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EXPLORING SMALL INTERFERING RNAS EFFETCS ON GENE EXPRESSION REGULATION IN MAIZE LEAVES

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Small interfering RNAs (siRNAs) form the most abundant class of sRNAs in maize. They derive from the processing of double-stranded RNA precursors by Dicer-like nucleases activity and are recruited in DNA silencing processes. 21 and 22-nt siRNAs function to establish the silencing of newly activated transposable elements (TEs). TEs reactivation happen in endosperm and vegetative nucleus of pollen and has been observed in somatic cells during environmental stresses and in mutants of the Pol IV-RNA-directed DNA methylation (RdDM) pathway. 24-nt siRNAs are involved in the stabilization and maintenance of TEs silencing, through RdDM. During this process Pol IV transcribes the newly silenced methylated DNA into precursors of siRNAs, which drive the deposition of repressive chromatin marks to the target TEs. It has been proposed that deeply silenced TEs are not transcribed at all and the C methylation at these loci is maintained entirely by the DNA methyltransferares.

In the maize genome TEs are enriched in the chromosomes arms, particularly in genes flanking regions, where they tend to engage RdDM. When RdDM is impaired the release of the epigenetic silencing of TEs can occur. A newly activated TE can function as a *cis*-regulatory element, altering the expression regulation of proximal or distant genes, or as a *trans*-regulatory element, misregulating the expression of genes through the action of TE-derived siRNAs.

In our work we employ the Pol IV mutant, *rmr6,* to investigate the effects of siRNAs lack on gene expression. Expression profiles of siRNAs and genes in wild type and *rmr6* plants (B73 line) are obtained through Illumina sequencing of juvenile maize leaves samples. Mutants show a significant decrease in siRNAs production from >75400 loci, mainly 23-24-nt sRNAs, and a significant increase from >350 loci, predominantly 21-22-nt sRNAs. Despite the global loss of 23-24-nt siRNAs we do not observe big changes in genome transcription levels in mutants: 777 loci are upregulated and 234 are downregulated. As it has been described in others RdDM mutants, the loss of siRNA production does not alter the general genome homeostasis, so at the vast majority of loci subjected to RdDM, siRNAs alone are not sufficient to maintain DNA repression. Upregulated siRNA loci are not associated with differentially expressed (DE) genes and TEs, so we are now investigating their possible effects in *trans* on RNA expression levels. Downregulated siRNA loci are found in proximity or inside both upregulated and downregulated genes and TEs, so we are now investigating the biological significance of this association, with the aim to identify the direct targets of RdDM where siRNAs act in *cis*. At these loci it will be useful to study other RdDM features, as DNA methylation and histones repressive marks, to fully understand how RdDM process is altered when siRNAs are missing.