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ORIGINAL PAPER

Diversity of Endophytic Fungal Community in Leaves of *Artemisia argyi* Based on High-throughput Amplicon Sequencing

QIU-FANG WU, LING-MIN HE, XIN-QIANG GAO, MEI-LING ZHANG,
JING-SHUN WANG and LI-JIANG HOU*

College of Biology and Food Engineering, Anyang Institute of Technology, Anyang, China

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Abstract

To investigate the community structure and diversity of endophytic fungi in the leaves of *Artemisia argyi*, leaf samples were collected from five *A. argyi* varieties grown in different cultivation areas in China, namely, Tangyin Beiai in Henan (BA), Qichun Qiai in Hubei (QA), Wanai in Nanyang in Henan (WA), Haiai in Ningbo in Zhejiang (HA), and Anguo Qiai in Anguo in Hebei (AQA), and analyzed using Illumina high-throughput sequencing technology. A total of 365,919 pairs of reads were obtained, and the number of operational taxonomic units for each sample was between 165 and 285. The alpha diversity of the QA and BA samples was higher, and a total of two phyla, eight classes, 12 orders, 15 families, and 16 genera were detected. At the genus level, significant differences were noted in the dominant genera among the samples, with three genera being shared in all the samples. The dominant genus in QA was *Erythrobasidium*, while that in AQA, HA, and BA was *Sporobolomyces*, and that in WA was *Alternaria*, reaching a proportion of 16.50%. These results showed that the fungal community structure and diversity in QA and BA were high. The endophytes are of great importance to the plants, especially for protection, phytohormone and other phytochemical production, and nutrition. Therefore, this study may be significant with the industrial perspective of *Artemisia* species.

Keywords: *Artemisia argyi*, endophytic fungus, leaf, Illumina, next-generation sequencing

Introduction

Mugwort (*Artemisia argyi*) is found in Europe (Artemisia vulgaris), Africa (Artemisia vulgaris), India (Artemisia vulgaris), Asia (Artemisia argyi), and America (Artemisia douglasiana). Early humans may have transported this plant throughout the world for its medicinal and food value (Adams et al. 2012). Its leaves are rich in essential oils, flavonoids, sugars, and other major components with pharmacological properties, such as bacteriostatic, insect-resistant, anti-inflammatory, antitussive, expectorant, soothing, antiallergic, antioxidant, and antitumor compounds, etc. Jiang et al. 2019a, 2019b). They are widely used in traditional Chinese medicine as well as in pharmaceutical products, animal feed, disinfectants, and other everyday products. With the increasing use of mugwort products, research on the quality and yield of mugwort cultivation to meet the growing global demand has increased.

Recent studies have shown that endophytic fungi, as a natural constituent of the plant micro-ecosystem, live in the tissues of healthy plants without causing any disease symptoms. These fungi have the functions of promoting plant growth, increasing plant disease resistance, inhibiting pathogenic bacteria, and even affecting the yield and quality of plants (Chu et al. 2020). Furthermore, endophytic fungi in plant leaves can not only directly or indirectly affect the synthesis of major therapeutic constituents of medicinal plants (Jiang et al. 2008). However, they can also have a significant influence on the internal microenvironment of roots, stems, and leaves, as well as affect the quality of these plants (Zhang 2017).

In a previous study, Zhang et al. (2011) isolated 19 strains of endophytic Actinomycetes from the leaves of *A. argyi* using plate culture screening method combined with crude extract of fermentation broth. Among these isolates, 11 strains presented extracellular

* Corresponding author: L.-J. Hou, College of Biology and Food Engineering, Anyang Institute of Technology, Anyang, China; **e-mail: hlj2013@nwsuaf.edu.cn**
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amylase activity and exhibited protease activity, and eight strains showed cellulase activity. Besides, two strains had antagonistic activity against pathogenic bacteria, and three strains presented antagonistic activity against penicillin-resistant *Staphylococcus aureus*. Furthermore, Shi et al. (2014) isolated and screened ten strains of Actinomycetes from the collective site of *A. argyi* rhizome by combining the primary screening method of confrontation culture with the rescreening method of fermentation broth, and noted that 70% of the isolates presented different degrees of antibacterial activity. Liu et al. (2019) used the plate culture method to isolate 13 strains of endophytic fungi belonging to three genera from the stems and leaves of North *A. argyi* grown in Tangyin, China and detected six species and three genera of endophytic fungi in the leaves. However, all these studies had employed traditional culture techniques, which cannot support the growth of unculturable microorganisms in the stems and leaves of *A. argyi*. To date, studies on the diversity of the endophytic fungal community in *A. argyi* based on high-throughput sequencing have not yet been reported. Therefore, to evaluate the influence of endophytic fungi on the growth and quality of *A. argyi*, the present study employed high-throughput amplicon sequencing technology to compare and analyze the diversity and composition of endophytic fungal community structure in the leaves of *A. argyi* cultivated in different regions in China. Besides, the degree of health and biocontrol application potential of *A. argyi* was also examined. The results obtained can provide a scientific basis and guidance for large-scale cultivation of *A. argyi* and its application for the biocontrol of plant diseases and pests.

Experimental

Materials and Methods

Overview of the test site. The experimental site was located in the *A. argyi* cultivation base of Anyang Institute of Technology in Henan Province, China (N36°0′, E114°35′). The region has a warm, temperate, continental monsoon climate with mild weather conditions and four distinct seasons. The annual average temperature is 14.9°C, the annual sunshine duration is 2,500 h, and the annual average precipitation is 538.4 mm, with 206 days of frost-free period and sandy loam soil.

Sample collection. *A. argyi*, commonly known as Chinese mugwort, is widely distributed in Henan, Hebei, Hubei, Anhui, and Zhejiang in China. Different ecological species have different biological flora. The leaves of 2-year-old *A. argyi* without diseases and pests on its surface were collected in June 2020 at the *A. argyi* cultivation base in Anyang Institute of Technology. Five kinds of *A. argyi* leaves were collected from Tangy in Beiai in Henan (BA), Qichun Qiai in Hubei (QA), Wanai in Nanyang in Henan (WA), Haiai in Ningbo in Zhejiang (HA), and Anguo Qiai in Anguo in Hebei (AQA). The experimental field was weeded manually without the use of herbicides. Fertilizers were not applied, and two crops were cultivated in a year.

During sampling, the third leaf of *A. argyi* was selected, and 15 samples (three biological replicates per *A. argyi* variety and five *A. argyi* varieties in total) were put into sterile plastic bags with labels (indicating the sample number, name, place, date, and collector), transported to the laboratory, and stored in a –80°C refrigerator for the subsequent isolation of DNA.

Extraction and electrophoresis of endophytic fungi total DNA from *A. argyi* leaves. Firstly, the samples were surface-washed with 70% ethanol solution three times, then washed with 1 × PBS solution three times, dried, and then extracte with liquid nitrogen grinding or tissue disrupter. Then, the endophytic fungi total DNA was extracted with the OMEGA kit, qualitatively detected using agarose gel electrophoresis, quantified by nucleic acid quantitative spectrophotometer (Nanodrop, USA), and stored in a refrigerator at –20°C for the subsequent analysis.

PCR amplification and sequencing of ITS1-ITS2 region of 18S rRNA. The extracted total DNA was used as a template, and the internal transcribed spacer (ITS) region of fungi (ITS1-ITS2 region) was amplified using specific PCR primers (ITS1F: 5’-CTTGGTCATTTAGAGGAAGTAA-3’; ITS2: 5’-GCTGCGTTCATCGATGC-3’). The reaction system for PCR comprised the following: Phusion Master Mix (2×), 15 μl; primer (2 μmol/l), 3 μl; DNA (1 ng/μl), 10 μl; and ddH2O, 2 μl. The PCR conditions were as follows: pre-denaturation at 95°C for 3 min, followed by 25 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s, and a final extension at 72°C for 7 min. The PCR products were qualitatively detected by electrophoresis using 2% agarose gel in 1 × TAE solution, purified, quantified, and a test library was constructed using Qubit 3.0. Subsequently, the test DNA library was subjected to high-throughput amplicon sequencing performed by Sangon Bioengineering Co., Ltd. (Shanghai) using the Illumina MiSeq sequencing platform (Liao et al. 2020). The raw data of high-throughput amplicon sequencing was uploaded to the NCBI SRA database, and could be downloaded from website (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA714493) and the clean data was obtained by quality control and filtering from the raw data.

Bioinformatics analysis. UPARSE was used for operational taxonomic unit (OTU) classification of the representative sequences at 97% similarity level, and the fungal community composition in each sample was

determined at different classification levels. Mothur software was used to analyze the dilution curves, and Shannon index, Simpson index, species richness index (ACE), and Chao1 index were employed to determine the microbial ecological diversity. The sequences were compared with the functional genes in the NCBI NT database using BLAST. The optimized sequences were identified at the phylum, class order, family, and genus levels according to the reference sequences in the database, and the community composition, abundance, and diversity were compared and analyzed. Ramette's method was used for principal component analysis (PCA), Excel (2007) was utilized to construct a histogram, and SPSS (19.0) software was employed to statistically analyze the class group test data of endophytic fungi in *A. argyi*.

FUNGuild functional analysis. The fungi classification and functional analysis was completed by FUNGuild (Fungi Functional Guild) software. It is a tool for classification and analysis of fungal communities through microecological guild depending on the currently published literature or authoritative website data.

Results

Qualitative analysis of endophytic fungi in *A. argyi* leaves. The optimal sequence and information about the genus or species number (OTUs) of the endophytic fungal community in *A. argyi* leaves were obtained using high-throughput amplicon sequencing (Table I). After merging and filtering of double-ended reads, the clean tags for endophytic fungi in WA, AQA, HA, BA, and QA samples were 53,504.0, 69,364.0, 71,661.0, 81,561.0, and 89,829.0, respectively. Species classification based on similarity level ≥ 97% revealed 709.0 OTUs , and the number of OTUs in WA, AQA, HA, BA, and QA samples was 165.0, 205.0, 230.0, 224.0, and 285.0, respectively (Fig. 1). QA presented the highest sequence number and species classification, which was mostly consistent with the values of alpha-diversity index. The dilution curve for the endophytic fungi in the five samples is shown in Fig. 2. When the sequencing data reached 50,000, the number of OTUs in the five samples remained flat, indicating that the sequencing depth of the samples was essentially reasonable and that the obtained data could reflect the composition of

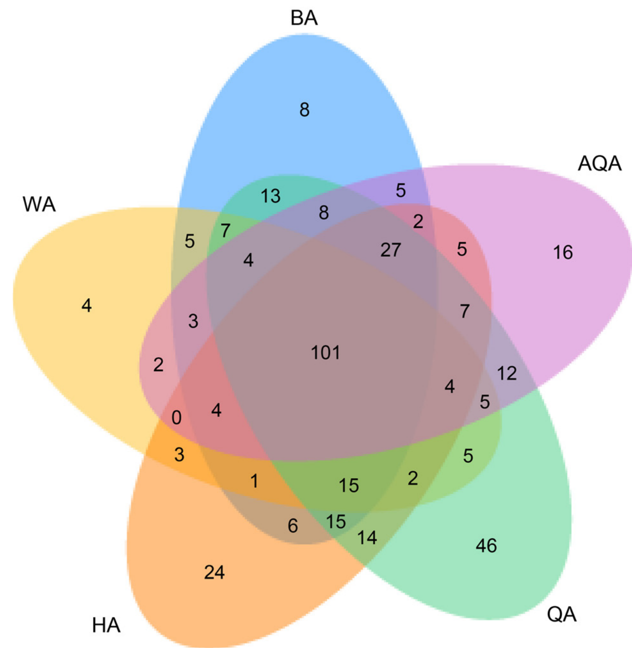

Fig. 1. Venn graph of OTUs distribution in the samples.

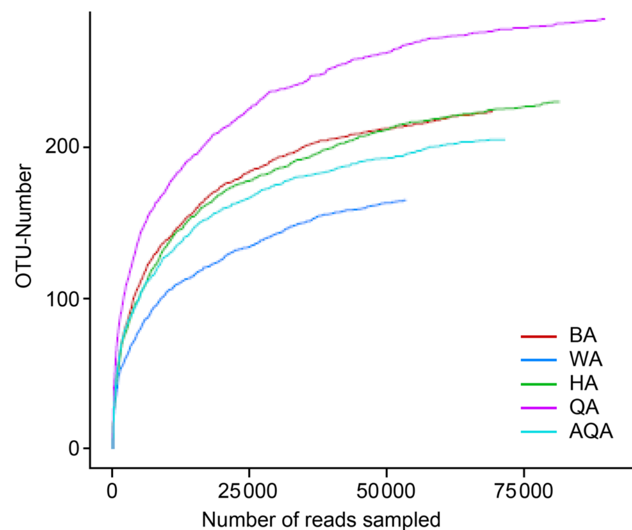

Fig. 2. Rarefaction curve of the samples.

Table I
Richness and diversity of endophytic fungi in *A. argyi* leaves.

Treatments	No. of reads	Shannon index	Chao1 index	ACE index	Simpson index
WA	53504.0	2.09 ± 0.01a	180.53 ± 15.03a	193.54 ± 8.34a	0.25 ± 0.000a
AQA	69364.0	2.19 ± 0.01a	223.91 ± 9.32a	226.58 ± 11.53a	0.24 ± 0.000a
HA	71661.0	2.57 ± 0.02a	239.38 ± 10.02a	242.84 ± 10.02a	0.14 ± 0.000a
BA	81561.0	2.62 ± 0.04a	255.54 ± 8.39a	253.33 ± 9.14a	0.08 ± 0.000a
QA	89829.0	2.99 ± 0.03a	291.73 ± 15.12a	297.24 ± 12.02a	0.12 ± 0.001a

Note: Same lowercase letters indicate no significant difference among the samples ($p > 0.05$).

the fungal community structure in the samples under natural conditions more tangibly and comprehensively.

Analysis of endophytic fungal diversity in *A. argyi* leaves. The values of soil microbial alpha-diversity were all > 100% based on the Coverage values (Table I). The microbial community diversity increased with the increasing values of Shannon, Chao1, and ACE indices and decreasing values of Simpson index. The Shannon, Chao1, and ACE indices for the samples exhibited the following trend: WA < AQA < HA < BA < QA, which indicated that the diversity of endophytic fungi in QA was the highest. The Simpson index presented the following trend: BA < QA < HA < AQA < WA, suggesting that BA showed the highest fungal diversity. The alpha-diversity index revealed that the microbial diversity of BA and QA was the highest.

Composition of endophytic fungi in *A. argyi* leaves. High-throughput amplicon sequencing detected two phyla, Ascomycota and Basidiomycota, in the five samples (WA, AQA, HA, BA, and QA) (Fig. 2). In WA, Ascomycota was predominant, accounting for 28.34%, followed by Basidiomycota with a relative abundance of 17.85% (Fig. 3). In AQA, HA, BA, and QA samples, Basidiomycota was dominant with a relative abundance of 33.45%, 41.41%, 40.64%, and 64.72%, respectively (Fig. 3), followed by Ascomycota with a relative abundance of 10.10%, 17.12%, 24.48%, and 16.27%, respectively (Fig. 3). In addition, unclassified fungal group with the relative abundance of 52.83%, 50.98%, 41.33%, 34.40%, and 18.27% was also detected in WA, AQA, HA, BA, and QA, respectively (Fig. 3), accounting for a large proportion of endophytic fungi in *A. argyi* leaves.

Furthermore, differences in the community composition and abundance of endophytic fungi at the class, order, family, and genus levels were noted in the five *A. argyi* samples. In WA and BA, Dothideomycetes belonging to Ascomycota was predominant, accounting for 20.96% and 22.33%, respectively, followed by Microbotryomycetes belonging to Basidiomycota, accounting for 8.36% and 17.25%, respectively. In AQA, Microbotryomycetes was dominant (15.52%), followed by Cystobasidiomycetes belonging to Basidiomycota (12.90%). In HA, Cystobasidiomycetes was dominant (19.10%), followed by Microbotryomycetes (15.92%). In QA, Cystobasidiomycetes was predominant (41.96%), followed by Dothideomycetes (12.68%). The dominant order in WA was Pleosporales, accounting for 17.73%, whereas that in AQA, HA, and BA was Sporidiobolales, with the relative abundance of 15.40%, 15.52%, and 15.25%, respectively. In QA, Erythrobasidiales was predominant with a relative abundance of 31.57%. The dominant family in WA was Pleosporaceae, accounting for 16.50%, while that in AQA, HA, and BA was Spordiobolaceae with a relative abundance of 15.39%, 15.52%, and 15.26%, respectively. In QA, Erythrobasidiaceae was predominant, with a relative abundance of 31.57%.

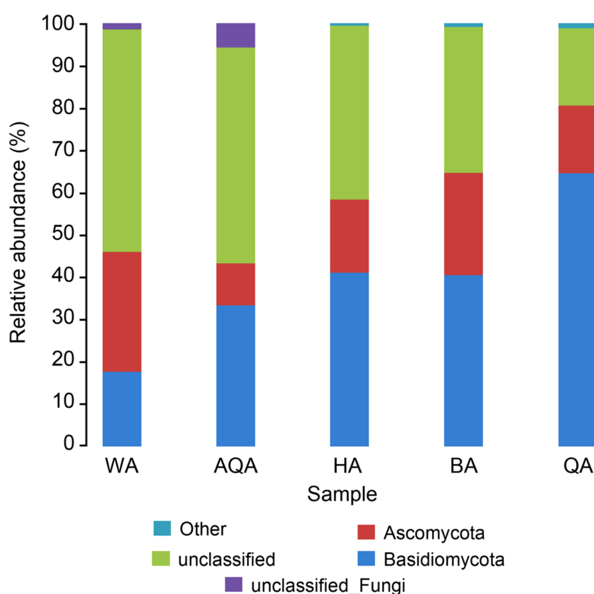

Fig. 3. Fungal community composition in the samples at the phylum level.

A total of 16 genera, including *Erythrobasidium*, *Sporobolomyces*, *Alternaria*, *Symmetrospora*, *Aureobasidium*, *Cryptococcus*, *Filobasidium*, *Papiliotrema*, *Coniothyrium*, *Dioszegia*, *Kondoa*, *Sphaerulina*, *Leucosporidium*, unclassified_*Phaeosphaeriaceae*, and unclassified_*Ascomycota*, were found in the samples (Fig. 4). Besides, other unclassified fungi were also detected. The common genera detected in all five samples were *Sporobolomyces*, *Erythrobasidium,* and *Alternaria*. While both AQA and QA contained *Cryptococcus*, which was not detected in the other samples, *Sphaerulina*, *Aureobasidium*, *Symmetrospora,* and unclassified_*Ascomycota* were found in WA, HA, and BA. Furthermore, *Papiliotrema* and unclassified_*Phaeosphaeriaceae* were detected in HA, but not in WA. Ten genera, including *Symmetrospora*, *Aureobasidium*, *Filobasidium*, *Papiliotrema*, *Sphaerulina*, unclassified_*Ascomycota,* and three genera found in all the five samples, were common in BA and QA. However, *Leucosporidium* was detected only in BA, whereas *Coniothyrium*, *Dioszegia*, *Kondoa*, *Cryptococcus*, unclassified_*Phaeosphaeriaceae* were found only in QA. According to the sequencing results, the richness of the endophytic fungal community structure in the five samples presented the following trend: QA > BA > HA > WA > AQA.

Analysis of phylogenetic relationship of the endophytic fungi in *A. argyi* leaves. The phylogenetic relationship of the endophytic fungi among the five *A. argyi* leaves samples was investigated based on the heat map of the genetic distance between the samples (Fig. 5). The color block represents the distance value, with the distance between the samples decreasing with the increasing grayness. Three branches can be observed in

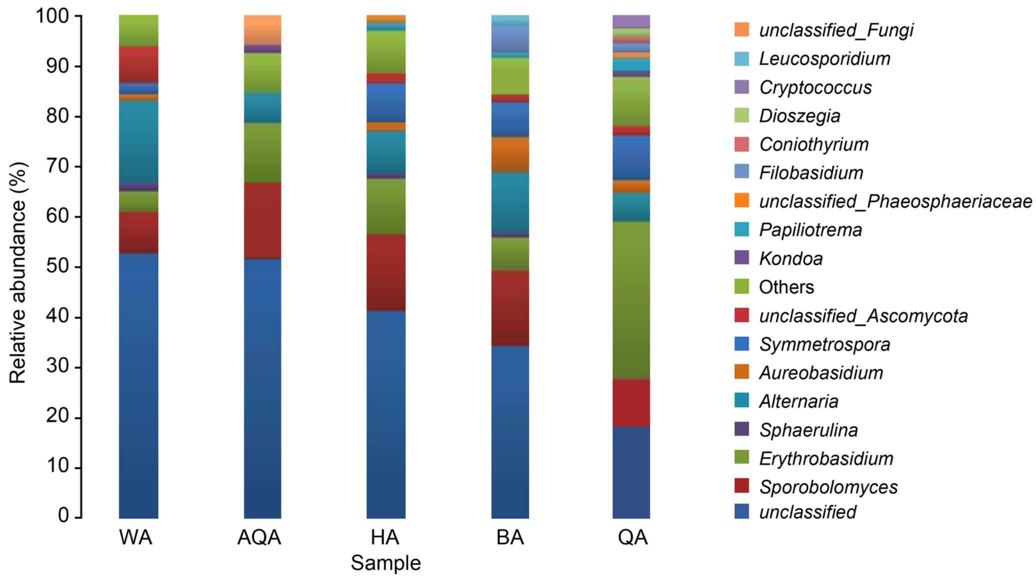

Fig. 4. Fungal community composition in the samples at the genus level.

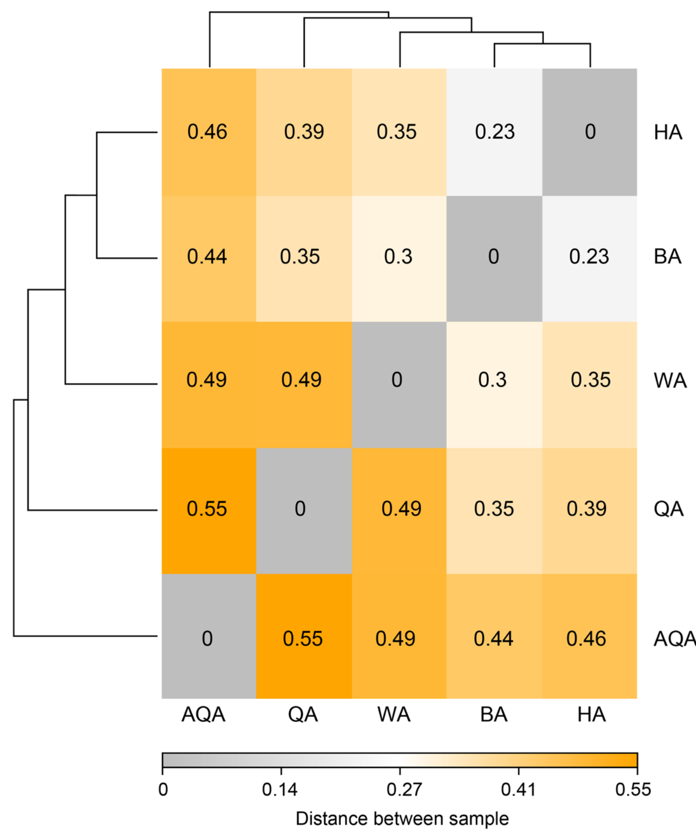

Fig. 5. Heatmap of genetic distance between the samples.

the figure, with HA and BA, which presented a closer genetic relationship, merging into one branch, WA forming another branch, and AQA forming another branch. In addition, QA exhibited a systematic branch, indicating the distant genetic relationship between QA and the other samples.

PCA analysis. PCA can reduce the dimension of data based on linear algebra. The original high-dimensional data are transformed and projected into the spatial coordinate system with lower dimensions (i.e., principal components) to show the natural distribution of samples. Each point represents a sample, and the shorter the distance is between the two points, the higher is the similarity in the microbial community structure between the two samples, and the smaller is the difference. The percentages on the axis parentheses

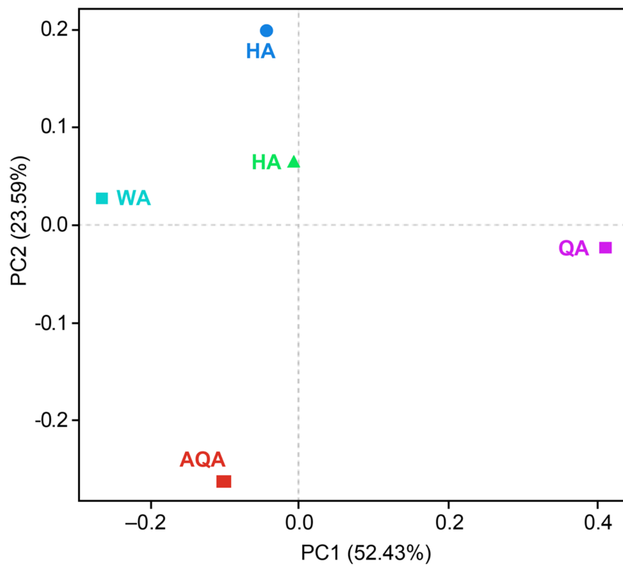

Fig. 6. PCA of the samples.

represent the percentage of variance in the original data that the corresponding principal component can explain. If PCA is 50%, then the variance of the X-axis can explain 50% of the overall analysis results. As shown in Fig. 6, the distance between QA and the other four samples was long, indicating that the similarity in the fungal communities between these samples was very low, whereas the difference was considerable.

Similarly, the distance between AQA and the other four samples was very long, implying that the difference in the fungal communities between the samples was very large. In contrast, the distance between HA and BA was relatively short, followed by that between HA and WA, which suggested that the similarity in the fungal communities between these samples was relatively higher, whereas the difference was more negligible when compared to former ones. Furthermore, the distance between BA and QA and AQA was long, indicating that the similarity in the fungal communities between these samples was low, but the difference was large, and consistent with the results of the heat map analysis. Thus, it can be concluded that the endophytic fungal community structure in the five *A. argyi* leaves samples was significantly different.

Discussion

In this study, 18 S rRNA amplicon sequencing of endophytic fungi in the leaves of *A. argyi* was conducted using a high-throughput sequencing platform based on fungal ITS1-ITS2 sequences. The variation in the sequencing reads was 53,504.0–89,829.0, with QA presenting the highest number of sequencing reads. The variation in the number of OTUs was between 165 and 205, with QA exhibiting the maximum number of OTUs. Furthermore, OTUs analysis showed the presence of common fungal populations, variations in the fungal populations, as well as unique OTUs among the five samples. Moreover, the diversity of the endophytic fungal community in the leaves of *A. argyi* was lower than that of *A. argyi* roots. The alpha-diversity, Shannon, Chao1, and ACE indices showed that the diversity of endophytic fungi in QA leaves was the highest, whereas the Simpson index revealed that the diversity of endophytic fungi in BA leaves was the highest.

Furthermore, QA and BA presented the highest alpha-diversity index. These results revealed that the diversity of the endophytic fungal community in *A. argyi* leaves was low, consistent with the findings of Liu et al. (2019). High-throughput amplicon results detected two phyla, five classes, nine orders, nine families, and nine genera in WA; two phyla, six classes, eight orders, eight families, and eight genera in AQA; two phyla, six classes, 10 orders, 11 families, and 11 genera in HA; two phyla, eight classes, 13 families, and 13 genera in BA; and two phyla, 10 classes, 13 orders, 16 families, and 15 genera in QA, indicating that the diversity of endophytic fungi was the highest in QA, followed by that in BA.

The finding that Basidiomycota was the dominant phylum in BA is not in agreement with that reported by Liu Miao et al. (2019). At the genus level, significant differences were noted with respect to the dominant genus of endophytic fungi among the five samples. The predominant genus of endophytic fungi in WA, AQA, HA, and BA was unclassified, with a relative abundance of 52.83%, 50.98%, 41.33%, and 34.40%, respectively, whereas *Erythrobasidium,* with the relative abundance of 31.35%, was dominant in QA. In contrast, Liu Miao et al. (2019) isolated three genera and six species of endophytic fungi from the leaves of *Artemisia* sp. by using traditional culture, isolation, and molecular identification methods and found that *Alternaria* sp. was the dominant genus. These inconsistent results could be owing to the different sampling times, cultivation climates, and soil types. *Erythrobasidium* has been noted to infect the leaf veins of sugar orange, causing citrus yellow shoot and has been detected in a significantly higher proportion in the diseased plants. In the present study, the abundance of *Erythrobasidium* was the highest in QA, and its ecological function needs to be further investigated.

Analysis of the fungal genera and species detected in the present study showed 33 pathogenic fungi, three symbiotic fungi, 34 saprophytic fungi, eight major plant pathogenic fungi, four biological control fungi, 39 environmental fungi, and 19 other fungi in the leaves of *A. argyi* (Table II). While the overall proportion of the detected beneficial biological control fungi, including

Table II
FUNGuild Functional analysis.

Generic	Type	Generic	Type
Aspergillus_intermedius	biocontrol fungi- saprotroph	*Phanerochaete*	pathotroph
Aspergillus_sydowii	biocontrol fungi-saprotroph	*Phlebia*	pathotroph-saprotroph-symbiotroph
Penicillium_catenatum	biocontrol fungi-saprotroph	*Phoma*	saprotroph-symbiotroph
Penicillium_oxalicum	biocontrol fungi-saprotroph	*Podosphaera*	pathotroph-saprotroph-symbiotroph
Botryosphaeria	pathotroph-saprotroph-symbiotroph	*Ramichloridium*	pathotroph-saprotroph
Botrytis	pathotroph-saprotroph	*Rhodotorula*	saprotroph
Cladosporium	pathotroph-saprotroph-symbiotroph	*Sarocladium*	pathotroph-saprotroph
Clonostachys	pathotroph-saprotroph	*Schizophyllum*	pathotroph-symbiotroph
Curvularia	saprotroph	*Sphaerulina*	saprotroph
Cyphellophora	pathotroph-saprotroph-symbiotroph	*Sporisorium*	saprotroph
Cystobasidium	saprotroph	*Sporobolomyces*	pathotroph-symbiotroph
Diaporthe	pathotroph-saprotroph-symbiotroph	*Stagonospora*	saprotroph
Dothidea	saprotroph	*Stemphylium*	saprotroph
Edenia	pathotroph	*Tilletiopsis*	saprotroph
Entocybe	pathotroph	*Trametes*	pathotroph-saprotroph
Entodesmium	pathotroph	*Tricharina*	pathotroph-saprotroph-symbiotroph
Erythrobasidium	pathotroph	*Trichomeriaceae*	pathotroph
Filobasidium	pathotroph-saprotroph-symbiotroph	*Trichosporon*	pathotroph-saprotroph-symbiotroph
Fusarium	pathotroph-saprotroph	*Verticillium*	pathotroph
Gibellulopsis	pathotroph-saprotroph	*Alternaria*	pathotroph-saprotroph
Herpotrichiellaceae	pathotroph-saprotroph-symbiotroph	*Amphisphaeriaceae*	pathotroph-saprotroph-symbiotroph
Knufia	pathotroph	*Amphobotrys*	pathotroph-saprotroph-symbiotroph
Leptosphaeria	pathotroph	*Anthracocystis*	pathotroph
Limonomyces	saprotroph	*Apiotrichum*	pathotroph
Microdochium	pathotroph-saprotroph-symbiotroph	*Articulospora*	pathotroph
Mycosphaerella	pathotroph-symbiotroph	*Ascochyta*	pathotroph
Myrmecridium	saprotroph	*Aurantiporus*	pathotroph
Myrothecium	symbiotroph	*Aureobasidium*	pathotroph
Neosetophoma	pathotroph-saprotroph-symbiotroph	*unclassified*	other
Occultifur	saprotroph	*unclassified_Ascomycota*	other
Paraconiothyrium	pathotroph-saprotroph	*unclassified_Fungi*	other
Paraphoma	pathotroph-saprotroph	*unclassified_Phaeosphaeriaceae*	other
Phaeosphaeria	pathotroph-saprotroph	Others	other

Penicillium catenatum, *Penicillium oxalicum*, *Aspergillus intermedius*, *Aspergillus sydowii,* was not high, environmental fungi formed a large proportion of the fungal population detected in this study. These environmental fungi could develop the symbiotic association with plants and transform the inorganic materials that plants cannot utilize into organic matter, degrade toxins such as phenol, decompose lignin, provide nutrients to plants, and promote plants' growth and development, and improve the contents of medicinal components in plants. Nevertheless, further studies are needed to determine whether the detected beneficial biocontrol fungi and environmental fungi are directly related to the incidences of *A. argyi* diseases. Besides, research on the ecological functions of many other fungi detected in the present study, whose effects on plants are still unclear, could be crucial for healthy plant growth and development and the biocontrol of pathogens.

Sporobolomyces usually exist on the surface of leaves and accumulate useful metabolites, such as essential oil, a carotenoid pigment, fungal polysaccharide, and extracellular enzymes, which have wide applications in food and medicine, cosmetics, and breeding industries. Besides, *Sporobolomyces* can also remove chromium in sludge and degrade cellulose and lignin (Wei et al. 2014). *Alternaria*, which is widely distributed in soil and plants, is a biocontrol agent with potential applications (Chu et al. 2020). Some species of this genus

isolated from *Artemisia annua* have been noted to present antitumor and antioxidant effects (Li et al. 2020), while some *Alternaria* spp. could control tobacco red root disease (Wang et al. 2001). Ma et al. (2017) found that the endophytic fungus *Cryptococcus* J22 in the leaves of Nuoli contains high content of total phenols and total flavonoids and ABTS, a strong scavenger of free radicals. Moreover, the total flavonoid contents in the leaves of *A. argyi* cultivated in Hubei Province have been reported to be relatively high, possibly owing to the relatively high proportion of *Cryptococcus* (Dong et al. 2016; Gong et al. 2019). *Filobasidium* is mainly found in the body and surface of plants (Ma et al. 2018; Liu et al. 2019), and could produce a variety of extracellular enzymes, such as α-amylase, which help in disease control and have a broad application prospect in the field of medicine (Wang et al. 2015). Thus, the antitumor, antimicrobial, and antioxidant properties of *A. argyi* might possibly be attributed to the presence of *Sporobolomyces*, *Alternaria*, *Cryptococcus,* and *Filobasidium,* and requires further investigation. Besides, the characteristics and functions of unclassified and other fungi found within the leaves of *A. argyi* should also be studied.

Endophytic fungi live in healthy plant tissues but do not infect or damage the host plants. Strobel et al. (2003, 2004) showed that the growth of endophytic fungi in medicinal plants produced natural medicinal products with therapeutic effects (Wang et al. 2016). In the present study, a minor proportion of the endophytic fungi in the leaves of *A. argyi* was found to have potential antitumor and antioxidant properties, which could have significant application prospects in medicine and health.

The present study is the first to use high-throughput amplicon sequencing to investigate the fungal community structure in the leaves of *A. argyi* grown in five different regions in China. The results showed that QA and BA had rich fungal community structure and diversity, presenting differences, although not significant, in the fungal species and distribution. The PCA results revealed that the phylogenetic relationship of the five *A. argyi* leaves samples was distant. Moreover, the majority of the fungal species were detected in the leaves of *A. argyi*, with few major pathogenic fungi and very few beneficial biocontrol fungi. These results can provide a theoretical basis for accomplishing the healthy growth and quality of perennial root plants.

Acknowledgment

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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