Physiological Responses of Piedmont Plants on the Northern Slope of the Tianshan Mountains to Drought Stress (Postprint)

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Abstract

To screen suitable plant species for vegetation restoration in the front-range zone of the northern Tianshan Mountains, Cara gana frutex, Hippophae rhamnoides, Rosa, and Xanthoceras sorbifolia were selected as experimental materials under two restoration methods (contour trenches and fish-scale pits). The temporal variation of soil moisture after irrigation was observed, and the trends in leaf relative water content, chlorophyll content, protective enzyme activities, and osmotic adjustment substances of the four plant species under varying soil moisture conditions were analyzed to comprehensively evaluate their drought resistance. The results showed that with decreasing soil moisture, both leaf relative water content and chlorophyll content decreased significantly, with the decline rate of chlorophyll content in the four plants under fish-scale pits being higher than that under contour trenches. The SOD and POD enzyme activities of the four plants exhibited a characteristic pattern of initial increase, followed by decrease, and finally returning to initial levels with intensifying water stress, though the timing of peak activity differed among species. The contents of soluble protein, malondialdehyde, and proline in the four plants showed varying degrees of upward trends with increasing water stress, with significant differences in their changes. Comprehensive evaluation of drought resistance capacity based on principal component analysis of seven physiological indicators for the four plants indicated that contour trenches are more suitable for vegetation restoration in this region, and Cara gana frutex and Rosa showed better adaptability to drought conditions.

Full Text

Abstract

To screen suitable plant species for vegetation restoration in the front mountain zone of the Northern Piedmont of the Tianshan Mountains, we conducted a field experiment using *Caragana frutex*, *Hippophae rhamnoides*, *Rosa*, and *Xanthoceras sorbifolia* as test materials under two restoration methods: level furrow and fish-scale pit. We observed temporal changes in soil moisture after irrigation and analyzed trends in leaf relative water content, chlorophyll content, protective enzyme activities, and osmotic adjustment substances in response to soil moisture variation, thereby evaluating the drought resistance of the four plant species. The results demonstrated that with decreasing soil moisture, both leaf relative water content and chlorophyll content decreased significantly, with the decline rate of chlorophyll content in fish-scale pit treatments exceeding that in level furrow treatments. The activities of superoxide dismutase (SOD) and peroxidase (POD) in the four plant species initially increased, then decreased, and finally returned to initial levels as water stress intensified, though the timing of peak activity varied among species. Soluble protein, malondialdehyde (MDA), and proline contents showed varying degrees of upward trends with increasing water stress, with significant differences observed. Principal component analysis (PCA) of seven physiological indicators revealed that level furrow restoration is more suitable for this region, while *Caragana frutex* and *Rosa* exhibited superior drought resistance suitability.

Keywords: vegetation restoration; drought resistance; Northern Piedmont of the Tianshan Mountains; plant physiology; restoration methods; drought resistance evaluation

Introduction

The front mountain zone of the Northern Piedmont of the Tianshan Mountains constitutes an important component of the mountain ecosystem in Xinjiang, encompassing the area from the coniferous forest line to the oasis foothills on the northern slopes. This region is characterized by sparse forest resources, simple species composition, poor soil conditions, exposed bedrock, and concentrated precipitation that often leads to torrential erosion during spring and summer. In recent years, severe anthropogenic disturbances have impaired the ecosystem's self-repair capacity, significantly compromising its water conservation and soil retention functions, which in turn threatens the sustainable development of the ecological environment in this critical area. As vegetation restoration efforts in Xinjiang have expanded to challenging sites such as gobi, barren hills, and deserts beyond oasis peripheries, the front mountain zone of the Tianshan Northern Piedmont is poised to become a key focus for large-scale vegetation restoration initiatives. Implementing scientific experiments and demonstrations through artificial interventions in this region will not only increase forest vegetation coverage in national forest protection project areas, thereby improving regional ecological conditions and enhancing forest ecosystem services, but also alleviate grazing pressure on natural grasslands, gradually restoring and improving their ecological functions. This approach is of significant scientific importance for enhancing the stability of the mountain grassland-forest ecosystem and fully realizing its soil conservation, windbreak, sand fixation, and water source 涵养 functions.

Current research on ecological restoration methodologies in arid regions primarily focuses on vegetation recovery in mountainous and plain areas with difficult site conditions. A critical step in vegetation ecological restoration engineering involves slope preparation measures, such as level terraces, level furrows, level steps, reverse-slope terraces (triangular level furrows), fish-scale pits, and hole planting. These structures intercept precipitation and surface runoff to meet plant survival and growth requirements. While fish-scale pits possess water retention capacity, their small size limits drought resistance effectiveness. Level furrows can intercept substantial runoff and conserve water and soil, but excessively close spacing often causes collapse pits. Previous studies indicate that *Pinus sylvestris* var. *mongolica* seedlings grow better under level furrow conditions than in fish-scale pits. Different restoration conditions produce varying plant growth performance and drought tolerance characteristics. Plant drought resistance research primarily focuses on physiological responses to water variation, as drought stress causes reactive oxygen species accumulation that damages plants. Increased malondialdehyde (MDA) content reflects cell membrane damage, while proline accumulation prevents cellular dehydration. Most existing drought resistance studies rely on pot experiments monitoring physiological indicators under different drought stresses, employing various statistical methods such as cluster analysis, membership function analysis, and principal component analysis for comprehensive evaluation. However, few studies have investigated the dynamic changes in plant physiology and biochemistry throughout an entire growing season under different vegetation restoration methods through field plot experiments, particularly under drought stress. No reports have addressed plant drought resistance in the unique climatic environment of the Tianshan Northern Piedmont front mountain zone.

1 Materials and Methods

1.1 Study Area Overview

The study area is located in the front mountain zone of the Northern Piedmont of the Tianshan Mountains, within the jurisdiction of the Manas South Mountain Branch of the Tianshan Eastern State-owned Forest Management Bureau, specifically at the Xiangya Terrace slope of the Shimenzi Management Station (86°06 08.74 -86°06 14.61 E, 43°53 31.48 -43°53 40.61 N). The region features a typical temperate continental arid climate with an average annual temperature of 7.8°C, January mean temperature of -24.7°C, July mean temperature of 17.7°C, maximum temperature of approximately 38°C, annual precipitation of 134 mm, and a frost-free period of 165–172 days. Vegetation coverage is low, dominated by *Caragana frutex*, *Berberis amurensis*, *Spiraea hypericifolia*, and *Rosa*. The soil type is chestnut soil with a field capacity of 23.4% and wilting coefficient of 8.7%.

1.2 Experimental Materials

We selected sites with similar altitude, slope, and aspect for establishing experimental plots using two restoration methods: level furrow and fish-scale pit, with *Caragana frutex*, *Hippophae rhamnoides*, *Rosa*, and *Xanthoceras sorbifolia* as study subjects. The slope between different restoration methods ranged from 5° to 10°. All test plants were 2-year-old seedlings of each species, from which we selected normally growing, pest-free, representative individuals for sampling. For each species, three seedlings were selected, and 30 healthy, intact leaves were collected from consistent positions (from the top of branches downward) during each sampling period.

Both restoration methods were constructed as illustrated in [Figure 1: see original paper]. Level furrows were dug along contour lines in relatively flat areas, with excavated soil piled on the downhill edge of the furrow. The furrow sides were shaped into slopes, cross-dams were built within the furrows, and the slopes and cross-dams formed a double-slope water collection area. Level furrow specifications were 150 cm × 80 cm × 80 cm (length × width × depth). Fish-scale pits were excavated along contour lines using small machinery, with excavated soil piled on the downhill edge to form a semi-circular water retention berm. Fish-scale pit specifications were 150 cm × 80 cm × 50 cm (top width × depth). In early June, plots were fully irrigated and allowed to dry naturally. Soil moisture was measured monthly, and plant leaves were collected simultaneously and immediately frozen in liquid nitrogen for preservation. In this study, soil water content on June 15 was designated as normal level (T1), July 15 as early stress stage (T2), August 15 as mid stress stage (T3), and September 15 as late stress stage (T4).

1.3 Experimental Methods

1.3.1 Soil Water Content Observation Soil volumetric water content served as the standard for drought classification. After field investigation, we found that plant root depth distribution ranged from 0–70 cm, with main root zones at 0-25 cm, 25-50 cm, and 50-75 cm. Continuous observations were conducted in these layers, with probes installed at each gradient. Daily soil water content values were calculated as the average of measurements from all probes.

1.3.2 Physiological Index Measurement Leaf relative water content (RWC) was measured using standard methods. Chlorophyll content was determined using the ethanol extraction colorimetric method. Superoxide dismutase (SOD) activity was measured using the nitroblue tetrazolium method, and peroxidase (POD) activity using the guaiacol colorimetric method.

Malondialdehyde (MDA) content was determined using the thiobarbituric acid method, proline content using the acidic ninhydrin colorimetric method, and soluble protein content using the Coomassie brilliant blue method.

1.4 Data Analysis

Data were analyzed using Excel 2016 and SPSS 24.0 software. One-way ANOVA and Duncan's multiple comparison tests were used to examine differences among restoration methods and physiological indicators under different water stresses, with significance level set at $\alpha = 0.05$. To comprehensively evaluate plant drought resistance, principal component analysis (PCA) was applied to seven physiological indicators. Raw data were standardized (Z-score method) before analysis. Bartlett' s test was conducted to verify applicability, revealing significant correlations among drought resistance indicators and confirming the suitability of PCA. Based on principal component scores weighted by variance contribution rates, comprehensive drought resistance scores were calculated, with higher scores indicating stronger drought resistance.

2 Results

2.1 Characteristics of Soil Moisture Changes in Different Plant Root Zones

Soil water content changes after water control are shown in [Figure 2: see original paper]. No significant differences in soil moisture changes were observed among different plants immediately after irrigation ($P > 0.05$). However, as the release time extended, significant differences in water content emerged throughout the growing season ($P < 0.05$). Soil moisture in the root zones of all four plant species showed an overall decreasing trend with time under both restoration methods. Under level furrow conditions, *Caragana frutex* and *Rosa* showed the smallest soil water content decline, while *Xanthoceras sorbifolia* in fish-scale pits showed the largest decline. Level furrow treatments exhibited water content reductions of 27-31%, with *Rosa* showing the smallest decline; fish-scale pit treatments showed reductions of 33-43%, with *Caragana frutex* showing the largest decline. Under T4 treatment, level furrow soil moisture decreased by 21-28%, while fish-scale pit moisture decreased by 19-27%, with *Caragana frutex* in level furrows showing the maximum decline and *Rosa* in fish-scale pits showing the minimum. Overall, level furrow soil moisture decline (72-76%) was lower than that of fish-scale pits (68-70%), and at the end of the water stress period, level furrow soil moisture content was higher than in fish-scale pits.

2.2 Changes in Plant Physiological Indicators Under Water Stress

2.2.1 Changes in Leaf Relative Water Content and Chlorophyll Content Changes in leaf relative water content of the four plant species under different water stresses are shown in [Figure 3: see original paper]. With prolonged water stress, leaf relative water content showed varying degrees of decline

(P < 0.05), particularly evident during T1–T2 treatment (P < 0.05). Level furrow *Caragana frutex* maintained relatively stable leaf water content throughout the stress cycle (P > 0.05), while level furrow *Rosa* showed the most significant changes. At T4 treatment, leaf relative water content of all four species was higher in level furrows than in fish-scale pits.

Chlorophyll content changes under different water stresses are illustrated in [Figure 4: see original paper]. Chlorophyll content in all four species showed an overall decreasing trend with intensifying water stress. *Caragana frutex* showed no significant differences throughout the growing season under either method (P > 0.05), while level furrow *Rosa* exhibited the most pronounced chlorophyll decline. Overall, chlorophyll content changes differed significantly between restoration methods (P < 0.05). Under level furrow, chlorophyll content decreased by 37.3%, 42.7%, 96.5%, and 63.6% for the four species respectively; under fish-scale pit, decreases were 32.6%, 34.3%, 78.6%, and 54.8% respectively. The percentage decline in chlorophyll content was significantly higher in fish-scale pits than in level furrows (P < 0.05).

2.2.2 Changes in Osmotic Adjustment Substances Malondialdehyde (MDA) content in all four species under both restoration methods showed fluctuating increasing trends (P < 0.05), with significant differences between methods (P < 0.05). Peak MDA content occurred at different times: *Caragana frutex* and *Hippophae rhamnoides* peaked at T3, while *Rosa* peaked at T2. *Xanthoceras sorbifolia* showed consistently increasing MDA content, rising by 44–48%.

Proline content exhibited fluctuating patterns under different restoration methods, with significant differences between level furrow and fish-scale pit treatments (P < 0.05). Under normal conditions (T1), proline content in fish-scale pits was 1.5 times that in level furrows, with significant differences (P < 0.05). *Caragana frutex*, *Hippophae rhamnoides*, and *Xanthoceras sorbifolia* showed increasing trends, peaking at T3 before gradually declining. *Rosa* showed an increasing trend, peaking at T2. Under T3 treatment, proline content increases in level furrow *Hippophae rhamnoides*, *Rosa*, and *Xanthoceras sorbifolia* reached 707.03%, 891.94%, and 591.94% respectively.

Soluble protein content changes are shown in [Figure 7: see original paper]. *Caragana frutex* and *Rosa* peaked at T2, while *Hippophae rhamnoides* reached maximum at T3, after which all gradually decreased. Level furrow *Xanthoceras sorbifolia* peaked at T3, while fish-scale pit *Xanthoceras sorbifolia* peaked at T2. Overall, when facing drought, the four species in level furrows showed smaller changes in soluble protein content than those in fish-scale pits, indicating that plants in level furrows could adapt to drought stress through minor adjustments in protein content.

2.2.3 Changes in Antioxidant Enzyme Activities The effects of water stress on SOD activity are presented in [Figure 8: see original paper]. SOD activity in all four species showed an initial increase followed by a decrease, with significant differences among species ($P < 0.05$). *Rosa* exhibited the most significant changes, with level furrow *Rosa* reaching a maximum of 707.03 $U \cdot g^{-1}$ at T3. When subjected to drought stress, SOD activity in *Hippophae rhamnoides* and *Xanthoceras sorbifolia* under both methods showed negative growth rates.

POD activity changes are shown in [Figure 9: see original paper]. POD activity in all four species showed an initial increase followed by a decrease, with significant differences between restoration methods ($P < 0.05$). *Caragana frutex* and *Rosa* in both methods peaked at T3, while *Hippophae rhamnoides* peaked at T2. Level furrow *Hippophae rhamnoides* gradually decreased with prolonged drought, while fish-scale pit *Hippophae rhamnoides* increased after T2. *Xanthoceras sorbifolia* in both methods showed an initial increase followed by a decrease, with level furrow peaking at T3 and fish-scale pit at T2.

2.3 Comprehensive Evaluation of Plant Drought Resistance Under Two Restoration Methods

Principal component analysis of seven physiological indicators under different water stresses yielded eigenvalues, contribution rates, and cumulative contribution rates for each plant species (Table 2). The first two principal components explained 84.94% of the total variance, indicating that extracting these two components could comprehensively represent the information from the seven drought resistance indicators.

Analysis of the principal component expressions revealed that all indicators except average chlorophyll content and average POD activity showed positive loadings on PC1, with leaf relative water content, chlorophyll content, proline content, and SOD activity having high loadings. This indicates that PC1 primarily reflects information about these indicators. The highest loading in PC1 was for average leaf relative water content (X_1), suggesting PC1 mainly reflects leaf water status information. PC2 showed the highest loading for average soluble protein content (X_7), indicating it primarily reflects soluble protein information.

Using the ratio of each principal component's eigenvalue to the sum of extracted eigenvalues as weights, we established a comprehensive evaluation model (Table 3). The comprehensive scores showed that under both restoration methods, the drought resistance ranking was: *Caragana frutex* > *Rosa* > *Hippophae rhamnoides* > *Xanthoceras sorbifolia*. However, the comprehensive score for level furrow (0.45) was higher than for fish-scale pit (0.27), demonstrating that level furrow restoration is more suitable for this region, with *Caragana frutex* and *Rosa* showing superior drought resistance adaptability.

3 Discussion

3.1 Selection of Vegetation Ecological Restoration Methods

Both level furrow and fish-scale pit can intercept slope runoff and erosion sediment, but level furrows demonstrate superior water and soil conservation effects.

Our results indicate that across the entire growing season, soil moisture decline in level furrows was lower than in fish-scale pits, and at the end of the water stress period, level furrow soil moisture content remained higher. Level furrows have larger volume and stronger surface runoff interception capacity, while furrow walls reduce soil water evaporation. Fish-scale pits, being semi-circular, are more suitable for afforestation and vegetation restoration in areas with low annual precipitation. In our study area, although the volumes differed, the greater depth of level furrows significantly improved water retention capacity. Level furrows also altered the local microenvironment, reducing surface soil nutrient loss compared to fish-scale pits, which perform better on steeper slopes.

3.2 Relationship Between Plant Physiological Indicators and Water Stress

Plant physiological indicators are sensitive to environmental changes and life cycles. The increase or decrease of some physiological indicators in mid-June plants (particularly in Xinjiang mountainous areas) may not be entirely caused by water stress but could be related to decreasing environmental temperatures. Our study showed that with prolonged water stress, leaf relative water content of the four species decreased to varying degrees under both restoration methods. *Caragana frutex* showed the greatest variation in leaf relative water content throughout the stress cycle, yet maintained higher levels than other species at the end of the drought period, suggesting strong drought resistance. Chlorophyll content reflects photosynthetic capacity and stress resistance. Numerous studies show that drought stress reduces chlorophyll content, as confirmed by our results where *Hippophae rhamnoides* showed the greatest chlorophyll decline. The rapid decrease may result from severe water loss weakening chlorophyll synthesis, while reactive oxygen species accumulation damages chloroplast membrane structures.

MDA, proline, and soluble protein are important osmotic adjustment substances. Our results showed that these compounds increased with drought stress duration. This aligns with findings that proline content correlates positively with drought resistance. The fluctuating patterns of SOD and POD activities—initially increasing then decreasing—reflect plant defense mechanisms. During early drought stress, plants enhance antioxidant enzyme activities to prevent oxidative damage and maintain normal physiological function. However, prolonged severe stress disrupts oxidative metabolism, accelerating reactive oxygen generation and accumulation, which intensifies membrane lipid peroxidation and damages membrane integrity, consequently reducing enzyme activities.

3.3 Screening of Drought-Resistant Plants

Plants employ biological characteristics to prevent water loss, maintain water balance, and rapidly accumulate osmotic substances like proline, MDA, and soluble protein to maintain cellular osmotic pressure. They also regulate antioxidant enzyme activities to scavenge reactive oxygen radicals, sustaining normal physiological activities to resist drought. Our comprehensive evaluation using PCA indicated that *Caragana frutex* and *Rosa* possess better drought resistance, consistent with previous studies using membership function and PCA methods. However, this study analyzed drought resistance based on limited physiological indicators, which presents certain limitations. Comprehensive evaluation should incorporate phenotypic traits, growth dynamics, and photosynthetic characteristics for more scientific assessment, providing a robust theoretical basis for vegetation restoration in the Tianshan Northern Piedmont front mountain zone.

4 Conclusions

Based on our study of drought resistance in four plant species under level furrow and fish-scale pit restoration methods, we conclude:

1) Throughout the growing season, significant differences in physiological indicators emerged between the two restoration methods. Level furrows exhibited lower soil moisture decline than fish-scale pits, and maintained higher soil moisture content at the end of the water stress period.

2) As soil moisture decreased, leaf relative water content and chlorophyll content decreased significantly. The percentage decline in chlorophyll content was greater in fish-scale pits than in level furrows. Antioxidant enzyme activities initially increased then decreased, eventually returning to initial levels with prolonged drought stress. Osmotic adjustment substances showed varying degrees of increase with intensifying drought stress.

3) Principal component analysis of seven physiological indicators for comprehensive drought resistance evaluation revealed the ranking: *Caragana frutex* > *Rosa* > *Hippophae rhamnoides* > *Xanthoceras sorbifolia*. Level furrow restoration proved more suitable for this region, with *Caragana frutex* and *Rosa* demonstrating superior drought resistance adaptability.

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