

INVESTIGATING THE CONTRIBUTION OF RMR6/POL IV AND HDA108 TO THE TRANSCRIPTIONAL REGULATION IN MAIZE LEAVES

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DNA cytosine methylation and histone post-translational modifications are conserved epigenetic mechanisms important for regulation of gene expression, developmental processes and genome stability. DNA cytosine methylation occurs at CG, CHG and CHH sites and is often associated with transcriptional gene silencing, while the functional consequences of histone modifications (acetylation, methylation, phosphorylation as well as ubiquitination) can be direct, causing structural changes to chromatin, or indirect, acting through the recruitment of effector proteins. A tight interplay between histone deacetylation and DNA methylation has been documented in both plants and mammals, suggesting that histone deacetylation and DNA methylation may act coordinately in gene silencing.

In Arabidopsis, multisubunit RNA polymerases IV and V (PolIV and PolV) orchestrate RNA-directed DNA methylation (RdDM) and transcriptional silencing through siRNA biogenesis, but the specification of the loci to be silenced requires HISTONE DEACETYLASE 6 (HDA6) activity upstream of PolIV recruitment and siRNA biogenesis. Despite the role of HDA6, PolIV and PolV on gene, transposon and transgene silencing, gross morphological defects are not apparent in single *hda6*, *polIV*, or *polV* null mutants, while *hda6/polIV* double mutants are dwarfed and have curled leaves. On the contrary, we showed that maize mutants for the PolIV and HDA6 orthologs (*rmr6* and *hda108*, respectively) present several defects during both vegetative and reproductive development, together with a progressive transgenerational degradation in plant quality. In addition, our efforts to recover viable *hda108/rmr6* double mutants were unsuccessful, indicating that mutations in these two epiregulators have a stronger phenotypic effect in maize than in Arabidopsis.

To investigate the contribution of both epiregulators on maize gene and transposon expression, we conducted total RNA-Seq and sRNA-Seq analyses on *hda108* and *rmr6* loss of function mutants and their respective wild-type siblings. In both mutants an increase in the portion of genome actually transcribed was highlighted. This transcriptional increase is associated to the derepression of intergenic, transposon-associated, regions and coding genes as well. Loci that are similarly derepressed in both *hda108* and *rmr6* single mutants will be presented and characterized. Results will be compared to those obtained in Arabidopsis, in the light of the different developmental phenotypes observed in the two plant species.