

## Effects of Saline Stress on the Germination of Durum Wheat and Barley

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Article Details: Received: 2025-09-20 | Accepted: 2026-03-16 | Available online: 2026-06-30

<https://doi.org/10.15414/afz.2026.29.01.178-186>



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Soil salinity poses a significant challenge to plant productivity and is a major issue for agriculture in Algeria. Consequently, developing varieties that can tolerate high salinity levels is a sustainable way to expand cereal cultivation, particularly in semi-arid regions. This study compares two durum wheat (*Triticum durum* Desf.) varieties and two barley (*Hordeum vulgare* L.) varieties with contrasting salt stress sensitivities. The experiment was conducted during the germination and early vegetative stages, assessing morphological, physiological and biochemical parameters. Specifically, the impact of escalating salinity levels (0–20 g/l NaCl) on the germination and initial growth of durum wheat (Bousselem and Waha) and barley (Tichedrett and Saida) was evaluated. The results revealed a highly significant, dose-dependent inhibitory effect of salinity. Increasing salt concentrations markedly reduced the final germination percentage (FGP), slowed germination kinetics and increased the mean germination time ( $T_{50}$ ). Morphological analyses showed severe inhibition of root and epicotyl growth at concentrations of 15 g/l and above. Of the tested genotypes, wheat variety Bousselem exhibited the greatest tolerance, maintaining an FGP of 28% under severe stress (20 g/l), while barley variety Tichedrett demonstrated the fastest germination rate. An important finding was that germination recovery occurred following exposure to moderate salinity, but not after severe stress (18 to 20 g/l). Overall, durum wheat displayed greater salt tolerance than barley. Furthermore, significant intraspecific variability was identified in Bousselem and Tichedrett, making them promising salt-tolerant genotypes worthy of further evaluation.

**Keywords:** NaCl effects, durum wheat, barley, germination, physiobiochemical parameters

### 1 Introduction

Soil and water salinisation are among the most severe abiotic stresses limiting agricultural productivity worldwide, particularly in arid and semi-arid regions. The main causes of salinity, including high evaporation rates, low rainfall and unsustainable irrigation practices, are well documented (Munns and Gilliam, 2015). Salinity currently affects over 20% of irrigated agricultural land, posing a significant threat to global food security (FAO, 2021). Climate change exacerbates this problem by contributing to rising sea levels and increased evapotranspiration rates (Isayenkov and Maathuis, 2019).

Soil salinisation significantly constraints cereal production in Algeria, particularly for durum wheat (*Triticum durum* L.) and barley (*Hordeum vulgare* L.), which are both key components of the national food supply. Salinity

adversely affects plant growth throughout the life cycle, with germination and the early stages of seedling establishment being particularly sensitive periods. During these phases, salinity imposes osmotic stress, limiting water uptake, as well as ionic toxicity resulting from the excessive accumulation of  $\text{Na}^+$  and  $\text{Cl}^-$  ions. These effects can disrupt metabolism and delay or inhibit germination (Van Zelm et al., 2020). Salt tolerance is strongly influenced by developmental stage and genetic background, with significant variation observed between and within cereal species, particularly during germination, which is a critical phase for crop establishment and final yield (Deinlein et al., 2014). Barley is generally considered to be more salt-tolerant than durum wheat; however, substantial intraspecific variability exists in both species, providing opportunities to select tolerant genotypes (Munns and Gilliam, 2015; Tavakoli et al., 2016).

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Plants employ various physiological and biochemical mechanisms to cope with salinity. These include maintaining ion homeostasis via  $\text{Na}^+/\text{H}^+$  exchangers (NHXs), salt overly sensitive (SOS) pathways, and high-affinity  $\text{K}^+$  transporters (HKTs); adjusting osmolality through compatible solutes, such as proline and soluble sugars; and activating antioxidant defences to mitigate oxidative stress (Zörb et al., 2019; Zhao et al., 2020). Consequently, early-stage screening based on germination-related traits has become a rapid, efficient and valuable method for identifying salt-tolerant varieties, complementing traditional agronomic evaluations (Acosta-Motos et al., 2017).

As conventional soil desalination techniques are not widely feasible, developing salt-tolerant cereal varieties is the most sustainable and economically viable strategy for enhancing productivity in saline environments (Fita et al., 2015; Roy et al., 2014). Against this background, the present study aims to evaluate the impact of escalating NaCl concentrations on germination behaviour and initial growth in selected durum wheat and barley varieties. By comparing interspecific and inter-varietal responses under controlled conditions, the study will identify differences in salt sensitivity at the germination stage and provide insights into the relevant physiology that could inform breeding programmes targeting the saline conditions prevalent in regions such as Algeria.

## 2 Material and Methods

### 2.1 Plant Material

This study used seeds from two durum wheat (*Triticum durum* Desf.) varieties (Waha and Bousselem) and two barley (*Hordeum vulgare* L.) varieties (Tichedrett and Saida). These seeds were obtained from the Cereals Science Leisure Center (CSLC) in Batna, Algeria, during the harvest of the year 2023.

### 2.2 Experimental Protocol

Germination tests were carried out using different concentrations of sodium chloride (NaCl). For each variety, 120 seeds per treatment were surface-sterilised using a 0.5% sodium hypochlorite solution. These were then thoroughly rinsed with tap water to remove any residual sterilising agent and given a final rinse with distilled water. The seeds were placed in 10 cm diameter Petri dishes lined with moist cloth, sterile filter paper, and were incubated for seven days. The control group was given 20 ml of distilled water, while the experimental groups were given 20 ml of NaCl solution at the following concentrations: 5 g/l, 10 g/l, 15 g/l, 18 g/l and 20 g/l. The Petri dishes were then incubated in the dark at a constant temperature of 25 °C. All reagents were

obtained from Sigma-Aldrich (Germany). Germination was defined as the radicle emerging from the seed coat to a length of at least 2 mm (Sayar et al., 2010). The experimental groups were organised as follows:

- Group A: The control (0 g/l) and the N1 level (5 g/l), across all genotypes.
- Group B: The N2 level (10 g/l), across all genotypes.
- Group C: The N3 (15 g/l) and N4 (18 g/l) levels, across all genotypes.
- Group D: The N5 level (20 g/l), across all genotypes.

### 2.3 Studied Parameters

#### 2.3.1 Final Germination Rate (FGR)

This parameter is a key indicator for identifying the saline concentration that represents the physiological limit for seed germination. It is expressed as the ratio of germinated seeds to total seeds (Côme, 1970).

#### 2.3.2 Germination Kinetics

In order to better understand the physiological significance of germination behaviour in the varieties under study, the number of germinated seeds was counted daily until day 7 of the experiment (Hajlaoui et al., 2007).

#### 2.3.3 Germination Speed

This metric reflects the germination energy responsible for depleting the seed's reserves. Germination speed can be expressed as Median Germination Time ( $T_{50}$ ), which is the time required to reach 50% of the final germination percentage (Côme, 1970), or Mean Daily Germination (MDG).  $T_{50}$  is calculated using the following formula:

$$T_{50} = T_1 + \left[ \frac{(0.5 \cdot N - G_1)}{(G_2 - G_1)} \right] \cdot (T_2 - T_1)$$

where:  $N$  – the final number of germinated seeds;  $T_1$  – the last day when the cumulative germination was less than 50%;  $T_2$  – the first day when the cumulative germination was equal to or greater than 50%;  $G_1$  – the cumulative number of seeds germinated by day  $T_1$ ;  $G_2$  – the cumulative number of seeds germinated by day  $T_2$

Mean Daily Germination (MDG) was calculated according to Osborne et al. (1993), the MDG is calculated as the final germination percentage divided by the number of days to reach final germination.

#### 2.3.4 Root and Shoot Lengths

The length of the primary root and the epicotyl (the embryonic shoot) were measured using a millimeter ruler to evaluate the impact of stress on plant growth.

### 2.3.4 Reversibility of Salt Stress

This parameter is useful for determining whether the salt's inhibitory effect is osmotic and/or toxic. Briefly, to assess the reversibility of salt stress, seeds were germinated in the presence of different concentrations of NaCl for four days. On day four, any seeds that had not germinated were rinsed three times to remove any residual sodium chloride. They were then transferred to new Petri dishes containing only distilled water and left for a further four days (Hajlaoui et al., 2007).

### 2.4 Statistical Analyses

Data interpretation was performed using a two-way analysis of variance (ANOVA) with fixed factors (salt concentration and variety) in the statistical software 'XLSTAT 2014'. Homogeneous group separation among means was conducted using the Newman-Keuls post-hoc test at a significance level of  $\alpha = 5\%$  ( $p < 0.05$ ). Histograms of the various studied variables were created using Microsoft Excel 2007.

## 3 Results and Discussion

### 3.1 Effect of Saline Stress on the Germination of the Studied Genotypes

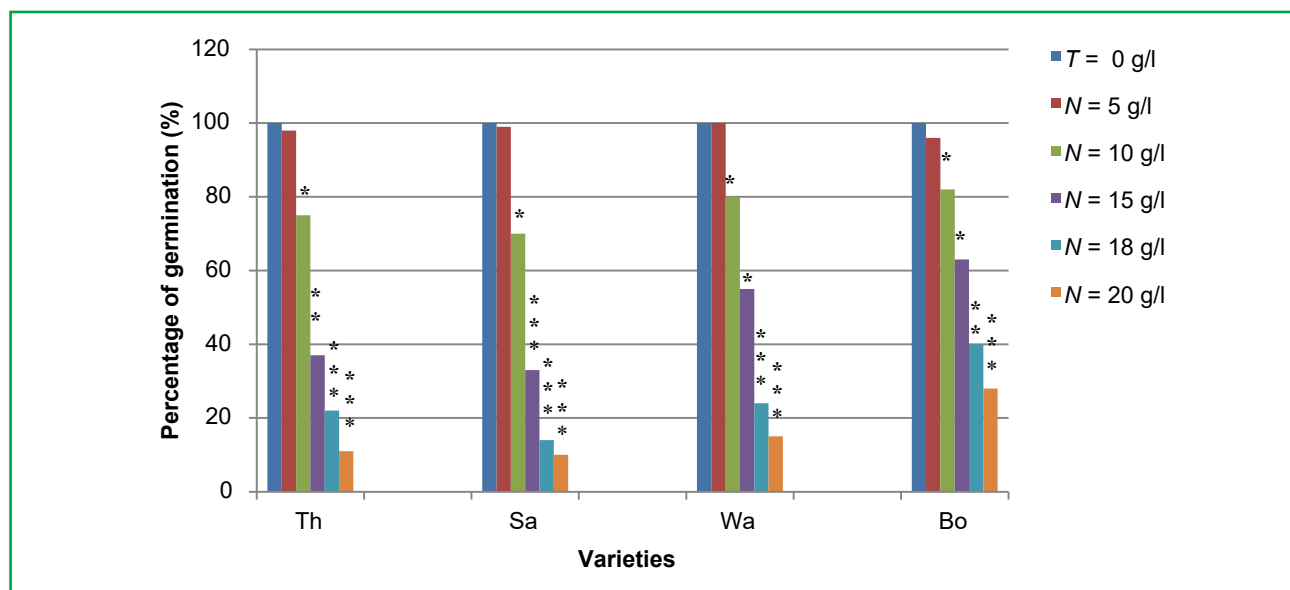
#### 3.1.1 Final Germination Percentage (FGP)

Although germination under saline stress does not fully reflect natural conditions, it still provides an indication of how varieties perform (Naceur et al., 2001). As shown in Figure 1, germination decreased progressively as

the salt concentration increased. In the control treatment using distilled water, germination rates were almost 100%. Under moderate stress (5 g/l), most varieties germinated at rates similar to the control, although small differences between varieties were apparent. However, at higher salt levels (10 and 15 g/l), germination declined significantly and, under severe stress (18 and 20 g/l), this reduction became even more pronounced. Of the wheat varieties, Bousselem (Bo) demonstrated the greatest tolerance, achieving 28% germination at the highest salt level, while Waha (Wa) and Saida (Sa) exhibited the greatest sensitivity. Among the barley varieties, Tichedrett (Tch) performed best under saline conditions. Overall, wheat varieties demonstrated better germination than barley, revealing clear differences in salt tolerance between species and varieties.

Separation of homogeneous groups using the Newman-Keuls test at  $\alpha = 5\%$  revealed two distinct groups with respect to the variety factor for the final germination rate. Specifically, group B comprised the barley varieties Saida and Tichedrett at all salinity levels, while group A included the durum wheat varieties Bousselem and Waha at all stress levels.

ANOVA provided further statistical evidence of the effects of salinity, showing a highly significant effect of NaCl on germination ( $F(5, 24) = 769.48, p < 0.0001$ ). Notably, germination at 0 g/l was significantly higher than at all other concentrations ( $p < 0.001$ ). Furthermore, as the concentration of NaCl increased (from 5 to 20 g/l), germination declined progressively, with significant



**Figure 1** Final germination percentage of durum wheat (Wa – Waha, Bo – Bousselem) and barley (Sm – Saida, Tm – Tichedrett) varieties exposed to increasing NaCl concentrations  
 \* $p \leq 0.05$ : significant; \*\* $p \leq 0.01$  – highly significant; \*\*\* $p \leq 0.001$  – very highly significant, compared to the control group (0 g/ml) in every variety

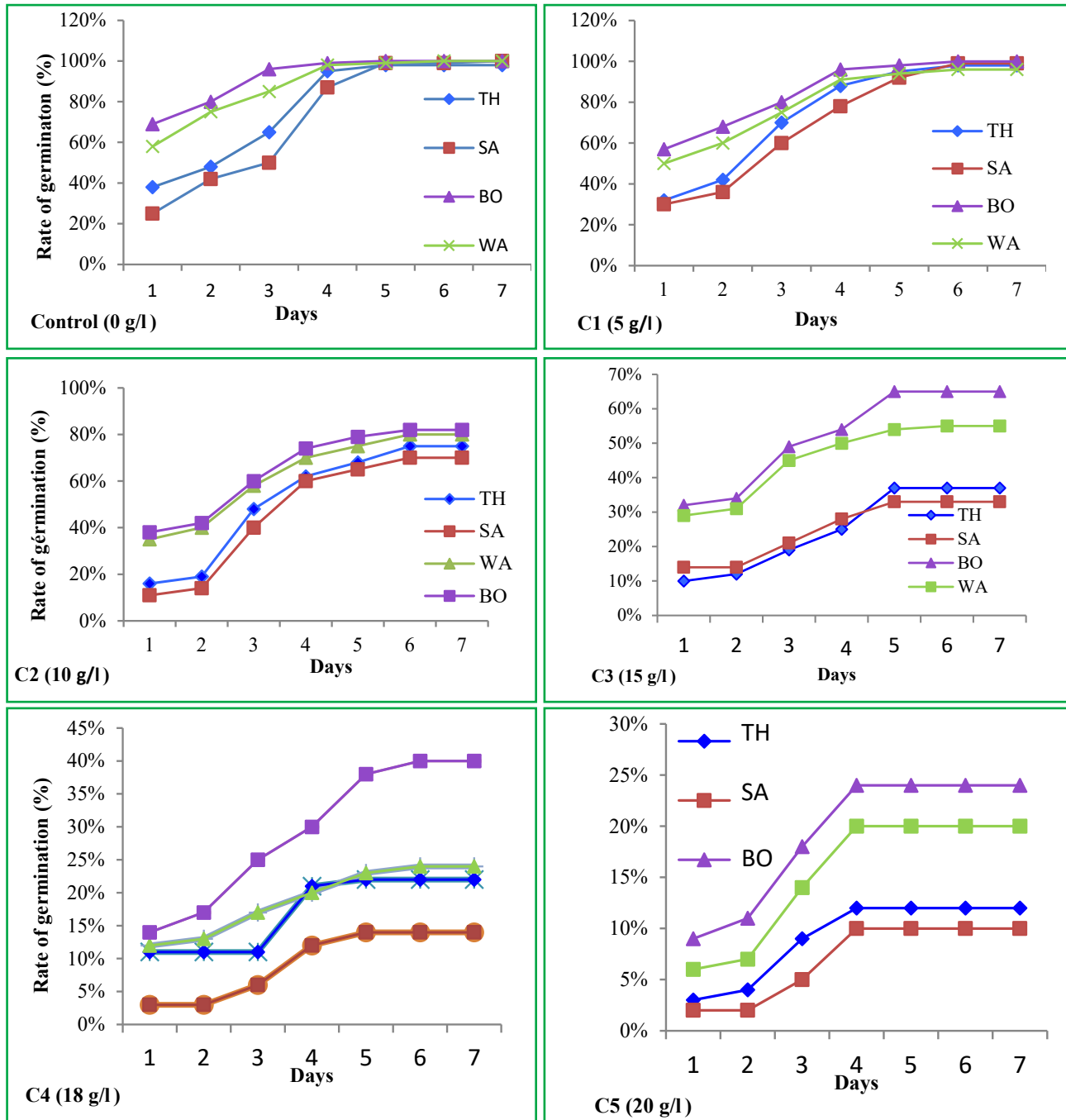
differences observed between most pairs ( $p < 0.05$  to  $p < 0.001$ ). Overall, increasing NaCl concentrations strongly and significantly inhibited seed germination.

### 3.2 Effects of Different NaCl Concentrations on the Germination Kinetics of Various wheat and Barley Varieties

Figure 2 presents the progression of germination for the four wheat and barley varieties over time across

all treatments. The results showed that the curves representing the germination rates of treated (stressed) seeds are situated below those of the control curves and approach zero as the NaCl dose increases.

Under control conditions (C0), all varieties germinated rapidly and consistently, achieving high or complete germination by day 7. Low NaCl levels (C1) caused only slight delays, resulting in final germination percentages similar to those of the control. Moderate concentrations



**Figure 2** Effects of different NaCl concentrations (C0 to C5) on the germination kinetics of various wheat and barley varieties over the 7-day saline stress application period

(C2 and C3) slowed germination noticeably, delaying the onset of germination and reducing the final percentage of germinated seeds compared to the control. High NaCl levels (C4 and C5) strongly inhibited germination, indicating a clear, concentration-dependent negative effect of NaCl on seed germination.

The germination curves reveal three distinct phases:

- A latency phase: necessary for the first germinations to emerge. During this phase, the germination rate remains low and its duration varies with the concentration of NaCl. This phase is short or even absent in control plants and in plants irrigated with a 5 g/l NaCl solution. However, it is prolonged, particularly in plants subjected to the 20 g/l NaCl treatment, for which this phase can last up to three days.
- A near-linear phase: then follows, during which the germination rate increases rapidly and proportionally over time, at least for control plants and those subjected to a 5 g/l NaCl solution. For higher concentrations, this phase is very short, which explains the reduced germination rate due to the inhibitory effect of salt on germination.
- A third phase: corresponds to a plateau representing the final germination percentage, reflecting the germination capacity of each variety at each concentration. This germination capacity appears to decrease for all varieties studied, but by different degrees depending on the species and the stress applied. The results of this study showed that the Bo variety is the most salt-tolerant and progresses more rapidly than the other varieties, whereas the Sa variety is the most sensitive. The remaining varieties exhibit *intermediate* behavior.

To rigorously demonstrate and justify the effect of salinity on the germination kinetics of wheat and barley, we analysed our results using a two-way ANOVA with repeated measures (factors: variety and salt concentration). We then performed a Newman-Keuls post-hoc test at a significance level of  $\alpha \leq 5\%$ . According to the test, the probability is  $p < 0.0001$ , indicating that salt concentrations have a highly significant effect on germination. Furthermore, the test revealed that time significantly affects the progression of germination under both saline and non-saline (control) conditions. Primary synthesis of the results from the Newman-Keuls test classified the salt concentrations into four homogeneous groups.

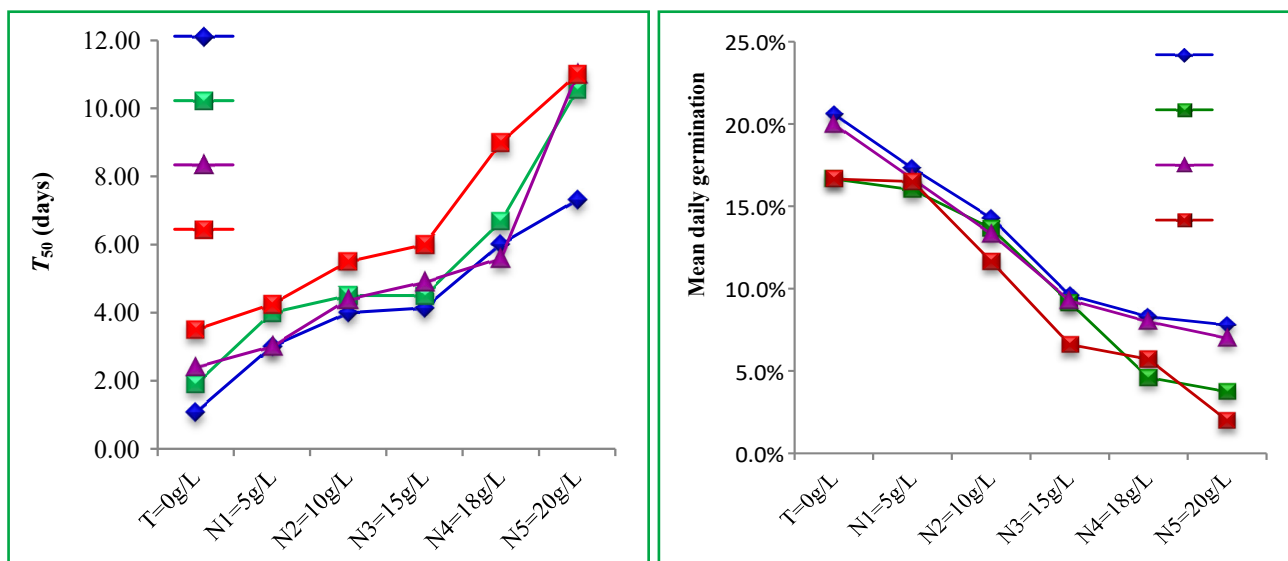
- Group A: represented by the control (0 g/l).
- Group B: represented by the concentration of 5 g/l.
- Group C: represented by the concentration of 15 g/l.
- Group D: represented by the concentrations of 18 and 20 g/l.

A second analysis using the Newman-Keuls test also classified the varieties into three groups:

- Group A: the Bo variety.
- Group B: the Wa variety.
- Group C: the barley varieties Sa and Tch.

### 3.3 Germination Speed and Mean Daily Germination

Figure 3 illustrates the impact of increasing NaCl concentrations on the mean germination time ( $T_{50}$ ) and the mean daily germination (MDG) of the examined wheat and barley varieties.  $T_{50}$  increased significantly ( $p < 0.05$ ) at moderate and high NaCl concentrations (10–15 g/l), indicating slower germination; meanwhile, MDG decreased significantly at these concentrations.



**Figure 3** Effects of different NaCl concentrations on the mean germination time ( $T_{50}$ ) and the mean daily germination (MDG) of the studied wheat and barley varieties

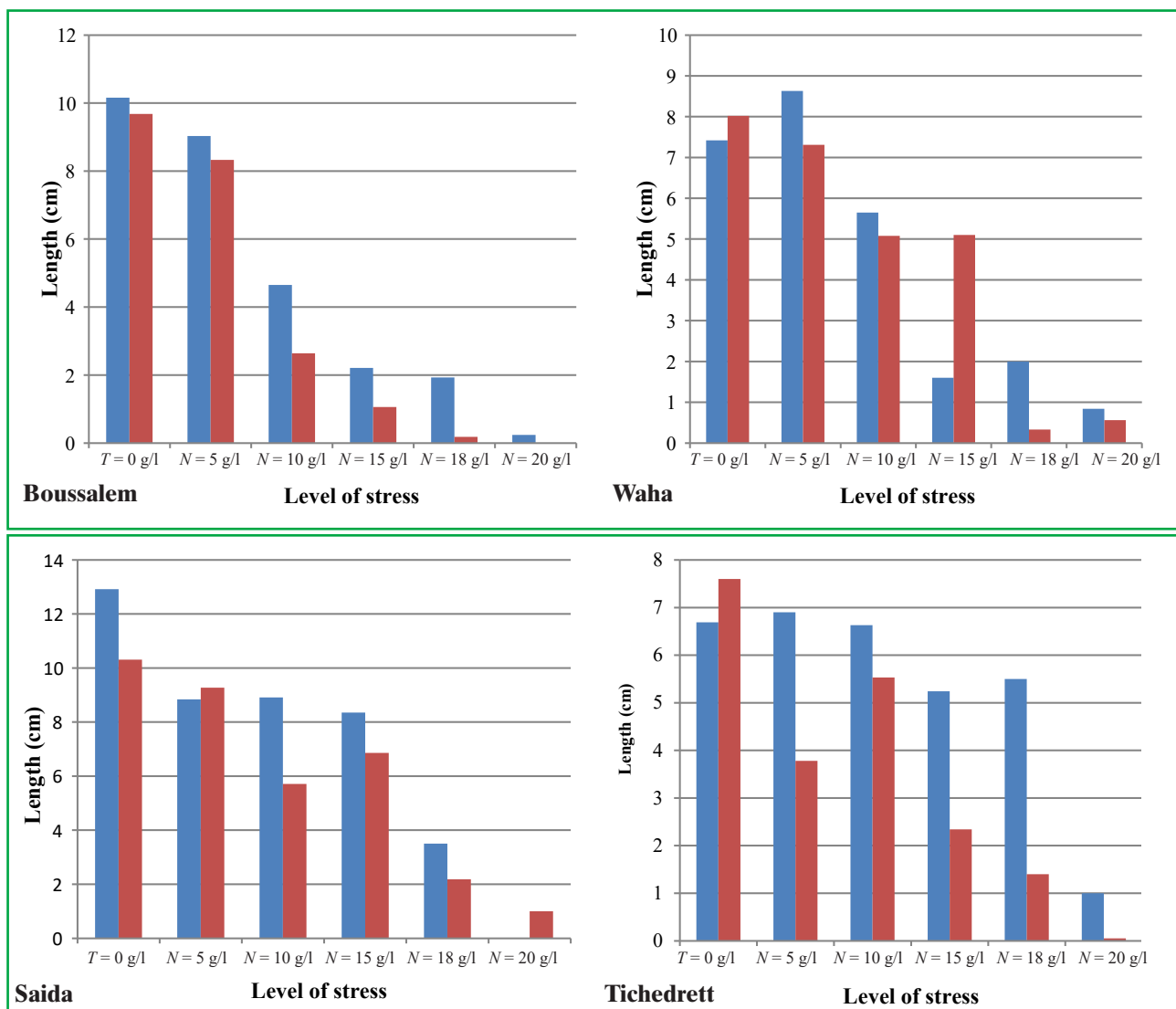
Low salinity (5 g/l) had a minimal effect, showing no significant difference from the control.

Among the varieties, Tch exhibited the fastest germination, with significantly lower  $T_{50}$  and higher MDG than the other varieties under all treatments. These results suggest that salinity significantly inhibits the speed and efficiency of seed germination, with this effect becoming more pronounced at higher NaCl concentrations.

### 3.4 Root and Epicotyl Lengths

Figure 4 illustrates the impact of escalating NaCl concentrations on root and epicotyl lengths. Moderate salinity caused only slight reductions, whereas severe stress (15, 18 and 20 g/l) had a strong inhibitory effect

on growth. A two-way ANOVA revealed a highly significant effect of salinity on root and epicotyl length ( $p < 0.0001$ ). Lengths under saline treatment were significantly shorter than in the control group ( $p < 0.05$  to  $p < 0.0001$ ). Newman-Keuls analysis classified the NaCl concentrations into six homogeneous groups: A (0 g/l), B (5 g/l), C (10 g/l), D (15 g/l), E (18 g/l) and F (20 g/l). Varietal differences also classified the genotypes into two categories: barley varieties (Saida and Tichedrett) and durum wheat varieties (Waha and Bousselem), reflecting the significant effects of genotype on root and epicotyl growth. Overall, both salinity level and genotype had a strong influence on seedling development, with higher NaCl concentrations producing the greatest growth inhibition.



**Figure 4** Variation in root and epicotyls lengths of the four wheat and barley varieties as a function of NaCl concentration (Blue color: Roots; Red color: Epicotyls values are means  $\pm$ SD ( $n = 10$ ); \* $p \leq 0.05$  – significant; \*\* $p \leq 0.01$  – highly significant; \*\*\* $p \leq 0.001$  – very highly significant, \*\*\*\* $p \leq 0.0001$  – extremely different, compared to control group (0 g/ml) in every variety

### 3.5 Reversibility of Salt Stress

Seeds that did not germinate in the 15 g/l NaCl treatment resumed germination when transferred to distilled water. However, their final germination rate was lower than that of seeds that germinated directly on the control medium. By contrast, seeds from the higher salinity treatments (18 and 20 g/l) showed almost no recovery after transfer, indicating near-total inhibition of germination (Figure 5).

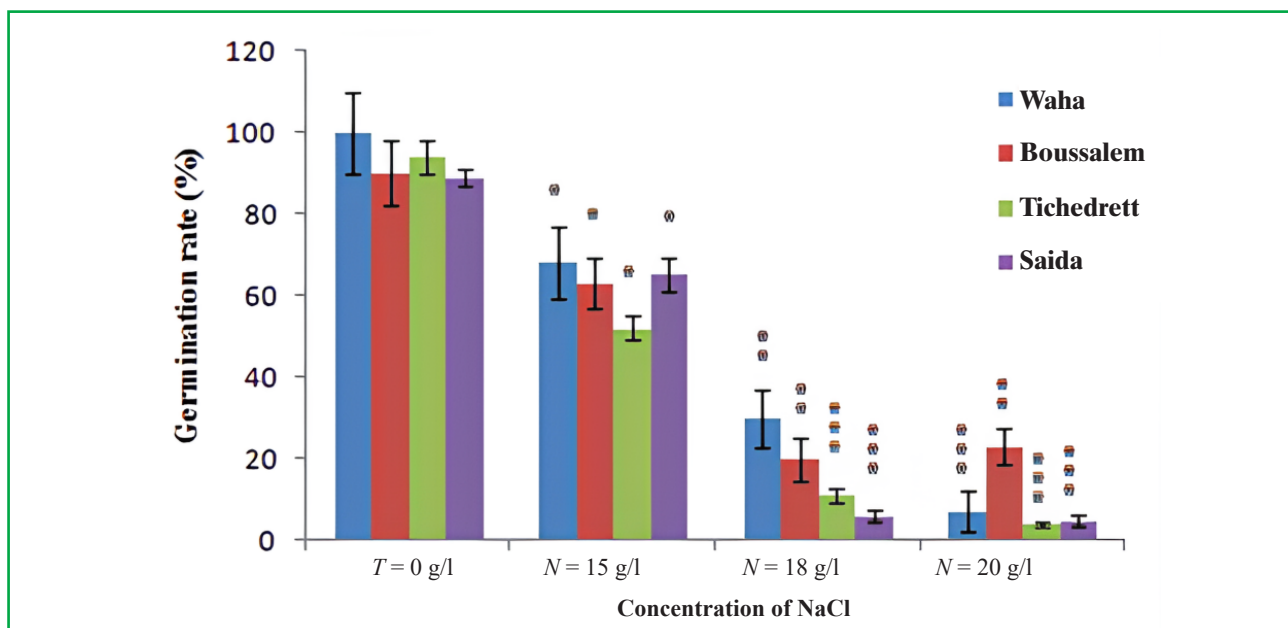
Final germination percentages after four days of recovery were significantly affected by NaCl concentration (one-way ANOVA:  $F(3, 12) = 75.4, p < 0.0001$ ). Post-hoc Tukey's HSD test revealed three distinct groups: the control group (0 g/l) exhibited the highest germination rate ( $93.3 \pm 4.9\%$ ), the 15 g/l group showed a moderate reduction ( $62.0 \pm 7.2\%$ ), and the 18 and 20 g/l groups exhibited severe inhibition ( $16.8 \pm 10.7\%$  and  $9.8 \pm 8.9\%$  respectively), with no significant difference between them ( $p < 0.05$ ). These results suggest that moderate salinity enables partial germination recovery, whereas extreme salinity results in irreversible inhibition.

Soil salinity is becoming an increasingly serious constraint on crop production, particularly in arid and semi-arid regions where cereals such as wheat and barley are crucial for food security. Seed germination and early seedling establishment are the most sensitive stages of the plant life cycle and are therefore highly vulnerable to salt stress. This study evaluated the impact of rising NaCl concentrations on the success and speed of germination, early seedling growth and recovery potential in four wheat and barley varieties.

We examined the effects of NaCl concentrations ranging from 0 to 20 g/l in two durum wheat varieties (Bousselem and Waha) and two barley varieties (Tichedrett and Saida). Previous studies have documented the inhibitory effects of salinity on cereal germination (Azmi and Alam, 1990). Building on these findings, the present study focused on modern wheat and barley varieties to better understand their responses to saline stress. Traits such as germination speed, uniformity and resilience are essential for successful seedling establishment and ultimately influence crop productivity (Bouatrous, 2012). Our results confirm that increasing salinity significantly reduces these attributes.

During the initial stages of exposure, seeds primarily experience osmotic stress, which impedes water uptake and delays the initiation of germination (Van Zelm et al., 2020). This effect may be reversible if the stress is removed rapidly (Julkowska and Testerink, 2015). However, prolonged exposure to saline conditions can lead to ionic toxicity due to the accumulation of sodium ( $\text{Na}^+$ ) ions in plant tissues, which disrupts cellular ion balance and metabolic processes (Munns and Gilliam, 2015). Such disturbances could explain the reduction in root and epicotyl elongation observed in our experiments, since radicle growth is crucial for water and nutrient uptake during the early stages of seedling development (Deinlein et al., 2014).

The recovery tests conducted in this study provide further clarification of this mechanism. Seeds exposed to high salinity partially resumed germination when



**Figure 5** Germination recovery rate (%) of stressed seeds following transfer to a salt-free medium across studied varieties values are means  $\pm$ SD ( $n = 10$ ); \* $p \leq 0.05$  – significant; \*\* $p \leq 0.01$  – highly significant; \*\*\* $p \leq 0.001$  – very highly significant, compared to the control group (0 g/ml) for each variety

transferred to distilled water, although full recovery was not achieved. This indicates that early osmotic inhibition can be alleviated when favourable conditions are restored; however, prolonged ionic stress results in more persistent physiological damage. At the molecular level, excess sodium ( $\text{Na}^+$ ) ions disrupt the  $\text{Na}^+/\text{K}^+$  balance, affecting enzyme activity and cellular metabolism (Assaha et al., 2017). Additionally, salinity alters phytohormone regulation, particularly by increasing abscisic acid (ABA) levels. Elevated ABA levels suppress germination-related gene expression and inhibit the mobilisation of energy reserves from the endosperm (Vishwakarma et al., 2017; He et al., 2018). Consequently, seeds reduce their metabolic activity as a survival response under stressful conditions.

Our results also suggest that germination speed is a more reliable indicator of salt tolerance than the percentage of seeds that ultimately germinate. Seeds that germinate rapidly may be better able to overcome early stress and establish seedlings successfully, making this parameter particularly valuable in breeding programmes aimed at improving stress tolerance (Rahaman et al., 2015). Furthermore, salt tolerance is stage-specific, meaning mechanisms conferring tolerance during germination may differ from those during vegetative or reproductive growth (Zhang et al., 2020; Gupta et al., 2020). Genetic variability also significantly contributes to these differences, with some species or genotypes being more sensitive during germination and others displaying greater resilience at this stage (Johanna, 2006; Zebro and Jae-Yun, 2024).

In line with this genetic variability, the present study revealed clear differences in salt tolerance among the tested wheat and barley varieties. Similar genotypic variation in germination and early seedling growth under saline conditions has been widely reported. For instance, Wu et al. (2025) observed significant variations in wheat germplasm's germination performance and seedling development under salinity, noting that certain genotypes exhibited greater germination capacity and growth potential. Similarly, Asif et al. (2019) demonstrated that increasing concentrations of NaCl delay germination and inhibit early growth, underlining the importance of varietal screening to distinguish between tolerant and sensitive genotypes. In our study, the durum wheat variety Bousselem demonstrated the best performance under severe salinity conditions, maintaining relatively high levels of germination and seedling growth. In contrast, the barley variety Saida proved to be the most sensitive, exhibiting strong inhibition and limited recovery at high NaCl concentrations. These findings confirm that early germination traits and seedling growth parameters are reliable indicators of salt tolerance, supporting the selection of cereal genotypes adapted to saline environments.

## 4 Conclusions

This study demonstrates that increasing salinity progressively reduces germination and early seedling growth in durum wheat and barley. Higher NaCl concentrations slowed germination, lowered final germination percentages, and restricted root and epicotyl elongation. The durum wheat variety Bousselem showed the highest tolerance, while the barley variety Saida was the most sensitive. Moderate salinity caused primarily osmotic stress, whereas high salinity induced near-irreversible ionic damage, limiting recovery. Mean germination time ( $T_{50}$ ) and early growth kinetics proved more reliable indicators of salt tolerance than final germination alone. These results highlight the value of combining germination speed, recovery potential, and seedling growth in selecting salt-tolerant genotypes to enhance cereal resilience in saline soils.

## Conflict of Interest

All authors declare that they have no conflicts of interest relating to this work.

## Author Contributions

Conceptualisation, L.N. and S.C.; Methodology, L.N. and H.F.; Validation, F.M. and S.C.; Formal Analysis, M.K. and F.M. Data Curation, M.K. and S.C.; Writing – Original Draft Preparation, M.K. and F.M.; Writing – Review & Editing, F.M. and M.K.; Visualization, L.N., H.F. and S.C.; Project Administration, L.N.; Funding Acquisition, no funding.

## AI and AI-Assisted Technologies use Declaration

Artificial intelligence was used only to edit and improve the English language in this document.

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