

Fungal endophytes of three sand dune plant species of west coast of India

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Beena, K. R., K. Ananda & K. R. Sridhar (2000). Fungal endophytes of three sand dune plant species of west coast of India. – *Sydowia* 52(1): 1–9.

Roots of three plant species (*Ipomoea pes-caprae*, *Launaea sarmentosa* and *Polycarpaea corymbosa*) established on the coastal sand dunes of west coast of India were examined for the presence of endophytic fungi by plating on malt extract agar and using damp incubation techniques. From 180 root segments, 220 fungal isolates were recovered that belonged to 31 filamentous fungal species (19 Deuteromycetes, six Ascomycetes and six sterile fungi). Plating consistently yielded more fungal isolates as well as species than damp incubation. *Chaetomium globosum* and *Torula caligans* were more frequently recovered on MEA, and *Fusarium* sp. after damp incubation. Among the endophytes 13% belonged to marine fungal taxa (*Monodictys pelagica*, *Periconia prolifica*, *Verruculina enalia* and *Zalerion maritimum*). Even though arenicolous fungi have adapted to coastal sand dune ecosystem, none of them were recovered as root endophytes. A maximum of 21 and up to seven fungal species per segment were recorded from *I. pes-caprae*. Based on the rarefaction index, the expected number of species was higher in *I. pes-caprae* as compared to other two plant species. In this pilot trial *Acremonium* was extensively isolated. Endophytic *Acremonium* is known to decrease the arbuscular mycorrhizal fungal colonisation and reproduction, but there seem to be no such effects on coastal sand dune plant species studied.

Keywords: endophytes, dune plants, sand dunes, coast, India.

Fungal endophytes have been isolated from a wide variety of plants (Wilson & Carroll, 1994), but most work on endophytic fungi is confined to temperate regions (Petrini, 1986; 1991). Studies on endophytic fungi have only recently been initiated in tropical region (Rodrigues & Petrini, 1997). A broad range of ascomycetes and anamorphic fungi are known as endophytes of non-graminaceous hosts. Because of the symptomless association of endophytic fungi with their host, this association is presumed to be mutualistic. The endophytic association of grass endophytes with their hosts results in many advantages to the host plant species such as increased plant growth (Clay, 1987), protection from fungal pathogens (Christensen

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& Latch, 1991; White & Cole, 1985), inhibition of herbivory (Clay, 1991; Johnson & Whitney, 1994) and increased tolerance to drought conditions (West & al., 1990). A delay of senescence through production of growth regulators (Petrini & al., 1992) and endophyte-directed decomposition of senescent or dead plant tissues (Rodrigues & Petrini, 1997) have been postulated for non-graminaceous endophytes.

Growth, establishment, survival and succession of coastal sand dune plants depends upon arbuscular mycorrhizal (AM) fungi (Koske & Polson, 1984). A literature search revealed no studies on the association of non-AM fungal endophytes with coastal sand dune plants. Therefore, the present study was undertaken to assess the extent of variability of root colonisation of three coastal sand dune plants by non-AM fungal endophytes.

Materials and methods

Five plants (about 10–20 m apart) each of *Ipomoea pes-caprae* (L.) R. Br. (Convolvulaceae), *Launaea sarmentosa* (Willd.) Alston (Asteraceae) and *Polycarpaea corymbosa* (L.) Lam. (Caryophyllaceae) growing in a mixed stand were chosen from the maritime sand dunes of Someshwara in the south-western part of Karnataka State (12° 47' N, 74° 52' E) during the post-monsoon season (October–December, 1998). The roots were dug out and brought to the laboratory in separate sterile polythene bags, rinsed thoroughly in running distilled water to remove the sand and debris, and processed within 4 h of collection.

From each plant twelve root segments of one cm length and four mm thickness were cut. They were surface sterilised by immersion in 96% ethanol (1 min), sodium hypochlorite (6% available chlorine, 3 min) and 96% ethanol (0.5 min) (Fisher & al., 1986). Immediately after surface sterilisation the root segments were rinsed thrice in sterile distilled water. Six segments per plant were placed horizontally on separate Petri dishes containing 1.5% malt extract agar (MEA) supplemented with antibiotics (streptomycin sulphate, 0.4 mg/ml; penicillin G, 0.4 mg/ml).

For damp incubation, autoclaved dune sand was aseptically spread into sterile Petri dishes and wetted with sterile distilled water. The remaining six surface sterilised root segments of each plant were horizontally placed over wetted sand in the Petri dishes, each of which was replenished once a week with 3 ml of sterile distilled water. All plates were incubated at $23 \pm 2^\circ \text{C}$ for 7–28 days depending on the growth rates of emerging fungi. Isolation from the MEA plates was by transfer of mycelium to MEA without antibiotics to obtain pure cultures for identification. From the root fragments incubated

on sand, identifications could either be made from fruiting structures which developed on the root surfaces or by mycelial transfer as described above.

Colonisation rates by endophytic fungi were calculated as percent frequency of occurrence (number of root segments colonised by a specific fungus divided by total number of root segments plated or damp incubated x 100). To compare the number of expected species [E(s)] among the isolates obtained on plating and damp incubation of root segments of three plant species, rarefaction indices (Ludwig & Reynolds, 1988) were calculated.

Results

From 180 segments of roots of three plant species 220 fungal isolates were recorded representing 31 species of filamentous fungi (Tab. 1, 2). Altogether six ascomycetes, 19 deuteromycetes and six sterile fungi were recovered. Only five species: *Chaetomium globosum*, *Fusarium* sp., two unidentified deuteromycetes and a sterile fungus (SF 3) were common to all plant species. Plating consistently yielded more isolates and species as compared to damp incubation. Most of the fungal species were recovered by plating on MEA. The frequency of occurrence of *Chaetomium globosum* and *Torula caligans* was highest (36.7%) on MEA, while *Fusarium* sp. was most frequent (20%) after damp incubation. One marine ascomycete (*Verruculina enalia*) and three marine deuteromycetes (*Monodictys pelagica*, *Periconia prolifica* and *Zalerion maritimum*) were isolated in this pilot study. Except for *Aspergillus* sp., *Fusarium* sp. and *P. prolifica* the overall frequency of occurrence of fungi was higher on MEA plates than after damp incubation.

Based on the frequency distribution, 17–30% segments after plating and 0–40% segments after damp incubation did not yield any fungi. All damp incubated segments of *P. corymbosa* yielded fungal isolates. A maximum of seven species were recovered per segment of

Tab. 1. – Number of isolates (out of 30 segments) and species of endophytic fungi recorded from the roots of three coastal sand dune plants (P, plated; D, damp incubated). A: Ascomycetes; D: Deuteromycetes; S: Sterile mycelia.

Host		Total Isolates	Total Species	A	D	S
<i>Ipomoea pes-caprae</i>	P	65	18	2	10	6
	D	16	6	0	6	0
<i>Launaea sarmentosa</i>	P	42	11	4	5	2
	D	16	8	3	4	1
<i>Polycarpaea corymbosa</i>	P	60	14	3	10	1
	D	21	12	2	10	0

I. pes-caprae, and only five species from other substrates. According to the rarefaction curves, more species were recovered after plating on MEA medium than after damp incubation (Fig. 1). The expected number of species was highest in *I. pes-caprae* than in other plant species.

Tab. 2. – Frequency of occurrence (%)* of endophytic fungi by plating (P) and damp incubation (D) of the root segments of three coastal sand dune plants.

Taxon		<i>Ipomoea</i>	<i>Launaea</i>	<i>Polycarpha</i>
		<i>pes-caprae</i>	<i>sarmentosa</i>	<i>corymbosa</i>
<i>Chaetomium globosum</i> Kunze ex Fr.	P	6.7	36.7	10
	D	0	6.7	6.7
<i>Fusarium</i> sp.	P	13.3	0	6.7
	D	20	6.7	3.3
Unidentified Deuteromycete (hyaline ovoid conidia)	P	0	13.3	16.7
	D	3.3	3.3	3.3
Unidentified Coelomycete (hyaline sigmoid conidia)	P	6.7	0	20
	D	0	3.3	6.7
SF 3 (brown mycelia)	P	6.7	16.7	3.3
	D	0	0	0
<i>Aspergillus</i> sp.	P	10	10	0
	D	13.3	0	0
SF 2 (green colonies)	P	26.7	0	0
	D	0	3.3	0
<i>Verticillium</i> sp.	P	16.7	10	0
	D	0	0	0
<i>Acremonium</i> sp.	P	13.3	10	0
	D	3.3	0	0
SF 4 (white mycelia)	P	13.3	6.7	0
	D	0	0	0
<i>Petriella sordida</i> (Zukal) Barron & Gilman	P	13.3	0	26.7
	D	0	0	10
<i>Zalerion maritimum</i> (Linder) Anastasiou	P	0	0	6.7
	D	6.7	0	3.3
<i>Periconia prolifica</i> Anastasiou	P	0	0	0
	D	6.7	0	3.3
<i>Minimidochium setosum</i> Sutton	P	0	10	16.7
	D	0	0	6.7
<i>Alternaria</i> sp.	P	0	0	13.3
	D	0	6.7	3.3
Unidentified Ascomycete	P	0	0	6.7
	D	0	3.3	0
SF 5 (dark mycelia)	P	20	0	0
	D	0	0	0
<i>Scolecobasidium terreum</i> Abbott	P	16.7	0	0
	D	0	0	0

Taxon		<i>Ipomoea pes-caprae</i>	<i>Launaea sarmentosa</i>	<i>Polycarpaea corymbosa</i>
<i>Phoma</i> sp.	P	13.3	0	0
	D	0	0	0
SF 1 (grey colonies)	P	13.3	0	0
	D	0	0	0
<i>Pseudobotrytis terrestris</i> (Timonin) Subram.	P	10	0	0
	D	0	0	0
<i>Cladosporium</i> sp.	P	6.7	0	0
	D	0	0	0
SF 6 (dark-brown mycelia)	P	6.7	0	0
	D	0	0	0
<i>Asteromella</i> sp.	P	3.3	0	0
	D	0	0	0
<i>Emericella nidulans</i> (Eidam) Vuill.	P	0	16.7	0
	D	0	20	0
<i>Verruculina enalia</i> (Kohlm.) Kohlm. & Volk.-Kohlm.	P	0	10	0
	D	0	0	0
<i>Melanospora zamiae</i> Corda	P	0	3.3	0
	D	0	0	0
<i>Torula caligans</i> (Batista & Upadhyay) Ellis	P	0	0	36.7
	D	0	0	13.3
<i>Brachysporium nigrum</i> (Link) Hughes	P	0	0	26.7
	D	0	0	6.7
<i>Monodictys levis</i> (Wiltshire) Hughes	P	0	0	6.7
	D	0	0	0
<i>Monodictys pelagica</i> (Johnson) Jones	P	0	0	3.3
	D	0	0	3.3

* Percent frequency of occurrence = Number of root segments colonized by a specific fungus divided by total number of root segments plated or damp incubated X 100; SF 1-6, Sterile fungi.

Discussion

The coastal sand dunes along the west coast of India harbour a varied strand vegetation. Among them the mat-forming and tree plant species are of considerable importance in dune stabilization (Rao & Meher-Homji, 1985). Since Nicolson (1959) first reported the association of AM fungi with sand dune plants, several studies have been carried out on AM fungal association with coastal sand dune plant species, but only little information is available on the non-AM endophytic fungal association with coastal sand dune vegetation. The results suggest that roots of strand vegetation are colonised by several non-AM fungal species.

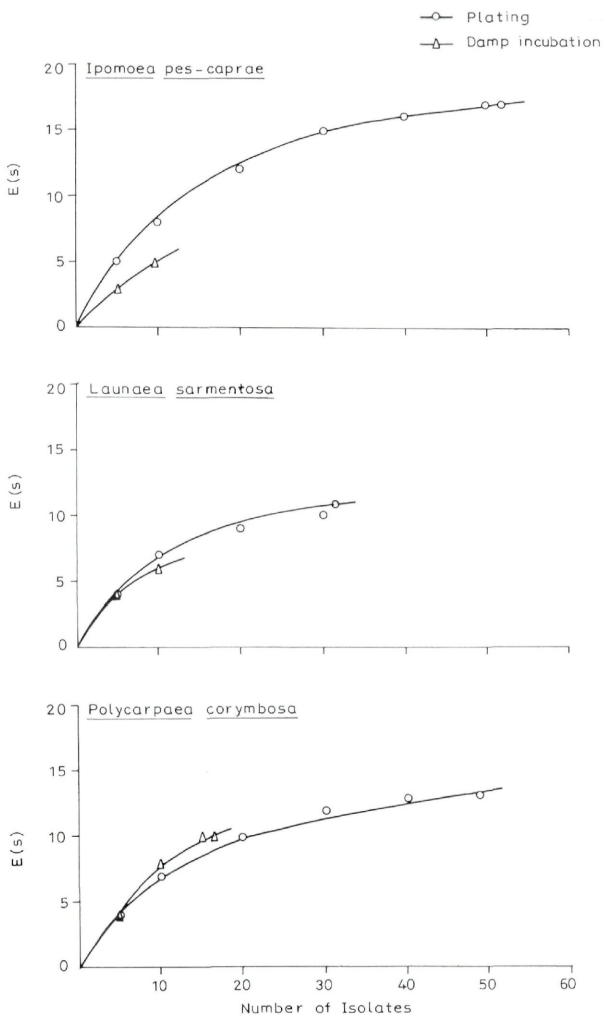


Fig. 1. - Rarefaction curves on the occurrence of non-AM fungal endophytes [E(s): expected number of species] of the isolates obtained from three coastal sand dune plants of west coast of India.

Ipomoea pes-caprae being one of the major mat-forming plant species of coastal sand dunes in tropics, it is of considerable interest to record its endophytic fungal components. *Ipomoea pes-caprae* harboured many species of endophytic fungi in comparison to *L. sarmentosa* and *P. corymbosa*. It has been observed that these plants showed least susceptibility to pathogens and insect herbivores on the west coast dunes of India (K. R. Sridhar, unpubl. observations). It is known that in addition to deter the herbivores, non-mycorrhizal grass endophytes are capable of altering the plant-plant competition and drought resistance in a similar way to that of mycorrhizae (Clay White, 1992). In the present study, *I. pes-caprae* and *L. sarmentosa* were colonised by *Acremonium* sp. during post-monsoon season with a frequency of occurrence up to 13.3%. The co-occurrence of both AM fungi and endophytic *Acremonium* in these plants may be one of the reasons for their increased resistance to pathogens and insect herbivores. On the other hand, Chu-Chou & al. (1992) and Guo & al. (1992) suggested that endophytic *Acremonium* may reduce the colonisation and reproduction of AM fungi. Beena (1999) observed that the AM fungal colonisation increased from summer to post-monsoon (*I. pes-caprae* from 28.7 to 93.6%; *L. sarmentosa* from 42.2 to 90.6%; *P. corymbosa* from 0 to 20%). The peak of AM fungal colonisation during post-monsoon coincides with the occurrence of endophytic *Acremonium*. Therefore, the present observations contradict the belief that endophytic *Acremonium* decreases the AM fungal colonisation and reproduction. Simultaneous assessment of AM and non-AM fungal endophytes in the field and greenhouse may reveal more about their contribution to the coastal sand dune vegetation. On the other hand, the present pilot study has been conducted only in a single geographical location and the sample size is quite small: thus, no firm conclusions can be drawn.

On the dunes of west coast of India, *Polycarpaea corymbosa* is established in mixed stand (along with other plants) and in pure stand. These plants belong to the so-called non-mycorrhizal family, Caryophyllaceae. *Polycarpaea corymbosa* plants in mixed stands have been shown to be colonised by AM fungi in percentages ranging from 5-23% (Beena, 1999). In the present pilot trial the root segments of *P. corymbosa* from mixed stand did not show the presence of *Acremonium*, thus ruling out the possibility of *Acremonium*-induced negative response for AM fungi. Although the rhizosphere of *P. corymbosa* in pure stand contains considerable AM fungal spores, they failed to get colonised by AM fungi (Beena, 1999). Further observations on the endophytic fungi of *P. corymbosa* in both pure and mixed stands may reveal more about their dependence on endophytic fungi.

It is interesting to note that 13% of the endophytes isolated belonged to marine fungi. The woody debris accumulated on the sea shore may be colonised by several marine fungi (Kohlmeyer & Kohlmeyer, 1979), in a similar way the living roots of sand dune plant species may also constitute one of the ecological niches for marine fungi. Arenicolous fungi, being adapted to coastal sand ecosystem, are of considerable interest among marine fungi, but in this study none of the arenicolous fungi were recorded as endophytes. Further studies are required to understand the importance of non-AM fungal endophytes in the ecology of sand dune plant species.

Acknowledgments

Appreciation is expressed to N. S. Raviraja and A. Arun Bhagwath, Department of Biosciences, Mangalore University for helpful suggestions. The authors are grateful to the reviewers for their constructive suggestions to improve the manuscript. This study was supported by Mangalore University under minor research grant to KRS.

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(Manuscript accepted 20th November 1999)

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Autor(en)/Author(s): Beena K. R., Ananda K., Sridhar K. R.

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