EFFECT OF SIX SINGLE SALT STRESSES ON GERMINATION OF ALFALFA (*MEDICAGO SATIVA*)

GAO, Z. W.[1†] – LIU, J.[2†] – ZHU, Q. A.[3] – LI, Q. A.[1] – LIU, J. Y.[1] – CUI, Y. H.[1] – MU, Y. G.[4*] – RASHEED, A.[5]

[1]*Tourism and Geographical Science Institute, Baicheng Normal University, Baicheng 137000, China*
(e-mail: gzw@bcnu.edu.cn; 577557902@qq.com; q2043858@163.com; 435839166@qq.com)

[2]*Agricultural Technology Extension Station of Dongliao County, Dongliao 136600, China*
(e-mail: liujing20221129@163.com)

[3]*Institute of Grassland Science, College of Life Sciences, Northeast Normal University Changchun 130024, China*
(e-mail: zhuq072@nenu.edu.cn)

[4]*School of Life Sciences, Jilin Normal University, Siping 136000, Jilin Province, China*
(e-mail: gaozw126@126.com)

[5]*College of Agronomy, Hunan Agricultural University, Changsha 410128, China*
(e-mail: adnanrasheed@hunau.edu.cn)

[†]*These two authors contributed equally to this work.*

[*]*Corresponding author*
e-mail: gaozw126@126.com

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Abstract. This article deals with the changes of germination index and physiological index under six single salts calcium chloride ($CaCl_2$), sodium chloride (NaCl), sodium sulfate (Na_2SO_4), sodium bicarbonate ($NaHCO_3$) magnesium chloride ($MgCl_2$), sodium bi-carbonate (Na_2CO_3). The single salt has a concentration gradient ranging from 25-150 mmol L^{-1} and the material for the experiment is alfalfa (*Medicago sativa*). The result of the experiment showed that with the rise of the concentration, the germination rate, germination vigor, germination index and vitality index declines ($P < 0.01$). Also, the length of the embryo, radicle and the weight of biomass declines extremely prominently ($P < 0.01$). The proline accumulation amount increases significantly under salt stress ($P < 0.01$). Alfalfa seeds have different tolerances to the six single salts. The order of tolerance to positive ions is $Mg^{2+} > Ca^{2+} > Na^{+}$. The order of tolerance to negative ions is $Cl^{-} > SO_4^{2-} > HCO_3^{-} > CO_3^{2-}$. The study also shows alkaline salt is more harmful than saline neutral salt. The result provides a reference framework for planting alfalfa according to the types of saline soil and the levels of concentration.
Keywords: *alfalfa, germination, proline, seedling growth, seedling vitality*

Introduction

The salinization of soil is an important factor which hinders the development of agriculture (Aouz et al., 2023; Khan et al., 2023a). The global population is increasing which is increasing the concentration of salts in our soils owing different activities. It has been reported that 50% soils around the globe will be converted into salt affected soils by the end of 2050 (Bhattarai et al., 2020; Khan et al., 2023b). Salinization has become an important environmental factor, which greatly affect growth and development of plants

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and severely threatened agricultural productivity and development (Yusnawan et al., 2021; Bouras et al., 2022; Nawaz et al., 2022). Currently, it is highly urgent and necessary to develop and employ large areas of salinized land and use salt-resistant plants to develop eco-farming and livestock raising (Chattha et al., 2022, 2023; Slimani et al., 2023; Dang et al., 2023). Alfalfa (*Medicago sativa*), as a perennial herb, belongs to the bean family, which has strong root system with nitrogen fixation ability. Also, it is cold and drought-resistant and has good regeneration ability (Zhang et al., 2019). It's a comparatively salt-resistant fodder crop, which is highly nutritious and is favored by livestock and poultry. Due to its good regeneration ability, it is also used as green fertilizer for improving soil conditions, and also called "King of the Fodders" (Mbarki et al., 2020; Hakl et al., 2021; Wan et al., 2022). If planted widely, it can enhance the development of agriculture and the ecosystem as well. The first trouble people encounter when planting alfalfa in saline-alkaline soil which inhibit the seed germination. However, due to regional differences of main saline and alkaline components in soils, the effect of planting alfalfa also differs greatly. Currently, most studies focus on one or two main salt stresses on the growth of seedlings under the effect of namely NaCl, Na_2SO_4 stresses (Gisbert et al., 2000; Sun et al., 2012). And these studies lack systematically, without relative and comparative research. Therefore, further study on salt tolerance of alfalfa is of great importance and urgency. Though many studies are available about the effect of salts stress on alfalfa. However, effect of various ionic stresses induced by different salts ($CaCl_2$, NaCl, Na_2SO_4, $NaHCO_3$, $MgCl_2$, Na_2CO_3) is not studied. Thus, this study was conducted to explore the influence of different salt stress sources on seed germination of alfalfa.

Materials and Methods

Cultivation of the materials

The alfalfa seeds of No.1 Gongnong (*M. sativa* L. cv. No.1 Gongnong) variety were taken from Jilin Academy of Agricultural Science. The seeds of alfalfa were disinfected in 99% ethanol solutions for 30 seconds. Further, petri dishes were deionized with water and disinfect in an oven at 120 °C oven for more than 3 hours. The concentration of different salts ($CaCl_2$, NaCl, Na_2SO_4, $NaHCO_3$, $MgCl_2$, Na_2CO_3-) were made by adding h distilled water. The salts were divided into following group: A, B, C, D, E, F; and different concentration of each salt (25, 50, 75, 100, 125, 150 $mmolL^{-1}$) was made. Moreover, distilled water was used as control group and experimental details are given in *Table 1*.

Sowing of seeds

The seeds were grown on filter papers in petri dishes. Two layers of filter paper were placed in each petri plate and 10 ml of different concentration of salts were added according to treatments. After that 100 seeds were placed on filter paper of each petri-plate and then petri plates were placed in incubator at 20°C under a light and darkness per of 12 hours respectively. The petri-dishes were regularly seen and water lost was measured by weighing and then changed level was maintained by adding salts and water.

Germination index

The number of seeds germinated on each day were counted and rate of them was determined with following equation: $Gr= n/N\times100\%$, n, number of germinations, N, number of seeds. Further germination vigor was determined on fourth day after sowing

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and it was determined with following formula: Gi=∑Gt/Dt (Gt, number of germination/days; Dt, corresponding number of days). Moreover, radical and embryo lengths were measured on 8th day by selecting ten complete embryos and radicle. Additionally, these radicals and embryos were oven dried (105°C) for 10 minutes 80°C until constant weight and later used to determine proline concentration. Ten seedlings were taken from each petri dish and their lengths from bottom to top were measured and average was taken.

Table 1. *Salt composition and molar ratio of various treatments and the stress factor*

Groups	Salt concentration	[Ca^{2+}]	[Mg^{2+}]	[Na^{+}]	[Cl^{-}]	[SO_4^{2-}]	[HCO_3^{-}]	[CO_3^{2-}]
A_1	50.0	25.0	0.0	0.0	50.0	0.0	0.0	0.0
A_2	100.0	50.0	0.0	0.0	100.0	0.0	0.0	0.0
A_3	150.0	75.0	0.0	0.0	150.0	0.0	0.0	0.0
A_4	200.0	100.0	0.0	0.0	200.0	0.0	0.0	0.0
A_5	250.0	125.0	0.0	0.0	250.0	0.0	0.0	0.0
A_6	300.0	150.0	0.0	0.0	300.0	0.0	0.0	0.0
B_1	25.0	0.0	0.0	25.0	25.0	0.0	0.0	0.0
B_2	50.0	0.0	0.0	50.0	50.0	0.0	0.0	0.0
B_3	75.0	0.0	0.0	75.0	75.0	0.0	0.0	0.0
B_4	100.0	0.0	0.0	100.0	100.0	0.0	0.0	0.0
B_5	125.0	0.0	0.0	125.0	125.0	0.0	0.0	0.0
B_6	150.0	0.0	0.0	150.0	150.0	0.0	0.0	0.0
C_1	25.0	0.0	0.0	50.0	0.0	25.0	0.0	0.0
C_2	50.0	0.0	0.0	100.0	0.0	50.0	0.0	0.0
C_3	75.0	0.0	0.0	150.0	0.0	75.0	0.0	0.0
C_4	100.0	0.0	0.0	200.0	0.0	100.0	0.0	0.0
C_5	125.0	0.0	0.0	250.0	0.0	125.0	0.0	0.0
C_6	150.0	0.0	0.0	300.0	0.0	150.0	0.0	0.0
D_1	25.0	0.0	0.0	25.0	0.0	0.0	25.0	0.0
D_2	50.0	0.0	0.0	50.0	0.0	0.0	50.0	0.0
D_3	75.0	0.0	0.0	75.0	0.0	0.0	75.0	0.0
D_4	100.0	0.0	0.0	100.0	0.0	0.0	100.0	0.0
D_5	125.0	0.0	0.0	125.0	0.0	0.0	125.0	0.0
D_6	150.0	0.0	0.0	150.0	0.0	0.0	150.0	0.0
E_1	50.0	0.0	25.0	0.0	50.0	0.0	0.0	0.0
E_2	100.0	0.0	50.0	0.0	100.0	0.0	0.0	0.0
E_3	150.0	0.0	75.0	0.0	150.0	0.0	0.0	0.0
E_4	200.0	0.0	100.0	0.0	200.0	0.0	0.0	0.0
E_5	250.0	0.0	125.0	0.0	250.0	0.0	0.0	0.0
E_6	300.0	0.0	150.0	0.0	300.0	0.0	0.0	0.0
F_1	25.0	0.0	0.0	50.0	0.0	0.0	0.0	25.0
F_2	50.0	0.0	0.0	100.0	0.0	0.0	0.0	50.0
F_3	75.0	0.0	0.0	150.0	0.0	0.0	0.0	75.0
F_4	100.0	0.0	0.0	200.0	0.0	0.0	0.0	100.0
F_5	125.0	0.0	0.0	250.0	0.0	0.0	0.0	125.0
F_6	150.0	0.0	0.0	300.0	0.0	0.0	0.0	150.0

Determination of proline concentration

The proline concentration was determined by the methods of Zhang (2003). The plants roots and shoots were taken and washed and then oven dried (105°C) until constant weight. After wards concentration of proline was determined by acid-ninhydrin method. The dried samples (0.1 g) were ground with 3% sulfosalicylic acid and extract was obtained

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and placed in boiling water for 10 minutes. Then this extract was at 3000 rpm and proline concentration was determined at 520 nm with spectrophotometer (Hitachi U-2001, Tokyo, Japan).

Statistical analysis

The collected data was analyzed using the cohort Co-state 6.4 software. Analysis of variance (ANOVA) was performed to statistically analyze the recorded data. The variance was performed by using SPSS10.0 software. In *Table 2*, P stands for the significant level of difference. The smaller the value of P, the greater the difference is. F= variance (MS)/ Error and the greater the value of F, the greater the difference.

Table 2. *The influence of six single salts with different concentration gradients on the germination*

Different indicators	Different treatment	$CaCl_2$	NaCl	Na_2SO_4	$NaHCO_3$	$MgCl_2$	Na_2CO_3
Germination rate	0	60.00±3.61a	60.00±3.61a	60.00±3.61a	60.00±3.61a	60.00±3.61a	60.00±3.61a
	25	70.67±7.57a	59.67±6.03a	52.67±4.16a	56.67±2.52a	72.00±2.65a	30.33±4.51b
	50	70.33±3.21a	59.67±6.43a	51.00±0.00a	42.67±4.16ab	63.67±3.79a	0.00±0.00c
	75	67.00±1.00a	50.00±5.57a	49.67±5.58a	7.67±1.53c	71.00±3.45a	0.00±0.00c
	100	63.33±5.51a	52.33±6.66a	37.00±5.29ab	0.00±0.00c	71.00±1.00a	0.00±0.00c
	125	63,67±7.23a	49.00±7.00a	29.33±2.31b	0.00±0.00c	64.00±4.36a	0.00±0.00c
	150	43.67±8.96ab	444.67±2.08a	10.67±2.08c	0.00±0.00c	57.33±2.89b	0.00±0.00c
Germination potential	0	53.00±1.00a	53.00±1.00a	53.00±1.00a	53.00±1.00a	53.00±1.00a	53.00±1.00a
	25	0.019±0.004a	0.016±0.001a	52.67±4.16a	56.67±2.52a	61.33±3.06a	30.00±4.58b
	50	0.020±0.004a	0.016±0.001a	51.00±4.58a	42.00±3.46ab	55.00±7.00	0.00±0.00c
	75	0.019±0.004a	0.014±0.002a	47.33±7.09ab	7.33±0.00c	55.67±4.93a	0.00±0.00c
	100	0.019±0.004a	0.015±0.001a	29.67±5.51c	0.00±0.00c	59.67±0.58a	0.00±0.00c
	125	0.018±0.002a	0.014±0.001ab	20.67±0.58c	0.00±0.00c	52.67±4.16a	0.00±0.00c
	150	0.018±0.000b	0.015±0.001b	7.00±1.00cd	0.00±0.00c	39.3±0.58ab	0.00±0.00c
Germination index	0	26.76±1.42a	26.76±1.42a	26.76±1.42a	26.76±1.42a	26.76±1.42a	26.76±1.42a
	25	25.79±2.06a	21.86±2.25a	19.13±1.83a	19.91±1.90a	28.22±0.61a	9.83±2.08a
	50	26.08±1.20a	20.35±1.88a	14.96±1.64b	13.37±1.11b	24.37±1.67a	0.00±0.00b
	75	23.09±1.77a	15.38±2.06b	12.40±2.02b	2.08±0.37c	24.19±2.69ab	0.00±0.00b
	100	18.46±1.91b	14.85±1.89b	7.95±1.15c	0.00±0.00c	25.35±1.61b	0.00±0.00b
	125	16.34±1.37b	12.95±2.18b	5.33±0.39c	0.00±0.00c	22.40±2.98bc	0.00±0.00b
	150	10.04±2.36c	11.04±1.18bc	1.87±0.35cd	0.00±0.00c	16.10±0.94c	0.00±0.00b

Note: Different lower-case letters after the same column indicate significant differences between treatments (P =0.05)

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Results

The influence of six single salts with different concentration gradients on the germination

The germination rate in each group tends to decline with rise in concentration of salts. $CaCl_2$ at its low concentration between 25 and 75 mmol·L^{-1}, improved the germination rate as compared to control, which shows that $CaCl_2$ at this concentration can enhance the germination (see *Figure 1A*). However, at concentration $CaCl_2$ significantly inhibited the germination of alfalfa. The application of $MgCl_2$ at lower concentrations (25-75 mmol·L^{-1}) increased the germination rate, however, higher concentration (125 mmol·L^{-1}) of $MgCl_2$ inhibited the germination. When the saline concentration was more than 50 and 100 mmol· L^{-1} under $NaHCO_3$ stress, then seed did not germinate.

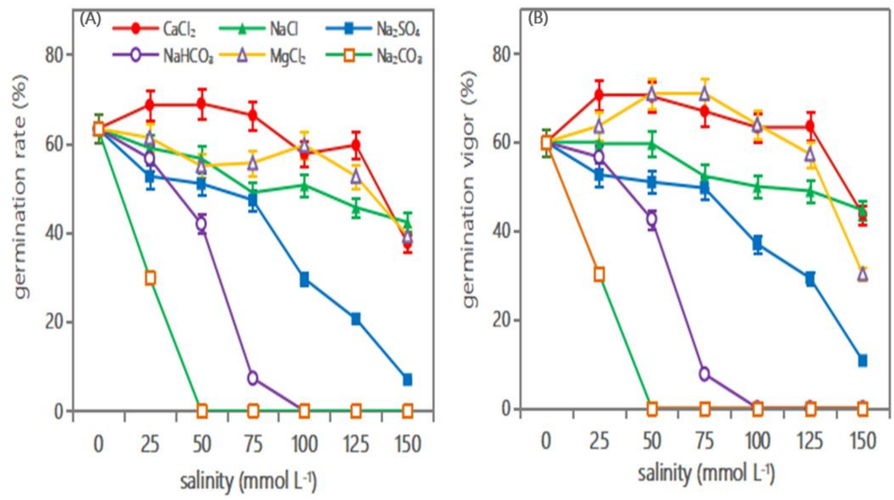

***Figure 1.** Effects of various concentrations of salt stress on seed germination (A) and germination vigor (B) of alfalfa. The values given in the figures are the means of three replicates with ± SE*

Different concentration of salt stress levels showed a significant impact on germination rate (see *Figure 1A)*. With the rise of concentration, germination vigor showed a declining tendency. Different salts like $CaCl_2$, NaCl, Na_2SO_4, $NaHCO_3$ and $MgCl_2$ are at their low concentration of 25 mmol·L^{-1}, showed that no obvious impact germination vigor, however, $MgCl_2$ and $CaCl_2$ at concentration between 25 and 75 mmol·L^{-1} increased the germination. Na_2CO_3 has a very obvious influence on germination vigor (see *Figure 1B)*.

At low concentration, it inhibited germination vigor considerably and seed was not germination at 50 mmol·L^{-1} Na_2CO_3 concentration. From the comparison of curve $CaCl_2$, NaCl and $MgCl_2$, it can be seed that Na^+ has a greater influence on germination rate than Ca^{2+}, Mg^{2+}. However, when the concentrations of $CaCl_2$, $MgCl_2$ were 150 mmol·L^{-1} it showed more toxic impacts as compared to NaCl. From the comparison of curve NaCl, Na_2SO_4, $NaHCO_3$ and $MgCl_2$, we noticed that when Na^+ contents were the same, the general influence extent of four kinds of negative ions on germination was recorded as: CO_3^{2-}> HCO_3^->SO_4^{2-}>Cl^-. This indicates that alkaline salt stresses more negatively impact the alfalfa as compared to neutral stresses.

The germination of alfalfa was inhibited to some extent under different concentration of all stresses (see *Figure 2A*). The rise of saline stress concentration decreased extent of

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germination index. The sensitivity of the germination index of alfalfa seeds was obvious, and different salts inhibited the germination of alfalfa seeds on different scales. With the rise of concentration, the vitality index showed a declining tendency (see *Figure 2B*). But $CaCl_2$ at the low concentration between 25 and 50 mmol·L^{-1}, increased vitality index of the seeds. However, $CaCl_2$ at the high concentration (≥75 mmol·L^{-1}) decreased vitality index of the seeds. The vitality index of all alfalfa seeds is inhibited to some extent and Na_2CO_3 has the greatest stress extent on vitality index.

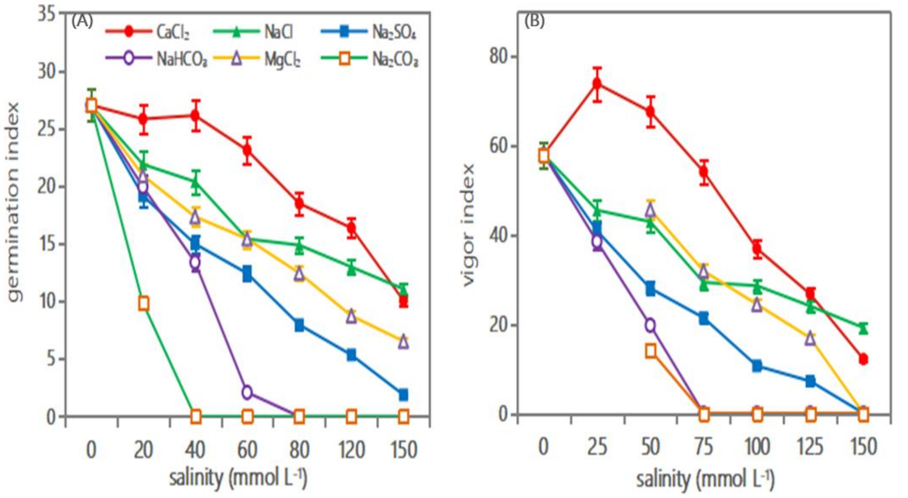

Figure 2. *Effects of various concentrations of salt stress on seed germination index (A) and vigor index (B) of alfalfa. The values given in the figures are the means of three replicates with ± SE*

The influence of six salts with concentration gradients on the growth and biomass of the embryos and of the radicles

The lower concentrations (25 and 50 mmol·L^{-1}) of $CaCl_2$ and $MgCl_2$ clearly enhanced the growth of the embryos, while the other four salts inhibited the growth (see *Figure 3A*). With the rise of concentration, the radicle length of alfalfa radicles decreased (see *Figure 3B*). At the concentration between 25 and 50 mmol·L^{-1}, $CaCl_2$ enhanced the growth of the radicles, while other groups of salts inhibited the growth (*Table 3*).

Under the stress of salts with different concentration gradients, at the low concentration between 25 and 50 mmol·L^{-1}, treatment solutions in $CaCl_2$, NaCl, $MgCl_2$ enhanced the growth of alfalfa seedlings, (see *Figure 4A* and *B*). At the concentration between 25 and 50 mmol·L^{-1}, the ratio of the root to the shoot in group $CaCl_2$ showed significant impact as compared to control, which indicates that within this extent of concentration, $CaCl_2$ has a greater impact on the growth of embryo than on the growth of the radicles. At the concentration between 25 and 75 mmol·L^{-1}, the ratio of the root to the shoot in NaCl was bigger than the control, which indicates that within this extent of concentration, NaCl has a greater enhancing effect on the radicles than on the embryos. When the concentration of NaCl was greater than 75 mmol·L^{-1} it inhibited growth of alfalfa, and root to shoot ration.

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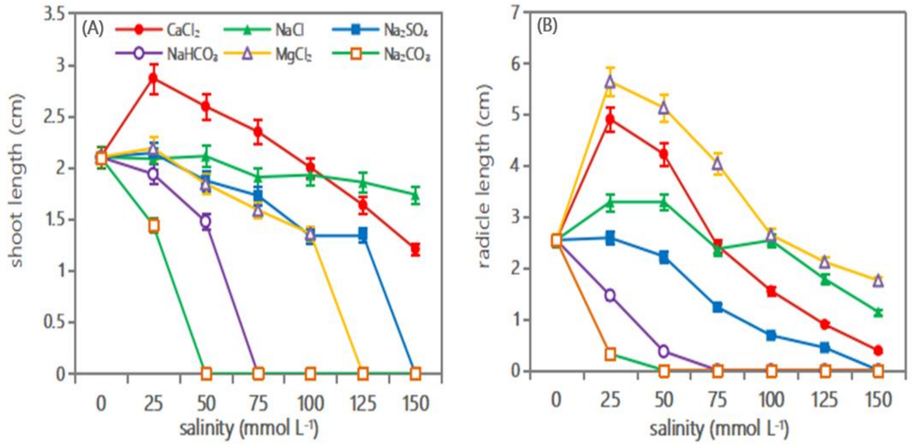

Figure 3. *Effects of various concentrations of salt stress on shoot (A) and radicle (B) length of alfalfa. The values given in figures are the means of three replicates with ± SE*

Table 3. *The influence of six salts with concentration gradients on the growth and biomass of the embryos and of the radicles*

Different indicators	Different treatment	$CaCl_2$	NaCl	Na_2SO_4	$NaHCO_3$	$MgCl_2$	Na_2CO_3
Embryo length	0	2.08±0.02a	2.08±0.02a	2.08±0.02a	2.08±0.02a	2.08±0.02a	2.08±0.02a
	25	2.87±0.39a	2.08±0.07a	2.14±0.06a	1.94±0.12a	2.47±0.33a	1.44±0.07b
	50	2.59±0.11a	2.11±0.07a	1.87±0.05a	1.48±0.08ab	2.83±0.05a	0.00±0.00c
	75	2.35±0.18a	1.91±0.13a	1.73±0.14a	0.00±0.00c	2.24±0.20a	0.00±0.00c
	100	2.00±0.08ab	1.93±0.07a	1.34±0.08ab	0.00±0.00c	1.82±0.05ab	0.00±0.00c
	125	1.64±0.05b	1.86±0.05a	1.35±0.09b	0.00±0.00c	1.54±0.04b	0.00±0.00c
	150	1.21±0.11bc	1.74±0.07a	0.00±0.00c	0.00±0.00c	1.37±0.02b	0.00±0.00c
Radicle length	0	2.54±0.30b	2.54±0.31a	2.54±0.32a	2.54±0.33a	2.54±0.34c	2.54±0.35a
	25	4.91±0.69a	3.29±0.43a	2.59±0.34a	1.46±0.18b	3.17±0.80a	0.32±0.06b
	50	4.23±0.96a	3.29±0.36a	2.23±0.17a	0.37±0.07c	2.31±0.51a	0.00±0.00b
	75	2.44±0.38b	2.37±0.11a	1.23±0.07b	0.00±0.00c	1.80±0.45b	0.00±0.00b
	100	1.55±0.06bc	2.54±0.28a	0.68±0.16b	0.00±0.00c	0.83±0.09c	0.00±0.00b
	125	0.89±0.08c	1.78±0.21ab	0.44±0.09b	0.00±0.00c	0.57±0.04c	0.00±0.00b
	150	0.38±0.06cd	1.14±0.28b	0.00±0.00bc	0.00±0.00c	0.38±0.04c	0.00±0.00b
biomass	0	0.017±0.001a	0.017±0.001a	0.017±0.001a	0.017±0.001a	0.017±0.001a	0.017±0.001a
	25	0.019±0.004a	0.016±0.001a	0.017±0.001a	0.018±0.002a	0.016±0.002b	0.012±0.004a
	50	0.020±0.004a	0.016±0.001a	0.018±0.001a	0.016±0.001a	0.018±0.004b	0.000±0.000b
	75	0.019±0.004a	0.014±0.002a	0.018±0.001a	0.000±0.000b	0.018±0.002b	0.000±0.000c
	100	0.019±0.004a	0.015±0.001a	0.016±0.001a	0.000±0.000b	0.019±0.004b	0.000±0.000c
	125	0.018±0.002a	0.014±0.001a	0.018±0.001a	0.000±0.000b	0.021±0.003b	0.000±0.000c
	150	0.018±0.000a	0.015±0.001a	0.000±0.000a	0.000±0.000b	0.021±0.004b	0.000±0.000c

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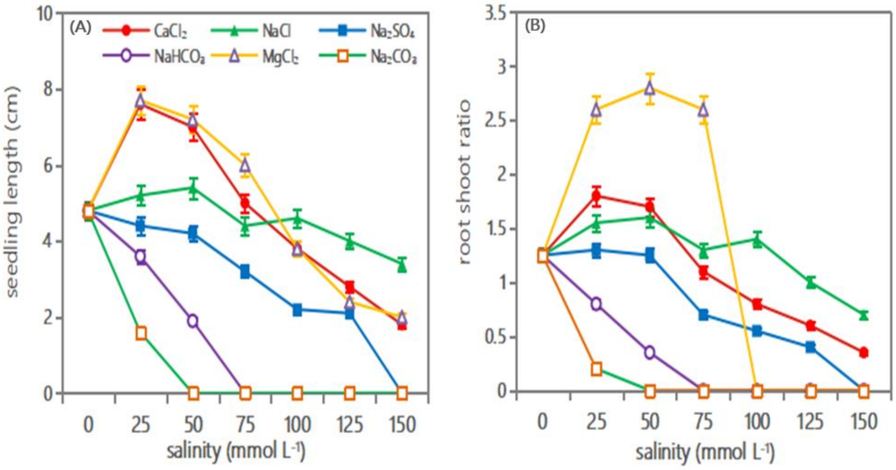

Figure 4. *Effects of various concentrations of salt stress on the seeding length (A) and root shoot ratio (B) in alfalfa. The values given in the figures are the means of three replicates with ± SE*

From the comparison of curves NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3, it can be seen that the influence extent of the four negative ions on the ratio of the root to the shoot ratio was recorded as: CO_3^{2-}>HCO_3^->SO_4^{2-}>Cl^-. Comparing curve of $CaCl_2$, NaCl and $NaHCO_3$, it can be seen that negative ion contents are the same within certain range, but Na^+ has a greater influence on the ratio of the root to the shoot than Ca^{2+}, Mg^{2+} (see *Figure 4B*). The influencing extent order on seeds is $MgCl_2$>$CaCl_2$>NaCl. Under the stresses of different salts with different gradients, solution $CaCl_2$ has an enhancing effect on the biomass of the whole plant. However, enhancing effect of NaCl showed continuous decline. Moreover, enhancing effect of solution Na_2SO_4 rises first and then declines; at the concentration between 25 and 50 mmol·L^{-1}, solution $MgCl_2$ has an enhancing effect on the biomass (see *Figure 5A*), after which the effect declines drastically due to the germination rate. Additionally, $NaHCO_3$ at the concentration of 25 mmol·L^{-1}, has an enhancing effect (see *Figure 4A*).

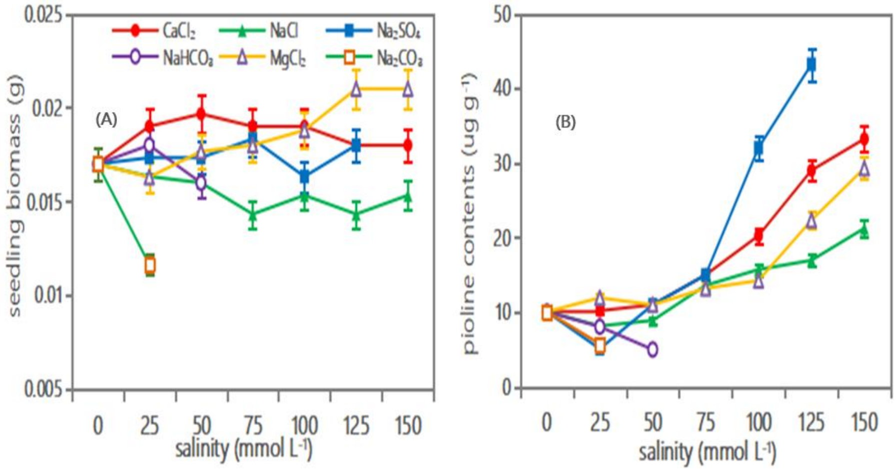

Figure 5. *Effects of various concentrations of salt stress on seedling biomass (A) and proline contents (B) of alfalfa. The values given in the figures are the means of three replicates with ± SE*

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The influence of the six single salt stresses on the proline content in the seedlings

Proline is a stress index which shows the responsive sensitivity of plant to environmental stresses. Generally, it is believed that the main function of proline is to work as an organic permeation accommodation matter, which protect the vitality of large biomolecules in the cell. At the low concentration of 25 mmol·L^{-1}, proline content in alfalfa treated with six salts declines (see *Figure 5B*). There is no obvious difference among different salts. At the concentration of more than 25 mmol·L^{-1}, with the rise of the saline concentration, the accumulation of the proline content in alfalfa treated with six salts increased and the difference was obvious (*Tables 4,5*).

Table 4. *The influence of the six single salt stresses on the proline content in the seedlings*

Different indicators	**Different treatment**	**$CaCl_2$**	**NaCl**	**Na_2SO_4**	**$NaHCO_3$**	**$MgCl_2$**	**Na_2CO_3**
Proline content	0	10.07±1.17b	10.07±1.17a	10.07±1.17c	10.07±1.17a	10.07±1.17b	10.07±1.17a
	25	10.2±3.24b	8.16±3.29a	5.09±1.05c	8.06±1.58a	11.93±1.31b	5.76±0.58a
	50	11.22±2.36b	8.97±2.96a	10.94±1.52c	5.45±1.24a	11.22±3.28b	0.00±0.00a
	75	10.39±1.88b	13.60±2.09a	13.13±4.07b	0.00±0.00b	13.20±3.11b	0.00±0.00a
	100	20.27±4.59a	15.73±1.35a	32.05±5.76b	0.00±0.00b	14.25±3.17b	0.00±0.00a
	125	29.02±7.07a	16.96±5.35a	49.24±32.16a	0.00±0.00b	22.34±0.15a	0.00±0.00a
	150	33.24±5.49	21.26±1.30a	0.00±0.00c	0.00±0.00b	29.31±11.77a	0.00±0.00a
Soluble sugar	0	0.87±0.06a	0.87±0.06a	0.87±0.06a	0.87±0.06a	0.87±0.06a	0.87±0.06a
	25	1.29±0.12a	0.80±0.36a	1.03±0.57a	1.02±0.02a	0.86±0.07a	0.81±0.26
	50	1.09±0.18a	0.87±0.18a	0.86±0.09a	0.77±0.05a	0.65±0.04a	0.00±0.00b
	75	0.97±0.15a	0.96±0.11a	0.79±0.32a	0.00±0.00b	0.68±0.09a	0.00±0.00b
	100	0.88±0.09a	1.04±0.06a	1.05±0.08a	0.00±0.00b	0.80±0.17a	0.00±0.00b
	125	1.07±0.3a	0.86±0.11a	1.22±0.05a	0.00±0.00b	0.00±0.00b	0.00±0.00b
	150	0.78±0.2ab	1.05±0.07a	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b

Table 5. *Variance analysis of different index changes in the alfalfa germination process under the stresses of six salts with different concentration gradients*

	df	**Germination rate**		**Germination vigor**		**Germination index**		**Vitality index**	
		F	P	F	P	F	P	F	P
Salt Type	5	428.749	0.000	118.705	0.000	332.311	0.000	242.032	0.000
Salt concentration	6	272.716	0.000	303.562	0.000	505.443	0.000	376.935	0.000
	df	Embryo length		Radicle length		Seedling length		ratio of the root to the trunk	
		F	P	F	P	F	P	F	P
Salt Type	5	527.920	0.000	154.231	0.000	362.528	0.000	48.651	0.000
Salt concentration	6	3229.410	0.000	137.272	0.000	315.052	0.000	46.895	0.000
	df	biomass		Proline content					
		F	P	F	P				
Salt Type	5	200.270	0.000	20.897	0.000				
Salt concentration	6	105.259	0.000	4.552	0.000				

※0.000 stands for the extreme significance of difference which is less than 0.001

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Discussion

Influence of salts with different concentration gradients on the germination of the seeds

Saline stress is one of the major adverse environmental factors that influence the growth of the plant and reduce the production. For a long time, the mechanism of plants' salt tolerance and the ways to improve their salt tolerance has become a major concern (Al-Dakhil et al., 2023; Aragão et al., 2023; Shao et al., 2023). Due to the geographical differences in different regions, the major constituent of salinized soils differs. Wang et al. (2006) found that alfalfa seeds treated in NaCl solution can germinate and grow well in conditions with no or little salt content. The salt with low concentration enhances the growth of alfalfa. The salt with relatively high concentration will not do fatal harm to the plant, but it slows down the germination and growth of the seeds. The salt with much too high concentration causes the seeds to decay and die. It also causes the seedling to die and the radicles to root growth (Purwestri et al., 2023; Zhou et al., 2023). On the one hand, the energy that should be used for growth is otherwise consumed by plants living in saline-alkaline soil conditions. The energy originally should be used for photosynthesis which helps the plants to grow, but in order to survive in saline-alkaline soil conditions, the plants have to spend much energy on the transportation and absorption of ions. On the other hand, in saline-alkaline soil conditions, the accumulation of large amounts of salts in the cell walls leads to the decline of turgor pressure, which in turn inhibits the growth of the plants. Hadjadj et al. (2023) concluded from their studies that the stresses of NaC1, Na_2SO_4 can inhibit germination of seeds extremely prominently, with the germination index and germination vigor declining the same way. During germination, the extreme concentration of the two salts that wormwood can tolerate is between 1.0％and 2.0％, with the harm of NaC1 greater than that of Na_2SO_4. When the concentration is kept to the same level, of the two negative ions-SO_4^{2-}, Cl^- has a greater inhibiting action on the germination, which shows that different negative ionic salts may have different toxic actions on the plants. The results of this experiment show that SO_4^{2-} has a greater inhibiting action on the germination index than Cl^-. Li (2002) and some other people argue that the germination pattern of halophyte and non-halophyte are basically the same. Although halophyte has a better accommodation to saline environment, the germination will also be inhibited.

The foremost organs that encounter the stresses are the radicles. They feel the changes in the environment first, and then signal the message to the above ground parts. Meanwhile, the roots are constantly influenced under the stresses of salts. The result also proves that germination of alfalfa seeds treated in different salts is influenced by the concentration of the salts and by different ions. Alkaline salts $NaHCO_3$, Na_2CO_3 take on a comparatively prominent inhibiting action on the germination, which may attribute to CO_3^{2-}, HCO_3^- change the pH value of the environment, thus changing the vitality of enzyme. Different salts have different influences on the growth of alfalfa seedlings. The stresses of salts inhibit the growth of alfalfa seedlings. With the rise of the saline concentration, the alkaline content increases, which lead to a greater inhibiting action. In order to survive, alfalfa changes the above- and underground growth. At the low concentration stresses of $CaCl_2$, NaCl, $MgCl_2$, the ratio of the root to the trunk is greater than that in the control, which suggests the three salts within this range of concentration enhance the growth of the roots. The ratio of the root to the trunk decreases after the

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concentration surpasses certain point, which indicates that after the concentration of salts reaches a certain point, the inhibiting action on the root's increases.

Influence of salts with different concentrations on the permeation accommodation matters

Under the different salt stresses and salts with different concentrations, plants accommodate themselves to the environment through permeation. In the process, cells synthesize and accumulate solutes that are vigorous and nontoxic with regard to permeation. The solutes include inorganic ions and organic matters. The results of the experiment show that alfalfa performs permeation accommodation by accumulating proline so that it can accommodate to the high permeating environment formed by saline and alkaline matters ($P < 0.001$). This result partially agrees with the result obtained by Ahmad et al. (2014) who found that plants with Na_2CO_3 had higher proline content than the control. This result basically agrees with the result obtained by Shi (2002), who used *Leymus chinensis* and sunflower seedlings as materials and discovered the variation pattern of proline. With the rise of alkaline salt concentration, the proline content does not vary very much. This may be owing to the decrease of the ability for proline to synthesize enzyme/ the vitality decrease in the proline synthetical enzyme. The unique stress element in alkaline salts is the high level of pH value. For example, the major stress element in Na_2CO_3 is not permeation stress and ionic toxicity but the high level of pH value. One of the plants accommodations to environment with high pH value is to accumulate saline metabolites (organic acid, critic acid, and proline) which as acts as buffer. These metabolites will adjust the pH value. The process is, however, energy consuming. Although it can decrease the pH value in the cells and inhibits the growth of the plants. Therefore, under alkaline stress, proline content is lower than that under neutral salt stress. This study shows that with the rise of saline concentration, proline content increases, but there is no significant difference among different salts.

Conclusion

The result of this experiment shows that during germination of alfalfa seeds, with the rise of concentration, germination rate, germination vigor, germination index and vitality index showed a declining tendency. The tolerance order of alfalfa seeds to the six salts used in this experiment was recorded as: $MgCl_2 > CaCl_2 > NaCl > KNO_3 > Na_2SO_4 > NaHCO_3 > Na_2CO_3$. The experiment also showed that stress extent of ions on the alfalfa seeds was recorded as: $Na^+ > Ca^{2+} > Mg^{2+}$ $CO_3^{2-} > HCO_3^- > SO_4^{2-} > Cl^-$. The rice in concentration of salts also inhibited the growth and alkaline salt has a greater inhibiting action than the neutral salt.

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