

MicroRNAs (miRNAs) regulate target gene expression by mediating target gene cleavage or inhibition of translation at transcriptional and post-transcriptional levels in higher plants. Until now, many grapevine microRNAs (Vv-miRNAs) have been identified and quite a number of miRNA target genes were also verified by various analysis. However, global interaction of miRNAs with their target genes still remained to perform more research. We reported experimental validation of a number of miRNA target genes in table grapevine that had been previously identified by bioinformatics in our earlier studies. To verify more predicted target genes of Vv-miRNAs and elucidate the modes by which these Vv-miRNAs work on their target genes, 31 unverified potential target genes for 18 Vv-miRNAs were experimentally verified by a new integrated strategy employing a modified 5'-RLM-RACE (RNA ligase-mediated 5' rapid amplification of cDNA ends), 3'-PPM-RACE (poly(A) polymerase-mediated 3' rapid amplification of cDNA ends) and qRT-PCRs of cleavage products. The results showed that these Vv-miRNAs negatively regulated expression of their target messenger RNAs (mRNAs) through guiding corresponding target mRNA cleavage, of which about 94.4% Vv-miRNAs cleaved their target mRNAs mainly at the tenth nucleotide of 5'-end of miRNAs. Expression levels of both miRNAs and their target mRNAs in eight tissues exhibited inverse relationships, and expressions both of cleaved targets and miRNAs indicated a cleavage mode of Vv-miRNAs on their target genes. Our results confirm the importance of Vv-miRNAs in grapevine growth and development, and suggest more study on Vv-miRNAs and targets can enrich the knowledge of miRNA mediated-regulation in grapevine.