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Biochemical Responses of Mulberry Varieties V1 and G4 to Different Spacing Conditions

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ABSTRACT: Mulberry plants compete with one another for few resources like air, light, soil moisture, and nutrients as a result of the lack of available space. The study focused on assessing the impact of various spacing configurations on biochemical parameters in two tree-type mulberry varieties, V1 and G4. Among the biochemical parameters measured, including chlorophyll-a (1.96 mg g^{-1}), chlorophyll b (0.81 mg g^{-1}), total chlorophyll (1.77 mg g^{-1}), carotenoids (0.80 mg g^{-1}), crude protein (23.16%), total sugars (15.61%), moisture content (78.34%), moisture retention capacity (64.14%), soluble protein(28.49 mg g-1), total carbohydrate (19.84 mg g^{-1}), and macronutrients such as nitrogen (4.16%), phosphorus (0.32%), and potassium (1.77%), it was consistently observed that the 6ft × 6ft spacing for the V1 variety yielded the highest values compared to the other spacing configurations used for the G4 variety. These results suggest that the cultivation of tree-type mulberries is particularly well-suited to a spacing of 6' × 6'.

Keywords: Biochemical, Chlorophyll Spacing, Mulberry, Moisture.

INTRODUCTION

Mulberry, as the exclusive host plant for *Bombyx mori* L. and a pivotal economic crop cultivated specifically for silkworm rearing, plays a vital role in the sericulture industry. It accounts for 38.20% of the successfully produced cocoons. Maintaining mulberry in optimal condition necessitates precise management, including the application of appropriate quantities of both organic and inorganic fertilizers. This is particularly important because mulberry is a perennial crop that undergoes periodic pruning (Rahman *et al.*, 2020; Vijay *et al.*, 2023). Mulberry, belonging to the *Morus* spp., is characterized by its fast growth, woody nature, and perennial status, often pruned to take on the form of bushes or dwarf trees. The quality of mulberry leaves is of paramount importance as they serve as the primary food source for silkworms (*Bombyx mori* L.), directly influencing the sustainability and profitability of the sericulture sector. Furthermore, the plantation system employed has a substantial impact on the quality of mulberry leaves (Sekhar *et al.*, 2015). Spacing plays a significant role in determining leaf yield, influencing various aspects of plant growth such as plant height, the number of branches per plant, shoot length, the number of leaves per plant, and ultimately, the leaf yield per plant.

In sericulture, the production of mulberries constitutes more than 60% of the total expenses associated with cocoon production, as highlighted by Wani *et al.* (2014). Enhancing mulberry quality and yield relies heavily on the yield of leaves and various related criteria. The factors contributing to mulberry leaf yield, such as genotype and agronomic techniques, as well as characteristics like plant leaf yield, internodal spacing, and the quantity and length of shoots, all exert a substantial influence, as discussed by Pawan *et al.* (2017). Different spacing affect the pest and disease incidence of mulberry and lowest incidence was reported in 6ft × 6ft. Spacing directly influence the growth and yield of mulberry such as highest single shoot length (133.10 cm), minimum internodal length (3.95 cm), length of the longest shoot (128.47 cm), total shoot length (1267.18 cm), physiological attribute like leaf area (137.75 cm^2), and yield attributes like single leaf weight of 5.35 g, weight of 100 leaves (518.50 g), number of leaves per shoot (61.75), and leaf yield per plant (3.00 kg) were found in 6ft ×6ft (Vijay *et al.*, 2023). The limited availability of space leads to competition among mulberry plants for essential resources like air, light, soil moisture, and nutrients. This competition, in turn, negatively impacts yield, as observed in the study by Setua *et al.* (2011). To address these dynamics, the current research was undertaken to evaluate the effects of different spacing arrangements in tree-type mulberry plantations on biochemical characters contributing to yield.

MATERIAL AND METHODS

Collection of samples. To investigate biochemical parameters, leaves were collected from the tree-type mulberry at 90 days after transplanting (DAT), after subjected to oven drying. The resulting dried samples were finely ground into a powder and then stored in an airtight container for subsequent analysis. The analysis was conducted in the PG laboratory located at the Department of Sericulture, Forest College and Research Institute, Mettupalayam.

Treatments

T1: Plot with 5'× 5' spacing
T2: Plot with 6' × 6' spacing
T3: Plot with 7' × 7' spacing

Biochemical parameters

Leaf moisture content

The moisture content in mulberry leaves was assessed by utilizing two parameters: the weight of the fresh leaves and the weight of the dry leaves. The results were expressed as a percentage, following the method.

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

Moisture retention capacity. The moisture retention capacity in the leaves was evaluated based on three parameters: the weight of the fresh leaves, the weight of the leaves after a six-hour period, and the dry weight of the mulberry leaves. The results were expressed as a percentage to quantify the extent of moisture retention, as per the method employed.

Moisture Retention Capacity

$$= \frac{\text{Weight of leaves after 6 hours - Dry weight}}{\text{Fresh weight - Dry weight}} \times 100$$

Chlorophyll content. In each replication, freshly harvested leaves from the middle section of various varieties were collected to measure their chlorophyll content. The determination of chlorophyll a, chlorophyll b, and total chlorophyll content in these leaves followed the procedure outlined by Zhang *et al.* (2023). This involved using a spectrophotometer to measure absorbance at wavelengths of 645 nm and 663 nm.

Carotenoid content. The carotenoid content in mulberry leaf extract was quantified using the following formula and expressed in units of milligrams per gram of fresh leaf weight.

Carotenoid = $(7.6 \times OD^{@480nm}) - (1.49 \times OD^{@510nm}) \times V/W \times 1000$

Total Carbohydrate: The analysis aimed to determine the total carbohydrate content in the collected leaf samples, and the outcomes were expressed in milligrams per gram of fresh leaf weight.

Crude Protein: To determine the crude protein content in mulberry leaf samples, the method proposed by Jones (1931) was employed. This calculation involved multiplying the nitrogen content (%N) by a factor of 6.25 to establish the crude protein composition.

Soluble Protein: The quantification of soluble protein levels in mulberry leaf samples was conducted according to the procedure outlined by Lowry *et al.* (1951). The results were quantified and reported in milligrams per gram of fresh leaf weight.

Total sugars. The total soluble sugar content was estimated through the utilization of the phenol sulfuric acid reagent method, a procedure detailed by Dubois *et al.* (1951). The outcomes were expressed as a percentage relative to the total sugar content.

Total nitrogen. Total nitrogen composition in mulberry sample was determined by micro-kjeldahl method as prescribed by Humphries (1956) and expressed in percentage.

Total phosphorus. To determine total phosphorous level in the mulberry sample, a process suggested by Jackson *et al.* (1973) was followed and unit was given in terms of per cent.

Total potassium. The potassium levels in plant sample was estimated as recommended by Jackson *et al.* (1973) and given in terms of percentage.

Statistical analysis. The data collected from above experiments were critically analysed by adopting Factorial Randomized Block Design (FRBD) as described by Fisher. During statistical analysis of data, the treatments which were found significant, the critical differences were calculated and analysed at five per cent level of probability. AGRES software package (version 0.74) was used to analyse the stage wise data.

RESULTS AND DISCUSSION

Notable variations were evident with respect to chlorophyll- a when mulberry plants planted with different spacing (Table 1). Among these spacing, highest chlorophyll-a of 1.96 mg g^{-1} was found to be in 6ft × 6ft leaves followed by 7ft × 7ft (1.80 mg g^{-1}). On comparing mulberry varieties, V1 was observed to be with higher level of chlorophyll-a (1.85 mg g^{-1}) than variety G4. In interaction between different spacing and different mulberry varieties, significantly more chlorophyll-a content of 2.01 mg g^{-1} was registered in leaves of 6ft × 6ft planted V1; whereas, lower content of 1.67 mg g^{-1} was recorded in 5ft × 5ft planted G4. Chlorophyll b in leaves significantly varied between spacing with higher values of 0.81 mg g^{-1} in 6ft × 6ft (Table 5). The next better treatment was 7ft × 7ft (0.77 mg g^{-1}) which was on par with (0.73 mg g^{-1}). Among mulberry varieties, highest chlorophyll b content of 0.80 mg g^{-1} was registered in V1 followed by G4 (0.75 mg g^{-1}). In case of interaction studies, there was no significant difference in the chlorophyll b in response to spacing and mulberry varieties. Sudhakar *et al.* (2018) reported that, 8x5 spacing of tree type mulberry with different irrigation systems showed the variation in the Chlorophyll a (2.31 mg g^{-1}), Chlorophyll b (0.70 mg g^{-1}), Total chlorophyll (3.01 mg g^{-1}) and moisture content (75.62%).

Total chlorophyll varied significantly between spacing with higher values of 1.77 mg g^{-1} in 6ft × 6ft leaves (Table 1). The next better treatment was 7ft × 7ft (1.65 mg g^{-1}) which was on par with 5ft × 5ft (1.60 mg g^{-1}). Among mulberry varieties, highest chlorophyll b content of 1.74 mg g^{-1} was registered in V1 followed by G4 (1.60 mg g^{-1}). In case of interaction studies, there was no significant difference in the total chlorophyll in response to spacing and mulberry varieties. Bharathi (2020) found that spacing 2.4 × 1.2 m showed very good content of moisture (73.13 - 79.71%), crude protein (25.56 - 29.53%), total sugars (16.34 - 17.37%), total chlorophyll (3.076 - 3.266 mg/g), total minerals (9.79 - 10.67%) and crude fibre (11.07 - 11.77%).

There was significant increase in the macronutrients namely nitrogen, phosphorus and potassium due to the wider spacing and tree type mulberry (Fig. 1). Among the different spacing's, the plants with 6ft × 6ft registered significantly highest nitrogen (4.16 %), phosphorus (0.32 %) and potassium (1.77 %) content. The next better treatment was 7ft × 7ft which recorded 3.98, 0.27 and 1.67 per cent, N, P and K respectively. On comparing different mulberry varieties, V1 recorded significantly highest macronutrients content (4.07%, 0.28%, and 1.74%) over other variety G4. The interaction between various concentrations and mulberry varieties showed that more quantity of macronutrients *viz.,* nitrogen, phosphorus and potassium of 4.24, 0.34 and 1.85 per cent, respectively were obtained from 6ft × 6ft spacing of V1 variety, whereas, lesser amount was observed in 5ft × 5ft (3.70%, 0.25% and 1.52%, respectively) in variety G4. Ahmed *et al.* (2022) observed that nutrient elements favored in increasing growth, leaf yield, improving quality of mulberry leaves, suppresses foliar diseases as well as improved silk cocoon productivity.

Carotenoids in mulberry leaves significantly varied between spacing with higher values of 0.80 mg g^{-1} in 6ft × 6ft (Fig. 2). The next better treatment was 7ft × 7ft (0.73 mg g^{-1}) which was on par with 5ft × 5ft (0.70 mg g^{-1}). Among mulberry varieties, highest carotenoids content of 0.77 mg g^{-1} was registered in V1 followed by G4 (0.71 mg g^{-1}). In case of interaction studies, there was no significant difference in the carotenoids in response to spacing and mulberry varieties. Significantly higher crude protein and total sugars of 23.16 % and 15.61 % were obtained in 6ft × 6ft mulberry leaves followed by 7ft × 7ft (21.01 % and 15.19 %). In respect to mulberry varieties, more amount of crude protein and total sugars (21.74 % and 15.45 %) were noticed in V1than G4. In case of interaction studies, there was no significant difference in the crude protein and total sugars in response to spacing and mulberry varieties. Sun *et al.* (2023) documented that increased in the sugar and chlorophyll content in the different stress condition. V1 had maximum photosynthetic pigments *viz.,* Chlorophyll-a (3.12 mg g^{-1}), chlorophyll-b (0.85 mg g^{-1}), total chlorophyll (3.23 mg g^{-1}) and carotenoids (1.02 mg g^{-1}); higher moisture content (75.82 %) and moisture retention capacity (72.65), soluble protein (31.21 mg g^{-1}) and total carbohydrate (20.61 mg g^{-1}); high amount of macronutrients as nitrogen (4.73 %), phosphorus (0.51 %) and potassium (3.51 %) were reported by Roja Kaliappan *et al.* (2021).

6ft × 6ft planted mulberry leaves recorded the highest moisture content and moisture retention capacity of 78.34 and 64.14 per cent, respectively, which was followed by 7ft × 7ft (75.33 % and 62.87 %, respectively) (Fig. 3). Among the mulberry varieties, V1 was found to be significantly superior in moisture content (77.41 %) and moisture retention capacity (63.56 %) of mulberry leaves than G4. In respect of interaction effect, maximum moisture content (79.56 %) and moisture retention capacity (65.14 %) were noticed in 6ft × 6ft planted V1 variety and minimum in the 5ft × 5ft planted G4 variety (72.34 % and 60.12 %, respectively). Magadum and Singh (2021) reported that, moisture content (72.17%) and moisture retention capacity (70.66 %) from different genotypes of mulberry with spacing of 90cm × 90cm.

Among the various spacing, best spacing was 6ft × 6ft, which recorded the highest soluble protein (28.49 mg g^{-1}) and total carbohydrate (19.84 mg g^{-1}) content compared to other spacing (Table 2). Regarding the different mulberry varieties, V1 recorded high amount of soluble protein (28.27 mg g^{-1}) and total carbohydrate (15.45 mg g^{-1}) compared to G4. In the interaction between different spacing and mulberry varieties, 6ft × 6ft in V1 recorded the maximum level of soluble protein (29.56 mg g^{-1}) and total carbohydrate (20.10 mg g^{-1}) and the minimum level was in 5ft × 5ft of G4 (25.13 mg g^{-1} and 16.89 mg g^{-1}). Yogananda Murthy *et al.* (2013) found that spacing 4 × 4 recorded highest soluble proteins (4.62%), total sugars (2.30%), chlorophyll-a (2.42 mg/g), chlorophyll-b (1.26mg/g), total chlorophyll (3.74mg/g), moisture content (66.18%) and moisture retention capacity (60.57%).

Table 1: Effect of different spacing on chlorophyll a (mg/g), chlorophyll b (mg/g) and total chlorophyll (mg/g) of tree type mulberry.

Treatments		Chlorophyll a (mg/g)			Chlorophyll b (mg/g)			Total chlorophyll (mg/g)		
		V1	G4	Mean	V1	G4	Mean	V1	G4	Mean
5ft × 5ft		1.72	1.67	1.69	0.76	0.71	0.73	1.92	1.80	1.86
6ft × 6ft		2.01	1.92	1.96	0.85	0.78	0.81	2.40	2.21	2.30
7ft × 7ft		1.83	1.78	1.80	0.79	0.76	0.77	2.12	1.98	2.05
Mean		1.85	1.79	1.81	0.80	0.75	0.77	2.14	1.99	2.06
CD (P=0.05)	T	0.11**			0.03**			0.15**		
	V	0.08**			0.03**			0.13**		
	T × V	1.01*			NS			NS		

*Significant, ** Highly Significant, NS – Non Significant; each value is mean of six replications and pooled mean of two crops

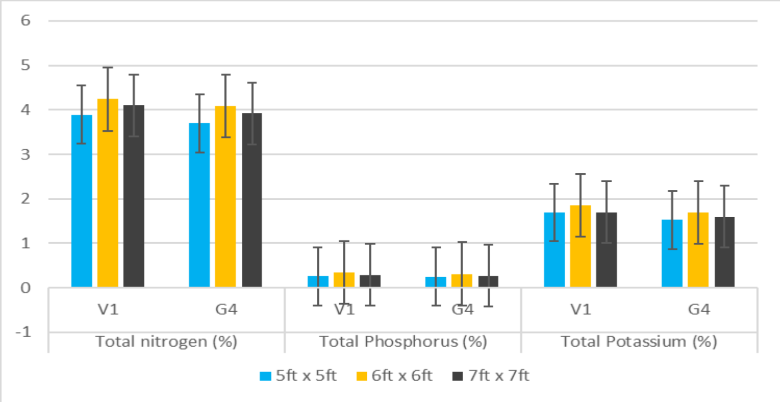

Fig. 1. Effect of different spacing on total nitrogen (%), total phosphorus (%) and total potassium (%) of tree type mulberry.

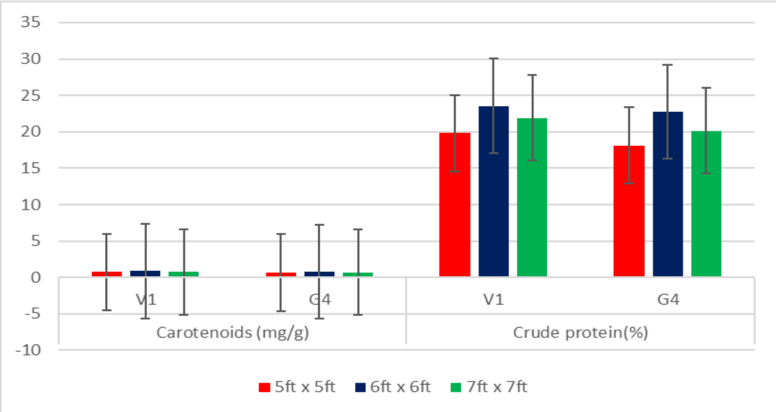

Fig. 2. Impact of different spacing on carotenoids (mg/g) and crude protein (%) of tree type mulberry.

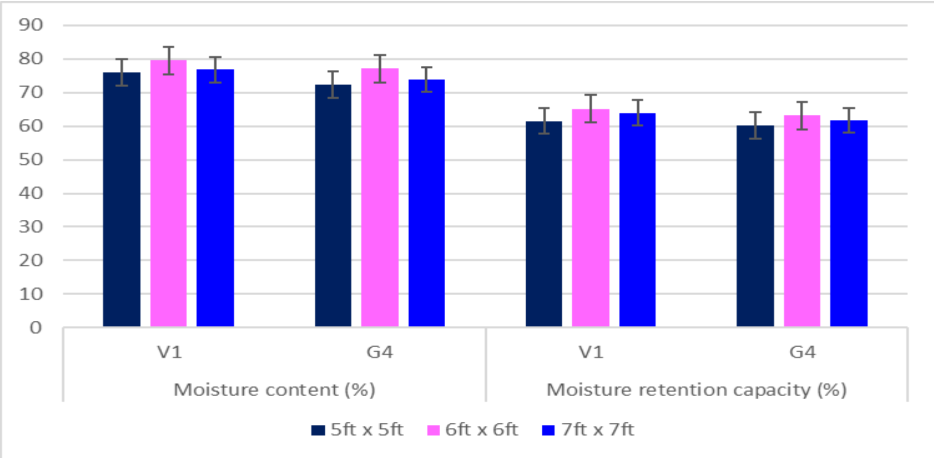

Fig. 3. Impact of different spacing on moisture content (%) and moisture retention capacity (%) of tree type mulberry.

Table 2: Effect of different spacing on soluble protein (mg g^{-1}), total carbohydrate (mg g^{-1}) and total sugars (%) of tree type mulberry.

Treatments		Soluble protein (mg g^{-1})			Total carbohydrate (mg g^{-1})			Total sugars (%)		
		V1	G4	Mean	V1	G4	Mean	V1	G4	Mean
5ft × 5ft		27.14	25.13	26.13	17.24	16.89	17.06	14.78	13.89	14.33
6ft × 6ft		29.56	27.43	28.49	20.10	19.59	19.84	16.14	15.09	15.61
7ft × 7ft		28.11	26.67	27.39	18.56	17.13	17.84	15.43	14.95	15.19
Mean		28.27	26.41	27.43	18.63	17.87	17.90	15.45	14.64	15.23
CD (P=0.05)	T	1.49**			1.80**			1.10**		
	V	1.21**			1.01**			0.08**		
	T × V	2.18*			2.01*			NS		

*Significant, ** Highly Significant, NS – Non Significant; each value is mean of six replications and pooled mean of two crops

CONCLUSIONS

According to the findings of this study, among the various spacing, 6ft x 6ft was recorded higher in all the biochemical parameters. These findings indicate that various yield-related factors performed more favourably in the 6ft × 6ft spacing plantation systems compared to narrower spacing alternatives. This phenomenon may be attributed to the spacing's influence, wherein wider spacing facilitates more robust growth by reducing competition for nutrients and space among the plants. Furthermore, the moisture content was found to be highest in the 6ft × 6ft spacing arrangement. This can likely be attributed to the ample spacing allowing for improved aeration and nutrient uptake, ultimately leading to higher moisture retention in the mulberry leaves.

FUTURE SCOPE

The promising spacing identified in this study can be exploited commercially to increase mulberry and cocoon cocoon.

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Conflict of Interest. None.

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