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Leaf and anatomical traits in relation to physiological characteristics in mulberry (*Morus* sp.) cultivars

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Abstract: Micromorphological and anatomical traits in relation to physiological characteristics were studied in the leaves of 4 mulberry (*Morus* sp.) cultivars (V_1, TR_{10}, S_{34}, and Mysore local) by scanning electron microscope. The results revealed that leaf thickness was lowest (124.42 ± 2.21 µm) in the TR_{10} genotype and highest in V_1 (263.77 ± 5.17 µm). Cultivar S_{34} ranked second in respect to leaf thickness (203.57 ± 1.98 µm), followed by Mysore local (127.94 ± 2.19 µm). The thickness of palisade parenchyma was 143.66 ± 2.42 µm in V_1, 64.95 ± 1.60 µm in TR_{10}, 83.92 ± 1.43 µm in S_{34}, and 62.69 ± 1.36 µm in Mysore local. The ratio for the character of palisade parenchyma thickness among the cultivars was 2.30:1.34:1.04:1 for V_1, S_{34}, TR_{10}, and Mysore local, respectively; differences among the 4 mulberry cultivars studied were significant. The thickness of spongy parenchyma differed significantly among the 4 mulberry cultivars studied, and the greatest thickness recorded was 72.61 ± 1.48 µm in S_{34}; it was lowest (34.04 ± 1.03 µm) in TR_{10}. The values of spongy parenchyma thickness were more than double in V_1 and S_{34} when compared to TR_{10}. The experimental data revealed a maximum photosynthetic rate of 27.39 ± 0.65 µmol m^{-2} s^{-1} in V_1 followed by 24.66 ± 1.33, 19.76 ± 0.81, and 17.02 ± 0.71 µmol m^{-2} s^{-1} in TR_{10}, S_{34}, and Mysore local, respectively, and differences among the genotypes were statistically significant. Similarly, leaf pigment content (SCMR values) also exhibited significant intergenotypic differences, ranging from 35.23 (Mysore local) to 42.13 (V_1) and correlating positively with chlorophyllous palisade tissue in the mesophyll and photosynthetic rates. Ultimately, this manifested in leaf yields.

Key words: Anatomy, mulberry cultivars, physiological characteristics, SEM

Introduction

Mulberry is an important crop plant in sericulture, and its foliage is the exclusive food of domesticated silkworm *Bombyx mori* L., which produces the natural silk used in textile industries. To a great extent, increasing the production of raw silk depends on higher yield and quality in mulberry leaves. Leaf yield in mulberry is a polygenic character influenced by several quantitative characters (Vijayan et al., 1997) and is the cumulative consequence of various physiological and biochemical processes. Different morphophysiological parameters are considered for developing high-yielding mulberry varieties during the evaluation and selection of parental lines for breeding programmes. Earlier studies were conducted to investigate the interrelationship of yield components from a mainly morphological point of

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view (Das & Krishnaswamy, 1969; Susheelamma et al., 1988; Sahu et al., 1995). The literature shows that photosynthesis, the prime physiological parameter and basis for biological yield, is correlated to stomatal frequency for gas exchange and thus has a direct effect on crop production. Morphologies of the various plant parts have been studied from a taxonomical point of view in the recent past (Fagundez & Izco, 2011; Kaya et al., 2011). Menon and Srivastava (1984) reported that biomass production and the leaf yield of different crops depend primarily on photosynthetic CO_2 assimilation. Hence, there has been considerable interest in the possibility of increasing biomass production by identifying genotypes/cultivars with higher rates of photosynthesis. In light of the importance of photosynthesis in enhancing mulberry production, the current study evaluates leaf micromorphology and anatomy of some popular mulberry cultivars by scanning electron microscopy (SEM). This may help identify certain intrinsic characters for mulberry improvement programmes and lead to improved commercial exploitation in different agroclimatic regions of India.

Materials and methods

Cultivation of mulberry: Based on high, medium, and low yield potential for the present study, 4 popular mulberry cultivars (V_1, TR_{10}, S_{34}, and Mysore local) were selected from the demonstration plots of the Central Sericultural Research and Training Institute, Mysore, India. These mulberry cultivars were established in randomised block design in subplots (5.4 × 3.6 m) under irrigated conditions with standard fertilizer (300:120:120 kg NPK ha^{-1} $year^{-1}$) and manure application (20 t compost ha^{-1} $year^{-1}$), as described by Dandin et al. (2000). The latest field recommended paired row system spacing, (150 + 90) × 60 cm between rows and plants, was followed. In 4 different subplots, 3 replications were maintained per cultivar. The garden soil was red loamy (pH 7.25-7.65) with electrical conductivity ranging from 0.11 to 0.13 S cm^{-1}. The fully mature mulberry leaves of all 4 cultivars were collected between days 65 and 70 in the crop growth period, as per standard sericultural practice, and subjected to physioanatomical evaluation.

Procedure for SEM: In order to study leaf thickness, palisade and spongy parenchyma, stomatal frequency, trichomes, and idioblasts by SEM, samples of 3 × 3 mm and 3 × 1 mm in size were fixed for 4 h in glutaraldehyde prepared in 0.2 M sodium cacodylate buffer (pH 7.2). The fixed samples were washed 3 times in sodium cacodylate buffer and then dehydrated in an alcohol-acetone series. The dehydrated samples were dried in a critical point drier (EMS-850, Electron Microscopy Sciences, Hatfield, PA, USA) using CO_2 as a transition fluid. The dried samples were mounted on copper stubs using double-sided cellophane tape. In order to observe a cross-sectional area, the leaf tissues of 3 × 1 mm were mounted vertically, exposing their cut surfaces. The dried samples were gold-coated (20 nm in thickness) with a sputter coater (EMS-550, Electron Microscopy Sciences). The coated samples were observed under a scanning electron microscope (JEOL 100CX II, JEOL, Tokyo, Japan) with an attached scanning device (ASID-4D, JEOL) at 20 kV, and photographs were taken at different magnifications. From each genotype, 5 samples were examined for confirmation of the results. Thickness of leaf and mesophyll tissue was recorded from SEM micrographs and statistically analysed.

Photosynthetic rate: The photosynthetic rate was measured in all 4 genotypes (V_1, TR_{10}, S_{34}, and Mysore local) for 10-12 h under cloud-free natural sunlight conditions using a portable photosynthesis system (Model-LI 6200, LI-COR Biosciences, Lincoln, NE USA). The photosynthetically active radiation (PAR) was around 1500 μmol m^{-2} s^{-1} during gas exchange measurements. The fully matured and indexed 12th leaf from the top of the shoot was selected, and gas exchange data were recorded at the time of harvest (days 65-70 of crop growth).

Leaf pigment composition: Using a SPAD chlorophyll meter (SPAD 502, Konica Minolta, Tokyo, Japan) the fully matured indexed leaves of all mulberry varieties were measured instantly under field conditions for in situ leaf pigment composition and expressed as SPAD chlorophyll meter reading (SCMR) values, as described by Pushpanathan et al. (2004).

Statistical analysis: The data were subjected to statistical analysis by analysis of variance (ANOVA) to ascertain the significance of various physioanatomical characters.

Results and discussion

Unlike other field crops, mulberry is cultivated for its leaves, which are the sole feed for silkworm *Bombyx mori* Linn. The association of a particular character in relation to other traits that contribute to mulberry leaf growth is of paramount importance. In the present study, various leaf physioanatomical characters such as leaf thickness, thickness of palisade parenchyma, thickness of spongy parenchyma, percentage of palisade parenchyma, photosynthetic rate, and relative leaf pigment content of 4 popular mulberry cultivars were studied, and the data are presented in Table 1. The data for leaf micromorphological traits such as the number of stomata, trichomes, and idioblasts per square millimetre are shown in Table 2. The results of the present study reveal that leaf thickness is lowest (124.42 ± 2.21 µm) in TR_{10} and highest in V_1 (263.77 ± 5.17 µm). The cultivar S_{34} ranked second in respect to leaf thickness (203.57 ± 1.98 µm), followed by Mysore local (127.94 ± 2.19 µm). The ratio among the 4 mulberry cultivars (TR_{10}, Mysore local, S_{34}, and V_1) was 2.12:2.06:1.29:1 for leaf thickness character, which reveals significant genetic differences. However, there is no significant difference between the TR_{10} and Mysore local cultivars (Table 1) (Figure 1). Recently, Jalaja et al. (2003) and Babu

Table 1. Variation in mesophyll characters, leaf pigment composition, and rate of photosynthesis in 4 different popular mulberry varieties.

Genotypes	Leaf thickness (µm)	Thickness of palisade parenchyma (µm)	Thickness of spongy parenchyma (µm)	Percentage of palisade parenchyma in mesophyll (µm)	Photosynthetic (µmol m^{-2} s^{-1})	Leaf pigment composition (SCMR values)	Leaf yield (g/ plant)
V_1	263.77 ± 5.17	143.66 ± 2.42	71.83 ± 1.24	67.97 ± 1.48	27.39 ± 0.65	42.13 ± 0.76	735.65 ± 40.89
TR_{10}	124.42 ± 2.21	64.95 ± 1.60	34.04 ± 1.03	65.75 ± 1.53	24.66 ± 1.33	40.09 ± 0.72	698.22 ± 42.76
S_{34}	203.57 ± 1.98	83.92 ± 1.43	72.61 ± 1.48	52.45 ± 0.96	19.76 ± 0.81	36.58 ± 0.59	512.70 ± 29.99
Mysore local	127.94 ± 2.19	62.69 ± 1.36	56.44 ± 1.86	50.63 ± 0.81	17.02 ± 0.71	35.23 ± 1.09	380.94 ± 35.51
F test	**	**	**	**	**	**	**
Standard error	3.60	1.88	1.23	1.25	0.93	0.81	36.66
CD at 5% level	11.09	5.79	3.81	3.85	2.86	2.49	14.57

±: Standard error, **: Significant at 0.01 level, SCMR: SPAD chlorophyll meter readings

Table 2. Variation in micromorphology of 4 mulberry varieties.

Genotypes	Number of stomata (per mm^2)	Number of trichomes (per mm^2)	Number of idioblasts (per mm^2)
V_1	1063.80 ± 15.41	55.00 ± 1.00	41.20 ± 1.16
TR_{10}	581.20 ± 8.78	220.00 ± 3.54	23.60 ± 1.50
S_{34}	1266.60 ± 16.68	35.60 ± 1.57	21.60 ± 1.50
Mysore local	563.00 ± 8.57	155.00 ± 5.67	20.00 ± 1.41
F test	**	**	**
Standard error	13.59	3.86	1.45
CD at 5% level	41.90	11.89	4.46

±: Standard error, **: Significant at 0.01 level

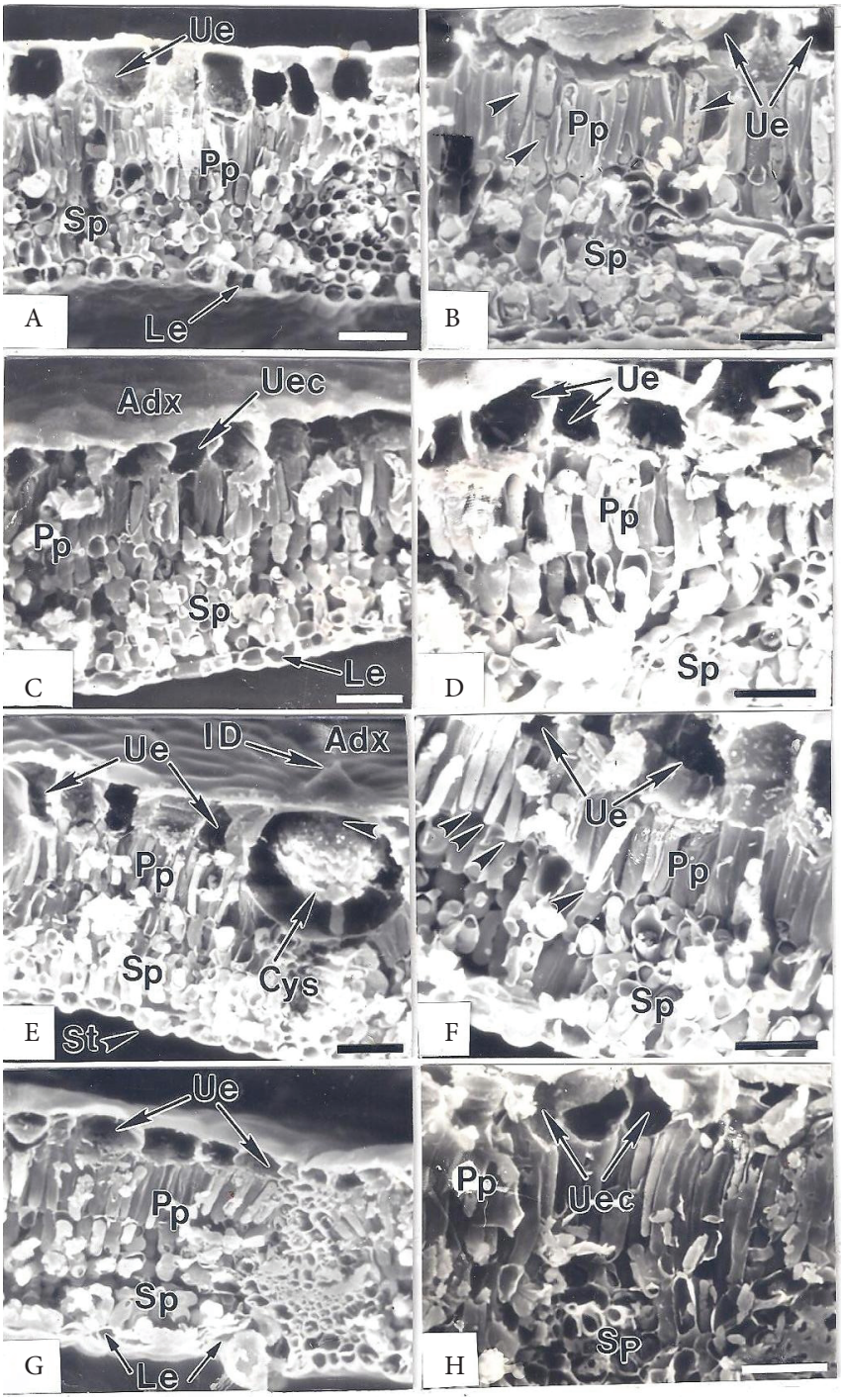

Figure 1. Scanning electron microphotographs of transverse section of mulberry leaves of 4 mulberry genotypes (V_1, TR_{10}, S_{34}, and Mysore local) showing anatomical features such as variation in leaf thickness, palisade tissue, and spongy parenchyma. A and B: microphotographs of the transverse section (TS) of mulberry leaf, V_1 genotype; C and D: TS of mulberry leaf, TR_{10} genotype; E and F: TS of mulberry leaf, S_{34} genotype; G and H: TS of mulberry leaf, Mysore local genotype. Scale bars: A, C, E, G = 12 μm; B, D, F, H = 10 μm. Ue = upper epidermis; Pp = palisade parenchyma; Sp = spongy parenchyma; Le = lower epidermis; Uec = upper epidermis cells; Adx = adaxial surface of leaf; ID = idioblast; St = stomata; Cys = cystolith.

et al. (2006) also reported anatomical adaptations of a few mulberry varieties under induced moisture stress and shade conditions. SEM observations in the present study also revealed that palisade parenchyma cells are localized on the upper surface of the blade (adaxial), and spongy parenchyma is located on the lower surface (abaxial). It was also noted that in all mulberry cultivars investigated, the palisade parenchyma is made up of elongated cells that are densely packed and arranged perpendicularly to the epidermis. The spongy parenchyma cells appear in different forms and constitute the major part of the mesophyll with 8-10 layers of cells arranged in an irregular fashion, with a smaller number of chloroplasts than palisade cells and large intercellular spaces (Figure 1). In addition, abaxial epidermis cells contained the stomata whereas the adaxial leaf surface was observed without stomata. Stomata are important for the diffusion of gases and water vapour, which, in turn, determine the metabolism of crop plants. The arrangement and frequency of stomata varied widely among mulberry cultivars (Table 2, Figure 2). Hsiao (1973) reported that photosynthesis is largely dependent on stomatal regulation. The palisade parenchyma, which is the chlorophyllous tissue, is an important character of the leaves of any plant as it reflects the ability of a genotype to capture solar energy and accounts for photosynthetic efficiency. The thickness of the palisade parenchyma was 143.66 ± 2.42 μm in V_1, 64.95 ± 1.60 μm in TR_{10}, 83.92 ± 1.43 μm in S_{34}, and 62.69 ± 1.36 μm in Mysore local (Table 1, Figure 1). The ratio for the character of thickness of palisade parenchyma among the cultivars was 2.30:1.34:1.04:1 for V_1, S_{34}, TR_{10}, and Mysore local, respectively, demonstrating significant differences among the 4 mulberry cultivars studied (Table 1). Furthermore, the values of the palisade ratios were highly significant compared to all other mesophyll tissue characters and directly correlated with photosynthetic rates (Table 1). The thickness of spongy parenchyma differed significantly among the 4 mulberry cultivars studied, and the greatest thickness recorded was 72.61 ± 1.48 μm in S_{34}; the lowest (34.04 ± 1.03 μm) was found in TR_{10}. The values of the thickness of spongy parenchyma were more than double in V_1 and S_{34} when compared to TR_{10} (Table 1, Figure 1). The structure of the mesophyll is associated with the photosynthetic performance of leaves via the regulation of internal light and carbon dioxide profiles; the percentage of palisade parenchyma is an important parameter in the mesophyll tissue of mulberry. The highest percentage of palisade parenchyma was recorded in V_1 (67.97 ± 1.48 μm) and the lowest percentage was found in Mysore local (50.63 ± 0.81 μm). However, there was no significant difference between S_{34} and Mysore local in the percentage of the palisade parenchyma parameter. Nevertheless, both cultivars individually exhibited significant differences when compared to V_1 and TR_{10} (Table 1).

Photosynthesis is the key to dry matter production by which the plants use the energy from sunlight to produce sugars, which cellular respiration converts into metabolic energy, i.e. ATP. The conversion of unusable solar energy into usable chemical energy is associated with the actions of the green pigment chlorophyll. Therefore, increasing photosynthetic efficiency is the most important way of increasing crop productivity (Gupta, 1994). The experimental data revealed a maximum photosynthetic rate of 27.39 ± 0.65 μmol m^{-2} s^{-1} in V_1, followed by 24.66 ± 1.33, 19.76 ± 0.81, and 17.02 ± 0.71 μmol m^{-2} s^{-1} in TR_{10}, S_{34}, and Mysore local, respectively, showing statistically significant differences among the genotypes (Table 1). Similarly, leaf pigment content (SCMR values) showed significant intergenotypic differences (Table 1), with values ranging from 35.23 (Mysore local) to 42.13 (V_1) and positively correlating with chlorophyllous palisade tissue in mesophyll and photosynthetic rates, ultimately manifesting in leaf yields. This positive relationship is corroborated in various crops (Gosh et al., 2003).

It is concluded from the present study that mulberry cultivars V_1 and TR_{10} are superior under irrigated conditions due to the association of favourable physiological and anatomical characteristics: high photosynthetic rates, leaf photoreceptor chlorophyll pigments and a higher ratio of chlorophyllous palisade tissue, leaf thickness, and stomatal frequency, all of which are directly correlated with high leaf yield in mulberry. These cultivars can be exploited in the field for the benefit of sericultural farmers.

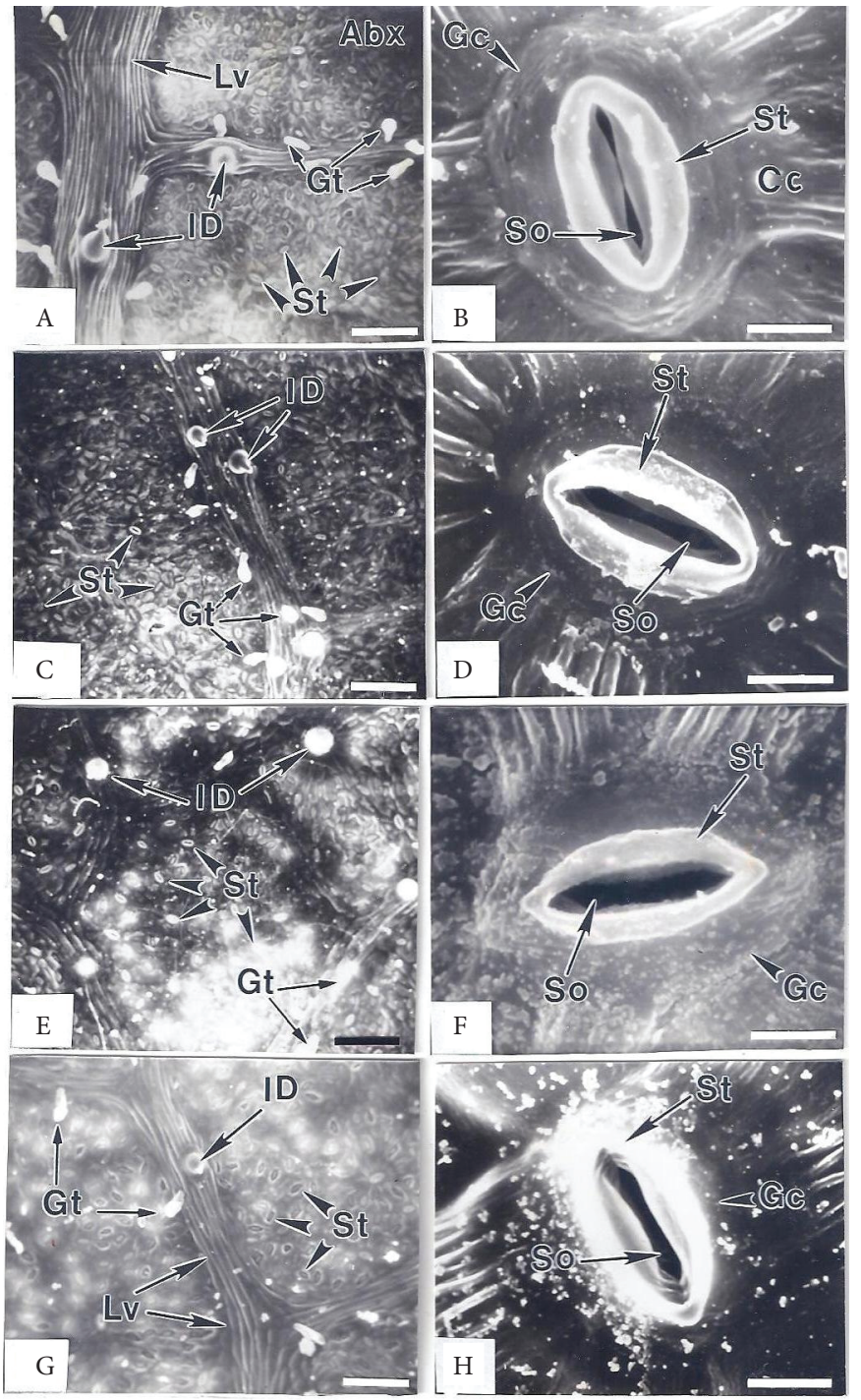

Figure 2. Scanning electron microphotographs of 4 mulberry genotypes (V_1, TR_{10}, $S_{34,}$ and Mysore local) showing the comparison of abaxial (Abx) leaf surface of mulberry. A: Abx leaf surface of mulberry, V_1 genotype; B: magnified view of stomata, V_1 genotype; C: Abx of mulberry, TR_{10} genotype; D: microphotograph of TR_{10} genotype; E: Abx of mulberry, S_{34} genotype; F: magnified view of stomata, S_{34} genotype; G: Abx of mulberry, Mysore local genotype; H: magnified view of stomata, Mysore local genotype. Scale bars: A, C, E, G = 30 µm; B, D, F, H = 2 µm. Lv = leaf vain; ID = idioblast; St = stomata; Gt = glandular trichome; Gc = guard cell; Cc = companion cell; So = stomatal opening.

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