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DNA Extraction and Molecular Analysis of V1 Mulberry (*Morus alba*) Leaves at Different Developmental Stages

*Ritwik Giri and Neha Mahato
CSB – CTRTI Ranchi, Jharkhand, India
*Corresponding Author's email: ritwikagriculture@gmail.com

Mulberry (*Morus alba*) is the primary host plant for silkworm rearing, and its genetic and physiological quality directly influences silk production. The present study aimed to extract and analyze genomic DNA from V1 mulberry leaves at different developmental stages (young, mature, and senescent) to assess variation in DNA yield, purity, and integrity. A modified CTAB method was employed for DNA extraction, followed by spectrophotometric analysis and agarose gel electrophoresis. Results indicated that young leaves yielded the highest quality DNA with superior purity (A260/A280 ~1.8), whereas mature leaves showed moderate yield, and senescent leaves exhibited degraded DNA due to increased phenolic compounds. Gel electrophoresis confirmed intact high-molecular-weight DNA in young leaves, while smearing was observed in older tissues. The study demonstrates that leaf developmental stage significantly influences DNA extraction efficiency and downstream molecular applications. These findings are useful for optimizing sampling strategies in mulberry molecular research.

Keywords: Mulberry, DNA extraction, CTAB method, Leaf stage, Molecular analysis, Morus alba

Introduction

Mulberry (*Morus alba*) plays a crucial role in sericulture as the sole food source for the silkworm *Bombyx mori*. The productivity and quality of mulberry leaves directly influence silkworm growth and silk yield. In recent years, molecular approaches have been increasingly used to study genetic variability, stress tolerance, and disease resistance in mulberry. DNA extraction is the first and most critical step in molecular studies. However, plant tissues, especially in species like mulberry, contain high levels of secondary metabolites such as polyphenols and polysaccharides, which interfere with DNA isolation. The efficiency of DNA extraction can vary significantly depending on the physiological stage of the leaf. Young leaves generally contain lower levels of inhibitory compounds and higher cellular activity, making them ideal for DNA extraction. In contrast, mature and senescent leaves accumulate phenolics and other compounds that can degrade DNA or reduce its purity. The present study aims to compare DNA yield and quality from V1 mulberry leaves at different developmental stages and to standardize an efficient extraction protocol.

Materials and Methods

Sample Collection

Leaf samples were collected from V1 mulberry plants at three developmental stages:

- Young leaves (top tender leaves)
- Mature leaves (fully expanded middle leaves)
- Senescent leaves (older bottom leaves)

Samples were collected early in the morning and stored in ice to prevent degradation.

DNA Extraction (Modified CTAB Method)

Fresh leaf tissue (0.5 g) was ground in liquid nitrogen using a sterile mortar and pestle. The powdered tissue was transferred to extraction buffer containing:

- ***CTAB (2%)***
- ***NaCl (1.4 M)***
- ***Tris-HCl (100 mM, pH 8.0)***
- ***EDTA (20 mM)***
- ***β-mercaptoethanol (0.2%)***

The mixture was incubated at 65°C for 30 minutes with occasional mixing. After incubation, chloroform:isoamyl alcohol (24:1) was added and centrifuged to separate phases. The aqueous phase was collected and DNA was precipitated using cold isopropanol. The DNA pellet was washed with 70% ethanol, air-dried, and dissolved in TE buffer. RNase treatment was performed to remove RNA contamination.

DNA Quantification and Quality Analysis

DNA concentration and purity were measured using a spectrophotometer at 260/280 nm ratio. DNA integrity was assessed by agarose gel electrophoresis (0.8% agarose gel).

Results

Table 1: DNA Yield and Purity

Leaf Stage	DNA Yield (µg/g tissue)	A260/A280 Ratio	Quality
Young	850	1.82	High
Mature	620	1.70	Moderate
Senescent	400	1.45	Low

Table 2: Gel Electrophoresis Observation

Leaf Stage	DNA Band Quality	Observation
Young	Sharp band	Intact DNA
Mature	Slight smear	Partial degradation
Senescent	Heavy smear	Degraded DNA

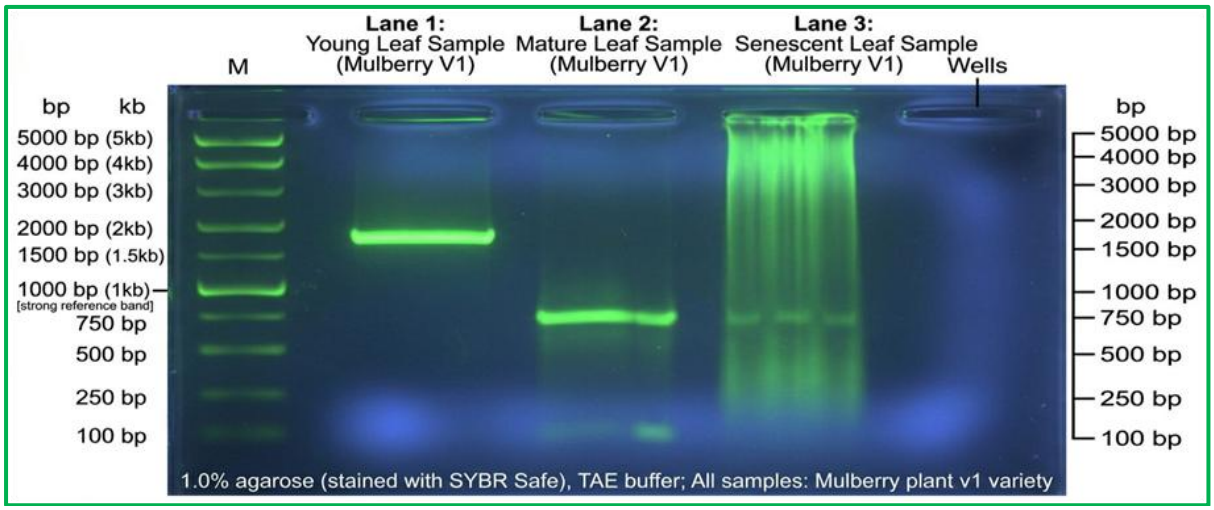

Fig :- Gel Electrophoresis Observation

Discussion

The results clearly indicate that the developmental stage of mulberry leaves significantly affects DNA extraction efficiency. Young leaves yielded the highest quantity and quality of DNA, which can be attributed to lower levels of phenolic compounds and higher cellular activity. Mature leaves showed moderate DNA yield and purity, indicating the presence of some inhibitory substances. Senescent leaves exhibited poor DNA quality due to the accumulation of secondary metabolites such as polyphenols, which interfere with DNA isolation and cause degradation. The A260/A280 ratio further confirmed these findings, with values close to 1.8 indicating pure DNA in young leaves, while lower values in older leaves

suggested contamination. Gel electrophoresis results supported the spectrophotometric data, showing intact DNA in young leaves and degraded DNA in senescent leaves. These findings are consistent with previous studies on plant DNA extraction. The study highlights the importance of selecting appropriate plant material for molecular analysis. Using young leaf tissue can significantly improve the efficiency and reliability of downstream applications such as PCR, sequencing, and genetic analysis.

Conclusion

The study concludes that young leaves of V1 mulberry are the most suitable source for DNA extraction due to their high yield, purity, and integrity. Mature leaves can be used with moderate success, while senescent leaves are not recommended for molecular studies. The modified CTAB method proved effective for mulberry DNA extraction, though optimization is required for older tissues.

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