Comparison of Cold Resistance between Aboveground and Underground of *Sedum aizoon* L. Seedling with Sprout under Low Temperature

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Abstract. The difference of cold resistance between aboveground and underground parts was compared by measuring the growth and cold-resistance physiology of dormancy-released *Sedum aizoon* L. seedling with sprout at -5, 0 and 5 degrees Celsius respectively. The results showed that with the decreasing temperature, the plant height and root length all showed a downward trend, and the plant height decreased more than root length. The aboveground fresh weight showed an upward trend, while the underground fresh weight showed a downward first and then upward trend. The relative electrical conductivity (REC), tissue water content, total soluble sugar and free proline content all showed an upward trend, while malondialdehyde (MDA) content showed a downward trend in leaves. The content of soluble protein showed a downward first and then upward trend in leaves. The changing trend of roots was the same as leaves in REC, total soluble sugar and soluble protein content, but the REC of roots increased less than leaves. There was no significant change in tissue water content, free proline and MDA content in roots. At the same low temperature, roots were all higher than leaves in the content of total soluble sugar and soluble protein, while the tissue water content and MDA content were the contrary. In conclusion, the cold resistance of the underground part was stronger than aboveground part in *Sedum aizoon* L. seedling with sprout.

1. Introduction

*Sedum aizoon* L. is a perennial fleshy root and facultative crassulacean acid metabolism plant of Sedum family. It is distributed in the Yangtze River Basin, the Yellow River Basin and even northeast China[1]. The underground part of *Sedum aizoon* L. can withstand the lowest temperature at -2℃ in summer[2]. At present, the research on *Sedum aizoon* L. is mainly focused on urban greening, chemical composition extraction and identification, pharmaceutical research, etc[3-5]. However, it has not been reported about the comparative study on cold resistance between aboveground and underground of *Sedum aizoon* L. seedling with sprout under low temperature. This experiment mainly studied the growth index, number and distribution of mucilage cells, chilling injury index, root vigor, photosynthetic pigments, water content of tissues, REC, MDA, total soluble sugar, soluble protein and free proline of *Sedum aizoon* L. seedling with sprout under different low temperatures and their relationship with cold resistance. The difference of cold resistance provides a reference for revealing its unique cold resistance mechanism.
2. Material and methods

2.1. Experimental materials
The experimental material was *Sedum aizoon* L. seedling with sprout, and *Suddum aizoon* L. was provided by Horticulture College of Sichuan Agricultural University.

2.2. Material preparation
In July, the upright and healthy *Suddum aizoon* L. with a diameter of 2 to 3 mm were selected to cut and propagate. The cuttings were cut from the top bud downward with a length of 6 to 7 cm, retained 3 to 4 leaves at the top bud, and removed the remaining leaves. Finally, the cuttings were retained with a length of 4 to 5 cm from the top bud to the incision. The cuttings were inserted into medium about 2 to 3 cm in 72 cell plug trays. The medium was comprised of nutrient soil, perlite and vermiculite, which was mixed in proportion according to 4:1:1 ratio. Watered once every 3 days after cuttage, the cutting seedlings could take root and recover growth after two weeks. After 30 days, the cutting seedlings with the same growth condition were transplanted in 12×13 cm (bottom × high) black nutrition bowl. Each bowl was only planted a cutting seedling and loaded with 250 g medium (The medium was the same as the above). The cutting seedlings continued to grow outdoors with uniform water and fertilizer management until the end of November in the same year. The aboveground parts were cut off uniformly before beginning the test at seven days, remaining seedling with sprout for reserving.

2.3. Experimental design
The prepared *Sedum aizoon* L. seedling with sprout were divided into the experimental and control groups. And then the experimental and control groups materials that was only watered once were placed in the artificial climate chamber for 30 days with 10 000 lux, 70% relative humidity, -5°C/0°C/5°C/25°C and 12 hours / 12 hours (Day / Night). At the end of the experiment, the growth parameters were measured immediately. Fresh samples of leaves and roots were used to determine REC, tissue water content, and to preserve some leaves or roots at -80°C for determining soluble protein and MDA content. The remaining aboveground and underground parts were dried at 105°C for 30 minutes in oven and then at 70°C until constant weight (recorded before and after drying weight), which were used to determine the contents of total soluble sugar and free proline.

2.4. Data processing and analysis
Data were analyzed with one-way ANOVA by Microsoft Excel 2007 and Origin Pro 2016 software, and compared the significant difference by new multiple range test (*P* < 0.05).

3. Results and Discussions

3.1. Growth performance
Under natural conditions, winter is the season for *Sedum aizoon* L. to show strong cold resistance. At this time, the vigorous growth of the aboveground part has withered and fallen off, replaced by new sprouts with strong cold resistance. In the experiment, the decreases of plant height, chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents were greater than those of root length and root vigor (Table 1.) with the decrease of temperature from 5°C to -5°C, which was similar to the results of Xu et al.[6] on low temperature stress of *Sedum aizoon* L. during the vigorous growth period in summer. This data indicated that the aboveground part was more sensitive to low temperature than the underground part in *Sedum aizoon* L. seedling with sprout.

| Temperature (°C) | Plant height (cm) | Root length (cm) | Aboveground fresh mass (g) | Underground fresh mass (g) |
|-----------------|------------------|-----------------|---------------------------|--------------------------|
| 25              | 2.08±0.15a       | 15.33±0.89a     | 0.73±0.064a               | 0.75±0.068b              |
3.2. Changes of cell membrane stability in leaves and roots

The water content of organs and tissues were higher at low temperature, the plants were easier to freeze[7]. The temperature was lower, and the decrement of tissue free water was more difficult. At different temperatures, the water content of leaf was higher than that of root in *Sedum aizoon* L. seedling with sprout. With the decrease of temperature, the former increased gradually, but the latter changed insignificantly, indicating that leaf was more vulnerable to low temperature injury than root. Leaf could reduce the injury that caused by intracellular icing by lowering tissue water content to the greatest extent, however, the root was able to be maintained the lower tissue water content to prevent freezing injury at low temperature (Fig 1.). Under low temperature stress, the stability of plant cell membranes was worse, the increment of their permeability was faster, and the damage of cell membranes was greater[8]. With the decrease of temperature, the REC of leaves and roots increased gradually, and the rate of increase in leaves was greater than that of roots, indicating that the stability of cell membrane of leaves was lower than that of roots at low temperature (Table 2.). In addition, MDA content in leaves decreased gradually with the decrease of temperature, and MDA content in roots did not change significantly. MDA content in roots was lower than that in leaves at all temperatures, which also showed that the stability of root cell membrane was higher than that in leaves (Table 2.).

![Fig 1. Changes of water content in leaf and root of *Sedum aizoon* L. seedling with sprout under low temperature.](image)

Note: Different lowercase letters in the figure indicate significant differences exist among different temperatures under the same index. The same below.

| Temperature (℃) | Relative electrical conductivity (%) | MDA content (nmol·g⁻¹ FW) |
|----------------|--------------------------------------|---------------------------|
|                | Leaves | Roots | Leaves | Roots | Leaves | Roots |
| 25             | 1.792±0.13b | 20.544±1.28c | 52.107±0.64c | 77.633±3.71a |
| 5              | 2.916±0.24c | 43.839±2.83b | 100.075±5.22a | 54.128±2.46b |
| 0              | 4.945±0.29b | 53.430±2.07b | 77.333±6.92b | 50.774±3.98b |
| -5             | 6.248±0.11a | 53.563±1.82a | 68.904±4.44b | 49.105±2.34b |

Note: Data were analyzed by new multiple range test and different lowercase letters in the same column meant significant difference among treatments at 0.05 level. The same below.
3.3. Changes of osmotic adjusting materials in leaves and roots

In most cases, soluble sugar acts as one of the osmotic adjusting materials in cells, which is positively correlated with the cold resistance of plants[9]. With the decrease of temperature, the total soluble sugar content increased gradually in leaves and roots of *Sedum aizoon* L. seedling with sprout. At various temperatures, the total soluble sugar content in roots was always higher than that in leaves (Fig 2a.). Therefore, the ability of roots to alleviate low temperature injury was stronger than that of leaves by increasing the total soluble sugar content. Researchers have not yet formed a unified understanding of the relationship between soluble proteins and plant cold resistance. Zhu et al.[10] considered that after low temperature stress, the soluble protein content of tea decreased first and then increased, which was the performance of tea plants gradually adapting to low temperature. The varieties with strong cold resistance had relatively high soluble protein content. Xu et al.[6] believed that the ability of *Sedum aizoon* L. to adapt to low temperature was limited in summer. After low temperature stress, the soluble protein content in leaves increased first and then decreased, and the soluble protein content could not always maintain a high state. In this experiment, the *Sedum aizoon* L. seedling with sprout was the state of cold-resistant in winter. The soluble protein content in leaves and roots decreased first and then increased with the decrease of temperature (Fig 2b.). At each temperature, the soluble protein content in roots was higher than that in leaves, which indicated that the ability of roots to reduce low temperature injury was stronger than that in leaves by increasing the soluble protein content. Like soluble proteins, it has been widely reported about the relationship between free proline and cold resistance of plants, and researchers have not yet formed a unified understanding. Zhou et al.[11] reported that the content of free proline in the leaves of *Festuca arundinacea*, which had strong cold resistance, had no significant change after low temperature treatment at 10℃. Chen et al.[12] took spring and winter wheat as the research material. The results showed that the content of free proline was relatively high in leaves of varieties with strong cold resistance, and the rate of increment was faster at low temperature. With the decrease of temperature, the free proline content of the leaves increased gradually, but that of the roots did not change significantly in *Sedum aizoon* L. seedling with sprout (Fig 2c.). This data indicated that the roots prevented low temperature injury by maintaining high content of free proline all the time, while the leaves alleviated low temperature injury by rapidly increasing the content of free proline.
Fig 2. Effects of low temperature on total soluble sugar, soluble protein and free proline content in leaf and root of *Sedum aizoon* L. seedling with sprout.

4. Conclusion
The cold resistance of underground part is stronger than that of aboveground part in *Sedum aizoon* L. seedling with sprout. The effect of low temperature on aboveground part is greater than that on underground part. The REC of leaf is greater than that of root. The content of soluble protein and total soluble sugar is always lower than that of root. The water content and MDA content of leaf tissue are always higher than that of root. In addition, the content of free proline in leaves increased gradually with the decrease of temperature, and the content of free proline in roots was also stable.

References
[1] Zhang, W.J., Liu, X.P., Wen, J.L., Mao, Z.Y. (1987) Seasonal shifts of photosynthesis and induction of CAM in *Sedum aizoon*. Acta Phytophysiologica Sinica, 13: 236-241.
[2] Zhao, G., Yuan, H.Y., Wei, L.Z., Tang, R.X., Zhang, X.F., Lu, X.P. (2011) A study of stress tolerance of a candidate plant-*Sedum aizoon* L. for green roof. Acta Agriculturae Universitis Jiangxiensis, 33: 335-339.
[3] Han, R.C., Wang, B. (2007) Study on the chemical constituents of the essential oil from *Sedum sarmentosum* Bunge. Journal of Liaoning University of Traditional Chinese Medicine, 9: 73-74.
[4] Habibi, G., Hajiboland, R. (2011) Comparison of water stress and uv radiation effects on induction of cam and antioxidative defense in the succulent *Rosularia Elymaitica* (Crassulaceae)[J]. Acta Biologica Cracoviensis Series Botanica,53: 15-24.
[5] Guo, J.J., Gui, M.Y., Zhang, P.P., Wang, J., Wang, H.B., Li, J.Y. (2017) Sensitivity of Sedum aizoon to the enhanced ultraviolet B radiation. Journal of Henan Agricultural University, 51: 71-81.
[6] Xu, D.M., He., Z.Q., Zhao, Y.P., Wang, Y.M., Long, S.J. (2016) Effects of arbuscular mycorrhizal fungi on growth and physiological characteristics of *Sedum aizoon* under low temperature stress. Pratacultural Science, 33: 2452-2464.
[7] Yang, Y.Z., Chen, G., Peng, F.R., Wang, G.X., Luo, Q., Ma, X. (2014) Differences in water and osmoregulation substance in Toona sinensis from different provenances under low temperature stress and their correlation to cold tolerance. Journal of Plant Resources and Environment, 23: 47-54.
[8] Yang, C.F., Zhao, Y., Liu, B.H., Yang, X.J. (2011) Study on relationship between cell membrane permeability of leaves of winter wheat under the low temperature treatment and cold resistance. Journal of Anhui Agricultural Sciences, 39: 11416-11417.
[9] Tian, L.P., Xue, L. (2007) Research progress on physiology and biochemistry of plant cold hardiness. Tianjin Agricultural Sciences, 17: 10-13.
[10] Zhu, Z., Jiang, J.Y., Jiang, C.J., Li, W. (2011) Effects of low temperature stress on SOD activity, soluble protein content and soluble sugar content in Camellia sinensis leaves. Journal of
[11] Zhou, Q.P., Yan, H.B., Liu, W.H. (2009) Study on resistance cold of four species of Festuca L.in seedlings stage. Acta Agrestia Sinica, 39: 4-8.

[12] Chen, X., Li, J.Y., Ma, J., Zhang, F.C. (2007) Effect of low temperature stress on change of free proline content in the leaves of spring and winter wheat. Xinjiang Agricultural Sciences, 44: 553-556.