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Physiological and biochemical profiling of different mulberry genotypes developed for Eastern and North Eastern India

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ABSTRACT

Physiological and biochemical profiling of mulberry varieties in Eastern and North Eastern (E & NE)India is important for identification of markers associated with leaf quality and stress tolerance while undertaking future breeding programs. Thirteen mulberry genotypes (two local varieties, eight released varieties and three advance breeding lines) were assessed on physiological and biochemical basis under irrigated conditions of West Bengal. Lipid peroxidation occurs due to production of reactive oxygen species (ROS) during biotic and abiotic stresses. Lower thiobarbituric acid reacting substances (TBARS) revealed lower lipid peroxidation activity indicating higher level of stress tolerance of variety.The check variety S-1 recorded lowest levels of TBARS followed by C-2028, Tr-10 and Kajli. Secondary metabolites not only enhances the mulberry leaf quality for silkworm (Bombyx mori) consumption, but also act as antioxidants for remediating the biotic and abiotic stresses faced by the crop plant; thereby helping to curb the ROS production leading to better leaf yield. Higher secondary metabolites (TPC, TFC and TTC) content were recorded in C-2028 and C-2060 indicating higher tolerance towards various stresses. Leaf quality parameters such as TSS, TSP, CCI and NRA of the test varieties were at par with S-1;while C-2060 recorded 12% higher yield over C-2038, the ruling mulberry variety of the zone. Overall, Multiple Trait Evaluation Index (MEI) reveals that genotypes C-2060, C-2028 and C-1730 were physiologically and biochemically superior exhibiting better traits for tolerance to biotic and abiotic stresses and might be more useful for developing climate resilient mulberry genotypes.

Keywords: Mulberry, physiological traits, biochemical traits and multiple trait evaluation index

Sericulture is a very successful enterprise with the farming communities as it provides year-round employment and continuous income. Mulberry silk contribution is 71.45% (25345MT) and 28.55% are wild silks *viz*., Eri (6750MT), Tasar (2981MT) and Muga (233MT) in India (Seri states of India, 2019). Mulberry sericulture activities not only create opportunities of silk production, but also ensure forces to drive the ecological/ biodiversity, soil/water conservation, employment generation, rural poverty alleviation, social empowerment and environmental protection.

Mulberry (*Morus* spp.), the sole food plant of mulberry silk worm (*Bombyx mori* L.) is a hardy perennial plant adaptable to varied agro-climatic conditions. Mulberryis a heterozygous, open-pollinated plant exhibiting wide variability with different ploidy levels (2n = 28-308). Silkworm rearing success is directly associated with the continuous supply of quality mulberry leaves and mulberry variety alone contributes to about 50% to silk cocoon production. Increased production of silk depends to a great extent on increased mulberry leaf yield (Chaluvachari and Bongale, 1995; Caccam and Mendoz, 2015; Masthan *et al*., 2017 and Alipanah *et al*., 2020). Introduction of new silkworm hybrids, more number of crops in a year, demand for high quality silk production has intensified the demand for superior mulberry varieties. To develop improved varieties suitable for different agro-climatic zones, the evaluation process should be continuous and obligatory. The present scenario of mulberry sericulture demands new varieties for enhancement of profitability and also to produce superior quality silk. Genetic enhancement of mulberry is possible only with sufficient genetic variability and appropriate evaluation strategies. Evaluation of mulberry varieties for better quantitative and qualitative traits is essential to identify suitable parental stocks for a plant breeding program.

Development of superior varieties is a continuous challenge for the breeders to sustain the sericulture industry. The incessant process of selection and hybridization has converted the low yielding (8-10MT/ ha/yr) traditional mulberry genotype of 1960s into high yielding triploid (44 MT/ha/yr) variety i.e.S-1635 (MI-0173) in recent times (Sarkar and Ghosh, 2005). The quest for high yielding genotypes is everlasting particularly in the context of ever shrinking arable land and stiff competition from other food crops. A series of new mulberry varieties (high yielding: C-2038; drought tolerant: C-1730; water logging tolerant: C-2028; soil acidity tolerant: TR-23; cold tolerant: RG-76) have been

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developed by Central Sericulture Research and Training Institute, Berhampore which are suitable for different agro-climatic conditions for the benefit of E and NE farmers. C-2038 variety is very promising; developed from hybridization between C-763 and CF110 (a Chinese accession) followed by stringent selection and evaluation. It is necessary to analyse the physiological and biochemical association of genetic resources (parental lines) before utilization in a crop improvement programme to achieve targeted traits.Growth and development as well the quality and quantum of cocoons produced are largely influenced by leaf quality, since the mulberry leaf contributes 38.20 per cent of silk production (Miyashitha, 1986). Bose *et al*. (1991) and Neog *et al*. (2011) revealed that mulberry varieties differed significantly in the composition of nutrients. The protein content (soluble and crude) of mulberry leaves is the paramount nutritional factor in determining the life cycle performance of silkworm (Pillai and Jolly, 1985). Carbohydrates are very important for maintaining healthy growth of young silkworm larvae. Fats or lipids are particularly the main forms of energy reserves and are important for the proper development of wild silkmoth, *Antherae-aassama* (Kataky and Hazarika, 1997). Plant produces some volatile compounds by the process of secondary metabolism which is responsible for the host specificity of silkworms and also enhances leaf quality for feeding silkworms (Jyothi *et al*., 2018; Sunita, 2019). Hence, the present study was designed to profile mulberry genotypes for physiological and biochemical traits and categorizing them on the basis of multiple trait evaluation.

MATERIALS AND METHODS

The present investigation was carried out during summer (April to June) season of 2019 with thirteen genotypes (Table 1) planted in the Museum Block of Mulberry Breeding and Genetics division at Central Sericultural Research and Training Institute, Berhampore, West Bengal, India (34° 0' 28" North, 71° 34' 24" East, 19 m above MSL with humid sub-tropical climate). Climatic condition during the period of experiment was hot and humid with an average max. temperature of 39.85^{0}C and 92% relative humidity. The experiment was conducted using complete randomized block design with two replications. Well established plantation of mulberry varieties/genotypes (323.7 m^2) with thirty plants in each row (60cm x 60cm) was utilized. Nine plants of same age and uniform size were selected randomly from two rows of each genotype and were tagged for recording observations on various physiological and biochemical parameters at 65 days after pruning.Cultural operations and application of inputs were followed as per the recommended package of practices for irrigated condition.

Physiological and biochemical parameters

Fresh weight and dry weight of three (top, bottom and middle) leaves from the longest shoot were recorded. Leaf moisture content (LMC) was calculated using the formula, LMC = [(Fresh wt. - Dry wt.) / Fresh wt.] x 100 (Vijayan *et al*., 2000). Relative water content (RWC) was estimated by recording the turgid weight of 0.5 g fresh leaf sample kept in water for 4 h, followed by drying in an hot air oven till constant weight was achieved; RWC = [(Fresh wt. - Dry wt.) / (Turgid wt. - Dry wt.)] x 100 (Weatherley,1950). The chlorophyll content index (CCI) of top, bottom and middle positioned leaves of longest shoot was determined by chlorophyll meter (CCM-200).Total chlorophyll content (TCC) was estimated by acetone method and absorbance was recorded at 645 and 665 (Arnon,1949).

Lipid peroxidation(TBARS: thiobarbituric acid reacting substances)was determined by the method of Heath and Packer (1968). Leaf tissue (0.5 g) was homogenized in 10 ml 0.1% trichloroacetic acid (TCA; w/v) and centrifuged for 20 min at 10000 rpm. One ml of supernatant was mixed with 4 ml 0.5% thiobarbituric acid (TBA) and diluted with 20% TCA. The mixture was incubated in a water bath at 95°C for 30 min and then cooled in an ice bath; absorbance was measured at 532 and 600 nm. Lipid peroxidation was determined using the formula TBARS = A × (1/155) × (vol. of TCA / vol. of extract) × (1 / wt. of tissue sample); where, A denotes the difference in absorbance and 155 m M^{-1} cm^{-1} is extinction coefficient. TBARS was expressed as nmols malondialdehyde (MDA)/g DW.

Secondary metabolites were extracted from the 1.5 g (1:20; w/v) leaf sample at room temperature with 85% aqueous methanol under agitation using a magnetic stirrer for 30 min. The mixtures were centrifuged at 2500g for 10 min and the supernatants were collected. The residues were re-extracted twice under the similar conditions and made up to a final volume of 50 ml. Total phenolic content (TPC) of extracts were determined using Folin–Ciocalteu reagent (Singleton *et al*., 1999). Catechol was used as standard and TPC was expressed as mg catechol equivalent per g FW.Total flavonoid content (TFC) was measured calorimetrically (Wu and Ng, 2008); absorbance was measured at 510 nm and expressed as mgquercet in equivalent (QE) per g FW. Total tannin content (TTC) was determined according to Ragan and Jensen (1977); absorbance was measured at 760 nm, quantified using a standard curve prepared with tannic acid and expressed as tannic acid equivalent in mg/g FW.

Leaf total soluble protein (TSP) was measured by Lowry's method (Lowry *et al*., 1951) using Folin-Ciocalteu reagent. The optical density was measured using a spectrophotometer at 750nm and TSP was expressed as mg/g FW. Total soluble sugar (TSS) was estimated by anthrone method (Sadasivam and Manickam, 1992); absorbance was measured at 630 nm and TSS was expressed as mg/g FW. NitrateReductase Activity (NRA) was recorded as per the method described by Klepper *et al*. (1971) using sulfanilamide and NEDD; absorbance recorded at 540 nm and NRA was expressed in$nmol\ NO^2\ g^{-1}\ FW\ h^{-1}$. Epicuticular wax content (ECW) was estimated by chloroform method (Juliana *et al*., 2006), transmission (%) was recorded at 590nm and ECW was expressed as $\mu g\ cm^{-2}$.

Growth and yield parameters

Leaf area (LA) was recorded using leaf area meter (LiCOR 3000, Lincon Nebraska, USA). Dry weight of leaves (LDW) was recorded after harvesting and drying the mulberry leaves in oven at 65^0C. Specific leaf area (SLA) and specific leaf weight (SLW) was calculated (SLA = LA / LDW; SLW= LDW/LA) and expressed as $cm^2\ gm^{-1}$and mg cm^{-2} respectively (Gardner *et al*., 1985).After 70 days of pruning leaf fall (%) was estimated by dividing the number of nodes without leaf by buds with leaf and leaf yield per plant (g) was calculated along with other traits like longest primary shoot (LLS), total shoot length (TSL) and number of leaves per meter shoot length (LPMS) utilizing standard procedures(Vijayan *et al.,* 2000).

Multiple trait evaluation index (MEI) for the studied parameters was performed using the formula: EI = [(A-B)/C] × 10 + 50 (Mano *et al*., 1993). The evaluation index value for negative traits *viz*., TBARS and SLA was computed separately by using the modified formula: [(B-A)/C] × 10 + 50 (Talebi and Subramanya, 2009); where, A = Value of a particular variety for particular trait, B = Mean value for a particular trait of all the varieties, C = Standard Deviation of a particular trait for all the varieties, 10 = Standard unit, 50 = Fixed value. The average EI value fixed for selection of a breed/ genotype is >50; genotypes with a score of >50 were considered to have greater potential. Correlation and path analysis were conducted for physio-biochemical and morphological traits using INDOSTAT version 8.5 software.

RESULTS AND DISCUSSION

Physiological and biochemical traits

Comparative analysis of mulberry varieties is important for identifying the future breeding genetic materials. The mean performance of mulberry varieties for the physiological and biochemical traits in the present study is presented in Table 2. Mulberry varieties differed in chlorophyll and protein content, nitrate reductase activity and secondary metabolites, which usually determine the mulberry leaf quality for silkworm rearing. Secondary metabolites also act as antioxidants and provide tolerance to plants against different abiotic (temperature, water) and biotic stresses (diseases/pests); mulberry varieties respond differently affecting leaf yield under various climatic conditions. According to Murthy *et al.* (2013), leaf moisture content is an important attribute for growth and development of silkworms.Among the thirteen varieties, leaf moisture content varied from 72.75% to 77.17% and was highest in Tr23 (77.17%). Relative water content ranged from 72.87% to 90.07 % and the highest was recorded in Tr10 (90.07%) followed by C2060 (89.51) and C2017 (88.85%).

Chlorophyll content index recorded from fully expanded leaf by chlorophyll meter (CCM-202) varied from 14.62 to 23.93 and the varieties, C1730 and Tr 23 recorded higher values. Total chlorophyll content (mg g^{-1} fw) recorded was highest in Bombai (3.99) followed by S1635 (3.93) and Tr10 (3.89).Chattopadhyay *et al.* (1996) reported significant correlation of chlorophyll content and photosynthetic rate in five Chinese mulberry varieties grown under tropical conditions. Similarly, Das *et al.* (1997) observed higher CO_2 fixation in mulberry genotypes having higher chlorophyll content; while Zelitch (1982) reported a close relationship among chlorophyll content, photosynthesis and crop yield in mulberry.

Proteins and sugars play an important role in determining the quality of mulberry leaf which in turn influences growth and development of silkworms (Ghosh *et al.,* 2006). Leaf total soluble sugar content (mg g^{-1}FW) was highest in C1730 (29.65) followed by Bombai (27.32), S1 (27.13) and the least in C2038 (21.83). Murthy *et al*. (2013) reported that protein content in different mulberry varieties have a direct bearing on larval growth particularly in the silk gland development and cocoon characteristics (shell ratio and silk recovery). The total soluble protein content (mg g^{-1}FW)in tested varieties varied from 20.40 to 40.99 and the varieties *viz*., C2038, S1635, C1730 and C2060 recorded significantly higher protein content over the variety, S1.

The mulberry genotypes possessing higher nitrate reductase activity (NRA) might be with more nitrogen utilization efficiency as NRA enhances the protein content in leaves. Nitrate reductase activity varied among the varieties from 1.58 to 8. 82 nmoles $NO_2^-\ g^{-1}$ FW hr^{-1} and C2016 variety recorded the maximum followed by C1730 and the minimum was recorded in

Table 1: Profile of mulberry varieties

Year of Recommendation	Varieties	Pedigree	Recommended areas
-	Kajili	-	Local variety mainly found in E and NE India
-	Bombai	-	Local variety mainly found in E and NE India
2000	S1 (Check)	OPH of Mandalaya	Irrigated and rain fed conditions of Eastern India
2000	S-1635	OPH of CSRS1, Triploid	Irrigated and high rainfall areas of E and NE India.
2000	Tr-10	(*M. indica*xMandalaya) [4x] x Philippines [2x]	Rainfed hills of Eastern India. Rainfed hills of Eastern India.
2000	BC_2-59	Kosen × Matigara (backcrossing)	Drought prone low rainfall red and lateritic soils of Eastern India
2012	C1730	T25 × S162	
2012	C2028	China White × S1532	Waterlogged areasin E and NE India.
2017	Tr23	T20 (4x) × S162 (2x)	Rainfed hills of E and N India.
2017	C-2038	CF110 × C763	Irrigated and high rainfall areas of E and NE India.
-	C2017*	Hosur × S-162	Irrigated areas in E and NE India
-	C2016*	Hosur × S-162	Rainfed areas in E and NE India
-	C2060*	Kajili OP × V1.	Irrigated condition in E and NE India

**Under pipeline for release as variety; OPH: Open Pollinated Hybrid*
(Source: CSR&TI, Berhampore, Central Silk Board, GOI)

Table 2: Physiological and biochemical characteristics of mulberry varieties

Genotype	LMC	RWC	CCI	TCC	TSP	TSS	ECW	NRA	TBARS	TPC	TFC	TTC
Kajili	72.9	72.87	19.6	3.63	20.4	23.8	293.7	5.88*	746.5	17.6	6.48	3.77*
Bombai	73.2	80.2*	17.6	3.99*	28.2	27.3	254.3	1.58	935.1*	19.3*	7.04*	3.11
S1635	75.7*	85.7*	20.1	3.93*	31.2	24.9	284.3	5.88*	1268.0*	18.6*	6.56	3.16
C2038	76.3*	84.6*	18.6	3.78	32.1	21.8	269.3	5.25*	899.8*	20.9*	7.60*	3.08
Tr10	74.9*	90.1*	16.4	3.89	34.6*	23.5	249.4	4.12*	718.7	11.3	6.64	3.06
$BC_2$59	76.2*	83.4*	16.5	3.81	35.4*	22.6	278.5	5.12*	947.2*	19.0*	7.92*	4.08*
Tr23	77.1*	77.8*	20.5	3.49	28.8	25.8	282.3	4.56*	969.5*	23.5*	5.28	4.41*
C1730	73.4	85.42*	23.9	3.82	40.9*	29.7	276.3	8.16*	1028.0*	16.2	6.56	4.92*
C2028	73.5	87.67*	19.1	3.41	36.4*	25.6	296.4	5.88*	677.9	27.9*	9.12*	5.99*
C2017	74.3*	88.85*	17.8	3.45	27.1	23.8	305.5*	5.37*	923.2*	23.6*	8.08*	2.77
C2016	72.6	87.65*	19.0	3.41	34.9*	23.2	317.3*	8.82*	890.2*	13.2	6.87	3.67*
C2060	75.9*	89.51*	14.6	3.34	35.1*	23.2	305.4*	6.79*	930.9*	22.5*	9.60*	5.43*
S1 (Check)	72.8	77.17	26.3	3.87	28.4	27.1	296.4	3.23	523.9	18.2	6.88	3.52
LSD (0.05)	0.93	0.28	2.47	0.02	4.33	2.84	0.51	0.02	292.8	0.15	0.09	0.01

Note: LMC = Leaf moisture content (%), RWC = Relative water content (%), CCI = Chlorophyll content index, TCC = Total chlorophyll content (mg g^{-1}FW), TSP = Total soluble protein (mg g^{-1}FW), TSS = Total soluble sugar (mg g^{-1}FW), ECW = Epicuticular wax content (µg cm^{-2}), NRA = Nitrate reductase activity (nmol NO^2 g^{-1} FW h^{-1}), TBARS = Thiobarbituric acid reacting substances (nmoles g^{-1}DW), TPC = Total phenol content (mg g^{-1}FW), TFC = Total flavanoid content (mg g^{-1}FW) and TTC = Total tannin content (mg g^{-1}FW).

Bombai. It was reported that any factor which influences nutrient uptake affects the leaf NRA and would directly affect the protein content (Ghosh *et al.,* 1994). The nitrate reductase is believed to be the limiting factor in overall assimilation of nitrate in plants (Beevers and Hageman, 1969).

Silkworm larval growth and the cocoon economic traits depend on mulberry leaf biochemical characteristics (Jha *et al*., 2016) which are inversely associated with excessive production of reactive oxygen species (ROS) during stress condition. ROS leads to peroxidation of lipids in the cellular membranes during stress conditions leading to the disruption of functionality of various enzymes; which ultimately could disturb various metabolic processes in the plant (Juan, 2019). Thiobarbituric acid reacting substances (TBARS) are produced in the plants as a result of lipid peroxidation. TBARS varied significantly among the varieties studied from 523.9 to 1268 nmoles g^{-1} DW. C2028 (677.91) recorded the least TBARS followed by Tr10 (718.7) and Kajili (746.5).Plants produce antioxidants during stress to ameliorate or curb the

Table 3: Growth and yield characteristics of mulberry varieties

Genotype	FLW	LA	SLA	TLP	LMS	LLS	LFH	LYP
Kajili	1.01	58.68	211.51	73.33	40.87*	83.09	21.56	370
Bombai	1.70	113.86	249.27*	97.33	30.85	150.16*	18.16	437
S1635	3.74*	201.68*	227.00	81.67	33.22	141.61	25.52*	1022*
C2038	4.48*	231.91*	226.96	72.33	27.69	133.52	11.35	1158*
Tr10	3.30*	161.93*	240.15*	87.00	27.10	145.41	10.34	682*
$BC_2$59	2.59*	185.95*	242.28*	76.00	29.92	134.45	17.65	723*
Tr23	2.50*	165.85*	247.93*	90.67	28.27	153.22*	16.97	821*
C1730	3.14*	152.05*	222.86	85.00	29.59	137.62	30.53*	760*
C2028	2.91*	186.31*	224.74	95.67	30.24	142.01	19.81	874*
C2017	2.41*	173.21*	247.85*	106.67*	31.91	144.32	13.27	832*
C2016	2.29*	153.21*	219.40	99.67	36.95*	132.42	14.34	864*
C2060	4.15*	193.58*	254.28*	108.00*	37.64*	150.83*	17.44	1301*
S1 (Check)	1.65	125.80	227.16	96.67	34.06	145.62	17.78	549
LSD (0.05)	**0.40**	**14.73**	**11.31**	**7.65**	**2.24**	**9.91**	**5.13**	**0.93**

Note: FLW = Fresh leaf weight (g), LA = Leaf area (cm^2), SLA = Specific leaf area (cm^2g^{-1}), TLP = Total leaves per plant (No's), LMS = Leaves per meter shoot length (Nos.), LLS = Length of longest shoot (cm), LFH = Leaf fall during harvest (%) and LYP = Leaf yield per plant (g)

**: Significant at 5% level*

effects of ROS. Secondary metabolites also act as antioxidants (Tasiu, 2019) and provide tolerance to the plants. C2028 and C2060 recorded maximum secondary metabolites (mg g^{-1}FW).C2028 recorded highest TPC (27.87) and TTC (5.99).C2060 recorded highest TFC(9.60) content. Iqbal *et al.*(2012) reported similar significant differences in the antioxidant content in different mulberry varieties with good nutritive values.

Growth and yield parameters

The importance of growth associated traits in mulberry for better leaf yield and quality were described by Sori and Gebreselassie (2016). The mean performance of the thirteen varieties for different growth and yield attributing parameters are presented in Table 3. Fresh leaf weight (g) ranged from 1.01 to 4.48 and C2038 recorded the highest fresh leaf weight (4.48) followed by C2060 (4.15) and S1635 (3.74) and most of the mulberry varieties recorded higher leaf weight over the check variety (S1). The enhanced photosynthesis can be attributed to the increased leaf area (Chattopadhyay *et al*.,1996) and the leaf area (cm^2) recorded was maximum in C2038 (231.96) followed by S1635 (201.68), C 2060 (193.58) and small sized leaves were noticed in Kajili (58.68), which is actually a multi-lobed leaf. The area of fresh leaf (cm^2) ranged from 58.68 to 231.96 and ten varieties recorded higher leaf area over S1. The larger leaf weight and size was noticed in the improved varieties as compared to local/ land races. The specific leaf area (cm^2g^{-1}) ranged from 211.51to 254.28 with less variation; C2060 recorded the highest (254.28) and Kajili recorded the least (211.51).

Growth parameters are very important criterion for selection of high yielding mulberry varieties (Subramaniam *et al*., 2012). Leaves per meter shoot length indirectly indicate the distance between the leaves; while the smaller inter-nodal distance indicates more number of leaves per meter length of shoot. Mean values for leaves per meter shoot varied from 27.10 to 40.87 and Kajili recorded the maximum (40.87) followed by C2060 (37.64) and C2016 (36.95). Growth of mulberry is assessed by longest and total shoot length and length of the longest shoot(cm) was observed in Tr23 (153.22) followed by C2060 (150.83) and Bombai (150.16), which is significant over the S1 variety. Leaf fall (%)during the harvest ranged from 10.34 to 30.53 and C1730 recorded the highest (30.53) followed by S1635 (25.52), Kajili (21.56).Biomass production and leaf yield of plants are the outcome of physio-biochemical processes (Blum, 1996). Leaf yield per plant (g) ranged from 370 to 1301; C2060 recorded the maximum (1301) followed by C2038 (1158), S1635 (1022) and nine varieties were found to be with significantly higher leaf yield over S1.

Correlation among morpho-physiological and biochemical traits in mulberry varieties

Correlation of a particular trait with other characteristics contributing to the leaf yield is of great

Table 4: Phenotypic correlation of morpho-physiological and biochemical traits with leaf yield in mulberry varieties

Traits	LMC	TSP	TSS	ECW	NRA	TBARS	TPC	TFC	TTC	LA	SLA	LLS	LFH	FLW	LYP
LMC	**1**	0.133	-0.406*	-0.228	-0.068	0.380*	0.266	0.012	0.026	0.249	0.614***	0.303	0.015	0.476**	0.665
TSP		**1**	0.077	-0.073	0.431**	0.098	-0.097	0.289	0.457**	0.404*	-0.052	0.379*	0.227	0.640***	0.491
TSS			**1**	-0.142	-0.117	-0.110	0.014	-0.272	0.209	-0.178	-0.105	0.251	0.418**	-0.127	-0.436
ECW				**1**	0.586***	-0.068	0.284	0.330*	0.312	-0.029	-0.197	-0.161	0.058	-0.189	0.165
NRA					**1**	0.225	-0.129	0.150	0.406*	-0.113	-0.353*	-0.257	0.188	0.275	0.349
TBARS						**1**	0.010	-0.127	-0.088	-0.255	0.291	0.128	0.130	0.262	0.325
TPC							**1**	0.491**	0.463**	0.148	0.259	0.219	0.181	-0.049	0.256
TFC								**1**	0.440**	0.287	0.193	0.157	0.037	0.220	0.508
TTC									**1**	0.024	-0.101	0.082	0.222	0.157	0.268
LA										**1**	0.061	0.428**	-0.132	0.169	0.480
SLA											**1**	0.504**	-0.067	0.251	0.335
LLS												**1**	-0.011	0.466**	0.289
TLF													**1**	0.089	0.107
FLW														**1**	0.570
LYP															**1**

*Statistically significant correlation at p d" 0.05; ** statistically significant correlation at p d" 0.01 and ***statistically significant correlation at p d" 0.001

Note: LMC = Leaf moisture content (%), TSP = Total soluble protein (mg g^{-1}FW), TSS = Total soluble sugar (mg g^{-1}FW), ECW = Epicuticular wax content (µg cm^{-2}), NRA = Nitrate reductase activity (nmol NO^2 g^{-1} FW h^{-1}), TBARS = Thiobarbituric acid reacting substances (nmoles g^{-1}DW), TPC = Total phenol content (mg g^{-1}FW), TFC = Total flavanoid content (mg g^{-1}FW), TTC = Total tannin content (mg g^{-1}FW),LA = Leaf area (cm^2), SLA = Specific leaf area (cm^2g^{-1}), LLS = Length of longest shoot (cm), LFH = Leaf fall during harvest (%),FLW = Fresh leaf weight (g) and LYP = Leaf yield per plant (g).

Table 5: Multiple trait evaluation of mulberry varieties

Genotype	TBARS	TFC	TTC	TPC	ECW	NRA	CCI	TSP	TSS	LLS	LYP	MEI	MEI Rank
Kajili	746.5	6.48	3.8*	17.6	293.7	5.9*	19.6	20.4	23.8	83.1	370	42.3	XIII
Bombai	935.1*	7.04*	3.1	19.3*	254.3	1.6	17.6	28.2	27.3	150.2*	437	45.4	XI
S1635	1268.0*	6.56	3.2	18.6*	284.3	5.9*	20.1	31.2	24.9	141.6	1022*	51.8	IV
C2038	899.8*	7.60*	3.1	20.9*	269.3	5.3*	18.6	32.1	21.8	133.5	1158*	48.7	IX
Tr10	718.7	6.64	3.1	11.3	249.4	4.1*	16.4	34.6*	23.5	145.4	682*	43.1	XII
$BC_2$59	947.2*	7.92*	4.1*	19.0*	278.5	5.1*	16.5	35.4*	22.6	134.5	723*	48.9	VIII
Tr23	969.5*	5.28	4.4*	23.5*	282.3	4.6*	20.5	28.8	25.8	153.2*	821*	50.7	VI
C1730	1028.0*	6.56	4.9*	16.2	276.3	8.2*	23.9	40.9*	29.7	137.6	760*	56.1	III
C2028	677.9	9.12*	5.9*	27.9*	296.4	5.9*	19.1	36.4*	25.6	142.0	874*	56.3	II
C2017	923.2*	8.08*	2.8	23.6*	305.5*	5.4*	17.8	27.1	23.8	144.3	832*	50.3	VII
C2016	890.2*	6.87	3.7*	13.2	317.3*	8.8*	19.0	34.9*	23.2	132.4	864*	51.1	V
C2060	930.9*	9.60*	5.4*	22.5*	305.4*	6.8*	14.6	35.1*	23.2	150.8*	1301*	56.6	I
S1(Check)	523.9	6.9	3.5	18.2	296.4	3.2	26.3	28.4	27.1	145.6	549	48.7	X
LSD (0.05)	**292.8**	**0.09**	**0.01**	**0.15**	**0.51**	**0.02**	**2.47**	**4.33**	**2.84**	**9.91**	**0.93**		

Note: TBARS = Thiobarbituric acid reacting substances (nmoles g^{-1}DW), TFC = Total flavanoid content (mg g^{-1}FW), TTC = Total tannin content (mg g^{-1}FW),TPC = Total phenol content (mg g^{-1}FW), ECW = Epicuticular wax content (µg cm^{-2}), NRA = Nitrate reductase activity (nmol NO^2 g^{-1} FW h^{-1}), CCI = Chlorophyll content index, TSP = Total soluble protein (mg g^{-1}FW), TSS = Total soluble sugar (mg g^{-1}FW), LLS = Length of longest shoot (cm), LYP=Leaf yield per plant (g)and MEI= Multi-trait evaluation index.

**: Significant at 5% level*

importance for the indirect selection of high yielding mulberry genotypes. Hence, knowledge of association between leaf yield and its component traits as well as among the component traits themselves can promote the efficiency of selection in mulberry breeding program. In fact, it is well established that correlation studies between leaf yield and yield components are pre-requisite in planning effective breeding programs. Quantitative traits like leaf yield expresses themselves in close association with many other traits. Change in the expression of one trait is usually associated with changes in the expression of many other traits. Therefore, the correlations obtained in the present study are useful in the selection of traits having direct and significant correlation in improving leaf yield (Table 4). Leaf yield per plant has positive correlations with all the traits except TSP (rp:-0.436). The highest statistically significant positive phenotypic correlation was observed between TSP and FLW (rp: 0.640) followed by LMC and SLA (rp:0.614); ECW and NRA (rp: 0.586). The highest statically significant negative phenotypic correlation was observed between TSS and LMC (-0.406) followed by NRA and SLA (rp:-0.353).

Significant positive correlation of TSP with NRA (0.431), TTC (0.457), FLW (0.640) and LLS (0.379); TSS with LFH (0.418); TPC with TFC (0.491), TTC (0.440); LA with LLS (0.428); TLF with FLW (0.466). NRA showed significant negative correlation with SLA (-0.353) and LMC with TSS (-0.406). Similar correlation studies for morpho-physiological parameters contributing to leaf yield in mulberry were reported by Sathyanarayana and Sangannavar (2020) and Saini *et al*. (2018). These observations indicate that improvements in each of the traits would lead to overall improvements of the genotypes. Such correlations help in making reasonable decisions in selecting traits controlled by multiple genes. Leaf yield as a quantitative trait, is polygenically controlled. These findings imply that efficiently leaf yield improvement depends on simultaneous improvements in all yield components. In fact, selection efforts based on leaf yield alone are often less effective and efficient (Mohammadi *et al.,* 2003). Selection needs to be made based on various traits of the crop at hand.

Multiple trait evaluation

Evaluation index is an index of multiple traits or as a performance index, which is a single valued measure of the multiple trait performance of a population (Mano *et al*., 1993). MEI (Table. 5) was calculated for significant parameters in all the thirteen genotypes and C2060 (56.6) performed best followed by C-2028 (56.3) and C-1730 (56.1). Sajgotra *et al*. (2018) has also ranked different mulberry varieties based on silkworm rearing traits after feeding the mulberry.This study concludes that mulberry varieties *viz*., C2060, C-2028 and C-1730 have better leaf quality (higher total soluble protein, sugar, leaf moisture, chlorophyll and secondary metabolites) and higher yield potential. As many of these traits (lipid peroxidation and secondary metabolites) are influencing factors for plant responses against the adverse conditions (abiotic and biotic stresses), they could as well be included as selection criteria for

developing improved mulberry varieties. However, further investigations on silkworm productivity are essential for better understanding of mulberry leaf quality on silkworm growth and development.

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