Identifying *Pulsatilla Chinesis (Bunge) Regel*, and *Potentilla Discolor Bunge* from Each Other using Restrictive Enzyme Dde I

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Abstract: For identifying *Pulsatilla chinesis (Bunge) Regel*, and *Potentilla discolor Bunge* from each other, a new method was established. We amplified the ITS regions of them, and sequenced the purified PCR products directly. We edited and compared the obtained sequences by Genetyx and BioEdit. The possible sites of restriction endonucleases were searched using PREMIER 5.0. It was found that Dde I can be used for their identification. In this case, we concluded that Dde I can be used effectively in identification of these plants.

1 INTRODUCTION

Because of its reliable therapeutic effects, *Pulsatilla chinesis (Bunge) Regel*, was included in *Pharmacopoeia of The People's Republic of China*, almost every edition (Chinese Pharmacopoeia Commisson, 2015). *Potentilla discolor Bunge*, has also the similar effects, but it was not included in *Pharmacopoeia of The People's Republic of China* since before. Currently, it was found to hold the function of curing diabetes, so it was started to be included in *Pharmacopoeia of The People's Republic of China* (Chinese Pharmacopoeia Commisson, 2015). With the lack of knowledge and morphological similarity, some regions in China still use them as each other (New Medical College of Jiangsu, 1985), affecting the collecting and even the correct usage, and more importantly, the therapeutic effects of the two materia medica. So how to identify the two materia medica becomes very important. Although researchers had created and even improved some methods such as those based on appearance, differences in structure under microscopy and differences in chemical components (Zhang et al., 2000), a thin-layer chromatography (Liu and Lei, 2005), but due to the similarities, it is hard to identify them from each other precisely with these methods. With the advance in molecular biology, authentication and identification using molecular biology techniques (Tang and Fu, 2000) becomes more and more popular in decades (Wang, 2001). And above all, they are very reliable. So, in this study, based on the fundamental techniques of molecular biology, we established a new method to identify *Pulsatilla chinesis* and *Potentilla discolour*.

2 MATERIALS AND METHODS

2.1 Plants

We collected *Pulsatilla chinesis* nearby Changchun, China. *Potentilla discolor* was purchased from YAODUBAICAOYANGSHENGTANG. *Pulsatilla chinesis* was authenticated by Professor Minglu Deng of Changchun University of Traditional Chinese Medicine and *Potentilla discolor,* Wenchang Guo of Jilin University (Table 1). We dried the plants with silica gel and preserved them in our laboratory. The leaves were used for the experiments.

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Table 1: Geological, purchasing information and the dates of sample collection.

Plants	Geological or purchasing information	Dates
Pulsatilla chinesis (Bunge) Regel	Collected at No.027 Country Road (4KM far from Tuding Town, Shuangyang, Changchun)	2013-6-2
Potentilla discolor Bunge	Purchased from YAODUBAICAOYANGSHENGTANG	2013-3-25

2.2 DNA Preparation

We took a small amount of leaf from every sample and cleaned them with cotton swabs to eliminate the impurities on the leaves with 70% alcohol in a culture dish. Then we dried the cleaned leaves at room temperature for a while and grinded them into powders with liquid nitrogen. We collected the powders and prepared genome DNA using Plant DNA Isolation Reagents (Takara Biotechnology) following the provider's instruction. The qualities of extracted DNA were checked in 1% agarose slab gels.

2.3 Primers

The primers of ITS reported in a former research (Takaiwa et al., 1985) were selected and synthesized by Takara Biotechnology Co., Ltd (Dalian, China).

2.4 PCR Reactions

Every PCR reaction was performed following the former research (Takaiwa et al., 1985) in a total amount of 50μL [1μL, each of the primers in 2.3, 5μL every genome DNA, 5μL Reaction Buffer, 5μLdNTPs, 1μL Taq DNA polymerase (Takara Biotechnology Co. Ltd)]. The PCR conditions are as follows: 94℃, 1 cylce,5 min, 35 cycles (denature at 94℃,1 min; annealing at 55℃ 2 min; extension at 72℃,2 min), 72℃, 1 cycle for 10 min. MiniCycler PTC-150(MJ Research Inc,) was used to perform the PCR reactions. PCR products were checked in 1% agarose slab gels.

2.5 Sequencing

2.4 PCR products were purified using PCR Filter Units (Millipore Corporation) then directly sequenced. We performed the sequencing reactions in a 10μL mixture for each sample using ABI BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) with every sequencing primer. The sequencing reactions conditions are as follows: 96℃, 1 cycle for 1 min, 25 cycles (denature at 96℃,30 sec, extension 50℃, 5 sec.), 60℃, 1 cycle, 4 min. We analysed the obtained sequences using 3130 Sequencer (Applied Biosystems.).

2.6 Comparisons and Editings

We used Genetyx-SV/RC version 11.0 and BioEidt version 7.0.9 to edit and compare the sequences.

2.7 Searching for Appropriate Restriction Endonuclease

We used Primer PREMIER (version 5.0, PREMIER Biosoft international, CA, USA) to search for a restrictive site that can be used, eventually, we found that Dde I can digest the two PCR products in different sites and can be recruited to identify them from each other.

2.8 Dde I Digestion

We used the purified the 2.4 PCR products for digestions. Dde I digestions were performed at 37℃ for 2h. We performed the reactions in MiniCycler PTC-150. Dde I was purchased from Biolabs (New England Biolabs.). We confirmed the digestions in a 2% agarose gel.

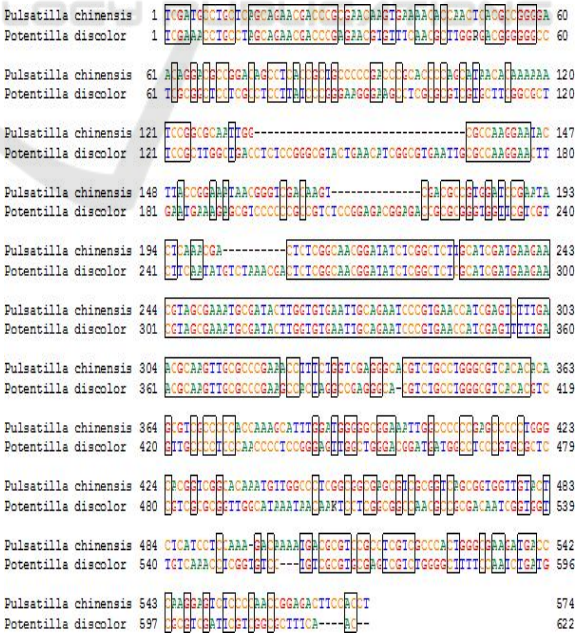
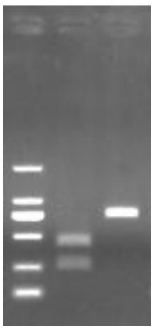


Figure 1: Differences in ITS sequences of *Pulsatilla chinesis* and *Potentilla discolor*.



Notes: Left to right, 50bp Marker; *Pulsatilla chinesis; Potentilla discolor*.

Figure 2: DdeI digestion.

3 RESULTS

Table 1 shows the geological, purchasing information and the dates. For each species, three different individual plants were put into use for sequencing.

Figure 1. shows the differences of ITS sequences in two plants.

Electrophoresis of the digests (Figure 2).

4 CONCLUSIONS AND DISCUSSION

We for the first time sequenced and reported *Pulsatilla chinesis* (Zhang et al. 2017) and *Potentilla discolor* ITS sequences (Zhang et al. 2015). Using these sequences, we established a new simple method to identify them from each other, that can ensure the correct use of those two drugs, especially in case they are used to cure different disease (for example, diabetes). As shown above, the new method we created in this study (first amplifying the ITS regions and then digesting them with Dde I) is very simple and reliable, so even a kit for identification is reasonable. It can used in the procedures such as acquisition, quality control etc. of the rude drugs. Although, further experimentation and confirmation are necessary.

Although we can use ITS sequences themselves directly to identify these two plants. But sequencing itself is a complicated technique and it need expensive equipments, like sequencer, to conduct the experiments. So, this new method should be a more practical one to be put in use.

Pulsatilla chinesis and *Potentilla discolor,* both have wide distributions in China, so differences in samples of different area can be predicted. For precise identification, enlargement of analysis in samples of different area and accumulation of knowledge are necessary.

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