Ann. For. Res. 68(2): 55-66, 2025
https://doi.org/10.15287/afr.2025.3877

ANNALS OF FOREST RESEARCH
www.afrjournal.org

Evaluation of gene diversity and gain in *Eucalyptus camaldulensis* seed orchards of two generations

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Varghese M., Kamalakannan R., Suraj P. G., 2025. Evaluation of gene diversity and gain in *Eucalyptus camaldulensis* seed orchards of two generations. Ann. For. Res. 68(2): 55-66.

Abstract This paper is a comparative synthesis of the gene diversity and gain based on published results of two *Eucalyptus camaldulensis* breeding programmes (BP 1&2) in India. The dynamics and genetic gain of four first generation (F_1) seedling seed orchards (SPA) of BP1 were compared with three second generation (F_2) SPAs and two clonal seed orchards (CSO) of BP2. Three F_1 orchards (F_1 SPAs 1,3&4) of BP1 had low flowering (30%) and high fertility variation (sibling coefficient, Ψ = 5-11) whereas F_1 SPA2 had 73% fertile trees (Ψ = 2.27). No significant gain was obtained in BP1 when the four F_1SPA seed crops were evaluated at two locations in genetic gain trials - to estimate the gain obtained in comparison to native provenance and commercial clone checks. For infusing improved seed from BP1 to BP2, three F_2SPAs were developed, of which two (F_2 SPA 1-2) were thinned genetic gain trials that incorporated bulked seed lots from four F_1 SPAs of BP1, and the third (F_2 SPA 3) originated from bulked seed of only one F_1SPA. Two clone trials of F_1 progeny selections were converted to clonal seed orchards (F_2 CSO 1&2). Flowering was low (26%) in the F_2 SPAs also with high fertility variation (Ψ, 9-14). The CSOs had high flowering (81%) but fertility was highly skewed in CSO2 (N_s = 2) compared to CSO1 (N_s = 11). The F_2 SPAs 1&2, which originated from four F_1 SPAs in genetic gain trials, had higher effective population size (N_s, 95 and 74) than the F_2 SPA3 (N_s = 39) and CSOs, and better progeny performance than the native provenance at 3 years. CSO2 had the lowest gene diversity and survival than the other taxa. Genetic composition and fertility status of the orchards affected the performance and genetic diversity of progeny.

Keywords: clonal seed orchard, fertility, provenance, breeding programme, progeny trial.

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Manuscript: received October 12, 2024; revised July 17, 2025; accepted September 22, 2025.

Introduction

India has about 4 million hectares of eucalypt plantations (GIT 2008) established by forest departments, forest development corporations, pulp companies, and farmers to meet the domestic fuel, pulp and timber requirement. *Eucalyptus camaldulensis* Dehnh. is the major species grown in the low rainfall regions of the country. Mysore gum (a natural hybrid of *E. terticornis* and other eucalypt species), the local eucalypt land race is grown in vast areas by small holder farmers even though the productivity is low (7 m^3 ha^{-1} yr^{-1}) compared to the native *E. camaldulensis* (12-25 m^3 ha^{-1} yr^{-1}) provenances (Chandra et al. 1992). Clones of higher yield (20-25 m^3 ha^{-1} yr^{-1} - Kulkarni, 2005) are promoted by the pulp industry in about a quarter of the planted area, but the commercial eucalypt clones have low diversity, and are beyond the reach of small farmers. It is necessary to produce improved seed to replace the low yielding Mysore gum plantations and increase plantation productivity for meeting the acute shortage of raw material faced by the wood-based industries.

Superiority of the northern Australian provenances from Queensland like Petford, Katherine, Laura River and Kennedy River (Chaturvedi et al. 1989) was evident from the IUFRO international provenance trials of *E. camaldulensis* (Kumaravelu et al. 1995). The early introductions from southern temperate localities of Australia were inferior to the northern tropical regions where the climatic conditions closely resemble the areas available in India (Boland 1981). 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 increasing the productivity of eucalypts. He emphasised the need for a short-term programme to achieve early genetic gain and a long-term programme to maintain sufficient diversity needed to sustain the gain in advanced generations.

A systematic breeding program for *E. camaldulensis* (Doran et al. 1996) was implemented in India in 1995 by the Institute of Forest Genetics and Tree Breeding - IFGTB, Coimbatore (BP1). Unpedigreed first generation seedling seed orchards (F_1 SPAs) were established in this breeding programme with a seed mix of more than 500 open pollinated families from 11 native Australian provenances for ensuring a broad genetic base needed for long-term improvement (Varghese et al. 2008). The first-generation (F_1) orchards were evaluated for fertility and predicted diversity of the seed crop (Kamalakannan et al. 2007). The genetic gain obtained from the F_1 orchard seed lots was estimated in genetic gain trials established at two diverse sites with replicated 49-tree plots in randomized complete block design, to compare the performance of the seed crops with the best native provenance and commercial clone checks, and the local land race (Varghese et al. 2009a). Seed was supplied from the F_1 SPAs of BP1 after selective thinning of the F_1 orchards for planting from the year 2000 (Krishnakumar et al. 2014) as per the breeding plan.

A second breeding programme for *E. camaldulensis* was initiated in 2007 by ITC Ltd (BP2), a major pulp and paper company in India. Open pollinated half pedigreed seed lots of four superior natural provenances were imported from CSIRO Australia to establish the breeding population. Open pollinated family seed lots were infused from second generation (F_2) orchards developed with the F_1 orchard seed of BP1 to increase the productivity and diversity of the breeding population (Ferreira et al. 2024). The performance of the F_2 orchard crops was evaluated in progeny trials along with the introduced native provenance families and commercial clone checks (Varghese et al. 2017).

This paper aims to develop new insights for successful domestication of eucalypts based on a comparative synthesis of the orchard dynamics, diversity, gain and mating pattern of *E. camaldulensis* seed orchards from published information of two breeding programmes. The study critically evaluates the factors that influence gain in breeding programmes, and the strategies to be employed for sustaining diversity and gain during domestication.

Materials and Methods

In BP1 four unpedigreed F_1 seedling seed orchards (F_1 SPA 1-4) were established with bulked seed of over 500 native provenance trees (Table 1).

To infuse improved germplasm to the second breeding programme (BP2) three F_2 SPAs (1-3) and two F_2 clonal orchards (CSO 1-2) were developed from BP1 selections. The F_1 genetic gain trials that incorporated bulked seed lots from four F_1 SPAs of BP1 were thinned and converted to F_2 SPAs (1&2) while a plantation of the high fertility F_1 SPA2 progeny was converted to F_2 SPA3. To improve the productivity in BP2, two clonal seed orchards (F_2 CSO 1-2) were developed based on selections from plantations of BP1 orchard seed.

Fertility variation and gene diversity were estimated in four F_1 seed orchards of BP1 by Kamalakannan et al. (2007) and five F_2 orchards of BP2 (Table 2) by Suraj et al. (2019). The genetic gain obtained from F_1 orchards over the native-provenance base population was estimated by Varghese et al. (2009a) and in F_2 orchards by Varghese et al. (2017). The genetic diversity (H_e) and number of alleles per locus in F_2 orchards was estimated by Suraj et al. (2019) from leaf samples of randomly selected seedlings of each *Eucalyptus* taxa using SSR markers. Mating system analysis was also done in F_1 SPA2 by Varghese et al. (2009a) and in selected families of the F_2 orchards by Suraj et al. (2019). The change in diversity and gain across two generations of orchards in the two breeding programmes is analysed to develop strategies necessary for sustaining diversity with generation advancement in breeding programmes.

In BP1 a single plantation was strategically used to combine the base, breeding and propagation populations by retaining only the best trees in the SPA after evaluation and thinning at four years. The BP2 was designed to maintain separate breeding and production populations. In BP2 separate production populations were developed after evaluating the breeding population of 183 introduced native provenance families and 48 F_2 families infused from BP1 orchards in three progeny trials (Varghese et al. 2017). The infused F_2 seed lots were evaluated for growth and fertility along with the introduced native provenance families in progeny trials at three locations.

The BP2 production populations were developed as three sublines by capturing the diversity of top families from the breeding population at three agroclimatic regions (from three progeny trials) by grafting the best fertile tree from the top 50 families at each site. The subline orchards were evaluated for fertility (% flowering trees) and fecundity (number of fruits/tree). The dynamics of the seed orchards was evaluated using the following parameters assuming that the parent trees were unrelated,

Table 1 Details of the seed orchards established in BP1*and BP2.

Breeding program	No. of seed orchards	Base population (No. of open pollinated families)	Composition of orchards		
			SPA	CSO	Sublines (SL)
BP1 – IFGTB	Four F_1 SPAs (1- 4)	500 families Bulked	11 Native provenances	-	-
BP2 – ITC	Three F_2 SPAs (1-3) Two F_2 CSOs (1 & 2)	183 Native + 48 families infused from 3 F_2 SPAs and 2 F_2 CSOs	F_2 SPA (1&2): Bulked seed from two genetic gain trials of 4 F_1 SPA seedlots F_2 SPA (3): Bulked seed of F_1 SPA 2	F_2 CSO (1&2): 21 clones selected from F_1 SPA seedlots	SL (1-3): one top tree grafted from 51-69 top families in three progeny trials

*(Kamalakannan et al. 2007), #(Suraj et al. 2019).

as the breeding population had large number of wild native families and only one tree was selected from each family in the sublines.

Sibling Coefficient (Ψ): the probability that two genes originate from the same parent, compared to a panmictic situation, was estimated from the number of fertile trees in the orchard (N) and individual fertility of each tree (Kang *et al*., 2003), to quantify the fertility difference between orchard genotypes.

Effective Population Size (N_s): was used to compute the number of unrelated trees that contribute equally to the gene pool (Lindgren & Mullin 1998) based on the contribution of the individual genotype to the gamete pool and the total number of trees (N) in the orchard.

Relative population size (N_r): was used to compare the effective number of trees that contribute to random mating (N_s), with the actual number of trees in the orchard (N).

Expected gene diversity (*GD*): was estimated in terms of the group coancestry (Kang et al. 2003).

The methodology used to estimate the orchard dynamics, productivity, genetic gain and genetic diversity of seed orchards of BP1 and BP2 are explained in detail in the cited references.

Results

Fertility variation and gene diversity of seed orchards

The F_1 SPAs of introduced native Australian provenances of BP1 showed distinct variation in fertility across sites in southern India. The proportion of flowering trees was significantly higher (73%) in F_1 SPA 2, but less than 30% in the other orchards (Table 2). The fecundity was also at least two to three times higher in SPA 2. The proportion of flowering trees remained more or less the same (23-26%) in F_2 SPAs developed from F_1 orchards, and the fecundity also did not increase with advancement of one generation. The F_2 CSOs however had higher proportion (81%) of flowering trees, but fecundity varied drastically in two clones across the two sites. The average fecundity (1500 fruits/ tree) was more or less similar in the other clones across the sites.

Fertility variation (Ψ) between trees was about 3-6% lower in the high fertility F_1 SPA2 than the other orchards. In F_2 SPAs the Ψ was high (9-14) indicating no improvement in fertility variation with one generation advancement. The F_2 CSO 1 had significantly lower fertility variation (Ψ = 1.9) than the F_2 SPAs but the high fecundity of two clones caused large fertility variation in F_2 CSO 2 (Ψ = 10.6). As the effective population size (N_s) is a function of the total number of orchard trees (N), the N_s was fairly similar (60-80) in three F_1 SPAs. In F_2 SPAs (1&2) also the N_s was around 70-90 but significantly lower (39) in the F_2 SPA3 that had about 2.5 times lower stocking than the other F_2 SPAs. The F_2 CSO 1 had a lower effective population size (N_s = 11) as the number of clones was only 21. The F_2 CSO 2 was over represented by two high fertility clones resulting in a low N_s of 2. The effective contribution of orchards was in the range of 7-15% in the low flowering F_1 SPAS and 7-11% in F_2 SPAs, and 10% in F_2 CSO2. Higher effective contribution was observed in the high fertility F_1 SPA2 (N_r = 0.45) and F_2 CSO 1 (N_r = 0.52). The gene diversity was generally high (*GD*=0.99) in the F_1 and F_2 SPAs as seed was introduced from large number of native Australian trees (500) and the orchard trees were assumed to be unrelated. The *GD* values were lower in F_1 SPA 4 (*GD* = 0.96), F_2 SPA 3 (*GD*=0.98) and F_2 CSOs (*GD*=0.91 to 0.96).

The sublines developed in BP2 (SL 1-3) had a different trend for most traits as all trees were fertile, but the stocking was lower (51-69 trees) than the SPAs. Fecundity was almost 8 times higher in SL 1 than the other two sublines that had similar fecundity as the SPA trees. The fertility variation in the sublines was low (Ψ = 1.5 to 2.1) compared to most SPAs resulting in greater effective contribution (N_r = 0.46 to 0.69) of trees. The GD was however slightly lower (0.98) than the SPAs as there were only 32-36 effectively contributing trees.

Table 2 Dynamics of seed orchards in BP1 and BP2.

Trait	IFGTB Breeding program (BP1)				ITC Breeding program (BP2)							
	Breeding/Production population (4 years)*				Germplasm infused into Breeding population (5 years)**					Production population (5 years)***		
	F_1 SPA (Native seedlots)				Seedlots infused from F_1 Orchards					Subline (SL) (Native + infused)		
	F_1 SPA-1	F_1 SPA-2	F_1 SPA-3	F_1 SPA-4	F_2 SPA-1	F_2 SPA-2	F_2 SPA-3	F_2 CSO -1	F_2 CSO-2	SL-1	SL-2	SL-3
N	525	182	478	192	1029	1050	369	21	21	69	60	51
Fertile trees (%)	26	73	30	23	26	23	26	81	81	100	100	100
Fruits per tree	1120	3850	1580	318	638	984	136	1518	14330 (1471)‡	8496	826	1118
Ψ	6.7	2.2	8.0	13.4	10.8	14.1	9.4	1.9	10.6	2.1	1.6	1.5
N_s	78	81	60	14	95	74	39	11	2	32	36	35
N_r	0.15	0.45	0.13	0.07	0.09	0.07	0.11	0.52	0.10	0.46	0.60	0.69
GD	0.994	0.992	0.993	0.967	0.995	0.993	0.987	0.966	0.915	0.984	0.986	0.986

Ψ: Sibling coefficient, N_s: Effective population size, N_r: Relative status number, GD: Predicted gene diversity
‡ Excluding two high fertility clones; *(Kamalakannan et al. 2007), **(Suraj et al. 2019), ***(Varghese et al. 2024).

Genetic gain from seed orchards

The growth (dbh) of the high fertility F_1 orchard seed lot of BP1 (F_1 SPA 2) at three years was on par with the native provenance and commercial clone controls tested in genetic gain trials at two sites, and about 5% less than the native seedlot and 8% less than the clone in the genetic gain trial at site 1 (Table 3). The survival (%) of the orchard progeny was on par with the native seed lots at the three sites. Thus, there was no significant gain in growth (dbh) over the native provenance after a generation of *E. camaldulensis* breeding in BP1. There was however 15% improvement in growth compared to the eucalypt land race that is commonly planted in southern India.

The F_2 orchard seed lot (F_2 SPA1) infused into BP2 had 9% better growth than the best native provenance seed lot (Kennedy River) in the progeny trial at site 1. The commercial clones had similar growth and survival as the orchard seed lot at this site. The gain in growth over the native provenance was comparatively less (2-5%) at the other two sites but the growth (11-18%) and survival were significantly better than the commercial clones at these sites.

Impact of genetic diversity on the seed orchard crop

From Fig. 1, it can be seen that the number of alleles has a positive impact on genetic diversity in the seed orchards ($R^2 = 0.56$). The number of alleles is higher in the SPAs than the CSOs. However, it can be seen that the number of alleles in F_2 SPA 3 (6.7) is lower than that of the F_2 SPAs 1& 2 (7.1& 7.4) and the native provenance (7.1), whereas in CSOs it was less (5-6) than the native provenance.

Genetic diversity was positively correlated with survival of the orchard progeny in the field (R^2=0.9). Survival was lowest in CSO2 (54%) which had the least diversity (0.65), and highest in SPA 1 (76%) with the highest genetic diversity. Native provenance (NAT), SPA 3 and CSO 1 had intermediate genetic diversity values (0.68) and intermediate survival (65-72%).

Table 3 Performance of orchard seedlots and control in BP1 and BP2.

Breeding program	Site	Native provenance		Orchard seedlot		Local land race		Commercial clone	
		Dbh (cm)	Survival (%)	Dbh (cm)	Survival (%)	Dbh (cm)	Survival (%)	Dbh (cm)	Survival (%)
BP1* (F_1 SPA2)	Site 1	7.6	96	7.2	94	-		7.8	91
	Site 2	6.3	85	6.2	89	5.4	89	-	-
	Site 3	6.3	86	6.2	87	-	-	6.4	91
BP2# (F_2 SPA1)	Site 1	6.8	89	7.4	94	-	-	7.3	66
	Site 2	8.3	86	8.5	91	-	-	7.2	81
	Site 3	5.8	75	6.1	80	-	-	5.5	46

*(Varghese et al. 2009a), #(Varghese et al. 2017).

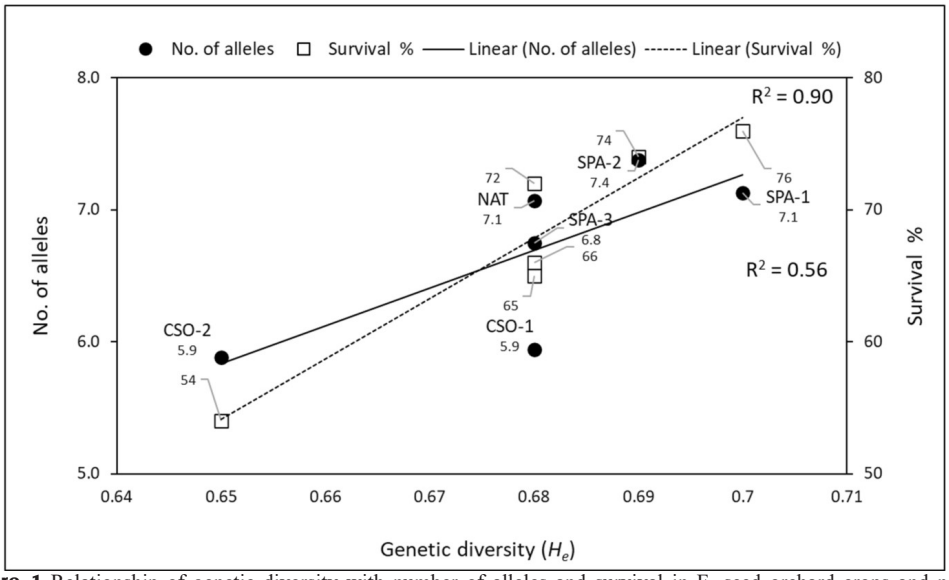

Figure 1 Relationship of genetic diversity with number of alleles and survival in F_2 seed orchard crops and native provenance (NAT) of *E. camaldulensis* (Suraj et al. 2019).

Discussion

Need for conserving diversity during domestication

When selection is done to maximize gain, safeguards have to made to retain diversity for sustaining gain in advanced generations. Adequate diversity is necessary for transferring genes from the orchard parents to the seed crop. If thinning is done without considering fertility, unequal flowering can reduce the diversity (Kamalakannan et al. 2016). The gene diversity loss in F_2 CSO2 (N_s =11) may be low since an effective number of 10 non-inbred clones with equal number of ramets can sustain the diversity with generation advancement (Kang et al. 2001a). But diversity of clonal seed orchards is however lower than the SPAs as the number of genotypes is less which affects the adaptability and survival of progeny in diverse sites (Suraj et al. 2019). Hence a low input strategy (Lindgren 2003) of unpedigreed SPAs would help to restrict gene loss in the initial stages of a breeding programme. Despite poor flowering and genetic drift, the unpedigreed F_1 SPAs of BP1 were effective in producing seed on par with

the native provenance (Varghese et al. 2009a). A high-intensity programme that focuses more on gain may lead to substantial genetic erosion in the first generation as it has fewer parents and a smaller breeding population than a seedling orchard.

Breeding populations generally have higher diversity than the native base population (Jiabin et al. 2020) when mating occurs between different provenances (Lefevre 2004). The BP1 SPAs had representation from 11 native provenances to promote mating between populations that are widely separated geographically in their natural range whereas in BP2, open pollinated families were introduced from only four major provenances. In a species with large geographical distribution like *E. camaldulensis*, when widely separated seed lots are crossed, the adaptability of some seed lots can be combined with the vigorous growth of other seed lots. Synchrony in flowering is however necessary to get the desired improvement which may be an issue when large number of provenances are included in the breeding population as was the case in BP1 (Varghese et al. 2017).

Progeny originating from orchards of the same origin can vary depending on the flowering status of trees. Excessive fertility of a few trees can lead to relatedness among progeny. The problem can be very acute if the number of flowering trees is very low as observed in some F_1 SPAs. Very little information is available on the fertility of improved populations (Anjos et al. 2023) or the flowering synchrony in eucalypt seed orchards (Spencer et al. 2020). As fertility and tree growth may not be correlated, fertility variation has to be assessed for evaluating the genetic quality of seed produced from seed orchards.

With the advancement of each generation, it is necessary to conserve the alleles, on par with the native provenance for ensuring the adaptability and productivity of the seed crop. Reduction in genetic diversity is manifested in the poor overall fitness and survival of the seed crops of F_2 CSO 2 and F_2 SPA 3 (Breed et al. 2014), compared to the native (NAT) and other F_2 SPA (1&2) seed lots of higher genetic diversity. Inbreeding in forest trees becomes evident with environmental stress (Griffin et al., 2019), generally associated with reduced growth and survival. In *E. nitens*, progeny of high fertility orchards (>40% flowering trees) had better growth than low flowering orchards (<20%) in South Africa (Swain et al. 2013). A positive association is reported between outcrossing rate and growth in *E. globulus* families with different levels of inbreeding (Faia et al. 2022). Growth differences among *E. camaldulensis* families from the Petford region, in progeny trials in Thailand (Pinyopusarerk et al. 1996), is found to be associated with differences in the outcrossing rate (Butcher & Williams 2002). Thus, a major requirement of domestication is to conserve diversity by eliminating inbred individuals from the breeding population and ensure that outcrossing is maintained among the retained genotypes in the breeding orchard.

Strategies for conserving diversity in breeding programmes

As many eucalypt species fructify at a young age seedling seed orchards are used as cost-effective means of producing an assured supply of genetically improved seed. However, when native seed lots are introduced in breeding populations they have to be carefully monitored for fertility, fecundity and flowering synchrony. It is preferable to restrict introduction from a few top native provenances as in the case of BP2 for getting uniformity in fertility and synchrony. When introduced to a new location seedling orchards help to release the 'neighbourhood inbreeding' prevalent due to mating between related trees in the natural habitat. Seedling orchards are therefore expected to produce improved seed compared to native provenances.

As envisaged in BP1, seedling seed orchards (SSOs) are very useful in combining the three

functions - genetic evaluation, breeding, and seed production, in a single plantation. But while SSOs may be useful in genetic evaluation of native seed lots and removing their inbreeding effects, if fertility variation is high as in the F_1 and F_2 SPAs they may not be very efficient in seed production. This problem was identified in many tropical SPAs by Pinyopusarerk and Harwood (2003) who recommended seed collection only from orchards with at least 50% flowering trees. Most of the F_1 and F_2 SPAs in the current study would not meet this criterion recommended for quality seed production. The issue can be resolved by maintaining separate breeding and production populations as in BP2. It is evident that the eucalypt F_2 CSOs established with grafts have greater fertility than SPAs. However, heterozygosity and genetic diversity of CSO progeny are lower than the SPAs (Leite et al. 2008). During domestication it is important to ensure that the heterozygosity prevailing in native stands is increased or at least maintained in the next generation.

After three generations of recurrent breeding in China (Lu et al. 2018) genetic diversity of *E. urophylla* orchards was maintained on par with the native populations. In Brazil, Leite et al. (2008) infused large number of trees of low genetic similarity from an improved SPA to maintain the diversity in a multi provenance base population of *E. grandis*. Heterozygosity and allelic diversity are influenced greatly by the effective population size of the orchard. In F_2 CSO 1 mating was close to random (Ψ=1.95) but low N_s impacted the allelic diversity which in turn affected the fitness and adaptability of the offspring (Suraj et al. 2019). Switching to the CSO route may be effective in improving fertility but the N_s has to be brought close to that of SPAs. The sublines developed in BP2 were effective in combining the high fertility of clonal orchards, and the higher effective population size of seedling orchards (Varghese et al. 2024). Since the subline orchards have representation from more than 50 unrelated families, they maintain three times higher *Ns* than the F_2 CSOs. Xie et al. (1994) reported that at least 25 unrelated clones would be needed in a second-generation orchard if fertility difference is not high, but the orchard size would be important if the number of clones was less than 50. The subline orchards meet the above requirement of adequate number of clones and low Ψ needed for maintaining diversity.

Despite not being typical seed orchards, the progeny of F_2 SPAs performed on par or better than the commercial clones and native seed lots at diverse sites (Varghese et al. 2017). Further improvement in advanced generations will depend on conservation of diversity by restricting inbreeding and co-ancestry. Constitution of the orchard and mating pattern are therefore two important factors that influence the gain and diversity, as evidenced by the two F_2 SPAs (SPA 1&2) that were originally genetic gain trials of four F_1 orchards. Even though fertility variation persisted in the second-generation SPAs, two F_2 SPAs (SPA 1&2) that had representation from four F_1 orchards (in genetic gain trials) had more alleles and genetic diversity than the F_2 SPA3 (which originated from one single F_1 SPA). The F_2 SPAs (1&2) had more grandparents than F_2 SPA3 (Varghese et al. 2009a) as they had representation from four first generation orchards. Danusevicius and Lindgren (2010) recommended restriction in number of parent trees per grandparent in an orchard for sustaining diversity and gain. Restriction in number of trees per family enables retention of alleles and high effective population size in seed crop (Silva et al. 2018). Since the F_2 SPA (1&2) seed lots had higher diversity, they had good adaptability to diverse sites (Varghese et al. 2017) with long (survival 67-72 %) and short dry spells (75-88 %). The multilocus outcrossing rate (t_m, 0.91-1.0) also increased with one generation advancement (Suraj et al. 2019) in the two F_2 SPAs (1&2) compared to the high fertility F_1

SPA 2 (t_m, 0.86, Varghese et al. 2009a), and is manifested in terms of higher field survival than the other F_2 seed lots of lower diversity (Funda & El-Kassaby 2012). Populations with narrow genetic base are more sensitive to environmental changes due to the inability to compete in heterogeneous conditions (Resende et al. 2018).

The factors like poor fertility and lower outcrossing rate of the F_1 SPAs (Butcher & Williams 2002) affected the adaptability and gain of the seed crop after a generation of breeding in BP1 (Varghese et al. 2009a). To rectify the drawbacks faced in BP1, new introduction was made in BP2 from only four superior provenances (NAT).

Management of eucalypt seed orchards for conserving diversity

Management strategies are necessary to correct the imbalance in fertility and capture more diversity in seed crop. In *E. camaldulensis* the percentage of fertile trees can be increased with application of Paclobutrazol™ (Varghese et al. 2009b) and other nutrients (high N + PK). Maintenance of adequate number of pollen parents and constant seed collection from superior trees (Kang et al. 2001b) can be used to reduce the genetic drift and correct the imbalance in fertility and mating (Chaix et al. 2007) when large number of genotypes are introduced to an exotic location, in the early stages of domestication.

Fertility variation is generally higher in seed stands than clonal orchards (Kang et al. 2003), but fertility variation can have a greater impact in a clonal orchard than a seedling orchard due to the lower number of genotypes contributing to the gene pool. Fecundity of trees can have a big impact on the orchard dynamics especially in clonal orchards as evidenced by the Ψ values in two F_2 CSOs (1.9 & 10.5) in the current study. Suitable strategies can be used to optimise the gain and effective clone number in a CSO of *E. camaldulensis* (Kamalakannan et al. 2016) to buffer the skew in fertility as in F_2 CSO 2. Mixing seeds from two consecutive harvests helps to enhance the overall fertility, status number, relative population size and gene diversity of the seed crop than that of either harvest. Mixing the seeds harvested in two consecutive years is reported to reduce the sibling coefficient by 31% and increase the relative contribution of trees by 45% in *E. camaldulensis* and *E. tereticornis* orchards in southern India (Kamalakannan & Varghese 2008).

When fertility variation is high, genetic thinning should be done only after evaluating fertility of the orchard trees (Park et al. 2017). Constrained seed collection has a greater impact on genetic diversity by significantly reducing the fertility variation, than the strategy of mixing seed from different seed years (Kamalakannan & Varghese 2008), but it is often not very feasible when large quantity of seed is required. A combination of constrained seed collection and mixing seed of different years would be very effective in improving diversity. Suitable designs can be used to manage the mating pattern and gene flow within and between populations in the seed orchard (Lstiburek & El-Kassaby 2010). Molecular markers can be used to modify the mating pattern of the orchard by screening the orchard genotypes for their inbreeding status, outcrossing rate and correlated paternity (Kullan et al. 2024).

Conclusions

Two breeding programmes of *E. camaldulensis* were implemented in India to produce improved seed compared to the imported native provenance base population. Productivity improvement depends on the genetic gain, and genetic diversity which impacts the adaptability of the seed crop to diverse environments. Low fertility of introduced native germplasm resulted in high fertility variation in the first and second-generation seedling seed orchards of the two breeding programmes.

A comparative analysis of eucalypt seed orchards of two generations highlights the need for careful management of the composition and diversity of breeding and production populations, for achieving the expected gain. The strategy of combining the base, breeding and production populations in a single plantation resulted in drastic diversity erosion in BP1. The problem was addressed in BP2 by developing separate production populations after evaluating the breeding populations. Grafted production orchards can be designed as in BP2, to combine the high fertility of clonal orchards, and the high effective population size of seedling orchards. The gain and diversity can be sustained in advanced generations by mating superior genotypes of unrelated families selected from multiple breeding populations for seed production in grafted production orchards.

Conflict of interest

The authors declare no financial or personal interests could influence the work presented in this paper.

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