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Research Article

Assessment of Epicuticular Wax and Physiological Traits in Mulberry under Limited Input Conditions

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Abstract

Mulberry (*Morus* spp.) leaf yield and quality is greatly influenced by various abiotic stresses. Among these, drought/moisture stress is the major limiting factor in mulberry for quality leaf production. Under water limited conditions, different physio-biochemical processes in the plants are altered and which inhibits the optimal growth and yield production in most of the crop plants. The biochemical constituents (proteins, carbohydrates, vitamins, *etc.*) and leaf moisture content are the primary components of the leaf quality parameters. Leaf moisture and leaf moisture retention capacity are the two key factors contributing for the palatability and digestion of leaves by silkworms, which in turn support the healthy growth of the silkworms. Epicuticular wax on the leaf surface significantly contributes to the prevention of post-harvest water loss in mulberry leaves by increasing the moisture retention capacity (MRC). Hence, the present study focuses on the physiological evaluation of 20 germplasm accessions and 2 check varieties in field for epicuticular wax, moisture retention capacity (MRC) and chlorophylls under limited input conditions of irrigation (60% Field Capacity: FC) and fertilizers (60% RDF: Recommended Dose of Fertilizers) to select physiological adaptive traits and tolerant genotypes for sub-optimal conditions. Among the genotypes, MRC ranged from 50.63% to 76.33% and epicuticular wax varied from 2.13 µg cm^{-2} to 11.46 µg cm^{-2}. Present study also indicated the high moisture retention capacity and epicuticular wax content in MI-0108, MI-0046, MI-0168, MI-0736, MI-0128 and MI-0577.

Keywords: Epicuticular wax, Limited input, Moisture retention capacity, Mulberry, Physiological efficiency

Introduction

Mulberry (*Morus* spp.) is an economically important crop plant and its foliage is the sole food source for silkworm, *Bombyx mori* L. Leaf quality plays a key role in determining the quality cocoon production (Kumar *et al.*, 2022). Abiotic factors such as temperature, soil pH, elevated CO_2, drought, water logging *etc.* leads to the significant reduction in leaf yield and quality in mulberry (Suresh *et al.*, 2026). Moisture content in mulberry leaves is very much important for enhancing nutrition levels, which improves the palatability and digestion of leaves by silkworms, as well as proper silkworm growth and development and cocoon quality (Koul *et al.*, 1996; Murthy *et al.*, 2013). It is a genetic character that is influenced by available soil moisture and root proliferation nature of mulberry variety (Sahu and Yadav, 1997). Post harvest water loss in mulberry leaves is an important aspect in silkworm rearing due to the time gap observed between the time of leaf harvest and silkworm feeding and it will negatively influence the silkworm growth (Mamrutha *et al.*, 2010). Retention of moisture content in the harvested leaves help in maintaining leaf quality in the detached leaves. The cuticle is a lipophilic layer, comprising cutin and cuticular waxes and functioning as a physical barrier between the plant surface and its external environment and which reduces the non-stomatal water loss (Kerstiens, 1996; Dhanyalakshmi *et al.*, 2019). Leaf surface waxes are

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compounds or mixtures of organic substances which are insoluble in water and it forms a hard glossy film on plant surface. These waxes are synthesized in epidermis and secreted into the cuticle and called as epicuticular wax (Koch and Barthlott, 2006). Higher chlorophyll content in leaves even during the moisture and drought conditions is a stress tolerance response and considered as an indication of plant's relative physiological stability and higher photosynthetic rate (Pallavolu *et al.*, 2023).

Although several studies have reported the importance of leaf moisture, epicuticular wax and chlorophyll content individually in relation to drought tolerance in mulberry, comparative evaluation of diverse mulberry germplasm under simultaneous reductions in irrigation and fertilizer inputs under field conditions remains limited. Identification of physiological traits associated with adaptation to such limited-input environments is therefore important for developing resilient mulberry genotypes suitable for sustainable sericulture. Therefore, the present study focused on the physiological evaluation of 20 (twenty) diverse mulberry germplasm accessions and two check varieties (V1 and AGB8) under 60% Field Capacity (FC) irrigation and 60% Recommended Dose of Fertilizers (RDF) to identify physiological adaptive traits and promising genotypes suitable for limited-input cultivation.

Materials and Methods

The field experiment was conducted at the Mulberry Physiology field, Central Sericultural Research and Training Institute (CSRTI), Central Silk Board, Srirampura, Mysuru. The study location is geographically positioned at Latitude 12.25° N and Longitude 76.62° E. The local climate is tropical, characterized by an average annual rainfall of 710 mm and ambient temperatures ranging from a winter minimum of 18 °C to a summer maximum of 36 °C. The study was carried out during March-May 2025, representing a standard crop cycle of 90 days after pruning. The experiment was laid out in a Randomized Block Design with three biological replications. Standard agronomic practices were followed uniformly throughout the experimental period to minimize environmental variation among treatments.

Plant Material

Diverse set of 20 mulberry germplasm accessions (MI-0168, ME-0071, MI-0520, MI-0290, MI-0268, MI-0494, MI-0736, MI-0128, MI-0108, MI-0046, MI-0027, MI-0753, MI-0019, MI-0226, MI-0718, MI-0504, MI-0285, MI-0577, MI-0006 and MI-0028) and 2 check varieties (V1 and AGB8) were selected for physiological evaluations under limited irrigation (60% Field Capacity) and fertilizer (60% Recommended Dose of Fertilizers) conditions.

Mulberry experimental field established in RBD (3 replications) with 20 germplasm accessions and 2 check varieties (V1 and AGB8) were maintained under 60% Field Capacity (FC) irrigation and 60% Recommended Dose of chemical fertilizers (210:84:84 kg NPK ha^{-1} year^{-1} in 5 splits) and Farm Yard Manure (FYM) (12 MT FYM ha^{-1}year^{-1} in two splits) at the experimental plot at CSB-CSRTI, Mysore. Intercultural operations were carried out in the experimental plot on a regular basis during the crop period and plant protection measures were undertaken uniformly across all treatments. Soil moisture was monitored regularly using soil moisture tensiometers installed in the field at depths of 15 cm, 30 cm and 45 cm, representing the active root zone of mulberry plants. Tensiometer readings were recorded on a daily basis to assess soil moisture status. Soil water tension values obtained from the tensiometers were used as an indicator of soil moisture availability. Fresh leaves (3 samples replication^{-1}) were harvested from all these genotypes during 45^{th} to 55^{th} day after pruning and the data represented in the present study are the average of one crop data.

Figure 1: Recording of chlorophylls using SPAD meter

Leaf Moisture Content

The fresh leaf samples were harvested from the field (20-25 leaves plant^{-1}) and fresh weight was recorded separately for each 20-25 leaf sample. Leaves were dried at room temperature for 24 hours followed by oven drying at 80 °C for 48 hours and immediately dry leaf weight was recorded separately for each sample. Leaf moisture content was determined by finding the difference between fresh leaf weight and dry leaf weight.

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

Moisture Retention Capacity (MRC)

Moisture Retention Capacity (MRC) of freshly harvested leaves was calculated by recording of the fresh weight,

weight after 6 hours and dry weight of leaves.

$$\text{Moisture Loss (\%)} = \frac{\text{Fresh Weight - Fresh weight after 6 hours}}{\text{Fresh weight}} \times 100$$

Moisture Retention Capacity (MRC) (%) = 100 - Moisture Loss (%)

Chlorophyll

Relative chlorophyll content in fresh leaves were recorded by a non-destructive method using SPAD (Soil Plant Analysis Development) meter. It also indicates the greenness of the leaves by measuring the difference in light absorption, as chlorophyll absorbs only red light but not infrared (Figure 1).

Epicuticular Wax

Epicuticular wax can be extracted from the freshly harvested leaves with redistilled chloroform and quantified using a calorimetric method (Ebercon *et al.*, 1977). To the extract, 5 mL wax reagent was added and heated for 30 minutes. After cooling, deionised water (12 mL) was added and the OD was recorded at 590 nm (Figure 2). This method is based on the colour change produced by the reaction of wax with acidic $K_2Cr_2O_7$. Carnauba wax was used as a standard for epicuticular wax quantification.

Statistical Analysis

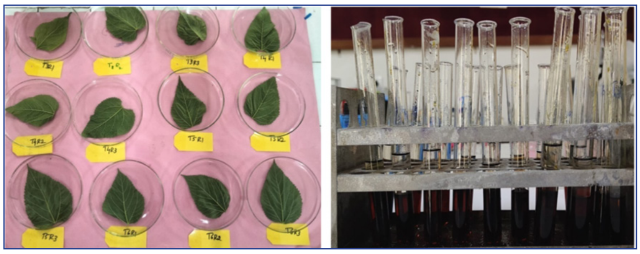

Figure 2: Extraction and estimation of epicuticular wax in freshly harvested leaves of different mulberry genotypes

Using SPSS (version 16.0; Chicago, IL, USA), one-way ANOVA (analysis of variance) and the Duncan multiple range test were performed at a significance level of $p<0.05$. Data obtained from three biological replications were analyzed using one-way analysis of variance (ANOVA) and treatment means were separated using Duncan's Multiple Range Test (DMRT) at $p<0.05$. Results are presented as mean ± standard error.

Results and Discussion

Data on leaf moisture content among 20 genotypes showed variation and moisture percentage ranged from 67.90% to 74.67%. The genotypes, MI-0028 (74.28%), MI-0168 (73.52%), MI-0128 (72.74%) showed higher moisture content among all 20 test genotypes. The check variety AGB8 and V1 indicated 74.67% and 72.12%. Low moisture content was recorded in MI-0736 (67.90%) and MI-0019 (68.85%) (Figure 3). Under limited irrigated conditions, the genotype MI-0028 (74.28%) showed relatively higher leaf moisture percentage. The difference in moisture percentage, moisture loss and consequent withering of leaves may vary with genotypes and environmental conditions in mulberry (Vijayan *et al.*, 1997; Jayaramaiah *et al.*, 2025).

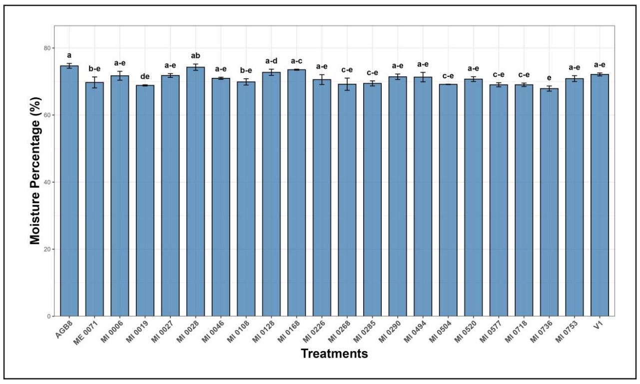

Figure 3: Variation in leaf moisture content among different mulberry genotypes [Values represent mean ± SE (n = 3)]

Moisture retention capacity (MRC) was recorded in all 20 genotypes and the data indicated the MRC ranged from 50.63% to 76.33%. Higher MRC recorded in MI-0108 (76.33%), MI-0046 (74.90%), MI-0504 (69.97%), MI-0168 (68.98%), ME-0071 (68.70%), MI-0006 (68.04%), MI-0226 (67.52%) and MI-0736 (67.19%). Low MRC observed in MI-0019 (50.63%) and MI-0285 (55.34%). In check varieties MRC was 69.57% (AGB8) and 67.98% (V1) (Figure 4). The observed variation among genotypes indicates substantial genetic diversity for post-harvest moisture conservation, suggesting that moisture retention capacity may serve as a useful physiological indicator for selecting drought-tolerant mulberry genotypes under limited-input cultivation.

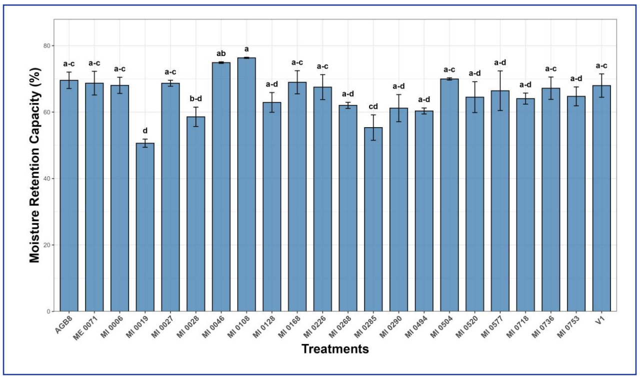

Figure 4: Variation in leaf moisture retention capacity (MRC) among different mulberry genotypes [Values represent mean ± SE (n = 3)]

Hence, epicuticular wax in leaf surface also extracted and quantified in selected genotypes and the result indicated the variation in the wax content among the genotypes. It ranged from 2.13 µg cm^{-2} to 11.46 µg cm^{-2}. Higher wax content was observed in MI-0736, MI-0108, MI-0577, MI-0128, MI-0168 and MI-0046. Least content was observed in MI-0028 as per the preliminary data (Figure 5). The comparatively higher epicuticular wax deposition observed in selected genotypes may have contributed to reduced cuticular transpiration, thereby improving post-harvest leaf water conservation under moisture-limited conditions. Epicuticular wax acts as a barrier for leaf cuticular transpiration and hence reduces the moisture loss in leaves (Zhang *et al.*, 2021). Genotypes with high epicuticular wax content showed high moisture retention capacity and leaf moisture content during the postharvest phase also (Mamrutha *et al.*, 2010; Gayathri and Babulal, 2024). Hence, mulberry leaves with high epicuticular wax and high MRC is a desirable trait in mulberry which will improve the leaf quality parameters and in turn will enhance the silkworm growth and development.

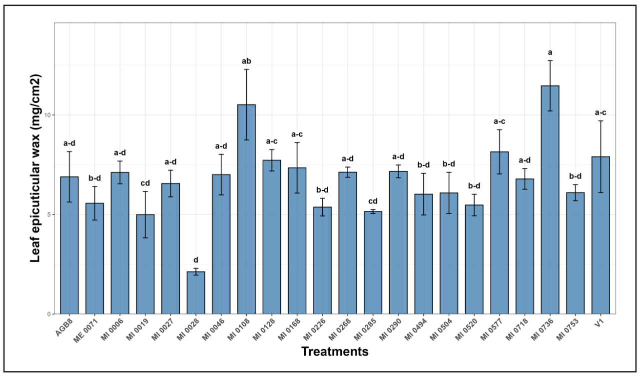

Figure 5: Variation in epicuticular wax content among different mulberry genotypes [Values represent mean ± SE (n = 3)]

The higher leaf moisture retention capacity was related with the higher epicuticular wax content. The stomatal aperture on the abaxial surface of cultivars with high wax amount was smaller than that of cultivars with low wax amount (Askanbayeva *et al.*, 2024). Total wax content was shown to be negatively correlated with net photosynthetic rate (P_N), transpiration rate (E) and stomatal conductance (gs) while positively correlated with moisture retention capacity (MRC) (Meena and Nataraja, 2022). Both cuticular wax and stomatal factor may be involved in regulating water loss in mulberry leaves in field conditions, as per the study of Ni *et al.* (2015). Induction of cuticle lipids was linked with reduced cuticle permeability and might be important for plant acclimation to subsequent water-limited conditions (Kosma *et al.*, 2009).

SPAD value ranged from 30.96 to 42.22 among the genotypes and higher SPAD value recorded in MI-0027 (42.22), MI-0226 (41.51), ME-0071 (40.46), MI-0108 (40.22) and MI-290 (38.46). Least SPAD value observed in MI-0285 (30.96) and whereas, in check variety AGB8 and V1 it was 32.50 and 35.08 respectively (Table 1). Higher SPAD value indicated the higher chlorophyll content and physiological efficiency under limited input conditions (Pallavolu *et al.*, 2023). Maintenance of relatively higher chlorophyll content under reduced irrigation and fertilizer supply suggests improved photosynthetic stability and enhanced tolerance to environmental stress in these genotypes. Moisture stress inhibits the chlorophyll synthesis and thereby decreasing the net photosynthetic rate and leaf biomass (Song *et al.*, 2026). Physio-biochemical markers were identified under moisture stress conditions in mulberry (Gayathri *et al.*, 2024) and further screening of the selected genotypes can be done using the various biochemical and morpho-physiological parameters.

Table 1: Variation in SPAD reading among different genotypes

Genotypes	SPAD value
AGB8	32.50±1.36 (f-h)
ME 0071	40.46±0.43 (a-c)
MI 0006	34.81±0.97 (d-h)
MI 0019	36.10±0.63 (c-g)
MI 0027	42.22±0.63 (a)
MI 0028	30.30±1.36 (h)
MI 0046	32.49±0.39 (f-h)
MI 0108	40.22±0.36 (a-c)
MI 0128	36.71±0.17 (b-f)
MI 0168	36.34±0.84 (c-f)
MI 0226	41.51±1.00 (ab)
MI 0268	36.54±0.97 (c-f)
MI 0285	30.96±0.58 (h)
MI 0290	38.46±1.16 (a-d)
MI 0494	34.80±1.10 (d-h)
MI 0504	33.11±0.83 (e-h)
MI 0520	34.21±0.86 (d-h)
MI 0577	31.27±1.28 (gh)
MI 0718	38.28±0.80 (a-d)
MI 0736	33.66±0.65 (d-h)
MI 0753	37.60±0.78 (a-e)
V1	35.08±0.81 (d-h)
SEm ±	0.33
CD (5%)	0.95
CV (%)	4.34

Conclusion

Epicuticular wax and leaf moisture retention capacity are the two significant physiological traits for assessing the wild mulberry germplasm and choosing the genotypes that are highly tolerant to drought/moisture stress. Mulberry leaves' nutritional and biochemical components are the primary determinants of leaf quality. Mulberry leaf quality will be affected by differences in the amount of crude proteins, soluble sugars and vitamins in the leaves. Furthermore, silkworms' ability to digest and consume food is directly impacted by leaf moisture content, which is regarded as a crucial leaf quality parameter. Therefore, a key factor in silkworm growth and cocoon production is the moisture content of the leaves. A key element influencing mulberry leaf quality is the leaves' ability to retain moisture after harvest. Under various stress conditions, such as salinity and water deficit, plants generally showed a significant increase in the amount of cuticular wax per unit area of leaves to prevent the excess water. Present study also indicated the high moisture retention and epicuticular wax content in the genotypes such as MI-0108, MI-0046, MI-0168, MI-0736, MI-0128 and MI-0577. Higher SPAD value is recorded in few genotypes such as MI-0027, MI-0226, ME-0071 and MI-0108. The identified physiological traits may serve as useful selection criteria in future mulberry breeding programmes aimed at developing cultivars adapted to water-limited and low-input production systems. The field evaluation is presently going on for yield estimation and identifying the morpho-physiological adaptive traits and better performing genotypes under limited irrigation (60% FC) and fertilizer (60% RDF) conditions for sustainable crop production.

Ethical Statement

The authors declare that no generative artificial intelligence tools were used in drafting or preparing this manuscript. The manuscript was written, interpreted and revised solely by the authors, who take full responsibility for its content.

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References

Askanbayeva, B., Janová, J., Kubásek, J., Zeisler-Diehl, V.V., Schreiber, L., Muir, C.D., Šantrůček, J., 2024. Amphistomy: Stomata patterning inferred from ^{13}C content and leaf-side-specific deposition of epicuticular wax. *Annals of Botany* 134(3), 437-454. DOI: https://doi.org/10.1093/aob/mcae082.

Dhanyalakshmi, K.H., Soolanayakanahally, R.Y., Rahman, T., Tanino, K.K., Nataraja, K.N., 2019. Leaf Cuticular Wax, a trait for multiple stress resistance in crop plants. In: *Abiotic and Biotic Stress in Plants*. (Ed.) de Oliveira, A.B. IntechOpen. DOI: https://doi.org/10.5772/intechopen.84565.

Ebercon, A., Blum, A., Jordan, W.R., 1977. A rapid colorimetric method for epicuticular wax content of sorghum leaves. *Crop Science* 17(1), 179-180. DOI: https://doi.org/10.2135/cropsci1977.0011183X001700010047x.

Gayathri, T., Babulal., 2024. Epicuticular wax and post-harvest water loss in mulberry. Shahatoot ki pattiyon mein nami ka samvardhan. *ICAR-Phal Phool* September-October, 25-26.

Gayathri, T., Rajashekar, K., Doss, S.G., Sarkar, T., Babulal., 2024. Evaluation of mulberry genotypes under moisture stress for the identification of physio-biochemical markers of stress tolerance. *Journal of Plant Growth Regulation* 43, 1999-2013. DOI: https://doi.org/10.1007/s00344-024-11241-x.

Jayaramaiah, V.C., Siddu, C.B., Kirankumar, R., Ramesh, S., 2025. Phenotypic characterization and identification of elite mulberry (*Morus* spp.) cultivars for desirable leaf quality. *Mysore Journal of Agricultural Sciences* 59(3), 103-113.

Kerstiens, G., 1996. Cuticular water permeability and its physiological significance. *Journal of Experimental Botany* 47(12), 1813-1832. DOI: https://doi.org/10.1093/jxb/47.12.1813.

Koch, K., Barthlott, W., 2006. Plant epicuticular waxes: Chemistry, form, self-assembly and function. *Natural Product Communications* 1(11), 1067-1072. DOI: https://doi.org/10.1177/1934578X0600101123.

Kosma, D.K., Bourdenx, B., Bernard, A., Parsons, E.P., Lu, S., Joubes, J., Jenks, M.A., 2009. The impact of water deficiency on leaf cuticle lipids of Arabidopsis. *Plant Physiology* 151(4), 1918-1929. DOI: https://doi.org/10.1104/pp.109.141911.

Koul, A., Sing, D., Gupta, S.P, 1996. Seasonal fluctuations in leaf moisture characters and dry matter in mulberry in subtropics. *Journal of Sericultural Science* 4, 23-27.

Kumar, S.R., Swathiga, G., Ramamoorthy, R., Vijayalakshmi, D., 2022. Effects of high temperature stress on physiological and yield parameters of mulberry varieties. *Research Biotica* 4(2), 58-61. DOI: https://doi.org/10.54083/ResBio/4.2.2022/58-61.

Mamrutha, H.M., Mogili, T., Lakshmi, K.J., Rama, N., Kosma, D., Kumar, M.U., Jenks, M.A., Nataraja, K.N., 2010. Leaf cuticular wax amount and crystal morphology regulate post-harvest water loss in mulberry (*Morus* species). *Plant Physiology and Biochemistry* 48(8), 690-696. DOI: https://doi.org/10.1016/j.plaphy.2010.04.007.

Meena, H.S., Nataraja, K.N., 2022. An approach to improve the post-harvest leaf quality in Indian mulberry (*Morus alba* L.). *Mysore Journal of Agricultural Sciences* 56(4), 184-191.

Murthy, V.N.Y., Ramesh, H.L., Lokesh, G., Munirajappa., Yadav, B.R.D., 2013. Leaf quality evaluation of ten mulberry (*Morus*) germplasm varieties through phytochemical analysis. *International Journal of Pharmaceutical Sciences Review and Research* 21(1), 182-189.

Ni, Y., Sun, Z., Huang, X., Huang, C., Guo, Y., 2015. Variations of cuticular wax in mulberry trees and their effects on gas exchange and post-harvest water loss. *Acta Physiologiae Plantarum* 37(6), 112. DOI: https://doi.org/10.1007/s11738-015-1856-1.

Pallavolu, L.A., Pasala, R., Kulasekaran, R., Pandey, B.B., Virupaksham, U., Perika, S., 2023. Analysing the SPAD dynamics of water-stressed vs. well-watered sesame (*Sesamum indicum* L.) accessions and establishing their relationship with seed yield. *PeerJ* 11, e14711. DOI: https://doi.org/10.7717/peerj.14711.

Sahu, P.K., Yadav, B.R.D., 1997. Genotypic differences in moisture content and moisture-retention capacity of leaf in mulberry (*Morus* species). *The Indian Journal of Agricultural Sciences* 67(11), 536-538.

Song, F., Yang, Q., Huang, J., Guo, Z., Li, Y., Deng, W., 2026. Plant drought stress: Physiological, biochemical and molecular mechanisms. *Plant Stress* 19, 101153. DOI: https://doi.org/10.1016/j.stress.2025.101153.

Suresh, G., Saha, D., Kumari, A., Palsaniya, M., Garhwal, P.C., Singh, V.P., Dadrwal, B.K., 2026. Abiotic stress-induced biochemical alterations in mulberry (*Morus* spp.) and their implications for silkworm nutrition. *Plant Cell Biotechnology and Molecular Biology* 27(3-4), 109-123. DOI: https://doi.org/10.56557/pcbmb/2026/v27i3-410369.

Vijayan, K., Reghunath, M.K., Das, K.K., Tikader, A., Chakaraborthi, S.P., Roy, B.N., Qadri, S.M.H., 1997. Studies on leaf moisture of mulberry germplasm varieties. *Indian Journal of Sericulture* 36(2), 155-157.

Zhang, Y., Du, Z., Han, Y., Chen, X., Kong, X., Sun, W., Chen, C., Chen, M., 2021. Plasticity of the cuticular transpiration barrier in response to water shortage and resupply in *Camellia sinensis*: A role of cuticular waxes. *Frontiers in Plant Science* 11, 600069. DOI: https://doi.org/10.3389/fpls.2020.600069.