Isolation of AM fungi

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Mycorrhiza

- Mycorrhizae are ultimate in reciprocal parasitism (symbiosis), wherein the fungus supplies mineral nutrients, plant growth hormones; and protects the root against pathogens. Higher plants supplies fungus with energy substrates.

- Partners in association are mostly from the fungal groups Basidiomycetes, Ascomycetes and Zygomycetes and most vascular plants.

- 97% of young roots of most plants have this association.

Mycorrhizal types

Endomycorrhizae – i) Vesicular Arbuscular
ii) Ericoid
iii) Orchidaceous

Ectomycorrhizae

Ectendomycorrhizae/ Ectoendomycorrhizae/ Arbutoid

Monotropoid (Special category)

Vesicular Arbuscular Mycorrhizae (VAM)

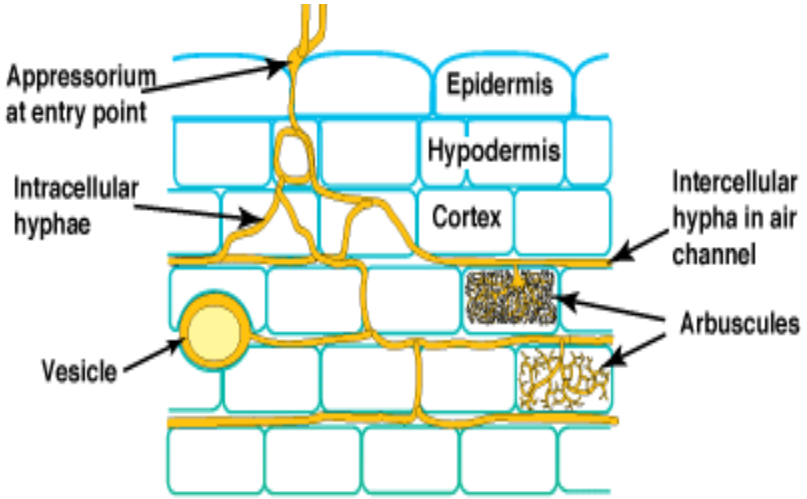
VAM are virtually ubiquitous, being present in tropical, temperate and arctic regions.

Greater impact in tropical agriculture as here PO_4 deficient and PO_4 fixing soils are present, phosphatic fertilizers are in short supply and temperature is congenial for their activity.

Apart from well known effect of VAM fungal associations on P uptake, there are also reports that it increases uptake of Zn, S, cytokinins and chlorophyll content in some plants

Vesicular-arbuscular mycorrhiza (VAM) is mostly formed by the symbiotic association between certain phycomycetous fungi and angiosperm roots.

The fungus colonizes the root cortex forming a mycelial network and characteristic vesicles (bladder-like structures) and arbuscules (branched finger-like hyphae).



Isolation of AM (Arbuscular Mycorrhizae/ VAM) fungi

Wet sieving method & Sucrose gradient method

Since VAM fungi are obligate endo-symbionts and have not been cultured, the only means of identifying these fungi is to collect resting spores from soil and determine the morphological characteristics.

By staining roots following appropriate methods, the morphology of vesicles and arbuscules can be understood which also serves as diagnostic characteristic to define spores.

Several techniques have been used for the isolation of VAM fungal spores from the soil such as floating technique, decanting and sieving, gradient centrifugation, monoclonal antibodies and polyclonal antibodies methods etc.

Wet sieving method

- Also known as wet sieving and decanting method (Gerdemann and Nicolson, 1963). Developed to isolate different size of spores.

- The soil near the root system is collected and an aqueous suspension is passed through different sieves to collect spores of different sizes.

- The wet sieving and decanting is one of the popular technique when compare to other techniques. This technique is used for sieving the coarse particles of the soil and retaining AMF spores and organic particles on sieves of different sizes.10 g of soil was mixed with 100ml of water in the 500 ml conical flask. The soil mixture was agitated vigorously to free the AMF spores from soil and allowed to settle for 15-45 minutes and the supernatant was decanted through standard sieves. By using a dissecting microscope, spores were picked by means of pipette or needle.

- Earlier, Gerdemann (1955) devised the first useful technique for extracting spores from soil. A soil sample was suspended in four times its volume of water, heavier particles were allowed to settle for a few seconds, then the liquid was decanted through a sieve with 1mm mesh. Whatever passed through this sieve was then poured through another sieve with 0.25 mm mesh. Material retained by this sieve was washed and transferred to a petridish, and the spores picked out by hand under a dissecting microscope.

- Technique given by Gerdemann (1955) was slightly refined by Gerdemann and Nicolson (1963) who used the following series of sieves: 1.0 mm ; 710 µm; 420 µm; 250 µm; 149 µm; 105 µm; 74 µm; and 44 µm.

- They found that most of the desired spores fell in the 420- 149 µm range, and they used this fraction for their study.

Source: https://www.slideshare.net/NawabKhatoon/mycorrhyzae-association

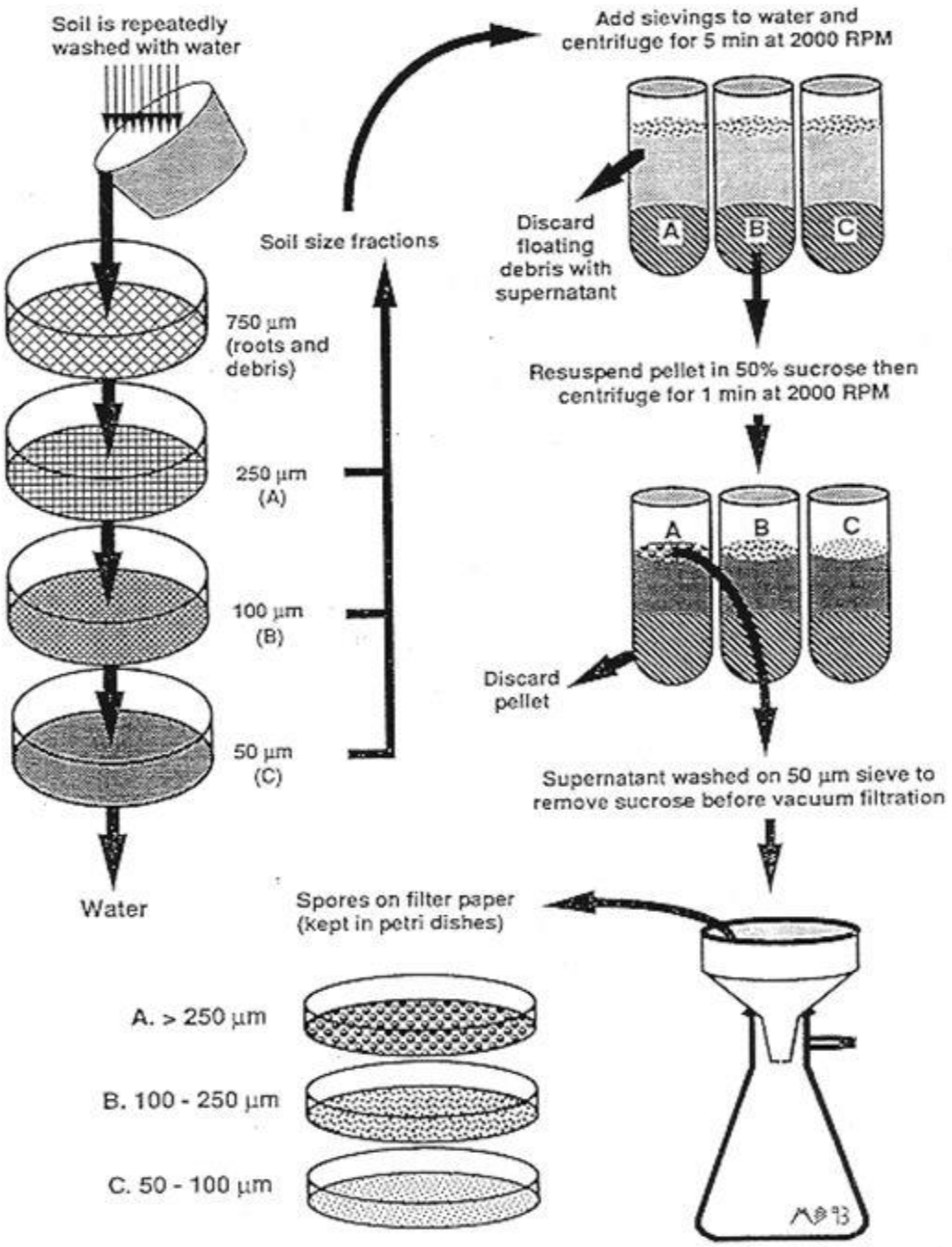
Sucrose gradient method

- Developed by Daniel and Skipper (1982); commonly used technique for AM spore extraction. Requires prior sieving and decanting.

- This gradient centrifugation method is result of many modifications, right from Ohms (1957), Mosse and Jones (1968), Mertz et al. (1979) etc.

- Spores were purified by re-suspending the sieving in the 40% sucrose solution and centrifugation was carried out. Centrifugation was carried out at 1750 rpm for 5 minutes. The supernatant was removed and poured into the sieves. The spores that hold on the sieves are carefully rinsed with tap water. The spores were collected by using dissecting microscope.

Spores extraction



wet-sieving and sucrose centrifugation method (Modified)

Brundrett et al. 1994. Practical methods in mycorrhiza research.

Belowground Research

- Mertz et al. (1979) protocol needs a special mention.

- They used discontinuous sucrose gradients to recover large number of spores from massive soil samples. They decanted and wet sieved 18 kg of soil with cold water, and found that most spores were present in the 425-250 µm fraction.

- Spores were separated from most of the remaining debris using discontinuous 30% (w/v) aqueous sucrose gradients. The sieved material was layered on 600 ml water over 200 ml sucrose in 1 L beaker. After settling, the spores and debris that collected at the interface were removed by vacuum aspiration, rinsed in cold water, and centrifuged for 1- 5 min at 1600 X g in clinical centrifuge on a second gradient (15 ml water over 20 ml sucrose in a 50 ml tube), the duration of centrifugation being determined by the kind and amount of debris present.