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Full Length Article

Effect of Salt Stress on Photosynthetic and Antioxidant Characteristics in Purslane (*Portulaca oleracea*)

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Abstract

Purslane (*Portulaca oleracea* L.), a typical C_4 plant, has the characteristic of high saline-alkali tolerance and strong adaptability. With the wild purslane as materials, photosynthetic physiological characteristics of purslane were studied under the different concentrations of NaCl (0, 50, 150, 200 mmol·L^{-1}). The results showed that with the increase of NaCl concentration, the intercellular CO_2 concentration of purslane and the values of chlorophyll a/b increased, while transpiration rate, net photosynthesis rate, stomatal conductivity and chlorophyll contents decreased. The small reduction in photosynthetic capacity of purslane caused by 50 mmol·L^{-1} NaCl stress was considered having no big effect on the growth of the purslane. The proline and Malondialdehyde (MDA) contents and the activity of Peroxidase (POD) in leaves of purslane increased with the increase of salt concentrations. However, the activities of catalase (CAT) and superoxide dismutase (SOD) have a maximum under 50 mmol·L^{-1} NaCl concentrations. Purslane had stronger antioxidant capacities at 50 mmol·L^{-1} NaCl concentration. In brief, the high photosynthetic efficiency and antioxidant characteristics of purslane were adapted to salt stress, which could provide technical reference for further improving saline-alkali soil and developing salt-resistant plant germplasm resources. © 2020 Friends Science Publishers

Keywords: Antioxidant enzymes; Physiological characteristics; *Portulaca oleracea*; Salt stress

Introduction

Purslane (*Portulaca oleracea* L.) is a kind of common weeds on the earth (Jin *et al.* 2016; Library 2018). Purslane is rich in proteins, polysaccharides, organic acids, mineral elements, and has unique nutritional value. Purslane is an annual herb, rich in omega-3 fatty acids and other components, and is an important medicinal plant (Jin *et al*. 2015). Purslane, as halophyte in the Haloph database, grows readily in soils that may be arid and saline (Yazici *et al*. 2007).

Salinity is the most serious abiotic factor of abiotic stresses (Mansour 2000). Soil salinization is one of the major limitations of growth and development and produce toxicity to plants, which is a worldwide problem. Purslane has strong adaptability to climate, soil and other environmental conditions, and has a certain salt tolerance. After purslane seedlings were treated by 140 mmol·L^{-1} NaCl for 18 days, the activity of glutathione reductase was 3.5 times higher than that of the control (Yazici *et al.* 2007), indicating that purslane has strong salt tolerance. Kafi and Rahimi (2011) found that K^+ content in leaves and stems of purslane decreased by the salt stress. Purslane was able to grow under salt stress. Parvaneh *et al.* (2012) showed that under 150 mmol·L^{-1} and 200 mmol·L^{-1} NaCl stress, the chlorophyll contents of purslane was increased, and the corresponding increase of proline and sugar was beneficial to plant photosynthesis (Yazici *et al*. 2007).

The mechanisms of salt stress such as metabolism, photosynthesis and growth of plant under salt stress have been extensively studied. Salt stress affects chloroplast components of the main organs of plant photosynthesis, such as pigments, enzymes, proteins and membrane lipids (Parida *et al.* 2003; Zaman *et al.* 2018), which reduces plant photosynthesis and consequently reduces plant productivity. Thus, the antioxidant physiological characteristics of purslane under salt stress were systematically studied in this paper, which could provide technical reference for further improving saline-alkali soil and developing salt-resistant plant germplasm resources.

Materials and Methods

The seeds of purslane (*P. oleracea* L.) were collected from the beach of Yancheng, Jiangsu province, China. The plants of purslane (*P. oleracea* L.) were identified by the professor Jiao Demao from Institute of germplasm resources and biotechnology, Jiangsu Academy of Agricultural Sciences, China. Seeds were sown into the pots, grown in an illuminating and incubated with 30/23ºC of day/night, 70% relative humidity.

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The seeds of purslane were germinated in vermiculite and sprayed with tap water every day. Seedlings of purslane grew in pots in moistened sand. Different levels of NaCl (0, 50, 150, 200 mmol·L^{-1}) were applied to the pots. Thereafter, the seedlings were watered with tap water every two days and with corresponding salt solution every three days. Measurements were taken 30 days after salinity treatments. Fresh weights of roots and shoots were measured. Purslane seedlings were dried in an oven at 75ºC for 72 h, and the dry weight was determined.

Measurement of gas exchange parameters and chlorophyll contents

The gas exchange parameters of 4–5 branches of seedlings were measured using a CIRAS-2 *PPS* (PP Systems, Ltd.) at the 1000 μmol·m^{-2}·s^{-1} light intensity, the CO_2 concentration of 380 μmol·mol^{-1} and 60% relative humidity. The leaf chamber was a PLC6 (U) standard chamber (2.5 cm^2). In this study, the leaf area was measured by LI-3000C Portable Area Meter (Li-Cor Biosciences, Lincoln, NE, USA). The fresh weight of the leaves was determined with a balance with a sensitivity of 0.1 mg.

Chlorophyll pigment contents were extracted from purslane leaves with methanol (30 mg fresh weight·mL^{-1}). The extraction process was carried out in darkness at room temperature for 24 h. The chlorophyll content is expressed as the fresh weight mg·g^{-1} by Wellburn (1994).

Indexes of active oxygen metabolism

The activity of superoxide dismutase (SOD), which can be expressed as the ability to inhibit the photochemical reduction of nitroblue tetrazolium, was determined according to Giannopolitis and Ries (1977). Peroxidase (POD) activity was measured according to the method of Kochba *et al.* (1992). A unit of peroxidase activity was expressed as the change in absorbance per minute. The activity of catalase (CAT), lipid peroxidation and the free proline contents were measured according to Cakmak and Marschner (1992), Heath and Packer (1968) and Bates *et al*. (1973), respectively.

Statistical analysis

The data in this test are mean ± standard deviation (SD). LSD test was used for data analysis ($P < 0.05$). The diffirent letters of each point in the charts and Figures indicated that there are significant differences between groups at the level of 0.05.

Results

Effect of salt stress on the growth parameters of purslane

From Table 1 and 2, it can be seen that the germination rate of purslane decreased gradually under a certain low concentration of salt water (50 mmol·L^{-1}). From the data of root and stem growth, it can be seen that, the growth of stem and root of purslane was significantly inhibited at high salt concentration . The dry weight of purslane reached the maximum at the 50 mmol·L^{-1} NaCl concentration. The plant height and dry weight did not decrease significantly, which had little effect on the normal growth of purslane. The higher the NaCl concentration, the shorter the plant height (Fig. 1) and root length, the more serious the water loss of leaves. Purslane can not adapt to the salt stress environment at this time. It can be inferred that if NaCl concentration is further increased, it may cause plant death.

With the increase of NaCl concentration, the more parts of purslane root tips stained blue by Trypan Blue (Fig. 2). This phenomenon indicates that under salt stress, the content of dead cells in root tip cells increased due to the damage of salt to root tip cells. When the NaCl concentration increased, the resistance of purslane to salt stress gradually decreased.

Effect of salt stress on the gas exchange parameters of purslane

According to Fig. 3, chlorophyll a/b increased slightly ($P > 0.05$) at NaCl concentration of 0–100 mmol·L^{-1}, while chlorophyll a/b ratio increased at NaCl concentration of 100–200 mmol·L^{-1}. The contents of chlorophyll a+b decreased due to the increase NaCl concentration. When NaCl concentration was 50 mmol·L^{-1}, the chlorophyll contents did not decrease significantly ($P > 0.05$), but had little effect on the photosynthetic capacity of purslane. When salt concentration reached 100 mmol·L^{-1}, the chlorophyll contents decreased significantly ($P < 0.05$), which had a significant effect on the normal development and growth of purslane and was not suitable for the growth of purslane.

When the NaCl concentration increased, the net photosynthetic rate (Pn) (Fig. 4A) and transpiration rate (Tr) (Fig. 4B) of purslane decreased gradually. When the NaCl concentration reached 200 mmol·L^{-1}, the net photosynthetic rate of purslane was negative, indicating that high salt stress significantly inhibited the photosynthesis and transpiration of purslane. With the increase of seawater concentration, intercellular CO_2 concentration (Ci) (Fig. 4C) showed a trend opposite to net photosynthetic rate. As can be seen from Fig. 4D, stomatal conductance (Gs) was inhibited by the salt ions, which was not conducive to the absorption of carbon dioxide in photosynthesis. With the increase of NaCl concentration, the transpiration rate, the stomatal conductance and net photosynthetic rate all decreased, due mainly to the utilization of CO_2 reduction.

Antioxidant characteristics of purslane under the salt stress

From Fig. 5A, it can be seen that the SOD activity of purslane was affected by different concentrations of salt

Table 1: The seedling growth and seed germination of purslane under salt stress

seed germination index	NaCl concentrations (mmol.L^{-1})				
	0	50	100	150	200
Prcentage of germination (%)	95.25±4.17^{a}	90.25±5.13^{a}	55.33±3.55^{b}	25.33±2.62^{c}	5.25±0.65^{d}
Germination energy (%)	65.33±3.21^{a}	50.50±4.68^{b}	25.50±3.45b^{c}	5.33±0.91^{d}	2.12±0.55^{e}
Shoot length(mm)	7.92±0.74^{a}	6.44±0.92^{b}	3.83±0.64^{c}	2.55±0.71^{d}	1.02±0.36^{e}
Root length(mm)	8.43±1.08^{a}	4.46±0.68^{b}	2.34±0.42^{c}	1.16±0.18^{d}	0.57±0.12^{e}

Table 2: The growth of purslane seedlings under salt stress

NaCl concentration (mmol.L^{-1})	Fresh (FW) weight (mg)	Dry (DW) weight (mg)	Plant length (cm)	Leaf relative water content (RWC)%
0	3225±127^{a}	267±31^{a}	11.2±1.4^{a}	73.2±6.3^{a}
50	2932±108^{a}	281±27^{a}	10.5±0.8^{a}	68.4±6.9^{a}
100	1012±79^{b}	196±16^{b}	8.1±0.8^{b}	59.3±4.1^{b}
150	863±82^{c}	157±15^{b}	7.4±0.6^{bc}	53.2±5.2^{b}
200	562±50^{d}	92±7^{c}	6.7±0.5^{c}	36.3±2.8^{c}

Values are mean±S.E. based on three replicates (n = 3)

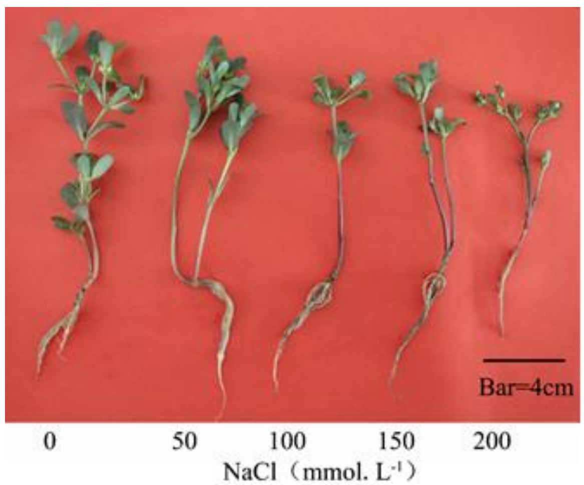

Fig. 1: Effect of salt stress on purslane phenotype. The age of seedling is 30 d

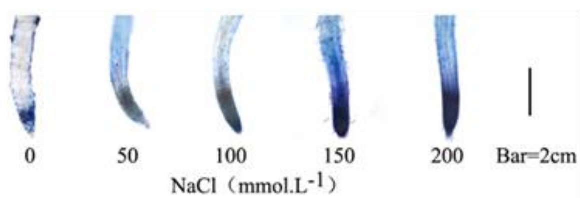

Fig. 2: Trypan blue staining of cells in root tips of purslane under different NaCl concentrations

stress. At the 50 mmol·L^{-1} NaCl concentration, the SOD activity reached its maximum, and then showed a downward trend. POD activity of Portulaca oleracea increased significantly as the results of NaCl stress (Fig. 5C). The MDA content of purslane increased (Fig. 5D), which indicated that the lipid peroxidation of cell membrane of purslane increased and the damage of cell membrane was more serious. The change of CAT activity after salt treatment was consistent with the trend of SOD (Fig. 5B). It showed that low concentration of salt ion induced the activities of protective enzymes to increase in order to remove the active oxygen and MDA (a lipid peroxidation product of membrane) accumulated under stress. Soluble protein and proline are both related to osmotic potential regulation of plant cells. Owing to the NaCl sress, soluble protein and proline increase significantly, which caused the damage of membrane and the enhancement of reactive oxygen. So the growth of purslane was inhibited.

Discussion

Plant growth and yield are directly related to photosynthetic performance. Chlorophyll is the main photosynthetic pigment and plays an important role in plant photosynthesis

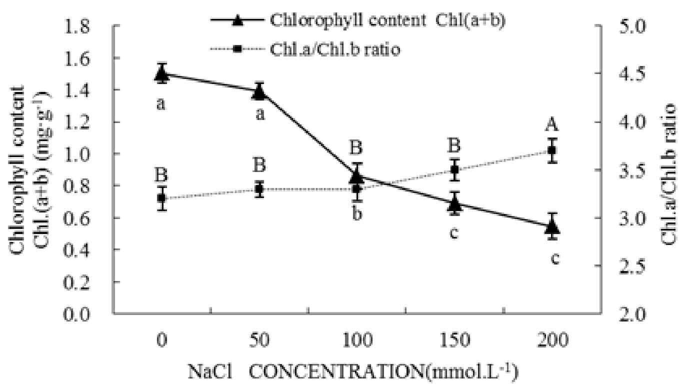

Fig. 3: Effects of NaCl stress on chlorophyll contents and *chlorophyll a/b* in purslane

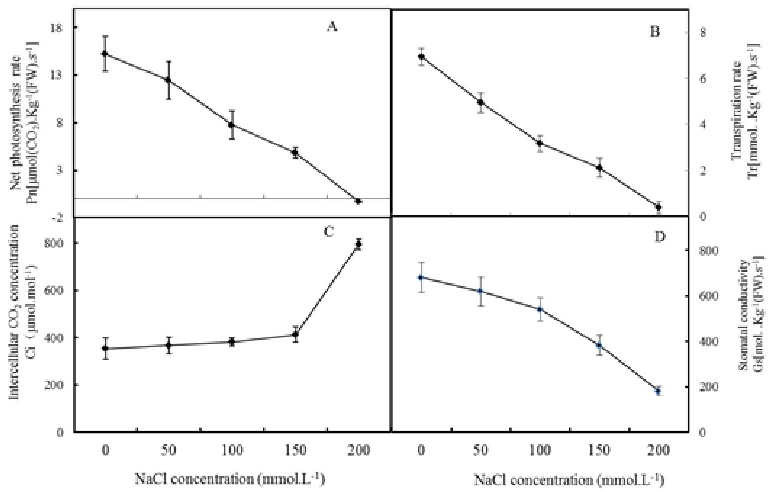

Fig. 4: Effect of NaCl stress on net photosynthetic rate (Pn) (A), transpiration rate (Tr) (B), intercellular CO_2 concentration (Ci) (C) and stomatal conductance (Gs) (D) in purslane

(Zhang *et al.* 2009). Salt stress decreased chlorophyll synthesis and inhibited photosynthesis (Barhoumi *et al.* 2007). With the increase of salt stress, the degradation of chlorophyll in purslane leaves aggravated in the present study. *Chlorophyll a* was more susceptible to salt stress than *chlorophyll b*. The increase of a/b showed that the decrease of *chlorophyll b* content was one of the main reasons for the decrease of photosynthesis in purslane.

Purslane is a typical C_4 plant with vascular bundle sheath cells and kranz type structure. The roots of purslane also have developed ventilation tissue (Ding *et al.* 2012), which ensures the metabolic needs of purslane. The result of purslane root tips stained by Trypan Blue showed that purslane had a certain adaptability and tolerance to salt environment. When the concentration of salt in nutrient solution was 50 $mmol \cdot L^{-1}$, Pn and Gs of purslane decreased slightly, the water use efficiency ,chlorophyll contents and photosynthesis changed little. At the 100 $mmol \cdot L^{-1}$ NaCl concentration, the photosynthesis of purslane was greatly affected, the physiological functions of various aspects were limited, and the normal development of purslane was slowed down. Purslane had high photosynthetic capacity under low salt stress, alleviated the damage of salt stress to the light system, thus maintaining a higher net photosynthetic rate, which may be a protective mechanism of purslane adapting to salt environment.

Under salinity and other stress conditions, the distribution of membrane lipids and proteins in plant cell membranes would be disturbed, and the activity of plant peroxidase will increase. Soluble substances, such as MDA, protein and proline, were accumulated to reduce the intracellular osmotic potential and ensure the supply of water (Valentina *et al*. 2002; Zhang *et al*. 2005). The protective enzymes SOD, CAT and POD in purslane increased to eliminate the damage caused by NaCl stress, and a large amount of protein and proline were accumulated (Fig. 5), which helped maintain the low osmotic potential of plant cells and resist the stress caused by stress. It can be seen that purslane still has strong photoinhibition and photooxidation resistance under salt stress, and maintains a higher photosynthetic capacity, thus reducing the damage of salt stress on plant growth. By studying the response

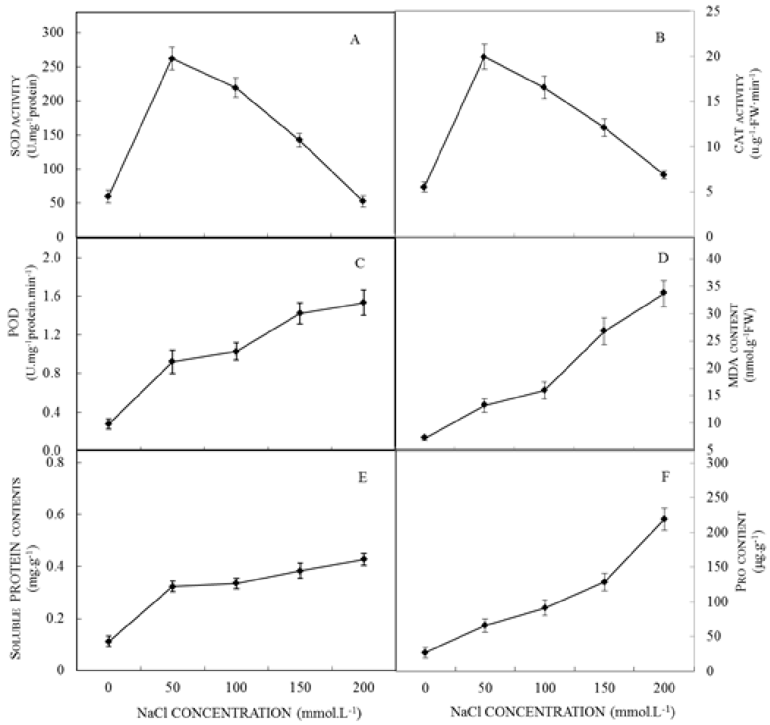

Fig. 5: The activities of superoxide dismutase (SOD) (A), peroxidase (POD) (B), and calatase (CAT) (C) and malonyldialdehyde (MDA) (D), soluble protein (E) and proline (Pro) (F) contents in leaves of purslane under NaCl stress

characteristics of key enzymes in C_4 efficient carbon assimilation pathway of purslane under above salt stress, the mechanism of high photosynthetic efficiency of purslane was clarified, which was of great significance for the improvement of saline-alkali land and the sustainable development of recycling.

Purslane is known as one of the most promising medicinal and edible plants in the 21st century (Kafi and Rahimi 2011; Jin *et al.* 2015; Zaman *et al.* 2018). Because of its strong salt tolerance, purslane could be a promising candidate to be used in ecosystem restoration in arid and semi-arid regions (Jin *et al.* 2016). It was of significant strategy that purslane was planted on coastal beach and developing saline soil agriculture for further accelerating the sustainable development of agricultural economy and expanding the space for agricultural development in China.

Conclusion

These findings suggest that Purslane plants responded to NaCl stress by enhancing their antioxidative capacity and proline accumulation. Under salt stress, purslane had high photosynthetic capacity, alleviated the damage of salt stress to the light system, thus maintaining a higher net photosynthetic rate. This study provides theoretic basis and technical approach for studying and exploring salt-tolerant plant resources.

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Author Contributions

Tang Ning and Yang Ping conceived and designed the experiments; Tang Ning performed the experiments; Wang Like and Yang Ping analyzed the data; Chen Quanzhan contributed materials; Zhang Bianjiang wrote the paper.

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