Indian J. Genet., **62**(3): 193-196 (2002)

DNA fingerprinting of Mysore Local and V-1 cultivars of mulberry (*Morus* spp.) with RAPD markers

V. Girish Naik, A. Sarkar and N. Sathyanarayana

Central Sericultural Research and Training Institute, Srirampura, Mysore 570 008

(Received: January 2001; Revised: July 2002; Accepted: July 2002)

Abstract

DNA fingerprinting was successfully applied to distinguish two popular mulberry (*Morus* spp.) cultivars, *viz.*, Mysore Local and V-1. RAPD analysis of 12 collections of each of these two cultivars derived from clones of different sources using 12 oligonucleotide random primers generated 73 applicons of which 40 were monomorphic and the rest 33 were polymorphic (45%). All the primers produced typical banding profiles for each of the cultivar suggesting the usefulness of the technique in DNA fingerprinting and cultivar identification. The genetic distance between these two cultivars based on the RAPD data set was estimated as 0.292, which is low in comparison to the morpho-agronomical difference, suggesting a narrow genetic base of the crop.

Key words : Mulberry, DNA fingerprinting, RAPD marker, cultivar identification

Introduction

Mulberry (*Morus* spp.) is mostly cultivated for its foliage, which is the sole food source for domesticated silkworm, *Bombyx mori* L. In tropical climate, mulberry is propagated asexually through stem cuttings at farmers' level, which maintains the purity of the genotype. Mysore Local is the oldest known cultivar in South India and still grown traditionally in some parts of Karnataka and Andhra Pradesh. Though low yielder, the cultivar is known for its hardiness and thrives well in dry climate. Since 1960, the Central Sericultural Research and Training Institute, Mysore has successfully developed a number of high yielding superior mulberry varieties like Kanva-2, S-54, S-36, S-13, S-34 and V-1 of which, the last one has an outstanding record in terms of leaf yield and quality [1]. The leaf yield potentiality of V-1 is around 65-70 Mt/ha/year under favorable conditions whereas the cultivar, Mysore Local yields only 18-20 Mt/ha/year under similar conditions. Yield of the other varieties mentioned above ranged between 35-48 Mt/ha/year.

Even though, many of these mulberry cultivars exhibit distinct agronomical features, it is cumbersome to delineate them under different agro-climatic conditions based on the phenotype. In contrast, DNA fingerprinting techniques are quick and accurately reveal the genetic difference among the varieties/cultivars without being influenced by environmental factors. This approach also provides significant advantage in discrimination, reliability, timeliness at a reduced cost [2]. DNA fingerprinting studies with RAPD markers have already been conducted for identification of cultivars in many crop plants including fruit plants [3-5], ornamentals (6-7) and cereals [8-10]. The present study was undertaken to establish the DNA fingerprinting technique for identification of mulberry cultivars utilizing Mysore Local and V-1 with RAPD markers and to ensure such technique for claiming intellectual property right (IPR) by the mulberry breeder. Further, the study also aimed at estimation of the genetic relatedness of these two morpho-agronomically contrasting genotypes.

Materials and methods

Mysore Local and V-1 cultivars were utilized for the study. A collection of Mysore Local from different sources is maintained in the Gene Bank of the Central Sericultural Research and Training Institute, Mysore. The cultivar, V-1 which has been bred in this institute and cultivated in many plots under different packages was included under this programme. Twelve plants each from Mysore Local and V-1 cultivars were selected randomly. Morphological and agronomical parameters were recorded to highlight the contrasting nature of these two cultivars.

Fresh young leaf samples were collected from the selected plants and genomic DNA was extracted using Nucleon Phytopure Kit (Amersham Life Science, UK) according to the manufacturer's instruction [11]. The genomic DNA was quantified on 0.8% agarose gel and diluted to a uniform concentration of 10 ng/µl for RAPD analysis.

PCR reactions were performed according to the protocol of Williams *et al.*, [12]. The PCR amplifications were carried out in 0.2 ml tube in MJ Research Thermal

Cycler PTC 200, 20 µl reaction volume containing 20 mM Tris-Cl (pH 8.4), 50 mM KCl, 2mM $MgCl_2$, 0.2µM primer, 0.1 mM each dATP, dTTP, dCTP and dGTP, 0.5U Taq polymerase (CCMB, Hyderabad) and 20ng

Table 1. List of random primers used in the study and markers generated

Sl. No.	Primer	Sequence	No. of markers generated*
1	OPI-06	5'-aaggcggcag-3'	7(4)
2	OPAI-14	5'-tggtgcactc-3'	10(4)
3	OPK-17	5'-cccgctacac-3'	3(1)
4	OPAW-14	5'-ggttctgctc-3'	8(6)
5	OPM-02	5'-acaacgcctc-3'	7(1)
6	OPAB-01	5'-ccgtcggtag-3'	3(1)
7	OPAM-02	5'-acttgacggg-3'	7(1)
8	OPAD-03	5'-tctcgcctac-3'	5(2)
9	OPI-12	5'-agagggcaca-3'	10(5)
10	OPAJ-15	5'-gaatccggca-3'	7(6)
11	OPC-05	5'-gatgaccgcc-3'	3(1)
12	OPAE-03	5'-catagagcgg-3'	3(1)

*Figures in the parenthesis indicate the number of polymorphic markers.

of template DNA. Twelve random primers (Table 1) from Operon Technologies Inc., Alameda, USA were used in the study. Amplification reactions were carried out following cycle profile: 1 cycle at 94°C for 5 min followed by 40 cycles at 94°C for 1 min, 35°C for 1 min, 72°C for 2 min and a final extension of 5 min at 72°C. PCR products were electrophoresed in 1.5% agarose confirmed gel [13] in 1X TBE buffer (89 mM Tris-borate, 2mM EDTA, pH 8.0) stained in ethidium bromide solution and gel images were recorded using Gel Documentation System (UVP, UK).

DNA banding patterns generated by RAPD were recorded as '1' for presence of the RAPD marker and '0' for absence. Genetic distance between these two cultivars was calculated based on the RAPD marker data set as per Nei and Li [14]. RAPD markers were identified by the source of the primer (OP = Operon), kit letter, the primer number and approximate size in base pairs.

Results and discussion

Mean morpho-agronomical data of Mysore Local and V-1 are presented in Table 2. A persual of Table 2 indicates that the two genotypes exhibit contrasting nature with respect to most of the characters studied.

A total of 12 oligonucleotide random primers were used for RAPD analysis against all the 12 collections of each of Mysore Local and V-1. All the primers produced distinct polymorphic banding pattern between the two cultivars. The RAPD profile generated by three primers, *viz.*, OPI-72, OPAW-14 and OPC-05 are shown in Fig. 1. A total of 73 markers were generated of which 40 were monomorphic and rest, 33 were polymorphic (45%). The size of the amplified markers ranged from 500-2500 bp with 3-10 markers per primer. As expected of asexually propagated material, no difference in the banding pattern was observed within the collections of the same cultivar. Similar result was obtained by Mulcahy *et al.*, [15] in vegetatively propagated apple and concluded that the different accessions of same cultivar yielded identical fingerprints. They observed that profiles of eight cultivars were highly reproducible with no significant variation among

Table 2. Mean morpho-agronomical data of Mysore Local and V-1 variety of mulberry

Sl. No.	Parameter	Mysore Local	V-1
1	Branching Nature	Erect	Erect
2	Phyllotaxy	1/3	2/5
3	Lobation type	Medium lobed	Unlobed
4	Leaf nature	Heterophyllous	Homophyllous (entire)
5	Leaf texture	Charactaceous	Coriaceous
6	Leaf apex	Acuminate	Acuminate
7	Leaf margin	Serrate	Serrate
8	Leaf base	Cordate	Cordate
9	Leaf shape	Ovate	Ovate lanceolate
10	Easiness for harvesting	Hard to harvest	Easy to harvest
11	Leaf length (cm)	14.35	25.40
12	Leaf width (cm)	11.64	17.50
13	Leaf area (sq. cm)	85.30	228.85
14	Length of the longest shoot (cm)	125.55	155.10
15	Total shoot length (cm)	1127.50	1605.00
16	Girth of the stem (cm)	6.86	10.55
17	Internodal distance (cm)	3.59	5.86
18	Yield/plant (g)	400.77	695.36
19	Moisture content of the leaf (%)	68.14	74.66
20	Resistance to leaf spot	Moderately resistant	Resistant
21	Resistance to powdery mildew	Highly susceptible	Moderately resistant
22	Resistance to leaf rust	Highly susceptible	Moderately resistant
23	Resistance to bacterial blight	Highly susceptible	Moderately resistant
24	Resistance to root knot diseases	Highly susceptible	Moderately resistant
25	Resistance to mealy bug	Susceptible	Resistant

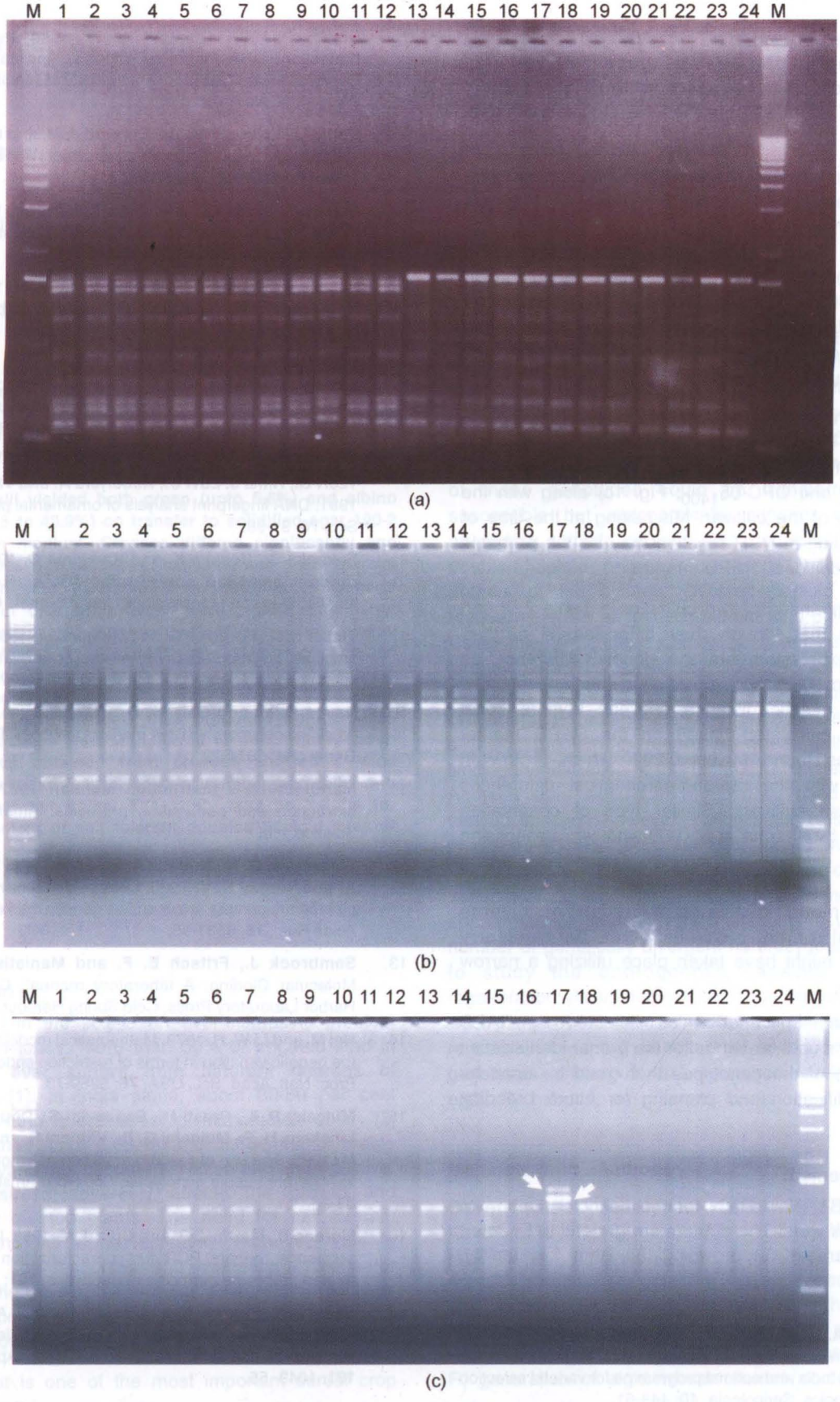

Fig. 1. RAPD fingerprints of Mysore Local collections (lane 1-12) and V-1 collections (lane 13-24) using the Oligonucleotide primers: (a) OPI-12; (b) OPAW-14; (c) OPC-05. Arrow marks in (c) indicates the additional markers amplified in collection No. 17 of V-1 variety. M is the molecular weight marker

the accessions. Moreno *et al.*, [16] studied 12 accessions of the clonally propagated grapevine varieties "Garnacha" from different sources using 12 ISSR primers and observed no variation in the DNA profiles produced.

Nybom *et al.*, [5] analyzed several cultivars of different blackberry and raspberry by DNA fingerprinting and concluded that all cultivars had unique fragment patterns, and no variation within cultivar was encountered. The present study also confirms the usefulness of the DNA fingerprinting technique in mulberry cultivar identification and development of cultivar specific molecular ID cards for use in protection of plant breeders right (PBR) as well as possible use in the plant variety registration.

Additional RAPD marker were detected in V-1 cultivar only in case of two primers, i.e., in collection No. 15 (OPAJ-14_{1200}) and collection No. 17 (OPC-05_{1500} and OPC-05_{1400}; Fig. 1c) along with the typical profile of the cultivar. Mis-pairing at the time of primer annealing in PCR reaction may be the probable reason for additional RAPD marker.

On comparison of two cultivars in Table 2, it can be observed that though they differ largely in their phenotypic expression, but the genetic difference as observed from DNA fingerprints was found to be low. Further, the genetic distance between Mysore Local and V-1 as calculated based on the RAPD marker data set was found to be 0.292; which is low in comparison with the morpho-agronomical differences between these cultivars. Similar type of observation was reported by Sharma *et al.*, (17) who, while assessing the genetic diversity in mulberry germplasm using florescence based AFLP markers observed high similarity among cultivated varieties of diverse geographical origin indicating that the mulberry improvement might have taken place utilizing a narrow genetic base.

The genetic variation as detected by RAPD analysis opens up the avenue for the proper identification and selection of the genotypes that could be used for varietal identification and planning for future breeding programme.

Acknowledgement

We thank DBT, New Delhi for the financial support to undertake this work. The authors are grateful to Director, CCMB, Hyderabad for providing the laboratory facility.

References

1. **Sarkar A., Jalaya S. Kumar and Datta R. K.** 2000. Gradual improvement of mulberry varieties under irrigated condition in South India and optimal programme for varietal selection in the tropics. Sericologia, **40**: 449-61.
2. **Smith S.** 1997. Cultivar identification and varietal protection. DNA markers-Protocols, Applications, and Overviews. *In*: Caetano-Anolles and Gresshoff (ed.). Wiley-VCH, New York.
3. **Koller B., Lehmann A., McDermott J. M. and Gessler C.** 1993. Identification of apple cultivars using RAPD markers. Theor. Appl. Genet., **85**: 901-4.
4. **Harada T., Matsukawa K., Sato T., Ishikawa R., Niizeki M. and Saito K.** 1993. DNA-RAPDs detect genetic variation and paternity in *Malus*. Euphytica, **65**: 87-91.
5. **Nybom H., Schaal B. A. and Rogstad S. H.** 1989. DNA "fingerprints" can distinguish cultivars of blackberries and raspberries. *In*: 5th International Symposium on *Rubus* and *Ribes* Acta Hortic., **262**: 305-10.
6. **Wold K. and Peters-van Rijn J.** 1993. Rapid detection of genetic variability in chrysanthemum (*Dendroanthema grandiflora* Tzvelev) using random primers. Heridity, **71**: 335-41.
7. **Tzuri G., Hillel J. Lavi U., Haberfeld A. and Vainstein A.** 1991. DNA fingerprint analysis of ornamental plants. Plant Sci., **76**: 91-98.
8. **Fukuoka S., Hosaka K. and Kamijima O.** 1992. Use of random amplified polymorphic DNAs (RAPDs) for identification of rice accessions. Jpn. J. Genet., **67**: 243-52.
9. **Tinker N. A., Fortin M. G. and Mather D. E.** 1993. Random amplified polymorphic DNA and pedigree relationships in spring barley. Theor. Appl. Genet., **85**: 976-84.
10. **Devos K. M. and Gale M. D.** 1992. The use of random amplified polymorphic DNA markers in wheat. Theor. Appl. Genet., **84**: 567-572.
11. **Manufacturer's Instruction Manual.** 1997. Nucleon extraction and purification protocols. Amersham Life Science., UK.
12. **Williams J. G. K., Kubelik A. R., Livak K. J., Rafalski J. A. and Tingey S. V.** 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., **18**: 6531-35.
13. **Sambrook J., Fritsch E. F. and Maniatis T.** 1989. Molecular Cloning: A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbour, New York.
14. **Nei M. and Li W. H.** 1979. Mathematical model for studying the genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci., USA, **76**: 5269-73.
15. **Mulcahy D. L., Cresti M., Sansavini S., Douglas G. C., Linskens H. F., Mulcahy G. B., Vignani R. and Pancaldi M.** 1993. The use of random amplified polymorphic DNAs to fingerprint apple genotypes. Sci. Hort. Amsterdam, **54**: 89-96.
16. **Moreno S., Martin J. P. and Ortiz J. M.** 1998. Inter-simple sequence repeats PCR for characterization of closely related grapevine germplasm. Euphytica, **101**: 117-25.
17. **Sharma A., Sharma R. and Machii H.** 2000. Assessment of genetic diversity in a *Morus* germplasm collection using florescence based AFLP markers. Theor. Appl. Genet., **101**: 1049- 55.