Arbuscular Mycorrhizal and Root colonizing Dark Septate Endophyic Fungal Associations in *Urginea indica* and *Urginea wightii* accessions.

ABSTRACT

Urginea indica belongs to Hyacinthaceae family.It is also known as Indian squill,commonly called as wild Onions.The over exploitation and habitat degradation has resulted in the loss of habitat andit has caused genetic depletion and loss of genetic diversity.Immediate Measures have to be undertaken for conservation , to save this economically important medicinal plant.The present investigation is an attempt to highlight the occurance of arbuscular mycorrhizal (AM) and Dark Septate Endophyte (DSE) fungal associations found in the root bulbs of urginea indica.8 Accessions of Urginea was examined in *Urginea indica kunth* and *Urginea wightii* accessions,collected from various regions of Karnataka and south India.Fungal Symbiosis was present in all the accessions,with 100 % root infection.Urginea has DSE fungal association and AM Association.

This is the first report on the DSE fungal association in *Urginea indica kunth.*In addition to the prevalance of AM fungal symbiosis. The role of DSE is still in infancy.Though noclear relationship between AM and DSE fungal colonizations was recognized.Our studies suggests the coexistence of AM and DSE fungal colonizations are common terrestrial habitats especially the xerophytic environment.DSE-plant symbiosis should not be limited to nutritional uptake but mediates other parameters such as drought resistance ,stress tolerance and herbivore resistance .Our results show 100% infection by AM and DSE fungal colonies. Infection levels were high throughout the year, the highest levels occurring in the most nutrient-stressed situations. Their occurence and variaton in different accessions is discussed below.

Key words: Arbuscular Mycorrhiza,Dark Septate Endophytic fungi ,Urginea

INTRODUCTION

Urginea indica belongs to Hyacinthaceae family.It is also known as Indian squill,commonly called as wild Onions,(vernacular names Van Pyaz,Kadu erulli).It isa perennial bulbous plant with roots measuring about 8-10 inches in length.(Vishal R.Kamble .2012).It is endemic to India ,Africa and Meditteranian regions.(Shiva Kameshwari 2012)The Genus Urginea (Syn.Drimia).Ethno-medicinally bulbs of U.indica has proved to be antiulcerous, antinematodal, antitumerous, anthelminteic,antiarthrites properties and is used to cure skin diseases like warts, abscesses, boils,cardic diseases,antidote to sorpion sting(M.S. Chitoor 2012). The bulbs contain many compounds that defend cells against free radicals by blocking the development of heart diseases,cancer,dropsy,edema,Dog bites,cut,wounds,infertility in man and numerous other ailments.Due to these many medicinal properties of Urginea indica bulbs has found its place in Brtish and European Pharmacopeias.(M.N.Shiva kameshwari.G,Paramasivan 2012).As per IUCN criteria,the threat status of Urginea indica is VULNERABLE for Chattisgharh and Madhya Pradesh (K.C Joshi ,M.S Negi 2010).Hence conservation through germ plasm and awarness is necessary for the sustainable utilization of this medicinally important plant.It has been established that the presence of mycohrrhiza (AM) fungi is important for coastal sand dune vegetation.(K R. Beena 2000),(V.Jaiswal 2001) (Vishal kamble 2012).

There are a number of studies carried on VAM Fungi they have shown that vesicular-arbuscular mycorrhizal (VAM) infection can significantly improve the phosphorus nutrition and yield of plants grown in soils of low fertility (Mosse, 1973). Crop plants have been widely studied and relatively little information has accumulated about the significance of VA mycorrhizal infection in natural and semi-natural wild variety of plant communities.Recently, there has been an increasing awareness on another group of anamorphic Ascomycetous fungi, which also frequently colonize roots of plants growing in various habitats.These fungi termed as dark septate endophyte (DSE) fungi produce dark septate or hyaline hyphae and microsclerotia. 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 seerangan and Muthukumar 2014).As part of an experimental study on the ecological role of VAM, a quantitative analysis was carried out to study the infection level in *Urginea indica and Urginea wightii* species growing in semi-natural,dry arid and wet soil. The other most studied groups of fungal root endophytes, the so-called Dark Septate Endophytes (DSE), are a polyphyletic aggregate of fungi belonging to Class 4 of non-clavicipitaceous endophytes (Rodriguez R, White 2009) which is broadly defined by the endophytic life strategy and presence of intraradical dark septate hyphae. Dark septate endophytes are an ubiquitous group of hyaline or darkly pigmented, sterile, septate endophytic fungi that colonize living plant organs, especially roots without causing any apparent or negative effects to the host plant(Jumpponen and Trappe 1998). These fungi usually form in root cortical cells clusters of inflated, rounded, thick-walled cells called microsclerotia (Jumpponen and Trappe 1998). There associations has been found in different plant species, suggesting the lack of host specificity (Jumpponen and Trappe 1998). Dark septate endophyte (DSE) fungi often contain melanin, which is helpful under unfavourable or stressful conditions like extreme temperature,drought, etc. (Jumpponen and Trappe 1998). The role played by DSE fungi is currently unresolved, recent studies indicate their potential to function as plant growth promoters both under favourable (Wu and Guo 2008; Andrade-Linares et al. 2011) and unfavourable conditions (Barrow and Osuna 2002; Jumponen 2001).

MATERIALS AND METHODS

The intensity of vesicular-arbuscular mycorrhizal infection was assessed in over 10 Accessions of Urginea indica collected from various parts of Karnataka and across South India.
Urginea indica accessions were collected from Udupi, Sithampundi, Kerala, Shimoga, Karwar, Magadi.Urginea whitii 4 acessions collected from Nagarhole, Yediyur, Gulbarga, and Bidadi.

The study was carried in two parts. In the first, a general survey of the infection and the percentage of infection was done number of vegetation and in 3 soil samples. In the second, Urginea grown as experimental plant in Sterilized soil grown in green house for a year and the infection levels in roots were analysed for AM and DSE fungal colonization.

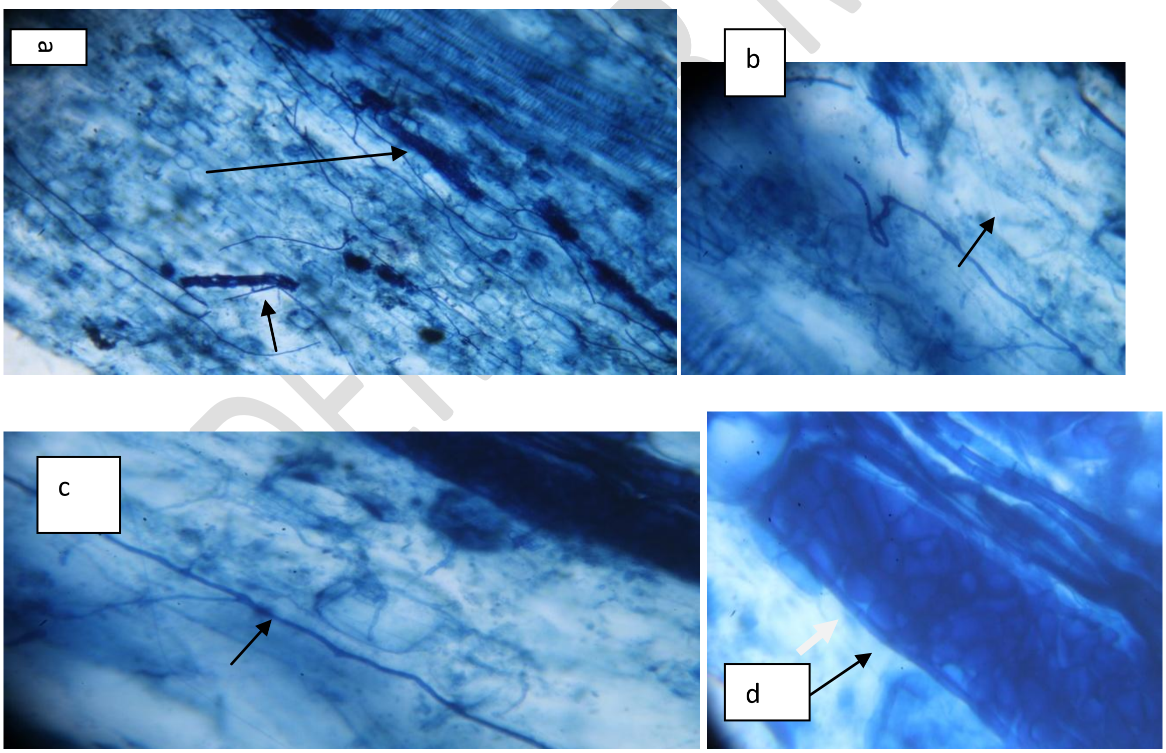
Evaluation of AM Fungal colonization
Freshly collected root samples were washed gently and made free from soil particles and cut into small segments of approximately 0.5cm. varying from 5 to 10 pieces,depending on the size of the sample.The roots were fixed in FAA for 24 hours.Roots were then cleared in 10% KOH and autoclaved (heated),once cooled they were acidified with (1N) HCL for 10 to 15 minutes.Later they were and stained in Trypan blue (Phillips and Hayman, 1970) the concentration of Trypan blue was reduced to (0.2 % in lacto glycerol) prepared in Lactoglycerol and the stained roots were again heated /autoclaved 15 minutes under 60 pressure (lbs). The stained roots were mounted on a glass on slides and examined under

Magnus compound microscope for theAM and DSE fungal structures. The presence of characteristic darkly pigmented or hyaline septate hyphae,and, when present, microsclerotia or moniliform cells were used to characterize DSE fungal colonization. Only root samples possessing arbuscules or arbusculate coils were considered to be arbuscular mycorrhizal.

Root colonization:

The percentage of total root length colonization and root length with different fungal structures for AM fungi (aseptate hyphae, hyphal structures)The percentage of AM infection was estimated by the root-slide technique of Nicolson (1955). All infected and uninfected segments were counted. The percentage of infection was calculated using the formula

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



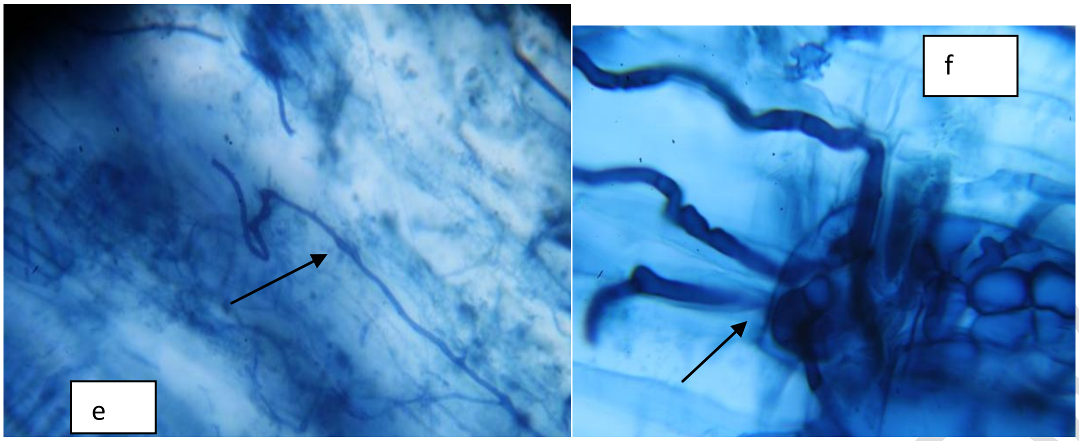
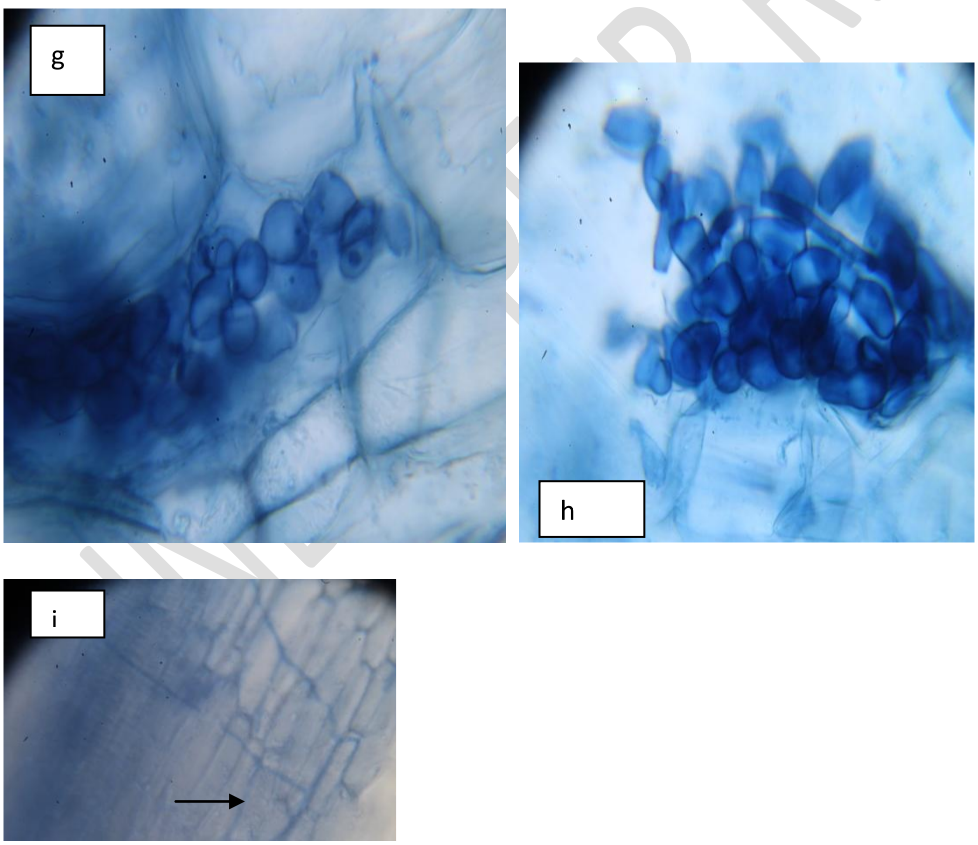
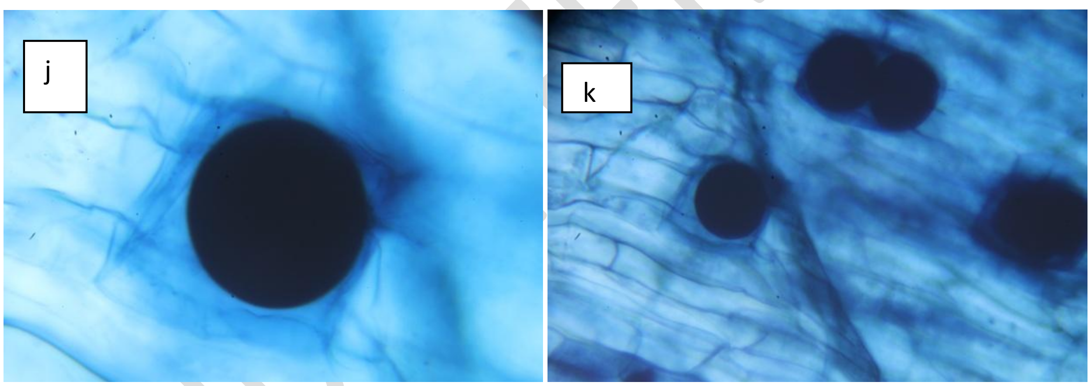


Figure 1: Mycorrhizal fungi, dark septate endophytes and other endophytic fungi inhabiting the same roots og *Urginea indica* accessions and *Urginea wightii* accessions

a) And d) clusterd intracellular microsclerotia b) ,c) and e) dark septae hyphae



g) and h) aggregation of vesicles i)the presence of arbuscules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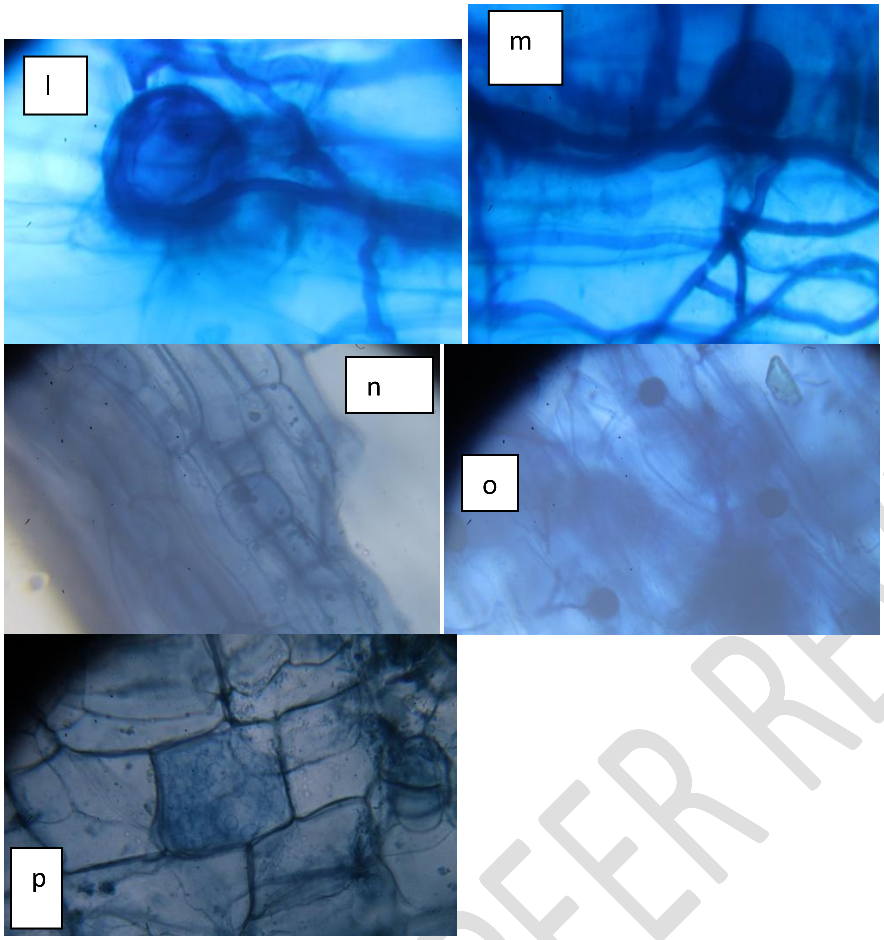

Figure 2)
j) and k) Terminal vesicles.l) and m) intradical hyphae and terminal vesicles. . O) distribution of vesicles P) arbuscules.

RESULTS and Discussion

Occurrence of AM and DSE Fungal Association

Fungal endophytes are defined as mycobionts which live inside living plant tissues, lack localized interfaces or specialized hyphae for nutrient transfer, their development is not synchronized with plant development and the plant does not nutritionally benefit from the symbiosis [Brundrett M.2006]. The present investigation in 10 accessions of *Urgine indica and whiti*i Accessions.were assessed for AM and DSE fungal association.of this all of them showed 100 % infection of Arbuscuar Mycorrhizal and Dark Septate endophytes,the presence of ectomycorrhizae,in few Accessions.The presence of Hyphal structures (hyphae,inter & intra cellular)was dominant, presence of vesicles and spores were present in all the acessions.Infection levels were high ,throughout the year, the highest levels of infection occurring in the most nutrient-stressed situations as observed in Thiruchendur Accession.

Roots were studied at different lenth cms about an average of 10 segments (0.5cms) .It was observed that there was extensive hyphae formation in 0.5-2 cms of the roots. Hartings Net was observed 0.2-0.4 the presence of Vesicles,clusterd vesicles and occurence of spores was seen.predominant occurence of spores was seen beyond 3.5 cms of the root lenth from the soil. It was It was also observed that the fungus g rows throughout the cortex,but not the endodermis and the stele.The fungus penetrates from

cell to cell forming a new coil.The Intracellular hyphae were usually found in the intermediate layers of the cortical cells of parenchyma.
The unresolved ecophysiological significance of Dark Septate Endophytes (DSE)may be in part due to existence of morphologically indistinguishable cryptic species in them (Tereza Lukešová et al 2015) The main objective in this study was to studyand report the presence of AM fungus of Dark septate mycorrhize

Conclusion:

The beneficial effect of indigenous AM fungi on the nutrition of agricultural plants depends on the abundance and type of fungi present in the soil.(Abbott and Robson,1982)The presence of any mycorrhizal associations found the roots of vascular plants plays an important role on sustainable agriculture its managemen.But the potential for employing AM fungi and the role of Dark septate mycorrhizae in agricuture and protection of its habitat requires more attention.In the present study indigenous AM fungi and dark septae mycorrizal association present in different 8 accessions were studied.From our studies we would like to conclude that DSE are prevalent in various habitat,not much understanding has been achived on DSE fungus,it cannot be overlooked as it has been stated to be mutifunctional ,such as drought resistance,environmental tolerance.The production of melanin tissues may deter mamalians and other pathogen root infections.The dynamics of this plant community,its host response under natural conditions may be difficult to determine.in a simple and controlled preliminary experiment.Urginea being a xerophyte,subjected to unfavourable, arid and dry environment, it can be clearly drawn to conclusion that the presence of the fungal associations is nesessary tosustain itself.Exactly how it does ,associations are symbiotic or not, still investigation has to be done,if so the role of each component is still yet to be confirmed. Our experiment suggests that DSE are abundant and their ecological signifance, in relation to AM fungi and other ectomycorrizae has to be significantly understood.this is a one small step before a giant leap.

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