Research Article

Leaf Quality Evaluation of Ten Mulberry (*Morus*) Germplasm Varieties through Phytochemical Analysis

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ABSTRACT

Ten mulberry varieties viz., Tr_8, Tr_{12}, Tr_{20}, S_{1708}, MS_5, C_6, C_{10}, *Matigara black*, *Morus nigra* and M_5 were analysed for their leaf quality through phytochemical tests. Results revealed that, total proteins, total sugars and amino acids were high in tender followed by medium and coarse leaves. Total proteins, total sugars and amino acids were high in S_{1708} leaves and total proteins and total sugars were low in M_5 leaves and amino acids were low in C_6 leaves. Phenols, prolines and chlorophyll contents were high in medium followed by coarse and tender leaves. Phenols were high in M_5 and least in *Matigara black* leaves. Prolines were high in *Matigara black* and low in M_5 leaves. S_{1708} leaves recorded highest total chlorophyll, chlorophyll-a and chlorophyll-b whereas C_6 recorded lowest total chlorophyll, chlorophyll-a and Chlorophyll-b. Moisture contents were high in tender followed by medium and coarse leaves. Moisture content and moisture retention capacity were significantly high in S_{1708} and lowest in C_6 leaves.

Keywords: Amino acids, Chlorophyll, Moisture, Mulberry, Proteins, Sugars.

INTRODUCTION

It is well-established fact that, in sericulture, more than 60% of total cost of cocoon production goes towards mulberry production alone. Hence, in recent years maximum attention has been given for the improvement of mulberry both in terms of quality and quantity. Growth and development of silkworm *Bombyx mori* L. and cocoon crop are mainly influenced by yield and nutritional quality of mulberry leaf used as feed. Nutritive value of mulberry (*Morus* spp.) leaf is a key factor besides environment and technology adoption for better silkworm cocoon crop. Among the various factors influencing silkworm growth, leaf quality plays a major role. It is a fact that leaf quality differs among mulberry varieties which in turn responsible for the difference in silkworm rearing performances.[1] Quality of mulberry leaf was highly influenced by varieties, cultivation practices, preservation techniques, age and position of leaf and leaf quality was determined based on moisture content. Higher moisture content of mulberry leaves has a direct effect on growth and development of silkworm by favouring the ingestion, digestion and assimilation of nutrients. Mulberry leaves containing more water, total sugar and soluble carbohydrate and less mineral are best relished by silkworms. Nutritive requirement of silkworm larvae vary with the maturity of leaves fed. Chawki silkworms required leaves of high moisture content as it is easy to digest and late age silkworms required mature leaves with less moisture content as late age silkworms have the strength to digest mature leaves. On the other hand too much mature leaves do not contain sufficient biochemical contents and moisture content is not suitable to feed silkworms.[2] Keeping in view, the importance of nutritional value of mulberry leaves, present study aims to evaluate better performing mulberry varieties through quantitative estimation of phytochemical parameters and identify the well suited mulberry variety to Kolar agro climatic conditions as it is the premier districts and traditional sericulture belt of Karnataka, alone accounts for 40% of total raw silk produced in state.

MATERIALS AND METHODS

Present experiment was carried out in the existing agro climatic conditions at Bethamangala village of Bangarpet taluk in Kolar district, Karnataka during 2007-2011. Ten mulberry varieties viz., TR_8, TR_{12}, TR_{20}, S_{1708}, MS_5, C_6, C_{10}, *Matigara black*, *Morus nigra* and M_5 selected from germplasm bank maintained at CSGRC, CSB, Hosur, Tamil Nadu were used in the investigation. Mulberry variety M_5 is used as a check variety for comparison purpose. Experiment was carried out in RBD method with 4 replications/variety. Mulberry leaves from three years old plants were used to test phytochemical parameters from time to time in different season's viz., summer, rainy and winter. Average values were tabulated in table 1 and values expressed are means of four replicate determinations. Data collected on various parameters were subjected to statistical analysis by adopting 'Method of Analysis of Variance' appropriate to the experiment design.[3]

Collection of Mulberry Leaf Samples

Healthy mulberry leaves were collected from the plants in experimental garden. Since silkworm feeds on tender, medium and coarse leaves at various developmental stages, quantification of phytochemical compounds has

been carried out in tender, medium and coarse leaves separately. Leaf samples collected were washed thoroughly with tap water followed by distilled water, then wiped and dried under shade followed by oven drying at 60°C-65°C till constant weight was attained. Completely dried leaf samples were pulverised separately and used for analysis.

Total Proteins

Total proteins were measured using Folin-phenol reagent.[4] 50mg of dry leaf powder was homogenised in 80% ethanol using mortar and pestle and homogenate was centrifuged at 5000rpm for 20 minutes. Supernatant was discarded and pellet was suspended in 10ml of 10% trichloro acetic acid (TCA) for 30minutes to precipitate proteins, centrifuged at 5000rpm for 10minutes and supernatant was discarded. Pellet was then washed with 5%TCA to remove interfering amino acids and phenols. Protein precipitate was then dissolved in 1N sodium hydroxide by allowing for 30minutes in hot water bath. Extracted proteins in 1N NaOH were diluted 10 times with distilled water. 1ml of protein sample was taken in a test tube, 5ml of alkaline copper reagent was added. Mixture was allowed to stand at room temperature for 10 minutes. 0.5ml of folin-phenol reagent was rapidly added and mixed. After 30 minutes absorbency was measured at 750nm. Protein content was calculated by preparing standard curve with bovine serum albumin (BSA).

Total Sugars

Total sugars were estimated using anthrone reagent.[5] 25mg of dry leaf powder was crushed thoroughly in 10ml of hot ethanol using a mortar and pestle. The leaf tissue was exhaustively extracted twice or thrice using small quantity of ethanol cooled and filtered through a whatman filter paper. The final volume of filtrate was made to 10ml either by adding or evaporating the ethanol. 1ml of ethanol extract was pipetted into a test tube and 4ml of anthrone reagent was added and mixture was incubated at 100°C in a boiling water bath for 10minutes. Then mixture was removed, cooled to room temperature in running water, and absorbance of resultant blue-green solution was measured at 625nm. Amount of sugar present in the extract was calculated using a standard curve prepared from glucose.

Amino Acids

Amino acids were measured by modified ninhydrin method.[6] 50mg of dry leaf powder was homogenised in 5ml of 80% methanol using mortar and pestle. Homogenate was centrifuged at 5000rpm for 10minutes. The supernatant was partitioned with an equal volume of petroleum ether in order to remove chloroplast pigments. The methanolic layer was used for amino acid estimation. 1ml of sample was taken in a test tube, to which 0.5ml of 0.2M methyl cellosolve ninhydrin solution was added and reaction mixture was kept over a boiling water bath for 20minutes. Later, it was cooled under running water. Volume of the reaction mixture was made up to 5ml with 60% ethanol reagent and absorbance was measured at 570nm. Amount of amino acids were calculated using a standard curve prepared with glycine.

Phenols

Phenols in fresh leaf were estimated by standard method.[7] 100mg of leaf sample was extracted with 10ml of distilled water in a clean mortar and pestle. Homogenate was centrifuged at 3000rpm for 20 minutes; supernatant was collected and used for analysis. To 1ml of the extract, 1ml of folin-phenol reagent was added followed by 2ml of 20%sodium carbonate solution. Mixture was shacked vigorously and placed on boiling water bath for one minute, then cooled under running water. Solution was diluted with distilled water to make up the volume to 25ml and optical density was measured at 650nm and Caffeic acid was used as standard.

Prolines

Prolines were estimated by standard method.[8] 200mg of dried leaf powder was homogenised in 3% sulphosalycillic acid and mixture was filtered through whatman No.1 filter paper. Filtrate was made up to 10ml by adding 3%sulphosalycillic acid. 2ml of filtrate was pipetted into test tube and 2ml of glacial acetic acid and 2ml of acid ninhydrin regent was added. Mixture was kept in a boiling water bath for 1hour. Then cooled to room temperature, add 4ml of toluene and mixed thoroughly. Finally colorless fraction was discarded and intensity of scarlet color formed was measured at 520nm. Proline content was calculated using the following equation.

$$\frac{35.892 \times \text{optical density} \times \text{volume of the extract}}{\text{Volume of sample} \times \text{weight of tissue (g)}} = \mu g/g$$

Where 35.892 is μ factor derived from standard curve for pure proline.

Chlorophyll Content

Total chlorophyll contents from fresh mulberry leaves without maceration were determined using Dimethyl sulphoxide (DMSO).[9] 50mg of leaf tissue was taken in a test tube and add 7ml of DMSO to it and kept the mixture in an electric oven for 3 hours at 65°C. Finally, extract was transferred to a measuring cylinder and made up volume to 10ml with DMSO and assayed immediately or transferred to vials and stored below 4°C until required for analysis.

Estimation of Chlorophyll Content

Total chlorophyll, chlorophyll-a and chlorophyll-b contents were estimated.[10] 3ml of chlorophyll extract was transferred to a cuvette and optical density was recorded at 645nm and 663nm against DMSO blank in systronics-105 (MK-1) spectrophotometer. Chlorophyll contents were calculated using following equations.

$$\text{Total chlorophyll} = \frac{20.2\,(D\,645) + 8.02\,(D663) \times 1000 \times \text{weight of leaf (g)}}{V} = \ldots \text{mg/gram}$$

$$\text{Chlorophyll-a} = \frac{(12.7 \times D663) - 2.67 \times D645) \times 1000 \times \text{weight of leaf (g)}}{V} = \ldots \text{mg/gram}$$

$$\text{Chlorophyll-b} = \frac{(22.9 \times D645) - (4.68 \times D663) \times 1000 \times \text{weight of leaf (g)}}{V} = \ldots \text{mg/gram}$$

Where V = Volume of the extract, D = Optical density.

Determination of Leaf Moisture Content and Moisture Retention Capacity

Leaf moisture content and moisture retention capacity were determined on fresh weight basis.[11] For each maturity, 25 leaves/replicate/variety were harvested separately from a longest shoot and leaves were wiped with a muslin cloth to remove dust particles and fresh weight was recorded immediately. Then leaves were kept in normal environmental conditions (26°C±1°C temperature; 70%±5% relative humidity) for 6hours. After 6hours, leaves were weighed for calculating water retention capacity. Then leaves were dried in hot air oven at 80°C for 48hours till constant weight was attained and dry weight was recorded. Leaf moisture content of tender, medium and coarse leaves was calculated separately by using following formula and expressed in percentage (%).

$$\text{Leaf moisture content (\%)} = \frac{\text{Fresh weight of leaves - Dry weight of leaves}}{\text{Fresh weight of leaves}} \text{X100}$$

Leaf moisture retention capacity was calculated by using following formula and expressed in percentage (%).

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

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

RESULTS AND DISCUSSION

Deficiency or imbalance of plant nutrients in mulberry leaves causes changes in the metabolic activity of silkworm larva. Nutritional contents of mulberry leaf greatly influence the growth and development of silkworm larvae, which in turn affects the quality and quantity of silk production.[12,13] Leaf quality is an important parameter used for evaluation of mulberry varieties while selecting best varieties for silkworm rearing and biochemical composition of mulberry leaves varies depending on variety, season, soil, water and cultural practices.[14,15]

Total Proteins

Total proteins in tender leaves of S_{1708}(27.31%) and Tr_8(25.81%) recorded higher values and significantly lower in M_5(15.91%) tender leaves. In medium leaves S_{1708}(20.92%) followed by Tr_8(20.10%) recorded highest protein content, did not differ significantly. M_5(12.94%) variety medium leaves revealed lesser protein content. Protein content was found highest in coarse leaves of S_{1708}(15.58%) followed by Tr_8(13.50%), and least was recorded in C_6(10.14%) and M_5(9.84%) (Table 1). Leaf protein is a major determinant of nutrient quality for many Lepidopteron larvae. It is known fact that, nearly 70% of protein content of raw silk namely fibroin and sericin are directly biosynthesized from mulberry leaf protein and remaining 30% is derived from silkworm body tissue and haemolymph protein, emphasizing the importance of leaf protein in silkworm nutrition.[16-18] Protein content present in different mulberry varieties had a direct bearing on larval growth particularly in silk gland development and cocoon characters of silkworm.[19] Similar studies were conducted and observed a significant varietal difference in protein content.[20,21] Biochemical composition of eight different mulberry varieties leaves S_{54}, C_1, MR_2, Kitchili, Roso, Kosen, Japanese and M_5 indicated variation in total protein content. However, except C_1 and MR_2 varieties other varieties did not showed significant variations.[22] 14 selected mulberry germplasm varieties also established maximum protein content in tender leaves which gradually depleted in medium and coarse leaves. Similar observations were also reported on different mulberry varieties.[23,24] Same trend was observed in present investigation. Studies on diploid and triploid mulberry varieties revealed that, higher crude protein content in triploids than diploids and also recorded variations in total protein content in many mulberry varieties.[25,26] 6 mulberry varieties were evaluated and results revealed that, S_{1635} variety recorded highest leaf protein (26.91%) content.[27] Proteins were higher in BC_{259}, S_{30}, S_{36}, S_{41} and C_1 varieties among the selected mulberry varieties.[28] TG mulberry variety had high protein content (23.58%) followed by DD (23.57%) and minimum in Jatinuni (19.92%) among the 23 elite varieties evaluated.[29] Maximum protein content was observed in mulberry variety *Chak majra* and minimum in Sujanpur variety.[30]

Total Sugars

Total sugar content was found to vary both in respect of leaf maturity and varieties studied. Tender leaves of S_{1708}(16.02%) recorded highest total sugars followed by Tr_{12}(15.05%) and lowest in C_6(10.85%). In medium leaves also similar trend was noticed. Highest was noticed in S_{1708}(14.10%) followed by Tr_{12}(13.52%) and less in C_6(9.73%) leaves. Course leaves of S_{1708}(11.74%), Tr_{12}(11.11%) and Tr_8(10.73%) recorded significantly higher level of total sugars lowest was recorded in C_6(8.02%) leaves (Table 1). Sugars play an important role in determining the quality of leaf that in turn influence healthy growth and development of silkworms. Sugars are utilized as the main source of energy apart from inducing the silkworms to bite the leaves (biting factor) and cherish it well. Tender leaves of S_{54} mulberry genotype showed higher total sugar content (13.05%) when compared to M_5 genotype (10.95%). Maximum total sugar content was recorded in tender leaves of 14 mulberry genotypes, which gradually declined with increasing growth periods and maturity levels. Higher sugar content in tender leaves could be attributed to the translocation of more photosynthetic products to upper

leaves (actively growing) than lower leaves and sugar depletion among varieties of different maturity levels could be attributed to genotypic characters. Similar observations were also noticed in the present investigation. Highest percentage of total sugars was recorded in S_{41} variety compared to other five indigenous varieties studied. S_{54} mulberry leaves contain more amount of total sugars compared to K_2 variety leaves.[31] Soluble sugar contents were higher in Kitchili and Japan varieties followed by S_{54} and M_5 mulberry varieties. Maximum total sugar content was found in Kairyoroso, an exotic variety among 7 mulberry varieties and also it was observed that, total sugars decrease with increased maturity of leaves from top to bottom.[32] Study reported that, maximum percentage of sugars in *Chinese white* variety and minimum in Sujanpur variety. It was observed that, maximum leaf sugar content in OPH_3 (11.60%) followed by Ber. C_{799} (11.28%) while minimum was found in $Sujanpur_5$ (9.22%). Mulberry variety S_{1635} (12.44%) showed maximum sugar content when compared to S_{13}, S_{34}, S_1, Vishwa and Mysore local varieties. It was recorded that, mulberry variety S_{1708} under 60cm x 60cm plant spacing showed maximum leaf sugar content among all the varieties studied. Exotic mulberry cultivar S_{799} had highest total sugars (12.66%) when compared to the varieties studied.[33] It was observed that, total sugar content was significantly higher in S_{36} mulberry variety among the taxa studied.[34]

Amino Acids

Amino acids recorded highest in tender leaves of mulberry variety S_{1708} (68.43 μmole/g) followed by Tr_8 (64.90 μmole/g) and lowest in C_6 (45.94 μmole/g) leaves. Significantly highest amino acids were noticed in medium leaves of S_{1708} (69.97 μmole/g) followed by Tr_8 (59.83 μmole/g) and lowest in C_6 (42.02 μmole/g) leaves. In course leaves, amino acid was highest in M_5 (33.11 μmole/g) followed by S_{1708} (33.01 μmole/g), Tr_8 (27.80 μmole/g), MS_5 (27.71 μmole/g), C_{10} (26.17 μmole/g), Tr_{12} (25.60 μmole/g), Tr_{20} (23.87 μmole/g), *Morus nigra* (23.04 μmole/g), *Matigara black* (22.52 μmole/g) and lowest was recorded in C_6 (21.88μmole/g) (Table 1). Mulberry leaves are quite rich in amino acid content (18.6%), forms an important constituent for silkworm nutrition.[35,36] Considerable amount of amino acids utilised for the formation of haemolymph, development of silk glands and cocoon production. Amino acids are important for phytophagous insects help them in food selection.[37] 12 mulberry genotypes were examined and results revealed that, there was no significant difference among the genotypes except a marginal difference of amino acid content in BC_{259}, S_{30}, S_{36} and G_5 mulberry genotypes (20.44mg/g-24.23mg/g). It was observed that, when mulberry genotypes are grown under drought condition, free amino acid contents varied among the genotypes.[38] Higher amount of free amino acid content recorded in S_{34} variety compared to other varieties studied. It was observed that, a significant difference in free amino acid content in S_{54} and MR_2 varieties, further Kitchili, Roso, Japan varieties did not differed and C_1 and Kosen varieties showed very low free amino acid content. Mulberry variety S_{41} recorded maximum amino acid content among the varieties tested and variation in amino acids is influenced by age of the plants, growth stage and maturity.

Phenols

Phenolic content in tender leaves of M_5 (7.51mg/g) recorded significantly high followed by *Morus nigra* (7.40mg/g) and lowest in *Matigara black* (6.10mg/g). In medium leaves also higher phenolic content was recorded in M_5 (8.98mg/g) followed by *Morus nigra* (8.54mg/g) and lower phenolic content was found in Tr_{20} (7.32mg/g). Course leaves of M_5 (8.00mg/g) registered comparatively higher phenolic content and lower was registered in *Matigara black* (6.79mg/g). It is also observed that, phenolic content is more in medium leaves followed by coarse and tender leaves (Table 1). Phenolic compounds are responsible for disease resistance in plants and function as hydrogen donors or acceptors in oxidation/reduction reactions. A small change in the host-phenol metabolism severely disrupts many processes, which are essential for normal growth and development of plants. Increase of phenolic compounds may result from either synthesis of new aromatic compounds and/or the acceleration of accumulative phenols from neighboring cells.[39] Several workers have reported that, disease resistant varieties possess higher phenol content compared to that of susceptible ones.[40] Presence of higher concentrations of phenolic compounds is considered to be one of the major factors for an incompatible host pathogen interaction.[41] Phenols possess a wide spectrum of biological activities and results show that mulberry extracts could be good sources of these natural constituents.[42]

Prolines

Prolines were more in medium leaves followed by coarse and tender leaves. Tender (2.36μg/g), medium (3.53μg/g) and coarse (2.95μg/g) leaves of *Matigara black* recorded higher prolines. Least values were recorded in tender (1.23 μg/g), medium (3.01 μg/g) and coarse (2.28 μg/g) leaves of M_5 (Table 1). Prolines forms important amino acid residues accumulated in higher concentration particularly in drought and disease tolerant plant species. Increased level of proline in mulberry plants under water stress has reported.[43] Accumulation of prolines was high in bean plant under water stress and accumulation level varied with duration of stress.[44] Similar observations were made in mulberry genotypes and reported that, mulberry variety Tr_4 (4.05μg/g) showed highest prolines followed by S_{34} (3.32μg/g), S_{13} (3.20μg/g), MR_2 (2.05μg/g), BC_{259} (1.63μg/g), M_5 (1.52 μg/g) and lowest in Kosen (1.10μg/g). High free prolines were recorded in BC_{259}, S_{30}, S_{36}, S_{41} and C_1 mulberry genotypes among the genotypes studied. Prolines were higher in mulberry variety Anantha than RFS_{175} and S_{30} clearly indicated that, relative

tolerance of Anantha to water stress as evidenced by increased levels of pralines.[45]

Chlorophyll Content

Mulberry variety S_{1708} recorded highest total chlorophyll content in tender (4.49mg/g), medium (6.38mg/g) and coarse (5.53mg/g) leaves followed by Tr_{12} (3.74mg/g, 6.04mg/g, 4.88mg/g respectively) and lowest total chlorophyll content was recorded in C_6(2.18mg/g, 3.27mg/g, 2.79mg/g respectively). With respect to chlorophyll-a, higher values were recorded in tender (2.89mg/g), medium (4.16mg/g) and coarse (3.68mg/g) leaves of S_{1708} followed by Tr_{12} (2.41mg/g, 3.95mg/g, 3.22mg/g respectively) and lower values were noticed in C_6(1.24mg/g, 2.03mg/g, 1.62mg/g respectively). Chlorophyll-b content recorded highest in tender (1.60mg/g), medium (2.22mg/g) and coarse (1.84mg/g) leaves of S_{1708} and lowest was recorded in C_6(0.76mg/g, 1.02mg/g, 0.98mg/g respectively) (Table 1). Chlorophyll content is very important for quantifying the photosynthetic efficiency of plant and is an essential constituent in assessing quality of foliage. Total chlorophyll content of fresh mulberry leaves ranged from 0.14% to 0.35% in weight and top and bottom leaves contains lesser amount of chlorophyll compared to middle order ones.[46] Chlorophyll contents were high in triploids than in tetraploids and diploids.[47] It was observed that, total chlorophyll content was much higher in MR_2 mulberry variety followed by Japan, Roso, C_1 and Kosen varieties. It was reported that, among different F1 hybrids of mulberry significant differences were noticed for total chlorophyll, chlorophyll-a and chlorophyll-b.[48] Total chlorophyll, chlorophyll-a and chlorophyll-b contents were maximum in Berhampore and S_1 mulberry varieties. Several scientists observed highest total chlorophyll, chlorophyll-a and chlorophyll-b in mulberry variety S_{1635} followed by S_1 among the varieties studied.[49] Among the selected mulberry varieties total chlorophyll (4.32mg/g) and chlorophyll-a (2.88mg/g) were highest in Tr_4 and least in OPH_3(2.46mg/g, 1.71mg/g respectively) and chlorophyll-b was highest in Tr_{10}(1.49mg/g) and least in S_{30} (0.70mg/g).

Table 1: Biochemical composition of different mulberry varieties

Mulberry varieties	Leaf maturity	Total Proteins (%)	Total Sugars (%)	Amino acids (μ mole/g)	Phenols (mg/g)	Prolines (μg/g)
Tr_8	T	25.81	13.81	64.90	7.02	2.33
	M	20.10	13.36	59.83	8.42	3.39
	C	13.50	10.73	27.80	7.37	2.84
Tr_{12}	T	23.98	15.05	60.99	7.26	2.06
	M	17.95	13.52	53.44	8.82	3.27
	C	11.67	11.11	25.60	7.80	2.77
Tr_{20}	T	23.77	13.67	53.65	6.32	2.02
	M	17.63	13.02	46.08	7.32	3.20
	C	11.43	09.61	23.87	6.87	2.68
S_{1708}	T	27.31	16.02	68.43	7.16	1.61
	M	20.92	14.10	62.97	8.59	3.20
	C	15.58	11.74	33.01	7.82	2.44
MS_5	T	22.12	12.08	49.03	6.75	1.88
	M	15.63	10.58	44.96	7.71	3.17
	C	11.04	08.76	27.71	7.21	2.38
C_6	T	19.18	10.85	45.94	6.89	1.96
	M	14.80	09.73	42.02	8.24	3.28
	C	10.14	08.02	21.88	7.29	2.54
C_{10}	T	22.00	12.00	48.00	6.58	1.28
	M	15.32	10.33	44.18	7.94	3.10
	C	10.84	08.55	26.17	6.94	2.48
Matigara black	T	20.82	11.10	47.93	6.10	2.36
	M	15.15	10.10	43.08	7.61	3.53
	C	10.41	08.11	22.52	6.79	2.95
Morus nigra	T	21.03	11.14	46.33	7.40	2.08
	M	15.19	10.77	43.76	8.54	3.48
	C	10.59	08.36	23.04	7.84	2.91
M_5	T	15.91	13.09	55.44	7.51	1.23
	M	12.94	11.90	52.58	8.98	3.01
	C	09.84	09.73	33.11	8.00	2.28
CD @ 5%		3.31	1.70	9.54	1.01	0.83

Table 1: Biochemical composition of different mulberry varieties (Continued...)

Mulberry varieties	Leaf maturity	Total Chlorophyll (mg/g)	Chl – a (mg/g)	Chl – b (mg/g)	Moisture Content (%)	Moisture retention After 6 hours (%)
Tr_8	T	3.42	2.16	1.28	78.10	80.16
	M	5.52	3.60	1.92	76.41	78.15
	C	4.56	2.95	1.62	74.03	75.61
Tr_{12}	T	3.74	2.41	1.32	76.80	78.55
	M	6.04	3.95	2.09	75.73	76.13
	C	4.88	3.22	1.68	73.68	75.29
Tr_{20}	T	2.72	1.86	0.84	72.89	74.60
	M	4.21	3.02	1.18	71.14	72.32
	C	3.49	2.47	1.03	68.78	70.78
S_{1708}	T	4.49	2.89	1.60	80.64	81.56
	M	6.38	4.16	2.22	78.19	79.54
	C	5.53	3.68	1.84	75.74	77.19
MS_5	T	2.44	1.53	0.94	70.77	72.04
	M	3.51	2.29	1.23	69.36	68.14
	C	3.07	2.01	1.08	66.84	67.26
C_6	T	2.18	1.24	0.76	66.72	68.26
	M	3.27	2.03	1.02	64.77	66.84
	C	2.79	1.62	0.98	63.16	64.78
C_{10}	T	2.34	1.43	0.81	68.70	70.14
	M	3.41	2.10	1.16	66.71	68.37
	C	2.91	1.82	1.00	64.93	66.90
Matigara black	T	2.70	1.74	1.08	69.05	71.08
	M	4.28	2.93	1.29	67.24	69.12
	C	3.44	2.30	1.14	65.16	67.00
Morus nigra	T	2.64	1.60	1.05	67.19	69.14
	M	4.15	2.83	1.23	65.04	67.32
	C	3.32	2.20	1.10	63.17	66.08
M_5	T	2.86	1.82	1.08	70.59	72.57
	M	4.36	2.98	1.38	69.32	70.93
	C	3.58	2.42	1.19	67.57	69.11
CD @ 5%		0.73	0.60	0.85	5.07	5.14

T: Tender, **M:** Medium, **C:** Coarse

Moisture Content and Moisture Retention Capacity

Mulberry varieties revealed variations in moisture content and moisture retention capacity. Moisture content was significantly higher in tender (80.64%), medium (78.19%) and coarse (75.74%) leaves of S_{1708} and lower was recorded in leaves of C_6(66.72%, 64.77%, 63.16% respectively). Moisture retention capacity was highest in tender, medium and coarse leaves of mulberry variety S_{1708}(81.56%,79.54%,77.19% respectively) and lowest was recorded in C_6 (68.26%, 66.84%, 64.78% respectively) (Table 1). In mulberry leaves, moisture content plays a vital role in improving nutrition levels which in turn improve the palatability and digestibility of leaves by silkworms as well as normal growth and development of silkworms and cocoons quality.[50] It is a genetic character and influenced by available soil moisture and root proliferation nature of mulberry variety.[51] Availability of moisture content in leaves enhances feeding efficiency of silkworm larvae which in turn increases growth rate.[52] Importance of dietary moisture content in relation to silkworm growth was emphasized that, decrease in leaf moisture content influenced different energetic parameters such as assimilation and conversion efficiency of food which decreases with decreasing dietary moisture content of leaf. It is a well-established fact that, moisture content of mulberry leaves decreased gradually with corresponding increase in leaf growth and varieties.[53] Moisture percentage was maximum in S_{54} and $Kanva_2$ mulberry varieties and minimum in Mysore local variety. Triploid mulberry varieties leaves were significantly higher in moisture content and similar results were recorded in the present experiment. Mulberry varieties S_{36} and DD recorded significantly higher leaf moisture content than M_5 variety under closer plant spacing of 60cmx60cm.[54] Moisture retention capacity plays an important role

because leaves with high moisture remain fresh and acceptable to silkworms for longer time and it is related to moisture content in leaves. Further, stomatal size and frequency play a major role in moisture retention in mulberry leaves. Moisture content and moisture retention capacity was superior in S_{36} mulberry variety followed by S_{41}, K_2 and Mysore local. Mulberry variety S_{1708} showed maximum leaf moisture content grown under 60cmx60cm spacing among all other varieties examined. Mulberry genotype S_{1635} possesses higher leaf moisture content and leaf moisture retention compared to other genotypes studied.[55] Maximum moisture content and moisture retention ability was found in Tr_{10} (76.94% and 71.41%) and minimum in $Sujanpur_5$ (64.04% and 57.39%).

CONCLUSION

Experiment revealed that, mulberry variety S_{1708} established its nutritional superiority with respect of total proteins, total sugars, amino acids, prolines, chlorophyll contents including leaf moisture and moisture retention capacity. Next to S_{1708}, triploid mulberry varieties viz., Tr_8, Tr_{12} and Tr_{20} recorded comparatively higher values in respect of phytochemical constituents. From the results, it is clear that mulberry variety S_{1708} turns out to be a superior in leaf biochemical chemical tests compared to other varieties studied under the same agro climatic conditions and it may be recommended for commercial silkworm rearing purpose at field level for better cocoon yield as well as for the sustainable growth and development of sericulture industry.

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