Methods for vegetative propagation: Theory

Lecture handout for SII Training workshop on *Allanblackia* domestication 23-27 October 2006, ICRAF, Nairobi, Kenya

Tchoundjeu, Z.; Asaah, E ; Atangana, A.
ICRAF-WCA/HT, BP 16317 Yaounde, Cameroon.

Fast track summary

Title: Methods for vegetative propagation: theory

Learning objectives

Participants will be able to:

- Understand the principle of vegetative propagation of agroforestry trees
- Understand the rationale for propagating agroforestry trees vegetatively
- Explain vegetative propagation techniques
- Apply vegetative propagation in the domestication of agroforestry trees

Instructional methods

Thirty minutes classroom presentation (PowerPoint) and thirty minutes discussions.

Instructional materials

'Lecture' note, copy of PowerPoint presentation, bibliographic references.

Summary

Asexual propagation or reproduction from vegetative parts of the original plant, is possible because every cell of the plant contains the genetic information necessary to regenerate the entire plant. Reproduction can occur through the formation of adventitious roots and shoots or through the uniting of vegetative parts by grafting or budding. 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.

Many agroforestry tree species are dioecious and farmers often report that when a tasty or large fruit from a superior fruit tree for example is planted, the seedling does not perform up to their expectations. In most cases they are interested only in the female, fruit-bearing individuals and sometimes retain a few male pollinator trees around. It is difficult if not impossible to predict in juvenile plants their sex expression. In this context, vegetative propagation of the female trees could help increase the number of fruit bearing trees on farm. Some of the benefits that could accrue from the application of vegetative propagation will include among others:

- multiplying 'true-to-type' elite material.
- controlling male to female tree ratios on farms,
- propagation of seedless plants
- avoidance of long juvenile periods
- control of growth form
- combination of clones
- Economics: elimination of the juvenile phase thus shortening the time to reach the reproductive maturity.

Vegetative propagation methods include:

- rooting of cuttings,
- layering or marcotting,
- grafting,
- micropropagation.

Introduction

Propagation is the natural mechanism by which plants regenerate. Propagation by seeds is the main method by which plants reproduce in nature. The method is also efficient and widely used in tree propagation. As a matter of fact, all trees produced seeds, except those individuals bearing only male flowers in some species. The process of seed production begins during flowering; when the pollen is transferred from the male part (anther) to the female part (stigma) of the flower-seed being the end product of a long, complex and physiological process.

In certain plant families such as *Burceraceae, Clusiaceae* and *Euphobiaceae*, it is common to find dioecious plants (separate male and female trees). This implies some plant (male trees) have functional stamens with anthers (male part) in their flowers while others (female trees) have functional pistile with stigmas (female parts) in the flowers. Examples include; *Allanblackia* spp, *Canarium schwweinfurthii* (Black fruit) and *Ricinodendron heudelotii* (njangsang) in which only trees bearing female flowers produce fruit and ultimately seeds. It is often very difficult to distinguish between male and female trees except when they are in flower. Another phenomenon common in some plant families such as *Sterculiaceae*, *Burceraceae* is hermaphroditism in which case plants will possess male and hermaphrodite flowers. The two types of flowers occur mixed on the same inflorescences, though occasionally on different inflorescences or trees as in *Cola nitida* and *Cola acuminata* (Opeke, 1977) and in *Dacryodes edulis* (Kengue, 1998). Studies on the floral biology of *Cola* for example has indicated incompatibility in the fertilisation of hermaphrodite flowers with pollen from male flowers from within or between trees exist. Thus in the propagation of such species by seeds, at least 5 seeds should be planted to increase the chances of having a female tree. However, the surest way of obtaining either a female or male tee is through vegetative propagation.

Vegetative propagation of agroforestry trees

Vegetative propagation of plants is essentially the reproduction of plant material from vegetative organs so that the offspring will contain the exact characteristics of the parent plant with regards to genotypes and health status (Macdonald, 1996). This is possible because plants unlike animals have meristimatic, undifferentiated cells that can differentiate to the various organs necessary to form a whole new plant. Thus a piece of plant shoot, root, or leaf can grow to form a new plant that contains the exact genetic information of its source plant.

While sexual reproduction has been responsible for the wide genetic diversity observed in many tropical tree species as a result of constant recombination of genes, vegetative propagation aims at the identical reproduction of plants with desirable features such as high productivity, superior quality, resistance, high tolerance to biotic and abiotic stress and therefore plays an important role in continuing preferred traits over generations. Vegetative propagation has been used in fruit tree improvement since biblical times, and continues to still relevant today in agroforestry tree domestication efforts.

Methods of vegetative propagation

Cuttings are severed uninodal leafy shoot or root fragments that can develop adventitious roots and shoots respectively. Uninodal leafy shoot cuttings are usually place into a suitable rooting substrate and kept under high humidity in propagators until roots and shoots are formed. Propagation by cuttings is a relatively easy method and can provide a large number of propagules in a very short time. Cuttings have been widely used in clonal propagation of forest trees where large numbers of planting stocks are required and recently in the production of quality germplasm of local fruit trees (*Irvingia gabonesis, Ricinodendron hedelotii, Dacryodes edulis, Allanblackia* spp), medicinal plants (*Prunus africana, Pausinystallia johimbe*).

Grafting is a technique used to untie 'parts' of different plants by bring the cambium of each into contact and then creating a situation under which the cut surfaces can unite and grow away together. There are many grafting methods, some very specific for a particular species. They depend on seize and shape of the scions and the place on the rootstock onto which they are grafted; they can be carried out at various times throughout the year. The technique involves two important stages: the preparation of the grafting surfaces and the procedures for aftercare. In addition, the technique also requires grafting skill first, then extended experience to become familiar with the aftercare procedure without which newly grafted plants might be lost due to poor aftercare.

Definitions of terms used in grafting.

Scion -The part of the graft that will provide the new system. The scion maybe united either at the apex or side of the rootstock.

Rootstock – the lower part of the graft. It normally posses a root system that will support the subsequent shoot development from the scion.

Layering (marcotting) technique is similar to cuttings with the advantage that the propagule is detached from the parent plant only after development of roots. Its multiplication rate is lower than that of cuttings though the plants are larger. Methods of layering are the oldest used for vegetative propagation. Three 'classes' of layering methods, depending on the soil and stem relationship can be distinguished:

- soil is mounded to the stem (mound layering, trench layering)
- stems are lowered into the soil (simple layering, tip layering)
- stems are not covered by soil (air layering)

Air-layering is the technique that has been widely used in the domestication of indigenous fruit trees in west and central Africa in capturing the attributes of elite trees within genetically diverse wild populations, so avoiding the long, slow process of tree breeding.

Micropropagation is a specialized method of propagation in which small pieces of plant tissues are regenerated on an artificial medium under sterile conditions. It embraces the regeneration from shoot and root tips, callus tissue, leaves, seed embryo, anthers and even single cells. Micropagation has following the applications in woody plant multiplication.

- As a means to remove viruses from plants, thereby improving clones of plants in clonal improvement programmes.
- As a very effective and rapid method to multiply high quality clonal material (bulking-up of plants).

Micropapagtion has its limitations and should not be regarded as the answer to all propagation problems. Some limitations are summarized below:

- The initial capital investment in the laboratory and equipment can be high. The financial commitment necessary has been quoted in the range of $15,000 to $250,000 (US), depending on the size of the laboratory and the refinement of the procedures desired.
- Micropropagation requires skill, usually specially trained laboratory manager and skilled disciplined support staff. All these entail additional cost.

Not withstanding, micropropagation should be seen as a technology to use in conjunction with conventional propagation techniques. Macropropagation is therefore, a "tool" to refine the production of some high value crops, to produce healthier plant material. Till date attempts on micropropagation of agroforestry species - *Dacryodes edulis* are still at the laboratory level (Youmbi and Benbadis 1998)

Rationale for propagating agroforestry trees vegetatively

Maintaining superior genotypes

Most tropical agroforestry tree species are outbreeders – *Allanblackia* spp, *Canarium schwweinfurthii* (Black fruit) and *Ricinodendron heudelotii* (njangsang) *Cola* spp; implying that through the recombination of genes during sexual reproduction, many important characteristics might disappear. If a superior individual tree/ideotype has been identified by farmers or researchers, its genetic information can be 'fixed' through vegetative propagation, thus allowing the reproduction of the same superior individual in the next generation.

Problematic seed germination and storage

Some tree species produce seedless fruits (e.g. off-season *Dacryodes edulis*,) and need to be propagated vegetatively, others bear fruit very scarcely or erratically (*Prunus africana)*; and some bear fruits abundantly but due to some form of physiological dormancy germination is very slow and the rates are quiet low *Allanblackia* spp,. Many tropical tree species have recalcitrant seeds that require special and often cumbersome seed handling procedures. In these cases, vegetative propagation might be a suitable and cheaper alternative to seedling production.

Shortening time to flower and fruit

An important reason for vegetative propagation is the shortening of the reproductive cycle of a tree. This is particularly important when the flowers, fruits or seeds are the desired products. Most vegetative propagation is done with scions or cuttings from mature trees, which maintain the characteristics of maturity after grafting or rooting as will be explained above. For instance *Irvingia gabonesis, Dacryodes edulis, Ricinodendron heudelotii* and *Cola spp* marcotts will fruit from 3 years after field planting. Grafts of *Irvingia* spp, *Garcinia kola* and *Allanblackia* have been observed to fruit in nursery 6 months after grafting.

Combining more than one genotype in one plant

Grafting is a unique way of combining desired characteristics from two or more plants into a single one. Scions with particular fruit characteristics can be grafted onto rootstocks with other desirable characteristics such as disease resistance and adaptability to environmental constraints. Another possibility is the grafting of more than one cultivar or species onto the same stem, for example *Irvingia gabonensis* (sweet fruits) grafted to an *Irvingia wombolu* (bitter fruits) rootstock or to extend the period of bearing by grafting early and late varieties on a single tree. Sometimes, it could even be the introduction of a pollinator branch onto a female tree in the case of dioecious species like *Ricinodendron heudelottii, Canarium schwweinfurthii* and *Allanblackia* spp.

Uniformity of plantations

For many commercially grown species - *Citurs* spp, *Mangifera indica,* uniformity of growth form or fruiting season is important economically. This phenomenon will sooner or later be relevant with the increasing demand for agroforestry tree products - *Irvingia kernel*, *Dacryodes edulis* fruits, *Cola* nuts and *Allanblackia* seed.

Box 1: When is it appropriate to use vegetative propagation

When the species in question:

- is an out breeder;
- is dioecious;
- has recalcitrant seeds;
- has low germination rates;
- flowers and fruits erratically and;
- to capture their genetic diversity.

Application of vegetative propagation in domestication of agroforestry trees

Many tree species are dioecious. Farmers are more interested in the female, fruit-bearing individuals and need only a few male pollinator trees around. It is difficult if not impossible to predict in small seedlings, whether they are female or male, and often trees change their sex expression with age. In these cases, vegetative propagation of the female trees could help increase the number of fruit bearing trees on farm.

Selection (capture of desirable traits)

Layering was developed, by the Chinese 4-5000 years ago. Although the layering techniques are quite simple, their commercial use today is restricted to specific applications. The reason for this is that the productivity of layers is far smaller than that of cuttings. However, the use of air layering technique has been widely used in the domestication of indigenous fruit trees in west and central Africa – *Irvingia gabonesis, Dacryodes edulis, Ricinodendron hedelotii, Cola* spp (*Anegbeh et al*; 2005 a,b, ICRAF-WCA/HT unpublished data).

Grafting has become the most used way to improve high-valued fruit trees like citrus, mango, or avocado. This is most likely attributed to the opportunities they offer to combine different traits in the different plant parts. ICRAF-WCA/HT is currently researching on the application of grafting techniques in the improvement of economically important local fruit trees of west and central Africa. Grafting has successfully been adapted to *Irvingia gabonesis* (bush mango), *Ricinodendron heudelotii* (Njangsang), *Garcina kola* (bitter cola), *Allanblackia* spp with grafted plants promising to fruit under 5 years as opposed to at least 10 years in the wild [ICRAF-WCA/HT unpublished data].

Despite the potential of grafting in the domestication of agroforestry trees, it also has some limitations. For example, graft incompatibilities arise when rootstock and scion do not readily join. Therefore, in order to produce successful and sustainable grafts, compatible clones have to be identified. This is a resource-consuming process that is inevitable for the success of the technique. Also, grafting techniques demand some skill.

Mass propagation

Vegetative propagation by cuttings of several local fruit trees and medicinal plants has received considerable, attention in the last decade from ICRAF-WCA/HT research programme on domestication of agroforestry trees. Salient factors (growth hormones, leaf area, cutting volume, rooting media) influencing the rooting ability of local fruit trees: *Irvingia gabonesis* (bush mango), *Dacryodes edulis* (safou, African plum), *Chrysophyllum albidum* (star apple), *Ricinodendron heudelotii* (njangsang), medicinal plants *Pausinystalia johimbe* (yohimbe), *Prunus africana* (pygeum), oil plants (*Allanblackia* spp) and wild vegetable *Gnetum africanum* (eru, okok) have been identified [Shiembo, 1996, Kengue, 1998, Mialoundama *et al*.; 2002, Tchoundjeu *et al*.; 2002, Ngo Mpeck *et al*.; 2003, Atangana *et al;* in press].
Propagation by cuttings, in low-cost 'non-mist' poly-propagators made of local material is rapidly being adopted by ICRAF partners, (NARS, NGOs and farmers) in Cameroon, Nigeria, Equatorial Guinea, Gabon, Democratic Republic of Congo and Ghana and Tanzania.

Tree improvement

Farmers often report that when a tasty or large fruit from a superior fruit tree for example is planted, the seedling does not perform up to their expectations. Scientific reasons advanced to explain these observations stems from the fact that, the pollinator of the seeds was a different fruit tree though of same species and thus the seeds have a different gene combination than the mother tree. Therefore, the ability to produce large and tasty fruits was not inherited. On the other hand, the application of vegetative propagation techniques can provide an exact copy of the mother tree from which the seed was harvested and retain the desired fruit quality in the following generation if the traits are heritable.

Box 2: Advantages and disadvantages of vegetative propagation

Vegetative propagation of *Allanblackia* is aimed at:

- developing an alternative method for the multiplication to seed (particularly important in the context of very slow seed germination),
- controlling male to female tree ratios on farms,
- reducing the time taken to fruiting for planted trees,
- multiplying 'true-to-type' elite material.

The necessity that *Allanblackia* germpalsm be:

- clearly documented
- of multiple provenances per species, where possible;

Vegetative propagation of Allanblackia species

Methods have been developed for the vegetative propagation of *Allanblackia floribunda* using single-node leafy stem cuttings in non-mist propagators (Atangana *et al*., in press). Factors identified to influence the rooting of cuttings are rooting medium, genetic origin (clone) and presence/absence of leaf. Best rooting percentages (68.7%) were obtained in leafy cuttings from one clone inserted in sand.

Experiments are also in progress for development of grafting protocols in *Allanblackia*. Preliminary results indicate that *Allanblackia gabonensis* trees are amenable to grafting, the best practice being side-tongue graft (80% success).

Specific issues in Allanblackia *vegetative propagation*

A. floribunda is a hard-to-root species, as cuttings develop roots at least 10 weeks after insertion in the propagator. Best rooting percentages are obtained after 24 weeks, what constitute an issue to overcome before scaling-up protocols of propagation. As a bonus, genetic origin of cuttings (clone) strongly influences the rooting ability in *Allanblackia* cuttings, indicating that the ability to clone particular trees will be an important factor in the selection that should be done following the ideotype concept, using ideotypes combining fruit and nut traits, as well as ability to root.

Protocol of propagation of Allanblackia *using rooting of cuttings*

1. Choice of cuttings

As one of the objectives is to massively propagate plus trees, an option would be to harvest cuttings from a recently felled trees known to produce fruits with desired characteristics (high productivity, heavy fruits with many seeds…). Juvenile cuttings should be harvested from coppicing stumps, sprayed with water and stored in moist polythene bags (to reduce the stress) and brought to the nursery.

2. Preparation of cuttings

Using a sharp blade, pest and disease-free shoots are cut into single-node cuttings of 3-4 cm length. The leaf lamina of each cutting should be trimmed to 12.5, 25 or 50 cm2 with scissors.

3. Non-mist propagators

High-humidity, non-mist propagators consisting of a wooden frame enclosed in clear polythene sheets so that the base of the propagator is watertight (3 m x 1 m x 1 m), and the lid is hermetically sealed, should be used. Sand should be the rooting medium, treated with fungicide (Ridomil Plus (metalaxyl-M and cupper) and Caocobre (cupper): 50 g each dissolved in 15 l of water) and insecticide (Cyperdim 220 EC 200g/l dimethoate and 20g/l cypermethrine: 40 ml dissolved in 1.5 l of water) 3 days prior to the beginning of the experiment.

4. Insertion of cuttings in the propagators, and follow-up

Three-hundred cuttings can be inserted in one non-mist propagator. Caocobre can be applied to the rooting medium when necessary (fungal attacks) during the rooting process. Each morning (very early), water level in the propagator should be monitored, and corrections made when excess or deficit. Cuttings should then be sprayed with a fine jet of water. Assessments of rooting success should start 10 weeks after cuttings' insertion in the propagator, by lifting the cuttings from the rooting medium. A cutting is considered to be rooted when it has one or more roots exceeding 1 cm. Rooted cuttings should be removed from the propagator a potted in black polythene bags (1 l) containing a 2:1 mixture of forest soil and sand.

Cuttings are then brought to a giant propagator for weaning (2 weeks). After 2 weeks, cuttings are removed from the giant propagator and keep in the nursery (shade) before transplantation that can occur 6 months later (cuttings should be transplanted in a shade place, otherwise, their leaves will be burnt by sun).

Protocol for* Allanblackia *grafting

Materials you need for grafting

- Pruning shears (pruners)
- Budding – knife or well sharpened knife
- Whetstone or fire
- Fastening film
- Protective plastic

Once the material is available, grafting of *Allanblackia* can be made using the '**side-tongue**' graftting which has proven to give the best success rate (80%) in *Allanblackia gabonensis*, as follows:

1. Choice of scion

   The choice of a scion is a very important step through grafting of *Allanblackia*, as grafting consists in uniting a selected tree with a rootstock. Hence, scions should be harvested from trees with known characteristics (female trees with heavy and many-seeded fruits…).

2. Preparing the scion

   - Gather (15-20cm long) 6-12 months old branches that can produce buds.
   - Choose a scion whose diameter matches that of the rootstock.
   - Trim the base of the branches with a bevel.

3. Preparing the rootstock

   - Choose a plant, with size of a pencil about 8mm.

   The plant diameter will depend on the growing speed of the species which can reach this size anytime between 3 and 12 months.

   - Remove the leaves and branches over 30 cm above the collar
   - Make a side cut of 2-3 cm.

4. Joining the rootstock and the scion

   - Insert the trimmed base of scion in the cut of the rootstock adjusting the edges of the scion to those of the rootstock.
   - Fasten the graft from bottom to top with fastening film.
   - Protect the scion entirely with a transparent plastic.

Cleft grafting could also be used, as it has the advantage of still grafting unsuccessfully grafted plants using the side-tongue graft, as the entire shoot of the plant is cut off between 10 and 30 cm before grafting with scion. Cleft grafting is done as follows:

Preparation of the rootstock

- Eliminate leaves and branches over 15-20cm above the collar when the rootstock has size of pencil (8mm).
- Cut the rootstock 15 or 20cm above the collar.
- Make an incision of 2-3cm from the apex.

Preparation of the scion

- Cut branches of 15-20cm long and matches that during buds.
- Choose a scion whose diameter matches that of the rootstock.
- Trim the base of the scion with a bevel.

Joining the Rootstock and Scion

- Insert the trimmed base of the scion in the incision of the rootstock adjusting the edges of the scion to those of the rootstock.
- Fasten the graft from bottom to top.
- Cover the scion with a transparent plastic.

Taking care of grafts

- Undo the plastic once the bud cover develops (14 days after the grating) on the scion.
- Gradually cut the rootstock each week until it is 1cm from the ligature.
- Cut the fastening film when the shoot has 7 leaves.

- Water, fertilize, weed and treat with a fungicide or an insecticide.
- Eliminate all other shoots on the rootstock other that the scion.

Precautions

- Graft as soon as the branches are taken.
- Avoid damaging the plants accidentally with grafting equipment.
- The cut part must be clean.
- Secure the ligature tightly to ensure good contact.
- Use sharp instruments and disinfect them with spirit or fire.
- Repeat the grafting operation if the scion or shield bud dies (two weeks after grafting).

Protocol for* Allanblackia *marcotting

Marcotting is a form of vegetative propagation that consists of inducing rooting of branches still attached on a tree. After rooting the branch is cut and transferred to a nursery to enable it to grow leaves and become an independent plant. Like all other vegetative propagation technique marcotting allow for the reproduction of exact copies of the mother tree and for early fructification as compared to seedling plant.

On the three main existing classes or marcotting, air layering is the one currently being experienced in *A. floribunda*. It consists of inducing the rooting on a branch of a tree having desirably characteristics a farmer will want to reproduce. After rooting, the branch is cut off, potted and placed in a humidity chamber in the nursery.

Air layering is done as follows:

a) Selection, Placing and Monitoring of Marcotts

- Select your best tree (good, taste, off-season tree, big-fruit yield tree).
- During April, May and June climb up the trees and choose suitable orthotropic branches with a diameter between 3 to 5 cm with the aid of a knife, strip the bark over a length of between 5 cm to 10 cm (choose a reasonable number of branches 10 maximum such that after rooting, the tree would not be over trimmed).
- Scratch the stripped part of the branch to remove the cambium (the slippery part) if not it will reconstitute the bark.
- Sheath the wound with well decomposed sawdust or moist infloresence of palm.
- The sheath is made with transparent polythylene paper so that roots can be seen when it develop.
- If there are many ants and squirrels in the area, check marcotts regulary (once every, month) and adjust those sheath that are about to be destroyed by ants and squirrels.

b) Harvesting

- Roots generally appear depending upon the species two to four months after the marcott is set. Cut the rooted marcotts to a length of 40 cm to 50 cm .
- Label and moisture the marcott and make sure that it remains permanently fresh from the place of harvest to the nursery.

c) Potting rooted marcotts

- Prepare a substrate composed of 1/3 sand and 2/3 black soil or comprising a mixture of 1/3 sand 1/3 black soil, 1/3 compost.
- Dip the stripped marcott in a basin of water.
- Free the roots by carefully removing the substrate between them.
- Insert the marcotts into pots with a lot of care avoid damaging the roots.
- With bee-wax, wood-ash, or mastic, cover the wounded part of the marcott to limit infections.
- Water the marcott and place it in the humidity chamber.
- Water it regulary without flooding it.
- When buds appear, remove the marcott immediately and place it under slight shade with temperature and moisture similar to the outside environment to permit it develop and harden-off.
- One to two months later, the marcotts can be transplanted once the leaves are fully developed
- Once the marcott is on the farm, it can be given any other phytosanitary treatment and care as other plants.

Conclusion

The application of vegetative propagation in tree domestication will ensure the production of uniform planting materials with desirable fruit characteristics (taste, seize, colour), disease resistance, tree morphology in the case of fruit tree and bark characteristics (thickness, active ingredient content) in the case of medicinal plants. However, it is important to inform partners/clients that improved germplasm deteriorates within a few generations when open pollinated. The alternatives are to buy *new* improved material or to take on vegetative propagation to maintain the good germplasm that was released. Also, it should be noted that in a clonal mixture, some clones are more vigorous and some easier to propagate than others within a given time. It is thus advisable to continue releasing and/or accepting new germplasm as the case maybe.

Key References

Anegbeh, P.O. Tchoundjeu, Z, Iruka, C.G. and Nkirika, C.N.(2005)a. Vegetative propagation of indigenous fruit trees :influence of defoilation on survival of rooted marcots (air-layered plants) for Irvingia gabonensis and Dacryodes edulis in onne, Niger Delta Region of Nigeria. *International Journal of Agriculture and rural Development* 6: 119-125.

Anegbeh, P.O., Ladipo, D.O. and Tchoundjeu, Z. (2005)b. Using marcotting technique for fruit development in the African pear *Dacryodes edulis*, *Scientia Africana* 4 (1&2): 102-108.

Atangana AR, Tchoundjeu Z., Asaah EK, Simons AJ and Khasa PD. In press. Domestication of *Allanblackia floribunda*: Amenability to vegetative propagation. *Forest Ecology and Management* **0: 000-000.**

Kengue, J, Tchio F., Ducelier D**.** (1998). Le marcottage aérien, une Technique pour la multiplication du safoutier. Dans C Kapseu et G. J. Kayem (eds): 2e séminaire int. sur la valorisation du safoutier pp 123 – 135.

Macdonald, B. (1996). Practical Woody Plant Propagation for Nursery Growers. Volume 1. Timber Press Inc. 669p.

Mialoundama F, Avana M L, Yombi E, Mampouya PC, Tchoundjeu Z, Mbeuyo M, Galamo GR, Bell JM, Kopguep F, Tsobeng AC, and Abega J (2002) Vegetative propagation of *Dacryodes edulis* (G.Dons) H.J. Lam by marcots, cuttings and micropropagation. Forest, Trees and Livelihoods, Vol. 12, 85-96.

Ngo Mpeck, M-L., Tchoundjeu, Z. and Asaah, E. (2003) Effects of indole-3-butyric acid concentration, cutting stem length and node position on vegetative propagation of *Pausinystalia johimbe. Propagation of Ornamental Plants* Volume 3 No. 2 11 -18.

Shiembo, P.N., Newton, A.C., and Leakey, R.R.B. (1996). Vegetative propagation of *Irvingia gabonensis*, a West African fruit tree. *Forest Ecology and Management* 87: 185-192.

Tchoundjeu, Z., Avana, M. L., Leakey, R.R.B., Simons, A. J., Asaah, E., Duguma, B., and Bell, J.M. (2002). Vegetative propagation of *Prunus africana* : effects of rooting medium, auxin concentrations and leaf area. *Agroforestry Systems* **54** : 183-192.

Tchoundjeu, Z.; Asaah, E.K; Anegbeh, P.; Degrande, D.; Mbile, P.; Facheux, C.; Tsobeng, A.; Atangana, R.A.; Ngo-Mpeck, M.L.; and Simons, A.J. (2006). Putting participatory domestication into practice in West and Central Africa. *Forests, Trees and Livelihoods*. Vol. 16, pp. 53–69

Youmbi, E. and Benbadis, A. (1998). Callogenese et rhizogenese sur le cotyledons de *Dacryodes edulis* cultives *in vitro*. In 2nd International workshop on African pear improvement and other new sources of vegetable oils. Eds Kapseu, C and Kayem G.J. pp 55-60.

Wiesman, Z. and Jaenicke, H. (2002). Introduction to Vegetative tree Propagation. *In Vegetative Tree Propagation in Agroforestry*. Training guidelines and references. Editors: Janenicke, H. and Beniest, J. pp 5-6.