

Incidence of Arbuscular Mycorrhizal Fungi in Indian Squill: *Drimia indica* from Coastal Sand Dunes of Konkan, India

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Abstract: *Drimia indica* (Roxb) Jessop. (Family: Hyacinthaceae), is also known as Indian squill. Due to over exploitation and anthropogenic pressures such as habitat degradation many populations of *D. indica* have been lost and caused genetic depletion as well as loss of genetic diversity. Hence, it is necessary to initiate new means and approaches are to be worked out for germplasm conservation and sustainable utilization of this economically important medicinal plant. In present paper an attempt has made to study remarkably ignored Arbuscular mycorrhizal (AM) fungi with *D. indica*. Mycorrhizal colonization percentage in *D. indica* samples was varied in different populations in the coastal sand of Konkan region of Maharashtra. In present paper besides the roots, incidence of mycorrhizal colonization and spores inside the *D. indica* scale cells is reported for first time.

Keywords: *Acaulospora scrobiculata*, AM fungi, CSDs, *Drimia indica* (Roxb) Jessop. *Glomus aggregatum*.

I. Introduction

Drimia indica (Roxb) Jessop (Family: Hyacinthaceae) (= *Urginea indica* Kunth.) is commonly known as Indian squill. It is a perennial bulbous geophyte with fibrous roots of six to ten inches of length. It is endemic to India, Africa and Mediterranean Regions [16]. The genus *Drimia* (Syn. *Urginea*) has been extensively studied for biosystematics [16], [32], [33]; cytological [3], [28], [29], [30], [36], [39]; cytotaxonomical-genetical aspects [4], [31] and very recently for DNA bar-coding as well as for molecular markers (ITS and *matK*) [5], [20]. Ethno-medicinally bulbs of *D. indica* has proved antiulcerous, antinematodal, antitumorous, anthelmintic antiarthrities properties and also used to cure skin diseases like warts, abscesses, boils, cardiac diseases, antidote to scorpion sting [18]. The bulbs contain literally hundreds of compounds that defend cells against attack by Marauding free radicals by blocking the development of heart diseases, cancer, Rheumatism dropsy, edema, gout, Asthma, Dog bites, cut wound, infertility in man and numerous other life shortening ailments. Due to these medicinal properties of *D. indica* bulbs have found its place in British and European Pharmacopeias [17].

Unfortunately, many populations of Indian squill, have been lost due to unawareness and over exploitation. As per IUCN criteria, Threat status of *D. indica* is VULNERABLE/R (A 1a, 1c, 1d) for Chhattisgarh and Madhya Pradesh [11]. Although, according to Gokhale et. al. [19], *D. indica* occurs frequently along sand dunes and sandy beaches of Maharashtra, the threatened status of this important plant cannot be ignored at any cost. Hence, it is necessary to initiate awareness, conservation and cultivation of Indian squill. Anthropogenic pressures such as habitat degradation are largely responsible for genetic depletion and loss of genetic diversity [16]. There is urgent need of new means and approaches are to be worked out for germplasm conservation and sustainable utilization of this economically important medicinal plant. It is now well established that, the understanding of mycorrhizal associations in coastal sand dune (CSD) plants and their distribution in the soil is necessary for the sustainable management of these habitats [1]. Although Arbuscular mycorrhizal (AM) fungi are important to the persistence of coastal sand dune vegetation, inventory on coastal sand dune AM fungi in India is very recent [12], [13], [34], [37], [38], [41]. However, little is known about the diversity of AM fungi in medicinal plants from Indian coastal sand dune habitats in general with few exceptions particularly from CSDs of Maharashtra [40], [42]. The objective of the study was to assess the presence and diversity of AM fungi associated with the Indian squill from coastal sand of Konkan region, India.

II. Material And Methods

The traditional taxonomy methods, based on root screening, and spore morphology were used to identify the AM fungal species associated with *D. indica* found in, two sandy beaches and coastal sand dunes adjoining to them from Konkan region of Ratnagiri District, Maharashtra (India) viz., Madban (16°34'41" North 73°20'39" East) and Are Ware (17°02'09"North 73°17'45" East) respectively during 2010-2011.

2.1 AM fungal colonization status

10-20 underground bulbs of plants were removed from sand and after collecting the desired roots or scales again buried to nurture in their natural habitat. Root samples, scales on bulbs surface and rhizospheric sand sample (500 g) per each plant were brought to the laboratory. Roots and scales were made free from sand and debris to process and stain with slight modifications in techniques of Phillips & Hayman [9] and Koske & Gemma [25]. Stained roots were observed under a binocular microscope to evaluate mycorrhizal colonization [15].

2.2 AM fungal species identification

Spores were isolated from Rhizospheric sand of plant with the help of wet sieving and decanting method [10]. The morphological properties of spores is based on observations of specimens mounted in polyvinyl alcohol-lactic acid-glycerol (PVLG) [24], in a mixture of PVLG and Melzer's reagent [14], a mixture of lactic acid to water at 1:1, Melzer's reagent, and in water. Spores mounted in Melzer's reagent were crushed in order to observe the staining of the different spore wall layers. Identification of AM fungi is followed after the original descriptions [21], [35]. Spores mountings in PVLG and the mixture of PVLG and Melzer's reagent (air-dried slides) were deposited at Bhavan's Mycorrhizal herbarium (BMH) Mumbai.

III. Results And Discussion

Although, the Indian squill: *D. indica* has been investigated for various parameters [3], [4], [5], [16], [20], [28], [29], [30], [31], [32], [33], [36], [39] however the AM fungal status in it has been found quite ignored with very few exceptions. Earlier studies of Parameswaran and Augustine [22], suggested AM fungal distribution and ecology of *Urginea indica* in scrub jungle from Tamil Nadu and also in few populations by Shiva Kameshwari [17] in non-coastal sand dune regions. However, these studies are not enough to provide specific details of mycorrhizal colonization pattern or morpho-taxonomical details of AM fungal spores. Although, most studies on AM fungi of coastal dunes are confined to temperate regions, and subtropical regions [23], and few were performed from the tropical coast of Hawaiian Islands [26], [27] and Singapore [6]. These studies have added significant data on various plant species but the identity of AM fungal species in *D. indica* is not yet known. Thus, present paper provides comprehensive information on incidence of AM fungi in *D. indica* found in coastal sand dunes of Konkan region of Maharashtra, India and hence makes first report for global AM fungal data base. Results obtained suggests that, AM fungi were colonized in the roots of all samples (Fig. 1) studied from site 1-Madban beach and site 2-Are Ware (henceforth, referred as *S1* and *S2* respectively). The samples of *D. indica* from both the study sites exhibited all components of mycorrhiza (vesicles, arbuscules and hyphae) with variation in occurrence intensity (Table 1). There was considerable variation in percentage colonization of AM fungi in *D. indica* root samples from both the sites. Although, *S2* was showing higher colonization but *S1* rhizosphere was comparatively enriched with higher spore count. Hence, to find out any correlation or to make any conclusion for these variations is very difficult; however, the most probable justification for it may be certain ecological or other factors. Besides the normal pattern of vesicles, grape bunch like clustered vesicles (cv) were also colonizing cortical cells of root in both the samples, their occurrence was also confirmed in scales of *S2* samples. Scale cells were also colonized with other endophytic fungi (oef) particularly in some *S1* samples. Scales of *S2* were also more frequently encountered with spores.

Previously, the occurrence of AM fungi in roots has been reported from an exceptionally wide range of plants. Besides roots, colonization has been reported in other plant parts [2]. Colonization has also been reported from scales of *Colocasia antiquorum*, *Elettaria cardamomum*, *Musa paradisiaca*, *Sansevieria trifasciata*, garlic, and ginger [7], [8]. In present paper, incidence of mycorrhizal colonization and spores inside the *D. indica* scale tissue is reported for first time. In present study, AM fungal species observed in rhizosphere samples of *D. indica* were belonging to four genera viz., *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora* which represents 9 species. The spores of all nine AM fungal species are presented in Fig. 2 from morpho-taxonomical point of view. From *S1* rhizospheres collectively 367 spores belonging to six species were recorded. Whereas, *S2* rhizospheres collectively exhibited 270 spores representing all nine species (Table 2). Amongst the nine species *A. scrobiculata* and *G. aggregatum* were dominating the rhizosphere of *D. indica* in the coastal sand dune habitat from Konkan region of Maharashtra. The prevalence of *G. aggregatum* was also recorded in the scales of *S2* samples. The results obtained also suggests that *Glomus* species were most frequently encountered (58%) followed by *Acaulospora* (29%) *Gigaspora* (11%) and *Scutellospora* spp. (2%) in *D. indica* rhizosphere samples.

I. FIGURES AND TABLES

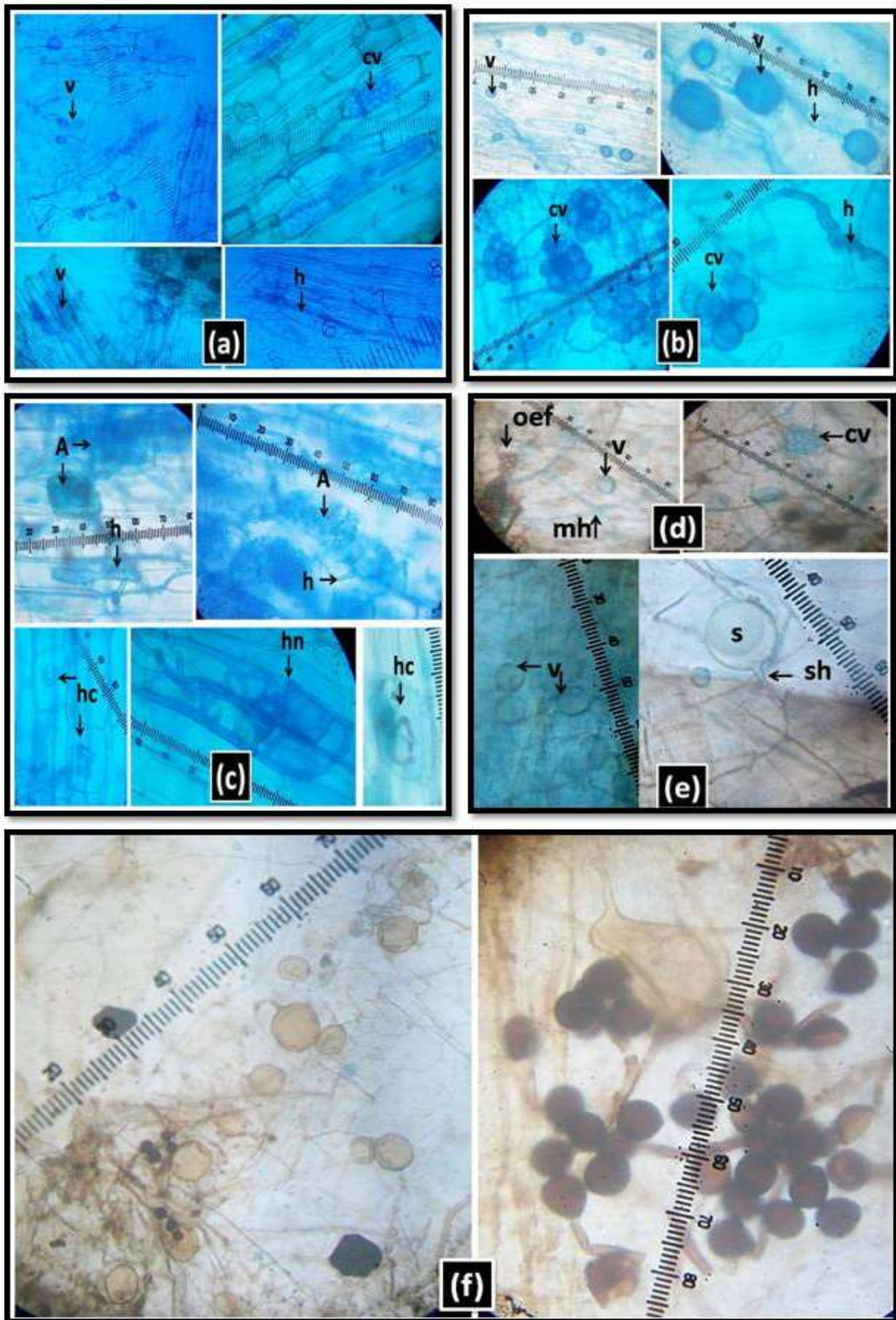


Figure 1

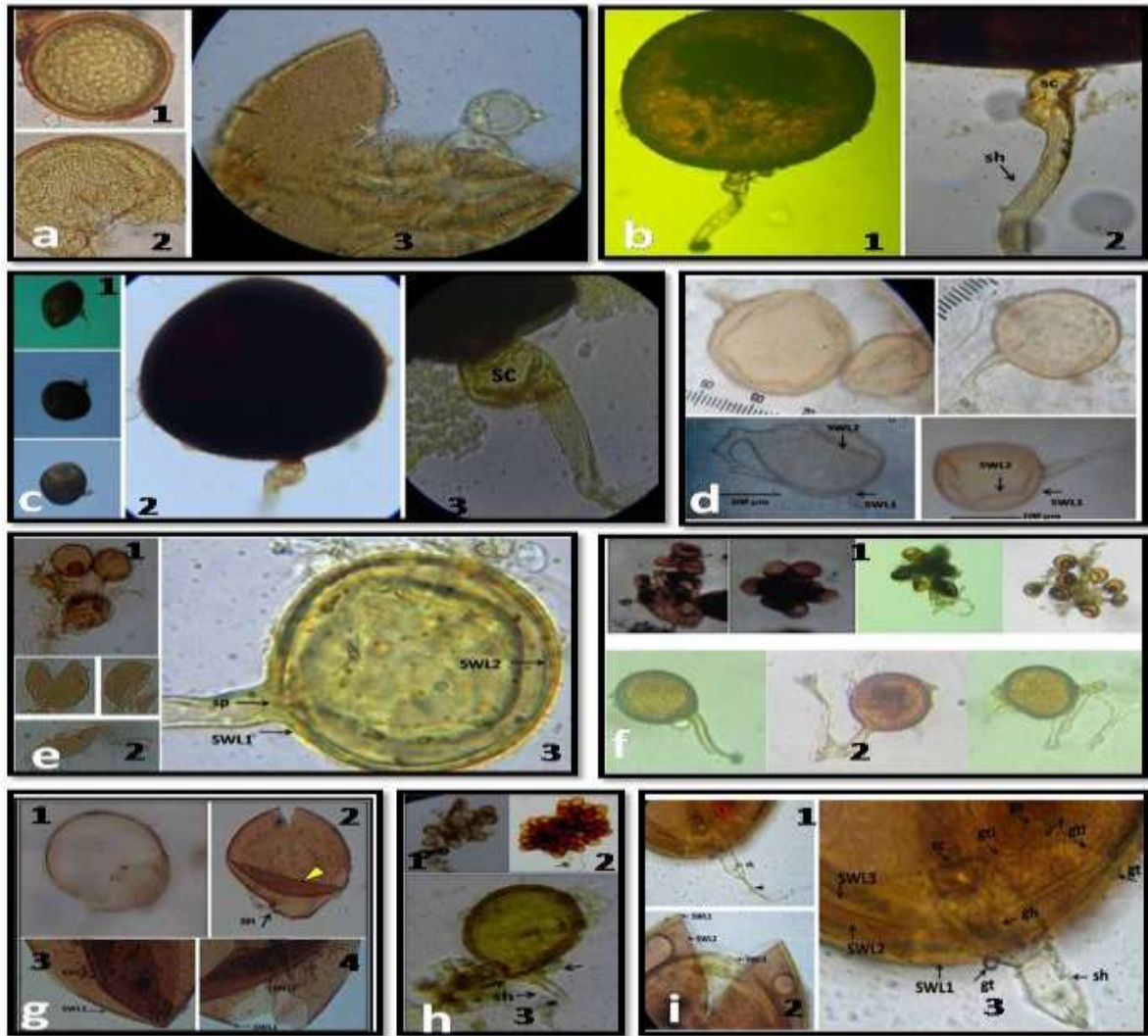


Figure 2

Figure 1. Mycorrhizal colonization in *Drimia indica*: Roots (a-c) and Scales of bulbs (d-f) showing: A: arbuscules; cv: clustered vesicles; h: hyphae; hc: hyphal coiling; hn: hyphal net; mh: mycorrhizal hyphae; oef: other endophytic fungi; s: chlamydo spores; sh: subtending hyphae of spore and v: vesicles. [Note spore cluster of *Glomus aggregatum* (f); S1 samples (a & d), S2 samples (b, c, e & f)].

Figure 2. Morpho-taxonomical details of AM fungal species associated with *Drimia indica* from Coastal Sand Dunes of Konkan, India: (a) *Acaulospora scrobiculata* Trappe: 1. Young spore, 2. Broken spore, 3. Enlarged view of spore; (b) *Gigaspora gigantea* (Nicol. & Gerd.) Ger. & Trappe: 1. Single spore formed terminally on a bulbous sporogenous cell, 2. Enlarged view of sporogenous cell (sc) and subtending hypha (sh); (c) *Gigaspora rosea* Nicol. & N.C. Schenck: 1. Different spores produced singly on the apex of bulbous suspensor cell, 2. Enlarged view of spore with globose to subglobose suspensor cell, 3. Enlarged view of spores base with bulbous suspensor cell (sc). (d) *Glomus aggregatum* Schenck & Smith: Spores with subtending hyphae and spore walls (SWL1 & SWL2); (e) *Glomus ambisporum* Smith & Schenck: 1. Spores with subtending hyphae 2. Crushed spores, 3. Enlarged spore with two wall layers (SWL1 & SWL2) and a small, bridging septum (sp); (f) *Glomus formosanum* Wu & Chen.: 1. Spores in broken sporocarps, 2. Enlarged view of spore showing attachments of 1-4 subtending hyphae; (g) *Glomus goensis* Khade: 1. Young spore with hypha, 2. Mature spore showing the insertion of hypha (SH) at base and sloughed spore wall from the base of the spore towards the apex (arrow headed), 3-4. Enlarge view of spore walls; (h) *Glomus pachycaulis* (Wu & Chen) Almeida & Schenck: 1-2. Young golden yellow sporocarp with ellipsoidal chlamydo spores borne terminally on subtending (arrow) non septate hypha (1- PVLG mount, 2- PVLG + Melzer's reagent), 3. A golden yellow young spore partially enclosed in a thin network of tightly oppressed hypha (arrows), short subtending hypha (sh) with lumen (3); (i) *Scutellospora* spp. (Unidentified): 1. Single spore formed on cylindrical sporogenous cell (sc) which is terminally formed on the subtending hypha (sh) that arises from mycelia hypha (black arrow headed), and also

note germ shield (red bordered arrow headed). 2. Crushed spore showing: one outer (SWL1), one middle (SWL2) and one inner (SWL3) spore wall, 3. Enlarged view of spore showing: spore walls (SWL1–SWL3); yellow-brown to brown germination shields (gs) showing 4–6 compartments, separated by large folds (f); often one initial germ hole (gh); germ tubes initiations (gti) are rarely visible from where the germ tubes (gt) may arise (germination visible in figure); bulbous sporogenous cell (sc) and subtending hypha (sh).

Table 1: AM fungal colonization in Indian squill: *D. indica* from CSDs of Konkan

Sr No.	<i>Drimia indica</i> samples-sites	#Occurrence intensity of AM fungi			Percentage colonization (%)	Net Total of spores encountered in rhizosphere
		Vesicles	Arbuscule	Hyphae		
1.	S1- Madban (16°34'41" N 73°20'39" E)	++	+	++++	41.66	367
2.	S2 - Are Ware (17°02'09" N 73°17'45" E)	++++	++++	++++	74	270

[# (+) 1-25%: Poor; (++) 25-50%: Moderate; (+++) 50-75%: good; (++++) more than 75%: Excellent]

Table 2: AM fungal species associated with Indian squill: *D. indica* from CSDs of Konkan

Sr. No.	AM fungal species	#Incidence of AM fungal species		No. of AM fungal spores 10 ⁻¹ g sand		Gross Total of spores (S1+S2)
		*S1	*S2	*S1	*S2	
1.	<i>Acaulospora scrobiculata</i> Trappe	√	√	120	65	185
2.	<i>Gigaspora gigantea</i> (Nicol. & Gerd.) Ger. & Trappe	√	√	32	17	49
3.	<i>Gigaspora rosea</i> Nicol. & N.C. Schenck	√	√	03	20	23
4.	<i>Glomus aggregatum</i> Schenck & Smith	√	√	101	105	206
5.	<i>Glomus ambisporum</i> Smith & Schenck	x	√	00	03	03
6.	<i>Glomus formosanum</i> Wu & Chen.	√	x	79	00	79
7.	<i>Glomus goaensis</i> Khade	√	√	32	15	47
8.	<i>Glomus pachycaulis</i> (Wu & Chen) Almeida & Schenck	x	√	00	35	35
9.	<i>Scutellospora</i> spp.	x	√	00	10	10
Net Total of spores encountered in <i>D. indica</i> rhizosphere				367	270	637

[@: (√) existing, (x) lacking; *S1-Site 1: Madban, *S2-Site 2: Are Ware]

IV. Conclusion

The findings in present study, has added significant information to existing data and hence proposing that Indian squill found in coastal sand dunes are enriched with AM fungal species. Thus with the help of potential of AM fungi in stabilizing the disturbed habitats and in conservation of vulnerable species, like *D. indica* is possible in future. Hence, initiation of isolation of such native AM fungal species in pure form is the need of time. New means and approaches are to be worked out for germplasm conservation and sustainable utilization of this economically important medicinal plant for which native AM fungal isolates can serves functional role. Hence extension of research program is required to obtain native AM fungal inoculum in future on urgent basis.

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