Biodiversity of endophytic fungi in *Kigelia pinnata* during two different seasons

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Endophytic fungi of inner bark and leaf tissue of *Kigelia pinnata* (Lam) Benth., also called sausage tree, from South India, have been studied during two different seasons. Four hundred bark and leaf segments were analysed and a total of 732 isolates representing 28 taxa, including 3 morphotypes were isolated. The endophytic fungal population is prevalent during winter on both the tissues and the colonization frequency and isolation frequency were greater in the leaves than bark tissue. Some endophytic fungi were common to both hosts, although more appeared to be tissue-specific.

Keywords: Bark and leaf tissue, endophytic fungi, *Kigelia pinnata*.

IN 1971, Ainsworth[1] defined an endophyte as 'a plant living inside another organism'. Endophytic fungi cause no harmful effects to the living host plant and they can be isolated from various parts of the plant[2–4]. All vascular plants harbour endophytic organisms[5]. There have been extensive studies[6–8] on endophytes of various plants in several countries over the past 15 years. Though endophytic fungi in plants became popular in the last two decades, studies related to angiosperm trees were limited[9–11] and those related to the family Bignoniaceae were especially less. The present study reports endophytic mycoflora of the sausage tree, *Kigelia pinnata* from South India.

There has been a great interest in endophytic fungi as potential producers of novel, biologically active products[12–14]. Endophytes are now considered as an important component of biodiversity as the distribution of endophytic mycoflora differs with the host. With the above knowledge it is quite promising to explore the endophytic fungi of the bark and leaf tissue of *K. pinnata*.

Kigelia pinnata is commonly called as sausage tree due to the shape of the fruit. The wood is pale brown or yellowish, undifferentiated and not prone to cracking[15]. It is an evergreen tree, but deciduous where there is a long dry season. The fruits, bark, leaves and roots are used to treat disorders of the blood vascular system, digestive system, infection, infestation, injuries, neoplasms, skin disorders, etc.

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Leaf and bark tissues were collected from mature trees (approximately 15 m) growing in the tropical reserved forest at Chengalpattu (12°41′N, 79°58″E), 50 km south of Chennai, South India. Twenty trees were selected for this study and 100 leaves were collected from the lower part of the crown of each tree about 3 m above the ground and bark peelings were collected from each tree 3 m above the ground. The samples were transported to the laboratory in closed, sterile polythene bags and processed within 24 h of collection[3,16]. Four hundred segments, approximately 5×5 mm^2 were cut from the upper, middle and lower portion of healthy leaves and bark[17]. The segments were surface-sterilized in 70% ethanol (Merck, Germany (30 sec)), immersed in 4% sodium hypochlorite (Sigma, USA (90 sec)) and rinsed in autoclaved double-distilled water for 5 sec (refs 17 and 18). Four hundred segments were placed in potato dextrose agar (PDA) medium with 100 mg of chloramphenicol in order to inhibit the growth of bacteria. Inoculated petri plates were incubated at 27°C under 12 h white fluorescent light : 12 h dark cycles[19]. The petri plates were observed daily for 3–4 weeks. Fungi growing out from sterile segments were transferred to fresh slants. Sterile isolates that could not be identified using morphological characters were given codes using culture characteristics such as growth rate, colony surface texture, colony margin and pigmentation[20,21].

Colonization rate (CR) was calculated as the total number of plant tissue segments infected by fungi divided by the total number of segments incubated[22]. Isolation rate (IR) was determined as the number of isolates obtained from plant segments divided by the total number of segments incubated. Colonization frequency (CF) was calculated as the number of plant segments colonized by a single endophyte divided by the total number of segments observed $\times$ 100. Isolation frequency (IF) was calculated as the total number of isolates of one species divided by the total number of isolates in that sample $\times$ 100. Simpson dominance index and Shannon–Wiener's diversity index were calculated for fungal diversity[23,24].

A total of 732 isolates of endophytic fungi were recovered from 800 samples of bark and leaf tissues of 20 *K. pinnata* trees of Bignoniaceae. CR of bark and leaf tissue (53.0% and 61.0%) as well as IR of bark and leaf tissue (0.61 and 1.22) indicate the wide variation between two tissues (Table 1). The overall fungal composition included 17 species in bark and 27 species in leaf tissues and they comprise 8.19% ascomycetes, 31.42% coelomycetes, 53.0% hyphomycetes, 4.64% zygomycetes and 2.73% sterile fungi (Table 2). Earlier studies showed that hyphomycetes dominate the endophytic assemblages both in bark and leaf tissues during both the seasons[25–27] and the incidence of zygomycetes appears to be low[28]. This is true for the present study also, as the zygomycetes population is 5.05% and basidiomycetes were totally absent; they are usually isolated in very low numbers in endo-

phyte research[3]. Isolation frequency on both the tissues indicates *Colletotrichum* sp. 2, *Pestalotiopsis* sp., *Trichoderma aeroviride*, *Botryodiplodia theobromae*, *Alternaria alternata*, *Alternaria tenuissima*, *Curvularia lunata*, *Curvularia geniculata* and *Drechslera hawaiiensis* were the common isolates, whereas *Aspergillus* sp., *Penicillium* sp. and morphotypes were restricted only to the leaf tissues (Figure 1).

Endophytic fungi were more prevalent on leaf tissue (66.66%) than on bark tissue (33.33%) (Table 2) and this may be accounted for by their anatomical structure. Tissue specificity has been widely observed[29,30]. Leaves contain mainly parenchymatous cells that are thin-walled, with chloroplasts and rich in starch, whereas bark tissue is covered with periderm which mainly strengthens the plant than supplying nutrient elements on which the endophyte depends. These differences may be a reflection of tissue recurrence in individual species and may reflect their capacity for utilizing or surviving within a specific substrate[31,32]. A comparative study of twig and leaf-associated endophytes in *Quercus ilex*[33] and the commonest endophytes, were found only in leaves, whereas the remainder were found in both leaves and

Table 1. Colonization and isolation rate of endophytic fungi of *Kigelia pinnata*

	Bark	Leaf	Total
No. of samples	400	400	800
No. of samples yielding fungi	212	244	456
No. of isolates	244	488	732
Colonization rate (%)	53.0	61.0	57.0
Isolation rate	0.61	1.22	0.91

Table 2. Endophytic fungi isolated from leaf and bark of *K. pinnata*

Endophyte	Bark	CF (%)	Leaf	CF (%)
Ascomycetes				
Chaetomium globosum	11	2.7	15	3.75
Nodulisporium sp.	14	3.5	20	5.0
Coelomycetes				
Botryodiplodia theobromae	30	7.5	46	11.5
Colletotrichum gloeiosporiodes	0	-	22	5.5
Colletotrichum sp. 2	32	8.0	24	6.0
Pestalotiopsis sp.	20	5.0	32	8.0
Phomopsis sp. 1	6	1.5	14	3.5
Phomopsis sp. 2	2	0.5	–	–
Phoma chrysanthemicola	–	–	2	0.5
Hyphomycetes				
Alternaria alternata	8	2.0	20	5.5
Alternaria tenuissima	12	3.0	26	6.5
Aspergillus flavus	–	–	8	2.0
Aspergillus niger	–	–	24	6.0
Aspergillus sp.	–	–	6	1.5
Curvularia lunata	16	4.0	38	9.5
Curvularia geniculata	18	4.5	42	10.5
Drechslera hawaiiensis	24	6.0	26	6.5
Fusarium solani	2	0.5	4	1.0
Nigrospora oryzae	8	2.0	26	6.5
Nigrospora sphaerica	–	–	6	1.5
Penicillium sp. 1	–	–	2	0.5
Penicillium sp. 2	–	–	2	0.5
Trichoderma aeroviride	30	7.5	40	10.5
Zygomycetes				
Mucor pusillus	2	0.5	6	1.5
Rhizopus oryzae	9	2.25	17	4.25
Mycelia Sterilia				
Morphotype 1	–	–	8	2.0
Morphotype 2	–	–	10	2.5
Morphotype 3	–	–	2	0.5

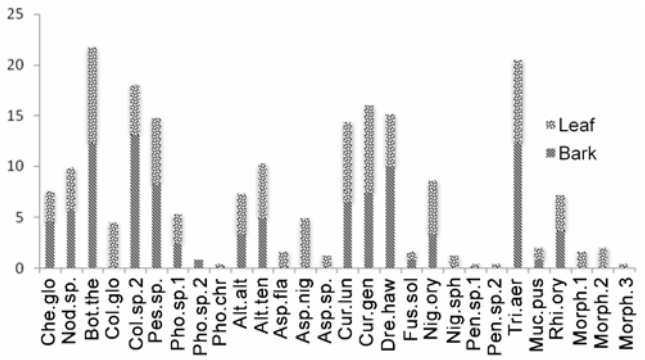

Figure 1. Isolation frequency of endophytes in bark and leaf of *Kigelia pinnata*. Che.glo, *Chaetomium globosum*; Nod.sp., *Nodulisporium* sp.; Bot.the, *Botryodiplodia theobromae*; Col.glo, *Colletotricum gloeiosporiodes*; Col.sp., *Colletotricum* sp.; Pes.sp., *Pestalotiopsis* sp.; Pho.sp.1, *Phomopsis* sp. 1; Pho.sp.2, *Phomopsis* sp.2; Pho.chr, *Phoma chrysanthemicola*; Alt.alt, *Alternaria alternata*; Alt.ten, *Alternaria tenuissima*; Asp.fla., *Aspergillus flavus*; Asp.nig., *Aspergillus niger*; Asp.sp., *Aspergillus* sp.; Cur.lun, *Curvularia lunata*; Cur.gen, *Curvularia geniculata*; Dre.haw, *Drechslera hawaiiensis*; Fus.sol, *Fusarium solani*; Nig.ory, *Nig.oryzae*; Nig.sph., *Nigrospora sphaerica*; Pen.sp.1, *Penicillium* sp.1; Pen.sp.2, *Penicillium* sp. 2; Tri.aer, *Trichoderma aeroviride*; Muc.pus., *Mucor pusillus*; Rhi.ory., *Rhizopus oryzae*; Morph. 1, Morphotype 1; Morph. 2, Morphotype 2 and Morph. 3, Morphotype 3.

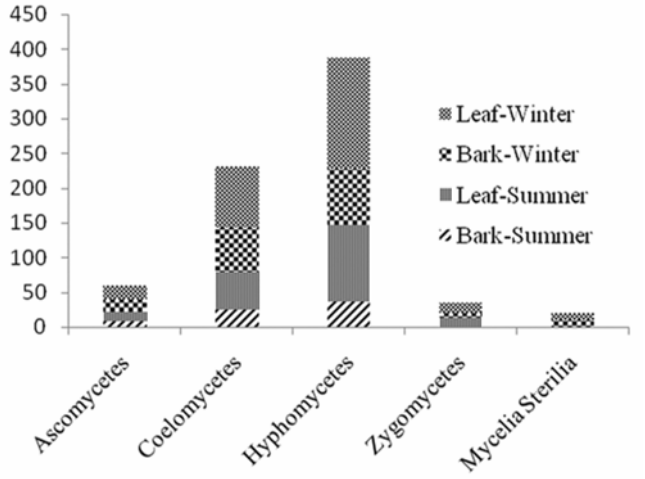

Figure 2. Fungal groups in summer and winter seasons.

Table 3. Fungal groups and colonization frequency during two seasons

Endophytes	Tissues of the host plant	Summer		Winter	
		Number of endophytes	CF (%)	Number of endophytes	CF (%)
Ascomycetes	Bark	8	2.96	17	3.67
	Leaf	14	5.18	21	4.54
Coelomycetes	Bark	25	9.25	65	14.06
	Leaf	54	20.00	86	18.61
Hyphomycetes	Bark	37	13.70	81	17.53
	Leaf	109	40.37	161	34.84
Zygomycetes	Bark	4	1.48	7	1.51
	Leaf	9	3.33	14	3.03
Mycelia sterilia	Bark	0	0	0	0
	Leaf	10	3.70	10	2.16

Table 4. Species diversity in terms of dominance, richness and evenness of endophytic assemblages in different tissues of *K. pinnata*

Tissue	Total no. of taxa	Total no. of isolates	Simpson index (1-D)	Shannon–Wiener index (Hs)	Evenness index
Bark	17	244	0.9144	2.593	0.7864
Leaf	27	488	0.9434	3.015	0.755

Table 5. Species diversity in terms of dominance, richness and evenness of endophytic assemblages of *K. pinnata* during different seasons

Tissue	Season	Total no. of taxa	Simpson index (1-D)	Shannon–Wiener index (Hs)	Evenness index
Bark	Summer	14	0.905	2.492	0.8631
	Winter	17	0.9035	2.56	0.7608
Leaf	Summer	24	0.9422	2.974	0.8154
	Winter	26	0.9424	2.998	0.771

twigs, but no twig-specific taxa were recovered. This interestingly coincides with the present study, as there were no bark-specific taxa in the overall isolates.

The overall endophytic population during summer was 36.88% and during winter was 63.11%, showing that the population of endophytes is prevalent during the wet season (Figure 2 and Table 3). In many instances leaves sampled during the wet season harboured more endophytes than those screened during the dry season[3,32,34]. Humidity and rainfall during winter were high in the present study and may have influenced CR and CF of fungal endophytes. Seasonal variation plays a major role in endophyte harvesting where environmental conditions pave the way for the symbiotic microbes to survive and explore; precipitation may be one of the major factors that influences the infection of endophytes. It has been reported that precipitation may influence the infection of endophytic fungi[35–37]. Seasonal influence on the distribution of endophytic fungi in the plant tissues is higher in spring compared to autumn due to the greater amount of rainfall might promote more effective dispersal of fungal spores[36].

Shannon–Wiener index (Table 4) indicates that the diversity of endophytes is more in the leaves (3.015) due to more number of species than the bark (2.593). Simpson dominance is comparatively higher in the leaves than in the bark which expresses the species specificity with the leaves as *Aspergillus niger* and *Rhizopus oryzae* have been studied widely with more isolates (24 and 17) respectively, whereas *A. niger* is completely absent in bark tissue. Though the dominance and diversity indices are high in the leaves than in the bark, the evenness index is higher in bark and lower in the leaves due to the frequent isolation of *B. theobromae*, *Colletotrichum* sp., *Pestalotiops*is sp., *D. hawaiiensis* and *T. aeroviride* and rare appearance of other taxa in the leaf tissues. In the case of bark, there is even distribution of species in spite of their lesser number compared to the leaves, as reported by Santamaria and Diez[38]. Evenness values varied mainly due to the unequal distribution of individuals and account

for rare species and measure the equitability between the species. Diversity index of bark tissue during summer and winter is low when compared to the leaf tissue during the same seasons (Table 5). Though Simpson diversity does not show much variation during the two seasons in the tissues, there is variation in evenness index, especially during summer in both the tissues indicating that uniform occurrence of various species is prevalent in summer than winter.

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