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Original Research

Unveiling Salinity Tolerance during Germination in Tunisian Barley Genotypes: Implications for Arid-Zone Agriculture

Faiza Boussora[1, 2]*, Sihem Ben Ali[1], Tebra Triki[1], Amna Ghanmi[1], Ali Ferchichi[3], Kamel Nagaz[1], Ferdaous Guasmi[1]

[1]Institute of Arid Lands of Medenine (IRA), Medenine Tunisia
[2]Higher Institute of Arts and Crafts of Tataouine, BP 47, Cite Elmahrajene – 3234 – Tataouine Tunisia
[3]National Institute of Agronomic Research of Tunis (INAT), Tunis Tunisia

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Abstract

Salinity stress is a major abiotic constraint that adversely affects seed germination and early seedling development, particularly in crops such as barley (*Hordeum vulgare* L.). As a critical initial phase of the plant life cycle, germination plays a key role in ensuring the successful establishment of the plant in its environment. In this context, the present study evaluates the impact of different NaCl concentrations (6 g/L and 12 g/L) on the germination performance and tolerance indices of five barley genotypes (Ardhaoui, Kounouz, Lemsi, Manel, and Rihane). Germination kinetics revealed a sigmoidal pattern, with a prolonged lag phase and a reduced final germination percentage under increasing salt stress. At 12 g/L NaCl, significant reductions in cumulative germination rates were observed, particularly for the Lemsi genotype, which displayed the highest sensitivity. In contrast, Ardhaoui maintained a higher germination percentage, indicating superior salt tolerance. Coleoptile and radicle elongation were severely inhibited by salinity, with reductions of up to 60% and 70%, respectively, at the highest NaCl concentration. Statistical analysis confirmed significant genotype-by-treatment interactions for both germination and elongation traits. Germination stress tolerance index (GSI) and tolerance index (TI) analyses further demonstrated that Ardhaoui displayed the highest resilience to salinity, while Lemsi was the most affected. These findings highlight substantial genetic variation in salinity tolerance among barley genotypes, positioning Ardhaoui as a promising candidate for cultivation in saline-prone environments. This study underscores the importance of selecting salt-tolerant genotypes to ensure sustainable barley production under increasing soil salinization conditions.

Keywords: barley, NaCl-induced stress, germination indices, elongation, salt tolerance screening

*e-mail: be.feiza@gmail.com
Tel.: +216 75 633 005
Fax: +216 75 633 006

Introduction

Seed germination marks the initial and critical transition from a dormant to an actively growing plant. It is a finely regulated process involving a cascade of physiological and metabolic activities, culminating in radicle protrusion [1, 2]. This developmental milestone is vital for seedling establishment, crop stand formation, and ultimately agricultural productivity [3]. However, germination is highly susceptible to environmental constraints, particularly salinity stress, which significantly affects both germinative success and early seedling vigour [4]. Salinity stress is one of the most detrimental abiotic factors limiting crop productivity worldwide, especially in arid and semi-arid regions where irrigation practices and high evapotranspiration intensify soil salinization. It is estimated that salinity affects over 20% of irrigated lands globally, with this percentage projected to increase due to climate change and unsustainable land management [5]. In Tunisia, this problem is particularly acute in central and southern regions, where poor irrigation water quality and groundwater overexploitation exacerbate the issue [6].

During germination, salinity imposes both osmotic stress and ionic toxicity on seeds. Osmotic stress, due to the lowered water potential in saline soils, restricts water uptake, delays or inhibits the imbibition phase, and creates a pseudo-drought condition [7]. This early water deficit compromises cellular turgor, inhibiting cell expansion and radicle emergence, the first visible sign of germination [8]. Ionic stress, on the other hand, results from excessive accumulation of sodium and chloride ions, which disturb ionic homeostasis, displace essential nutrients like potassium and calcium, and impair key enzymatic and metabolic functions [9]. These effects compromise not only the water balance but also reserve mobilization, an essential process during germination. High salt concentrations inhibit the activity of hydrolytic enzymes such as α-amylases and proteases, which are required to degrade starch and proteins stored in the endosperm. Consequently, the energy supply needed for embryonic axis elongation is curtailed [9]. Furthermore, salinity affects the expression and function of membrane transporters involved in nutrient and solute distribution, leading to restricted resource allocation to the growing embryo [10].

Salinity-induced stress also disrupts cellular redox homeostasis, leading to the overproduction of reactive oxygen species (ROS), such as superoxide radicals and hydrogen peroxide. These molecules can cause oxidative damage to lipids, proteins, and nucleic acids, further inhibiting germination and seedling growth [11]. In early stages, these combined effects result in lower germination percentages, reduced seedling length, poor root system development, and compromised vigour, key parameters for early crop establishment and yield potential [12].

In cereals like barley (*Hordeum vulgare* L.), the negative effects of salt stress on seed germination are well documented. Studies have consistently reported that high salinity levels reduce radicle and coleoptile growth, delay the onset of germination, and decrease final germination rates [13]. These physiological constraints directly impact crop establishment in the field, especially under rainfed conditions, where plants are already exposed to multiple abiotic challenges [14]. Barley is one of the oldest domesticated cereals and remains a staple in many regions due to its broad adaptability and short growth cycle. Its ability to grow under marginal conditions makes it a key crop for food and fodder security in arid and semi-arid agroecosystems. Beyond its agronomic resilience, barley grains are highly valued for their nutritional properties, including their richness in minerals, proteins, dietary fiber, and a wide array of vitamins. These attributes make barley a staple ingredient in many traditional foods and beverages, thereby playing a key role in both the local diet and cultural practices. Additionally, barley serves as a fundamental component of animal feed, thereby supporting the livestock sector. In Tunisia, especially in the southern regions, barley is the most widely cultivated cereal and represents a crop of substantial economic and industrial relevance. Its exceptional tolerance to arid conditions and saline soils underscores its importance as a dependable agricultural resource, ensuring livelihood security for farmers operating in environmentally harsh areas. In Tunisia, barley is cultivated on over 500,000 hectares, primarily in rainfed areas [15]. However, increasing soil salinity severely limits its productivity, particularly during germination and early growth stages.

To cope with salinity, plants have evolved a wide array of adaptive strategies that operate at morphological, physiological, biochemical, and molecular levels. These mechanisms are crucial for minimizing the detrimental effects of salt stress and maintaining cellular homeostasis under high salinity conditions. One of the primary responses is osmotic adjustment through the accumulation of compatible solutes like proline and glycine betaine, ion exclusion, and compartmentalization of toxic ions into vacuoles [16]. These responses help preserve cell turgor, maintain enzyme activity, and prevent damage to metabolic pathways. Maintaining a high K^+/Na^+ ratio is particularly critical for enzymatic function and signal transduction under salt stress. In addition to these mechanisms, salinity tolerance is also supported by the activation of antioxidant defense systems, stress-responsive transcription factors, and hormonal adjustments, particularly involving abscisic acid (ABA), ethylene, and jasmonic acid, which regulate gene expression, stomatal closure, and resource allocation during stress [17].

Given the agronomic importance of barley and the increasing salinity challenges in Mediterranean and semi-arid regions, identifying and characterizing salt-tolerant genotypes is a key research priority. Germination represents a particularly vulnerable stage and an ideal target for selection. Salt-tolerant genotypes are expected to maintain higher germination rates, faster radicle emergence, and more efficient metabolic activation under salinity conditions. This study aims to evaluate the germination responses of five Tunisian barley genotypes, Ardhaoui, Kounouz, Lemsi, Manel, and Rihane, under varying levels of NaCl-induced salinity. By comparing germination indices and stress tolerance traits, we aim to identify genotypic differences in salinity response and highlight promising candidates for breeding programs focused on improving resilience to salt-affected environments. We hypothesize that some genotypes will show greater tolerance, reflected in superior germination performance and early vigour under saline conditions.

Materials and Methods

Plant Material

Five barley genotypes were used in this study: one local landrace from southern Tunisia (Ardhaoui), two improved Tunisian cultivars (Kounouz and Manel), and two introduced varieties (Lemsi and Rihane). All genotypes were provided by the gene bank of the Arid Land Institute (IRA Gene Bank) and were selected for their contrasting genetic backgrounds, agronomic traits, and stress responses. As summarized in Table 1, these genotypes differ in adaptation zones, growth cycles, and levels of tolerance to key biotic and abiotic stresses such as salinity, drought, and lodging. Ardhaoui, a six-row spring-type landrace, is well known for its high drought and salinity tolerance and is widely cultivated in the arid regions of southern Tunisia. Farmers in these areas continue to favor it due to its long-standing adaptation to harsh environments. However, this heavy reliance on a single genotype may limit opportunities for improving resilience and productivity. Therefore, the inclusion of alternative cultivars such as Kounouz (semi-arid adapted, moderately disease-resistant), Manel (early-maturing, high fertility), Lemsi (late-maturing, high fertility), and Rihane (lodging-resistant, high-yielding) offers a valuable opportunity to explore genotypic diversity. This diversity provides a solid basis for evaluating differential responses to salinity stress at the germination stage and identifying cultivars with promising traits for broader use in similar agro-ecological zones.

Germination

Grain Sterilization and Germination

The experiment was conducted under sterile conditions in a laminar flow hood, and the grains were soaked in sterile water. Two levels of salt stress were applied (6 g/L NaCl and 12 g/L NaCl), using NaCl as the stress agent. The barley grains were disinfected by soaking in a 10% sodium hypochlorite solution for 20 minutes, followed by four rinses with distilled water. The purpose of surface sterilization was to eliminate any external fungal or bacterial contamination. The seeds were then germinated at a rate of 20 grains per Petri dish, with four replicates for each genotype and each treatment. The Petri dishes, lined with two layers of filter paper, were soaked in distilled water for the controls and in 6 and 12 g/L of NaCl for the treated seeds. To ensure proper imbibition, the Petri dishes were placed at 4ºC for 24 hours. Germination was carried out at an average temperature of 25ºC in darkness, and the germinated grains were counted daily for 7 days, with radicle emergence being the indicator of germination.

Studied Parameters

Germination was monitored for 7 days (after which the germination rate remained constant), and germinated seeds were counted daily to establish the kinetics that allowed for the determination of T50 (hours), which corresponds to the time required for 50% of the seeds to germinate. T50 values were evaluated using the equations $y = a\ x + b$ derived from the tangents of the germination curves [18].

The corrected germination percentage (GC%) was calculated on the 7th day using the formula of [19]:

$$GC = \frac{Nx}{N0} x\ 100 \quad (1)$$

Where Nx represents the number of germinated seeds on the 7th day in a medium containing x g/L of NaCl, and N0 represents the number of germinated seeds after 7 days in the control medium.

To characterize the germination rate of barley seeds subjected to stress, the results were also expressed in terms of the Germination Stress Tolerance Index (GSI), which was calculated after determining the Promptness Index (PI) using the following formula [20]:

Table 1. Effect of salt stress on the average germination time (T50) of seeds from the five Studied Barley genotypes.

	Ardhaoui	Kounouz	Lemsi	Manel	Rihane
Control	3,06 h	3,06 h	3,34 h	3,05 h	3,04 h
T1 (6 g/L NaCl)	4,1 h	4,14 h	5,22 h	4,29 h	4,16 h
T2 (12 g/L NaCl)	5,65 h	5,64 h	8,97 h	6,70 h	5,70 h

The values are derived from the data in Fig. 1. Each value represents the mean of four replicates.

$$\text{GSI \%} = \frac{\text{PIS}}{\text{PIC}} \text{x } 100 \qquad (2)$$

Where PIS is the Promptness Index of stressed seeds and PIC is the Promptness Index of control seeds.

The Promptness Index (PI) was calculated as follows:

$$\text{PI} = \text{nD1}\,(7 - \text{D1}) + \dots + \text{nD6}\,(7 - \text{D6}) \qquad (3)$$

Where nD1 is the number of seeds germinated on day 1, nD6 is the number of seeds germinated on day 6, and D1 to D6 represent the respective days of germination.

The radicle length was measured on the 7th day of germination for seeds from the five genotypes germinated in both control and NaCl-containing media. Measurement was performed using a cotton thread to account for radicle curvature.

Radicle elongation was assessed after measuring the radicle length on the 7th day of germination (168 hours of imbibition). The obtained length was divided by the elongation period, calculated as 168 hours minus T50. The elongation rate (E) was expressed in mm/ hour [18].

The Tolerance Index (TI) was calculated based on radicle length, following [21]:

$$\text{TI\%} = \frac{\text{Average radicle length of treated seeds}}{\text{Average radicle length of control seeds}} \times 100 \qquad (4)$$

This index enables the comparison of the plants' ability to germinate in a saline medium relative to those germinating in a control medium.

Statistical Analysis and Graphical Representations

The results were statistically analyzed using SPSS software. Prior to performing the general linear model (GLM) analysis, data normality was assessed using the Shapiro-Wilk test ($\alpha = 0.05$), and homogeneity of variances was verified with Levene's test ($\alpha = 0.05$) to ensure the validity of applying the GLM. The analysis of variance (ANOVA) was then conducted to evaluate the main effects of genotype, treatment, and their interactions.

Results

Germination Percentage

Effect of Saline Stress on the Cumulative Germination Percentage

The germination curve typically follows a sigmoidal shape. Analysis of the germination curves of barley at different NaCl concentrations confirms that this sigmoidal pattern is maintained. Fig. 1 illustrates the cumulative germination percentages of the studied barley genotypes, showing that most treatments follow a characteristic sigmoidal curve, which can be divided into three phases:

Lag phase: Lasting approximately 2 days for both control and treated samples across all studied genotypes.

Linear phase (rapid increase phase): The rate of germination varies depending on the treatment concentration and genotype, with a sharp rise in germination percentage.

Plateau phase: Represents the final cumulative germination percentage, indicating the maximum germination capacity, which varies according to treatment concentration and genotype (Fig. 1).

The maximum germination of seeds grown in H_2O is considered 100%, and germination rates under different stress conditions are expressed relative to this reference. Among the different genotypes, the germination percentage increases at varying rates depending on the salt concentration before stabilizing and reaching its maximum after 7 days. A progressive decline in germination kinetics is observed with increasing salinity, with a significant delay becoming evident at 12 g/L NaCl. However, NaCl treatment does not appear to affect the initiation of germination, which consistently begins on the second day across all genotypes. At 6 g/L NaCl, cumulative germination rates exhibited a slight reduction across all five genotypes but remained relatively comparable to control values. In contrast, exposure to 12 g/L NaCl led to a pronounced decline in the corrected germination percentage, particularly in the Lemsi genotype, indicating its higher sensitivity to salt stress. Conversely, the Ardhaoui genotype showed the highest cumulative germination rate, suggesting superior salt tolerance.

The average germination time (T50), determined from the slopes of the germination kinetics, was delayed by several hours in stressed seeds compared to the control. This delay varied according to both salt concentration and genotype (Table 1).

At a concentration of 12 g/L NaCl, the germination kinetics of the studied barley genotypes slow down. However, only the Lemsi genotype shows a significant impact from the 12 g/L NaCl concentration, with the T50 increasing from 3.34 hours in the control to 8.97 hours in the treated seeds. In contrast, the germination capacity of the other genotypes remains notable and higher than that of the Lemsi genotype.

Effect of Saline Stress on the Final Germination Percentage

Fig. 2 displays the corrected final germination percentages of the five barley genotypes under various NaCl concentrations, using radicle emergence as the germination criterion. This parameter was calculated using Equation (1), as presented in the Experimental section. The figure illustrates that the genotypes respond differently to saline stress, influenced by both stress intensity and genotype. Notably, NaCl progressively

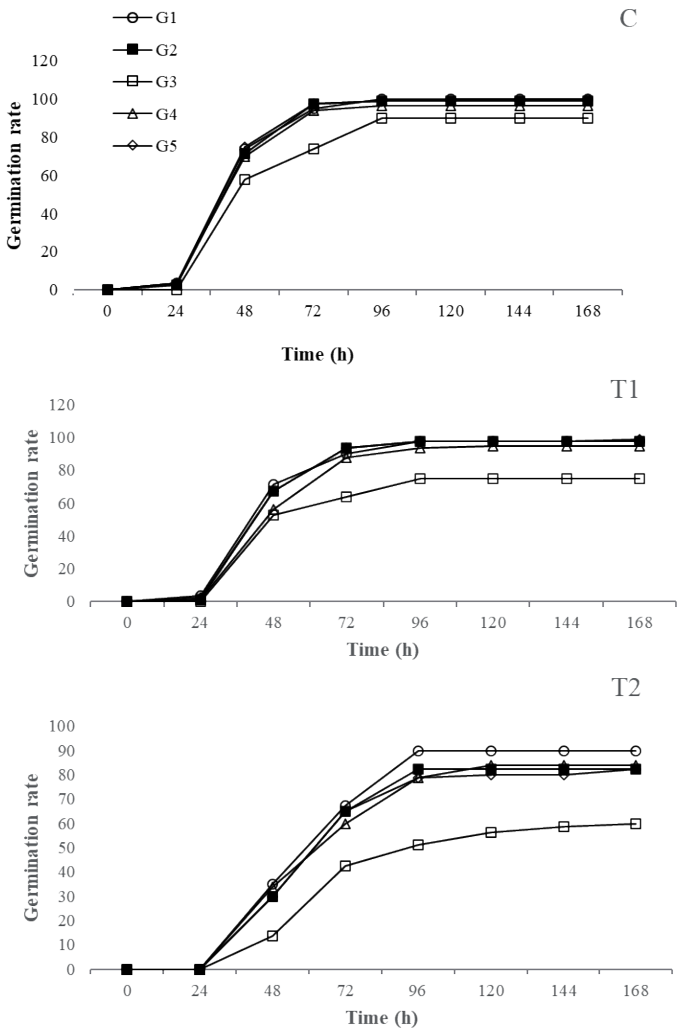

Fig. 1. Evolution of the cumulative germination percentage of barley seeds from different genotypes over time at various NaCl concentrations. Each value represents the mean of four replicates. T1 (6 g/L NaCl) and T2 (12 g/L NaCl) represent the salt stress treatments, while G1 (Ardhaoui), G2 (Konouz), G3 (Lemsi), G4 (Manel), and G5 (Rihane) correspond to the studied barley genotypes.

decreases the final germination rate of barley seeds, with a more significant reduction at higher salt concentrations.

At the lower NaCl concentration of 6 g/L, the cumulative germination rates for the genotypes "Ardhaoui", "Kounouz", "Manel", and "Rihane" showed only minor decreases, remaining relatively close to the control values, with a reduction of about 5%. In contrast, the Lemsi genotype experienced a more pronounced decrease, with a reduction of approximately 20% compared to the other genotypes.

These decreases became more pronounced at the 12 g/L NaCl concentration, leading to a significant drop in corrected germination percentage, especially for the Lemsi genotype (around a 40% reduction). This suggests that Lemsi is the most salt-sensitive genotype, showing low tolerance, particularly at higher salt levels. Specifically, statistical analysis ($p<0.05$) indicates that the corrected germination percentage of Lemsi under the 12 g/L NaCl treatment (T2) is significantly lower than both the control and the 6 g/L NaCl treatment (T1), as indicated by the distinct lettering ('a' for control, 'a' for T1, and 'd' for T2) in Fig. 2. While other genotypes also show a reduction in germination at 12 g/L NaCl, the effect is most pronounced and statistically significant in Lemsi.

Elongation

Effect of Salt Stress on Coleoptile Length

Fig. 3a) presents the results of the aerial system analysis under varying NaCl concentrations, demonstrating a significant impact of salinity on coleoptile length across all five barley genotypes.

Exposure to moderate (6 g/L NaCl) and high (12 g/L NaCl) salt concentrations significantly reduced coleoptile length in all genotypes studied. Specifically, germination in 6 g/L NaCl resulted in an approximate 50% reduction in coleoptile length across all genotypes. Ardhaoui and Manel exhibited the shortest coleoptiles under these conditions, ranging from 4 to 5 cm,

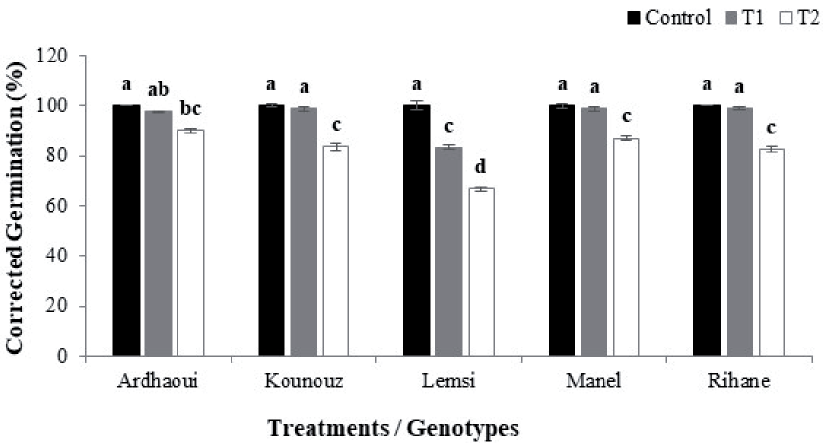

Fig. 2. Impact of salt stress on corrected germination percentage across barley genotypes.
Each value represents the mean of four replicates. T1 (6 g/L NaCl) and T2 (12 g/L NaCl) represent the salt stress treatments. Data are presented as means±standard error. Different letters indicate significant differences (p<0.05).

compared to Rihane, which maintained an average coleoptile length of approximately 8 cm relative to control conditions. A more severe reduction in coleoptile length (approximately 60%) was observed at 12 g/L NaCl, with statistically significant decreases across all genotypes relative to their respective controls.

These results demonstrate a clear inverse relationship between NaCl concentration and coleoptile length, indicating a dose-dependent effect of salinity on early seedling development. Supporting these observations, Fig. 3 shows that under control conditions, coleoptile length varies significantly among genotypes, with Rihane exhibiting significantly longer coleoptiles compared to Ardhaoui and Manel (p<0.05). The imposition of salt stress (T1 and T2) diminishes these genotypic differences, converging towards similarly reduced coleoptile lengths, particularly at the highest NaCl concentration (12 g/L).

Effect of Salt Stress on Radicle Length and Elongation

The root length is the most significantly affected parameter under both moderate and severe stress conditions in all five barley genotypes. Under control conditions (Fig. 3b)), Ardhaoui and Manel exhibited shorter root lengths (approximately 17 cm), while Lemsi showed significantly shorter roots (approximately 12 cm) compared to Ardhaoui, Kounouz, and Rihane, based on statistical analysis (p<0.05).

At the moderate NaCl concentration (T1), the radicle lengths of all genotypes were significantly reduced compared to their respective controls, and Ardhaoui (approximately 10 cm) exhibited significantly shorter radicle length compared to Kounouz and Rihane (approximately 12 cm, p<0.05). At the higher NaCl concentration (T2), the reduction in radicle length was even more pronounced, with a 70% decrease across all

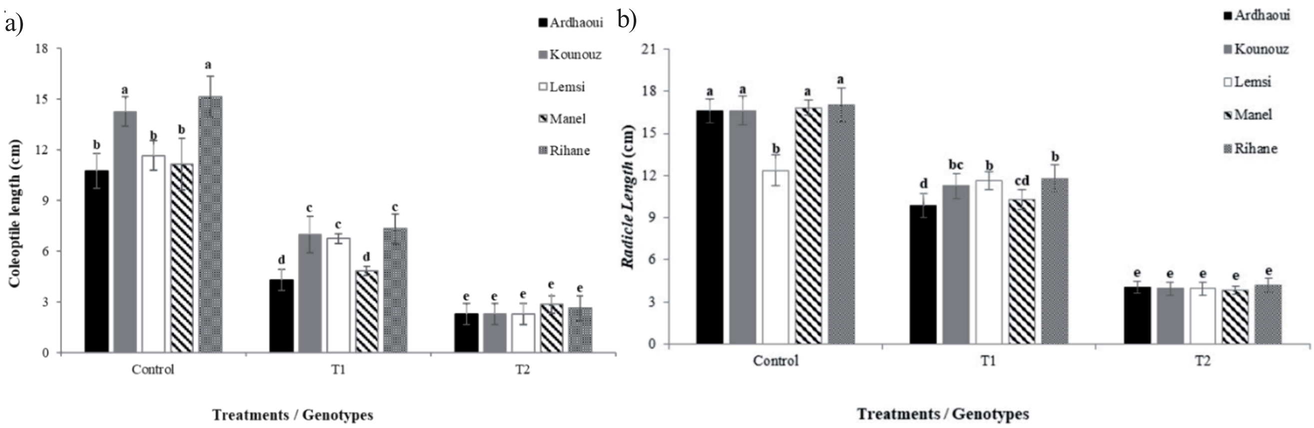

Fig. 3. a) Effect of NaCl on coleoptile length in different barley genotypes after 7 days of seed germination. b) Effect of NaCl on radicle length in different barley genotypes after 7 days of seed germination. Radicule and coleoptile length of five barley genotypes (Ardhaoui, Kounouz, Lemsi, Manel, and Rihane) after 7 days of germination under control conditions and two NaCl treatments (T1 and T2). Each value represents the mean of four replicates. Error bars represent standard error. Different letters indicate significant differences (p<0.05) based on a post-hoc test.

genotypes compared to the controls ($p<0.05$). Notably, under severe salt stress (T2), there were no statistically significant differences in root length between the genotypes, as evidenced by the shared lettering in Fig. 3b), indicating a convergence to similarly stunted root growth. In general, radicle length decreased as NaCl concentration increased, affecting all genotypes at every concentration. The results of the root system analysis under varying NaCl concentrations, presented in Fig. 3b), confirm that the radicle length is influenced by the presence of NaCl in the imbibition medium for all five genotypes.

Elongation refers to the stretching of cells that allows the root to grow. Therefore, maintaining root elongation in saline conditions is one of the factors contributing to salinity tolerance. Results analysis shows that elongation is significantly impacted by salt stress, with a more pronounced effect at higher NaCl concentrations across all five barley genotypes. Both tolerant and sensitive genotypes exhibited a similar response to salt stress, as elongation rates decreased with increasing salinity. At 6 g/L NaCl, elongation declined by approximately 40%, from an average of 1.00 mm/ hour under control conditions to 0.60 mm/h across all genotypes. At 12 g/L NaCl, elongation was further reduced by nearly 75%, reaching an average of 0.25 mm/ hour. While all genotypes exhibited a comparable trend, minor variations suggest potential differences in salt tolerance, with Lemsi showing a slightly slower decline at 6 g/L. These results indicate a strong inhibitory effect of salinity on grain elongation, regardless of genotype.

Tolerance Indices

Effect of Salt Stress on Germination Stress Tolerance Index (GSI)

The tolerance of the studied genotypes to the applied stress is also assessed using the Germination Stress Index (GSI), a key indicator of a genotype's ability to maintain germination under saline conditions. This parameter was calculated using Equations (2) and (3), as presented in the Experimental section. The following figure (Fig. 4a)) illustrates the variation in GSI across different salinity levels and genotypes. GSI is calculated as the ratio of germination percentage under stress conditions to that under control conditions, providing a quantitative measure of salt tolerance. A higher GSI indicates better germination resilience, while a lower GSI reflects greater sensitivity to salinity. The observed variations in GSI among the five barley genotypes highlight their differential responses to increasing NaCl concentrations, offering insights into their adaptive capacity under salt stress.

These results indicate a strong inhibitory effect of salinity on germination, with significant variations among genotypes. The progressive decline in GSI with increasing NaCl concentrations suggests that higher salinity levels impair seed viability and early seedling establishment. While all genotypes experienced reductions in GSI, the degree of impact varied, highlighting differences in salt tolerance. Ardhaoui, which maintained a GSI close to 80% under severe stress, appears to be the most resilient genotype, suggesting a better adaptive capacity to saline conditions. In contrast, Lemsi exhibited the highest sensitivity, with a drastic 50% reduction in GSI at 12 g/L NaCl, indicating greater susceptibility to salt stress. The remaining genotypes demonstrated intermediate responses, with GSI values ranging between 60% and 70% of their respective controls. Overall, the variation in GSI among genotypes under increasing salinity levels provides insight into their differing salt tolerance mechanisms. The relative tolerance observed in Ardhaoui suggests that it may possess physiological or biochemical traits that enhance germination under stress conditions, whereas the marked sensitivity of Lemsi underscores its vulnerability to saline environments.

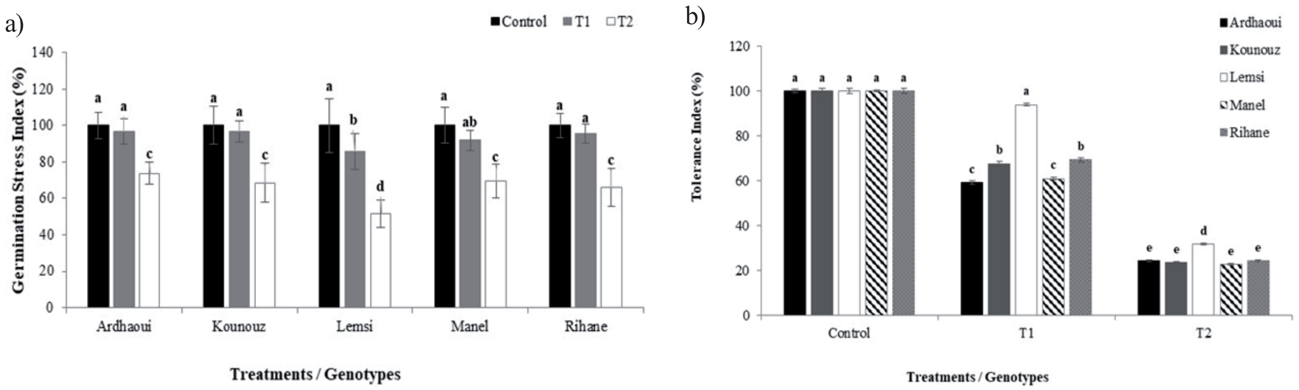

Fig. 4. a) Variation in the Germination Stress Index (GSI%) among different barley genotypes after seven days of grain germination under saline conditions. b) Variation in the Tolerance Index (TI%) among different barley genotypes after seven days of grain germination under saline conditions. Each value represents the mean of four replicates. Error bars represent standard error. Different letters indicate significant differences ($p<0.05$) based on a post-hoc test.

Effect of Salt Stress on the Tolerance Index

The Tolerance Index (TI) is a key parameter used to evaluate the ability of plants to sustain growth under stress conditions. It is typically expressed as a percentage, comparing plant growth under stress to that under control conditions. A higher TI value indicates greater stress tolerance, whereas a lower TI reflects higher susceptibility to adverse conditions.

The Tolerance Index data for the five barley genotypes (Ardhaoui, Kounouz, Lemsi, Manel, and Rihane), calculated using Equation (4) presented in the Experimental section, reveal significant differences in their ability to withstand salt stress (Fig. 4b)). Under control conditions, all genotypes exhibit a TI of 100%, indicating uniform performance in terms of germination and growth. However, under moderate salt stress (T1), Ardhaoui and Kounouz demonstrate the highest tolerance levels, whereas Lemsi, Manel, and Rihane show significantly lower indices, with Rihane being the most sensitive. Under severe salt stress (T2), the tolerance index declines across all genotypes (Fig. 4b)). Among them, Ardhaoui exhibits the least reduction in tolerance, indicating a superior capacity to withstand high salinity levels. In contrast, Lemsi, Manel, and Rihane display the lowest tolerance indices, highlighting their reduced ability to germinate and grow under saline conditions.

Multivariate Analysis

The results of a multivariate analysis, combining ANOVA and Principal Component Analysis (PCA), are presented to evaluate the germination and seedling growth responses of five barley genotypes under salt stress conditions (Fig. 5). ANOVA was used to assess the statistical significance of differences between genotypes, while PCA helped to identify patterns of variation and relationships between the traits studied.

The ANOVA results show that both the treatment (T) and genotype (G) have highly significant effects ($p<0.001$) on all measured traits, including root length, coleoptile length, tolerance index, corrected germination, germination stress index, and germination rate after 7 days. The interaction between treatment and genotype ($T \times G$) also has a significant effect on most traits, with root length, coleoptile length, and tolerance index showing strong significance. However, the interaction is less significant for the germination stress index ($p = 0.004$) and germination rate (7 days), indicating that the impact of treatment on these traits may be influenced by the genotype. The error values show some variability, but overall, the results suggest that both salinity treatment and genotype play critical roles in determining the salt tolerance and germination performance of the barley genotypes.

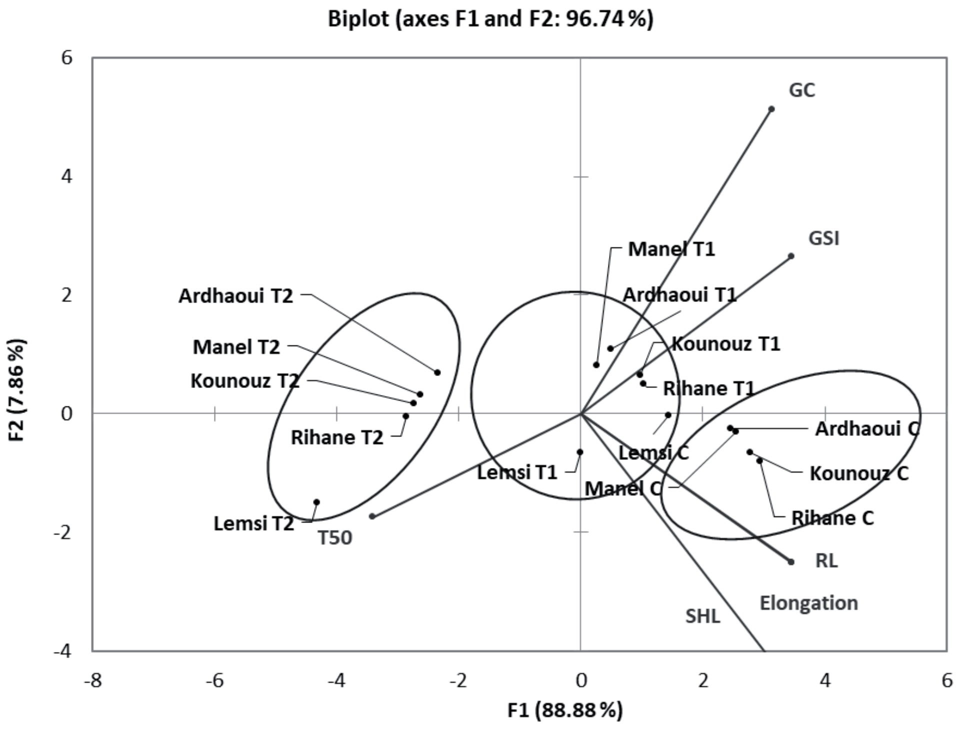

Fig. 5. Principal Component Analysis (PCA) of germination and seedling growth traits in five barley genotypes under salt stress. The plot illustrates the variation in germination performance and seedling growth responses to different levels of salinity across the genotypes, highlighting the distinct patterns of tolerance. Each point represents a genotype under a specific salinity treatment, with the axes indicating the primary sources of variation in germination and seedling growth traits. Genotypes: Ardhoui; Kounouz; Lemsi; Manel; Rihane. Parameters: GC: corrected germination; SHL: coleoptile length; RL: Radicle length; GSI: Germination tolerance Index. Treatments: C: Control; T1: 6 g/L NaCl; T2: 12 g/L NaCl.

The biplot illustrates the responses of various barley genotypes (Ardhaoui, Kounouz, Lemsi, Manel, and Rihane) to different salt stress treatments (Control, T1, T2), capturing 96.74% of the total variance with axes F1 (88.88%) and F2 (7.8%). Under Control conditions, all genotypes cluster closely, indicating similar growth metrics. However, under T1 (moderate salt stress), Ardhaoui and Kounouz maintain favorable growth, while Lemsi and Manel show less impact. The most significant shift occurs in T2 (severe salt stress), where Rihane and Lemsi cluster negatively, indicating poor performance. The red vectors representing growth parameters, such as Germination Speed Index (GSI), Growth Capacity (GC), root length (RL), and shoot length (SHL), highlight that genotypes like Ardhaoui display positive correlations with effective germination and growth, while Rihane and Lemsi demonstrate increased susceptibility to high salinity, confirming Ardhaoui as the most resilient genotype.

Discussion

The main objective of this study was to evaluate the impact of salinity stress on the germination potential and early seedling growth of five Tunisian barley (*Hordeum vulgare* L.) genotypes. By comparing their physiological responses under controlled salt stress conditions, we aimed to identify genotypic differences in salt tolerance during the critical early stages of plant development, to select candidate varieties suitable for cultivation in saline-prone environments of southern Tunisia. Salinity is one of the most detrimental abiotic stresses affecting germination, particularly in arid and semi-arid regions, where soil salinization is intensifying due to poor quality irrigation water and evapotranspiration [22]. The germination phase is extremely sensitive to salt stress because it is the point at which seeds transition from dormancy to active metabolic growth, relying heavily on internal nutrient reserves and water availability [9].

Salt stress disrupts germination through two primary mechanisms: osmotic stress and ionic toxicity. Osmotic stress occurs first, immediately after seed imbibition, when high salt concentrations in the surrounding medium reduce the water potential, hindering water uptake [23]. This impedes the rehydration of seed tissues and delays the activation of key metabolic processes such as the synthesis of hydrolytic enzymes [24]. As a result, reserve mobilization is impaired, particularly the breakdown of stored carbohydrates, proteins, and lipids in the endosperm that are essential to nourish the growing embryo [9]. Subsequently, ionic toxicity, mainly due to Na^+ and Cl^- accumulation, leads to cellular imbalances, enzyme inhibition, and oxidative damage [25]. These ions can accumulate in sensitive embryonic tissues, disrupt membrane integrity, and interfere with DNA replication and protein synthesis, further delaying or even arresting embryo growth. In some genotypes, these effects result in low germination speed, poor emergence, and stunted radicle and coleoptile elongation [26].

In the present study, the tested barley genotypes showed significant differences in their germination percentage, mean germination time, and early seedling vigor under salinity stress. These genotypic differences suggest that the ability to tolerate salt stress during germination is, at least partially, genetically controlled. Genotypes exhibiting faster germination rates and longer radicle or coleoptile lengths under salinity are likely to possess more efficient osmotic adjustment mechanisms, such as the accumulation of compatible solutes (e.g., proline, glycine betaine) that help maintain cellular turgor and protect macromolecules [27].

Moreover, these genotypes may have a better ability to exclude toxic ions or compartmentalize them into vacuoles through specialized transport proteins, thereby preserving cellular homeostasis. Hormonal regulation also plays a crucial role: salt stress is known to increase abscisic acid (ABA) levels, which suppress germination, while reducing gibberellic acid (GA), which promotes radicle emergence and embryo expansion [28]. Genotypes with greater hormonal balance and signaling efficiency may be better equipped to withstand the inhibitory effects of salinity.

The observed variability among genotypes could also be attributed to their ecological origins and genetic background. Landraces from southern Tunisia, for instance, have evolved under harsh environmental conditions and may have undergone natural and farmer-led selection for traits such as salt and drought tolerance. These varieties potentially harbor adaptive alleles associated with early vigor, stress resilience, and reserve mobilization efficiency, traits that are critical for seedling establishment in saline soils. Particularly, root and shoot elongation are key indicators of seedling vigor under stress. In our study, some genotypes, such as Ardhaoui and Rihane, maintained greater elongation of radicles and coleoptiles, suggesting their ability to overcome the initial osmotic stress and sustain cell division and expansion. This is important because early root growth allows seedlings to access deeper soil layers with lower salt concentrations, while rapid shoot elongation supports quicker emergence and autotrophic growth. The differences in these parameters highlight distinct physiological strategies among genotypes, ranging from escape mechanisms to actual physiological tolerance.

In saline environments, germination speed and early seedling growth are not only indicators of stress response but also crucial agronomic traits. Rapid germination allows plants to establish before salt accumulates to critical levels in the upper soil layers due to evaporation [29]. In summary, salinity negatively affects germination through osmotic inhibition, ion toxicity, and hormonal imbalance, leading to delayed or poor seedling establishment. However, the differential responses among the five barley genotypes underscore the importance of genotypic variability in shaping tolerance strategies.

Conclusions

This study highlights the detrimental impact of salinity stress – particularly at NaCl concentrations above 6 g/L – on the germination dynamics of five Tunisian barley genotypes. Although final germination percentages were only moderately affected in most genotypes, significant delays in germination speed were observed, underscoring the sensitivity of early developmental processes to osmotic stress and ion toxicity. Among the tested varieties, Ardhaoui stood out for its superior performance under saline conditions, exhibiting both a relatively high germination rate and better seedling vigor, in contrast to more sensitive genotypes such as Lemsi. These findings confirm barley's inherent resilience to saline environments, yet they also reveal substantial genotypic variability in early-stage stress tolerance. The ability of Ardhaoui to maintain better physiological performance under salt stress makes it a promising candidate for cultivation in marginal, salt-affected areas and a valuable resource for breeding programs focused on enhancing salinity tolerance in barley. Beyond the academic insights, this research carries practical importance for sustainable agriculture in arid and semi-arid regions, particularly in the context of climate change and increasing soil salinization due to poor irrigation practices. Identifying and promoting salt-tolerant genotypes such as Ardhaoui can support the development of resilient cropping systems and sustain rural livelihoods in regions facing growing environmental challenges.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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