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Research Article

Study of biodiversity of Arbuscular Mycorrhizal Fungi (AMF) in the Rhizosphere of *Withania somnifera* (L.)

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Abstract

Species diversity of Arbuscular Mycorrhizal Fungi (AMF) was investigated from the rhizosphere of *Withania somnifera* growing in Sanjay Gandhi National Park,Borivali, Mumbai. The rhizosphere soil was collected and trap culture was established using saplings of *Withania somnifera* for a period of five months. The species diversity was studied by isolating the AM fungal spores from the rhizospheric soil in the pots and staining roots of the plants. The spore density in trap culture was 53.66±6.03/10g soil in five months period. On the basis of morphological characteristics, a total of nine AMF species belonging to three genera *Viz.,Glomus, Acaulospora* and *Gigaspora* were identified.The trap culture study revealed a very high root colonization percentage(80%) showing fungal hyphae, vesicles,arbuscules , intraradical spores and other endophytes. Hyphal and vesicular colonization was high compared to arbuscules.

INTRODUCTION

Arbuscular mycorrhizas are symbiotic relationships between the plant roots and soil fungi belonging to phylum Glomeromycota (Schussler *et al*., 2000). The ubiquity of Arbuscular Mycorrhizal Fungi (AMF) at the interface between soil and plant roots makes them a key functional group of soil biota. AMF are known to benefit plant establishment by increasing resistance to environmental stresses, enhancing plant nutrient acquisition, water relations, disease resistance and improving soil quality (Smith and Read, 2008). The biochemical and antioxidant activities were observed to be increased due to inoculation of mycorrhizal fungi and *Trichoderma* (Doley *et al.*, 2014). These beneficial effects of mycorrhiza are mainly attributed to the fungal hyphae spreading through the soil beyond the rhizosphere, which enables more efficient soil exploitation for nutrients (Li *et al*., 2006). AM Fungi have been described as keystone mutualists in ecosystems due to their unique position at the root-soil interface (Kumar *et al.*, 2010).Since biodiversity has been a major research topic in terrestrial ecology, it has been largely ignored in terms of soil biota mainly in the tropical regions (Moreira *et al.*, 2006).

Diversity of AMF species is measured mainly by extracting, counting and identifying their field collected asexual spores, the fungal propagule that possess morphological characters to define species in this group of organisms(Morton *et al.*,1995) although molecular techniques have been revealed as useful tool for characterization and identification of AMF (Kowalchuk *et al.*, 2002) .

Field-collected spores, however, are found in some circumstances in low numbers, parasitized lacking informative taxonomic characteristics impairing a more accurate identification as components of spore walls are susceptible to alteration and deterioration by a wide sort of agents in the soil. Establishment of trap cultures using bulk soil or by mixing rhizosphere soil and root pieces

with sterilized diluents and growing with suitable hosts, represents a strategy to yield a large number of healthy spores which can be readily identifiable and supplement the assessment of local species diversity in different ecosystems (Bever *et al.,* 1996). Trap cultures have been widely used to access AMF diversity and isolate indigenous fungi (Patrícia L *et al.,* 2009).

Withania somnifera (Ashawagandha) is very revered herb of the Indian Ayurvedic system of medicine as a Rasayana (tonic). It is used for various kinds of disease processes and especially as a nervine tonic. (Singh *et al.,* 2011),It belongs to family solanaceae. Since the diversity of AMF was not studied from this plant earlier from this region,the present study was undertaken to understand AMF diversity of Withania under trap culture conditions.

MATERIALS AND METHODS

Soil sampling : Root samples and rhizosphere soil of *Withania somnifera* was collected from Borivali National Park , Mumbai and preserved in sterile polythene bags and stored in refrigerator at 4^0 C until use. Soil sample upto 20 cm depth was collected.

Trap Culture: The trap culture was set up as follows:

a) Rhizosphere soil was collected from the rhizosphere of *Withania somnifera* and mixed with sterilized sand in the ratio of 1:1. b) This mixture was filled in 15cm diameter pots which were sterilized by wiping with alcohol. c). Small plants of *Withania* were planted in two pots.(Plants propagated from seeds) d) The pots were watered thrice a week for 5 months and no other nutrient medium was added. e) After 5 months the plants were uprooted and root colonization checked in the roots. f) The soil from the pots was dried and Spore density checked in the soil (Rodrigues and Muthukumar, 2009).

Spore extraction

The soil samples were subjected to wet-sieving and decanting technique (Gerdeman and Nicolson,1963) for the isolation of spores. The isolated spores were picked up with the needle under a dissecting microscope and were mounted in polyvinyl lactoglycerol and observed under compound microscope. The spore number was counted by Gaur and Adholya method, 1994.

Taxonomic identification of spores up to species level was done by descriptions provided by the INVAM website: http://invam.caf.wvu.edu., website: www.zor.zut.edu. and manual of Shenck and Perez, 1990.

Root Colonization of AM Fungi

Root samples were subjected to root clearing and staining technique **(**Philips and Hayman,1970**)** in which the root samples were cut into 1cm bits and then cleared with 10% KOH for one hour, rinsed with distilled water and cleared with 5N HCl for 3min, and stained with 0.05% trypan blue in Lactophenol and percentage of root colonization was calculated by Read *et al.*,1976.

RESULTS AND DISCUSSION

Table 1: AM Fungal status of *Withania somnifera*

	Mean Spore Density	**Percent Root Colonization**	**Mycorrhizal Spores isolated and Species Identified**	**AM Structures Observed**			**Other Endophytes**
				Arbuscules	**Vesicles**	**Hyphae**	
Trap Culture data	53.66±6.03/10g Soil	80%	*Glomus macrocarpum, G.intraradices, G.multicaule, Glomus badium ,Gigaspora albida, Acaulospora soloidea Acaulospora gerdemannii A.bireticulata, A. myriocarpa*.	++ Arum type (Linear)	+++	+++	+

+Poor, ++Moderate, +++Good, +++ Excellent, - Absent

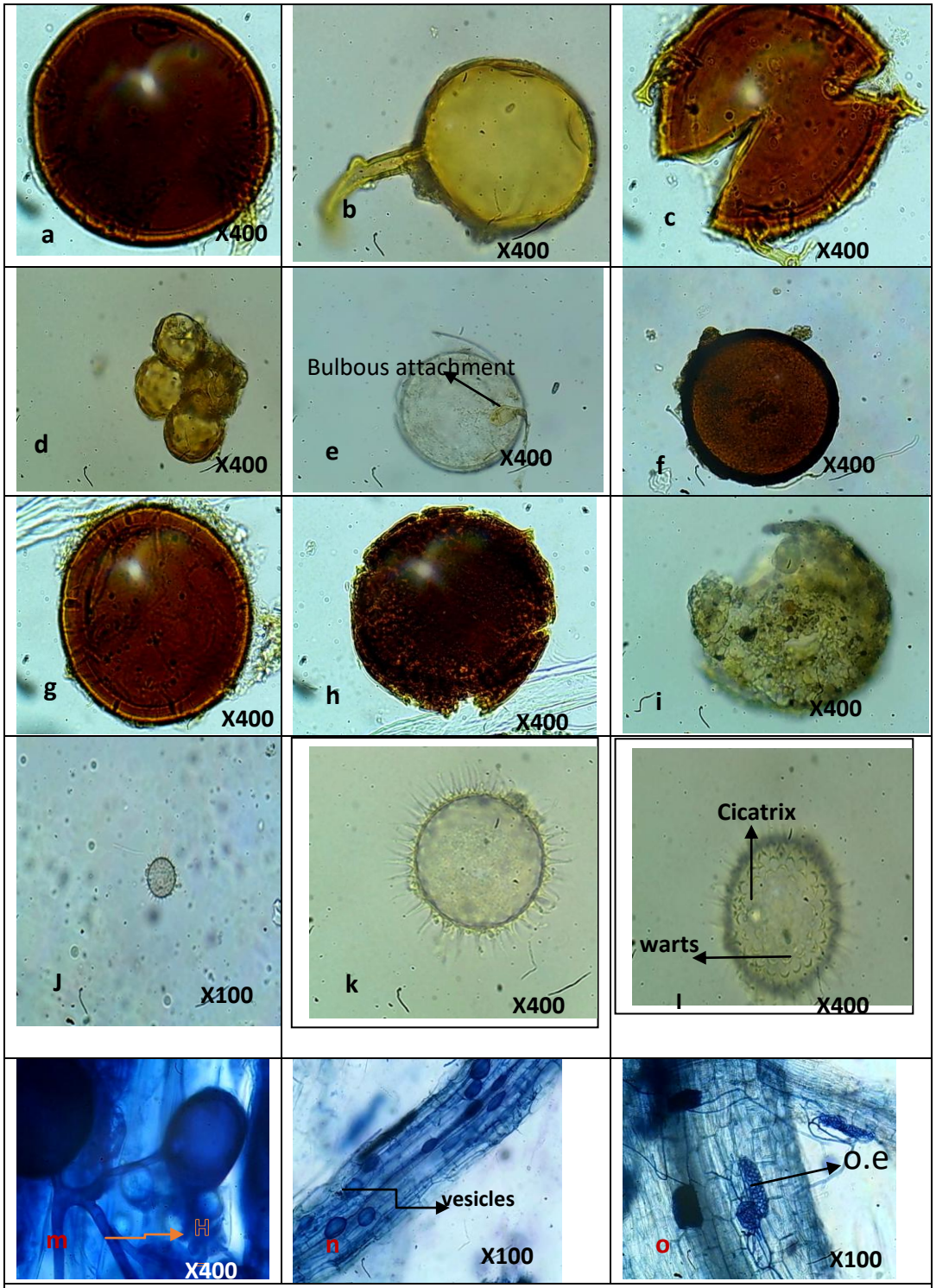

Fig. 1: A**rbuscular Mycorrhizal Fungi morphotypes associated with the rhizosphere of *Withania somnifera*L. in Trap culture.**

a. *Glomus macrocarpum* b. *Glomus intraradices* c.*Gomus multicaule* d. *Glomus sporocarp* e. *Gigaspora albida* f. *Glomus badium*, g. *Acaulospora gerdemannii* h. *Acaulospora bireticulata* i. *Acaulospora myriocarpa* jkl. *Acaulospora soloidea* AMF-colonized roots showing different internal structures indicated by arrows: m. H-Shaped hyphae and intra radical spores n. Vesicles o. Other endophytes.

Root Colonization

The mycorrhizal root colonization was 80% in *Withania somnifera*. Yaseen and Ibrar, 2011 observed 94-98% root colonization in *Withania* from University of Peshawar and Bhadar Baba. In our study, the mycorrhizal structures present in the roots included mycelium, vesicles and arbuscules as shown in Fig.1. Mycelia of various type like Y-shaped, H-shaped and parallel mycelia were observed. Vesicles were of elliptical shape.The arbuscules were of arum type (Linear). Other endophytes were also observed.

Species Composition of *Withania somnifera*

In the present study, spore diversity was studied by trap culture. Occurrence of additional AMF species in the traps is a well documented phenomenon, justifying the use of trap cultures for more complete AMF surveys than direct isolation of spores from the field soils (Oehl *et al*., 2004). The AM spore density was 53.66±6.03/10g soil in the trap culture study. The spores observed were *Glomus macrocarpum, Glomus intraradices ,Gomus multicaule, Gigaspora albida, Glomus badium, Acaulospora soloidea , Acaulospora gerdemannii, Acaulospora bireticulata* and *Acaulospora myriocarpa* as shown in table 1.

The spores were identified on the basis of their morphological characteristics using descriptions provided by the INVAM website: http://invam.caf.wvu.edu., website www.zor.zut.edu. and manual of Shenck and Perez,1990.

The most dominant species were *Glomus* followed by *Acaulospora* and *Gigaspora* The photographs of the spores isolated are shown in Figure 1 . *Glomus* species are known to be widely distributed and are commonly found in different geographical regions (Stutz *et.al*, 2000). Furthermore, *Glomus* species are more adaptable to adjustment of sporulation patterns in varied environmental conditions (Stutz *et al.*, 1996) resulting in dominance.

The spores of *Acualospora soloidea* described by Vaingankar and Rodrigues were pale brown to brown (Vaingankar and Rodrigues,2011), whereas the spores obtained by us had colourless to hyaline colour. Since *Withania* is highly medicinal plant, the knowledge of mycorrhizal status will be of immense importance to the researchers.

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