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A Comparative Biochemical Study of Mulberry (*Morus spp.*) Mini Clones Over Conventional Stem Cuttings

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Authors' contributions

This work was experimented in collaboration among all authors. All authors approved the final manuscript.

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ABSTRACT

The mulberry (*Morus* spp.) plant holds significant value in the sericulture industry, as its foliage serves as a vital source of food for the mulberry silkworm (*Bombyx mori* L.). A research study was undertaken to figure out which mulberry variety V1 and MR2 propagated both by stem cuttings and

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apical cuttings was best performed locally. The investigation was based on biochemical composition including leaf moisture, moisture retention capacity, chlorophyll-a, chlorophyll-b, carotenoid, total chlorophyll, nitrogen, phosphorous, potassium, soluble protein, total carbohydrates, crude protein, total sugar content present in mulberry leaves. The nutritional status of different mulberry varieties is determined by its biochemical composition present. The findings showed that the mulberry mini-clones 60DAP-AC (V1) and 60DAP-AC (MR2) have registered enhanced nutritional parameters than 90DAP-SC (V1) and 90DAP-SC (MR2) and were therefore best suited for raising silkworms to obtain good cocoon parameters.

Keywords: Apical cuttings; biochemical; mulberry; stem cutting; variety.

1. INTRODUCTION

Sericulture involves indoor rearing of domesticated mulberry silkworm, *Bombyx mori* L. which feeds primarily on Mulberry (*Morus* sp.). Genus *Morus* comprises of more than 70 species and majority of them are confined to the Asian continent. Mulberry belongs to the family Moraceae and special character of this family is the presence of idioblasts in the upper epidermis portion of the leaf. Most of the cultivated and commercially exploited species in the genus *Morus* are diploid in nature having chromosomal number 2n=28. Production and cultivation of triploid plants are important because of high-quality leaves and adaptation to varied climatic conditions. Most of the mulberry varieties in India belong to *Morus indica* [1]. Mulberry is said to be originated in the Indo-china border and is widespread along the Sub-Himalayan region up to an altitude of 3300MSL [2]. Most of the mulberry varieties evolved by the selection, a selection from open pollination, mutation breeding and controlled hybridization techniques. Among different species *M. nigra, M. latifolia, M. laevigata, M. alba and M. indica* are the five common species that are distributed widely across India [3].

In sericulture, 65% of total cost of cocoon production goes to mulberry production. Nutritional quality of mulberry leaves have much higher influence in development and growth of mulberry silkworm *Bombyx mori* L [4]. Leaf quality of mulberry (*Morus* spp.) is greatly influenced by moisture content. Mulberry leaf quality varies with position and age of leaf, varieties, preservation techniques etc [5]. Nutritive value of mulberry has considerable impact on silkworm cocoon production [6] and [7]. Growth and development of silkworm was considerably influenced by moisture content of mulberry leaves which favours digestion, ingestion and assimilation of nutrients from mulberry leaves. Therefore, the present study was undertaken to evaluate biochemical composition of mulberry leaves taken from apical and stem propagated cuttings.

2. MATERIALS AND METHODS

2.1 Experimental Location

The research was conducted within the period of October 2021 to June 2022 at Department of Sericulture and Clonal complex at Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam, Coimbatore district, Tamil Nadu (11˚20‘N, 76˚55’E, 300 meters above mean sea level with average rainfall of 800mm).

2.2 Source of the Study Material

The parent material for stem cutting and apical cutting propagation of commercial mulberry varieties V1 and MR2 were collected from Main field (J-block) at Department of Sericulture, Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam.

2.3 A Comparative Biochemical Study of Mulberry Mini Clones Over Conventional Stem Cuttings

2.3.1 Effect of Different Transplanting Days on Biochemical Parameters of Apical Cuttings Under Field Conditions

At nursery level, the best performed and healthy mini clones were selected and used for the study. Different days of transplanting as treatments *viz.* 50 DAP, 60 DAP, 70 DAP, 80 DAP and 90 DAP of mini clones and 90 days old mulberry sapling generated using stem cutting method of propagation were used as check to evaluate the mini clones (apical cuttings) and stem cuttings of both V1 and MR2 mulberry

varieties. Main field was levelled and ploughed to bring soil to fine tilt. Plants were planted at wider spacing 10 feet x 10 feet to avoid interaction between plants and to evaluate the full potentiality of mini clones under field conditions. Mini clones and stem cuttings were transplanted at appropriate planting dates. Irrigation was done once in five days. Weeding was done 60 days after first treatment transplantation. The experiments were conducted in Factorial Randomized Block Design (FRBD) with three replications and ten plants per replication following regular package of practices [8].

2.3.2 Characterize the Nutritional Quality of Mini Clonal Propagated Mulberry Leaves

From the above best performing treatments were selected and leaves from the mini clones were harvested at 90 DAT followed by oven drying for further analysis. The dried sample was ground into fine powder and kept aside in an airtight container. Analysis was carried out in PG laboratory at Department of Sericulture, Forest College and Research Institute, Mettupalayam, India.

2.3.2.1 Leaf moisture content

Moisture content in mulberry leaves was determined by using two attributes *i.e.* fresh leaf weight and dry leaf weight and expressed in terms of percentage [9].

$$\text{Moisture content in leaf (\%)} = \frac{\text{Fresh weight of leaves} - \text{Dry weight of leaves}}{\text{Fresh weight of leaves}} \text{X } 100$$

2.3.2.2 Moisture retention capacity

Moisture retention capacity in the leaves was estimated using three attributes *i.e.* fresh leaf weight, leaf weight after 6 hours and dry weight of the mulberry leaves and expressed in percentage [10].

$$\text{Moisture retention capacity} = \frac{\text{Weight of leaves after 6 hours} - \text{Dry weight of leaves}}{\text{Fresh weight of leaves} - \text{Dry weight of leaves}} \text{X } 100$$

2.3.2.3 Chlorophyll content

In each replication, fresh leaves from middle part of best performing mini clones were harvested for measuring chlorophyll content. The chlorophyll a, b and total chlorophyll content in leaves were determined by procedure suggested by Cock *et al* [11] at wave lengths of 645 nm and 663 nm using spectrophotometer and calculated as follows,

Chlorophyll a = (12.7 x OD @ 663nm) – (2.69 x OD @ 645nm) x V/W x 1000
Chlorophyll b = (2.69 x OD @ 645nm) – (4.68 x OD @ 663nm) x V/W x 1000
Total Chlorophyll = OD @652nm x 1000/34.5 x V/W x 1000

Where,
OD - Optical Density @ particular absorbance
V - Final volume of supernatant liquid
W - Weight of leaf sample taken for study

2.3.2.4 Carotenoid content

Carotenoid content in mulberry leaf extract was determined using following formula and expressed in terms of mg g-1 fresh leaf weight.
Carotenoid = (7.6 x OD@480nm) – (1.49 x OD@510nm) x V/W x 1000

2.3.2.5 Total carbohydrate

Total carbohydrate composition in leaf sample collected was estimated as recommended by Yemm and Willis [12] and results expressed as mg per g of fresh leaf weight.

2.3.2.6 Crude protein

Jones and Breese [13] suggested a formula to calculate crude protein composition in mulberry leaf sample by multiplying a factor 6.25 with 'N' content (%).

2.3.2.7 Soluble protein

Soluble protein level in mulberry leaf sample was calculated by following the process prescribed by Lowry *et al.* [14] and given in terms of mg/g of fresh leaf weight.

2.4.2.8 Total sugars

The amount of total soluble sugars was estimated by phenol sulphuric acid reagent method [15]. The quantity of total sugar was expressed in percentage.

2.4 Analysis of Major Nutrients in Mulberry Mini-Clone Leaf Sample

2.4.1 Total nitrogen

Total nitrogen composition in mulberry sample was determined by micro-kjeldahl method as prescribed by Humphries [16] and expressed in percentage.

2.4.2 Total phosphorus

To determine total phosphorous level in the mulberry sample, a process suggested by Jackson [17] was followed.

2.4.3 Total potassium

The potassium levels in plant sample was estimated as recommended by Jackson (17) and given in terms of percentage.

3. RESULTS AND DISCUSSION

Chlorophyll a, chlorophyll b and total chlorophyll content significantly differed among the treatments of both the leaves of V1 and MR2 mulberry variety. In 60 DAP-AC (V1) mulberry leaves have registered chlorophyll a (1.85mg/g), chlorophyll b (0.76 mg/g) and total chlorophyll content (2.12 mg/g) and 90 DAP-SC (V1) recorded chlorophyll a (1.78 mg/g), chlorophyll b (0.73 mg/g) and total chlorophyll content (1.92 mg/g) (Table 1). In MR2 variety, 60 DAP-AC (MR2) mulberry leaves have registered chlorophyll a (1.67 mg/g), chlorophyll b (0.70 mg/g) and total chlorophyll content (1.81 mg/g) and 90 DAP-SC (MR2) recorded chlorophyll a (1.67 mg/g), chlorophyll b (0.70 mg/g) and total chlorophyll content (1.70 mg/g) (Table 2). Increase in chlorophyll content in mulberry leaves clearly indicates enhanced photosynthetic activity. But, chlorophyll a, chlorophyll b and total chlorophyll content of 60 DAP-AC (V1) and 90 DAP-SC (V1) mulberry leaves are on par with each other which is statistically proven indicates similar level of photosynthetic activity. The present findings was supported by Sudhakar *et al.* [18] who reported total chlorophyll content of 2.74 (mg/g) in V1 mulberry at 180DAP. It was further supported by Hadimani *et al.* [19] who also reported chlorophyll a (1.56 mg/g), chlorophyll b (0.73 mg/g), total chlorophyll content of 2.27 (mg/g) in V1 mulberry. The research findings also correlated with the findings of Geetha *et al.,* [20] who recorded total chlorophyll content (1.65 mg/g) in MR2 mulberry variety.

Different days of hardening have a significant impact on moisture content (%) and moisture retention capacity (%) among the treatments of both V1 and MR2 leaf foliage. Leaf quality is an important factor to consider in silkworm rearing which is influenced by moisture content in the leaves. Moisture content can be influenced by variety and available soil moisture [21]. Leaf moisture retention capacity gives an idea about moisture content available in preserved mulberry leaves kept aside to feed silkworms later. In 60 DAP-AC (V1) mulberry leaves have found moisture content (75.32%), moisture retention capacity (61.92%) and 90 DAP-SC (V1) mulberry leaves have found moisture content (69.19%), moisture retention capacity (60.32 %) (Table 5). In MR2, 60 DAP-AC (MR2) mulberry leaves have found moisture content (71.91%), moisture retention capacity (59.82%) and 90 DAP-SC (MR2) mulberry leaves have found moisture content (67.87%), moisture retention capacity (57.72%) (Table 6). Similarly, the findings derive support from Kalaivani *et al.* [22] who estimated that moisture content (72.0%), moisture retention capacity (61.71%) in MR2 variety and Kiruthika *et al.* [23] who estimated moisture content (76.0%) in V1 variety.

Macro nutrient analysis of different treatments showed significant difference in NPK content in both V1 and MR2 leaves. These variations may be due to different growth period following different days of transplantation. In MR2, 60 DAP-AC (MR2) mulberry leaves have found nitrogen content (3.32%), phosphorous content (0.28%) and potassium content (1.59%) followed by 90 DAP-SC (MR2) mulberry leaves have found nitrogen content (2.79%), phosphorous content (0.25%) and potassium content (1.52%) (Table 4). In 60 DAP-AC (V1) mulberry leaves have found nitrogen content (3.46%), phosphorous content (0.29%) and potassium content (1.67%) followed by 90 DAP-SC (V1) mulberry leaves have found nitrogen content (2.88%), phosphorous content (0.27%) and potassium content (1.64%) (Table 3). NPK content of mulberry leaves can be influenced by the dosage of application of Farm Yard Manure [4]. Increased NPK composition in leaves of V1 and MR2 was due to enhanced efficiency in uptake of nutrients from the soil by the mini-clones. The result obtained are in line with Kiruthika *et al.* [23] who noticed nitrogen content (3.93%), phosphorous content (0.34%) and potassium content (1.76%) in V1 and Bheevi *et al.* [24] in an experiment registered nitrogen content (3.05%), phosphorous content (0.25%) and potassium content (1.50%) in MR2.

Nitrogen content in leaves have an impact on crude protein composition. The nitrogen content among the treatments varied significantly due to different dates of transplantation. Similar trend were obtained in crude protein levels. The crude protein content of 60DAP-AC (V1) and 90 DAP-

SC (V1) was 20.15% and 19.08% respectively (Table 7). In MR2 variety, crude protein content of 60 DAP-AC (MR2) and 90 DAP-SC (MR2) was 18.67% and 17.76 % respectively (Table 8). This was supported by Srivastava *et al*. [25] who recorded crude protein 15.32 % in *M alba*. The readings was further supported by Kiruthika *et al.* [23] who revealed crude protein content in V1 is 24.4% and Geetha *et al.* [20] who registered crude protein content levels from 12 to 20 % in MR2.

According to Ramamoorthy *et al.* [26], total carbohydrate content in V1 and MR2 was found to be 18.2 mg/g and 16.9 mg/g respectively. The utilization of carbohydrate increases with age of the plant for their growth and development. In present study, total carbohydrate levels of 60DAP-AC (V1) and 90 DAP-SC (V1) was 18.52 mg/g and 17.23 mg/g respectively (Table 7). In MR2 variety, total carbohydrate levels of 60 DAP-AC (MR2) and 90 DAP-SC (MR2) was 16.89 mg/g and 16.83 mg/g respectively (Table8).

Table 1. Effect of different transplanting days on chlorophyll-a, b (mg/g), total chlorophyll (mg/g) and carotenoid content (mg/g) of variety V1 mini-clones

	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)	Carotenoid (mg/g)
DAP	**V1**	**V1**	**V1**	**V1**
T1-60D (AC)	1.85^{b}	0.76^{bc}	2.12^{b}	0.73^{b}
T2-70D (AC)	1.84^{b}	0.79^{b}	2.18^{b}	0.75^{b}
T3-90D (AC)	2.01^{a}	0.85^{a}	2.40^{a}	0.82^{a}
T4-90D (SC)	1.78^{b}	0.73c	1.92^{c}	0.63^{c}
SE(d)	**0.05**	**0.01**	**0.07**	**0.02**
CD(0.05)	**0.11****	**0.03****	**0.15****	**0.06****

***Highly significant, *Significant; Each value is the mean of four replications*
Mean followed by same alphabets are on par with each other

Table 2. Effect of different transplanting days on chlorophyll-a, b (mg/g), total chlorophyll (mg/g) and carotenoid content (mg/g) of variety MR2 mini-clones

	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)	Carotenoid (mg/g)
DAP	**MR2**	**MR2**	**MR2**	**MR2**
T1-60D (AC)	1.67^{c}	0.70^{b}	1.81^{bc}	0.62^{b}
T2-70D (AC)	1.80^{b}	0.73^{b}	1.94^{b}	0.65^{b}
T3-90D (AC)	2.01^{a}	0.81^{a}	2.23^{a}	0.71^{a}
T4-90D (SC)	1.67^{bc}	0.70^{ab}	1.70^{c}	0.60^{b}
SE(d)	**0.07**	**0.03**	**0.06**	**0.02**
CD(0.05)	**0.15****	**0.06****	**0.14****	**0.05****

***Highly significant, *Significant; Each value is the mean of four replications*
Mean followed by same alphabets are on par with each other

Table 3. Effect of different transplanting days on macronutrient composition of variety V1 mini-clones

	Nitrogen (%)	Phosphorous (%)	Potassium (%)
DAP	**V1**	**V1**	**V1**
T1-60D (AC)	3.46^{c}	0.29^{b}	1.67^{b}
T2-70D (AC)	3.89^{b}	0.30^{b}	1.69^{b}
T3-90D (AC)	4.32^{a}	0.33^{a}	1.88^{a}
T4-90D (SC)	2.88^{d}	0.27^{b}	1.64^{b}
SE(d)	**0.13**	**0.01**	**0.05**
CD(0.05)	**0.29****	**0.02****	**0.11****

***Highly significant, *Significant; Each value is the mean of four replications*
Mean followed by same alphabets are on par with each other

Table 4. Effect of different transplanting days on macronutrient composition of variety MR2 mini-clones

	Nitrogen (%)	Phosphorous (%)	Potassium (%)
DAP	**MR2**	**MR2**	**MR2**
T1-60D (AC)	3.32^{c}	0.28^{bc}	1.59^{bc}
T2-70D (AC)	3.67^{b}	0.29^{b}	1.61^{ab}
T3-90D (AC)	4.21^{a}	0.31^{a}	1.68^{a}
T4-90D (SC)	2.79^{d}	0.25^{c}	1.52^{c}
SE(d)	**0.09**	**0.01**	**0.03**
CD(0.05)	**0.20****	**0.03****	**0.07****

***Highly significant, *Significant*
Each value is the mean of four replications
Mean followed by same alphabets are on par with each other

Table 5. Effect of different transplanting days on moisture content (%) and moisture retention capacity (%) of variety V1 mini-clones

	Moisture content (%)	Moisture retention capacity (%)
DAP	**V1**	**V1**
T1-60D (AC)	75.32^{a}	61.92^{ab}
T2-70D (AC)	75.81^{a}	62.05^{ab}
T3-90D (AC)	78.24^{a}	64.52^{a}
T4-90D (SC)	69.19^{b}	60.32^{b}
SE(d)	**2.68**	**1.29**
CD(0.05)	**5.85***	**2.81****

***Highly significant, *Significant*
Each value is the mean of four replications
Mean followed by same alphabets are on par with each other

Table 6. Effect of different transplanting days on moisture content (%) and moisture retention capacity (%) of variety MR2 mini-clones

	Moisture content (%)	Moisture retention capacity (%)
DAP	**MR2**	**MR2**
T1-60D (AC)	71.91^{a}	59.82^{b}
T2-70D (AC)	72.43^{a}	60.05^{ab}
T3-90D (AC)	74.26^{a}	62.51^{a}
T4-90D (SC)	67.87^{b}	57.72^{b}
SE(d)	**1.80**	**1.20**
CD(0.05)	**3.93***	**2.63****

***Highly significant, *Significant*
Each value is the mean of four replications
Mean followed by same alphabets are on par with each other

The protein content of mulberry leaves had contributed to 70% of silk protein synthesized by silkworm [1]. The experimental results have showed that in V1, leaf protein content of treatment 60DAP-AC (V1) and 90 DAP-SC (V1) was 27.12 mg/g and 26.54 mg/g respectively (Table 7). In MR2 variety, protein levels of 60 DAP-AC (MR2) and 90 DAP-SC (MR2) was 23.17 mg/g and 22.61 mg/g respectively (Table 8). The present results corroborate with the findings done by Sudhakar *et al.* [27] estimated 20.48% soluble protein content and also supported by Ramamoorthy *et al.* [26] who recorded soluble protein content in leaf was around 24.8 mg/g and 20.0 mg/g in V1 and MR2 respectively.

In the study, total sugar levels in 60DAP-AC (V1) and 90 DAP-SC (V1) was 13.42 % and 12.23 % respectively (Table 7). In MR2 variety, total sugar content in 60 DAP-AC (MR2) and 90 DAP-SC (MR2) was found to be 12.27 % and 12.05 % respectively (Table 8).

Table 7. Effect of different transplanting days on proximate composition of variety V1 mini-clones

	Soluble protein (mg/g)	Total carbohydrate (mg/g)	Crude protein (%)	Total sugars (%)
DAP	**V1**	**V1**	**V1**	**V1**
T1-60D (AC)	27.12^{b}	18.52^{ab}	20.15^{bc}	13.42^{b}
T2-70D (AC)	27.57^{b}	18.84^{a}	20.73^{b}	14.69^{b}
T3-90D (AC)	29.52^{a}	19.80^{a}	22.66^{a}	15.52^{a}
T4-90D (SC)	26.54^{b}	17.23^{b}	19.08^{c}	12.23^{c}
SE(d)	**0.75**	**0.62**	**0.57**	**0.07**
CD(0.05)	**1.64****	**1.36****	**1.25****	**0.15****

***Highly significant, *Significant*
Each value is the mean of four replications
Mean followed by same alphabets are on par with each other

Table 8. Effect of different transplanting days on proximate composition of variety MR2 mini-clones

	Soluble protein (mg/g)	Total carbohydrate (mg/g)	Crude protein (%)	Total sugars (%)
DAP	**MR2**	**MR2**	**MR2**	**MR2**
T1-60D (AC)	23.17^{bc}	16.89^{b}	18.67^{bc}	12.27^{bc}
T2-70D (AC)	24.23^{b}	17.10^{b}	19.48^{b}	12.22^{b}
T3-90D (AC)	26.42^{a}	19.25^{a}	21.30^{a}	14.16^{a}
T4-90D (SC)	22.61^{c}	16.83^{b}	17.76^{c}	12.05^{c}
SE(d)	**0.54**	**0.43**	**0.60**	**0.06**
CD(0.05)	**1.17****	**0.95****	**1.31****	**0.14****

***Highly significant, *Significant*
Each value is the mean of four replications
Mean followed by same alphabets are on par with each other

High sugar contents may be due to increased metabolic activity which may in turn be responsible for additional synthesis of sugar. These findings are in line with Dandin and giridhar [28]. The results were further supported by Sudhakar *et al.* [18] who registered total sugar content of 16.4% in V1 variety.

4. CONCLUSION

Mini clones 60 DAP-AC (V1) and 60 DAP-AC (MR2) showed mean chlorophyll a content of 1.85 and 1.67 mg/g respectively. Similarly, chlorophyll b content was 0.76 mg/g and 0.70 mg/g of 60 DAP-AC (V1) and 60 DAP-AC (MR2) mini clones respectively. Treatments 60 DAP-AC (V1) and 60 DAP-AC (MR2) recorded total chlorophyll content of 2.12 and 1.81mg/g respectively. Treatments 60 DAP-AC (V1) and 60 DAP-AC (MR2) showed carotenoid content of 0.73 mg/g and 0.62 mg/g respectively.

Mini clones of 60 DAP-AC (V1) recorded maximum nitrogen (3.46%), phosphorous (0.29%) and potassium (1.67%). Similarly, mini clones of 60 DAP-AC (MR2) showed highest nitrogen (3.32%), phosphorous (0.28%) and potassium (1.59%) content when compared to check. Similar trend was observed with respect to moisture content and moisture retention capacity. Mini clones of 60 DAP-AC (V1) recorded moisture content and moisture retention capacity of 75.32 per cent and 61.92 per cent. Likewise, mini clones of 60 DAP-AC (MR2) showed 71.91 per cent and 59.82 per cent moisture content and moisture retention capacity respectively. Higher soluble protein (27.12mg/g), total carbohydrate (18.52mg/g), crude protein (20.15%) and total sugar content (13.42%) were recorded in V1 mini clones on 60 DAP. Similarly, MR2 mini clones showed maximum of soluble protein (23.17mg/g), total carbohydrate (16.89 mg/g), crude protein (18.67%) and total sugar content (12.27%) on 60 DAP compared to check. Mini clones 60 DAP-AC (V1) and 60 DAP-AC (MR2) performed better in biochemical parameters compared to their respective check.

Hence, Mini clonal leaves can be used to feed silkworm to get enhanced silk parameters.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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