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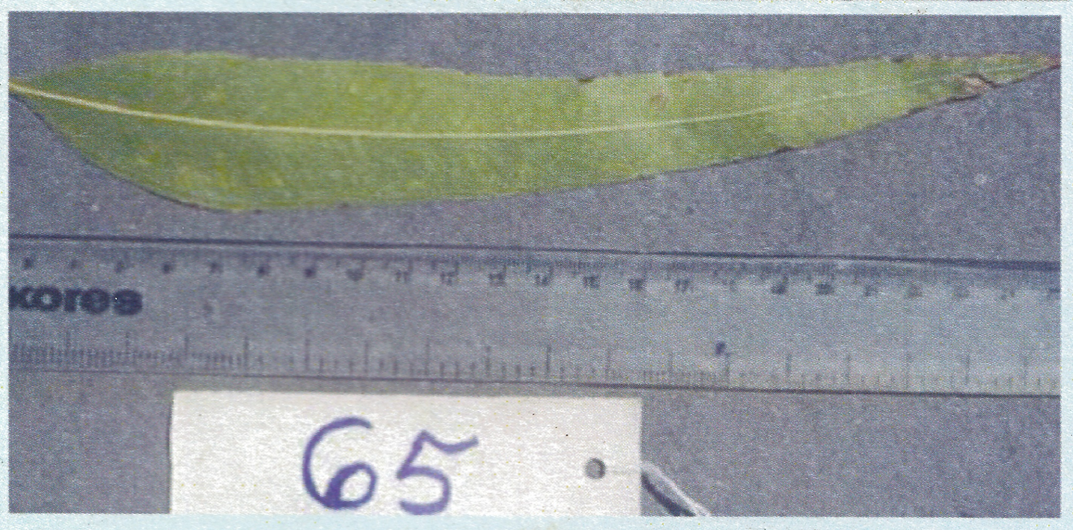
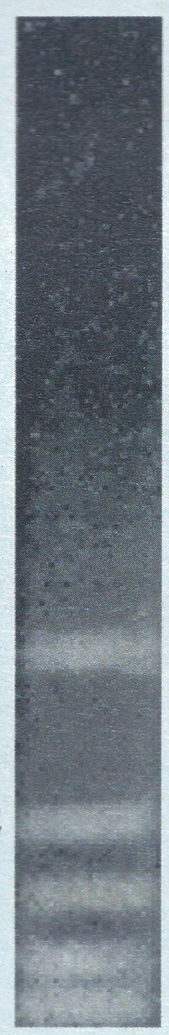
Genetic Diversity and DNA Finger Printing of Eucalypt and Acacia Clones



OPA 13



OPA 02

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August 2005

KFRI

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(Final Report of the Project KFRI 369/2001)

Genetic Diversity and DNA Finger Printing of Eucalypt and Acacia Clones

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August 2005

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ABSTRACT OF THE PROJECT PROPOSAL

1. Project code and title : KFRI 369/2001 Genetic Diversity and DNA Finger Printing of Eucalypts and Acacia Clones

2. Investigators : M. Balasundaran
E.J. Maria Florence
J.K. Sharma

3. Research Fellow : Binu C. Nair

4. Objectives :

i. To develop DNA finger prints of eucalypt and acacia clones being planted by KFD

ii. To estimate the genetic diversity between clones of each species by comparing RAPD markers using statistical software

5. Date of commencement : June 2001

6. Date of completion : May 2003

7. Funding agency : Forest Development Fund

Acknowledgement

We are thankful to Dr. K.S.S. Nair, former Director, KFRI and Dr. J.K. Sharma, Director for their keen interest and constant encouragement. We acknowledge with thanks the help provided by Dr. T.B. Suma, Smt. Binu C. Nair and Ms. Raakhee Vijayaraghavan during the laboratory work and data analysis. Thanks are also due to Smt. V. Rekha, who provided some of the photographs of KFRI clones. We gratefully acknowledge the financial help rendered by Kerala Forest Department (Development Fund) for carrying out the work.

B. Balasundaran

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ABSTRACT

RAPD finger printing and estimation of genetic diversity of 15 KFRI eucalypt clones and 7 mangium clones have been carried out. These clones are used by Kerala Forest Department for raising plantations. OPA primers, OPA 02, OPA 13 and OPA 18 were used for carrying out RAPD finger printing of nine *Eucalyptus tereticornis* clones and six *E. camaldulensis* clones. OPB 01, OPB 04, OPB 05 and OPB 17 were the primers used for mangium clones. It was possible to differentiate the clones using the RAPD finger prints. The DNA finger prints of *E. tereticornis* clones are provided in the report along with photographs of clones showing their morphological features. The finger prints of *E. camaldulensis* and mangium clones are presented separately for each primer. Analysis for polymorphism showed that 76.32 per cent of *E. tereticornis* loci, 71.88 per cent of *E. camaldulensis* loci and 56 per cent of *A. mangium* loci were polymorphic. Genetic diversity analysis showed that genetic distance coefficient values between clones were very low indicating high genetic similarity between the clones, especially with respect to mangium clones. UPGMA dendrogram was constructed based on the genetic distance coefficients. Genetically similar clones have been grouped in one cluster. The importance of keeping adequate distance between genetically similar clones grouped within the same cluster during field planting is emphasized.

1. Introduction

Trees in a plantation derived from a single clone are genetically identical. All these trees will look alike morphologically (phenotypically) except for the changes brought about by local environmental conditions such as water availability, soil characters, etc. Due to this peculiar reason, clonal plantations are highly vulnerable to disease and pest epidemic, even though utmost care is usually taken to release disease and pest resistant clones. Hence, in a clonal forestry programme, it is always safe to plant as many genetically divergent clones as possible.

1.1. Genetic characterization and DNA finger printing

Genetic diversity forms the base of biodiversity hierarchy (Namkoong *et al.*, 1996) and the diversity serves as the resource for survival and future evolution of species (Frankel *et al.*, 1995). In the past, forest genetic research involved classical approaches for the assessment of genetic variation by using either morphological or physiological traits. Recently, DNA based markers such as RFLPs, RAPDs, AFLPs and microsatellites have been applied for the detection of genotypic polymorphism in plants (Lisitsyn *et al.*, 1993).

Even though, the importance of estimation of genetic diversity between clones selected to raise large scale plantations was recognized earlier, it was not possible to estimate the diversity within a short time. But now it is easy to estimate the genetic diversity of clones through molecular markers.

Right of commercial exploitation of plant varieties and clones can be protected through Plant Varieties Protection Act and the new clones can be registered with government agencies. With the discovery of molecular markers, it is now possible to provide the unique DNA profiles to identify clones facilitating their registration.

Random amplified polymorphic DNA (RAPD) markers are one of the easiest among the molecular markers used for DNA finger printing and for studying the genetic diversity of plants. RAPD markers constitute a class of genetic markers which produce arbitrary fragment length polymorphisms and utilize single, arbitrary decamer DNA oligonucleotide primers to amplify regions of the genome using polymerase chain reaction (Williams *et al.*, 1990). Priming sites are thought to be randomly distributed throughout the genome, and polymorphism in these regions results in amplification products differing in size. RAPD analysis with agarose gel electrophoresis has been used successfully to identify and discriminate species, varieties, hybrids and cultivars (Dawson *et al.*, 1996; Ortiz *et al.*, 1997). Compared to other markers, they provide a fast and cheap means of generating large numbers of loci randomly spread throughout the genome (Rafalski and Tingey, 1993). The reproducibility of RAPD markers and their ability to differentiate between individual genotypes have been utilized in verification, identification and characterization of clones of forest trees (Lange *et al.,* 1993; De Lala *et al.*, 2000).

KFRI has identified about 106 candidate plus trees (CPTs) of eucalypt species and 80 CPTs of Acacia species. Of these, 22 clones of eucalypts and three clones of acacia species have been released to the Kerala Forest Department for large scale planting. Even though, the clones are developed from different provenances, identification of clones based on morphological characters may not be very reliable. Rekha (2000) studied the foliar characters of 16 *E. tereticornis* clones. She concluded that clones of the same provenance cannot be distinguished from each other based on a single character. However, it may be possible to distinguish the clones based on DNA markers, because each clone is genotypically different. The objective of the present study is DNA finger printing and genetic diversity studies of the KFRI clones being planted by the Kerala Forest Department on a large scale.

2. Materials and Methods

For the present study, 15 eucalypt clones and five *Acacia mangium* clones were used. The list of clones and the origin of their ortets (mother trees) are provided in Tables 1 and 2.

Table 1. List of Eucalypt clones selected for DNA finger printing and genetic diversity studies

Sl. No.	Clone No.	Provenance/origin of clone
E. tereticornis		
1	KFRI 14	Kennedy River, Qld[1]
2	KFRI 15	Morehead River, Qld
3	KFRI 16	Morehead River, Qld
4	KFRI 21	Kennedy Ck Pen Dev Road, Qld
5	KFRI 28	80 Km NNW Cook town, Qld
6	KFRI 38	East of Kupiano, PNG
7	KFRI 43	Ravenshoe, Qld
8	KFRI 49	Morehead River, Qld
9	KFRI 65	Kennedy Creek Pen Dev Road, Qld
E. camaldulensis		
1	KFRI 10	Cape River, Qld
2	KFRI 23	West of Normanton, Qld
3	KFRI 24	Daly Waters, Nt
4	KFRI 25	Katherine, Nt
5	KFRI 54	Cape River, Qld
6	KFRI 68	Cape River, Qld

Qld: Queensland; Nt: Northern territory; PNG: Papua New Guinea

Table 2. List of *Acacia mangium* clones selected for DNA finger printing and genetic diversity studies

Sl. No.	Clone No.	Provenance/origin of clone
1	KFRI 38B-1	Wipim Oriomo
2	KFRI 38B-2	Wipim Oriomo
3	KFRI 38B-3	Wipim Oriomo
4	KFRI 38B-4	Wipim Oriomo
5	KFRI 38B-5	Wipim Oriomo
6.	KFRI 38B 6	Wipim Oriomo
7	KFRI 38B 7	Wipim Oriomo

2.1. Sample collection

Leaf samples were collected for extraction of DNA from four ramets of each eucalypt clone raised in pots for this purpose in KFRI campus. Leaf samples of *Acacia mangium* were collected from clones maintained in the KFRI clonal nursery at Kottappara in Kodanad Forest Range, Malayattoor Forest Division. Twigs with tender leaves were excised from plants, kept in buckets containing water, covered with polythene bags and transported to KFRI laboratory.

2.2. DNA extraction

Genomic DNA was extracted from the juvenile leaf samples of eucalypts and *A. mangium* (from 200 mg approx.) following the method of Doyle and Doyle (1990). Samples were homogenised in hot (65^0C) CTAB buffer containing 2 per cent CTAB, 100mM Tris pH 8.0, 20mM EDTA pH 8.0, 1.4M NaCl and the homogenates were incubated at 60^0C on a water bath for half an hour. The samples were extracted with chloroform:isoamyl alcohol (24:1) followed by centrifugation for 20 min and the aqueous phase eluted out. To the aqueous layer, double the volume of ice-cold absolute alcohol was added and incubated at -20^0C for 12

hours. The DNA was precipitated by centrifuging for 10 min at low speed (3000g). The DNA pellet was further purified, air dried and dissolved in 100µl of double distilled water and checked for purity on agarose gels.

2.3. Polymerase chain reaction

Twenty decamer oligonucleotides from operon kit A and B (Operon technologies, USA) were screened for genetic polymorphism analysis and DNA finger printing. Based on repeatability and the number of polymorphic bands obtained, the primers OPA 02, OPA 13 and OPA 18 were used for eucalypts and OPB 01, OPB 04, OPB O5, and OPB17 were used for *A. mangium* DNA amplification and finger printing. DNA was amplified in 25 µl reaction mixtures containing 30-50ng of template DNA, 200µM each of dATP, dTTP, dCTP and dGTP, 1.5 units of Taq DNA polymerase, 20 picomoles of each primer and 5µl Taq buffer with 1.5mM $MgCl_2$ (Genei, Bangalore). The incubation mixture was overlaid with one or two drops of mineral oil (Genei, Bangalore) and subjected to 45 cycles of amplification in PTC-150 Minicycler (MJ Research Inc., USA), each of 60 seconds denaturation (94°C), 60 seconds annealing (36^0C) and 120 seconds extension (72^0C). The last cycle was followed by incubation for 10 minutes at 72^0C.

2.4. Separation and visualization of amplified products

The PCR amplification products were electrophoresed on Wide Mini-Sub Cell GT and Mini-Sub Cell GT (Biorad, USA) in 1.5 per cent horizontal agarose gel (Biorad, USA) in TBE buffer (40 mM Tris-borate, 1mM EDTA, pH 8.0). The gels, after the completion of electrophoresis, were stained with ethidium bromide and bands were compared with a 100bp DNA ladder (Genei, Bangalore). The gel pictures were documented using Kodak Digital Science Electrophoresis Documentation and Analysis system 120 (Kodak, USA).

2.5. Data analysis

The DNA fragment sizes were estimated comparing DNA size markers run on the same gel and the data scored for RAPD analysis. The bands were scored '1' for their presence and '0' for absence in each DNA sample to create binary data matrices. The data matrices were entered into the POPGENE version 1.31 computer package and genotype comparisons were made (Yeh *et al.*, 1999). The observed number of alleles (na), expected number of alleles (ne), per cent of polymorphic loci (polymorphic bands per total bands) and gene diversity index were determined. Nei's genetic distance and similarity indices were calculated for constructing UPGMA dendrogram.

3. Results and Discussion

The study provides RAPD fingerprints of nine *E. tereticornis* clones, six *E. camaldulensis* clones and seven *A. mangium* clones (Plates I - V). The DNA finger prints of these clones are also used to determine the degree of genetic variation between the clones belonging to each species.

3.1. DNA finger prints of eucalypt species

The DNA fingerprints of all the clones together are given in Plates I, II and III. Four ramets of each clone were initially used for finger printing in order to confirm consistency of the result. The DNA fingerprint of each clone from *E. tereticornis* is given along with clone morphology in plates VI, VII, VIII, IX and X. Some of the bands were found specific to the clone as indicated in the plate. These DNA bands can be used as marker for identification of the clone when the specific primer is used for DNA amplification. The banding pattern obtained when the specific primer is used for the finger printing is the RAPD fingerprint of the clone.

3.2. Genetic diversity

Thirty eight RAPD bands were obtained when nine clones of *E. tereticornis* were screened for variation using the three selected primers based on the scoring of bands for presence or absence. For the six clones of *E. camaldulensis*, 32 RAPD bands were obtained. The PCR amplification products generally ranged from 100-3000 base pairs in size. Assuming that each RAPD band represented a single locus, 76.32 per cent of the loci showed polymorphism (29 out of 38 loci.) in *E. tereticornis*. In *E. camaldulensis,* out of the total 32 loci resolved, 23 loci (71.88 %) showed polymorphism.

The estimation of the gene diversity index (h) at the locus level showed that many of the loci were genetically similar with an average diversity index of 0.27

for both *E. tereticornis* and *E. camaldulensis*. This indicates that the clones are genetically similar to a great extent within the species.

Among *E. tereticornis* clones, genetic similarity indices (Nei, 1978) showed that clones 21 and 43 are the most identical ones (D=0.0267) and clones 14 and 65 are the most distant ones (D= 0.6931) (Table 3). Among *E. camaldulensis* clones, clones 10 and 24 are the most genetically similar (D=0.0645) and clones 25 and 10, and 23 and 68 are the genetically distant ones (D=0.6325) (Table 4). The UPGMA dendrogram, constructed based on the genetic distance coefficients, revealed the genetic relatedness of the clones in both the species. One main cluster with 7 clones comprising clones 21, 43, 65, 16, 15, 28 and 38 and a single separate cluster with two clones 49 and 14 were observable in the dendrogram of *E. tereticornis* (Fig. 1). The dendrogram of *E. camaldulensis* showed two main clusters, each with three clones. The first one consisted of clones 68, 10 and 24 and second consisted of clones 23, 25 and 54 (Fig. 2).

The information on genetic distance between the clones will be useful in deciding field deployment of clones more scientifically. It is always safe to plant genetically most distant clones together or in nearby plots as far as pest and disease problems are concerned. Insects and pathogens can multiply and spread rapidly in epidemic proportions if the clones are genetically similar. Hence, clones within a cluster such as clone 21, 43 and 65 (Fig. 1) should not be planted in adjacent plots because the genetic distance between these clones is very low (distance coefficients (D)= 0.0267 to 0.0541). It will be safer to plant clones 14 and 65 in nearby plots because they are genetically more distant (D=0.6931) than other clones.

Table 3. Nei's unbiased measures of genetic distance coefficients for *E. tereticornis* clones

Clone	KFRI 21	KFRI 43	KFRI 65	KFRI 49	KFRI 14	KFRI 16	KFRI 15	KFRI 28	KFRI 38
KFRI 21	****								
KFRI 43	**0.0267**	****							
KFRI 65	0.0541	0.0267	****						
KFRI 49	0.5931	0.5465	0.5931	****					
KFRI 14	0.5931	0.6419	**0.6931**	0.3795	****				
KFRI 16	0.3795	0.3417	0.3795	0.4187	0.5021	****			
KFRI 15	0.3417	0.3054	0.3417	0.3795	0.3054	0.1411	****		
KFRI 28	0.3417	0.3054	0.3417	0.3795	0.4595	0.2036	0.1112	****	
KFRI 38	0.5021	0.4595	0.5021	0.4595	0.6419	0.4187	0.3054	0.2364	****

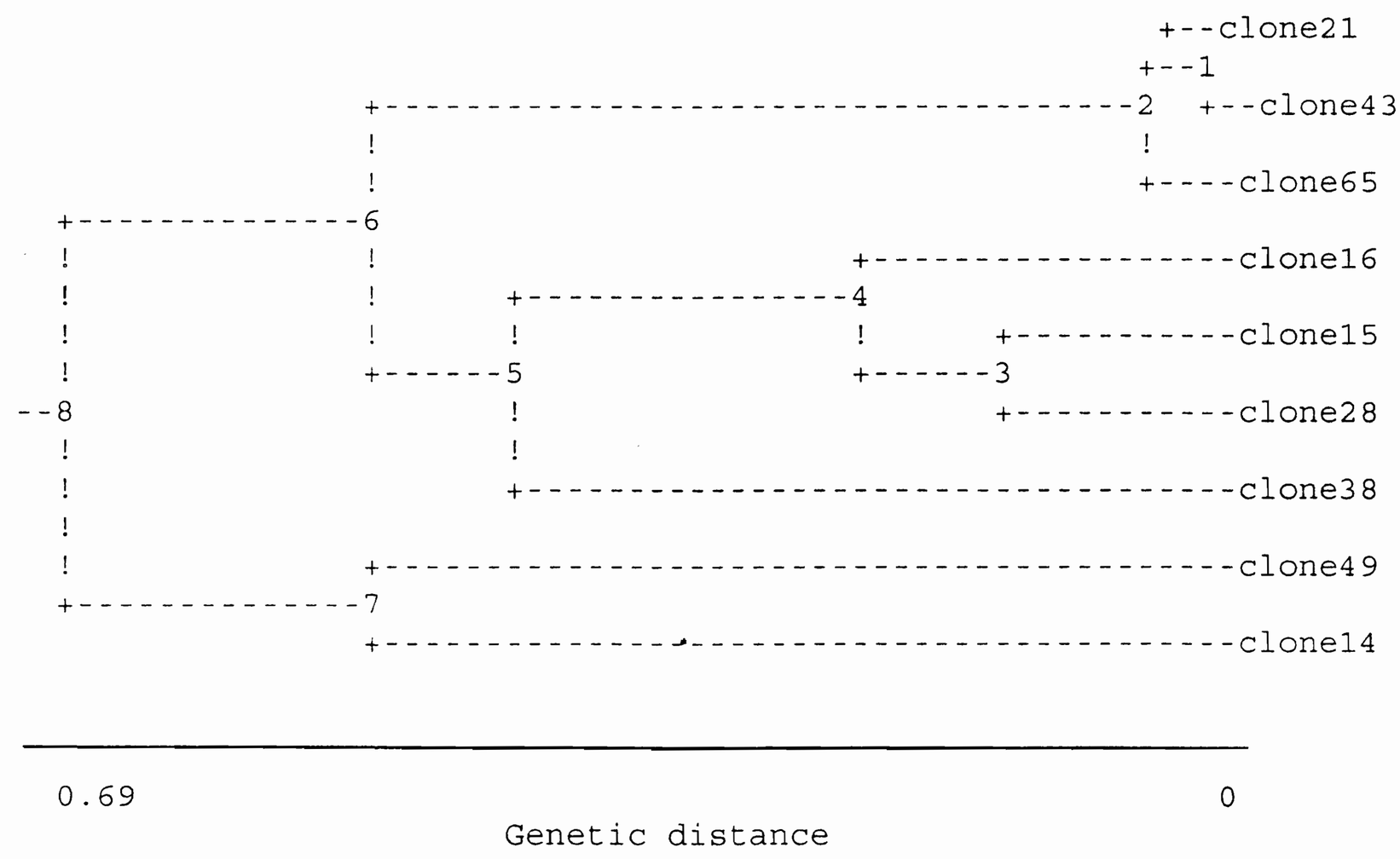


Fig. 1. Dendrogram based on Nei's (1978) genetic distance for *E. tereticornis* clones

Table 4. Nei's unbiased measures of genetic distance coefficients for *E. camaldulensis* clones

Clones	KFRI 68	KFRI 23	KFRI 25	KFRI 10	KFRI 24	KFRI 54
KFRI 68	****					
KFRI 23	**0.6325**	****				
KFRI 25	0.5754	0.2469	****			
KFRI 10	0.4212	0.4700	**0.6325**	****		
KFRI 24	0.3302	0.3747	0.5213	**0.0645**	****	
KFRI 54	0.4700	0.3302	0.2877	0.3302	0.3302	****

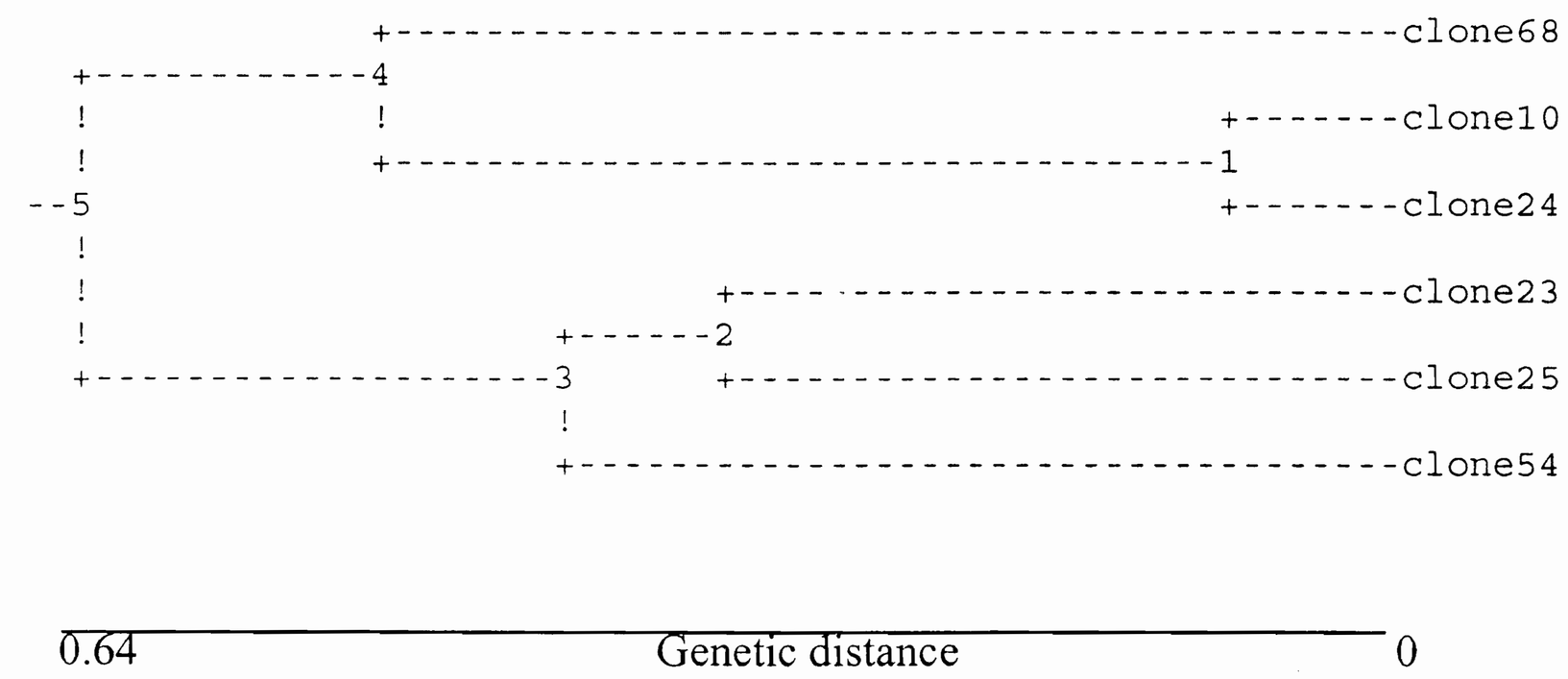


Fig. 2. Dendrogram based on Nei's (1978) genetic distance for *E. camaldulenis* clones

3.3. Acacia mangium

Seven clones of *A. mangium* were used for DNA finger printing and for estimating genetic variation using the four selected primers (OPB 01, OPB 04, OPB 05 and OPB 17) out of 40 tested. Twenty five RAPD bands were scored for presence or absence. The size of the bands generally ranged from 100 to more than

1000 base pairs. Assuming that each RAPD band represented a single locus, all the loci (viz. 7 loci for OPB 01, 5 for OPB 04, 8 for OPB 05 and 5 for OPB 17 (Plates IV and V) were found to show 56 per cent polymorphism (14 out of 25 loci.).

The various genetic diversity measures viz. per cent of polymorphism, number and frequency of alleles and gene diversity indices are sufficient enough to discriminate the seven genotypes of the species. Genetic similarity shows that clone 1 and clone 7 are the genetically most distant ones (D=0.654) and the clone 1 and clone 4 are the genetically similar ones (D=0.128). The UPGMA dendrogram constructed based on the genetic distance coefficients revealed the genetic relatedness of the seven genotypes. Two main clusters with clear separation were observable in the dendrogram. The first cluster consisted of genotypes 1, 4, 2 and 3 and the second cluster consisted off genotypes 5, 6 and 7.

The genetic similarity values of the seven genotypes of *A. mangium* showed that the values (0.52-0.88) were significantly higher than the genetic distance values (0.128-0.654). The high amount of genetic similarity may be due to the common origin of the clones from Australian provenance, Wipim Oriomo.

Hence, while deploying the clones, it is better to avoid clones which come together within the same cluster as seen in Figure 3. For example, field deployment of clones 1 and 4, 2 and 3, and 5 and 6 should be avoided because they are genetically more identical than others and hence there is an increased chance for faster spread of disease or pest epidemic in genetically similar clones.

Table 5. Nei's (1972) unbiased measures of genetic distance between the seven clones of *A. mangium*

Clone	1	2	3	4	5	6	7
KFRI 38B-1	***						
KFRI 38B-2	0.274	***					
KFRI 38B-3	0.387	0.174	***				
KFRI 38B-4	0.128	0.223	0.223	***			
KFRI 38B-5	0.223	0.329	0.446	0.174	***		
KFRI 38B-6	0.446	0.329	0.446	0.387	0.174	***	
KFRI 38B-7	0.654	0.511	0.386	0.446	0.328	0.329	***

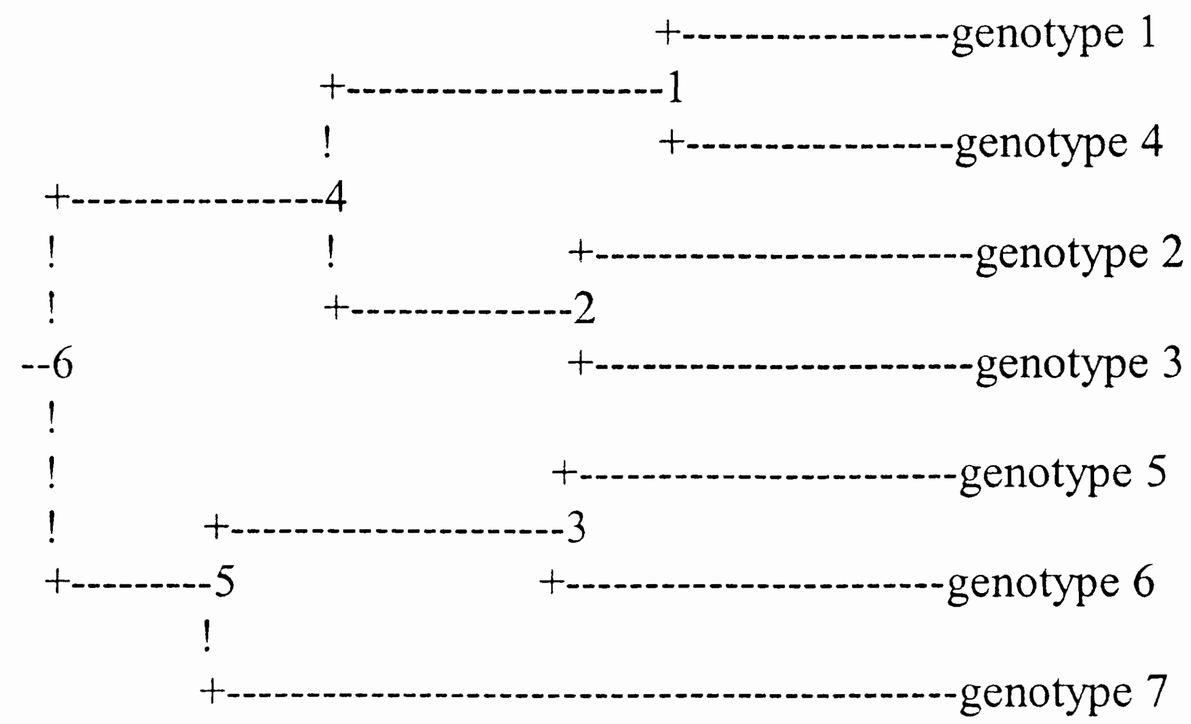


Fig. 3. Dendrogram of seven clones of *A. mangium* based on Nei's (1972) genetic distance

4. Conclusion and Recommendation

RAPD finger printing and estimation of genetic diversity of 15 KFRI eucalypt clones and seven mangium clones have been carried out. It is possible to differentiate the clones using RAPD finger prints using the same primer and PCR conditions. The DNA finger prints will be useful for registration of clones. Genetic diversity analysis shows that genetic distance coefficient values between clones are very low indicating high genetic similarity between the clones, especially with respect to mangium clones. The UPGMA dendrogram constructed based on the genetic distance coefficients has grouped the clones into different clusters. Clones within a cluster are genetically similar. Planting genetically similar clones together runs the risk of disease and pest epidemic. This point has to be borne in mind during field planting of the clones. Obviously, clones included within a cluster have to be planted apart keeping sufficient distance between them. Ramets of such clones should not be mixed while planting.

The RAPD fingerprints of each clone obtained in the present study can be used for registering the clones along with their morphological characters.

Only a few primers have been used in the present study. For obtaining more accurate results, more number of primers have to be used for genetic diversity studies. It may also be possible to get still further accurate results if microsatellite markers are employed for DNA finger printing and genetic diversity studies.

5. References

Dawson, I.K., Simons, A.D., Waugh, R. and Powell, D. 1996. *Gliricidia sepium* x *G. maculata* in Meso-America revealed by PCR based assays. *Mol. Ecol.* **5**: 89-98.

De Lala, M.L., Gomes, E.A., Esbrisse, E.J. and De Araujo, E.F. 2000. Random amplified polymorphic DNA (RAPD) analysis of genotypic identities in *Eucalyptus* clones. *Silvae Genet.* **49**: 239-243.

Doyle, J.J. and Doyle, J.L. 1990. Isolation of DNA from fresh plant tissue. *Focus* **12**: 13-15.

Frankel, O.H., Brown, A.H. and Burds, J.J. 1995. The conservation of plant biodiversity, Cambridge University Press. Cambridge.

Lange, W.J., Wingfield, B.D., Viljoen, C.D. and Wingfield, M.J. 1993. RAPD fingerprinting to identify *Eucalyptus grandis* clones. *South Afric. For. J.* **167**: 47-50.

Lisitsyn, N., Lisitsyn, N. and Wigler, M. 1993. Cloning the differences between two complex genomes. *Science* **259**: 946-956.

Namkoong, G., Boyle, T., Gregorius, H.R., Joly, H.Y., Savolainen, O., Ratnam, R. and Young, A. 1996. Testing criteria and indicators for assessing the sustainability of forest management: genetic criteria and indicators. CIFRO, Bogor, Indonesia, p. 12.

Nei, M. 1972. Genetic distance between populations. *American Naturalist*, **106,** 283-292.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**: 583- 590.

Ortiz, A., Renaud, R., Calzada, I. and Ritter, E. 1997. Analysis of plum cultivars with RAPD markers. *J. Hort. Sci.* **72**: 1-9.

Rafalski, T.A. and Tingey, S.T. 1993. Genetic diagnostics in plant breeding-RAPDs, microsatellites and machines. *Trends in Genet.* **9**: 275-280.

Rekha, V. A. 2000. A comparative study and taxonomic evaluation of foliar characters of some Australian clones of *Eucalyptus tereticornis* Sm. M. Phil. Dissertation in Botany. Bharathiar University, Tamil Nadu.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, L.A. and Tingey, S.V. 1990. DNA polymorphism amplified by arbitrary primers is useful as genetic markers. *Nucleic Acids Res.* **18**: 6531-6535.

Yeh, F.C., Yang, R. and Boyle, T. 1999. POPGENE Version 1.31: Microsoft Window-based Freeware for Population Genetic Analysis. A Quick User's Guide. A joint project developed by Centre for International Forestry Research and University of Alberta, Canada, p. 25.

PLATE I

RAPD finger prints of Eucalypt clones using Primer OPA 02

Clone numbers KFRI 21 to KFRI 54

M = Marker - Low range DNA ruler (Genei, Bangalore) 100bp – 3000bp

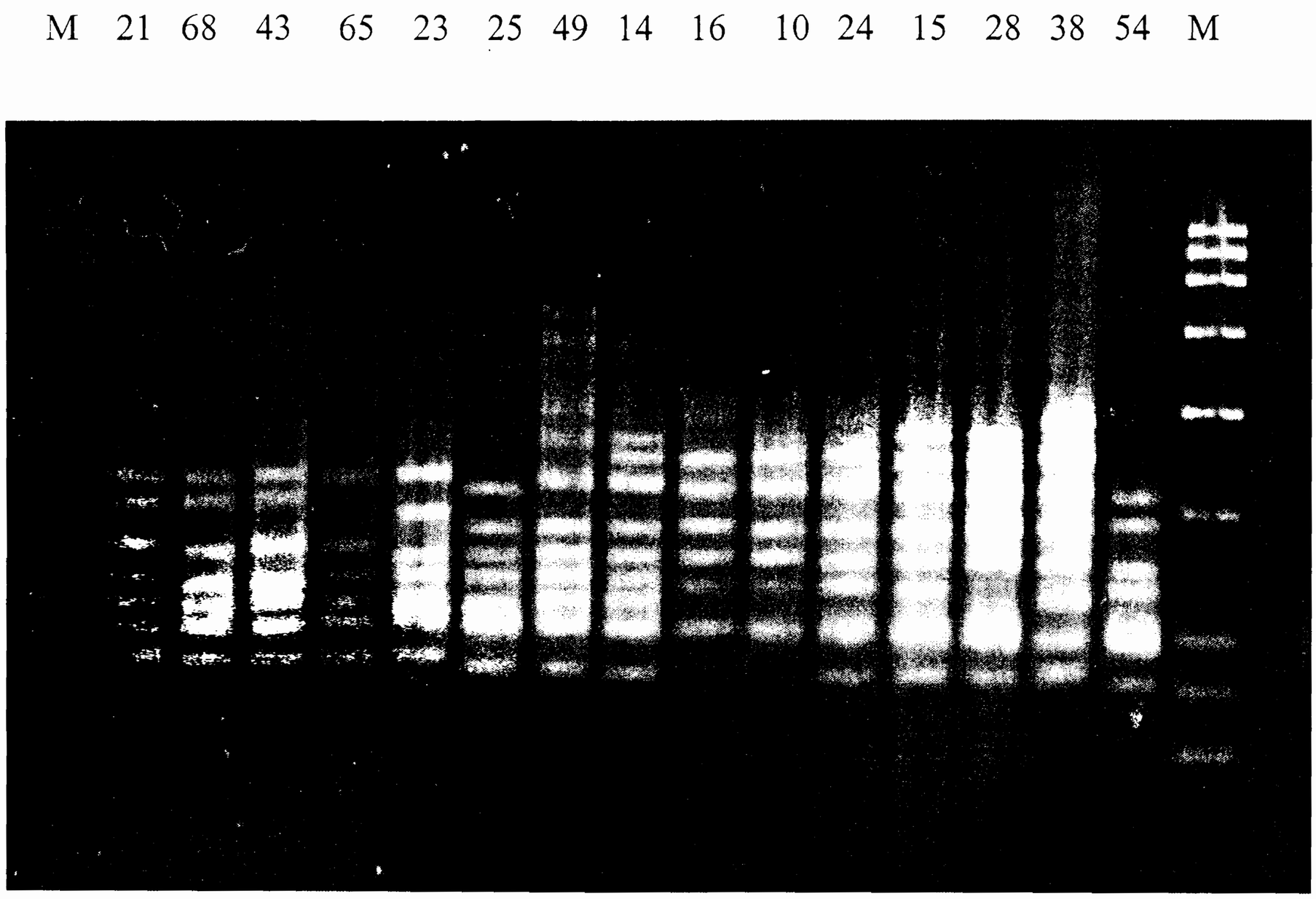


Plate II

RAPD finger prints of Eucalypt clones using Primer OPA 13

Clone numbers KFRI 21 to KFRI 54

M = Marker - Low range DNA ruler (Genei, Bangalore) 100bp – 3000bp

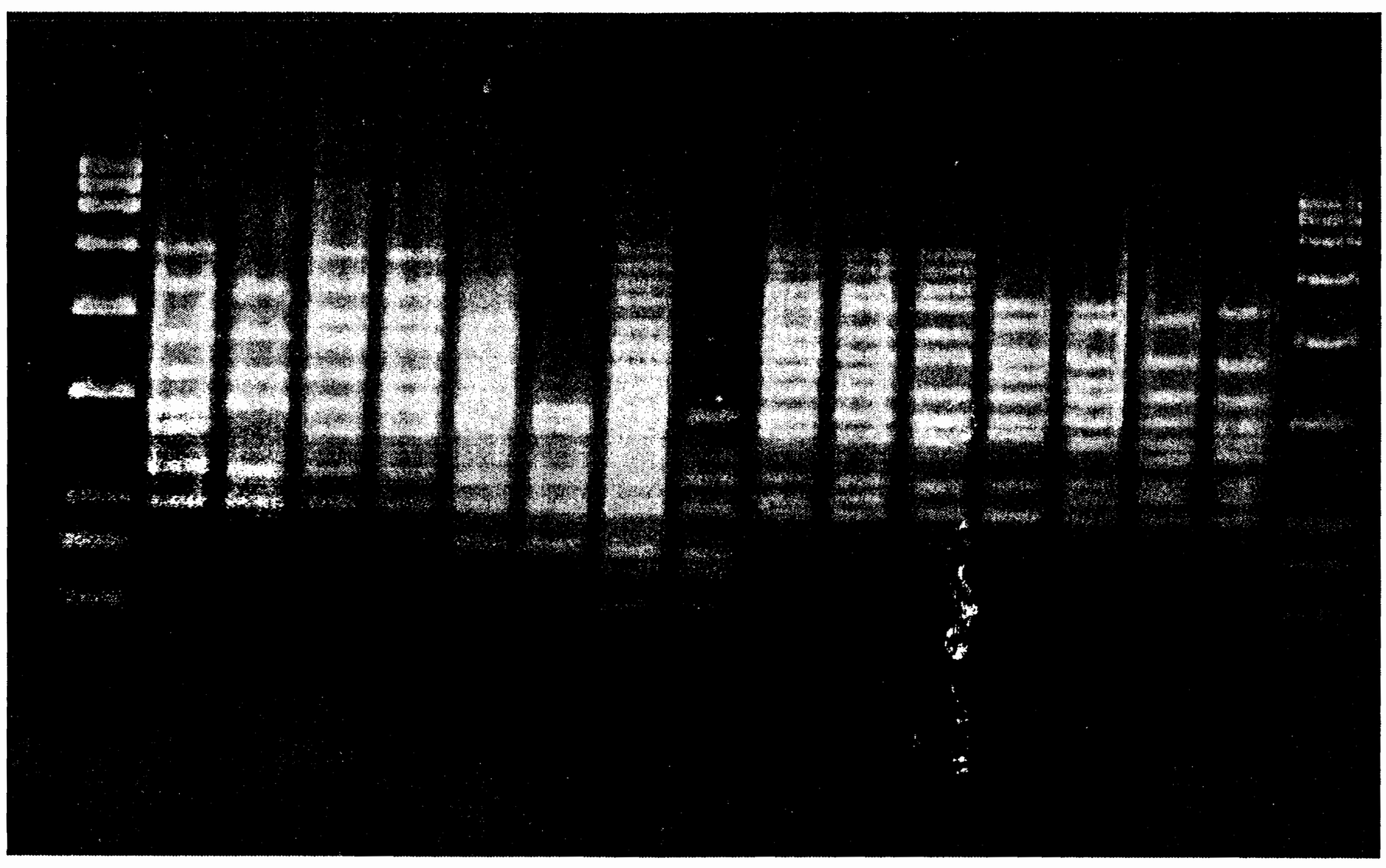


PLATE III

RAPD finger prints of Eucalypt clones using Primer OPA 18

Clone numbers KFRI 21 to KFRI 54

M = Marker - Low range DNA ruler (Genei, Bangalore) 100bp – 3000bp

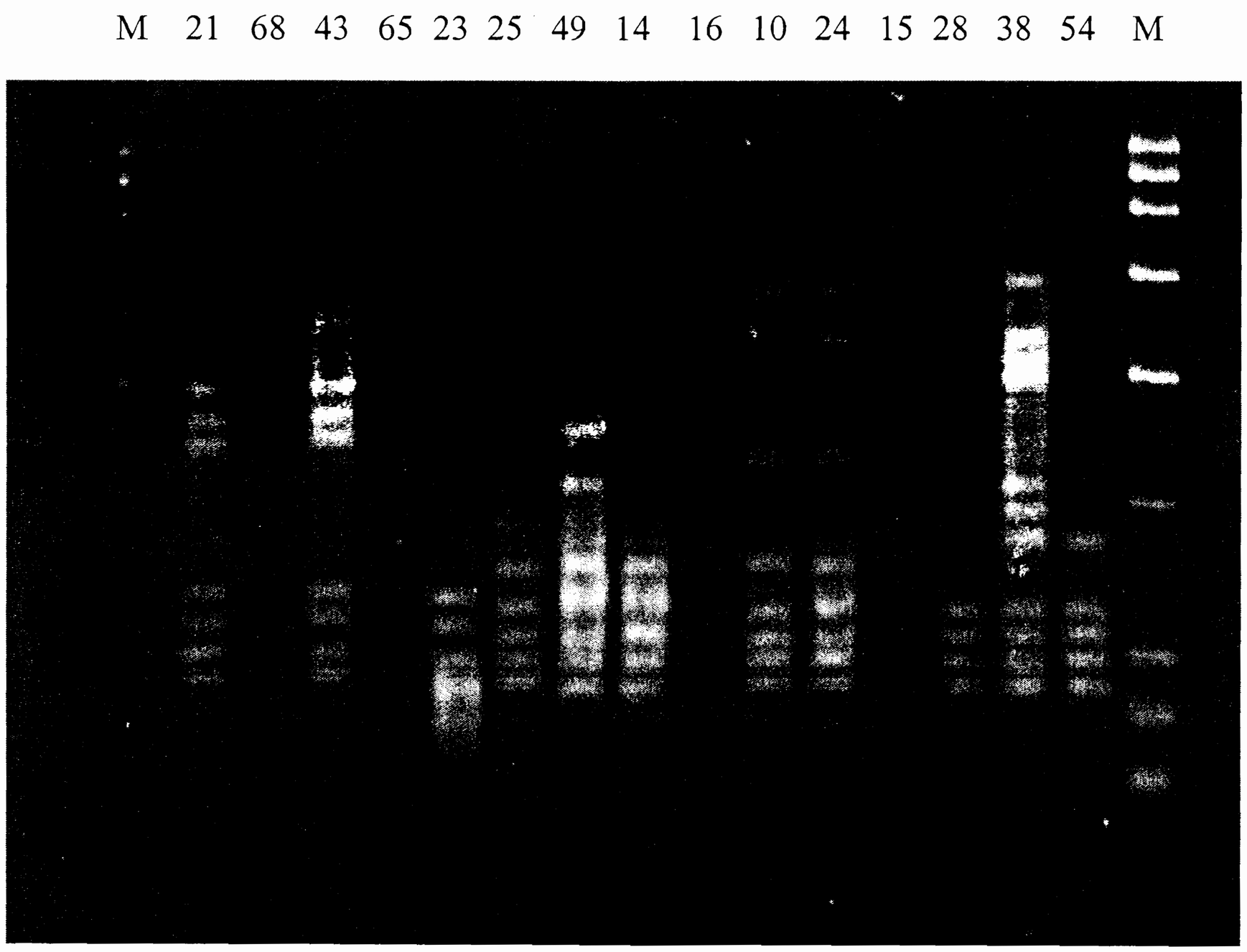


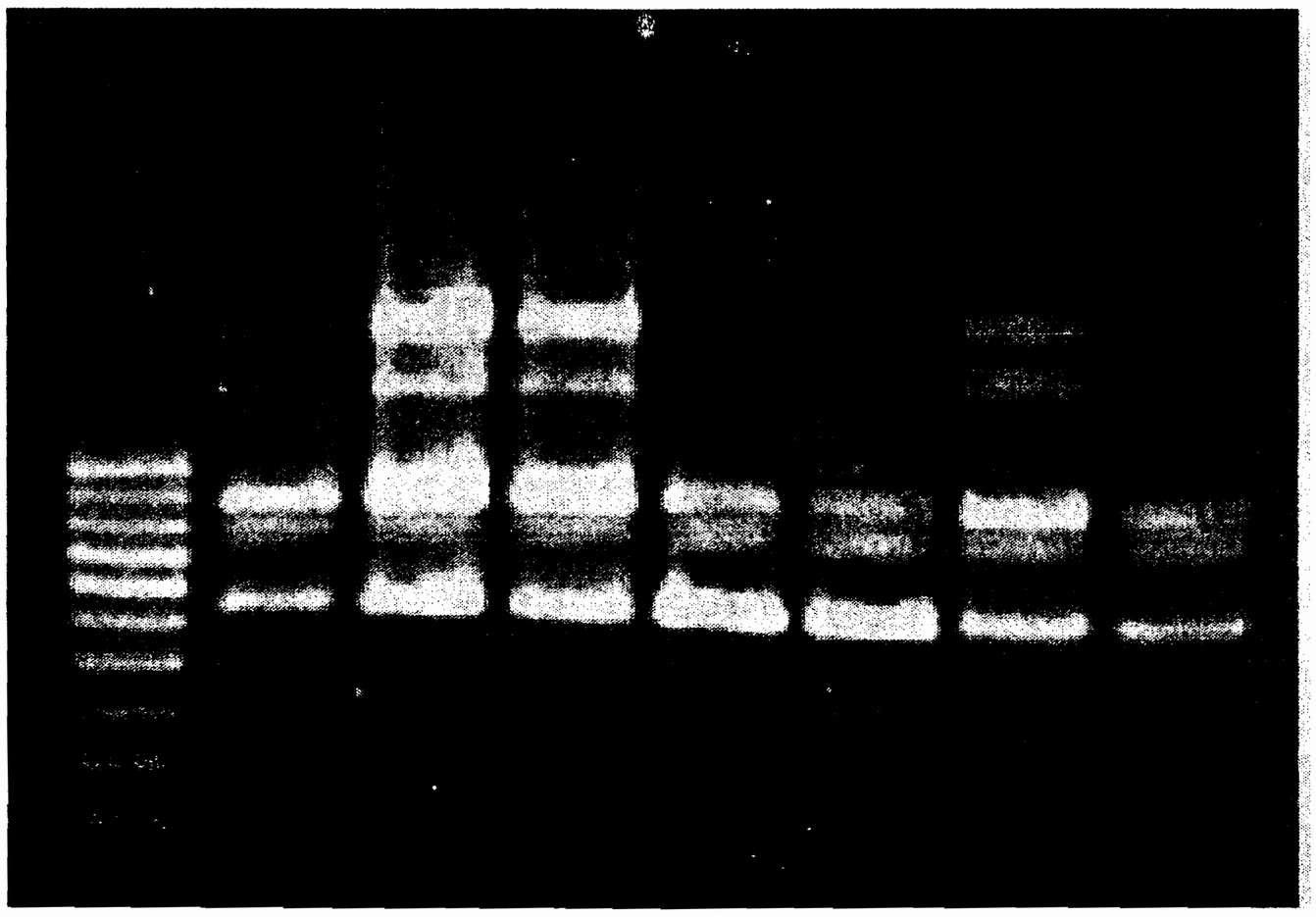
PLATE IV

RAPD finger prints of *Acacia mangium* clones using Primer OPB 01

KFRI Clone number KFRI 38 B-1 to KFRI 38 B-7

M = Marker (100 bp DNA ladder)

M 1 2 3 4 5 6 7



Primer OPB 04

M 1 2 3 4 5 6 7

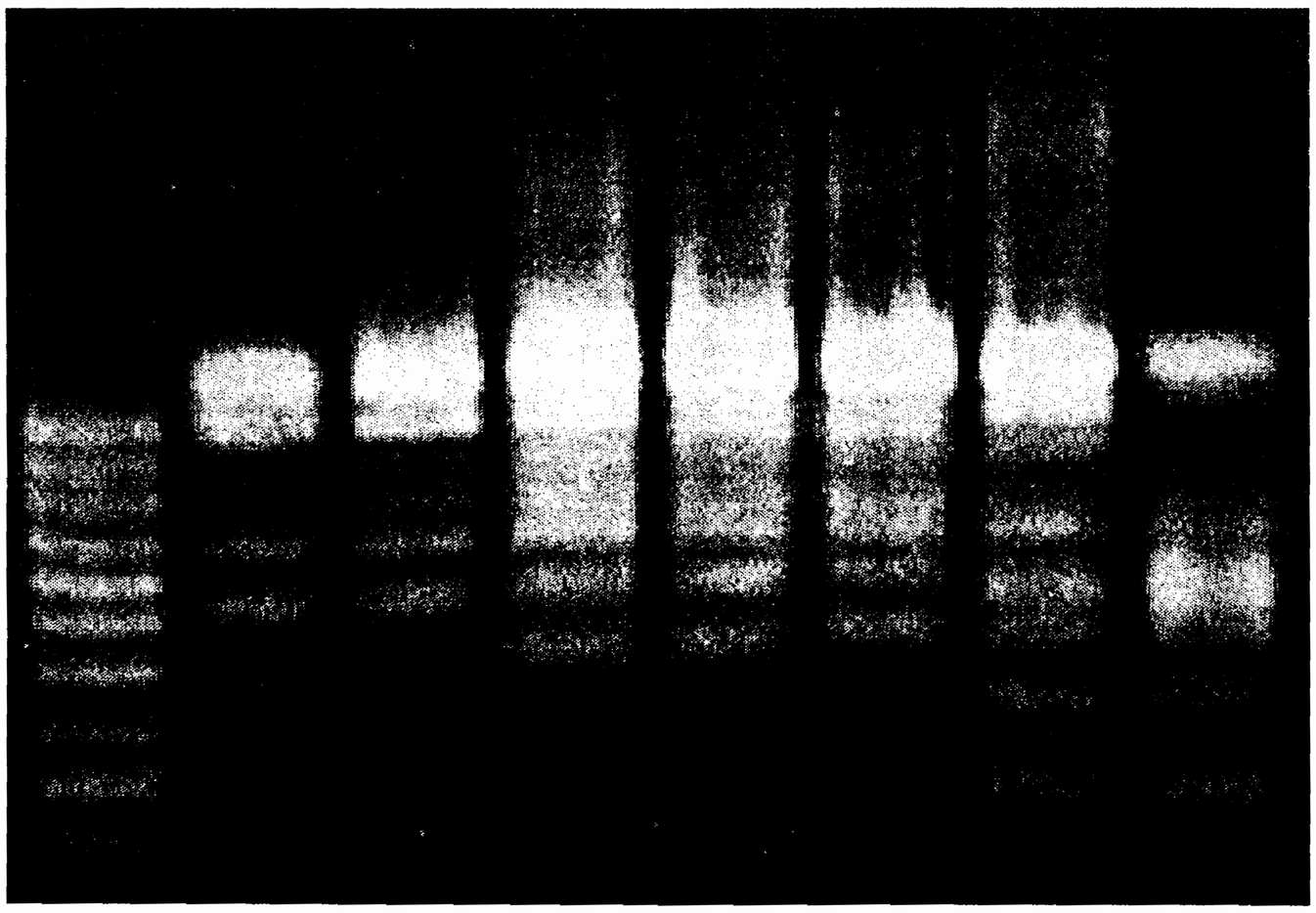


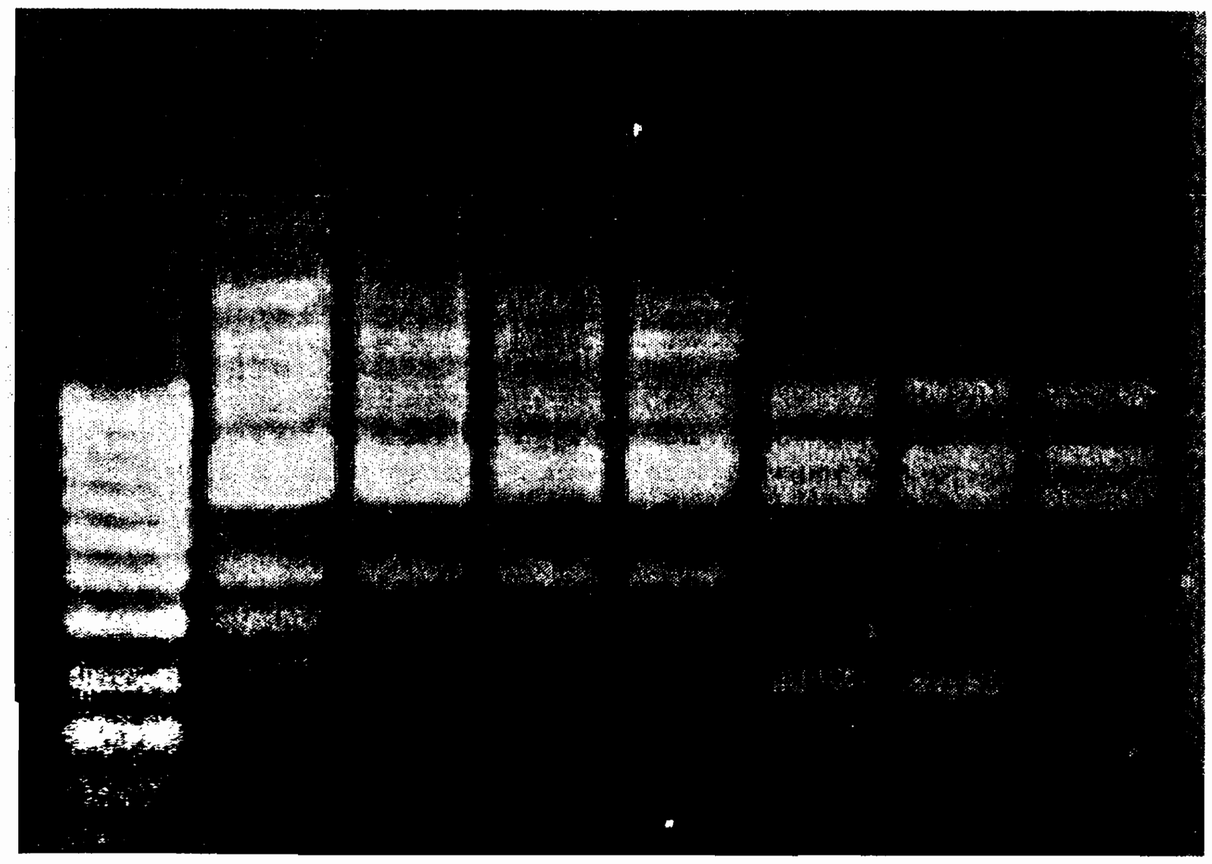
PLATE V

RAPD finger prints of *Acacia mangium* clones using Primer OPB 05

KFRI Clone number KFRI 38 B-1 to KFRI 38B-7

M = Marker (100 bp DNA ladder)

M 1 2 3 4 5 6 7



Primer OPB 17

M 1 2 3 4 5 6 7

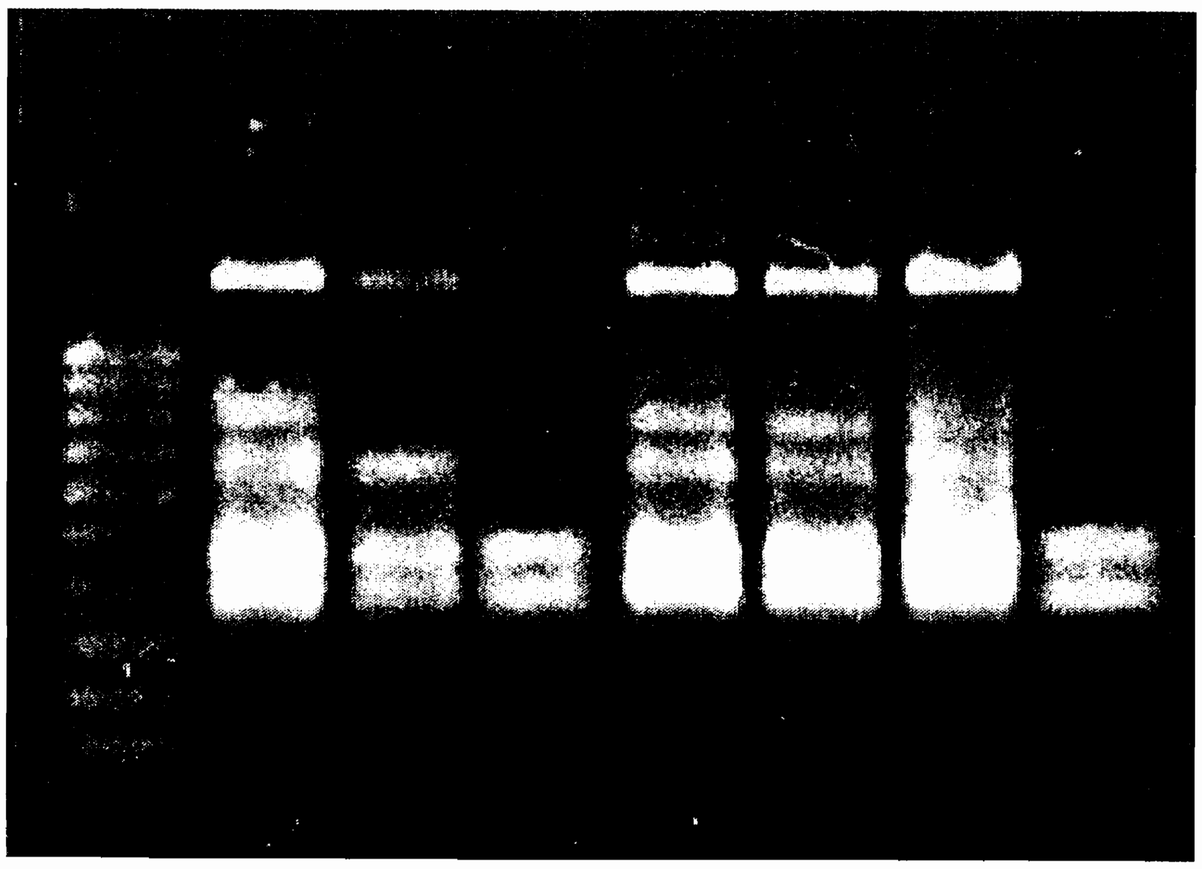


PLATE VI

DNA finger prints of *E. tereticornis* clones

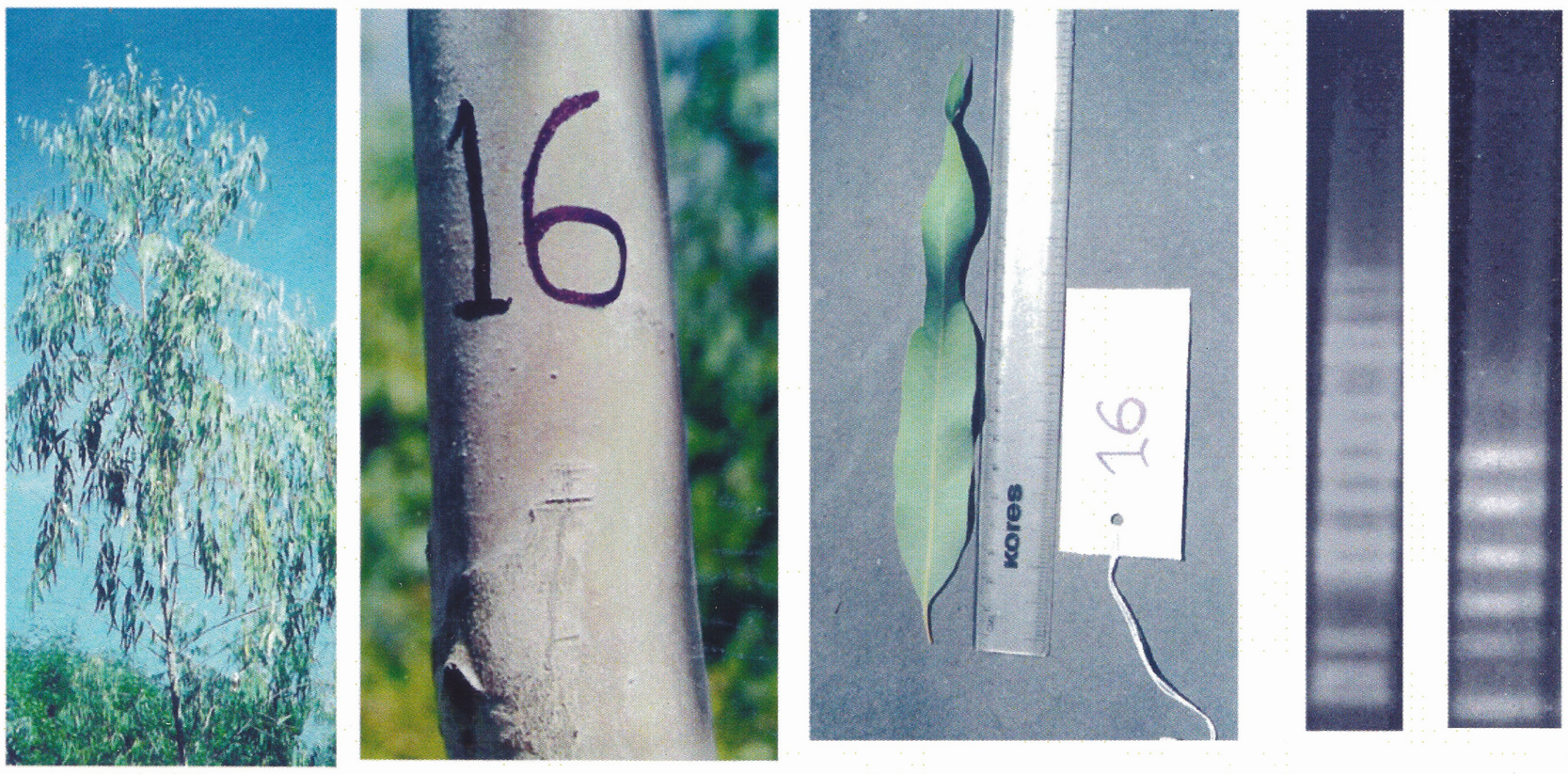
KFRI 14 OPA 13 OPA 02 OPA 18

KFRI 15 OPA 13 OPA 02

PLATE VII

DNA finger prints of *E. tereticornis* clones

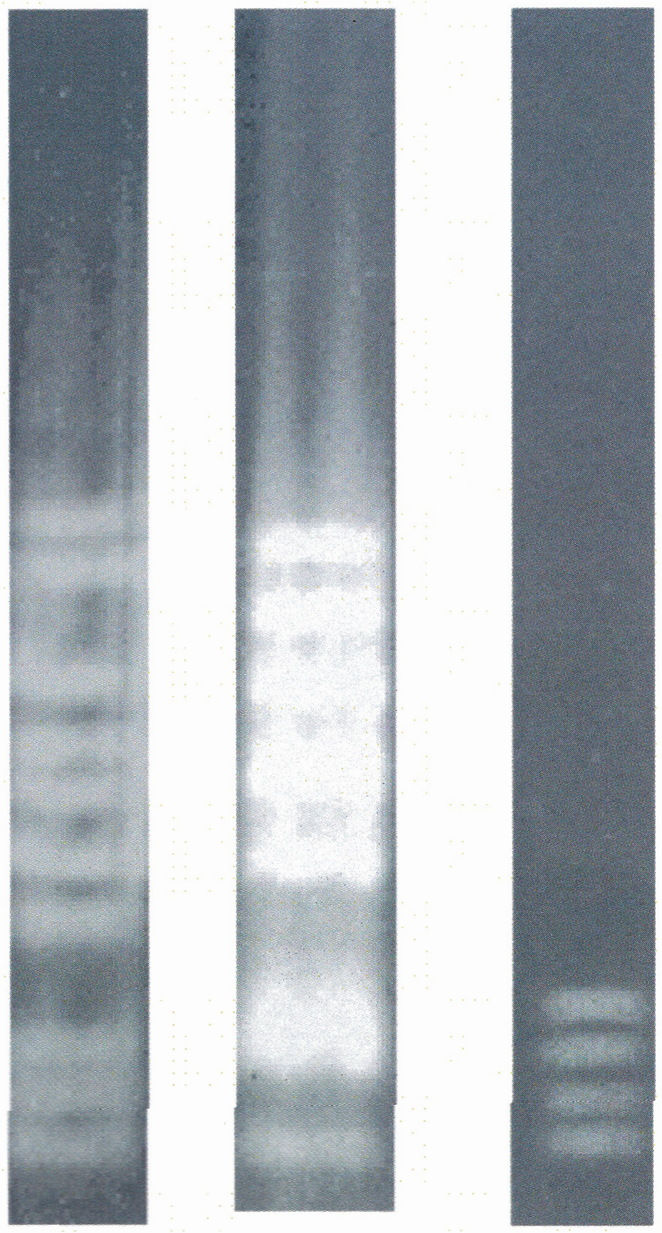
KFRI 16 OPA 13 OPA 02



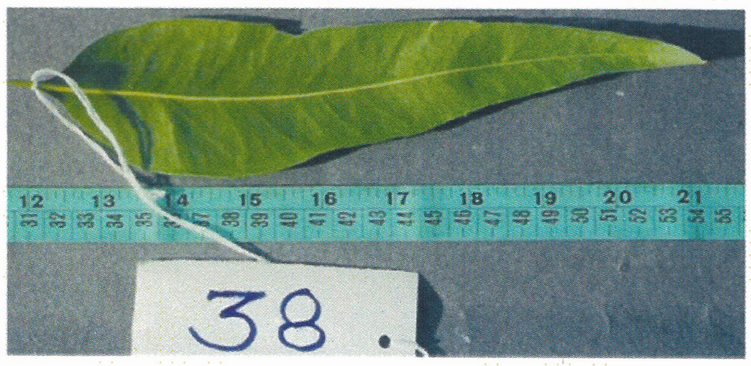
KFRI 21 OPA 13 OPA 02 OPA 18

PLATE VIII
DNA finger prints of *E. tereticornis* clones

KFRI 28



KFRI 38



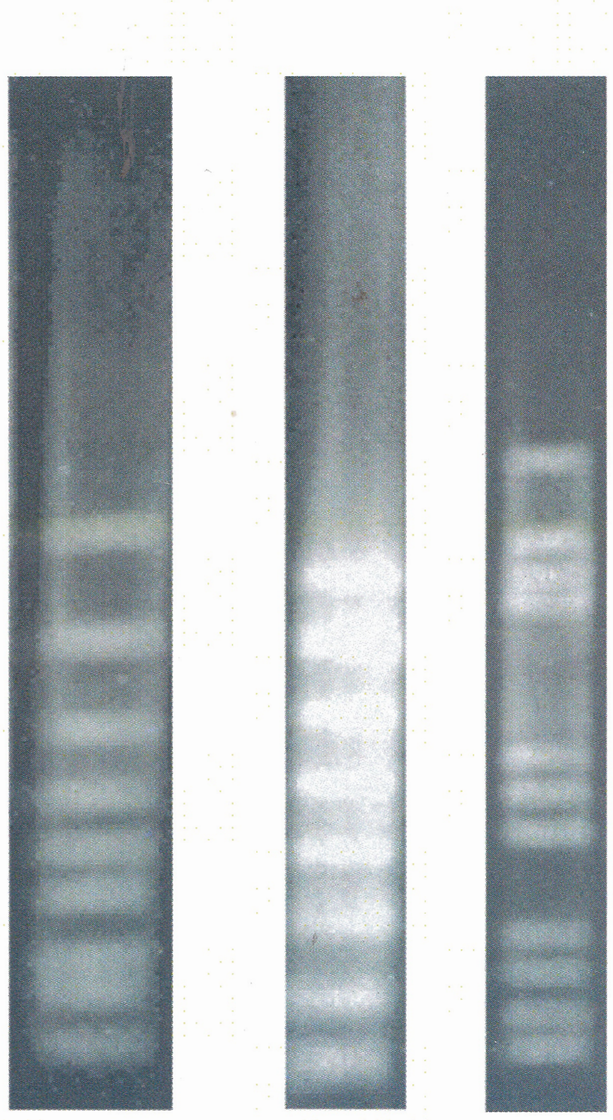
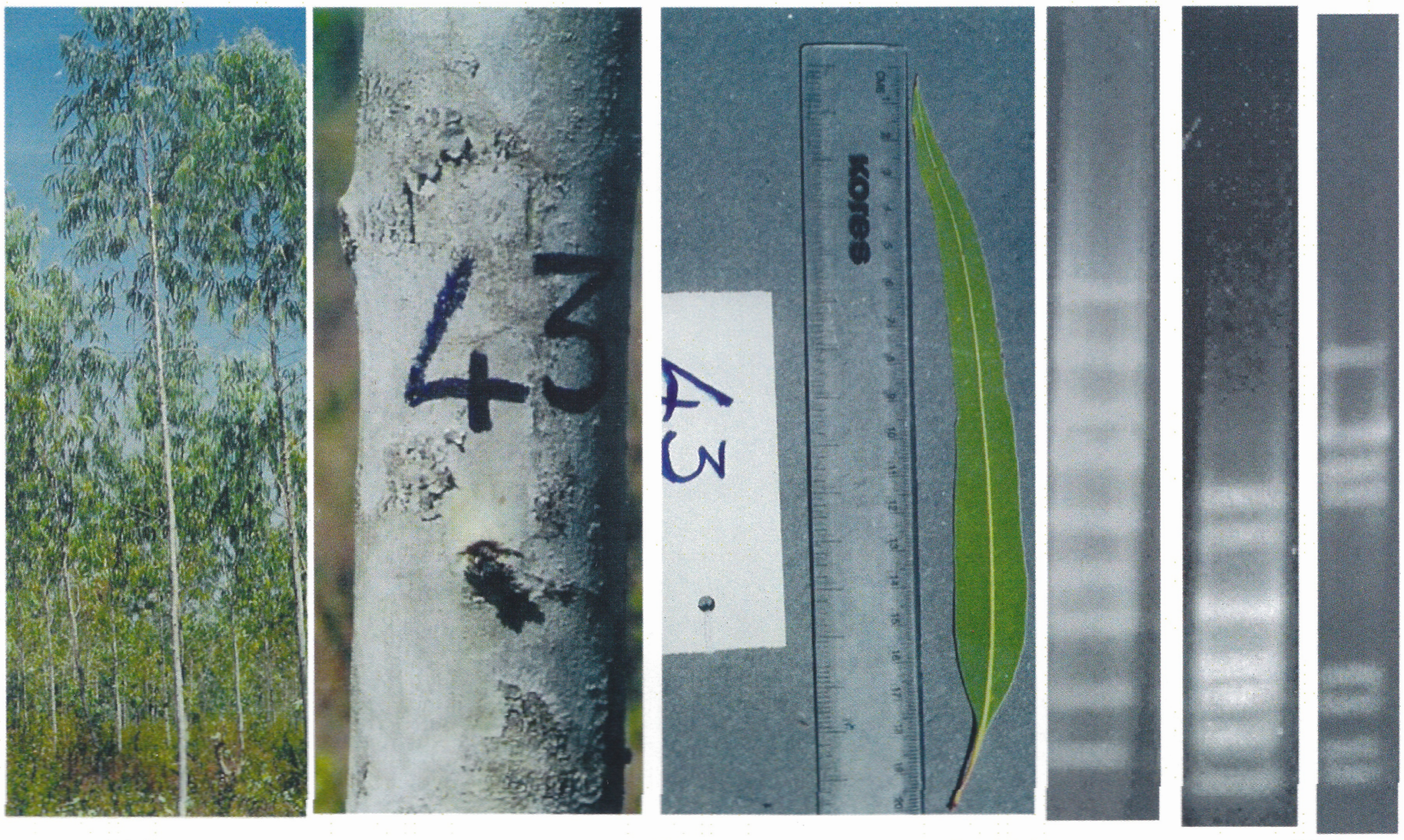


PLATE IX

DNA finger prints of *E. tereticornis* clones

KFRI 43 OPA 13 OPA 02 OPA 18



KFRI 49 OPA 13 OPA 02 OPA 18

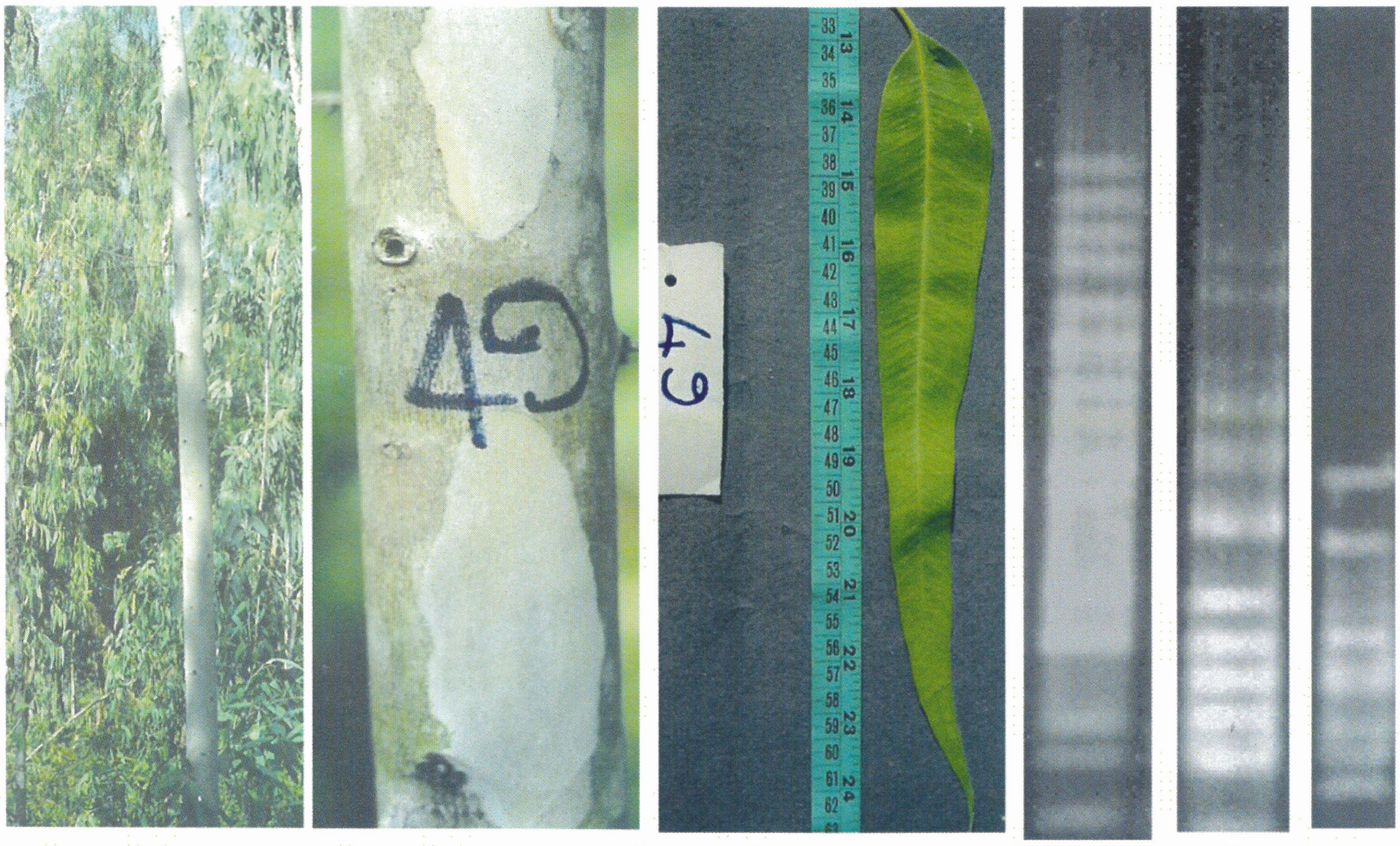


PLATE X

DNA finger prints of *E. tereticornis* clones KFRI 65