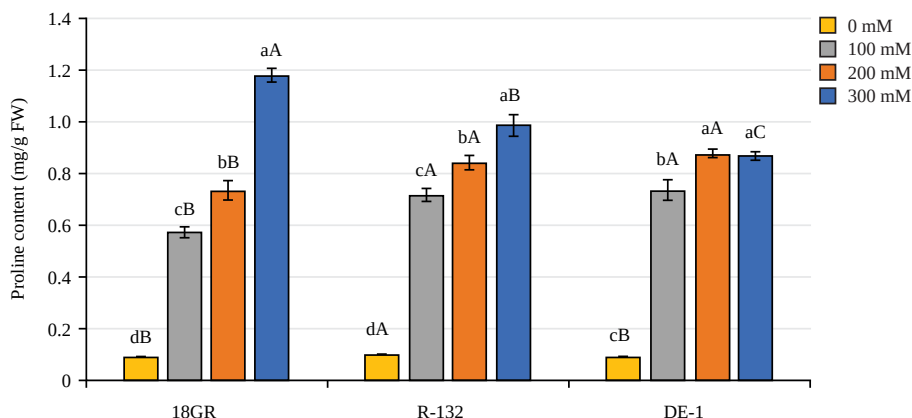


Fig. 12: NaCl stress effect on roots proline content mg/g of fresh weight

Each histogram represents the mean value $\pm$ the confidence interval of three independent replicates. (a, b, c, d, A, B, C): means followed by the same letters are not significantly different at $P < 0.05$ according to Duncan's test

Radicle proline content was correlated positively and significantly with MGT ($r = 0.891$; $p < 0.01$), for 100 and 200 mM ($r = 0.772$; $p < 0.01$) NaCl concentrations. Germination rate was negatively and significantly ($p < 0.05$) correlated with MGT (from 100 to 300 mM) (Table 3). Under 300 mM NaCl treatment, correlation between MGT and proline content changed to be negatively significant ($r = -0.887$; $p < 0.01$), indicating that at these conditions' seeds explain difficulty to germinate and only germinated seeds produce proline. Proline contents recorded an increase compared to the control by 12, 10 and 9 times, respectively for 18GR, R-132 and DE-1. These results agree with several studies on other varieties of quinoa^{15,46}. For example, Stoleru *et al.*¹⁵ found that only the cultivar Vikinga exhibits good adaptation to salt stress by increasing proline synthesis with increasing NaCl concentration. 18GR performed higher proline content than R-132 and DE-1, indicating a more efficient mechanism in up-regulation of the proline biosynthesis pathway.

CONCLUSION

This work complements many studies assessed under salinity conditions (from 100 to 750 mM of NaCl) done for quinoa as a halophyte, adding to our comprehension of how quinoa reacts to salt stress in germination and seedling stages. In this study, the response was dependent on accessions, treatment and their interaction effect. All studied parameters revealed important variability between assessed accessions. Accession 18GR was the most tolerant, easily germinating at increasing concentrations of NaCl up to 300 mM, representing only 10% GP reduction, keeping its MGT at less than 24 hrs to

start germination and preserving good radicle growth and proline synthesis. Salt treatments significantly increased the accumulation of H_2O_2 and MDA in radicles for the three quinoa accessions. In moderate salt stress, a clear and positive connection was noted between oxidative stress indicators (H_2O_2 and MDA) and defense enzymes (CAT, POD and APX). However, this relationship vanished when stress intensified to 300 mM.

From an applied perspective, accession 18GR is recommended for field evaluation in the center-western saline zones of Tunisia, particularly at soil salinities equivalent to 100-200 mM NaCl (10-20 mS/cm), where it maintains germination above 90% and robust radicle development. Future research should prioritize: (i) Transcriptomic profiling of the proline biosynthesis pathway (P5CS gene expression) in 18GR to identify molecular markers of salt tolerance; (ii) Field trials under realistic agronomic conditions in North African semi-arid soils; and (iii) Investigation of epigenetic regulation underlying the observed geographic origin-dependent antioxidant strategies.

Appendix 1: Means comparison of each accession, treatment within seven days of quinoa seeds germination kinetics (supplement to Fig. 1).

Appendix 2: Means comparison between roots length of each accession, treatment within seven days (supplement to Fig. 5).

Appendix 3: Pearson's Correlation between germinative parameters (GP, RL), oxidative markers (H_2O_2 , MDA), proline content (PC) and antioxidant enzymes (CAT, POD, APX).

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES

During the preparation of this work, the authors used Claude (Anthropic, claude-sonnet-4-6) to improve manuscript language, grammar, and structure. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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APPENDIX

Appendix 1: Means comparison of each accession, treatment within seven days of quinoa seeds germination kinetics (supplement to Fig. 1)

Days/treatments		T0	T100	T200	T300
1	18GR	73.33±6.53 ^a	73.33±6.5 ^a	66.66±6.53 ^a	66.6±6.53 ^a
	R-132	0 ^c	0 ^c	0 ^b	0 ^b
	DE-1	43.3±6.532 ^b	13.33±6.5321 ^b	0 ^b	0 ^b
2	18GR	100 ^a	96.6±6.53 ^a	73.33±6.53 ^a	70 ^a
	R-132	76.6±6.53 ^b	53.3±6.53 ^b	40 ^b	16.66±6.53 ^b
	DE-1	63.3±6.5 ^c	26.6±6.53 ^c	0 ^c	0 ^c
3	18GR	100 ^a	100 ^a	76.3±6.53 ^a	83.3±6.53 ^a
	R-132	80±11.31 ^b	76.6±6.53 ^b	63.33±6.53 ^b	40±11.31 ^b
	DE-1	80 ^b	46.6±6.53 ^c	0 ^c	0 ^c
4	18GR	100 ^a	100 ^a	86.66±6.53 ^a	83.3±6.53 ^a
	R-132	86.6±6.53 ^b	86.6±6.53 ^b	63.33±6.53 ^b	53.3±6.53 ^b
	DE-1	93.33±6.53 ^b	66.6±6.53 ^c	13.3±6.5 ^c	0 ^c
5	18GR	100 ^a	100 ^a	90 ^a	90 ^a
	R-132	86.6±6.53 ^b	86.6±6.53 ^b	73.33±6.53 ^b	60±11.31 ^b
	DE-1	93.33±6.53 ^b	66.66±11.53 ^c	33.3±6.5 ^c	23.3±6.5 ^c
6	18GR	100 ^a	100 ^a	93.33±6.53 ^a	90 ^a
	R-132	96.6±6.53 ^{ab}	96.6±6.53 ^a	76.66±6.53 ^b	60±11.31 ^b
	DE-1	93.33±6.53 ^b	70 ^b	40 ^c	23.33±6.53 ^c
7	18GR	100 ^a	100 ^a	93.33±6.53 ^a	90 ^a
	R-132	96.6±6.53 ^{ab}	96.66±6.53 ^a	76.66±6.53 ^b	60±11.31 ^b
	DE-1	93.33±6.53 ^b	70 ^b	40 ^c	33.3±6.53 ^c

Means comparison of the cumulative rates of germinated seeds in each treatment, based on the Duncan test was calculated between accessions (a,b,c)

Appendix 2: Means comparison between roots length of each accession, treatment within seven days (supplement to Fig. 5)

Days/treatments		T0	T100	T200	T300
1	18GR		0.53±0.06 ^a	0.5 ^a	0.56±0.13 ^a
	R-132		0 ^b	0 ^b	0 ^b
	DE-1		0.66±0.32 ^a	0.5 ^a	0 ^b
2	18GR		1.23±0.28 ^b	1.5±0.56 ^a	1.66±0.86 ^a
	R-132		2.26±0.28 ^a	1.1±0.11 ^a	1.16±0.17 ^b
	DE-1		1 ^c	1.16±0.32 ^a	0 ^c
3	18GR		2.5±0.56 ^b	2.6±0.4 ^a	2.9±0.74 ^a
	R-132		5.16±0.17 ^a	2.26±0.28 ^{ab}	2.26±0.28 ^b
	DE-1		2.5±0.56 ^b	2.16±0.32 ^b	0 ^c
4	18GR		4±0.56 ^b	5±0.56 ^a	4.5±0.56 ^a
	R-132		5.7±0.29 ^a	3.63±0.17 ^b	3.23±0.28 ^b
	DE-1		4.16±0.32 ^c	3.16±0.32 ^c	0.66±0.32 ^c
5	18GR		5.53±0.57 ^b	6.16±0.32 ^a	5.36±0.36 ^a
	R-132		6.53±0.28 ^a	4.46±0.51 ^b	3.72±0.16 ^b
	DE-1		4.83±0.32 ^c	3.5±0.5 ^c	1.33±0.32 ^c
6	18GR		8.6±0.59 ^a	7.16±0.32 ^a	7.33±0.32 ^a
	R-132		6.73±0.28 ^b	4.73±0.72 ^b	4.13±0.13 ^b
	DE-1		5.15±0.32 ^c	3.8±0.32 ^c	1.6±0.32 ^c
7	18GR		14.66±1.30 ^a	8.43±0.45 ^a	8.33±0.32 ^a
	R-132		7.26±0.28 ^b	5.5 ^b	4.63±0.26 ^b
	DE-1		6.16±0.32 ^c	4.66±0.32 ^c	2.33±0.32 ^c

Means comparison between roots length of each accession in each treatment, based on the Duncan test was calculated between accessions (a,b,c)

Appendix 3: Pearson's Correlation between germinative parameters (GP, RL), oxidative markers (H₂O₂, MDA), proline content (PC) and antioxidant enzymes (CAT, POD, APX)

18GR								
	H ₂ O ₂	MDA	CAT	POD	APX	GP	RL	PC
H ₂ O ₂	1							
MDA	0.871**	1						
CAT	0.948**	0.913**	1					
POD	0.897**	0.853**	0.922**	1				
APX	-0.122	-0.345	-0.311	-0.484	1			
GP	-0.775**	-0.667*	-0.750**	-0.730**	0.127	1		
RL	-0.901**	-0.721**	-0.817**	-0.832**	-0.029	0.640*	1	
PC	0.958**	0.849**	0.933**	0.971**	-0.282	-0.719**	-0.927**	1
R-132								
	H ₂ O ₂	MDA	CAT	POD	APX	GP	RL	PC
H ₂ O ₂	1							
MDA	0.914**	1						
CAT	-0.510	-0.369	1					
POD	0.695*	0.729**	0.150	1				
APX	-0.831**	-0.785**	0.190	-0.883**	1			
GP	-0.908**	-0.875**	0.350	-0.791**	0.879**	1		
RL	-0.942**	-0.861**	0.255	-0.768**	0.833**	0.854**	1	
PC	0.739**	0.734**	0.161	0.793**	-0.728**	-0.718**	-0.898**	1
DE-1								
	H ₂ O ₂	MDA	CAT	POD	APX	GP	RL	PC
H ₂ O ₂	1							
MDA	0.846**	1						
CAT	-0.901**	-0.781**	1					
POD	0.337	0.148	-0.243	1				
APX	-0.439	-0.644*	0.461	0.584*	1			
GP	-0.297	-0.437	0.445	0.731**	0.862**	1		
RL	-0.961**	-0.854**	0.834**	-0.366	0.388	0.230	1	
PC	0.744**	0.633*	-0.530	0.635*	0.016	0.248	-0.859**	1

Significance levels; *p≤0.05, **p≤0.01. GP: Germination pourcentage, RL: Root lenght, H₂O₂: Hydrogen peroxide, MDA: Malondialdehyde, PC: Proline content, CAT: Catalase, POD: Peroxidase and APX: Ascorbate peroxidase