



A COMPARATIVE STUDY OF FOLIAR MICRO-MORPHOLOGY OF SIX GENOTYPES OF *MORUS ALBA* L. FROM DARJEELING DISTRICT, WEST BENGAL, INDIA

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Abstract

Morus alba is one of the most important genus under the family Moraceae distributed in tropics and sub-tropics. *Morus alba* is the only food source for silk worm larvae, beside this it also bears other commercial importance. Selection of appropriate genotype of mulberry is an important criterion because it will put significant impact on economic parameters of mulberry dependent industries. Present work was focused on studying comparative foliar micro-morphology of six mulberry genotypes. The work was aimed to study micro-morphological characteristics that can be used to identify superior genotype. The obtained results were statistically studied through ANOVA, Correlation, PCA and Dendrogram analysis. Result suggests that on the basis of morphological and statistical analysis S1 and V1 genotype are most suitable for silk industry because of its smaller stomatal aperture and less trichome density. S1635 and Guangdong are represented by large foliar size, with high frequency of stomata and trichome, while TR10 and BC259 bears the intermediate characters.

Keywords: Mulberry, genotype, leaf architecture, stomata, dendrogram

Introduction

Moraceae commonly known as Mulberry family or fig family is one of the economically important family of flowering plants. The family of around 1100 species, representing 38 genera (Christenhusz & Byng, 2016) are geographically distributed in the tropical and sub tropical regions and mostly concentrated in Asia and the Pacific Islands. Moraceae is a very complex family and due to its extremely diverse morphology and habitat range. All the species of this family has milky sap.

Datta (2002) stated that the genus *Morus* includes 68 species out of which one is *Morus alba* (white mulberry). *Morus alba* is one of the most economically important genus of the family Moraceae. Bentham and Hooker (1885) placed *Morus alba* L. under the order Rosales in the family Moraceae. It is small to medium sized tree which attains height up to 20 meter. The species is native to northern China (Zhou *et al.*, 2013) and widely cultivated elsewhere for fodder and silkworm rearing and timber production (Gupta, 1993). The species are

generally deciduous. Leaves are deeply lobed, cordate at base, apex acuminate and margin serrated. Inflorescence catkin; male and female flowers are usually on separated trees, sometimes they may occur on the same tree. Male flowers are small, 4 stamens, and the filament is inflexed. Female flowers are inconspicuous, perianth 4, free. Fruit achene, ovoid, pedunculate, red when immature, blackish purple to purple or greenish-white when matures (Tutin, 1964). Radjabi *et al.* (2010) stated that for proper growth and development of silk worm larvae, nutritional level of mulberry genotypes play an important role. Not only in silk industry, white mulberry bears multipurpose application in various commercial fields like in pharmaceutical, paper, organic fertilizer and many other industry (Łochyńska and Oleszak, 2015).

White mulberry bears wide environmental adaptability (Peris *et al.*, 2014), leading to numerous morphological variability according to environmental condition (Cordell *et al.*, 1998). Gray (1990) stated that morphological variability among mulberry genotype helps them to survive under various environmental conditions. Datta (2000)

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reported that in India about 282,244 hector area is used for mulberry cultivation which covers the land from tropical states like Karnataka, Andhra Pradesh, Tamil Nadu to sub-tropical states of West Bengal, Assam, Himachal Pradesh etc. In West Bengal mulberry is cultivated in almost all the districts covering an area of 21,358 hectars except Howrah and East Midnapore district. As mulberry cultivation is an out-door practice so it requires large open land for cultivation. One of the prime reasons for introduction of different genotypes of mulberry time to time is to select the better one with superior quality and which produces large number of leaves.

Since time many cultivars of white mulberry was developed for fulfilling the demand of silk and other industries where it act as an economic crop but proper comparative study of this cultivars was lacking through which most economic one can be identified. Micro-morphological characters of leaves can act as an effective tool for identifying superior or better genotype of white mulberry for cultivation purpose. In present study an attempt has been made to present a comparative account of micro-morphology of six mulberry genotypes (S1635, TR10, S1, BC259, V1, and Guangdong) commonly available in Darjeeling district of West Bengal, India for selecting the superior one on the basis of morphological characteristics.

Materials and Methods

Plant sample collection

Six genotypes of white mulberry namely S1635, TR10, S1, BC259, V1, and Guangdong were collected for present study from Matigara Sericulture Farm, Siliguri, West Bengal in the month of September to October, 2017. The area was located at 26°70'40"N and 88°35'37"E.

Non-microscopic data collection of the genotypes

The leaf length, lamina length, lamina breadth, petiole length and internodal length of all the six genotypes were determined with the help of a scale and written down in the field notebook and were tabulated as such in the excel sheet where further calculations were done.

Light microscopic study of genotypes

Light microscopic study was done by taking pilling of leaves equidistant from the leaf margin and midrib following the method of Funmilola Mabel *et al.* (2014). To remove chlorophyll from leaves, the cut leaves were boiled in 80% alcohol two to three times. If after alcohol treatment leaves remained opaque then the leaf was allowed to boil in lactic acid to achieve the desired level of clearing (Lama 2004). The clear specimens were then

mounted in glycerine and observed under light microscope (Olympus trinocular microscope) to compare the observable foliar characters, if required the clear specimens are stained with safranin for better observation of characters.

Under microscope different quantitative anatomical characters like vein islets, vein termination, palisade ratio, palisade diameter, stomatal frequency, stomatal index, trichome length and breadth etc were evaluated. Camera lucida drawing was made under compound microscope for required characters.

Statistical analysis

Results were expressed as Mean \pm SEM where n = 50 for general morphological characters and 20 for microscopic characters. Difference between different attributes are indicated with different letters (a, b, c, etc.) differ significantly at p = 0.05 by Duncan's Multiple Range Test (DMRT). The data were subjected to analysis of variance (ANOVA) to determine the level of significance of different attributes under study. To determine direct or inverse relationship between different attributes under study correlation study was done using XLSTAT 2017 software. Principle Component Analysis (PCA) of different genotypes and variables under study was performed using XLSTAT 2017 software. Similarity Dendrogram was prepared using XLSTAT 2017 Software to clusters genotypes with similar characters.

Results and Discussion

The mean values of different morphological parameters *viz.* lamina length, lamina breadth, petiole length, internodal length and leaf surface area were enlisted in table 1. Significant difference was observed among the six genotypes on comparing the parameters. Among all the genotypes large and small lamina length and breadth was observed in Guangdong (195.04 mm) and TR10 (138 mm) genotype respectively, while on comparing petiole length it was found that the petiole length of Guangdong was the smallest. Thus the genotype Guangdong bears large lamina with small petiole indicating its advanced morphological form. The increasing order of leaf surface area among the genotypes was as follows S1 < V1 < TR10 < BC259 < S1635 < Guangdong. Menon and Srivastava (1984) reported that carbon dioxide assimilation by plant through photosynthesis and the leaf biomass was directly associated. As Guangdong bears large surface area so it may attain greater biomass through photosynthesis which might favour its yield.

In table 2, different quantitative microscopic characters such as vein islet, vein termination, palisade ratio, palisade diameter, stomatal frequency and stomatal

Table 1: Variation in length and breadth of lamina, length of internode and surface area of six mulberry genotype

CULTIVAR	Lamina Length (mm)	Lamina Breadth (mm)	Petiole Length (mm)	Internodal Length (mm)	Leaf surface area (Sq mm)
S1635	166.30±8.16 ^c	131.10±4.93 ^c	47.70±5.70 ^a	78.90±4.11 ^a	174936.30±207.20 ^b
TR10	138.00±9.06 ^f	88.40±4.00 ^f	44.20±5.54 ^b	58.32±6.61 ^b	60370.30±258.36 ^d
S1	150.40±5.65 ^d	114.00±6.02 ^d	37.60±3.11 ^c	45.32±6.90 ^d	26586.60±93.35 ^f
BC 259	172.30±6.89 ^b	158.20±7.30 ^b	36.20±2.76 ^d	38.40±3.41 ^e	71370.40±201.42 ^c
V1	140.40±8.37 ^e	95.40±4.00 ^e	33.40±3.66 ^e	33.00±3.38 ^f	36686.50±154.88 ^e
GUANGDONG	195.40±9.16 ^a	177.80±7.07 ^a	32.10±4.47 ^f	52.60±5.41 ^e	202694.70±307.68 ^a

Results are expressed as Mean ± SEM. Values with different letters (a, b, c, etc.) differ significantly ($p=0.05$) by Duncan's Multiple Range Test (DMRT)

Table 2: Variation in quantitative microscopic traits of six mulberry genotype under study

CULTIVAR	Vein Islet (per mm ²)	Vein Termination (per mm ²)	Palisade ratio (per mm ²)	Palisade diameter (per mm ²)	Stomatal frequency (per mm ²)	Stomatal Index (%)
S1635	7.20±0.84 ^c	16.20±2.59 ^b	9.83±1.42 ^{abc}	10.00±0.62 ^a	242.42±42.86 ^c	22.62±2.45 ^a
TR10	5.08±0.49 ^c	6.70±1.05 ^c	9.04±0.21 ^{bc}	5.88±0.33 ^b	476.00±52.13 ^a	16.42±2.17 ^b
S1	17.40±2.30 ^b	9.20±1.79 ^c	12.51±2.16 ^a	5.00±1.49 ^b	238.05±32.23 ^c	16.43±0.64 ^b
BC 259	20.20±2.59 ^b	16.40±2.07 ^b	8.73±0.35 ^c	5.80±1.42 ^b	458.90±117.36 ^a	12.53±2.01 ^c
V1	6.20±0.84 ^c	15.20±2.28 ^b	10.13±1.39 ^{abc}	10.00±1.15 ^a	382.98±121.28 ^b	12.45±2.42 ^c
GUANGDONG	28.52±2.22 ^a	19.62±1.11 ^a	12.00±2.72 ^{ab}	6.66±0.75 ^b	470.20±39.98 ^a	18.05±0.95 ^b

Results are expressed as Mean ± SEM. Values with different letters (a, b, c, etc.) differ significantly ($p=0.05$) by Duncan's Multiple Range Test (DMRT)

Table 3: Variation in stomatal attributes and its associated cells of six mulberry genotype under study.

CULTIVAR	Stoma Length (µm)	Stoma breadth (µm)	Length of Stoma + Guard cell (µm)	Breadth of Stoma + Guard cell (µm)	Length of Stomata + Subsidiary cell (µm)	Breadth of Stomata + Subsidiary cell (µm)
S1635	19.01±3.44 ^a	3.86±0.27 ^a	24.47±1.73 ^a	14.05±2.92 ^a	29.35±3.73 ^a	43.33±4.42 ^{ab}
TR10	11.16±1.57 ^b	3.20±0.40 ^{ab}	18.60±3.56 ^b	12.56±1.12 ^{ab}	21.86±2.56 ^{bc}	38.88±2.58 ^{bc}
S1	7.00±1.39 ^c	1.99±0.75 ^c	17.33±2.91 ^b	9.93±1.91 ^b	19.29±1.91 ^c	32.89±3.21 ^d
BC 259	10.86±1.58 ^b	3.80±0.49 ^a	18.31±2.40 ^b	14.24±2.23 ^a	24.15±2.40 ^b	34.47±3.21 ^{cd}
V1	10.69±2.50 ^{bc}	2.57±1.15 ^{bc}	18.54±2.45 ^b	10.88±1.81 ^{ab}	22.42±2.45 ^{bc}	28.89±3.21 ^d
GUANGDONG	10.96±1.61 ^{bc}	3.30±0.14 ^{ab}	19.60±1.42 ^b	10.64±1.69 ^{ab}	23.88±0.42 ^b	45.77±2.01 ^a

Results are expressed as Mean ± SEM. Values with different letters (a, b, c, etc.) differ significantly ($p=0.05$) by Duncan's Multiple Range Test (DMRT)

Table 4: Variation in trichome and idioblast attributes of six mulberry genotype under study

CULTIVAR	Trichome Length (µm)	Trichome Breadth (µm)	Trichome density (per mm ²)	Idioblast density (per mm ²)
S1635	223.00±7.28 ^b	30.34±3.21 ^b	38.28±1.39 ^c	21.77±1.11 ^d
TR10	156.00±3.26 ^d	24.04±2.96 ^c	34.26±0.96 ^d	18.55±0.55 ^d
S1	253.00±11.89 ^a	43.66±1.38 ^a	28.36±2.41 ^e	28.24±1.81 ^c
BC 259	89.40±5.12 ^e	15.20±2.24 ^d	55.08±0.89 ^a	32.51±2.09 ^b
V1	216.00±9.63 ^b	28.24±2.59 ^{bc}	47.86±1.55 ^b	35.89±2.14 ^b
GUANGDONG	189.00±7.05 ^c	26.03±3.01 ^{bc}	37.24±1.72 ^{cd}	45.29±2.56 ^a

Results are expressed as Mean ± SEM. Values with different letters (a, b, c, etc.) differ significantly ($p=0.05$) by Duncan's Multiple Range Test (DMRT)

index (fig 1) were enlisted, that may serve as an effective tool in distinguishing the six genotypes under microscopic study. The number of vein islet was recorded highest in S1 genotype (17.04/mm²) while Guangdong bears the

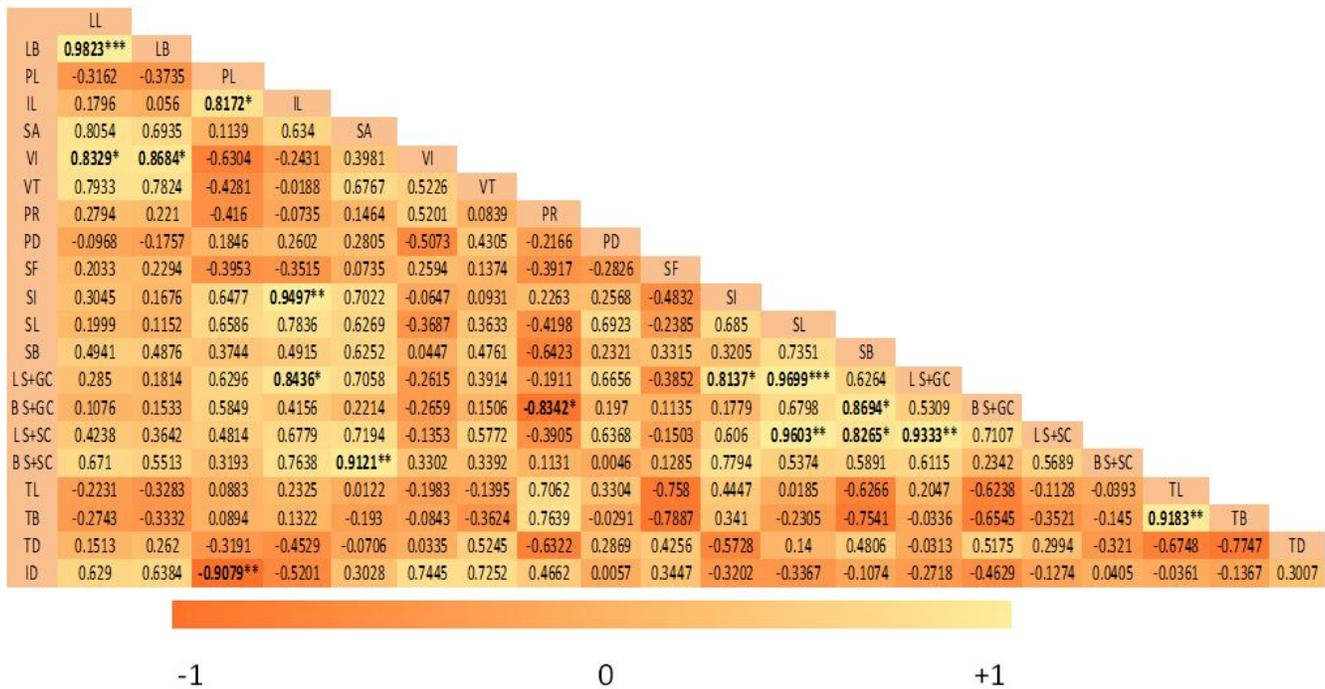
largest number of vein termination (19.62/mm²). The palisade diameter in S1 genotype was the smallest of all and thus its palisade ratio is highest. In all mulberry varieties stomata are present on abaxial surface (Kumar

Table 5: ANOVA analysis representing the significance level among different parameters under study

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Mulberry genotype	5.2E+10	20	2.6E+09	9.808575	4.1E-16	1.671357
Morphological traits	2.78E+10	105	2.65E+08			
Total	7.98E+10	125				

Significant at p<0.01 level

Table 6: Pearson correlation coefficient matrix of different traits under study.



*, **, ***; Significant at p = 0.05, 0.01, 0.001 respectively

LL = Lamina Length, LB = Lamina Breadth, PL = Petiole Length, IL = Internodal Length, SA = Surface Area, VI = Vein Islet, VT = Vein Termination, PR = Palisade Ratio, PD = Palisade Diameter, SF= Stomatal Frequency, SI = Stomatal Index, SL = Stroma Length, SB = Stroma Breadth, L S+GC = Length of Stroma and Guard Cell, B S+GS = Breadth of Stroma and Guard Cell, L S+SC = Length of stomata and subsidiary cell, B S+SC = Breadth of Stomata and subsidiary cell, TL = Trichome Length, TB = Trichome Breadth, TD = Trichome Density, ID = Idioblast Density

et al., 2012). Fagundez and Izco (2011) stated that stomata were one of the fundamental taxonomic characters of leaves that take part in transpiration and gaseous exchange during photo-assimilation. In the present study TR10 genotype bears the highest frequency of stomata (476/mm²) while S1 bears least number of stomata per millimetre square of microscopic field. Susheelamma and Jolly (1988) observed that the mulberry variety that bears lower stomatal frequency and least pour size is better adapted in tropical countries, as S1 genotype bears the least frequency of stomata (238.05/mm²) which may be the reason for its better adaptability and wide acceptability in tropical and sub-tropical region. On comparing the stomatal index it was observed that S1635 tops the list (22.62%) while V1 lowers the list (12.45%), the increasing order of stomatal index was as follows V1 < BC259 <

TR10 < S1 < Guandong < S1635.

The size of stomata, stroma opening and its adjoining cells play important role in proper adaptation of different varieties at different location. Susheelamma and Datta (1993) reported that the mulberry genotype that bears small stomatal size can retain the moisture content of leaves more efficiently to that of those that bears large stomatal openings. Beside this, photosynthetic efficiency is dependent to some extent over stomatal frequency and size of stomata present on leaf surface (Maghsoudi and Moud, 2008). Table 3 comprises different stomatal attributes which are useful for proper adaptation of a genotype at a particular location, as well as in distinguishing different genotypes. The present study reveals that among the six genotypes S1 bears smallest stroma length (7 μm) and breadth (1.99μm). When the length and breadth of

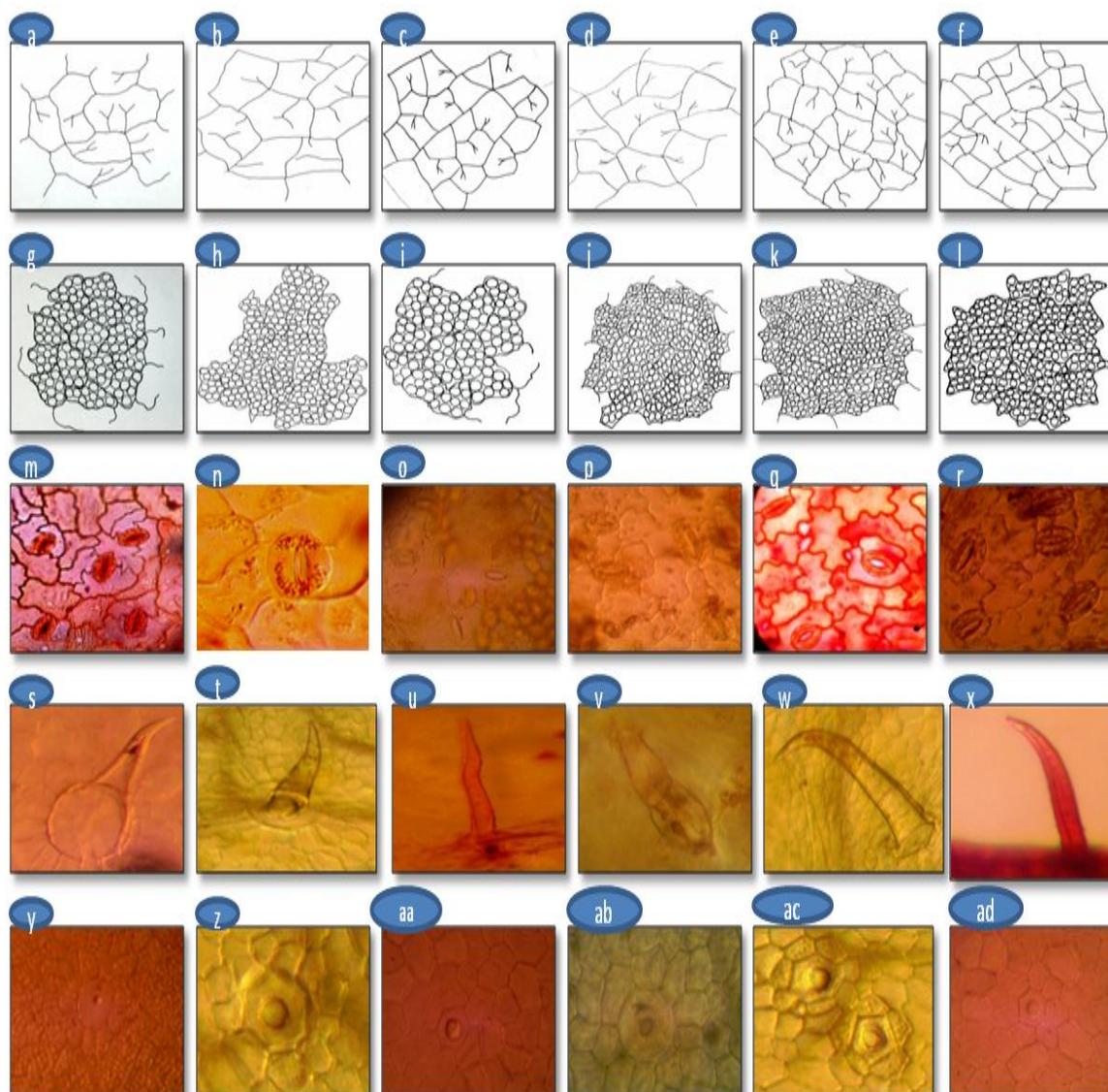
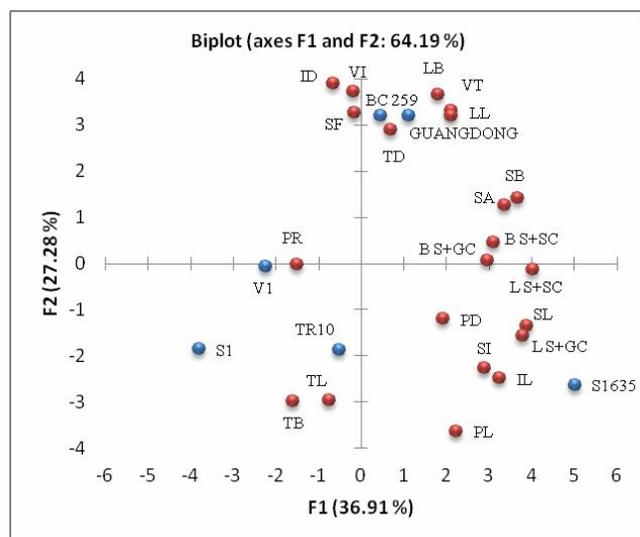


Fig. 1: Photo plate representing images of different micro-morphological foliar parameters under study of six studied genotypes namely S1635, TR10, S1, BC259, V1, Guangdong respectively. **a-f.** Camera lucida drawing representing vein islet and vein termination; **g-l.** Camera lucida drawing representing palisade cells; **m-r.** Light microscopic image of stomata; **s-x.** Light microscopic image of trichome; **y-ad.** Light microscopic image of idioblast

both stroma and guard cell together was compared, it was observed that S1 was lowest. Thus it may be stated that as frequency of stomata is least in S1 genotype, beside this it also bears least size of stroma and guard cell thus it may be able to retain the moisture content in a better way as compared to other genotypes helping in its greater adaptability in tropical and sub-tropical areas.

Present study also reveals wide variation in trichome length and breadth in all the genotypes (table 4). Presence of both glandular and non-glandular trichomes was noted. BC259 had the shortest trichomes (89.40 μm) while the genotype S1 was observed with longest trichomes (253 μm). Trichome exhibits mechanical barrier against herbivory caused by insects (Baur *et al.*, 1991). On

comparing trichome and idioblast density it was observed that the genotype BC259 and Guangdong bears the highest number of trichome and idioblast per square millimetre of microscopic field respectively while S1 and TR10 bears the least number of trichomes and idioblasts respectively. Kesavacharyulu *et al.* (2004) stated that type and density of trichome present in a particular genotype puts effect on overall acceptability of leaves by silkworm larvae and it has also been reported that acceptability of leaves by larvae decreases with the increase in trichome density. Singhal *et al.*, (2010) stated that in sericulture industry acceptability of leaves by silkworm larvae is most important than any other parameters. As S1 genotype bears least trichome density,



LL = Lamina Length, LB = Lamina Breadth, PL = Petiole Length, IL = Internodal Length, SA = Surface Area, VI = Vein Islet, VT = Vein Termination, PR = Palisade Ratio, PD = Palisade Diameter, SF = Stomatal Frequency, SI = Stomatal Index, SL = Stroma Length, SB = Stroma Breadth, L S+GC = Length of Stroma and Guard Cell, B S+GS = Breadth of Stroma and Guard Cell, L S+SC = Length of stomata and subsidiary cell, B S+SC = Breadth of Stomata and subsidiary cell, TL = Trichome Length, TB = Trichome Breadth, TD = Trichome Density, ID = Idioblast Density

Fig. 2: Principal component analysis of six mulberry genotype under study (blue dots) along with variable morphological traits (red dots)

it may be stated that it will be more acceptable in silk industry than any other genotypes.

To determine the degree of significance between different attributes under study ANOVA analysis was preformed. From ANOVA analysis (table 5) it can be said that different micro-morphological leaf attributes were significantly related with different genotypes under study at $p < 0.01$ level. From table 6 it was observed that lamina length was positively and significantly correlated with lamina breadth ($r = 0.9823^{***}$) and vein islets ($r = 0.8329^*$). Lamina breadth was also positively and significantly associated with vein islet ($r = 0.8684^*$). Petiole length have positive correlation with internodal length ($r = 0.8172^*$) and negative correlation with idioblast density ($r = -0.9079^{**}$). Internodal length bears positive and significant association with stomatal index ($r = 0.9497^{**}$) and length of stroma + guard cell ($r = 0.8436^*$). Surface area correlate positively with breadth of stomata + subsidiary cell ($r = 0.9121^{**}$). Palisade ratio was negatively correlated with breadth of stroma + guard cell ($r = -0.8342^*$). Stomatal index was positively and significantly correlated with stroma breadth ($r = 0.8137^*$). Stroma length was positively and significantly correlated

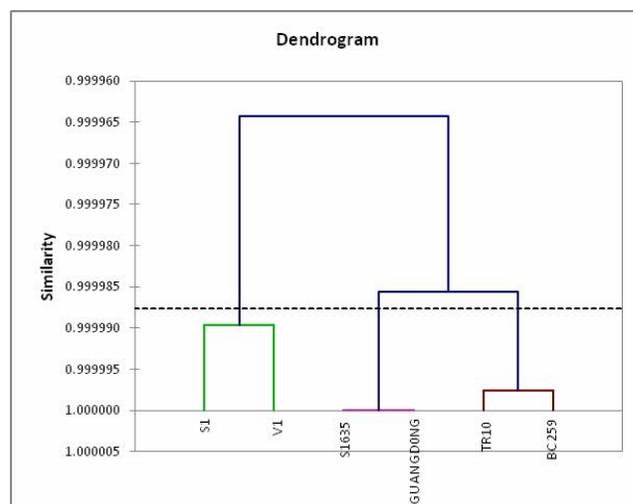


Fig 3: Dendrogram representing similarity clusters of six mulberry genotype under study with respect to overall parameters

with length of stroma + guard cell ($r = 0.9699^{***}$) and length of stomata + subsidiary cell ($r = 0.9603^{**}$), while stroma breadth was positively correlated with breadth of stroma + guard cell ($r = 0.8694^*$) and length of stomata + subsidiary cell ($r = 0.8265^*$). Positive and significant correlation was also observed between length of stroma + guard cell with length of stomata + subsidiary cell ($r = 0.9333^{**}$) and trichome length with trichome breadth ($r = 0.9183^{**}$). Thus from these outcome it may be stated that most significant attributes are positively correlated with each other. Lamina length was directly correlated with lamina breadth, similarly stomata and trichome attributes were also positively correlated with each other. The positive correlation between internodal lengths with petiole length is very significant which suggest that developing leaves get sufficient space for proper growth; besides this the upper whorled leaves was not overlapping the lower leaves due to which photosynthetic efficiency might be enhanced leading to accumulation of more photosynthate. Vein islet is responsible for distribution of photosynthetic products and it was positively correlated with length and breadth of lamina, which reveals that greater the leaf size more is the number of vein islet and thereby greater the distribution of sugar leading to the development of healthy leaves which may serve the industrial requirement. These findings are significant as it may help to identify proper cultivar for proper purpose.

For better understanding the relationship between variables with clustering groups Principal Component Analysis (PCA) was performed. The first two principal components account for 36.91% and 27.28% of the data variance (fig 2). The scoring system of first principal component (PC1) is dominated by internodal length,

surface area, palisade ratio, palisade diameter, stomatal index, stroma length, stroma breadth, combine length and breadth of stroma and guard cell and combine length and breadth of stomata and subsidiary cell. The first axis is dominated by a group of 3 species namely S1635, S1, V1. The scoring system of second principle component (PC2) is dominated by lamina length and breadth, petiole length, vein islet, vein termination, stomatal frequency, trichome length and breadth, trichome density and idioblast density. In second axis a group of 3 species TR10, BC259 and Guangdong occupy their position. PCA analysis reveals that lamina length and breadth are positively correlated, while both are directly correlated with stomatal frequency, stomatal index and stoma length and breadth both singly or in association with length of guard cell and subsidiary cell. This finding suggest us the fact that with increase in lamina size, stomatal attributes will also increase simultaneously. From this it may be stated that mulberry genotype with smaller leaves are more preferable because with increase in size of leaves, number of stomata also increases which might reduce the moisture level of leaves thereby decreasing leaf quality.

Fig 3 represents agglomerative hierarchical clustering (AHC) of different genotypes with respect to the micro – morphological attributes used in the present study. AHC was performed using Pearson similarity dendrogram. AHC study reveals that S1 genotype shows high degree of resemblance with V1 genotype and thus grouped together. Besides these two more clusters were formed: one is by S1635 and Guangdong genotype while another group was formed by TR10 and BC259.

Conclusion

Thus from the above study it was observed that all the six genotypes of mulberry bears considerable variation with respect to each other. Morphological variation clusters the six genotypes into three groups. The first group is represented by S1 and V1 genotype which is characterized by small size leaves with small stomatal aperture and less trichome density. The second group is formed by S1635 and Guangdong which is represented by large size leaves with greater internodal distance, large stomatal aperture and high stomatal frequency. The last group was represented by TR10 and BC259 moderate leaf size with high stomatal frequency. This trait can be utilized for screening out better mulberry genotype which will improve silk worm rearing system and many other industrial purposes.

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