Genetic Diversity Analysis and RAPD Marker Inheritance in F_1 Progenies of Mulberry (*Morus spp*.)

Jyoti Prakash[1], V. G. Naik[2], A. Sarkar[2] and R. K. Salgotra[1*,]

[1]School of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha, Jammu-180 009, J and K, INDIA
[2]Molecular Biology Laboratory, Central Sericultural Research and Training Institute, Central Silk Board, Srirampura, Mysore, Karnataka-570 008, INDIA

**Corresponding author: R. K. Salgotra, rks_2959@rediffmail.com*

Abstract

The present study attempts to understand the pattern of RAPD markers inheritance in the F_1 progenies of a cross Mysore Local and V-1 of mulberry (*Morus spp*.). RAPD marker analysis was carried out using 5 decamer random primers. Out of 33 amplicons generated, 20 were of monomorphic and rest 13 were polymorphic (39:39%). The size of the amplified products ranged from 500-3200 bp. The genetic distance (GD) between the parents based on RAPD data set is 0.186, which is low in comparison with morpho-agronomical divergence. In some progenies, GD calculated was higher than the parents suggesting OPA-02_{2900}, OPA-02_{900}, OPA-02_{800} RAPD markers showed expected Mendelian pattern of inheritance. Besides three other markers, OPA-04_{1450}, OPA-04_{1000} and OPA-07_{1000} showed Mendelian segregation pattern for 1:1 ratio. The finding proves that the dominant status of the RAPD markers may not be a limiting factor in genetical analysis of a heterozygous tree like mulberry, where F_1 tends to behave like a segregating population. The polymorphic RAPD markers in parents, segregates as independent alleles in the F_1 progenies and showed the heterozygotic status of mulberry varieties.

Keywords: *Morus spp*, RAPD markers inheritance, molecular diversity.

The mulberry (*Morus spp*.) is an economically important plant, as it is the only food source of the domesticated Silkworm, *Bombyx mori* L. Mulberry is reported to be distributed in 30 countries. According to Western historian, mulberry culture spread to India from China through Kotar (Tibet) by about 140 B.C. Since then the sericulture industry has widely grown and today it provides livelihood to approximately seven million people in the country. The genus *Morus* belongs to family Moraceae and it comprises of many species and varieties. There is considerable amount of confusion in the taxonomy of the genus as adopted by different taxonomist. The classification of mulberry more relined on the external

characters like the leaves, flowers, fruits etc. According to Koidzumi (1917), mulberry has 24 species and a variety under species. The classification of Indian varieties of mulberry as in other countries is also not clear. Brandis (1874) described four species, *Morus alba* Linn., *M. laevigata* (*M. glabrata* Wall.), *M. Indica* and *M. serrata* Roxb. (*M. pabularia* Dacne). Hooker (1885) recorded *M. indica* Linn., *M. serrata* Roxb., and *M. laevigata* Wall, and *M. atropurpurea* Roxb., a chinese species, closely allied to *Morus alba*. Several varieties like V-1, S-1, S-36, K-2, S-13, S30, Mysore Local, C-776 etc. are being cultivated in the country. Most of the Indian varieties of mulberry belong to *M. indica* or *M. alba.*

Mulberry is a perennial, heterozygous, dioecious and cross-pollinated tree plant. Genetic improvement of mulberry cultivars by adopting the conventional breeding methods, like other agricultural or horticultural plants, is not been so successful because of meager genetic information for important economical traits and their linkages. Typical constraints associated with tree crop like long reproductive cycle, difficulty in characterization and non-availability of genetic information are hindering the success of mulberry important. Homozygous lines are not available consequently very little is known about the inheritance pattern in mulberry. As a result, the selection of parents for hybridization has become increasingly difficult. Identification of divergent parents for hybridization programme through conventional method has not yielded satisfactory results. Morphological traits cannot serve as an unambiguous marker because of environmental influence (Wang and Tanksley, 1989). So, there is a need to apply recent biotechnological approaches, which can help to overcome the problems and give acceleration and directionality to the mulberry variety improvement efforts. The field of biotechnology has a wide expense of strong potentiality in achieving good nutrition, high leaf potentiality of mulberry and a biotic-abiotic stress resistance by transfer of superior gene pools of multiple novel traits from one plant to another plant of different taxonomical relationships. So, biotechnology is the most appropriate for scientific community in short circuiting conventional breeding for sustainable moriculture (Chakravorty and Suryanarayana, 1993). Employing modern molecular methods to study the phylogenetics and consanguinity relationship in mulberry and to interpret the genetic difference between the varieties at molecular level will be very helpful to the geneticists and mulberry breeders.

With the development of PCR-based markers such as random amplification of polymorphic DNA (RAPD) (Williams *et al.,* 1990), amplified fragment length polymorphism (AFLP) (Vos *et al.,* 1995), microsatellite or simple sequence repeat (SSR) (Powell *et al.,* 1996), sequence characterized amplified region (SCARs) (Paran and Michelmore, 1993), cleaved amplified polymorphic sequences (CAPS) (Konieczny and Ausubel, 1993), and direct amplification of length polymorphism (DALP) (Desmarais *et al.,* 1998) it becomes possible to conduct rapid and efficient polymorphism analysis of plant genomic DNAs (John *et al.,* 1990, Lou, 1998) and

to construct a genetic maps (John *et al.,* 1990, Soniski *et al.*, 1996, Mudge *et al.,* 1996) which is necessary for the genetic study of a plant. RAPD technique is simple, faster and cost effective in comparisons to RFLP (Restriction Fragment Length Polymorphism) and does not involve the use of radioactivity. RAPD markers can be generated in large numbers and can complement traditional characters that may be limited in availability. They provide a valuable new resource for phylogenetic studies (Hoey *et al.,* 1996). So these molecular techniques are increasingly used in DNA fingerprinting for cultivar identification (Kollar *et al.*, 1993) diversity estimation (Chalmers *et al.,* 1992), population genetic studies (Huff *et al.,* 1993) and marker aided selection in breeding (Barua *et al.,* 1993). The present study was carried out to the studies the inheritance of RAPD markers and to know the molecular diversity of parents as well as progenies. The work will also provide the DNA profiles of these two economically important mulberry varieties and the progenies derived from the cross of Mysore Local x V-1.

Materials and Methods

Plant material

The study was carried out with two mulberry varieties namely, Mysore Local and V-1. The variety Mysore Local has been maintained in gene bank of Central Sericultural Research and Training Institute (CSR and TI), Mysore. V-1 was cultivated in the number of plots for evaluation trials and leaf production. The F_1 progenies of Mysore local (♀) x V-1 (♂) was maintained in small plots of molecular biology section following standard package of practices. Morphological data of both parents and progenies were recorded following the norms of mulberry germplasm characterization.

Genomic DNA isolation and quantification

Fresh young leaves from the plants were collected for DNA extraction using the standard Cetyl Trimethyl Ammonium Bromide (CTAB) method (Doyle and Doyle, 1990). To obtain high molecular weight and pure DNA from mulberry, tissue (0.5g) was ground in liquid nitrogen with the help of mortar and pestle. The powder was transferred to the oak-ridge tubes containing 10ml of extraction buffer [1M Tris-HCI (pH-8.0); 5M NaCl; 0.5M EDTA; 10% SDS (w/v); 10% PVP (w/v) and 2% β-mercaptoethanol (w/v)]. The slurry was incubated at 65°C for 30 minutes and then at room temperature for 15 minutes. The mixture was extracted with equal volume of SEVEG Chloroform: Isoamylalcohol (24:1) and centrifuged at 7500 rpm for 10 minutes. The supernatant was transferred to a fresh tube and repeated the SEVEG Chloroform: Isoamylalcohol (24:1) until the white interphase disappeared. The aqueous phase was extracted once with Phenol : Chloroform : Isoamylalcohol (25 : 24 :1) mixture and centrifuged at 7500 rpm

for 10 minutes and finally once again extracted with SEVEG. The supernatant was transferred to sterile glass corex tubes and DNA was precipitated with 2.0 volume of chilled ethanol. The DNA was recovered by spooling. The recovered DNA was washed with 70% ethanol and air dried and resuspended in 500 µl of TE-buffer [10mM Tris-HCl (pH-8.0),1nM EDTA] and stored at 4°C until used. The DNA was quantified both by spectrophotometric method and on 0.8% agarose gel. The stock solution was diluted to uniform concentration of 10ng/µl.

DNA marker analysis

PCR reactions were performed according to the protocol of Williams *et al.,* (1990). The PCR amplification was carried in a 0.2 ml tube in Gene Amp 9700 PCR system (Applied Biosystems, U.S.A.) with 20µl reaction volumes containing 20 mM Tris-HCl (pH-8.4), 50mM KCl, 2mM $MgCl_2$, 0.2 µM primer, 0.1 mM of each of dATP, dTTP, dCTP, dGTP, 0.5U of taq DNA. The random polymerase (Bangalore Genei) and 20ng of template DNA was used. Five random primers covering most of the chromosomes were obtained from Operon Technologies Ltd., Almada, U.S.A. (Table 1). Amplification reactions were carried out by following cycle profiles: 1 cycle at 93°C for 2 min followed by 45 cycles at 93°C for 1min. 35°C for 1 min., 72°C for 2 min. and a final 7 min. extension at 72°C. PCR products were electrophoresed on 1.5% agarose gel (Sambrook *et al.,* 1989) in 1x TAE, stained in ethidium bromide and the gel image was recorded using the gel-documentation system.

Table 1: List of RAPD primers used in the study and marker polymorphism

Primer	Sequence (5' – 3')	Total no. of amplicons	No. of Polymorphic amplicons	% Poly-morphism
OPA – 01	CAGGCCCTTC	8	3	37.5
OPA – 02	TGCCGAGCTG	7	2	28.6
OPA – 03	AGTCAGCCAC	8	4	50.0
OPA – 04	AATGGGGCTG	6	3	50.0
OPA – 07	GAAACGGGTG	4	1	25.0
Total		**33**	**13**	**39.39**

Inheritance and diversity analysis

The bending patterns generated by RAPD markers were scored as '1' for the presence of amplicons and '0' for the absence. All RAPD markers were performed twice and only reproducible bands in the range of 3500 to 500 were scored. A similarity matrix was generated using the equation s=2Nxy/(Nx+Ny), where Nxy is the number of shared amplications between x and y entries. 'Nx' is the total

number of amplicons in x entry and 'Ny' is the total number of amplicons in y entry. Genetic distance (1-s) was calculated and a dendrogram was constructed based on the distance matrix data sets by applying unweighed pair group method and arithmetic average (UPGMA) using PHYLIP (Phylogeny inference package) version 3.6 (alpha 2). RAPD amplicon markers were identified by the source of primers (OP=Operon) kit letter, the primer no. and its approximate size in base pairs. Goodness of fit to the 3:1 and 1:1 pattern of segregation of RAPD markers, as predicted for the Mendelian characters in a monohybrid cross and test cross respectively, was determined by the Chi-square test.

Results and Discussion

Mysore Local (♀) and V-1 (♂) represented a pair of contrasting genotypes for different morpho-agronomical characters ideally suited for genetically analysis (Table 2). In general, Mysore Local is a low yielder but hardy and nutritionally less superior. Whereas, V-1 is a recently evolved variety with high leaf production,

Table 2: Morpho-agronomical characters of Mysore Local and V-1

Name of the Parameters	Mysore Local	V-1
Color of the matured shoot	Brown	Grey
Phyllotaxy	1/3	2/5
Lentical density/sq.cm	11.0	13.0
Lobation type	Medium Lobed	Unlobed
Lobation number	0-4	0
Leaf Nature	Heterophyllous	Homophyllous
Leaf texture	Chartaceous	Coraceous
Leaf shape	Ovate	Ovate-Lanceolate
Easiness for harvesting	Hard to harvest	Easy to harvest
Leaf length(cm)	14.35	25.40
Leaf width(cm)	11.64	17.50
Petiole length (cm)	4.03	5.5
Petiole width(cm)	0.28	0.4
Length of the longest shoot(cm)	125.55	155.10
Total shoot length(cm)	1127.50	1605.00
Girth of the stem(cm)	6.86	10.55
Internodal distance(cm)	3.59	5.86
Yield/Plant(g)	400.77	695.36
Moisture% of leaf	68.14	74.66
Resistance of leaf spot	Moderately resistant	Resistant
Resistance to Powdery Mildew	Highly suspecptible	Moderately resistant

Table 3: Morphological characterization of F_1 progenies

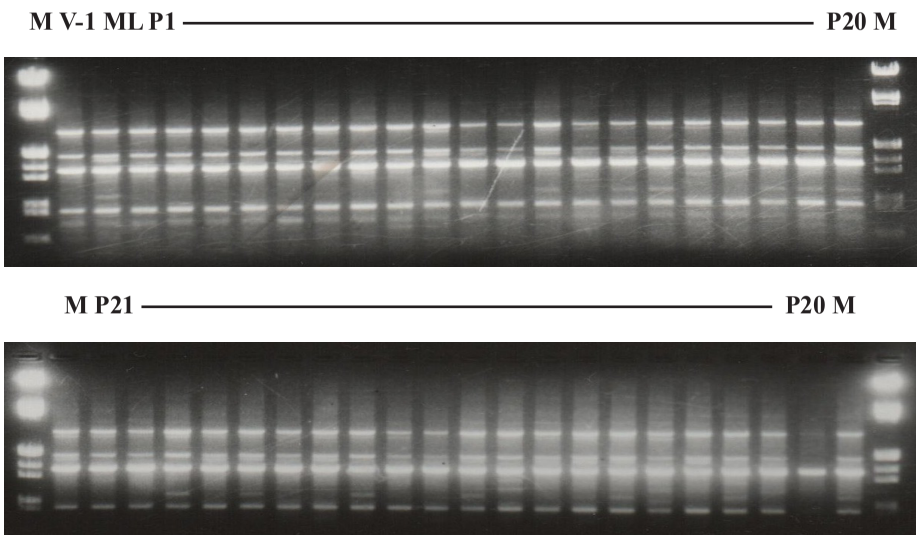
Progenies	Phyllotaxy	Lobation		Leaf colour	Leaf texture	Leaf shape	Leaf margin	Leaf apex	Leaf base
		Nature	Type						
P_1	1/3	Unlobed	..	Green	Chartaceous	Wide Ovate	Crenate	Acuminate	Cordate
P_2	2/5	Unlobed	..	Green	Chartaceous	Wide Ovate	Dentate	Acuminate	Truncate
P_3	2/5	Unlobed	..	Light green	Chartaceous	Wide Ovate	Dentate	Acuminate	Cordate
P_4	2/5	Unlobed	..	Deep green	Chartaceous	Wide Ovate	Crenate	Acuminate	Cordate
P_5	1/3	Unlobed	..	Deep green	Chartaceous	Wide Ovate	Dentate	Acuminate	Cordate
P_6	1/3	Unlobed	..	Green	Chartaceous	Wide Ovate	Serrate	Acuminate	Truncate
P_7	1/3	Unlobed	..	Deep green	Chartaceous	Wide Ovate	Serrate	Acuminate	Cordate
P_8	1/3	Unlobed	..	Green	Chartaceous	Wide Ovate	Dentate	Acuminate	Cordate
P_9	2/5	Unlobed	..	Deep green	Chartaceous	Wide Ovate	Dentate	Acuminate	Cordate
P_{10}	2/5	Unlobed	..	Deep green	Chartaceous	Wide Ovate	Crenate	Acuminate	Cordate
P_{11}	Mixed	Unlobed	..	Green	Coriaceous	Ovate	Crenate	Acuminate	Truncate
P_{12}	1/3	Lobed	Medium	Light green	Chartaceous	Wide Ovate	Dentate	Acuminate	Cordate
P_{13}	2/5	Lobed	Shallow	Light green	Coriaceous	Ovate	Crenate	Acuminate	Truncate
P_{14}	1/3	Unlobed	..	Light green	Coriaceous	Wide Ovate	Crenate	Acuminate	Truncate
P_{15}	2/5	Unlobed	..	Green	Chartaceous	Wide Ovate	Serrate	Acuminate	Cordate
P_{16}	2/5	Lobed	Medium	Deep green	Chartaceous	Wide Ovate	Dentate	Acuminate	Cordate

Contd.

Progenies	Phyllotaxy	Lobation		Leaf colour	Leaf texture	Leaf shape	Leaf margin	Leaf apex	Leaf base
		Nature	Type						
P_{18}	2/5	Unlobed	..	Green	Chartaceous	Wide Ovate	Crenate	Acuminate	Truncate
P_{19}	2/5	Lobed	Shallow	Green	Coriaceous	Wide Ovate	Serrate	Acuminate	Truncate
P_{20}	2/5	Lobed	Shallow	Green	Chartaceous	Wide Ovate	Dentate	Acuminate	Cordate
P_{21}	2/5	Unlobed	..	Deep green	Chartaceous	Wide Ovate	Crenate	Acuminate	Truncate
P_{22}	2/5	Lobed	Medium	Deep green	Coriaceous	Wide Ovate	Dentate	Acuminate	Cordate
P_{23}	2/5	Unlobed	..	Deep green	Chartaceous	Ovate	Dentate	Acuminate	Truncate
P_{24}	2/5	Unlobed	..	Green	Chartaceous	Wide Ovate	Crenate	Acuminate	Cordate
P_{25}	1/3	Lobed	Medium	Deep green	Chartaceous	Wide Ovate	Serrate	Acuminate	Cordate
P_{26}	2/5	Lobed	Medium	Light green	Chartaceous	Wide Ovate	Crenate	Acuminate	Truncate
P_{27}	2/5	Unlobed	..	Deep green	Chartaceous	Ovate	Dentate	Acuminate	Cordate
P_{28}	2/5	Unlobed	..	Deep green	Chartaceous	Wide Ovate	Dentate	Acuminate	Truncate
P_{29}	2/5	Unlobed	..	Light green	Chartaceous	Ovate	Dentate	Acuminate	Truncate
P_{30}	1/3	Unlobed	..	Deep green	Chartaceous	Wide Ovate	Dentate	Acuminate	Truncate
P_{31}	2/5	Lobed	Medium	Green	Chartaceous	Wide Ovate	Crenate	Acuminate	Truncate
P_{32}	2/5	Unlobed	..	Green	Chartaceous	Wide Ovate	Dentate	Acuminate	Cordate
P_{33}	2/5	Unlobed	..	Light green	Chartaceous	Wide Ovate	Dentate	Acuminate	Truncate
P_{34}	2/5	Unlobed	..	Light green	Chartaceous	Wide Ovate	Dentate	Acuminate	Truncate
P_{35}	2/5	Lobed	Medium	Green	Chartaceous	Ovate	Crenate	Acuminate	Truncate

Contd.

Progenies	Phyllotaxy	Lobation		Leaf colour	Leaf texture	Leaf shape	Leaf margin	Leaf apex	Leaf base
		Nature	Type						
P_{37}	1/3	Unlobed	..	Deep green	Chartaceous	Ovate	Crenate	Acuminate	Truncate
P_{38}	Mixed	Unlobed	..	Deep green	Chartaceous	Wide Ovate	Crenate	Acuminate	Cordate
P_{39}	Mixed	Lobed	Medium	Deep green	Chartaceous	Ovate	Serrate	Acuminate	Cordate
P_{40}	2/5	Unlobed	..	Deep green	Chartaceous	Wide Ovate	Crenate	Acuminate	Cordate
P_{41}	2/5	Unlobed	..	Light green	Coriaceous	Wide Ovate	Dentate	Acuminate	Cordate
P_{42}	1/3	Lobed	Medium	Green	Coriaceous	Wide Ovate	Serrate	Acuminate	Cordate



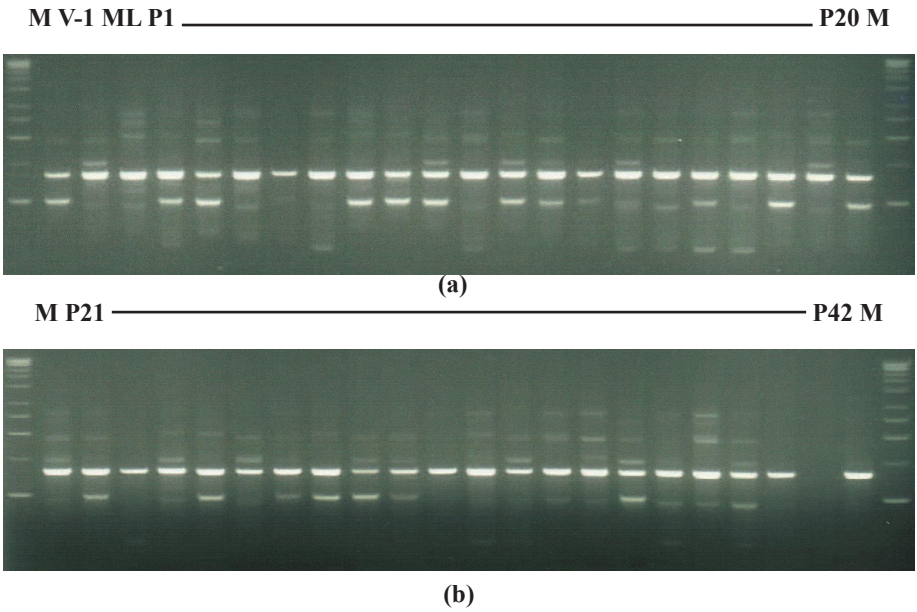


Fig. 1: RAPD marker inheritance pattern in Mysore Local (ML) x V-1 cross. (a) Inheritance pattern in progenies (P1 – P42) using OPA-01 and (b) Inheritance pattern in progenies (P1 – P42) using OPA-07. M is the kilobase DNA marker.

with nutritionally superior leaf and respond very well to agronomical inputs. Of the about 200 seeds raised from the cross, 57 progenies were finally survived and transplanted in a small plots. Forty-two progenies were found suitable with respect to their growth and availability of the leaf material for DNA extraction. Mysore Local has light green leaf compared to the deep green colour of V-1. Among the progenies, 10 showed light green leaf colour, 14 showed intermediately green colour and 18 showed deep green colour as in V-1. Similarily, the character inheritance with respect to lobation type, leaf margin and phyllotaxy (Table 3). However, these characters were recorded in the young plants (juvenile stage), which need to be observed again when the plants reach correct stage of maturity.

RAPD analysis was carried out using five Operon primers. All the RAPD reactions were performed twice and only reproducible bands in the range of 500 to 3500 bp were considered for scoring. A total of 33 amplicons were generated of which 20 were monomorphic and rests 13 were polymorphic (33.33%) (Table 1). The RAPD amplification profiles of V-1, Mysore Local and F_1 progenies was carried out using primers OPA-01, OPA-02, OPA-03 and OPA-07 (Figure 1). Out of the 13 polymorphic markers 12 were considered for segregation analysis, as the markers under consideration were present in either one of the parents or in both. The segregation pattern of RAPD markers of Mysore Local x V-1 cross F_1 progenies showed expected Mendelian pattern of inheritance (3:1 ratio) in 3 RAPD

markers namely OPA-02_{2900}, OPA-03_{900} and OPA-03_{800} (Table 4). Besides, three other RAPD markers OPS-04_{1450}, OPA-04_{1000} and OPA-07_{1000} showed Mendelian segregation for 1:1 ratio with non-significant Chi-square value of probability. The banding intensity in the RAPD profiles varied from intense to normal and feeble. However, no attempt was made to assign the genotype of the parents and F_1 progenies because minor deviation in the RAPD reaction and electrophoresis can adversely effect the interpretation. The genetic distance in comparision with female parent (Mysore Local) was maximum in case of progenies P_{41} (0.2) and minimum (0.017) was in case of P_{26} (Table 5). Similarly genetic distance in comparison with male parent (V-1) was maximum in case of progenies P_{12} and P_{19} (0.177) and minimum was in case of P_{36} (0.040). Among the progenies the maximum genetic distance was recorded between the progenies P_4 and P_{38} (0.276) and no genetic distance as per RAPD marker data was observed in between two pairs of progenies namely P_4 and P_{38}.

Table 4: F_1 Segregation pattern of RAPD markers in Mysore Local X V-1 cross

RAPD Primer	Marker Size (in bp)	F1 Segregation		Total	X2 (3:1)	X2 (1:1)
		Presence (1)	Absence (0)			
OPA – 01	3100	5	37	42	89.17*	24.38*
	1800	9	33	42	64.29*	13.71*
	1050	14	28	42	38.89*	4.67*
OPA – 02	2900	29	13	42	0.79NS	10.76*
OPA – 03	900	29	13	42	0.79NS	10.76*
	800	33	9	42	0.28NS	24.47*
	700	17	25	42	26.7*	26.00*
	600	17	25	42	26.7*	26.00*
OPA – 04	1450	18	24	42	23.14*	0.86NS
	1300	11	31	42	53.36*	9.52*
	1000	19	23	42	19.84*	0.38NS
OPA – 05	1000	16	26	42	30.51*	2.38*

The genetic distance between parents based on the RAPD data set is calculated 0.186, which is low in comparison with morpho-agronomical differentiation. This may be attributed to the screening of a small fraction of genome by RAPD primer, which may not reflect the genetic divergence between the varieties accurately. But

low genetic divergence of varieties can be appreciated in the background of tree gene flow that might have occurred in the course of mulberry varietal selection and breeding. Almost all the varieties under different species intercross with each other to produce fertile hybrids obscuring the species delimitation at varietal level. In fact, most of the mulberry varieties are the selection from open-pollination hybrids. This view has been confirmed by Sharma *et al.,* (2000) while assessing the genetic diversity in mulberry germplasm using florescence-based AFLP markers showed high similarity among cultivated mulberry varieties of diverse geographic origin. The study also confirmed that the mulberry improvement has taken place utilizing a narrow genetic base.

The varied genetic distance values in comparison with parents and progenies to progenies are explained in terms of recombinations and independent assortment of those RAPD markers. The genetic distance values between Mysore Local and progeny 41(P_{41}) was higher than that of parental combination (0.200). Comparison of V-1 with progenies revealed a lower genetic distance than its parental combination. Among progenies P_4 and P_{38} exhibited highest genetic divergence (GD) of 0.278. Few of the progenies were having similar genetic make-up (GD - 0) based on the limited number of RAPD markers under consideration.

Heterozygosity among cultivated mulberry varieties due to out-breeding behavior has been well recorded as in the case of most of the tree species (Tikadar *et al.,* 1999). Evolution of homozygous lines through selfing has not been successful party due to dioecious nature of mulberry varieties. Even in case of monoecious varieties, the selfed lines could not be maintained beyond S_2 or S_3 generations probably because of the expression of deleterious genes and intrinsic requirement of heterozygosity for mulberry growth and development. To attain reproductive maturity mulberry plant takes quite some time (anywhere between 2 to 3 years) and hence breeding is somewhat time consuming and laborious. Based on these facts and assumptions, molecular markers are thought as an alternative for generation of genetic information in mulberry and can be used as a tool to study its inheritance pattern.

RFLP and SSR markers are co-dominant and are only advantageous where F_2 generation can be easily obtained. As discussed, generation of F_2 population in mulberry is quite demanding or even impossible. RAPDs are known to be dominant markers, which can seriously restrict its use in segregation analysis. However, parental mulberry varieties have been originated from open crosses for a long time and hence it shows immediate segregation when crossed. This is an evidence for very high level of heterozygosity in parents. Perhaps lack of co-dominance with RAPD markers might not represent such a disadvantage for crosses involving heterozygotes. Carlson *et al.,* (1991) showed the appropriateness of this technique in the study of segregation of RAPD markers in F_1 progeny of conifers. He also

Table 5: Dissimilartiy Coefficient values among Mysore Local, V-1 and its progenies

	ML	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20
V-1	0.19	0.06	0.13	0.06	0.10	0.10	0.08	0.08	0.12	0.10	0.06	0.10	0.18	0.08	0.14	0.10	0.10	0.10	0.12	0.18	0.16
ML	0.00	0.17	0.11	0.17	0.16	0.09	0.11	0.11	0.11	0.12	0.13	0.09	0.05	0.11	0.05	0.09	0.09	0.09	0.11	0.09	0.11
P1		0.00	0.06	0.09	0.08	0.13	0.06	0.06	0.10	0.16	0.04	0.13	0.16	0.11	0.16	0.13	0.08	0.08	0.10	0.16	0.14
P2			0.00	0.11	0.06	0.06	0.04	0.04	0.08	0.22	0.06	0.10	0.14	0.08	0.10	0.10	0.14	0.10	0.12	0.18	0.14
P3				0.00	0.13	0.08	0.11	0.06	0.06	0.12	0.04	0.04	0.12	0.06	0.12	0.08	0.08	0.13	0.10	0.08	0.08
P4					0.00	0.04	0.02	0.10	0.06	0.15	0.08	0.08	0.15	0.06	0.08	0.12	0.04	0.00	0.06	0.15	0.18
P5						0.00	0.06	0.10	0.06	0.15	0.08	0.04	0.15	0.02	0.04	0.08	0.04	0.08	0.10	0.12	0.14
P6							0.00	0.08	0.08	0.18	0.06	0.10	0.14	0.08	0.10	0.10	0.06	0.06	0.08	0.18	0.20
P7								0.00	0.08	0.17	0.02	0.10	0.13	0.08	0.10	0.10	0.06	0.10	0.12	0.13	0.15
P8									0.00	0.13	0.06	0.10	0.13	0.08	0.10	0.14	0.06	0.10	0.12	0.10	0.12
P9										0.00	0.15	0.17	0.11	0.14	0.11	0.15	0.15	0.17	0.13	0.08	0.10
P10											0.00	0.08	0.12	0.06	0.12	0.08	0.04	0.08	0.10	0.12	0.14
P11												0.00	0.12	0.02	0.04	0.08	0.04	0.08	0.10	0.08	0.14
P12													0.00	0.14	0.11	0.06	0.13	0.13	0.10	0.08	0.10
P13														0.00	0.06	0.06	0.02	0.06	0.08	0.10	0.10
P14															0.00	0.06	0.10	0.13	0.10	0.08	0.13
P15																0.00	0.08	0.08	0.10	0.08	0.10
P16																	0.00	0.04	0.06	0.12	0.14
P17																		0.00	0.02	0.12	0.14
P18																			0.00	0.10	0.12
P19																				0.00	0.06
P20																					0.00
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	P21	P22	P23	P24	P25	P26	P27	P28	P29	P30	P31	P32	P33	P34	P35	P36	P37	P38	P39	P40	P41	P42
V-1	0.08	0.10	0.06	0.10	0.08	0.17	0.06	0.14	0.13	0.08	0.08	0.08	0.06	0.10	0.17	0.04	0.10	0.06	0.16	0.13	0.09	0.15
ML	0.10	0.10	0.13	0.09	0.11	0.02	0.17	0.09	0.11	0.11	0.11	0.11	0.09	0.13	0.15	0.11	0.12	0.17	0.07	0.19	0.20	0.07
P1	0.10	0.10	0.08	0.12	0.10	0.15	0.04	0.08	0.15	0.06	0.11	0.06	0.13	0.13	0.11	0.14	0.16	0.09	0.10	0.11	0.07	0.18
P2	0.12	0.12	0.10	0.10	0.12	0.10	0.11	0.06	0.17	0.12	0.04	0.08	0.10	0.14	0.11	0.16	0.14	0.11	0.12	0.13	0.09	0.15
P3	0.10	0.10	0.04	0.12	0.06	0.15	0.09	0.17	0.11	0.10	0.06	0.06	0.13	0.04	0.67	0.06	0.12	0.00	0.14	0.11	0.07	0.14
P4	0.13	0.10	0.12	0.12	0.14	0.11	0.13	0.08	0.11	0.06	0.62	0.10	0.04	0.16	0.15	0.18	0.15	0.28	0.10	0.15	0.11	0.13
P5	0.13	0.10	0.08	0.12	0.10	0.11	0.13	0.12	0.14	0.10	0.02	0.10	0.04	0.12	0.11	0.14	0.17	0.08	0.10	0.15	0.11	0.20
P6	0.12	0.12	0.10	0.14	0.12	0.10	0.11	0.06	0.13	0.08	0.04	0.08	0.06	0.14	0.13	0.16	0.14	0.11	0.12	0.13	0.09	0.15
P7	0.08	0.08	0.06	0.10	0.08	0.04	0.10	0.10	0.16	0.08	0.04	0.04	0.06	0.10	0.13	0.12	0.13	0.06	0.17	0.17	0.13	0.15
P8	0.11	0.08	0.10	0.13	0.17	0.13	0.14	0.14	0.16	0.08	0.08	0.08	0.10	0.10	0.13	0.12	0.17	0.06	0.17	0.17	0.13	0.26
P9	0.09	0.13	0.19	0.11	0.13	0.14	0.11	0.19	0.10	0.10	0.18	0.13	0.12	0.08	0.14	0.13	0.11	0.12	0.13	0.18	0.15	0.13
P10	0.06	0.06	0.04	0.08	0.06	0.11	0.08	0.12	0.14	0.06	0.06	0.02	0.12	0.08	0.11	0.10	0.12	0.14	0.10	0.15	0.11	0.13
P11	0.10	0.06	0.08	0.08	0.10	0.11	0.13	0.16	0.10	0.06	0.06	0.03	0.04	0.12	0.06	0.10	0.08	0.08	0.06	0.15	0.11	0.06
P12	0.09	0.13	0.12	0.08	0.10	0.04	0.16	0.12	0.16	0.13	0.14	0.10	0.15	0.08	0.10	0.16	0.11	0.12	0.10	0.14	0.15	0.09
P13	0.12	0.08	0.06	0.10	0.08	0.13	0.11	0.14	0.13	0.08	0.04	0.08	0.06	0.10	0.09	0.12	0.10	0.06	0.08	0.13	0.09	0.08
P14	0.13	0.13	0.12	0.08	0.13	0.07	0.16	0.15	0.10	0.10	0.06	0.10	0.04	0.15	0.10	0.10	0.11	0.12	0.06	0.18	0.15	0.06
P15	0.13	0.10	0.04	0.08	0.10	0.08	0.08	0.12	0.06	0.14	0.06	0.06	0.12	0.08	0.11	0.06	0.12	0.08	0.10	0.11	0.11	0.06
P16	0.10	0.06	0.04	0.08	0.10	0.11	0.13	0.12	0.14	0.06	0.06	0.06	0.08	0.12	0.11	0.14	0.12	0.08	0.06	0.15	0.11	0.10
P17	0.13	0.06	0.08	0.12	0.10	0.11	0.08	0.08	0.10	0.06	0.10	0.10	0.08	0.12	0.15	0.14	0.15	0.13	0.10	0.11	0.11	0.10
P18	0.15	0.08	0.10	0.10	0.12	0.04	0.10	0.10	0.08	0.08	0.12	0.08	0.10	0.14	0.17	0.12	0.17	0.14	0.08	0.13	0.13	0.08
P19	0.13	0.09	0.08	0.11	0.10	0.11	0.12	0.19	0.06	0.10	0.14	0.09	0.12	0.08	0.10	0.02	0.15	0.08	0.95	0.14	0.15	0.06
P20	0.15	0.11	0.10	0.17	0.08	0.13	0.10	0.14	0.12	0.15	0.16	0.12	0.18	0.06	0.13	0.08	0.13	0.10	0.12	0.13	0.13	0.11
P21	0.00	0.17	0.10	0.06	0.08	0.09	0.14	0.13	0.15	0.08	0.12	0.08	0.13	0.10	0.12	0.11	0.06	0.10	0.11	0.20	0.17	0.14
P22		0.00	0.11	0.09	0.08	0.12	0.10	0.13	0.15	0.08	0.12	0.08	0.13	0.10	0.16	0.11	0.13	0.10	0.11	0.16	0.17	0.11
P23			0.00	0.12	0.02	0.11	0.04	0.12	0.10	0.10	0.06	0.06	0.12	0.04	0.11	0.06	0.12	0.04	0.14	0.11	0.11	0.20
P24				0.00	0.13	0.07	0.16	0.15	0.10	0.10	0.10	0.06	0.12	0.08	0.10	0.10	0.08	0.06	0.06	0.18	0.15	0.09
P25					0.00	0.09	0.04	0.10	0.12	0.12	0.08	0.08	0.14	0.02	0.13	0.08	0.10	0.06	0.15	0.13	0.13	0.11
P26						0.00	0.15	0.08	0.10	0.13	0.10	0.09	0.11	0.11	0.14	0.09	0.11	0.15	0.09	0.18	0.18	0.09
P27							0.00	0.08	0.11	0.10	0.11	0.10	0.13	0.08	0.11	0.10	0.16	0.09	0.14	0.24	0.17	0.14
P28								0.00	0.11	0.14	0.10	0.14	0.16	0.12	0.15	0.18	0.15	0.17	0.14	0.11	0.11	0.17
P29									0.00	0.12	0.13	0.12	0.10	0.10	0.09	0.04	0.14	0.11	0.12	0.09	0.09	0.08
P30										0.00	0.12	0.08	0.06	0.14	0.13	0.12	0.13	0.10	0.08	0.17	0.13	0.11
P31											0.00	0.12	0.06	0.10	0.09	0.12	0.10	0.06	0.12	0.13	0.09	0.12
P32												0.00	0.14	0.10	0.13	0.08	0.13	0.06	0.08	0.17	0.13	0.11
P33													0.00	0.16	0.11	0.14	0.12	0.13	0.10	0.15	0.11	0.10
P34														0.00	0.11	0.06	0.12	0.04	0.18	0.17	0.11	0.13
P35															0.00	0.08	0.10	0.02	0.08	0.09	0.15	0.12
P36																0.00	0.13	0.06	0.12	0.13	0.13	0.08
P37																	0.00	0.12	0.13	0.14	0.15	0.09
P38																		0.00	0.14	0.11	0.07	0.14
P39																			0.00	0.17	0.13	0.08
P40																				0.00	0.05	0.12
P41																					0.00	0.17

showed that because of high levels of heterozygosity most of the situation represented a test cross and followed the 1:1 segregation in F_1 progenies of Douglas-fir. Besides, from theoretical considerations it would appear that lack of co-dominance should not preclude the usefulness of the RAPD markers in genome mapping studies with tree species (Allard, 1956).

Studies in analysis of segregation of RAPDs (Echt *et al.,* 1992, Roy *et al.,* 1992) have put forth two assumptions concerning these markers: (1) each band is considered to represent a single locus and is (2) a dominant marker for the locus. On this basis, the presence of fragment in a parent is considered to be either in the homozygous condition or in the heterozygous condition. It is not generally possible to distinguish homozygosity from heterozygosity at a locus from the presence of a RAPD band or marker. (Carlson *et al.,* 1991). However, attempts can be made to assign the genotype based on the marker status and its segregation in progenies. Huang *et al* (2000) estimated the allelic frequency indirectly using the homozygous null genotypes (-/-) and correlated for dominance according to Lynch and Milligan (1994). An estimate can be made about the homo or heterozygotic status of the RAPD locus based on the relative band intensity, which may not be always accurate. A parent without the RAPD band is assigned the recessive genotype. When a marker present in one parent but absent in the other (*Pp x pp or pp x Pp*) the marker will segregate in 1:1 in the F_1 generation. When a marker is present in both the parents the parents (*Pp x Pp*) the expected segregation ratio for that marker to the progeny is 3:1. In this study the data were analyzed within the framework of these assumptions. Segregating 12 RAPD markers were considered for analysis of Chi-square tests were performed for each of the markers to determine the segregation difference from the expected ratio of 3:1 and 1:1. Out of the 12 RAPD loci observed in the parents 3 markers followed the segregation pattern of 3:1 and another 3 markers followed the segregation ratio of 1:1 at a significance level of P=0.05. The deviation of other RAPD markers from Mendelian pattern of inheritance may be attributed to the availability of limited progenies for the study where no sampling strategy could be applied. Segregation analysis may strictly follow the expected Mendelian pattern of inheritance. Similarly Mendelian inheritance pattern of RAPD have been obtained in broccoli and cauliflower (Hu and Quiros, 1991), Lettuce (Michelmore *et al.,* 1991), tomato (Giovannoni *et al.,* 1991) and Betula (Roy *et al.,* 1992).

In three progenies (namely, P_{37}, P_{40} and P_{42}) a new marker $OPA\text{-}02_{1200}$ not present in either of the parents appeared. The probable reasons may be : (1) as a result of recombination that produced new binding sites for the primers in the genomic DNA of the F_1 progeny, (2) imperfect matching and binding of the primers to template DNA (William *et al.,* 1990), Rafalski *et al* 1991). (3) Competition for the primer-binding sites is an important factor controlling, which RAPD fragments are sufficiently amplified to be visible.

Lack of genetic information and availability of markers have severely restricted the mulberry-breeding programme. The study showed RAPD markers can be a valuable tool for mulberry breeding programme. The study established an accurate and reproducible profiling pattern for mulberry for plant variety identification and protection of Plant Breeders Rights. The present study has made no attempt to link the RAPD markers with the traits for breeders' interest. The study needs to be further strengthened by segregation analysis of RAPD markers in large number of progenies and screening of many more primers for the development of molecular linkage map. The progenies also need to be characterized for important morpho-agronomical traits accurately. The establishment of correlation between the amplification products and the morpho-agronomical traits by bulk segregation analysis will help the breeders in identification of markers for the traits of their interest. However, the present work has laid a foundation for future research in this direction. The polymorphic RAPD markers in parents segregate as independent alleles in the F_1 progenies and also throwing some light on the heterozygotic status of mulberry varieties. The findings suggested that RAPD can be used as a technique for marker assisted selection (MAS) in mulberry breeding.

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