

Identification of rust on Berberis and preliminary analysis of genetic structure based on ITS molecular markers

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Abstract – Berberis is the alternate host of wheat stripe rust and wheat stem rust. It plays an important role in the variation, occurrence, and prevalence of wheat stripe rust and wheat stem rust. Berberis is widely distributed in Yunnan Province. In this study, 230 samples of rust on Berberis collected from different regions of Yunnan Province in 2017 were analyzed by combining molecular markers with morphological observation. The results showed that the rust of Berberis in Yunnan Province can be divided into five groups, namely, wheat stripe rust, 97% of the rust with the similarity to the stem rust of bluegrass in a dry land, 96% to 98% of the rust with the similarity to the stem rust, 93% to 94% of the rust with the similarity to the stem rust of wheat and 93% to 94% of the rust with the similarity to the stem rust of wheat. There is genetic differentiation among rust fungi of the same group. There are great differences in the depth of rust organ invasion into the host leaf, the ratio of the depth of invasion into the host leaf to the thickness of the leaf, and the size of rust spore. According to the morphological characteristics of rust organ and rust spore, different rust fungus groups on Berberis can be distinguished.

Keywords: Berberis; rust fungus; rust organ; rust spore; identification

1. Introduction

Berberis belongs to the family Berberidaceae of Ranunculaceae, which has a wide range of species and distribution. There are about 500 species in the world, mainly distributed in Asia, North America, Europe and Africa [1]. About 250 species are found in Asia's China, mainly in the West and Southwest [2]. There is an abundance of *Berberis* in Yunnan Province, especially in the northwest of Yunnan Province, where the population density is relatively large.

A large number of studies have shown that many cereal rust fungi have complex life cycle. According to the morphological characteristics of spores, the life cycle can be divided into five stages: Summer spore, winter spore, rust spore, basidiospore and sexual spore, which brings great difficulties to the overall and comprehensive study of cereal rust [3]. In 1866, De et al. found that there was a phenomenon of transposition of stem rust on *Gramineae* plants and *Berberis*, and confirmed that *Berberis* was the transposition host of wheat stem rust [4-8]. At present, it has been confirmed that more than 90 species or varieties of *Berberis*, ten mahonia and their hybrid progeny can be infected by stem rust, but ten Mahonia plants and more than 20 kinds of *Berberis* have some resistance to stem rust [9]. In 2010, it was reported that *P. brachypodii*, *P. pygmaea*, *P. montanensis*, *P. brachypodii-phoenicoidis* formed rust organ in *Berberis*. In the same year, Jin et al. confirmed that *Berberis* was the host of wheat stripe rust [10]. In addition, a large number of studies have reported that more than 30 species of rust can infect *Berberis* for sexual reproduction [11]. In 2011, it was detected that the rust of *Berberis shensiensis* Ahrendt and *Berberis potaninii* Maxim belong to *Puccinia striiformis* by molecular means [12]. The rust spores of *Berberis shensiensis* Ahrendt are the closest to those of *P. striiformis*, with a similarity of 95%. It is 94% similar to the rust spores of *Berberis emerald carousel* and *Poa pratensis* L. striped stem rust reported by Jin et al.; the matching rate of rust spores on *Berberis oligodentata* was 97% and 93%

respectively, compared with that of winter spores of wheat stripe rust reported by Jin et al. [10,12]. In 2013, Berlin et al. analyzed the rust organ of *Berberis* in Sweden with the method of molecular biology and morphology. The results showed that the rust on *Berberis* in Sweden can be divided into three different groups: oat specific type, wheat or rye specific type and oat grass specific type [13]. In 2016, Zhao Jie et al. studied and analyzed the *Berberis* in Linzhi, Tibet. According to the different sizes of the rust organ on the *Berberis*, it showed that there were many kinds of rust bacteria in this area [14]. This studies show that the rust on *Berberis* is very complex.

In the study of pathogen morphology, the classification of rust is mostly based on the morphology of winter spores [15,16]. However, the related studies also showed that the other spore stages besides the winter spores also have morphological characteristics, which can be used for taxonomic and phylogenetic studies [17]. For example, the arrangement and shape of spores and the morphological characteristics of Chardonnay [18], Morphological characteristics of rust organ and sporophyte [16,19, 20]. In addition, different molecular markers are used to sequence the target fragments of specific molecular markers. According to the similarity of the target fragments, different pathogen species can be identified, and even lower level fungi can be identified. However, people also hope that the differences of molecular markers can be reflected in the morphology. Therefore, even in the era of rapid development of molecular markers, it is necessary to combine morphology with molecular markers to identify different rust species.

Berberis in Yunnan Province is widely distributed, with high reserves and high density. *Berberis* Resources are very rich. In most areas, the climate is mild and humid, and there are many kinds of plants, which are suitable for the occurrence, spread and prevalence of a variety of pathogens. In such a complex ecological environment, up to now, there is no report on the systematic study of rust species on *Berberis*. Therefore, this study systematically analyzed 230 samples of rust collected from *Berberis* in 2017 by using the method of combining molecular and morphological, which is of great significance to understand the types of rust on *Berberis* and the relationship between rust on *Berberis* and other plants.

2. Materials and methods

2.1 Test materials

The rusts on *Berberis* used in this study were collected from Kunming (Western hills, Wild-cat Mountain) and Qujing (Yiche and Nagu) in 2017. When collecting samples, fresh samples of rust organ should be selected for collection, and self-made sample collection bag should be used for packaging separately after collection to avoid mutual pollution between different products. The samples were taken back to the laboratory and stored in - 20 °C refrigerator.

Table 1. Rustfungus Sample Collection on *Berberis*

year	Collection location	Number of samples	Number of cloned and sequenced samples	<i>Berberis</i> species	time	Sample Number
2017	Yiche	35	25	<i>Berberispruinosa</i>	2017.6	YC-1~35
	Nagu	50	30	<i>Berberispruinosa</i>	2017.6	NG-1~50
	Western mountain	35	30	<i>Berberispruinosa</i>	2017.5-6	XS-1~35
	Wildcat mountain	35	35	<i>Berberispruinosa</i>	2017.4	YMS-135
	Wildcat mountain	75	68	<i>Berberisgracilis</i>	2017.4	YMS-36~110
	Total	230	193			

2.1.1 Primers

The primers used for molecular markers of rust in this experiment were general primers for fungi: ITS1-TCCGTAGGTGAACCTGCGG and ITS4-TCCCTCCGCTTATIGATATGC [21].

2.1.2 Instruments, equipment and reagents used in the test

Test equipment: ultra clean workbench, high pressure steam sterilizer, drying box, electronic balance, HH-4 digital constant temperature water bath pot, Microfuge18 centrifuge, Blue Shield 522 visible light gel electrophoresis transmission instrument, ABI-PCR instrument, 78HW-1 constant temperature heating magnetic stirrer, GeneQuant Pro protein nucleic acid analyzer, Imagequant-300Gel imaging system, different range of pipettes, Leica fluorescence microscope and body microscope, etc.

Test consumables: Omega fungal extraction kit, 2x high fidelity enzyme, agarose, TAE, anthocyanin, gel Recovery Kit, flat end clone kit, ddH₂O, centrifuge tube, etc.

2.2 Methods

2.2.1 Sample collection

The samples used in this experiment were collected from Kunming (Western hills and Wildcat hills) and Qujing (Yiche and Nagu) from April to June 2017. The incidence of *Berberis* was observed from the end of March. After the rusty organ on *Berberis* developed obviously, fresh rusty organ was selected for collection. In order to prevent mutual contamination between samples, a single sample was installed in the collection bag, and then brought back to the laboratory for preservation at -20 °C. It is used for morphological observation and DNA extraction.

2.2.2 DNA extraction of rust on *Berberis*

The genomic DNA of rust samples was extracted with the fungal DNA extraction kit of Omega company, and the specific methods are as follows: (1) Scrape the rust spores on the plant leaves into the 1.5mL centrifuged tube which has been sterilized by high temperature with the sterilized blade (half of each lesion is scraped, the other half is used for the observation of rust organ and rust spore morphology in the later stage), add the liquid nitrogen to grind fully; (2) Immediately add 500µL of CPL buffer and 10 µL of β - mercaptoethanol, and mix well on the vortex oscillator; (3) Water bath at 65 °C for 15 min. during the water bath, the centrifuge tube was reversed up and down to mix the samples twice; (4) Add 500 µL of chloroform / isopentyl alcohol with the proportion of 24:1 to 1.5mL centrifuge tube, shake and mix it on the vortex meter, and centrifuge it at 13000g for 10min; (5) Transfer 300 µL of supernatant to a new 1.5mL centrifuge tube; (6) Add 150 µL CXD buffer and 300 µL absolute ethanol, shake and mix well; (7) Take HiBind collection column and put it in a 2.0mL collection tube, transfer all the mixed solution to HiBind collection column, centrifuge at 10000g for 1min, and collect DNA samples; (8) Transfer the collection column to another new collection tube, add 650 µL SPW buffer (diluted with anhydrous ethanol) into the collection column to wash the collection column, centrifuge at 10000g for 1min, discard the waste liquid, and repeat this step once; (9) Place the collection column in the collection tube and centrifuge it at 10000g for 2min; (10) Transfer the dried collection column to 1.5mL centrifuge tube, add 50-100 µL solution buffer (before use, take a warm bath at 60 °C), place it at room temperature for 2min, centrifuge it at 10000g for 1min to elute the DNA, and repeat this step once with the collection body. The liquid collected in the centrifuge tube is the DNA solution of fungus.

2.2.3 PCR detection

The total PCR volume of ITS molecular marker was 50 µL, including 2 × phanta Max × Buffer 25 µL, dNTPs 1.0 µL, phanta 1.0 µL, primer ITS1 and ITS4 2 µL, ddH₂O 18µL, template DNA (25ng / µL) 1 µL. ddH₂O negative control was set up for each reaction. The amplification was carried out on ABI-PCR. The reaction procedure was: pre denaturation at 95 °C for 3min; denaturation at 95 °C for 15s, annealing at 57 °C for 15s, 72 degrees 30s, 35 cycles, 72 degrees 5min, 4 PCR; after PCR, the 3µL PCR product was mixed with 2µL containing anthocyanin Loading-buffer (1mL Loading buffer + 10µL Anthocyanin). The agarose gel was used for electrophoresis under 120 loading. PCR products were cloned and sequenced after gel recovery.

2.2.4 Cloning and sequencing of ITS markers of rust fungus

The PCR product fragment with the same size was purified with the gel recovery kit of Kunming Shuoqing Biotechnology Co., Ltd. The specific operation are as follows:(1) Use the blade to cut the Dan into 1.5ml centrifuge tube;(2) Add 500 μ L sol solution to the centrifuge tube containing the target DNA, and bathe in water at 65 °C for 10 minutes until the glue is completely dissolved;(3) The sol solution was completely transferred into the DNA binding column, and stored at room temperature for 2 minutes ;(4) Centrifuge 10000g at room temperature for 1min, and discard the waste liquid;(5) Add 750 μ LSPW buffer (diluted with anhydrous ethanol) and leave it at room temperature for 2min;(6) Centrifugation of 10000g at room temperature for 1min, waste liquid and 10000g at room temperature for 2min(7) 30-50 mL of ddH₂O was added into the DNA binding column, and the column was kept at room temperature for 2min;(8) After centrifuging at room temperature for 2 min, the obtained liquid is the purified DNA. The purified DNA was cloned with the flat end clone kit of Adlai Biotechnology Co., Ltd.:(1) Add 8 μ Lof purified DNA sample, 1 μ L of Ptopo-Blunt Simple Vectr and 1 μ L of 10xEnhancer to the PCR tube, and connect them at room temperature for 5min; (2) Transfer the connection solution completely into the centrifuge tube containing 100 μ L of cells, and place it at room temperature for 5min;(3) Add 300ml of LB culture medium into the centrifuge tube in the previous step, and culture on a shaking table at 37 °C and 180rpm for 10min;(4) Take 200 μ L bacterial solution and spread it evenly on LB medium added with ampicillin for overnight culture;(5) Single colony was selected and cultured in 5mL centrifuge tube with 2mL LB medium at 37 °C and 180rpm for 5h;(6) The positive clones were sent to shuoqing Biotechnology Co., Ltd. for sequencing.

2.2.5 Data analysis

Morphological study: the single spot of all rust fungi was observed by means of body microscope, and the observation of single rust organ was first made into frozen section, which was cut into 12 μ m by freezing microtome and placed on the slide for observation under Leica fluorescence microscope, and the depth of single rust organ invading the host plant and the thickness of the leaves of the host plant were measured by measuring tools in Leica fluorescence microscope. The rust spores were observed and measured under Leica fluorescence microscope. In each population, 14-50 rust organs (14 of wheat stripe rust) and 100 rust spores were observed.

Sequence analysis of rust ITS: after the vector sequence is removed from the cloned sequence, sequence alignment analysis is carried out on NCBI website to find the sequence related information. The results showed that the sequence of rust was the target sequence of this study. After getting the target sequence, use MAGE5.1 for sequence comparison, align the sequence of each gene, and use the neighbor joining method in the software to construct phylogenetic tree, with a repetition of 1000 times.

3. Results and analysis

3.1 Symptoms of rust on *Berberis*

In order to observe the overall morphological characteristics of the rust organs on *Berberis*, *berberis* rust sample were numbered and photographed with camera and body microscope to observe the morphological characteristics of rust organ. The morphological characteristics of rust organ on *Berberis* are shown in Fig. 1: the symptoms of rust fungus on *Berberis* are various, each leaf forms at least one spot and up to 10 spots about, each spot consists of 3 or more rust organs (Fig. 1). Some of the diseased spots are distributed in the middle or edge of the leaves on both sides of the main vein (Fig. 1a-j), some are distributed in the main vein of the leaves (Fig. 1K), and some are infected with young stems or fruits (Fig. 1L).



Fig.1. Samples of rust on *Berberis* collected from different areas.(Note: the scale in the figure is 100 μ m, A-F is the sample collected from yemaoshan, G-I is the sample collected from Xishan, J-L is the sample collected from Yiche and Nagu)

3.2 ITS sequence analysis of the rust organ of *Berberis* in Yunnan Province

In order to analyze the species of rust on *Berberis* in Yunnan Province, the ITS sequences of 193 rust on *Berberis* from different places were compared on NCBI website, and the target sequences were obtained by cloning and sequencing. In addition, 18 sequences with high similarity to the target sequence were downloaded from NCBI website, and one sequence of scabbard rust was used as the reference sequence. The genealogical tree analysis was constructed with MAGE5.1, and the results are as follows:

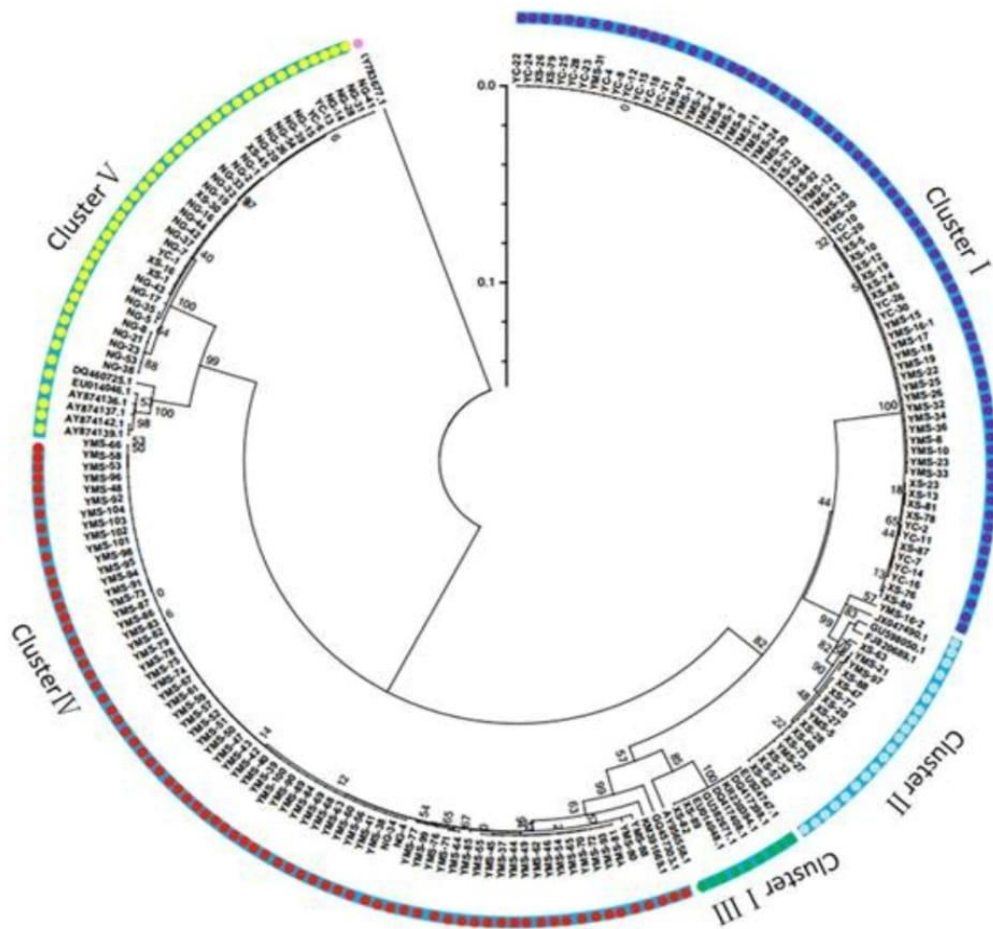


Fig 2 : Genealogical tree Analysis of Rust fungus from *Berberis*

(Note: cluster I: the similarity with *Pucciniastriformis* f. sp is 93-94%; cluster II: the similarity with *Pucciniastriformis* 96-98%; cluster III: the similarity with *Pucciniastriformis*f. spis 100%; Cluster IV: the similarity with *Puccinia brachypodii* is 97%; cluster V: the similarity with *Puccinia graminis*f. sp is 93-94%.

It can be seen from Figure 2 that 193 ITS sequences obtained by cloning and sequencing in this experiment are divided into 5 groups: 35 of them are *Puccinia graminis*f.sp, accounting for 18.13% of the total; 70 of them are *Puccinia brachypodii*, accounting for 36.27% of the total; 69 of them are *Pucciniastriformis*f. sp. with 93%-94% similarity; accounting for 35.75% of the total; 17 of them are *Pucciniastriformis*with 96%-98% similarity, accounting for 8.8% of the total; 2 of them are *Pucciniastriformis* f. sp.with 100% similarity, accounting for 1.04% of the total. And there is abnormal differentiation of rust in the same group (Fig. 2).

3.3 Morphology of rust organ and rust spore on *Berberis*

In order to clarify the relationship between the morphological characteristics of rust organ of different groups in Figure 1, this study selected some individuals from each group to observe the morphology and size of rust organ and rust spore. The results are shown in Figure 3:

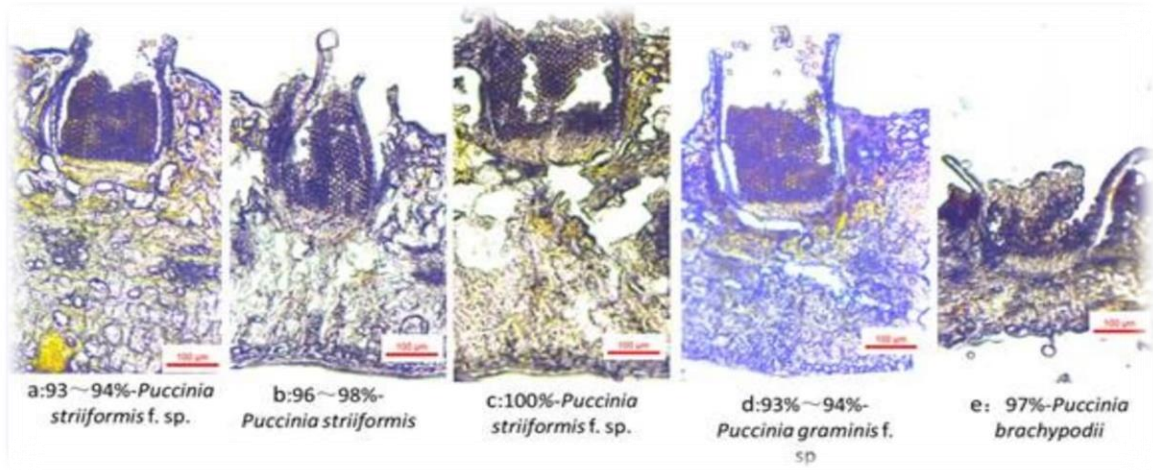


Figure 3. Morphological characteristics of rust organ of rust fungus on *Berberis*

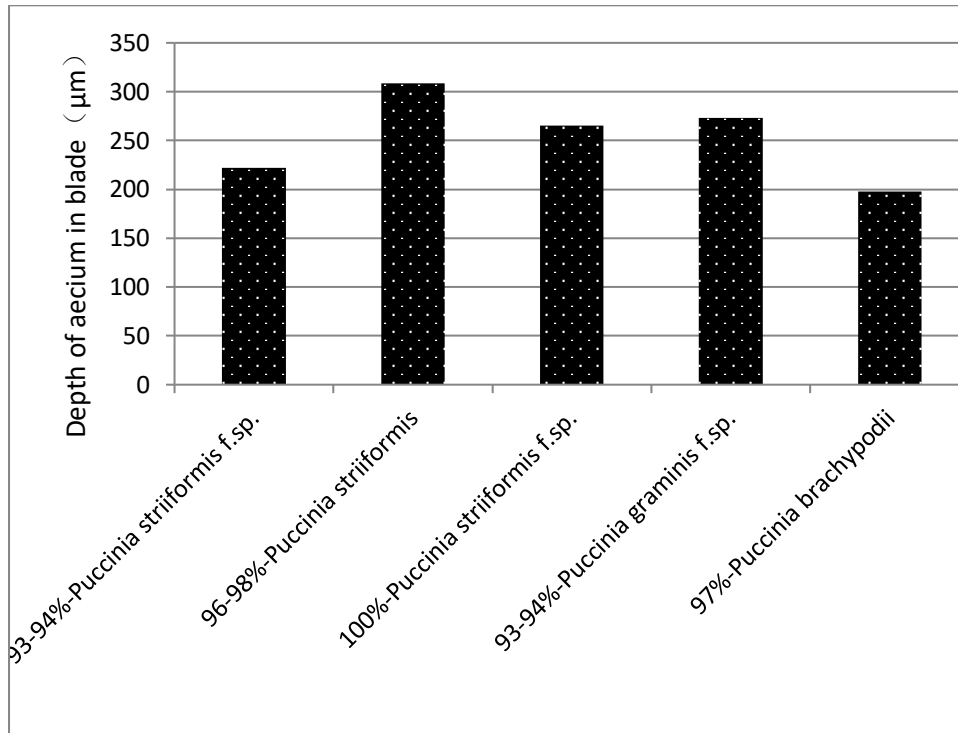


Fig. 4. The depth of aecium invading leaves on *Berberis*

In order to compare the morphological characteristics of different rust organs, in this study, in addition to 14 rust organs of wheat stripe rust, 50 rust organs were randomly selected from the samples collected at the same time to measure the depth of rust organs invading into the host *Berberis*. The results showed that the depth of the rust organ which was 96% - 98% similar to that of the stripe rust was between 281-365 µ m, and the average depth was 308.83 µ m, which was the deepest among the five kinds of rust, followed by 93% - 94% similar to that of the wheat stem rust. The depth of the rust organ which was 221-305 µ m, and the average depth was 273.16 µ m. The average depth of wheat stripe rust was 265.32 µ m, which was 93% - 94% similar to that of wheat stripe rust. The average depth of wheat stripe rust was 222.20 µ m, which was 180-277 µ m. The shortest time for rust organ to invade the host plant is 97% of the rust similar to that of bluegrass stem rust. The depth of rust organ to invade the leaves is between 150-226 µ m, and the average depth is 198.05 µ m (Fig. 4).

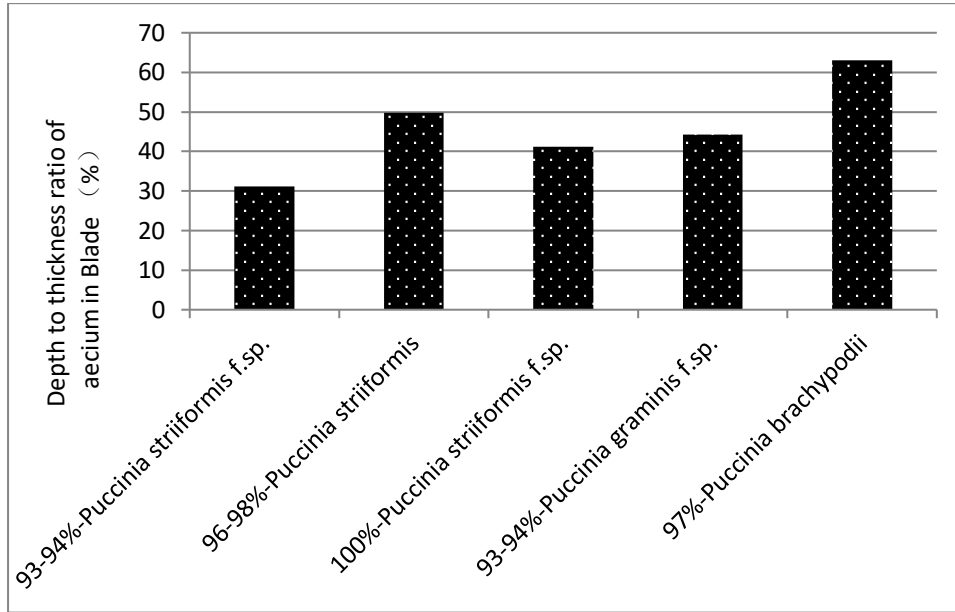


Figure 5. The ratio of the depth of aecium invading the leaves to the thickness of *Berberis* leaves

In order to compare the ratio of the invasion depth of aecium on *Berberis* to the thickness of the leaves of the host plants, this experiment measured the invasion depth and the thickness of the leaves of 14 aeciums of two wheat stripe rust, and the other rust groups measured the invasion depth and the thickness of the leaves of 50 aeciums, as shown in Figure 3. Among the five rust species, the ratio of the depth of aecium invading the host plant to the thickness of the leaves of the host plant was the highest, which was 97% similar to that of bluegrass stalk rust. The average ratio was 63.06%. The second is rust with a similarity of 96% - 98% with stripe stem rust. The ratio of the depth of aecium invading host plant to the thickness of host plant leaves is 38.50% - 66.55%, the average ratio is 49.74%, and the similarity with wheat stem rust is 93% - 94%. The ratio of the depth of rusty organ invading the host plant to the thickness of its leaves was 35.09% - 57.36%, with an average ratio of 44.22%. The ratio of the depth of wheat stripe rust invading the host plant to the thickness of the host plant leaves is 33.1% - 50.36%, with an average ratio of 41.14%. The ratio of the depth of wheat stripe rust invading the host plant to the thickness of the host plant leaves is 22.49% - 48.78%, with an average ratio of 31.12% (Figure 5).

3.4 Morphology of rust spores of rust on *Berberis*

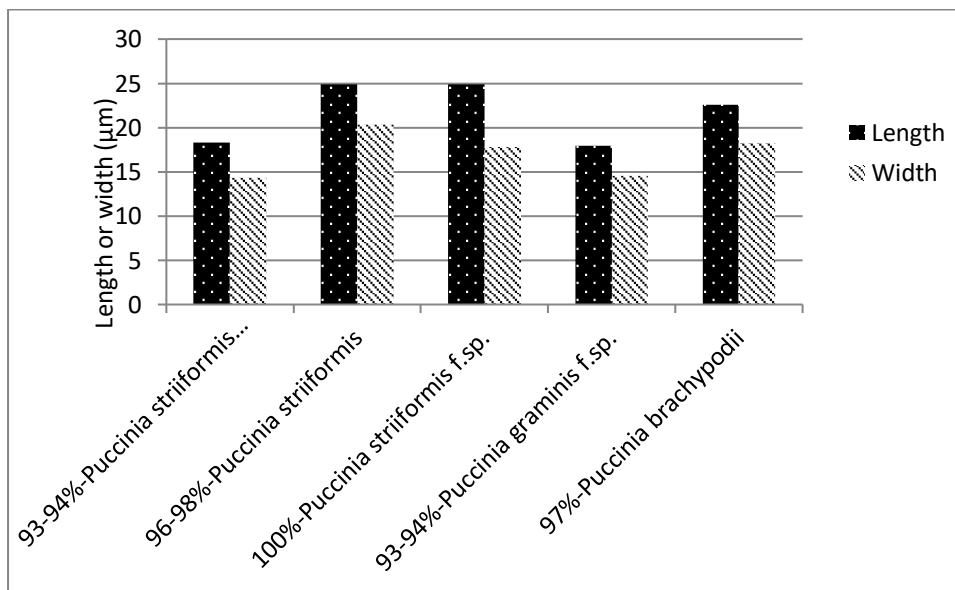


Fig. 6. Length and width of rust spores on *Berberis*

After the difference of aecium on *Berberis* was observed, the rust spores of each type were observed under Leica fluorescence microscope in this experiment, and it was found that there were also great differences among rust spores of different groups (Fig. 7). After the difference of morphological characteristics of rust spores was observed, 100 rust spores of each group were randomly selected to measure their length and width, as shown in Fig. 6. The length of rust spores of wheat stripe rust and rust were 24.86 μm and 24.89 μm , respectively. The similarity was 96%-98% of that of stem rust, which were the longer aecium of several kinds of rust. Secondly, the length of rust spores was 22.60 μm , 18.32 μm and 17.94 μm , respectively. The longest rust spore is the rust with a similarity of 96% - 98% and a width of 20.34 μm , followed by the rust with a similarity of 97% to Stem rust of *Poa pratensis* in dry land, 93% - 94% to wheat stem rust and 93% - 94% to wheat stripe rust. The width of the rust spore is 18.21 μm , 17.78 μm , 14.54 μm and 14.30 μm respectively (Fig. 6 and Fig.7).

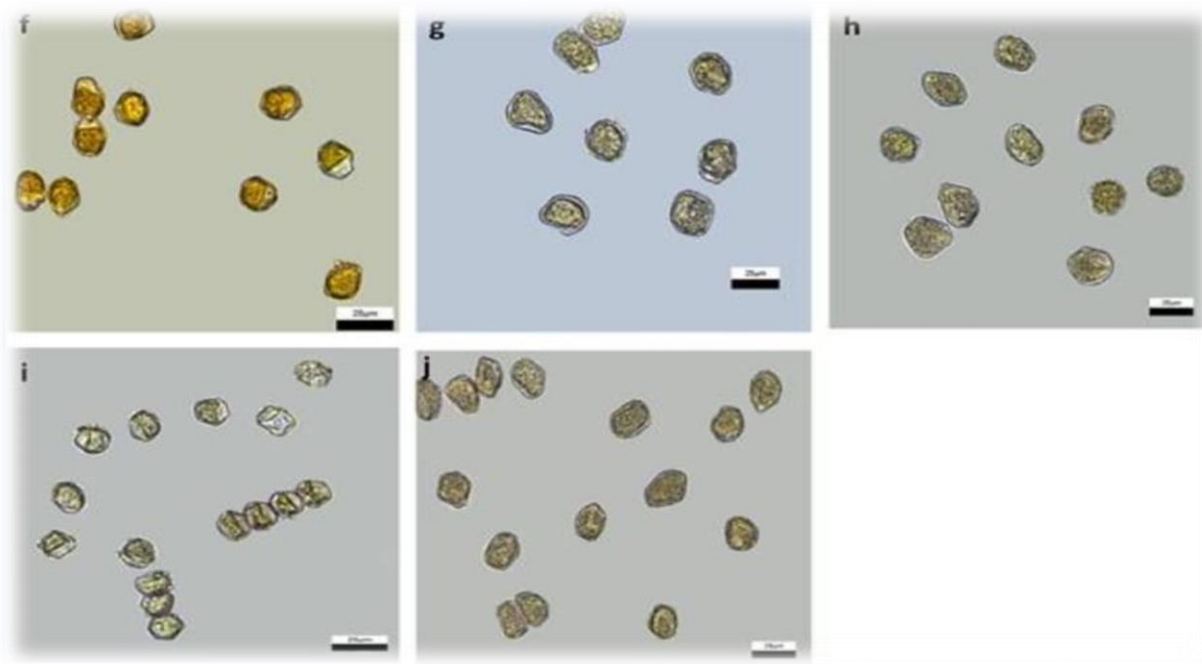


Fig. 7. Rust spores on *Berberis*,

(Note: the scale in the figure represents 25 μm . f is the rust spore with the similarity of 93% - 94%; g: the rust fungus with the similarity of 96% - 98%; h: the rust fungus with the similarity of 93% - 94%; j: the rust fungus with the similarity of 97 %.)

4. Discussion

Yunnan Province has a special geographical location, unique climate and abundant *Berberis* resources. In this study, 230 samples of *Berberis* aecium collected from different areas of Yunnan Province in 2017 were identified and analyzed by a combination of molecular biology and morphology. The strains with the highest similarity and coverage were selected on the NCBI website for comparison with this experiment. The results showed that the rust on the *Berberis* collected in this study belonged to the genus stipularia (Table 2). By using MEGA5.1 software to compare and analyze its sequences of rust on *Berberis*, different sequences are divided into five groups: rust with the similarity of *Puccinia grainini* f. sp. of 93-94%, rust with the similarity of *Puccinia brachypodii* of 97%, rust with the similarity of *Puccinia striiformis* f. sp. of 93-94%, rust and *Puccinia striiformis* f. sp. with the similarity of *Puccinia striiformis* of 96-98%. There are great differences in the morphology of aecium and rust spore among different rust groups. Some rust species can be distinguished according to the morphology of aecium and rust spore. Similar to the research results of Berlin et al. On the rust of *Berberis* in Sweden in 2013, the research of Berlin found that there was more than one kind of rust on *Berberis* in more than 50% areas [22]. In this study, it is found that there are many kinds of rust on *Berberis* in each area (Fig. 8). Due to the limited number of samples collected and identified, in this study, there may be other rust on *Berberis* that have not been collected and identified, which shows that the rust on *Berberis* is very complex.

It is reported that the same rust can also infect more than 80 species of *Berberis*, and the more common *Puccinia graminis* can infect more than 80 species of *Berberis*[22], but different species of *Berberis* have different resistance to the same rust [23]. In this study, only 97% of *Puccinia brachypodii* similar to *Puccinia brachypodii* was detected in the *Berberis* collected from yemaoshan. However, in *Berberis farinæ*, 93-94% of the rust similar to *Puccinia striiformis* f. sp. and 96-98% of the rust similar to *Puccinia striiformis* were detected. In Western mountain area, the rust with a similarity of 93-94% with *Puccinia graminis* f. sp., 96-98% with *Puccinia striiformis* f. sp., 93-94% with *Puccinia striiformis* f. sp., 96-98% with *Puccinia striiformis* f. sp. and *Puccinia striiformis* f. sp. were detected. In the *Berberis* of Xishan area, 93-94% - *Puccinia striiformis* f. sp. had the most rust, which was 19, accounting for 51.35%. The second is the rust of 96-98% - *Puccinia striiformis*, which is 12, accounting for 32.24%; the fourth is 93-94% - *Puccinia graminis* f. sp. and the second is 100% - *Puccinia striiformis* f. sp., which accounts for 10.81% and 5.4% respectively. No rust of 97% - *Puccinia brachypodii* was detected (Fig. 8). It shows that the same kind of *Berberis* has different resistance to rust of different groups.

In 2013, Berlin et al. from Sweden used the method of combining molecular biology and morphology to study the rust fungi collected from *berberis*. The results showed that different rust fungicould bedistinguished and identified according to the morphological characteristics of aecium and rust spore [22]. In this experiment, the observation of aecium and summer spore morphology of different groups of rust fungi also had great differences. However, in this study, it is also found that there are large differences in the size of aecium on *Berberis* at different stages of disease (Fig. 1). Although the samples used to observe aecium and rust spore in the experiment are collected from the same time and the same place, it is not guaranteed that the same time of disease can be observed. Therefore, in the presence of such uncontrollable factors, although there are differences in the size of aecium and rust spore of different rust samples, the differences in the size of aecium and rust spore among some rust fungi are relatively small (Fig. 6). In 2013, Berlin et al. Also found that two rust fungi with similar morphology can be divided into different species after molecular marking[22]. Therefore, the identification of different rusts only by the morphology of aeciums and rust spores will inevitably lead to identification errors for various reasons. In order to accurately identify different types of rust on *Berberis*, it is necessary to use the combination of molecular markers and morphology.

In 2005, Abbasi et al. found that *P.graminis* had obvious monophylicity, but it was also a complex species with high genetic variability [24]. In addition, a large number of studies have also shown that some other fungi of *Puccinia* are also monophyletic, and there is a high degree of genetic variation in rust in different groups [22,25,26]. The results of its marker cloning and sequencing in this experiment also showed that there would be differences in its sequences in the same group of rust, indicating that there was variation in the same group of rust, but the variation degree of different rust groups was different (Fig. 2). This may be due to the variation in the process of forming aecium by the combination of sex clasp and fertilized silk. Since Jin et al. Confirmed that *Berberis* is the host of wheat stripe rust in 2010 [10], a breakthrough has been made in sexual reproduction and Toxicity Variation of wheat stripe rust in China. However, in Northwest Sichuan, Yunnan, Guizhou, Xinjiang, Qinghai and other provinces and regions, *Berberis* are widely distributed, and the occurrence of *Berberis* rust in spring is very serious. At present, it has not been determined whether there is sexual reproduction of wheat stripe rust in these areas [11]. In this study, two of the 35 samples of *Berberis* rust collected in Western mountain of Kunming were identified as wheat stripe rust (Fig. 2 and table 2). It is confirmed that *Berberis* can become the host of wheat stripe rust under the natural conditions of Yunnan Province, and there is sexual reproduction of wheat stripe rust. In this study, the rust on *Berberis* in Yunnan Province was systematically studied, which laid a good foundation for revealing the diversity of rust on *Berberis* and further studying the sexual generation of rust such as wheat stem rust and wheat stripe rust.

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