Physiological Response of *Vitis Amurensis* Rupr. Seedlings to Drought Stress and Re-watering

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Abstract. In order to study drought resistance in *Vitis amurensis* Rupr. seedling and to provide some theoretical bases for water management in *Vitis amurensis* Rupr. production, using potted ‘Beta’ tissue culture seedlings as materials, effects of natural drought stress for different durations and re-watering treatment on physiological indexes of leaves. The results showed that drought stress can cause cells dehydration of *Vitis amurensis* Rupr., and this reaction intensifies with time. Especially after 20 d drought stress re-watering, the relative water content of leaves has no obvious change compared with drought treatment, that is, it has caused loss to cell membrane, while the effect of short- and medium-term drought (5 d, 10 d) is relatively small. The content of proline and soluble sugar increased first and then decreased with the extension of drought stress time, and the osmotic regulator increased relatively steadily in the short time (5 d, 10 d), which indicated that it could maintain metabolic balance through its own osmotic regulation. The accumulation of proline and soluble sugar decreased under prolonged stress (20 d), the accumulation of proline and soluble sugar was significantly higher than the control, which still had some protective effect on the cells. Under short drought stress, the activity SOD, POD, CAT grape increased steadily, but with the prolongation of stress time, the activity of the three protective enzymes varied greatly. The SOD activity reached a peak d stress 15, and the POD, CAT activity reached a peak in 20. After re-watering, the three protective enzyme activities of *Vitis amurensis* Rupr. decreased, and after 10 d of stress, the protective enzyme activity of re-watering could be restored to the control level. It shows that the grape has a certain ability to recover after drought. To sum up, this study suggests that *Vitis amurensis* Rupr. seedlings can withstand 10 d of drought stress because of their high osmotic regulation and antioxidant capacity to maintain normal physiological metabolism.

1. Introduction
Water is an important environmental factor that determines the structure and function of arid and semi-arid ecosystems [1]. China is one of the driest countries in the world, and the arid and semi-arid area accounts for 52.5% of the land area [2]. Water deficit is one of the most important factors limiting crop growth, which will not only cause leaf wilting, but also change the permeability of plant cell membrane, affect the normal life activities of plants, and even cause plant death [3-4]. The drought resistance of plants is not only manifested in growth, reproduction or viability, but also in the ability to recover quickly after drought relief [5]. Chen et al. [6] proposed the concept of drought adaptability,
that is, the overall performance of crops in drought and rehydration process becomes drought adaptability, which includes both drought resistance and recovery ability after rehydration [7].

Most of the high-quality grapes producing areas are located in arid and semi-arid areas in China. The lack of water not only limits the growth and development of grapes, but also affects the quality and yield of grapes, especially the quality of wine. *Vitis amurensis* Rupr. also known as northeast *Vitis amurensis* Rupr., which has been used as a raw material for wine making for more than 70 years, is the main raw material for wine brewing in Northeast China [8]. In recent years, with the continuous expansion of cultivation area, the planting trend of *Vitis amurensis* Rupr. in arid and semi-arid areas has been increasing [9-10]. In this study, through simulating natural drought stress and rehydration, we studied the physiological response mechanism of *Vitis amurensis* Rupr. variety 'Beta', in order to provide theoretical basis and technical support for *Vitis amurensis* Rupr. cultivation.

2. Materials and methods

2.1. Test materials
Selection of *Vitis amurensis* Rupr. variety 'Beta' as test material. Cuttaged breeding in spring 2019, when the seedlings grow to 7~8 pieces, the selection of growth is basically the same, no pests and diseases plant transplanting diameter 15 cm, bottom diameter 7 cm, high 8 cm bottom breathable flowerpot. The flowerpot is covered with soil-separated sponges, filled with dry soil and vermiculite, with a ratio of 3:1.

2.2. Test methods
The material was cultured in the solar greenhouse of the demonstration park of modern agricultural management of the Heilongjiang Academy of Agricultural Sciences. The soil moisture content reached saturation after the experimental materials were fully watered, then natural drought was carried out. The functional leaves with growth consistent were selected at 5 d, 10 d, 15 d and 20 d for each physiological index determination. Each determination was repeated three times, and the control was observed to replenish water at 9 am every day. To avoid interference from other conditions, the test materials were put in artificial climate boxes with temperature 25±2 ℃ and light intensity 0~70 µmol•m−2•s−1. After drought stress tests were taken, the re-watering test was carried out immediately (according to the water outflow at the bottom of the flowerpot). The re-watering 5 d took the leaves that recovered the growth, and each physiological index was measured separately.

The relative water content of leaves was measured by dry weighing method [11], chlorophyll content was determined by 95% ethanol extraction method [12], proline content was determined by acid ninhydrin method [13], soluble sugar content was determined by thiobarbitt method [13], SOD activity was determined by nitrogen blue tetrazolium (NBT) photoreduction method [13], POD activity was determined by guasol method [13], CAT activity was determined by ultraviolet spectrophotometer [13].

3. Statistical analysis
Excel 2003 was used to sort out and graph all the data. SPSS 20.0 was used for ANOVA.

4. Results

4.1. Effects of drought stress on the leaves relative water content of *Vitis amurensis* Rupr.

The result showed that the relative water content of *Vitis amurensis* Rupr. leaves decreased gradually with the prolongation of drought stress time (figure 1). The relative water content of *Vitis amurensis* Rupr. was 85.51%, 75.41%, 65.52% and 56.38% at 5 d, 10 d, 15 d and 20 d, which compared with the control decreased by 4.32%, 15.71%, 27.01% and 37.13% (P<0.05), respectively. The relative water
content of drought stress 5 d quickly recovered to the control level after re-watering 5 d (P>0.05). The relative water content of drought stress 10 d, 15 d recovered to 94.17% and 79.82% of the control level, respectively. But the relative water content of drought stress 20 d did not change significantly with drought treatment, indicating that long-term drought stress, *Vitis amurensis* Rupr. plants were seriously damaged and could not recover in the short term.

4.2. Effects of drought stress on the chlorophyll content of *Vitis amurensis* Rupr.
With the extension of drought stress time, the content of chlorophyll increased significantly (P<0.05) (figure 2). The chlorophyll content of *Vitis amurensis* Rupr. was 0.34 mg·g·1·FW, 0.38 mg·g·1·FW, 0.42 mg·g·1·FW, 0.57 mg·g·1·FW at 5 d, 10 d, 15 d and 20 d, which compared with the control increased by 13.33%, 31.03%, 40.00% and 90.00%, respectively. It is may be caused by the relative increase of chlorophyll content due to the decrease of leaf water content [14]. The chlorophyll content of drought stress 5 d and 10 d were decreased after re-watering 5 d, which no significant difference compared with the control (P>0.05). The chlorophyll content of drought stress 15 d, 20 d also recovered slightly, but they were still significantly higher than that of control (P<0.05).

4.3. Effects of drought stress on the proline content of *Vitis amurensis* Rupr.
With the prolongation of drought stress time, the proline content of *Vitis amurensis* Rupr. showed the trend of first rising and then decreasing (figure 3). The proline content in drought stress 5 d was not
significantly different from control (P>0.05), and the proline content in other time points were significantly higher than that in control (P<0.05). At 10 d, 15 d and 20 d, the proline content of Vitis amurensis Rupr. under drought stress was 26.83 μg·g⁻¹, 36.14 μg·g⁻¹, 33.47 μg·g⁻¹, which compared with the control increased by 7.92%, 43.07 and 31.93%, respectively. The proline content of stress 15 d, 20 d decreased significantly after re-watering 5 d (P<0.05), and the proline content of stress 5 d, 10 d did not change significantly (P>0.05).

![Figure 3. Effects of drought stress on the proline content of Vitis amurensis Rupr.](image)

4.4. Effects of drought stress on the soluble sugar content of Vitis amurensis Rupr.
With the extension of drought stress time, the soluble sugar content of Vitis amurensis Rupr. at first and then decreased (figure 4). There was no significant difference in soluble sugar content at 5 d under drought stress (P>0.05), and the soluble sugar content of other time points were significantly higher than that of control (P<0.05). At 15 d, the soluble sugar content of Vitis amurensis Rupr. was reached a peak of 35.48%, which compared with the control increased 65.33%. The soluble sugar content of drought stress 10 d, 15 d, 20 d were decreased significantly after re-watering 5 d (P<0.05), and the soluble sugar content of drought stress 5 d did not change significantly (P>0.05).

![Figure 4. Effects of drought stress on the soluble sugar content of Vitis amurensis Rupr.](image)
4.5. Effects of drought stress on the protection enzyme system of *Vitis amurensis* Rupr.

With the extension of drought stress time, SOD activity increased at first and then decreased, while POD and CAT activity increased gradually (figure 5-7). The SOD, POD, CAT activities were increased significantly at 10 d (P<0.05), the SOD activity reached a peak at 15 d, and then began to decrease; however, the POD, CAT activities continued to increase and reached a peak at 20 d. It shows that the response of the protective enzyme system to drought stress is different, and the three play a synergistic role. After re-watering 5 d, the SOD, POD, CAT activities were decreased, and the SOD, POD, CAT activities of 10 d was not significant compared with the control.

![Figure 5](image1.png)

**Figure 5.** Effects of drought stress on the SOD activity of *Vitis amurensis* Rupr.

![Figure 6](image2.png)

**Figure 6.** Effects of drought stress on the POD activity of *Vitis amurensis* Rupr.
Figure 7. Effects of drought stress on the CAT activity of *Vitis amurensis* Rupr.

5. Discussion
Water is the basis for maintaining the normal life activities of plants. The relative water content of leaves directly reflects the water deficit in plants, and the relative water content of leaves under drought stress directly reflects the water retention ability of plants [15-17]. The relative water content of white clover [18] and raspberry [19] all decreased with drought stress time prolonged. In this study, with the extension of drought stress time, the relative water content of *Vitis amurensis* Rupr. leaves decreased gradually. Indicating that drought stress can cause cells dehydration of *Vitis amurensis* Rupr., and this reaction intensifies with time. Especially after 20 d drought stress re-watering, the relative water content of leaves has no obvious change compared with drought treatment, that is, it has caused loss to cell membrane, while the effect of short- and medium-term drought (5 d, 10 d) is relatively small.

Plants adapt to drought stress through regulation of photosynthetic rate, while chlorophyll content is closely related to photosynthetic rate [20]. Some studies have shown that chlorophyll content in plants is positively correlated with drought stress time [21-22]. In this study, the chlorophyll content of *Vitis amurensis* Rupr. increased with the prolongation of stress time. However, some studies have shown that with the prolongation of drought time, the chlorophyll content of hosta [23] showed a decreasing trend, and the chlorophyll content of *amorpha fruticosa* [24] showed a trend of first increasing and then decreasing. This difference may be related to stress time, intensity and the biological characteristics of the plant itself; however, chlorophyll content increased may be caused by the decrease in relative water content of leaves, the increase in dry weight per gram of leaves and the number of cells, and the concentration of chlorophyll [25].

Osmotic regulation refers to the role of cells in regulating osmotic potential by increasing or decreasing solute [26]. Osmotic regulatory substances can reduce water potential of cells and are important responses for plants to adapt to long-term drought [27]. Studies have shown that the large accumulation of proline and soluble sugar in the lower reaches of drought stress can reduce the intracellular osmotic potential and water potential, which is beneficial to absorb water under drought stress [28-29]. Drought stress can cause proline accumulation in plants such as *Phyllostachys edulis* [26] and *Medicago ruthenica* [30]. The results showed that the content of proline and soluble sugar increased first and then decreased with the increase of drought stress time, and the osmotic regulator increased relatively steadily in the short time (5 d, 10 d), which indicated that it could maintain metabolic balance through its own osmotic regulation. Although the accumulation of proline and soluble sugar decreased in the long-time stress (20 d), the accumulation of proline and soluble sugar was significantly higher than control, which still had some protective effect on the cells. After re-watering 5 d, the accumulation of osmotic regulatory substances also decreased, similar results were obtained with Cai *et al* [30].
Drought stress can cause the accumulation of harmful substances such as reactive oxygen species in plants, which can cause cell membrane damage, and the metabolism of reactive oxygen species is out of tune [31]. The accumulation of harmful substances such as reactive oxygen species can make the corresponding protective enzyme system in plants produce response defense. The protective enzyme systems of different plants respond differently to drought responses. As the drought stress time was prolonged, the SOD, POD, CAT activities of camphora leaves [22] all showed a tendency to increase first and then decrease. In this study, with the extension of drought stress time, the SOD activity showed a trend of increasing first and then decreasing, and the POD, CAT activities showed a trend of increasing gradually. Short time drought stress, the SOD, POD, CAT activities of *Vitis amurensis* Rupr. increased more smoothly, but with the prolongation of stress time, the activities of three protective enzymes changed significantly, the SOD activity reached a peak at 15 d, the POD, CAT activities reached a peak at the 20 d. After re-watering 5 d, the three protective enzyme activities were decreased, the protective enzyme activity of 10 d drought stress could be restored to control level, which indicating that the *Vitis amurensis* Rupr. have a certain ability to recover after drought.

To sum up, this study suggests that *Vitis amurensis* Rupr. seedlings can withstand 10 d of drought stress because of their high osmotic regulation and antioxidant capacity to maintain normal physiological metabolism.

**Acknowledgements**

This work was supported by the Ministry of Science and Technology's special project of Basic Investigation - Investigation of wild Economic plant Resources in the Forbidden Forest of Northeast China (nos. 2019FY100503-5) and Heilongjiang Academy of Agricultural Sciences' special Project of Agricultural Science and Technology Innovation - Research on excellent germplasm Resources and Key Cultivation Techniques of Fruit trees (nos. HNK2019CX11).

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