Development of SSR Markers and Genetic Diversity Evaluation of Mycocentrospora Acerina Causing Round Spot of Panax Notoginseng in Yunan Province, China

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Abstract

Sanqi round spot, which is caused by *Mycocentrospora acerina*, is a destructive disease limits the production of *Panax notoginseng* in Yunnan province of China. However, the disease has not been studied comprehensively. In the current study, we identify *M. acerina* polymorphic microsatellite markers using CERVUS 3.0 and compare the genetic diversity of its isolates from *P. notoginseng* round spot using Simple Sequence Repeat (SSR) markers and polyacrylamide gel electrophoresis. Thirty-two SSR markers with good polymorphism were developed using MISA and CERVUS 3.0. The genetic diversity of 187 *M. acerina* isolates were evaluated using 14 representative SSR primers, and the polymorphic information content values of 14 sites ranged from 0.813 to 0.946, with a total of 264 alleles detected at 14 microsatellite loci. The average expected heterozygosity was 0.8967. The genetic diversity of *M. acerina* in Yunnan province does not reflect geographic specificity.

Background

Sanqi [*Panax notoginseng* (Burk.) F. H. Chen], a member of the Araliaceae family (Siciliano *et al.,* 2008), is mainly distributed in Yunnan Province, China. *P. notoginseng* is a valuable traditional Chinese medical herb with multiple pharmacological applications (Duan *et al.,* 2017). Ginsenosides and amino acids have various positive effects on the circulatory system, cardiocerebral vascular system, central nervous system, and endocrine system, and reduces inflammation (Michael *et al.,* 2001).

P. notoginseng is a perennial herb with more than 400-year cultivation history. With increasing demand in recent years, its cultivation area has increased rapidly (**Xia *et al.,* 2014**). However, large scale cultivation has led to the emergence of severe diseases in production, such as root rot, which is caused by *Cylindrocarpon destructans* var. crassum (Long *et al.,* 2015), *Fusarium oxysporum* (Ma *et al.,* 2019) and *F. solani* (Tang *et al.,* 2019), round spot caused by *Mycocentrospora acerina* (Lu *et al.,* 2005), and dark speckle caused by *Alternaria panax* (Chen *et al.,* 2019). Such severe diseases have limited the cultivation and production of Sanqi in Yunnan (Jiang, Qin and Ye, 2011). Round spot is the most severe disease affecting the leaves, and it spreads extremely rapidly in rainy season, causing 20–50% yield losses (Lan, Long and Li, 2018).

Round spot mainly occurs on the leaves, and develops further into a brown spot with a transparent point in the middle, finally forms gray-white mold layer and produces conidia of *M. acerina* (Dai *et al.,* 2017). The disease is caused by Ascomycotina, Dothideomyceta, Mycosphaerellaceae, Mycocentrospora, *Mycocentrospora acerina (Hartig) Deighton* (Wall and Lewis, 1980), which is a quarantine pest in China. There are 23 species of fungi belonging to the *Mycocentrospora* genus. (http://www.speciesfungorm.org/), all of which are plant pathogenic fungi. Moreover, *M. acerina* infects more than 20 plant species globally, including several economically valuable crops such as *Daucus carota* L. var. sativa Hoffm (Davies, Lewis and Day, 1981) and *Paeonia lactiflora* (Garfinkel and Chastagner, 2019), and Acer L (Ellis, 1974). It is worth noting that *M. acerina* can infect nearly 23 kinds of weeds, mainly including blue pansy, scorpion, chamomile, bidens(Hermansen, 2010). Although several reports have explored *M. acerina* disease prevalence and its biological characteristics (Dai *et al.,* 2017), only a few studies have investigated its molecular biology.

M. acerina was reported to infect Asarum in Liaoning Province in 1990, causing leaf blight of Asarum, and this is the first report of this pathogen in China（Wang, Fu, Wang, 1992）. The fungus was reported to infect *P. notoginseng* in Yunnan Province of China in 1997 (Chen, Chen, and Yu, 1997). So far, *M. acerina* has only been found to infect these two kinds of medicinal cash crops in China, and the impact on *P. notoginseng* is more serious. *P. notoginseng* is a unique Chinese herbal medicine, which can be cultivated only in Yunnan and Guangxi provinces of China. The planting area in Yunnan Province accounts for about 99%. *P. notoginseng* round spot is the main leaf disease of *P. notoginseng*. If the control is not timely, the incidence rate of *P. notoginseng* round spot can reach 100%, causing serious economic losses, especially in the rainy season, it often causes large-scale epidemic and transmission. In the field, the disease was mainly transmitted by the conidia on the leaves of *P. notoginseng* through rain splashing.

In the early 1820s, plant pathologists began to pay attention to the relationship between changes in the genetic structure of pathogen populations and plant diseases. The pathogen population defined by geneticists early is a collection of individuals of the same species, including all genotypes in the population. The genotype is affected by the growth and extinction of the host, gene drift, environment, and reproductive methods. Therefore, the absolute pathogen population is difficult to define. Therefore, in actual research, the sum of the sampling of pathogen populations in a limited time and a certain space is usually analyzed as a population(Wang and Shan, 1998). The genetic structure of plant pathogen population reflects the evolutionary potential and evolutionary history of pathogen(Yu, 1979). Therefore, the ultimate goal of studying pathogen population genetics is to determine the factors that play a major role in the population evolution of pathogenic fungi, and to grasp the rules of interaction of these evolutionary factors.

In the history of agricultural production practice progress, plant pathogens have important economic and social impacts on humans, and the knowledge of the genetic diversity of plant pathogens will help people understand and control the agricultural ecosystem. The pathogenic fungus population is changing and will adapt to changes in the control methods and its living environment. Eventually, the genetic structure of the pathogen population changes, causing plants to lose resistance(Salama *et al.,* 2000). The speed of strain evolution is mainly reflected by the number of genetic variation in the pathogen population. This result will help the judgment of the effective maintenance time of disease prevention measures in agriculture. And pathogen populations with complex genetic structure can often adapt faster to the host's disease resistance or the sensitivity of fungicides. Therefore, the understanding of the genetic structure variation and distribution of phytopathogenic fungi populations will have important guiding significance for disease resistance breeding, the rational distribution of disease resistance genes, and the rational use of fungicides in production. The rapid development of molecular biology based on DNA carrying genetic information has made it a reality to accurately detect the genetic variation of plant pathogen populations(Zhou *et al.,* 2018).

DNA-based pathogen population genetic diversity research methods mainly include SCAR (sequence characterized amplification regions), AFLP (amplification fragment length polymorphism), and restriction fragment length polymorphism. RFLP (Restriction Fragment length polymorphism), SSR (simple sequence repeat), ISSR (inter-simple sequence repeat), RAPD (random amplification polymorphism), SNP (Single Nucleotide Polymorphism). Among them, the RFLP operation is more complicated, time-consuming, and expensive; RAPD is poor in stability and cannot provide complete biological genetic information; SCAR is mostly used for gene location and molecular marker assisted selection; AFLP is expensive and complicated; ISSR can better reflect genetic diversity, but does not have species specificity; SNP mainly refers to the polymorphism of the DNA sequence caused by single nucleotide variation at the genome level of the same individual or closely related populations, which reflects the ratio of polymorphism The low mutation frequency of SSR markers makes it more suitable for the population genetics of distantly related segregation(Bridge, 20); therefore, SSR has rich polymorphism and can better reflect the level of genetic diversity. With the gradual publication of biological genome data, SSR is more convenient in the research of species genetic diversity.

Simple sequence repeat (SSR) is the simplest among numerous molecular marker methods used to evaluate levels of diversity in species. With the publication of genomic databases, SSR is convenient in the study of genetic diversity (Barus, Bayu and Hanafiah, 2020; Adjebeng-Danquah, 2020). Pathogenic fungal populations exhibit variations across different locations as adaptations to changes triggered by control methods and diversity in their living environments (Salama, 2000). The genetic structure of a pathogenic fungus population can also change and lead to loss of resistance in host plants (Colling and Matthies, 2004). Development of molecular markers for *M. aceria* could offer a more comprehensive genetic basis for *M. acerina* studies, which would enhance efforts to control Sanqi round spot. Therefore, understanding the variation in genetic structure and distribution of plant pathogenic fungi populations could enhance our understanding of the distribution of genetic informatin and facilitate the formulation of appropriate disease control strategies (Jordan 2010; Fernández, 2006).

In this study, we analyzed the SSR characteristics in the *M. acerina* genome and developed SSR primers from *M. acerina.* Moreover, the effectiveness of the primers in analyzing population genetic diversity structure in *M. acerina* was analyzed.

Methods

Strain isolation and observation

M. acerina strains were collected from six major *P. notoginseng* production regions, including Honghe, Wenshan, Qujing, Kunming, Lijiang, and Puer in Yunnan Province (Table 1). The geographical distribution of the samples shown in Figure 8. *M. acerina* were obtained using tissue isolation (Wang *et al.,* 2015) but with a few modifications (Zietkiewicz, Rafalsk and Labuda, 1994). The junction between healthy and diseased tissue was washed using sterilized water and then immersed in alcohol (75%) for 2–3 min and washed again using sterilized water and dried on sterilized filter paper. Afterward, the samples were transferred into PDA medium and cultivated at 20°C for 4d. Then, we verified the isolates based on Koch postulates and pured by hyphal-tipped. *M. acerina* was identified based Morphological characteristic and ITS sequence.

DNA extraction and polymerase chain reaction amplification of internal transcribed spacer (ITS1/4) regions

Genomic DNA was extracted from 0.2 g of mycelium using the Omega Fungi DNA Kit (Kunming Shuoqing Biological Engineering Technology Co. Ltd., Kunming, China) according to the manufacturer's instructions. Amplification reactions were performed in a 20 μL volume containing 1 μL template DNA, 10 μL mix (DNA polymerase, Buffer, dNTP), 1 μL primer ITS1 (TCCGTAGGTGACCTGCGG), 1 μL primer ITS4 (TCCTCCGCTTATTGATATGC), and 7 μL ddH_2O (Chen *et al.,* 2010). Polymerase chain reaction (PCR) was performed using a T1 thermocycler (Biometra, Germany), with initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 45 sec, 60°C for 45 sec, and 72°C for 90 sec, and a final extension at 72°C for 10 min. Amplification products were separated by electrophoresis on 1% agarose gels in a 0.5× TAE buffer, using a 2000-bp DNA ladder as a DNA molecular weight marker. The PCR products were sequenced at Kunming Shuoqing Biological Engineering Technology Co. Ltd. Molecular Evolutionary Genetics Analysis (MEGA 5.1) was used to construct a phylogenetic tree based on the neighbor-joining method. Bootstrap values were evaluated using 1,000 replications.

Simple sequence repeat screening

SSRs were screened using MISA (http://pgrc.ipk-gatersleben.de/misa/) based on the whole genome data of *M. acerina* (Wang *et al.,* 2018; Wang, Chen and Zhao, 2016). MISA is a script written in Perl language, which can identify SSRs from genome FASTA files (Xu *et al.,* 2017), MISA is simple to run without networking, and does not require high hardware. It has become the tool of choice for most SSR researchers. In the SSR parameter settings, we defined that six, five, four, three, two, and one base were repeated five, five, five, five, six, and ten times.

The genome used in this study was sequenced using Hiseq 2500 from Illumina(unpublished). After sequencing, two sets of 101bp long and short paired-end short sequence data were generated. *M.acerina* produced a total of 20.502 Gb original sequence, after filtering quality control, remove the linker sequence, low-quality base sequence and low sequence complexity sequence. The K-mer parameter is 75, and the final assembly result is obtained after automatic assembly and gap filling using SOAP denovo software (BGI, Shenzhen, China). The assembled size of *M. acerina* is 39Mb in total. The N50 index is an evaluation index for the continuity of genome assembly, this value is calculated by sorting the contig sequence length from largest to smallest. The larger the N50 value, the better the continuity of the contig generated by the assembly. In this study, *M. acerina* genome assembly contig N50 is 151kb, scaffold N50 is 567kb, the assembly quality is credible.

Primer design

SSR primers for the whole genome of *M. acerina* were designed in the PRIMER 3.0 (http://Frodo.wi.mit.edu/primer3) website (Hou *et al*., 2018) based on the screened SSR results. The primers were synthesized by Shuoqing Biological Engineering Technology Co. Ltd.

For the initial screening, 24 isolates from different sources, 118 SSR primers were designed and amplified with 20-μL PCR mixtures. All the amplification products were separated on 2.5% agarose gels in 0.5× TAE buffer, using a 100-bp DNA ladder as a DNA molecular marker (Raju, Sheshumadhav and Murthy, 2008). The primers with clear, specific, and target bands

(100~500 bp) could be tested by automatic electrophoresis apparatus (Qsep^{100TM}). Finally, the polymorphism of primers was assessed using CERVUS 3.0 (Azam *et al*., 2018), and the primers in which PIC exceeded 0.5 were retained for use genetic diversity analyses (Zhou *et al*., 2019).

SSR analysis of *Mycocentrospora acerina* genome

Based on the initial results, 14 primer pairs with high polymorphism in *M. acerina* populations were selected (MP30, MP36, MP47, MP50, MP51, MP56, MP61, MP62, MP63, MP68, MP84, MP92, MP113, and MP114) for use in amplification reactions. Stable and clear fragments ranging in size between 100 bp and 800 bp were transformed into a binary character matrix (1 = presence, 0 = absence) (Zeng *et al*., 2013; Komy, Saleh and Molan, 2012).

Statistical analysis

Genetic diversity parameters of each geographic population, including observed number of alleles, effective number of alleles, Nei's diversity index, Shannon's information index, total gene diversity, intrapopulation gene diversity, the coefficient of gene differentiation, and gene flow (Saad *et al*..2009), were calculated using POPGENE 32 (version 1.32) (Liu *et al*. 2010). NTSYS-pc (version 2.0) was used to calculate genetic similarity coefficient (Liu and Yang, 2010; Gaikwad *et al.,* 2008). The phylogenetic tree was analyzed using unweighted pair-group algorithm with arithmetic averages clustering analysis (Wang *et al*., 2014).

Results

Isolation and identification of strains

All the isolates were identified based on their colonies and conidium (Figure 1A, B), and symptoms on inoculated plants were similar to those of plants growing in the field (Figure 2C, D). Phylogenetic trees, constructed based on internal transcribed spacer (ITS1/4) sequences, showed that the isolates were grouped with *M. acerina* (Figure 3).

Genomic SSR analysis

A total of 8250 microsatellite sequences with 1 to 6 base repeats were obtained from the *M. acerina* genome. The average length of SSRs was 26 bp. SSR lengths of different repeat types were varied (Figure 4).

Among the SSRs, there were 3379 mono-nucleotide repeats, which accounted for 40.96% of the total repeats. Among the mono-nucleotide repeats, 113 repeats were repeated more than 30 times. In addition, there were 2137 tri-nucleotide SSRs (25.90%) and 179 penta-nucleotide repeats, which accounted for the lowest proportion (2.17%), with hexa-nucleotide repeats accounting for 3.71% of the total repeats. The maximum repetition times of each of the SSRs were 81, 40, 155, 217, 72, and 144 (Figure 5). Such SSRs with abundant repeats are beneficial to the development of molecular markers.

Based on the proportions of mono-nucleotide SSRs of *M. acerina*, T or A bases existed in single nucleotide SSR, and the number of poly T or A bases was 2449, accounting for 72.47% of the bases. There were 930 repeats with C or G bases, which was far less than those with T or A bases. Among the di-nucleotide repeats, there were four types of SSR sequences: AC, AG, AT, and CG. AG/CT repeats accounted for 49.84% of all the di-nucleotide repeats, followed by AC/GT and AT/AT. CG/CG type repeats were the least (9%). Among the tri-nucleotide repeats, AAC/GTT, AAG/CTT, and AAT/ATT were the main types. Overall, AAG repeats were the most abundant, accounting for 22.28%, and CCG content was the least (3.89%) (Figure 6).

The numbers and proportions of tetra-, penta-, and hexa-nucleotide repeats in the *M. acerina* genome are listed in supplementary table 1, 2, and 3. There were 28 types of tetra-nucleotide repeats, among which ATCC had the highest content (12.48%), and CCCG had the lowest content (0.16%). In addition, there were 61 penta-nucleotide repeats, AACAC and AATCC accounting for the largest proportions. Hexa-nucleotide repeats were the most abundant (103), AACCCT was the most common, with 44 motifs.

Polymorphism of SSR Primers

Thirty-two pairs of primers had highly polymorphic loci ([PIC] > 0.5) and could be used as SSR markers in the construction of an *M. acerina* genetic map and for genetic diversity analyses. PIC is commonly used to assess the degree of gene variation. A locus can be considered a highly polymorphic marker when its PIC value exceeds 0.5. According to the SSR primer data (Table 2), the average PIC was 0.6492, the average allele number per locus was 5.147, the average proportion of locus types was 1.00, and the average expected heterozygosis (He) was 0.7212.

Genetic diversity within populations

A total of 148 polymorphic bands were amplified from 187 populations of *M. acerina* using 14 SSR primers. The PIC of 14 loci ranged from 0.813 (MP56) to 0.946 (MP114), with an average PIC value of 0.8852, all of which were highly polymorphic. A total of 264 alleles were detected at 14 microsatellite loci, out of which 14 alleles were detected on MP61 with the least number. Thirty-one alleles with high diversity were detected on MP114. The average number of alleles per locus was 18.857. The observed heterozygosis (Ho) of 14 loci was 0, and He ranged from 0.831 to 0.951, with an average of 0.8967.

Diversity between populations

Nei's genetic diversity (0.0896) and Shannon's information index (0.1712) were the highest in the Honghe population (HH), followed by in the Puer population (LC); and the lowest in Lijiang, at 0.0842 and 0.143, respectively (Table 3). The genetic diversity in different populations was relatively low.

The average observed allele number (Na) was 2.00; the average effective allele number was 1.11; the Nei' gene diversity (h) was 0.0908; and the average Shannon diversity index was 0.1761. In addition, the total genetic diversity (Ht) was 0.0909; the intra-population genetic diversity (Hs) was 0.0884, and the genetic differentiation index (Gst) was 0.0277, which indicates that there was very low genetic variation of 2.77% variation among populations. The estimated level of gene flow (Nm) was 17.5757, which indicates that there were numerous changes to genes attributed to gene flow in different regions, and that gene flow was not the primary factor influencing genetic diversity in the population.

Among the six populations, the populations from Kunming and Honghe had the greatest genetic similarity (0.9988) and the least genetic distance (0.0012). In addition, Lijiang and Lancang had the least genetic similarity (0.9931) and the largest genetic distance (0.007) (Table 4). Generally, the genetic distances between the populations above were small, and their genetic similarity coefficients were close to 1, which indicated that the genetic relationships between strains in each population were close, and there was low genetic differentiation among different populations.

Cluster analysis

Cluster analysis revealed that the maximum similarity coefficient was 0.97 and the minimum was 0.83 among 187 *M. acerina* strains collected from 12 counties of 6 prefectures (cities) in Yunnan Province (Figure 8).

Discussion

The biology of *M. acerina* and round spot of *P. notoginseng* in Yunnan

In the 20th century, there were many studies on *M. acerina*, mainly focusing on the host diversity and transmission methods (Wall and Lewis, 1978; Catherine, 1980; Hermansen, 1992). In the 21st century, few studies on *M. acerina.* Sébastien Louarn studied the influence of *M. acerin*a on the polyacetylenes and 6-methoxymellein in organic and conventionally cultivated carrots (*Daucus carota*) during storage (Louarn *et al.,* 2012). Since it was discovered in 1997 that *M. acerina* can infect the important economic crop of Chinese medicinal material *P. notoginseng* in Yunnan Province, our laboratory (Key Laboratory of Agricultural Biodiversity and Pest Control of the Ministry of Education) has examined the biological characteristics of *M. acerina* and a lot of research has been done on the trait of spread in the field, and it is found that *M. acerina* is a kind of low

temperature-loving fungus. When the temperature exceeds 32℃, its conidia will lyse, and the optimum growth temperature is 14-22℃, same as many hosts, such as cyclamen, pansy, lettuce and sanqi. The latest measured length of *M. acerina* is (137.36~486.24μm) × (4.35~16.46μm) (n=100), and a single conidia can cause infection (Supplementary Figure 1 and 2). *M. acerina* cause initial infection through chlamydospores stored in the soil, and spread in the field through conidia on the leaf surface of infected leaves, causing re-infection. Conidia are mainly spread by rain splash. *P. notoginseng* has a serious problem of continuous cropping, it will take at least 10 years to replant. Therefore, it was only planted in Wenshan in the 1990s, and it has now spread to Kunming, Honghe, Lijiang and Jianshui. No matter where the *P. notoginseng* plants, *P. notoginseng* round spot disease will follow. It is not known whether it is because the *M. acerina* originally existed in the local area or the pathogen spreads with various media. The results of this study indicate that the genetic distances between the *M. acerina* populations in different regions are relatively close, and the similarity is high, which may indicate that there are frequent exchange activities between *M. acerina* in different regions, such as seedlings. Cross-regional transportation and other media dissemination. In addition, the prevention and treatment of *P. notoginseng* round spot is mainly concentrated in the rainy season (June to September). Using the technology of facility cultivation to build a rain-proof film in the rainy season can prevent *P. notoginseng* round spot and reduce the use of chemical pesticides(Supplementary Figure 3). The reduction in the amount of chemical pesticides can reduce the survival pressure of *M. acerina*, which can also affect the genetic relationship between populations in different regions.

Features of SSR loci in *M. acerina*

A total of 8250 repeats were obtained from the screened SSR sequences, which indicated that the number of SSRs was high in the *M. acerina* genome compared to in some eukaryotes (Luo *et al.,* 2015; Sharopova *et al.,* 2002). The analysis of microsatellite sequences in *M. acerina* could enhance our understanding of its genome structure, especially the composition of non-coding regions, and the mechanisms of pathogenicity and its heredity in *M. acerina* at the genome level (Kirungu *et al.,* 2018). Among all SSR types, A and T are abundant, which is consistent with the SSR loci results in most eukaryotic genomes, probably due to the transformation of methylated C residues into T residues (Toth, 2000). According to Velascor (2007), the presence of a large number of short repeat sequences indicates that a species has a high mutation frequency, while species with high proportions of long repeat motifs generally have relatively short evolutionary times or low mutation frequencies (Velasco *et al.,* 2007). A large number of short repeats of single, dibasic, and tribasic bases were observed in the genome of *M. acerina*, suggesting that *M. acerina* had a relatively high mutation frequency or a relatively short evolutionary time (Sia *et al.,* 1997).

With advancements in genome sequencing technologies, molecular marker studies have become more cost-effective (Mccouch *et al.,* 2002). Based on genomic data, we obtained 8250 SSRs, which accounted for 0.55% of the whole genome sequences. In the *Fusarium graminearum* genome, SSR sequences obtained accounted for 0.27% of the whole-genome sequences (Cho *et al.,* 2000) and 0.21% in the *Sphacelotheca reilianm* genome (Lu and Brewbaker, 1999). In this study, more than 100 pairs of primers were designed, out of which merely 32 pairs were polymorphic, probably because most of the selected primers existed in the coding regions of the genome, with only a few located in the non-coding regions. Studies have demonstrated that SSRs in coding regions often exhibit low polymorphism, and SSR markers should be designed as much as possible within non-coding regions, because coding regions have much greater selection pressure than non-coding regions and are relatively conserved in the course of species evolution, while non-coding regions are more likely to evolve or mutate (Liu, Li and Qin, 2020; Wei, *et al.,* 2011).

Genetic diversity of *M. acerina* in Yunnan

In the current study, the PIC of polymorphic loci ranged from 0.53 to 0.8, which was high when compared to the PIC in other eukaryotes. For example, PIC ranged from 0.3 to 0.4 in *Dactylis glomerata* L. (Xie *et al.,* 2009), 0 to 0.756 in *Magnaporthe oryzae* (Zhou *et al.,* 2018), and 0.305 to 0.726 in *Panonychus citri* (Sun *et al.,* 2014). According to the results based on primer polymorphism, 14 SSR loci were used to analyze the genetic diversity of *M. acerina* populations. the PIC of screened primers was higher; however, after population analysis, the genrtic diversity of *M. acerina* does not reflect geographic specificity. The

potential reason is that the SSR primer loci are within the coding regions of the genome, which have high degrees of conservation (Sun *et al.,* 2015). Judicious selection of primers could improve the accuracy of results. The genetic diversities of *Pyricularia oryzae* Cav. and *Puccina striiformis* f. sp. tritici in Yunnan Province have been reported to be high (Liu, *et al.,* 2016; Iwano, Lee J.Y and Lee C.Y, 1990). Therefore, the genetic diversity obtained for *M. acerina* in the study could be due to the single genetic background of *M. acerina* as a quarantine pest in China (Wu *et al.,* 2015) or its stable survival in areas with highly homogenous ecological environments for prolonged periods.

The automatic nucleic acid protein analyzer used in study is a novel instrument that can be applied in population genetics analyses. Compared with polyacrylamide gel electrophoresis, its operation is relatively simple and time saving, and it can simulate the electrophoretogram and directly read bands, which facilitates analysis procedures. In addition, it can be used directly for DNA and protein sample analysis (Mao, *et al.,* 2020; Li, *et al.,* 2014).

Some of the primary factors influencing the evolution of the population genetic structures of pathogenic fungi include population size, reproductive mode, and genetic drift (Ferrucho *et al.,* 2013; Cumagun, 2007). In this study, 189 *M. acerina* strains were selected, and the population number was moderate. The mode of reproduction of *M. acerina* in the field is asexual reproduction (Evenhuis, Verdam and Zadok, 2010), which, to a certain extent, is not conducive to its genetic variation and the evolution of its populations. Pathogenic fungi are small individuals and easily experience genetic drift by natural or artificial means, and gene drift is generally considered to hinder the evolution of organisms (Mcdermott and Mcdonald, 1993).

In the current study, there were no significant correlations in genetic diversity among strains from different geographical sources. Continuous selection and mutation of pathogenic genes will lead to homozygous individual genes, thus reducing the genetic diversity level of *M. acerina* population. These factors can partly explain that the isolates from the same region cannot be totally clustered into a group, and some isolates from different regions have very high genetic similarity coefficient. Another possible explanation is that the host *P. notoginseng* is merely grown in Yunnan and Guangxi, China, and Wenshan in Yunnan is the place of origin. The rest of the sampling points in the paper have been gradually planting *P. notoginseng* in the past 5-6 years. Current research show that due to the low genetic diversity of maple populations, we can effectively prevent the occurrence of diseases in this area through timely cleaning of diseased leaves, rain-proof cultivation, and alternate use of chemicals (Wang et al., 2019).

Conclusion

In this study, we developed 14 SSR primers of *M.acerina* can be used in diversity analysis and identification of *M. acerina* and its closely related species. Genetic diversity of *M. acerina* in Yunnan province does not reflect geographic specificity.

Abbreviations

M.acerina*: Mycocentrospora acerina*

P.notoginseng*: Panax notoginseng* (Burk.) F. H. Chen

SSR: Simple sequence repeat

SCAR: Sequence characterized amplification regions

AFLP : amplification fragment length polymorphism

RFLP : Restriction Fragment length polymorphism

ISSR: inter-simple sequence repeat

RAPD: random amplification polymorphism

SNP: Single Nucleotide Polymorphism

ITS: Inter transcribed spacer

PCR: Polymerase chain reaction

MP: Primer of *M. acerina*

Declarations

Ethics approval and consent to participate

Not applible

Consent for publication

All the authors agreed to publish the paper in Phytopathology research.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

Wang huiling, Yang kuan, Zhu youyong and He xiahong contributed to the conception of the study;

Wang huiling, Luo lifen, He chi and Li xiang performed the experiment;

Wang huiling and Yang kuan performed the data analyses and wrote the manuscript;

Wang huiling, Guo liwei, Huang huichuan, Liu yixiang, Yang min and Zhu shusheng helped perform the analysis with constructive discussions.

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References

1. Adjebeng-Danquah, J., Manu-Aduening, J., Asante, I. K., Agyare, R.Y. and Offei, S.K. (2020) Genetic diversity and population structure analysis of ghanaian and exotic cassava accessions using simple sequence repeat (SSR) markers. *Heliyon*, http: //doi. org/ 10.1016/j.heliyon.2019.e03154
2. Barus, H., Bayu, E. and Hanafiah, D. (2020) Identification genetic of soybean mutant (*Glycine* max L. Merril) based on fatty acid characters using simple sequence repeat (SSR) markers. *The 3rd International Conference Community Research and Service Engagements*, http://doi. org/10.4108/eai. 4-12-2019.2293861
3. Bridge, P.D., Singh, T., Arora, D.K. (2003) The application of molecular markers in the epidemiology of plant pathogenic fungi. *Mycology*, 10.1201/9780203913369.ch5
4. Catherine J.W., Lewis B.G. (1980) Infection of carrot plants by *Mycocentrospora acerina*. Transactions of the British Mycological Society, 74(3), 587-593.
5. Chen, K., Chen, S.X., Yu, Z.W. (1997) A new disease on sanqi. Plant Protection, 1, 49.
6. Chen, X.Y., Sui, C., Gan, B.C., Wei, J.H. and Zhou, Y.K (2010) First report of *corynespora* leaf spot on patchouli caused by *Corynespora cassiicola* in China. *Plant Disease,* 94(12), 1508.3.
7. Chen, Y.J., Wang, Y., Feng, G.Q. and Liu, Y.Z. (2005) Biological characteristics of *Alternaria panax* whetz isolated from *Panax notoginseng.Acta Phytopathologica Sinica*, 3, 267–269.
8. Cho, Y.G., Ishii, T., Temnykh, S., Chen, X., Lipovich, X.L., Mccouch, S.R., Park, W.D., Ayres, N. and Cartinhour, S. (2000) Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativaL.*). *Theoretical & Applied Genetics,* 100, 713–722.
9. Colling, G. and Matthies, D. (2010) The effects of plant population size on the interactions between the endangered plant *Scorzonera humilis*, a specialised herbivore, and a phytopathogenic fungus. *Oikos*, 105(1), 71–78.
10. Cumagun, C. J. R. (2007) Population genetic analysis of plant pathogenic fungi with emphasis on *Fusarium* species. *Philippine Agricultural entist*, 90(3), 244–256.
11. Dai, L., Xu, Y.L., Long, Y.J., Du, L.S., Du, Y.L. and He. X.H. (2017) The growth characteristic of mycelia and conidia for *Mycocentrospora acerina*. *Journal of Yunnan Agricultural University* (Natural Science), 32(1), 27–35.
12. Duan, L., Xiong, X.J., Hu, J.Y., Liu, Y.M., Li, J. and Wang, J. (2017) *Panax notoginseng* saponins for treating coronary artery disease: a functional and mechanistic overview. *Frontiers in Pharmacology,* 8, 702.
13. Davies, W.P., Lewis, B.G. and Day, J.R. (1977) Infection of stored carrot roots by *Mycocentrospora acerina* (hartig) deighton. *Transactions of the British Mycological Society*, 77(1), 139–151.
14. Ei-Komy, Saleh and Molan, A. (2012) Molecular characterization of early blight disease resistant and susceptible potato cultivars using RAPD and SSR markers. *African Journal of Biotechnoogy,* http://doi. org/10.5897/AJB11.2709
15. Ellis, M.B. (1974) Dematiaceous hyphomycetes in Britain. *Transactions of the British Mycological Society,* http://doi. org/10.1016/S0007-1536(74)80031-8
16. Evenhuis, A., Verdam, B. and Zadoks, J.C. (2010) Splash dispersal of conidia of *Mycocentrospora acerina* in the field. *Plant Pathology*,46(4), 459–469.
17. Fernández-Ortuño, D., Pérez-García, A., López-Ruiz, F., Romero, D., Vicente, A.D., and Torés, J.A. (2006) Occurrence and distribution of resistance to Qoi fungicides in populations of *podosphaera fusca* in south central Spain. *European Journal of Plant Pathology*, 115(2), 215–222.
18. Ferrucho, R.L., Ceresini, P.C., Ramírez-Escobar, U.M., Mcdonald, B.A. and García-Domínguez, C. (2013) The population genetic structure of rhizoctonia solani AG-3PT from potato in the Colombian andes. *Phytopathology*,103(8), 862–869.
19. Garfinkel, A.R. and Chastagner, G.A. (2019) Strategies to address emerging fungal diseases in peony (*Paeonia lactiflora*) in the united states. *Acta horticulturae,* 199–206.
20. Gaikwad, A.B., Behera, T.K. Singh, A.K. Chandel, D., Karihaloo J.L. and Staub, J.E. (2008) Amplified fragment length polymorphism analysis provides strategies for improvement of bitter gourd (*Momordica charantia* L.). *Acta Horticulturae,* 871, 71–78.

21. Hermansen A.(2010) Weeds as hosts of *Mycocentrospora acerina*. *Annals of Applied Biology*, 121(3):679-686.
22. Hou, L.X., Wei, A.Z., Wang, L.H. and Liu, Y.L. (2018) Analysis of SSR loci and development of molecular markers in *Zanthoxylum bungeanum* transcriptome. *Journal of Agricultural Biotechnology,* 26(7), 1226–1236.
23. Iwano, M., Lee, J.L. and Lee, C.Y. (1990) Distribution of pathogenic races and changes in virulence of rice blast fungus, *pyricularia oryzae* cav. in Yunnan province, china. *Jarq* 23(3), 241–248.
24. Jinqing, X.U., Tengfei, X., Lei, W., Handong, W., Huaigang, Z., Dengcai, L. *et al.* (2017). Characterization and gene function analysis of SSR sequences in *Hulless barley* transcriptome. *Journal of Triticeae Crops,* 37(2), 175–184.
25. Jiang, N., Qin, L.Y. and Ye, Y.F. (2011) Research advances in diseases of *Panax notoginseng*. *Journal of Southern Agriculture,* 2, 149–153.
26. Jordan, V.W.L. (2010) Estimation of the distribution of *Verticillium* populations in infected strawberry plants and soil. *Plant Pathology*, 20(1), 21-24.
27. Joy, K., Yanfeng, D., Xiaoyan, C., Richard, M., Zhongli, Z., Xingxing, W. *et al.* (2018) Simple sequence repeat (SSR) genetic linkage map of d genome diploid cotton derived from an interspecific cross between *Gossypium davidsonii* and *Gossypium klotzschianum*. *International Journal of Molecular ences,* 19(1), 204.
28. Kaye, C., Milazzo, J., Rozenfeld, S., Lebrun, M.H. and Tharreau, D. (2003) The development of simple sequence repeat markers for *Magnaporthe grisea* and their integration into an established genetic linkage map. *Fungal Genetics and Biology*, 40(3), 207–214.
29. Liu, C., Li, J. and Qin, G. (2020) Genome-wide distribution of simple sequence repeats in pomegranate and their application to the analysis of genetic diversity. *Tree Genetics & Genomes*, https://doi.org/10.1007/s11295-020-1428-4
30. Li, Y., Long, S. and Li, X. (2018). Occurrence of root rot of *Panax notoginseng* caused by *Fusarium oxysporum* in China. International *Journal of Agriculture and Biology*, 20(10), 2175–2180.
31. Li, Y.F., Zhang, X.L., Liao, X.G. and Zhang, D. (2014) Evaluation study of multiplex PCR assay for detection diarrheagenic *Escherichia* coli. *Henan Journal of Preventive Medicine*, https://doi.org/10.13515/j.cnki.hnjpm.2014.02.040
32. Liu, X.F., Yuan, W.Y., Liang, D., Shi, X.W. and Ma, Z.H. (2016). Population genetic structures of *Puccinia striiformis* f. sp.tritici in Yunnan and Guizhou province. *Journal of Yunnan Agricultural University* (Natural Science), 49(6), 95–100.
33. Liu R. and YANG J.S. (2010) Genetic diversity of some *Dendranthema* spp. based on RAPD analysis. *Journal of Agricultural University of Heb*, 33(1), 60-65+83.
34. Long, Y.J., Mao, Z.S., Zhu, S.S., Chen, Z.j., Wei, F.G. and Yin, Z.B. *et al.* (2015). The pathogen of *Panax notoginseng* root rust spot.*Mycosystema*, 34(2), 177–185.
35. Louarn S, Nawrocki A, Edelenbos M, Dan F.J., Jensen O.N., Collinge D.B. (2012) The influence of the fungal pathogen Mycocentrospora acerina on the proteome and polyacetylenes and 6-methoxymellein in organic and conventionally cultivated carrots (Daucus carota) during post harvest storage. Journal of proteomics, 75(3), 962-77.
36. Lu, N., Chen, Y.J., Lu, H.J. and Liu, Y.L. (2005). Biological characterization of round spot pathogen on *Panax notoginseng*. *Journal of Yunnan Agricultural University,* 2, 193–195.
37. Lu, X.W. and Brewbaker, J.L. (1999) Molecular mapping of QTLs conferring resistance to *Sphacelotheca reiliana* (kühn) clint. *Maize Genetics Cooperation Newsletter*, 73, 36.
38. Luo, H.Y., Wang, X.J., Zhan, G.M., Wei, G.R., Zhou, X.L., Zhao, J. *et al.* (2015) Genome-wide analysis of simple sequence repeats and efficient development of polymorphic SSR markers based on whole genome re-sequencing of multiple isolates of the wheat stripe rust fungus. *Plos One*, http://doi. org/10.1371/journal.pone.0130362
39. Ma, Y.N., Chen, C.J., Li, Q.Q., Xu, F.R., Cheng, Y.X. and Dong, X. (2019). Monitoring antifungal agents of *Artemisia annua* against *Fusarium oxysporum* and *Fusarium solani*, associated with *Panax notoginseng* root-rot disease. *Molecules*, http://doi. org/10.3390/molecules24010213
40. Mao, Y.X., Zhang, X.L., Hu, Z.J. and Zhao, Y. (2020) Genetic diversity of *Trachycarpus fortunei* (Hook.) H. Wendl germplasm resources in Guizhou by SRAP molecular markers analysis. *Journal of Southern Agriculture,* 51(1), 27–35.

41. Mccouch, S.R., Teytelman, L., Xu, Y., Katarzyna, B.L., Karen, C. and Mark, W. *et al. (2002)* Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.) (supplement). *DNA Res*. 9(6), 257–279.
42. Mcdermott, J.M. and Mcdonald, B.A. (1993) Gene flow in plant pathosystems. *Annual Review of Phytopathology*, 31(1), 353–373.
43. Nina, S., Karen, G., Timothy, K.M., Gavin, S., Lucy, T. and Stanley, F. (2000) A whole-genome microarray reveals genetic diversity among *Helicobacter pylori* strains. Proceedings of the National Academy of Sciences of the United States of America, **97**(26), 14668–14673.
44. Raju, K.S., Sheshumadhav, M. and Murthy, T. G. K. (2008) Molecular diversity in the genus *Nicotiana* as revealed by randomly amplified polymorphic DNA. *Physiology & Molecular Biology of Plants*, 14(4), 377–382.
45. Richard, S. and Leonard, N. (2000) Microsatellites in different eukaryotic genomes : survey and analysis. *Genome Research,* 10(7), 967–981.
46. Saad, Y.M. (2009) Analysis of genetic variations in two *Sarotherodon galilaeus* sexes using popgene. *Global Veterinaria*, 3(1), 22–25.
47. Salama N., Guillemin K., Mcdaniel T.K., Sherlock G., Tompkins L. and Falkow S. (2000) A whole-genome microarray reveals genetic diversity among *Helicobacter pylori* strains. *Proc Natl Acad Sci USA*, 97(26):14668-14673.
48. Sharopova, N., McMullen, M.D., Schultz, L. Steve, S., Hector, S.V. and Jack, G. *et al.* (2002) Development and mapping of SSR markers for maize. *Plant Mol Biol*, 48, 463–481.
49. Sia, E.A., Kokoska, R.J., Dominska, M., Greenwell, P. and Petes, T.D. (1997) Microsatellite instability in yeast: dependence on repeat unit size and DNA mismatch repair genes. *Molecullar and Cellular Biology*. 17(5), 2851–2858.
50. Sun, T., Kong, L.W., Wang, M.M., Jin, P.Y., and Hong, X.Y. (2014) Development and characterization of novel EST-microsatellites for the citrus red mite, *Panonychus citri* (Acari: Tetranychidae), *Systematic and Applied Acarology*, 14(9), 499.
51. Sun, T.T., Wang, D.W., Chen, L., Wang, W.F., Gong, D.P. and Chen, Y.Q. *et al.* (2015). Characteristics and distribution of SSR loci in the coding region of potato genome and functional analysis of their encoding proteins. *Molecular Plant Breeding,* 13(01), 171–177.
52. Tang, B.F., Li, X., Pu, L.M., Zhao, Q., Cui, X.M. and Ge, F. *et al.* (2019) A pathogenesis-related protein 10 gene pnpr10–3 was involved in molecular interaction between *Panax notoginseng* and *Fusarium solani*. *Australasian Plant Pathology,* 48, 447–456.
53. Tiziana, S., Pier, L.C., Mauro, P. and Alessandra, B. (2008) Flower buds and seeds essential oils of *Panax notoginseng* (burk.) f.h. chen (araliaceae). *Journal of Essential Oil Research*. 20(1), 63–65.
54. Velasco, R., Zharkikh, A., Troggio, M., Cartwright, D.A., Cestaro, A., Pruss, D. *et al.* (2007) A high quality draft consensus sequence of the genome of a *Heterozygous grapevine* variety, http://doi. org/10.1371/journal.pone.0001326
55. Wall, C.J. and Lewis, B.G. (1980) Infection of carrot plants by *Mycocentrospora acerina*. *Transactions of the British Mycological Society*, 74(3), 587–593.
56. Wang, C.W., Fu, J.F., Wang, W.K. (1992) Brief report on the study of a new disease- the leaf withering disease of Asarum. Journal of Shenyang Agricultural University, 23(4), 355-356.
57. Wang, H.L., Wang, F., Jin, B.H., Zhang, H., Yang, K., Wang, W.P., Yang, M., Zhu, S.S., He, X.H. (2019) Sensitivity and fitness analysis of round spot of *Panax notoginsen Mycocentrospora acerina* to azoxystrobin, prochloraz and difenoconazole*g. Chinese Journal of Pesticide Science,* 21(3), 273-278.
58. Wang, X., Chen, L. and Zhao C.L. (2016) Mining SSR Molecular Marker Sites with MISA Tool for Different Types of Sequences. *Chinese Agricultural Science Bulletin*, 32(10), 150-156.
59. Wang, W.Y., Zhao, C.L., Chen, Z.J., Wen, G.S., Wei, F.G., Long, T.J. *et al.* (2015). Studies on the isolation, identification and in vitro growth rates of the three pathogenic fungi from *Panax notoginseng* cultivated in Wenshan eparchy. *Agricultural Science Technology*, 16(6), 1165.

60. Wang, Z., Zheng, Q.M., Cheng, W.T., Mao, Y.Y., Cai, Y.Q. and Ma, Y.H. (2018) Information analysis and molecular marker development of transcriptome SSR in *Pitaya*. *Guizhou Agricultural sciences,* 46(07), 1–5.
61. Wang, Z.J., Wu, W., Hu, B., Zhang, H.L., Bai, X. and Zhao, J.J. *et al.* (2014). Molecular epidemiology of Aleutian mink disease virus in China. *Virus Research*. 184, 14–19.
62. Wang, Z.H., Shan, W.X. (1998) An underdstanding of the population genetics of plant pathogenic fungi: its scope and role. *Acta Phytopathologica Sinica*, 28(1):5-9.
63. Wei, H.L. and, Edo, K. (2011) Evolutionary pressures on simple sequence repeats in prokaryotic coding regions. *Nucleic Acids Research*, 6, 6.
64. White, C.M., Fan, M.C., Song, J., Tsikouris, J.P. and Chow, M. (2001) An evaluation of the hemostatic effects of hydrophilic, alcohol, and lipophilic extracts of *notoginseng*. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, 21, 773–777.
65. Wu, H., Liu, B.L., Wang, W.H., Shi, Y.M., Wei, S.F. and Zhou, H. (2015) Genetic diversity analysis of male sterile lines for three-line hybrid rice mainly applied in Guangxi. *Journal of Southern Agriculture,* 46(04), 550–554.
66. Xia, P.G., Zhang, S.C., Liang, Z.S., and Qi, Z.H. (2014) Research history and overview of chemical constituents of *Panax notoginseng*. *Chinese Traditional and Herbal Drugs*, 45(17), 2564–2570.
67. Xie, W.G., Zhang, X.Q., Ma, X., Peng, Y. and Huang, L.K. (2009) Genetic variation and relationship in orchard grass (*Dactylis glomerata* l.) germplasm detected by ssr markers. *Hereditas (Bjing),* 31(6), 654–662.
68. Yu, D.B. (1979) Population genetics of plant pathogens. *Plant Protection*, 1, 1-7.
69. Zeng, X.Q., Wang, Y.L., Xu, Q.J., Yuan, H.J., Zaxi, L. and Ni, M. (2013) Assessment of genetic diversity in tibetan hulless barley germplasm(*Hordeum vulgare* L. var. nudum hk.f.) by ssr primers. *Journal of Triticeae Crops,* 33(02), 260–267.
70. Zhou, H., Ren, Z.H., Liu, P.K. Zhang, Z.Y., Wang, H.H. and Liu, M.R. (2018) Analysis of genetic diversity of Magnaporthe oryzae in rice blast nursery in Taojiang of Hunan by SSR markers, *Southwest China Journal of Agricultural Sciences*, 31(04), 725-730.
71. Zhou, X.J., Wang, H.L., Li, F.L., Zhang, K., Wang, Y.N. and Ya, H.Y. *et al.* (2019) Development of polymorphic ssr markers in *Rhododendron henanense* subsp. lingbaoense based on rad-seq. *Journal of Agricultural Biotechnology,* 27(01), 55–62.
72. Zhou, Y.Y., Wang, L.Q., Fu, Y.L., Yang, L.Q. (2017) A review of research on biological genetic resources and traditional knowledge. *Journal of Northeast Agricultural University*(Social Science Edition), 16(01), 60-70.
73. Zietkiewicz, E., Rafalski, A., and Labuda, D. (1994) Genome fingerprinting by simple sequence repeat (ssr)-anchored polymerase chain reaction amplification. *Genomics*, 20(2), 176–183.

Tables

TABLE 1 *Mycocentrospora acerina* populations examined in the simple sequence repeat analysis.

Location	ID	Population	Isolates	Latitude (N)	Longitude (E)
Honghe (HH)	SC	Shaochong, Honghe	4	23° 54' 30"	102° 27' 53"
	LP	Longpeng, Honghe	4	23° 58' 12"	102° 34' 13"
	JS	Jianshui, Honghe	15	23° 24' 22"	102° 49' 58"
	LX	Luxi, Honghe	15	24° 41' 14"	103° 51' 10"
	MZ	Mengzi, Honghe	3	23° 37'	103° 40 '
Puer (PE)	L	Lancang, Puer	15	22° 40' 29"	99° 50' 49"
Wenshan (WS)	YS	Yanshan, Wenshan	2	23° 23' 4"	104° 16' 24"
	WS	Wenshan, Wenshan	3	23° 14' 30"	104° 5' 0"
	DM	Dumeng, Wenshan	6	23° 15' 42"	104° 8' 42"
	QL	Qiubei, Wenshan	7	23° 56' 25"	103° 48' 21"
	QX	Qiubei, Wenshan	16	23° 50' 2"	104° 6' 10"
	QS	Qiubei, Wenshan	6	23° 50' 23"	104° 6' 34"
Qujing (QJ)	SZ	Shizong, Qujing	16	24° 75' 44"	103° 91' 78"
	LS	Luoping, Qujing	16	24° 47' 47"	104° 17' 36"
Kunming (KM)	SL	Shilin, Kunming	12	24° 48' 31"	103° 38' 16"
	XD	Xundian, Kunming	20	25° 44' 48"	103 ° 21'
Lijiang (LJ)	LJ	Lijiang	7	26°86'	100°25'

TABLE 2 Primers of the whole genome of *Mycocentrospora acerina*

Locus	Repeat Motif	Primer sequence	Size (bp)	Expected Heterozygosity (He)	Polymorphic Information Content (PIC)	Annealing Temperature (Tm)
MP40	$(CTA)_{27}$	CACATGCTCAGTCATTTGTGG	243	0.681	0.599	56
		GGTGCAATCGGAAAGAATTG				
MP42	$(TC)_{19}$	AAGCGCACTTGCCTATTGAT	225	0.609	0.53	58.4
		GGTGAGTGTTGCTGACGAAA				
MP2	$(CAT)_{14}$	CGTCCATCTTCCTCTTCACC	200	0.71	0.622	56.4
		GCTCATGTTCGATGGATGTG				
MP49	$(GA)_{25}$	GGAAGGAAATCCAGGTGTGA	200	0.594	0.505	56
		CCCACTTCCTGTTTGCTTGT				
MP54	$(CAA)_{19}$	GTTGTTGCCAGCAAGAGT02GA	199	0.623	0.552	58.4
		AACAACCCTGGCACTACTCG				
MP55	$(TTGA)_{19}$	TTCCTCTCCCTCTCCCTCTC	214	0.623	0.552	59
		ATGCTGCAAGTCTGTTGACG				
MP56	$(TAG)_{25}$	TGTGTGTGTGTTGTTGTTGTTG	228	0.87	0.812	59.5
		TGACAAGCAAGTAGATTTTTACGTTT				
MP4	$(GACA)_{6}$	AGGGTAGCTCAAAGCCACTG	273	0.725	0.665	58.4
		CTTTCCAAGCTGAGGGTGAG				
MP58	$(GA)_{25}$	TCGTTTTTGGAGCGTTCTTT	209	0.739	0.659	54.4
		TGGACGCACTCCTTCTTTTC				
MP52	$(CCA)_{11}$	GCTTCGGTGTCTGGAATCAT	179	0.609	0.53	56.4
		AAACTTCAATGTCGCCAAGG				
MP51	$(TTG)_{19}$	CGTCTCTGTTATTGCTGCTTT	159	0.826	0.76	55.7
		CGCACAACCAATGAGAAACA				
MP 13	$(AGT)_{12}$	CACGTCACGGAGCAAGTAGA	211	0.696	0.622	57.4
		TGATGAGGTCCAACGGAGAT				
MP3	$(GT)_{13}$	CATGTGCATTGCTGTGTTGT	170	0.725	0.644	61.4
		CAGCGAGTGAATGGAAGTGA				
MP50	$(CTGT)_{19}$	GCTTTACTTTGCCCGTCTGT	200	0.768	0.701	55.4
		TGCATCTCCTCACATCCATC				
MP65	$(CA)_{22}$	ACCTCCACACCTGCACCTAC	245	0.609	0.53	59.5
		GCGGGCTTGTAGTCGTAGAG				
MP68	$(CAT)_{7}$	GGATATGCCTCACCATTTGC	166	0.797	0.739	55.4
		ATATGGAAGGCCGCAGTGTA				

Locus	Repeat Motif	Primer sequence	Size (bp)	Expected Heterozygosity (He)	Polymorphic Information Content (PIC)	Annealing Temperature (Tm)
MP83	$(CTT)_{12}$	TGAGCAGGGGCCAAATACTA	156	0.779	0.702	54.4
	$(ATGA)_{10}$	TTAAATTCCCATCCCCATCC				
MP36	$(CAT)_{17}$	ATCTGTCACCACCATCACCA	193	0.87	0.812	59
		AGCTCGCGATCTAAACATCC				
MP39	$(AGTG)_{24}$	ATGTGTGTGTGTGCCTGGAT	247	0.594	0.505	60
		TATATGCCCATTCCCATTCC				
MP46	$(CACT)_{10}$	TTCCTCTGACGCATCCTCTT	207	0.638	0.535	60
		TGGGCATGTAATGAGTGGTG				
MP47	(CAGG)7	GATTGTAAGCCGCAGAAGGT	247	0.754	0.68	60
		TCACGACTCCATCACTCCAA				
MP20	$(TACA)_{11}$	TGTGTCGCTCACTCACTCAA	239	0.754	0.671	59
		GGAAGGAGTGGAGTTGATGG				
MP90	$(TC)_{18}$	TCAAAACCGAAACCCAGAAA	191	0.551	0.503	55.4
		GGGAGAAGAAGGGCAGAGG				
MP108	$(TCG)_{10}$	TCACTACCCCTACCCCCTTT	237	0.681	0.599	57.4
	$(TCA)_5$	CGGTCGGCATAGGGTATTTA				
MP92	$(CTA)_{31}$	ACCCCAACACTCAATCATCC	219	0.71	0.643	54.7
		TCTGGCAAGAAGAAGAAATGC				
MP62	$(CTA)_{20}$	CAGAAAATCCTAGCTACTGCTGCT	174	0.725	0.644	56.5
		TGCAGTCTCTTCACCCTGTTT				
MP84	$(AGA)_{18}$	TTCAATCGTGCAAGGTGTGT	167	0.739	0.686	58
		GAGAGGAGCAGGGCATGTAG				
MP115	$(AC)_7(TC)_{12}$	TCTGCTGCCATGTAGTGCTC	246	0.768	0.692	55.4
		ATGTGATTTTGGGGGAAACA				
MP63	$(TC)_{21}$	CAGACTTCCCAGTCACCACA	195	0.797	0.726	55.5
		TTGGCTACTACTGCACCAAAAA				
MP113	$(TG)_7(AG)_{10}$	CATCTCTCATCTCCCCAGGA	225	0.812	0.746	57.4
		AATCCCATCACACGCTTCTC				
MP114	$(CTC)_9$	GATGTGCAGAGTTTCGGTCA	232	0.913	0.862	55.4
	$(TTC)_8$	GGAAGCTGATTCATCCCAGT				
MP61	$(CA)_{36}$	TGGTGGCTAGTTGGTTGGAT	212	0.928	0.878	56.4

Locus	Repeat Motif	Primer sequence	Size (bp)	Expected Heterozygosity (He)	Polymorphic Information Content (PIC)	Annealing Temperature (Tm)
		GGTCGTCACTGTTGCTTGAA				

TABLE 3 Genetic diversity of geographic populations of *Mycocentrospora acerina*

Population	Na	Ne	Nei's genetic diversity	Shannon's information index
Qujing	1.6892	1.1108	0.0888	0.1658
Honghe	1.8041	1.1089	0.0896	0.1712
Kunming	1.7297	1.1103	0.0891	0.1674
Puer	1.6892	1.1099	0.0893	0.1676
Lijiang	1.4122	1.1187	0.0842	0.143
Wenshan	1.777	1.1099	0.0893	0.1693

TABLE 4 Nei' genetic identity (above diagonal) and genetic distance (below diagonal) of geographic populations of *Mycocentrospora acerina*

popID	Honghe	Lijiang	Puer	Kunming	Wenshan	Qujing
Honghe	****	0.9938	0.9976	0.9988	0.9982	0.9977
Lijiang	0.0062	****	0.9931	0.9937	0.9959	0.9946
Puer	0.0024	0.007	****	0.9975	0.998	0.9978
Kunming	0.0012	0.0064	0.0025	****	0.9979	0.9975
Wenshan	0.0018	0.0041	0.002	0.0021	****	0.9983
Qujing	0.0024	0.0054	0.0022	0.0025	0.0017	****

Figures

Figure 1

The geographical locations of six *Mycocentrospora acerina* populations in Yunnan(green region).

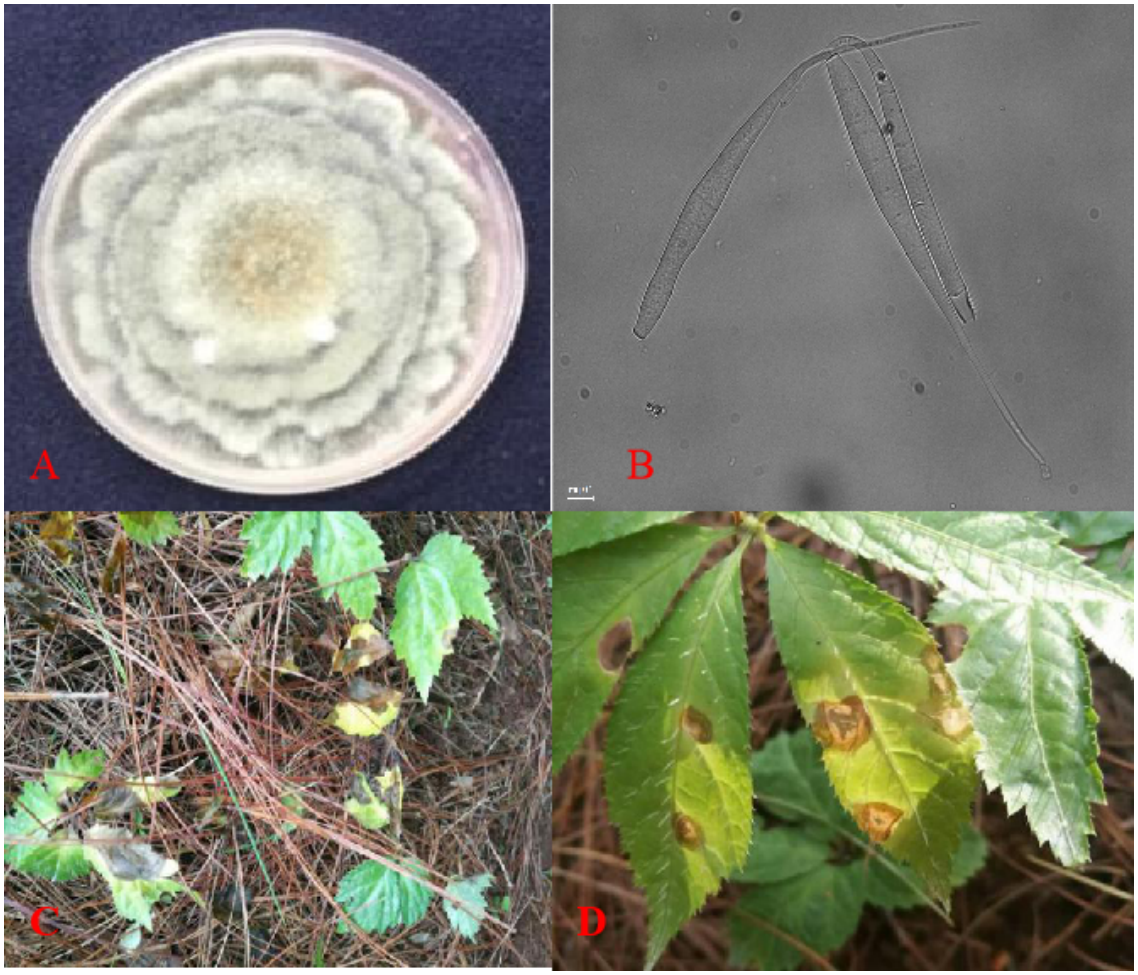

Figure 2

Mycocentrospora acerina colonies (A), conidia (B), and symptoms (C, D) in the field.

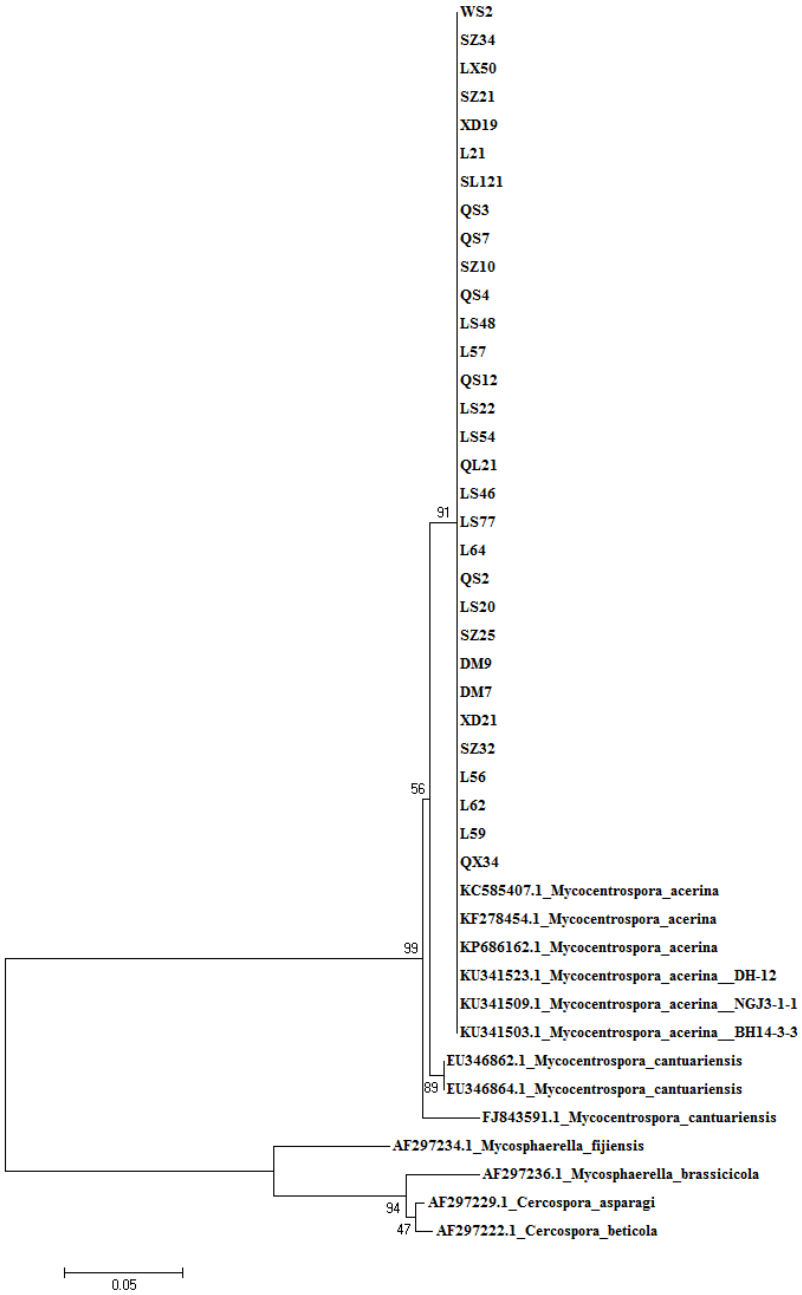

Figure 3

Phylogenetic tree constructed based on internal transcribed spacer1 (ITS1)sequences.

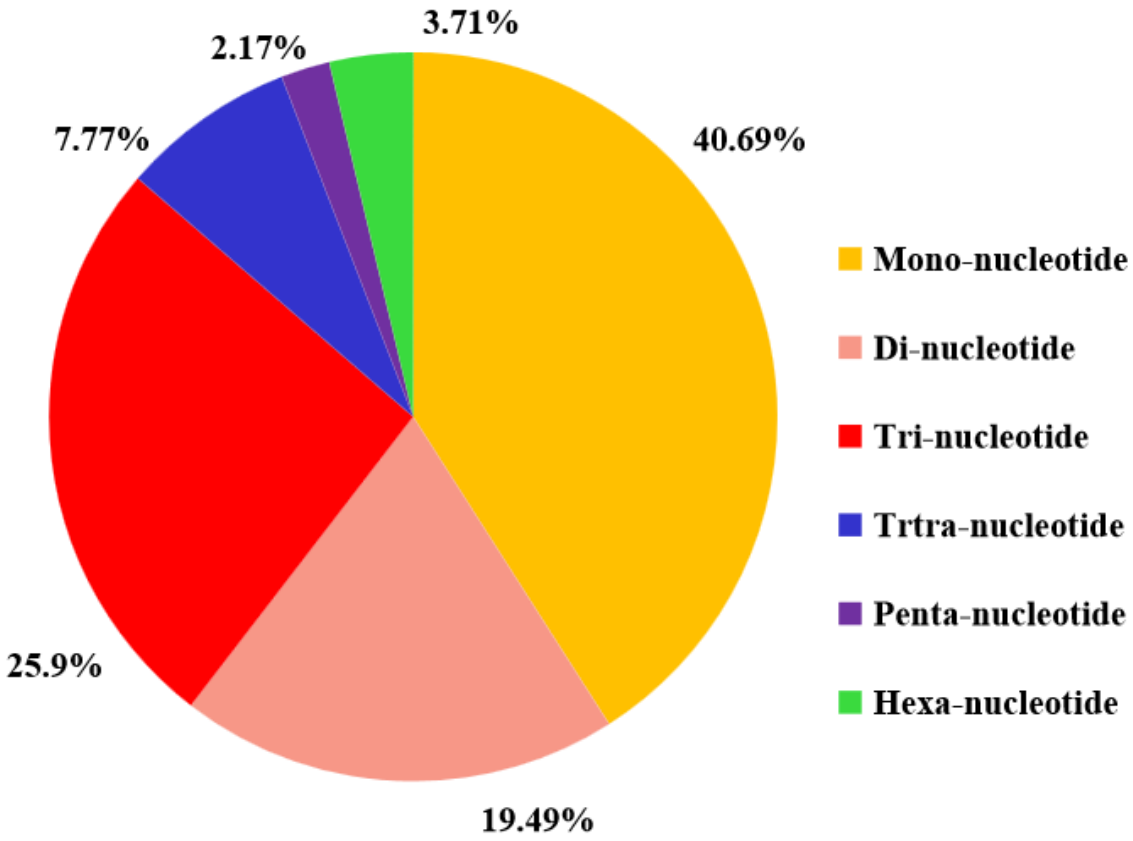

Figure 4

Summary of simple sequence repeats (SSRs) identified in the *Mycocentrospora acerina* genome.

Figure 5

Distributions and frequencies of simple sequence repeats (SSRs) in *Mycocentrospora acerina*.mono-nucleotide repeats, DNR: di-nucleotide repeats; TNR: tri-nucleotide repeats, Te_NR: tetra-nucleotide repeats, PNR: penta-nucleotide repeats, HNR: hexa-nucleotide repeats.

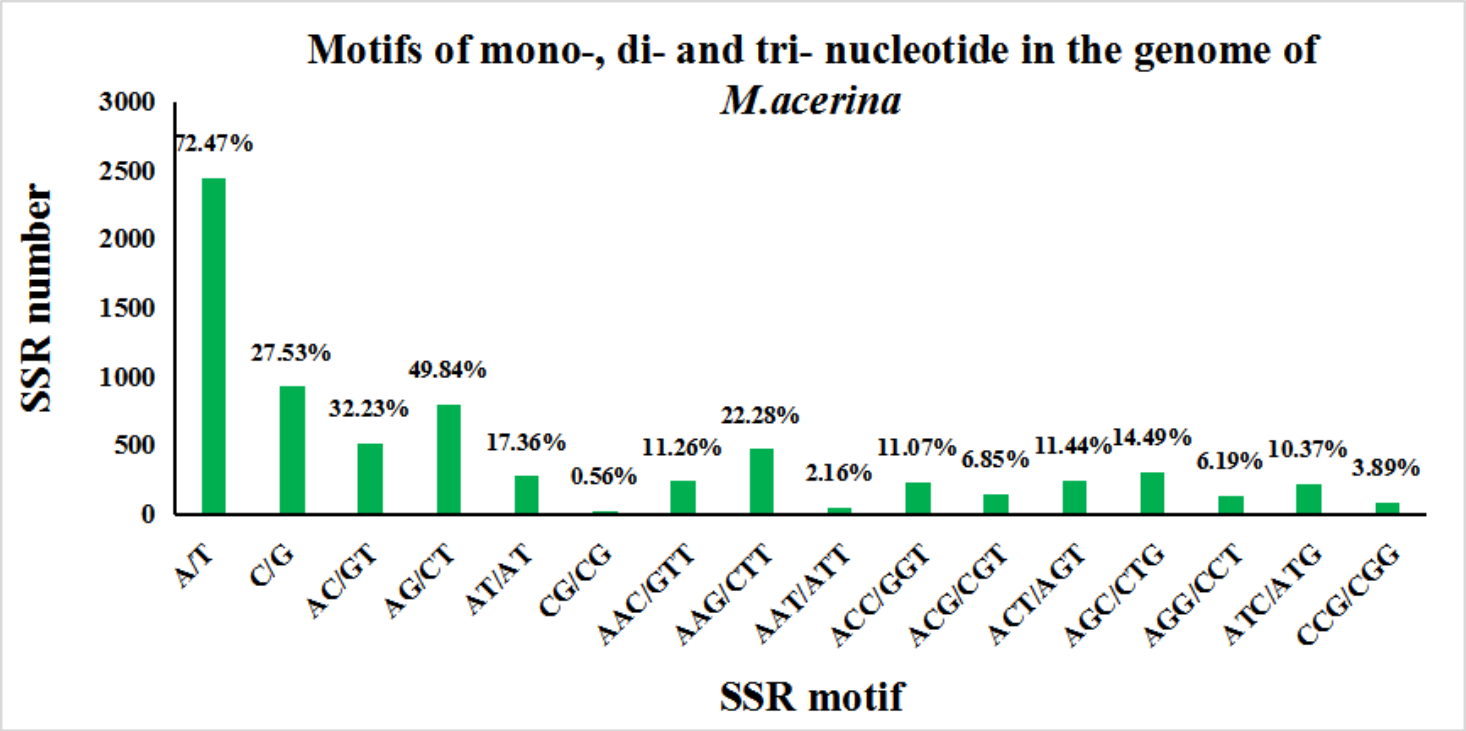

Figure 6

Motifs of mono-, di-, and tri-nucleotide simple sequence repeats (SSRs) in the whole genome of *Mycocentrospora acerina.*

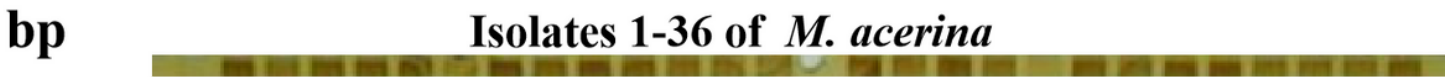

Figure 7

Primer MP56 for polyacrylamide gel electrophoresis of amplified products based on 36 *Mycocentrospora. acerina* strains.

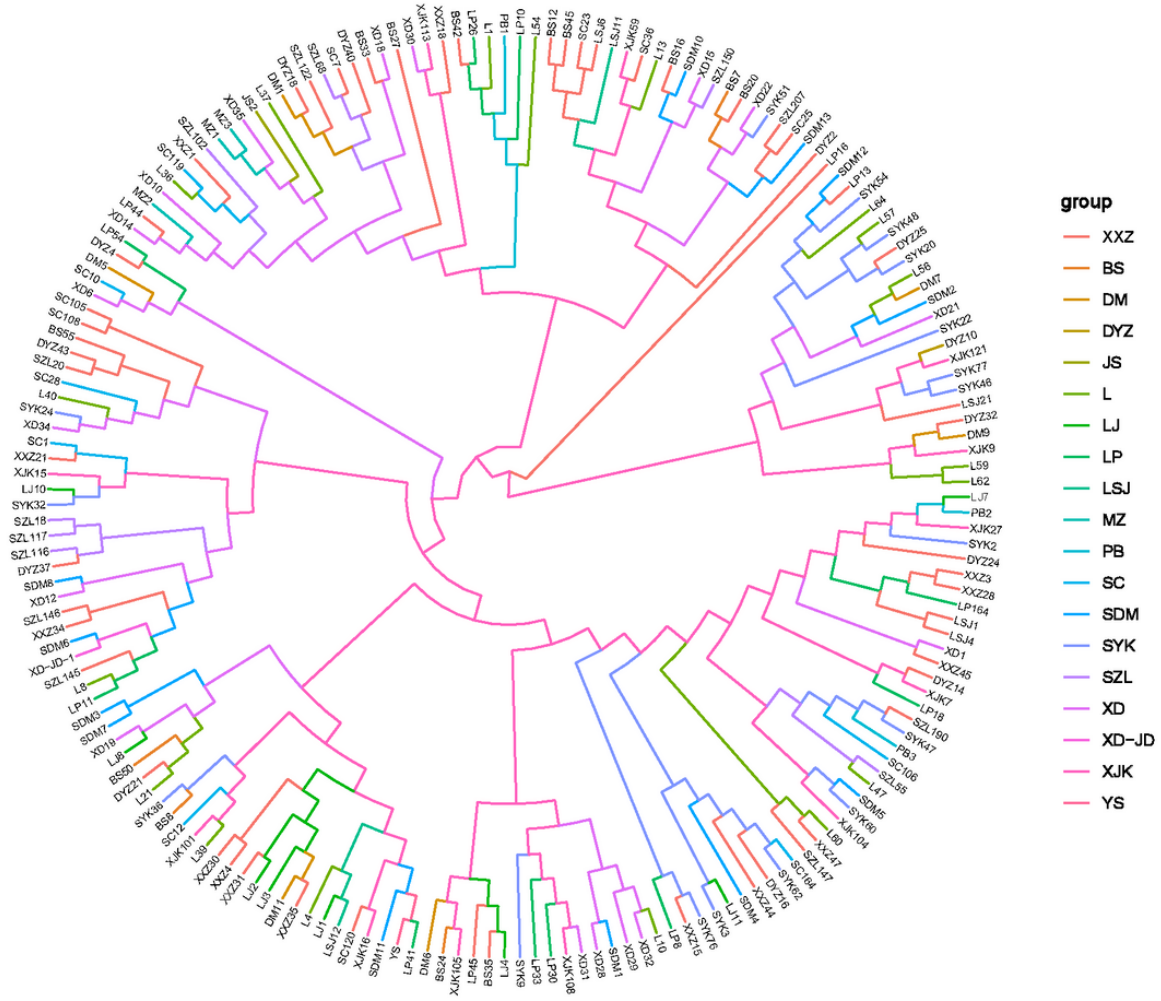

Figure 8

Cluster analysis of 187 *Mycocentrospora acerina* strains in Yunnan province based on genetic similarity coefficient. Different color meas different sampling location, SZL(Jianshui), BS(Luxi), JS(Jianshui), MZ(Mengzi), LP (Longpeng) and SC(Shaochong) were the isolates from Honghe, YS(Yanshan), WS(Wenshan), LSJ(Qiubei), SDM(Qiubei), XXZ(Qiubei), DM(Dumeng) and PB(Wenshan) were the fungis from Wenshan, DYZ and SYK were the isolates from Qujing, XD(Xundian), XD-JD(Xundian) and XJK(Shilin) were the fungis from Kunming, LJ prensents Lijiang.

Supplementary Files

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