Study on the relationship between mucilage cells and low temperature tolerance of *Sedum aizoon* L.

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Abstract. Mucilage cells were generally distributed in the vegetative organs of dicotyledons. We tried to analyse the relationship which was unknown between mucilage cells and the frost resistance by plant tissue staining technique, semi-lethal temperature and freezing injury index in *Sedum aizoon* L. The results showed that the number of mucilage cells was significantly larger than control group in the leaves and stems with experimental group. The semi-lethal temperatures were -3.34℃ and <-5℃, respectively, when the aboveground parts grew at 25℃ and 5℃. Further, the freezing injury investigation was consistent with the above results, indicating the close relationship between mucilage cells and the frost resistance in aboveground parts of *Sedum aizoon* L.

1. Introduction

*Sedum aizoon* L. is a perennial and facultative crassulacean acid metabolism herb of Crassulaceae, which is widely distributed in Fujian, Jiangsu and Henan provinces of China [1]. As a new type of health care wild vegetable, *Sedum aizoon* L. is rich in active ingredients such as flavonoids, triterpenoids, sterols, arbutin, alkaloids, etc. It has antioxidant, protects cerebrovascular, promotes blood circulation and reduces Blood lipids. [2, 3]. *Sedum aizoon* L. is easy to be propagated by cuttings with high survival rate and suitable growth temperature of 18℃ to 25℃. The lower part of -30℃ can safely overwinter, and the upper part of 5℃ can grow slowly. *Sedum aizoon* L. has a broad application prospect in the cultivation of the health care vegetable and city greening because of its potential health care functions and outstanding frost resistance.

At present, the research hot spots focus on the similar frost resistance, involving abundant research materials and data types [4]. On the one hand, a large number of studies have shown that after low temperature treatment, eggplant, pepper and cassava have similar changes in their physiological and biochemical processes, showing high activity of SOD, POD and CAT, high content of proline, soluble protein, Soluble sugar, lower malondialdehyde content and relative conductivity, compared with the control group, the plant after low temperature treatment showed stronger cold tolerance [5-7]. Mucilage cells may be related to the frost resistance of *Sedum aizoon* L.

Mucilage cells which are generally found in the vegetative organs of dicotyledons mainly relate to store carbohydrate and water, reduce transpiration, and protect plants from intensive radiation or phytophagous insects [8]. When Mastroberti and Mariath studied the development of mucilage cells in the leaves of angustifolia (Araucariaceae), it was speculated that mucus cells might be involved in resisting hypothermia [9]. Matthews and Endress investigated the distribution of mucilage cells in various plant flower organs and leaves, and the results showed that mucilage cells were common in the
leaf epidermis of sedum plants [10]. Wang Qian et al. pointed out that the low temperature tolerance characteristics of the genus Sedum plant, Moss and Sedum, may be related to the number of mucilage cells distributed in the respective leaves [11]. *Sedum aizoon* L. is the same as the sedum plant, whether mucilage cells are distributed in the vegetative organs, and the correlation between mucilage cells and their outstanding low temperature tolerance ability has not been reported.

This experiment was carried out by studying the low temperature treatment of *Sedum aizoon* L. at 25°C and 5°C for 30 days. The semi-lethal temperature of the aboveground part was determined by Logistic equation, and the distribution of mucous cells in the vegetative organs of plants was observed by paraffin section.

2. Materials and methods

2.1 Plant materials

Preparation of annual *Sedum aizoon* L. Annual *Sedum aizoon* L. was planted in the experimental base of Sichuan Agricultural University. Softwood cuttings was used to propagate annual *Sedum aizoon* L. Green tip was selected with a diameter of 2 mm to 3 mm from plant without diseases and insect pests. Then the green tip would be treated by cutting off a length of 6-7 cm, retaining 3-4 leaves from the top. The green tip was inserted into substrate about 2-3 cm in a 72 holes plug tray. The substrate was comprised of nutrient soil, perlite and vermiculite. Watered once three days after cuttage, the cutting seedlings could take root and recover growth about two weeks. When the roots of the seedlings grown to 6-7 cm, they could be transplanted with soil. The cutting seedlings with the same growth condition were planted in nutrition bowl. The cutting seedlings continued to grow for one year. Before the experiment, selected the *Sedum aizoon* L. that has the same growth, and cut the upper part of the withered yellow. Left the newly grown buds and relaxed the seedlings for 7 days.

The test was carried out in an artificial climate chamber with a light intensity of 10,000 LX, a humidity of 80 %, and a 12 h light. Set the control temperature to 25°C, low temperature treatment 5°C, no day and night temperature difference, 3 times for each treatment. When the *Sedum aizoon* L. was cultured for 30 days, the above-mentioned semi-lethal temperature and the distribution of mucous cells in the vegetative organs of the plants were measured and analyzed.

2.2 Determination method.

2.2.1 The determination of semi-lethal temperature and frost damage index

The test was carried out in an artificial climate chamber. Refer to S.-E. Jacobsen's method to determine the semi-lethal temperature of *Sedum aizoon* L.[12]. First, *Sedum aizoon* L. acclimated at a low temperature of 5°C for 24 hours and then subjected to the low temperatures treatment at 0°C, -1°C, -2°C, -3°C, -4°C and -5°C of 30 minutes each. Fitting with the logistic equation to find the semi-lethal temperature.

The freezing injury index would refer to the method of Kaw and Khush [13]. The ability of frost resistance was contrary to the change of freezing injury index. The standard of freezing injury index was divided into 5 grades. Level 0 was flourishing leaves without softening. Level 1 was less than 1/3 of the lower leaves without wilting or softening. Level 2 was less than 1/2 of the lower leaves without wilting or softening. Level 3 was more than 1/2 of the lower leaves and the stems with all softening and watery lesions. Level 4 was all the stems and the leaves with softening and watery softening.

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\text{Freezing injury index} = \frac{\sum (gi2 \times Ld2 \times 100)}{gi1 \times Ld1}
\]

\(gi2\) = number of plants with each freezing injury grade; \(Ld2\) = value of each freezing injury grade; \(gi1\) = number of total plants; \(Ld1\) = highest value of freezing injury grade.

2.2.2 Histochemical staining method of paraffin section.

According to Soukup A, Tylová E [14], paraffin section was commonly used to observe the morphological structure of cell tissue. Mucilage cells were stained with safranin O and fast green.
2.2.3 Statistical analysis.
Data was processed by Origin 2016, DP Controller and Adobe Photoshop CS6.

3. Results

3.1 The semi-lethal temperature and the freezing injury index of Sedum aizoon L.
The relative electrical conductivity data fitting Logistic equation is \( y = 76.22888 - 60.55184/(1 + (x/3.2611)^{10.6783}) \), \((R^2 = 0.99978)\), and the semi-lethal temperature of the above ground parts is -3.34°C. The numerical value of the relative electrical conductivity shows a decreasing-rising-declining trend with decreasing temperature in the range of 0 % to 20 %, and the semi-lethal temperature of Sedum aizoon L. is less than -5°C. (Fig. 1).

![Graph showing relative electrical conductivity and subzero temperature](image)

Fig 1. The relative electrical conductivity data of experimental and control group fitting Logistic equation, respectively
Note: The semilethal temperature is expressed by the temperature corresponding to 50% of the relative conductivity of leaves, then the Logistic equation was fitted to the relative conductivity data of leaves at 25°C.

3.2 Microstructure of mucilage cells in vegetative organs
The distributions of mucilage cells can be shown with the results of safranin O and fast green staining (Fig. 2). The components of mucilage cells can be shown with the results of Schiff’s reagent and fast green staining (Fig. 3).
Fig 2. The distributions of mucilage cells in vegetative organs of *Sedum aizoon* L. at two temperatures (Safranin O and fast green staining)

Note: The micrograph of the vegetative organs of *Sedum aizoon* L. at 25℃ (Root: A1, A2; Stem: C2, C2; Leaf: E1, E2); The micrograph of the vegetative organs of *Sedum aizoon* L. at 5℃ (Root: B1, B2; Stem: D2, D2; Leaf: F1, F2); Scale bars: A1 to F1 = 200 μm; A2 to F2 = 50 μm; MC: mucilage cell.
Fig 3. The distribution of mucopolysaccharides in vegetative organs of *Sedum aizoon* L. at two temperatures (Schiff's reagent and fast green staining)

The micrograph of the vegetative organs of *Sedum aizoon* L. at 25℃ (Root: G1, G2; Stem: I1, I2; Leaf: K1, K2); The micrograph of the vegetative organs of *Sedum aizoon* L. at 5℃ (Root: H1, H2; Stem: J1, J2; Leaf: L1, L2); Scale bars: G1 to L1 = 200 μm; G2 to L2 = 50 μm; Chl: Chloroplasts; ICA: the intermediate between chloroplast and amyloplast.

4. Discuss

In conclusion, this experiment preliminarily verified that the low temperature tolerance of *Sedum aizoon* L. is closely related to the distribution of mucilage cells in its vegetative organs. Its unique low temperature resistance mechanism has a development value that requires in-depth research. In addition, we speculated that the proteinoplast and amyloplast might be related to the formation of mucilage cells according to the three staining results of mucilage cells in the roots.

Reference

[1] Lin, Z., Fang, Y., Huang, A., Chen, L., Guo, S., & Chen, J. (2014). Chemical constituents from *Sedum aizoon* and their hemostatic activity. *Pharmaceutical Biology*. 52(11), 1429–1434.
[2] Li, W.L., Luo, Q.Y., Wu, L.Q., Two new prenylated isoflavones from *Sedum aizoon*. *Fitoterapia*. 2011, vol.82, pp.405-407.
[3] Xu, T.Y., Wang, Z.H., Lei, T.L., Lv, C.N., Wang, J., Lu, J.C., New flavonoid glycosides from *Sedum aizoon*. *Fitoterapia*. 2015, vol.101, pp.125-132.
[4] Xu, C.X., Research progress on the mechanism of improving plant cold hardiness. *Acta Ecologica Sinica*. 2012, vol.32, pp.7966-7980. (In Chinese)
[5] Dong, A.L., Effects of cold acclimation on the physiological of eggplant seedlings to low temperature stress. *Gansu: Gansu Agricultural University*, 2016, pp.25-29. (In Chinese)
[6] Filatova, A.G., Volkov, I.O., Investigating the microstructure and surface composition of corn starch cryogel treated with Fe Cl3, solution. *Bulletin of the Russian Academy of Sciences: Physics*, 2010, vol. 74 (7), pp.1037-1038.

[7] Back, K.H., Skinner, D.Z., Alteration of antioxidant enzyme gene expression during cold acclimation of near-isogonics wheat lines. *Plant Science*, 2003, vol.165, pp.1221-1227.

[8] Mary, G., Pieter, B., A survey of mucilage cells in vegetative organs of the dicotyledons. *Israel Journal of Botany*, 1989, vol.389, pp.125-174.

[9] Mastroberti A.A., Mariath J.E.D.A., Development of mucilage cells of Araucaria angustifolia (Araucariaceae), *Protoplasma*, 2008, vol.232, pp.233.

[10] Matthews, M.L., Endress, P.K., Floral structure and systematics in four orders of rosids, including a broad survey of floral mucilage cells, *Plant Systematics & Evolution*. 2006, vol.260, pp.199-221.

[11] Wang, Q., Guan, X.L., Hu, Z.H., Lu, C.F., Leng, P.S., Relationship between cold tolerance and leaf structure of the three species of Sedum, *Chin J Appl Environ Biol*, 2013, vol.19, pp.280-285. (In Chinese)

[12] Jacobsen S E, Monteros C, Corcuera L J, et al. Frost resistance mechanisms in quinoa (Chenopodium quinoa Willd.)[J]. European Journal of Agronomy, 2007, 26(4):471-475.

[13] Kaw, R., Khush, G.A., modified technique of screening for cold tolerance in rice, *International Rice Research Newsletter*, 1985, vol.10, pp.15-16.

[14] Soukup A, Tylová E. Essential methods of plant sample preparation for light microscopy[J]. Methods in Molecular Biology, 2014, 1080:1.