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GENETIC DIVERSITY ANALYSIS BASED ON MORPHOLOGICAL CHARACTERS IN MULBERRY (*MORUS* SPP.)

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Abstract

Mulberry genetic resource is increasingly being recognized as one of the basic key component for sustainable silk production under changing climatic condition. In this investigation, analysis of multivariate was done to assess the diversity in 20 mulberry genotypes (includes indigenous and exotic) for leaf yield and its growth attributes. The analysis of variance (ANOVA) showed the presence of significant variation among genotypes for the parameters measured. Wide range and variance among the genotypes indicated the presence of variability for the traits on which selection can be practiced. For cluster analysis classified 20 genotypes into four divergent groups and greater genetic distance was detected among the members of cluster I and II and cluster II and III. The members of these divergent clusters may be combined in future breeding programmes to obtain genotypes with combined leaf yield and more branches per plant. The results showed that the germplasm having a wide genetic diversity thus the genotypes *viz.*, BSRM64, BSRM66, BSRM63, BSRM65, BSRM45 and BSRM56 can serve as a promising donors for improving the leaf productivity of mulberry.

Key words: Cluster analysis, Genetic diversity, Mulberry, Yield traits

Introduction

Mulberry (*Morus* spp.) is a perennial heterozygous plant originated from China, which is the primary center of origin (Vavilov 1926). Leaves of Mulberry plant are the primary and only food of silkworm (*Bombyx mori* L.) belongs to family Moraceae. The plants are cultivated under both tropical and temperate climatic conditions of different regions in Bangladesh. As leaf productivity is one of the principal factors that decide the sustainability and profitability of sericulture, good quality mulberry leaf increases the cocoon productivity and quality of silk (Ashiru 2002, Doss et al. 2012). To develop high yielding with superior cultivars is a major challenge and goals for the breeders. For this purpose, the exotic collection was introduced into Bangladesh from different countries as a result, it is very difficult to know the exact geographical distribution of Bangladesh mulberry varieties and behavior of the plants in respect to expression of various traits. Evaluation of any crop is a continuous manner to conform new varieties suitable for unique zones for commercial utilization. The present scenario of sericulture enterprise needs new varieties appropriate for different agro-climatic situations. Suitable parent material needs to be identified from large number of germplasm accessions for the purpose. Bangladesh Sericultural Research Germplasm is presently maintaining 32 exotic mulberry genotypes that were collected from different countries and are being maintained in germplasm bank under tropical dry climatic condition. Earlier the performance of different

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exotic accessions was highlighted by various authors (Cappellozza et al. 1995 & 1996, Tikader and Roy 1999a, Tikader and Rao 2002a). Moreover, estimates of genetic diversity and relationship between various collections from diverse origin helps in efficient management and utilization of germplasm (Rabbani et al. 1988). Several studies have already been highlighted the variability of mulberry germplasm (Thangavelu et al. 2000, Tikader and Rao 2002a) and association of different agronomical traits was also studied in detail (Vijayan et al.1997, Tikader and Roy 1999a).

Genetic diversity is one of the key elements in tailoring the effective breeding programme in any crop. Success of hybridization followed by selection depends largely on the selection of parents with high genetic variability for different characters. The genetically diverse parents are likely to produce heterotic effects and desirable segregates. Several workers have emphasized the importance of genetic divergence for the selection of desirable parents (Archana et al. 2018). Genetic diversity forms the basis of agriculture and the usefulness of a genetically diverse gene pool in plant breeding cannot be overemphasized (CGR 2005). Moreover, genetic diversity within and among the population is the backbone of conservation of plant genetic resources for both present and future use (Quedraogo 2001). Mulberry breeder's will require as much genetic diversity as possible from which to select and recombine favorable traits through cross breeding (Tikader and Dandin 2007a, Tikader and Dandin 2008 a&b) to develop varieties that are adopted to Bangladeshi environment. Improvement through breeding or clonal selection depends on the extent of magnitude of diversity between the genotypes. The process requires grouping the genotypes into different clusters and select for utilization. Different authors highlighted grouping and selection of accessions from different clusters for crop improvement (Rajan et al.1997, Fotedar and Dandin 1998, Vijayan et al.1999, Tikader et al.1999b & 2003, Tikader and Roy 2001 & 2002). The exotic collection from temperate climate is superior in quality aspects like leaf moisture content, moisture retention and biochemical parameters which can be incorporated in locally adopted varieties through breeding. Such reports are available and produced a good number of varieties in India (Tikader and Kamble 2007). In India four mulberry species are reported and sixty eight recognized species of mulberry in world, practically there is less crossing barrier among the species and within the species (Das and Swami 1965, Dwivedi et al. 1989, Tikader and Dandin 2007 & 2008). Thus the present study was conducted to know the performance of indigenous and exotic mulberry germplasm genotypes on growth and yield traits and group them using divergence analysis for effective utilization in improvement.

Materials and Methods

Twenty mulberry genotypes *viz*. BSRM5, BSRM16, BSRM18, BSRM19, BSRM20, BSRM24, BSRM34, BSRM39, BSRM40, BSRM45, BSRM50, BSRM54, BSRM55, BSRM56, BSRM58, BSRM59, BSRM63, BSRM64, BSRM65 and BSRM66 were collected and maintained in the Germplasm bank of Bangladesh Sericulture Research and Training Institute (BSRTI), Rajshahi, Bangladesh for these study (Table 1).

Data were recorded and evaluated their efficiency during four cropping seasons for final yield trial (FYT) in 2017-2018. The plantation was made with 12 plants in each replication with 90 × 90 cm spacing under randomized block design (RBD) with 3 replications. Recommended cultural practices about 4-crop schedule were followed by standard methods of Bangladesh Sericulture Research and Training Institute (BSRTI) (Quader et al. 1992) and irrigation was provided as and when required.

Table 1. List of mulberry genotypes used for the study

Sl. No.	Genotypes	Cultivar's/local name	Place of origin	Remarks (developed as)
1.	BSRM 5	V-5/BM-1	Bangladesh	Indigenous
2.	BSRM 16	BM-4	Bangladesh	Indigenous
3.	BSRM 18	S-799/BM-2	India	Exotic
4.	BSRM 19	S-1/BM-3	India	Exotic
5.	BSRM 20	S-54	India	Exotic
6.	BSRM 24	BM-5	Bangladesh	Indigenous
7.	BSRM 34	BM-7	Bangladesh	Indigenous
8.	BSRM 39	S-13	India	Exotic
9.	BSRM 40	S-30	India	Exotic
10.	BSRM 45	BM-6	Bangladesh	Indigenous
11.	BSRM 50	*M. multicaulies*	Japan	Exotic
12.	BSRM 54	Diploid F_1	China	Exotic
13.	BSRM 55	Triploid	China	Exotic
14.	BSRM 56	BM-8	Bangladesh	Indigenous
15.	BSRM 58	BM-9	Bangladesh	Indigenous
16.	BSRM 59	OP-146/BM-12	Bangladesh	Indigenous
17.	BSRM 63	CPH-91/BM-10	Bangladesh	Indigenous
18.	BSRM 64	CPH-167/BM-11	Bangladesh	Indigenous
19.	BSRM 65	S-1635	India	Exotic
20.	BSRM 66	THAI-1	Thailand	Exotic

Data from the middle five plants were recorded on various growth and yield attributing traits such as total branch number (TBN), total branch height (TBH) (cm), nodes/meter (N/M), internodal distance (IND) (cm), length of longest shoot (LLS) (cm), leaf length (LL) (cm), leaf width (LW) (cm), petiole length (PL) (cm), apex length (AL) (cm), 10 fresh leaves weight (FLW) (g), leaf petiole ratio (LPR) (cm), total shoot weight (TSW) (g), leaf yield (LY) (g) and moisture content (MC) (%).

For analysis of genetic divergence, the genetic distance between the different pairs of genotypes was calculated, employing the generalized Mahalanobis distance as a measure of genetic dissimilarity among the cultivars (Mahalanobis 1936). To estimate this distance, the averages were computed for each of the variables for each cultivar, and then the residual covariance matrix was established, the data transformation matrix, the variance of transformed variables, the averages of uncorrelated variables, and finally the pivotal

condensation technique for resolving the dispersion matrix (Araújo et al. 2014). From the dissimilarity matrix a cluster analysis was performed using the hierarchical methods of single linkage (nearest neighbor), of Ward, of complete linkage (furthest neighbor), of the median, the average linkage within a cluster and the average linkage between clusters, allowing dendrogram to be produced. To validate clusters, that is, to verify the ability of the dendrogram to reproduce the dissimilarity matrix (Araújo et al. 2014).

For statistical analysis data were analyzed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using a commercially available statistics software package (SPSS® for Windows, V. 22.0, Chicago, USA). Multivariate techniques including K-mean cluster analysis was done by SPSS version 22. The cluster analysis as a nonparametric multivariate method classifies genotypes into categories and data were analyzed to determine Euclidean distance based on paired group method to determine dissimilar groups of the accessions. Pair wise distances between the accessions based on Mahalanobis distances were recorded (Mahalanobis 1936). Ward's minimum variance cluster analysis (Ward 1963) was used to group the tested mulberry germplasm genotypes.

Results and Discussion

Experimental findings based on the data pooled over four seasons. All the seasons selected for evaluation had significant effect on the expression of all the traits. Variance analysis of 14 growth and yield traits indicated that significant variation exists among the genotypes. Significant difference at 1% level was observed among the majority traits like total branch height, nodes/meter, Internodal distance, leaf length, leaf width, petiole length, apex length, 10 fresh leaves weight, leaf petiole ratio and moisture content (Table 2).

Table 2. Analysis of variance (ANOVA) subjected to variation of different parameters of growth and yield parameter in mulberry (*Morus* spp.) on the basis of 20 mulberry genotypes

Yield attributing traits	Source of variation		
	Genotypes (Mean SS)	Error (Mean SS)	F-test
Total branch number	11.854	3.747	3.164***
Total branch height	247831.498	100696.408	2.461^{NS}
Nodes per meter	44.330	12.644	3.506***
Internodal distance	0.819	0.261	3.145***
Length of longest shoot	1636.171	1425.681	1.148^{NS}
Leaf length	38.039	0.355	107.068***
Leaf width	37.401	0.383	97.558***
Petiole length	1.210	0.137	8.813**
Apex length	14.387	0.068	212.810***
10 Fresh leaves weight	285.354	46.867	6.089***
Total shoot weight	36252.211	67624.402	2.222^{NS}
Leaf petiole ratio	2.335	0.163	14.301***
Leaf yield	57585.494	114765.807	2.196^{NS}
Moisture content	6.624	1.305	5.076***

** = significant at p≤0.01, *** = significant at p≤0.001 and NS = Non-significant.

Ward's minimum variance cluster analysis based on Mahalanobi's distance grouped 20 mulberry genotypes into 4 clusters (Table 3 and Fig.1). The grouping pattern showed 7 genotypes in cluster III followed by 5 genotypes each in cluster II and IV, whereas includes 3 genotypes in Cluster I. The cluster means values are presented in Table 4. Cluster I had the highest mean value for total branch number, total branch height, length of longest shoot, petiole length and total shoot weight per plant with shorter node per meter and moisture content. Cluster II showed highest mean values for 10 fresh leaves weight, leaf width, leaf apex length, leaf petiole ratio, leaf yield and moisture content per plant. This cluster included most of the indigenous developed lines and varieties like BSRM64, BSRM66, BSRM63 and BSRM45. Cluster III showed lowest mean values for 10 fresh leaves weight, leaf length, leaf width, petiole length, apex length and leaf petiole ratio. Cluster IV showed highest mean values for nodes per meter with lowest total branch number, total branch height, internodal distance, length of longest shoot, total shoot weight and leaf yield. These genotypes can be utilized to induce more branches and higher leaf weight in breeding populations. The inter cluster distances is presented in Table 5. The maximum inter cluster distance was observed between cluster I and II (14.516) whereas minimum between II and III (4.366) indicates that the accessions grouped in these clusters are genetically divergent and similar respectively. The second most diverse clusters having highest genetic distance was cluster II and III with the distance of 10.555. Members of these divergent clusters can be utilized in transgressive breeding programmes. The genotypes are distributed equally in different clusters. The entire cluster groups have both exotic and indigenous developed genotypes. Clustering analysis based on morphological and leaf yield traits grouped 20 mulberry genotypes into four different clusters (Table 3) and indicates that these genotypes exhibited notable genetic divergence in terms of morphological and yield traits. Therefore, classification in this study based on morphological traits is in agreed with previous report. Previous reports on clustering in mulberry by various authors also indicated that the exotic and indigenous accessions could be grouped in same cluster (Fotedar and Dandin 1988, Rajan et al.1997, Tikader et al. 2003a) and the present study also supports the previous findings. The clustering pattern clearly indicates that genetic diversity and geographical distribution possess no relation and the mulberry genotypes collected from different sources grouped as per their performance based on agronomical traits. In all the clusters other than cluster II, the combination of genotypes joined with each other based on close affinity and genetic value. The breeders have the opportunity to select suitable genotypes for further utilization. Tikader and Kamble (2008) studied the genetic diversity of indigenous and exotic accessions using Mahalanobis D^2- technique for leaf yield and eight agronomic traits. By using Ward's minimum variance cluster analysis they grouped the fifty indigenous and exotic accessions into 9 clusters. The role of exotic genotypes is very essential and the result revealed that a good number of exotic genotypes performed well with indigenous genotypes. The introduced exotic genotypes have been used for development of sericulture and presently the important commercial variety has been developed by using it as one of the parents in hybridizations. Hence, the indigenous × exotic derivatives from cluster I should be crossed with the genotypes of cluster II to increase the number of branches. High yielding genotypes from cluster III could be further tested for their combining ability. Thus the genotypes present in different clusters can be hybridized to assemble desirable traits with higher heterotic potential. Similar types of results are obtained by Suresh et al. (2018).

Table 3. Classification of 20 mulberry genotypes into different clusters on the basis of divergence

Clusters group	No. of genotypes	Genotype name
I	3	BSRM5, BSRM19, BSRM56
II	5	BSRM16, BSRM45, BSRM63, BSRM64, BSRM66
III	7	BSRM18, BSRM34,BSRM39, BSRM40, BSRM55, BSRM58, BSRM65
IV	5	BSRM20, BSRM24, BSRM50, BSRM54, BSRM59

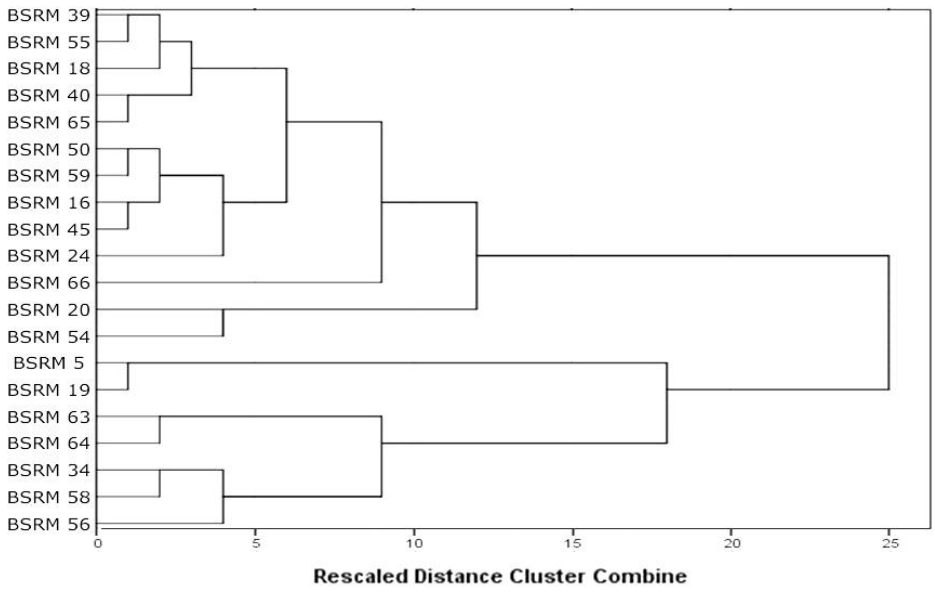

Fig. 1. Dendrogram produced using Ward's minimum variance cluster analysis based on D^2 matrix demonstrating to the association among 20 mulberry genotypes of Bangladesh.

Table 4. Cluster means for different traits in 20 mulberry genotypes

Cluster	No. of genotypes	TBN	TBH	N/M	IND	LLS	FLW	LL	LW	PL	AL	TSW	LPR	LY	MC
I	3	12.93	1757.07	25.09	4.01	159.07	29.54	14.87	11.94	3.72	2.97	693.01	4.01	809.58	74.50
II	5	11.04	1218.08	25.16	4.03	130.25	41.83	14.40	12.57	3.05	3.19	607.75	4.79	919.89	76.74
III	7	11.50	1363.84	26.34	3.94	140.50	29.18	11.07	8.58	2.87	1.19	590.16	3.96	771.80	76.23
IV	5	9.52	1040.90	26.97	3.83	129.09	37.95	14.13	11.47	3.17	2.38	474.67	4.44	720.39	75.82

TBN = Total branch number, TBH = Total branch height, N/M = Nodes per meter, IND = Internodal distance, LLS = Length of longest shoot, FLW = 10 Fresh leaves weight, LL = Leaf length, LW = Leaf width, PL = Petiole length, AL= Apex length, TSW = Total shoot weight, LPR = Leaf petiole ratio, LY = Leaf yield, MC = Moisture content.

Table 5. Mean inter cluster distance (D^2) among 20 mulberry genotypes

	Distances between final cluster centers			
Cluster	1	2	3	4
1	-	14.516	4.366	8.623
2	14.516	-	10.555	6.049
3	4.366	10.555	-	4.510
4	8.623	6.049	4.510	-

The experiment was conducted in partial lattice design with 20 diverse mulberry germplasm genotypes for fourteen growth and yield traits. Highly significant differences of growth and yield traits were observed (Tikader and Kamble 2008c). The combined analysis indicates the superiority of some genotypes over the other tested accessions (Tikader and Kamble 2008c). The relationship of different growth and yield traits were worked out and found association with leaf yield. Several authors reported the similar type's findings (Vijayan et al. 1997, Tikader and Roy 1999, Tikader and Dandin 2005 & 2008a). The highly correlated traits that is number of branches per plant and total shoot length should considered during selection. The cluster analysis grouped the genotypes into four clusters irrespective of geographic origin. The breeders have the opportunity to select a suitable group of germplasm for further utilization. The genetic diversity is being assessed by single trait leaf yield which is the end product used by farmers for silkworm rearing to produce cocoon. This confirms the importance of understanding how individual trait or farmers use group of traits to identify different genotypes. Morphological identity along with growth and yield traits has the direct relevance to the farmers as well as plant breeders to select, utilize and conservation of germplasm (Tikader and Kamble 2008c). It is important to note that growth and yield traits have a number of limitations to express in different environmental condition and influence the performance either positive or negative (Tikader and Kamble 2008d). Breeders also used as one of the parent for breeding and developed improved varieties. Thus the exotic mulberry genotype, which has performed well, may be suitable for further utilization and conservation of genetic resources.

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