1. Introduction
Domestication of a tree species is necessary for sustained productivity in plantations. Domestication essentially involves a sequence of repeated selections and mating carried out to bring about change in the gene frequencies (Eldridge et al., 1993). This technique, often referred as ‘tree improvement’, is used to alter the genetic makeup of a species with the objective of improving its utility. A tree improvement programme is normally planned and organized for a species to improve the growth rate or form of the species, resistance to diseases and pests, suitability to the environment and its utility. The main objective of a breeding programme is to enhance productivity, genetic diversity is also important for sustaining the productivity over generations. Most of the tropical tree species are outcrossed and adequate diversity is a prerequisite for production of outcrossed progeny. After identifying high yielding provenances, which is the first step in an improvement programme, concerted efforts are made to locate orchards in ideal environments for good flowering and seed production. Compared to agricultural crops, domestication of forest trees has a very short history and even the most manipulated forest species is only a few generations removed from their wild ancestors. The domestication process has been slow because of the long rotation period, irregularity in flowering and fruiting, and high levels of outbreeding with consequent loss of genetic gain in subsequent generations. Eucalypt, the widely planted forest tree, was introduced as an exotic in many countries, including India. Tree improvement programmes were initiated in many countries, (Davidson, 1998) including India, using the extensive plantations originated from these early introductions which were often haphazard and suspected to have originated from a restricted genetic base of few native trees. The early exotic plantations may not be having many useful alleles of the original population and there would also be deleterious effects of inbreeding. Eldridge (1978) suggested that in such situations it would be wise to obtain fresh seeds from natural stands and enlarge the base.
About 4 Mha of eucalypt plantations were raised (GIT, 2008) by forest departments, forest development corporations, pulp and paper companies, and farmers in India. Eucalypt plantations are raised to meet the fuel, pulp and timber needs without disturbing the natural forest cover. *Eucalyptus camaldulensis* and *E. tereticornis* are the major species grown in arid regions of the country. *E. grandis* is grown in over 3,000 ha in high elevations (Kerala and Tamil Nadu) and *E. urophylla* and *E. pellita* in about 15,000 ha in the high rainfall regions of Karnataka (Amanulla, pers. comm.). The wide spread of the genus across the country was made possible with the emergence of ‘Mysore gum’, a land race evolved from the early introduction of *E. tereticornis* and six other eucalypts and inter-specific hybrids of *E. tereticornis* in Karnataka state (Kaikini, 1961). Over the years, a highly variable land race (widely referred as *Eucalyptus* hybrid) evolved which was able to adapt to the different agro-climatic regions of the country; often quite distinct from the native locations in Australia (Davidson, 1998). This land race is considered a mixture of pure *E. tereticornis* as well as some genetic segregates of interspecific hybrids.

Wide segregation and variation in morphological traits were noticed in most of the *Eucalyptus* hybrid plantations and attempts were made to have some control on seed collected for establishing plantations. As early as in 1965, Venkatesh and Kedharnath advocated selection of good trees in different regions and establishing seed production areas for supplying seeds for use in that area. They hoped that this strategy would help in restricting suitable adapted genotypes within a region and avoiding movement of seeds across regions with differing environments. Selective harvest of seeds from good trees resembling *E. tereticornis* was recommended to reduce segregation. There was, however, no control on seed collection and vast areas came under plantations of low yielding *Eucalyptus* hybrid origin.

The early introductions of *E. camaldulensis* and *E. tereticornis* to India were from southern temperate localities in Australia rather than the northern tropical regions where the climatic conditions closely resemble the areas available in India (Boland, 1981) because of the inaccessibility and difficulties in collecting seeds. Boland (1981) observed shorter opercula typical of the southern provenances in the eucalypt land race in India compared to the longer, horn shaped opercula typical of the north Australian provenances. From his observations on the seed morphology and coppice shoots in *Eucalyptus* hybrid plantation, Boland felt there was a mixture of *E. camaldulensis* genes in these plantations. This led to further degradation and wide variation in growth and leaf morphology in the plantations. The yield is poor because of the hybrid breakdown compared to the pure species of *E. camaldulensis* and *E. tereticornis* (Davidson, 1998; Varghese et al., 2000b).

IUFRO coordinated international provenance trials for *E. camaldulensis* and *E. tereticornis* (Kumaravelu et al., 1995) were initiated during 1980s and suitable
provenances were identified for various regions. Results from different provenance trials indicated the superiority of the northern provenances of eucalypts to the southern provenances (Ghosh et al., 1977). The local *Eucalyptus* hybrid seed lots were inferior in comparison to the ‘Petford’ and ‘Katherine’ provenances of *E. camaldulensis* and ‘Laura River’ and ‘Kennedy River’ seedlots of *E. tereticornis* (Chaturvedi et al., 1989). The yield of the local land race averages around 7 m³ ha⁻¹ yr⁻¹ (Chandra et al., 1992) which is much lower to the pure species lines of *E. tereticornis* and *E. camaldulensis* (12-25 m³ ha⁻¹ yr⁻¹). Tree improvement was attempted in the existing *Eucalyptus* hybrid plantations. Plus trees and seed stands were selected (Rathinam and Surendran, 1981; T.N.F.D, 1993) in virtually every state and progeny trials conducted (Kedharnath and Vakshaya, 1978; Vinaya Rai et al., 1980; Krishnaswamy et al., 1984) to estimate the genetic parameters and clonal seed orchards were also established. Inter specific hybrids, both naturally occurring (Kedharnath, 1980) and artificial (FRI-4 and FRI-5, Venkatesh and Sharma, 1978), were raised to exploit the hybrid vigour. Vegetative propagation of trees intensively selected from plantations (Lal et al., 1993, Tripathi et al., 1996 and Kulkarni, 2005) has helped to increase the yield considerably (20-25 m³ ha⁻¹ yr⁻¹, Lal et al., 1993) in dry regions. In high rainfall regions of Karnataka, *E. pellita* and *E. urophylla* were identified for planting from 22 species evaluated in field trials (Satishchandra and Madhav, 2002). Progeny (Satishchandra, 2007) and clones (Amanulla, 2007) selected from offspring of superior trees in the species trials were used for further planting. Provenance tests were conducted for *E. grandis* in high elevations of southern India (Subramanian et al., 1992). However in the absence of a planned breeding programme, with the best identified natural populations, these useful breeding techniques when employed separately, result in a dead end situation (Boland, 1981) and the gain obtained is not sustainable.

Davidson (1998), who studied the tree improvement status of eucalypts in Asia-Pacific countries, observed that eucalypt tree improvement in India has remained uncoordinated and weakly supported from the point of view of domestication and breeding. In his report, Davidson (1998) provided different approaches that can be adopted for a sustained increase in productivity of eucalypts. He emphasised the need for a short-term programme to achieve immediate genetic gain from the existing variation as well as a long-term programme to maintain sufficient diversity essential for success of selection in future generations. He advocated several short term improvement strategies that would reduce the inbreeding effects and supply improved seeds for immediate planting requirement like retaining good plantations of identified provenances or establishing seedling seed orchards from identified trees.

There has been a taxonomic revision and the best performing provenances identified in the provenance trials of *E. tereticornis* (Chaturvedi et al., 1989) like
Laura River, Kennedy River and Morehead River are included under *E. camaldulensis* now (Brooker and Kleinig, 1994; Doran and Burgess, 1993). Encouraged by the improved performance of these provenances, the provenance trials were thinned to remove inferior trees and use it as a seed stand to meet the immediate requirement of planting stock. The new plantations established over extensive areas with seed collected from a few trees by agencies like Tamil Nadu Forest Plantation Corporation (TAFCORN) were definitely superior to Mysore gum trees. Seed from good trees selected in provenance trials was also used to establish seedling seed orchards to produce improved seed (Varghese et al., 2001). These orchards are not suited as a starter material for a long-term improvement programme as the genetic base of the seed lots in the provenance trial is often as narrow as five to ten trees. Nevertheless they serve as a useful short-term approach to meet immediate seed requirement of planting agencies. These orchards were useful until seeds were available from the large breeding seedling orchards and unpedigreed orchards established as part of a eucalypt breeding programme implemented in southern India (Doran et al., 1996).

2. Breeding Programme for *E. camaldulensis* and *E. tereticornis*

A comprehensive breeding programme which recognises the changes in classification was implemented in southern India with greater emphasis on *E. camaldulensis*. Domestication of eucalypt was given a new thrust in India with new seed introductions for improving the productivity. Bulked seed and family identified seed lots of best provenances identified in provenance trials were obtained from CSIRO, Australia (Doran et al., 1996) for implementing the programme.

2.1 Short-Term Strategy

Unpedigreed orchards were established in southern India with mass selected seed through open pollination without retaining family identity. Seeds were obtained from a large number of unrelated trees of suitable provenances from natural stands or from selected plantations of known origin. Stands were raised with a mixture of equal amount of seed from all trees and thinned heavily, sufficiently early to promote seed production in retained trees.

An unpedigreed orchard is relatively cheap and simple to establish and provide a reliable source of fairly improved seed. Unpedigreed orchards of broad genetic base after selective thinning can be used as interim source of seed during the initial stages of a breeding programme. Even when a pedigreed breeding population is established for long-term breeding, it is important to have an unpedigreed orchard as they offer a quick means of improved seed at an early stage of improvement programme and will be quite useful in case the subsequent generations of the breeding programme fail to take off. Unpedigreed seedling orchards or seed production areas (SPAs) were established as an interim measure...
to provide short-term gain, until the main strategy involving the establishment of large, family identified progeny trials was raised, evaluated and progressively thinned for seed production. Bulked seed from over 500 trees each of *E. camaldulensis* and *E. tereticornis* of identified superior provenances were used for establishing four unpedigreed seedling orchards (Varghese et al., 2009a). Small quantities of seed from other provenances were also included to maintain a broad genetic base. The orchards were established (Table 1, Fig. 1) at a dry site, Pudukkottai in Tamil Nadu (SPA 1 and 3) and a moist site, Panampally (SPA 2 and 4) in Kerala state (Kamalakannan, 2007). These orchards bring about a chance of mating between populations that are widely separated geographically in their natural range. In a species like *E. camaldulensis* with large geographical distribution, when widely differing seed lots are crossed, the adaptability of some seed lots to diverse environments can be combined with the vigorous resilience of others.

<table>
<thead>
<tr>
<th>Orchard</th>
<th>SPA-1</th>
<th>SPA-2</th>
<th>SPA-3</th>
<th>SPA-4</th>
<th>SSO-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>Pudukkottai</td>
<td>Panampally</td>
<td>Pudukkottai</td>
<td>Panampally</td>
<td>Erumana</td>
</tr>
<tr>
<td>Species</td>
<td><em>E. camaldulensis</em></td>
<td><em>E. camaldulensis</em></td>
<td><em>E. tereticornis</em></td>
<td><em>E. tereticornis</em></td>
<td><em>E. tereticornis</em></td>
</tr>
<tr>
<td>Latitude</td>
<td>10° 51′ N</td>
<td>10° 32′ N</td>
<td>10° 53′ N</td>
<td>10° 52′ N</td>
<td>11° 00′ N</td>
</tr>
<tr>
<td>Longitude</td>
<td>78° 49′ E</td>
<td>78° 46′ E</td>
<td>78° 49′ E</td>
<td>78° 46′ E</td>
<td>78° 38′ E</td>
</tr>
<tr>
<td>Altitude (m)</td>
<td>180</td>
<td>400</td>
<td>320</td>
<td>400</td>
<td>420</td>
</tr>
<tr>
<td>Annual rainfall (mm)</td>
<td>650</td>
<td>1,400</td>
<td>650</td>
<td>1,400</td>
<td>850</td>
</tr>
<tr>
<td>Mean temp (°C)</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Area (ha)</td>
<td>1.2</td>
<td>0.5</td>
<td>1.2</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Fig. 1. Map showing location of seed orchards and genetic gain trial sites in southern India.
growth of other seedlots. Synchronization in flowering is, however, a factor that has to be managed to get the desired improvement. Provenance resource stands (PRS) of three Queensland provenances of *E. camaldulensis*, Kennedy River, Morehead River and Laura River (Doran and Burgess, 1993) were also established at Pudukkottai, Tamil Nadu, using bulked seed from individual provenances. The SPAs and PRS were selectively thinned to remove phenotypically inferior trees (Varghese *et al.*, 2000a).

Thinning was undertaken in two stages at two and four years in SPAs to avoid competition between trees and, for production of large crowns. Since pedigree information was not available, phenotypic information was used for selecting good trees to be retained for seed production (Harwood *et al.*, 1996). Thinning is needed to provide the optimum spacing necessary for a tree to express its genetic potential and for good seed production. A suitable method was devised to retain 150-200 best trees per hectare in the seed stand. The thinning procedure helps to remove inferior trees in unpedigreed orchards by excluding site heterogeneity and ensuring uniform distribution of retained trees in the stand (Hegde and Varghese, 2002). Index selection, that combines information on several traits of interest into a single index, was used since it enables the breeder to assign a total score to each individual (Zobel and Talbert, 1984). In a finite base population of progenies of selected trees, index selection is useful for simultaneous selection on multiple traits as well as to predict the gain for each trait (White and Hodge, 1989). After dividing the plantation into 10 tree blocks, block averages and block adjusted values were determined for each individual tree for each trait as per Cotterill and Dean, 1990. Heterogeneity of site affects the accuracy in selection of trees as evidenced by the skewed distribution of selected trees in certain patches when large blocks of 90 trees were used. 10 tree blocks reduced the effect of site with emphasis on genetic potential of the tree, as every tree is compared with nine adjacent trees within each block. Among the different selection indices attempted, ‘correlation index’ that takes into account economic weights for traits of interest, correlation between traits, and block adjusted values of individual tree (Cotterill and Dean, 1990) for computing the ‘index’ was found to be ideal (Hegde, 2003). After final thinning at four years, seven to eight kg of seed can be collected annually from about 25 trees in each orchard (Pinyopusarerk and Harwood, 2003b) so that the trees get enough time to recover for next seed collection. Seed is supplied from these orchards to farmers to replace Mysore gum plantations (Varghese *et al.*, 2004b).

### 2.2. Long-Term Strategy

The main breeding strategy followed is recurrent selection for general combining ability with open pollination in single populations retaining family identity (Eldridge *et al.*, 1993). A single plantation that was laid out initially as a progeny trial was
strategically used to combine the base, breeding and propagation populations in each site. The progeny trials provide information on family merit which is used as a basis for selection and breeding for the next generation as well as thinning for conversion to commercial seed orchards. These orchards are a source of improved seed of wide diversity and adaptability to regions with similar agro-climatic conditions (Doran et al., 1996). Selection and mating are the key activities in this breeding programme. They accumulate genes which influence yield and adaptation, increasing over successive generations the frequency of the superior trees. A progeny trial with large number of families of identified provenances after conversion to a seedling seed orchard is expected to give about 20 per cent more gain than that obtained from the best provenance. The gain from a thinned seed production area is expected to be the same as that obtained from a pedigreed first generation seedling seed orchard. Gains beyond the first cycle will normally depend on the accuracy in selecting the best genotypes and management of inbreeding in orchards. Gains will be cumulative in the first and subsequent cycles (Doran et al., 1996).

First generation progeny trials were established as per the programme at three locations, namely Panampally (Kerala), Pudukkottai (Tamil Nadu) and Sathyavedu (Andhra Pradesh) with 188 open-pollinated families of *E. camaldulensis* belonging to 18 natural provenances representing three distinct geographical regions of Australia (11 provenances from Queensland, 4 from Western Australia and 3 from Northern Territory) in 1996 (Hegde, 2003). Queensland provenances, particularly Petford, Kennedy River, Gilbert River, Laura River and Morehead River showed clear superiority over provenances from the other two Australian regions at Pudukkottai. The differences among Australian provenances were less pronounced at Sathyavedu and Panampally, where there was no marked advantage of Queensland provenances over those from the Northern Territory and Western Australia. Provenances from all three regions in Australia displayed generally better growth than the Mysore gum selections (Varghese et al., 2008a). After evaluation, 10 per cent of the worst families were thinned out at two years. The best trees of the top 90 per cent of the families selected from the first generation seedling orchard and fresh infusion families will be the base for the second generation orchards. Family performance data from second generation orchards will facilitate ranking of parents retained in the first generation orchard thus helping in roguing by backward selection and enhancing the genetic merit of the seed produced.

*E. camaldulensis* and *E. tereticornis* intergrade in parts of their natural range in Queensland (Doran and Burgess, 1993). Superior provenances such as Laura and Morehead Rivers, now classified as *E. camaldulensis* subsp. simulata by Brooker and Kleinig (1994) and also Kennedy River and Petford provenances are among the best-performing natural provenances in arid regions of southern India. The clear superiority of Queensland seed lots at Pudukkottai in Tamil Nadu (Varghese et al.,
2008a) clearly indicated that *E. camaldulensis* breeding population for this dry region and similar regions should comprise primarily of selections from the best Queensland provenances. Petford (Queensland) provenance was reported to perform well in provenance trials across several sites conducted by the Eucalyptus Research Centre in Andhra Pradesh where the annual rainfall is around 800 mm (Chaturvedi et al., 1989). The same observation was made by Ginwal *et al.* (2004a) and Singh and Prakash (2002) in field tests in Haryana and Punjab. This provenance is also found suitable in other countries like Bangladesh (Davidson and Das, 1985) and Brazil (Moura, 1986) due to its inherent capacity for fast growth and stability across a wide range of climatic conditions (Ginwal *et al.*, 2004a). Some planting agencies in India import native seed from Petford region for routine planting. Thus, it is important to establish good orchards of North Queensland provenances of *E. camaldulensis* with sufficient genetic base to produce quality seed for planting in India. Selections from seed lots of other regions in Australia (northern territory and western Australia) and *E. tereticornis* could be retained in breeding populations for regions receiving higher rainfall.

Native seed lots of *E. tereticornis* (91 families representing 13 native provenances of Australia and Papua New Guinea were introduced in northern India and tested at three diverse sites in Uttarakhand and West Bengal. North Queensland provenances performed better and, in particular, two provenances, viz., Walsh River and Burdekin River, Queensland ranked the best in that region (Ginwal *et al.*, 2004b). In southern India, 37 open pollinated families of *E. tereticornis* belonging to 13 provenances were evaluated in Tamil Nadu. Families from Helenvale provenance of Queensland and Mt. Garnet showed superior growth on par with Kennedy River provenance at four years of age (Ravi, 2008).

3. Balancing Gain and Diversity in Seedling Orchards

Seed orchards are expected to generate superior quality seed compared to other unimproved sources. Selection methods for increasing gain are often opposed to strategies for improving diversity of the seed crop. Different selection strategies can be employed for enhancing gain. In combined index selection, weights are given based on individual and family values (Falconer and Mackay, 1996) whereas phenotypic selection ranks the individual based on its phenotypic value without considering its family merit. An ideal strategy would be one that combines gain and diversity monitored in terms of a quantified effective population number (Lindgren *et al.*, 1996) with adequate representation from different families for production of outcrossed seed. When thinning is done in a half-sib progeny trial, the relationship between genetic gain and diversity must be well understood (Kamalakannan *et al.*, 2007a). These factors must be assessed to adequately manage inbreeding in plantations and to plan seed collection. Breeding values are normally used for culling inferior families and individuals to maximise gain. Fertility of trees also has to be considered as it ultimately decides the
transfer of genes to the seed crop. The quality of seed from an orchard will be judged from the possible gain and the diversity of the deployed crop. Genetic value of seed will be determined by the breeding values and the maternal and paternal gametes produced by the orchard trees. Loss of diversity and increase in relatedness are expected in advanced stages of improvement. Improvement in gain is at the cost of diversity with each advance in generation. It is, however, important to monitor the consequences of selection and thinning done to enhance gain. As relatedness increases beyond a certain point, depending on the deviation from random mating, much of the desired benefits may not be achieved. Seed collection has to be planned to prevent informal collection and dissemination which will soon lead to genetic deterioration. When a species is introduced to a new location, there is a risk that the proportion of selfing will increase if seed is collected from isolated trees or from small plots where only a few trees have commenced flowering. Inbreeding can also build up if seeds from only a very few mother trees are used to establish a stand so that the next generation will be derived from mating between close relatives.

4. Role of Seedling Seed Orchards in Domestication

Seedling seed orchards (SSOs) are cost-effective means of making available an assured supply of genetically improved seed. Orchards established with introduced seed should be intensively managed for abundant seed production and be isolated from other stands by at least 100 m to reduce contamination. Thinning should be done to retain outstanding trees. After introduction to a new location seedling orchards help to release the ‘neighbourhood inbreeding’ in the native genotypes as the parent trees in their natural habitat are usually surrounded by related trees and mating is normally restricted among them. This affects the quality of seed collected from these stands. But in a seedling seed orchard this is overcome by planting several unrelated trees in a small area and mating takes place between individuals that would not have come together in nature. Thus seedling seed orchards function as breeding populations from where new favourable recombinants can be obtained. Seedling seed orchards are very useful in combining seed production, genetic test and breeding for new recombination. SSOs are highly flexible, easy to establish, cost-effective and very effective in short rotation eucalypt. Seed orchards offer the advantage of capturing years of improvement done by an agency and deploying the same to a new location by importing advanced generation seed. After one round of testing and elimination in the new orchards, selections can be made for different agro-climatic regions.

Seedling seed orchards facilitate low-intensity breeding (Lindgren, 2003). Low-intensity breeding techniques are relevant in developing countries like India where large quantities of seed are required by resource poor farmers. It can be a back-up to aggressive high-intensity breeding programmes with genetically narrow breeding stock (Cotterill, 1989). Seedling seed orchards also conserve genetic resources.
producing seed of reasonable gene diversity, genetic variability and adaptability to
the new location. They preserve options to initiate a breeding programme and support
introduction and evaluation in new locations. A low-intensity breeding programme
will generally meet these goals, with more emphasis on genetic diversity than on
economic traits. It is effective when resources may be too small and when the alternative
is the local genetic stock of low trait and genetic diversity. A high-intensity programme
that focuses more on gain may lead to substantial genetic erosion in the first generation
as it accommodates fewer parents and a smaller breeding population. In tree breeding
programmes there is a risk that methods suitable for large investment programmes are
often implemented with the intention of enhancing gain in small farmer holdings. A
robust low-intensity strategy is able to provide reasonable gain and keep inbreeding
in check in community orchards managed by poor farmers. In ideal situations (especially
for E. camaldulensis in arid locations) these orchards are quite attractive, as the
investment for planting stock production is low and gain obtained is comparable to
that of commercially available clones (Varghese et al., 2009a).

5. Evaluation of Orchard Dynamics for Domestication

Seedling seed orchards of eucalypts after evaluation and thinning, are expected to yield
adequate quantity of improved seed and enhance the productivity of plantations. Though
suitable provenances have been identified in provenance tests at various sites, very
little information is available on the fertility of natural provenances or the suitability of
sites for locating eucalypt seed orchards. Since fertility and tree growth may not be
correlated, fertility variation has to be assessed for predicting the genetic quality of seed
produced from seed orchards. Progeny originating from orchards of the same seed
origin could vary depending on the flowering status and the fertility variation between
trees. Excessive fertility of a few trees can lead to relatedness among progeny. Loss of
diversity occurs from increase in coancestry levels in the orchard as a result of variable
flowering among trees. The problem can be very acute if the number of flowering trees is
very low. Poor and irregular flowering is often observed in orchards that are not located
on good flowering sites, and even on good sites, micro-site influences are important
(Sweet, 1992). Genetic quality of seed crop from an orchard can be estimated based on its
predicted composition. The genetic value, relatedness and fertility of parental genotypes
strongly determine the genetic gain and diversity of progeny. Prediction of the gain and
diversity of progeny can be done based on fertility contribution and the number of seed
and pollen parents in the orchard (Kang et al., 2001b). The following parameters can be
used to evaluate the orchard dynamics and predict the quality of orchard progeny.

Sibling Coefficient ($\psi$), which is the probability that two genes originate from the
same parent, compared to a panmictic situation, can be used to quantify the fertility
differences between orchard genotypes. Sibling coefficient is calculated from the
number of fertile trees in the orchard \( (N) \) and individual fertility \( (p_i) \) of each tree (Kang et al., 2003).

\[
\psi = N \sum_{i=1}^{N} p_i^2
\]  \[1\]

**Effective Population Size \( (N_e) \) or Status Number** (Lindgren and Mullin, 1998) is used to characterize an orchard based on the number of unrelated trees that contribute equally to the gene pool. In an ideal situation when the trees in an orchard are assumed to be non-inbred and unrelated, status number is calculated as

\[
N_s = \frac{1}{\sum_{i=1}^{N} p_i^2}
\]  \[2\]

Where \( p_i \) is the contribution from individual genotype \( i \) to the gamete pool and \( N \) the total number of trees in the orchard.

For an ideal seed orchard where the fertility of the trees is uniform and there is no inbreeding or relatedness or migration, effective population size \( (N_e) \) of the seed orchard crop and total number of trees \( (N) \) is the same.

The relative population size \( (N_r) \) is used to compare the effective number of trees that contribute to random mating, with the actual number of trees in the orchard.

\[
N_r = \frac{N_s}{N}
\]  \[3\]

**Group Coancestry** \( (\Theta) \) is the probability that two genes taken at random from the gene pool of the expected seed orchard crop will be identical by descent. The group coancestry of the orchard trees becomes the expected inbreeding in the seed crop. Group coancestry can be regarded as a measure of gene diversity lost during tree breeding operations. When all the trees in the orchard are unrelated and non-inbred, group coancestry can be calculated according to Lindgren and Mullin (1998).

\[
\Theta = 0.5 \sum_{i=1}^{N} p_i^2
\]  \[4\]

Where \( p_i \) is the probability that genes sampled at random from the gamete pool originate from genotype \( i \).

The fecundity of each tree can be used as the probability of contribution of each genotype in determining the group coancestry \( (\Theta) \), which can be obtained by adding all possible pairings of gametes from orchard trees.
6. Impact of Fertility Variation in Orchards

6.1. Unpedigreed Orchards

Fertility status of unpedigreed orchards (SPA 1 to 4) of *E. camaldulensis* and *E. tereticornis* established at a dry site (Pudukkottai) in Tamil Nadu and a moist site (Panampally) in Kerala (Table 2, Fig. 1) was evaluated at four years of age (Kamalakannan, 2007). The two eucalypt species differed considerably in fertility status in the moist site. More than 73 per cent of *E. camaldulensis* trees were fertile at the moist site (SPA-2) in contrast to less than 25 per cent fertile trees in *E. tereticornis* (SPA-4). At the low rainfall site around 25 per cent trees were fertile in both species. The number of fruits produced per tree also followed a similar pattern. Fruit production was quite high (4,000-5,000 per tree) in *E. camaldulensis* at the high rainfall site whereas it was quite low (average 300 fruits per tree) in *E. tereticornis* (Kamalakannan et al., 2007b). In the dry location both species had the same fecundity (around 1,000 fruits per tree). Tree diameter had poor correlation with fruit production.

Fertility variation was low in the *E. camaldulensis* (SPA-2) at Panampally as indicated by the sibling coefficient value ($\psi = 2$) whereas it was unusually high for *E. tereticornis* in the same site ($\psi = 13$). In the dry Pudukkottai site both species had more or less similar fertility variation ($\psi = 7$-$8$). Even though there were almost three times more trees in the dry location, the effective population size in *E. camaldulensis* orchards was almost the same at both sites since almost 45 per cent of the trees contributed effectively to seed production in the moist site in comparison to a mere 15 per cent effective contribution at the other site. Both species had more or less the same effective population size at Pudukkottai (SPA-1 and SPA-3). Since many *E. tereticornis* trees did not flower at the moist site, there were only about 14 effectively contributing trees resulting in a low relative population size (7%) and high genetic drift. In spite of the fertility variation three of the orchards had low coancestry values and adequate predicted genetic diversity (except in the *E. tereticornis* orchard, SPA-4 at the moist site in Kerala).

<table>
<thead>
<tr>
<th>Trait</th>
<th><em>E. camaldulensis</em></th>
<th><em>E. tereticornis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>SPA-1</td>
<td>SPA-2</td>
<td>SPA-3</td>
</tr>
<tr>
<td>$N$</td>
<td>525</td>
<td>182</td>
</tr>
<tr>
<td>Fertile trees (%)</td>
<td>26</td>
<td>73</td>
</tr>
<tr>
<td>$\psi$</td>
<td>6.7</td>
<td>2.2</td>
</tr>
<tr>
<td>$N_r$</td>
<td>78</td>
<td>81</td>
</tr>
<tr>
<td>$N_s$</td>
<td>0.15</td>
<td>0.45</td>
</tr>
</tbody>
</table>

* $N$ - No. of trees; $\psi$ - Sibling coefficient; $N_r$ - Effective population size; $N_s$ - Relative population size
6.2. Pedigreed *E. tereticornis* Orchard

In family identified progeny trials fertility status assumes great importance. Fertility variation was investigated in a progeny trial of *E. tereticornis* in Tamil Nadu before thinning. Thirty-seven open pollinated families of *E. tereticornis* belonging to 13 provenances representing distinct geographical regions of Australia and Papua New Guinea (28 from Queensland and nine from Papua New Guinea) were evaluated for growth and fertility at four years of age. Four selected families and one routine seedlot of Mysore gum land race were used as control. Fertility was high in Mysore gum trees so that 12 per cent of the trees, all from the land race produced 52 per cent of the fruits before thinning (Fig. 2). Based on phenotypic selection thinning was done to retain 20 per cent superior trees in the seed orchard (SSO-1). After thinning the fecundity was more skewed as 12 most fertile trees of the land race contributed almost 81 per cent of the fruits (Varghese et al., 2003). Sibling coefficient was high ($\theta=17.4$). This causes a big shift in the really contributing trees in relation to the actual number of trees in the orchard. In effect many outstanding trees of the superior native provenances would not be represented in the next generation thus reducing gene diversity and gain considerably. The seed crop will have over-representation from more productive local trees.

6.3. Role of Fertility in Gene Diversity of Seed Crop

Fertility is an important trait for consideration in first generation introductions of natural provenances (Varghese et al., 2003). Poor fertility has been reported in

![Fig. 2. Fecundity variation in pedigreed *E. tereticornis* seed orchard.](image-url)
E. tereticornis stands in tropical moist environments (Arnold, 1996; Pinyopusarerk and Harwood, 2003a) in Asian countries. Fertility variation is expected to be lower in seed orchards than in seed stands (Kang et al., 2003) in a domesticated species or for a species in its native location. Kang et al. (2003) reported an average $\psi$ value of 2.62 for seed orchards of broad-leaved species. Generally sibling coefficient values may be high in young orchards and during poor flowering years (Kang et al., 2003). It is observed that the moist location at Panampally, Kerala receiving about 1,400 mm rainfall is suitable for locating E. camaldulensis seed orchards ($\psi = 2.2$) but not E. tereticornis ($\psi = 13.4$). This point has been confirmed by the near similar pattern of fruit production in two consecutive years (Kamalakannan et al., 2007b). Very poor flowering and seed production have been reported in locations receiving higher precipitation (>2,000 mm) in Philippines and Vietnam and are thus unsuitable for establishing E. camaldulensis orchards (Pinyopusarerk and Harwood, 2003b). Both species seem to have similar fecundity at dry Pudukkottai site.

If there are not many flowering trees in the orchards, there would be a low effective population size compared to the actual population size, which would lead to loss of diversity in seed crop (Varghese et al., 2004a). Thus fertility observations need to be recorded periodically to ensure adequate diversity and outcrossing in the seed crop. Genetic quality and diversity of progeny from high fertility orchards like E. camaldulensis at Panampally (SPA-2) is expected to be better than that of E. tereticornis orchard at the same site (SPA-4) with low effective population size and predicted gene diversity. The progeny originating from such low flowering orchards would suffer from inbreeding due to related mating. Land races with high fecundity tend to dominate the seed crop as seen in pedigreed orchard (SSO-1) of E. tereticornis and hence only a small proportion of the expected genetic resources will be captured in the next generation. Though the relative population size ($N_r$) in unpedigreed and pedigreed E. tereticornis orchards (SPA-4 and SSO-1) is similar, the composition of progeny would be very different since the expected inbreeding is higher in the pedigreed orchard due to high fecundity of many related trees of the local land race.

It is advisable to have large number of trees at sites where flowering is poor to maintain an acceptable effective population size. There is a strong negative relationship between sibling coefficient and fecundity of trees (Fig. 3). From a survey of several eucalypt seed orchards in Asian countries, Pinyopusarerk and Harwood (2003b) recommended that seed collection could be done in orchards where 50 per cent of the trees were fertile. When fertility is low, big orchards with large number of trees can be effective in ensuring diversity in the seed crop. High stocking will ensure a high effective population size as seen in E. camaldulensis orchard (SPA-1, 525 trees) at Pudukkottai, which is almost on par with the corresponding high fertility orchard at Panampally (SPA-2). Differences in fertility between trees could be genetic (Eriksson et al., 1973) or influenced by environmental factors (Hedegart, 1976) and
management of orchard (Zobel and Talbert, 1984). As seeds are often in practice collected only from a few good trees for plantation establishment, large orchards ensure sufficient outcrossed seed even in poor flowering years.

6.4. Outcrossing Rate in Seed Orchard
It is well known that the extent of inbreeding affects growth performance in *Eucalyptus*. Inbred individuals, especially selfed individuals, display inbreeding depression of growth (Eldridge *et al*., 1993). The rates of out crossing and inbreeding in seed orchards, therefore, have a crucial impact on the quality and performance of seed delivered to growers. Petford provenance is reported to have very high out crossing rate (mean multilocus outcrossing rate $t_m = 0.95$) in its natural range (Butcher and Williams, 2002). Hence seed from this provenance is known to perform well in trials across several diverse locations. It is important to use superior germplasm (high outcrossing rate) as starter material for a breeding programme to ensure sustained productivity with domestication. There are not many reports of outcrossing rates in seedling seed orchards of *E. camaldulensis*. Butcher and Williams (2002) presented evidence that selection against homozygotes may be operative in *E. camaldulensis*.

![Fig. 3. Relationship between sibling coefficient ($\psi$) and fruits/tree in eucalypt orchards (SPAs 1-4; fertility at 8 and 9 years of age).](image)

$R^2 = 0.7754$
Mating system was assessed in the high fertility orchard (SPA-2) at Panampally (Fig. 1). Outcrossing rate was estimated in 15 individual trees of SPA-2, located at different positions across the orchard, using allozymes following procedures of Moran and Bell (1983). Variation at each of the nine most variable allozyme loci in *E. camaldulensis* (*Aat-1, Aat-2, Aat-3, Idh-1, Gpi-2, Mdh-2, Pgd-1, Ugp-1, Ugp-2 and Ugp-3*), representing six enzyme systems, were scored for 20 progeny from each of the 15 parent trees. Multi-locus and single-locus outcrossing rates were estimated.

The multi-locus estimate of the out-crossing rate (in SPA-2) was 0.86. The mean single-locus estimate was 0.88. The multi-locus estimates of out-crossing rates for the 15 individual mother trees ranged from 0.41 to 1.00, with selfing detected in five of the 15 families. Higher outcrossing rates are usually expected in seed orchards due to the absence of neighbourhood inbreeding as closely related trees are not planted adjacent to each other (Moran *et al.*, 1989). However, there was not much difference between single- and multi-locus outcrossing estimates in the Panampally stand, indicating that inbreeding in the stand is primarily due to selfing rather than neighbourhood inbreeding. The multi-locus estimate of 86 per cent for the out-crossing rate is acceptable for operational seed production, but the selfing rate of 14 per cent is higher than desirable. Outcrossing was higher (95%) in progeny trials of *E. camaldulensis* in Thailand (Butcher and Williams, 2002), and *E. camaldulensis* seed orchards in north Queensland that incorporated working beehives (94%) (Moncur *et al.*, 1995).

Differences in flowering time among trees in the Panampally stand may account for the low outcrossing rates of some of the sampled trees. Trees which flower out of synchrony with the others in the stand (early or late flowerers) would be less likely to receive outcross pollen. A further point of interest arising from the allozyme study in SPA-2 was the revelation that despite 73 per cent of the trees being fertile, 22 per cent of offspring were full-sibs, indicating that some or all of the 15 open pollinated seed families sampled in this study received pollen from relatively few pollen parents (Varghese *et al.*, 2009a). Since the orchard originated from a seed mix of 514 trees of 14 natural provenances (Doran *et al.*, 1996), it is necessary to examine the timing of flowering in different trees to detect the extent of asynchrony in flowering. A similar study on a pedigreed *E. camaldulensis* orchard of similar origin in Thailand revealed very high outcrossing rate ($t_m = 0.95$) and nearly all progeny grown from that seed orchard were derived from cross-fertilisation (Butcher and Williams, 2002). The quality of seed produced by the Panampally seed production area could be enhanced by monitoring the phenology of individual trees and avoiding collecting seed from trees that flower out of synchrony with the others. Positioning honey bee (*Apis mellifera*) hives near the stand during peak flowering times can also be considered. A significant increase in seed yield and quality ($t_m = 0.75$ no bees to $t_m = 0.94$ with bees) was observed when hives were placed in natural stands of *E. camaldulensis* in north Queensland (Moncur *et al.*, 1995).
Estimates of outcrossing rates for individual trees can be quite variable as reported by Jones et al. (2008) in *E. grandis* ($t_m=0.64-1.00$) where some seedlots showed complete outcrossing with no self-pollination events. Campinhos et al. (1998) reported variation in individual outcrossing rates ($t_m=0.33-0.99$) among genetically identical clones of *E. grandis*, that was entirely due to environmental influences. Another study of *E. grandis* in natural populations showed a large variation in outcrossing rates, with $t_m$ values ranging from 0.34 to 1.48 for individual trees (Burgess et al., 1996). Variation in individual outcrossing rates in natural populations has also been reported in other *Eucalyptus* species (Griffin et al., 1987; Peters et al., 1990). The variation could be due to pollinator density (Moncur et al. 1995), age of trees (Moran and Brown, 1980) or position in the tree canopy (Patterson et al., 2001). The time of flowering during a season may also affect individual tree outcrossing rates, as trees flowering at the beginning and end of a season may have less opportunity for outcrossing (Fripp et al., 1987). In *E. grandis* Jones et al. (2008) reported an average pollen dispersal distance of 57.96 m, with the longest pollination distance being 192 m and 51 per cent of pollen within the seed orchard travelled less than 50 m, and 77 per cent less than 100 m. Despite the variation in maximum pollen dispersal distances in eucalypt species, the majority of pollen is dispersed locally with fewer occurrences of long-distance dispersal (Potts and Wiltshire, 1997). Strategies may be developed and implemented to reduce the potential for gene flow from plantations. These may include use of buffer zones and guard rows of non-compatible trees to catch the majority of dispersed pollen. Isolation from other compatible trees has proved to be effective in reducing pollen contamination in seed production areas. Buffer zones of native rainforest were used to isolate *E. grandis* and *E. urophylla* seed orchards from production plantations in Brazil. Isolation distances of 400 m (Campinhos et al., 1998) and 800 m (Junghans et al., 1998) resulted in pollen contamination rates of 14.2 and 2.8 per cent, respectively. Other methods of reducing the level of gene flow by pollen include manipulation of phenological patterns and collecting seed when orchard trees flower asynchronously with other surrounding compatible plantation trees.

7. Genetic Gain and Diversity of Orchard Seedlots
The justification for any tree-breeding programme is the genetic gain actually realised in improved plantations and the financial return on the investment in breeding (Eldridge et al., 1993). The gain is not limited to wood volume as a result of improved growth rate but also covers a range of other traits including stem and branch form, wood quality, and resistance to pests and diseases. For an estimate of realised gain, it is necessary to establish comparative trials to determine whether and by how much the plantations have been improved.
To evaluate the gain obtained from orchards, seed was collected from 25 trees of each orchard in five orchards (SPA 1 to 4 and SSO-1) at four years of age. Equal quantity of seed from each mother tree was combined to give five orchard bulk seedlots that were tested in genetic gain trials at three locations in southern India (Table 3, Fig. 1). A single bulked seedlot of three Australian provenances of *E. camaldulensis* (Morehead River, Laura River, and Kennedy River) was included as a control in each trial. A commercial clone and ‘Mysore gum’ seedlot were also used as control (Varghese et al., 2009a). Seedlots from the four unpedigreed orchards (SPA 1 to 4) did not differ substantially in growth and survival in any of the trials at three years, whereas the *E. tereticornis* pedigreed orchard (SSO-1) had significantly lower survival than the SPA seedlots and controls. Survival of this seed lot was at least 14 per cent lower than that of the other orchards at two test sites, and at least 50 per cent lower at the third site (Varghese et al. 2009a). The local Mysore gum seedlot had lower growth than the progeny of SSO-1, but it had excellent survival (89%), so its predicted volume production on a per hectare basis was much better than that of SSO-1. The natural bulk seedlot comprising Kennedy, Laura and Morehead River provenances of *E. camaldulensis* displayed very similar growth and survival as those of the SPAs at the three trial sites. The commercial clones had high survival but were only slightly higher in productivity than the natural-provenance seedlots. Though the SPA seedlots did not show much genetic improvement compared to the natural provenance, their growth and survival is satisfactory for operational planting in southern India with similar growth as the best commercially planted clones and substantially greater volume per hectare than Mysore gum. The pedigreed *E. tereticornis* orchard (SSO-1) had poor survival and growth compared to the SPAs since the pollen pool was dominated by the local land race. The growth rates of SPA seedlots achieved in the three performance trials are very similar to those obtained in tests of candidate clones at Karunya reported by Varghese et al. (2008a).

Table 3. Diversity and fecundity of orchard progeny (3 years) in genetic gain trials

<table>
<thead>
<tr>
<th>Seedlot</th>
<th>Polymorphism (%)</th>
<th>Fruits/Tree</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Kulwally (site 1)</td>
</tr>
<tr>
<td>SPA-1</td>
<td>30.7</td>
<td>244</td>
</tr>
<tr>
<td>SPA-2</td>
<td>29.8</td>
<td>256</td>
</tr>
<tr>
<td>SPA-3</td>
<td>28.1</td>
<td>246</td>
</tr>
<tr>
<td>SPA-4</td>
<td>-</td>
<td>72</td>
</tr>
<tr>
<td>SSO-1</td>
<td>26.2</td>
<td>249</td>
</tr>
<tr>
<td>Native seedlot</td>
<td>17.8</td>
<td>27</td>
</tr>
<tr>
<td>Local seedlot</td>
<td>18.8</td>
<td>-</td>
</tr>
<tr>
<td>Clone</td>
<td>-</td>
<td>16</td>
</tr>
</tbody>
</table>
This shows that seed collections from unpedigreed SPAs provide a low-cost option for mass production of superior eucalypt planting stock which is now quite popular among farmers in southern India.

Despite no significant difference between SPA seedlots in overall growth, fertility variation in parent orchards had an impact on variability in growth and fertility of progeny. Variability in tree height, measured in terms of coefficient of variation (C.V.), was lowest in SPA-2 and highest in SSO-1 at all the test sites (Varghese, 2009). There was a significant positive correlation between fertility variation in the orchards (sibling coefficient) and variability in progeny (C.V. for height) at all the test locations (Fig. 4). At three years, the fecundity of orchard progeny (in genetic gain trials) was comparatively higher in one location (Kulwally, 750 mm rainfall) compared to the other two test sites (Table 3) which highlights the need for site selection in locating new orchards. In this location fruit production per tree was significantly higher (~250) in progenies of three SPAs and SSO-1. It was comparatively low in *E. tereticornis* SPA-4 (72) and quite low in the natural provenance seedlot (27) and the clone (16). SPA seedlots had low fertility at Karunya (1,000 mm rainfall) but the local land race had high fecundity (358) followed by that of SSO-1 (104). At the third site, Bhadravati (800 mm rainfall) fecundity was low at three years but by five years of age, the fecundity increased significantly in three of the orchard seedlots (SPA1-3) and followed a similar trend as that in Kulwally. The clone, natural provenance seedlots and the progeny of SPA-4 had low fecundity in all the test locations. One generation of domestication in the seed orchards increased the fertility of the orchard progeny, relative to the natural provenances, as shown by fruit counts in the genetic gain trials. It appears that precocious flowering is a highly heritable trait, and it has responded to selection in the first-generation SPAs, as reported in *E. globulus* (Chambers et al., 1997). Fecundity will tend to increase in advanced generations but the status number (effective population size) would decrease if genetic erosion is not addressed.

First generation plantations or orchards are thus the critical entry points to a newly introduced location, where rapid loss of alleles can occur (Varghese et al., 2003). When assembling seed collections from superior trees for advanced generation breeding, the relatively low levels of flowering observed in the first generation orchards even at nine years (Kamalakannan et al., 2007b) will substantially reduce the genetic diversity passed on to the progeny. Large orchards located at sites conducive to heavy flowering, would be required to capture most of the newly introduced alleles in open-pollinated breeding populations (Zobel et al., 1988). In *E. nitens*, Swain et al. (2013) reported improved performance of progeny from higher fertility orchards (>40% flowering trees) than low flowering orchards (<20%). After three successive generations of breeding Verryn et al. (2009) reported 14 per cent improvement in tree volume with each generation in
E. grandis, in line with the classical breeding assumptions. Gain obtained will also depend on the planting strategy. Gain may vary when clones are planted in monoclonal blocks, where there is competition between individuals of the same genotype, compared to mixed planting of different clones (Stanger et al., 2011). The actual gain has to be evaluated at an appropriate scale rather than in small tree plots to avoid wrong predictions while evaluating large number of entries (Callister et al., 2013).

7.1. Molecular Diversity of Orchard Crop
In recent years molecular markers have been widely used in genetic studies of tree populations (Newton et al., 2002; Peng et al., 2003). Generally seed orchard populations often show a high percentage of polymorphic loci and alleles per locus (Chaix et al., 2003). The percentage of polymorphic loci (Kimura and Crow, 1964) in seed orchard crops was estimated and principal component analysis carried out using ISSR molecular markers (Kamalakannan et al., 2009) to evaluate the extent of genetic variation transmitted from seed orchards to the next generation crop of new recombinants.

Principal coordinates analysis (Fig. 5) revealed separate clustering of E. camaldulensis and E. tereticornis seedlots and separation between the orchard seedlots indicating differences in genetic composition of seed crops (Kamalakannan et al., 2009). The local seedlot clustered midway between the two species indicating a mix of genes of both species. The natural population grouped separately from the orchard crops indicating that mating between different provenances within the orchard has resulted in new combinations in the seed crop. Though the SPAs originated from the same parent seedlots, fertility variation has resulted in seed crops that differ in genetic composition but still maintain adequate diversity and gain for establishing future plantations compared to the local seedlot.

8. Management to Modify Fertility Status of Orchards
Fertility plays a very important role in converting the genetic value of the selected trees to gain in the next generation. From a diversity stand point it is better to have a fertile tree of slightly lower genetic value than to have a superior tree that does not contribute to seed production. Unequal fertility can also cause deviation in the mating pattern. Thus the major concern would be to have random mating of maximum number of trees of known genetic potential. This would be especially true in a first generation seedling seed orchard where the species is initiated into domestication process.

Application of flowering promoter chemical Paclobutrazol™ as soil drench is effective in increasing fecundity and the number of flowering trees in E. camaldulensis and E. tereticornis orchards. The percentage of fertile trees increased three to four times with its application, but the impact gradually reduced
Fig. 4. Relationship between sibling coefficient (fertility variation) in seed orchards and variability (tree height) in progeny.

Fig. 5. Grouping of four orchard seedlots and control (native and local seedlot) using principal component analysis.
(though significantly higher than control) in four years. Capsule production was higher in *E. camaldulensis* (two times) and *E. tereticornis* (five times) than control even after three years of application (Varghese et al., 2009b).

### 8.1. Constrained Seed Collection and Mixing of Seeds

Two management strategies that can be used to reduce the impact of fertility variation on diversity of seed crop are constrained seed collection and mixing seed crops from two consecutive years. Constrained seed collection (selective harvest) can be done by restricting number of fruits from each parent tree (Bila, 2000; Kang et al., 2003; Varghese et al., 2006b). Equal seed collection helps to have uniform female contribution and remove impact of excessive fertility of few individual trees. Increasing the number of male parents enhances the effective number (Lindgren and El-Kassaby, 1989), which, however, lowers the genetic value of the crop, as the additional male parents selected are genetically inferior to the trees selected for merit. A combination of the two strategies would serve to achieve both objectives as seed collection is limited to the superior trees and higher diversity levels are maintained by contribution from males. This strategy ensures reduced drift and inbreeding in advanced generations.

Mixing seeds from two consecutive harvests helps to enhance the overall fertility and cumulative contribution in the seed crop than that of either harvest (Fig. 6). The status number, relative population size and gene diversity can be enhanced with this strategy in *E. camaldulensis* and *E. tereticornis* SPAs (Kamalakannan and Varghese, 2008). Mixing seeds harvested at eight and nine years of age helped to reduce the sibling coefficient to 2.21 from the first and second year values of 2.24 and 3.19 in *E. camaldulensis* orchard (SPA-2) at Panampally. As a result the relative contribution of trees increased from 31 per cent to 45 per cent. Mixing two consecutive seed crops in the low flowering *E. tereticornis* orchard (SPA-4) at the same site reduced the sibling coefficient by 44 per cent and increased relative population size by 57 per cent. At the arid Pudukkottai site, mixing two seed crops reduced the sibling coefficient by 36 per cent and 50 per cent and enhanced the status number by 56 per cent and 95 per cent in *E. camaldulensis* and *E. tereticornis*, respectively. Constrained seed collection would substantially reduce sibling coefficient and enhance status number in the orchards.

Selective harvest and genetic thinning and a combination of both are orchard management options that can be used to increase genetic gain while maintaining genetic diversity in seed orchards (Lindgren and El-Kassaby, 1989). The practice of selective harvest improves only the genetic contribution of seed parents, while both seed and pollen parents are improved with genetic thinning. When fertility variation is very high in orchards, genetic thinning should be done only after fertility evaluation of the trees. Constrained seed collection is very effective in reducing the impact of fertility variation but is often not very feasible when large quantity of seed is required.
Mixing seed crops from different harvests is an easy option which does not require much technical inputs for implementation. Seed orchard trees contribute more equally to the seed crop if seed crops from consecutive years are mixed. This strategy would be very effective in species where the trees show alternate bearing.

Fig. 6. Cumulative contribution in *E. camaldulensis* (SPA-2) and *E. tereticornis* (SPA-4) orchards.
tendency (Varghese et al., 2008b). Since the relative contribution of trees varies between years, the composition of seed crops vary and gene diversity of the seed mix would be equal to or more than that of the best crop. This strategy will be very beneficial in domesticating a newly introduced exotic species as in the case of *E. tereticornis* in tropical humid regions (Varghese et al., 2003). A similar observation was made by Kang et al. (2005) in seed orchards of *Pinus thunbergii* where fertility variation for the combined seed crops was lower than that observed for any single year, implying that the genetic diversity of seed crop would increase if seeds collected from different years are pooled. Diversity of seed from orchards of the same origin at different locations could vary, since the fertility of trees vary between locations as seen in the case of *E. tereticornis* orchards (SPA 3 and 4). Mixing seed from different orchards would, however, affect the performance at either site as the seed from an orchard is best suited to a similar site.

9. Implication of Diversity in Eucalypt Domestication

Modification of a species to suit an exotic site involves adaptability to survive and perform well in the new location. The problems are acute in first generation introductions and suitable strategies must be employed to reduce genetic erosion. Lack of awareness of this factor may result in unnoticed erosion in genetic base of plantations. Balancing gain and genetic diversity is important in eucalypts because of the fast turnover resulting from short rotations. A seedling orchard provides opportunities for correcting fertility problems and producing seed for different requirements. Effective number of contributing trees may increase with generations as seeds are collected from fertile trees, but the status number may decrease as inbreeding increases. The effective number of mothers can intentionally be forced to become more similar to the real number by extracting seeds tree wise and discarding excess seeds from mothers contributing most, or the prolific seed bearers may not be completely harvested. This principle of equal seed contribution is widely used in establishing what are known as extensive seedling seed orchards (Nikles et al., 1984; Varghese et al., 2000a).

Great emphasis is placed on gain and hence clonal plantations are becoming extremely popular (Lal et al., 1993). Though seed still remains the major planting material in India, clonal option (which is often a short-term strategy in most breeding programmes) has gained priority that massive investment is made on establishing propagation systems for mass multiplication and deployment. A few clones are (based on preference of farmers) are often planted over extensive areas which can reduce the genetic diversity of the planting stock substantially. Low diversity can result in outbreak of pest and disease epidemics as seen recently when some popular clones were devastated by gall wasp infestation (Jacob, 2010). In a time bound breeding programme the top performing progenies can be cloned at the end of each
generation of breeding. When a large number of vigorous progenies are cloned, they can be tested at different locations for deployment as clones and for developing clonal seed orchards for increased gain. It is desirable to have large breeding populations of families and pedigree identified clones in each generation. The breeding populations then get converted to seedling and clonal seed orchards that provide fair diversity in deployed seed, enhanced gain in the form of clones, and information on progeny performance of parents in the previous generation.

9.1. Deployment of Clones for Enhancing Gain

The currently available commercial eucalypt clones originated as selections from a narrow base like provenance trials or from local Mysore gum plantations (Kulkarni, 2005). Progeny trials and unpedigreed plantations of native seedlots offer an opportunity to select phenotypically outstanding trees of wide genetic base and tap early benefit from a breeding programme by deploying first generation clones of known origin and diversity. Top trees identified in family trials and SPAs (refer Section 2, Table 1) were cloned based on phenotypic superiority. As many as 78 clones of \(E. \text{camaldulensis}\) and 27 clones of \(E. \text{tereticornis}\) were vegetatively propagated from basal coppice of the selected ortets and tested at three diverse locations differing in annual precipitation in southern India. There was significant site-by-clone interaction that only about 10 per cent clones were superior to natural Kennedy River provenance seedlot at one site whereas 96 per cent of the clones outperformed the control seedlot at another site (Varghese et al., 2008a). The clone trials can be thinned to retain the top performing clones at each site for conversion to clonal seed orchards. Selections from orchard progeny at different locations can be cloned and evaluated for suitability to each site. Seed orchards can thus be the base for regular infusion of new clones to specific sites. The gains from deploying the best 10 per cent of clones (from each trial) after evaluation was seen to vary (25-109%) depending on the suitability of the clone to the site (Varghese et al., 2008a).

Clones popular in low rainfall regions when tested in a high rainfall location in Kerala were inferior to natural Kennedy River seedlot, which clearly showed that it was necessary to first introduce a seedlot and select clones rather than shift clones across contrasting sites (Harwood, 1999).

Clonal testing is suggested to achieve the greatest benefit in long-term breeding of eucalypt species (Danusevicius and Lindgren, 2003). The gains tapped from clonal selection can be used to achieve enhanced gain in subsequent generations through clonal orchards. It is desirable to have representation from large number of clones as it offers more flexibility in using selective harvesting; to increase the effect of later thinnings; to get a more efficient overlap in phenology and to decrease the amount of selfing. Lindgren et al. (1989) developed linear deployment methods for balancing the options for gain with that for gene diversity by deploying unequal
number of clones. This strategy helps to obtain as much gain as possible by deploying clones with high breeding value, while maintaining desirable levels of diversity with representation from clones with moderate breeding value in low proportion, to maintain diversity in an orchard.

A study was done to optimise thinning in clone evaluation trials of *E. camaldulensis* by using variable thinning intensity in different clones for conversion to clonal seed orchards (Ravi, 2008). Since these clones were intensively selected from large number of entries in first generation seedling seed orchards, they offer a short term strategy, an opportunity for substantial improvement after a generation of testing in breeding populations. Linear deployment ensures that the number of ramets retained per clone is linearly related to the breeding value (Lindgren and Matheson, 1986) of the clone. The average gain obtained from the clone is the product of the breeding value and number of copies of the clone deployed. There has to be a balance in the number of ramets that can be included beyond which the benefit obtained is reduced resulting from inbreeding and loss of diversity. The easiest thinning option would be to eliminate the low ranking clones completely with truncation selection which also maximises the genetic gain. It is, however, not suitable for the first thinning in an orchard as it reduces the options for further thinning to address fertility issues. This calls for a balanced selection between maximum gain by selecting very few best clones and the need for diversity by including representations from more entries using linear deployment strategies. The essential difference between the thinning strategies is the cut off limit, the intercept, which decides the lowest value of clones to be selected. When the intercept is chosen, the slope of the selection line decides the number of ramets in the lower ranking clones resulting in a smooth line with less skew in distribution of the selected ramets.

Thinning strategies were evaluated at three years in clone trials of 87 clones with 15 ramets each. Linear thinning when compared with truncation selection (removing all clones below the trial mean for tree height), would give greater gain and diversity. Since the primary objective of managing a seed orchard is to enhance gain, linear thinning is preferred since it addresses both gain and diversity concerns at the same time. Simple mass selection based on phenotypic value is also an effective method of thinning clone trials as it ensures adequate representation of clones and moderate gain. Phenotypic selection gives higher representation of clones than truncation selection but linear thinning is the ideal strategy for maximum gain and adequate diversity (Varghese et al., 2006a; Ravi, 2008). In advanced generations Kang et al. (2001a) recommended an effective clone number of at least 10 with an equal number of ramets per clone.

10. Domesticating Eucalypts for Sustained Productivity

Though enhancing gain is the primary objective of any improvement programme, a broad genetic base would be necessary for initiating a domestication programme. Low variability and high adaptability of outstanding provenances of *E. camaldulensis*
Kennedy, Laura and Morehead Rivers and Petford to several test sites indicates adequate outcrossing and gene flow in the native stands. These natural seedlots, which are the starting material for domestication, may, however, have very poor fecundity in newly introduced sites (Varghese et al., 2009a). Thus extreme care has to be taken to prevent rapid gene loss, at the first level of domestication, in a newly introduced site (Varghese et al., 2003).

We have seen in earlier sections of this chapter that despite high fertility variation ($\psi = 7-8$) and poor flowering (30% fertile trees) in $E.\ camaldulensis$ and $E.\ tereticornis$ orchards in an arid location, the effective population size was comparable to that in a moist location which had high fertility (73% fertile trees), and the mean growth rate of the orchard seedlots also did not differ significantly. This is because, despite low flowering, mating between genetically divergent provenances gives rise to inter-provenance hybrids, which is stable across generations (due to a complementary effect), and does not breakdown in subsequent generations (Nikles and Griffin, 1992). But a high relative proportion of genes will be carried forward to the next generation only in the high flowering orchards. There would be related mating happening in next generation among progeny of low flowering orchards. Orchards with higher proportion of fertile trees and relative population size will have offspring with higher diversity, growth and survival compared to orchards with lower fertility as reported in $Eucalyptus\ regnans$ (Eldridge and Griffin, 1983).

Breeding populations for high and low rainfall sites will have different genotypes selected for adaptability, and should be separately maintained after evaluation of first generation introductions at the respective locations (Varghese et al., 2008a). Fertility is to be monitored before seed collection to ascertain the coancestry levels and effective population size in high rainfall locations as recommended by Pinyopusarerk and Harwood (2003a). Breeding orchards and production orchards need to be clearly identified based on the diversity of the seed crop. A production orchard can be stocked with the best or a few outstanding provenances to maximize gain. However, for sustained productivity a breeding orchard should have a broad diversity to carry forward a high proportion of unrelated genes. Keeping sibling coefficient levels below three would ensure acceptable effective population sizes in seed stands (Kang et al., 2003). For a successful domestication program, seed orchards should have acceptable levels of fertility variation and effective population size. The orchards should be evaluated for predicted inbreeding and molecular diversity of the seed crop; and the realised genetic gain relative to currently planted controls (Varghese et al., 2009a), before seeds are released for commercial planting.

11. Designing Seed Orchards Using Molecular Markers

In long-term breeding programme, the intensity of selection applied over breeding populations restricts the number of genotypes retained in the final orchard, thereby
decreasing genetic diversity and increasing the risk of inbreeding depression over successive generations. Although molecular markers can reveal genetic diversity, they are not fully exploited to assess genomic diversity in traditional tree breeding programme (Glenn and Chaparro, 1996; Gaiotto and Grattapaglia, 1997; Grattapaglia, 2000; Marcucci Poltri et al., 2003). In open-pollinated eucalypt seed orchards male parentage is assumed to be random and derived from outcrossing. As a result, the male parentage contribution within and among the different families is unknown and relationships among the progeny (half-sib or full-sib) are unknown. Molecular markers allow accurate estimation of genetic distance and genetic diversity.

Zelener et al. (2005) proposed a selection strategy using genetic diversity information measured at the DNA level in individuals pre-selected based on their fitness. This strategy helps to decide whether to increase genetic gain (with loss of genetic diversity), or to preserve a high genetic diversity (at the expense of genetic gain), or to combine both scenarios. Individuals carrying unique allele variants would be preserved in the seedling seed orchard and genotypes are separated into different populations based on similarity indices. It is ensured that all alleles in the parent population are retained in the seed orchard. This methodology for orchard design emphasizes individual and family selection, because the largest proportion (about 80 per cent) of the total molecular variation in the population is found in individuals within families, with a moderate proportion (15-18%) of the total genetic variation among families within provenances. Thus, almost 90 per cent of the families will be represented in the selected seed orchard in different proportions. Mating pattern, including pollen dispersal and gene flow within and between populations, will be managed to maintain the genetic structure of seed crop (Levin and Kerster, 1974), which in turn will influence the breeding population size and seed orchard design.

Differential family performance in open-pollinated progeny trials can be verified for possible inbreeding depression resulting from selfing or mating between relatives (Matheson and Mullin, 1987; Hodge et al., 1996) before conversion to seed orchards. Direct comparison of growth rates of self-pollinated and outcrossed eucalypts have demonstrated negative effects of inbreeding on seed viability and growth (Hodgson, 1976; Griffin and Cotterill, 1988; Hardner and Potts, 1995). A positive association between outcrossing rate and growth has also been reported in *Eucalyptus grandis* using families selected on the basis of different levels of prior inbreeding (Burgess et al., 1996). Differences in growth among families from the Petford region in provenance/progeny trials in Thailand (Pinyopusarerk et al., 1996) were found to be associated with differences in outcrossing rate (Butcher and Williams, 2002). Based on the superior growth performance of populations of *E. camaldulensis* occurring between 14°S and 18°S latitudes and 143°E and 145°E in north-east Queensland, the Petford region has become one of the most important seed sources for plantations in the wet/dry tropics (Midgley et al., 1989; Doran and Burgess, 1993). Thus the major challenge in
domestication is to first eliminate inbred individuals from the introduced population and ensure that high outcrossing is maintained among the retained genotypes in breeding orchard. Molecular information on diversity and genetic distance helps to improve the genetic base in breeding programme. Molecular markers can be used to evaluate redundancy or deficiency in germplasm collections. Based on the levels of heterozygosity the genetic base of a breeding population can be improved by introducing material with high genetic variability from natural populations. Leite et al. (2002) evaluated genetic variability in a base population of *E. urophylla* before initiating a breeding programme in Brazil. Muro-Abad et al. (2001) used RAPD markers as a tool to direct the hybridisation programmes in Brazil by choosing the appropriate genotypes based on genetic distances for crossing clones of *E. grandis* and *E. urophylla* and markers can be used to analyze the progeny of interspecific crosses (between *E. grandis* and *E. urophylla*) to develop stable and productive genotypes and maintain genetic diversity during selection.

References
Campinhos, E.N.; Peters-Robinson, I.; Bertolucci, F.L. and Alfenas, A.C. 1998. Interspecific hybridization and inbreeding effect in seed from a *Eucalyptus*
grandis x E. urophylla clonal orchard in Brazil. Genetics and Molecular Biology, 21(3): 369-374.


Doran, J.C.; Pinyopusarerk, K.; Arnold, R and Harwood, C.E. 1996. Breeding plan for Eucalyptus camaldulensis in Tamil Nadu. UNDP/FAO Regional Project on


Domestication strategies for eucalypts in India


