The Role of Vegetative Propagation in CSIRO Forestry and Forest Products

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Vegetative propagation by means of cuttings, grafting, and micropropagation is playing an increasingly important role in research projects in CSIRO Forestry and Forest Products. It is being used as part of an integrated tree improvement program that includes selection of superior clones as parents in seed orchards and for the production of interspecific hybrids. Techniques have been developed to manipulate flowering to allow rapid turnover of generations, reliable selection from field trials, and to change selection criteria over time. Particular attention is being paid to the development of forest trees adapted to planting on farms in the lower rainfall areas of southern Australia. CSIRO Forestry and Forest Products is also a partner in a number of overseas aid projects and joint ventures that involve vegetative propagation of superior clones for plantations.

INTRODUCTION

CSIRO Forestry and Forest Products played a major role in the early development of *Pinus radiata* cuttings for planting of selected clones (Eldridge and Spencer, 1986; Eldridge and Owen, 1986; O'Regan and Sar, 1989). Cuttings of superior clones and families of *P. radiata* now represent a large proportion of current plantings. More recently our work has concentrated on eucalypts and other species.

Vegetative propagation MUST form part of a genetic improvement program. It is not an alternative to breeding and selection but a valuable tool, as it enables trees with unique genes and gene combinations to be included in the program. One-off gains can be achieved by selection of superior clones but conventional breeding may soon overtake these gains or leave the plantation exposed to unacceptable risks as too few clones are planted.

Two of several projects in the Division demonstrate how vegetative propagation plays an important part in the future domestication and breeding of forest trees. The two projects are:

1) Tree improvement for the lower rainfall areas of southern Australia. The aim is to breed trees adapted to planting on farms in the drier regions of southern Australia.
2) South Pacific Regional Initiative on Forest Genetic Resources (SPRIG) where tree improvement for several South Pacific countries is underway.

Vegetative propagation plays an important role in both of these projects. Some of the techniques used are:

- Grafting to bring selected trees into a breeding arboretum for manipulation of flowering and cross pollination. Grafting is also

used to bring superior parents into seed orchards, e.g., *Eucalyptus maculata* [syn. *Corymbia maculata*].
- Cuttings to produce trees for field trials.
- Micropropagation as an alternative to cuttings.
- Microcuttings as a means of high-density propagation.

This research is part of the larger research program of Tree Improvement and Genetic Resources Program of the CSIRO Forestry and Forest Products and involves participation from many CSIRO staff and collaborators. The Tree Improvement and Genetic Resources Program is 70% externally funded and its projects are closely linked to the demands of our clients.

DOMESTICATION OF TREE SPECIES

In all but a few commercial species (*P. radiata, Eucalyptus globulus, Araucaria cunninghamii*) our forest trees have had no selection for adaptation to site or to produce particular products. In many cases we are still at the stage of determining the best species to plant in a particular area.

In order to determine the best trees for particular sites in the shortest possible time, well conducted field trials are essential. CSIRO has simplified trial design and statistical analysis by developing two, user-friendly computer packages. The packages CycDesigN (Whitaker et al., 1997) and DataPlus (Williams et al., 1999) cover the complete range of procedures from trial design, allocation of treatments to trial plots, data entry into spread sheets, preliminary analysis of data, and then links into the Genstat statistical package for data analysis. Feedback from many training programs in several countries was used to modify the packages.

CSIRO is not a plantation agency, it does not own any forests so we need to work with forest growers and plantation agencies to get our results tested in the field. Our collaborators include state forest services, farm forestry networks, private companies in Australia, and overseas joint venture partners.

TREE IMPROVEMENT FOR THE LOWER RAINFALL AREAS OF SOUTHERN AUSTRALIA

This is a new initiative for the genetic improvement of trees adapted to the lower rainfall regions of southern Australia (Harwood and Arnold, 1999). This project involves CSIRO and State agencies with support from the Rural Industries Research and Development Corporation - Joint Venture Agroforestry Project.

Superior species and provenances adapted to the lower rainfall areas of southern Australia will be selected and incorporated into genetic improvement programs (Harwood and Arnold, 1999). Several seed orchards have been established, some from field trials of provenances of particular species. All of these field trials were established with collaborators in both Government and private organisations. Table 1 lists the seedling seed orchards that have been established.

Table 1. Eucalypt seedling seed orchards established in the lower rainfall areas of southern Australia.

Eucalyptus species	Orchards (no.)	Date planted
badjensis	1	1996
benthamii	4	1994, 1995
camaldulensis	4	1996, 1998
dunnii	3	1995
grandis	2	1996, 1997
maculata [syn. *variegata*], *henryi*	4	1995
cladocalyx	1	1998
occidentalis	2	1998

The above seed orchards represent only a part of an extensive network of seed orchards that the Division has established throughout Australia. All of these seed orchards have been established with collaborators.

These seed orchards will provide superior seed of particular species to growers in the next few years. In addition, CSIRO is also developing hybrids between species for testing alongside the pure species in field trials. Hybrid eucalypts being tested are: *E. saligna* × *E. grandis, E. saligna* × *E. tereticornis*, and *E. grandis* × *E. camaldulensis* (from CSIR in South Africa).

Additional hybrid combinations are being developed using selected *E. grandis* and *E. camaldulensis* parents. Both interspecific and intraspecific hybrids will be created. Vegetative propagation plays an essential part in all of these research projects. Grafting is used to maintain the parents for cross pollination. The most successful grafts have been tip-cleft grafts using scions about 4 to 5 mm in diameter and containing 2 to 4 nodes. Other grafting techniques tried include splice-grafts done with the aid of a graft guide (Brennan and Mudge, 1998), side-cleft, patch-budding, and micrografting. Micrografting skills are important, as they allow rapid multiplication of selected scions from scarce material. Not all of our grafts have been successful. In a few cases overgrowth or poor growth of both the scion and the rootstock has occurred, implying graft rejection or a poor union.

Once successful grafts are established it is necessary to encourage the trees to flower as soon as possible. Placing the plants in a breeding arboretum where treatments such as water stress, cold, and Paclobutrazol, can be applied to grafted trees to encourage flowering. In the breeding arboretum the plants are in large movable pots so that they can be transferred to different environments. Once selected parents flower they are cross pollinated using standard techniques.

The production of large amounts of hybrid eucalypt seed is costly in terms of both time and labour. Even after hybrids are produced they need to be tested in the field to ensure that only the best trees are propagated for commercial planting. It is common for many seedlings from a cross to be abnormal.

Hybrid seedlings are grown on under greenhouse conditions and screened for abnormalities, for rooting ability, and other parameters important in selecting trees suitable for planting as clones. Selection parameters include:

- Production rate of ramets from mother plants.
- Rooting ability and survival to field planting.
- Performance of cuttings in field relative to seedlings.

The importance of selecting clones that are easy to propagate cannot be overemphasised. All of the successful clonal eucalypt plantation programs overseas such as in Brazil, South Africa, and Morocco always select clones that are easy to vegetatively propagate. Only a small proportion of trees initially selected in the field get through this final barrier of being suitable for large-scale, commercial vegetative propagation (Zobel, 1993).

The main technique used for propagation of clones is stem cuttings from hedges maintained under greenhouse conditions. Cuttings are rooted in Aircell containers (BCC, Sweden) that allow aeration of the medium, root pruning, and sorting of the cuttings after rooting.

Other techniques that are being tested are microcuttings produced from mother plants grown under greenhouse conditions and under hydroponics. Microcuttings offer high rates of production of cuttings per unit area. The method is being extensively used in Brazil with eucalypt hybrids. Rates of production of over 1000 rooted cuttings per month, per square metre of mother plants have been reported.

Micropropagation will also be used to propagate some clones. Simple techniques of micropropagation developed at CSIRO involve growing the mother plants in vitro as rooted plants rather than as multiple shoots in shoot cultures. The advantages of this system are:

- High rates of production of shoot explants suitable for transfer to a rooting medium.
- Shoot explants with normal leaf development and greater vigour.
- Plants that are easier to acclimatise.
- Easier maintenance of production stock and scheduling of production.

Simpler and therefore cheaper methods of micropropagation offer the advantages of high-density propagation under sterile conditions and under conditions where many factors can be controlled compared to cuttings. A recent example is the work of Dr. Tatsuya Shiraishi (pers. comm.) where he promoted rooting of recalcitrant clones using specific strains of mycorrhizal fungi in vitro.

Once results from field trials are analysed, the next step is to transfer this technology to the field. This may involve joint ventures or licensing nurseries to propagate superior seedlings and clones. Royalty payments will form part of these arrangements.

SOUTH PACIFIC REGIONAL INITIATIVE ON FOREST GENETIC RESOURCES (SPRIG).

This is a collaborative regional project between countries the South Pacific (Fiji, Vanuatu, Samoa, the Solomon Islands, and Tonga) with three Australian consortium partners (CSIRO Australian Tree Seed Centre, Queensland Forest Research Institute, and Fortech). AusAid provides funding for the project as part of the Australian Government's overseas aid program.

The aim of SPRIG is to improve the sustainable development of forest tree resources in the South Pacific. It has three main components:

1) **Conservation.** Develop strategies for conserving genetic resources of priority indigenous species. Both Forest Department and community views (via rapid rural appraisal) are evaluated and priority species and varieties are selected.
2) **Tree improvement.** Collection, distribution, and exchange of germplasm, field trials to select superior genotypes and propagation.
3) **Strengthen institutions.** Improve capability of institutions to continue this work in the future. This involves supply of computers, database programs to manage germplasm, seed collection gear, and staff training.

Species to study were selected by a regional meeting of Pacific experts. They suggested a number of indigenous species and one exotic species (mahogany, *Swietenia macrophylla*). About 10 priority species have been selected for collection in each country.

Extensive collections of *Toona australis* (Australian red cedar) have been made in Australia and of *Endospermum medullosum* (whitewood) in Vanuatu. *Toona australis* is in the same plant family (Meliaceae) as mahogany and has performed well in Tonga where it is highly regarded.

Vegetative propagation has an important part to play in the Pacific because many of the species present difficulties in propagation from seed but are easy to vegetatively propagate. Some are already planted as stump-cuttings and used as living fences. Species such as *Pometia pinnata* have recalcitrant seed that is difficult to store for long periods.

Using cuttings from seedlings, success has been achieved with sandalwood, *Toona australis*, mahogany, and *Endospermum* (Walker, Collins and Haines, 1999).

The Queensland Forest Research Institute developed a versatile portable mist propagator to assist the vegetative propagation program. These units provide battery-operated mist propagation units, for use in areas where power supply is either unavailable or unreliable. The units are also used as part of the training programs.

An important advantage to Australia is that the trees from the extensive collection of *Toona australis* (Larmour, 1999) will be grown in field trials in Australia to screen for resistance to the cedar tip moth (*Hypsipyla robusta*), which has devastated previous attempts to grow plantations.

FUTURE ISSUES

These two projects illustrate the importance of vegetative propagation as a valuable component to tree improvement programs. Vegetative propagation is also important with many other projects in which CSIRO is involved.

The ability to graft mature selected scions will always have a role in a genetic improvement program. Similarly, the ability to propagate cuttings of selected trees assists in evaluating genotypes in field trials.

However, if vegetative propagation is to play a role in providing planting stock for large-scale commercial planting then major challenges still remain in reducing the cost of each ramet. All vegetative propagation techniques require much more labour compared to the production of seedlings. Microcuttings or a combination of simple forms of micropropagation and microcuttings may provide a solution.

Attempts at reducing the labour costs of vegetative propagation of forest trees by using robotics and automation have so far failed. This is particularly so where robots have tried to emulate the human propagator. Propagators rapidly make decisions on selection of suitable shoots, where to cut the shoots in relation to the node, and plant the shoot at a set position and depth in the propagation medium. Such decisions are very complex for a machine to handle as they include vision, handling, and many decisions need to be made rapidly. Such systems are neither easy nor cheap to emulate by robotics.

Alternative approaches to automation such as the RITA system (INRA, France), simple bioreactors (Osmotek - Rehovot, Israel), and meristematic nodules (Ziv et al., 1994) do not involve such complex decision making procedures. These systems may be more suitable for large-scale propagation in the future. However, each system needs to be evaluated under commercial conditions.

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