An analogous study of cytidine methylation during leaf rust pathogenesis in *Triticum aestivum* L.: A peek into the epigenetics of host-pathogen interaction.

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INTRODUCTION

- *Puccinia triticina* is a biotrophic fungi that causes leaf rust disease in *Triticum aestivum* L. (bread wheat).
- It has been concluded that DNA methylation has an active involvement in gene expression (Saripalli et al., 2019).
- It has also been inferred that DNA methylation occurs in a tissue or organ-specific manner in wheat (Ndiave et al., 2020 and can inactivate the expression of genes (gene expression regulation).

AIM

- Understanding the cross-talk between the gene pool of wheat and cytidine methylation during leaf rust pathogenesis.
- Building conceptual knowledge and combating the virulence of the pathogen.
- Understanding the importance of epigenetics during plant-pathogen interaction.

METHODOLOGY

Wheat leaf sample collection

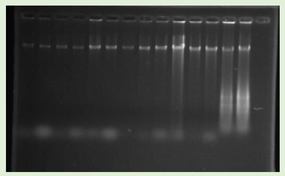
NILs of wheat variety HD2329 and HD2329+*Lr24* grown at National Phytotron Facility, IARI, New Delhi

Infection induced with urediniospores of *Puccinia triticina* , pathotype 77-5

Infected leaf samples collected at different time points i.e.,0hpi, 24hpi, 48hpi, 72hpi, 120hpi, and 168hpi

DNA isolation and acid hydrolysis

DNA Bands

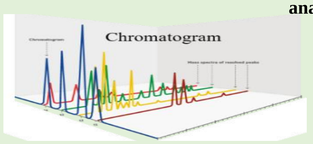


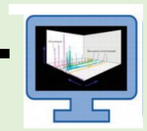
After Hydrolysis

HPLC analysis for detection of dC and 5mdC

Sample

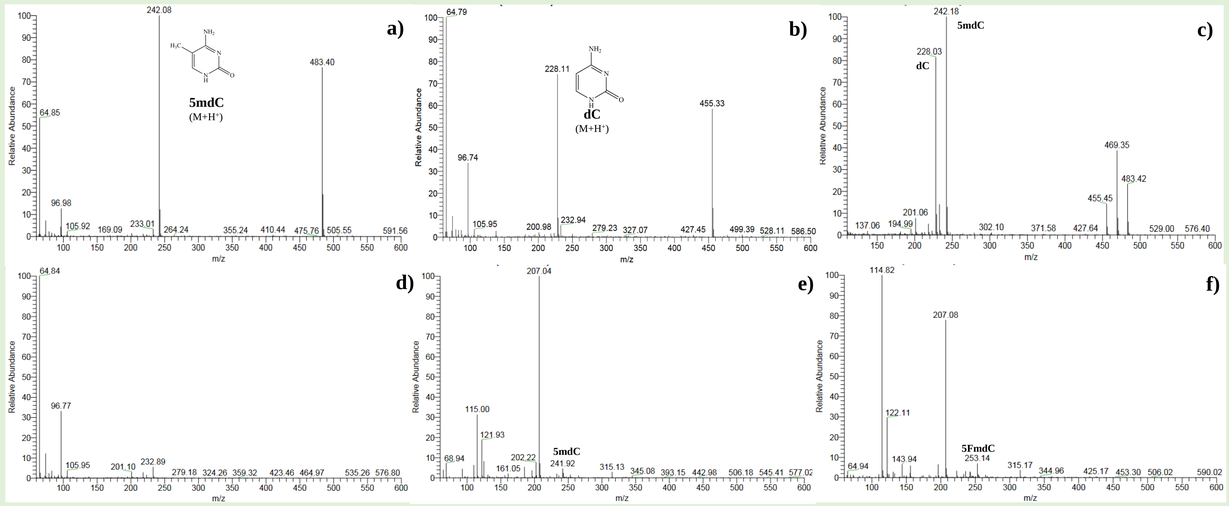
UHPLC system (Ultimate 3000, USA) coupled with a LTQ-XL ion trap mass analyzer





Flowchart of protocol followed for detection of genomic DNA methylation level of the wheat leaf samples through UHPLC technique (Li et al., 2016).

RESULTS



LC-MS chromatographs of standard solutions a) 5'- methyl -2'- Deoxycytidine (5mdC), b) 2'- Deoxycytidine and c) the standard solution mix of 5mdC and dC and samples d) mock, e) the susceptible wheat variety at 48hpi where the product ions of dC after fragmentation can be seen with m/z 207 and 114 along with 5mdC and f) resistant wheat variety at 48hpi showing the fragmentation product of dC along with the probable intermediate product of demethylation, 5- Formyl-methyl-deoxycytidine.

Processing of obtained data and the fragmentation patterns for the samples of all time points of infection in susceptible and resistant wheat varieties for the quantification of genomic DNA methylation is under process.

CONCLUSION AND FUTURE SCOPE

- The complete study would help in the analysis of the collision-induced dissociation (CID) of dC and the intermediate products of methylation/demethylation.
- It would help in enlightening us with the potential role of this epigenetic modification during biotic stresses and might serve as a boon for inducing methylation in wheat varieties against rust diseases to increase its annual yield.
- The stability of methylation during and after stress-induced environmental conditions can also be studied thoroughly validating its stability through generations.

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