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Effects of Salt Stress on the Enzyme Activity and Chlorophyll Content of Bush Cinquefoil (*Potentilla fruticosa L.*)

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Abstract: *In order to studied the effect of salt stress onantioxidant enzyme system and photosynthetic pigments in the leaves of Bush Cinquefoil (Potentilla fruticosa L.),and the correlation between the important physiological characteristics of salt tolerance. Bush Cinquefoil (Potentilla fruticosa L.) cutting seedlings was selected as the material, through watering NaCl and Na_2SO_4 solution of different concentration. The results showed that: with the constant enhancement of salt stress, SOD activity, chlorophyll content of Potentilla fruticosa L. leaves decreased; POD activity showed a rising trend; the carotenoid content decreased; the chlorophyll content of leaves was positive correlation with SOD, negatively correlated with POD, so SOD had certain protective effect on chlorophyll. Based on the analysis, Potentilla fruticosa L. has a certain tolerance to salt, suitable for planting in areas with low degree of salinization.*

Keywords: *salt stress; Potentilla fruticosa L.; antioxidant enzyme system; photosynthetic pigments*

1. INTRODUCTION

Bush Cinquefoil (*Potentilla fruticosa L.*) also known as Prunus humilis, is a deciduous shrub of Potentilla of Rosaceae. Dense branches and leaves, yellow flowers, shaped like plum blossoms, flowering from June to July, suitable for garden ornamental shrubs or hedgerows [1]. Leaves and fruits contain tannin, which can be used to extract tannin extract. Tender leaves can be drunk on behalf of tea leaves. Flowers and leaves are used as medicines and have the effects of invigorating spleen, eliminating dampness, clearing summer heat, and regulating menstruation [2]. Bush Cinquefoil (*Potentilla fruticosa L.*), resistant to low temperatures of - 50 ℃ and likes moist soil with good drainage. it is resistant to drought and barren. Bush Cinquefoil is an excellent greenery and medicinal plant, which has broad application prospect. However, the research and promotion of Bush Cinquefoil is still in its primary stage, mainly focusing on medicinal and reproductive aspects, while the research on salt resistance of Bush Cinquefoil is relatively few, and mainly under NaCl stress. Chloride and sulfate are the main salt damage components in the saline-alkali land in the arid region of northern China, which severely restricts the economic development in the arid region of northern China [3]. In order to expand the application of Bush Cinquefoil in saline-alkali regions in north China, especially in arid regions in north China, this project studied the effects of different concentrations of NaCl and Na_2SO_4 solution treatment on antioxidant enzyme system and chlorophyll content of Bush Cinquefoil (*Potentilla fruticosa L.*) seedlings, in order to provide reference for improving the salt tolerance of Bush Cinquefoil and its application scope in north China.

2. MATERIALS AND METHODS

2.1. Materials

The seedlings tested were 2 - year - old Bush Cinquefoil cuttage seedlings. seedlings with the same growth and strong plants were selected from the nursery of Hebei agricultural university to be planted in a ceramic basin with a diameter of 21 cm and a depth of 25 cm. the cultivation medium was mainly ordinary garden soil in. During the period of normal management, experiment. was carried out when the young plant of Bush Cinquefoil was about 50 cm tall. 10 plants in each group and 30 plants in each treatment set up 3 repeated. 0.2 %, 0.4 %, 0.6 %, 0.8 % and 1.0 % NaCl and Na_2SO_4 solutions were respectively applied into the basin in 1l three times to ensure sufficient salt absorption, and distilled water was used as the control (CK).

The test materials of Bush Cinquefoil were planted in a ceramic flowerpot with a diameter of 21 cm and a depth of 25 cm. the cultivation medium was mainly., a common garden soil. Every 10 pots are one group, and every 30 pots are treated, i.e. 3 repeated. Pour clear water into flowerpots as control (CK), and place trays at the bottom of flowerpots to prevent salt loss from.

2.2. Test Methods and Determination Methods

After 7 and 13 days of NaCl and Na_2SO_4 stress, 20 mature leaves were randomly taken from plants at the same stress level between 7: 00 and 8: 00 in the morning to measure the physiological indexes of salt tolerance. The determination of chlorophyll content is based on the method of the Bo chin[4], the determination of superoxide dismutase (SOD) activity is based on azoblue tetrazole photochemical reduction method[3], and the determination of peroxidase (POD) activity is based on guaiacol method [5]

The test data were analyzed by SPSS 19.0 statistical software for variance and correlation between indicators and excel 2007 was used to tabulate.

3. Results and Analysis

3.1. Effect of Salt Stress on Antioxidant Enzyme System of Bush Cinquefoil

As can be seen from table 1, under salt stress, SOD activity in leaves of Bush Cinquefoil decreased with the increase of salt concentration in. Except for NaCl stress of 0.2 % salt concentration for 7 days, SOD activity had no significant difference with CK, and SOD activity of other treatment levels had significant difference with CK. After 13 days of stress, all treatments with different concentrations of NaCl reached significantly different levels of. After 7 days and 13 days of Na_2SO_4 stress, the treatment with different concentrations reached significant difference level with CK, and after 13 days of stress, there was significant difference between the treatment concentrations of. In comparison, SOD activity under NaCl stress was lower than that under Na_2SO_4 stress, which indicated that SOD activity in leaves of Bush Cinquefoil was more sensitive to Na_2SO_4 stress in.

With the increase of NaCl and Na_2SO_4 stress levels, pod activity in leaves of Bush Cinquefoil showed an overall upward trend, and the values were higher than CK. After 7 days of stress, POD activity and CK in leaves treated with 0.8 % and 1.0 % NaCl reached significantly different levels of. After 13 days of stress, all treatments with different NaCl concentrations reached significantly different levels of. from CK except for Na_2SO_4 stress of 0.2 % salt concentration for 7 days, SOD activity had no significant difference with CK, and SOD activity at other treatment levels had significant difference with CK. After 13 days of Na_2SO_4 stress, there were significant differences between different treatments.

In comparison, under the same treatment time and salt concentration stress, the SOD activity and pod activity of NaCl treatment were lower than that of Na_2SO_4 treatment, which indicated that the effect of NaCl stress on antioxidant enzyme system of Bush Cinquefoil was stronger than that of Na_2SO_4 stress on.

Table1. *The effect of salt stress on SOD enzyme activities、POD enzyme activities of Potentilla fruticosa L. leaves*

	%	Days of treatment (d)			
	Salt	NaCl		Na_2SO_4	
	Concentration	7d	13d	7d	13d
SOD activity (enzyme activity unit·g^{-1}FW)	CK	98.000c	85.600f	102.289d	90.510f
	0.20	98.005c	77.600e	98.333c	79.400e
	0.40	93.543b	49.800d	97.815c	54.800d
	0.60	96.067c	29.067c	96.954c	40.867c
	0.80	58.694a	27.200b	82.823b	32.600b
	1.00	58.886a	17.133a	69.354a	26.867a
POD activity (A470·min^{-1}·g^{-1}FW)	CK	14.318a	17.955a	16.126a	18.822a
	0.20	17.647ab	28.313b	17.833ab	30.333b
	0.40	18.833bc	52.133c	19.311b	53.833c
	0.60	15.815ab	49.100c	20.631b	56.667d
	0.80	21.968c	60.256d	27.462c	62.000e
	1.00	28.126d	58.293d	30.314c	71.833f

Note: *Analysis of variance is at the 0.05 level. a, b, c…… is significant difference between different salt concentrations（in line）.It is same in thereinafter.*

3.2. Effect of Salt Stress on Photosynthetic Pigment Content in Leaves of Bush Cinquefoil

As shown in table 2, with the increase of salt concentration, chlorophyll a, chlorophyll b and total chlorophyll content in leaves of Bush Cinquefoil decreased significantly by. after different salt stress treatments. However, under the same stress intensity, the content of chlorophyll treated with NaCl was higher than that treated with Na_2SO_4, which indicated that chlorophyll synthesis in leaves of Bush Cinquefoil was more destroyed under Na_2SO_4 stress, and its light energy capture, transmission and conversion were all affected to a greater extent by.

Under salt stress, the carotenoid content of all salt treatments reached significant difference level, but the change was not significant, floating. above and below CK Under the same stress time and concentration, the content of carotenoids under NaCl stress was lower than that under the reduced Na_2SO_4 stress, which indicated that the content of carotenoids in leaves of Bush Cinquefoil was more sensitive to NaCl stress in.

The chlorophyll a/b value in leaves of Bush Cinquefoil increased first and then decreased with the increase of NaCl concentration and reached the maximum value of. When the salt concentration was 0.4 % (after 7 days of stress) and 0.6 % (after 13 days of stress) Under Na_2SO_4 stress, chlorophyll a/b value showed a decreasing trend first and then increasing trend in. The ratio of total chlorophyll to carotenoids showed a downward trend with the increase of salt concentration, which indicated that the effects of NaCl and Na_2SO_4 stress on the total chlorophyll content were higher than those on the carotenoid content in.

Table2. *Effect of salt stress on photosynthetic pigments content of Potentilla fruticosa L. leaves*

	%	(d) days of treatment			
	Salt	NaCl		Na_2SO_4	
	Concentration	7d	13d	7d	13d
($\mu g \cdot g^{-1}$) Chla content	CK	22.908f	18.636e	21.575e	18.636f
	0.20	20.877e	19.841f	15.841d	15.447e
	0.40	20.080d	15.266c	15.492d	12.231d
	0.60	18.151c	16.217d	13.308c	10.741c
	0.80	14.394b	12.911b	10.877b	10.577b
	1.00	10.650a	7.201a	9.820a	6.526a
($\mu g \cdot g^{-1}$) Chlb content	CK	4.595f	4.553e	4.795f	4.919f
	0.20	4.174e	3.895d	3.770e	4.342e
	0.40	3.603d	2.819c	3.589d	3.138d
	0.60	3.315c	2.620b	3.012c	2.335c
	0.80	2.779b	2.476b	1.958b	1.942b
	1.00	2.329a	2.047a	1.450a	0.830a
($\mu g \cdot g^{-1}$) Chl(a+b) content	CK	27.503f	23.189e	26.370f	23.555f
	0.20	25.051e	23.736f	19.612e	19.789e
	0.40	23.683d	18.085c	19.082d	15.369d
	0.60	21.466c	18.837d	16.321c	13.076c
	0.80	17.173b	15.387b	12.836b	12.519b
	1.00	12.979a	9.248a	11.270a	7.356a
($\mu g \cdot g^{-1}$) Car content	CK	5.863f	6.417f	5.863a	6.417e
	0.20	4.836b	5.105e	6.354b	6.769f
	0.40	5.216d	4.638d	6.542d	4.420a
	0.60	4.874c	4.258a	6.629e	5.034b
	0.80	4.037a	4.378b	6.777f	5.881c
	1.00	5.267e	4.572c	6.472c	6.106d
Chl a/b	CK	4.985c	4.093e	4.499c	3.789d
	0.20	5.002c	5.094d	4.202c	3.558e
	0.40	5.573a	5.415b	4.317c	3.898d
	0.60	5.475ab	6.190a	4.418c	4.600c
	0.80	5.180bc	5.214c	5.555b	5.446b
	1.00	4.573d	3.518f	6.772a	7.863a

Chl(a+b) /Car	CK	4.691b	3.614d	4.498a	3.671a
	0.20	5.180a	4.650a	3.087b	2.923b
	0.40	4.540bc	3.899c	2.917c	3.053b
	0.60	4.404cd	4.424b	2.462d	2.223c
	0.80	4.254d	3.515d	1.894e	2.832b
	1.00	2.464e	2.023e	1.741f	1.205d

3.3. Correlation Analysis of Chlorophyll Content with Superoxide Dismutase and Peroxidase Activities in Leaves under Salt Stress

As shown in table 3, after 7 days of NaCl stress, SOD was significantly negatively correlated with pod, chlorophyll a, chlorophyll b, and chlorophyll total were significantly positively correlated with SOD, chlorophyll b was significantly negatively correlated with pod, and chlorophyll a, chlorophyll total were significantly negatively correlated with pod. After 13 days of NaCl stress, SOD activity and pod activity reached a very significant negative correlation with; Chlorophyll a and total chlorophyll were significantly and positively correlated with SOD, while chlorophyll b was significantly and positively correlated with SOD in. The correlation between pod and chlorophyll a was not significant, but extremely significant negative correlation with chlorophyll b, and significantly negative correlation with total chlorophyll.

Sod and pod reached extremely significant negative correlation. in all stages of Na_2SO_4 stress After 7 days of Na_2SO_4 stress, chlorophyll a and chlorophyll content were significantly positively correlated with SOD and negatively correlated with pod in; Chlorophyll b was positively correlated with SOD and negatively correlated with pod in. After 13 days of Na_2SO_4 stress, chlorophyll a, chlorophyll b, total chlorophyll and SOD were significantly and positively correlated with each other in; Chlorophyll a was negatively correlated with pod, while chlorophyll b and chlorophyll total were negatively correlated with pod in.

Table1. *The coefficient of correlation of morphological and physiological characters of Potentilla fruticosa L. leaves under salt stress*

	days of treatment /d		X1	X2	X3	X4	X5
NaCl	7	X1	1				
		X2	-0.880*	1			
		X3	0.916*	-0.924**	1		
		X4	0.862*	-0.861*	0.969**	1	
		X5	0.911*	-0.918**	0.999**	0.978**	1
	13	X1	1				
		X2	-0.941**	1			
		X3	0.838*	-0.785	1		
		X4	0.976**	-0.976**	0.830*	1	
		X5	0.884*	-0.839*	0.995**	0.881*	1
Na_2SO_4	7	X1	1				
		X2	-0.974**	1			
		X3	0.825*	-0.895*	1		
		X4	0.924**	-0.973**	0.973**	1	
		X5	0.852*	-0.917*	0.999**	0.984**	1
	13	X1	1				
		X2	-0.986**	1			
		X3	0.964**	-0.981*	1		
		X4	0.982**	-0.976**	0.985**	1	
		X5	0.971**	-0.982**	0.999**	0.992**	1

*** Correlation is significant at the 0.01 level (2-tailed)*

** Correlation is significant at the 0.05 level (2-tailed)*

Note: X1 SOD, X2 POD, X3 Chla, X4 Chlb, X5 Chl(a+b)

4. DISCUSSION

4.1. Effect of Salt Stress on Enzyme Activity

Under adverse circumstances, plants need to rely on the balance of active oxygen metabolism to maintain their normal growth and development of. Salt stress can induce plants to produce a large

amount of reactive oxygen species, and excessive accumulation of reactive oxygen species will produce oxygen stress on seedlings [6]. Antioxidant enzymes can remove excess active oxygen in plants and reduce the damage of salt stress to seedlings [7,8]. In this experiment, with the increase of salt stress intensity, SOD and pod activities showed a downward trend, and SOD activities were significantly different between different treatment concentrations after 7 days of NaCl stress, 7 days of Na_2SO_4 stress, and 13 days of stress. pod activities were significantly different between different treatments after 13 days of salt stress. Compared with NaCl, the changes of SOD and pod activities under Na_2SO_4 stress were smaller than those under NaCl stress, which indicated that the antioxidant enzyme system of Bush Cinquefoil (*Potentilla fruticosa L.*) was more sensitive to NaCl stress in.

4.2. Effects of Salt Stress on Photosynthetic Pigments

Pigment is an important substance in plant photosynthesis. its content directly affects the intensity of photosynthesis and is an important indicator of plant salt tolerance. In this study, with the increase of salt concentration, a large amount of Na^+ and Cl^- accumulated in Bush Cinquefoil, resulting in a significant decrease in chlorophyll a, chlorophyll b, and chlorophyll content in. This indicates that the accumulation of Na^+ and Cl^- in the body of Bush Cinquefoil destroys the ion balance in the cell, leading to the relaxation of protein binding between chlorophyll and thylakoid membrane [7] and the enhancement of chlorophyllase activity [8], accelerating the decomposition of. by chlorophyll. When plants grow in saline environment, the contents of Na^+ and Cl^- accumulate in their bodies, which leads to a decline in photosynthesis. this has been confirmed in a large number of literatures. however, for different plants, there is a certain difference in the degree of decline in the dominant ion and photosynthetic rate [9]. In this study, under the same stress intensity, the decrease of chlorophyll content under NaCl stress was significantly smaller than that under Na_2SO_4, which indicated that chlorophyll in leaves of Prunus humilis was more sensitive to Na_2SO_4 stress, Na^+ concentration might be the main ion affecting photosynthesis of Prunus humilis, and. needs to be further verified.

Carotenoids can not only absorb and transmit light energy, but also quench active oxygen to protect [10]. for photosynthesis. Carotenoids in leaves of Bush Cinquefoil did not change much under salt stress, floating up and down in CK. the results were consistent with those of Chen shigang[11]research of salt stress on carotene species of poplar tree. The carotenoid content was higher than CK after 7 days of Na_2SO_4 stress and reached the maximum value at 0.2 % salt concentration after 13 days of stress, and higher than CK. other salt treatment values were lower than CK, suggesting that carotenoids played a protective role under short-term (7 days) of Na_2SO_4 and low-salt stress, but under long-term (13days) and high-salt stress, the carotenoid decomposition rate accelerated or synthesis was hindered by. In addition, under the same stress time and concentration, the content of carotenoids under NaCl stress is lower than that under Na_2SO_4 stress, which shows that carotenoids are more sensitive to NaCl stress. however, under the medium and low salt stress in this study, Na_2SO_4 stress is more harmful to chlorophyll production. it can be seen that carotenoids have certain protective effect on chlorophyll, but they do not play a key role in.

Under salt stress, the carotenoid content of all salt treatments reached significant difference level, but the change was not significant, floating. above and below CK Under the same stress time and concentration, the content of carotenoids under NaCl stress was lower than that under the reduced Na_2SO_4 stress, which indicated that the content of carotenoids in leaves of Bush Cinquefoil was more sensitive to NaCl stress in.

Chlorophyll a/b in leaves of Bush Cinquefoil increased first and then decreased with the increase of NaCl concentration, which indicated that chlorophyll a was higher than chlorophyll b under NaCl stress and the change trend of chlorophyll a/b in leaves of Cinnamomum camphora Seedlings was the same in studies such as Han haozhang[12]; However, the concentration of Na_2SO_4 showed an upward trend, which indicated that the degree of damage to chlorophyll b caused by Na_2SO_4 stress was greater.

Under NaCl and Na_2SO_4 stress, the total chlorophyll/carotenoid ratio decreased with the increase of salt concentration, which indicated that salt stress had a greater influence on the total chlorophyll content and had a smaller influence on the carotenoid content, which was consistent with the research conclusions of Rao and Han haozhang [12-13].

4.3. Correlation Analysis

After 7 days of NaCl stress, SOD was negatively correlated with chlorophyll a, chlorophyll b and chlorophyll content in; Under Na_2SO_4 stress for 7 days, SOD was significantly and positively correlated with chlorophyll a and chlorophyll total, and was significantly and positively correlated with chlorophyll b in; Under NaCl stress for 13 days, SOD was significantly and positively correlated with chlorophyll a and chlorophyll total, and was significantly and positively correlated with chlorophyll b in; Under Na_2SO_4 stress for 13 days, SOD was significantly and positively correlated with chlorophyll a, chlorophyll b, and total chlorophyll content, which indicated that SOD could provide long-term protective effect on chlorophyll under salt stress, especially with chlorophyll b, and the protective effect was strongest in.. However, under NaCl and Na_2SO_4 stress, pod was negatively correlated with chlorophyll a, chlorophyll b and total chlorophyll, which indicated that pod did not play a protective role on chlorophyll.

5. CONCLUSION

To sum up, under NaCl stress, SOD activity, pod activity and carotenoid content are lower than those under Na_2SO_4 stress, while chlorophyll content is higher than that under Na_2SO_4 stress, which indicates that NaCl stress has a greater influence on the oxidase system of Prunus humilis, and Na_2SO_4 stress has a greater influence on photosynthesis of Prunus humilis. in order to fully analyze the reasons, further research on. is needed in terms of the distribution of salt ions in the body and the influence of salt ions on the microstructure of plants. In addition, this project only analyzed the results of pot seedlings of Bush Cinquefoil under single salt stress, and whether the results of field multi-salt stress experiments can be consistent is still to be further studied in.

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