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Characterization and cluster analysis of some indigenous and exotic mulberry collections based on random DNA marker assay

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ABSTRACT

Groups of mulberry (*Morus* spp.) germplasm were selected from the field gene bank of Central Sericultural Research and Training Institute, Mysore based on accessions having identical names and similar origin or descent. These germplasm were characterized using DNA marker technology with intent of assessing the genetic identity of accessions and diversity available in the collection. RAPD fingerprinting using 23 informative primers generated 294 polymorphic markers out of the total of 332 markers. As many as 6 primers showed 100% marker polymorphism indicating high genetic variability among the germplasm. Dice similarity based on the DNA markers revealed coefficient value ranging from 1.000 (perfect match) to 0.624 (divergent). Clustering by un-weighted group method of arithmetic averages resolved in to 10 groups with cluster I comprising largest number of germplasm (10 Nos.). Even though few germplasm showed closer genetic relationship, all the accessions were considered unique except the pair S-796 and S-1096. The pair was assigned as genetically redundant and hence to be treated as duplicate accessions by the gene bank curators. The study explains the possibility of using the outcome in context of developing a core sub-set of mulberry germplasm. The core subset will be rationalized collection which can be used by the mulberry breeders for in-depth evaluation for novel genes and traits. A smaller collections having all the alleles will be more attractive to breeders in crop improvement programs.

KEYWORDS

Mulberry germplasm;
DNA markers;
Characterization;
Cluster analysis;
Genetic redundancy;
Core sub-set.

INTRODUCTION

Mulberry (*Morus spp*.) is a perennial plant and of economic importance in sericulture. Mulberry is cultivated for its foliage which is the sole source of food for the silk producing domesticated silkworm-*Bombyx mori* L. Sericulture is practiced in different climatic regions of the country, which necessitates the cultivation of productive mulberry varieties suited for the area. Further, there is constant demand for genetic improvement of the crop due to threats from environmental vagaries and biotic factors. Germplasm are the reservoirs of the novel genes and traits

for crop improvement programs. Large number of germplasm have been assembled in field gene banks of different sericultural institutes through exploration and introduction of exotic collections[1-3]. Lack of accurate passport information, absence of well planned collection strategies and non-existent pre-introduction screening methods in mulberry germplasm have resulted in unnecessary genetic redundancy causing of burden on management in terms of resource requirements and achieving the desired objectives[4]. Further, unmanageable increase in the number of collection has resulted in superfluous characterization and evaluation of the germplasm adding little or no value in utilization. Germplasm characterization and classification provide essential information for genetic improvement of crops[5]. Characterization of perennial mulberry tree by phenotypic markers lacks accuracy and reliability due to quantitative nature of the traits and larger effect of genotype x environment[6]. In comparison, the advanced molecular marker technology overcomes the limitations posed by phenotypic markers with added advantage of speed and automation to the characterization process.

The discovery of Polymerase Chain Reaction (PCR) based DNA amplification has resulted in the development of many marker technologies for characterization of germplasm resources[7]. Among the marker techniques, simple sequence repeat (SSR) motifs often termed as microsatellites are most popular among the geneticists because of co-dominant nature and high degree of reproducibility across laboratories. The use of SSR loci as polymorphic markers has expanded considerably over the past decade both in number of studies and in number of organisms, primarily due to their facility and power for population genetic analysis[8]. However, the only constraint in using microsatellites is the lack availability in sufficient numbers and huge cost and time factor involved in the development of this marker. In crops, where not much genetic information is forthcoming or poorly studied, the anonymous/random markers provide an opportunity to characterize the germplasm quickly with ease and at comparatively low cost. Neutral markers like Random Amplified Polymorphic DNA (RAPD) are expected to reflect demographic and genetic history of populations[9]. The abundance of large number of RAPDs makes it possible to use in the crop systems with unknown genetic structure. RAPDs have been used in mulberry cultivar identification[10], germplasm characterization[11], identification of duplicates in germplasm[4] and genome mapping[12].

The present study is an attempt to characterize a collection of 36 indigenous and exotic mulberry germplasm short-listed based on groups having identical names or common origins and/or descent using RAPD markers. Because of extensive introduction of mulberry germplasm as well as collection through exploration, many accessions with same or similar name of location and synonyms exist due to indiscriminate assignment of names by different groups involved in the germplasm collection at different centers. The main objective of the study is to assess the genetic diversity prevailing in these collections as well to assess the genetic redundancy to aid towards a larger interest of a construction of a core subset mulberry. The study also will depict accurate interrelationships among the collection from diverse source to predict the development of varieties in different regions.

EXPERIMENTAL

Plant materials

The plant materials utilized in the study were available in the field gene bank of Central Sericultural Research and Training Institute (CSRTI), Mysore, India. These germplasm were basically assembled from within the country from different locations as well from outside the country namely, France and Italy. The list of germplasm along with pedigree information is provided in the TABLE 1. In the field gene bank, each germplasm accession is represented by nine plants and maintained as a medium bush under standard package of practices recommended by the Institute.

Genomic DNA isolation

Fresh tender leaves from mulberry germplasm were collected for extraction of high molecular weight DNA using HiPurA Plant genomic DNA Purification Spin Kit (HiMedia Laboratories Pvt. Ltd., India). The DNA was quantified on 0.8% agarose gel after staining with ethidium bromide solution. The stock DNA solution was diluted to uniform concentration of 10mg/µl for PCR amplification.

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TABLE 1 : The list of mulberry germplasm accessions utilized in the study

Sl. No.	Germplasm	CSRTI Acc. No.	Origin/Species/Pedigree
1	Furcata	446	France, collection
2	Furcata	391	France, collection
3	Cattaneo	380	Italy, *M. alba* L.
4	Cattaneo	129	Italy, *M. alba* L.
5	S-31	207	Dehradun, Uttarakand, India
6	S-30	156	*M. indica*, Mysore, India
7	S-523	209	Dehradun, Uttarakand, India
8	S-642	210	Dehradun, Uttarakand, India
9	S-741	211	Dehradun, Uttarakand, India
10	S-796	212	Dehradun, Uttarakand, India
11	S-1096	213	Dehradun, Uttarakand, India
12	S-1301	215	Dehradun, Uttarakand, India
13	S-1531	216	Dehradun, Uttarakand, India
14	MS-1	253	Majra, India, Selection
15	MS-2	227	Majra, India, Selection
16	MS-3	254	Majra, India, Selection
17	MS-5	228	Majra, India, Selection
18	MS-5	272	Majra, India, Selection
19	MS-6	255	Majra, India, Selection
20	MS-7	229	Majra, India, Selection
21	MS-8	256	Majra, India, Selection
22	MS-9	230	Majra, India, Selection
23	Morus F1	388	France, Collection
24	Morus F2	389	France, Collection
25	Morus F3	390	France, Collection
26	Morus F4	402	France, Collection
27	Morus F5	426	France, Collection
28	Morus F6	395	France, Collection
29	Morus F7	431	France, Collection
30	Mahadeva Farm	330	Uttar Pradesh, India, Collection
31	Mahadeva	331	Uttar Pradesh, India, Collection
32	Mahadeva Farm	322	Uttar Pradesh, India, Collection
33	Mahadeva Farm	323	Uttar Pradesh, India, Colletion
34	Mahadeva Farm	318	Uttar Pradesh, India, Collection
35	Mahadeva	328	Uttar Pradesh, India, Collection
36	Mahadeva Farm	325	Uttar Pradesh, India, Collection

PCR amplification

PCR reactions were performed according to the protocols of Williams et al[13]. The PCR amplification was carried in a 0.2ml non-sticky PCR tube in PTC-200 DNA engine (MJ Research, U.S.A.) with 20µl reaction volumes containing 20mM Tris-HCl (pH 8.4), 50mM KCl, 2.0mM $MgCl_2$, 0.2µM primer, 0.1mM of each dNTPs, 0.5U of Taq DNA polymerase (Genei, Bangalore) and 20ng of template DNA. The random primers (TABLE 2) were obtained from Operon Technologies Inc., Almeda, USA. Amplification reactions were carried out by following cycle profiles: initial denaturing cycle at 93°C for 2 min followed by 40 cycles at 93°C for 1min, 35°C for 1min, 72°C for 2 min and a final 7 min extension at 72°C. Products amplified by the PCR reaction were electrophoresed on 1.5% agarose gel[14] in 1x TAE stained in ethidium bromide and the gel image was recorded using the gel documentation system (Syngene, U.K.).

TABLE 2 : Random primers used in the study and details of marker amplification

Sl. No.	Primer	Primer Sequence 5' - - - - - - - - - - 3'	No. of markers	No.of Polymorphic markers	% Of polymorphism
1	OPL-18	-ACCACCCACC-	14	11	78.5
2	OPN-19	-GTCCGTACTG-	15	15	100
3	OPN-20	-GGTGCTCCGT-	12	9	75
4	OPQ-01	-GGGACGATGG-	18	17	94.4
5	OPQ-02	-TCTGTCGGTC-	13	12	92.3
6	OPR-01	-TGCGGGTCCT-	21	20	92.2
7	OPR-03	-ACACAGAGGG-	9	9	100
8	OPR-04	-CCCGTAGCAC-	13	11	84.6
9	OPR-05	-GACCTAGTGG-	7	7	100
10	OPR-06	-GTCTACGGCA-	12	11	91.6
11	OPR-07	-ACTGGCCTGA-	13	11	84.6
12	OPR-08	-CCCGTTGCCT-	18	18	100
13	OPR-09	-TGAGCACGAG-	11	9	81.8
14	OPR-10	CCATTCCCCA-	22	22	100
15	OPR-11	-GTAGCCGTCT-	15	12	80
16	OPR-12	-ACAGGTGCGT-	18	17	94.9
17	OPR-13	-GGACGACAAG-	15	13	86.6
18	OPR-14	-CAGGATTCCC-	22	20	90.9
19	OPR-15	-GGACAACGAG-	22	21	95.4
20	OPR-16	-CTCTGCGCGT-	10	8	80
21	OPR-17	-CCGTACGTAG-	15	15	100
22	OPS-01	-CTACTGCGCT-	6	5	83.3
23	OPS-04	-CACCCCCTTG-	11	10	90.9
		Total	**332**	**294**	**88.55**

Data analysis

Well resolved and consistently reproducible DNA bands in the range of 350-4300 bp generated by RAPD were selected and scored as "1" for presence of marker and "0" for the absence. Bands with the same migra-

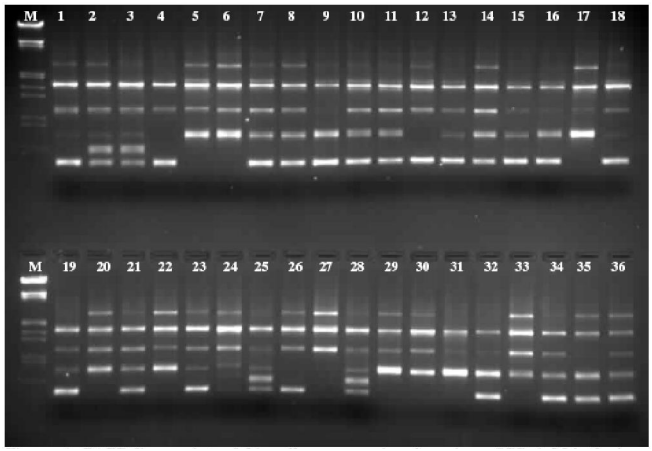

Figure 1 : RAPD fingerprints of 36 mulberry accessions by primer OPR-6. M is the λ *EcoR*1+*Hind* III double digest molecular weight marker. Lane 1 to 36 corresponds to the mulberry germplasm accessions in the TABLE 1

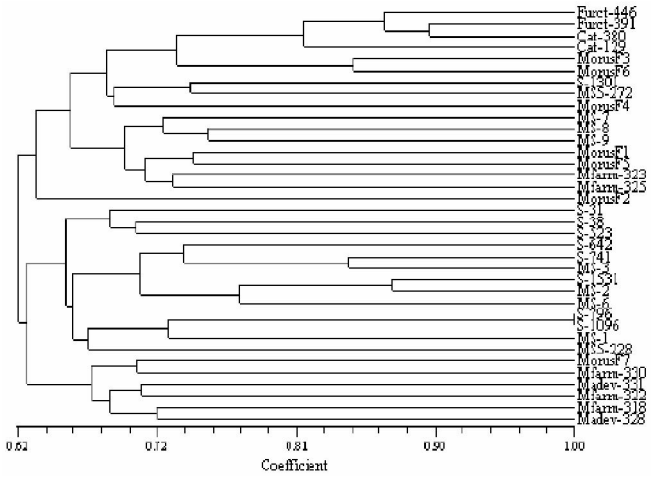

Figure 2 : Genetic interrelationship among mulberry germplasm collections by UPGMA clustering based on the RAPD marker similarity coefficient values

tion distance were considered homologous. A pair wise similarity matrix was computed and analyzed with NTSys[15] Version 2.0. Dice similarity matrix was generated using the equation, s=2Nxy/(Nx +Ny), where Nxy is the number of shared markers between 'x' and 'y' entries. Nx is the total number of markers in 'x' entry and Ny is the total number of markers in 'y' entry[16]. Genetic distance (1-s) was calculated and a dendrogram was constructed based on the similarity matrix data set by applying un-weighted pair group method of arithmetic averages (UPGMA). The similarity values were used to construct tree plot using NTSYS 2.02i statistical software. RAPD markers were identified by the source of primers (OP for Operon), kit letter, the primer number and its approximate size in base pairs.

RESULTS

Selection and grouping of germplasm

The germplasm collections utilized in the study originated from different locations. The germplasm were initially identified from a larger collection based on the similarity in names or origin or pedigree. Majority of the collections were indigenous and have originated from the state of Uttarakand. The S series germplasm have been evolved by selection at RSRS, Dehradun except S-30, which is evolved at CSRTI, Mysore. The MS series was represented by nine germplasm collection evolved by RSRS, Majra. *Morus* F1 to F7 were the mulberry germplasm collections obtained from France. In addition, two collections by same name, Furcata with two different accession numbers (Acc. No. 391 and 446) were introduced from France. The study also selected two collections from Italy by the same name, i.e., Cattaneo (*M. alba*). In addition, there were seven indigenous collections by name, Mahadeva Farm/ Mahadeva with different accession numbers.

Genetic polymorphism assay with RAPD

In the present study, totally 23 informative RAPD primers identified from the screening experiments (unpublished data) were used for DNA marker amplification. Among the primers, as many as 6 primers showed 100% polymorphism with overall mean polymorphism of 88.55%. A representative DNA fingerprints of the 36 germplasm produced by the random primer OPR-06 is shown in Figure 1. A total of 332 markers were amplified and detected on the agarose gel of which 294 were polymorphic. The size of the amplified markers ranged from 350 to 4300bp. TABLE 2 provides the details of primer-wise DNA marker amplification. Primers, OPR-10, 14 and 15 amplified maximum number (22) markers and the least number (6) of markers were observed in the DNA fingerprints generated by OPS-01. Dice similarity coefficient (TABLE 3) was calculated using the semiqual module in the NTSYS pc program and a matrix was generated using DNA marker scores. Based on the similarity coefficient, the maximum (1.000) and the perfect match were seen between germplasm S-796 and S-1096. But, least similarity (0.624) was recorded between the collection *Morus* F3 and Mahadeva.

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TABLE 3 : DNA maker based Dice similarity coefficient matrix of 36 mulberry germplasm accessions

	cc1	cc2	cc3	cc4	cc5	cc6	cc7	cc8	cc9	cc10	cc11	cc12	cc13	cc14	cc15	cc16	cc17	cc18	cc19	cc20	cc21	cc22	cc23	cc24	cc25	cc26	cc27	cc28	cc29	cc30	cc31	cc32	cc33	cc34	cc35	cc36
Furct-446	1.000																																			
Furct-391	0.882	1.000																																		
Cat-380	0.856	0.900	1.000																																	
Cat-129	0.813	0.819	0.812	1.000																																
S-31	0.660	0.664	0.626	0.664	1.000																															
S-38	0.647	0.639	0.627	0.625	0.691	1.000																														
S-523	0.667	0.658	0.647	0.639	0.678	0.703	1.000																													
S-642	0.671	0.693	0.658	0.706	0.650	0.706	0.729	1.000																												
S-741	0.649	0.660	0.617	0.660	0.640	0.616	0.685	0.743	1.000																											
S-796	0.629	0.615	0.616	0.621	0.632	0.665	0.622	0.698	0.661	1.000																										
S-1096	0.627	0.613	0.614	0.619	0.630	0.663	0.620	0.696	0.659	1.000	1.000																									
S-1301	0.709	0.712	0.695	0.673	0.613	0.628	0.629	0.689	0.669	0.698	0.696	1.000																								
S-1531	0.645	0.637	0.613	0.662	0.642	0.599	0.681	0.733	0.696	0.675	0.673	0.684	1.000																							
MS-1	0.641	0.639	0.615	0.594	0.632	0.715	0.689	0.711	0.617	0.726	0.723	0.698	0.644	1.000																						
MS-2	0.660	0.658	0.634	0.678	0.644	0.606	0.754	0.762	0.718	0.665	0.663	0.680	0.875	0.677	1.000																					
MS-3	0.630	0.673	0.610	0.641	0.620	0.628	0.698	0.726	0.845	0.667	0.665	0.656	0.715	0.660	0.764	1.000																				
MS5-228	0.608	0.614	0.622	0.579	0.618	0.667	0.647	0.684	0.689	0.667	0.665	0.662	0.645	0.680	0.640	0.735	1.000																			
MS5-272	0.701	0.704	0.720	0.686	0.667	0.590	0.660	0.677	0.644	0.697	0.695	0.739	0.734	0.629	0.737	0.688	0.695	1.000																		
MS-6	0.648	0.646	0.634	0.639	0.688	0.612	0.667	0.664	0.642	0.601	0.605	0.634	0.780	0.599	0.764	0.655	0.619	0.662	1.000																	
MS-7	0.645	0.636	0.638	0.606	0.590	0.636	0.545	0.642	0.604	0.575	0.573	0.624	0.585	0.622	0.606	0.610	0.667	0.554	0.667	1.000																
MS-8	0.655	0.639	0.648	0.639	0.602	0.646	0.599	0.612	0.615	0.614	0.612	0.676	0.644	0.640	0.660	0.621	0.655	0.649	0.699	0.705	1.000															
MS-9	0.630	0.642	0.630	0.608	0.655	0.655	0.583	0.615	0.605	0.604	0.602	0.637	0.653	0.623	0.642	0.617	0.657	0.638	0.657	0.737	0.751	1.000														
MorusF1	0.682	0.699	0.675	0.660	0.619	0.621	0.635	0.677	0.662	0.579	0.577	0.623	0.645	0.635	0.647	0.643	0.601	0.611	0.662	0.696	0.739	0.705	1.000													
MorusF2	0.669	0.647	0.669	0.627	0.599	0.576	0.578	0.603	0.561	0.561	0.559	0.643	0.562	0.559	0.569	0.559	0.555	0.605	0.566	0.616	0.607	0.658	0.630	1.000												
MorusF3	0.709	0.740	0.772	0.650	0.578	0.554	0.597	0.597	0.619	0.538	0.536	0.632	0.573	0.556	0.587	0.577	0.595	0.619	0.607	0.703	0.652	0.684	0.730	0.646	1.000											
MorusF4	0.664	0.676	0.686	0.634	0.640	0.578	0.635	0.614	0.637	0.575	0.573	0.671	0.660	0.608	0.711	0.671	0.621	0.705	0.687	0.646	0.687	0.674	0.735	0.643	0.722	1.000										
MorusF5	0.685	0.655	0.651	0.648	0.619	0.635	0.649	0.628	0.624	0.570	0.569	0.616	0.626	0.629	0.655	0.644	0.615	0.631	0.650	0.677	0.693	0.659	0.740	0.610	0.684	0.674	1.000									
MorusF6	0.726	0.762	0.786	0.689	0.592	0.589	0.597	0.648	0.632	0.585	0.583	0.671	0.581	0.577	0.608	0.618	0.616	0.658	0.621	0.706	0.693	0.667	0.732	0.664	0.849	0.725	0.723	1.000								
MorusF7	0.568	0.597	0.576	0.574	0.621	0.644	0.624	0.630	0.633	0.618	0.616	0.577	0.588	0.658	0.623	0.639	0.667	0.579	0.615	0.649	0.615	0.633	0.653	0.570	0.590	0.654	0.640	0.612	1.000							
Mfarm-330	0.617	0.630	0.625	0.636	0.628	0.643	0.598	0.679	0.626	0.669	0.667	0.658	0.595	0.630	0.603	0.632	0.671	0.626	0.636	0.691	0.657	0.674	0.664	0.632	0.655	0.667	0.701	0.694	0.704	1.000						
Madev-331	0.582	0.548	0.561	0.566	0.592	0.637	0.603	0.643	0.639	0.618	0.616	0.618	0.601	0.651	0.636	0.612	0.645	0.605	0.637	0.649	0.637	0.669	0.589	0.528	0.582	0.594	0.640	0.597	0.686	0.704	1.000					
Mfarm-322	0.608	0.595	0.602	0.593	0.591	0.601	0.602	0.634	0.631	0.662	0.660	0.630	0.673	0.682	0.662	0.631	0.594	0.624	0.701	0.616	0.664	0.616	0.644	0.534	0.610	0.644	0.623	0.624	0.676	0.660	0.706	1.000				
Mfarm-323	0.651	0.643	0.664	0.623	0.587	0.590	0.566	0.611	0.671	0.587	0.585	0.632	0.589	0.605	0.596	0.625	0.637	0.632	0.601	0.705	0.692	0.680	0.711	0.632	0.662	0.667	0.715	0.687	0.648	0.673	0.627	0.611	1.000			
Mfarm-318	0.606	0.599	0.593	0.591	0.610	0.578	0.580	0.671	0.656	0.634	0.632	0.594	0.664	0.613	0.667	0.649	0.613	0.622	0.669	0.621	0.655	0.664	0.641	0.546	0.557	0.664	0.621	0.580	0.652	0.657	0.667	0.708	0.671	1.000		
Madev-328	0.563	0.550	0.564	0.568	0.565	0.617	0.585	0.617	0.627	0.599	0.597	0.558	0.622	0.631	0.610	0.640	0.618	0.607	0.616	0.619	0.623	0.655	0.612	0.524	0.569	0.610	0.647	0.585	0.657	0.690	0.701	0.662	0.648	0.717	1.000	
Mfarm-325	0.688	0.664	0.667	0.643	0.590	0.608	0.602	0.615	0.639	0.617	0.615	0.652	0.634	0.630	0.621	0.625	0.637	0.611	0.659	0.719	0.742	0.632	0.695	0.596	0.672	0.692	0.714	0.713	0.679	0.691	0.672	0.684	0.727	0.697	0.694	1.000

Clustering based on RAPD marker similarity

Clustering based RAPD marker similarity using UPGMA analysis revealed 10 distinct groups. Among different clusters (Figure 2), Cluster I was the largest with 9 collections followed by the cluster II (7 collections), Cluster V (6 collections) etc. The remaining clusters were smaller in size with number of collection ranging from 1 to 3.

DISCUSSION

Accurate genetic characterization of the germplasm collection is very important for utilization in crop improvement program and conservation in the gene bank. Morphological markers have been routinely used for

this purpose. However in recent times, due to rapid advancement in DNA marker technology, researches are opting for the later for characterization and assessment of genetic diversity of crop germplasm due to accuracy, reliability and speed. Introduction of germplasm resource to a gene bank is a complex process involving many scientific and technical personnel over a period of time[17]. The germplasm accumulated by different groups of researchers during different times looses rationality. Limitations in the methodology involved in the introduction of germplasm in the form of clonal materials as is the case of mulberry often leads to genetic redundancy in the gene bank[6]. In the present study, a group of 36 mulberry germplasm having similar origin and names were identified and short-listed for assessment of genetic diversity with a focus on the assessment of genetic redundancy in these germplasm.

Genetic polymorphism observed in the current study is almost in concurrence with earlier studies undertaken in other mulberry germplasm accessions[18-20]. However, the polymorphism detected in this study was slightly higher possibly due to better representation of germplasm from different sources. The clustering procedure we used resulted in grouping of 36 collections into 10 clusters. The largest of all was the clusters I with 25% entries of the total collection. Two collections from France with identical name (Furcata) were suspected to be redundant. However, the molecular marker analysis suggested a close relationship but, can not be treated as redundant (s=0.882) and hence to be retained as unique accessions in the gene bank. Similarly, the two Italian collections with identical name Cattaneo were also not duplicates but with a similarity value of 0.812. The seven collections with Mahdeva Farm/Mahadeva collected from same place in the state Uttar Pradesh are quite dissimilar and grouped in as many as four divergent clusters based on UPGMA analysis. The result justifies the inclusion of these collections with separate accession numbers in the germplasm bank. The genetic analysis of collections *Morus* F1 to F7 from France based on the DNA marker show uniqueness and clustering analysis categories them in 4 distinct groups. Similar results were also obtained in case of MS series of germplasm collection. Even the collection with identical names (i. e., MS-5) showed complete divergence suggesting the error might have crept due to mislabeling the two collections in any one of the stages of introduction to the germplam bank. The S series of collections includes as many as eight accessions which have originated from the RSRS, Dehradun. The variety S-30 was developed by selection at Central Sericultural Research and Training Institute, Mysore. Among the S-series of Dehradun valley, S-796 and S-1096 were identified as duplicates based on the DNA fingerprinting analysis. The two collections showed 100% similarity among the markers amplified. Naik and Dandin (2006) while suggesting a strategy for identification of duplicates in the mulberry field gene bank statistically derived a minimum of 66 polymorphic markers or 9 informative primers as the criterion for comparison. Similarly[21], successfully used RAPD analysis for detection of genetic redundancy in the rice germplasm collection at International Rice Research Institute, Manila. Probably error during the selection program or at the time of introduction to the gene bank might have resulted in the genetic duplication in the case of S-796 and S-1096. Lack of clear-cut clustering based on the geographic origin suggests the possibility of genetic mixing primarily due to out breeding behavior and utilization of diverse gene source during the development of improved lines. Similar conclusion was drawn by[19] while analyzing important and promising mulberry varieties of India using RAPD and ISSR analysis.

The study reports the case of duplication in the germplasm (S-799 and S-1099) and issue needs to be addressed by the germplasm managers/curators. Even though, passport information suggested a possibility of higher levels of genetic redundancy of the germplasm collection, the molecular marker analysis proved the uniqueness of these collections except in one case. However, there is need to minimize the number of germplasm which can effectively represent the entire variability present in the group. In other words, the molecular analysis can be used for development of core set of germplasm. The core subset will be rationalized collection which can be used by the mulberry breeders for in-depth evaluation for novel genes and traits. A smaller collections having the all the alleles will be more attractive to breeders in crop improvement programs. The present study is a part of the larger program of developing core collection of mulberry germplasm for effective utilization in breeding. The downsized collec-

tion of core can be maintained by all the breeders involved in mulberry genetic improvement in different research institute as working germplasm at a reduced cost and higher efficiency.

CONCLUSION

Characterization of groups of mulberry germplasm with identical names and similar origin or descent using DNA markers resulted in establishment of genetic uniqueness of all the accessions except one pair. The germplasm S-796 and S-1096 were confirmed redundant. Clustering based on marker similarity coefficient suggest a closer relationship among few of the accessions calling for the development of a core subset containing the entire variability of the whole collection. The core subset of mulberry germplasm can be easily subjected intensive evaluation and their utilization can be enhanced in crop improvement programs.

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