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File: ■ Hops (*Humulus lupulus*, Cannabaceae)
■ MicroRNAs
■ *Citrus Bark Cracking Viroid*

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RE: MicroRNA Expression in Hops Is Altered by Pathogenic Viroid Infection

Mishra AK, Duraisamy GS, Matoušek J, Radisek S, Javornik B, Jakse J. Identification and characterization of microRNAs in *Humulus lupulus* using high-throughput sequencing and their response to *Citrus bark cracking viroid* (CBCVd) infection. *BMC Genomics*. November 15, 2016;17:919. doi: 10.1186/s12864-016-3271-4.

Small ribonucleic acids (sRNAs) modulate transcription and post-transcription processes, thereby regulating gene expression. MicroRNAs (miRNAs) are non-coding sRNAs that modulate myriad aspects of plant development, such as responses to stress and micronutrient deficits. Hops (*Humulus lupulus*, Cannabaceae) is an economically important plant used in beer production, with several previously reported bioactive properties. Hops and other plants are susceptible to viral infections, including *Citrus bark cracking viroid* (CBCVd), which has recently been discovered to cause severe illness in hops. Viral RNA can imitate the actions of miRNA during infection, disrupting normal plant physiology and leading to yellowing, premature flowering, decreased cone size, and other abnormalities. This basic research study compared sRNAs from infected and control hops to identify differences in miRNA profiles.

Samples of the hops cultivar "Celeia" were procured from the Slovenian Institute of Hop Research and Brewing (Žalec, Slovenia) and from local farms overseen by the institute. CBCVd infection was identified by real-time polymerase chain reaction (RT-PCR) analysis. Previously generated sRNA sequence data were processed using the FASTX toolkit or the CLC Genomics Server, and sequences of types of RNA other than miRNAs were identified using databases (including GenBank) and excluded. A hops transcriptome database available through the National Center for Biotechnology Information (NCBI) was used to predict candidate miRNAs. To find potential target genes for miRNAs, 2 µg of RNA were extracted from hops leaves; PCR was used to amplify targets for further analysis. The read count frequency of individual miRNA transcripts in hops tissues was normalized as transcripts per million using a specified formula. Quantitative RT-PCR was used to measure the expression levels of selected target genes.

Resultant sRNA libraries from the leaves, roots, and cones of control and infected hops were sequenced. The size of most sRNAs was 20-24 nucleotides (nt); 21 and 24 nt were the most common lengths. Matched miRNAs were 28.07% of the sequence reads of the control hops library and 17.88% of reads in the infected hops library. The unique miRNAs comprised 0.78% of the control hops library and 0.39% of the infected hops library. Database comparison revealed 67 conserved miRNAs (found both in hops and in other previously studied plants) in the control and infected hops libraries. The conserved miRNAs belonged to 40 miRNA "families"; it was observed that different families had varying expression levels. Many of these conserved miRNAs were significantly different between the libraries of control and infected hops ($P<0.05$). Also, 36 conserved miRNAs were "CBCVd-responsive" in the infected hops library and assumed to be involved in plant defense processes. In further analysis, the miRNA MIR167 family showed a large fold change in infected hops as compared to control, indicating a potential prominent role in response to infection.

Nine novel miRNAs were found to be more highly expressed in leaves than roots and cones, and two were expressed to a higher degree in cones as compared with leaves and roots. It was also found that 93.89% of miRNA targets were thought to be modulated by cleavage and 6.10% by repression of translation. This study also identified highly conserved miRNAs; included were those involved with plant growth, flowering, and possibly stress adaptation. For ten miRNAs identified as CBCVd-responsive, quantitative RT-PCR showed that the target gene expression was negatively correlated with miRNA expression. The authors suggest this observation as evidence of molecular response to CBCVd infection.

In conclusion, there were notable differences in the libraries of control and infected hops. The 21-nt size of sRNAs was more common in the infected hops library; it is mentioned that this might suggest infection-induced expression of these sRNAs or repression of those at 24 nt. This observation agrees with other literature and is interpreted as evidence of potential silencing or crosstalk pathways. This also may indicate differences in DNA methylation. Further work will ideally investigate downstream implications of observed differential expression of miRNAs between control and infected hops. One of the authors (S. Radisek) is employed by the Slovenian Institute of Hop Research and Brewing.

—Amy C. Keller, PhD

Referenced article can be accessed at https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-016-3271-4.