Effects of Saline-Alkali Stress on Osmotic Adjustment and Antioxidant Enzyme System in Jerusalem Artichoke (Postprint)

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Date: 2024-03-01T00:00:00+00:00

Abstract

To elucidate the physiological responses of plants under saline-alkali stress, Jerusalem artichoke (Helianthus tuberosus) was selected as the experimental material. Three different treatments were established: a nutrient soil group (CK), a mild saline-alkali soil group (LS), and a moderate saline-alkali soil group (MS). Changes in physiological indices such as organic osmotic adjustment substances (soluble sugars, soluble proteins, proline), malondialdehyde (MDA) content, and antioxidant enzyme system activities [superoxide dismutase (SOD), peroxidase (POD), catalase (CAT)] in Jerusalem artichoke were investigated under different saline-alkali stress intensities. The results showed that: (1) The contents of organic osmotic adjustment substances, including soluble sugars, proline, and soluble proteins, in Jerusalem artichoke leaves increased under different saline-alkali stress intensities. (2) There was no significant difference in MDA content among groups; however, the activities of antioxidant enzyme system indicators, including SOD, POD, and CAT, in Jerusalem artichoke leaves showed an upward trend with increasing saline-alkali stress intensity. Compared with the CK group at the same period, after 150 days of saline-alkali stress, SOD activities in the LS and MS groups increased significantly by 22.13% and 26.49%, respectively; CAT activities in the LS and MS groups increased significantly by 81.66% and 92.38% compared with the CK group ($P<0.05$); POD activity in the MS group remained significantly higher than that in the CK group throughout the measurement period. These results indicate that under saline-alkali stress, Jerusalem artichoke enhances its resistance by increasing the contents of osmotic adjustment substances (soluble sugars, soluble proteins, proline) and activating the antioxidant enzyme system (SOD, CAT, POD), demonstrating strong saline-alkali tolerance.

Full Text

Abstract

To reveal the physiological response of plants to saline-alkali stress, we selected Jerusalem artichoke (*Helianthus tuberosus*) as our research object and established three treatment groups: a nutrient soil control group (CK), a light saline-alkali soil group (LS), and a moderate saline-alkali soil group (MS). We investigated changes in physiological indicators including organic osmoregulatory substances (soluble sugar, soluble protein, proline), malondialdehyde (MDA) content, and antioxidant enzyme system activities [superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT)] in Jerusalem artichoke under different saline-alkali stress intensities. The results demonstrated that: (1) The content of organic osmoregulatory substances in Jerusalem artichoke leaves—including soluble sugar, proline, and soluble protein—increased under varying intensities of saline-alkali stress. (2) No significant differences in MDA content were observed among groups. However, with increasing saline-alkali stress intensity, the activity indices of antioxidant enzyme systems (SOD, POD, and CAT) in Jerusalem artichoke leaves exhibited an upward trend. After 150 days of saline-alkali stress, SOD activity in the LS and MS groups increased significantly by 22.13% and 26.49%, respectively, compared to the CK group. CAT activity in the LS and MS groups increased significantly by 81.66% and 92.38%, respectively, relative to the CK group ($P < 0.05$). Furthermore, POD activity in the MS group remained significantly higher than in the CK group throughout the measurement period. These findings indicate that *Helianthus tuberosus* can adapt to saline-alkali environments by increasing osmoregulatory substance content (soluble sugar, soluble protein, proline) and activating its antioxidant enzyme system (SOD, CAT, and POD), demonstrating strong saline-alkali tolerance.

Keywords: saline-alkali stress; *Helianthus tuberosus*; organic osmoregulatory substances; antioxidant enzyme systems

Introduction

Salinized soil represents an important reserve cropland resource, and its development and utilization hold significant strategic importance for ensuring national and regional food security. The Hetao Irrigation District in the Wuliangsuhai watershed of Inner Mongolia is located in an arid and semi-arid region of northwestern China. Due to unique climatic and hydrogeological conditions, secondary soil salinization occurs frequently in this area, continuously reducing cultivated land productivity and affecting sustainable agricultural development. High salt content in saline-alkali soils elevates soil solution concentration, inhibiting plant water and nutrient uptake and severely impacting normal plant growth. The adverse effects of saline-alkali stress caused by excessive salt accumulation can be broadly categorized into two types: rapid osmotic stress and gradual ion toxicity [**?**]. The former primarily manifests as physiological drought leading to stomatal closure, reduced transpiration, decreased water con-

sumption, increased diffusion resistance, and limited leaf photosynthetic rates, while ion toxicity hinders new leaf growth and reduces biomass [**?**].

Soil salinization affects global agricultural production and ecological environments, currently covering approximately 20% of terrestrial area. Some scholars predict that the proportion of salinized cultivated land worldwide may reach 30% by 2050 [**?**]. Rozema and Flowers [**?**] noted that biotechnology, particularly the cultivation of salt-tolerant plants, can help address global salinization issues. Therefore, studying the physiological effects of saline-alkali stress on plants can enhance understanding of resistance mechanisms and facilitate the development of salt-tolerant plants and rational improvement of saline lands.

When plants encounter abiotic stress, they primarily achieve physiological adaptation through osmotic adjustment. Osmotic adjustment refers to the process by which plants synthesize organic substances to lower cellular water potential, thereby maintaining osmotic pressure stability to some extent, increasing cytoplasmic concentration, reducing osmotic potential, and promoting water absorption and utilization to help plants withstand adverse environments and ensure normal physiological activities [**?**]. Additionally, when plants suffer from stress, cell membranes are the first to be damaged, leading to malondialdehyde (MDA) accumulation and resulting in oxidative damage with excessive reactive oxygen species (ROS) production [**?**]. MDA, as the final decomposition product of lipid peroxidation, is an important indicator reflecting lipid peroxidation reactions in plants under stress [**?**]. Higher MDA content indicates more severe damage to plants [**?**].

Under normal conditions, ROS production and scavenging in plants maintain dynamic equilibrium at low levels, causing no damage to cell membranes [**?**]. However, under saline-alkali stress, excessive reduction of electron transport chains in plant mitochondria and chloroplasts generates large amounts of ROS, causing oxidative stress. To maintain ROS balance and alleviate saline-alkali damage, plants develop an antioxidant enzyme-based ROS scavenging system [**?**]. Superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) can effectively remove ROS, reduce damage from external stress, and enhance plant resistance [**?**].

Jerusalem artichoke (*Helianthus tuberosus*) is a herbaceous plant in the Asteraceae family. Its underground tubers are rich in starch and inulin, making it edible as a food crop that the Food and Agriculture Organization has designated as a "21st-century crop for shared human and livestock use." It is also a biomass energy plant that can serve as raw material for starch and ethanol production. In recent years, research on the saline-alkali tolerance characteristics of Jerusalem artichoke has gradually attracted scholars' attention. For example, Han et al. [**?**] conducted proteomic analysis of Jerusalem artichoke planted in farmland, light saline-alkali grassland, and heavy saline-alkali grassland, finding that metabolic balance regulation plays an important role in sprouting Jerusalem artichoke' s response to saline-alkali soil stress. Gao et al. [**?**] studied germination rates and seedling growth under different saline-alkali stresses, finding that low salt

concentrations promote germination, but plant height, leaf number, leaf weight, and leaf area gradually decrease with increasing salt concentration. Currently, few studies have reported on the variation patterns of leaf osmoregulatory substances and antioxidant enzyme activities in Jerusalem artichoke under saline-alkali stress, particularly in the Wuliangsuhai watershed of Bayannur City, Inner Mongolia. To investigate whether Jerusalem artichoke responds to and adapts to saline-alkali stress by increasing internal osmoregulatory substance content and antioxidant enzyme activity, this study employed pot simulation experiments. We measured organic osmoregulatory substances (soluble sugar, soluble protein, proline) in Jerusalem artichoke leaves to study synthesis and consumption patterns; measured MDA content changes to understand lipid peroxidation levels; and simultaneously measured antioxidant enzyme (SOD, POD, CAT) activities to explore regulatory mechanisms under saline-alkali stress. This research provides theoretical guidance and reference for bioremediation approaches in saline lands of the Hetao Irrigation District and Wuliangsuhai watershed.

Materials and Methods

1.1 Experimental Materials

Saline-alkali soil samples were collected from abandoned saline-alkali cropland in the Wuliangsuhai watershed of Bayannur City, Inner Mongolia (108°43 E, 40°46 N). The 0–20 cm soil layer was used as the experimental soil. This region features a typical temperate continental climate with abundant light and heat resources, large diurnal temperature variations, concentrated rainfall, and concurrent rainfall and heat. Located downstream of the Hetao Irrigation District, the area experiences prominent soil salinization and secondary salinization issues due to groundwater levels, climatic conditions, and irrigation methods. The physical properties of the experimental soil are shown in Table 1 .

The experiment was conducted at the Sanqingyuan Nursery of Beijing Forestry University. Jerusalem artichoke tubers of uniform size were obtained from a planting base and planted on April 25, 2021, with general watering management to ensure normal growth. The cultivation containers were plastic pots (22 cm height × 18 cm diameter), with planting depth at 10 cm and soil depth at 15 cm. The culture medium consisted of commercially purchased nutrient soil and collected saline-alkali soil.

1.2 Experimental Design

Three treatment groups were established: a nutrient soil group (CK), a light saline-alkali soil group (LS) prepared by mixing saline-alkali soil with nutrient soil at a 1:1 ratio, and a moderate saline-alkali soil group (MS) using only the collected saline-alkali soil. Each treatment had 15 replicates. The actual soil salt content was 0.05% in CK, 0.18% in LS, and 0.33% in MS. Jerusalem artichoke enters the mature period after approximately 120 days of saline-alkali stress. Sampling was conducted twice at 120 days and 150 days post-stress. The third

to fifth fully expanded leaves from top to bottom were collected, immediately wrapped in aluminum foil, placed in a liquid nitrogen tank for freezing, and then stored at ultra-low temperature for determination of osmoregulatory substance content and antioxidant enzyme system activity. All indicators were calculated based on fresh leaf weight.

1.3 Measurement Methods

1.3.1 Determination of Osmoregulatory Substances Approximately 0.2 g of Jerusalem artichoke leaves were weighed and ground with 5 mL of 0.05 mol·L^{-1} phosphate buffer in an ice bath to form a homogenate. The homogenate was centrifuged at 10,000 r·min^{-1} for 15 min at 4°C, and the supernatant was used as the enzyme solution for determination of soluble sugar, soluble protein, proline, and MDA content. Soluble sugar content was measured using the anthrone colorimetric method, soluble protein content using the Coomassie brilliant blue G-250 method, free proline using the acidic ninhydrin method [**?**], and MDA content using the thiobarbituric acid (TBA) method [**?**].

1.3.2 Determination of Antioxidant Enzyme Activities Approximately 0.3 g of Jerusalem artichoke leaves were weighed and ground with 5 mL of phosphate buffer (pH 7.8, containing 5 mmol·L^{-1} EDTA-Na_2, 2 mmol·L^{-1} ascorbic acid, and 2% PVP) in an ice bath to form a homogenate. The homogenate was centrifuged at 12,000 r·min^{-1} for 20 min at 4°C, and the supernatant was used as the enzyme solution for SOD, POD, and CAT activity measurements. SOD activity was determined using the nitroblue tetrazolium (NBT) photochemical reduction method [**?**], POD activity using the guaiacol method [**?**], and CAT activity using the ultraviolet absorption method [**?**].

1.4 Statistical Analysis

Experimental data were compiled using Excel 2021. SPSS 23.0 software was used for one-way ANOVA on data from different treatment groups, with Duncan' s method employed for multiple comparisons ($P < 0.05$). Origin 2021 software was used for graphical analysis.

Results

2.1 Effects of Saline-Alkali Stress on Organic Osmoregulatory Substance Content in Jerusalem Artichoke Leaves

During the mature period (120–150 days of saline-alkali stress), soluble sugar content in all groups showed a slight upward trend. At 120 days, soluble sugar content in both LS and MS groups was significantly higher than in the CK group ($P < 0.05$), with increases of 19.75% and 27.73%, respectively. At 150 days, soluble sugar content in the LS group increased by 14.76% compared to CK, while the MS group increased by 79.44% relative to CK, with significant

differences between all groups ($P < 0.05$). The overall trend was MS > LS > CK [Figure 1: see original paper].

Similar to soluble sugar content, soluble protein content also showed a gradually increasing trend over the experimental period. At 120 days, soluble protein content in the LS and MS groups increased by 16.67% and 17.19%, respectively, compared to CK, though these differences were not significant. By 150 days, significant differences emerged among all three groups ($P < 0.05$), with LS and MS groups showing increases of 9.62% and 16.21%, respectively, relative to CK. The overall trend throughout the measurement period was MS > LS > CK [Figure 1: see original paper].

Proline content in all groups showed a more pronounced increasing trend. At 120 days, proline content in the LS and MS groups increased by 6.02% and 93.88%, respectively, compared to CK, with significant differences between groups ($P < 0.05$). By 150 days, the LS and MS groups showed increases of 82.59% and 89.33%, respectively, relative to CK, with all inter-group differences being significant ($P < 0.05$) [Figure 1: see original paper].

2.2 Effects of Saline-Alkali Stress on MDA Content and Antioxidant Enzyme System Activities

Comparison of MDA content changes revealed no significant differences among any experimental groups during the measurement period. At 120 days, MDA content in the LS and MS groups increased by 15.60% and 18.08%, respectively, compared to CK, but these differences were not significant. Similarly, at 150 days, no significant inter-group differences were observed [Figure 2: see original paper].

SOD activity in all groups increased gradually with continued stress. Compared to CK, SOD activity at 120 days increased significantly by 24.89% in LS and 22.13% in MS ($P < 0.05$). By 150 days, LS and MS groups showed significant increases of 23.07% and 26.49%, respectively, relative to CK ($P < 0.05$) [Figure 2: see original paper].

POD activity changes differed from those of SOD. At 120 days, POD activity in the LS and MS groups increased by 3.57% and 5.90%, respectively, compared to CK, but these differences were not significant. However, by 150 days, POD activity in the MS group was significantly higher than in both CK and LS groups ($P < 0.05$), increasing by 84.29% relative to CK, while the LS group increased by 67.20% [Figure 2: see original paper].

CAT activity showed the most dramatic increases. At 120 days, CAT activity in the LS and MS groups increased significantly by 81.66% and 92.38%, respectively, compared to CK ($P < 0.05$). This trend continued at 150 days, with LS and MS groups showing significant increases of 25.20% and 22.77%, respectively, relative to CK ($P < 0.05$). Throughout the measurement period, CAT activity in both saline-alkali stress groups remained significantly higher than in

CK [Figure 2: see original paper].

Discussion

When plants experience saline-alkali stress, they can synthesize various osmoregulatory substances to resist the adverse conditions. Soluble sugars, soluble proteins, and proline are common osmoregulatory substances that play important roles in regulating osmotic balance and protecting cell structure [**?**]. This study demonstrates that after 150 days of saline-alkali stress, soluble sugar and proline content in Jerusalem artichoke leaves were significantly higher than in CK. As soil salt concentration increased, both soluble sugar and proline content increased correspondingly, with proline showing more pronounced increases, indicating that Jerusalem artichoke can regulate cellular osmotic potential through proline accumulation and enhanced soluble sugar synthesis to protect cell structure and function. This aligns with the trend observed in willow (*Salix matsudana*), where soluble sugar and proline content increased with saline-alkali stress concentration [**?**]. Zhang et al. [**?**] reported similar conclusions in alfalfa (*Medicago sativa*) research, suggesting that soluble sugar and proline content can serve as physiological indicators of saline-alkali tolerance to some extent.

Soluble protein is an important osmoregulatory substance in plants, essential for cellular osmotic adjustment. It can improve water retention in leaf cell tissues to resist external stress [**?**]. Unlike soluble sugar and proline content, soluble protein content in this study showed an increasing trend with saline-alkali stress intensity, but differences were not significant at 120 days. This resembles results in *Zelkova serrata*, where soluble protein content increased slowly under different saline-alkali stress levels without significant differences between treatment groups [**?**], suggesting that saline-alkali stress at this stage did not promote soluble protein synthesis in Jerusalem artichoke. However, at 150 days, inter-group soluble protein content increased significantly with stress intensity ($P < 0.05$), indicating that prolonged saline-alkali stress promoted soluble protein synthesis to mitigate damage.

Adversity stress causes plants to generate numerous oxygen free radicals, and plants correspondingly develop a complete antioxidant enzyme system to defend membrane structures against ROS damage and maintain membrane integrity. SOD converts oxygen free radicals to H_2O_2, while POD and CAT primarily scavenge peroxides [**?**]. Analysis of Jerusalem artichoke leaf antioxidant enzyme systems revealed that after a period of saline-alkali stress, SOD, POD, and CAT activities increased significantly, with greater enhancement under higher saline-alkali intensity. This resembles findings in alfalfa [**?**] and wintersweet (*Chimonanthus praecox*) [**?**]. Comparing treatment groups revealed that these three enzyme activities varied with stress intensity and growth stage.

MDA is a lipid peroxidation product that adversely affects cells. This study found no significant differences in MDA content among groups at 120 days, but at 150 days, MDA content in the MS group showed a clear increasing

trend compared to other groups. Liang et al. [**?**] reported that under low-concentration saline-alkali stress, MDA content increase was small in chufa (*Cyperus esculentus*), while high-concentration stress caused significant MDA increases, consistent with changes in rice (*Oryza sativa*) and sorghum leaves [**?**, **?**]. This suggests that plant membrane structures suffer minor damage under low-concentration stress but severe damage under high-concentration stress. In this study, Jerusalem artichoke membrane structures experienced minor damage at 120 days but severe damage at 150 days under MS conditions.

The increased amplitude of CAT activity in the MS group showed a decreasing trend compared to the LS group, suggesting that prolonged stress under high-salinity environments may limit CAT regulatory function. Current research shows that plant antioxidant enzyme systems function within certain limits, and when stress intensity exceeds plant capacity, enzyme activities decrease, showing a pattern of initial increase followed by decrease [**?**].

Conclusion

Under saline-alkali and other adversity stresses, the dynamic balance of active oxygen metabolism in plants is disrupted, promoting lipid peroxidation represented by MDA. Plants increase antioxidant enzyme activities (SOD, POD, CAT) to scavenge oxygen free radicals, which is crucial for plant adaptation and damage reduction. This study found that under different saline-alkali stress intensities, Jerusalem artichoke leaves showed increased content of soluble sugar, proline, and soluble protein, with significant differences at 150 days. As stress intensity increased, SOD, POD, and CAT activities also increased. However, with prolonged growth under stress, the increasing amplitude of CAT activity in the MS group showed a decreasing trend, indicating that long-term, high-intensity saline-alkali stress may limit CAT regulatory function.

In summary, under saline-alkali stress, Jerusalem artichoke can maintain water balance between plant cells and the external environment and preserve membrane structure integrity through regulation of organic osmotic substances and antioxidant enzyme system activities, thereby resisting impacts on plant metabolism and ensuring normal physiological activities. However, when stress intensity exceeds plant capacity, Jerusalem artichoke' s regulatory ability becomes inhibited to some extent.

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