LINEAR THINNING IN A CLONAL TEST OF *EUCALYPTUS CAMALDULENSIS* FOR CONVERSION TO A CLONAL SEED ORCHARD

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VARGHESE, M., LINDGREN, D. & RAVI, N. 2006. Linear thinning in a clonal test of *Eucalyptus camaldulensis* for conversion to a clonal seed orchard. In this study, we used linear deployment technique to carry out genetic thinning of clones and ramets in a three-year-old clonal test of *Eucalyptus camaldulensis* for converting it to a seed orchard. Four different strategies, namely, truncation selection and three linear thinning options, were employed to have a balanced representation of clones in the orchard. Three linear strategies were considered, namely, to retain the same ramet number, to keep the gain same or to keep the diversity same as with truncation selection. The gain and diversity that can be achieved with the different options were studied using suitable intercept and slope values for the linear deployment line. Linear deployment retaining the same ramet number was efficient in conserving diversity compared with the second strategy of keeping genetic gain the same as truncation selection. The third strategy which provided maximum gain at the same diversity as truncation selection was suggested to be the most balanced method of thinning the orchard as it provided high gain and reasonable diversity and adequate options for further thinning based on later observations including fertility. Simple mass selection for height growth was as effective as the second linear strategy in terms of both genetic gain and diversity.

Keywords: Linear deployment, truncation selection, genetic thinning, genetic gain, diversity, mass selection

VARGHESE, M., LINDGREN, D. & RAVI, N. 2006. Penjarangan linear dalam ujian klon *Eucalyptus camaldulensis* untuk penukaran kepada kebun biji benih klon. Dalam kajian ini kami menggunakan teknik pengerahan linear bagi menjalankan penjarangan genetik klon dan ramet dalam ujian klon *Eucalyptus camaldulensis* yang berusia tiga tahun. Tujuannya adalah untuk menukarnya kepada kebun biji benih. Empat strategi berlainan, iaitu pilihan pemangkasan dan tiga pilihan penjarangan linear, dilaksanakan untuk mendapatkan gambaran seimbang klon di dalam kebun. Tiga strategi linear dipertimbangkan iaitu untuk mengekalkan bilangan ramet yang sama, untuk mengekalkan keuntungan yang sama, dan yang akhirnya untuk mengekalkan kepelbagaian sama seperti pilihan pemangkasan. Keuntungan dan kepelbagaian yang boleh dicapai menggunakan pilihan berlainan dikaji. Ini dilakukan menggunakan pintasan dan cerun yang sesuai bagi garis pengerahan linear. Pengerahan linear yang mengekalkan bilangan ramet yang sama didapati efisien dalam memulihara kepelbagaian berbanding dengan strategi kedua iaitu yang mengekalkan keuntungan genetik agar sama dengan pilihan pemangkasan. Strategi ketiga yang memberi keuntungan maksimum pada kepelbagaian yang sama dengan pilihan pemangkasan dicadangkan sebagai cara terbaik bagi penjarangan kebun. Ini kerana strategi ini memberi keuntungan dan kepelbagaian yang tinggi serta pilihan yang mencukupi bagi penjarangan selanjutnya berdasarkan pemerhatian kemudian, termasuk kesuburan. Pilihan pukal ringkas untuk pertumbuhan ketinggian adalah sama efisiennya dengan strategi linear kedua dari segi keuntungan genetik dan kepelbagaian.

INTRODUCTION

A genetic improvement programme for *Eucalyptus camaldulensis* was initiated in India by systematically introducing germplasm of a wide genetic base from selected natural provenances in Australia. (Doran *et al.* 1996). Both long-term and short-term strategies have been employed for improving yield in plantations. Clonal testing was suggested to achieve the greatest benefit in

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long-term breeding of *Eucalyptus* species (Danusevičius & Lindgren 2003). Clonal selections from breeding populations can be used to achieve improvement in subsequent generations.

Clonal seed orchards, after thinning based on clonal performance, are predicted to offer considerable gains even at low number of ramets and weak heritability (Rosvall *et al.* 1998, Shelbourne 1992). Breeders are faced with a need to choose between high gain from intensive selection and to sustain reasonable diversity. Methods to balance the need for gain and gene diversity was developed by deploying unequal numbers of ramets per clone (Lindgren & Matheson 1986, Lindgren *et al.* 1989). The objective to obtain as much gain as possible could be achieved by deploying clones with high breeding value while maintaining desirable levels of diversity. Compared with truncation selection, it is often favourable to deploy larger number of clones with moderate breeding value in low proportion to maintain diversity. Linear deployment thinning has been suggested to provide a solution based on a comparison with truncation selection at constant stocking (Bondesson & Lindgren 1993).

The aim of this study was to evaluate genetic gain and diversity under different thinning options (desired stocking, desired effective number and desired gain) and optimize roguing scheme in a clonal trial of *E. camaldulensis* when the trial was converted to a clonal seed orchard.

MATERIALS AND METHODS

Seed orchard and data collection

A three-year-old clonal test of *E. camaldulensis* established at Coimbatore (11° 00' N, 76° 58' E, 400 m altitude, 900 mm rainfall) in southern India was used for the study. The clonal trial comprised 87 clones which were selected from seven seedling seed orchards and commercially available clones. The trial was converted to a clonal seed orchard after assessment. The trial was established with 15 ramets of each clone at a spacing of 3 × 2 m in three plots with five replications in a resolvable incomplete block alpha design (Williams & Talbot 1993) with a row column structure for providing incomplete blocking within replicates.

The heights of the trees in the plots were measured and the mean height for each clone was used as parameter for thinning options. There was an initial stocking of 1157 trees with an average of 13 ramets per clone. The mean heights of the clones varied from 4.8 to 8.4 m with an overall mean value of 7.08 m. Mean value of a trait (e.g. height) estimated from several genetic copies of clones gives fairly precise information on the genetic value (Shelbourne 1992), which is a rather good estimate of the breeding value (Namkoong 1981).

Theoretical background

Gain estimation

The merit of a clone is assumed to be linearly dependent on its breeding value. The average gain obtained from a clone is the product of the breeding value and number of copies of the clone deployed. There is, however, a number of ramets that can be included beyond which the benefit obtained is reduced as a result of inbreeding and loss of diversity. Genetic thinning can be made optimal using linear deployment technique in the sense that at a given effective number, gain is maximized if ramet number is proportional to breeding value. In this study, the genetic value of clones was determined from clonal averages observed on the trial site itself.

The average breeding value (G) obtained from different clones weighted with their occurrence can be expressed as (Lindgren & Matheson 1986)

$$G = \sum r_i g_i \,/\, \sum r_i \qquad (1)$$

where
g_i = breeding value of clone i
r_i = ramets of clone i after thinning

Diversity estimation

The effective number of clones (N_c) in the seed orchard crop is defined as (Kang *et al.* 2001)

$$N_c = \frac{\left[\sum p_i\right]^2}{\sum \left[p_i\right]^2} \qquad (2)$$

where
p_i = the proportion of ramets of each clone.

N is influenced by the total number of clones (N) as well as the number of ramets of each clone. For the same number of clones, N_c is inversely proportional to the variance in the number of ramets. The effective number of clones will be highest for the unrogued orchard when all clones have equal number of ramets. The removal of genetically inferior ramets or clones increases the gain but lowers the effective number of clones. Definition (2) corresponds to status number of the seed orchard crop when there is no fertility variation among clones and the clones are unrelated and random mating (Kjaer 1996, Lindgren & Mullin 1998).

The relative effective clone number (N_r) was computed to compare the effective number of clones with the actual number of clones retained in the orchard (Kang *et al.* 2001)

$$N_r = \frac{N_c}{N} \qquad (3)$$

Genetic thinning options

Truncation selection and different options for thinning the clonal trial were compared using an Excel workbook (http://www.genfys.slu.se/staff/dagl). The four approaches to thin ramets of different clones in a conventional clonal trial to convert it to a seed orchard were applied and compared as follows:

(1) truncation selection
(2) linear thinning Option 1—same ramet number as truncation selection
(3) linear thinning Option 2—same genetic gain as truncation selection
(4) linear thinning Option 3—same diversity as truncation selection.

Truncation selection means thinning away all ramets of clones, which have the mean height below a certain value (intercept). Linear deployment means that the number of ramets per clone is used proportional to their breeding value (Lindgren & Matheson 1986). If the suggested number of ramets by this procedure is larger than the existing number of ramets (slope), no ramets are rogued. The linear techniques were compared with simple mass selection of the same number of phenotypically superior ramets as that of the best linear option.

RESULTS

Truncation selection

The average of breeding values of all clones is set as the intercept and the slope is infinity, which essentially means that all the ramets of the clones with breeding values above the intercept will be selected. This is the conventional method of selecting clones for a clonal seed orchard. This strategy resulted in 51% loss in effective number of clones compared with the original population (N_c= 85.7) while providing 7% improvement in height growth (Table 1). The N_r value, however, remained the same since the number of selected ramets in each clone remained the same too. This truncation selection was used as the reference for comparison of linear deployment alternatives discussed below.

Linear deployment

Attempts were made to apply linear deployment methods to achieve a better balance for loss of diversity per unit gain.

Option 1

Compared at the same stocking as truncation selection, average height reduced by almost 1% and genetic gain dropped by 14% (from 7.2 to 6.2%), although there was 35% improvement in the effective number of clones. The number of clones preserved increased by 67%.

Option 2

Compared at the same genetic gain as the truncation selection, the number of ramets dropped by 25%, the number of clones preserved raised by 63% and the effective number of clones increased by 19%.

Option 3

Compared with truncation selection at the same effective clone number, the average height increased by 1.2% and genetic gain by 18%. The number of ramets reduced by 31% even though the number of clones retained increased by 44%.

The number of ramets of low ranking clones decreased in the three deployment alternatives respectively from Options 1 to 3 (Figure1). The relative effective number of clones also decreased from Options 1 to 3 in response to the increase in intercept value and the consequent variation in the number of ramets of selected clones.

Simple mass selection

Compared with truncation selection, this method resulted in an increase in effective clone number by 23% and a marginal reduction in average tree height (0.5%). There was an increase of 72% in the number of clones retained.

DISCUSSION

A very important aspect to be considered while roguing a seed orchard is genetic gain. Since these clones have been intensively selected from large number of entries in first generation seedling seed orchards, they offer a short-term strategy for assembling the best trees from different orchards. This provides an opportunity for substantial improvement after a generation of testing in breeding populations. Even for long-term breeding of eucalypts, clonal testing has been reported to be the best approach to evaluate parent trees compared with phenotype testing and progeny testing (Danusevičius and Lindgren 2003). Preserving a large number of clones maintains genetic diversity. The fertility of the clone is important for transferring the gain to the next generation. A clone may later be found desirable based upon its fertility, even though it may be slightly inferior to other clones.

A high effective number of clones is desirable if it is considered important to have genetic diversity in forest plantations. Keeping large numbers of clones maintains flexibility in roguing and the value of later thinnings can be increased by producing a more efficient overlap in phenology and decreasing the amount of selfing.

Options for thinning the clonal trial

The easiest thinning option would be to completely eliminate the low ranking clones with truncation selection. Given the stocking, this method also maximizes genetic gain. This may be acceptable if the selection is done from a very large number of clones at the beginning of a selection programme and diversity is not a big concern. It is not suitable for first thinning in an orchard because it reduces the options for further thinning. First thinnings call for a balanced selection between maximum gain (by selecting very few best clones) and the need for diversity (by including representatives from more entries using linear deployment strategies) (Lindgren *et al.* 1989). The Bondesson and Lindgren (1993) algorithm combines effective number of clones and genetic gain in an optimal way. In most optimization methods we can fix the value of one of them and maximize the other. Linear deployment focuses on intercept and slope where we can use different values of these parameters to arrive at suitable values of G and N_c. Linear deployment will still be optimal as long as clones are not related or equally related. The essential difference between the thinning strategies is the cut-off limit, the intercept, which determines 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

Table 1 Different options for genetic thinning in clonal trial of *Eucalyptus camaldulensis*

Parameter	Before thinning	Mas selection	Truncation selection	Linear thinning Option 1	Option 2	Option 3
No. of clones	87	74	43	72	70	62
N_e	85.7	52.1	42.4	57.3	50.5	42.4
N_r	0.985	0.704	0.986	0.796	0.721	0.684
Ramets	1157	396	573	573	429	396
Intercept (m)	$-\infty$	7.50	7.08	6.50	6.60	6.81
Slope (ramets/m)	0	∞	∞	11.2	9	12
Height (m)	7.05	7.52	7.56	7.49	7.56	7.65
Gain (%)	100.0	106.67	107.2	106.2	107.2	108.5

Note: The intercept for mass selection means a height below which no ramets were selected while for the other values it means that no ramets were selected from clones below that height.

smooth line with less skew in distribution of the selected ramets.

The linear deployment strategies are roughly characterized by the intercept values; at lower intercept, the thinnings tend to create more variance in ramets per clone among the selected clones. The intercept increased from Options 1 to 3 resulting in selection of comparatively fewer low ranking clones in the three deployment alternatives respectively (Figure 1). Increasing the intercept value increased the gain; the slope, however, ensured adequate ramet distribution among clones. Increase in the intercept values resulted in a decrease in the relative clone number (from Options 1 to 3) indicating a reduction in representation of the low ranking clones. N_r values before thinning and with truncation selection were the same indicating infinite slope and selection of all ramets of the represented clones. Hence the number of clones (N) and effective number of clones (N_c) were almost the same for truncation selection, while they differed in the linear selection and mass selection methods depending on the variance in number of ramets of selected clones. Simple mass selection provides an intermediate thinning strategy similar to that of Option 2 by providing adequate diversity and gain close to truncation selection (as indicated by the relative clone number and average height of selected clones).

Linear deployment can be seen as a special case of a broader method that is optimal for unrelated clones when diversity is not dependent on relatedness and there are a large number of ramets. Linear deployment will not be optimal when the number of ramets is low because of truncation errors when transforming decimals to the integer number of ramets. Numerical runs were made to evaluate the magnitude of this truncation problem and the deviations were neglectable. Linear deployment focuses on an essential aspect in a seed orchard; the fertility of the clones as controlled by the number of ramets, rather than as discrete individuals.

Orchard stocking (number of ramets)

A subjective balance between maintaining adequate diversity and enhanced gain in Option 1 resulted in the same number of ramets as truncation selection, and eliminated only 17% the clones by allowing low representation of inferior clones (Figure 1). It was, therefore, efficient in maintaining diversity. The second option (constant gain) eliminated 20% of the clones and had a reduction in stocking of 25% from truncation selection. There was also a need to reduce the number of ramets of each clone in three tree plots in each replication. Considering the above two alternatives, only 14 clones in Option 2 had more than 10 ramets, requiring that at least two ramets be retained in each plot in each replication. This was a better option than having 23 clones or 41 clones with more than 10 ramets in Option 1 and truncation selection respectively. When diversity was kept constant, Option 3 also achieved the same distribution pattern of ramets as that of Option 2 but had a 31% reduction in total stocking. This would result in wider spacing, better conditions for

Figure 1 Number of ramets selected as a function of the clone value flowering and seed set and a reduced chance of selfing compared with truncation selection.

Thus, linear deployment produces a smooth line rather than the infinite slope that is the result of truncation selection (Figure 1), balancing the number of ramets in clones and balancing the benefits of gain and diversity. For *Eucalyptus* clonal seed orchard, 396 trees per hectare (achieved from Option 3) would be considered as high stocking, but it would be acceptable in the third year because the trees are still young and could be thinned further to produce trees with large crowns for seed production. In the first thinning, clone diversity and gain would be greater concerns than orchard stocking. At the same stocking, truncation selection has the disadvantage that diversity is considerably reduced because the emphasis is on gain. The extra gains provided by truncation selection may not be sustained when the fertility of trees and seed production are considered.

Maximize gain at a given diversity level

Optimal selection maximizes gain with adequate safeguards on diversity. Maintenance of diversity increases options for anticipated thinnings, increases the diversity of the seed crop (unequal flowering can reduce diversity), and permits orchards to better function as clonal archives. In advanced generation orchards the loss of gene diversity is high when the effective number is less than 10 non-inbred clones with equal numbers of ramets (Kang *et al.* 2001). Thus, if fertility differences are not high, at least 25 clones would be needed in a second-generation orchard, and the orchard size would be important if the number of clones was less than 50 (Xie *et al.* 1994). Truncation selection resulted in an effective number of 42 clones, a number considered adequate, but it would be desirable to have more clones after the first thinning by permitting a higher number of lower ranking clones to be retained in low proportion and, thus, keeping diversity constant (N_c = 42). Option 3 gave an acceptable solution, providing maximum gain with representation from 62 clones while preserving options for subsequent thinning. Option 3 preserved a more balanced representation of ramets than the other selection methods.

Compared with truncation selection, only Option 3 gave greater gain and diversity. Though Option 2 provided greater diversity, the gain was the same as that of truncation selection. Since the primary objective of managing a seed orchard was to enhance gain, Option 3 was preferred since it addressed both gain and diversity concerns at the same time. Simple mass selection was also an effective method of thinning a clonal trial as it ensured adequate representation of clones and moderate gain. It had higher diversity and slightly lower gain than that of linear Option 2 (Table 1). It allowed considerably higher representation of clones than truncation selection and can easily be done without much expertise. Option 3 would, however, be the preferred option when emphasis is on gain since it provided maximum gain with adequate diversity.

Considerations for seed orchard management

Three-tree rows are sometimes preserved as an outcome of the roguing options discussed here. This result will tend to increase selfing, but it is probably not a critical concern as the orchard will be subjected to a second thinning at the age of six to address the fertility of the trees. Linear deployment will somewhat reduce the remaining proportion of three-ramet rows, while truncation selection would aggravate the situation. Option 3, which provided high gain at a fixed diversity level, ensured that this concern was reduced to a minimum. Thus, Option 3 would be particularly suitable for the current seed orchard, which will be thinned again within a few years. If stocking is too high, the ramets suppress each other and there is insufficient space for management operations. If the stocking is too low, the land is used inefficiently and weed control is a problem but these factors have a marginal impact on seed output because ramets develop wide crowns with increased spacing if thinning is done early.

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