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Genetic improvement of Teak

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ABSTRACT OF PROJECT PROPOSAL

Code : KFRI 319/99

Title : Genetic improvement of Teak

Objectives : 1. Assessment of status of plus trees selected in Kerala
2. Selection of new plus trees
3. Selection (grouping) of plus trees for progeny trials through isozyme studies
4. Identification of elite trees through progeny trials

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E.M.Muralidharan (Objective 3)

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ABSTRACT

Fifty plus trees of teak and 750 ha of seed stands were selected in main teak growing Forest Divisions of Kerala during 1980-`82 in cooperation with the Forest Department. Three pilot teak seed orchards were also established in the northern, central and southern parts of Kerala in a total area of 6.1 ha. A production seed orchard in 28 ha was also established by Forest Department with the technical expertise from KFRI. Though clonal seed orchard establishment progressed very well, low flowering and low fruit production hampered the teak improvement programmes. However, as an interim measure, improved seeds from seed stands are made available. Some of the plus trees selected earlier were felled or fallen and hence, the number of trees are not enough to have a broad genetic base. Also, progeny trial establishment and evaluation of plus trees were not attempted in a systematic way.

This project was initiated to select a few more plus trees and to establish a progeny trial for evaluation of plus trees for their breeding value. This report is on the first phase of the twenty year Teak improvement programme planned for increasing the productivity. Thirty three of the total 50 teak plus trees selected in Kerala during early 1980s were relocated and marked. Sixty two new plus trees were selected from plantations which had crossed half the rotation period and also from natural forests. On an average the selection differential estimated is about 37 per cent for total height and clear bole height and about 32 per cent for girth at breast height. At least 5 to 15 per cent increase in volume is expected by selecting the best families or individuals. A progeny trial was established at Nilambur with 74 families so as to evaluate the plus trees for their breeding value. In the nursery, progenies of plus trees from Konni area have shown better performance, but in the field trial, the same trend was not seen. However, it is too early for an evaluation. The maintenance of the field trial and its evaluation are still continuing in the second phase of the genetic improvement programme. This trial can be converted to a seedling seed orchard after proper evaluation and thinning or can be retained as a breeding population.

Isozyme analysis was initiated with limited number of plus tree clones. The study shows that there is no diversity between the few selected plus trees of Nilambur origin.

INTRODUCTION

The important objective of genetic improvement programme for teak is to improve the growth rate and tree form so that higher volumes with larger length of clear bole are available in short rotation. The loss of timber due to branch knots, flutes, bumps, spiral grains and blisters also is to be reduced. Resistance to insect pests is also desired. Concurrent improvement in the aforementioned features cumulatively contribute to increased productivity of plantations and improved quality of timber for market.

Genetic improvement programmes in teak were initiated in India by the Forest Research Institute (FRI), Dehra Dun in 1962. Based on the methodology developed, action programmes were started to select plus trees, establish seed stands and clonal seed orchards, evaluate plus trees and establish elite seed orchards. As a result, plus tree selection, establishment of seed stands and clonal seed orchards progressed well in the country.

In Kerala, 1500 ha seed stands and 50 plus trees were selected by KFRI scientists in cooperation with the Kerala Forest Department. Three pilot teak seed orchards with 17, 20 and 25 clones in a total area of 6.1 ha were also established (Venkatesh *et al.*, 1986) and technical support was provided to the Forest Department to establish production teak seed orchard in 28 ha.

Though clonal seed orchard establishment progressed very well, low flowering and low fruit production hampered the teak improvement programmes (Indira, 2004). Another obstacle is the low seed germination percentage in teak which comes around 20 to 25 (Indira *et al.*, 1996). The various reasons for low fruit productivity were analysed in detail. It is found that lack of effective pollinators, self incompatibility and fungal attack contribute much for low fruit productivity (Indira and Mohanadas, 2002., Mohanadas *et al.*, 2002)

Evaluation of plus trees, to prove their breeding value, could not be done so far. The overall expression (phenotype) of an organism is the sum total of its genetic constitution and the environment. Through progeny trials it is possible to estimate the genetic diversity and heritability for each character and thereby help the breeders to select further breeding strategies. Through these trials selection of best families also could be done. Hence, this project was taken up as the first phase of the twenty year teak improvement programme with the following objectives:

1. Assessment of status of plus trees selected in Kerala
2. Selection of new plus trees
3. Selection (grouping) of plus trees for progeny trials through isozyme studies
4. Identification of elite trees through progeny trials.

Isozyme analysis has been a powerful tool in biology for several decades. Isozymes are multiple molecular forms of enzymes that have different electrophoretic mobility but which share a common substrate. When tissue extracts are subjected to gel electrophoresis and treated with enzyme specific stains, the enzyme products can be visualised as bands. Genetic interpretation of the number and mobility of these bands can reveal single or multilocus genotypes. The relative simplicity of the technique has made isozymes popular as single gene markers. Isozymes are however only a small proportion of the total number of proteins in an organism and they are influenced by the cellular environment.

An attempt was made in this study to understand the relationship among the plus trees of teak in Kerala using isozyme analysis. The measure of relatedness among the plus trees is expected to help in devising a strategy for conservation, selection and genetic improvement programmes by grouping together of related plus trees. A standardisation of the technique was first required to undertake the study including selection of suitable tissues for extraction, the appropriate isozymes and buffers, electrophoretic technique and visualisation procedure.

REVIEW OF LITERATURE

Conventional breeding programmes in teak is 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). Wellendorf and Kaosa-ard (1988) estimated the possible volume gain from using the seeds from seed production areas at 5-12 per cent. Kjaer and Foster (1996) estimated the gain from one conventional breeding cycle, which probably exceeds 10 per cent compared to using the best available seed sources from SPAs. Since selection differential is one of the two factors, which determines the genetic gain or success of improvement work, intensive selection criteria for plus trees will definitely increase the possible genetic gain.

Plus tree evaluation through progeny trials was not carried out effectively in India because screening of plus trees is a long term process and moreover seed collection from individual plus trees is a difficult task. However, few experiments were conducted, but they were all with very few families. Out of the total 94 plus trees tested so far (including 9 families at Lohara, Maharashtra, 9 families at Mohagata, Maharashtra, 25, 16 and 8 families all at Dhandatopa , Orissa and 10 families at Nilambur, Kerala) 31 have been reported to be good general combiners (Mandal and Chawhaan, 1999). However, these trials did not cross even half or one third rotation to get a clear picture. 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.

Phenotypic variance (σ^2P) is due to the combination of genotypic variance (σ^2G) and environment variance (σ^2E). Two types of genes viz., additive genes (A) and non - additive (NA) genes contribute to the total genetic variance. Clonal forestry allows full use of non additive gene effects like dominance, epitasis, over dominance (hybrid vigour) and also frequent favourable segregants since there is possibility of tapping the true to the parental genotype. 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).

Isozymes have become very popular as genetic markers in many facets of plant breeding and genetics, including studies of gene flow, mating system, levels of genetic variability and systematics and phylogeny (El-Kassaby and Ritland, 1998). The usefulness of isozymes as gene markers in population genetic studies was first recognized during 1960s. These biochemical markers were used as genetic markers in forest tree species from early 1970s, when several groups of forest geneticists began to study within population genetic diversity and also genetic differentiation among tree populations (Bergmann and Hattemar, 1998). A number of studies on isozyme genetic variation in broad leaved forest tree species appeared from early 1980s onwards. Researchers have successfully used isozyme analysis on teak for identification of clones (Kumaravelu, 1979) and for understanding the mating system and genetic structure of teak provenances (Kertadikara and Prat, 1995). In the study conducted by Kumaravelu (1979) the isozyme Esterase was used to characterize four clones of *Tectona grandis*.

MATERIALS AND METHODS

The variability present in a population determines the success of selection. Plus trees are the best phenotypes/performers in an existing population. They help to direct the improvement programme in a desired direction by supplying materials for further selection, hybridization or gathering or conserving favourable genes. Hence, selection of plus trees was the first and foremost step in this programme.

Plus tree selection and progeny trial

Teak plantations in the various forest divisions and a few natural teak populations were surveyed and outstanding trees with desirable characters without any defect were marked. The characters evaluated for selection of these plus trees were as follows,

i. Superiority in height and length of clear bole,
ii. Superiority in girth at breast height (gbh),
iii. Straightness,
iv. Absence of bumps, flutes, epicormic shoots, buttresses, twisting, etc.,
v. Narrow compact crown with light branches and
vi. Free from diseases and other defects

On the first cruise, trees with outstanding characters mentioned above were marked with a yellow band around the trunk at breast height. Measurements of height, gbh, length of clear bole, etc. were taken. These trees were designated as candidate plus trees. The superiority of these trees was assessed by comparing with five co-dominant trees (trees of comparison) within a radius of 25-50 m so as to ascertain their genetic superiority. From the candidate plus trees the best were selected and they were given two yellow bands. The trees of comparison were also marked and measured. Selection was intensive so that a very high selection differential was obtained. Selection differential and heritability are the factors which determine the genetic gain or success of improvement work. The details of the plus trees and the trees of comparison including approximate location-map were recorded in the plus tree register.

Seeds were collected from plus trees individually without mixing. The floor around the plus trees was cleaned and gunny bags were spread. After reaching the top of the tree the tree climber collected the seeds by pulling and shaking the branches. The seeds were cleaned and weighed and number of seeds per 100 g was counted. Nursery was established both at Peechi and Nilambur. Three replications were maintained for each seed lot. Germination and growth measurements were taken in the nursery.

Progeny trial was established in 3.5 ha area in Karulai Range, Nilambur South. Seedlings from 74 plus trees were field planted to assess the genetic superiority of the plus trees and to identify the elite trees. Lattice design was adopted because a large number of families can be tested with a fairly high degree of experimental precision and the layout allows to overcome the unexpected damages which may occur in the forest area. One of the advantage in this layout is that, if due to any unforeseen causes, a portion of the experimental crop is affected, still the results from the remaining blocks give ample scope for analysis and valid deduction of conclusion (Krishnaswamy and Hrishi, 1975). Half sib progenies of 64 plus trees were planted in a Triple Lattice design with 15 plants per replication in 3 m x 3 m spacing. Progenies of 10 other plus trees, which had few seedlings, were planted in a randomized block design with three blocks and 10 plants per replication for each family. Mixed seeds from Parambikulam and local Nilambur seeds were also planted as control. Observations on survival and growth were taken.

Biochemical studies

The extraction and staining protocols were standardized using teak tissues collected from trees and young seedlings. Young dormant buds, green opened leaves, reddish brown unopened leaves and inner bark of the trees were collected from the trees to standardise the method. Epicormic shoots induced on branch cuttings of plus trees maintained in mist chamber were also used in a few cases. Plus trees T2, T10 , T38, T41, T47, T52 and T57 were used directly and fresh epicormic shoots from branch cuttings were used for T2, T10, T 41, T55 and T57. The plus trees assembled in the teak seed orchard at Palappilly were also included in the study.

Polyacrylamide gel electrophoresis (PAGE) was adopted for isozyme separation. Methods reported by Wendel and Weeden (1989) and Sadasivam and Manickam (1992) were followed for the running of the gels and visualization of the bands.

The following 10 enzyme systems were tested for suitability as markers:
Malate dehydrogenase (MDH), Superoxide dismutase (SOD), Malic enzyme (ME), Esterase (EST), Peroxidase, Shikimic acid dehydrogenase (SAD), Alcohol dehydrogenase, Catalase, Glutamate dehydrogenase (GDH) and Glucose 6 phosphate dehydrogenase (G6PDH).

The following extraction buffers with modifications were tested to select the best one. Young dormant shoot buds were extracted and stained for MDH.

1.	Phosphate buffer 50 mM (pH 7.5) Sucrose 5% DTT 0.1%	2.	Phosphate buffer 50 mM Sucrose 5% DTT 0.1% PVP 40 5%
3.	Tris HCL 50 mM (pH 7.5) Sucrose 5% DTT 0.1%	4.	Tris HCL 50 mM (pH 8.0) Sucrose 5% PVP 0.1%
5.	Sodium tetra borate 50 mM (pH 8.3) DTT 0.1% PVP 2% Sucrose 0.14 M Sodium metabisulphate 20 mM	6.	Sodium tetra borate 50 mM (pH 8.3) DTT 0.1% PVPP 10% Sucrose 0.14 M Sodium etabisulphate 20 mM

Extraction

Fresh plant material (500 mg) was ground in 1.2 ml of the ice cold buffer using a pre-chilled mortar and pestle, centrifuged at 15000x g for 20 min. in a refrigerated centrifuge and the supernatant transferred to an eppendorf tube.

Gel Electrophoresis

A 10 per cent separating gel (composition below) was cast with a 6 per cent stacking gel over it. The electrophoresis was carried out using a vertical mini dual Electrophoresis system (Bangalore Genie). Approximately 20-35 µl of sample was loaded into each well with tracking dye and gels were subject to electrophoresis for 1.5 hrs at 10-15 mA at 4°C inside a refrigerator. Gels were transferred to a staining tray and development of banding patterns monitored. Bands were either scored in diagrammatic sketches or photographed on a white light Transilluminator.

Separating Gel (10%)		**Stacking Gel (6%)**	
Water	2.4 ml	Water	2.1 ml
Tris HCl 1.5 M (pH 8.8)	1.5 ml	Tris HCl 0.5 M (pH 6.8)	1.25 ml
Acrylamide (30%)	2.0 ml	Acrylamide (30%)	1.0 ml
Ammonium per sulphate	45 µl	Ammonium per sulphate	25 µl
TEMED	5 µl	TEMED	5 µl
Total volume	6 ml	Total volume	5 ml

RESULTS AND DISCUSSION

Assessment of status of plus trees selected in Kerala

During 1980-82, 50 plus trees were selected, of which 17 are fallen, dried or felled. Hence, 33 plus trees are still in the field.

Selection of new Plus trees

Information on Teak plantations above 30 years old (half the rotation age) were collected. Approximately 1500 ha of teak plantations and natural forests in Thenmala, Konni, Malayattur, Thrissur, Parambikulam, Nilambur, Wynad, Vazhachal and Kannur Divisions were surveyed and 200 plus tree candidates were identified. From 200 plus tree candidates, 61 new plus trees were selected after comparing with the trees of comparison. At present 94 plus trees are available in Kerala including 33 trees selected during 1980-82. Their characters were measured and recorded. Details of plus trees are provided in Table1. The selection differential for each plus tree is also estimated and given in Table 2. On an average the selection differential estimated is around 37 per cent for total height and clear bole height and around 32 per cent for girth at breast height.

Progeny trial

Collection of seeds from the plus trees was quite challenging since experienced plus tree climbers were not available. Only with three to four visits (to each area in the same or consecutive years) we could collect seeds from almost all the plus trees because each tree had different seed years. Some of the plus trees had not produced enough seeds and hence, we could not collect seeds from them.

Nursery was raised both at Nilambur and at Peechi. Number of seeds per kg and germination percentage are varied from year to year which may be due to climatic variations or due to seed maturity variation (Table 3). The number of seeds per kg varied from 1160 to 3600 and germination varied from 1.2 to 31.42 per cent. Seeds from some of the plus trees had very poor germination rate and hence could not be field planted due to lack of enough seedlings.

Table 1. Details of plus trees

New Plus trees selected during 1998-2001							
Tree No.	**Forest Division**	**Range**	**Locality**	**Year of planting**	**Ht in m**	**Clear bole in m**	**GBH in cm**
101	Kannur	Kanjangad	Kottamala	1877	30.4	14.2	117
102	Wyanad	Begur	4th Mile	1926	35.8	17.2	198
103	Wyanad	Begur	That Road	1957	31	16.6	171
104	Wyanad	Begur	Bhoothackal	1942	45.4	24.4	150
105	Kannavam	Kannavam	Kuttappalam	1945	45.4	25.6	203
106	Kannavam	Kannavam	Research Plot	1921	45.4	26.2	234
107	Kannavam	Kannavam	Nedumpoil	1941	49.6	27.4	225
108	Nilambur	Nilambur	Chaliyar Mucku	1948	45.4	28.6	314
109	Nilambur	Nilambur	Chaliyar Mucku	1948	49.6	32.8	172
110	Nilambur	Karulai	Poolackapara	1950	34	21.4	161
111	Nilambur	Karulai	Kanjirakadav	1951	37.2	18.4	152
112	Nilambur	Karulai	Mundankadav	1963	45.4	29.2	158
113	Nilambur	Karulai	Kallamthodu	1969	32.8	22.6	135
114	Nilambur	Vazhikkadavu	Nellikutha	1943	49.6	28	180
115	Nilambur	Vazhikkadavu	Nellikutha	1943	46.6	26.2	240
116	Nilambur	Nilambur	Aravallikavu	1934	42.1	22.6	195
117	Nilambur	Edavanna	Vadapuram	1923	45.4	26.2	231
118	Nilambur	Edavanna	Vadapuram	1923	45.4	31	200
119	Nilambur	Vazhikkadavu	Nellikutha	1944	41.2	24.4	183
120	Parambikulam	Sungam	Parambikulam	1926	48.1	25.6	229
121	Parambikulam	Sungam	Parambikulam	1945	48.1	28	191
122	Parambikulam	Sungam	Parambikulam	1945	41.2	22.6	189
123	Parambikulam	Sungam	Parambikulam	1945	45.4	22.6	227
124	Parambikulam	Sungam	Amakundu	1925	46.6	30.4	203
125	Parambikulam	Sungam	Thunakadavu	1943	43.6	27.4	186
126	Parambikulam	Sungam	Preserv. plot	Natural	49.6	24.4	376
127	Parambikulam	Sungam	Preserv. plot	Natural	46.6	21.4	390
128	Parambikulam	Sungam	Thekkady	Natural	39.4	20.2	278
129	Parambikulam	Sungam	Thekkady	Natural	31.6	16.6	250
130	Malayattoor	Kuttampuzha	Malakkappara	Natural	41.2	22.6	710
131	Malayattoor	Kuttampuzha	Malakkappara	Natural	39.1	22.6	592
132	Konni	Konni	Aruvapalam	1946	46.6	29.2	245
133	Konni	Konni	Kanjirappara	1956	39.4	25.6	160
134	Konni	Konni	Kanjirappara	1956	35.8	23.2	145
135	Konni	Konni	Avolikuzhy	1968	31	18.4	167
136	Konni	Konni	Umayamkuppa	1955	43.6	25.6	202

137	Konni	Mannarappara	Chempala	1943	46.6	23.2	266
138	Konni	Mannarappara	Kadampupara	1941	45.4	26.2	210
139	Konni	Mannarappara	Chittar	1947	44.2	26.2	216
140	Konni	Mannarappara	Aruthala	1945	45.4	26.2	168
141	Konni	Naduvathmuzhy	Kiliyara	1954	46.6	32.8	183
142	Konni	Naduvathmuzhy	Vayakkara	1959	44.2	29.2	173
143	Konni	Naduvathmuzhy	Manneera	1955	46.6	30.4	257
144	Konni	Naduvathmuzhy	Manneera	1956	49.6	34	206
145	Aryankavu	Aryankavu	Thalappara	1968	39.1	21.4	155
146	Aryankavu	Aryankavu	Thalappara	1968	34.6	20.2	155
147	Aryankavu	Aryankavu	Edappalayam	1968	35.8	26.2	140
148	Aryankavu	Aryankavu	Edappalayam	1968	39.1	26.2	163
149	Aryankavu	Aryankavu	Edappalayam	1968	37	26.2	183
150	Aryankavu	Aryankavu	Chenagiri		42	19	210
151	Aryankavu	Aryankavu	Chenagiri		42	28	210
152	Trichur	Peechi	Peechi	Natural	35	18	285
153	Trichur	Peechi	Peechi	Natural	29	20	261
154	Trichur	Peechi	Peechi	Natural	24	14	308
155	Trichur	Peechi	Peechi	Natural	44	22	218
156	Trichur	Peechi	Peechi	Natural	48	28	297
157	Trichur	Pattikkad	Pattikkad	Natural	28	15	207
158	Trichur	Pattikkad	Pattikkad	Natural	28	19	201
159	Trichur	Pattikkad	Pattikkad	Natural	30	18	213
160	Trichur	Pattikkad	Pattikkad	Natural	30	18	214
161	Trichur	Pattikkad	Pattikkad	Natural	43	24	182
162	Trichur	Peechi	Peechi	Natural	35	20	200
Old Plus trees selected during 1980-82							
1	Nilambur	Nilambur	Aravallikkavu	1920	46.6	25.6	268
2	Nilambur	Nilambur	Nellikutha	1930	41.2	24.4	183
3	Nilambur	Edavanna	Edacode	1924	42.7	26.2	230
4	Nilambur	Edavanna	Edacode		39.4	21.4	221
5	Nilambur	Karulai	Karulai		44.2	25.6	322
6	Nilambur	Karulai	Karulai	Dried	49.6	41.2	249
7	Nilambur	Edavanna	Edacode		49	34	238
8	Nilambur	Edavanna	Edacode	Fallen			
9	Nilambur	Karulai	Cherupuzha	Fallen			
10	Nilambur	Nilambur	Chathanpara	1944	48.1	28	223
11	Nilambur	Nilambur	Chathanpara	1944	49.6	32.8	231
12	Nilambur	Vazhikkadavu	Nellikuttha	1930	43.6	31	172
13	Nilambur	Vazhikkadavu	Nellikutha	1930	43.6	29.2	237
14	Konni	not relocated					
15	Konni	not relocated					

16	Konni	not relocated					
17	Konni	Naduvathmuzhy	Naduvathmuzhy	1950	41.2	29.2	182
18	Konni	Naduvathmuzhy	Naduvathmuzhy	1950	35.8	20.2	204
19	Konni	Naduvathmuzhy	Naduvathmuzhy	1950	49.6	25.6	251
20	Konni	not relocated					
21	Konni	not relocated		1960	49.6	27.4	181
22	Wynad	Begur	Ayyappanpara	**Fallen**			
23	Konni	not relocated					
24	Nilambur	Edavanna	Edacode		37	21.4	220
25	Nilambur	Vazhikkadavu	Nellikutha		49	30	246
26	Aryankavu	Aryankavu	Aryankavu	1911	45	22.6	236
27	Aryankavu	Aryankavu	Aryankavu	1911	35.8	22.6	234
28	Aryankavu	Aryankavu	Aryankavu	1911	35.3	21.4	259
29	Aryankavu	Aryankavu	Aryankavu	**Felled**			
30	Aryankavu	Aryankavu	Aryankavu	**Felled**			
31	Aryankavu	Aryankavu	Aryankavu	**Felled**			
32	Aryankavu	Aryankavu	Aryankavu	**Felled**			
33	Aryankavu	Aryankavu	Anakuthy	1930	44.2	29.2	214
34	Aryankavu	Aryankavu	Anakuthy	1930	48.1	27.4	210
35	Aryankavu	Aryankavu	Anakythy	**Felled**			
36	Aryankavu	Aryankavu	Anakuthy	1930	42.7	22.6	187
37	Aryankavu	Aryankavu	Anakuthy	1930	48.1	28	250
38	Aryankavu	Aryankavu	Anakuthy	1930	37	22.6	272
39	Aryankavu	Aryankavu	Anchumucku	**Felled**			
40	Aryankavu	Aryankavu	Anchumucku	**Felled**			
41	Aryankavu	Aryankavu	Anchumucku	**1915**	26.2	11.8	195
42	Aryankavu	Aryankavu	Anchumucku	1915	45.4	23.2	203
43	Konni	Naduvathmuzhy	Naduvathmuzhy		49.6	27.4	191
44	Aryankavu	Aryankavu	Anchumucku	**Felled**			
45	Aryankavu	Aryankavu	Anchumucku	**Felled**			
46	Aryankavu	Aryankavu	Anakuthy	1930	19.4	25.6	174
47	Aryankavu	Aryankavu	Anakuthy	1930	42.7	26.2	214
48	Aryankavu	Aryankavu	Anchumucku	1915	183	28	41.2
49	Wyanad	Begur	Tholpetty	1922	34	21.4	200
50	Wyanad	Begur	Tholpetty	1922	32.8	20.2	190

Table 2. Teak plus trees and selection differential (in %)

			Height			**Clear bole**			**Girth at Breast height**		
Sl. No.	**Tree No.**	**Locality**	**PT**	**TC**	**SD**	**PT**	**TC**	**SD**	**PT**	**TC**	**SD**
1	T 1	Nilambur	35	32.2	8.69	19	16.2	17.28	235	206.8	13.6
2	T 2	Nilambur	40	38.8	3.09	22	17.6	25.00	236	185	27.4
3	T 3	Nilambur	37	32.4	14.19	22	17	29.4	218	189.2	15.2
4	T 4	Nilambur	36	35.4	1.69	24	18.6	29.03	165	166.20	0.7
5	T 5	Nilambur	38	35.4	7.34	28	16.2	72.83	240	220.8	8.6
6	T 6	Nilambur	43	36	19.44	26	21 .o	23.8	282	234.25	20 0
7	T 7	Nilambur	33	27.6	19.56	20	14.8	35.1 3	180	138.6	29.8
8	T 8	Nilambur	36	33.8	6.5	23	19.6	17.34	187	154.4	21.1
9	T 9	Nilambur	38	31.8	19.49	26	18.8	38 29	180	133.4	34.9
10	T 10	Nilambur	40	32.2	24.22	23	18.8	22.34	192	146.6	30.9
11	T 11	Ni lambur	32	28.6	11.88	18	14.4	25	152	115.20	31.9
12	T 12	Nilambur	41	37.8	8.46	23	16.8	36 00	216	192	12.5
13	T 13	Nilambur	36	31.2	15.38	21	15.4	36.36	161	127.2	26.5
14	T 14	Konni	32	27.4	16.78	20	14.4	26.66	146	148.8	1.8
15	T 15	Konni	31	29.6	4.72	19	15 0	53.33	132	128.6	2.6
16	T 16	Konni	31	29.6	4.72	23	15	48.14	137	134 00	6.5
17	T 17	Konni	33	31.2	5.76	24	16.2	10.52	140	149.6	4.4
18	T 18	Konni	39	34.4	13.37	21	19	21.05	196	149.6	31.0
19	T 19	Konni	35	34.4	1.74	23	19 0	106.89	152	100 60	1.6
20	T 20	Konni	34	27.4	24.08	24	11.6	23.59	134	106.2	33.2
21	T 21	Konni	36	29.8	20.8	22	17.8	54.92	132	140.6	24.2
22	T 22	Wynad	32	27	31.57	22	14.2	50.68	173	89.6	23
23	T 23	Konni	35	26.6	19.88	22	14.6	50	139	134.2	55.1
24	T 24	Nilambur	37	34.2	18.51	24	16	38.88	191	128.6	42.32
25	T 25	Nilambur	45	37.2	20.96	26	19	35.28	230	195.4	17.7
26	T 26	Arien kavu	37	29.8	24.16	19	16	17.28	199	197	1.0
27	T 27	Arienkavu	34	33.8	0.59	22	21	5.76	201	201.4	0.1
28	T 28	Arienkavu	34	33.8	0.59	17	16	8.56	195	201.4	3.1
29	T 29	Arienkavu	33	27.4	20.43	20	18	13.63	138	11 6.80	18.1
30	T 30	Arienkavu	34	28.8	18.05	22	16	34.14	174	150.4	15.6
31	T 31	Arien kavu	32	31.8	0.62	20	18	9.89	170	139.2	22.1
32	T 32	Arienkavu	32	29.6	8.1	23	17	38.55	172	145.2	18.4
33	T 33	Arienkavu	34	32.4	4.93	23	16	41.97	182	162	12.3
34	T 34	Arienkavu	33	32.4	1.85	21	16	29.62	165	162	1.8
35	T 35	Arienkavu	36	30.6	17.64	25	17	47.05	169	140.2	20.7
36	T 36	Arienkavu	32	31.2	2.56	17	17	0	165	160.6	3.1
37	T 37	Arienkavu	39	31.2	25	19	17	11.76	229	160.6	42.5
38	T 38	Arienkavu	35	32	9.37	22	18	25	178	142.8	24.6
39	T 39	Arienkavu	35	32.4	8.02	17	17	2.4	203	158.2	28.3
40	T 40	Arienkavu	34	32.8	3.65	18	17	8.43	151	141.6	6.6

			Height			Clear bole			Girth at Breast height		
Sl. No.	Tree No.	Locality	PT	TC	SD	PT	TC	SD	PT	TC	SD
41	T 41	Arienkavu	39	36.6	6.55	21	15 60	34.61	176	137.8	27.7
42	T 42	Arienkavu	44	32.2	36.64	24	15	62.16	21 7	139.8	55.2
43	T 43	Konni	34	32	6.25	18	17	7.1 4	144	137.49	1.8
44	T 44	Arienkavu	36	31.O	16.1 2	25	17	48.8	183	146.4	25
45	T 45	Arienkavu	30	28.4	5.63	21	17	25	152	131.4	15.6
46	T 46	Arienkavu	40	33	21.21	21	18	19.31	243	166.6	45.8
47	T 47	Arienkavu	32	31.2	2.56	18	17	5.88	184	160.6	14.5
48	T 48	Arienkavu	34	32.8	3.04	19	17	14.45	166	141.6	17.2
49	T 49	Wynad	28	22.8	22.8	17	11	60.37	183	142.4	28.5
50	T 50	Wynad	29	26.6	9.02	19	13	50.79	174	166.6	4.3
1	T 101	Kanjangad	30.4	23.44	29.69	14.2	9.88	43.7	117	73.4	59.4
2	T 102	Wynad	35.8	35.54	0.73	17.2	20.3	--	198	172.2	15.0
3	T 103	Wynad	31	25.24	22.82	16.6	12.2	36.0	171	133.8	27.8
4	T 104	Wynad	45.4	34.48	31.7	24.4	21.1	15.0	150	137	9.5
5	T 105	Kannavam	45.4	32.92	37.9	25.6	14.08	81.8	203	136.6	48.6
6	T 106	Kannavam	45.4	45.64	0	26.2	25.24	3.8	234	175.8	33.1
7	T 107	Kannavam	49.6	43.9	13.0	27.4	16.48	66.2	225	142.8	57.6
8	T 108	Nilambur	45.4	40.18	13.0	28.6	17.44	64.0	314	268.2	17.0
9	T 109	Nilambur	49.6	43.36	14.4	32.8	22.12	48.2	172	130	32.3
10	T 110	Nilambur	34	32.08	59.9	21.4	18.6	15.0	161	163.2	
11	T 111	Nilambur	37.2	31.66	17.5	18.4	15.28	20.4	152	139	9.4
12	T 112	Nilambur	45.4	32.68	59.9	29.2	15.88	83.9	158	112.8	40.0
13	T 113	Nilambur	32.8	27.64	18.7	22.6	12.16	85.8	135	93.4	44.5
14	T 114	Nilambur	49.6	39.7	24.93	28	23.56	18.8	180	137	31.4
15	T 115	Ni lambur	46.6	42.4	9.9	26.2	20.44	28.1	240	212	13.2
16	T 116	Nilambur	42.1	34.16	23.2	22.6	20.56	99.0	195	140.4	38.9
17	T 117	Nilambur	45.4	38.1	19.1	26.2	24.04	9.0	231	169.2	36.5
18	T 118	Nilambur	45.4	42.22	2.5	31	21.76	42.5	200	154.4	29.5
19	T 119	Vazhachal	41.2			24.4			183		
20	T 120	Parambik	48.1	43	11.9	25.6	19.36	32.2	229	207.6	10.3
21	T 121	Parambik	48.1	44.2	8.8	28	22.36	25.2	191	159.2	19.9
22	T 122	Parambik	41.2	41.56		22.6	18.88	19.7	189	156.2	20.9
23	T 123	Parambik	45.4	37.16	22.1	22.6	20.44	10.6	227	147.8	53.6
24	T 124	Parambik	46.6	45.7	1.9	30.4	22.6	34.5	203	204.6	
25	T 125	Parambik	43.6	39.1	11.5	27.4	17.92	52.9	186	166.8	11.5
26	T 126	Parambik	49.6			24.4			376		
27	T 127	Parambik	46.6			21.4			390		
28	T 128	Parambik	39.4			20.2			278		
29	T 129	Parambik	31.6			16.6			250		
30	T 130	Malayattur	41.2	41.2		22.6	22.6		710	710	

			Height			Clear bole			Girth at Breast height		
Sl. No.	**Tree No.**	**Locality**	**PT**	**TC**	**SD**	**PT**	**TC**	**SD**	**PT**	**TC**	**SD**
31	T 131	Malayattur	39.1	39.1		22.6	22.6		592	592	0
32	T 132	Konni	46.6			29.2			245	157.8	55.26
33	T 133	Konni	39.4	34.6	13.9	25.6	23.44	9.2	160	130	23.0
34	T 134	Konni	35.8	27.16	31.8	23.2	12.88	80.1	145	111.4	30.1
35	T 135	Konni	31			18.4			167		47.5
36	T 136	Konni	43.6	40.24	8.3	25.6	19.72	23.8	202	167.4	20.66
37	T 137	Konni	46.6	38.12	22.2	23.2	16.96	36.8	266	198.6	33.9
38	T1 38	Konni	45.4	43.3	0.48	26.2	20.56	27.4	210	172	22.0
39	T 39	Konni	44.2	35.14	25.8	26.2	17.8	47.1	216	144	50.0
40	T 140	Konni	45.4	41.56	9.9	26.2	23.08	13.5	168	117	43.6
41	T 141	Konni	46.6	35.74	30.4	32.8	19.96	64.3	183	130.6	40.1
42	T1 42	Konni	44.2	39.1	13.0	29.2	25.48	14.6	173	160.2	8.0
43	T 143	Konni	46.6	44.2	5.4	30.4	27.16	11.9	257	178.2	76.9
44	T144	Konni	49.6	43.42	14.2	34	25. 6	32.8	128.	128.2	60.7
`45	T 145	Arienkavu	39.1	31	26.1	21.4	15.04	42.3	155	117	32.5
46	T 146	Arienkavu	34.6	29.68	16.6	20.2	15.64	29.2	155	149.2	3.9
47	T 147	Arienkavu	35.8	30.64	6.8	26.2	18.4	42.4	140	136	2.9
48	T1 48	Arienkavu	39.1	34.22	14.3	26.2	24.16	8.4	163	128.8	26.6
49	T 149	Arienkavu	37	32.44	14.0	26.2	18.04	45.2	183	124.8	46.6
50	T 150	Arienkavu	42	42.7		19	20.2		210	210	
51	T 151	Arienkavu	42	42.7		28	29.2		210	210	
52	T 152	Peechi	35			18			285		
53	T 153	Peechi	29			20			261		
54	T 154	Peechi	24			14			308		
55	T 155	Peechi	44			22			218		
56	T 156	Peechi	48			28			297		
57	T 157	Pattikkad	28			15			207		
58	T 158	Pattikkad	28			19			201		
59	T 159	Pattikkad	30			18			213		
60	T 160	Pattikkad	30			18			214		
61	T 161	Pattikkad	43			24			182		
62	T 162	Peechi	35			20			200		

PT-Plus tree TC- Trees of comparison SD- Selection differential

*** Selection differential for Plus trees 1 to 50 was estimated during 1984**

Table. 3 Number of seeds/kg and germination percentage of Plus tree seeds

Serial No.	Plus tree number	Number of seeds/ kg		Germination percentage	
		Year 2000	Year 2001	Year 2000	Year 2001
1	1	1590		19.38	
2	2		2290		11.7
3	3				
4	4	1560		14.14	
5	5	1970		11.22	
6	6	1900		15.7	
7	7				
8	10	2650		17.18	
9	11	1530		24.46	
10	12				
11	13				
12	17	2240		20.97	
13	18		2170		15.1
14	19	2070		14.25	
15	21	1760	1980	27.48	11.6
16	24	1820		15.38	
17	25				
18	26	1670		29.08	
19	27	2280		20.53	
20	28				
21	33	1440		19.71	
22	34	1520		31.42	
23	36	1300		4.28	
24	37	1220	1850	1.31	9.6
25	38	1700		21.36	
26	41	1640	2290	15.74	6.3
27	42	1490		21.06	
28	43	1680		11.38	
29	46	1350		23.11	
30	47	1600		10.4	
31	48	1280		17.09	
32	49	2340		21.28	
33	50	1980		17.61	
34	101	2060		11.36	
35	102	2270		9.78	
36	103	1160		12.91	
37	104	1870		13.41	
38	105	3600		5.03	
39	106	1960		14.07	
40	107	2830		18.14	
41	108	2000		12.32	

Serial No.	Plus tree number	Number of seeds/ kg		Germination percentage	
		Year 2000	**Year 2001**	**Year 2000**	**Year 2001**
42	109	1960		16.21	
43	110		2420		10.9
44	111	1480		14.08	
45	112				
46	113	2370		26.51	
47	114	1590		8.31	
48	115		2140		13.0
49	116	2090		12.62	
50	117				
51	118		3190		1.7
52	119				
53	120	1320		16.84	
54	121	1380		16.18	
55	122	1170	1710	12.14	8.2
56	123	1240		21.73	
57	124	1570		25.99	
58	125	1320		17.44	
59	126	2030		5.88	
60	127	1440		20.57	
61	128	2100		14.29	
62	129		1890		3.4
63	130	2200		12.75	
64	131	2140		5.45	
65	132	1650		29.08	
66	133	1710		6.6	
67	134	1670		11.03	
68	135	2440		2.92	
69	136	2410		5.93	
70	137	1760		11.36	
71	138	1890		5.74	
72	139	2030		16.67	
73	140	1600		18.09	
74	141	1740		19.64	
75	142	1360	2620	4.02	1.2
76	143	1250		13.39	
77	144	1590	2360	3.84	11.4
78	145	1510		14.61	
79	146	1550		12.19	
80	147	1600		11.13	
81	148	1700		22.95	
82	149	1590		15.39	
83	150	2720	3120	6.25	9.1

Serial No.	Plus tree number	Number of seeds/ kg		Germination percentage	
		Year 2000	Year 2001	Year 2000	Year 2001
84	151	1650	2230	6.44	3.1
85	152		3220		2.9
86	153		2710		2.3
87	154		2000		2.6
88	155		1860		0.0
89	156		1900		2.0
90	157		2400		4.7
91	158				
92	159		2350		6.9
93	160		2630		3.8
94	161				
95	162				
96	163Kannimara		2240		10.1
97	Shenkottai kolteak	1800		0.0	

Growth performance

The mean stem diameter of the families at the end of one year in the nursery is given in Table 4 and the mean height after six months of field planting is given in Table 5. Ten families, which had very good growth as well as ten families, which had very poor growth are listed in Table 6. In the nursery, progenies of plus trees from Konni have shown better performance, but in the field trial, there was no such differentiation between areas of origin. However, it is too early for an evaluation. The maintenance of the field trial and its evaluation are still continuing in the second phase of the genetic improvement programme.

Isozymes

Methods were standardized for 10 enzymes (Malate dehydrogenase, Superoxide dismutase, Malic enzyme,Esterase, Peroxidase, Shikimic acid dehydrogenase, Alcohol dehydrogenase, Catalase, Glutamate dehydrogenase and Glucose 6 phosphate dehydrogenase). Visualization of bands was possible with varying degrees of success. The best results were obtained in MDH followed by the other enzymes in the order given above.

Dormant buds were found to be the best material for extraction compared with newly opening leaves and fully opened leaves. Four buffers were screened for their suitability and Sodium tetra borate buffer with 10 per cent PVPP was found to give better results with most enzymes. A seasonal variation in isozyme expression was noticed with bands being visualized in the summer and absence of bands during the rains.

No polymorphism could be found in the material collected from different clones in the clonal seed orchard at Palapilly or from stem cuttings of seven plus trees of Nilambur (Fig. 2). On the other hand, occasionally polymorphism was seen in randomly selected trees growing in different areas of Thrissur. Further testing of protocols especially of different buffers is therefore needed.

In recent times molecular markers like RAPD, AFLP and microsatellites have been preferred by many researchers because of the reliability of the techniques and particularly since these methods are not very dependant on the type of tissues used or the environmental conditions. In teak too, such methods are being applied at KFRI and other organizations and hence, it would be prudent to compare the results of such studies so as to arrive at a suitable methodology.

Table 4. Mean stem diameter (in cm) of the families at the end of one year in the nursery

Tree No.	**Diameter**	**Tree No.**	**Diameter**	**Tree No.**	**Diameter**	**Tree No.**	**Diameter**	**Tree No.**	**Diameter**
T135	0.876	**T150**	0.703	**T140**	0.622	**T116**	0.572	**T49**	0.518
T137	0.798	**T130**	0.693	**T36**	0.622	**T143**	0.572	**T125**	0.518
T37	0.787	**T149**	0.682	**T128**	0.619	**T103**	0.566	**T142**	0.513
T113	0.785	**T131**	0.665	**T27**	0.615	**T114**	0.566	**T109**	0.512
T138	0.783	**T41**	0.663	**T122**	0.605	**T26**	0.562	**T10**	0.512
T126	0.768	**T151**	0.658	**T101**	0.602	**T106**	0.561	**T123**	0.505
T120	0.762	**T146**	0.656	**T47**	0.60	**T17**	0.56	**T141**	0.505
T121	0.762	**T111**	0.652	**T43**	0.596	**T42**	0.56	**T11**	0.503
T134	0.759	**T104**	0.649	**T48**	0.587	**T19**	0.558	**T124**	0.501
T133	0.746	**T144**	0.648	**T105**	0.585	**T108**	0.556	**T1**	0.488
T136	0.744	**T24**	0.641	**T21**	0.581	**T102**	0.538	**T4**	0.481
Mixed	0.725	**T33**	0.634	**T34**	0.581	**T132**	0.532	**T5**	0.475
T127	0.72	**T148**	0.632	**T6**	0.578	**T46**	0.53	**T107**	0.452
T139	0.714	**T145**	0.625	**T38**	0.573	**T147**	0.525	**T50**	0.435

Table 5. Mean height (in cm) at 6 months after field planting

Tree No.	Height	Tree No.	Height	Tree No.	Height	Tree No.	Height	Tree No.	Height
T33	57.25	**T140**	35.87	**T1**	32.37	**T11**	28.11	**T145**	25.36
T132	55.54	**T36**	34.19	**T108**	32.0	**T38**	28.10	**T136**	25.34
T101	52.00	**T127**	34.13	**T150**	31.99	**T143**	28.06	**T126**	25.17
T34	45.97	**T19**	34.11	**T121**	31.97	**T41**	27.67	**T142**	25.00
T6	44.22	**T151**	34.03	**T104**	31.89	**T5**	27.45	**T46**	24.28
T49	44.06	**T26**	33.96	**T116**	30.0	**T128**	27.31	**T110**	23.66
T144	43.33	**T43**	33.44	**T131**	29.67	**T103**	27.17	**T27**	23.50
T21	43.07	**T146**	33.43	**T141**	29.63	**T42**	27.10	**T149**	23.09
T133	40.56	**T124**	33.33	**T130**	29.62	**T120**	26.67	**T125**	22.90
T47	39.50	**T106**	33.28	**T147**	29.25	**T153**	26.42	**T160**	22.50
T50	39.21	**T37**	33.23	**T10**	29.19	**T24**	26.33	**T113**	20.40
T122	38.64	**T148**	33.09	**T123**	29.00	**T139**	26.08	**T111**	18.75
T48	38.61	**T137**	32.98	**T4**	28.88	**T138**	26.00	**T134**	18.67
T109	37.66	**T105**	32.57	**T17**	28.56	**T107**	25.82	**Kanni mara**	17.25
T135	36.07	**T102**	32.44	**T114**	28.11	**T159**	25.5		

Table 6. Families showing very good and very poor growth

Sl. No.	**Best families**		**Poor families**	
	for stem diameter	**for height**	**for stem diameter**	**for height**
1	T135-Konni	T33- Arienkavu	T50-Wynad	Kannimara
2	T137- Arienkavu	T132-Konni	T107-Kannavam	T134-Konni
3	T113-Nilambur	T101-Kannur	T5-Nilambur	T111-Nilambur
4	T138 - Konni	T34-Arienkavu	T4-Nilambur	T113-Nilambur
5	T126-Konni	T6-Nilambur	T1-Nilambur	T160-Thrissur
6	T121-Parambiklm	T49-Wynad	T124 - Parambikulam	T125-Parmbikm
7	T120-Parambiklm	T144-Konni	T11- Nilambur	T149-Arienkavu
8	T134-konni	T21-Konni	T123 - Parambikulam	T27-Arienkavu
9	T133-Konni	T133-Konni	T141 - Konni	T110-Nilambur
10	T136-Konni	T47-Arienkavu	T10 &T109-Nilambur	T46-Arienkavu

Fig. 1. Plus tree from Arienkavu

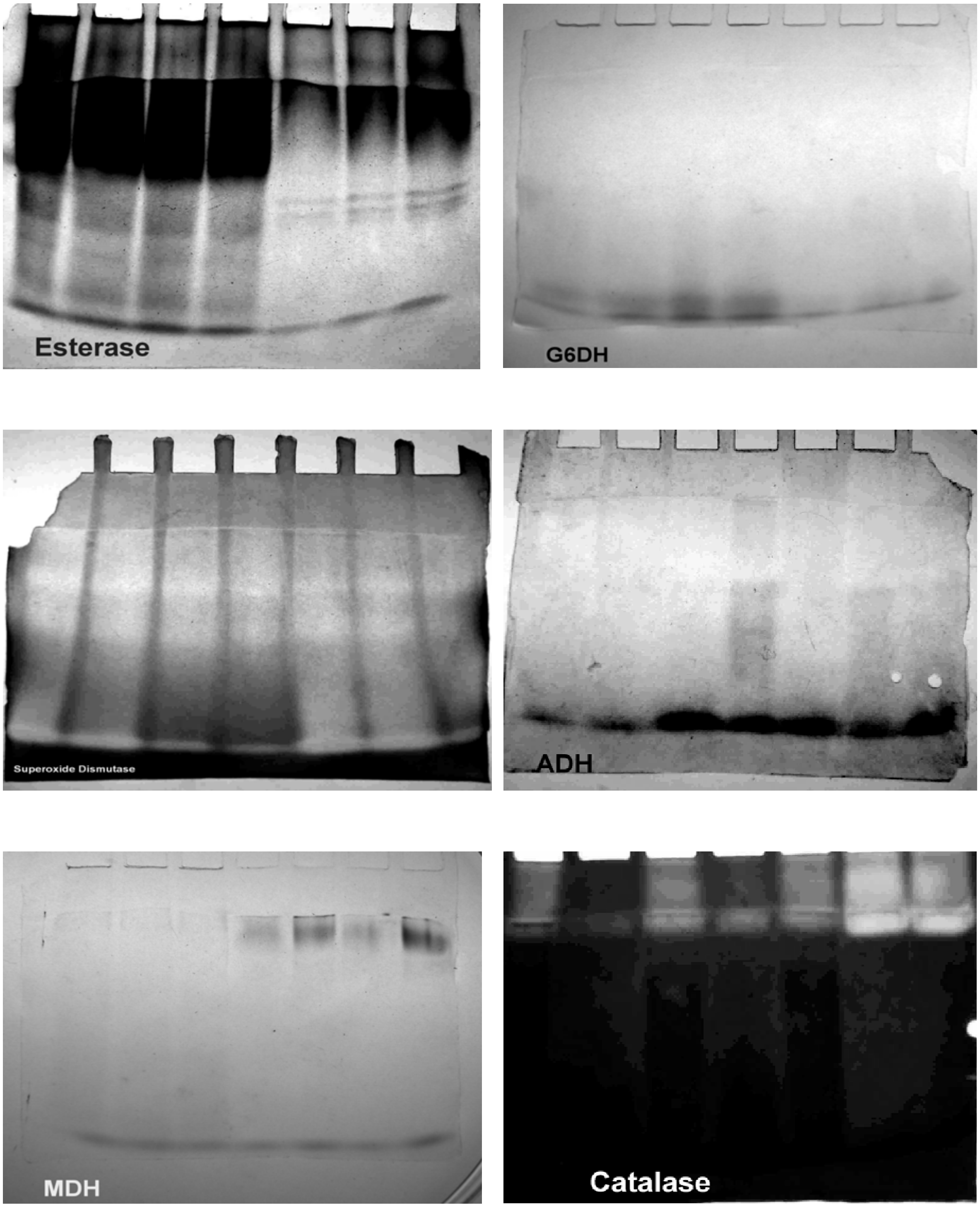

Fig. 2. Banding pattern of six isozyme systems.
The different lanes are of different plus trees

CONCLUSIONS

This project was initiated to assess the status of plus trees selected during 1979 to 1982, select few more plus trees and to establish a progeny trial for evaluation of plus trees for their breeding value. Many of the plus trees selected earlier were fallen or felled and only 33 out of 50 are left behind. Sixty two new plus trees were selected from plantations which crossed half the rotation period and from natural forest in Kerala. On an average the selection differential estimated is around 37 per cent for total height and clear bole height and around 32 per cent for girth at breast height. By selecting the best families or individuals, at least 5 to 15 per cent increase in volume is expected. A progeny trial was established at Nilambur with 74 families so as to evaluate the plus trees. This trial can be converted to a seedling seed orchard after proper evaluation and thinning or can be retained as a breeding population.

Phenotypic variance (σ^2P) is due to the combination of genotypic variance (σ^2G) and environment variance (σ^2E). Two types of genes viz., additive genes (A) and non - additive (NA) genes contribute to the total genetic variance. Clonal forestry allows full use of non additive gene effects like dominance, epistasis, over dominance (hybrid vigour) and also frequent favorable segregants since there is possibility of tapping the true to the parental genotype. Both half sib and full sib progeny trials allow additive gene effects and so new recombinants are possible to occur. Hence, through exploiting the general and specific combining ability, improved high productive seeds can be produced. Hence, half sib and full sib progeny trials pave way for further improvement whereas cloning helps to tap the parental genotype.

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