Phenotypic Characterization and Identification of Elite Mulberry (*Morus* Spp.) Cultivars for Desirable Leaf Quality

V. C. Jayaramaiah[1], C. B. Siddu[2], R. Kirankumar[3] and S. Ramesh[4]
[1]Department of Sericulture, Bangalore University, Bengaluru
[2]ICAR-Indian Institute of Horticultural Research, Hessaraghatta Lake Post, Bengaluru
[3&4]Department of Genetics and Plant Breeding, College of Agriculture, UAS, GKVK, Bengaluru - 560 065
e-Mail : siddubc16@gmail.com

Authors Contribution

V. C. Jayaramaiah : Conceptualization, design, material preparation, conduct of field experiments and data collection

C. B. Siddu : Curation, analysis of data and tabulation of results

R. Kirankumar : Manuscript preparation and draft writing

S. Ramesh : Supervision and guidance

Corresponding Author :
V. C. Jayaramaiah

Received : January 2025
Accepted : Feburary 2025

Abstract

Mulberry is the sole food source for the silkworm (*Bombyx mori*) and plays a pivotal role in sericulture, which supports the livelihoods of millions of resource-poor farmers in India. Farmers prefer mulberry cultivars with high leaf yield coupled with high carbohydrate, protein contents and high moisture retention capacity. Under this premise, the objective of the study aimed at assessing leaf yield and quality traits in mulberry cultivars (which include hybrids, mutants and selections) developed and released over the past 20 years and to identify those with high leaf yield coupled with high quality traits. These cultivars were filed-evaluated in a three-replicated randomized complete block design. The data were collected over five leaf harvests spanning three years for eight quantitative traits and leaf biochemical constituents. Analysis of variance revealed significant differences among the cultivars and environments, indicating the role of genetic and environmental factors for these trait's expression. Higher magnitude of genetic variability observed for traits such as leaf yield, moisture content and leaf weight indicated further scope for the improvement of these traits. Variety-2 had higher nitrogen, chlorophyll and protein contents, while variety-7 was rich in phosphorus and potassium. Additionally, variety-3 excelled in total soluble sugars. Leaf quality traits such as moisture content and protein significantly influenced silkworm growth, nutrient assimilation and cocoon productivity. Cultivars with higher protein and carbohydrate content are more suitable for silkworm performance and silk yield. Cultivars 2 and 7 are recommended for breeding programs targeting improved leaf quality and yield.

Keywords : Sericulture, Genetic variability, Moisture retention capacity, Carbohydrate, Protein, Improvement

Sericulture is an important agro based industry that plays a major role in employment generation. Sericulture involves raising of the silk worms by feeding on their specific host plants. Among the four different kinds of silks produced in India, mulberry silk occupies a lion share with a production of 27,654 MT from an area of 2.53 lakh hectares (Sushmitha *et al.,* 2024, 2022-23). About 92 per cent of the total Indian silk production comes from mulberry (*Morus* sps.). Mulberry can be grown in a wide range of agro-climatic conditions both in rainfed and irrigated areas. Its foliage is the sole diet for monophagous silkworm *Bombyx mori* (Sushmitha *et al.,* 2024). Four mulberry species (*M. indica*, *M. alba*, *M. laevigata*, *M. serrata*) have been reported from India. Mulberry a deep-rooted persistent crop continues to grow and generate leaves throughout the year in tropics. India is the second largest producer of mulberry silk with an estimated annual production of about 16,000 metric tons. Indeed, mulberry is an important source of livelihood for several millions of resource-poor farmers in India (Checker *et al.,* 2012).

Maximization of mulberry leaf yield per unit area would lead to the realization of two most important objectives namely reduced cost of production and increased cocoon production per hectare (Krishnaswami, 1986).

Farmers prefer mulberry varieties with high leaf yield coupled with high carbohydrate and protein contents and high moisture retention capacity (Meena and Nataraja, 2022). Silkworm is a highly sensitive insect which respond sharply to changes in the mulberry quality evident traits such as carbohydrate, protein and moisture retention capacity. A rather high marketable cocoon harvest depends on several factors such as silk worm breed, mulberry variety and rearing environment. Good quality cocoons can be harvested when silkworms are fed with nutritionally superior leaves which results in improved silk production (Shilpashree *et al*., 2015 and Kaushal Kumar *et al.,* 2018). Therefore, programmes aimed at breeding mulberry focus on high leaf yield coupled with high water, protein and carbohydrate contents. In this backdrop, the present study aimed at unravelling the variability among the released mulberry varieties for selection of those for further improvement of mulberry for its leaf yield and quality traits.

Material and Methods

The experimental materials used in the present study consisted of nine mulberry (*Morus indica*) varieties namely Var-1 (CPH-1), Var-2 (CPH-2), Var-3 (OPH-3), Var-4 (OPH-4), Var-5 (OPH-5), Var-6 (a mutant reverted to wild type Mysore local), Var-7 (Mysore local), Var-8 [Kanva-2 (M5)] and Var-9 [Victory-1 (V_1)]. The planting material of the varieties *viz*, Var-7, Var-8 and Var-9 were procured from Central Sericultural Research and Training Institute (CSR & TI), Mysore where the varieties were bred. The rest of the six varieties were bred at the Department of Sericulture, Bangalore University, Jnanabharathi campus, Bangalore. These varieties have been bred over last 40 years. The salient features of these nine varieties are described in Table 1 and photographs of the varieties are depicted in Fig. 1 and Fig. 2.

Table 1

Mulberry varieties used in the present study

Variety	Pedigree/origin	Ploidy	Salient features
Var-1 Controlled pollinated Hybrid (CPH-1)	Colchicine treated Mysore local B& × colchicine treated Kanva -2 @& at Jnanabharathi Bangalore University	Aneuploid	Bear spread shoots with closely spaced large leaves and faster growth
Var-2 Controlled Pollinated Hybrid (CPH-2)	Gamma Ray Irradiated Mysore local B& × Colchicine treated Kanva-2@& at Jnanabharathi Bangalore University	Aneuploid	Bear erect shoots with closely arranged thick dark green unlobed leaves fast growing and early maturity
Var-3 Open Pollinated Hybrid (OPH-3)	Colchicine Treated Kanva-2 selected erect shoots with longest inter-nodal at Jnanabharathi Bangalore University	Aneuploid	Fast growing with big trunk and innumerable distance and bears large unlobed leaves borne on long petioles
Var-4 Open Pollinated Hybrid (OPH-4)	Colchicine Treated Kanva-2 Gamma Ray Irradiated, selected at Jnanabharathi Bangalore University	Aneuploid	Vertically growing sturdy shoots with shortest intermodal distance and bears dark green unlobed leaves
Var-5 Open Pollinated Hybrid (OPH-5)	Colchicine Treated Kanva-2 Ethyl Methane sulfonate treated selected at Jnanabharathi, Bangalore University	Aneuploid	Bear erect shoots with dark green and leathery unlobed leaves

Continued....

TABLE 1 Continued....

Variety	Pedigree/origin	Ploidy	Salient features
Var-6 (Local)	7.5KR Gamma-ray irradiated clonal selection at Bangalore University, Jnanabharathi campus	Aneuploid	Display vigorous growth innumerable sturdy spreading shoots with reduced intermodal distance. Bears heart shaped unlobed closely arranged thick dark green shining leaves
Var-7 (Mysore Local)	Clonal selection from a local collection at CSRTI, Mysore	Diploid	Drought tolerant and recommended for rainfed ecosystem
Var-8 (Kanva-2)	Open pollination Hybrid selection made at Kanva Government silk Farm, Channapatna, Karnataka	Diploid	Bear erect spread sturdy shoots with reduced intermodal distance, shiny unlobed leaves with prolonged moisture retention capacity. Recommended for irrigated production ecosystem
Var-9 (Victory-1)	S-30 × Berc.776. at CSRTI, Mysore	Diploid	Fast growing sturdy shoots bearing boat shaped elongated dark green leaves

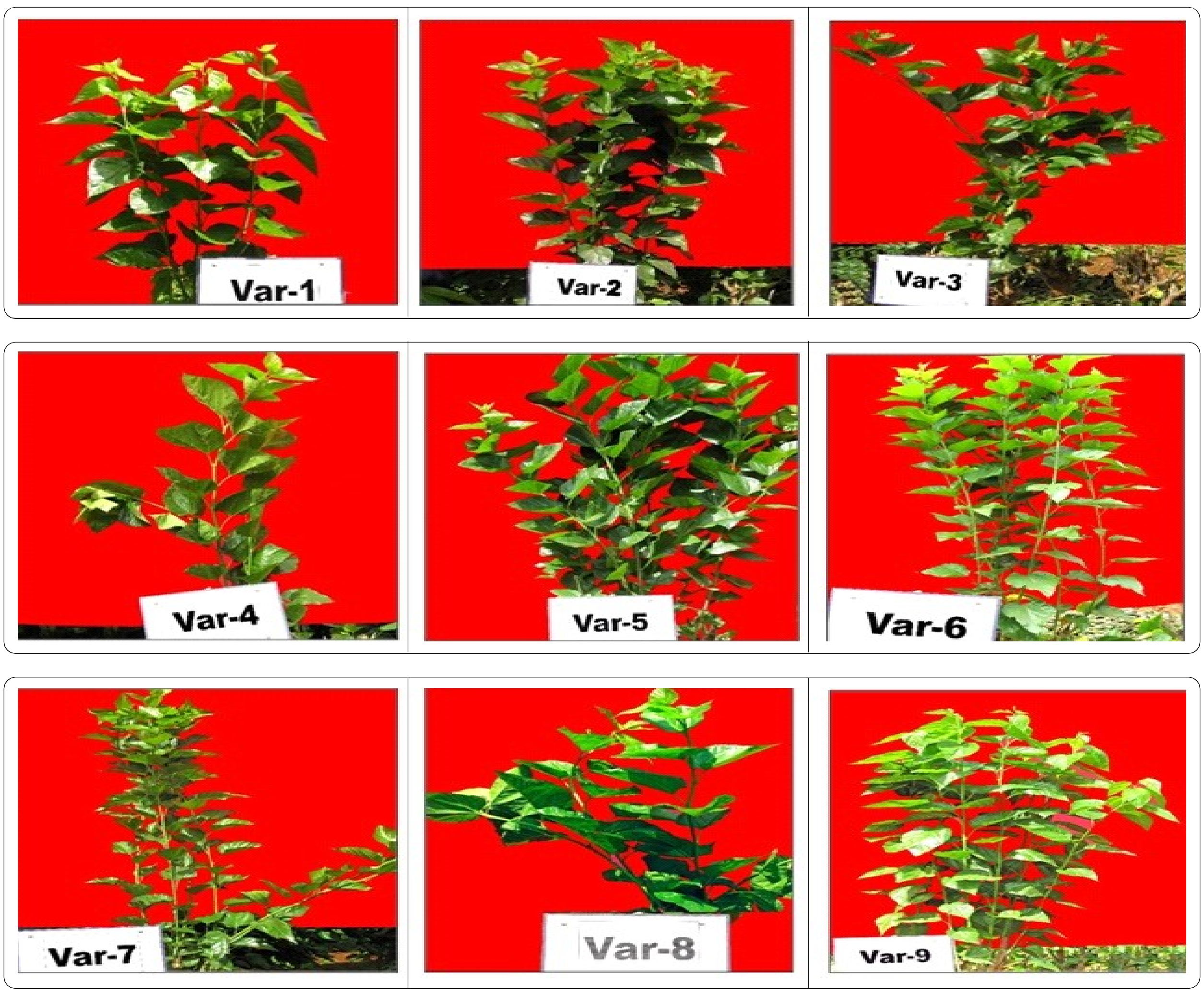

Fig. 1 : Photographs of different varieties in field condition

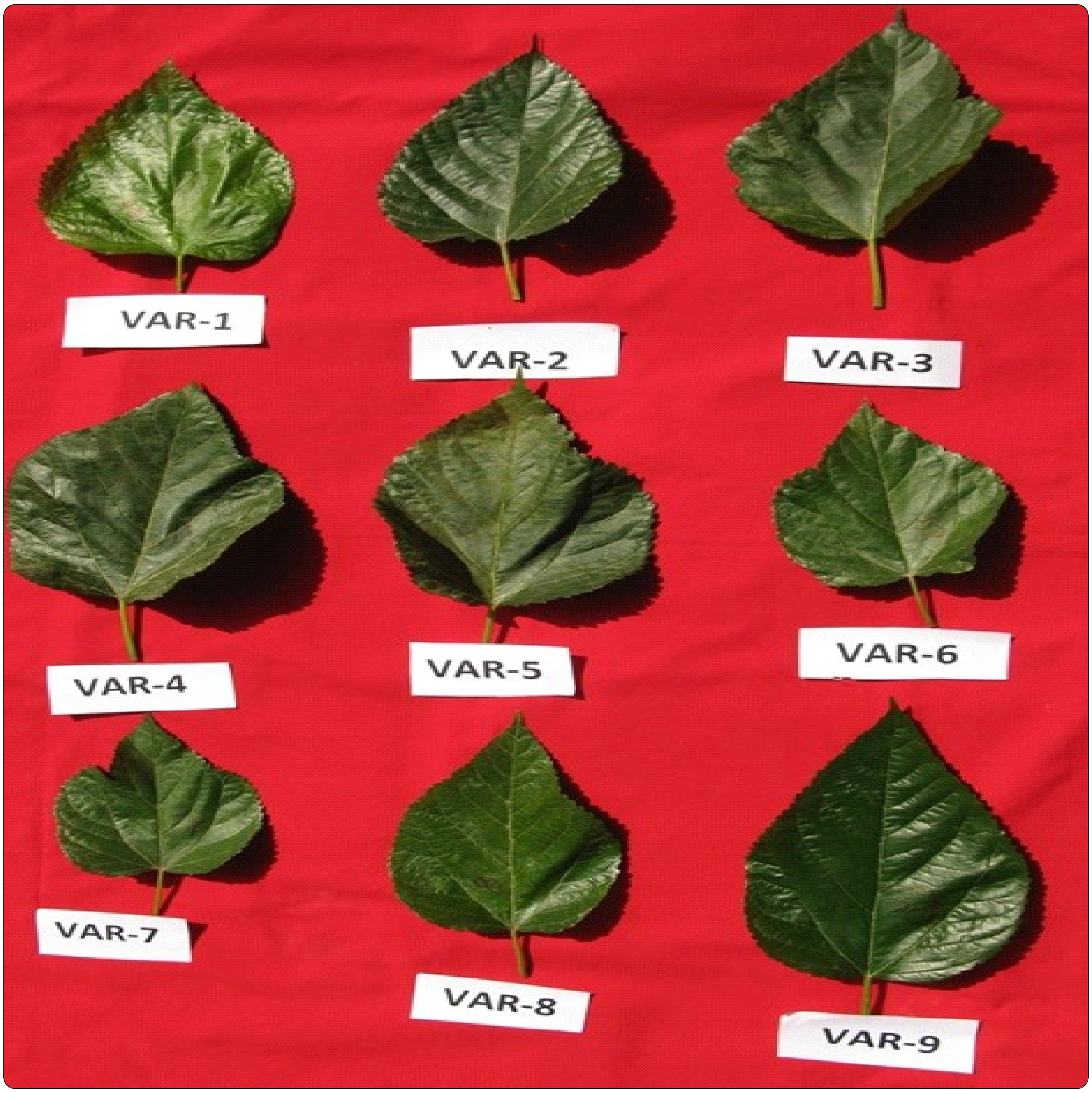

Fig. 2 : Photographs of leaves of different mulberry varieties

The varieties were evaluated in a randomized complete block design (RCBD) with four replications. The cuttings of each of the nine varieties were planted with a spacing of 2' × 2' consisting nine plants per replication during 2012 rainy season (September). Thus, the total experimental area measuring 1298 sq ft. consisted of 324 plants (9 varieties × 9 plants × 4 replications). The plants in experimental area were bottom pruned (plants were cut above 0.20m from the soil surface) after each leaf harvest by leaf picking method during 2013 rainy season. Plants were pruned using the same procedure for the second time (90 days after first pruning) during 2014 after leaf harvest. After second harvest and pruning, three more leaf harvests were made followed by bottom pruning 90 days after previous harvest. Thus, during three-year schedule, a total of five leaf harvests were made at 90 days intervals. Farmyard manure at 20 tonnes/ha/year was applied at the time first planting and after every harvest and pruning. In addition to this, inorganic fertilizers supplying N, P and K at 280:120:120 kg/ha, respectively was applied in five split doses, corresponding to the number of harvests. Irrigation of about 1.5-acre inches was given once in 8 - 10 days during non-rainy periods. The frequency of irrigation varied during rainy seasons depending on the requirement. The healthy crop was raised following other recommended packages for production of mulberry leaves. The annual rainfall ranged from 1068.4 mm (2012) to 1117mm (2017) during the experimental period. The experimental plot consisted of red sandy loam soil.

Sampling and Data Collection

The leaves were harvested manually from the nine varieties during 2013 July, 2014 January, 2014 September, 2015 July and 2016 April. Data were collected on eight quantitative traits from nine randomly selected plants/replicate/variety, after 90^{th} day after each pruning (Tikader and Kamble, 2009). The procedure for recording data is described in Table 2.

Leaf Biochemical Analysis

Samples of tender, medium and coarse leaves were collected to estimate biochemical contents. The powdered leaf samples were stored in newly opened plastic covers and used for estimation of chlorophyll a (Arnon, 1949) chlorophyll b (Arnon, 1949), total chlorophyll (Arnon, 1949), total soluble sugars (Yemm and Willis, 1954), soluble proteins (Lowry *et al*., 1952) total nitrogen (Lowry *et al*., 1952), phosphorus (Bray and Kurtz, 1945) and potash (Hanway and Heidel, 1952).

RESULTS AND DISCUSSION

Analysis of Variance

ANOVA is a diagnostic step to ascertain the presence of different sources of variation of mulberry varieties

TABLE 2

Procedure of recording the data on eight quantitative traits

Trait	Procedure for recording the data on the traits
Leaf yield per plant (g)	The leaves harvested from each variety and in each replication were weighed and expressed as leaf yield per plant (g)
Moisture content in 100 composite lamina (%) weight	Fresh (composite) 100 lamina were collected between 9-11 a.m. and weighed in grams. They were dried in hot air oven at 80^0cand dry weight was recorded. Moisture% was calculated by using the following formula - fresh lamina weight - dry fresh lamina weight/fresh lamina multiplied by 100
Moisture content in six 24 hoursand dry formula - lamina	Fresh (composite) 100 lamina were collected between 9-11 a.m. and weighed in grams after 100 composite lamina hours at room temperature. They were dried in hot air oven at 80^0cfor after six hours of weight was recorded. Moisture% was calculated by using the following harvest (%) weight (after 6 hrs) - dry lamina weight/ lamina weight (after 6 hrs) multiplied by 100.
Leaf moisture retention (LWR) capacity (MRC) hrs–	It is the capacity of leaves to retain moisture after 6 hrs. from harvest. The moisture retention capacity was calculated using the following formula.MRC % = (100 lamina weight after 6 (%) 100 oven dry lamina weight)/ (100 fresh lamina weight – 100 oven dry lamina weight) × 100.
Fresh weight of 100 composite leaves (g)	100 fresh leaves were harvested per plant from 9 plants per replication and weighed in grams and mean vales were calculated and expressed as 100 leaf weight
Fresh weight of 100 composite lamina (g)	After recording the fresh weight 100 leaves petioles were separated from leaves and lamina were weighed in grams and expressed as average 100 lamina weight
Laminar index (LI)	LI was calculated using the formula (weight of lamina/weight of leaf × 100)
100 composite lamina to petiole ratio by weight (g)	100 composite lamina and petioles from which the leaves were harvested were weighed and expressed as 100 composite lamina to petiole ratio by weight (g)

for different quantitative traits. It partitions the total variation into different components attributable to genetic and non-genetic sources. Pooled ANOVA across all the five environments indicated significant differences among varieties and environments for all the eight traits. Varieties also interacted significantly with environments as evidenced by the significant mean squares attributable to varieties × environment interaction (Table 3).

The differences among varieties could be attributed to difference in the plant architecture driven by (i) differences in the chromosomal complement and (ii) history of genesis of the varieties. While varieties 1 to 6 are aneuploid, varieties 7, 8 & 9 are diploid, variety 1, 3, 4 & 5 are polyploids. While the varieties 1, 2 & 9 are hybrids developed by controlled pollination, varieties 3 & 8 are selections from open pollinated hybrids. While varieties 4 & 6 are gamma ray induced mutants, variety 5 is an *Ethyl methane sulfonate* (EMS) induced mutant. Variety 7 is a selection from landrace from Mysore local, a traditional belt of sericulture. The varieties interacted significantly with temporal environments indicating their differential performance across environments. Such differential performances are attributed to variability in weather parameters such as day length, sunshine hours, rainfall and relative humidity which are known to affect photosynthesis, hence leaf yield and its contributing characteristics. Several previous

TABLE 3

Pooled ANOVA of nine mulberry varieties for eight quantitative traits

Source of variation	Degrees of freedom	Mean sum of squares			
		Leaf yield plant^{-1} (g)	Moisture content in 100 composite fresh lamina (%)	Moisture content in 100 composite lamina after 6 hours of harvest (%)	Leaf moisture retention (LWR) capacity (MRC) (%) of 100 lamina
		1	2	3	4
Environment	04	5038763.00 **	7582.00 **	1724.30 **	6653.10 **
Replication (Environment)	15	149392.00 ***	287.00	33.40	88.40
Varieties	08	966067.00 **	2403.00 **	142.40 **	417.10 **
Varieties × Environment	32	157976.00 **	2871.00 **	91.10 **	208.80 **
Error	120	54127.00	176.00	33.00	105.40

Source of variation	Degrees of freedom	Mean sum of squares			
		Fresh weight of 100 composite leaves (g)	Fresh weight of 100 composite lamina (g)	Laminar index	100 composite lamina - petiole ratio by weight (g)
		5	6	7	8
Environment	04	44039.00 **	29460.00 **	66.20	13.82
Replication (Environment)	15	994.00	1149.00	46.20	0.35
Varieties	08	173171.00 **	127067.00 **	96.00	17.78
Varieties × Environment	32	9800.00 **	6964.00 **	61.90	2.15
Error	120	1401.00	1199.00	62.30	0.63

*** Significant at P = 0.001; ** Significant at P = 0.01; * Significant at P = 0.05

TABLE 4

Estimates of components of variability among nine mulberry varieties for different quantitative traits

Parameter	Environment				
	2013 Rainy season	2013-14 Post rainy season	2014 Rainy season	2015 Rainy season	2016 Summer season
Leaf yield plant^{-1} (g)					
Variety variance	67328.32	12144.24	28315.97	79260.68	142889.37
Environment variance	21828.44	69290.42	28521.44	87658.28	59590.38
Phenotypic variance	89156.76	81434.66	56837.41	166918.96	202479.75
Moisture content in 100 composite fresh lamina (%)					
Variety variance	10.72	3236.85	1×10^{-6}	1.49	07.00
Environment variance	05.82	0745.62	67.38	8.18	50.57
Phenotypic variance	16.54	3982.47	69.38	9.67	57.57
Moisture content in 100 composite lamina after 6 hours of harvest (%)					
Variety variance	15.43	01.82	13.43	01.03	60.38
Environment variance	71.99	27.86	14.51	14.61	24.53
Phenotypic variance	87.42	29.68	27.94	15.64	84.91
Leaf moisture retention (LWR) capacity (MRC) (%) of 100 lamina					
Variety variance	28.52	23.87	66.01	19.59	53.39
Environment variance	60.64	40.84	43.80	33.68	337.93
Phenotypic variance	89.16	64.71	109.81	53.27	391.32
Fresh weight of 100 composite leaves (g)					
Variety variance	14644.70	14062.88	5954.69	7074.68	9863.68
Environment variance	01633.30	01551.27	1761.36	1217.37	0675.94
Phenotypic variance	16278.00	15614.15	7716.05	8292.05	10539.62
Fresh weight of 100 composite lamina (g)					
Variety variance	10331.90	10661.53	4417.88	5438.59	6428.15
Environment variance	01112.42	01292.82	1405.39	0879.97	1163.97
Phenotypic variance	11444.32	11954.35	5823.27	6318.56	7592.12
Laminar index					
Variety variance	086.55	2.61	02.77	0.22	07.36
Environment variance	277.17	6.78	21.17	12.31	32.37
Phenotypic variance	363.72	9.39	23.94	12.53	39.73
100 composite lamina - petiole ratio by weight (g)					
Variety variance	1.73	1.44	0.36	0.65	1.62
Environment variance	0.53	0.94	0.88	0.51	0.32
Phenotypic variance	2.27	2.37	1.24	1.15	1.94

Table 5a
Estimates of chlorophyll a, chlorophyll b and total chlorophyll in tender (T), medium (M) and coarse (C) oven-dried lamina of nine mulberry varieties

Varieties	Chlorophyll a				Chlorophyll b				Total chlorophyll			
	T	M	C	Mean	T	M	C	Mean	T	M	C	Mean
V1	0.72	1.75	0.15	0.87	2.34	3.21	4.35	3.30	2.21	3.03	4.20	3.15
V2	4.10	5.34	4.62	4.69	0.65	0.21	0.60	0.49	1.89	5.27	13.01	6.72
V3	0.15	0.14	0.04	0.11	3.03	2.98	1.53	2.51	2.87	2.96	1.48	2.44
V4	1.12	1.05	1.04	1.07	1.05	1.05	1.69	1.26	1.11	2.61	2.72	2.15
V5	0.77	0.88	1.00	0.89	1.14	1.87	1.57	1.53	1.87	2.01	2.62	2.17
V6	0.78	0.82	0.66	0.75	1.15	2.11	0.93	1.40	1.93	2.11	1.59	1.88
V7	0.76	0.77	0.43	0.65	1.03	0.98	0.52	0.84	1.81	1.73	0.96	1.50
V8	0.01	0.04	0.03	0.03	3.59	3.92	3.09	3.53	3.52	3.06	3.12	3.23
V9	9.92	0.03	0.03	3.33	3.32	3.33	2.74	3.13	3.29	4.02	2.71	3.34

researchers such as Pawan *et al*. (2018), Suresh *et al*. (2019), Ahalya *et al*. (2020) and Serajurand Shahinul (2020) reported significant variability among mulberry varieties for important agronomic traits.

Components of Variability

By and large, the magnitude of variability attributable to varieties was higher than that of environment for five of the eight traits such as, leaf yield per plant, moisture content in 100 composite fresh lamina, fresh weight of 100 composite leaves, fresh weight of 100 composite lamina and 100 composite lamina - petiole ratio. For rest of the three traits, namely, moisture content in 100 composite lamina after 6 hours of harvest, leaf moisture retention capacity and laminar index (Table 4) Thus, relative magnitude variability attributable to genetic (varieties) and non-genetic (temporal environments) sources varied with the trait. This variation may be due to the expression of different traits at different development stages. Therefore, it is quite likely that relative contribution of genes and the environment on the expression of the traits would vary.

Identification of Mulberry Varieties with High Nutritional Value

Among the leaf quality traits, by and large, estimates of the contents of chlorophyll a (Table 5a), total chlorophyll (Table 5a), soluble proteins (Table 5b) and nitrogen (Table 5c) in the tender, medium and coarse leaves and average of variety 2 were greater than those in other varieties. On the other hand, contents of total soluble sugars (Table 5b) were higher in tender, medium and coarse leaves and average of variety 3 than those in other varieties. Both phosphorous and potassium contents (Table 5c) were higher in tender, medium and coarse leaves and average were higher in variety 7 than those in other varieties. Thus, while nitrogen, one of the major nutrients was higher in variety 2, phosphorous and potassium, other two major nutrients were higher in variety 7. Further, barring total soluble sugars, variety 2 and variety 7 were superior for other leaf biochemical constituents. Among the leaf quality evident traits, leaf water content positively influences silkworm growth and development by increasing leaf intake and digestion and assimilation of nutrients because it has gustatory and olfactory stimulant effects (Purohit and Kumar, 1996 and Sori & Gebreselassie, 2016). Water intake of silkworm is directly related to the moisture content of mulberry leaf and the amount of leaf intake (Rahmathulla *et al.,*2006). The total soluble sugars in mulberry leaves influence the silkworm growth and also the cocoon production. About 70 per cent of the silk protein is derived directly from the mulberry leaf protein. The correlation

TABLE 5b

Estimates of soluble sugars and soluble protein in tender (T), medium (M) and coarse (C) oven-dried lamina of nine mulberry varieties

Varieties	Total soluble sugars				Soluble proteins			
	T	M	C	Mean	T	M	C	Mean
V1	0.55	1.99	1.00	1.18	1.23	0.19	0.79	0.74
V2	3.80	1.46	2.06	2.24	1.39	1.24	0.79	1.14
V3	6.63	4.92	5.94	5.83	0.86	0.23	0.72	0.60
V4	4.24	5.21	5.00	4.82	0.51	0.62	0.37	0.50
V5	0.92	0.57	1.74	1.08	1.13	1.16	0.59	0.96
V6	0.93	7.25	2.02	3.40	0.99	0.55	0.90	0.81
V7	4.78	6.08	4.98	5.28	0.77	0.61	0.46	0.61
V8	4.59	4.00	5.62	4.74	0.59	0.26	0.52	0.46
V9	3.95	1.61	4.98	3.51	1.09	0.73	0.63	0.82

TABLE 5c

Estimates of nitrogen, phosphorus and potassium contents in in tender (T), medium (M) and coarse (C) oven-dried lamina of nine mulberry varieties

Varieties	Nitrogen (%)				Phosphorus (%)				Potassium (%)			
	T	M	C	Mean	T	M	C	Mean	T	M	C	Mean
V1	3.17	3.24	2.81	3.07	0.42	0.64	0.49	0.53	0.99	1.42	1.11	1.17
V2	4.23	4.07	3.21	3.84	0.76	0.58	0.34	0.60	0.58	1.03	1.01	0.87
V3	4.11	3.4	2.93	3.48	0.52	0.57	0.33	0.61	0.70	0.96	0.90	0.85
V4	3.75	3.96	3.01	3.57	0.61	0.52	0.30	0.56	1.06	1.28	0.86	1.07
V5	4.27	3.68	2.93	3.63	0.41	0.55	0.33	0.52	0.60	1.00	0.93	0.84
V6	4.19	3.65	3.09	3.64	0.74	0.51	0.34	0.55	0.68	0.89	0.86	0.81
V7	4.07	3.48	3.44	3.66	0.88	0.52	0.34	0.66	0.98	0.99	0.91	0.96
V8	3.52	3.32	4.03	3.62	0.59	0.37	0.39	0.59	0.97	0.34	0.39	0.57
V9	4.39	3.92	2.73	3.68	0.89	0.65	0.44	0.63	1.03	1.06	1.11	1.07

between leaf protein content and cocoon production efficiency of silkworm depends on the amount of mulberry leaves consumed by the silkworm. Therefore, an increase in protein content of mulberry leaves is expected to increase cocoon productivity (Machii & Katagiri, 1991 and Machii *et al.,* 2000). In general, young silkworms require tender mulberry leaves with higher leaf water, proteins, carbohydrates, lower fiber and mineral contents. On the other hand, older silkworms require progressively mature leaves with lower leaf water content (Krishnaswami, 1990).

Acknowledgements : The senior author gratefully acknowledges Bangalore University for providing research fund under the 'University Funded Research Project on Genetic improvement of Mulberry through

In-vivo mutagenesis and hybridization' for conducting research pursuing Ph.D. degree program at University of Agricultural Sciences, Bangalore, India.

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