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8 Clonal Propagation of Multipurpose and Fruit Trees Used in Agroforestry

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1. INTRODUCTION

One of the keys to the widespread adoption of agroforestry (AF) is the availability of high quality planting stock of appropriate multipurpose and fruit trees (MPFT). Although zygotic seed (i.e., sexual propagation) is often the easiest, cheapest, and most common means of propagating most MPFTs, in this review we have chosen to emphasize the alternative, asexual propagation, as both a traditional and increasingly important contemporary approach to plant propagation. The terms *vegetative* and *clonal propagation* will be used synonymously with *asexual propagation*. The reader is referred to several recent publications on critical aspects of MPFT production from seed, including germplasm conservation and collection, germination, dormancy, storage, distribution, evaluation, etc. (Turnbull, 1990; Sedgley and Griffin, 1989a; Glover and Adams, 1990; Venkatesh, 1988).

Vegetative propagation has traditionally played an essential role in the production of certain MPFT species, and is becoming increasingly important as the scientific discipline of AF focuses on genetic improvement of MPFTs. The purpose of this review is to assess the state of indigenous and more recent knowledge of the art and science of asexual propagation of MPFTs used in AF. Although the review emphasizes woody legumes and other taxa in the broad category of multipurpose trees and shrubs, we also include fruit trees; hence the term MPFT. Asexual propagation of fruit trees will be limited to examples relevant to small-scale AF systems, as opposed to high intensity monocultural orchard production systems. MPFTs are defined broadly to include species like banana (*Musa* sp.) and papaya (*Carica papaya*), which, although lacking secondary growth, function as "trees" in AF systems.

We will discuss the factors involved in determining the most appropriate methods of propagation for a given species, and associated constraints and limitations. Current research on asexual propagation will be reviewed, and outcomes that have or may impact the implementation of modern AF systems will be identified. Last, we will consider areas where future research is most needed for the improvement of AF systems. Table 1 is a glossary of technical terms used throughout the chapter.

2. LIMITATIONS OF SEXUAL PROPAGATION

Across many MPFTs and agroforestry systems, seed is the easiest and cheapest method of propagation and is generally considered the default approach, unless other considerations apply. However, there are a number of circumstances that may limit or even eliminate the option to propagate MPFTs from seed.

2.1 SEED AVAILABILITY

The most obvious limitation to seed propagation is the unavailability of seed. The most extreme case would be the need to propagate tree species, hybrids, or selections which are biologically

TABLE 1
Glossary of terminology used in the text. Definitions are, in some cases, specific to the context of agroforestry.

Adventitious	Formation of a root or shoot (bud) from other than naturally occurring sequential branching of a seedling root or shoot system, respectively; i.e., adventitious roots are those which develop on stems or leaves, while adventitious shoots are those which arise on roots (suckering), or on stems, from other than lateral buds. Either adventitious roots and/or buds also may develop *in vitro* from undifferentiated callus tissue in some micropropagation systems.
Allele	The same gene on multiple (two, in the case of diploids) chromosomes within a cell; alleles may be homozygous (identical)or heterozygous (dissimilar)
alleles	One of a pair of genes
aseptic	Plant tissue and associated environment free of microorganisms; characteristic of most micropropagation systems
De novo	Newly developed in response to some external stimulus; not naturally occurring (e.g., root formation on a cutting)
Heterozygosity/ Heterozygous	A preponderance of genes in a plant genome with dissimilar alleles; giving rise to high level of variability in the next generation of seedlings
Homozygosity/homozygous	A preponderance of genes with identical alleles
In vitro	In a tissue culture/micropropagation system; literally "in glass."
Inbreeding	Deliberate self pollination, through several generations, of a naturally outcrossing species, giving rise to increasingly homozygous genotype
Marcottage	Air layering
Meiotic	Cell division giving rise to haploid sex cells (gametes)
Mitotic	Cell division giving rise to genetically identical, diploid, somatic cells, i.e., non gamete cells of the plant body
Ortet	Stock plant from which propagules are obtained for asexual propagation
Precocity	Development of reproductive maturity (ability to flower) sooner than is typical for the species
Propagule	Portion (ramet) of a stock plant (ortet) removed for asexual propagation; i.e., cutting, scion, explant
Ramet	Propagule; portion of an ortet detached for asexual propagation; i.e., cutting, scion, rooted layer, or explant
Recalcitrant	Seed which is intolerant of drying and, in the case of temperate recalcitrant seed, also intolerant low temperature; hence limited storage life.
Scion	Upper portion of a grafted plant which gives rise to the above ground shoot system
Stock (rootstock, understock)	Lower portion of a grafted plant (including the root system) onto which the scion is grafted
Stooling	A type of layering in which rooting occurs at the base of ground level coppice shoots mounded with a moist soil or other medium (e.g., sawdust)
systemic	Occurring throughout the plant body, with the exception of primary shoot and root meristems
vivipary (seed)	Extreme lack of dormancy characterized by germination within the fruit (pod) while still attached to the parent tree.

seedless. Insufficient supply of high quality seed to meet existing demand is a less drastic, though perhaps a more important long term problem. The latter can result from irregular or seasonal (phenological) periodicity of flowering. Monocarpic bamboo (e.g., *Dendrocalamus*) is an extreme example of this since some species require 20 to 50 years of growth to flower and subsequently die. Even annual cycles of seed production combined with poor seed storage characteristics for recalcitrant seeded species like neem (*Azadirachta indica*) may result in seed unavailability.

2.2 SEEDLESSNESS

Infertility resulting in seedlessness has arisen in a number of cultivated plant taxa either as a result of domestication in the distant past, or through more recent hybridization. In cultivated banana, seedlessness arose from natural hybridization between species with different ploidy levels (Purseglove, 1972). The resultant increase in palatability contributed to banana's domestication but it necessitated asexual propagation (by division). Similarly, seedless selections of breadfruit (*Artocarpus altilis*), which are more palatable than the seeded varieties of this species, are propagated by root cuttings (Purseglove, 1972). While there are few other naturally seedless MPFTs, there are instances of deliberate selection of seedlessness in tree crop for which either the seed or the fruit is not considered essential to the utility of the tree. For example, seedlessness has been selected in Washington navel orange (*Citrus sinensis*) (Purseglove, 1968), and several ornamental cultivars of honeylocust (*Gleditsia triacanthos*) (Dirr, 1990); both of which are propagated by bud grafting. In such cases selection for seedlessness has been possible because of asexual alternatives to seed propagation.

In the genus *Leucaena*, seedlessness due to triploidy from interspecific hybridization has been exploited in Indonesia. Brewbaker (1988) reported that seedless hybrids are bud grafted for use as shade over coffee (*Coffea* sp.) and tea (*Camellia sinensis*) because they are not prone to weediness, as is the *L. leucocephala* (Hughes and Styles, 1987; Brennan, 1990). Such hybrids are sterile because they are the triploid offspring of crosses between tetraploid (*L. leucocephala*) and diploid species (*L. diversifolia, L. esculenta, and L. pulverulenta*, etc.).

2.3 INSUFFICIENT SEED PRODUCTION TO MEET DEMAND

In some instances, adoption of AF may be hampered by an insufficient supply of MPFT seed (Mugo, 1997). Ironically, the most serious seed shortages may occur with the species that are least prone to weediness and thus more suitable in AF systems (e.g., *Glircidia sepium*, *Inga* spp.). Even in the genus *Leucaena*, which is widely known for prolific seed production and weediness, the demand for seeds of improved varieties exceeds supply. For example, in a 1997 seed catalog, the average price for seed of several available *Leucaena* species and hybrids exceeded $100 per kilo (Agroforester Tropical Seed, Holualoa, Hawaii).

2.4 SEASONAL LIMITATIONS

The seed of most MPFTs are at least somewhat tolerant of drying and cool temperatures, which are characteristics necessary for moderate to long term seed storage. Consequently, these species can be harvested and stored for later use locally or elsewhere. On the other hand, MPFTs with recalcitrant seed (intolerant of low temperature and/or drying, and hence store poorly; e.g., mango (*Mangifera indica*), neem, citrus, etc.) are only available during a narrow window of time. The woody leguminous genus *Inga* is another example. Several species of *Inga* are used extensively throughout tropical America for fuel wood, coffee shade, and fruit, however seed *recalcitrance* and *vivipary* restrict their broader distribution (transport). Vegetative propagation could provide an alternative, which would permit propagation of *Inga* spp. throughout the year and possibly facilitate distribution of propagules outside of their native range.

2.5 GENETIC VARIABILITY IN SEED PROPAGATED TREES

Sexual outbreeding through cross pollination has evolved as the dominant natural reproductive strategy (breeding system) in trees because it ensures genetic recombination, *heterozygosity*, and the concomitant seedling variation on which natural selection may act to bring about adaptations favorable to survival (Jain, 1976). Domestication of normally outcrossing species, on the other hand, depends on increasing uniformity, not only for its own sake (e.g., synchronous harvest), but also because it makes possible the selection of improved genotypes. Genetic gains associated with deliberate selection of superior genotypes of normally outcrossing species can be captured immediately, via cloning of the selected individual, avoiding the loss of the selected trait in subsequent seedling generations due to segregation of *alleles*.

3. RELATIONSHIPS BETWEEN NATURAL REPRODUCTIVE SYSTEMS AND DEVELOPMENT OF DELIBERATE PROPAGATION STRATEGIES FOR CROP SPECIES

Woody plants have evolved an impressive array of reproductive strategies which have contributed to their survival and proliferation (Hancock, 1993). Several distinct breeding systems have evolved in higher plants including, in order of importance: predominant outcrossing, predominant inbreeding, apomixis, and other vegetative propagation strategies (Jain, 1976). The most common of these in natural populations is outcrossing, which assures the genetic variability on which natural selection acts to bring about adaptive evolutionary change. On the other hand, a shift towards self pollination is regarded as a characteristic of the domestication syndrome of crop species which has resulted in increasing homozygosity, less seedling variability and hence greater crop uniformity (Hancock, 1992). *Inbreeding* has facilitated the fixing of improved genotypes by unconscious or deliberate selection by early agriculturists. This trend has continued in more modern times. Many fruit trees such as apple (*Malus domestica*), grape (*Vitis vinifera*), and citrus, and woody ornamentals such as *Rhododendron* sp. and *Bougainvillea* sp.), and some, elite lines of forest tree species such as Douglas fir (*Pseudotsuga menziesii*) and *Eucalyptus* spp., have been greatly improved genetically as a result of artificial selection programs which have depended, at least in part, on vegetative propagation. Furthermore, the successful future application of genetic engineering to tree crop improvement ultimately will depend on regeneration of intact transformed plants via *in vitro* asexual propagation. Other factors which contribute to the relatively difficult domestication and improvement of MPFT species by classical (sexual) breeding include their long generation times and irregularity in flowering and fruiting (Leakey, Newton and Dick, 1994). In such cases, vegetative propagation can be a powerful tool for capturing genetic gains.

Although sexual reproduction of woody plants has been the dominant theme both evolutionarily and throughout the history of human agriculture, woody plants have evolved a diverse array of vegetative reproductive strategies as well (Sedgley and Griffin, 1989b). Human understanding and modification of these natural vegetative reproductive strategies has been essential to the development of traditional and modern agricultural systems. Throughout the history of crop domestication, farmers and modern plant breeders have inadvertently and/or deliberately selected for ease of propagation. Widespread cultivation of vegetatively propagated crops may actually predate cultivation of seed propagated crops because of the relative ease of propagation of the former (Hancock, 1992).

The relatively "wild," undomesticated state of most MPFTs (Burley, 1993) compared to the world's major seed propagated food crops, such as rice (*Oryza sativa*), maize (*Zea mays*), and beans (*Phaseolus* sp.), has some bearing on the relative difficulty of seed propagation of MPFTs, and hence the relative usefulness of vegetative propagation. With respect to major seed propagated crop species, the domestication syndrome described by Hancock (1992) includes features which facilitate seed collection, like lack of shattering, and rapid germination due to the absence of seed dormancy. On the other hand, the relatively undomesticated MPFT species tend to retain sexual reproductive traits, associated with adaptations to their natural environments (e.g., dormancy, shattering of dehiscent fruit, etc.), that render them more difficult to propagate from seed in sufficient quantity for use in AF systems. For example, whereas seed dormancy has been almost entirely selected against in agronomic legumes like *Phaseolus* beans, seed coat-associated dormancy is a

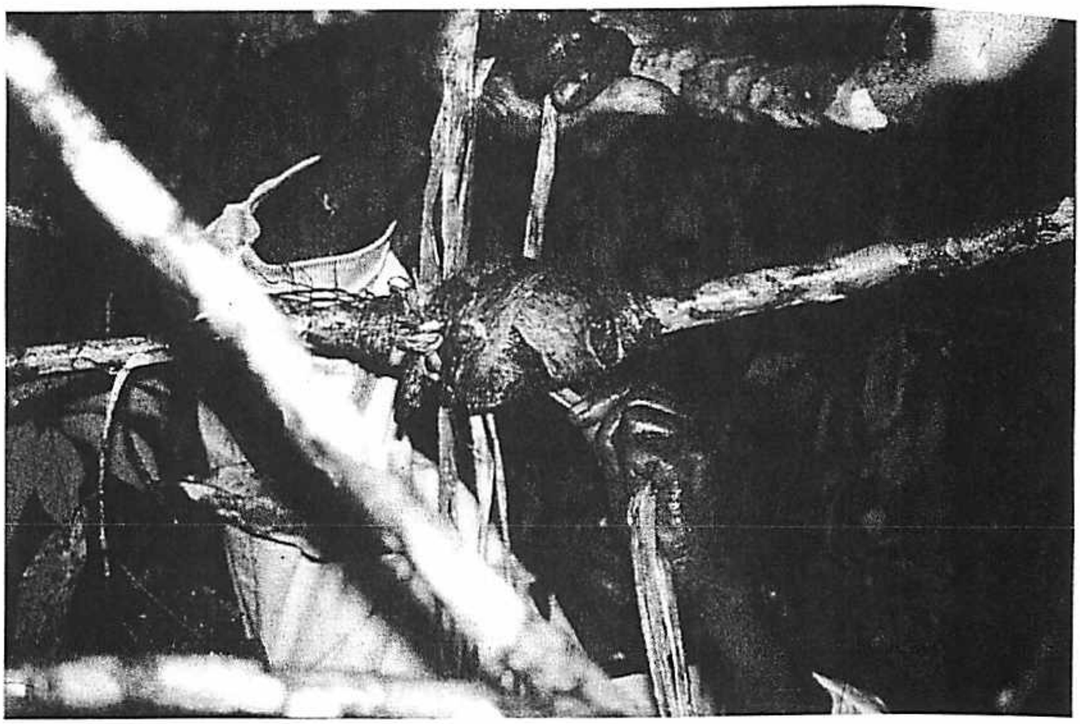

FIGURE 1. Traditional method of marcottage (air layering) of lychee in the Ranomafana region of Madagascar. A woody branch is wounded by girdling with a knife, and the girdled area is packed with a mixture of cow dung and mud. This is wrapped with the dried leaf of raffia palm and tied with cordage made from the inner bark of haftra (*Dombeya* sp.).

common characteristic of many woody legumes adapted to xeric and/or cold environments (e.g., *Acacia, Leucaena, Robinia*). While such dormancy is advantageous in terms of long-term natural seed bank "storage" or deliberate storage of seed, it may interfere with prompt, uniform germination under cultivation. As a result, a major focus of MPFT seed propagation research has focused on pregermination treatments to overcome dormancy. On the opposite extreme are MPFTs, usually from the humid tropics, which exhibit little if any seed dormancy, and are intolerant of drying and/or low temperature. Such seeds, termed recalcitrant, have very low storage potential (Bonner, 1990; Chin, 1989). Some tropical MPFTs, which produce recalcitrant seeds, include mango, citrus, rubber (*Hevea brasiliensis*), jackfruit, avocado (*Persea americana),* coffee, cocoa (*Theobromo cacao*), *Inga* spp., and neem. Examples of temperate recalcitrant species include nut trees such as hickories (*Carya* sp.*)*, pecans (*Carya pecan*), filbert (*Corlyus avellana*), walnut (*Juglans* sp.), and oak (*Quercus* sp.*)*.

The observation and utilization by early agriculturists of naturally evolved asexual reproductive strategies must have played an important role in the development of deliberate vegetative propagation techniques. For example, natural rooting of attached vegetative structures (e.g., natural layering of epiphytic *Ficus* species) could easily lead to deliberate *marcottage* (Figure 1) and other layering methods. Cuttage propagation is likely to have arisen from observation of the rooting of wind thrown branches of riparian species, such as willow. Natural division of perennial organs (e.g., tubers, corms, and runners) is the biological basis for vegetative propagation of important crops like cassava (*Manihot esculenta*), potato (*Solanum tuberosa*), banana, etc. Observation of natural grafting at points of branch-to-branch contact may have inspired early attempts approach grafting (Figure 2) and led subsequently to detached scion grafting and budding of fruit and other tree species.

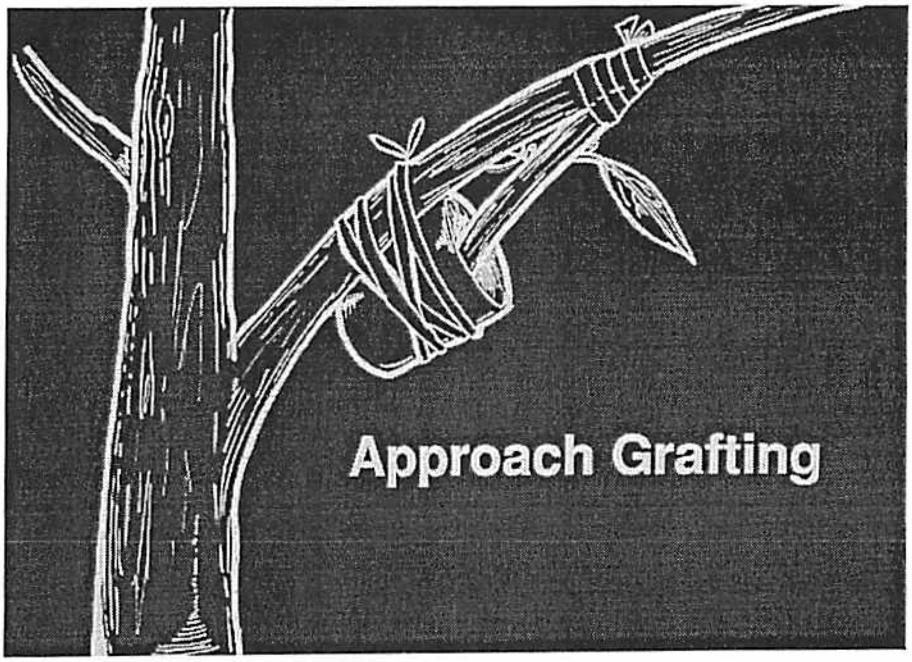

FIGURE 2. Approach grafting. This ancient intact-scion method of grafting allows the graft union to form with minimal water stress because the scion remains attached to its own root system (main tree). After graft union formation, the scion has an alternative source of water and nutrients from the root system of the understock plant in the small pot. It can then be cut away from the main tree (scion donor), with a minimum of water stress.

4. ASEXUAL PROPAGATION STRATEGIES

Asexual propagation may be defined as regeneration of a new individual from a portion (*ramet*) of a stock plant (*ortet*) by processes involving *mitotic* (not *meiotic*) cell division, and subsequent regeneration of complementary cells, tissues, and/or organs or entire plant to replace those "missing" from the ramet. Shoot cuttage, layering and shoot micropropagation involve replacement of roots by the process of *adventitious* root formation, while root or leaf cuttage and some types of micropropagation involve replacement of shoots by adventitious shoot (bud) formation. In the case of grafting, the missing part is replaced by fusing the ramet (*scion*) to a compatible *stock*. "Replacement" may occur via asexual (somatic) embryogenesis in some micropropagation systems. Apomixis is a special case of naturally occurring asexual reproduction in which seed is produced that contains an asexual (not zygotic) embryo which arose from mitotic division of maternal cells in flower-associated tissues, rather than by fertilization of a maternal egg cell by pollination.

4.1 LAYERING

Layering involves induction of adventitious roots along a portion of the shoot of an intact plant so that the newly rooted shoot section can be subsequently detached and transplanted. This may involve root induction on a shoot in the aerial portion of the tree (marcottage), or on a shoot at the base of a tree, either naturally or deliberately brought into contact with soil (tip layering, mound layering, etc.). Since the shoot undergoing rooting has a continuous supply of water from its preexisting root system, layering involves less moisture stress to the shoot being layered than propagation from shoot cuttings. Layering occurs naturally in some species, including purple raspberry (*Rubus occidentalis x idaeus*), many figs (*Ficus*), strawberry (*Fragaria x ananaassa*) (roots associated runner plantlets), some vines (e.g., English ivy, *Hedera helix*), etc. In a broad sense, root suckering could be considered the inverse of shoot layering since it involves adventitious formation of a complementary organ (shoot) on an intact organ (root). In either case, deliberate wounding may or may not be required to induce the process of adventitious root or (bud) formation. In the case of shoot layering (*stooling*, marcottage, tip layering), rooting is facilitated by light deprivation (etiolation) of the shoot by covering a portion of it with a moist medium (e.g., soil, moss, sawdust, etc.

4.2 CUTTAGE

Unless specified otherwise, cuttage refers to induction and/or elongation of adventitious roots at the basal end of a shoot cutting (ramet), detached from an intact stock plant (ortet). In the case of leaf and root cuttings, both adventitious shoot and adventitious root formation must occur to complement the ramet. With most species, rooting of a shoot cutting involves induction of *de novo* adventitious roots in response to wounding and/or other stimuli. In some species, formation of a new root system may involve elongation of preformed adventitious roots initiated during an earlier stage of normal shoot development (e.g., *Ficus*, bamboo, English ivy). Induction of *de novo* adventitious roots may only require the stimulus of wounding (severance from the ortet) for easy-to-root species, whereas more difficult-to-root species may require the additional stimulus of auxin type plant hormone or growth regulator, and/or additional post severance wounding, moisture, or etiolation. Especially in more difficult-to-root species, avoidance of water stress is critical for rooting cuttings, necessitating precise management of moisture, irradiance, and temperature of the propagation environment.

4.3 GRAFTING

Grafting involves placing two similar or dissimilar plant organs (stem/stem, stem/root, or root/root) from genetically compatible plants in intimate contact, with sufficient pressure and cambial alignment to induce the formation of an anatomically and physiologically functional graft union between the scion and stock. Just as the presence or absence of an intact root system affects the degree of moisture management required in air layering and cuttage respectively, intact scion grafting such as approach grafting (Figure 2) or inarching requires less intensive moisture management than detached scion grafting or budding. This is because both stock and scion have an intact root system in the case of approach grafting or inarching.

4.4 MICROPROPAGATION

In the broad sense, micropropagation refers to *in vitro* plant regeneration involving the use of a relatively small propagule, referred to as an explant, on an artificial nutrient medium within an *aseptic* environment. Development leading to multiplication may involve stimulation of normal (non-adventitious) elongation and/or branching of the original explant (shoot or root), and or *de novo* induction of adventitious organs (shoot and/or root). In the more narrow sense, micropropagation refers specifically to axillary or nodal shoot culture, which is the most common/important type of *in vitro* plant propagation. Shoot culture involves the use of growth regulators (cytokinins and/or auxins) during a multiplication stage to promote repeated cycles of shoot elongation and/or branching, followed by an indefinite number of cycles of subdivision and reculture on fresh medium. Proliferated shoots are then rooted as microcuttings either *in vitro* or *ex vitro*. Rooting may or may not require exogenous auxin-stimulated root induction. Other less common and generally less desirable *in vitro* plant multiplication systems involve proliferation adventitious buds or somatic embryos directly on an explant or from secondary undifferentiated callus or liquid cell suspension cultures.

4.5 APOMIXIS

Apomixis, or apomictic embryo formation, as described above, is a natural asexual seed formation process, which, unlike more typical zygotic seed arising sexually, involves only maternal (not paternal) genes. Hence, apomictic seedlings are genetically identical (clonal) to the maternal parent. In some cases (e.g., citrus, mango), one or more apomictic embryos may co-occur in the same seed with a zygotic (sexual) embryo. This is known as mixed (zygotic and apomictic) polyembryony. Apomictic seedlings, like sexual seedlings are rejuvenated, capable of flowering only after a somewhat protracted period of maturation, and hence delayed in flowering/fruiting compared to clonal plants arising from cuttage, grafting, or layering. Because of their clonal uniformity, apomictic seedlings are used widely as rootstocks for citrus and mango.

5. PROPAGATION IN TRADITIONAL AND/OR LOW TECHNOLOGY AGROFORESTRY SYSTEMS

Traditional AF systems are defined here as the historical and ongoing integrated use of trees and crops by indigenous peoples using low technology approaches developed more or less *in situ.* To some extent this is the same as defining traditional practices as those which are indigenous, i.e., practiced prior to the relatively recent introduction of "modern" technologies consisting of materials and/or services not available previously. Important propagation-related technologies categorized as modern include those which require the use of electricity, plumbed water and synthetic materials like polyethylene and synthetic rooting hormone (auxin) formulations. As implied above, the MPFTs used as components of traditional AF systems tend to be those which were chosen not only for one or more desirable products or services, but also selected, as least inadvertently, for relative ease of propagation. Many traditional practices involving asexual propagation, on the other hand, have developed for MPFTs, which do not produce seed readily, or at all, such as cassava, banana, or bamboo.

One of the simplest traditional vegetative propagation systems is collection of root suckers, sometimes referred to as wildings. Root suckers originate from adventitious bud formation on roots, either spontaneously, or as a result of wounding roots. Many species naturally root sucker such as *Faidherbia albida* (Ajee and Duhoux, 1994), *Inga feuillei* (Mudge, personal observation), *Robinia pseudoacacia, Ocotea usambarensis* and *Melia volkensii* (Teel, 1984), *Acacia* spp., *Chlorophora* spp, *Cordia alliodora,* and *Melia azedarach* (Longman, 1993). Traditional propagation by division of basal shoots, sometimes referred to as "suckers," but of axillary rather than adventitious origin, is practiced with the tree-like MPFTs, banana and bamboo.

Cuttage is another traditional method of vegetative propagation which requires minimal specialized equipment or skill. The semi-woody perennial root crop cassava is typically propagated from leafless shoot cuttings taken at the time of root harvest (Purseglove, 1968). In many traditional AF systems, large cuttings known as stake or pole cuttings are rooted directly in their final field location for use as living fences, for soil regeneration and production of firewood, medicinals or other products (Gautier, 1995; Jolin and Torquebian, 1992; MacDicken, 1990). For direct field planting, large cuttings are preferred over seedlings and smaller nursery-rooted cuttings, because they are often quicker to produce useful products and services. MPFTs commonly rooted from large stake cuttings include species in the relatively ease-to-root genera *Ficus, Erythrina* (Gautier, 1995; Jolin and Torquebian, 1992), *Glircidia*, and *Hibiscus* (MacDicken, 1992). Martin (1997) presents an extensive list of species suitable for living fences, many of which are amenable to propagation from stake cuttings. Farmers in Cameroon use seven species of *Ficus*, two of *Erythrina*, and eight other species as hedge rows, for demarcation of boundaries, and containment of livestock (Gautier, 1995). Jolin and Torquebian (1992) observed stake cutting propagation of five species of *Erythrina* and 37 other species in various tropical locations in Central and South America, Africa, and India, as well as *Salix sp., Robinia, Populus, Morus, Cupressus,* and *Castanea* in temperate locations in Europe and North America. Interestingly, several of the genera reported to be successfully rooted from stake cuttings, including *Leucaena leucocephala* (Jolin and Torquebian, 1992; MacDicken, 1990) in the tropics, and the temperate species *Robinia, Tilia, Castanea,* and *Picea* (Jolin and Torquebian, 1992), are generally regarded as moderately difficult to root. It should be noted that in some cases, stake cuttings may erroneously appear to have rooted based on initial shoot growth, however, subsequent decline and excavation may indicate a lack of root formation (Brennan, personal observation).

Propagation from stake cuttings usually begins with pollarding (Gautier, 1995) or coppicing (Jolin and Torquebian, 1992) of established plants, followed by new shoot growth over three or four years, and subsequent harvest and planting of the stakes during the rainy season. The length (0.5–2.5 m) and diameter (5–15 cm) of stake cuttings may vary significantly with species and country (Jolin and Torquebian, 1992; Gautier, 1995). Leaves are removed and stakes may be "conditioned" before planting. This is done by stacking them in a shady location for one to several weeks. Successful rooting of stake cuttings is typically greater than 70% (Jolin and Torquebian, 1992; Gautier, 1995). With some species (*Erythrina burana*) before planting large (2 m long × 10 cm diameter) stake cuttings, the bark is removed from the portion that is to be buried (Teketay, 1990).

Vegetative propagation of MPFTs by methods other than cuttage have been practiced where an advantage can be gained by clonal rather than sexual propagation. Lychee (*Litchi chinensis*), for example, is propagated by marcottage (described below) because it cannot be rooted from cuttings and when planted from seed it takes several years to flower and is not true-to-type.

Grafting is being used in some traditional AF systems. A combination of grafting and cuttage is practiced by Indonesian farmers, to achieve an unusually "intimate" tree/crop association between the Ceara rubber tree (*Manihot glaziovii*) and cassava (*Manihot esculenta*). De Bruijn and Dharmaputra (1974) describe the invention, in 1952, of a unique method of cassava production by a Javanese farmer, Mukibat. Over the last several decades "Mukibat grafting" has been adopted not only by Javanese farmers, but also in Sumatra as detailed by Foresta, Basri and Wiyono (1994). The multipurpose Ceara tree, though displaced for rubber production by *Hevea brasiliensis* (para rubber), is used by farmers as a source of oil for cooking, edible leaves and fodder. Mukibat grafting involves grafting a shoot of Ceara (scion) onto a cassava shoot (understock) which is then rooted. The resulting grafted plant produces the Ceara shoot products while the cassava rootstock produces more than twice the yield of a normal cassava plant (de Foresta et al. 1994). The basis for the increase in cassava yield is the extension of the growing season due to the lack of dormancy of Ceara (E. Fernandes, pers. com.). Cassava, on the other hand, has an annual period of dormancy. This extension of the growing season is inversely analogous to the dwarfing of apple trees which is achieved by the use of selected clonal rootstocks. In that case dwarfing is achieved by a combination of earlier cessation and diminished rate of growth of the fruit variety when grafted onto a dwarfing rootstock (J. Cummins, pers. com.). Tamarind (*Tamarindus indica*) is another example of a traditional grafting practice. In Thailand, farmers have selected numerous sweet tamarind varieties, which are approach grafted onto sour tamarind rootstocks. In addition to approach grafting, some farmers air layer sweet cultivars and then inarch graft several rootstocks onto the transplanted layer for increased stability in windy sites (Brennan, personal observation). Other reports of traditional grafting include the decades-old Indonesian practice of bud grafting seedless Leucaena species and hybrids for use as shade in coffee and tea plantations described by Brewbaker, 1988.

In some cases, minor modifications of a traditional technique may have profound effects on the success of a propagation technique. For example, Tanala farmers in Madagascar have practiced marcottage to clonally propagate lychee, but the traditional practice is only marginally successful (Mudge, personal observation) (see Figure 1). "Modern" air layering, typically involves wrapping a polyethylene moisture barrier around pre moistened moss which surrounds a wounded stem

(Brennan and Mudge, 1997a). The traditional Tanala practice involves packing the wounded stem with a mixture of mud and dung, wrapping it with dried leaves of raffia palm, and securing it with cordage from the inner bark of haftra (*Dombeya sp.*), an indigenous tree. Because the raffia leaf wrapping provides little resistance to evaporation, the dung packing material has a tendency to dry out during periods of low rainfall. Due to repeated wetting and drying of the packing, success is low (<50%), and marcotts which root take from six months to a year before they are sufficiently well rooted to be severed from the parent plant. The recent introduction of polyethylene sheeting, as a moisture barrier, increased the success rate from <50% to >80%, and decreased time to harvest of rooted marcotts from >six months to as little as eight weeks. Access by farmers, world wide, to polyethylene has had a profound effect on their ability to asexually propagate plants. The use of polyethylene for moisture management in cutting propagation is discussed below.

6. CONSIDERATIONS IN THE SELECTION AND USE OF ASEXUAL PROPAGATION TECHNOLOGIES

6.1 APPROPRIATE TECHNOLOGY

The various propagation strategies can be classified along a continuum from passive to active environmental modification, ranging from those that take advantage of natural processes with minimal technological inputs, to those requiring extreme and sophisticated technological intervention. Hence, from low to high technological input, the continuum would be as follows:

harvest of natural suckers or layers < deliberate layering
< cuttage (hardwood < semihard wood < softwood)
< grafting (approach < detached scion)
< micropropagation < *in vitro* embryogenesis.

The point of this ranking is to emphasize the importance of choosing a method of propagation for a given MPFT species that will not only achieve the intended outcome, but also be appropriate for the level of technology available and expertise of the propagator. For example, because of the need for aseptic conditions, it is unlikely that tissue culture techniques will be appropriate for on-farm or nursery production. On the other hand, it is likely that tissue culture will become an increasingly important tool for MPFT breeding/tree improvement programs. Moisture management of leafy cuttings is restricted to the use of polyethylene water vapor barriers when plumbed water and electricity is not available for mist propagation. The specific environmental and physiological constraints, which apply to each method of propagation, will be discussed in Section 6.4.

6.2 GROWTH PHASE CONSIDERATIONS

The ontogenetic development of a woody plant from the point of seed germination to eventual flowering involves a physiological change in receptivity to flower induction by environmental conditions (photoperiod, temperature, etc.). A seedling begins its life cycle in the juvenile phase characterized by its inability to flower regardless of environmental conditions. Subsequently, after a period of as little as a few months to as much as several to many years, the tree undergoes a gradual transition to the adult phase, characterized by the ability to flower under inductive environmental conditions (Hackett, 1985). Obviously, this process of reproductive maturation is essential to the agricultural production of flowers, fruits, or seeds. On the other hand, vegetative propagules (cuttings, scions, explants for micropropagation, etc.) taken from adult phase tissues are significantly more difficult to propagate by cuttage, grafting, layering, or by micropropagation, than juvenile phase propagules.

Recognition and understanding of the temporal and spatial distribution of the juvenile and adult phases of the sexual life cycle has important implications with respect to the outcome of asexual propagation. In this regard, it is important to understand that reproductive maturation (transition from the juvenile to the adult phase) does not occur in differentiated tissues (stems and leaves), but in primary apical and lateral shoot meristems (Zimmerman, 1972). The chronological age of a given meristem at the time of its phase transition is not fixed, but rather it is proportional to its rate of node production (i.e., growth rate) (Hackett, 1985). Hence, a more rapidly growing terminal shoot undergoes meristem maturation (phase change) sooner than a dormant or slower growing lateral or basal shoot meristem. Epicormic buds are buds initiated early in shoot development that lie dormant indefinitely unless renewed bud growth is triggered by coppicing or other stimulus. Epicormic buds laid down early in the development of tree seedlings are juvenile and remain so as long as the buds remain dormant, but the process of reproductive maturation resumes if the buds are stimulated to grow (Kramer and Kozlowski, 1979). In this sense epicormic buds may constitute a reserve of juvenile meristems on an otherwise mature tree. To fully appreciate the significance of growth phase to successful propagation, it is important to understand that shoot tissues (xylem, phloem, vascular cambium, dormant buds) laid down by a juvenile meristem remain juvenile, even after the meristem from which they developed has undergone its phase transition and begins to produce new adult phase shoots. Hence, a given seed derived tree will simultaneously have juvenile tissue (wood, epicormic buds, etc.) near the base of the tree, produced while the meristem was juvenile, while more distal shoots and buds, produced after meristem phase change, will be in the adult phase. The simultaneous existence of both phases on the same tree, known as cyclophysis (Hartman et al., 1997) has important implications for the selection of vegetative propagules from a stock plant. Cyclophysis dictates that propagules taken from the base of a seed grown tree will be easier to propagate asexually, but will flower later than propagules taken from the distal portion of the same tree. Adventitious buds arising from roots (suckering) are apparently also in the juvenile phase, and hence give rise to easily rooted juvenile shoots.

In a minority of woody plant species there are easily recognizable morphological differences between juvenile and adult leaves (heterophylly) and shoots which can facilitate propagule selection. A classic example, which has been the subject of a great deal of growth phase-related research is *Hedera helix*. Juvenile portions of the plant have lobed leaves, a vine growth habit, and performed adventitious shoot born roots, whereas *H. helix* in the adult phase has entire leaves, an upright growth habit and lacks stem born performed adventitious roots (Hackett, 1985). In the case of the Australian phyllodinous acacias (e.g., *A. koa, A. melanoxlon, A. saligna*) juvenile leaves are bipinnately compound whereas adult phase leaves are reduced to a broad flattened petiole (phyllode) without true leaflets (Purseglove, 1968). Juvenile shoots of some citrus species and the temperate woody legume, *Gleditsia triacanthos*, bear spines, whereas adult shoots are spineless. All of these phase-related characteristics can be used by the propagator to select propagules of the appropriate growth phase (juvenile for ease of rooting; adult for rapid onset of flowering) in the relatively few species which exhibit phase-related dimorphisms. Propagule selection for most other species, which do not exhibit phase dimorphism, must be based on the positional considerations described above.

It should be pointed out that a natural process of rejuvenation occurs during embryogenesis, during the formation of zygotic or apomictic seed, or *in vitro* somatic embryos. Apparent rejuvenation associated with micropropagation and serial grafting will be discussed below.

6.3 CLONING AS A TOOL FOR TREE IMPROVEMENT

6.3.1 Exploration, Selection, and Domestication of New MPFTs

In efforts to bring about MPFT improvement through plant exploration and collection of new germplasm, for subsequent evaluation, and eventually domestication, the propagule of choice, collected in the field, is usually seed. This is true for a variety of reasons including the ease of transport and storage, and also it suits the objective of collecting as much genetic variation as possible. Nevertheless, seed availability does not always coincide with the timing of collection trips and hence collection of vegetative propagules may be necessary.

As part of a tropical tree improvement program, Leakey and co-workers at the Institute for Tropical Ecology (ITE) have focused on vegetative propagation technology, mainly via cuttings, for selection of improved genotypes. Many of the species they have worked with are currently or potentially important components of AF systems (Leakey, Newton, and Dick, 1994). This research at ITE, described below, is especially significant due not only to the breadth of species investigated but also to its contribution to our understanding of the physiological factors affecting rooting and the development of techniques for optimizing the propagation environment.

6.3.2 Cloning as a Tool for Tree Seed Orchard Production

With temperate forest species, clonal seed orchards have utilized grafting for several decades (Sweet, 1995) as a means of asexually propagating otherwise difficult to root species such as Douglas fir. One example of asexual propagation being used as a tool for the genetic improvement of MPFTs comes from work with the genus *Leucaena*. At the University of Hawaii, several interspecific *Leucaena* F1 hybrids, produced by hand pollination, have performed well in field trials (Sorensson and Brewbaker, 1994), including psyllid resistance (Wheeler and Brewbaker, 1990). As mentioned earlier, interspecific triploid hybrids between the tetraploid *L. leucocephala* and the diploid species *(L. esculenta, L. diversifolia, L. pulverulenta*, etc.) are seedless and hence free of the problem of self-weediness due to excessive seed production. However, due to the difficulty of clonal propagation there are no large-scale orchards established to produce seed of interspecific F1 hybrid *Leucaena* through open pollination. One approach to achieving this objective would be to exploit the gametophytic self-incompatibility that is characteristic of all diploid species of *Leucaena* and the tetraploid *L. pallida* (Pan, 1985; Sorensson and Brewbaker, 1994). Self-incompatibility (SI) is a mechanism that ensures obligate outcrossing, whereby all individuals comprising a clone of a fertile hermaphroditic genotype are unable to produce seed by self-pollination (Richards, 1986). Since SI operates only within a clone, vegetative propagation is necessary to exploit the SI mechanism which theoretically allows only interspecific F1 hybrid seed production on the SI parent, under open-pollination conditions (Bray, 1984; Brewbaker and Sorensson, 1994; Sorensson and Brewbaker, 1994; Toruan-Mathius, 1992). Wheeler (1991) suggested that grafting could be used to produce seed of interspecific hybrids in open-pollinated orchards. In such an orchard a single clone of a SI species, such as *L. pallida* or diploid *L. diversifolia,* would presumably set seed only from cross-pollination with an interplanted cross compatible species like *L. leucocephala*.

Two studies have reported cloning self incompatible *Leucaena* species by grafting for use in the open pollinated production of interspecific hybrid seed (Bray and Fulloon, 1987; Brennan, 1995). In the former study (Bray and Fulloon, 1987), the cloned species was a diploid (*L. pulverulenta*) which was interplanted with the pollen donor species, (tetraploid *L. leucocephala*) to produce triploid seed for subsequent use as sterile hybrid trees. In the latter study Brennan (1995) cloned tetraploid *L. pallida* by grafting and interplanted these with the pollen donor *L. leucocephala* to produce the fertile F1 hybrid seed (Figure 3). In each case the authors reported only partial success in obtaining hybrid seed production. In both cases vegetative propagation by grafting was necessary to take advantage of intraclonal self-incompatibility. Given to the common occurrence of self-incompatibility that promotes outcrossing among trees of many species, it is likely that similar strategies may be useful in future production of F1 hybrid seed of other MPFT species. One possibility is *Inga*, another genus of tropical woody legumes that exhibits SI (Popenoe et al., 1989). In the first report of asexual propagation of this genus, Brennan and Mudge (1997a) found that *Inga feuillei* rooted well (up to 86%) from leafy cuttings, and 97% by marcottage. Espinal de Rueda (1996) successfully rooted five other species of *Inga* from cuttings.

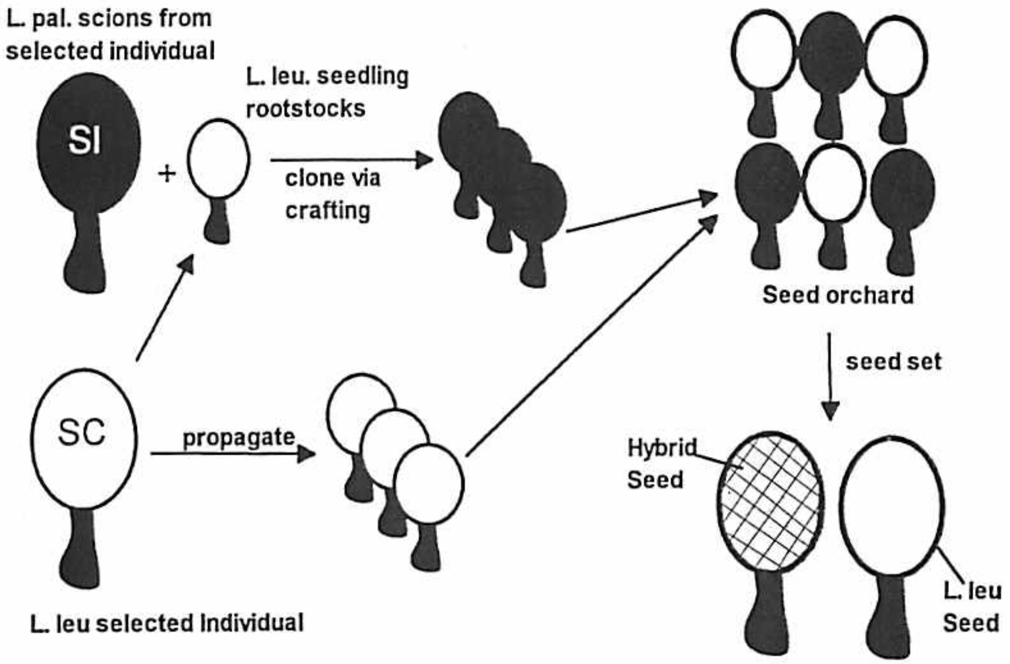

FIGURE 3. Strategy for interspecific hybrid seed production between the intraclonally self incompatible (SI) *Leucaena pallida* and *L. leucocephala*. The *L. pallida* clone, which is propagated by grafting onto seedling understocks of *L. leucocephala*, should produce only F1 hybrid seed.

6.4 LIMITATIONS OF ASEXUAL PROPAGATION

Although the purpose of the foregoing discussion has been to highlight the advantages and reasons for asexual propagation of MPFTs, there are certain disadvantages associated with cloning. These must, of course, be taken into consideration when formulating a strategy for propagation of MPFTs for any purpose.

6.4.1 Difficult-to-Propagate Asexually

Based on current technology, asexual propagation cannot be accomplished effectively, or at all, for certain taxa. MPFT taxa which are considered difficult to clone include, but are not limited to, *Prosopis, Leucaena, Acacia*. However, due to a lack of research, little is known about the feasibility of clonal propagation of many MPFT species. This was the case with many of the tropical forest species (e.g., *Khaya ivorensis, Triplochiton scleroxylon*) currently under investigation at ITE (Leakey, Newton, and Dick, 1994). Such research has resulted in further gains in the range of species, which can be asexually propagated.

6.4.2 Insufficient Genetic Variability

Another concern which has been raised about asexual propagation is that it will result in a narrowing of the gene pool, which could render a population of a given species more vulnerable to biotic (diseases and pests) or physical stresses. Barnes and Burley (1982) discuss the essential safeguard of using multiple clonal genotypes in any large-scale clonal tree introduction program, and point out that the number of clones necessary may be relatively few. Longman (1993) recommends 10–30 clones in a stand for tropical hardwood species.

6.4.3 Quality Considerations

Questions have been raised from time to time about the quality of clonally propagated planting stock compared to seedlings, especially with regard to root system quality and its impact on subsequent plant performance. Although there has been little research with MPFTs along these lines, Longman (1993) supports the view that cutting root system quality is as high as that of seedlings, if the cuttings have been properly handled in the nursery. Based on anatomical and physiological comparisons, Blake and Filho (1988) found that *Eucalyptus grandis* cuttings were more likely to exhibit drought stress than seedlings, and Sasse and Sands (1996) made a similar finding for *Eucalyptus globulus*. Mohammed and Vidaver (1991) reported that micropropagated Douglas fir *(Pseudotsuga menziesii)* were more prone to water stress than seedlings. Patel et al. (1987) found anatomical irregularities in the root-to-shoot junction of micropropagated black and white spruce (*Picea* sp.*)* compared to seedlings. On the other hand, Harrision et al. (1989) found that peach (*Prunus persica*) cuttings and seedlings responded similarly to water stress. Regarding temperature stress, Ritchie et al. (1992) reported that Douglas fir cuttings were at least as cold hardy as seedlings. Bender et al. (1987) reported that nutrient uptake and translocation was similar in micropropagated plantlets and seedlings of *Thuja occidentalis*.

6.4.4 Pathogen Transmission

Plant viruses, MLOs (mycoplasma-like organisms), and xylobacteria pose serious problems in many agricultural species. The effects of viral infection on host plants range from outright disease symptoms to non-symptomatic decline in vigor and crop yield. Since viruses, MLOs, and xylobacteria are systemic, they are present in (most) vegetative propagules (ramets), and hence any plant(s) regenerated from an infected ramet is also infected. A virus moves throughout the tree as it grows, including across a graft union. For example, as much as half of the dwarfing effect of M9 clonal apple rootstock on a fruiting variety grafted onto it, is due to viral infection (Ferree and Carlson). Overall, however, the transmission of viruses and MLOs as a result of clonal fruit and nut tree propagation is considered a serious problem world wide on many tree crops including citrus, pome fruits (apple, peach, etc.), walnut (*Juglans nigra*), avocado (*Persia americana*), etc. (Rom and Carlson, 1987). Fortunately part of the solution to this overall problem is associated with the caveat noted above. Viruses occur systemically through *most* of an infected tree, with the important exception of the primary meristems and incompletely differentiated shoot tissue for a short distance behind the meristem per se (generally less than 1 mm). Hence, *in vitro* meristem tip culture can be used to regenerate a plant free of specific viruses. In fact, virus elimination via meristem tip culture is an important component of tree crop improvement programs for many species world wide (e.g., apple, citrus, etc.) (Rom and Carlson, 1987).

7. CURRENT AND FUTURE RESEARCH IN ASEXUAL PLANT PROPAGATION

7.1 CUTTAGE

As noted above, while a few popular MPFT species (e.g., *Ficus, Erythrina, and Gliricidia*) are traditionally propagated from cuttings, most others are seed propagated. However, reports of successful cuttage propagation with species usually seed propagated are worth noting. Some of these include *Acacia* (Badji et al., 1991; Nilum and Verma, 1995; Dick and East, 1992), *Albizia, Calliandra* (Dick et al., 1996) *Leucaena* (Hu and Liu, 1981); Bristow, 1983), *Inga* (Brennan and Mudge, 1997a; Espinal de Rueda, 1996), *Faidherbia* (Danthu, 1991; Nikiema and Tolkamp, 1992), *Prosopis* (Arya et al., 1993; Dick et al., 1994; Felker, 1994; Goel and Behl, 1995; Nilum and Verma, 1995), *Cassia* (Khanna and Arora, 1984), *Azadirachta indica* (Kamaluddin and Ali, 1996; Chander et al., 1996), and Grewia (Shamet and Dhiman, 1991).

Given the relatively undomesticated state of many MPFT species used in tropical AF and forestry systems, research on cuttage propagation of a given species is often ground breaking. When cutting propagation technologies are developed for MPFT production in the rural tropics, one of the primary goals should be to develop propagation systems that are appropriate for small-scale farmers and local nurseries. A comprehensive program should involve optimizing components of the propagation system at each stage including stock plant management, cutting selection, post severance preparation of cuttings prior to sticking, post sticking management of the propagation environment and finally post rooting nursery stock management. As mentioned above, researchers at ITE have investigated factors controlling the rooting of cuttings of several tropical hardwood species, but most intensively with *Triplochiton scleroxylon*. With this particular species their research covers nearly the full range of cutting propagation system components listed above from stock plant related factors to environmental and physiological considerations during rooting. Similar, though less intensive experiments have been performed with other species at ITE including *Acacia tortillis* (Dick and East, 1992), *Prosopis juliflora* (Dick et al., 1994), *Calliandra calothyrsus* (Dick et al., 1996), *Khaya ivorensis* (Tchoundjeu and Leakey, 1996), *Eucalyptus grandis* (Hoad, and Leakey, 1996*), Terminalia spinosa* (Newton et al., 1996), *Milicia excel*sa (Ofori et al., 1996), and *Gnetum africanum* (Shiembo et al., 1996). Much of this research is summarized in a set of general recommendations for cutting propagation of tropical hardwoods (Leakey et al., 1990; Longman, 1993; Leakey et al., 1994).

7.1.1 Stock Plant Management

Several stock plant related factors affecting the subsequent rooting of cuttings include general plant health (vigor, mineral nutrition, pests and diseases), ontogenetic age (juvenile and adult growth phase), growing environment, and position from which the cutting originated. Environmental factors include light (irradiance and spectral quality), temperature, moisture and soil fertility. The influence of these factors on rooting is mediated by their integrated effects on the anatomy and physiology of the cuttings eventually taken from the stock plant, including, but not limited to photosynthesis, carbohydrate metabolism, phenology, water relations and the level and activity of endogenous substances (including phytohormones).

7.1.1.1 Irradiance

In growth chamber experiments, Leakey and Storeton-West (1992) reported that a higher percentage of *T. scleroxylon* cuttings rooted from stock plants grown at lower (250 umol/m^2/s) than at higher irradiance (650 umol/m^2/s). In contrast, 69% of *Prosopis* cuttings rooted when stock plants were grown at 520 umol/m^2/s compared to only 10% at 150 umol/m^2/s (Klass et al., 1985). The positive effect of reduced irradiance on rooting of some MPFTs is consistent with findings from most experiments involving a broad range of herbaceous and woody species (primarily non MPFTs) although there were some exceptions (reviewed by Moe and Andersen, 1988; Maynard and Bassuk, 1988).

7.1.1.2 Etiolation

Another interesting and potentially useful manipulation of the stock plant light environment is the absence of light. Etiolation refers to plant growth in the absence of light, and the same term has been applied to the deliberate practice of growing stock plants in near darkness in order to increase subsequent rooting of cuttings. This practice can have dramatic effects on rooting of cuttings, and is one of the more exciting technological developments to emerge from propagation research in many years, although the practice has been tested with only a few tropical MPFT species. As reviewed by Maynard and Bassuk (1988), and Howard (1994) the practice usually involves enclosing the stock plant in a black cloth or plastic tent for as little as one week, during the period of a new growth flush. In temperate species the treatment is usually applied just as stock plants are emerging from winter dormancy. New shoot growth, which occurs during the dark treatment, is pale yellow due to the absence of chlorophyll development. Etiolated shoots are very tender and cuttings would be easily desiccated if transferred directly to a lighted propagation environment. This is avoided by gradually regreening stock plants in the light, for one to two weeks before taking cuttings. Stock plant etiolation, followed by regreening, dramatically promotes rooting of a number of difficult-to-root temperate species such as apple, filbert (*Corylus avellana*), chestnut (*Castanea s*), oak (*Quercus s*), hornbeam (*Carpinus s*), maple (*Acer s*), and many others. Etiolation-promoted rooted has also been reported for a few tropical species including mango, avocado, and jack fruit (Maynard and Bassuk, 1988). A modification of the etiolation technique, known as banding involves placing a Velcro strip, at the beginning of the regreening period, over the portion of the stock plant stem which will subsequently become the base of the cutting. For some species including avocado, apple, and hibiscus, etiolation plus banding is more effective than etiolation alone. Considering the potential benefits of this low technology approach to rooting of cuttings, additional studies with difficult-to-root tropical MPFTs, especially legumes, are warranted.

7.1.2 Stock Plant Phenology

Another factor influencing the rooting of cuttings is stock plant phenology as influenced by season (time of year). For example, rooting of *Prosopis* cuttings is strongly seasonal, with spring cuttings rooting substantially better that those taken in the fall (Felker and Clark, 1981). Klass et al. (1985) independently investigated two of the components of seasonal variation (temperature and photoperiod), and found that stock plant temperature of 35°C and an optimum photoperiod was best for subsequent rooting of *Prosopis*. The optimum stock plant photoperiod for subsequent rooting of cuttings was only 12 h for rooting in contrast to an optimum of 18 h for the cuttings themselves in the post severance propagation environment. Of course, photoperiod is not the only factor in seasonal differences in rooting of cuttings. Arya et al. (1993) considered seasonal effects from the standpoint of temperature and rainfall. They reported that *Prosopis cineraria* rooted best during the cool/dry period (February–May) which corresponds to the period of most active shoot growth, rather than during the warm/rainy period. Surprisingly, the cuttings taken during the optimal months of February–May had approximately equivalent rooting despite considerable variation in photoperiod and temperature. Low rainfall occurred during these optimal months, suggesting that the limiting stock plant-associated factor controlling seasonal variation in rooting was rainfall rather than photoperiod or temperature. Similarly, Danthu (1992), and Nikiema and Tolkamp (1992) reported that *Faidherbia* cuttings from older (adult phase) trees rooted best when taken at the time of bud break (dry season). However, juvenile cuttings of *Faidherbia albida* rooted best when taken during the period of maximum leaf fall (rainy season) Nikiema and Tolkamp (1992). This suggests an interaction between stock plant phenology and cutting growth phase. *Faidherbia* is well known for its unusual "reverse" phenology of active shoot growth during the dry season and defoliation during the rainy season. *Acacia senegal*, which has more typical phenology, rooted better during the rainy season than the dry season (Badji et al., 1991).

7.1.3 Cutting Selection

The position on the stock plant from which a cutting is taken can influence the rooting of that cutting and growth form in several ways. The position within the canopy from which a cutting is taken will influence the degree of shading under which the cutting develops, which may affect rooting via irradiance and light quality (R/FR). (See Chapter 6.)

Stock plant position from which a cutting is taken will also influence potential rooting via growth phase effects as described earlier, since a seed grown woody plant can simultaneously have both juvenile (basal) and adult (distal) growth phases (cyclophysis) in different locations. The relative ease of rooting of juvenile vs. adult phase cuttings is extensively documented in the woody plant propagation literature (Hackett, 1985), as well as the literature on MPFTs. For example, Nikiema and Tolkamp (1992) compared the rooting of *Faidherbia* cuttings taken from juvenile stump sprouts (coppice shoots) with that of cuttings from adult phase branches and found 53–93% and 27% rooting, respectively. These finding are consistent with those of Danthu (1992) who also worked with *Faidherbia*. For *Prosopis juliflora*, Goel and Behl (1995) reported 61% rooting of cuttings from 1-year-old stock plants, as compared to only 10% from the adult portion of 8-year-old trees.

7.1.4 Post Severance/Pre Sticking Treatment of Cuttings

7.1.4.1 Auxin Application

The discovery of the root promoting activity of the synthetic auxin analogs IBA and NAA (Zimmerman and Wilcoxon, 1935) was undoubtedly one of the most important advances in asexual plant propagation in the twentieth century. Auxins act by triggering early cell division of the root primordia that will eventually develop into adventitious roots (Hartman et al., 1997). Hence, the primary effect of auxin application to cuttings is to increase the number of roots per cutting. Reliance on this criterion for judging the effectiveness of auxin application (on any treatment) may be misleading, however. For example, *Inga feuillei* cuttings were observed to form more than 30 roots per cutting when treated with 20,000 ppm IBA, compared with less than half as many roots per cutting on cuttings treated with 4000 ppm IBA. Roots of the former, however, were short, thickened (brittle) and unbranched, in contrast to longer, narrower, more branched roots on cuttings treated with the lower concentration (Mudge, unpublished observation). In such cases, evaluation of overall root system quality (root number, length, and branching) is more useful than quantification of treatment effect based on roots per cutting alone. Other responses to auxin application which have been noted include reduced time required for satisfactory rooting and increased percentage rooting (Blazich, 1988). In moderate and difficult-to-root species this latter response may ultimately be the most useful benefit. All three responses to applied auxin (roots per cutting, time to rooting, and percentage rooting) were evident in the African mahogany (*Khaya ivorensis*) (Tchoundjeu, and Leakey, 1996).

The most commonly used synthetic auxins, (IBA then NAA) are generally more effective than IAA (Hartman et al., 1997; Nikiema and Tolkamp, 1992), although Shamat and Dihman (1991) found that *Grewia optiva* cuttings rooted better with IAA than IBA or NAA. Combinations of IBA plus NAA are often more effective than either applied separately (Arya et al.1993).

Although the literature on plant propagation contains references to innumerable species where auxin has enhanced rooting, not all species respond, and the response is often somewhat variable depending on interactions with other factors such as phenology and growth phase. The optimal auxin concentration appropriate for a given species varies with the time of year and hardness of wood. Softwood cutting typically require 0.05 to 0.3% (w/w in powder formulation; w/v in quick dips). Semihardwood cuttings respond best in the range of 0.1 to 0.5%, and hardwood cuttings require 0.25 to 1.0%, and occasionally several times higher (Hartman et al., 1997). Seasonal variation in the response of cuttings to auxin is seen in the results of experiments with *Acacia senegal* treated with none, 0.2% NAA, 1.0% IAA, 0.2% or 8.0% IBA at 4 times during the year including the wet and dry seasons. This species is unusual since the most effective auxin treatment was 8.0% IBA, which is extraordinarily high compared to nearly all other reports in the literature. Arya et al. (1993) reported that untreated cuttings of *Prosopis cineraria* did not root, whereas cuttings treated with individual auxins (IAA, IBA, NAA), combinations (IAA+IBA+2,4-D), or auxins combined with the vitamin thiamin (NAA+IBA+thiamine) all resulted in at least 25% rooting. The most effective treatments (>50%) were from the two mixtures. Felker and Clark (1981) also reported that thiamine combined with auxin increased rooting of *Prosopis* cuttings over auxin alone. Auxin-stimulated rooting has been reported with numerous other MPFTs including *Cordia alliodora, Vochysia hondurensis, Albizia guachapele* (Leakey et al. 1990), *Triplochiton scleroxylon, Khaya ivorensis* (Longman, 1993), and *Inga* spp. (Brennan and Mudge, 1997a; Espinal de Rueda, 1996).

cuttings is to a very great extent a matter of avoiding excessive moisture loss (Loach, 1988), although in some species, CO^2 uptake is a consideration as well, as discussed above.

Maintenance of favorable cutting water relations depends on management of three critical environmental parameters — irradiance, humidity, and temperature. These must be carefully managed regardless of whether cuttings are propagated in a high tech greenhouse with sophisticated modern environmental controls, or in simpler systems that use polyethylene moisture barriers and/or shading. A practical understanding of the physiological response of leafy cuttings to these parameters may make the difference between success and failure.

Water loss from leafy cuttings via transpiration is controlled primarily by the interactive effects of atmospheric moisture (approximated by relative humidity), temperature, and light. If water loss by the cutting exceeds water uptake, water stress will occur, and, if severe enough, the cutting will die and/or not root (Loach, 1989). The potential rate of water loss increases as the difference in atmospheric moisture between the interior of the leaf and the surrounding air increases. Similarly, water loss increases as the difference in air temperature between the leaf and the surrounding air increases. Consequently, the principal management strategies for minimizing moisture loss from cuttings are increasing the relative humidity of the propagation and decreasing the temperature, and particularly decreasing the leaf to air temperature difference. The effect of light on moisture loss from cuttings is mediated by its effect on leaf and air temperature, and on stomatal aperture. In practical terms water loss is reduced by decreasing light levels because of the reduced leaf and air temperatures associated with shading. At higher light levels, leaf temperature is often somewhat warmer than surrounding air, in some cases by as much as several degrees (Mudge et al., 1995; Loach, 1989), creating a considerable driving force for water loss even at high atmospheric humidity.

Humidity is managed in most propagation systems either by the use of a polyethylene barrier or intermittent mist. Although polyethylene is often more appropriate when electricity and plumbed water are lacking, one must be careful to manage the system to avoid a temperature increase. In polyethylene systems, shade must be used to control temperature. Grange and Loach (1983) recommended sufficient shading to give a maximum irradiance of 100 W/m^2 which is $\geq 70\%$ shading under "summer" (warm, high light) conditions. Under mist, on the other hand, the mist itself achieves cooling, so that higher levels of irradiance are tolerable.

Due to the adverse effect of light on leaf temperature, polyethylene systems are generally more effective than mist systems under relatively cool, low light (Loach, 1977; Mudge et al. 1995) than under warmer, higher irradiance conditions where mist systems are favored (Loach, 1988).

Another factor, which may influence the effectiveness of polyethylene and mist systems in cool climates, is their effect on night temperatures. Although minimizing leaf and air temperature during the daytime is a key consideration in successful cutting propagation, nighttime temperatures can have an important but opposite effect. In the relatively cool Kenya highlands, near the equator where daytime irradiance levels were high, hibiscus cuttings rooted better under polyethylene than under mist, even though daytime leaf and air temperatures were several degrees higher under polyethylene than under mist (Mudge et al., 1995). At night, however, leaf and air temperatures decreased to as low as 6°C for cuttings which had been misted during the day (mist off at night). In contrast leaf and air temperatures were higher by approximately 4°C under polyethylene. The lower rooting under the mist treatment, compared to those under polyethylene, was probably due, at least in part, to suboptimal night temperatures for the former.

Leakey et al. (1990) described an easily constructed polyethylene propagation chamber approximately 2 to 4 m long × 1 m wide × 0.5–1.0 m high consisting of a wooden frame with a hinged cover for access to the cuttings. One drawback of this relatively large raised polyethylene system is that the relative humidity inside the chamber dropped considerably upon opening, and reequilibrated slowly due to the large chamber volume. Brennan and Mudge (1997a) described a smaller (modular) raised polyethylene system made of a 10 × 36 × 51 cm plastic flat, with an attached wire frame which are enclosed in a clear polyethylene bag. This system requires opening less frequently since it is usually filled to capacity with cuttings initially and so is not reopened subsequently for addition of new cuttings as a larger system might be. Consequently, humidity remains more uniform, and when the bag is opened, humidity equilibrates faster due to its lower volume.

An even simpler polyethylene system, refereed to as contact polyethylene, does not require any frame. It involves merely placing a sheet of polyethylene on top of the cuttings and sealing the edges with a rubber strip or lath strip. Contact polyethylene has been shown to improve the rooting of cuttings compared to raised polyethylene systems (Loach, 1977; Mudge et al., 1995; Grange and Loach, 1983,). Grange and Loach (1983) speculated that the improved rooting under contact polyethylene compared to raised polyethylene was due to the lower leaf to air temperature difference, which they observed in the former. Mudge et al. (1995) also reported higher rooting with contact compared to raised polyethylene system despite the fact that there was no difference in the leaf to air temperature gradient between the two systems.

7.2 GRAFTING

Research on propagation of fruit trees by grafting has proceeded steadily, ever since its adoption by early agriculturists several thousand years ago. Mango, which is traditionally propagated in India by approach grafting (Kanwar and Bajwa, 1974), has received considerable attention as a subject of grafting research. Unlike the majority of grafting techniques which involve detaching the scion (ramet) from the stock plant (ortet), approach grafting has the advantage of the scion remaining attached to its own root system until the graft union is formed (see Figure 2). Approach grafting allows a relatively low rate of multiplication due to the labor and spatial constraints inherent in tying seedling rootstocks into the canopy of established scion donor trees. Research on detached scion grafting for mango has focused on side grafting (Kanwar and Bajwa, 1974), bench grafting (Majumdar and Rathore, 1970), epicotyl grafting (Chakrabarti and Sadhu, 1983), and saddle grafting (Thomas, 1981), and the effects of defoliation and bud stick storage on veneer grafting (Singh and Srivastava, 1979). Grafting is used extensively for clonal propagation of a wide range of temperate fruit and nut species including apple, pear (*Pyrus communis*), grape (*Vitis vinifera*), and stone fruits including peach, cherry (*Prunus avium*), almond (*P. dulcis*), and for several important tropical fruit species including mango, citrus, avocado, etc. The primary focus of research related to grafting of these important fruit crops is on selection of clonal or seedling rootstock for adaptation to particular soil characteristics (moisture content, salinity, pH, etc.), as well as disease and pest resistance (Rom and Carlson, 1987).

Another interesting use of grafting is in papaya breeding. Although papaya is commercially propagated from seed, bud grafting has been used in papaya breeding (Sookmark and Tai, 1975) because it allows selection of clones not only of known genotype but also gender, since the seedling gender of this dioecious species is unknown prior to flowering (Singh et al., 1985).

Compared to tree species grown primarily for their fruit, grafting is seldom used to propagate multipurpose trees grown for other purposes. However, there are a few cases where grafting has been used to clone desirable selections of MPFTs such as seedless *Leucaena* (Brewbaker, 1988) and elite Haitian selections of *Prosopis juliflora* (Wojtusik et al., 1993). Wojtusik and Felker (1993) compared several different grafting techniques and reported invigorating effects of specific rootstocks on scion biomass production and a wide range of interspecific graft compatibility within the genus *Prosopis*.

As described above in Section 6.3.2, grafting has shown promise as a method of propagating selected genotypes to take advantage of self-incompatibility in leucaena seed orchards. *Leucaena* species have been successfully grafted using several standard methods including whip and tongue, and cleft grafting (Versace, 1982), approach grafting (Brennan, 1992) and more recently by a somewhat novel technique called single bud splice grafting (Brennan and Mudge, 1997b). This technique relies on a plexiglass grafting tool that allows grafting of small (3–5 mm diameter) single node scions onto two- to three-month-old seedling rootstocks. Using this method, the authors reported a high degree of interspecific graft compatibility between 18 Leucaena scion species and 2 rootstock species.

There are few reports on grafting other woody legume species with the exception of the temperate species *Gleditsia triacanthos*. A thornless variety *G. triacanthos* and selected seedless cultivars (e.g., Moraine) are routinely bud grafted for use as ornamentals (Dirr, 1990). Thornlessness and seedlessness are characteristics that could be useful in temperate AF systems involving this genus.

Acacia spp. and the closely related *Faidherbia albida*, are examples of tropical MPFTs, which have proven extremely difficult to propagate asexually, particularly from adult trees. In such cases approach grafting may be possible, as has been the case with *Acacia koa* (Brennan, 1995, unpublished data). In addition, there are reports on successful micrografting performed *in vitro* (tissue culture) (Palma, 1996; Detrez, et. al, 1992; Detrez, 1995; Monteuuis, 1996) or *ex vitro* (post micropropagation) using micropropagated scions (Palma et al., 1996). The success of these *in vitro* methods is related to the apparent rejuvenation of reproductively mature tissue resulting from *in vitro* culture. *In vitro* micrografting will be further discussed below in the section on micropropagation.

Aside from the selection and use of clonal rootstocks for few species of fruit trees including apple, citrus, and some stone fruits, most tree grafting, including most MPFTs, involves the use of seedling rootstocks. Because of the high degree of heterozygosity associated with naturally out-crossing tree species, seedling rootstock effects tend to be more variable than clonal rootstocks. Nevertheless, Wojtusik and Felker (1993) reported that scions of several *Prosopis* spp. were invigorated (produced more biomass) when grafted onto seedling rootstocks of species other than themselves. Brennan (1995) evaluated the effects of two lines *of Leucaena leucocephala* (weedy and the giant type, K636) and *L. diversifolia* as rootstocks for several scion species (*L. leucocephala, L. diversifolia, L. esculenta, L. pallida* and the hybrid KX3). Although the effects of rootstock genotype on scion growth were complicated by significant genotype x environment interactions, there were significant effects of rootstock genotype on growth of several scions. Although these early investigations of rootstock genotype effects on scion growth and development of *Prosopis* and *Leucaena* are encouraging, they are far from the sophisticated use of rootstock genotype to influence scion vigor, root system adaptation to specific soil types, and other characteristics in apple and citrus. It is obvious that research on MPFT rootstocks is in its infancy. Clonal rootstock selection for MPFTs is an area for future research, which could contribute significantly to MPFT improvement. For example, it may be possible to select for MPFT rootstock architecture that minimizes below ground competition with interplanted crops. Another important area well worth investigating is selection of MPFT rootstocks for adaptation to specific soil types and soil born pests and diseases.

7.3 MICROPROPAGATION

Micropropagation has been useful for the large-scale commercial production and genetic improvement of a few traditional forestry species such as eucalyptus, pine (*Pinus*), and Douglas fir, as well as several temperate woody ornamentals such as *Rhododendron* sp., lilac (*Syringa vulgaris*), and *Kalmia sp.* Despite the intriguing research results with microprogation of MPFTs during the past two decades, it seems unlikely that micropropagation will be used for the routine production of MPFTs for direct use in AF systems, given the emphasis on the use of resource appropriate technologies. Micropropagation may, however, be useful in MPFT breeding/genetic improvement programs where lower technology propagation approaches are less suitable.

Most research on the micropropagation of MPFTs has focused on explant selection and manipulation of the auxin and cytokinin phytohormones to optimize *in vitro* shoot and subsequent root production, respectively. Trigiano et al., (1992) recently reviewed the status of micropropagation of both tropical and temperate woody legumes. The majority of research on micropropagation of tropical woody legumes has involved the genera *Acacia* and *Leucaena*. More than 10 different species of *Acacia* have been investigated, while most research on *Leucaena* has focused on L. *leucocephala* (Table 2).

TABLE 2
Research reports of micropropagation of multipurpose and fruit trees used or potentially useful in agroforestry.

Species	Approach[1]	Growth Phase	Regeneration	Reference
A. saligna	ASC	adult	Yes	Barakat et al., 1992
A. saligna	ASC	7 mo.	Yes	Jones et al., 1990
A. nilotica	ASC	juvenile	Yes	Dewan et al., 1992
A. senegal	C	ND	No	Hustache et al., 1986
A. senegal	MG	4 yr.	Yes	Palma et al., 1996
A. senegal	ASC	juvenile	Yes	Badji et al., 1993
A. koa	C	juvenile	Yes	Skolmen and Mapes, 1976
A. nilotica	SE	juvenile (3N)	Yes	Garg et al., 1996
A. mangium	ASC	juvenile	Yes	Galiana et al., 1991
A. mangium	MG	adult	Yes	Monteuuis, 1996
A. mearnsii	ASC	juvenile	Yes	Huang, 1994
A. bivenosa	ASC	7 mo.	Yes	Jones et al., 1990
A. holosericea	ASC	7 mo.	Yes	Jones et al., 1990
A. salicina	ASC	7 mo.	Yes	Jones et al., 1990
A. sclerosperma	ASC	7 mo.	No	Jones et al., 1990
A. catechu	SE	juvenile	Yes	Rout et al., 1995
A. melanoxylon	C	juvenile	Yes	Meyer and van Staden, 1987
A. auriculiformis	ASC	ND	Yes	Reddy et al., 1995
A. auriculiformis	C	juvenile	Yes	Ranga Rao and Prasad, 1991
A. auriculiformis	ASC	juvenile	Yes	Mittal et al., 1989
Faidherbia albida	ASC	juvenile	Yes	Duhoux and Davies, 1985
Leucaena leucocephala	DO, C	juvenile	No	Nagmani and Venketeswaran, 1983
L. diversifolia	DO, C	juvenile	No	Nagmani and Venketeswaran, 1983
L. retusa	DO, C	juvenile	No	Nagmani and Venketeswaran, 1983
L. leucocephala	C	juvenile	No	Mazari and Rubluo, 1984
L. leucocephala	ASC, DO	juvenile	Yes	Hossain et al., 1993
L. leucocephala	ASC	14 mo.	Yes	Goyal and Felker, 1985
L. leucocephala	ASC	juvenile	No	Glovak and Greatbatch, 1981
L. leucocephala	ASC	adult	Yes	Dhawan and Bhojwani, 1987
L. leucocephala	ASC	juvenile	Yes	Ravishankar et al., 1983
L. leucocephala	ASC	juvenile/adult	No	Toruan-Mathius, 1992
L. pallida	ASC	juvenile/adult	No	Toruan-Mathius, 1992
L. Kx3	ASC, SE	juvenile/adult	No	Toruan-Mathius, 1992
Prosopis cineraria	ASC	ND	ND	Goyal and Arya, 1984
Albizia procera	ASC, DO	juvenile	Yes	Hossain et al., 1993
Albizia lebbeck	C	adult	Yes	Gharyal and Maheshwari, 1990
Cassia fistula	C	adult	Yes	Gharyal and Maheshwari, 1990
C. siamea	C	adult	Yes	Gharyal and Maheshwari, 1990
Robinia pseudoacacia	C	adult	Yes	Han et al., 1990
Gymnocladus	?	juvenile/adult.	ND	Smith and Obeidy, 1991

[1] Abbreviations: ASC = axillary shoot culture, C = callus, DO = direct organogenesis, SE = somatic embryogenesis, MG = micrografting, ND = no data

In an extensive survey of woody plant taxa including 126 species across 67 genera, 33 families, 16 orders and six subclasses, Einset (1991) found that species in the order Fabales (including Leguminosae) tended to exhibit a positive *in vitro* growth response to cytokinins. Because of considerable interspecific differences and even lab-to-lab differences, few generalizations can be made about experimental attempts to optimize cytokinin-induced axillary shoot proliferation, except that benzyl adenine (BA) and kinetin (Kin) in the range of 1 to 10 μM are most commonly employed for this purpose. Two other cytokinins, zeatin and thiadiazuron (TDZ), are regarded as more potent cytokinins than either BA or kinetin in a wide range of species (Heutteman and Preece, 1993) including legumes (Chalupa, 1987; Yusnita et al., 1990; Smith and Obeidy, 1991). For the micropropagation of ericaceous species, a third alternative cytokinin, isopentynl adenine (2iP), is preferred to BA or Kin. These three alternatives to BA and Kin have been tested in only a few woody legumes. Toruan-Mathiius (1992) found BA to be more effective for axillary shoot production than either Kin or 2iP in *L. leucocephala, L. pallida*, or the *Leucaena* hybrid, KX3. Similarly, shoot production in *Acacia nilotica* was higher in response to BA than either Kin or zeatin. Considering their usefulness in other plant taxa, evaluation of TDZ and zeatin as possible alternatives to the more traditionally used BA or Kin, is advisable across a wider range of other MPFTs.

The ease of micropropagation, like any asexual propagation approach, declines with increasing ontogenetic age of stock plant; i.e., adult phase explants are more difficult to micropropagate than juvenile explants. From Table 2, it is apparent that most woody legume micropropagation systems have involved culture initiation from juvenile explants, in most cases from seedlings or ungerminated zygotic embryos. When starting from older, reproductively mature stock plants of seedling origin, successful micropropagation can be facilitated by explant selection from the juvenile portion of a tree. As explained earlier, such juvenile tissue may be present in the form of dormant epicormic buds at the base of an otherwise mature stock plant. Epicormic shoots arising from coppicing (or natural environmental stress) give rise to juvenile shoots. Meyer and van Staden (1987) reported that shoot cultures established from coppice shoot explants of *Acacia melanoxylon* proliferated more rapidly and gave rise to organogenic callus to a greater extent than cultures initiated from adult shoot explants. Similarly, Smith and Obeidy (1991) reported that *Gymnocladus* shoots initiated from juvenile basal trunk explants proliferated comparably to seedling explant-derived cultures. Similarly, Sanchez and Vietez (1991) reported that explants from juvenile basal sprouts of chestnut exhibited higher establishment, growth and proliferation than explants from the adult phase canopy. Since adventitious buds formed on roots of mature trees are presumed to be in the juvenile growth phase (Hackett, 1985), explants from root suckers might be expected to perform better than adult explants from other locations on the same tree. Barghchi (1987) successfully initiated cultures from juvenile adventitious shoots arising on root cuttings of *Robinia pseudoacacia*. These root sucker explants proliferated and rooted better than explants from shoots of adult phase trees. Other MPFTs which have a natural propensity to sucker from intact roots include *Faidherbia albida* (Ajee and Duhoux, 1994), *Inga feuillei* (Mudge, personal observation), breadfruit, etc. In such species, root suckers could potentially be used as a source of juvenile shoot explants for a shoot culture-based micropropagation systems. On the other hand, Gassama (1989, cited in Detrez et al., 1992) reported that microcuttings of *Faidherbia albida* from root suckers of adult phase trees were difficult to root. Han et al. (1997) reported that there was no difference in *in vitro* performance of basal (juvenile) and more distal (adult) explants of *R. pseudoacacia*. These later two studies suggest that the generalization that juvenile explants perform better than adult explants does not always hold true.

Root organ culture (ROC) is another related approach to micropropagation of species, which have a natural tendency to sucker. ROC involves culturing root explants, usually in the presence of auxin (without cytokinin) to encourage more or less normal root growth and development (including branching of lateral roots). ROC, has the potential advantage that subculture, by cutting one root into several pieces, requires less precision and time than subdivision of multiple shoot cultures into individual microshoots. In contrast to shoot organ culture, which is dependent on plantlet formation via adventitious root formation, ROC requires adventitious shoot formation. This is much more easily accomplished with the relatively few species that have a natural tendency to form root suckers *in situ*, like raspberry, and poplar (Borgman and Mudge, 1984 and references cited there in), and *Faidherbia albida* (Ahee and Duhoux, 1993). Given the relatively few woody species with the habit of root suckering, the *in vitro* ROC approach would seem to be less broadly applicable across MPFT species than the more common shoot culture-based micropropagation systems. ROC has been attempted with *Faidherbia* albida, which suckers naturally, and for which *in vitro* shoot culture has had only limited success (Duhox and Davies, 1985; Gassama and Duhox, 1987; Ahee and Duhoux, 1993). Although seedling radicle-derived ROC of *Faidherbia* was successfully initiated, it could only be subcultured for a limited period. Nonetheless, during that period, adventitious shoot formation did occur, and rooted plantlets were established *ex vitro* (Ahee and Duhoux, 1993).

Another *in vitro* technique that may be useful for overcoming the difficulty of clonal propagation of adult phase explants is micrografting. *In vitro* micrografting is reported to bring about the rejuvenation of adult phase explants (Jonard, 1986). The technique involves grafting small adult phase scions onto an *in vitro* germinated seedling rootstock (Trigiano et al., 1992). This approach has potential advantages over either direct micropropagation (without grafting) or conventional *in situ* grafting (without micropropagation). *In vitro* micrografting was originally used to eliminate viruses by culturing citrus shoot tip explants that would otherwise not survive *in vitro* (Jonard, 1986). Subsequently, *in vitro* micrografting of adult phase explants onto seedling rootstocks has been used as a means of cloning Douglas Fir (Monteus, 1995) and *Acacia mangium* (Monteus, 1996). In both cases, a single explant yielded only one grafted individual upon *ex vitro* reestablishment, i.e., there was no multiplication per se. Nonetheless this is analogous to the 1:1 ratio of scion to new plant achieved by conventional grafting.

A particularly interesting use of micrografting has been used to take advantage of the apparent rejuvenating effects of *in vitro* culture per se (Trigiano, 1992), and/or the rejuvenating influence of juvenile seedling understock on adult scions (Hartman et al., 1997). Detrez et al., (1992) achieved rapid multiplication of *Faidherbia albida*, by micrografting adult shoot tip explants onto seedlings. Using what they called chain micrografting, explants were grafted onto seedlings. The shoot explants (microscions) elongated into multinode shoots, which could then be cut into individual nodes for regrafting onto a new *in vitro* seedling understock. Repeating the process over three cycles, they found that the multiplication coefficient (the number of single node scions harvested from a multinode shoot grown from one original single node scion) increased from 5 with the first cycle to 8.5 and 10.2 with the second and third cycles, respectively. This increase in multiplication was attributed to rejuvenation. Similarly, Perrin et al. (1994) showed that adult scions of *Hevea brasiliensis* micrografted onto seedling rootstocks could be multiplied *in vitro* by successive subculturing of new scion growth, but only if a small piece of the original seedling rootstock was included with each subculture. Subsequent rooting of these subcultured shoots (originating from an adult phase microscion explant) was as high as microshoots grown from a seedling (juvenile) explant, suggesting that rejuvenation had occurred. In an experiment with *A. senegal,* Palma et al. (1996) reported that conventional shoot micropropagation of adult explants resulted in *in vitro* proliferation of microshoots, which could then be successfully grafted *in situ* (nursery). These encouraging results from *in vitro* micrografting of several MPFT species suggest that it is an approach worthy of further exploration as means of achieving rejuvenation and multiplication of MPFTs which are otherwise difficult to propagate asexually (e.g., *Prosopis, Leucaena*, etc.).

Micropropagation is becoming an important tool for conservation of plant species including MPFTs. *In vitro* germplasm conservation may be especially important for MPFTs with recalcitrant seeds, and may save space compared to conventional *in situ* collections. Cryopreservation techniques which hold tissue cultures, and even intact buds at reduced (>0°C), and even ultralow (−196°C) temperatures, also may be useful in conservation of MPFTs (Sakai, 1985). In addition, transport of germplasm across international boundaries can be facilitated by *in vitro* cultures since they are soil-free (Engmann, 1995) and often free of pests and diseases.

Since the beginning of the use of *in vitro* culture for clonal propagation, there have been concerns about the loss of genetic fidelity via mutation and/or induction of aneuploidy, and/or "physiological decline" resulting from *in vitro* culture (Evans and Bravo, 1986). These deleterious changes are more likely when explants have gone through an undifferentiated stage, i.e., callus or suspension culture, prior to adventitious organogenesis (Muashige, 1974). There have been claims that even callus-free shoot culture of banana results in off types (Smith and Drew, 1990). Krikorian et al. (1993) reinvestigated this situation and found that off types were associated with the pre-severance condition of the shoot from which the explants were collected, rather than the micropropagation process, per se. Clearly, future exploitation of micropropagation for MPFT improvement will require careful attention to problems of unintended variation *in vitro*.

An application of *in vitro* clonal propagation that is becoming extremely important is its use as a component of plant genetic engineering. This refers to recombinant DNA technology involving the transfer of a (useful) gene or genes, promoters, etc., from one organism (plant, bacterium, fungal or even animal) to another plant, in order (eventually) to confer some desirable genetic trait such as disease or pest resistance, altered ripening, drought tolerance, etc. Essentially all current transformation strategies including plasmid, electroporation [biolistic (gene gun) mediated] involve *in vitro* culture and subsequent regeneration of transformed plants. In the last decade many woody species and herbaceous perennial species have been successfully transformed including (but not limited to) apple (Korban and Chen, 1992), poplar (Confalonieri et al., 1995), grape (Scorza et al., 1995a), Juneberry (Hajela et al., 1993), pine (Aronen et al., 1995), papaya (Tennant et al., 1994), apricot (Camara et al., 1992), plum (Scorza et al., 1995b), almond (Archilletti and Damiano, 1995), *Allocasuarina* (Phelep et al., 1991), spruce (Hood et al., 1990), and eucalyptus (Macrae and Van Staden, 1993).

Although, in most cases, woody plant transformation research has not yet yielded agriculturally useful improvements, this technology will probably make important future contributions to the genetic improvement of tree crops, including some species useful in agroforestry. As practical woody plant transformation systems begin to come on line, there are concerns about the potentially undesirable consequences of widespread unintended dispersal ("escape") of foreign DNA from the transformed crop species to related wild species through pollen transfer. One solution to this problem may be deliberate genetic engineering of (male) sterility into otherwise transformed crop species, rendering them incapable of pollen-mediated gene transfer (Strauss et al., 1995).

8. CONCLUSION

Clonal propagation has played an important role in traditional agroforestry practices, particularly for staple food crops like cassava, banana, taro, potato, sweet potato and others. In addition to these herbaceous components, asexual methods have been used to propagate some of the woody components of agroforestry systems as well. Farmers have employed easily rooted tree species as boundary plantings, and grafting and layering have been used for fruit tree propagation and selection for centuries. Important advances in cutting propagation technology over the last several decades include the introduction of polyethylene and automatic mist systems for moisture management, and the use of synthetic auxins for promotion of adventitious rooting. Although these have been widely utilized to expand the palate of woody species and improved cultivars used for ornamental purposes (especially in temperate areas), they have only recently begun to be applied to the genetic improvement and nursery production of MPFTs and for the domestication of new species. Recent systematic approaches to optimization of pre and post severance environmental conditions for the rooting of cuttings has demonstrated that cutting propagation is feasible for a much broader range of MPFT species than previously considered. Likewise, recent research in micropropagation is beginning to have an impact on genetic improvement of MPFTs. Regardless of asexual propagation technique, the difficulty of propagating adult phase plant material will have to be overcome so that selection of improved genotypes can reach its full potential. New approaches like etiolation of cuttings and *in vitro* micrografting have shown great promise for improved asexual propagation of a wide range of difficult-to-root adult phase woody crops, and the experimental evaluation of these methods with many as yet untested MPFT species is likely to bring further success. *In vitro* micropropagation will have an increasingly important role as genetic transformation systems for more MPFT species are developed. Continued improvements at the low technology end of the spectrum, such as improved moisture management systems for cutting propagation, and natural alternatives to commercial rooting hormones should not be neglected, so that farmers will have access to improved clonal selections locally. As advances are made at both the low and the high end of the technological spectrum, asexual propagation will increase in importance as a powerful tool for the development of sustainable AF systems.

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