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AM Fungal Status in *Ketaka: Pandanus fascicularis* From Coastal Region of Konkan, Maharahshtra

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Abstract: *Present paper deals with assessment of Arbuscular mycorrhizal fungi (AM) associated with different populations of Pandanus fascicularis found in coastal region of Konkan Maharashtra. All samples of P. fascicularis roots were colonized by AM fungi. The mean percentage of root length colonization ranged from 39% to 74%. Amongst the thirteen AM fungal morphospecies Kuklospora colombiana was the most widely distributed species. Species richness of AM fungi ranged from 3 to 6. Based on spore density and relative abundance, three species were dominant viz., Acaulospora bireticulata, A. scrobiculata, and K. colombiana. Details of AM fungal status in P. fascicularis are discussed in present paper.*

Keywords - *Arbuscular Mycorrhizal Fungi, Pandanus fascicularis, Relative abundance, Species diversity, Spore density.*

I. INTRODUCTION

More than 500 species are known to occur in genus *Pandanus*, out of these 36 species have been recorded in India [1]. The Ayurvedic drug *Ketaka* is *Pandanus fascicularis* Lam. (Synonyms: *P. tectorius, P. odoratissimus*) belonging to the family Pandanaceae [1-2]. Vernacular names of this plant are: Sansktit- Ketaki; Hindi-Keura, Kewda, Gagandhul; Tamil- Tazhai; Telugu-Mugali; Kannada-Tale Mara; English-Screw pine [3-4]. It grows wildly in coastal regions of India and Andaman islands [5]. Its distribution is documented over coastal Districts of Orissa, Andhra Pradesh, Tamil Nadu, and to some extent in parts of Uttar Pradesh [6]. In Konkan region of Maharashtra its distribution is confined with backshore zone of coastal area especially at Raigad, Ratnagiri and Sindhudurg District. The fragrant spadices of staminate inflorescence are more frequently used by Indian women for hair dressings in Maharashtra. Kewda attar and water are used for flavoring various foods, sweets, syrups and soft drinks. They are popular in north India, especially on festive occasions.

It is now well documented that, different plants are widely used in various traditional systems of medicine like Ayurveda, Siddha and Unani to treat rheumatic fever, rheumatism and rheumatoid arthritis and *P. fascicularis* is a good example of it which has proved its analgesic activity [5]. In Ayurveda *Ketaka* used for headache, anorexia, indigestion, constipation, eye diseases, leprosy, hypoglycemic activity [7-8]. Recently Madhavan *et al.* [9] suggested that *P. fascicularis* root possesses the potent antihyperglycemic activity which may be due to the presence of phenolic compounds, tannins, and flavonoids. Ecologically, the *P. fascicularis* is also important as its root system binds soil and checks soil erosion. On sandy soils along the sea coast, it fixes sand dunes, which act as a barrier against encroachment of the sea and protect human settlements along the coast line [10].

The cultivation practices, marketing and trading of Kewda has proved positive impact on the local economy at Ganjam District, Orissa, India [6], where it constitutes the backbone of the local economy [10]. Although Maharashtra has a coastline of 720 Km which is composed with either sandy or rocky area, it has wide scope for cultivation of *P. fascicularis.* However, for such practices extensive study of coastal region is necessary which includes physical, geological and biological aspects like microbial association with coastal flora. Although, beach ecosystems in India particularly from Goa have been studied for first two aspects and for restoration of degraded coastal dunes very well [11-13], unfortunately such efforts have not made in Maharashtra. The microbial association (especially mycorrhizal) in coastal vegetation has been always remained unexplored in Maharashtra.

It is confirmed that, mycorrhizal colonization benefits soil rehabilitation and erosion control by stimulating soil aggregation [14-15]. The extramatrical mycelium of Arbuscular Mycorrhizal fungi (AM) during symbiosis with the plant also provides a large surface area on colonized root and thus help in maximum absorption of orthophosphate from bulk soil [16]. Hence in present paper it was thought worthwhile to assess the AM fungal diversity associated with different populations of *P. fascicularis* which is established as very dense vegetation in Konkan region of Maharashtra.

II. Material And Methods

2.1 Sample collection:

Roots and rhizosphere soil samples of different populations of *P. fascicularis* found in coastal region of Konkan Maharashtra were collected during 2010-2012. Study area comprises five sites belonging to three Districts *viz.*, Raigad: Revdanda beach; Ratnagiri: Murud beach Dapoli, Ganpatipule beach; Sindhudurg: Munge beach and Tambaldeg beach respectively. At each of the field sites, 5 soil core samples per tree were taken at a depth of 5-30 cm using a soil digger and spade. Approximately 1 kg total of rhizosphere soil from each site was collected. Soil samples were mixed into composite samples and root samples were removed from the soil and preserved in 70% ethanol for each tree.

2.2 AM fungal root colonization assessment:

In the laboratory, roots of each plant from every location were made free from sand and soil-debris by washing, after clearing and staining technique of Phillips and Hayman [17]. Roots fixed in 70% ethanol were cleared in 10% (w/v) KOH solution and autoclaved at 121°C and 15 lb/inch^2 for 15 minutes. Then, roots were washed with distilled water to remove KOH, stained with 0.05% cotton blue dye for overnight. They were observed under a binocular microscope using lactophenol to evaluate mycorrhizal colonization [18]. Fifty stained roots (each about 1 cm in length) were assessed for colonization percentage using the intercept method [19] under a Olympus compound microscope.

2.3 AM fungal spore isolation and species identification:

After root removal, the sand samples from each location were combined to obtain a single sample per location. AM fungal spores occurring in the rhizosphere soil samples were extracted directly by using the wet sieving and decanting method of Gerdemann and Nicolson [20]. 100 g of each soil sample was suspended in 500 ml of water and stirred for 10 mins. Sieve sizes of 250, 210, 120, 75 and 45 µm, were used for spore collection. The spores retained on each sieve were transferred to filter paper and subsequently examined under a Magnus Stereomicroscope at a magnification of up to 400X and identified based on spore morphology. Each spore morphotype was mounted in polyvinyl-lactoglycerol (PVLG) and PVLG mixed with Melzers reagent in 1:1 (v/v) ratio [21]. Identification of AM fungal species was based on original descriptions of Schenck and Pérez [22] and also using current species descriptions and identification manuals (International Culture Collection of Vesicular and Arbuscular Endomycorrhizal Fungi [http:// invam.caf.wvu.edu/Myc_Info/Taxonomy/species.htm]).

2.4 AM fungi species status:

2.4.1 Spore density (SD) is the number of spores in 100 g soil. Relative abundance (RA) was defined as the percentage of spore numbers of a species divided by the total spores observed [23]. The **frequency isolation** (IF) of each AM fungal species was calculated by the percentage of the number of the samples in which the species or genus was observed. The dominant AM fungal species according to **relative abundance** (RA > 6%) and spore density in 100 g soil (spore density higher than 40 spores) and species richness were determined for each sampling site.

2.4.2 Diversity index and concentration of Dominance AM fungal diversity was evaluated using the Shannon-Weiner diversity index which has two main components, evenness and number of species [24]. The Shannon–Weiner index (H') was calculated according to the formula $H' = -\Sigma(ni/N) \log_2 (ni/N)$, where ni represents individuals of a species and N represents the total number of species. Concentration of dominance (C) was also measured by the Simpson's index [25] using the formula $C = \Sigma(ni/N)^2$, where ni and N are the same as for Shannon–Weiner diversity index.

III. Results And Discussion

All samples of *P. fascicularis* roots were colonized by AM fungi (Fig. 1a-f). The mean percentage of root length colonization ranged from 39% in *Pf3* to 74% in *Pf2* ($P < 0.05$), and generally exceeded 45% (Table 1). Significant differences in percentage of root colonization occurred between sampling sites ($P < 0.05$). In addition to regular components of AM fungi other fungal endophytes (*ofe*) were also recorded in *Pf1*, *Pf2* and *Pf5*. Whereas, chlamydospore (*s*) were observed in *Pf1*. Thus, *P. fascicularis* appears to be readily colonized by AM fungi under a coastal soil conditions. Recently Kamble and Mulani, [26] reported Arum-type of AMF colonization in the roots of *P. fascicularis* from a sandy beach at Murud Dapoli in Maharashtra. As a part of its extension, in present work we have investigated AM fungal species associated with those samples which were recently collected by Kamble and Mulani, [26].

In present work, total, 1275 AM fungal spores were derived using wet sieving and decanting technique rhizosphere soil samples of *P. fascicularis* from five study sites (*Pf1-Pf5*). Spore density in samples ranged from 39 to 890 spores 100 g^{-1} soil (Table 1). Maximum spore density was observed in *Pf 1* (890±7.4) and minimum in CM1 (39±2.7). There was a significant difference ($P < 0.05$) in spore density between many of the sites (Table 1).

Thirteen morphospecies of AM fungi were identified using spore characteristics. Species richness of AM fungi ranged from 3 to 6 (average 4.4). Species were distributed as follows: 3 species from *Pf3*, 4 from *Pf1 & Pf4*, 5 from *Pf2*, and 6 species from *Pf1* (Table 2). *Acaulospora* and *Kuklospora* occurred most frequently and overall, were the most prevalent, containing 5 and 1 species, respectively. There were 2 species in *Glomus*, 1 species in *Ambispora, Diversispora, Entrophospora* and *Scutellospora* across the 5 sampling sites (Table 2).

In present study, *Kuklospora colombiana* was the most widely distributed species. It was found in 4 out of 5 sampling sites, namely *Pf1, Pf2, Pf3* and *Pf4* (80% IF). The next most widely distributed taxon was *Acaulospora scrobiculata* which appeared in 3 sites, *Pf1, Pf2* and *Pf3* (60% IF). *Acaulospora bireticulata* and *Glomus etunicatum* both were appeared in 2 sites, *viz., Pf4 & Pf5* (40% IF). Out of thirteen, 8 species were only found at one site (20% IF), for example, *Acaulospora laevis* in *Pf3*, *Acaulospora* spp 1, *Acaulospora* spp 2 & *Ambispora callosa* in *Pf3*, *Diversispora versiformis* in *Pf4*, *Entrophospora baltica* in *Pf1*, *Glomus heterosporum* in *Pf2* and *Scutellospora spp* in *Pf2* (Table 2). Although, *Scutellospora projecturata* was also appeared in 3 sites, *Pf1, Pf2* and *Pf5* (60% IF), its relative abundance (0.549 RA) was comparatively low (Table 3). In present study AM fungal species *viz., Acaulospora callosa, Entrophospora baltica, Kuklospora colombiana* and *Scutellospora projecturata* have been first time recorded for any other known coastal sand dune plant from Maharashtra. Thus it makes new addition of these four AM fungal species for Maharashtra with reference to *P. fascicularis.*

Based on spore density and relative abundance, three species were dominant (> 40 spores 100 g-1soil, RA ≥ 6%); *Acaulospora bireticulata* (112 spores, 8.8%), *Acaulospora scrobiculata* (467 spores, 36.6%), and *Kuklospora colombiana* (429 spores, 33.6%). Although, *Diversispora versiformis* and *Entrophospora baltica* were encountered with more than 40 spores 100 g^{-1} soil, however relative abundance was 4.862 and 5.019 respectively (RA ≤ 6%). Hence, these two species were considered as non-dominant (Table 3). The morphological characteristics of AM fungal spores recovered during study are illustrated in Fig. 2.

Species diversity was calculated using two indices. The Shannon–Weiner diversity index ranged from 0.915 to 1.471. The highest occurred in *Pf5* (*H′* = 1.471) and the lowest in *Pf1* (*H′* = 0.915). Similarly, the Simpson's index ranged from 0.209 to 0.575, the highest was in *Pf5* and the lowest was in *Pf1* (Table 1).

IV. Tables

Table 1. AM fungal root colonization, Spore density (SD), Shannon-Weiner index (*H′*) and Simpson's index (*D*) in *P. fascicularis* at each sampling site.

Samples and Collection site	AM colonization*[1] (%)	SD[2]	*H′*	*D*
Raigad District				
***Pf1* [Revdanda beach :18°33'28"N 72°55'16"E]**	$50.33\pm1.9_a$	890±7.4	0.915	0.209
Ratnagiri District				
***Pf2* [Murud beach Dapoli: 17°45′32"N 73°11′8" E]**	$74.66\pm1.3_b$	39±2.7	1.053	0.419
***Pf3* [Ganpatipule beach:17°8'47"N 73°15'53"E]**	$39\pm1.2_c$	74±2.6	1.084	0.555
Sindhudurg District				
***Pf4* [Munge beach 16°14'2"N 73°25'19"E]**	$51.66\pm1.8_a$	118±7.2	1.007	0.408
***Pf5* Tambaldeg beach [16°16'56"N 73°24'31"E]**	$48.33\%\pm1.9_a$	154±7.3	1.471	0.575

[*The same letter in the column indicates that there is no significant difference at α = 0.05; [1]mean±SD, n = 50; [2]mean±SD, n = 2]

Table 2. AM fungal Spore density (S) and relative abundance (RA) at each sample site of *P. fascicularis*.

AM fungal Species	Sample site*									
	Pf1		*Pf2*		*Pf3*		*Pf4*		*Pf5*	
	S	RA	S	RA	S	RA	S	RA	S	RA
Acaulospora	**424**	**47.64**	**23**	**58.97**	**50**	**67.57**	**42**	**35.59**	**114**	**74.03**
A. bireticulata	-	-	-	-	-	-	42	35.59	70	45.45

A. laevis	-	-	-	-	30	40.54	-	-	-	-
A. scrobiculata	424	47.64	23	58.97	20	27.03	-	-	-	-
Acaulospora spp 1	-	-	-	-	-	-	-	-	32	20.78
Acaulospora spp 2	-	-	-	-	-	-	-	-	12	7.79
Ambispora	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**23**	**14.93**
A. callosa	-	-	-	-	-	-	-	-	23	14.93
Diversispora	**0**	**0**	**0**	**0**	**0**	**0**	**62**	**52.54**	**0**	**0**
D. versiformis	-	-	-	-	-	-	62	52.54	-	-
Entrophospora	**64**	**7.19**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
E. baltica	64	7.19	-	-	-	-	-	-	-	-
Glomus	**0**	**0**	**11**	**28.20**	**0**	**0**	**12**	**10.17**	**13**	**8.44**
G. etunicatum	-	-	-	-	-	-	12	10.17	13	8.44
G. heterosporum	-	-	11	28.20	-	-	-	-	-	-
Kuklospora	400	**44.94**	**03**	**7.69**	**24**	**32.43**	**02**	**1.74**	**0**	**0**
K. colombiana	400	44.94	03	7.69	24	32.43	02	1.74	-	-
Scutellospora	**02**	**0.22**	**02**	**5.12**	**0**	**0**	**0**	**0**	**04**	**2.60**
S. projecturata	02	0.22	01	2.56	-	-	-	-	04	2.60
Scutellospora spp	-	-	01	2.56	-	-	-	-	-	-
Total spores	**890**	**100**	**39**	**100**	**74**	**100**	**118**	**100**	**154**	**100**
Species richness (SR)	**4**		**5**		**3**		**4**		**6**	

Table 3. Identified AM fungi with their spore density (S), isolation frequency (IF) and relative abundance (RA) in *P. fascicularis* rhizosphere (dominant species are in bold).

AM fungal species	S	IF	RA
***Acaulospora bireticulata* Rothwell & Trappe.**	**112**	**40**	**8.784**
Acaulospora laevis Gerd &Trappe.	30	20	2.351
***Acaulospora scrobiculata* Trappe**	**467**	**60**	**36.626**
Acaulospora spp 1 (Unidentified)	32	20	2.509
Acaulospora spp 2 (Unidentified)	12	20	0.941
Ambispora callosa (Sieverd.) C. Walker, Vestberg & A. Schüssle	23	20	1.803
Diversispora versiformis (Karst.) Oehl, silva & Sieverd.	62	20	4.862
Entrophospora baltica Blaz., Madej & Tadych	64	20	5.019
Glomus etunicatum Becker & Gerd.	25	40	1.960
Glomus heterosporum Smith & Schenck	11	20	0.861
***Kuklospora colombiana* (Spain & Schenck) Ohel & Sieverd**	**429**	**80**	**33.646**
Scutellospora projecturata Kramadibrata & Walker	07	60	0.549
Scutellospora spp (Unidentified)	01	20	0.078
Total: AM fungi 13 species	1275	-	100

V. Figures

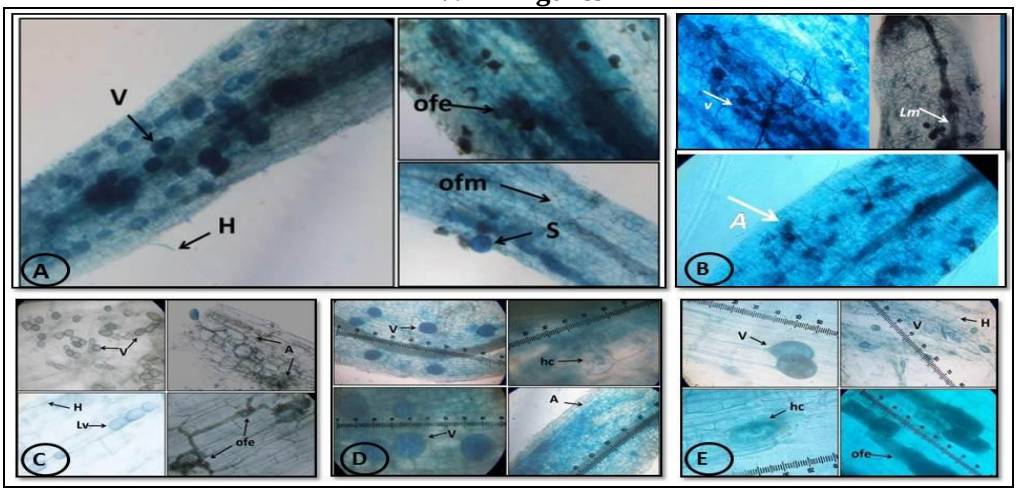

Fig. 1(A-E): AM fungal root colonization in different samples (*Pf1-Pf5* respectively) of *P. fascicularis* [A-Arbuscule, H- Hyphae, hc -Hyphal coiling, Lm- Linear mycelium, Lv- Linear Vesicles, Ofe-Other fungal endophyte, ofm- Other fungal mycelium, S- Chlamydospore, V- Vesicle].

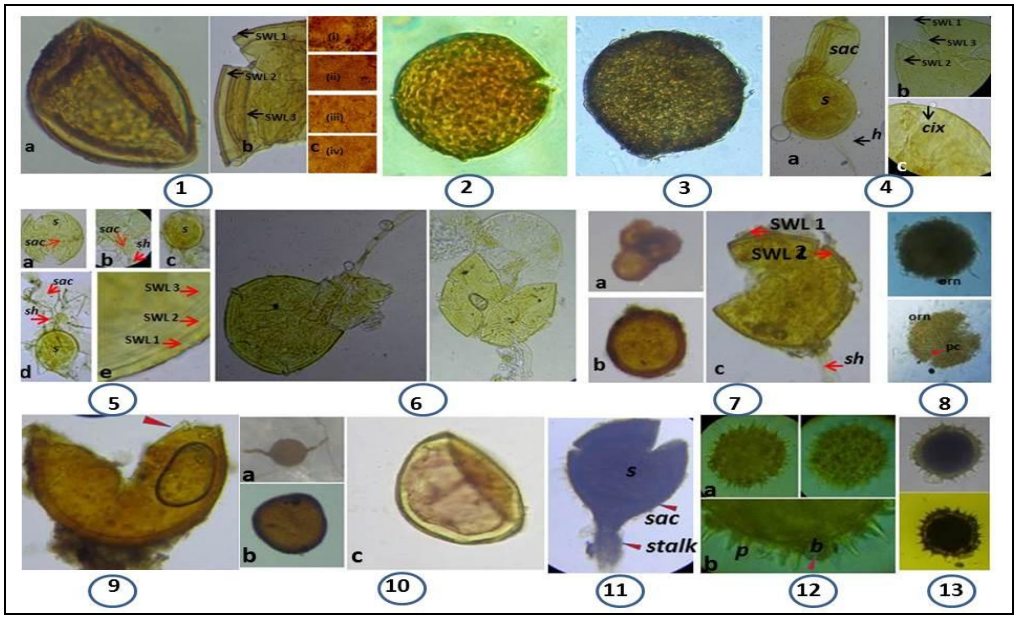

Figure 2. The morphological characteristics of AM fungal spores recovered from different samples of *P. fascicularis:* 2.1. *Acaulospora bireticulata* Rothwell & Trappe:. [a] Spore, [b] Enlarged view of spore wall showing three layers (SWL), [c] Enlarged view of the surface ornamentation (i-iv); **2.2. *Acaulospora laevis* Gerd &Trappe.; 2.3. *Acaulospora scrobiculata* Trappe**; **2.4. *Acaulospora* spp 1 (Unidentified): [**a] Spore (s) and sporiferous sacule (sac); subtending hypha (h), [b-c] Enlarged part of spore showing spore walls (SWL1-SWL3) and cicatrix (*cix*); **2.5. *Acaulospora* spp 2 (Unidentified):** Spore (s) showing disintegrating sporiferous sacule (sac) and subtending hypha (sh) in water mount [a-b] and in PVLG & Melzer's reagent [c-d]; Enlarged part of spore showing spore walls (SWL1-SWL3) [e]; **2.6. *Ambispora callosa* (Sieverd.) Walker, Vestberg & Schüssle:** Spores crushed in PVLG with Melzer's reagent, showing the rust-coloured reaction of the outer wall component, the yellow reaction of the contents exuded through the break, and the creasing resulting from the pliable nature of the wall components. **2.7. *Diversispora versiformis* (Karst.) Oehl, Silva & Sieverd.: [**a] Spores, [b] Enlarged spore, [c] Spore with short, fragile subtending hypha (*sh*) that is principally continuous with semi-persistent outermost spore wall layer (SWL1) but not with structural layer SWL2; **2.8. *Entrophospora baltica* Blaz., Madej & Tadych:** Mature spores with characteristic hyphal mantle (orn) and round cicatrix (pc) formed after detaching sacule; **2.9. *Glomus etunicatum* Becker & Gerd.** spore with sloughing layer patchily stained with Melzer's reagent; **2.10. *Glomus heterosporum* Smith & Schenck:** Spore mount in- water [a], PVLG [b], PVLG & Melzer's reagent [c]; **2.11. *Kuklospora colombiana* Spain &**

Schenck) Ohel& Sieverd.: Spore (s) showing sporiferous sacule (sac) and stalk; **2.12. *Scutellospora projecturata* Kramadibrata and Walker.: [**a] Spores, [b] Enlarged view of spore showing prominent protuberances (*p*) of various shapes that give the species its name, and arrow headed bulbous base (*b*) and **2.13. *Scutellospora spp* (Unidentified).**

VI. CONCLUSION

Besides to aromatic and medicinal potential of Ayurvedic drug *Ketaka: P. fascicularis*, ecologically this plant is also important as its root system binds soil and checks soil erosion, it fixes sand dunes, which act as a barrier against encroachment of the sea and protect human settlements along the coastline line. Present paper has provided sound data on AM fungal association and species diversity with *P. fascicularis.* Thus, an establishment of AMF association with *P. fascicularis* apparently helps to strengthen the ecological efficacy in relation with coastal region. However, for introducing the coastal eco-agriculture practices of *P. fascicularis* at mostly underestimated regions in Konkan region of Maharashtra or so, application of this potential mycorrhiza on large scale is the most effective method. For this purpose production of native AM fungal inoculum or consortium on large scale is the need of near future and hence extension of work is required.

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