Studies on Physiological, Anatomical and Biochemical aspects of some mulberry genotypes during different seasons under Kashmir climatic conditions

Shaista Mehraj
(2019-885-D)

Division of Basic Sciences and Humanities

College of Temperate Sericulture

Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir

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Shaista Mehraj
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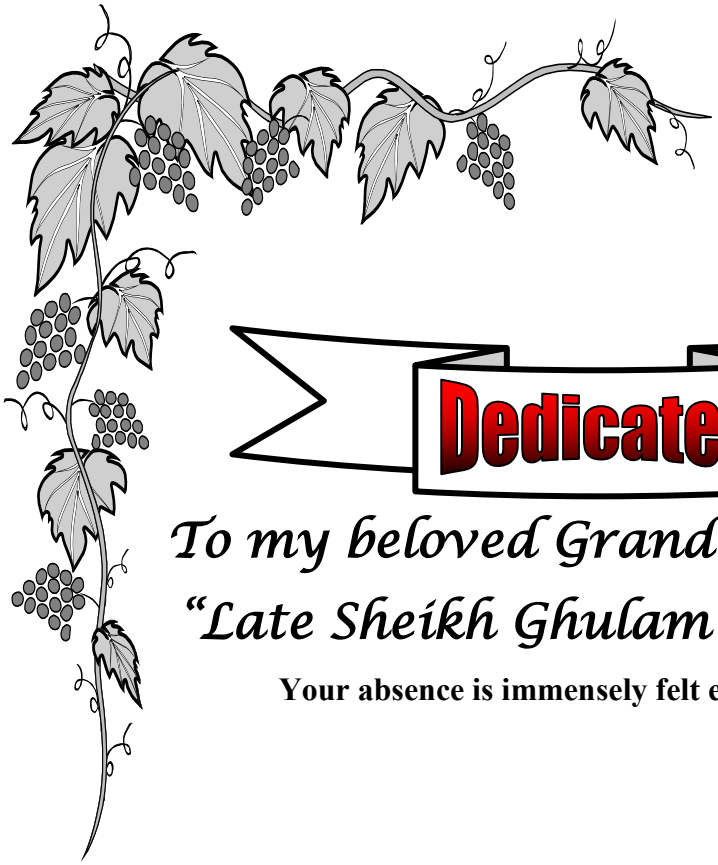

Dedicated

To my beloved Grand father

"Late Sheikh Ghulam Nabi"

Your absence is immensely felt every moment…

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Basic Sciences and Humanities,
College of Temperate Sericulture, Mirgund, Baramulla

Certificate – I

This is to certify that the thesis entitled **"Studies on Physiological, Anatomical and Biochemical aspects of some mulberry genotypes during different seasons under Kashmir climatic conditions"** submitted in partial fulfilment of the requirements for the award of **Doctor of Philosophy in Sericulture**, to the College of Temperate Sericulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, is a record of bonafide research work carried out by **Ms. Shaista Mehraj (Regd. No. 2019-885-D)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that information received during the course of investigation has duly been acknowledged.

(Dr. Mushtaq Rasool Mir)
Chairman
Advisory Committee

Endorsed

Head
Division of Basic Sciences and Humanities,
College of Temperate Sericulture, Mirgund,
SKUAST-Kashmir

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Basic Sciences and Humanities,
College of Temperate Sericulture, Mirgund, Baramulla

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We, the members of the Advisory committee of **Ms. Shaista Mehraj (Regd. No. 2019-885-D)**, a candidate for the degree of **Doctor of Philosophy in Sericulture**, have gone through the manuscript of the thesis entitled, **"Studies on Physiological, Anatomical and Biochemical aspects of some mulberry genotypes during different seasons under Kashmir climatic conditions"** and recommend that it may be submitted by the student in partial fulfilment of the requirements for the award of degree.

Advisory Committee

Chairman

Dr. Mushtaq Rasool Mir
Professor and Head,
Division of Basic Sciences and Humanities, CTS, Mirgund

Members

Dr. Nisar Ahmad Ganie
Associate Professor & Head,
Division of Cocoon Crop Protection, CTS, Mirgund

Dr. Farooq Ahmad Khan
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Dr. Shoukat Ara
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Division of Environmental Sciences, FoH Shalimar
(Dean's Nominee)

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Basic Sciences and Humanities,
College of Temperate Sericulture, Mirgund, Baramulla

Certificate – III

This is to certify that the thesis entitled, **"Studies on Physiological, Anatomical and Biochemical aspects of some mulberry genotypes during different seasons under Kashmir climatic conditions"** submitted by **Ms. Shaista Mehraj (Regd. No. 2019-885-D)** to the College of Temperate Sericulture Mirgund, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy in Sericulture** was examined and approved by the Advisory Committee and external examiner on

Chairman
Advisory Committee

External Examiner
Dr. Pankaj Tewary
Director (Rtd.)
Central Sericultural Research and Training Institute, Mysore, CSB, Ministry of Textiles, Govt. of India

Prof. & Head
Division of Basic Sciences and Humanities,
College of Temperate Sericulture, Mirgund,
SKUAST-Kashmir

Associate Dean
College of Temperate Sericulture, Mirgund,
SKUAST-Kashmir

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Basic Sciences and Humanities,
College of Temperate Sericulture, Mirgund, Baramulla

Name of the student : **Shaista Mehraj**

Registration No. : 2019-885-D

Major subject : Sericulture

Minor subjects : Plant Physiology

Major advisor : **Dr. Mushtaq Rasool Mir**
Professor and Head,
Division of Basic Sciences and Humanities,
College of Temperate Sericulture, Mirgund

Title of the Thesis : **"Studies on Physiological, Anatomical and Biochemical aspects of some mulberry genotypes during different seasons under Kashmir climatic conditions"**

ABSTRACT

Mulberry (*Morus* sp.) is cultivated for its leaf which is the only food to silkworm (*Bombyx mori* L.). Qualitative as well as quantitative improvement in mulberry leaf is a pre requisite for the development of sericulture as mulberry leaf has been found to contribute the maximum (38.2%) towards the success of a cocoon crop (Miyashita *et al.*, 1986). The plant is capable of thriving under varied agro climatic conditions and has a high degree of morphological, anatomical and physiological adjustments to changes in environment. The mulberry gives two flushes of leaf, first in May-June (spring crop of silkworm rearing) and the second in August-September (Late summer/autumn crop). The two flushes of mulberry leaf produced at two different seasons experience varied conditions in terms of climate, phenology and physiology and as such are expected to differ in various quality parameters and different genotypes can behave differently in the two seasons. Ten mulberry genotypes- Goshoerami, Ichinose, KNG, Rokokoyoso, Kokuso-20, Kokuso-21, Limoncina, Ensatakasuke, Chinese white and Kairyoroso were studied for various Physio-biochemical and anatomical parameters during spring and autumn seasons and correlations among different parameters were worked out. The genotypes depicted great variation as far as the parameters are concerned. During both the seasons, Goshoerami had the highest fresh weight of hundred leaves (520.90 and 606.91g), leaf moisture content at chawkie (78.85%

&75.71%) , late age of silkworm rearing (76.61%&73.09%), moisture retention capacity after 6 (93.45 % &91.66%), and 12 hours (87.97% &81.35%) of harvest, Absolute Growth Rate (2.65, 5.98, 7.20 g/day during I, II, III interval of spring &3.28, 6.85, 9.81 g/day during I, II, III interval of autumn), Leaf Net Assimilation rate (0.9445, 0.9703, 0.8262 mg/cm^2/day during I, II, III interval of spring & 0.9741, 1.0055, 0.8473 mg/cm^2/day during I, II, III interval of autumn) single leaf area (343.65 &375.19 Cm^2) and the yield per plant (6.750&7.690Kg), KNG registered the highest nitrogen (3.92% &3.77%), phosphorus (0.331% &0.314%), potassium (1.538%& 1.517%), calcium (13021.63 &13122.52 ppm), nitrate reductase activity (14.48 &10.64 µmol/ml/hr), total soluble sugar (2.03% & 2.33%) and total soluble carbohydrate (3.95% & 3.41%) whereas Kokuso-21 recorded the highest chlorophyll "a" (2.32 &1.99 mg/g), chlorophyll "b" (1.76 & 1.29 mg/g), total chlorophyll (4.08 &3.28 mg/g), magnesium (6378.00 &6399.83 ppm) and iron (203.53 &195.82 ppm). Ensatakasuke, a fruiting genotype, recorded the highest specific leaf weight (0.789 & 0.794 mg/cm^2) and total carotenoids (562.83 &535.50 µg/g).

As far as the leaf anatomical parameters recorded during autumn are concerned, Goshoerami had the highest upper cuticular thickness (11.362 µm), palisade thickness (65.447 µm), spongy proportion (41.968%) and leaf thickness (194.858 µm). Chinese white registered the highest Stomatal length (24.135), breadth (11.781 µm) and palisade proportion (41.485%) where as Rokokoyoso registered the highest Stomatal frequency (711.061/ mm^2), stomatal index (23.572%) and lower cuticular thickness (5.978 µm).

During spring, leaf yield per plant had positive correlations with all the traits except TSLPP (r: -0.63), NSPP (r: -0.55) and SLA (r:-0.17). Likewise during autumn, it had positive correlations with all the traits except TBLPP (r: -0.08), NBPP (r: -0.47) and LNAR (r:-0.56). Leaf moisture content and moisture retention capacity showed positive correlation with most of the physiological and biochemical parameters. However, they were negatively correlated with total phenol content. Amongst the various leaf anatomical features, upper epidermal and cuticular thickness, spongy thickness and spongy proportion showed a highly positive correlation with most of the biochemical and physiological parameters. The study has generated information on the anatomical, physiological and biochemical aspects of different mulberry genotypes and their correlations and can be useful in future breeding programmes.

Key Words: Mulberry, Genotypes, Growth, Yield, correlation, Physiological, Anatomical, Biochemical

Signature of Student

Dated: _____

Signature of Major Advisor

Dated: _____

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Dated: _____

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Chapter- 1

INTRODUCTION

Mulberry (*Morus* sp.) is cultivated for its leaf which is the only food to silkworm (*Bombyx mori* L.). Qualitative as well as quantitative improvement in mulberry leaf is a pre-requisite for the development of sericulture as mulberry leaf has been found to contribute the maximum (38.2%) towards the success of a cocoon crop (Miyashita *et al.*, 1986). The plant is capable of thriving under varied agro climatic conditions. This clearly indicates that mulberry has high degree of morphological, anatomical and physiological adjustments to changes in environment. Mulberry can be trained as bush, dwarf or tree and all these types can be maintained with different cultural practices.

Science of mulberry cultivation is an applied science which involves a detailed study of morphological, anatomical, physiological and ecological features of mulberry. The study encompasses cultivation techniques suited for these features so as to produce the best mulberry leaves for the rearing of silkworms and to obtain rich harvest of such leaves from a unit cultivation area in a reasonably economic manner.

The systematic position of mulberry is as under:

Kingdom	: Plantae
Division	: Spermatophyta
Class	: Angiospermae
Sub- class	: Dicotyledoneae
Order	: Urticales
Family	: Moraceae
Genus	: Morus

The genus *Morus* contains about 64 species (Anonymous, 2022), most of them are important from sericulture point of view; others are cultivated for fruit and timber. It is a perennial, woody, deep rooted plant, either monoecious or dioecious. The plant after proper establishment within four to five years can come to full yielding capacity and continue to yield for over seventeen years without any significant deterioration in the leaf yield (Kumeresan and Prakash,2001). However, under temperate conditions, mulberry grows luxuriantly for 50-60 years. The productivity and profitability of sericulture depends mainly on maximization of leaf yield per unit area at economically viable cost.

Mulberry leaf protein is the only source for silkworm (*Bombyx mori* L.) to synthesize the commercial silk. It is impossible for the silkworm to complete the growth in the absence of mulberry. The host specificity of silkworm is not only due to the nutritional superiority of leaves but primarily due to the presence of some attractants in the leaves to which the insect gets lured. The leaves are highly nutritious with high protein, sugar, carbohydrate and mineral content. Since silkworm is monophagus and depends solely upon mulberry for nutrition, the quality of mulberry leaf has a direct bearing with its health and growth and also with the quantity and quality of silk.

In Kashmir, mulberry is mostly grown as scattered trees under rainfed conditions and hardly gets any attention as far as input application and other protective measures are concerned. The winter buds sprout during March-April and the leaf increases gradually in size till it is fed to the silkworms reared during the spring (1st week of May-15 June). The leaves for feeding the worms are collected by individual plucking during the initial stages of the worms but the leaf consumption during the fifth stage of rearing (spring crop) is so tremendous that it is not possible to go for individual leaf plucking and the leaves are harvested along with the branches. This besides preserving the leaf moisture for longer durations, helps to reduce labour. This coincides with the only annual pruning in mulberry trees. After pruning in June, the lower most buds left on the trees sprout

and the branches attain a length of about 2 meters till the end of growing season (mid October) and bear luxuriant leaves. Thus mulberry gives two flushes of leaves, first in May-June (spring crop of silkworm rearing) and the second in August-September (Late summer/autumn crop). In spite of this the silkworm rearing is conducted at farmer's level only once during May-June and farmers are reluctant to devote their land exclusively to mulberry cultivation. Even if they agree, they plant mulberry on non-productive soils as a single crop per year as it seems less economic. Multiple cropping by taking at least two cocoon crops in a year can help popularize sericulture in Kashmir but in sericulture this has not picked up because of many factors and scarcity of quality leaf in mulberry in season(s) other than spring seems to be a major factor. The growth pattern in mulberry is such that the plant though receives least attention still has a distinction of producing two flushes of leaves in a year as against only one in all the other tree species of this region.

Mulberry is a sensitive plant responding very well to the changes in edaphic and climatic factors. It shows reduced growth when moisture and plant nutrients are limiting. Leaf yield and quality in mulberry is governed by many factors like genetic, cultural, physiological and biochemical aspects of the plant. Leaf yield is contributed by the branching behaviour of the genotype concerned, length of branches, internodal distance, leaf weight and other various yield attributing characters. The photosynthetic performance of plants could be used for the prognosis of plant productivity. The ecological behaviour of the plants is determined by photosynthetic productivity, coupled with the use of photosynthetic products in growth and development. Photosynthesis and its related physio-biochemical parameters have long been considered for determining the leaf yield of a crop (Menon and Srivastava, 1984; Ghosh *et al.,* 2006).

Higher moisture content in mulberry leaf 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 its retention

capacity for longer durations in mulberry genotypes have positive influence on the growth and development of silkworm. Different genotypes are said to vary in the leaf moisture content and its retention after harvesting the leaf. Besides, environmental factors and leaf anatomical parameters like stomatal frequency, stomatal index, mesophyll tissue, cuticle thickness and leaf thickness also influence the moisture content of the leaf and its retention capacity (Mir *et al.* 2012). Moisture content and its retention in excised leaves are proposed as indices in screening mulberry for drought resistance. 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 a given water potential. This gains more importance under Kashmir climatic conditions where mulberry is mostly grown under rainfed conditions.

Evaluation of genotypes for anatomical, physiological and biochemical parameters will help in the development of cultivars for adverse environments. This will help to take up mulberry plantation in the belts, otherwise known as unfit for mulberry cultivation due to lack of irrigation facilities and water availability. Consequently, there will be an increase in mulberry wealth in the region resulting in better cocoon crop and higher silk production.

The two flushes of mulberry leaves produced at two different seasons experience varied conditions in terms of climate, phenology and physiology and as such the leaves raised are expected to differ in various quality parameters even in the same genotype and different genotypes can behave differently in the two seasons. Thus, some genotypes will be better during the spring crop (Ist flush) where as others can be better during late summer/ autumn crop (2^{nd} flush).

Lot of studies have been taken up to investigate the interrelationship of the yield components from morphological point of view (Sahu *et al.*, 1995, Mir *et al.*, 2012). But very few studies have been conducted to examine the influence of physio-biochemical parameters like absolute growth rate, net assimilation rate,

specific leaf area, specific leaf weight, leaf chlorophyll content, leaf protein and sugar content on overall leaf yield and its quality in mulberry especially under Kashmir climatic conditions. It has been reported that photosynthetic efficiency may be directly related to biomass production and therefore, photosynthesis index be included in breeding protocol (Sankar *et al.,* 1991). Chen (1992) has established the linear relationship between irradiance saturated net photosynthetic rate (P_{max}) and the nitrogen content in mulberry leaf. A primary objective of the mulberry improvement programme in Kashmir is to increase yield well matched with the quality of leaf for two cocoon crops, thus making it imperative to study the inter relationship among different physio biochemical parameters that are supposed to influence photosynthetic rate as well as biomass production. Under Kashmir climatic conditions, the identification of physiologically efficient genotypes gains paramount importance given the inputs and the available photoperiod in the region. Since no such work was carried out in the region till now, the study entitled, **"Studies on Physiological, Anatomical and Biochemical aspects of some mulberry genotypes during different seasons under Kashmir climatic conditions''** was taken up with the following objectives:

- To evaluate different mulberry genotypes for yield and yield attributing traits under temperate climatic conditions.
- To study the physiological and anatomical characteristics of different mulberry genotypes under temperate climatic conditions.
- To estimate the biochemical components of mulberry leaf of different genotypes.
- To workout the correlation between physiological, anatomical, yield and biochemical parameters in mulberry.

Chapter 2

REVIEW OF LITERATURE

The review of literature has been considered under the different heads highlighting the role of various findings and their relevance towards the present study and improvement of mulberry sericulture through selection of mulberry varieties which possess promising qualities as well as quantitative traits.

2.1 Evaluation of growth and yield

Mulberry is grown in different agro climatic regions chiefly for its foliage to rear silkworm (*Bombyx mori* L.). The leaf yield is a complex parameter which is attributed to several component factors. Dandin and Jolly (1986) grouped the economic attributes in four major categories depending on their contribution to propagation, growth, yield and quality. Accordingly four phases of evaluation *viz*: individual character assessment, yield evaluation, quality test and screening for local adaptability have been suggested. With the development of sericulture industry and the recent increase in the technical knowledge, it has become necessary to isolate or evolve mulberry varieties which give a higher leaf yield for the benefit of silkworm rearers.

Rahman and Islam (2020) evaluated twenty mulberry genotypes *viz*., BSRMS5, BSRMS16, BSRMS18, BSRMS19, BSRMS20, BSRMS24, BSRMS34, BSRMS39, BSRMS40, BSRMS45, BSRMS50, BSRMS54, BSRMS55, BSRMS56, BSRMS58, BSRMS59, BSRMS63, BSRMS64, BSRMS65, BSRMS66 for their genetic variability and correlation studies among morphological, phenotypical and yield attributing characteristics and reported that phenotypic co- efficient of variation (PVC) was higher (97.68%) than genotypic co-efficient of variation (GVC, 96.99%).Further, leaf yield showed significantly positive phenotypic and genotypic correlation with all the other growth traits *viz*., total shoot weight (0.817), length of longest shoot (0.600), total branch height

(0.596) and fresh weight of 10 leaves (0.425) except total number of branches and nodes per meter.

Tikadar and Kamble (2008) evaluated twenty five mulberry genotypes *viz*., MI-0029,MI-0080, MI-0154, MI-0252, MI-0290, MI-0296, MI-0301, MI-0308, MI-0310, MI-0312, MI-0313, MI-0324, MI-0326,MI-0346, MI-0349, MI-0369, MI-0370, MI-0376, MI-0388, MI-0400, MI-0415, MI-0416, MI-0431, MI-0437, MI-0439 for growth and yield traits and reported that leaf yield performance showed significant positive correlation with the other traits. He further observed that coefficient of variation was maximum for single leaf weight (36.32%) and minimum for leaf moisture content (2.08%).

Mir *et al.* (2012) evaluated seven mulberry genotypes *viz*: Goshoerami, Ichinose, KNG, Tr-10, Chinese white, Rokokoyoso and Chattatul Zangir growing as tree for their yield and yield attributing characters and found that leaf yield per plant (7.198 kg), fresh weight of 100 leaves (533.56 g) and leaf area (360.67 cm^2) was highest in Goshoerami during spring crop under Kashmir climatic conditions.

Tikadar and Roy (2006) undertook evaluation of mulberry germplasm based on morphological and yield attributes for selection. The results indicated a wide range of association and provided more scope to select the characters based on ranking correlation for crop improvement.

Mir *et al.* (2003) conducted evaluation of different mulberry genotypes under rainfed conditions in Kashmir. The study was conducted on dwarf type of plantation which ultimately led to the identification of some promising genotypes like Goshoerami, Togowase, Kairyoroso, Kasuga, Tomeiso, Ichinose, Kokuso-27, KNG, Rokokoyoso and Kokuso-20 for such type of plantation.

Satyanarayana and Rajahekhargouda (2002) studied the Victory –I variety of mulberry for all plant growth and yield parameters on medium black soils under Dharwad conditions and ascertained the superiority of V-I over S-36 in all growth and yield parameters.

Fotedar (2002) studied the correlation and path analysis in mulberry. The correlation and path analysis when considered together indicated that shoot length is the best criterion for assessing the leaf yield potentiality of a genotype.

Eswar Rao *et al.* (2002) conducted evaluation of tetraploid and triploid mulberry genotypes for propagation, growth and yield parameters under irrigated conditions in South India and found that higher leaf yield per plant per harvest was recorded in triploids followed by diploids.

Ananda Rao and Tikadar (2002) studied variability of growth parameters in mulberry (*Morus* sp.) germplasm. Higher mean values were recorded for leaf characters in exotic accessions, whereas, for shoot characters such values were found in indigenous accessions. Significant positive association was observed between leaf yield per plant with number of shoots, length of longest shoot, total shoot length and internodal distance.

Fotadar and Dandin (1997) conducted preliminary screening of genotypes from the gene pool under temperate conditions. Results indicated that most of the desirable traits are present in the cultivars Kokuso-27 and Kairyoroso though being poor rooting genotypes.For improving the rootability, controlled hybridization of these cultivars with Chinese white and Rokokoyoso was suggested.

Vijayan *et al.* (1998) conducted a correlation study with leaf yield and morpho-anatomy. Correlation between leaf yield and various morpho anatomical characters revealed a wide spectrum of variation in triploid varieties. Positively significant correlation were found between leaf yield and number of primary branches per plant, leaf thickness, spongy layer thickness and weight of 100 dry leaves.

Vijayan *et al.* (1997) conducted correlation studies in mulberry and reported that leaf yield in mulberry is a polygenic character influenced by several

characters and is the cumulative consequence of various physiological and biochemical processes.

Susheelamma *et al.* (1998) worked on exploitation of promising mulberry genotypes for yield potentiality. The study resulted in recommendation of Acc-203 at farmer's level in tropical conditions.

Menon and Srivastava (1984) reported that biomass production and leaf yield of different crops depend primarily on photosynthetic CO_2 assimilation.

Dandin *et al.* (1983) evaluated 161 mulberry accessions for yield components namely nature of leaf, growth rate, internodal distances, fresh weight 0f 100 leaves, percentage of consumable portion of leaf, sprouting behaviour, rooting capacity, root proliferation rate by length and weight, moisture content and moisture retaining ability. Depending upon the nature of the character suitable evaluation methods have been evolved and applied. Varieties S-1, RFS-135 and English black showed higher values for six characters.

Das and Krishnaswamy (1969) worked on estimation of components of variation of leaf yield and its related traits in mulberry. Average plant height and number of branches per plant were considered superior characters in the selection of mulberry genotypes under irrigated conditions.

2.2 Leaf moisture studies

The quality of mulberry leaf plays a vital role in silkworm rearing. Many factors like season, variety, irrigation, manure application, pruning and harvesting are considered to determine the quality of leaves. Inspite of growing the mulberry under ideal conditions, the leaves lose moisture to a considerable extent during the period the leaves are harvested and actually fed to the worms. The loss of moisture and withering of harvested leaves varies with different varieties and environmental temperatures (Leopold and Kriedlmann, 1975).The moisture content in mulberry in relation to varietal effect, foliar characteristics and its

influence on silkworm rearing has been considered by different workers from time to time and is reviewed briefly as:

Malik (2023) evaluated leaf of five mulberry genotypes *viz*., Goshoerami, KNG, Mukey, Kokuso-20 and Botatul to determine the moisture content, moisture retention capacity after 6 hours and moisture retention capacity after 12 hours during autumn season and reported that the highest moisture content (73.90%) was recorded in Goshoerami where as the highest moisture retention capacity after 6 hours (86.25%) and 12 hours (78.81%) was registered by KNG.

Islam *et al.* (2022) evaluated leaf of four mulberry varieties *viz*., Kokuso-21, SKM-33, Goshoerami and Ichinose for moisture content and its retention capacity after 6 hours during spring season and reported that the highest moisture (77.50%) was recorded in Ichinose where as the highest moisture retention capacity after 6 hours (79.21%) was recorded in Goshoerami.

Ahalya *et al*. (2020) analysed fifteen mulberry genotypes *viz*., ME-03, ME-06, ME-27, ME-18, ME-18, ME-67, ME-95, MI-79, ME-052, MI-66, MI-143, ME-224, MI-012, MI-517, M5 and S-13 to determine the moisture content and moisture retention capacity with regard to seasons and found significant variations among the mulberry varieties. Me-052 recorded the highest moisture content (83.03%) and M5 recorded highest moisture retention capacity after 6 and 9 hour (74.15% and 68.70%) in rainy season.

Biradar and Narayanswamy (2020) studied 37 mulberry genotypes *viz*.,V1, ML, M5, RFS175, MR2, DD, S-34, S-36, S-41, AR-12, S13, DW, UP, HL, MS3, ME05, ME-95, ME-107, MI-494, ME-65, MI-0158, MI-0510, ME-169, MI-240, ME-0142, ME-143, MI-233, MI-139, MI-231, MI-516, MI-32, MI-565 and MI-491 to determine relative water content and moisture content parameters. Among these mulberry genotypes highest relative water content and moisture content of 98.7% and 77.28% was recorded in MI-516 and ME-143 respectively.

Shah *et al.* (2019) observed the moisture content and moisture retention capacity of five mulberry varieties namely S146,SI635,G_4, Vishala and C2038 and found that among these mulberry varieties highest evaluation index for moisture content (64.61%) and moisture retention capacity (61-79%) was recorded in C2038 followed by Vishala with(53.61% and 46.94%) moisture content and its retention capacity respectively.

Ramamoorthy *et al*. (2018) evaluated different mulberry genotypes *viz*., V1, MR2, G_4, S36 and found highest moisture (78.5± 2.50 %) and protein content (24.8±0.22%) in the leaves of V1.

Kumar *et al*. (2018) conducted biochemical analysis of ten mulberry varieties *viz*., BC-259,K-2RFS-175,S-1,S-146,S-776,S-1635,S-1531,TR-8 and UP-1 and observed highest moisture content (80.6%), moisture retention capacity (81.6%) and total protein (27.31%) in case of S1635 variety.

Sanchez- Salcedo *et al.* (2017) evaluated mulberry clones of *M. alba* (MAI, MA2, MA3 and MA4) and *M. nigra* (MN1, MN2 and MN3) with respect to chemical constitution of leaves and reported highest moisture (66.9 ± 1.8%) and protein (19.4 ± 0.7%) content in MA3 followed by 59.7±1.4% and 18.7 ±0.7%in MN2.

Kumar *et al*. (2018) studied some mulberry varieties *viz*., S-1635, Sujanpur-Local, V1, S-146, TR-10 and Chinese white and reported that moisture content and its retention capacity showed no significant difference among these mulberry varieties.

Shivashankar (2015) analysed mulberry varieties *viz*., V1, S-34,M-5, S-13,S-146, Karanahalli local and Mysore local and reported that among these mulberry genotypes V1 recorded the highest moisture content (75.34%)and moisture retention capacity (93.09%) after six hours during spring season.

Mir *et al*. (2003) evaluated seven mulberry genotypes *viz*., Goshoerami, Ichinose, KNG, Tr-10, Chinese white, Rokokoyoso and Chattatul Zangir for their

leaf moisture content and reported that Goshoerami registered the highest moisture content of 77.14 percent during spring and 75.11 percent moisture content during autumn season under Kashmir climatic conditions.

Ninge Gowda and Sudhakar (2002) reported that anatomical characters such as stomatal size, stomatal frequency, mesophyll tissue, cuticle thickness and leaf thickness in mulberry influence the moisture content and moisture retention capacity.

Vijayan *et al.* (1997) conducted studies on leaf moisture of mulberry germplasm under West Bengal conditions and observed that moisture content and moisture retention capacity were under the influence of both genetic and environmental factors.

Wang and Clarke (1993) tried to find out relationship of excised leaf water loss and stomatal frequency in wheat and found that stomatal frequency is perhaps one of the several factors influencing observed genotypic differences in rate of water loss.

Hatalii *et al.* (1993) studied the interrelationship of stomatal frequency, interveinal distance and yield under depleting soil moisture regimes in wheat genotype and found that a reduction in stomatal frequency was found to be a drought resistant character.

Moglii *et al.* (1992) worked on physiological effects of stomatal characters in mulberry at three ploidy levels and found that stomatal frequency and transpiration rates decreased with increase in ploidy levels.

Paul *et al.* (1992) while studying the impact of dietary moisture on nutritional indices and growth of *Bombyx mori* L. reported that absolute consumption of feed and growth rate of larvae increased with increasing levels of leaf moisture content.

Bose *et al.* (1991) reported the highest moisture content (73.31%) in S-52 and the lowest (70.10 %) in local variety. Total minerals ranged from 8.91 percent

in S-41 to 12.74 percent in local whereas crude protein ranged from 7.96 percent in K-2 to 9.88 percent in S-36 and S-41.

Sastry *et al.* (1988) worked on the varietal differences in the loss of moisture from harvested mulberry leaves and found that varieties belonging to S series are better than K-2 and local varieties and this makes them more suitable to fit well for cultivation in tropical sericultural areas.

Susheleema and Jolly (1986) studied morpho physiological parameters associated with drought resistance in mulberry and found negative correlation between stomatal frequency and moisture retention capacity.

Sikdar *et al.* (1983) undertook evaluation of morpho physiological parameters associated with drought resistance in mulberry. Stomatal size and moisture retention capability were found negatively correlated whereas, leaf thickness, cuticle thickness and moisture retention capacity of detached leaves reflected positive correlation.

Bongale and Chaluvachari (1995) have reported that leaves characterized by higher leaf moisture and moisture retention capacity were identified as superior quality leaves.

Sengupta *et al.* (1971) reported that leaf moisture content and moisture retention capacity of genotypes have positive influence on the growth and development of silkworm.

Parpiev (1968) concluded that leaf moisture content has favourable effect on the palatability and assimilability of nutrients and is considered as one of the criteria in estimating the leaf quality.

Mandal and Krishnaswami (1965) studied the changes in the nutritive value of mulberry leaves on storage and found that for successful rearing, the maintenance/retention of sufficient moisture content in harvested leaf for prolonged periods is of immense importance.

2.3 Anatomical studies

Manjunatha *et al.* (2021) while studying the morpho biometric and cytogenetic analysis of clonally evolved three mulberry cultivars *viz*., Talaghattapura-1, Anantha and Vishala along with their mother plants *viz*., M-5, RFS-135 and S-1635 reported that the highest leaf thickness (316.45 ±1.22 µm) was in Vishala, highest cuticular thickness (14.61±3.00 µm) in Anantha and the thickest palisade layer (121.51± 5.31 µm) in S-1635 cultivar.

Manjunatha *et al.* (2021) conducted the stomatal studies of six mulberry varieties *viz*., Talaghattapura-1, Anantha, Vishala, M-5, RFS-135 and S-1635 and reported that S-1635 mulberry variety recorded the highest stomatal frequency (677.84 /mm^2), largest stomata size (512.36 µm) and the least number of chloroplast/ stomata (10.26).

Shivashankar (2015 a) conducted leaf moisture studies of six mulberry varieties namely V1, S13, S146, S34, M5 and Karanahalli local as influenced by foliar spray of paras and reported that the V1 variety recorded the maximum leaf moisture content and moisture retention capacity after 6 and 12 hours which is due to more thickness of upper cuticle cum epidermis, stomata of smaller dimensions and more thickness of leaf.

Shivashankar (2015b) conducted leaf anatomical studies of six mulberry varieties namely V1, S13, S146, S34, M5 and Karanahalli local as influenced by foliar spray of paras and reported that the V1 variety recorded the highest upper cuticle thickness (10.38 µm) and upper epidermal thickness (22.38 µm).

Mir *et al.* (2012) studied the moisture status of seven mulberry genotypes namely *viz*: Goshoerami, Ichinose, KNG, Tr-10, Chinese white, Rokokoyoso and Chattatul Zangir growing as trees under rain fed conditions in Kashmir and reported that Goshoerami recorded the highest moisture content (74.67%), moisture retention capacity after 6 hours (92.21%) and 12 hours (84.43%) which may be due to more thickness of upper cuticle (11.38µm), upper epidermis (23.68

µm), stomata of smaller dimensions (20.78 x 10.83 µm) and more thickness of leaf (194.95 µm).

Mir *et al*. (2012) studied the foliar anatomy of seven mulberry genotypes namely *viz*: Goshoerami, Ichinose, KNG, Tr-10, Chinese white, Rokokoyoso and Chattatul Zangir and reported that Goshoerami recorded the highest upper and lower cuticle thickness (11.38 µm and 5.19 µm) respectively and highest palisade thickness (65.35 µm).

Laltanmawii and Roychowdhuri (2010 a) studied the effect of chromosomal variation on morphology and leaf anatomical behaviour in six mulberry genotypes *viz*., S-1, V-1, S-1635, C-1730, T-1 and T-23 and reported that thickness of upper cuticle was significantly highest (10.52 µm) in tetraploids followed by triploids (6.28 µm) and diploids (6.27 µm).They further reported that the thickness of lower cuticle was the highest in diploids (6.24 µm).

Laltanmawii and Roychowdhuri (2010 b) evaluated six mulberry genotypes *viz*., S-1, V-1, S-1635, C-1730, T-1 and T-23 to study the effect of chromosomal variation on morphology and leaf anatomical traits and reported that the triploids (2n=3x=42) were superior to the diploids and tetraploids. He also reported that upper cuticle(r = 0.77 µm), palisade (r = 0.71 µm) and spongy parenchyma (r = 0.74 µm) layers of leaf showed positive correlation with the increase in number of chromosomes.

Vijayan *et al.* (2008) studied morpho-biochemical and anatomical features of five mulberry genotypes *viz*., English black, C776, *Rotun diloba*, Mandalaya and Tollygunj and reported that English black registered the highest leaf thickness(149 µm), palisade thickness(52.9 µm),spongy thickness(56.7 µm) but the least stomatal frequency (737/mm^2).

Mahajan and Dhillon (2004) worked on anatomical resistance mechanisms of Muskmelon leaf against *Pseudopernospora cubensis* (Berkeley and Curtis) and found anatomical characteristics (more number of palisade layers, significantly

higher thickness of epidermis cum cuticle, cuticle and palisade tissue, comparatively thinner spongy parenchyma and significantly higher palisade proportion) have been found to act as structural barriers against the penetration and invasion by *Pseudoperonospora cubensis* (Berkeley and Curtis).

Raja (2003) screened various mulberry varieties as per disease intensity and also worked on correlation between disease intensity and stomatal frequency of various mulberry genotypes. Correlation between percent disease intensity and stomatal frequency had positive but non significant effect at 5% level of significance.

Grewel *et al*. (1999) studied the role of stomata in relation to kernel bunt caused by *Neovossia indica* (Mitra) and *Telltia indica* (Mitra) in bread wheat and found that the resistant lines had lower stomatal frequency than the susceptible lines. However, no significant differences in stomatal frequency were observed between susceptible and resistant lines of durum wheat and *Triticale.*

Philip *et al*. (1991) conducted study on the anatomical characteristics of the mulberry leaf in relation to resistance/ susceptibility to various pathogens. The average thickness of cuticle cum epidermal wall was significantly higher in resistant varieties than that in susceptible varieties.

Kour and Dillon (1988) worked on pre infection anatomical defense mechanisms of groundnut leaf against *Cercosporidium personatum* (Berk and Curt) and found that the most resistant variety of ground nut had significantly highest frequency of trichomes, calcium oxalate crystals and significantly thickest epidermis cum cuticle and palisade layers.

Vijayan *et al.* (1998) conducted a correlation study between leaf yield and morpho anatomical characters of triploid mulberry varieties with high bush conditions and reported positive significant correlations between leaf yield and number of primary branches per plant, leaf thickness, spongy thickness, spongy layer thickness and weight of 100 dry leaves.

Milkyan and Babyan (1971) studied anatomical characteristics of some mulberry varieties in relation to the feeding value. Varieties with thinner cuticle and also fewer cystoliths in the upper epidermis were found to be more palatable to the silkworms.

Philip and Govindaiah (1996) have found that factors like cuticular wax, surface layer thickness, stomatal frequency and thickness and compactness of palisade layer play an important role in providing defence to mulberry plants against *Cercospora moricula* (Cooke).

Mayee and Apet (1995) compared five rust resistant and two susceptible genotypes of groundnut for morphological and anatomical features and found that the resistant genotypes had less and smaller stomata, compact palisade layer, thick epidermis cum cuticle as compared to the susceptible genotypes.

2.4 Physiological studies

Malik (2023) evaluated leaf of five mulberry genotypes *viz*., Goshoerami, KNG, Mukey, Kokuso-20 and Botatul for their macronutrient and cholorophyll content and reported that KNG registered the highest nitrogen content (3.351%) and the highest phosphorus content (0.249%). They also reported that the chlorophyll content was the highest (3.886 mg/g) in KNG.

Islam *et al.* (2022) evaluated leaf of four mulberry varieties *viz*., Kokuso-21, SKM-33, Goshoerami and Ichinose to determine their chlorophyll content and reported that Goshoerami leaf had the highest total chlorophyll content (0.576%), chlorophyll “a” (0.370%) and chlorophyll “b” content (0.209%).

Mosharraf *et al.* (2022) conducted studies on determination of the best time for total chlorophyll and carotenoid extraction from mulberry leaves. The study revealed that spring season is the best time to get the maximum total chlorophyll (5.68 µg/ml) and carotenoid content (0.57 µg/ml) in mulberry leaf.

Levickiene *et al.* (2018) evaluated *Morus alba* L. leaves for their macronutrient content during different seasons and reported that calcium content

is higher during autumn season as compared to spring season while as potassium content is more during spring season. They also reported that the phosphorus and magnesium content are more during the autumn season as compared to spring.

Rodriguez *et al.* (2018) conducted agronomical, physiological and biochemical characterization of some Chinese mulberry cultivars and reported that three mulberry cultivars *viz*. Guisang You 62, Guandong 11 and Guisang You 12 exhibited an anisohydric physiology, exerting low stomatal control which permits a substantial decrease in leaf water potential. He also reported that these plants were able to develop active osmotic adjustment to confront water stress, thus maintaining leaf turgor.

Kumar *et al.* (2018) evaluated 10 mulberry clones *viz*., BC-259, K-2, RFS-175, S-1, S-146, S-776, S-1635, S-1531, Tr-8 and UP-1 through phytochemical analysis and reported that total proteins, total sugars and amino acids were highest in tender leaves followed by medium and coarse leaves. They also reported that total proteins, total sugars and total amino acids were the highest in S-1635 followed by K-2 and the lowest in UP-1.

Paul *et al*. (2017) conducted the measurements of stomatal density and stomatal index on leaf surfaces of five crops *viz*: cotton, groundnut, mung bean, tomato and sorghum and reported that the highest stomatal index was found in mung bean with value of 29.4%.

Murthy *et al.* (2013) evaluated ten mulberry varieties for leaf quality through phytochemical analysis and reported that total proteins, total sugars and amino acids were higher in tender leaves followed by medium and coarse leaves but the total phenols, prolines and chlorophyll content were highest in medium leaf followed by coarse and tender leaves. They also reported that the moisture content was highest in tender leaf followed by medium and coarse leaves.

Sethua *et al.* (2012) evaluated 20 mulberry genotypes for their photosynthetic efficiency through physio-biochemical analysis and reported that

out of the genotypes, T-30 was superior with respect to photosynthetic rate (14.18µmol $m^{-2}s^{-1}$), total chlorophyll (19.1mg g^{-1}fresh weight), total soluble sugar (47.7 mg g^{-1} fresh weight), leaf moisture content (81.06%) and leaf area (103.4 cm^2).

Yigit *et al.* (2017) studied the elemental composition of three mulberry species *viz*; white mulberry, red mulberry and black mulberry and reported that the highest Calcium content (20171.1 ppm) was recorded in white mulberry where as the highest Magnesium content (5632.5 ppm) was recorded in red mulberry. They further reported that the highest iron content (77.7 ppm) was recorded in case of black mulberry.

Ghosh *et al.* (2006) evaluated ten mulberry varieties for physiological and biochemical parameters and reported that among the selected varieties, S1635 showed highest leaf area (305.70 cm^2), net photosynthetic rate (14.66 µ mol m^{-2} $s^{-1)}$, chlorophyll content (2.13 mg g^{-1} fresh weight), total soluble sugar (48.44 mg g^{-1} fresh weight) and total soluble protein (39.63 mg g^{-1} fresh weight).

Sarkar *et al.* (2003) studied the variability in specific leaf weight in 165 mulberry accessions and reported that greater SLW provides more photosynthetic potential per unit area of leaf and has been frequently considered to be correlated with photosynthesis in plants. The mean SLW ranged from 35.3 to 72.3 g/m^2 in the collection of 165 mulberry accessions.

Patil *et al.* (2002) tested the leaf nutritive quality status in improved genotypes of mulberry (*Morus alba* L.) and found S-1635 had higher leaf water content, leaf water retention, chlorophyll a, chlorophyll b, total chlorophyll, soluble sugars and total nitrogen content then the rest and hence recommended the genotype for commercial cultivation under Karnataka conditions.

Chaitanya *et al.* (2002) conducted studies on photosynthetic rate and biomass productivity among four mulberry cultivars *viz*. K-2, MR-2, BC2-59 and S-13 and reported the highest net photosynthetic rate (P_N) in BC2-59. They also

found that P_N and activities of photosynthetic enzymes are significantly correlated with growth and biomass production in mulberry cultivars under study.

Singhvi *et al.* (2001) have worked on the cause and relationship of leaf yield in mulberry with its morphological and physiological traits. Path co-efficient analysis undertaken to evaluate relationship between physiological and morphological traits with leaf yield suggested that cumulative shoot length, leaf area, stem yield and the rate of photosynthesis are the major components for leaf yield and may be given maximum weightage while deciding about selection criteria for improvement of mulberry yield under irrigated conditions.

Bongale *et al.* (1991) conducted a study on soil fertility and mulberry leaf nutrient contents (macro and micro) in different areas of Karnataka and observed significant variability in the soil nutrient status and the leaf nutrient content of the areas under study.

Shivaprakash *et al.* (2000) while studying nitrogen uptake in three improved varieties of mulberry under irrigated field cultivation observed seasonal variation in nitrogen content and its uptake.

Bari and Quader (2000) have observed seasonal variations in nutritional values of mulberry leaves and have reported better nutritional values during May and June than other seasons.

Das *et al.* (1999) evaluated seventy two mulberry accessions and reported that photosynthetic rate had highest significant positive correlation with protein content followed by sugar and total chlorophyll content in the leaf. He also found that relative water content and water deficit percent in leaf influence the photosynthetic rate vis-à-vis leaf yield in mulberry.

Davidson and Campbell (1984) showed that the relative growth rate (RGR) in wheat was initially high and decreased with time to negative values during the soft dough stage. However, crop growth rate (CGR) increased with time until

about the anthesis before decreasing rapidly to zero at the soft dough stage and to negative values thereafter.

Machii (1984) while analysing the shoot development and growth in the mulberry species, *Morus acidosa* Ariff concluded that the larger production of dry matter occurred in the secondary shoots in July and this phenomenon continued until September. Relative growth rate (RGR) increased with the growth of the plant and reached maximum value in the late June. He also concluded that net assimilation rate (NAR) was highest in May and leaf area ratio (LAR) was the highest in July.

Sikdar *et al.* (1983) undertook evaluation of morpho-physiological parameters associated with drought resistance in mulberry. Stomatal size and moisture retention capacity were found to be negatively correlated whereas leaf thickness, cuticle thickness, length of the root, dry weight of the root and moisture retention capacity of detached leaves reflected positive correlation.

2.5 Biochemical studies

Malik (2023) evaluated leaf of five mulberry genotypes *viz*., Goshoerami, KNG, Mukey, Kokuso-20 and Botatul for their biochemical constituents and reported that KNG recorded the highest total protein content (20.96%), highest carbohydrate content (20.11%) and the highest phenol content (4.85 mg GAE/g).

Islam *et al*. (2022) evaluated leaf of four mulberry genotypes *viz*., Kokuso-21, SKM-33, Goshoerami and Ichinose for their biochemical composition and reported that Goshoerami registered the highest total phenol content (0.73%), highest total carbohydrate content (11.01%) and the highest protein content (13.78%).

Tantray *et al.* (2021) evaluated leaf of three mulberry genotypes namely Goshoerami, Ichinose and Kokuso-21 for their protein and carbohydrate content at three different stages (tender, medium and coarse) and reported a decreasing trend in terms of protein and carbohydrate content from tender to coarse textured

leaves of the three genotypes. They also reported that Goshoerami had the highest protein content of 25.34% whereas highest carbohydrate content (16.91%) was found in Kokuso-21.

Hadimani *et al.* (2019) investigated biochemical constituents of 18 mulberry genotypes *viz*: TG, English Black, MR2, V-1, S-54, OPH-1, S-36, M5, Viswa (DD0, TR-8, RFS-175, BR-8, S-1635, Vishala, *Morus multicaulis*, TR-10,Mysore local and S-41 and reported that leaf of V-1 mulberry variety has the highest total soluble proteins (24.13%), carbohydrate (24.12%), chlorophyll (2.278 mg/g) and total soluble sugars (14.02%).

Krajnc *et al.* (2019) studied biochemical constitution of mulberry genotypes from different geographical regions of Slovenia and reported the highest protein content of 20.2 g/100g in leaves of A40 mulberry genotype (*M. alba*).

Yu *et al.* (2018) worked on nineteen mulberry varieties *viz*., D10, DHS, GS8, HYS, HM, THY, LYD, LJ504, NS14, QS1, TP2, TS, X7920, XGS, XYZL, XG, XS, Y237 and ZS5801 and found that HYS variety recorded the highest crude protein (37.36 ± 0.59 g/100g dry weight) and highest total soluble sugar (150.31 ± 4.12 mg/g) followed by other varieties. The lowest crude protein (27.63 ± 0.06 g/ 100g dry weight) and total soluble sugar (102.65 ± 4.23 mg/g) were found in case of XS mulberry variety.

Suman *et al.* (2018) studied variation in biochemical constituents of different clones of *M.alba viz.*, TR10, Mandaley, S36, S799, S30, Kanva 2, Chinese white, Philipino, S146, K2MS, S1531, Berhampore, S1307, ME-65 and Nauni in different seasons and found highest crude protein content (17.82 %) in rainy season and lowest in autumn season (14.34%).

Sanchez- Salcedo *et al. (*2017) evaluated mulberry clones of *M. alba* (MAI, MA2, MA3 and MA4) and *M. nigra* (MN1, MN2 and MN3) with respect to chemical constitution of leaf and reported highest moisture (66.9 ± 1.8%) and

protein (19.4 ± 0.7%) content in MA3 followed by MN2 with (59.7±1.4% and 18.7 ±0.7% respectively).

Sori and Gebreselassie (2016) carried out biochemical estimation of eleven mulberry genotypes namely Saja, M4, Kumbl, K2, S13, Melkassa, Hijaji, Awassa, Gatama, Ano and Bedele and found highest moisture content (78.73%) and soluble protein (8.31 mg/g) in Kumbl genotype.

Jha *et al.* (2016) carried out biochemical analysis of seven mulberry varieties *viz*., Mandalaya, Jaysree, S1, V1, K2, S1635 and Bombay and recorded highest protein content of 24.98 ± 0.29 mg/g in the leaves of S1635 variety. It was further reported that protein content decreased with increase in the leaf maturity.

Shyla *et al. (*2016) carried out biochemical estimation of V1 mulberry leaves after application of nutrients given through fertilizers and recorded highest moisture, carbohydrate and soluble protein content of 81.75%, 25.26% and 10.04% respectively at 150% concentration of fertilizers as compared to control.

Abdelmegeed (2016) carried out biochemical estimation in leaves of *M. nigra* and *M. alba* and recorded highest total carbohydrate (3.19%), protein (21.00%), phenol (1.16%) and crude fibre (13.74%) in leaves of *M. nigra* than *M. alba*.

Shivashankar (2015) analysed mulberry varieties *viz*., V1, S-34, M-5, S-13, S-146, Karanahalli local and Mysore local and reported that among these mulberry genotypes V1 recorded the highest moisture content (75.34%) and moisture retention capacity (93.09%) after six hours during spring season.

Dimitrova *et al.* (2015) carried out the total carbohydrate estimation in leaves and fruits of three mulberry species namely *M.alba, M.rubra* and *M. nigra* and recorded the highest total carbohydrate content in leaves (4.1± 1.0 g/100g) and fruits (9.8 ±0.9 g/100g) of *M. rubra* than the other two species.

Murthy *et al.* (2013) investigated ten mulberry varieties *viz*., TR-8, TR-12, TR-20, S17908, MS5, C6, C10, Matigara black, *M.nigra* and M5 and reported

that S1708 contained the highest protein content of 27.31 %. They also evaluated mulberry germplasm varieties through phytochemical analysis and reported that Tr_{20} variety recorded the highest total sugar content (16.02%) as compared to other varieties.

Subhan *et al.* (2013) evaluated mulberry species namely *M. alba*, *M. latifolia*, *M. rubra* and *M. nigra* with regard to selected biochemical constituents. Highest protein content of 21.06% was recorded in *M. nigra* followed by *M. latifolia* and *M.rubra* with protein content of 19% and 18.85% respectively. Highest carbohydrate content of 37.25% was recorded in *M. nigra* followed by *M. latifolia* and *M. alba* which recorded carbohydrate content of 36.55% and 35.47% respectively.

Khan and Naik (2012) conducted chemo assay of some mulberry cultivars *viz*., Mysore local, V1 and S1635 and reported that the highest protein (238.84 mg/g), carbohydrate (40.71 mg/g) and moisture content (78.32 %) in S1635.

Iqbal *et al.* (2012) investigated mulberry species namely *M. alba*, *M. rubra* and *M. nigra* with regard to biochemical constitution of leaves and recorded highest protein (24.63 ± 0.86%) and ash (11.73 ± 1.09%) content in *M. rubra*.

Kumar and Chauhan (2011) carried out study on the biochemical constituents of leaves of some mulberry varieties *viz*., S-13, S-146, S-1,S-54,BR-2,AR-12 and S-36 and reported highest protein content (0.317 mg/g), carbohydrate (0.624 mg/g) and total lipid (0.923 mg/g) content in S-36, AR-12 and S-13 respectively.

Srivastava and Elangovan (2011) analysed some mulberry varieties namely S-1, S-13,S-1635, S-146,TR-10,K-2 and Mysore local and found highest protein content in the tender leaves of K-2 variety (0.196 mg/g) followed by S-1635 (0.168 mg/g) and Mysore local (0.165 mg/g).

Cheema *et al.* (2011) estimated nutrient content of leaves of *Morus alba, Acacia nilotica, Ziziphus jujube* and *Syzgium cumuni* and recorded highest crude protein content of 23 % in *M. alba* followed by *A. nilotica* (13.2%).

Imran *et al.* (2010) carried out biochemical estimation of four mulberry species *viz*., *M. alba* (white mulberry), *M. laevigata* (large black fruit), *M. laevigata* (large white fruit) and *M. nigra* (black mulberry) with regard to fruit and recorded highest protein (1.73±0.10 g/100g) and total carbohydrate (17.96 ± 1.54 g/100 g) content in *M. laevigata* (large black fruit) as compared to other species.

Thirumalaisamy *et al.*(2009) studied the biochemical composition in the leaves of six mulberry genotypes namely V1, ML, S13, S30, S36 and RFS-135 and reported that V1 had the highest total protein, total sugar and total phenol content of 26.72%, 16.72% and 4.56% respectively.

Adeduntan and Oyerinde (2009) studied mulberry varieties *viz*., Obeche, S36, S54 and K2 with regard to biochemical constitution of leaves and reported the highest protein content of 34.31 ± 0.0% in Obeche.

Ramachandra *et al.* (2008) evaluated five mulberry varieties namely V1, M5, S54, S36 and DD with for biochemical constitution of leaf and reported the highest moisture (77.41%), total protein (29%) and total sugar (12.86) content in V1.

Shah *et al.* (2007) while evaluating different mulberry varieties namely Husang China, Japan Early and Chinese Evergreen reported the highest protein content (36%) in Husang china as compared to the other varieties.

Khan *et al*. (2007) evaluated seven mulberry varieties *viz*., SKM-20, SKM-27, SKM-33, SKM-36, SKM-48, Goshoerami and Ichinose through chemo and bioassay and found that mulberry varieties differ significantly in their chemical constituents that influence the economic parameters of the silkworm. They further reported that maximum moisture content was found in Ichinose (74.46%) which was at par with Goshoerami (73.82%) and SKM-27 (73.80%) and moisture

retention capacity was maximum in case of Goshoerami (67.94%) followed by SKM-27.

Malik and Reddy (2007) evaluated three mulberry varieties namely S36, V1 and M5 with regard to moisture content and total protein and found significant differences among these varieties. The highest moisture content (76.20%) and total protein content (233.2 mg/g) was recorded in V1 variety.

Ghosh *et al.* (2006) studied some mulberry genotypes namely V1, C2016, C2017, anantha, RFS-175, C1730, Thallaghatapura, Vishala, S1 and S1635 and found that among these varieties S1635 recorded the highest total soluble protein (39.63 mg/g) as compared to the other varieties.

Srivastava *et al.* (2006) investigated biochemical composition in leaves of six mulberry genotypes namely TR-8, CM, Haldwani local, S-34, S-146 and K-2 and reported the highest crude protein content of 30.91 ± 0.06% in leaves of CM followed by S-146.

Sinha *et al.* (2003) studied seven mulberry varieties *viz*., V1, S1635, RFS175, MR2, SV1, Jatuni and JRH for the estimation of moisture, total carbohydrate and crude protein content and reported significant variations among these genotypes. Highest moisture and total carbohydrate content of 73.04% and 22.83% respectively was recorded in S1635 genotype whereas, highest crude protein content (26.40%) was recorded in JRH genotype.

Goyal *et al*. (2003) carried out biochemical estimation of mulberry varieties namely Kanva -1 and Mawapa and recorded the highest crude protein, calcium and phosphorus of 18.6%, 2.4% and 0.24% respectively in Mawapa variety.

Venkataramana *et al*. (2002) carried out study on moisture and moisture retention capacity in leaves of mulberry varieties namely V1, Kanva-2 and S-36 and reported the highest moisture (71.84%) and moisture retention capacity (70.17%) in V1.

Narahari *et al*. (2001) carried out biochemical estimation of M5 mulberry variety under irrigated conditions and reported that the leaves of this mulberry variety contain moisture, protein and sugar content of 75.8%, 24.47% and 11.10% respectively.

Narayananswamy *et al.* (1996) reported that protein and carbohydrate are prime components of nutrition in leaf and their quantities greatly influence the growth of silkworms. They further reported that mulberry leaf quality depends on the age, variety and leaf position within the same species.

Krishnaswami *et al.* (1970) reported that silkworm larval growth, development and cocoon production is directly proportional to the nutritional and biochemical quality of mulberry leaves.

Chapter 3

MATERIAL AND METHODS

The present study was under taken on well-established mulberry genotypes maintained in the old Germplasm Bank of College of Temperate Sericulture, Mirgund, SKUAST-K under rainfed conditions. The experiment was conducted as per the following details:

Target crop	:	Mulberry
Experimental site	:	College of Temperate Sericulture (CTS), Mirgund
Experimental lab	:	CTS, Mirgund, Basic Sciences and Humanities, Faculty of Horticulture, Shalimar & Centre of Research for Development, University of Kashmir, Srinagar.
No. of genotypes	:	10

- G_1: Goshoerami
- G_2: Ichinose
- G_3: KNG
- G_4: Rokokoyoso
- G_5: Kokuso-20
- G_6: Kokuso-21
- G_7: Limoncina
- G_8: Ensatakasuke
- G_9: Chinese white
- G_{10}: Kairyoroso

No. of years : 02

- Y_1 : 2021
- Y_2 : 2022

No. of seasons : 04

- S_1: Spring 2021
- S_2: Autumn 2021
- S_3 : Spring 2022
- S_4 : Autumn 2022

No. of replications : 03

No. of plants per replication : 03

Design of experiment : RCBD

The plantation was properly maintained as per the package of practices recommended for temperate conditions of Kashmir (Ahsan *et al.,* 1990). Each year pruning was given only once, in the first week of June by cutting the branches right from the crown base. The following parameters were recorded in both spring (1st crop) and late summer/ autumn (2nd crop) during both the years.

3.1 Growth and yield parameters

The growth and yield parameters were recorded coinciding with the 5th stage of silkworm rearing during spring (1st week of June) and autumn (2nd week of September) crops during both the years. The parameters considered were:

3.1.1 No of shootlets or branches per plant

During spring because of frost damage of apical portion, leaf is present on shootlets whereas during autumn it is borne on main branch. The number of shootlets or branches were counted manually in three plants per replication per treatment.

3.1.2 Total shootlet length or branch length per plant (cm)

For total shootlet length or branch length per plant (cm), all the shootlets or branches of three plants from each replication were measured with the help of measuring tape and the total shootlet or branch length calculated by adding the values.

3.1.3 Internodal distance (cm)

During spring, as the shoot lets were short, middle 50 cm were taken to calculate the internodal distance whereas during autumn middle one meter was taken for the purpose as the branches had a very good length. Total buds were counted in the selected portion and internodal distance calculated.

3.1.4 Fresh weight of 100 leaves (g)

For fresh weight of leaves, composite samples of 100 leaves comprising of almost equal number of tender, medium and coarse leaves were taken and weighed immediately in the morning.

3.1.5 Leaf yield per plant (kg)

Leaf yield was taken by harvesting fully the leaves of a plant and weighing it on a digital balance.

For each parameter three observations from each genotype were recorded to take the mean values.

3.2 Leaf moisture studies (%)

Moisture content of the leaf was estimated during chawkie (1st week of May and 2nd week of August respectively for spring and autumn) and fifth instar (1st week of June and 2nd week of September) for spring and autumn. It was determined on dry weight basis. One hundred fresh leaves comprising of tender, medium and coarse in approximately equal proportion were harvested early in the morning and weighed immediately. They were stored at room temperature (22-23^{0}C) and weighed again after 6 and 12 hours. The leaves were then completely dried in a hot air oven at 60° C for 48 hours (Ninge, Gowda and Sudhakar, 2002)

Plate 1. The dry weight was recorded and the moisture content and moisture retention capacity (MRC) calculated as:

3.2.1 Moisture content in leaf (chawkie and late age): It was calculated as:-

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

3.2.2 Moisture retention capacity after 6 hours (5^{th} stage)

$$\text{MRC after 6 hours (\%)} = \frac{\text{(Weight after 6 hours–Dry weight)}}{\text{(Fresh weight – Dry weight)}} \times 100$$

3.2.3 Moisture retention capacity after 12 hours (%) (5^{th} stage)

$$\text{MRC after 12 hours (\%)} = \frac{\text{(Weight after 12 hours–Dry weight)}}{\text{(Fresh weight – Dry weight)}} \times 100$$

3.3 Anatomical studies

The studies were carried out during autumn season only. 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) which was prepared as under:

70% ethyl alcohol	:	90 ml
Glacial acetic acid	:	05 ml
Formalin	:	05 ml

The material was kept in the fixative for 20 hours to:-

- Kill the cells suddenly and uniformly so that they retain, as far as possible, the same appearance which they possess in life.
- Preserve the cells and tissues by inhibition of putrification and autolytic changes.
- Render the cell constituents resistant to the subsequent processes such as dehydration and staining etc. prior to their examination under the microscope.

Plate 1: Collection and processing of leaf samples for moisture studies

- Facilitate the proper staining of tissues.

The fixed material was later transferred to 70% ethanol (C_2H_5OH) for preservation (Plate 2). For the fixed material, dehydration, infiltration and embedding was done properly and thin sections (10-20 µm) cut mechanically with the help of microtome. Standard procedures for staining and embedding were followed as per Dwivedi and Singh (1990) later modified by Khasim (2002) and the sections were mounted in DPX. The palisade and spongy proportion was calculated as per Tiwari *et al.* (1986). The measurement of following tissues was done through micrometry.

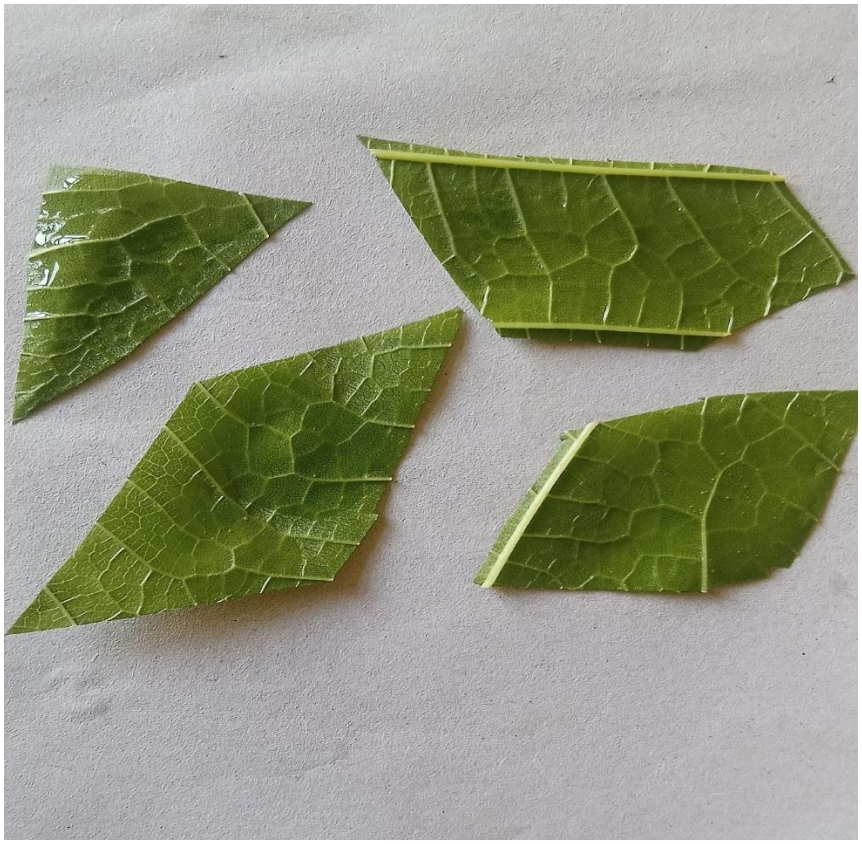
1. **Upper cuticle thickness (µm)**
2. **Lower cuticle thickness (µm)**
3. **Upper epidermis thickness (µm)**
4. **Lower epidermis thickness (µm)**
5. **Palisade thickness (µm)**
6. **Palisade proportion (%)**
7. **Spongy thickness (µm)**
8. **Spongy proportion (%)**
9. **Stomatal frequency**:

For stomatal study, lower and upper epidermal peels were taken as per the method of Pohl (1967) later modified by Ghouse and Yunus (1972). The stomatal frequency was determined by adopting nail polish impression method (Slatyer, 1964). Stomatal size was recorded using ocular micrometre by taking length and width of the stomata (Plate 3)**.**

10. **Stomatal index:**

Slides prepared for stomatal frequency were also used for determination of stomatal index by using the formula given by Mallick *et al. (*2016).

Plate 2: Fixation and preservation of leaf samples for Anatomical studies



Preparation of an epidermal impression by coating the abaxial leaf surface with nail varnish

Preparation of an epidermal impression by coating the adaxial leaf surface with nail varnish

Plate 3: Preparation of epidermal impression

$$\text{Stomatal index (\%)} = \frac{\text{Stomatal density}}{\text{Stomatal density + epidermal cell density}} \times 100$$

3.4 Physiological studies

Following physiological parameters were recorded:

3.4.1 Absolute growth rate (g/day):

For calculating the AGR four identical branches were identified and marked as 1, 2, 3 and 4. On the first day of first interval, leaves of 1st branch were harvested and weighed on digital balance. The leaves so harvested were allowed to dry completely so as to get the dry weight. After 15 days leaves of 2nd branch were harvested and same procedure was followed for 3rd and 4th branch also**.** The AGR of leaves was calculated by using the following formula (Gregory,1926) :

$$\text{AGR(g/day)} = \frac{W2-W1}{T2-T1}$$

Where,

W2= Weight at Time 2

W1= Wight at Time 1

T2-T1 = Time interval in days (15 days)

3.4.2 Leaf net assimilation rate (mg/cm^2/day):

The LNAR directly indicates thr rate of net photosynthesis. For calculating the LNAR four identical branches were identified and marked as 1, 2, 3 and 4. On the first day of first interval, leaves of 1st branch were harvested and weighed on digital balance. The leaf area of the leaves was calculated. The leaves so harvested were allowed to dry completely so as to get the dry weight. After 15 days leaves of 2nd branch were harvested and same procedure was followed for 3rd and 4th branch also. It was calculated using the formula (Gregory,1926) as:

$$\text{LNAR(mg/cm}^2\text{/day)} = \frac{W2-W1\ (\text{Ln } L2-\text{Ln } L1)}{T2-T1\ (L2-L1)}$$

Where,

L1 and W1 are the leaf area and dry weight of leaf at times T1

L2 and W2 are the leaf area and dry weight of leaf at times T2.

T2-T1 = Time interval in days (15 days).

Ln L2 and Ln L1 are natural logs of leaf areas.

3.4.3 Leaf area (cm^2):

The single leaf area was recorded with the help of a graph paper.

3.4.4 Specific leaf weight (mg/cm^2):

The SLW was calculated by using the following formula:

$$SLW(mg/cm^2) = \frac{\text{Leaf weight}}{\text{Leaf area}}$$

3.4.5 Specific leaf area (cm^2/mg): The SLA was calculated by using the following formula:

$$SLA(cm^2/mg) = \frac{\text{Leaf area}}{\text{Leaf weight}}$$

3.4.6 Chlorophyll content (mg/g):

Total Chlorophyll content was determined using dimethyl sulphoxide (DMSO) as described by Hiscox and Israelstam (1979). Total chlorophyll content from fresh mulberry leaf without maceration was determined using Dimethyl suphoxide (DMSO). 50 mg of leaf tissue was taken in a test tube and 7 ml of DMSO added to it. The mixture was kept in an electric oven for 3 hours at 65^0C. Finally the extract was transferred to a measuring cylinder and made up to a volume of 10 ml with DMSO and assayed immediately or transferred to vials and stored below 4^0C till used for analysis. Total chlorophyll, chlorophyll "a" and chlorophyll"b" contents were estimated. 3 ml of chlorophyll extract was transferred to cuvette and absorbance was recorded at 645nm and 663 nm against DMSO blank using spectrophotometer (Plate 4). Chlorophyll contents were calculated using following equations:

Plate 4: Processing of leaf samples for chlorophyll estimation

$$\textbf{Chlorophyll "a" (mg/g)} = \frac{[12.7(A\ 663)\ 2.69(A\ 645)] \times V}{(1000 \times W)}$$

$$\textbf{Chlorophyll "b" (mg/g)} = \frac{[22.9\ (A\ 645)\ 4.68\ (A\ 663)] \times V}{1000 \times W}$$

$$\textbf{Total Chlorophyll (mg/g)} = \frac{[20.2\ (A\ 645) + 8.02\ (A\ 663)] \times V}{1000 \times W}$$

Where,

V = Volume of solvent

W = fresh weight of tissue extracted

3.4.7 Nitrate Reductase activity (µmol/ ml/ hr):

The nitrate reductase activity was estimated by following the method of Paliwal and Ilangoran (1990). 200 mg of finely cut leaf tissue was taken after washing in a test tube containing 8 ml of phosphate buffer, 1 ml of 0.1 M KNO_3 and 1 ml of 5% propanol. Test tubes were incubated at 30^0C for 30 minutes. After this, test tubes were placed in a boiling water bath for 2 minutes. They were cooled and 1 ml of extract was taken from it and to which 1 ml of extract, 1 ml of NED was added which resulted in the development of pink colour. Absorbance was measured at 540 nm against distilled water as blank. The amount of nitrite was calculated from standard curve by using different concentration of KNO_3. KNO_3.was dissolved in distilled water and diluted to 0.2, 0.4, 0.8 and 1. To each test tube, 1 ml of 1% sulphanilamide and 1 ml of NED was added. The test tubes were incubated for 20 minutes to develop pink colour. The intensity of pink colour was measured at 540 nm. Absorbance values were plotted on y axis against the concentration of standard KNO_3 solution on x axis to get a standard curve.

3.4.8 Nutrient content:

1. Collection of leaf samples:

Composite leaf samples *i.e.* tender, medium and coarse leaves of each genotype were used for N, P and K estimation.

2. Processing of leaf samples:

The fresh samples were washed with tap water to decontaminate leaves from dust and other adhering material, followed by washing with acidified distilled waters to remove metallic contaminants. The samples were finally washed with distilled water thoroughly. The samples were then dried on filter papers, packed in paper bags and dried in oven at 70^0C for 24 hours. The samples were separately homogenized in a stainless steel blender to pass through 2 mm mesh sieve and were stored in air tight polythene bags for chemical analysis.

3. Analysis of leaf samples for nutrient estimation:

The processed leaf samples were subjected to nutrient analysis for recording the following parameters:

4. Nitrogen (%):

The nitrogen content was determined by Microkjaldal's method as described by Jackson (1973). 0.5 g of mulberry leaf sample was taken in a digestion tube to which 4-5 g of digestion mixture (10 parts of potassium sulphate and 0.5 parts of Copper sulphate) was added and digestion unit was allowed to run for 3 hours. The tube was then left undisturbed overnight and next day tube was fitted in the condensation unit. The contents were collected in a receiving flask which was then titrated against 0.1N Hydrochloric acid and nitrogen was calculated using the following formula:

$$\text{Nitrogen (\%)} = \frac{\text{(TV-Blank) x Normality of acid x DF}}{\text{Weight of sample}} \times 100$$

Where,

TV = Titration Value

DF = Digestion Factor

5. Phosphorus (%):

The phosphorus content in mulberry leaf was determined by Vandomolybedate phosphoric acid yellow colour method (Jackson, 1973). 0.5 g of mulberry leaf sample was taken in a flask and then 10 ml of di- acid (mixture of nitric acid and perchloric acid) in the ratio of 9:4 was added to it. The flask was then kept undisturbed overnight. Next day, it was placed on a hot plate at 115-120^{0}C for digestion till a watery transparent aliquot was obtained. The digested samples were then filtered and diluted with double distilled water to make up final volume to 100 ml. The contents were filtered through Whatman filter paper No. 1. The phosphorus in the extract was estimated by developing blue colour with ammonium molybedate using ascorbic acid as reluctant. Colour intensity was measured at 660 nm in spectrophotometer.

6. Potassium (%): Leaf potassium content was determined by flame photometry method using corning flame photometer, U.K.(Jackson,1973).Di-acid digestion of mulberry leaf samples was carried out in the same manner as described for estimation of phosphorus content in leaf. The digest was then introduced in the flame photometer to get the concentration of potassium expressed in per cent (Plate 5).

7. Calcium and Magnesium content (ppm):

The calcium and magnesium content was determined on Atomic absorption spectrophotometer (ECIL Model). The diacid digested leaf extract was filtered using Whatman No.1 filter paper. Then the extract was fed to atomic absorption spectrophotometer with acetylene cylinder for analysis of calcium and magnesium.

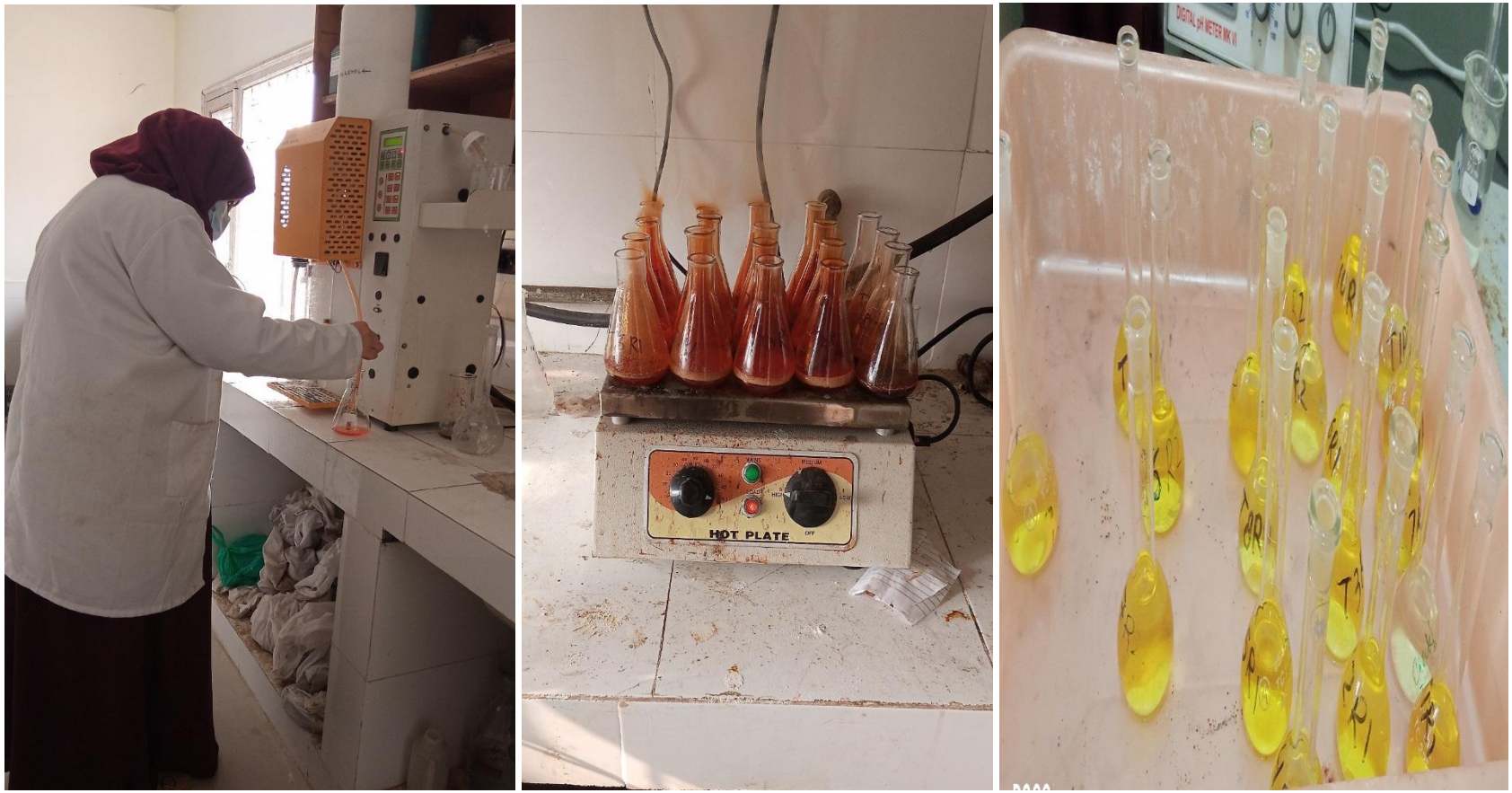


Plate 5: Nutrient estimation in mulberry leaves

8. Iron content (ppm):

The iron content was determined on Atomic absorption spectrophotometer (ECIL Model). The diacid digested leaf extract was filtered using Whatman No.1 filter paper. Then the extract was fed to atomic absorption spectrophotometer with acetylene cylinder for analysis of iron.

3.5 Biochemical studies

Following biochemical parameters were recorded :

3.5.1 Total soluble sugars (g/100g):

The total soluble sugar content in mulberry leaf of different genotypes was determined by the method of Morris (1948). For this, 0.5g of the leaf sample was homogenized in 10ml of 80% hot ethanol using a mortar and pestle. The resulting solution was centrifuged at 13000 rpm for 10 minutes and the supernatant was preserved. The residue was again extracted three times with hot 80% ethanol, centrifuged and the supernatants were pooled together. For determination of sugars, 500 µl of the extract was combined with 2.5 ml distilled water and 4ml anthrone reagent. This was followed by heating for exactly 8 minutes and then by rapid cooling at room temperature. The absorbance was recorded at 630 nm and amount of sugars present in each sample was calculated using glucose as standard and expressed as g equivalents per 100g leaves on fresh weight.

3.5.2 Total carotenoids (µg/g):

The total carotenoid content in the extract of leaf samples of the mulberry genotypes was determined as per method of Hiscox and Israelstam (1979). 2g of each leaf sample was homogenized in 20 ml of methanol followed by filtration on a Buchner funnel through Whatman No, 42 filter paper. The extraction was continuously repeated until the sample was free from pigments. The collected filtrates were pooled and saponified with 12% ethanolic potassium hydroxide (@ 1ml for every 10ml of extract) in a water bath at 60^0C for 30 minutes. This was followed by the addition of an equal amount of water to the resulting solution and

partitioning three times with an equal volume of petroleum ether in a separating funnel. The petroleum ether layers so obtained were combined and evaporated to dryness in a rotatory evaporator at 25^0C. The residue thus obtained was then dissolved in 5ml of ethanol and the absorbance was recorded at 450nm. A calibration curve was also established using ß- carotene as standard. The total carotenoid content in each sample was then calculated and the results were expressed as µg beta carotene equivalents per g leaf sample on dry weight basis.

3.5.3 Total soluble proteins (g/100g):

The total soluble protein content of leaves of different mulberry genotypes was estimated by the method of Lowry *et al.* (1951). 0.5g of each leaf sample was homogenized in 10ml of freshly prepared Sodium phosphate buffer (pH 7.4) centrifuged at 10,000 rpm for 10 minutes and the supernatant was collected. This step was repeated thrice for complete extraction. The supernatants were pooled and the final volume of each extract was made upto 50 ml.To 500µl of each sample extract, 4.5ml of reagent I (48ml of 2% Na_2CO_3 prepared in 0.1N NaOH + 1 ml of 1% sodium potassium tartarate + 1ml of 0.5% copper sulphate) was added and incubated for 15 minutes. This was followed by the addition of 500µl of freshly prepared reagent (Folin- Colcalteu reagent) to each sample and further incubation for 30 minutes in dark. The absorbance was then measured at 660 nm against reagent blank. A calibration curve was established using BSA equivalent/ g on fresh weight.

3.5.4 Total soluble carbohydrates (g/100g):

The total carbohydrate content was estimated by Anthrone method (Morris, 1948).100 mg of the leaf sample was weighed into a boiling tube, hydrolyzed by keeping it in boiling water bath for 3 hours with 5 ml of 2.5 N HCl and cooled to room temperature, it was then neutralized with sodium carbonate until effervescence ceased. Volume was made up to 100 ml and centrifuged and supernatant was collected. About 0.2- 1 ml of the sample was used for analysis. 1

ml of water served as blank.Volume was made up to 1 ml in all the tubes with distilled water, then 4 ml of anthrone reagent was added, heated for 8 minutes in a boiling water bath, cooled rapidly and the change in colour from green to dark green was read at 630 nm. A standard graph was drawn by taking the concentration of glucose on x- axis and spectrophotometer reading on y axis. From the graph, the concentration of the glucose in the sample was calculated.

3.5.5 Total phenols (g/100g):

The total phenol content of leaves of different mulberry genotypes was estimated by the method described by Malick and Singh (1980). Approximately, 500 mg of dried mulberry leaf powder was homogenized in ten times the volume of 80% ethanol and centrifuged at 13000 rpm. The supernatant was collected and the residue was again re extracted with five times the volume of 80% ethanol. The pooled supernatants were evaporated to dryness and finally reconstituted in known volume of double distilled water (5 ml). To determine the total phenol content, 500 µl of the reconstituted extract was first combined with 2.5 ml of double distilled water. Immediately, 0.5ml of Folin-Cioucalteau reagent was added to it. The mixture was incubated for 3 minutes after which 2ml of 20% sodium carbonate was added to each sample, vortexed and boiled in a water bath for exactly one minute. The absorbance of each sample was recorded at 650 nm against the reagent blank. Catechol was used as a standard to establish a standard curve and the concentration of phenol in each sample was determined accordingly (Plate 6).

The observations recorded were compiled and analysed as per “R” statistical package and correlations worked out.

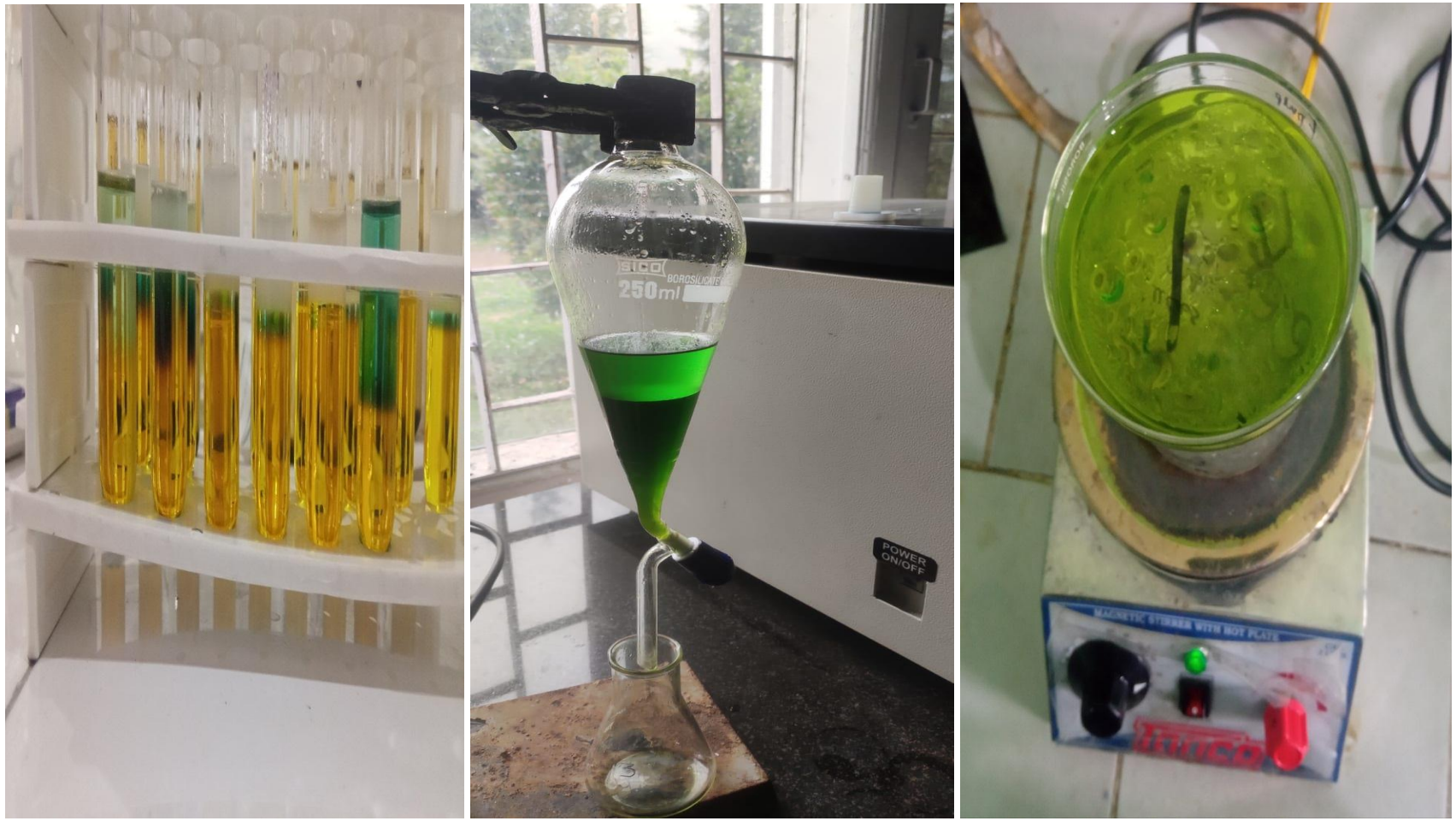


Plate 6: Processing of leaf samples for Biochemical analysis

Chapter 4

EXPERIMENTAL FINDINGS

4.1 Growth and yield parameters

The growth and yield parameters recorded during the study period (2021-2022) are furnished below:

4.1.1 Number of shootlets/ branches per plant

As a result of winter frost, the apical portion of the branches is damaged and the lower buds get chance to grow resulting in the formation of lateral shootlets during the spring crop. During the course of the present study, the number of shootlets per plant was the highest (65.00) in Chinese white being significantly higher than the rest of the genotypes. It was followed by 50.50 shootlets per plant in Rokokoyoso where as the least number of shootlets per plant (30.66) was recorded in Ichinose.

Number of branches per plant during autumn was the highest (48.33) in Rokokoyoso which was statistically higher than the rest of the genotypes. The number of branches per plant ranged from 31.50 in Ichinose to 45.83 in Chinese white (Table-1).

4.1.2 Total shootlet / branch length(cm)

Total shootlet length per plant during spring was the highest (2524.78 cm) in Chinese white which was statistically significant over the rest of the genotypes. It was, however, followed by Limoncina with 2462.76 and Rokokoyoso with 2413.63 centimetres shootlet length. The least shootlet length per plant (1260.80 cm) was registered in Ichinose.

During autumn, the total branch length per plant was the highest (6861.75 cm) in Rokokoyoso which was found to be statistically significant over the rest of the genotypes. The total branch length per plant ranged from 4437.20 in Ichinose to 6545.56 centimetres branch in Kairyoroso. (Table -2).

Table 1: Number of shootlets or branches per plant of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	40.33	40.66	40.50	35.41	35.62	35.50
G_2: Ichinose	31.00	30.33	30.66	30.93	32.07	31.50
G_3: KNG	38.66	40.00	39.33	34.29	34.35	34.32
G_4: Rokokoyoso	50.66	50.33	50.50	48.31	48.35	48.33
G_5: Kokusu-20	36.33	36.33	36.33	32.75	32.91	32.83
G_6: Kokuso- 21	33.66	33.00	33.33	31.68	31.68	31.33
G_7: Limoncina	43.67	44.66	44.16	41.76	41.90	41.83
G_8: Ensatakasuke	31.67	32.00	31.83	28.85	29.15	29.00
G_9: Chinese White	64.66	65.33	65.00	45.79	45.87	45.83
G_{10}: Kairyoroso	49.00	48.33	48.66	45.07	45.25	45.16
C.D ($P \leq 0.05$)	**1.345**	**1.359**	**1.392**	**1.312**	**1.314**	**1.309**

Table 2: Total shootlet or branch length per plant (cm) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	1919.93	1954.38	1937.16	6513.93	6530.73	6522.33
G_2: Ichinose	1260.63	1260.96	1260.80	4422.13	4452.26	4437.20
G_3: KNG	1315.16	1338.66	1326.91	5806.13	5837.86	5822.00
G_4: Rokokoyoso	2381.70	2445.56	2413.63	6853.73	6869.76	6861.75
G_5: Kokusu-20	1821.33	1852.60	1836.96	5962.53	5961.33	5961.85
G_6: Kokuso- 21	1815.83	1830.06	1822.95	5672.36	5679.40	5675.88
G_7: Limoncina	2457.30	2468.23	2462.76	6383.10	6344.83	6363.96
G_8: Ensatakasuke	1658.26	1656.50	1657.38	4537.06	4557.40	4547.23
G_9: Chinese White	2517.34	2532.22	2524.78	6467.10	6531.96	6499.53
G_{10}: Kairyoroso	2342.23	2338.40	2340.31	6526.53	6564.60	6545.56
C.D ($P \leq 0.05$)	**32.027**	**31.397**	**32.434**	**53.115**	**50.052**	**58.385**

4.1.3 Internodal distance (cm)

During spring, the internodal distance was the highest (5.46 cm) in Goshoerami being significantly higher than the rest of the genotypes except Rokokoyoso and Chinese white registering 5.40 cm internodal distance each. It was the least (4.00 cm) in Ensatakasuke.

During autumn, the internodal distance was the highest (6.90 cm) in Goshoerami being statistically at par with Kokuso-21 registering 6.87 centimetres internodal distance and significantly higher than the rest of the genotypes. Limoncina, on the other hand, registered the shortest internodal distance of 4.33 centimetres. (Table-3)

4.1.4 Fresh weight of 100 leaves (g)

The fresh weight of hundred leaves was more in autumn as compared to spring season. During spring, it was the highest (520.90 g) in Goshoerami being significantly higher than all the other genotypes. Limoncina, on the other hand registered the least fresh weight of hundred leaves (205.91 g).

During autumn, Goshoerami again registered the highest fresh weight of hundred leaves (606.91g) being statistically significant over the rest of the genotypes. The fresh weight of hundred leaves was the least (279.71 g) in Limoncina.in the rest of the genotypes it ranged from 320.66 grams in Rokokoyoso to 393.27 grams in Kokuso-20 (Table -4).

4.1.5 Leaf yield (kg)

The leaf yield per plant was the highest (6.75 kg) in Goshoerami being statistically significant over the rest of the genotypes. It was, however, followed by KNG with 4.83 kg leaf per plant where as the least yield per plant (2.27 kg) was recorded in in Limoncina.

The leaf yield per plant was in general more in autumn as compared to spring season. During autumn leaf yield per plant was the highest (7.69 kg) in Goshoerami being statistically significant over the rest of the genotypes. It was however followed by KNG, Kokuso -20 and Ichinose yielding respectively 5.61, 5.38 and 5.14 kg of leaf per plant. The yield per plant was again the least (3.17 kg) in Limoncina (Table-5).

Table 3: Internodal distance (cm) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	5.43	5.47	5.46	6.80	6.93	6.90
G_2: Ichinose	4.27	4.33	4.30	4.87	5.07	4.97
G_3: KNG	5.00	5.13	5.07	6.23	6.37	6.30
G_4: Rokokoyoso	5.43	5.37	5.40	5.17	5.27	5.21
G_5: Kokusu-20	4.33	4.50	4.42	6.40	6.37	6.38
G_6: Kokuso- 21	4.20	4.23	4.22	6.83	6.97	6.87
G_7: Limoncina	4.60	4.53	4.57	4.47	4.87	4.67
G_8: Ensatakasuke	3.90	4.10	4.00	4.23	4.43	4.33
G_9: Chinese White	5.37	5.43	5.40	5.10	5.20	5.15
G_{10}: Kairyoroso	4.20	4.40	4.30	4.70	4.90	4.80
C.D ($P \leq 0.05$)	**0.139**	**0.146**	**0.135**	**0.181**	**0.176**	**0.183**

Table 4: Fresh weight of hundred leaves (g) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	519.73	522.07	520.90	604.23	609.60	606.91
G_2: Ichinose	286.54	284.07	285.31	376.56	378.90	377.73
G_3: KNG	303.59	307.90	305.74	339.42	340.29	339.85
G_4: Rokokoyoso	259.93	259.71	259.82	320.29	321.03	320.66
G_5: Kokusu-20	292.08	291.83	292.11	392.57	393.97	393.27
G_6: Kokuso- 21	285.71	285.96	285.83	584.66	584.78	389.76
G_7: Limoncina	204.76	207.06	205.91	278.89	280.89	279.71
G_8: Ensatakasuke	267.06	266.43	266.74	427.29	428.23	427.76
G_9: Chinese White	254.61	254.22	254.42	323.67	323.51	323.59
G_{10}: Kairyoroso	257.68	256.62	257.15	320.61	320.78	320.69
C.D (P≤0.05)	**2.815**	**2.401**	**1.786**	**2.320**	**2.000**	**1.478**

Table 5: Leaf Yield per Plant (kg) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	6.69	6.82	6.75	7.67	7.71	7.69
G_2: Ichinose	4.31	4.39	4.35	5.17	5.12	5.14
G_3: KNG	4.78	4.87	4.83	5.66	5.56	5.61
G_4: Rokokoyoso	3.47	3.49	3.48	4.55	4.54	4.55
G_5: Kokusu-20	4.60	4.65	4.62	5.34	5.42	5.38
G_6: Kokuso- 21	4.24	4.30	4.27	5.01	5.03	5.02
G_7: Limoncina	2.33	2.22	2.27	3.15	3.19	3.17
G_8: Ensatakasuke	4.21	4.28	4.25	4.81	4.82	4.83
G_9: Chinese White	2.39	2.42	2.41	3.27	3.29	3.28
G_{10}: Kairyoroso	3.21	3.33	3.27	4.28	4.35	4.32
C.D ($P \leq 0.05$)	**0.459**	**0.405**	**0.458**	**0.416**	**0.449**	**0.490**

4.2 Leaf Moisture Studies

The moisture content in fresh leaf and its retention capacity after 6 hours and 12 hours of harvest recorded during the study period (2021-2022) are furnished below:

4.2.1.1 Moisture content of fresh leaf at chawkie stage (35 days after sprouting) of silkworm rearing:

During spring, the moisture content in fresh leaf at chawkie stage of silkworm rearing was the highest (78.85%) in Goshoerami being significantly higher than the rest of genotypes. The moisture content in fresh leaf at chawkie stage of silkworm rearing ranged from 73.29 % in Limoncina to 76.84 % in KNG.

The moisture content in fresh leaf was in general more during spring as compared to autumn season. It was the highest (75.71%) in Goshoerami being statistically significant over rest of the genotypes. It was followed by KNG with 72.22 % moisture content. Kairyoroso and Limoncina recorded the leaf moisture content of 69.92% and 69.90% respectively (Table-6).

4.2.2 Moisture content of fresh leaf at late age (65 days after sprouting) of silkworm rearing:

During spring, the moisture content in fresh leaf at late age of silkworm rearing was again the highest (76.61%) in Goshoerami, being statistically significant over the remaining genotypes. It was the least (70.11 %) in Limoncina.

During autumn, the moisture content in fresh leaf at late age of silkworm rearing was again the highest (73.09%) in Goshoerami being significantly higher than the remaining genotypes. The moisture content of the leaf was the least (65.18%) in Limoncina (Table-7).

Table 6: Leaf Moisture content (%) at chawkie stage of silkworm rearing of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	78.53	79.16	78.85	75.53	75.89	75.71
G_2: Ichinose	74.25	74.32	74.28	71.14	71.16	71.15
G_3: KNG	76.88	76.80	76.84	72.25	72.19	72.22
G_4: Rokokoyoso	74.57	74.78	74.67	70.15	70.17	70.16
G_5: Kokusu-20	75.18	74.98	75.08	71.64	71.91	71.77
G_6: Kokuso- 21	74.81	74.68	74.74	71.39	71.36	71.37
G_7: Limoncina	73.33	73.24	73.29	69.93	69.87	69.90
G_8: Ensatakasuke	74.24	74.29	74.26	70.29	70.31	70.30
G_9: Chinese White	73.46	73.85	73.65	70.05	70.13	70.09
G_{10}: Kairyoroso	74.23	74.24	74.24	69.89	69.95	69.92
C.D (P≤0.05)	**0.166**	**0.141**	**0.105**	**0.413**	**0.445**	**0.471**

Table 7: Leaf moisture content (%) at late age of silkworm rearing of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	76.40	76.82	76.61	73.05	73.14	73.09
G_2: Ichinose	72.13	72.26	72.19	70.67	70.85	70.76
G_3: KNG	73.43	73.31	73.37	71.78	70.61	71.19
G_4: Rokokoyoso	72.92	72.73	72.82	69.96	69.88	69.92
G_5: Kokusu-20	73.84	73.94	73.89	70.86	70.94	70.89
G_6: Kokuso- 21	72.78	72.65	72.71	70.79	70.71	70.75
G_7: Limoncina	70.09	70.14	70.11	69.42	60.95	65.18
G_8: Ensatakasuke	72.68	72.81	72.74	70.21	70.25	70.23
G_9: Chinese White	72.09	72.40	72.24	69.24	69.26	69.25
G_{10}: Kairyoroso	73.59	73.82	73.70	69.53	69.65	69.59
C.D ($P \leq 0.05$)	**0.652**	**0.612**	**0.626**	**0.575**	**0.678**	**0.534**

4.2.3 Moisture retention capacity after 6 hours

During spring, the moisture retention capacity of mulberry leaf after 6 hours was the highest (93.45%) in Goshoerami, being significantly higher than the remaining genotypes where the values ranged from 77.60 percent in Limoncina to 87.21 percent in Ichinose.

During autumn, the moisture retention capacity of leaf after 6 hours was the highest (91.66%) in Goshoerami being significantly higher than the rest of the genotypes. The moisture retention capacity after 6 hours was the least (80.35 %) in Limoncina (Table-8).

4.2.4 Moisture retention capacity after 12 hours

During spring, moisture retention capacity after 12 hours followed the same trend as that after 6 hours of harvest with the highest (87.97%) in Goshoerami being statistically significant over the rest of the genotypes where the values ranged from 69.06 percent in Limoncina to 81.46 percent in Kokuso-20.

During autumn, the highest moisture retention capacity of leaf after 12 hours (81.35%) was registered by Goshoerami being statistically significant over rest of the genotypes. It was the least (71.27 %) in Limoncina (Table-9).

Table 8: Moisture retention capacity (%) of leaf after 6 hours of harvest of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	93.22	93.67	93.45	91.38	91.95	91.66
G_2: Ichinose	87.14	87.29	87.21	87.34	87.47	87.40
G_3: KNG	86.58	86.36	86.47	84.86	84.75	84.80
G_4: Rokokoyoso	80.45	80.39	80.42	82.11	82.94	82.52
G_5: Kokusu-20	85.85	85.98	85.91	82.78	82.95	82.87
G_6: Kokuso- 21	84.72	84.88	84.80	81.10	81.31	81.21
G_7: Limoncina	77.54	77.66	77.60	80.29	80.39	80.35
G_8: Ensatakasuke	81.34	81.61	81.48	80.81	80.99	80.91
G_9: Chinese White	78.25	78.70	78.47	80.64	80.76	80.70
G_{10}: Kairyoroso	80.27	80.49	80.38	81.75	81.95	81.85
C.D ($P \leq 0.05$)	**0.735**	**0.825**	**0.763**	**0.121**	**0.171**	**0.584**

Table 9: Moisture retention capacity (%) of leaf after 12 hours of harvest of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	87.91 (9.43)	88.04 (9.44)	87.97 (9.43)	81.18	81.52	81.35
G_2: Ichinose	81.09 (9.06)	81.41 (9.08)	81.25 (9.07)	77.19	77.32	77.25
G_3: KNG	78.89 (8.94)	79.05 (8.95)	78.97 (8.94)	75.31	75.56	75.44
G_4: Rokokoyoso	69.29 (8.38)	69.41 (8.39)	69.35 (8.39)	71.45	71.53	71.49
G_5: Kokusu-20	81.38 (9.07)	81.55 (9.08)	81.46 (9.08)	77.47	77.65	77.56
G_6: Kokuso- 21	77.68 (8.87)	77.86 (8.88)	77.77 (8.87)	75.08	75.23	75.15
G_7: Limoncina	68.95 (8.36)	69.16 (8.38)	69.06 (8.37)	71.21	71.32	71.27
G_8: Ensatakasuke	73.60 (8.64)	73.77 (8.65)	73.68 (8.64)	76.90	77.22	77.06
G_9: Chinese White	70.92 (8.48)	71.12 (8.49)	71.02 (8.49)	77.65	77.85	77.75
G_{10}: Kairyoroso	69.42 (8.39)	69.91 (8.42)	69.66 (8.41)	77.11	77.24	77.18
C.D (P≤0.05)	0.171	0.171	0.117	0.749	0.184	0.961

*Values in parenthesis are square root transformed values

4.3 Anatomical studies

The leaf is dorsiventral with reticulate venation. The upper epidermis usually consists of a single layer of compactly arranged cells. Their continuity is broken at certain places due to enlargement of the cells in the form of idioblasts. The outer walls of the epidermal cells are often cutinized and a thick layer of cuticle is formed. Stomata are absent but cystoliths are quite common. In surface view, the epidermal cells form a sheath (1to 2 layered) sometimes stellate around the base of trichome.

The lower epidermis is much like the upper epidermis but with a thinner cuticle. Idioblasts are absent. Stomata are quite common and are of ranunculaceous type. In Rokokoyoso, some enlarged stomata are noticed in addition to the normal ones.

Mesophyll is clearly differentiated into palisade and spongy tissues. The palisade is 1-2 layered and confined to the upper surface. The cells are cylindrical, thin walled and compactly arranged with their long axis at right angles to upper epidermis. Chloroplasts are numerous and marginal. Sometimes it occurs in more than one layer. Spongy parenchyma consists of isodiametric or slightly elongated cells with fewer chloroplasts. The cells are loosely packed leaving large intercellular spaces and respiratory cavities near the stomata. The observations recorded in respect of various foliar anatomical features are furnished below:

4.3.1 Upper and lower cuticular thickness (µm)

The thickness of upper cuticle was the highest (11.362 µm) in Goshoerami being significantly higher than the rest of the genotypes. It was the least (5.413 µm) in Chinese white.

The lower cuticle thickness was the highest (5.978 µm) in Rokokoyoso which was statistically at par with Kairyoroso having 5.935 µm thick lower cuticle. In the remaining genotypes, the thickness ranged from 4.177 µm in KNG to 5.192 µm in Goshoerami (Table-10).

Table 10: Upper and lower cuticular thickness (µm) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Upper cuticular thickness		Pooled over Spring	Lower cuticular thickness		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	11.373	11.350	11.362	5.183	5.200	5.192
G_2: Ichinose	7.710	7.713	7.712	4.333	4.337	4.335
G_3: KNG	7.967	7.977	7.972	4.173	4.180	4.177
G_4: Rokokoyoso	5.913	5.920	5.917	5.977	5.980	5.978
G_5: Kokusu-20	7.620	7.617	7.618	4.207	4.217	4.212
G_6: Kokuso- 21	7.687	7.683	7.685	4.233	4.237	4.235
G_7: Limoncina	5.417	5.417	5.417	4.543	4.547	4.545
G_8: Ensatakasuke	5.963	5.967	5.965	4.253	4.260	4.257
G_9: Chinese White	5.413	5.413	5.413	4.523	4.540	4.532
G_{10}: Kairyoroso	5.873	5.880	5.877	5.933	5.937	5.935
C.D ($P \leq 0.05$)	**0.832**	**0.810**	**0.829**	**0.733**	**0.632**	**0.780**

4.3.2 Upper and lower epidermal thickness (µm)

The upper epidermis in all the genotypes is thicker than the lower epidermis. The upper epidermis was thickest (24.733 µm) in KNG being statistically significant over the rest of the genotypes. On the other hand, the thickness was the least (20.160 µm) in Chinese white.

The lower epidermis on the other hand, was the thickest (13.315µm) in Ichinose being statistically at par with that of Kokuso-21 (12.977 µm) and Kokusu-20 (12.935 µm) and significant over the remaining genotypes. The lower epidermal thickness was the least (7.408 µm) in Goshoerami (Table-11).

4.3.3 Palisade thickness (µm) and palisade proportion (%)

The palisade thickness was the highest (65.447µm) in Goshoerami being significantly higher than the rest of the genotypes. The palisade thickness was the least (57.600 µm) in KNG.

Chinese white registered the highest palisade proportion of 41.485 percent being statistically at par with Limoncina registering 41.463 percent palisade proportion and significant over rest of the genotypes. Palisade proportion was the least (31.652 %) in Ichinose (Table-12).

4.3.4 Spongy thickness (µm) and Spongy proportion (%)

The spongy thickness, in general, was more than palisade thickness. It was the highest (81.790 µm) in Goshoerami being statistically significant over the rest of the genotypes where it ranged from 42.278 µm in Limoncina to 77.889 µm in Kokusu-21.

The spongy proportion was the highest (41.968%) in Goshoerami being statistically at par with Ichinose (41.688%) and significant over the rest of the genotypes with the least (29.818 %) in Limoncina (Table-13) Plate 7-8.

Table 11: Upper and lower epidermal thickness (µm) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Upper epidermal thickness		Pooled over Spring	Lower epidermal thickness		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	23.663	23.676	23.670	7.413	7.403	7.408
G_2: Ichinose	23.167	23.180	23.173	13.313	13.317	13.315
G_3: KNG	24.730	24.736	24.733	12.743	12.753	12.748
G_4: Rokokoyoso	22.867	22.877	22.872	7.540	7.557	7.548
G_5: Kokusu-20	23.417	23.423	23.420	12.930	12.940	12.935
G_6: Kokuso- 21	23.447	23.457	23.452	12.970	12.983	12.977
G_7: Limoncina	20.183	20.203	20.193	10.557	10.563	10.560
G_8: Ensatakasuke	23.067	23.077	23.072	11.250	11.267	11.258
G_9: Chinese White	20.163	20.157	20.160	10.513	10.517	10.515
G_{10}: Kairyoroso	22.843	22.853	22.848	7.526	7.520	7.523
C.D ($P \leq 0.05$)	**0.513**	**0.371**	**0.545**	**0.493**	**0.425**	**0.496**

Table 12: Palisade thickness (µm) and proportion (%) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Palisade thickness(µm)		Pooled over Spring	Palisade proportion (%)		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	65.400	65.493	65.447	33.570	33.600	33.585
G_2: Ichinose	57.653	57.713	57.683	31.650	31.653	31.652
G_3: KNG	57.560	57.640	57.600	32.413	32.370	32.392
G_4: Rokokoyoso	62.197	62.527	62.362	37.540	37.620	37.580
G_5: Kokusu-20	58.900	59.043	58.972	32.147	32.177	32.162
G_6: Kokuso- 21	62.630	62.710	62.670	33.180	33.173	33.177
G_7: Limoncina	58.776	58.817	58.797	41.470	41.457	41.463
G_8: Ensatakasuke	59.260	59.023	59.142	33.267	33.103	33.185
G_9: Chinese White	59.577	59.647	59.612	41.407	41.563	41.485
G_{10}: Kairyoroso	61.263	61.333	61.298	37.513	37.527	37.520
C.D ($P \leq 0.05$)	**0.119**	**0.171**	**0.157**	**0.118**	**0.172**	**0.109**

Table 13: Spongy thickness (µm) and proportion (%) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spongy thickness (µm)		Pooled over Spring	Spongy proportion (%)		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	81.757	81.823	81.790	41.970	41.967	41.968
G_2: Ichinose	75.973	76.063	76.018	41.660	41.717	41.688
G_3: KNG	70.393	70.770	70.582	39.640	39.747	39.693
G_4: Rokokoyoso	61.190	61.350	61.270	36.930	36.910	36.920
G_5: Kokusu-20	76.150	76.250	76.200	41.560	41.553	41.557
G_6: Kokuso- 21	77.810	77.967	77.889	41.220	41.243	41.231
G_7: Limoncina	42.247	42.310	42.278	29.813	29.823	29.818
G_8: Ensatakasuke	73.660	73.733	73.697	41.353	41.347	41.350
G_9: Chinese White	43.147	43.213	43.180	30.100	30.117	30.108
G_{10}: Kairyoroso	59.863	59.907	59.885	36.653	36.653	36.653
C.D ($P \leq 0.05$)	**0.406**	**0.405**	**0.408**	**0.362**	**0.374**	**0.377**

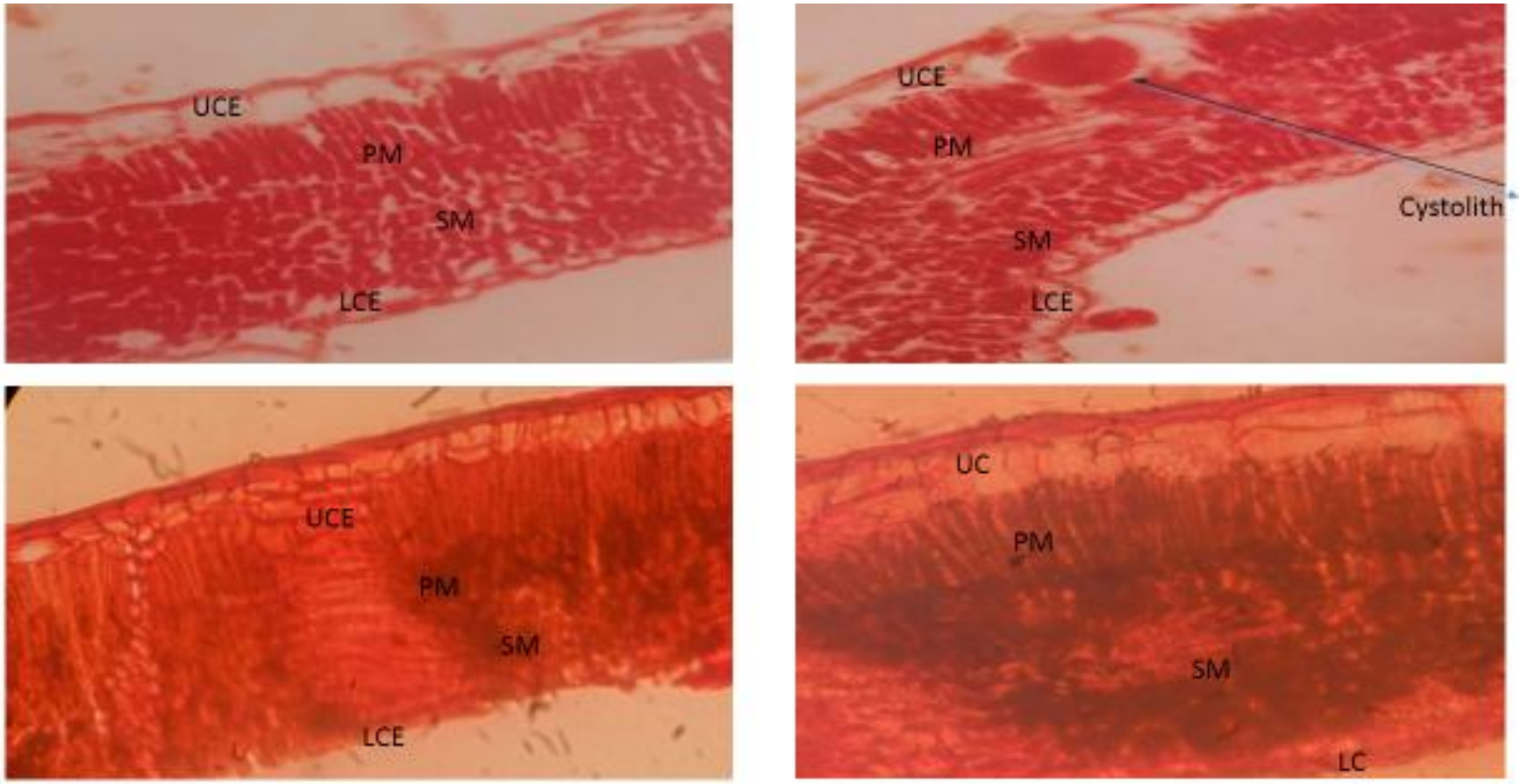


Plate 7: Cross sections of mulberry leaf showing various tissues

UCE= upper cuticle and epidermis, LCE =Lower cuticle and epidermis, PM= Palisade mesophyll, SM = Spongy mesophyll, UC = Upper cuticle, LC = Lower cuticle.

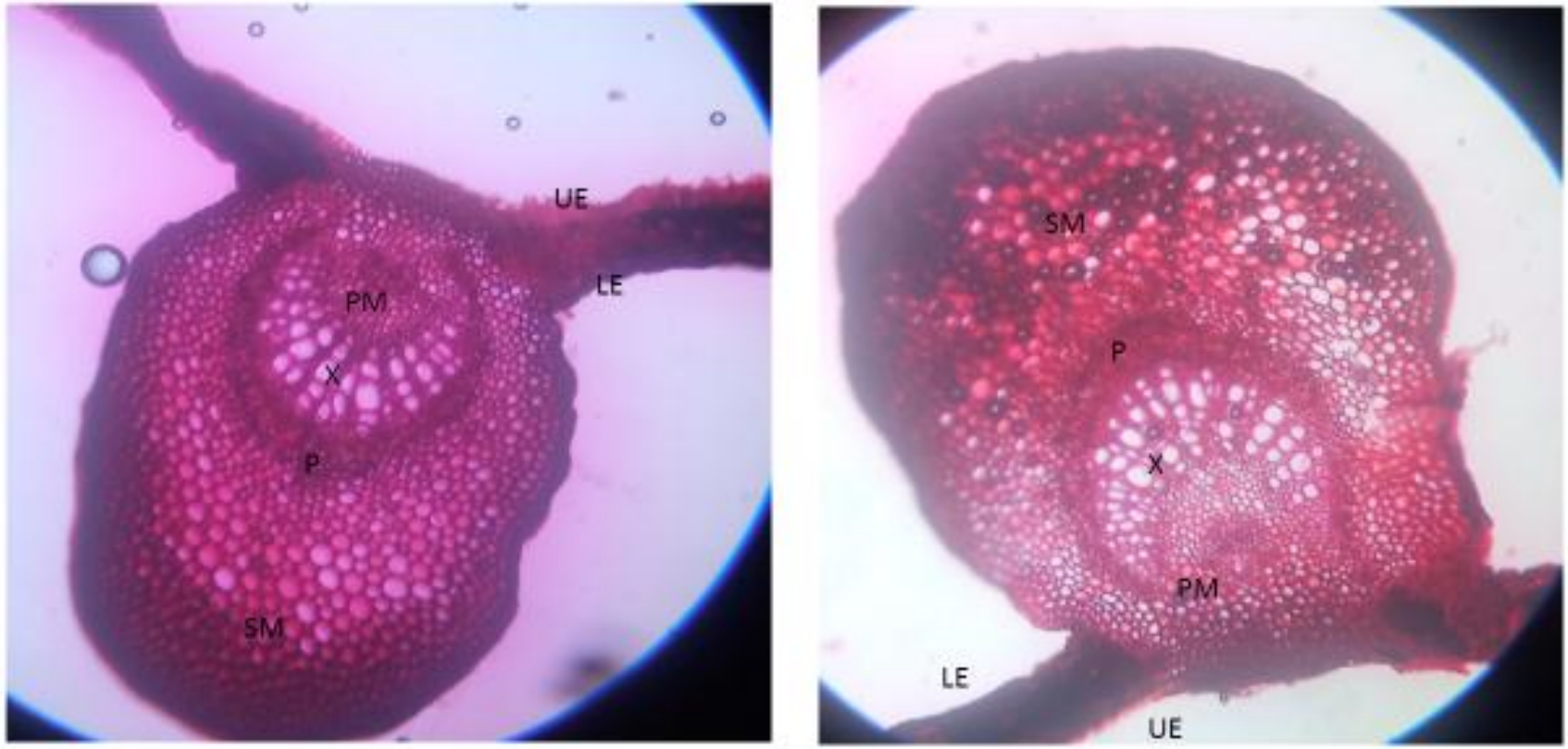


Plate 8: Transverse section of mulberry leaf showing various tissues

UE= Upper epidermis, LE =Lower epidermis, PM= Palisade mesophyll, SM = Spongy mesophyll, X=xylem, P= Phloem

4.3.5 Total leaf thickness (µm)

The total leaf thickness was the highest (194.858 µm) in Goshoerami being significantly higher than the rest of the genotypes. In the remaining genotypes, it ranged from 141.790 µm in Limoncina to 188.907 µm in Kokusu-21 (Table-14).

4.3.6 Stomatal frequency (mm^2) and Stomatal index (%)

The stomatal frequency was the maximum (711.061 stomata per mm^2) in Rokokoyoso being statistically significant over the rest of the genotypes where the frequency ranged from 432.020 stomata per mm^2 in KNG to 698.183 stomata per mm^2 in Goshoerami.

The stomatal index was the highest (23.572%) in Rokokoyoso being significantly higher than rest of the genotypes. It was the least (18.168%) in KNG (Table-15).

4.3.7 Stomatal length and Stomatal width (µm)

The stomatal length was the highest (24.135 µm) in Chinese white which was statistically at par with 24.047 µm in Limoncina and significant over the rest of the genotypes. It was the least (19.807 µm) in Goshoerami.

The stomatal width too was the highest (11.781 µm) in Chinese white being statistically at par again with Limoncina registering 11.718 µm stomatal width and significant over the rest of the genotypes. The least stomatal width (9.958 µm) was again recorded in Goshoerami (Table-16) Plate 9-10.

Table 14: Leaf thickness (µm) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Autumn 2021	Autumn 2022	Pooled over Seasons
G_1: Goshoerami	194.790	194.927	194.858
G_2: Ichinose	182.150	182.323	182.237
G_3: KNG	177.567	178.057	177.812
G_4: Rokokoyoso	165.683	166.210	165.947
G_5: Kokusu-20	183.223	183.490	183.357
G_6: Kokuso- 21	188.776	189.037	188.907
G_7: Limoncina	141.723	141.857	141.790
G_8: Ensatakasuke	178.120	178.327	178.223
G_9: Chinese White	143.337	143.500	143.418
G_{10}: Kairyoroso	163.303	163.430	163.367
C.D (P≤0.05)	**0.619**	**0.606**	**0.617**

Table 15: Stomatal frequency (No of stomata/mm²) and stomatal index (%) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Genotype \ Season	Stomatal frequency (No. of stomata/mm²)		Pooled over Spring	Stomatal index (%)		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	698.697	697.670	698.183	22.317	22.330	22.323
G_2: Ichinose	617.220	618.157	617.688	20.377	20.383	20.380
G_3: KNG	432.003	432.037	432.020	18.130	18.207	18.168
G_4: Rokokoyoso	710.693	711.430	711.061	23.537	23.607	23.572
G_5: Kokusu-20	652.287	653.347	652.817	21.697	21.767	21.732
G_6: Kokuso- 21	657.780	657.906	657.843	21.883	21.893	21.888
G_7: Limoncina	466.350	466.143	466.247	18.410	18.443	18.427
G_8: Ensatakasuke	472.910	473.720	473.315	19.417	19.423	19.420
G_9: Chinese White	450.987	451.030	451.008	18.350	18.367	18.358
G_{10}: Kairyoroso	688.733	687.877	688.305	22.097	22.153	22.125
C.D (P≤0.05)	**0.749**	**0.744**	**0.756**	**0.414**	**0.402**	**0.417**

Table 16: Stomatal length and stomatal width (µm) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Stomatal length(µm)		Pooled over Spring	Stomatal width (µm)		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	19.793	19.820	19.807	9.947	9.970	9.958
G_2: Ichinose	20.570	20.597	20.583	10.680	10.693	10.686
G_3: KNG	20.050	20.053	20.052	9.983	10.00	9.992
G_4: Rokokoyoso	20.787	20.813	20.800	11.050	11.070	11.060
G_5: Kokusu-20	20.333	20.360	20.347	10.660	10.660	10.660
G_6: Kokuso- 21	19.910	20.607	20.258	10.533	10.520	10.526
G_7: Limoncina	24.037	24.057	24.047	11.677	11.76	11.718
G_8: Ensatakasuke	20.650	20.670	20.660	10.740	10.760	10.750
G_9: Chinese White	24.127	24.143	24.135	11.780	11.783	11.781
G_{10}: Kairyoroso	22.023	22.047	22.035	10.830	10.840	10.835
C.D ($P \leq 0.05$)	**0.137**	**0.148**	**0.153**	**0.490**	**0.481**	**0.499**

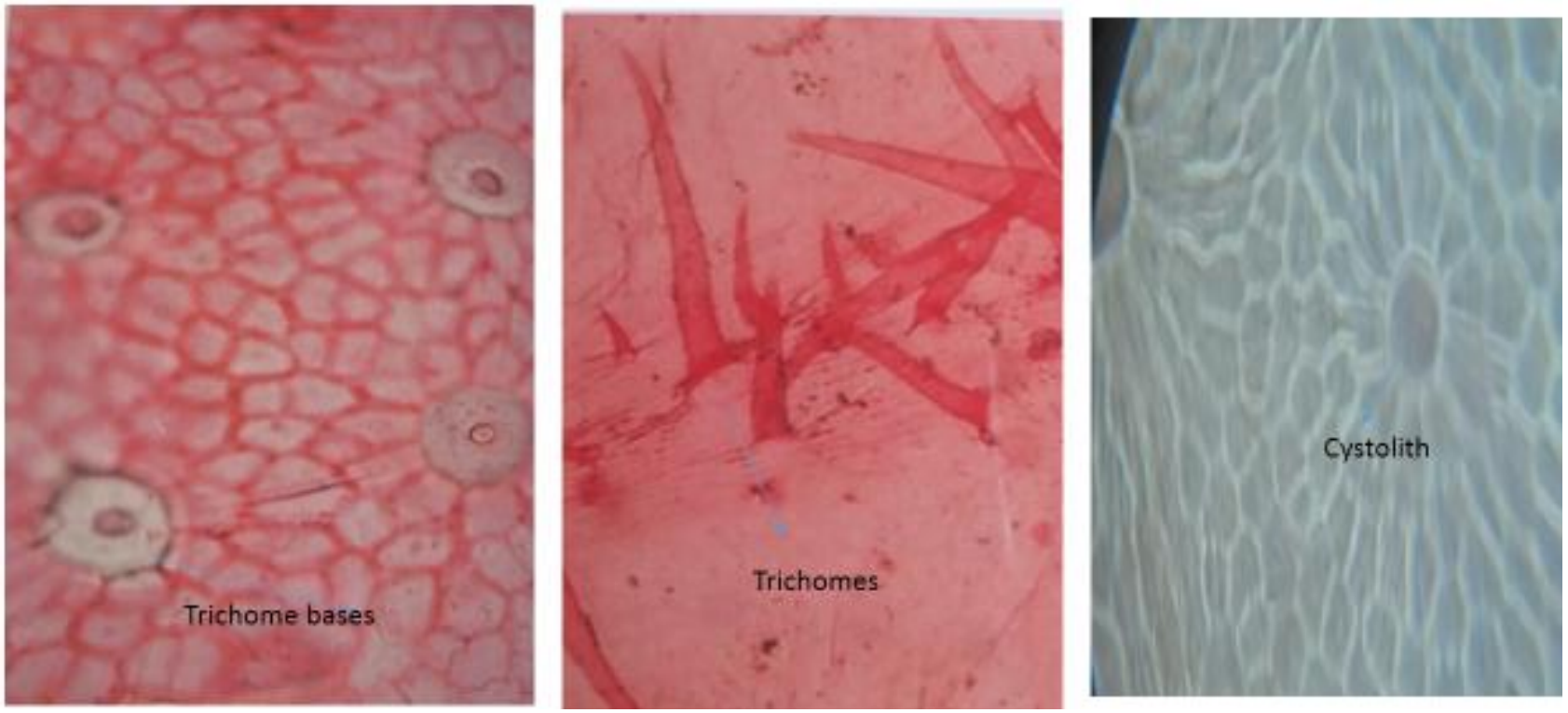


Plate 9: Upper epidermal peel showing Trichome bases, trichomes and cystoliths

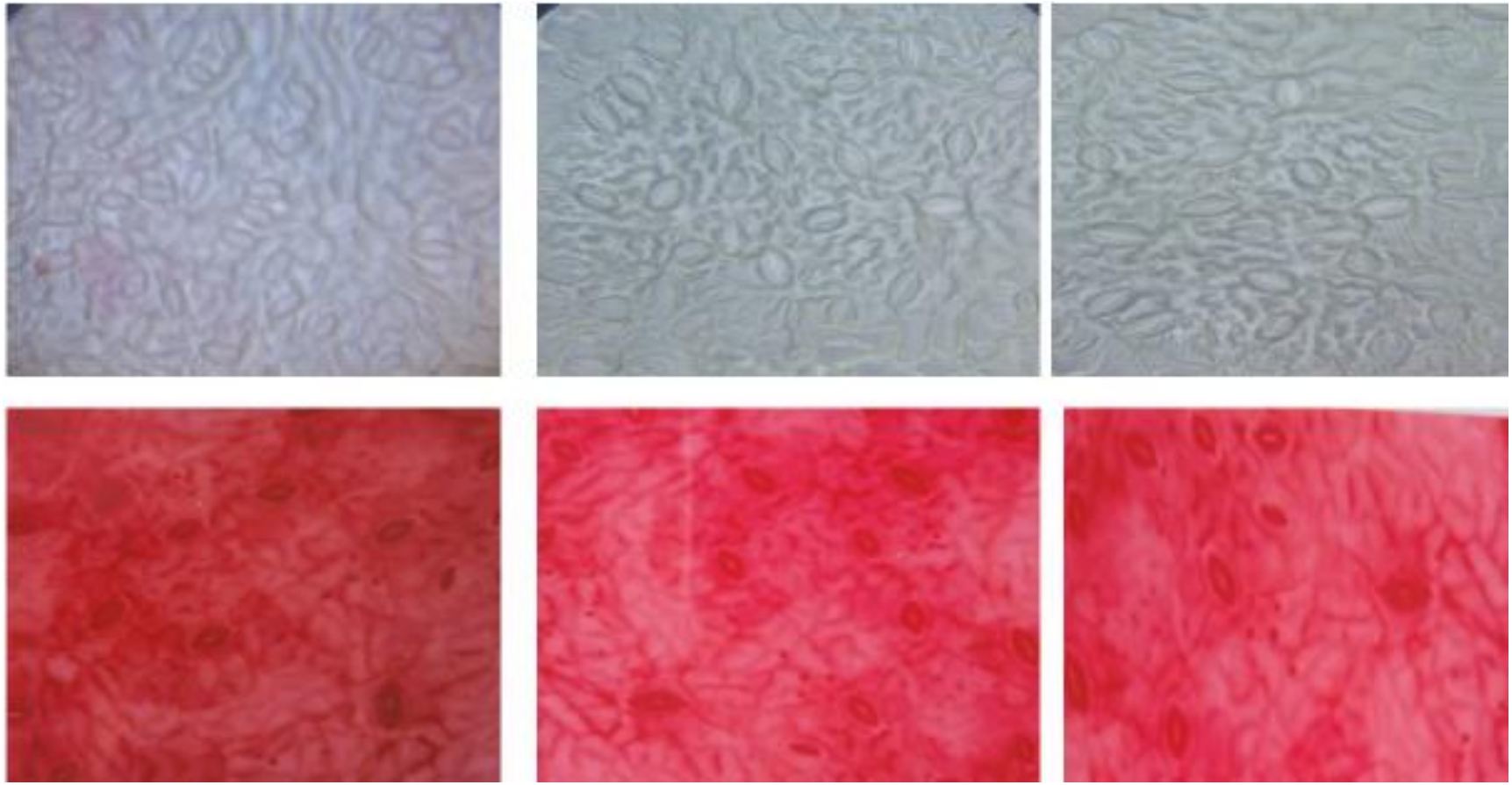


Plate 10: Lower epidermal peel showing stomata

4.4 Physiological studies

The observations recorded during the course of study (2021-2022) are briefly described below:

4.4.1 Leaf Absolute Growth Rate (g/day):

Absolute Growth Rate recorded thrice at an interval of 15 days during spring was significantly the highest in case of Goshoerami with 2.65 grams, 5.98 grams and 7.20 grams per day for the three intervals respectively whereas, it was the least (1.57, 2.77 and 3.68 grams per day for the three intervals respectively) in case of Chinese white. The Absolute Growth Rate of all the genotypes showed an increasing trend from the first interval to the third interval (Table-17).

Absolute Growth Rate recorded for three intervals during the 2nd crop i.e. 1st August -15th September too was the highest in case of Goshoerami with 3.28, 6.85 and 9.81 grams per day where as it was the least in Chinese white with 1.90 grams per day during the first interval (1st -15th August), 3.81 grams per day during the 2nd interval (16th -31 August) and 4.42 grams per day during the 3rd interval (1st -15th September). During autumn crop also the leaf Absolute Growth Rate showed an increasing trend from the first to the third interval (Table-18).

4.4.2 Leaf net assimilation rate (mg/cm^2 /day)

During the three intervals of spring crop, Goshoerami reported the maximum leaf net assimilation rate with 0.9445, 0.9703 and 0.8286 mg/cm^2 /day respectively for the 1st (10 -25th April), 2nd (26 April-10 May) and 3rd (11-25th May) intervals being statistically at par with KNG and Kokuso-21 and significant over the rest of the genotypes. It was the least in Chinese white with 0.8741,0.8907 and 0.7525 mg/cm^2 / day for the 1st (10 -25th April), 2nd (26 April-10 May) and 3rd (11-25th May) intervals respectively (Table-19).

Table 17: Absolute Growth Rate (g/day) at an interval of 15 days of mulberry genotypes during spring season (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring 2021			Spring 2022			Pooled over Spring		
	10 April-25 April	26April-10 May	11 May-25 May	10 April-25 April	26April-10 May	11 May-25 May	10 April-25 April	26April-10 May	11 May-25 May
G_1: Goshoerami	2.63	5.96	7.19	2.67	6.00	7.21	2.65	5.98	7.20
G_2: Ichinose	1.63	4.73	6.47	1.67	4.83	6.49	1.65	4.78	6.48
G_3: KNG	2.21	3.46	5.61	2.25	3.52	5.69	2.23	3.49	5.65
G_4: Rokokoyoso	2.46	4.15	5.76	2.48	4.19	5.80	2.47	4.17	5.78
G_5: Kokusu-20	1.56	4.14	6.37	1.58	4.18	6.39	1.57	4.16	6.38
G_6: Kokuso- 21	2.42	3.65	5.62	2.46	3.69	5.68	2.44	3.67	5.65
G_7: Limoncina	1.67	3.17	4.56	1.69	3.21	4.60	1.68	3.19	4.58
G_8: Ensatakasuke	1.85	4.15	5.56	1.89	4.19	5.90	1.87	4.17	5.88
G_9: Chinese White	1.54	2.74	3.64	1.60	2.80	3.72	1.57	2.77	3.68
G_{10}: Kairyoroso	1.85	3.47	4.66	1.91	3.53	4.70	1.88	3.50	4.68
C.D (P≤0.05)	**0.101**	**0.125**	**0.230**	**0.104**	**0.128**	**0.132**	**0.103**	**0.127**	**0.231**

Table 18: Absolute Growth Rate (g/day) at an interval of 15 days of mulberry genotypes during autumn season (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring 2021			Spring 2022			Pooled over Spring		
	1-15 August	16-30 August	1-15 Sept.	1-15 August	16-30 August	1-15 Sept.	1-15 August	16-30 August	1-15 Sept.
G_1: Goshoerami	3.25	6.82	9.79	3.31	6.88	9.83	3.28	6.85	9.81
G_2: Ichinose	1.94	5.01	8.12	1.96	5.05	8.16	1.95	5.03	8.14
G_3: KNG	2.59	3.95	6.21	2.63	3.99	6.25	2.61	3.97	6.23
G_4: Rokokoyoso	2.49	4.88	6.79	2.53	4.92	6.83	2.51	4.90	6.81
G_5: Kokusu-20	1.91	4.95	7.29	1.93	4.99	7.31	1.92	4.97	7.30
G_6: Kokuso- 21	2.91	4.29	6.31	2.95	4.31	6.35	2.93	4.30	6.33
G_7: Limoncina	2.02	3.97	5.22	2.06	4.10	5.26	2.04	3.99	5.24
G_8: Ensatakasuke	2.35	4.48	6.07	2.39	5.20	6.09	2.37	5.00	6.08
G_9: Chinese White	1.88	3.79	4.40	1.92	3.83	4.44	1.90	3.81	4.42
G_{10}: Kairyoroso	2.32	4.63	5.90	2.38	4.67	5.94	2.35	4.65	5.92
C.D (P≤0.05)	**0.116**	**0.142**	**0.261**	**0.120**	**0.144**	**0.262**	**0.118**	**0.145**	**0.264**

Table 19: Leaf Net assimilation rate (mg/cm^2/day) at an interval of 15 days of mulberry genotypes during spring season (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring 2021			Spring 2022			Pooled over Spring		
	10 April-25 April	26April-10 May	11 May-25 May	10 April-25 April	26April-10 May	11 May-25 May	10 April-25 April	26April-10 May	11 May-25 May
G_1: Goshoerami	0.9442	0.9701	0.8259	0.9448	0.9705	0.8265	0.9445	0.9703	0.8262
G_2: Ichinose	0.9385	0.9662	0.8175	0.9391	0.9666	0.8179	0.9388	0.9664	0.8177
G_3: KNG	0.9385	0.9663	0.8174	0.9389	0.9667	0.8176	0.9387	0.9665	0.8175
G_4: Rokokoyoso	0.9336	0.9662	0.8156	0.9340	0.9666	0.8162	0.9338	0.9664	0.8159
G_5: Kokusu-20	0.9395	0.9655	0.8179	0.9397	0.9657	0.8183	0.9396	0.9656	0.8181
G_6: Kokuso- 21	0.9394	0.9633	0.8165	0.9398	0.9637	0.8171	0.9396	0.9635	0.8168
G_7: Limoncina	0.9198	0.9568	0.8110	0.9202	0.9572	0.8114	0.9200	0.9570	0.8112
G_8: Ensatakasuke	0.9254	0.9445	0.8084	0.9258	0.9447	0.8088	0.9256	0.9446	0.8086
G_9: Chinese White	0.8970	0.9305	0.7523	0.8972	0.9309	0.7527	0.8971	0.9307	0.7525
G_{10}: Kairyoroso	0.9200	0.9412	0.8101	0.9204	0.9416	0.8105	0.9202	0.9414	0.8103
C.D ($P \leq 0.05$)	**0.161**	**0.198**	**0.363**	**0.163**	**0.201**	**0.364**	**0.163**	**0.200**	**0.365**

During the autumn crop, leaf net assimilation rate was again the maximum in Goshoerami registering 0.9741, 0.1055 and 0.8473 mg/cm^2 /day respectively during the 1st (1st-15th August), 2nd (16-31st August) and 3rd (1st-15th September) intervals.it was statistically at par with the values recorded in KNG and Kokuso-21 and significant over the rest of the genotypes where it was the least with 0.9164 mg/cm^2 /day during 1st interval, 0.9363 mg/cm^2 /day and 0.7597 mg/cm^2 during the 3rd interval in Chinese white (Table-20).

The leaf net assimilation rate showed an increasing trend between first and the second interval where as a decreasing trend during the 3rd interval during both the seasons in all the genotypes under study.

4.4.3 Single leaf area

The Single leaf area was the highest (343.65 cm^2 during spring and 375.19 cm^2 during autumn crop) in Goshoerami being statistically significant over the rest of the genotypes and the least (133.01cm^2 and 141.41 cm^2 respectively during spring and autumn crop) in Limoncina. The differences among the genotypes were, however, significant (Table-21).

4.4.4 Specific leaf weight

The specific leaf weight on the other hand was the highest in Ensatakasuke with 0.789 g/cm^2 during spring and 0.794 g/cm^2 during autumn where as it was the least (0.443 g/cm^2 during spring and 0.452 g/cm2 during autumn) in case of Kokuso-20 (Table-22).

4.4.5 Specific leaf area

The specific leaf area was the highest (226.11cm^2/g in spring and 224.21cm^2 /g during autumn) in Kokuso-20 where as it was the least (126.59 cm^2/g during spring and 125.85 cm^2/g during the autumn crop) in Ensatakasuke (Table-23).

Table 20: Leaf Net assimilation rate (mg/cm^2/day) at an interval of 15 days of mulberry genotypes during Autumn season (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Autumn 2021			Autumn 2022			Pooled over Autumn		
	10 April-25 April	26April-10 May	11 May-25 May	10 April-25 April	26April-10 May	11 May-25 May	10 April-25 April	26April-10 May	11 May-25 May
G_1: Goshoerami	0.9442	0.9701	0.8259	0.9448	0.9705	0.8265	0.9445	0.9703	0.8262
G_2: Ichinose	0.9385	0.9662	0.8175	0.9391	0.9666	0.8179	0.9388	0.9664	0.8177
G_3: KNG	0.9385	0.9663	0.8174	0.9389	0.9667	0.8176	0.9387	0.9665	0.8175
G_4: Rokokoyoso	0.9336	0.9662	0.8156	0.9340	0.9666	0.8162	0.9338	0.9664	0.8159
G_5: Kokusu-20	0.9395	0.9655	0.8179	0.9397	0.9657	0.8183	0.9396	0.9656	0.8181
G_6: Kokuso- 21	0.9394	0.9633	0.8165	0.9398	0.9637	0.8171	0.9396	0.9635	0.8168
G_7: Limoncina	0.9198	0.9568	0.8110	0.9202	0.9572	0.8114	0.9200	0.9570	0.8112
G_8: Ensatakasuke	0.9254	0.9445	0.8084	0.9258	0.9447	0.8088	0.9256	0.9446	0.8086
G_9: Chinese White	0.8970	0.9305	0.7523	0.8972	0.9309	0.7527	0.8971	0.9307	0.7525
G_{10}: Kairyoroso	0.9200	0.9412	0.8101	0.9204	0.9416	0.8105	0.9202	0.9414	0.8103
C.D ($P \leq 0.05$)	0.161	0.198	0.363	0.163	0.201	0.364	0.163	0.200	0.365

Table 21: Leaf Area (cm^2) of mulberry genotypes (Pooled over two seasons of 2021 & 2022).

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	342.26	345.05	343.65	374.28	376.10	375.19
G_2: Ichinose	173.63	176.38	175.00	189.33	190.33	189.83
G_3: KNG	235.80	237.81	236.80	259.17	259.41	259.29
G_4: Rokokoyoso	140.94	143.49	142.21	148.60	151.10	149.85
G_5: Kokusu-20	246.02	249.37	247.70	278.86	279.58	279.22
G_6: Kokuso- 21	225.65	227.35	226.50	235.35	238.72	237.03
G_7: Limoncina	131.62	134.39	133.01	140.00	142.81	141.41
G_8: Ensatakasuke	146.66	147.45	147.06	151.76	153.45	152.61
G_9: Chinese White	137.22	138.50	137.86	144.05	147.15	145.60
G_{10}: Kairyoroso	138.60	140.31	139.45	143.03	146.01	144.52
C.D ($P \leq 0.05$)	**1.156**	**0.653**	**0.641**	**1.014**	**0.254**	**0.505**

Table 22: Specific leaf weight (mg/cm²) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	0.632	0.665	0.648	0.623	0.655	0.639
G_2: Ichinose	0.717	0.719	0.718	0.730	0.732	0.731
G_3: KNG	0.453	0.445	0.449	0.475	0.462	0.469
G_4: Rokokoyoso	0.588	0.559	0.573	0.590	0.594	0.592
G_5: Kokusu-20	0.438	0.449	0.443	0.441	0.462	0.452
G_6: Kokuso- 21	0.579	0.561	0.570	0.561	0.565	0.563
G_7: Limoncina	0.581	0.586	0.583	0.595	0.599	0.597
G_8: Ensatakasuke	0.786	0.793	0.789	0.800	0.788	0.794
G_9: Chinese White	0.483	0.484	0.483	0.477	0.476	0.477
G_{10}: Kairyoroso	0.541	0.540	0.540	0.547	0.558	0.553
C.D (P≤0.05)	**0.116**	**0.113**	**0.115**	**0.138**	**0.143**	**0.141**

Table 23: Specific Leaf area (cm^2/mg) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	157.53	150.82	154.17	159.86	152.59	156.22
G_2: Ichinose	139.32	137.63	138.47	136.86	136.57	136.71
G_3: KNG	220.32	224.42	222.37	221.09	221.19	221.14
G_4: Rokokoyoso	171.04	181.44	176.24	182.79	172.59	177.69
G_5: Kokusu-20	229.80	222.42	226.11	224.17	224.25	224.21
G_6: Kokuso- 21	172.82	168.07	170.45	173.26	161.69	167.48
G_7: Limoncina	172.19	170.55	171.37	172.34	166.32	169.33
G_8: Ensatakasuke	127.09	126.10	126.59	124.84	126.85	125.85
G_9: Chinese White	206.86	206.87	206.86	209.38	209.92	209.65
G_{10}: Kairyoroso	184.70	185.14	184.92	182.52	179.16	180.84
C.D ($P \leq 0.05$)	**11.350**	**10.166**	**7.354**	**21.445**	**13.031**	**10.454**

4.4.6 Chlorophyll "a" content

During spring chlorophyll "a" content was the highest (2.32 mg/ml) in case of Kokusu-21 being at par with the values recorded in KNG (2.28 mg/ml and Goshoerami (2.23 mg/ml) but significantly higher than the other genotypes. On the other hand, it was the least (1.69 mg/ml) in Limoncina.

During autumn chlorophyll "a" was lower as compared to chlorophyll a content during spring. It was the highest (1.99 mg/ml) again in Kokusu-21 being at par with the values recorded in KNG (1.94 mg/ml) and Goshoerami (1.90 mg/ml) and significant over the rest of the genotypes. The least chlorophyll "a" (1.56 mg/ml) was recorded in case of Limoncina (Table-24).

4.4.7 Chlorophyll "b"content

Chlorophyll "b" during spring too was the highest (1.76 mg/ml) in Kokuso-21 being at par with the values recorded in KNG (1.74 mg/ml) and Goshoerami (1.72 mg/ml) and significant over the rest of the genotypes. It was the least (1.05 mg/ml) in Limoncina.

During autumn chlorophyll "b" was lower as compared to chlorophyll "b" content during spring. It was the highest (1.29 mg/ml) again in Kokusu-21 being at par with the values recorded in KNG (1.26 mg/ml) and Goshoerami (1.23 mg/ml) and significant over the rest of the genotypes. The least chlorophyll "b" (1.05 mg/ml) was recorded in case of Limoncina (Table-25).

4.4.8 Total Chlorophyll content

Total chlorophyll during spring was the highest (4.08 mg/ml) in Kokuso-21 statistically at par with KNG (4.02 mg/ml) and Goshoerami (3.95 mg/ml). It was statistically significant in the rest of the genotypes with the least (2.82 mg/ml) in Limoncina.

During autumn also Kokuso-21 had the highest value (3.28 mg/ml) for leaf chlorophyll being statistically at par with KNG (3.19 mg/ml) and Goshoerami (3.13 mg/ml). It was statistically significant in the rest of the genotypes with the least (2.63 mg/ml) in Limoncina (Table-26).

Table 24: Leaf chlorophyll "a" content (mg/g) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	2.21	2.25	2.23	1.87	1.94	1.90
G_2: Ichinose	2.08	2.14	2.11	1.73	1.75	1.74
G_3: KNG	2.27	2.29	2.28	1.93	1.95	1.94
G_4: Rokokoyoso	1.87	1.87	1.87	1.62	1.70	1.66
G_5: Kokusu-20	2.09	2.13	2.11	1.68	1.76	1.72
G_6: Kokuso- 21	2.31	2.34	2.32	1.97	2.01	1.99
G_7: Limoncina	1.67	1.71	1.69	1.55	1.57	1.56
G_8: Ensatakasuke	1.98	2.02	2.00	1.70	1.73	1.72
G_9: Chinese White	1.68	1.72	1.70	1.56	1.60	1.58
G_{10}: Kairyoroso	1.84	1.94	1.89	1.67	1.70	1.69
C.D ($P \leq 0.05$)	**0.141**	**0.144**	**0.145**	**0.253**	**0.252**	**0.255**

Table 25: Leaf chlorophyll "b" (mg/g) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	1.70	1.74	1.72	1.22	1.25	1.23
G_2: Ichinose	1.63	1.65	1.64	1.12	1.15	1.13
G_3: KNG	1.73	1.75	1.74	1.24	1.27	1.26
G_4: Rokokoyoso	1.41	1.45	1.43	1.05	1.09	1.07
G_5: Kokusu-20	1.67	1.68	1.68	1.17	1.19	1.18
G_6: Kokuso- 21	1.75	1.76	1.76	1.28	1.30	1.29
G_7: Limoncina	1.12	1.14	1.13	1.04	1.06	1.05
G_8: Ensatakasuke	1.53	1.56	1.54	1.12	1.15	1.14
G_9: Chinese White	1.22	1.23	1.22	1.06	1.09	1.07
G_{10}: Kairyoroso	1.46	1.47	1.46	1.12	1.15	1.14
C.D ($P \leq 0.05$)	**0.102**	**0.105**	**0.104**	**0.081**	**0.084**	**0.085**

Table 26: Leaf total chlorophyll content (mg/g) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	3.91	3.99	3.95	3.08	3.19	3.13
G_2: Ichinose	3.71	3.80	3.75	2.85	2.89	2.87
G_3: KNG	4.01	4.04	4.02	3.17	3.22	3.19
G_4: Rokokoyoso	3.28	3.32	3.30	2.76	2.70	2.73
G_5: Kokusu-20	3.76	3.82	3.79	2.92	2.88	2.90
G_6: Kokuso- 21	4.06	4.11	4.08	3.26	3.31	3.28
G_7: Limoncina	2.80	2.85	2.82	2.61	2.64	2.63
G_8: Ensatakasuke	3.51	3.58	3.54	2.83	2.88	2.85
G_9: Chinese White	2.90	2.94	2.92	2.62	2.64	2.63
G_{10}: Kairyoroso	3.29	3.41	3.35	2.84	2.82	2.83
C.D (P≤0.05)	**0.250**	**0.253**	**0.256**	**0.228**	**0.230**	**0.231**

4.4.9 Nitrate reductase activity

Nitrate reductase activity was the highest with 14.48 µmol/ml/hr during spring and 10.64 µmol/ml/hr during autumn in Kokuso-21 being statistically at par with KNG with the value of 13.83 and 10.35 µmol/ml/hr during spring and autumn respectively and significant over rest of the genotypes. Limoncina, on the other hand, depicted the least nitrate reductase activity with 10.12 and 6.91 µmol/ml/hr during spring and autumn season respectively (Table-27).

4.4.10 Nitrogen

Leaf nitrogen content during spring was the highest (3.92 %) in KNG being statistically at par with the values 3.78% and 3.67% recorded respectively in Kokuso-21 and Goshoerami and significantly higher than the rest of the genotypes with the least (2.85%) in Limoncina.

During autumn, the leaf nitrogen content was in general lower as compared to spring season. It was the highest (3.77%) again in KNG being at par with 3.59% and 3.44% leaf nitrogen recorded in Kokuso-21 and Goshoerami respectively but significantly higher than the rest of the genotypes. It was the least (2.71%) in Limoncina. (Table-28)

4.4.11 Phosphorus

Leaf phosphorus during spring content was the highest (0.331%) in KNG being statistically at par with the values 0.286 percent and 0.258 percent recorded respectively in Kokuso-21 and Goshoerami and significantly higher than the rest of the genotypes with the least (0.148%) in Limoncina.

During autumn, the leaf phosphorus content was in general lower as compared to spring season. It was the highest (0.314%) again in KNG being at par with 0.274 percent and 0.226 percent leaf nitrogen recorded in Kokuso-21 and Goshoerami respectively but significantly higher than the rest of the genotypes. It was the least (0.130%) in Limoncina. (Table-29)

Table 27: Nitrate Reductase activity (µmol/ml/hr) of leaf of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	12.93	12.99	12.96	9.50	9.56	9.53
G_2: Ichinose	12.53	12.49	12.51	9.26	9.17	9.21
G_3: KNG	14.49	14.47	14.48	10.61	10.69	10.64
G_4: Rokokoyoso	10.26	10.32	10.29	8.19	8.31	8.25
G_5: Kokusu-20	12.65	12.75	12.70	9.45	9.51	9.48
G_6: Kokuso- 21	13.81	13.85	13.83	10.32	10.38	10.35
G_7: Limoncina	10.05	10.19	10.12	6.88	6.94	6.91
G_8: Ensatakasuke	10.18	10.29	10.23	8.11	8.19	8.15
G_9: Chinese White	10.22	10.20	10.21	7.24	7.18	7.20
G_{10}: Kairyoroso	10.62	10.72	10.67	8.22	8.34	8.28
C.D (P≤0.05)	**0.295**	**0.292**	**0.294**	**0.285**	**0.292**	**0.289**

Table 28: Leaf Nitrogen content (%) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	3.67	3.67	3.67	3.42	3.46	3.44
G_2: Ichinose	3.32	3.37	3.34	3.11	3.13	3.12
G_3: KNG	3.91	3.93	3.92	3.77	3.78	3.77
G_4: Rokokoyoso	3.13	3.16	3.15	2.98	2.98	2.98
G_5: Kokusu-20	3.44	3.45	3.45	3.38	3.38	3.38
G_6: Kokuso- 21	3.77	3.79	3.78	3.58	3.61	3.59
G_7: Limoncina	2.85	2.86	2.85	2.70	2.73	2.71
G_8: Ensatakasuke	3.11	3.12	3.11	2.93	2.95	2.94
G_9: Chinese White	2.91	2.91	2.91	2.72	2.75	2.73
G_{10}: Kairyoroso	3.15	3.16	3.16	2.97	2.98	2.97
C.D (P≤0.05)	**0.228**	**0.232**	**0.258**	**0.164**	**0.168**	**0.181**

Table 29: Leaf Phosphorus content (%) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	0.257	0.259	0.258	0.225	0.228	0.226
G_2: Ichinose	0.199	0.202	0.201	0.186	0.187	0.186
G_3: KNG	0.328	0.334	0.331	0.313	0.316	0.314
G_4: Rokokoyoso	0.185	0.191	0.188	0.173	0.176	0.174
G_5: Kokusu-20	0.284	0.289	0.286	0.273	0.276	0.274
G_6: Kokuso- 21	0.226	0.228	0.227	0.231	0.239	0.235
G_7: Limoncina	0.148	0.149	0.148	0.128	0.132	0.130
G_8: Ensatakasuke	0.243	0.247	0.245	0.224	0.229	0.227
G_9: Chinese White	0.145	0.147	0.146	0.128	0.129	0.128
G_{10}: Kairyoroso	0.173	0.175	0.174	0.145	0.146	0.145
C.D ($P \leq 0.05$)	**0.041**	**0.044**	**0.043**	**0.039**	**0.040**	**0.039**

4.4.12 Potassium

Leaf potassium content during spring was the highest (1.538%) in KNG being statistically at par with the values 1.501 percent and 1.458 percent recorded respectively in Kokuso-21 and Kokuso-20 and significant over rest of the genotypes with the least (1.103%) in Limoncina.

During autumn, the leaf potassium content was in general lower as compared to spring season. It was the highest (1.517%) again in KNG being at par with 1.485 percent and 1.436 percent leaf potassium recorded in Kokuso-21 and Kokuso-20 respectively and significantly higher than the rest of the genotypes. It was the least (1.077 %) in Limoncina (Table-30).

4.4.13 Calcium

During spring, calcium content in leaf was the highest (13021.63 ppm) in KNG being significantly higher than the rest of the genotypes. However, it was the least (11181.05 ppm) in Ensatakasuke.

In general calcium content was more in autumn as compared to spring. During autumn however, leaf calcium was the highest (13122.52 ppm) in KNG being statistically significant over the values recorded in other genotypes ranging from 11218.31 ppm in Ensatakasuke to 12602.52 ppm in Limoncina (Table-31)

4.4.14 Magnesium

Leaf magnesium during spring was the highest (6378.00 ppm) in KNG being significantly higher than the rest of the genotypes with magnesium content ranging from 4391.83 ppm in Limoncina to 5620.67 ppm in KNG.

During autumn, leaf magnesium content was the highest (6399.83 ppm) again in KNG being statistically significant over the values ranging from 4419.00 ppm in Limoncina to 5651.00 ppm KNG (Table-32).

Table 30: Leaf Potassium content (%) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	1.398	1.407	1.403	1.384	1.386	1.385
G_2: Ichinose	1.185	1.192	1.188	1.165	1.167	1.166
G_3: KNG	1.535	1.542	1.538	1.516	1.519	1.517
G_4: Rokokoyoso	1.279	1.285	1.282	1.265	1.266	1.265
G_5: Kokusu-20	1.456	1.459	1.458	1.435	1.437	1.436
G_6: Kokuso- 21	1.499	1.502	1.501	1.484	1.486	1.485
G_7: Limoncina	1.133	1.135	1.134	1.115	1.118	1.116
G_8: Ensatakasuke	1.291	1.291	1.291	1.276	1.229	1.252
G_9: Chinese White	1.104	1.102	1.103	1.074	1.081	1.077
G_{10}: Kairyoroso	1.151	1.151	1.151	1.125	1.131	1.128
C.D (P≤0.05)	**0.079**	**0.084**	**0.081**	**0.082**	**0.083**	**0.081**

Table 31: Leaf Calcium content (ppm) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	11742.00	11746.67	11744.33	11894.00	11904.67	11899.33
G_2: Ichinose	11733.33	11741.00	11737.17	11792.67	11797.67	11795.17
G_3: KNG	13017.00	13026.33	13021.67	13118.67	13126.33	13122.50
G_4: Rokokoyoso	11391.00	11304.33	11392.67	11433.00	11456.00	11444.50
G_5: Kokusu-20	12468.33	12471.33	12469.83	12503.67	12530.00	12516.83
G_6: Kokuso- 21	12471.00	12475.00	12473.00	12534.00	12546.67	12540.33
G_7: Limoncina	12568.00	12576.33	12572.17	12598.67	12606.33	12602.50
G_8: Ensatakasuke	11179.67	11182.33	11181.00	11209.67	11227.00	11218.33
G_9: Chinese White	12565.67	12570.67	12568.17	12588.33	12605.67	12597.00
G_{10}: Kairyoroso	11384.67	11393.33	11389.00	11422.00	11438.00	11430.00
C.D ($P \leq 0.05$)	**5.571**	**5.647**	**5.616**	**6.326**	**7.463**	**6.547**

Table 32: Leaf Magnesium content (ppm) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	5605.00	5613.33	5609.17	5642.33	5637.00	5639.67
G_2: Ichinose	5126.67	5132.67	5129.67	5155.33	5156.33	5155.83
G_3: KNG	5613.67	5627.67	5620.67	5651.33	5650.67	5651.00
G_4: Rokokoyoso	4428.00	4433.00	4430.50	4472.00	4472.00	4472.00
G_5: Kokusu-20	5143.67	5152.00	5147.83	5171.67	5171.00	5171.33
G_6: Kokuso- 21	6373.67	6382.33	6378.00	6392.00	6407.67	6399.83
G_7: Limoncina	4388.67	4395.00	4391.83	4411.67	4426.33	4419.00
G_8: Ensatakasuke	5118.67	5124.33	5121.50	5138.00	5147.67	5142.83
G_9: Chinese White	4392.00	4397.33	4394.67	4420.00	4422.67	4421.33
G_{10}: Kairyoroso	5107.33	5123.33	5115.33	5133.67	5150.00	5141.83
C.D (P≤0.05)	**4.433**	**4.433**	**4.429**	**4.533**	**4.452**	**4.504**

4.4.14 Iron

Iron content in leaf was the highest in Kokuso-21 with 203.53 ppm during spring and 195.82 ppm during autumn being significantly higher than the values recorded in other genotypes during both the seasons. Chinese white registered the lowest values with 161.61 ppm during spring and 157.58 ppm during autumn season (Table-33).

4.5 Biochemical constituents

Leaf biochemical constituents with respect to total soluble sugars, total soluble proteins, total soluble carbohydrates, total phenol and total carotenoid content during spring and autumn season are furnished below:

4.5.1 Total soluble sugars

Total soluble sugar during spring was the highest (2.03 g/100g fw) in KNG being statistically at par with 1.99 g/100g fw in Goshoerami and 1.96 g/100g fw in Kokuso-21 and significant over rest of the genotypes. it was the least (1.55 g/100g fw) in Limoncina.

During autumn, total soluble sugar again was the highest (2.33 g/100g fw) in KNG being statistically at par with 2.24 g/100g fw in Goshoerami and 2.18 g/100g fw in Kokuso-21and significant over rest of the genotypes. The total soluble sugar was the least (1.79 g/100g fw) in Limoncina (Table-34).

4.5.2 Total carotenoid

The carotenoid content during spring was the highest in Ensatakasuke with 562.83 µg/g in spring and 535.50 µg/g in autumn being significantly higher than the values recorded in other genotypes during both the seasons. However, it was the least (503.17 µg /g fw during spring and 487.83 µg /g fw during autumn) in Limoncina (Table-35).

Table 33: Leaf Iron content (ppm) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	186.63	186.94	186.78	180.23	180.88	180.56
G_2: Ichinose	179.17	179.55	179.36	174.31	174.52	174.41
G_3: KNG	198.58	198.71	198.64	193.44	193.89	193.67
G_4: Rokokoyoso	157.82	158.09	157.96	151.68	151.95	151.81
G_5: Kokusu-20	198.26	198.57	198.42	193.17	194.13	193.65
G_6: Kokuso- 21	203.33	203.73	203.53	195.71	195.93	195.82
G_7: Limoncina	161.37	161.85	161.61	157.40	157.76	157.58
G_8: Ensatakasuke	177.05	177.35	177.20	171.25	171.59	171.42
G_9: Chinese White	160.28	160.46	160.37	156.28	156.78	156.53
G_{10}: Kairyoroso	156.26	156.78	156.52	150.81	151.16	150.99
C.D ($P \leq 0.05$)	**0.136**	**0.131**	**0.133**	**0.334**	**0.471**	**0.326**

Table 34: Total soluble sugars in leaf (g/100g fw) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	1.98	2.00	1.99	2.21	2.27	2.24
G_2: Ichinose	1.73	1.71	1.72	1.97	1.99	1.98
G_3: KNG	2.00	2.06	2.03	2.31	2.35	2.33
G_4: Rokokoyoso	1.58	1.62	1.60	1.80	1.86	1.83
G_5: Kokusu-20	1.89	1.95	1.92	2.02	2.06	2.04
G_6: Kokuso- 21	1.93	1.99	1.96	2.16	2.20	2.18
G_7: Limoncina	1.56	1.54	1.55	1.75	1.83	1.79
G_8: Ensatakasuke	1.66	1.62	1.64	1.95	1.91	1.93
G_9: Chinese White	1.54	1.58	1.56	1.85	1.81	1.83
G_{10}: Kairyoroso	1.60	1.62	1.61	1.82	1.88	1.85
C.D ($P \leq 0.05$)	**0.104**	**0.106**	**0.104**	**0.103**	**0.105**	**0.107**

Table 35: Total carotenoid content in leaf (µg/ g fw) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	533.00	536.67	535.67	504.67	507.00	505.83
G_2: Ichinose	538.33	542.33	540.33	522.33	525.33	523.83
G_3: KNG	538.33	542.67	540.50	508.67	513.67	511.17
G_4: Rokokoyoso	554.67	557.00	555.83	527.33	531.67	529.50
G_5: Kokusu-20	547.67	548.67	548.17	516.33	521.67	519.00
G_6: Kokuso- 21	549.67	552.33	551.00	525.33	526.67	526.00
G_7: Limoncina	502.67	503.67	503.17	485.33	490.33	487.83
G_8: Ensatakasuke	561.33	564.33	562.83	530.00	541.00	535.50
G_9: Chinese White	509.33	511.67	510.50	493.67	495.00	494.33
G_{10}: Kairyoroso	504.67	511.00	507.83	492.33	498.67	495.50
C.D (P≤0.05)	**2.963**	**2.966**	**2.964**	**2.339**	**2.345**	**2.342**

4.5.3 Total soluble proteins

Total soluble proteins depicted almost the same trend as in total soluble sugar. During spring, it was the highest (2.30 g/100g fw) in Goshoerami being statistically at par with 2.23 g/100g fw in KNG and 2.21 g/100g fw in Kokuso-20. Limoncina on the other hand, depicted the least (1.76 g/100g fw) total soluble protein.

During autumn, total soluble protein was again the highest (2.74 g/100g fw) in Goshoerami being at par with 2.71 g/100g fw in KNG and 2.69 g/100g fw in Kokuso-20. On the other hand, it was the least (2.01 g/100g fw) in Limoncina (Table-36).

4.5.4 Total soluble carbohydrates

Total soluble carbohydrates was the highest in KNG with 3.95g/100g fw during spring and 3.41g/100g fw during autumn being statistically at par with Kokuso-21 with the value of 3.71 g/100g fw during spring and 3.26 g/100g fw during autumn and significant over rest of the genotypes during both the seasons. It was the least (3.04 g/100g fw during spring and 2.82 g/100g fw during autumn) in Chinese white. (Table-37)

4.5.5 Total phenol

Total phenol content was the highest (4.34 g/100g fw) in Limoncina being statistically at par with 4.25 and 4.22 g/100g fw in Chinese white and Rokokoyoso respectively but significant over rest of the genotypes. It was the least (3.47 g/100g) in KNG.

During autumn also Limoncina had the highest value of phenol content (4.17 g/100g fw) being statistically at par with the values 4.12 g/100g fw in Chinese white and 4.08 g/100g in Rokokoyoso and significant over rest of the genotypes with the least (3.30 g/100g) phenol content in KNG.(Table-38)

Table 36: Total soluble proteins in leaf (g/100g fw) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	2.29	2.31	2.30	2.72	2.76	2.74
G_2: Ichinose	2.15	2.21	2.18	2.54	2.60	2.57
G_3: KNG	2.21	2.25	2.23	2.71	2.71	2.71
G_4: Rokokoyoso	1.84	1.90	1.87	2.32	2.40	2.36
G_5: Kokusu-20	2.21	2.21	2.21	2.64	2.74	2.69
G_6: Kokuso- 21	1.93	1.97	1.95	2.38	2.34	2.36
G_7: Limoncina	1.73	1.79	1.76	2.03	1.99	2.01
G_8: Ensatakasuke	1.89	1.91	1.90	2.27	2.31	2.29
G_9: Chinese White	1.75	1.79	1.77	2.04	2.06	2.05
G_{10}: Kairyoroso	1.81	1.87	1.84	2.14	2.10	2.12
C.D ($P \leq 0.05$)	**0.103**	**0.105**	**0.102**	**0.102**	**0.103**	**0.101**

Table 37: Total soluble carbohydrates in leaf (g/100g) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	3.58	3.65	3.62	3.23	3.26	3.24
G_2: Ichinose	3.29	3.36	3.33	3.05	3.10	3.07
G_3: KNG	3.94	3.96	3.95	3.40	3.42	3.41
G_4: Rokokoyoso	3.16	3.19	3.18	2.93	2.96	2.94
G_5: Kokusu-20	3.46	3.56	3.51	3.16	3.22	3.18
G_6: Kokuso- 21	3.68	3.76	3.72	3.25	3.26	3.26
G_7: Limoncina	3.09	3.17	3.13	2.86	2.91	2.89
G_8: Ensatakasuke	3.23	3.27	3.25	3.00	3.04	3.02
G_9: Chinese White	2.99	3.09	3.04	2.82	2.83	2.82
G_{10}: Kairyoroso	3.14	3.23	3.19	2.92	2.93	2.92
C.D (P≤0.05)	**0.216**	**0.232**	**0.222**	**0.153**	**0.158**	**0.156**

Table 38: Total phenol content in leaf (g/100g fw) of mulberry genotypes (Pooled over two seasons of 2021 & 2022)

Season / Genotype	Spring		Pooled over Spring	Autumn		Pooled over Autumn
	Spring 2021	Spring 2022		Autumn 2021	Autumn 2022	
G_1: Goshoerami	3.52	3.50	3.51	3.30	3.36	3.33
G_2: Ichinose	3.62	3.64	3.63	3.44	3.42	3.43
G_3: KNG	3.45	3.49	3.47	3.33	3.27	3.30
G_4: Rokokoyoso	4.24	4.20	4.22	4.05	4.11	4.08
G_5: Kokusu-20	3.59	3.59	3.59	3.46	3.28	3.42
G_6: Kokuso- 21	3.55	3.53	3.54	3.35	3.39	3.37
G_7: Limoncina	4.32	4.36	4.34	4.15	4.19	4.17
G_8: Ensatakasuke	4.02	4.06	4.04	3.96	3.94	3.95
G_9: Chinese White	4.26	4.24	4.25	4.14	4.10	4.12
G_{10}: Kairyoroso	4.05	4.09	4.07	3.99	3.99	3.99
C.D (P≤0.05)	**0.254**	**0.251**	**0.254**	**0.116**	**0.119**	**0.118**

4.6 Correlation studies

Correlation of a particular trait with other characteristics contributing to the leaf yield is of great importance for the indirect selection of high yielding mulberry genotypes. Therefore, the correlation obtained in the present study are useful in the selection of traits having direct and significant correlation in improving leaf yield.

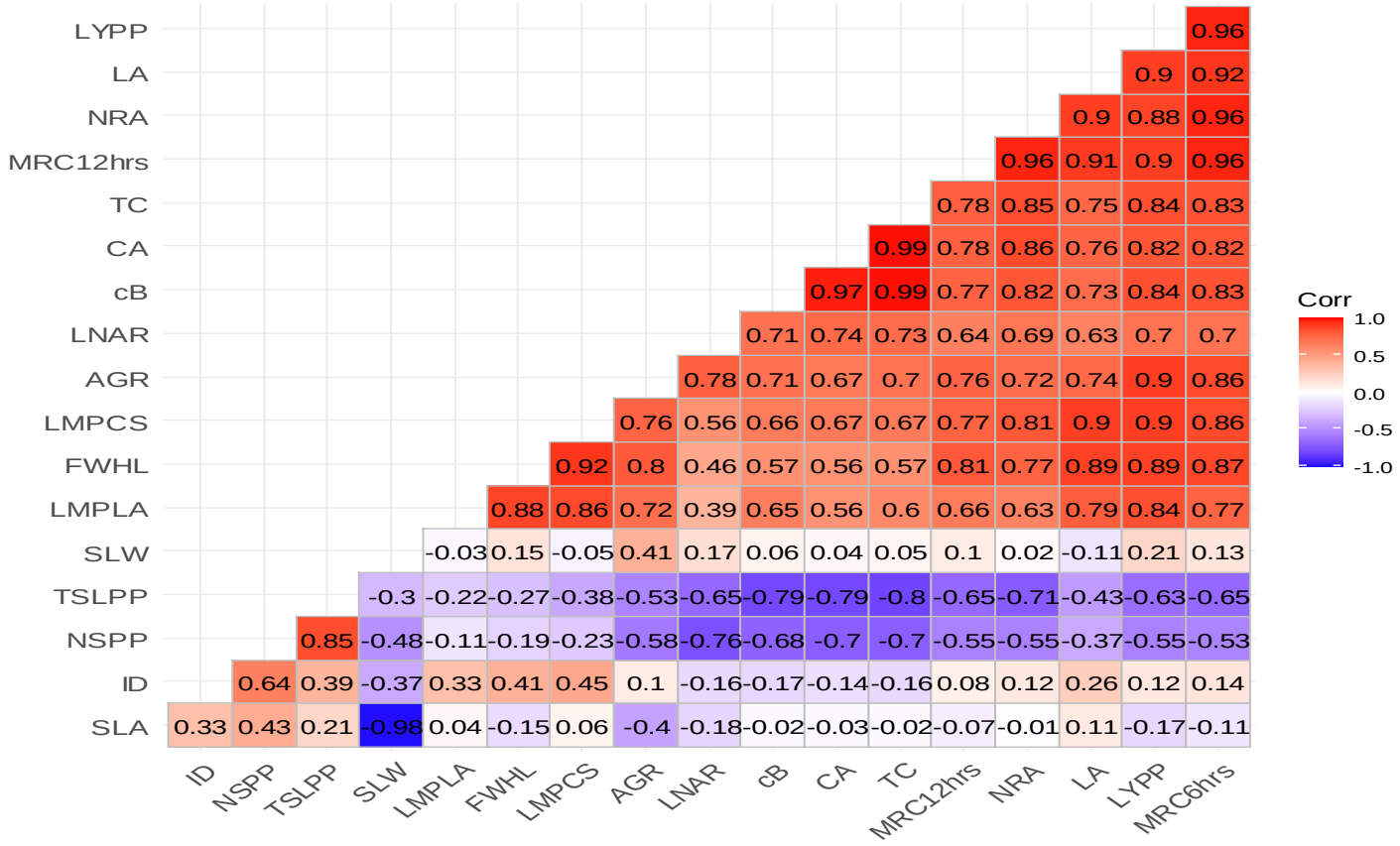
4.6.1 Leaf yield, growth parameters, leaf moisture and physiological traits

During spring, leaf yield per plant had positive correlations with all the traits except TSLPP (r: -0.63), NSPP (r: -0.55) and SLA (r:-0.17).The highest statistically significant positive correlation was observed between CA and TC (r: 0.99), CB and TC (r: 0.99) followed by CA and CB (r: 0.97), MRC12 hours and NRA (r: 0.96), FWHL and LMPCS (r: 0.92).However, the least statistically significant positive correlation was observed between FWHL and LNAR (0.46) followed by FWHL and CA (0.56); LMPLA (0.56). Conversely, the highest statistically significant negative correlation was observed between SLA and SLW (-0.98); TSLPP and TC (-0.80), followed by TSLPP and CA (-0.79); TSLPP and CB (-0.79).The least statistically significant negative correlation was observed between NSPP and SLW(r: -0.48) followed by NSPP and MRC 6hrs (r:-0.53). However, there was no correlation between SLA and the other traits except SLW (-0.98); SLW and other traits except SLA (-0.98); ID and other traits except NSPP (r: 0.64) and LMPCS (r: 0.45). The correlation matrix of the various traits are depicted in Table-39.

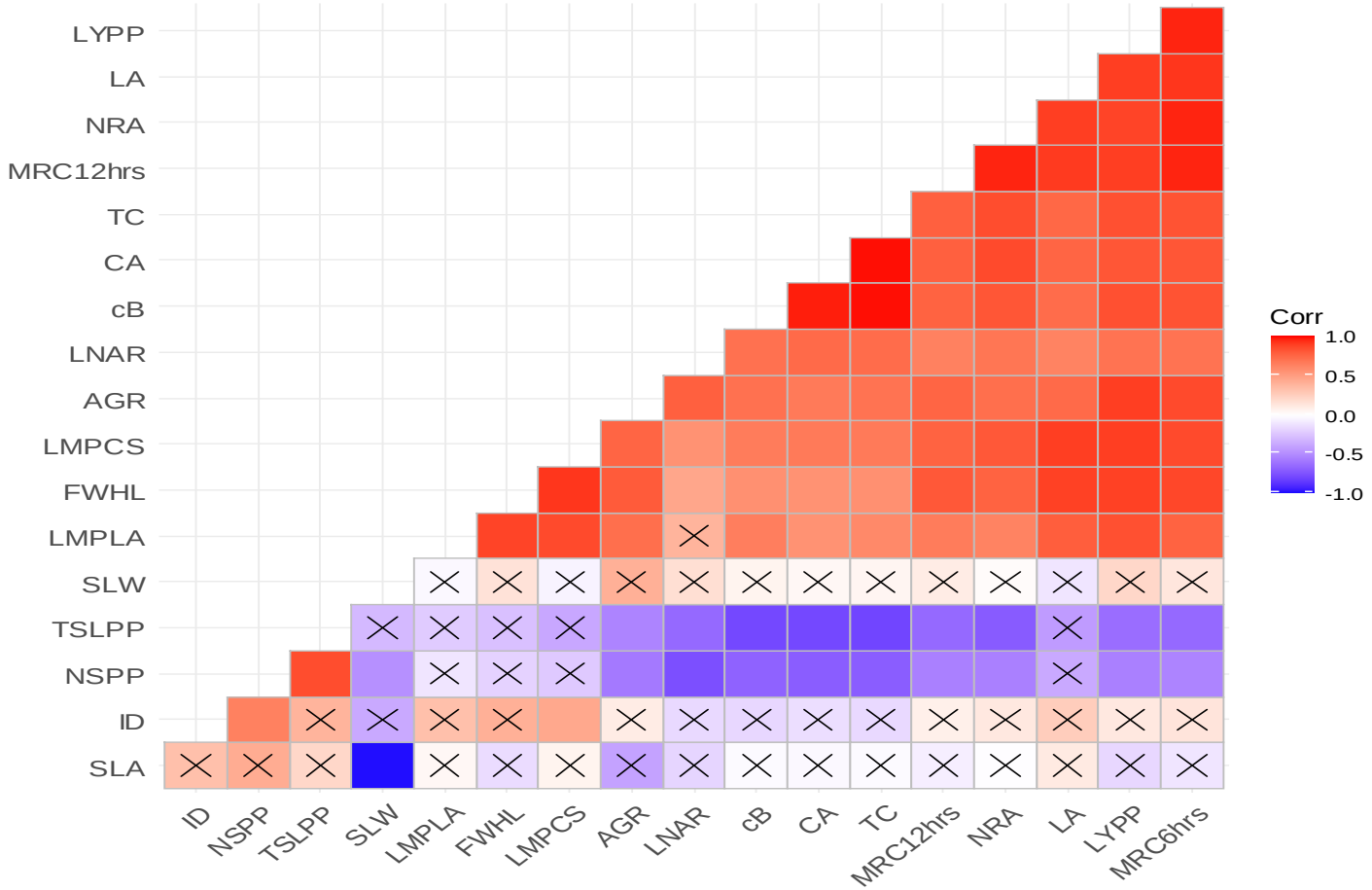
During autumn, leaf yield per plant had positive correlations with all the traits except TBLPP (r: -0.08), NBPP (r: -0.47) and LNAR (r:-0.56).The highest statistically significant positive correlation was observed between CA and TC (r: 0.98), CB and TC (r: 0.97) followed by CA and CB (r: 0.97), LA and LMPCS (r: 0.97).However, the least statistically significant positive correlation was observed between AGR and CA,CB (r: 0.47) followed by TC and MRC6hrs(0.45).

Conversely, the highest statistically significant negative correlation was observed between SLA and SLW (r: -0.97) which was followed by LNAR and FWHL (r: -0.91); LNAR and LMPCS (r: -0.84). The least statistically significant negative correlation was observed between LNAR and NRA(r: -0.44) followed by NBPP and SLW(r:-0.48). However, there was no correlation between TBLPP and the other traits except SLW (r: -0.67), SLA (r: 0.53); SLW with SLA (-0.97).The correlation matrix of the various traits are depicted in Table-40.

Table 39: Correlation between Leaf yield, growth parameters, leaf moisture and physiological traits during spring season

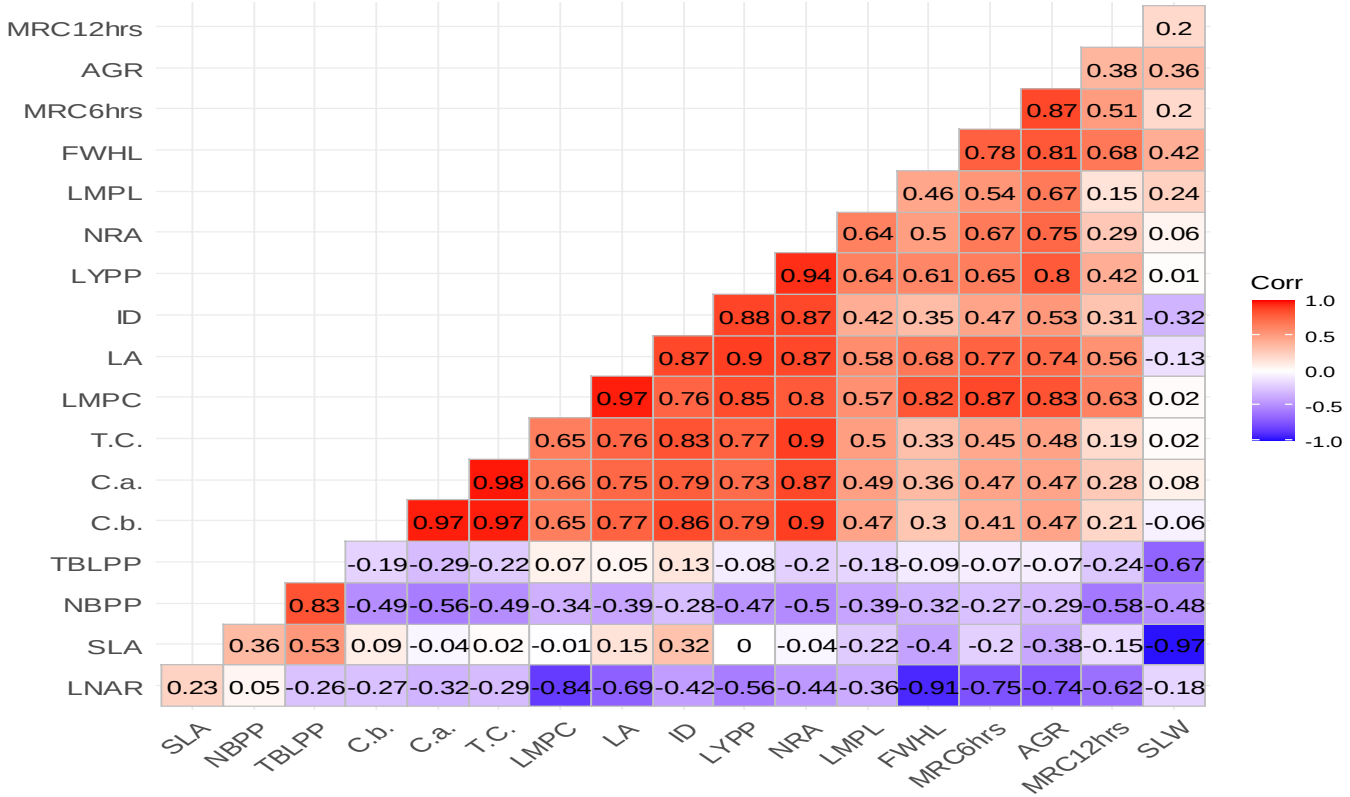


LYPP = Leaf yield per plant, ID = Internodal distance, FWHL= Fresh weight of hundred leaves, TSPP = Total shoot lets per plant, TSLPP= Total shootlet length per plant, LMPCS= Leaf moisture percentage at chawkie stage, LMPLA= Leaf moisture percentage at late age, MRC6 hrs = Moisture retention capacity after 6 hours, MRC12hrs= Moisture retention capacity after 12hours, AGR = Absolute growth rate, LNAR= Leaf net assimilation rate, LA= leaf area, SLA= Specific leaf area, SLW= Specific leaf weight, CA= Chlorophyll "a", cB= Chlorophyll "b", TC= Total chlorophyll, NRA = Nitrate reductase activity.

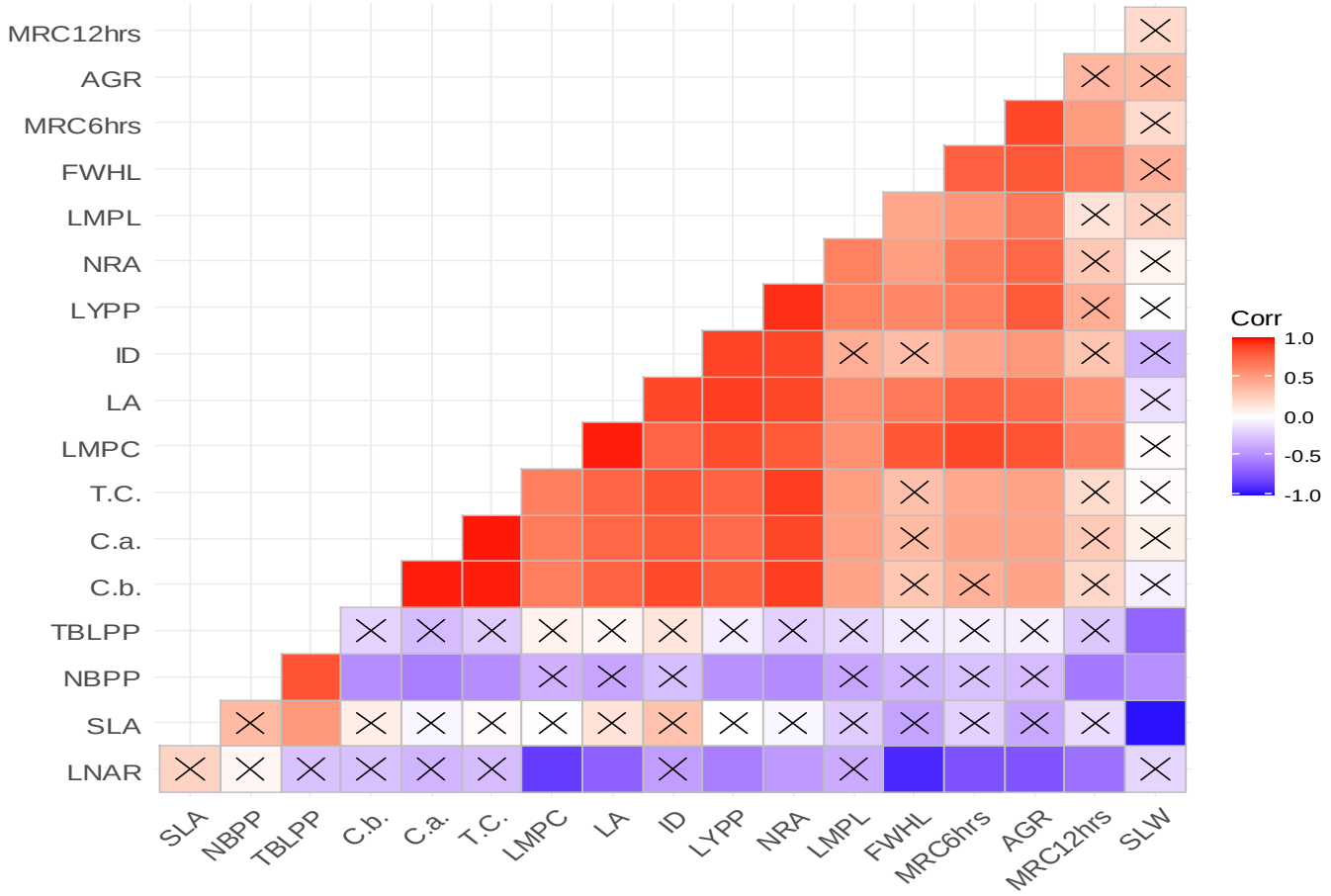


LYPP = Leaf yield per plant, ID = Internodal distance, FWHL= Fresh weight of hundred leaves, TSPP = Total shoot lets per plant, TSLPP= Total shootlet length per plant, LMPCS= Leaf moisture percentage at chawkie stage, LMPLA= Leaf moisture percentage at late age, MRC6 hrs= Moisture retention capacity after 6 hours, MRC12hrs= Moisture retention capacity after 12hours, AGR = Absolute growth rate, LNAR= Leaf net assimilation rate, LA= leaf area, SLA= Specific leaf area, SLW= Specific leaf weight, CA= Chlorophyll "a", cB= Chlorophyll "b", TC= Total chlorophyll, NRA = Nitrate reductase activity.

Table 40: Correlation between Leaf yield, growth parameters, leaf moisture and physiological traits during autumn season



LYPP = Leaf yield per plant, ID = Internodal distance, FWHL= Fresh weight of hundred leaves, TBPP = Total branches per plant, TBLPP= Total branch length per plant, LMPC= Leaf moisture percentage at chawkie stage, LMPL= Leaf moisture percentage at late age, MRC6 hrs= Moisture retention capacity after 6 hours, MRC12hrs= Moisture retention capacity after 12hours, AGR = Absolute growth rate, LNAR= Leaf net assimilation rate, LA= leaf area, SLA= Specific leaf area, SLW= Specific leaf weight, ca= Chlorophyll "a", cb= Chlorophyll "b", TC= Total chlorophyll, NRA = Nitrate reductase activity.



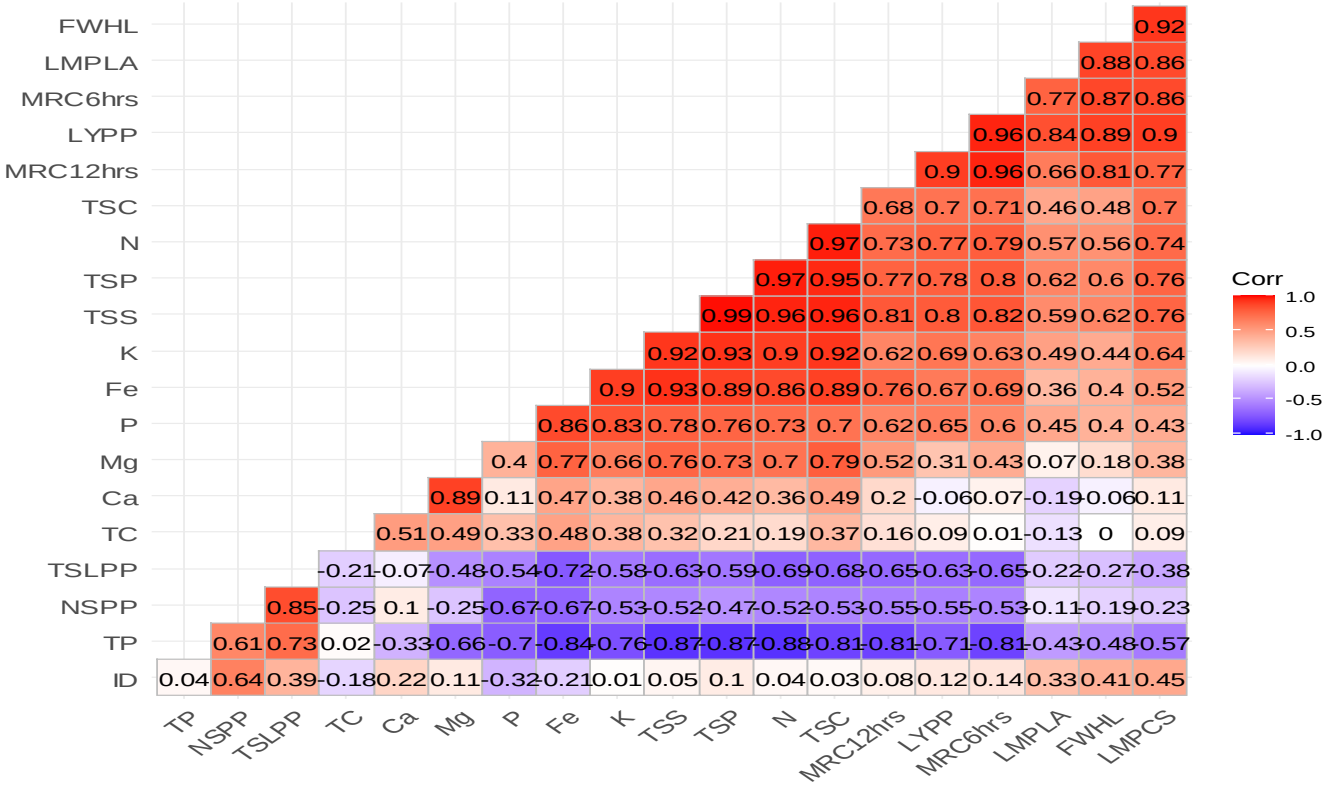
LYPP = Leaf yield per plant, ID = Internodal distance, FWHL= Fresh weight of hundred leaves, TBPP = Total branches per plant, TBLPP= Total branch length per plant, LMPC= Leaf moisture percentage at chawkie stage, LMPL= Leaf moisture percentage at late age, MRC6 hrs= Moisture retention capacity after 6 hours, MRC12hrs= Moisture retention capacity after 12hours, AGR = Absolute growth rate, LNAR= Leaf net assimilation rate, LA= leaf area, SLA= Specific leaf area, SLW= Specific leaf weight, ca= Chlorophyll "a", cb= Chlorophyll "b", TC= Total chlorophyll, NRA = Nitrate reductase activity.

4.6.2 Leaf yield, growth parameters, leaf moisture, nutrient status and biochemical traits

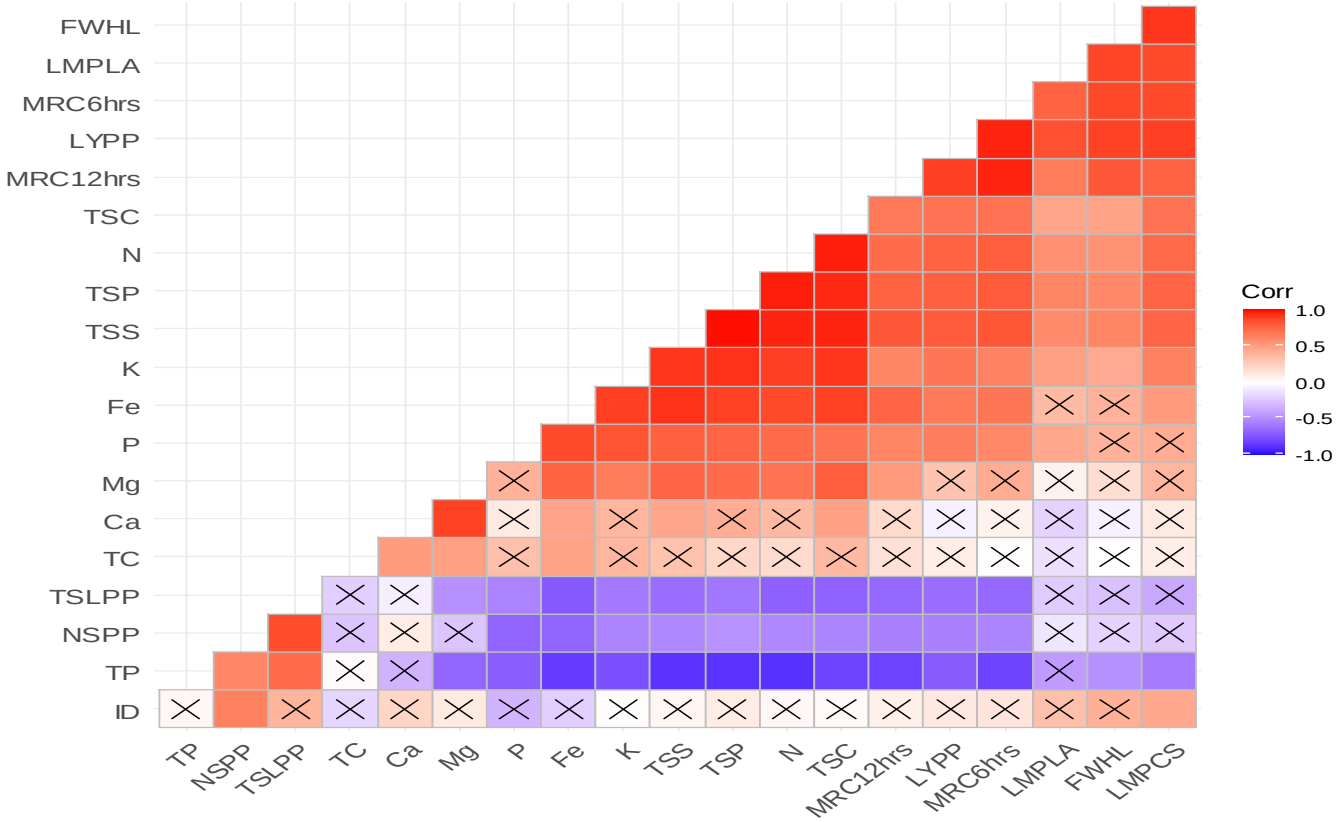
During spring, the highest statistically significant positive correlation was observed between TSP and TSS (r: 0.99) which was closely followed by TSP and N (r: 0.97), TSC and N (r: 0.97), MRC6hrs and MRC12hrs (r: 0.96), LYPP and MRC6hrs (r: 0.96), TSS and N (r: 0.96). On the other hand, least statistically significant positive correlation was observed between P and LMPLA (r: 0.45), ID and LMPCS (r: 0.45) followed by TSC and LMPLA (r: 0.46). Conversely, the highest statistically significant negative correlation was observed between TP and N (r:- 0.88) closely followed by TP and TSS (r:- 0.87), TP and TSP (r: -0.87) and TP and Fe (r:-0.84).The least statistically significant negative correlation was observed between NSPP and TSP (r:- 0.47) followed by TSLPP and Mg (r:-0.48). However, no correlation was observed between TC and other traits except Ca (r: 0.51), Mg(r: 0.49) and Fe(r: 0.48). The correlation matrices are depicted in Table-41.

During autumn, the highest statistically significant positive correlation was observed between TSC and N (r: 0.98) which was closely followed by TSS and N (r: 0.97), TSS and N (r: 0.97) and TSP and N (0.97). On the other hand, least statistically significant positive correlation was observed between FWHL and LMPLA (r: 0.46) followed by LMPL and Fe(r: 0.47) and CA and Fe(r: 0.48). Conversely, the highest statistically significant negative correlation was observed between TP and N (r: 0.88) closely followed by TP and TSC (r: 0.87) and TP and Fe (r: 0.85).The least statistically significant negative correlation was observed between NBPP and LYPP (r: -0.47). However, no correlation was observed between TC and other traits except Ca (r: 0.5). The correlation matrices are depicted in Table-42.

Table-41: Correlation between leaf yield, growth parameters, leaf moisture, nutrient status and biochemical traits during spring season

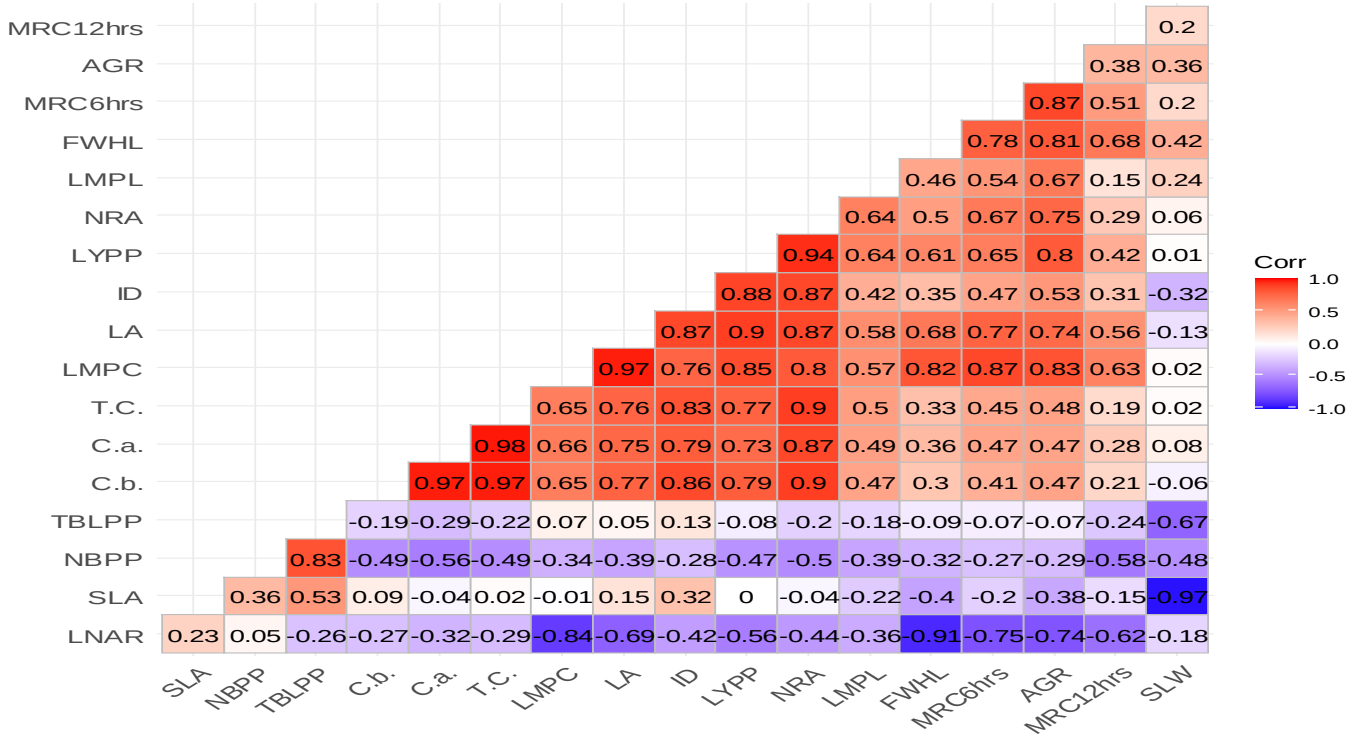


LYPP = Leaf yield per plant, ID = Internodal distance, FWHL= Fresh weight of hundred leaves, NSPP = Total shoot lets per plant, TSLPP= Total shoot let length per plant, LMPCS= Leaf moisture percentage at chawkie stage, LMPLA= Leaf moisture percentage at late age, MRC6 hrs = Moisture retention capacity after 6 hours, MRC12hrs= Moisture retention capacity after 12hours, N = Nitrogen, P= Phosphorus, K= Potassium, Ca= Calcium, Mg= Magnesium, Fe= Iron, TSS= Total soluble sugar, TSP= Total soluble protein, TSC= Total soluble carbohydrate, TC=Total carotenoid, TP= Total phenol

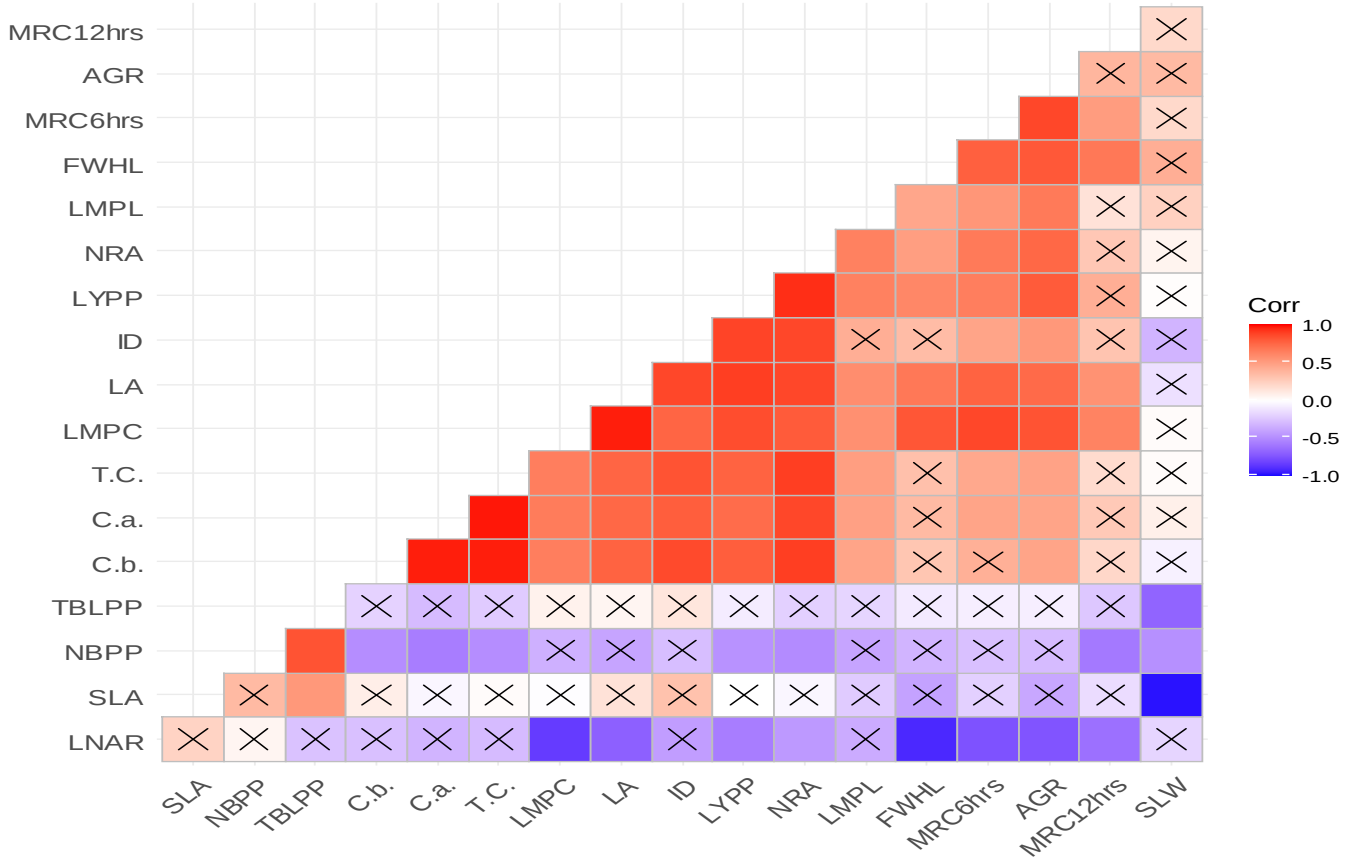


LYPP = Leaf yield per plant, ID = Internodal distance, FWHL= Fresh weight of hundred leaves, NSPP = Total shoot lets per plant, TSLPP= Total shoot let length per plant, LMPCS= Leaf moisture percentage at chawkie stage, LMPLA= Leaf moisture percentage at late age, MRC6 hrs = Moisture retention capacity after 6 hours, MRC12hrs= Moisture retention capacity after 12hours, N = Nitrogen, P= Phosphorus, K= Potassium, Ca= Calcium, Mg= Magnesium, Fe= Iron, TSS= Total soluble sugar, TSP= Total soluble protein, TSC= Total soluble carbohydrate, TC=Total carotenoid, TP= Total phenol

Table-42: Correlation between leaf yield, growth parameters, leaf moisture, nutrient status and biochemical traits during autumn season



LYPP = Leaf yield per plant, ID = Internodal distance, FWHL= Fresh weight of hundred leaves, NBPP = Number of branches per plant, TBPP = Total branch length per plant, LMPC= Leaf moisture percentage at chawkie stage, LMPL= Leaf moisture percentage at late age, MRC6 hrs = Moisture retention capacity after 6 hours, MRC12hrs= Moisture retention capacity after 12hours, AGR = Absolute growth rate, LNAR= Leaf net assimilation rate, LA= leaf area, SLA= Specific leaf area, SLW= Specific leaf weight, ca= Chlorophyll "a", cb= Chlorophyll "b", TC= Total chlorophyll.



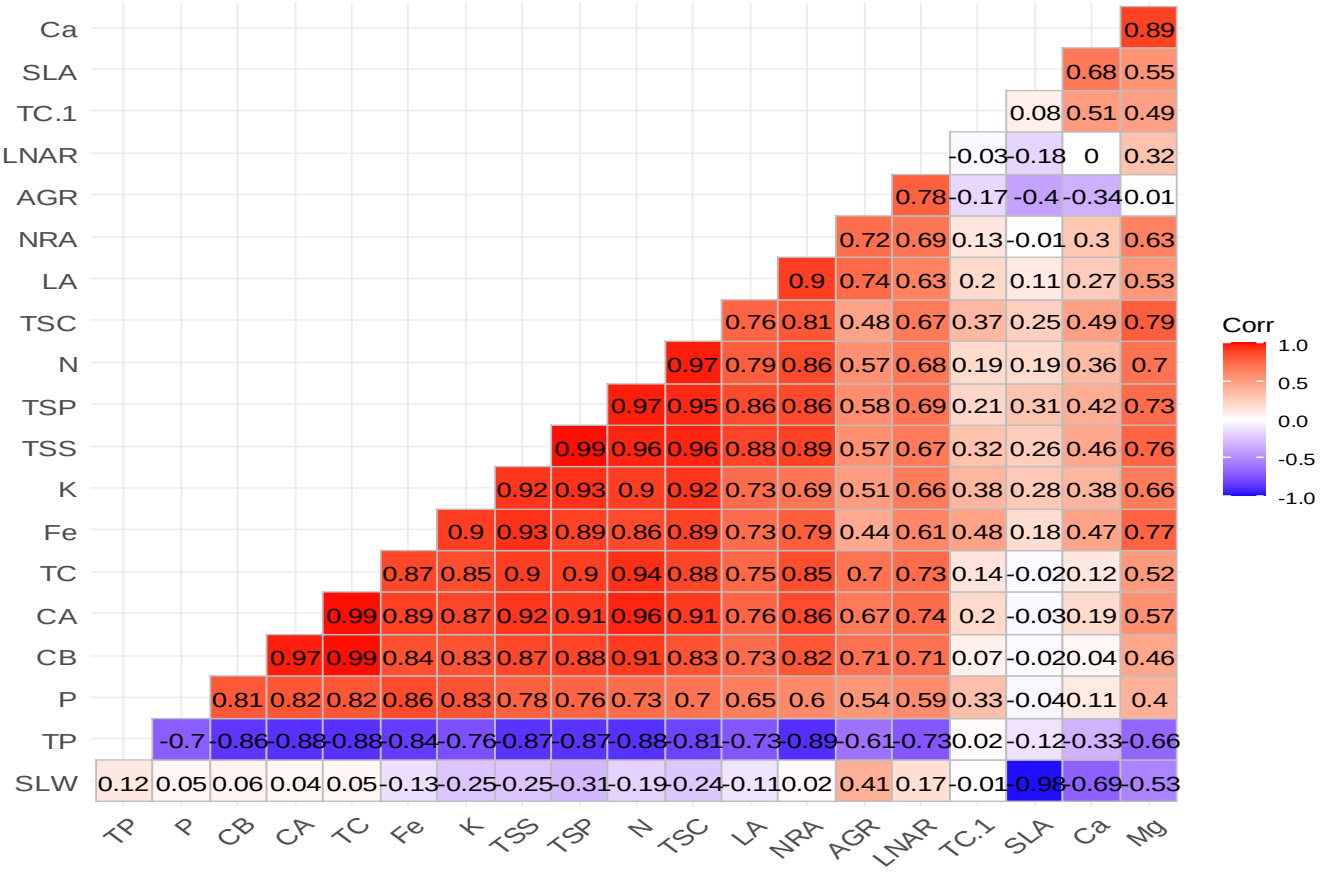
LYPP = Leaf yield per plant, ID = Internodal distance, FWHL= Fresh weight of hundred leaves, NBPP = Number of branches per plant, TBPP = Total branch length per plant, LMPC= Leaf moisture percentage at chawkie stage, LMPL= Leaf moisture percentage at late age, MRC6 hrs = Moisture retention capacity after 6 hours, MRC12hrs= Moisture retention capacity after 12hours, AGR = Absolute growth rate, LNAR= Leaf net assimilation rate, LA= leaf area, SLA= Specific leaf area, SLW= Specific leaf weight, CA= Chlorophyll“a”, cb =Chlorophyll“b”, TC= Total chlorophyll.

4.6.3 Physio-biochemical traits and nutrient status

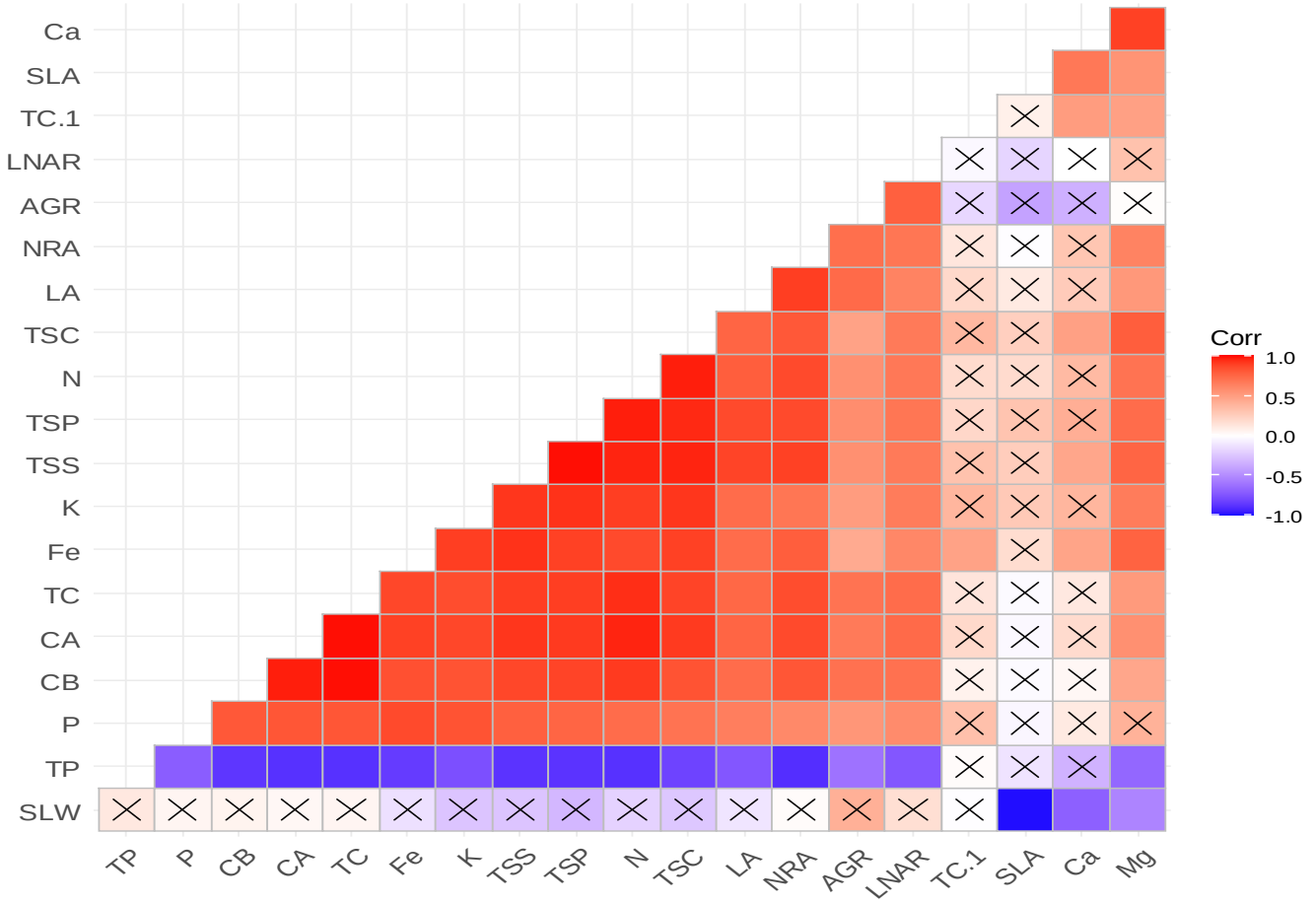
During spring**,** the highest statistically significant positive correlation was observed between NRA and LA (r: 0.90) closely followed by NRA and TSS (r: 0.89); TSS and LA (r: 0.88); NRA and TSP (r: 0.86).However, the least statistically significant positive correlation was observed between Fe and AGR (r: 0.44) followed by TSC and AGR (r: 0.48); K and AGR (r: 0.51). Conversely the highest statistically significant negative correlation was observed between NRA and TP (R:-0.89) closely followed by TP and LNAR (r:-0.73). On the other hand the least statistically significant negative correlation was observed between SLW and Mg (r:-0.53) closely followed by TP and Mg (r: -0.66). However, there was no correlation between TC1 and other parameters except Fe (r: 0.48). The correlation matrices are depicted in Table-43.

During autumn, the highest statistically significant positive correlation was observed between CA and TC (r: 0.98) closely followed by TSC and TSS (r: 0.97) and CA and CB (r: 0.97).However, the least statistically significant positive correlation was observed between AGR and CA (r: 0.47), AGR and CB (r: 0.47) followed by TC and AGR (r: 0.48);Mg and AGR (r: 0.47). Conversely the highest statistically significant negative correlation was observed between SLW and SLA (r: -0.97) NRA and TP (R:-0.91) closely followed by TP and TSC (r:-0.87). On the other hand the least statistically significant negative correlation was observed between SLW and Mg (r:-0.48) closely followed by LNAR and NRA (r: -0.44). However, there was no correlation between TC1 and other parameters except Ca (r: 0.50). The correlation matrices are depicted in Table-44.

Table 43: Correlation between Physio-biochemical traits and nutrient status during spring season

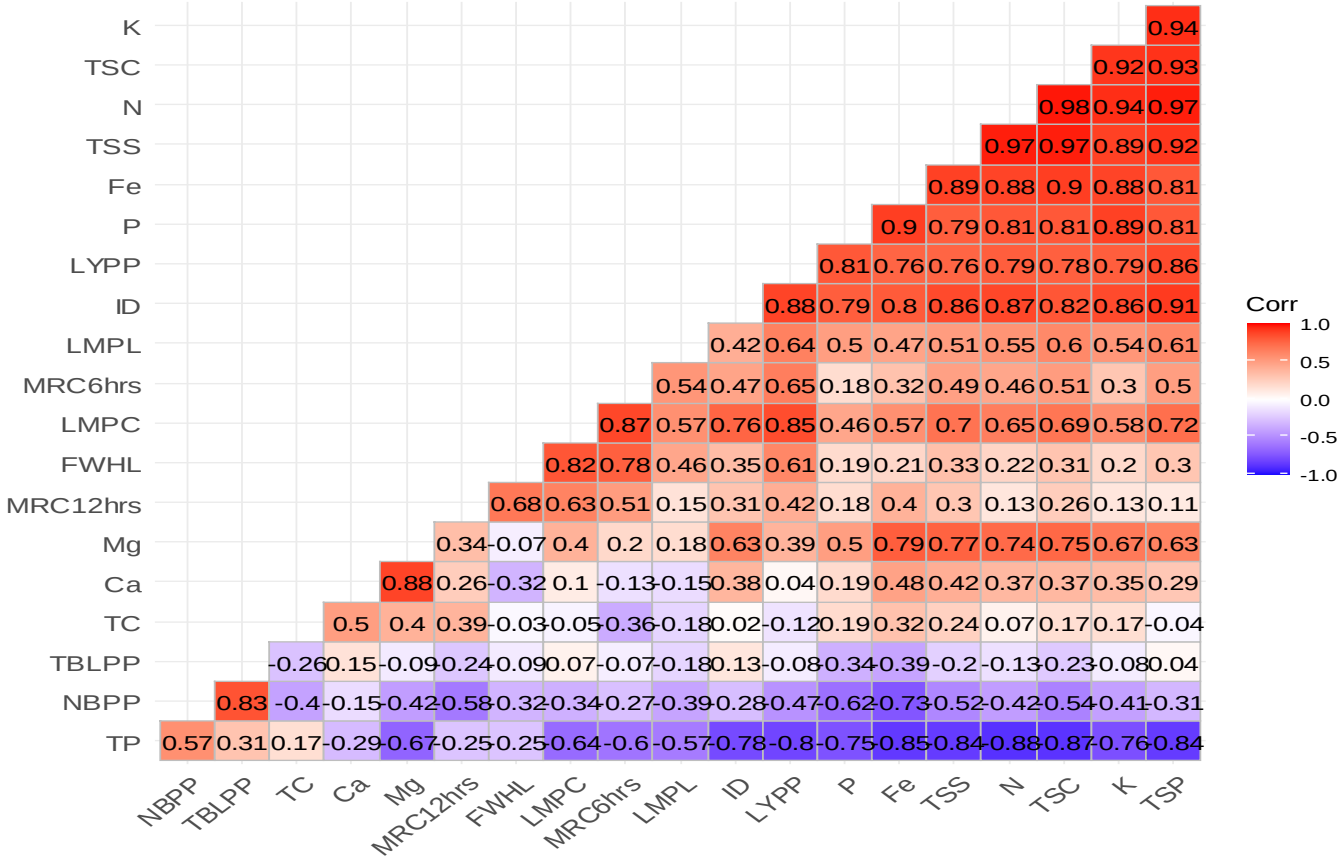


AGR = Absolute growth rate, LNAR= Leaf net assimilation rate, LA =Leaf area, SLA= Specific leaf area, SLW= Specific leaf weight, CA= Chlorophyll “a”, CB= Chlorophyll “b”, TC.1= Total chlorophyll, N = Nitrogen, P= Phosphorus, K= Potassium, Ca= Calcium, Mg= Magnesium, Fe= Iron, TSS= Total soluble sugar, TSP= Total soluble protein, TSC= Total soluble carbohydrate, TC=Total carotenoid, TP= Total phenol, NRA = Nitrate reductase activity.

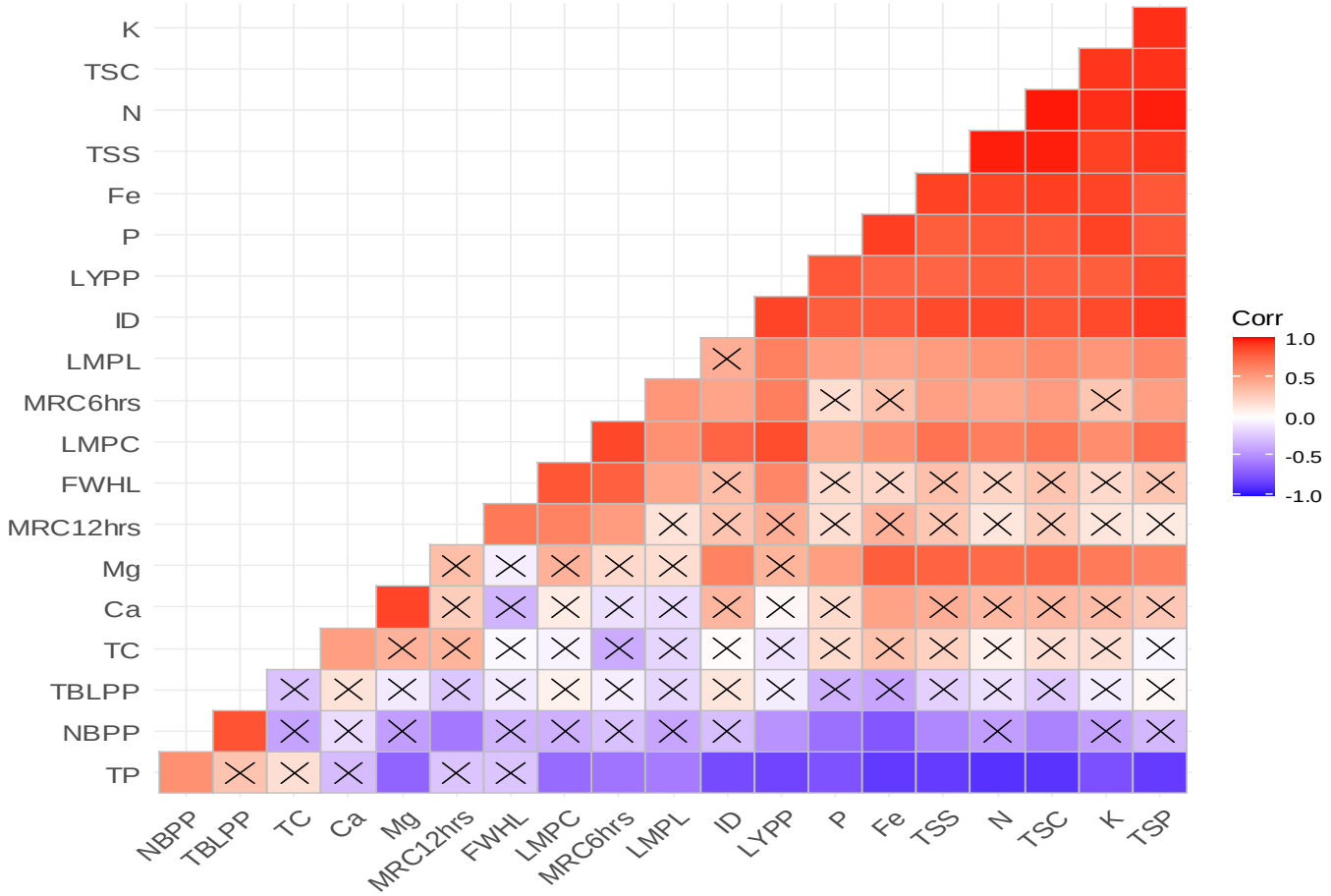


AGR = Absolute growth rate, LNAR= Leaf net assimilation rate, LA =Leaf area, SLA= Specific leaf area, SLW= Specific leaf weight, CA= Chlorophyll “a”, CB= Chlorophyll “b”, TC.1= Total chlorophyll, N = Nitrogen, P= Phosphorus, K= Potassium, Ca= Calcium, Mg= Magnesium, Fe= Iron, TSS= Total soluble sugar, TSP= Total soluble protein, TSC= Total soluble carbohydrate, TC=Total carotenoid, TP= Total phenol, NRA = Nitrate reductase activity.

Table 44: Correlation between Physio-biochemical traits and nutrient status during autumn season



LYPP = Leaf yield per plant , ID = Internodal distance, LMPL = Leaf moisture percentage at late age, MRC 6 hrs = Moisture retention capacity after 6 hours, MRC 12 hrs = Moisture retention capacity after 12 hours , LMPL = Leaf moisture percentage at late age, FWHL = Fresh weight of hundred leaves, TBLPP= Total branch length per plant, NBPP = Number of branches per plant, ca= Chlorophyll “a”, cb= Chlorophyll “b”, TC = Total chlorophyll, N = Nitrogen, P= Phosphorus, K= Potassium, Ca= Calcium, Mg= Magnesium, Fe= Iron, TSS= Total soluble sugar, TSP= Total soluble protein, TSC= Total soluble carbohydrate, TC=Total carotenoid, TP= Total phenol.



LYPP = Leaf yield per plant , ID = Internodal distance, LMPL = Leaf moisture percentage at late age, MRC 6 hrs = Moisture retention capacity after 6 hours, MRC 12 hrs = Moisture retention capacity after 12 hours , LMPL = Leaf moisture percentage at late age, FWHL = Fresh weight of hundred leaves, TBLPP= Total branch length per plant, NBPP = Number of branches per plant, ca= Chlorophyll “a”, cb= Chlorophyll “b”, TC = Total chlorophyll, N = Nitrogen, P= Phosphorus, K= Potassium, Ca= Calcium, Mg= Magnesium, Fe= Iron, TSS= Total soluble sugar, TSP= Total soluble protein, TSC= Total soluble carbohydrate, TC=Total carotenoid, TP= Total phenol.

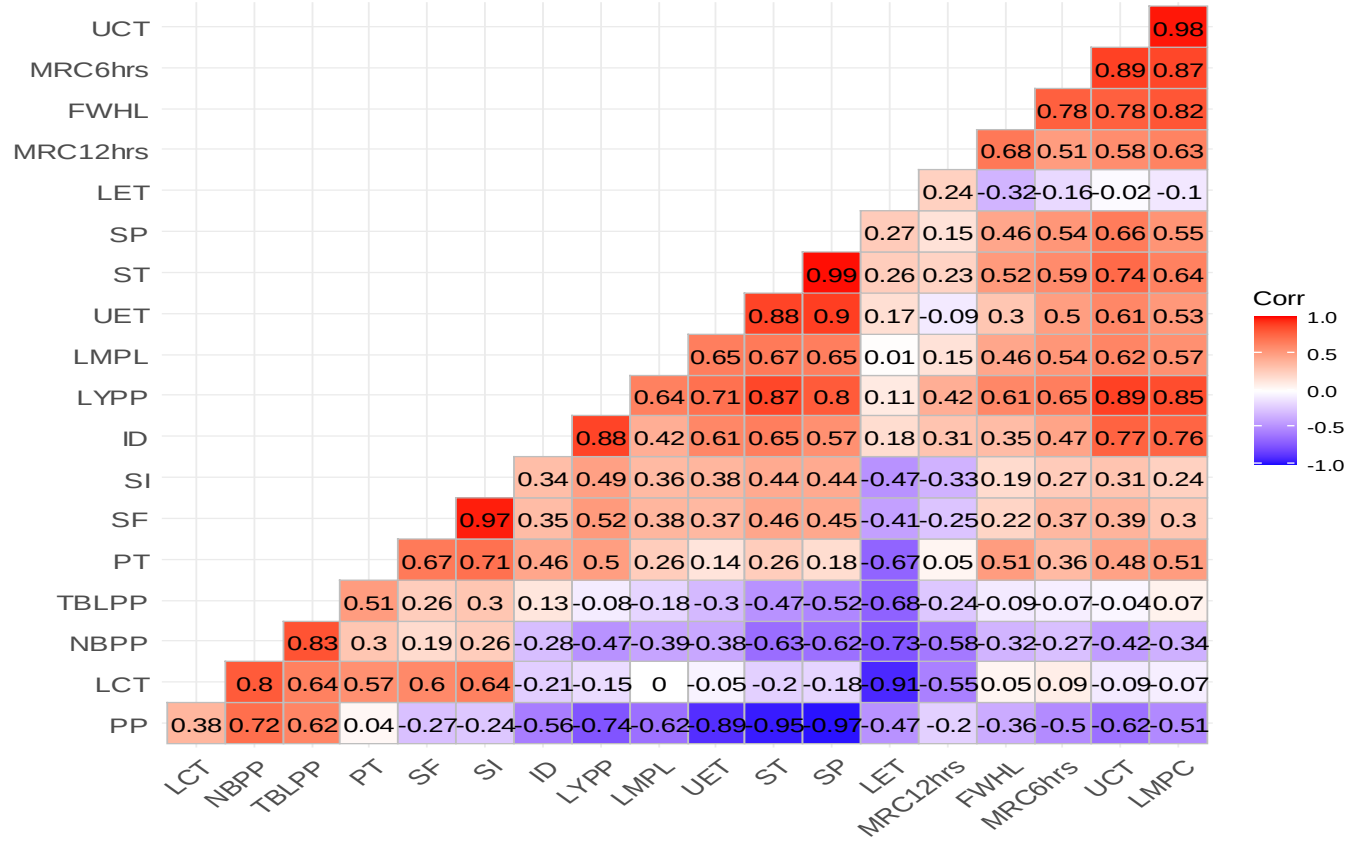
4.6.4 Growth, yield and moisture status with anatomical features

The highest statistically significant positive correlation was observed between ST and SP (r: 0.99) which was closely followed by UCT and LMPC (r: 0.98); SF and SI (r: 0.97); UET and ST (r: 0.88) ; ID and LYPP(r: 0.88) where as the least statistically significant positive correlation was observed between SI and ST (r: 0.44) and SI and SP (r: 0.44) which was followed by SF and SP (r:0.45) ; SF and ST (r: 0.48). Conversely, the highest statistically significant negative correlation was observed between PP and SP (r: -0.97) followed by PP and ST (r:-0.95); LCT and LET(r:-0.91).On the other hand the least statistically significant negative correlation was observed between SI and LET (r: -0.47); PP and LET (r: -0.47); PP and LMPC (r: -0.51).). However, there was no correlation between MRC12hrs and other parameters except NBPP (r:-0.58) and LCT (r:-0.55). The correlation matrices of these traits are depicted in Table-45.

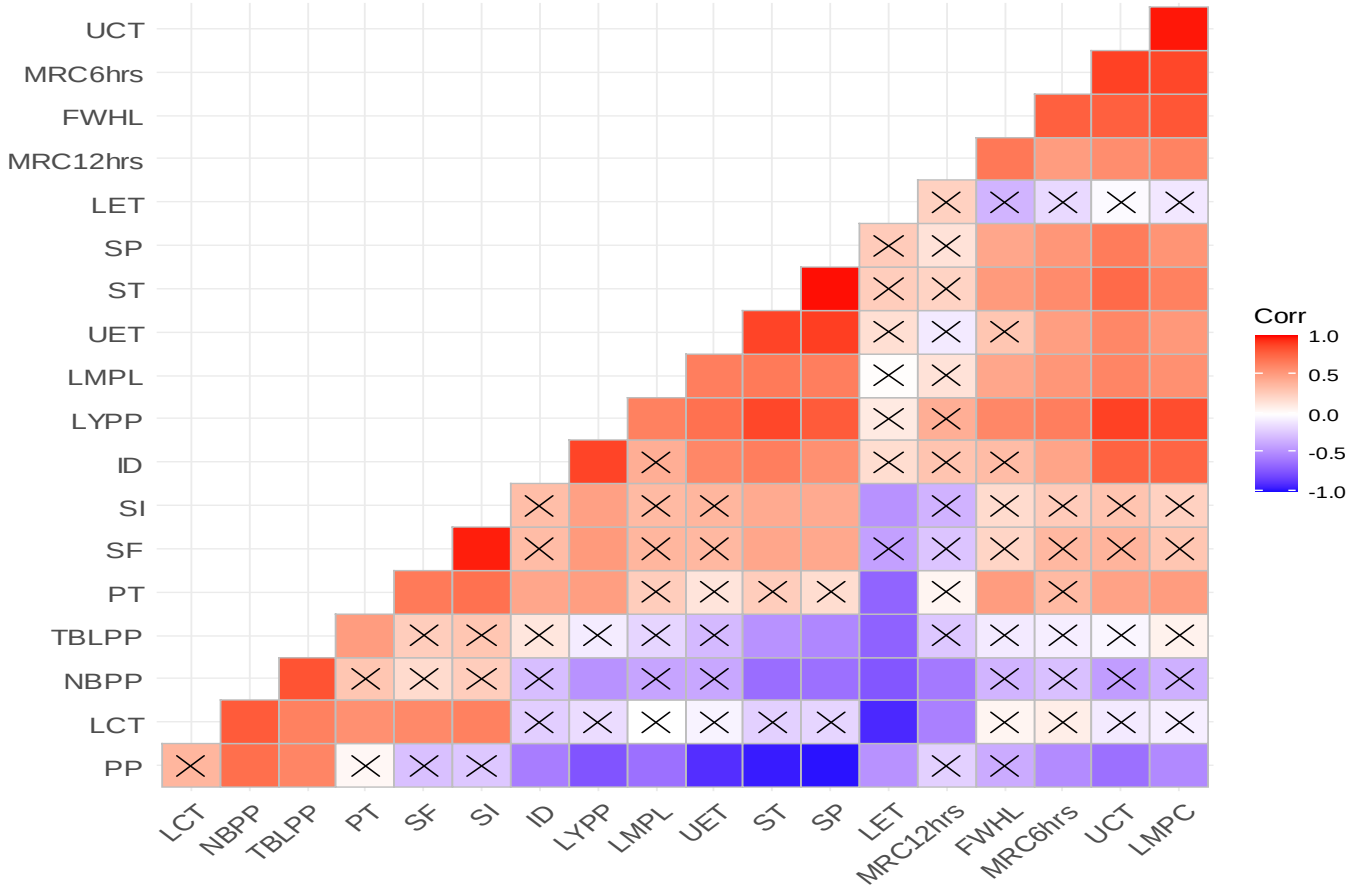
4.6.5 Leaf anatomical features with physiological traits

The highest statistically significant positive correlation was observed between NRA and SP (r: 0.91) closely followed by NRA and UCT (r: 0.88); AGR and UCT (r: 0.87); SP and NRA (r: 0.86) where as the least statistically significant positive correlation was observed between NRA and SF (r: 0.47); UCT and PT (r: 0.48); LET and LNAR(r: 0.51). Conversely, the highest statistically significant negative correlation was observed between NRA and PP (r: -0.83) followed by UCT and LNAR (r:-0.76; PT and LNAR (r: -0.71). On the other hand the least statistically significant negative correlation was observed between NRA and LNAR (r: -0.44).However no correlation was observed between LET and other traits except LCT(r: - 0.91), PT(r:-0.67) and SI(r:-0.47). The correlation matrices of the traits are depicted in Table-46.

.Table 45: Correlation between leaf growth, yield, moisture and Leaf anatomical features.

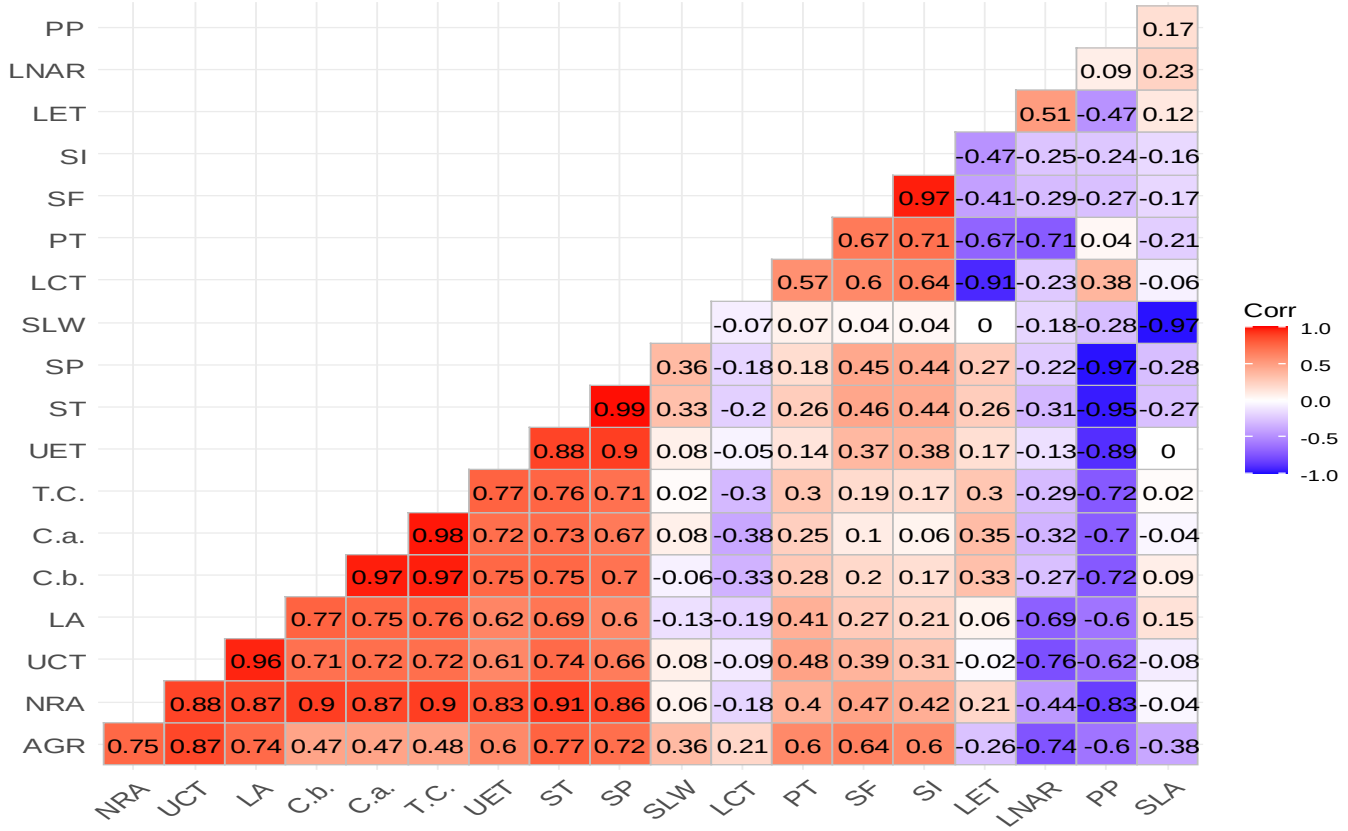


LYPP = Leaf yield per plant, ID = Internodal distance, FWHL= Fresh weight of hundred leaves, NBPP = Number of branches per plant, TBLPP= Total branch length per plant, LMPC= Leaf moisture percentage at chawkie stage, LMPL= Leaf moisture percentage at late age, MRC6 hrs = Moisture retention capacity after 6 hours, MRC12hrs= Moisture retention capacity after 12hours, UCT= Upper cuticular thickness, LCT= Lower cuticular thickness, UET= Upper epidermal thickness, LET= Lower epidermal thickness, ST= Spongy thickness, SP= Spongy proportion, PT= Palisade thickness, PP= Palisade proportion, SF= Stomatal frequency, SI = Stomatal index.

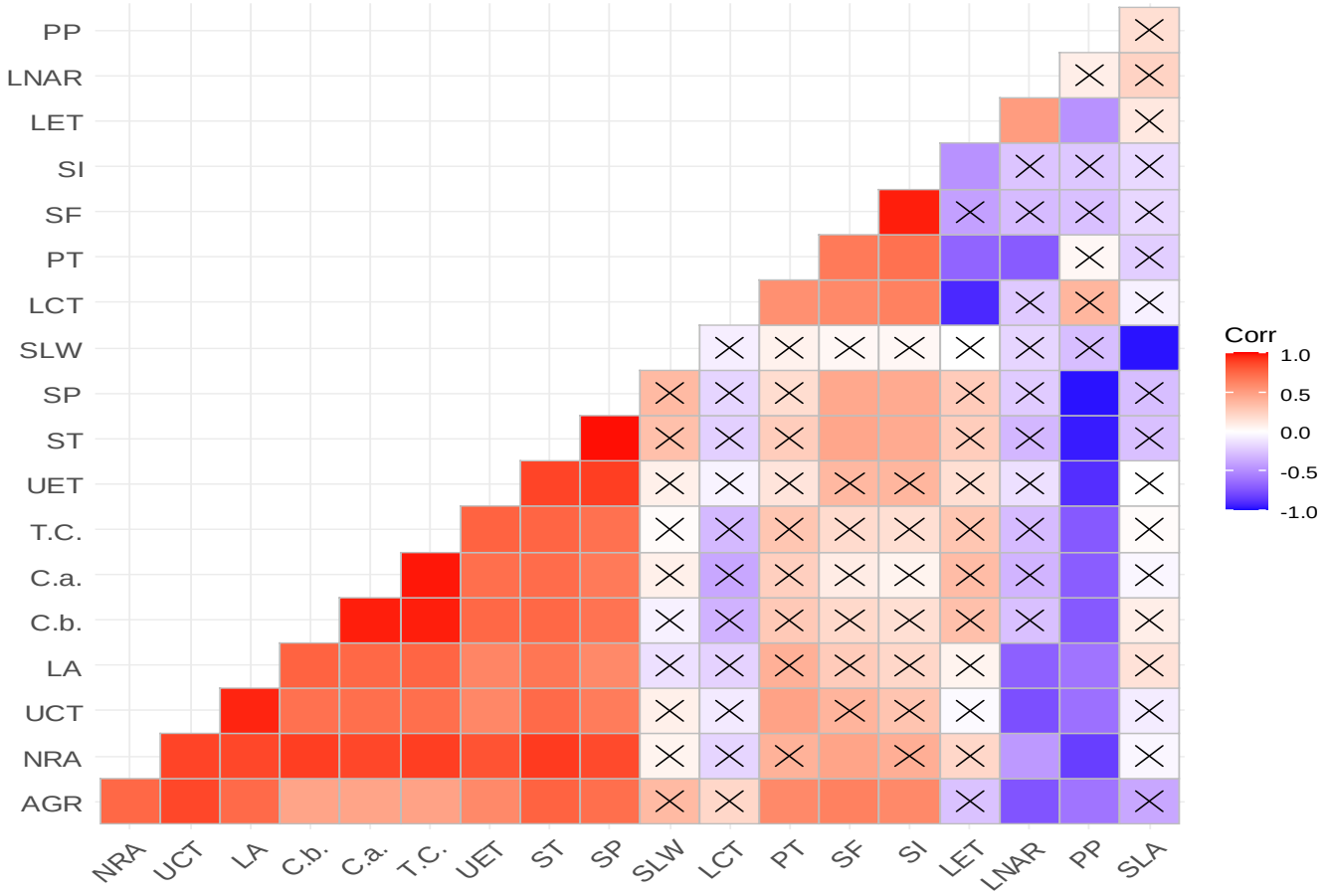


LYPP = Leaf yield per plant, ID = Internodal distance, FWHL= Fresh weight of hundred leaves, NBPP = Number of branches per plant, TBLPP= Total branch length per plant, LMPC= Leaf moisture percentage at chawkie stage, LMPL= Leaf moisture percentage at late age, MRC6 hrs = Moisture retention capacity after 6 hours, MRC12hrs= Moisture retention capacity after 12hours, UCT= Upper cuticular thickness, LCT= Lower cuticular thickness, UET= Upper epidermal thickness, LET= Lower epidermal thickness, ST= Spongy thickness, SP= Spongy proportion, PT= Palisade thickness, PP= Palisade proportion, SF= Stomatal frequency, SI = Stomatal index.

Table 46: Correlation among anatomical features with physiological traits



UCT= Upper cuticular thickness, LCT= Lower cuticular thickness, UET= Upper epidermal thickness, LET= Lower epidermal thickness, ST= Spongy thickness, SP= Spongy proportion, PT= Palisade thickness, PP= Palisade proportion, SF= Stomatal frequency, AGR = Absolute growth rate, LNAR= Leaf net assimilation rate, LA= Leaf area, SLA= Specific leaf area, SLW= Specific leaf weight, CA= Chlorophyll "a", CB= Chlorophyll "b", TC= Total chlorophyll, SI = Stomatal index, NRA = Nitrate reductase activity.



UCT= Upper cuticular thickness, LCT= Lower cuticular thickness, UET= Upper epidermal thickness, LET= Lower epidermal thickness, ST= Spongy thickness, SP= Spongy proportion, PT= Palisade thickness, PP= Palisade proportion, SF= Stomatal frequency, AGR = Absolute growth rate, LNAR= Leaf net assimilation rate, LA= Leaf area, SLA= Specific leaf area, SLW= Specific leaf weight, CA= Chlorophyll "a", CB= Chlorophyll "b", TC= Total chlorophyll**,** SI = Stomatal index, NRA = Nitrate reductase activity.

Chapter 5

DISSCUSSION

The results of the study entitled **"Studies on Physiological, Anatomical and Biochemical aspects of some mulberry genotypes during different seasons under Kashmir climatic conditions"** are discussed under the following heads:

5.1 Growth and yield

Goshoerami had the highest yield per plant during both spring (6.75 kg) and autumn (7.69 kg) where as Limoncina yielded the least with 2.27 and 3.17 kilograms leaf yield per plant during spring and autumn respectively. Goshoerami recorded the highest internodal distance during both spring (5.46 cm) and autumn (6.90cm) where as Ensatakasuke had the least internodal distance during spring (4.00 cm) and autumn (4.33cm). Chinese white though had significantly higher values for number of shoot lets per plant (65) and total shoot let length per plant (2524.78 cm) during spring and Rokokoyoso with the highest number of branches (48.33 cm) and total branch length (6861.75 cm) per plant during autumn lagged behind Goshoerami and some other genotypes like Ichinose, KNG, Kokusu-20, Kokuso-21 as far as leaf yield is concerned. The importance of growth associated traits in mulberry for better yield and quality have been documented by Sori and Gebbreselassie (2016). The knowledge of association between leaf yield and component traits can help in easy and efficient selection for future improvement programmes. Genotypic variations in yield and yield attributing characters were also reported by Magadum and Singh (2021), Baksh *et al*.2001, Mir *et al.* (2003), Mir (2012). Bigger leaf size, more leaf area and higher weight of 100 leaves are regarded as dependable characters associated with leaf yield (Rangaswami *et al.* ,1976), Susheelamma *et al*. (1988) and Bindroo *et al.* (1990). Pandit and Koul (2003) too have reported that leaf yield is controlled by a number of quantitative

characters with fresh leaf weight being a major component. The findings of the above authors are in line with the findings of present research programme.

5.2 Leaf moisture status

Moisture content in the mulberry leaf and its retention capacity play a crucial role in improving the palatability of these leaves to silkworm. Yokoyama (1975) and Paul *et al.* (1992) have highlighted the beneficial effects of leaf moisture content on its palatability and digestibility by the silkworm. Leaves should retain sufficient moisture for longer durations avoiding withering as many chemical changes occur on withering during storage. The results of the current study revealed that all the genotypes under study had more than 70% moisture content during spring at both chawkie (35 days after sprouting) and late age (60-65 days after sprouting) silkworm rearing. The moisture content during autumn was in general less as compared to the moisture content during spring season. Goshoerami recorded the highest leaf moisture content at chawkie stage of silkworm rearing during both the seasons (78.85% and 75.71% in spring and autumn respectively) where as it was the least in Limoncina during both the seasons (73.29 % and 69.90 % in spring and autumn respectively). The leaf moisture percentage at late age of silkworm rearing too was the highest in Goshoerami during both the seasons (76.71% and 73.09% in spring and autumn respectively) where as it was the least (70.11% during spring and 65.18% during autumn) in Limoncina. Better moisture content during spring could be because the spring season follows the winter season which is characterised by heavy precipitation in the form of rain and snow that might improve the water table and the moisture status of the leaves of mulberry. Krishnaswami (1970) and Hamamura *et al.* (1962) have reported that high leaf moisture content and its retention capacity have a positive influence on the growth and development of silkworm.

Moisture retention capacity is yet another very important parameter that decides the keeping quality of leaf. Moisture content in fresh leaf and its retention

capacity for longer durations besides higher yield in the genotypes Goshoerami, Ichinose, KNG, Kokuso-20 and Kokuso-21 may have had an important role in popularising these genotypes at farmers level under Kashmir conditions as majority of the farmers do not have sufficient mulberry plantation of their own and have to collect and transport the leaf over longer distances before feeding to their worms. The results of the current study revealed that the moisture retention capacity after 6 hours was the highest in Goshoerami during both the seasons (93.45% and 91.66% in spring and autumn respectively) where as it was the least in Limoncina (77.60% and 80.35% in spring and autumn respectively). Moisture retention capacity after 12 hours again was the highest in Goshoerami during both the seasons (87.97% and 81.35% in spring and autumn respectively) where as it was the least in Limoncina (69.06% and 71.27% in spring and autumn respectively). The variation in moisture content and its retention can be attributed to leaf anatomical and morphological features including stomatal frequency, cuticle thickness and mesophyll thickness. Similar results were reported by Mir *et al.* (2012), Shivashanker *et al.* (2015). The findings of the above authors are in line with the findings of present research programme.

5.3 Anatomical studies

Leaf anatomical characters are key functional and adaptive traits determining plant capacity to thrive in specific environments, in particular, because these traits have important implications for foliage potential, photosynthesis capacity (Scafaro *et al.,* 2011; Terashima *et al.,* 2006), disease resistance and structural defense mechanism [Juniper and Cox (1973), Sarwar *et al.* (1996) and Mir *et al.* (2012)], drought tolerance and moisture retention [Clarke and Mccaig (1982), Mir *et al.* 2012)].The genotypes depicted a great variation as far as the leaf anatomical features namely upper cuticle thickness, lower cuticle thickness, upper epidermal thickness, lower epidermal thickness, spongy thickness, palisade thickness, leaf thickness, stomatal length, stomatal width, stomatal index and stomatal frequency are concerned. The genotypes in general

had thicker upper cuticle as compared to lower cuticle except Rokokoyoso and Kairyoroso which can be the reason for comparatively less moisture content in these genotypes. Kurtz (1950) found that the formation of wax which has been considered to play an important role in water economy of plants was proportional to the thickness of the cuticle. Usually the genotypes with more upper cuticle cum epidermal thickness had better moisture content than those having lower thickness. Similar results have also been reported by Shivashankar (2015) and Mir *et al.* (2012). Drought tolerance adaptation involving dehydration tolerance would allow a range of plants to produce maximum leaf growth at a given water potential (Ninge, Gowda and Sudhakar, 2002). Leaf thickness and stomatal density have also been reported to be the most important leaf anatomical traits for coffee leaf photosynthesis. The results of the current study revealed that Goshoerami recorded the highest upper cuticular thickness (11.362µm), palisade thickness (65.447 µm), spongy thickness (81.790 µm), spongy proportion (41.968 %) and the leaf thickness (194.58%). This genotype performed the best as far as its yield, chlorophyll content and moisture status are concerned which may be because high photosynthetic rate, chlorophyll pigments, higher palisade thickness, leaf thickness and stomatal frequency are all directly correlated with high leaf yield in mulberry (Kumar *et al*. 2012). The lower cuticular thickness was the highest (5.978 µm) in Rokokoyoso. The upper epidermal thickness was the highest (24.733 µm) in KNG whereas Ichinose recorded the highest lower epidermal thickness (13.315 µm).The palisade proportion was the highest (41.463%) in Chinese white.

As far as the stomatal studies are concerned, the highest stomatal frequency (711.061 stomata /mm^2 was recorded in case of Rokokoyoso where as it was the lowest (432.020 mm^2) in KNG. The stomatal index too was the highest (23.572 %) in Rokokoyoso and the lowest (18.168%) in KNG. The highest stomatal length (24.135 µm) was recorded in case of Chinese white where as it was the least (19.807 µm) in Goshoerami. The stomatal width was the highest

(11.781 µm) in Chinese white where it was the least (9.958 µm) in Goshoerami. The results are in conformity with those of Erarslan *et al.* (2021), Kumar *et al.* (2012) and Mir *et al.* (2012). The findings of the above authors are in line with the findings of present research programme.

5.4 Physiological studies

The absolute growth rate (AGR) in both the seasons showed an increasing trend in all the genotypes from 1st to last interval. In a growing plant where the photosynthetic tissues make the major contribution to dry matter production, the absolute growth rate at any time is the product of net assimilation rate and the amount of leaf present. Absolute growth rate recorded thrice at an interval of 15 days during spring was significantly the highest in case of Goshoerami with 2.65 grams, 5.98 grams and 7.20 grams per day whereas, the it was the least (1.57, 2.77 and 3.68 grams per day) in case of Chinese white. Absolute growth rate recorded for three intervals during the autumn crop i.e. 1 August -15th September too was the highest in case of Goshoerami with 3.28, 6.85 and 9.81 grams per day where as it was the least in Chinese white with 1.90 grams per day during the first interval (1st -15th August), 3.81 grams per day during the 2nd interval (16-31 August) and 4.42 grams per day during the 3rd interval (1-15th September). Absolute growth rate may be responsible for the variation in leaf area and leaf yield as the genotypes with the higher yield showed relatively higher AGR. These results are also supported by the findings of Ghule *et al.* (2013) in BT cotton, Neilson *et al*. (2015) and Blum *et al.* (1989) in sorghum. The variation in AGR during the two seasons can be attributed to variation in temperature and photoperiod. The findings of the above authors are in line with the findings of present research programme.

Leaf net assimilation rate is the increase in dry biomass per unit leaf area and is a complex physiological variable associated with photosynthetic and respiration rates and the most important predictor of relative growth rate across a wide range of woody and herbaceous crops. During the course of present

investigation, the data revealed that during the three intervals of spring crop, Goshoerami reported the maximum leaf net assimilation rate with 0.9445, 0.9703 and 0.8286 mg/cm^2 /day respectively for the 1st (10 -25th April), 2nd (26 April-10 May) and 3rd (11-25th May) intervals. During the autumn crop, leaf net assimilation rate was again the maximum in Goshoerami registering 0.9741, 1.0055 and 0.8473 mg/cm^2 /day respectively during the 1st interval (1st-15th August), 2nd (16-31st August) and 3rd (1st-15th September) interval. It was however the least in Chinese white with 0.9164 mg/cm^2 /day during 1st interval, 0.9363 mg/cm^2 /day during 2nd interval and 0.7597 mg/cm^2 /day during the 3rd interval. As against the AGR, the leaf net assimilation rate showed an increasing trend between first and the second interval where as a decreasing trend during the 3rd interval in all the genotypes under study. These findings are in agreement with the findings of Brewster and Branes 1981, Walters *et al.* 2012, Shipley (2002), Poorter *et al.* (2012). Leaf net assimilation rate showed an increasing trend during the first two intervals and a decreasing trend in the last interval. This might be because leaf net assimilation rate decreases towards maturity of the leaf. Net assimilation rate and specific leaf area may vary with light availability and interspecific differences in specific leaf area generating variation in relative growth rate (Shipley *et al.* (2002). Quero *et al.* (2008) found leaf net assimilation rate to be dependent on mass and area based photosynthetic rates and strongly associated with daily carbon gain among four *Quercus* species. The findings of the above authors are in line with the findings of present research programme.

Chlorophyll content is very important for quantifying the photosynthetic efficiency of plant and is an essential constituent in assessing quality of foliage (Murthy *et al*. 2013). Based on the results obtained, chlorophyll content was in general more in spring as compared to autumn season. Chlorophyll "a" content was the highest in Kokuso-21 during both the seasons with 2.32 mg/g in spring and 1.99 mg/g in autumn where as it was the least in Chinese white during both the seasons (1.69mg/g in spring and 1.56 mg/g in autumn). Chlorophyll "b"

content too was the highest in Kokuso-21 during both the seasons with 1.76 mg/ml in spring and 1.29 mg/g in autumn where as it was the least in Limoncina during both the seasons (1.13 mg/g in spring and 1.05 mg/g in autumn). Total chlorophyll content was again the highest in Kokuso-21 during both the seasons with 4.08 mg/g in spring and 3.28 mg/g in autumn where as it was the least in Limoncina during both the seasons (2.82 mg/g in spring and 2.63 mg/g in autumn). Islam *et al.* (2022) while evaluating four mulberry genotypes for their chlorophyll content reported variations in chlorophyll content among mulberry varieties with the highest chlorophyll content (0.576%) in Goshoerami. Ghosh *et al.* (2006) too have reported variations in the chlorophyll content of some mulberry varieties in the gangatic alluvial soils. In general, chlorophyll content and carotenoids were more during spring as compared to autumn in all the genotypes. The studies are in conformity with Mosharraf *et al.* (2022) who have reported more chlorophyll and carotenoids in the mulberry leaf during spring than autumn. Demarez *et al.*(1999) while working on seasonal variation of leaf chlorophyll content of a temperate forest have reported that leaf chlorophyll concentration increases strongly at the beginning of the growing season (from April to May), remains stable during several months (from June to August) and decreases strongly when the leaves are senescent (from September to October/November). The findings of the above authors are in line with the findings of present research programme.

In the present investigation, the differences among the mulberry genotypes in terms of carotenoid content in leaves were significant being the highest in Ensatakasuke with 562.83 µg/g in spring and 535.50 µg/g in autumn whereas, it was the least (503.17 µg /g fw during spring and 487.83 µg /g fw during autumn) in KNG. These results are in line with that of Malik *et al*. (2023) who studied the varietal differences in carotenoid content among various mulberry genotypes under Kashmir climatic conditions. In general carotenoid content is more in spring as compared to autumn. Tan *et al*. (2019) while working on seasonal variation of

total carotenoids content in golden scallops (*Chlamys nobilis)* reported that the total carotenoid content was the highest in March – April and the lowest in September – October. Oliveira *et al.* (2018) while studying the seasonal variation of chlorophyll and carotenoids in leaves of *Hancornia speciosa* and reported that the total chlorophyll and total carotenoid content was the highest in spring (March – April) and the lowest in autumn (September – November). The findings of the above authors are in line with the findings of present research programme.

The Single leaf area was the highest (343.65 cm^2 during spring and 375.19 cm^2 during autumn crop) in Goshoerami and the least (133.01cm^2 and 141.41 cm^2 respectively during spring and autumn crop) in Limoncina. Sethua *et al.* (2012) studied the varietal differences in leaf area among 20 mulberry genotypes and reported the highest leaf area (103.40 cm^2) in T-30 genotype. Mir *et al*. (2012) while working on seven mulberry genotypes reported that Goshoerami recorded the highest single leaf area (360.67 cm^2 and375.70 cm^2) in spring and autumn respectively. This is in conformity with observations of Magadum *and Singh* (2021), Shyla (2016) and Ananya (2014). A genotype having more leaf area is more efficient in utilizing the natural resources i.e. light, water and minerals to maximize leaf production. This might be due to the sufficient sunlight, aeration and also abundant supply of the nutrients and moisture. The findings of the above authors are in line with the findings of present research programme.

Specific leaf weight and specific leaf area are considered as one of the important physiological parameters under growth analysis (Causton and Venus., 1981). Specific leaf weight and area are the desired characteristic for a better foliage quality (Nelson and Schweitzer (1988). Based on the results obtained, the specific leaf weight was the highest in Ensatakasuke with 0.789 g/cm^2 during spring and 0.794 g/cm^2 during autumn where as it was the least (0.443 g/cm^2 during spring and 0.452 g/cm^2 during autumn) in case of Kokuso-20. The specific leaf area was the highest (226.11cm^2/g in spring and 224.21cm^2 /g during autumn) in Kokuso-20 where as it was the least (126.59 cm^2/g during spring and 125.85

cm^2/g during the autumn crop) in Ensatakasuke. Genotypic variations in specific leaf weight and area in mulberry have also been found by Rahaman *et al.* (1995). These results are also in conformity with those of Ghosh *et al.* (2006), Sathyanarayana and Sangannavar (2020). High SLW in conjugation with other growth parameters is expected to increase the yield of leaves (Sarkar *et al*., 2003). The findings of the above authors are in line with the findings of present research programme.

Nitrate reductase activity determines future of actively growing plant particularly in terms of yield and biomass. The results of the current study revealed that nitrate reductase activity was the highest with 14.48 µm/ml/hr during spring and 10.64 µm/ml/hr during autumn in KNG. Limoncina, on the other hand, depicted the least nitrate reductase activity with 10.12 µm/ml/hr and 6.91 µm/ml/hr during spring and autumn season respectively. Ghosh *et al.* (2006) studied the varietal differences in nitrate reductase activity among ten mulberry varieties and reported that S1635 variety had the highest nitrate reductase activity (13.25 µmol/hr/g fw). Hatam and Hume (1976) while studying the relations between nitrate reductase activity and nitrogen accumulation in soybeans reported that the NR activity of field grown crops was proportional to nitrate uptake and might be used to determine amounts and seasonal patterns of nitrate activity by soybean plants. The results are in confirmation with those of Sethua *et al.* (2012) and Harijan *et al*. (2021). In the present study, the genotypes with higher nitrate reductase activity showed higher leaf protein content and leaf yield, which may be due to their better nutrient uptake and more in built nitrogen utilization efficiency. The findings of the above authors are in line with the findings of present research programme.

High nitrogen content in the leaf tissue allows the plant to have more chlorophyll, RuBisCo and triggers a higher rate of photosynthesis (Osaki *et al*., 1993). The results of the present study revealed that the leaf nitrogen content was the highest in KNG (3.92 % and 3.77% during spring and autumn respectively)

whereas it was the least in Limoncina (2.85% and 2.71% in spring and autumn respectively). Similar trend was recorded in nitrate reductase activity and may be the reason for variation in nitrogen content of the mulberry genotypes. Phosphorus is an element that directly affects the process of photosynthesis and it has also been reported to affect the dark reactions of photosynthesis, the apparent quantum efficiency and starch accumulation but the rate of electron transport and stomatal conductance is not affected. In the current study, the leaf phosphorus content was the highest in Kokuso-21 (0.331% in spring and 0.314% in autumn) where as it was the least (0.146% in spring and 0.128% in autumn) in Chinese white. Potassium is an element that is directly involved in translocation of photosynthates from source to sink. The results of the present study revealed that leaf potassium content was the highest in KNG (1.538% in spring and 1.517% in autumn) where as it was the least (1.103 % in spring and 1.077% in autumn) in Chinese white. NPK content in the leaf of mulberry genotypes was more during spring as compared to autumn. Parveen *et al.* (2022) and Malik *et al.* (2023) have also reported variations in the NPK content of some mulberry genotypes under Kashmir climatic conditions. Similar findings were also reported by Noor ul din *et al.* (2015). Higher content of P and K during spring as compared to autumn might be due to the fertilizer doze in vogue in the region which involves the application of full P and K as basal dose during spring and no application during autumn. (Baksh *et al.,* 2001). The differences in the NPK content in the leaf of the mulberry genotypes under study could be due to a variability in the nutrient use efficiency of these genotypes. (Bijarnia *et al*. 2017). The findings of the above authors are in line with the findings of present research programme.

Calcium is a structural component of cell wall. It activates enzymes, influences water movement in cell and is necessary for cell growth and division. In the present investigation, calcium content in leaf was the highest in KNG in both the seasons (13021.63 ppm in spring and 13122.52 ppm in autumn) and the least in Ensatakasuke (11181.05 ppm in spring and 11218.31 ppm in autumn).

Magnesium is a critical structural component of the chlorophyll and is necessary for the functioning of plant enzymes in the production of carbohydrates, sugars and fats. Leaf magnesium during both the seasons was the highest (6378.00 ppm and 6399.83 ppm in spring and autumn respectively) in Kokusu-21and it was the least (4391.83 ppm and 4419.00 ppm in spring and autumn respectively) in Limoncina. Iron content in leaf was the highest in Kokuso-21 with 203.53 ppm during spring and 195.82 ppm during autumn and least in Limoncina with the value of 161.61 ppm during spring and 157.58 ppm during autumn season. Ca, Mg and Fe content was more during autumn as compared to spring. Levickiene *et al.* (2018) have reported seasonal variations in the calcium and magnesium content of some mulberry genotypes. Khan *et al*. (2007), Rafiqui *et al.* (2021), Parveen *et al*. (2022) also have reported variations in the micro nutrient content of some mulberry genotypes under Kashmir climatic conditions. The increase in calcium content during the autumn season could be contributed to deposition of calcium as calcium pectate in middle lamella and replacement of starch deposits by calcium oxalate crystals which help in sequestering calcium in the leaves (Shear,1975). Variations in magnesium content in the leaf of mulberry genotypes might be responsible for the variations (depicted by the genotypes) in chlorophyll content as magnesium is an important constituent of chlorophyll and is directly involved in its synthesis (Tranknera *et al.,* 2018). The increase in magnesium content may be contributed to decrease in potassium content of leaves as these ions are strong antagonists, thus the cation magnesium is accumulated at the expense of potassium decrease (Loue, 1964; Nooruldin *et al*.2015). Manolikaki *et al.* (2022) have reported seasonal variation in the iron content of olive leaves. The findings of the above authors are in line with the findings of present research programme.

5.5 Biochemical studies

Sugars play an important role in determining the quality of leaf that in turn influence healthy growth and development of silkworms. Sugars are utilized as

the main source of energy apart from inducing the silkworms to bite the leaves (biting factor) and cherish it well. The results of the present investigation revealed that the highest total soluble sugar content was recorded in the leaves of KNG (2.03 and 2.33 g/100g fw in spring and autumn respectively) and it was the least (1.55 and 1.79 g/100g fw in spring and autumn respectively) in Limoncina. It was more in autumn as compared to spring. Genotypic differences in sugar content of mulberry genotypes have also been reported by Bose and Bindroo (2001) where in Chinese white had the highest value as compared to other genotypes. Varietal differences in total soluble sugar content in mulberry leaf have also been reported by Sethua *et al.* (2012) and Sori and Gebreselassie (2016). Souza *et al.* (2010) and Moraes *et al.* (2002) too have observed the seasonal variations in soluble sugars and starch and reported higher soluble sugar content during autumn as compared to spring and summer. Variation in the leaf sugar content could be attributed to the translocation of more photosynthetic products as reported by Hadimani *et al.* (2019). The findings of the above authors are in line with the findings of present research programme.

Carbohydrate content in mulberry leaves is maximum utilized by the chawkie silkworm larvae for physiological combustion and for making fat of the silkworm body. The results of the current study revealed that the highest total soluble carbohydrate content was observed in the leaves of KNG (3.95 and 3.41 g/100g fw in spring and autumn respectively) where as the lowest total soluble carbohydrate content (3.04 and 2.82 g/100g fw in spring and autumn respectively) was observed in Chinese white. Varietal differences in carbohydrate content in mulberry leaf have also been reported by Malik (2023) and Islam *et al.* (2022) in Kashmir climatic conditions and Harijan *et al.* (2021) and Hadimani *et al.* (2019) in tropical climatic conditions. Khan and Naik (2012) while evaluating three mulberry genotypes for their biochemical studies observed that S1635 genotype had the highest carbohydrate content as compared to other genotypes during the spring season which eventually decrease towards the summer season. Similar

observations were reported by Sivaci and Duman (2014). The findings of the above authors are in line with the findings of present research programme.

Leaf protein is a major determinant of nutrient quality for many lepidopteron larvae. It is a known fact that, nearly 70% of protein content of raw silk is directly biosynthesized from mulberry leaf protein and remaining 30% is derived from silkworm body tissue (Bose *et al*., 1991). In the present study, the highest total soluble protein was recorded in the leaves of Goshoerami (2.30 and 2.74 g/100g fw in spring and autumn respectively) where as it was the least (1.76 and 2.01 g/100g fw in spring and autumn respectively) in Limoncina. It was more in autumn as compared to spring season. Ayoub (2021) conducted biochemical studies of eight mulberry genotypes and reported that the highest protein content (23.62%) was recorded in the leaves of KNG. Sethua *et al.* (2012) while evaluating different mulberry genotypes for their physio- biochemical studies reported that the highest total soluble protein content (33.92 mg/g fw) was recorded in the leaves of T-36 as compared to other genotypes. Similar studies were conducted by Parveen (2022), Harijan *et al.* (2021) Sori and Gebreselassie (2016) and Murthy *et al.* (2013). Vasant and Nitin (2019) reported the seasonal variation in leaf protein content of *Aegle marmelos* (Bael) and reported that the highest protein content (5.995 mg/g) was recorded in autumn season as compared to spring (4.258 mg/g). The findings of the above authors are in line with the findings of present research programme.

Phenols are responsible for disease resistance in plants and function as hydrogen donors or acceptors in oxidation / reduction reactions. A small change in the host phenol metabolism severely disrupts many processes, which are essential for normal growth and development of plants. Increase of phenolic compounds may result from either synthesis of new aromatic compounds or the acceleration of accumulative phenols from neighbouring cells. The results of the current study revealed that the highest total phenol content was observed in the leaves of Limoncina (4.34 and 4.17 µg/g fw in spring and autumn respectively)

where as it was the least in KNG (3.47 and 3.30 µg/g fw in spring and autumn respectively). It was more in spring season as compared to autumn. Ayoub *et al.* (2021) conducted biochemical studies of eight mulberry genotypes and reported that the highest phenol content (4.22%) was recorded in the leaves of Kanva-2. Zou *et al*. (2012) reported that the mulberry leaves harvested in spring season had comparatively more phenol content as compared to summer and autumn season. Similar results were also reported by Murthy *et al.* (2013) and Harijan *et al.* (2021). The findings of the above authors are in line with the findings of present research programme.

5.6 Correlation among growth and yield, moisture content, physio-biochemical parameters and anatomical features

Correlation of a particular trait with other characteristics contributing to the leaf yield is of great importance for the indirect selection of high yielding mulberry genotypes. Therefore, the correlation obtained in the present study could be useful in the selection of traits having direct and significant correlation in improving leaf yield in mulberry. The results of the present investigation revealed that during spring, the highly significant positive correlation was observed between CA and TC (r: 0.99), CB and TC (r: 0.99), TSP and TSS (r: 0.99), CA and CB (r: 0.97), TSP and N (r: 0.97), MRC12 hours and NRA (r: 0.96), TSC and N (r: 0.97), MRC6hrs and MRC12hrs (r: 0.96), LYPP and MRC6hrs (r: 0.96), TSS and N (r: 0.96), NRA and LA (r: 0.90), NRA and TSS (r: 0.89); TSS and LA (r: 0.88); NRA and TSP (r: 0.86) where as the least significant positive correlation was observed between FWHL and CA (0.56), LMPLA (0.56), K and AGR (r: 0.51), TSC and AGR (r: 0.48),FWHL and LNAR (0.46), TSC and LMPLA (r: 0.46), P and LMPLA (r: 0.45), ID and LMPCS (r: 0.45), Fe and AGR (r: 0.44). The highly significant negative correlation was observed between SLA and SLW (-0.98), NRA and TP (R:-0.89), TP and N (r: 0.88), TP and TSS (r: 0.87), TP and TSP (r: 0.87), TSLPP and TC (-0.80),TSLPP and CA (-0.79); TSLPP and CB (-0.79), TP and Fe (r:0.84),TP and LNAR (r:-0.73) whereas the least significant

negative correlation was observed between NSP and SLW(r: -0.48) followed by NSPP and MRC 6hrs (r:-0.53), NSPP and TSP (r:- 0.47), TSLPP and Mg (r:- 0.48), SLW and Mg (r:-0.53),TP and Mg (r: -0.66).

During autumn, the highly significant positive correlation was observed between TSP and TSS (r: 0.99), ST and SP (r: 0.99), CA and TC (r: 0.98), UCT and LMPC (r: 0.98); SF and SI (r: 0.97), TSC and TSS (r: 0.97), CA and CB (r: 0.97). CB and TC (r: 0.97), LA and LMPCS (r: 0.97), TSP and N (r: 0.97), TSC and N (r: 0.97), MRC6hrs and MRC12hrs (r: 0.96), LYPP and MRC6hrs (r: 0.96), TSS and N (r: 0.96), NRA and SP (r: 0.91), ID and LYPP(r: 0.88),); UET and ST (r: 0.88), closely followed by NRA and UCT (r: 0.88); AGR and UCT (r: 0.87); SP and NRA (r: 0.86).However, the least significant positive correlation was observed between LET and LNAR(r: 0.51), TC and AGR (r: 0.48), SF and ST (r: 0.48), UCT and PT (r: 0.48), AGR and CA (r: 0.47), AGR and CB (r: 0.47), Mg and AGR (r: 0.47), NRA and SF (r: 0.47), TSC and LMPLA (r: 0.46), TC and MRC6hrs (0.45), P and LMPLA (r: 0.45), ID and LMPCS (r: 0.45), SI and ST (r: 0.44) and SI and SP (r: 0.44), SF and SP (r: 0.45). Conversely, the highly significant negative correlation was observed between SLA and SLW (r: -0.97), SLW and SLA (r: -0.97), PP and SP (r: -0.97), PP and ST (r:- 0.95); LCT and LET(r:-0.91)LNAR and FWHL (r: -0.91), NRA and TP (R:-0.91), TP and N (r: 0.88), TP and TSC (r:-0.87), TP and Fe (r: 0.85).On the other hand the least significant negative correlation was observed between PP and LMPC (r: -0.51), SLW and Mg (r:-0.48), NBPP and SLW(r:-0.48), NBPP and LYPP (r:- 0.47), LNAR and NRA (r: -0.44), SI and LET (r: -0.47), PP and LET (r: -0.47), LNAR and NRA(r: -0.44). Magadum and Singh (2022) while studying correlation and path coefficient analysis of growth and yield contributing characters in mulberry reported that leaf yield per plant has a positive and significant correlation with number of leaves per plant, internodal distance, total shoot length, fresh weight of 10 leaves, leaf area and leaf moisture content and its retention capacity. Harijan *et al*. (2021) studied the correlation among morpho-physiological and biochemical

traits in mulberry and reported that positive and significant correlation among TSP and FLW(r: 0.640), LMC AND SLA (r: 0.614), NRA and SLA (r: 0.353), TSP and NRA (r: 0.431). Sathyanaryana and Rajshekhargouda (2020) reported positive and significant correlation between total carbohydrate content, total protein content, nutrient content and yield. The results are also in conformity with those of Magadum and Singh (2021), Sathyanaryana and Rajshekhargouda *et al.* (2020), Saini *et al*. (2018), Biradar and Narayanswamy (2020), Laltanmawii and Roychowdhuri (2010).

Chapter 6

SUMMARY AND CONCLUSION

Growth and yield parameters in mulberry showed significant differences among genotypes in both the seasons with Goshoerami having highest yield during both the seasons as compared to the other genotypes.

The genotypes under study depicted variations as far as moisture content in leaf at chawkie (35 days after sprouting) as well as late age (60 days after sprouting) and its retention capacity. All the genotypes had more than 70% moisture content during spring. High leaf moisture content and its retention capacity in Goshoerami may be due to more thickness of upper cuticle cum epidermis, stomata of smaller dimensions and more thickness of leaf. Leaf moisture content in general was more in spring than in autumn.

Absolute growth rate was more in autumn as compared to spring. Absolute growth rate showed an increasing trend during all the three intervals in all the genotypes in both the seasons and differences in absolute growth rate may be responsible for the variation in leaf yield in the genotypes. Further higher yield during autumn may be attributed to higher absolute growth rate as compared to spring.

Leaf net assimilation rate showed an increasing trend during the first two intervals and a decreasing trend in the last interval. This might be because leaf net assimilation rate decreases towards maturity of the leaf.

Chlorophyll content also depicted variations among the genotypes with the maximum in Kokuso-21 and the least in Limoncina. It was higher during spring season in all the genotypes in comparison to autumn. Possible reason could be that chlorophyll has a tendency to decline more rapidly when plants are heading towards leaf scenesense.

Carotenoid content was more during spring (fruiting season) than autumn and comparatively higher in Ensatakasuke and Rokokoyoso (two fruiting genotypes) in both the seasons as compared to other genotypes.

NPK content in the leaf of Goshoerami, Kokuso-21 and KNG was higher than the other genotypes which may be due to better nitrate reductase activity in these genotypes. More Nitrogen content during spring as compared to autumn might be attributed to higher nitrate reductase activity where as the higher Phosphorus and Potassium content might be attributed to fertilizer schedule in vogue in the region.

Variation in magnesium may be due to variation in potassium content and may be responsible for variation in chlorophyll content among the genotypes. The increase in calcium content during the autumn season could be due to deposition of calcium oxalate crystals towards the fall.

The variation in leaf sugar content among the genotypes and between the seasons could be due to varied translocation rates of photosynthetic products. Carbohydrate content was more during spring which decreased towards the late summer/ autumn. Leaf protein was more during autumn as compared to spring. Phenols were more in Chinese white and the phenol content was more during spring.

The genotypes depicted great variation in different leaf anatomical features and the genotypes in general had thicker upper cuticle as compared to lower cuticle which can be the reason for better leaf moisture in these genotypes. The reason behind Goshoerami faring the best in terms of yield and leaf moisture status might be due to higher photosynthetic rate, chlorophyll content, palisade thickness, leaf thickness and stomatal frequency.

Leaf moisture content and moisture retention capacity showed positive correlation with most of the physiological and biochemical parameters. However, they were negatively correlated with total phenol content.

Amongst the various leaf anatomical features, upper epidermal and cuticular thickness, spongy thickness and spongy proportion showed a highly positive correlation with most of the biochemical and physiological parameters. However, there was no correlation between lower epidermal and cuticular thickness and other physiological and biochemical parameters.

The study has generated information on the anatomical, physiological and biochemical aspects of different mulberry genotypes and their relationship with leaf yield and quality. The information generated can be useful in future breeding programmes.

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Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Basic Sciences and Humanities,
College of Temperate Sericulture, Mirgund, Baramulla

CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner **Dr. Pankaj Tewary**, Director (Rtd.) Central Sericultural Research and Training Institute, Mysore, CSB, Ministry of Textiles, Govt. of India during viva-voce examination held on **31-10-2023** have been incorporated in the manuscript entitled **"Studies on Physiological, Anatomical and Biochemical aspects of some mulberry genotypes during different seasons under Kashmir climatic conditions"** submitted by **Ms. Shaista Mehraj (Regd. No. 2019-885-D).**

(Dr. Mushtaq Rasool Mir)
Chairman
Advisory Committee