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HIERARCHICAL CLUSTER ANALYSIS IN EXOTIC INTRODUCTIONS OF MULBERRY

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The natural variability of germplasm samples is often very high and their crossing offers a simple way to recover large number of new combinations. Fifty-six exotic entries of different geographic origin varying in their yield potential were subjected to hierarchical cluster analysis to classify the variability. Twenty one agronomic traits viz., leaf yield, stomatal frequency, stomatal size, cuticle thickness, total leaf thickness, palisade to spongy parenchyma ratio, length of the root, dry weight of the root, root to shoot ratio by length and by weight, moisture retention capacity, moisture percentage, number of primary branches, length of the shoot, inter-nodal distance, number of leaves per meter length of the shoot, leaf area, weight of 100 leaves, percentage of sprouting and rooting and total chlorophyll content were measured. Studies revealed that 56 mulberry entries fell in five clusters. Considerable genetic diversity was observed among the entries studied. On the basis of inter and intra-cluster distances, parents could be selected for breeding purpose.

Key words : Mulberry, agronomic traits, hierarchical clustering, genetic divergence

Hanson (1972) stressed the importance of diverse germplasm pools in increasing the genetic diversity for selection and in improving the frequency of genes for potentially valuable plant characteristics. The efficient utilisation of genetic resources required that, they be adequately classified and evaluated. Germplasm collections that have been established for many crop species generally contain a large number of entries. Studies on several crops have pointed out that an incredible amount of variation occurs in these collections (Ashri 1973; Narayan and Macfield 1976; Hussaini *et. al.*, 1977; Camussi, 1979 and Tolbert *et al.*, 1979). All the desirable materials cannot easily be evaluated or located from large collections (Holden, 1984). Only a relatively small number of the collection has been fully described for morphological and physiological characters in mulberry (Susheelamma and Jolly, 1986). This is an important prerequisite for effective and efficient utilization of germplasm collections in breeding programmes (Duvick, 1884).

Since, univariate approaches to exploit data on the germplasm lack precision principally because of G × E interactions, hierarchical cluster analysis could become a useful tool for the management of the variation and also to estimate the segregation potential of germplasm in crosses (Peters and Martinelli, 1989). Multivariate statistical techniques have been suggested to measure genetic and phenotypic divergence among entries to aid in planning crosses among genotypes belonging to different clusters (Bhatt, 1970; Camussi *et al.*, 1983).

The multivariate approach increases the precision and at the same time decreases the complexity introduced otherwise by increasing replications of estimates for a given variable or by increasing the number of variable and decreasing the characteristics of the sample. The established computer algorithms developed in the field of multivariate statistics allow the mixing of both qualitative and quantitative data thereby, facilitating the usage of all available data on an entry. A difficulty in using this statistics is the choice of the algorithm. Sokal (1986) and Rohlf Wooten (1988) have reported that UPGMA clustering analysis (group average or average linkage) generally yield most accurate results, although there are other reports stating that the results obtained from six of the most commonly used methods were nearly identical (Lebeda and Jendrulik, 1987).

Mulberry is a tropical East Asian genus distributed in temperate, sub-tropical and tropical regions of the world. It is distributed to north and west Asia, European, South and Central American continents of the world (Sastry, 1984). Fifty-six germplasm entries representing 12 countries spread over Europe, Asia, Australia, South America and the Far East, being maintained at the germplasm bank, CSRTI, Mysore, India were studied with respect to twenty-one agronomic traits. The entries were clustered on the basis of their degree of relatedness with regard to twenty-one agronomic traits measured from the Germplasm samples, enabling to use them with more accuracy in directed breeding programme.

MATERIALS AND METHODS

In the present study, fifty-six entries collected from diverse geographic countries viz., Japan, Bangala Desh, Indonesia, Myanmar, China, erstwhile U.S.S.R., France, Italy, Australia, Paraguay and Brazil were critically analysed for twenty-one agronomic traits. All the germplasm entries, which were utilised for evaluation, were maintained in the field under identical conditions at Central Sericultural Research and Training Institute, Mysore, India. The spacing maintained between rows and plant to plant was 150 cm and 120 cm respectively. The field was maintained following package of practices as recommended by Krishnaswami (1978). All the plants were pruned during the month of June and were allowed to grow. After 70 days of pruning (i.e., during August-rainy season), five plants were selected at random and growth observations contributing to yield were recorded. During the subsequent two seasons of the year (December-Winter season, April-Summer season) yield attributes viz., length of the shoot, inter-nodal distance, number of primary branches per plant, 100 leaves weight, number of leaves per meter length of the shoot, and leaf yield were further recorded. For stomatal studies, 5^{th}, 6^{th} and 7^{th} leaves from the top of three months old shoots of randomly selected plants from each entry were collected. Stomatal impressions were peeled out and frequency was determined. Twenty microscopic fields were observed for each entry. The average length and breadth of stomatal aperture was determined on 20 measurements from all the three leaves for final consideration. For total leaf and cuticle thickness, free hand sections of tender, medium and mature leaves were used. Thickness of leaf, cuticle and ratio of palisade parenchyma to spongy parenchyma were measured (in μm). In all the cases, a minimum of 20 observations were taken during three seasons and the mean of calculated. To determine the moisture content (MC) and moisture retention capacity (MRC), 100 leaves from all the age groups were collected at 9 A.M. in polythene bags and fresh weight (FW) noted. Thereafter, the leaves were kept in the open at laboratory temperature for 12h and the weight (IW) was recorded. Then the leaves were dried in oven for 72 hr. at 80°C for complete dryness and dry weight (DW) was recorded. Moisture content

Table 1. Mean and range estimates of the twentyone agronomic traits in fiftysix exotic introductions used for clustering analysis

Variable	Mean	Std. Dev.	Minimum	Maximum	C.V (%)
AYPY	2.60	1.33	0.20	5.31	51.15
STOFRE	636.60	189.69	306.46	1244.00	29.00
STOSI	206.20	43.66	118.57	313.00	21.17
CUTTHI	4.85	2.20	1.67	10.47	45.34
TOTHI	152.81	25.24	114.97	247.90	16.51
PALI	1.38	0.34	0.88	2.50	25.34
ROOL	23.57	14.44	5.09	62.00	61.37
DWROOT	1.78	1.74	0.10	8.64	97.37
RSRL	0.91	0.59	0.20	3.33	64.96
RSRW	0.45	0.14	0.08	0.80	31.80
MRC	60.21	4.64	47.73	68.28	7.71
NPBP	12.27	6.93	2.00	37.00	56.49
SHOOTL	153.96	53.68	57.60	303.68	34.87
IND	4.14	0.80	2.25	5.86	19.29
NLPML	24.35	4.46	17.00	36.00	18.31
LAREA	154.66	22.72	105.38	220.25	14.69
W100L	455.12	191.53	137.46	940.00	42.08
MP	69.85	3.04	62.28	76.86	4.35
SPRP	34.56	27.46	2.00	96.60	79.44
ROOTP	37.00	27.71	2.00	95.40	74.89
TCC	2.25	0.43	1.49	3.62	18.97

AYPY: Avg. leaf yield; STOFRE : Stomatal frequency; STOSI : Stomatal size; CUTTHI : Cuticle thickness; TOTHI : Total thickness of leaf; PALI : Palisade parenchyma ratio; ROOL : Length of the root; DWROOT : Dry Wt. of the root; RSRL : Root to shoot ratio by length; RSRW : Root to shoot ratio by Wt.; MRC : Moisture retention capacity; NPBP: No. of primary branches; SHOOTL : Shoot length; IND : Internodal distance; NLPML : No. of leaves/Mtr. of length; LAREA : Leaf area; W100L : Wt. of 100 leaves; MP : Moisture percentage; SPRP : Sprouting percentage; ROOTP : Rooting percentage; TCC : Total chlorophyll content.

(MC) and moisture retention capacity (MRC) was calculated using the formula :

$$MC = \frac{FW - DW}{FW} \times 100$$

$$MRC = \frac{IW - DW}{IW} \times 100$$

Table 2. Analysis of variance for 21 agronomic variables as realised from the operation of SPSS/PC+" Quick cluster programme". (The programme was executed after Z-transformation of the estimates of variables)

Variable	Cluster MS	Error MS	DF	F	Prob
ZAYPY	0.8206	1.0141	51.0	0.8092	0.525
ZSTOFRE	3.7392	0.7852	51.0	4.7623	0.002
ZSTOSI	1.4261	0.9666	51.0	1.4754	0.223
ZCUTTHI	7.1077	0.5210	51.0	13.6434	0.000
ZTITHI	3.6487	0.7923	51.0	4.6055	0.003
ZPALI	1.4727	0.9629	51.0	1.5294	0.208
ZROOL	4.6083	0.7170	51.0	6.4271	0.000
ZDWROOT	8.3118	0.4265	51.0	19.4873	0.000
ZRSRL	2.9771	0.8449	51.0	3.5235	0.013
ZRSRW	3.2891	0.8205	51.0	4.0088	0.007
ZMRC	5.8297	0.6212	51.0	9.3845	0.000
ZNPBP	4.5719	0.7199	51.0	6.3512	0.000
ZSHOOTL	7.6653	0.4772	51.0	16.0620	0.000
ZIND	4.6828	0.7112	51.0	6.5848	0.000
ZNLPML	4.0830	0.7582	51.0	5.3851	0.001
ZLAREA	2.6809	0.8682	51.0	0.0880	0.024
ZW100L	7.1488	0.5177	51.0	13.8076	0.000
ZMP	1.7428	0.9417	51.0	1.8507	0.134
ZSPRP	7.8581	0.4621	51.0	17.0049	0.000
ZROOTP	8.1702	0.4376	51.0	18.6690	0.000
ZTCC	1.1903	0.9851	51.0	1.2083	0.319

For root proliferation studies, equal sized cuttings from mature shoots of all the accessions were planted in earthen pots of 80 × 40 × 40 cm (height × breadth × width) dimension and were allowed to grow for three months. These were watered once in four days. The three months saplings were uprooted without damaging the root system. Roots were washed thoroughly and the length of the root and shoot, and the fresh weight of the root and shoot were recorded which later

were dried for 72 hr. at 80°C in oven for complete dryness and weight of dried roots and shoots were recorded separately. Root to shoot ratio by length and weight were calculated. To determine the percentage of sprouting and rooting, one hundred cuttings prepared out of ten months old shoots were taken from each entry and planted separately in nursery beds. After 20 days of planting, sprouting percentage was recorded and the percentage of rooting was recorded after three months of planting on the basis of survival rate. The data on leaf yield were collected for two years from five plants, which were selected at random. Total chlorophyll content was estimated following the colorimetric method of Arnon (1949). Leaf area was recorded by using LICOR-3100 leaf area meter. All the observations were recorded on twenty-one agronomic traits were further analysed.

Clustering methodology

The data on the twenty-one characters computed and agglomerative hierarchical clustering was done using SPSS/PC + software (Microsoft ware Dept. SPSS Inc., Chicago III), employing the method of average linkage between groups (Romesburg, 1984) under UPGMA (unweighted pair-group method using arithmetic averages). The clustering is based on the squared Euclidean distances with distance $(x, y) = \Sigma_i (x_i - y_i)^2$. The average linkage between groups is taken as the average of the distance between all pairs of cases with one member of each group.

RESULTS AND DISCUSSION

Five groups were obtained through clustering, based on twenty-one variables. The degree of variability is evident from the data presented in Table-1. Among the variables studied, maximum variability was observed in case of dry weight of root (CV % 97.37) followed by percentage of sprouting (CV% 79.44) and percentage of rooting (CV% 74.89). Minimum variability was recorded for moisture percentage (CV% 4.35). A persual of Table-1 indicates, that leaf yield per plant per year ranged between 0.20-5.31 kg in different entries studied. Stomatal frequency, stomatal size, cuticle thickness, total leaf thickness and palisade parenchyma to spongy parenchyma ratio ranged from 306.46-1244 per mm^2, 118.57-313 μm^2, 1.67-10.47 µm, 114.97-247.90 µ, and 0.88-2.50 respectively. Length of the root, dry weight of the root, moisture retaining capacity and moisture content ranged between 5.09-62.00 cm, 0.10-8.64 g, 47.73-68.28 per cent and 62.28-76.86 per cent respectively, where as number of primary branches per plant, inter-nodal distance, 100 leaves weight and total chlorophyll content ranged between 2.00-37.00, 2.25-5.86 cm, 137.46-940.00 g and 1.49-3.62 mg/g fresh weight respectively.

Application of quick clustering

The quick cluster provision of SPSS/PC + was used with a five cluster command. The analysis of variance for the twenty-one agronomic variables and the final cluster centers for these variables are presented in Table 2. Except in the case of average leaf yield (AYBY), stomatal size (STOST), ratio of palisade parenchyma to spongy parenchyma (PALI), moisture percentage in leaf (MP) and total chlorophyll content of the leaf (TCC), in all the other variables the cluster variance was found to be substantially higher than the error variance. A persual of Table- 4 indicates that cluster 4 has the highest indices for leaf yield (AYPY), length of the root (ROOL), dry weight of the root (DWROOT), inter-nodal distance (IND), whereas, cluster 3 has the highest indices for 100 leaves weight (W100L), palisade parenchyma to spongy parenchyma ratio (PALI) and total chlorophyll content (TCC). On the other hand, cluster-2 has the highest indices for moisture percentage (MP), moisture retention capacity (MRC), number of primary branches per plant (NPBP) and root to shoot ratio by length (RSRL). Cluster-1 has the highest indices for total

Table 3. Result of quick cluster with five cluster command showing final cluster centers for twentyone variables (With z-transformation) as realised for five clusters

Cluster	ZAYPY	ZSTOFRE	ZSTOSI	ZCUTTHI
1	–0.0188	0.4058	–0.2503	1.2383
2	–0.3973	1.2135	–0.6447	0.0095
3	0.1957	0.0813	–0.1313	–0.1679
4	0.6974	0.0777	0.0948	–0.8123
5	–0.1695	–0.5832	0.3824	–0.5507
Cluster	ZTOTHI	ZPALI	ZROOL	ZDWROOT
1	0.7814	–0.1223	–0.4664	–0.7144
2	0.6040	–0.2495	0.8340	–0.0987
3	–0.1207	0.4991	–0.4638	–0.1383
4	–0.3553	–0.4730	1.6334	2.9319
5	–0.4789	–0.1989	0.2624	0.1549
Cluster	ZRSRL	ZRSRW	ZMRC	ZNPBP
1	0.0499	–0.3385	–1.0560	0.1583
2	1.4466	–0.8341	1.0031	1.2572
3	–0.4625	–0.3220	0.4441	–0.7605
4	–0.0697	0.5432	0.7377	0.8260
5	0.0587	0.5630	0.0198	0.1301
Cluster	ZSHOOTL	ZIND	ZNLPML	ZLAREA
1	1.2107	0.7086	–0.6426	0.3895
2	0.3757	0.3512	–0.4804	–0.2715
3	–0.7724	–0.7370	0.7201	0.2764
4	–0.5767	0.9774	–0.7511	0.7338
5	–0.1577	–0.0879	0.0504	–0.5300
Cluster	ZW100L	ZMP	ZSPRP	ZROOTP
1	–0.9578	0.0008	0.6140	0.6847
2	–0.8575	1.1221	0.5623	0.6189
3	0.9207	0.0971	–0.9298	–0.9555
4	0.2274	–0.0272	1.9463	1.8537
5	0.0234	–0.2985	–0.0597	–0.0825
Cluster	ZTCC			
1	0.1374			
2	–0.0904			
3	0.3645			
4	–0.4551			
5	–0.294			

thickness of leaf (TOTHI), cuticle thickness (CUTTHI) and length of the shoot (SHOOTL). The cluster membership is presented in Table 4. Five clusters have clearly been identified. The cluster-1 comprises thirteen genotypes viz, three entries from Japan, three from Indonesia, five from Pakistan and remaining two entries, one each from Myanmar and Bangala Desh. Cluster-2 comprises four entries, one each from Japan, Indonesia, Paraguay and Australia. Cluster-3 has sixteen entries. Out of them, fourteen are from Japan and remaining two from Brazil and France. Cluster-4 has the minimum membership of three entries, one each from Japan, China and France. Cluster-5 has the maximum entries consisting of twenty genotypes viz., eleven belonging to Japan, two from erstwhile U.S.S.R., two from Bangala Desh, two from Paraguay and of the remaining three, one each from China, Italy and Myanmar. It is also clear from the data in Table-5, that all the high yielding genotypes have fallen in Cluster-3 and low yielding genotypes in clusters-1 and 2. However, Japanese genotypes were found in all five clusters and genotypes from Indonesia, France, Myanmar and Paraguay in more than one cluster. The inter-cluster distances are presented in Table 5. It is evident that the diversity is the highest between the clusters 3 and 4 as reflected by the inter-cluster distance of 6.46 followed by the distance between clusters 1 and 4 (6.0675).

The present study pointed out to the existence of enormous variability in the germplasm and establishes that, in order to manage collections more effectively, the clustering method can be employed. The presence of entries from Japan, Myanmar, erstwhile USSR, China, Italy, Bangala Desh and Paraguay in a single cluster suggests that genetic diversity in not directly related to geographic distribution although; it is generally associated with geographic diversity. Similar findings have been reported by Singh and Singh (1979), Singh and Gupta (1968) and Murthy

Table 4. Number of entries by country of origin in an exotic collection of Mulberry. The five cluster groups are based on quick cluster with five cluster command

Acc. No.	Origin	Cluster	Distance	Acc No.	Origin	Cluster	Distance
246	Pakistan	1	0.996	168	Japan	3	4.985
245	Japan	1	2.880	128	Japan	3	6.021
247	Pakistan	1	3.254	126	Japan	3	6.138
248	Pakistan	1	3.479	101	Japan	3	6.436
239	Myanmar	1	4.321	160	Japan	3	6.536
250	Pakistan	1	4.364	122	Japan	4	1.119
233	Indonesia	1	4.418	104	China	4	5.512
236	Indonesia	1	4.565	132	France	4	5.884
257	Japan	1	5.268	134	USSR	5	1.285
178	Bangala Desh	1	5.848	131	Myanmar	5	4.299
235	Indonesia	1	6.091	167	Japan	5	4.360
249	Pakistan	1	6.330	176	Itali	5	4.383
231	Japan	1	6.778	164	Japan	5	4.471
136	Japan	2	0.918	174	Japan	5	4.570
125	Paraguay	2	5.247	184	Japan	5	4.575
220	Australia	2	5.558	129	Japan	5	4.657
234	Indonesia	2	6.772	149	Japan	4	4.658
169	Japan	3	1.079	190	Japan	5	4.664
186	Brazil	3	2.702	189	Paraguay	5	4.695
173	Japan	3	4.325	133	USSR	5	5.178
171	Japan	3	4.325	238	Japan	5	5.330
192	France	3	4.508	124	Japan	5	5.386
127	Japan	3	4.522	180	Bangala-Desh	5	5.387
182	Japan	3	4.552	137	Japan	5	5.388
181	Japan	3	4.696	147	China	5	4.451
177	Japan	3	4.959	179	Bangala-Desh	5	5.466
183	Japan	3	4.962	191	Paraguay	5	5.521
102	Japan	3	4.963	205	Japan	5	6.954

and Arunachalam (1966). The study also confirms the superiority of multivariate approaches in studying the relationships between genotypes by bringing together genotypes, which show wide diversity in more than one character. The usefulness of the method is further realised by the fact that the agronomical, physiological and quality related traits could be taken into consideration at a time, for grouping the collections.

The groupings of collections from Bangladesh, Pakistan in 5th and 1st cluster respectively suggest the closeness among the genotypes. At the same time, the presence of entries from geographically distant and diverse countries in the same cluster indicates the fact that the correspondence between genetic divergence and geographic diversity is obscured when the species have been the object of intense breeding effort (Jain and Wu, 1977; Tolbert *et al.*, 1979). This material probably has been exchanged among breeders of different countries so that, the difference between germplasm from countries with sharply contrasting agro-ecological conditions have been consistently reduced. This might be the cause for finding genotypes from the erstwhile USSR and Bangala Desh; two geoecologically diverse countries in the same cluster. This is in confirmation with the findings of Zohary (1970) and Spagnoletti Zeuli and Qualset (1987) who reported that the geographical position does not correspond with the phenotypic grouping. The present findings

Table 5. Matrix of cluster distances as realised from quick clustering with a five cluster command

Cluster	1	2	3	4
1	0.0			
2	3.8519	0.0		
3	4.8567	5.1341	0.0	
4	6.0675	5.2356	5.4600	0.0
5	3.9456	4.1074	3.0093	4.8478

have clearly illustrated that, sampling from different clusters would be more effective in achieving great allelic diversity for hybridization, rather than confining to selection based on geographic diversity or based on limited number of many agronomical characters. Frankel and Brown (1984) have opined that computer facilitated data reduction can simplify sampling of large collections for breeding or research purpose or in establishing 'core collections' to study genetic variation.

A systematic search based on combining ability among the exotic germplasm and improved varieties is likely to uncover yet unknown yield promoting genetic resources (Frankel and Brown, 1984). Choosing parents from distant clusters showing highest cluster indices for the particular desired quantitative agronomic character would be highly beneficial in the directed breeding programme.

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