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Effects of Salt Stress on Seed Germination and Embryo Growth of *Oxytropis coerulea*

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ABSTRACT

Background: *Oxytropis coerulea* is considered to be an exceptional forage. The salinization of soil is a prevalent ecological issue worldwide. In order to study the effects of saline solution on seed germination and embryo growth of *O. coerulea*.

Methods: An experiment was carried out using the culture dish filter paper method. Three salt stresses, namely, Na_2CO_3, NaCl and double salt were set, each of which was set to 0%, 0.3%, 0.6% and 0.9% concentration.

Result: The results indicated that as the concentration of salt increased, there was a decrease in the germination rate, germination index and embryo growth. The activities of SOD, POD and CAT, as well as soluble protein content, initially increased and then decreased with increasing salt stress. The toxic effects of the three salts ranged from strong to weak as Na_2CO_3 > double salt > NaCl. The salt resistance indexes of 0.3% Na_2CO_3, NaCl and double salt were 53.87, 57.00 and 38.73, respectively and the salt resistance index of 0.6% NaCl was 50.21, indicating a high salt-tolerant degree. It showed certain resistance to 0.3% Na_2CO_3, 0.6% NaCl and 0.3% double salt. In short, the various soluble mechanisms of *O. coerulea* in different stages of salt stress and different salt stress levels have different reaction mechanisms, which reflect the salt stress of *O. coerulea* seeds. *O. coerulea* seeds can be planted in a suitable concentration of saline-alkali land, which can utilize the feed value and medicinal value of *O. coerulea* to improve economic benefits and utilize saline soil to improve the ecological environment.

Key words: Antioxidant enzyme activity, Embryo growth, Germination rate, *Oxytropis coerulea*, Salt tolerance.

INTRODUCTION

Salinization of soil is a worldwide ecological problem. Soil salinity affects approximately 800 million hectares of arable land worldwide (Yuan *et al*., 2016). The total saline land is about 1125 million hectares in the world (Hossain, 2019). China is one of the world's largest saline-alkali land. The saline-alkali lands are about $9.913.7\times10^7$ hm^2 (Zhang and Wang, 2021), of which the saline-alkali cultivated land is about 6.6×10^6 hm^2 and there is also a 2.0×10^7 hm^2 saline-alkali wasteland waiting to be utilized (Liu and Wang, 2021). Salinity is the most important abiotic stress factor that negatively affects agricultural production and quality (Majeed and Muhammad, 2019). Some crop varieties are difficult to exert their yield potential due to salt damage of salinized soil. Therefore, it is a long-term economic benefit to study the salt tolerance and salt tolerance mechanism of plants and the selection of plant varieties resistant to salt.

Oxytropis *coerulea* is a perennial herb of the family Leguminosae. It is cold-tolerant and drought-tolerant with thick roots, abundant leaf content, high nutritional value and strong regenerative capacity. It can be used repeatedly during the growing season and it has good palatability as a high-quality forage (Fan *et al*., 1999). In addition, its plants also have medicinal value of qi-fixing, detoxification and dehydration. The flowers are fresh and elegant with a long flowering period and they also have high ornamental value. In recent years, wild flowers have been widely used in garden landscaping due to the unique ornamental effect, leading to an increase in research on the ecological value and appreciation of *O. coerulea* (Dong *et al*., 2016). It can be concluded that *O.coerulea* can effectively maintain the carbon-oxygen balance of the garden environment if it is distributed with large-sized trees. *O. coerulea* is a grazing

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type grass that is tolerant to grazing, trampling and has strong regeneration ability. It can be used multiple times during the growing season. *O. coerulea* has good palatability and is often selected from grass herds for grazing by cattle, sheep and horses. They are considered as fattening forage. The thick, fleshy main roots of *O. coerulea* contain abundant nutrients. Cultivating on barren gravel slopes is a promising forage for improving degraded mountain grasslands. In the rainy season, whether it is shallow sowing in ditches or driven by sheep and trampling after sowing, seedlings are easy to emerge and the germination rate of seeds is generally above 40%.

However, the salt tolerance of *O. coerulea* has not been previously reported. In comparison to other stages of plants life, plant seeds exhibit lower resistance and tolerance to environmental stress during the germination stage. The germination performance of seeds and the protective enzyme system within the plant are crucial indicators that determine the ability of plants to survive and establish themselves in a new environment (Zhou, 2001). Therefore, this experiment aimed to investigate the tolerance of *O. coerulea* to various concentrations of salt solution and provide a theoretical foundation for the future cultivation of *O. coerulea* under saline conditions.

MATERIALS AND METHODS

Seed source

This experiment utilized wild *O. coerulea* seeds that were collected from Laiyuan County of Baoding City, Hebei Province in October 2021. Following the cleaning process, the seeds were stored in a refrigerator at a temperature of 4°C. Thousand seed weight was measured to be 2.3 g and the germination rate was determined to be 93.5%.

Test design and method

An experiment was carried out using the culture dish filter paper method. The seeds of *O. coerulea* were soaked in hot water at 98°C for 30 minutes to remove the hard seeds. The 2% sodium hypochlorite solution was sterilized for 10 minutes, then washed with sterile deionized water. 50 pieces of *O. coerulea* seeds with the same size were placed in a petri dish with double-layered sterile filter paper and 5 ml of different salt solutions were added respectively. Na_2CO_3, NaCl and double salt (main components are 50% NaCl, 30% Na_2SO_4, 10% $NaHCO_3$, 10% Na_2CO_3) were used as three salt stresses, each set at 0%, 0.3%, 0.6% and 0.9% concentration. This resulted in a total of 10 treatments, with 4 replicates per treatment. The seeds of *O. coerulea* were placed in a 25±5°C incubator, with a light intensity set to 6000 lux. The day and night periods were each 12 hours long. The number of seeds germinated for each treatment was observed and recorded every day and the water in the petri dish was supplemented for 3 consecutive days until the seeds no longer continued to germinate. On the 7^{th} and 13^{th} day after seed germination, the antioxidant enzyme activity, MDA content, soluble protein content and cell membrane permeability were measured. After seed germination, several indicators were measured, including the length of the germ, hypocotyl and radicle, as well as 100 grains of fresh weight and relative chlorophyll content.

Indicators and methods

Germination per cent =

$$\frac{\text{Number of germinated seeds}}{\text{Number of seeds kept for germination}} \times 100\%$$

(Riis *et al*. 1995)

Germination emergence =

$$\frac{\text{Number of seeds to be tested within 12 days of germination}}{\text{Normal number of seeds sprouted}} \times 100\%$$

(Murillo *et al*. 2002)

$$Gi = \Sigma(Gt/Dt)$$

Gt: Number of germinated seeds per day.

Dt: Corresponding sprouting days) (Carpıcı *et al*., 2009).

$$Vi = S \bullet Gi$$

S: Average fresh weight of 100 seeds.

Gi: Germination index) (Zhang *et al*. 2015).

$$GV = \Sigma(Gt \bullet Dt)/\Sigma Gt$$

Gt: Number of seeds germinated.

Dt: Corresponding germination days) (Kaya and Day, 2008).

SPAD: SPAD-502 Plus Portable chlorophyll meter.

Antioxidant activity

Determination of superoxide dismutase activity by nitroblue tetrazolium (NBT) method; determination of peroxidase activity by guaiacol method; reaction solution for determination of catalase activity including 25 mmol/L phosphoric acid Buffer (pH = 7.0), 10 mmol/L H2O2 and enzyme extract were determined by UV spectrophotometry at 240 nm (Patra *et al*., 1978).

Malondialdehyde content

Thiobarbituric acid method (Soleimanzadeh *et al*., 2010).

Soluble protein content

Coomassie brilliant blue solution (Doganlar *et al*., 2010).

Cell Membrane Permeability

Conductivity Method (Wiser and Blom, 2016).

Salt tolerance index =

$$\frac{\text{Average relative germination rate+Average radicle length+Relative germination potential}}{3}$$

(Zhang *et al*., 2010)

Salt damage index =

$$\frac{\text{Control germination rate-Germination rate of treatment group}}{\text{Control germination rate}} \times 100\%$$

Statistical analysis

The experimental data were recorded by Microsoft Excel software and plotted into a histogram. All data were subjected to a one-way analysis of variance and the mean differences were compared by the lowest significant difference (LSD). Comparisons with $p<0.05$ were considered significantly different.

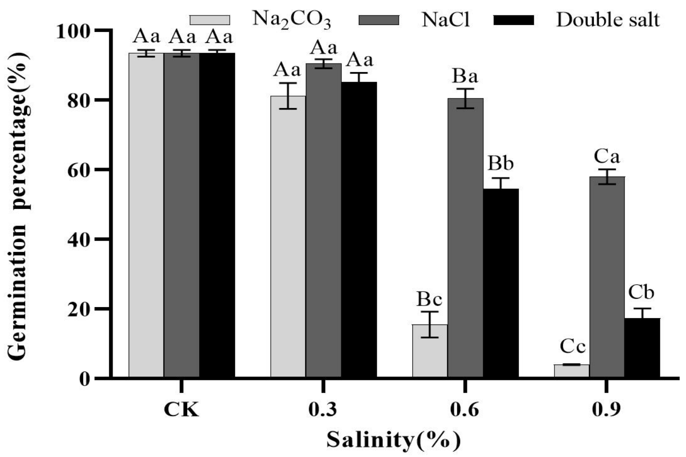
RESULTS AND DISCUSSION

Effect of salt stress on germination rate of *O. coerulea* Seeds

The germination rate of *O. coerulea* seeds decreased with the increase in salt solution concentration (Fig 1). The germination rate decreased sharply when treated with 0.6% Na_2CO_3 or 0.9% double salt. The germination rate has dropped steadily by NaCl treatment increased with concentration. The germination rate of *O. coerulea* seeds were significantly inhibited by the concentration of 0.6%, 0.9% Na_2CO_3 and double salt. When the salt concentration is the same, the order of germination rate from high to low is NaCl > double salt > Na_2CO_3.

Germination potential, germination index, germination vitality index, average germination days

The germination potential, germination index and germination vigor index of the seeds all decreased with the increase in the concentration of each salt stress (Table 1). Seed treatment with 0.6% Na_2CO_3 or 0.9% NaCl and double salts significantly declined the germination index. Under the same concentration of different salt treatment groups, the germination index seems to be highest in NaCl



Note: a-c: salt concentration differences of the same species; A-D differences in salt concentrations of the same type.

Fig 1: Effects of Na_2CO_3, NaCl and double salt on germination percentage of *Oxytropis coerulea* at four different concentrations.

Table 1: Effects of Na_2CO_3 , NaCl and double salt on germination emergence (GE), Germination index (GI), germination vitality index (GVI) and average germination days/d at four different concentrations of *Oxytropis coerulea* seeds.

Salt	Concentration /%	Germination emergence/%	Germination index	Germination vitality index	Average germination days/d
	0 (CK)	93.0±1.36A	64.3±3.52A	67.7±6.21Aa	12.9±0.2Bc
Na_2CO_3	0.3	80.0±7.37Ba	45.7±4.60Ba	6.9±0.46Bc	13.5±0.1ABa
	0.6	18.5±3.70Cc	12.0±2.90Cc	1.9±0.57Bc	13.5±0.3ABb
	0.9	4.0±0.12Dc	4.0±0.11Cc	0.4±0.00Bb	15.0±1.0Aa
NaCl	0.3	78.5±4.13Ba	39.1±3.90Ba	41.5±3.10Ba	14.0±0.3Ba
	0.6	69.0±3.12Ca	34.5±2.54Ba	32.7±1.04Ba	14.5±0.2ABa
	0.9	38.5±0.55Da	22.9±1.32Ca	13.8±0.86Ca	15.0±0.5Aa
Double salt	0.3	59.5±4.82Bb	34.8±2.67Ba	33.7±2.13Bb	13.8±0.2Aa
	0.6	48.5±4.11Cb	24.9±2.35Cb	9.3±1.52Cb	14.1±0.2Aab
	0.9	16.5±2.51Db	7.1±1.30Db	1.7±0.14Cb	14.4±0.2Aa

Note: Each value is the mean of the four replicates. For each treatment group, values not followed by the same letter within a column are significantly different at $p<0.05$. Different small letters indicate significance among different salts at the same concentration; Different capital letters indicate significance among different salt concentrations at the same salt.

treatment followed by double salt and Na_2CO_3. The germination toxicity of Na_2CO_3 treatment was significantly greater than other salt treatments. The average germination days of *O. coerulea* seeds increased with increasing levels of salinity and the difference in salt treatment of the same concentration was not significant.

Embryo length, 100 fresh weight and relative chlorophyll content

The germ, hypocotyl, radicle length, 100-gravity fresh weight and relative chlorophyll content of *O. coerulea* seeds decreased with increasing treatment concentration (Table 2). The seeds of *O. coerulea* treated with 0.6%, 0.9% Na_2CO_3 and 0.9% double salt declined in germination index, were significantly affected by salt stress and the leaves were fragile. After washing, they were broken and some indicators could not be measured. Under the same concentration of different salt treatment groups, the experimental index seems to be the highest in NaCl treatment, followed by double salt and Na_2CO_3. It indicated that the normal growth and development of seeds were seriously hindered by the high concentration of Na_2CO_3. The 0.3% NaCl treatment was not significant when compared to the control and the damage caused by NaCl was the smallest among the three salts.

Antioxidant enzyme activity

Superoxide dismutase (SOD) activity

The SOD activity of the three salt-treated *O. coerulea* seeds gradually decreased as the germination time increased (Fig 2). The SOD activity of Na_2CO_3 and double salt treatment initially increased and then decreased with increasing concentration. On the 7th day of germination, the activity of SOD was the highest when the double salt concentration of Na_2CO_3 was 0.3% and the activity became smaller as the concentration increased (Fig 2-A). Under 0.6% NaCl treatment, it can react to produce higher SOD. On the 13th day of germination, the activity of Na_2CO_3 and NaCl was the highest at the concentration of 0.3% and the activity of Na_2CO_3 and double salt was the highest at the concentration of 0.6% (Fig 2-B). The SOD treatment at 0.9% Na_2CO_3 was less than the control, which indicated that the range of protective enzyme tolerance of *O. coerulea* seeds may have been exceeded.

Peroxidase (POD) activity

The POD activity of *O. coerulea* seeds treated with double salt and Na_2CO_3 generally decreased with the prolongation of germination time (Fig 3). On the 7th day of germination, the activity of POD in Na_2CO_3 and double salt treatment first increased and then decreased with the increase of

Table 2: Effects of Na_2CO_3, NaCl and Double salt on shoot length(cm), hypocotyl length (cm), radicle length (cm), hundred kernel fresh weight (g) and relative chlorophyll content/SPAD at four different concentrations of Oxytropis coerulea Seeds.

Salt	Concentration /%	Shoot length/cm	Hypocotyl length/cm	Radicle length/cm	Hundred kernel fresh weight/g	Relative chlorophyll content/SPAD
	CK	0.7±0.03Aa	1.2±0.06Aa	3.0±0.12Aa	1.1±0.06Aa	35.3±1.14Aa
Na_2CO_3	0.3	0.5±0.04Bb	0.7±0.11Ba	0.4±0.06Bb	0.3±0.10Bb	26.8±2.38Bb
	0.6	-	-	-	0.2±0.02Bc	-
	0.9	-	-	-	0.1±0.01Bc	-
NaCl	0.3	0.7±0.04Aa	1.0±0.06Aa	2.0±0.29Ba	0.9±0.09Aa	33.0±1.02Aa
	0.6	0.7±0.05Aa	0.9±0.09Aa	1.1±0.05BCa	0.9±0.06Aa	26.1±1.60Ba
	0.9	0.6±0.08Aa	0.7±0.09Ba	0.6±0.15Ca	0.6±0.05Ba	25.3±3.61Ba
Double salt	0.3	0.7±0.06Aa	1.0±0.12Aa	1.5±0.14Ba	0.8±0.16Aa	31.0±1.38Bab
	0.6	0.5±0.05Bb	0.6±0.07Bb	0.3±0.07Cb	0.4±0.05Bb	24.3±0.71Ca
	0.9	-	-	-	0.3±0.02Bb	-

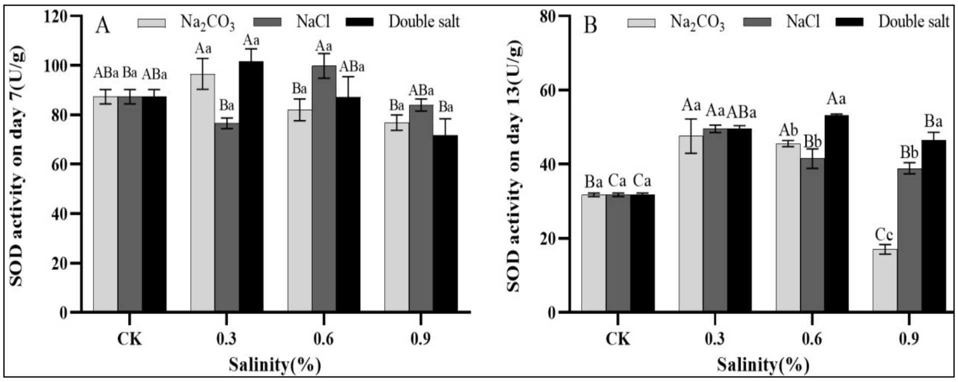


Fig 2: Effects of the 7th day (A) and 13th day (B) on SOD activity of *Oxytropis coerulea* seeds.

concentration (Fig 3-A). The concentration of NaCl was lower than that of the control. The POD concentration was highest when the concentration was 0.3% under Na_2CO_3 and double salt treatment. When the concentration was 0.9%, the POD activity of NaCl was the highest at the same concentration. On the 13th day of germination, the activity of POD in NaCl and double salt treatment first increased and then decreased with the increase of concentration (Fig 3-B). The treatment of 0.3%-0.9% Na_2CO_3 was smaller than the control value and decreased continuously with increasing concentration. At concentrations of 0.3%-0.6%, the POD activity of NaCl and double salt treatment was significantly higher than that of Na_2CO_3. At a concentration of 0.9%, the POD activity of NaCl treatment was significantly higher than other treatments and the order of POD activity was NaCl > double salt > Na_2CO_3.

Activity of catalase (CAT): The CAT activity and the SOD activity of the three salt-treated *O. coerulea* seeds were nearly identical (Fig 4). On the 7th day of germination, the CAT activity was highest under the salt stress treatments when the concentration was 0.3%. When the concentration was 0.6% and 0.9%, the CAT activity was highest under NaCl treatment. On the 13th day of germination, the CAT activity of 0.6%-0.9% Na_2CO_3 treatment was lower than the

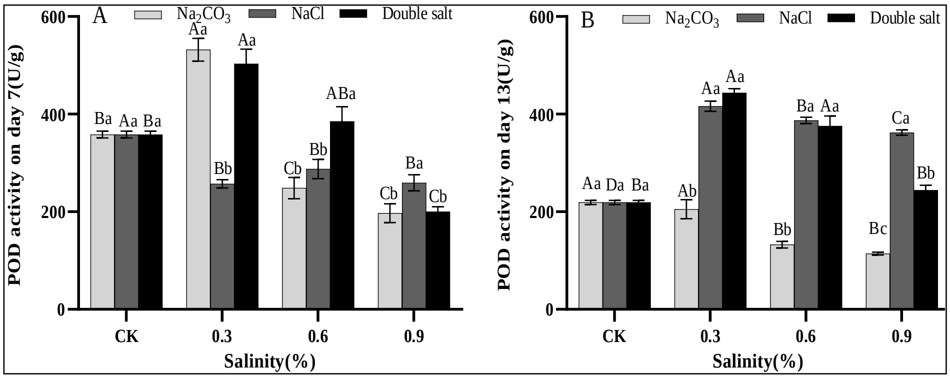


Fig 3: Effects of the 7th day(A) and 13th day (B) on POD activity of *Oxytropis coerulea* seeds.

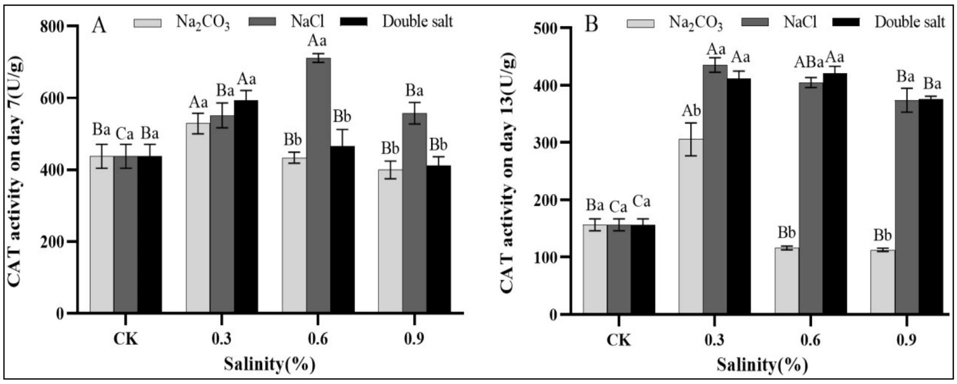


Fig 4: Effects of the 7th day(A) and 13th day (B) on CAT activity of *Oxytropis coerulea* seeds.

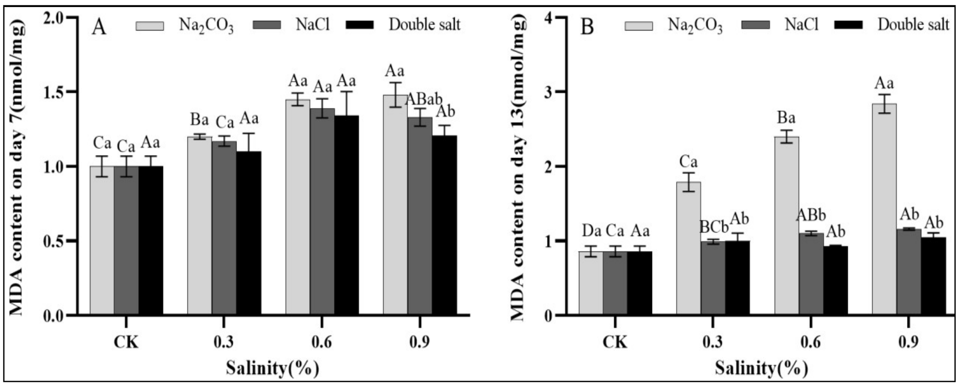


Fig 5: Effects of the 7th day (A) and 13th day (B) on MDA activity of *Oxytropis coerulea* seeds.

control value, suggesting that the tolerance range of *O. coerulea* seed protective enzymes may have been exceeded at this time. When the concentration of each salt was between 0.3% and 0.9%, the CAT activity of NaCl and double salt treatment was significantly higher than that of Na_2CO_3.

Malondialdehyde content

On the 7th day of *O. coerulea* seed germination, the malondialdehyde (MDA) content of *O. coerulea* seeds increased with the increase of $N_{a2}C_{O3}$ concentration. Additionally, it increased initially and then decreased with the increase of NaCl and double salt concentration (Fig 5-A). On the 13th day of O. *lanceolata* seed germination, the MDA content increased with the increase of $N_{a2}C_{O3}$ and NaCl concentrations. However, the double salt concentration initially decreased and then increased (Fig 5-B)., the difference between the NaCl and double salt results was not significant when compared to the control. Furthermore, the MDA content of *O. coerulea* seeds in the Na_2CO_3 treatment group increased significantly over time.

Soluble protein content

On the 7th day of seed germination, the soluble protein content increased as the salt concentration increased (Fig 6-A). When the concentration was the same, the NaCl treatment had the highest soluble protein content. On the 13th day of *O. coerulea* seed germination, the soluble protein content of *O. coerulea* seeds decreased with the increasing Na_2CO_3 concentration and increased with increasing NaCl

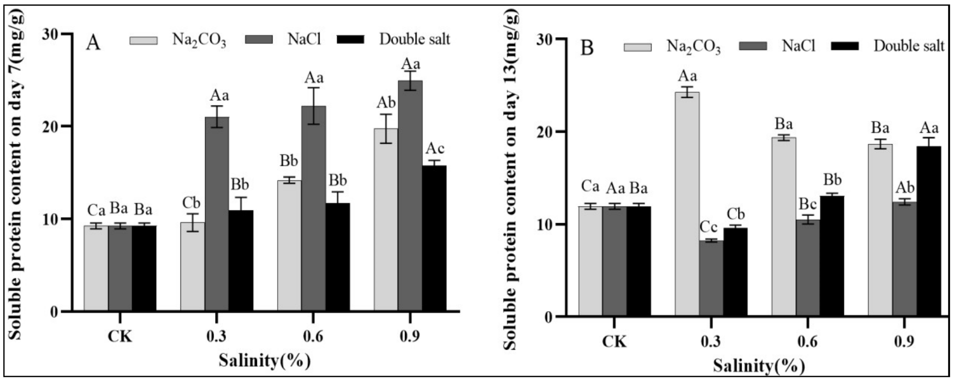


Fig 6: Effects of the 7th day(A) and 13th day (B) on soluble protein content of *Oxytropis coerulea* seeds.

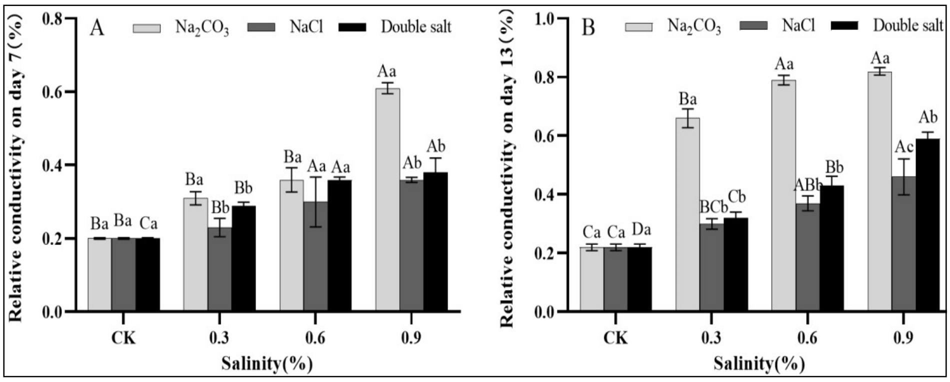


Fig 7: Effects of the 7th day(A) and 13th day (B) on conductivity of *Oxytropis coerulea* seeds.

Table 3: Effects of salt stress on *Oxytropiscoerulea* and its salt tolerance.

Salt	Concentration/ %	Salt tolerance index	Saline-alkali index/%	Saline-alkaline tolerance degree	Saline-alkaline tolerance grade
Na_2CO_3	0.3	53.87	13.00	High resistance	1
	0.6	11.33	80.25	sensitive	5
	0.9	2.67	96.00	sensitive	5
NaCl	0.3	57.00	3.25	High resistance	1
	0.6	50.21	14.00	High resistance	1
	0.9	32.37	38.00	Resistance	2
Double salt	0.3	38.73	9.00	High resistance	1
	0.6	34.43	41.45	Moderate resistance	3
	0.9	11.25	81.50	sensitive	5

and double salt concentration (0.3%-0.6%). The soluble protein content of NaCl and 0.3% double salt was lower than that of the control group (Fig 6-B). Comparing the data on the 7th day and the 13th day of *O. coerulea* seed germination in the figure, it can be observed that the protein content of *O. coerulea* seeds in 0.9% Na_2CO_3, 0.3%-0.9% NaCl and 0.3% double salt treatment groups decreased over time.

Cell membrane permeability

The electrical conductivity of *O. coerulea* seeds increased with increasing concentration and prolonged stress time. On the 7th day, the seed conductivity of 0.9% Na_2CO_3 treatment increased greatly (Fig 7-A). On the 13th day, the seed conductivity of different concentrations of Na_2CO_3 treatment increased significantly and the conductivity value of NaCl treatment was small and stable (Fig 7-B).

Salt tolerance evaluation

As can be seen from Table 3, the salt tolerance index of the same salt solution decreased as the concentration increased. Na_2CO_3 has the largest reduction between 0.3% and 0.6%, NaCl has the largest reduction between 0.6% and 0.9% and the double salt has the largest reduction between 0.6% and 0.9%. Under the salt stress of different concentrations, the salt tolerance index of NaCl was the largest. The salt tolerance index is 0.97 for 0.9% Na_2CO_3 and 57.00 for 0.3% NaCl.

The salt-alkali damage index of the same salt solution increased with increasing concentration. The highest salt tolerance index is 96.00% at 0.9% Na_2CO_3 and the smallest is 3.25% at 0.3% NaCl. The salt tolerance index of *O. coerulea* seeds was classified according to the salinity index (Table 4). The salt-tolerant grade 1 (high-resistance) included 0.3% Na_2CO_3, NaCl, double salt and 0.6% NaCl. The salt-tolerant grade 2 (resistance) included 0.9% NaCl and the salt-tolerant grade 3 (moderate-resistance) included 0.6% double salt. The remaining salt and alkali resistance grade is 5 (sensitive).

Through the salt stress of different species and different concentrations of *O. coerulea* seeds, the experimental results and application suggestions for germination index, enzyme activity, physiological index and physiological salt tolerance range were discussed.

1. The germination rate reflects the germination ability of the seed. The higher the salt concentration, the lower the germination rate of the seed. When the salt concentration is the same, the germination rate may be the highest in NaCl treatment, followed by double salt and Na_2CO_3. This is similar to the test results on *Brassica Napus* (Kandil *et al*., 2012) and Canola seed germination (Bybordi, 2010).
2. The germination potential and germination index decreased as the increase of salt concentration increased. The average germination days of *O. coerulea* seeds also increased with the increase in salt concentration. However, there was no significant difference in the salt treatment with the same concentration, suggesting that the average germination days were mainly affected. This finding is consistent with the research results to Anbarasu Mariyappillai on on *Vigna mungo* under NaCl stress (Mariyappillai and Kulanthaivel, 2024). The seed germination rate of *Vicia sativa* also decreased with the increase of salt concentration (Zhao *et al*., 2022) The embryo length of *O. coerulea* treated with 0.3% and 0.6% NaCl was greater than that of the control group. Further verification is needed to determine whether the low concentration of NaCl solution has the effect of increasing the length of the germ.
3. Under unfavorable conditions, the active oxygen species generated by plants have a detrimental impact on the cell membrane. This, in turn, triggers the activation of the protective enzyme system to eliminates surplus free radicals from the body. SOD, POD and CAT are crucial elements of antioxidant defense systems and can be used as indicators of tissue damage (Kumar *et al*., 2021).

The results indicated that the SOD, POD and CAT of *O. coerulea* seeds exhibited regular changes under different salt stresses. In the various salt treatments, the SOD activity in a low concentration NaCl solution was lower than that in the control in the short term, specifically under the 0.6% treatment. It was significantly higher than other concentrations, suggesting that a short-term treatment of 0.3% NaCl concentration, is insufficient to cause damage to plant cells. The SOD activity increased in the other treatments at a low concentration of 0.3% and then steadily decreased steadily with an increase in concentration. This indicates that the seeds initially caused damage to the cells under the stress of other low-concentration salts, triggering the defense system and increasing SOD activity to limit the damage caused by free radicals to cells. Under long-term salt stress, the SOD activity decreased. When the concentration of Na_2CO_3 was 0.9%, the SOD activity decreased significantly, indicating that the damage to cells exceeded its self-protection range, resulting in irreparable damage to the plant body (Sinky *et al*., 2024).

The changes in POD and CAT activities were almost identical to those of SOD activity. Under long-term treatment stress with a NaCl solution, the activity of POD increased significantly over time, possibly due to an increasing ability to protect itself as salt stress accumulated. During long-term stress, the CAT activity in the seed increased significantly with increasing of concentration and then decreased significantly below the control level. This indicates that in extreme environment, the seed can quickly

Table 4: Saline-alkaline stress damage grading table.

Level	Saline-alkali index	Salt tolerance
1	0.00~20.00	High resistance
2	20.10~40.00	Resistance
3	40.10~60.00	Moderate resistance
4	60.10~80.00	Moderate sensitive
5	80.10~100.00	Sensitive

react and activate its protective system, but when the stress exceeds its tolerance range, its CAT activity drops rapidly. Therefore, in the long-term evolution of plants, peroxidase in the defense system plays a role in maintaining the dynamic balance of the intracellular environment and improving the ability of seeds to resist external environmental disturbances.

Under salt stress, plant growth inhibition is related to the destruction of the cell membrane system. The change in plasma membrane permeability can be measured to determine the extent of cell membrane damage (Mansour, 2013). Malondialdehyde is a product of membrane lipid peroxidation in plant cells and can be used as an indicator of plant aging and stress (Hnilickova *et al*., 2021). As time and concentration increase, the MDA content and cell membrane permeability of *O. coerulea* seeds in treatment group significantly increase. The results indicate that Na_2CO_3 causes the greatest damage to *O. coerulea* seed and the NaCl damage was the smallest, which was the result of germination. This result is similar to the study of *Lycium barbarum* (Zhang *et al*., 2019).

Most of the soluble proteins in plants are enzymes that participate in metabolic activities and their content reflects the overall metabolic activity of plant tissues (Taffouo *et al*., 2017). In the short-term, exposure to Na_2CO_3, NaCl and double salt stress can increase the protein content and increase the intracellular osmotic potential by promoting the synthesis of new resistance protein. However, in the long-term Na_2CO_3 stress, *O. coerulea* seeds may inhibit protein synthesis. The storage of proteolysis is accelerated to enhance salt resistance and has this process operates through different mechanisms depending on the type of salt.

The test measures only the growth index of the seed during germination. The salt tolerance of the plant was fully reflected during the growth process. Therefore, it is necessary to comprehensively judge a number of biological indicators at different growth stages. In the future, several concentration gradients and various salt solutions may be set. It is also necessary to study other salt types such as potassium salts and salt damage to plants. It is also possible to investigate the recovery of germination ability after seed salt stress is relieved.

CONCLUSION

The results showed that all three salts at different concentrations inhibited seed germination of *O. coerulea*. In this study, it was further confirmed that the tolerance degree to three kinds of salt stress in the germination stage of *O. coerulea* seed was Na_2CO_3, double salt and NaCl in order from large to small. With the increase of salt concentration, the physiological indexes and growth indexes of *O. coerulea* seeds generally showed a downward trend. During the germination, they changed the antioxidant enzyme activity and protein content in different periods and different salt stresses. The reaction mechanism works together to resist salt stress.

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Conflict of interest

All authors declare that they have no conflict of interest.

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