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Study on the Seeds of Magnolia sibiricum Treated by Natural Thermostratification

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Abstract: In this paper, 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 chinensis seeds, ARF5, breeding.

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1. INTRODUCTION

Seed dormancy and germination are important events in the individual development of higher plants, which are related to the survival and development of populations[1]. At the same time, it is also a biological characteristic formed by plants in order to adapt to various factors such as climate change and temperature difference to maintain their own reproduction and development, which has important ecological significance. Seed dormancy refers to an internal state in which seed germination is inhibited under sufficient moisture, temperature and oxygen conditions[2]. This means that as long as the inhibition is removed, the seeds can germinate under a wide range of environmental conditions. Germination is defined as the beginning of seed swelling, to the elongation of the hypocotyl and the penetration of the tissue around the embryo by the radicle. Seed dormancy and germination have always been key research issues[3]. The dormancy and germination phenomenon of plant seeds is a feature of plant seeds to maintain their survival and adapt to changes in the external environment. It is subject to the regulation of many genes and the influence of ecological environmental factors. Using quantitative genetics methods and mutants and other methods, in-depth research on the dormancy and germination characteristics of plant seeds has begun to attract people's attention, but so far, there is no particularly detailed mechanism that can fully reveal the dormancy and germination of plant seeds[4-5]. In recent years, with the rapid development of molecular biology technology, the research on plant seed dormancy and germination has also reached the molecular level, and great progress has been made. The application of molecular biology technology, especially the application of gene expression and genome sequencing and proteomics analysis, has become more and more extensive. With the completion of the sequence analysis of certain plant genomes, the focus of research has begun to shift from revealing all the genetic information of life. To the function of plant genes at the molecular level. These advances at the molecular level provide new tools and new directions for the study of plant seed dormancy and germination[6].

Phytohormones are a class of organic substances produced by the plant's own metabolism, and are also called plant natural hormones or plant endogenous hormones. Although its content is very low, it can regulate the entire process of plant life activities, such as the expression of plant genes, the basic laws of growth and development, and the regulation of metabolic processes. At present, there are six categories of recognized plant hormones, namely: auxin (IAA), gibberellin (GA), cytokinin (CTK), abscisic acid (ABA) and ethylene (ETH), and brassinosteroid (BR) . The study of endogenous hormones in plants is one of the important contents in the field of plant physiology. The distribution position of phytohormones in plants and their content are of special significance for research in the fields of plant life activities and crop genetic breeding and cultivation. At the same time, because plant hormones are also the focus of regulating plant seed dormancy and germination, the study of the relationship between plant endogenous hormones and seed dormancy and germination has always been a hot spot in the study of seed physiology and biochemistry.

2. MATERIAL AND METHODS

Using Omniplant RNA Kit (DNase I) all-round plant RNA extraction kit (Kangwei Century Company) method. The specific method is as follows: take seed samples of various periods, remove the black seed coats, freeze with liquid nitrogen, grind them into powder in a sterilized and frozen mortar for

homogenization. Transfer the supernatant to the filter column of the collection tube by centrifugation, and then centrifuge in the centrifuge tube, add 0.5 times the volume of the supernatant absolute ethanol, transfer the resulting solution and precipitate to the adsorption column, add 350μL to the adsorption column Buffer RW1, discard the waste solution. Add 80μL of DNasel mixture directly to the adsorption column and incubate at 20-30℃ for 15min. Add 500μL of Buffer RW2 to the adsorption column RM and centrifuge to load the adsorption column RM into new RNase-Free Centrifuge Tubes (1.5mL), add 30-50μL RNase-Free Water to the middle part of the adsorption membrane and leave it at room temperature for 2 minutes After centrifugation for 1 min, RNA solution was obtained. The RNA quality was measured by 1% agarose gel electrophoresis, and the RNA concentration was measured by the NanoDrop 2000 (Thermo Fisher Technology Co., Ltd.) spectrophotometer. The RNA solution is quick-frozen in liquid nitrogen and stored in an ultra-low temperature refrigerator at -70℃ to prevent degradation.

The first-strand cDNA synthesis by reverse transcription adopts the method of TaKaRa (Dalian Bao Biotechnology Co., Ltd.) kit. Using the total RNA of Magnolia sylvestris as a template, using TransScript RT/RI Enzyme Mix and 2×TS Reaction Mix to efficiently synthesize the first-strand cDNA, first add the following reagents: 50μg total RNA, 1μL Anchored Oligo(dT)18(0.5μg/μL), 10μL 2×TS Reaction Mix, 1μL TransScript RT/RI Enzyme Mix, and then add RNase-free Water until the total volume is 20μL. After gently mixing, heat at 85℃ for 5s to inactivate TransScript RT/RI Enzyme Mix to obtain cDNA. Store the reverse transcription product in a refrigerator at -20 ℃.

SYBR Green I chimeric fluorescence method was used for fluorescence quantification. 2×SuperReal PrelMix Plus, 50×ROX Reference Dye, and the above primers without primer dimer and cDNA template were selected to form a reaction system. The reaction system included 0.4μL Forward Primer (10 μM), 0.4μL Reverse Primer (10 μM), 10μL 2×TransStart Top/Tip Green qPCR SuperMix, 0.4μL Passive Reference Dye (50x), and then add cDNA template and ddH2O to the entire reaction system to 20μL. The reaction system was placed in the ABI StepOne Plus real-time fluorescent quantitative PCR instrument, and the Magnolia magnolia actin gene was used as the internal reference gene. The qPCR reaction was carried out using a three-step method.

Fig. 1 Phase analysis of arrangement

3. RESULTS

3.1 Extraction and quality inspection

The quality of RNA is usually represented by two indicators: one is agarose gel electrophoresis detection, which can reflect the degree of RNA degradation, whether there is protein contamination, genetic contamination, etc. The second is RNA concentration and OD value. The measured RNA

concentration is convenient for later reverse transcription, and the detection of OD value can identify whether it meets the fluorescence quantitative requirements.

The RNA of Magnolia sylvestris seed layered at 0, 15d, 30d, 45d, 60d, 75d, 90d was extracted and tested by agarose gel electrophoresis. The results are shown in Figure 1. Among them, the RNA of the seeds of Magnolia sylvestris at 0 and 75d The bands are clearest and have the highest brightness.

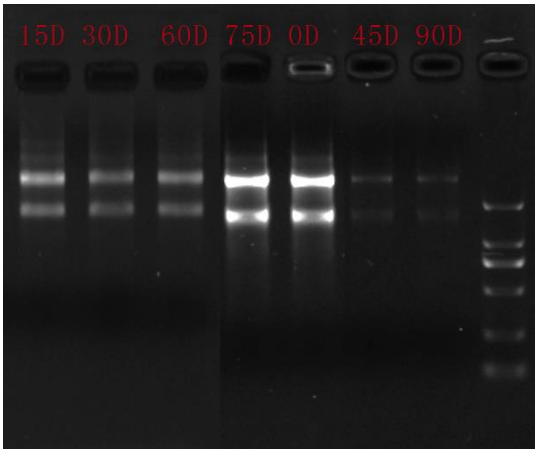

Fig.1 RNA gel electrophoresis of Magnolia sibiricum seeds in different periods

The extracted RNA was tested, and the RNA content of Magnolia sibiricum seeds in different periods was shown in Table 2. OD 260/OD280 is an indicator of nucleic acid purity. The OD 260/OD280 value with better purity should be between 2.0 and 2.2. If it is too low or too high, it means that there is the influence of protein and phenolic substances. The RNA test results show that although the concentration of Magnolia sibiricum seeds RNA varies in different periods, its OD 260/OD 280 values meet the fluorescence quantitative requirements, so the extracted RNA at all stages can be used.

3.2 Primer test results

The synthesized primers are tested, and the size of the target band is required to be consistent, without primer dimer, that is, a single band has no miscellaneous bands. It was detected by agarose gel electrophoresis, and the results are shown in Figure 2. The results showed that the first three primers ARF5q, ARF5M, and 49380-1 had no bands, indicating that they were not suitable for fluorescence quantification. The fourth primer 49380 2 has an obvious single band without other miscellaneous bands, and according to the fluorescence quantification requirements, the size of the target fragment is the same as predicted. It is concluded that primer 49380-2 is applicable. The figure shows that the primer 49380-2 is obvious, but the brightness is not high. It may be because the marker is too much, which makes it too bright. The comparison shows that the primer band is darker. It is also possible that the primer is a mixed DNA, which works when the expression level is low.

The results show that the conserved domains of the fourth primer are relatively scattered, and alternative splicing sites may exist. Therefore, there may not be many genes in the fluorescent quantitative primers, and the gene structure is more complicated.

The melting curves are all single peaks, indicating that the amplification products have good specificity, no primer dimers and non-specific amplification. And the annealing temperature at the peak position is consistent with the setting. Therefore, the amplified fluorescent fragments are the products we need, and the experimental results are valid.

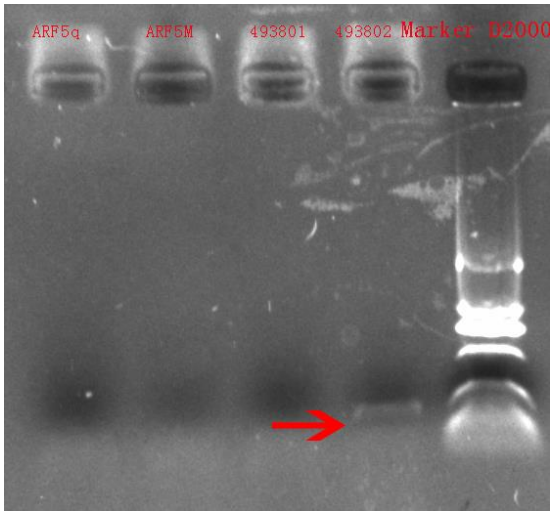

Fig.2 Primer agarose gel electrophoresis detection chart

4. CONCLUSION

Fluorescence quantitative analysis and endogenous auxin content determination of ARF5 gene in Magnolia sibiricum seeds at different stages of the germination process showed that the relative expression of ARF5 gene and the change trend of endogenous auxin content were roughly the same under dry seed state and At 90 days of stratification, the relative expression of ARF5 gene and the content of endogenous auxin were higher than other stages, but at 30 days of stratification, the content of endogenous auxin increased slightly, but the auxin response factor ARF5 gene did not change significantly. The relative expression of ARF5 gene increased to some extent until 45 days of stratification. We infer that this phenomenon may be caused by the delay in the auxin response. In the remaining stages of the germination process, the endogenous auxin content showed a fluctuating change, which was about the same trend as the relative expression of the ARF5 gene. From this we inferred that ARF5 gene can respond to changes in endogenous auxin to promote the germination of Magnolia sieboldii seeds.

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