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Pre-breeding Efforts to Utilize Wild *Morus* Species and DNA Fingerprinting of F_1 Hybrids Using RAPD

T. Amalendu and K. Chandrakant Kamble
Central Sericultural Germplasm Resources Centre, Hosur –635 109, India

Abstract: *Morus laevigata* and *Morus serrata are* the wild *Morus* species of mulberry available in India possess unique features of bigger leaf size, higher leaf moisture and moisture retention, higher protein and carbohydrate with high adaptability to adverse climatic condition. In an effort to transfer these traits to cultivated species, inter-specific hybridization was effected between *M. indica* with *M. laevigata* and *M. indica* with *M. serrata*. After repeated trial of hybridization, successful F_1 seeds were obtained among the crosses. The F_1 hybrids showed better performance than female parent in most of the characters while it was better than male parent for few characters in *M. indica* with *M. laevigata*. In another cross, the F_1 hybrids showed better performance than both the parents for most of the characters (*M. indica* x *M. serrata*). The crosses are expected to carry some genetic load, as the wild species were genetically and geographically distant and carry valuable genes. Polymorphism of genomic DNAs of nine parents and their seven hybrids were obtained from two wild and four cultivated species. Twelve arbitrary primers of Random Amplified Polymorphic DNA (RAPD) unraveled abundant polymorphism. Of the F_1 hybrids, the banding pattern indicates similar like their respective parents. However, a few hybrids showed unique bands, which are different from respective parents. The RAPD technique demonstrated that hereditary variability occurred in between wild and cultivated *Morus* species at inter and intra-specific levels.

Key words: Crossing behaviour, inter and intra-specific hybrids, molecular tools and mulberry

INTRODUCTION

The new genomic constitutions can be obtain through interspecific hybridization and breeders considered it as a useful tools for mulberry crop improvement (Tikader and Dandin, 2007). Rich resources of wild relatives of mulberry are reported to occur in India in tropical and sub-tropical Himalayan belt (Hooker, 1885). The wild relatives of mulberry could be used as bridges for the transfer of useful agronomic traits from the wild species to the cultivated ones. Presently, the Indian mulberry genetic resources have enriched due to continuous introduction and influx from the temperate region. Yet desired material for different agro-climatic zones are in demand for higher leaf production. The mulberry is generally propagated through stem cuttings and monoculture of a desirable variety in a specific area makes the mulberry plantations almost homogeneous and vulnerable to diseases and pests (Tikader and Kamble, 2008). *M. serrata* and *M. laevigata* possess several agronomic important traits including resistance to abiotic stresses like drought and frost (Tikader and Thangavelu, 2003). Earlier attempts of inter-specific cross-involving *M. laevigata* and *M. serrata* with other cultivated mulberry species showed reproductive barrier. Some authors tried to study the crossability among different *Morus* species and its inheritance pattern (Basavaiah and Dandin, 1989; Das and Krishnaswami, 1965; Dandin *et al*., 1987; Tikader and Dandin, 2001; Tikader and Rao, 2002). All the reports are of preliminary in nature. Moreover, continuous use of similar material, genetic base has narrowed. Thus, in order to broaden the narrow genetic base, new gene pools need to be incorporated into the cultivated forms of mulberry (Vijayan *et al*., 2004). Employing modern molecular tools, relationships in mulberry parents and their offspring are possible to interpret and classify at molecular level. Molecular markers such as RAPD (Lou *et al*., 1996, 1998; Chatterjee *et al*. 2004; Zhao and Pan, 2004; Vijayan *et al*., 2006; Vijayan, 2007) were used to create molecular identity.

The present investigation was conducted to assess the performance of F_1 hybrids obtained from crosses of wild and cultivated species and also to find out the new genomic constituents through molecular tools.

MATERIALS AND METHODS

Plant material: Six female and 3 male parents of different mulberry species were used to get the F1 hybrids (Table 1).

1. *M. indica* (var. Kanva-2) a popular commercial variety in South India with smooth, medium size, with pale green colour leaves, unisexual (female), diploid, good rooting, suitable for silkworm rearing.
2. *M. indica* (var. Laciniata) popularly known as Kajli, highly dissected leaves, widely used for silkworm rearing by local silkworm farmers, unisexual (female), diploid and good rooter.

Corresponding Author: T. Amalendu, Central Sericultural Germplasm Resources Centre, Hosur –635 109, P.B. No.44 Krishnagiri, Tamil Nadu India

Table 1: List of accessions used in the study

Accessions	Accession name	Sex	Species	Status
MI-0014	Kanva-2	Female	*M. indica Linn.*	Cultivar
MI-0364	Lamia bay	Male	*M. laevigata* Wall.	Wild
MI-0608	Kanva-2 x Lamia bay hybrid	Female	*M. laevigata* Wall.	Hybrid
MI-0068	Kajli	Female	*M. indica* Linn.	Cultivar
ME-0081	*M. serrata*	Male	*M. serrata* Roxb.	Wild
MI-0831	Kajli x *M. serrata* hybrid	Male	*M. indica* Linn.	Hybrid
MI-0400	Krishnaswami-2	Female	*M. indica* Linn.	Cultivar
MI-0833	Krishnaswami-2 x Lamia bay hybrid	Female	*M. indica* Linn.	Hybrid
MI-0437	Baragarh-2	Female	*M. indica* Linn.	Cultivar
MI-0829	Baragarh-2 x Lamia bay hybrid	Female	*M. indica* Linn.	Hybrid
MI-0379	Urgam - 4	Female	*M. serrata* Roxb.	Wild
MI-0378	Urgam - 2	Male	*M. serrata* Roxb.	Wild
MI-0646	Urgam-4 x Urgam-2	Female	*M. serrata* Roxb.	Hybrid
MI-0365	Doomarnali	Female	*M. laevigata* Wall.	Wild
MI-0673	Laevigata hybrid	Female	*M. laevigata* Wall.	Hybrid

3. *M. indica* (var. Krishnaswamy-2) an improved variety collected from sericulturist house, smooth, dark green, medium size leaf, suitable for silkworm, diploid, unisexual (female), good rooter.
4. *M. indica* (var. Baragarh-2) a collection from Uttaranchal through survey and exploration, medium size, dark green, smooth leaf, suitable for silkworm rearing, diploid, good rooter, adjustable in various agro-climatic conditions and unisexual (female).
5. *M. laevigata* (var. Lamia Bay) a wild collections from Andaman and Nicobar Islands, with smooth, big size, dark green colour leaves, unisexual (male), diploid, leaf palatable to silkworm.
6. *M. laevigata* (var. Doomarnali) a wild collections from Andaman and Nicobar Islands, smooth, big size leaf, dark green, unisexual (female), diploid, poor rooter, leaf palatable to silkworm.
7. *M. serrata* (var. ME-0081) a wild collections having thick velvety dark green leaf, unisexual (male), poor rooter, tetraploid, not suitable for silkworm rearing but have the plasticity to adjust in adverse climatic condition i.e., frost, drought and biotic stress.
8. *M. serrata* (var. Urgam - 2) a wild collections from Urgam valley, Chamoli, Uttaranchal, thick velvety dark green leaf, unisexual (female), poor rooter, tetraploid, not suitable for silkworm rearing but can be fed only to 5th stage matured worms. The accessions has the capacity to tolerate the adverse climatic condition i.e., frost, drought and biotic stress.
9. *M. serrata* (var. Urgam - 4) a wild collections from Urgam valley, Uttaranchal, thick velvety dark green leaf, unisexual (male), poor rooter, polyploid, not suitable for silkworm rearing but can be fed to 5th stage matured worms. The accession has the capacity to tolerate the adverse climatic condition i.e., frost, drought and biotic stress.

In the hybridization programme, crosses were effected during normal flowering season i.e., February – March. Before hybridization, flowering of both parents were synchronized to match for effective breeding. Both the parents produced sufficient flowers to carry out the experiment. The female catkins of the parents were covered with pergamin paper bags after anthesis and within a week, stigma becomes receptive as evidenced by its white colour. Pollen was collected from the male inflorescence in a petriplate and kept under cover just before the dehiscence of the anthers. The pollen grains were dusted over the receptive stigmas with the help of a camel hairbrush. Pollination was repeated 2-3 times for one week to pollinate the flowers of all ages inside the paper bags (Tikader and Thangavelu, 2002). In each cross, 20 female inflorescences were pollinated.

The cross becomes effective if the pollen fertility is higher. Pollen fertility was studied by standard 2% acetocarmine staining method (Tikader and Rao, 2001; Vijayan *et al*., 2004). The pollen that had taken stain was considered as fertile and those that had not stained properly and with irregular shape were considered as sterile. The observation was made on more than 350 pollens. Hanging drop method in 2% sucrose solution was used to test pollen germination (Kulkarni *et al*., 2002)

The seeds collected from the cross after 4-5 weeks were germinated and planted in nursery to get seedlings. After establishment, a comparative assessment of parents and F_1 was made. The observations were recorded for parents and wherever possible for F_1 plant. The morphological parameter was recorded based on visual observation (Das *et al*., 1970). Leaf anatomical parameters were recorded as per the standard procedure. The growth traits were recorded following standard procedure for mulberry germplasm (Tikader and Rao, 2002).

Twelve RAPD markers used in the present study are presented in Table 2.

Genomic DNA isolation: Genomic DNA was isolated from pooled fresh young leaves of ten individual plants of each parent and F_1 hybrid plants using Nucleon Phytopure Kit, Amersham Life Sciences, UK as per manufacturers' instruction (1997). The quantity and purity of isolated genomic DNA were assessed by UV spectrophotometer and on 0.8% agarose gel, respectively. The genomic DNA samples were diluted to a uniform concentration of 10 ng/µl for polymerase chain reaction (PCR).

RAPD amplification: PCR was performed according to the protocol of Williams *et al.* (1990). DNA amplifications were carried out in 0.2 ml tubes in Gene

Table 2: List of RAPD random primers used in the study

Sl no.	Primers	Sequence (5' – 3')
1	OPC – 01	-ttc gag cca g-
2	OPC – 05	-gat gac cgc c-
3	OPC – 06	-gaa cgg act c-
4	OPC – 08	-tgg acc ggt g-
5	OPC – 09	-ctc acc gtc c-
6	OPC – 10	-tgt ctg ggt g-
7	OPC – 11	-aaa gct gcg g-
8	OPC – 14	-tgc gtg ctt g-
9	OPB – 06	-tgc tct gcc c-
10	OPB – 07	-ggt gac gca g-
11	OPB – 12	-cct tga cgc a-
12	OPB – 17	-agg gaa cga g-

Amp 9700 PCR system (Applied Biosystems, USA) in 20µl reaction volume containing 20mm Tris - Cl (pH 8.4), 50mM KCl, 2mM $MgCl_2$, 0.2 µM primer, 0.1 mM each dATP, dTTP, dCTP and dGTP, 0.5 U Taq DNA polymerase (Genie, Bangalore) and 20 ng of template DNA. The random primers were obtained from Operon Technologies Inc. Alameda, USA. Amplification reactions were carried out with following cycle profiles: 1 cycle at 93ºC for 2 min followed by 40 cycles at 93ºC for 1 min, 35ºC for 1 min, 72ºC for 2 min and a final extension of 7 min at 72ºC. PCR products were electrophoreses on 1.5% agarose gel in 1 x TAE buffer and stained in ethidium bromide solution and gel images were recorded using Gel Documentation Systems (Syngene, UK). Each reaction was repeated twice and the bands in the range of 300 - 3400 bps were scored. DNA banding patterns generated by RAPDs were scored as '1' for the presence of the fragment and '0' for the absence.

RESULTS

The F_1 hybrids of interspecific crosses (MI-0014 x MI-0364), (MI-0068 x ME-0081), (MI-0400 x MI-0364), (MI-0437 x MI-0364) and intraspecific crosses (MI-0365 x MI-0364), (MI-0378 x MI-0379) were made. The morphological character of F_1 hybrid is like *M. indica* (var. Kanva-2) and *M. laevigata* (var. Lamia Bay). The hybrid showed female flowers like mother plant but the fruit size and colour changes to pink unlike black colour of mother plant. The growth parameters i.e., leaf yield and moisture % showed higher values than parents and intermediate values for other growth traits (Table 3).

The performance of *M. indica* (var. Kajli) and *M. serrata* (var. ME-0081) was observed in F_1 hybrid (Table 3). The morphological variability in hybrid was like parents except branching nature. The growth traits indicate intermediate values of both parents for single leaf weight; leaf area, moisture % and leaf yield per plant.

The result of F1 hybrid of *M. indica* (var. Krishnaswami-2) and *M. laevigata* (var. Lamia bay) showed the dominancy of female parent characters in respect of morphological traits. The growth traits showed intermediate results hybrid (Table 3).

The performance of hybrid of *M. indica* (var. Baragarh-2) and *M. laevigata* (var. Lamia bay) showed the morphological parameters like female parent. The performance of growth parameters presented in Table 3.

The morphological parameter of intraspecific hybrids of *M. laevigata* x *M. laevigata* showed erect branch, green and smooth leaf like parents. Reproductive behaviour observed in hybrid shows intermediate size of inflorescence, profuse fruiting, colour of fruit changes to pink. The growth traits indicate superiority over female parent (Table 3).

The performance of intra-specific hybrid (*M. serrata* x *M. serrata*) showed dark green, smooth and serrate leaf like parents. The growth traits showed hybrid vigour in F_1 plants than parents. Earlier breeding behaviour of some wild species was tested at CSGRC, Hosur and reported the similar findings (Tikader and Dandin, 2001). This is the first pre-breeding effort and the F_1 hybrid can be further utilized for crop improvement. The results of pre-breeding in cotton were reported by Kulkarni *et al.,* (2002).

The scoring of the amplification products with primer OPC-09 and OPC-10 are presented in Fig. 1 and 2, respectively. The amplified product showed abundant variation in banding pattern when different primer and materials were used. The maximum band was 18 and minimum 1. The largest fragment was about 2000 bp, the smallest 500 bp in light of molecular weight marker indicating that the primers used have multi-combining sites in DNA of 300-3400 bp in length. Discriminative RAPD loci, the bands that were common to the F_1 hybrid and only one parent, were either male or female.

The results of the amplification of the total RAPD *loci* (monomorphic and discriminative) in different hybrid combinations are presented in Fig.1 and 2.

Morus indica x Morus laevigata (MI-0014 x MI-0364) hybrid: Among the 12 primers tested, all primers amplified and a total of 77 discriminative RAPD loci, specific for the F_1 hybrid and parents were obtained. The primer OPC-14 and OPB-12 showed the highest percentage of discriminative RAPD *loci* (100%) for the F_1 hybrid and parents. Although the results were similar, the percentages of discriminative loci were higher for the F_1 hybrid and parents (79.89%). The banding pattern of hybrid is like both the parents and showed intermediate characters.

Morus indica x Morus serrata (MI-0068 x ME-0081) hybrid: A total 12 primer tested, six primer (OPC-01, OPC-05, OPC-06, OPC-11, OPC-14 and OPB-12) showed 100% amplification and discriminative RAPD *loci* observed (91.96%) which is specific for the F_1 hybrid and parents. The results indicate 23.07% discriminative RAPD *loci* (D %). The F_1 hybrid showed resemblances like female parent in morphological appearance.

For confirmation of the effectiveness of the interspecific and intraspecific crosses, molecular tools like RAPD was used to know the polymorphism of F_1 hybrid and parents. The salient features of F_1 hybrid resemblances either of the parents. In some cases additional band observed in F_1 hybrid which is not present in any of the parents (Fig. 1 and 2). Similar results were

Table 3: Comparative performances of parents and F_1 hybrids of inter and intra specific crosses in different *Morus* species.

Characters	Parent ♀	Parent ♂	Hybrid ♀ / ♂	Percentage heterosis over ♀	♂
	M. indica x M. laevigata (MI-0014 x MI-0364)				
Sex	♀	♂	♀	---	---
Leaf length (cm)	15.00	32.00	25.00	+ 66.67	-21.88
Leaf width (cm)	13.00	28.00	23.00	+ 76.92	-17.86
Single leaf wt. (g)	4.00	12.00	10.00	+ 150.00	-16.67
Leaf area (cm^2)	275.00	790.00	650.00	+ 136.36	-13.33
Leaf moisture (%)	70.00	71.00	78.80	+ 12.57	+11.00
Leaf yield / plant (kg)	1.35	1.70	2.00	+ 83.25	+17.65
	M. indica x M. serrata (MI-0068 x ME-0081)				
Sex	♀	♂	♂	---	---
Leaf length (cm)	17.00	10.75	15.07	-11.35	+40.18
Leaf width (cm)	14.40	7.80	13.04	-6.95	+71.79
Single leaf wt. (g)	1.39	4.00	2.37	+70.50	-40.75
Leaf area (cm^2)	127.00	240.00	185.00	+45.66	-22.92
Leaf moisture (%)	65.75	71.00	78.88	+19.96	+11.10
Leaf yield / plant (kg)	0.65	0.90	0.95	+46.15	+ 5.55
	M. indica x M. laevigata (MI-0400 x MI-0364)				
Sex	♀	♂	♀	---	---
Leaf length (cm)	20.40	32.00	22.10	+8.33	-29.51
Leaf width (cm)	16.90	28.00	20.00	+18.34	-20.95
Single leaf wt. (g)	6.70	12.00	7.10	+5.97	-40.83
Leaf area (cm^2)	330.00	790.00	570.00	+72.73	-27.85
Leaf moisture (%)	72.00	71.00	70.00	-2.78	-1.41
Leaf yield / plant (kg)	1.21	1.70	1.48	+22.31	-12.94
	M. indica x M. laevigata (MI-0437 x MI-0364)				
Sex	♀	♂	♀	---	---
Leaf length (cm)	22.00	32.00	25.00	+ 13.64	- 21.88
Leaf width (cm)	18.00	28.00	21.00	- 16.67	- 25.00
Single leaf wt. (g)	7.25	12.00	7.50	+ 3.45	- 37.50
Leaf area (cm^2)	390.00	790.00	750.00	+ 92.31	- 5.06
Leaf moisture (%)	75.00	71.00	75.00	---	+ 5.63
Leaf yield / plant (kg)	1.90	1.70	1.75	- 7.89	+ 2.94
	M. laevigata x M. laevigata (MI-0365 x MI-0364)				
Sex	♀	♂	♀	---	---
Leaf length (cm)	21.00	32.00	29.00	+ 38.09	- 9.38
Leaf width (cm)	15.50	28.00	24.00	+ 54.84	- 14.29
Single leaf wt. (g)	7.80	12.00	10.50	+34.62	- 14.29
Leaf area (cm^2)	320.00	790.00	675.00	+ 110.93	- 14.56
Leaf moisture (%)	70.00	71.00	72.00	+ 2.86	+ 1.41
Leaf yield / plant (kg)	1.40	1.70	2.25	+ 60.70	+ 32.35
	M. serrata x M. serrata (MI-0379 x MI-0378)				
Sex	♀	♂	♀	---	---
Leaf length (cm)	18.80	17.80	20.50	+ 9.04	+ 15.17
Leaf width (cm)	15.75	16.30	19.00	+ 20.63	+ 16.56
Single leaf wt. (g)	6.10	5.90	7.49	+ 22.79	+ 26.95
Leaf area (cm^2)	189.00	193.00	275.00	+ 45.50	+ 42.49
Leaf moisture (%)	71.71	69.53	77.76	+ 8.44	+ 11.84
Leaf yield / plant (kg)	1.29	0.75	1.52	+ 17.83	+ 102.87

reported by Lou *et al.,* (1998) in hybrids of *Morus* species. DNA fingerprinting of F_1 interspecific hybrids from the Triticeae tribe using molecular tools (ISSR's) was reported (Martin *et al*., 1999).

***Morus indica x Morus laevigata (MI-0400 x MI-0364) hybrid*:** The highest number of discriminative RAPD *loci* (15) has been amplified in the F_1 'Krishnaswami-2 x *M. laevigata*' hybrid and its parents. The primer OPC-14 (63.6%) and OPC-11 (61.5%) showed the highest percentage of discriminative RAPD *loci* corresponded for the F_1 'Krishnaswami-2 x *M. laevigata*' hybrid and its parents. The banding pattern is different for both the parents.

Morus indica x Morus laevigata (MI-0437 x MI-0364) hybrid: The discriminative RAPD *loci* (47) have been amplified in the F_1 'Baragarh-2 x *M. laevigata*' hybrid and its parents. The primer OPC-11 showed the highest percentage of discriminative RAPD *loci* (75.00%) for the F_1 'Baragarh-2 x *M. laevigata*' hybrid and its parents. The banding pattern showed resemblances with the female parent for the morphological parameters.

Morus serrata x Morus serrata (MI-0379 x MI-0378) hybrid: The intra specific hybrid showed discriminative RAPD *loci* (88) and amplified in the F_1 'Urgam-2 x Urgam-4' hybrid and its parents. Among the 12 primers, seven primers showed 100% discrimination among the F_1 hybrid and its parents. The banding pattern resemblances for both the parents.

Morus laevigata x Morus laevigata (MI-0365 x MI-0364) hybrid: A total discriminative RAPD locus (89) has been amplified in the F_1 'Doomarnali x Lamia bay' hybrid and its parents. A total of five primers showed

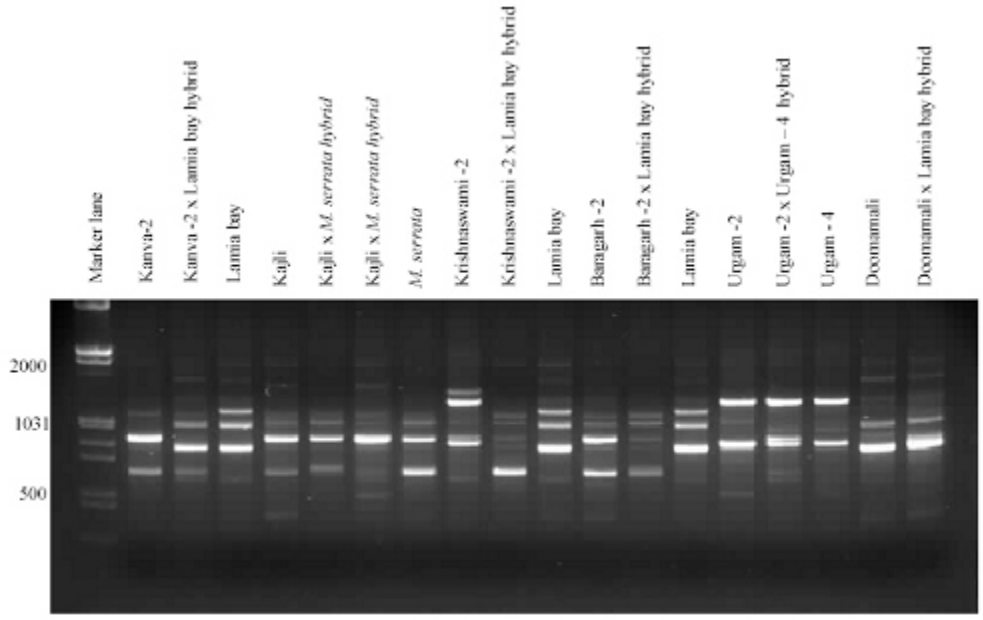

Fig 1: PCR fingerprint of different mulberry parents and hybrids with RAPD primer OPC09 resolved on 1.5% agarose gel

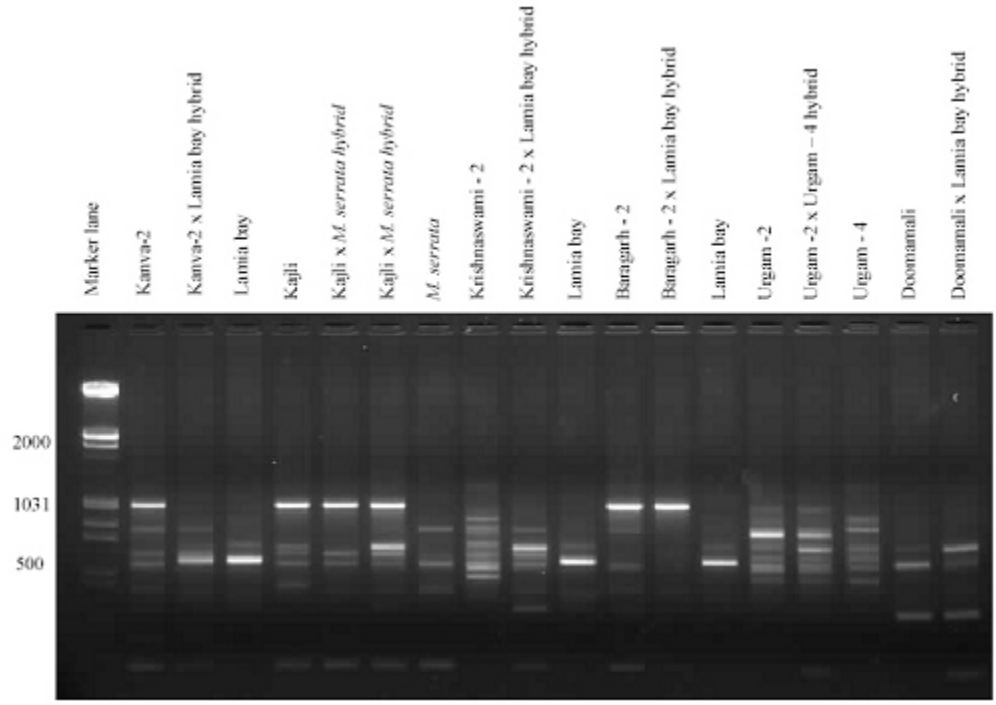

Fig.2: DNA fingerprints of different mulberry parents and hybrids with RAPD primer OPC-10 resolved on 1.5% agarose gel

100% discrimination for the F_1 hybrid and its parents. The percentage of discriminative RAPD loci was higher for the parent (83.60%). The banding pattern showed resemblances with the female parent in morphological and reproductive parameters.

For all the 12 primer tested, OPC-09 and OPC-10 are mentioned in the Fig. 1 and 2 to understand the polymorphisms available in different parents and its hybrids both in interspecific and intra-specific cross. Twelve RAPD primers were used to know the polymorphism in the hybrids and parents and generated 175 markers of which 14 were monomorphic and a total of 156 were polymorphic. The percentage of polymorphism calculated was 89.14. The marker per

primer ranges from 1-16. The marker size ranges from 300 bps to 3400 bps and average markers per primers was 14.6.

DISCUSSION

Breeding behaviour of some wild species was tested at CSGRC, Hosur and reported the findings (Tikader and Dandin, 2001). Similar finding was also reported involving *M.laevigata* and *M.serrata* by Tikader and Dandin, (2007). Thus the pre-breeding effort highlights the possibility of using wild *M. laevigata* and *M. serrata* effectively and efficiently. Similar result was also reported in cotton (Kulkarni *et al*., 2002). The possibility of getting recombinants through back cross of the hybrids for various traits is quiet possible in mulberry. The F_1 plants also showed the characters of high biomass, vigorous growth, profuse fruit formation, timber yield that can be exploited for non-sericulture purposes. The inheritance of traits in the inter-specific and intra-specific crosses was tested using RAPD to know the polymorphism of F_1 hybrid and parents. The banding pattern of F_1 hybrid progeny does not resemble either of the parents completely or shows an intermediate band. In some cases additional band was observed in F_1 hybrid, which is not present in any of the parents (Tikader and Dandin, 2008). The polymorphism in mulberry hybrids and respective parents was also reported by Lou *et al*., (1998). Li *et al*., (1996) applied RAPD - PCR amplification technique to identify the offspring parent and the determination of somatic hybrid in hybrid rice. The study was also reported by Martin *et al*. (1999) using RAPDs for the polymorphic *loci* detection in the *T. tauschii* x *Agropyron cristatum* amphiploid and respective parents. In total 94 RAPD loci inter-specific crosses of Kanva-2 x Lamia bay hybrids, 98 for Kajli x *M. serrata* hybrid, 105 for Kajli x *M. serrata* hybrid, 117 for Krishnaswami x Lamia bay hybrid and 95 for Baragarh-2 x Lamia bay hybrid and respective parents were amplified. In case of intra-specific crosses similar observations were recorded *i.e*., Urgam-2 x Urgam-4 hybrids (100) and Doomarnali x Lamia bay hybrids (106).

The morphological observation was recorded for parents and hybrids, which was similar to female parents and the discrepancies between the crosses 'Kajli x *M. serrata* hybrid and Baragarh-2 x Lamia bay hybrid suggest an effect of female parent dominance as the band pattern is similar with the corresponding parents. Another cross (Kanva-2 x Lamia bay hybrids) suggests the effect of both the parents and the hybrid was intermediate. In crosses Kajli x *M. serrata* hybrid and Krishnaswami x Lamia bay hybrid showed that F_1 hybrids are different from both the parents and specific fragment appeared in the hybrids. Mulberry is a heterozygous perennial plant and showed lot of variation in the hybrids. The similar result was reported by Lou *et al*., (1998). In intra-specific crosses 'Urgam-2 x Urgam-4' hybrids showed resemblances with both the parents, whereas 'Doomarnali x Lamia bay hybrids' showed dominance of female parent.

The fingerprints of genomic DNA described above indicated that DNA fragments and deletion in the corresponding region of hybrid genomes did have taken place, which led to similarity and specific fragment of parent-offspring yielded with one primer was quite different depending on hybrid combinations, which is probably related to the unequal contribution of parental genetic substances in the process of fertilization, different speed of DNA replication, complementary loci formed after genomic recombination, etc. Moreover, the differences of RAPDs obtained in the same hybrid with different primers are probably related to the degree of homology, between primer sequences and genomic DNA. Therefore primers of high homology and rich complementary loci with genomic DNA should be screened. Wang *et al*. (1994) and Tanaka (1994) considered that RAPDs was simple method of high efficiency when they analyzed parent-offspring relationship in hybrid rice and tea with RAPD finger printings. Ko *et al*., (1996) considered that RAPD was a useful tool for establishing genetic diversity in Australian wildflower. The result obtained in the present study indicated that mulberry parents and their resulting hybrids had abundant DNA polymorphism. RAPD-PCR technique discriminated the genetic variability and relationship between parents and its offspring effectively and proved to be a valuable fingerprint system for all F_1 hybrid combinations and respective parents. Moreover, the RAPD was able to establish the parent-offspring relationship both in inter and intra-specific hybrids of mulberry. Therefore, RAPD analysis can interpret the genomic DNA differences of breeding materials and selection can be made from inter-specific and intra-specific hybrid materials. The complementary use of molecular markers and conventional breeding could allow a more accurate evaluation and selection of superior hybrids.

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