Plantation Improvement Using Technically Improved Clonal Propagation – An Overview of the Latest Technology in India.

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Variation in tree species, and improvement options

Cloning is a propagation technology that can be very effective in making practical use, via 'clonal forestry', of proven superior individuals occurring in nature or developed by breeding.

The alternative (traditional) propagation route to improved plantations, 'seedling forestry', involves mass selection for various kinds of seed orchards that in turn provide improved seed. Although this approach has often served well in the past, and will continue to do so in appropriate circumstances (usually where requisite resources for clonal forestry are lacking, or profitability of it would be marginal), clonal forestry is currently showing great potential in the forestry industry.

This paper outlines the conditions under which clonal forestry is an appropriate option and, as background to enable a better understanding of clonal propagation, a simplified explanation of variation in tree species and improvement options has been provided,

Clonal Propagation

What is a clone?

In simple terms, a clone comprises identical copies of an individual. That is to say, the members of a clone have the same genetic constitution except insofar as mutation has occurred during propagation. In forestry and horticulture, a clone comprises all the descendants of a single plant, produced by vegetative propagation.

A clonal forestry plantation, produced from the same parent material, consists of trees that are all expected to be genetically identical to each other. Traditional seedling plantations however consist of trees that are similar to each other but each individual tree is different to every other tree owing to, in the majority of cases, the combinations of their parents (male and female) being different. Seedling plantations (where there has been no special selection or breeding of the parents) can contain vigorous plants and runts, fast growers and slower growers, tall straight trees and short crooked ones.

Care, however is still needed when propagating clonal material from different parts of the same parent plant as they may have different properties, depending for instance whether the shoots are orthotropic (vertically growing) or plagiotropic (non-vertical to horizontally growing) or from the root.

Plantation Improvement

In a nutshell, relative uniformity is a feature of clonal plantations whereas variation to a greater or lesser degree is a feature of seedling plantations.

The extent of the variation in seedling plantations can be reduced by using genetically-improved and graded seed, rigorous culling prior to planting, and by silvicultural thinning in the field.

The clonal approach to plantation improvement is to identify a small number of individual trees, by replicated tests in target environments conducted over several years, that are superior in a desired combination of such traits as tree form, wood quality, speed of growth, tolerance of salinity, etc., and to reproduce them vegetatively, to create a plantation of trees all identical to these superior individuals.

Clonal forestry plays a significant role in many forestry plantation operations around the world, and is now common practice in Acacias, Eucalyptus, & pine plantations. The Australian, South African, Brazilian and Indonesian hardwood industry is a notable exception.

Why do many timber companies around the world plant clonal plantations?

Well-executed clonal plantations confer many advantages over traditional seedling plantations.
Clonal plantations are based on superior individuals that have been selected by the plant breeder for their superior genetic qualities.

Qualities selected by breeders for the mass-production of elite clones may include:

- Increased growth rate
- Superior vigour
- Superior form/shape (eg apical dominance for sawn log production)
- Increased timber volume/yield
- Superior timber qualities (eg strength, durability, density, paper quality)
- Pest and disease resistance
- Drought resistance
- Suitability to specific soil types
- Suitability to specific climatic zones
- Salt tolerance

The tree breeder usually selects for a limited number of the most important traits as little progress can be made if selection is attempted for a large number of traits.

As well as genetic advantages, clonal plantations offer additional flow-on benefits such as:

- Relative uniformity
- Low levels of runts and deaths
- Lower initial stocking rates
- Lower establishment costs
- Cheaper harvesting costs
- Increased profitability

Elite clones supplied to plantations by nurseries are uniform for two reasons:

- The rooted cuttings that each comprises, are identical genetically; and
- In the case of SKB, the production process used incorporates extensive sorting for age, development, height, vigour etc of the individual plants produced.

This uniformity results in the following advantages to the plantation owner:

- All plants supplied are of plantable quality and the planting team does not need to sort, select or discard, thus resulting in more efficient planting and cost savings during the planting process;
- 100% of the plants delivered are virtually identical in size, vigour and growth characteristics including growth rate. This means that there are few runts or deaths in the plantation, and therefore no re-planting (as a result of poor genetic performance) is usually necessary, resulting in considerable cost savings at the planting stage.
- Barring a low natural mortality, all plants grow into good quality trees in the plantation. There is little or no need for thinning of runts as the plantation grows.
- It is generally considered that clonal plantations do not require the same degree of early thinning, and so they are usually planted at a lower rate per hectare than seedling plantations.1
- Since growth of all trees across the plantation is uniform, except due to differences in local sites, harvest operations are easier, more efficient and more cost effective.
- All plants in a clonal plantation have been selected for superior qualities, which in most cases result in higher return at harvest.

Replanting is to be avoided 'at all costs' as it is very expensive since it requires the purchase of replacement plants, and expenditure on additional, more costly, labour to replant replacements located usually at random all through the plantation. Usually there is less need for replanting in clonal plantations.

Price considerations

After superior clones have been identified within seedling-derived populations (which can be very expensive, especially if breeding is continued to develop even better clones), the only additional cost in establishing a clonal hardwood plantation compared to a seedling plantation is the higher cost of a clonally produced plant.

For example, a typical seedling eucalypt plantation may be planted at 5000 stems/ha are compared to a clonal plantation at the same site planted at 2250 stems/ha. The lower stocking of clonal plantations may remove the need for the early thinning of poor quality trees. However, stocking densities at different plantation ages (maintained by appropriate thinning) will still need to be determined by requirements to maintain 'site capture' (to avoid invasion of unwanted growth), to meet particular log specifications and markets appropriate to the growth stage of the plantation, the end use of the timber and when a market may be available.

Clonal propagation of hardwoods, especially eucalypts, can be a totally different scenario and at the other extreme end of the spectrum regarding difficulty, technology and capitalisation. This impacts on price, and so at face value there is a perceived price barrier (by the grower) working against the establishment of hardwood clonal plantations, even though a closer study of the full economic picture may show otherwise.

In general, the price of rooted cuttings of clones is about three to five times the price of mass produced seedlings, due to the high labour costs in the production process, the high level of technical skill required and the high level of capitalisation required by the nursery. Whereas a wholesale eucalypt seedling may cost, say, Rs.1, a *Eucalyptus* rooted cutting of a selected clone will cost, say, Rs. 6.

Depending on the total cost of seedlings relative to other establishment costs and the cost to a company of the developed and chosen clones, the additional cost of purchasing the plants (as rooted cuttings) for a clonal plantation may be quite small and may be offset many times over by the flow-on savings. Clonal plantations can therefore be a very cost-effective forestry operation, particularly for shorter rotation crops or where the use of clonal material significantly shortens the rotation.

- Average productivity of these clones at 5 years rotation is 20 to 25 m3 /ha-1 /yr-1
- Very high productivity of 50 m3 /ha-1 /yr-1 has been obtained by many farmers who planted these unique clones.

New Cloning Techniques

Since the first developmental stage until its recognition as an operational method of reproducing superior trees, cloning of *Eucalyptus* species is undergoing a continuous process of improvement through incorporation of new technical concepts and technologies at different phases of the process. Campinhos & Ikemori, 1983; Zobel & Ikemori, 1983; Ferreira & Santos, 1997; Denison & Keitzka, 1993.

The interest of intensively managed container-grow has largely made its evidences for the propagation by rooted cuttings of number of tropical forest species, (Beaujard *et al* 2000, Urchin *et al* 1996, Monteuuis and Bon 1987, Monteuuis 1988). The propagation by micro-cutting and mini-cutting represents the most modern concept of commercial cloning of *Eucalyptus*. The terminology of these two techniques has been standardized as mini-cutting,

Nursery establishment and stock plant area maintenance costs were too high for unexpectedly variable and overall low rooting rate scores. Replacing the traditional field-grown stock plants by intensively managed container-grown ones resulted in easier and more economical maintenance, while increasing the number and improving the cuttings quality.

New generation cuttings are short orthotropic shoots, lately formed with an active shoot apex, several internodes and leaf pairs underneath. The traditional cuttings were more lignified stem portion with one pair of trimmed leaves only, and collected from field produced shoots after coppice. The new generation cuttings speed up the clonal multiplication process and hence shorten breeding and selection cycle duration, while improving the cutting quality issued from the intensively managed planting stock.

Rooting stem-cutting was not suitable for a large number of economically important species, including those important for energy and charcoal, like *E. citriodora, E. maculata, E.*

paniculata and *E. cloeziana*, and an important number of clones of rootable species had problems for commercial propagation. Most of the problems were associated with accelerated maturation process causing rapid loss of rooting-predisposition, and manifestation of topophytic effect. The phenomena of topophysis affects clones in different intensities, and is the main cause of intra-clone differences in growth and reduction of rooting ability. Franclet *et al.* (1987) emphatically pointed out that topophysis induced physiological differences and these differences can result in sufficient intra-clonal variability that can suppress the potential advantages of cloning. Another limitation of stem cutting was associated with alterations of root system architecture, leading to root deformation. In many clones such deformations prevented their full genetic expression, consequently reducing the ratio between selected trees and number of clones effectively used. Because such limitations of rooting stem-cutting, alternative methods were developed for commercial cloning of *Eucalyptus* species.

Mini-Cutting

The idea came up with the observations that rooting ability of stem-cuttings decreases with ontogenetic aging and the decline may be faster than reported in the literature. In *E. grandis* for example, the rooting competence decreased from the fourteenth node up Patton & Willing, (1974), while it took longer in the *E. deglupta*. Assis *et al.* (1992) observed that clones of *E. saligna, E. grandis* and *E. urophylla* that had equally high proportion of stem-cutting rooting *in vitro*, showed differential levels of decline in the rooting percentage when managed in clonal hedges. This indicated that some factor related to clone growth, encompassing period between planting and cutting harvest (6 months), could be responsible for these differences. Preliminary tests done at Klabin Riocell (unpublished) showed, independent the species, almost 100% rooting of the very young mini-cuttings obtained from the cotyledonary leaf pair and the same tendency was maintained in the difficult to root species like *E. citriodora, E. cloeziana, E. paniculata, E. dunnii*, and *E. globulus*. However, with age, ranging from few days to some months, the cuttings harvested from such young plants showed a marked reduction in their rooting ability and in some cases such ability was totally lost. These observations suggested that the rooting potential reaches the maximum value at high juvenility level and is similar in all species tested. But the decrease in the rooting ability with seedling age differed among species, which was similar to that found in the older materials in the field. This suggests that, at some stage, part of the juvenility obtained through rejuvenation process *in vitro* (Gonçalves *et al*. 1986) and/or on basal sprouts of cut adult tress (Hartney, 1980) is being gradually eroded during the growth of the clones in the clonal hedges.

Results were obtained from trials established to define substrate, growth substances, environmental conditions for rooting etc. One of the most significant findings of this new technology was complete elimination of the use of growth substances usually required for the rooting of stem-cutting (Assis *et al*. 1992). These substances did not increase rooting of micro-cuttings, instead in some cases reduced it, indicating that the endogenous auxin concentration in the juvenile tissues was sufficient to promote rooting. Based on these results, a super-intensive system of *Eucalyptus* propagation *ex vitro* was established.

The main feature of the technique is the use of juvenile plants or plants rejuvenated in vitro, as source of vegetative propagules. Shoot apices are used as micro-cuttings, which are placed to root in a green house equipped with temperature and humidity control. The actual size of micro-cuttings is about 7 to 8 cm with two to three leaf-pairs. Presence of the shoot apex is important for quality of the root system, because its presence induces taproot-like system. The micro-stumps left after micro-cutting harvest, sprout rapidly producing new micro-propagules, which can be harvested for use within a period of 15 days in the summer and 30 days in the winter.

Since its first use, the micro-cutting technique is improving continuously by incorporating new research findings. The evidence of evolution of this technique are well documented in publications of Assis *et a*l. (1992); Xavier & Comerio (1996); Assis (1997); Wendling *et al* (2000); Higashi *et al*. (2000), and Campinhos *et al*. (2000). The technical contributions, reciprocally exchanged through a pre-competitive development model and intense information exchange, were the bases for the fast evolution of this new concept of cloning *Eucalyptus* in large scale.

Advantages of Mini-Cutting

Compared to the traditional stem-cutting rooting, mini-cutting has many advantages leading to operational, technical, economical, environmental and quality benefits can be summarized as follows:

- Choice of a culture substratum allowing the production of the best cuttings, independently of local soils characteristic.
- Operationally, the labor demanded and cost is markedly reduced, due to elimination of labor intensive treatment in smaller indoor areas at much lower costs.
- Reduction of risks by the pathogenic whose have an expansion is very difficulty controllable in field-grown stock plants, and limited to the intensively managed grown resulting in reduced fungicide application.
- The rooting speed of micro-cuttings is usually reduced to half compared to rooting stem-cutting, thus considerably improve the use of the facilities
- Mini-cuttings produce better quality root system with a tendency for a taproot-like system in contrast to the predominant lateral root growth habit in the stem-cutting system.
- Best controls of stock plant environment, less exposed to the sun and other environment regime variations of field-grown stock plants;
- The area culture and installation proximity, reducing stress risks between the instant of the crop and the installation of the cuttings in rooting conditions.
- Increasing of stock plants density and their productivity/surface.
- Best physiological conditioning of vegetative material allowing increasing appreciably rooting rate even for clone reputed refractory in traditional conditions.

Methodology

Construction of raised sand beds.

Fiber glass or Concrete troughs of 1 ft height are placed on raised benches. The length of the troughs can vary according to the requirement. Bottom 5 cm is filled with pebbles or stones for proper drainage, and on the top of it river sand is filled to a height of 20 cm. The raised sand beds are protected under poly houses with sides is covered with insect proof mesh to protect the plants from rain, disease and pest.

For an area of 100 sq.Mt at a spacing of 10 cm X 10cm 10,000 plants can be planted. Weekly thrice fertilization is practiced with a solution of NPK 13-13-21 (5g/plant/week) and weekly micro nutrient spray (foliar soluble fertilizer with Magnesia and oligo - elements in concentrations of 8-12-24, +4+31) for 1g/plant/week

These rooted cuttings are subject to reiterated successive cut-back of the main axis to shorten them and to maintain them near the root system,

Cutting production on managed container-grown stock plant

The entire short orthotropic shoot newly formed, with an active shoot apex and an average number of three entire leaf pairs and 5 to 7 cm of long are harvested, 20 days after pillared. Each entire shoot harvested are immediately planted in the containers filled of a substratum compounded Vermiculite and Perlite in proportions 8/2 (v/v). The containers containing cuttings are then placed in mist chambers on heightened tabels, for 30 days in the rooting area , and submit to watering (15 seconds all 10min the 15 first days, and 15 seconds all hours them 10 next days).

Preventive treatments are practiced each week with fungus (Chlorothalonil to 550 g/l and Carbendazim to 100 g/l using 0,5 ml/l) and Cypercal 50 EC insecticide (Cyperméthrine to 50 g/l) to 10 ml/l doses.

Weaning

At the end of rooting stage, plantlets are transferred from mist chamber to 50 % shade for 4 days and changing of watering duration, from 15 seconds all hours to 15 seconds all 2 hours . Pest and disease protection are the same than the rooting phase.

Acclimation of rooted cuttings:

After weaning, cases containing young rooted cuttings are placed in acclimation conditions during approximately 45 days, in the total absence of shade and a watering of 15mn one to twice per day according to the sunning. During this phase, the fertilization is reduced to the utilization of granular NPK 13-13-21 once by week always to 5g/plant/week. Pest and disease protection are the same.

Results

Morphological comparison between different material (stool plant cuttings and rooted cuttings) according to their origin field-grown stock plants or intensively managed container-grown:

Photo1: Cutting from field-grown stock plant

Photo2 Cutting from intensively managed sand bed grown

Traditional cuttings issued from field-grow stool plant (**Photo1**) are more lignified stem portion of 5 to 8 cm length, taken in the median part of the stem, with 2 halved and opposed leaves, and two buds in dormant condition. Cuttings issued from intensively managed container-grown (**Photo2**) are entire short orthotropic shoots newly formed with 5 to 7 cm of long, with active shoot apex and with an average number of three entire leaf pairs. These entire shoots are harvested to its basis on the remaining fragment of the main axis of the stool plant after cut-back.

(a) (b)

Photo3: Rooted Cutting from field-grown bed

(a) (b)

Photo4: Rooted cutting from sand

The field-grown stock plants rooted cuttings (**photo3**) comes from improvement of one of two lateral buds of lignified stem portion. Root's system developed by this cuttings is less dense (**photo4**) with 1 to 3 main roots and very few secondary roots.

Propagules Productivity

Compared to the field orchards, the capacity of producing vegetative propagules in the super-intensive systems is highly expressive, consequently, more cuttings can be produced per year per unit constructed area of mini hedge, besides they are ease of handle, manage and they have a lower cost per plant.

Certain practices of management of mini-stumps in the sand beds produce vegetative propagules similar to the macro-cutting system. The sprouts are allowed to grow to bigger sizes, and after collection each sprout is divided into three mini-cuttings (basal, middle and apical). This system produces higher number of propagules/m^2 but it loses the best characteristic of the mini-cutting provided by the presence of shoot apex, responsible for producing high quality root system (taproot-like system, discussed earlier)

One question that arises from the spacing in mini-hedges is about the influence of close spacing on the rooting of mini-cuttings and the mini-stump productivity. Trials showed that the spacing of 5 x 5 cm between plants does not interfere significantly in the rooting capacity of the mini-cuttings, but wider spacing permits higher survival rate of mini-stumps. But the final results are better with narrower spacing, since there is more than proportional compensation by existence of higher number of plants/m^2.

Table1 Comparison of the cuttings production between intensively managed sand bed-grown and the field-grown stock plants in 100sq mt. area.

Stages	Number of cutting obtained	
	Using field-grown stock plants spaced at 1mt x1mt.	Using intensively managed sand bed -grown at 10cm X10cm
At the beginning	100	10000
After 3 months	100	90000
After 6months	100	225000
After 1 year	100	495000
After 2 year	35000	1035000

- Based on cuttings/mini-cuttings/stump considering 12 months of effective production, without discounting the replacement of dead plants.

Replacing the traditional field-grown stock plants by intensively managed container-grow ones resulted in easier and more economical maintenance, while increasing the number and improving the quality of the cuttings, having productivity 30 folds higher than the traditional system.

At an interval of 20 days each plant can give 3 cuttings. 10 lakh cuttings can be obtained in 2years from 100 sq. mt. Area assuming 100% survival. At the same time field grown plant coppiced at an interval of 2years will yield around 35000 cuttings @ 35 cuttings per tree for 30 days interval from 100 sq mt. Area

Discussion & Conclusion

The precocity of stool plants installed with cutting comes from precedent intensively managed sand bed-grown, expressed by early harvesting of cuttings; show the interest of the utilization of the increasingly juvenile vegetative material.

In fact, leaf axis of rooted cuttings coming from field-grown stock plants results of the improvement of one of two lateral dormancy buds of lignified stem portion. The level of bud's dormancy differs from a stem to another in the same stump, and from a cutting to another within a same stem. It leads to consider that physiologic stage is very different between intensively managed stool plants and field-grown stool plants. Physiologic stage of intensively managed stool plants is susceptible physiological stage to determine an "ability state of to the vegetative propagation" (Monteuuis 1988).

Nevertheless, the intensively managed rooted cuttings is an orthotropic seedling coming from the phylotaxique improvement of the apex, harvested to the basis of the stool plant, rejuvenated by reiterated successive cut-back of the main axis. Cuttings produced by this system constitute physiologically younger vegetative material than cutting fashioned from stem of field-grown stump. The effect age of origin plant on the behaviour of the future stool plant has been observed by Walker (1985), Beaujard *et al* (2000), Girouard (1974), Monteuuis & Bon M.C., (1987), and Farmer (1974). The important differences noted in term of productivity between managed container-grown stool plant and field-grown stool plant can therefore be explained by the physiological state difference and particularly by juvenility in favour of managed sand bed -grown young material.

Our results show a strong production of the roots biomass of the managed sand bed-grown rooted cuttings, comparatively to the field-grown rooted cuttings. Saydat and Yahia (1995) have shown that, rooted cuttings from managed sand bed-grown preserve their advantage to the field because they develop there, rooting system more important than rooted cuttings from field grown stock plant. Experimentations would have to be led in this aim on *Eucalyptus* research.

The morphology of the intensively managed cuttings predisposes it to an organogenesis oriented essentially to the rooting, due to the pre-existence of leaves and apex, the distribution of leaves on the stem, a good mass of roots, and finally a best juvenile state. The existence of apex confers to the cuttings its orthotropic form, due to the lateral growth inhibition which is implied the terminal bud. Several works mention the importance of the morphology of the cutting, especially their orthotropy on the rooting (Frohlich 1961, Marien 1991, Adendorff 1991, Fournioux, 2000). The effect of cut-back on the adventitious rooting and improvement of stool plants productivity has been observed by Girouard (1970), Monteuuis (1984) Monteuuis and Bon (1987). Cuttings coming from field-grown stock plants are handicapped by the absence of apex, due to the bad spatial leaf distribution and the absence of secondary roots.

The clonal variability of rooting (percentage, mass, structure) has been pointed out on several varieties by several authors. Factors being able to influence this variability can be linked to genetic differences, to climatology, to seasonal variations, to vegetative material juvenility and to clones bank management (Adendorff and Schon 1991). The evolution of the productivity with stool plants age can be explained by the progressive improvement of its rooting with age, entailing a best exploration of roots and a best absorption of mineral elements contents in the substratum.

The rooting rate is used in clonal selection programs as first order criteria after which others variables can have an influence in the selection (Wignall *et al* 1991). The study of the rooting of cuttings in the different phases of propagation deserves to be deepened, notably by the study of the rooting kinetic.

Intensive management technique in sand-grown for propagating *Eucalyptus* is an easy technique to popularize. Its exponential increase of cuttings production increases its interest, in particular for the developing countries which often do not have means to acquire expensive technologies.

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