International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 27-01-2021; Accepted: 23-02-2021: Published: 27-02-2021
www.botanyjournals.com
Volume 6; Issue 1; 2021; Page No. 641-644

Dark septate endophyes, arbuscular mycorrhizal associations and spores studies in the roots of *Urginea indica* and *Urginea wightii*

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Abstract
Urginea indica and *Urginea wightii* is also known as Indian squill, commonly called as wild onions.It belongs to the family Asperagaceae. It is an important medicinal plant with many medicinal properties. The present study, which is in continuation of the previous investigation, is an attempt to re-highlight the occurance of arbuscular mycorrhiza (AM) and dark septate endophyte (DSE) fungal associations to detemine the diversity of mycorrizal colonizing spores inside the root cells of *Urginea indica* and *Urginea wightii* species. The study revealed the occurence of auxillary cells and formation of young vesicles was noticed. The rhizosphere analysis for spore distribution indicated the fungal spores as a regular component of soil microflora and AM fungal spore population of *Glomus* species was dominant among the recovered spores in most of the localities. The presence of spores of three imprtant genus namely *Glomus*, *Acaulospora* and *Gigaspora* species have found to be common in the three accessions of *Urginea indica kunth* and *Urginea wightii*.

Keywords: arbuscular mycorrizal fungi, dark septate endophytes, vesicles, microsclerotia, moniliform hyphae, *glomus, acaulospora, gigaspora*

Introduction
Endophytes are microorganisms that live within plant tissues without causing any symptoms of diseases. Anton de bary, German botanists who is considered the father of plant pathology coined the term endophytes in 1880 to micoorganisms that colonize the roots. The potential importance of endophytic fungi became clear in 1975, when Charles Bacon discovered the endophytes in pasture grasses. Endophytes Protect plants against heavy metal toxicity and increase the uptake of nutrients and enhance metabolic activity of plants to overcome stress. Endophytic cyanobacteria are involved in nitrogen fixation (Thangavelu muthukumar, 2014). The DSE fungi often coexist with different types of mycorrhizal fungi, including the AM fungi. It is therefore essential to understand the interaction of these fungi as they inhabit the same niche within plant roots. (Kumar sreerangan and Muthukumar 2014). *Urginea indica* is a rare, threatened and endagered medicinal plant.It is one of the extremely interesting poly typic genera with about 9 species represented in India (Hemadri. K and Swahari. S (1982). In the present study 13 accessions of *Urginea indica* and *Urginea wightii were* collected from various regions of Karnataka and south India and maintained in the germplasm in the department of botany, Bangalore university. These accessions was examined for mycorrhizal associations. *Urginea indica* accessions from kanakapura, trichy, ramnagar, thiruchendur, madanapalli, pilali and annegudda was examined and 6 accessions of *Urginea wightii* belonging to bellary, gubbi, ranganathitu, gorur, bukhapatna and chenamalipura was studied.

Materials and Methods
Collection of materials
The intensity of vesicular-arbuscular mycorrhizal infection was assessed in 13 accessions of *Urginea indica* and *Urginea wightii* collected from various regions of karnataka and south India were maintained in the germplasm in the department of botany, Bangalore University. These accessions was examined for mycorrhizal associations. *Urginea indica* accessions from kanakapura, trichy, ramnagar, thiruchendur, madanapalli, Pilali and annegudda was examined.6 accessions of *Urginea wightii* collected from bellary, gubbi, ranganathitu, gorur, bukhapatna and chenamalipura was studied. *Urginea indica* accessions of magadi and *Urginea wightii* accessions of yediyur and gulbarga was examined for the precence of arbuscular mycorrizal spores.
Freshly collected root samples from the bulbs were washed gently and made free from soil particles and cut into small segments of approximately 0.5cm on 5 to 10 pieces, depending on the size of the sample. The roots were fixed in FAA for 24 hours. Roots were then cleaned in 10% KOH and autoclaved (heated), Once cooled they were acidified with (1N) HCL for 10 to 15 minutes. Later they were stained in trypan blue (Phillips and Hayman, 1970) the concentration of trypan blue was reduced to 0.2 % in lacto glycerol. The stained roots were again heated /autoclaved 15 minutes under 60 pressure (lbs). The stained roots were mounted on a glass slide and examined under Magnus compound microscope for the AM and DSE fungal structures.

Evaluation of root colonization
The percentage of total root length colonization and root length with different fungal structures for AM fungi and the percentage of AM infection was estimated following the methods employed by Nicolson (1955). All infected and uninfected segments were counted. The percentage of infection was calculated using the formula.

$$\text{Per cent of mycorrhizal colonization} = \frac{\text{Number of root segments colonized}}{\text{Total number of root segments examined}} \times 100$$

Arbuscular Mycorrizal spore separation

Soil samples were determined In an aqueous solution of soil: water (1 : 1, v : v) isolation, soil pH was slightly acidic (6.7-6.9). The rhizosperic soil of *Urginea indica* from thatguni and udupi accessions and *Urginea wighti* of yediyur accessions was obtained from natural habitat was taken as sample. The AM fungal spores was analysed by wet seiving and decanting method by Gerdeman and Nicolson 1963 [10]. The spores were retrieved and the isolated AMF spores were quantified, using Grid Line Intersect Method (Gaur and Adholeya, 1994) The total number of spores obtained by wet sieving was referred to as Spore density. All intact AM fungal spores were counted. Spores were considered as intact when they were in living condition with cytoplasmic content and free from any parasitic attack. Spores were observed on slides containing alcohol-lactoglycerol.

Results

Root colonization by Arbuscular Mycorrizae and Dark septate mycorrizae

In the present investigation in continuation from the previous results, the occurrence of arbuscular mycorrizae and dark septate endomycorrizal fungal association was present in all the accessions showing 100 % AM and DSE fungal infection. The fungal entry into the roots was characterized by the formation of an appressorium originating from the extraradical hyphae on the root surface (K. Seerangan, 2014). The spread of hyphae was intra-cellular hyphal coils, arbusculate coils were present in all roots of the plant (Lewis 2016) [16]. The DSE fungal colonization was characterized by the presence of hyaline or darkly pigmented, regularly septate hyphae with or without microsclerotia or moniliform cells. Arbuscular mycorrizae indicated by the presence of a highly branched shrubby structure called arbuscule. Though it appears that the arbuscule occupies the root cell, it actually occurs between the root cell wall and the cell membrane within. The cell membrane fits over the arbuscule like a rubber glove over your hand. Thus the fungus never comes into direct contact with the root cell nucleus, mitochondria or other cell structures. The exchange of nutrients between the two partners, minerals from fungus to plant and sugars from plant to fungus, takes place at the cell membrane-arbuscule interface (Wanxiao Wang 2017) [15]. Root samples possessing arbuscules or arbusculate coils were considered to be arbuscular mycorrhizae. Vesicles were present in all the accessions. Formation of vesicles was observed in tatguni accessions and empty vesicles was also observed in Gulbarga accessons.

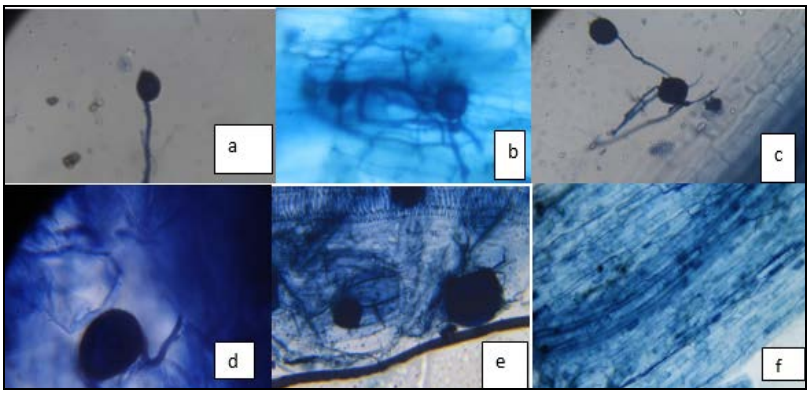

Fig 1: Spores found in *Urginea indica* and *Urginea wightii* species. a) Germinating spore of *Acaulospore* species. (b) Spore germination of *Glomus* species showing tangled hyphae. (c) Spores of *Scutellopsora* species. (d) Spore of *Glomus fasiculatum* species. (e) Spores of *Glomus geosporum*. (f) Spore distribution in root of *Urginea indica*.

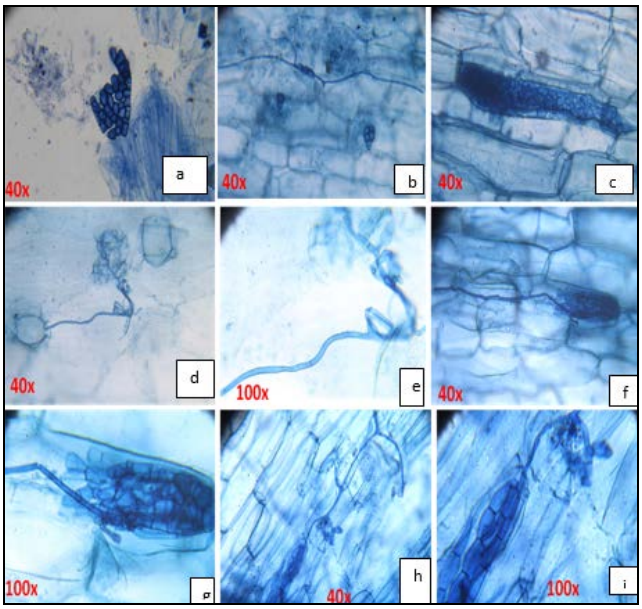

Fig 2: a) formation of vesicle. (b) Mycorrizal hyphae. (c) Micro sclerotia. (d) Empty vesicle as skeleton e) hyphal growth. (E, f, g and h) hyphal growth from vesicles leading to arbuscules formation. (i) Hyphal coils of dark septate mycorrihzae and arbusular mycorrihzae.

Identification of AMF spores

The spores of AM fungi were identified, using the guidelines given by the earlier studies (Morton and Benny, 1990; Mukerji, 1996; Schenck and Perez, 1990; Morton and Redecker, 2001; Sharma *et al*., 2009; Kumar *et al*., 2009). The identification of these fungi was done, following the manual of Schenck and Perez (1990) and was also compared with reference species description demonstrated by INVAM (International culture collection of vesicular arbuscular mycorrhizal fungi).For the characterization of AM fungi, various characteristics of spores, such as, morphology, shape, color and size, were studied. For examining the spore size and shape up to 50 spores were taken in a drop of lactic acid.

Results

The preliminary studies on AM spores in tatguni and udupi accessions belonging to *Urginea indica* and gulbarga accessions belonging to *Urginea wightii* species shows the occurance of predominant occurance of *Glomus* species, *Acaulospora* species and *Gigaspora* species. The occurance of *Sclerocystis* species was relatively lesser than *Glomus* species. Limiting its precence to only 1 accession. In the present study, only genus *Sclerocystis* has been reported to be present in high adaptation with ornamental plants. While in tatguni accession more than one appressorium was observed located at an entry point. In most cases, adjacent appressoria probably results from the branching of single external hyphae before or after contact with the root. Brundrett *et al.,* 1985 described characteristic branching of the patterns of the internal hyphae of *Glomus species.*

Discussion

Arbuscular Mycorrhizal Fungi (AMF) have existed unchanged morphologically for atleast 460 million years, despite lacking sexual reproduction (Redecker D, 2000) [1]. AMF are coenocytic organisms that have evolved to be multi genomic, possessing a large amount of genetic variation for ribosomal DNA. Reproduction occurs by asexual spores that contain hundreds or even thousands of nuclei and these spores are the only form under which species can be identified morphologically, the ability of spores to germinate is a prerequisite for the establishment of mycorrhizal symbiosis (Julie Marleau, Yolande Dalpé, 2011). The AMF are a group of root-inhabiting, symbiotic organisms that are widely distributed geographically and are among the most common soil fungi. AMF form symbioses with the roots of approximately 80% of all vascular plant species (Muthukumar, 2014). The AM fungi form mutualistic association with majority of land plants and provide several advantages to host plants (Krishnakumar *et al*., 2013).These fungi offer a wide variety of host benefits, the most well-known being an increase of mineral uptake, particularly of phosphorus (Beena, 2000). Drought tolerance through increased water uptake and a higher resistance to root pathogens (Muthukumar and Udaiyan 2013). Mycorrhizal plants also experience improved nodule function in the case of legumes and better soil structure, due to the ability of the fungi to bind soil particles and decrease soil erosion (J.W. Geredeman 1965). AMF are obligate biotrophs that play an important role in natural and grass ecosystems by enhancing plant growth (Hayman and Tauares 1985)

Conclusion

The main objective was to study the available accessions of the 2 species of *Urginea indica* and *Urginea wightii* and to confirm and report the presence of AMF and DSE. Roots were studied as of earlier studies and the results obtained confirmed 100% mycorrhizal infection which has led to the reconfirmation of the coexistence of AM fungi and DSE in the two species of *Urginea indica* and *Urginea wightii* species. Their occurence was predominantly high in plants growing in highly stressed, arid and dry condition, when compared to less stressed habitat and in controlled conditions, with water and nutrients supplied. This group of fungi are plant-root symbionts, ubiquitous in most ecosystems, which reproduce asexually via multinucleate spores for which sexuality has not yet been observed (Julie Marleau *et al*, Yolande dalphe, 2011). No relationship was found between pH and spore numbers (D. Khanam 2006) [17]. AMF spores are said to contain a high heterogeneity in the number of nuclei among sister spores and shows the high levels of genetic variation within individuals combined with the large number of nuclei in AMF spores may thus be the evolutionary strategy adapted by AMF in order to reconcile their multi genomic organization with the need to remain adaptable to diverse micro environmental changes. Leading to vast and diverse symbiosic association (Julie Marleau *et al*, Yolande dalphe, 2011).

Acknowledgments

The author conveys sincere thanks and gratitude to her late Guide Dr. Shivakameshwari, Associate Professor Department of Botany, Bangalore University.

The author conveys sincere thanks to UGC for providing funding through Rajiv ghandhi national fellowship.

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