GUIDELINE ON CLONAL PROPAGATION OF *MELIA VOLKENSII*

GUIDELINE ON CLONAL PROPAGATION OF *MELIA VOLKENSII*

Jason G. Kariuki[1], Hisaya Miyashita[2], Taiki Kobayashi[2], James K. Ndufa[1] and Bernard M. Kamondo[1]

[1]Kenya Forestry Research Institute, P. O. Box 20412 Nairobi 00200, Kenya

[2] Forest Tree Breeding Center, Forestry and Forest Products Research Institute,
3809-1 Ishi, Juo, Hitachi, Ibaraki 319-1301, Japan

November 2021

Cover Photos: Clockwise: Young covered grafts of *Melia volkensii*, Grafted seedlings of *M. volkensii*, Fruiting M*. volkensii* clonal tree in orchard, Kibwezi *M. volkensii* clonal orchard (Photographs by J. Kariuki)

Citation:
Jason G. Kariuki, Hisaya Miyashita, Taiki Kobayashi, James K. Ndufa and Bernard M. Kamondo (2021). Guideline on Clonal Propagation of *Melia volkensii.* KEFRI and FTBC

Published by: Forest Tree Breeding Center, Forestry and Forest Products Research Institute
3809-1 Ishi, Juo, Hitachi, Ibaraki 319-1301, Japan

First published on November 10, 2021

ISBN 978-4-86693-554-6

Layout & Design: International Cooperation Division, Forest Tree Breeding Center, Forestry and Forest Products Research Institute

Printed by: SANKEISHA CO., LTD. 2-24-1, Chumaru-cho, Kita-ku, Nagoya, Aichi 462-0056, Japan

Foreword

Melia volkensii is a fast growing drought tolerant and termite resistant tree found only in eastern Africa. The species produces high quality timber which can be harvested in 10 to 15 years. Apart from its highly valuable timber, it is also a good source of fodder, hence it has a high potential for improving livelihoods of dryland rural communities. Melia is readily accepted for planting in eastern drylands of Kenya but can also do well in a number of areas in coast region. Although the acceptance of *M. volkensii* is high, actual planting is still low but increasing and its seedlings production has depended on seeds collected from general sources.

In 2012, Kenya Forestry Research Institute (KEFRI) with support from Japanese International Cooperation Agency (JICA) under the project on 'Development of Drought Tolerant Trees for Adaptation to Climate Change in Drylands of Kenya' developed and started implementing a Melia breeding strategy that constituted, among others, selection of candidate plus trees, establishment of seed orchards and progeny trials using the selected material. The breeding objective was production of fast growing trees for timber production that will be drought tolerant and adaptable to the environment that prevails in Melia growing areas and in drier sites. These activities were continued under Capacity Development Project (CADEP) from 2017 to 2021. The Kenya government and some non-governmental organizations and private sector are promoting Melia planting in the drylands.

KEFRI, with support from JICA, as part of the Melia breeding programme, has established two 11 hectare (total 22 ha) of clonally propagated Melia seed orchards in Tiva and Kibwezi for production of improved seed for raising quality Melia plantations in the drylands. Besides use of improved Melia seed, KEFRI and FTBC recognize the potential of using principles of clonal forestry in further improvement of the species and establishment of commercial plantations of the species. This guideline gives insights on the clonal propagation methods that form the basis for promoting forest tree breeding of *M. volkensii*. The clonal propagation methods tested so far involve raising of *M. volkensii* from shoot cuttings and air layering/marcotting.

The potential of use of root cuttings and air layering/marcotting as means of propagating the species for establishment of the Melia clonal seed orchards and potential for raising improved plantations are also explored. Development of these clonal propagation methods for *M. volkensii* is still under development and this guideline will be updated as new results are obtained. This manual, developed from the practices and experiences gained by KEFRI and Forest Tree Breeding Centre (FTBC-Japan) researchers during the two phases of the project, will be useful as a reference in establishing clonal orchards and plantations of *M. volkensii* and other similar species.

Joshua K Cheboiwo (PhD)

.....

Director, KEFRI

Tohru Nakashizuka (Toru Asano)

.....

Director General of FFPRI

Table of Contents

List of Tables

List of Figures

List of Plates

1. Introduction

Melia volkensii is an indigenous drought tolerant and multipurpose tree species endemic to the Arid and semi-arid areas of eastern Africa. Its common names are Melia, tree of knowledge (English); Mukau (Kamba, Tharaka and Mbeere), Tile (Boran) Boba, Mbamba (Somalia, Oromo) Maramarui (Samburu), and Kirumbutu (Taita). Melia is a deciduous tree that attains a height of 6 to 20 m and an average diameter of about 25 cm. The crown is open while the bark is grey and fairly smooth. Leaves are bright green, up to 35 cm long, compound, with 3-7 deeply lobed leaflets that are densely hairy when young. The flowers are small, white and fragrant, arranged in loose inflorescences. Mature fruits are yellow later developing brownish patches due to the deposit of cork. The fruit is an ovoid drupe 3 to 4 cm long with each fruit containing one to five seeds that are enclosed in a very hard and thick endocarp (nut). The seeds are oval, about 2 cm long and 0.5 cm wide. There are about 200 seeds per kg of nuts, with the average number of seeds per kg of extracted seed ranging from 3000 to 3,500. At the radicle end of the seed is an appendage called the caruncle.

M. volkensii occurs naturally in the semi-arid zone of Ethiopia, Somalia, Kenya and Tanzania at altitudes between 100 m and 1700 m, in areas with mean annual rainfall of 300-1000 mm and temperature range of 26 - 38 ^{o}C (Orwa *et al*., 2009) (Figure 1). The species is found in deciduous bushlands in association with Acacia-Commiphora vegetation. The species grows well mostly on sandy soils, sandy loams, or sandy clay soils with good drainage.

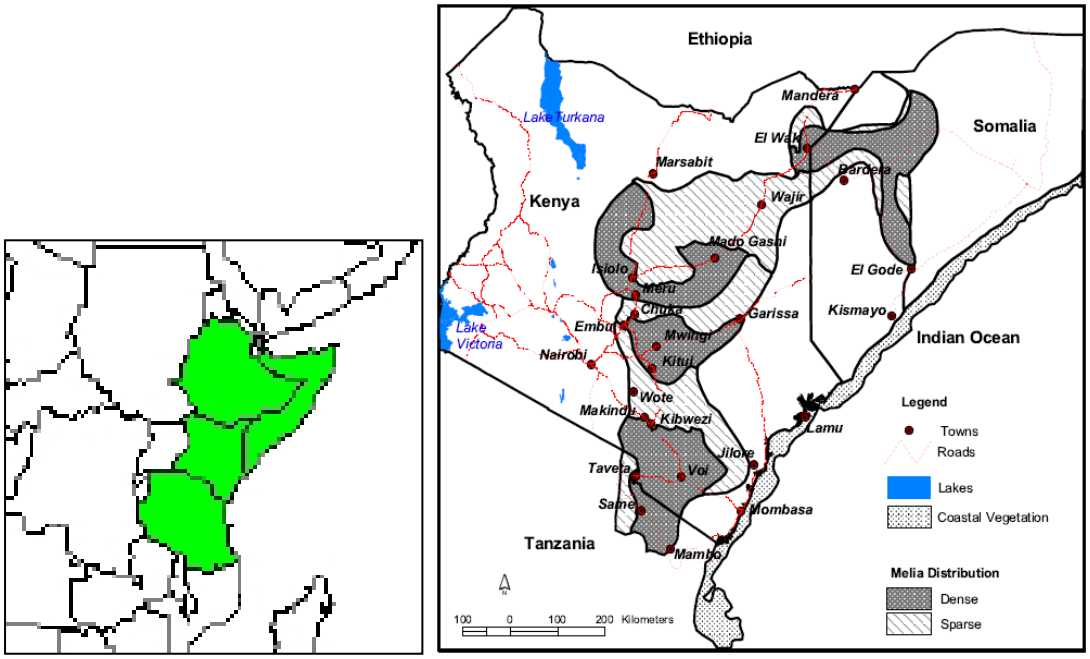

Figure 1: Distribution of *Melia volkensii* in eastern Africa

Melia volkensii is a fast growing tree species which coppices well with a rotation of 8-15 years. The species is preferred because of its high value timber used for construction and furniture. The wood is durable and termite resistant and easy to work and shape and therefore used to make log hives. Twigs, leaves and fruits are used as fodder for goats, cattle and sheep during the dry season. The species also contains compounds that are toxic to insects and aqueous extracts of the fruits are traditionally used to control fleas and ticks.

In an effort to leverage on the species potential, Kenya Forestry Research Institute (KEFRI) with support from Japanese International Cooperation Agency (JICA) developed and implemented a Melia breeding programme from 2012 that constituted among others, selection of Candidate Plus Trees (CPTs), collection of scions from the CPTs, raising of grafted seedlings that were used in establishment of clonal seed orchards.

2. The role of clonal propagation in Melia breeding

In the field, selection of Candidate Plus Trees (CPTs) from dry and semi-arid areas was based on the following tree characteristics: Excellent growth and tree form; trees not damaged by pests and are drought tolerant. The CPTs are important breeding stock and genetic resource for promoting improved forest establishment and tree breeding in future. However, since the CPTs are not tested, it is not clear whether the observed characteristics are genetically determined or are due to environmental influences, until progeny tests are carried out to confirm their genetic worth.

In order to preserve the original genetic composition, the individual CPTs were clonally propagated and planted in two locations (Tiva and Kibwezi) as 1st generation clonal seed orchards. The clonally propagated material in the orchards also serves as conservation stands of the CPTs.

3. Purpose of the Guideline

This guideline gives insights on the clonal propagation methods that form the basis for promoting forest tree breeding of *Melia volkensii*. The clonal propagation methods involve raising of *M. volkensii* from shoot cuttings, root cuttings and air layering/marcotting as means of propagating the species for establishment of the Melia clonal seed orchards and potential for raising improved plantations. Development of clonal propagation methods for *M. volkensii* are still under development and this guideline will be updated as new results are obtained. This guideline also presents review of methods of clonal propagation methods, the application of clonal propagation in establishment of orchards and potential of other methods for clonal propagation of Melia.

4. Review of clonal propagation methods

The main goal of clonal propagation is to reproduce plants with the identical genotype to the parent plant. In forestry, several methods such as grafting, cuttings, air layering/marcotting and tissue culture have been developed for clonal propagation of trees. We have tested some of these methods for *Melia volkensii* propagation. Although the preferred method for the species' propagation is through seed, clonal propagation methods are important in the breeding process such as in clonal seed orchard establishment and deployment of hybrids.

4.1 Grafting

Grafting is one the basic methods of vegetative propagation of trees whereby the scion of the target tree is joined onto a seedling rootstock of a compatible tree species. There are several methods of grafting including bark grafting, cleft grafting and whip grafting. A successful graft is achieved through fusion of the cambium layers of both the scion and the rootstock. After grafting, callus (the healing tissue that forms in the wound of a plant) fuses the cambium of rootstock with the cambium of the scion allowing passage of water and nutrients from the rootstock to the scion

and carbohydrates produced by photosynthesis to the rootstock. Top cleft grafting is the mostly used method for grafting of *M. volkensii*.

Successful grafting process requires the following: (1) Timely planning/scheduling of work, (2) Adequate preparation of rootstock, (3) Use of appropriate techniques and skills acquired through training (Table 1).

Table 1: Grafting process and detailed activities in each task

Task	**Details**
Timely work	• For grafting it is desirable to collect the scion for grafting during the dry season (dormancy period), maintain freshness of collected scions, fast delivery to grafting site, graft within three days of collection and refrigeration where when necessary. • The best time to plant Melia in the ASALs of Kenya is December, just after the long-term rainy season. Therefore, the grafting work must be completed by the end of September in order to allow at least 4 weeks to raise the grafted seedlings in the nursery. It is desirable to carry out grafting work in August to September.
Preparation of rootstock	• By the time the grafting work starts, rootstocks that match the diameter of the scions must have been raised. In order to grow Melia rootstocks to the size of the scion diameter, it is desirable to sow seed at least 4 months earlier. If possible, use seedlings from the same mother tree as the rootstock or rootstock of comparable growth rate of the scion tree.
Cleft Grafting method	• It is important to match the cambiums of the scion and the rootstock. The cambium is located between the xylem and the phloem. The cambium of the scion and the rootstock are joined at a slight angle, and both cambium intersect at some point. The joined surfaces need to be as smooth as possible in order to bring the joints into close contact. Therefore, the joint surfaces of the scion and the rootstock are cut with a very sharp blade or scalpel.
Fixing the graft joint	• Immediately after grafting, it is necessary to form callus promptly and inosculation as soon as possible. Therefore, tie the grafted section tightly to avoid any shifting of joined parts. For this, a grafting tape or a suitable alternative such as parafilm are used. After confirming inosculation of the joint, it is necessary to promptly remove the tape so that the growth at grafting joint is not hindered by tightening tape as the joint grows and expands.
Prevention of drying graft	• To prevent the joint from drying out, apply a glue based reagent containing a fungicide such as Topsin or wood glue containing a fungicide to any cut surfaces on the scion. Also, cover the upper part from the joint (scion) with a polyethylene bag to maintain humidity. Make an incision off the top corner of the bag to prevent it from becoming over-humidified due to the sealed state.
Prevention of sprouts on rootstock	• Immediately after grafting, the rootstock is normally aggressive in sprouting and it is important to remove any sprouts of buds from the rootstock part of the grafted seedling. If allowed to grow, the sprouts overwhelm and kill of any growth from the scion part.

Task	Details
Raising of grafted seedlings	• Immediately after grafting, use a 50% shade net cloth to partially block sunlight and wind. The purpose of shading is to moderate photosynthesis and transpiration in the scion and promote the inosculation of the scion and the rootstock. Wind protection is intended to prevent excessive drying of the joints. • About two weeks after the grafting, the scion and the rootstock will have inosculated and the joints will not come off easily. Therefore, remove the polyethylene bag covering the scion and also replace the 50% shade net with a 30% one to increase the light and commence acclimatization. • After about 4 weeks after the grafting, the scion and the rootstock are fully inosculated and the joined parts become woody. At this stage, remove the shade net completely to expose seedlings to prevailing weather elements and allow acclimatization.

Plate 1: Effects of not matching growth of scions and rootstock

4.2 Cuttings

Stem cuttings is a vegetative propagation method whereby small branches of the trees are cut and used as scions for root induction. The scions are sterilized and inserted into rooting media that is highly porous such as sand, coco peat and vermiculite. Rooting hormones such as IBA are used to stimulate development of roots. In most cases, callus develop at the inserted cut end of the scion before rooting (Plates 2 and 3). However, in most cases the rooting process is difficult for most tree species and few tree species are amenable to vegetative propagation by cuttings. In addition, the rooting rate differs depending on the families and varieties even within tree species that are possible to propagate by cuttings. In general, rooting rate decreases as the age of the tree increases. It is therefore vital to understand the tree characteristics that enhance rooting such as the correct timing of scion collection, type of cutting, age of the tree and cuttings rooting environment (temperature, light intensity and humidity) in order to use this method of propagation.

Plate 2: Propagation of *Melia volkensii* using cuttings

Plate 3. Propagation of *Melia volkensii* using various media

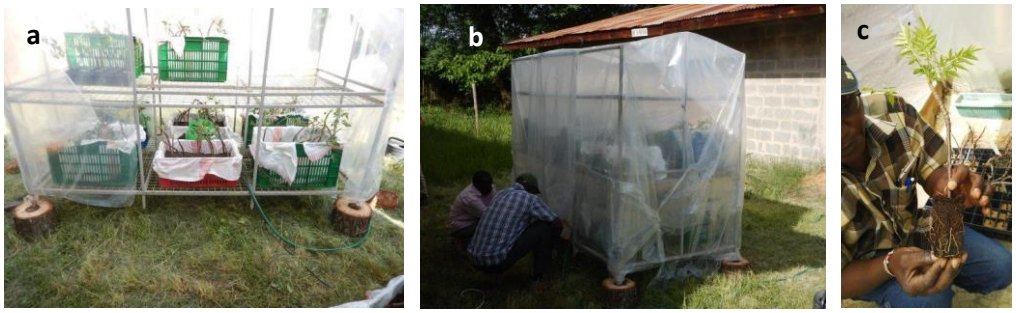

Plate 4. Propagation of *Melia volkensii* using various media

5. Application of clonal propagation in establishment of *Melia volkensii* clonal seed orchards

Establishment of clonal seed orchards was done using the selected Candidate Plus Trees as sources of reproductive material (scions). The process of clonal seed orchard establishment involves, raising of rootstock, collection of scions and grafting, raising of grafts in the nursery, identification of suitable land/site for orchard establishment land preparation and fencing, orchard layout design, staking, pitting and finally planting (Figure 2). A suitable site for Melia orchard establishment should be fairly dry, slightly slopping with well-drained deep soils devoid of rocks.

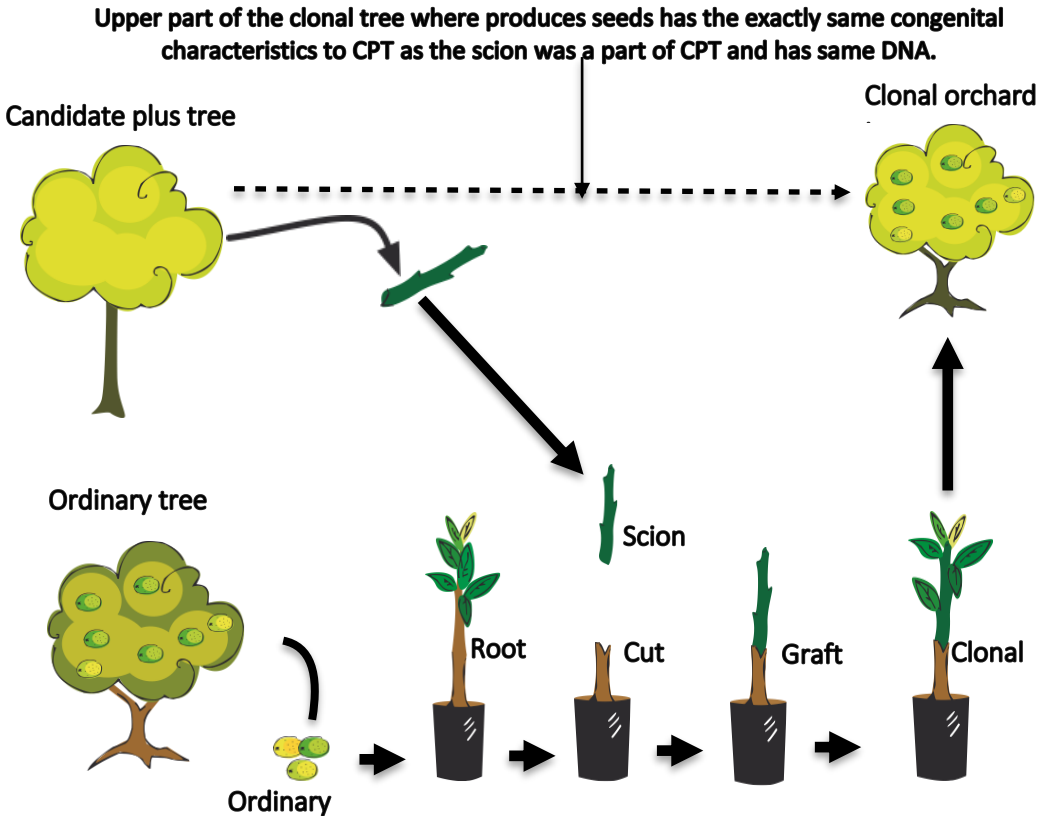

Figure 2: Concept of seed orchard establishment

5.1 Rootstock potting soil preparation

The soil for raising rootstock seedlings should rendered weed free. This was done by collecting and storing the soil for one month to allow germination of weed seeds. The soil was fumigated using Nemasol fumigant (Mentam sodium 510 GH) then covered completely with a polythene sheet for 10 days before potting. The potting mixture was made of forest soil, sand and manure in a ratio of 2:1:1 (Figure 3).

(a)

SUGGESTED STANDARD MIX

Forest soil (Loamy clay)	River sand	Manure (decomposed)
2	1	1

Test the mixture by rolling a damp sample in your hand.
A good mixture should roll and hold its shape
but it breaks if the roll is bent.
If it does not hold add 1 part Forest soil (Clay).

(b)

Figure 3: (a) Soil potting mixture for drylands and (b) Forest soil, sand and manure

5.2 Melia Seed pre-treatment and sowing for raising rootstock

For the clonal seed orchards scions are grafted onto Melia rootstock. The rootstocks should be minimum of 4 months old before grafting. However, rootstock raised in the nursery for up to one year can also be used provided frequent root pruning is done. The time of seed sowing to raise the rootstocks is therefore determined by the projected grafting time. For example, in establishing

the first generation orchards rootstock seed were sown in in April 2012 for grafting in September 2012.

For successful germination, Melia seed is pretreated as follows

- Nipping of seed by breaking the caruncle (Plate 5a) and soaking in cold water mixed with fungicide e.g. Ridomil or Benlate (5 g/l) for 12 to 24 hours.
- After soaking, the seeds are slit longitudinally starting at the point of nipping to the other end of the seed using a sterilized razor blade or scalpel (Plate 5b). The slit should be restricted to the seed coat. Slitting allows leaves to be released on germination.
- For optimum germination, Melia seed should be sown on sterilized river sand (Plate 6). The sand is sterilized by drenching it using 450 ml of sodium hypochlorite solution (e.g. JIK) per 20 litres of water and also sprayed with a fungicide (e.g. Ridomil). The sterilized sand is placed in germination bed, a non-mist propagator or a plastic basin. Seed are sown by placing the seed on the sterilized sand and covering with a layer of sand equal to double the length of the seed. The sand is watered thoroughly using sterilized water. After sowing, the germination media is covered with a clear polythene sheet. A space of six inches between the polythene sheet and media should be allowed to avoid contact of the tender germinating seedlings which cause mortality. Watering of the bed after sowing is done only when necessary by observing the wetness of the germination media. Melia will germinate in 3 to 6 days.
- The germinated seeds are then transplanted into pots filled potting mixture.

(a) Nipping


(b) Slitting


(c) Soaking

Plate 5: *Melia volkensii* seed pretreatment

Normal tree nursery management practices should be undertaken to ensure a healthy root stock. The rootstock is managed in the nursery through watering, weeding, root pruning where necessary and pest control.

(a) See pre-treatment (b) Sowed seed (c) Geminated/pricked out (d) Ready rootstock

Plate 6: Seed Pretreatment, sowing and nursery management

5.3 Collection of scions

In Melia, scions should be collected and grafted within 72 hours. The size of the scions collected should be matched to the size of the rootstocks for best union. The number of scions collected should allow for 25% above the number to be grafted. Also, the number of grafted seedlings should be 20% more than what is needed for planting to allow for any beating up. In the first generation clonal orchards, the scion collection from the 100 selected CPTs was as follows:

(i) Scion identification - This was precisely done to ensure that scions collected were appropriate to the size of the rootstock in the nursery. Clean, healthy and mature scions were identified and cut from the CPTs with a secateur/sickle type knife mounted on 8 to 12 m extended rods and about 6 inch scions cut using a secateur (Plate 7a).

(ii) About 75% of the leaves were removed and the scions drenched with clean water to keep them moist (Plate 7b).

(iii) They were then wrapped with newspapers, fastened with masking tape and labeled. A batch from one plus tree was placed in a polythene bag and labeled again and packed in a cool box (Plate 7c).

(iv) The scions should be delivered to the grafting centre in the soonest time possible to avoid dessication but not later than 2 days after collection.

(a) Scion collection

(b) Scion drenching

(c) Scion packaging

(c) Transport scions in coolbox

Plate 7: Scion collection and processing

The equipment used in scion collection, packaging and transportation were: Ladder, standard secateurs, long handled secateurs (8 -12 m long) or looping shears, cool boxes and cooling ice blocks, labeling material, packaging material and sanitizers (sodium hypochlorite, surgical spirit) for cleaning the equipment. The equipment should be cleaned after scion collection from any single CPT.

5.4 Grafting

Grafting is done such that the cambium layer of the scion matches or rests on the cambium layer of the rootstock. Cambium is a thin layer of living cells between the bark and the wood from which bark and wood tissues are formed. It is the source of all girth growth in woody stems. As the cambium cells divide, bark is produced to the outside and wood to the inside. During the healing of the wound incurred during grafting, the cambium produces callus cells which will join the scion and the rootstock. Eventually, the callus cells differentiate to form the vascular connections between the rootstock and scion (Plate 9). The best practice recommended for grafting in Melia was followed in grafting of the scions collected from CPTs. Of the 80 scions collected from each CPT, 72 were grafted onto Melia rootstocks at the KEFRI's Kitui nursery in September 2012 using the top grafting method (Plate 8). Proper documentation and labeling of grafted seedlings using allocated CPT code should be done so that each grafted seedling has a source identity.

The following equipment is required for grafting:

- Grafting knife (or surgical blades); Grafting tape; Secateurs, Grafting paste
- Chairs/table and small stool, basin/bucket
- Fungicide and 70% surgical spirit
- Cotton wool and gloves
- Labeling material
- Shade nets 30% and 50%

- Polythene sheet

A high level of hygiene should be maintained at all times. Grafting knives/blades should be dipped periodically into methylated spirits to sterilize them during grafting.

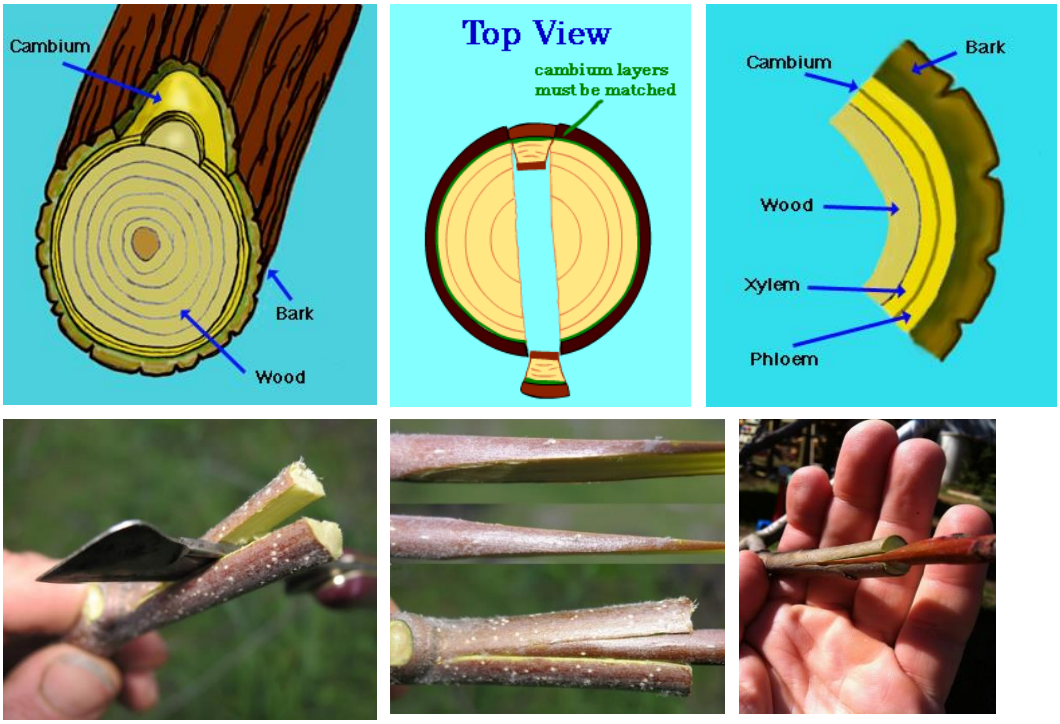

Plate 8: Cambium layer and grafting cross section

The grafting process involved watering the rootstocks soil to saturation an hour before grafting. The grafting equipment were assembled, cleaned and sterilized using 70% surgical spirit. Grafting of Melia was done using the following steps:

- Sterilize the scions by dipping the scions into water mixed with appropriate amount of fungicide for 5 to 10 minutes.
- Using a secateurs, make a clean horizontal cut and discard at least the top half of the rootstock (Plate 9a).
- On the remaining rootstock, use a sharp grafting knife or surgical blade to make a vertical cut (wedge) at the top centre. The wedge should be 2 to 2.5cm deep.
- On the scion, make 2 clean cuts to shape the lower part of the scion into a balanced V shape using a sharp grafting knife or surgical blade. The size of the cut on the scion should match the size of the wedge on the rootstock for a perfect fit (Plate 9b).
- Insert the V shaped part of the scion into the rootstock wedge. Ensure proper contact of scion and rootstock. It is important that the graft union be a clean, snug fit with intimate contact of the cambium layers in both the scion and root stock. Wrapping the union with grafting tape or parafilm provides this intimate contact as well as providing support for the scion on the root stock.
- Completely cover the top part including stem of the grafted seedling with a polythene bag (Plate 9e) and secure at the base of the seedling using a cotton twine.

- Place the grafted seedlings in nursery beds covered with a 50% shade net (Plate 9f). Reduce shading to 30% after one month. Remove the polythene bag covering the grafts once a graft has developed leaves.
- Two weeks before planting, expose the seedlings to full sunlight for acclimatization. In case of heavy rains, cover the grafts with a polythene sheet supported by a frame.
- Manage the grafts in the nursery through watering, root pruning and disease control for about 4 months until they are ready for planting.

Plate 9: The *Melia volkensii* grafting process:
(a) Cutting off rootstock (b) Fixing scion on rootstock (c) Parafilm tape (d) Tied graft using parafilm (e) Cover graft using Polythene (f) Shading of new grafts

5.5 Management of grafts

This is a crucial stage of the grafting process necessary to maximize survival of grafted seedlings. For Melia, it is important to maintain a moist environment around the grafted wound and the reason why the grafts are enclosed in polythene to ensure high humidity that promotes wound healing. On the other hand, overwatering damages Melia grafts. Watering should be done directly to the potted soil and not the leaves (once the polythene paper is removed). The rootstock portion of the graft should be checked for buds and sprouts frequently and removed as they can grow and suppress the grafted scion development and growth.

(a) Grafting (b) Covered grafts (c) Growing grafts

Plate 10: Grafting and graft management


Plate 11: Grafting of seedlings at Kibwezi

6. Overview of *Melia volkensii* clonal propagation methods under development

This section describes other methods of vegetative propagation such as stem cuttings, root cuttings and air-layering/marcotting which are still under development for *M. volkensii* and results provided are preliminary. We have also described methods tried for *Melia azedarach* in Japan for possible adoption in *M. volkensii*.

6.1 Cuttings

6.1.1 Adoption of Melia azedarach propagation method for M. volkensii propagation

Melia azedarach is a species of the same genus as *M. volkensii*, with a distribution in Japan, Taiwan, China and the warm regions of Himalayas. It is also widely planted in Argentina and Paraguay in South America, and is used for furniture. *M. azedarach* is as a fast-growing tree that can earn income in the short period in Japan.

Preliminary series of tests were conducted in Japan included tests on timing (season) of cuttings, length of scions and media for propagation. In Kumamoto, the original method of de-budding has been developed by Kumamoto Prefectural Forest Research Center since 1997. It has been reported that it is difficult to propagate the species by cuttings, and seedlings are mainly produced through seed sowing.

M. azedarach cuttings study was conducted in FTBC (Ibaraki, Central of Japan in June 2017 as a reference for the development of *M. volkensii* cuttings propagation methods in Kenya. The study was conducted by the method commonly used for *Cryptomeria japonica* (Conifer, Japanese cedar) during the rainy season, which is the appropriate season for cutting propagation in Japan. As a result, about 40% rooting rate was obtained. However, this trial was using scions from young seedlings and not from mature trees (e.g. Candidate Plus Tree). The outline of the study was as follows:

- The scions for use in cuttings were collected from the sprouted branches generated from seedlings that had been cultivated in the nursery and glasshouse at FTBC Japan. The collected scions were immersed in slow running water as well as in Indole Acid diluted solution for 24 hours and 96 hours respectively, in order to restore the vitality of the scions. The length of the scions used were 20 cm and 40 cm, because longer scions are better for rooting even in *C. japonica* in Japan. Pumice as well as forest soil in FTBC were used for the rooting medium, the cutting beds were placed in the greenhouse or outside in the cypress forest.

After one (1) month, 22 (39%) of the cuttings immersed for 24 hours rooted, with no difference between running water and Indole Acid diluted solution. Most of the rooted cuttings were in pumice. 12 of the cuttings (21%) immersed for 96 hours rooted (Table 2, Plate 12). From the results, it can be seen that immersion for 24 hours, with pumice as the rooting medium, and raising the cuttings in a greenhouse (temperature control) are effective for rooting of *M. azedarach*.

Table 2. Result of the *Melia azedarach* cutting study

Cutting propagation test (*Melia adzedarach*) Jul. 2017 FTBC Hitachi

Treatments		Soil for rooting	Storage space	Scions		Result	Rooting rate
Immersion	Period			Length	Number	Rooting	
running water	24H.	Kanuma	Green house	40cm	10	5	50%
				20cm	8	4	50%
		Soil	Outside	40cm	10	2	20%
Indolyl Acid diluted solution		Kanuma	Green house	40cm	10	5	50%
				20cm	8	6	75%
		Soil	Outside	40cm	10	0	0%
			Total		56	22	39%
running water	96H.	Kanuma	Green house	40cm	10	3	30%
				20cm	8	5	63%
		Soil	Outside	40cm	10	1	10%
Indolyl Acid diluted solution		Kanuma	Green house	40cm	10	3	30%
				20cm	8	0	0%
		Soil	Outside	40cm	10	0	0%
			Total		56	12	21%

* Rooting was comfirmed 1 month after cutting.

Plate 12 Rooted cuttings

6.1.2 Cuttings of M. volkensii

Preliminary trial of rooting of cuttings of *M. volkensii* was started in 2016 in FTBC and in KEFRI Kitui in 2020. Several factors were considered in designing the trial.

- *Determination of the appropriate time for collecting cuttings*

In order to determine the optimum time for collecting Melia cuttings, we commenced testing in which cuttings were done at monthly intervals. The trial started in February 2020 in KEFRI Kitui, but was suspended due to the effects of COVID-19. Using 15 families with good growth, 9 scions of each family were used in the trial. Preliminary results show that use of old coco peat as rooting media for cuttings, was good for rooting.

- *Size of scions for cuttings*

The size of scion for cuttings must take into account the content of internal nutrients and plant hormones. In the case of the cuttings of Cedar in Japan, scions of 40 cm or more are used in warm areas in order to shorten the seedling raising period in the nursery and reduce costs. However, 20 cm scions are usually used. In the case of *M. volkensii*, the appropriate size of scions is still under investigation. However, we recommend use of 25-30 cm scions.

- *The current results*

The formation of callus and rooting with several roots was observed in a number of cuttings, but the conditions and reasons for low rooting are under investigation.

The trials so far have provided information on best type of media for cuttings (old coco peat is best), most appropriate container (multi-cavity containers), and the control of temperature and

humidity. In the future, we will test differences in rooting properties depending on the scion collection time and families.

Since the purpose of tests on propagation through cuttings was to preserve the original genetic materials, the purpose would have been achieved, whether 30 cuttings produce 10 seedlings or 100 cuttings produce 10 seedlings. A summary of the findings and chronology of activities are shown in Table 3. The detailed of the trials are shown in Table 4:

Plate 13: Cuttings of *Melia volkensii* in 2016
(a) at start (b) successful roots developed)

Table 3: Trials of root cuttings and summary of findings

Period	**Conditions**
2013 / August (Dry season)	• We examined the type of soil, the size of the scion, and the condition of the scion in dry season. • The container used a plant pot and was covered with polyethylene-sheet. • Type of soil used: Sand or forest soil • Size of scion; large (40cm) or small (20cm). • Check the condition of scions (leaf settling, green or brown, etc.). • No rooting hormones used except fungicide on rooting media. • ***Results*****:** Kitui, Greenhouse, Sand, Large size; Rooting was observed from one scion. Conclusion: Large scion is better.
2016 / February (Dry season)	• We used the chemical agent for promoting rooting and examined the type of soil. • ***Result*****:** Kitui, Greenhouse, Sandy soil used, Large size scion, IBA used; Rooting was observed from one scion. Conclusion: Using IBA.
2016 / October (Dry season)	• We collected scions from ten (10) families from Tiva clonal seed orchard. • ***Result*****:** Kitui, Greenhouse, Sandy soil, Large size, IBA; Rooting was observed from one scion. Conclusion: Continue to collect scion from well-growing Melia CPTs clone.
2018 / February (Dry season)	• Preliminary test in Japan, the process of immersing the scions in running water for 24 hours to refresh the scions is important, so do the same for Trial in Kenya. • Since the use of plastic pots has been banned, use biodegradable pots. • Long pot with a small diameter is easy to handle. • ***Result*****:** Not observed the rooting**.** Point: Continue the trial.

Period	Conditions
2018 / April (Rainy season)	• In preliminary tests in Japan, the good results have been obtained in the rainy season. • ***Result*:** No observed rooting. Conclusion: Low temperatures during the rainy season not conducive to rooting.
2018 / November (Rainy season)	• Continued the trial in the rainy season. • Small polythene house covered with black shade net. • Long scions (40cm), immerse in running water, IBA, Long plant pots used. • ***Result*:** No observed rooting. Conclusion: The temperature inside the greenhouse was over 50 degrees Celsius, hence could have affected rooting.
2019 / August (Dry season)	• Crate boxes with 40cm depth and holes at the bottom used. • 3 types of media used: Sand, Pumice and Old coco peat. • Scions collected from three (3) families. • Long scions (40cm), immersed in running water for 12 hours and dipped in IBA powder before planting in media. • The crate box was placed in the middle of the plant shelf in the greenhouse. • Irrigation; once a day, two hours. • ***Result*:** At Kitui greenhouse, Old coco peat media, Large sized scions (40cm); Rooting was observed from two scions**.** Conclusion: Old coco peat is better.
2019 / November (Dry season)	• Using irrigator • Using Rock wool plant container. • ***Result***: Not observed the rooting. Conclusion: Humidity was too high.
2020 / February (Dry season)	• The trial of cutting was started and was to be repeated at monthly intervals to test effects of cuttings season on rooting. • Collect scion from fifteen (15) varieties. Collecting scion will have done in Tiva clonal seed orchard. • Nursery crates 40cm depth used to hold cuttings media. • Type of soil used: Old coco peat. • Long scions used (40cm), immersed in slow running water for 12 hours, IBA rooting powder used. • Irrigation; once a day, two hours.

Table 4: Details of trials of root cuttings, treatments and conditions

Date	Season	Site	Place	Sunshade	Type of soil	Container	Collect scion Tree age	Size of scion	Chemical agent	Refreshing
2013/Aug.	Dry	Kitui	Greenhouse Nursery	Shade net	Sand Forest soil	Plant pot	Tree age 2	Large (40cm) Small (20cm)		
2013/Aug.	Dry	Kibwezi	Nursery	Shade of tree	Sand Forest soil	Plant pot	Tree age 2	Large (40cm) Small (20cm)		
	Result :	Kitui, Greenhouse, Sand, Large size; Rooting was observed from one scion								
		Kitui, Nursery, Sand, Large size, not covered sheet; Rooting was observed from one scion								
		Kibwezi; No rooting observed								
2016/Feb.	Dry	Kitui	Greenhouse	Shade net	Sandy soil	Plant pot	Tree age 5	Large (40cm)	Auxins; (IBA)	

Date	Season	Site	Place	Sunshade	Type of soil	Container	Collect scion Tree age	Size of scion	Chemical agent	Refreshing
2016/Feb.	Dry	Kitui	Nursery	Shade of tree	Sandy soil	Propagator	Tree age 5	Large (40cm)	Auxins; (IBA)	
	Result :	Kitui, Greenhouse, Sandy soil, Large size, IBA; Rooting was observed from one scion								
2016/Oct.	Dry	Kitui	Greenhouse	Shade net	Sandy soil	Plant pot	Tree age 5	Large (40cm)	Auxins; (IBA)	
	Result :	Kitui, Greenhouse, Sandy soil, Large size, IBA; Rooting was observed from one scion								
2018/Feb.	Dry	Kitui	Greenhouse	Shade net	Sandy soil	Long pot	Tree age 7	Large (40cm)	Auxins; (IBA)	running water (24h)
	Result :	Not observed the rooting								
2018/Apr.	Rainy	Kitui	Greenhouse	Shade net	Sandy soil	Long pot	Tree age 7	Large (40cm)	Auxins; (IBA)	running water (24h)
	Result :	Not observed the rooting								
2018/Nov.	Rainy	Kitui	Vinyl house	Shade net	Sandy soil	Long pot	Tree age 7	Large (40cm)	Auxins; (IBA)	running water (24h)
	Result :	Not observed the rooting								
2019/Aug.	Dry	Kitui	Greenhouse	Shade net	Sand Pumice Old coco peat	Crate	Tree age 8	Large (40cm)	Auxins; (IBA)	running water (24h)
	Result :	Kitui, Greenhouse, Old coco peat, Large size; Rooting was observed from two scions								
2019/Nov.	Rainy	Kitui	Green house	Shade net	Sand Pumice Old coco peat	Crate	Tree age 8	Large (40cm)	Auxins; (IBA)	running water (24h)
	Result :	Not observed the rooting								
2020/Feb.	Dry	Kitui	Greenhouse	Shade net	Old coco peat	Crate	Tree age 9	Large (40cm)	Auxins; (IBA)	running water (24h)
	Collected scions from fifteen (15) families. Collecting scion done at Tiva clonal seed orchard.									
	The trial of cuttings started and done at monthly intervals, but was suspended due to the effects of COVID-19.									

6.1.3 Proposed treatment to promote rooting by hardening

The following treatments are reviewed for possible application in rooting of *M. volkensii*

(a) Cold treatment

By gradually exposing cuttings to low temperature, polysaccharides are accumulated in the cells of the plant. Use the mechanism of acclimatization to low temperature that is used in rooting plants in high latitude areas use to overwinter. Gradually lower the temperature to acclimatize cuttings to a low temperature, and finally store at 4 °C for about 2 weeks. This treatment is expected to accumulate polysaccharides in cells and promote rooting.

(b) Wilting treatment

When the water absorption from the roots is restricted in the plant, the water absorption amount becomes smaller than the transpiration amount from the leaves, and the plant wilting. Wilted plants recover their water absorption and shift to a state where the air humidity is increased to reduce the amount of transpiration. Utilizing this temporary wilting phenomenon, it is temporarily placed in a dry state just before the cutting, and after the cutting, the osmotic pressure of the cuttings is used to suck up the water and cause the water to flow into the cells. This treatment expects the effect of promoting roots.

(c) Bottom heat treatment

It is a method developed in the horticulture to promote rooting of cuttings such as flowers and vegetables. It is also a combination with cold treatment, and it is a method that expects rooting promotion by warming the rooting site at the bottom while keeping the upper part of the scions in a low temperature state. It is a method that can be done by simple hydroponics.

6.1.4 Clonal propagation protocol

The following is the interim recommended protocol for vegetative propagation in *M. volkensii* based on the preliminary results and observation in both *M. volkensii* and *M. azedarach* rooting trials (Table 5).

Table 5: Protocol for *Melia volkensii* vegetative propagation through cuttings

Activity	**Details**
1. Scion collection, transport and refrigeration	• At the time of scion collection, scion larger than the scion of cuttings are collected from the branches. • Cover the cut end of scions with wet newspaper to prevent it from drying out. • Put the scion in a large polyethylene bag that can hold everything, and wrap it with tape to prevent it from drying out. • Place in a cooler box with a polyethylene bag of ice and refrigerate. • Promptly transport to the processing laboratory/nursery.
2. Refreshing scions	• It is also important to immerse the scions in running water before performing the rooting promotion treatment with auxins. To prevent the scions from wilting between the time they are collected and planting, soak them in slow running water for about 24 to 72 hours to refresh the scions.
3. Adjustment of scions	• Use a sharp knife to smooth the cutting edge of the scion. In addition, to cut the cutting edge of the scion on angle. In order to stabilize, the cutting edge of the scion should be wider.

Activity	Details
4. Rooting promotion treatment using chemical agents	• Rooting promotion treatment using auxins, which are plant hormones, is common. Auxins Indole-3-acetic acid (IAA) and Indole-3-butyric acid (IBA) are often used for rooting promotion treatment of trees. Auxins are for softwoods and IBA for hardwoods such as Melia.
5. Rooting promotion treatment	• For rooting promotion treatment with IBA solution, soak in a solution diluted to the specified concentration (in label) for 24 hours. Alternatively, it may be immersed in an undiluted solution of IBA solution for 3 seconds and used for cuttings. In that case, do it just before cutting planting.
6. Media for planting cuttings	• The media for cuttings planting, it is necessary to select a media with high porosity or aeration in order to avoid the retention of water. In the case of Melia, Sand, Pumice, Coco peat (old, Fermented) has been tried and Coco peat found to be best.
7. Moisture management of planting media	• It is important to consider mist irrigation time (morning or evening), the number of irrigation times, and the continuous irrigation time. Further, to consider a method of humidity control by sealing with polyethylene sheet to avoid evaporation of water. • It is necessary to change the conditions for moisture management and humidity control at the time when the cuttings are planted and during the subsequent growing stage. For example, after confirming rooting, it is necessary to gradually reduce the amount of irrigation to promote root growth.
8. Control of light environment	• It is necessary to avoid photosynthesis in order to suppress the elongation and rapid growth of scion and encourage rooting. Therefore, immediately after the planting the cuttings, a 50% shade net is used to block excessive sunlight. At the same time, it is necessary to pay sufficient attention to the control of temperature and humidity. • After rooting, in order to promote growth, the degree of shading of the shade net should be reduced to 30% shade net. In this way, the rooted cuttings gradually acclimatize to the existing.
9. Raising of cutting's seedlings	• After about a month and a half, raise seedlings in the open so that they can adapt to the environment. The seedling should be grown to 30 cm or more in height, and the bottom of the rooting should be visually confirmed.

6.2 Root cuttings

Root cutting is one of the general clonal propagation method, and its use have been reported in various species such as *Populus tremuloides* (Snedden *et al*., 2010), *Paulownia tomentosa*, *Paulownia fortunei* (Ede *et al.,* 1997), *Prunus avium* (Ghani and Cahalan, 1991). Other examples include the mangroves. In general, trees that send up suckers from their roots are the best candidates for propagation through root cuttings. However, with root cuttings one will be propagating the root stock variety.

Grafting is used as the clonal propagation method in *M. volkensii*. However, the method requires investments in time and effort to prepare the plant materials, and a practiced technique and dedicated tools are also required. Therefore, root cutting method for clonal propagation was investigated for *M. volkensii* (Hanaoka *et al.* 2016).

In Melia, root size was found to be important for the successful formation of adventitious buds, with larger roots forming adventitious bud at the rate of 77% compared with 37.2% for smaller roots (Hanaoka *et al.* 2016). The recommendation is to use root with a cut edge larger than 15 mm and fresh weight greater than 20 g. Cross-sectioned root without root tip is also usable, and therefore, two or more vegetative propagules can be obtained from one tap root. The adventitious buds are formed on cut edge of proximal side (Plate14). Therefore, distal end of root has to be on the bottom side when cross-sectioned roots are planted. One has an easier time identifying the cut end later on if you make your cuts at an angle. It is best to collect the root when trees are dormant as at this phase, the tree's growth energy is stored in the roots.

Plate 14: Shoot formation from root (proximal tip at the bottom) in *Melia volkensii*

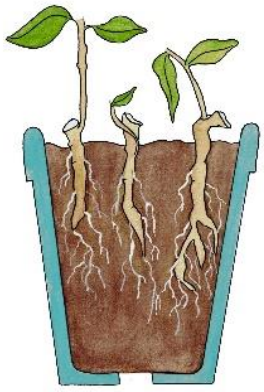

Figure 4: Illustration of root cuttings propagation

6.3 Air layering/ Marcotting

This was tried at KEFRI's Tiva Melia seed orchard site in 2019 (Plate 15). After two months it was observed that the marcotted branches retained leaves. All the branches callused but these did not develop into roots. It is difficult to pinpoint the cause of the failure. Therefore, in order to test effects of the rainy season and the dry season, the activity will be repeated to test the effects of season on rooting.

Plate 15. Marcotting of *Melia volkensii* at Tiva;
(a) Incision to remove bark, (b) Removed bark (c) Media preparation (d and e) Tying, (f) Marcots ready and tied with foil

7. References

Ede, F. J.; Auger, M. and Green, T. G. A. 1997. Optimizing root cutting success in *Paulownia Spp*. J. Hort. Sci. Biotechnol. 72:179-186.

Ghani, A. K. M. O. and Cahalan, C. M. 1991. Propagation of *Prunus avium* from root cutting. Forestry 64:403-409.

Snedden, J.; Landhausser, S. M.; Lieffers, V. J. and Charleson, L. R. 2010. Propagating trembling aspen from root cuttings: impact of storage length and phenological period of root donor plants. New Forest. 39:169-182.

Hanaoka, S.; Ohira, M.; Matsushita, M.; and Kariuki, J. 2016. Optimizing the size of root cutting in *Melia volkensii* Gürke for improving clonal propagation and production of quality planting stock. African Journal of Biotechnology, 15(29), 1551-1558.

Forest Tree Breeding Center, Forestry and Forest Products Research Institute
3809-1 Ishi, Juo, Hitachi, Ibaraki 319-1301, Japan
E-mail: ikusyu@ffpri.affrc.go.jp ♦ Website: www.ffpri.affrc.go.jp/ftbc/en/index.html

Kenya Forestry Research Institute
Head Office: Muguga off Nairobi-Nakuru Highway
P.O. Box 20412-00200, Nairobi Kenya
Tel: +254-724-259781/2, +254-722-157414, +254-734-251888
E-mail:director@kefri.org ♦ Website: www.kefri.org

ISBN 978-4-86693-554-6 C3061