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Development of Core Collection for Perennial Mulberry (*Morus* spp.) Germplasm

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ABSTRACT

Improvement of mulberry (crop) depends on the availability of suitable parents and breeders' thorough knowledge on the database of mulberry germplasm. To develop suitable varieties which are tolerant to stress condition in different agro climatic regions and resistant to pest and diseases, broad spectrum genetic variability is a pre-requisite. In this study, a total of 628 mulberry accessions were collected from 23 different countries and classified into 5 geographical groups, based on the qualitative and quantitative database. The grouping represented tropical wet (237), tropical dry (121), sub-tropical humid (215), semi-arid (10), arid (04), highland (25) and others (16). A core collection was made to compare the data, following the combined cluster analysis. The K-clustering procedure was followed to classify the mulberry accessions and 10 clusters were formed. The results indicated that cluster VIII had more number of accessions (125), whereas cluster II was minimum (27). In other clusters, mulberry accessions were distributed randomly irrespective of geographical origin and genetic diversity. Based on the geographical origin, genetic diversity and avoiding repetitiveness, a core collection was developed for the utilization of mulberry crop improvement programme in the future.

Keywords: Mulberry germplasm, core collection, geographical distribution, statistical analysis

INTRODUCTION

The germplasm collections are many and large that they diffuse and discourage effective evaluation of the accessions and thus hinder their utilization. As a solution to the problem, Frankel and Brown (1984) suggested that a core collection could be developed from the available germplasm. The core collection is the representative with the minimum repetitiveness of genetic diversity of a crop species and its relatives. The accessions, which are not included in the core, are retained as a reserve sub set for future need and searching for rare alleles (Basigalup *et al.*, 1995). The first core collection was developed from the Australian collection of perennial *Glycine* spp. Subsequently, the core collection was also developed in other crops (Holbrook *et al.*, 1993). Prior to develop a core collection, decision regarding the size of the core, sampling procedure, maintenance and improvement of the designated core has to be made (Brown, 1989). It was suggested that the number of entries in the core should be about 10 % of the total collection.

CSGRC, Hosur is maintaining 1100 mulberry accessions at present, which is large and hinders a thorough evaluation of its potential for improving important traits. The objective of this research was to evaluate and characterize the accession to designate a possible core collection for the evaluation and breeding programmes in the future.

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MATERIALS AND METHODS

The study was carried out at the Central Sericultural Germplasm Resources Centre, Hosur, in Tamil Nadu. It is situated around 12.45° N and 77.15° E, with an altitude of 942m above the mean sea level, and a dry tropical climatic condition. The average rainfall ranges from 1000 mm per anum. The mulberry plantations, at the field gene bank, are maintained as dwarf trees, spacing 2.4 x 2.4 m with two prunings/ year. The recommended agronomical package of practices was followed to maintain the plantation (Tikader *et al.*, 1999, 2000). The experiment was set up in an augmented block design to accommodate more number of mulberry plants. Each accession was represented by 4 plants and the materials used for this study represented 23 countries accessions including India (*see* Table 1). The data on growth behaviour and related growth traits were recorded after 90 days of plant pruning. The intercultural operation and pruning were conducted after the plants were attained at two years of age. The leaf moisture and the retention capacity of the leaf moisture of the harvested leaf were calculated as per standard procedure (Vijayan *et al.*, 1997).

The observation carried out on the important growth traits was recorded at the field gene bank for two seasons per year, over a period of three years. The data was computed and the multivariate statistical methods i.e., the analysis of variance, correlation, combined cluster analysis based on correlation, principal component analysis within each geographical group with random selection of entities in each cluster, were applied. A stratified random sampling procedure was used to divide the collection into a number of non-overlapping strata. The mulberry accessions were grouped based on the requirement such as the geographical origin, sexual behaviour, as well as qualitative and quantitative traits. As such, 455 indigenous and 173 exotic accessions were grouped based on origin in different zones (Johnson, 1978). The accessions were placed into different zones, i.e., tropical wet, tropical dry, sub-tropical humid, semi-arid, arid, high land and others. The multivariate analysis was done to group the mulberry accessions (628) using the Statistical Package for Social Science (SPSS).

RESULTS AND DISCUSSION

The mulberry accessions, representing different geographic regions, are presented in Table 1. The geographical distribution indicates that the maximum accession fall in the Tropical wet (237 acc.), followed by the sub-tropical humid (215 acc.), tropical dry (121 acc.), high land (25 acc.), and others (16 acc.); while the minimum in the semi-arid zone (10 acc.). The analysis of variance, indicating a high degree of variability and the relationship of different growth traits, were established through the correlation coefficient analysis (Table 2). The leaf yield of mulberry is a complex trait and it depends on several factors. The correlation matrix indicated that the leaf yield was highly associated with a number of primary branches (0.60**), total shoot length (0.74**), length of the longest shoot (0.64**), 100-leaf weight (0.22**), total biomass yield (0.99**), leaf moisture content (0.25**) and leaf moisture retention capacity (0.25**). Nevertheless, the leaf yield showed a negative significant association with the leaf shoot ratio (-0.29**) and laminar index (-0.18**). Other traits were also found to associate with each other and showed a complex relationship. Similar results had also been reported earlier by several authors (Tikader *et al.*, 1999; Vijayan *et al.*, 1997).

There are different ways which can be used to classify and group the mulberry accessions. For this, different authors have worked on mulberry and grouped the accessions in different ways, according to the clustering packages (Tikader *et al.*, 1999; Tikader and Rao, 2001a;

TABLE 1
Geographic distribution of mulberry accessions (628 accessions) of different countries

Zones	States/ Territory of India	Accessions	Country	Accessions
	Andaman & Nicobar Islands	02	Bangladesh	05
	Assam	10	Burma (Myanmar)	06
	Kerala	48	France	15
	West Bengal	125	Indonesia	05
Tropical wet			Italy	08
			Papua New Guinea	01
			Philippines	01
			Portugal	01
			Spain	02
			Thailand	04
			Venezuela	01
			Vietnam	03
Total		**185**		**52**
	Gujarat	01	Pakistan	07
	Karnataka	68	Zimbabwe	09
Tropical dry	Maharastra	02		
	Madhya Pradesh	12		
	Tamil Nadu	22		
Total		**105**		**16**
	Arunachal Pradesh	05	Australia	02
	Manipur	12	China	11
	Meghalaya	21	Cyprus	01
Sub-tropical humid	Nagaland	01	Hungary	01
	Uttar Pradesh	91	Japan	59
			Paraguay	04
			Russia	01
			South Korea	06
Total		**130**		**85**
	Punjab	05		
Semi-arid	Rajasthan	05		
Total		10		(–)
	Himachal Pradesh	02		
High land	Jammu & Kashmir	22		
	Sikkim	01		
Total		**25**		**(–)**
Others				16
Grand total		455		173

TABLE 2
Correlation coefficient matrix for different growth parameters in mulberry germplasm

Trait	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12
X1	—											
X2	0.93**	—										
X3	-0.19**	-0.08	—									
X4	-0.14**	-0.08	0.47**	—								
X5	-0.35**	-0.23**	0.54**	0.48**	—							
X6	0.55**	0.71**	0.06	-0.04	-0.03	—						
X7	-0.01**	0.07	0.13**	-0.13**	0.32**	0.36**	—					
X8	-0.36**	-0.25**	0.51**	0.45**	0.94**	-0.01	0.37**	—				
X9	-0.43**	-0.44**	0.19**	0.12**	0.16**	-0.51**	-0.11**	0.17**	—			
X10	0.60**	0.74**	0.25**	0.25**	0.22**	0.64**	0.08*	0.18**	-0.29**	—		
X11	-0.15**	-0.13**	-0.08	-0.13**	0.01	-0.07	0.18**	0.16**	0.05	-0.18**	—	
X12	0.64**	0.78**	0.22**	0.23**	0.18**	0.67**	0.07	0.15**	-0.35**	0.99**	-0.17**	—

X1 = No. of the primary branches, X2 = Total shoot length, X3 = Leaf moisture retention capacity, X4 =Leaf moisture content , X5 = 100 leaf weight, X6 =Length of the longest shoot X7 = Internodal distance, X8 = Lamina weight, X9 = Leaf shoot ratio, X10 = Leaf yield, X11 = Laminar index, X12 = Total biomass weight

Tikader and Roy, 2001b, 2002). The accessions were grouped using the K clustering method in the SPSS package (version 11). The accessions were distributed into ten clusters, with the representative of different regions (Table 3). The cluster-wise distribution of mulberry indicated that the maximum accessions were grouped in cluster VIII (125 acc.), followed by VII (89 acc.), VI (76 acc.), III (70 acc.); I (62 acc.), IX (54 acc.), X (35 acc.) and IV (29 acc.). The minimum accessions were grouped in cluster II (27). The mulberry accessions, which were grouped with each other irrespective of their geographical distribution and genetic diversity. The distance between the final cluster centres are shown in Table 4. The distances between the final cluster centres indicated the maximum inter cluster distance between clusters IV and X (8.81), suggesting that the accessions grouped in these clusters are genetically divergent. The minimum inter-cluster distance was observed between cluster III and IV (1.97), indicating the accessions which were grouped in these cluster are genetically similar. The intra-cluster distances showed no variation between them. The different cluster groups provided a scope for the selection of accessions for further mulberry crop improvement programme (Tikader and Roy, 2002). During cluster analysis. the variability was also partitioned and presented character wise (Table 5). However, the F - test should be used only for descriptive purposes because the clusters were chosen to maximize the differences between the cases in the different clusters. The observed significance levels were not corrected for this, and could not be interpreted as the test of hypothesis that the cluster means were equal.

Two methods were found suitable to be used for designating the core collection; these are (a) combined cluster analysis based on correlation, PCA within each geographical group with a random selection of entries with each cluster, and (b) direct selection from the entries within each geographical group. Nevertheless, to run the multivariate statistical package, complete set of data is required. On the other hand, the principal component and cluster analysis can be performed according to a complete set of data and these are excellent tools to be used in grouping the accessions by degree of similarity (Brown, 1989; Smith *et al.*, 1995: Peeters and Martinelli, 1989).

TABLE 3
Region-wise distribution of mulberry accessions in different clusters

Region	Mulberry accessions in each cluster										
	I	II	III	IV	V	VI	VII	VIII	IX	X	Total
Tropical dry	18	8	14	4	13	8	11	38	4	3	121
Tropical wet	23	8	26	17	29	34	22	52	24	2	237
Sub-tropical (Humid)	14	8	22	7	13	33	48	24	23	22	214
Semi-arid	–	–	–	–	–	–	2	7	1	–	10
Arid	–	1	–	–	1	–	–	–	–	2	4
High land	5	–	7	–	1	1	5	1	1	4	25
Unknown	2	2	1	1	4	–	1	3	1	2	17
Total	62	27	70	29	61	76	89	125	54	35	628

TABLE 4
Distances between the final cluster centres

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X
I	00	3.74	3.09	5.94	3.05	3.79	2.49	3.49	2.59	4.50
II		00	6.41	6.95	4.64	6.94	5.18	6.24	5.22	6.34
III			00	7.13	4.60	1.97	2.54	3.60	3.54	4.49
IV				00	3.37	6.17	6.60	3.78	5.16	8.81
V					00	4.33	3.46	2.53	2.35	5.94
VI						00	3.52	2.59	3.51	5.94
VII							00	3.79	2.54	3.72
VIII								00	2.90	6.39
IX									00	5.12
X										00

TABLE 5
Variability partitioned during cluster analysis

	Cluster		Error		F – value
Characters	Mean square	df	Mean square	df	
Z score (NBR)	48.745	9	0.305	618	159.988
Z score (TSL)	54.320	9	0.223	618	243.044
Z score (MRC)	38.073	9	0.460	618	82.747
Z score (MC)	31.352	9	0.558	618	56.188
Z score (HUNLF)	48.013	9	0.315	618	152.254
Z score (LLS)	40.761	9	0.421	618	96.830
Z score (INTNOD)	26.487	9	0.629	618	42.12
Z score (WTLAM)	50.309	9	0.282	618	178.456
Z score (LSR)	45.133	9	0.357	618	126.324
Z score (YIELD)	52.637	9	0.248	618	212.249
Z score (TBIO)	54.382	9	0.223	618	244.306
Z score (LAMINDEX)	24.207	9	0.662	618	36.566

NBR = No of primary branches, TSL = Total shoot length, MRC = Leaf moisture retention, MC =Leaf moisture content, HUNLF = 100 leaf weight, LLS = length of the longest shoot, INTNOD = Internodal distance, WTLAM = Lamina weight, LSR = Leaf shoot ratio, YIELD = Leaf yield, TBIO = Total biomass weight, LAMINDEX = Laminar index.

Based on its geographical origin and variability, a core collection was developed. This collection consists of 135 accessions which represent different countries, geographical regions, variability and avoid repetitiveness for further mulberry improvement programme (Table 6). The core collection was also developed for the perennial *Medicago* plant introductions, following a similar procedure (Basigulap *et al.*, 1995; Diwan *et al.*, 1994).

TABLE 6
List of the core collection of mulberry based on its geographical origin and variability for 12 traits (135 accessions)

Sl.	Accessions	Country	Sl. no	Accessions	Country	Sl. no	Accessions	Country
1	ME-0132	Zimbabwe	46	MI-0275	India	91	MI-0121	India
2	MI-0018	India	47	MI-0322	India	92	MI-0160	India
3	MI-0107	India	48	ME-0053	Japan	93	MI-0287	India
4	MI-0141	India	49	ME-0147	Italy	94	MI-0291	India
5	ME-0003	Burma	50	MI-0366	India	95	MI-0305	India
6	ME-0006	Indonesia	51	ME-0157	Unknown	96	MI-0319	India
7	MI-0090	India	52	MI-0042	India	97	ME-0007	Bangladesh
8	MI-0188	India	53	MI-0155	India	98	ME-0033	Thailand
9	MI-0199	India	54	MI-0056	India	99	ME-0084	Bangladesh
10	ME-0018	Indonesia	55	MI-0091	India	100	MI-0024	India
11	MI-0142	India	56	MI-0112	India	101	MI-0039	India
12	MI-0345	India	57	MI-0163	India	102	MI-0074	India
13	MI-0156	India	58	MI-0187	India	103	MI-0102	India
14	MI-0299	India	59	MI-0193	India	104	MI-0220	India
15	MI-0330	India	60	MI-0203	India	105	MI-0267	India
16	ME-0144	France	61	MI-0106	India	106	MI-0274	India
17	MI-0364	India	62	MI-0113	India	107	MI-0312	India
18	MI-0427	India	63	MI-0122	India	108	MI-0344	India
19	ME-0030	Pakistan	64	MI-0128	India	109	MI-0398	India
20	MI-0057	India	65	MI-0137	India	110	MI-0412	India
21	MI-0450	India	66	MI-0151	India	111	ME-0027	Japan
22	ME-0055	Cyprus	67	MI-0066	India	112	MI-0004	India
23	MI-0048	India	68	MI-0400	India	113	MI-0145	India
24	MI-0103	India	69	ME-0082	France	114	MI-0154	India
25	MI-0147	India	70	MI-0209	India	115	MI-0349	India
26	MI-0030	India	71	MI-0236	India	116	MI-0370	India
27	MI-0053	India	72	MI-0449	India	117	MI-0017	India
28	ME-0020	Burma	73	ME-0040	Japan	118	MI-0319	India
29	MI-0060	India	73	ME-0049	Japan	119	ME-0129	Zimbabwe
30	MI-0087	India	75	ME-0054	China	120	ME-0052	Papua New Guinea
31	MI-0183	India	76	ME-0068	Japan	121	MI-0045	India
32	MI-0234	India	77	ME-0114	Japan	122	MI-0150	India

Cont. Table 6

33	MI-0301	India	78	ME-0139	South Korea	123	MI-0244	India
34	ME-0065	Burma	79	ME-0154	South Korea	124	MI-0333	India
35	MI-0214	India	80	MI-0373	India	125	ME-0057	Paraguay
36	ME-0156	China	81	MI-0431	India	126	MI-0034	India
37	MI-0254	India	82	MI-0439	India	127	MI-0139	India
38	MI-0310	India	83	MI-0206	India	128	MI-0437	India
39	ME-0155	Unknown	84	MI-0222	India	129	MI-0438	India
40	MI-0292	India	85	ME-0012	Pakistan	130	ME-0166	France
41	MI-0298	India	86	MI-0012	India	131	ME-0047	Japan
42	MI-0308	India	87	MI-0014	India	132	ME-0061	Russia
43	MI-0266	India	88	MI-0016	India	133	ME-0078	Japan
44	MI-0245	India	89	MI-0031	India	134	ME-0094	Japan
45	MI-0264	India	90	MI-0036	India	135	ME-0153	France

CONCLUSIONS

Any statistical package requires a complete database for a proper analysis and interpretation to be done. In other words, the number of accession is to be reduced to designate a core collection. Moreover, a limited number of accessions will not represent the geographical zones. A collection of 15 – 20 % of most variable, representing different geographical regions and core group, can be made and used for further evaluation and breeding purposes. The new collection/ introductions are dependent on variability, which can be added to the present core collections from time to time, considering 15 - 20% of the samples.

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