ISOLATION OF ARBUSCULAR-MYCORRHIZAL FUNGI

Arbuscular mycorrhizal fungi (AMF) is an important component of biodiversity particularly in tropical & sub-tropical ecosystems. About 80% of the total plant species are associated with AMF and are thus, potential factors determining diversity in the ecosystems. They can modify the structure and functioning of a plant community. Many reports document the occurrence of AM in diverse crops of intensively cultivated arable soils. These fungi can increase plant growth under low-fertility conditions and of particular interest for well-fertilized agricultural soils, can improve tolerance towards different kinds of stress such as drought or resistance towards root pathogens. These fungi can enhance mineral nutrient acquisition in host plants. Phosphorus, nitrogen, zinc, and copper acquisition are most commonly reported being enhanced by AMF in plants.

Sample collection for AM fungal analysis

Collecting mycorrhizal fungi should be undertaken in a wall-planned and ordered fashion to achieve a high standard of retrievable information.

Equipments and Reagents:

Sampler
Spade
Zipped polythene bags
Scissors
Blotting sheets

Methodology

Divide the sample field into four blocks. Take the samples from the rhizosphere of host plants at a depth of 0-30 cm in each block. Number of samples from each block should be decided on the basis of the site topography. The number can vary from 3-15. Air dry the sample to a point where there is no free moisture and then fill the sample in plastic bags, sealed and stored at 40°C in cold room until it is further processed. These samples then can be screened for mycorrhizal parameters (spore count, species richness, percent root colonization, infectivity potential of AMF, etc.) or can be used for initiating the trap cultures for their further multiplication.

Soil samples collected for isolation of AM fungi have different propagules consisting of chlamydospores or azygospores, vesicles and mycelium or infected root pieces. Used together, as they occur in soil, these propagules may be termed mixed inoculum as compared with spores that have been separated from soil and represent a ‘pure’ inoculum. Before a propagule recovery technique can be selected, the desired form of propagule must be determined.

Various techniques are used to recover AM propagules from soil. The most basic of these is wet sieving and decanting to remove the clay and sand fractions of the soil while retaining spores and other similar-sized soil and organic matter particles on sieves of various sizes.

Wet sieving method

Spores of AM fungi in soil can be collected by the wet sieving method. The gravity of spores is a little lighter than that of soil particles. Successive decantation of soil suspension followed by sieving with fine mesh can concentrate the spores from soil. Since the spores are globular or sub–globular in 50–500 μm in diameter, they, in sievings can be recognized under a dissecting microscope.

Equipments:

1) Sieve: Sieves with various mesh size. At least the following mesh size are required; 1 mm, 100 μm and 50 μm. Other sizes such as 500μm and 250μm are preferable. Stainless steel sieves are commercially available. However, it is possible to make a plastic sieve with PVP tubes and nylon mesh by yourself. 2) Fine glass pipettes: Tip of disposable glass Pasteur pipette (1 ml) is softened with flame of gas burner and is sharpened. Various sizes of tips fitting to sizes of spores can be prepared. 3) Forceps: Light fine tweezers is preferable. Tips of the tweezers should be sharpened with a fine file or sandpaper and polished with abrasives. 4) Dissecting microscope: Stereoscopic zoom microscope with bifurcated illuminator of fiber arm is advisable. Transmitted illumination system is also needed. 5) Compound microscope: Biological compound microscope is needed. Nomarsky's DIC illumination system is advisable.

Procedure:

Ten to 50 g of freshly collected soil sample is put into 1 to 2 litters of plastic beakers. Usually rhizosphere soils are rich in AM fungal spores. Beaker size can be changed depending on the soil sample size. Soil is suspended with about 500 ml to 1 litter of tap water. Soil macro-aggregates should be crushed with hand. After 10-30 seconds of settling down of soil particles, the upper layer of soil suspension is poured into the sieving (Fig. 3). The procedure should be repeated until the upper layer of soil suspension is transparent. The sievings on the fine mesh is collected into a small beaker and dispersed with ultra sonication. Weak sonication (i.e. 30W 30 sec) is enough, and strong sonication may destroy fungal spores. Then the dispersed sample is again passed through the sieve. Depending on toughness of soil aggregate, the sonication process can be repeated. Usually AM fungal spores are collected on 100 μm. Some small spores are on 50 μm. To collect large spores such as *Gigaspora margarita*, 250 μm sieve is efficient.

Sucrose density layer method

Equipment and Reagent

Stalking sieves with nylon or stainless steel mesh and a large range of pore sizes for isolating spores from the soil sample 40-50 micron (0.04 mm) for small sized spores100 micron (0.10 mm) for medium sized spores 250 micron (0.25 mm) for very large spores and sporocarps.
Wash bottles containing water
Jars for collecting the sieving
Stereo zoom (stereomicroscope)
Petri dishes (11 cm) for observing the sieving under stereomicroscope
Micropipettes for spore picking
Sodium hexametaphosphate
Centrifuge

Methodology

Collect the sievings in jars. Transfer the sievings into centrifuge tubes and centrifuge for 5 minutes at 1750 rpm in a horizontal rotor. Decant the supernatant liquid carefully and re-suspend pellet in 60% sucrose solution. Again centrifuge for 2 –5 minutes. Pour the supernatant (with spores) onto a 300BSS sieve size and rinse with water to remove the sugar. Transfer the sieving onto the grided petridishes/plate and observe it under stereomicroscope. Count the number of spores in plate/dish and express it as spores/g of the soil sample.