

# ESTIMATION OF OUT-CROSSING RATE IN THE FIRST AND SECOND GENERATION ORCHARDS OF *ACACIA AURICULIFORMIS*



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## Introduction

*Acacia auriculiformis* A.Cunn.ex Benth is a fast growing multipurpose plantation species. The plantations have enriched the soil through litter, nitrogen fixation and increase soil pH in acidic soils. The Institute of Forest Genetics & Tree Breeding (IFGTB), Coimbatore have been established Seedling Seed Orchards in *Acacia auriculiformis* in Southern States (Tamil Nadu and Kerala) of India with the technical collaboration of Australian Tree Seed Center, CSIRO, Australia.

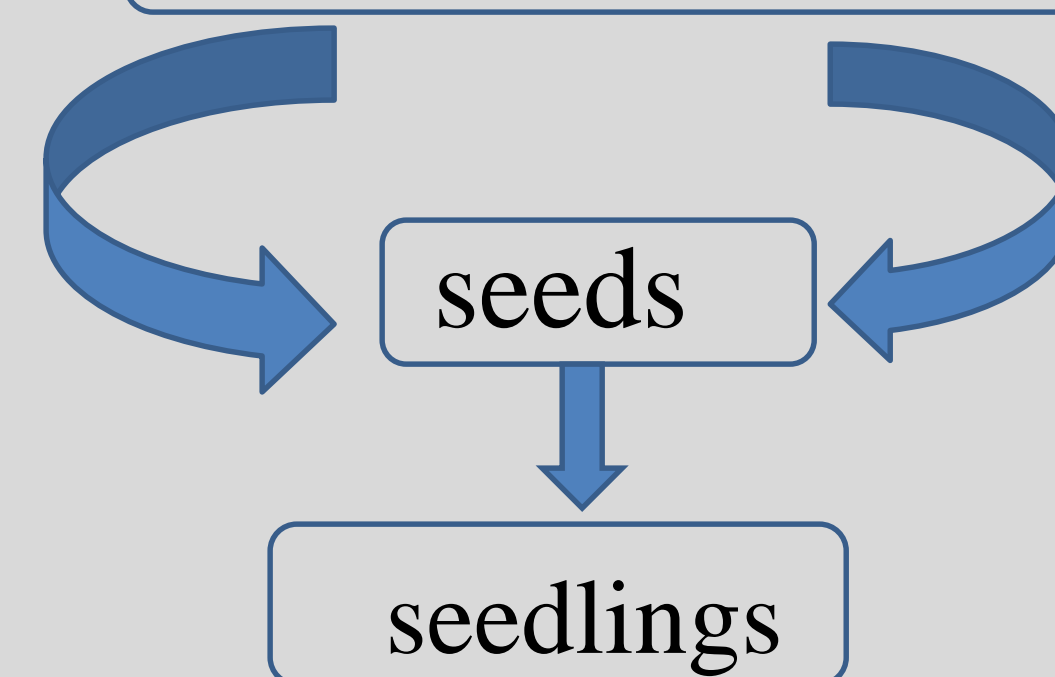
- The Second generation seed orchards were also assembled by selecting the best trees in the best families from the progenies of the first generation seed orchard trees.
- Seeds produced from these orchards would produce seedlings with even better potential for good growth than the first generation orchards.
- Few families in the orchard population has a problem in seed setting. To address this problem a study on mating system in the orchards were undertaken through DNA markers.
- Microsatellite marker is the most popular genetic marker which is co-dominant and highly polymorphic suitable for the study of population genetics.

## Materials and Methods

### Seedling Seed Orchard



### Orchards (IG&IIG)



DNA extraction(Quagen Kit) in leaves

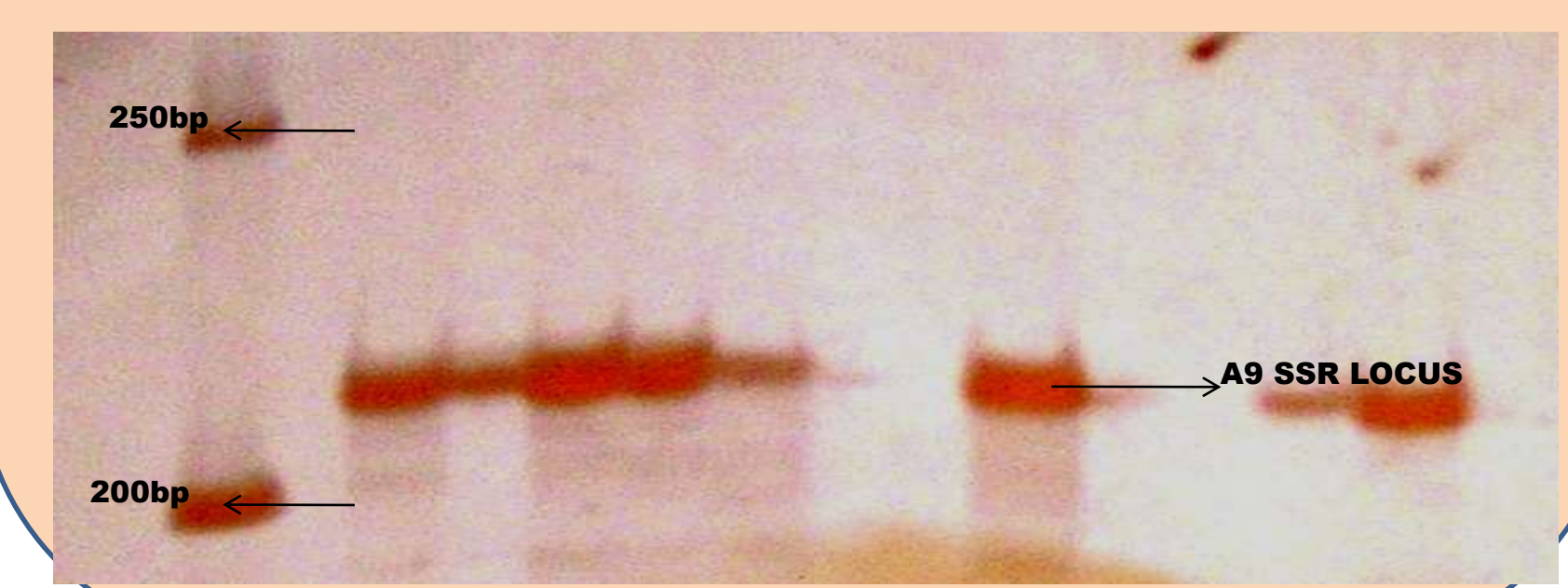
### PCR

PCR reaction conditions were optimized for five polymorphic microsatellite primers. The amplicons were analysed on silver staining assay of Polyacrylamide gel electrophoresis. The sizes of alleles indicated as homozygous & heterozygous. Multi locus out-crossing rate was estimated using MLTET programme.



### SSR amplification in *Acacia auriculiformis*

Sl. No.	Name of the SSR Loci	Accession No.	Amplicon size	Annealing temperature
1.	AH2-2(F) & (R)	HQ1108 63.1	150-200bp	56°C
2.	AH2-6(F)&(R)	HQ1108 66.1	150-200bp	59°C
3.	AH2-17(F)&(R)	HQ1108 74.1	150-200bp	59°C
4.	AH3-1(F) & (R)	HQ1108 75.1	200-250bp	59°C
5.	AH3-17(F) & (R)	HQ1108 80.1	200-250bp	59°C



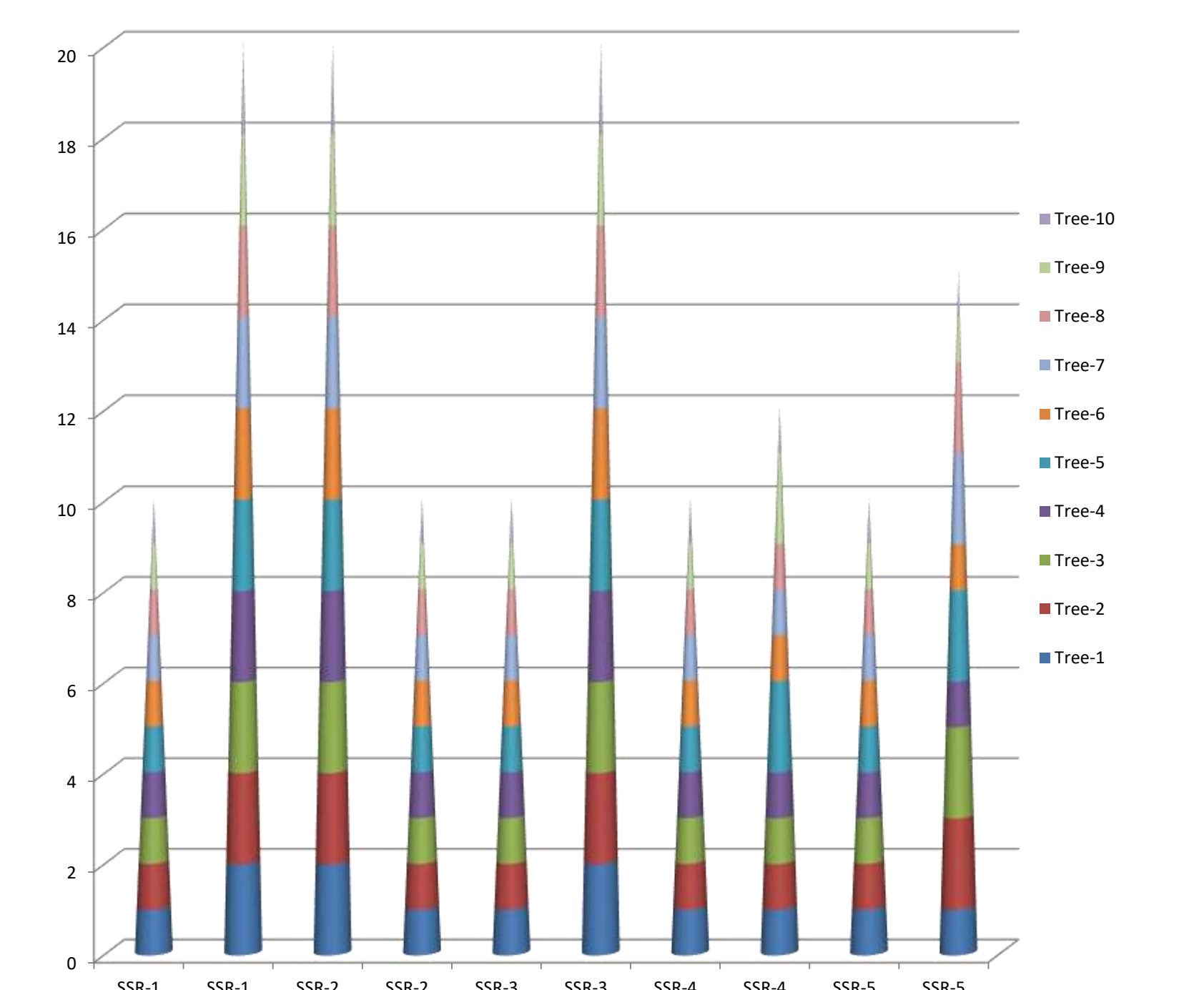
## Results

- The microsatellite region of the genome product sizes were ranged between 150bp to 250 bp in all the five primers.
- High out-crossing rates in both generation orchards population and it reflects that this species is highly out-crossed.
- The shared alleles of pollen to ovule were two to three among the families of the population. But there was no significant variation in the pollen and ovule allele frequency estimates in both the generations.

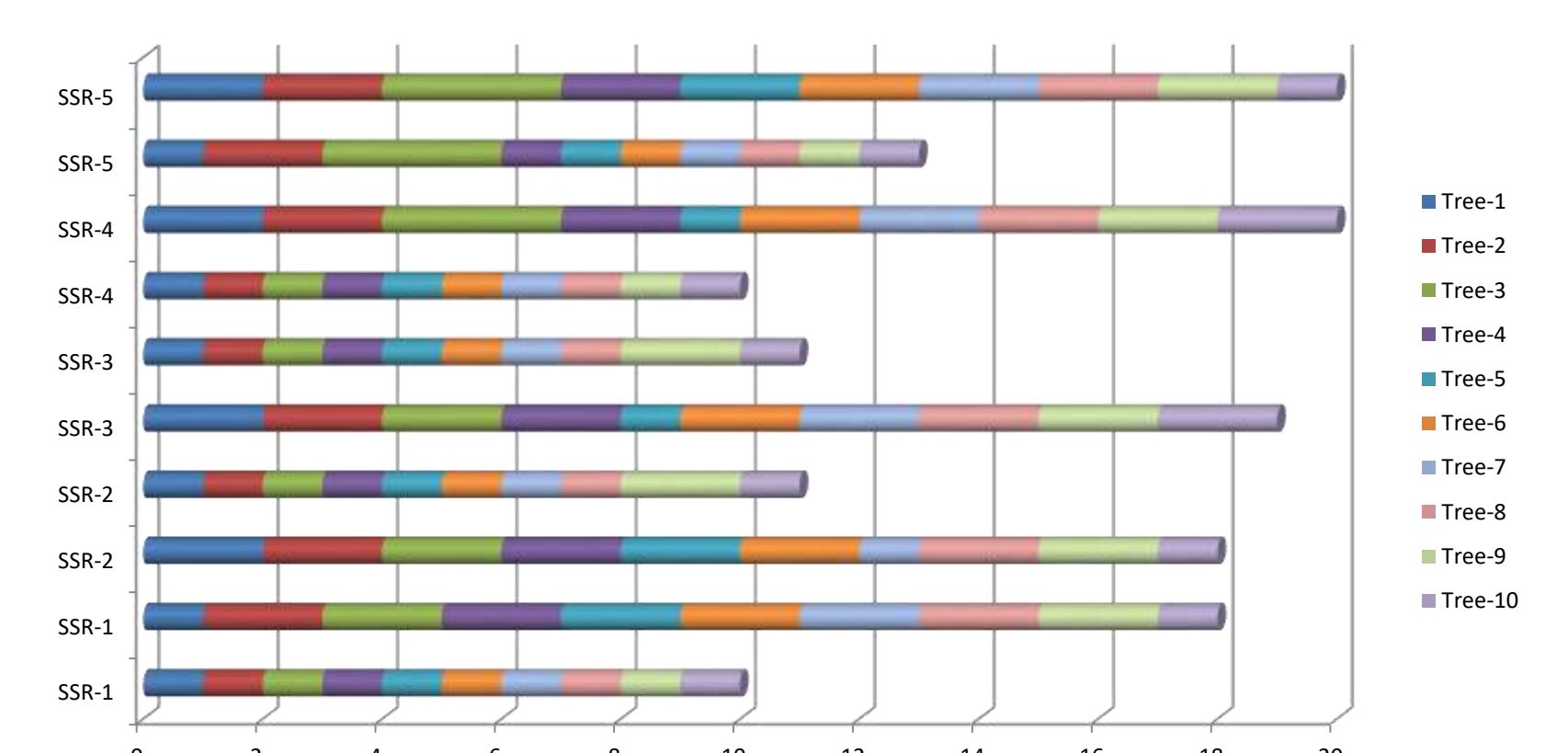
### Gene Frequency Estimate (SD) in First generation progenies

Locus	allele	pollen		Ovule	
		allele	pollen	allele	ovule
ssr-1	1	0.617(0.021)	0.617(0.021)	1	0.644 (0.066)
	2	0.383 (0.021)	0.383(0.021)	2	0.356 (0.066)
ssr-2	2	0.458 (0.025)	0.458(0.025)	2	0.420 (0.069)
	1	0.542 (0.025)	0.542(0.025)	1	0.580 (0.069)
Ssr-3	1	0.531(0.031)	0.531(0.031)	2	0.336 (0.057)
	2	0.464(0.031)	0.464(0.031)	1	0.610 (0.057)
Ssr-4	1	0.563 (0.028)	0.563(0.028)	1	0.647 (0.054)
	3	0.118 (0.010)	0.118(0.010)	3	0.109 (0.031)
	2	0.319 (0.024)	0.319(0.024)	2	0.244 (0.045)
Ssr-5	1	0.548(0.032)	0.548(0.032)	1	0.483 (0.042)
	3	0.182(0.012)	0.182(0.012)	2	0.272 (0.038)
	2	0.267(0.020)	0.267(0.020)	3	0.098 (0.028)

### Maternal genotypes in first generation progenies



### Maternal genotypes in second generation progenies



Family	Family level estimates in First Generation Progenies				Family level estimates in second Generation progenies			
	ts	SE(ts)	tm	SE(tm)	ts	SE(ts)	tm	SE(tm)
Tree1	1.039	0.021	1.021	0.001	0.817	0.134	1.095	0.001
Tree2	1.118	0.168	1.002	0.100	1.053	0.017	1.044	0.001
Tree3	1.031	0.022	1.023	0.001	1.236	0.050	1.068	0.001
Tree4	0.888	0.122	0.946	0.091	1.074	0.014	1.044	0.001
Tree5	1.045	0.024	1.020	0.001	0.643	0.092	0.936	0.082
Tree6	0.903	0.133	0.885	0.086	1.059	0.011	1.044	0.001
Tree7	1.041	0.010	1.036	0.001	0.957	0.168	0.996	0.062
Tree8	0.799	0.172	0.843	0.174	1.057	0.009	1.044	0.001
Tree9	1.056	0.009	1.047	0.001	1.099	0.125	1.022	0.051
Tree10	1.057	0.009	1.041	0.001	1.100	0.025	1.015	0.001
Mean	0.99	0.069	0.98	0.045	1.00	0.06	1.03	0.02

## Significance of the study

High level of multi locus out-crossing rates in first (tm = 0.98 ; SE = 0.05) and second generation ( tm= 1.0; SE = 0.02) orchards. The orchard population in both generation shows the massive out-crossed genotypes can be very helpful for advance breeding programme

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