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Screening of mulberry (*Morus* sp.) germplasm for quality traits

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Abstract

The present study was carried out to screen mulberry germplasm accessions for quality trait through biochemical analysis and rearing performance of silkworm, *Bombyx mori* L. Fifteen mulberry accessions viz., MI-0783, MI-0665, MI-0065, MI-0789, MI-0669, ME-0095, ME-0174, MI-0532, MI-0632, MI-0497, MI-0828, MI-0845, MI-0657, ME-0174, MI-0300 belonging to different species were screened for qualitative traits. V1 mulberry variety was utilized as check variety in the present study. Among the fifteen mulberry accessions, MI-0669 exhibited significantly higher moisture content (77.23%), crude fibre (12.75%) and protein content (25.42%). MI-0665 recorded significantly higher total carbohydrates (22.10 g/100 g), ash content (11.59%), chlorophyll a (3.92 mg/g), chlorophyll b (1.87 mg/g), and total chlorophyll (5.84 mg/g) content. The leaves of the above mentioned fifteen accessions and check variety were simultaneously screened for rearing performance of double hybrid silkworm viz., (CSR6 x CSR26) x (CSR2 x CSR27). Economic traits of silkworm viz., Mature larval weight (61.89 g/10 larvae) effective rate of rearing (92.44%), single cocoon weight (2.206 g), single shell weight (0.482), shell ratio (21.84%) silk filament length (1297 cm), silk productivity (6.881 g/day), silk filament weight (410 mg) and raw silk percent (18.59%) were significantly maximum when the leaves of MI-0669 accession were fed to the silkworm. The estimation of biochemical constituents play a crucial in selection of mulberry cultivars with good quality. The rearing traits of silkworm, silk productivity and silk quality is improved when the silkworm is fed with the leaves of cultivars with high moisture content and nutritional profiles.

Keywords: Mulberry, germplasm, accessions, qualitative traits, biochemical constituents, rearing performance

Introduction

Mulberry leaf quality is one of the key factors influencing the growth and development of silkworm, *B. mori*. The silkworm being a monophagous insect derives all the nutrients from mulberry leaf. Since the production of good quality cocoons depend on providing good quality leaves to silkworm, the development of superior quality leaf has become one of the prime objective in mulberry breeding programme. The leaf quality intrin is determined by the number of factors viz., mulberry variety, ecological factors, agronomical practices, method of harvest and preservation etc. Different quality traits such as leaf moisture content, proteins, carbohydrates, nitrogen, amino acids and chlorophyll are responsible for leaf quality (Bongale *et al.*, 1991) [21].

Varieties possessing high nitrogen and amino acids contents in leaves are nutritively superior and promote growth and development of silkworm (Machii and Katagiri, 1991; Suryanarayanan and Shivashankar Murthy, 2002) [21, 33]. According to some scientists, the combination of two traits i.e., nitrogen and total amino acid contents at higher quantity determines the leaf quality (Machii and Katagiri, 1991 and Suryanarayana and Sivashankar Murthy, 2002) [21, 33]. Higher chlorophyll content in leaves indicates the photosynthetic efficiency; therefore it can be used as one of the criteria for quantifying photosynthetic rate in mulberry (Sujathamma and Dandin, 2000) [31].

More than 60 percent of the total cost of cocoon production is incurred for mulberry leaf production alone. Thus, improvement of mulberry for qualitative and quantitative trait is essential to sustain the profitability in sericulture. All the quality traits or nutrients may not be available at highest level in a genotype (Sujathamma and Dandin, 2000) [31], as there are several factors, such as nutrients uptake, absorption of water, nitrogen utilization efficiency

etc., which are involved and differ from accession to accession. Therefore, it is most essential to identify accessions possessing some of the important quality traits together, which may help to improve the growth and cocoon characters of silkworm. The effect of feeding of different mulberry varieties on larval growth, cocoon yield and other economic characters has been reported by number of investigators (Krishnaswami *et al.*, 1970; Dar *et al.*, 1988; Ninge Gowda and Sudhakar, 2002; Jalaja Kumar and Ram Rao, 2008) [17, 10, 23, 15]. Since the production of fine quality cocoons depends on providing good quality leaves to silkworm, the development of superior quality leaf has become one of the prime objective in mulberry breeding programme. Appropriate selection of the tropical cultivars based on their effect on growth and cocoon yield parameters of *B. mori* races in different agro-climatic conditions is essential to select and exploit promising cultivars for better sericulture practices. The present investigation was undertaken to screen mulberry accessions for qualitative traits by analyzing important biochemical traits and rearing traits for fifteen mulberry accessions using ruling cultivated variety V1 as check variety.

$$\text{Leaf moisture percent (\%)} = \frac{\text{Fresh weight of leaves} - \text{Dry weight of leaves}}{\text{Fresh weight of leaves}} \times 100$$

Total carbohydrate content was determined by phenol sulphuric method (Sadasivam and Manickam, 1996) [28]. Crude fibre and ash content were estimated by the method of AOAC (1955) [4].

The chlorophyll was estimated by following the procedure outlined by Hiscox and Israelstam (1979) [11] at the wave lengths of 645 nm and 663 nm using spectrophotometer. The chlorophyll a, chlorophyll b and total chlorophyll content of leaf was computed using the formula suggested by Arnon (1949) [3] and expressed in mg/g of leaf tissue. Lowry's method (1959) was used to quantitatively measure the protein content.

Economic traits of silkworm, *Bombyx mori* L.,

Silkworms (*B. mori* L.) were reared by feeding leaves of individual mulberry accession during favorable commercial crops in three replications. To identify the accessions with superior leaf quality, rearing performance study was conducted with the silkworm double hybrid (CSR6 x CSR26) x (CSR2 x CSR27).

Rearing traits viz., mature larval weight, effective rate of rearing, cocoon weight, shell weight, shell ratio were recorded. Silk quality parameters viz., silk productivity, silk filament length, filament weight, raw silk percent, denier and renditta were recorded.

The experiment was repeated twice and average data on different leaf quality traits for biochemical constituents and economic traits of silkworm were statistically analyzed by completely randomized design (Cochran and Cox, 2000) [9].

Results and Discussion

Biochemical Constituents

1. Moisture Content

Significant variations were observed among the mulberry germplasm accessions with respect to moisture content (Table.1). Among the accessions, significantly high

Materials and Methods

The leaf quality characters of fifteen mulberry accessions viz., MI-0783, MI-0665, MI-0065, MI-0789, MI-0669, ME-0095, ME-0174, MI-0532, MI-0632, MI-0497, MI-0828, MI-0845, MI-0657, ME-0174, MI-0300 belonging to different species were screened for quality traits through biochemical analysis and silkworm rearing. V1 mulberry variety was utilized as check variety in the present study.

Estimation of Biochemical Constituents

Leaf moisture content was estimated by following the method of Vijayan *et al.* (1997) [36]. The apical, middle and bottom leaves of several mulberry leaf samples were collected. After gently removing the water from the leaf surface with tissue paper, the collected leaf samples were cleaned under running tap water to get rid of dust and other residues. The fresh weight of each sample were recorded separately. Following a 24-hour drying period at ambient temperature and a 12-hour oven drying period at 60 °C, the dry leaf weight of each sample was promptly recorded. Using the following formula, the moisture content of the apical, middle, and bottom leaves were estimated and expressed as a percentage (%).

moisture content was recorded in MI-0669 (77.23%), whereas in V1 variety moisture content was 78.26 per cent. The result of the present study is in conformity with the findings of Banerjee *et al.*, (2007) [5] and Ahalya *et al.*, (2020) [2]. Leaf moisture content is a genetic character and influenced by available soil moisture and root proliferation nature of mulberry variety. Feeding capacity of the silkworm larvae is improved by the presence of available moisture in the leaves, which accelerates their growth rate (Murthy *et al.*, 2013) [22]. Most researchers noticed that, moisture content of mulberry leaves decreased gradually with corresponding increase in leaf growth. Thangamani and Vivekanandan (1984) [35] observed wide range of variation in eight varieties of mulberry for moisture content (63.67 to 70.60%) and total sugars (8.64 to 15.58%). Paul *et al.* (1992) [26] observed in their studies that availability of moisture content in leaves enhances the feeding efficiency of the larvae which inturn increases the growth rate.

2. Total Carbohydrates (g/100 g)

Significantly maximum carbohydrate content was recorded in MI-0665 (22.10 g/100 g) followed by MI-0669 (21.86 g/100 g) while, V1 variety recorded 23.75 g/100 g. Current study results were enlightened by Ramamoorthy *et al.*, (2018) [27] who reported highest protein content (24.8±0.22%) in the leaves of V1 variety. The V1 mulberry variety has highest level of total sugar (16.72%) and total protein (26.72%) when compared to the other five mulberry types, according to Kumar *et al.*, (2009) [19]. Carbohydrate was reported to be chief source of energy for silkworm (Horie, 1978) [12]. Carbohydrates are estimated based on the amount of sugar and starch content available in leaves (Bose and Bindroo, 2001) [8]. If the leaves have high carbohydrate content, silkworm gain more energy and inturn may enhance the synthesis of silk protein.

3. Crude fibre (%)

Significantly higher crude fibre content was observed in leaves of MI-0669 (12.75%) followed by MI-0828 (12.54%) and it was 13.02 percent in V1 variety (Table 1). Several workers have evaluated mulberry varieties and different species of mulberry for the crude fibre content. Salcedo *et al.* (2017) [29] reported that *Morus nigra* accessions showed higher crude fibre content (8.4 g/100 g) than *Morus alba* (7.1 g/100 g). Similarly, Iqbal *et al.* (2012) [13] reported highest crude fibre content in *Morus nigra* (12.3 g/100 g) and *Morus alba* (10.1 g/100 g). (Adeduntan and Oyerinde 2010) [1] reported crude fibre content in S36 (10.8%), Obeche (20.73%), S54 (13.70%) and in K2 (8.74%).

4. Ash Content (%)

Total ash content was significantly maximum in leaves of MI-0669 (12.73%) followed by MI-0665 (11.87%) it was 13.06 percent in V1 variety. (Table 1). Iqbal *et al.* (2012) [13] studied that nutritive composition in the leaves of three indigenous species of mulberry namely, *Morus alba*, *M. nigra* and *M. rubra* and reported that the ash content varied between 8.91±0.51 percent to 11.73±1.09 percent.

5. Chlorophyll Content

Among the mulberry accessions, MI-0665 recorded the highest chlorophyll a (3.92 mg/g), chlorophyll b (1.87 mg/g) and total chlorophyll content (5.84 mg/g) (Table 1). The check variety V1 exhibited chlorophyll a, chlorophyll b and total chlorophyll content of 1.64, 1.95 and 5.53 mg/g, respectively. Chlorophyll content is important for quantifying the photosynthetic efficiency of plant and is an essential constituent in assessing quality of foliage. Yadhav *et al.*, (2022) reported that the highest total chlorophyll content was observed in S146 mulberry leaves. The silkworm fed with S146 mulberry leaves showed better cocoon performance effective rate of rearing (ERR%), cocoon weight, pupa weight, shell weight and filament length compared to other mulberry varieties.

6. Total Proteins

Among the fifteen mulberry accessions, MI-0669 (25.42%) recorded significantly maximum protein content followed by MI-0632 (24.77%) and V1 check variety recorded leaf protein content of 26.93 percent (Table 1). For many Lepidopteron larvae, the quality of the nutrients is mostly determined by the protein content in the leaves. It is well known that mulberry leaf protein directly biosynthesizes about 70 percent of the protein content of raw silk, specifically fibroin and sericin, with the remaining 30 percent coming from silkworm body tissue and hemolymph

protein. This indicates the significance of leaf protein in the silkworm diet. Protein content present in different mulberry varieties had a direct influence on larval growth particularly in silk gland development and cocoon characters of silkworm (Shah *et al.*, 2007 & Islam *et al.*, 2023) [30, 14]. Similar studies were conducted by Bongale and Chaluachari (1993) [6] observed a significant difference in protein content of eight different mulberry varieties leaves S54, C1, MR2, Kitchili, Roso, Kosen, Japanese and M5.

Economic traits of silkworm, *Bombyx mori* L.,

Economic traits of silkworm viz., mature larval weight (61.89 g/10 larvae) effective rate of rearing (92.44%), single cocoon weight (2.206 g), single shell weight (0.482), shell ratio (21.84%) silk filament length (1297 cm), silk productivity (6.881 g/day), silk filament weight (410 mg) and raw silk percent (18.59%) were significantly maximum when the leaves of MI-0669 accession were fed to the silkworm (Table 2). The variation in larval weight may be due to the difference in nutritional composition of the leaf. The present results are comparable with the findings of Tayade *et al.* (1988) [34], who observed significant differences in weight of larvae when the silkworm larvae were fed on mulberry varieties viz., Kanav-2, Kosen, LM-1, LM-2, Mysore Local, S-30, S-36, S-41 and S-54. Patil *et al.* (2001) [25] reported that higher levels of leaf water content, total soluble sugars, soluble proteins, total chlorophyll and leaf water retention increased larval weight.

Growth and development of silkworm (*Bombyx mori* L.) and the cocoon crop yield are influenced largely by the varietal difference and nutritional quality of mulberry (*Morus* spp.) leaf used as food (Parpiev, 1968 and Krishnaswami *et al.*, 1970) [24, 17]. Differences in silk quality parameters between the accessions might be due to difference in the nutritional contents in leaves of different accessions and larval duration during fifth instar. These results are in conformity with the findings of Sujathamma *et al.* (2001) [32] who reported significant differences all the characters viz., cocoon weight, shell weight, shell ratio, average filament length and filament denier, when the larvae were fed on different mulberry varieties. The food quality influences the weight of the cocoon, silk yield and physicochemical properties of silk thread. Kahn *et al.* (2007) evaluated seven mulberry genotypes through bio-assays and reported that the highest filament length was recorded in Ichinose (1206 m). Sujathamma and Bose *et al.* (1995) reported that succulent mulberry leaves with less fibre and high mineral content stimulate the metabolic activities in silkworm resulting in quantitative improvement of cocoon and silk.

Table 1: Biochemical composition of different mulberry germplasm accessions

Sl. No.	Accession Number	Moisture content (%)	Total Carbohydrates (g/100 g)	Crude fibre (%)	Ash Content (%)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)	Protein Content (%)
1.	MI-0783	67.96	17.89	9.75	9.77	1.63	0.74	2.38	19.78
2.	MI-0665	76.54	22.10	11.78	11.59	3.92	1.87	5.84	19.01
3.	Mi-0065	66.64	17.46	9.19	9.15	1.84	0.67	2.61	20.57
4.	MI-0789	70.82	21.08	10.08	10.08	1.92	0.73	1.69	20.05
5.	MI-0669	77.23	21.86	12.75	12.73	3.48	1.85	5.32	25.42
6.	ME-0095	75.67	20.14	12.08	12.10	2.27	1.65	3.98	24.06
7.	ME-0174	70.26	19.04	9.54	9.57	1.99	1.17	3.10	20.46
8.	MI-0532	71.69	19.67	10.58	10.55	1.95	1.15	3.18	21.98
9.	MI-0632	76.66	20.93	11.98	11.94	2.73	1.41	4.49	24.77
10.	MI-0497	72.88	20.04	10.04	10.06	2.09	1.59	3.89	23.87
11.	MI-0828	75.05	21.56	12.54	12.53	2.96	1.69	4.03	23.41
12.	MI-0845	73.42	19.85	10.84	10.83	2.66	1.45	4.01	22.08
13.	MI-0657	75.51	18.93	11.27	11.26	2.77	1.60	4.15	23.66
14.	ME-0174	70.26	18.14	9.64	9.63	1.98	1.24	3.35	21.15
15.	MI-0300	69.91	18.53	10.08	10.10	1.72	1.31	3.00	21.00
16.	V1	78.26	23.75	13.02	13.06	1.64	1.95	5.53	26.93
SE(d)		0.3974	0.1809	0.1758	0.2090	0.0699	0.0631	0.0742	0.1644
CD at 5%		0.8095	0.3686	0.3581	0.4257	0.1423	0.1286	0.1511	0.3349

Significant at 5% level

Table 2: Rearing performance of double hybrid silkworm (CSR6 x CSR26) x (CSR2 x CSR27) fed with leaves of 13 different mulberry accessions

Sl. No.	Accession Number	Mature Larval weight (g/10 larvae)	ERR (%)	Single cocoon weight (g)	Single shell weight (g)	Shell Ratio (g)	Silk Filament length (zm)	Silk Productivity (cg/day)	Silk Filament weight (mg)	Raw Silk percent (%)
1.	MI-0783	56.14	85.29	1.852	0.385	20.81	1129	5.505	309.00	16.69
2.	MI-0665	60.89	91.94	2.194	0.480	21.89	1267	6.862	386.00	17.59
3.	Mi-0065	56.41	87.04	1.909	0.389	20.36	1135	5.552	306.67	16.06
4.	MI-0789	57.47	88.81	2.108	0.415	19.69	1157	5.929	319.33	15.15
5.	MI-0669	61.89	92.44	2.206	0.482	21.84	1297	6.881	410.00	18.59
6.	ME-0095	60.12	91.17	2.217	0.455	20.53	1204	6.505	381.33	17.20
7.	ME-0174	58.04	89.87	2.136	0.423	19.79	1158	6.038	323.00	15.12
8.	MI-0532	58.52	89.78	2.163	0.427	19.72	1167	6.095	327.00	15.11
9.	MI-0632	60.92	91.84	2.238	0.477	21.31	1237	6.809	347.67	15.54
10.	MI-0497	58.69	90.38	2.212	0.434	19.62	1171	6.200	332.33	15.02
11.	MI-0828	59.79	90.48	2.288	0.456	19.93	1186	6.514	376.33	16.44
12.	MI-0845	59.51	90.73	2.263	0.435	19.23	1167	6.219	356.33	15.74
13.	MI-0657	60.22	91.49	2.255	0.474	21.04	1218	6.776	379.67	16.84
14.	ME-0174	57.53	87.47	1.982	0.407	20.56	1149	5.819	315.67	15.93
15.	MI-0300	56.69	87.16	1.935	0.395	20.40	1141	5.638	310.33	16.04
16.	V1	63.49	93.61	2.216	0.495	22.33	1324	7.071	443.67	20.02
SE(d)		0.3182	0.3493	0.0118	0.0027	0.1625	4.7668	0.0388	4.7987	0.2468
CD at 5%		0.6481	0.7114	0.0240	0.0055	0.3311	9.7634	0.0791	9.7747	0.5028

Significant at 5% level

Conclusion

Screening of fifteen mulberry accessions for quality traits revealed that MI-0669 and MI-0665 superior with respect to biochemical constituents and economic traits of silkworm. The silkworms fed on leaves of accession MI-0669 and MI-0665 were able to consume and digest more food which in turn led to increased larval growth. Hence, the silkworms which derived more nutrition from food produced good quality of cocoons with maximum cocoon and shell weights, shell ratio, productivity, filament length and denier. These findings provide a valuable foundation for selecting suitable genotypes for breeding mulberry varieties with enhanced nutritional profiles to optimize sericulture practices and increase silk yield.

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