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SEM Studies on the Leaf Surface of Promising Mulberry (*Morus* spp.) Genotypes

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Mulberry (*Morus* spp.) leaf quality has a great role in silkworm rearing which in turn affects the overall silk yield. In the recent past, many varieties of mulberry have been evolved considering the morphological characters, growth, yield, and quality parameters based on bioassay. The present investigation was carried out on ten promising mulberry genotypes *viz.* Tr-10, K-2, S-36, S-54, S-1, V-1, Mysore local, S-13, S-34, and RFS-135 to characterize stomatal size and frequency, trichomes and idioblasts using SEM. These new parameters will provide useful information for cultivars identification as well as for selecting mulberry genotypes adapted to different eco-climatic conditions and assessing the feeding quality of leaf for silkworm rearing.

Keywords: SEM, leaf surface, promising genotypes, *Morus* spp.

Mulberry (*Morus* spp.) is exploited for feeding silkworms (*Bombyx mori* L.) which produce silk fibre. Mulberry is grown in various eco-climatic conditions of temperate and tropical countries like Japan, China, and India. India ranks second internationally in terms of silk production. The comparatively low production and inferior leaf quality is mainly due to the absence of mulberry genotypes suited for specific eco-climatic zones.

The mulberry leaf surface determines the plant's adaptation to different environments, exchange of water vapour, CO_2 and O_2 through stomatal pores, and palatability to the silkworm. The hairy cover of the trichomes on the epidermis determines the epidermal transpiration and the energy budget of the leaves. Peter et al. (1995) have reported that plant trichomes have a role in insect resistance, and growth and development of large-bodied insects may be retarded due to feeding hindrance. Many lepidopteran moths prefer pubescent plant surfaces for oviposition, but early instar larvae may suffer higher mortality. The chemicals of glandular trichomes may impede an insect's ability to feed. A dense pubescence of plant trichomes changes the optical properties of the leaf surface and may reflect or absorb certain wavelengths of light (Southwood, 1986). Another possible physiological function is that by trapping a layer of air against the leaf surface, trichomes could conserve heat and moisture. The trichomes have been correlated with succulency in mulberry genotypes by Raju et al. (1980). Fujita and Uchikawa (1986) have used trichomes and idioblasts for identification of Japanese mulberry genotypes. Katsumata (1972) found idioblasts to be a useful feature for the classification of mulberry trees. Melikyan and Babyan (1971) found that idioblasts have a significant role in determining the feeding quality of leaf to the silkworm. Though numerous mulberry varieties have been evolved, no attempt has been made to study the leaf surface. Therefore, the present study was undertaken with ten promising mulberry genotypes to determine variation in stomata, trichomes, and idioblasts using SEM.

MATERIALS AND METHODS

Ten promising mulberry genotypes *viz.* Tr-10, K-2, S-36, S-54, S-1, V-1, Mysore local, S-13, S-34, and RFS-135 grown in red loamy soil of pH 7.35 under a pit system of plantation were used for the study. The genotypes Mysore local, S-13, S-34, and RFS-135 were rainfed selections while K-2, S-36, S-54, S-1, and V-1 were developed for irrigated areas. Tr-10 was a triploid genotype cultivated in hilly areas.

Plots of 3 × 5 m were laid out in RBD with 60 × 60 cm spacing in between the plants. The tenth leaf from the first fully opened leaf from the top of different genotypes was cut into pieces 3 mm^2 in size and fixed in 2.5% glutaraldehyde (prepared in 0.2 M cacodylate buffer, pH 7.2) for 2 h, then washed twice in buffer, postfixed for 3 h in 2% osmium tetroxide, and dehy-

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drated in an alcohol-acetone series at room temperature. The dehydrated material was dried in a critical-point drier (EMS-850) using CO_2 as the transition fluid. The dried samples were mounted onto 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). The coated samples were examined under electron microscope (JEOL 100 CX II-ASID 4D) at 20 kV. Stomatal size and frequency, and length of trichomes and idioblasts were measured in 25 fields each for 10 different leaf samples from different plants for each genotype. Means and standard error were calculated.

RESULTS AND DISCUSSION

Based on the scanning electron microscopic observations, the stomatal size of all the genotypes studied can be grouped into three categories: 1. Largest-sized stomata (13.251 ± 0.005 µm length and 5.527 ± 0.008 µm width) in genotype Tr-10, 2. medium-sized stomata (ranging between 10.010 - 10.014 µm length and 4.533 - 5.047 µm width) in genotypes K-2, S-36, S-54, S-1, and V-1, and 3. small-sized stomata (ranging between 8.021 - 8.513 µm length and 3.046 to 5.753 µm width) in genotypes Mysore local, S-13, S-34, and RFS-135 (Table 1, Plate I, Figs.1-10).

Further, based on the number of stomata in an area of 133.2 µm² the genotypes can again be grouped into 3 categories: 1. Genotypes Tr-10 and V-1 with 3 to 4 stomata, 2. genotypes K-2, Mysore local, S-13, S-36, S-34, S-54, and S-1 with 5 to 7 stomata, and 3. genotype RFS-135 with an average of 8 stomata (Table 1, Plate II, Figs. 1-10).

There seems to be an adaptive correlation between size and the number of stomata among different genotypes. Genotype Tr-10 had the largest stomata but the fewest in number. This genotype is a triploid and it is reported that the large size of the stomata is an indication of the higher ploidy level in mulberry. Conversely, the rainfed genotype RFS-135 had the smallest stomata but the highest in number. Genotype S-1 had medium-sized as well as a medium number of stomata. However, in genotype V-1, though the stomata were medium in size, there were fewer stomata per unit area. Genotype S-13 had very small stomata and also a moderate number per unit area. This genotype is reported to be quite adaptive for rainfed areas (Susheelamma and Datta, 1993) and may be utilized for further exploitation in different eco-climatic zones. The highest photosynthetic rate has been recorded in this genotype by Chakraborti et al. (1996). Banerjee (1991) found the highest moulting percentage in multivoltine- and bivoltine-hybrid silkworm races fed with leaves of this genotype.

The trichomes and idioblasts of all ten genotypes are presented in Table 1, Plate III, Figures 1-10. In all the genotypes studied, the trichomes found were glandular and the trichomes of genotype S-54 were longest at 21 ±0.66 µm. In another category, trichomes were medium in length, ranging from 20.09 ±0.27 to 23.21 ±0.57 µm in genotypes S-34, Mysore local, S-1, RFS-135, and Tr-10. In a third category, trichomes length ranged between 16.64 ±0.43 to 19.47 ±0.43 µm in the genotypes K-2, S-13, S-36, and V-1. Thus, genotype RFS-135 had the smallest but the most stomata and the highest average trichome length. Genotype Tr-10 had the largest stomata, the fewest in number, and an average trichome length of 23.21 ±0.57 µm. Genotype S-54 had the highest average trichome length of 26.21 ±0.66 µm.

Idioblasts were hemispherical with microtubercles all around the surface. Idioblasts found were either

Table 1. Stomatal size, frequency, size of trichomes and idioblasts in different genotypes of mulberry.

Name of genotype	Stomatal Length (µm)	Stomatal Width (µm)	Stomatal Number per 133.2 µm² area	Length of trichomes (µm)	Length of idioblasts (µm)
Tr-10	13.251±0.005	5.527±0.008	3.80±0.24	23.21±0.57	16.62±0.58
K-2	10.013±0.004	5.047±0.006	7.00±0.20	16.64±0.43	24.67±0.43
S-36	10.010±0.006	4.561±0.006	6.80±0.34	19.40±0.43	17.17±0.60
S-54	10.014±0.006	4.752±0.004	5.90±0.22	26.21±0.66	19.83±0.57
S-1	10.013±0.005	4.533±0.007	6.90±0.22	20.45±0.43	21.62±0.79
V-1	10.012±0.006	4.548±0.004	4.00±0.24	19.47±0.43	12.84±0.32
Mysore local	8.067±0.004	3.046±0.007	5.00±0.20	20.23±0.57	16.45±0.51
S-13	8.034±0.004	4.751±0.007	5.90±0.22	18.24±0.32	25.26±0.67
S-34	8.513±0.009	5.753±0.004	6.00±0.24	20.09±0.27	31.24±0.61
RFS-135	8.021±0.006	4.506±0.003	8.00±0.20	21.21±0.57	21.08±0.63

± = Standard Error

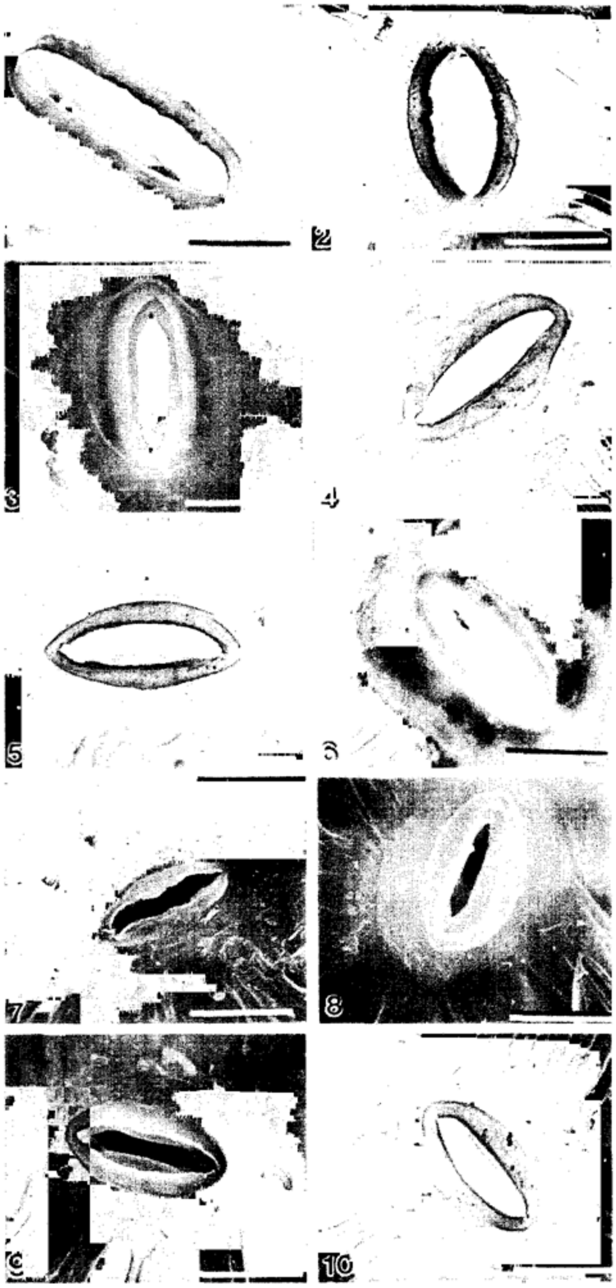

Plate I. Different size of stomata in the mulberry genotypes Tr-10 (Fig. 1), K-2 (Fig. 2), S-36 (Fig. 3), S-54 (Fig. 4), S-1 (Fig. 5), V-1 (Fig. 6), Mysore local (Fig. 7), S-13 (Fig. 8), S-34 (Fig. 9), and RFS-135 (Fig. 10). Scale bar = 5 µm.

Plate II. Number of stomata in the mulberry genotypes Tr-10 (Fig. 1), K-2 (Fig. 2), S-36 (Fig. 3), S-54 (Fig. 4), S-1 (Fig. 5), V-1 (Fig. 6), Mysore local (Fig. 7), S-13 (Fig. 8), S-34 (Fig. 9), and RFS-135 (Fig. 10). Scale bar = 10 µm.

long- or short-notched. In this regard, genotype S-34 displayed a very long idioblast (31.24 ± 0.61 µm) while short idioblasts of 12.84 ± 0.32 to 19.83 ± 0.57 µm were observed in genotypes V-1, Mysore local, Tr-10, S-36, and S-54. The idioblasts in genotypes S-13, K-2, S-1, and RFS-135 were intermediate in length (21.08 ± 0.63 to 25.26 ± 0.67 µm) (Table 1, Plate III, Figs. 1-10). Fujita and Uchikawa (1986) found variation in trichomes and idioblasts of many Japanese mulberry cultivars and so used trichomes and idioblasts as a tool for cultivar identification. Shah and Kachroo (1975) expressed the opinion that the information about trichomes may throw light on the preference for certain foliage by the silkworm. Idioblasts

Plate III. Idioblasts and trichomes in the mulberry genotypes Tr-10 (Fig. 1), K-2 (Fig. 2), S-36 (Fig. 3), S-54 (Fig. 4), S-1 (Fig. 5), V-1 (Fig. 6), Mysore local (Fig. 7), S-13 (Fig. 8), S-34 (Fig. 9), and RFS-135 (Fig. 10). Scale bar = 20 µm.

have also been found to have a significant role in determining the feeding quality of leaf to the silkworm by Melikyan and Babyan (1971), however, early instar larvae may suffer from higher mortality (Peter et al., 1995).

The present investigation may be useful in cultivars identification, the selection of genotypes adapted to different eco-climatic conditions and the assessment of the feeding quality of leaf for silkworm rearing.

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