



# Phytochemical Investigation on Assessment of Leaf Quality of Different Mulberry Germplasm Genotypes

Muzafar Ahmad Bhat <sup>a\*</sup>, Amita Kiran <sup>a</sup>,  
Suraksha Chanotra <sup>a</sup>, Shawani Devi <sup>a</sup>, Ariba Zehra <sup>a</sup>,  
Rakesh Panjalia <sup>b</sup>, Abdul Aziz <sup>c</sup> and Mohd. Azam <sup>d</sup>

<sup>a</sup>P. G. Department of Sericulture, Poonch Campus, University of Jammu, India.

<sup>b</sup> P. G. Department of Zoology, University of Jammu, India.

<sup>c</sup> J&K Revenue Department, India.

<sup>d</sup> Government CSM Degree College, Mendhar, University of Jammu, India.

## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

The growth and development of the silkworm, *Bombyx mori* and the cocoon crop are influenced by the yield and nutritional value of the mulberry leaf used as feed. It is true that there are disparities in leaf quality between mulberry genotypes, which explain the variations in silkworm rearing. The present study used mulberry genotypes of S1635, S164, S13, Chinese White, Chak Majra, C4, G4, C2038, V1 and K2 to estimate several biochemical components in tender mulberry leaves. Lowry's method was used to calculate the amount of protein, Anthrone method [1] was used to calculate the amount of total carbohydrates, Ninhydrin method was used for estimation of total proline, the amount of chlorophyll was calculated by Arnon method and direct method was used for estimation

\*Corresponding author: E-mail: [mbhat62@gmail.com](mailto:mbhat62@gmail.com);

of moisture content in tender leaves of different mulberry varieties. V1 tender leaves (26.30 mg/g) had considerably lower total protein contents compared to S13 (49.05 mg/g) and Chinese white (48.55 mg/g) tender leaves. Tender leaves from S13 (10.40 mg/g) and V1 (7.55 mg/g) had greater total carbohydrate levels than tender leaves from S1635 (2.55 mg/g). The levels of total chlorophyll in tender leaves from S13 (2.59 mg/g) and S1635 (2.56 mg/g) were higher than those in tender leaves from C2038 (1.74 mg/g). Tender leaves from S164 (3.90 mg/g) and S13 (3.26 mg/g) had greater amounts of total proline than tender leaves from C2038 (2.00 mg/g). Total proline levels were greater in Chinese white (80.15%) and C4 (78.60%) tender leaves than in S164 tender leaves (61.51%).

**Keywords:** Phytochemical; assessment; mulberry; germplasm; genotypes.

## 1. INTRODUCTION

Due to the monophagous nature of silkworms makes sericulture completely dependent on mulberry for the overall output. It is a well-known statistic in sericulture that mulberry production accounts for more than 60% of overall cocoon manufacturing costs. Mulberry leaves are high in protein, carbohydrates, chl a, chl b, total carotenoids, ascorbic acid and various mineral elements. Changes in the composition of metabolic activity of silkworm larval body are caused by nutritional deficiencies or imbalances in leaves. Mulberry leaf meal's amino acid profile suggests that it is an excellent supply of essential amino acids, particularly lysine (1.80%) and leucine (2.58%). Mulberry contains around 8.01 to 13.42 g of carbohydrate, 4.72 to 9.96 g of crude protein, 0.64 to 1.51% crude fat, 4.26 to 5.32 g of total ash, and 69 to 86 kcal/100 g of energy [2]. However, fewer efforts have been made to evaluate the biochemical constituents available in different parts of mulberry. Therefore, the present investigation was conducted to evaluate biochemical composition in tender leaves of mulberry.

## 2. MATERIALS AND METHODS

Mulberry varieties selected for the study comprised of S1635, S164, S13, Chinese white, Chakmajra, C4, G4, C2038, V1 and K2, maintained at germplasm bank of P. G. Department of Sericulture, Poonch campus, University of Jammu.

### 2.1 Protein Estimation

Lowry's method was used to calculate the amount of protein in samples derived from various mulberry leaf genotypes [3] and values were recorded at 660 nm in spectrophotometer.

### 2.2 Carbohydrate Estimation

The Anthrone method [1] was used to calculate the amount of total carbohydrates in tender leaves of mulberry. The amount of carbohydrate in the sample was calculated using the method and expressed in mg/ml.

$$\begin{aligned} &\text{Con. of the sample} \\ &\quad \text{Optical density of the sample} \\ &= \frac{\times \text{Concentration of the standard}}{\text{Optical density of the standard}} \end{aligned}$$

### 2.3 Estimation of Proline

Ninhydrin method was used for estimation of total proline in tender leaves of mulberry. The chromophore-containing toluene was separated from the aqueous phase using a separating funnel, and the absorbance was measured in a spectrophotometer at 520 nm [4].

### 2.4 Estimation of Chlorophyll

According to [5], the amount of chlorophyll was calculated. A known amount of leaf tissue (100 mg) was suspended in 10 ml of 80% acetone, thoroughly mixed and stored at 4°C in the dark overnight. After centrifugation at 5000 rpm, the supernatant was removed and the absorbance measured in a Spectrophotometer at 663 nm.

### 2.5 Estimation of Moisture Content (Direct Method)

Each sample of fresh leaves were weighed and recorded as  $W_f$  before being dried for 2 hours at 104°C.  $W_d$  was assigned to the dry matter weighed. The following equation was used to determine the leaf water content:

$$\text{Water content(\%)} = \frac{(W_f - W_d)}{W_f} \times 100$$

Where, Wf = fresh weight and  
Wd= dry weight.

Triplicates of each sample were taken.

## 2.6 Data Analysis

All the data derived from three replications of different parameters were used to draw mean values along with standard deviation and significant variations employing one-way ANOVA using SPSS version 20.

## 3. RESULTS

### 3.1 Protein Concentration (mg/g) in Tender Leaves of Different Mulberry Varieties

Total protein concentration were higher in S13 (49.05 mg/g) and Chinese white (48.55 mg/g) tender leaves, but substantially lower in V1 (26.30 mg/g) tender leaves. Mulberry varieties S1635, S164, Chakmajra, C4, G4, C2038 and K2 had total protein content of 31.59, 41.93, 46.60, 45.26, 44.95, 28.59, 43.56 mg/g (Fig. 1). The differences in protein content in leaves across the mulberry cultivars are significantly significant ( $P>0.01$ ) (Table 1).

### 3.2 Carbohydrate Concentration (mg/g) in Tender Leaves of Different Mulberry Varieties

Tender leaves from S13 (10.40 mg/g) and V1 (7.55 mg/g) had greater total carbohydrate

concentrations than tender leaves from S1635 (2.55 mg/g). Total carbohydrate content of mulberry types S1635, S164, Chinese white, Chakmajra, C4, G4, C2038, and K2 was 2.69, 3.26, 2.61, 3.31, 5.57, 3.90, 2.59 mg/g (Fig. 2). The differences in total carbohydrates content in leaves between the mulberry varieties are highly significant ( $P>0.01$ ) (Table 1).

### 3.3 Chlorophyll Content (mg/g) in Tender Leaves of Different Mulberry Varieties

Total chlorophyll contents were higher in tender leaves from S13 (2.59 mg/g) and S1635 (2.56 mg/g) than tender leaves from C2038 (1.74 mg/g). Mulberry kinds S1635, S164, Chinese white, Chakmajra, C4, G4, C2038, and K2 have total chlorophyll content of 2.51, 2.29, 2.01, 2.32, 2.85, 2.00, 1.85 mg/g (Fig. 3). There are substantial changes in chlorophyll content in leaves amongst mulberry cultivars ( $P>0.01$ ) (Table 1).

### 3.4 Proline Content (mg/g)

Tender leaves from S164 (3.90 mg/g) and S13 (3.26 mg/g) had greater total proline levels than tender leaves from C2038 (2.00 mg/g). Total proline content of mulberry varieties S1635, Chinese white, Chakmajra, C4, G4, V1, S164, and K2 is 2.59, 2.69, 2.51, 2.29, 2.85, 2.32, 2.56 mg/g (Fig. 4). The proline content of leaves varies significantly among mulberry cultivars ( $P>0.01$ ) (Table 1).

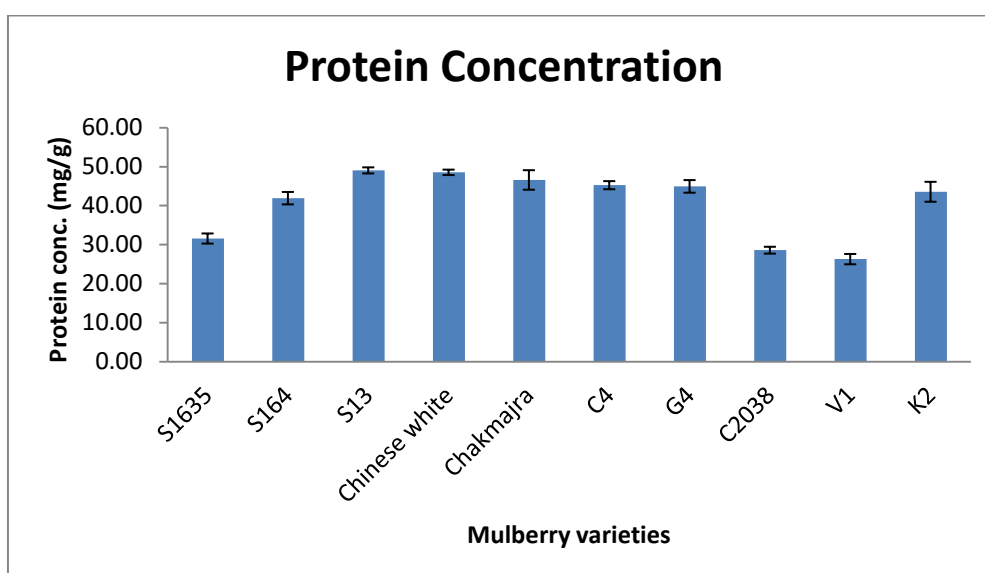


Fig. 1. Protein concentration (mg/g) in tender leaves of different mulberry varieties

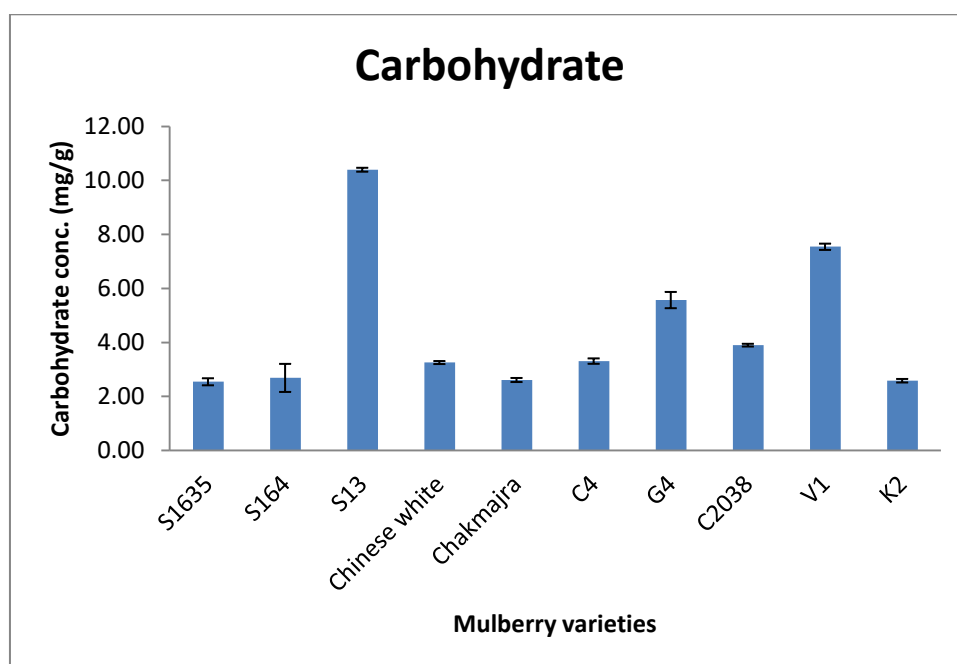


Fig. 2. Carbohydrate concentration (mg/g) in tender leaves of different mulberry varieties

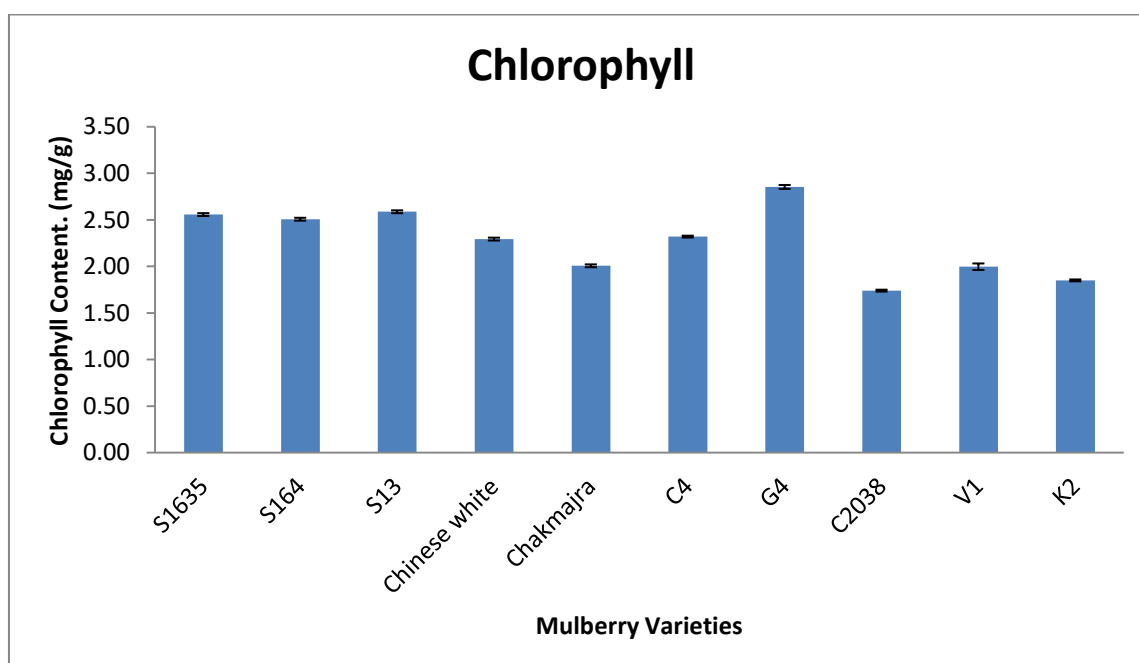


Fig. 3. Chlorophyll concentration (mg/g) in tender leaves of different mulberry varieties

### 3.5 Moisture Content (%)

Tender leaves from Chinese white (80.15 %) and C4 (78.60 %) had greater total proline levels than tender leaves from S164 (61.51 %). Total proline content of mulberry varieties S1635, Chinese white, Chakmajra, C4, G4, V1, S164, and K2 is 74.67, 73.38, 76.75, 78.60, 73.71, 76.60, 75.93,

77.55 % (Fig. 5). Mulberry cultivars differ considerably in terms of leaf moisture content (percentage) ( $P > 0.01$ ) (Table 1).

## 4. DISCUSSION

Phytochemical tests were used to assess the leaf quality of ten mulberry varieties: S1635, S164,

S13, Chakmajra, Chinese white, C4, G4, k2, V1 and C2038. S13 (49.05 mg/g) and Chinese white (48.55 mg/g) tender leaves had greater total protein concentrations, however, V1 (26.30 mg/g) tender leaves had significantly lower total protein concentrations. S1635, S164, Chakmajra, C4, G4, C2038, and K2 mulberry varieties showed total protein content of 31.59, 41.93, 46.60, 45.26, 44.95, 28.59, 43.56 mg/g, respectively. According to Adeduntan and Oyerinde, in 2010, the largest protein content

was found in S-36, followed by S-54 and Kanva-2, which is consistent with the current findings. [6] screened total protein content of six mulberry leaf varieties, namely S-13, S-30, S-36, Mysore Local, V-1, and RFS-135 and found that V-1 had the highest total protein content (26.72 mg/gm), whereas the S-36 variety had the highest protein content (0.315 mg/gm) which are in contradict with the present findings. K-2 has the highest carbohydrate content, according to [7], which is not in agreement with the current study.

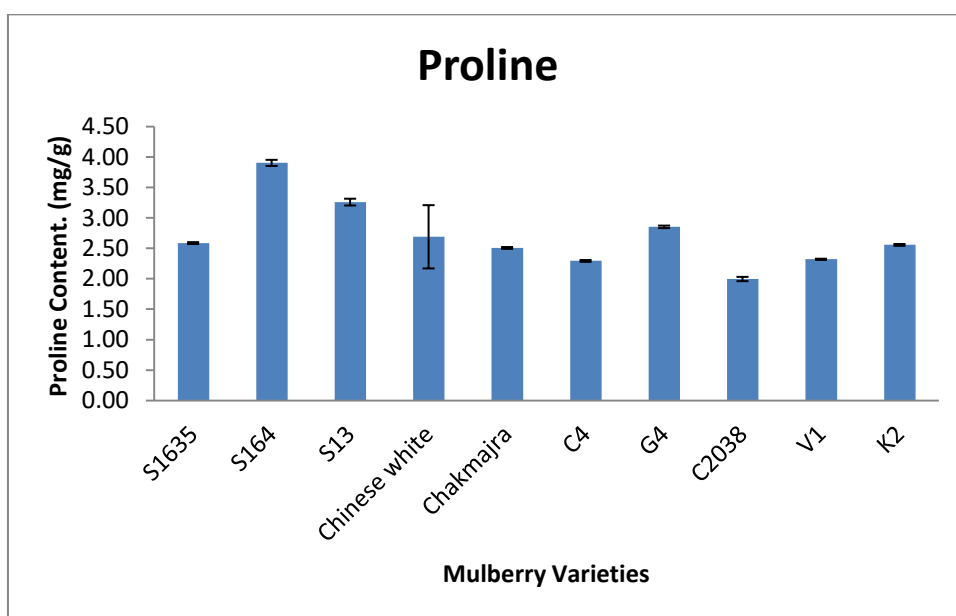


Fig. 4. Proline concentration (mg/g) in tender leaves of different mulberry varieties

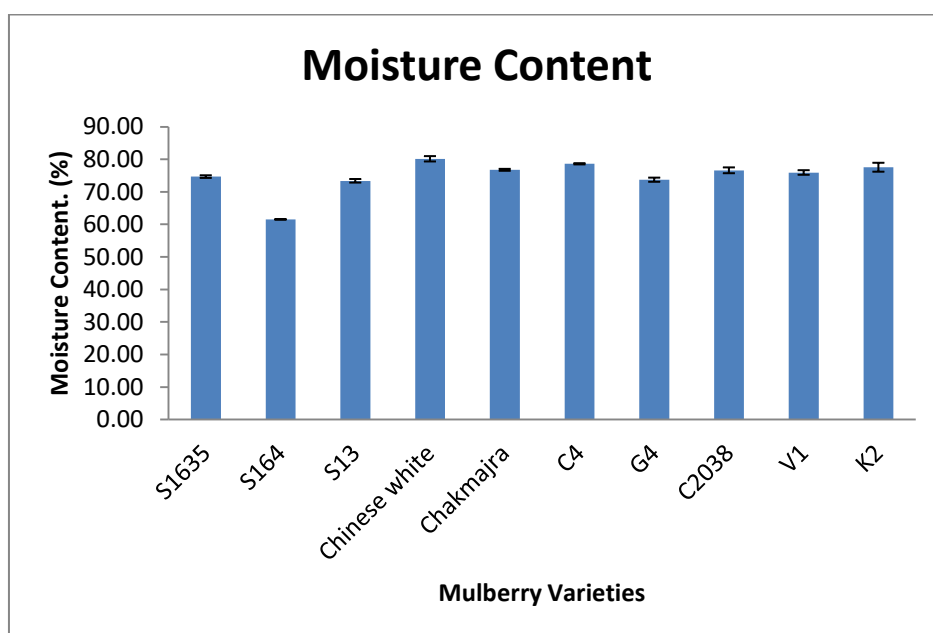


Fig. 5. Moisture content (%) in tender leaves of different mulberry varieties

**Table 1. Average values of total biochemical constituents and ONE-WAY ANOVA**

Parameters	Protein	Carbohydrates	Proline	Chlorophyll	Moisture
	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.
<b>Mulberry varieties</b>					
<b>S1635</b>	31.587±0.749	2.547±0.075	2.587±0.009	2.557±0.009	74.667±0.232
<b>S164</b>	41.927±0.921	2.690±0.300	3.903±0.029	2.507±0.009	61.510±0.056
<b>S13</b>	49.050±0.447	10.400±0.042	3.260±0.032	2.587±0.009	73.380±0.315
<b>Chinese white</b>	48.553±0.401	3.260±0.032	2.690±0.300	2.293±0.009	80.147±0.472
<b>Chakmajra</b>	46.597±1.446	2.613±0.043	2.507±0.009	2.007±0.009	76.750±0.172
<b>C4</b>	45.257±0.600	3.313±0.058	2.293±0.009	2.320±0.006	78.603±0.110
<b>G4</b>	44.950±0.934	5.573±0.174	2.853±0.012	2.853±0.012	73.710±0.368
<b>C2038</b>	28.590±0.507	3.903±0.029	1.997±0.020	1.740±10.006	76.600±0.517
<b>V1</b>	26.297±0.765	7.547±0.067	2.320±0.006	1.997±0.020	75.930±0.407
<b>K2</b>	43.563±1.474	2.587±0.038	2.557±0.009	1.850±0.006	77.547±0.791
C.D.	2.674	0.352	0.286	0.030	1.193
SE(m)	0.900	0.119	0.096	0.010	0.402
SE(d)	1.273	0.168	0.136	0.014	0.568
C.V.	3.837	4.622	6.187	0.779	0.929
F-Value	89.359	496.582	31.860	1258.737	164.336
Significance	0.000	0.000	0.000	0.000	0.000

Koyuncu [8] tender leaves from S13 (2.59 mg/g) and S1635 (2.56 mg/g) had greater total chlorophyll levels than tender leaves from C2038 (1.74 mg/g). The differences in protein content in leaves across the mulberry cultivars are significantly significant ( $P>0.01$ ). Total carbohydrates and crude protein revealed that K-2 apical leaves had the lowest protein value (0.196 mg/g). S-146 (0.122 mg/g) content in apical leaves, more protein content in K-2 (0.284 mg/g) in intermediate leaves, and least in apical leaves. The highest value of protein content in S146 (0.208 mg/g) and the lowest value of protein content in TR-10 (0.128 mg/g) and the most carbohydrate content were shown in the bottom leaves. TR-10 apical leaf carbohydrate content (0.292 mg/g). TR-10 (0.547 mg/g) has the highest carbohydrate content, while S-1635 (0.257 mg/g) has the lowest. S-1635 carbohydrate content (0.546 mg/gm) leaves [9].

## 5. CONCLUSION

Mulberry cultivars S1635, S164, S13, Chinese white, Chakmajra, C4, G4, C2038, V1 and K2 were used for the present investigation to study different biochemical profiles in tender leaves. S13 (49.05 mg/g) and Chinese white (48.55 mg/g) tender leaves had greater total protein concentrations, however, V1 (26.30 mg/g) tender leaves had significantly lower total protein concentrations. S1635, S164, Chakmajra, C4, G4, C2038 and K2 mulberry varieties showed

total protein content of 31.59, 41.93, 46.60, 45.26, 44.95, 28.59, 43.56 mg/g, resp. Differences in leaf protein content between mulberry cultivars are statistically significant ( $P>0.01$ ). Hence, based on the current results suitable mulberry varieties namely S1635, S164, S13, Chinese white, Chakmajra, C4, G4, C2038 and V1 could be recommended as nutritionally superior cultivars for enhancing growth of silkworm. This could also opens the doors for mulberry breeders for incorporating traits of such nutritious varieties to others for making profitable hybrids in near future.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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