



Research Article

Influence of Varying Salinity on Germination Indices and Threshold of Salt Tolerance in Alfalfa (*Medicago sativa* L.)

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Abstract | A laboratory experiment was conducted to evaluate the impact of varying NaCl concentrations on germination indices of alfalfa (*Medicago sativa* L.). The study aims to determine the threshold level of salinity tolerance in alfalfa. Six salinity levels (50, 100, 150, 200, 250, and 300 mM) and a control (distilled water) was arranged in a completely randomized design with three replicates. One-way ANOVA revealed that germination percentage was reduced by 80%, germination index was 98%, while germination energy and germination vigor index was declined by 95%. However, the mean germination time was increased significantly, which indicated a delay in germination at the higher salt concentration. Significant reduction in germination (30%) was at 250 mM with a lowest germination (10%) recorded at 300 mM. Pearson's correlation matrix for germination indices (germination energy, germination rate index, mean germination rate, and germination vigor index) revealed a strong negative correlation with salinity. In contrast, mean germination time was negatively correlated with all the germination indices, which indicated that at higher salt concentration the seeds would take more time in germination or could be permanently inhibited. Second order polynomial regression model showed a declined slope for germination %, germination energy, and germination index across varying salt concentrations with R^2 values of 0.93, 0.97, and 0.96, respectively, which showed that increase in salinity significantly reduced germination efficacy of alfalfa. However, an improved germination efficiency and germination vigor index was observed under low to moderate salinity level. These results showed the threshold limit of salinity and its damaging effect on alfalfa. In light of these findings it is concluded that alfalfa seeds could tolerate salinity stress ranged from 50 and 150 mM, beyond which germination and seedling growth substantially reduced. It is suggested that alfalfa can germinate and survive well under moderate to high salinity concentration. Therefore, alfalfa could be an alternative crop for fodder production in the saline regions.

Received | August 31, 2024; **Accepted** | January 16, 2025; **Published** | April 14, 2025

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Citation | Khan, M.A., M. Fawad, A. Jamal, A. Ali and R. Ahmad. 2025. Influence of varying salinity on germination indices and threshold of salt tolerance in alfalfa (*Medicago sativa* L.). *Sarhad Journal of Agriculture*, 41(2): 528-537.

DOI | <https://dx.doi.org/10.17582/journal.sja/2025/41.2.528.537>

Keywords | Alfalfa seeds, Germination indices, Germination variability, Salinity tolerance, Correlation analysis



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Introduction

Salinity is a major abiotic factor, causing significant yield losses in crops, and is regarded as one of the primary cause of land degradation globally (Munns and Tester, 2009; Bhattarai *et al.*, 2020). Salinization had posed threats to the agricultural productivity in most of the region of Pakistan. Soil salinization affects alfalfa productivity around the globe (Farissi *et al.*, 2011; Bertrand *et al.*, 2015). Regional variations in alkaline and saline components in soils affects the alfalfa cultivation. In saline regions cultivation of annual crops is challenging because of its short root system. However, alfalfa is perennial in nature with a deep-root system which is a good choice for saline areas. Alfalfa technically known as *Medicago sativa* L. belong to the family Fabaceae, with a rapid regeneration and growth. It is cultivated as green fertilizer for improving soil condition, and considered as King of the Fodders (Hakl *et al.*, 2021; Wan *et al.*, 2022). Alfalfa could survive under deficit irrigation supply, and prevent salt-laden groundwater from reloading the top-soil with salt ions. Cultivation of alfalfa in salt-affected area provide a continuous supply of protein-enriched feed to the livestock (Yuegao and Cash, 2009). However, over 8 ds/m of soil salinity, alfalfa germination and growth becomes more susceptible (Steppuhn *et al.*, 2012). According to the previous reports, alfalfa germination and seedling stages are most vulnerable to salt stress (Peel *et al.*, 2004).

Seed germination is a complex process that is very sensitive to several environmental factors. Once the salt concentration in soil exceeds the threshold, it results in a high-water potential compared to that of the seed and prevent water from being absorbed by the seed from the surrounding soil. However, if there is insufficient water content in the seed, the germination process would be inhibited. The high concentration of metal ions in saline soil could also be one of the reasons for poor seed germination, since metal ions have a toxic effect on seeds (Zhang *et al.*, 2011; Sattar *et al.*, 2010). Seed germination is a crucial stage of plant growth, which affect the final yield of crop. Germination efficiency is directly associated with the germination vigor of seeds. Healthy seeds germinate well as compared to less viable seeds. In soil, germination is directly affected by various biotic and abiotic factors. Plants in response to abiotic stress show certain tolerance mechanisms that

maintain growth and yield (Ibrahim, 2016). Majority of plants grow neither on saline nor in alkaline soil environments, and even a medium salt concentration of 100 mM NaCl would cause a steep decline in the yield (Cheeseman, 2015).

In the present study we have investigated the influence of different salinity concentration on alfalfa seeds to find the optimum range of salinity for maximum germination efficacy. In the past few decades, most of the researcher have worked on the effect of one or two primary salt (NaCl and Na₂SO₄) stress on germination and seedling growth of alfalfa (Gisbert *et al.*, 2000; Sun *et al.*, 2012). Despite the fact that there is numerous published work on the impact of salinity on alfalfa. However, there is still a lack of systematic and comparative research in these studies. Therefore, in the current study we have explored the correlation between germination indices and salinity concentration and to predict the threshold limit of salinity by using second order polynomial regression model.

Materials and Methods

Experiment description

A lab study on effect of different salinity (NaCl) concentration (50, 100, 150, 200, 250, 300mM) was investigated on growth and germination indices of alfalfa. Alfalfa seeds were placed into Petri plates (15 seeds per each) and moistened with the prepared solution. The experiment was carried out in June 2023 in a completely randomized design having three replicates.

Data collection procedure

Seeds was put in 21 sterile Petri plates containing a double layer of filter paper to retain moisture. Each Petri plate was subjected to their respective treatment and a control (distilled water) for comparison. A seed was considered to be germinated at the emergence of radical growth (Tobe *et al.*, 2000).

Detail data for each parameter was recorded by the following procedure.

Germination percentage (%)

Counting was started soon after the first seedling emerged in each of the Petri plates; and further data was recorded till execution of the experiment. Seeds were considered germinated when the visible radicle

length reached at 2 mm. The counting was continued when there was no further increase in the counting and final data were computed by using the following equation (Smith and Varvil, 1984).

$$\text{Germination \%} = \frac{\text{Total number of seeds emerged}}{\text{Total number of seed grown}} \times 100$$

Germination index (GI)

Germination index was calculated by using the following equation (Rice, 1960).

$$\text{Germination index} = \frac{\text{No. of emerged seeds at first count} + \dots + \text{No. of emerged seeds at final count}}{\text{Days of first count} + \dots + \text{days of final count}}$$

Mean germination rate (MGR)

Mean germination rate is the reciprocal of the mean germination time. MGR is computed by using the following equation Ranal and Santana (2006).

$$\text{Mean germination rate} = \frac{1}{\text{MGT}}$$

Germination rate index (GRI)

Germination rate index was computed by using the following formula:

$$\text{Germination rate index} = \frac{\text{Germination index}}{\text{Germination percentage}}$$

Energy of germination (EG)

Energy of germination is the percentage of seedlings that germination three days after sowing. EG was computed by using the following equation (Ruan et al., 2002).

Mean germination time (MET)

Mean germination time was computed by using the following formula (Ellis and Roberts., 1981).

$$\text{Mean germination time} = \frac{\sum Dn}{\sum n}$$

Whereas, n represents the number of seeds that germinated on a given day of observation (not the cumulative total), and D is the number of days counted from the start of germination.

Coefficient of variance of germination time (CoVGT)
Coefficient of variance of germination time was computed by the following equation (Ellis and Robert, 1981).

$$\text{Coefficient of variance of germination time} = \left(\frac{SD}{MGT} \right) \times 100$$

Where, SD is the standard deviation and MGT is the mean germination time.

Coefficient of velocity of germination (CoVelG)

The coefficient of velocity of germination was determined with the formula as outlined by Scott et al. (1984).

$$\text{Coefficient of velocity of germination} = \frac{\sum N_i}{NN_i}$$

Where, N represents the number of seeds germinated on day i, and T denotes the number of days from seeding corresponding to N.

Mean daily germination (MDG)

Mean daily germination was calculated using the formula from Scott et al. (1984):

$$\text{Mean daily germination} = \frac{\text{FGP}}{D}$$

Where, FGP represents the final germination percentage, and D is the day of maximum germination during the experimental period.

Root and shoot weight (mg)

To estimate fresh and dry weights, the shoot and root portions were separated and weighed. They were then oven-dried at 80°C for 24 hours, and the final dry weight was recorded.

Statistical analysis

All the experimental data were statistically analyzed using one-way analysis of variance (ANOVA) suitable for a completely randomized design with the Statistix 8.1 software (Analytical Software, Tallahassee, FL, USA) to test the significance of various treatments. Means data for all the variable was compared by using Least Significant Difference (LSD) test at a significance level of $\alpha = 0.05$. Second degree polynomial regression model was computed to predict the effect of varying salt concentrations on each germination variable, and Pearson's correlation matrix was carried out to find the association between germination variables and salinity by using Microsoft Excel 2013.

Results and Discussion

The statistical analysis of data indicates that varying salt concentrations significantly impacted germination percentage, germination energy, mean germination time, germination index, germination vigor index, mean daily germination, coefficient of variance in germination time, coefficient of velocity of germination, as well as average fresh weight of roots and shoots (mg) (Table 1).

Table 1: *F-Fisher values of different treatments resulting from analysis of variance (ANOVA).*

Source of Variation	Treatments
DF	6
Germination % (GP)	96.2***
Germination energy (GE)	34.5***
Germination rate index (GRI)	50.7***
Mean germination time (MGT)	44.5***
Mean germination rate (MGR)	41***
Mean daily germination (MDG)	96.2***
Coefficient of variance of germination time (CoVGT)	44.1***
Coefficient of velocity of germination (CoVelG)	21***
Root weight (mg)	6.82**
Shoot weight (mg)	25.6***
Germination vigor index (GVI)	59.8***

*F-Fisher values are referred to Bliss-transformed data; DF: degrees of freedom; ***, ** and * indicate statistical significance at $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$, respectively; ns: not significant.*

Data presented in Table 2 show that highest G% (90 ± 0), GE (66.7 ± 3.33), GI (66.7 ± 3.3), MET (10.08 ± 0.45), and GVI (9.41 ± 0.13) was recorded in the control treatment (water). Moreover, as the concentration increases there was a significant decrease

in the germination variables. However, a slight reduction in germination efficiency was recorded at 50 mM NaCl concentration which have statistically comparable affect to that of control. Similarly, MET, GE, MET values were statistically comparable at control 50, 100, and 150 mM NaCl levels respectively, whereas, germination (%) and germination vigor index was significantly affected by the concentration. There was a substantial decline in GP% (10 ± 0.0), GE (0.3 ± 0), GI (0.23 ± 0.05), MET (13 ± 0.29), and GVI (39.6 ± 2.6) at 300 mM NaCl concentration (Figure 1). However, a considerable variation in all variable in response to highest salt concentration particularly 250 and 300 mM was observed. Alfalfa showed some degree of stress toleration to various salt concentration whereas, 150 mM was noted as the tolerable limit of salt stress in alfalfa, however

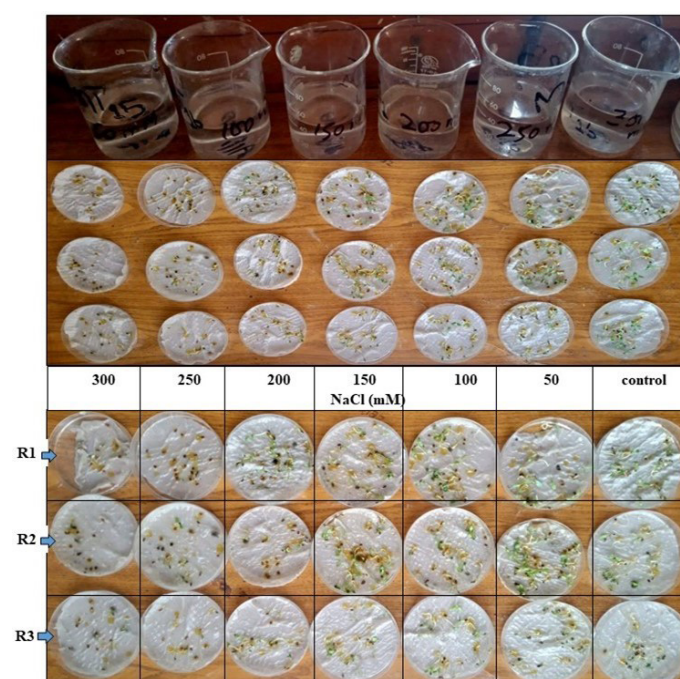


Figure 1: Germination of alfalfa seeds under varying NaCl concentrations

Table 2: *Effect of salinity concentrations on G (%), GE, GI, MGT and GVI of alfalfa.*

Salinity concentrations	Germination %	Germination energy (GE)	Germination index (GI)	Mean germination time (MET)	Germination vigor index (GVI)
Control (Water)	90 ± 0 a	66.7 ± 3.33 a	10.08 ± 0.45 a	9.41 ± 0.13 d	834 ± 51.3 a
NaCl (50 mM)	86.7 ± 3.33 ab	66.7 ± 3.33 a	10.08 ± 0.21 a	9.4 ± 0.05 d	763.6 ± 64.5 ab
NaCl (100 mM)	80 ± 0 b	53.3 ± 5.77 a	8.42 ± 0.34 a	9.58 ± 0.2 d	672.3 ± 77.1 b
NaCl (150 mM)	66.7 ± 6.67 c	33.3 ± 8.82 b	5.56 ± 1.28 b	9.75 ± 0.18 d	539.1 ± 35.5 c
NaCl (200 mM)	53.3 ± 3.33 d	20 ± 5.77 bc	3.43 ± 0.6 c	10.51 ± 0.3 c	413.1 ± 17.4 d
NaCl (250 mM)	30 ± 0 e	6.7 ± 3.33 cd	1.41 ± 0.08 d	11.14 ± 0.19 b	180.2 ± 8.7 e
NaCl (300 mM)	10 ± 0 f	0.3 ± 0 d	0.23 ± 0.05 d	13 ± 0.29 a	39.6 ± 2.6 f
LSD _(0.05)	9.36	14.30	1.73	0.60	116.97

Values (mean $\pm$ SE) presented with different alphabetically letters are statistically different at 5% level of probability.

Table 3: *Effect of salinity concentrations on MGD, CoVGT, CoVelG, average shoot and root weight (mg) of alfalfa.*

Salinity concentrations	Mean daily germination (MDG)	Coefficient of variance of germination time (CoVGT)	Coefficient of velocity of germination (CoVelG)	Average root fresh weight (mg)	Average shoot fresh weight (mg)
Control (Water)	6.4±0 a	0.04±0.002 a	17.1±0.31 b	2.6±0.28 a	6.6±0.28 a
NaCl (50 mM)	6.2±0.238 ab	0.03±0 a	17.5±0.25 b	2.5±0.28 a	6.3±0.32 ab
NaCl (100 mM)	5.7±0 b	0.03±0.003 a	20.2±0.61 b	2.4±0.53 a	6±0.45 ab
NaCl (150 mM)	4.8±0.476 c	0.02±0.004 b	34.1±7.25 b	2.3±0.24 a	5.9±0.23 ab
NaCl (200 mM)	3.8±0.238 d	0.02±0.001 b	44.7±5.28 b	2.1±0.27 a	5.7±0.21 b
NaCl (250 mM)	2.1±0 e	0.01±0.001 c	97.8±2.22 b	1.3±0.06 b	4.7±0.23 c
NaCl (300 mM)	0.7±0 f	0.01±0 d	05.6±0.2 a	1±0.1 b	3±0.16 d
LSD _(0.05)	0.67	0.005	18.43	0.72	0.75

Values (mean ± SE) for each parameter presented with different alphabetically letters are statistically different at 5% level of probability.

as the concentration increased from 150 there was a substantial decline in the germination indices of alfalfa. Kaiwen *et al.* (2020) confirmed a decline in alfalfa seed germination at 200 mM NaCl. Our results are consistent with the previous findings, that salt stress reduces alfalfa germination and vigor, particularly at higher salinity 200 mM (Lei *et al.*, 2018; Sandhu *et al.*, 2017).

Analysis of data revealed that different salt concentrations significantly affected MDG, CoVGT, CVG, average root and shoot fresh weight (Table 3). The data presented in Table 3 showed that higher values 6.4±0, 0.04±0.002 and 17.1±0.31 of MDG, CoVGT and CoVelG were observed, in the control (water), while the lowest data for these variables (0.7±0, 0.01±0 and 505.6±102.89) was found in the NaCl (300mM). The data further indicate that MDG was considerably varied across the treatments, however, CoVGT was statistically same at control, 50, 100 and 150 mM treatments. Similarly, CoVGT for control, 50, 100, 150, 200, and 250 mM was statistically at par with each other. However, there was a significant decline in CoVGT at salinity of 300mM. Likewise, highest average root and shoot weight was observed in the control which was statistically at par with NaCl 50, 100, 150, 200Mm, and the lowest was observed at 300mM (Figure 1). Interestingly, the seed germinated under all the salinity levels, however, seedling could not persistently survive under salt concentration of 300 mM. The data further revealed that alfalfa seeds could tolerate some degree of salinity stress. However, Li *et al.* (2022) found that high salt concentration disrupts the physiological function of alfalfa. Furthermore, salt stress also reduces the seedling biomass of alfalfa (Anower *et al.*, 2023; Postnikova *et al.*, 2023). This could be due to the seed physiological response to salinity. Studies

further showed that physiological responses of seeds to salinity involves disruption of ion homeostasis and increased reactive oxygen species (ROS) production, which causes oxidative stress during germination and seedling growth process (Gao *et al.*, 2019; Chen *et al.*, 2021).

Effect of varying salinity concentrations on germination indices and seedling growth of alfalfa

Second degree polynomial regression showed that germination variables were significantly affected by different salt concentrations (Figure 2). The model for alfalfa germination indicated a strong negative relationship with different salt levels ($Y = -0.0036x^2 + 1.0674x$, $R^2 = -0.949$) (Figure 2a). The inverted curve indicated that germination was increased to a threshold level of 148 mM, and then exhibited a sharp decline with further increase in salt concentration. However, germination energy indicated a weak negative relationship ($y = -0.0023x^2 + 0.6525x$, $R^2 = -0.505$) (Figure 2b). The curve, showed that maximum germination energy reached at 142 mM and then declined. Similarly, germination rate index had the same trend and show a weak relationship with salt concentration ($y = -0.0004x^2 + 0.1033x$, $R^2 = -0.567$) (Figure 2c). Mean germination time, show a slight linear increase in germination time with increase in salt concentration, however, the correlation was weak ($R^2 = -10.51$, $y = -0.0002x^2 + 0.1088x$) (Figure 2d). Likewise, germination vigor index negatively responded to salt concentration ($y = -0.005x^2 - 1.2285x + 837.66y$, $R^2 = 0.9956$) (Figure 2e). Mean germination rate, showed a highly consistent association with salt concentration ($y = -4E-07x^2 + 4E-05x + 0.1058$, $R^2 = 0.9913$) (Figure 2f). Conversely, the very low coefficients revealed that salt concentration has negligible but consistent effect on mean germination rate, furthermore, the germination

rate substantially increased at lower salt level, ranged from 50–60 mM. In a comparable study [Bhattarai et al. \(2022\)](#) found that salinity significantly affected germination rate and vigor of alfalfa seeds, however, the degree of salt stress depends on the genotype, they further found highest germination (73%) for ‘Halo’ cultivar, at 16 dS m⁻¹ salinity concentration. Interestingly, the growth of alfalfa increased when exposed to low concentrations of salt. Although a comparatively higher concentration reduced the speed of germination. However, too high salinity completely inhibited germination or even decay seeds and also cause the seedling to die ([Zhou et al., 2023](#)).

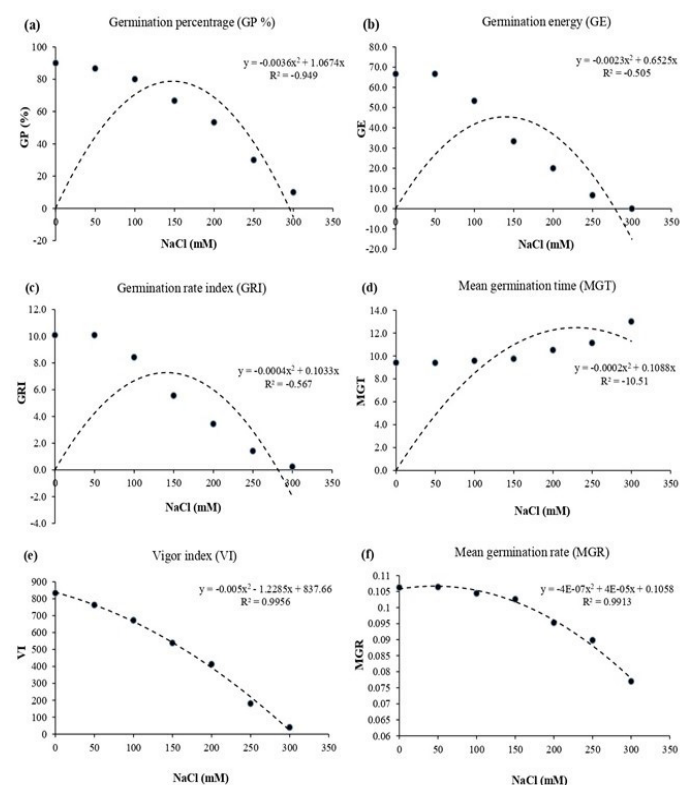


Figure 2: Effect of varying NaCl (mM) on germination variables of alfalfa: GP (a), GE (b), GRI (c), MGT (d), GVI (e), MGT (f). The curve exhibited a second order polynomial model, where Y show the germination variable at a salinity (mM) X ($p = 0.0000$). Data points are the mean of salinity level for three replicates.

Similarly mean daily germination, exhibited a strong relationship ($y = -6E-05x^2 - 0.0024x + 6.4512$, $R^2 = 0.9981$) with salinity levels (Figure 3a). Coefficient of variance of germination time, shows a considerably higher relationship with NaCl concentration ($y = -2E-07x^2 - 5E-05x + 0.0357$, $R^2 = 0.984$) (Figure 3b). However, coefficient of velocity of germination, reveals that germination velocity also decreased notably at higher salinity level with a critical threshold level at 72.88 mM ($y = 0.0089x^2 - 1.2977x$, $R^2 = 0.8025$) (Figure 3c). The model and line plot for the root weight in response to salinity levels showed a

threshold limit of 22.5 mM, afterward, the root weight substantially declined as the salinity level increased ($y = -2E-05x^2 + 0.0009x + 2.5645$, $R^2 = 0.97$) (Figure 3d). Contrary, shoot weight in response to the salinity, showed a weak association ($y = -0.0003x^2 + 0.0828x$, $R^2 = -4.37$) (Figure 3e). Previous studies showed that germination rates significantly reduced with increase in salinity levels ([Torabi et al., 2011](#); [Gao et al., 2023](#)). Moreover, high salt stress extend the mean germination time in alfalfa ([Aberchane et al., 2024](#)). Similarly, [Zhanwu et al. \(2022\)](#), found that salinity and alkaline stresses negatively affected germination rates and velocities. Apparently, alfalfa exhibited a sharp decline in germination % with the increase in salinity levels. Previous studies highlighted 100 mM NaCl as threshold level of salinity of alfalfa germination, however, the final germination (%) of the Ifrane cultivar reached to 98% but decreased at 150 mM to about 84%. Apparently, root growth of alfalfa increased at lower concentrations, while inhibited at higher concentrations ([Gao et al., 2023](#)). Similarly, alfalfa roots are sensitive to salinity stress but have negligible impact on the shoot growth. In a comparable study, shoot weight of some alfalfa genotypes was less affected than root weight under saline condition ([Aberchane et al., 2024](#)). In light of these findings, it is suggested that salinity level ranged from 50–150 mM could be tolerated by alfalfa, particularly during early seedling stages.

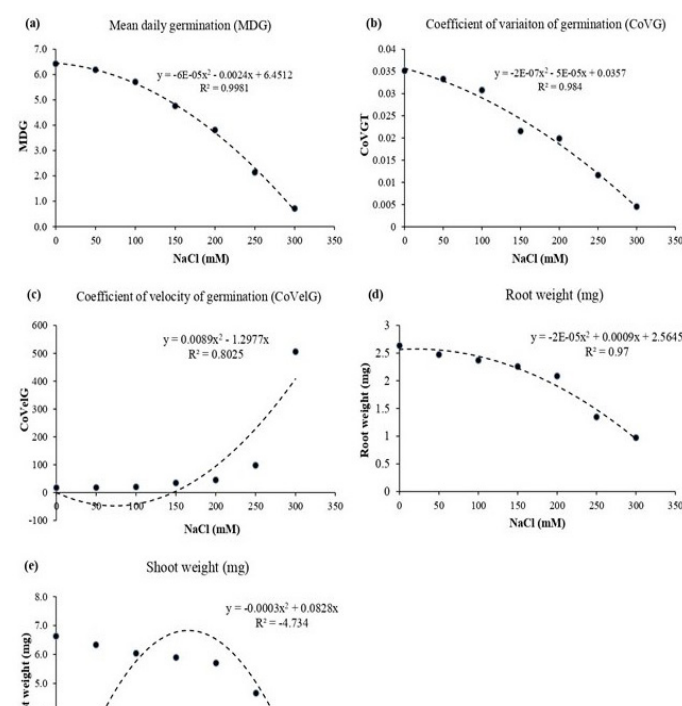


Figure 3: Effect of varying NaCl (mM) on germination variables of alfalfa: MDG (a), CoVGT (b), CoVelG (c), root weight (d), and shoot weight (e). The curve represents a second order polynomial model, where Y show the variables at a salinity (mM) X ($p = 0.0000$). Data points are the mean of salinity level for three replicates.

Pearson's correlation of germination variables

The analysis of the correlations among various germination indices of alfalfa seeds showed significant relationships with seed germination efficacy and vigor of seedlings under salt stress (Figure 4). The figure showed that germination % and germination energy exhibited a perfect positive correlation ($r = 1$). Similarly, GE correlates closely to the germination rate index (GRI, $r = 0.99$), which indicated that germination energy tend to be associated with faster germination rates. The mean germination rate (MGR) was highly correlated with the GRI ($r = 0.97$) and both these are critical for assessing the germination vigor. Contrary, the vigor index (VI) has a similar positive correlation with MGR, ($r = 0.97$), apparently show that the seedling VI depends on germination rates. CoVGT had a strong positive correlation with root weight ($r = 0.98$), because the germination of seeds accelerated by this factor that led to increased growth of root length. GP and GE are highly correlated to shoot and weight ($r = 0.98$ and $r = 0.98$, respectively). Interestingly, higher germination directly correlates with seedling vigor, which revealed that germination indices could predict the growth and vigor of the seedlings.

and efficiency that results in poor seedling growth. The correlation generally exhibits two clusters of variables germination efficiency for GP, GE, GRI, and MGR, showing the relative importance of the variables for germination performance and "Growth Efficiency" for VI, shoot and root weight, which show the association between germination efficiency and seedling growth. Apparently, salinity causes osmotic pressure, which hinders water uptake by the seed and reduced the germination %, which increase the time of germination (Debez *et al.*, 2004). Salinity stress affects various physiological and enzymatic function in seeds, since both of these factors are importance for effective germination (Zhanwu *et al.*, 2022). It is evident that high salinity stress impede germination due to increase in the osmotic pressure, which adversely affected the physiological function of seed germination.

Conclusions and Recommendations

In light of the findings, it is concluded that higher salinity stress drastically reduce the germination and early growth of alfalfa. The highest germination indices for GP, GE, GI, and, GVI was recorded in the control treatment. However, these values gradually decreased as the salinity level increased, with a substantial reduction in germination efficiency was observed at 300 mM NaCl. The damage was due to the harmful effects of high salinity stress. Apparently, alfalfa tolerated a moderate level of salinity stress (150 mM), beyond which GP and seedling growth were considerably inhibited. Salinity has high negative relationship with most of the germination traits, (%), germination energy, and germination vigor index. Based on the second order polynomial model, germination variables responded well at a threshold limit of salinity (150 mM), beyond which a significant reduction in germination and growth was observed at 200-300 mM, however, the seedling still survived at higher concentration (300 mM). Based on findings, it is suggested that salinity above 150 mM NaCl is not suitable for alfalfa cultivation. Future investigation should be focused on screening of salt-tolerant genotypes in terms of osmotic potential and oxidative stress.

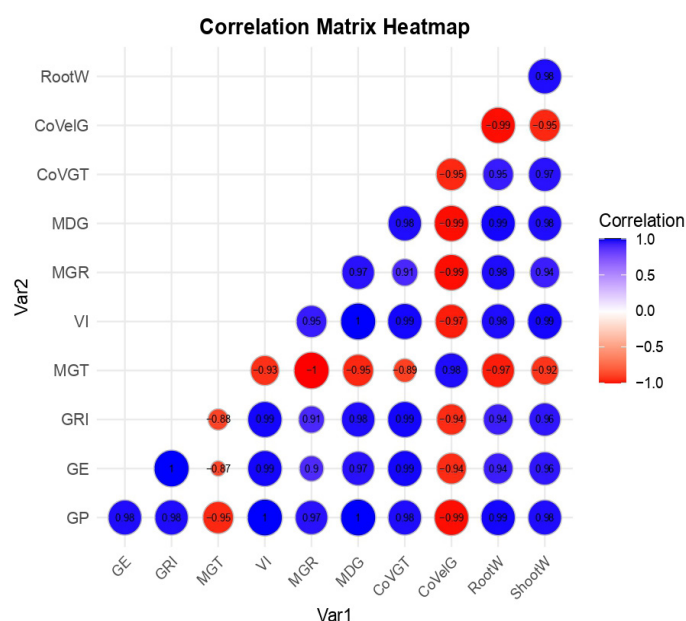


Figure 4: Correlation matrix heatmap of pairwise relations between various germination and seedling growth variables of alfalfa. The size and color of the circles show the strength and direction of correlation, where blue represents positive correlations and red represents negative correlations. Var1 and Var2 on the axes refer to the observed variables.

Furthermore, MGT has a negative correlation with GE, ($r = -0.93$), GP, ($r = -0.95$), GRI, ($r = -0.91$) and VI, ($r = -0.89$); which indicated that extended germination periods delay the energy, rate

Acknowledgements

The financial support of Higher Education Commission, Islamabad through Project No. HEC-

NRPU# 16722 entitled “Exploring the potential of wild plants in dry zones of southern Punjab and Khyber Pakhtunkhwa” is greatly acknowledged.

Novelty Statement

The present study highlight the critical threshold of alfalfa germination under varying salt concentration. Through an integrated approach of regression and correlation analysis, the precise limit of salt tolerance for maximum germination of alfalfa is 50mM, however alfalfa seeds showed an acceptable salinity tolerance up to 150 mM, beyond which germination variables showed a negative response. The present investigation lays down new guidelines to improve alfalfa germination under saline regimes and to develop a baseline information of salt tolerance limit.

Author's Contribution

Muhammad Azim Khan: Supervision, Investigation, Funding acquisition, manuscript editing proofreading.

Muhammad Fawad: Research conceptualization, performed research work, data collection, data entry and manuscript writing

Aftab Jamal: Data validation and statistical analysis

Arsalan Ali: Resources, Data validation, manuscript editing.

Rizwan Ahmad: Resources, manuscript review and proofreading.

Conflict of interest

The authors have declared no conflict of interest.

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