Effects of Soil Acid stress on Root Ultrastructure and Enzyme Protection System of *Sedum aizoon* L.

Luxi Yang¹,a, Yiming Wang¹,b, Shengju Long ¹,c, Zhongqun He¹,d*, Yan Chen¹,e, Yingpeng Zhao¹,f
¹College of Horticulture, Sichuan Agricultural University, Chengdu 611130, PR China
*Corresponding author’s e-mail: dhzqun328@163.com

Abstract. This experiment studied the effects of four different soil pH values (3.4, 4.4, 5.5, 6.6) on the growth and enzyme protection system of *Sedum aizoon* L. by simulating soil acidification. Results showed that: *Sedum aizoon* L. grew best in the control soil. With the decrease of soil pH, plant height and aboveground fresh were gradually inhibited, but mild stress (pH 5.5) could promote the growth of root length and increase the aboveground fresh, while moderate and severe stress could inhibit its increase. When the soil pH was in the range of 4.6 - 6.6, the activities of SOD, CAT, APX, GR, soluble sugar, soluble protein, proline and mad in the plant increased significantly, but the relative electrical conductivity of the root system was not significantly different from that of the control. After the soil pH was lower than 4.6, except APX activity continued to increase, SOD, CAT, GR activity and soluble sugar, soluble protein, proline content decreased significantly, and root relative conductivity and MDA content increased significantly, which indicated that severe soil acid stress would reduce root protective enzyme activity, cause osmotic adjustment imbalance, and lead to slow or even stop plant growth.

1. Introduction
Soil acidification is a phenomenon commonly found in nature[1]. Under the effect of rain, hydrogen ions in soil solution replace salt ions, resulting in leaching of base ions in the soil, reduced salt saturation, and decreased soil pH[2]. Over 40% of China's arable land soil is acidified, showing a large area of acidified soil, a wide range, poor acidified soil structure, low fertility, and high levels of toxic heavy metals, threatening food security and sustainable development of farmland[3].

Soil acidification will destroy the structure of soil aggregates, reduce soil ventilation and water permeability, and lead to soil cracking and hardening and other phenomena. With the decrease of soil pH, the activity of beneficial microorganisms such as nitrifying bacteria and ammoniated bacteria will gradually be inhibited[4]. Potassium, calcium, sodium, magnesium and other important basic ions will show a large number of leaching loss under the replacement of hydrogen ions, which will lead to the decline of soil fertility and seriously affect the growth of plants[5]. Affecting the functional relationship between membrane proteins and lipids, membrane proteins are subjected to peroxide stress[6], which severely threatens the normal growth of plant roots.

*Sedum aizoon* L., alias yangxin dishes, belong to crassulaceae stonecrop perennial herbs, rich in protein, carbohydrates, carotene, vitamin B and C, calcium, phosphorus, iron and other nutrients, has the function of preventing hypertension and heart disease, and strong adaptability, no disease and insect pest, easy cultivation management[7]. It has become a green health care vegetable with high economic value, and the cultivated area is increasing year by year in China [8].
With the gradual increase of soil acidification area in China and the rise of wild vegetable cultivation, exploring the effects of different degrees of acidified soil on the root ultrastructure and enzyme protection system of *Sedum aizoon* L., will help to provide some theoretical basis for the cultivation of *Sedum aizoon* L. in the future.

2. Materials and methods

2.1. Plant materials and Soil materials

The soil is from sichuan agricultural university (soil pH6.6, available nitrogen 53.6 mg·kg⁻¹, available phosphorus 44.1mg·kg⁻¹, available potassium 176.7mg·kg⁻¹, the organic matter 10.2 mg·kg⁻¹).

The plant material of this experiment is *Sedum aizoon* L., provided by the school of horticulture at sichuan agricultural university. All plant materials were inserted after 20d, and the matrix used in cutting propagation was perlite, vermiculite, and nutrient soil by volume ratio 1:1:4.

2.2. Experiment design

The experiment was carried out by pot culture method. the diluted concentrated sulfuric acid was used to adjust the acidity of the soil to obtain soil with four gradients of pH 3.4 (severe), pH 4.6 (moderate), pH 5.5 (mild) and pH 6.6 (CK). then *Sedum aizoon* L. with a plant height of 4 cm was transplanted into nutrition pots ( specification: 12× 13 cm ) filled with different soil ph and cultured. each nutrition pot was transplanted with 1 plant and 300 g of the treated soil. each treatment lasted 8 pots, one plant per basin, and was repeated 3 times for 96 pots. During the cultivation process, soil moisture content was measured with a soil three-parameter instrument to keep it at about 75 %.When measured in 28 d growth index (plant height, root length, dry aboveground fresh weight, fresh, root-shoot ratio of underground part), osmotic regulation substances (root relative electrical conductivity, soluble sugar content, free proline, soluble protein and MDA content), the antioxidant enzyme activity (SOD, POD, APX, GR).

2.3. Statistical methods

All data were analyzed with statistical software SPSS version 22.0 (IBM Corporation). Comparisons of the means used the least significant difference (LSD) at P ≤ 0.05.

3. Results

3.1. Effects on growth

It can be seen from table 1 that the plants growing in CK soil were the highest, and the plant height was gradually inhibited with the increase of soil acidification, and the plant height under severe stress was only 27.7% of CK. The root length was characterized by mild stress promoting root growth, moderate and severe stress inhibiting the root growth tendency, and the root length of plant under mild stress was significantly increased by 19.3% compared with CK. The change trend of ground fresh weight and underground fresh weight was consistent with the trend of plant height and root length. Under serious soil acid stress, the plant height did not increase, and root length was only 28.7% of the CK, which showed that severe stress had a serious effect on the root system of the plant, causing the plant to grow slowly or stop growing.

| Soil pH | Plant height (cm) | Root length (cm) | Ground fresh weight (g) | Underground fresh weight (g) | Root cap ratio |
|---------|-------------------|------------------|------------------------|-----------------------------|---------------|
| CK6.6   | 15.43±0.65c       | 13.8±0.95b       | 14.62±0.98a            | 2.01±0.18b                  | 0.13±0.01c    |
| pH5.5   | 10.60±0.37b       | 16.46±2.46a      | 8.20±1.49b             | 2.268±0.37a                 | 0.27±0.03b    |
| pH4.6   | 10.17±0.53b       | 9.01±0.68c       | 8.42±1.66b             | 1.19±0.27c                  | 0.14±0.02c    |
| pH3.4   | 4.28±0.17c        | 3.96±0.76d       | 1.62±0.53c             | 0.41±0.11d                  | 0.30±0.05a    |

Note: soil pH3.4 (severe stress), soil pH4.6 (moderate stress), soil pH5.5 (mild stress), soil pH6.6
The small-letter means significantly different at the P<0.05 level. The following tables are the same.

### 3.2. Effects on osmotic regulation

As can be seen from table 2, the relative conductivity and MDA content of plant roots gradually increased with the increase of soil acidification, while the three kinds of osmotic regulating substances showed a trend of increasing first and then decreasing. The relative conductivity and MDA content of root under severe stress obviously increased by 47.5% and 36.1%, while soluble sugar, soluble protein and proline content obviously decreased by 24.9%, 14.9% and 42.5% compared with CK, which was shown that the root infiltration regulation failure, root membrane permeability and cell membrane structure were seriously damaged under severe soil acid stress.

| Soil pH | Root relative conductivity (μmol/g) | MDA content (μmol/g) | Soluble sugar content (μg/mg) | Soluble protein content (μg/mg) | Proline content (μg/g) |
|---------|----------------------------------|---------------------|-----------------------------|-------------------------------|---------------------|
| CK 6.6  | 0.40±0.05<sup>b</sup>            | 0.36±0.02<sup>c</sup>| 432.12±27.23<sup>b</sup>    | 2.21±0.14<sup>b</sup>         | 60.61±4.95<sup>a</sup> |
| pH 5.5  | 0.44±0.05<sup>b</sup>            | 0.42±0.01<sup>b</sup>| 504.88±19.36<sup>a</sup>    | 2.65±0.11<sup>b</sup>         | 66.65±5.10<sup>a</sup> |
| pH 4.6  | 0.47±0.03<sup>b</sup>            | 0.43±0.01<sup>b</sup>| 486.76±26.57<sup>a</sup>    | 2.74±0.09<sup>a</sup>         | 67.05±4.49<sup>a</sup> |
| pH 3.4  | 0.59±0.04<sup>a</sup>            | 0.49±0.03<sup>a</sup>| 324.50±16.58<sup>c</sup>    | 1.88±0.11<sup>c</sup>         | 34.85±3.14<sup>b</sup> |

### 3.3. Effects on protective enzyme activity

It can be seen from table 3 that the activity of SOD, CAT and GR increased first and then decreased, and the activity of APX showed a trend of continuous increase. The activity of SOD, CAT and GR in the root system under moderate stress reached the maximum, and the activity of SOD, CAT and GR under severe stress was significantly reduced by 43.3%, 21.5% and 25% than that of moderate stress. The APX activity reached the maximum under severe stress and increased by 136.6% compared with CK. It is indicated that in severe stress, the activity of the root protection enzyme is reduced, resulting in the production of reactive oxygen free radicals that cannot be eliminated in time, thus causing severe oxidative stress to the root system.

| Soil pH | SOD activities (U/g FW) | CAT activities (U/g FW) | APX activities (U/g FW) | GR activities (U/g FW) |
|---------|------------------------|------------------------|------------------------|------------------------|
| CK 6.6  | 382.99±11.10<sup>c</sup> | 149.55±12.57<sup>b</sup> | 46.57±5.32<sup>c</sup>   | 8.07±0.53<sup>c</sup>   |
| pH 5.5  | 463.63±29.92<sup>b</sup> | 156.42±12.68<sup>b</sup> | 61.62±6.91<sup>b</sup>   | 16.27±0.64<sup>a</sup>  |
| pH 4.6  | 551.67±41.08<sup>a</sup> | 203.21±5.54<sup>a</sup> | 78.57±3.27<sup>b</sup>   | 17.73±1.49<sup>a</sup>  |
| pH 3.4  | 312.81±40.84<sup>d</sup> | 159.48±7.50<sup>b</sup> | 110.20±10.07<sup>a</sup>| 13.29±0.92<sup>b</sup>  |

### 3.4. Effect on root tip ultrastructure

As can be seen from Figure 1, soil acid stress 28d, the mitochondria and plasmid structure of the root cells under CK and light stress are intact, round or oval, the structure of cell nuclear membrane in CK is complete and clear. Under moderate stress, the mitochondria were slightly damaged, the nucleus was swollen and deformed, the nuclear membrane was loose, and the plastids were deformed to a certain extent, and the internal structure had a certain degree of failure. Under severe stress, the mitochondria are seriously damaged, the crista is swollen and deformed, the mitochondrial membrane is partially broken, and a large number of plastids are produced in the cells and there is a serious collapse.
4. Conclusions
Soil acidification causes the imbalance of nutrient elements such as nitrogen, phosphorus and potassium in soil, and has an effect on the growth of root system. With the decrease of soil pH, the cell membrane structure and intracellular organelles are gradually subjected to oxidative stress, but SOD, CAT, APX, GR and osmotic regulating substances can effectively remove the reactive oxygen species produced in the root system and maintain the balance of internal and external osmotic potential of the cell to ease the plant stress injury. But after soil pH below 4.6, antioxidant enzyme activity decreases gradually, and the generation of reactive oxygen free radicals exceeds the capability of the root system to remove the limit, the root of the cells and organelles membrane structure will suffer severe oxidative stress damage, cause osmotic regulation imbalance, root relative electric conductivity increases rapidly.

References
[1] Xu RK. (2012) Soil Acidification as Influenced by some Agricultural Practices. J. Journal of Agro-Environment Science, 21(5):385-388.
[2] Xie S S. (2013) Causes and improvement of soil acidification in China. J. Guangdong chemical industry, 40(24): 85-86.
[3] Yu TY, Sun XS, Shi CR, et al. (2014) Research progress on soil acidification hazards and prevention and control technology. J. Journal of ecology, 11: 377-3143.
[4] Wang AS, Angle JS, Chaney RL, et al. (2006) Changes in soil biological activities under reduced soil pH during Thlaspi caerulescens phytoextraction. J. Soil: Biology and Biochemistry, 38(6): 1451-1461.
[5] Yi JX, Lv LX, Liu GD. (2006) Research on soil acidification and acidic soil’s melioration. J. Journal of Tropical Biology, (01):23-28.
[6] Schwarzerová K, Zelenková S, Nick P, et al. (2002) Aluminuminduced rapid changes in the microtubular cytoskeleton of tobacco cell line. J. Plant & Cell Physiology, 43:207-216.
[7] Wang S, Ma J, Zhao W, et al,(2014) Research on optimization growing cultivation of Sedum aizoon L. in northwest Guizhou. J. Northern Horticulture, 11: 147-149.
[8] Dong QW. (2006)The cultivation techniques of vegetable and vegetable cultivation. J. Modern Agriculture, 11:27.