***Genome wide methylome and CRISPR/dCas9-based*** ***gene activation in Medicago truncatula***

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Tobacco retrotransposon, *Tnt1*, has been used to mutagenize and tag the whole genome of *Medicago truncatula*. We studied the somatic embryogenesis of *M. truncatula* R108 using leaf explants and explored the dynamic shifts in the methylation landscape from leaf explants to callus formation and finally embryogenesis. Robust cytosine methylation in all three contexts of CG, CHG and CHH patterns was observed during somatic embryogenesis compared to the controls. Differentially methylated promoter region analysis showed a stronger CHH methylation in embryogenesis samples when compared to CG and CHG methylation. Strong correlation (89.71 %) was identified between the differentially methylated regions and the site of *Tnt1* insertions in *M. truncatula* R108 and stronger hypermethylation of genes correlated with higher number of *Tnt1* insertions in all contexts of CG, CHG and CHH methylation.

We generated stable transgenic *M. truncatula* lines expressing the chimeric fusions of CRISPR/dCas9 with a transcriptional activator and single or multi-guide RNAs targeting the long-terminal repeats of *Tnt1* to activate the gene expression in the *M. truncatula Tnt1* mutants. These transgenic lines were crossed with *Tnt1* insertion lines wherein the insertions are in gene promoter regions. A significant upregulation of six-fold to 15-fold was observed in genes located at modular positions of ~500 bp upstream of the transcriptional start sites in the *Tnt1* insertion lines NF21042, NF11121 and NF8355. Overall, this novel approach could be utilized to overcome the necessity to overexpress individual genes that may result in misexpression of genes in undesired tissues when constitutive promoters are used.