

Methods To Process Isolation of Vam/ Am Fungi in Vascular Plants Roots and Rhizosphere Soil in Jaipur District (Raj.), India



Botany

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ABSTRACT

Vesicular-arbuscular endomycorrhizal fungi include among its members some of the majority widespread root symbionts species. Vesicular arbuscular mycorrhizae (VAM) are present in the roots of almost all vascular plants. These common, soil-borne fungi belong to the family, the Endogonaceae and generate fungal structures in the cortex of the roots. VAM play a decisive role in the mineral nutrition of plants by transferring phosphorus and other mineral deposits from the soil to the plant. Techniques for obtaining VAM samples from natural sources from cultivated land (summer and winter crops) around the Jaipur district are outlined. Methods of isolating spores, staining and clearing root samples and of identifying the fungal structures are discussed.

INTRODUCTION

The soil organisms that develop beneficial symbiotic associations with plants roots and contribute majorly to plant growth are called mycorrhizal fungi. The mycorrhizae are the feeder root of plant growing in natural world and are beneficial to their host plant. The plant's root with zone of powerful microbial metabolic activity occurring where, there is a high concentration of carbon is called the rhizosphere.^[1]

The most widespread and best known of these associations are the Vesicular Arbuscular Mycorrhizae (VAM) fungi. Vesicular Arbuscular Mycorrhizas are formed by aseptate mycelial fungi and are so-called because of the two characteristics structures-vesicles and arbuscules- found in roots with type of infection.^[2]

Arbuscular mycorrhizal fungi are known to enhance fertility of soil and inoculation of plants with them in waste land improves the absorption of uptake of water, minerals and particularly the poorly mobile ion phosphorus from the soil and appreciably increases the growth of plants.

Methods and techniques to isolation spores ('wet sieving and decanting method', Gerdemann and Nicolson, 1963)^[3] from plant rhizosphere soil and external hyphae of arbuscular mycorrhizal (AM) fungi colonization in roots are important tools in mycorrhizal research.

Arbuscular mycorrhizal fungi comprise intra- and extraradical structures. In Glomeromycota, intraradical hyphae be capable of penetrate the outer cell wall of root and grow between or inside of the root cell wall and plasma membrane where they develop the intraradical structures, arbuscules and vesicles. The extra radical structures are hyphae and spores that develop outside of the roots in the soil.^[4]

The hyphae grow within the plant root and make longer out into the surrounding soil acting as an extension of the root system. This relationship greatly increases the absorptive surface area of the root system and with the help of the fungus; the plant is able to obtain mineral nutrients from the soil. In return the plant provides carbohydrates and other nutrients to the fungus. The fungi utilize these carbohydrates to synthesize and emit molecules like Glycoprotein called 'Glomalin' which has a cementing capacity to maintain soil particles together and is mainly involved in soil aggregation.^[5]

A local variant of VAM species are more beneficial than an foreign VAM species to its host, this result suggests adaptation of the local VAM fungal species to environmental conditions at the site.^[6]

A number of interacting factors have an effect on the successful colonization of VAM fungi are pH, soil nutrients, organic matter, moisture, temperature, and the age of disturbed sites have shown correlation with VAM root colonization and diversity.^[7]

MATERIAL AND METHODS

Survey and Sampling Site Description

Jaipur is the capital city of the state of Rajasthan, India is situated in the eastern border of Thar Desert, a semi-arid land. It is located on the northwestern part of the subcontinent. The total length of Jaipur extending from east to west is about 180 km whereas the width from north to south is about 110 km.

Two sampling sites were selected for my research work:

(I) Farm lands on (near Chaksu) Tonk road, NH 12.

(II) Farm lands on (near Amer) Delhi road, NH 8.

Location:	26.92°N 75.82°E
Altitude:	431 m (1417 ft.) Above Sea Level
Climate:	Hot Semi-Arid Type
Summer temperature :	Minimum: 25° C, Maximum: 45° C
Winter temperature:	Minimum: 5° C, Maximum: 22° C
Annual rainfall:	650 mm

The varied kind of soils available in Jaipur (Raj.) are mostly sandy, saline, alkaline and chalky, loamy and black soils are also found in some areas.

Collection of Soil and Root Samples

Soil samples were collected from two different sites, Site 1: Tonk road (near Chaksu, NH12), Site 2: Delhi road (near Amer, NH 8) located in the Jaipur district (Rajasthan). In Each sites Selected Farmlands (Cultivated land) was divided into four different zones (Zone I), (Zone II), (Zone III) and (Zone IV). Roots and soil samples were collected from the rhizosphere of plants growing in that area.

From each zone of site, 3-4 healthy plants (Bajra in summer Season and Wheat in winter Season) were selected. The roots of plant (Bajra and Wheat) and rhizosphere soil was dug out with a trowel to a depth of 0-15 cm after scrapping away the top 1 cm layer of soil. Samples were collected randomly from different zone in each site, pooled and homogenized.

The collected samples were taken in sealed plastic bags, labelled and transported to the laboratory in an insulated container.

Before processing, all the samples were sieved (< 2 mm mesh size) to remove stones, coarse roots and other litter, and fine roots were collected from each sample. Soil samples were air-dried and stored at 4°C for further experiments.

Isolation of Vesicular Arbuscular Mycorrhiza Species from Plant Roots:

After sample collection next step was isolation of vesicular arbuscular mycorrhizal species collected from root samples.

The following steps were made:

1. Fine root samples were collected and then washed with running tap water and fixed in FAA (Formalin Acetic acid).
2. Roots were segmented into 1cm bits. Three replicates of 100 root bits each, selected at random were processed separately for determining the mycorrhizal intensity in the roots.
3. Root bits were treated with 10% KOH solution for 30 min at 40 °c temperatures. The concentration of KOH and time of incubation of roots depend upon the age and softness of the roots.

Pour off the KOH solution and rinse the roots well in a beaker using at least three complete changes of tap-water or until no brown colour appears in the rinse water.

4. After thorough washing, root bits were stained with trypan blue (0.01% trypan blue) for 24hrs at room temperature.

Stained root pieces were mounted in lactoglycerol and examined under microscope for the mycorrhizal colonization and its spore's structures study.

Isolation of VAM Spores from Rhizosphere Soil Mixtures

Spores were isolated from field-collected root-rhizosphere soil mixtures. Spores of arbuscular fungi were isolated by using the 'wet sieving and decanting method' described by Gerdemann and Nicolson, 1963.^[3]

The following steps were made:

In soil remove the coarse materials like straw, debris and rocks should be removed with a 2-mm sieve.



Figure 1: Soil sample mixed with tap water

1. 100 gm of air-dried root-rhizosphere soil mixture were placed into a glass container with 1000 ml of tap water.
2. The root-soil mixture was vigorously mixed with a glass rod for 30 seconds.



Figure 2: Soil suspension sieved

3. A 10-second pause enabled to settle heavier particles and organic material, the remaining soil-water suspension were slowly poured through a set of two sieves. The sieves used are those with pores of diameters of 0.5mm (the top one) and 0.045 mm (lowest one). Most spores retain on the 0.045 mm sieve.
4. The extracts were washed away and spores collected from the sieves in to Petri dishes.



Figure 3: The extracts washed away and spores collected in to Petri dishes

5. Using a microscope, spores and aggregates were picked by means of dropper and needle.



Figure 4: Isolated spore picked with dropper under microscope

6. Selected spores were separated with a needle. A drop or two of mountant (poly vinyl lacto glycerol) was spread on the centre of a clean and dry slide so as to hold cover slip. Spores were placed on the mountant and the cover slip was placed gently by avoiding air bubbles. Such prepared slides were labeled, allowed to dry in a dust free chamber for 3-5 days. The edge of the cover slip was sealed with clear nail polish to prevent the desiccation and entry of air bubbles. Spores were examined under microscope and photographs were taken.

Analysis of Soil Samples

Soils constitute the weathered surface of the earth's crusts which is mixed with organic material and in which microorganisms live and plants grow. Soil testing is one of the most important tools to determine the status of plant nutrients in a field.

The air dried and sieved Soil samples were analyzed for the concentration and presence of like pH, Organic carbon, macro and micro nutrients in the soil testing laboratory, Agriculture Research Center Durgapura, Jaipur. Soil characteristics of different sites are presented in table 1 and 2.

RESULTS AND DISCUSSION

Isolated of Vesicular Arbuscular Mycorrhiza Fungi-

Following photographs were taken of prepared plant roots slides-

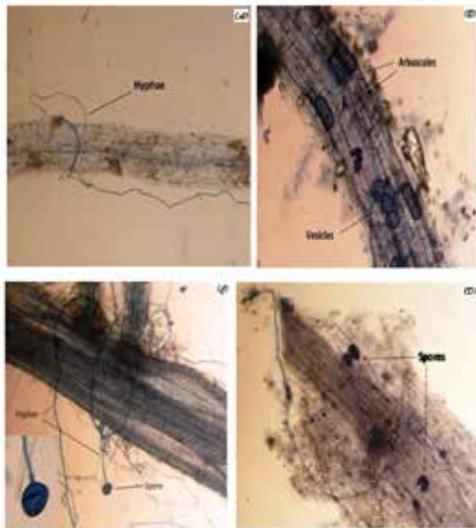


Figure 5: Isolated Vesicular arbuscular mycorrhizae fungi from roots slides showing these structures: (a) Hyphae (b) Arbuscules, vesicles (c) Hyphae, Spores (d) Spores

Isolated VAM Spores from Soil Sample

Following photographs were taken of prepared VAM (Glomus) fungi spore slides-

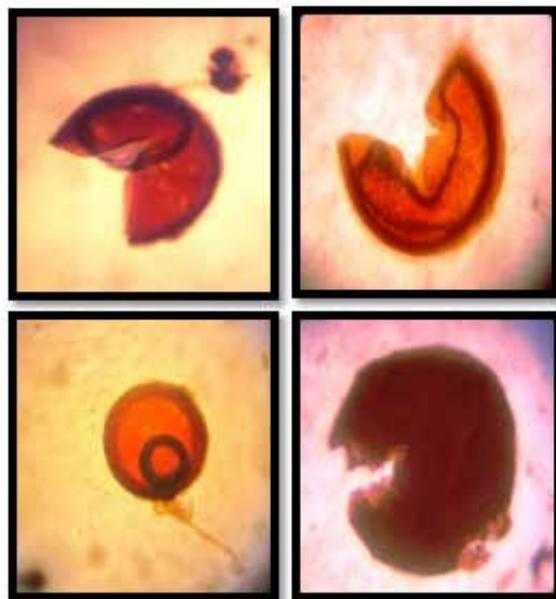


Figure 6: Isolated VAM (Glomus) fungi spores from soils

Analysis of Physico –chemical Properties of Soil

The data obtained from different sites soil analysis are given in below tables-

Table 1: Physico-chemical properties of different rhizosphere soils of Tonk road (Site I) region, Jaipur

Parameters	Samples	pH	Ec (dS/m)	Organic carbon (%) P (mg/g)	Macro and Micro nutrients					
					K (mg/g)	Zn (ppm)	Fe (ppm)	Cu (ppm)	Mn (ppm)	
Study site (I) Tonk road	Farm land(S1)	8.0	0.29	0.17	34	190	1.07	8.40	0.92	6.80
	Farm land(S2)	8.2	0.31	0.19	40	216	1.02	11.50	1.40	7.50

Table 2: Physico-chemical properties of different rhizosphere soils of Delhi road (Site II) region, Jaipur

Parameters	Samples	pH	Ec (dS/m)	Organic carbon (%) P (mg/g)	Macro and Micro nutrients					
					K (mg/g)	Zn (ppm)	Fe (ppm)	Cu (ppm)	Mn (ppm)	
Study site (II) Delhi road	Farm land(S1)	8.2	0.28	0.14	42	224	1.28	14.48	1.28	8.72
	Farm land(S2)	8.1	0.29	0.21	37	210	1.23	9.36	0.98	8.40

CONCLUSION

These methods are primarily used to isolation, identify mycorrhizal associations and measure the degree of root colonization. We hope that the present article will help the readers to choose an appropriate method to isolation and quantify AM fungi in roots and their rhizosphere soil for their specific experimental set-up. The management and strategic applications of VAM to enhance growth of food crops most especially in with an understanding of exploiting VAM benefits towards sustainable agricultural development is very important.

REFERENCE

1. Barea J., Pozo M.J. and Azcon-Aguilar C. (2005), "Microbial co-operation in the rhizosphere." Journal of Experimental Botany, 56(417), 1761-1778. | 2. Powell C.L. and Bagyaraj D.J. (1984), "VA Mycorrhiza." CRC Press, Inc. Schenck, N. C. | 3. Gerdemann J.W., Nicolson T.H. (1963), "Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting." Trans. Brit. Mycol. Soc., 46, 235-244. | 4. Smith S.E and Read D.J. (2008), "Mycorrhizal Symbiosis", 3rd Ed. Academic Press, San Diego, USA. | 5. Wright S.F and Upadhyaya A.(1996), "Extraction of an abundant and unusual protein from soil and comparison with hyphal protein from arbuscular mycorrhizal fungi." Soil Sci., 161(9), 575-586. | 6. Requena N., Jimenez L., Toro M. and Barea J.M. (1997), "Interactions between plant growth promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi and Rhizobium spp. in the rhizosphere of Anthyllus cytisoides, a model legume for revegetation in Mediterranean semi-arid ecosystem." New Phytol., 136, 667-677. | 7. Mukhopadhyay S. and Maiti S.K. (2009), "VAM fungi –A future prospect for biological reclamation of mine degraded lands" Int. Journal Env.P., 29(9), 801-809. |