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Effects of Different Salt Types on Seed Germination and Seedling Growth of Annual *Poa annua* (Postprint)

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Abstract

Using the petri dish method, this study investigated the effects of different salt types and concentrations on the seed germination characteristics of annual bluegrass (*Poa annua*), measuring indicators including relative germination rate, relative germination potential, relative germination index, relative vigor index, relative plumule length, relative radicle length, and relative salt injury rate, and analyzing the response of annual bluegrass seed germination and seedling growth to different salt types. The results showed that: (1) The seed germination and seedling growth characteristics of annual bluegrass from the Yongchang provenance were higher than those of the other three provenances. (2) Under the interactive effects of different salt types, salt concentrations, and main factors, the seed germination and germination-stage seedling growth of annual bluegrass were significantly inhibited; alkaline salts exhibited stronger toxicity than neutral salts, and compared with the plumule of seedlings at the germination stage, the radicle was more sensitive. (3) The salt injury intensity of different salt types on the seed germination stage of annual bluegrass was in the following order: $\text{Na}_2\text{CO}_3 > \text{NaHCO}_3 > \text{Na}_2\text{SO}_4 > \text{NaCl}$. The research results can provide a scientific basis for planting annual bluegrass in the ecological restoration of different types of saline-alkali land.

Full Text

Preamble

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Effects of Different Salt Types on Seed Germination and Seedling Growth of *Poa annua*

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Abstract

The effects of different salt types and concentrations on the germination characteristics of *Poa annua* seeds were investigated using the Petri dish method. Indices including relative germination rate, relative germination potential, relative germination index, relative vigor index, relative bud length, relative radicle length, and relative salt damage rate were measured to analyze the responses of *P. annua* seed germination and seedling growth to different salt types. The results showed that: (1) *P. annua* seeds from the Yongchang provenance exhibited superior germination and seedling growth characteristics compared to the other three provenances. (2) Seed germination and seedling growth during the germination period were significantly inhibited under different salt types, concentrations, and their interactions, with alkaline salts showing stronger toxicity than neutral salts, and radicles being more sensitive than buds during the germination stage. (3) The salt toxicity of different salt types during the seed germination period of *P. annua* ranked as follows: $\text{Na}_2\text{CO}_3 > \text{NaHCO}_3 > \text{Na}_2\text{SO}_4$. These findings provide a scientific basis for planting *P. annua* in ecological restoration of different types of saline-alkali lands.

Keywords: *Poa annua*; salt type; germination characteristics; seedling growth; salt tolerance

Introduction

Soil salinization is a worldwide problem that seriously threatens ecological environments and the sustainable development of agriculture. China is a large country with saline-alkali land, and the area of salinized land is approximately $3,630.53 \times 10^4$ ha, mainly distributed in Northeast China, North China, and Northwest inland regions [citation]. It is predicted that in the coming decades, much land will be abandoned due to salinization [citation], which has drawn increasing attention to the problem of salinization. Particularly in Northwest China, the salt composition of saline soils is dominated by neutral salts (Na_2SO_4) and alkaline salts (NaHCO_3 , Na_2CO_3) [citation]. The salinized area in Northwest China has reached $2,506.33 \times 10^4$ ha, accounting

for nearly 69.03% of the national salinized area, becoming an important factor restricting the development of green industries and ecological restoration in Northwest China. Soil salinization has also become one of the challenges in urban turf establishment and maintenance, as turf planted on high-salt soils is prone to bald patches, delayed spring green-up, early autumn senescence, poor growth, and even large-scale death, increasing management difficulty and reducing turf quality [citation]. Currently, how to rationally develop and utilize saline-alkali land has become an important issue facing agricultural and pastoral development in arid regions. Studies have shown that herbaceous plants play a crucial role in the ecological restoration of saline-alkali land [citation]. Therefore, selecting appropriate herbaceous plants for biological improvement of different types of saline-alkali land is particularly important.

Plants exhibit different tolerance levels to saline-alkali environments at different growth stages, and the seed germination stage is the most sensitive period to saline-alkali stress [citation], representing a critical period that determines whether plant seeds can successfully establish in saline-alkali soils. Different salt types have different effects on plant seed germination and seedling growth, and the same plant species shows distinct responses under different salt concentrations. Yin Lili et al. [citation] conducted treatments with three salts at different concentrations on mung bean seeds and found that all three salt stresses inhibited seed germination, with the highest sensitivity to Na_2CO_3 stress. Huang Lihua et al. [citation] demonstrated that sodium salts had significantly greater inhibitory effects on seed germination and root-bud growth of *Leymus chinensis* than potassium salts, alkaline salts were more toxic than neutral salts, and divalent salts were more inhibitory than monovalent salts. Lei Chunying et al. [citation] investigated the effects of different sodium salt stresses on seed germination and seedling growth of *Kalidium foliatum*, revealing that *K. foliatum* showed significant differences in response to Na_2CO_3 salt, and at the same salt concentration, seedling roots were more sensitive to salt than seedling buds, with alkaline salts being more toxic than neutral salts. Wu Luguang et al. [citation] studied the effects of two single-salt stresses on seed germination of *Bothriochloa ischaemum*, showing that Na_2SO_4 had a low-salt promotion and high-salt inhibition effect, while Na_2CO_3 had a more obvious inhibitory effect on seed germination.

Poa annua is a cool-season turfgrass in the Poaceae family, characterized by a short life cycle, strong reproductive capacity, trampling resistance, mowing tolerance, strong stress resistance, and easy establishment, making it an excellent material for short-term ornamental turf in winter-spring seasons or for overseeding warm-season turfgrass areas [citation]. Due to its well-developed survival strategies, *P. annua* plays an important role in the productivity, species diversity, and ecological functions of saline-alkali ecosystems [citation]. Therefore, establishing *P. annua* turf in saline-alkali land can both improve the land and generate ecological benefits. Currently, research on herbaceous plant responses to salt stress has mainly focused on neutral salts [citation], while studies on the effects of alkaline salts on seed germination and seedling growth of wild *P. an-*

nua are rarely reported. In view of this, this study used Na_2SO_4 , NaHCO_3 , and Na_2CO_3 solutions to simulate different salt and alkali environments at various concentrations, comparing the effects of different salt types and sodium salt concentrations on seed germination and seedling growth of *P. annua*. The aim was to explore the impacts of different salt stresses on *P. annua* seed germination and seedling growth, providing references and guidance for planting *P. annua* in different types of saline-alkali land in Northwest China.

1. Materials and Methods

1.1 Test Materials

Seeds of four wild *P. annua* accessions were collected in September 2021 from different locations in Gansu Province, all being local species. Their geographic locations and climatic conditions are shown in Table 1 .

1.2 Experimental Design

Full, uniform *P. annua* seeds were used as experimental materials and placed in Petri dishes lined with two layers of filter paper. The experiment employed a completely randomized design with three salt types (Na_2SO_4 , NaHCO_3 , and Na_2CO_3) at multiple concentration levels. Distilled water served as the control (CK). Each treatment was replicated four times, with 5 mL of the corresponding salt solution added to each Petri dish. The dishes were incubated at 25°C under a 12 h light/12 h dark photoperiod. The weighing method was applied daily to replenish evaporated water and maintain stable salt concentrations.

1.3 Measurement Indicators

The germination criterion was defined as radicle length reaching 0.2 cm. The number of germinated seeds was recorded daily, germination potential was counted on day 7, final germination rate was calculated, and bud and radicle lengths were measured. The following indices were calculated:

Germination rate (GR) = (Number of normally germinated seeds / Total seeds tested) \times 100%

Germination potential (GP) = (Number of normally germinated seeds at final count / Total seeds tested) \times 100%

Germination index (GI) = Σ (Number of normally germinated seeds each day / Corresponding germination day)

Vigor index (VI) = GI \times Radicle length

Relative germination rate (RGR) = (GR under salt stress / GR of control) \times 100%

Relative germination potential (RGP) = (GP under salt stress / GP of control) \times 100%

Relative germination index (RGI) = (GI under salt stress / GI of control) \times 100%

Relative vigor index (RVI) = (VI under salt stress / VI of control) \times 100%

Relative bud length (RBL) = (Bud length under salt stress / Bud length of control) \times 100%

Relative radicle length (RRL) = (Radicle length under salt stress / Radicle length of control) \times 100%

Relative salt damage rate (RSDR) = (GR of control - GR under salt stress) / GR of control \times 100%

1.4 Statistical Analysis

Data were analyzed using SPSS 20.0 statistical software for one-way ANOVA and Duncan's multiple range test ($\alpha = 0.05$) to compare differences among treatments. Multi-way ANOVA was used to analyze interaction effects among factors. Origin 2021 was used for graphing. Following Chen et al. [citation], membership function values [$\mu(X_j)$], weight coefficients (W_j), and comprehensive evaluation values (D) were calculated for each accession:

Membership function value (for positive correlation with salt tolerance): $\mu(X_j) = (X_j - X_{\min}) / (X_{\max} - X_{\min})$

Membership function value (for negative correlation with salt tolerance): $\mu(X_j) = (X_{\max} - X_j) / (X_{\max} - X_{\min})$

Weight coefficient: $W_j = V_j / \Sigma V_j$

Comprehensive evaluation value: $D = \Sigma[\mu(X_j) \times W_j]$

where X_j is the measured value of index j , X_{\min} and X_{\max} are the minimum and maximum values of index j , V_j is the contribution rate of index j , and D is the comprehensive evaluation value. Higher D values indicate stronger salt tolerance.

2. Results and Analysis

2.1.1 Effects of Salt Stress on Seed Germination and Seedling Growth of *P. annua* from Different Provenances

Seed germination and seedling growth of *P. annua* were significantly affected by salt type, salt concentration, and their interactions ($P < 0.01$). Provenance also showed extremely significant effects on these parameters ($P < 0.01$). Other main factors and interactions had no significant effects ($P > 0.05$). The Yongchang provenance exhibited significantly higher relative germination rate, relative germination potential, relative germination index, and relative vigor index compared to other provenances ($P < 0.05$). All three salt types significantly inhibited these indices, with Na_2CO_3 showing the strongest inhibitory effect ($P <$

0.05). Salt concentration also had a significant inhibitory effect. At $50 \text{ mmol} \cdot \text{L}^{-1}$, seed germination rate and potential were significantly reduced by 44.73% and 44.23% compared to the control, respectively. The interaction between salt type and concentration showed that at $50 \text{ mmol} \cdot \text{L}^{-1}$, seeds were significantly inhibited by all three salts ($P < 0.05$). At $250 \text{ mmol} \cdot \text{L}^{-1}$, germination rate and potential under Na_2CO_3 stress were significantly reduced ($P < 0.05$).

2.1.2 Relative Germination Rate and Germination Potential Characteristics

The relative germination rate and relative germination potential of Yongchang seeds were significantly higher than those of other provenances ($P < 0.05$). All three salt types inhibited seed germination, with Na_2CO_3 showing the most significant inhibitory effect ($P < 0.05$). Salt concentration also had a significant inhibitory effect on these parameters. At $50 \text{ mmol} \cdot \text{L}^{-1}$, seed germination rate and potential were significantly reduced by 44.73% and 44.23% compared to the control, respectively. The interaction between salt type and concentration showed that at $50 \text{ mmol} \cdot \text{L}^{-1}$, seeds were significantly inhibited by all three salts ($P < 0.05$). At $250 \text{ mmol} \cdot \text{L}^{-1}$, germination rate and potential under Na_2CO_3 stress were significantly reduced ($P < 0.05$).

2.2 Seedling Characteristics of *P. annua* During Germination Period

Yongchang seedlings exhibited significantly higher relative bud length and relative radicle length compared to other provenances ($P < 0.05$). Different salt types had significantly different effects on *P. annua* seedlings during the germination period (Figure 3 [Figure 3: see original paper]). Na_2CO_3 and NaHCO_3 stresses completely inhibited seedling growth at $100 \text{ mmol} \cdot \text{L}^{-1}$, while Na_2SO_4 showed significantly less inhibition ($P < 0.05$). With increasing salt concentration, relative bud length and relative radicle length decreased significantly ($P < 0.05$), with reductions of 25.27%, 54.69%, 83.58%, and 96.72% at 50, 100, 200, and $250 \text{ mmol} \cdot \text{L}^{-1}$, respectively. The interaction between salt type and concentration showed that at $50 \text{ mmol} \cdot \text{L}^{-1}$, seedlings were significantly inhibited by all three salts ($P < 0.05$), with relative radicle length showing greater sensitivity than relative bud length.

2.3 Relative Salt Damage Rate Characteristics

The relative salt damage rate of Yongchang seeds was significantly lower than that of other provenances ($P < 0.05$). Na_2CO_3 stress caused the highest salt damage rate among all salt types ($P < 0.05$). As salt concentration increased, the relative salt damage rate increased significantly. At $50 \text{ mmol} \cdot \text{L}^{-1}$, seed germination was significantly inhibited, with the salt damage rate increasing by 94.3% compared to the control. At $250 \text{ mmol} \cdot \text{L}^{-1}$, the salt damage rate under Na_2CO_3 stress was significantly higher than the control ($P < 0.05$). The interaction between salt type and concentration showed that at $50 \text{ mmol} \cdot \text{L}^{-1}$,

all three salts significantly increased the salt damage rate ($P < 0.05$), with Na_2CO_3 showing the strongest effect.

2.4 Evaluation of Salt Toxicity of Different Salt Types on *P. annua* Seed Germination

The salt toxicity of different salt types on *P. annua* seeds during germination ranked as follows: $\text{Na}_2\text{CO}_3 > \text{NaHCO}_3 > \text{Na}_2\text{SO}_4$.

3. Discussion

Seed germination is the most important stage in plant growth and also the most sensitive period for plants to respond to stress. Successful germination under salt stress is key for plants to complete their life cycle and establish populations [citation]. Germination indices are essential for testing seed quality [citation] and are important for breeding, seed storage improvement, and germplasm resource protection [citation]. Zhang Yingying [citation] found that provenance had extremely significant effects on germination behavior of Poaceae species, with seed germination being inherently and closely related to life form. Our results showed that Yongchang provenance *P. annua* had significantly higher seed germination and seedling growth characteristics than the other three provenances, indicating that plants affect seed production through divergent adaptation to produce different life forms, thereby causing differences in germination capacity and seedling growth.

Chlorides, sulfates, and carbonates are the main salts in saline soils, and their inhibition of seed germination involves both ion toxicity and osmotic stress effects [citation]. Pinheiro et al. [citation] found that salt stress had significant inhibitory effects on seed germination, while other studies have found that low concentrations promote seed germination [citation]. Our study measured the effects of neutral and alkaline salts on *P. annua* seed germination and seedling growth, finding that salt stress significantly inhibited these processes, mainly by reducing relative germination rate, delaying germination time, increasing relative salt damage rate, and inhibiting bud and radicle growth. These results are similar to those of Zhang Xiuling et al. [citation] and Xu Man [citation]. *P. annua* seed germination and seedling growth showed significant differences in response to different salt types, concentrations, and their interactions, with alkaline salts being more toxic than neutral salts. The relative germination rate of *P. annua* decreased with increasing salt concentration, and the degree of inhibition varied among different salt types at the same concentration, consistent with the results of Wang Weihua et al. [citation].

Different plant tissues and organs show distinct responses to saline-alkali stress. Croser et al. [citation] believed that salt had more obvious inhibitory effects on aboveground parts than underground parts, while Liu Jie et al. [citation] thought that underground parts were more sensitive to salt when roots were relatively tender. Our results showed that under different salt types, concentrations, and

their interactions, the decrease in radicle length was significantly greater than that in bud length, which is more consistent with the view that underground parts are more sensitive to salt when roots are relatively tender. This may be related to the direct contact between radicles and salt solution. Therefore, compared with germination rate and bud length, radicle length can better reflect the saline-alkali tolerance of *P. annua* during the germination period.

The salt toxicity of different salt types on *P. annua* is a comprehensive performance affected by multiple factors, and a single index cannot objectively and truly reflect plant salt toxicity. Therefore, this study used the fuzzy membership function method to comprehensively evaluate the salt toxicity of different salt types based on relative germination rate, relative germination potential, relative germination index, relative vigor index, relative bud length, relative radicle length, and relative salt damage rate. The results showed that the salt toxicity of different salt types on *P. annua* seeds during germination ranked as $\text{Na}_2\text{CO}_3 > \text{NaHCO}_3 > \text{Na}_2\text{SO}_4$, consistent with the results of Huang Lihua et al. [citation]. However, Wang Zhichun et al. [citation] believed that carbonates and bicarbonates generally cause greater harm to plants than chlorides and sulfates, and that chlorides cause greater harm than sulfates, which is inconsistent with our results. This discrepancy may be related to salt concentration, plant species, and interaction effects, which require further study.

4. Conclusion

The germination and seedling growth of four wild *P. annua* accessions were inhibited to varying degrees under different salt types and concentrations. Among them, Yongchang provenance showed superior germination characteristics compared to other provenances, alkaline salts were more toxic than neutral salts, and radicles were more sensitive than buds during the germination period. The salt toxicity of different salt types on *P. annua* seeds during germination ranked as: $\text{Na}_2\text{CO}_3 > \text{NaHCO}_3 > \text{Na}_2\text{SO}_4$. Therefore, when using *P. annua* for turf greening and application promotion in saline-alkali land, the type of saline-alkali land should be considered.

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