CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Institute of Tropics and Subtropics

Department of Crop Sciences and Agroforestry

Vegetative propagation of tropical trees

Bachelor thesis

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Declaration

I declare that I have elaborated my thesis independently and quoted only quotations listed in the references.

Prague, May 6, 2012

Michaela Jedličková

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Abstract

To domesticate tree species, it is important to find a way of vegetative propagation. The phenotype of tree propagated by seed is unpredictable and generative period is too long. In case of vegetative propagation, it is unnecessary to wait till the production of seeds. There are 5 ways of tree vegetative propagation, which are cuttings, grafting and budding, layering, root suckers and micropropagation. The aim of the thesis was to describe those techniques and to review the experiments done with 12 species preferred in Peruvian Amazonia. But there is not reported the way of vegetative propagation of all 12 tree species, just 7 of them. Most of them were propagated by cuttings and *in vitro*. WPM medium and ½ and ¾ MS medium with some supplements was tested successfully. Softwood, semi-hardwood, hardwood cuttings and air-layering was made with IBA successfully, too. It is also possible to construct artificial seeds *in vitro*. It was successfully tested on some species. But there exists some unsuccessful experiments. To find out the best method of vegetative propagation is needed to make further research on every species.

Key words: vegetative propagation, cuttings, grafting, layering, *in vitro*, tropical trees

Abstrakt

Abychom mohli domestikovat různé druhy stromů, je potřeba nejdříve nalézt způsob, jak je rozmnožit vegetativně. Fenotyp stromu rozmnoženého semenem je nepředvídatelný a generativní doba je moc dlouhá. V případě použití vegetativního rozmnožování není nutné čekat na vytvoření semen. Existuje 5 způsobů vegetativního množení stromů, je to řízkování, roubování a očkování, hřížení, kořenové výhonky a mikropropagace. Cílem této práce bylo popsat tyto jednotlivé techniky a shrnout experimenty udělané s 12 druhy stromů preferovanými v Peruánské Amazonii. Ale nebyl oznámen způsob vegetativního rozmnožování všech 12 stromů, ale jen 7 z nich. Většina byla rozmnožena řízkováním nebo *in vitro*. WPM médium a ½ a ¾ silné MS médium s doplňky bylo úspěšně použito. Bylinné, polodřevité, dřevité řízky a hřížení bylo úspěšně použito za pomoci IBA. Také je možné vytvořit *in vitro* umělá semena. Tento způsob byl úspěšně testován na několika druzích. Ale také existují neúspěšné experimenty. K zjištění nejlepší metody vegetativního rozmnožení zvolených stromů je potřeba provedení dalšího výzkumu.

Klíčová slova: vegetativní rozmnožování, řízkování, roubování, hřížení, *in vitro*, tropické stromy

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1. Introduction

The tropical region is situated between the Tropic of Cancer (23° 26’ latitude north) and the Tropic of Capricorn (23° 26’ latitude south) with the equator in the centre. The term “tropics” is derived from Greek meaning “turning”. It used to be used in astronomy to indicate the southern and northern limits of the ecliptic, where the sun appears to turn after reaching these limits (Soanes and Stevenson, 2006). Tropical climate is influenced by many factors which make it very diverse, but we can generalize, that the annual sum of temperatures is higher than elsewhere. At the equator, photoperiod is about 12 hours and it varies very little. The increase in difference between the longest and shortest day at low latitudes is about 7 minutes per degree (Paull and Duarte, 2011). The majority of trees is propagated by seeds here, because sustainable way of vegetative propagation of local species is not found yet and these species are not domesticated. Phenotype of generatively propagated trees is unpredictable. Domestication of indigenous tree species is high priority in tropics. It is partly because wild varieties are not as profitable as domesticated cultivars with desirable attributes. Another reason is that cultivation of domesticated trees can decrease deforestation of wild ones. Vegetative propagation is simple pathways to domestication and it can provide the exact characteristics farmers and markets demand. Vegetatively propagated trees can also be used to reforestation.

The Ucayali region in Peru is an important source of timber, so the deforestation there is high. Most of its production is consumed domestically. However, local people of Ucayali benefit little (White *et al*, 2005). In this region, a research was made to explore priority tree species among local farmers. It is important to find out, how these trees can be propagated vegetatively, but techniques of veg. propagation were reported only for 7 of them.

2. Literature review

2.1 Propagation

Domestication is a pathway to improve the quality and productivity of priority trees. First of all, in developing a domestication strategy for certain tree species, we decide if we use the vegetative or generative propagation (Akinnifesi *et al*, 2008). Vegetative or asexual propagation involves mitotic cell division. It consists in removing off a part of plant and induction of adventitious root or shoots formation. Genetically, new plant is identical as the donor plant. This process is possible thanks to totipotency[1] of plants. Generative or sexual propagation of plants involves male and female reproductive organs, reciprocal meiotic cell division and finally the creation of progeny with genetic information different from parents. Most woody plants are highly heterozygous[2]. It is a cause of relatively high amount of genetic variation. Sexual reproduction is the propagation by seed (Scianna *et al*, 2001). However, the lack of genetically superior seed sources is very often (Akinnifesi *et al*, 2008) and highly variable plant material cannot be used on large orchards if we want high yield and consistent quality, which is requested by market. Some aims are achieved by selection of superior wild trees and following vegetative propagation. The breeding programme shows results too. It consists in sowing of numerous seeds and comparing their quality with their parents. In many cases, superior mutants or special characteristics are found. The retention of these characteristics is made by vegetative propagation and creates new cultivars or varieties (Paull and Duarte, 2011). Generally, foresters use seed-based tree breeding. On the contrary, horticulturalists adopted clonal vegetative propagation and the development of cultivars (Akinnifesi *et al*, 2008).

2.2 Methods of vegetative propagation

There are several reasons why and in which situation to use vegetative propagation (Akinnifesi *et al*, 2008)

- In a wild population, combination of desirable traits is rare.
- Clones offer market specifications as high uniformity of products.
- The species to be propagated is a shy seeder.[3]

[1] Ability of a cell to give a rise to any cell type (Soanes and Stevenson, 2006)
[2] A heterozygous individual have two different alleles of a particular gene or genes. (Soanes and Stevenson, 2006)
[3] It does not flower and fruit every year or produces only a very small seed crop (Akkinnifesi *et al*, 2008)

- The propagation material is limited.
- The propagation material is sterile. This happens especially to hybrids.
- Quick multiply of superior material (trees have a long juvenile period to wait to see the superiority).
- The seeds have a short period of viability.
- Farmers do not have time or knowledge to use the breeding programme
- Cuttings from a mature tree are mature too. They can flower even in the nursery. This is advantage meanly in fruit business

Veg. propagation has some disadvantages, too (Akinnifesi *et al*, 2008)

- Many diseases are transmitted by clonal propagation and new disease spread on every clone. Planting of different cultivars together minimise that risk.
- Clonal trees have a much weaker root system. They are expected to produce fruit rather than wood.
- Cloning is expensive compared to production of seedlings

The choice between juvenile and mature tissue as donor is very important. The biggest advantage of mature tree is that it already demonstrated its qualities, but there are disadvantages as well. Propagation by mature stem cuttings is very difficult. On contrary, propagate a juvenile tissue is easy. Mature tissue is capable of reproductive process, so it will flower and fruit soon. (Juvenile tissues flower after 3 to 8 years.) This effect is useful in case of fruit trees, which became profitable sooner. Plants propagated from mature tissues will have a lower stature. It is also useful in case of fruit trees but not in case of timber trees. If the treetops are smaller, we can plant more trees on hectare and the yield on hectare will be bigger. And lower fruit trees do not use energy to be higher, but to create fruits. On the other hand, timber production requires the vigour and form associated with juvenile trees. So for timber trees, juvenile tissue is used. The sources of juvenile tissues are seedlings, coppice shoots and root suckers. The advantage of coppice shoots from the stumps of felled timber trees is that these trees have proven to be superior. Seedlings offer more genetic diversity for screening. However, the most important is good rooting ability (Akinnifesi *et al*, 2008).

There are several methods of vegetative propagation:

- Cuttings

- Grafting and budding
- Root suckers
- Layering
- Micropropagation

For every method, we need sterile and sharp knife to cut the wood, not to split it (Longman, 1993).

2.2.1 Cuttings

Two types of cuttings can be used to multiply the plant material: (i) leafy stem cuttings and (ii) leafless stem cuttings.

Leafy stem cuttings

Leafy stem cuttings are also called greenwood, softwood or summer cuttings. More than 80% of tropical trees (so far tested) can be rooted as leafy stem cuttings (Longman, 1993). The rooting of softwood cuttings depend on the presence of a leaf. Cuttings without a leaf die very quickly. Rooting ability is maximised when cutting is photosynthetically active and produces assimilates and when the leaf is not suffering water stress (Leakey, 2004). Loss of water is minimized by trimming one third to one half of leave (Horynová *et al*, 1969). It is shown on figure 1. It is important to keep cuttings in a poly-propagator or under mist until they root. It prevents them from dry out (Longman, 1993). Softwood cuttings consist of succulent and new growth tissue and they are gathered from early to late summer. True softwood cuttings generally produce adventitious roots faster and have higher rooting ability than the second type called hardwood cuttings (Scianna *et al*, 2011). Softwood cuttings need very high air humidity, moderately low intensity of light, equable temperature and protection from wind, heavy raindrops, diseases and pests. All of these conditions can be proved by mist or poly-propagator, although it is important to prevent the dry out anyway. Evaporation from leaves and stems of unprotected leafy cuttings is high even in the humid tropics and at first, the cuttings have no root system, so water uptake is slow. We can keep cuttings from drying out by shading them, keeping them moist with a hand–sprayer (even while preparation) and trimming the leaves to limit the evaporation. In any case, conditions should be checked regularly (Longman, 1993). The use of growth regulators is appropriated to higher the rooting ability. They are sold like a powder or liquids (Horynová *et al*, 1969). Few millimetres of the cuttings could be put in the powder or the liquid form could be dissolved in alcohol and the cut quickly dipped in.

It could be dissolve at water too, but the cuttings need to stand in it for more than 4 h (Longman, 1993). The summer cuttings should be long around 10 cm. The part coming to the ground should not include any leaves and the cut at an angle is taken just under the bud (Horynová *et al*, 1969).

Figure 1, Adjustment of leafy cutting (Nečas, 2004)

The use of propagators

Poly-propagator (figure 2) is actually a simple wooden frame covered by clear or white strong polyethylene sheeting. It contains a reserve of water under a moist rooting medium. As mentioned before, the poly-propagator should be uniformly shaded. Temperature varies here between 28-30 °C. In hot dry zones, frequent watering can reduce high temperatures. The frame should be made of durable wood. It is better when the parts resting on the ground are termite resistant. Alternatively, we can treat the wood frame with preservative, which does not damage the vegetative material. The stones under the rooting medium should be from 30 to 120 mm big, gravel from 5 to 10 mm and all that material should be washed before use. The base of poly-propagator is made of a double piece of polyethylene covered by sand to prevent piercing or stretching of polythene. Sand should be spread on the ground and under the rooting medium too. We need to make sure that propagator stands horizontal because of the water level. To control and refill it without soaking the rooting medium, we put a piece of plastic pipe or bamboo (25-30 cm long and about 5 cm in diameter) in the corner. All should be add carefully without piercing the sheet. Holes in it markedly reduce humidity (Jaenicke and Beniest, 2002).

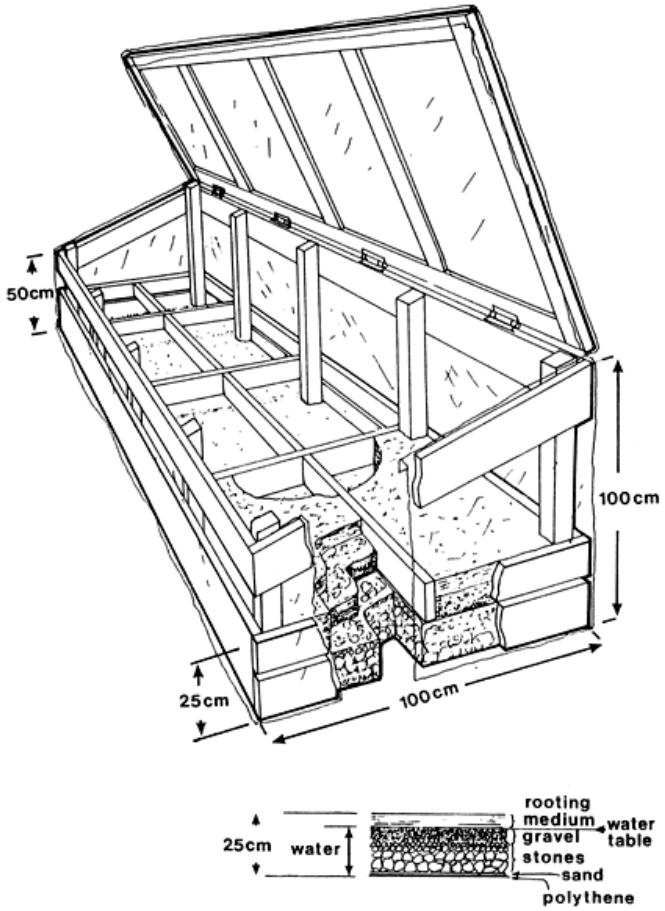

Figure 2, poly-propagator 1x3x1 m (Longman, 1993)

In mist propagator, cuttings are set in well-drained rooting medium. It is repeatedly moistened by fine mist. This mist is produced by water at high pressure which passes through special mist jets. Above the whole propagating area should be a plastic shadecloth offering 50-70 % shade. The mist is produced by water passing under pressure through mist jets. Mist jets are very small holes. To prevent block of the holes with dirt, the mist jets should consist a filtration system (Longman, 1993). High-pressure mist with a fine fog is better than low pressure systems with larger drops. It provides more complete coverage (Felker, 2008). A complete system consist also a mist controller with a timer that allows an arrangement of the length of burst of mist (2-15 s) and the frequency of bursts (every 2-60 min). The possibility to set different regime for day and night is needed too. The approximate times to start are: 6:30-18:30 – 10 s bursts every 4 min and 18:30-6:30 – 5 s bursts every 45 min. Too much water can make the cuttings rot but it is needed to keep some water drops on leaves (Longman, 1993). To simplify work, we can track evaporative demand. Best way is a non-mechanical method to prevent breaks down. We can use for example a system in which frequency between mists is proportional to the light intensity.

Mist propagator needs piped water and electricity. That makes it unacceptable for developing countries. But these problems were exceeded by electrical system connect to truck battery permanently charged by solar panel and by connection to water reservoir (figure 3). This system is capable of operating a mist bench (2,2 x 2,2 m) at high-pressure for about 8 h (Felker, 2008).

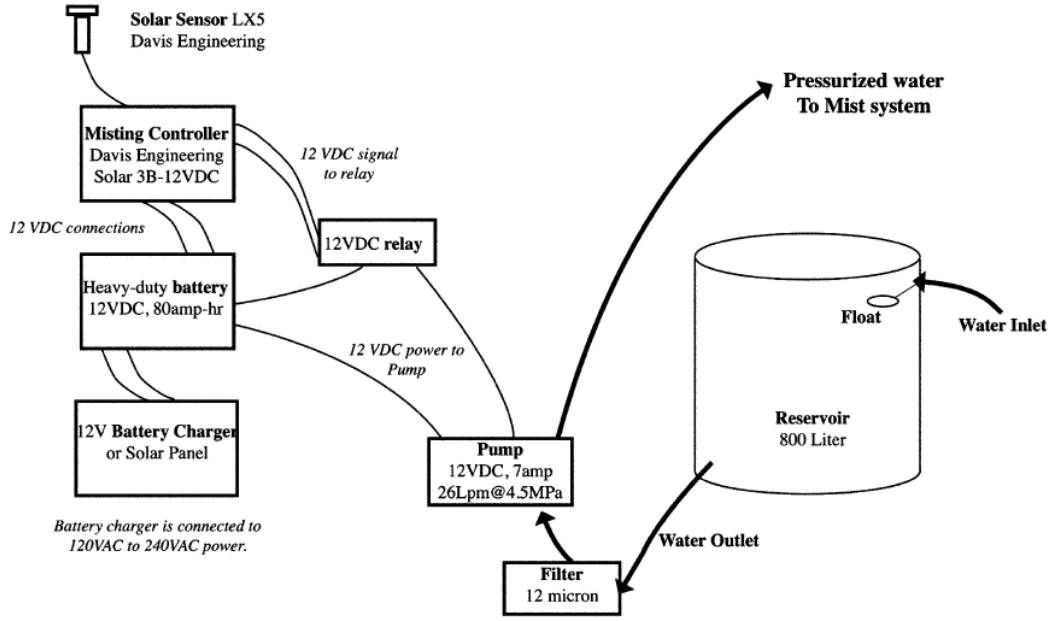

Figure 3, Schematic diagram for a light intensity controlled, high-pressure mist system with DC backup power for rooting cuttings (Felker, 2008)

Which is the best method depends on environment. Poly-propagators are cheaper, easy to build, do not need piped water under pressure (this point is very important in tropics). They could be used either for small or large scale propagation. Their construction and management is not hard even for beginners. But some species root better when they are covered with drops of water. That is a case to use mist propagator. It is also needed for research at well-equipped centres (Longman, 1993).

Leafless stem cuttings

Hardwood or leafless stem cuttings dry up very slowly. They can survive in moist soil. It is big advantage, because we do not need any special expensive equipment. But we need plenty of parent plants. Hardwood cuttings are taken before the coming of the first frost. They should have all buds inactive. Annual mature and not to thick shoot is cut. It can be stored in deep hot-bed or in river sand in cellar or it can be used directly. The cuttings should be from 10 to 20 cm long. Top is cut away 1 cm above the bud and lower part is cut away right under the bud (Horynová *et al*, 1969). These cuttings are planted at

an angle (it increase rooting) to the highest bud. Care consists in weeding out, watering and aerating (Nečas, 2004). Sometimes we can create live fences and live poles by planting 1-2 m long poles that will take root and produce new shoots (Longman, 1993).

Factors affecting rooting process

One of the most important aspects is determination of a rooting substrate. Most tropical trees require a light medium without water logging. Satisfying substrates are for example fine river sand, gravel, vermiculite, even rotted saw dust. We can use some mixtures of enumerated substrates: river sand and saw dust (1:1) and gravel with saw dust (1:1). Before use, substrate should be washed properly if not sterilized (Jaenicke and Beniest, 2002).

After cutting a shoot from a plant, it is not capable of taking up the water, so it is essential to ensure high humidity to prevent withering. But too high humidity can cause rotting. Water is very important factor affecting viability of cuttings (Jaenicke and Beniest, 2002).

Plant hormones affect rooting ability, some decrease it others increase. (See chapter about micropropagation)

Light and temperature also influence the rooting process. Tropical trees are habituated to 12 hours of photoperiod. Change of day length and irradiance affect rooting too, but every species is different. Right conditions can be found out only by experimentation (Jaenicke and Beniest, 2002).

Potting

After successful rooting of cuttings, we can pot every plant into its own container. In this phase, we can lose many plantlets. Right substrate (light but nutrient-rich), shade and humidity are essential to their survival. Firstly, carefully wet the soil, because it allows easy removal of plants. Plant should be lift with a flat piece of wood to protect roots. Shake of the rooting substrate. For potting, we select only healthy strong plants. If roots are too long, they can be pruned with a sharp and clean knife. Then, place the cutting into a container already partly filled with substrate, cover the roots with substrate, press it around the cutting and water. Place the container in a humid, well-shaded environment until shoot growth commence. Watering should be done with a sprayer with fine drops of water (Jaenicke and Beniest, 2002).

2.2.2 Grafting and budding

These are vegetative propagation techniques in which a part of cultivar is attached to a rootstock plant. In budding, single bud is used; in grafting we use a whole stem as a scion (Elam, 1997). The scion is attached to the rootstock in way that the two parts unite and grow together. The rootstock provides the lower part as roots and a short part of the trunk. The scion grows to become the rest of the trunk and branches. The most important thing is to unite the rootstock with the scion successfully. Healing process in a plant stars with a formation of callus or scar tissue. It is formed by cambium, thin layer of cells between the bark and wood. Annual growth is originated there. This means that the cambium of scion and rootstock must be in contact as on figure 4 (Lewis and Alexander, 2008).

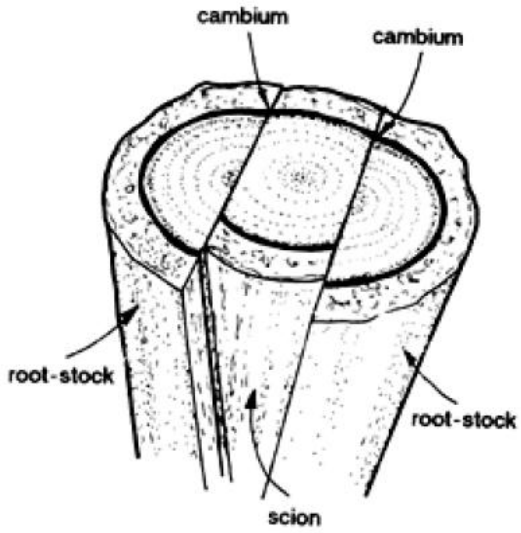

Figure 4, Contact of cambiums (Longman, 1993)

Only closely related plants or plant of the same species offers the compatibility between rootstock and scion. Often, seedlings are used as rootstocks. They are cheap, have better root system and the production is easy. However, because of the genetic variability, rootstocks are also vegetatively propagated. For many trees, rootstock is selected for its resistance or tolerance to the environment or diseases. The scion is selected for the superiority in production of major product. It is important to ensure to choose healthy plants (Elam, 1997). In budding, the advantage is that less scion wood is used and this operation is quicker than grafting (Lewis and Alexander, 2008).

Best time to graft or bud is the spring or autumn. It is the time when the bark is easily separated from the wood. But we must plan it late enough so that the bud will not

begin to grow and that the callus does not grow too much so it would cover the bud itself. Another important aspect is weather. Warm weather ensures a good union of the scion and rootstock (Elam, 1997).

Whip grafting

This is the best grafting technique for small-diameter. Whop grafts allow the plan to develop more rapidly than the budding thanks to the use of more scion wood (Elam, 1997). This grafting is easy to do when the diameter of scion and rootstock matches. So, we should choose straight part of the rootstock about the same diameter as the scion. Than make a sloping cut right through the rootstock shoot and another one through the scion. One cut should match to the other one as on figure 5. Surfaces of the cuts have to be as flat as possible to ensure good contact of their cambial regions. Clump two parts together and firmly wrap it with budding tape, starting from below and finishing above the join. Budding tape is made of plastic without any glue. It is tie on by slipping the end under the last turn and pulling tight. Scion must be protected from drying until the union is complete by mist, shading or covering it with a plastic bag (Lewis and Alexander, 2008).

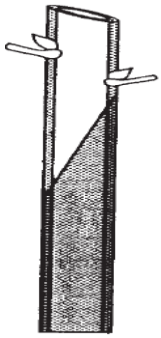

Figure 5, Complete whip graft (Lewis and Alexander, 2008)

Wedge or cleft grafting

This type of graft is named for the shapes of the cuts made. We make the cleft to the rootstock and the scion is shaped like a wedge. This type is a good choice for species that callus easily. As before, it is the best to find the same diameter of shoots and to choose the straight ones. After cutting them, we make a cut straight down the middle of the rootstock shoot. The depth of it should be about three times the diameter of the scion. The base of the scion is cut to a long wedge and insert into the slit in the rootstock. The top of the wedge is leaved exposed. It serves as a source of callus to help heal the top of the rootstock. If we work with different diameters, the cambial areas of scion and rootstock

have to match as on figure 6. As before, we use the budding tape and plastic sac until the unification (Lewis and Alexander, 2008).

Figure 6, Complete wedge or cleft graft (Lewis and Alexander, 2008)

Whip and tongue grafting

It is simple whip graft with addition of a tongue to help hold the graft in position. This makes it easier to tie (Lewis and Alexander, 2008). Firstly, we make long sloping cuts on the rootstock and on the scion like with simple whip graft. Than we make a matching tongue on both parts (figure 7). After, we tie the wound up and protect the scion against the dry out (Elam, 1997).

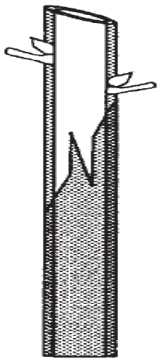

Figure 7, Complete whip and tongue graft (Lewis and Alexander, 2008)

Bark grafting

This technique is the best for large-diameter trees or branches. The rootstock must be freshly cut. Than we cut vertical slits about 6 cm long through the bark. There should be space left between these slits about 10 cm. After, the sloping cut on the scions are made, we insert the scion into the slit with cut surface of the scion facing the wood as on figure 8 (Elam, 1997). Insert must be done carefully to do not destroy the cambial cells. We fix the scions by tree tape and prevent the dry out with plastic bag (Lewis and Alexander, 2008).

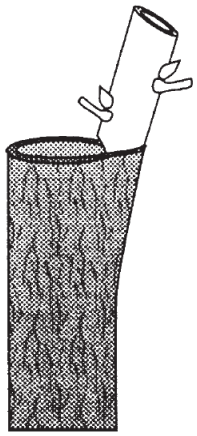

Figure 8, Complete bark graft (Lewis and Alexander, 2008)

Approach grafting

For approach graft, we need two complete plants with its own shoot and root system. This technique is the best for plants difficult to graft, because both plants stay intact until they are successfully healed and longer grafting cuts offer better chance to unify rootstock with scion. Firstly, we select long straight plants, one as rootstock and the other as scion. The best is to plant them close. We cut all shoots and leaves in way. Next, we cut a strip up to 30 cm long from the side of the rootstock and the scion. It should be about two-thirds the width of shoot. Finally, we bind it together with grafting tape. After healing process is complete, we cut the base of scion and the top of rootstock and remove the tape (Lewis and Alexander, 2008).

T-budding

We make T-shaped cut on the rootstock about 30 cm above the ground avoiding any buds. We cut the bark bud not the wood. The vertical part should consume the part of bud wood under the bud, so the length is around 3 cm. Horizontal cut is long as one-third of the circumference. Bud is horizontally cut through the bark and into the wood. This piece is long 4 cm with the bud situated in upper third. This bud is gently sliced in the t-shaped cut. It is important to put it in rightly oriented. Then wrap it with the grafting tape. Leave the bud exposed and do not cover it with any wax or sealant. After the bud has healed, the tape is unwrapped and remaining shoots of rootstock are cut 30 cm above the bud. This nurse branch protects the bud. After the bud wood has grown a few leaves, the nurse branch is cut just above the bud union (Elam, 1997).

Chip budding

If the bark does not lift or slip easily from the wood, it is better to use the chip budding than the t-budding. It is also a better choice if we proceed in cooler climates, where callusing is slower. Choose scion wood that is about the same diameter as the rootstock or a little bit smaller. Make the cut on select rootstock directly across the stem slightly angled down. Do not cut where the buds are. The second cut start about 2 cm above the first. Cut out the chip. Make those cuts again in the scion with the bud in the centre of the chip. Size of the chip should be the same (figure 9). Fit the scion chip into the rootstock and bind it tightly with grafting tape. Make sure that at least one side of cambial areas matches and do not cover the bud with the tape (Lewis and Alexander, 2008).


Figure 9, Shape of cuts and the chip bud (Lewis and Alexander, 2008)

Patch budding

This technique is used on thin-barked trees when we cannot use t-budding. To successfully patch bud, it is essential to cut two patch of the same size. For that reason, double-bladed knife was made. It allows to make a perfectly parallel cuts distant about 2,5 cm. But we can manage work without it. Firstly, we make two parallel horizontal cuts, then two parallel vertical cuts in the bark of rootstock. This makes a square 2,5 x 2,5 cm which is removed. This same square is made on the bud wood with the bud in the centre (figure 10). Remove this patch from the shoot by pushing sideways. Pulling it from the stick could destroy the bud by pulling out the core of the bud. The patch is inserted in rootstock and wrap with the grafting tape. Do not cover the bud. After the union is complete, remove the tape and when the bud starts to grow, we can cut the rootstock just above this bud (Trinklein, 2009).

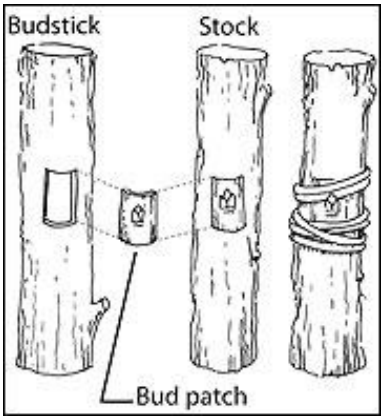

Figure 10, Patch bud (Trinklein, 2009)

2.2.3 Root suckers

It is possible to separating shoots that have been produced on roots. If we remove a section of root, on the part of the root which is not connected to the tree will grow a new sucker. After that, we dig up the sucker with the root and cut out the inconvenient part of the root (figure 11) and we have a new individual with same genotype like the parent tree (Longman, 1993). This way is not suitable for grafted trees. Suckers would be from rootstock, not entire cultivar, but they can be used as rootstocks for next grafting.

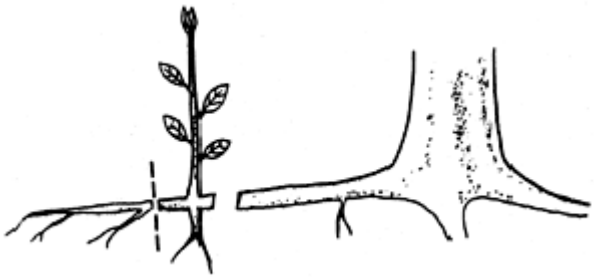
Figure 11, Root sucker (Longman, 1993)

2.2.4 Layering

When stems are still attached to their parent plant, they may form roots in contact with a rooting medium. This method is successful because the water stress is minimized. After formation of roots, the layer (rooted stem) is detached from the parent plant. Like this, we can get new plants with the same superiority as the donor plant (Evans and Blazich, 1999).

Simple layering

By bending a low growing, flexible stem to the ground, we can accomplish to do the simple layer (Evans and Blazich, 1999). Usually, it is done with many-stemmed shrubs, because they produce long, soft shoots near ground. After the end of the dormant season, the developing young shoots are bent down and part of it is buried leaving about 20 cm of

the tip above the soil as on figure 12. During the season, shoot grow and produce roots in the lower point in the soil. It is better to fix the shoot in the ground with wire or wood stakes. Wounding the lower side of the bent branch can help the rooting process. But it is important to avoid any disease in the substrate and kept it moist. After about one season, rooted stem is dug, cut from the parent plant and plant in a shade (Jaenicke and Beniest, 2002).

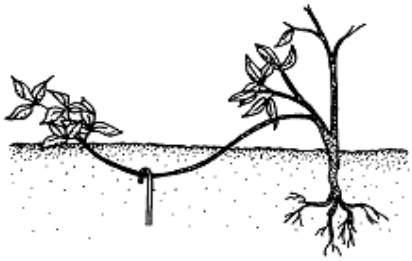

Figure 12, Simple layer (Evans and Blazich, 1999)

Air layering

Air layering or marcotting is much more useful for trees than the simple layering, because of the lack of low shoots. It has been successful as a mean propagation technique of the more difficult-to-root plants. For optimum rooting, air layers are made on no older than annual shoots and on woody plants, stems of pencil size or larger are the best. First, we make two parallel cuts 4 cm apart around the stem. The cuts are made through the bark and cambium layer. Afterwards, connect these two cuts with one long cut and remove the ring of bark. Now, the inner woody tissue is exposed. Apply on the wound handful of moist sphagnum moss and cover it entirely. The sphagnum moss should be soaked few hours and squeezed before use to insure the right quantity of water. We can tie it in place with string to keep it in position. To prevent drying out, the ball of sphagnum moss should be wrapped in polyethylene (figure 13). Important is to use polyethylene without any holes and to draw both ends around the stem and secure it with electricians tape. This tape adheres to the stem and to polyethylene. For assurance, support the shoot with stake to prevent breakage at the wounded area. After the penetration of new roots through the moss ball, the rooted branch may be removed from the parental plant. The cut is made just below the ball of moss and roots. Then the polyethylene is carefully removed. This new plant is planted without remove of the moss or disturbance of roots. Keep the plant moist and avoid direct sunlight (Everett).

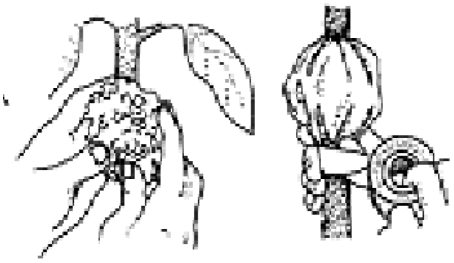

Figure 13, Air layer (Evans and Blazich, 1999)

Mound layering

Mound layering or stooling is the best way of layering for plants that naturally produce many strong coppice shoots and were severely cut lowly above soil level (Jaenicke and Beniest, 2002). The plant is cut to 2,5 cm above the soil in the dormant season. Dormant buds will produce new shoots in the spring. In spring, as the new shoots grow, mound soil over them. Substrate has to be kept moist and free of pathogens. This will stimulate developing of roots at the bases of the young shoots as on figure 14 (Evans and Blazich, 1999). But if they are covered too high, leaves may be cover. This leads to weakening of the shoot (Jaenicke and Beniest, 2002). Layers are removed in the dormant season and planted as separate plats (Evans and Blazich, 1999).

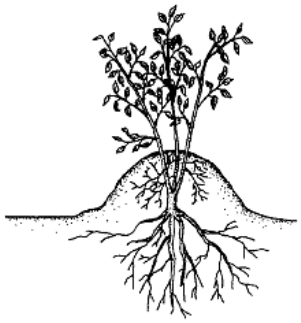

Figure 14, mound layers (Evans and Blazich, 1999)

2.2.5 Micropropagation

Micropropagation, tissue or *in vitro* culture is a relatively new vegetative propagation technique. It is based on plant's totipotency. Plants are capable of regenerate a complete new plant from small amounts of living tissue, but it requires an investment in laboratory, equipment and materials. The most vulnerable aspect of this technique is the necessity of cleanness of laboratory and aseptic (even sterile) environment. Plant material propagated *in vitro* in controlled condition is very susceptible to attacks by bacteria and fungi (Jaenicke and Benies, 2002). The micropropagation of woody plants is more difficult than that of herbaceous plants. Their sterilization is harder because of the surface and within-explants contaminants. Initiation of roots, their development and habituation are

also problematic with some tree species (Ishii *et al.*, 2006). It has many advantages as uniformity and reversion to juvenility and it is valuable for the rapid multiplication of species that are difficult to propagate and rare. The common method of tissue culture is shoots arising from axillary buds. It is called axillary shoot culture (Jain and Ishii, 2003). There are also other types of micropropagation as protoplast culture, nodal culture, shoot tip culture, cryopreservation, viral elimination, micrografting, somatic embryogenesis and genetic transformation (Pijut *et al*, 2012).

Medium for tissue culture

The selection of a culture medium is vital for the success of process. The inorganic salt formulation of media varies. In most cases, MS medium[4] is used. Other commonly used medium for the micropropagation of woody plants is WPM[5]. The use of diluted concentrations of MS salts is not rare either due to the high total salt concentration, because it was originally defined for tobacco. But the only way to defined several factors responsible for the success of tissue culture of different species (as medium, growth regulators and other additives) is through experimentation (Jain and Ishii, 2003). Culture media contain several compounds: macronutrients, micronutrients, vitamins, amino acids, source of carbon (sugar), other organic supplements and solidifying agent (agar). If needed, hormones can be added (Anonymous, 2003). Generally, if we add too much of a growth regulator, it has opposite effect and a plant (Jaenicke and Beniest, 2002). The cytokinins stimulate cell division, induce shoot formation and axillary shoot proliferation. It inhibits root formation. BAP (6-benzylaminopurine) and kinetin are the most frequently used cytokinins. The auxins stimulate callus production and cell growth and they initiate roots. The most commonly used auxins are IAA (indole 3-acetic acid) and IBA (indole-3-butyric acid). They are two other occasionally used growth regulators: gibberellins and abscisic acid. Certain species require these hormones for enhanced growth. GA_3 (gibberellins) are added to encourage the growth of low-density cell cultures and to elongate stunted or dwarfed plantlets. It elongates the stem. ABA (abscisic acid) stimulates callus growth in some species. In another species, it inhibits it. It also enhance shoot and bud proliferation. Some plants can produce toxic phenolic compounds during culture. If we are propagating these plants, we can add activated charcoal to the medium, because of its ability of binding them. However, activated charcoal is able to bind the growth regulators

[4] Murashige & Skoog medium
[5] Woody plant medium

from the culture medium, too. It can absorb NAA (1-napthaleneacetic acid), kinetin, BA (6-benzylaminopurine) IAA and 2iP (6-γ-γ-dimethylallylaminopurine) (Anonymous, 2003).

General techniques of micropropagation

In vitro usually involves four stages: initiation of aseptic culture, multiplication, rooting of formed shoots and transfer of plants to greenhouse of field conditions. Sometimes, the stage 0 is introduced (Bhojwani and Razdan, 1996).

Stage 0 involves the preparation of mother plants. To reduce the contamination problem in next stage, it is better to grown the mother plants in a glasshouse and do not overflow them. It minimizes the infection and reduces the need of harsh sterilization in stage 1. Stage 0 also includes exposing the plants to suitable light and temperature. In woody plants suitable temperature treatments should help in breaking bud dormancy, which ensures more responsive explants[6] (Bhojwani and Razdan, 1996).

Stage 1 is called the initiation of cultures. Firstly, we need to prepare the medium and then explants. The nature of explants depends on the method used to multiply shoots. These methods are shoot multiplication by callusing, adventitious bud formation and enhanced axillary branching (which is the best to use, especially for our purpose). To enhance axillary branching, we do not really need any hormones, so it is cheaper and simpler. For enhanced axillary branching, we must make explants which carry a preformed vegetative bud. It is better to use pathogen-free plants, because if we want to produce virus-free plants from infected one, it is obligatory to start with sub-millimetre shoot tips. The most suitable explants from virus-free plant are nodal cuttings. Small shoot-tip explants have a low survival rate and the initial growth is slow. It is also known that sub-terminal and older segments withstand the toxicity of sterilization much better than terminal cuttings. Terminal cuttings also have higher chance of contamination. The physiological state of the mother plant at the time of explant taking influence the response of the buds. Actively growing shoots at the beginning of the growing season generally give the best results. This aspect can be minimized by cultivation of plants in conditions required for continual growth (as temperature and light) in glasshouse. We cannot forget that everything must be sterilized. Plant tissue culture media support the growth of plants as many microorganisms. Fungi and bacteria generally grow much faster than explants and

[6] Any plant segment collected from parent plant for use in tissue culture (Jaenicke and Beniest, 2002)

kill it. Contaminants also exude metabolites toxic to plant tissue. That means that the completely aseptic environment inside the culture is essential (Bhojwani and Razdan, 1996). There are several possible sources of contamination of the medium: the culture vessel, the medium, the explant, the environment of the transfer area, the instruments, the environment of the culture room and the operator. The operating surface needs to be wiped down with alcohol. It is important to remember that any surface we touch is no longer sterile. Spores are present in air, so we need to protect material from fallout. To sterilize the culture vessel, the medium and the instruments, we use an autoclave. It provides steam heating under pressure (121 °C). To sterilize the medium in culture vessel (glasses with cover) 20 min in autoclave are enough. Other materials are there for 40 min. After every contact, instrument's tip should be put in ethanol and then in fire to burn every microorganism. To provide sterile environment of the transfer area, we use flowbox. The operator must wash his hands with soap and water before starting any work. To sterilize the explants, submerge them for 1-3 min in 70% ethanol. Tissues are then submerged in 20% commercial bleach (like SAVO) for 5 min. After, rinse 3x in sterile, distilled water. Insert the explants in containers and cover them as quickly as possible (Gamborg and Phillips, 1995).

Stage 2 (multiplication) is the most critical stage. Most of the failures occur here. There were already mentioned three approaches possible to follow in this point: through callusing, adventitious bud formation and enhanced axillary branching. Callus[7] cultures are genetically instable. That makes them inconvenient for purpose of multiplication of superior trees. Buds formed at any place other, than shoot apex or leaf axil, are called adventitious buds. Adventitious bud formation has the advantage that only a small piece of root or leaf is needed, no axillary bud. In many crop plants propagation through bud formation from root and leaf cuttings is standard horticultural practice. In this case, it is better to use grow hormones to ensure the success of bud formation (Bhojwani and Razdan, 1996). Cytokinins and gibberellins start the differentiation of shoot tissue (Jaenicke and Beniest, 2002). In the axil of each leaf, bud is present and every bud has the potential to develop into a shoot. In nature, for few periods these buds remain dormant. Sometimes to stimulate the growth of axillary bud, it is necessary to remove the terminal bud. This happens to species with a strong apical dominance. In tissue culture, we use only

[7] Undifferentiated cells, they can be influenced by hormones to differentiate into various plant organs. (Jaenicke and Beniest, 2002)

a nodal segment with axillary bud which will grow to a shoot. This process can be supported with cytokinins (Bhojwani and Razdan, 1996).

In stage 3, we need plants to develop roots. Adventitious and axillary shoots in medium with the presence of cytokinin generally do not form roots. To develop them, we need to transfer them into a rooting medium. This medium does not contain cytokinins or gibberellins but auxins. It is common practice to root shoots in vitro but very expensive. It is possible to root plants in vivo. Ex vitro (in vivo) rooting offers many advantages. In vivo rooting combines the rooting and acclimatization stages, so it reduces aseptic handling and the possibility of non-success. Roots formed in vivo are of better quality. Roots formed in vivo frequently die or collapse after remove from cultures and new are formed during acclimatization (Bhojwani and Razdan, 1996).

In stage 4, we transplant explants from culture to acclimate them. The unique set of grow conditions induce structural and physiological abnormalities. Plants have poor control of water loss and heterotrophic mode of nutrition, so gradual acclimatization is necessary for them to survive in greenhouse or on field. During transfer of plants from culture, the medium is gently washed from their lower part. Plants are transferred to potting soil and watered with low concentration of inorganic nutrients, ¼ strength of used in medium is sufficient. For the first 10 to 15 days, it is necessary to maintain high humidity (around 90 %). The humidity is then gradually reduced to the surrounding humidity for 2 to 4 weeks. The simplest way is to cover plants with plastic with small holes and to reduce the humidity, enlarge them or make the new ones. (Bhojwani and Razdan, 1996) In stage 3, plantlets can be hardened by placing containers on a cooled place. Than condensation is directed towards the bottom so the humidity of the culture is reduced. This improves survival rate during acclimatization (Pollard and Walker, 1990). After successful acclimatization, plantlets are potted in their own containers like in chapter about cuttings.

2.3 Peruvian Amazonia

Peru is divided in three topographical units: La Costa, La Sierra and La Selva. La Selva is a continental area of lowland humid tropical forest occupying almost 60% of Peru. It is considered part of the Amazon. It can be seen on figure 15 as green area. This large area is very different from the rest of the country. Western zone of sierra is cooler and coastal regions are very dry. La Selva is ecologically important. Thanks to near Ands, primary forests here are more biodiverse, than any other part of the Amazon. Peruvian

Amazonia contains an exceptional total of endemic plants and birds. The varied altitude and annual rainfall offer conditions for many species (White *et al*, 2005). However, timber production is concentrated here. There are tree laws to regulate deforestation but without big success (Harcourt *et al*, 1996). About 2 million people live here and they practice land-extensive economic activities with potential consequences on biodiversity and environment. The most visible activity is deforestation by timber extraction or slash-and-burn agriculture (White *et al*, 2005). Rivers provide fish and fertile land for farmers. Annual level of river changes up to 10 m (Lojka, 2011). In Peruvian Amazon is very suitable climate. Precipitations fall all year long, meanly during spring and autumn. Annual rainfall amounts from 2 000 to 4 000 mm and relative air humidity moves around 90 %. Mean temperature is around 26 °C. Annual change is not noticeable, maximal amplitude is 3 °C. Even the mean temperature of the coldest month is higher than 18 °C (Valíček, 1989).

Figure 15, Peru (Anonymous, 2012)

3. Objectives and methodology

Huml (2011) made a research in Ucayali region in Peru, which trees are priority to local farmers. 12 top-ranking native species were selected. It is important to search which way is possible to vegetatively propagate those 12 species. The objective of this thesis was to collect scientific information about vegetative propagation of priority tropical tree species from Peruvian Amazon. From those 12 species, vegetative propagation was reported only on 7 of them: *Swietenia macrophylla* (Meliaceae), *Inga edulis* (Fabaceae), *Cedrela odorata* (Meliaceae), *Guazuma crinita* (Sterculiaceae), *Calycophyllum spruceanum* (Rubiaceae), *Dipterix odorata* (Fabaceae) and *Simarouba amara* (Simaroubaceae)

This study has a form of literature review. I collected the information from internet database Scopus (www.scopus.com), ScienceDirect (www.sciencedirect.com) and SpringerLink (www.springerlink.com). I also used several scientific books and articles about the experiments.

4. Results

4.1 Swietenia macrophylla King (Meliaceae)

Big-leaf mahogany is a fast-growing, light-demanding, canopy tree species considered very valuable for timber. This species requires persistent growing space for optimal early growth (Grogan *et al*, 2010). It is distributed from Mexico to the Amazonia, but there is a risk of its extinction. It is difficult to regenerate naturally because of *Hypsipyla grandella* which affects all Meliaceae. It destroys the apical meristems and induces structural deformations (Schottz *et al*, 2007).

4.1.1 Sexual propagation

Mature fruits can be collected directly from trees, placed in ventilated, shaded area and separated by beating it against a hard surface. Then seeds are dried to 6-8%. No seed pre-treatment is required. Germination rate is high when seeds are fresh (over 90%), germination itself commences 10-17 days after we sow them (ICRAF).

4.1.2 *In vitro* possibilities

Leaf and root fragments were used as explants for induction of adventitious buds. 90% of leaf explants formed callus on $^{3}/_{4}$ strength MS medium supplemented with 4,4 μM BA and 0,54 μM NAA or 8,9 μM BA and 0,11 μM NAA or 0,54 μM NAA. 55% of root explants formed callus on MS medium with 2,2 μM BA and 0,54 μM NAA. Then adventitious roots were regenerated from leaf callus on medium with 1,2 μM kinetin and 2,7 μM or 5,4 μM NAA (Pijut *et al*, 2012). Jain and Ishii used for adventitious bud formation could epicotyl segments in half strength MS medium with addition of BA. It was find out that maximum respond was to 4 mg/l BA (2003).

For nodal segments was used MS medium with half strength of nitrates and 0,89 μM BA (Pijut *et al*, 2012). Schottz *et al* (2007) studied the effect of BAP in combination with 2iP in MS medium or QL medium[8] on juvenile nodal explants. The highest multiplication rate was in the presence of 18,51 μM BAP and 2,2 μM 2iP on MS medium. Multiplication was reduced on QL medium.

In 1997, there was also reported the use of WPM medium supplemented with 10 μM zeatin by Maruyama and Ishii for multiplication stage (Schottz *et al*, 2007). Other vegetative propagation was not reported.

[8] Quoirin and Lepoivre medium

4.2 *Inga edulis* Mart. (Fabaceae)

This tree natively grows in Bolivia, Peru, Ecuador, Colombia and Brazil. It grows rapidly on poor soils and survives on floodplains waterlogged for 2-3 month per year. It is resistant to cold and drought, but demands light (ICRAF). It is a *Leguminosae*, so it is nitrogen fixing tree species. It is used as shade for coffee in Latin America, it provide fuel wood, and timber. *Inga edulis* is appreciated for the sweet edible aril surrounding seeds. The seeds are cooked and eaten as vegetables (Brennan and Mudge, 1998).

4.2.1 Sexual propagation

Inga trees are commonly propagated by seed. It could be sow directly in the field. To avoid desiccation, it must be watered often or sow during a season of regular rainfall. Better way is to sow it in a plastic bag 2 cm below the soil surface. The seeds germinate in 2-3 days. Seedlings stay in the nursery for 2-3 month and then they are transplanted on the field (ICRAF). Bud seeds of *Inga* trees are recalcitrant[9]. Also seeds sometimes germinate before detaching from the parent plant. This induces unavailability of seed during much of the year. Without the use vegetative propagation, it is an obstacle to domestication of this tree (Brennan and Mudge, 1998).

4.2.2 Vegetative propagation

Vegetative propagation of *Inga edulis* was not reported, but propagation of *Inga feuillei* (which is very close to *I. edulis*) was, so we can suppose that same technique will function for *Inga edulis* too.

The effect of IBA on rooting of leafy cuttings of *I. feuillei* was made in 1998. Leafy cutting were treated with 0,3 or 0,8% or nothing. One third of untreated cuttings and cuttings treated with 0,3% IBA rooted, while more than two thirds of those treated with 0,8% IBA rooted (Brennan and Mudge, 1998).

Effect of auxin treatments on rooting of softwood cuttings were experimented also under fog. Single-node softwood terminal cuttings were made 10-12 cm long and their leaves were trimmed to a single pair of leaflets. Three auxin levels were used: 0, 0,3 and 0,8% IBA in powder. All cuttings were placed under fog. The best response was from cuttings treated with 0,3% IBA with a survival rate of 78% (Brennan and Mudge, 1998).

[9] Seed cannot survive drying or chilling, so it cannot be stored for more then a few weeks (Brennan and Mudge, 1998)

Other experiment was done with semi-hardwood cuttings. Cuttings were 12 to 15 cm long with a single dormant axillary bud at the distal end. One part was leafless and other had trimmed all leaves except the rachis and the basal pair of leaflets. All of them were treated with auxin. Basal end of cutting was dipped in 0 or 0,3 or 0,8% IBA. These cuttings were in greenhouse under mist or under polyethylene. Results showed that semi-hardwood cuttings need leaves to survive. The best result showed leafy semi-hardwood cuttings treated with 0,8% IBA under mist with survival rate equal to 78%. Untreated cuttings under mist had higher survival rate than treated with 0,8% IBA under polyethylene (Brennan and Mudge, 1998).

Air Layering of *Inga feuillei* was done with or without IBA on semi-hardwood stems. Three weeks after air layering, 97% of treated layer and 76% of untreated layers had visible roots. Five weeks after air layering, 100% of both possibilities had roots. They were all harvested after eight weeks. There were no differences in the number of roots between the two treatments. All of them were transplanted successfully (Brennan and Mudge, 1998).

4.3 Cedrela odorata L. (Meliaceae)

It is a tropical tree valuable for its wood, but it is also affected by *Hypsipyla grandella.* Due to logging, this species faces high risk of extinction in the wild and plantations are troublesome by the borer. Natural populations are genetically diverse, so it is possible to find resistant individuals, so vegetative propagation and rejuvenation technologies are ways to domesticate these resistant trees for timber production or forest restoration (Pena-Ramirez *et al*, 2010).

4.3.1 Sexual propagation

Transplanting of naturally regenerated seedlings is very common, but seeds are used too. Fresh seeds germinate gladly, but if we store them, they rapidly lose its viability. Germination takes 2-4 weeks and seeds need to be adequately moisturised. Seeds are sown in separate containers in nursery and then transplanted on the field (ICRAF).

4.3.2 Vegetative propagation

The effect of BA and NAA on *Cedrela odorata* was tested *in vitro*. Half strength MS medium was used with different concentrations of growth regulators. Hypocotyl segments produced adventitious buds only in the presence of BA and NAA together. The

highest total number of adventitious buds was obtained with 4mg/L BA and 1 mg/L NAA (Jain and Ishii, 2003). Hypocotyl segments were also used in other experiment. 20% coconut water was added to TY17 medium[10]. On this medium was observed the adventitious shoot regeneration. Coconut water increased the number of adventitious buds per explant. No studied cytokinin provided similar results. The response of juvenile tissue was more efficient than the mature tissue due to the number of formed buds (Pena-Ramirez *et al*, 2010). There was also reported the possibility of construction of artificial seeds of *C. odorata.* Shoot-tip explants and nodal segments were encapsulated into single or double layered alginate gel beads containing medium at different concentrations. They were regenerated under aseptic and non-aseptic conditions. Rooting of encapsulated shoot-tips was much better than that of encapsulated nodal explant. The best results showed double layered beads containing 1000% concentrate of medium with 0,5% activated charcoal in the inner layer and 100% medium in the outer layer. For non-aseptic conditions is better to use double layered capsules with activated charcoal in the inner layer. However, the plant regeneration rate decreased considerably in comparison with aseptic conditions. But when artificial seeds were incubated *in vitro* for 1 week before sowing on non-aseptic substrate, the plant regeneration rate was a little increased (Maruyama *et al*, 1997).

Grafting was reported as a possibility of rejuvenation. There were collected shoots of *C. odorata* mature elite trees and they were immediately grafted onto juvenile rootstocks of the same species. The grafting process was applied by side wedge cuttings on the young trees (rootstocks) and "V" cuttings on the shoots of mature trees (scions). Grafted plants were kept in greenhouse at 35°C and 80% humidity for 5 weeks. After 1 week, the apical shoots of rootstock were cut to induce shoot formation. Then for 3 weeks, responding axillary buds from rootstock were cut out. This process induced shoot formation on the scion (Pena-Ramirez *et al*, 2010). In other experiment, *C. Odorata* was successfully grafted on *Toona ciliata.* After introduction of *T. ciliata* to Brazil, it shows absence of attacks by *H. gandella*, but the induction of resistance to the grafted plant is just speculations (Paula *et al*, 1997).

Branch and stem cuttings are the most common methods of propagation. Budding and air-layering methods are also successful (Paula *et al*, 1997).

[10] Full-strength WPM plus half-strength MS vitamins ()

4.4 Guazuma crinita Mart. (Sterculiaceae)

This specie is a fast-growing tree. It has a soft-light wood which is used for light construction, furniture, matches, moulding, cases and other. It shows great adaptability including degraded areas and poorly drained heavy soils. It is usually propagated by seeds. There was not developed cheaper effective vegetative propagation method than *in vitro* (Maruyama *et al*, 1996).

4.4.1 Sexual propagation

The fruits are collected from the tree or gathered from the ground after they fall. They are covered by hair which needs to be cut before sowing. We can sow the fruits or try to release the seeds from it. The seeds need to germinate as soon as possible. Germination rate is only 50% and germination takes longer than in case of other species. The seeds are sow to separate containers and then transplanted on the field (Rollo, 2009).

4.4.2 Vegetative propagation

In 1996, first micropropagation with *G. crinita* was done. Shoots were multiply from aseptically germinated seedlings. There was examined the effect of different basal media and growth regulators. Rapid clonal propagation of shoot-tips was achieved with WPM supplemented with 10 μM of trans-zeatin. After 45 days, shoots were transplanted on WPM with 1 μM of kinetin to elongate and root them. High rooting rate was obtained and all plantlets were acclimatized successfully (Maruyama *et al*, 1996). There was also done the same experiment like with *C. odorata*: construction of artificial seeds. For *G. crinita* was best the same choose of media concentration, but it showed higher rates of bud emergence and shoot growth. When artificial seeds of *G. crinita* were incubated *in vitro* for 1 week before sowing on non-aseptic substrate, plant regeneration increased on 100% unlike in case of *Cedrela odorata* (Maruyama *et al*, 1997). Micropropagation of *Guazuma crinita* was also done by root and petiole culture. There was examinated the effect of types of explants, cytokinins and photon flux density on adventitious bud formation. Segments of roots near the stem were used as root explants (1-2 cm long). Petiole explants (1-2 cm long) with or without *laminae* were obtained from younger leaves. All were then cultivated on WPM supplemented with BA or zeatin at concentration 1 or 10 μM and under low (65 μmol m^{-2} s^{-1}) or high (135 μmol m^{-2} s^{-1}) photon flux density for 16 hours per day. 45 days after, 65 to 100% of the root segments and petiole explants with *laminae* developed small adventitious buds on WPM with zeatin. Lower percentage was obtained

with 1 μM and 100% with 10 μM. But petiole explants without *laminae* showed a low ability of development of buds (12-20%). Generally, *laminae* induces the clusters of adventitious buds and petiole explants without it induces callus. In case of *G. crinita*, presence of *laminae* had stimulative effect. Photon flux density affected just BA. In a presence of 1 μM of BA under low photon flux density, 50% of petiole with the *laminae* and 5% of root segments produced buds, bud under high density, only 40% of petiole with *laminae* and 0% of root explants produced buds. In case of presence of 10 μM of BA, the differences are more noticeable. Under low photon flux density, 20% of petiole with *laminae* and 40% of root segments produced adventitious buds, bud under high density no explant produced buds (Ishii *et al*, 1997).

Rollo made a research on the possibility of use of leafy cuttings. These cuttings were made from seedlings. They had 1 leaflet and 2 nodes and they were long about 14 cm. One half of cuttings were treated in fresh 100% coconut milk for 16,5 h. Untreated cuttings were put for the same time in a water. Then the cut was disinfected with $KMnO_4$. Again, two groups consisting both treated and untreated cuttings were placed one in the nursery bed and the other one in poly-propagator. Nursery bed was watered every day and poly-propagator twice a week. The treated cuttings rooted better in poly-propagator and their mortality was higher nursery bed. Untreated cuttings rooted better in poly-propagator, they rooted even better, than treated cuttings and their mortality was higher in nursery bed. The experiment showed that treatment has no influence on rooting in nursery bed (Rollo, 2009).

4.5 Calycophyllum spruceanum (Bentham) Hooker f. Ex Schum. (Rubiaceae)

It is a fast-growing tree that colonizes the floodplains. It is species which likes lighted area. The tree is used for constructions, firewood, charcoal and bark. *C. spruceanum* has ability to regenerate its bark that is used in local medicine to treat abscesses, cancer, cuts, diabetes, malaria, pellagra, skin parasites and others (Lipenský, 2010).

4.5.1 Sexual propagation

C. spruceanum is usually propagated by seeds. Its epigeal germination takes 6-60 days and requires sandy substrate, often watering and sunlight. It is better to sow seeds in

nursery to increase the germination rate, transplant them in their own containers and then to the field (Lipenský, 2010).

4.5.2 Vegetative propagation

Lipenský made the experiment on rooting cuttings of *C. spruceanum* in fine sand as rooting medium. It took place in Ucayali region in Peru. Semi-hardwood cuttings were taken from seedlings. There was made with one leaf. All cuttings were treated for 15 min in fungicide. Each cutting of each group was treated by one of the concentration of IBA for 3 sec: 0, 1000, 2000, 4000 and 5000 ppm. Whole experiment was made in poly-propagator. After 21 days, evaluation was made. The best IBA treatment was with 2000 ppm. 80% of cuttings rooted. Treatment also minimized mortality rate from 36% to 10% (2010).

4.6 Dipterix odorata (Aubl.) Willd (Fabaceae)

Dipterix odarata is extended from Central America to Amazonian basin. This timber tree is indifferent to soil conditions and it grows mainly on dryland. It prefers short day photoperiod and lighted area. Its wood is used for building and naval construction, because it is resistant to rot and very heavy (Rollo, 2009).

4.6.1 Sexual propagation

This species is commonly propagated by seeds, but their seeds are recalcitrant. This fact inhibits longer storage. If we use whole fruits as sow material, the germination rate is low, but if the seeds are release, the germination rate is higher. Germination takes 3-5 weeks. At the beginning, they grow at the nursery and then the seedlings are transplanted to the field (Rollo, 2009).

4.6.2 Vegetative propagation

Rollo made apical and basal leafy cuttings of semi-hardwood shoots from seedlings. They had 1 leaflet and 2 nodes and they were long about 7-9 cm. All cuttings were treated with fresh 100% coconut milk as growth regulator for 16,5 h. Then the treated cut was disinfected 0,5 h with 2% $KMnO_4$. One group of apical and basal leafy cuttings was put in nursery bed and the other group of apical and basal cutting was put in poly-propagator. Nursery bed was irrigated every day and the poly-propagator twice a week. The mortality of apical leafy stem cuttings were higher in nursery bed, but rooting of basal stem cuttings were better in nursery bed. This experiment took place in Pucallpa (2009).

4.7 Simarouba amara Aublet (Simaroubaceae)

It is dioeceous tree spread in Central America and Amazon. It needs very humid conditions. *S. amara* is cultivated for wood, bark and leaves. Durability of the wood in forest is low, so it is used for interior purposes, like furniture, music instruments, matches, for paper and others. The bark and leaves are used in the local medicine in tropics. It is used to treat fevers, malaria, diarrhoea, to stimulate digestion and to stop bleeding (Lipenský, 2010).

4.7.1 Sexual propagation

This tree is usually propagated by seeds. Silt soils provide higher germination rate than other soils. The viability of frees seeds is decreasing quickly. It is better to sow *S. amara* in nursery and pot it in its own container (Lipenský, 2010).

4.7.2 Vegetative propagation

With *Simarouba amara*, Lipenský made the same experiment like with *C. spruceanum.* But in case of *S. amara*, there was 98% rate of mortality of treated and untreated cuttings. There are no reports about vegetative propagation of *S. amara* (2010).

Techniques of vegetative propagation suitable for other mentioned species have not been reported.

5. Discussion

I made a review on vegetative propagation of 12 preferred tree species in Peruvian Amazon. Most of them are used for timber, but there are some fruit and medicinal species, too. However, vegetative propagation was reported only for 7 species of those 12 and in case of *Simarouba amara,* it was not successful. On table 1 is a summary of successful reported propagation. *Swietenia macrophylla* was propagated *in vitro*. WPM medium and ½ and ¾ MS medium was tested successfully with some supplements. Veg. propagation of *Inga edulis* was not reported, but of *Inga feuillei*, which is very close, was. Softwood, semi-hardwood, hardwood cuttings and air-layering was made successfully with IBA. *Cedrala odorata* was propagated *in vitro* in ½ MS medium, by construction of artificial seeds and by grafting with *C. odorata* and *Toona ciliata* as rootstocks. The propagation of *Guazuma crinita* was tested *in vitro* on WPM medium, artificial seeds were constructed too and leafy cuttings also rooted successfully. In case of *Calycophyllum spruceanum,* only semi-hardwood cuttings were tested. They rooted successfully. *Dipterix odorata* was successfully propagated by leafy apical cutting in nursery bed and leafy basal cuttings in poly-propagator. *Simarouba amara* was propagated by cuttings, too, but with 98% of mortality. That means unsuccessfully. We can see that cuttings are reported for most species, but more experiments were done on micropropagation. WPM and half strength MS medium are the most used media for tree cultures. The use of growth regulators changes with species. To find out how the type and concentration affects the explants is usually one of the aims of experiments on *in vitro*. Air-layering was reported for *Cedrela odorata* and *Inga feuillei,* but for *C. odorata* was not reported the technique as the use of treatment or rooting rate. The same case happened with budding of this tree even without the report on possible rootstocks. Grafting was reported only for *C. odorata*, as a possibility of rejuvenation and formation of resistance against the borer.

Table 1, Summary of successful reported propagation of preferred trees from Peruvian Amazon

Scientific name and family	**generative propagation**	**vegetative propagation**			
		in vitro	*Cuttings*	*grafting*	*layering*
Swietenia macrophylla King (Meliaceae)	by seeds, but they quickly lose its viability	1/2 and 3/4 MS, WPM (QL medium)			
Inga edulis Mart. (Fabaceae) not reported, but *I. feuillei* was	by seeds, but they are Recatcitrant		leafy with 0,8% IBA semi-hardwood with 0,8% IBA softwood with 0,3% IBA		air-layering, rooting quicker with IBA
*Cedrela odorata*L. (Meliaceae)	naturally regenerated seedlings by seeds, but they quickly lose its viability	1/2 MS medium TY17 medium Artificial seeds		*C. odorata* and *Toona ciliata* as rootstock *C. odorata* as scion	air-layering possible, but technique unreported
Guazuma crinita Mart. (Sterculiaceae)	by seeds, low germination Rate	WPM Artificial seeds	leafy in poly-propagator		
Calycophyllum spruceanum (Benth.) Hook. f. ex K. Schum. (Rubiaceae)	by seeds		semi-hardwood with 2000 ppm = 0,2% of IBA		
Dipterix odorata (Aubl.) Willd (Fabaceae)	by seeds, but they are Recatcitrant		apical leafy in poly-propagator basal leafy in nursery bed, both treated in 100% coconut milk		
Simarouba amara Aublet (Simaroubaceae)	by seeds				

6. Conclusion

In case of many species, there is a problem to get seeds beyond the harvest. Some quickly lose viability, some cannot be stored. However, farmers in Peruvian Amazon propagate trees mainly by seeds. So the possibility of vegetative propagation is a great breakthrough. But I found out, that many techniques of vegetative propagation of chosen species are not experimented. There is no report, which one of those techniques could be successful for those 12 species. But there is also no report about any plant species that would be impossible to propagate *in vitro*. It means that micropropagation could be used for every species after finding suitable medium and growth regulators. However, this technology requires expensive equipment and materials and specific conditions have to be met at workplace. It is not easy to process and it requires experienced and skilled workers, so it is not really a technology suitable for farmers. It is better to propagate trees by cuttings or air-layering. But as shown in a case of *Simarouba amara*, those techniques could be unsuccessful. The possibility of acquired resistance against pests through grafting is also interesting. To conclude, many further experiments and researches are needed to continue in tree improvement.

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