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Biochemical insights into selective mulberry (*Morus* sp.) genotypes

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Abstract
The biochemical assessment of six mulberry genotypes *viz*., MI-0543, MI-0615, MI-0651, MI-0663, MI-0685 and MI-0718 in comparison with two cultivated varieties *viz*., V1 and G4 was carried out in the present study. The contents of chlorophyll (chlorophyll-a, chlorophyll-b, and total chlorophyll), total protein, carbohydrate levels leaf moisture and moisture retention capacity were estimated for the above-mentioned mulberry genotypes and cultivated varieties. The results of the study revealed a notable variation among the genotypes and varieties with respect to biochemical constituents. V1 variety exhibited the highest levels of total protein (26.63%), total carbohydrate (17.53%), chlorophyll content (chlorophyll a- 4.31, chlorophyll b- 2.23, total chlorophyll- 6.15), leaf moisture content (80.54%) and moisture retention capacity (81.05%). MI-0685 and MI-0663 performed the best for majority of the criteria among the six genotypes, whereas MI-0615 recorded minimum values for biochemical constituents. These biochemical constituents estimated in this study play a crucial in selection of mulberry cultivars with good quality. The cultivars with high moisture content and nutritional profiles when fed to the silkworm, improves the rearing traits and increases the silk productivity and improves the silk quality.

Keywords: Mulberry, total chlorophyll, total carbohydrate, leaf moisture content, silk productivity

Introduction
Many species and genotypes of the genus *Morus* exhibit notable differences in their morphological, physiological, and biochemical characteristics (Banerjee *et al*., 2007) [3]. Biochemical composition varies among mulberry cultivars, affecting their aptitude for sericulture (Biasiolo *et al*., 2004 and Dandin *et al*., 2021) [4, 7]. The leaves of some genotypes may exhibit high carbohydrates content but less chlorophyll, while others might be high in proteins but lacking in specific minerals (Bongale *et al*., 1997; Yadav *et al*., 2022) [6, 24]. According to Doss *et al*., (2012) [8], choosing the best genotypes for particular environmental circumstances and production objectives requires an understanding of these biochemical variations. Aside from being the catalyst for photosynthetic activity, which enables the plant to produce vital nutrients, chlorophyll also acts as a gauge of the general health and nutritional value of the plant. The nutritional value of mulberry leaves is directly related to the amount of chlorophyll present, which affects the amount of protein, carbohydrates and minerals they contain.

The biochemical components of mulberry cultivars differ and these nutrients directly affect the growth, development, disease resistance and silk yield of silkworms. (Haque *et al.*, 1990; Horie, 1980; Islam *et al.*, 2023; Ito, 1961) [10, 11, 13]. The quantity of proteins and amino acids found in the mulberry leaves was significantly positively correlated with the weight of the cocoon shell (Shah *et al.*, 2007) [21]. Mulberry leaves with a higher moisture level favour nutritional absorption, digestion and assimilation, which directly affects silkworm growth and development. Silkworms prefer mulberry leaves that contains more water, total and soluble carbohydrates, and less minerals. According to Raman *et al.*, (1995) [20], the water content of mulberry leaves affects the leaves' palatability and digestibility for silkworms, which has a direct impact on their metabolic and feeding efficiency. In the present study, mulberry genotypes with better growth traits were evaluated for phytochemical characteristics and compared with cultivated varieties.

Increased productivity, efficiency and environmental sustainability of silkworm results by mulberry genotype selection based on an understanding of the important biochemical characteristics.

Materials and Methods

Six mulberry genotypes viz., MI-0543, MI-0615, MI-0651, MI-0663, MI-0685, MI-0718 and two check cultivated varieties *viz*., V1, and G4 were subjected to biochemical studies. The moisture content of the leaves, their ability to retain moisture and other biochemical parameters *viz*., total protein, total carbohydrate, total chlorophyll-a, chlorophyll-b and total chlorophyll were estimated in the present study.

Estimation of moisture content

Leaf moisture content was calculated as per the standard procedure to estimate leaf moisture content by Vijayan *et al.*, (1997). The apical, middle and bottom leaves of several mulberry leaf samples were collected. After gently removing the water from the leaf surface with tissue paper, the collected leaf samples were cleaned under running tap water to get rid of dust and other residues. The fresh weight of each sample were recorded separately. Following a 24-hour drying period at ambient temperature and a 12-hour oven drying period at 60°C, the dry leaf weight of each sample was promptly recorded. Using the following formula, the moisture content of the apical, middle, and bottom leaves were estimated and expressed as a percentage (%).

$$\text{Leaf moisture per cent (\%)} = \frac{\text{Fresh weight of leaves} - \text{Dry weight of leaves}}{\text{Fresh weight of leaves}} \times 100$$

Moisture retention capacity

The following formula was used to assess the leaf moisture retention capacity, which was then reported as a percentage (%).

$$\text{Leaf moisture loss (\%)} = \frac{\text{Fresh wt. of Leaves} - \text{Wt. of leaves at 6 hours after harvest}}{\text{Fresh weight of leaves}} \times 100$$

Leaf moisture retention (%) = 100 - Leaf moisture loss

Chlorophyll content (mg/g)

The Arnon (1949) method was used to estimate the chlorophyll content in the leaves. After homogenizing 1g of leaf samples with 80% acetone, the supernatant solution was extracted and centrifuged for 5 minutes at 5000 rpm. Then the absorbance readings were recorded at 663 and 645 nm in a spectrophotometer. Using the following calculations and spectrophotometer readings, the quantity of chlorophyll was calculated and expressed in mg.

$$\text{Chlorophyll a} = 12.7\ (OD663) - 2.69\ (OD645) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll b} = 22.9\ (OD645) - 4.68\ (OD663) \times \frac{V}{1000 \times W}$$

$$\text{Total chlorophyll} = 20.2\ (OD\ 645) + 8.02\ (OD663) \times \frac{V}{1000 \times W}$$

Where, V = Volume of chlorophyll extract; W = Weight of leaf sample taken.

Total carbohydrate content

The Anthrone Reagent method was used to quantitatively estimate the amount of carbohydrates in the apical, middle, and bottom samples of mulberry leaves (Dubios M.K., 1956). To estimate the amount of carbohydrates in 0.2 g of leaves from various mulberry varieties were ground in distilled water using a mortar and pestle and the clear supernatants were collected in separate test tubes. Four milliliters of Anthrone reagent was added to the leaves to produce a green color and the absorbance of the green color was measured using a U-V Spectrophotometer set to 625 nm. The standard carbohydrate solutions (Dextrose L) were used to calculate the amount of carbohydrates.

Total proteins

Lowry's method (1959) was used to quantitatively measure the protein content. Approximately 0.5 gm of mulberry leaf samples from the apical, middle, and bottom of different genotypes were thoroughly cleaned to remove surface dust and other residues before being crushed and Trichloroacetic acid solution (5 ml) was used to grind the material. The resulting clear supernatants were then collected in different test tubes and centrifuged at 4000 rpm for 15 minutes. The solution turned blue and the absorbance of the blue colour was recorded using a U.V. Spectrophotometer set to 650 nm.

Result and Discussion

Moisture Content and Moisture Retention Capacity (%)

The results revealed variations among the mulberry genotypes with respect to moisture content and moisture retention capacity (Fig.1). Moisture content was significantly higher in apical (80.54%), middle (78.10%) and bottom (76.80%) leaves of V1 and significantly lower in leaves of MI-0615 (66.72%, 63.96%, 63.96% respectively). Moisture retention capacity was highest in apical, middle and bottom leaves of mulberry variety V1 (81.05%,79.65%,78.02% respectively) and lowest in MI-0615 (69.16%, 66.15%, 64.78% respectively) (Table 1). The result of the present study is in conformity with the findings of Banerjee *et al.*, (2007) [3] and Ahalya *et al.*, (2020) [1]. Leaf moisture content is a genetic character and influenced by available soil moisture and root proliferation nature of mulberry variety. Feeding capacity of the silkworm larvae is improved by the presence of available moisture in the leaves, which accelerates their growth rate (Bongale *et al.*, 1997, Murthy *et al.*, 2013) [6, 18]. Most researchers noticed that, moisture content of mulberry leaves decreased gradually with corresponding increase in leaf growth. Likewise, in the current study, moisture percentage was maximum in apical leaves of V1, G4 and MI-0685 and minimum in bottom leaves of MI-0615 variety. Similar results were obtained by Khan, M. M., & Sankar Naik, S. (2012) [14] and reported that highest leaf moisture content was recorded in tender leaves of S-1635 (78.32%) on the other hand, lowest value was observed in coarse leaves of Mysore local (70.47%). Venkataramana *et al.*, (2002) [22] carried out study on moisture and moisture retention capacity in leaves of mulberry varieties and recorded highest moisture (71.84 %) and moisture retention capacity (70.17 %) in V1 followed by the S-36 variety, which was comparable to the current findings.

Chlorophyll Content

Fig. 2 shows that the mulberry variety V1 recorded the highest total chlorophyll content in the apical (4.33 mg/g), middle (6.15 mg/g), and bottom (5.39 mg/g) leaves, followed by G4 (4.11 mg/g, 5.53 mg/g, and 4.98 mg/g, respectively), and MI-0718 (2.01 mg/g, 3.58 mg/g, and 2.91 mg/g, respectively). With respect to chlorophyll-a, higher values were recorded in apical (2.83 mg/g), middle (4.31 mg/g) and bottom (3.80 mg/g) leaves of V1 followed by G4 (2.69 mg/g, 4.02 mg/g, 3.32mg/g respectively) and lower values were noticed in MI-0718 (1.12 mg/g, 2.20 mg/g, 1.42 mg/g respectively). Chlorophyll-b content was recorded highest in apical (1.54 mg/g), middle (2.23 mg/g) and bottom (1.71 mg/g) leaves of V1 and lowest in MI-0685 (0.68 mg/g, 1.11 mg/g, 0.74 mg/g respectively) (Table 1). Chlorophyll content is important for quantifying the photosynthetic efficiency of plant and is an essential constituent in assessing quality of foliage. Yadhav *et al.,* (2022) reported that the highest total chlorophyll content was observed in S146 mulberry leaves. The silkworm fed with S146 mulberry leaves showed better cocoon performance effective rate of rearing (ERR %), cocoon weight, pupa weight, shell weight and filament length compared to other mulberry varieties. Apical and bottom leaves contain less amount of chlorophyll compared to middle order ones. Among the selected mulberry varieties, total chlorophyll (6.38mg/g) and chlorophyll-a (4.16mg/g) were highest in V1 and least in (2.01mg/g, 1.12mg/g respectively) and chlorophyll-b was highest in G4 (2.31mg/g) and least in MI-0685 (0.76 mg/g).

Total Proteins

Among the six mulberry genotypes and check varieties, V1 variety recorded highest leaf protein (26.63%) content. Proteins were higher in MI-0543 (22%), MI-0663 (23.77%), MI-0718 (21.03%) and MI-0685 (20.82%) varieties among the selected mulberry varieties (Fig.3). Total proteins in leaves of V1 (26.63%) and G4 (24.10%) recorded higher values and significantly lower in MI-0651 (17.63%) followed by MI-0615(19.18%). For many Lepidopteron larvae, the quality of the nutrients is mostly determined by the protein content in the leaves. It is well known that mulberry leaf protein directly biosynthesizes about 70 per cent of the protein content of raw silk, specifically fibroin and sericin, with the remaining 30 per cent coming from silkworm body tissue and hemolymph protein. This indicates the significance of leaf protein in the silkworm diet. Protein content present in different mulberry varieties had a direct influence on larval growth particularly in silk gland development and cocoon characters of silkworm (Shah *et al.*, 2007 & Islam *et al.,* 2023) [21]. Similar studies were conducted by Bongale and Chaluachari (1993) [5] observed a significant difference in protein content of eight different mulberry varieties leaves S54, C1, MR2, Kitchili, Roso, Kosen, Japanese and M5.

Total Carbohydrate

Total carbohydrate content of different mulberry genotypes was studied. Maximum leaf carbohydrate content was significantly maximum in V1 (17.53%) followed by G4 (16.02%) while, minimum in MI-0615 (11.11%). Apart from check varieties, MI-0718 (14.10%) showed maximum carbohydrate content when compared to MI-0543, MI-0615, MI-0651 and MI-0663. Current study results were enlightened by Ramamoorthy *et al.,* (2018) [19] who reported highest protein content (24.8±0.22 %) in the leaves of V1 variety. The V1 mulberry variety has highest level of total sugar (16.72%) and total protein (26.72%) when compared to the other five mulberry types, according to Kumar *et al*., (2009).

Table 1: Biochemical composition of different mulberry genotypes

Mulberry genotypes		Chl-a (mg/g)	Chl –b (mg/g)	Total chlorophyll (mg/g)	Moisture content (%)	MRC (%)	Total carbohydrates (%)	Total proteins (%)
MI-0543	A	1.62	0.92	2.70	71.14	73.22	12.08	22.00
	M	2.41	1.40	3.27	69.61	72.02		
	B	1.96	1.19	2.94	65.16	67.26		
MI-0615	A	1.74	1.13	2.86	66.72	69.16	11.11	19.18
	M	2.89	1.77	4.36	63.96	66.15		
	B	2.03	1.23	3.23	62.10	64.78		
MI-0651	A	2.18	1.25	3.32	69.36	71.33	13.02	17.63
	M	3.22	1.83	4.15	67.20	69.54		
	B	2.74	1.49	3.58	64.77	66.08		
MI-0663	A	2.29	1.20	3.51	70.72	72.24	13.81	23.77
	M	3.68	1.96	4.28	68.88	70.78		
	B	2.93	1.54	3.70	66.72	68.14		
MI-0685	A	1.53	0.68	2.64	76.80	79.11	13.67	20.82
	M	2.16	1.11	3.49	74.17	76.13		
	B	1.82	0.74	3.07	72.89	74.20		
MI-0718	A	1.12	1.05	2.01	73.15	75.13	14.10	21.03
	M	2.20	2.02	3.58	70.02	72.04		
	B	1.42	1.26	2.91	68.78	70.93		
V1	A	2.83	1.54	4.33	80.54	81.05	17.53	26.63
	M	4.31	2.23	6.15	78.10	79.65		
	B	3.80	1.71	5.39	76.80	78.02		
G4	A	2.69	1.33	4.11	78.91	80.16	16.02	24.10
	M	4.02	2.31	5.53	75.98	77.19		
	B	3.32	1.66	4.98	73.68	75.33		
CD @ 5%		0.63	0.81	0.71	5.13	5.20	1.62	3.20

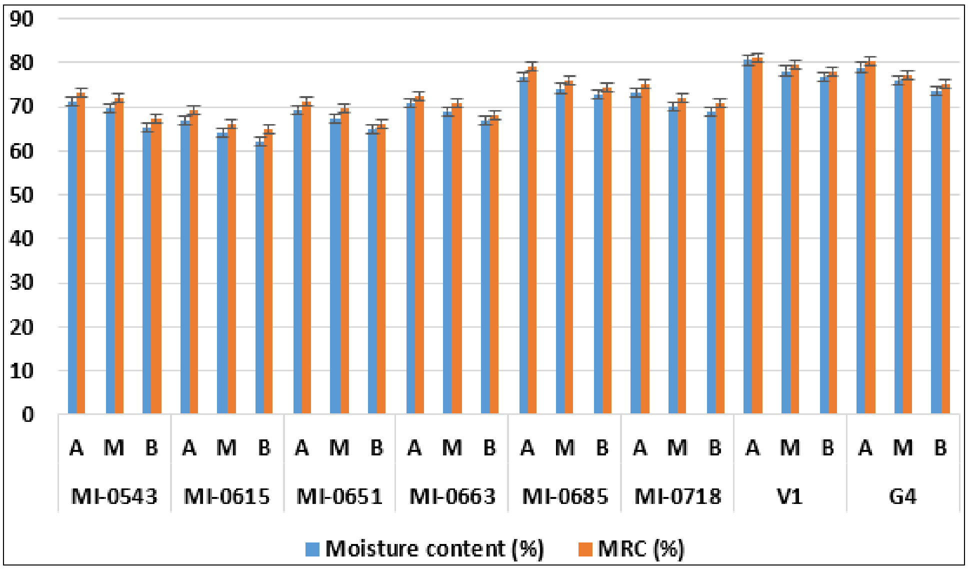

Fig 1: Leaf moisture content and Moisture retention capacity of mulberry genotypes

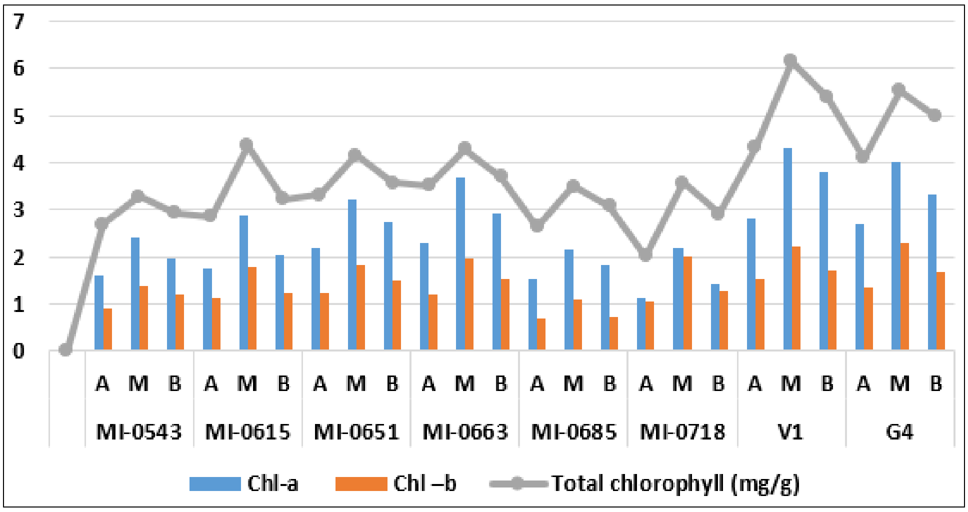

Fig 2: Chlorophyll concentrations of mulberry genotypes

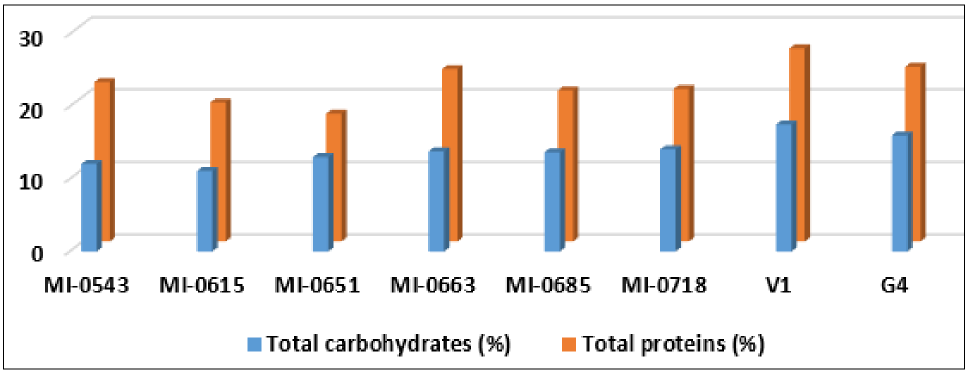

Fig 3: Total protein and total carbohydrate content of mulberry genotypes

Conclusion

The biochemical evaluation of six mulberry genotypes and two check cultivars revealed notable differences in key parameters such as moisture content, moisture retention capacity, chlorophyll content, protein and carbohydrate levels. The V1 cultivar and MI-0685, MI-0663 genotypes consistently demonstrated superior performance, making it a promising genotype for improving silk production. The results emphasize the critical role of biochemical properties in determining mulberry leaf quality and their direct impact on silkworm nutrition, growth and cocoon production. These findings provide a valuable foundation for selecting suitable genotypes for breeding mulberry varieties with enhanced nutritional profiles to optimize sericulture practices and increase silk yield.

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