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PHYTOPLASMA-ASSOCIATED REVERSE METAMORPHOSIS: A COMPARATIVE DNA METHYLOME ANALYSIS IN *SESAMUM INDICUM*

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Abstract

Phytoplasma infection in *Sesamum indicum* induces severe morphological abnormalities such as phyllody, characterized by the transformation of floral organs into leaf-like structures. This phenomenon represents retrograde metamorphosis, where reproductive organs revert to vegetative identity. The present study investigates genome-wide DNA methylation changes associated with phytoplasma infection using Whole Genome Bisulfite Sequencing (WGBS) and methylation-sensitive PCR approaches. Comparative methylome profiling between healthy and infected plants revealed global hypomethylation, particularly in the CHH context, along with locus-specific hypermethylation in genes associated with development and Défense. Differentially methylated cytosines (DMCs) were predominantly located in intergenic and promoter regions, suggesting epigenetic regulation of gene expression. The findings highlight the critical role of DNA methylation in phytoplasma-induced phenotypic plasticity and provide insights into molecular mechanisms underlying retrograde metamorphosis.

Keywords - Phytoplasma, Phyllody, DNA methylation, WGBS, Retrograde metamorphosis, *Sesamum indicum*, Epigenetics.

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1-Introduction

Sesamum indicum L. (sesame) is one of the oldest cultivated oilseed crops, valued for its high oil content, antioxidant compounds, and nutritional importance. In India, sesame contributes significantly to edible oil production, particularly in states like Uttar Pradesh, Rajasthan, and Gujarat. However, sesame productivity is severely constrained by phytoplasma-associated diseases, especially phyllody[1,2].

Phytoplasmas are wall-less, phloem-restricted prokaryotes belonging to the class Mollicutes. They are transmitted by insect vectors such as leafhoppers and cause a wide range of plant developmental abnormalities[3]. In sesame, phytoplasma infection leads to symptoms including floral virescence, witches' broom, little leaf, and most prominently phyllody, where floral organs transform into leaf-like structures[4].

This transformation represents retrograde metamorphosis, a phenomenon where reproductive structures revert to vegetative identity. At the molecular level, this is associated with disruption of floral meristem identity genes, particularly MADS-box transcription factors (e.g., APETALA1, LEAFY homologs). Phytoplasma-secreted effector proteins, such as SAP54, are known to degrade these transcription factors, thereby reprogramming plant development[5,6].

In recent years, epigenetics has emerged as a crucial layer of gene regulation in plant-pathogen interactions. Among epigenetic modifications, DNA methylation plays a central role in regulating gene expression, transposable element silencing, and genome stability. In plants, DNA methylation occurs in three sequence contexts: CG, CHG, and CHH (where H = A, T, or C). These methylation patterns are dynamically regulated in response to environmental and biotic stresses[7].

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Losses in economic production are a common consequence of phytoplasma associated symptoms which are essentially the result of severe changes to the normal developmental programme of the host plant. The extent to which this has a deleterious effect on the genetic control of the host plants is not well known nor is the mechanism by which this occurs[8]. The insertion of a methyl group to the 50-a position of cytosine bases in DNA is one of the epigenetic methods used to regulate gene expression (-CH3). Disruption of DNA methylation has been linked to aberrant development in plants. DNA methylation has been described in plant pathogen interaction as playing a role in biotic stress[9].

Previous studies suggest that pathogen infection can induce genome-wide methylation changes, leading to activation or repression of stress-responsive genes. However, the role of DNA methylation in phytoplasma-induced retrograde metamorphosis in sesame remains largely unexplored[10].

Therefore, the present study aims to:

- Perform comparative DNA methylome analysis between healthy and infected sesame plants
- Identify differentially methylated cytosines (DMCs) and genes (DMGs)
- Correlate methylation changes with developmental reprogramming and phyllody symptoms

This study provides novel insights into the epigenetic basis of phytoplasma pathogenesis and floral reversion.

2-Materials and Methods:

2.1-Study Area

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The study was conducted in sesame-growing regions of Jaunpur district, located in the eastern part of Uttar Pradesh under the Varanasi division. The region lies between latitude 24.24°N and longitude 82.70°E, characterized by a subtropical climate with hot summers and moderate rainfall (~1000–1200 mm annually).

Agricultural fields in this region frequently report phytoplasma infection in sesame crops, making it an ideal site for sampling. The soil type is predominantly alluvial, suitable for sesame cultivation.

2.2-Plant Material Collection

Four categories of plant samples were collected:

- Healthy flowering plants (HF)
- Healthy vegetative plants (H1)
- Phyllody-infected plants (I1)
- Little leaf-infected plants (LL)

Plants were identified based on visible symptoms, and samples were collected during the flowering stage. Leaves and floral tissues were immediately frozen in liquid nitrogen and stored at −80°C.

Fig-1 *Sesamum indicum* plant and seeds

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2.3-Phytoplasma Detection and Confirmation

- DNA was extracted using CTAB method
- Nested PCR performed using universal phytoplasma primers (P1/P7 followed by R16F2n/R16R2)
- Amplified products (~1.2 kb) were visualized on agarose gel
- Sequencing confirmed similarity with 16SrI-B phytoplasma group

2.4-DNA Isolation and Quality Assessment

- Genomic DNA extracted from all samples
- Quality assessed using spectrophotometry (A260/A280 ratio ~1.8)
- Integrity confirmed by agarose gel electrophoresis

2.5-Whole Genome Bisulfite Sequencing (WGBS)

- DNA treated with sodium bisulfite
- Library preparation using Illumina-compatible kits
- Sequencing performed on high-throughput platform
- ~150 million reads generated per sample

2.6-Bioinformatics Analysis

- Reads mapped to *Sesamum indicum* reference genome
- Methylation levels calculated for CG, CHG, CHH contexts
- Differentially methylated cytosines (DMCs) identified using statistical thresholds
- Annotation performed using gene databases

2.7-Validation of Methylation

- McrBC digestion followed by qPCR (qAMP method)
- Selected genes validated for methylation changes

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3-Result and Discussion:

Table - 1 Global DNA Methylation Patterns

The analysis revealed that overall methylation levels ranged between 40–46% across all samples. However, infected plants showed distinct methylation alterations.

Sample Type	Total Methylation (%)	Interpretation
HF	45.2	High methylation stability
H1	44.8	Baseline control
I1	41.3	Reduced methylation
LL	42.5	Moderate reduction

Although global methylation levels appear similar, subtle reductions in infected plants indicate epigenetic reprogramming. The decrease is more pronounced in specific sequence contexts.

Table – 2 Context-Specific Methylation Changes

Context	HF (%)	I1 (%)	Observation
CG	70–80	Slightly reduced	Stable
CHG	40–50	Slight increase	Moderate shift
CHH	20–30	Strong reduction	Major change

CHH methylation is primarily regulated by RNA-directed DNA methylation (RdDM) pathway. Its reduction suggests disruption of gene silencing mechanisms, leading to activation of genes responsible for abnormal development.

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Table – 3 Distribution of DMCs Across Genome

Region	% of DMCs	Biological Significance
Intergenic	50–60	Regulatory elements
Promoter	20–25	Gene expression control
Exon	10–15	Coding region
Intron	5–10	Minor role

Higher DMC density in intergenic and promoter regions indicates that regulatory sequences are more affected than coding regions, influencing transcriptional regulation.

Table – 4 Hypomethylation vs Hypermethylation

Type	Percentage	Role
Hypomethylated	~79%	Gene activation
Hypermethylated	~21%	Gene suppression

Dominant hypomethylation suggests activation of vegetative pathways and suppression of floral identity, leading to phyllody.

Table – 5 Functional Analysis of DMGs

Gene	Function	Methylation Change	Implication
STOREKEEPER	DNA binding	Hypo	Growth regulation
PP2-B15	Phloem protein	Hypo	Pathogen interaction
OMT	Metabolism	Hyper	Stress response
STP7	Transport protein	Hyper	Development

Genes involved in development, transport, and defense are epigenetically regulated, contributing to disease symptoms.

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The present study demonstrates that phytoplasma infection induces extensive epigenetic reprogramming in *Sesamum indicum*, leading to retrograde metamorphosis. The most significant observation is the widespread hypomethylation, particularly in the CHH context, which is closely associated with gene activation.

DNA methylation is a key regulator of transcriptional activity. In plants, CHH methylation is maintained by the RdDM pathway, which is highly sensitive to stress conditions. The observed reduction in CHH methylation suggests that phytoplasma infection disrupts this pathway, leading to derepression of genes involved in vegetative development.

One of the hallmark features of phyllody is the conversion of floral organs into leaf-like structures. This transformation is regulated by MADS-box genes, which define floral organ identity. Hypomethylation in promoter regions of these genes may lead to their misexpression, contributing to floral reversion.

Additionally, phytoplasma effector proteins such as SAP54 are known to target and degrade floral transcription factors. The combined effect of effector-mediated protein degradation and epigenetic deregulation amplifies developmental abnormalities.

The predominance of DMCs in intergenic regions suggests involvement of transposable elements and regulatory DNA sequences. Hypomethylation of these regions may activate transposons, further destabilizing genome structure and gene expression.

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Furthermore, genes involved in phloem transport (e.g., PP2-B15) showed hypomethylation, indicating enhanced susceptibility to pathogen colonization. Conversely, hypermethylation of metabolic genes may suppress defense responses, facilitating disease progression.

The results also highlight the dynamic nature of epigenetic regulation in plant-pathogen interactions. Unlike genetic mutations, epigenetic changes are reversible, suggesting potential for developing epigenetic-based disease management strategies.

Overall, this study provides strong evidence that DNA methylation acts as a molecular switch controlling developmental fate in phytoplasma-infected sesame plants.

Conclusion

This study provides comprehensive insights into the epigenetic mechanisms underlying phytoplasma-induced retrograde metamorphosis in *Sesamum indicum*. Comparative methylome analysis revealed that:

- Phyllody is associated with global hypomethylation, especially in CHH context
- Differential methylation primarily affects developmental and defense genes
- Intergenic regions serve as hotspots for epigenetic variation
- DNA methylation plays a critical role in regulating gene expression during infection

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Overall, DNA methylation emerges as a key regulatory layer in phytoplasma pathogenesis and symptom expression. These findings open new avenues for developing epigenetic-based strategies for disease resistance in sesame.

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