

Impact of Viral Infection on the Structure and Diversity of Endophytic Fungal Communities in Areca Palm (*Areca catechu* L.) Leaves

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Abstract [Objectives] To elucidate the community composition of endophytic fungi in virus-infected areca palm (*Areca catechu* L.) leaves, and to explore the potential correlations between viruses associated with areca palm yellow leaf disease (YLD) and these endophytic fungi.

[Methods] Leaf samples from healthy, yellowing, and stunted areca palms were systematically collected in August 2025 from an orchard affected by yellow leaf disease (YLD) at Dongsheng Farm, Qionghai City, Hainan Province. The infection rates of areca palm velarivirus 1 (APV1), areca palm yellow leaf-associated ormycovirus (APYLaOMV), and areca palm latent totivirus 1 (APLTV1) were determined using RT-PCR assay. Endophytic fungi were isolated and purified through the tissue separation method, and species identification was conducted based on ITS sequencing and homology comparison using the NCBI database. [Results] The detection rates of APV1, APYLaOMV, and APLTV1 in areca palm diseased leaves were 60%, 50%, and 30%, respectively. A total of 81 endophytic fungal strains were isolated from 13 samples and identified as belonging to 14 genera and 37 species, all within the phylum Ascomycota. Community structure analysis revealed that the endophytic fungal communities in healthy leaves were relatively simple at the class, order, and family levels, with *Nigrospora* as the dominant genus. In contrast, fungal diversity increased significantly in diseased leaves, where *Phyllosticta* emerged as the dominant genus. Furthermore, the composition of endophytic fungi varied markedly among different leaf tissues. In the mesophyll tissue of diseased leaves, *Phyllosticta* and *Colletotrichum* were co-dominant genera, whereas *Fusarium* predominated in the vein tissues. In healthy leaves, *Nigrospora* was the dominant genus in both mesophyll and vein tissues. [Conclusions] This study identifies the predominant viral species associated with YLD in areca palms and characterizes the community structure of leaf endophytic fungi in Qionghai City. It provides essential data and theoretical foundations for further elucidating the complex pathogenic mechanisms underlying YLD in areca palms, screening effective biocontrol fungi, and developing integrated management strategies.

Key words Yellow leaf disease (YLD), APV1, APYLaOMV, APLTV1, Endophytic fungi

1 Introduction

Areca palm (*Areca catechu* L.), recognized as one of the four major traditional southern medicines in China^[1], is a tropical economic crop characterized by the highest output value per unit area in Hainan Province. The cultivation area extends to 200 000 hm², with its output value contributing over 80% to the local economy^[2–3]. However, since the initial identification of yellow leaf disease (YLD) in areca palms in Tunchang County, Hainan Province, in 1981^[4], the disease has diffused throughout the primary areca-producing regions on the island. Currently, the affected area encompasses 38 300 hm², representing more than 33.27% of the total planting area^[5]. This disease results in the weakening of plants, a significant decline in yields, and even causes dieback and mortality within 5–7 years^[6], thereby becoming the principal challenge to the high-quality and sustainable development of the areca industry.

Early studies indicated that areca palm yellow leaf phytoplas-

ma (AYLP) was the primary pathogenic factor responsible for YLD in areca palms^[7]. However, with advances in virological research, Yu Hongmei *et al.*^[8–9] identified a novel virus, areca palm velarivirus 1 (APV1), from YLD-affected samples using viral metagenomic techniques, and established its association with YLD in areca palms. Subsequent studies have further confirmed that APV1 can be transmitted in a non-cyclic semi-persistent manner by *Ferrisia virgata* and *Pseudococcus cryptus*^[10–11]. High detection rates have been reported in major areca production areas, including Wanning, Qionghai, Sanya, and Tunchang in Hainan^[12], thereby confirming APV1 as a principal pathogen responsible for YLD in areca palms. Following the identification of APV1, Tan *et al.*^[13] detected a species of *Totivirus* in arecanut samples while investigating the pathogenic factors of YLD in areca palms using RNA sequencing (RNA-seq) technology. They designated this virus as areca palm latent totivirus 1 (APLTV1). Previous studies have showed that APLTV1 is present in certain asymptomatic areca palms, with an average detection rate of 30% in YLD-affected samples, indicating a potential association with the development of YLD^[14–15]. Moreover, by performing high-throughput sequencing on samples from areca palms affected by YLD as well as on healthy samples, our research team successfully isolated and identified a novel virus, designated as areca palm yellow leaf-associated ormycovirus (APYLaOMV)^[14]. Subsequent RT-PCR detection and analysis showed that APYLaOMV was specifically

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present in the YLD-affected samples, providing initial evidence for its association with YLD in areca palms.

Endophytic fungi are a group that inhabits plant tissues without causing disease to their hosts^[16]. As a vital component of the host microecosystem, these fungi establish long-term mutualistic symbiotic relationships with plants^[17–18]. Areca palm, a tropical economic crop, contains a diverse array of endophytic fungal species within its tissues. Previous research has isolated and identified several genera, including *Penicillium*, *Fusarium*, *Phyllosticta*, *Trichoderma*, and *Colletotrichum*, from the roots, leaves, flowers, and fruits of areca plants^[19–20]. Furthermore, the diversity of endophytic fungi varies among different tissue types, with leaf tissues exhibiting greater species richness and more even distribution compared to other parts^[19]. Nevertheless, there is a lack of systematic studies on how viral infection reshapes endophytic fungal communities in areca palm leaves, as well as the joint involvement of endophytic fungi and viruses in YLD development. This gap in knowledge restricts a comprehensive understanding of the complex pathogenic mechanisms underlying YLD in areca palms. In this study, healthy and YLD-infected areca palm leaves from Dongsheng Farm in Qionghai, Hainan, were selected as subjects. The infection status of APV1, APYLaOMV, and APLTV1 was systematically assessed. Additionally, endophytic fungi from different leaf tissues were isolated and identified, and the differences in community composition were analyzed. This research provides foundational data for elucidating the relationships between viral and fungal communities, exploring potential biocontrol resources, and developing integrated prevention and control systems. These findings hold practical significance for ensuring the sustainable development of the areca industry in Hainan.

2 Materials and methods

2.1 Materials

2.1.1 Areca palm leaf samples. In August 2025, at the areca YLD plantation of Dongsheng Farm, Qionghai City, Hainan Province, healthy areca palms with normal phenotypes (fully green foliage) and diseased palms showing characteristic yellowing or bunchytop symptoms were selected. From each plant, three samples of the middle leaves were collected in a triangular pattern. Following collection, the samples were immediately placed in sealed bags, labeled with identification numbers, and stored in low-temperature containers for preservation. Subsequently, the samples were transported to the laboratory for virus identification as well as the isolation and purification of endophytic fungi.

2.1.2 Culture media, main reagents and instruments. Potato dextrose agar (PDA) was prepared by dissolving 200 g of potatoes and 20 g of glucose in 1 000 mL of deionized water, followed by the addition of 15–20 g of agar. The mixture was then sterilized by autoclaving at 121 °C for 20 min.

The primary reagents used in this study included the

RNAprep Pure polyphenol polysaccharide plant total RNA extraction kit, the reverse transcription cDNA first strand synthesis kit, the DNA extraction kit, and the 2 × *Taq* PCR Mix, all of which were procured from Tiangen Biotech (Beijing) Co., Ltd.

The main instruments included a high-speed low-temperature tissue grinder (KZ-III-F), a centrifuge (JIDI-17R), a Bio-Rad T100 PCR instrument, and a gel electrophoresis apparatus.

2.2 Methods

2.2.1 Sample preparation and total RNA extraction. Approximately 0.1 g of areca palm leaves were weighed and placed into a 2 mL enzyme-free and sterile grinding tube. Three 4 mm enzyme-free and sterile steel balls were added, and the tube was then placed in a tissue grinder pre-cooled to –10 °C. The grinding program was set as follows: operated at a frequency of 70 Hz, running for 40 sec per cycle, with 5 sec pauses between cycles, repeated three times. Following grinding, total RNA was extracted according to the protocol provided with the RNAprep Pure polyphenol polysaccharide plant total RNA extraction kit.

2.2.2 RT-PCR assay of samples. First-strand cDNA was synthesized using a reverse transcription cDNA first-strand synthesis kit, with total RNA serving as the template. The reaction mixture (20 µL) comprised 4 µL of total RNA, 4 µL of 2 × reverse transcription mix, and 12 µL of RNase-free ddH₂O. The reaction conditions were 42 °C for 15 min, followed by 95 °C for 3 min. The resulting cDNA products were subsequently stored at –20 °C.

PCR amplification was conducted using cDNA as the template and virus-specific primers (Table 1). The reaction mixture (20 µL) comprised 2 µL of cDNA, 12.5 µL of 2 × *Taq* PCR MasterMix, 0.5 µL each of upstream and downstream primers, and 4.5 µL of RNase-free ddH₂O. The conditions were as follows: pre-denaturation at 94 °C for 3 min; 36 cycles of denaturation at 94 °C for 30 sec, annealing at 54 °C for 30 sec, and extension at 72 °C for 1 min; followed by a final extension at 72 °C for 5 min. The PCR products were subsequently detected and analyzed by electrophoresis on a 1% agarose gel.

Table 1 Specific detection primers for APV1, APYLaOMV and APLTV1

Virus	Primer	Sequence (5'-3')	Fragment size //bp
APV1	F	GAAAGATCTGACCAGTAATGG	495
	R	TACACATACAAGTTAGCAGGAGG	
APYLaOMV	F	GAATTACCCACGCCTCCAG	630
	R	GAGCTTTCCAGGACATGCC	
APLTV1	F	GGCAATAGAAACCCCTTATTCATTT	840
	R	CCACGCATCTAAATTAGGGATC	

2.2.3 Isolation and purification of endophytic fungi. Endophytic fungi from areca palm leaves were isolated using the tissue separation method. The collected areca palm leaves were rinsed with running water to remove surface dust, after which the leaves were placed in a sterile petri dish. Under a laminar flow hood, sterile

scissors were employed to cut the leaf veins and mesophyll into strips measuring approximately 0.3 cm × 0.2 cm and small pieces of approximately 0.3 cm × 0.3 cm, respectively. The leaf tissues were sequentially disinfected by immersion in 75% ethanol for 30 sec, followed by treatment with a 1% sodium hypochlorite solution for an additional 30 sec. Subsequently, the tissues were rinsed three times with sterile water and placed on sterile filter paper to remove surface moisture. The disinfected leaf tissues were then inoculated onto PDA medium in the following order: healthy veins, healthy mesophyll, diseased veins, and diseased mesophyll. Each group was appropriately labeled. After sealing the culture dish, it was inverted and incubated at a constant temperature of 25 °C. Once mycelial growth was visible at the colony margin, mycelial tips were aseptically excised using a sterile inoculation needle and subcultured onto fresh PDA medium for purification.

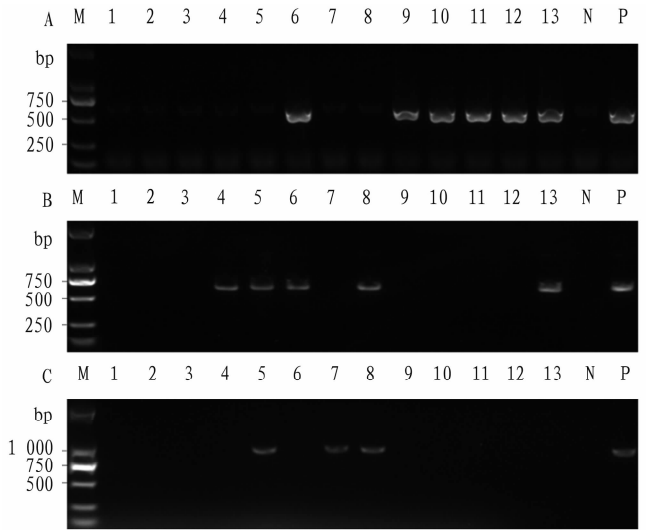
2.2.4 Molecular identification of endophytic fungi. The purified strain was inoculated onto PDA medium and incubated at 25 °C for 5–7 d. Subsequently, the mycelium was aseptically scraped off using a sterile blade and transferred into a 1.5 mL centrifuge tube that had been sterilized at high temperature. Two to three steel beads were added, and the sample was homogenized using a low-temperature tissue grinder. Genomic DNA was then extracted from the strain following the protocol provided with the fungal DNA extraction kit.

PCR amplification was conducted using the universal fungal primers ITS1 (5'-TCCGTAGGTGAACCTGC-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The PCR reaction mixture (50 µL) comprised 2 µL of DNA template, 25 µL of 2 × *Taq* PCR Master Mix, 2 µL each of forward and reverse primers (10 µmol/L), and 19 µL of RNase-free ddH₂O. The amplification protocol included a pre-denaturation at 94 °C for 3 min; followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 55 °C for 50 sec, and extension at 72 °C for 1 min; with a final extension at 72 °C for 10 min. The PCR products were analyzed by electrophoresis on a 1% agarose gel and subsequently sent to Shanghai Bioengineering Co., Ltd. for sequencing.

3 Results and analysis

3.1 Virus detection results and detection rates Three virus-specific primers, APV1, APYLaOMV, and APLTV1, were employed to perform RT-PCR assays on healthy areca palm leaves (No. QJ1-QJ3) and 10 diseased leaves (No. QB1-QB10). The amplification products were analyzed via agarose gel electrophoresis to determine the virus species (Fig. 1). The statistical results of the specific virus detections are presented in Table 2. The results indicated that none of the three target viruses were detected in healthy leaves. In contrast, APV1, APYLaOMV, and APLTV1 were identified in the diseased leaves. The detection rates of APV1, APYLaOMV, and APLTV1 in the diseased leaves were 60%, 50%, and 30%, respectively. In addition, co-infection with multiple viruses was observed. The co-infection rates of APV1 and APYLaOMV, as well as APYLaOMV and APLTV1,

were both 20%.



NOTE A. APV1 detection; B. APYLaOMV detection; C. APLTV1 detection; M. D2000 DNA marker; 1-3. QJ1-QJ3; 4-13. QB1-QB10; N. Blank control; P. Positive control.

Fig. 1 RT-PCR assay of three viruses

Table 2 RT-PCR detection of three viruses in areca palm leaf samples

Sample No.	APV1	APYLaOMV	APLTV1
QJ1	-	-	-
QJ2	-	-	-
QJ3	-	-	-
QB1	-	+	-
QB2	-	+	+
QB3	+	+	-
QB4	-	-	+
QB5	-	+	+
QB6	+	-	-
QB7	+	-	-
QB8	+	-	-
QB9	+	-	-
QB10	+	+	-

NOTE "+" denotes a positive test result, whereas "-" signifies a negative test result.

3.2 Isolation, purification and identification of endophytic fungi Thirteen collected areca palm leaves were subjected to isolation and purification processes, resulting in the acquisition of 81 endophytic fungi. Among these, 10 isolates originated from the mesophyll of healthy leaves (No. QJ1R-QJ3R), 21 from the veins of healthy leaves (No. QJ1M-QJ3M), 20 from the mesophyll of diseased leaves (No. QB1R-QB10R), and 30 from the veins of diseased leaves (No. QB1M-QB10M). Following sequencing and homologous sequence alignment using the NCBI database, 81 endophytic fungi were identified, representing 37 species (including some unidentified species) across 14 genera, as detailed in Table 3.

Table 3 Identification results of endophytic fungi in areca palm leaves

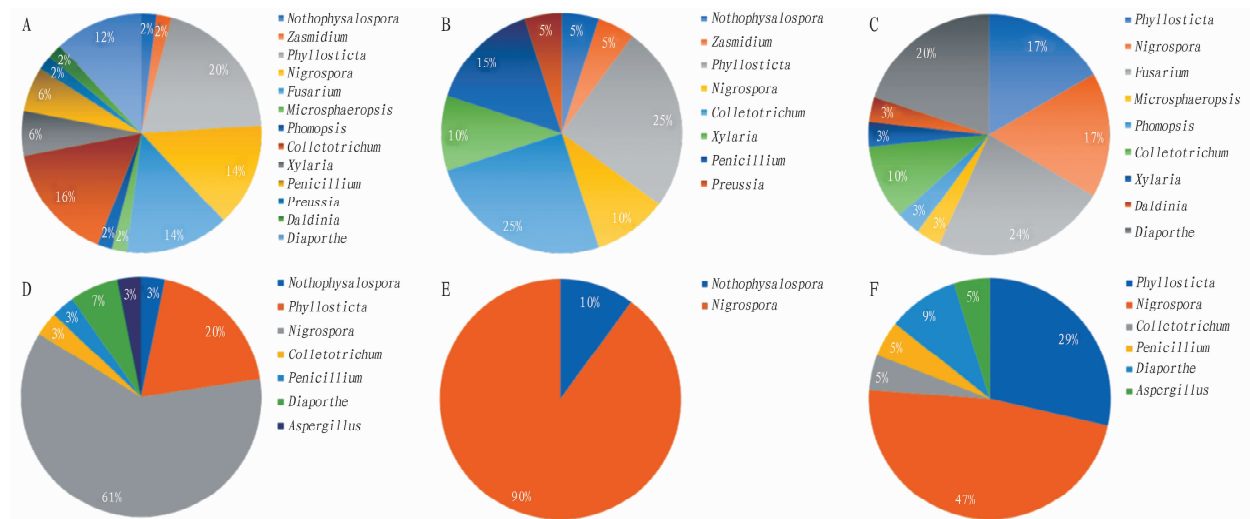
Tissue	Strain No.	Genus	Species	Homologous similarity // %	Strain number
Veins of healthy leaves	QJ1M-1,4,5,6,7	<i>Phyllosticta</i>	<i>P. capitalensis</i>	99.68 – 100	5
Veins of diseased leaves	QB5M-2			100	1
Mesophyll of diseased leaves	QB6R-9			99.85	1
Veins of healthy leaves	QJ1M-9		<i>Phyllosticta</i> sp.	99.84	1
Veins of diseased leaves	QB2M-3,5		<i>P. aloicola</i>	99.85 – 99.90	2
Mesophyll of diseased leaves	QB8R-5,6/QB10R-5			99.85 – 99.90	3
Veins of diseased leaves	QB1M-1/QB3M-3		<i>P. fallopiae</i>	99.55 – 100	2
Mesophyll of diseased leaves	QB10R-9			100	1
Mesophyll of healthy leaves	QJ2R-1,2,3,4/QB7R-5/QB10R-6	<i>Nigrospora</i>	<i>N. sphaerica</i>	99.63 – 100	6
Veins of healthy leaves	QJ2M-1,3,4/ QJ3M-6			100	4
Veins of diseased leaves	QB5M-6, QB9M-3			99.33/100	2
Veins of healthy leaves	QJ2M-2,5,7, QJ3M-1		<i>Nigrospora</i> sp.	99.62 – 100	4
Mesophyll of healthy leaves	QJ3R-3,6			100	2
Veins of diseased leaves	QB1M-3,4			99.83/100	2
Veins of healthy leaves	QJ2M-9		<i>N. lacticonia</i>	100	1
Mesophyll of healthy leaves	QJ3R-1,2		<i>N. macarangae</i>	99.46/99.64	2
Mesophyll of healthy leaves	QJ3R-4		<i>N. oryzae</i>	99.64	1
Veins of diseased leaves	QB8M-2			100	1
Veins of healthy leaves	QJ3M-7		<i>N. pyriformis</i>	100	1
Veins of healthy leaves	QJ3M-2	<i>Aspergillus</i>	<i>A. fumigatus</i>	100	1
Veins of diseased leaves	QB1M-5	<i>Fusarium</i>	<i>F. begoniae</i>	99.66	1
Veins of diseased leaves	QB2M-4, QB5M-3/5		<i>F. verticillioides</i>	99.32/99.68/99.49	3
Veins of diseased leaves	QB3M-6, QB9M-4		<i>F. proliferatum</i>	99.50/100	2
Veins of diseased leaves	QB5M-4		<i>F. temperatum</i>	100	1
Veins of diseased leaves	QB1M-9	<i>Microsphaeropsis</i>	<i>M. arundinis</i>	100	1
Veins of diseased leaves	QB3M-4	<i>Phomopsis</i>	<i>Phomopsis</i> sp.	99.18	1
Veins of healthy leaves	QJ3M-9	<i>Colletotrichum</i>	<i>C. gloeosporioides</i>	99.65	1
Veins of diseased leaves	QB5M-1		<i>C. citri-maximae</i>	99.35	1
Mesophyll of diseased leaves	QB6R-6		<i>C. cordylinicola</i>	99.35	1
Veins of diseased leaves	QB8M-1			99.51	1
Mesophyll of diseased leaves	QB8R-2, QB9R-7		<i>C. aotearoa</i>	100	2
Mesophyll of diseased leaves	QB9R-2, QB10R-8		<i>C. siamense</i>	99.51/100	2
Veins of diseased leaves	QB7M-1			100	1
Mesophyll of diseased leaves	QB6R-5, QB8R-4	<i>Xylaria</i>	<i>X. feejeensis</i>	99.66/100	2
Veins of diseased leaves	QB7M-5			100	1
Mesophyll of healthy leaves	QJ3M-4	<i>Penicillium</i>	<i>Penicillium</i> sp.	99.83	1
Mesophyll of diseased leaves	QB7R-1,4		<i>P. shearii</i>	100	2
Mesophyll of diseased leaves	QB7R-3		<i>P. citrinum</i>	99.66	1
Mesophyll of healthy leaves	QJ1R-3	<i>Nothophysalospora</i>	<i>N. agapanthi</i>	97.74	1
Mesophyll of diseased leaves	QB2R-2			98.12	1
Mesophyll of diseased leaves	QB3R-7	<i>Zasmidium</i>	<i>Zasmidium</i> sp.	99.48	1
Mesophyll of diseased leaves	QB8R-1	<i>Preussia</i>	<i>Preussia</i> sp.	100	1
Veins of diseased leaves	QB6M-2	<i>Daldinia</i>	<i>D. eschscholtzii</i>	100	1
Veins of healthy leaves	QJ2M-6	<i>Diaporthe</i>	<i>D. phaseolorum</i>	100	1
Veins of diseased leaves	QB9M-6			99.67	1
Veins of healthy leaves	QJ3M-5		<i>Diaporthe</i> sp.	100	1
Veins of diseased leaves	QB8M-3		<i>D. chamaeropicola</i>	100	1
Veins of diseased leaves	QB8M-5		<i>D. lithocarpi</i>	100	1
Veins of diseased leaves	QB8M-7		<i>D. limonicola</i>	98.87	1
Veins of diseased leaves	QB9M-5		<i>D. tulliensis</i>	99.83	1
Veins of diseased leaves	QB10M-2		<i>D. miriciae</i>	100	1

The results indicated that all isolated strains were classified within the phylum Ascomycota. The endophytic fungal communities in healthy areca palm leaves exhibited relatively simple compositions at the class, order, and family taxonomic levels. In contrast, the fungal communities present on diseased leaves were more diverse and exhibited greater abundance. The endophytic fungi isolated from healthy leaves were classified into three classes: Sordariomycetes, Dothideomycetes, and Eurotiomycetes. At the order level, six groups were identified: Amphispheariales, Botryosphaerales, Apiosporales, Glomerellales, Eurotiales, and Diaporthales. At the family level, seven groups were recognized: Clypeophysalosporaceae, Phyllostictaceae, Apiosporaceae, Glomerellaceae, Aspergillaceae, and Diaporthaceae. The endophytic fungi present in diseased leaves were classified into three classes: Sordariomycetes, Dothideomycetes, and Eurotiomycetes. At the order level, these fungi encompassed 10 groups, including Amphispheariales, Mycosphaerellales, Botryosphaerales, Apiosporales, Hypocreales, Pleosporales, Diaporthales, Glomerellales, Xylariales, and Eurotiales. Besides, at the family level, 11 groups were identified, namely Clypeophysalosporaceae, Mycosphaerellaceae, Phyllostictaceae, Apiosporaceae, Nectriaceae, Didymosphaeriaceae, Diaporthaceae, Glomerellaceae, Xylariaceae, Aspergillaceae, and Sporormiaceae.

Significant differences were observed in the composition of endophytic fungi across different tissue parts. At higher taxonomic ranks (classes, orders, and families), the richness pattern between tissues exhibited distinct characteristics. At the genus level, regardless of the health status of the leaves, the endophytic fungal community in the veins was more abundant than that in the mesophyll. The endophytic fungi isolated from the mesophyll of

healthy areca palm leaves were classified into two classes (Sordariomycetes, Dothideomycetes), two orders (Amphispheariales, Apiosporales), and two families (Clypeophysalosporaceae, Apiosporaceae). The endophytic fungi isolated from the veins of healthy areca palm leaves belonged to three classes (Sordariomycetes, Dothideomycetes, Eurotiomycetes), five orders (Botryosphaerales, Apiosporales, Glomerellales, Eurotiales, Diaporthales), and five families (Phyllostictaceae, Apiosporaceae, Glomerellaceae, Aspergillaceae, Diaporthaceae). The endophytic fungi isolated from the mesophyll of diseased leaves were classified into three classes (Sordariomycetes, Dothideomycetes, Eurotiomycetes), eight orders (Amphispheariales, Mycosphaerellales, Botryosphaerales, Apiosporales, Pleosporales, Glomerellales, Xylariales, Eurotiales), and eight families (Clypeophysalosporaceae, Mycosphaerellaceae, Phyllostictaceae, Apiosporaceae, Glomerellaceae, Xylariaceae, Aspergillaceae, Sporormiaceae). The endophytic fungi isolated from the veins of diseased leaves belonged to two classes (Sordariomycetes, Dothideomycetes), four orders (Hypocreales, Pleosporales, Diaporthales, Xylariales), and four families (Nectriaceae, Didymosphaeriaceae, Diaporthaceae, Xylariaceae).

Significant differences were observed in the dominant genera of endophytic fungi across various sample types (Fig. 2). Specifically, *Phyllosticta* was the predominant genus in diseased leaves, constituting 20% of the fungal community. Within the mesophyll of diseased leaves, both *Phyllosticta* and *Colletotrichum* were dominant genera, each representing 25%. In the veins of diseased leaves, *Fusarium* was the dominant genus, accounting for 24%. Conversely, *Nigrospora* was the dominant genus in healthy leaves and their respective tissues, comprising 61% of the fungal community in healthy leaves overall, 90% in the mesophyll, and 47% in the veins.



NOTE A. Diseased areca palm leaves; B. Mesophyll of diseased leaves; C. Veins of diseased leaves; D. Healthy leaves; E. Mesophyll of healthy leaves; F. Veins of healthy leaves.

Fig. 2 Proportion of endophytic fungi in different tissues of areca palm leaves at the genus level

4 Conclusions and discussion

In this study, RT-PCR was employed to detect three viruses

(APV1, APYLaOMV, APLTV1) in both healthy and YLD-infected leaves collected from plantations in Qionghai City, Hainan

Province. The results indicated that none of the target viruses were present in the healthy leaves. Among the diseased leaves, the detection rates for APV1, APYLaOMV, and APLTV1 were 60%, 50%, and 30%, respectively. The detection rate of APV1 was somewhat lower than the 75%–100% range reported in previous studies^[12], whereas the detection rate of APLTV1 aligned with findings reported by Niu *et al.*^[14]. These results suggest that multiple viruses may collectively contribute to the manifestation of YLD in areca palms. Furthermore, the data indicate that these three viruses can infect areca palm plants either individually or in combination under natural field conditions, implying that the disease may result from co-infection by multiple viruses.

A total of 81 endophytic fungi were isolated from areca palm leaves and identified as 37 species across 14 genera, all of which belong to the phylum Ascomycota. This finding aligns with previous reports that the endophytic fungi associated with areca primarily derive from the phylum Ascomycota^[18–19]. In healthy leaves, the endophytic fungal community was relatively uniform, with *Nigrospora* identified as the dominant genus. In contrast, the diversity of endophytic fungi in diseased leaves increased significantly, exhibiting greater taxonomic richness at the class, order, and family levels. These results suggest that viral infection may influence the community structure of endophytic fungi in areca palm leaves. This phenomenon confirms the findings of Ma Rui *et al.*^[20], who reported that YLD markedly alters the structure of the phyllosphere microbial community in areca palms. Notably, the present study identified significant differences in the composition of endophytic fungi between the mesophyll and veins of diseased leaves. Fungal diversity was greater in the veins compared to the mesophyll, with the veins harboring a richer fungal community. Specifically, *Phyllosticta* and *Colletotrichum* were the predominant genera in the mesophyll, whereas *Fusarium* was the dominant genus in the veins. This phenomenon may be attributed to variations in the microenvironment across different tissues and the limited distribution of the virus within the phloem. Members of the Closteroviridae family predominantly localize in the host's phloem, exhibiting phloem-restricted infection^[21–22]. This characteristic may contribute to the observed differentiation in the structure of endophytic fungal communities between the veins and the mesophyll. *Colletotrichum* and *Fusarium*, common plant pathogenic fungi, can accumulate extensively in diseased leaves, potentially accelerating disease progression. This observation suggests that viral infection may facilitate the colonization of opportunistic pathogenic fungi by altering the host's microecological environment^[23]. Furthermore, this study identified that in certain diseased samples, only the single virus APV1 was detected; nevertheless, the diversity of endophytic fungi remained significantly higher than in healthy samples. This finding displays that APV1 infection alone may substantially influence the microecology of areca palm leaves.

In conclusion, the incidence of YLD in areca palms is closely associated with the co-infection of multiple viruses and notable successions in the structure of endophytic fungal communities. The

findings of this study provide fundamental data for elucidating the complex pathogenic mechanisms underlying YLD in areca palms and offer a theoretical foundation for the subsequent screening of biocontrol fungi as well as the development of integrated prevention and control strategies.

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tol strategies and rigorously managing all aspects of cultivation, economic losses caused by pests and diseases can be effectively reduced, thereby ensuring the yield and quality of under-forest *A. auricula* and promoting the green and sustainable development of this industry.

4 Conclusions

The under-forest cultivation model of *A. auricula*, recognized as an environmentally sustainable and efficient planting approach, has exhibited significant developmental potential. Forest ecosystems naturally provide shaded and humid conditions, which are advantageous for the cultivation of *A. auricula*. Utilizing these natural benefits not only conserves land resources and reduces production costs but also fosters economic development within forest areas^[10]. Nevertheless, to achieve high-quality yields in under-forest *A. auricula* production, cultivators must possess comprehensive techniques, including site selection, mushroom stick production, spawn running management, and ear emergence management, and effectively address key challenges such as pest and disease control, environmental regulation, and the enhancement of both yield and quality.

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