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A New Record: Incidence of Arbuscular Mycorrhizal Fungi (AMF) in *Eleocharis konkanensis* an Endemic Sedge of Maharashtra (India)

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ABSTRACT

Family Cyperaceae, members are commonly known as sedges. This family has ambiguity about mycorrhizal or non-mycorrhizal nature. So far, many sedges have been investigated globally for mycorrhizal occurrence interest. However, a sedge genus, *Eleocharis* has been not paid more attention for mycorrhizal investigations. Present work is the first attempt to evaluate mycorrhizal status in a recently discovered endemic sedge species: *Eleocharis konkanensis* Chandore, Borude, Kambale & S.R. Yadav which grows in a Lateritic rocky plateaus habitat. This study makes a new report on mycorrhization in an endemic sedge: *E. konkanensis* The roots of *E. konkanensis* from lateritic platue are microscopically examined for colonization of AMF. The study revealed that the roots of all the plants were colonized in the range of 21 - 98% by AM fungal components. Overall colonization percentage (*OCp*) in *E. konkanensis* is 56.55. Analysis of mychorrhizal roots suggests there is great variation found in occurrence intensity (*OI*) expression for vesicles (*MCp50± 1.15)*, arbuscles (*MCp21.43± 0.65*[p]*)*, and hyphae (*MCp 98.21± 1.70*). Besides, extraradical and intraradical spores of AMF have been recorded to roots. During present work, 12 AMF species belonging to four Glomeromycota families are recorded in association with *E. konkanensis*. These AMF species are *viz., Aculospora dilatata*, *A. scrobiculata*, *A. spinosa.*, *A. sporocarpa*, *Acaulospora* spp, *A. tuberculata*; *Diversispora epigaea*; *Scutellospora arenicola*, *Glomus albidum*, *G. ambisporum*, *G macrocarpum* and *Septoglomus constrictum*. Thus, *E. konkanensis* is the first representative of genus *Eleocharis* ever studied by any Indian mycologist with reference to root colonization followed by spore's identification of AMF at species level. Till the present work, no data was available on AMF species of genus *Eleocharis.* Hence, *E. konkanensis* is proposed here as the first representative of genus *Eleocharis* ever studied by any mycologist. Based on the relative abundance and spore density the native AMF species of endemic *E. konkanensis* are *viz.*, *Diversispora epigaea*, *Aculospora dilatata* and *Aculospora scrobiculata.* Finally, on the basis of in-depth analysis we propose that *Eleocharis konkanensis* an endemic sedge of Maharashtra is a new mycorrhizal plant to the science.

Introduction

The members of the Cyperaceae are commonly known as sedges. The genus *Eleocharis* Brown is distributed worldwide. According recent checklist of Cyperaceae (Govaerts *et al.,* (2016), *Eleocharis* comprises about 300 species at global data. As far as concern with present context, till date 26 species of *Eleocharis* are known to India. It includes *E. setifolia* (A. Rich) Raynal which is recorded for India by Wadoodkhan (2015); three new species recently described for Indian states are *viz., E. khandwaensis* Mujaffar, Chandore and S.R. Yadav from Madhya Pradesh (Mujaffar *et al.,* 2014), *E. konkanensis* Chandore, Borude, Kambale and S.R. Yadav (Chandore *et al.*, 2016) and *E. neglecta* Borude, Chandore, Gholave and S.R. Yadav (Borude *et al.*, 2016) from Maharashtra.

Geographically, *Eleocharis konkanensis* distributed endemically at 200–300 ft from MSL on Low level Ferricrete (LLF)- Lateritic rocky plateaus of Konkan region specifically at Sindhudurg and Ratnagiri district of Maharashtra in India.

Several species of *Eleocharis* are investigated for their allelopathic effects on other aquatic and wetland plants (Wooten and Elakovich 1991; Pedersen, 2002).

Although it is assumed that, Cyperaceae members are lacked with mycorrhizal association or it is very rare (Hirsch and Kapulnik 1998); however, several reports have produced supportive records for mycorrhization is predominantly of AMF type in sedges (see Harley and Harley 1987a; Tester *et al.,* 1987). However a sedge genus *Eleocharis* has been not paid more attention for mycorrhizal investigations. Because, arbuscular mycorrhizal fungi (AMF) are a ubiquitous soil-inhabiting obligate biotrophic root fungi (Smith and Read, 2008) and they plays significant role in ecosystem functioning.

The AMF diversity data relies primarily in studies performed in natural ecosystems. Nevertheless, little is known about AMF diversity in extreme human-impacted ecosystems such as Lateritic rocky plateaus associated ephemerals.

Review of documents on AMF of *Eleocharis* species for last seven decades (Dowding, 1959 to de la Providencia *et al.,* (2015) including contribution of Muthukumar (2004) and Hossler (2010) showed that (Table 1), genus *Eleocharis* comprises three categories of plant species such as mycorrhizal, non-mycorrhizal and mycorrhizal as well as non-mycorrhizal. Eleven species of *Eleocharis* were reported as mycorrhizal *viz., E. acutangula*, *E. atropurpurea*, *E. dulcis, E. geniculata*, *E. obtusa*, *E. ovata*, *E. palustris*, *E. pusilla*, *E. quinqueflora*, *Eleocharis sp.,* and *Eleocharis spp.* Whereas, non-mycorrhizal *Eleocharis* species were *viz., E. aff. pachycarpa*, *E. congesta*, *E. elliptica*, *E. erythropoda*, *E. geniculata*, *E. quinqueflora*, *E. scheuchezeri*, *Eleocharis sp.*, *E. tenius*, *E. triste*, and *E. vaginatum* (Table 1). From this survey it is evident that, two species *viz., E. geniculata* and *E. quinqueflora* were recorded as dual performers i e mycorrhizal as well as non-mycorrizal. Thus out of twenty *Eleocharis* species about 50% are known to be distinctly mycorrhizal and rest is non-mycorrhizal.

All the 20 species so far investigated were associated with wetland ecosystem at global scenario. Present work is the first attempt to evaluate mycorrhizal status in a recently discovered *E. konkanensis* grown in Lateritic rocky plateaus habitat. This study makes a new report on mycorrhization in an endemic sedge *E. konkanensis*.

Materials and Methods

Site description and sample collection

Sampling was performed at LLF-Lateritic rocky plateaus of Hativale, Abasaheb Marathe College Campus, Vikhare Gothane, (16.6572^0 North and 73.5211^0 East) 12 km from Rajapur which falls under Ratnagiri district. *E. konkanensis* samples were collected during July 2016 (SSL and MMK) and identification was validated by ANC. The dry preservation is deposited at Botany department herbarium, BCA (Figure 1A).

E. konkanensis is a small, perennial ephemeral herb, grows along the edges of natural ponds, on roadside and on the open places of lateritic plateaus having considerably less soil layer. As, it grows in association with other ephimerals such as: *Cyperus pulchellus* and *Eleocharis atropurpurea*, *Habenaria grandifloriformis*, *Utricularia reticulata* and *Trithuria konkanensis*. Therefore roots excavation of *E. konkanensis* and its soil collection was done randomly from about 25-30 such a plants where associating ephimerals were less crowded. This precaution was taken to avoid unwanted collection of AMF biota of unwanted vegetation and thereby to prevent misleading results on AMF spores. Altogether, around 250g soil was randomly collected for AMF spore extraction procedure and to determine the soil properties.

The plants along with the soil samples and roots were collected in different collection bags and transported from field to laboratory which immediately refrigerated at 4°C subsequent to arrival. The roots were processed immediately. All the rhizosphere soil samples were homogenized prior to remove coarse roots segments, stones and adhered particles through sieving procedure (2 mm mesh size). Subsamples of soil were air dried and used for estimation of physico-chemical properties

Physicochemical parameters of soil:

Physicochemical parameters were tested for soil: i) texture, moisture (Jackson, 1967); ii) pH (van Reeuwijk, 2002); iii) organic carbon (Walkley and Black, 1938); iv) carbonate (Piper, 1966) and available phosphorus (Olsen *et al.*, 1954).

Status of AM fungal colonization in roots

It was determined by assessing roots for percentage of colonization and occurrence intensity of three mycorrhizal components as given below:

Percentage colonization

The randomly selected and stained 100 root segments of of *E. konkanensis* were observed under a light microscope and subjected to intercept method (Brundrett, *et al.*, 1996), AMF colonization percentage,(Phillips and Hayman 1970). The overall colonization percentage (*OCp)* by AMF was calculated on the basis of observed values for mean colonization percentage (*MCp)* of three component i) vesicles (*V*), ii) arbuscules (*A*) and iii) hyphae (*H*)**.** The occurrence of other fungal endophytes (*Ofe*), such as dark septate endophytes (DSE) was also screened. microscopically.

Occurrence intensity

All the three components of AMF were interpreted for occurrence intensity under categories such as, *poor* (1-25%), *moderate* (25-50%), *good* (50-75%) and *excellent* (>75%) which was denoted as '*p, m, g* and *e'* respectively. To interpret occurrence intensity (*OI*) of fungal structures, mean colonization percentage (*MCp)* for each fungal structure *V, A* and *H* was determined separately. Finally pattern of AMF colonization for *E. konkanensis* was determined. Any other special structure of mycorrhizal colonization

(Smc) if present in root piece was also recorded.

AM fungal spore extraction

AMF spores were extracted by following sieving and decanting technique of Gerdemann and Nicolson (1963) with limited quantity of rhizospheric soil (10g). Total spore number was estimated following by Gaur and Adholeya (1994). Apparently healthy spores were counted under stereomicroscope (Olympus 003421) and examined under Binocular Light microscope using different objectives like 10×, 40× and 100×. Slides were digitally documented with the help of Canon IXUS 155 Camera.

AM fungal species identification

Taxonomical placements of AM fungal spores and sporocarps up to species level were done using bibliographies by Schenk and Perez (1990). The identification is purely based on the synaptic keys (Hall and Fish, 1979; Pacioni, 1992) and also after consulting AMF species descriptions provided by International Culture Collection of Vesicular and Arbuscular Endomycorrhizal Fungi [http://invam.caf.wvu.edu/Myc_Info/Taxonomy/species.htm]. The species codes were followed after Schenk and Perez (1990).

The voucher slides containing the isolated spore specimens were assigned accession codes '$BCA{:}MH_{SSL}{}^{n}$' [where, *BCA:MH* is Bhavan's College Andheri: Mycological Herbarium; *SSL:* initials of second Author and *'n'* is accession number assigned]. All the slides and corresponding photo-micrographic plates have been deposited in the slide collection section of Mycorrhizal Research Laboratory of Department. The *Spore density* (S) was considered as the number of spores in 10g soil. *Relative abundance* (RA) value for AMF species (*RAspp*) was equivalent to the spore percentage of a species (Dandan and Zhiwei, 2007). Similarly, RA of AMF family (*RAfam*) was considered as spore percentage of a family. Based on RA and S values (*RAspp* > 6%) and spore density (S ≥ 7 spores) the dominant AMF species were determined. Similarly dominating Glomeromycota families of *E. konkanensis* rhizospere were determined (*RAfam* > 40%) and spore density (S ≥ 10 spores).

The standard errors of means were statistically analyzed for percentage colonization, spore density and relative abundance of AMF species by using Microsoft excel 2010.

Results and Discussion

Physico-chemical parameters of soil of E. konkanensis

Nature of soil needed for each plant and its rhizospheric microbes is varies from species to species and hence for habitat restoration and conservation program the soil properties should be taken into consideration. The soil of study area is lateritic and reddish due to excess natural iron content. Soil sampled from *E. konkanensis* rhizosphere has a relatively loose texture and its characteristics are shown in Table 2. These soils often have up to 40% gravel and slightly acidic with pH 6.4. Organic Carbon (5.70 %) and Organic matter (9.80%) calculated is higher in the topsoil of *E. konkanensis* Whereas, Carbonate content recorded is quite higher (19.09±0.01mg.kg^{-1}); while phosphorus content is poor 1.04 ± 0.02mg.kg^{-1}

Percentage colonization and occurrence intensity of AMF in roots of E. konkanensis

Based on tabulated references (Table 1), out of twenty *Eleocharis* species nine were either distinctly mycorrhizal or non-mycorrhizal, and remaining two were of dual nature showing mycorrhizal as well as non-mycorrhizal association. All the earlier known mycorrhizal *Eleocharis* species were reported on the basis of interception method only and not by AMF spores identification technique. In present work we have assessed the mycorrhizal nature of endemic sedge *E. konkanensis* based on both root interception method and AMF spores extraction followed by identification up to species level.

Percentage colonization

The roots of *E. konkanensis* from lateritic platue were microscopically examined for presence of AMF (Table 3). In present assessment, roots of *E. konkanensis* were assessed for presence of AMF colonization (Table 3) and denoted by *MCp i. e.* mean colonization percentage for individual components. The study revealed that the roots of all the plants were colonized in the range of 21 - 98% by AM fungal components. The degree of AMF components colonization was found varied i. e. vesicles 50% arbuscules 21.43% and hyphae 98.21%. However, overall colonization percentage (*OCp*) in *E. konkanensis* was 56.55.

Table.1 Tabulated review of documents* on mycorrhizal incidence in genus *Eleocharis*

***Eleocharis* species**		**Mycorrhizal status**			**References**
		M	**NM**	**PR** (cm)	
1.	*E. acutangula*	√ M	-	0.1	M[16, 18, 19, 22, 29]
2.	*E. aff. pachycarpa*	-	√ NM	-	NM[12,]
3.	*E. atropurpurea*	√ M	-	0.23	M[2]
4.	*E. congesta*	-	√ NM	0	NM[6]
5.	*E. dulcis*	√ M	-	-	M[21]
6.	*E. elliptica*	-	√ NM	0	NM[30]
7.	*E. erythropoda*	-	√ NM	0	NM[27]
8.	*E. geniculata*	√ M	√ NM	0	M[8, 29] ; NM[16, 21, 25]
9.	*E. obtusa*	√ M	-	0–0.75	M[10, 15]
10.	*E. ovata*	√ M	-	0–0.9	M[8, 29]
11.	*E. palustris*	√ M	-	0–0.75+	M[1, 3, 5, 13, 14, 15, 16, 24, 29]
12.	*E. pusilla*	√ M	-	0–0.56	M[7, 16]
13.	*E. quinqueflora*	√ M	√ NM	+	M[11, 16]; NM[13, 14, 29]
14.	*E. scheuchezeri*	-	√ NM	-	NM[4, 16, 28]
15.	*Eleocharis sp.*	√ M	-	-	M[8]
16.	*Eleocharis sp.*	-	√ NM	-	NM[12]
17.	*Eleocharis spp.*	√ M	-	0–0.7	M[8]
18.	*E. tenius*	-	√ NM	0	NM[9]
19.	*E. triste*	-	√ NM	-	NM[4]
20.	*E. vaginatum*	-	√ NM	-	NM[26]

After analyzing [20]Muthukumar (2004) and [15]Hossler (2010)
PR: A proportional range of root length (cm) colonized by AMF is provided when available; however, some studies reported only '+' or '-', so these indicators are also included.

Reference superscripts are for citation referral with √Mycorrhizal: M and √Non-mycorrhizal: NM.[[1]Anderson *et al.*, (1984); [2]Aziz *et al.*, (1995); [3]Beck-Nielsen and Madsen (2001); [4]Bledose *et al.*, (1990); [5]Bohrer *et al.*, (2004); [6]Chaubal *et al.*, (1982); [7]Clayton and Bagyaraj (1984); [8]Cooke and Lefor (1998); [9]Cornwell *et al.*, (2001); [10]de la Providencia *et al.*, (2015); [11]Dowding (1959); [12]Fontenla *et al.*, (2001); [13]Harley and Harley. (1987a); [14]Harley and Harley. (1987b); [15]Hossler (2010); [16]Khan and Belik (1995); [17]Khon and Stasovski (1990); [18]Muthukumar (1996); [19]Muthukumar and Udaiyan (2000); [20]Muthukumar *et al.*, (2004); [21]Ragupathy and Mahadevan (1993); [22]Ragupathy *et al.*, (1990); [23]Silva *et al.*, (2001); [24]Søndergaard and Laegaard (1977); [25]Thoen (1987); [26]Thormann *et al.*, (1999); [27]Turner *et al.*, (2000); [28]Väre *et al.*, (1992); [29]Wang and Qiu (2006); [30]Weishampel and Bedford (2006)]

Table.2 Physicochemical properties of *E. konkanensis* soil

Sr.No.	Parameters	Status
1.	Colour	Red
2.	Soil texture	≈ 40% gravel,
3.	PH	6.44 ± 0.02
4.	Organic Carbon	5.70 %
5.	Organic Matter	9.80 %
6.	Carbonate	19.09 ± 0.01 $mg.kg^{-1}$
7.	Phosphorus	1.04 ± 0.02 $mg.kg^{-1}$

(±) Standard error of mean

Table.3 Incidence of AMF colonization in roots of *E. konkanensis*

Particulars	***AMF colonization***		
Fungal structures	***Vesicles***	***Arbuscules***	***Hyphae***
MCp (%)	50± 1.15 ^{m}	21.43± 0.65^{P}	98.21± 1.70 ^{e}
OCp (%)	56.55 ±1.16^{g}		
OI	Moderate	Poor	Excellent
OCI	Good		
Smc Features	Formation of: A, Av, Ch, H, Hc, ERs, IRs & S (Figure 1 B-C)		
Pmc	VAH		
Other fungal endophytes	Ofe-Present: Dsh & Scl (Figure 1D)		

(MCp) mean colonization percentage*;* ***(OI)***Occurrence intensity **[*(p)*** *1-25%,* ***(m)*** *25-50%,* ***(g)*** *50-75%,* ***(e)****>75%*]**;** ***(Smc)***Structures of Mycorrhizal colonization **[*(A)*** *Arbuscules*, ***(Av)*** Aggregated vesicles, ***(Ch)*** Chlamydospore, ***(H)*** Hyphae; ***(H)*** Hyphal coiling; ***(ERs)***Extraradical spore, ***(IRs)***Intraradical spore and ***(S)*** young spore]**;** ***(OCI)*** Overall colonization intensity **[**range of values is same as ***OI***]**;** ***(OCp)*** Overall colonization percentage*;* ***(Pmc)*** Pattern of Mycorrhizal colonization; ***(VAH)*** *Vesicular- Arbuscular-hyphal type;* ***(ofe)*** *Other fungal endophytes,,* [***(Dsh)*** dark septate hyphae and ***(Scl)***sclerotia

Table.4 AMF species associated with *E. konkanensis* and their spore density (S) and relative abundance (RA)

Specimen Accession Code	AMF species	S	RA	
			RAspp	***RAfm***
Family: Acaulosporaceae			54.901	
BCA:MH$_{SSL}$01	*Aculospora dilatata* Morton.	7	13.725	54.901
BCA:MH$_{SSL}$02	*Acaulospora scrobiculata* Trappe	7	13.725	
BCA:MH$_{SSL}$03	*Aculospora spinosa*. Walker and Trappe	2	3.921	
BCA:MH$_{SSL}$04	*Aculospora sporocarpa* Berch	6	11.764	
BCA:MH$_{SSL}$05	*Acaulospora* spp1	2	3.921	
BCA:MH$_{SSL}$06	*Aculospora tuberculata* Janos and Trappe	4	7.843	
Family: Diversisporaceae				
BCA:MH$_{SSL}$07	*Diversispora epigaea* (B.A. Daniels & Trappe) C. Walker & A. Schüßler	**9**	**17.647**	**17.647**
Family: Gigasporaceae				
BCA:MH$_{SSL}$11	*Scutellospora arenicola* Koske & Halvorson	2	3.921	3.921
Family: Glomeraceae				
BCA:MH$_{SSL}$08	*Glomus albidum* C. Walker & L.H. Rhodes	4	7.843	23.529
BCA:MH$_{SSL}$09	*Glomus ambisporum* G.S. Sm. & N.C. Schenck	1	1.960	
BCA:MH$_{SSL}$10	*Glomus macrocarpum* Tul. & C. Tul.	6	11.764	
BCA:MH$_{SSL}$12	*Septoglomus constrictum* G.A. Silva & Oehl	1	1.960	
Total	**12**	**51**	**100**	**100**

Figure.1 Fig 1A-D *Eleocharis konkanensis* Chandore, Borude, Kambale and S.R. Yadav and root colonization. Fig 1A Specimen; Fig 1B-C Root colonization showing mycorrhizal components *viz.* Arbuscules [*A*], Aggregated vesicles [*Av*]; Chlamydospore [*Ch*]; Hyphae [*H*], Hyphal coiling [*H*c], Extrararadical spore [*ERs*]; Intrararadical spore [*IRs*], young spore [*S*] etc; Fig 1D Root colonization with other fungal endophyte [*Ofe*] components *viz.*, dark septate hyphae [*Dsh*] and Sclerotia [*Scl*]

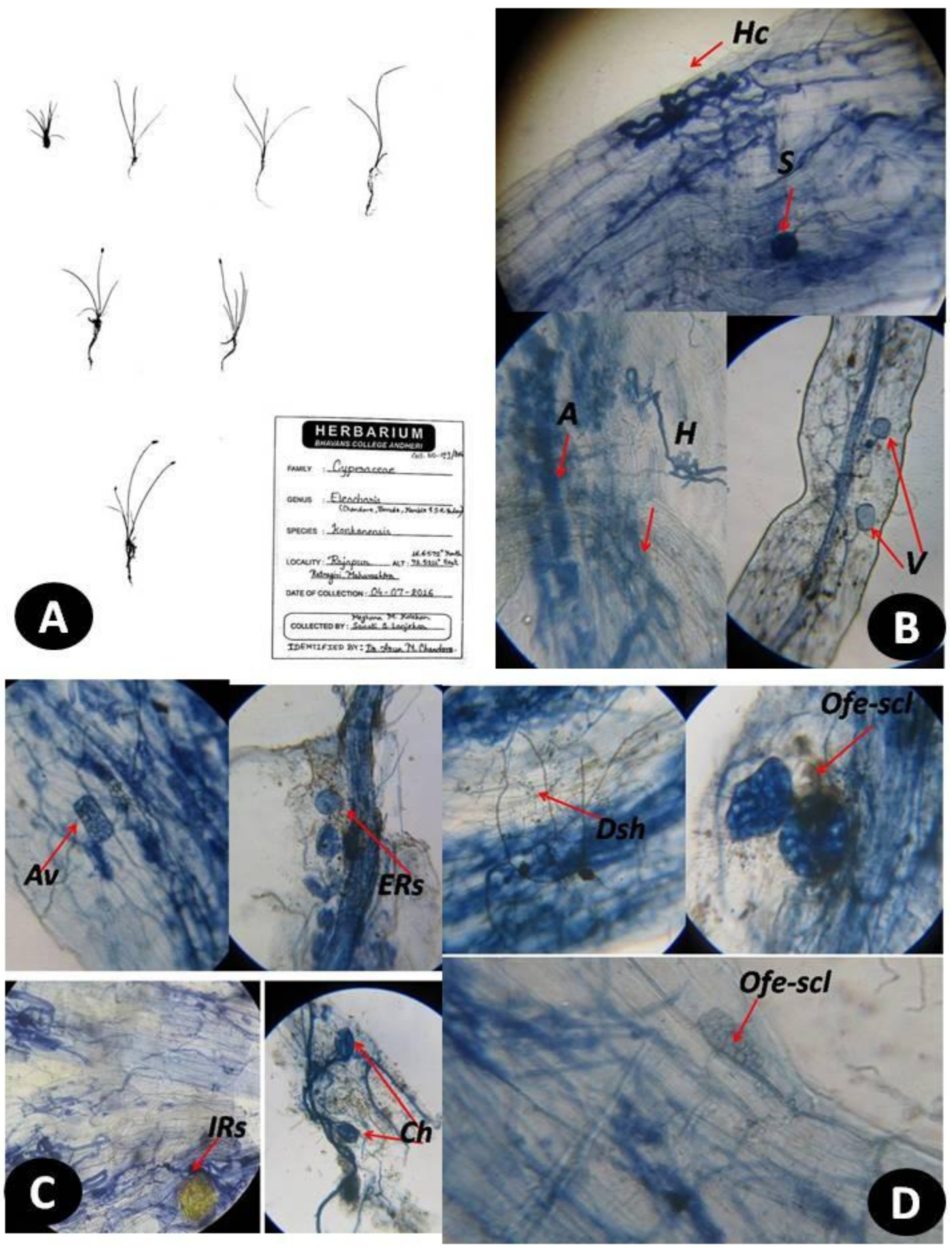


FIGURE 1

Figure.2A-D. AMF spores associated with *Eleocharis konkanensis* showing morphotype details. Fig.2A *Acaulospora dilatata* Morton.; Fig.2B *Acaulospora scrobiculata* Trappe; Fig.2C *Aculospora spinosa* Walker and Trappe and Fig.2D *Aculospora sporocarpa* Berch [Fig.2A-D(a) Spore mounting in different media like water, Melzer's Reagent and PVLG; Fig 2A-C(b) Entire or crushed spore; Fig.2D(b) Crushed spore showing Circatrix (arrow); 2A-D(c) Magnified view showing spore wall and surface details]

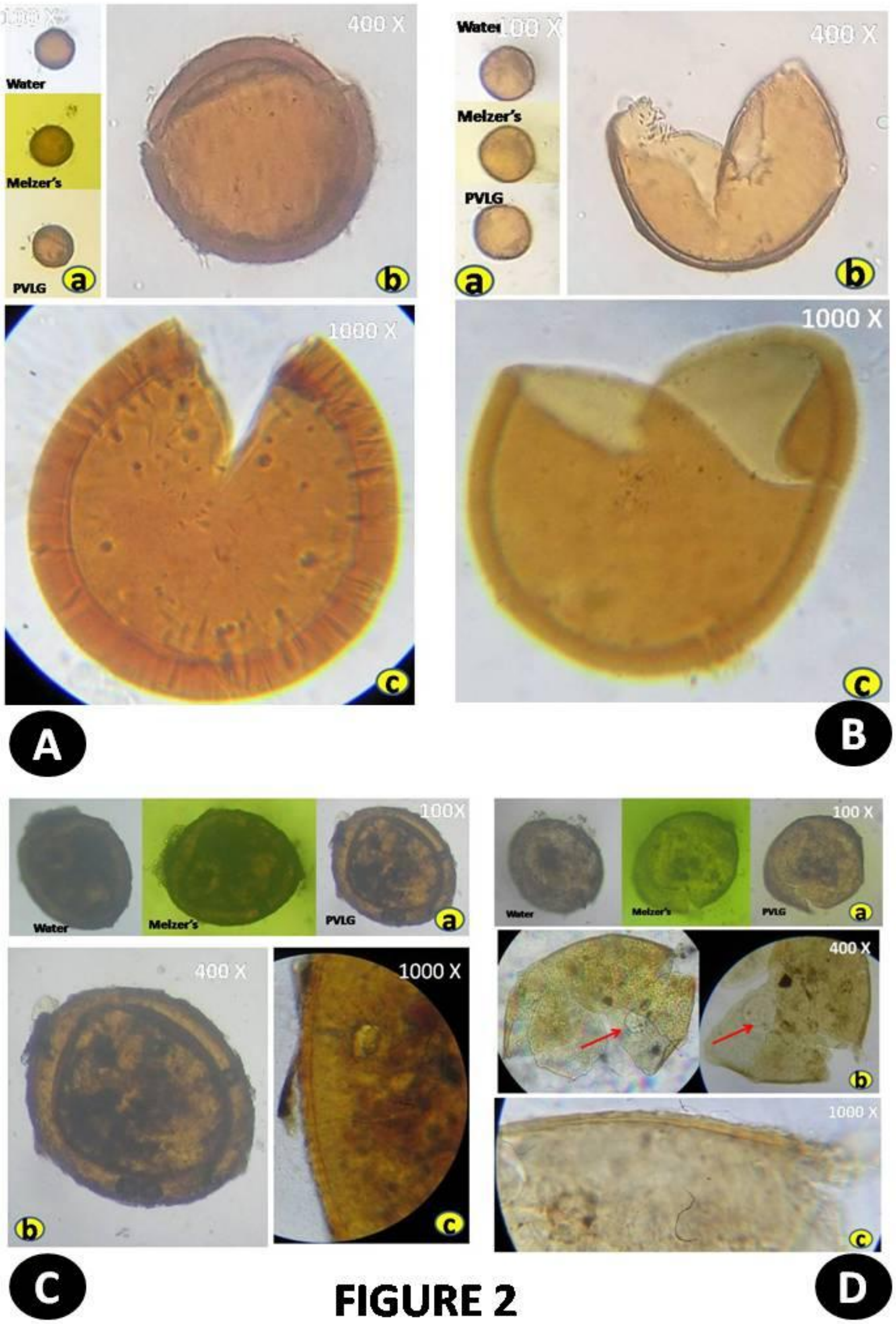


Figure 3A-D. AMF spores associated with *Eleocharis konkanensis* showing morphotype details. Fig.2A *Aculospora spp1*; Fig.3B *Aculospora tuberculata* Janos and Trappe; Fig.3C *Diversispora epigaea* (B.A. Daniels and Trappe) C. Walker and A. Schüßler and Fig.3D Magnified view of entire spores of (a) *Glomus albidum* C. Walker and L.H. Rhodes (b) *Glomus ambisporum* G.S. Sm. and N.C. Schenck; (c) *Glomus macrocarpum* Tul. and C. Tul. [Fig.3A-C(a) Spore mounting in different media like water, Melzer's Reagent and PVLG; Fig.3A-C(b) Entire or crushed spore; 3A-C(c) Magnified view showing spore wall and surface details.]

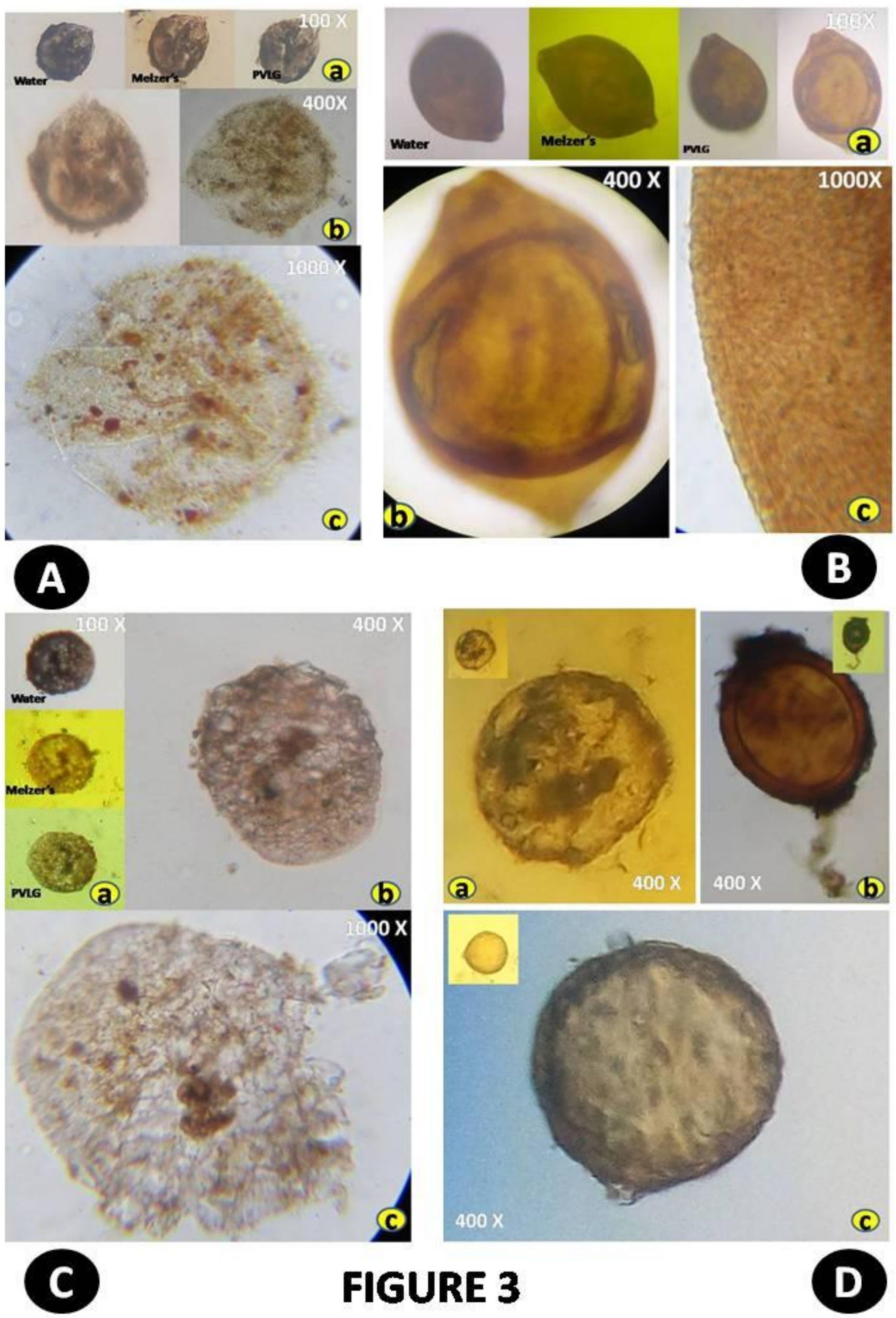
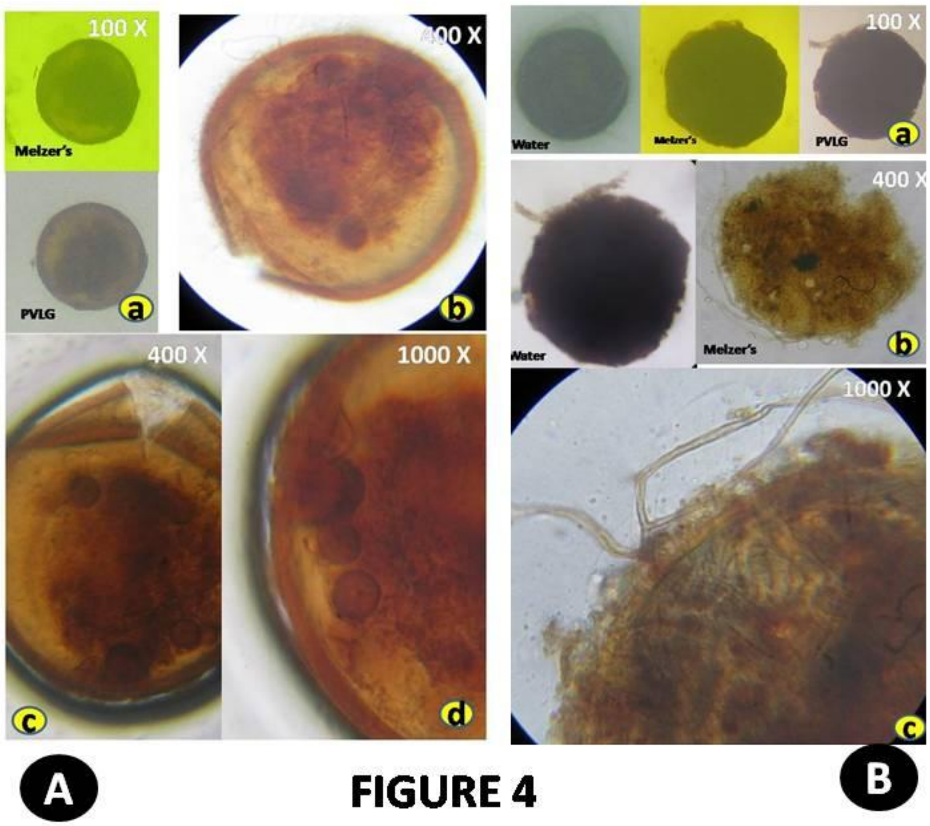


Figure.4A-B AMF spores associated with *Eleocharis konkanensis* showing morphotype details. Fig.4A *Scutellospora arenicola* Koske and Halvorson [Fig.4A(a) Spore mounting in different media like Melzer's Reagent and PVLG; Fig.4A(b-c) crushed spore showing spore wall and surface details.; 4A(d) Magnified view showing inner spore wall details.]; and Fig.4B *Septoglomus constrictum* G.A. Silva and Oehl; [Fig.4B(a-b) Spore mounting in different media like Water, Melzer's Reagent and PVLG; Fig.4B(c) Magnified view showing spore wall and surface hyphae]



Occurrence intensity (OI)

The occurrence intensity (*OI*) observed is qualitative expression of corresponding *MCp* values which are also presented in Table 3. Analysis of mychorrhizal roots suggests there is great variation found in *OI* expression for vesicles (*MCp50± 1.15)*, arbuscles (*MCp21.43± 0.65*^{p}*)*, and hyphae (*MCp 98.21± 1.70*); Thus, arbuscles *OI* was comparatively poor.In present investigation, although the arbuscular colonization was poor but it is quite common in sedges. According to Muthukumar *et al.,* (2004), "reports on arbuscule occurrence are limited and the nutritional benefits of mycorrhizal association in sedges possessing arbuscules is yet to be ascertained". However, our results showed establishment of vesicular-arbuscular-hyphal VAH type colonization. In fact it was makes general agreement with Jalonen *et al.*, 2013, stated that, total AM colonization is often dominated by hyphal colonisation (Table 3).

It was also confirmed with Muthukumar *et al.,* (2004), that, intra radical vesicles and hyphae are frequently encountered AMF structures in sedge roots. During present investigation aggregated form of vesicles (*Av*), hyphal coiling (*Hc*), chlamydospores

(*Ch*) are clearly observed (Figure 1B-C). Furthermore AMF spores have been recorded both attached to roots *i e* extrararadical spore (*ERs*) like Muthukumar *et al.*, (1997); Cooke and Lefor (1998) and within roots *i e* intraradical spore (*IRs*), as that of Miller *et al.*, (1999) (Figure 1C). These observations decisively demonstrate that endemic sedges like *E. konkanensis* would greatly improve the persistence and survival of AMF propagules in the soil.

In earlier study range of proportional root length (cm) colonized by AMF was found varied for different species (Table 1) *i.e. E. obtusa* and *E. palustris* were colonized 0–0.7 and 0–0.6cm respectively; whereas, arbuscular colonization was 0–0.1 and 00cm respectively, it was lacked of hyphal coiling but showed presence of sepatate endophytes (Hossler (2010). However, our observations on *E. konkanensis* suggests presence of other fungal endophytes (*Ofe)* dark septate hyphae *(Dsh)* (Figure 1D) along with AMF colonization. It is evident that, dark septate fungi can extensively colonizes the roots under such an environmental condition where AMF fail to proliferate (Khon and Stasovski, 1990; Treu *et al.*, 1996). The role of *Dsh* in most of the plant is unclear but there is growing evidence that these endophytes may play roles similar to those of AMF in enhancing host growth and nutrition uptake (Barrow and Aaltonen, 2001). It often to see dual colonization of dark septate endophytes with AMF under less extreme environments (Read and Haselwandter, 1981; Bledose *et al.*, 1990; Khon and Stasovski 1990; Väre *et al.*, 1992; Treu *et al.*, 1996). In present report such dual colonization is appeared but AMF colonization is dominating over dark septate endophytes.

In recent study, de la Providencia *et al.*, (2015) performed molecular diversity exploration using high-throughput PCR, cloning and sequencing of 18S rDNA on *E. obtusa* spontaneously inhabiting in extremely petroleum hydrocarbon polluted sediments. Results observed were found with unexpected diversity of AMF associated. Hence these associated AMF were considered as potentially important microbial candidates in bioremediation of oil-contaminated soils. Therefore to tap the commercial and environmental potential of sedges like Eleocharis more attention is needed toward the mycorrhizal diversity by using molecular tools along with classical method in AMF research.

Assessement of AM fungal species

In genus *Eleocharis* till the date no data is available on AMF species. Hence, present paper provides significant details on AMF species associated with any *Eleocharis* species ever studied before. In present study total twelve species of AM fungi under four families of Glomeromycetes such as: Acaulosporaceae, *Diversisporaceae*, Gigasporaceae and Glomeraceae were identified from the lateritic soil samples of *E. konkanensis* distributed over five genera viz., *Acaulospora*, *Diversispora*, *Scutellospora*, *Glomus* and *Sclerocystis.* The spores of all 12 species are presented in Table 4. The details of all 12 AMF species spore morphotypes are well illustrated in present paper (Figures 2-4). Amongst the 12 species, genus *Acaulospora* and *Glomus* represented six and three species (50% and 25%) respectivelly. represented two species (11.11%). Whereas, remaining three genera *viz.*, *Diversispora* and *Scutellospora and Septoglomus* represented only one species (8.33%).

During present work, AM fungal species associated with *E. konkanensis* are identified up to species level (Table 4). These species are *viz.*, *Aculospora dilatata* Morton. [*BCA:MH*$_{SSL}$*01*], *Acaulospora scrobiculata* Trappe [*BCA:MH*$_{SSL}$*02*], *Aculospora spinosa.*

Walker and Trappe [*BCA:MH*$_{SSL}$*03*], *Aculospora sporocarpa* Berch [*BCA:MH*$_{SSL}$*04*], *Acaulospora* spp1 [*BCA:MH*$_{SSL}$*05*], *Aculospora tuberculata* Janos and Trappe [*BCA:MH*$_{SSL}$*06*]; *Diversispora epigaea* (B.A. Daniels and Trappe) C. Walker and A. Schüßler [*BCA:MH*$_{SSL}$*07*]; *Scutellospora arenicola* Koske and Halvorson [*BCA:MH*$_{SSL}$*11*]; *Glomus albidum* C. Walker and L.H. Rhodes [*BCA:MH*$_{SSL}$*08*], *Glomus ambisporum* G.S. Sm. and N.C. Schenck [*BCA:MH*$_{SSL}$*09*], *Glomus macrocarpum* Tul. and C. Tul.[*BCA:MH*$_{SSL}$*10*] and *Septoglomus constrictum* G.A. Silva and Oehl [*BCA:MH*$_{SSL}$*12*]. Thus, *E. konkanensis* is proposed as the first representative of genus *Eleocharis* ever studied by any Indian mycologist with reference to root colonization followed by spore's identification of AMF at species level.

Spore density and relative abundance

The total number of AM fungal spores recovered from lateritic rhizospere soil samples of *E. konkanensis* was 51 and encountered at the rate of 1-9 spores 10^{-1} g soil. The spore density (S) of all 12 AM fungi were determined and expressed as number of spores per 10g of soil of *E. konkanensis* which is presented in Table 3.

After analyzing the RA and S values for native AMF species of *E. konkanensis i e RAspp* > 6% and S ≥ 7 spores the dominant AMF species are *Diversispora epigaea* (*RAspp*: 17.647; S:9), *Aculospora dilatata* and *Aculospora scrobiculata* (*RAspp*: 13.725; S:7 each). Whereas, dominant AMF Glomeromycota family recognized after comparing values: *RAfam* > 40% and S ≥ 10 spores. The dominating AMF Glomeromycota family is Acaulosporaceae**.** Although, Diversisporaceae is apparently expressed highest number of spores (9 spores 10^{-1} g soil) which is contributed by *Diversispora epigaea* alone. However, Acaulosporaceae has expressed spores number ranging (2-7 spores 10^{-1} g soil) but cumulatively 28 spores 10^{-1} g soil are encountered. It is difficult at present to explain the probable reason for this differential expression of spore numbers by two families. Probably, the inter-generic competition between two genera *i.e. Diversispora* (single species) *vs Aculospora* (6 species) could led the underestimating effect over representing family (Diversisporaceae) by Acaulosporaceae. However, to support this hypothesis, such kind of diversified data must be processed from various AMF community associations with different host. At present, we conclude Acaulosporaceae members are dominatingly associated with an endemic sedge *E. konkanensis* from lateritic habitat. Finally, on the basis of in-depth analysis we propose that *Eleocharis konkanensis* an endemic sedge of Maharashtra is a new mycorrhizal plant to the science.

In present paper, documentary evidences proved that, genus *Eleocharis* is less studied for AMF colonization in general; more precisely it is poorly investigated with reference to AMF species. Hence, present paper provides significant details on AMF species associated with any *Eleocharis* species ever studied. Ambiguity of sedges or Cyperaceae members with reference to their mycorrhizal or non-mycorrhizal merit is still unresolved. Because, colonization in sedges may be strongly influenced by variation in environmental conditions. However, based on the numerically available references, currently it can be concluded that Cyperaceae is not strictly a non-mycorrhizal family (Muthukumar *et al.,* 2004). To support it, present report on mycorrhizal nature of an endemic sedge *E. konkanensis* could stand as one more encouraging evidence. Nevertheless, threatened inhabitants flora of lateritic plateaus from Konkan region is yet to investigate for their natural symbionts like mycorrhiza. Hence, inventories are needed for many more such endemic plant species associated with poorly known lateritic soil-plant - mycorrhiza interaction.

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