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Seasonal and Habitat Dependent Variations in Culturable Endophytes of *Camellia sinensis*

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Abstract

Seasonal and habitat dependent variations in the endophytes of *Camellia sinensis* are investigated in this study. Stems and leaves of *C. sinensis* from naked and under-forestry tea garden have been collected for isolating endophytic fungi in January, April and October, respectively, as different seasons. Twenty-one endophytic species including a new species, are observed in this study. It is confirmed that species in under-forestry tea garden are more related to the naked tea garden. However, both habitats have similar evenness indices and Shannon-Wiener indices. Shannon-Wiener indices in spring are highest in all compared seasons. The diversity of species in stem is higher than in leaf. In all seasons, the evenness indices are slightly higher in leaf than stem. The number of endophytic fungi in autumn is the smallest, while the largest in winter. Furthermore, the frequencies of *Neurospora crassa, Phomopsis* sp4., *Trichoderma viride*, *Phomopsis* sp2., *Pleosporales* sp., *Pestalotiopsis microspora*, *Glomerella* sp., *Colletotrichum gloeosporioides, Botryosphaeria* sp., *Penicillium sclerotiorum* and *Rosellinia* sp. vary significantly in different habitats. The composition and diversity of endophytic fungi are different between leaves and stems. *Guignardia mangiferae, T. viride, P. sclerotiorum, Pleosporales* sp., *Phomopsis* sp4., *C. gloeosporioides, P. clavispora, Glomerella* sp. and *N. crassa* show remarkable organizational preference in tea plants.

Keywords: *Camellia sinensis;* Endophytes; Season; Habitat; Organizational preference

Introduction

Endophytes are organisms inhabiting the living plant organs at some time during their lives, without causing apparent harm to the host [1]. Many studies have studied various geographical and climatic zones which are ubiquitous, and occurred within all examined plants, including a broad range of host orders, families, genera and species in various ecosystems [2].

The diversity of microbial symbionts associated with plants plays an important role in the nutrition and the adaptation of hosts to biotic and abiotic stress, thus evolving the holobiont. The genetic diversity of the microbial consortium can extend the range of the environment where hosts will survive [3,4]. Endophytic fungi may influence the plant metabolic state through communication and transduction, and contribute genes and relevant bioactive products [5]. Tea polyphenols (TPs) content in tea plant, which would change tea quality, may be influenced by endophytes. Recent literature reported that climate anomalies could affect tea production, and endophytes may play important roles in enhancing the tolerance of tea plant to the changing environments.

Endophytic fungi have a wide range of species, and have close ecological relationships with other organisms, which are usually considered as a potential source of natural active substance. It's well known that secondary metabolites in tea, e.g. TPs, caffeine and theanine, which are important antioxidant and anticancer agent [6]. Current methods of extracting these metabolites from tea plant were high-cost and low-output. Endophytic fungi isolated from tea plant may be another way to obtain these bioactive products by fermentation.

Few studies about endophytic fungi of *Camellia sinensis* have been investigated, compared with other important economic crops. In 1925, mycorrhizal in tea plant was firstly discovered [7]. Thereafter, the studies about endophytes in tea plant have been concentrated on the mycorrhizal for a long time [8-10]. Systematic research of endophytic fungi in tea plant did not start, till the beginning of 21 century. Some preliminary studies have shown that there is organizational preference of endophytic fungi in tea plant [11-13]. *The Guignardia sp., Pestalotiopsis sp., Colletotrichum sp*. and *Aspergillus niger* were considered as the dominant species of endophytic fungi in tea garden of Fujian province, China [14,15]. Adding TPs into potato dextrose agar medium (PDA medium) can inhibit the growth of some species of endophytic fungi from tea plant, thus the chemical composition of tea leaves may be an important factor that affects the distribution of endophytes [15]. There was no report on seasonal and habitat dependent variations in the endophytic fungi of *Camellia sinensis*. However, the accumulation levels of secondary metabolites, such as polyphenol and caffeine, which have an impact on microorganism propagation under different seasons and environment, were in huge variant. In this study, endophytic fungi variation observed in *Camellia sinensis*, under different environment from different tissues, were performed.

Materials and Methods

Study site

The study site was chosen in the Zijin hill of Nanjing City (31° N, 148°49′ E, 150 m a.s.l.), China. The annual average temperature is 16°C, and the annual precipitation is 1106 mm.

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Sample collection

Healthy plants of *C. sinensis* were collected from naked tea-garden and under-forestry tea-garden (planted over 10 years), by alternate sampling method. The canopy density of forest is 57.3%, surveyed by system sampling method. One branch about 4-5 mm in diameter, and included leaves was collected while sampling. 100 tea plants were collected from each tea garden every time, and time intervals were September 2010, December 2010 and April 2011. A stem from each branch, with the length of 7 cm and diameter of 3 mm, which was fully lignified, was intercepted. A mature material that had 4-5 leaves was chosen from the terminal bud. Both stems and leaves were used for isolating endophytic fungi after surface sterilization.

Fungal isolation and initially identification

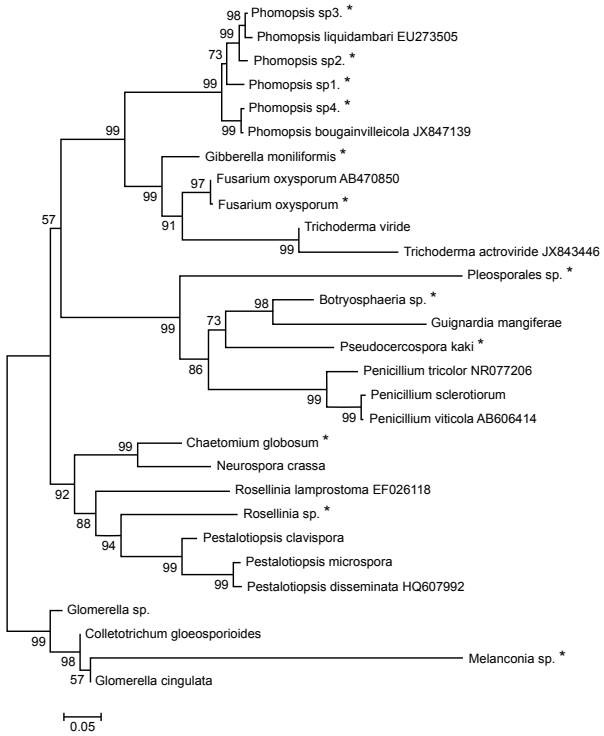
For surface sterilization, samples were immersed in water with saturated detergent for 0.5 hr, then washed with tap water and irrigating for 2 hr. Then, the samples were soaked with 75% ethanol for 30 sec and rinsed three times with sterile distilled water, then disinfect with 1.5% sodium hypochlorite (leaves soaked for 3 min, while the stems soaked for 8 min). Before use, they were rinsed with sterile distilled water 5 times.

Cut a leaf square (side length is 0.5 cm, and away from the petiole of whole leaf's 1/3 length, and one side is cut along the main vein) from each leaf with sterile scalpels and surgical blades. Stem sections were intercepted from the middle of each stem (about 1 cm in length, and spitted in half along the central longitudinal). Samples were plated on PDA medium. 200 μL sterile water was used for rinsing plant tissue to a blank PDA plates as CK-W, to determine whether the surface sterilization completely. The unused plant tissue were placed on blank PDA plate and frictional contact for 2 min. Remove the plant tissue and marked these plates as CK-L (leaf blot) and CK-S (stem blot), to determine whether the materials have contaminated in aseptic.

The plates were incubated at 25°C for 7 days in dark. Purify fungal isolates by Single-spore method, and hyphal-tip method for those species that are hard to get spore. Use micro-morphological observations to make a preliminary division of these fungi.

Fungal DNA extraction, PCR amplification of ITS region, electrophoresis and identification

The pure cultures (subcultured for 2-3 times), were used for the DNA extraction. Fungal DNA was extracted from 0.2 g semidry mycelia, according to the method of Stirling [16]. The target rDNA region, including Internal Transcribed Spacer 1, Internal Transcribed Spacer 2 regions and 5.8S gene was amplified using primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) (Figure 1). Amplifications were performed in a total reaction volume of 25 μL, containing 1×PCR buffer, 2.5 mM $MgCl_2$, 2 mM dNTP, 0.5 unit of ExTaq polymerase, 10 pM of each primer (Genescript Corp., China), and 50 ng of template DNA. PCR amplification was performed in a thermal cycler (Takara Corp., Japan), programmed as: 94°C for 8 min; 94°C for 30 s, 55°C for 40 s, 72°C for 60 s, 35 cycles; final extension at 72°C for 10 min. The amplification products were separated by electrophoresis on 1.2% (W/V) agarose gel stained with ethidium bromide (0.5 μg/mL), and visualized under 300 nm UV light and photographed. DL2000 marker (Takara Co., Japan) was used as reference. Amplification products obtained from PCR reaction which extracted from agarose gel by AxyPrep DNA Gel Extraction Kit (Axygen Corp. USA), were used for sequencing. Sequencing reaction was carried out by ABI 3730 Avant Genetic analyzer (Genescript Corp. Nanjing City, Jiangsu Province, China).



Note: *means the fungus is a rare species in tea plant

Figure 1: Neighbour-joining analysis of ITS and 5.8S rDNA sequences of endophytic fungi isolated from *Camellia sinensis*.

DNA sequence obtained for each strain from each forward and reverse primer (ITS1 and ITS4) were inspected individually for quality. Both strands of the DNA were assembled to produce a consensus sequence for each strain, using BioXM software v2.6.0 (Nanjing Agricultural University, China). The sequences were submitted to the NCBI (National Centre for Biotechnology and Information), and accession numbers were obtained, then aligned with closely matching GenBank and UNITE databases by BLASTn search. Identification of these fungi to species or genus was performed, according to the results of micro-morphological observations and molecular alignment.

Statistical analysis

The Infection Rate (IR%) of a single species was calculated as the percentage of the number of plant tissue containing the species, divided by the whole tissue in a same tested [17]. The Relative Abundance (RA%) of a single species was calculated as the percentage of the number of this species isolated, divided by the number of whole species of fungus isolated in a same tested.

Shannon-Wiener index was used to evaluate the diversity of tea-plant's endophytic fungi in different habitats at different seasons [18]: $H' = -\sum_{i=1}^{s} P_i \ln P_i$, where S is the number of the species in tested, P_i is the number of species i isolated, divided the number of all species of fungus isolated.

Pielou index was used to evaluate the evenness:

$J=H'/H_{max}$, where $H_{max}=\ln S$.

Margalef index was used to evaluate the species richness:

$R=(S-1)/\ln N$, where N is the number of all species of fungus isolated.

Coefficient of community (Cc) was calculated to examine the similarity of fungal assemblages:

$Qs=2a/b+c$, where a is the number of common species, and b and c are the numbers of species to two different tested respectively [18].

The analyses were performed on DPS (Data Processing System) v7.05 software (Refine Information Tech. Co., LtD, China) [19].

Phylogenetic relationship analysis

Sequence alignments were performed using Clustal X software version 2.0, with default settings [20]. The phylogenetic tree was constructed by the Neighbour-joinning method, using MEGA 4.0 [21]. Test of inferred phylogeny used bootstrap with 1000 replicates, and all positions containing gaps and missing data were eliminated by complete deletion option. P-distance of nucleotide was chosen as the substitution model [22].

Results

Infection rate and species of endophytic fungi

A total of 1,258 endophytic fungi were isolated from 568 tea plant tissue, and 21 endophytic species investigated in this study, including a new species which has not been reported (GeneBank JQ809664-JQ809684). More than 25% difference exist in the ITS section between this species, and its most similar known fungi. We tentatively considered it as a new species of *Melanoconiales*, but further information was required to determine its genus and species. Another species was identified as a new species of *Gloeosporium*, which was firstly isolated from tea plant, which only isolated from Rainforest Plants before (GeneBank JN418782). This endophyte has 97% identities compared with *Colletotrichum gloeosporioides* or *Glomerella septospora*, tentatively named as *Gloeosporium sp*.

Infection rate of endophytic fungi of stem, no matter in under-forestry or naked tea-garden is 100%, but varied significantly with season and habitat in leaf. In autumn and spring, under-forestry tea-garden showed a higher infection rate of endophytic fungi, compared with naked tea-garden (97.73% and 72.22% in autumn, 80.77% and 62.00 in spring), and no significant differences were found in winter (97.92% and 95.83%), while the number of species showed the similar trend.

The total number of 151 fungi isolated from leaf was observed a highest value in winter (79 from under-forestry tea-garden and 72 from naked tea-garden). However, total of 342 fungi was observed from stem in spring (214 from under-forestry tea-garden, and 128 from naked tea-garden). It is the only test in which the fungi isolated from naked tea-garden is more than in under-forestry tea-garden (192>159) (Table 1).

Variation in non-rare species

10 fungal species were considered as not-accidental endophytic fungus (Table 2). The frequencies of *Neurospora crassa, Phomopsis sp4., Trichoderma viride, Phomopsis sp2., Pleosporales sp., Pestalotiopsis microspora, Glomerella sp., Colletotrichum gloeosporioides, Botryosphaeria sp., Penicillium sclerotiorum* and *Rosellinia sp.* varied significantly with habitat. Frequencies of *Gloeosporium* (including *G. cingulata* and *Glomerella sp*.) were higher in under-forestry habitat, while the frequencies of *N.crassa, T.viride* and *P. sclerotiorum* were higher in naked habitat. The varied of the frequencies of *P. clavispora* should be attended; this fungus was frequent in stems from under-forestry tea-garden, but rare in stems from naked tea-garden and rare in leaves.

Differences exist in composition and diversity of endophytic fungi between in leaves and stems. *Phomopsis sp4*., most frequent in stems, while infection rates of *Glomerella sp*., *C. gloeosporioides* and *N. crassa* are also higher than 40%. The infection rate and relative abundance of *Glomerella sp*. in stems from under-forestry tea-garden were significantly more than in stems from naked tea-garden. However, *N. crassa* showed a contrary trend. These result indicated that not only organizational preference, but also habitat preference were remaining in these two species fungi. *C. gloeosporioides* was most frequent in leaves from under-forestry tea-garden (27.78%). Only infected were 12.69% leaves from naked tea-garden. *Phomopsis sp4*. (20.83%) was the second most frequent species in leaves from under-forestry tea-garden. *P. sclerotiorum* was most frequent in leaves from naked tea-garden (32.09%), and *T.viride* (17.91%) was the second frequent species. *P. sclerotiorum* was only infected 20.14% leaves from under-forestry tea-garden.

Various numbers of endophytic fungi were obtained from different seasons. Overall, the total number of fungi isolated in autumn was significantly less than in winter and spring, of which the largest number of fungi isolated in winter. This is different from previous studies [13,23]. The most dominant species in different seasons are *Phomopsis*

		Leaf					Stem					Total		
Season	**Habitat**	**No. of samples**	**No. of samples yielding fungi**	**No. of isolates**	**No. of species**	**Infection Rate (%)**	**No. of samples**	**No. of samples yielding fungi**	**No. of isolates**	**No. of species**	**Infection Rate (%)**	**No. of isolates**	**No. of species**	**Infection Rate (%)**
Oct.	Under-Forestry	44	43	76	13	97.73	44	44	94	10	100.00	170	15	98.86
	Naked	36	26	44	8	72.22	48	48	83	10	100.00	127	13	88.10
	Total	80	69	120	13	86.25	82	82	177	12	100.00	297	15	95.83
Jan.	Under-Forestry	48	47	79	11	97.92	48	48	159	11	100.00	238	13	98.96
	Naked	48	46	72	11	95.83	48	48	192	11	100.00	264	12	97.92
	Total	96	93	151	11	96.88	96	96	251	12	100.00	502	13	98.44
Apr.	Under-Forestry	52	42	74	14	80.77	52	52	214	16	100.00	288	18	90.38
	Naked	50	31	43	10	62.00	50	50	128	14	100.00	171	15	81.00
	Total	102	73	117	14	71.57	102	102	342	17	100.00	459	18	85.78
Total	Under-Forestry	144	132	229	16	91.67	144	144	467	17	100.00	696	18	95.83
	Naked	134	103	159	12	76.87	146	146	403	15	100.00	562	15	88.93
	Total	278	235	388	16	84.53	280	280	770	17	100.00	1058	18	93.09

Table 1: Effects of season, habitat, and plant tissue on the infection and isolation rates of endophytic fungi.

	Season	Under-foresty				Naked				Total	
		Leaf		Stem		Leaf		Stem			
		IR (%)	RA (%)	IR (%)	RA (%)	IR (%)	RA (%)	IR (%)	RA (%)	IR (%)	RA (%)
Phomopsis sp4.	Oct.	27.27	18.18	40.91	19.78	5.56	5.13	50.00	30.00	32.56	20.29
	Jan.	22.92	14.86	72.92	23.18	6.25	4.29	85.42	22.91	46.88	18.99
	Apr.	13.46	13.21	92.31	26.23	4.00	5.41	84.00	35.59	48.53	25.32
	Total	20.83	15.54	70.14	23.76	5.22	4.79	73.29	28.38	43.13	21.47
Neurospora crassa	Oct.	11.36	7.58	22.73	10.99	16.67	15.38	29.17	17.50	20.35	12.68
	Jan.	35.42	22.97	39.58	12.58	20.83	14.29	89.58	24.02	46.35	18.78
	Apr.	5.77	5.66	42.31	12.02	4.00	5.41	22.00	9.32	18.63	9.72
	Total	17.36	12.95	35.42	12.00	13.43	12.33	46.58	18.04	28.52	14.20
Trichoderma viride	Oct.	2.27	1.52	N/A	N/A	N/A	N/A	2.08	1.25	1.16	0.72
	Jan.	10.42	6.76	4.17	1.32	31.25	21.43	20.83	5.59	16.67	6.75
	Apr.	13.46	13.21	13.46	3.83	18.00	24.32	8.00	3.39	13.24	6.91
	Total	9.03	6.74	6.25	2.12	17.91	16.44	10.27	3.98	10.74	5.35
Pestalotiopsis clavispora	Oct.	4.55	3.03	6.82	3.30	N/A	N/A	4.17	2.50	4.07	2.54
	Jan.	N/A	N/A	56.25	17.88	N/A	N/A	8.33	2.23	16.15	6.54
	Apr.	3.85	3.77	36.54	10.38	N/A	N/A	8.00	3.39	12.25	6.39
	Total	2.78	2.07	34.03	11.53	N/A	N/A	6.85	2.65	11.09	5.52
Pestalotiopsis microspora	Oct.	2.27	1.52	2.27	1.10	N/A	N/A	2.08	1.25	1.74	1.09
	Jan.	14.58	9.46	16.67	5.30	6.25	4.29	18.75	5.03	14.06	5.70
	Apr.	5.77	5.66	17.31	4.92	N/A	N/A	10.00	4.24	8.33	4.35
	Total	7.64	5.70	12.50	4.24	2.24	2.05	10.27	3.98	8.27	4.12
Glomerella cingulata	Oct.	2.27	1.52	N/A	N/A	8.33	7.69	N/A	N/A	2.33	1.45
	Jan.	8.33	5.41	10.42	3.31	6.25	4.29	16.67	4.47	10.42	4.22
	Apr.	7.69	7.55	15.38	4.37	8.00	10.81	10.00	4.24	10.29	5.37
	Total	6.25	4.66	9.03	3.06	7.46	6.85	8.90	3.45	7.92	3.94
Glomerella sp.	Oct.	N/A	N/A	95.45	46.15	N/A	N/A	27.08	16.25	31.98	19.93
	Jan.	14.58	9.46	60.42	19.21	8.33	5.71	70.83	18.99	38.54	15.61
	Apr.	5.77	5.66	76.92	21.86	2.00	2.70	60.00	25.42	36.27	18.93
	Total	6.94	5.18	77.08	26.12	3.73	3.42	52.74	20.42	35.74	17.79
C. gloeosporioides	Oct.	54.55	36.36	9.09	4.40	11.11	10.26	29.17	17.50	26.74	16.67
	Jan.	25.00	16.22	54.17	17.22	22.92	15.71	60.42	16.20	40.63	16.46
	Apr.	7.69	7.55	53.85	15.30	4.00	5.41	32.00	13.56	24.51	12.79
	Total	27.78	20.73	40.28	13.65	12.69	11.64	40.41	15.65	30.63	15.25
Penicillium sclerotiorum	Oct.	4.55	3.03	29.55	14.27	22.22	20.51	22.92	13.75	19.77	12.32
	Jan.	20.83	13.51	N/A	N/A	39.58	27.14	N/A	N/A	15.10	6.12
	Apr.	32.69	32.08	1.92	0.55	32.00	43.24	2	0.85	17.16	8.95
	Total	20.14	15.03	9.72	3.29	32.09	29.45	8.22	3.18	17.25	8.59
Guignardia mangiferae	Oct.	40.91	27.27	N/A	N/A	44.44	41.03	N/A	N/A	19.77	12.32
	Jan.	2.08	1.35	N/A	N/A	4.17	2.86	2.08	0.56	2.08	0.84
	Apr.	5.77	5.66	1.92	0.55	2.00	2.70	N/A	N/A	2.45	1.28
	Total	15.28	11.40	0.69	0.24	14.18	13.01	0.68	0.27	7.57	3.77

Table 2: Infection rates (IR) and relative abundance (RA) of non-rare endophytic fungi species in tea plant.

sp4. The order of the other dominant species has difference in different seasons. *Glomerella sp*. was the second dominant species in autumn and spring. However, its relative abundance was only 14.74% in winter. On the contrary, the relative abundance of *N. crassa* is increased greatly in winter, and become to the second dominant species. Some fungi of Melanconiaceae (including *P. clavispora, Glomerella sp*. and *C. gloeosporioides*) showed the same trend in infection rates. This may be related to the Melanconiaceae fungus, prone to produce spores, and the spores have a high tolerance of extreme environment. Compared with in autumn, *T. viride, P. clavispora, P. microspora* and *G. cingulata* have a higher infection rates, and relative abundance in winter and spring. It indicates that these fungi might be sensitive to high temperature, and high temperature is might one of the main factors of their growth and infection. In contrast, *G. mangiferae* have the highest infection rate and relative abundance in autumn, and a higher temperature might be more suitable for its colonization and proliferation.

Diversity and similarity of endophytic fungi

The Shannon-Weaver diversity indices of endophytic fungi in these tests, from high to low, were under-forestry tea-garden (3.6922)>naked tea-garden (3.1893) in habitats, and Apr. (2.4562)>Jan. (2.2413)>Oct. (2.1657) in seasonal. In addition, there was much higher Shannon-Weaver diversity indices of endophytic fungi in stems, than in leaves of these habitats or seasons (Tables 3 and 4).

Coefficients of endophytic fungi community were 0.5263-1.0000, among the tests with different seasons, different habitats and different plant organizations. There was the highest similarity (1.0000) of endophytic communities, between the leaves from different habitats in winter, and the lowest similarity (0.5263) between winter's stems from under-forestry tea-garden and spring's leaves from naked tea-garden. There always have a high similarities (>0.75), when only one condition changes. Among the different seasons, the coefficient community are

	Leaf				Stem				Total			
	Oct.	Jan.	Apr.	Total	Oct.	Jan.	Apr.	Total	Oct.	Jan.	Apr.	Total
Shannon-Wiener index (H')	1.9586	2.1699	2.4096	2.4390	1.6261	1.9963	2.3102	2.2017	2.1281	2.2010	2.5265	3.6922
Evenness index (J)	0.7636	0.9049	0.9131	0.8797	0.7062	0.8325	0.8332	0.7771	0.7858	0.8581	0.8741	1.2774
Richness index (S)	13	11	14	16	10	11	16	17	15	13	18	18
Total Isolated	76	79	74	229	94	159	214	467	170	238	288	696

Table 3: Shannon-Wiener indices of endophytic fungi from Camellia Sinensis in under-forestry tea-garden.

	Leaf				Stem				Total			
	Oct.	Jan.	Apr.	Total	Oct.	Jan.	Apr.	Total	Oct.	Jan.	Apr.	Total
Shannon-Wiener index (H')	1.8147	2.0157	1.8828	2.1487	1.8567	2.0060	1.9944	2.0730	2.1369	2.1772	2.2563	3.1893
Evenness index (J)	0.8727	0.8406	0.8177	0.8647	0.8063	0.8366	0.7557	0.7655	0.8331	0.8762	0.8332	1.1777
Richness index (S)	8	11	10	12	10	11	14	15	13	12	15	15
Total Isolated	44	72	43	159	83	192	128	403	127	264	171	562

Table 4: Shannon-Wiener indices of endophytic fungi from Camellia Sinensis in naked tea-garden.

0.8387-0.9286, and are 0.9091-0.9600 between different habitats, and 0.8000-0.8696 between leaves and stems in the same season.

Molecular phylogenetic analysis

Endophytic fungi were rich in molecular diversity in tea-plant. The *Sphaeropsidaceae* and *Melanconiaceae* were the most abundant families. The *Phomopsis* was the most abundant genus. Two new species (*Melaconia* sp. and *Pleosporales* sp.), and known species have a large genetic distance. Another new species, *Glomirella* sp., was similar to other known *Glomirella* species. These three species might be the kind of specific endophytic fungi that symbiosed and coevolved with tea plant.

Discussion

The colonization rates (62.00-100.00%) of endophytic fungi in *C. sinensis* are high in the present study, and similar results were obtained in previous reports [14,24]. This can be due to the fact that the *C. sinensis* is a kind of woody perennials plants.

Some endophytes and some pathogenic fungi of tea plant are the same species, such as *Phomopsis* and *Gloeosporium*. But, it is found that ITS sequences of these fungi are not exactly same with a few Single-nucleotide polymorphism between endophytes and pathogenic fungi. The fact is reminiscent of the symbiotic compromise established by tea plant and pathogenic fungi. The result is consistent with previous report.

It is found that the colonization rates of endophytic fungi are higher in stems than in leaves. A possible reason is that the plant organizational substrates contain different components, like polyphenols. The antimicrobial activity may influence the colonization and distribution of endophytic fungi [25-27].

Compared with naked tea-garden, under-forestry tea-garden has good diversity and richness of species. However, there are similar evenness indices and Shannon-Wiener indices. The high similarity between the number of endophytic fungi species isolated from under-forestry tea-garden and naked tea-garden might be related with the long process of evolution and cultivation. Tea plant and its endophytic fungi might form a stable relationship during the evolution. These factors contribute to the diversity and stability of endophytic fungi in *C. sinensis*.

Shannon-Wiener indices in spring are higher than winter and autumn. The difference is more in stem than in leaf. Generally, endophytic fungi in leaf have a better diversity then in stem. But, we cannot rule out the case that some of dominant species from stem grow too fast in medium and inhibited some fungi which grow weak, and leading the result of isolated less fungal species. Endophytic fungi in leaf and stem, both show higher Evenness indexes in winter, while they are lowest in autumn. But in all seasons, the Evenness indexes always slightly higher in leaf than in stem.

Seasonal changes of fungal population may reveal the endophytic fungi infection, growth condition in different seasons at certain level. Spring is the active season of infection; during this period, a large number of fungal species colonized in tea-plant, and gradually to be in a dynamic equilibrium through continuous interspecific competition and the role of environmental factors. In this process, some fungal species disappeared, while the dominant position of dominant species was strengthened gradually. So, the diversity indices and envenness indices were low in autumn. Endophytic fungi, mainly in dormant state during winter, the number of fungi isolated were the maximum in this study during this period. The possible reason is that the sclerotium and spores were in large quantities in winter, and they were difficult to lose activities during the surface disinfection. Interspecific competition is not active in winter, because of the reduced physiological activites of endophytic fungi. This was the foundation for a new round of fungal infection and competition in spring. However, this inference requires further research to validate. The activity of secondary metabolites is different throughout the year, which is also an important factor affecting of the endophytes' seasonal variation.

Many endophytic fungi have shown the organizational preference in tea plant. *G. mangiferae, T. viride, P. sclerotiorum* and *Pleosporales sp*. preferred to colonize in leaves, while *Phomopsis sp4*., *C. gloeosporioides, P. clavispora, Glomerella sp*. and *N. crassa* showed a strong preference in stems. Habitat preference also expressed in some endophytic fungi. *Pleosporales sp., P. clavispora, Phomopsis sp2*., *P. microspora, Glomerella sp.* and *C. gloeosporioides* showed a strong preference in under-forestry tea garden; on the contrary, *N. crassa, Botryosphaeria sp*., *P. sclerotiorum* and *T. viride* prefer to colonize in naked, more than under-forestry tea-garden. These kinds of preferences may be contributed by the fungal characteristics of wet and shade preferences, synthesis and accumulation of secondary metabolites in tea might be another reason, which depends on different light and temperature conditions. The different trends may manifest in unequal habitats, because of the forestry canopy density is changing in different seasons. Although the incidence and diversity of endophytes was consistent among season, tissue type and habitat, and the affection of habitat is not as strong as season and tissue type, which is same as other publications.

The mechanism of endophyte fungal distribution and composition

might be related to environmental conditions, organizational structures and nutrient substances, interactions between endophytic fungi and host plants. So, the further research about the mutual influence between *Camellia sinensis* and the diversity and composition of the endophytic fungi is necessary in the future study.

Collectively, we assume that the new species may be the host-specific endophyte of tea plant, and to be the evidence that endophytes represent formerly uncharted fungal lineages and comprise vast amounts of fungal diversity on a global scale [28]. Of course, to substantiate this assumption, further work about taxonomic and their various characteristics are required.

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