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Leaf Quality Evaluation of Eight Mulberry (*Morus* spp.) Genotypes through Biochemical Analysis

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ABSTRACT: Silkworm, *Bombyx mori* L. derives all the nutrients required for its growth and development from mulberry leaves which is the sole source of food for the insect. Therefore, silk production is directly influenced by the nutritional quality of leaves. The present study evaluated the biochemical composition of leaves from eight mulberry genotypes—Goshoerami, Ichinose, Koksu-21, Koksu-20, KNG, Enshutakasuke, Kanva-2 and Kairiyoroso. The experiment was laid in a randomized block design with three replications assessing the following parameters: nitrogen (%), phosphorus (%), potassium (%), total protein (%), total carbohydrate (%), total chlorophyll (%) and total phenol (%). Results indicated significant variations among the genotypes in macronutrient content. KNG exhibited highest nitrogen (3.78%), protein (23.62%), carbohydrate (20.27%) and potassium (1.50%) levels while Kokso-21 displayed the highest phosphorus (0.31%) and chlorophyll (0.35%) content. In contrast, Kanva-2 showed the lowest nitrogen (2.82%), protein (17.63%), carbohydrate (15.87%) and chlorophyll (0.26%) content. The highest phenol content was observed in Kanva-2 (4.22%) while Koksu-21 had the lowest (3.28%). KNG outperformed all other genotypes in most of the parameters and was found to be the most nutritive. These differences highlight genotypic variability and variation in nutrient absorption and hence suggest that the selection of genotype plays an important role in determining the nutritional quality of mulberry leaves which will directly impact the silkworm growth and silk yield.

Keywords: Mulberry, genotype, biochemical, analysis, quality.

INTRODUCTION

Sericulture, the art and science of rearing silkworms on scientific basis with an aim of producing quality silk, is an old-age practice with global significance. The industry encompasses three activities *viz.*, silkworm rearing, mulberry cultivation and silk reeling. However, more than 60% of total cost of cocoon production goes towards mulberry production alone (Lakshmanan, 2010). In order to safeguard the health and robustness of silkworm, the nutritional quality of mulberry leaf is critical asmulberry (*Morus* spp.) leaves are the primary and exclusive food source for the silkworm, *Bombyx mori* L. (Rafiqui *et al.*, 2023). The biochemical composition of mulberry leaves directly influences the growth and development of silkworms which in turn influences the quantity and quality of cocoons produced by them. The key biochemical constituents such as proteins, carbohydrates, lipids, minerals and secondary metabolites contribute to various physiological and metabolic processes essential for optimal silkworm performance (Alipanah *et al.*, 2020). Studies indicate that silkworms fed on high quality mulberry leaves exhibit better growth rates and higher cocoon production compared to those fed on lower quality leaves (Hamzah, 2018).

The quality of leaf among different varieties of mulberry is significantly influenced by its genome, cultivation practices, preservation techniques, age and position of leaf which is turn responsible for the difference in silkworm rearing performances (Rohela *et al.*, 2020). Macronutrients such as nitrogen, phosphorus and potassium directly influence mulberry leaf quality and silkworm health (Sarkhel *et al.*, 2020). Nitrogen aids protein synthesis, supporting growth and cocoon quality while phosphorus facilitates energy metabolism and nutrient utilization and potassium enhances enzyme activation, digestion, immunity and overall health (Ruth *et al.*, 2019). Similarly, leaf protein provides essential amino acids for silkworm growth, silk protein synthesis and cocoon formation (Urbanek Krajnc *et al.*, 2022). Carbohydrates and dietary fibers support energy metabolism (Chundang *et al.*, 2020) while phytochemicals like flavonoids and phenolics enhance immunity and reduce oxidative stress (Hao *et al.*, 2022). Furthermore, the chlorophyll, influenced by nitrogen, plays a crucial role in photosynthesis, improving leaf nutrient content and silkworm nutrition (Ahmed *et al.*, 2022).

Since there is existence of wide variability in the biochemical composition of mulberry leaves across genotypes and nutritional profiling is necessary for understanding their potential impact on silkworm rearing and silk yield. Although the present study does not involve direct silkworm rearing, it aims to evaluate eight mulberry genotypes through biochemical evaluation of leaf which can aid the selection of nutritionally superior mulberry varieties thereby enhancing sericulture productivity and sustainability.

MATERIAL AND METHODS

The study was carried out during autumn season on well-established mulberry germplasm of College of Temperate Sericulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Mirgund, maintained as per the recommended package of practices (Anonymous, 2003). Leaf samples were collected from three plants of each genotype of each replication. The samples were then shade-dried, packed in paper bags and dried in oven at 70ºC till constant weight was obtained. The samples were separately homogenised in a stainless-steel grinder/blender to pass through 2 mm mesh sieve and were stored in airtight polythene bags for chemical analysis. The biochemical analysis of leaf was conducted following standard procedures. The experiment was laid in randomized block design with three replications for each treatment. The estimation of different biochemical constituents from healthy leaves of eight mulberry genotypes – Goshoerami, Ichinose, Koksu-21, Koksu-20, KNG, Enshutakasuke, Kanva-2 and Kairiyoroso was undertaken at Research Centre for Residue and Quality Control Analysis laboratory, SKUAST-Kashmir for the following parameters:

The observations on following parameters were recorded:

Leaf nitrogen (%). The nitrogen content was determined by Microkjaldal's method as described by Jackson (1973).

Leaf phosphorus (%). The phosphorus content was determined by Vandomolybedate phosphoric acid yellow colour method (Jackson, 1973).

Leaf potassium (%). The potassium content was determined by Flame photometry method using corning flame photometer, U.K. (Jackson, 1973).

Total protein (%). The total protein content was determined by Kjeldhal method as described by Ranganna (1997).

Total carbohydrate (%). The total carbohydrate was determined by Anthrone method as per the procedure outlined by Hedge and Hofreiter (1962).

Total chlorophyll (%). The total chlorophyll content of the mulberry leaf was determined by Sadasivam and Manickam (1992) method.

Total phenol (%). The total phenolic content of the mulberry leaf was determined by modified method reported by Malik and Singh (1980).

RESULTS

Macronutrients (N, P, K) (%). Optimum levels of major nutrients in leaves enhance their nutritional quality, energy availability for silkworms, improves digestion and overall silkworm health which ultimately enhances silk yields (Iqbal *et al.*, 2012). Nitrogen is a fundamental component of amino acids which are essential for protein synthesis in silkworms (Lim *et al.*, 2013). Similarly, phosphorus plays a vital role in energy metabolism and is crucial for the growth and development of silkworms (Chakrabarti *et al.*, 1997). Potassium is essential for various physiological processes including enzyme activation, stress resistance and osmoregulation which are critical for silkworm health and digestion (Goudar and Kaliwal 2001).

In the present investigation, the nitrogen concentration among the mulberry genotypes exhibited considerable variation ranging from 2.82% to 3.78%. KNG stood out with the highest nitrogen content of 3.78% followed by Kokso-21 at 3.58% and Goshoerami at 3.44%. In contrast, the lowest nitrogen levels were recorded in Kanva-2 at 2.82% followed by Enshutakasuke at 2.94% and Kairiyoroso at 2.98% (Table 1). Similarly, phosphorus levels also exhibited notable differences, ranging from 0.14% to 0.31%. The highest phosphorus content was found in Kokso-21 at 0.31% followed by Kokso-20 at 0.27% and KNG at 0.23%. The genotypes with the lowest phosphorus levels were Kairiyoroso at 0.14% and Kanva-2 at 0.15% (Table 1). Potassium content in the mulberry genotypes ranged from 1.12% to 1.50%. KNG exhibited the highest potassium content at 1.50% followed closely by Kokso-21 at 1.48% and Kokso-20 at 1.43%. The lowest potassium levels were recorded in Kairiyoroso at 1.12% and Ichinose at 1.16% (Table 1).

Table 1: Variation in the macronutrients (N, P, K) content among leaves of different mulberry genotypes.

Genotype	Nitrogen (%)	Phosphorus (%)	Potassium (%)
Goshoerami	3.44	0.20	1.39
Ichinose	3.11	0.18	1.16
Kokso-21	3.58	0.31	1.48
KNG	3.78	0.23	1.50
Enshutakasuke	2.94	0.22	1.27
Kanva-2	2.82	0.15	1.31
Kokso-20	3.38	0.27	1.43
Kairiyoroso	2.98	0.14	1.12
C.D ($p \leq 0.05$)	0.098	0.04	0.07

Fig. 1. Macronutrient estimation of different mulberry genotypes at RCRQA laboratory, Shalimar.

Total Protein (%). Proteins are vital for growth and development in silkworms and higher protein content in mulberry leaves can lead to enhanced growth rates and silk production as they provide essential amino acids for larval growth and silk synthesis (Urbanek Krajnc *et al.*, 2022)

In the present investigation, the total protein content varied considerably among the genotypes ranging from 17.63% to 23.62%. KNG exhibited the highest protein content at 23.62%, followed by Kokso-21 at 22.37% and Goshoerami at 21.50%. The genotypes with the lowest protein content were Kanva-2 at 17.63% and Enshutakasuke at 18.37% (Table 2).

Total Carbohydrate Content (%). Since carbohydrates are the primary energy source for silkworms, and higher carbohydrate levels in mulberry leaves can promote better vitality in the silkworm larvae and promote silk yield (Rafiqui *et al.*, 2023).

In the present investigation, the total carbohydrate content ranged from 15.87% to 20.27%. The highest carbohydrate levels were found in KNG at 20.27%, followed by Kokso-21 at 19.82% and Goshoerami at 19.74%. The lowest carbohydrate content was observed in Kanva-2 at 15.87%, followed by Enshutakasuke at 16.38% (Table 2).

Total Chlorophyll Content (%). Higher chlorophyll content is indicative of better photosynthetic efficiency, which can enhance the overall quality of mulberry leaves and their nutritional value for silkworms (Srichaikul *et al.*, 2011).

In the present investigation, chlorophyll content varied across genotypes, ranging from 0.26% to 0.35%. Kokso-21 had the highest chlorophyll content at 0.35%, followed by KNG at 0.34% and Goshoerami at 0.32%. The genotypes with the lowest chlorophyll levels were Kanva-2 at 0.26% and Kairiyoroso at 0.28% (Table 2).

Total Phenol Content (%). Phenolic compounds are known for their antioxidant properties, which can enhance the health of silkworms by protecting them from oxidative stress (Hao *et al.*, 2022).

Fig. 2. Biochemical estimation of different mulberry genotypes at RCRQA laboratory, Shalimar.

In the present investigation, the total phenol content among the genotypes ranged from 3.28% to 4.22%. Kanva-2 exhibited the highest phenol content at 4.22%, followed by Enshutakasuke at 4.09%. The lowest phenol levels were recorded in Kokso-21 at 3.28%, with Goshoerami and KNG showing intermediate levels of 3.41% and 3.36%, respectively (Table 2).

Table 2: Variation in the biochemical content among leaves of different mulberry genotypes.

Genotype	Total Protein (%)	Total Carbohydrate (%)	Total Chlorophyll (%)	Total Phenol (%)
Goshoerami	21.50	19.74	0.32	3.41
Ichinose	19.44	17.15	0.31	3.68
Kokso-21	22.37	19.82	0.35	3.28
KNG	23.62	20.27	0.34	3.36
Enshutakasuke	18.37	16.38	0.29	4.09
Kanva-2	17.63	15.87	0.26	4.22
Kokso-20	21.13	19.12	0.31	3.47
Kairiyoroso	18.63	16.04	0.28	3.98
C.D ($p \leq 0.05$)	0.61	0.30	0.02	0.09

DISCUSSION

The variation in the biochemical constituents among different mulberry genotypes has been recorded by many researchers across the mulberry growing regions (Kumar *et al.*, 2010; Mahadeva and Nagaveni 2012; Murthy *et al.,* 2013; Sharma *et al.,* 2015; Priya and Sujathamma 2020; Rafiqui *et al.*, 2023). The observed variations can be attributed to several factors including genetic diversity, environmental conditions and cultivation practices. However, genetic variation is considered as one of the significant contributor to the differences in biochemical composition among mulberry genotypes. Awasthi *et al*. (2004) reported substantial genetic diversity in mulberry revealing that different genotypes exhibit distinct biochemical profiles due to their genetic makeup. This genetic diversity influences the synthesis of various metabolites including proteins, phenolics and carbohydrates, which are crucial for the plant's growth and nutritional value for silkworms. Geçer *et al*. (2016) found significant differences in the content of sugars, organic acids and phenolic compounds between black and white mulberry accessions underscoring the influence of genotype on biochemical composition. The wild and cultivated *Morus* species exhibit differences in biochemical properties (Weiguo *et al.*, 2006) indicating that the genetic background is a key determinant of the biochemical composition (Vijayan and Chatterjee 2003). Krishnan *et al.* (2014) conducted a microsatellite marker analysis that revealed significant genetic diversity among mulberry accessions which correlates with variations in biochemical traits. Similarly, the plant's adaptability to different climatic conditions also resulted in substantial variations in secondary metabolites which are essential for the plant's bioactivity. Yang *et al.* (2017) reported that climatic variations can lead to differences in the accumulation of phenolic compounds in mulberry fruits which are known for their health benefits.

The methods adopted in cultivating mulberry can also lead to variations in its biochemical constituents. Sugiyama *et al*. (2016) demonstrated that the application of nitrogen affects the functional components of mulberry leaves indicating that fertilization practices can significantly influence the biochemical composition. The variation in leaf nutrient content among different genotypes also suggests differences in their ability to absorb nutrients from the soil. This can be attributed to variations in root proliferation, the density and efficiency of their root systems and the availability of nutrients in the soil.

CONCLUSIONS

The study evaluated different biochemical constituents of leaf which are essential for silkworm growth and silk production among eight mulberry genotypes. Among them, KNG was found to be the most nutritive, containing the highest levels of nitrogen, protein, carbohydrate and potassium. Kokso-21 had the highest phosphorus and chlorophyll content while Kanva-2 had the lowest levels of essential nutrients but the highest phenol content which may help in plant defence. The variations in nutrient composition can be attributed to genetic differences, environmental factors and cultivation practices. These results highlight the importance of selecting the suitable mulberry genotype with improved leaf quality for better sericulture productivity. Therefore, targeted breeding and improved cultivation practices are needed to develop better mulberry varieties for sustainable sericulture. Future research should focus on how these genotypes affect silkworm growth, cocoon yield and silk quality to confirm their usefulness in sericulture.

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Conflict of Interest. None.

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