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Germination and seedling growth responses of the weed *Sesbania cannabina* to salinity and drought under iso-osmotic conditions

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Abstract

Sesbania cannabina (Retz.) is identified as a troublesome invasive weed in certain agricultural systems. This study quantified and compared its ecophysiological responses to iso-osmotic salinity (NaCl) and drought (PEG 6000) during germination and seedling establishment. Two independent experiments were conducted in a completely randomized design with osmotic potentials ranging from 0 to −10 bar at the Agronomy Laboratory of the University of Jiroft. Evaluated traits included germination indices, root and shoot length, and root and shoot dry weight. ANOVA revealed highly significant effects ($P \leq 0.01$ p \le 0.01 $P \leq 0.01$) for stress type, osmotic potential, and their interaction across all traits. Mean comparisons ($P \leq 0.05$ p \le 0.05 $P \leq 0.05$) showed that mild stress (up to −2 bar) had marginal impacts, whereas potentials below −4-4−4 bar sharply reduced seedling growth. Non-linear regression models (Weibull, Logistic, and Gompertz) accurately fitted the stress-response curves ($R^2 \geq 0.97$). To quantify tolerance limits, the EC50 (osmotic potential causing a 50% trait reduction) was calculated. An independent t-test confirmed a highly significant divergence ($P \leq 0.01$) between the two stress regimes. Drought severely inhibited root elongation (EC50 = −5.3 bar). Conversely, roots exhibited exceptional salt tolerance (EC50 > −10.38 bar). In conclusion, under the tested laboratory conditions, *S. cannabina* seedlings demonstrate significantly greater physiological resilience to salt toxicity than to purely osmotic water deficit.

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Introduction

Invasive and aggressive weed species are among the major challenges in agricultural production systems, as they can reduce crop productivity and complicate crop management practices. One such species is *Sesbania cannabina* (Retz.) Pers., which has recently become an important weed in cotton (*Gossypium hirsutum* L.) production systems(Iqbal et al., 2019). Although this species is native to central and northern Australia, it has gradually spread to several other regions, including China, India, Indonesia, Malaysia, Papua New Guinea, and some Pacific Islands. Its expansion in agricultural areas has raised increasing concern because of its rapid establishment and strong competitive ability (Cunningham, 2011). In cotton fields, *S. cannabina* competes with the crop during much of the growing season. Severe infestations can lead to considerable reductions in cotton lint yield. Moreover, dense populations of this weed may interfere with mechanical harvesting, which can further reduce both the quantity and quality of the harvested lint (Iqbal et al., 2021). Seed germination and seedling establishment are critical stages in the life cycle of weeds and play an important role in their successful establishment in cropping systems. These processes are influenced by several environmental factors such as light, temperature, soil pH, and osmotic stresses including salinity and drought (Gaba et al., 2017). Such factors can affect embryo activity, nutrient balance, and hormone regulation,

which ultimately influence germination and early seedling growth (Bajwa et al., 2018). A better understanding of the germination ecology of *S. cannabina* can help explain its ability to adapt to different environments and spread in agricultural systems. This knowledge is also important for developing effective strategies to manage this weed and limit its further spread (Flores et al., 2016). Within the genus *Sesbania*, previous studies have shown that freshly dispersed seeds of several species, including wand riverhemp (*Sesbania virgata* (Cav.) Poir.) and *S. cannabina*, may exhibit innate dormancy (Souza et al., 2010). In addition, seedlings of these species have demonstrated considerable tolerance to waterlogged or saturated soil conditions (Ren et al., 2017). Treatments involving boiling water, H_2 SO_4 , or physical abrasion of the seed coat have been reported as effective methods for breaking seed dormancy in wand *Sesbania sesban* (Wang & Hanson, 2008). Assessing the adaptability of this plant in different environments therefore requires evaluating its tolerance to osmotic stresses such as salinity and drought, which are among the most common abiotic constraints affecting plant establishment (Chen & Jiang, 2010). The germination and early establishment stages are particularly sensitive to environmental stresses. Because plant growth begins with seed germination and successful establishment determines subsequent survival and adaptation, this stage plays a decisive role in the final establishment of plant populations (Finch-Savage & Bassel, 2016). Consequently, germination indices are widely used to evaluate plant tolerance to environmental stresses. Numerous studies have investigated plant responses to salinity and drought stresses. For instance, research by Qu et al. (2008) on *Halocnemum strobilaceum* revealed that the highest germination percentage occurred at a salinity level of 100 mM NaCl, while the maximum germination rate was observed up to 200 mM. Additionally, the highest germination under drought stress was recorded up to −2 Mpa (Qu et al., 2008). Khammari et al., (2007) investigated the salinity thresholds of *Carthamus tinctorius* (Khammari et al., 2007).

Although previous studies have provided some information on dormancy and environmental adaptability in *Sesbania* species, comparative information on the germination responses of *S. cannabina* to salinity and drought under equivalent osmotic conditions remains limited. In particular, little is known about how these stresses affect germination and early seedling growth of this species under iso-osmotic conditions. A better understanding of these responses could help explain the ability of *S. cannabina* to establish in different environments and may also contribute to improving management strategies for this weed. Therefore, the present study aimed to investigate the effects of salinity and drought stress under iso-osmotic conditions on the germination and early seedling growth of *S. cannabina*.

Material and Methods

Experimental site, design, and treatments

To evaluate the tolerance of *Sesbania cannabina* to salinity and drought stresses during seed germination and early seedling growth, two independent laboratory experiments were conducted at the Faculty of Agriculture, University of Jiroft. Both experiments were arranged in a completely randomized design (CRD) with three replications. Identical equivalent osmotic potentials of 0 (control), −2, −4, −6, −8, and −10 bar were generated using sodium chloride (NaCl) for salinity stress and polyethylene glycol 6000 (PEG 6000) for drought stress. The desired osmotic potentials for the NaCl solutions were calculated using the Van't Hoff equation (Asnin & Stepanova, 2024; Luo & Roux, 2010) (Equation 1):

(Equation 1): $\Psi_s = -c \times i \times R \times T$

where Ψs is the osmotic potential (bar), C is the molar concentration of the solute (mol L^{-1}), i is the van't Hoff factor (2 for NaCl), R is the ideal gas constant (0.08314 L bar K^{-1} mol^{-1}), and T is the absolute temperature (Kelvin degree: K).

Alternatively, the osmotic potentials for the PEG 6000 solutions were determined based on the Michel–Kaufmann equation (Michel & Kaufmann, 1973) (Equation 2):

$$\Psi_s = -(1.18 \times 10^{-2})C - (1.18 \times 10^{-4})C^2 + (2.67 \times 10^{-4})CT + (8.39 \times 10^{-7})C^{2T}$$

where Ψs represents the osmotic potential (bar), C is the concentration of PEG 6000 (g lit^{-1}), and T is the ambient temperature (centigrade degree: C).

Seed preparation and germination assays

Prior to the germination trials, *Sesbania cannabina* seeds were immersed in boiling water for 15 seconds to overcome physical dormancy. The seeds were then surface-sterilized in a 1% (v/v) sodium hypochlorite solution for one minute and thoroughly rinsed three times with sterile deionized water. For each experimental unit, a cohort of 25 seeds was evenly distributed within a 9-cm-diameter petri dish on a double layer of Whatman No. 1 filter paper. The filter papers were saturated with 5.0 ml of either sterile

deionized water (control) or the designated treatment solution. To tightly control ambient humidity and minimize evaporative loss, the petri dishes were sealed with Parafilm and enclosed in transparent plastic containers. The samples were then incubated for 14 days in a germinator set at a constant temperature of 25◦C under a 14-hour light and 8-hour dark photoperiod.

Data collection and seedling traits

Following the methodology of international seed testing association (Matthews et al., 2012), the number of germinated seeds was recorded daily. Seed germination was visually confirmed when the radicle reached approximately 2 mm in length. At the end of the 14-day incubation period, Germination Percentage (GP) (equation3) and Germination Rate (GR) (equation4) were calculated using standard methodologies (Matthews et al., 2012). The formulas are defined as follows:

(Equation 3): $$GP = \left(\frac{N_i}{N}\right) * 100$$

where GP is the germination percentage, Ni represents the cumulative number of germinated seeds by the final day of the assay, and N is the total number of seeds evaluated.

(Equation 4) $$GR = \sum_{i=1}^{k} \frac{n_i}{d_i} = \frac{n_1}{d_1} + \frac{n_2}{d_2} + \cdots + \frac{n_k}{d_k}$$

where GR is the germination rate (expressed as seeds per day), n_i the number of newly germinated seeds recorded on day d_i is the number of days elapsed since the onset of the experiment, and k denotes the total number of days for the assay (Matthews et al., 2012).

Upon completion of the experiment, seedling growth parameters, including root length, shoot length, and seedling biomass, were recorded. The seedlings were subsequently partitioned, and fresh samples were oven-dried at 70°C for 48 hours. The dry weights of the root and shoot were recorded using a high-precision analytical balance. Furthermore, the root to shoot dry weight ratio was calculated to determine the allometric coefficient, providing insights into biomass allocation under stress conditions.

To accurately quantify and compare the sensitivity of different plant traits to salinity and drought, the EC50 index (representing the effective osmotic potential causing a 50% reduction in a given trait compared to the control) was calculated. Rather than relying solely on mean comparisons, EC50 values were determined through regression analysis. For each measured parameter, the trait's response data across different stress levels were fitted to an appropriate regression model. The exact osmotic potential corresponding to a 50% decline was then derived via interpolation. This regression-based approach provided a standardized threshold, enabling a precise, trait-by-trait evaluation of stress vulnerability.

Statistical analysis

Prior to statistical analysis, the normality of residuals and homogeneity of variances were verified using the Shapiro-Wilk and Levene's tests, respectively. Given the independent experimental setups, data from the NaCl and PEG 6000 treatments were subjected to separate one-way analyses of variance (ANOVAs). Differences among treatment means were evaluated using Duncan's multiple range test at alpha = 0.05. All statistical procedures were conducted using SAS software, version 9.4 (Lenz, 2015). Treatment levels resulting in complete inhibition (zero variance across all replicates) were excluded from the analysis of variance to meet the assumption of homogeneity of variances. It should be noted that a combined factorial analysis was precluded by the distinct physiological mechanisms of the stress agents. Specifically, the dual osmotic and ionic toxicity induced by NaCl, contrasted with the pure osmotic stress of PEG, inherently generates unequal variance distributions (heteroscedasticity). Such violations of ANOVA assumptions would lead to biased Mean Square Errors (MSE) and invalid F-tests. Therefore, to ensure statistical rigor, the effects of NaCl and PEG were analyzed via separate one-way ANOVAs. Furthermore, to differentiate the specific effects of ion toxicity from pure osmotic stress, mean comparisons of germination traits between NaCl and PEG at equivalent osmotic potentials were conducted using independent samples t-tests and regression analysis.

Results

Drought stress

The results indicated that drought stress had a significant effect at the 1% probability level on the traits of germination percentage and rate, radicle and shoot length, radicle and shoot dry weight, and the radicle-to-shoot dry weight ratio (Table 1). Mean comparison results showed that germination percentage significantly decreased ($P \leq 0.05$) compared to the control as the level of drought stress increased from -4 bar to -10 bar. The germination rate index was similarly affected by drought stress as the germination percentage (Table 1), with a 94% reduction in germination rate as the osmotic potential decreased to -10 bar. However, the reduction in this index compared to the control was not significant

up to -2 bar, and a significant decrease in germination rate was observed from -4 bar onwards. The results of fitting the Gompertz model (Fig. 1a, b) indicated that the decreasing trend in germination percentage due to the drop in osmotic potential was very slow and negligible up to the −2 bar level. Subsequently, a sharp exponential decline was observed between the −4 and −6 bar levels, ultimately reaching minimum values under severe stress conditions (−8 and −10bars) (R^2=0.991). A similar pattern was recorded for the germination rate, where, after an initial resistance up to −2 bar, the rate decreased with a steep slope and almost ceased under severe stress conditions (R2=0.989).

Table 1. Analysis of variance (mean squares) for the effect of drought stress on germination characteristics and seedling growth of *Sesbania cannabina*

Sources of variations	df	Germination percentage	Germination rate	Root length	Shoot length	Root dry weight	Shoot dry weight	Allometric[1] Coefficient
Treatment (Drought)	5	4219.4**	6.28**	17.19**	19.55**	0.011 **	0.105 **	0.76**
Error	12	24.94	0.047	0.076	0.075	0.00088	0.0004	0.058
C.V. (%)	-	8.58	13.91	16.74	15.83	16.16	15.31	19.54

ns, *, and ** indicate non-significant and significant differences at $P \leq 0.05$ and $P \leq 0.01$ respectively; 1: Allometric coefficients at -8 and -10 bar are mathematically undefined (0/0) due to lack of growth. These were treated as zero in the ANOVA based on biological rationale (ND).

Changes in radicle and shoot length

Radicle length decreased by 69% and shoot length by 93%.as indicated in table 2 when the stress intensity increased from zero to -6 bar osmotic potential, in fact, shoot growth exhibited greater sensitivity to drought stress conditions compared to radicle growth. Furthermore, the results of this experiment showed that seedling growth was almost completely inhibited at the -8 and -10 bar treatment levels, although seed germination was observed at these levels. Fitting the Weibull model to the longitudinal growth data demonstrated (Fig.1:c and d) a difference in the sensitivity of these two organs. Radicle length remained stable up to the −2bar level and then declined with a steep exponential slope up to −6 bar (R^2=0.991). In contrast, shoot length showed significantly higher sensitivity to drought stress; its decreasing trend began immediately and steeply from the −2 bar level, reducing to near-zero values at an osmotic potential of −6 bars (R^2=0.98).

Changes in radicle and shoot dry weight

Changes in radicle and shoot dry weight under different levels of osmotic potential are presented in Table 1. The results demonstrated that shoot dry weight decreased by 60.4%, 85.4%, 93.7%, 100%, and 100% in the -2, -4, -6, -8, and -10 bar treatments, respectively, compared to the control. Similarly, radicle dry weight decreased by 40%, 65%, 79.3%, 100%, and 100% in the -2, -4, -6, -8, and -10 bar potentials, respectively, compared to the control. The changes in dry weight for both radicle and shoot were significant ($P < 0.01$) at all levels compared to the control. Variations in biomass production were also well described by the Weibull model (Fig.1, e and 1, f). Dry matter accumulation in the radicle exhibited a mild decline up to a potential of −2 bar, but after crossing this threshold, it decreased sharply up to the −6 bar level (R^2=0.986). For shoot dry weight, the slope of decline was much steeper, with a substantial drop initiating from the very early stages of stress (between 0 and -2 bars), confirming the higher vulnerability of shoot growth compared to the root under osmotic stress (R^2=0.992). The allometric coefficient (root-to-shoot length ratio) of *Sesbania cannabina* seedlings exhibited a highly significant increase ($P \leq 0.01$) in response to decreasing osmotic potential (Table 2). The lowest value was recorded under normal, non-stressed conditions (control), whereas the peak value was observed at the -6bar osmotic potential. This upward trend highlights a key adaptive morphological mechanism; under moderate to elevated water deficit, the seedlings strategically prioritize biomass and carbon allocation towards root elongation rather than shoot growth, thereby enhancing their capacity for water foraging. However, under severe osmotic stress levels (-8 and -10 bar), this adaptive response failed. The excessive stress exceeded the seedlings' tolerance threshold, leading to a complete cessation of growth and rendering the allometric coefficient unmeasurable (recorded as Not Defined/ND).

Table 2. Effects of PEG-induced drought stress on germination indices and seedling growth traits of *Sesbania cannabina*

Osmotic potential (bar)	Germination percentage (%)	Germination rate (day^{-1})	Root length (cm)	Shoot length (cm)	Root dry weight (g)	Shoot dry weight (g)	Allometric coefficient
0	96 a	3.18 a	3.59 a	6.50 a	0.160 a	0.48 a	0.33 c
-2	95 a	2.92 a	3.17 a	2.77 b	0.096 b	0.19 b	0.50 b
-4	82 b	2.50 b	2.12 b	0.70 c	0.056 c	0.07 c	0.74 b
-6	41 c	0.39 c	1.11 c	0.45 cd	0.033 d	0.03 d	1.33 a
-8	20 d	0.20 c	0.00 d	0.00 d	0.000 e	0.00 d	0e
-10	14 d	0.19 c	0.00 d	0.00 d	0.000 e	0.00 d	0e

Means followed by the same letter(s) within each column are not significantly different ($P \leq 0.05$) according to Duncan's Multiple Range Test; ND (Not Determined): The allometric coefficient could not be calculated at -8 and -10 bars because both root and shoot dry weights were zero. Mathematically, the ratio of zero to zero (0/0) is undefined.

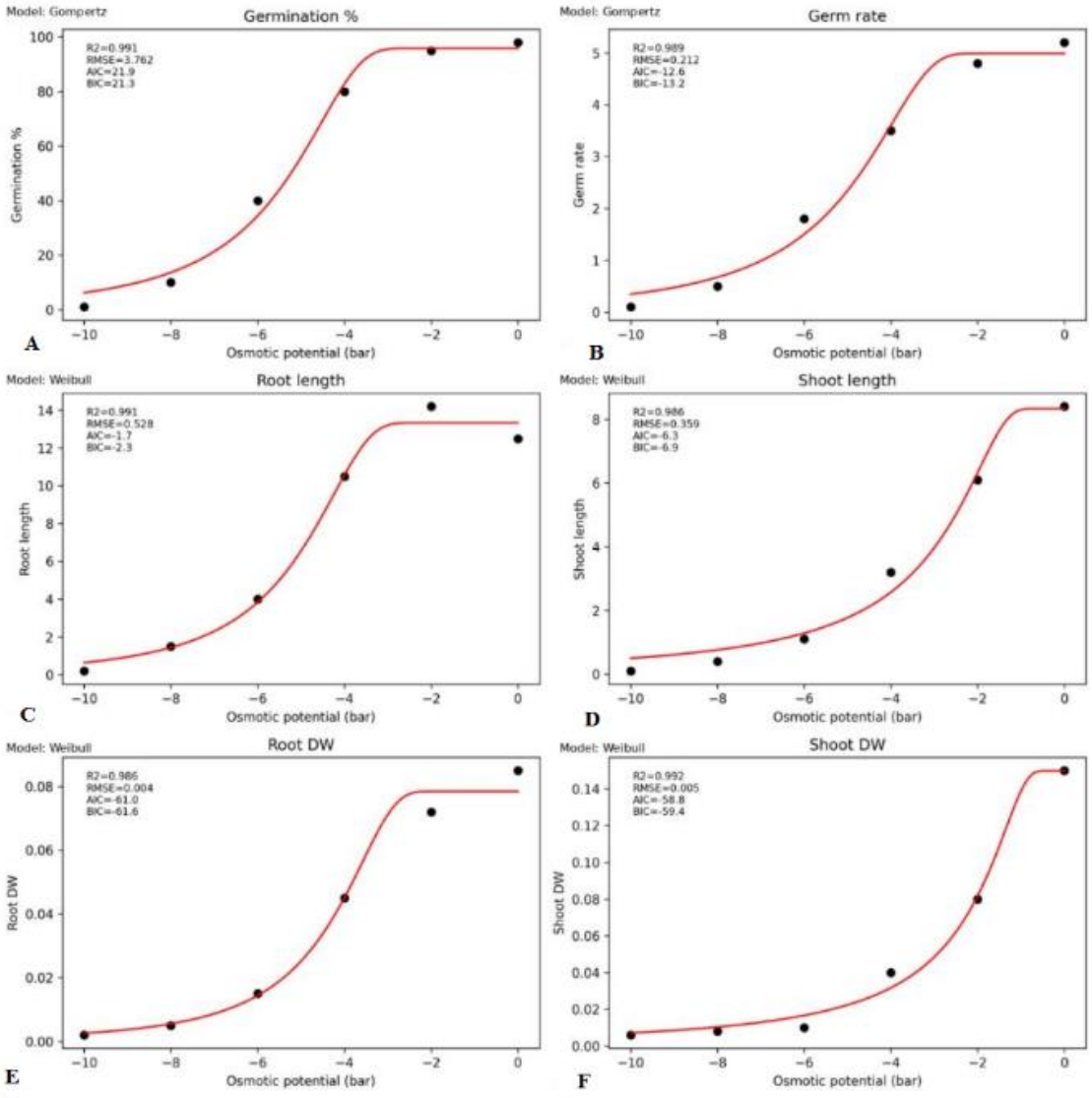

Fig. 1. Non-linear regression of germination and seedling growth traits of *Sesbania cannabina* under PEG6000-induced osmotic stress ranging from 0 to −10 bar. Panels (A) and (B) illustrate the response of Germination percentage and Germination rate, respectively, modeled using the Gompertz function. (C), (D), (E), (F) depict the response of Root length, Shoot length, Root dry weight, and Shoot dry weight, respectively, fitted using the Weibull model. Black data points represent the observed experimental values, and solid lines indicate the predicted nonlinear regression curves. Goodness-of-fit statistics for each model, including the coefficient of determination (R^2), Root Mean Square Error (RMSE), are displayed within each panel to demonstrate model robustness and parsimony).

Salinity stress

In this study, to compare the plant's resistance to salinity and drought conditions, salinity stress levels were investigated correspondingly and similarly to drought stress, based on osmotic potential (bar). Traits including germination percentage and rate, radicle and shoot length, radicle and shoot dry weight, and the radicle-to-shoot ratio were significantly affected (at the 1% level) by different salinity stress levels (Table 3). With increasing salinity stress intensity (decreasing osmotic potential), the germination percentage significantly decreased. Specifically, at osmotic potentials of -2, -4, -6, -8, and -10 bar, this component decreased by 17%, 37%, 54%, 64%, and 74% relative to the control, respectively (Table 4). Examination of the germination rate index revealed that a decrease in osmotic potential (increase in salinity level) up to -2 bar had no significant effect on germination rate. However, from -4 bar onwards to -10 bar, the germination rate significantly ($P < 0.01$) decreased, with this index showing a 56% reduction compared to the control at the -10 bar level. The trend of the decreasing changes in germination percentage was slow up to the -4 bar level and then decreased exponentially.

A shift in osmotic potential toward non-stressed conditions led to a substantial improvement in the final germination percentage. The robust fit of the Weibull model (R^2=0.99) indicates that the response of GP to salinity is strictly non-linear and threshold-oriented. This pattern suggests that while germination remains relatively stable under mild stress, a distinct and progressive decline is triggered once the osmotic potential passes a specific physiological threshold.

Germination rate exhibited the highest sensitivity to increasing salt stress, with its decline most accurately captured by the Logistic model. The sigmoidal pattern of this response indicates that while seeds maintain synchronized metabolic activation under mild stress, germination capacity drops sharply once a critical osmotic threshold is reached. Increasing the salinity stress intensity from 0 to -2 bar had no significant effect on root length (Table 3). However, from -2 bar to -10 bar, however, root length decreased significantly from −2 to −10 bar. Differences among the −4, −6, −8, and −10 bar treatments were not statistically significant. Shoot length was significantly affected by salinity stress (Table 3). It decreased from 0 to −6 bar, with no significant differences among the −6, −8, and −10 bar treatments (Table 4). Overall, with increasing salinity stress intensity up to -10 bar, the lengths of the root and shoot decreased by 49% and 88%, respectively. Shoot growth was more reduced than root growth under salinity stress. While salinity stress severely inhibited root elongation, a rapid recovery in growth was observed as the osmotic potential increased. The application of the Weibull model for this trait reveals a non-linear, threshold-based response. This implies that roots possess a higher compensatory growth potential at lower stress intensities, allowing for rapid soil volume exploration once osmotic constraints are partially lifted. Shoot length followed a developmental pattern similar to root length, albeit with a slightly different sensitivity profile. According to the Weibull model, shoot length was strongly constrained under high salinity levels but increased under milder conditions (Fig. 2 c, d). This clear non-linear response highlights that shoot elongation is more sensitive to the onset of osmotic stress than to its absolute magnitude. The effects of salinity stress on root and shoot dry weight are shown in Table 3. Root dry weight was not significantly affected at −2 bar (Table 4). However, beyond -2 bar, root dry weight significantly decreased, with this index showing approximately an 84% reduction compared to the control at the -10 bar concentration (Table4). As salinity stress intensity increased, shoot dry weight also significantly decreased, reaching its minimum at -10 bar, with a reduction of about 80% compared to the control. Based on these results, shoot dry weight appears to be more affected by salinity stress than root dry weight. The accumulation of root biomass was best described by the Gompertz model, which characterized the response as a slow and gradual progression (Fig. 2, e). This growth kinetic suggests that increasing osmotic potential—particularly within the median range toward zero—effectively bolsters the seedling's capacity for dry matter allocation to the roots, strengthening the plant's structural foundation. Similar to the roots, shoot dry matter accumulation was strictly limited by salinity, yet showed an accelerated gain as stress levels decreased. The high goodness-of-fit of the Gompertz model confirms that biomass partitioning to the aerial parts occurs non-linearly and with a distinct lag phase. This delay reinforces the high vulnerability of shoot tissues to unfavorable osmotic environments during the early heterotrophic-to-autotrophic transition. The analysis of the allometric coefficient showed that its highest value was observed in the -6 bar treatment (Table 4), and its lowest value was recorded in the -10 bar treatment. Consistent with Table 4, as the stress intensity increased from zero (control) to -6 bar, the allometric coefficient increased. However, with further decreases in osmotic potential, the magnitude of this

component significantly decreased. The increase in this ratio up to -6 bar indicates that the shoot is more sensitive to increased stress intensity than the root.

Table 3. Analysis of variance for the effects of salinity stress on germination and seedling growth traits of *Sesbania cannabina*.

Sources of variations	df	Germination percentage	Germination rate	Root length	Shoot length	Root dry weight	Shoot dry weight	Allometric coefficient
linity levels	5	2419**	2.63 **	1.97 **	20.7 **	0.005 **	0.078 **	0.023 **
rror	12	20.7	0.012	0.20	0.11	0.00008	0.0003	0.0008
C.V. (%)	-	7.75	3.7	15.6	10.2	12.1	6.6	9.44

ns, *****, and ****** indicate non-significant and significant differences at $P \leq 0.05$ and $P \leq 0.01$ respectively.

Table 4. Mean comparison of the effect of salinity stress on germination and early seedling growth characteristics of *Sesbania cannabina*.

Stress levels (Bar)	Germination percentage	Germination rate	Root length(cm)	Shoot length(cm)	Root dry weight(g)	Shoot dry weight(g)	Allometric coefficient
0	99.3 a	3.94 a	4.03 a	6.75 a	0.13 a	0.50 a	0.25 bc
-2	82.6 b	3.81 a	3.74a	5.75 b	0.11 a	0.38 b	0.29 b
-4	62.7c	3.52 b	2.57 b	4.45 c	0.09 b	0.30c	0.30 b
-6	45.6 d	2.94 c	2.52 b	1.33 d	0.06 c	0.14 d	0.47a
-8	35.5 e	2.02d	2.30 b	1.03 d	0.04 d	0.13 de	0.31 b
-10	25.7 f	1.72 e	2.06 b	0.80 d	0.02 d	0.10e	0.21c

Means followed by the same letter(s) within each column are not significantly different ($P \leq 0.05$) according to according to Duncan's Multiple Range Test.

Table 5. Half maximal effective concentration (EC50 (bar)) for germination and growth traits of *Sesbania cannabina* under drought and salinity stress.

Trait	Drought EC50 (bar)	Salinity EC50 (bar)
Germination percentage (%)	−5.5	−5.5
Germination rate (seed/day)	−5.1	−8.3
Root length (cm)	−5.3	>−10.0 *
Shoot length (cm)	−3.3	−4.7
Root dry weight (g)	−4.2	−5.7
Shoot dry weight (g)	−2.3	−4.6

* Note: Under salinity stress, the root length at -10 bar (2.06 cm) was still slightly above 50% of the control (2.015 cm), indicating exceptional salt tolerance in root elongation. The EC50 is mathematically extrapolated to approximately -10.3 bar.

Comparison of *Sesbania* seedling resistance to salinity and drought stress

To precisely quantify and compare the differential sensitivity of *Sesbania cannabina* to salinity and drought, the effective osmotic potential required to cause a 50% reduction in each evaluated trait "half maximal effective concentration" (EC50) was calculated (Table 5). The resulting thresholds revealed a distinct hierarchy in stress tolerance among the measured parameters. Under both stress conditions, shoot dry weight and shoot length emerged as the most sensitive traits. However, their 50% reduction occurred at notably milder stress levels under drought (−2.3 and −3.3bars, respectively) compared to salinity (−4.6 and −4.7 bars, respectively). Conversely, the most resilient traits varied depending on the stressor type. Under drought stress, germination percentage exhibited the highest tolerance, with an EC50 value of −5.5 bars. Under salinity, root parameters proved to be the most resilient, with root length and root dry weight withstanding osmotic potentials as low as >−10.0 and −5.7 bars, respectively. This specific hierarchical response particularly under

salinity, where above-ground traits decline faster than root parameters, highlights the plant's strategic resource allocation. By prioritizing root maintenance, *S. cannabina* likely enhances its water uptake capacity through active osmotic adjustment, thereby delaying the severe impacts of ion toxicity and water deficit in the shoots. Furthermore, the paired t-test results (Table 6) highlighted a crucial physiological divergence in the plant's response to the two stress types. While the overall germination percentage did not differ significantly between salinity and drought stresses (P = 0.93), indicating a high intrinsic viability and initial stress resilience of the seeds, the indices related to post-germination growth (e.g., germination rate, root length, and seedling length) were significantly higher under salinity stress ($P \leq 0.05$). Based on these statistical analyses, it can be concluded that while *Sesbania* maintains its basic germination capacity under pure osmotic stress, its post-germination growth is significantly more resilient to NaCl-induced salinity at equivalent osmotic potentials), likely due to the plant's capacity for osmotic adjustment via ion uptake in saline environments.

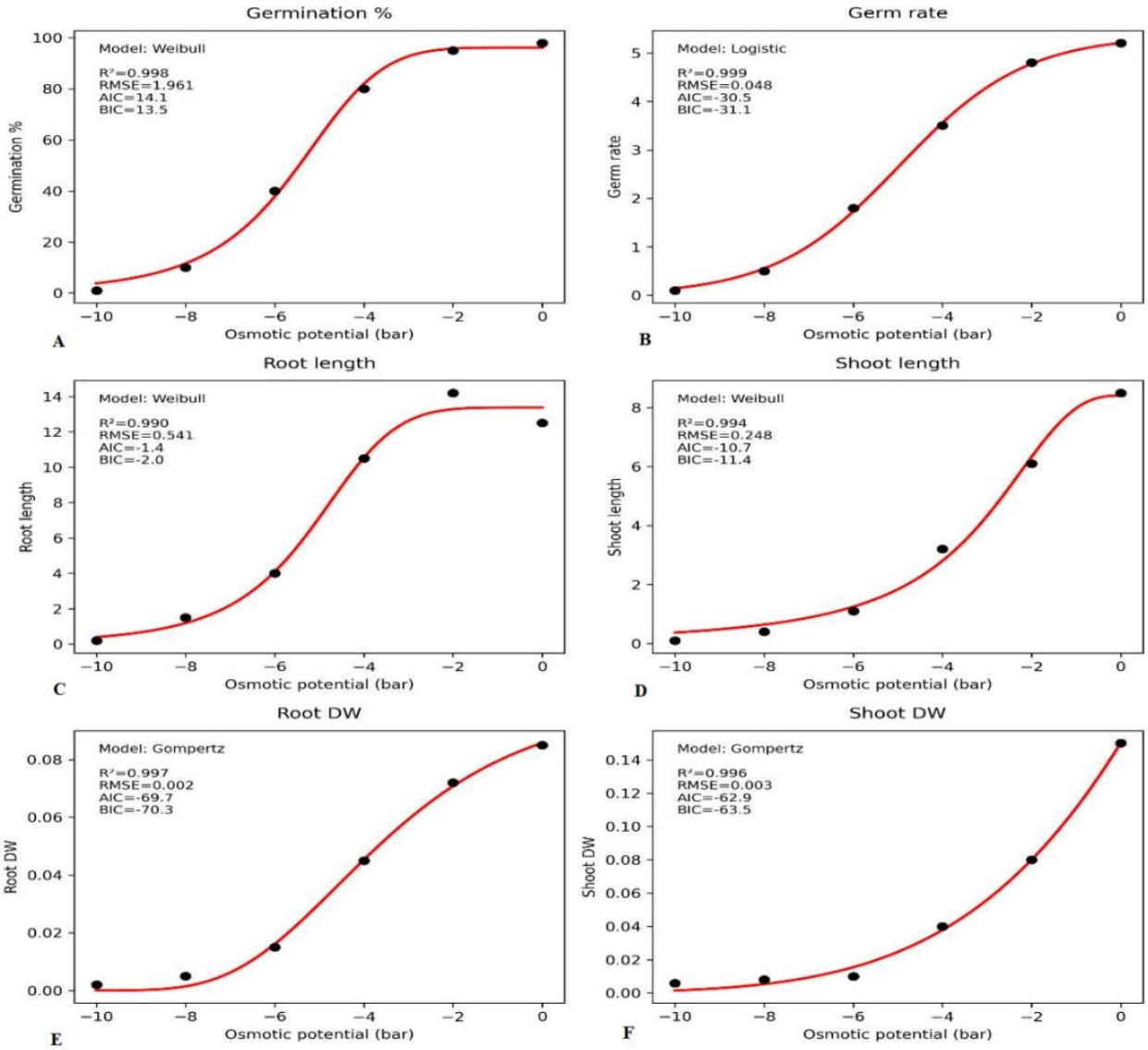

Fig. 2. Non-linear regression of germination and seedling growth traits of *Sesbania cannabina* under salt-induced osmotic stress ranging from 0 to −10 bar. (A, B) Germination percentage (GP), (C) Root length (RL,), and (D) shoot length (SL) were fitted using the Weibull three-parameter model, germination rate (GR) using the logistic three-parameter model, and root (E) and shoot (F) dry weights (RDW and SDW) (E and F) using the Gompertz three-parameter model. Black circles show observed mean values, while red curves indicate fitted model predictions. The high R^2 values and low RMSE across traits confirm the suitability of these nonlinear models for describing the response of *S. cannabina* to increasing osmotic stress.

Table 6. Comparison of *Sesbania* germination and seedling growth characteristics between salinity and drought stress conditions using an unpaired t-test.

	Germination percentage (%)	Germination rate	Root length (cm)	Shoot length (cm)	Root dry weight (g)	Shoot dry weight (g)
Difference in stresses	0.51	1.31	1.22	1.62	0.02	0.13
T	0.09	4.41	3.64	2.82	1.75	4.34
Pvalue	0.93^{ns}	0.01^{**}	0.01^{**}	0.04^{*}	0.14^{ns}	0.01^{**}

Mean Difference represents the average difference between salinity and drought treatments. ∗ Significant at $P < 0.05$. ∗∗ Significant at $P < 0.01$; ns non-significant.

Discussion

The present results showed that decreasing water potential inhibited both germination and early seedling growth of *Sesbania cannabina*, although the response varied among traits and stress types. The decline in germination under drought and salinity was mainly associated with reduced water uptake by the seeds, which delays the metabolic reactivation required for germination to proceed. This interpretation agrees with Hmissi et al. (2023), which identified restricted imbibition and impaired early metabolism as major causes of poor germination under osmotic stress (Hmissi et al., 2023). Under salinity, however, the response of germination was not uniform across traits. Final germination percentage declined progressively with increasing stress and was reduced by about 74% at −10 bar, whereas germination rate remained close to the control up to −2 bar. This suggests that *S. cannabina* seeds can tolerate mild salinity during the initial phase of germination, but this tolerance is gradually lost as stress intensity increases. At higher salinity levels, inhibition is probably caused by the combined effects of osmotic limitation and ion toxicity. Similar patterns have been reported by Rani et al. (2024), which showed that moderate salinity may have limited effects on the early kinetics of germination, while higher salt concentrations reduce the final proportion of seeds able to complete germination. Seedling growth was more sensitive to stress than germination itself (Rani et al., 2024). Radicle and shoot elongation declined sharply under both osmotic treatments, with stronger reductions under drought. Under salinity, radicle and shoot lengths decreased by up to 49% and 88%, respectively, while under drought the reductions reached approximately 69% and 93%. This difference is physiologically meaningful. Once germination has occurred, continued seedling growth depends on sustained water uptake, cell expansion, and meristematic activity. These processes are highly sensitive to loss of turgor pressure. Farooq et al. (2017) described cell expansion as one of the earliest growth processes affected by water deficit (Farooq et al., 2017). Othmani, 2022 similarly reported that seedling elongation is often restricted before germination is completely suppressed (Othmani, 2022). This interpretation is also consistent with (Gul et al., 2019) and (Suleiman et al., 2023), which emphasized that early seedling growth is generally more vulnerable than germination because it depends directly on turgor maintenance, osmotic adjustment, and the continuation of metabolic activity after radicle protrusion. The present results therefore suggest that, in *S. cannabina*, the transition from germination to seedling establishment is a more vulnerable stage than radicle protrusion alone.

The dry weight responses support this interpretation. Increasing drought stress progressively reduced both radicle and plumule dry weight, and severe drought completely inhibited plumule biomass formation. Such a response indicates that stress did not merely reduce organ length, but also limited the conversion of seed reserves into new seedling tissue. This may result from reduced reserve mobilization, impaired assimilate transport, and lower metabolic activity under limited hydration. Under salinity, growth reduction may also involve ionic and oxidative components. Excessive accumulation of Na+ and Cl− can disturb ionic balance, inhibit enzyme function, and damage membranes, thereby restricting seedling biomass production (Khammari et al., 2007; Hmissi et al., 2023; Suleiman et al., 2023). A decrease in germination rate and percentage due to increased drought stress intensity has also been reported in various plants, (Khammari et al., 2007; Qu et al., 2008; Luo & Roux, 2010; Suleiman et al., 2023).

A notable result was that drought caused stronger inhibition than salinity when both were compared at equivalent osmotic potentials. The estimated EC_{50} was about −5.3 bar under drought, whereas under salinity it exceeded −10.38 bar. This indicates that *S. cannabina* seeds were more tolerant to salinity-induced osmotic stress than to drought-induced water deficit. One explanation is that, under saline conditions, seeds

and young seedlings may partially adjust osmotically through ion uptake, compartmentalization, or accumulation of compatible solutes, helping to maintain water uptake at lower external potentials. Such mechanisms have been discussed by Rani et al. (2024) as important contributors to salinity tolerance during germination and early growth. In contrast, drought imposes a more direct limitation on water availability and does not provide external solutes that could contribute to osmotic adjustment, which may explain the lower tolerance threshold observed under drought.

Nonlinear modeling helped clarify how *Sesbania cannabina* responded to increasing drought and salinity stress. The Gompertz model described germination traits more effectively, suggesting that germination remained relatively stable at lower stress levels but declined sharply after a critical threshold. This pattern is in line with Bradford's (2002) hydrotime concept, which explains germination behavior in terms of water potential limits within the seed population(Bradford, 2002). The comparatively higher EC50 values for germination also suggest that this stage was less sensitive to stress, possibly because seeds were still able to maintain basic metabolic activity and some degree of osmotic adjustment under moderate stress conditions (Parihar et al., 2015). By contrast, seedling growth was better fitted by the Weibull model and showed a much steeper decline as stress increased. This finding indicates that the post-germination stage was more vulnerable than germination itself, which is physiologically reasonable because early seedling growth depends directly on cell expansion, turgor maintenance, and continued water uptake(Yuan et al., 2025). Once these processes are restricted, growth is reduced more rapidly than germination (Bhattacharya, 2021). The lower EC50 values for root and shoot traits further support this interpretation and point to early seedling establishment as the more sensitive stage under both drought and salinity.

Overall, *S. cannabina* showed some capacity to germinate under mild salinity, but its seedling growth was strongly restricted by decreasing water potential, especially under drought. These findings indicate that successful establishment of this species in the field is likely to depend less on the ability of seeds to initiate germination and more on whether sufficient moisture is available to support early seedling growth after emergence.

Conclusion

In general, both drought and salinity stresses significantly reduced all germination characteristics, including germination percentage and rate, root and shoot length, and root and shoot dry weight. Our findings clearly show that *Sesbania cannabina* is remarkably more tolerant to salt stress than to drought. When faced with challenging conditions, the plant relies on a simple but highly effective survival strategy: it limits shoot growth and channels its energy into expanding its root system to search for water. The most interesting takeaway is how differently the plant reacts to these two stresses. While drought immediately limits growth, the plant actually manages to hold its ground against salinity for much longer. It does this by absorbing salt ions and using them to maintain its internal water balance—a process known as active osmotic adjustment. This clever mechanism keeps the roots growing even at extreme salinity levels (with an EC50 exceeding −10.38 bar) and delays the harmful effects of salt toxicity. In short, *S. cannabina* is a tough, highly resilient plant. Its natural ability to thrive and establish roots in harsh, salty soils makes it an excellent candidate for restoring degraded lands and producing forage where standard crops would simply fail.

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Ethical Approval and Consent

This article does not contain any studies with human participants or animals performed by any of the authors.

CRediT author statement

M. Jowkar Tang-Karami: Designed the project, Provided laboratory support, Data analysis, Writing – review & editing. **J. Taei Semiromi:** Designed the project, Provided laboratory support, Data analysis, Writing – review & editing. **M. Roozkhosh:** Designed the project, Provided laboratory support, Data analysis, Writing – original draft, Writing – review & editing.

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Data Availability

All data generated or analyzed during this study are included in the published article.

Conflict of interest

The authors declare that they have no conflict of interest.

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