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ISOLATION AND IDENTIFICATION OF FUNGAL ENDOPHYTES FROM VELAMEN ROOTS OF EPIPHYTIC ORCHIDS

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ABSTRACT

In the present investigation, different epiphytic orchids such as *Acampe praemorsa, Arachnis flos-aeris, Dendrobium aphyllum* and *Rhynchostylis retusa* were studied for morphological and fungal identifications of velamen roots. Diverse endophytic fungal species were detected, isolated and identified from velamen roots through microscopic and macroscopic features. They include *Aspergillus niger, Curvularia geniculata, Cylindrocarpon sp, Cercospora sp, Fusarium sp and Mucor sp*. Percentage and Colonization frequency of endophytic fungi and the percentage of dominant fungi were calculated. Scientific approaches on the diversity of endophyte, their mutualistic interaction with plants remains scanty and more studies are needed to explore their full potentiality in plant protection sector.

INTRODUCTION

Orchids grow in a wider range of habitats mainly in tropical and subtropical climates. They exhibit large variations in their habitat, life forms, floral features and tropical patterns (Gardes, 2003). Indian orchids are found at varying altitudes & climatic conditions (Singh, 2001). Several orchids are used in ayurvedic formulations for their rejuvenating and restorative properties (Hoffman, 2011). Nearly 70% species are epiphytic orchids which grow on other plants in tree canopies have well developed velamenous roots and fleshy leaves (Zotz, 2013).

Mycorrhizal interactions present in orchids to persist in less than ideal habitats and has led to their occurrence worldwide. Orchids have several unique properties and much of their diverse presence may be attributed to their relationship with mycorrhizal fungi (Smith & Read, 2008). The production of minute seeds with minimal nutrient reserves leads the orchids to be dependent upon mycorrhizal fungi for some resources necessary for germination and growth during early stages of plant development (Rasmussen, 1995; Arditti and Ghani, 2000; Rasmussen and Whigham, 2000). The present study is an attempt to isolate and identify fungal endophytes from velamen roots of epiphytic orchids in Kerala.

MATERIALS AND METHODS

Plant material

In the present study velamen roots of different epiphytic orchids such as *Acampe praemorsa, Arachnis flos-aeris, Dendrobium aphyllum and Rhynchostylis retusa* were collected randomly from Central Travancore region in Kerala during the month of November to July of the year 2015-2016 which were used in fungal isolation and identification.

Systematic position

Kingdom : Plantae
Division : Phanerogamae
Class : Monocotyledonae
Series : Microspermae
Family : Orchidaceae

I. Morphological Studies

Twenty different velamen roots of each orchid were collected and thoroughly investigated. Average number of velamen roots per plant, average diameter and number of branched velamen roots were noted and recorded for morphological studies.

ii. Isolation of Fungal Endophytes

Healthy velamen roots were collected and transported to the laboratory in sterile polythene bags and processed within 24 hours of collection. Surface sterilization of the explants is done according to standard procedures (Lodge et al., 1996; Schulz et al., 1993). The segments were inoculated into Potato Dextrose Agar (PDA), which was supplemented with Amoxicillin to inhibit bacterial growth. Petri plates were sealed with parafilm and incubated at room temperature for 7-10 days. The fungi that grew out from the segment were periodically exalted and subcultred. Hyphae taken from the segment were stained using lactophenol cotton blue and were identified according to their macroscopic and microscopic characteristics such as colour of the culture, morphology of fruiting body and spore morphology (Singh, 2000).

RESULTS

In the present investigation morphological characters of the velamen roots of four different epiphytic orchids *Acampe praemorsa, Arachnis flos-aeris, Dendrobium aphyllum* and *Rhynchostylis retusa* were carried out. By analysis, it was found that in *Acampe praemorsa* average length of velamen roots were 30 cm, average breadth is 1 cm, average number of branched root is 9 and average number of velamen roots per plant is 16. But in *Arachnis flos-aeris average* length of the velamen roots were 22.5 cm, average breadth is 1.06 cm, and average number of branched root is 6, average number of velamen roots present in per plant is 11.6 (Table 1). In *Dendrobium aphyllum* average length of the velamen roots were 8.8 cm, average breadth is 0.12 cm, and average number of branched root is 8, average number of velamen roots present in per plant is 15 (Table 1). *Rhynchostylis retusa* shows that average length of the velamen roots were 30.4 cm, average breadth is 1.3 cm, average number of branched roots per plant is 5, average number of velamen roots present per plant is 13.5 (Table 1). The endophytic fungal presence and colonization frequency of fungi in tested orchids are given in Fig 1 & 2 respectively.

Table 1: Morphological details of the velamen roots collected from orchids

Serial no.	Name of the orchids	Average number of roots per plant	Average length of roots (cm)	Average breadth of roots (cm)	Average number of branched roots per plant
1	*Acampe praemorsa*	16	30	1.08	9
2	*Arachnis flos-aeris*	11.6	22.5	1.06	6
3	*Dendrobium aphyllum*	15	8.8	0.12	8
4	*Rhynchostylis retusa*	13.5	30.4	1.56	5

Table 2: Colonization frequency of fungi isolated from velamen roots of orchids under study.

Serial No.	Name of orchids	Fungal isolates	Colonization frequency (%)
1	*Acampe praemorsa*	*Aspergillus niger*	83.3
		Cylindrocarpon sp.	74
		Fusarium sp.	45.4
2	*Arachnis flos-aeris*	*Aspergillus niger*	46
		Cercospora sp.	78.6
		Cylindrocarpon sp.	50
3	*Dendrobium aphyllum*	*Aspergillus niger*	74
		Curvularia geniculata	84
		Fusarium sp.	41
		Mucor sp.	53
4	*Rhynchostylis retusa*	*Aspergillus niger*	86
		Cercospora sp.	78
		Cylindrocarpon sp.	83.3
		Gliocladium sp.	87
		Mucor sp.	64.5

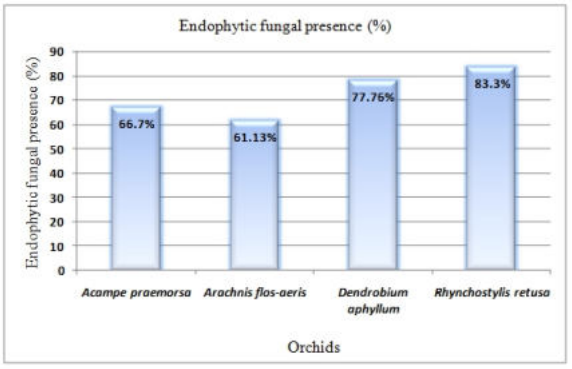

Fig 1: Presence of endophytic fungi (%)

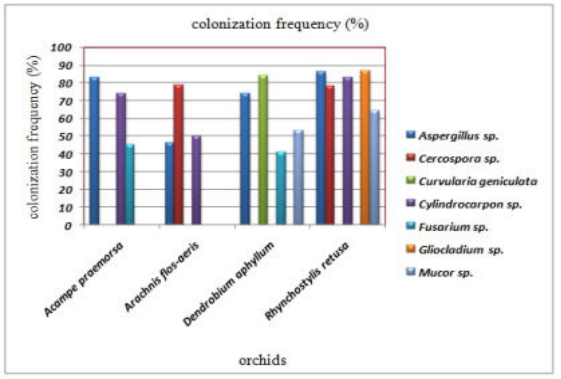

Fig 2: Colonization frequency of fungi in tested orchids (%)

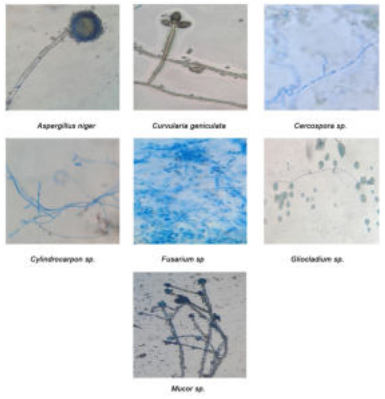

Fig 3: Isolated fungal species.

DISCUSSION

In the present investigation different epiphytic orchids such as *Acampe praemorsa, Arachnis flos-aeris, Dendrobium aphyllum and Rhynchostylis retusa* were studied for morphological and fungal identification of velamen roots. Morphologically the velamen roots vary in their length, breadth, and number depends up on the species. Average number of velamen roots per plants vary from 11.6 to 16; highest is in *Acampe praemorsa* and lowest is in *Arachnis flos-aeris*. A function attributed to the velamen cell wall thickening, is to provide mechanical support, avoiding cellular collapse during dehydration (Noel, 1974).

Diverse endophytic fungal species were detected, isolated, sub cultured and identified from velamen roots of orchid species through microscopic and macroscopic features. *Aspergillus niger, Cylindrocarpon,* and *Fusarium* from *Acampe praemorsa; Curvularia geniculata, Aspergillus niger, Mucor,* and *Rhizopus from Dendrobium aphyllum; Gliocladium, Aspergillus niger, Cercospora, Cylindrocarpon,* and *Mucor* from *Rhynchostylis retusa; Aspergillus niger, Cylindrocarpon* and *Cercospora from Arachnis flos-aeris* were identified. The dominant fungi in *Acampe praemorsa, Arachnis flos-aeris, Dendrobium aphyllum* and *Rhynchostylis retusa* were *Aspergillus niger* (41.09%), *Aspergillus niger* (46.64%), *Curvularia geniculata* (33.3%), and Gliocladium (21.81%) respectively.

Endophytic organisms have received considerable attention as they are found to protect their host against pest, pathogens and even domestic herbivorous (Weber, 1981). Currently endophytes are considered as unexplored source of bioactive natural compounds. They have been found to play a crucial role in the production of beneficial chemical compounds as secondary metabolites.

CONCLUSION

The present study was undertaken to elaborate the taxonomic diversity of fungal isolates from velamen roots of epiphytic orchids. Fungal endophytes possess beneficial effect on the host plants and also act on the key source of water and minerals to the orchids. Endophytic fungi are one of the most creative groups of secondary metabolite producers for exploitation in the pharmaceutical industry, agriculture and in environmental application. Understanding of this interaction is essential for the development of proper bio control strategy.

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