Effects of Saline-Alkali Stress on Growth and Osmotic Adjustment Substance Content in Willow Seedlings (Postprint)

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Abstract

Using Salt Willow No. 1 as the experimental material, two neutral salts NaCI, Na2SO4 and two alkaline salts NaHCO3, Na2CO3 were mixed in different proportions to form three combinations: A (NaCl:Na2SO4:NaHCO3:Na2CO3=1:2:1:0), B (NaCl:Na2SO4:NaHCO3:Na2CO3=1:9:9:1), and C (NaCl:Na2SO4:NaHCO3:Na2CO3=1:1:1:1), with four concentration gradients (50, 100, 150, and 200 mmol/L), simulating 12 different saline-alkali conditions with varying salinity and alkalinity to treat Salt Willow No. 1 seedlings. Four indicators were measured: plant height growth increment, malondialdehyde (MDA) content, proline content, and soluble sugar content. The results showed that with increasing salt concentration and proportion of alkaline salts, the plant height growth increment of Salt Willow No. 1 seedlings showed a decreasing trend, while leaf MDA, proline, and soluble sugar contents increased to varying degrees. The plant height growth increments of groups A, B, and C decreased by 61.32%, 68.67%, and 73.02% relative to CK at a salt concentration of 200 mmo/L, respectively, with differences reaching significant levels ($P < 0.05$). In treatment group A (pH value of 8.04), the increasing trend of leaf MDA, proline, and soluble sugar contents was not significant with increasing salt concentration; in treatment group B (pH value of 8.66), leaf MDA content showed a significant increasing trend when salt concentration exceeded 100 mmol/L, while proline and soluble sugar contents increased sharply when salt concentration exceeded 150 mmol/L; in treatment group C (pH value of 9.47), all leaves of Salt Willow No. 1 seedlings turned withered and yellow when salt concentration exceeded 150 mmol/L. Comprehensive analysis results indicated that under mixed saline-alkali stress, the growth of Salt Willow No. 1 seedlings was inhibited to varying degrees; Salt Willow No. 1 could tolerate the three-component saline-alkali stress not exceeding 100 mmol/L, and saline-alkali conditions with salt concentration > 150 mmol/L and pH > 9.51 were unsuitable for the growth of Salt Willow No. 1.

Full Text

Preamble

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Effects of Saline-Alkali Stress on Growth and Osmotic Adjustment Substances in Willow Seedlings

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Abstract

This study investigated the physiological resistance characteristics of willow (*Salix psammophila* 'Yanliu No. 1') under complex saline-alkali stress to provide a theoretical basis for willow cultivation in saline-alkali soils. Using 'Yanliu No. 1' seedlings as experimental material, we examined the effects of 12 types of saline-alkali stress with different salinity and alkalinity levels on growth and osmotic adjustment substances. The stress conditions were simulated by mixing two neutral salts (NaCl and Na_2SO_4) and two alkaline salts ($NaHCO_3$ and Na_2CO_3) in various proportions: A (NaCl:Na_2SO_4:$NaHCO_3$:Na_2CO_3 = 1:2:1:0), B (NaCl:Na_2SO_4:$NaHCO_3$:Na_2CO_3 = 1:9:9:1), and C (NaCl:Na_2SO_4:$NaHCO_3$:Na_2CO_3 = 1:1:1:1), at concentrations of 50, 100, 150, and 200 mmol/L. Plant height increment and leaf contents of malondialdehyde (MDA), free proline, and soluble sugar were measured. Results showed that salt concentration was the main factor driving growth in 'Yanliu No. 1' . With increasing salt concentration and proportion of alkaline salts, plant height increment showed a decreasing trend, whereas leaf MDA, proline, and soluble sugar content increased to varying degrees. At 200 mmol/L, plant height increment under treatments A, B, and C was significantly lower ($P < 0.05$) than that under the control treatment (CK, 0 mmol/L), decreasing by 61.32%, 68.67%, and 73.02%, respectively. Under treatment A (pH 8.04), leaf MDA, proline, and soluble sugar content in 'Yanliu No. 1' did not differ significantly with increasing salt concentration. Under treatment B (pH 8.66), leaf MDA content showed a significantly increasing trend at salinity >100 mmol/L, whereas proline and soluble sugar content increased considerably at salinity >150 mmol/L. Under treatment C (pH 9.47), leaf MDA, proline, and soluble sugar content showed small variation at 100 mmol/L, whereas leaves withered at salinity >150 mmol/L. Comprehensive analysis indicated that: (1) the growth of *S. psammophila* 'Yanliu No. 1' was subject to different degrees of inhibition under complex saline-alkali stress; (2) 'Yanliu No. 1' could not tolerate stress at concentrations >100 mmol/L of the three mixed saline-alkali solutions; and (3) the saline-alkali condition with salinity >150 mmol/L and pH >9.51 was not

suitable for the growth of 'Yanliu No. 1'.

Keywords: saline-alkali stress; *Salix psammophila* 'Yanliu No. 1'; growth; osmotic adjustment substances

Introduction

Soil salinization is a critical factor limiting plant growth and productivity. Planting species adapted to saline-alkali soils represents an effective measure for ecological restoration of these areas. Previous salt stress research has primarily focused on single-salt stress; however, in China, most saline-alkali lands contain complex mixtures of multiple cations and anions, with salinization and alkalinization often occurring simultaneously. The ecological damage from these composite salts is greater than that from single salts. Researchers have simulated complex saline-alkali conditions by mixing NaCl, Na_2SO_4, $NaHCO_3$, and Na_2CO_3 in different proportions to study responses in oats, mung beans, *Reaumuria soongorica*, mulberry, sunflower, and other plants. Salt stress and alkali stress are distinct stresses with synergistic effects, where salt stress dominates at low alkali intensity and alkali stress dominates at high intensity. Buffer capacity and salinity are the main factors determining plant stress under complex conditions.

Willow (*Salix*), belonging to Salicaceae, is an important species for landscaping, timber production, and farmland shelterbelts. Previous studies on willow salt tolerance have mainly addressed neutral salt or alkali stress separately, with limited research on responses to complex saline-alkali stress. 'Yanliu No. 1' is a tree variety developed from the desert shrub *Salix psammophila* through more than a decade of research and breeding at the Shandong Forestry Research Institute. This study examined the effects of complex saline-alkali stress on the growth and osmotic adjustment substance content of 'Yanliu No. 1' seedlings to explore their saline-alkali tolerance range and physiological characteristics.

Materials and Methods

1. Plant Material Cultivation

The experimental material consisted of willow clones provided by the Shandong Forestry Research Institute. Uniform willow cuttings were planted in plastic pots (15 cm diameter × 15 cm height) filled with washed river sand and appropriate perlite. After cutting establishment, plants were irrigated with 1/2 Hoagland nutrient solution, sterilized with carbendazim, and cultivated in a greenhouse with day/night temperatures of 25/18°C. Willow seedlings with relatively uniform growth were selected for the experiment.

2. Simulated Saline-Alkali Treatment Design

NaCl, Na_2SO_4, $NaHCO_3$, and Na_2CO_3 were mixed in different proportions to create three treatment groups (A, B, C) with gradually increasing alkaline salt ratios. Each group had four concentration gradients (50, 100, 150, and 200 mmol/L total mixed salt concentration). The pH values corresponding to treatments A, B, and C were 8.04, 8.66, and 9.47, respectively. To avoid salt shock, salt concentration was increased incrementally by 50 mmol/L every 3 days until reaching the target concentration. Treatment intervals were 3 days, with 100 mL of solution applied per treatment. Regular quantitative watering was maintained, and functional leaves were collected for index determination after treatment.

shows the salt composition, molar ratios, and corresponding pH values for each treatment solution.

3. Measurement Indices and Methods

Plant Height Increment Measurement: Plant height was measured before and after stress treatment. Height increment was calculated as: post-stress height - pre-stress height.

Malondialdehyde (MDA) Content Determination: Leaf samples (0.5 g) were ground and homogenized with 2 mL of 10% trichloroacetic acid (TCA). The homogenate was centrifuged at 3000 r/min for 10 min. Two mL of the supernatant was mixed with 2 mL of 0.5% thiobarbituric acid (TBA) in a stoppered glass tube, reacted in a boiling water bath for 10 min, rapidly cooled, and centrifuged again. The supernatant absorbance was measured at 532 nm, 600 nm, and 450 nm.

Proline Content Determination: Plant samples (0.5 g) were placed in stoppered glass tubes with 5 mL of 3% sulfosalicylic acid solution, extracted in a boiling water bath for 30 min (with frequent shaking), cooled, and filtered. Two mL of the filtrate was mixed with 2 mL glacial acetic acid and 2 mL acidic ninhydrin reagent in a stoppered glass tube, heated in boiling water for 30 min until a red color developed. After cooling, 4 mL toluene was added, the mixture was shaken for 30 s, and the upper red toluene layer was carefully transferred for colorimetric measurement at 520 nm using toluene as a blank.

Soluble Sugar Content Determination: Leaf samples (0.3 g) were ground and extracted in boiling water for 30 min, filtered into 25 mL volumetric flasks, and brought to volume. Sample extract (0.5 mL) was placed in a stoppered glass tube with 1.5 mL distilled water, 0.5 mL anthrone-ethyl acetate reagent, and 5 mL concentrated sulfuric acid. Tubes were immediately placed in a boiling water bath for 1 min, cooled to room temperature, and absorbance was measured at 630 nm using a blank as reference.

4. Data Processing

Experimental data were processed and analyzed using Excel 2016 and SPSS 19.0. One-way ANOVA was used for significance analysis of different salt concentrations and pH values on physiological indices, with Duncan' s multiple comparison test applied.

Results

1. Effects of Saline-Alkali Stress on Willow Seedling Height Increment

Plant height after salt stress is the most direct indicator of salt tolerance, representing a comprehensive manifestation of various metabolic responses. Under complex saline-alkali stress, the growth of 'Yanliu No. 1' seedlings was inhibited to varying degrees. With increasing salt concentration, height increment in all groups showed a decreasing trend. Compared with the control (CK), height increment in group A decreased by 27.20%, 45.48%, 53.18%, and 61.32% at 50, 100, 150, and 200 mmol/L, respectively; group B decreased by 29.65%, 46.82%, 63.77%, and 68.67%; and group C decreased by 31.22%, 55.96%, 66.33%, and 73.02%. All differences were significant ($P < 0.05$). At concentrations above 150 mmol/L, leaves in group C turned completely yellow. At the same salt concentration, there were no significant differences in height increment among groups A, B, and C ($P > 0.05$).

[Figure 1: see original paper] shows the effects of complex saline-alkali stress on willow seedling height increment.

2. Effects of Saline-Alkali Stress on Malondialdehyde (MDA) Content in Willow Seedlings

Under saline-alkali stress, reactive oxygen species metabolism becomes unbalanced in plants, and cell membranes suffer lipid peroxidation from reactive oxygen attacks, leading to functional damage. With increasing salt concentration, MDA content in groups A, B, and C showed an overall increasing trend, with values higher than or equal to the control. Under treatment A, MDA content showed small increases with no significant differences from the control ($P > 0.05$). Under treatment B, MDA content increased significantly at salt concentrations >100 mmol/L ($P < 0.05$). Under treatment C, MDA content increased significantly at salt concentrations >50 mmol/L ($P < 0.05$), reaching maximum values at 150 and 200 mmol/L.

[Figure 2: see original paper] illustrates the effects of complex saline-alkali stress on MDA content in willow seedling leaves.

3. Effects of Saline-Alkali Stress on Proline Content in Willow Seedlings

Free proline is a soluble protein with strong water adsorption capacity and a primary substance involved in plant osmotic adjustment. Under saline-alkali stress, free proline accumulated to varying degrees in all treatment groups. With increasing salt concentration, proline content in groups A, B, and C showed a trend of initial increase followed by decrease, reaching maximum values at 150, 50, and 100 mmol/L, respectively, with relative increases of 23.82%, 154.29%, and 41.83% compared with the control ($P < 0.05$). Overall, proline content in group A showed small increases with no significant differences from the control ($P > 0.05$). In group B, proline content increased slowly below 100 mmol/L but increased sharply under high-salt stress. In group C, proline content increased significantly at salt concentrations >100 mmol/L ($P < 0.05$), reaching maximum values at 150 and 200 mmol/L, with increases of 72.58% and 158.75% compared with the control ($P < 0.05$). At the same salt concentration, there were no significant differences in proline content among the three groups ($P > 0.05$).

[Figure 3: see original paper] presents the effects of complex saline-alkali stress on proline content in willow seedling leaves.

4. Effects of Saline-Alkali Stress on Soluble Sugar Content in Willow Seedlings

With increasing salt concentration, soluble sugar content in groups A, B, and C showed intermittent accumulation. In group A, soluble sugar content increased then decreased, reaching a maximum at 100 mmol/L, with no significant differences from the control ($P > 0.05$). In group B, soluble sugar content began to increase significantly at 150 mmol/L ($P < 0.05$), reaching a maximum at 200 mmol/L with a 313.99% increase compared with the control ($P < 0.05$). In group C, soluble sugar content increased significantly at salt concentrations >100 mmol/L ($P < 0.05$), reaching a maximum at 150 mmol/L with a 48.45% increase compared with the control ($P < 0.05$). At the same salt concentration, there were no significant differences in soluble sugar content among the three groups ($P > 0.05$).

[Figure 4: see original paper] demonstrates the effects of complex saline-alkali stress on soluble sugar content in willow seedling leaves.

5. Variance Analysis of Stress Indicators with Salt Concentration and pH

Variance analysis showed that different salt concentrations and pH values, as well as their interactions, significantly affected the physiological indices of 'Yanliu No. 1' seedlings. Salt concentration had highly significant effects on plant height increment, MDA content, proline content, and soluble sugar content ($P < 0.01$). pH had highly significant effects on plant height increment ($P < 0.01$) but not on proline content ($P > 0.05$). The interaction between salt concentration and pH had no significant effects on plant height increment or proline content ($P > 0.05$). The effect of salt concentration on each stress indicator was significantly greater than that of pH.

summarizes the effects of salinity, pH, and their interactions on different stress indices of willow seedlings.

Discussion and Conclusion

Plant growth is inhibited under environmental stress, with complex saline-alkali stress having particularly significant effects. In this study, the growth of 'Yanliu No. 1' seedlings was inhibited to varying degrees, with height increment decreasing as salt concentration increased. Leaves turned completely yellow under high-salt stress, especially in treatment C with higher alkaline salt proportions (C3, C4). This indicates that under complex saline-alkali stress, 'Yanliu No. 1' seedlings were affected not only by salt concentration but also by high pH, consistent with results from studies on *Rubus crataegifolius* seedlings.

When plants suffer stress, cell membranes are the first to be damaged. MDA is a product of membrane lipid peroxidation, and higher MDA content indicates greater plant injury. This study found that leaf MDA content in 'Yanliu No. 1' seedlings increased to varying degrees with increasing salt concentration, indicating that oxygen free radicals induced membrane lipid peroxidation and caused varying degrees of cell membrane damage. Under treatment A, the small increase in MDA suggests minor plasma membrane damage. Under treatment B, MDA increased sharply at >100 mmol/L, indicating intensified membrane lipid peroxidation and increased plant injury. Under treatment C, MDA increased significantly at >150 mmol/L, suggesting that 'Yanliu No. 1' seedlings had lost their stress response capability.

Salt stress causes osmotic stress in plants, leading to loss of turgor pressure. Plants increase cytoplasmic concentration by accumulating osmotic adjustment substances to balance osmotic potential and prevent the vacuole from drawing water from the cytoplasm. Proline and soluble sugar are important osmotic adjustment substances in plants. This study showed that with increasing saline-alkali stress concentration, leaf proline and soluble sugar content in 'Yanliu No. 1' seedlings increased. Under low-salt stress (100 mmol/L), proline and soluble sugar content in groups A, B, and C showed minimal changes. Under high-salt stress (150 mmol/L), proline and soluble sugar content in group A increased insignificantly, while in groups B and C they increased sharply to maximum values. When seedlings in group C had completely yellow leaves (C3, C4), proline and soluble sugar content decreased, indicating that 'Yanliu No. 1' has certain saline-alkali tolerance but accumulates large amounts of proline and soluble sugar to alleviate damage when tolerance limits are exceeded, eventually succumbing to high-salt and high-alkali stress.

Complex saline-alkali stress is not a simple additive effect of salt and alkali stresses but exhibits synergistic effects. Under low alkalinity (pH 8.04), increases in proline and soluble sugar content were minimal. Under low salinity (50 mmol/L), although salt concentration increased from 50 to 200 mmol/L, changes in proline and soluble sugar content were not significant, indicating that pure salt stress had limited effect. However, when salt and alkali stresses acted together, the synergistic effect caused greater damage to plants. 'Yanliu No. 1' seedlings could grow normally under conditions with salinity <100 mmol/L and pH <9.51. When salinity exceeded 150 mmol/L and pH exceeded 9.51, leaves turned completely yellow (C3, C4), indicating these conditions were unsuitable for growth. The results demonstrate that under conditions of high salt with low alkali or high alkali with low salt, the physiological changes in 'Yanliu No. 1' were minimal, whereas mixed saline-alkali stress >100 mmol/L significantly affected its physiology.

References

[References section would be formatted according to journal standards, preserving all citation numbers and details as in the original]

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