

**Breeding Strategies, Mating Systems and Future Perspective of
Indigenous Tree Species Improvement in Kenya:
A Case Study of *Melia volkensii***

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Breeding Strategies Mating Systems and Future of Indigenous Tree Species Improvement in Kenya: A Case Study of *Melia volkensii*

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Preface

Forest tree breeding is the application of genetic, reproductive biology, ecological and economics principles to produce genetically improved tree materials. Therefore, it is important to understand the diverse mating systems of plants and methods to promote crossing for genetic improvement. In this book, these topics are briefly explained, and practical results of recent studies in *Melia volkensii* presented. This book introduces *Melia volkensii* as an example case study and aims to promote and encourage further tree breeding of insect-pollinated "non-model" tree species in tropical regions. We would be grateful if the results and suggestions in this book are useful for development of genetic improvement activity for tropical forest trees in Africa.

The studies described in this book were originally done as lectures for “Capacity Development Project for Sustainable Forest Management in the Republic of Kenya”, supported by the Japan International Cooperation Agency (JICA)’s project. The studies and publication of this book would have been impossible without the support of JICA. We are deeply grateful to JICA for its kind support. We also appreciate Tree breeders and scientists at the Kenya Forestry Research Institute and Forest Tree Breeding Center for their inputs and contributions in this series of studies.

25 October 2021

Michinari MATSUSHITA

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Chapter 1: General Introduction to Tree Breeding in Kenya

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1.1 Introduction

Breeding is the practice of producing genetically improved materials, by combining and applying biological, ecological and economic principles. The history of forest tree breeding is relatively recent, compared to the long breeding histories sometimes more than 1,000 years, of livestock, crops, and fruit trees. However, the breeding history of major forest tree species dates back to mid-20th century, mainly for coniferous tree species of Northern temperate and boreal forests. Tree breeding for broad-leaved tree species started much later than coniferous trees. However, poplar and birches in northern forests are important species and their breeding programs are on-going relatively well. Breeding programs for tropical tree species also started much later, with the exceptions of *Eucalyptus* and *Acacia*. Unfortunately, few tropical forest tree species have been put under well designed breeding programs, even though they are important both economically and ecologically. The breeding program for *Melia volkensii*, an endemic species in the drylands of eastern Africa commenced in 2012, and much progress has been made in the species' improvement. *M. volkensii* breeding work has provided valuable experience in breeding of indigenous tree species.

1.2 History of tree breeding in Kenya

Introduction of exotic species in Kenya started with cypress (*Cupressus lusitanica*) in 1910 while systematic tree improvement started in 1936 with the establishment of registered seed areas from existing plantations. Species trials started in 1940s, focusing on introduction and testing of fast growing exotic species such as Pines and Eucalypts.

In 1948, the East African Agriculture and Forestry Research Organization (EAAFRO) was established in Muguga, to conduct research in forestry in the three East African countries under the East African Community. Subsequently, tree improvement activities were expanded in early 1950s to include identification and selection of plus trees and establishment of progeny trials of exotic species with special focus on pines and cypress. In 1962, The Forest Research Coordinating Committee of EAAFRO recommended a tree improvement programme to be developed and prioritized for the three East African Countries of Tanzania, Uganda and Kenya. The programme was to be implemented for 18 years.

The priority tree species recommended by EAAFRO's Forest Research Coordinating Committee for initial improvement were highland tree species namely: *Pinus patula*, *Cupressus lusitanica* and *Pinus radiata*. The programme emphasized the general principles such as species-site matching, provenance trials, plus tree selection, phenology studies, development of vegetative propagation techniques, establishment of seed orchards and progeny trials. The ultimate objective of the programme was to establish adequate improved seed sources to enhance planting and productivity of major commercial plantation tree species in the region.

The key elements of the tree improvement programme in Kenya have been species prioritization, identification and expansion of base populations; selection and testing of the plus trees through

progeny trials and establishment of seed orchards. Although some gains have been made in terms of wood volume increment and establishment of seed sources, there is need to upscale tree improvement activities in order to obtain higher outputs.

In 1977, the East African Community was disbanded and forestry research became the responsibility of individual countries, with forestry research remaining as a Department within the then Kenya Agricultural Research Institute (KARI). Subsequently, Kenya Forestry Research Institute (KEFRI) became a fully-fledged institute in 1986 and has continued with the tree improvement programme.

In addition to priority tree species identified earlier, KEFRI undertook research in other trees species in order to diversify commercial forest plantation species. While from the outset, tree improvement programme in Kenya initially focused on three highland fast growing exotic timber species, subsequent expansion of the programme included lowland pines and later involved diversification of priority species such as *Eucalyptus grandis*, *E. urophylla*, *E. camaldulensis*, *Grevillea robusta*, *Markhamia lutea* and *Gmelina arborea*. In 2012, two indigenous species; *Melia volkensii* and *Acacia tortilis* were included in tree improvement programme.

1.3 *Melia volkensii* description and growth characteristics

Melia volkensii, locally known as Mukau or commonly as Melia is a broad-leaved tree species of the Meliaceae family, native to arid and semi-arid areas of eastern Africa with geographical distribution in Ethiopia, Kenya, Somalia and Tanzania. In Kenya, it is naturally found in the arid and semi-arid regions within agro-ecological zones IV-VI. The family Meliaceae contains many economically important tree species that produce high quality wood. For example, Mahogany: genus *Swietenia* (in Central America). Neem: *Azadirachta indica* (in India). Cedro: *Cedrela odorata* (in South America). Genus *Khaya* known as African mahogany. Genus *Toona* (in Asia to Australia), known as red cedar, also including *T. sinensis* (Chinese mahogany). Genus *Melia*: including *M. azedarach* known as chinaberry (in East Asia). The natural distribution range of *Melia volkensii* is about 100-1600 m above sea level and is characterized by dry bush land, woodland and wooded grassland (Kimondo and Kiamba, 2004; Muchiri *et al.*, 2016).

Melia volkensii is a deciduous, broad-leaved, insect-pollinated and monoecious tree species that grows to a height of up to 15 to 20 m (Orwa *et al.* 2009). The species sheds and flushes leaves in the dry and rainy season respectively. Since Kenya has two distinct seasons each year (rainy and dry season), new leaves are produced twice a year. Flowers and fruits are also produced twice a year, and fruits are ripe at the end of each dry season.

Due to *Melia*'s fast growth, excellent wood quality and termite resistance (Kariuki *et al.* 1990; Mulanda *et al.* 2015), the species is recognized as a valuable tree for forestry and agroforestry. The wood is good for making furniture, acoustic drums, containers, mortars, door and window frames and door shutter rafters. The tree is also used for production of beehives, wood carving and as mulch,

green leaf manure, fodder, bee forage and provision of environmental services (Luvanda *et al.* 2015).

The tree is valued for timber yields in short rotations of 10 to 15 years, thus considerably fast-growing compared to other hardwood trees. These qualities, combined with low tree-crop competition when properly managed have made *Melia* a popular species in the drylands where farmers have intercropped the tree with agricultural crops for years.

1.4 Project on breeding of *Melia volkensii*

In 2012, KEFRI with support from Japan International Cooperation Agency (JICA) under the project on ‘Development of Drought Tolerant Trees for Adaptation to Climate Change in Drylands of Kenya’ developed and commenced implementation of a *Melia* breeding strategy. The strategy included selection of Candidate Plus Trees (CPTs), collection of scions from the CPTs for establishment of 1st generation clonal seed orchards, establishment and evaluation of progeny tests. The breeding objective is to develop fast growing, drought tolerant and healthy trees for timber production that will be and adaptable to both *Melia volkensii* growing areas ASALS (ecological zones IV and V) and more extreme arid areas (zone VI). The tree breeding activities continued under the Capacity Development Project for Sustainable Forest Management (CADEP-SFM) project implemented in 2017 to 2021. Activities included evaluation of progeny tests and selection of second generation trees so as to promote the current breeding population and genetic resources of *Melia volkensii*.

Eighty CPTs were selected in arid and semi-arid areas, (Mutha-Inyali, Katulani-Kavisuni, Voi-Mwatate, Voi-Galana, Embu-Ishiara-Gatunga, Embu-Dams, Mwea Special, Mwingi-Nuu, Mwingi-Tseikuru, Isiolo-Meru, Garissa-Bangale, Garba-Wamba and Wamba-Marsabit) and twenty CPTs 20 from very arid areas (Garissa, Wamba, Garbatulla and Marsabit). The clonal material obtained from the CPTs were used to establish two large clonal seed orchards each 11 hectatres, in Kitui and Kibwezi between 2012 and 2014. In addition, seed obtained from the orchards were used to establish eight F1 progeny trials with four main progeny tests sites located in Tiva (Kitui), Kibwezi, Marimanti and Kasigau and 4 sub-progeny tests sites at Makima, Gaciongo, Ikithuki and Voi. The genetic resources represented in the CPTs, orchards and progeny tests constituted a base of genetic resources for improvement of *Melia volkensii*

References

- Kariuki, J. G.; Mulaya, J.; Kimondo, J.; Ogawa, S. and Muchiri, D. 1990. Genetic improvement of *Melia volkensii*: Evaluation of candidate plus trees. <http://10.10.20.22.8080/handle/123456789/502>
- Kimondo, J. M. and Kimba, K. 2004. An overview of natural distribution, propagation and management of *Melia volkensii*. In Kamondo, B.M.; Kimondo, J.M.; Mulatya, J. M. and Muturi, G. M. Research and Technology Development of Mukau (*Melia volkensii* Gurke). Pp 7-11.
- Luvanda, A. M.; Musyoki, J.; Cheboiwo, J.; Wekesa, L. and Ozawa, M. 2015. An assessment of the socio-economic importance of *Melia volkensii* based enterprises in Kenya. KEFRI/JICA Project.

- Muchiri, G.; Ndufa, J. K. and Takeda, Y. 2016. Chapter 1. Introduction. In: KEFRI (ed) Guideline on Production, Distribution and Use of Improved Melia Seed and Seedlings in the Drylands of Kenya. pp. 1
- Mulanda, E. S.; Chuhila, Y.; Awori, R. M.; Adero, M. O.; Amugune, N. O.; Akunda, E. and Kinyamario, I. 2015. Morphological and RAPD-marker characterization of *Melia volkensii* (Gurke) in vitro plants regenerated via direct and indirect somatic embryogenesis. African Journal of Biotechnology 14: 1261-1274
- Orwa, C.; Mutua, A.; Jamnadass, R. and Simons A. (2009) Agroforestry database: a tree reference and selection guide version 4.0. <http://www.worldagroforestry.org/af/treedb/>

Chapter 2: Breeding Strategies and Mating Systems of Plant Species

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2.1 Introduction

Mating systems in plants evolved diversely, and there are several options of pollination methods (crossing) which are more useful and effective for breeding of plant species. This chapter describes the diversity of mating systems and breeding strategies.

Plant mating system can generally be categorized within the range between perfectly self- and perfectly cross-pollinating. Goodwillie *et al.* (2005) found that about 10 to 15% of plants were predominantly self-pollinating and more than 40% being mainly cross-pollinating. The rest show both self- and cross-pollination. More specifically, mating systems are classified into five categories: (1) Predominantly selfing, (2) Predominantly outcrossing, (3) Mixed selfing and outcrossing, (4) Facultative or obligate apomixis and (5) Intra-gametophytic or haploid selfing (Liengsiri *et al.*, 1998). Selfing, outcrossing and mixed systems are usually observed in most flowering plants.

2.2 Self-pollination

Self-pollination occurs when the pollen grains from the anther of a flower pollinate the stigma of the same flower or another flower of the same plant. This essentially means that the plant mates with itself. Self-pollinating plants have bisexual flowers or perfect flowers that have both female (stigma) and male sexual organs (stamen) and functions within each flower. Therefore, inbred offspring of the self-fertilizing plant receive all genes from this single parent, hence the offspring are genetically similar to the parent.

Although selfing can sometimes be harmful due to inbreeding depression, selfing may also provide great advantages. Importantly, self-pollination provides an assurance of reproductive success. If self-pollination is possible, pollen transfer is not necessary, and even one individual can successfully produce progeny. Plants generally need ‘mediators’ (pollinators) for pollen transfer such as wind, insects, birds, and animal agents. Self-pollinating plants may do require agents to transfer their pollen. In any pollination event, strongly self-pollinating plants rely almost completely on self-fertilization to reproduce.

Many crop plants show strong self-pollinating mechanisms. Rice, wheat, and oats, common beans and tomato are typical examples. The perfect flowers of these plants have evolved mechanisms of accelerating self-pollination. The petals of such plants are typically closed when their stigma are receptive to pollen, and this system allows self-pollen access to the stigma from the anthers within a same flower. Mechanisms promoting self-pollination include:

- i. Bisexuality: Presence of male and female organs within the same hermaphrodite flower.
- ii. Homogamy: Anthers and stigma of a flower maturing at the same time.
- iii. Cleistogamy: Pollination and fertilization occur within an unopened flower.
- iv. Chasmogamy: Opening of flowers only after the completion of pollination.
- v. Another arrangement surrounding stigma: Stigmas are surrounded very closely by

anthers.

2.3 Cross-pollination

In cross-pollinating species, a plant mates with another plant of the same species. Cross-pollination occurs when the sperm cell from a pollen grain transferred from an individual plant fertilizes an ovule of another plant of the same species. The offspring are produced by two genetically different parents. The main advantage of cross-pollination is that it can produce a new genotype as the offspring that has genetic potential to adapt to various environments and conditions. Cross-pollination that recombines different sets of genes of parents will be able to produce more appropriate gene combinations and increase the likelihood of having more superior traits adaptable to new environmental conditions. As plants are not able to move directly to transfer their pollen, they are constantly disadvantaged due to unreliable pollen transfer system, relying on fickle “strangers”, such as insects, birds and animals and wind. Despite this difficulty, many plants have evolved to promote cross-pollination.

Mechanisms promoting cross-pollination includes:

1. Dicliny: Having unisexual flowers
 - i) Dioecy: An individual plant having only male flowers and another individual plant having only female flowers (male and female plants exist in a species),
 - ii) Monoecy: Male and female flowers are separate but present in the same plants
2. Dichogamy: Maturation of anthers and stigma of the same flowers at different times, promoting cross pollination even in the hermaphrodite species, including
 - i) Protogyny: Pistil matures before anthers, or
 - ii) Protandry: Anthers mature before the pistil
3. Heterostyly: Different length of styles and filaments within a flower
4. Self-incompatibility: The inability of fertile pollens to fertilize the same flower
5. Male sterility: There are pollen grains but they cannot function

2.4 Breeding strategies for self- and cross-pollinating plants

The mode of plant reproduction, mating system and genetic composition are important factors to consider while deciding on suitable breeding and selection strategy. Knowledge of a species’ reproductive and mating system, genetic diversity of germplasm, degree of inbreeding depression, maturity and longevity, and ease of vegetative propagation are essential for artificial manipulation through crossing to improve the genotypes.

Breeders therefore make enormous efforts to develop methods of efficiently optimizing plant breeding programs. To obtain the highest breeding benefit per given time, optimization of the programs can vary largely depending on: Species; regions and populations; breeding strategies and methods; genetic structure, diversity, and variation; and, trait heritability (i.e. degree of inheritance).

Such programs are explored and selected at several hierarchical levels: i) Breeding strategy (selection intensity, breeding population size and structure, maintenance of genetic diversity), ii) Breeding methods (mating type, testing and selection methods, testing population size and time) and iii) Production methods for market use (seeds from orchards or clonal plantations). Considering genetic gain, diversity, cost and time components, breeding strategies should be compared and checked to enhance progress in long-term breeding programs.

Self-pollinating plants require less effort in isolation to avoid unintended cross-pollination. The less efforts in isolation make it easier to manage several breeding trials in a limited space and time, and will allow breeders to conduct several breeding projects without threat of cross-contamination. However, removal of male organs from a perfect flower (emasculation) is required. Generally, labour is much more needed to make crosses in self-pollinating plants than in cross-pollinating ones. In this context, cross-pollinating plants can be handled with simple techniques.

Breeding of self-pollinating plants allows breeders to easily create uniform varieties. Once such varieties are developed, they are much easier to maintain. In self-pollinating plants, subsequent generations of self-pollination rapidly fixes groups of genes, whereas cross-pollinating plants easily outcross and recombine their genes.

Most importantly, self-pollinated plants suffer less from inbreeding depression. In a breeding program for such plants, this allows breeders to start from relatively fewer plant materials, compared to breeding of cross-pollinating plants. Self-pollination leads to each plant deriving separate families as “lines”. After several generations, the plants in each family will become more uniform within the family, while stronger differences will arise between families, and the genotypes of each family become fixed. Therefore, in self-pollinated plants, many lines must be created to find a line that has all the preferable traits. In contrast, in cross-pollinating plants, by eliminating the inferior families, the gene pool of the breeding populations can be gradually, sometimes slowly improved per generation, but it will also allow for production of next-generation populations with combinations that can simultaneously contain several preferable traits.

Based on these differences, plant breeding strategies are categorized as: Line breeding (mainly in self-pollinating plants); Population-based breeding (mainly in cross-pollinating plants) and hybrid breeding.

2.4.1 Self-pollination breeding approaches in plants

During the evolution process over time, certain limitations triggered the development of self-fertilization mechanisms in a plant species. Some of the reasons why a self-fertilization mode of reproduction is very effective include the efficiency of reproduction, and thus the fixation of highly adapted genotypes. The key point of breeding for self-pollinating plants is that almost no inbreeding depression occurs. Basic breeding strategies for self-pollinating plants are follows.

2.4.1.1 Mass selection

Mass selection is selection based on plants' phenotypic characters. Mass selection is used to improve the overall population, but it is relatively limited in the recent breeding programs of self-pollinating plants. In mass selection, seeds are collected from phenotypically "good" individuals, and the next generation is planted from the mixed seeds. Mass selection has been traditionally used to improve old "land" varieties of crops, and is the basic, simplest and lowest-cost breeding program.

2.4.1.2 Line selection

Generally, line selection follows several steps: first, superior appearing plants are selected from genetically diverse populations. Next, progenies of the individual selections are planted and evaluated, by simple observation. Third, following the evaluation based on simple observations alone, more extensive trials are undertaken to check whether the remaining progenies after the second step show superior performance. Then, the progenies that are superior compared to the base variety are recognized as a new pure-line variety. While this method has diminished in importance in the breeding of major cultivated plants in recent years, the line selection method has provided important genetic resources.

2.4.1.3 Pedigree breeding

Pedigree breeding starts with producing the hybrid progeny of the first generation (F₁), by crossing two parental genotypes, each of which has preferable characters absent in the other one. Superior genotypes are selected in successive generations and a record is maintained of parent–progeny relationships. The F₂ generation (progeny of the crossing of the two F₁ individuals) becomes the first population for selection in pedigree programs. In the F₂ generation, individuals carrying non-preferable genes are eliminated. The hybrid condition gives way to pure breeding as a result of natural self-pollination, and families derived from different F₂ plants begin to display their unique character. After a few generations, homozygosity becomes extensive, indicating nearly pure-breeding condition. After several generations before last step to releasing a new variety for commercial production, precise evaluations to test the variety's performance are conducted.

2.4.1.4 Back-crossing

An outstanding variety can often be improved by transferring some specific preferable character that it lacks. This can be accomplished by first crossing a plant of the superior variety to a plant of the donor variety, and then mating the progeny back to a plant having the genotype of the superior parent. This breeding technique is known as backcrossing. After several back-crossing steps, the progeny will form a hybrid of the character being transferred but will be like the original parent for all other genes. The advantages of the backcrossing are its rapidity, the small number of plants required, and the predictability.

2.4.2 Cross-pollination breeding approaches in plants

Populations of cross-pollinating plants are often characterized by a high level of heterozygosity in

genetic variation. The key points in breeding cross-pollinated species are population-based selection such as mass selection, and development of crossed varieties. Since cross-pollinated plant species are natural hybrids (heterozygous), they normally lose vigour as they become pure-bred (homozygous), known as inbreeding depression. To balance selection intensity and preserving genetic diversity, there have been several trials to determine effective breeding strategies and methods for out-crossing plants.

Genetic diversity is affected by several factors that affect species' history and characteristics, such as mode of reproduction (degree of outcrossing), past and current population size, population bottlenecks (rapid decline of population size), natural selection, mutation rates and migration rates. The current state of genetic diversity has important implications for potential fitness and adaptation, and also genetic improvement potential for breeding.

Reduced fitness as a result of inbreeding (inbreeding depression) is one of the greatest genetic risk, due to reduction of heterozygosity and carrying high proportion of deleterious recessive alleles. It is thus important to maintain a stable breeding population size to keep high levels of genetic diversity. A decrease in population size will lead to a decrease of heterozygosity through generations. Even if environmental changes occur, genetic diversity will be important in environmental adaptability; the possibility of finding some adaptive genotypes will be high in populations with higher genetic diversity.

Once genetic diversity in a population is lost, it is often difficult to recover. Basically, genetic diversity should be a key consideration in breeding for out-crossing plants, especially for tree breeding programmes. Sufficient numbers and genetic diversity should be maintained in breeding populations. Breeding generally selects a limited number of individuals or families showing superior performances and inevitably it decreases genetic diversity. A balance between selection intensity and retention of genetic diversity is important for a sustainable breeding program. If a small number of very superior varieties is selected, the genetic gain from the 1st to 2nd generation will be very high. However, genetic gains a few generations after the first selection will be much lower, because of crossing among genetically related individuals. The selection limit will be higher under relaxed selection intensity, even though genetic gain per each generation is not so high. Genetic diversity in each generation should be managed and should be considered in the selection intensity.

Many forest tree species are basically cross-pollinating and only small amount of selfing rates are known to occur naturally. In these species, controlled selfing is possible, but self-compatibility and inbreeding depression vary largely among individuals, populations and species. Basically, breeding for cross-pollinating plants include: mass selection, recurrent selection, and hybridization.

2.4.2.1 Mass selection

In cross-pollinating plants, mass selection takes a large number of phenotypically superior

individual plants (genotypes) that are selected in bulk, and the seeds used to produce the next generation. It is basic but simple and easier method of selection where individual plants are selected based on their phenotypic performance. Such bulk selections are often effective in improvement at the initial breeding stages. Mass selection has traditionally been effectively applied in improving quantitative traits, such as yield and volume, despite the low heritability of such traits. This selection does not provide any control over the pollen parents (i.e. fathers), while effective selection is only limited to female parents. Since only maternal plants are used to harvest seeds, whereas the pollen source is not known, the estimates for heritability and genetic performances are limited.

2.4.2.2 *Recurrent selection*

Recurrent selection is a refined version of mass selection. Based on progeny tests, the poorer progenies are dropped. It should be noted that recurrent selection is based not only on the appearance of parent plants but also on the performance of their progeny. Progeny selection can provide more effectiveness than solely phenotypic selection for quantitative traits of low heritability. However, testing progenies requires an extra generation, and thus gain per cycle of selection must be double that of simple phenotypic selection to achieve the same rate of gain per unit time.

Based on progeny tests, progenies showing superior performances are phenotypically selected from the base population, and then the selected individuals are crossed with each other to produce seeds and seedlings for the next breeding population. This recurrent selection consists of selecting parents and crossing them to produce next generation populations for reselection (Penny *et al* 1963). Family means will be improved, but a large variation within a family should be maintained. Selection of progeny individuals is based on their performance in progeny tests, rather than just the mean performance of parents. Next cycles of breeding can be made as long as satisfactory improvement continues. Most tree improvement programs are based on recurrent selection and its developed variation.

Depending on the ease of crossing, the methods to produce offspring are selected based on full-sib or half-sib basis. If controlled pollination is easier, a number of full-sib families, each produced by making crosses between the different parental plants from the base breeding population are evaluated in trials. On the other hand, if the effort for conducting controlled pollination is relatively limited, seeds from selected half-sibs, which have been pollinated by random pollen from the parental population (meaning that only the maternal plants are known and selected) is grown for progeny tests. Because of the limited combination pairs of crossing parents to produce full-sibs, genetic diversity of breeding populations can narrow over generations, and hence a large genetic diversity of base breeding population is preferred at the start if the process is to continue.

2.4.2.3 *Hybridization*

One possible technique of genetic improvement is hybrid breeding. Most of the successful hybrids have been interspecific hybrids. For example, hybrid larch is one of well-known examples for

conifer hybrids. Japanese larch (*Larix kaempferi*) originated in Japan, and has been introduced widely in East Asia, Europe and North America. *Larix kaempferi* often shows superior growth compared to other larch species (e.g. *L. decidua* and *L. laricina*) and has therefore been widely used in hybrid breeding. Hybrids between *L. kaempferi* and other larches have been used commercially in North America, Europe and Asia. Other larch hybrids include *L. leptolepis* x *decidua*. For other conifers, there are also several hybrid examples such as: hybrids between *Pinus coulteri* and *P. jeffreyi* in California (Zobel 1981) and hybrids of *P. serotina* and *P. taeda* in North Carolina (Saylor and Kang 1973), and *Pinus rigida* x *taeda* in Korea (Hyun 1976). For broad-leaved trees, there are hybrid poplars (*Populus* spp), hybrid *Acacia* (Kato *et al.* 2012) and hybrid *Eucalyptus* (Horsley *et al.* 2010; Randall *et al.* 2015). Many interspecific hybrids show better growth than their parental species, and this phenomenon is known as “hybrid vigor”. Heterosis (hybrid vigor) is an interesting scientific topic, but well-documented literature for the mechanism is limited.

2.5 Differences in breeding strategies for crops and forest trees

The genetic improvement of forest trees has some similarities to crop breeding, especially of cross-pollinating ones. Most of the concepts are similar, but there are some large differences between crop breeding and forest tree breeding. For example, field crops reach reproductive maturity even in a few months within a year, while most trees require many years. Moreover, harvesting occurs a few months for crops, whereas in forest trees, wood production occurs between 10-100 years. Therefore, breeding cycle for crops can be completed in 2-3 years, whereas the tree breeding cycle often takes 8 to 10 years, or much more.

Secondly, crop breeding is usually uses varieties that have historically been domesticated and are already genetically homogeneous. Hence, current crop breeding usually starts with well-known varieties that are genetically pure. In contrast, forest tree breeding generally starts with “wild” materials, i.e. initial selection from natural forests and plantations.

Sites for planting are also largely different. While crops will be planted on flat, uniform, often well-watered farms, trees may be planted on difficult sites, sometimes with little or no site preparation. Environmental adaptation is important: crop varieties are sometimes specified in terms of suitable soils, watering, and climatic conditions. On the other hand, trees may be planted over a much wider range of soils and climatic zones.

In tree breeding, due to high outcrossing rates, relatively high inbreeding depression, longer rotation period and potential planting sites in various environments, there are potential risks of uncertainty of unpredictable space and time. Therefore, breeding program for many forest trees are typically population-based to balance improving genetic gain and maintaining genetic diversity. Most tree breeding program follow recurrent selection schemes, consisting of multiple levels, including base populations where initial phenotypic selections are made, breeding populations where crosses among the selected individuals are created and tested, and production populations (El-Kassaby and

Lstibůrek 2009).

References

- Danusevičius, D. and Lindgren, D. 2002. Efficiency of Selection Based on Phenotype, Clone and Progeny Testing in Long-term Breeding. *Silvae Genetica* 51:19-26.
- El-Kassaby, Y. and Lstibůrek, M. 2009. Breeding without breeding. *Genetics Research* 91(2): 111-120.
- Goodwillie, C.; Kalisz, S. and Eckert, C. G. 2005. The evolutionary enigma of mixed mating systems in plants: Occurrence, theoretical explanations, and empirical evidence. *Annual Review of Ecology Evolution and Systematics* 36: 47-79.
- Horsley, T. N.; Johnson, S.D. and Myburg, A. A. 2010. Comparison of different control-pollination techniques for small-flowered eucalypts. *New Forests* 39:75–88.
- Hyun, S. K. 1976. Interspecific Hybridization in Pines with the special Reference to *Pinus rigida* x *taeda*. *Silvae Genetica* 25: 188-191.
- Kato, K.; Yamaguchi, S.; Chigira, O.; Ogawa, Y. and Isoda, K. 2012. Efficient establishment of Acacia hybrids by artificial pollination using stored pollen. *Journal of Tropical Forest Science* 24: 209-216.
- Kita, K.; Fujimoto, T.; Uchiyama, K.; Kuromaru, M. and Akutsu, H. 2009. Estimated amount of carbon accumulation of hybrid larch in three 31-year-old progeny test plantations. *Journal of Wood Science* 55: 425–434.
- Lindgren, D. and Prescher, F. 2005. Optimal clone number for seed orchards with tested clones. *Silvae Genetica* 54: 80-92.
- Pâques, L. E. 2004. Roles of European and Japanese larch in the genetic control of growth, architecture and wood quality traits in interspecific hybrids (*Larix* × *eurolepis* Henry). *Annals of Forest Science* 61: 25–33
- Randall, B. W.; Walton, D. A.; Lee, D.J. and Wallace, H. M. 2015. Comparison of three pollination methods for *Eucalyptus argophloia*, a small-flowered eucalypt. *Annals of Forest Science* 72: 127-133.
- Saylor, L. C. and Kang, W. W. 1973. A study of sympatric populations of *Pinus taeda* and *Pinus serotina* in NC. *Journal of the Elisha Mitchell Science Society* 89 (142): 101–110.
- White, T. L.; Adams, W. T. and Neale, D. B. 2007. *Forest Genetics*, CABI.
- Zobel, B. J. 1981. Wood quality from fast grown plantations. *TAPPI* 64(1): 71–74.
- Zobel, B. and Talbert, J. 1984. *Applied Forest Tree Improvement*. North Carolina State University.

Chapter 3: Field Methods for Controlled Pollination

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3.1 Controlled pollination (hand pollination)

In tree breeding, controlled pollination (hand pollination) is one of the essential procedures, for controlling selfing and outcrossing. The exact procedures used to ensure self or cross-pollination will depend on the floral structure and manner of pollination. In general, cross-pollination for self-pollinating plant species is more difficult than *vice-versa*, as preventing self-pollination inside unopened flowers is cumbersome.

Skills for preventing contamination and preparing viable pollens are essential. A study checking self-compatibility or producing breeding materials (progenies) by artificial pollination may be required. The basic techniques and flow in controlled pollination include; emasculation, carrying out the pollination bagging, and marking and labelling.

3.2 Emasculation

Emasculation is a technique removing stamens and anthers or inactivating undesirable pollen. In hermaphrodite flowers, emasculation is essential to prevent self-pollination. In monoecious plants, male flowers within an inflorescence or male flowers very adjacent to female flowers within a plant should be removed. In species with large flowers, hand emasculation is accurate and it is adequate while in small flowers suction method may be applied.

3.2.1 Hand Emasculation

In species with large flowers, removal of anthers is possible with the help of forceps. Anthers are removed before they dehisce, generally done in morning. The corolla of the selected flower is opened with the help of forceps and the anthers are carefully removed with the help of forceps. Sometimes corolla may be totally removed along with epipetalous stamens e.g. gingelly. In all cases, gynoecium should not be injured. An efficient emasculation technique should prevent self-pollination and produce high percentage of seed set on cross pollination.

3.2.2 Suction Method

This method is useful in species with small flowers. Emasculation is done in the morning immediately after the flowers open. A thin rubber or a glass tube attached to a suction hose is used to suck the anthers from the flowers.

Pollen grains are generally more sensitive to environmental factors than female reproductive organs. In emasculation using hot water, the temperature and duration of treatments vary among species. In the case of rice, it is adequate to soak in hot water for 10 minutes at a temperature of 40-44 °C. Treatment should be conducted before the anthers dehiscence and prior to the opening of the flower. Ensure whole inflorescence is immersed in hot water.

3.2.3 Alcohol

This method is not commonly used. The method consists of immersing the inflorescence in alcohol of suitable concentration for a brief period followed by rinsing with water. Studies indicated that

for Lucerne, when the inflorescence was immersed in 57% alcohol for 10 seconds the method was highly effective. It is better method of emasculation than suction method.

3.2.4 Hormonal ingredient

It has been known that some hormonal ingredient, such as BAP, can change the primordium from male to female organs which increases the number of female flowers.

3.2.5 One-Stop Pollination (OSP)

Harbard *et al.* (2000) developed a method for emasculation of flowers called One Stop Pollination (OSP). OSP requires only one visit to the flower to emasculate and immediate pollination of stigmas to induce receptivity, followed by bagging. Horsley *et al.* (2010) compared various methods of Eucalyptus controlled pollination and showed that the Artificially Induced Pollination (AIP) method is the most promising technique due to high seed yield and lower labor cost whereas OSP method had the lowest genetic contamination.

3.3 Carrying out the pollination

The pollen grains collected from a desired male plants should be used to pollinate the emasculated female flower. This is done in the early morning. The flowers are bagged immediately after artificial crossing.

3.4 Bagging

Immediately after emasculation, the flower or inflorescence is enclosed with suitable bags of appropriate size to prevent random cross-pollination. Bagging also prevents pollen contamination. Seed set is sometimes reduced in enclosed in bags due to excessive temperature and humidity inside the bags.

3.5 Marking and labelling

The flowers or inflorescences should be marked properly including details of both parents and the pollination date.

Chapter 4: Preliminary Results of Artificial Pollination of *Melia volkensii*

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4.1 Introduction

A hybrid is a cross between two or more discrete genetic sources. A hybrid can be inter-specific (between two species) or between two different provenances or even genotypes (Zobel and Talbert 1994). Hybrids are appealing their potential to produce genotypes with special combinations of properties that may either increase the value of the genetic resource or final product e.g. specific timber properties, disease resistance or they may be developed to increase the growth potential of the genotype e.g. greater vigour on specific sites compared to pure families.

The principal reasons for producing hybrids are: To combine complementary traits of two parents; to exploit hybrid vigour (heterosis) and to increase adaptability for afforestation into marginal areas. Furthermore, hybridization gives the opportunity to combine characteristics which cannot be easily obtained in individual families.

Intraspecific crossing between genotypes aims at creating hybrids of high productivity and adaptability to marginal sites. Hybrids may also show vigour on sites that are marginal to both hybridized genotypes. Heterosis occurs when the hybrid grows with more vigour than the better of the two parents or has better adaptive characteristics. This is common in cereal and crop breeding. Hybrid vigour usually relates to traits of size, mostly volume production, disease and drought resistance. Hybrid vigour can be measured as the difference between F1 and the mid-parent values although useful heterosis is referred to as “amount by which F1 exceeds the better parent line”. The main area of interest will be the useful heterosis rather than the mid-parent values.

Responses of hybrids may be incompatibility, vigorous and homogenous progeny, and heterogeneous progeny with high performance trees, heterogeneous, slow growing and abnormal forms. It is recommended to use at least five different trees (non-related) for each genotype.

Artificial crossing of *Melia volkensii* started in 2018 with the objective of producing second generation varieties that are drought tolerant and fast growing. The first step was to develop a suitable technique for crossing, taking into account seasonality and stage of flowering, i.e. early, mid and late flowering.

4.2 Materials and equipment needed for artificial pollination

The following materials will be required for use in pollination;

- Micro-sieves
- Silica gel and 100ml bottles
- Hand gloves
- Make-up brushes for pollination
- Storage bags
- Hand sprayers
- Surgical blades and surgical spirit
- Galvanized wire

4.3 Pollen collection and processing of *M. volkensii*

For pollen extraction, ripe unopened flowers, often yellow in colour were collected and kept in 100ml plastic bottles. Ripe unopened flowers were collected as whole umbels and stored in bags containing silica gel to hasten drying. Drying is necessary for the anthers to release pollen. The released pollen is refined using a pollen sieve (Plate 1). Pollen will be processed and stored in airtight containers.



Plate 4.1: Sieve used for pollen processing (Hubbard Scientific Co. Northbrook ILL.)

After drying, the anthers were sieved through a mesh to remove debris and a second mesh to sieve the pollen. The resulting pollen was placed into polypropylene vials, sealed in glass bottles containing silica gel and stored in a fridge at -4 °C until needed. Pollen were kept separate per tree.

4.4 Artificial pollination of *M. volkensii*

For *Melia volkensii*, the One Stop Pollination method was used. This involved selection of ripe unopened flowers often yellow in colour. The selected flowers from the female parents were emasculated (removal of all anthers) and sprayed with clean water using a high pressure hand spray to remove any pollen and avoid contamination. The stigma was then sliced diagonally and pollen applied in between the slice.

The pollinated bunch of flower on a branch were then covered with a pollination bag made of bridal veil with a fabricated galvanized steel wire of the appropriate diameter and length being used to form a skeleton support system for the isolation bags. Accurate labeling of the female recipient trees of *M. volkensii* and the source male pollen used and date of pollination was done. As with the conventional method, these isolation bags were removed 2 weeks after pollination. The pollinated flowers were counted, monitored and surviving number documented until harvesting of fruits.



Plate 4.2: Melia pollination: A: Pollen collection, B: Processing, C: Pollination, D: Bagging



Plate 4.3: A: Bagged flowers, B-D: Developing hybridized fruits up to 3 months - Kibwezi

References

Zobel, B. and Talbert, J. 1984. Applied Forest Tree Improvement. North Carolina State University, John Wiley and Sons, Inc.

Chapter 5: Genetic Analysis Can Reveal Mating System of *Melia volkensii*

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5.1 Introduction

Genetic diversity is one of the components of biodiversity, and it provides the basis for adaptation and resistance to unexpected stress and climate change (Booy *et al.* 2000). Therefore, importance of genetic diversity is often emphasized in conservation and management of plant populations. In breeding programs as well, it is important to always manage the balance between genetic gain and loss of genetic diversity within the target species. In tree breeding, the magnitude of genetic diversity is one of the criteria to be considered in selection intensity.

Genetic diversity is important for environmental adaptability, and reflects the potential of evolution. If genetic diversity decreases, the potential of future adaptability of the species to environmental changes will be reduced. Once genetic diversity decreases over generations (time), its recovery is generally difficult. Since breeding is one of the processes of artificial evolution, the principle of genetic diversity also applies to tree breeding. Therefore, in tree breeding programs, when breeders plan crossing designs and selection approaches, management of genetic diversity has to be very carefully considered.

Breeding is a process of artificial evolution to select only a limited number of ‘superior’ individuals or families. Consequently, there is a delicate balance between decreasing genetic diversity and strong selection intensity (selecting only limited numbers of very good progenies). Therefore, balancing selection intensity and loss of genetic diversity is important for sustainable breeding activity. If a small number of superior trees or varieties is selected, the genetic gain from the 1st to 2nd generation will be high, while reduction of genetic diversity is high. After several breeding generations, the genetic gains over time will be low because the frequency of mating among genetically related individuals will increase. To sustainably improve target traits in tree species, use of breeding materials that are not only just superior but also genetically diverse is important.

Genetic diversity is affected by species’ characteristics and history, such as reproduction system (selfing or outcrossing), past and current population size, population bottlenecks (rapid decline of population size), natural selection, mutation rates, migration rates, and the interactions of these factors. Analysis of genetic diversity of a species will reveal the foregoing species characteristics, which have important implications for species’ adaptation to environments.

In nature, inbreeding depression reduces genetic diversity over several generations. Therefore, plant species have evolved diversely having several mating system in order to avoid inbreeding and maintaining genetic diversity. Inbreeding depression is one of the future risks associated with tree breeding.

5.2 Genetic study can reveal Plant mating system

Mating system is an important determinant of genetic diversity and evolutionary potential of flowering plant populations (Barrett and Harder 2017; Hodgins and Yeaman 2019), and can affect expected genetic gain through breeding (Ivetic *et al.* 2016; Tambarussi *et al.* 2018). As described in

Chapter 2, mating systems are classified into five models: (1) predominant selfing, (2) predominant outcrossing, (3) mixed mating systems (selfing and outcrossing), (4) facultative or obligate apomixis and (5) intra-gametophytic or haploid selfing (Liengsiri et al. 1998). First three models, selfing, outcrossing and mixed pattern, are usually observed in most flowering plants.

Many flowering plants show predominant outcrossing in natural conditions, and the system has advantages of maintaining genetic diversity within populations. Selfing has advantage of ensuring reproduction under conditions of pollen limitation, but it may increase genetic load within a population (Darwin 1876). More specifically, the risk of seed discounting, reduction of germination rate and/or poor growth of progenies etc. may occur due to inbreeding depression (Care 2020). DNA markers enable the estimation of mating systems directly or indirectly, and recent studies using DNA markers have revealed that various ecological and environmental condition influence the outcrossing and selfing rates. As a result, mating systems often vary not only among species but also among populations within a species (Whitehead *et al.* 2018; Tani *et al.* 2015; Eckert *et al.* 2009). Therefore, in breeding and conservation programs, mating system should be carefully evaluated in each plant population.

While outcrossing rate is the principal determinant of genetic diversity in seed, the diversity of pollen donors (mating patterns) also affects the seed's genetic diversity. Therefore, it is recommended to evaluate both mating systems and diversity of mating patterns in seed orchards. If pollen cloud diversity is low in seed orchards, we should reconsider tree arrangement considering genetically controlled traits such as flowering phenology.

5.3 On-going study of *Melia volkensii* mating system

Melia volkensii is a monoecious, insect-pollinated tree species distributed in semi-arid region of eastern Africa with low stand density (Orwa et al. 2009). There is no empirical study to reveal mating systems of *M. volkensii*, however, the species is believed to be self-compatible (Orwa *et al.* 2009). Hanaoka *et al.* (2012) estimated genetic parameters in three natural populations of *M. volkensii*, and positive values of fixation index (Wright 1951) were evident in most of SSR loci implying the presence of mixed mating patterns in the species. Use of outcrossed seeds (progenies) is ideal for both forestry and tree breeding to avoid effects of inbreeding depression. It is therefore very important to understand the actual mating patterns in *M. volkensii* seed orchards to ensure production of good quality seed for plantation forestry. We therefore have embarked on a study to evaluate the mating systems and patterns in *M. volkensii* seed orchards established under KEFR/JICA cooperation project. The findings of the study will be useful in designing strategies for establishment and management of the seed orchards and future breeding programme of the species.

References

- Barett, S. C. and Harder, L. D. 2017. The ecology of mating and its evolutionary consequences in seed plants. Annual Review of Ecology, Evolution and Systematics 48: 23.
- Booy, G.; Hendriks, R. J. J.; Smulders, M. J. M.; Groenendaal, J. M. and Vosman, B. 2000. Genetic diversity

- and the survival of populations. *Plant Biology* 2: 379-395
- Care, O.; Gailing, O.; Muller, M.; Krutovsky, K. V. and Leinemann, L. 2020. Mating system in a native Norway spruce (*Picea abies* [L.] KARST) stand-relatedness and effective pollen. *Diversity* 12: 266.
- Eckert, C. G.; Kalisz, S.; Geber, M. A.; Sargent, R.; Elle, E.; Cheptou, P. O.; Goodwillie, G.; Johnston, M. O.; Kelly, J. K.; Moeller, D. A.; Porcher, E.; Ree, R. H.; Vallejo-Marin, M. and Winn, A. A. 2009. Plant mating systems in a changing world. *Trends in Ecology and Evolution* 25: 35-43
- Hodgins, K. A. and Yeaman, S. 2019. Mating system impacts the genetic architecture of adaptation to heterogeneous environments. *New Phytologist*. 224: 1201-1214
- Liengsiri, C.; Boyle, T. J. B. and Yeh, F. C. 1998. Mating system in *Pterocarpus macrocarpus* Kurz in Thailand. *The American Genetic Association* 82: 216-221
- Ivetic, V.; Devetakovic, J.; Nonic, M.; Stankovic, D. and Sijacic-Nikolic, M. 2016. Genetic diversity and forest reproductive material – from seed source selection to planting. *iForest* 9: 801-812
- Orwa, C.; Mutua, A.; Kindt, R.; Jamnadass, R. and Simons, A. 2009. Agroforestry database: a tree reference and selection guide version 4.0.
(http://apps.worldagroforestry.org/treedb/AFTPDFS/Melia_volkensii.PDF)
- Tani, N.; Tsumura, Y.; Fukasawa, K.; Kado, T.; Taguchi, Y.; Lee, S. L.; Lee, C. T.; Muhammad, N.; Niiyama, K.; Otani, T.; Yagihashi, T.; Tanouchi, H.; Ripin, A. and Kassim, A. R. 2015. Mixed mating system are regulated by fecundity in *Shorea curtisii* as revealed by comparison under different pollen limited condition. *PLOS ONE*. 10: e0123445
- Whitehead, M. R.; Lanfear, R.; Mitchell, R. J. and Karron, J. D. 2018. Plant mating systems often vary widely among populations. *Frontiers in Ecology and Evolution*. 6: 38.
- Wright, S. 1951. The genetic structure of populations. *Annual Eugenics* 15: 323-345.

Chapter 6: Types of Pollination Methods for Next Generation Breeding

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6.1 Introduction

Controlling self- and out-crossing pollination are basic and essential techniques in tree breeding. It is important that breeders master these techniques. The crossing procedure adopted depends largely on the species characteristics i.e. mating system of target plant species as described in Chapter 5, floral biology, structure of inflorescences and an individual flower. The procedure adopted also depends on:

- The physiological and biochemical characteristics of pollen grains,
- The difficulty of sampling flowers and pollen grains,
- The ease of stimulating flower bud initiation,
- The flowering phenology, and
- The mast seeding habit

The structure and characteristics of flowers, phenology, and pollen grains determines the efficiency and success of control pollination. For these reasons, breeders take great efforts to establish such characteristics the target plant species before undertaking controlled pollination.

6.1.1 Important principles in controlled pollination

It is important to understand the mode of pollination and mating patterns of plant species. If the extent of outcrossing under natural condition is relatively large, then the flowers should be protected by bagging. On the other hand, there is no need for emasculation if a species does not show any self-compatibility or shows only limited level of self-pollination. Genetic studies using molecular markers have proven useful in estimation of the extent of outcrossing and selfing rates (i.e. selfing rates) and the pollen dispersal patterns (Chapter 3).

For plants that have large flowers and easily distinguishable reproductive organs (stigma and stamen) that can be rapidly handled manually, no special equipment and skills for emasculation are required. On the other hand, some equipment such as plastic tubes with a moderate inner diameter are key tools for storing pollen grains and efficiently conducting artificial pollination for creating hybrids in species such as *Acacia*, that have typically very small flowers on a linear inflorescence (Kato *et al.* 2012). Controlled crossing (hand pollination) with well-considered mating designs can potentially provide accurate assessment of genetic parameters and selection of next breeding generations. However, it is also one of the greatest challenge in tree breeding, requiring much time and labour in collecting enough pollen and conducting very-delicate manipulation.

6.2 Types of controlled pollination

6.2.1 Normal controlled pollination

Controlled pollination can evoke an image of tedious and delicate manipulation using tweezers, magnifying glasses, and small brushes, but is just the basic and fundamental way for most plant breeding programs. The manipulation of controlled pollination will largely depend on the size of

each individual flower, and whether the species has perfect flowers (having both stamen and pistils). If self-compatible plants have perfect flowers, breeders generally first need to emasculate the flowers by removing the stamen. There is no need to emasculate, if the plant shows incompatibility or has unisexual flowers, that is, flowers only having either male or female organs. After collecting pollen from male plants, it is then transferred to the emasculated female flower. Before and after conducting such hand pollination, some covers such as non-woven bags should be used to exclude other pollen from contaminating the cross.

Using controlled pollination, mating designs are considered essential for creating pedigree families for progeny tests, facilitating accurate assessment of genetic parameters (e.g. heritabilities, breeding values, genetic gain), and the selection of elite genotypes for advanced breeding (Lambeth *et al.* 2001; El-Kassaby and Lstibůrek 2009). For these objectives, breeders select to use several types of mating design, such as the factorial mating design, circular mating design and partial diallel mating design. However, conducting large numbers of artificially controlled crossing is one of the greatest challenges for breeding, requiring much time and labour to complete.

6.2.2 *Poly-crosses (pollen mixes)*

Poly-cross is a type of controlled crossing, in which the maternal plant of a seed is known but the paternal plant of a seed is unknown, because a mixture of pollen grains of several pollen donors is used to pollinate each female. For a progeny test, its identities can be maintained only for the maternal parents, i.e. half-sibs. When using fingerprinting with DNA markers, pedigree reconstruction of offspring can be done to determine full-sibs.

6.2.3 *Open pollinations*

Open pollination (known as blind crosses) are done just like in natural pollination i.e. by making crosses without any attempt to ensure that pollen is only transferred from certain paternal plants. This is accomplished by bringing all of the plants together and allowing them to freely cross-pollinate. Outside pollen is often excluded either by isolation distance or by caging the plants. The open pollination is not intended for strict control of outside pollen, but can be the simplest and lowest-cost crossing procedures.

6.2.4 *Supplemental mass pollination*

Supplemental mass pollination (SMP) has been used to effectively promote out-crossing (Funda *et al.* 2016). Even when in an open-pollination setting, supplemental mass pollination can reduce pollen contamination, and is used to introduce planned parents into a crossing population at a reasonable cost (Eriksson and Wilhelmsson 1991) and also to increase the genetic diversity of the crossing offspring (El-Kassaby and Ritland 1986; Lai *et al.* 2010). Requirements for success of SMP are. First, the enhancement of crossing achieved by SMP must be clear. Second, phenology should be monitored carefully in order to apply SMP prior to maximum pollen shedding. Third, fresh pollen or stored pollen with high viability should be used. The extent of success of SMP can be monitored by using genetic markers such as SSR markers (e.g. Moriguchi *et al.* 2010; Funda *et*

al. 2016).

6.2.5 Isolation tent system (mating booth)

Other attempts to promote crossing with reduced pollen contamination are using physical isolation of ramets, such as: storing large containerized grafts and bringing them back when no conspecific pollen is present in the outside environment (Eriksson and Wilhelmsson 1991); moving grafts into a greenhouse (Hörnsten et al. 1997); or using physical cover as a mechanical hinder to pollen contamination (Lindgren 1994).

The isolation tents (crossing booths) system has been used in scots pine, through creation of a physical barrier against contaminating pollen which at the same time, accelerated the reproductive phenology of trees within the tent and induced temporal reproductive isolation between trees inside and those growing outside (Funda *et al.* 2016). Due to the limited number of available fathers in each tent, their seed lots exhibited low fathers' effective population sizes and higher rates of biparental inbreeding, but SMP can substantially improve the seed lots' status. Isolation tent system and mating booths provide an option to suitable environments for crossing, without the need for controlled crossing (hand pollination) and spending a lot of time and efforts.

6.2.6 Breeding without breeding (BwB)

BwB is one of the strategies in modern breeding programs, providing an effective and economic method to breed outcrossing species (El-Kassaby and Lstibůrek 2009). This method is accomplished without any controlled pollination and experimental field testing altogether: both considered to be the most resource demanding activities in breeding programs, so called “breeding without breeding” (El-Kassaby and Lstibůrek 2009). The pedigree reconstruction approach is extended to open-pollinated breeding populations for reconstruction of mating relationships, without making crosses and using the result to estimate genetic parameters for parental genetic evaluation and selection of superior individuals. It has been suggested that BwB is applicable within zones of lesser economic value that do not warrant the setups of a fully designed program such as minor species. According to El-Kassaby and Lstibůrek 2009, the BwB allows the capture of 75–85% of the genetic response to selection attained through conventional program without the need to do any controlled pollination and simplified or possibly no experimental field testing. BwB combines the use of genotypic or phenotypic pre-selection of superior individuals, DNA markers for fingerprinting and pedigree reconstruction of offspring to assemble naturally created full- and half-sib families, resulting from mating among selected parents, and quantitative genetic analyses to identify elite trees for further genetic improvement.

References

- El-Kassaby, Y. A.; Ritland, K. 1986. The relation of outcrossing and contamination to reproductive phenology and supplemental mass pollination in a Douglas-fir seed orchard. *Silvae Genetica* 35: 240–244.
- El-Kassaby, Y. and Lstibůrek, M. 2009. Breeding without breeding. *Genetics Research* 91(2): 111-120.

- Eriksson, U. and Wilhelmsson, L. 1991. Slutrapport till skogsbrukets forskningsfond - För anslag nr. 128 - "Styrd pollinering och kontrollerad fröframställning". Institutet för skogsförbättring, Arbetsrapport 243, 34 pp. [in Swedish]
- Funda, T.; Wennström, U.; Almqvist, C.; Gull, B. A. and Wang, X. R. 2016. Mating dynamics of Scots pine in isolation tents. *Tree Genetics & Genomes* 12: 112.
- Hörnsten, L.; Hannertz, M.; Eriksson, U. and Almqvist, C. 1997. Kontrollerad fröproduktion i växthusplantager. Skogforsk, Arbetsrapport 359, 21pp. [in Swedish].
- Kato, K.; Yamaguchi, S.; Chigira, O.; Ogawa, Y. and Isoda, K. 2012. Efficient establishment of Acacia hybrids by artificial pollination using stored pollen. *Journal of Tropical Forest Science* 24: 209-216.
- Lai, B. S. K.; Funda, T.; Liewlaksaneeyanawin, C.; Klapste, J.; Van Niejenhuis, A.; Cook, C.; Stoehr, M. U.; Woods, J. and El-Kassaby, Y. A. 2010. Pollination dynamics in a Douglas-fir seed orchard as revealed by pedigree reconstruction. *Annals of Forest Science* 67: 808.
- Lambeth, C.; Lee, B. C.; O'Malley, D. and Wheeler, N. 2001. Polymix breeding with parental analysis of progeny: an alternative to full-sib breeding and testing. *Theoretical Applied Genetics* 103: 930–943.
- Lindgren, D. 1994. Effect of tree cover on Scots pine pollination and seeds. *Forest Genetics* 1:73–80.
- Moriguchi Y.; Taira, H.; Tani, N. and Tsumura, Y. 2004. Variation of paternal contribution in a seed orchard of *Cryptomeria japonica* determined using microsatellite markers. *Canadian Journal of Forest Research* 34:1683–1690.

Chapter 7: Selection of the 2nd Generation *Melia volkensii* from Progeny Trials

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7.1 Introduction

The objectives of tree breeding programs include improvement of stand volume and wood properties, adaptation to environments and, breeding for pest and disease resistance. Such improvements are performed through several progeny and clonal-based tests. A tree breeding program typically starts with selection of “phenotypically” superior genotypes as “candidate plus trees” from a natural or planted forests. This is a first-step of mass selection. Progenies are obtained from selected candidate plus trees and grown in test plantations, also known as “progeny test sites” that act as genetic trials. Based on these tests, the best genotypes can be selected. Selected trees are typically vegetatively propagated through grafting or use of cuttings, and second generation seed orchards established for improved seed production.

If clonal propagation is easier, scion gardens comprising the best genotypes are established, and their cuttings are mass produced and used directly in clonal plantations. The seed orchard system is widely used, while the clonal plantation systems are typically applied commercially in some broadleaved trees such as Poplars and Eucalypts.

7.2 Progeny test sites of *Melia volkensii*

Candidate plus trees were selected from the northern, central and southern distribution range of Kenya, and have been planted as 1st generation clonal seed orchards in Kitui and Kibwezi. The orchards produced seed in the second year, the seed collected, seedlings raised in the nursery. The seedlings were used to establish first generation progeny test sites (PTS) in 2014 and 2015 in order to develop superior varieties of *M. volkensii*. The genetic analysis of the progeny trials gave results of genetic parameters used to enhance the Melia breeding activities.

Initially, 25 candidate sites for Progeny Trial Site (PTS) establishment were selected, and their climate conditions were compared by cluster analysis (Ward method). Finally, 12 sites were selected based on climate conditions (based on data from the WorldClim v1.4 databases). These sites were: Kibwezi, Marimanti, Gaciongo, Makima, Tiva, Ikithuki, Kasigau and Voi some being main and some sub-progeny trials (Figure 7.1).

Climate conditions varied at each test site:

- PTS-Kibwezi have a comparatively high temperature and high seasonal variability in temperature, with intermediate precipitation in dry seasons and low precipitation in the wet seasons.
- The PTS-Marimanti and Sub-PTS at Gaciongo (also in Marimanti area) have somewhat high temperature and high seasonal variability in temperature, with low precipitation in both dry and wet seasons.
- Sub-PTS-Makima has comparatively low temperature and high seasonal variability in temperature, with low precipitation in the dry seasons and high precipitation in the wet seasons.

- The PTS-Tiva (in Kitui) has slightly low temperature and intermediate seasonal variability in temperature, with somewhat low precipitation in the dry seasons and high precipitation in the wet seasons.
- Sub-PTS-Ikithuki has slightly low temperature and low seasonal variability in temperature, with low precipitation in both dry and wet seasons.
- PTS-Kasigau and Sub-PTS-Voi have comparatively low temperature, and low seasonal variability of temperature, with low precipitation in both dry and wet seasons.

Each PTS and Sub-PTS site includes seedlings produced by mother trees derived from the northern, central and southern *Melia* distribution areas, and therefore their performance can be tested in various climatic conditions. If certain levels of variation in survival rate and/or growth performance (e.g. variation among provenances > variation among families) are detected, cautious rules about seed and seedling distribution range should be considered. Feedback from regular research at test sites will be important.



Figure 7.1. *Melia volkensii* Progeny Test Sites

(From North to South: Gacigongo Sub, Marimanti Main, Makima Sub, Kitui Main, Ikithuki Sub, Kibwezi Main, Voi Sub and Kasigau Main PTS)

7.3 Evaluation of traits in PTS

Since setting up of the PTSs, their assessment was undertaken twice a year in January - February

and July – August. Traits measured were tree height, diameter, fecundity, healthiness, and stem form (Figure 7.2). Wood property was also assessed at year 5.

From the statistical analysis on growth and seed production, superior clones of 1st generation *Melia* that exhibited superior growth and good seed production were identified. Results of the PTS indicated that:

- There was a significant growth variation among families, despite large between- and within- site growth variation.
- Based on the breeding analysis, performances of both growth and seed production were predicted to improve in the future.
- The progenies of “good” families generally showed faster growth across sites.

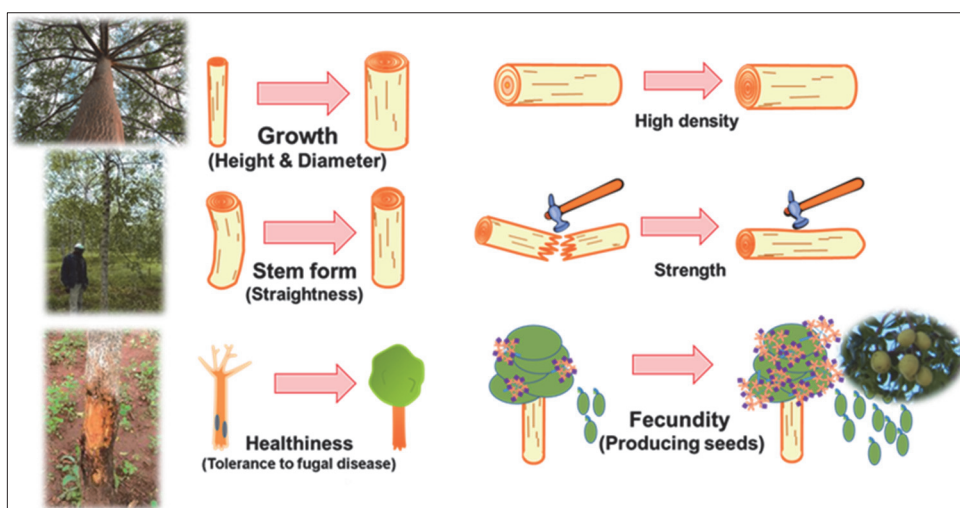


Figure 7.2. Summary of measured traits in PTS



Plate 7.1. Planted *Melia volkensii* trees in PTS

7.4 Selection of 2nd generation *Melia volkensii*

Four hundred (400) superior trees were selected from the PTSs as F2 forward breeding population. In 2019, based on PTS assessment data, a step toward selection for next-generation were taken

using statistical analysis.

We analyzed PTS data and estimated genetic performance of *Melia* trees in PTS. Based on estimated breeding value as an indicator of the genetic performance for each individual tree, the order of priority in traits rankings were; 1. Volume, 2. Stem straightness and healthiness, 3. Fecundity. Progenies were selected in such a way that estimated genetic performance in each trait was kept as high as possible. Using data of multiple traits, improving multiple traits simultaneously was done.

As discussed in Chapter 5, it is important to balance improving multiple traits and maintaining genetic diversity because the original breeding population size of *Melia volkensii* is relatively small (100 CPTs of 1st generation). Using these principles, selection of 2nd generation *Melia* from PTS was carried out. About top 10% of planted trees in each PTS were selected. Since the results of early selection was based on four-years-old *Melia* selection of the breeding population for next-generation was based on genetic gains estimated at 14% for stem volume. These activities were followed by discussion on the future *Melia* breeding plan setting up 100 2nd gen individuals in respective eco-region (which were thought to be manageable breeding genetic resources in Kenya).

It was proposed that the 2nd generation orchard and PTS be set –up in the four eco-regions with each ecoregion having 100 2nd generation individuals (Figure 7.3)

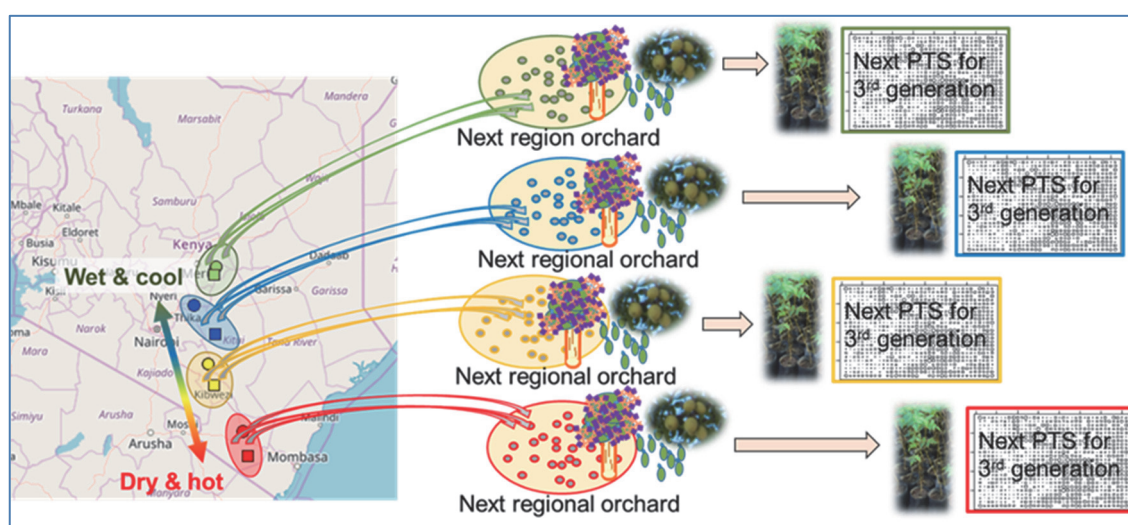


Figure 7.3 Proposed distribution of 2nd generation trees

References

- Kariuki, J. G.; Mulaya, J.; Kimondo, J.; Ogawa, S. and Muchiri, D. 1990. Genetic improvement of *Melia volkensii*: Evaluation of candidate plus trees. <http://10.10.20.22.8080//handle/123456789/502>
- Kimondo, J. M. and Kimba, K. 2004. An overview of natural distribution, propagation and management of *Melia volkensii*. In Kamondo, B. M.; Kimondo, J. M.; Mulatya, J. M. and Muturi, G. M. Research and Technology Development of Mukau (*Melia volkensii* Gurke). Pp 7-11.
- Luvanda, A. M.; Musyoki, J.; Cheboiwo, J.; Wekesa, L. and Ozawa, M. 2015. An assessment of the socio-economic importance of *Melia volkensii* based enterprises in Kenya. KEFRI/JICA Project
- Muchiri, G.; Ndufa, J. K. and Takeda, Y. 2016. Chapter 1. Introduction. In KEFRI (ed) Guideline on Production, Distribution and Use of Improved Melia Seed and Seedlings in the Drylands of Kenya. Pp. 1
- Steven, J. P.; Robert, P. A. and Robert, E. S. 2006. Maximum entropy approach to species distribution modelling. Ecological Modeling 190: 231-259.

Chapter 8. Future Directions in *Melia volkensii* breeding

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8.1 Introduction

Melia volkensii is a high value indigenous and drought tolerant tree species found in Arid and Semi-Arid Lands (ASALs) of eastern Africa. It grows well mostly on sandy soils, sandy loams, or sandy clay soils with good drainage. The species is fast growing (up to 20 meters in 10 to 15 years), multipurpose tree providing high quality termite resistant timber that is used in making high quality furniture. Other products of *Melia* include poles, posts, fodder, medicine, firewood, and bee forage.

The immense potential of *M. volkensii* as a drylands plantation species is threatened by over-exploitation of natural stands for timber production (Stewart and Blomley, 1994) and habitat fragmentation in settled areas that are associated with genetic erosion and dysgenic selection (Runo *et al*, 2004). Selection of good quality trees, their ex-situ conservation and breeding is the most promising intervention towards sustainable development of *M. volkensii* as plantation species in the semi-arid-areas. In 2012, KEFRI started the *Melia* improvement programme in collaboration with JICA and FTBC. The initiative will make available genetically improved seed to be distributed for commercial plantations in the drylands

The breeding objective for the *Melia volkensii* is production of fast growing trees for timber production that will be drought tolerant and adaptable to both the common *Melia* growing areas and drier sites. The desirable traits include good stem form, growth and branching habit, and apparent resistance to pests and diseases.

8.2 Current status of *Melia* breeding

Breeding programme for *Melia volkensii* was initiated in 2012. The first generation of the breeding process involved selection of Candidate Plus Tree (CPTs), scion collection from the CPTs and grafting, establishment of clonal seed orchards, progeny test establishment, data collection, analysis and evaluation of genetic parameters and selection of second generation trees from the progeny tests. The achievements of these activities are summarized below:

Activity	Achievement
Candidate Plus Tree(CPT) selection	• 100 CPTs selected in 13 transects across the <i>Melia</i> natural range from 80m to 1,700m above sea level in: Galana, Voi, Mutha, Kitui, Embu, Tharaka-Nithi, Isiolo, Samburu, Marsabit and Bangale
Scion collection and grafting	• 80 scions were collected from each CPT and grafted onto rootstock at KEFRI Kitui nursery. Seedlings managed and cared for 4 months before transplanting
Clonal Seed Orchard establishment	• Two 11- hectare 1st generation clonal seed orchards established at Tiva and Kibwezi

Activity	Achievement
Progeny Test establishment and data collection	<ul style="list-style-type: none"> • Eight progeny tests planted in diverse regions of the <i>Melia</i> range. To assess the genetic worth of parent trees based on performance of their progeny • Data collected from one (1) month age and thereafter every twice a year for 6 years • Wood properties assessment done in all Progeny test sites
Data analysis for evaluation of genetic parameters	<ul style="list-style-type: none"> • Data analyzed and used for: <ul style="list-style-type: none"> ○ Selection of 2nd generation candidate trees ○ Development of a traits table for use in further improvement of <i>M. volkensii</i> ○ To be used for improving 1st generation seed orchard through genetic rogueing

8.3 Future direction in *Melia volkensii* breeding

The *Melia volkensii* breeding program is advancing well and there is a growing need for improved seed. The strategy is to move the breeding population forward through establishment of F2 clonal seed orchards, which will be followed by establishment of F2 progeny tests. In addition, the results of genetic analysis of F1 progeny tests will be used in improvement of existing seed orchards through rogueing. We also intended to broaden genetic base of the current breeding population to at least 150 CPTs families through new infusions.

Initial indications are that the families perform consistently relative to one another over the target sites. Should the plant physiological studies indicate a need to develop a specific drought tolerant sub populations/seed orchard, this can be added to the overall plan.

8.4 Summary of way forward

1. The existing seed orchards will be improved through 30% rogueing in 4 of the 6 blocks, based on the progeny performance observed in the series of progeny trials.
2. Establish F2 clonal seed orchards using 400-tree F2 forward breeding population selections identified from joint genetic analysis of the 2015 main progeny and sub progeny trials. Integrate wood properties data in further evaluation of progeny tests.
3. The progeny and sub-progeny trials will undergo 20% thinning per family after enough data is collected in order to enrich pollen cloud and use the trials for seed production. The purpose of this action is to prepare the trials for production of improved next generation seed (F2 seed).
4. At least 50 additional *Melia* CPTs will be selected from areas of *Melia* occurrence to broaden the genetic base of the breeding population. Since valuable genetic diversity exists in the

extremes of the natural distribution of a species, collections from the extremities of *Melia* distribution (southern Ethiopia and northern Tanzania) will be added as the new infusions, and in combination with existing CPTs, used to form a breeding archive/foundation stock. The proportion of the arid/semi-arid/very arid selections will be informed by the analysis of the progeny trials.

5. Genetic gain trials will be established in order to monitor the relative improvement of the various seed sources and generations. Potential entries in the genetic gains trials include seed from each of the current clonal seed orchards; seed from the same orchards after rogueing, seed from the orchards arranged according to zone of origin, and clones of the F2 seed collected from the progeny trials.

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