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Effect of Low temperature treatment on dormancy release of *Sedum aizoon* L.

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Abstract: The dormant *Sedum aizoon* L. seedlings were treated with different days (1d, 3d, 5d and 7d) of low temperature (5°C), and restored to grow for 40d (25°C). The growth indexes, photosynthetic characteristics and carbohydrate metabolism indexes were measured to compare the dormancy relieving effects of the seedlings. The results showed that *Sedum aizoon* L. was physiologic dormancy, and the effect was the best when it was treated at 5°C for 5 days, and the dormancy was completely relieved.

1. Introduction

Sedum aizoon L. is a genus of *Sedum* in the *Sedum* family, which is distributed in all provinces of The Yangtze River Basin and the Yellow River Basin and even the northeast of China. In recent years, *Sedum aizoon* L., as a new health vegetable, has attracted more and more attention due to its outstanding nutrition and health function. *Sedum aizoon* L. is rich in carbohydrates, protein, glucose, amino acids, vitamin B1, vitamin B2, calcium, phosphorus, iron and other substances needed by the human body^[1]. In addition, the active ingredients such as caffeine, flavonoids and oleanolic acid contained in this vegetable have health functions such as refreshing, anti-oxidation, protecting cardiovascular and cerebrovascular, promoting blood circulation and lowering blood glucose. However, there is bud dormancy phenomenon in *Sedum aizoon*, the growth period is from March to September, and the suitable growth temperature is 15°C~25°C. After November, the upper part of the growth under the appropriate temperature gradually turns yellow and falls off, and dormant buds replace it^[2], which is difficult to meet the requirements of product marketing and seriously restricts the supply of *Sedum aizoon*.

This experiment studied the influence of different low temperature treatment time on the dormancy release of *Sedum aizoon*, aiming to select the most suitable low temperature treatment time for the dormancy release of dormant buds, and to provide products for the annual market supply of *Sedum aizoon*. In addition, this study can provide some reference to the study on the mechanism of the dormancy release of *Sedum aizoon*.

2. Materials and Methods

The experimental material is the sprout of *Sedum aizoon* L., which is provided by College of Horticulture, Sichuan Agricultural University. Cutting propagation was carried out in July. After 30 days, the cuttings with the same growth were selected and transplanted to a black nutrient pot 12cm×13cm (bottom × high), with one plant for each pot. The seedlings were placed outdoors to continue growing until October, entering a dormant state.



The prepared buds were placed in an artificial climate room and treated in darkness at 5°C for 1d, 3d, 5d and 7d respectively, with the environmental relative humidity of 70%. They were watered once before the test, and were not watered during the 0d to 7d treatment. Each treatment was set to repeat for 3 times, a total of 60 plants. After the treatment, the materials of the test group and the control group were immediately placed in an artificial climate chamber with light intensity of 10000LX, relative humidity of 70%, temperature of 25°C, and 12h/12h (day/night) for 40 days. The plants were watered once every 3~4d, with the watering capacity of 50mL per plant. At the end of the experiment, the growth indexes of all treated plants were measured immediately. The chlorophyll fluorescence parameters of plants were measured by PAM-2500 fluorescence analyzer. The photosynthetic pigment content, sugar content and metabolism-related enzyme activities were measured by fresh samples from functional leaves at the same site.

3. Determination of indicators

3.1. Growth index

The plant height, root length, stem thickness and leaf thickness were measured by ruler and vernier caliper. Weigh the fresh weight of the aboveground and underground parts of the plant with a analytical balance.

3.2. Determination of chlorophyll fluorescence parameters

The chlorophyll fluorescence kinetic parameters were determined by a portable modulated chlorophyll fluorescence analyzer (PAM-2500)

3.3. Determination of sugar content in leaves

The reducing sugar content was determined by 3, 5-dinitrosalicylic acid (DNS) method. The glucose content was determined by the glucose oxidase method. The fructose content was determined by the resorcinol method. The determination of sucrose content was based on the method of Yang, Q.N.^[3]

3.4. Determination of enzyme activity related to Carbohydrate metabolism.

Enzyme activity was determined by reference to Zhi Shan's method^[4]. (Amylase, Soluble acid invertase, Cell wall bound invertase, Neutral invertase, Adp-glucose pyrophosphorylase, Starch phosphorylase)

4. Results

4.1. Growth

As can be seen from Table 1, with the extension of days under low-temperature (5°C), the single leaf area of sedum aizoon presented an increasing trend during the low-temperature treatment. The plant height, stem diameter and aboveground fresh weight increased first and then decreased. The increase of plant height, stem diameter and aboveground fresh weight in 5 days was obviously higher than that of other treatments.

Table 1. Effect of low temperature (5°C) treatment with different durations on biomass of Sedum aizoon L.

Duration (d)	Plant height (cm)	Stem diameter (mm)	Leaf thickness (mm)	Single leaf area (cm ²)	Aboveground fresh mass (g)
0	3.12±0.10d	1.73±0.08d	0.78±0.049a	6.15±0.31e	1.65±0.12d
1	4.36±0.23c	2.11±0.09c	0.79±0.067a	8.10±0.54d	3.42±0.23c
3	5.32±0.16b	2.31±0.09b	0.80±0.079a	9.41±0.24c	5.11±0.24ab
5	5.70±0.08a	2.46±0.04a	0.77±0.051a	10.61±0.41b	5.40±0.53a

7	5.54±0.12ab	2.18±0.03c	0.74±0.060a	11.328±0.67a	4.62±0.31b
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4.2. Chlorophyll fluorescence parameter

As can be seen from Table 2, with the extension of days under low-temperature(5°C), the Fv/Fm values of sedum aizoon in each treatment did not change significantly, ranging from 0.852 to 0.861, indicating that the photochemical reactions of the plants were in a normal activated state. Fo, Fm and NPQ values first increased and then decreased. After 3 days low-temperature treatment, Fo, Fm and NPQ are gradually reduced, while Φ_{PSII} , qP and ETR values are increased after lower first, After 3 days low-temperature treatment Φ_{PSII} , qP and ETR value begin to gradually increases, but 5th and 7th days data (Φ_{PSII} , qP and ETR) had no obvious difference.

Table 2. Effects of low temperature (5°C) treatment with different durations on chlorophyllII fluorescence parameters in leaves

Fluorescence parameters	0d	1d	3d	5d	7d
Fo	0.374±0.022a	0.318±0.006c	0.346±0.011b	0.325±0.016b	0.321±0.005c
Fm	2.525±0.09a	2.283±0.05b	2.430±0.04a	2.292±0.07b	2.275±0.05b
Fv/Fm	0.852±0.004b	0.861±0.000a	0.858±0.002a	0.858±0.003a	0.859±0.002a
Φ_{PSII}	0.561±0.012c	0.625±0.015b	0.602±0.016b	0.636±0.017ab	0.657±0.003a
NPQ	0.608±0.031a	0.388±0.033c	0.509±0.043b	0.353±0.016c	0.214±0.015d
qP	0.739±0.016c	0.795±0.025ab	0.775±0.021b	0.803±0.022ab	0.816±0.003a
ETR	90.5±1.92c	100.8±2.40b	97.1±2.60b	102.6±2.85ab	106.0±0.58a

It can be seen that all parameters of chlorophyll fluorescence of sedum aizoon began to return to normal state after 3 days of low temperature treatment.

4.3. Carbohydrate content

As can be seen from Figure 1, with the extension of days under low-temperature(5°C), the reducing sugar and fructose content of sedum aizoon first decreased and then increased. After 1 day of low-temperature treatment, the contents of reducing sugar and fructose in sedum aizoon showed changes, showing signs of breaking dormancy. After 5 days of low-temperature treatment, the contents of all kinds of sugar reached to completely remove dormancy, while the changes of glucose content may have no significant relationship with the dormancy of sedum aizoon.

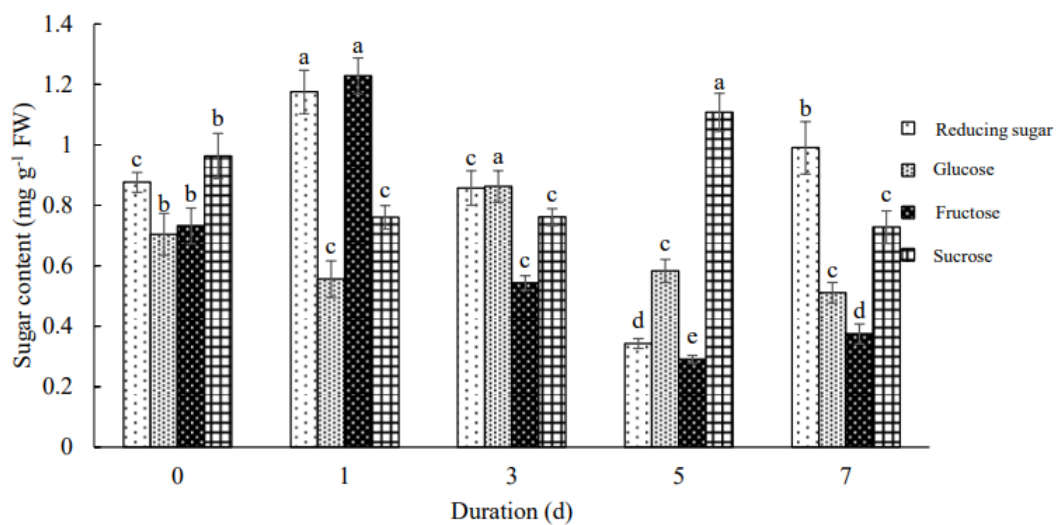


Fig.1. Effects of low temperature (5°C) treatment with different durations on sugar content of *Sedum aizoon* L.

4.4. Carbohydrate metabolic related enzymes activity

As can be seen from Table 3, with the extension of the low-temperature (5°C) treatment time, the activity of AMY in the leaves of *Sedum aizoon* was first decreased and then increased, the enzyme activity of STP in the leaves was first increased and then decreased, and the enzyme activities of SAI, CWI and NI were first decreased and then increased. After 1 day of low temperature treatment, the enzyme activities of AMY, SAI, CWI and NI all changed, showing signs of breaking dormancy. At 5 days of low temperature treatment, the enzyme activities of AMY, SAI, CWI and NI all reached the state when the dormancy was completely lifted, which was consistent with the changing law of reducing sugar, fructose and sucrose content.

Table 3. Effects of low temperature (5°C) treatment with different durations on carbohydrate metabolic related enzymes in leaves

Carbohydrate metabolic related enzymes	0d	1d	3d	5d	7d
AMY (mg·Maltose·g ⁻¹ ·h ⁻¹)	8.144±0.71a	8.201±0.64a	7.194±0.68a	3.820±0.35c	5.147±0.37b
STP (μg·Pi·g ⁻¹ ·min ⁻¹)	4.043±0.20a	2.811±0.27b	3.042±0.14b	2.041±0.07c	1.668±0.09d
AGPase (U·g ⁻¹)	25.721±0.98c	26.829±1.79c	31.300±0.89b	32.195±3.10b	35.772±1.79a
SAI (mg·Glucose·g ⁻¹ ·h ⁻¹)	28.255±1.08c	36.280±1.54a	32.717±0.89b	29.058±1.22c	31.739±0.48b
CWI (mg·Glucose·g ⁻¹ ·h ⁻¹)	1.420±0.09d	2.632±0.15b	2.562±0.16bc	2.384±0.09c	2.841±0.04a
NI (mg·Glucose·g ⁻¹ ·h ⁻¹)	5.181±0.32d	7.633±0.34a	6.486±0.25b	5.779±0.25c	5.978±0.24bc

5. Discussion

In nature, plants have dormancy. According to the conditions of dormancy termination, dormancy is divided into two types, namely forced dormancy and physiological dormancy^[5]. Physiological

dormancy can only be released and restored to normal growth after the dormancy plant has been treated with light, high temperature, low temperature, hormone, etc.^[6]. This study showed that plant height, stem diameter, leaf area and aboveground fresh weight were significantly increased compared with the control group in the low-temperature treatment from 1d to 5d. Growth index of sedum aizoon reached the maximum at 5d, and dormancy of seedlings was completely relieved. During the process of plant dormancy release, there was no significant difference in Fv/Fm between the experimental group and the control group, and it fluctuated from 0.852 to 0.861, indicating that Fv/Fm had no correlation with the degree of dormancy. Low temperature treatment for 5d, sedum aizoon seedling photosynthetic ability restore the fastest, chlorophyll fluorescence parameters Φ_{PSII} , qP and ETR all returned to normal, continue to low temperature treatment Φ_{PSII} , qP and ETR had no significant increase. The restorative growth of sedum aizoon increased the photosynthetic activity (qP value) to enhance the leaf's utilization efficiency of light energy and promote photosynthetic accumulation^[7]. In this experiment, the low temperature treatment before 5d, Sedum aizoon seedling only reducing sugar, sucrose, fructose content in leaf and AMY, SAI, CWI and activity of NI a downward trend. This is similar to the results of Sato A's study^[8]. continue to low temperature treatment, sedum aizoon leaves' reducing sugar, fructose content, total AMY, SAI, CWI and NI enzymes are gradually rise, sucrose content gradually decline, suggests that excess low temperature may restart the dormancy of sedum aizoon bud.

6. Conclusions

The above results prove that *sedum aizoon* L. has physiological dormancy, and treatment at low temperature of 5°C for 5 days has the best effect on relieving dormancy, which can be completely relieved.

Acknowledgments

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