

Mechanism of ARF5 Gene Promoting Seed Germination of *Magnolia Sibiricum*

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Abstract

Magnolia is one of the rare and precious ornamental plants in our country. It has important ecological, economic and social benefits. However, the germplasm resources are already endangered, and the long dormant period of its seeds is an important obstacle to afforestation, introduction and breeding. Therefore, it is of great significance to explore the mechanism that promotes the germination of *Magnolia chinensis* seeds. In this experiment, the natural stratification treatment of *Magnolia sylvestris* seeds was used as the research object to study the relationship between plant endogenous auxin and auxin response factor ARF5 gene and seed germination during the seed germination of *Magnolia serrata*. Through the determination of the relative water content, endogenous auxin content and other physiological indicators of the seeds at different stratification times, the extraction of RNA, and the quantitative expression of the ARF5 gene were studied.

Keywords

Magnolia; Relative water content; Endangered; RNA.

1. Introduction

Magnolia sylvestris, also known as *Magnolia vulgaris*, *Magnolia sylvestris*, and Peony, is a small deciduous tree belonging to the genus *Magnolia* of the Magnoliaceae (Magnoliaceae). It is listed as a rare and endangered plant under national key protection in the "Red Book of Chinese Plants" [1]. The geographical distribution is discontinuous. It is mainly distributed in Ji'an and Tonghua in Jilin Province. It is the northernmost species of Magnoliaceae in China, and its taxonomic position is extremely important. The flower is named after the white petals, purple stamens, charming fragrance, and the shape of a celestial lady. *Magnolia* is a rare flower and tree, a precious tree species that survived the ice age, and a world-famous plant under my country's key protection. The leaves are like green carvings and the flowers are like jade carvings. The fertile soil under the shade forest is a broad-leaved subarbor with a height of up to ten meters. Its flowers and leaves are beautiful and rich in fragrance. It is an important landscaping tree species and light industrial raw material for extracting spices.

Wood can be used to make agricultural tools, and flowers can be extracted from aromatic oils. The flowers are beautiful, with long pedicels, and they show up in the wind. They are well-known garden ornamental trees at home and abroad. The flowers are used as medicine and can

be used to make extracts. At present, some scholars have conducted research on the form of *Magnolia sylvestris* [2], resource status [3-4], cultivation techniques [5-6], the chemical composition of essential oils [7], and the idea of establishing protected areas. Ornamental, aromatic and medicinal value, it is a kind of rare wild woody plant resource with great development prospects. Judging from the current distribution of *Magnolia sylvestris* in my country, the germplasm resources are already in an endangered state. The main reason is its ecological and biological characteristics. Closely related, the long dormancy period of seeds is an important obstacle to afforestation, introduction and breeding. Seed dormancy is an aspect of growth suspension. It is an important stage in the process of plant growth and development. It is beneficial to the reproduction of populations and has important ecological significance, but it gives us seeds and seedlings breeding, cultivation, and research work. It brings inconvenience, so it is of great significance to deeply understand and explore the mechanism that promotes the germination of *Magnolia sylvestris* seeds.

2. Materials and Methods

2.1. Test Materials and Preliminary Treatment

Disinfect the naturally dried magnolia seeds with 0.2% KMnO₄ solution for 30min. After washing with clean water, mix the seeds with wet sand (60% water content) at a ratio of 1:3 for alternating temperature stratification to accelerate germination: low temperature stratification (0 -10°C, 30d), diurnal stratification (8-10°C in the day, -5-0°C in the night, 15d), high temperature accelerating germination (constant temperature 15°C) until germination, check regularly to maintain the humidity of the seed sand. During the stratification process, samples were taken every 15 days until germination. We regard the radicle to break through the seed coat and exceed 1/2 of the seed length as germination. The relative water content of the above-mentioned samples is measured, and part of the liquid nitrogen is quick-frozen and stored in a refrigerator at -80°C for endogenous auxin content determination and nucleic acid extraction.

2.2. Determination of Endogenous Growth Hormone Content

The determination of endogenous auxin content uses the method of enzyme-linked immunoassay kit (Hailian Biological Company). The method is as follows: Take a sample of the treated seed and grind it into a certain amount of phosphate buffered saline solution, pH 7.4. Quickly freeze and store with liquid nitrogen for later use. After the sample is thawed, the homogenate is centrifuged and the supernatant is collected. Add sample and add the sample and enzyme label reagent to the bottom of the plate well. Incubate at 37°C for 60 min. Remove the sealing film, discard the liquid, and spin dry. Fill each well with washing liquid, let it stand for 30 seconds and discard it, repeat 5 times, and pat dry. Add 50μL of developer A to each well, and then add 50μL of developer B, shake gently to mix, and develop color at 37°C in the dark for 15 minutes. Add 50μL of stop solution to each well for the stop reaction (the blue turns to yellow at this time). Within 15 minutes of adding the stop solution, zero the blank hole, and measure the absorbance (OD value) of each hole in sequence with a wavelength of 450 nm. According to the obtained results, the endogenous auxin content of *Magnolia sibiricum* seeds at different stages was calculated by standard curve.

3. Results and Analysis

3.1. Changes in Relative Water Content During Seed Germination

The relative moisture content of seeds refers to the percentage of the moisture content in the seeds to the seed mass. As one of the quality indicators of seeds, seed water content can be used to detect the germination process of seeds. Samples of *Magnolia sylvestris* seeds were sampled

regularly to observe the germination of the seeds. The results showed that the seeds of *Magnolia sylvestris* became white when stratified for 75 days and germinated on 90 days. Record the dry weight and fresh weight of the seed samples in different periods, and calculate the relative water content changes during different stratification times during the germination process of *Magnolia chinensis* seeds, as shown in Figure 1.

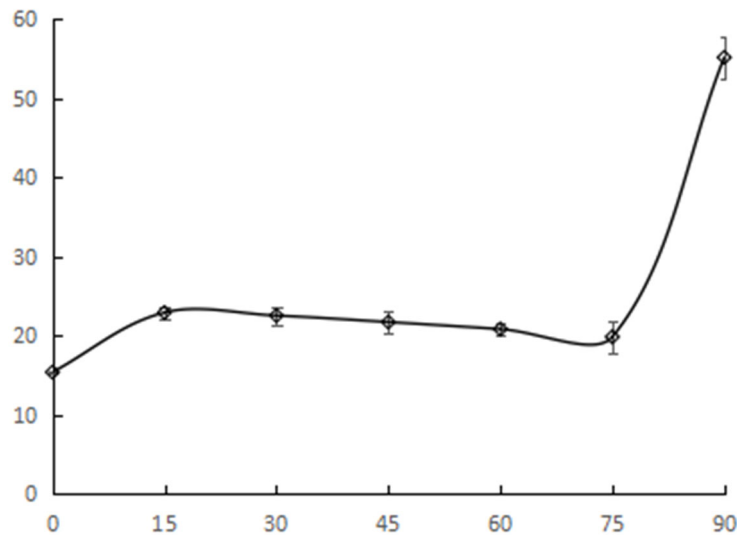


Figure 1. Changes in relative water content during seed germination

We generally believe that the germination of a seed starts from the swelling of the seed. The swelling of the seed is regarded as the seed germination. When the seed germinates, the first is to absorb water. Therefore, the relative water content is low before the seed germination, especially the lowest in the state of dry seeds. As the number of stratification days increases, the relative water content of the seeds fluctuates in a small range. During the seed germination process, its dry weight, fresh weight and relative water content are all dynamically changing, which is an important parameter to detect the seed germination process. Generally speaking, in the process of germination, metabolism continues to increase, free water increases, and the ratio of free water to bound water increases. Fresh weight increases. Due to the strong respiration during the seed germination process, the dry weight will be reduced by consuming organic matter such as glucose. The relative water content will also increase.

From this we can verify that the seed water absorption process is divided into three stages, the first stage 0-15d is the seed swelling stage, which mainly involves the seed absorbing water expansion and the reactivation of the internal physiological mechanism of the seed; the second stage 15-75d is the seed water content stability. In this stage, the main physiological and biochemical activities such as the release of seed dormancy are carried out, and the water content changes little; the third stage is the rapid water absorption stage from 75d to the seed germination, and the seeds enter the rapid growth stage and quickly absorb water to ensure seed germination.

3.2. The Content and Change of Endogenous Auxin During Germination

As shown in Figure 2, *Magnolia grandiflora* contains a high concentration of auxin in the state of dry seeds, with a content of $8.664\mu\text{mol/L}$. During the seed germination process, as the number of stratification days increases, the endogenous auxin content begins to drop sharply. From $8.664\mu\text{mol/L}$ to the lowest $1.106\mu\text{mol/L}$, with the extension of the stratification time to 30d, the endogenous auxin content in the seeds began to rise again to $3.835\mu\text{mol/L}$. At 45d, 60d and 75d, the stratification remained relatively stable at $1.849\mu\text{mol/L}$, $1.963\mu\text{mol/L}$, and

1.844 μ mol/L, respectively. The stratification started to rise sharply to 7.678 μ mol/L when the seeds germinated at 90d.

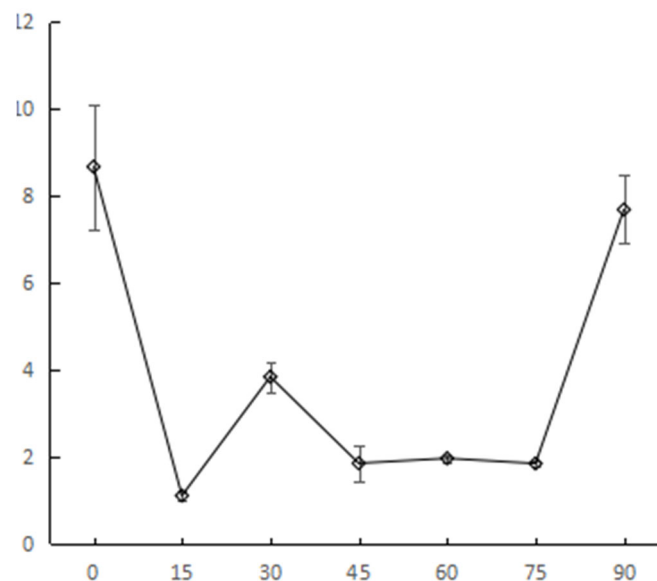


Figure 2. Measured and predicted principal component model comparison

According to the results, the high content of endogenous auxin in dry seeds of *Magnolia sylvestris* may be the cause of the dormancy of the seeds. Excessive concentration of endogenous auxin inhibited the germination of seeds. The subsequent decrease is because the seeds germinate through the process of swelling and absorb a large amount of water, resulting in a sharp decrease in the content of endogenous auxin. Throughout the stratification stage, the physiological activities inside the seeds lead to fluctuating changes in the content of endogenous auxin. From 75 days to 90 days of stratification, the embryos grow rapidly and the endogenous auxin content increases again. From this, we infer that the mechanism of endogenous auxin regulating the seed germination of *Magnolia sieboldii* is that high concentration inhibits its germination, and low concentration promotes its germination. In the stage of seed germination, endogenous auxin performs different functions. Inside the seeds of *Magnolia sylvestris*, 15-45d auxin participates in the release of seed dormancy and promotes seed germination. When the seeds enter the germination stage at 75-90d, auxin improves the elasticity of the cell wall, promotes cell water absorption and cell enlargement, thereby promoting the rapid growth of seed embryos. This result is consistent with the result of seed moisture content.

4. Conclusion

During the germination process, the relative water content of the seeds of *Magnolia sylvestris* increased first and then steadily, and then increased rapidly. The seeds absorb water from 0-15d, the water content of the seeds is relatively stable at 15-75d, and the seeds quickly absorb water at 75-90d to ensure seed germination. At this stage, the endogenous auxin content is the highest when it is not stratified, which inhibits the germination of seeds. Due to the transient increase in embryo growth at 15-45 days, the embryos enter the post-ripening stage at 45-75 days, and the auxin content weakens within 75-90 days. The sharp increase in source auxin content promotes the germination of seeds. The auxin response factor ARF5 gene was up-regulated in the three samples at 0, 45 and 90 days, which was consistent with the changes in endogenous auxin content.

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