Integrated Assessment of Drought, Saline, and Alkaline Stress Responses During Seed Germination of Sesamum indicum L. Using PCA and Membership Function Analysis

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Integrated Assessment of Drought, Saline, and Alkaline Stress Responses During Seed Germination of *Sesamum indicum* L. Using PCA and Membership Function Analysis

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Abstract

Soil salinization, alkalization, and drought increasingly threaten crop establishment in arid regions. This study evaluated the effects of drought, salt, and alkali stress on the germination and early growth of *Sesamum indicum* L. to identify tolerance differences among these stresses. Four osmotic potentials (−0.09, −0.17, −0.23, and −0.34 MPa) were established using polyethylene glycol (PEG 4000) to simulate drought, NaCl to induce salt stress, and Na_2CO_3 to represent alkali stress, with distilled water as the control. Low salt stress (−0.09 MPa NaCl) did not significantly affect germination, whereas higher stress levels (−0.34 MPa) under all treatments markedly suppressed germination. Among the three stresses, Na_2CO_3 caused the strongest inhibition. The germination index decreased progressively with declining osmotic potential, while the average germination time increased by one to two days compared with the control. Drought, salt, and alkali stresses also restricted seedling growth, though mild salt and drought stress slightly enhanced dry matter accumulation before severe inhibition occurred. Principal component analysis and membership function evaluation classified sesame responses into three tolerance categories: high, moderate, and sensitive. The combined analysis indicated that sesame exhibits greater tolerance to salt stress than to drought or alkali stress. Overall, Sesamum indicum adapts to mild osmotic stress by sustaining germination and nutrient mobilization, but higher stress levels disrupt germination dynamics and seedling development. These findings provide a comparative framework for screening sesame genotypes suited to saline environments and for improving stress-resilient cultivation strategies.

Keywords: seed germination; Sesame; NaCl stress; Na_2CO_3 stress; PEG stress

1. Introduction

Soil salinization, alkalization, and drought are among the most severe abiotic stresses limiting agricultural productivity in arid and semi-arid regions (Dierickx, 2009; Rengasamy et al., 2022). Global climate change and unsustainable irrigation practices have accelerated the accumulation of soluble salts and carbonates in soils, resulting in reduced soil fertility, impaired seedling establishment, and yield decline in major crops (Li D et al., 2018). Understanding how plants respond to these combined stresses during germination is critical, as germination represents a vulnerable phase in the plant life cycle where tolerance mechanisms are first expressed (Qu and Zhao, 2004; Guo et al., 2009).

Salinity and alkalinity exert different but often overlapping physiological constraints on plants. Salinity stress, mainly caused by neutral salts such as NaCl, reduces water potential and induces ionic toxicity through excessive Na^+ and Cl^- accumulation (Kefale et al., 2024). In contrast, alkalinity stress, generally associated with Na_2CO_3 and $NaHCO_3$, combines osmotic imbalance with elevated soil pH, leading to nutrient deficiency, membrane destabilization, and enzyme inactivation (Qu and Zhao, 20004; Guo et al., 2009). Drought further compounds these problems by creating low water availability, impairing seed imbibition, and reducing metabolic activity (Dissanayake et al., 2019). Hence, simultaneous evaluation of these stresses provides a more realistic understanding of plant performance in marginal soils where such conditions coexist.

Seed germination is a key determinant of plant establishment and population persistence, and it directly reflects the plant's physiological capacity to overcome osmotic and ionic stress (El Harfi et al., 2016; Gholamhoseini and Dolatabadian, 2004). Germination behavior under drought, salt, and alkali conditions varies widely among species and genotypes. For example, differential sensitivity to salinity and alkalinity during early growth has been reported across crop species, with post-germination growth often more sensitive than germination itself (Zhou et al., 2023; Mahdavi, 2016). Similarly, low concentrations of polyethylene glycol (PEG) may have limited effect on seed germination, but higher osmotic potentials significantly inhibit water uptake and enzyme activation (Dissanayake et al., 2019; Zhang et al., 2017). In some species, mild saline or osmotic stress can transiently promote early growth before inhibitory effects dominate, reflecting stress-induced shifts in biomass allocation (Yu et al., 2019; Zhang et al., 2017). These variations highlight the complexity of plant stress responses and the necessity of species-specific evaluation.

Sesamum indicum L. (sesame) is generally regarded as moderately drought tolerant and capable of growing in slightly acidic to slightly alkaline soils; however, its performance is frequently challenged by salinity and alkalinity in major production regions such as South Asia, Africa, and parts of China, where soil degradation and irrigation-induced salt accumulation are increasingly common (Ashri and Singh, 2006; Dawar et al., 2025). Despite its agronomic importance, few studies have compared sesame responses to different abiotic stresses under controlled iso-osmotic conditions. Most existing research has focused on single stress factors, limiting understanding of how drought, salt, and alkali stresses differentially influence sesame seed germination and early seedling development (El Harfi et al., 2016; Gholamhoseini and Dolatabadian, 2024). Addressing this gap is crucial for selecting and breeding genotypes better adapted to saline and drought-prone environments (Zhang et al., 2019).

In this context, the present study investigates the germination behavior and early growth responses of *Sesamum indicum* under simulated drought (PEG 4000), salt (NaCl), and alkali (Na_2CO_3) stresses. Four osmotic potential levels (−0.09, −0.17, −0.23, and −0.34 MPa) were used to mimic varying stress intensities, while distilled water served as the control. The experiment was designed to quantify changes in germination rate, germination index, average germination time, radicle and hypocotyl length, and seedling dry matter accumulation. To obtain a comprehensive understanding of sesame tolerance, multivariate analyses, including principal component analysis (PCA) and membership function evaluation, were performed.

The study is driven by three central considerations. First, drought, salinity, and alkalinity stress occur simultaneously in semi-arid agroecoystems, yet comparative data for sesame remain scarce. Second, identifying stress-specific tolerance thresholds across stresses could provide guidance for sesame cultivation in saline-alkaline soils and water-limited regions. Third, determining the most responsive germination indicators can support rapid screening of stress-tolerant genotypes.

Accordingly, the hypothesis of this study is that *Sesamum indicum* exhibits differential tolerance to drought, salt, and alkali stresses during germination, with salt stress expected to impose less inhibition than alkali or drought stress due to its lower pH effect and osmotic balance capacity (Qu and Zhao, 2004; El Harfi et al., 2016).

The specific objectives were therefore: (i) to compare the effects of drought, salt, and alkali stresses on sesame seed germination, germination dynamics, and seedling growth characteristics, (ii) to determine tolerance thresholds and identify critical osmotic potentials for germination inhibition under each stress, (iii) to evaluate comprehensive stress tolerance using PCA and membership function analysis to classify sesame responses into tolerance categories. This integrative approach aims to clarify the relative influence of osmotic, ionic, and pH stresses on sesame germination and to provide a theoretical basis for enhancing stress resilience in sesame cultivation through genotype selection and soil management strategies.

2. Materials and Methods

2.1 Experimental Design

The experiment was conducted to evaluate the effects of drought, salt, and alkali stress on the germination and early growth of *Sesamum indicum* L. (cv. Luyinzhi 1). The seeds of this variety, which are known for their high sesamin content, high calcium content, and saline-alkali tolerance, were provided by Shandong Shilixiang Sesame Co., Ltd. Uniform and plump seeds were selected and surface-sterilized in 5% sodium hypochlorite for 5 minutes, followed by thorough rinsing with distilled water to remove residual chlorine.

Germination tests were carried out in 90-mm Petri dishes lined with two layers of Whatman filter paper. Thirty seeds were evenly placed in each dish, and 10 mL of treatment solution was added. Distilled water was used for the control (CK). Polyethylene glycol (PEG 4000), sodium chloride (NaCl), and sodium carbonate (Na_2CO_3) solutions were prepared to simulate drought, salt, and alkali stresses, respectively. For each stress treatment, four osmotic potential gradients (−0.09, −0.17, −0.23, and −0.34 MPa) were established. Each treatment was replicated three times.

The osmotic potential of the solutions was determined using a WP4C dew point potentiometer (Decagon Devices, USA), while pH values were measured with a Sartorius PB-10 pH meter. The concentrations, osmotic potentials, and pH values of all treatment solutions are presented in Table 1. All experiments were performed in an artificial climate chamber under controlled environmental conditions: 12-hour photoperiod, photosynthetic photon flux density ≥ 56.6 μmol m^{-2} s^{-1}, day/night temperature of 23 ± 1 °C, and relative humidity between 60% and 80%. Germination was monitored every two hours during the first 24 hours and every six hours thereafter. Seeds with visible radicle protrusion were considered germinated.

After seven days of incubation, the radicle and hypocotyl lengths of all seedlings were measured, and the seedlings were oven-dried at 105 °C for 30 minutes, followed by drying at 75 °C to constant weight for dry matter determination. To evaluate stress tolerance, principal component analysis (PCA) and membership function analysis were performed to calculate the comprehensive evaluation value (D value). Based on the D-value ranking, sesame seed tolerance under different treatments was categorized into three levels: high tolerance, moderate tolerance, and sensitivity.

Table 1 Concentration, osmotic potential, and pH of solutions under different stress treatments

Stress	Treatment	Concentration (mmol L^{-1})	Osmotic potential (MPa)	pH
Control	Distilled water	0	0	6.76
Salinity	NaCl	20	-0.10	6.77
		40	-0.19	6.76
		60	-0.26	6.75
		80	-0.34	6.74
Alkalinity	Na_2CO_3	20	-0.11	11.48
		30	-0.17	11.49
		40	-0.24	11.51
		60	-0.36	11.52
Drought	PEG 4000	40	-0.09	6.77
		70	-0.17	6.75
		90	-0.23	6.72
		140	-0.34	6.69

2.2 Measurement and calculation of seed germination indicators

2.2.1 Germination rate

Germination rate (GR) represents the percentage of germinated seeds relative to the total number of tested seeds, with the formula as follows:

$$\mathrm{GR} = \left(\frac{\mathrm{N}}{\mathrm{S}}\right) \times 100\% \quad (1)$$

Where N represents the number of germinated seeds in the experiment, and S is the total number of seeds tested.

Tolerance threshold and tolerance limit values were used to describe the critical and extreme points of sesame seed tolerance under stress (Matías et al., 2014; Hao and Wang, 2025). Tolerance threshold refers to the osmotic potential corresponding to a 50% reduction in the germination rate relative to the control. The tolerance limit value was defined as the osmotic potential at which the germination rate decreased by 75% (only 25% of seeds germinated).

2.2.2 Germination Index

The germination index (GI) was used to evaluate seed vigor and the speed of germination. It was calculated according to the method of Abdul-Baki and Anderson (1973):

$$GI = \Sigma(\frac{Nt}{t}) \quad (2)$$

Where Nt represents the number of seeds germinated on day t, and t denotes the corresponding number of days for germination.

2.2.3 Average germination time

Average germination time (GT) reflects the mean time required for seeds to complete germination and indicates the rate of germination progress (Božena Š., 2023). It was calculated using the following formula:

$$GT = \Sigma\frac{(Nt\times t)}{S} \quad (3)$$

Where Nt represents the number of seeds germinated on day t, t represents the corresponding germination day, and S is the total number of seeds to be tested.

2.2.4 Dry matter mass

At the end of the 7-day germination period, seedlings from each Petri dish were collected and oven-dried. Samples were initially heated at 105 °C for 30 minutes to inactivate enzymes, followed by drying at 75 °C until a constant weight was achieved. The dry matter mass (g plant^{-1}) was recorded using an analytical balance (accuracy ± 0.0001 g).

2.2.5 Length of embryonic root and hypocotyl

The lengths of the embryonic root (radicle) and embryonic axis (hypocotyl) were measured using a digital caliper. Ten randomly selected seedlings per replicate were measured, and mean values were used for analysis. These parameters were employed to assess early seedling growth responses under different osmotic potentials.

2.3 Data Analysis

Data were analyzed using Microsoft Excel 2019 and SPSS 24.0 (IBM Corp., USA). ANOVA was performed to determine the effects of stress type and osmotic potential on germination parameters. Significant differences among means were tested using least significant difference (LSD) at $p < 0.05$. Correlation analysis was conducted to evaluate relationships among germination and growth parameters.

Principal component analysis (PCA) and membership function analysis were performed to comprehensively evaluate stress tolerance (Tang, 2010). The following equations were used:

$$U(X_j) = \frac{(X_i - X_{i,min}}{X_{i,max} - X_{i,min}} \quad (4)$$

where $U(X_j)$ represents the membership function value of the j-th germplasm, X_i is the i-th principal component value, and $X_{i,min}$ and $X_{i,max}$ are the minimum and maximum values of that component, respectively.

The weight (w_i) of each principal component was determined by its contribution rate (P_i) according to the equation:

$$w_i = \frac{P_i}{\sum P_i} \quad (5)$$

The comprehensive evaluation (D) representing overall stress resistance was calculated as:

$$D = \sum[w_i \times U(X_j)] \quad (6)$$

The D-values were then used to classify sesame seed responses into three tolerance categories: high tolerance, moderate tolerance, and sensitive, following Tang (2010).

3 Results

3.1 The effect of PEG, NaCl, and Na_2CO_3 stress on the germination rate of sesame seeds

The germination rate of sesame seeds showed a gradual decline with decreasing osmotic potential under all stress treatments (Fig. 1). Under the control (CK) condition, the germination rate reached 97.8%. In NaCl treatments at −0.09, −0.17, and −0.25 MPa, no significant difference was observed compared with the control. However, PEG and Na_2CO_3 treatments at the same osmotic potentials resulted in lower germination rates than CK.

At identical osmotic potentials, the germination rate under Na_2CO_3 stress was significantly lower than that under PEG stress. At −0.34 MPa, germination decreased by 10%, 55.6%, and 97.8% under NaCl, PEG, and Na_2CO_3 treatments, respectively, compared with CK. These results indicate that Na_2CO_3 stress exerted the strongest inhibitory effect on seed germination, followed by PEG and NaCl.

Linear regression analysis was used to estimate tolerance thresholds and limits for each stress (Table 2). The osmotic potential corresponding to 50% germination was considered the tolerance threshold, and that corresponding to 25% germination was defined as the tolerance limit. Under NaCl stress, these values were −0.605 MPa and −0.720 MPa, respectively; under PEG stress, −0.318 MPa and −0.384 MPa; and under Na_2CO_3 stress, −0.142 MPa and −0.220 MPa. Sesame seeds therefore exhibited the greatest tolerance to NaCl stress, moderate tolerance to PEG-induced drought, and the least tolerance to Na_2CO_3 stress.

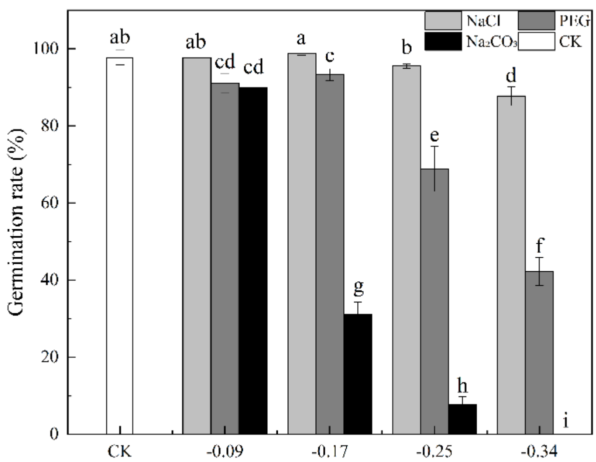


Fig. 1 The effect of NaCl, PEG, and Na_2CO_3 treatments at different osmotic potentials on the germination rate of *Sesamum indicum* seeds. Bars represent mean ± standard deviation (n=3). Different letters denote significant differences among treatments at $p < 0.05$ (LSD test).

Table 2: Regression equations and tolerance thresholds for *Sesamum indicum* seed germination under different stress conditions.

Stress type	Germination rate regression equation	R^2	50% threshold (MPa)	25% limit (MPa)
NaCl	$y = -1.9377x^2 + 0.3929x + 0.9721$	0.9607	-0.605	-0.720
PEG	$y = -6.0136x^2 + 0.4069x + 0.9802$	0.9679	-0.318	-0.384
Na_2CO_3	$y = 3.5184x^2 - 4.5064x + 1.0701$	0.9191	-0.142	-0.220

3.2 Effect of PEG, NaCl, and Na_2CO_3 stress on the germination index

The germination index of sesame seeds decreased progressively with declining osmotic potential in all treatments (Fig. 2). A significant linear correlation was observed between the germination index and osmotic potential for each stress treatment. For every 1 MPa decrease in osmotic potential, the germination index decreased by 27.57, 62.91, and 87.39 under NaCl, PEG, and Na_2CO_3 treatment, respectively. Among them, the smallest reduction occurred in NaCl treatments, while Na_2CO_3 treatment showed the largest decrease. This result is highly consistent with the response of seed germination rate to NaCl, PEG, and Na_2CO_3 stresses.

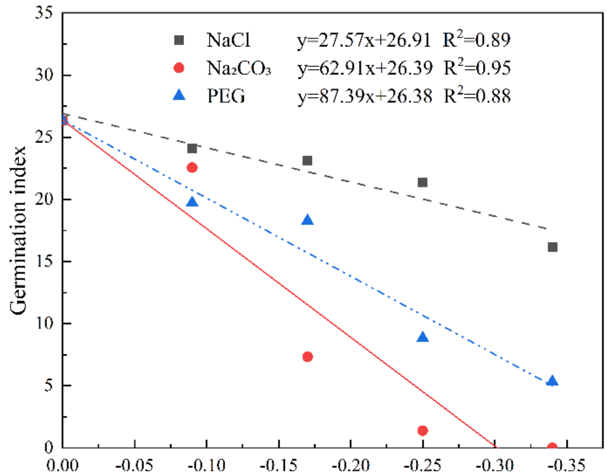


Fig. 2 Germination index of *Sesamum indicum* seeds under NaCl, PEG, and Na_2CO_3 stress at different osmotic potentials. Each marker represents the mean of 3 replicates. Regression equations describe the relationship between osmotic potential (x) and germination index (y) for each stress treatment. R^2 values indicate the strength of fit for each regression line.

3.3 Effect of PEG, NaCl, and Na_2CO_3 stress on average germination time

The average germination time of sesame seeds under control treatment was 1.2 days (Fig. 3). As osmotic potential decreased, germination time increased progressively under all stresses. Under NaCl, PEG, and Na_2CO_3 treatments, germination took significantly longer than in the control, while under PEG treatment was longer than under NaCl and Na_2CO_3 treatments. When the solution osmotic potential was -0.09 MPa and -0.34 MPa, the average germination time of sesame seeds under PEG treatment was 1.6 days and 2.5 days, respectively, which were 34% and 111% longer than that under the CK ($p<0.05$). At osmotic potentials of -0.09 MPa and -0.17 MPa, no significant difference in germination time was observed between NaCl and Na_2CO_3 treatments.

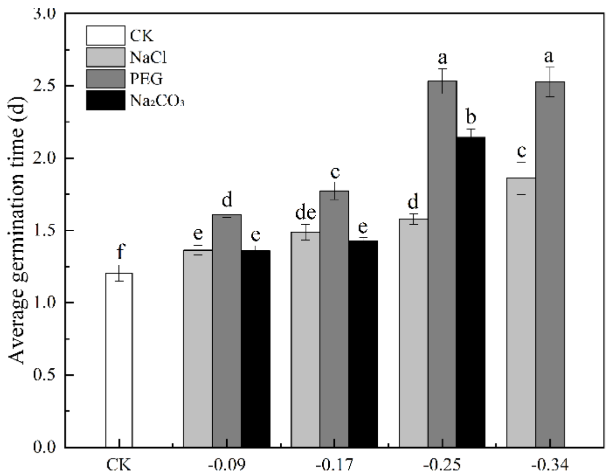


Fig. 3 Average germination time of *Sesamum indicum* under NaCl, PEG, and Na_2CO_3 stress at different osmotic potentials. Bars represent mean ± standard deviation (n=3). Different letters denote significant differences among treatments at $p < 0.05$ (LSD test).

3.4 Effects of PEG, NaCl, and Na_2CO_3 stress on seedling root and hypocotyl length

Under the control condition, the hypocotyl and radicle lengths were 10.07 mm and 49.05 mm, respectively (Fig. 4). Both parameters decreased with declining osmotic potential under all stress treatments. At -0.09 MPa NaCl, the radicle length decreased by 3.3% (not significant, $p>0.05$), while the hypocotyl length decreased by 23.2% ($p<0.05$). Under PEG stress, hypocotyl and radicle lengths decreased by 34.3% and 31.1%, respectively, compared with the CK treatment ($p<0.05$). Under severe stress levels (-0.34 MPa NaCl, -0.25 and -0.34 MPa PEG, and all Na_2CO_3 concentrations), most seedlings exhibited no emergence or decay of hypocotyl and radicle of sesame seedlings, and no measurements were recorded.

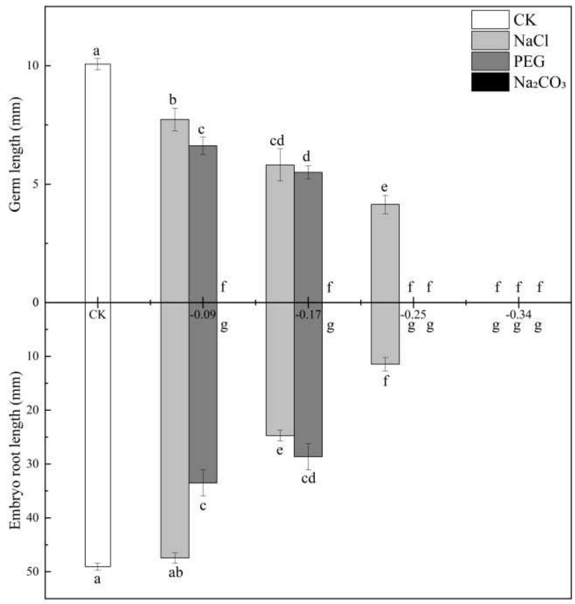


Fig. 4 Hypocotyl and radicle lengths of Sesamum indicum under NaCl, PEG, and Na_2CO_3 stress at different osmotic potentials. Bars represent mean ± standard deviation (n=3). Different letters denote significant differences among treatments at $p < 0.05$ (LSD test).

3.5 Effects of PEG, NaCl, and Na_2CO_3 stress on dry matter mass

The dry weight of sesame seedlings under CK was 0.1026 g (Fig. 5). Under NaCl and PEG stress, dry matter initially increased slightly at low stress (-0.09 MPa) and then decreased at higher osmotic stress levels. In contrast, Na_2CO_3 stress caused a continuous decline in dry matter. At -0.09 MPa, under the NaCl and PEG treatments, the dry matter weight of sesame seedlings was 49% and 5.2% higher, respectively, than under the CK. In the Na_2CO_3 solution, the dry matter weight of sesame seedlings was 0.083 g, which was 19% lower than that of the CK.

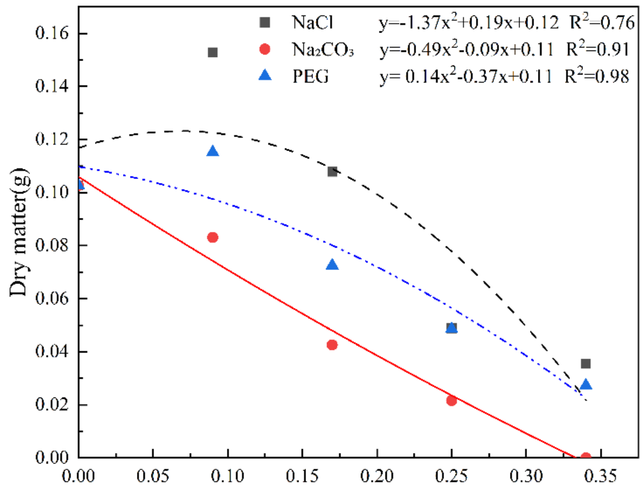
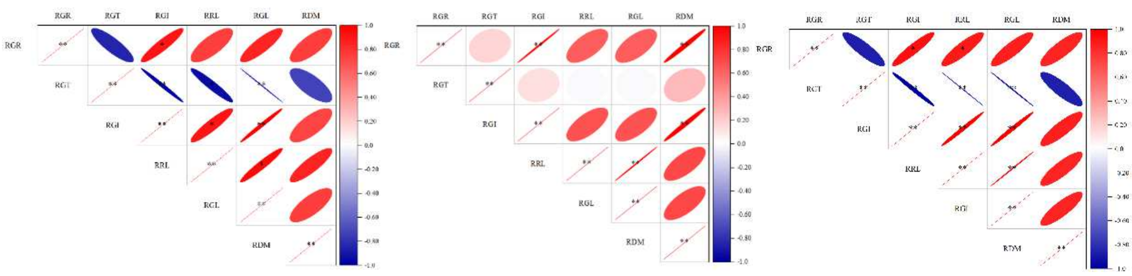


Fig. 5 The effects of different osmotic potentials induced by NaCl, PEG, and Na_2CO_3 treatments on the dry matter mass of sesame seedlings

3.6 Correlation analysis

Correlation analysis revealed strong relationships among germination and seedling growth parameters under salt and drought stress (Fig. 6a, 6c). The relative germination time (RGT) was highly negatively correlated with growth-related indicators, such as relative radicle length (RRL) and relative hypocotyl length (RGL) ($r \leq -0.95$). Under alkali stress (Fig. 6b), correlations were generally weaker. Only the relative germination rate (RGR) and relative germination index (RGI) showed strong positive correlations with relative dry mass (RDM) and RGL ($r \geq 0.99$). Additionally, RGI showed significant or highly significant positive correlations with growth-related indicators (RRL, RGL) under all three stresses.



a) Salt stress b) Alkaline stress c) Drought stress

Fig. 6 Correlation heatmap among germination and growth parameters under (a) NaCl, (b) Na_2CO_3, and (c) PEG stress. RGR: Relative germination rate; RGT: Relative germination time; RGI: Relative germination index; RRL: Relative radicle length; RGL: Relative hypocotyl length; RDM: Relative dry matter mass. * and ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively.

3.7 Principal Component Analysis of Various Indicators

Principal component analysis (PCA) was performed on six germination and growth indicators to reduce variable redundancy. Two principal components met the selection criteria of eigenvalue ≥ 1 and cumulative contribution ≥ 80% [18]. The first and second components explained 69.4% and 16.26% of total variance, respectively, accounting for 85.66% cumulatively (Table 3).

The factor loading matrix (Table 4) indicated that the first component (CI_1) was strongly associated with the germination index (r = 0.931), while the second component (CI_2) was most correlated with germination time (r = 0.952). These results suggest that germination index and germination time are the most representative indicators for assessing stress tolerance at the germination stage.

Table 3. Variance contribution rate of principal components derived from sesame germination indices

Principal component	Eigenvalue	Contribution (%)	Cumulative (%)
1	4.858	69.399	69.399
2	1.138	16.264	85.662
3	0.499	7.125	92.788
4	0.353	5.047	97.835
5	0.127	1.812	99.647
6	0.016	0.234	99.88
7	0.008	0.12	100

Table 4. Principal component load matrix of germination and seedling growth indicators of *Sesamum indicum*

Principal component	RGR	RGT	RGI	RRL	RGL	RDM
1	0.864	-0.111	0.931	-0.917	0.925	0.929
2	0.353	0.952	0.123	0.200	-0.187	0.046

3.8 Comprehensive evaluation of stress tolerance

3.8.1 Membership function analysis

The membership function value $U(X_j)$ of the comprehensive index was calculated according to Formula (4). As shown in Table 5, the lowest $U(X_j)$ was recorded under Na_2CO_3 stress at -0.36 MPa, indicating high sensitivity to alkali conditions. The highest $U(X_j)$ occurred under NaCl at -0.09 MPa, representing the strongest stress tolerance.

Table 5. Comprehensive evaluation of sesame seed stress resistance based on principal component and membership function analysis

Treatment	Osmotic Potential (MPa)	CI_1	CI_2	$U(X_1)$	$U(X_2)$	*D*-value	Evaluation	Ranking
CK	0	2.95	-0.80	0.99	0.48	0.88	High tolerance	2
NaCl	-0.10	3.02	-0.43	1.00	0.57	0.91	High tolerance	1
	-0.19	1.75	0.02	0.78	0.67	0.76	High tolerance	4

	-0.26	0.50	0.25	0.57	0.73	0.60	Moderate tolerance	6
	-0.34	-0.78	0.81	0.35	0.86	0.46	Moderate tolerance	8
Na_2CO_3	-0.11	0.09	0.25	0.50	0.73	0.55	Moderate tolerance	7
	-0.17	-1.78	-0.42	0.18	0.57	0.26	Sensitive	11
	-0.24	-2.61	0.27	0.04	0.73	0.18	Sensitive	12
	-0.36	-2.83	-2.83	0.00	0.00	0.00	Sensitive	13
PEG 4000	-0.09	1.86	-0.05	0.80	0.66	0.77	High tolerance	3
	-0.17	1.11	0.25	0.67	0.73	0.69	Moderate tolerance	5
	-0.23	-1.29	1.49	0.26	1.02	0.42	Sensitive	9
	-0.34	-1.99	1.17	0.14	0.95	0.31	Sensitive	10
W_i				0.788	0.212			

3.8.2 Weight determination

The weight values (W_j) of different comprehensive indicators were derived from their variance contributions, with Cl_1 and Cl_2 accounting for 0.788 and 0.212, respectively (Table 5).

3.8.3 Comprehensive evaluation

The comprehensive evaluation value (D value) of sesame seed stress resistance under different treatments was calculated using Formula (6), and the stress resistance was ranked by strength based on the D values. Among all treatments, the NaCl treatment at -0.09 MPa had the largest D value, indicating the strongest resistance to this treatment; the Na_2CO_3 treatment at -0.36 MPa had the smallest D value, indicating the weakest resistance to this treatment (Table 6). All treatments were ranked according to the magnitude of D values. Specifically, the four NaCl treatments all reached a tolerance level above moderate tolerance, while the four Na_2CO_3 treatments showed moderate tolerance or sensitivity. This indicates that sesame seeds have greater resistance to salt stress than alkali stress during the germination stage.

4 Discussion

4.1 Stress-specific inhibition mechanisms

This study demonstrates that sesame germination is strongly shaped by stress type, even when osmotic potential is comparable. Germination declined progressively with decreasing osmotic potential across all treatments, but the magnitude of inhibition differed markedly, following the order Na_2CO_3 (alkali) > PEG (osmotic drought) > NaCl (salt). At −0.34 MPa, Na_2CO_3 nearly eliminated germination (97.8% reduction), whereas PEG allowed partial germination and NaCl caused only a minor decline (10%). This contrast indicates that alkalinity suppresses germination through mechanisms extending beyond osmotic limitation, reflecting the added burden of elevated pH and carbonate chemistry.

A notable feature of the dataset is the stability of germination under mild NaCl stress. Treatments up to −0.25 MPa showed no significant deviation from the control, suggesting that early germination in sesame is relatively resilient to moderate ionic stress. Under neutral salinity, limited ion entry can contribute to osmotic adjustment during imbibition, supporting water uptake and metabolic reactivation without immediately disrupting membranes or enzymes (Hu et al., 2018). Such buffering, however, is transient and becomes less effective as stress intensity increases or as development progresses (Zhang and Zhao, 2011).

In contrast, PEG imposed a stronger constraint on germination dynamics at comparable osmotic potentials, reflected by sharper declines in germination index and pronounced delays in germination time. Because PEG does not penetrate seed tissues, it restricts water availability directly and persistently, limiting hydration-driven processes required for enzyme activation and reserve mobilization. Iso-osmotic comparisons across species consistently show stronger inhibition under PEG than NaCl, including in sesame and quinoa, where PEG delays germination and early emergence at less negative water potentials than NaCl (El Harfi et al., 2016; Dissanayake et al., 2019; Zhang et al., 2017). These patterns align closely with the present results.

The strongest inhibition occurred under Na_2CO_3, reflecting the compound nature of alkaline stress. Beyond osmotic limitation, elevated pH and carbonate/bicarbonate ions disrupt membrane electrochemical gradients, nutrient solubility, and proton-dependent transport (Riaz et al., 2020; Dolui et al., 2021). These constraints directly interfere with respiration restart, enzyme activation, and coordinated radicle protrusion (Chen et al., 2021; Zhao et al., 2014). Classic studies emphasize that alkaline salts impose qualitatively different constraints from neutral salinity, particularly through pH-driven disturbances in membrane function and metabolic regulation (Qu and Zhao, 2004; Guo et al., 2009).

From a broader physiological perspective, alkalinity tends to induce wider metabolic disturbance than neutral salinity. Evidence from metabolomic and transcriptomic analyses shows that alkaline stress disrupts core metabolic networks more extensively than NaCl, pushing cells beyond a narrow osmotic-stress response (Bai et al., 2023). Applied to germination, this implies that the cost of maintaining pH stability, ionic balance, and redox homeostasis under Na_2CO_3 may exceed the seed's compensatory capacity during the transition from quiescence to active metabolism, resulting in near-complete inhibition rather than delayed germination (Liu et al., 2010; Patil et al., 2012; Wang et al., 2022).

4.2 Physiological basis of higher salt tolerance

Across analyses, sesame exhibited higher tolerance to NaCl during germination than to PEG or Na_2CO_3. Germination rate remained stable under mild NaCl stress, and regression-based thresholds showed that comparable reductions in germination required more negative osmotic potentials under NaCl. This ranking was reinforced by the membership function–based comprehensive resistance index: at −0.36 MPa, NaCl produced a high D value (0.91), whereas Na_2CO_3 at the same osmotic potential resulted in D = 0, indicating extreme sensitivity.

This integrated ranking mirrors patterns reported in other crops (Li D et al., 2018), suggesting that higher tolerance to neutral salinity at germination may reflect a broadly conserved physiological response. Under NaCl stress, limited ion entry can support osmotic adjustment and water uptake, provided that ions are effectively compartmentalized and that stabilizing ions such as K^+ and Ca^{2+} remain sufficient to protect membranes and enzymes (Qu and Zhao, 2004). In contrast, PEG restricts hydration directly, delaying enzyme activation and reserve mobilization despite equivalent osmotic potential.

The developmental stage further modulates tolerance. Evidence from soybean indicates that germination can proceed at tissue Na^+ concentrations that already inhibit seedling growth, reflecting lower ionic sensitivity during pre-germination than during active expansion (Hosseini et al., 2007). This stage-specific buffering likely operates in sesame as well, allowing relatively stable germination under mild salinity while seedling growth becomes increasingly constrained as metabolic demand intensifies.

Correlation patterns support this interpretation. Under NaCl stress, germination traits remained strongly coordinated with seedling growth parameters, and relative germination time showed a consistent negative association with growth. Such coordination suggests that neutral salinity, within the tested range, permits a relatively synchronized transition from germination to early growth, unlike alkaline stress, where physiological integration deteriorates.

4.3 Alkalinity as a compound stress (osmotic + pH effects)

Alkaline stress emerged as the dominant limiting factor for sesame germination and early development. Na_2CO_3 caused sharp reductions in germination even at relatively mild osmotic potentials, with nearly complete inhibition at −0.34 MPa. Comprehensive evaluation consistently ranked alkali stress as the most detrimental condition across germination and growth traits.

This response reflects the compound nature of alkalinity. Elevated pH suppresses proton-dependent transport, destabilizes intracellular pH regulation, and reduces nutrient availability, while carbonate ions intensify ionic imbalance. These effects directly interfere with energy-dependent processes essential for radicle emergence and sustained growth (Qu and Zhao, 2004; Guo et al., 2009; Guo et al., 2010). Alkalinity also commonly amplifies oxidative stress, increasing membrane damage risk during imbibition and metabolic restart.

Physiological evidence in sesame supports this interpretation. Under increasing alkalinity, reductions in germination and seedling vigor are accompanied by higher Na^+ accumulation, elevated Na^+/K^+ ratios, and increased lipid peroxidation, despite concurrent accumulation of osmoprotectants such as proline and soluble sugars (Guo et al., 2010; Mahdavi, 2016). These responses indicate that protective adjustments are activated but are insufficient to counteract combined pH and ionic constraints.

Mild NaCl and PEG slightly increased seedling dry mass, potentially reflecting concentration effects, altered biomass allocation, or transient stimulation of reserve mobilization under moderate stress, a phenomenon previously reported under mild salinity and drought conditions (Yu et al., 2019; Zhang et al., 2017). In contrast, Na_2CO_3 caused a continuous decline in dry matter, consistent with stronger metabolic inhibition and a reduced capacity for biomass formation under alkaline conditions. Similar reductions in dry matter accumulation under alkaline stress have been linked to impaired carbon metabolism, ionic imbalance, and oxidative damage that collectively constrain growth processes (Mahdavi, 2016; Qu and Zhao, 2004).

4.4 Germination index and germination time reflected as superior screening traits

Germination index and germination time emerged as the most informative indicators of stress response in this study. Germination index declined consistently with decreasing osmotic potential across all treatments and captured stress intensity more sensitively than final germination percentage, particularly under Na_2CO_3 stress. Multivariate analysis further supported this distinction by identifying germination index as the primary contributor to variability (CI_1), with germination time forming a complementary axis (CI_2). Similar conclusions have been drawn in stress-response studies where germination dynamics provided stronger discrimination among stress levels than final germination alone (Zhou et al., 2023; Dissanayake et al., 2019).

These traits integrate multiple early physiological processes, including hydration, membrane repair, enzyme activation, and reserve mobilization. Disruption at any of these steps is rapidly expressed as delayed or slowed germination, whereas final germination percentage can remain relatively high even under substantial physiological stress, particularly under neutral salinity, where osmotic adjustment can partially compensate for stress effects (Gholamhoseini and

Dolatabadian, 2024; El Harfi et al., 2016). Consequently, germination index and germination time provide a more sensitive and predictive assessment of early-stage stress tolerance than germination percentage alone.

4.5 Implications for sesame establishment in saline–alkaline soils

From an establishment perspective, the present findings indicate that sesame may successfully germinate under mild salinity, but effective stand establishment depends primarily on post-germination performance under drought and alkaline conditions. Seedling growth was consistently more sensitive than germination, particularly under PEG and Na_2CO_3 stress, indicating that germination alone may overestimate establishment potential in stressful soils. Comparable stage-dependent sensitivity has been reported in sesame and other crops, where early seedling growth imposes greater physiological demands than radicle emergence (El Harfi et al., 2016; Zhou et al., 2023).

Because germination index remained strongly associated with early growth across stress types, it represents a practical screening trait for identifying materials with improved establishment potential (Sedghi, 2025). Importantly, the strong contrast between NaCl and Na_2CO_3 responses underscores that tolerance to neutral salinity does not imply tolerance to alkaline conditions. Similar distinctions between salt and alkali stress tolerance have been emphasized in crop studies, highlighting the need to consider high-pH effects explicitly rather than extrapolating from NaCl-based assessments alone (Qu and Zhao, 2004; Li D et al., 2018). Therefore, screening and management strategies for sesame in saline–alkaline environments should explicitly incorporate alkaline stress to more accurately predict field establishment and performance.

5 Conclusion

This study demonstrates that sesame germination and early seedling growth are strongly dependent on stress type and intensity, even under comparable osmotic potentials. Mild salinity (−0.09 MPa NaCl) did not affect germination, indicating substantial tolerance to low ionic stress. In contrast, hypertonic conditions (−0.34 MPa) imposed by PEG, NaCl, and especially Na_2CO_3 significantly inhibited germination, with alkali stress causing the most severe reduction. Regression analysis confirmed distinct tolerance thresholds, showing that sesame seeds tolerate salt stress across a wider osmotic range than drought and alkali stress. Germination index declined progressively with increasing stress intensity and was most strongly suppressed under Na_2CO_3, highlighting its sensitivity to early physiological disruption. All stresses significantly delayed germination, with PEG causing the greatest delay, reflecting the dominant role of water limitation in slowing metabolic activation. Seedling growth was more sensitive than germination across all stresses. Mild salinity and drought temporarily promoted dry matter accumulation, whereas alkaline stress consistently reduced biomass formation. Comprehensive evaluation using principal component analysis and membership function values classified treatments into tolerant, moderate, and sensitive groups, confirming that sesame exhibits significantly higher tolerance to salt stress than to drought and alkali stress at the germination stage. These results emphasize the need to distinguish among stress types when evaluating sesame establishment in saline–alkaline environments.

Declaration:

Ethics approval and consent to participate

All participants provided written informed consent before participation, ensuring they understood the purpose of the study, their rights, and confidentiality measures.

Consent for publication

All authors have given their consent for the publication of this manuscript.

Data Availability

The materials used in this research and protocols are available upon request.

Competing interests

The authors declare that they have no competing interests that could have influenced the results There are no financial, personal, or professional conflicts of interest related to this research.

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Authors' contributions

Zhu Hai: Conceptualization, Investigation, Formal Analysis, Writing – Original Draft. Sun Chitao: Conceptualization, Methodology, Supervision, Funding Acquisition, Writing – Review & Editing. Shahbaz Khan, Writing – Review & Editing. Xu Jiayi: Investigation, Software, Visualization, Data Curation. Wang Xiaohan: Methodology, Formal Analysis, Validation. Zhang Kai: Resources, Data Curation. Bao Jianping: Funding Acquisition, Resources, Project Administration.

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