

LEAF SURFACE SCANNING ELECTRON MICROSCOPY OF 16 MULBERRY GENOTYPES (*Morus* spp.) WITH RESPECT TO THEIR FEEDING VALUE IN SILKWORM (*Bombyx mori* L.) REARING

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ABSTRACT

Mulberry (*Morus* spp.) is the only silkworm (*Bombyx mori* L.) food plant. In Indian sub tropics, S-146 is the only popular and ruling mulberry genotype for silkworm rearing. As a result, mulberry leaf availability is always the limiting factor, and therefore, sub tropics are contributing less than 1% of the country's total silk production compared with more than 60% under tropical conditions. Besides climatic conditions, this is due to a very limited number of mulberry genotypes available in this region for silkworm rearing. However, in the mean time, 15 mulberry genotypes viz. 'Tr-10', 'Chinese White', 'K-2', 'Sujanpur Local', 'BC₂-59', 'S-1635', 'C-1730', 'Mandalaya', 'S-30' (Vishala), 'RFS-175', 'Anantha', 'C-2016', 'C-2017', 'S-41' and 'V-1' were also introduced in the sub tropics, but remained unexplored. In sericulture, leaf surface is also an important parameter for, both, the silkworm's acceptability of not having any feeding impediment and the mulberry improvement programs. The objective of this study was to explore the possibilities of using these 16 mulberry genotypes for their leaf surface characteristics by scanning electron microscopy and using them for sericulture. Based on leaf yield, stomatal size, stomatal number per unit of area and trichomes and idioblasts length, these genotypes were grouped into different categories. The mulberry genotype 'Mandalaya', in addition to the ruling genotype 'S-146' excelled because of their higher leaf yield and desired leaf surface characteristics. Furthermore, the genotypes 'K-2', 'S-41' and 'Sujanpur Local' are also suggested to develop high yield mulberry genotypes in the Indian sub tropics.

Key words: *Morus indica*, scanning electron microscopy, stomata, trichomes, idioblasts, leaf yield, sericulture.

INTRODUCTION

Mulberry (*Morus* spp.) is the sole food plant of silkworms (*Bombyx mori* L., Lepidoptera: Bombycidae) and its leaf quality has a great role in silkworm rearing, which in turn affects the overall silk production (Singhal *et al.*, 2001; 2006). In India, sericulture is mostly practiced in tropical region which is contributing more than 60% of the total country's silk production. It is mainly due to congenial climatic conditions and many high yielding mulberry genotypes evolved time to time. These genotypes have been characterized for leaf surface parameters under tropical climatic conditions (Singhal *et al.*, 1999) which is serving

as a useful tool for mulberry improvement programs. But, the sub-tropical India still contributes to only less than 1% of the total country's silk production due to very limited number of mulberry genotypes available for silkworm rearing. For this purpose, 'S-146' is the only popular and most commonly used mulberry genotype exists so far, and as a result, there is always shortage of mulberry leaf for silkworm rearing. But, meanwhile, many more mulberry genotypes viz. 'Tr-10', 'Chinese White', 'K-2', 'Sujanpur Local', 'BC₂-59', 'S-1635', 'C-1730', 'Mandalaya', 'S-30' (Vishala), 'RFS-175', 'Anantha', 'C-2016', 'C-2017', 'S-41' and 'V-1' have also been introduced in this region but remained unexplored. To exploit these genotypes for sericulture in Indian sub tropics, the present study was undertaken for their leaf surface characteristics which may serve as guideline information for evolving high yielding mulberry genotypes, besides having their role in feeding behavior to the silkworms.

Southwood (1986) opined that mulberry leaf surface determines its adaptation to varying eco-climates,

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exchange of water vapor, CO₂ and O₂ through stomatal pores and the feeding quality to the silkworms. Susheelamma and Jolly (1986) have correlated leaf stomatal size and frequency with adaptation of mulberry in tropical India. Kesavacharyulu *et al.* (2004) reported that higher density of trichomes on mulberry leaf surface reduces the leaf acceptability to the silkworms, even if the leaf quality is biochemically superior. Therefore, the leaf surface trichomes have been considered as very significant for the mulberry breeders in identifying potential genotypes as an early selection process without going for actual rearing test. Peter *et al.* (1995) found that plant trichomes also have a role in insect resistance, since, growth and development of large bodied insects may be retarded due to feeding hindrance by the trichomes. Their high density causes mulberry foliage's unacceptability to the insects due to physical hindrance (Singh *et al.*, 1971; Levin, 1973). Idioblasts have also been found to have a significant role in determining feeding quality of the mulberry leaf to the silkworms (Melikyan and Babyan, 1971). Mulberry leaf surface trichomes and idioblasts have been used for genotypes identification in Japan by Fujita and Uchikawa (1986), whereas Katsumata (1972) has classified mulberry trees on the basis of idioblasts. Shah and Kachroo (1975) stated that the information about trichomes may throw light on the preference for certain foliage by the silkworm. Biasiolo *et al.* (2004) found micro morphological features of 10 mulberry cultivars as an important tool for mulberry description.

Therefore, in Indian sub tropics, where only one genotype namely S-146 is used for silkworm rearing, there is a scope of increasing the silk production from at present of 1% to more, but, only when more number of mulberry genotypes are explored for sericulture to meet the overall leaf requirement. In this regard, the present study was undertaken to evaluate potential mulberry genotypes out of 16 genotypes introduced in Indian sub tropics for higher leaf yield and the feeding quality to the silkworms.

MATERIALS AND METHODS

The present study was carried out in 16 genotypes of mulberry (*Morus indica* L.): 'S-30' (Vishala), 'S-1635', 'S-146', 'Anantha', 'Mandalaya', 'C-1730', 'C-2016', 'C-2017', 'RFS-175', 'Tr-10', 'V-1', 'S-41', 'K-2', 'Chinese White', 'BC₂-59' and 'Sujanpur Local' grown under bush type of plantation at 0.9 x 0.9 m spacing in between plants and rows at experimental farm of Regional Sericultural Research Station (32°43' N, 74°54' E; 250 m.a.s.l.), Miran Sahib, Jammu, India. The type of

soil was sandy clay loam, pH 7.0 (66.65% sand, 24.26% clay and 9.09% silt with fertility level of 180.8 kg N ha⁻¹, 16.0 kg P ha⁻¹ and 165.0 kg K ha⁻¹). The leaf samples for scanning electron microscopy were prepared according to the method of Bozzola and Russell (1992). The tenth leaf from the first fully opened top leaf of the longest branch of each genotype was cut into 3 mm² pieces and subsequently fixed in 2.5% glutaraldehyde (prepared in 0.2 M sodium cacodylate buffer, pH 7.2) for 2 h. The samples were then postfixed for 3 h in 2% osmium tetroxide and dehydrated in an alcohol-acetone series at room temperature of 25 ± 2 °C. The dehydrated materials were then dried in a critical point drier (EMS-850, Electron Microscopy Sciences, Hatfield, Pennsylvania, USA) using CO₂ as the transition fluid. The dried samples were mounted on the copper stubs, keeping the abaxial leaf surface up by using double-sided sticky tape, and then coated with gold (20 nm thickness) in a sputter coater (EMS-550, Electron Microscopy Sciences, Hatfield, Pennsylvania, USA). The coated samples were examined under scanning electron microscope (JEOL 100 CX II-ASID 4D, Tokyo, Japan) at 20 kV. The leaf surface features *viz.* stomatal size and their frequency, length of trichomes and idioblasts were measured in 25 fields of each 10 different leaf samples from different plants in each genotype. The leaf yield data were also recorded during spring season (main silkworm rearing season during March-April contributing 90% of the total silk production in sub tropics) from the sample of 96 plants at 0.9 x 0.9 m spacing in between plants and rows in a plot area of 94.77 m² replicated three times in randomized block design (total area 284.31 m²) and calculated for 1 ha in each genotype. Data collected for 3 yr were averaged. All data were calculated for means and standard error.

RESULTS AND DISCUSSION

The pedigree and leaf yield of 16 mulberry genotypes, namely, 'S-30' (Vishala), 'S-1635', 'S-146', 'Anantha', 'Mandalaya', 'C-1730', 'C-2016', 'C-2017', 'RFS-175', 'Tr-10', 'V-1', 'S-41', 'K-2', 'Chinese White', 'BC₂-59' and 'Sujanpur Local' grown in Indian sub tropics are presented in Table 1. The mulberry genotype S-30 (Vishala) revealed highest leaf yield of 7373.80 kg ha⁻¹ season⁻¹ followed by 6764.34 kg ha⁻¹ season⁻¹ in 'S-1635'. The lowest leaf yield (3249.38 kg ha⁻¹ season⁻¹) was recorded in the genotype 'Sujanpur Local' followed by 'BC₂-59' (4038.27 kg ha⁻¹ season⁻¹). When mulberry genotypes were categorized on the basis of range in leaf yield, the genotypes 'C-1730', 'Mandalaya', 'Anantha', 'S-146', 'S-1635' and 'S-30' (Vishala) were found with higher leaf yield, more than 6000 kg ha⁻¹ season⁻¹

Table 1. Pedigree and leaf yield potential of different mulberry genotypes under sub tropics.

Mulberry genotype	Origin	Pedigree	Leaf yield per season kg ha ⁻¹
S-30 (Vishala)	Indigenous (Mysore, India)	Natural selection	7373.80
S-1635	Indigenous (Berhampore, India)	Selection from seedlings of open pollinated seeds collected from mother plant CSRS 1	6764.34
S-146	Indigenous (Berhampore, India)	Open pollinated hybrid selection	6476.54
Anantha	Indigenous (Ananthpur, India)	Selection from the population of mulberry var. RFS-135	6352.31
Mandalaya	Indigenous (Berhampore, India)	Clonal selection	6211.31
C-1730	Indigenous (Berhampore, India)	Triploid cross T ₂₅ tetraploid mother and S-162 diploid male parent	6121.31
C-2016	Indigenous (Berhampore, India)	Open pollinated hybrid selection	5954.94
C-2017	Indigenous (Berhampore, India)	Open pollinated hybrid selection	5736.27
RFS-175	Indigenous (Mysore, India)	Open pollinated hybrid selection	5722.88
Tr-10	Indigenous (Berhampore, India)	Cross between colchicine induced tetraploid of (<i>Morus indica</i> x S-1) x Philippine (2x)	5681.48
V-1	Indigenous (Mysore, India)	Hybrid of mulberry genotypes S-30 x Berhampore C776	5516.42
S-41	Indigenous (Mysore, India)	Ethyl methane sulfate (EMS) seed mutant	5241.47
K-2	Indigenous (Mysore, India)	Selection from the seedlings population	5037.56
Chinese white	Indigenous (Pampore, India)	Clonal selection	4659.87
BC ₂ -59	Indigenous (Berhampore, India)	Back crossing of female plant (<i>M. indica</i> var. Matigari x Kosen) and with recurrent parent Kosen	4038.27
Sujanpur Local	Indigenous (Jammu, India)	Open pollinated hybrid selection	3249.38
Critical Difference at 5%			391.40

under category I, while other genotypes ranked between categories II and III being the lowest leaf yield of 3249.38 kg ha⁻¹ season⁻¹ in Sujanpur Local (Table 2).

Likewise, all genotypes differed in the stomatal size (Figures 1 and 2), stomatal number per 1333.2 μm^2 area (Figures 3 and 4) and trichome and idioblast lengths (Table 3, Figures 5 to 6). Based on four categories of

variation in stomatal size, a very small size of stomata (15.609 μm) was observed in the genotype Sujanpur Local under category I. On the other side, the genotype BC₂-59 was observed with exceptionally very large size of stomata (99.638 μm) falling under category IV (Table 4). Similarly, stomatal number per 1333.2 μm^2 area varied between 8 and 16.

Table 2. Categories for variation in leaf yield potentiality of different mulberry genotypes in Indian sub tropics.

Category	Range in leaf yield potentiality	Mulberry genotypes
I	> 6000 kg ha ⁻¹ season ⁻¹	C-1730, Mandalaya, Anantha, S-146, S-1635 and S-30 (Vishala)
II	5000-6000 kg ha ⁻¹ season ⁻¹	K-2, S-41, V-1, Tr-10, RFS-175, C-2016 and C- 2017
III	< 5000 kg ha ⁻¹ season ⁻¹	Chinese white, BC ₂ -59 and Sujapur Local

When categorized, the genotypes BC₂-59, C-1730, Mandalaya, Tr-10, C-2017, K-2 and Sujapur Local were found with less than 10 stomata in 1333.2 μm^2 area falling under category I (Table 5). However, the genotypes RFS-175, Anantha, V-1, S-30 (Vishala) and S-41 were recorded with more than 15 stomata falling under category III. The genotype BC₂-59, which revealed largest stomatal size had less than 10 stomata per unit of area. Most of the genotypes *viz.* S-41, K-2, Tr-10, C-2017, S-30 (Vishala), Mandalaya, V-1, Chinese white and C-2016 ranged under the category II of size 25-50 μm . In this regard, Susheelamma and Datta (1993)

have correlated small stomatal size with leaf moisture retention capacity maintaining high plant water potential in mulberry. It is therefore the reason for very poor leaf yield of BC₂-59 (4038.270 kg ha⁻¹ season⁻¹), as maximum water might be transpiring through very large stomata. In Indian sub tropics, where mulberry is cultivated only under rainfed conditions, the genotype may not be of much use for cultivation practices being poor leaf yielder with undesirable leaf surface characteristics.

The present study also revealed a wide variation in trichome and idioblast lengths in all the genotypes. The trichomes were found glandular and idioblasts were

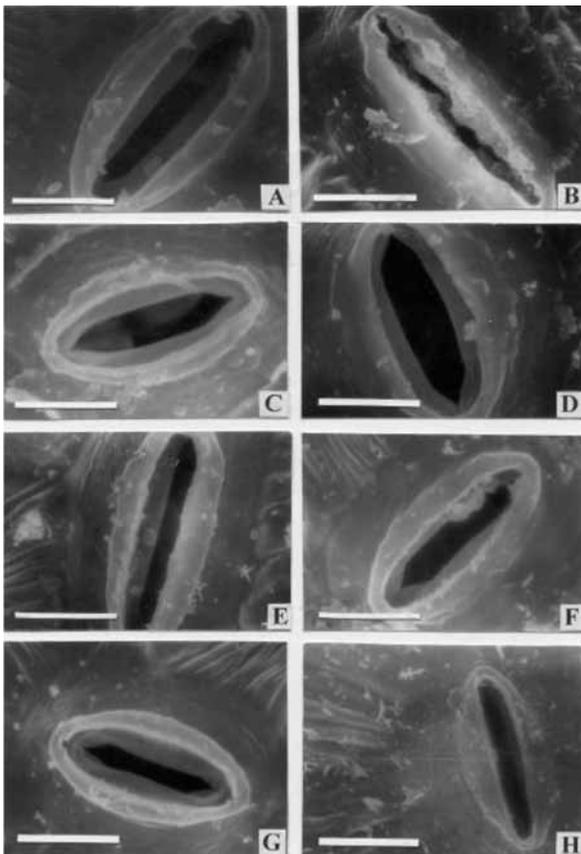


Figure 1. Stomatal size in mulberry genotype BC₂-59 (A), S-1635 (B), RFS-175 (C), C-1730 (D), S-146 (E), Anantha (F), C-2016 (G), Chinese white (H). Scale bar = 5 μm . Magnification = 4000X.

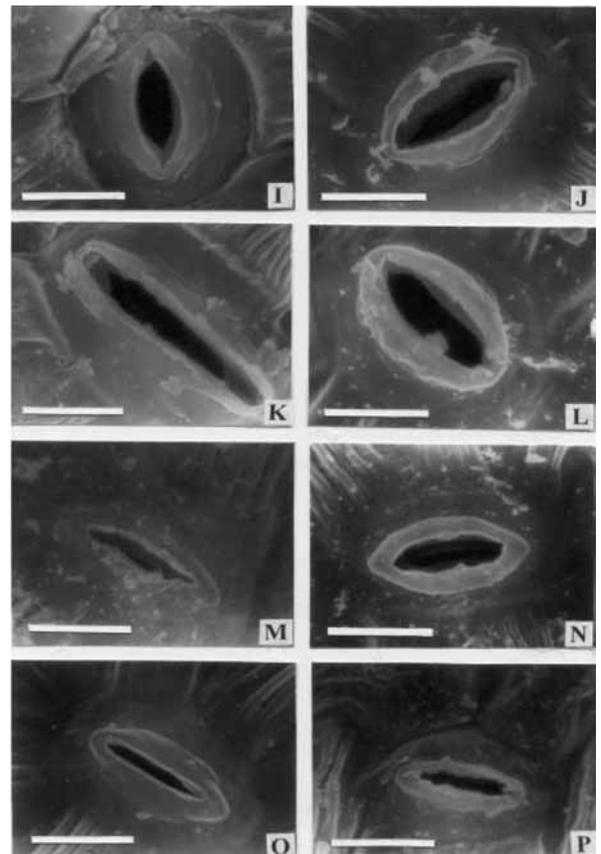


Figure 2. Stomatal size in mulberry genotype V-1 (I), Mandalaya (J), S-30 (Vishala) (K), C-2017 (L), Tr-10 (M), K-2 (N), S-41 (O) and Sujapur Local (P). Scale bar = 5 μm . Magnification = 4000X.

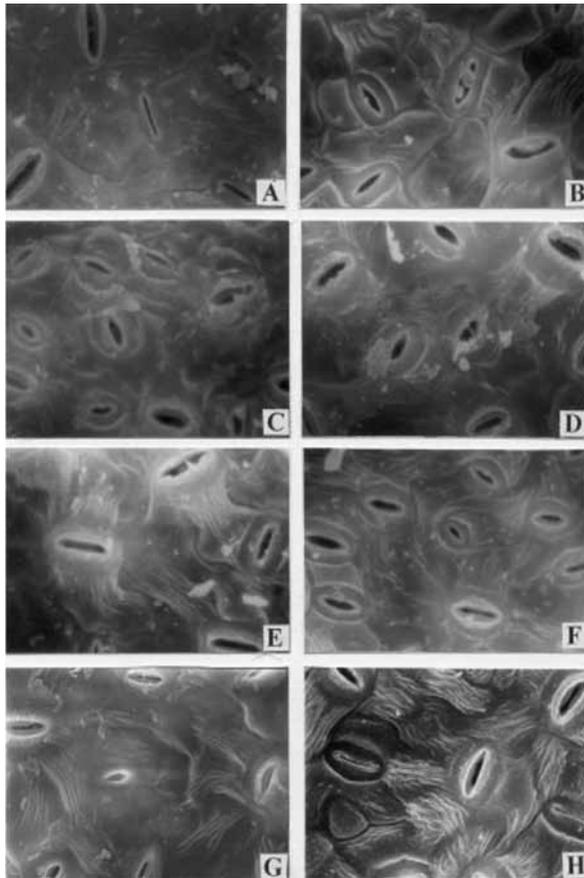


Figure 3. Number of stomata per $1333.2 \mu\text{m}^2$ area in mulberry genotype BC₂-59 (A), S-1635 (B), RFS-175 (C), C-1730 (D), S-146 (E), Anantha (F), C-2016 (G), Chinese white (H). Magnification = 1500X.

long or short notched, with hemispherical shape, having microtubercules all around their surface. The genotypes S-146, Mandalaya, K-2, S-41 and Sujapur Local had short trichomes as well as idioblasts (Tables 6 and 7) falling under the category I. The genotype S-30 (Vishala) was observed with longest trichomes as well as idioblasts falling under the category III. It clearly indicates that though this genotype had highest leaf production, may have poor acceptability to the silkworms in accordance with Kesavacharyulu *et al.* (2004). As is the complex situation in sericulture, leaf acceptability to the silkworms is more important than any other parameter. Further, the genotypes BC₂-59, V-1 and RFS-175 may not be desirable for Indian sub tropics because of poor leaf yield and leaf surface characteristics having long trichomes and idioblasts. Among all genotypes, Mandalaya seems

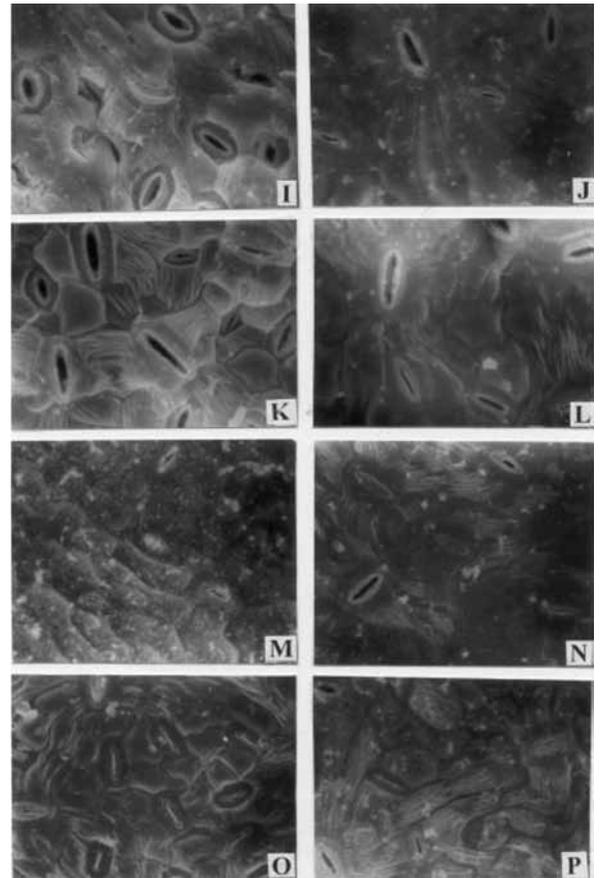


Figure 4. Number of stomata per $1333.2 \mu\text{m}^2$ area in mulberry genotype V-1 (I), Mandalaya (J), S-30 (Vishala) (K), C-2017 (L), Tr-10 (M), K-2 (N), S-41 (O) and Sujapur Local (P). Magnification = 1500X.

to be very promising having higher leaf yield with small stomatal size, less number of stomata per unit of area and shorter trichome and idioblast lengths.

CONCLUSIONS

The present study suggests Mandalaya as the most promising mulberry genotype with higher leaf yield and shorter trichome and idioblast lengths for silkworm rearing in addition to the only ruling genotype S-146 presently used under sub tropical conditions. The study reveals that the mulberry genotypes S-146, Mandalaya, K-2, S-41 and Sujapur Local can be exploited for mulberry improvement programs considering favorable leaf surface characteristics in Indian sub tropics.

Table 3. Variation in stomatal size and their frequency and trichomes and idioblasts length in different mulberry genotypes of Indian sub tropics.

Mulberry genotypes	Stomatal size (L x W)	Stomatal number per 1333.2 μm^2 area	Trichomes length	Idioblasts length
	— μm —		— μm —	
BC ₂ -59	99.638 ± 0.085	08.001 ± 0.024	29.124 ± 0.193	17.997 ± 0.126
S-1635	61.829 ± 0.103	11.017 ± 0.015	24.207 ± 0.088	24.783 ± 0.133
RFS-175	60.414 ± 0.110	15.116 ± 0.042	21.142 ± 0.081	24.314 ± 0.248
S-146	57.573 ± 0.091	11.131 ± 0.017	18.958 ± 0.104	11.704 ± 0.249
C-1730	56.476 ± 0.094	08.023 ± 0.027	21.141 ± 0.055	22.056 ± 0.077
Anantha	51.367 ± 0.103	15.017 ± 0.044	22.315 ± 0.078	14.018 ± 0.157
C-2016	47.638 ± 0.123	10.288 ± 0.017	19.147 ± 0.075	18.401 ± 0.078
Chinese white	47.542 ± 0.146	11.996 ± 0.034	20.075 ± 0.217	11.995 ± 0.163
V-1	47.211 ± 0.089	15.101 ± 0.045	21.101 ± 0.088	15.120 ± 0.238
Mandalaya	46.623 ± 0.115	08.025 ± 0.166	17.074 ± 0.148	13.997 ± 0.052
S-30 (Vishala)	46.276 ± 0.082	16.101 ± 0.050	25.781 ± 0.178	30.118 ± 0.088
C-2017	40.103 ± 0.133	09.344 ± 0.087	22.085 ± 0.166	14.034 ± 0.105
Tr-10	36.968 ± 0.079	08.125 ± 0.019	22.926 ± 0.189	21.005 ± 0.164
K-2	31.924 ± 0.116	09.212 ± 0.007	19.077 ± 0.165	14.005 ± 0.163
S-41	28.489 ± 0.124	15.012 ± 0.088	16.974 ± 0.023	12.011 ± 0.141
Sujanpur Local	15.609 ± 0.088	08.005 ± 0.073	16.351 ± 0.164	11.712 ± 0.116

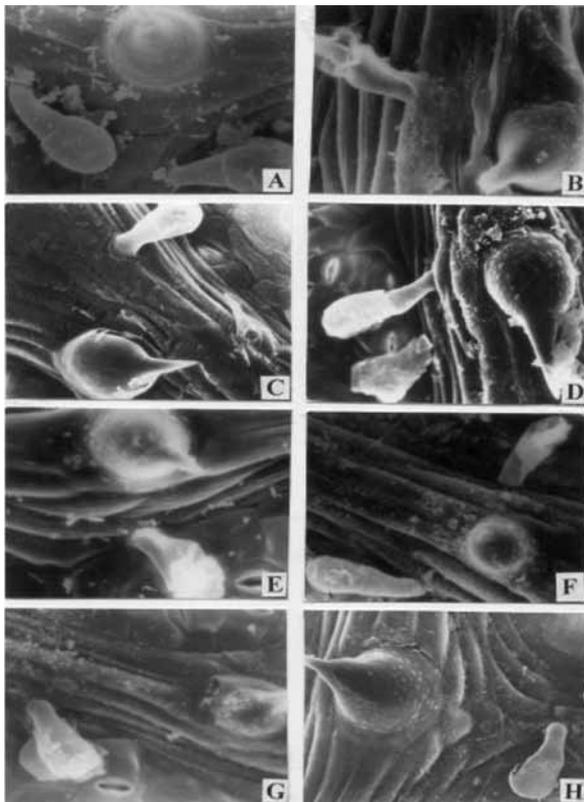


Figure 5. Trichomes and idioblasts in mulberry genotype BC₂-59 (A), S-1635 (B), RFS-175 (C), C-1730 (D), S-146 (E), Anantha (F), C-2016 (G), Chinese white (H). Magnification = 1000X.

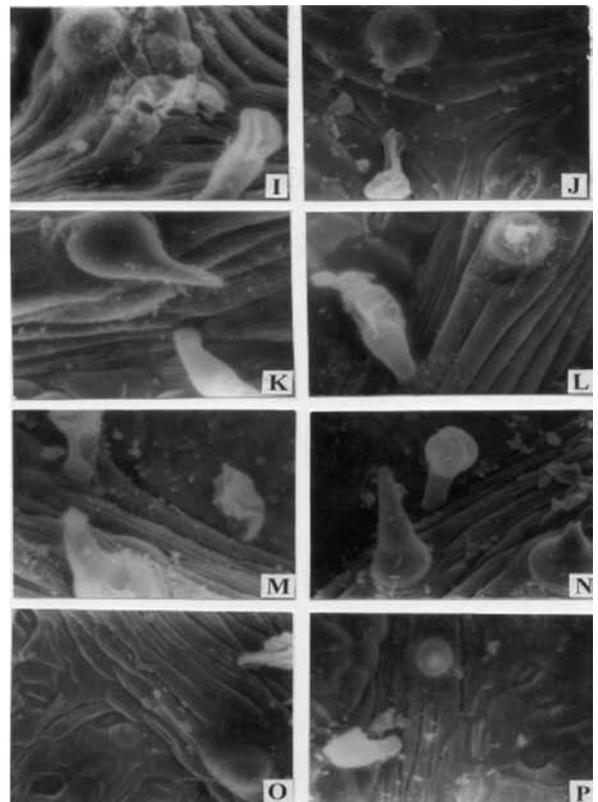


Figure 6. Trichomes and idioblasts in mulberry genotype V-1 (I), Mandalaya (J), S-30 (Vishala) (K), C-2017 (L), Tr-10 (M), K-2 (N), S-41 (O), and Sujanpur Local (P). Magnification = 1000X.

Table 4. Different categories for variation in stomatal size of different mulberry genotypes in Indian sub tropics.

Category	Range of stomatal size (L x W)	Mulberry genotypes
I	Very small size (< 25 μm)	Sujanpur Local
II	Small size (25-50 μm)	S-41, K-2, Tr-10, C-2017, S-30 (Vishala), Mandalaya, V-1, Chinese white, and C-2016
III	Medium size (50-75 μm)	Anantha, C-1730, S-146, RFS-175, and S-1635
IV	Large size (> 75 μm)	BC ₂ -59

Table 5. Different categories for variation in stomatal number per 1333.2 μm^2 area of different mulberry genotypes in Indian sub tropics.

Category	Range of stomatal number	Mulberry genotypes
I	Low (< 10)	BC ₂ -59, C-1730, Mandalaya, Tr-10, C-2017, K-2, and Sujanpur Local
II	Medium (10-15)	S-1635, S-146, C-2016, and Chinese white
III	High (> 15)	RFS-175, Anantha, V-1, S-30 (Vishala), and S-41

Table 6. Different categories for variation in trichome length of different mulberry genotypes in Indian sub tropics.

Category	Range of trichome length	Mulberry genotypes
I	Short (< 20 μm)	S-146, C-2016, Mandalaya, K-2, S-41, and Sujanpur Local
II	Medium (20-25 μm)	S-1635, RFS-175, C-1730, Anantha, Chinese white, V-1, C-2017, and Tr-10
III	Long (> 25.00 μm)	BC ₂ -59 and S-30 (Vishala)

Table 7. Different categories for variation in idioblast length of different mulberry genotypes in Indian sub tropics.

Category	Range of idioblasts length	Mulberry genotypes
I	Short (< 15 μm)	S-146, Anantha, Chinese white, Mandalaya, C-2017, K-2, S-41, and Sujanpur Local
II	Medium (15-25 μm)	BC ₂ -59, C-1635, RFS-175, C-1730, C-2016, V-1, and Tr-10
III	Long (> 25 μm)	S-30 (Vishala)

RESUMEN

Microscopía electrónica de barrido de la superficie foliar de 16 genotipos de *Morus* spp. en relación a su valor alimenticio para crianza del gusano de la seda (*Bombyx mori* L.). La morera (*Morus* spp.) es la única planta de alimento para el gusano de la seda (*Bombyx mori* L.). En los sub-trópicos de la India, 'S-146' es el único genotipo popular y predominante de morera para criarlo. Como resultado, la disponibilidad de hojas es siempre el factor limitante y, por lo tanto, los sub-trópicos contribuyen con menos de 1% del total de la producción nacional de seda comparado con más de 60%

bajo condiciones tropicales. Además de las condiciones climáticas, esto se debe al número muy limitado de genotipos de morera disponible en esta región para la crianza de gusanos de seda. Se introdujeron 15 genotipos de morera (Tr-10', 'Chinese White', 'K-2', 'Sujanpur Local', 'BC₂-59', 'S-1635', 'C-1730', 'Mandalaya', 'S-30' (Vishala), 'RFS-175', 'Anantha', 'C-2016', 'C-2017', 'S-41' and 'V-1') a los sub-trópicos, pero permanecieron inexplorados. En sericultura, la superficie foliar es también un parámetro importante para la aceptación por parte del gusano por no tener inconvenientes alimentarios y para los programas de mejoramiento de morera. El objetivo del estudio fue explorar las posibilidades de utilizar estos 16

genotipos para sericultura evaluando las características de su superficie foliar por microscopía electrónica de barrido. Los genotipos se agruparon en diferentes categorías según producción foliar, tamaño y número de estomas por unidad de área, y longitud de tricomas e idioblastos. Destacó 'Mandalaya' junto al popular 'S-146' por su producción foliar y características deseadas de superficie foliar. Además, basado en las características de sus hojas, los genotipos 'K-2', 'S-41' y 'Sujanpur Local' también se sugieren para desarrollar genotipos de morera de alto rendimiento en los sub-trópicos de la India.

Palabras clave: *Morus indica* L., microscopía electrónica de barrido, estomas, tricomas, idioblastos, producción foliar, sericultura.

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