Postprint: Simulated Effects of Salt Stress in Sandy Soil on Physiological Growth of Tiger Nut (Cyperus esculentus) Seedlings

Authors: Ma Xingyu

Date: 2023-01-17T00:00:00+00:00

Abstract

To understand the physiological and growth adaptability of Cyperus esculentus seedlings to salt stress in sandy desertified land, this study conducted pot experiments using aeolian sandy soil as the culture substrate under greenhouse conditions, subjecting C. esculentus seedlings to different concentrations of NaCl stress, and analyzed the growth, physiological and biochemical characteristics, and Na+ and K+ balance after 30 d and 50 d of salt stress. The results showed that: (1) After 30 d and 50 d of salt stress, the aboveground and underground parts of C. esculentus seedlings grew well at NaCl concentrations $ $0.5 g・kg-1, but plant height was significantly reduced; at NaCl concentrations $ $1.0 g・kg-1, the aboveground and underground dry weights, plant height, and leaf area all decreased to varying degrees. (2) At 30 d of salt stress, the seedlings primarily alleviated salt stress through massive accumulation of proline, while at 50 d, they mainly enhanced their osmotic adjustment capacity through large accumulation of soluble protein and soluble sugar to adapt to salt stress. (3) With increasing NaCl concentration, Na+ content and Na+ /K+ ratio in both leaves and roots increased significantly, indicating that their ionic balance was disrupted. (4) The membership function comprehensive evaluation results showed that the integrated physiological and growth performance of C. esculentus seedlings did not change significantly at NaCl concentrations $ $0.5 g・kg-1, but decreased significantly when exceeding 1.0 g・kg-1, indicating that C. esculentus seedlings possess certain salt tolerance and can grow normally under mild salt stress conditions in sandy land.

Full Text

Simulation Study on the Effects of Salt Stress on the Physiological Growth of Cyperus esculentus Seedlings in Sandy Soil

MA Xingyu[1,2,3,4], HUANG Caibian[1,2,3], ZENG Fanjiang[1,2,3], LI Xiangyi[1,2,3], ZHANG Yulin[1,2,3,5], DING Ya[1,2,3,5], GAO Yanju[1,2,3,4], XU Mengqi[1,2,3,4]

[1] Xinjiang Key Laboratory of Desert Plant Roots Ecology and Vegetation Restoration, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, Xinjiang, China

[2] State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, Xinjiang, China

[3] Cele National Station of Observation and Research for Desert Grassland Ecosystem in Xinjiang, Cele 848300, Xinjiang, China

[4] University of Chinese Academy of Sciences, Beijing 100049, China

[5] Xinjiang University, Urumqi 830046, Xinjiang, China

Abstract

To understand the physiological and growth adaptability of Cyperus esculentus seedlings to salt stress in desertified land, we conducted a pot experiment in a greenhouse using aeolian sandy soil as the culture medium. Different concentrations of NaCl stress were applied to C. esculentus seedlings to analyze their growth, physiological and biochemical characteristics, and Na^+/K^+ balance after salt stress. The results showed: (1) When the NaCl concentration was $0.5 g\cdot kg^{-1}$, seedlings grew well but plant height decreased significantly. After 30 days of stress, both aboveground and belowground parts of C. esculentus seedlings were significantly affected at NaCl concentrations $1.0 g\cdot kg^{-1}$, with decreases in aboveground and belowground dry weight, plant height, and leaf area. (2) On day 30 of salt stress, seedlings primarily content and Na^+/K^+ ratio in leaves and roots increased significantly with increasing salt concentration, indicating disrupted ion balance. (4) The comprehensive evaluation using membership function showed that when NaCl concentration was $0.5 g\cdot kg^{-1}$, the comprehensive physiological and growth performance of C. esculentus seedlings did not change significan 1}$. This indicates that C. esculentus seedlings possess certain salt tolerance and can grow normally under mild salt stress conditions in sandy soil.

Keywords: Cyperus esculentus; salt stress; seedling growth; osmotic adjustment; ion content

Introduction

China currently has 3.69$\times 10^{7}$ hm^2 of various types of utilizable saline soils, of which approximately 1.07$\times 10^{7}$ hm^2 has agricultural utilization prospects, accounting for 22.01% of the total saline soil area nationwide. Xinjiang is one of the regions most severely affected by salinization in China, primarily characterized by oasis and desertified saline soils, which represent promising saline lands with good agricultural development value. Effective management and development of utilizable saline soils can not only expand regional cultivated land resources and agricultural development space, but also alleviate food security pressure. Introducing and planting salt-tolerant plants can significantly increase soil organic matter and biodiversity while substantially improving the micro-ecological environment of saline soils. Therefore, introducing plants suitable for growth in saline lands, especially crops with high comprehensive utilization value, represents an important measure for saline soil improvement and productivity enhancement.

Cyperus esculentus is a perennial herb of the Cyperaceae family (cultivated as an annual crop in agricultural production), integrating multiple uses including grain, oil, forage, feed, medicine, and ornamental landscaping. It is a high-yield economic crop with strong stress resistance, wide adaptability, and tolerance to poor soil conditions. In recent years, C. esculentus has been trial-planted in both northern and southern Xinjiang oases and wind-sand areas. However, limited knowledge exists regarding whether C. esculentus can be successfully cultivated on desertified lands with varying degrees of salinization, and effective theoretical guidance for large-scale planting in desertified saline soils is lacking. Therefore, studying the response and adaptation mechanisms of C. esculentus to salt stress in desertified land is of great significance for its large-scale cultivation and efficient land utilization in Xinjiang' s sandy saline soils.

Salt stress damages plant growth and development primarily through secondary responses including osmotic stress, ion toxicity, nutrient imbalance, and oxidative damage. To adapt to salt stress, plants have evolved a series of physiological and biochemical protective mechanisms, such as synthesis of osmotic adjustment substances, enhanced antioxidant enzyme activity, and upregulated expression of salt tolerance genes. Different plants exhibit different salt tolerance and response mechanisms. For example, salt-tolerant wheat (Triticum aestivum) seedlings alleviate salt stress through strong K^+/Na^+ regulation capacity and antioxidant enzyme systems. Quinoa (Chenopodium quinoa) seedlings adapt to salt stress by increasing soluble sugar and proline contents, enhancing antioxidant enzyme activity, and reducing malondialdehyde (MDA) content. Primula forbesii seedlings resist salt damage by increasing soluble protein and proline contents and superoxide dismutase and peroxidase activities. Tang et al. studied the short-term salt tolerance of C. esculentus seedlings using vermiculite as the culture medium and found that they enhance salt tolerance by increasing antioxidant enzyme activity in leaves and accumulating proline and soluble sugar. However, it remains unclear whether C. esculentus seedlings maintain strong salt tolerance under prolonged stress. Therefore, this study used wind-sand soil from the oasis-desert transition zone in the southern Tarim Basin as the culture medium, conducted pot experiments in a greenhouse, and monitored plant growth performance, Na^+ and K^+ contents, and changes in leaf organic osmotic adjustment substances and MDA content under different salt stress durations and concentrations. The objective was to analyze the growth and physiological responses and salt tolerance of C. esculentus seedlings to salt stress in desertified soil, providing theoretical reference for large-scale promotion and cultivation of C. esculentus in Xinjiang' s sandy saline lands.

1. Materials and Methods

1.1 Material Cultivation and Treatment The experiment was conducted in a greenhouse at the Urumqi Botanical Garden, Xinjiang. The tested C. esculentus variety was "Xinke No. 1." Before the experiment, a seed germination pre-test was performed, achieving a germination rate of 95%. Full, non-moldy, mechanically undamaged, and uniformly sized seeds were selected and soaked in distilled water for 24 hours before sowing.

The test soil was collected from the 0-20 cm soil layer in the Cele oasis-desert transition zone on the southern edge of the Tarim Basin (80°03 E, 37°00 N). The soil type was aeolian sandy soil with salt content of 0.108%, pH 7.8, organic matter content of 4.40 $mg \cdot kg^{-1}$, available nitrogen content of 7.44 $mg \cdot kg^{-1}$, available phosphorus content of 2.16 $mg \cdot kg^{-1}$, and available potassium content of 128 $mg \cdot kg^{-1}$. According to investigations, C. esculentus planting time in Xinjiang generally occurs in mid-to-late April, when temperatures are not yet stable with average temperatures not exceeding 15°C. Therefore, during seedling growth, the greenhouse daily average temperature was controlled at 15.64$±1.74°*C, averagehumidityat*68.18±7.93±$3399.09 lux, and daylight duration at 12 hours.

Air-dried soil was placed in plastic pots (23 cm upper diameter, 20 cm height, 34 cm bottom diameter), with 10 kg of soil per pot. Pots were placed on trays to allow later water absorption. Base fertilizer was applied at 0.08 $g \cdot kg^{-1}$ urea and 0.12 $g \cdot kg^{-1}$ potassium dihydrogen phosphate. Five seeds were sown per pot, followed by 300 mL of water. When seedlings reached the three-leaf-and-one-heart stage (30 days after sowing), they were thinned to three seedlings per pot with uniform growth. Salt stress treatments began on May 30, 2021.

Soil salt stress gradients were set according to Huang et al.' s classification standards for salinization degree based on salt content (Table 1). Five NaCl concentration gradients were established: control (CK, 0 $g \cdot kg^{-1}$), mild salt stress (S1, 0.5 $g \cdot kg^{-1}$), moderate salt stress (S2, 1.0 $g \cdot kg^{-1}$), severe salt stress (S3, 2.0 $g \cdot kg^{-1}$), and salt soil (S4, 3.5 $g \cdot kg^{-1}$), with six replicates per treatment. To avoid salt base effects, NaCl was dissolved in water and applied to the soil. The CK treatment received only water. Based on measured field capacity of 23.6% in pot soil, the S1 treatment received NaCl solution at 0.5 $g \cdot kg^{-1}$ daily increments, S2 at 1.0 $g \cdot kg^{-1}$, S3 at 2.0 $g \cdot kg^{-1}$, and S4 at 3.5 $g \cdot kg^{-1}$. Soil water content was maintained at 70% of field capacity using the weighing method.

1.2 Measurement Items and Methods **Growth index measurement:** On day 30 of salt treatment, plant height was measured for each treatment with six replicates. Then, three pots were randomly selected per treatment (each pot as one replicate). All leaves were cut, brought to the laboratory, washed with water, dried, and scanned using a scanner (HP G3110). Leaf area was calculated using ImageJ software. The remaining aboveground parts and underground parts of C. esculentus seedlings were washed and oven-dried at 105°C for 30 minutes, then at 80°C to constant weight to obtain dry weights of each part. Aboveground parts included stems and leaves, while underground parts included roots and tubers. Root-shoot ratio was calculated as: underground dry weight/aboveground dry weight.

Leaf relative water content was measured using the saturated weighing method. Three additional pots were selected per treatment. Mature leaves from the same position were cut, stored in a portable refrigerator, brought to the laboratory, rapidly rinsed with distilled water, and surface water was blotted before measuring fresh weight. Leaves were then immersed in 15 mL test tubes filled with distilled water (avoiding leaf damage) for 24 hours in the dark. After saturation, surface water was gently blotted and saturated fresh weight was measured. Leaves were then oven-dried at 105°C for 30 minutes and dry weight was measured. Relative water content was calculated as: (fresh weight - dry weight)/(saturated fresh weight - dry weight) × 100%.

Physiological indicators and ion content measurement: For each treatment, three pots were randomly selected and mature leaves were cut. MDA content was measured using the thiobarbituric acid method. Proline content was measured using the sulfosalicylic acid extraction method. Soluble sugar content was measured using the anthrone colorimetric method. Soluble protein content was measured using the Coomassie brilliant blue G-250 colorimetric method. Proline, soluble sugar, and soluble protein contents were calculated based on leaf fresh weight. Na^+ and K^+ contents in leaves and roots were extracted and measured according to Wang et al., with contents calculated based on dry weight.

1.3 Comprehensive Evaluation of Salt Tolerance Based on measured indicators of C. esculentus seedlings after salt stress (growth, physiological and biochemical indicators, and leaf Na^+/K^+ content), the fuzzy membership function method was used to calculate membership function values for salt tolerance evaluation, where larger values indicate stronger salt tolerance:

For indicators positively correlated with salt tolerance: R(Xi) = (Xi - Xmin)/(Xmax - Xmin)

For indicators negatively correlated with salt tolerance: R(Xi) = 1 - (Xi - Xmin)/(Xmax - Xmin)

Where R is the membership function value, Xi is the measured value of each indicator, Xmin is the minimum measured value of the indicator, and Xmax is the maximum measured value.

1.4 Data Statistical Analysis Data were processed using Microsoft Excel 2019 and statistically analyzed using IBM SPSS Statistics 23 software. One-way ANOVA and Duncan' s multiple comparison tests were used to examine differences in growth and physiological indicators under different salt concentrations ($P < 0.05$). Pearson correlation analysis was used to examine relationships among indicators. Origin 2021 software was used for graphing.

2. Results

2.1 Effects of Salt Stress on C. esculentus Seedling Growth After 30 days of salt treatment, aboveground dry weight showed no significant difference between CK and S1 treatments, but both were significantly higher than other treatments. Differences among S2, S3, and S4 treatments were not significant, but all were significantly lower than CK and S1. Belowground dry weight was highest under S1 treatment, with no significant difference from CK but significantly higher than other treatments. Plant height decreased significantly after salt treatment, being lowest under S4 treatment but not significantly different from S3. Leaf area also decreased significantly under all salt treatments, with reductions of 25.3%, 64.9%, 65.8%, and 72.6% compared to CK for S1-S4, respectively.

After 50 days of salt treatment, aboveground dry weight in CK-S4 treatments increased by 32.1%, 36.2%, 29.8%, and 27.6%, respectively, compared to day 30. Belowground dry weight decreased under all treatments except S1, with reductions of 47.2%, 18.2%, and 38.9% for S2-S4, respectively. Plant height increased by 40.9%, 19.5%, 15.1%, and 17.0% for CK-S4, respectively. Leaf area increased under CK treatment but decreased under salt treatments, with reductions of 32.9%, 27.8%, 30.1%, and 39.3% for S1-S4, respectively.

After 30 days of salt stress, leaf relative water content increased slightly under S1 treatment and was significantly higher than other salt treatments. Root-shoot ratio increased under all salt treatments but not significantly. After 50 days of salt stress, leaf relative water content was highest under S1 treatment but not significantly different from other treatments. Root-shoot ratio decreased under all salt treatments, with no significant decrease under S1 but significant decreases under S2-S4. Compared to day 30, leaf relative water content increased under CK and S1 treatments at day 50, with minimal changes under other treatments. Root-shoot ratio increased under CK treatment at day 50, but decreased under all salt treatments.

2.2 Effects of Salt Stress on Leaf Organic Osmotic Adjustment Substances **Proline content:** After 30 days of salt treatment, leaf proline content was higher under all salt treatments than CK, with no significant difference between CK and S1, but S2-S4 were significantly higher than CK, increasing by 35.6%, 40.5%, and 24.6%, respectively. After 50 days, leaf proline content was highest under S4 treatment, significantly higher than other treatments. Compared to day 30, proline content under S1-S4 treatments at day 50 decreased by 18.3%, 19.4%, 14.6%, and 20.3%, respectively.

Soluble sugar content: After 30 days of salt treatment, soluble sugar content was highest under S2 treatment, not significantly different from S1 but significantly higher than other treatments. After 50 days, soluble sugar content increased significantly under all salt treatments compared to CK, with the greatest increase under S2 treatment (20.3%). Under both stress durations, leaf soluble sugar content showed a trend of first increasing then decreasing with increasing salt stress.

Soluble protein content: After 30 days of salt treatment, soluble protein content decreased under S1 treatment and increased under S2-S4 treatments, but differences among treatments were not significant. After 50 days, soluble protein content increased significantly under all salt treatments compared to CK, rising linearly with salt stress intensity. Compared to day 30, soluble protein content under S1-S4 treatments at day 50 increased by 53.2%, 51.6%, 68.4%, and 23.5%, respectively.

2.3 Changes in MDA Content of C. esculentus Leaves Under Salt Stress After 30 days of salt stress, MDA content increased with salt stress intensity, peaking at S2 treatment and then decreasing. MDA content under CK treatment was significantly lower than other treatments. After 50 days, MDA content was highest under S4 treatment, significantly higher than other treatments. Compared to day 30, MDA content under CK treatment at day 50 decreased by 25.2%, while other treatments showed varying degrees of increase, with S4 treatment increasing by 278.2%.

2.4 Effects of Salt Stress on Na^+, K^+ Content and Na^+/K^+ Ratio in C. esculentus Plants **Leaf Na^+, K^+ content and Na^+/K^+ ratio:** After 30 days of salt treatment, leaf Na^+ content increased with salt stress, with S2-S4 treatments significantly higher than CK (increasing by 62.6%, 100.3%, and 112.7%, respectively). Leaf K^+ content decreased significantly under all salt treatments compared to CK, with reductions of 20.7%, 24.1%, 27.8%, and 30.7% for S1-S4, respectively. The leaf Na^+/K^+ ratio increased significantly with salt stress, being 13.5%, 36.0%, 39.7%, and 31.2% higher under S1-S4 treatments than CK, respectively.

After 50 days of salt treatment, leaf Na^+ content was significantly higher under all salt treatments than CK, with S2-S4 treatments showing no significant differences among themselves but all significantly higher than S1. Leaf K^+ content decreased under all treatments except S1, with S2-S4 treatments decreasing by 25.1%, 16.9%, and 30.4%, respectively. The leaf Na^+/K^+ ratio increased significantly with salt stress intensity, with S1-S4 treatments increasing by 40.9%, 47.6%, 41.7%, and 49.0%, respectively, compared to CK.

Root Na^+, K^+ content and Na^+/K^+ ratio: After 30 days of salt treatment, root Na^+ content under S1-S4 treatments increased by 43.3%, 55.4%, 44.3%, and 49.0% compared to CK, respectively, with no significant differences among salt treatments. Root K^+ content decreased significantly under all salt treatments compared to CK. The root Na^+/K^+ ratio increased significantly with salt gradient, with no significant difference between S3 and S4 treatments but both significantly higher than other treatments.

After 50 days of salt treatment, root Na^+ content was significantly lower under all salt treatments than CK, with no significant differences among salt treatments. Root K^+ content was significantly lower under S2-S4 treatments than CK, decreasing by 30.1%, 41.7%, and 12.1%, respectively. The root Na^+/K^+ ratio increased significantly with salt stress intensity, with no significant difference between S3 and S4 treatments but both significantly lower than S2 treatment.

2.5 Membership Function Values and Salt Tolerance Evaluation of C. esculentus Under Different Salt Concentrations Comprehensive evaluation using the membership function method showed that under both stress durations, the membership function values of C. esculentus followed the order: CK > S1 > S2 > S3 > S4. Salt tolerance decreased with increasing salt stress intensity. Under mild salt stress, C. esculentus showed strong adaptability and good growth performance. The comprehensive evaluation values at day 50 were lower than at day 30 under all treatments, indicating that prolonged salt stress in sandy soil is detrimental to C. esculentus seedling growth.

3. Discussion

3.1 Effects of Salt Stress on C. esculentus Growth Salt damage in plants typically results in stunted growth, reduced leaf area, and decreased root biomass, though responses vary among species with different salt tolerance levels. Studies on Echinochloa frumentacea, Agropyron cristatum, and other plants have found that low salt stress can promote plant height and biomass accumulation, while high salt concentrations are inhibitory. This study showed that mild salt stress (S1) had no significant effect on aboveground dry weight and leaf area of C. esculentus after 30 days, but significantly reduced plant height. With prolonged stress, aboveground dry weight remained relatively unaffected, but plant height and leaf area decreased significantly, and belowground dry weight was also inhibited. Under moderate and severe salt stress, aboveground and belowground dry weights, plant height, and leaf area were all significantly inhibited. This indicates that short-term low salt stress does not negatively affect C. esculentus, but prolonged stress inhibits root growth and leaf area expansion. Similar findings were reported for wheat seedlings, where short-term salt stress of varying degrees promoted growth, but prolonged stress inhibited seedling development. This demonstrates that both stress intensity and duration affect C. esculentus seedlings.

Many studies suggest that root growth is more sensitive to salt stress than aboveground parts, with plants reducing root growth under high salt concentrations to decrease salt absorption and transport to shoots as a defense mechanism, resulting in decreased root-shoot ratio. However, other studies indicate that roots are less sensitive than shoots, with plants increasing biomass allocation to roots to enhance water and nutrient acquisition, leading to increased root-shoot ratio. This study showed that root-shoot ratio increased slightly after 30 days of salt stress but decreased to varying degrees after 50 days. This suggests that C. esculentus root sensitivity to salt stress may be delayed, but long-term salt stress is detrimental to root growth, leading to decreased root-shoot ratio.

3.2 Effects of Salt Stress on Leaf Osmotic Adjustment Substances in C. esculentus Osmotic stress is the primary challenge for plants in saline soils, causing physiological drought and directly affecting plant growth. To counteract osmotic stress caused by salt accumulation, plants accumulate osmotic adjustment substances such as soluble sugars, soluble proteins, and proline to enhance cellular osmotic adjustment capacity and maintain normal water uptake and metabolic activities. Proline is a high-quality amino acid with osmotic adjustment function that can effectively alleviate salt stress damage and maintain membrane structure stability. Soluble proteins are enzymes involved in various metabolic processes that protect plant cell membranes and metabolism-related enzymes. Soluble sugars serve as carbon skeletons and energy sources for synthesizing other organic solutes, stabilizing cell membranes and protoplasm colloids.

This study showed that after 30 days of salt stress, leaf proline, soluble sugar, and soluble protein contents in C. esculentus were all higher than CK. However, with increasing salt stress intensity, proline content increased most significantly, soluble sugar content first increased significantly then decreased, while soluble protein content showed minimal change. This indicates that in the early stage of salt stress, C. esculentus primarily alleviated salt stress through massive proline accumulation in leaves. With prolonged stress, proline content first decreased significantly then increased significantly with salt stress intensity, soluble sugar content increased significantly to a certain level then remained stable, while soluble protein content showed a linear significant increase trend. This demonstrates that under long-term salt stress, C. esculentus primarily enhances osmotic adjustment capacity through massive accumulation of soluble protein and soluble sugar, with proline also accumulating substantially under high salt conditions.

Most studies show that proline, soluble sugar, and soluble protein contents increase with salt stress intensity, though some report decreases under excessively high salt concentrations, consistent with the soluble sugar trend observed here. Different organic osmotic adjustment substances may respond differently to salt stress, possibly related to their respective contribution rates. Long-term salt stress leads to accumulation of more harmful substances requiring synthesis of more enzymes for detoxification, making soluble protein play a greater role in cellular osmotic adjustment and become the main osmotic adjustment substance in C. esculentus leaves, consistent with research on Dianthus chinensis. Additionally, under severe salt stress (S4) at this stage, the massive accumulation of MDA also reflected severe damage to C. esculentus cell membranes, and the sharp increase in proline content may reflect the need for more proline accumulation to maintain membrane structure stability. This demonstrates that C. esculentus possesses strong osmotic adjustment capacity under salt stress conditions.

3.3 Effects of Salt Stress on Na^+, K^+ Content and Na^+/K^+ Ratio in C. esculentus Na^+ and K^+ metabolism is crucial for plant adaptation to saline environments. Imbalance between them adversely affects physiological processes. Excessive Na^+ uptake causes osmotic stress, inhibits enzyme activity, and disrupts cellular metabolic processes. K^+ is involved in turgor pressure control, photosynthetic metabolism, and enzyme activation, and its content decline leads to growth inhibition. This study showed that with increasing salt stress intensity, Na^+ content in C. esculentus leaves and roots increased significantly, while K^+ content decreased significantly, consistent with most research findings. Leaf K^+ content was higher than in roots, and both maintained relatively stable levels under moderate and severe stress, indicating that C. esculentus possesses strong selective K^+ absorption capacity. Transporting more K^+ to leaves under salt stress helps reduce osmotic stress in leaves, and with accumulation of proline, soluble protein, and other osmotic adjustment substances, the water loss trend in plant cells is slowed, maintaining relatively stable leaf relative water content even under high salt stress. Therefore, appropriate K^+ accumulation can enhance C. esculentus growth adaptability.

Maintaining an appropriate Na^+/K^+ ratio is an important indicator of plant salt tolerance. The Na^+/K^+ ratio is necessary for normal stomatal function and some physiological processes, and can serve as a typical indicator of salt ion damage in plant tissues. Lower Na^+/K^+ ratios typically indicate stronger salt tolerance, as salt-tolerant plants often absorb sufficient K^+ to cope with salt damage. This study showed that with increasing salt concentration, Na^+/K^+ ratios in leaves and roots increased significantly, indicating that while Na^+ increased in cytoplasm, K^+ leaked out, disrupting ion balance and inhibiting C. esculentus growth, consistent with most research. With prolonged salt stress, Na^+/K^+ ratios in roots and leaves increased to varying degrees under moderate and severe salt stress, indicating that longer stress duration makes it more difficult to maintain appropriate Na^+/K^+ ratios, mainly due to more severe Na^+ accumulation in C. esculentus cytoplasm. Therefore, mild salt stress may be more conducive to C. esculentus seedling growth.

3.4 Comprehensive Salt Tolerance Evaluation of C. esculentus Based on Membership Function Plant salt tolerance is influenced by multiple factors, and evaluating salt tolerance based on a single indicator may lead to biased results. Using fuzzy mathematics membership function to evaluate C. esculentus salt tolerance helps eliminate such limitations. The comprehensive evaluation showed that salt tolerance of C. esculentus in sandy soil under different salt stress levels followed the order: CK > S1 > S2 > S3 > S4. Salt tolerance decreased with increasing salt stress intensity. Under mild salt stress, C. esculentus showed strong adaptability and good growth performance. The salt tolerance evaluation values at day 50 were lower than at day 30 under all treatments, indicating that prolonged salt stress in sandy soil is detrimental to C. esculentus seedling growth.

Salinization and alkalization coexist in saline soils. Studies have found that mild saline sandy soil is more conducive to C. esculentus seed germination, but it can also grow normally in alkaline sandy soil. The sandy soil used in this study was also alkaline, and the good growth of C. esculentus seedlings under mild salt stress may be due to the alleviating effect of alkaline conditions on low salt stress. Additionally, sandy soil is loose, which is beneficial for C. esculentus root and tuber growth and later harvest. Therefore, C. esculentus can be promoted for cultivation in Xinjiang' s mildly saline sandy land and alkaline sandy soil.

4. Conclusion

By investigating the growth and physiological responses of C. esculentus seedlings under different salt concentrations, this study explored the feasibility of cultivating C. esculentus as an economic crop in sandy saline soils. Based on comprehensive analysis of growth and physiological indicators under salt stress:

1. Under mild salt stress ($ 0.5$g \cdot kg^{-1}), both aboveground and belowground parts of C. esculentus seedlings grew well, but were significantly inhibited with increasing stress intensity and duration.

2. In the early stage of salt stress, C. esculentus seedlings primarily alleviated salt stress through massive proline accumulation, while in the later stage they mainly enhanced osmotic adjustment capacity through massive accumulation of soluble protein and soluble sugar. With increasing salt stress intensity, Na^+ content and Na^+/K^+ ratio in leaves and roots increased significantly, while K^+ content decreased significantly and then remained stable when NaCl concentration exceeded 1.0 $g \cdot kg^{-1}$.

3. Massive accumulation of osmotic adjustment substances resulted in minimal changes in leaf relative water content under salt stress. These results indicate that C. esculentus seedlings can adapt to salt stress in sandy soil by maintaining high leaf water-holding capacity and osmotic adjustment ability, but high salt stress should not persist for extended periods.

This study provides important guidance for the promotion and cultivation of C. esculentus in Xinjiang' s sandy saline lands.

References

[1] Yang Jinsong, Yao Rongjiang, Wang Xiangping, et al. Research on salt-affected soils in China: History, status quo and prospect[J]. Acta Pedologica

Sinica, 2022, 59(1): 10-27.

[2] Yang Jinsong, Yao Rongjiang, Wang Xiangping, et al. Halt soil salinization, boost soil productivity[J]. Science, 2021, 73(6): 30-34.

[3] Hu Mingfang, Tian Changyan, Zhao Zhenyong, et al. Salinization causes and research progress of technologies improving saline-alkali soil in Xinjiang[J]. Journal of Northwest A&F University (Natural Science Edition), 2012, 40(10): 111-117.

[4] Yun Xuexue, Chen Yusheng. International development of saline-alkali land and its enlightenment to China[J]. Territory & Natural Resources Study, 2020(1): 84-87.

[5] Yang Zhenle. Characteristics and research progress of Cyperus esculentus[J]. Northern Horticulture, 2017(17): 192-201.

[6] Pascual B, Maroto J V, López-Galarza S, et al. Chufa (Cyperus esculentus L. var. sativus Boeck.): An unconventional crop. Studies related to applications and cultivation[J]. Economic Botany, 2000, 54(4): 439-448.

[7] Liu Yalan, Zhao Yue, Xu Mengqi, et al. Effect of row spacing on the growth of Cyperus esculentus and soil properties in extremely arid region[J]. Acta Agrestia Sinica, 2021, 29(11): 2486-2493.

[8] Yang Min, Tian Liping, Xue Lin. Quality and production potential of different chufa varieties in arid climate region of Xinjiang[J]. Chinese Journal of Oil Crop Sciences, 2013, 35(4): 451-454.

[9] Saitiniyazi M, Kadeer M. Analysis and prediction of climate change characteristics in Xinjiang in recent 30 years[J]. China Southern Agricultural Machinery, 2020, 51(17): 90-91.

[10] Zelm E V, Zhang Y, Testerink C. Salt tolerance mechanisms of plants[J]. Annual Review of Plant Biology, 2020, 71: 403-433.

[11] Deinlein U, Stephan A B, Horie T, et al. Plant salt-tolerance mechanisms[J]. Trends in Plant Science, 2014, 19(6): 371-379.

[12] Qi Qi, Ma Shurong, Xu Weidong. Advances in the effects of salt stress on plant growth and physiological mechanisms of salt tolerance[J]. Molecular Plant Breeding, 2020, 18(8): 2741-2746.

[13] Cai Xiaofeng, Hu Tixu, Ye Jie, et al. Molecular mechanisms of salinity tolerance in plants[J]. Journal of Huazhong Agricultural University, 2015, 34(3): 134-141.

[14] Tang Rong, Liang Peixin, Guo Chenli, et al. Effect of saline-alkali stress on growth and physiological characteristics of Cyperus esculentus[J]. Journal of Zhejiang Agricultural Sciences, 2022, 63(3): 528-533.

[15] Meng Fanhao, Wang Cong, Xu Shoujun. Advances in research on effects of salt stress on plant and the mechanism of plant salt tolerance[J]. Journal

of Inner Mongolia University for Nationalities (Natural Science Edition), 2014, 29(3): 315-318.

[16] Huang Shaowen, Gao Wei, Tang Jiwei, et al. Total salt content and ion composition in tillage layer of soils in the main vegetable production regions of China[J]. Journal of Plant Nutrition and Fertilizers, 2016, 22(4): 965-977.

[17] Yi Liangpeng, Wang Zuwei. Root system characters in growth and distribution among three littoral halophytes[J]. Acta Ecologica Sinica, 2011, 31(5): 1195-1202.

[18] Mauchamp A, Mésleard F. Salt tolerance in Phragmites australis populations from coastal Mediterranean marshes[J]. Aquatic Botany, 2001, 70(1): 39-52.

[19] Salter J, Morris K, Bailey P C E, et al. Interactive effects of salinity and water depth on the growth of Melaleuca ericifolia Sm. (Swamp paperbark) seedlings[J]. Aquatic Botany, 2007, 86(3): 213-222.

[20] Arndt S K, Arampatsis C, Foetzki A, et al. Contrasting patterns of leaf solute accumulation and salt adaptation in four phreatophytic desert plants in a hyperarid desert with saline groundwater[J]. Journal of Arid Environments, 2004, 59(2): 259-270.

[21] Dou Xintong, Wang Yingjie, Wang Huazhong, et al. Physiological response and tolerance difference of two wheat varieties to NaCl stress[J]. Acta Ecologica Sinica, 2021, 41(12): 4976-4992.

[22] Yang Farong, Liu Wenyu, Huang Jie, et al. Physiological responses of different quinoa varieties to salt stress and evaluation of salt tolerance[J]. Acta Prataculturae Sinica, 2017, 26(12): 77-88.

[23] Jia Yin, Xiang Yuanfen, Song Yilin, et al. Physiological responses of Primula forbesii seedlings to NaCl stress and alleviation effect of exogenous calcium[J]. Chinese Journal of Grassland, 2022, 44(1): 10-19.

[24] Huang Yong, Guo Meng, Zhang Hongrui, et al. Effects of salt stress on seed germination and seedling growth of carnation[J]. Acta Prataculturae Sinica, 2020, 29(12): 105-111.

[25] Liu B, Kang C, Wang X, et al. Physiological and morphological responses of Leymus chinensis to saline-alkali stress[J]. Grassland Science, 2015, 61(4): 217-226.

[26] Lu Anqiao, Zhang Fengju, Xu Xing, et al. Effects of salt stress on growth and physiological characteristics of Echinochloa frumentacea seedlings[J]. Acta Prataculturae Sinica, 2021, 30(5): 84-93.

[27] Xu Jing, Dong Kuanhu, Gao Wenjun, et al. Growth and physiological responses of Agropyron cristatum seedlings under NaCl and Na_2SO_4 stress[J]. Chinese Journal of Grassland, 2011, 33(1): 36-41.

[28] Wang Shufeng, Hu Yunxue, Sun Haiqing, et al. Effects of salt stress on growth and root development of two oak seedlings[J]. Acta Ecologica Sinica, 2014, 34(4): 1021-1029.

[29] Wang Juan, Li Dequan. The accumulation of plant osmoticum and activated oxygen metabolism under stress[J]. Chinese Bulletin of Botany, 2001(4): 459-465.

[30] Mišić D, Šiler B, Nestorović Živković J, et al. Contribution of inorganic cations and organic compounds to osmotic adjustment in root cultures of two Centaurium species differing in tolerance to salt stress[J]. Plant Cell, Tissue and Organ Culture, 2012, 108(3): 389-400.

[31] Zhang Ya, Shi Shuqian, Li Yaping, et al. Osmotic regulation and chlorophyll fluorescence characteristics in leaves of wheat seedlings under different salt stresses[J]. Chinese Journal of Applied Ecology, 2021, 32(12): 4381-4390.

[32] Flowers T J, Munns R, Colmer T D. Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes[J]. Annals of Botany, 2015, 115(3): 419-431.

[33] Zhao Yingxue. Effects of NaCl stress on physiological and biochemical basis of root and leaf of Allium mongolicum Regel seedlings[D]. Hohhot: Inner Mongolia Agricultural University, 2019.

[34] Jiang Ying, Zhou Meng, Wu Yue, et al. Evaluation of salt tolerance and its underlying physiological mechanisms in different oats[J]. Pratacultural Science, 2018, 35(12): 2903-2914.

[35] Guo Rui, Li Feng, Zhou Ji, et al. Physiological responses of linseed (Linum usitatissimum) to salt and alkali stresses[J]. Chinese Journal of Plant Ecology, 2016, 40(1): 69-79.

[36] Liu Ling, Luo Jianhong, Li Bingwen, et al. Effect of saline water drip irrigation on salt ions distribution for the Haloxylon ammodendron-Cistanche deserticola association and quality of Cistanche deserticola[J]. Arid Zone Research, 2022, 39(3): 908-915.

[37] Maathuis F J M, Amtmann A. K^+ nutrition and Na^+ toxicity: the basis of cellular K^+/Na^+ ratios[J]. Annals of Botany, 1999, 84(2): 123-133.

[38] Tian Xiaoxia, Wei Xiaofeng, Wei Hao, et al. Comprehensive evaluation of drought tolerance of six forage species at the seedling stage[J]. Arid Zone Research, 2022, 39(3): 978-985.

[39] Xu Jieliang, Zhang Fenghua, Li Bianbian, et al. Effect of fertilization on the characteristics of soil microbial community in the rhizosphere of Cyperus esculentus in the sandy area of Xinjiang[J]. Arid Zone Research, 2021, 38(6): 1741-1749.

[40] Wang Yizhen, Ding Guodong, Cui Xinran, et al. Effects of saline-alkali stress on the growth and photosynthetic characteristics of Cyperus esculentus

and the responses of protective enzymes[J]. Journal of Arid Land Resources and Environment, 2022, 36(5): 146-152.

[41] Wang Zichen, Zhang Bing, Guan Yongxiang, et al. Study on physiology of chufa cultivation in solonchak[J]. Soils, 2017, 49(6): 1126-1131.

[42] Zhang Xiaoyan. Main high-yield cultivation techniques of Cyperus esculentus[J]. Bulletin of Agricultural Science and Technology, 2009, 38(6): 165-166.

[43] Rehman S, Harris P J C, Bourne W F. The effect of sodium chloride on the Ca^{2+} and Na^{+} concentrations of the seed coat and embryo of Acacia tortilis and A. coriacea[J]. Annals of Applied Biology, 1998, 133(2): 269-279.

[44] Xu Y, Xiao H Y, Zheng N J, et al. Progress on responding of free amino acid in plants to salt stress[J]. Environmental Science & Technology, 2016, 39: 40-47.

[45] Gao Qian, Li Yi, Su Shiping, et al. Effects of salt stress on physiological characteristics of Reaumuria soongorica seeds during imbibition[J]. Journal of Desert Research, 2014, 34(1): 83-87.

[46] Lei Chunying, Ji Xiaomin, Peng Muzhi, et al. Effect of sodium salinity stress type on the germination of Kalidium foliatum seeds and its young seedling growth[J]. Arid Zone Research, 2021, 38(5): 1436-1441.

[47] Li Ruyu, Chai Weiwei, Guo Xiaonong, et al. Effect of salt treatment on osmotic adjustment of Apocynum venetum seedlings[J]. Molecular Plant Breeding, 2020, 18(12): 4105-4110.

[48] Wang Xinying, Shi Junhui, Liu Maoxiu, et al. Response of main osmotic adjustment substances to NaCl stress accumulation in Atriplex canescens[J]. Arid Zone Research, 2012, 29(4): 621-627.

[49] Kosugi H, Kikugawa K. Thiobarbituric acid reaction of aldehydes and oxidized lipids in glacial acetic acid[J]. Lipids, 1985, 20(12): 915-922.

[50] Zou Qi. Instructional on Plant Physiology[M]. Beijing: China Agriculture Press, 2000: 11-12.

[51] Li Hesheng. Experimental Principle and Techniques of Plant Physiology and Biochemistry[M]. Beijing: Higher Education Press, 2000: 195-197, 258-260.

[52] Li Ling. Experimental Guidance of Plant Physiology Module[M]. Beijing: Science Press, 2009: 54-55.

[53] Wang Baoshan, Zhao Kefu. Comparison of extractive methods of Na^{+} and K^{+} in wheat leaves[J]. Plant Physiology Communications, 1995, 31(1): 50-52.

[54] Mao Shuang, Zhou Wanli, Yang Fan, et al. Research progress on mechanism of plant roots response to salt-alkali stress[J]. Bulletin of Soil and Water Conservation, 2021, 30(10): 1991-2000.

Note: Figure translations are in progress. See original paper for figures.