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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