*Indian J. Genet.*, **66**(4): 287-292 (2006)

# Identification of duplicate collections in the mulberry (*Morus* spp.) germplasm using RAPD analysis

**V. Girish Naik and S. B. Dandin**

Molecular Biology Laboratory, Central Sericultural Research and Training Institute, Mysore 570 008

(Received: January 2006; Revised: September 2006; Accepted: October 2006)

## Abstract

**Mulberry (*Morus* spp.) is the only source of food for the domesticated silkworm; *Bombyx mori* L. Large numbers of mulberry germplasm have been conserved in the field gene banks, many of which are suspected to be duplicates. PCR based markers like RAPDs are neutral to environmental effects and can be efficiently utilized along with passport and morphological data for identification of duplicate collection in a gene bank. A close examination of passport and morphological data became a basis for identification of four suspected group of duplicates along with a closely related genotype of suspected duplicate Group I. Two sets of true duplicates (Mysore Local and V-I) were used as controls. A total of 31 random primers were used for PCR amplification, generating 357 markers of which, 228 (63.9%) were polymorphic. The DNA marker profiles of true duplicates were identical demonstrating the reliability of the technique. The closely related genotype RFS-135 was discriminated from the suspected duplicate Group I (Anantha and RFS-175) with a similarity of 94.4%. Group I, II, and IV were unambiguously confirmed duplicate sets and clustered at 100% similarity within the group. But the suspected duplicate collection in the Group III comprising of Kousen and Xuan-10 were discriminated by 12 primers and 16 markers. The result obtained from the study predicted a minimum requirement of 100 markers or 9 primers for detection of at least one difference for discrimination of closely related collections.**

**Key words:** Mulberry, duplicate collections, gene bank, RAPD markers

## Introduction

Mulberry (*Morus* spp.) is an economically most important crop plant in sericulture. Its foliage is the only source of food for the domesticated silkworm, *Bombyx mori*, which produces natural silk - the Queen of Textiles. Mulberry is a perennial, dioecious and heterozygous tree. Vegetative propagation is the only means to maintain the cultivars' identity. Long history of cultivation for sericulture and recent efforts by traditional breeding for mulberry improvement has resulted in a number of varieties grown in different regions. Conservation efforts by countries involved in sericulture have resulted in the accumulation of approximately 5000 accessions in the field gene banks. A large portion of these collections is suspected to be duplicates or redundant. An efficient strategy is required to establish the genetic identity of the collections/accessions and reduce the redundancy for minimization of management and operational cost of the field germplasm conservation programme of mulberry.

The filed gene bank at Central Sericultural Research and Training Institute (CSRTI), Mysore, holds 480 mulberry collections as working germplasm for utilization in the breeding programme. Our routine study based on passport information and morphological observation has suggested the existence of duplicate collections in the field gene bank. However, one can never be sure of this assessment, as most of the morphological characters are subjected to environmental influence. At International Potato Center (CIP), Lima, Peru, duplicate identification in the field gene bank has reduced the size of collection from 1939 to 909 accessions [1]. RAPD analysis is now being routinely used in identification of duplicate collections in the sweet potato germplasm. RAPD analysis was used for accurate discrimination of rice germplasm that also included true and suspected duplicates as well as closely related accessions [2]. DNA fingerprinting has been carried out successfully and shown that no intra-cultivar variability exists in RAPD marker profiles of clonally propagated mulberry cultivars [3]. RAPD marker profiles were highly consistent and useful in establishing the cultivar identity. Even though a number of other DNA based marker systems are available for discrimination of duplicate collections in mulberry germplasm, RAPD is simple, economical and large number of samples can be processed at a time. Further, the technique requires comparatively less quantity of DNA and no usage of radioisotope.

In the present study, a set of two true duplicates along with four groups of suspected duplicates and a closely related genotype of a suspected duplicate group were considered based on the passport and

morphological data. The work was mainly aimed to establish an unambiguous method for identification of duplicate collection in mulberry germplasm by RAPD analysis.

## Materials and methods

*Plant materials*: Ten mulberry collections suspected to represent four duplicate groups including a closely related genotype (RFS-135) of a suspected group I (RFS-175 and Anantha) were identified from the field gene bank of CSRTI, Mysore based on the passport and morphological data. Besides, two pairs of true duplicates were sampled from the popular mulberry cultivars (Mysore Local and V-I) from two different sources. Mulberry collections utilized in the study along with the origin and pedigree is given in the Table 1.

**Table 1.** List of true and suspected duplicate mulberry collections utilized in the study

| Sl. No. | Name of the collection | Origin | Pedigree | Remarks |
|---|---|---|---|---|
| 1. | Mysore Local (M-6) | Unknown | Land race | True duplicate (Group I) |
| 2. | Mysore Local (M-7) | Unknown | Land race | True duplicate (Group I) |
| 3. | V-I (M-13) | CSRTI, Mysore | S-30 × C-776 | True duplicate (Group II) |
| 4. | V-I (M-17) | CSRTI, Mysore | S-30 × C-776 | True duplicate (Group II) |
| 5. | RFS-135 | CSRTI, Mysore | OPH of K-2 | Closely related to RFS-175 |
| 6. | Anantha | RSRS, Ananthapur | - | Suspected duplicate (Group I) |
| 7. | RFS-175 | CSRTI, Mysore | OPH of K-2 | Suspected duplicate (Group I) |
| 8. | S-146 | CSRTI, Berhampore | OPH selection | Suspected duplicate (Group II) |
| 9. | Check Majra | RSRS, Dehradun | Clonal selection | Suspected duplicate (Group II) |
| 10. | DD | KSSRDI, Bangalore | Collection from Dehradun | Suspected duplicate (Group II) |
| 11. | Kousen | Japan | Selection | Suspected duplicate (Group III) |
| 12. | Xuan-10 | China | Collection | Suspected duplicate (Group III) |
| 13. | *M. multicaulis* (Acc. 235) | Indonesia | Collection | Suspected duplicate (Group IV) |
| 14. | *M. multicaulis* (Acc. 212) | Indonesia | Collection | Suspected duplicate (Group IV) |

*Morphological data*: Morphological data of mulberry collections in the field gene bank were recorded as a part of routine characterization of germplasm at CSRTI, Mysore. However, the present study considered only nine characters, which are qualitative in nature and neutral to environmental influence *viz.*, branching nature, phyllotaxy, leaf color, leaf texture, leaf shape, leaf margin, leaf base, sex and fruit color.

*Genomic DNA isolation*: Genomic DNA from pooled fresh young leaves of ten individual plants of each collection was isolated using Nucleon Phytopure Kit, Amersham Life Sciences, UK as per manufacturers' instruction [4]. The purity and the quantity of isolated genomic DNA were assessed by UV-Vis spectrophotometer as well as on 0.8% agarose gel. 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 developed by Williams *et al.*, [5]. The PCR amplifications were carried out in 0.2 ml tubes in Gene Amp 9700 PCR system (Applied Biosystems, USA) in 20 µl reaction volume containing 20 mM Tris-Cl (pH 8.4), 50 mM KCl, 2 mM $MgCl_2$, 0.2 µM primer, 0.1 mM each dATP, dTTP, dCTP and dGTP, 0.5 U Taq polymerase (Genei, Bangalore) and 20 ng of template DNA. The random primers (Table 2) were obtained from Operon Technologies Inc., Alameda, USA. Amplification reactions were carried out with following cycle profiles: 1 cycle at 93°C for 2 min followed by 40 cycles at 93°C for 1 min, 35°C for 1 min, 72°C for 2 min and a final extension of 7 min at 72°C. PCR products were electrophoresed on 1.5% agarose gel in lx TAE buffer and stained in ethidium bromide solution and gel images were recorded using a Gel Documentation System (Syngene, UK).

*Data analysis*: Morphological characters were numerically coded for the character state and simple matching coefficient was calculated [6]. The similarity matrix based on morphology was computed for the construction of UPGMA tree. RAPD amplification with each primer was performed twice and bands in the range of 250-3500 bp were scored. DNA banding patterns generated by RAPDs were recorded as '1' for the presence of the fragment and '0' for the absence. Dice similarity coefficient(s) between true and suspected duplicate collections were calculated based on the RAPD data set [7]. The RAPD markers were identified by the source of the primer (OP = Operon), kit letter, the primer number and approximate size in base pairs. Genetic relationships among the collections were established by UPGMA clustering based on Dice similarity matrix of RAPD markers. The two similarity matrices used for UPGMA clustering based on

morphological and RAPD marker were tested for association by Mantel test [8].

## Result and discussion

*Morphological analysis*: Clustering of true and suspected duplicate mulberry germplasm collections based on the similarity matrix of morphological characters is presented in the Fig. 1. The analysis indicated all the true and suspected mulberry germplasm collections paired together at 100% similarity in the respective clusters except, RFS-135. The collection RFS-135 was identical in its phenotype with that of RFS-175 and Anantha except for arrangement of leaves on the shoot (phyllotaxy). The former had a 1/2 and 1/3 type of leaf arrangement compared to the 1/3 and 2/5 in the latter, the first suspected duplicate group.

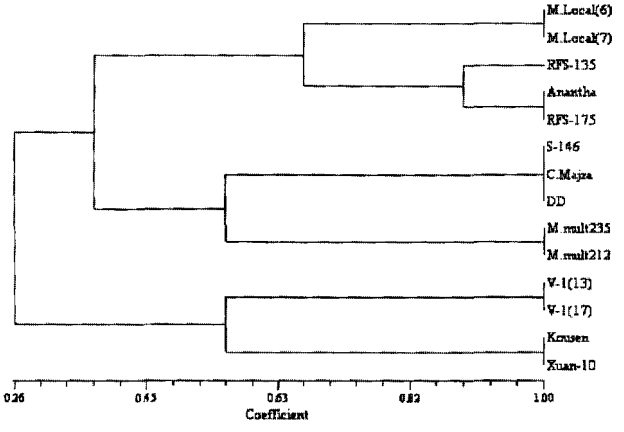


**Fig. 1. UPGMA clustering of true and suspected duplicate mulberry germplasm collections based on morphological characters**

*RAPD analysis*: The genomic DNA of all the collections was subjected to PCR amplification using 33 random primers sequentially from OPA-01 to OPA-20 and OPC-01 to OPC-13 without bias. Only two primers namely, OPA-05 and OPA-08 did not show polymorphic amplification profiles among the different collections and hence not considered for further analysis. A total of 357 markers (amplicons) were generated by amplification with 31 primers out of which, 228 (63.9%) were polymorphic (Table 2). The marker size ranged from 250 to 3500 bp and on an average, 11.5 markers was generated per primer. Some of the primers were more informative than others; for example, a single primer OPA-07 could distinguish between all the duplicate sets. DNA profiles generated by the primers, OPA-07 and OPA-09 are shown in Fig. 2. RFS-135, which is closely related to RFS-175 but not identical (both are open pollinated hybrids of cultivar K-2) could be discriminated from the latter in the presence of OPA-$03_{450}$, OPA-$04_{1450}$, OPA-$04_{1000}$, OPA-$07_{1450}$, OPA-$07_{1100}$, OPA-$07_{450}$ etc., and absence of OPA-$09_{1900}$, OPA-$10_{850}$, OPA-$14_{2300}$ etc., RAPD markers and showed high similarity (94.4%). The genetic similarity among the different collections considered for the study ranged from 76% (between V-I and RFS-175/Anantha) to 100% (among duplicate collections). However, the third suspected group of duplicate collection namely, Kousen and Xuan-10 did not show complete similarity in DNA amplification profiles. The former differed from the latter in having additional markers *viz.*, OPA-$01_{1900}$, OPA-$02_{1400}$, OPA-$02_{450}$, OPA-$07_{1100}$, OPA-$17_{1800}$, OPA-$17_{1250}$, OPA-$17_{900}$, OPA-$18_{1300}$, OPC-$01_{500}$, OPC-$07_{800}$ and OPC-$13_{1300}$ and absence of OPA-$14_{3400}$, OPA-$14_{300}$ OPA-$20_{1800}$, OPA-$09_{900}$ and OPC-$10_{3500}$ markers.

**Table 2.** List of random primers used in the study and marker polymorphism

| Sl. No. | Primer | Sequence (5'-3') | Total no. of amplicons | No. of polymorphic amplicons | % of ploymorphism |
|---|---|---|---|---|---|
| 1. | OPA-01 | -caggcccttc- | 08 | 04 | 50.0 |
| 2. | OPA-02 | -tgccgagctg- | 15 | 11 | 73.3 |
| 3. | OPA-03 | -agtcagccac- | 13 | 06 | 46.2 |
| 4. | OPA-04 | -aatggggctg- | 13 | 08 | 61.5 |
| 5. | OPA-06 | -ggtccctgac- | 04 | 01 | 25.0 |
| 6. | OPA-07 | -gaaacgggtg- | 18 | 14 | 77.8 |
| 7. | OPA-09 | -gggtaacgcc- | 12 | 10 | 83.3 |
| 8. | OPA-10 | -gtgatcgcag- | 14 | 10 | 71.4 |
| 9. | OPA-11 | -caaatcgccgt- | 13 | 07 | 53.9 |
| 10. | OPA-12 | -tcggcgatag- | 10 | 08 | 80.0 |
| 11. | OPA-13 | -cagcacccac- | 09 | 03 | 33.3 |
| 12. | OPA-14 | -tctgtgctgg- | 12 | 07 | 58.3 |
| 13. | OPA-15 | -ttccgaaccc- | 15 | 12 | 80.0 |
| 14. | OPA-16 | -agccagcgaa- | 14 | 10 | 71.4 |
| 15. | OPA-17 | -gaccgcttgt- | 11 | 08 | 72.7 |
| 16. | OPA-18 | -aggtgaccgt- | 10 | 04 | 40.0 |
| 17. | OPA-19 | -caaacgtcgg- | 10 | 05 | 50.0 |
| 18. | OPA-20 | -gttgcgatcc- | 13 | 08 | 61.5 |
| 19. | OPC-01 | -ttcgagccag- | 12 | 07 | 58.3 |
| 20. | OPC-02 | -gtgaggcgtc- | 14 | 08 | 57.1 |
| 21. | OPC-03 | -gggggtcttt- | 06 | 06 | 100.0 |
| 22. | OPC-04 | -ccgcatctac- | 10 | 04 | 40.0 |
| 23. | OPC-05 | -gatgaccgcc- | 14 | 09 | 64.3 |
| 24. | OPC-06 | -gaacggactc- | 12 | 11 | 91.7 |
| 25. | OPC-07 | -gtcccgacga- | 11 | 08 | 72.7 |
| 26. | OPC-08 | -tggaccggtg- | 14 | 07 | 50.0 |
| 27. | OPC-09 | -ctcaccgtcc- | 12 | 04 | 33.3 |
| 28. | OPC-10 | -tgtctgggtg- | 13 | 11 | 84.6 |
| 29. | OPC-11 | -aaagctgcgg- | 11 | 06 | 54.6 |
| 30. | OPC-12 | -tgtcatcccc- | 07 | 06 | 85.7 |
| 31. | OPC-13 | -aagcctcgtc- | 07 | 05 | 71.4 |
| | | Total average | 357 | 228 | 63.9 |

Cluster analysis (UPGMA) was performed based on the Dice similarity matrix (Table 3) generated by computing polymorphic as well as monomorphic markers

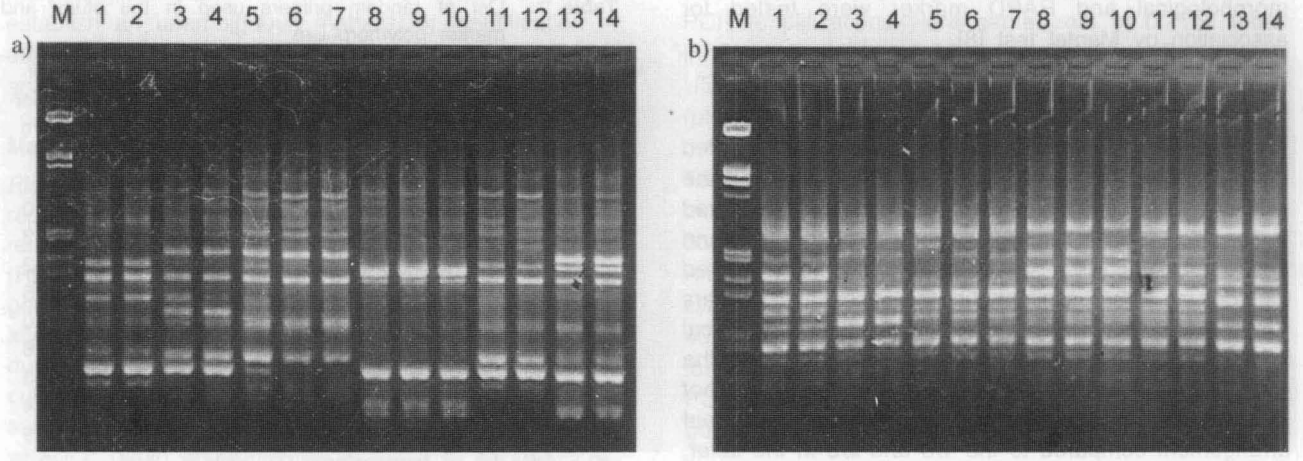


**Fig. 2. DNA fingerprints of true and suspected duplicate mulberry germplasm collections generated using random primers OPA-07 and, b) OPA-09. 1) Mysore Local (6), 2) Mysore Local (7), 3) V-I (13), 4) V-I (17), 5) RFS-135, 6) Anantha, 7) RFS-175, 8) S-146, 9) Check Majra, 10) DD, 11) Kousen, 12) Xuan-10, 13) *M. multicaulis* (235), 14) *M. multicaulis* (212). M is the λ. DNA EcoRI+HindIII double digest molecular size marker.**

**Table 3.** Dice similarity coefficient values based on RAPD marker data set of true and suspected duplicate collections of mulberry

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Mysore local (6) | 1.000 | | | | | | | | | | | | | |
| 2. | Mysore local (7) | 1.000 | 1.000 | | | | | | | | | | | | |
| 3. | V-I (13) | 0.814 | 0.814 | 1.000 | | | | | | | | | | | |
| 4. | V-I (17) | 0.814 | 0.814 | 1.000 | 1.000 | | | | | | | | | | |
| 5. | RFS-135 | 0.798 | 0.798 | 0.792 | 0.792 | 1.000 | | | | | | | | | |
| 6. | Anantha | 0.775 | 0.775 | 0.760 | 0.760 | 0.944 | 1.000 | | | | | | | | |
| 7. | RFS-175 | 0.775 | 0.775 | 0.760 | 0.760 | 0.944 | 1.000 | 1.000 | | | | | | | |
| 8. | Check Majra | 0.811 | 0.811 | 0.784 | 0.784 | 0.775 | 0.768 | 0.768 | 1.000 | | | | | | |
| 9. | S-146 | 0.811 | 0.811 | 0.784 | 0.784 | 0.775 | 0.768 | 0.768 | 1.000 | 1.000 | | | | | |
| 10. | DD | 0.811 | 0.811 | 0.784 | 0.784 | 0.775 | 0.768 | 0.768 | 1.000 | 1.000 | 1.000 | | | | |
| 11. | Kousen | 0.855 | 0.855 | 0.805 | 0.805 | 0.838 | 0.815 | 0.815 | 0.830 | 0.830 | 0.830 | 1.000 | | | |
| 12. | Xuan-10 | 0.857 | 0.857 | 0.794 | 0.794 | 0.831 | 0.826 | 0.826 | 0.824 | 0.824 | 0.824 | 0.966 | 1.000 | | |
| 13. | *M. multicaulis* (235) | 0.777 | 0.777 | 0.770 | 0.770 | 0.761 | 0.762 | 0.762 | 0.762 | 0.762 | 0.762 | 0.807 | 0.818 | 1.000 | |
| 14. | *M. multicaulis* (212) | 0.777 | 0.777 | 0.770 | 0.770 | 0.761 | 0.762 | 0.762 | 0.762 | 0.762 | 0.762 | 0.807 | 0.818 | 1.000 | 1.000 |

to construct the dendrorgram as shown in Fig. 3. The two pairs of true duplicates (Mysore Local collection No. 6 and 7 and V-I collection No. 13 and 17) and three sets of suspected duplicates (RFS-175 and Anantha; S-146, Check Majra and DD; *M. multicaulis* accession No. 235 and 212) were clustered together at 100% similarity. However, two other collections (Kousen and Xuan-10) of suspected duplicates could be differentiated from each other at 96.6% similarity. Further, the closely related collections namely, RFS-135 and RFS-175 also could be discriminated unambiguously at 94.4% similarity.

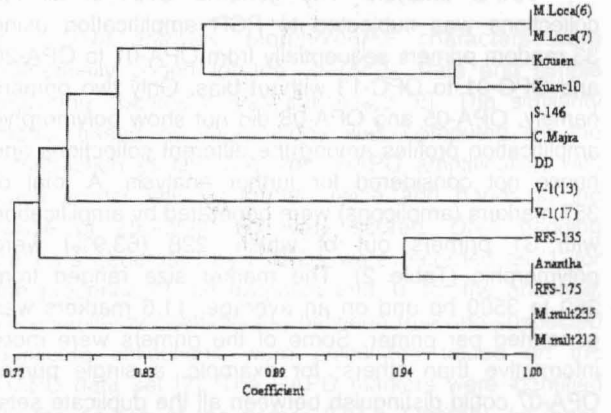


**Fig. 3. UPGMA clustering of true and suspected duplicate mulberry collections based on RAPD marker similarity**

The results obtained from the study was utilized to predict the approximate number of primers, total number of markers and number of polymorphic markers needed to detect a difference between a suspected duplicate pair of mulberry collections. Out of the total of 31 primers utilized, 12 primers could discriminate between the suspected duplicate pair namely, Kousen and Xuan-10. Therefore, the number of primers (n) needed to detect at least one difference between a

pair of suspected duplicate with 99% confidence is calculated as $(19/31)^n = 1\text{-}0.99$, which is about 9. Similarly, the total number of marker (m) and the number of polymorphic markers (p) needed for the purpose is calculated as $(341/357)^m = 0.01$ (100) and $(212/228)^p = 0.01$ (64), respectively.

The two similarity matrices developed independently out of morphological and RAPD marker data were tested for association by Mantel test and the matrix correlation (r) was found to be 0.66 at p = 0.01 level. Therefore, the test for matrix correspondence was good fit and suggested a close association, which in turn supports the two step strategy (first morphological and then followed by RAPD analysis) adopted for identification of duplicates in the mulberry germplasm.

It is estimated that a substantial number of mulberry germplasm collections have been assembled mainly in the field gene banks of sericultural countries such as China (2600), Japan (1300), India (1050) etc. A sizable number of them are suspected to be duplicates. As mulberry is highly heterozygous, the collections are propagated and maintained vegetatively in the gene bank to retain the genetic identity. Field gene banks have finite capacity and hence need an efficient conservation programme with minimum redundancy to reduce the management and operational costs.

The present study was undertaken in the above direction, for exploring the possibility of utilization of molecular marker approaches and developing an accurate procedure for designating a set of collections as duplicates. The study utilized two sets of true duplicates serving as controls that can be regarded as replicate samples. The reliability of the RAPD marker technique was demonstrated by the absence of any banding difference between the replicated control samples. Naik *et al.*, [3] have demonstrated the effectiveness of DNA fingerprinting technique in mulberry cultivars with random primers and confirmed the absence of intra-cultivar variation in mulberry. However, to enhance the reliability, the study utilized pooled samples from ten individual plants of a collection.

The diversity pattern of mulberry collections ascertained by RAPD markers closely resembled that of morphological marker analysis. The investigation utilized nine morphological traits, which are qualitative in nature and hence least influenced by environmental effects. The numbers of such traits are few in mulberry and quantitative traits are vastly influenced by climate, agronomical inputs and soil conditions besides showing continuous variability and ideally not suited for establishing the accurate genetic relationship. The study included four suspected groups which were preliminarily identified based on the passport and morphological data and also included a closely related genotype of RFS-175, *i.e.*, RFS-135 (both OPH of cultivar K-2) to test the discriminatory power of the molecular technique. The result showed ample number of marker variation and the genetic distance was measured at 0.056 (1-s), confirming the utility of the technique in resolving the closely related genotypes. All the collections within the four suspected duplicate groups had identical morphology and DNA profiles except the group III consisting of Kousen and Xuan-10, which also showed identical morphology but, difference in 16 RAPD markers of 12 primers out of the total 357 scored. Table 1 suggests the diverse origin of collections (*i.e.*, Kousen and Xuan-10) but opinion among researchers point towards a common origin. The similarity based on the RAPD markers among these two collections is only 96.6% and hence cannot be considered duplicate. However, the collections in suspected duplicate group I (Anantha and RFS-175); group II (S-146, Check Majra and DD) and group IV (*M. multicaulis* 235 and *M. multicaulis* 212) are confirmed duplicates based on the result obtained from the present investigation. However, theoretically one can never prove two collections be genetically identical unless entire genome is sequenced which is practically not a viable proposition. Hence, there is a need to arrive at a practical limit with technologies available today.

Hintum and Knupffer [9] suggested that the first step in the identification of probable duplicates is based on the available passport data. Dobrovolskaya *et al.*, [10] while working towards rationalization of wheat germplasm collections identified twelve potential duplicate groups consisting of three to nine accessions with identical names/numbers and analyzed with microsatellite markers. A bootstrap approach based on re-sampling of both microsatellite markers and alleles within marker loci was used to test homogeneity. Although several homogenous groups were identified, it became clear that cultivar name identity alone did not allow the determination of duplicates. Virk *et al.*, [2] have discussed two procedures for designating the duplicates among the collections held in the rice gene bank. In both the procedures, they suggested that the potential duplicates would be first detected from the germplasm after a close examination of the passport data. First procedure requires initial morphological characterization of suspected duplicates. Those, which could not be separated, will be subjected to a full 100 RAPD marker analysis. These collections that could not be distinguished in the suspected group would be then designated as duplicates. The second procedure involves the pre-screening of suspected duplicates with 2-3 primers and those which could not be discriminated would be subjected to full scale 100 RAPD marker

analysis and again those which could not be discriminated would be assigned as duplicates. The first procedure mentioned above can be adopted for identification of duplicate collections/accessions in the mulberry gene bank. The procedure is much more straightforward in mulberry as it is propagated mainly by vegetative means unlike rice, which is through sexually produced seeds. Besides, Zhang *et al.*, [1] also suggested the identification of suspected duplicates based on a good morphological characterization, then RAPD profiling can be conducted on a limited number of accessions and comparison can be made on the same gel, so that operational error will be minimized because of cross plate and cross gel comparisons. Faniza *et al.*, [11] showed a stable clustering of the *Vitis vinifera* genotypes based on genetic distance measures derived from large number of RAPD markers. However, to reduce the cost and time for such analysis, they tested minimum number of markers required to obtain such a stable cluster. Their study indicated that, some rearrangement of the genotypes in the dendrogram was observed as the number of markers decreased; below 100-150 markers, the clustering got completely rearranged. The study thus highlights the minimum number of RAPD markers essentially needed for assessment of genetic distances and interrelationship among the *Vitis vinifera* genotypes.

The comparison of relative effectiveness of RAPD and morphological markers is useful when considering the strategy to be adopted before full-scale molecular marker analysis. A significant correlation ($r = 0.66$ at $\dot{p} = 0.01$) suggests a close association between the two matrices based on similarity [8] indices used in the cluster analysis. The statistically validated consideration of 100 RAPD markers as criteria for identification of duplicates in rice by Virk *et al.*, [2] has been analyzed with reference to mulberry system. Incidentally, an identical number of RAPD markers (*i.e.*, 100 nos.) were estimated to be the requisite number of markers for designating the duplicates in mulberry also. Therefore, the study supports the scheme procedure that was suggested by Virk *et al.*, [2] for designation of duplicate collections.

The number of collections/accessions in Indian field gene bank of mulberry germplasm collections in different Research Institutes is growing steadily. However, there is a finite capacity for accommodation of germplasm collections in terms of space, resources, manpower etc. Therefore, identification of duplicate based on morphological and RAPD analysis offers a better management option for mulberry germplasm. According to the assessment made by Zhang *et al.*, [1], the identification of redundancy in sweet potato germplasm at CIP, Peru will eventually save about 30-40% of maintenance cost of the gene bank. The development of core germplasm collection is one of the important requirements at present in mulberry improvement. The downsized collection after identification of duplicates will provide a much better base for construction of core collection.

**Acknowledgement**

Authors thank Dr. A. Sarkar, CSRTI, Berhampore and Dr. Ramesh K. Agarwal, CCMB, Hyderabad for support provided during the work. The authors also acknowledge Mrs. M. Rekha, CSRTI, Mysore for help rendered in some of the statistical considerations. The work has been undertaken with the financial support received from the Department of Biotechnology, Govt. of India, New Delhi.

**References**

1. **Zhang D. P., Ghislain M., Huaman Z., Rodrguez F. and Cervantes J. C.** 1996. Identifying duplicates in sweetpotato germplasm using RAPD. *In*: International Potato Center Program Report 1995-96, International Potato Center, Lima, Peru, pp. 90-96.
2. **Virk P. S., Newbury H. J., Jackson M. T. and Ford-Lloyd B. V.** 1995. The identification of duplicate accessions within a rice germplasm collection using RAPD analysis. Theor Appl Genet., **90**: 1049-1055.
3. **Naik V. G., Sarkar A. and Sathyanarayana N.** 2003. DNA fingerprinting of Mysore Local and V-I mulberry (*Morus* spp.) with RAPD markers. Indian J. Genet., **62**: 193-196.
4. **Manufacturers Instruction Manual.** 1997. Nucleon extraction and purification protocols. Amersham Life Sciences, UK.
5. **William J. G. K., Kubelik A. R., Livak K. J., Rafalski J. A. and Tingey S. V.** 1990. DNA polymorphism by amplified arbitrary primers are useful as genetic markers. Nucleic Acids Res., **18**: 6531-6535.
6. **Sneath P. H. A. and Sokal R. R.** 1973. Numerical Taxonomy - The principles and practice of numerical classification. W. H. Freeman and Company, San Francisco.
7. **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**: 5269-5273.
8. **Mantel N.** 1967. The detection of disease clustering and a generalized regression approach. Cancer Res., **27**: 209-220.
9. **Hintum T. J. L. and Knupffer H.** 1995. Duplication within and between germplasm collection. I. Identifying duplication on the basis of passport data. Gen. Res. Crop Evol., **42**: 1127-1133.
10. **Dobrovolskaya O., Saleh U., Malysheva-Otto L., Rodder M. S. and Borner A.** 2005. Rationalization of germplasm collections: a case study of wheat. Theor. Appl. Genet., **111**: 1322-1329.
11. **Fanizza, G., Colonna G., Resta P. and Ferrara G.** 1999. The effect of the number of RAPD markers on the evaluation of genotypic distances in *Vitis vinifera*. Euphytica, **107**: 45-50.