

Alignment analysis of the Vv-miRNAs identified from various grapevine cultivars indicates that over 30% orthologous Vv-miRNAs exhibit a 1-3 nucleotide discrepancy only at their ends, suggesting that this sequence discrepancy is not a random event, but might mainly derive from divergence of cultivars. With advantages of miR-RACE technology in determining precise sequences of potential miRNAs from bioinformatics prediction, the precise sequences of Vv-miRNAs predicted computationally can be verified with miR-RACE in a different grapevine cultivar. This presents itself as a new approach for large scale discovery of precise miRNAs in different grapevine varieties.

Among 88 unique sequences of Vv-miRNAs from bioinformatics prediction, 83 (96.3%) were successfully validated with MiR-RACE in grapevine cv. 'Summer Black'. All the validated sequences were identical to their corresponding ones obtained from deep sequencing of the small RNA library of 'Summer Black'. Quantitative RT-PCR analysis of the expressions levels of 10 Vv-miRNA/target gene pairs in grapevine tissues showed some negative correlation trends. Finally, comparison of Vv-miRNA sequences with their orthologs in Arabidopsis and study on the influence of divergent bases of the orthologous miRNAs on their targeting patterns in grapevine were also done.

The validation of precise sequences of potential Vv-miRNAs from computational prediction in a different grapevine cultivar can be a new way to identify the orthologous Vv-miRNAs. Nucleotide discrepancy of orthologous Vv-miRNAs from different grapevine cultivars normally does not change their target genes. However, sequence variations of some orthologous miRNAs in grapevine and Arabidopsis can change their targeting patterns. These precise Vv-miRNAs sequences validated in our study could benefit some further study on grapevine functional genomics.