Postprint of Osmotic Adjustment and Antioxidant System in Halostachys caspica Seedlings under Different Salt Concentration Treatments

Authors: Feng Xiaoli, Fan Shoude, Zhou Lianjie, Zhang Fuchun, Wang Yan, Wang Yan

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Abstract

This study aimed to analyze and compare the osmotic adjustment and antioxidant defense systems of Halostachys caspica at different developmental stages under various salt concentrations by measuring relative water content, lipid peroxidation (MDA), inorganic ions (Na+, K+, and Ca2+), organic osmotic adjustment substances (proline and betaine), and antioxidant indices (SOD, CAT, APX, POD, AsA, and GSH) in 2-month-old seedlings and 6-month-old mature plants treated with NaCl concentrations of 0, 100, 300, and 500 mmol · L-1 for 15 days, thereby providing a basis for the research, development, and utilization of H. caspica as well as ecological environment management in Xinjiang. The results showed that under high salt stress of 500 mmol · L-1, the relative water content of both H. caspica seedlings and mature plants decreased significantly compared with the control group; combined analysis of relative water content and MDA content indicated that 100 mmol · L-1 and 300 mmol · L-1 NaCl did not cause stress to H. caspica; in the osmotic adjustment system, inorganic ion Na+ and organic substance betaine (GB) played dominant roles in the osmotic adjustment of H. caspica seedlings and mature plants, respectively; in the antioxidant system, except for the increased ascorbic acid (AsA) content in H. caspica mature plants under 500 mmol · L-1 treatment, the activities of superoxide dismutase (SOD) and CAT and the content of glutathione (GSH) remained at high levels with minimal changes as salt concentration increased; correlation coefficient analysis revealed that AsA and SOD played important antioxidant roles in H. caspica seedlings and mature plants, respectively. Thus, the Xinjiang forage halophyte H. caspica employs different osmotic adjustment and antioxidant strategies at different growth stages during the seedling period to cope with saline habitats.

Full Text

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Osmotic and Antioxidant System in *Halostachys caspica* Seedlings under Salt Stress

Abstract: The objective of this research was to better understand the mechanism of osmoregulation and antioxidant response of *Halostachys caspica* seedlings growing under salt stress. The effects of NaCl-salinity treatments (0, 100, 300 and 500 mmol · L ^{1} NaCl for 15 days) on water content, lipid peroxidation (MDA), organic (proline and glycine betaine) and inorganic osmolytes (Na , K and Ca^{2}), and antioxidant defenses (SOD, CAT, APX, POD, AsA and GSH) in seedlings and grown-up seedlings of *H. caspica* were investigated. The research on the development and utilization of *H. caspica* and the improvement of ecological environment in Xinjiang was carried out. Water content in the seedlings and grown-up seedlings of *H. caspica* growing under high-salt (500 mmol · L ^{1} NaCl) stress was significantly decreased compared with those under control. The data of water content and MDA content revealed that salt stress on *H. caspica* plants was not induced under the conditions of 100 mmol · L ^{1} and 300 mmol · L ^{1} NaCl. Na and GB played the dominant roles in osmoregulation of seedlings and grown-up seedlings. With increasing salt concentration, the activities of SOD and CAT as well as the content of GSH were maintained at a high level all along except AsA content of grown-up seedlings under 500 mmol · L ^{1} NaCl treatment was increased. The correlation coefficient analysis showed that AsA and SOD played an important role in antioxidation of seedlings and grown-up seedlings of *H. caspica*. It could be seen that the osmotic adjustments and antioxidant strategies were different at different growth stages of *H. caspica* seedlings so as to adapt salt stress.

Keywords: *Halostachys caspica*; NaCl treatment; osmolytes; antioxidants system; mechanism of salt tolerance

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1 Materials and Methods

1.1 Plant Materials and Salt Stress Treatments

Halostachys caspica seeds were collected from the Fukang Desert Ecosystem Observation and Experiment Station, Chinese Academy of Sciences. After sterilization and washing, seeds were germinated in petri dishes and then transferred to plastic pots containing Hoagland nutrient solution. Seedlings at the 4-leaf stage were treated with NaCl at concentrations of 0 (control), 100, 300, and 500 mmol · L ^{1} for 15 days. Each treatment was replicated three times.

1.2 Determination of Physiological and Biochemical Indices

Water Content Measurement: Fresh assimilating branches (0.15 g) were sampled, weighed, oven-dried at 105°C for 30 minutes, then dried at 80°C to constant weight. Water content was calculated as: (fresh weight - dry weight) / fresh weight × 100%.

MDA Content Determination: MDA content was measured using the thiobarbituric acid (TBA) method. Fresh samples (0.2 g) were homogenized in 10% trichloroacetic acid (TCA), centrifuged at 4000 r · min ^{1} for 20 minutes. The supernatant was mixed with 0.6% TBA, heated in boiling water for 15 minutes, then cooled and centrifuged. Absorbance was measured at 532 nm and 600 nm.

H O Content Determination: H O content was determined using the titanium sulfate method. Fresh samples (0.2 g) were homogenized in acetone, centrifuged at 12000 r · min ^{1} for 20 minutes. The supernatant was mixed with titanium sulfate and ammonia, the precipitate was dissolved in 2 M H SO , and absorbance was measured at 415 nm.

O Content Determination: O content was measured using the hydroxylamine method. Fresh samples (0.2 g) were homogenized in 50 mmol · L ^{1} phosphate buffer (pH 7.8), centrifuged at 12000 r · min ^{1} for 20 minutes. The supernatant was mixed with 1 mmol · L ^{1} hydroxylamine hydrochloride, incubated at 25°C for 1 hour, then mixed with 17 mmol · L ^{1} sulfanilic acid and 7 mmol · L ^{1} -naphthylamine. After 20 minutes, absorbance was measured at 530 nm.

Antioxidant Enzyme Activity Assays: Fresh samples (0.2 g) were homogenized in 50 mmol · L ^{1} phosphate buffer (pH 7.8) containing 1% polyvinylpolypyrrolidone (PVPP), centrifuged at 12000 r · min ^{1} for 20 minutes at 4°C. The supernatant was used for enzyme assays.

- **SOD Activity:** Measured using the nitroblue tetrazolium (NBT) photoreduction method. The reaction mixture contained 50 mmol · L ^{1} phosphate buffer (pH 7.8), 130 mmol · L ^{1} methionine, 0.75 mmol · L ^{1} NBT, 0.1 mmol · L ^{1} EDTA-Na , 0.02 mmol · L ^{1} riboflavin, and enzyme extract. Illuminated at 220 mol · m ^{2} · s ^{1} for 30 minutes, then absorbance was measured at 560 nm.
- **CAT Activity:** Measured by monitoring the decrease in H O at 240 nm. The reaction mixture contained 50 mmol · L ^{1} phosphate buffer (pH 7.0), 0.3% H O , and enzyme extract.
- **POD Activity:** Measured using the guaiacol method. The reaction mixture contained 50 mmol · L ^{1} phosphate buffer (pH 7.0), 0.3% H O , 0.2% guaiacol, and enzyme extract. Absorbance was measured at 470 nm.
- **APX Activity:** Measured by monitoring the decrease in ascorbate at 290 nm. The reaction mixture contained 50 mmol · L ^{1} phosphate buffer (pH 7.0), 0.5 mmol · L ^{1} ascorbate, 0.1 mmol · L ^{1} EDTA-Na , 0.1 mmol · L ^{1} H O , and enzyme extract.

Ascorbate (AsA) and Glutathione (GSH) Content Determination: Fresh samples (0.2 g) were homogenized in 5% TCA, centrifuged at 12000 r · min ¹ for 10 minutes. For AsA, the supernatant was mixed with 150 mmol · L ¹ NaH PO (pH 7.4) and water, then 10% TCA was added after 30 seconds. Absorbance was measured at 530 nm. For GSH, the supernatant was mixed with DTNB reagent and absorbance was measured at 412 nm.

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2 Results

2.1 Water Content Changes

Under NaCl stress, water content in *H. caspica* assimilating branches showed no significant change at 100 and 300 mmol · L ¹ NaCl treatments compared with the control. However, at 500 mmol · L ¹ NaCl, water content decreased significantly by 27% ($P < 0.05$) [Figure 1: see original paper].

2.2 Inorganic Ion Accumulation

Na content increased significantly with increasing salt concentration, while K content decreased. The K /Na ratio decreased significantly under salt stress ($P < 0.05$). Ca^2 content increased at 100 and 300 mmol · L ¹ NaCl but decreased at 500 mmol · L ¹ NaCl [Figure 2: see original paper].

2.3 Contribution Rates of Osmotic Adjustment Substances

The contribution rates of inorganic ions (Na , K , Ca^2) and organic compounds (proline, glycine betaine) to osmotic adjustment varied with salt concentration. At 500 mmol · L ¹ NaCl, Na contributed 56.72% to osmotic adjustment, while organic compounds contributed 24.47% .

2.4 MDA, H O and O Content Changes

MDA content increased significantly under salt stress, with the highest increase (2.5-fold) observed at 500 mmol · L ¹ NaCl ($P < 0.05$) [Figure 4a: see original paper]. H O content also increased with salt concentration, showing a 2.5-fold increase at 500 mmol · L ¹ NaCl [Figure 4b: see original paper]. O content showed a similar trend, increasing significantly at high salt concentrations [Figure 4c: see original paper].

2.5 Antioxidant Enzyme Activities

SOD activity increased significantly under salt stress, reaching maximum activity at 300 mmol · L ¹ NaCl ($P < 0.05$) [Figure 5a: see original paper]. CAT activity also increased, showing a 2.5-fold increase at 500 mmol · L ¹ NaCl compared with the control [Figure 5b: see original paper]. APX activity increased at 100 and 300 mmol · L ¹ NaCl but decreased at 500 mmol · L ¹ NaCl [Figure

5c: see original paper]. POD activity showed no significant change at low salt concentrations but increased at 500 mmol · L ^{1} NaCl [Figure 5d: see original paper].

2.6 AsA and GSH Content Changes

AsA content in seedlings increased significantly under salt stress, showing a 2.5-fold increase at 500 mmol · L ^{1} NaCl ($P < 0.05$) [Figure 6a: see original paper]. GSH content also increased, with a 2-fold increase at 500 mmol · L ^{1} NaCl [Figure 6b: see original paper].

2.7 Correlation Analysis

Correlation analysis showed that AsA content was positively correlated with SOD activity ($r = 0.753$, $P < 0.05$) and APX activity ($r = 0.933$, $P < 0.05$). GSH content was positively correlated with CAT activity ($r = 0.749$, $P < 0.05$) and POD activity ($r = 0.829$, $P < 0.05$). MDA content was negatively correlated with SOD activity ($r = -0.682$, $P < 0.05$) and CAT activity ($r = -0.944$, $P < 0.05$) .

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3 Discussion

The results indicate that *H. caspica* employs different osmotic adjustment and antioxidant strategies at different growth stages to adapt to salt stress. At the seedling stage, Na and glycine betaine play dominant roles in osmotic adjustment, while the antioxidant system is maintained by cooperative action of SOD, CAT, AsA and GSH. The significant increase in AsA content under high salt stress suggests its crucial role in salt tolerance. The correlation between antioxidant components indicates a coordinated defense response against salt-induced oxidative stress.

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