

Diversity of Arbuscular Mycorrhizae Fungi from Orchard Ecosystem

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Abstract

Arbuscular mycorrhizal fungi (AMF), a symbiotic microorganism survives in both soil and roots. 80% of the plant roots acts as the host for the AMF and they are known as the component of soil and functional links between soil and plant. Orchard ecosystem was selected for the study since they are having a diversified plant flora. Roots and rhizosphere soil samples were collected randomly from *Rosa indica*, *Citrus lemon*, *Emblia officinalis*, *Punica granatum*, *Mangifera indica* orchard located at Gandhigram Rural Institute-Deemed University, Gandhigram, Dindigul, Tamil Nadu, India. The samples were processed for the presence of AMF in both roots and soil. Wet sieving and decanting method was followed for the isolation of AMF from soil and trypan blue staining was carried out for roots. The mean percent colonization in roots and mean spore population was calculated for a period four months from December 2011 to March 2012. The monthly variation in AMF spore population and root colonization were recognized from the data obtained. The abundance of AMF is from two families viz., *Glomus* (*G. aggregatum*, *G. fasciculatum*, *G. mosseae* and *Acaulospora* (*Acaulospora* sp)).

Keywords: Orchard ecosystem; Diversity; *Glomus* sp; *Acaulospora* sp.

Introduction

In recent years, there has been increasing evidence that the microbial communities of soil and plants have an important role in the development of sustainable agriculture. Among the microorganism living in the rhizosphere of plants, the arbuscular mycorrhizal fungi have been found to be essential components of sustainable soil-plant systems. AMF are one of the most abundant underground symbioses, since AMF could colonise more than 80% of the higher plants [1].

The mycorrhizal symbiosis is a natural relationship between plant roots and fungi that can enhance plant growth, reduce plant nutrient requirements, increase survival rate and development of plants, improve plant resistance to abiotic and biotic stresses, enhance crop uniformly and increase fruit production [2,3].

AMF are common root colonizing fungi that form symbiotic association with higher plants [4-6]. Although these fungi are obligate symbionts they are not host specific and one species may be found to be associated with various plants in the same locality. Also one host plant can support mixed populations of AMF species [7].

The importance of mycorrhizal fungi diversity and ecosystem functioning is now being recognized, particularly with respect to their potential to control plant diversity and productivity [8]. There is also a growing understanding in the role of the plant community in determining the structure of mycorrhizal fungal communities [9,10]. Hence the present work focuses on the diversity of arbuscular mycorrhizae fungal diversity in orchard ecosystem. Since orchard ecosystem consists of diversified plants the work was carried out with the diverse samples which would fulfill the relationship between plant and mycorrhizal fungal communities which play a fundamental role in

determining the species composition and diversity of both plant and fungal communities.

The objective of the study is to collect random samples viz., roots and rhizosphere soil and processing of the same for the diversity studies from five plants viz., *Rosa indica*, *Citrus lemon*, *Emblia officinalis*, *Punica granatum*, *Mangifera indica* during the period December 2011 to march 2012.

Materials and Methods

Collection of sample

Random sampling method was employed. Roots and all associated rhizosphere soil samples were collected from five different plants viz., *Rosa indica*, *Citrus lemon*, *Emblia officinalis*, *Punica granatum*, *Mangifera indica* at a depth of 5 cm to 10 cm. The number of samples collected was based on the number of dominant crops in the field. The collected root samples were washed with water and fixed in FAA (Formalin-Acetic acid-Alcohol) solution and soil samples were transferred in aseptic container to the laboratory, Department of Biology, Gandhigram Rural Institute-Deemed University, Gandhigram, Dindigul, Tamil Nadu.

Estimation of Arbuscular Mycorrhizae Fungi colonization in roots

The roots fixed in FAA were washed thoroughly in tap water but not vigorously enough to detach the external hyphae. The roots were placed in 250 ml beaker and 10% KOH was added to it. This mixture was boiled at 90°C for 30 minutes to 1 hr. After boiling the KOH solution was poured off and the roots were thoroughly washed with tap water for three times. The roots were immersed 30% H₂O₂ for 3-5 minutes until the roots get bleached. Then the roots were washed with tap water followed by acidification with 5 N HCl for 3-4 minutes and

stained with 0.05% trypan blue. After staining the root samples were cut into equal pieces of 1 cm length, 10 bits per slide was kept using lactophenol cotton blue as mounting medium and examined under low power microscopy for hyphae and high power microscopy for vesicles and arbuscles [11-13].

Isolation of AMF from Rhizosphere soil

Common technique for isolation of the arbuscular mycorrhizae spores from soil [14] is based on wet sieving and decanting [15]. In order to isolate the fungal spores, a series of sieves were used [16]. 50 g of rhizosphere soil samples was air dried, 500 ml of water was added and a magnet was placed on a magnetic stirrer for 5 minutes. The flask was kept in stasis for 45 seconds until the heavier particles are deposited and immediately, the supernatant was poured (3 times to completely drain the contents of the flask) on a series of sieves, with mesh sizes of 25, 50 and 100. The slag's on the sieve were washed with sufficient amount of water. Eventually, the remainder of the top sieve 100, 50 and 25 micrometers were collected separately and poured onto the graded filter paper No. 1 and spores were counted [15]. The shape and frequency of spores were observed using binocular microscope and were preliminarily identified using INVAM website and Research results [17]

Identification of AMF

Identification of AMF from the rhizosphere soil samples were done with the standard keys.

Taxonomy of VAM fungi [18,19]

Manual for the identification of VAM fungi [14]

Taxonomy of VAM fungi, classification, nomenclature & identification [18]

Revised classification of AMF [19]

Results

Roots and rhizosphere soil samples from the orchard was collected from Gandhigram Rural Institute-Deemed University, Gandhigram, Dindigul, Tamil Nadu, India during the period of December 2011-March 2012. The five samples collected were processed for recording percent root colonization and spore population. The results were tabulated in table.

S.No	Sample	December 2011(%)	January 2012(%)	February 2012(%)	March 2012(%)
1	<i>Rosa indica</i>	48.8	17	49.7	47.9
2	<i>Citrus lemon</i>	40	21	53.2	52.1
3	<i>Emblica officinalis</i>	49.1	22.2	63.1	53.7
4	<i>Punica granatum</i>	50.2	25.2	52.7	50.4
5	<i>Mangifera indica</i>	46.8	26.9	54.7	51.7
Total		234.9	112.3	273.4	255.8
Mean		46.98	22.46	54.68	51.16

Table 1: Mean percent root colonization of AMF in the root samples

The rhizosphere soil samples collected under five different root zones in the orchard, GRI, Gandhigram, Dindigul, Tamil Nadu were processed for identification of AMF population and the results were presented in the Tables 1-3.

S.No	Sample	December 2011 (%)	January 2012(%)	February 2012(%)	March 2012 (%)
1	<i>Rosa indica</i>	38.3	40.7	87.6	73.4
2	<i>Citrus lemon</i>	44.6	23.9	64.3	59.8
3	<i>Emblica officinalis</i>	46.0	35.1	70.9	69.4
4	<i>Punica granatum</i>	34.2	20.9	72.4	71.2
5	<i>Mangifera indica</i>	28.0	29.2	78.9	63.7
Total		191.1	149.8	374.1	337.5
Mean		38.22	29.96	74.82	67.5

Table 2: Mean spore population of AMF in the rhizosphere soil sample

S.No	AM fungi	Family
1	<i>Glomus aggregatum</i>	Glomaceae
2	<i>Glomus fasciculatum</i>	Glomaceae
3	<i>Glomus mosseae</i>	Glomaceae
4	<i>Glomus sp</i>	Glomaceae
5	<i>Acaulospora sp</i>	Acaulosporaceae

Table 3: List of AMF recorded in orchard ecosystem

Discussion

In the present study diversity of mycorrhizae fungal flora in the orchard ecosystem, Gandhigram Rural Institute-Deemed University, Gandhigram, Dindigul was investigated. Genus and species level identifications were made which results in five different species of AMF recorded during December 2011-March 2012. The study revealed that all the root samples and rhizosphere soil samples had AMF association but varied in percent colonization and population. Maximum percent root colonization was recorded by *Emblica officinalis* (63.1) during Feb. 2012-Mar 2012 followed by *Mangifera indica* (54.7) during Feb. 2012-Mar 2012 and the maximum mean spore population of AMF in rhizosphere soil was recorded by *Rosa indica* (87.6) during Feb. 2012 to Mar 2012 followed by *Mangifera indica* (78.9) during Feb. 2012-Mar 2012. The result infers that the root samples and rhizosphere soil samples had maximum AMF population in all the five samples screened during Feb 2012-Mar 2012.

This observation obviously showed the occurrence of AM fungi within the roots and the mean percentage root colonization of AMF increased from February to march, which corroborates previous studies in other crops such as cassava [20,21]. Seasonal changes affected AM spore numbers which were observed to be higher in the dry season and lower during the rainy season. The level of spore

numbers is known to be affected by soil moisture content was also reported [5]. AMF survivals are known to be resistant to adverse environmental conditions [22], it is expected that more spores will be found in soils under such unfavorable climatic conditions and hence the present works observation was maximum during the dry season.

There were four dominant species (*G. aggregatum*, *G. fasciculatum*, *G. mosseae*) in *Glomus* and one species in *Acaulospora* respectively. Though the species diversity of *Acaulospora* (1 species) was less than *Glomus* (4 species), high isolation frequency and abundance was noticed in the orchard ecosystem. *G. aggregatum*, frequently occur inside roots [23], also this fungus has been known for its frequency in occurrence [24]. *G. fasciculatum* indicates that some species are facultative symbionts [25]. *G. mosseae* is a frequent component of communities of arbuscular mycorrhizal fungi associated with plants of different regions [26]. *Acaulospora sp* are probably a widely distributed arbuscular mycorrhizal fungus in the world in both cultivated and uncultivated soils [27].

Arbuscular mycorrhizae (AM) are ubiquitous symbioses between plant roots and AM fungi in terrestrial ecosystems. AM fungi extend their waded mycelial network to everywhere, which combines plenty of plant individuals to make a large functional organism. The mycelial network of AM fungi provides a greater absorptive surface than root hairs and thus is helpful for the plants to absorb nutrition in soil, particularly phosphorus. The AM fungal community influences the course of plant succession, affects plant community diversity and productivity (and has a strong influence on ecosystem function) [28].

The dominance of AM fungal genera in the orchard ecosystem may be related to their sporogenous characteristics. *Glomus* and *Acaulospora* possess the smallest size spores in AM fungi taxa. Small spores are easy to distribute and produce a large number of spores in a short time [29]. Though *Glomus* constitutes the most fungal species (4 species of 5) in this study, one species in *Acaulospora* are distributed widely in the sampling area and produce more spores.

Wide distribution and a strong capacity of sporulation usually exist concurrently in dominant species [30,31]. But in this study, the dominant species of *G. aggregatum*, *G. fasciculatum*, *G. mosseae* were found to be widely distributed in root samples rather than their abundance in soil. It has been reported that, because of their strong mycelium network that spreads among host plants in a large area and producing fewer spores, some *Glomus species* spores may be relatively infrequent in the soil but root colonization by them is abundant [32,33].

Unevenness of AM fungal distribution is ubiquitous in natural ecosystems. Large variation in spore density and species richness of AM fungi in different rhizosphere and roots has also been reported at many sites [34-38]. There are several possible reasons for this. Firstly, disparity in sporulation ability of different AM fungi species can result in unevenness of spore density [39]. Secondly, the occurrence of interspecific competition among AM fungi is possible in the soil and within roots [40]. Also seasonality, spatial and temporal variation, complex below ground structure, host-preference and disturbance are generally thought to coincide with the variation of AM fungi distribution and community structure [41-45]. For this study, sporulation ability, host-preference and complex below ground structure seem to be more influential in the distribution of mycorrhizal spores and community structure in orchard ecosystem.

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