Fungal Biotec 2(2): 1–15 (2022) ISSN 2774-0323

www.fungalbiotec.org **Article – special issue**
Doi 10.5943/FunBiotec/2/2/1

The endophytic fungi of *Aquilaria sinensis* from southern China

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Du TY, Karunarathna SC, Hyde KD, Mapook A, Wariss HM, Aluthwattha ST, Wang YH, Mortimer PE, Xu JC, Tibpromma S 2022 – The endophytic fungi of *Aquilaria sinensis* from southern China. Fungal Biotec 2(2), 1–15, Doi 10.5943/FunBiotec/2/2/1

Abstract

Agarwood, a dark resin heartwood, is known as one of the most expensive natural products on the planet. In China, agarwood is mainly produced by *Aquilaria sinensis* (Lour.) Spreng. (Thymelaeaceae). Fungal inoculation is the primary mechanism to artificially induce agarwood production. In this study, endophytic fungi were collected and isolated from the branches of three samples distributed over three agarwood plantations originating from the same maternal plant. Through whole chloroplast genome analyses, it was confirmed that the three agarwood-producing trees from the three plantations belong to *A. sinensis*. Fungi isolated from *A. sinensis* samples belonged to five genera viz. *Annulohypoxylon*, *Crassiparies*, *Diaporthe*, *Fusarium*, and *Lasiodiplodia* that are common in all three plantations, while *Fusarium* was the dominant genus in all three plantations. Interestingly, even though the quality of agarwood from the three different plantations (*A. sinensis* GDA1, GDA2 and GDA3) are the same, *A. sinensis* GDA1 contained the least number of endophytic fungal strains when compared with *A. sinensis* GDA2 and GDA3. To determine whether there is a direct relationship between the diversity of fungal community and the quality of agarwood, further research is required.

Keywords – Agarwood – *Fusarium* – Guangdong Province – Thymelaeaceae

Introduction

Agarwood, or Chenxiang (沉香) in Chinese, is well known as a fragrant dark resin heartwood (Naef 2011, Cui et al. 2013). Agarwood is produced in several plant genera belonging to Thymelaeaceae viz. *Aetoxylon*, *Aquilaria*, *Gonystylus*, and *Gyrinops* (Rasool & Mohamed 2016). *Aquilaria* is the main genus that produces agarwood, and *A. sinensis* is the only species cultivated in China (Persoon & Beek 2008, Uddin et al. 2008, Azren et al. 2018, Lv et al. 2019). *Aquilaria* is mainly distributed across Southeast Asian countries, while in China, it is most widely distributed in

Submitted 22 November 2021, Accepted 24 January 2022, Published 28 January 2022
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Guangdong, Guangxi, Hainan, and Yunnan (Blanchette 2003, Cui et al. 2013, Subasinghe et al. 2019). Agarwood is widely used in perfumes, traditional medicine, religious activities, aromatic food ingredients, and incenses (Liu et al. 2013, Chhipa et al. 2017).

Agarwood cannot be produced in healthy trees, however, wounds or infections can trigger the self-defense system of trees to produce secondary metabolites that protect trees from injury (Faizal et al. 2020). The natural production of agarwood is thus rare and slow, as only 7–10% of old trees can produce agarwood, resulting in high agarwood prices on the global market (Xu et al. 2013, Azren et al. 2018, Naziz et al. 2019).

To date, more than 300 compounds have been extracted from agarwood resin, mainly sesquiterpenes and chromones, which are important components of agarwood (Wang et al. 2018). They are important factors of the aroma of agarwood resin, and have important development and application potentials in medicinal fields, e.g., as sedatives and antitumor compounds (Rasool & Mohamed 2016, Wang et al. 2018).

The high economic and medicinal value of agarwood has increased market demand, leading to the overharvesting of agarwood resources and destruction of natural *Aquilaria* forests (Xu et al. 2013, Thanh et al. 2015). Currently, eight species of *Aquilaria* are listed in the International Union for Conservation of Nature's (IUCN) Red List (Yang et al. 2016, IUCN 2021, Tibpromma et al. 2021). To meet market demand and reduce the destruction of naturally occurring agarwood trees, establishing agarwood plantations and artificial induction technology are on the rise in Southeast Asia (Azren et al. 2018).

In China, the artificial induction of agarwood dates to the Song Dynasty (Liu et al. 2013). To date, several methods have been used to produce artificially induced agarwood (Faizal et al. 2017, Azren et al. 2018). Agarwood resins produced by introducing physical and mechanical injury in *Aquilaria* trees result in low-quality product (Faizal et al. 2017). Chemical induction methods harm the environment, and the agarwood yield is not high (Faizal et al. 2017). In artificial induction of agarwood via fungi, some wounds are created in the tree first, and then the fungi are inoculated into the wound (Pojanagaroon & Kaewrak 2005, Rasool & Mohamed 2016). Artificial agarwood induction using fungi was first introduced in 1929 by Tunstall, while it was first introduced to China in 1976 (Gibson 1977, Chen et al. 2017). Researchers have shown that agarwood induction by fungi is effective, and the quality of artificially induced and naturally produced agarwood is similar (Azren et al. 2018, Subasinghe et al. 2019). In addition, most fungi isolated from agarwood resin are endophytic fungi that are also a source of secondary metabolites (Monggot et al. 2017).

Some fungi are known to have the potential to induce the production of agarwood viz. *Aspergillus niger* (Subasinghe et al. 2019), *Fusarium* spp. (Mohamed et al. 2010, Tian et al. 2013, Chen et al. 2017, Faizal et al. 2017, Sen et al. 2017, Subasinghe et al. 2019), *Lasiodiplodia theobromae* (Chen et al. 2017, Huang et al. 2017), *Nemania aquilariae* (Tibpromma et al. 2021), and *Rigidoporus vinctus* (Chen et al. 2018).

In this study, *A. sinensis* samples with agarwood resins and fresh leaves were collected from three plantations in Guangdong. Fresh leaf samples of agarwood-producing trees were used for chloroplast DNA extraction, sequencing, and analysis to identify agarwood tree species from three different plantations. The endophytic fungi were isolated from branches and identified based on ITS sequence data. Furthermore, endophytic fungi profiles from three *A. sinensis* plantations were compared.

Materials & methods

Sample collection

A branch with dark resin and fresh leaves were collected from a representative tree in each of three plantations on 10 December 2020 from Beijing Zhongke Qinan Technology Co., Ltd., Guangdong Province, China. The agarwood-producing trees of the three plantations were propagated from the same maternal plant (*A. sinensis*), and the trees of three plantations were 4-year-old. The three samples were labelled as GDA1, GDA2, and GDA3 ("GDA" refers to *A.*

sinensis samples from Guangdong, while the number after the letters refers to different plantations). Samples were packed in plastic bags, brought to the laboratory in ice boxes and stored in a refrigerator at 4 ℃, until fungal isolations were performed. Collected tree branches with dark resin were used to isolate endophytic fungi, while fresh leaves were used to sequence the chloroplast whole genome of *A. sinensis*.

Surface sterilization of samples and isolation of endophytic fungi

Branches were cut into five short (5 cm) woodblocks using a sterilized wood cutter. The first, third, and fifth short woodblocks were numbered A, B, and C, respectively (Fig. 1). Each short woodblock was cleaned with sterile distilled water with the bark removed, and then moved into the laminar flow cabinet. Wood blocks were cut into 40 pieces (0.5 × 0.5 cm) using a sterilized blade. Surface sterilization was carried out based on Chen et al. (2017) and Tibpromma et al. (2021). Four sterilized wood pieces were placed on a 9 cm potato dextrose agar (PDA) plate, and the spacing between small wood pieces were kept at 4 cm. PDA plates with wood pieces (10 plates) were incubated at 25±2 ℃, and from the second day onwards, fungal colonies were monitored. The viable hyphae at the edge of fungal colonies were selected with sterilized needles and transferred to a new PDA plate to obtain pure cultures. Pure cultures were used for DNA extraction and deposited in the Kunming Institute of Botany Culture Collection (KUMCC), China.

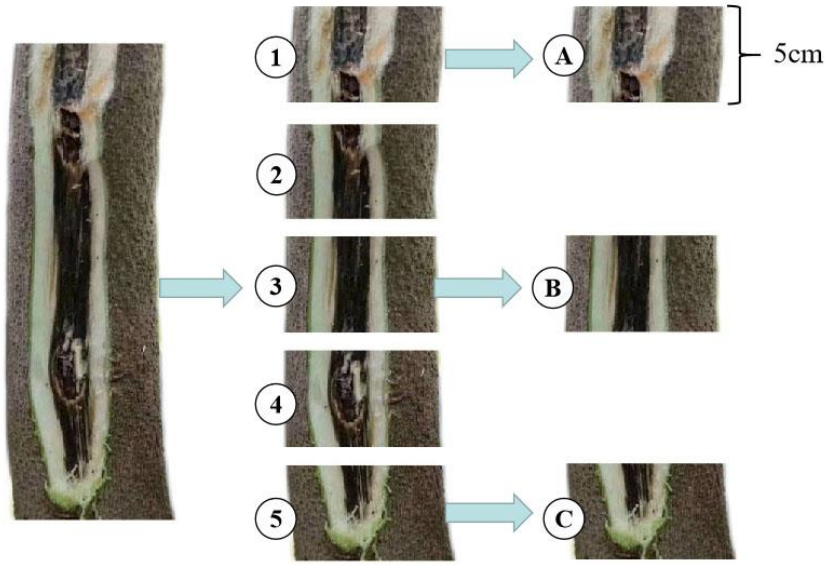

Fig. 1 – Each *Aquilaria sinensis* wood block was cut into five short blocks and the first, third, and fifth blocks were used.

DNA extraction, PCR amplification, and endophytic fungal distribution

DNA was extracted from one-week-old fungal mycelium from pure cultures using the Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux®, P.R. China) following manufacturer instructions. DNA was amplified with primers ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). The total volume of the PCR mixture for amplification was selected following the methods described in Du et al. (2021). The thermal cycling program was performed as 3 min initial denaturation at 95 °C, followed by 35 cycles of 30 s denaturation at 95 °C, 50 s primer annealing at 55 °C, 30 s extension at 72 °C, and a final 10 min extension at 72°C. Purification and sequencing of PCR products were carried out by Qinke Biotech Co., Yunnan, China. All fungal ITS sequences were blasted in GenBank (http://www.ncbi.nlm.nih.gov) for preliminary identification.

Chloroplast genome sequencing and assembly

Fresh leaves collected from *A. sinensis* plantation in Guangdong Province were dried using silica gel. Total genomic DNA of *A. sinensis* was extracted using Biospin Plant Genomic DNA Extraction Kit-BSC13S1 (BioFlux®, P.R. China) by Tiangen Huada Gene Technology Co., Shenzhen, China. The genes with chloroplast genome are listed in Table 1. Illumina Hi-Seq 2500 was used to generate the 150 bp pair-end reads. GetOrganelle pipeline (https://github.com/Kinggerm/GetOrganelle) was used for de novo assembling the chloroplast genome, using SPAdes as the assembler (Bankevich et al. 2012), then visualized in Bandage (Wick et al. 2015) to determine paths of the plastome. We used PGA (Qu et al. 2019) to annotate the chloroplast genome automatically, manually adjusted and visualized in Geneious Prime v.2019.1.3 (Kearse et al. 2012). A final circular chloroplast genome map was drawn using OGDRAW (Lohse et al. 2013).

Table 1 Details of genes/loci with chloroplast genome in *Aquilaria sinensis*.

Category of genes	Group of gene	Category of genes
Self-replication	Small subunit of ribosome	rps2, rps3, rps4, rps7, rps8, rps11, rps12, rps14, rps15, rps16, rps19
	Large subunit of ribosome	rpl2, rpl14, rpl20, rpl22, rpl23, rpl32, rpl33, rpl36
	DNA-dependent RNA polymerase	rpoA, rpoB, rpoC1, rpoC2
	Ribosomal RNA genes	rrn4.5, rrn5, rrn16, rrn23
	Transfer RNA genes	trnA-UGC, trnC-GCA, trnD-GUC, trnE-UUC, trnF-GAA, trnfM-CAU, trnG-GCC, trnG-UCC, trnH-GUG, trnI-CAU, trnK-UUU, trnL-CAA, trnL-UAA, trnL-UAG, trnM-CAU, trnN-GUU, trnP-UGG, trnQ-UUG, trnR-ACG, trnR-UCU, trnS-GCU, trnS-GGA, trnS-UGA, trnT-GGU, trnT-UGU, trnV-GAC, trnV-UAC, trnW-CCA, trnY-GUA
Genes for photosynthesis	Subunits of NADH dehydrogenase	ndhA, ndhB, ndhC, ndhD, ndhE, ndhG, ndhG, ndhH, ndhI, ndhJ
	Large subunit of Rubisco	rbcL
	Subunits of photosystem II	psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL, psbM, psbN, psbT, psbZ
	Subunits of photosystem I	psaA, psaB, psaC, psaI, psaJ, ycf3, ycf4
	Subunits of ATP synthase	atpA, atpB, atpE, atpF, atpH, atpI
	Subunits of cytochrome	petA, petB, petD, petG, petL, petN
Other genes	C-type cytochrome synthesis gene	ccsA
	Envelope membrane protein	cemA
	Subunit of acetyl-CoA	accD
	Maturase	matK
Genes of unknown function	Conserved open reading frames	ycf15

Results

Comprehensive classification based on ITS sequences data

The results of the BLAST search of endophytic fungi sequences in this study are shown in Table 2 and the diversity of endophytic fungi communities is shown in Figure 2.

In GDA1, endophytic fungi were grouped across six genera. In block A: *Annulohypoxylon* sp. (1 strain), *Fusarium* sp. (8 strains), and *Lasiodiplodia* sp. (1 strain). In block B: *Fusarium* sp. (2

strains), and *Trichosporon* sp. (1 strain). In block C: *Curvularia* sp. (1 strain), *Diaporthe* sp. (2 strains), and *Fusarium* sp. (10 strains). In total, twenty strains of *Fusarium* were isolated from all blocks.

In GDA2, the endophytic fungi were grouped in 13 genera. In block A: *Annulohypoxylon* sp. (1 strain), *Diaporthe* sp. (1 strain), *Fusarium* sp. (3 strains), *Lasiodiplodia* sp. (1 strain), *Phlebiopsis* sp. (1 strain), and *Phomopsis* sp. (3 strains). In block B: *Crassiparies* sp. (2 strains), *Daldinia* sp. (2 strains), *Fusarium* sp. (2 strains), *Hypoxylon* sp. (1 strain), *Lasiodiplodia* sp. (2 strains), *Nigrospora* sp. (2 strains), *Phomopsis* sp. (1 strain), *Pseudopithomyces* sp. (1 strain), *Talaromyces* sp. (1 strain), and *Xylaria* sp. (2 strains). In block C: *Diaporthe* sp. (9 strains), *Fusarium* sp. (3 strains), and unidentified endophytic fungi (3 strains). Eight strains of *Fusarium* were isolated from all blocks.

In GDA3, endophytic fungi were grouped in 16 genera. In block A: *Cladosporium* sp. (1 strain), *Colletotrichum* sp. (3 strains), *Corynespora* sp. (1 strain), *Curvularia* sp. (5 strains), *Daldinia* sp. (1 strain), *Diaporthe* sp. (4 strains), *Hypoxylon* sp. (2 strains), *Periconia* sp. (4 strains), and unidentified endophytic fungi (3 strains). In block B: *Annulohypoxylon* sp. (1 strain), *Colletotrichum* sp. (1 strain), *Crassiparies* sp. (4 strains), *Diaporthe* sp. (1 strain), *Massaria* sp. (1 strain), *Neopestalotiopsis* sp. (1 strain), *Paracamarosporium* sp. (3 strains), *Periconia* sp. (1 strain), and unidentified endophytic fungi (3 strains). In block C: *Massaria* sp. (1 strain), *Crassiparies* sp. (2 strains), *Fusarium* sp. (2 strains), *Hermatomyces* sp. (1 strain), *Lasiodiplodia* sp. (1 strain), and *Paracamarosporium* sp. (4 strains). Two strains of *Fusarium* were isolated from block C.

A total of 116 fungal strains were isolated from all three sample blocks, including 26 strains of six genera in GDA1, 39 strains of 13 genera in GDA2, and 51 strains of 16 genera in GDA3. *Fusarium* was the most abundant genus (30 strains), followed by *Diaporthe* (17 strains). Among them, *Annulohypoxylon*, *Crassiparies*, *Diaporthe*, *Fusarium*, and *Lasiodiplodia* were found in GDA1, GDA2, and GDA3 samples.

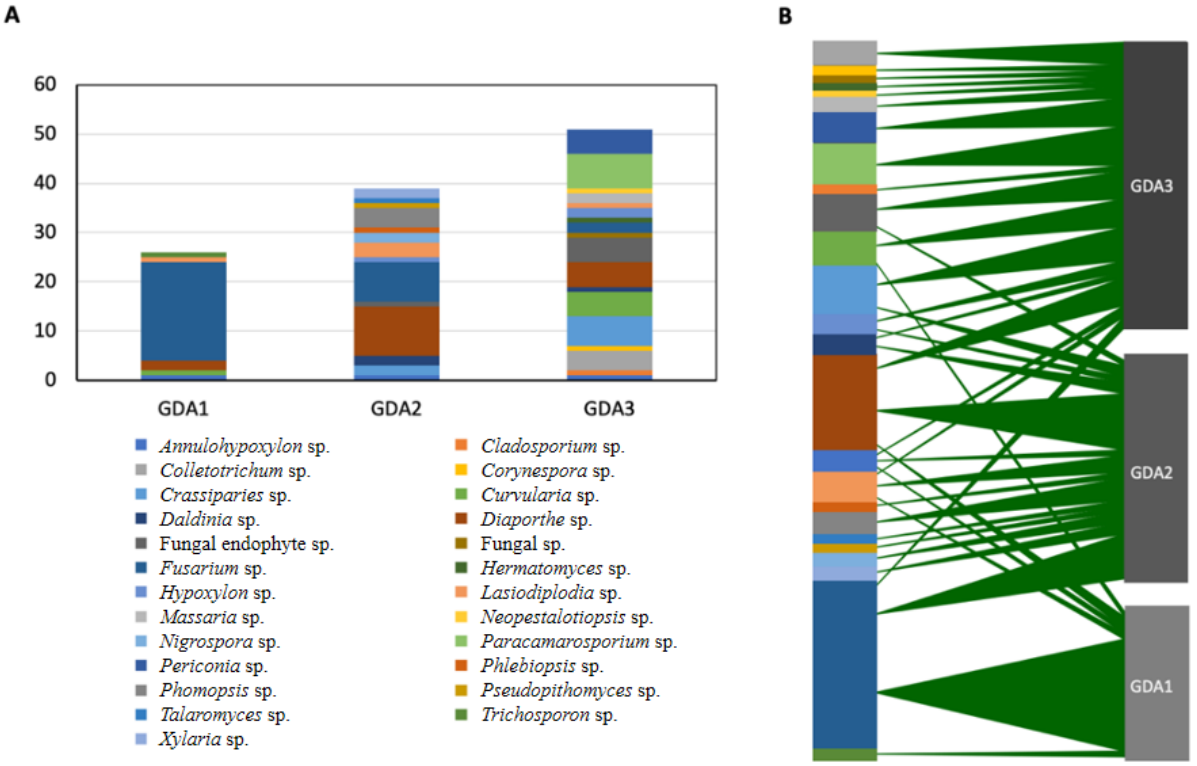

Fig. 2 – Endophytic fungi distribution in *Aquilaria sinensis* plantations. A. Fungal diversity in three different plantations (GDA1, GDA2, and GDA3). B. The fungal communities in three different plantations.

Table 2 Blast results of endophytic fungi in *Aquilaria sinensis* based on ITS.

Isolate number	Culture collection number	GenBank accession number	BLAST search results				
			Closest match	Isolate number	GenBank accession number	Identity	Query coverage
GDA1-A1	KUMCC 21-0218	OL548883	*Fusarium verticillioides*	SMFP6	MT371376	100.00%	100%
GDA1-A2	KUMCC 21-0219	OL548884	*Fusarium verticillioides*	SMFP6	MT371376	100.00%	100%
GDA1-A3	KUMCC 21-0220	OL548885	*Fusarium verticillioides*	3-F5	MT594370	100.00%	100%
GDA1-A4	KUMCC 21-0221	OL548886	*Fusarium verticillioides*	3-F5	MT594370	100.00%	100%
GDA1-A5	KUMCC 21-0222	OL548887	*Fusarium verticillioides*	3-F5	MT594370	100.00%	100%
GDA1-A6	KUMCC 21-0223	OL455016	*Annulohypoxylon thailandicum*	P8	MW488220	100.00%	97%
GDA1-A7	KUMCC 21-0224	OL548888	*Lasiodiplodia theobromae*	ZW 50-1	MT644474	99.78%	100%
GDA1-A8	KUMCC 21-0225	OL548889	*Fusarium verticillioides*	3-F5	MT594370	100.00%	100%
GDA1-A9	KUMCC 21-0226	OL548890	*Fusarium verticillioides*	3-F5	MT594370	100.00%	100%
GDA1-A10	KUMCC 21-0227	OL548891	*Fusarium verticillioides*	3-F5	MT594370	100.00%	100%
GDA1-B1	KUMCC 21-0228	OL455770	*Fusarium verticillioides*	3-F5	MT594370	100.00%	100%
GDA1-B2	KUMCC 21-0229	OL455771	*Fusarium verticillioides*	3-F5	MT594370	100.00%	100%
GDA1-B3	KUMCC 21-0230	OL455772	*Trichosporon asahii*	CU12015.6	MT482659	100.00%	100%
GDA1-C1	KUMCC 21-0231	OL455773	*Diaporthe* sp.	Di7	MT102322	100.00%	100%
GDA1-C2	KUMCC 21-0232	OL455774	*Diaporthe* sp.	Di7	MT102322	100.00%	100%
GDA1-C3	KUMCC 21-0233	OL455775	*Fusarium verticillioides*	3-F5	MT594370	100.00%	100%
GDA1-C4	KUMCC 21-0234	OL455776	*Curvularia* sp.	CCCT 17.73	MN192995	100.00%	100%
GDA1-C5	KUMCC 21-0235	OL455777	*Fusarium verticillioides*	3-F5	MT594370	100.00%	100%
GDA1-C6	KUMCC 21-0236	OL455778	*Fusarium verticillioides*	3-F5	MT594370	100.00%	100%
GDA1-C7	KUMCC 21-0237	OL455779	*Fusarium verticillioides*	3-F5	MT594370	100.00%	100%
GDA1-C8	KUMCC 21-0238	OL455780	*Fusarium verticillioides*	3-F5	MT594370	100.00%	100%
GDA1-C9	KUMCC 21-0239	OL455781	*Fusarium verticillioides*	3-F5	MT594370	100.00%	100%
GDA1-C10	KUMCC 21-0240	OL455782	*Fusarium verticillioides*	3-F5	MT594370	100.00%	100%
GDA1-C11	KUMCC 21-0241	OL455783	*Fusarium verticillioides*	3-F5	MT594370	100.00%	100%
GDA1-C12	KUMCC 21-0242	OL455784	*Fusarium verticillioides*	3-F5	MT594370	100.00%	100%
GDA1-C13	KUMCC 21-0243	OL455785	*Fusarium verticillioides*	3-F5	MT594370	100.00%	100%
GDA2-A1	KUMCC 21-0244	OL455786	*Phomopsis* sp.	LF20	KX510126	100.00%	100%
GDA2-A2	KUMCC 21-0245	OL455787	*Diaporthe miriciae*	LF9	KX398059	100.00%	100%
GDA2-A3	KUMCC 21-0246	OL455788	*Annulohypoxylon annulatum*	C4	FJ481150	97.25%	100%

Table 2 Continued.

Isolate number	Culture collection number	GenBank accession number	BLAST search results				
			Closest match	Isolate number	GenBank accession number	Identity	Query coverage
GDA2-A4	KUMCC 21-0247	OL455789	*Phomopsis* sp.	A0616	KF494825	100.00%	100%
GDA2-A5	KUMCC 21-0248	OL455791	*Fusarium verticillioides*	3-F5	MT594370	100.00%	100%
GDA2-A6	KUMCC 21-0249	OL455792	*Phlebiopsis* sp.	Wu 890805_1	MT561711	100.00%	100%
GDA2-A7	KUMCC 21-0250	OL455793	*Phomopsis* sp.	A0616	KF494825	100.00%	100%
GDA2-A8	KUMCC 21-0251	OL455794	*Fusarium verticillioides*	3-F5	MT594370	100.00%	100%
GDA2-A9	KUMCC 21-0252	OL455795	*Lasiodiplodia pseudotheobromae*	UILRZ4	MT565288	100.00%	100%
GDA2-A10	KUMCC 21-0253	OL455796	*Fusarium verticillioides*	C302	KR350649	100.00%	100%
GDA2-B1	KUMCC 21-0254	OL455797	*Lasiodiplodia* sp.	A10	MT571538	100.00%	100%
GDA2-B2	KUMCC 21-0255	OL455798	*Lasiodiplodia theobromae*	ZW 50-1	MT644474	100.00%	100%
GDA2-B3	KUMCC 21-0256	OL455799	*Hypoxylon* sp.	C.W. Hsieh CHD109	MH777050	99.83%	100%
GDA2-B4	KUMCC 21-0257	OL455800	*Daldinia eschscholtzii*	117_01_02	MT507862	100.00%	100%
GDA2-B5	KUMCC 21-0258	OL455809	*Nigrospora chinensis*	jpsk11	MT561422	100.00%	100%
GDA2-B6	KUMCC 21-0259	OL455810	*Nigrospora chinensis*	jpsk11	MT561422	100.00%	100%
GDA2-B7	KUMCC 21-0260	OL455811	*Fusarium redolens*	JH10-3	MT563395	100.00%	100%
GDA2-B8	KUMCC 21-0261	OL455812	*Daldinia eschscholtzii*	117_01_02	MT507862	100.00%	100%
GDA2-B9	KUMCC 21-0262	OL455813	*Talaromyces* sp.	YBS1-7	MN518430	99.80%	100%
GDA2-B10	KUMCC 21-0263	OL455814	*Phomopsis* sp.	A0616	KF494825	100.00%	100%
GDA2-B11	KUMCC 21-0264	OL455815	*Xylaria* sp.	ZJ12-6B	FJ487924	100.00%	100%
GDA2-B12	KUMCC 21-0265	OL455816	*Fusarium redolens*	JH10-3	MT563395	100.00%	100%
GDA2-B13	KUMCC 21-0266	OL455817	*Crassiparies quadrisporus*	KT 2986	LC271244	100.00%	100%
GDA2-B14	KUMCC 21-0267	OL455818	*Pseudopithomyces maydicus*	SS_20.1	MT497428	100.00%	100%
GDA2-B15	KUMCC 21-0268	OL455829	*Xylaria berteroi*	G1417	MK247857	99.59%	100%
GDA2-B16	KUMCC 21-0269	OL455830	*Crassiparies quadrisporus*	KT 2986	LC271244	100.00%	100%
GDA2-C1	KUMCC 21-0270	OL455831	*Diaporthe* sp.	G1565	MK247830	100.00%	100%
GDA2-C2	KUMCC 21-0271	OL455832	*Diaporthe discoidispora*	NKDL-1-6	MN816410	100.00%	100%
GDA2-C3	KUMCC 21-0272	OL455833	*Fusarium redolens*	JH10-3	MT563395	99.79%	100%
GDA2-C4	KUMCC 21-0273	OL455834	*Diaporthe* sp.	G1565	MK247830	100.00%	100%
GDA2-C5	KUMCC 21-0274	OL455835	*Diaporthe tulliensis*	FJ13-1	MW504754	99.80%	100%
GDA2-C6	KUMCC 21-0275	OL455836	*Diaporthe* sp.	G1565	MK247830	100.00%	100%

Table 2 Continued.

Isolate number	Culture collection number	GenBank accession number	BLAST search results				
			Closest match	Isolate number	GenBank accession number	Identity	Query coverage
GDA2-C7	KUMCC 21-0276	OL455837	*Diaporthe* sp.	G1565	MK247830	99.80%	100%
GDA2-C8	KUMCC 21-0277	OL455838	Fungal endophyte sp.	PP51	EF467438	100.00%	100%
GDA2-C9	KUMCC 21-0278	OL455819	*Fusarium solani*	YMZ1	KY245947	99.79%	100%
GDA2-C10	KUMCC 21-0279	OL455820	*Diaporthe* sp.	G1565	MK247830	100.00%	100%
GDA2-C11	KUMCC 21-0280	OL455821	*Diaporthe* sp.	G1565	MK247830	100.00%	100%
GDA2-C12	KUMCC 21-0281	OL455822	*Fusarium redolens*	JH10-3	MT563395	99.79%	100%
GDA2-C13	KUMCC 21-0282	OL455823	*Diaporthe* sp.	G1565	MK247830	100.00%	100%
GDA3-A1	KUMCC 21-0283	OL455824	*Curvularia* sp.	CCCT 17.73	MN192995	100.00%	100%
GDA3-A2	KUMCC 21-0284	OL455825	*Colletotrichum* sp.	CAYPB44	MT611204	99.79%	100%
GDA3-A3	KUMCC 21-0285	OL455826	*Diaporthe* sp.	USTCMS4001	KY856938	100.00%	99%
GDA3-A4	KUMCC 21-0286	OL455827	Fungal endophyte sp.	PP51	EF467438	100.00%	100%
GDA3-A5	KUMCC 21-0287	OL455828	*Curvularia* sp.	CCCT 17.73	MN192995	100.00%	100%
GDA3-A6	KUMCC 21-0288	OL455839	*Periconia macrospinosa*	1D0102	KT385709	100.00%	87%
GDA3-A7	KUMCC 21-0289	OL455840	*Colletotrichum* sp	CAYPB44	MT611204	99.79%	100%
GDA3-A8	KUMCC 21-0290	OL455841	*Diaporthe* sp.	USTCMS4001	KY856938	100.00%	98%
GDA3-A9	KUMCC 21-0291	OL455842	*Curvularia lunata*	JP89B-1X	MG649266	100.00%	100%
GDA3-A10	KUMCC 21-0292	OL455843	*Curvularia* sp.	CCCT 17.73	MN192995	100.00%	100%
GDA3-A11	KUMCC 21-0293	OL455844	*Daldinia eschscholtzii*	111_03_02	MT507848	99.79%	100%
GDA3-A12	KUMCC 21-0294	OL455845	Fungal sp.	I12F-01373	KC131333	100.00%	94%
GDA3-A13	KUMCC 21-0295	OL455846	*Periconia macrospinosa*	1D0102	KT385709	100.00%	88%
GDA3-A14	KUMCC 21-0296	OL455847	*Curvularia* sp.	CCCT 17.73	MN192995	100.00%	100%
GDA3-A15	KUMCC 21-0297	OL455848	*Diaporthe eucalyptorum*	C12	MK247579	99.80%	100%
GDA3-A16	KUMCC 21-0298	OL455849	*Diaporthe* sp.	ZHKUCC20-0007	MT355677	100.00%	100%
GDA3-A17	KUMCC 21-0299	OL455850	*Periconia macrospinosa*	1D0102	KT385709	100.00%	88%
GDA3-A18	KUMCC 21-0300	OL548882	*Hypoxylon investiens*	FS57	MF770837	99.83%	100%
GDA3-A19	KUMCC 21-0301	OL455851	*Colletotrichum* sp.	CAYPB44	MT611204	99.79%	100%
GDA3-A20	KUMCC 21-0302	OL455852	*Corynespora cassiicola*	CICR-NCS	MN945374	100.00%	100%
GDA3-A21	KUMCC 21-0303	OL455853	*Cladosporium* sp.	18BPLE031	MT645944	100.00%	100%
GDA3-A22	KUMCC 21-0304	OL455854	*Hypoxylon pseudefendleri*	MFLU 15-1200	NR_155173	98.49%	100%

Table 2 Continued.

Isolate number	Culture collection number	GenBank accession number	BLAST search results				
			Closest match	Isolate number	GenBank accession number	Identity	Query coverage
GDA3-A23	KUMCC 21-0305	OL455855	Fungal endophyte sp.	PP51	EF467438	100.00%	100%
GDA3-A24	KUMCC 21-0306	OL455856	*Periconia macrospinosa*	1D0102	KT385709	100.00%	87%
GDA3-B1	KUMCC 21-0307	OL455857	*Diaporthe* sp.	CBS 115584	KC343208	99.38%	100%
GDA3-B2	KUMCC 21-0308	OL455859	*Periconia macrospinosa*	1D0102	KT385709	100.00%	88%
GDA3-B3	KUMCC 21-0309	OL455017	*Massaria pyri*	CBS 125644	NR_157426	94.81%	24%
GDA3-B4	KUMCC 21-0310	OL455860	*Paracamarosporium hawaiiense*	HQD55	KX631728	100.00%	100%
GDA3-B5	KUMCC 21-0311	OL455861	Fungal endophyte sp.	PP51	EF467438	100.00%	100%
GDA3-B6	KUMCC 21-0312	OL455862	*Colletotrichum* sp.	CAYPB44	MT611204	99.79%	100%
GDA3-B7	KUMCC 21-0313	OL455863	*Neopestalotiopsis saprophytica*	YLWB-FBR01	MT576586	99.78%	100%
GDA3-B8	KUMCC 21-0314	OL455864	Fungal endophyte sp.	PP51	EF467438	100.00%	100%
GDA3-B9	KUMCC 21-0315	OL455865	Fungal endophyte sp.	PP51	EF467438	100.00%	100%
GDA3-B10	KUMCC 21-0316	OL455866	*Crassiparies quadrisporus*	KT 2986	LC271244	100.00%	100%
GDA3-B11	KUMCC 21-0317	OL455867	*Crassiparies quadrisporus*	KT 2986	LC271244	100.00%	100%
GDA3-B12	KUMCC 21-0318	OL548892	*Paracamarosporium hawaiiense*	HQD55	KX631728	100.00%	100%
GDA3-B13	KUMCC 21-0319	OL548893	*Annulohypoxylon stygium*	GAB070	KY250375	99.87%	90%
GDA3-B14	KUMCC 21-0320	OL548894	*Crassiparies quadrisporus*	KT 2986	LC271244	100.00%	100%
GDA3-B15	KUMCC 21-0321	OL548895	*Crassiparies quadrisporus*	KT 2986	LC271244	100.00%	100%
GDA3-B16	KUMCC 21-0322	OL548896	*Paracamarosporium hawaiiense*	HQD55	KX631728	100.00%	100%
GDA3-C1	KUMCC 21-0323	OL455015	*Massaria pyri*	CBS 125644	NR_157426	94.81%	23%
GDA3-C2	KUMCC 21-0324	OL548897	*Lasiodiplodia gonubiensis*	PSJ26	KY052901	100.00%	100%
GDA3-C3	KUMCC 21-0325	OL548898	*Paracamarosporium hawaiiense*	HQD55	KX631728	100.00%	100%
GDA3-C4	KUMCC 21-0326	OL548899	*Fusarium verticillioides*	3-F5	MT594370	100.00%	100%
GDA3-C5	KUMCC 21-0327	OL548900	*Fusarium verticillioides*	3-F5	MT594370	100.00%	100%
GDA3-C6	KUMCC 21-0328	OL548901	*Paraconiothyrium* sp.	EF30200031_EF30200031	MT887393	100.00%	97%
GDA3-C7	KUMCC 21-0329	OL548902	*Crassiparies quadrisporus*	KT 2986	LC271244	100.00%	100%
GDA3-C8	KUMCC 21-0330	OL444759	*Paracamarosporium hawaiiense*	HQD55	KX631728	100.00%	100%
GDA3-C9	KUMCC 21-0331	OL548903	*Hermatomyces tectonae*	C371	MK347772	100.00%	100%
GDA3-C10	KUMCC 21-0332	OL548904	*Crassiparies quadrisporus*	KT 2986	LC271244	100.00%	100%
GDA3-C11	KUMCC 21-0333	OL548905	*Paracamarosporium hawaiiense*	HQD55	KX631728	100.00%	100%

Results of chloroplast genome assembly

Genome features

The complete chloroplast genome of *A. sinensis* is 174,909 bp, displaying a typical quadripartite structure of angiosperms. It consists of a large single copy (LSC) region of 85,570 bp and a small single copy (SSC) region of 5,131 bp, and two inverted repeat (IR) regions of 42,103 bp. It harbors 133 genes, including 78 protein-coding genes, 38 tRNA genes and eight rRNA genes. The overall GC content of the chloroplast genome was 36.7%. The three assembled chloroplast genomes had the same number of genes and introns as well as the same gene order (Fig. 3, Table 1). Figure 3 shows that although GDA1, GDA2, and GDA3 come from different plantations, they are all *A. sinensis*. The chloroplast genome sequences have been uploaded to GenBank under the accession numbers GDA1: OL334991, GDA2: OL334992, and GDA3: OL334993.

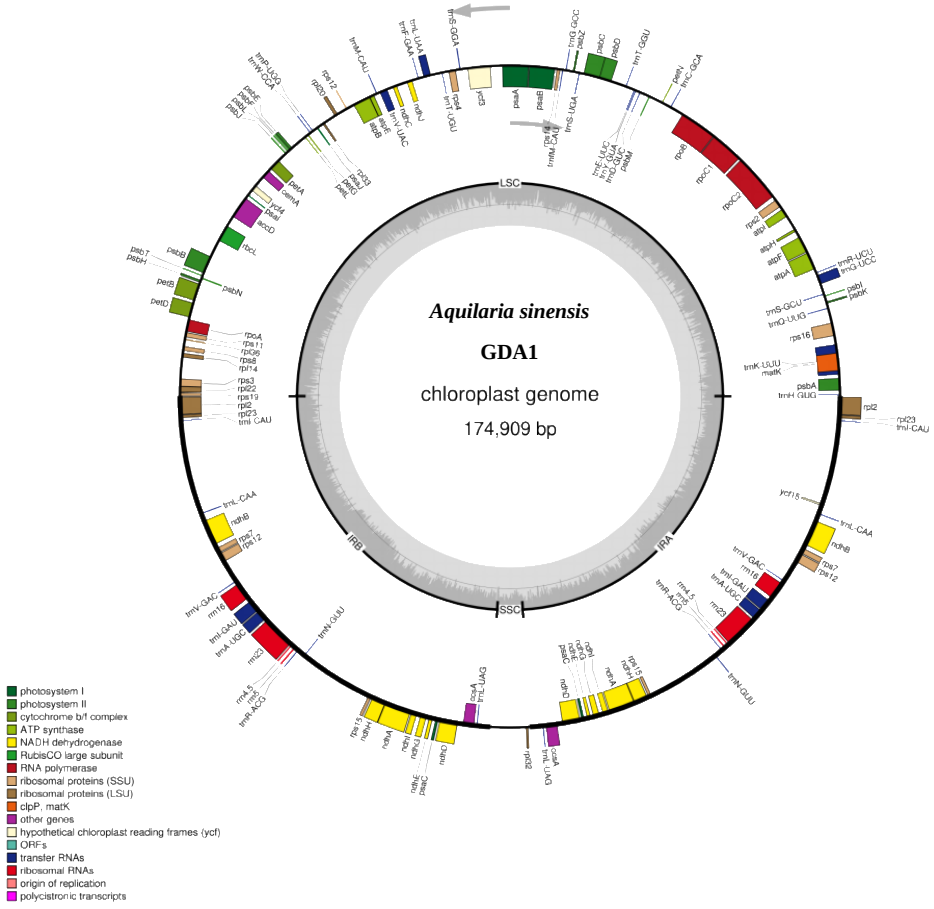

Fig. 3 – Gene map of GDA1, GDA2, and GDA3 chloroplast (cp) genome sequence. Organization of the cp genome of *A. sinensis* showing annotated genes. Genes drawn inside the circle are transcribed clockwise, and those outsides are counterclockwise. Genes belonging to different functional groups are color-coded. The inner circle shows the locations of the large single-copy region, small single-copy, and the pair of inverted repeats (IRa and IRb). The darker grey region in the inner circle corresponds to GC content, whereas the lighter grey region corresponds to AT content.

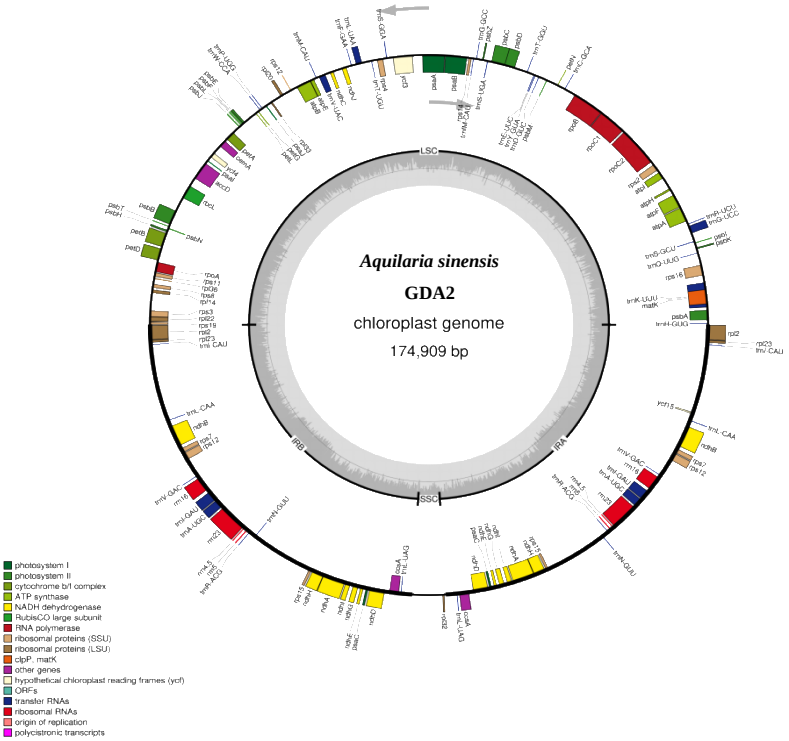

Fig. 3 – Continued.

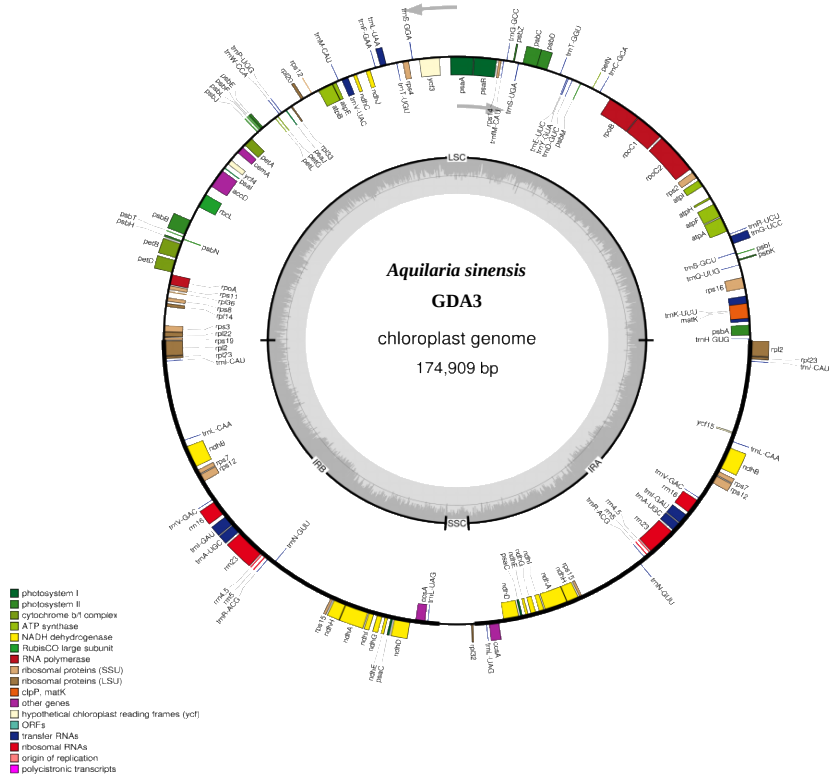

Fig. 3 – Continued.

Discussion

In this study, we collected samples from three *A. sinensis* plantations, derived from the same *A. sinensis* maternal plant, while the trees of the three plantations are 4-year-old. Through analyses of the chloroplast genome, the plants of these three different plantations were identified as *A. sinensis* (Fig. 3).

Through the isolation and identification of endophytic fungi from three samples, we found that the fungal genera *Annulohypoxylon*, *Crassiparies*, *Diaporthe*, *Fusarium* and *Lasiodiplodia* are present in all the three samples, while *Fusarium* is the most dominant genus, followed by *Diaporthe* (Fig. 2). *Fusarium* includes a variety of species that are pathogenic to plants, humans, and animals, and they produce a variety of bioactive secondary metabolites (Leslie & Summerell 2006, Kvas et al. 2009, Proctor et al. 2013, Costa et al. 2021). *Diaporthe* includes important plant pathogens, endophytes, or saprobes with a wide host range (Udayanga et al. 2011, Gao et al. 2017, Mapook et al. 2020).

It has been reported that many fungi in *Fusarium* have the potential to induce the production of agarwood viz. *F. bulbigenium*, *F. lateritium*, *F. moniliforme*, *F. oxysporum*, *F. proliferatum*, *F. sambucinum*, *F. solani*, and *F. tricinctum* (Santoso 1996, Tamuli et al. 2000, Budi et al. 2010, Ma et al. 2012, Faizal et al. 2017). In this study, we assume that the fungal genus *Fusarium* is associated with agarwood, since *Fusarium* was found to be most dominant in GDA1, GDA2, and GDA3.

According to the agarwood company that manages the plantations in Guangdong, the quality of agarwood from GDA1, GDA2, and GDA3 is equivalent. However, our results show that GDA1 has the lowest diversity of endophytic fungi when compared to GDA2 and GDA3. Therefore, whether there is a direct relationship between the quality of agarwood and endophytic fungal community needs further research. In future research, dominant *Fusarium* strains isolated from GDA1, GDA2, and GDA3 should be identified to the species level and their secondary metabolites should be analyzed. In addition, *Fusarium* species isolated in this study will be used for inoculating *A. sinensis* trees to observe whether they can induce agarwood production.

Acknowledgments

Saowaluck Tibpromma thanks the International Postdoctoral Exchange Fellowship Program (number Y9180822S1), CAS President's International Fellowship Initiative (PIFI) (number 2020PC0009), China Postdoctoral Science Foundation and the Yunnan Human Resources, and Social Security Department Foundation for funding her postdoctoral research. Samantha C. Karunarathna thanks the CAS President's International Fellowship Initiative (PIFI) young staff under the grant number: 2020FYC0002, and the National Science Foundation of China (NSFC) under the project code 31851110759. Kevin D. Hyde thanks the Thailand Research Funds for the grant "Impact of climate change on fungal diversity and biogeography in the Greater Mekong Subregion (RDG6130001)". Peter E. Mortimer thanks the National Science Foundation of China (NSFC), project codes 41761144055 and 41771063 for financial support.

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