Knowledge Consortium of Gujarat
Department of Higher Education - Government of Gujarat
Journal of Science - ISSN : 2320-0006

Continuous Issue - 15 | March – May 2018

RdDM mediated epigenetics and its biological significance in plants

Introduction:

DNA methylation refers to the addition of a methyl group to the cytosine bases of DNA. In both prokaryotes and eukaryotes, DNA methylation occurs in the contexts of CG, CHG, and CHH *(H = A, C, or T). Methylation mostly occurs in centromeric and pericentomeric regions as well as other repetitive DNA sequences. DNA methylation in plant is governed by different factors viz., aging, nutrient intake, genetic, metal exposure and environmental pollution.

RNA-Directed DNA methylation:

RNA-mediated Transcription Gene Silencing in plants is mainly governed by RNA- directed DNA methylation. This term was coined in 1994 by Sanger and his colleagues. RNA-directed DNA methylation is a nuclear process in which siRNAs mediate the methylation at cytosine residues of DNA sequence and thus inhibit the expression of coding DNA.

Molecular Mechanism of RdDM:

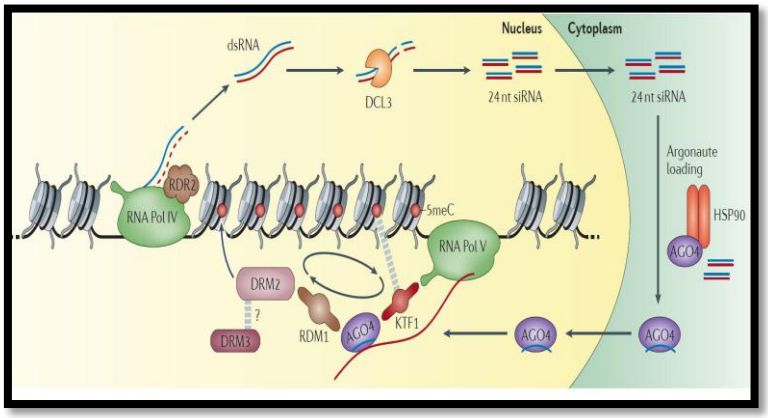

Fig. 1: RdDM pathway in plants

RNA-dependent DNA methylation is epigenetic modification of siRNA. For the action of RdDM, active transcription site is a prime requirement. It makes promoter regions inaccessible for the transcription initiation complex. Modifications in methylation patterns introduced by RdDM are inheritable and it passed on to the next generation.

Biological significance of RNA-directed DNA methylation in plants:

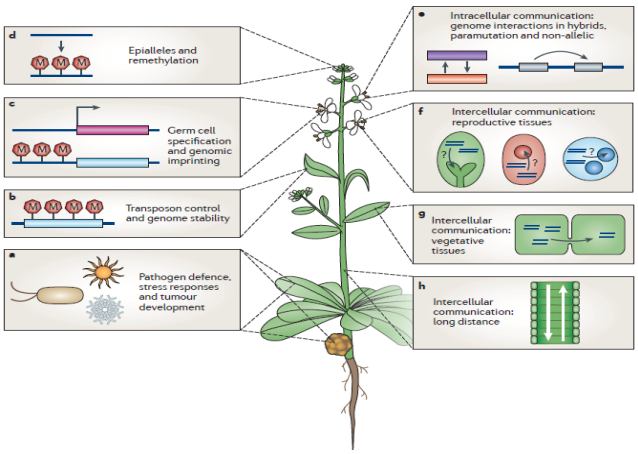

Fig. 2: Biological processes involving RdDM components.

RdDM is important for paramutation, genetic imprinting, regulation of genes and in different plant developmental processes as well as environmental stress responses. The RdDM pathway has been implicated in transposition silencing, defense against pathogen, inter and intercellular communication.(Matzke et al.,2014)

Molecular Techniques for detection of RdDM:

There are different bisulfite based and DNA digestion based methods for the molecular detection of of RdDM. It includes Bisulfite Sequencing, MassARRAY, Pyrosequencing, Real-time MSP MethyLight, Methylation specific PCR, combined bisulphate restriction analysis and southern blot hybridization. Most widely utilizes technique is bisulphate sequencing. In this technique DNA is denatured to single strand and treated with sodium bisulphate. Sodium bisulphate convert the non-methylated cytokines into uracils leaving 5' methylcytosine untouched in order to calculate methylation percentage. Converted DNA is then amplified with PCR and cloned with adaptors followed by sequencing.

Case Studies:

Ding et al. (2012) studied RNA-directed DNA methylation involved in regulation of photoperiod-sensitive male sterility (PSMS) in rice. PSMS is a precious germplasm for hybrid rice breeding. Mutant Nongken 58S variety encodes a long non-coding RNA termed as Psi–LDMAR which is essential for normal male sterility of the long day rice plants. In Nongken 58S, increased methylation in the promoter of LDMAR reduced the expression of LDMAR leading to male sterile conditions under long-day plants. Over expression of AK111270 in mutant variety, greatly enriched Psi–LDMAR showed pollen and spikelet sterility under short-day conditions.

Jochen et al. (2013) studied DNA methylation mediated controlled gene expression in Arabidopsis thaliana for critical development of crown gall tumors. A crown gall tumor formation is due to expression of the oncogenes such as IaaH, IaaM and Ipt, which are encoded by Agrobacteriam T-DNA that is integrated into the plant genomic DNA. Promoter methylation at IGR1 and IGR2 (confirmed by bisulfite sequencing) leads to transcriptional gene silencing and ultimately reduced tumor weight. The reduced tumor size is directly co related with reduced relative transcript number of IaaH, IaaM and Ipt and altered methylation pattern of coding genes. This experiment studies provide evidence that RNA directed DNA methylation regulates the development of crown gall tumors.

Poggin et al. (2003) checked RNAi targeting of DNA virus in plants. dsRNA construct targeted to geminivirus promoter to cure Vigna mungo plants from the viral disease.PCR with viral DNA specific primers detected much less viral DNA in the leaves bombarded with transgene construct than in those bombarded with blank control. Transcriptome analysis of control and treated plant showed down regulation of viral genes. Methylation pattern studies of the promoter region of control and treated plant revealed hypermethylation in the treated plants.

Kawakatsu et al. (2012) studied RNA silencing induced by an artificial sequence in rice. mGLP-1 (Glucagon like peptide-1) sequence act as an RSIS in rice. The transcripts containing RSIS become templates for ds RNA synthesis in nucleus. This is followed by siRNA production and targeted methylation of seed storage proteins. The non functioning of seed storage proteins is coupled with methylation at the promoter site of seed storage proteins. Silencing efficiency is dependent on construct sequence and size.

Liu et al. (2015) studied DNA demethylase governs fruit ripening in tomato. Transgenic plants with delayed ripening showed reduced fruit ripening gene expression. Bisulfite sequencing and heat map presentation showed altered methylation pattern at the promoter site of fruit ripening genes. During the ripening stage higher demethylase (DML-2) activity was found. Demethylation at the promoter site of ripening genes leads to active transcription and translation of ripening genes that ultimately leads to ripeness in tomato.

Groszmann et al. (2011) studied differential siRNA levels in Arabidopsis hybrids leads to an epigenetic contribution to hybrid vigor. Hybrids between the Arabidopsis thaliana accessions C24 and Landsberg erecta showed strong heterosis in few vegetative traits such as rosette diameter, leaf number and biomass. The reciprocal hybrids show a decreased level of 24-nt small RNA (sRNA) relative to both the parents.

Conclusion:

- From ongoing discussion it can be concluded that RNA-Directed DNA methylation is a complex epigenetic pathway requiring chromatin remodelling proteins, different transcription factors and RNA binding proteins.
- This process also regulate physiological process and gene expression in development of crown gall tumors, epigenetic contribution to hybrid vigor, photo period sensitive male sterility, targeting of DNA viruses in plant, fruit ripening and transcription termination.

Future thrusts:

- Development of cereal species which are sprouting tolerant.
- Epigenetic variation in plant hybrid and their role in heterosis.
- Identifying to consensus promoter motifs for Pol IV and Pol V.

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