RESEARCH ARTICLE

Biodiversity of Arbuscular Mycorrhizal fungi in Kaas plateau, Satara, Maharashtra, India

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ABSTRACT

Arbuscular Mycorrhizal fungi, previously known as Vesicular–Arbuscular-Mycorrhizal fungi (VAM) are soil microbes , forming obligate symbiotic association with the roots of 87% of the land plants. The Kaas plateau is situated in the western ghat of Sahyadri ranges 22km from Satara city in Maharashtra. This plateau is famous for its flowering plant diversity. The objective of the present study was to study the biodiversity of AM Fungi in five plants most commonly occurring and flowering in the month of September i.e. *Eriocaulon manoharanii, Pogostemon deccanensis, Senecio grahamii, Impatiens oppositifolia* and *Dipcadi montanum.* These plants were screened for arbuscular mycorrhizal (AM) spore number and root colonization. The average mycorrhizal root colonization was found to be 79.2±6.1 percent. *Dipcadi montanum* showed maximum root colonization. The spore number ranged from 210±10 to 618±17.5 per 5 gm. The highest mycorrhizal spore count was found in *Dipcadi montanum* and lowest in *Eriocaulon manoharanii*. It was found that number of spores in the rhizosphere of plant was not related to the intensity of AM root colonization. The dominant AM species found were *Acaulospora scrobiculata*, *Glomus albidum* and *Glomus macrocarpum*. The average spore density of AM fungi in Satara Kaas plateu is found to be 330 spores per 5gm. Type of infection found in the roots was in the form of arbuscules, vesicles, spores (intra radical and extra radical spores), hyphae and sporocarps. Another type of potentially beneficial fungi associated with roots was observed, namely, dark septate endophytic fungi (DSEF). Dark septate endophytic fungi were identified in *Pogostemon deccanensiss* and *Senecio grahamii* .

Keywords: Arbuscular Mycorrhizal Fungi, Kaas Plateau, Dark septate endophytic fungi (DSEF).

INTRODUCTION

Many microorganisms form symbiosis with plants that range, on a continuous scale, from parasitic to mutualistic. Among these, arbuscular mycorrhizal (AM) fungi are ubiquitous plant root symbionts that can be considered as 'keystone mutualists' in terrestrial ecosystem, forming a link between biotic and abiotic ecosystem components via. carbon and nutrient fluxes that pass between plant and fungi in the soil (O'Neill *et al*., 1991).It is estimated that about 87% of terrestrial plants form mycorrhizal associations (Stoyke and Currah, 1993). In addition to mycorrhiza, the roots of plants may get frequently colonized by fungi with dematiaceous septate hyphae which are often sterile in culture conditions (Newsham, 1999). Such fungi are collectively called as dark septate endophytic fungi (DSEF). Dark septate endophytic fungi (DSEF) belong to ascomycetous and anamorphic fungi. Once DSEF colonized the roots of the plants, they form characterized inter and intracellular structures like net of hyphae, microsclerotia and occasionally, a partial mantle (Jumpponen and Trappe, 1998). DSEF can be easily distinguished from AM fungal hyphae by their dark red brown to dark brown colour, thick lateral wall and frequent septa. So far DSEF have been reported mostly from arctic (Jumpponen and Trappe, 1998), alpine environments (Read and Haselwandter, 1981), neotropical cloud forests (Rains *et al*., 2003) and grassland ecosystem (Mathew and Malathy, 2007). AM Fungi were not documented from Kaas plateau and hence this study was carried out in some of the dominant plants which were flowering in the month of September 2015 to study the diversity of AM Fungi.

MATERIALS AND METHODS:

Soil sampling: Rhizosphere soil of five plants viz; *Eriocaulon manoharanii, Pogostemon deccanensis, Senecio grahamii, Impatiens oppositifolia, Dipcadi montanum* was collected from Kaas plateau, Satara, Maharashtra in the second week of September 2015 and preserved in sterile polythene bags and stored at 4°C until use. Soil samplesupto 20cm depth was collected. Root samples were collected from the rhizospheric soil during sieving and decanting technique as uprooting the plants is prohibited from Kaas plateau.

***Spore Extraction*:** The soil samples were subjected to wet sieving and decanting technique for the isolation of spores by Gerdeman and Nicolson's method,(1963). The isolated spores were picked up with needle under stereo zoom microscope and were mounted in polyvinyl lactoglycerol and observed under compound microscope.

***Taxonomic Identification of spores*:** This was done using the identification manual by Shenck and Perez, 1990, Rodrigues and Muthukumar,2009 and descriptions provided by the international collection of VAM www.invam.in and www.zor.zut.edu.

***Spore Density counting*:**
The quantification of AM spores was done by 'Grid Line Intersect Method of Adholeya and Gaur (1994).

***Root Colonization of AM fungi*:**
Phillips and Hayman, (1970) Root samples isolated from the rhizosheric soil were processed and stained by 'Rapid Clearing and Staining Method' following Phillips and Haymann (1970). Per cent root colonization was observed taking 10 randomly selected root pieces. Qualitative characteristics of AM fungi i.e. external hyphae, vesicles, arbuscules and endospores were observed in stained root samples. The percentage of mycorrhizal root colonization was determined by the following equation:

$$\text{Percentage AM root colonization} = \frac{\text{Total number of root segments colonized}}{\text{Total number of root segments examined}} \times 100$$

RESULTS AND DISCUSSION

AM fungi are ubiquitous and ecologically important root symbionts of most terrestrial plants. In the present study, status of AM fungi associated with five plants most commonly occurring in Kaas plateau was studied. The results of the rhizosphere soil assessment of five plant species have been presented in the table. The mycorrhizal root colonization ranged from 70% in *Senecio grahamii* to 86% in *Dipcadi montanum*.

Root samples of all the plant species showed a wide range of variation in terms of AM root colonization. The mycorrhizal structures present in the roots included mycelium, vesicles and arbuscules. Mycelia of various type like Y-shaped, H-shaped and parallel mycelia were reported in the roots. Vesicles of different shapes like elliptical, round, globose, oval and elongated were observed. Paris type (Coiled) of arbuscules were observed in *Senecio grahami* and in the rest four plants it was arum type (Linear).

The AM spore density ranged from 210± 10 to 618.3± 17.5. The highest spore population was recorded in the rhizospheric soil of *Dipcadi montanum* and it was followed by *Pogostemon decanens*is and *Senecio grahamii.* The lowest was recorded in *Eriocaulon manoharani*. We observed highest spore density in family Asparagaceae. Kumar (2013) reported highest spore count in the family Asteraceae followed by Boraginaceae while Liliaceae was observed with least spore count. *Asparagus* is placed in Liliaceae in Bentham and Hookers classification. *Pogostemon* showed maximum species diversity as shown in the table and least was shown by *Impatiens.* Coexistence of DSEF and AMF was detected in two plants *Pogostemon* and *Senecio.*

Table: Status of different structures and spore density of AMF and DSEF in the plants of Kaas Plateau.

Sr. No	Botanical Name	Common name	Family	Type of Infection			AM spore count/ 5gm. of soil	% AM Root Colonization	AM Fungi Identified	Dark Septate Endophytic Fungi
1	*Eriocaulon manoharanii*	Dwarf Pipewort	Eriocaulaceae	+	+	+	210 ±10	80 ±5	*Glomus constrictum, Acaulospora* x, *Acaulospora y*	–
2	*Pogostemon deccanensis*	Jambhli Manjiri	Lamiaceae	+	+	+	318.3 ±16	83.6 ±5.1	*Gigaspora, Glomus albidum, Glomus macrocarpum, Glomus* x, *Acaulospora scrobiculata, Acaulospora* x *Spore in spore syndrome*	+
3	*Senecio grahamii*	Sonki	Asteraceae	+	+	-	260 ±11.3	70 ±5	*Glomus macrocarpum, Acaulospora x*	+
4	*Impatiens oppositifolia*	Lal terda	Balsaminaceae	+	+	+	240 ±19	77.66 ±8.5	*Glomus, Acaulospora* x	–
5	*Dipcadi montanum*	Dalzell	Asparagaceae	+	+	-	618.3 ±17.5	86 ±5.2	*Acaulospora spinosa, Acaulospora* x, *Acaulospora* y, *Glomus glomerulatum*	–

Tripathi (2014) have reported DSEF from the deciduous forests of Central India. Mandyam and Jumpponen (2005) speculated that DSE fungi would be prevalent in various habitats and colonize a substantial proportion of the species present in mixed plant communities. This group of fungi cannot be overlooked while assessing the fungal communities of any ecosystem, as their abundance may equal or even exceed that of the VAM fungi.

We could not find any significant correlation between root colonization and spore density in our study. The variation in spore density and colonization of AMF associated with different host plant species may be generated by a variety of potential mechanisms, including biological characteristics of rhizosphere under host species, variation in host species, mycorrhizal dependency, host plant-mediated alterationof the soil micro environment,or other unknown host plant traits, as described by Lorgio *et al*. (1999) and Eom *et al*., 2000. However, some researchers found a positive relationship between VAMF colonization and spore density (Sigüenza *et al*., 1996), whereas others found a negative relationship (Fontenla *et al*., 1998).

CONCLUSION

The plants selected for study of AM Fungal diversity were *Eriocaulon manoharanii, Pogostemon deccanensis, Senecio grahamii, Impatiens oppositifolia* and *Dipcadi montanum.* The study was carried out in mid September. The spores isolated from the plants belonged to three genera*Glomus, Acaulospora, Gigaspora*.The dominant AM species found were *Acaulospora scrobiculata*, *Glomus albidum* and *Glomus macrocarpum*. *Pogostemon deccanensis* and *Senecio graham* also showed DSEF in the roots . The root colonization showed presence of mycorrhizal structures - Arum type (Linear) of arbuscules in four plants and Paris type(Coiled) of arbuscules in *Senecio grahami. Pogostemon deccanensis* showed maximum species diversity as shown in the table and least was shown by *Impatiens oppositifolia.*

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