III. NURSERY ESTABLISHMENT AND MANAGEMENT

3.1 Advantages of setting-up a nursery

There are many advantages for setting-up nursery for forest tree species, some tree species produced very fine seeds, e.g. *Eucalyptus* spp. and *Melaleuca leucadendron*, that is not possible to be sawn directly to the field. Many other tree species produce recalcitrant seeds which can not be stored easily without loosing viability, and this condition was combined with the seeds production which is not at the same time as the seeds required for plantation establishment. For example, *Pinus merkusii* produced abundance seeds in February, while plantation establishment is started on October. Some tree species, especially *Shorea* spp. which produce seeds only every 4 – 10 years, and also *Pheronema canescense* are usually propagated using tip or stem cutting which need special treatments and facilities. Therefore, it is not possible to plant these species directly to the field. Besides, compared with agricultural crops, seeds of forest trees are commonly available in limited amount, lower germination rates and the price is higher; therefore, maximum rate of germination and growth is very important.

By setting-up nursery, foresters could give the best condition for seeds to germinate uniformly, and seedlings to grow vigorously. During the early establishment young seedlings of most tree species need shade, it is possible only in the nursery. Seedlings of *Shorea* spp. need shade for early establishment or otherwise the growth of the seedlings is stunted and the leaves become yellowish.

3.2 Pointers in nursery site selection

There is several consideration to select nursery site, i.e. it should be closer to the source of water, nursery workers, the area is relatively flat, good accessibility, no extreme condition (very hot, very cold, strong wind, dry or flooding etc.), and if possible close to the planting sites. The continuity of water supply is essential for seedling production in the nursery. In Indonesia nursery usually established close to the river, canal, or man made lake or pond. Careful consideration needs to be made, since seedlings production is conducted during the dry season to be ready for planting in the following rainy season. Therefore, fluctuation of water flow in the river or canal and water availability in the lake or pond needs to be observed before the establishment of a nursery.

Nursery activities, especially the large-scale seedling production, need many workers for seed sawing, watering, over spinning, media preparation, fertilizer and pesticides application etc. Some nurseries sometimes need to be established away from source of workers; therefore, the company has to build camps for the workers.

The area should be flat to reduce cost of cut and fill during the establishment of the nursery. However, in some cases a nursery is established on a terrain, especially short-term nursery. Terrace is made to ease the arrangement of the seedlings.

Good accessibility is a requirement for a nursery area, so that delivery of nursery materials and transportation of seedlings to the planting sites is easy and safe. Beside, controlling and monitoring the nursery by the higher rank officer could be done routinely and easily.   Area with possible extreme climatic condition should be avoided to reduce the lost of seedlings. Climatic data need to be collected before deciding the area will be used for nursery.

To reduce the cost of seedlings transportation, nursery may be established as close as possible to the planting site. However, in the case of permanent nursery it is not always possible. For example, the teak nursery of Perum Perhutani, a state forest company in Java, supply teak seedlings to the planting site that is located more than 200 km from the nursery. And even they send the seedlings to other island, such as Kalimantan.

3.3 Types of nurseries

There are basically two types of nurseries, i.e. permanent nursery and short-term nursery. The permanent nursery is established for a long-term seedling production and for high quantity, such as 1 – 3 million seedlings per year. Short-term nursery is usually established for one period of planting with seedling production of several thousand seedlings only.

3.4 Nursery physical (structural) requirements

To support nursery operation several infrastructures are needed depending on the nursery types. There is no physical requirement for a short-term nursery, but for a permanent nursery needs more representative facilities. Facilities needed commonly are office, water storage and watering installation, electricity, communication equipments, media preparation house, storage house to store equipments, fertilizer, pesticides etc., green house, propagation chambers, shaded nursery beds and open nursery beds.

3.5 Production of planting stocks in the nursery

3.5.1 Seed (or sexual) propagation

3.5.1.1 Preparation of media

Media being used in the nursery varies between forestry companies; however the most common media is a mixture of topsoil, sand and compost with variable composition. Perum Perhutani uses potting media from a mixture of topsoil from under pine stand (50%), compost (30%) and rice husk (20%) to produce pine seedlings. In outside Java, where peat is abundant, other company uses potting media of a mixture of peat (70%) and topsoil (30%). Since peat is commonly acidic, liming is needed accordingly. Before materials of media are being mixed, they are sieved to pass 4 mm sieve size. Figure 3.1 show the preparation of media for nursery. The media-mix then are filled into containers, such as polytubes, pot trays, or most commonly polybags and arranged in nursery beds of 5 x 1 m^2 (Figure 3.1).

3.5.1.2 Pregermination treatment of seeds

Indonesian forest tree seeds are commonly orthodox; these include *T. grandis*, *A. mangium*, *P. falcataria*, *M. eminii*, *G. arborea*, *M. leucadendron*, *S. oleosa*, and *M. azedarach*. However, many of them are recalcitrant seeds, such as *A. lorantifolia*, *P. merkusii*, *Shorea* spp., *S. macrophylla*, *S. walichii*, *T. sureni*, and *K. anthoteca*. Recalcitrant seeds do not need special treatments prior to sowing, except *P. merkusii* seeds that need 12 hr soaking in fresh water. The soaking is to select seeds with high viability, i.e. those which are sunken. Floating seeds are commonly half-full or empty seeds. Meanwhile, orthodox seeds need pre germination treatments prior to sowing. The treatments varies among species. *Acacia mangium* and *P. falcataria* could be simply soaked in boiling water for 5 mins and then transferred to fresh water for overnight. The volume of the water is at least 3 times of that of the seeds. The swelling seeds than could be sown on sandy media to germinate. *T. grandis* seeds in general have very low germination rates (30 – 60%) due to difficulties to remove seed dormancy. One most recommended treatment is by soaking in running water over night and then sun dried for a day, and this sequence is repeated three times before the seeds are then germinated. There is no special pregermination treatment for *M. eminii*, *G. arborea*, *M. leucadendron*, *S. oleosa*, and *M. azedarach*.

Figure 3.1 Preparation of media in a nursery. Top-soil and other components of media-mix are sieve to obtain relatively uniform size of grain.

Figure 3.2 Arrangement of polybags containing media in nursery bed.

3.5.1.3 Sowing and germination

Sowing and germination of seeds are normally conducted in plastic boxes (38 x 30 x 12 cm^3) (Figure 3.3), or in wooden boxes of 5 x 1 x 20 cm^3. Since germination process do not require nutrient, the media that is needed not necessarily fertile. However, the media should be porous and sterile from pathogens. The media that is commonly used for germination is sand, combination of sand and top-soil (1 : 1 v/v), and top-soil. *T. grandis* is usually germinated on sand, meanwhile no specific media to germinate *A. mangium*, *P. falcataria*, *M. eminii*, *G. arborea*, *M. leucadendron*, *S. oleosa*, *M. azedarach*, *A. lorantifolia*, *P. merkusii*, *Shorea* spp., *S. macrophylla*, *S. walichii*, *T. sureni*, and *K. anthoteca*. *P. merkusii* is very sensitive to dumping-off; therefore, sterilization of seeds and media is very important. The seeds of *P. merkusii* are soaked in 2 - 2.5 g/l solution of fungicide (Dithane M-45) for 12 hr. After sowing the seeds in germination boxes, the media is wetted with the dose of fungicide solution.

For fine seeds, such as *Eucalyptus* and *M. leucadenron* the sowing beds need to be protected from direct rainwater and sunlight. Watering has also needed to be done very carefully to avoid disturbing the germinating seeds. By contrast, large seeds with high germination rate, such as *Shorea* spp., could be sown directly into polybags.

Figure 3.3 Seeds germination in plastic boxes in placed in a green house.

3.5.1.4 Watering

Watering is commonly conducted twice a day, i.e. at 06.00-09.00 o'clock and at 16.00-18.00. Watering is done by using sprinklers or manually. The size of water droplets should be kept as fine as possible to avoid disturbance to germinating seeds.

3.5.1.5 Potting or transplanting

Transplanting of germinating seeds are started when the seedling has already had two leaves with exceptions are for *P. merkusii*, *Eucalyptus* spp., and *M. leucadenron*. *P. merkusii* seedlings are transplanted to containers when the cotyledon is still intact with the needles, and the needles has yet opened. Only good quality seedlings, germinating normally and healthy are transplanted to the container. This is the first step of selection of good quality planting materials.

3.5.1.6 Growing

The transplanted seedlings are raised in the nursery beds under suitable shading net for 1 – 2 months. Newly transplanted seedlings are sensitive to direct sunlight with full light intensity; therefore, providing shading is necessary (Figure 3.4).

3.5.1.7 Use of biofertilizers

Biofertilizers such as rhizobium for nitrogen fixation on leguminous plants, ecto- and endomycorrhiza to enhance acquisition of phosphorous and other nutrients by plants have been known. However, only ectomycorrhiza has been applied regularly in the nursery of *P. merkusii* and *Shorea* spp. These two plant species form symbiotic relationships with ectomycorrhizal fungi (Figure 3.5 and 3.6).

Figure 3.4 Growing seedlings of (a) *P. merkusii*, (b) *A. lorantifolia*, (c) *T. grandis*, and (d) *S. macrophylla* under shade. *P. merkusii* seedlings are grown in polytubes, while the rest of species are grown in polybags.

Figure 3.5 Fruit bodies of *Scleroderma* sp., an ectomycorrhiza forming fungus, that can be use to inoculate seedlings of *P merkusii*, *Eucalyptus* spp., and *Shorea* spp.

Figure 3.6 Mycelia of ectomycorrhizal fungus covering the roots and media of *P. merkusii*.

Other beneficial soil microbes, such as rhizobium and arbuscular mycorrhizal fungi (AMF), have not been put into practice. *A. mangium* and *P. falcataria* are important leguminous forest tree species which form symbiotic relationships with rhizobium and form root nodules (Figure 3.7). These species also form symbiotic relationships with AMF (Figure 3.8). Arbuscular mycorrhizal fungi colonize roots of most plants including forest tree species.

Figure 3.7 Root nodules on roots of *Paraserianthes falcataria*.

Figure 3.8 Arbuscular mycorrhizal fungi *Gigaspora rosea* form symbiotic relationships with roots of *P. falcataria* and establish hyphal network in the media.

3.5.1.8 Protection from pests and diseases

Intensive pest and disease control has been reported in the nursery of *Pinus merkusii* at Pongpoklandak, West Java (Christiani, 2002). Dumping-off is the main problem in the nursery of *P. merkusii*. There are several technique to overcome this disease, i.e. using fungicide, removing infected seedlings, opening shading net, control watering, and replacing media. The fungicides being used so far are Previcur, Dithane, Benlate and Antracol, which is applied 5 days after transplanting the seedlings to polybags and repeated every 5 days for 1 month.

3.5.1.9 Hardening-off

Hardening-off is needed to train the seedlings to adapt to adverse condition in the planting sites. This is conducted by cutting the overgrown roots, reducing watering intensity, opening shading nets, and no fertilizer application.

3.5.2 Clonal (or asexual/vegetative) propagation

The concept of vegetative propagation is that an exact copy of the genome of a matter plant is made and continued in new individuals. This is possible because plants, - unlike animals or humans, - have meristematic, undifferentiated cells that can differentiate to the various organs necessary to form a whole new plant.

A piece of plant shoot, root, or leaf, can therefore, grow to form a new plant that contains the exact genetic information of its source plant.

Whereas sexual reproduction by seeds provides opportunity for variation and evolutionary advancement, vegetative propagation aims at the identical reproduction of plants with desirable features such as high productivity, superior quality, or high tolerance to biotic and/or abiotic stresses, and as such, plays a very important role in continuing preferred trait from one generation to the next.

The most important vegetative propagation techniques for tree species are the propagation by cuttings, layering, budding, grafting and micro propagation. The most important reasons for vegetative propagation are :

- Maintaining superior genotypes
- Problematic seed germination and storage
- Shortening time to flower and fruit
- Combining desirable characteristics of more than one genotype into single plant.
- Controlling phases of development.
- Uniformity of plantations.

3.5.2.1 Cuttings

Cuttings are severed plant pieces with at least one node. Various plants organs can be used for cuttings: stem, shoot, root or leaf cuttings. Cuttings are usually placed into a suitable rooting substrate and kept under high humidity until roots and shoots haved formed. Plant propagation by cuttings can yield a high multiplication rate and produces plants with their own root system.

Taking stem and shoot cuttings is perhaps the most common way to vegetatively popagate trees. The process is relatively simple requiring only a limited area for reproduction, whilst a single mother- or stock plant can yield many cuttings.

On the other hand, species which have been successfully developed with shoot cuttings are *T. grandis, P. merkusii, A. mangium, Shorea* spp*., S. macrophylla, Eucalypthus* sp. and *K. anthoteca*; and with stem cuttings are *P. canescens* and *Shorea* spp.; and with root cuttings is *D. latifolia*.

Rooting Process

The rooting of cuttings is a complex process resulting from a combination of many factors. The success of taking cuttings starts with the status of the stock- or motherplants and this is affected by several endogenous and exogenous factors. Once the cuttings are harvested from the mother plant. Several measures need to be taken to ensure proper conditions for the rooting process. This starts with a healing process, the formation of new cells, the induction of root formation, the linking up or bridging of these roots with existing vascular tissue of the cutting stem, elongation of these newly formed roots and finally the development of a new functional plant from the cut stem pieces. Again, several exogenous factors influence the success of this process.

Factors affecting the rooting process

Factors affecting the rooting process are the rooting substrate, humidity, hormones, leaf area, light and temperature, and plant hygiene.

Rooting substrate

Determination of appropriate substrates is essential for the rooting of stem cuttings. Most tropical tree species require a light medium with good drainage to prevent waterlogging and subsequent rotting of the cuttings. The following substrates were found to satisfy these requirements:

- Soil
- fine river sand
- soil and river sand mixture
- peat
- compost
- soil and compost mixture
- vermiculite

In order to avoid pest and disease attacks, the substrates should be washed properly before use and sterilized if possible. They should be renewed at least once per year.

Humidity

As soon as a cuttings is removed from a stock plant, it will not be able to take up the water needed for its survival and development. It thus becomes critical to maintain an optimal level of ambient humidity to make sure that the cuttings will not wilt and dry out due to low humidity, or become diseased because of a too high humidity. Water is an important external factor affecting the success of rooting of the cuttings.

Hormones

As mentioned in the introduction, plant hormones are of paramount importance in the multiplication process. Certain hormones such as auxins (IBA, IAA, NAA) will influence root development, and others such as gibberellins will be influence stem elongation and bud development. Depending on the balance of these hormones in the mother plant and in the cuttings, the rooting process will be affected either positively or adversely. Therefore, it is sometimes necessary to increase the amount of root promoting hormones. Synthetic plant hormones can be applied to promote the root development process either through their direct action on the root development process or through an antagonistic action on root inhibiting hormones. The appropriate balance of hormones in the cuttings will affect wound healing, the development of root primordial, initial root development, root elongation, hardening and further development of the rooted cutting. The hormonal balance in the stock plant will influence that of the cuttings, and thus timing of taking cuttings is an important consideration in the cutting process. In general, it will be important to go through a set of experiments to determine appropriate auxin concentration for rooting unknown species.

Shoot cuttings of *T. grandis* usually apply concentration of 10 – 40 ppm IBA, *P. merkusii* apply 1500 ppm, *S. leprosula* and *S. selanica* apply 500 – 100 ppm IBA, *A. mangium* apply 500 – 1500 ppm IBA, *K. anthoteca* and *S. macrophylla* apply 100 – 500 ppm IBA, and *E. deglupta* apply 200 – 500 ppm IBA. However it must be noted that not all species require auxin for rooting, for example stem cuttings of *P. canescens* apply without hormone.

Leaf area

Plants need nutrients (nitrogen, phosphorous, potassium, etc.) and metabolites (proteins, lipids, carbohydrates) for their growth and development and thus it is important that mother plants and cuttings are in optimal condition as far as their nutrient and energy status is concerned. In cuttings, this metabolic activity takes place in the leaves remaining on the cutting.

The initiation of roots in a cutting elies on the photosynthetic activity of the leaf area of the cutting. It is therefore important to maintain a sufficiently large leaf area on a cutting so that the leaves can continue to reduce the metabolites necessary for root initiation through photosynthesis. At the same time, the cuttings will lose water through transpiration of this leaf area. The recommended leaf area will need to ensure that there is an optimal balance between these two processes and this will vary from species to species.

Light and temperature

Ambient light and temperature conditions will also influence the rooting process. Control of these factors often requires equipment and infrastructure that may not be readily available in all nursery (electricity, additional light or complete darkness, heating cables in the rooting substrate).

Preparing cuttings

Management of stoct plants

Some important rules for the management of stock plants are:

- Establish stock plants are close as possible to the propagation area.
- Prune the stock plants regularly (thrice a year) to encourage production of good shoots and maintain juvenility of the vegetative material. Always conserve one pair of feeding leaves on each plant.
- Use fertilizer to accelerate growth on nutrient deficient soils.

Taking cuttings

- Cuttings should be taken early in the morning before the sun is hot, as this will keep transpiration and thus drying out to minimum.
- Trim leaves before the shoots are detached from the stock plants as this reduces water-loss. Leaf areas for optimum rooting vary with species.
- Use a polyethylene bag that is moistened inside to carry the shoots.
- Keep the collected shoots under shade, without throwing or sequuzing the bags.

- If you are carrying the shoots over a longer distance, keep them in a cool box – but ensure that the shoots do not directly touch the cooling elements.

3.5.2.2 Air layering or marcoting

The term air layering or marcoting is used for all types of propagation in which roots are formed while the stem is still attached to the mother plant. Only after the root formation, the layer is detached and planted as a new plant.

Air layering or marcoting can be done with almost any woody plant and is an excellent method to propagate small numbers of individual trees. In principle, marcoting is conducted by removing (peeling) barks of the branch or stem up to the cambium layer. Afterwards, the peeling site is covered with moist medium and is tightly closed with plastict sheet, covered fibre or other available materials. Root primordial will emerge from the marcotted stem. To stimulate root growth, all peeled area are polished with growth hormone such as IAA, NAA, IBA, or Roone F.

In forestry, plant propagation using marcoting is considered less productive. However, if it is used for obataining cutting material, this technique is a proper stepping store to rejuvenate old plant tissue (rejuvenilization) so that the produced stem and shoot cutting is more easy to root. Several species which can be marcotted are *T. grandis, P. merkusii, S. macrophylla, P. falcataria, Shorea* spp., and *Eucalypthus* sp.

3.5.2.3 Grafting

This technique is very revolutionary in forestry, especially in tree improvement to obtain good progenies. Connection (joining) between root-stock and scion should be accurate to make the grafting successful. Accuracy of the connection is determined by the success in joining the cambium of root-stock and that of scion. If both united, each will develop callus which can tranlocate nutrition, water, and other materials needed for plant growth, from roots to growing points. The next stage is that each component will recover the wound of the other so that the tissue can function properly.

Several trees which have been successfully developed with grafting, especially for clonal seed orchard or clone bank are *T. grandis, P. merkusii, S. macrophylla*, and *K. anthoteca*.

The failure of grafting could be due to several things, such as:

- Disease attacking the joining (connection)
- Scion grows faster before the joining has been properly united
- Mechanical damage of phloem tissue
- Rootstock does contain enough carbohydrate to be translocated toward scion (unever distribution of carbohydrate).
- Family difference
- Different behavior between rootstock and scion.

3.5.2.4 Budding

In principle budding is attaching axillary bud to branch or stem which is desired from a rootstock. This technique has been developed on *T. grandis* for developing clone bank.

Factors which should be considered are among other things:

- Growth characteristics of bud which is attached (plagiotrop or orthotrop).
- Physiological status (dormant or active)
- Plant species
- Attack by pest and disease.

3.5.2.5 Micropropagation or tissue culture

Micropropagation or tissue culture are terms used for procedures to propagate plants from plant cells, tissues or organs under aseptic conditions in a controlled artifial environment.

Tissue culture techniques are based on the principle of omni- or totipotency, meaning that indefinite culture of plant cells is possible under ideal conditions. In recent years, the tissue culture of animal cells has also become possible. In order for cells to survive outside the organism, the surrounding environment must mimic the optimal conditionsin terms of light, temperature, humidity and supply of nutrients, vitamins and hormones. The balance of auxins and cytokinins enables the manipulation of shoot or root formation *in vitro*. Auxins are needed to promote the development of roots, an auxin-free substrate is necessary for maturation of tissues, and a substrate containing cytokinins and gibberellins is needed to start the differentiation of shoot tissue. By careful subculture an or in substrates containing different ratios of these hormones and increasing levels of nutrients, it is therefore possible to control the stages of development. Hardening of the young plantlets is similar to 'normal' seedlings in a greenhouse under the shade and high humidity conditions until strong to withstand field conditions.

Requirements

Micropropagation work requires a small number of well-trained and skilled workers, some laboratory space and time to produce large quantities of propagules.

Unlike the conventional vegetative propagation of trees in nursery, micropropagation requires an organized laboratory space:

- Preparation room
- Transfer or inoculation room
- Incubation or culture room
- Greenhouse

The most critical requirement for successful in vitro propagation is maintenance of aseptic conditions as well as the control of temperature and humidity during the entire process of micropropagating plants.

The regeneration of any explant (cell, tissue, organ) into entire new plant needs the balanced supply of hormones, nutrients and sugar suspended in either an inert solution or a solid supporting material e.g agar-agar. The critical hormones inducing development of shoots are cytokinins and auxins are needed for root development. These hormones have to be supplemented with basal salts of the essential mineral nutrients and carbohydrates from sugar e.g. sucrose. For some plants species the enhancement of growth and development of in vitro plants, requires additional supplementation of other hormones such as gibberellic acid and some vitamins. The basal salts medium most commonly used in tissue culture work is that of Murashige and Skoog (1962). Other modified media have been formulated e.g. the woody plant medium by McCown and Lloyd (1981).

Techniques

The following are the general steps in the micropropagation procedure that apply to all techniques described further on:

- Defining the purpose for carrying out micropropagation.
- Selecting appropriate techniques.
- Preparation of appropriate culture media, sterilized in an autoclave or pressure cooker.
- Collection of plant materials (leaves, stem. Roots, flowers or fruits) from selected field or greenhouse trees.
- Preparation of explants collected from the field for inoculation into the culture media: washing, cutting into small sections, sterilizing with sterilant e.g. sodium hypochlorite.
- Preparation of the laminar air flow hood for inoculation work: cleaning with disinfectant (e.g. alcohol) of the dissection stage, hood sides and air filters, disinfecting surgical tools by flaming.

Several forest tree species which have been produced by tissue culture are *T. grandis, A. mangium, P. falcataria* and *Eucalypthus spp*. Other species which are still developed at only in experimental scale are *P. merkusii* and *Shorea* spp.

3.5.3 Wildlings as planting materials

In many cases utilizing wildlings as planting materials is more visible than using seeds. This is true for trees with recalcitrance seeds where storing seeds is not possible without loosing seeds viability, and also the tree has ability to regenerate naturally. Planting material for *Shorea* spp. are mainly wildlings growing naturally under mature trees or stand. Wildlings are harvested and taken to the nursery, containerized and then pre-conditioned for one or two months under intensive care. Afterwards, the wildlings are ready to be out planted to the field. Similar condition is for *Agathis lorantifolia* (Figure 4.9), and *Schima walichii*.

Figure 3.9. Harvesting of wildlings of *Agathis lorantifolia* in Gunung Walat Educational Forest, Faculty of Forestry-Bogor Agricultural University at Sukabumi district.