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Comparative germination of *Moringa peregrina* and *Moringa stenopetala* under NaCl Stress: Screening evidence and implications for agroforestry in arid Algeria

Original Article

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Abstract:

This study conducted a comparative analysis of the germination strategies of *Moringa peregrina* and *Moringa stenopetala* under varying salinity stress levels (0%, 4%, 8%, 12%, and 16% NaCl) to evaluate their potential for agroforestry in arid regions of Algeria. Salinity significantly affected all germination parameters, including germination rate, speed, mean germination time, vigor index, and stress tolerance index ($p<0.001$). *Moringa peregrina* demonstrated higher tolerance, maintaining viable germination up to 8% NaCl, whereas *M. stenopetala* showed complete inhibition beyond 4% NaCl. Germination kinetics exhibited a sigmoidal pattern, characterized by a delayed onset and reduced final percentages under stress conditions. Principal component analysis indicated that *M. peregrina* displayed enhanced resilience, whereas *M. stenopetala* showed increased sensitivity to high salinity. These findings suggest that *M. peregrina* is more suitable for cultivation in saline-prone arid regions, whereas *M. stenopetala* requires additional strategies to mitigate salinity stress.

Key words:
seed vigor indices, abiotic stress tolerance, saline soils, arid-zone crops, agroecological adaptation

Apstrakt:

Komparativno klijanje vrste *Moringa peregrina* i *Moringa stenopetala* pod stresom NaCl: dokazi iz skrininga i iplikacije za agrošumarstvo u aridnom Alžiru

Ova studija je sprovela komparativnu analizu strategija klijanja *Moringa peregrina* i *Moringa stenopetala* pod različitim nivoima salinitetnog stresa (0%, 4%, 8%, 12% i 16% NaCl), kako bi se procenio njihov potencijal za agrošumarstvo u aridnim regionima Alžira. Salinitet je značajno uticao na sve parametre klijanja, uključujući stopu klijanja, brzinu klijanja, srednje vreme klijanja, indeks vigoroznosti i indeks tolerantnosti na stres ($p<0,001$). *Moringa peregrina* je pokazala veću tolerantnost, zadržavajući održivo klijanje do 8% NaCl, dok je kod *M. stenopetala* došlo do potpune inhibicije klijanja već iznad 4% NaCl. Kinetika klijanja je pokazala sigmoidni obrazac, koji se odlikuje odloženim početkom i smanjenim konačnim procentima pod stresnim uslovima. Analiza glavnih komponenti (PCA) je ukazala da *M. peregrina* ima povećanu otpornost, dok je *M. stenopetala* pokazala veću osetljivost na visok salinitet. Ovi rezultati sugerišu da je *M. peregrina* pogodnija za gajenje u aridnim regionima sklonim zaslanjivanju, dok *M. stenopetala* zahteva dodatne strategije za ublažavanje salinitetnog stresa.

Ključne reči:
indeks vigoroznosti semena, tolerancija na abiotički stress, zaslanjena zemljišta, usevi aridnih zona, agroekološka adaptacija

Introduction

Soil salinity is one of the most pressing abiotic constraints in agriculture, affecting more than 20% of irrigated land worldwide and expanding rapidly under climate change and unsustainable irrigation practices (Negacz et al., 2022; Singh, 2022). Elevated salt concentrations reduce osmotic potential and disturb ion homeostasis, severely impairing seed germination and early seedling

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establishment (Shi et al., 2021; Tang et al., 2022). Because germination is the most sensitive stage in the plant life cycle, it is widely used as a bioassay to evaluate salinity tolerance. Germination indices, such as germination percentage, mean germination time, velocity coefficient, and germination speed, are robust tools for quantifying seed vigor and stress resilience (Wang & Shi, 2024; Boulahia et al., 2023).

The genus *Moringa* (family Moringaceae), comprising 14 species, has gained global importance owing to its nutritional, medicinal, and agroforestry value (Kumar et al., 2021; Bekka et al., 2022). *Moringa peregrina* is distributed in North Africa and the Middle East, with cultivation records in Algeria, especially in southern regions such as Oued Souf (Benhedid et al., 2019). *Moringa stenopetala* is native to East Africa (Ethiopia and Kenya) but has been considered for introduction into North African drylands due to its high drought tolerance and socioeconomic potential (Bania et al., 2023). Both species are valued for their multipurpose use as sources of food, fodder, and medicine (Shumi et al., 2022; Ghoohestani et al., 2025). However, while *M. peregrina* has received some attention in arid North African ecosystems, *M. stenopetala* remains poorly studied outside its native range in Africa.

Soil salinization is a growing challenge in Algeria, mainly caused by the use of highly mineralized irrigation water and insufficient drainage infrastructure (Benhedid et al., 2019; Kholaiq et al., 2022). Therefore, developing or introducing alternative crops that tolerate saline soils is a priority for agroforestry and sustainable agriculture in the region. Previous studies have investigated the effects of salinity on the germination and physiology of *Moringa* species, notably *M. oleifera* and *M. peregrina* (Al-Shoaibi & Boutraa, 2021; Nouman et al., 2021), but comparative data for *M. peregrina* and *M. stenopetala* under controlled saline conditions remain limited.

The present study aimed to evaluate the effects of increasing NaCl concentrations on the germination behavior of *M. peregrina* and *M. stenopetala*. By assessing multiple germination indices and applying statistical approaches (ANOVA, GLM/GLMM, PCA), this study provides early-stage screening evidence of species-specific tolerance and adaptive strategies. Our findings offer insights into the potential of these species as candidates for agroforestry development in saline-prone arid regions of Algeria.

Material and Methods

This study was conducted at the Laboratory of Ecophysiology, Department of Ecology and Environment, Faculty of Natural and Life Sciences, University of Batna2, Algeria. *Moringa* seeds were collected from a forest conservatory nursery in the Wilaya of Oued Souf, southeast Algeria.

Seed preparation and experimental approach

This study was conducted at the Laboratory of Ecophysiology, Department of Ecology and Environment, Faculty of Natural and Life Sciences, University of Batna 2 (Algeria). Fully mature seeds of *M. peregrina* and *M. stenopetala* were collected on 15 April 2024 from a forest conservatory nursery in Oued Souf, southeast Algeria. The germination experiment was carried out from 05 November to 5 December 2025.

Seed preparation and experimental approach

Only fully ripe seeds (brown-tan tegument, firm endosperm, and negative float test) were selected for the experiment. Seeds were washed with tap water for 5 min, immersed in a sodium hypochlorite solution (10%) for 15 min, and rinsed three times with sterile distilled water. A total of 20 seeds per treatment per species (5 boxes × 4 seeds) were used, with 100 seeds per species across the five NaCl levels. Seed size was measured in a random subsample (n = 30). For *M. peregrina*, the seeds had a mean length of 10.0 ± 0.4 mm and a mean width of 13.0 ± 0.5 mm, with a 100-seed mass of 29.4 g. For *M. stenopetala*, the seeds were smaller, with a mean length of 4.0 ± 0.3 mm and a mean width of 13.0 ± 0.4 mm, while the 100-seed mass averaged 18.6 g.

Seeds were placed in plastic boxes (19.5 × 13.5 cm) lined with three layers of filter paper. Each box received 15 mL of NaCl solution corresponding to the designated treatment. Treatments included 0 (control), 4, 8, 12, and 16% NaCl (w/v); equivalent electrical conductivity (EC, $dS \cdot m^{-1}$) values were measured with a WTW Cond 3110 conductivity meter (Germany) and are reported in the Results.

Growth conditions

All boxes were incubated in the dark at a constant temperature of 25 °C, with relative humidity maintained at 65 ± 3% in the chamber (Memmert IPP260 incubator, Germany). The filter papers were rewetted as necessary to maintain consistent moisture throughout the experiment. The emergence of a visible 2 mm radicle was used as the criterion for germination, and daily counts were recorded for 7–8 consecutive days.

Germination parameters

Germination rate (GR). The final germination rate was calculated as (Abdelkader et al., 2023):

$$GR\ (\%) = (Gx / Gt) \times 100$$

Where Gx is the number of germinated seeds and Gt is the total number of seeds sown.

Germination speed (GS). Germination speed was determined as the ratio of cumulative germinated seeds to the weighted time of emergence:

$$GS = 100\ (N1 + N2 + \ldots + Nn) / (N1T1 + N2T2 + \ldots + NnTn)$$

Where Ni is the number of seeds germinated at time Ti.

Germination Speed Coefficient (GSC). The GSC was calculated following Khulal et al. (2022):

$$CVG = (N1 + N2 + \ldots + Nx) / 100 \times (N1T1 + N2T2 + \ldots + NxTx)$$

where N is the number of seeds germinated each day and T is the time in days

Mean germination time (MGT). MGT was calculated according to Kujur and Lal (2015):

$$MGT = \Sigma NiTi / \Sigma Ni$$

Where Ni is the number of seeds germinated at time Ti.

Germination index (GI). The germination index was computed as (Anwar et al., 2021):

$$GI = (7 \times N1) + (6 \times N2) + \ldots + (1 \times Nn)$$

Where weights decrease progressively with germination days.

Vigour index (VI). The germination vigour index was calculated following Abdul-Baki and Anderson (1973):

$$VI = [(a/1 + b/2 + c/3 + \ldots + z/n) \times 100] / S$$

where a, b, …, z are the number of seeds germinated on days 1 to n, and S is the total number of seeds tested.

Germination kinetics. To characterize germination dynamics, the number of germinated seeds was recorded daily for seven days (Hajlaoui et al., 2007).

*Germination stress tolerance index (GSTI).*Salinity tolerance during germination was quantified using GSTI (Bouslama & Schapaugh, 1984).

$$GSTI\ (\%) = (VI\ stressed / VI\ control) \times 100$$

Where VI represents the vigour index.

Statistical analyses

The experiment was conducted using a completely randomized design (CRD) with five replicates per treatment. A two-way analysis of variance (ANOVA) was conducted to assess the effects of species, NaCl concentration, and their interactions on germination parameters. Significant differences between treatments were identified using Tukey's post hoc test ($p<0.05$). Generalized Linear Models (GLM) and Generalized Linear Mixed Models (GLMM), with species as a random effect, were used to analyze the impact of salinity on germination rate, evaluating interspecific variance and model fit (AIC and REML criteria). Principal Component Analysis (PCA) was performed to explore the relationships between germination variables and identify the key factors driving responses to salinity stress. All statistical analyses were conducted using R software (version 4.2.1). Data are presented as mean ± standard deviation (SD).

Results

Germination rate

Salinity had a highly significant effect on the germination rates of *M. peregrina* and *M. stenopetala* ($p<0.001$). However, the effect of species was not significant ($p=0.477$), indicating that both species performed similarly under non-saline conditions. A marginally significant interaction ($p\approx0.068$) suggested a slight difference in the response to salinity. Both species exhibited high germination rates under control and low-salinity (T0–T1) conditions. At 8% NaCl (T2), *M. peregrina* maintained an 85% germination rate, whereas that of *M. stenopetala* dropped to 35% ($p\approx0.0004$). At 12% NaCl (T3), *M. peregrina* achieved 40% germination, whereas *M. stenopetala* failed to germinate. At the highest salinity level (16% NaCl; T4), no germination was observed in either species (**Fig. 1**). These findings indicate a moderate salt tolerance of up to 8% NaCl, with *M. peregrina* demonstrating greater resilience under severe salinity stress.

Germination speed

Germination speed was significantly influenced by species, salinity treatment, and their interactions (all $p<0.001$). *Moringa stenopetala* exhibited a significantly higher germination rate than *M. peregrina* (difference = +6.75; $p<0.001$), particularly under low-to-moderate salinity levels (4–12% NaCl). As shown in **Fig. 2**, the highest germination rate for *M. stenopetala* was recorded at 8% NaCl (T2; 25%), followed by 4% NaCl (T1; 24%) and the control (T0; 23%), suggesting that mild salinity does not inhibit germination rate in this species. In contrast, no germination was observed at 12% and 16% NaCl (T3–T4), indicating high sensitivity to severe salinity. *M. peregrina* showed a similar trend, with a peak germination rate at T2 (16%), followed by T0 and T1 (15%), and a decline at T3

(13%). Germination was completely inhibited by T4 treatment (0% germination). These findings indicate that *M. stenopetala* exhibits a higher germination rate under saline conditions than *M. peregrina*.

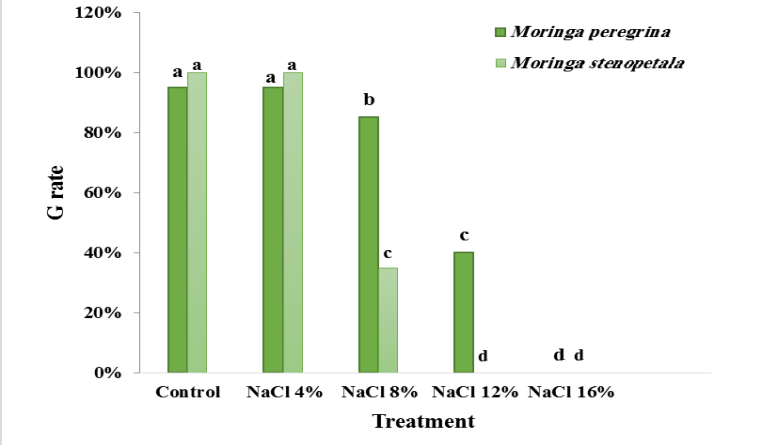

Fig. 1. Germination rate (%) of *Moringa peregrina* and *Moringa stenopetala* seeds under different NaCl concentrations (0, 4, 8, 12, and 16%) at 25 °C in the dark

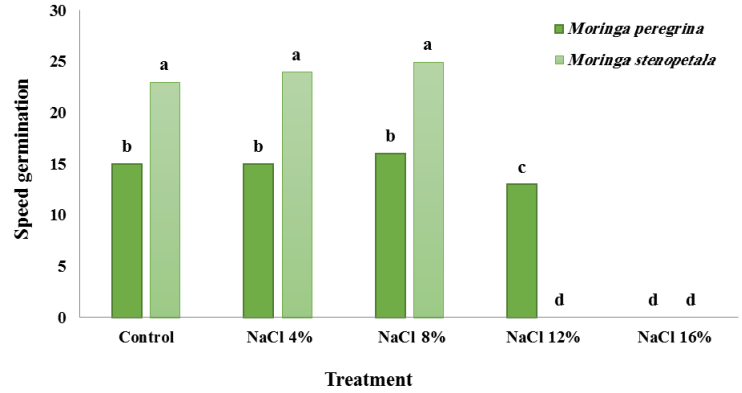

Fig. 2. Germination speed of *Moringa peregrina* and *M. stenopetala* seeds under different NaCl concentrations (0, 4, 8, 12, and 16 %) at 25 °C in the dark

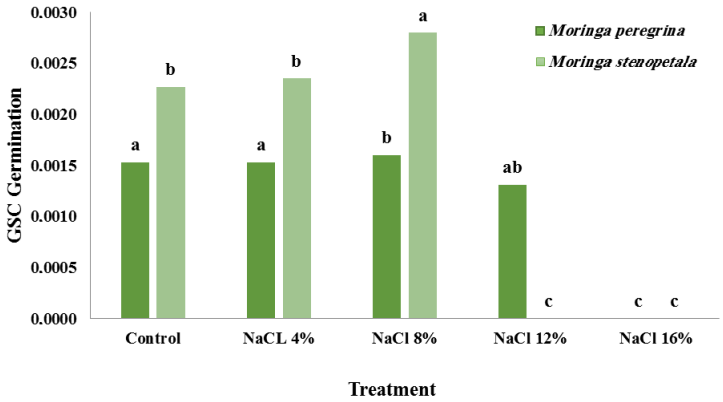

Fig. 3. Germination speed coefficient of *Moringa peregrina* and *Moringa stenopetala* seeds under different NaCl concentrations (0, 4, 8, 12, and 16%) at 25 °C in the dark

Germination Speed Coefficient (GSC)

Statistical analysis revealed that species, salinity treatment, and their interaction significantly influenced the germination speed coefficient (GSC), with *M. stenopetala* exhibiting a substantially lower GSC than *M. peregrina* ($p<0.001$), indicating a faster, more synchronized germination pattern in *M. peregrina*. Notwithstanding heterogeneity in the data (as indicated by a significant Levene's test, $p<0.001$), the use of balanced sampling ensured robustness of the statistical analysis. GSC declined progressively as NaCl concentration increased, reaching minimal values at severe salinity (T3–T4). Conversely, a peak at moderate stress (T2) suggests an optimal stimulatory effect (**Fig. 3**). *Moringa stenopetala* exhibits superior germinative resilience under mild to moderate salt stress conditions, as evidenced by its consistent outperformance of *M. peregrina* at low salinity levels (T0–T1).

Mean germination time

Mean germination time (MGT) was significantly influenced by species, salinity treatment, and their interactions. As shown in **Fig. 4**, MGT increased with increasing NaCl concentration (T0–T4), reflecting a delay in germination under saline conditions. In *M. peregrina*, the highest MGT values were recorded at T3 (7.6 days) and T2 (6.3 days), whereas lower values were observed under the control and low salinity treatments (T0–T1; 6.2 days). No germination was observed in T4 (16% NaCl concentration). In contrast, *M. stenopetala* maintained a stable MGT of approximately 5.0 days from T0 to T2, with no germination at higher salinity levels (T3–T4). Overall, *M. stenopetala* germinated significantly faster than *M. peregrina*, with a mean MGT difference of –1.30 d ($p<0.001$). This stability in MGT under moderate salinity (up to 12% NaCl) highlights the superior germinative efficiency and salt tolerance of *M. stenopetala*, whereas *M. peregrina* showed a longer delay before germination ceased under stress.

Germination Vigour Index (GVI)

The germination vigor index (GVI) was significantly influenced by species, salinity treatment, and their interaction (p<0.001 for all). As shown in **Fig. 5**, *M. peregrina* exhibited the highest GVI at 8% NaCl (T2; 33.25), indicating a stimulatory effect of moderate salinity, followed by values of 28.33 at T0, 27.33 at T3, and 20.74 at T1. No vigor was recorded with 16% NaCl (T4). In contrast, *M. stenopetala* showed a markedly lower GVI across all treatments, peaking at T0 (11.05) and decreasing progressively to 9.58 at T1 and 5.33 at T2, with no germination vigor observed at T3 and T4. The critical threshold for GVI reduction was between 8% and 12% NaCl, beyond which vigor declined sharply for both species. Although *M. peregrina* maintained higher vigor and showed enhanced performance under moderate salinity, *M. stenopetala* displayed greater sensitivity, with a significant reduction in GVI beyond 8% NaCl. At 16% NaCl, the GVI collapsed to near zero in both species, indicating complete physiological inhibition. These findings suggest that *M. peregrina* is better suited for cultivation under moderately saline conditions.

Germination Index (GI)

The germination index (GI) was significantly affected by species, salinity treatment, and their interaction (p<0.001). Overall, *M. peregrina* exhibited higher GI values than *M. stenopetala*, with an average difference of 15.79 units (p<0.001). In *M. peregrina*, the highest GI was recorded at 4% T1 NaCl (99), followed by T2 (81), before decreasing sharply at T3, with complete inhibition at T4. In *M. stenopetala*, the maximum GI was observed under control conditions (72), declining gradually to T1 (67) and T2 (28), with no germination at T3 or T4. The critical threshold for inhibition was 8% NaCl for both species, with *M. stenopetala* showing greater sensitivity from 4% NaCl onward (**Fig. 6**). These results confirm that *M. peregrina* maintains a more efficient germination process under moderate saline conditions, whereas *M. stenopetala* is more vulnerable to salt stress.

Germination Stress Tolerance Index (GSTI)

The germination stress tolerance index (GSTI) was significantly influenced by salinity treatment (p<0.001) and species (p=0.0068), with a notable

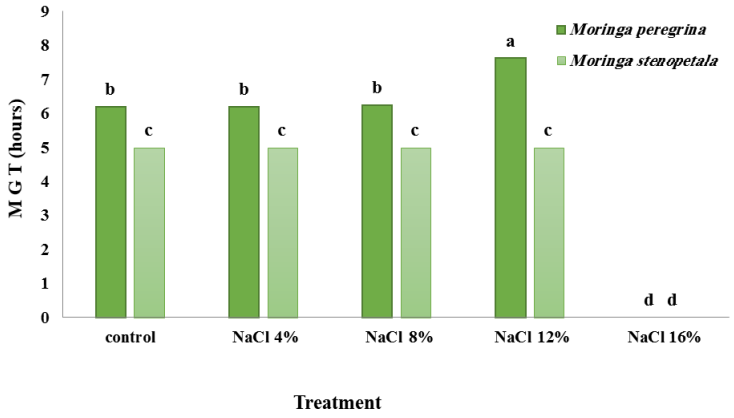

Fig. 4. Mean germination time of *Moringa peregrina* and *M. stenopetala* seeds under different NaCl concentrations (0, 4, 8, 12, and 16%) at 25 °C in the dark

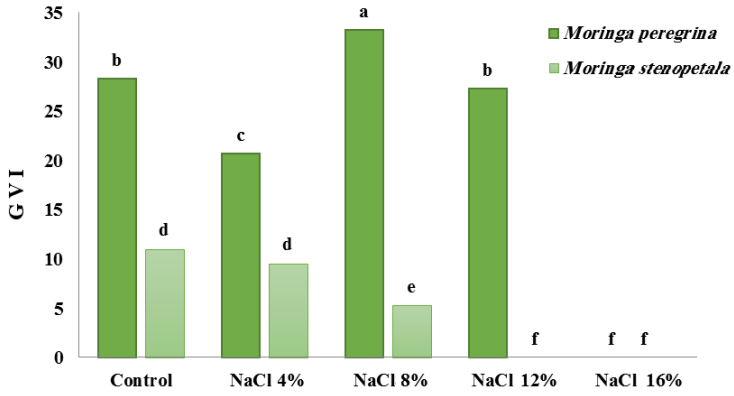

Fig. 5. Germination vigour index of *Moringa peregrina* and *M. stenopetala* seeds under different NaCl concentrations (0, 4, 8, 12, and 16%) at 25 °C in the dark

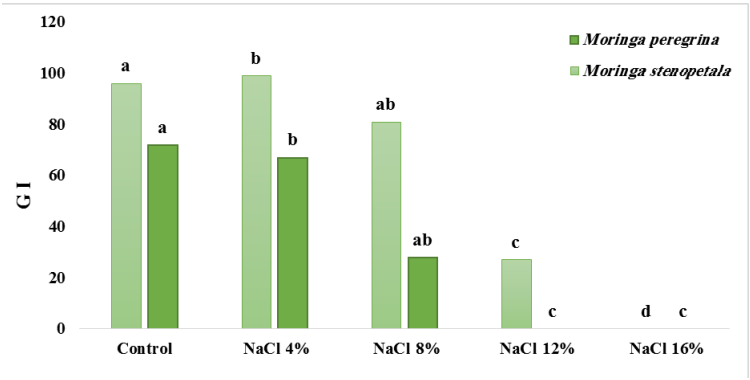

Fig. 5. Germination index of *Moringa peregrina* and *M. stenopetala* seeds under different NaCl concentrations (0, 4, 8, 12, and 16%) at 25 °C in the dark

species × treatment interaction. Both *M. peregrina* and *M. stenopetala* exhibited 100% GSI under control (T0) and low-salinity (T1) conditions. At 8% NaCl (T2), *M. peregrina* maintained a high tolerance (GSI = 89%), while *M. stenopetala* dropped sharply to 35%. At 12% NaCl (T3), *M. peregrina* had a moderate GSI (42%), whereas *M. stenopetala* showed no tolerance. GSTI values were null for both species at 16% NaCl (T4) concentration. The decline in GSI was dose-dependent, with a critical threshold emerging at 8% NaCl. Interspecific differences were most pronounced at moderate salinity levels, highlighting the greater resilience of *M. peregrina* to environmental stress.

Germination kinetics

Statistical analysis revealed that salinity had a highly significant effect on germination kinetics, whereas the effect of species alone was not significant (p=0.478). This indicates that *M. peregrina* and *M. stenopetala* responded similarly to increasing salt stress. However, the species × treatment interaction showed a trend toward significance (p=0.0686), suggesting a slightly differentiated response between species, particularly at intermediate NaCl concentrations. As **Fig. 8** shows, the germination kinetics curves of *M. peregrina* and *M. stenopetala* under varying NaCl concentrations exhibit a characteristic sigmoidal shape, consisting of three distinct phases: a Lag Phase: Minimal germination during the initial days. This phase lasted three days in *M. peregrina* (T0–T2) and four to five days in *M. stenopetala*, with a delay observed at T2 in the latter. No germination occurred at T4 in either species, indicating complete inhibition or extended dormancy. Exponential Phase: Rapid increase in the germination rate. This phase extended up to day 4 in *M. peregrina* (T0–T2) and up to day 6 in *M. stenopetala*, with a delayed onset at T2 in both species. No exponential growth was observed at T4, and only *M. peregrina* showed limited progression at T3. Plateau Phase: Stabilization of the germination rate. Both species showed reduced final germination percentages at T3, and no germination occurred at T4. These observations align with the statistical findings that germination kinetics deteriorated sharply at 8% NaCl, with a progressive yet pronounced decline. At 12% and 16%, germination was virtually absent, indicating strong physiological inhibition. Although both species were similarly affected by high salinity levels, *M. peregrina* demonstrated slightly greater tolerance, maintaining germination activity at T3, whereas *M. stenopetala* was fully inhibited beyond T2.

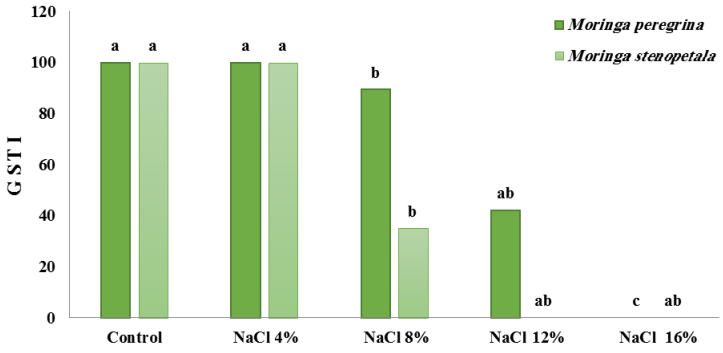

Fig. 7. Germination stress tolerance index of *Moringa peregrina* and *Moringa stenopetala* seeds under different NaCl concentrations (0, 4, 8, 12, and 16%) at 25 °C in the dark

GLM and GGLM model

Analysis using a Generalized Linear Model (GLM) and Generalized Linear Mixed Model (GLMM) demonstrated that salinity treatment had a highly significant effect on germination rate (Grate), with a notable decline starting at 8% NaCl (-10 points, p=0.00693) and near-complete inhibition at 16% NaCl (-95 points, $p< 2e^{-16}$) compared to the control. The main effect of species (*M. stenopetala* vs. *M. peregrina*) was not significant (p=0.17), although *M.*

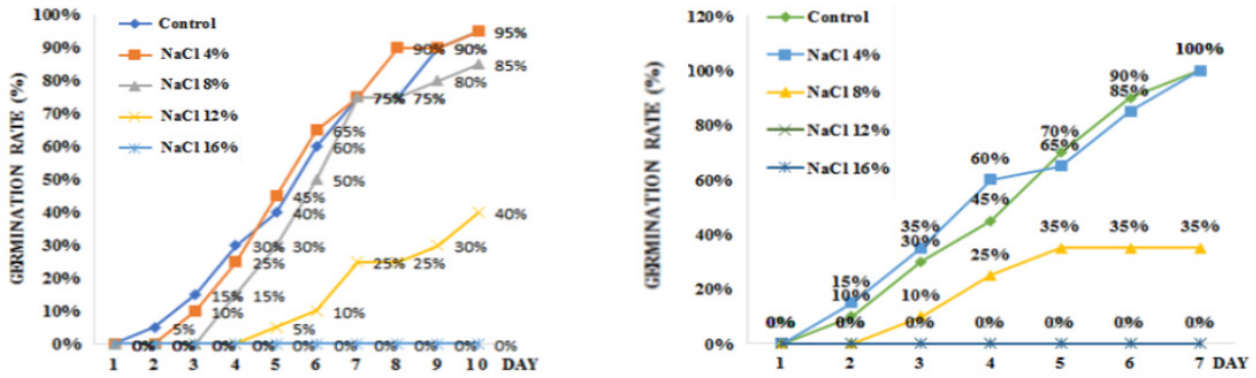

Fig. 8. Germination kinetics: left - *M. stenometala*, right - *M. peregrina*

stenopetala showed a slightly positive trend (+5 points). A significant interaction was observed at 8% NaCl (p=0.025), indicating greater sensitivity in *M. stenopetala*. The GLMM, which incorporated species as a random effect, confirmed these findings with low interspecific variance (0.065) and a slight improvement in fit (REML = 1076.4 vs. GLM AIC = 1135.4). Overall, salinity treatment was the dominant factor, with *M. stenopetala* being more vulnerable to moderate salinity. The GLMM refines the modeling of variability, offering guidance for cultivation strategies in saline environments.

Principal Component Analysis

The PCA biplot (**Fig. 9**) revealed that salinity stress significantly differentiated *M. peregrina* and *M. stenopetala* in terms of germination performance. *Moringa peregrina* demonstrated enhanced resilience, with consistent germination success (GSC), whereas *M. stenopetala* showed greater variability, suggesting potential stress sensitivity. The first principal component (Dim1) was driven by germination speed and index (G rate, GSTI, and Kinetic G), reflecting the overall performance. The second component (Dim2) is dominated by GSC and MGT, highlighting differences in success and timing. The correlation matrix supports these findings, demonstrating robust interrelationships among key germination variables, with G speed potentially indicating a distinct stress response mechanism. This analysis suggests that breeding or selection for salinity tolerance in moringa should focus on enhancing GSC and Kinetic G, particularly in *M. stenopetala*.

Discussion

Moringa peregrina and *M. stenopetala* are promising species within the *Moringa* genus, well adapted to arid and semi-arid environments owing to their nutritional, medicinal, and agroforestry value. Resilience to saline conditions is becoming increasingly important as soils in drylands are progressively salinized (Bekka et al., 2022). Our results show that *M. peregrina* exhibits greater tolerance, maintaining viable germination up to 12% NaCl, whereas *M. stenopetala* was completely inhibited above 4%. These contrasting responses reflect distinct physiological strategies of the two species.

Previous studies have confirmed that *M. peregrina* displays moderate salinity tolerance during germination, supported by osmotic adjustment and ion regulation (Al-Shoaibi & Boutraa, 2021). In contrast, empirical data on *M. stenopetala* remain scarce, although habitat modeling indicates a preference for slightly saline soils (1–2 dS·m^{-1}), consistent with its native range in East Africa (Bania et al., 2023). This aligns with our findings, where *M. stenopetala* was more sensitive to intermediate NaCl concentrations.

The overall effects of salinity on seed germination have been well documented. Increasing salt concentration delays germination, prolongs the mean germination time (MGT), and reduces vigor indices (VI, GSTI). Comparative studies on halophytes and glycophytes have shown that critical thresholds generally occur between 8 and 12% NaCl, a range consistent with our results (Khan et al., 2002; Farooq et al., 2015). Ashraf and Foolad (2013) further emphasized that interspecific differences in salt tolerance derive from mechanisms such as ion exclusion and osmoprotectant accumulation,

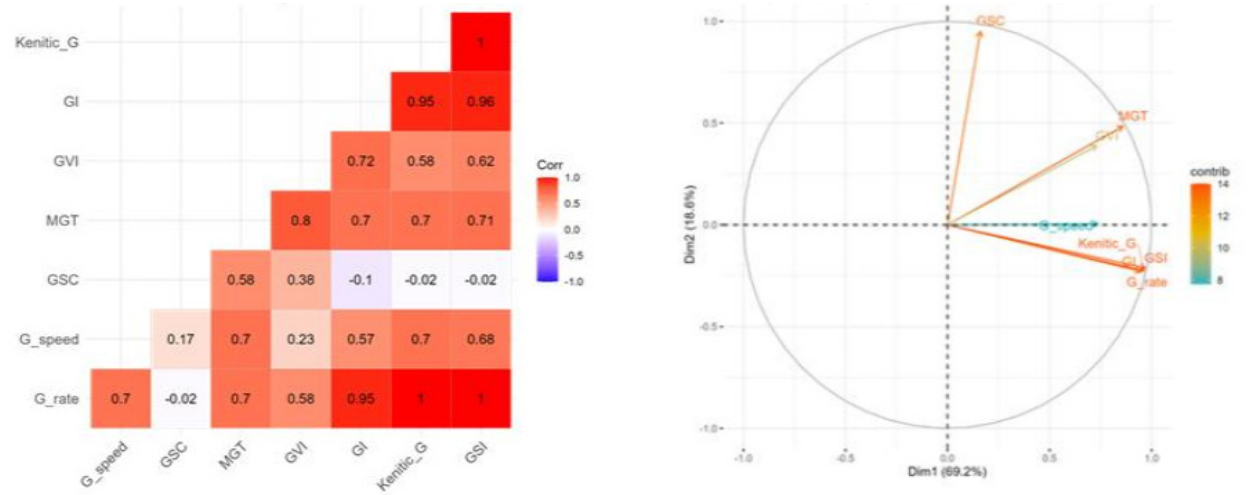

Fig. 9. Principal component analyses (left) and correlation matrix between germinative variable (right) of *M. peregrina* and *M. stenopetala* under saline conditions

which likely explain the superior resilience of *M. peregrina* in this study.

Our findings are also consistent with reports on *M. oleifera*, which retains partial germination under saline stress but suffers reduced growth and biomass at later stages (Nouman et al., 2021; Nóbrega et al., 2021). This suggests that early-stage tolerance in *M. peregrina* may provide a competitive advantage for its integration into saline-prone agroforestry systems in Algeria. Conversely, *M. stenopetala* may require supportive measures, such as seed priming, moringa leaf extract (MLE) application, or biostimulant treatments, to enhance germination and establishment under stress (Soliman et al., 2015; Farhat et al., 2023; Uddin, 2021).

Conclusion

In conclusion, salinity significantly reduced germination in both species, but *M. peregrina* maintained better performance under moderate stress, whereas *M. stenopetala* was highly sensitive and failed to germinate beyond 4% NaCl. These results highlight the potential of *M. peregrina* for agroforestry in saline-prone arid regions and indicate the need for targeted strategies to improve *M. stenopetala* establishment under such conditions.

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