MYCORRHIZAL INOCULATION TECHNOLOGY

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WHAT IS MYCORRHIZA?

- Mycorrhiza is the symbiotic association between a fungus and roots of plants. The fungi gets its food and nutrients from the host plant and in return offers several benefits to the host plant

TYPES OF MYCORRHIZA

- There are 6 types of mycorrhiza as described by Harley and Smith (1983) namely:

    1. Ectomycorrhiza
    2. Endomycorrhiza
    3. Ectendomycorrhiza
    4. Ericoid mycorrhiza
    5. Arbutoid and monotrpoid mycorrhiza
    6. Orchid mycorrhiza

- The six types of mycorrhiza differ in the kind of host plant they associate with a kind of fungi. The first two are most common in agroforest crops and will be discussed further.

- In **Ectomycorrhiza,** the infected roots are usually enlarged, the outer surface covered with a compact fungal mantle, with mycelia (rhizomorphs) radiating outward into the soil. The fungus invades the cortical tissues but are confined in between the cell walls, thus from the Latin word "ecto" meaning outside.

- The fungi are basidiomycetes producing typical fruiting bodies such as mushrooms or puffballs from which basidiospores may be extracted. Many of these fungi can be isolated into pure culture in agar media. This kind of symbiotic association is found in root of pines, eucalyptus and agoho.

- In **Endomycorrhiza** the infected roots are not enlarged. The roots have to be examined under a microscope to detect infection. The fungi are able to penetrate cortical cells, thus from the Latin word "endo" meaning inside.

- The fungus forms a loose network of hyphae on the root surface and may infect roots through root hairs or directly through epidermal cells. The fungi also penetrate cortical cells where they develop complex hyphal branching systems resembling small bushes called **arbuscules**. The arbuscule is the most significant structure because it is the **site for fungus/plant metabolite (food and nutrient) exchanges**.

- Another common feature in the endomycorrhiza is the production by some species of thin-walled spherical to ovate structures called **vesicles** which are terminal swellings of the hyphae which serves as **food storage** organs.

- Due to the presence of vesicles and arbuscules, this group of fungi are also called **vesicular-arbuscular mycorrhiza** or **VAM**.

- Endomycorrhiza is much more common than ectomycorrhiza. It is found in agricultural, horticultural and forest trees i.e. rice, corn, peanut, mungbean, papaya, citrus, guava, *Acacia mangium, A. auriculiformis, Leucaena leucocephala, Paraserianthes falcataria* and many others.

- A constraint in the utilization of VAM in crop production is its inability to be cultivated in culture medium. VAM inoculants are grown in the roots of living host plants.

BENEFITS OF MYCORRHIZAL ASSOCIATION

There are many benefits that can be derived in mycorrhizal associations, namely:

1. <u>Mycorrhiza aids in mineral nutrient absorption.</u>

- Soil fertility is very poor in marginal uplands. Many authors reported that mycorrhiza increases the absorption of essential nutrients such as phosphorus, potassium, calcium, nitrogen and several micronutrients. This is made possible by the substantial proliferation of hyphae within the soil capable of increasing the absorption of available and relatively unavailable nutrients.

- Mycorrhiza also produces the enzyme system necessary to facilitate the extraction of nutrients bound in mineral elements and organic matter. Mycorrhizal roots are more effective in nutrient absorption than root hairs.

- Through mycorrhiza partial replacement of chemical fertilizers is achieved. This is significant because most agroforest areas are done in marginal areas needing plentiful supply of chemical fertilizers, which are now very expensive.

2. <u>Mycorrhiza increases the drought resistance of plants.</u>

- Drought is one problem limiting the survival and growth of seedlings planted in the field. Mycorrhiza also aids in water absorption making mycorrhizal plants more drought tolerant than non-mycorrhizal plants.

3. Mycorrhiza controls pathogenic root infections.

- Survival and growth of plants are affected by soil-borne pathogens. Mycorrhiza has been shown to control some pathogenic fungi and nematodes. Mycorrhiza can do this by:

  a) Utilizing surplus carbohydrates and other chemicals thereby reducing amounts stimulating to pathogens.
  b) Providing a physical barrier against pathogens.
  c) Secreting antibiotic inhibitory or lethal to pathogens and
  d) Supporting a protective microbial rhizosphere population.

4. Mycorrhizal fungi produce growth hormones and growth regulating substances.

- In nature, growth regulating substances are produced by plants. Some mycorrhizal fungi may produce growth hormone including auxins, cytokinins, gibberellins and growth promoting substances such as vitamins. These fungal-produced compounds are then donated to the plant for the latter's growth.

5. Mycorrhiza promotes the activity of other beneficial organisms.

- The roots of plants also attract many beneficial organisms such as nitrogen-fixing or phosphate- solubilizing bacteria. Mycorrhiza may promote the activities of these organisms.

- For example, the Rhizobium bacteria which fixes nitrogen also requires phosphorus. In marginal areas phosphorus is unavailable due to fixation by iron, zinc, and other metals. Mycorrhiza may provide the phosphorus required by the nitrogen-fixing bacteria.

INOCULATION TECHNOLOGIES FOR AGROFORESTRY

Five mycorrhizal inoculation technologies are available for use in agroforestry areas. These are as follows:

1. MYCOGROE

- MYCOGROE is a tabletted form of mycorrhizal inoculant which contains spores of ectomycorrhizal fungi mixed in sterile soil as carrier. It contains no added chemicals and is a natural product. It is beneficial for both plants and soil. It is ideal for pines, eucalyptus and Casuarina (agoho) species.

- Upon inoculation, the spores germinate around the vicinity of the roots. The fungal mycelia eventually penetrate the roots where they become established and send forth numerous hyphae in the soil where they help in the absorption of nutrients and water. The hyphae also protect the roots, from harmful pathogens by completely covering the roots surface and secreting growth promoting substances for the host plant. In field trials, a single inoculation with MYCOGROE was found to replace from 60 to 85% of the chemical fertilizer requirement of the plant.

- One MYCOGROE tablet is sufficient for one plant. For best results, one tablet is incorporated into the plastic container during the bagging operation. In established seedlings, the MYCOGROE is placed 1- to 1.5-inch deep away from the base of the seedling. No re-inoculation is needed before the seedlings are outplanted in the field.

2. MYKOVAM -1

- MYKOVAM -1 is a soil-based bio-fertilizer containing spores, infected roots and propagules of beneficial endomycorrhizal fungi. The fungi incorporated has been screened for their effectiveness to promote growth, survival and yield of plants. MYKOVAM -1 has been tested effectively for agricultural crops (upland rice, corn, tomato, eggplant), fruit trees (papaya, citrus, guyabano, guava) and forest trees (*A. mangium, A. auriculiformis*, falcata, raintree). The non-host specificity of MYKOVAM -1 makes it ideal for use in many crops.

- MYKOVAM -1 is conveniently distributed in 1 kilogram packets. To inoculate containerized seeds/seedlings, about 1 teaspoon or 1 softdrink cup about 3-5 grams is added preferably during the bagging operations, directly beneath or near the seed/seedling. MYKOVAM-1 can also be layered in seed boxes for plants needing pre-germination treatments.

- For agricultural crops in the field, MYKOVAM -1 is added along the furrows at a rate of 100 grams per linear meter. MYKOVAM-1 can be incorporated or used as soil in marcotting operations.

- The spores and propagules germinate around the roots of the host plant. The fungi eventually invade and stabilize within the roots and produce numerous extensive external hyphae.

- These hyphae are more effective than root hairs in absorbing nutrients (particularly phosphorus) and water which later will be given to the host plant.

- MYKOVAM -1 can replace 60-80% of the chemical fertilizer requirement of the plants.

3. **MYKOVAM -2**

- MYKOVAM -2 is a granulated form of MYKOVAM -1 which was processed in a granulating machine. It basically contained the same ingredients as MYKOVAM -1 but appears similar to granular chemical fertilizer. It is easier to use due to its granular form. MYKOVAM -2 can be applied mechanically in tractor-drawn fertilizer applicators.

- MYKOVAM -2 is inoculated at the same rate and manner as MYKOVAM -1.

4. **DIRECT SEEDING BLOCKS (DSB)**

- DSB are compressed agricultural waste materials (i.e. coconut coir dust, sugarcane bagasse and sawdust) which has a hole in the center for mycorrhizal inoculum and seed placement.

- The DSB with the mycorrhizal inoculant and seeds can be sown directly in the field coupled with people land/site preparation.

- During the rainy season, the seeds germinate and form roots.

- The mycorrhizal spores germinate and invade the roots of the seedlings. The fungi later on produce numerous external hyphae which help in the absorption of water and nutrients.

- Later the roots grow out of the DSB to be established in the soil. The DSB, since it is biodegradable, can provide additional nutrients to the seedling.

- Since the DSB are directly sown in the field, they completely bypass the nursery operation. Thus, larger savings in plantation establishment cost can be realized.

- The size of the DSB may be varied depending on the size of the seeds. Furthermore, other probitions may be added such as insecticides may be incorporated in the DSB mixture to protect the seed from insects.

5. MYCOBEADS

- MYCOBEADS contain mycelia of ectomycorrhizal fungi produced in liquid fermentors and embedded in alginate beads. They are effective for reforestation particularly for pines, eucalyptus and agoho (Casuarina) species.

- MYCOBEADS are inoculated the same way as MYCOGROE tablets. One bead is good for one seedling inoculated preferably during the soil bagging operation. If planting stock is already established in plastic bag, one bead is inoculated about one inch from the base of the seedling at a depth of 1.5 to 2.0 inches.

- Upon inoculation, the fungal mycelia develop around the vicinity of the roots. They eventually infect and develop within the roots. Numerous external hyphae are produced by the fungi and help in the absorption of water and nutrients.

LITERATURE CITED

Harley, J.L. and S.E. Smith. 1983. Mycorrhizal Symbiosis. Academic Press. London, 483 p.

GENETIC RESOURCE CONSERVATION: IMPLICATIONS FOR STNM

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BASIC CONCEPTS AND PRINCIPLES

Definition of Terms

- Genetic resources -- include the biological, economic, scientific, or social values of the heritable materials contained within and between species

- Genetic resources conservation -- the management of human use of genetic resources so that they may yield the greatest sustainable benefit to present generation, while maintaining their potential to meet the needs and aspirations of future generations

- Biodiversity -- the variety and abundance of species, their genetic composition, and the communities, ecosystems and landscapes in which they occur. Also called biological diversity, it embraces the variability within and between all species of plants and animals and ecological systems they inhabit.

- Plant genetic resources -- genetic diversity found within the plant species which feed, shelter and provide medicine for the world's population; vital part of biodiversity.

- Ecosystem Management -- practices which are oriented towards the entire biological system instead of one particular species or commodity.

- Ecosystem approach recognizes that the interrelationships between organisms are critical and stresses ecological structure, function, and processes.

- Interrelationships between biodiversity and ecosystem management and their ecological importance:

  - High species diversity contributes to ecosystem stability. The more diverse an ecosystem is, the more stable it becomes.

  - Diverse ecosystems provide many services, e.g. maintenance of the atmosphere and climate, soil generation, recycling of wastes, and regulation of pests.

  - Conserving biodiversity preserves unique genetic information. Each species contains unique genotypes and therefore has ecological as well as potential economic value.

- Genotype - combination of genes that defines a particular trait or characteristic of a tree.

Three Levels of Biodiversity

- Genetic diversity - the number of genotypes within a species

- Species diversity - the number of species within a population and the number of these populations within a community or ecosystem

- Ecosystem diversity - the number of communities and ecosystems in a particular region

THREATS TO GENETIC DIVERSITY

- genetic erosion - loss and/or replacement of traditional varieties and wild relatives of crops

- genetic vulnerability - risk due to the lack of gene or the gene the variety carries

- genetic wipeout - rapid and wholesale destruction of genetic resources

FACTORS CONTRIBUTING TO LOSS OF PLANT GENETIC RESOURCES

- vegetation clearing
- habitat destruction and loss
- industrialization
- introduction of exotic species
- introduction of high-yielding varieties
- excessive use of pesticides
- natural calamities

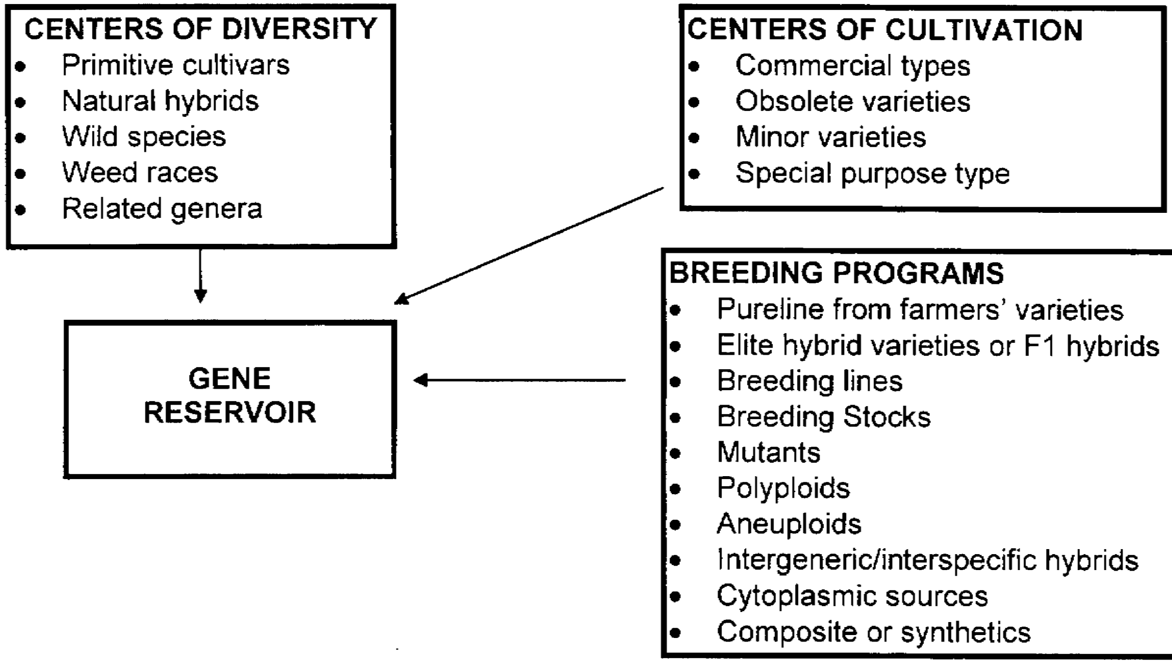

Figure 1. The spectrum of plant genetic resources

EXAMPLES OF THE DANGERS OF GENETIC UNIFORMITY

Year		
1840	famine in Ireland	potato late blight
1917	wheatless days in US	stem rust
1943	Bengal famine	rice brown spot
1945	complete elimination of oats derived from "Victoria" variety in the US	
1970-71	elimination of corn hybrids carrying T-cytoplasm	southern corn leaf blight
1974-76	Brown Plant Hopper (BPH) biotype shift in the Philippines and Indonesia	hopperburn
1982 - 83	Tungro infestation in IR-36 and IR-42 in the Philippines	rice tungro virus

To cope with problems arising from modern agriculture, it is essential to maintain genetic diversity.

SOME GENETIC CONSIDERATIONS IN FOREST NURSERY OPERATIONS

- Forest nursery management can affect three major attributes of genetic resources: diversity, adaptation and productivity. Diversity is necessary for adaptation to occur, and trees must be adapted to their environments to be productive.

- Job of forest nursery managers is to maintain or enhance genetic diversity and its adaptations in their desire to provide useful forest products and values.

    - It is important for forest nursery managers to know when they are making genetic decisions and what the implications of those decisions are.

- Forest nursery operations deal with trees in their reproductive stage where genes normally get distributed and recombined -- genes are "moved around" and discriminated for or against.

    - Gene sampling from a population during seed collection activities leading to genetic drift.

    - Movement of genes during planting operations -- gene flow may occur if planted trees have different gene frequencies with the natives.

- Seed collection is part of a gene sampling process.

    - Genetic diversity in planted stock is confined by the level of diversity in seeds collected.

    - Maximum diversity potential is reached in a seedlot.

- Genetic effects of each nursery practice in the sequence of nursery activities should be considered.

- Examples of nursery practices that may reduce genetic diversity:

    - collection of immature or low quality seeds
    - improper sowing and lifting dates
    - inappropriate selection of nursery sites
    - heavy grading which may eliminate homozygous inbreds or whole families from mixed lots
    - reinvigorating culled seedlings for next year's planting operations
    - inappropriate seedling processing and handling operations

- Forest nurseries affect biodiversity at all levels. Nurseries do not only preserve species but are also able to increase biodiversity.

- Forest and conservation nurseries can supply plant materials for a wide range of planting projects that will be generated by the biodiversity and ecosystem management issues.

- Nursery practices that maximize tree percent and seedling survival will preserve the maximum potential genetic diversity. Conversely, forest nurseries with large mortalities undergo the greatest genetic change.

- Site preparation and planting practices should also be evaluated. Seedling processing and handling combined with undesirable site conditions can cause losses in the field

- Nursery managers have the option of keeping seedlots separate by clone, family or stand at different stages in the nursery to maximize seedling percent. Later, mixing or "building up" can be done creating possible genetic combinations in the plantation.

- Seedlots collected in different years from the same seed zone may be mixed, provided the seeds have similar germination values or other performance qualities known from past experience with the individual seedlots. This practice widens the parental base in the combined lot.

- Seedlots from different zones may also be mixed if the environment at the planting site has been greatly changed by severe burning or damage by typhoons. As a general rule, however, at least 50% of the mixture should be of the local lot.

- Rapid global climate change is a potential threat to biodiversity. Such may trigger a series of events leading to major reductions in the natural range of many species. There may be accompanying pest outbreaks, wildfires, drought, reduced seed production and problems in natural regeneration. If these occur, gene banks will definitely assume great importance.

- Deployment of nursery stocks with greater diversity and planting at higher densities can help maintain or restore species in trouble.

- When adaptive ranges of species and seedlots are identified through testing over a wide range of sites, then appropriate mixtures could be developed for planting in almost any site.

- In general, concerns about maintaining genetic diversity and adaptation become more important as lesser known species are used. In most cases, they occur in small populations, have more inbreeding, and more restricted recombination. These are factors that facilitate the evolution of locally adapted gene complexes. Such species are likely to be more specifically adapted to local environments, require smaller seed zones, and may have more specific cultural requirements to maintain their diversity.

- The clonal propagation option can be used for these species and for those with unique genotypes having special adaptations to specific environments and when such are suitable for vegetative reproduction.

- Consider the genetic factors when conducting nursery studies for new species for new objectives of planting.

- Knowledge of the source and parentage of genetic stock can be useful for controlling the amount of unexplained variability in experiments dealing with propagation methods and cultural treatments.

FOREST NURSERIES AND BIODIVERSITY CONSERVATION

Current Uses of Nurseries in Biodiversity Conservation

- Gene conservation
- Propagation of rare species
- Preservation of local genotypes
- Resource protection and conservation
- Shelterbelts and conservation plantings
- Ecosystem restoration
- Enhancing wildlife habitat
- Rehabilitation of recreation sites
- Forest health maintenance
    - breeding pest resistant species
    - developing pest resistant forests

Some Genetic Considerations for Seed Collection

- 30-50 seed parents per provenance

- Individual trees should be well separated to represent adequately the desired genetic variability and to have a large enough genetic base.

- Sampling strategy should be such that collection of several provenances within each of the recognized seed zones (region of provenances) across the range of species attained.

- Collection of enough seed from each tree of each provenance to enable a nearly equal amount of seed per parent tree to be bulked and distributed.

- Collection of large amount of seeds from a large amount of trees per provenance region for the establishment of base populations in several areas/locations.

PLANT GENETIC RESOURCES CONSERVATION

- Best means to guarantee their availability for the use of present and future generations.

- Since no one can predict future environmental needs with certainty, it is important to conserve as wide a range of genetic variation within the species as possible.

STRATEGIES USED IN THE CONSERVATION OF PGR

A. According to the location of conservation efforts

1. Ex situ conservation - conservation outside of the natural habitat of the species

   a. Gene banks
   b. Botanic gardens, National parks, Arboreta

2. In situ conservation - conservation in the natural habitat of the species

   a. On-farm - in farmer's fields or farming communities
   b. Natural reserves

B. According to the methodologies used (applies to ex situ only)

1. Cold storage (seed, pollen)
2. Field maintenance (living plants)
3. In vitro conservation (micropropagation)
4. Cryopreservation
5. Ultra-dry seeds

C. According to the length of time materials are to be conserved (applies to ex situ only)

In case of seeds, the length of storage period depends on storage conditions:

- temperature - the lower the temperature, the longer the viability of seeds under storage.
- seed moisture content - the lower the seed moisture content (up to a certain level) the longer the viability of seeds under storage
- relative humidity - related to seed moisture content

In case of in vitro materials, the length of storage period depends on culture media and temperature.

1. Short-term:

seeds; 1-5 years
5 to 15°C

in vitro - 2-12 months interval between subculture cycles
22-25°C
media with or without growth retardant

2. Medium-term

seeds; 10-25 years
-5 to 5°C

in vitro - 1- 5 years
2-25°C depending on species and type of storage materials
modified media (growth retardant)

3. Long-term

seeds; 50 years or more
-10 to -20°C

in vitro - > 5 years
liquid nitrogen (-196°C)
cryoprotectant

D. According to the Availability of Germplasm Materials for distribution

1. Active collection - the germplasm materials are available for distribution to bonafide users

2. Base collection - the germplasm materials are not for general distribution; samples are withdrawn only for regeneration to replenish the base and/or active collections.

3. Duplicate collection - the germplasm materials are for safe-keeping as a back-up of the base collection, usually housed in an area as institution separate from the base collection.

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RE-ENTRY PLAN PREPARATION

RE-ENTRY PLAN PREPARATION

As the major output from the course, each participant is required to prepare a re-entry plan which is either a set of actions that will solve the pest problems in their respective areas, or a work plan integrating the learnings gained from the course in the present and future projects of their organization. The plan is presented before the other participants and a panel of resource persons for critiquing and further improvement.

The format for the re-entry plan is as follows:

A. GENERAL INFORMATION

Name of Participant(s): ______________________________

Title of Project: ______________________________

Project Location: ______________________________

Project Beneficiaries: ______________________________

Implementing Organization: ______________________________

Collaborating Agencies: ______________________________

B. BRIEF DESCRIPTION OF THE PROJECT

C. WORKPLAN

Objectives	Activity	Person(s)/ Group Responsible	Schedule or Time Frame	Expected Output	Remarks

D. AREAS FOR POSSIBLE COLLABORATION WITH IAF

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