RESEARCH ARTICLE

Terrestrial Orchid Mycorrhiza and Non- Mycorrhizal Endophytes from Kolhapur District (M.S.) - III

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ABSTRACT

Orchid Mycorrhiza (OM) is a specialized group of endophytes, mutually associated with roots of almost all terrestrial orchids. They play an important role, not only in germination of seeds, but also for nutrient uptake throughout the entire adult life of many orchids, so much so that they are identified as mycoheterotrophs. As many as 45 numbers of terrestrial orchids belonging to the genera *Habenaria*, *Nervelia*, *Malaxis*, *Peristylus*, *Pectilis*, *Geodorum*, *Zeuxine*, *Cheirostylus* and *Eulophia* are recorded from Kolhapur District of Maharashtra state. Till date there are no reports of studies on OM of terrestrial orchids in the study area. Fungi isolated from orchids until now belong to the genus *Rhizoctonia*, Agaricales and other Basidiomycetes. In the present study OM from roots of *Habenaria brachyphylla* (Lindl.) Aitch., *Habenaria commelinifolia* (Roxb.) Wall. Ex Lindl., *Habenaria diphylla* (Nimmo) Dalzell, *Habenaria digitata* Lindl., *Habenaria foliosa* A. Rich.,*Habenaria foetida* Blatt. and McCann, *Habenaria furcifera* Lindl., *Habenaria gibsonii* Hook.f., *Habenaria heyneana* Lindl., *Habenaria longicorniculata* J. Graham, *Habenaria longicornu* Lindl., *Habenaria marginata* Colebr.,*Habenaria plantaginea* Lindl.,*Habenaria rariflora* A.Rich., *Habenaria roxburghii* Nicolson, *Malaxis versicolor* (Lindl.) Abeyw., *Nervelia infundibulifolia* Blatt. and McCann, *Nervelia crociformis* (Zoll. and Moritzi) Seidenf, *Pecteilis gigantea* (Sm.) Raf., *Peristylus densus* (Lindl.) Sant. and Kapad., *Peristylus lawii* Wight., *Peristylus plantagineus* (Lindl.) Lindl. are isolated and identified for the first time from Maharashtra state. During the present study non-mycorrhizal endophytes have also been recorded.

Keywords: Orchid Mycorrhiza, *Habenaria*, *Nervelia, Peristylus, Pecteilis, Malaxis.*

INTRODUCTION

Orchidaceae is one of the largest family in the world consisting about approximately 600-800 genera and over 25000-35000 species, has a worldwide distribution and largest number of the species are in the tropics. As many as 45 number of terrestrial orchid species belonging to the genera *Habenaria, Nervelia, Malaxis, Peristylus, Pecteilis, Geodorum,*

Zeuxine, Cheiristylus and Eulophia are recorded from Kolhapur District of Maharashtra state. The seeds of orchids are minute and contain very less reserve food. Hence, colonization by compatible fungus is essential for germination and early seedling development.

Several Orchid species remain achlorophyllus during their entire life cycle , depending on fungi for carbon compounds derived either from the breakdown of organic matter in the soil or from mycorrhizal linkage with autotrophic plants. There are 3 groups of Mycorrhizal fungi,

1) *Rhizoctonia*
2) Mycelium radicis atrovirens (Non –Sporulating Hyphomycetes)
3) Hyphomycetes

Orchid Mycorrhiza are unique in that they occur only within the Family Orchidaceae which is one of the largest family of flowering plants. The diagnostic feature of OM is the formation of hyphal coils (pelotons) within host root cells. OM can, therefore, be considered within the broad category of endomycorrhizas. The present investigation of terrestrial orchids has focused on both mycorrhizal and non- mycorrhizal fungi. Orchid mycorrhiza is the well known symbiotic relationship between orchid roots and a variety of fungi belonging to Fungi Imperfecti like *Rhizoctonia* and Basidiomycetes like *Tulasnella, Sebacina, Ceratobasidium* and Thanetophorus, etc. Orchid seeds are very minute, dust like and need symbiotic association of the suitable fungi for their germination, further growth and development into protocorm like bodies as the seeds lack sufficient endosperm.

Endophytes belong to a wide range of organisms - Bacteria and Fungi, inhibiting the healthy plant tissues without causing visible pathological symptoms. Endophytic fungi are polyphyletic, functionally diverse and serve as; latent pathogens, mutualists (e.g. Mycorrhiza) saprophytes involved in decomposition, nutrient turnover anti-herbivory, symbiotic increase of host plant fitness to abiotic stresses and improve adaptability to various environmental conditions.

MATERIALS AND METHODS

33 species of 09 genera of terrestrial orchids were collected from Kolhapur district. Field photography was done with a Nikon Digital camera. Healthy Roots of orchids were collected and preserved in 4% Formalin solution.

Isolation of Mycorrhizal fungi:
Mycorrhizal fungi were isolated using a modification of Masuhara and Katsuya method. Root & root hair were used as inoculants. PDA, CDA, NDY, NA and OA was used for the growth of fungal cultures in petriplates and test tubes, incubated at room temp. (25º C). Pure cultures were maintained on. PDA,CDA,NDY,NA and OA medium.

Anatomical Studies:
Thin**,** free hand sections of the roots were taken and stained with trypan blue (0.1% Lactophenol) and observed under the research microscope for presence of fungal hyphae in cortical cells in the root.

OBSERVTATIONS AND RESULT

Table 1: Localities of collection

Sr. No.	Genera	Field visits					
		2012-13	Locality	2013-14	Locality	2014-15	Locality
1.	*Cheirostylis parvifolia* Lindl.	-	-	14/09/13	Tillari, Chandgad	03/08/14	Amba
2.	*Habenaria brachyphylla* (Lindl.) Aitch.	20/08/12	Kas, Thosegar, Bamnoli, Satara	08/10/13	Kas	19/08/14	Kas, Satara Bamnoli, Thoseghar
3.	*Habenaria commelinifolia* (Roxb.) Wall. ex Lindl.	08/09/12-09/09/12	Malshej Ghat, Bhimashankar, Vichitragad	16/08/13 - 17/08/13	Malshej, Vichitragad	15/08/14-18/08/14	Uran, Malshej

4.	*Habenaria diphylla* (Nimmo) Dalzell	08/09/12-09/09/12	Malshej Ghat, Bhimashankar, Vichitragad	08/10/13	Satara, Kas, Bamnoli, Thoseghar	14/09/14	Gaganbawada
		Sept 2012	Matheran, Uran, Lonavala	15/09/13	Gaganbawada		
		07/08/12	Kas, Thosegar, Bamnoli, Satara Thosegar	16/08/13 - 17/08/13	Malshej, Vichitragad		
5.	*Habenaria digitata* Lindl.	07/08/12	Kas, Thosegar, Bamnoli, Satara	08/10/13	Satara, Kas, Bamnoli, Thoseghar	19/08/14	Kas, Satara Bamnoli, Thoseghar
6.	*Habenaria foliosa* A. Rich.	05/08/12	Ugwai Devrai, Radhanagari	30/08/13	Amba		
		05/09/12	Gavase Devrai, Ajara	22/09/13	Patgaon		
		Sept 2012	Matheran, Uran, Lonavala	15/07/13	Radhanagari		
				02/10/13	Gavase Devrai Ajara		
7.	*Habenaria foetida* Blatt. and McCann	22/07/12	Ajara	30/08/13	Amba	10/08/14	Patgaon, Pal, Bhatwadi
		05/08/12	Ugwai Devrai, Radhanagari	15/07/13	Radhanagari	30/11/14	Radhanagari
		29/07/12	Patgaon	02/10/13	Gavase Devrai Ajara	19/08/14	Kas, Satara Bamnoli, Thoseghar
8.	*Habenaria furcifera* Lindl.	Sept.2012	Tillari	02/10/13	Ajara	29/07/14	Ajara, Tillari
		Oct.2012	Barki, Anuskura Ghat			12/10/14	Kasar Kandgaon
		12/09/12	Amba	30/08/13	Amba ghat	15/08/14-18/08/14	Uran, Malshej
		08/09/12-09/09/12	Malshej ghat, Bhimashankar,Vichitragad	13/10/13	Karanj, Mumbai	07/09/14	Patgaon, Pal
		Aug.- Oct.	Tillari	16/08/13 - 17/08/13	Malshej, Vichitragad	03/08/14	Amba
		Sept 2012	Matheran, Uran, Lonavala	22/09/13	Patgaon	29/07/14	Ajara, Tillari
		July2012	Katyayni Devrai, Kolhapur	28/07/13	Masai Pathar, Panhala	29/07/14	Kasar Kandgaon
		Oct.2012	Kas, Thosegar, Bamnoli, Satara	08/10/13	Satara, Kas, Bamnoli, Thoseghar	29/07/14	Ajara
9.	*Habenaria gibsonii* Hook.f.	Aug.- Oct.	Ratnagiri	15/07/13	Radhanagari	29/07/14	Kasar Kandgaon
				02/10/13	Gavase Devrai Ajara		
10.	*Habenaria heyneana* Lindl.	Oct.2012	Kas	15/07/13	Radhanagari	19/08/14	Kas, Satara Bamnoli, Thoseghar
		05/08/12	Ugwai Devrai, Radhanagari	Sept.2013	Amboli Choukul	21/09/14	Amboli Choukul
11.	*Habenaria longicorniculata* J. Graham	Aug.-Sept.	Vaibhavwadi	30/08/13	Amba	17/07/14	Radhanagari
		12/09/12	Amba	14/09/13	Tillari, Chandgad	19/08/14	Kas, Satara Bamnoli, Thoseghar
		08/09/12-09/09/12	Malshej ghat, Bhimashankar, Vichitragad	15/07/13	Radhanagari	07/09/14	Patgaon, Pal

		Aug.-Sept.	Tillari	16/08/13 - 17/08/13	Malshej, Vichitragad	15/08/14-18/08/14	Uran, Malshej
		05/09/12	Gawase Devrai, Ajara	08/10/13	Satara, Kas, Bamnoli, Thoseghar	10/08/14	Patgaon, Pal, Bhatwadi
		Sept 2012	Matheran, Uran, Lonavala	Sept.2013	Amboli Choukul	03/08/14	Amba
		Aug.- Oct.	Karanj, Mumbai	08/11/13	Bugate-Alur	29/07/14	Ajara, Tillari
		Aug.- Oct.	Bugate-Alur	16/08/13 - 17/08/13	Bhimashankar	29/07/14	Kasar Kandgaon
		08/09/12-09/09/12	Malshej ghat, Bhimashankar, Vichitragad			29/07/14	Ajara, Tillari
12.	*Habenaria longicornu* Lindl.	22/07/12	Ajara	16/08/13	Uran-Raigad	10/08/14	Patgaon, Pal, Bhatwadi
		Sept.2012	Kolhapur				
13.	*Habenaria marginata* Colebr.	Oct.2012	Kolhapur				
		Sept 2012	Matheran, Uran, Lonavala	20/08/13	Kolhapur	Jun-Oct.2014	Kolhapur
		05/08/12	Ugwai Devrai, Radhanagari	10/08/13	Patgaon,	21/09/14	Amboli Choukul
		Aug.-Sept.	Gaganbawada	08/10/13	Satara, Kas, Bamnoli, Thoseghar	03/08/14	Amba
		Aug.-Sept.	Amboli			30/11/14	Radhanagari
14.	*Habenaria multicaudata* Sedgw.	08/09/12-09/09/12	Malshej ghat, Bhimashankar, Vichitragad	02/10/13	Ajara	10/08/14	Patgaon, Pal, Bhatwadi
15.	*Habenaria ovalifolia* Wight					17/07/14	Radhanagari
		Aug.-Sept.	Tillari	14/09/13	Tillari, Chandgad		
16.	*Habenaria plantaginea* Lindl.	Sept 2012	Matheran, Uran, Lonavala	14/09/13	Tillari, Chandgad		
		Sept.- Oct.	Mahabaleshwar				
17.	*Habenaria rariflora* A.Rich.					03/08/14	Amba
						29/07/14	Ajara, Tillari
18.	*Habenaria roxburghii* Nicolson	Sept.2012	Sutagatii	09/09/13	Sutgatii Ghat	14/09/14	Sutakatti, Dist. Belgavi
19.	*Habenaria stenopetala* Lindl.	Oct.2012	Koyna, Nawaza	Oct.2013	Koyna, Nawaza	Oct.2014	Koyna, Nawaza
20.	*Liparis nervosa* (Thunb.) Lindl.	05/08/12	Ugwai Devrai, Radhanagari	15/09/13	Radhanagari, Patgaon, Ajara	29/07/14	Kasar Kandgaon
		Aug.-Sept.	Tillari				
21.	*Malaxis versicolor* (Lindl.) Abeyw.	Aug.-Oct.	Thosegar	30/08/13	Amba,	17/07/14	Radhanagari
		12/09/12	Amba	14/09/13	Tillari, Chandgad	19/08/14	Kas, Satara Bamnoli, Thoseghar
		Aug.-Oct.	Tillari	15/09/13	Radhanagari, Patgaon, Ajara	07/09/14	Patgaon Pal Devrai
		Oct. 2012	Barki, Anuskura Ghat			15/08/14-18/08/14	Uran, Malshej
		Sept 2012	Matheran, Uran, Lonavala			10/08/14	Patgaon, Pal, Bhatwadi

			Mahabaleshwar			03/08/14	Amba
						29/07/14	Ajara, Tillari
						29/07/14	Kasar Kandgaon
						29/07/14	Ajara, Tillari
22.	*Nervelia concolor* (Blume)Schltr. (= N. aragoana)	Aug.-Oct.	Thosegar	15/07/13	Radhanagari	17/07/14	Radhanagari
		20/08/12	Kas, Thosegar, Bamnoli, Satara	10/08/14	Patgaon, Ajara	19/08/14	Kas, Satara Bamnoli, Thoseghar
		08/09/12-09/09/12	Malshej ghat, Bhimashankar, Vichitragad			29/07/14	Ajara, Tillari
		Aug.- Oct.	Tillari				
		Oct.2012	Koyana nagar, Nawaza				
		Aug.-Oct.	Mahabaleshwar				
23.	*Nervelia infundibulifolia* Blatt. and McCann (=N. infundibliformis)	05/09/12	Gawase Devrai, Ajara	30/08/13	Amba,	12/10/14	Kasar Kandgaon
		05/08/12	Ugwai Devrai, Radhanagari	15/07/13	Radhanagari	21/09/14	Amboli Choukul
		12/09/12	Amba	02/10/13	Ajara	10/08/14	Patgaon, Pal, Bhatwadi
24.	*Nervelia crociformis* (Zoll. and Moritzi) Seidenf *(N.prainanana)*	Aug.-Oct.	Patagaon			17/07/14	Radhanagari
		05/09/12	Gawase Devrai, Ajara			07/09/14	Patgaon, Pal
		22/07/12	Chaloba Devrai, Ajara			19/08/14	Kas, Satara Bamnoli, Thoseghar
		Oct.2012	Kas			15/08/14-18/08/14	Uran, Malshej
		08/09/12-09/09/12 Aug.-Oct.	Malshej ghat, Bhimashankar, Vichitragad Karanj			10/08/14	Patgaon, Pal, Bhatwadi
						03/08/14	Amba
		Sept 2012	Matheran, Uran, Lonavala			29/07/14	Ajara, Tillari
						29/07/14	Kasar Kandgaon
						29/07/14	Ajara, Tillari
25.	*Pecteilis gigantea* (Sm.) Raf.	Aug.Sept.	Tillari	29/07/14	Tillari, Chaloba Devrai,	17/07/14	Radhanagari
		05/09/12	Gawase Devrai Ajara	15/07/13	Radhanagari	29/07/14	Ajara, Tillari
		05/08/12	Ugwai Devrai, Radhanagari	22/09/13	Patgaon, Ajara	29/07/14	Ajara, Tillari
		Aug.- Oct.	Amboli	02/10/13	Satara, Kas, Bamnoli, Thoseghar	10/08/14	Patgaon, Pal, Bhatwadi
		July 2012	Amba			19/08/14	Kas, Satara Bamnoli, Thoseghar
		20/08/12	Kas, Thosegar, Bamnoli,Satara			29/07/14	Ajara, Tillari
26.	*Peristylus densus* (Lindl.) Sant. and	05/08/12	Ugwai Devrai, Radhanagari	14/09/13	Tillari, Chandgad	29/07/14	Ajara, Tillari

	Kapad.	20/08/12	Kas, Thosegar, Bamnoli,Satara	22/09/13	Patgaon, Ajara	29/07/14	Ajara, Tillari
		05/07/12	Gawase Devrai Ajara	02/10/13	Satara, Kas, Bamnoli, Thoseghar	10/08/14	Patgaon, Pal, Bhatwadi
		Sept 2012	Matheran, Uran, Lonavala			17/07/14	Radhanagari
27.	*Peristylus goodyeroides* (D.Don) Lindl.	05/08/12	Ugwai Devrai, Radhanagari	15/07/13	Radhanagari	17/07/14	Radhanagari
		08/09/12-09/09/12	Malshej ghat, Bhimashankar, Vichitragad	02/10/13	Ajara	29/07/14	Ajara, Tillari
		Sept 2012	Matheran, Uran, Lonavala			03/08/14	Amba
						29/07/14	Ajara, Tillari
28.	*Peristylus lawii* Wight.	Sept 2012	Matheran, Uran, Lonavala			10/08/14	Patgaon, Pal, Bhatwadi
		Sept.-Oct.	Pratapgad				
29.	*Peristylus plantagineus* (Lindl.) Lindl.	29/07/12	Patgaon	15/07/13	Radhanagari	17/07/14	Radhanagari
				02/10/13	Gawase Devrai Ajara	29/07/14	Ajara, Tillari
30.	*Peristylus aristatus* Lindl.	05/08/12	Ugwai Devrai, Radhanagari				
		Aug.-Oct.	Tillari				
31.	*Zeuxine longilabris* (Lindl.) Trimen					29/07/14	Kasar Kandgaon

Table 2: : Fungi isolated

Sr No	Host	Media	Fungal Isolates.
1.	*Habenaria digitata* Lindl.	PDA	Vegitative mycelium
2.	*Habenaria foliosa* A. Rich.	PDA	*Aspergillus sp.*
3.	*Habenaria foetida* Blatt. & McCann	PDA	*Aspergillus sp.*
4.	*Habenaria heyneana* Lindl.	CDA	Vegitative mycelium
5.	*Habenaria longicorniculata* J.Graham	CDA	*Rhizoctonia solani* J. G. Kuhn
6.	*Habenaria marginata* Colebr.	NDA	*Rhizoctonia solani* J. G. Kuhn
7.	*Habenaria multicaudata* Sedgw.	PDA	*Rhizoctonia solani* J. G. Kuhn
8.	*Habenaria ovalifolia* Wight	PDA	*Rhizoctonia solani* J. G. Kuhn
9.	*Habenaria rariflora* A.Rich.	PDA	*Rhizoctonia solani* J. G. Kuhn
10.	*Habenaria roxburghii* Nicolson	PDA	*Rhizoctonia solani* J. G. Kuhn
11.	*Liparis nervosa* (Thunb.) Lindl.	PDA	*Aspergillus sp.*
12.	*Malaxis versicolor* (Lindl.) Abeyw.	PDA	*Aspergillus sp.*
13.	*Nervelia concolor.*(Blume)Schltr.	PDA	*Fusarium sp.*
14.	*Nervelia infundibulifolia* Blatt. &McCann	PDA	*Rhizoctonia solani* J. G. Kuhn
15.	*Nervelia crociformis* (Zoll.& Moritzi) Seidenf	PDA	Vegitative mycelium
16.	*Pecteilis gigantea* (Sm.) Raf.	PDA	*Rhizoctonia solani* J. G. Kuhn
17.	*Peristylus lawii* Wight.	PDA	*Rhizoctonia solani* J. G. Kuhn
18.	*Habenaria commelinifolia* (Roxb.) Wall. ex Lindl.	CDA	*Rhizoctonia solani* J. G. Kuhn
19.	*Habenaria brachyphylla* (Lindl.) Aitch.	CDA	Vegitative mycelium
20.	*Habenaria rariflora* A.Rich.	PDA	*Rhizoctonia solani* J. G. Kuhn
21.	*Peristylus goodyeroides* (D.Don) Lindl.	PDA	*Trichoderma , Gonytrichum& Glioladium*
22.	*Peristylus densus* (Lindl.) Sant. and Kapad.	PDA	*Rhizoctonia solani* J. G. Kuhn
23.	*Habenaria diphylla* (Nimmo) Dalzell	PDA	Vegitative mycelium

C. ***Habenaria crinifera*** **Lindl D.** ***Nervelia concolor*** **(Blume)Schltr E.** ***Pecteilis gigantea*** **(Sm.) Raf F.** ***Habenaria ovalifolia*** **Wight**
G. ***Habenaria commelinifolia*** **(Roxb.) H.** ***Malaxis versicolor*** **(Lindl.) Abeyw I.** ***Habenaria brachyphylla*** **(Lindl.) Aitch**

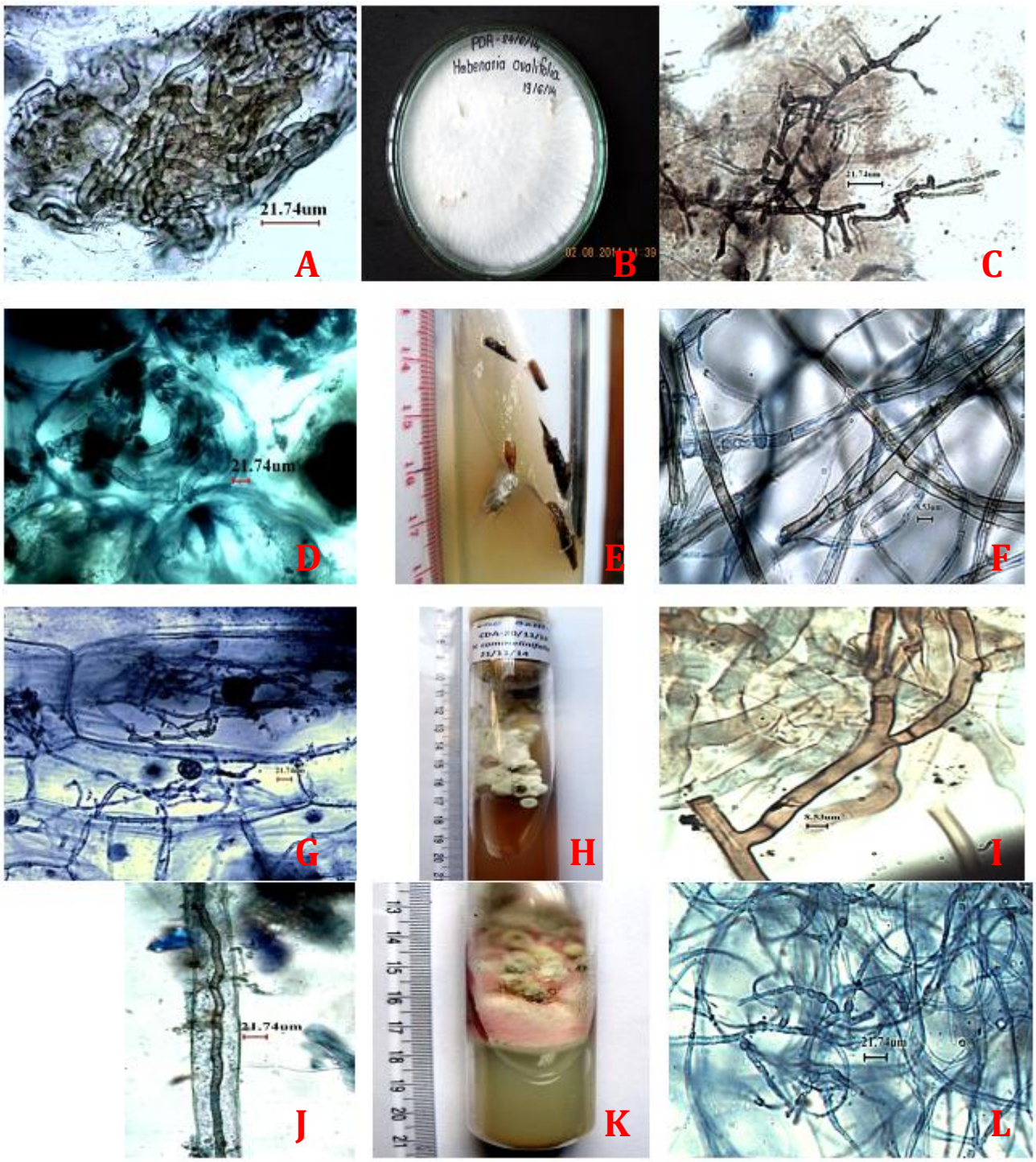

Fig. 2: A. *Rhizoctonia* peloton in *Habenaria roxburghii* Nicolson root cells; **B.** Pure isolate of *Rhizoctonia* sp.from *Habenaria ovalifolia* Wight roots; **C.** *Rhizoctonia* sp.(100X) from *Habenaria ovalifolia* Wight; **D.** *Rhizoctonia* peloton in *Habenaria marginata* Colebr root cells; **E.** Pure isolate of *Rhizoctonia* sp. from *Habenaria multicaudata* Sedgw. roots; **F.** Vegetative mycelium (100X) from *Malaxis versicolor* (Lindl.) Abeyw ;**G.** *Rhizoctonia* sp. peloton in *Nervelia plicata* (Andrews) Schltr. root cells; **H.** Pure isolate of *Rhizoctonia* sp. from *Habenaria commelinifolia* (Roxb.) Wall. ex Lindl. root cell; **I.** *Rhizoctonia* sp.(100X) from *Habenaria marginata* Colebr; **J.** *Rhizoctonia* sp. peloton in *Habenaria commelinifolia* (Roxb.) Wall. ex Lindl. root cell; **K.**Pure isolate of *Rhizoctonia* sp. from *Habenaria foetida* Blatt. and McCann root; **L.** Vegetative mycelium (40X) from *Habenaria multicaudata* Sedgw.

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