International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **3(7),** 40-48, July **(2014)** *Int. Res. J. Biological Sci.*

Phenomic analyses of indigenous and exotic accessions of Mulberry (*Morus* spp.)

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Available online at: www.isca.in, www.isca.me
Received 1st February 2014, revised 13th March 2014, accepted 14th April 2014

Abstract

Broadening of gene pool vis-à-vis development of superior genotypes is a continuous process for Mulberry (Morus spp.) since the silkworm Bombyx mori L. thrives only on its foliage. The objective of the present study was to understand the interrelationship between selected indigenous / exotic genotypes of the Mulberry germplasm collection of Central Sericulture Research and Training Institute (CSR&TI), Berhampore, West Bengal, India with a phenomic approach. The association between traits was further elucidated to identify appropriate marker assisted selection strategies. The phenogram and factorial analysis of ninety four genotypes was constructed on the basis of fourteen quantitative parameters. The improved genotypes were found to be clubbed in the smallest cluster while seventy four percent exotic germplasm were grouped together in the largest cluster of phenetic tree. Correlation analysis between twenty seven parameters (fourteen quantitative with additional thirteen anatomical) revealed forty six significant correlation values, of which twenty eight percent correlations were negative. The negative correlations between traits of interest were found in opposite principal coordinates.

Keywords *Mulberry* accessions, phenomic analysis, traits.

Introduction

Mulberry (*Morus* spp., family: Moraceae) is extremely important for the sericulture industry since its foliage are the sole food of the silkworm *Bombyx mori* L. Hence, the quality and yields of Mulberry leaf have direct consequence on silk production[1]. The genus *Morus* has a world wide distribution though its cultivation centers mainly in East, Central and South Asia for silk production. Apart from its importance in sericulture, fruits of few species of mulberry are edible (*M. alba, M. indica* and *M. laevigata*) while certain species are timber yielding (*M. laevigata* and *M. serrata*)[2]. Though Hou indicated a multi centered origin of Mulberry[3] but the general belief is that it has originated on the low slopes of the Himalayas bordering China and India. Mulberry essentially is a fast growing; perennial tree that is maintained as short or medium bushes by repeated pruning and conventional propagation is by vegetative means through stem cuttings.

Moriculture has expanded horizontally since traditional plant breeding has contributed to development of several commercially released Mulberry varieties with desirable agronomic traits. A series of high yielding Mulberry genotypes have been selected, developed and released till date and the 'hunt' for developing even superior genotypes is on, particularly targeting towards several traits such as leaf retention capacity, leaf size and weight, total biomass, resistance to pest and diseases, tolerance to drought, salinity, and cold stress. For this, a large gene pool of Mulberry, that is still untapped in repository or wild, can be of great aid.

Further challenge in Mulberry breeding is deciphering suitable markers, be it morphological or molecular, and to link those with important agronomic traits so that those can be helpful for early screening of promising recombinants in Marker Assisted Selection / Breeding (MAS/MAB) program. Though characterization through morphological features often faces criticism due to their non discreet nature as the expression of most of the phenotypic traits are regulated by developmental stages and/or environmental cues. However, few strictly genetically controlled traits like development of stomata, trichome, idioblast etc. and their density vis-à-vis diversity on Mulberry leaf surface may add some insight in MAB since leaf is the predominant selection criterion in Mulberry breeding. This will plausibly complement the segregation of molecular markers in the breeding population to find out association/linkage; and consequent upon a genetic map constructed from a population segregating for a trait of interest will be helpful for QTL (Quantitative Trait Locus/Loci) identification[4].

In the backdrop of these, the present work was envisaged with following objectives: i. To assess the diversity of selected indigenous/exotic Mulberry genotypes from the repository of CSR&TI, Berhampore, West Bengal, India along with the improved varieties considering quantitative traits following the Mulberry descriptor through a phenomic approach and to use the phenomic data set for understanding the interrelationship between indigenous/exotic genotypes by cluster analysis based phenogram construction and factorial analysis; and ii. to elucidate the association between traits in selected accessions /

genotypes by correlation and factorial analyses to identify future trait based selection strategies for development of further improved Mulberry genotypes.

Material and Methods

Plant material: Ninety four genotypes of Mulberry comprising of sixty three indigenous (number starting with MI – *Morus* Indigenous) and thirty one exotic (number starting with ME – *Morus* Exotic) accessions of CSR&TI at Berhampore, WB, India were taken for the present study[5, 6]. Four (C2038, GEN 1, Kajli-OP and C2028) indigenous and one exotic ($CF_1$10) genotypes are yet to be registered (table 1). The parentage of the known hybrids is mentioned since their species status is yet to be assigned (table 1). Apart from the germplasm collection of CSR&TI, the study material set also comprised of few improved genotypes (viz. S1635, C2028, V1, C2038, Kajli-OP and GEN-1; year of development being 1978, 1983, 1997, 1999, 2000 and 2008 respectively). The experimental site is located at 24º6´north latitude and 88º15´east longitude; 19.0 m above mean sea level. Mean maximum and minimum temperatures were 32.2ºC and 20.6ºC respectively. Average maximum and minimum relative humidity was 90% and 62% respectively, the rainy season was distributed between May to November (mean annual rainfall 1377 mm). The soil type was Gangetic alluvium having pH 6.9, EC 0.12 mmhos/cm and organic C 0.56%. The genotypes were planted at 60 cm x 60 cm spacing in a randomized complete block design with three replications and maintained as bushes with standard doze of N,P,K (kg $ha^{-1}yr^{-1}$) in a ratio of 336:180:112 along with Farm Yard Manure (20 t $ha^{-1}yr^{-1}$) and other recommended cultural practices. Observations were recorded on five randomly selected plants per genotype. Data were recorded for three years each having five consecutive harvests.

Descriptor and traits: Fourteen standard parameters (viz. growth and yield related ones like Leaf yield per plant (LY), Total shoot length (TSL), Fresh weight of 100 leaves (FW), Unit leaf area (ULA), Specific Leaf Weight (SLW), Net Photosynthetic Rate (NPR); Physiological parameters like Transpiration rate (TRP), Stomatal Conductance (SC), Relative Water Content (RWC), Leaf Moisture Content (LMC); and biochemical parameters like Total Chlorophyll (TC), Soluble Protein (SP), Soluble Sugar (SS), Nitrate Reductase Activity (NRA)), following the standard descriptor of Mulberry genetic resource evaluation were initially considered for all the genotypes under study. For further analysis in case of selected genotypes, thirteen anatomical descriptors (viz. STS: Stomatal Size, STF: Stomatal Frequency, IDL: Idioblast Length, IDW: Idioblast Width, IDF: Idioblast Frequency, PT: Palisade thickness, SPT: Spongy Thickness, UCT: Upper Cuticular Thickness, LCT: Lower Cuticular Thickness, UET: Upper Epidermal Thickness, LET: Lower Epidermal Thickness, LFT: Leaf Thickness, CHL: Chloroplast) were included.

Construction of phenogram and graphical representation of factorial analysis: The mean data with standard error ranges of fourteen quantitative parameters (original data not shown) were arranged in continuous class intervals and numeric was assigned accordingly to each class interval in case of every genotype. The table of character state (derived from numerical data) was imported to dedicated software and subsequent analysis was done considering continuous dissimilarity data to perform weighted neighbor-joining tree (WPGMA, graphical representation of cluster analysis) based on Euclidean distance calculation (through Bootstrapping) with the help of software, DARwin 5.0.128. The data set was further subjected to Factorial analysis on dissimilarity coordinate with the same software. The values of Eigenvectors of first three PC axes derived from non-Euclidean dissimilarity were taken in consideration in further data analysis[7].

Correlation and factorial analysis between traits: The same data matrix of character state (derived from fourteen parameters as described earlier) of the selected genotypes after necessary transposition (putting column values into rows and vice versa) along with thirteen anatomical parameters as stated before (quantitative in nature; data range was grouped in class intervals, then numeric was assigned) was used to study the correlation between traits. The analysis was done using statistical software MINITAB Release 13 Windows NT 4 © 2000 Minitab, Inc. The combined data matrix of traits in case of selected genotypes was subsequently subjected to Factorial analysis on dissimilarity coordinate with the help of software, DARwin 5.0.128 with the same consideration of Eigenvectors as stated above.

Results and Discussion

Phenogram and graphical representation of factorial analysis: The tree in its vertical topographical form (Figure 1) resolved into three major clusters, of which the central major one comprised of fifty three genotypes, which can be further demarcated by a number of sub and infra clusters; the second largest cluster comprised of twenty four genotypes, clearly divisible into two sub clusters; while the smallest cluster comprising of seventeen genotypes showed unique topology. Seventy four percent exotic germplasms (twenty three out of total thirty one) were placed in the central major cluster, while the second largest and smallest cluster accommodated five and three exotic germplasms respectively. The seventeen genotypes in the smallest cluster revealed a unique hierarchical descendant nature (figure 1). The genotypes of the smallest cluster were clearly identifiable in the Principal Co-ordinate (Factorial) analysis (figure 2) where the relative proximities between themselves became more obvious. The improved varieties developed at CSR&TI or developed / selected elsewhere be it indigenous or exotic were spatially distant (marked differentially in figure 2) from the six landraces (* marked in figure 2), which became obscure in the crowd of similar genotypes of the largest and the second largest clusters derived from neighbor-joining phenogram. Inertia values are 38.79% and 13.38% for factorial coordinates axes 1 and 2, respectively.

Table-1
Mulberry genotypes under study

Indigenous				
Sl. No.	Name	Species / hybrid	Source	Accession No.
1	Kakpillai	*M. indica* Lin.	Andhra Pradesh	MI - 0064
2	Assambola	*M. indica* Lin.	Assam	MI - 0011
3	Kaliakothai	*M. indica* Lin.	Assam	MI - 0035
4	Golaghat	*M. alba* Lin.	Assam	MI - 0057
5	Jatinuni	*M. indica* Lin.	Assam	MI - 0038
6	Surat	*M. indica* Lin.	Gujarat	MI - 0073
7	Kanva-2	*M. indica* Lin.	Mysore	MI - 0038
8	MS-1	*M. alba* Lin.	Mysore	MI - 0054
9	MS-5	*M. alba* Lin.	Mysore	MI - 0040
10	RFS-175	*M. indica* Lin.	Mysore	MI - 0066
11	OPH-1	*M. indica* Lin.	Mysore	MI - 0061
12	ACC-165	*M. indica* Lin.	Mysore	MI -0054
13	MS-6	*M. alba* Lin.	Mysore	MI - 0060
14	Mysore local	*M. indica* Lin.	Mysore	MI - 0062
15	MS-9	*M. alba* Lin.	Mysore	MI - 0065
16	MR-1	*M. sinensis* Hort.	Mysore	MI - 0028
17	MS-7	*M. alba* Lin.	Mysore	MI -0069
18	FGDTR-9	*M. indica* Lin.	Mysore	MI - 0070
18	MS-8	*M. alba* Lin.	Mysore	MI - 0071
20	V1	C776 x S30	Mysore	MI - 0008
21	S30	*M. alba* Lin.	Mysore	MI-0046
22	Nagaland local	*M. indica* Lin.	Nagaland	MI - 0167
23	Sujanpur	*M. alba* Lin.	Punjab	MI - 0222
24	Sujanpur-5	*M. alba* Lin.	Punjab	MI - 0051
25	Punjab local	*M. alba* Lin.	Punjab	MI - 0059
26	Jodhpur	*M. indica* Lin.	Rajasthan	MI -0072
27	Black cherry	*M. indica* Lin.	Unknown	MI - 0094
28	Almora local	*M. indica* Lin.	Uttar Pradesh	MI - 0015
29	Sultanpur	*M. indica* Lin.	Uttar Pradesh	MI - 0109
30	Kajli	*M. indica* Lin.	West Bengal	MI - 0068
31	Ber-A	*M. indica* Lin.	West Bengal	MI - 0089
32	Ber-B	*M. indica* Lin.	West Bengal	MI - 0003
33	Ber-6	*M. indica* Lin.	West Bengal	MI - 0179
34	Ber-20	*M. indica* Lin.	West Bengal	MI - 0116
35	M. indica-X	*M. indica* Lin.	West Bengal	MI - 0102
36	CSRS-2	*M. indica* Lin.	West Bengal	MI - 0010
37	Kolitha-3	*M. indica* Lin.	West Bengal	MI - 0108
38	Kolitha-7	*M. indica* Lin.	West Bengal	MI - 0096
39	Kolitha-8	*M. indica* Lin.	West Bengal	MI - 0056
40	Kolitha-9	*M. indica* Lin.	West Bengal	MI - 0091
41	Dudhia Red	*M. indica* Lin.	West Bengal	MI - 0088
42	Dudhia white	*M. indica* Lin.	West Bengal	MI - 0060
43	Bishnupur-4	*M. indica* Lin.	West Bengal	MI - 0092
44	Bishnupur-9	*M. indica* Lin.	West Bengal	MI - 0117
45	Tollygaunge A	*M. indica* Lin.	West Bengal	MI - 0098
46	Bush Malda A	*M. indica* Lin.	West Bengal	MI - 0095

47	Bush Malda B	*M. indica* Lin.	West Bengal	MI - 0075
48	Matigara white	*M. alba* Lin.	West Bengal	MI - 0100
49	Matigara Black	*M. indica* Lin.	West Bengal	MI - 0078
50	Tista valley	*M. indica* Lin.	West Bengal	MI - 0093
51	Kurseon	*M. indica* Lin.	West Bengal	MI - 0163
52	KPG-1	*M. indica* Lin.	West Bengal	MI - 0144
53	MI Black	*M. indica* Lin.	West Bengal	MI - 0097
54	KPG-2	*M. indica* Lin.	West Bengal	MI - 0105
55	MI HP	*M. indica* Lin.	West Bengal	MI - 0099
56	C763	English Black x *M. multicaulis*	West Bengal	MI - 0124
57	C2038	CF_1 10 x C763	West Bengal	---
58	C776	(English black *x M. multicaulis)*	West Bengal	MI-0158
59	GEN 1	V1 x Kajli (OP)	West Bengal	---
60	Kajli (OP)	*M. alba* Lin.	West Bengal	---
61	C2028	*M. alba* Lin.	West Bengal	---
62	S1635	*M. alba* Lin.	West Bengal	MI - 0173
63	Bombai local	*M. alba* Lin.	West Bengal	MI - 0112
Exotic				
Sl. No.	**Name**	**Species/hybrid**	**Source**	**Accession No.**
64	M. australis	*M. australis*	Australia	ME - 0001
65	Australia	*M. australis* Koitz.	Australia	ME - 0093
66	Bogura-1	*M. indica* Lin.	Bangladesh	ME - 0097
67	Bogura-4	*M. indica* Lin.	Bangladesh	ME - 0084
68	Shrim-2	*M. alba* Lin.	Bangladesh	ME - 0007
69	Shrim-5	*M. alba* Lin.	Bangladesh	ME - 0041
70	Shirim-8	*M. alba* Lin.	Bangladesh	ME - 0025
71	Burma-8	*M. indica* Lin.	Burma	ME - 0020
72	S1	*M. alba* Lin.	Burma	ME-0065
73	Mandalaya	*M. alba* Lin.	Burma	ME-0045
74	Monla-1	*M. alba* Lin.	Burma	ME - 0003
75	Monlai	*M. alba* Lin.	Burma	ME - 0090
76	Molai	*M. indica* Lin.	Burma	ME - 0003
77	China white	*M. alba* Lin.	China	ME - 0042
78	China Black	*M. alba* Lin.	China	ME - 0106
79	CF_1 10	(*M. multicaulis x M. indica* HP)	China	---
80	Multicaulis	*M. latifolia* Poir.	France	ME - 0046
81	Rotundiloba	*M. rotundiloba* Koidz.	France	ME - 0095
82	Cyprus	*M. alba* Lin	Greece	ME - 0055
83	M.multicaulis	*M. latifolia* Poir.	Indonesia	ME - 0006
84	M nigra	*M. nigra* Lin.	Indonesia	ME - 0008
85	M.cathyana	*M. cathyana* Hemsl.	Indonesia	ME - 0018
86	Italian Mulberry	*M. alba* Lin	Italy	ME - 0105
87	KPKSO 13	*M. bombycis* Koidz.	Japan	ME - 0021
88	Okinowasi	*M. bombycis* Koidz.	Japan	ME -0041
89	Calabrasa	*M. latifolia* Poir.	Paraguay	ME - 0023
90	Muraso	*M. bombycis* Koidz.	Paraguay	ME - 0122
91	Phillipine	*M. alba* Lin	Phillipines	ME - 0118
92	Thailand lobed	*M. alba* Lin	Thailand	ME - 0125
93	Thailand unlobed	*M. alba* Lin	Thailand	ME - 0082
94	FRNANDS	*M. alba* Lin	Unknown	ME - 0057

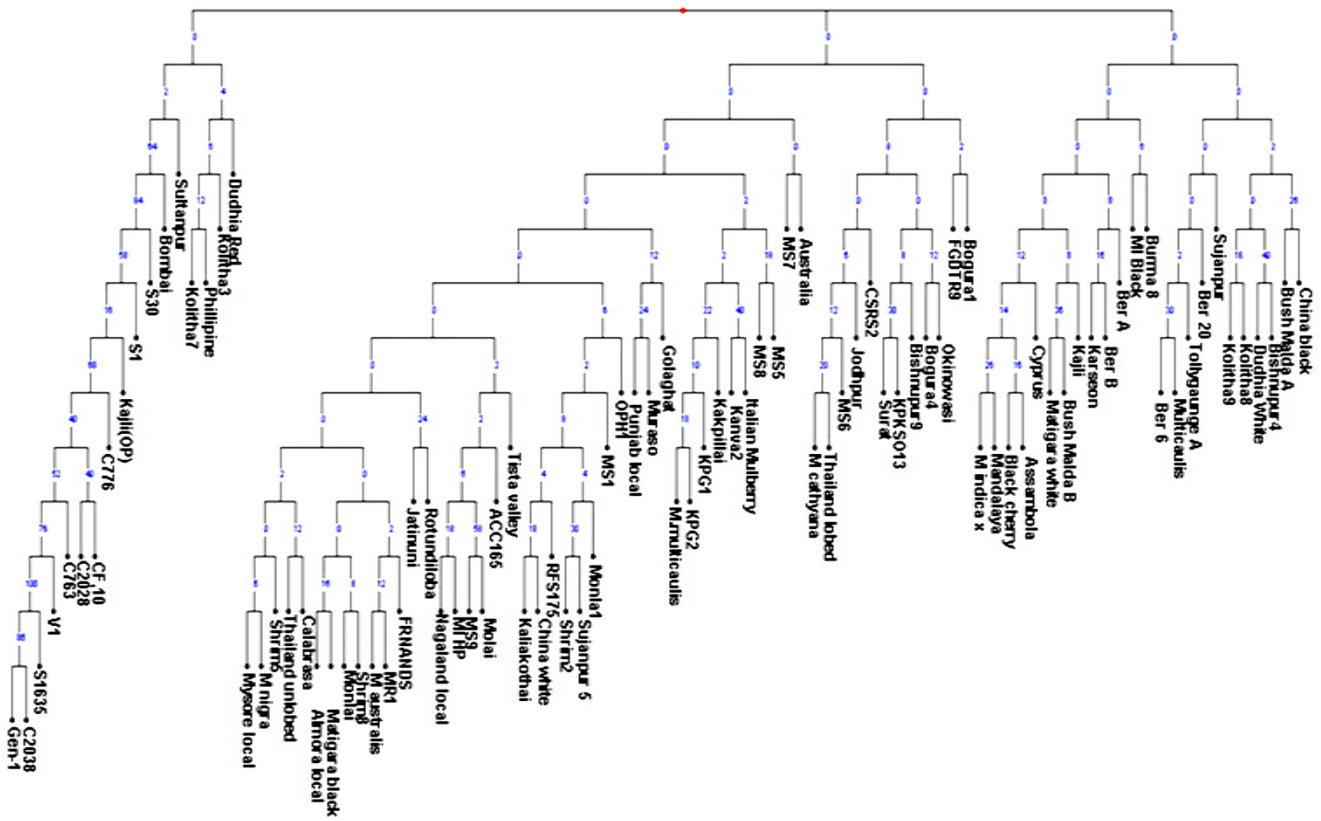

Figure-1

The Neighbour-joining (WPGMA) vertical topographical representation of phenogram (with Bootstrapping, values in blue) of ninety four genotypes of Mulberry constructed with software DARwin 5.0.128 showing clusters / sub / infra clusters

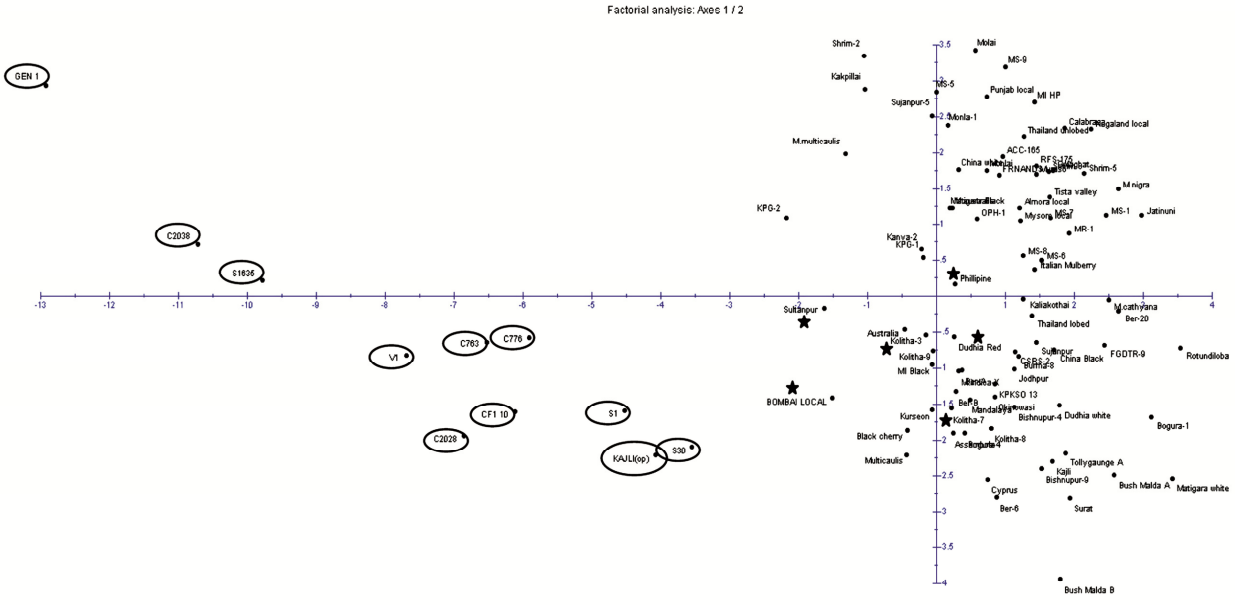

Figure-2

Principal Co-ordinate (Factorial) analysis of ninety four genotypes of Mulberry constructed with software DARwin 5.0.128

Development of superior genotypes is always an overwhelming task for the Mulberry breeders to keep parity with the feeding habit of the silkworms. This is even more challenging since the selection criterion in Mulberry is largely based on vegetative descriptors and centered on leaf[8], which is a highly plastic organ[9]. Though molecular marker information is gaining momentum in recent times[2,10-13] but the standard Mulberry literatures are relying till now on morphological descriptors along with conventional growth and leaf yield related agronomical parameters. The incessant selection by the breeders towards even superior Mulberry genotypes was reflected in all the forms of analyses. The improved genotypes were placed in a unique topologically distinct cluster terminating in two genotypes, Gen-1 and C2038 in neighbor joining tree (figure 1). In fact, Gen-1 is the latest developed variety of Mulberry by CSR&TI, Berhampore (table 1) and is a selection from the hybridization program between Kajli (OP) (♀) and V1 (♂). The relative position of the three genotypes, viz. Kajli (OP), V1 and Gen1 as separate tiers in the smallest cluster of the neighbor-joining tree (figure 1) is in accordance with their parental and hybrid nature. Similar accordance was also observed in other improved variety, C2038 and its two parents $CF_1$10 (♀) and C763 (♂). Of the three exotic genotypes (Philippine, S1 and $CF_1$10, from Philippines, Burma and China respectively) present in this cluster, two (S1 and $CF_1$10) have already been utilized in different breeding programs leaving the scope of utilization of the rest (Philippine) considering its proximity to the improved genotypes after necessary phenomic characterization for its desirable attributes. Similarly, the relative closeness of Kanva-2 with Italian Mulberry in the largest cluster plausibly gives a lead for further improvement of this (Kanva -2) highly popular Mulberry variety, which is widely cultivated in India and recently introduced to some of the South East Asian countries. The grouping of seventy four percent exotic germplasm in the largest cluster of neighbor joining phenetic tree indicate that these genotypes are yet to be explored totally and a thorough assessment of these along with the rest Indigenous landraces / local may help to look for desirable agronomic traits for future Mulberry improvement program. The proximity of certain Indian accessions along with the exotic ones probably can be explained from the postulated multi centered origin of Mulberry[3] as well as the manipulation of this extremely heterogeneous crop by the Mulberry breeders for centuries resulting in a steady gene flow among different geographical areas.

The interrelationship between Indigenous/exotic genotypes was apparently become more pronounced when the same data was subjected to Principal Coordinate Analysis (PCoA). It is a member of factorial analysis family working on distance matrices. It considers the space of high dimension defined by the distances between units two by two[14]. The relative proximities of the improved varieties from yet to be explored gene pool of exotic and indigenous landraces were revisited through the graphical form of this analysis (Figure 2). Factorial analysis and tree methods constitute two very different approaches for the representation of diversity structure. Factorial methods aim mainly to give an overall representation of diversity and are not really interested by individual effects. On the other hand, tree methods tend to represent individual relations faithfully and may be less accurate for the global structure. They are thus two different ways of viewing the data and must be considered complementary rather than concurrent prior finalizing future Mulberry breeding program.

Correlation between traits: Correlation analysis between twenty seven traits (fourteen standard and thirteen anatomical descriptors as mentioned in Materials and Methods) was performed in case of seventeen genotypes (members of smallest cluster showing unique topology; figure 1). The observed values (Pearson correlation) were subsequently considered following their level of significance (*p* values). Of the total three hundred fifty one values in the triangular matrix (upper / lower) only forty six combination between traits were found to be statistically significant ($p<0.05$). Twenty eight percent (thirteen out of forty six) significant correlations were found to be negative. The level of significance was further grouped in three ranges to understand the relative association between traits (figure 3).

The most important quantitative trait from the perspective of Mulberry breeding, i.e. leaf yield per plant showed positive correlation of differential values with Relative Water Content (RWC), total chlorophyll, Nitrate Reductase activity (NRA), stomatal size and number of chloroplast per stomata while the value was negative with idioblast length. Fresh weight of 100 leaves showed positive correlation of differential values with unit leaf area, stomatal conductance, Leaf Moisture Content (LMC), total chlorophyll and NRA while correlation was negative with soluble sugar. The correlation of unit leaf area was always positive with stomatal conductance, LMC, NRA and stomatal size. The correlation of specific leaf weight was always negative with lower cuticular thickness and upper epidermal thickness. The correlation between net photosynthetic rate and upper epidermal thickness was positive while the correlation of transpiration with both RWC and LMC was negative. Stomatal conductance showed positive correlation with LMC, total chlorophyll, NRA and stomatal size. RWC showed positive correlation with LMC but negative correlation with stomatal frequency, idioblast length and idioblast frequency. The correlation between LMC and NRA was positive and it was same between total chlorophyll and NRA. Stomatal frequency revealed negative correlation with soluble protein and sugar. Stomatal size showed positive correlation with NRA, upper cuticular thickness, upper epidermal thickness and number of chloroplast per stomata. The correlation between stomatal frequencies was positive with idioblast length while it was negative with lower epidermal thickness. Idioblast length showed positive correlation with idioblast frequency. Palisade thickness showed positive correlation with leaf thickness. Similarly the correlation between upper cuticular and epidermal thickness was positive (figure 3).

Success of any breeding program essentially depends on screening of recombinants through pre set desirable markers. Breeding of Mulberry centers on improvement of leaf - characteristics from the points of view of yield and acceptability / rejection of the silk worms. Selection often becomes problematic since major association between quantitative (yields) and qualitative phenotypic traits are difficult to sort out. Correlation and subsequent factorial analysis in selected genotypes were hence, performed taking in account of twenty seven parameters. Leaf yield per plant, the seemingly most desirable attribute as the selection criterion was critically assessed and this parameter showed predictable significant positive correlations with Relative Water Content and total chlorophyll (Figure 3). More water content obviously indicates juicy nature of leaves, which is preferred by silkworms while consuming it. Yield is governed by enhanced photosynthetic rate and hence, showing positive correlation with chlorophyll content, which in consequence is correlated with fresh weight of leaves. To maintain the optimum turgid condition of leaves transpiration rate needs to be properly regulated and the result of negative correlation between RWC and transpiration / RWC and stomatal frequency; specific leaf weight and upper epidermal thickness is, hence, explainable. Looking for significant association between quantitative parameters with anatomical descriptors provided some definite leads and interestingly, these relationships were of negative in nature. Yield was found to be negatively correlated with length of idioblast; which vis-à-vis its (idioblast) frequency were negatively correlated with RWC (Figure 3). Idioblasts are conspicuous cellular structures present in the adaxial leaf surface of almost all mulberry plants albeit in different form, structure and frequency; they were clearly distinguishable from epidermal, trichome and parenchyma cells. Site-specific cellular localization of Ca and Si within an idioblast has been reported earlier through electron microscopy and x-ray microanalysis[15,16]. High density of idioblasts causes physical hindrance to the tender mouthparts of the silkworms, which leads to rejection of mulberry foliages as feeds[17].

Factorial analysis between traits: Factorial analysis of twenty seven traits derived from seventeen genotypes identified five major components; the major three of these were responsible for 36.9, 20.57 and 15.32% of total variability (inset table for Figure 4). The principal coordinates divided the traits either into groups of 17 / 10 or groups of 12 / 15 (Figure 4). Significant negative correlations between traits of interest (RWC / idioblast length; specific leaf weight / upper epidermal thickness; RWC / idioblast frequency; RWC / transpiration; leaf yield per plant / idioblast length; RWC / stomatal frequency; fresh weight of 100 leaves / soluble sugar) as revealed from correlation analysis (Figure 3) was also reflected in factorial analysis as those were placed in opposite coordinates (differentially marked in Figure 4).

The results of correlation were further substantiated by PCoA since the graphical representation of this Factorial analysis provided clear cut evidence of minimum utilizable traits, on which the future selection strategy for Mulberry breeding program can centers around. Principal component analysis (PCA) has been erstwhile utilized in clarifying the relationship among traits in different plants[18] including Mulberry[19]. However, use of anatomical descriptors as selective traits, probably first of its kind indicated that a superior genotype of Mulberry can be redefined as having minimum idioblast and stomatal frequency with simultaneous thick epidermis in leaves.

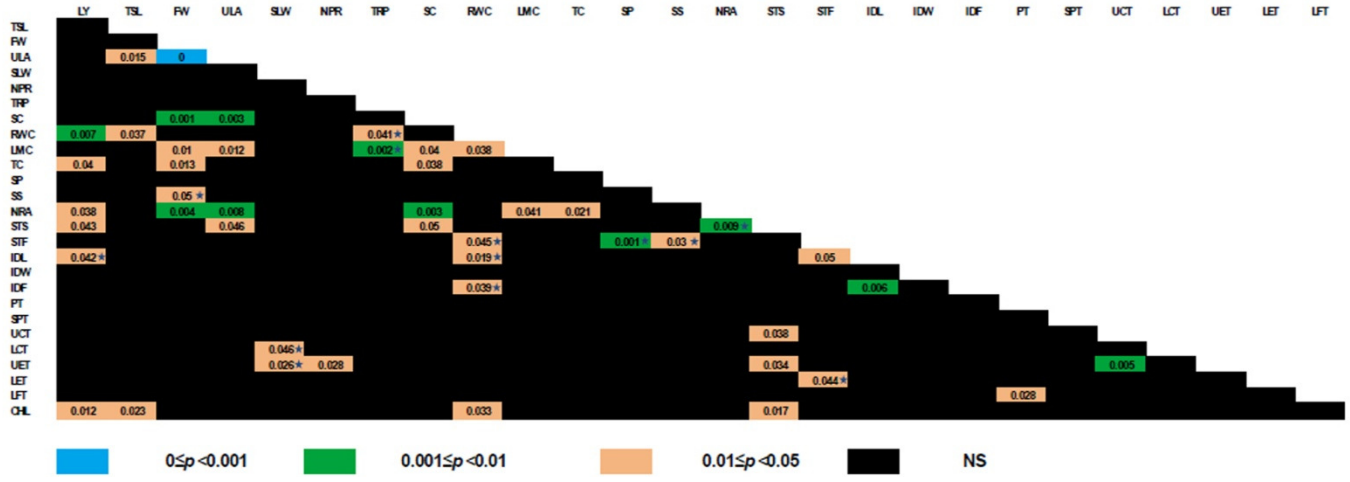

Figure-3

Correlation analysis between traits of seventeen Mulberry genotypes (members of smallest cluster of Figure 1) with software MINITAB Release 13 Windows NT 4 © 2000. LY: Leaf yield per plant, TSL: Total shoot length, FW: Fresh weight of 100 leaves, ULA: Unit leaf area, SLW: Specific Leaf Weight, NPR: Net Photosynthetic Rate, TRP: Transpiration, SC: Stomatal Conductance, RWC: Relative Water Content, LMC: Leaf Moisture Content, TC: Total Chlorophyll, SP: Soluble Protein, SS: Soluble Sugar, NRA: Nitrate Reductase Activity, STS: Stomatal Size, STF: Stomatal Frequency, IDL: Idioblast Length, IDW: Idioblast Width, IDF: Idioblast Frequency, PT: Palisade thickness, SPT: Spongy Thickness, UCT: Upper Cuticular Thickness, LCT: Lower Cuticular Thickness, UET: Upper Epidermal Thickness, LET: Lower Epidermal Thickness, LFT: Leaf Thickness, CHL: Chloroplast * - negative correlation, colour bars for range of level of significances, NS: Non significant.

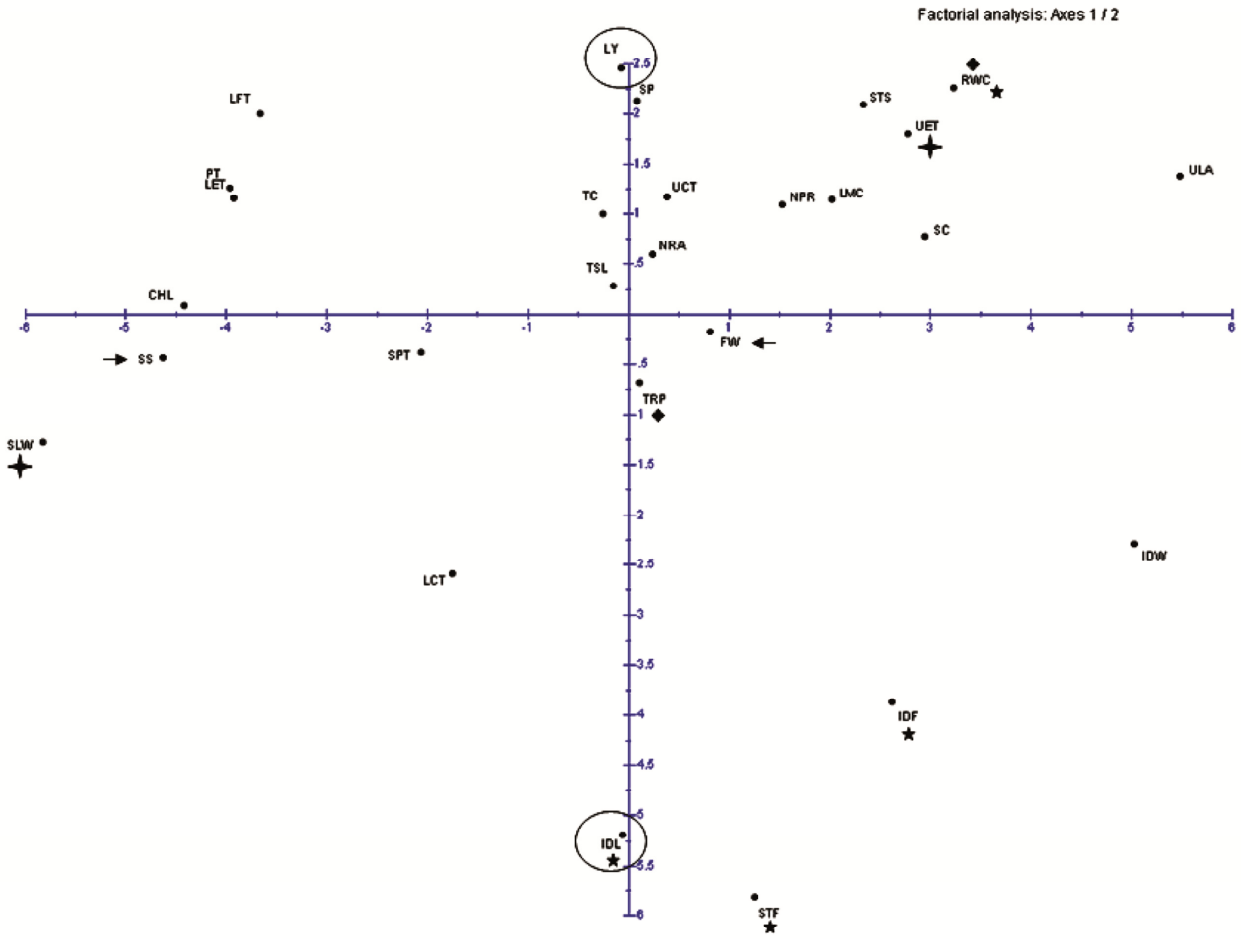

Figure-4
Principal Co-ordinate (Factorial) analysis (based on twenty seven traits) of seventeen selected genotypes of Mulberry constructed with software DARwin 5.0.128

Conclusion

The grouping of seventy four percent exotic germplasm in the largest cluster of neighbor joining phenetic tree indicate that these genotypes are yet to be explored totally and a thorough assessment of these along with the rest unexplored Indigenous landraces / local may help to look for desirable agronomic traits for future Mulberry improvement program. Use of anatomical descriptors as selective traits, probably first of its kind indicated that a superior genotype of Mulberry can be redefined as having minimum idioblast and stomatal frequency with simultaneous thick epidermis in leaves.

Acknowledgements

The financial assistance in form of research fellowship provided by UGC, India is acknowledged by the first author (VA).

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