Effect of Saline-Alkali and Drought Stress on Seed Germination of Haloxylon Ammodendron*

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Abstract. Based on the problems of desertification and salinization in the Qaidam Basin, this paper studies the coupled stress of saline-alkali and drought conditions on the seed germination of Haloxylon ammodendron from Qaidam provenance, and clarifies the comprehensive effect of salt, alkali and drought stress on Haloxylon ammodendron seed germination vigor. It provides a reference for the research on the resistance of Haloxylon ammodendron and provides a theoretical basis for improving the breeding efficiency of Haloxylon ammodendron population in the area of bad situation.

Keywords: Haloxylon Ammodendron, Seed Germination, Saline-Alkali Stress, Drought Stress

1. Introduction
Although the growth of all plants is inhibited under saline-alkali and drought stress, there are still some plants that have considerable ability to resist drought and salinization and can germinate and form colonies in a certain environment of saline-alkali and drought [1-5]. Haloxylon ammodendron is a plant of the genus Haloxylon of Chenopodiaceae, which is mainly distributed in Xinjiang, western Gansu, northwest Ningxia, northern Qinghai, Inner Mongolia and other places in China. It has strong adaptability, grows rapidly and has a strong covering ability at the same time [6, 7]. It also has certain feeding value and can be used as a fuel. It is a pioneer tree species for saline-alkali soil management, wind prevention and sand fixation [8, 9].

Through studying the response of Haloxylon ammodendron seed germination to multiple stress environments and understanding the mechanism of drought resistance and salt tolerance of Haloxylon ammodendron seed germination, this paper further supplements the data of drought resistance and salt tolerance adaptation strategies of Haloxylon ammodendron in the northwest of China.

2. Materials and Methods

2.1. Seed Source and Treatment
Haloxylon ammodendron seeds were collected in Qaidam Basin, Qinghai Province (36°04’N, 97°48’ E, altitude 2975m). After the seeds are naturally dried, the 1,000-grain weight is 5.381 ± 0.126 g and the
average particle size is 2.12 ± 0.54 mm.

Randomly select the full seeds, put 25 capsules on each dish evenly on the filter paper soaked with the treatment solution, cover them, and place them in a constant temperature incubator at 25 °C with 75% humidity. Record every 24h, take the broken chest as the germination and count the number of germinated seeds. If there is no new seed germination for 3 consecutive days, it is regarded as the end of germination. The experiment was repeated 5 times in each group. The germination rate and vigor index are calculated using the following formulas.

Germination rate: \( GP = \frac{n \times 100}{N} \)

Among them, \( n \) is the number of seed germination and \( N \) is the number of test seeds.

Vigor index: \( VI = \sum (Dt/ t) \times Lr \)

\( D_t \) is the number of germinated seeds in \( t \) days, and \( L_r \) is the radicle length.

2.2 Germination Test

Using distilled water as a control (0 mmol / L, CK), select NaCl, Na₂SO₄ and NaHCO₃, and according to the mass ratio of 2: 1: 0, 2: 1: 1, 2: 1: 2 prepare solutions whose pH (A) is respectively 7.06, 8.78, 9.04. PEG solution osmotic potential (unit MPa) (C) 0, \( \psi_1 \), \( \psi_2 \) correspond to mixed salt concentration (B) 0, 300mmol / L, 500mmol / L. There are 3 factors, and take the germination rate and vigor index the response values. Use Design-Expert 8.06 software, and use a custom response surface method to conduct a 3 factor 3 level response surface (RSM) analysis test.

2.3 Data Analysis

The comprehensive effect analysis of the interaction of pH, salinity and drought on seed germination was carried out in the software Design-Expert 8.06.

3. Results

3.1 Measurement of Solution Osmotic Potential

The measured values of the water potential of the mixed salt solution of each treatment group by the dew point water potential meter are shown in Table 1. The difference between the water potential groups of each concentration solution is significant (\( P < 0.05 \)). This shows that it is meaningful to set isotonic PEG treatment based on Table 1. The water potential of PEG-6000 solution simulated isotonic drought stress treatment group is 0, -1.3 MPa and -2.1 MPa respectively.

**Table 1.** The osmotic potential of salt solution and the corresponding concentrations of PEG – 6000 solutions

| Solution concentration / mmol/L | Treatment  | Water potential MPa | PEG-6000 concentration/ g/KgH₂O |
|---------------------------------|------------|---------------------|----------------------------------|
| 0                               | \( \psi_1 \) | 0                   | 0                                |
| 300                             | \( \psi_2 \) | 1.3±0.013c          | 341.093                          |
| 500                             | \( \psi_3 \) | 2.1±0.06e           | 440.625                          |

3.2 Results of the Response Surface Test

**Table 2.** Experimental design and result of response surface test

| Number | pH value | Osmotic-salt Mpa | Osmotic-PEG Mpa | Germination percentage | Vigor index |
|--------|----------|------------------|-----------------|------------------------|-------------|
| 1      | 7.06     | -2.1             | -2.1            | 15.9045                | 13.9416     |
As shown in Table 2, the seed germination rate (GP) and vigor index (VI) are measured, and the principal component analysis is performed to obtain regression equations about the comprehensive vigor index (GVI) against pH (A), salt penetration potential (B) and PEG penetration potential (C).

\[
\begin{align*}
GVI &= 0.503 \times GP + 0.503 \times VI \\nonumber
GVI &= -208.42 + 76.29 \times A + 5.45 \times B + 40.85 \times C + 2.08 \times AB - 1.08 \times AC + 14.88 \times BC - 5.01 \times A^2 - 3.58 \times B^2 - 0.19 \times C^2
\end{align*}
\]

Table 3. Variance analysis of the regression model

| Source | df | F value | P value  | Significance | Coefficients |
|--------|----|---------|----------|--------------|--------------|
| Model  | 9  | 177.81  | <0.0001  | **           |              |
| A      | 1  | 66.82   | <0.0001  | **           | -5.4         |
| B      | 1  | 472.98  | <0.0001  | **           | 14.84        |
| C      | 1  | 678.81  | <0.0001  | **           | 17.77        |
| AB     | 1  | 8.78    | 0.0087   | **           | 2.17         |
| AC     | 1  | 2.36    | 0.1427   | -1.12        |              |
| BC     | 1  | 433.87  | <0.0001  | **           | 16.4         |
| A^2    | 1  | 3.3     | 0.0868   | *            | -4.91        |
| B^2    | 1  | 10.57   | 0.0047   | **           | -3.95        |
| C^2    | 1  | 0.03    | 0.8643   |              | -0.21        |
| Residual | 17 | 46.0777 | 16.9172  |              |              |
| Cor Total | 26 |         |          |              |              |
note: Asterisks indicate a significant (** at 0.01 level, * at 0.05 level)

Figure 1. Interaction of pH and salt on comprehensive vigor index at drought stress
Figure 2. Interaction of pH and drought on comprehensive vigor index at salt stress
Figure 3. Interaction of osmotic in saline solution and PEG solution on comprehensive vigor index

Under saline-alkali stress, on the one hand, the germination vigor of Haloxylon ammodendron decreased with the increase of drought stress, but within a certain range of salinity (0 ~ -0.84Mpa), it increased with the increase of salinity (Figure 1). On the other hand, with the increase of pH, the seed germination vigor decreased sharply. When the pH value exceeds 8, the synergistic effect between the pH value and drought is strengthened, which is also due to the increase in stress caused by the increase in salt (Figure 2). Under drought stress, judging from the contour line, when the pH exceeds 7.55, the alkaline stress and salt stress strengthened synergistically. And with the increase of salinity, the comprehensive germination vigor of Haloxylon ammodendron seeds decreased (Figure 3).

Through regression equation calculation, the thresholds of Haloxylon ammodendron seeds' germination vigor to pH and osmotic potential under salt-alkali stress are 11.2 ~ 11.7 and -2.8 ~ -3.2Mpa respectively. Under salt and drought stress, the thresholds of Haloxylon ammodendron seeds' germination vigor to salt osmotic potential and drought osmotic potential are -3.24Mpa ~ -3.64Mpa and -2.01Mpa ~ -2.03Mpa respectively. And under the combined stress of the three, the thresholds of Haloxylon ammodendron seeds’ germination vigor to pH, salt osmotic potential and drought osmotic potential are respectively 9.3 ~ 9.6, -1.45 ~ -1.55Mpa and -2.35 ~ -2.42Mpa.

4. Conclusion
Studies have shown that the vigor index reflects the degree of seed germination uniformity and radicle growth to a certain extent, and has certain predictive significance for the seed colonization ability after seed germination [10].

In this study, low-intensity salt stress has a certain compensation effect on drought stress, indicating that Haloxylon ammodendron can quickly germinate roots under drought and low osmotic stress, which is also an adaptive germination strategy of Haloxylon ammodendron seeds from Qaidam provenance. Qaidam Haloxylon ammodendron seeds generally mature during September to October,
when the Qaidam region just entered the period of soil salinity fluctuations caused by reduced precipitation and groundwater level activities. For regions above the critical depth, that is obviously not conducive to seed germination and seedling growth of Haloxylon ammodendron, so Haloxylon ammodendron seeds that are inhibited under drought and saline stress and fail to germinate dormantly enter the seed bank. Under the conditions of low soil salinity in Qaidam area from May to June [11], precipitation begins to increase, the seeds germinate quickly and develop a strong root system, thereby increasing the probability of seedling survival and establishment after seed germination.

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