Vegetative Propagation of Trees

For Seedling Production

Technical Manual

Small and Large scale Plantation directorate

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Forestry provides various socio-economic benefits and uses by generating income to the rural and urban community. It is used as energy sources for fuel wood, construction material, timber, deliver ecosystem and environmental services. In order to sustainably develop the forest sector and increase its economic contribution to nation GDP, there are policy and strategies leading the sectoral activities in planned manner. The revised proclamation of the 2017 ensures sustainable development, conservation and utilization of forest resources in small holders, community, association and state owned forests. It provides various incentives and rights for forest growers’ in woodlots, plantation and conserving natural forest through participatory forest management approach. It is believed to draw the attentions and interest of investors engaged for establishing commercial plantations to produce industrial forest products. The production of seedlings for the establishment of plantations is basically done by seed method; however; based on the objectives of the plantation the mode of seedling production could be vegetative way; this could be done for the objective of getting vigorous tree stand which are used for timber and industrial uses. In this case the mother tree is selected based on the phenotypic performance in order to produce thousands of seedlings from a single tree. Even though; the initial investment of establishing the vegetative propagation infrastructure and training cost is high, once it is started then it will be a sustainable source of vigorous seedlings that are true to type with respect to the phenotypic characteristic which determine the wood quality and properties for multipurpose use of industrial products.

In Ethiopian context; tree seedling production using vegetative way is not as such extensively applied in all regions of the nation; however, there are some pilot investments started by the Ethiopian Environment, forestry research institute, projects and more dominantly some universities through joint research by doing the tissue culture technology. Moreover; there are huge projects in the commission of environment forest and climate change including the forest sector development program and REED+ project which may have potential taking the initiative and become pioneer in the extensive employment of asexual propagation of seedling using the vegetative way. There may be a chance that massive seedling production being started by the projects using vegetative propagation with root training facilities to hasten the commercial and industrial production of forest products. The future intensification of forestry for industrial input require the employment large scale establishment of plantation in which large mass of seedlings need to be produced asexually in the nursery sites which are true to type and vigorous. The asexual reproduction is less employed in Ethiopia because of lack of well-trained expertise and relevant technological infrastructure. The clonal technology is highly capital
intensive at the inception of the investment, it may be expected to be piloted by the national forestry programs and projects which are capable of introducing the technology. Hence, this technical manual gives oversight on the technology and infrastructure that would be helpful for the forestry experts at federal, regional, zonal and wereda level to increase the production and productivity of trees per unit area.

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1. Introduction

Deforestation is a widespread phenomenon at global scale, which declines of world’s forest resources and resulted in impoverished landscapes with respect to environmental, economic and aesthetical benefits (Davidson, 1989). In Ethiopian context, there are similar trends of deforestation and forest degradation because of proximate and underlying factors. The direct factors are extensive sectoral expansion of agriculture; infrastructure and investment which are the prominent; similarly the lack of harmonization between sectors policy issues and institutional weakness are also responsible for the continuous decline of forest resource. In order to overcome the effect of deforestation and degradation on climate change, climate resilient green economy is drawn as a master strategy of the country which is being implemented by different sectors through green economic pathway. In particular, the forest sector is one of the main pillar gives due emphasis on establishing new forest and sustainable management of the existing forest. In this regard, the national forest sector policy and strategy ensure the establishment of new forest and conserving the existing natural forest. The creations of new forestland give an opportunity for mitigation of the effect of climate change and contribute to growth of national GDP in realization of the dream to become middle income country by 2025. Among these policies and strategies the first and second growth and transformation plan, forest sector development programs have been implemented in line with the revised forest proclamation through creation plantations in afforestation and reforestation implemented by government institutions and national programs and projects.

At national scale; there are ample policy and strategies which have been drawn since the inception of Ministry of environment forestry and climate change in 2013; these policies, strategies and programs include a clear forest policy, forest proclamation national climate resilient green economy strategy, forest sector development program; revised forest proclamation, strategy of reducing emission from deforestation and degradation and growth and transformation plan 1&2 are the main which really bring immense change in the sectoral development of forestry. Forest sector in the CRGE strategy has national scope of establishing new and protecting the existing forest to meet the objectives of growth and transformation plan and realize the international commitment of restoration of 22 Mha of degraded land. Annually; it is planned to create new forest land of 0.6 ha by raising 4.27 billion seedlings. So far the reproduction of trees is done by means of seed which has its own evolved problem of poor quality seed system. Therefore, the introduction of new and effective technology of asexual reproduction in forest nursery establishment is crucial to increase scale of plantation in afforestation and to the extension of commercial plantation at large scale.
Hence, plantations are established using traditional way of using seed method. The employment of additional technology of asexual method inevitable for rising seedling which is the vigor, true to type, disease free and efficient way to meet the goal reaching up to 30% forest cover by 2030. Therefore; introducing the concept and methodology of asexual/vegetative production is prime important to produce millions of seedling which are healthy progeny, economically large number of seedlings. The technological transformation from seed bed preparation to root training infrastructure would have also additional advantages which avoids extra cost of seed bed preparation and enable maintaining of standard seedlings.

2. Role of Vegetative Propagation in Afforestation/Reforestation

Recent study and assessment of the forest resources indicated that the current forestry contribution to national GDP has grown from 8% to 12% which draws the attention of policy makers to give a due concern to the development of the forestry sector. The implementation of feasible policy and strategy that would really contribute towards the achievements targeted economic contribution in realization of green economic development. It is indicated that through the course of implementation the second growth and transformation plan; annually 4.27 billion healthy and standard seedlings are expected to be raised across the nation in all nurseries to be planted which maximum achievements on an area of 0.6 million hectare. The nurseries are managed by government as well as the projects and national programs; however; the production standard seeding materials with respect to appropriate quality is bottle neck for establishing seedlings to make new forest. In this regard the national mechanism of provision and regulation quality seeds in the supply and demand system is least organized and malfunctioned; hence the issue of provision of quality and standard of seedlings production become the prime factor for producing economically feasible seedlings to establish forest through afforestation and reforestation. Therefore, besides of seedling raising using quality seeds; role of vegetative propagation using clonal technology becomes important for implementation of successful plantation in afforestation and reforestation plan across the nation.

The existing forestry system has certain drawback in terms of producing vigorous and standard seedlings for massive plantation establishment in afforestation and reforestation that are being implemented by government which has low seedling establishment percentage and overcome the impact of existing situation of climate change. In this regard; the cost of producing a single vigor and standard seedling become an issue of cost effective activity for creation of new forests besides using to raise the seedling by seed or sexual method.
Hence, the application of asexual technologies would be inevitable to successful afforestation and reforestation program by piloting the technology in most of Ethiopian highland. Based on the recent assessment study done by forest sector development program; artificial plantation is expected to cover over one million hectare where the eucalyptus species account the highest proportion reaching up to 90% in the plantation. The role of vegetative mode of propagation in the forestry system especially producing seedlings by cutting and tissue need a great attention by decision makers to be well practiced. The use of seedling raining through cuttings for eucalyptus species are quite cost effective with respect to the economic, environmental and restoration perspective. It becomes the priority which supports the economic contribution of the forest sector and brings about sustainable plantations forests across the nation.

3. Objective of Employing Vegetative Propagation

The strategic objectives of using asexual/vegetative propagation of seedlings would emerge from the importance of establishing new forests and reforestation of degraded areas which comprehensively address the issues of policy, economic, social aspects of forest sector development. Hence besides the traditional way of establishing rising and establishing seedlings by seed method it is crucial to use additional technology of asexual method of seedling rising which is the genetically true to type, vigor, disease free and efficient way targeted to the achievements for reaching the anticipated forest cover to 30% by 2030. In this regard; asexual/vegetative propagation using the clonal technology has comprehensive impacts with respect;

- To increasing the knowledge gap of expertise at all levels of government institutional organization (research, university and projects)
- To promote the economic aspect of raising that are cheap and reliable in terms of producing vigor and healthy seedlings and enhance technical feasibility of establishing standard seedlings
- To increase and exploit the advantage of cloning technology includes the selection of desirable characteristics of cultivars for uniformity, fast growth, high yield and disease, pest and drought resistance.
- To extensively apply root training infrastructure which avoids the cost of nursery establishment
- To meet policy aspect the vast plantation establishment in national programs of afforestation and reforestation
- For Leveraging the resources from donors and private sectors to forestry sector
- For leveraging the technology of vegetative propagation and commercialization of forestry
4. Types of vegetative Propagation

In general, there are two broad categories of asexual reproduction for seedling production as macro and micro propagation where the former is broad category comprised cuttings, grafting, layers and offsets/apomixes that large number of genetically similar cultivars from the mother plant is produced while the latter is more technology requiring method of producing seedlings from tissue multiplication. Therefore, among the clonal technologies of seedling production cuttings and micro propagation or tissue culture is the core content of this technical manual. There are five main methods of vegetative propagation: cuttings, layering, budding, grafting and tissue culture. Propagation by cuttings is done by taking sections from the tree and manipulated to root and produce shoot in an appropriate medium. Propagation by grafting and budding involves fusing of scion from a tree that has the desired traits with the rootstock. Some of the benefits that could accrue from the application of vegetative propagation will be getting by multiplying ‘true-to-type’ elite material into millions of similar copy offspring.

The prime advantages of using asexual methods of plant propagation is employment on massive plantation materials or seedlings for afforestation and reforestation programs, it is evident that plants propagated by this method are true to type and uniform in growth, high yielding and form quality stands and fruits. Especially; while establishing agroforestry system some fruits such as banana, pineapple, seedless guava and seedless grape varieties can only be propagated through vegetative means. In the course of vegetative propagation of fruit tree the tree stands or orchards bear fruits earlier than seed propagated plants and assure the sustainable continuation of genetic configurations of the parents.

Plants produced by asexual propagation method also have manageable size and have uniform fruits making harvesting easy some diseases can be avoided in susceptible varieties by grafting them on a resistant rootstock. Another advantage of using asexual reproduction is described by the inferior quality crown of the existing plants can be improved. For example, side grafts and crown grafting in mango. It is possible to grow multiple varieties on the same plant. One can grow numerous varieties of roses and mangoes on different branches of the same stock. The number of plant per hectare is more due to its small canopy and restricted growth where the vegetative propagation helps for rapid multiplication using modern techniques like tissue culture and other micro propagation techniques.
5. Advantages of Using Asexual Propagation for Seedling Production

The practical use of vegetative propagation methods is based on two biological considerations and reasoning: the first and foremost reason is for keeping and maintenance of the physiological condition of the parent tree in the propagated part; maintenance of genetic constancy. That is, the part propagated is genetically identical to the original individual. The second important reason is the vegetative propagation is widely used in breeding. Literally, asexual propagation or reproduction of seedlings is done from any of vegetative parts of the original plant because every cell of the plant contains the genetic information necessary to regenerate into the entire plant. The reproduction resumes through the formation of adventitious roots and shoots or through the uniting of vegetative parts by grafting or budding. Through the course of propagation stem cuttings and layers have the ability to form adventitious roots, and root cuttings can regenerate a new shoot system. It is also possible for leaves to regenerate both new roots and new shoots while a stem and a root can be grafted together to form a single plant.

The concept of vegetative propagation is producing an exact copy of the genome of mother plant that is made and continued in new individuals. This is possible because plants, unlike animals or humans, have meristemtic, 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 is done by seeds provides opportunity for variation and

Figure 1 - Different types of propagation
evolutionary advancement of the offspring. Hence, vegetative propagation aims at creating the identical offspring/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. Among the most important vegetative propagation techniques for tree species are the propagation by cuttings, layering, budding, grafting and micro propagation. The reasons for importance of vegetative propagation would allow the offspring in maintaining superior genotypes; unlike the problem occurred in seed germination and avoids growth period by shortening time to flower and fruit; combining desirable characteristics of more than one genotype into single plant.

6. Disadvantages of Using Asexual Propagation for seedling production

There are some disadvantages of using asexual reproduction system while seedlings are raised by vegetative method. Literally; the seedlings which are raised using asexual method of reproduction often results plants that are not hardy and fall easy in performance to adverse conditions of soil, climate, diseases, pests, etc. The practical reason for this is not well articulated perhaps, it might be related to the narrow genetic diversity for adapting various environmental conditions. There are some difficult and more expensive methods of propagation in some plants like papaya, coconut, etc. Plants are generally not so vigorous and long lived as seedling plants and they require special skill for propagation. The other disadvantage is hybridization in these plants is not possible because there is no variation in the progeny; these methods are not suitable for development of a new variety. But the use of cutting and tissue culture in eucalyptus species is effective in establishing vigor and standard seedlings of true to type.

There is also another disadvantageous issue of using asexual reproduction method for seedling production is they are somehow capital intensive with regard to initial establishment of infrastructures. The method requires technological propagation infrastructures including green house, mist chamber with cooling pad, poly tunnels/screen house, tissue culture room with temperature and light control. The technical materials include root trainers (Composite/block/single cell), other containers, grafting machine, secateurs, cutting knife, scissors and vermiculite. Comparatively; having overcome the initial capital investment required for asexual method of seedling reproduction; especially for cutting and tissue culture technology it is possible to produce vigor seedlings which are true to type that are going to establish forest stand by increasing the productivity of a given tree species per unit area and improving the economic and environmental contribution of the plantation.
7. Production of seedlings through vegetative means

It was indicated in (Teshome Tessema, 2017) that the concept of vegetative propagation refers to the production of exact copy of the genome/individuals from a mother plant which is made and continued into new individuals. This is possible because plants have meristematic, undifferentiated cells that can differentiate to the various organs necessary to form a whole new plant (Teotônio, et. al., 2004). 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. There are different types of vegetative propagation-Macro and micro-propagation methods (Figure1)

7.2. Principles of Macro propagation by Cutting

In general, vegetative propagation involves the use of young or juvenile materials obtained from mother tree based on the superior phenotypic performance. Such superior genotypes could be faster growth, higher yield, resistance to pests, diseases. Therefore, a successful clonal program must have a sustainable source of supply of juvenile material from the mother garden or hedges. There are different types of macro propagation in the asexual method of seedling reproduction however; the reproduction of seedlings using cutting methods is the most important while we are thinking about massive establishment of plantation in afforestation and reforestation of eucalyptus species in large-scale industrial use. The asexual reproduction methods by cuttings are performed using a vegetative plant part, leaf, stem or root with at least one node, which is taken from the parent plant in order to regenerate itself, thereby it forms a whole new plant. In the course of performing vegetative propagation a single plant organs can be used for cuttings by stem, shoot, and root or leaf cuttings. Basically the cuttings are usually placed into a suitable rooting substrate and kept under high humidity until roots and shoots have formed as an out growth.

Plant propagation by cuttings can yield a high multiplication rate and produces plants with their own root system. Taking stem and shoot cuttings are the most common way to perform vegetative propagated seedlings and grow it into trees. The propagation process is conducted under small area. The most common species which have been successfully developed with shoot cuttings are T. grandis, P. merkusii, A. mangium, Shorea spp., S. macrophylla, Eucalyptus sp. and K. anthoteca; and with stem cuttings are P. canescens and Shorea spp.; and with root cuttings is D. latifolia.
There are four step or procedures in the process of making macro propagation by cutting; the first is preparation of the cutting part from the superior genotype mother tree using standard procedures and equipment; second the formation of shooting and rooting parts of the cutting materials using modern or local propagation mist chamber can also be constructed using locally available materials; third is the growing of seedling materials in green house for fast growth condition by manipulating the environmental factors and fourth and lastly using root trainers open air hardening using soil media. The local chamber can be constructed by external parts of the three dimensional propagator can be constructed using wooden frames. The infrastructures of asexual reproduction by cuttings include the green house facility. After mini-cuttings develop roots, they are transferred to greenhouse that is a less controlled environment as compared the mist propagation chamber, shoot development takes place with the growth reaching to plantable size.

7.2.1. Propagation of seedlings cutting

A cutting is a vegetative plant part which has at least one node taken from the parent plant in order to regenerate itself, thereby forming a whole new plant. Various plant organs can be used for cuttings: stem, root, rhizome fragments or leaf cuttings. Whereas the multiplication of plant parts by using the rooting of cuttings is probably the most common technique by which trees can be propagated vegetative way (Figure-2). Vegetative propagation is used in mass production of Eucalyptus seedlings for commercial purposes. Plant propagation by cuttings can yield a high multiplication rate and produces plants with their own root system.

Figure 2- Picture of stem and shot minincutting taken from eucalyptus species
The source of propagative materials is the mother garden which is established from plantlets of known phenotypic performance. Technically the clonal hedges are composed of individual stumps referred to as ramets. In the process of collecting propagative material a 6-year old rotation period is recommended to form ramets after which they should be replaced. Comparatively the young stumps or ramets produce better cuttings compared to older or mature stumps. The ramets are maintained by constant weeding and cleaning (removing unwanted shoots) Fertilizers are also recommended in ramets that more coppices will be expected from fertile sites compared to those that are infertile or have been degraded in one way or the other.

7.2.2. Steps in performing clonal technique

In the course of vegetative propagation, cutting with its selected tree germplasm are prepared from mother tree. Hence care should be taken with respect acquiring the right genetic quality while selecting planting stock for collecting cuttings from sources. The age of the stock plant should be carefully selected from which the propagating material is going to be taken and the proper conditions of the propagating material need also be considered to be (vigor and free of pest and disease damage). In stem cuttings the inherent capacity or regenerative capacity is crucial for successful propagation. A woody plant progresses property of growth stages as juvenility, maturity and senility. The potential asexual regenerative capacity during this cycle declines in a sigmoid curve fashion which is related to age. The highest level of this regenerative capacity occurs during the juvenile phase, declining only slightly as time progresses. At the onset of sexual maturity the capacity declines markedly and continues to decline steadily, until the senile phase is reached when this potential is of propagation is virtually lost. Thus, the inherent regenerating capacity varies significantly based on the type of species and is not the same for all woody plants.

a. Preparation by Cuttings.

Management of stock plants requires some important rules; the management of stock plants is to establish stock plants that 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. Moreover use fertilizer to accelerate growth in nutrient deficient soils.
a. Taking cuttings

The advantage of cloning depends on the selection of desirable characteristics of cultivars for uniformity, fast growth, high yield and disease, pest and drought resistance. Principally, cuttings should be taken early in the morning before the sun is hot, in order to resist transpiration and avoid drying out of the material. Procedurally, the leaves are trimmed and leave the shoot to reduce water-loss. Most often the sizes of leaf areas for optimum rooting vary with species. Therefore the use a polyethylene bag which moistened inside to carry the shoots. Keep the collected shoots under shade, without throwing or squeezing it in 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. Based on the prime objective of promotion of the asexual propagation of seedlings the eucalyptus species would be given due emphasis as it is highly applied to this species. This manual also considers propagation of cuttings is to be employed on eucalyptus species. Next the rooting process is triggered by plant hormones at the base of the cuttings. The presence of buds and leaves strongly affects rooting of stem cuttings the extent of rooting. During the rooting process there are factors affecting cloning these an optimal temperature determined using an alcohol thermometer under transparent 500 micron polthene next the light intensity to be reduced by 60% to 75% depending on the intensity of light in the area; next humidity to get an optimal humidity measured using a hydrometer; rooting media also another factor where the subsoil may be used on site. For the media use of vermiculite and cocoa peat is another option; the rooting hormone is used at 6% Hormoril or rootex (IBA) and finally the PH-optimal pH is maintained between 5.5 and 6.5.

Procedure for seedling production from mini-cuttings collected from stock plants

- Collect leaf branch cutting early in the morning 6:00- 7:00 AM, using clear polyethylene bags; spray water into cuttings to avoid water loss;
- Take to laboratory for further trimming to length ranging from 7 to 10 cm
- Subdivide the branches in to cutting that range from ca 7-10 cm long and diameter of ca 2-3mm.
- As cutting preparation progresses, spray the cuttings with water now and then.
- Hold a bunch of 5-7 cutting upside down b/n the fore and the middle fingers, and treat each cutting with just a drop of 0.2-0.4% IBA using a micropipette
- Immediately after applying the hormone ventilate the treated parts of the cutting manually or expose them for ca 60s to a cold stream of air generated by a pump
Cuttings were packed in clean polyethylene bags and were taken to the propagators to be inserted into the prepared rooting medium (sand) inserted in depth of ca 2-3 cm.

Maintain the Relative humidity of the propagator to within 75-80% by intermittent tap water sprays and optimal temp (ca 25-30°C) is critical for the initiation of root peimororida.

It takes 22 weeks, depending on the stock plant age.

The assessment on the survival, calloused, and rooted cuttings need to be made 4 weeks after inserting cuttings into the growing media and then every week during the subsequent periods.

7.2.3. Factors affecting the multiplication of cuttings

A. Rooting substrate
Literally, the 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 need to satisfy these requirements: it has the composition of soil, fine river sand, soil and river sand mixture, peat, compost, soil and compost mixture and vermiculite. In order to avoid pest and disease attacks, the substrates should be washed properly before use and sterilized. The media should be renewed at least once per year.

B. Humidity
As soon as a cutting 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 get wilted and dry out due to low humidity or not to become diseased because of a too high humidity. Water is an important external factor affecting the success of rooting of the cuttings.

C. Hormones
The plant hormones are of paramount importance to initiate the multiplication process. Certain hormones such as auxins (IBA, IAA, NAA) will influence root development, and others such as gibberellins will 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 cuttings and thus timing during taking of the cuttings is an important procedure to be followed in the cutting process.

D. 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 remained cutting. The initiation of roots in a cutting relies 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 the leaf area.

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 nurseries (electricity, additional light or complete darkness, heating cables in the rooting substrate).

7.2.4. Facilities required for producing seedlings from cuttings

Stock plant source
Stock plants or tree selected from genetically superior mother trees are considered as the stock plant source. So the stock plant is the vegetative source from which cuttings will be taken for mass production of seedlings. The source stock need to be established near the propagation area where the cuttings are being processed. Cuttings then will be collected, inserted into the root trainer or growing media and proceed to the sequential processes of seedling production.
Rooting chamber/mist chamber (controlled room for rooting of cuttings)

Mini-cuttings require high humidity and consistent temperatures to prevent excessive drying of young meristematic buds and at the same time enhancing root development during propagation. Hence they should be placed in appropriate growing media, with a root trainer with in a mist propagation chamber are kept (for few days Figure-3), and depending on the species till roots are developed. The mist propagation chamber is an area with controlled environmental conditions mainly temperature, moisture and air movement or wind. The propagation chamber can be constructed the same way modern tissue culture laboratory growth rooms are built and equipped with modern temperature and humidity control units (Figure-3). It can also be constructed using locally available materials, like what is produced for Juniperus procera.

Figure 3-Mist propagation chamber (rooting house) for cutting
Construction of the propagators (growing media) using locally available materials.

External parts of the three dimensional propagator can be constructed using wooden frames. The procedures are as follow:

- Cover the wooden frames with clear, colorless pieces of plastic sheets.
- Partition it internally in to six equal but self-contained compartments
- Overlay each compartment with double – layered plastic sheet such that the entire unit compartment will be watertight,
- Layer the bases of each compartment successively with stones (6-10cm), small stones (3-6cm) and gravel.
- Cover the remaining top most layers of the compartments with clean sand, sieved using 1mm mesh-sieve, and washed in running tap water to remove mud and debris,
- Erect a piece of plastic pipe, diameter 3 cm and length 40cm, in the front right corner of each compartment.
- The level of water in each compartment then will be maintained at a height of 20cm from the bottom of the compartment,
- Cover layer with clear sand with a depth of 5-7cm to serve as rooting medium for the treated cutting
- Keep all the propagators under 1.5m high wooden beds, cover them with leaves of phonex reclinata or other similar cover for obtaining up to 80% interception of direct sunlight

Green house

After mini-cuttings develop roots, they are transferred to greenhouse (Figure-4) that is a less controlled environment as compared the mist propagation chamber, for shoot development and further growth up to reaching plan table size. The green house facilities are capital incentive investment which is useful for large scale asexual reproduction of seedlings. Its climatic conditions are manipulated with respect to the temperature and humidity for the better shoot growth. These infrastructures are implemented in the regions through piloting in weredas in line with the seed production methods.
Hardening bench in the open air

After the greenhouse shoot development the seedlings are transferred into root trainer, in the due process of time after they reach into palatable size, seedlings are exposed to open air by transferring them from the green house and fixing them in a nursery bench (Figure-5) for hardening purpose.

Figure 4- Green house for growing rooted cuttings in to a full sized seedling

Figure 5- Hardening bench in the open air
8. Principle and Methods of Micro propagation

Micro-propagation is another method of asexual reproduction of vegetative propagation under in vitro conditions wherein plants are propagated using miniature plant tissues called explants grown aseptically in test tube or other container. Micro-propagation offers a rapid means for producing planting stock on a mass scale from a single nodal explant or seed or callus raised from explants. Micro-propagation has the advantages of small space requirement, high multiplication rate, freedom from seasonal influences and freedom from microbes. In micro-propagation, nodal shoot segments or shoot tips are exploited to form multiple shoots on appropriate nutrient medium. The growth controls that operate in an intact plant can be broken down or eliminated under in vitro conditions, leading to profuse production of shoots from a single initial explant (Figure-6).

Propagating trees using micro-propagation technique initially requires high investment, in terms of equipment and training. Therefore micro propagation only used for high value tree crops which have commercial importance. Micro propagation covers a wide range of methods and techniques to vegetative propagate relatively small parts of plant material in extremely controlled environments. In this method, all forms of tissue culture and micro propagation are combined. The characteristic of these techniques is that plants are developed from single cells or tissue, which is grown in aseptic culture media. Micro propagation allows a very high multiplication rate; from a single plant thousands of new ‘daughter’ plants can be produced. Tissue culture offers enormous potential in producing large quantities of genetically uniform proliferates of the desired material in a short time frame.

During the process very small pieces of a tree are washed to be free of all bacteria or fungi, then it is grown on a special nutrient medium in sterile culture and encouraged to produce many plantlets. Then the each plant material is then potted, grown on, weaned and hardened. However, it is essential that enough care is taken in selection of the initial material, production of the plants, nursery development and field plantation. Even though this is not a common vegetative propagation technique, it is important to be familiar with the overall concepts and principles, as to understand why this method can be considered in the broader context of plant propagation and tree domestication and multiplication.
The shoots can be separated and rooted to give rise to entire plantlet. Generally, micropropagation is approached in three ways-enhanced axillary bud break, adventitious bud differentiation and somatic embryogenesis. Regeneration from fascicular meristems has been achieved from juveniles as well as mature tissues in many species. Propagation via adventitious meristems involves the induction of unipolar shoots on explants followed by shoot excision and induction of root meristem.

8.1. General procedures of Tissue culture preparation

The collection and preparation of ex-plants is by taking the 'micro-cuttings include small pieces of tissue are taken from the selected tree with a sharp blade, and thoroughly washed in a disinfectant; Success has usually been achieved with strongly juvenile material, such as parts of newly germinated seedlings, but small, active buds and even flowering parts have also been used. Using the sterile-air bench and sterilized tools, a tube is opened and a single piece of tree tissue planted on the middle of the slope. The capacity for propagation by in vitro culture is limited by the establishment and maintenance of the tissue in an appropriate condition to induce the rapid division and subsequent differentiation of cells. Hence; the ex-plant must be kept in sterile conditions, and provided with macro- and micronutrients; a source of energy, usually sucrose; vitamins, amino-acids; the correct balance and sequence of plant growth regulators.

The techniques of preparing the media considers as there are several different media which have given good results which include; distilled water and agar are added, together with specific quantities of the plant growth regulators (hormones) that determine whether shoots or roots are formed. After autoclaving to kill spores of micro-organisms the medium is poured into sterile tubes on the sterile-air bench, closed with cotton-wool, and allowed to set at an angle, producing a sloping surface. The Shoot multiplication follows the aim of the first stage is to encourage the culture to form many small shoots. This may take a few weeks, or longer. The next step is sub-culturing which includes; once the shoots on the 'micro-stock plant' can be handled, they should be transferred to a fresh tube containing a medium that will promote rooting. Shoot/root formation is generally dependent on the cytokinin/auxin ratio in the nutrient medium. The rooting process include Transfer of plantlets to soil; Weaning; Re-potting; Hardening; Grading of seedlings for planting and Dispatching and transporting seedlings.
8.2. Selection of plant material to be propagated

The newly growing shoots have meristematic regions under favorable environmental conditions can grow in to plantlets. The age of the cultivar determines its potential and relative regenerative capacity. Secondly, the ability to recover regenerative capacity can be improved by manipulation of the parent plant, for instance by harvesting old parts and using propagules from new coppices.

8.3. Collection and preparation of ex-plants

Micro-cuttings are small pieces of tissue that are taken from the selected tree with use of a sharp blade and thoroughly washed in a disinfectant such as hydrogen peroxide (H2O2) or a solution of hypochlorite. Cuttings should be taken with strongly juvenile material, such as parts of newly germinated seedlings, but small, active buds and even flowering parts can be used. Setting the micro-cuttings is prepared by using the sterile-air bench and a sterilized tool with a tube is opened and a single piece of tree tissue planted on the middle of the slope. A little sterile water is added, the tube closed again and placed under the lights.

8.4. Preparation of growth media

The capacity for propagation by in vitro culture is limited by the establishment and maintenance of the tissue in an appropriate condition to induce the rapid division and subsequent differentiation of cells. The ex-plant must be kept in sterile conditions, and provided with (a) macro- and micronutrients, (b) a source of energy, usually sucrose, (c) vitamins, amino-acids, etc, and (d) the correct balance and sequence of plant growth regulators, co-factors, etc, to regulate the subcellular and cellular processes of cell division and differentiation of shoot, root, or embryo. Success will also depend on the osmotic pressure and pH of the medium, which can be a solid or liquid, and the physical environment.

8.5. Preparing the media:

There are several different media which have given good results and the basic chemicals can be obtained ready mixed. Distilled water and agar are added, together with specific quantities of the plant growth regulators (hormones) that determine whether shoots or roots are formed. (Note: Cytokinins tend to stimulate shoots, while auxins promote roots; but both are often needed). After autoclaving - to kill spores of micro-organisms - the medium is poured into
sterile tubes on the sterile-air bench, closed with cotton-wool, and allowed to set at an angle, producing a sloping surface. [Note: some heat-sensitive hormones must be added after heating, using a sterile syringe.

8.6 Multiplication and transplanting

Shoot multiplication. The aim of the first stage is to encourage the culture to form many small shoots (Figure-6). This may take a few weeks, or longer. Sub-culturing Once the shoots on the ‘micro-stockplant’ can be handled, they should be transferred to a fresh tube containing a medium that will promote rooting. The ability of plant tissues to form adventitious roots depends on the interaction of many different endogenous and exogenous factors. Skoog and Miller (1957) reported that the shoot/root formation is generally dependent on the cytokinin/auxin ratio in the nutrient medium. Jones (1978) demonstrated that the cytokinin inhibit rooting and also prevent the root growth. Therefore, it is better to culture the shoots on a PGR free medium before they are transferred to a rooting medium. If shoot multiplication is slow, the whole culture will need transferring to a new tube each month.

Rooting. In the second stage, each shoot forms a root system.
Transfer of plantlets to soil. The new plantlets need to be placed carefully into a sterilised potting soil in small pots, covered with a polythene bag and allowed to grow larger under the same conditions as the cultures. (Note: At this stage they are still very delicate, and need ‘intensive care)

Weaning The established plantlets need to be gradually accustomed to less humid conditions and somewhat brighter lighting. It is easy to lose them if this stage is done too rapidly, particularly when they are taken to a shaded nursery bed. However, if weaning is too slow the shoot may become drawn and weak.

Re-potting During the nursery part of weaning, transfer the plants carefully to larger pots, using unsterilized soil. The inoculum for mycorrhizas or nodules can be added to the potting mixture.

Hardening For light-demanding species, the shading can be gradually reduced after the plants have been fully weaned.
9. Summary and Practical applicability of vegetative propagation

The introduction of vegetative technology in Ethiopia is believed to have positive impact for the development of forest sector for successful accomplishment of plantations in afforestation and reforestation. The forest sector has five term plan with activities to meet goals set by the end of second growth and transformation plan. The commercialization of forest sector with the industrialization of forest products require the creation new plantations using seedling production system which does not only make the afforestation program successful; besides the use of vegetative propagation of seedlings is important to form forest stands that are true to type and vigorous. The use of vegetative propagation of seedlings production would increase the percentage of seedling establishment in woodlots and commercial plantation. The practical applicability of clonal technology in Ethiopia depends on the technical feasibility of employing the technology in some pilot areas using the nursery sites where eucalyptus are dominant; probably in most of highland areas; the technology may be introduced in the pilot form in some
selected project sites of forest sector development program, research sites, universities. The introduction of the technology may be started through piloting of capacity building and infrastructure development which are capital intensive operation. Piloting may be done in the existing operational districts/woreda of the project sites. The capacity building refers on the training of expertise working at all levels federal to regional and werdea for effective implementation of the technology and infrastructure. Once the activities piloted in the projects sites have become feasible and acceptable by private and community forestry developers it would be introduced to large scale in government institution for nursery establishment.

Therefore; the strategic institutional unit for feasible implementation of piloting the project is woreda level. For instance the forest sector development program can take the initiative with the capacity building through training and establishing the infrastructures of greenhouse, mist chambers and root trainers' facilities. The implementation of pilot project activities require huge amount of money for initial establishment of the clonal facilities and relevant infrastructures including root training facilities. Similarly, the REED+ project would take the start up through piloting in some project woredas as strategic unit. Once the technology is transferred through capacity building and infrastructure establishment on project nursery site it then transferred to nearby selected government institutions where there are nursery sites and plantations. More over; the starting of asexual propagation methods in the existing forest enterprises which are actively working in Oromia and amahara regions are also be entry points to introduce the technology where both sexual and asexual methods proportionally implemented for seeding production with the customization of relative advantages and disadvantages.
10. References
