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Production potential and leaf quality evaluation of selected mulberry (*Morus alba*) clones

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Abstract
The study was conducted under Uttar Pradesh Council of Agriculture Research funded project at C. S. Azad University of Agriculture and Technology, Kanpur to evaluate the leaf quality of 10 selected mulberry clones viz., BC-259, K-2, RFS-175, S-1,S-146,S-776, S-1635, S-1531,Tr8 and UP-1 through phytochemical analysis. It was apparent from the results of analysis that total proteins, total sugars and amino acids were high in tender leaves followed by medium and coarse leaves. Total proteins, total sugars and amino acids were high in S1635 leaves followed by K-2 and S-146 and total proteins and total sugars were low in UP-1 leaves. Moisture contents were high in tender followed by medium and coarse leaves. Moisture content and moisture retention capacity were significantly high in S1635 and lowest in CM leaves

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

Introduction
In sericulture industry, approximately 65% of total cost of cocoon production goes towards mulberry production alone. Hence, in last two decades 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 most important 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 (Bongale *et al*, 1997) [2]. Quantity and 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 (Krishswami *et al*., 1971) [11]. Keeping in view, the importance of nutritional value of mulberry leaves, present study leads to evaluate better performing mulberry varieties through quantitative estimation of phytochemical parameters and identify the well suited mulberry variety to Central Plain zone agro climatic zone conditions as it is the suitable region for raw silk production in Uttar Pradesh.

Materials and Methods
The experiment was carried out in the Central Plain zone agro climatic zone conditions as it is the suitable region for raw silk production in Uttar Pradesh during 2012-2013. Ten mulberry varieties viz., BC-259, K-2, RFS-175, S-1,S-146,S-776, S-1635, S-1531,Tr8 and UP-1 selected from germplasm bank maintained at C. S. Azad University of Agriculture and Technology, Kanpur were used in the investigation. Mulberry variety CM 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 three replications.

Observation data collected of various parameters were subjected to statistical analysis by adopting 'Method of Analysis of Variance' appropriate to the experiment design (Sunderal *et al.*, 1972) [22].

Collection of Mulberry Leaf Samples

Fresh and 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 (Lowry *et al*., 1951) [12]. 50 mg 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 30 minutes 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-phenolreagent was rapidly added and mixed. After 30 minutes absorbence was measured at 750nm. Protein content was calculated by preparing standard curve with bovine serum albumin.

Total Sugars

Anthrone reagent was used to estimate the total sugars (Plummer *et al*., 1971) [15]. 25 mg of dry leaf powder was crushed thoroughly in 10ml of hot ethanol by mortar and pestle. The leaf tissue was exhaustively extracted twice or thrice using small quantity of ethanol cooled and filtered by 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 (Moore *et al*., 1948). 50mg of dry leaf powder was homogenised in 5ml of 80% methanol using mortar and pestle. Homogenate was centrifuged at 5000 rpm for 10 minutes. 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 20 minutes. 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. Amino acids were calculated using a standard curve prepared with glycine.

Chlorophyll Content

Total chlorophyll contents from fresh mulberry leaves without maceration were determined using Dimethyl sulphoxide (Hiscox *et al*., 1979) [8]. 50 mg 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. Total chlorophyll contents were estimated (Arnon, 1951). 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.

$$\text{Total chlorophyll} = \frac{20.2(D645)+8.02\,(D663)\ \text{X}\ 1000\ \text{X weight of leaf (g))}}{V} = \text{mg/g}$$

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

Leaf Moisture Content

Leaf moisture content and moisture retention capacity were determined on fresh weight basis(Vijayan *et al*., 1997). 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 (%).

$$\text{Leaf moisture content (\%)} = \frac{\text{Fresh weight of leaves-Dry weight of leaves}}{\text{fersh eight of leaves}}\ \text{X}\ 100$$

Moisture Retention Capacity

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

$$\text{Leaf moisture loss (\%)} = \frac{\text{Fresh weight of leaves-weight of leaves at 6hours after harvest}}{\text{fersh eight of leaves}} \text{ X LMC}$$

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

Results and Discussion

Bio-chemical composition studies of leaves

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 (Purohit *et al*., 1996) [16]. 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 (Das *et al*., 2001) [5]. After phytochemical analysis of 10 mulberry clones, results presented in Table-8 indicated that cultivar S-1635 produced highest total protein, total sugar and total chlorophyll in leaves followed by K-2. Clone S-1 has registered third place in the bio-chemical composition of leaves. Almost similar trend were noticed in moisture content and moisture retention after 6 hours.

Total Proteins

Total proteins in tender leaves of S1635 (27.31%) and K-2(25.81%) recorded higher values and significantly lower in Sultanpur (15.91%) tender leaves. In medium leaves S-1635 (20.9%) followed by K-2 (20.1%) and S-146 (17.6%) recorded highest protein content, did not differ significantly. While minimum value of protein content was observed in UP-1 (12.90%). In coarse leaves, protein content was found highest in S-1635(15.6%) followed by K-2 (13.5%), and least was recorded in UP-1 (9.8%) and (Table 2). Leaf protein is a major determinant of nutrient quality for many Lepidopteron larvae. It is known fact that, nearly 69% of protein content of raw silk namely fibroin and sericin are directly biosynthesized from mulberry leaf protein and remaining 31% is derived from silkworm body tissue and haemolymph protein, emphasizing the importance of leaf protein in silkworm nutrition (Bose *et al*., 1991) [4]. Protein content present in different mulberry varieties had a direct bearing on larval growth particularly in silk gland development and cocoon characters of silkworm. Similar studies were conducted and observed by Murthy *et al*, (2013). Selected mulberry germplasm varieties also established maximum protein content in tender leaves which gradually depleted in medium and coarse leaves. (Rao *et al*., 2000) [17].

Total Sugars

Total sugar content was found to vary both in respect of leaf maturity and varieties studied. Tender leaves of S-1635 (16.2%) recorded highest total sugars followed by S-146 (15.1%) and lowest in BC-259 (10.6%). In medium leaves also similar trend was noticed. Course leaves of S-1635 (11.7%), S-146 (11.1%) and K-2 (10.7%) recorded significantly higher level of total sugars and lowest was recorded in RFS-175 (8.1%) (*c.f.* Table 2). 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 (Tiku, 1998) [24].

Chlorophyll Content

Mulberry clone S-1635 recorded highest total chlorophyll content in tender leaves (4.5 mg/g), medium (6.3 mg/g) and coarse (5.4 mg/g) followed by K-2 (3.6 mg/g, 6.0 mg/g, 4.7 mg/g, respectively) and lowest total chlorophyll content was recorded in BC-259 (2.2 mg/g, 3.3 mg/g, 2.7 mg/g, respectively) similar to (Hotta 1975). (c.f. Table 8). 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.

Moisture Content and Moisture Retention Capacity

Mulberry varieties revealed variations in moisture content and moisture retention capacity. Moisture content was significantly higher in tender leaves in S-1635 (80.6%), medium (78.2%) and coarse (75.6%) and lower was recorded in leaves of BC-259 (66.5%, 64.7%, 63.2%, respectively). Moisture retention capacity was highest in tender, medium and coarse leaves of mulberry clone S-1635 (81.6%, 79.5%, 77.2%, respectively) and lowest was recorded in BC-259 (68.1%, 66.8%, 64.8%, respectively) (Table 8). 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 (Koul *et al*., 1996) [10]. It is a genetic character and influenced by available soil moisture and root proliferation nature of mulberry variety (Sahu *et al*., 1997) [19]. Availability of moisture content in leaves enhances feeding efficiency of silkworm larvae which in turn increases growth rate. 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 (Pathak *et al*., 1988) [14].

Table 1: Leaf area, leaf yield, leaf-shoot ratio, moisture content and moisture retention capacity of the selected Mulberry clones

Clones	Leaf Area (cm^2)			Leaf Yield Kg $plant^{-1}$			Leaf shoot ratio		
	1st year	2nd year	Average	1st year	2nd year	Average	1st year	2nd year	Average
BC -259	257.33	260.32	258.83	1.06	1.12	1.09	1.62	1.87	1.75
CM	232.67	232.95	232.81	0.80	0.85	0.83	1.25	1.30	1.28
RFS-175	194.67	196.80	195.74	2.38	2.40	2.39	1.08	1.80	1.44
K-2	333.00	335.12	334.06	3.54	3.62	3.58	1.96	1.95	1.96
S-1	227.00	233.80	230.40	2.40	2.42	2.41	1.72	1.89	1.81

S-13	196.00	196.42	196.21	1.82	2.08	1.95	1.40	1.42	1.41
S-146	256.67	280.45	268.56	2.65	2.56	2.61	1.82	1.97	1.90
S-1531	229.67	231.42	230.55	1.49	1.58	1.54	1.15	1.50	1.33
S-1096	201.00	201.72	201.36	1.55	1.86	1.71	1.14	1.33	1.24
S-1635	413.67	414.04	413.86	3.89	3.97	3.93	1.96	2.02	1.99
S-34	226.33	228.02	227.18	1.71	1.79	1.75	1.40	1.42	1.41
S-36	213.67	211.52	212.60	1.77	1.72	1.75	1.54	1.62	1.58
S-41	201.67	200.12	200.90	1.14	1.32	1.23	1.28	1.45	1.37
S-54	201.67	211.82	206.75	0.81	0.89	0.85	1.72	1.65	1.69
S-776	176.33	176.35	176.34	2.58	2.35	2.47	1.32	1.70	1.51
S-799	219.33	221.12	220.23	2.25	2.30	2.28	1.13	1.45	1.29
TR-8	111.67	112.22	111.95	1.32	1.56	1.44	1.36	1.42	1.39
TR-11	204.67	204.78	204.73	1.44	1.56	1.50	1.29	1.30	1.30
Sultanpur	154.67	154.84	154.76	1.78	2.01	1.90	0.81	0.92	0.87
UP-1	180.33	181.22	180.78	1.84	1.87	1.86	0.91	0.82	0.87
SE diff.	19.43	19.83	19.63	0.27	0.32	0.30	0.25	0.23	0.24
CD (0.05)	39.33	40.14	39.74	0.54	0.64	0.59	0.51	0.47	0.49

DAP-Days after prunning

Table 2: Biochemical composition of leaves of selected mulberry varieties (March 2014)

Mulberry varieties	Leaf maturity	Total Proteins (%)	Total Sugars (%)	Total Chlorophyll (mg/g)	Moisture Content (%)	Moisture retention After 6 hours (%)
BC -259	T	19.2	10.6	2.2	66.5	68.1
	M	14.8	9.7	3.3	64.7	66.8
	C	10.2	8.2	2.7	63.2	64.8
K-2	T	25.8	13.8	3.4	78.1	80.2
	M	20.1	13.4	5.5	76.4	78.2
	C	13.5	10.7	4.6	74	75.6
RFS-175	T	20.8	11.1	2.7	69.1	71.1
	M	15.2	10.1	4.3	67.2	69.2
	C	10.4	8.1	3.4	65.2	67
S-1	T	23.8	13.7	2.7	72.9	74.6
	M	17.6	13.2	4.2	71.2	72.3
	C	11.4	9.6	3.5	68.8	70.8
S-146	T	23.9	15.1	3.6	76.8	78.6
	M	17.6	13.5	6	75.6	76.1
	C	11.7	11.1	4.7	73.6	75.3
S-776	T	20.8	11.1	2.7	69.1	71.1
	M	15.2	10.1	4.3	67.3	69.1
	C	10.4	8.1	3.4	65.2	67
S-1635	T	27.3	16.2	4.5	80.6	81.6
	M	20.9	14.1	6.3	78.2	79.5
	C	15.6	11.7	5.4	75.7	77.2
S-1531	T	22.2	12.8	2.4	70.8	72.1
	M	15.6	10.6	3.5	69.4	68.2
	C	11.2	8.8	3.1	66.8	67.3
TR-8	T	21.3	13.1	2.8	70.6	72.6
	M	15.2	11.9	4.4	69.3	70.9
	C	10.6	9.7	3.6	67.6	69.1
UP-1	T	15.9	11.2	2.6	67.2	69.2
	M	12.9	10.8	4.2	65.1	67.3
	C	9.8	8.4	3.3	63.2	66.1
CD @ 5%		3.3	1.7	0.6	5.1	5.2

T-tender, M-Medium, C-coarse

Conclusion

Experiment revealed that, mulberry variety S-1635 established its nutritional superiority with respect of total proteins, total sugars, chlorophyll contents including leaf moisture and moisture retention capacity. Next to S-1635, triploid mulberry clone viz., K-2, S-146, and recorded comparatively higher values in respect of phytochemical constituents. From the results, it is clear that mulberry variety S-1635 turns out to be a superior in leaf biochemical chemical tests compared to other clones studied under the same agro climatic conditions and it may be recommended for commercial silkworm rearing purpose at field level for better cocoon yield and for the sustainable growth and development of sericulture industry

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