

3. Mycorrhiza

3.1. Introduction

Mycorrhizal fungi are species of fungi that intimately associate with plant roots forming a symbiotic relationship, with the plant providing sugars for the fungi and the fungi providing nutrients such as phosphorus, to the plants. Mycorrhizal fungi can absorb, accumulate and transport large quantities of phosphate within their hyphae and release to plant cells in root tissue.



A mycorrhiza (“fungus – root”) is a type of endophytic, biotrophic, mutualistic symbiosis prevalent in many cultivated and natural ecosystems. There are three major groups of mycorrhiza: Ectomycorrhiza, Ectendomycorrhiza and Endomycorrhiza. Ectomycorrhiza and endomycorrhiza are important in agriculture



and forestry. In Thailand, endomycorrhiza biofertilizer has been investigated for ten years. Initially the mycorrhizal biofertilizer production is for economic crops such as fruit trees (durian, longan, sweet tamarind, mangosteen, papaya). Now the biofertilizer can be used for vegetables and rubber.

Endomycorrhiza (vesicular arbuscular mycorrhiza; VA mycorrhiza; now known as arbuscular mycorrhiza, AM) play a very important role on enhancing the plant growth and yield due to an increase supply of phosphorus to the host plant. Mycorrhizal plants can absorb and accumulate several times more phosphate from the soil or solution than non-mycorrhizal plants. Plants inoculated with endomycorrhiza have been shown to be more resistant to some root diseases.

Arbuscular Mycorrhizal (AM) fungi (or Vesicular-Arbuscular Mycorrhizal, VAM fungi), belonging to the Phylum Glomeromycota are symbionts with terrestrial plant roots. It is now generally recognized that they improve not only the phosphorus nutrition of the host plant but also its growth, which may result in an increase in resistance to drought stress and some diseases. Therefore, AM fungi offer a great potential for sustainable agriculture, and the application of AM fungi to agriculture has been developed. In fact, in some countries the AM fungal inocula have been commercialized. Since it is laborious and cost-consuming for production of AM fungal inocula because of their obligate biotrophic nature, the ways to increase the function of the indigenous AM fungi in soil have also been developed. In this manual, the introductory techniques with AM fungi are presented.

General procedure has been documented in various text books and web sites. In this manual some additional information is described together with the outline of procedure.

3.2. Benefits of Mycorrhizal Biofertilizer

Mycorrhiza plays a very important role on enhancing the plant growth and yield due to an increase supply of phosphorus to the host plant. Mycorrhizal plants can absorb and accumulate several times more phosphate from the soil or solution than non-mycorrhizal plants. Plants inoculated with endomycorrhiza have been shown to be more resistant to some root diseases.

Mycorrhiza increase root surface area for water and nutrients uptake. The use of mycorrhizal biofertilizer helps to improve higher branching of plant roots, and the mycorrhizal hyphae grow from the root to soil enabling the plant roots to contact with wider area of soil surface, hence, increasing the absorbing area for water and nutrients absorption of the plant root system. Therefore, plants with mycorrhizal association will have higher efficiency for nutrients absorption, such as nitrogen, phosphorus, potassium, calcium, magnesium, zinc, and copper; and also increase plant resistance to drought. Benefits of mycorrhizal biofertilize can be seemed as follows:

1. Allow plants to take up nutrients in unavailable forms or nutrients that are fixed to the soil. Some plant nutrients, especially phosphorus, are elements that dissolve were in water in neutral soil. In the extreme acidic or basic soil, phosphorus is usually bound to iron, aluminum, calcium, or magnesium, leading to water insolubility, which is not useful for plants. Mycorrhiza plays an important role in phosphorus absorption for plant via cell wall of mycorrhiza to the cell wall of plant root. In addition, mycorrhiza help to absorb other organic substances that are not fully soluble for plants to use, and also help to absorb and dissolve other nutrients for plants by storage in the root it is associated with.
2. Enhance plant growth, improve crop yield, and increase income for the farmers. Arising from improved water and essential nutrients absorption for plant growth by mycorrhiza, it leads to improvement in plant photosynthesis, nutrients translocation, and plant metabolism processes. Therefore, the plant has better growth and yield, reduce the use of chemical fertilizer, sometimes up to half of the suggested amount, which in turn increases income for the farmers. As in the trial involving mycorrhizal biofertilizer on asparagus it was observed that, when the farmers used suggested amount of chemical fertilizer together with mycorrhizal biofertilizer, it was found that the crop yield improved by more than 50%, and the farmers' income increased 61% higher than when chemical fertilizer alone was used.
3. Improve plant resistance to root rot and collar rot diseases. Mycorrhizal association in plant roots will help plant to resist root rot and collar rot diseases caused by other fungi.
4. It can be used together with other agricultural chemicals. Mycorrhiza are endureable to several chemical substances; for example; pesticide such as endrin, chlordane, methyl parathion, methomyl carbofuran; herbicide such as glyphosate, fuazifopbutyl; chemical agents for plant disease elimination such as captan, benomyl, maneb triforine, mancozed and zineb.

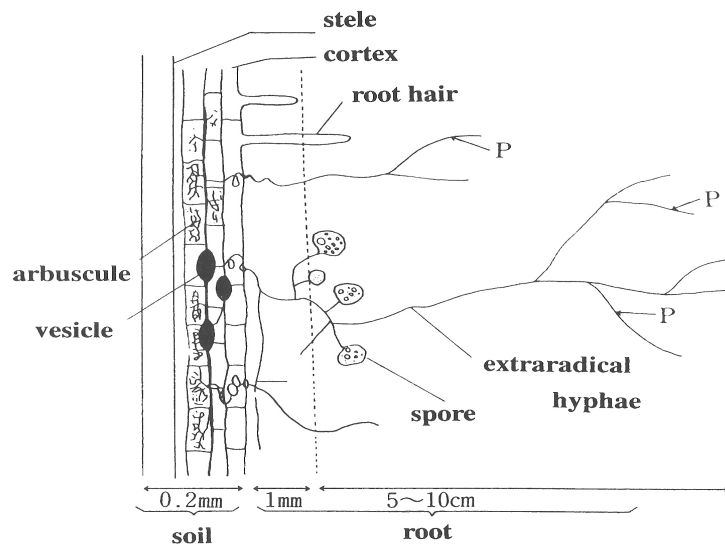


3.3. Isolation of Arbuscular Mycorrhizal Fungi

3.3.1. Taxonomy of AM fungi

AM fungi show the peculiar characteristics in morphology and physiology. Spores of AM fungi are generally formed in soil and their sizes (50-500 μm in diameter) are much larger than those of other fungi. There is no septum in their hyphae. No sexual growth-phase has been observed. Spores germinate when they are under favorable conditions, extend their hyphae and colonized plant roots. The fungi penetrate the hyphae into cortex layer of roots and form the hyphal organs, “vesicles” and “arbuscules” which are characteristics to AM fungi (Fig. 1). AM fungi belonging to Gigasporaceae are known not to form vesicles. Colonization on plant roots is essential for proliferation of AM fungi. AM fungi are thus recognized as obligate symbiotic fungi. The interaction between AM fungi and plants is generally mutualism based upon nutrient exchange.

Fig. 1: Schematic picture of arbuscular mycorrhizal fungi colonizing roots and their hyphal extension into soil.



Because of morphological characteristics such as no hyphal septum, AM fungi had long been recognized as a member of Zygomycota. Recent molecular phylogenetic studies showed that Zygomycota is poly-phyletic and that AM fungi should be separated from other Zygomycota. A new Phylum Glomeromycota has been proposed for AM fungi. Current classification system is summarized in Fig. 2. This classification is mainly based upon the sequence data of rRNA gene. However, some new genera have been raised with relatively small numbers of isolates, so further study may revise the present classification system.

In this manual, the morphological characteristics of representative genera are shown in the following sections. Although there is a recent trend that the sequence data of AM fungi is over-emphasized for the identification, the conventional morphological observation is still important and should not be neglected for identification.

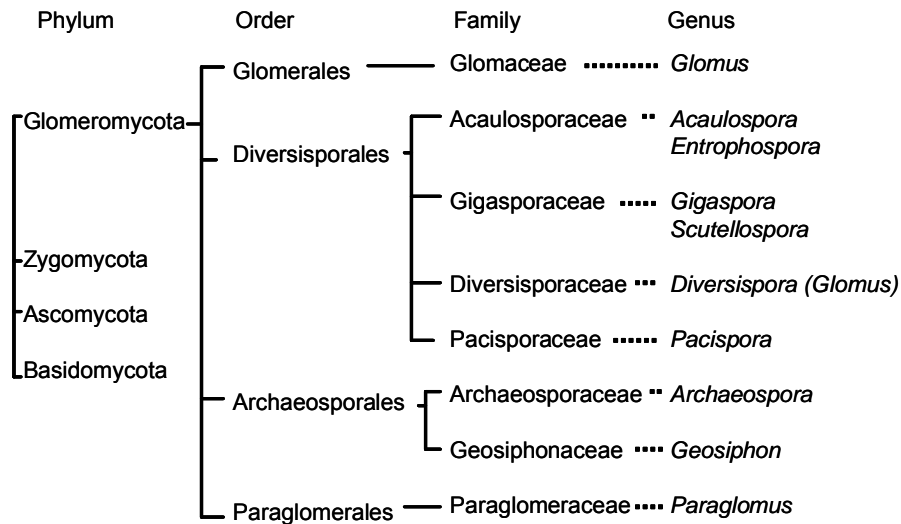


Fig. 2: Taxonomy of arbuscular mycorrhizal fungi.

<http://www.tu-darmstadt.de/fb/bio/bot/schuessler/amphylo/amphylogeny.html>

3.3.2. Observation of arbuscular mycorrhizal fungi in roots

Arbuscular mycorrhizal fungal structure in roots is usually not observed without appropriate staining. Freshly collected root samples should be washed gently and be free from soil particles. Ultrasonic treatment is effective to disperse soil particles closely adhered to roots.

Roots are treated with 10 % KOH solution for 30 min to 1-2 hours in a hot bath, depending on thickness of root structure. Treated roots are washed with water and treated with 2 % HCl solution. Acidified root samples are stained with 0.05 % trypan blue (or acid fuchsin) in lactic acid for 10-15 min in a hot bath or for a few hours without heating. The roots are destained with lactic acid or lacto-glycerol and are now ready for microscopic observation. The stained roots may be observed first under a dissecting microscope with transmitted illumination and then observed under a compound microscope. Fungal structures are stained and can be easily recognized.

3.3.3. Isolation of spores from soils and their observation for identification

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. Tweezers Model 113SA (OHM-Werkzeuge, Germany) is the

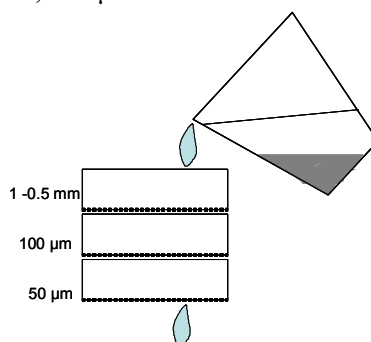
most convenient with good handling. Contact address: OHM-Werkzeuge, Flamerscheid 3a, P.O. Box 42791-41, D-42799, Leichlingen, Germany. Tips of the tweezers should be sharpened with a fine file or sandpaper and polished with abrasives.

- 4) Dissecting microscope: Stereoscopic zoom microscope with bifurcated illuminator or 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 liters 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 liter 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.

Fig. 3: Sieving of soil suspension.



Spores of AM fungi have characteristic shapes and colours, and so it is not difficult to discriminate the spores in organic debris collected on the sieves. However, it is recommended that those who have not yet observed the spores before should learn from the experts how the spores look like. Pictures of spores in the textbooks and the websites may be helpful to recognize AM fungal spores.

In the soil such as grassland soil rich in organic debris, it may be hard to find the spores hidden by the debris. In such a case, sucrose density centrifugation technique is often used to separate spores from the organic debris.

3.3.4. Morphological observation of spores for identification

Morphology of spores is a basis for identification of AM fungi, because the hyphae and the organs such as arbuscules and vesicles are not specific to species. Spores collected from soil often deteriorate so that they may be used only for tentative identification at genus level. For detailed observation, culturing the target AM fungus is required, and the spores from pot culture should be used. At least 30-50 spores from the same morphological spore type should be observed, and more observation is

recommended.

1) Observation of intact spores under dissecting microscope:

Spores collected from soil or culturing medium are put in a watchglass or a small Petri dish, and their shape, colour and the attachment to spores are observed. Spores should be classified into each spore type based upon morphology. For each spore type, detailed observation is conducted.

For color description, standard colour chart such as soil colour chart or “colour chart of glomalean fungi” (see INVAM web site) should be used. The colour chart should be under the same illumination as used for spore observation, because the colour itself is greatly affected by the characteristics of illumination.

It should be reminded that hyphal attachments such as sporiferous saccule for Acaulosporaceae and subtending hyphae for Gigasporaceae are often lost during collection of spores from soil.

2) Observation of spores mounted on slide glass under a compound microscope:

Spores are mounted with polyvinyl lactoglycerol (PVLG) on a slide glass. Several slides should be made. These are for intact spores mounted with PVLG, for crushed spores mounted with PVLG, for spores mounted with PVLG containing Meltzer’s reagent.

The morphological characteristic as indicated in Table 1 should be recorded. These characteristics may be helpful to identify genus of the target fungus (Fig. 4). However, some genera such as *Archaeospora* needs not only these morphological characteristics but also sequence data. For species identification, the characteristics should be compared with those in the species description in the original reference. Many species description and pictures are also available in INVAM website. Detailed information for morphological observation is available in review by Morton and his website. Species identification without enough expertise may cause trouble in scientific society, so those who wish to identify species of AM fungi should consult the experts in AM fungal taxonomy.

For spore size, at least 40-50 spores should be examined, and more examination is preferable.

PVLG (poly-vinyl lacto-glycerol):

Polyvinyl alcohol (polymerization 1000-1500), 1.66 g, is dissolved in 10ml of deionized water. Complete dissolution may need 6 hours at 80 °C. The dissolved polyvinyl alcohol is mixed with 10 ml of lactic acid and 1ml of glycerol. It can be used more than a day after preparation.

Fig. 4: Morphology of representative genera of arbuscular mycorrhizal fungi.

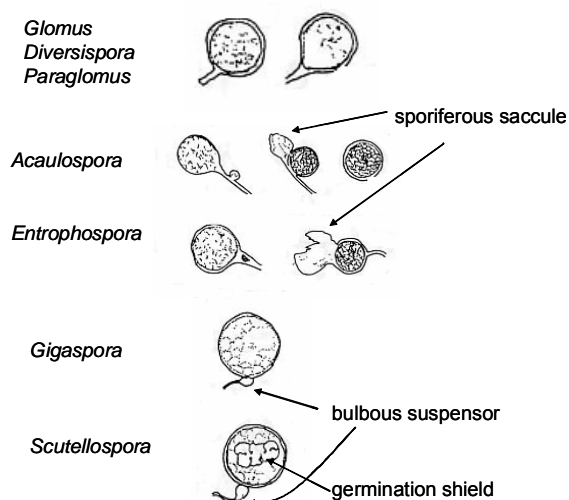


Table 1 Morphological character of spores of AM fungi

Shape:	(i.e. globular, spherical, irregular etc)
Size:	Globular: diameter (minimum – average – maximum) Irregular shape: length x width (minimum – average – maximum)
Colour:	(compare with Standard Colour Chart)
Hyphal attachment:	(i.e. sporiferrous saccule, bulbous suspensor etc) sporiferrous saccule = <i>Acaulospora</i> , <i>Entrophospora</i> , <i>Archaeospora</i> bulbous suspensor = <i>Gigaspora</i> , <i>Scutellospora</i>
Auxiliary cell:	(presence = <i>Gigaspora</i> , <i>Scutellospora</i> , none)
Sporocarp:	(presence, none)
Germination shield:	(presence = <i>Scutellospora</i> , absence)
Surface ornamentation:	(i.e. smooth, rough, reticulate etc)
Vesicle:	(presence or absence in mycorrhizal roots)

* These characters should be recorded with careful observation of many spores.

3.3.5. Culturing AM fungi

AM fungi need the symbiotic association with plants for proliferation. Therefore, culturing AM fungi is to inoculate AM fungi to host plant and to grow the inoculated plant. For the AM fungal inoculum, spores collected from soil can be used. However, spores in soil are not always active in colonizing plants. Therefore, trapping culture is often employed. Soil or sieving of soil is used as inoculum (Soil Trap Culture). To isolate AM fungi colonizing roots, mycorrhizal plants collected from field can be transplanted to potting medium as Plant Trap Culture (Murakoshi et al. 1998) (Fig. 5).

Potting medium: Sterile soil or soil-sand mixture is usually used. We prefer to use commercially available “Akadama-tsuchi” which is collected from subsoil of volcanic ash soil and is prepared for horticulture use (ref Saito NP or picture). Various potting materials for horticulture can be also used. However, the materials for potting medium should be low in available phosphate and preferably not rich in organic matter. In some cases the fungi isolated from some specific soils may need the specific soil properties for their growth.

Host plant: Various mycotrophic plants can be used: leguminous species (i.e. *Trifolium* spp., *Medicago* spp., *Lotus japonicus*) and grass species (i.e. *Lolium* spp., *Paspalum notatum*), and other herbaceous species (i.e. *Plantago* spp.). Onion and leek (*Allium* spp.) are also good hosts. AM fungi generally do not show host specificity but some species show host preference. Therefore, the plant species from which the target AM fungus is isolated can be used as a host plant.

Growth conditions: Any conditions, which support good growth of host plants, are acceptable. To avoid contamination, a growth chamber is preferable. If greenhouse is used, it should be kept clean. It should be reminded that cross-contamination or contamination from dust is inevitable under open-air conditions, even in growth chamber. To prevent cross-contamination from other pot culture in the same chamber, use of

plastic bag (SUNBAG, Sigma Co.) is advisable (Walker & Vestberg 1994).

Single spore isolation: To purify an isolated fungus, single spore isolation is needed. Even if the spores are morphologically identical, it often contains contaminants whose morphology is very similar. Successive pot culture of such multispore isolates would cause unexpected outbreak of the contaminant. Furthermore, even if the culture contains only one species, it may be composed of genetically diverse populations. For such a genetic studies or population genetics, the purification through single spore isolation is essential.

For single spore isolation, no specific equipment is needed. For efficient handling, two sets of dissecting microscopes are place side by side. One microscope is for picking up single spore from spores in a dish. Another is for inoculation of a spore on roots. Seedling placed in the pot is placed under another microscope. Under the first microscope, single spore is picked up and transferred under the second microscope. Under the second microscope, the spore is placed on fine roots or root tip of the seedling (Fig. 6). If culture is successful, the detailed morphological observation is required. Potting medium can be dried by stopping watering to the pot. After the host plant wilt, the dried soil containing spores can be stored for a year at 4-5°C. It is advisable that the isolated fungi are re-cultured every year. Flow of isolation and culture of AM fungi is summarized in Fig. 7.

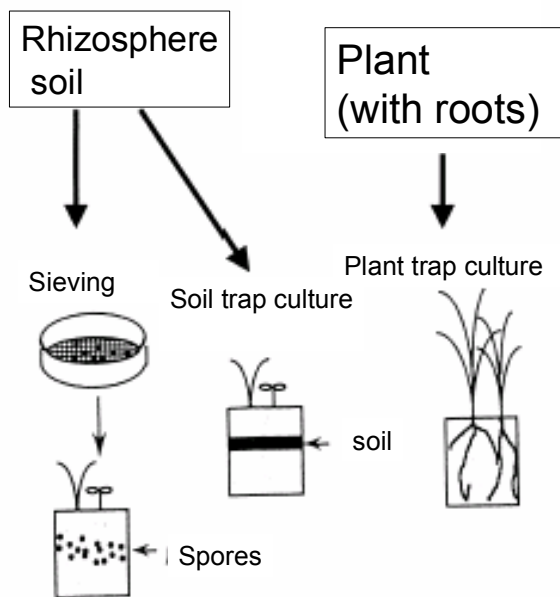


Fig. 5: Methods for trapping AM fungi.

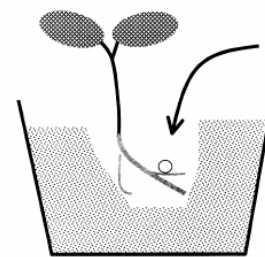


Fig. 6: Single spore isolation.

Throughout this procedure, the followings should be reminded.

- 1) Origin information of the isolated fungi should be recoded in detail as much as possible. (i.e. site description (latitude, elevation, vegetation, soil type, cropping history etc.), soil properties etc.)
- 2) At each culture step, voucher specimen of spores should be prepared and stored.

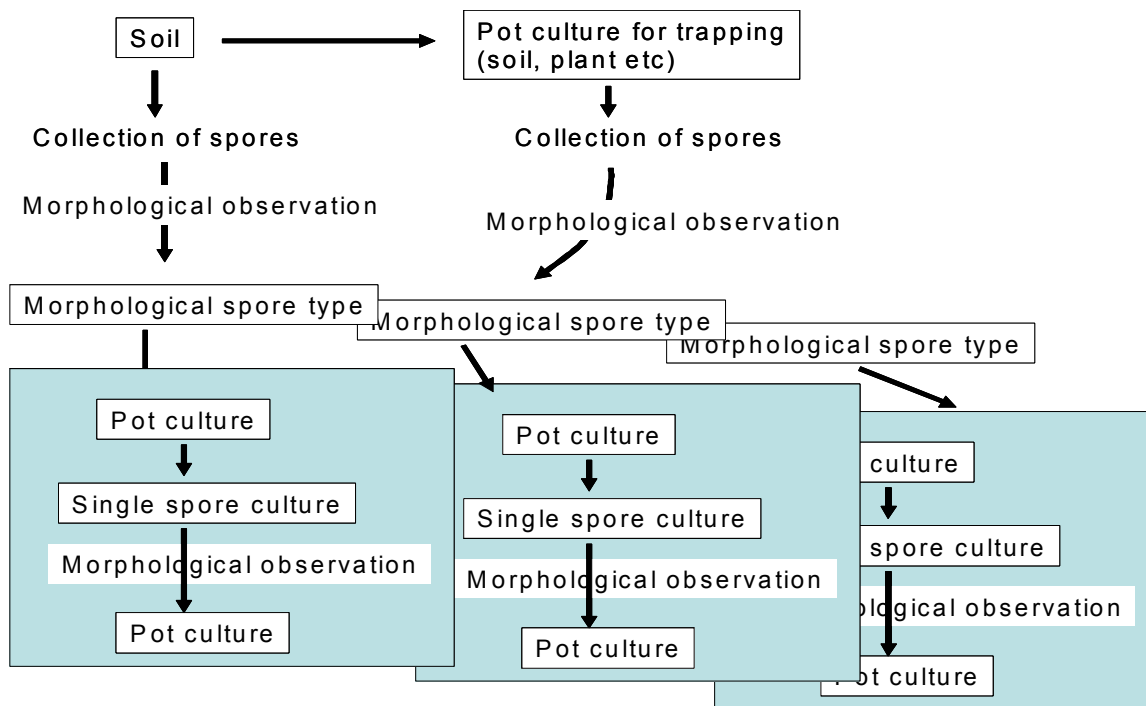


Fig. 7: Flow of isolation and culture of AM fungi.

3.3.6. DNA extraction from spores

Molecular phylogenetic information is essential for taxonomy of AM fungi. Sequence data for conserved genes such as rRNA is obtained by PCR amplification followed by sequencing with DNA extracted from spores. Many protocols for DNA extraction are reported. One of the protocols which we have used is described.

Cleaning of spores:

Clean spores are collected with tweezers or fine Pasteur pipette. Spores should wash several times with sterile water with sonication for 10-20 seconds. If spores show water repellency, surfactant such as Tween 80 can be used. Microplate with 6 or 12 wells is convenient for successive washing.

Crushing spores:

We use a cap of Eppendorf tube. The cap is cut from the tube and placed upside down on the stage of a dissecting microscope. Twenty μ l of Instagene (Biorad) is added to the cap. A spore or spores are put into the cap and crushed thoroughly with a micro-pestle or any fine rod. The tube itself is put on the cap with crushed spores. Then the tube is centrifuged for while for spinning down the reagent with crushed spores from the cap into the tube.

Extraction of DNA:

Follow the instruction provided by manufacture. Further purification of DNA with ethanol/chloroform precipitation is sometimes needed to remove inhibitor for PCR amplification.

PCR amplification and DNA sequencing:

Conditions of PCR amplification depend on the primers you will use. AM fungi are multi-nuclear organisms and often show polymorphisms in their sequence. It is advisable to sequence several clones from

the target fungus and to check the phylogenetic position of the sequence by constructing phylogenetic tree if the target genes are located within a reasonable clade of the tree (Sawaki et al. 1998).

References for section 3.3

1. Morton, J. B. & Redecker, D. (2001) Two families of *Glomales*, *Archaeosporaceae* and *Paraglomaceae*, with two new genera *Archaeospora* and *Paraglomus*, based on concordant molecular and morphological characters. *Mycologia* 93: 181-195.
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3. Saito, M. & Marumoto, T. (2002) : Inoculation with arbuscular mycorrhizal fungi: the status quo in Japan and the future prospects. *Plant Soil* 244: 273-279.
4. Sawaki, H., Sugawara K. & Saito M. (1998) Phylogenetic position of an arbuscular mycorrhizal fungus, *Acaulospora gerdemannii*, and its synanamorph *Glomus leptotichum*, based upon 18S rRNA gene sequence. *Mycoscience* 39: 477-480.
5. Schüßler, A., Schwarzott, D. & Walker, C. (2001) A new fungal phylum, the *Glomeromycota*: phylogeny and evolution. *Mycological Research* 105: 1413-1421.
6. Walker, C. & Vestberg, M. (1994) A simple and inexpensive method for producing and maintaining closed pot cultures of arbuscular mycorrhizal fungi. *Agricultural Science in Finland* 3: 233-240.

3.4. Inoculant Production

3.4.1. Preparation of trap culture inoculum of arbuscular mycorrhizal fungal spores

The following text is adapted from INVAM publication. For the establishment of inoculum from monospecific cultures trapping of healthy arbuscular mycorrhizal fungi (AMF) spores is often a necessity. Spore trap cultures can also aid in AMF identification. AMF spores sampled directly from a field plot may appear healthy but are not viable. The spores may appear differently, due to weathering and intrinsic soil environment effect, either physical, chemical or biological. Trap cultures are important in the following situations:

- When AMF colonization is high in roots of a plant community, but with little or no spores produced, especially in arid and hydric soil conditions.
- Where the soil has high microbial activity, especially in tropical environments, with relatively high temperature and humidity moisture). Organic matter content at these sites can be high. Under these environments, AMF spores may physically transform resulting in difficulty in species identification.
- To gather abundant healthy spores of different species and establish monospecific cultures for specific purposes.

Procedures

- i. Rhizosphere soil is collected, with shoots of trap plant cut at the crown, and roots are finely chopped and mixed with the soils using a sharp chopper.
- ii. The chopped roots and soil are mixed 1:1 (v/v) with autoclaved coarse sand in a mechanical mixer, or massaged well in a durable plastic bag.
- iii. The soil mix is then transferred to a 15 cm plastic pot.
- iv. Plant seeds of suitable trap plant such as tropical signal grass, *Brachiaria decumbens*, into the pot.
- v. The pot cultures are maintained in a greenhouse for at least 3 months, and check sporulation from time to time. By the fourth month AMF sporulation may be at the peak. Sanitary tests may also be carried out to ensure no contamination from parasitic fungi occurs.
- vi. Keep fertilizer application to a minimum, to encourage AMF proliferation.
- vii. Trap culture pots are later left to dry under shade for up to 2 weeks.
- viii. Harvest the spores using the sieving and decanting techniques or the density-gradient centrifugation technique.
- ix. The monospecific spores are ready for inoculation onto seedlings of the desired crops.

3.4.2. Inoculation of AMF

Two weeks before spore inoculation, the desired seedlings (e.g. oil palm, vegetable, pasture grass) are prepared in suitable containers filled with sandy loam soil.

- i. The seedlings are gently uprooted singly or in a small bunch, and have a gentle stream of water sprayed onto the roots so that they stick together.
- ii. Spores collected from 3.3.1 are suspended in water and about 200 μ l of the spore suspension are pipetted onto the moist roots.
- iii. The inoculated seedlings are immediately transplanted into containers of suitable size, containing sterilized soil.
- iv. The soil is topped with a sterile growth medium, watered gently under shade, before transferring into the greenhouse.
- v. To encourage colonization of AMF onto seedling, fertilizers are not given during the early growth stage of the seedlings.

3.4.3. Problems and potential for AMF inoculum production and utilization

- i. Situations where effective indigenous AMF population is low.
- ii. Inoculation is best for transplanted crops, where soil disturbances has reduces AMF inoculum potential.

3.5. Inoculant Application

1. Application rate of VA mycorrhiza biofertilizer is 10 g or 1 spoonful per plant.
2. VA mycorrhiza biofertilizer can be used at any stage of plant growth. However, for maximum benefits it should be applied during seedling stage or placed at the base of plant hole before planting. After two weeks of application, other suitable fertilizers can be applied.
3. For planting by stem cutting, the growing media are mixed with VA mycorrhiza biofertilizer prior to planting. The cutting stocks can be transferred to field one month after roots have developed.
4. For transplanting, simply sprinkle VA mycorrhiza biofertilizer adjacent to the plant roots and cover with soil.
5. For grown trees, soil under the plant canopy is trenched or the leaf litter under the tree is removed. About 10 g (1 spoonful) per plant of VA mycorrhizal biofertilizer is applied to the root hair system and then covered with soil.
6. VA mycorrhizal biofertilizer can be used in combination with several types of biofertilizers (e.g. *Rhizobium* biofertilizer, or PGPR).



3.6. Preservation and Precautions

1. Mycorrhizal biofertilizer can be kept under shade at room temperature. Normally AM fungi can live for 1-5 years, depending on the species.
2. Avoid using VA mycorrhizal biofertilizer on plants with root rot or stem rot. Mycorrhizal biofertilizer is more useful when applied prior the infection.
3. Avoid using VA mycorrhiza biofertilizer with some chemical products such as fosetyl, metalazyl and metalaxyl mancozeb since these substances can inhibit growth of VA mycorrhizal fungi.

References

Text books and other useful web-sites:

1. Smith, S.E. and Read, D.J. *Mycorrhizal Symbiosis*, 2nd edition, Academic Press (1997).
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3. Norris, J.R., Read, D.J. and Varma, A.K. (ed.) *Methods in Microbiology*, Vol. 24, *Techniques for the Study of Mycorrhiza*, Academic Press (1992)
4. Brundrett, M. et al. *Working with Mycorrhizas in Forestry and Agriculture*, Australian Centre for International Agricultural Research (1996).
5. *Working with Mycorrhizas in Forestry and Agriculture* <http://www.ffp.csiro.au/research/mycorrhiza/>
Various methodologies and many beautiful pictures are available.
6. International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi
<http://invam.caf.wvu.edu/>
7. Taxonomy and species description are available. Representative isolates are distributed upon request.
The International Bank for the Glomeromycota
<http://www.kent.ac.uk/bio/beg/englishhomepage.htm>
8. GINCO (Glomeromycota in vitro collection)
http://res2.agr.ca/ecorc/ginco-can/index_e.htm
9. Microorganisms Section of the NIAS Genebank,
National Institute of Agrobiological Sciences (NIAS), Japan
<http://www.gene.affrc.go.jp/micro/index.html>
10. These sites provide various information and their isolates are distributed upon request.
Mycorrhiza Information Exchange
<http://mycorrhiza.ag.utk.edu/>
11. The Mycological Society of Japan
http://wwwsoc.nii.ac.jp/msj7/english/index_e.html