EPIGENETIC INSIGHTS INTO VIROID PATHOGENESIS FROM GENOME-WIDE DNA METHYLATION ANALYSIS OF CBCVd-INFECTED HOP PLANTS (*HUMULUS LUPULUS* VAR. 'CELEIA')

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Viroids are small, single-stranded RNA molecules, that exploit host factors for propagation, causing severe diseases in agricultural crops. Notably, recent outbreaks such as the Citrus bark cracking viroid (CBCVd) in hop plants in Slovenia, Germany, and Brazil, emphasize the urgency to study the underlying mechanisms underscoring viroid pathogenesis and host response to infection.

Prevailing research delineates the intricate interplay between viroid RNA and host RNAi factors, orchestrating nuanced changes in gene expression, metabolic pathways, and the observable phenotype. Further investigation has discerned viroid-induced alterations in DNA methylation patterns via the RNA-directed DNA methylation pathway. Previous study has indicated consistent whole-genome DNA methylation levels between CBCVd-infected and healthy hop plants. Our current work, however, employs a comprehensive bisulfite sequencing approach, illuminating subtle yet significant shifts in specific parts of the DNA methylation landscape of the hop genome consequent to CBCVd infection. In addition, our study analysed important epigenetic marks of CBCVd-infected hop plants, providing novel evidence for host DNA methylation's role in defence against viroids. We found that genes linked to pathogen interaction pathways, such as MAPK signalling and LRR, exhibited hypomethylation, potentially enhancing host defence. Conversely, key RNA transcription genes like POL II, POL IV, and POL V displayed hypermethylation, highlighting DNA methylation's defensive significance. Additionally, our efforts were centred on establishing a vital connection between DNA methylation and pre-existing transcriptomic data, allowing us to gain a more comprehensive insight into plants' defence against viroids. Interestingly, the mediator subunit MED7a was upregulated and hypomethylated, suggesting impaired RNA transcription and emphasizing mediator complexes' role in viroid-infected plants.