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ORIGINAL ARTICLE

Comparison of cold resistance physiological and biochemical features of four *Herba Rhodiola* seedlings under low temperature

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Abstract To discuss the cold resistance performance of different *Herba Rhodiolae* and successfully transplant *Herba Rhodiolae* to the Gansu plateau area for nursing, domestication and planting, this paper systematically studies six physiological and biochemical features of *Rhodiola kirilowii*, *Rhodiola algida*, *Rhodiola crenulata* and *Herba Rhodiolae* that are closely associated with cold resistance features and concludes with the cold resistance capability of *Rhodiola kirilowii*. In the selected six main indexes of the *Herba Rhodiolae*, the POD, SOD and CAT activity and MDA and Pro content in the leaf are the main physiological and biochemical indexes to indicate the cold resistance performance of four *Herba Rhodiolae* seedlings and can be regarded as the preliminary indexes to assess the winter performance of *Herba Rhodiolae*. The research work will provide the theoretical basis for the wild variants of *Herba Rhodiolae* and GAPJ base construction.

1. Introduction

Herba Rhodiolae belongs to the herbaceous perennial plants *Rhodiola* type and is used in traditional Tibetan medicine. *Herba Rhodiolae*, which is slightly sweet and bitter in flavor, can adapt to cold, dry or damp environments. As for medicinal applications, it can be used to invigorate blood circulation, stop bleeding, regulate the flow of vital energy and remove obstructions and nourish the blood, support healthy energy levels, make the brain healthy and enhance intelligence.

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Besides, it can also help to nourish and build the body, relieve fatigue, and treat diseases like senile heart failure, asynodia, diabetes and liver diseases. (Zhang, 1984). The main functions include anti-anoxia, anti-fatigue, anti-aging, anti-toxicity, anti-radiation, anti-cancer and two-way adjustment of the nervus centralis and endocrine system (Bao and Li, 1995). Nowadays, researches on different *Herba Rhodiolae* mainly focused on the content of chemical compositions extracted in *Herba Rhodiolae* such as glycosides (Liu et al., 2005). Few research on the physiological and biochemical index measurements of cold resistance is reported, not to mention the comprehensive evaluation of cold resistance for *Herba Rhodiolae*. However, it is well known that wild *Herba Rhodiolae* requires rigorous environmental and weather conditions in transplanting, domestication and planting, thus the transplanted *Herba Rhodiolae* cannot adapt the new environments, leading to an extremely low survival rate (Ashraf et al., 2013a,b). With continuous discovery of the wonderful functions of *Herba Rhodiolae*, the importance of research on *Herba Rhodiolae* is emphasized continuously in medicine all over the world recently. At the same time, medicine, health products and foods produced by using *Herba Rhodiolae* are extensively applied for astronauts, pilots, athletes, divers and people working under special conditions. *Herba Rhodiolae* medicines and health products are available for sale in Japan, China and many western countries, however, only the wild *Herba Rhodiolae* cannot meet the market requirements. Therefore, it becomes more and more urgent to study the artificial planting of *Herba Rhodiolae*. To successfully transplant the *Herba Rhodiolae*, it is very necessary to deeply understand its cold resistance physiological and biological indexes. Here, in order to pave the way for the wide transplant and production of *Herba Rhodiolae*, out experiment studies systematically the physiological and biological indexes of four *Herba Rhodiolae* types under the simulated natural low-temperature conditions at the lab-level.

2. Materials and methods

2.1. Plant materials

Rhodiola kirilowii, *Rhodiola algida*, *Rhodiola crenulata* and *Herba Rhodiolae* seedlings are collected by High and Cold Ecology Institute of the Gansu Normal University for Nationalities from Zhuoni (over 2500 m elevation), Luqu (over 3400 m elevation), Gannan state, Gansu province, Yushu (over 3800 m elevation), Qinghai province, and Seqila Mountain (over 4000 m elevation), Tibet. These seedlings were planted in a 18 m high and Φ25 cm flowerpot on May 12, 2012. After the seedling grows 15 cm high, the leaves are collected.

2.2. Processing method

The *Herba Rhodiolae* leaves collected from the cultivated seedlings are placed inside a refrigerator, which simulates the low-temperature conditions under natural conditions and the temperature is set as −30 °C, −25 °C, −20 °C, −15 °C, −10 °C, −5 °C and 0 °C. The indoor CK is used as the contrast. The refrigerator is controlled to decrease at a rate of 1 °C. When the temperature reaches the required temperature, the test materials were placed inside the refrigerator for 24 h. Partial samples were taken out and placed under indoor temperature for 15 h for control group measurements. At the same time, other samples were further processed under the set gradient.

2.3. Instrument devices

Abbe refractometer, balance, oven, low-temperature procedure control refrigerator, VV-9200 spectrophotometer, electronic balance, mortar and Constant temperature water bath kettle.

2.4. Index measurement method

2.4.1. Measurement of cell membrane permeability of Herba Rhodiolae blade

Similar to the ultraviolet absorption method proposed by Xie and Xu (1986): firstly select fours kinds of *Herba Rhodiolae* blades, wash with tap water to remove dirt on the surface, then wash with distilled water 1–2 times, and use a clean gauze to extract the surface moisture, finally remove the large vein, and cut with the small blade round 1 cm^2 to place in refrigerators under 20 °C room temperature, −30 °C, −25 °C, −20 °C, −15 °C, −10 °C, −5 °C and 0 °C, respectively for low-temperature treatment. All samples were kept for 15–30 min, and then taken out. After adding 50 ml distilled water, the conductivity was measured with a conductometer under different temperatures.

2.4.2. Determination of proline content in Herba Rhodiolae blade

As mentioned by Zhi and Li (2000) using the acidic-ninhydrin developing method, the process is as follows: weigh 0.5 g of *Herba Rhodiolae* blades of all four kinds described above and put them into several big test tubes respectively, and then add 5 ml sulfosalicylic acid (concentration 3%). After extracting in a boiling water bath for 10 min and then cooling to room temperature, the filter liquor which is the proline solution, is collected using another batch of clean tubes. 2 ml extracting solution was transferred into another clean test tube, and 2 ml glacial acetic acid and 2 ml acidic ninhydrin reagent were added and subsequently heated in a boiling water bath for another 30 min, during which the solution become red in color. After cooling, 4 ml methylbenzene was added. Finally, the fast mixer (SK96-A) was used to extract for 20 s, then the upper liquid was removed into a 10 ml centrifuge tube after standing. The centrifuge was kept under 3000 r/min for 5 min. Meanwhile, methylbenzene was regarded as blank control, with upper red methylbenzene liquid absorbed for color contrast. The light absorption value was measured with the wave length of 520 nm. Here, Proline content is defined as (μg/g) = $5 \times X/(2 \times W)$, where X is the proline content checked with unit μg. W is the fresh weight of sample with unit g.

2.4.3. Measurement of SOD activity of Herba Rhodiolae blade

Refer to pyrogallol autoxidation method of Liu (1985): prepare hydrochloric acid of 10 mmol L^{-1}, pyrogallol of 50 mmol L^{-1} and K_2HPO_4-KH_2PO_4 (pH8.37) buffer solution of 0.05 mol L^{-1}, and add 28 μl guaiacol. The mixture was heated and stirred using a magnetic stirring apparatus till

guaiacol is dissolved. After the solution was cooled to room temperature, 19 μl of hydrogen peroxide (30%) was added and mixed uniformly. Finally, 4 kinds of *Herba Rhodiolae* materials with a weight of 1 g were added, then take appropriate quantities of liquid nitrogen to grind to a powder, and add appropriate quantities of phosphate buffer into the mortar to grind to a homogenate. After centrifuging for 15 min, 3 ml reaction mixture was added, and the absorbance value was measured with a spectrophotometer at a wave length of 470 nm.

Total activity of SOD (u/g·FW) = $[(A_c - A_E) \times V]/(1/2A_c \times W \times V_t)$, where A_c refers to the absorbance of the irradiation control tube. A_E refers to absorbance of the sample tube. V means the total volume of the sample solution (ml). V_t is the measured enzyme solution volume (ml, 30 μl). W refers to the fresh weight of the sample with unit g, which should be converted to mass of chloroplast mg in the measurement process. The unit of protein content is mg/g.

2.4.4. Measurement of POD activity of Herba Rhodiolae blade

Refer to the methoxyphenol method of Li and Gong (2008): prepare phosphate buffer of 0.3 mol/L (pH 6.0), mix uniformly 124 ml of Na_2HPO_4 and 876 ml of NaH_2PO_4. The obtained solution is exactly 1000 ml in quantity of mixed liquid of PBS (0.3 M, pH 6.0). Take 200 ml PBS (0.3 M, pH6.0), and add 0.075 ml liquid (original liquid) guaiacol (2-methoxyphenol). After dissolving by heating and stirring, 0.110 ml H_2O_2 (30%) was added after cooling. Weigh 2 g for all 4 kinds of *Herba Rhodiolae* materials and grind them to a powder respectively, and then add appropriate phosphate buffer in the mortar to grind to a homogenate. After centrifuging for 15 min, 5 ml reaction mixture and 35 μl enzyme fluid were added, taking PBS with zero adjustment as reference. The absorbance value was measured with a spectrophotometer under the wave length of 470 nm, which is,

$$\mathrm{POD(u/g\ min)} = (\Delta A_{470} \times V_t)/(W \times V_s \times 0.01 \times t)$$

where ΔA_{470} is the change of absorbance within reaction time. W refers to the fresh weight of the sample (g). t means the reaction time (min). V_t refers to the total volume of extracting enzyme fluid (1.6 ml). V_s is the volume of enzyme fluid taken for use when measured (ml, 30 μl).

2.4.5. Measurement of CAT activity of Herba Rhodiolae blade

Adoption of potassium iodide oxidation method (He, 2000): preparation of phosphate buffer of 0.16 mol/L (pH 7.0), by taking 457 ml (Na_2HPO_4) and 292 ml (NaH_2PO_4) to mix together, and then adding distilled water to obtain 1000 ml of mixed liquid PBS. Take 205 ml PBS (0.16 M, pH7.0), and add 0.3090 ml H_2O_2 (original liquid) into a shake well, thus getting mixed reaction liquid. Weigh 4 kinds of *Herba Rhodiolae* materials respectively amounting to 1.5 g, and take appropriate quantities of liquid nitrogen to grind to a powder, and then take appropriate quantities of phosphate buffer in the mortar to grind to a homogenate, after centrifuging for 12 min, add 5 ml mixed reaction liquid, and add 0.2 ml enzyme liquid. Make zero adjustment taking PBS as reference, and measure OD 240 (ultraviolet) (for 40 s).

$$\mathrm{CAT(u/g\ min)} = (\Delta A_{240} \times V_t)/(W \times V_s \times 0.01 \times t)$$

ΔA_{240}: change of absorbance within reaction time; W refers to fresh weight of the sample (g); t refers to reaction time (min); V_t refers to total volume of extracting enzyme fluid (ml, 1.6 ml); V_s refers to volume of enzyme fluid taken for use when measured (ml, 0.1 ml).

2.4.6. MDA measurement method: refer to the thiobarbituric acid method of Zhang et al. (2009)

1.5 g plant material was put into a mortar, and then 1.6 ml TCA (10%) and 2 ml TDA were added; followed by 9 ml of TCA until fully ground, and then centrifuged for 10 min properly, accordingly, the supernatant liquid is the sample liquid. Absorb 3 ml of sample liquid, and add 3 ml of TBA (0.8%) into the mixture and make sure it is uniformly distributed. Cover the test tube with a closed tube. Keep in boiling water for 15 min, and then rapidly cool and centrifuge. Take the supernatant liquid to measure the OD value at a wave length of 532 nm.

$$\mathrm{MDA\ concentration\ C\ (mol/L)} = 6.45 \times 10^{-6} \times A_{532} - 0.56 \times 10^{-6} \times A_{532}$$

$$\mathrm{MDA\ content\ (mol/g\ FW)} = C \times V/W$$

where V refers to volume of the extracting solution (3 ml), and W refers to the fresh weight of the sample (1.5 g).

2.5. Data processing

Microsoft Excel is used for piloting and DPS7.5 is used for statistical analysis.

3. Results

3.1. Variation of the cell membrane permeability for four *Herba Rhodiolae* leaves under low temperature

Research results (Fig. 1) indicate that the change trends of the relative electric conductivity for all four *Herba Rhodiolae* are different. Accompanied with the decreasing temperature, in the initial period, the electric conductivity of *Herba Rhodiolae* and *Rhodiola crenulata* continuously increase. On the contrary,

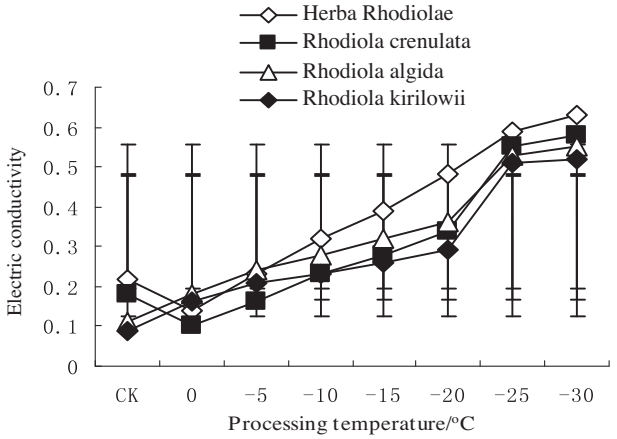

Figure 1 Trends of relative electric conductivity on cell membrane permeability of *Herba Rhodiolae* leaves.

the electric conductivity of the *Rhodiola kirilowii* and *Rhodiola algida* decrease during the same period. This is likely due to the fact that *Rhodiola kirilowii* and *Rhodiola algida* might generate a certain elastic threat in response to the low-temperature damage within 0 °C and −5 °C, therefore the functions and structure of the cell membrane recover. Moreover, the electric conductivity for all four *Herba Rhodiolae* increase when the temperature is decreased from −5 °C to −15 °C. The increment of *Herba Rhodiolae* is smaller, suggesting its strongest cold resistance capability. The increment of *Rhodiola crenulata* is secondary, which means the cold resistance capability is also secondary. This is followed by the increment of *Rhodiola algida*, demonstrating that *Rhodiola algida* belongs to the middle-strength summer resistance type. The increment of the *Rhodiola kirilowii* has a maximum value, which implies its weakest cold resistance capability the weakest compared with the other three groups. Besides, Fig. 1 also indicates that the percentage variation of the electric conductivity for these different types is over 50% under −25 °C, thus the permeability of the ionic electrolyte is extremely severe during this period. When the temperature decreases from −25 °C to −30 °C, the electric conductivity of four *Herba Rhodiolae* increases slowly.

3.2. Variation of free Pro content of four Herba Rhodiolae leaves under low-temperature threat

Fig. 2 shows that the Pro content *Herba Rhodiolae* and *Rhodiola crenulata* increases before the temperature decreases to −10 °C. Moreover, it is obvious that the Pro content of the *Herba Rhodiolae* increases much faster. Meanwhile, the Pro content for both *Rhodiola kirilowii* and *Rhodiola algida* decreases, with the former one decreasing much faster. With decreasing temperature, the Pro contents for all *Herba Rhodiolae* encounter an inductive increase within −10 °C and −20 °C. It is noteworthy that the Pro content of *Rhodiola kirilowii* reaches the maximum at −15 °C, which increases significantly by 25.29% ($P < 0.01$) compared to CK. Afterward, it was found to decrease. On the contrary, the Pro content of *Rhodiola algida* reaches the maximum at −20 °C, and then it decreases and increases again by 27.95% ($P < 0.01$) compared to CK. The Pro contents of *Herba Rhodiolae* and *Herba Rhodiolae* are under the growth state all the time and reach the maximum at −25 °C. They increase significantly by 48.89% ($P < 0.05$) and 34.44% compared to CK, respectively, after which they begin to decrease.

3.3. Variation of POD activity of four Herba Rhodiolae leaves under low-temperature threat

As shown in Fig. 3, after processing under different temperatures, the POD activity of four *Herba Rhodiolae* leaves change following a typical trend as "descend-ascend-descend". The POD activity of four *Herba Rhodiolaes* decrease differently in the initial period from 20 °C to −10 °C. The decreasing speed from highest to lowest are *Rhodiola kirilowii*, *Rhodiola algida*, *Rhodiola crenulata* and *Herba Rhodiolae*, respectively. When the temperature decreases from −10 °C to −20 °C, the POD activity of four *Herba Rhodiolae* leaves begin to increases, indicating that all *Herba Rhodiolaes* have the ability to reduce the damage due to the low temperature to the plants. Compared to CK, when the temperature decreases from −10 °C to −20 °C, the POD activity of the *Herba Rhodiolae* increases from 2834 U/mg FW to 266.23 U/mg·FW, with the increasing percent of 0.83%. Similarly, the POD activity of *Rhodiola kirilowii*, *Rhodiola algida* and *Rhodiola crenulata* increase by 22.73%, 19.58% and 9.39%, respectively, but they are lower than that under CK. When the temperature decreases from −20 °C to −30 °C, the POD activity content also decreases slowly. When the temperature is under −20 °C, more and more active oxygen will be generated, which results in severe destruction of the protective enzyme system.

3.4. Variation of SOD activity of four Herba Rhodiolae leaves under low-temperature threat

As shown in Fig. 4, all SOD activity of the four *Herba Rhodiolae* leaves firstly increase and then decrease with further decreased temperature. Specially, the SOD activity remarkably increases in the initial period from 20 °C to −15 °C. When the temperature decreases to −15 °C, the increase of SOD activity for all four *Herba Rhodiolaes* reaches up to the maximum values. The increase in speeds and peaks from highest to lowest

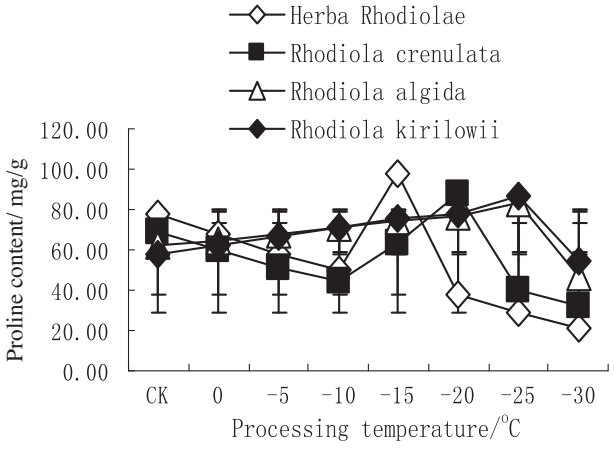

Figure 2 Influence of low-temperature threat on cell membrane permeability of *Herba Rhodiolae* leaves.

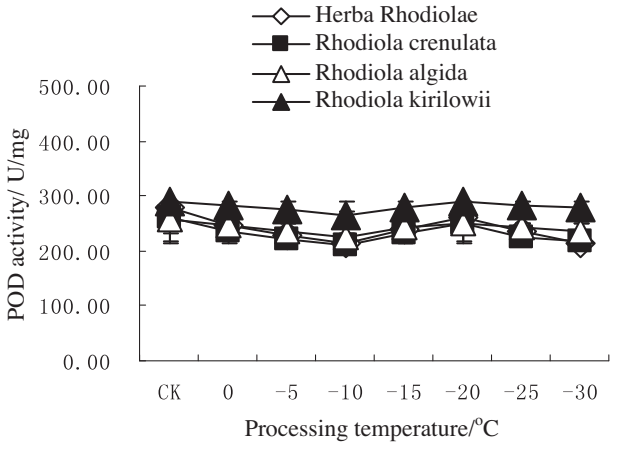

Figure 3 Influence of low-temperature threat on POD activity of *Herba Rhodiolae* leaves.

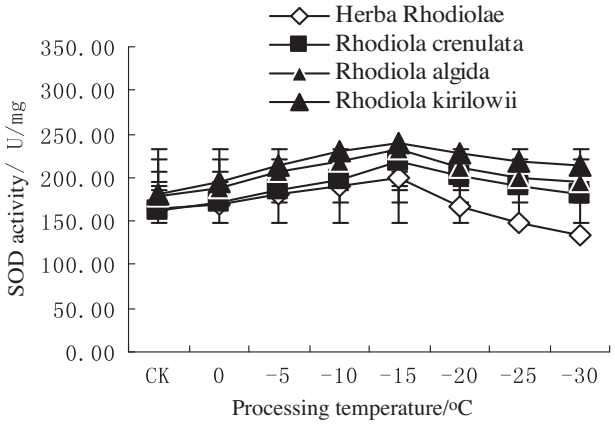

Figure 4 Influence of low-temperature threat on SOD activity of *Herba Rhodiolae* leaves.

are *Herba Rhodiolae*, *Rhodiola crenulata*, *Herba Rhodiolae* and *Rhodiola kirilowii*, respectably. In details, the SOD activity of *Herba Rhodiolae* increases quickly and the peak is 238.55, with the increase rate as high as 24.36%. The increase in speed of the *Rhodiola kirilowii* is minimal, with the peak increase rate only 0.02%. These results indicate that the cold resistance capability of *Herba Rhodiolae* becomes stronger in this period and the SOD activity of the leaves increases to a maximum. After −15 °C, the SOD activity starts to decrease.

3.5. Variation of CAT activity of four Herba Rhodiolae leaves under low-temperature threat

Fig. 5 indicates the fact that the change law of CAT activity for four *Herba Rhodiolae* is similar to each other under low-temperature threat. During the period when the temperature is decreased from CK to −30 °C, the CAT activity of *Rhodiola kirilowii* and *Rhodiola algida* presents a so-called "rising-dropping" trend while the former group increases quickly. However, the CAT activity of *Herba Rhodiolae* and *Rhodiola crenulata* demonstrates a "down–up–down" trend. Among them, during −10 °C to −20 °C, the CAT activity of *Herba Rhodiolae* encounters an inductive increase firstly, then a downward trend appears. During the range from −15 °C to −25 °C, with the decreased temperature, the CAT activity of *Rhodiola crenulata* encounters an inductive increase, afterward it begins to decrease. The CAT content of *Rhodiola algida* reaches the maximum under −25 °C and starts to decreases after that. When the temperature decreases to −30 °C, the CAT activity of *Herba Rhodiolae* reduces to the minimum.

3.6. Variation of MDA content of four Herba Rhodiolae leaves under low temperature threat

Fig. 6 indicates the MDA content of four *Herba Rhodiolaes*, which shows the increasing MDA content with the decreased temperature under low temperature threat. It demonstrates that the enhanced membrane lipid superoxide inside the body, which implies the peroxidating action will become strong with the decreasing of external temperature. Actually, MDA content starts to decrease in the initial decreasing period from 20 °C to −15 °C. When the temperature continuously decreases within −15 °C to −20/C, MDA content starts to increase, but change speed of the MDA content for four *Herba Rhodiolaes* is not very high. Here, the change speed of *Rhodiola crenulata* is highest, with the MDA content enhancing from 42.75 mmol/g·FW to 63.88 mmol/g·FW at −30 °C, which is 49.42% compared with CK. The MDA content of *Herba Rhodiolae* ranked the second position, with the change amount from 55.08 mmol/g·FW to 67.79 mmol/g·FW at −30 °C, followed by *Rhodiola algida* and *Rhodiola kirilowii*, with the MDA contents changing slowly from 53.93 to 39.62 mmol/g·FW, and from 48.11 to 49.14 mmol/g·FW at −30 °C, respectively.

3.7. PCA analysis of 6 physiological and biochemical indexes affecting cold resistance of Herba Rhodiolae

The average of 6 physiological and biochemical indexes that affect the cold resistance of *Herba Rhodiolae* are computed under 8 temperatures. After standardization, the PCA analysis results are given in Table 1. Specially, Table 1 indicates that the cold resistance capabilities of four *Herba Rhodiolae* ranked from highest to lowest are of order *Herba Rhodiolae*, *Rhodiola crenulata*, *Rhodiola algida* and *Rhodiola kirilowii*. Besides, the six physiological and biochemical indexes that affect cold resistance of *Herba Rhodiolae* from strongest to weakest are SOD

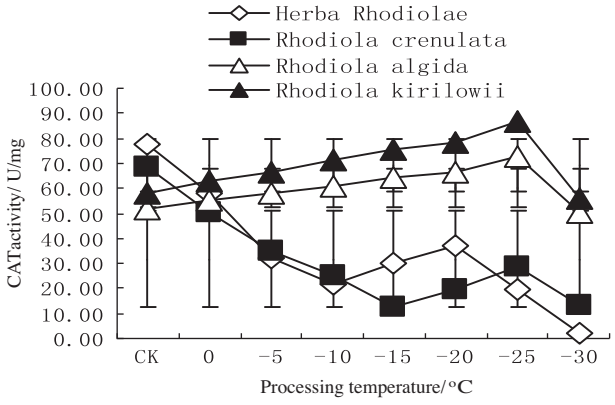

Figure 5 Influence of low-temperature threat on CAT activity *t* of *Herba Rhodiolae* leaves.

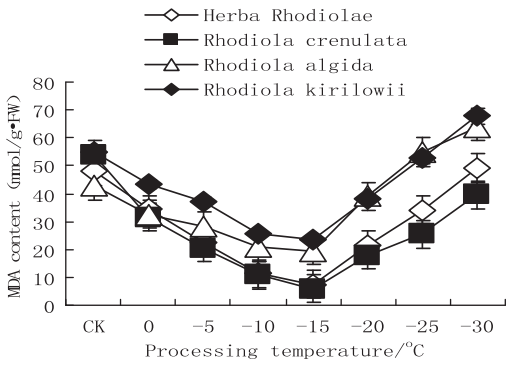

Figure 6 Influence of low-temperature threat on MDA content of *Herba Rhodiolae* leaves.

Table 1 PCA analysis of 6 physiological and biochemical indexes affecting cold resistance of *Herba Rhodiolae*.

Index	Type				
	Herba Rhodiolae	*Rhodiola crenulata*	*Rhodiola algida*	*Rhodiola kirilowii*	Index sorting
Electric conductivity (%)	−0.7876	−0.7969	−0.8562	−0.8328	6
Proline content /(mg/g)	−0.1361	−0.1444	−0.0863	−0.1358	5
POD activity (U/g FW)	2.0762	1.924	1.8815	1.9986	2
SOD activity (U/g FW)	1.2345	1.418	1.4493	1.3309	1
CAT activity (U/g FW)	−0.3745	−0.4294	−0.1812	−0.1347	4
MDA content (mmol/g FW)	−0.4488	−0.4982	−0.4319	−0.4025	3
Type sorting	4	3	2	1	

activity, POD activity, MDA content, CAT activity, proline content and electric conductivity.

4. Discussion

As a typical plant growing at high-elevation and cold areas, *Herba Rhodiolae* has the unique physiological mechanism adaptive to the special environment. This unique physiological mechanism indicates that the special plant physiological and biochemical indexes inside the body change according to the variation of the external temperature to adjust its own physiological modification and adapt to environmental conditions. The experimental results indicate that the change law of 6 physiological and biochemical indexes of four *Herba Rhodiolaes* is different.

When the permeability of cell membrane for certain plants is damaged due to low temperature, the membrane permeability will increase and the solutes inside the membrane will leak, leading to the increase of electric conductivity because of leaking liquid. Therefore, if the electric conductivity increases, the freezing of plants will become severe. It is indicated through a study on the relationship between cold resistance and cell membrane permeability of 4 kinds of *Herba Rhodiolae* that the cold resistance of *Rhodiola Fastigiata* is strongest while that of *Rhodiola kirilowii* is weakest. Proline is the main cytoplasm permeation adjustment agent. When the plants are threatened by a reverse environment such as low temperature, the accumulative Pro inside the body will quickly accumulate. When plants resist threats from low temperature, Pro can balance the cellular metabolism, thus the anti-reversion force of plants will be enhanced (Ashraf et al., 2011a,b,c). Research on the free proline change of four *Herba Rhodiolaes* under low temperature indicates that the withstanding capabilities of four *Herba Rhodiolaes* from highest to lowest are *Herba Rhodiolae*, *Rhodiola crenulata*, *Rhodiola algida* and *Rhodiola kirilowii*, respectively. The change trend of POD activity for four *Herba Rhodiolae* leaves show a typical "descend-ascend-descend" trend during the low-temperature processing. When the temperature decreases from −10 °C to −20 °C, four *Herba Rhodiolaes* can alleviate the damage resulting from the low temperature to the plants via self adjustment. The SOD activity of four *Herba Rhodiolae* leaves will firstly increase and subsequently decrease with the decreased temperature. It indicates that the cold resistance of four *Herba Rhodiolaes* ranking from strongest to weakest are *Herba Rhodiolae*, *Rhodiola crenulata*, *Herba Rhodiolae* and *Rhodiola kirilowii*. The CAT is an important protective enzyme in the plant tissue and its activity is significantly associated with the anti-reversion force in plants. Research on the change of CAT activity of four *Herba Rhodiolae* leaves indicates that the CAT activity of *Herba Rhodiolae* and *Rhodiola crenulata* increases firstly and then decreases with the further decrease of temperature (Ashraf et al., 2012). The CAT activity of four *Herba Rhodiolaes* reduces to the minimum when the temperature decreases to −30 °C. At this time, the low temperature leads to non-recoverable damage to all four *Herba Rhodiolaes*. It also indicates that the cold resistance of the *Herba Rhodiolae* is maximum while the cold resistance of the *Rhodiola kirilowii* is minimal. MDA is the membranous peroxidation product of the organ when the plant organs are undergoing adverse conditions or aging conditions, which indicates the degree of membranous peroxidation and the response strength of the plants to adverse conditions (Tabassum et al., 2014). If MDA increases, it means the membranous peroxidation action is introduced and anti-reversion force will be strengthened.

5. Conclusions

Based on the above analysis, six physiological and biochemical indexes that affect the cold resistance of four *Herba Rhodiolaes* are comprehensively evaluated. It is concluded that higher elevation of growth environment for all four *Herba Rhodiolaes* will lead to stronger cold resistance and cold withstanding ability. The strength ranked from highest to lowest for *Herba Rhodiolae*, *Rhodiola crenulata*, *Rhodiola algida* and *Rhodiola kirilowii*. 6 physiological and biochemical indexes that affect cold resistance of the *Herba Rhodiolae* ranked from strongest to weakest are SOD activity, POD activity, MDA content, CAT activity, proline content and electric conductivity. Based on the PCA analysis, POD, SOD and MDA contents are the main physiological and biochemical indexes reflecting the cold resistance difference of the *Herba Rhodiolae* seedling, which can be identified as the preliminary indexes to assess cold resistance of *Herba Rhodiolae* type. This research results will provide the theoretical basis and technical guidelines for transplanting, domestication and standardized cultivation of *Herba Rhodiolae* in future.

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