

Effect of Saline-alkali Stress on Seed Germination of the Halophyte *Halostachys caspica*

Rui Zhang

Xinjiang University

Huizhen zhang

Xinjiang University

lai wang

Xinjiang University

Youling Zeng (✉ zeng_ylxju@126.com)

Xinjiang University

Article

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Abstract

The process of soil salinization and the preponderance of saline water sources all over the world represent one of the most harmful abiotic stresses to plant growth. In nature, soil salt and alkali coexist, and the interaction of salt and alkali is an important feature of saline-alkali stress. *Halostachys caspica* is a salt-tolerant halophyte belonging to Amaranthaceae and widely distributed in arid and saline-alkali areas in Xinjiang, Northwest China. This paper studied the response of the germination of the desert halophyte *Halostachys caspica* to different types of salt stress, aiming to provide a theoretical basis for development and utilization of halophytes growing in saline-alkali soils. The results showed that the seed germination of *Halostachys caspica* was not significantly inhibited when the salt concentration was low (≤ 200 mmol/L), but with the increase of salt concentration, the germination rate of seeds dropped sharply, and the recovery germination rate at high concentration was higher than that at low concentration, when salt was removed. Step-by-step regression analysis showed that at the same concentration, the alkaline salt inhibited seed germination more severely than the neutral salts; the inhibitory effect of the mixed salt and mixed alkali on seed germination is heavier than that of the mono-salt and mono-alkali; under mixed salt alkali stress, the higher the alkaline salt content, the higher the degree of inhibition on seed germination; indicating that alkaline salt play a major role in mixed saline-alkali stress.

Introduction

Land salinization has become a worldwide ecological problem, and it has been found that its degree and area are increasing year by year, restricting the global agricultural development^{1–3}. It is estimated that approximately one-third of arable land throughout the world is affected by natural and secondary salinity to varying degrees^{4,5}. Xinjiang has the saline-alkali soil area of 2181.4×10^4 hm², accounting for 22.01% in China, is the area with the widest distribution of soil salinization, the most types of salinization, and the most severe accumulation of soil salt in China^{6,7}. Therefore, soil salinization is a widespread abiotic stress source and has become a major constraint and obstacle to global agricultural crop production and sustainable development⁸.

Saline alkali stress is the second largest abiotic stress factor that hinders the normal growth and development of crops after drought stress, causing great damage to agricultural production and land productivity⁹. Most of the salinized land in Xinjiang is mainly composed of NaCl, Na₂SO₄, Na₂CO₃ and NaHCO₃ compound salts. Salt stress is mainly caused by neutral salts such as NaCl and Na₂SO₄, pH value is between 7 and 8;alkali stress is mainly caused by alkaline salts such as NaHCO₃ and Na₂CO₃, High pH (more than 8.5) and mixed stress is caused by both neutral and alkaline salts in soils^{10,11}. Salt alkali stress will damage plant cell structure, the stability of cell osmotic pressure in vivo and plant nutrition mechanism^{12,13} and saline-alkali soils are also characterized by fragile ecological environment, harsh soil conditions, rare plant species and low biodiversity¹¹.

Seed germination stage is the most sensitive stage to determine plant seed establishment and survival^{14–16}, salt has a greater impact on plant seed germination and seedling growth, reducing plant growth at all stages of development¹⁷, even lead to the decline of plant yield. The ability to resist salinity at the seed germination stage can measure the ability of the plant to resist salinity. Salt tolerance is the result of complex adaptation of salt penetration and ion effect during seed germination. High germination percentage in hypersaline environments together with the ability to recover germination after the alleviation of salinity are indicators of salt tolerance¹⁸. Under the condition of high salinity (200 mmol/L NaCl), the seed germination of an annual halophyte *Cakile maritima*, which is common along the Mediterranean coast, is severely inhibited and delayed, and the seedling length is also limited¹⁹. At the same neutral salt concentration, the tolerance of *Haloxylon ammodendron* seeds to neutral salt Na₂SO₄ is greater than that to NaCl²⁰. However, a large number of studies have shown that alkaline salt stress is more destructive to plants than neutral salt stress^{21–24}. For example, after pepper seeds were treated with neutral salt and alkaline salt of the same concentration, the inhibition of alkaline salt on pepper seed germination rate was stronger²⁵. The study found that, plants are not only exposed to the stress of a single neutral or alkaline salt, but a complex environment of saline-alkali interaction. The harm of saline-alkali complex stress to plants is greater than that of single salt stress and alkali stress, but the harm of alkali stress is greater than that of salt stress^{22,26}. Studies have reported that different concentrations of salt stress have different effects on the growth and physiological characteristics of halophytes, suitable saline alkali stress is conducive to seed germination²⁷.

Halostachys caspica is a perennial halophytic shrub belonging to Amaranthaceae. It has highly succulent assimilating branches, which can reduce soil salt content and increase soil organic matter. This plant has a strong adaptability to the salt soils, is one of the main halophytes in the arid desert area of Xinjiang, and has the characteristics of drought resistance, saline alkali resistance, wind erosion resistance and sand burial resistance. It is a key species to prevent land desertification, and is also an important medicinal plant and good forage plant²⁸. At present, researches related to *Halostachys caspica* mainly focus on seed germination²⁹, analysis of medicinal components^{30–32}, feeding value³³, and study on stress response genes and salt tolerance mechanism^{34–36}. This experiment explored the effect of different types of saline-alkali stress on the seed germination of *Halostachys caspica*. The purpose of this study is to explore the tolerance and adaptability to saline-alkali stress during the seed germination period of *Halostachys caspica*, and to provide theoretical basis for the cultivation and management of *Halostachys caspica* in different types of saline-alkali land, and lay a foundation for improving the saline-alkali property of the land and further research on *Halostachys caspica* in the future.

Materials and methods

1 Materials

The *Halostachys caspica* seeds used in the experiment are preserved by our laboratory (were collected from plants grown wildly in the extremely saline-alkali and semi-desert regions located at the edge of

Gurbantunggut Desert in Xinjiang, Northwest of China), Select *Halostachys caspica* seeds with full and uniform grain size. The experimental research on the germination of *Halostachys caspica* and the collection of wild seeds of *Halostachys caspica* comply with relevant institutional, national, and international standards and legislation.

2 Reagent solution

NaCl, NaHCO₃, mixed neutral salt solution (NaCl and Na₂SO₄), mixed alkali salt solution (NaHCO₃ and Na₂CO₃), saline solution mixed (NaCl and NaHCO₃, at a 1:1, 1:3 and 3:1 molar ratio), distilled water, sterile water, sodium hypochlorite solution. All saline solution treatments were first configured as 1 mol/L stock solutions and diluted to different concentrations (50, 100, 200, 300, 400, 500, 600 mmol/L) for seed germination.

3 Test methods

3.1 Seed surface disinfection

The healthy and uniform seeds were selected for germination experiments, sodium hypochlorite and sterile water were used as mixed sterilization for 30 min, rinsed with distilled water 3 times. a clean filter paper was used to dry surface humidity of seeds.

3.2 Saline solution design

Salt stress was treated with single salt NaCl pH was adjusted to 5.4±0.3 and mixed neutral salts (NaCl and Na₂SO₄, at a 1:1 molar ratio pH was adjusted to 6.2±0.3); single alkali salt NaHCO₃ (pH was adjusted to 8.5±0.3) and mixed alkaline salts (NaHCO₃ and Na₂CO₃, at a 1:1 molar ratio, pH was adjusted to 9.5±0.3). Mixed saline-alkali stress was treated with NaCl and NaHCO₃, at a 1:1, 1:3 and 3:1 molar ratio pH was adjusted to 8.3±0.3.

3.3 Saline treatment

In a 90 mm petri dish containing two layers of filter paper, 5 mL of the corresponding saline solution was added and the sterilized seeds were sown on soaked filter paper. Germinated at 16 h/8 h at 25°C/15°C (light/dark), 60% relative humidity, 80% light. Seed germination takes radicle emergence as the standard (radicle emergence as the standard of seed germination). During the germination process, the seed germination rate was counted every day, and remove the germinated seeds. The saline solution was changed every 2 days, and the final germination rate was calculated. Distilled water treatment group was as a control, and each treatment was repeated 3 times, and 40 seeds each petri dish. The observation period of the experiment was set as 13 days, and the observation period of distilled water recovery was 7 days.

4. Evaluation of salt and alkaline resistance

The salt and alkaline tolerance level of seeds refers to the standard of (Wang et al., 2015)³⁷. The germination rate after NaCl stress, NaHCO₃ stress, and mixed stress treatment is used as the dependent variable (y), and the corresponding salt stress treatment concentration is used as the independent variable (x) to establish a regression equation. The salt alkali solution concentration corresponding to the germination rate of *Halostachys caspica* at 75%, 50%, and 25% is used as the moderate, threshold, and limit values for the salt alkali resistance of seeds during germination.

5. Data analysis

The indicators under stress were germination rate, recovery germination rate and total germination.

Stress germination rate= $A/C \times 100\%$

Recovery germination rate= $[B/ C-A] \times 100\%$

Total germination rate= $[A+B /C] \times 100\%$

A: Number of seeds germinated under stress

B: Number of seeds germinated after germinating seeds were transferred to distilled water

C: Total number of seeds per treatment

Relative salt damage rate= $[(CK \text{ germination rate} - \text{Germination rate of treatment group}) / CK \text{ germination rate}] \times 100\%$

The germination rate, the total germination rate and the recovery germination rate were converted into arcsines before the data analysis. All treatments were replicated three times, and the data were expressed as mean $\pm$ SE.

Data were analyzed by one-way analysis of variance (ANOVA) using the statistical software SPSS

20.0 (SPSS Inc. Chicago, IL, USA). Two-way analysis of variance (ANOVA) was performed to test the significance of single and interactive effects of salinity and pH on all variables. The treatment mean values were compared by post hoc least significant difference Tukey's HSD test. The term significant indicates differences for which $P \leq 0.05$. All figures were graphed by GraphPad Prism version 5.0 (GraphPad software, San Diego, CA, USA), and regression equations were carried out using the statistical software Excel 2019.

Results

1 Effect of neutral and alkaline salts on seed germination of *Halostachys caspica*

Table 1
The regression analysis of neutral and alkaline salt concentration with germination rate

Salt type	Regression equation	R ²	Moderate value (mmol/L)	Threshold (mmol/L)	Limit (mmol/L)
NaCl	$y = -0.0236x + 85.207$	0.5856	432.5000	1491.8220	2551.1440
NaHCO ₃	$y = -0.049x + 80.249$	0.9253	107.1224	617.3265	1127.5306
NaCl: Na ₂ SO ₄ = 1:1	$y = -0.0477x + 86.664$	0.7605	244.5283	768.6373	1292.7463
NaHCO ₃ : Na ₂ CO ₃ = 1:1	$y = -0.1052x + 89.622$	0.8989	138.9924	376.6350	614.2776

The type and concentration of salt, as well as their interaction, have a significant impact on the germination rate of *Halostachys caspica* seeds ($P < 0.05$). From Fig. 1A, it can be seen that with the increase of salt concentration, the germination rate of *Halostachys caspica* seeds significantly decreases; at the same time, under the same salt concentration stress, compared with neutral salt treatment, the seed germination rate significantly decreased under alkaline salt stress; moreover, the germination rate of *Halostachys caspica* seeds in the mixed neutral salt and mixed alkaline salt treatment groups was consistently lower than that in the single neutral salt and single alkaline salt treatment groups. Increasing the concentration of the mixed salt alkali solution had a greater stress effect on *Halostachys caspica* than increasing the concentration of the single salt alkali solution. This indicates that the seeds of *Halostachys caspica* have a certain salt tolerance, and low concentrations of neutral and alkaline salts have almost no inhibitory effect on the germination of *Halostachys caspica* seeds. The stress effect of alkaline salts on *Halostachys caspica* germination is greater than that of neutral salts.

Transferred the ungerminated *Halostachys caspica* seeds under salt alkali stress to distilled water for rehydration testing, and calculate the germination rate of the germinated seeds. As shown in Fig. 1B, with the increase of salt concentration, the recovery germination rate of seeds significantly increases. It indicates that under high concentration stress, the seed vitality is not damaged and only inhibits seed germination. When the pressure decreases, the seeds will recover quickly.

The relative salt damage rate shows a significant increasing trend as the stress increases. Under the same salt concentration stress, the relative salt damage rate is the highest under the mixed alkaline salt stress of NaHCO₃ and Na₂CO₃. At the same time, it was found that when the concentration of NaCl and NaCl mixed with Na₂SO₄ neutral salt treatment group was 100mmol/L, the relative salt damage rate was negative, indicating that this concentration had a promoting effect on the germination of *Halostachys caspica* seeds (Fig. 1C). This result is consistent with the phenotype, the seed germination rate of the neutral salt NaCl and NaCl mixed with Na₂SO₄ treatment group showed no difference compared to the control group, while the root length and aboveground parts were basically the same. The alkaline salt

Na₂CO₃ and NaHCO₃ mixed with Na₂CO₃ treatment group showed significant differences compared to the control group, and with the increase of alkaline salt concentration, the root length and aboveground part of the *Halostachys caspica* significantly decreased (Fig. 1D).

In addition, the linear regression equation shows a highly significant negative correlation between relative germination rate and salt alkali concentration (Fig. S1). According to the slope of the regression equation and the calculation of the appropriate salt concentration values, threshold and limit values, as well as salt tolerance levels corresponding to germination rates of 75%, 50%, and 25%, it is indicated that the tolerance order of *Halostachys caspica* to the four treatments is NaCl > NaCl: Na₂SO₄ = 1:1 > NaHCO₃ > NaHCO₃: Na₂CO₃ = 1:1 (Table 1 and S3).

2 Effect of saline-alkali mixed stress on seed germination of *Halostachys caspica*

Table 2
The regression analysis of saline-alkali mixed concentration with germination rate

Salt type	Regression equation	R ²	Moderate value (mmol/L)	Threshold (mmol/L)	Limit (mmol/L)
NaCl:NaHCO ₃ = 1:1	y=-0.1351x + 86.418	0.9112	84.5150	269.5633	639.6595
NaCl:NaHCO ₃ = 1:3	y=-0.1529x + 91.720	0.9451	109.3525	272.8581	599.8692
NaCl:NaHCO ₃ = 3:1	y=-0.0736x + 86.757	0.9152	159.7418	499.4158	1178.7636

Under the stress of saline-alkali mixture NaCl: NaHCO₃ at a 1:1 molar ratio, the germination rate of seeds treated with 50mmol/L was the fastest, and there was no significant difference compared to the control (CK) treatment (p > 0.05). The germination rate of seeds significantly decreased when the salt concentration was greater than 50mmol/L (Fig. 2A). When the alkaline salt content in the mixed salt alkali increased, it was found that its inhibitory effect on the germination of *Halostachys caspica* seeds was strengthened; When the neutral salt content in the mixed salt alkali increases, its inhibitory effect on seed germination decreases.

The germination recovery experiment showed that ungerminated seeds began to germinate within 2 days after being transferred to distilled water, and the peak period of germination recovery was from the 3rd to the 5th day. At the same time, under high concentration mixed salt alkali stress, the germination recovery rate of *Halostachys caspica* seeds is relatively high. With the increase of alkaline salt components in the mixed salt alkali, the germination recovery rate of seeds significantly decreases. (Fig. 2B). This indicates that salt alkali stress does not cause complete loss of vitality to plant seeds, and some seeds that have not germinated in the salt alkali solution can still resume germination after being transferred to distilled water.

The results of relative salt damage rate showed that the mixed treatment of neutral salt NaCl and alkaline salt NaHCO₃ had a significant degree of damage to the seeds of *Halostachys caspica*. When the mixed

salt alkali concentration is 500mmol/L, the relative damage rate to the seeds of *Halostachys caspica* is as high as 90%; When the neutral salt composition in the mixed salt alkali solution increases, the relative salt damage rate decreases (Fig. 2C, Table S4). The tolerance of *Halostachys caspica* to three different treatments is as follows: NaCl: NaHCO₃ = 3:1 > NaCl: NaHCO₃ = 1:1 > NaCl: NaHCO₃ = 1:3 (Table 2, Fig. S2). The phenotypic results of *Halostachys caspica* treated with mixed salt alkali were also consistent with this. Compared with the control, the NaCl: NaHCO₃ = 1:3 treatment group had a more significant inhibitory effect on the seeds of *Halostachys caspica*, while the NaCl: NaHCO₃ = 3:1 treatment group had no significant difference in root length and aboveground parts compared to the control, and was stronger than the NaCl: NaHCO₃ = 1:1 treatment group (Fig. 2D). This indicates that the inhibitory effect of mixed salt alkali treatment on the seeds of *Halostachys caspica* is mainly exerted by alkaline salts, that is, the larger the proportion of alkaline salts, the greater the inhibitory effect on seed germination.

Table 3
Two-way ANOVA of effects of salinity pH and their interactions on seed germination of *Halostachys caspica*

Variance source	Germination Rate
Salt Concentration	112.135**
PH	76.713**
Salt Concentration × PH	13.122*

*, 0.05;** , 0.01

From Table 3, it can be seen that the germination rate of *Halostachys caspica* seeds is significantly influenced by salinity (F = 12.135, P < 0.01), pH (F = 76.713, P < 0.01), and their interaction (F = 13.122, P < 0.05). From the results of the Two-way ANOVAs analysis of variance, it can also be seen that salt concentration is the decisive main factor, and pH value has a secondary impact on the germination of *Halostachys caspica* seeds.

Discussion

1 *Halostachys capsica* has strong salt and alkali resistance

In recent years, the mechanism of how salinity inhibits seed germination of halophytes in high salt environment has attracted much attention. Many halophytes show different responses to salinity during germination. *Halostachys caspica* is a small shrub, which can grow in saline and alkaline desert areas. It is important to study the inhibition of different types and concentrations of salt-alkali on the germination of halophyte seeds. The harm of salt alkali stress to plants is the result of a series of comprehensive effects, mainly affecting plant growth and development by reducing water absorption capacity, affecting ion balance, hypertonic stress, and damaging the photosynthetic system^{14,38}. In this experiment, although the germination rate of *Halostachys caspica* seeds gradually decreased and the relative salt damage rate increased with the increase of neutral and alkaline salt concentration (Fig. 1), it still had a certain

germination rate at a high concentration (600 mmol/L) of salt and alkali, indicating that *Halostachys caspica* had a strong salt and alkali tolerance.

2 Salt concentration and pH value are the main factors affecting the germination of *Halostachys caspica*.

The speed of seed germination depends on the speed of water absorption. Salinity slows down the speed of water absorption, making it difficult to repair the cell membrane during the absorption process, and aggravating the damage of the membrane structure, leading to the exudation of seed solute³⁹. With the increase of salinity, the degree of damage to the seed is more and more obvious, and ultimately affects the seed germination. The same conclusion was reached in this experiment, the germination rate of *Halostachys caspica* seeds was closely related to the salt concentration. In this experiment, when the salt free solution was used, the seeds had the largest germination rate. When the salt concentration was ≤ 200 mmol/L, the inhibition of seed germination was not significant, but when the salt concentration was greater than 200 mmol/L, the inhibition of seed germination was significant(Fig. 1A,2A); The results showed that the higher the salt concentration was, the greater the inhibition effect on the germination of *Halostachys caspica* seeds was. Therefore, salt is the most important factor affecting seed germination.

Many experimental data show that high pH value is the key to limit the growth and development of plants under alkaline conditions^{10,40}. The effect of high pH value on plant growth is different at different development stages. Soil alkaline pH stress can alter apoplastic pH and affect the intracellular pH environment^{41–43}, thereby regulating multiple metabolic and physiological processes, including photosynthesis⁴⁴, ionic homeostasis^{45,46}, membrane transport^{47,48} and reactive oxygen species (ROS) balance^{49,50}. Alkaline salts contain HCO_3^- and CO_3^{2-} , which increase the pH of water. Low concentrations of alkaline salts may not affect seed water absorption, but as the concentration of alkaline salts increases, the seed germination rate sharply decreases, indicating that high pH is one of the limiting factors for seed germination^{51–53}. In this study, under the same salt concentration, the germination rate of *Halostachys caspica* seeds significantly decreased with the increase of salt pH; and the influence of high pH increases with the increase of salinity. Therefore, in summary, salt concentration and pH value are the main factors affecting the germination of *Halostachys caspica* seeds.

3 The inhibitory effect of alkaline salt stress on *Halostachys caspica* is stronger than that of neutral salt stress

Salt stress and alkali stress have different degrees of damage to plants, and the tolerance to salt and alkali varies among different plants. The tolerance of the same plant under different stresses is also different. At present, a large number of studies have shown that alkaline salt stress has a greater inhibitory effect on plant growth than neutral salt stress⁵⁴. The reason may be that (1) alkali salt stress forces the pH of the root environment to increase, causing damage to normal root growth. At the same time, it can also cause metal elements to combine with OH^- to form precipitates, affecting the effective absorption of plant nutrients by plant roots, causing a serious lack of nutrients in the plant body, and also seriously reducing the respiratory function of the root system; (2) When plants are in a high pH saline

alkali environment, their roots may secrete some organic acids, which indirectly affect the normal growth of plants.

However, studies have also shown that low concentrations of neutral salt solutions can promote the germination of some plant seeds, such as *Astragalus membranaceus* (Fisch.) Bunge and *Solanum nigrum* L. In this experiment, it was also found that the low concentration (100mmol/L) neutral salt NaCl and mixed neutral salt NaCl and Na₂SO₄ treatment group could improve the germination rate of *Halostachys caspica* compared to the control (CK), and the relative salt damage rate of *Halostachys caspica* seeds was negative, indicating that this concentration can promote the germination of *Halostachys caspica* seeds (Fig. 1A,1B). The reason for this phenomenon may be that plant seeds absorb a small amount of inorganic ions in low concentration salt environments. With the increase of inorganic ions, plant seeds produce two ways of action to promote germination. One is to reduce the water potential inside the cells, promote the seeds to actively absorb water, and meet the water demand for normal seed germination; The second is that seeds promote the full hydrolysis of substances stored inside the seeds, which can also serve as osmotic substances or promote the synthesis of osmotic regulating substances. At the same time, it will activate some enzyme activities required for seed germination, thereby increasing respiratory intensity and ultimately promoting effective seed germination.

Therefore, in summary, alkaline salt stress has a stronger inhibitory effect on salt spike wood than neutral salt stress.

4 The inhibitory effect of mixed salt-alkali treatment on *Halostachys caspica* is stronger than that of single salt-alkali treatment.

Studies show that mixed salt alkali stress is different from salt or alkali stress alone, and more complex than salt or alkali stress alone^{55,56}. For the salt alkali mixed stress, the mutual enhancement of salt stress and alkali stress is its significant feature. In nature, high concentration of salt and high pH often occur at the same time, and their synergistic effect may have a greater impact on plant growth and development than any single stress. Mixed saline-alkali stress will lead to more serious nutrient ion imbalance, reduce osmoregulation ability, inhibit antioxidant system, and more serious plant growth inhibition⁵⁷⁻⁶⁰. In this experiment, it was found that the mixed saline alkali treatment group had greater inhibition on the germination rate of *Halostachys caspica* seeds than the single saline alkali treatment group (Fig. 1). It's widely common that saline-alkaline stress has negative impacts on plant growth and development mainly via ionic osmosis and high pH stresses, thus it is more harmful than salinity stress alone^{61,62}.

Conclusions

In terms of salt and alkali stress, the research on the response mechanism of plants to salt stress is the most, while the research on mixed salt and alkali stress is relatively few. In addition, many studies generally call salt and alkali stress salt stress, which is lack of science. Salinization and alkalization often occur at the same time, and there is interaction between them. Therefore, it is of great practical

significance to study the effects of mixed saline alkali stress on improving crop yield and environmental construction.

This experiment preliminarily analyzed the effects of different salt combinations on the seed germination of *Halostachys caspica* from the perspective of seed germination level, which laid the foundation for further elucidating the complex germination mechanism of *Halostachys caspica*. Further research should be carried out from the following aspects: (1) The effects of salt and alkali stress on the physiological metabolism of the seed germination of halophyte *Halostachys caspica* need to be further studied; (2) Although salt alkali stress will reduce plant yield, it will also improve the quality of some plant fruits, such as wheat⁶³, tomato⁶⁴, oats⁶⁵, etc. Therefore, it is a new breakthrough for future research to determine the salt concentration balance threshold that can make plant yield and quality reach the optimal level. (3) At present, most of the studies focus on the seed germination process and the seedling stage of plants, and there are few systematic and comprehensive studies on the whole life process of plants. It may be a new discovery to explore and compare the dynamic changes of various indicators and specific signal transduction processes from different parts of different periods of plant growth. (4) Salt and alkali resistance of plants is a complex process jointly regulated by multiple genes. From the perspective of differences in salt and alkali resistance between salt tolerant plants and salt sensitive plants, high-throughput difference data analysis was carried out to find out the root causes of the differences, and use cDNA chips, two-dimensional electrophoresis technology, agrobacterium mediated method to identify plant salt and alkali resistant genes biologically, achieve the transfer of salt resistance gene, and It is of great significance to comprehensively and systematically explore and explain the mechanism of plant salt tolerance from different disciplines for crop improvement, and it will also be a hot topic for future research.

Declarations

Data availability statement The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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Author contributions

R.Z. and Y.Z. designed the experiments and analyzed the data. R.Z. and Y.Z. wrote the manuscript text. R.Z. performed the experiments. All authors reviewed the manuscript.

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Figures

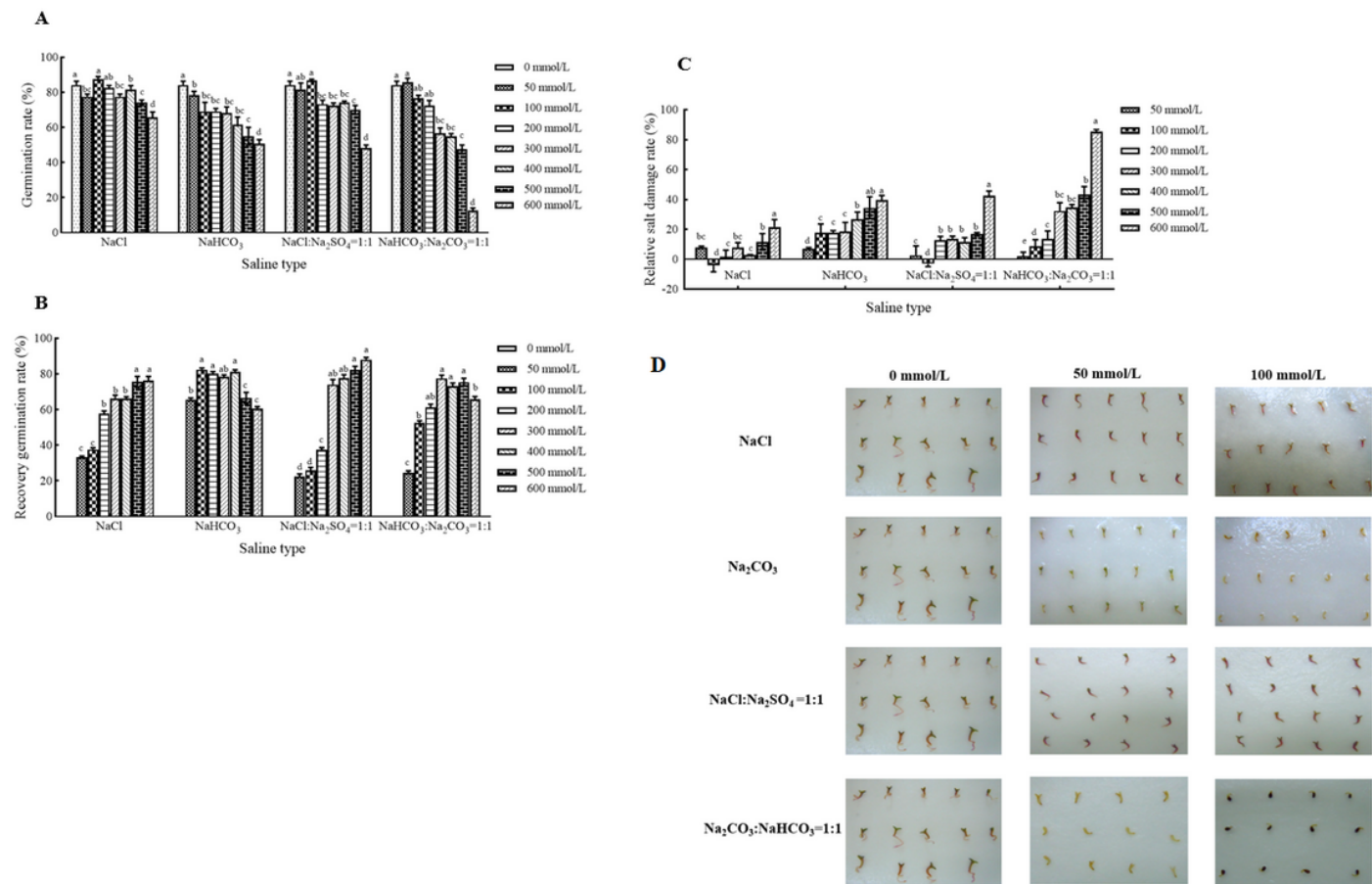


Figure 1

Seed germination of *Halostachys caspica* under neutral and alkaline salt stress. (A) Seed germination rate of *Halostachys caspica* under different stress. (B) The recovery germination rate of *Halostachys caspica* under different stress. (C) The relative salt damage rate of *Halostachys caspica* under different stress. (D) Phenotypes of *Halostachys caspica* seed germination under single saline and alkali stress. The same letters in the figure represent there is not significant ($p>0.05$), and the different letters represent there is significant ($p<0.05$).

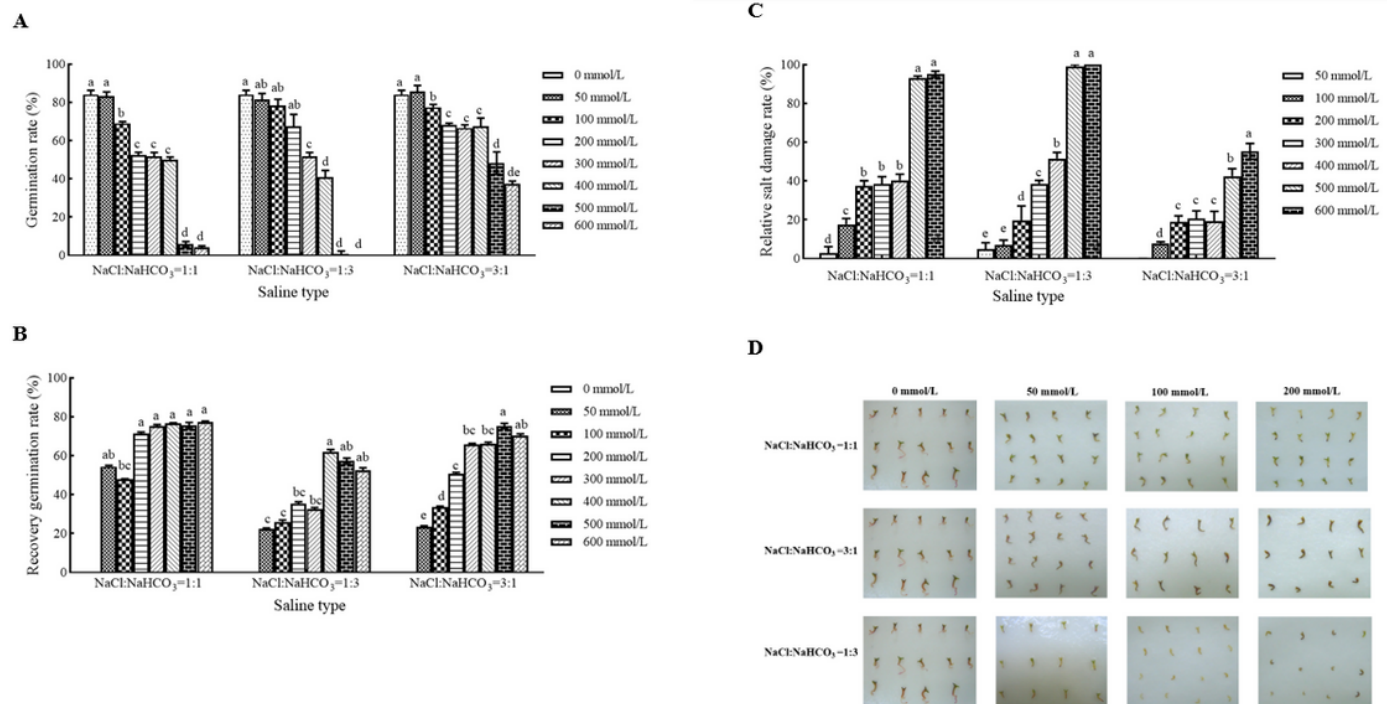


Figure 2

Seed germination of *Halostachys caspica* under saline-alkali mixed stress. (A) Seed germination rate of *Halostachys caspica* under saline-alkali mixed stress. (B) The recovery germination rate of *Halostachys caspica* under saline-alkali mixed stress. (C) The relative salt damage rate of *Halostachys caspica* under saline-alkali mixed stress. (D) Phenotypes of *Halostachys caspica* seed germination under saline-alkali mixed stress. The same letters in the figure represent there is not significant ($p>0.05$), and the different letters represent there is significant ($p<0.05$).

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