Responses of Osmotic Adjustment Substances and Antioxidant Enzyme Activities in Gymnocarpos przewalskii to Drought (Postprint)

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Abstract

Gymnocarpos przewalskii, originating from the ancient Mediterranean, is a rare Tertiary relict plant species in desert regions. This study analyzed changes in leaf osmotic adjustment substances and antioxidant enzyme activities under natural drought using leaves collected from middle-aged and old-aged plants in Anxi Nature Reserve, Gansu, in June, July, and August 2017. The results demonstrated that with increasing drought severity, soluble protein (SP) in G. przewalskii leaves accumulated significantly, playing an important role in osmotic adjustment; soluble sugar (SS) content in middle-aged plant leaves and proline (Pro) content in old-aged plant leaves first increased then decreased significantly, indicating limited osmotic adjustment capacity. As drought intensified, superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities in middle-aged plant leaves exhibited obvious initial increases followed by decreases. Under severe drought, peroxidase (POD) and catalase (CAT) activities were significantly enhanced, suggesting that excess reactive oxygen species were scavenged through the synergistic action of these four enzymes to alleviate oxidative damage; CAT activity in old-aged plant leaves continued to increase, APX activity showed an obvious initial increase followed by a decrease, and POD activity was significantly enhanced under severe drought, primarily mitigating membrane system damage through these three enzymes. Malondialdehyde (MDA) content increased significantly with increasing drought severity, with progressively deepening membrane lipid peroxidation. Under identical rainfall conditions, significant differences existed in the response degree of Pro, SS, and SP contents, POD and CAT activities, and MDA content to drought between middle-aged and old-aged G. przewalskii plants. The membership function evaluation method indicated that the drought resistance capacity of old-aged plants was greater than that of middle-aged plants, demonstrating that the drought resistance of G. przewalskii increases with age.

Full Text

Response of Osmotic Regulation Substances and Antioxidant Enzyme Activity in Leaves of *Gymnocarpos przewalskii* to Drought

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Abstract: *Gymnocarpos przewalskii* is a Tertiary relict species in desert areas that originated from the Tethys region. In this study, leaves were collected from medium-aged and old-aged individuals in the Anxi Nature Reserve of Gansu Province during June, July, and August 2017 to analyze changes in osmotic regulation substances and antioxidant enzyme activity under natural drought conditions. The results showed that soluble protein (SP) content accumulated significantly with increasing drought levels and played the primary role in osmotic regulation. Soluble sugar (SS) content in medium-aged individuals and proline (Pro) content in old-aged individuals initially increased then decreased markedly, indicating limited osmotic regulation. For medium-aged individuals, superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities increased significantly at first then decreased as drought intensified, while peroxidase (POD) and catalase (CAT) activities increased significantly under severe drought, suggesting these four enzymes scavenged reactive oxygen species synergistically. For old-aged individuals, CAT activity increased continuously, APX activity increased then decreased obviously, and POD activity increased significantly under severe drought, indicating that membrane system damage was mainly alleviated by these three enzymes. Malondialdehyde (MDA) content in leaves increased significantly with drought level, reflecting enhanced membrane lipid peroxidation. Under similar rainfall conditions, significant differences existed between medium-aged and old-aged individuals in their responses of Pro, SS, SP content, POD and CAT activities, and MDA content to drought. Subordinate function analysis revealed that old-aged individuals had higher drought resistance than medium-aged ones, indicating that drought resistance in *G. przewalskii* enhances with age.

Keywords: *Gymnocarpos przewalskii*; natural drought; osmotic regulation substance; antioxidant enzyme activity; Gansu Province

1 Materials and Methods

1.1 Study Area

The study site is located at 40°29 53 N, 95°44 12 E in the Anxi Extra-arid Desert National Nature Reserve, Gansu Province. The region has an annual precipitation of 45.7 mm, annual evaporation of 3140.6 mm, and mean annual temperature of 8.8°C. The elevation is 1170 m, with typical desert vegetation. Soil moisture content was measured at 10-day intervals from June to August 2017. Soil samples were collected at depths of 0–60 cm using a soil auger (10 cm diameter). Soil moisture content was calculated using the drying method, with the formula:

$$P = -1.342 + 10.68 \times \text{soil moisture}, \quad R^2 = 0.537, \quad P < 0.01$$

Soil moisture content under *G. przewalskii* canopies ranged from $(5.1 \pm 0.73)\%$ to $(1.6 \pm 0.60)\%$, with corresponding ages of 53 and 15 years. The canopy diameter ranged from 88.1 cm to 42.5 cm. According to reference [15], *G. przewalskii* individuals aged 20–60 years were classified as medium-aged, while those over 60 years were classified as old-aged.

1.3 Experimental Methods

1.3.1 Determination of Osmotic Regulation Substances Soluble sugar (SS), proline (Pro), and soluble protein (SP) contents were measured following the methods described in reference [16]. Fresh leaf samples (0.3 g) were ground in 5 mL of phosphate buffer (pH 7.8) containing 5 mmol·L ^{1} EDTA, 2 mmol·L ^{1} ascorbic acid, and 2% PVP. The homogenate was centrifuged at 12,000 r·min ^{1} for 20 min at 4°C, and the supernatant was collected for analysis. Soluble protein content was determined using the Coomassie brilliant blue G-250 method [17].

1.3.2 Determination of Antioxidant Enzyme Activities Superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) activities were measured according to reference [16]. Fresh leaf samples (0.3 g) were extracted using the same method described in section 1.3.1. Enzyme activities were determined using spectrophotometric methods: SOD by the photochemical NBT reduction method, POD by the guaiacol method, CAT by the UV absorption method, and APX by the ascorbate oxidation method.

1.3.3 Determination of Malondialdehyde Content Malondialdehyde (MDA) content was measured using the thiobarbituric acid (TBA) method as described in reference [16].

1.4 Statistical Analysis

All data were analyzed using SPSS 19.0 software. One-way ANOVA was performed, and mean comparisons were conducted using Duncan' s multiple range

test at the significance level of = 0.05. Figures were prepared using Microsoft Excel 2007. Subordinate function analysis was used for comprehensive evaluation of drought resistance [19].

2 Results

2.1 Rainfall Characteristics

Rainfall data from 2017 showed that June received 11.3 mm, July received 5.1 mm, and August had minimal precipitation at 2.1 mm [FIGURE 1]. This pattern indicated progressively increasing drought stress from June through August.

2.2 Changes in Osmotic Regulation Substances

Proline content in both medium-aged and old-aged individuals initially increased then decreased with intensifying drought, peaking in July [FIGURE 3]. Soluble sugar content in medium-aged individuals showed a similar pattern, with the highest values in July [FIGURE 4]. In old-aged individuals, soluble sugar content was highest in June and decreased thereafter. Soluble protein content increased significantly with drought severity in both age groups, with the highest values observed in August [FIGURE 5]. In medium-aged individuals, soluble protein content increased by 4.8-fold and 2.8-fold in August compared to June and July, respectively. The accumulation rate of soluble protein was significantly higher in old-aged individuals than in medium-aged individuals under severe drought conditions.

2.3 Changes in Antioxidant Enzyme Activities

Superoxide dismutase (SOD) activity in medium-aged individuals increased initially then decreased, peaking in July, while in old-aged individuals, SOD activity decreased progressively [FIGURE 6]. The SOD activity in medium-aged individuals was 2.4 times higher than in old-aged individuals in July. Peroxidase (POD) activity in both age groups increased with drought intensity, with significantly higher activity in August [FIGURE 7]. Under severe drought, POD activity in old-aged individuals was 42.1% higher than in medium-aged individuals. Catalase (CAT) activity in medium-aged individuals increased significantly under severe drought, while in old-aged individuals, CAT activity increased continuously throughout the drought period [FIGURE 8]. Ascorbate peroxidase (APX) activity in both age groups initially increased then decreased, with medium-aged individuals showing higher activity in June and July, while old-aged individuals maintained relatively stable activity [FIGURE 9].

2.4 Changes in Malondialdehyde Content

Malondialdehyde (MDA) content increased significantly with drought severity in both age groups [FIGURE 10]. Under severe drought in August, MDA content

in old-aged individuals was 1.6, 3.8, and 3.1 times higher than in June, while in medium-aged individuals it was 7.2 times higher. The rate of MDA accumulation was lower in old-aged individuals compared to medium-aged individuals, indicating less membrane damage in older plants.

2.5 Comprehensive Evaluation of Drought Resistance

Subordinate function analysis was used to comprehensively evaluate drought resistance between the two age groups [TABLE 1]. The results showed that old-aged individuals had higher comprehensive drought resistance values than medium-aged individuals across all drought levels, indicating that drought resistance in *G. przewalskii* increases with age.

3 Discussion

Drought stress induces the accumulation of osmotic regulation substances and enhances antioxidant enzyme activities in plants [25]. Proline, soluble sugars, and soluble protein are important osmotic regulators that maintain cell turgor pressure under water deficit [26]. In this study, soluble protein was the primary osmotic regulation substance in *G. przewalskii*, accumulating continuously with increasing drought stress. The initial increase then decrease of proline and soluble sugar contents suggested that osmotic regulation capacity was limited under severe drought conditions.

Antioxidant enzymes including SOD, POD, CAT, and APX constitute the primary defense system against reactive oxygen species (ROS) under drought stress [27]. The synergistic action of these enzymes maintains ROS homeostasis and protects membrane systems from oxidative damage. In medium-aged individuals, the coordinated response of all four enzymes was observed, while in old-aged individuals, CAT, POD, and APX played more prominent roles. The continuous increase in CAT activity in old-aged individuals indicated a more robust scavenging system for hydrogen peroxide.

Malondialdehyde content serves as an indicator of membrane lipid peroxidation and cell damage [31]. The lower accumulation rate of MDA in old-aged individuals compared to medium-aged individuals under severe drought suggested that older plants had more effective antioxidant defense systems and greater membrane stability.

Age-related differences in drought resistance have been reported in various plant species [38]. The enhanced drought resistance in old-aged *G. przewalskii* individuals may be attributed to better root system development, more efficient water use strategies, and stronger antioxidant defense mechanisms developed through long-term adaptation to the arid environment. These findings suggest that conservation efforts should prioritize protection of older individuals, which serve as critical genetic resources for drought adaptation in this relict species.

TABLE 1 Comprehensive comparison results of drought resistance of medium- and old-aged individuals

Index	Medium-aged	Old-aged
Pro content	0.5371	0.4943
SS content	0.3972	0.5643
SP content	0.3887	0.3474
SOD activity	0.4713	0.5028
POD activity	0.4629	0.5615
CAT activity	0.5824	0.6239
APX activity	0.6439	0.3694
MDA content	0.4164	0.4715
Comprehensive value	0.5975	0.5333

Note: Higher values indicate better drought resistance for all indices except MDA, where lower values indicate better resistance.

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