KFRI Project Report No. 421 **ISSN 0970-8103**

Improvement of Teak through genetic evaluation

E. P. Indira
Forest Genetics & Biotechnology Division

Kerala Forest Research Institute
An Institution of Kerala State Council for Science, Technology and Environment
Peechi – 680 653, Thrissur, Kerala, India

January 2012

KFRI Project Report No. 421 **ISSN 0970-8103**

Improvement of Teak through genetic evaluation

(Final Report of Project KFRI 438/04)

E. P. Indira
Forest Genetics & Biotechnology Division

Kerala Forest Research Institute
An Institution of Kerala State Council for Science, Technology and Environment
Peechi – 680 653, Thrissur, Kerala, India

KFRI

January 2012

ABSTRACT OF THE PROJECT PROPOSAL

a. Project number : KFRI 438/04

b. Title : Improvement of teak through genetic evaluation

c. Funding agency : KFRI Plan Fund

d. Principal Investigator : E.P. Indira

f. Duration : April 2004-March 2012

g. Objectives:

1. to study the genetic variability and inheritance pattern in economically important characters
2. to select elite trees/ clones
3. to establish a seedling seed orchard

h. Expected output:

1. Genetic variability and inheritance pattern in economically important characters will be estimated.
2. The elite trees/ clones can be identified.

ACKNOWLEDGEMENTS

Sincere thanks are due to Dr. J. K.Sharma, former Director and to Dr. K. V.Sankaran, Director, KFRI, for the support and encouragement. I express my gratitude to all the officials in the Kerala Forest Department for their co-operation during the field work. The assistance rendered and the courage shown by Mr. Dhanush, Dr. Pramod N. Nair and Mr.Sojan Jose during the strenuous field work in an elephant prone area is appreciable and noteworthy. The help rendered by Dr. Sabna Prabha during the laboratory work is also acknowledged. I am grateful to Dr. K.Swarupanandan, Research Coordinator for the support given during the project period and to Dr. P. Rugmini for statistical advice. My sincere thanks are due to Dr. K.V.Bhat, Dr. R.C.Pandalai and Dr. E.M.Muralidharan for their editorial comments for improving the manuscript.

CONTENTS

ABSTRACT

Selection and evaluation of plus trees through progeny trials are crucial steps in genetic improvement programmes. Half sib or full sib progeny trials allow the use of additive gene effects and hence, new recombinants can be identified and thereby paving way for further improvement.

A progeny trial plot was established under the present study at Nilambur with 64 families (progenies of 64 plus trees) to evaluate the breeding value of these plus trees. The trial was monitored for growth up to the age of five years after field planting. The results of the present study revealed that there is no influence of geographical origin of the families on growth performance. With respect to girth at breast height, the best cluster of families comprised progenies of plus trees T4, 6 and 24 from Nilambur, 19,136 and 137 from Konni, 105 and 106 from Kannavam, 48 and 150 from Arienkavu, 50 and 104 from Wynad and 121 from Parambikulam.

The phenotypic and genotypic coefficients of variation as well as family heritability were moderate for height and girth in general. The progeny trial can accomplish the purpose of a seed orchard after removing the poor trees and allowing a minimum of 10m spacing between trees.

The present study conducted using microsatellite markers, to determine the genetic similarity between plus tree clones and to exclude close relatives in further improvement programmes, showed possibility of demarcation of various clones from each other even with two microsatellite markers except in two clones where three markers were necessary. The study also showed good genetic variability between clones. The clustering of ten clones revealed that all the clones from Arienkavu belonged to one group. Clone T20 from Konni was closer to Arienkavu clones rather than Nilambur clones. Plus tree clones T1 and T10 from Nilambur formed one cluster while T3 from Nilambur stood separately.

1. INTRODUCTION

The important objective of genetic improvement programme for teak is to enhance the growth rate and tree form so that higher volumes with longer length of clear bole are available in short rotation. Selection and evaluation of plus trees through progeny trials followed by identification of elite trees/ clones are the crucial steps in genetic improvement. The overall expression (phenotype) of an organism is the sum total of its genetic constitution and the environment. Conventional breeding programmes in teak are expected to achieve a remarkable genetic gain of around 15 to 25 per cent in volume as well as tree form (Kjaer *et al*., 2000). Half sib or full sib progeny trials allow use of additive gene effects and hence, new recombinants can be identified and thereby paving way for further improvement (Zobel and Talbert, 1984). Through progeny trials it is possible to estimate the genetic diversity and heritability for each character whereby the breeders can select further breeding strategies. Through these trials selection of best families also could be done. A long term programme for twenty years was initiated in 1999 for the genetic improvement of teak and in the first phase, efforts were made for i) the assessment of status of plus trees selected in Kerala during early 1980s, ii) selection of new plus trees and iii) establishment of progeny trial.

Hence, 61 new plus trees were selected from different Forest Divisions in the first phase of the above programme making a total of 94 plus trees including the remaining 33 plus trees selected during 1980s (Indira and Muralidharan, 2005). Seeds were collected from almost all plus trees and a progeny trial plot was established at Nilambur with 64 families to evaluate the breeding value/ genetic worth of these plus trees for higher productivity and timber quality. This trial was supposed to be assessed up to half / one third of the rotation period so as to allow the plants to express the characters. This progeny trial can also be used for marker assisted selection in future. Measurements at regular intervals will show the long-term trends for evaluating the plus trees to select the elite trees/clones. It is also possible to estimate the inheritance pattern in economically important characters which will help us to chalk out detailed breeding programme for further genetic improvement.

The low genetic variability in breeding populations and seed orchards will hinder further genetic improvement programmes and will also lead to inbreeding depression and

related problems. Hence, the genetic variability between these plus trees (within and between populations) has to be estimated so as to avoid close relatives. Hence, the present project was initiated with the following objectives:

i) to study the genetic variability and inheritance pattern in economically important characters
ii) to select elite trees/ clones
iii) to establish a seedling seed orchard

2. MATERIALS AND METHODS

The variability present in a population determines the success of selection. As mentioned in the introduction, 111 plus trees were selected for teak in Kerala. They are the best phenotypes/performers in the particular populations surveyed. They help to direct the improvement programme in a desired direction by supplying materials for further selection, hybridization or gathering or conserving favourable genes.

Progeny Evaluation

A progeny trial was established in Karulai Range, Nilambur South, where seedlings from 64 plus trees (half sib progenies) were planted in the field. The details of the families (progenies of plus trees) are given in Table 1. They were planted in Randomized Block Design (RBD) with 15 plants per replication in 3 m x 3 m spacing.

Table 1. Details of the Families in the progeny trial

Sl.No.	Plus tree No.	Division	Range	Locality
1	T 1	Nilambur	Nilambur	Aravallikkavu
2	T 4	Nilambur	Edavanna	Edacode
3	T 5	Nilambur	Karulai	Karulai
4	T 6	Nilambur	Karulai	Karulai
5	T 10	Nilambur	Nilambur	Chathanpara
6	T 11	Nilambur	Nilambur	Chathanpara
7	T 17	Konni	Naduvathmuzhy	Naduvathmuzhy
8	T 19	Konni	Naduvathmuzhy	Naduvathmuzhy
9	T 21	Konni	Naduvathmuzhy	Naduvathmuzhy
10	T 24	Nilambur	Edavanna	Edacode
11	T 26	Arienkavu	Arienkavu	Arienkavu
12	T 27	Arienkavu	Arienkavu	Arienkavu
13	T 33	Arienkavu	Arienkavu	Anakuthy
14	T 34	Arienkavu	Arienkavu	Anakuthy
15	T 36	Arienkavu	Arienkavu	Anakuthy
16	T 37	Arienkavu	Arienkavu	Anakuthy
17	T 38	Arienkavu	Arienkavu	Anakuthy
18	T 41	Arienkavu	Arienkavu	Anchumucku
19	T 42	Arienkavu	Arienkavu	Anchumucku
20	T 43	Konni	Naduvathmuzhy	Naduvathmuzhy
21	T 46	Arienkavu	Arienkavu	Anakuthy
22	T 47	Arienkavu	Arienkavu	Anakuthy
23	T 48	Arienkavu	Arienkavu	Anchumucku

24	T 49	Wynad	Begur	Tholpetty
25	T 50	Wynad	Begur	Tholpetty
26	T 101	Kanjangad	Kanjangad	Kottamala
27	T 102	Wynad	Begur	4th Mile
28	T 104	Wynad	Begur	Bhoothackal
29	T 105	Kannavam	Kannavam	Kuttappalam
30	T 106	Kannavam	Kannavam	Research Plot
31	T 107	Kannavam	Kannavam	Nedumpoil
32	T 108	Nilambur	Nilambur	Chaliyar Mucku
33	T 109	Nilambur	Nilambur	Chaliyar Mucku
34	T 114	Nilambur	Vazhikkadavu	Nellikutha
35	T 116	Nilambur	Nilambur	Aravallikavu
36	T 120	Parambik	Sungam	Parambikulam
37	T 121	Parambikulam	Sungam	Parambikulam
38	T 122	Parambikulam	Sungam	Parambikulam
39	T 123	Parambikulam	Sungam	Parambikulam
40	T 124	Parambikulam	Sungam	Amakundu
41	T 125	Parambikulam	Sungam	Thunakadavu
42	T 127	Parambikulam	Sungam	Preserv. plot
43	T 128	Parambikulam	Sungam	Thekkady
44	T 130	Malayattur	Kuttampuzha	Malakkappara
45	T 132	Konni	Konni	Aruvapalam
46	T 133	Konni	Konni	Kanjirappara
47	T 134	Konni	Konni	Kanjirappara
48	T 135	Konni	Konni	Avolikuzhy
49	T 136	Konni	Konni	Umayamkuppa
50	T 137	Konni	Mannarappara	Chempala
51	T1 38	Konni	Mannarappara	Kadampupara
52	T 139	Konni	Mannarappara	Chittar
53	T 140	Konni	Mannarappara	Aruthala
54	T 141	Konni	Naduvathmuzhy	Kiliyara
55	T1 42	Konni	Naduvathmuzhy	Vayakkara
56	T 143	Konni	Naduvathmuzhy	Manneera
57	T144	Konni	Naduvathmuzhy	Manneera
58	T 145	Arienkavu	Arienkavu	Thalappara
59	T 146	Arienkavu	Arienkavu	Thalappara
60	T 147	Arienkavu	Arienkavu	Edappalayam
61	T148	Arienkavu	Arienkavu	Edappalayam
62	T 149	Arienkavu	Arienkavu	Edappalayam
63	T 150	Arienkavu	Arienkavu	Chenagiri
64	T 151	Arienkavu	Arienkavu	Chenagiri

Though the area was protected by electric fence by the Forest Department, some of the plants were destroyed by elephants. Hence, the percentage of survival could not be assessed. Observations on growth were recorded at intervals on height, basal girth and girth at breast height in randomly selected 10 intact plants per block. The data collected were statistically analysed through SPSS package (version 10.0). The analysis of variance (ANOVA) and the mean comparison test were also carried out. Family heritability was estimated following Wright (1976). The phenotypic and genotypic coefficient of variation was also estimated following Singh and Chaudhary (1985).

Genetic variability among plus tree clones through DNA analysis

Ten teak clones were selected at the teak clonal seed orchard established during 1981-82 by Kerala Forest Research Institute (KFRI) at Palappilly. The source materials of the clones were plus trees. The clones selected for the present study were KFRI T1, T3, T10, T12 (all from Nilambur), T20 (from Konni) and KFRI T26, T28, T31, T34, T39 (from Arienkavu).

The young leaf samples were collected from these 30 clonal grafts (10 clones x 3 ramets). DNA extraction was done following modified CTAB method (Doyle and Doyle, 1987). These samples were DNA fingerprinted along with the reference clones (having different alleles) used for an earlier study (Indira *et al*., 2010). Three microsatellite markers AC01, AC28 and AG14, (EMBL sequences) selected on the basis of polymorphism, were used for amplification. The amplified products were subjected to 4.5 % denaturing gel electrophoresis in polyacrylamide gel (PAGE) for one and a half hours at 90W in a Sequi-Gen GT Sequencing Cell (BIO-RAD, USA). The electrophoresis was carried out in 1X TBE buffer. After the electrophoresis, the gel was silver stained to visualize the bands clearly. Photographs of gels were taken.

The bands were scored for allelic polymorphism based on the position of the bands as well as for heterozygosity/ homozygosity. According to the position of bands, alleles were differentiated for each marker (locus) and numbers were given based on the reference clones from KFRI. Then the alleles were scored for each locus in all the 30 samples (all the three

ramets of each of the ten clones). After the identification of alleles, the total number of alleles was counted at each locus in each sample and overall samples.

The genetic distance between clones was estimated through Fstat and a dendrogram was prepared following UPGMA method of clustering.

3. RESULTS AND DISCUSSION

Progeny Evaluation

On analysis of early height growth, highly significant difference could be seen between families (Table 2). Up to third year there was no significant difference for basal girth and then at fourth and fifth years, there was significant difference. Families were not significantly different for GBH till 4th year while there was highly significant difference at 5th year. The mean performance of each family with regard to height at different ages is given in Table 3. A view of the performance of the progenies is given in Fig.1.

Table 2. Analysis of variance for growth parameters at different intervals

Source	DF	M.S.S.	F	Significance
Height 3 months after planting				
Family	63	298.286	2.331	0.000**
Replication	2	255.355	1.995	0.140
Height 1.5 years after planting				
Family	63	0.419	3.112	0.000**
Replication	2	0.192	1.426	0.244
Basal girth at 3 year				
Family	63	18.927	1.303	0.105ns
Replication	2	29.617	2.039	0.134
Basal girth at 3.5 year				
Family	63	20.948	1.363	0.072ns
Replication	2	11.366	0.739	0.480
Basal girth at 4th year				
Family	63	28.104	1.622	0.011*
Replication	2	24.880	1.436	0.242
Basal girth at 5th year				
Family	63	37.642	2.183	0.000**
Replication	2	10.044	0.582	0.560
GBH at 3.5 year				
Family	63	11.599	1.108	0.310ns
Replication	2	7.487	0.715	0.491
GBH at 4th year				
Family	63	15.624	1.228	0.165ns
Replication	2	8.525	0.670	0.513
GBH at 5th year				
Family	63	22.917	1.696	0.006**
Replication	2	6.567	0.486	0.616

**, * significant difference between families at 1% and 5 % level

Many of the families which had very good height three months after field planting have shown poor height growth at the age of 1.5 years. But T132 from Konni was best performer during both the periods. T128 from Parambikulam also showed maximum height growth along with T132 (Table 3 and Fig.2).

Regarding basal girth, T105 from Kannavam T42 from Arienkavu, T4 from Nilambur, T137 from Konni showed maximum growth (Table 4 and Fig.3). With respect to girth at breast height (GBH), again T105, T4 showed higher GBH (Table 5 and Fig.4) along with T150 (Arienkavu) and T121 (Parambikulam).

Fig.1. A view of the early performance of progenies

Table 3. Mean height growth of families after field planting

Height at 3 months (in cm)		**Height at 18 months (in m)**	
Family	**Mean**	**Family**	**Mean**
T 36	62.6[a]	T 132	3.51[a]
T 34	61.5[ab]	T 128	3.37[a]
T 132	59.67[abc]	T 127	3.01[ab]
T 33	58.1[abcd]	T 130	2.99[abc]
T 49	56.67[abcde]	T 50	2.47[bcd]
T 101	55 [abcdef]	T 4	2.46[bcd]
T 105	52.93[abcdefg]	T 133	2.44[bcde]
T 21	52.83[abcdefg]	T 33	2.39[bcde]
T 134	52.33[abcdefgh]	T 6	2.36[bcdef]
T 4	49.33[abcdefghi]	T139	2.35[bcdef]
T 50	49.3[abcdefghi]	T 5	2.34[bcdef]
T 106	48.87[abcdefghi]	T 11	2.32[bcdef]
T 151	48.53[abcdefghi]	T 19	2.3[cdef]
T 150	47.17[abcdefghij]	T 123	2.28[def]
T 102	47.1[abcdefghij]	T 147	2.25[def]
T 146	46.57[abcdefghij]	T 116	2.23[def]
T 104	46.37[abcdefghij]	T 134	2.23[def]
T 124	45.83[abcdefghij]	T 48	2.23[def]
T 148	43.7[abcdefghijk]	T 102	2.23[def]
T 41	42.43[abcdefghijk]	T 142	2.23[def]
T 149	42.33[abcdefghijk]	T 114	2.21[def]
T 6	42.23[abcdefghijk]	T 101	2.2[def]
T 19	42.17[abcdefghijk]	T 140	2.19[def]
T 133	41.93[abcdefghijk]	T 136	2.18[def]
T 136	41.67[abcdefghijk]	T 108	2.1767[defg]
T 127	40.6[abcdefghijk]	T 21	2.1733[defg]
T 144	40.13[abcdefghijk]	T 137	2.14[defg]
T 17	39.4[bcdefghijk]	T 149	2.13[defg]
T 26	39.27[bcdefghijk]	T 148	2.13[defg]
T 43	38.67[bcdefghijk]	T 109	2.11[defg]
T 1	38.33[bcdefghijk]	T 151	2.11[defg]
T 122	38.27[cdefghijk]	T 150	2.07[defg]
T 137	37.43[cdefghijk]	T 41	2.05[defg]
T 140	37.1[cdefghijk]	T 49	2.03[defg]
T 47	36.93[cdefghijk]	T 120	2.03[defg]
T 142	36.67[cdefghijk]	T 143	2.03[defg]

T 114	36.17^{defghijk}	T 27	2.01^{defg}
T 141	35.8^{defghijk}	T 34	1.99^{defg}
T 108	35.5^{defghijk}	T 24	1.98^{defg}
T 116	35.43^{defghijk}	T 144	1.98^{defg}
T 128	34.6^{efghijk}	T 1	1.98^{defg}
T 46	33.5^{efghijk}	T 107	1.95^{defg}
T 42	33.1^{fghijk}	T 122	1.95^{defg}
T 37	32.43^{fghijk}	T 124	1.93^{defg}
T 139	31.77^{fghijk}	T 47	1.93^{defg}
T 138	31.27^{ghijk}	T 46	1.92^{defg}
T 123	31.17^{ghijk}	T 42	1.91^{defg}
T 107	31^{ghijk}	T 121	1.88^{defg}
T 109	30.8^{ghijk}	T 36	1.87^{defg}
T 125	30.33^{ghijk}	T 38	1.86^{defg}
T 5	30.23^{ghijk}	T 135	1.85^{defg}
T 27	30.17^{ghijk}	T 141	1.83^{defg}
T 120	30^{ghijk}	T 104	1.8^{defg}
T 130	29.93^{ghijk}	T 106	1.79^{defg}
T 121	29.67^{ghijk}	T 125	1.75^{defg}
T 147	29.37^{ghijk}	T 26	1.75^{defg}
T 148	28.9^{hijk}	T 17	1.73^{defg}
T 135	27.93^{ijk}	T 146	1.71^{defg}
T 38	27.5^{ijk}	T 10	1.7^{defg}
T 143	26.53^{ijk}	T 43	1.7^{defg}
T 10	25.9^{ijk}	T 37	1.68^{efg}
T 24	24.43^{ijk}	T 138	1.67^{efg}
T 145	23.43^{ijk}	T 145	1.6^{fg}
T 11	21.7^{k}	T 105	1.41^{g}
Grand mean	**39.196**		**2.11**

Mean values superscribed with different letters are significantly different (Sig. 0.055)
Mean values with shading are lower than the grand mean

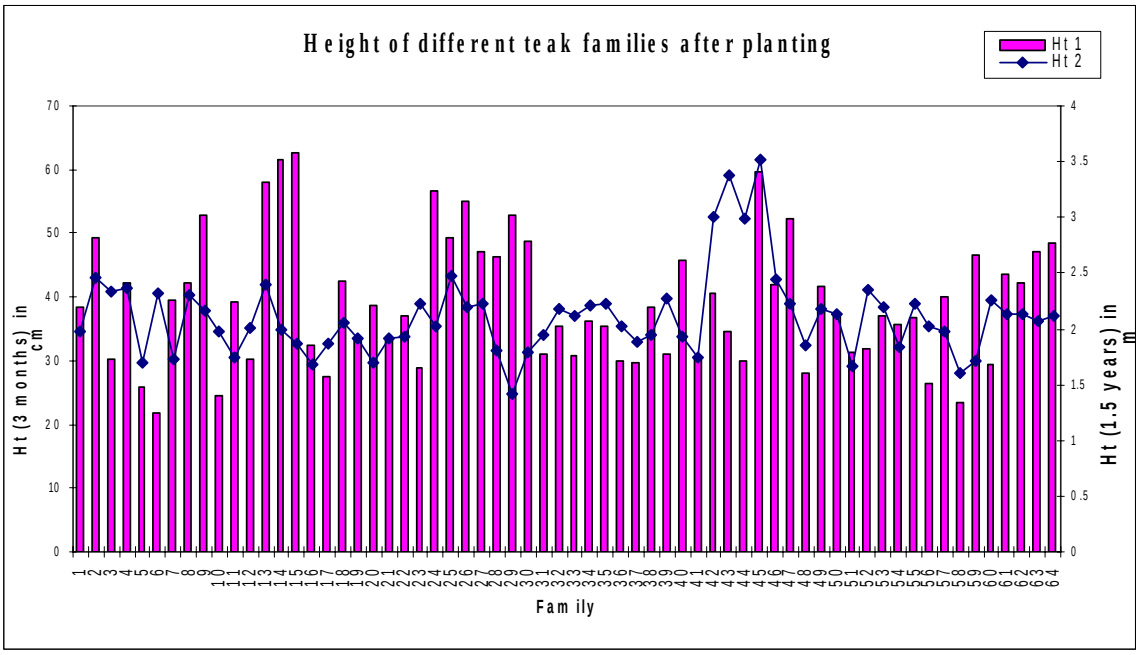


Fig.2. Height growth in different families after field planting

Table 4. Basal girth of different families at different ages

Basal girth at 3 year		Basal girth at 3.5 year		Basal girth at 4 year		Basal girth at 5 yr	
Family	**Mean**	**Fam.**	**Mean**	**Fam.**	**Mean**	**Fam.**	**Mean**
T150	19.50[a]	T105	22.00a	T42	26.00a	T105	31.00[a]
T105	18.33[ab]	T150	21.83ab	T4	25.83a	T137	30.67[ab]
T4	17.43[abc]	T4	21.00abc	T105	25.75a	T150	30.33[abc]
T114	16.83[abcd]	T121	21.00[abc]	T46	25.43[ab]	T106	29.50[abcd]
T49	16.67[abcde]	T42	21.00[abc]	T137	25.33[abc]	T36	29.17[abcde]
T42	16.67[abcde]	T136	20.50[abcd]	T150	25.33[abc]	T4	29.00[abcde]
T104	16.60[abcde]	T6	20.33[abcd]	T50	23.67[abcd]	T42	29.00[abcde]
T121	16.20[abcde]	T137	20.33[abcd]	T106	23.00[abcde]	T109	28.50[abcde]
T127	16.17[abcde]	T10	20.00[abcd]	T136	23.00[abcde]	T121	28.27[abcde]
T136	16.00[abcde]	T50	19.67[abcde]	T109	22.83[abcde]	T10	27.33[abcdef]
T19	15.50[abcde]	T106	19.67[abcde]	T104	22.50[abcde]	T24	27.33[abcdef]
T106	15.33[abcde]	T104	19.50[abcdef]	T24	22.50[abcde]	T17	27.17[abcdefg]
T144	15.13[abcde]	T24	19.00[abcdefg]	T121	22.27[abcdef]	T19	27.17[abcdefg]
T146	15.03[abcde]	T127	18.53[abcdefg]	T17	22.17[abcdef]	T124	27.00[abcdefgh]
T137	15.00[abcde]	T46	18.50[abcdefg]	T48	21.77[abcdef]	T34	27.00[abcdefgh]
T134	14.97[abcde]	T109	18.43[abcdefg]	T10	21.33[abcdefg]	T114	26.67[abcdefghi]
T6	14.87[abcde]	T120	18.33[abcdefg]	T6	21.27[abcdefg]	T135	26.17[abcdefghij]
T46	14.83[abcde]	T134	18.33[abcdefg]	T36	21.17[abcdefg]	T148	26.17[abcdefghij]
T10	14.67[abcde]	T33	18.33[abcdefg]	T135	21.17[abcdefg]	T50	26.00[abcdefghij]
T140	14.67[abcde]	T135	18.00[abcdefgh]	T149	21.10[abcdefg]	T6	25.67[abcdefghijk]
T33	14.20[abcde]	T43	18.00[abcdefgh]	T127	21.00[abcdefg]	T136	25.50[abcdefghijk]
T43	14.10[abcde]	T48	17.83[abcdefgh]	T33	21.00[abcdefg]	T149	25.50[abcdefghijk]
T132	14.00[abcdef]	T17	17.77[abcdefgh]	T134	20.83[abcdefg]	T33	25.33[abcdefghijk]
T138	13.97[abcdef]	T148	17.67[abcdefgh]	T148	20.83[abcdefg]	T46	25.33[abcdefghijk]
T17	13.87[abcdef]	T19	17.60[abcdefgh]	T114	20.67[abcdefg]	T5	25.27[abcdefghijk]

T139	13.77^{abcdef}	T140	17.33^{abcdefgh}	T124	20.50^{abcdefg}	T140	25.17^{abcdefghijk}
T34	13.77^{abcdef}	T141	17.33^{abcdefgh}	T140	20.50^{abcdefg}	T104	25.00^{abcdefghijk}
T149	13.67^{abcdef}	T132	17.17^{abcdefgh}	T34	20.50^{abcdefg}	T48	24.67^{abcdefghijk}
T41	13.50^{abcdef}	T49	17.00^{abcdefgh}	T49	20.43^{abcdefg}	T138	24.50^{abcdefghijk}
T109	13.33^{abcdef}	T124	17.00^{abcdefgh}	T145	20.00^{abcdefg}	T151	24.00^{abcdefghijk}
T24	13.20^{bcdef}	T37	17.00^{abcdefgh}	T37	20.00^{abcdefg}	T145	23.83^{abcdefghijk}
T5	13.00^{abcdef}	T36	16.93^{abcdefgh}	T5	19.83^{abcdefg}	T49	23.67^{abcdefghijk}
T124	13.00^{abcdef}	T114	16.87^{abcdefgh}	T19	19.83^{abcdefg}	T11	23.67^{abcdefghijk}
T147	13.00^{bcdef}	T5	16.83^{abcdefgh}	T43	19.67^{abcdefg}	T134	23.67^{abcdefghijk}
T37	13.00^{abcdef}	T144	16.83^{abcdefgh}	T132	19.33^{abcdefg}	T142	23.00^{abcdefghijk}
T48	13.00^{abcdef}	T139	16.67^{abcdefgh}	T151	19.17^{abcdefg}	T144	23.00^{abcdefghijk}
T21	12.80^{abcdef}	T34	16.67^{abcdefgh}	T144	19.10^{abcdefg}	T146	23.00^{abcdefghijk}
T130	12.60^{abcdef}	T146	16.53^{abcdefgh}	T11	19.00^{abcdefg}	T38	23.00^{abcdefghijk}
T36	12.53^{abcdef}	T149	16.17^{abcdefgh}	T41	18.80^{abcdefg}	T122	22.83^{abcdefghijk}
T133	12.43^{abcdef}	T116	16.00^{abcdefgh}	T141	18.67^{abcdefg}	T141	22.83^{abcdefghijk}
T120	12.33^{abcdef}	T145	16.00^{abcdefgh}	T21	18.67^{abcdefg}	T21	22.67^{abcdefghijk}
T148	12.23^{abcdef}	T41	15.90^{abcdefgh}	T138	18.50^{abcdefg}	T139	22.50^{abcdefghijk}
T141	12.10^{abcdef}	T11	15.67^{abcdefgh}	T146	18.50^{abcdefg}	T43	22.33^{abcdefghijk}
T135	12.00^{abcdef}	T130	15.67^{abcdefgh}	T142	18.33^{abcdefg}	T37	22.17^{bcdefghijk}
T145	12.00^{abcdef}	T133	15.67^{abcdefgh}	T120	18.27^{abcdefg}	T116	22.00^{bcdefghijk}
T151	12.00^{abcdef}	T138	15.67^{abcdefgh}	T130	18.17^{abcdefg}	T133	22.00^{bcdefghijk}
T27	12.00^{abcdef}	T142	15.67^{abcdefgh}	T133	18.17^{abcdefg}	T127	21.67^{cdefghijk}
T50	11.83^{abcdef}	T1	15.33^{abcdefgh}	T139	18.17^{abcdefg}	T147	21.67^{cdefghijk}
T142	11.67^{abcdef}	T151	15.33^{abcdefgh}	T116	17.67^{abcdefg}	T120	21.50^{defghijk}
T128	11.47^{bcdef}	T125	15.00^{abcdefgh}	T38	17.50^{abcdefg}	T27	21.50^{defghijk}
T116	11.43^{bcdef}	T143	15.00^{abcdefgh}	T27	17.00^{bcdefg}	T132	21.33^{defghijk}
T125	10.93^{bcdef}	T27	15.00^{abcdefgh}	T125	16.67^{cdefg}	T107	21.00^{defghijk}
T101	10.67^{bcdef}	T122	14.33^{abcdefgh}	T147	16.67^{cdefg}	T26	21.00^{defghijk}
T107	10.60^{bcdef}	T107	14.00^{abcdefgh}	T47	16.67^{cdefg}	T130	20.67^{efghijk}
T1	10.50^{bcdef}	T101	13.67^{bcdefgh}	T143	16.17^{defg}	T41	20.43^{efghijk}
T123	10.50^{bcdef}	T128	13.67^{bcdefgh}	T101	16.00^{defg}	T123	19.00^{fghijk}
T11	10.33^{bcdef}	T147	13.50^{cdefgh}	T107	16.00^{defg}	T125	18.67^{fghijk}
T143	10.33^{bcdef}	T38	13.50^{cdefgh}	T1	15.83^{defg}	T1	18.50^{ghijk}
T26	10.00^{cdef}	T47	13.33^{cdefgh}	T122	15.67^{defg}	T101	18.33^{hijk}
T122	9.33^{def}	T123	12.50^{defgh}	T26	15.33^{defg}	T108	18.33^{hijk}
T47	9.30^{def}	T102	11.67^{efgh}	T128	15.00^{defg}	T128	18.11^{ijk}
T102	8.77^{ef}	T26	11.33^{fgh}	T123	14.83^{efg}	T47	17.67^{jk}
T38	8.67^{ef}	T21	11.00^{gh}	T108	13.67^{fg}	T143	17.17^{k}
T108	6.07^{f}	T108	9.83^{h}	T102	13.00^{g}	T102	17.00^{k}
Mean	**13.19**		**16.81**		**19.74**		**23.89**

Mean values superscribed with different letters are significantly different
Mean values with shading are lower than the grand mean

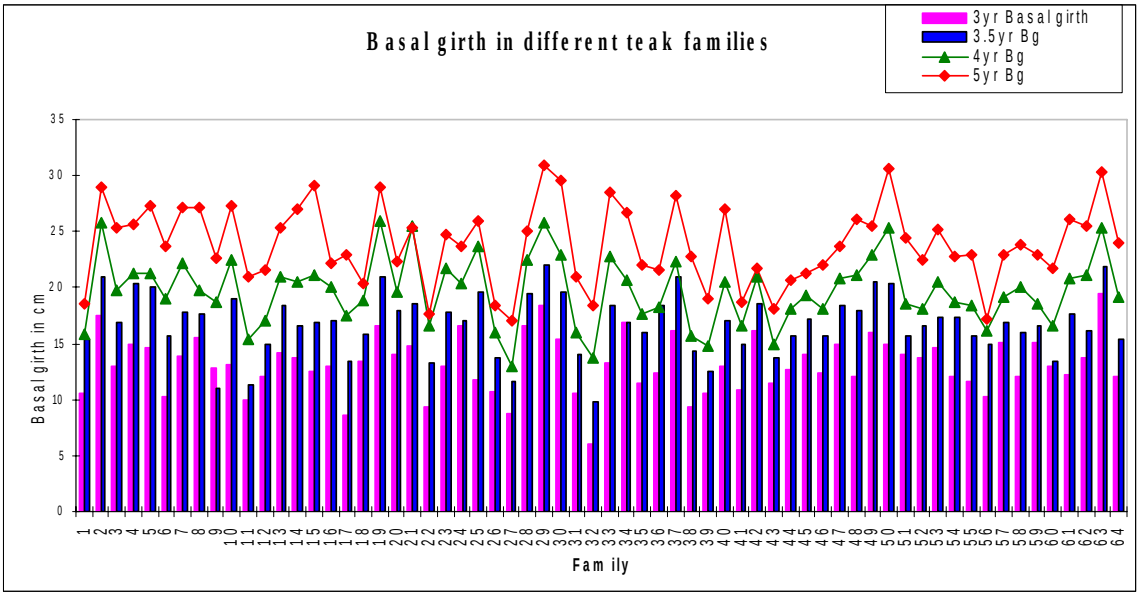


Fig. 3. Basal girth at various years in different families

Table 5. GBH in different families at various ages

GBH 3. 5year		GBH 4 year		GBH 5 year	
Family	Mean	Family	Mean	Family	Mean
T136	14.67[a]	T4	17.17[a]	T105	20.67[a]
T121	14.00[ab]	T136	16.67[ab]	T150	20.50[a]
T4	13.83[abc]	T137	16.17[abc]	T121	20.27[ab]
T6	13.67[abc]	T50	15.67[abcd]	T4	20.00[abc]
T127	13.00[abcd]	T105	15.67[abcd]	T24	19.83[abc]
T105	12.83[abcd]	T121	15.50[abcd]	T106	19.67[abcd]
T104	12.67[abcd]	T106	15.17[abcde]	T136	19.67[abcd]
T19	12.50[abcd]	T104	15.00[abcde]	T34	19.50[abcd]
T137	12.33[abcd]	T6	14.83[abcde]	T50	19.33[abcde]
T43	12.33[abcd]	T24	14.83[abcde]	T137	19.33[abcde]
T50	12.00[abcd]	T19	14.83[abcde]	T6	18.93[abcdef]
T145	12.00[abcd]	T140	14.67[abcde]	T19	18.50[abcdef]
T48	12.00[abcd]	T150	14.67[abcde]	T140	18.33[abcdef]
T106	11.67[abcde]	T127	14.50[abcde]	T145	18.33[abcdef]
T109	11.67 [abcde]	T48	14.43[abcde]	T42	18.33[abcdef]
T148	11.67[abcde]	T34	14.00[abcdef]	T10	18.00[abcdef]
T24	11.33[abcde]	T42	13.83[abcdef]	T48	17.83[abcdef]
T141	11.33[abcde]	T46	13.67[bcdef]	T124	17.67[abcdef]

T150	11.33^{abcde}	T49	13.50^{bcdef}	T135	17.50^{bcdef}
T10	11.00^{abcde}	T141	13.50^{abcdef}	T17	17.50^{abcdef}
T116	10.67^{abcde}	T145	13.50^{abcdef}	T148	17.50^{abcdef}
T144	10.57^{abcde}	T43	13.43^{abcdef}	T109	17.33^{abcdef}
T134	10.50^{abcde}	T134	13.33^{abcdef}	T141	17.33^{abcdef}
T135	10.50^{abcde}	T109	13.17^{abcdef}	T5	17.27^{abcdef}
T130	10.33^{abcde}	T17	13.00^{abcdef}	T46	17.17^{abcdef}
T132	10.33^{abcde}	T5	12.83^{abcdef}	T149	16.83^{abcdef}
T42	10.33^{abcde}	T135	12.83^{abcdef}	T104	16.67^{abcdef}
T120	10.17^{abcde}	T38	12.83^{abcdef}	T151	16.67^{abcdef}
T17	10.17^{abcde}	T11	12.67^{abcdef}	T49	16.50^{abcdef}
T46	10.17^{abcde}	T124	12.67^{abcdef}	T43	16.50^{abcdef}
T49	10.00^{abcde}	T149	12.67^{abcdef}	T36	16.43^{abcdef}
T114	10.00^{abcde}	T36	12.67^{abcdef}	T138	16.17^{abcdefg}
T151	10.00^{abcde}	T116	12.33^{abcdef}	T134	16.10^{abcdefg}
T5	9.83^{abcde}	T130	12.33^{abcdef}	T38	16.00^{abcdefg}
T41	9.83^{abcde}	T10	12.17^{abcdef}	T11	15.33^{abcdefg}
T11	9.67^{abcde}	T21	12.17^{abcdef}	T139	15.33^{abcdefg}
T139	9.67^{abcde}	T132	12.10^{abcdef}	T144	15.33^{abcdefg}
T21	9.67^{abcde}	T144	12.07^{abcdef}	T21	15.33^{abcdefg}
T149	9.67^{abcde}	T37	12.03^{abcdef}	T37	15.33^{abcdefg}
T146	9.50^{abcde}	T133	11.67^{abcdef}	T122	15.00^{abcdefg}
T37	9.50^{abcde}	T148	11.67^{abcdef}	T132	14.83^{abcdefg}
T107	9.33^{abcde}	T41	11.43^{abcdef}	T146	14.77^{abcdefg}
T1	9.33^{abcde}	T120	11.33^{abcdef}	T114	14.67^{abcdefg}
T138	9.33^{abcde}	T139	11.33^{abcdef}	T116	14.67^{abcdefg}
T140	9.33^{abcde}	T142	11.33^{abcdef}	T41	14.67^{abcdefg}
T142	9.33^{abcde}	T151	11.33^{abcdef}	T120	14.53^{abcdefg}
T143	9.33^{abcde}	T122	11.00^{abcdef}	T130	14.33^{abcdefg}
T36	9.33^{abcde}	T138	11.00^{abcdef}	T127	14.00^{abcdefg}
T34	9.00^{abcde}	T146	11.00^{abcdef}	T142	14.00^{abcdefg}
T133	8.83^{abcde}	T33	11.00^{abcdef}	T33	13.67^{abcdefg}
T101	8.67^{abcde}	T107	10.83^{abcdef}	T1	13.33^{abcdefg}
T33	8.67^{abcde}	T101	10.33^{abcdef}	T133	13.00^{abcdefg}
T47	8.67^{abcde}	T1	10.00^{abcdef}	T107	12.67^{bcdefg}

T38	8.33^{abcde}	T143	9.83^{abcdef}	T27	12.67^{bcdefg}
T124	8.00^{abcde}	T26	9.67^{abcdef}	T108	12.50^{cdefg}
T128	7.67^{bcde}	T27	9.67^{abcdef}	T123	12.50^{cdefg}
T27	7.50^{bcde}	T47	9.67^{abcdef}	T147	12.50^{cdefg}
T122	7.33^{bcde}	T114	9.17^{bcdef}	T101	12.33^{cdefg}
T26	7.33^{bcde}	T123	8.67^{cdef}	T128	11.94^{defg}
T108	7.17^{bcde}	T125	8.67^{cdef}	T26	11.67^{efg}
T125	7.00^{cde}	T128	8.67^{cdef}	T125	11.33^{fg}
T123	6.67^{de}	T108	8.50^{def}	T143	11.33^{fg}
T147	6.67^{de}	T147	7.83^{ef}	T47	11.33^{fg}
T102	5.00^{e}	T102	6.67^{f}	T102	8.67^{g}
Mean of all families	**10.11**		**12.36**		**15.90**

Mean values superscribed with different letters are significantly different
Mean values with shading are lower than the grand mean

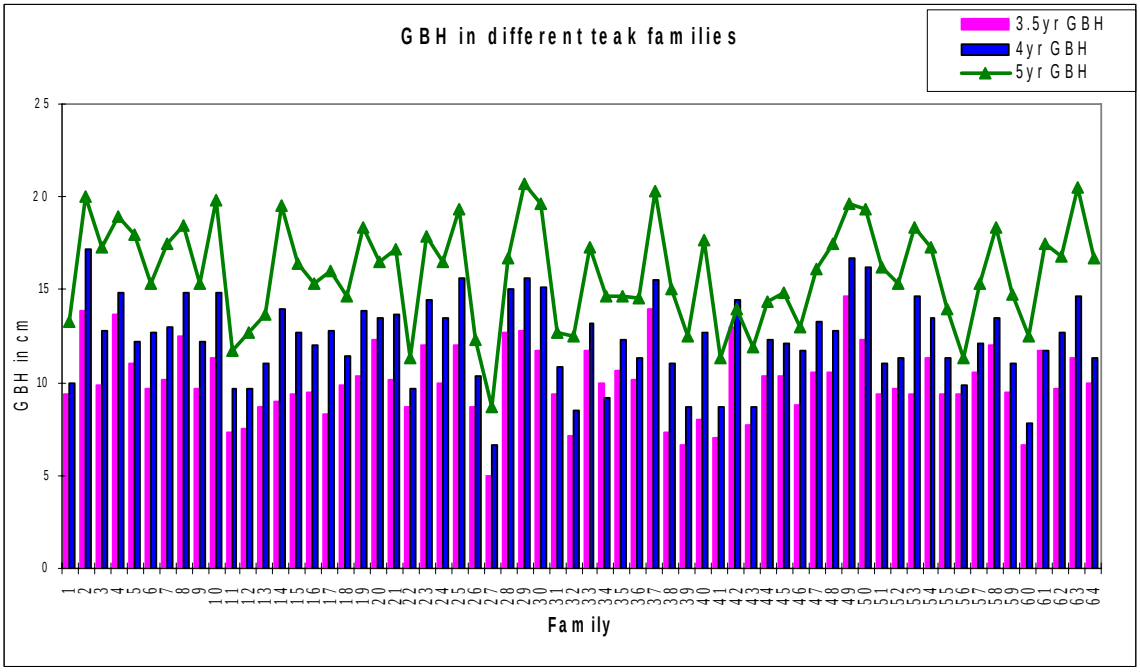


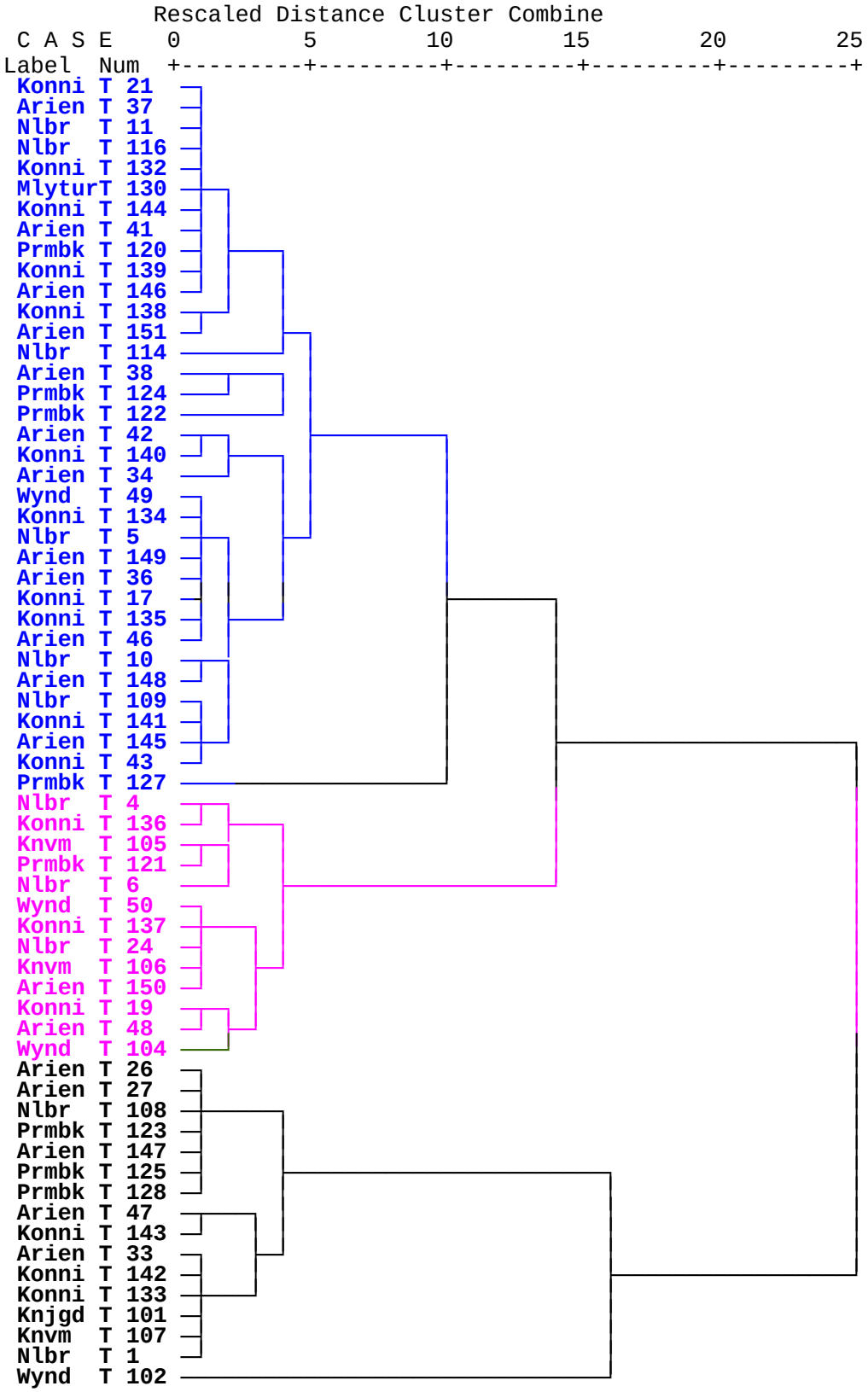
Fig. 4. GBH at different ages

Euclidean clustering using average linkage between groups with respect to GBH at three ages shows three groups on allowing 25% variability within a group. There was no influence of geographic location on this aspect as can be seen from the dendrogram (Fig. 5). The best cluster comprised families T4, 6 and 24 from Nilambur, 19,136 and 137 from

Konni, 105 and 106 from Kannavam, 48 and 150 from Arienkavu, 50 and 104 from Wynad and 121 from Parambikulam.

There was a negative correlation (-0.226) between height and GBH. The families which had a high value for early height growth did not show a high value for GBH and Vice Versa (Fig. 6).

Fig. 5. Dendrogram using average linkage between groups with respect to GBH



--- Good families, --- Moderate, --- Poor performers

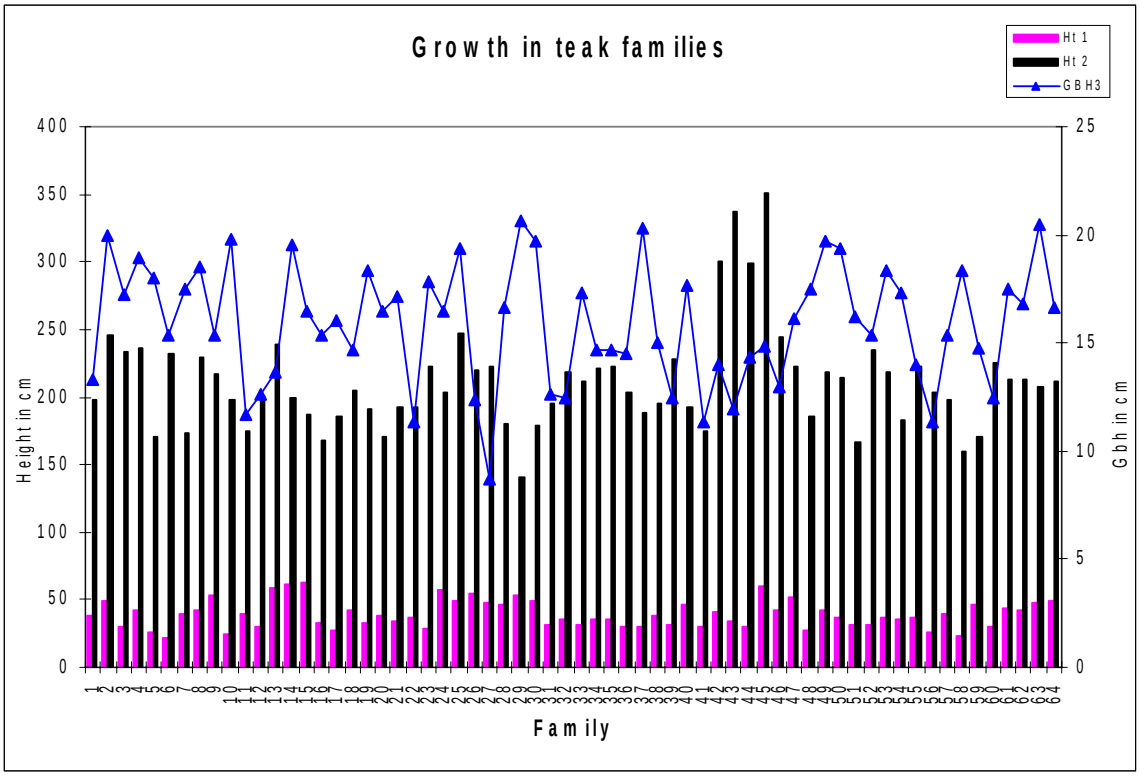


Fig. 6. Relation between height growth and GBH

The phenotypic and genotypic coefficients of variation (PCV and GCV) were moderate for all the characters under study. However, GBH at 5th year had high PCV (Table 6). The magnitudes of PCV were not very close to the corresponding magnitudes of GCV. This indicates the influence of environmental components on these characters especially on GBH.

The Family heritability estimated showed a moderate heritability for the growth characters under study. However, height had higher heritability than girth. On estimation of the heritability using 16 half-sib families up to an age of 8 years, Lakshmikantham *et al.* (1974) reported high family heritability for height and girth though decreasing with age.

Since teak exhibits high within population diversity and also shows genetic variation even between seeds of the same fruit (Indira *et al*., 2010), the progeny trial can accomplish the purpose of a seed orchard after removing the poor trees and allowing a minimum of 10m spacing between trees.

Table 6. Coefficient of variation and heritability

Genetic parameter	For Height 1	For Height 2	For basal girth 5 years	For GBH 5 years
PCV (%)	25.44	17.716	18.36	21.72
GCV (%)	19.21	14.58	12.696	14.63
Family heritability (%)	57.1	67.8	47.8	45.35

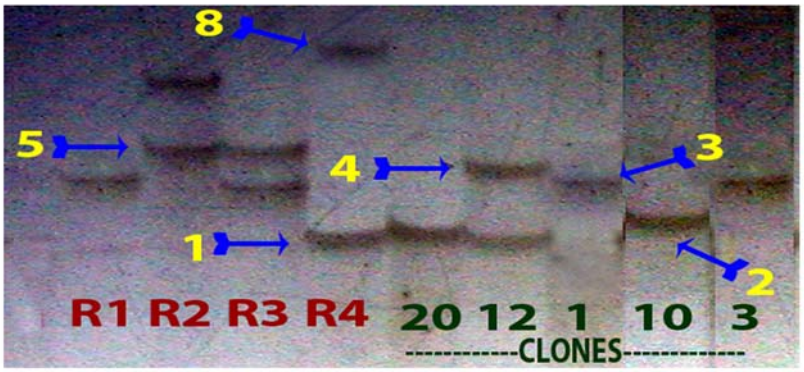
Genetic variation among plus tree clones through DNA analysis

Some of the ramets of clones did not give good results in PAGE (Polyacrylamide gel electrophoresis) and they were excluded from final results. The number of alleles found altogether for ten clones were 4, 4 and 7 for the three microsatellite markers, AC01, AG28 and AG14 respectively. From the analysis of DNA fingerprints, the alleles and the heterozygosity/ homozygosity for particular loci were identified in the ramets/clones (Table 7 and Figure 7).

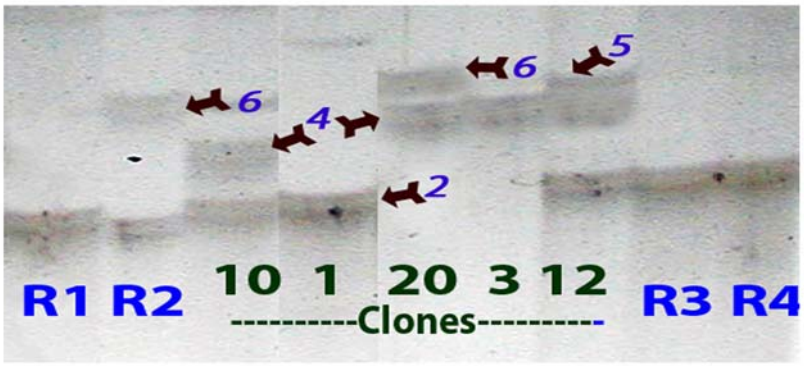
The genotypic data from 10 different clones revealed that all the alleles obtained in Konni clone were also present in Nilambur clones except one allele. Allele number 6 in AC28 present in Konni and Arienkavu was not seen in Nilambur. Allele 3, 4 and 7 for AG14 were seen only in Arienkavu.

Table 7. Alleles obtained for 10 different clones

Clone No.	Markers		
	AC01	AC28	AG14
T1 (Nilambur)	3,3	2,2	1,5
T3 (Nilambur)	3,3	4,4	6,6
T10 (Nilambur)	2,2	2,4	5,5
T12 (Nilambur)	1,4	4,5	2,5
T20 (Konni)	1,1	4,6	6,6
T26 (Arienkavu)	1,4	4,4	7,7
T28 (Arienkavu)	1,1	4,6	4,4
T31 (Arienkavu)	1,1	4,4	1,4
T34 (Arienkavu)	4,4	4,6	2,3
T39 (Arienkavu)	1,4	4,4	1,1
Total number of alleles	4	4	7



AC01



AC28

AG14

Figure 7. DNA fingerprints of clones in PAGE

(R1 to R5- reference clones for detecting the alleles & Clones 1, 3 etc. are Plus tree clones T1, T3)

The different combination of the 15 alleles could differentiate the ten clones very easily. In the present study it was possible to differentiate the various clones from each other even with two microsatellite markers except 2 clones namely T26 and T39. In *Populus grandidentata*, five clones could be differentiated with single locus microsatellite genotypes

(Rahman and Rajora, 2002). Rimbawanto *et al*. (2003) had used SCAR (Sequence characterized amplified region) markers for clonal identification of teak where, out of ten clones six clones had an identical banding pattern within the ramets, whilst the other four clones contained ramets that did not match with the other ramets within the clone. The successful application of molecular markers for distinguishing plant clones depends primarily on the extent of detectable interclonal genetic variability and the availability of suitable molecular markers (Rahman and Rajora, 2002). In the present study there was good genetic variability between clones. All these results indicated the efficiency of the markers for differentiating all the ten clones from each other.

The clustering done through UPGMA (Unweighted Pair Group Method with Arithmetic Mean) showed that all the clones from Arienkavu (T26, 28, 31, 34 and 39) belonged to one group (Fig.8). Clone from Konni was closer to Arienkavu clones rather than Nilambur clones. Clone T3 from Nilambur stood separately while T1 and T10 from Nilambur fell in another cluster.

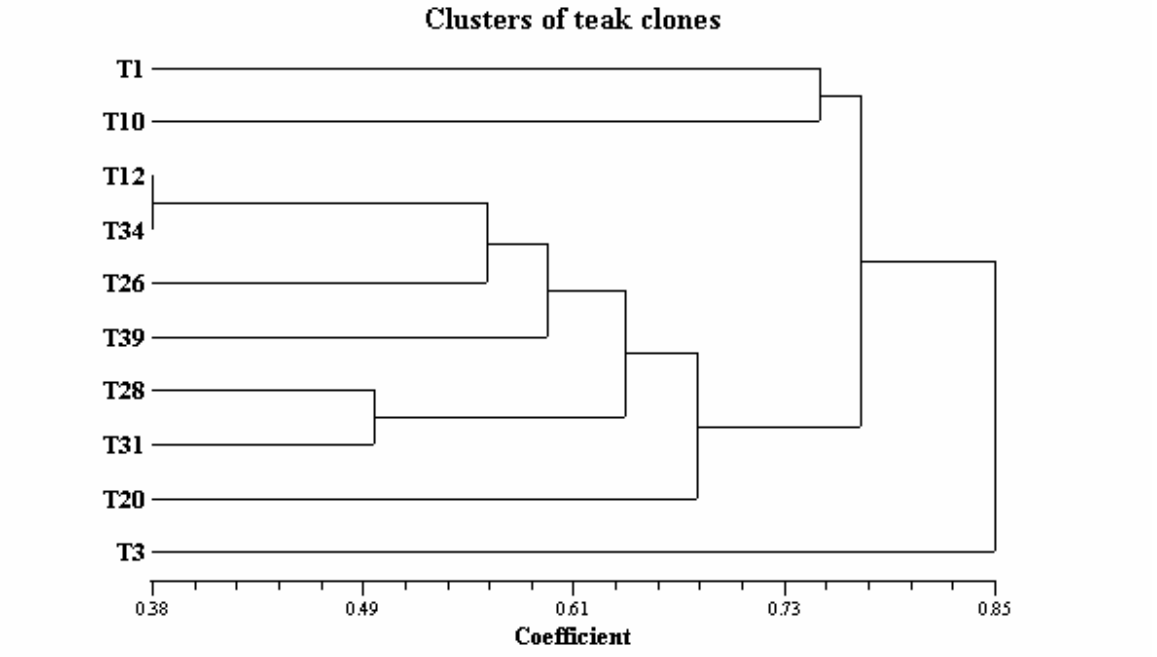


Fig. 8. Dendrogram using UPGMA method of clustering in teak clones

4. SUMMARY AND CONCLUSIONS

A total of 94 plus trees of teak are available in Kerala out of the 111 plus trees selected during early 1980s and 1990s by the Genetics Division of Kerala Forest Research Institute. A progeny trial was established at Nilambur with 64 families (progenies of 64 plus trees) to evaluate the breeding value of these plus trees for higher productivity and tree form. During the present project period this trial was monitored for growth up to the age of five years after field planting. The data were analysed for the comparative performance of the families and for the estimation of phenotypic and genotypic coefficients of variation and heritability.

The results revealed that there is no influence of geographical origin of the families on growth performance. The best cluster (for GBH) comprised plus tree families T4, 6 and 24 from Nilambur, 19,136 and 137 from Konni, 105 and 106 from Kannavam, 48 and 150 from Arienkavu, 50 and 104 from Wynad and 121 from Parambikulam. These plus trees, which are producing progenies with better growth performance, may be assembled in a seed orchard so that they would cross pollinate with each other to produce genetically improved seeds.

The phenotypic and genotypic coefficients of variation as well as family heritability were moderate for height and girth in general. The magnitudes of PCV were not very close to the corresponding magnitudes of GCV. This indicates the influence of environmental components on growth characters especially on GBH. There is a non significant negative correlation between height and girth at breast height.

In order to find out the genetic similarity between plus tree clones and to avoid close relatives in further improvement programmes, studies were conducted using microsatellite markers (DNA markers). The present study showed the possibility of demarcation of the different clones from each other even with two microsatellite markers except in two clones where three markers are necessary. The study also revealed good genetic variability between clones. The clustering of ten clones following UPGMA method showed that all the clones from Arienkavu belonged to one group. Clone from Konni is closer to the geographically nearer Arienkavu clones rather than Nilambur clones. Among the Nilambur clones, T1 and T10 are in one cluster while T3 stands separately.

5. LITERATURE CITED

Doyle, J.J. and Doyle, J.L. 1987. CTAB DNA extraction in plants. *Phytochemical Bulletin* 19, 11-15.

Indira, E.P. and Muralidharan, E.M. 2005. Genetic improvement of teak. *KFRI Research Report* No. 267. KFRI, Peechi. 27p.

Indira, E.P., Balasundaran, M. and Mohanadas, K. 2010. Developing know-how for the improvement and sustainable management of teak genetic resources. *KFRI Research Report* No. 339. KFRI, Peechi. 29p.

Lakshmikantham, D., Rawat, M.S. and Kedharnath, S. 1974. Half-sib analysis of genetic variance in teak. *Ind. J. Genet*. 34A: 413- 418.

Rahman, M.H. and Rajora, O.P. 2002. Microsatellite DNA fingerprinting, Differentiation, and genetic relationships of clones, cultivars, and varieties of six poplar species from three sections of the genus *Populus. Genome* 45: 1083-1094.

Rimbawanto, A., Isoda, K. and Suharyanto 2003. Clonal identification of teak using SCAR markers. *In*: Rimbawanto, A. and Susanto, M. (eds.) *Advances in Genetic Improvement of Tropical Tree Species.* Proc. of the International Conference, Yogyakarta, Indonesia, 1-3 October, 2002. pp. 77-80.

Singh, R.K. and Chaudhary, B.D. 1985. *Biometrical Methods in Quantitative Genetic Analysis.* Kalyani Publishers, New Delhi. 318p.

Wright, J.W. 1976. *Introduction to Forest Genetics*. Acad. Press, London. 463p.

Zobel, B.J. and Talbert, J.T. 1984. *Applied Forest Tree Improvement*. John Wiley and Sons, New York, USA. 505p.