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Profiling of small RNAs derived from cucumber mosaic virus in infected *Nicotiana benthamiana* plants by deep sequencing

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Highlights

- The profile of vsRNAs was characterized in CMV-m2b infected *Nicotiana benthamiana*.
- The NbRDR6 was confirmed to enhance the antiviral defense by amplifying vsRNAs.
- The NbAGO proteins bind different vsRNAs to promote the antiviral silencing.

ABSTRACT

In plants, RNA silencing is a conserved mechanism underlying antiviral immunity. To investigate antiviral responses in *Nicotiana benthamiana*, we analyzed the profiles of the virus-derived small RNAs (vsRNAs) in wild-type *N. benthamiana* and *NbRDR6* mutant plants infected with the cucumber mosaic virus (CMV) 2b-deficient mutant. We observed that NbRDR6 regulates RNA silencing by producing vsRNAs that trigger an effective antiviral response, while NbRDR1 may nonredundantly and synergistically function with NbRDR6 to mediate immune responses. The vsRNAs in *N. benthamiana* and *NbRDR6* mutant plants mainly comprised 21 or 22 nucleotides, and mostly consisted of a 5′-terminal adenine. Additionally, *NbAGO2* expression was significantly up-regulated in *N. benthamiana* and *NbRDR6* mutant plants, suggesting that NbAGO2 is closely associated with the antiviral activities of vsRNAs. The distribution of vsRNAs in the CMV genome was biased toward RNA sense strands in both *N. benthamiana* and *NbRDR6* mutant plants. These findings indicate the specific and conserved antiviral immunity in *Nicotiana benthamiana*.