



Original Research Article

Study on leaf moisture status of some Mulberry varieties as influenced by Foliar spray of Paras (Mulberry garden)

M. Shivashankar*

Department of Life Science, Janan Bharathi, Bangalore University, 560056-Bangaluru, India

*Corresponding author

ABSTRACT

Keywords

Mulberry,
Bombyx
mori L.
Leaf moisture,
Foliar spray,
Production of
cocoon

Mulberry is a sole source of food materials and an important perennial crop grown primarily for foliage to rear the silkworm *Bombyx mori* L. For the production of cocoons. Among many other constituents, moisture content in V1 mulberry leaf as influenced by foliar spray and its retention for longer durations play an important role in the successful production of cocoons. The study conducted on seven mulberry genotypes growing as trees under rain fed conditions in Kanakapura showed that the genotypes differ in the leaf moisture and its retention, with V1 varieties recoded the maximum values. The moisture retention capacity after six hours and 12 hours of harvest in spring and early autumn was also significantly higher in V1. This may be due to more thickness of upper cuticle cum epidermis, stomata of smaller dimensions and more thickness of the leaf and foliar spray of Paras.

Introduction

Mulberry leaves of bad quality will affect the silkworm health. Since silkworm is monophagous and depends solely upon mulberry for nutrition, the quality of mulberry has a direct influence on the health and growth of the silkworm. Mulberry is an important plantation crop grown primarily for foliage to rear the silkworm *Bombyx mori* L. It grows in different climatic conditions ranging from tropical to temperate and is unique in the expression of genetic characters under different environmental conditions. It is widely distributed in varied ecological and geographical zones from intensive

cultivation in temperate, sub-tropical and tropical areas to natural occurrence in forests. This clearly indicates that mulberry has a high degree of morphological and physiological adjustments to changes in the environment. Mulberry leaf protein is the only source for silkworm to synthesize the commercial silk. It is impossible for the silkworm to complete the growth in the absence of mulberry. The host specificity of *Bombyx mori* L. is not only due to the nutritional superiority of leaves but primarily due to the presence of any chemical factors in the leaves to which the insect gets lured. These include attracting,

biting and swallowing factors. The leaves are highly nutritious with high protein, sugar and mineral content. Higher moisture content in mulberry leaves is known to increase the amount of ingestion and digestion ability of silkworm as moisture acts as olfactory and gustatory stimulant (Ito, 1963). High leaf moisture content and moisture retention capacity of the mulberry genotypes have a positive influence on the growth and development of silkworm. For successful rearing the maintenance/retention of sufficient moisture content in the leaves for prolonged periods is of immense importance Legacy (1958), Hamamura *et al* (1962), Mandal and Krishnaswami (1965). Different genotypes are said to influence the leaf moisture content and its retention in harvested leaf. Besides, environmental factors, leaf anatomical parameters like stomatal size, stomatal frequency, mesophyll tissue, cuticle thickness and leaf thickness also influence the moisture content of the leaf and its retention capacity. Drought tolerance adaptation involving dehydration tolerance would be most advantageous in mulberry plants, in which leaf production is of primary importance as dehydration tolerance adaptation would allow a range of plants to produce maximum leaf growth at given water potential Ninge Gowda & Sudhakar, (2002). Further moisture content and its retention in excised leaves are proposed as indices in screening for drought resistance in wheat Clarke & Mccaig, (1982). The present investigation was, therefore, an attempt to see the differences in moisture content in fresh leaf, as influenced by foliar spray of Paras, its retention and the anatomical Leaf Moisture Status of Some Mulberry Genotypes features of mulberry leaf which have a direct bearing with it so that mulberry production is improved both qualitatively and quantitatively.

Materials and Methods

The present investigation was taken up on ten year old mulberry trees raised in Budiguppe village, Kankapuratq,, Ramanagara District located at 30° 12' North latitude and 75° 35 ' East longitude and 2000 meters above the sea-level. Mulberry genotypes namely V1, S-13, S-146, S-34, M-5, Karanahalli local and one local genotype namely Mysore local, maintained under rainfed conditions, were taken up for the study. The experiment was laid out in a Randomized Block Design (RED) with three replications for each genotype. Plants were raised at 9' x 8' spacing and number of plants per treatment per replication with foliar application of Paras 0.5% was kept uniform .The plantation was maintained as per the package of practices recommended for temperate conditions of Kashmir (Ahsan *et al.* 1990).

The moisture content of the leaf was determined on dry weight basis. One hundred fresh leaves, comprising of tender, medium and coarse leaves were harvested early in the morning and weighed immediately. They were then kept at room temperature and weighed again after 06 and 12 hours. The leaves were then dried in hot air oven at 60°C for 48 hours (Ninge Gowda & Sudhakar, 2002). The dry weight was recorded and the moisture content and moisture retention capacity calculated as per the following formulae:

$$\text{Moisture content (\%)} = \frac{(\text{Fresh weight} - \text{Dry weight})}{\text{Fresh weight}} \times 100$$

$$\text{Moisture retention} = \frac{(\text{Weight after 6 hrs} - \text{Dry weight})}{(\text{Fresh weight} - \text{Dry weight})(\%)} \times 100$$

$$\text{Moisture retention} = \frac{(\text{Weight after 12 hrs- Dry weight})}{\text{Capacity after 1 2 hours} \times 100} \times 100$$

(Fresh weight - Dry weight)

Three observations per treatment per replication were recorded and an average calculated. Data recorded during the two years was pooled.

For anatomical studies fully expanded leaves from three month old shoots were collected early in the morning. Soon after their collection, they were fixed in Formalin Acetic Alcohol (FAA). The material was kept in the fixative for 20 hours. The fixed material was later transferred to 70% ethanol (C₂H₅OH) for preservation. For Stomatal study and Idioblast frequency lower and upper epidermal peels were taken as per the method of Pohl (1967); Ghose and Yunus (1972). For the fixed material, dehydration, infiltration and embedding was done properly and thin sections (10-20 μ m) cut mechanically with the help of a microtome. Standard procedures for staining and embedding were followed as per Dwivedi and Singh (1990); Khasim (2002) and the sections mounted in DPX. Stomatal frequency was studied under (10 x 40) magnification. Idioblast frequency on the other hand was recorded under low magnification (10 x 10), so as to accommodate more number of idealists per microscopic field.

Number per microscopic field in both the cases was counted and frequency calculated on millimeter basis. Microscopic measurements of various leaf tissues were made at 10 different locations in five cross sections, using ocular micrometer. Measurements were made at 10 x 40 magnification. The palisade and spongy proportion were calculated as per Tiwari *et al.* (1986).

Analysis of variance (ANOVA) was done as per Singh and Choudhary (1977).

Results and Discussion

The observers recorded for two springs and two autumn crops during the study period were pooled and are shown in Table-1.

During spring moisture content in fresh leaf was maximum (75.34%) in V1 which was significantly higher than the remaining genotypes, whereas, it ranged from 71.66 percent in Mysore local to 72.46 percent in S34. During autumn crops also, the moisture content in fresh leaf was the highest (74.55%) in the V1 and least (69.89%) in Mysore local. In the remaining genotypes it ranged from 69.89 percent in Karanahalli local to 71.33 percent in S34. The moisture content in fresh leaf was more during spring (72.95% general mean) than autumn (70.83%). Moisture retention capacity (%) after six hours was the (93.09%) in V1, being significantly more than the remaining genotypes where the values ranged from 87.34 percent in S13 to 75.15 percent in Mysore local. During autumn crops, it was maximum (93.85%) in V1 being significant.

Moisture retention capacity after 12 hours again it was maximum in V1 in both the seasons, (75.34.85% and 93.85.00% during spring and autumn respectively). The values were significant over the rest of the genotypes. The least value in this regard was recorded in Mysore local (69.66%) during autumn and Karanahalli local (69.89%) during autumn.

The observations recorded in respect of various foliar anatomical features are furnished in Table-

The thickness of upper cuticle was maximum 10.38 μ m in V1 being significantly more than the other genotypes; whereas, the thickness was least (3.13 μ m) in Mysore local. In the remaining genotypes, it ranged from 4.14 μ m in S34 to

6.88 um in S146. The thickness of lower cuticle, however, was maximum (4.98 um) in M5 being significantly higher than the other genotypes except V1 (4.19 um). The thickness was least (1.13 um) in Karanahalli local. In Mysore local the thickness was (1.48 um).

The upper epidermis in all the genotypes is thicker than the lower epidermis. The upper epidermis is thickest (23.74 um) in V1 being statistically at par with that in S146 (22.68 um) and significant over the rest of the genotypes. The thickness is least (15.02 um) in Mysore local. The lower epidermis on the other hand is thickest (12.32 um) in S13 being statistically at par with that of S146 (11.75 um) and significant over the remaining genotypes. The thickness of lower epidermis is least (7.38 um) in V1.

The palisade thickness is maximum (73.38 um) in Mysore local which is significantly higher than the rest of the genotypes. The

thickness is least (56.55 um) in S146. The spongy tissue thickness is highest (96.29 um) in Karanahalli local being significantly higher than the remaining genotypes. The thickness is least (42.10 %) in M5.

Palisade proportion was maximum (39.89%) in Mysore local, closely followed by M5 (39.70%), whereas it was the least in S13 with 31.60 percent palisade proportion. In the remaining genotypes it ranged from 32.26 percent in S146 to 36.27 percent in Karanahalli. Spongy proportion, on the other hand was maximum (48.16%) in S34, whereas it was the least in M5 with only 28.48 percent Spongy proportion. In the remaining genotypes it ranged from percent in Rokokuyaso to 40.87 percent in V1.

The total leaf thickness is maximum (198.53 um) in S34 being statistically at par with that of Goshoerami (193.95 um) and significant over the rest of the genotypes. The leaf thickness is least in M5 (145.36 um).

Table.1 Leaf Moisture Status of Mulberry Varieties as Influenced by Foliar Spray of Paras

Table-1 Leaf Moisture Status of mulberry varieties as influenced by foliar spray of Paras									
genotype	Moisture contents %			Moisture retention capacity after 6 hours (%)			Moisture retention capacity after 12 hours (%)		
	Spring	Autumn	Mean	Spring	Autumn	Mean	Spring	Autumn	Mean
V1	75.34	74.55	74.94	93.09	91.33	92.21	93.85	89.22	91.53
S13	71.78	70.78	71.78	87.33	87.34	87.33	80.38	76.22	78.3
S146	72.33	70.67	71.50	86.47	84.78	85.63	77.74	74.11	75.93
S34	72.46	71.33	71.95	86.39	84.56	85.48	77.34	73.33	74.84
M5	72.11	70.44	70.78	78.31	80.67	79.49	70.4	76.78	73.59
Karanahalli local	71.33	69.89	71.11	79.62	82.56	81.09	68.48	70.56	69.52
Mysore local,	71.66	69.66	70.66	75.15	79.22	77.19	70.66	70.45	70.56
G-Mean	73.95	70.83		83.77	84.35	76.95	74.35		
C.D.(5%)	1.83	1.12	3.74	3.54		2.66	1.32		

Table.2 Leaf Anatomical Features in Mulberry Varieties Growing as Plants as Influenced by Foliar Spray of Paras

Genotype	Upper Cuticle thickness (um)	Lower cuticle thickness (um)	Upper epidermal thickness (um)	Lower epidermal thickness (um)	Palisade thickness (um)	Palisade proportion (%)	Spongy thickness (um)	Spongy Proportion (%)	Stomatal Size	Stomatal frequency (mm ²)
V1	10.38	4.19	22.38	6.38	64.35	32.66	80.74	40.88	19.78 x 10.83	697.25
S13	6.7	3.34	22.18	12.32	56.66	30.6	74.97	40.63	21.00 x 11.04	616.11
S146	6.98	3.18	23.74	12.75	56.55	31.26	89.38	38.51	18.78 x 10.73	430.14
S34	4.14	1.13	18.6	6.8	64.98	31.07	96.29	49.16	20.87 x 8.77	608.21
M5	4.4	3.53	19.16	9.52	58.55	39.7	42.1	28.48	23.11 x 9.98	449.44
Karanahalli local	4.92	4.98	21.88	6.55	61.18	36.27	60.17	35.69	19.04 x 11.077	709.66
	3.13	1.48	15.02	8.83	72.38	39.89	69.54	38.26	27.04 x 13.94	695.05
Mysore local,	5.81	3.18	20.47	8.88	62.09	34.63	70.46	38.66		600.84
G-Mean	0.17	0.8	0.59	0.09	1.92	1.32	2.75	2.07		44.22

The stomata were present in the lower surface of the leaf and in no case stomata were observed on the upper surface. The Stomatal frequency was maximum (709.66 per mm²) in Karanahalli local being non significant over that of V1 and Mysore local and significantly higher than the other four genotypes. The frequency was least (449.44 per mm²) in M5. The Stomatal size was maximum (27.04 um x 12.94 um) in Mysore local . In the remaining, it was 19.78 umx 9.83 um in V1 21.0 umx 10.04 um in white and 19.04 x 10.77 in Karanahalli local.

Due to foliar application of commercial fertilizers Paras in mulberry leaves moisture content and its retention for longer periods play a very important role in improving the palatability of these leaves to silkworm. Many workers have highlighted the favorable effect of high moisture content in different varieties of mulberry leaf on palatability and digestibility by silkworm (Waldbauer 1968; Yokoyama 1975 and Paul *et al.*, 1992). All the genotypes under study during spring possessed more than 70 percent moisture content which is regarded to be optimum for silkworm rearing (Jolly and Dandin, 1986).

However, two genotypes namely Karnahalli local and mysore local possessed less than 70 percent during autumn crop. Further, the leaf moisture content in general was two percent more during spring than autumn which may be attributed to the favorable climatic conditions especially rainfall during spring in this region. The resultsof the present investigations are in conformity with the findings of Fotedar and Dandin (1997), who have tested the leaf moisture (with out foliar spray) status of some tropical and temperate genotypes maintained as dwarf under irrigated conditions.

The two type of plantation has enabled the genotypes to possess fairly good moisture

content in their leaf even under rainfed conditions, which can be attributed to the extensive root proliferation in tree type of plantation. Moisture retention capacity, under foliar application of Paras yet another very important parameter deciding the keeping quality of leaf, also plays very important role as the leaves are transported over long distances and stored for at least 8-10 hours before feeding to the Silkworm worms.

Besides, the determination of moisture retention capacity in the excised leaves has revealed promise for differentiating drought resistance of mulberry genotypes (Susheelamma *et al.* 1992). Leaves should retain sufficient moisture longer, without withering as many concomitant chemical changes occur on wilting during storage (Friend, 1958). The genotypes showed more than 80% moisture retention capacity after 6 hours and more than 74% moisture retention capacity after 12 hours of harvest. The moisture retention capacity and initial moisture content in the leaf, in general, did not follow the same trend among the genotypes studied. The moisture retention capacity after 6 hours and 12 hours in both the seasons was significantly higher in V1 which may be due to more thickness of upper cuticle cum epidermis, stomata of smaller dimensions and more thickness of leaf and probably not to the initial moisture content in the leaf as proposed by Khan (2006).

Kurtz (1950) found that the formation of wax, which has been considered to play an important role in the water economy of plants, was proportional to the thickness of the cuticle. The fairly good moisture content in the fresh leaf and its retention for fairly longer periods in V1 makes this genotype an answer to solve the shortage of quality leaf in the region.

References

- Ahsan, M.M., Dhar, K.L. and Fotadar, R.K. (1990) Package of practices of mulberry cultivation under temperate conditions. *Indian Silk*, 29(2):7-12.
- Clarke, J.M. and Mccaig, T.N. (1982) Evaluation of techniques for screening for drought resistance in wheat. *Crop science*, 22:503-506.
- Dwivedi, J.N and Singh, R.B. (1990) *Essentials of plant techniques*, Tub. Scientific Publishers. Ratanada Roac LP.O.No.91, Jodhpur-342001 I, India.
- Fotadar, R.K. and Dandin, S.B. (1997) Chemical composition and feeding studies of different mulberry genotypes under temperate conditions. *Indian Journal of Sericulture*, 36(1):22-26.
- Friend, W.G. (1958) Nutritional requirements of polyphagous insects. *annu. Rev. entomol.* 3:57-73.
- Ghouse, A.K.M. and Yunus, M (1972) Preparation of epidermal peels from leaves of Gymnosperms by treatment with hot, 60% HNO₃. *Stain Technology*, 47:322-324.
- Ito, T. (1963) Nutrition in Silkworm. *Indian Journal of Sericulture*, 1:15-17.
- Hamamura, Y., Nayashiya, K., Naito, K., Matsura, K. and Nishida, J. (1962) Food selection by silkworm larvae-Nature, 183:1746-1747.
- Jolly, M.S. and Dandin, S.B. (1986) Collection, conservation and evaluation of mulberry (*Morus spp.*) germplasm Central Sericultural Research and Training Institute Mysore, India, 44.
- Khan I.L. (2006) Evaluation of some promising mulberry (*Morus spp.*) genotypes through chemo and bioassay. *M.Sc. thesis submitted to Sheri-e-Kashmir University of Agricultural Sciences and technology (Kashmir), Srinagar.*
- Khasim, S.M. (2002) *Botanical micro-techniques; Principles and practices*. Capital Publishing Company, New Delhi, P-27.
- Kurtz, E.B. (1950) The relation of the characteristics and yield of wax to plant age. *Plant Physiology*, 25:269-278.
- Legacy, J.M. (1958) Recent advances in silkworm nutrition. *Jinn. Rev. Ent.* 3:75-86.
- Mandal, L.N. and Krishnaswami, S. (1965) Changes in the Leaf Moisture Status of Some Mulberry Genotypes. nutritive value of mulberry leaves in storage after harvest. Paper presented in the world congress on silk production Bairut.
- Ninge Gowda, K.N. and Sudhakar (2002) Studies on the leaf moisture of some exotic mulberry varieties. *Advances in Sericulture Research* (Proceedings of the National Conference on Strategies for Sericulture Research and Development, 16-18, November, 2000.
- Paul, B.C., Subarao, G. and Deb, D.C. (1992) Impact of dietary moisture on nutritional indices and growth of *Bombyx mori* L. and concomitant larval duration. *J. Insect Physiology*. 38(3):229-235.
- Pohl, R.W. (1967) Controlled maceration of grass leaves in 40-80% Nitric acid for Preparation of epidermis for slides. *Stain technology*, 42:195-197.
- Singh R.K. and Choudhary, B.D. (1977) *Biometrical Methods in Quantitative Genetic Analysis*, Kalyani Publishers, New Delhi, India, pp.53-59.
- Susheelamma, B.N., Kumar, J.S., Sikdar, A.K., Dandin, S.B., Jolly, M.S. and Sengupta, K. (1992) Exploitation of promising mulberry genotypes for yield potentiality. *Serico/ ogza*, 32(2) :295-300.
- Tiwari, S.P., Sukumaran, N.P. and Khushu, C.L. (1986) Patho-coefficient analysis of leaf anatomical characters affecting frost injury in potato. *Jl. Breed.* 97:272-274.
- Waldbauer, G.P. (1968) The consumption and utilization of food by insects. *Advanced Insect Physiology*, 5:229-288.
- Yokoyama, T. (1975) *Text Book of Tropical Sericulture*. Japan Overseas Cooperation volunteers, Tokyo, 444-537.