Physiological Responses of Germinating Jerusalem Artichoke Tubers to Saline-Alkali Soil Stress (Postprint)

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Date: 2017-03-08T00:00:00+00:00

Abstract

Soil salinization represents a significant challenge to global agricultural production and ecological environments. Experimental plots were established in farmland, lightly saline-alkaline grassland, and severely saline-alkaline grassland for tuber planting of Jerusalem artichoke (Helianthus tuberosus). During the tuber germination stage in May of the subsequent year, tuber samples were collected for determination of malondialdehyde, free proline, and soluble sugar contents, as well as antioxidant enzyme activities, and subjected to proteomic analysis to investigate the physiological responses of germinating Jerusalem artichoke tubers to saline-alkaline soil stress. The electrical conductivity of the 0-20 cm soil layer, which characterizes soil soluble salt content, demonstrated a progressive intensification of saline-alkaline stress from farmland to lightly and severely saline-alkaline grasslands. Variations in malondialdehyde content reflected increasing damage severity in Jerusalem artichoke tubers, while the osmotic adjustment capacity based on free proline also exhibited a gradual enhancement. Proteomic analysis revealed that differentially expressed proteins associated with genetic information processing constituted the largest proportion (28.75%) and were predominantly up-regulated, suggesting that proteins involved in DNA replication and transcription, protein synthesis and folding play critical roles in the response to saline-alkaline stress. Additionally, substantial numbers of differentially expressed proteins were associated with carbohydrate and polysaccharide metabolism (15%), amino acid metabolism (11.25%), and energy metabolism (7.5%), indicating that modulation of metabolic homeostasis is crucial for germinating Jerusalem artichoke tubers to cope with saline-alkaline soil stress. These findings establish a foundation for elucidating the physiological mechanisms underlying the adaptation of germinating Jerusalem artichoke tubers to salt stress.

Full Text

Preamble

Acta Ecologica Sinica –ChinaXiv Partner Journal
Vol. 37, No. 4, Feb. 2017
DOI: 10.5846/stxb201610262181

Physiological Response of Sprouting Jerusalem Artichoke Tubers to Saline-Alkali Stress

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Abstract

Soil salinization represents a critical global challenge affecting agricultural production and ecological environments. Currently, saline-alkali soils occupy approximately one-third of Earth's land surface, and projections indicate that over 50% of arable land may become salinized by 2050. The combination of high salt content and elevated pH in these soils poses a major constraint to plant growth and crop productivity. This study investigated the physiological and molecular responses of Jerusalem artichoke (*Helianthus tuberosus* L.), a perennial herbaceous species valued for its high biomass and inulin-rich tubers, to saline-alkali stress during the critical tuber sprouting stage.

Tubers were planted in three distinct soil types: farmland (control), light saline-alkali grassland, and severe saline-alkali grassland, all located in a typical alkalinized grassland region of northeastern China. Sprouting tubers were harvested the following May for comprehensive analysis. We quantified malondialdehyde (MDA), free proline, and soluble sugar contents, measured antioxidant enzyme activities, and conducted proteomic profiling to elucidate the adaptive mechanisms of sprouting tubers to saline-alkali stress.

Soil electrical conductivity measurements (0–20 cm depth) confirmed a clear salinity gradient among the three sites, with farmland showing the lowest soluble salt content and severe saline-alkali grassland the highest. As soil salinity increased, MDA content in tubers rose significantly, indicating escalating membrane lipid peroxidation and stress damage. Concurrently, free proline accumulation increased progressively, demonstrating enhanced osmotic adjustment capacity. However, soluble sugar content showed no significant changes, suggesting it does not serve as a primary osmoticum during sprouting.

Proteomic analysis using two-dimensional gel electrophoresis (2-DE) identified over 1,000 reproducible protein spots. MALDI-TOF/TOF analysis successfully

identified 80 differentially expressed proteins (DEPs) between treatments. Among these, 42 DEPs were detected in light saline-alkali conditions and 38 in severe saline-alkali conditions. KEGG pathway annotation classified these proteins into eleven functional categories: genetic information processing (28.75%), carbohydrate and polysaccharide metabolism (15%), signal transduction (17.5%), amino acid metabolism (11.25%), energy metabolism (7.5%), secondary metabolite biosynthesis (3.75%), nucleotide metabolism (2.5%), transport and catabolism (2.5%), cell motility (2.5%), and unknown functions (8.75%).

Notably, proteins involved in genetic information processing were predominantly upregulated, including those associated with DNA replication, transcription, protein synthesis, and folding. This suggests that salt tolerance in sprouting tubers initiates at the gene expression level. Carbohydrate, energy, and amino acid metabolism-related proteins comprised one-third of all DEPs, highlighting the importance of metabolic homeostasis in stress adaptation. These findings provide novel insights into the molecular mechanisms underlying saline-alkali tolerance in Jerusalem artichoke during its critical establishment phase.

Keywords: Jerusalem artichoke (*Helianthus tuberosus*); sprouting tubers; physiological response; alkaline soil

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Introduction

Soil salinization constitutes one of the most pervasive abiotic stresses limiting global crop productivity and ecosystem stability. Saline-alkali soils currently occupy vast areas worldwide, with predictions that they may affect over half of all cultivated lands by mid-century. Developing salt-tolerant crops and vegetation is therefore essential for sustainable land use and food security. Jerusalem artichoke (*Helianthus tuberosus* L., Asteraceae) has emerged as a promising candidate due to its exceptional adaptability to marginal lands, substantial biomass production, and tubers rich in inulin—a valuable polysaccharide for bioenergy and biorefinery applications. The species exhibits remarkable tolerance to cold, drought, and nutrient-poor conditions, making it particularly suitable for ecological restoration of degraded saline-alkali grasslands.

Previous research has demonstrated that Jerusalem artichoke can naturally colonize moderately saline-alkali soils in the Songnen Plain of northeastern China, with select genotypes capable of growth and tuber production even under severe saline-alkali conditions. While the species primarily propagates vegetatively via tubers, sexual reproduction through seeds remains possible, though seed germination is highly sensitive to salt stress. Controlled experiments have shown that tuber sprouting exhibits superior salt tolerance compared to seed germination, and seedlings derived from tubers display greater stress resilience than those from seeds. This advantage likely stems from the abundant nutrient reserves stored in tubers. However, the underlying physiological mechanisms enabling

tuber sprouting under saline-alkali conditions remain poorly understood, particularly the molecular processes during this critical establishment phase.

Most previous physiological and ecological studies on salt stress in Jerusalem artichoke have focused on vegetative growth stages using seawater irrigation or coastal saline soil models. Research teams from Nanjing Agricultural University and the Yantai Institute of Coastal Zone Research have extensively investigated physiological responses and salt tolerance mechanisms across different ecotypes and cultivars. However, these studies have largely overlooked the tuber sprouting stage—a key bottleneck in population establishment. The present study addresses this knowledge gap by systematically analyzing the physiological and proteomic responses of sprouting Jerusalem artichoke tubers to gradient saline-alkali stress, aiming to elucidate the unique adaptive mechanisms that enable successful seedling establishment in hostile saline-alkali environments.

Materials and Methods

Study Site

The study was conducted at the Ecological Experimental Station of Zhaodong Jinyuan Animal Husbandry Co., Ltd., located in Taiping Village, Taiping Township, Zhaodong City, Heilongjiang Province (45°54 N, 125°55 E). The region experiences a cold temperate arid monsoon climate characterized by windy, dry springs; hot, rainy summers; and cold, dry winters. Mean annual precipitation ranges from 400–500 mm, with an average temperature of 3.1°C and 10°C accumulated temperature of 2700°C.

Experimental Design and Sample Collection

Three experimental plots were established based on vegetation status and soil salinity levels: farmland (control), light saline-alkali grassland, and severe saline-alkali grassland. Selected Jerusalem artichoke tubers were planted in each plot during spring 2013. The following May, at the tuber sprouting stage, sprouting tubers were excavated for analysis. Fresh tuber samples were collected for MDA, proline, soluble sugar, and antioxidant enzyme assays, while additional samples were frozen in liquid nitrogen for proteomic analysis. Soil samples were collected from 0–10 cm, 10–20 cm, and 20–30 cm depths, air-dried, and sieved through a 0.25 mm mesh for chemical characterization.

Soil Chemical Analysis

Soil salinity was characterized by measuring electrical conductivity (EC) of soil extracts using a DDS-307 conductivity meter. Soil pH was determined using a pH S-3C pH meter. Ion composition (CO^{2}, HCO, SO^{2}, Cl, Na, K, Ca^{2}, Mg^{2}) was analyzed by atomic spectrophotometry and ion chromatography.

Tuber Physiological Analysis

Fresh tuber samples were weighed (M1), oven-dried to constant weight (M2), and relative water content was calculated as [(M1-M2)/M1] × 100%. MDA content was determined using the method of Fei et al. [22]. Free proline content was measured according to Liu and Zhao [23]. Soluble sugar content was assayed following Zhang and Qu [24]. Antioxidant enzyme activities—superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD)—were measured using the protocols of Xia et al. [25].

Proteomic Analysis

Total soluble proteins were extracted from sprouting tubers using established laboratory methods [26]. Protein samples were separated by two-dimensional electrophoresis (2-DE) and gels were stained with Coomassie Brilliant Blue (CBB). Gel images were acquired using a Scanner III and analyzed with ImageMaster 2D Platinum software. Protein spots showing statistically significant differences ($p < 0.05$) with abundance ratios 1.5 were selected for identification.

Differentially expressed proteins were excised, digested, and identified by MALDI-TOF/TOF MS (Applied Biosystems/MDS Sciex, USA). Peptide mass fingerprinting data were searched against the MASCOT database (http://www.matrixscience.com). Identified proteins were functionally annotated and classified using KEGG pathway analysis (http://www.genome.jp/kegg/) and BLAST homology searches.

Statistical Analysis

All data were analyzed using SPSS Statistics v17.0 software. One-way ANOVA was performed to compare differences among treatment groups, with significance set at $p < 0.05$.

Results

Soil Chemical Properties

Soil electrical conductivity (0–20 cm depth) revealed significant differences in soluble salt content among the three plots, establishing a clear salinity gradient. The farmland soil exhibited the lowest EC, while the severe saline-alkali grassland showed the highest. The light saline-alkali grassland displayed intermediate values similar to farmland in the 10–20 cm layer but significantly lower than the severe saline-alkali plot. Since Jerusalem artichoke tubers and roots primarily inhabit the 0–20 cm soil profile, this layer' s average EC effectively characterized the stress gradient experienced by the plants.

Ion analysis showed that Na^+, K^+, Ca^{2+}, Mg^{2+}, CO_3^{2-}, HCO_3^-, SO_4^{2-}, and Cl^- concentrations followed similar trends, increasing progressively from farmland

to severe saline-alkali conditions. Soil pH values were correspondingly elevated in the saline-alkali plots compared to farmland.

[Figure 1: see original paper] Soil electrical conductivity and pH values in sampling plots

Physiological Responses of Sprouting Tubers

MDA, a product of membrane lipid peroxidation, serves as a reliable indicator of oxidative damage under stress. MDA content in sprouting tubers increased significantly with soil salinity, indicating progressively greater injury from farmland to light to severe saline-alkali conditions.

Free proline content exhibited a parallel increasing trend, demonstrating that Jerusalem artichoke tubers actively accumulate proline for osmotic adjustment to counteract salt and water stress during sprouting. In contrast, soluble sugar content showed no significant differences among treatments, suggesting that soluble sugars do not function as primary osmotic regulators during this developmental stage.

[Figure 2: see original paper] MDA, free proline, and soluble sugar contents in sprouting Jerusalem artichoke tubers

Salt stress induces oxidative stress in plant tissues, triggering changes in antioxidant enzyme activities to scavenge excess reactive oxygen species. We measured SOD, CAT, APX, and POD activities in sprouting tubers. While some differences were observed between light and severe saline-alkali plots, no clear consistent pattern emerged across all enzyme types.

[Figure 3: see original paper] Antioxidant enzyme activities in sprouting Jerusalem artichoke tubers

Proteomic Analysis of Sprouting Tubers

Proteomic comparison between saline-alkali plots and farmland identified numerous differentially expressed proteins. Using the criteria of $p < 0.05$ and abundance ratio 1.5, we identified 42 DEPs in light saline-alkali tubers (26 upregulated, 16 downregulated) and 38 DEPs in severe saline-alkali tubers (24 upregulated, 14 downregulated).

KEGG functional classification revealed that DEPs were distributed across eleven metabolic pathways. The largest functional group (28.75%) comprised proteins involved in genetic information processing, including DNA replication, transcription, translation, and protein folding. Most of these were upregulated, suggesting that enhanced protein synthesis and quality control are central to salt tolerance.

Carbohydrate and polysaccharide metabolism proteins (15%) included enolase (glycolysis), phosphogluconolactonase (pentose phosphate pathway), transketolase, and fructosyltransferase. Energy metabolism proteins (7.5%) comprised

ATP synthase subunits and oxidoreductases. Amino acid metabolism proteins (11.25%) included methionine synthase, cysteine synthase, and arginase. Signal transduction proteins (17.5%) featured auxin-induced proteins, dehydrins, and mitogen-activated protein kinases.

[Figure 4: see original paper] Differentially expressed proteins in sprouting Jerusalem artichoke tubers from alkaline grassland compared with farmland

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Discussion

The experimental design successfully established a salinity gradient, with soil electrical conductivity increasing progressively from farmland to light to severe saline-alkali grassland. This gradient effectively simulated natural conditions for investigating tuber responses to escalating stress.

The progressive increase in MDA content confirmed that membrane oxidative damage intensifies with salinity, while the concurrent rise in free proline indicates active osmotic adjustment. However, the proteomic data did not reveal upregulation of enzymes directly involved in proline metabolism, suggesting that proline accumulation may result from both enhanced synthesis and inhibited degradation, as reported in other species [17].

The predominance of DEPs related to genetic information processing is particularly noteworthy. Upregulation of aminoacyl-tRNA synthetases, elongation factors (EF-2), heat shock proteins (HSPs), and protein disulfide isomerases indicates that Jerusalem artichoke invests heavily in maintaining protein synthesis fidelity and proper folding under stress. Translation elongation factors are essential for ribosomal protein synthesis, and their increased expression likely preserves cellular function by ensuring continuous production of stress-responsive proteins. HSPs play crucial roles in protein quality control, assisting in folding nascent polypeptides and clearing damaged proteins, thereby contributing to osmotic stress tolerance.

Carbohydrate and energy metabolism proteins constituted another major functional group. Upregulation of glycolytic enzymes (enolase, phosphoglycerate mutase) and TCA cycle components (malate dehydrogenase) in saline-alkali plots suggests metabolic reprogramming to meet increased energy demands during stress adaptation. The observed upregulation of fructosyltransferase, which catalyzes fructan synthesis, aligns with findings in wheat where enhanced fructan production maintains cellular osmotic potential under salinity [30].

Amino acid metabolism proteins, including methionine and cysteine synthases, were differentially expressed, reflecting altered demands for protein synthesis and antioxidant production. The upregulation of dehydrins, a family of stress-responsive proteins, in severe saline-alkali conditions further indicates activation of protective mechanisms against cellular dehydration.

Overall, these results demonstrate that sprouting Jerusalem artichoke tubers employ a multi-faceted strategy to cope with saline-alkali stress, centered on maintaining genetic information processing integrity, reprogramming primary metabolism for energy and osmotic homeostasis, and activating protective protein networks. These findings lay a foundation for understanding the unique physiological mechanisms that enable Jerusalem artichoke establishment in saline-alkali soils and provide valuable targets for breeding improved salt-tolerant varieties.

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