

MicroRNA (miRNA) is a class of functional non-coding small RNA with 19-25 nucleotides in length while Amur grape (*Vitis amurensis* Rupr.) is an important wild fruit crop with the strongest cold resistance among the *Vitis* species, is used as an excellent breeding parent for grapevine, and has elicited growing interest in wine production. To date, there is a relatively large number of grapevine miRNAs (vv-miRNAs) from cultivated grapevine varieties such as *Vitis vinifera* L. and hybrids of *V. vinifera* and *V. labrusca*, but there is no report on miRNAs from *Vitis amurensis* Rupr, a wild grapevine species.

A small RNA library from Amur grape was constructed and Solexa technology used to perform deep sequencing of the library followed by subsequent bioinformatics analysis to identify new miRNAs. In total, 126 conserved miRNAs belonging to 27 miRNA families were identified, and 34 known but non-conserved miRNAs were also found. Significantly, 72 new potential Amur grape-specific miRNAs were discovered. The sequences of these new potential va-miRNAs were further validated through miR-RACE, and accumulation of 18 new va-miRNAs in seven tissues of grapevines confirmed by real time RT-PCR (qRT-PCR) analysis. The expression levels of va-miRNAs in flowers and berries were found to be basically consistent in identity to those from deep sequenced sRNAs libraries of combined corresponding tissues. We also describe the conservation and variation of va-miRNAs using miR-SNPs and miR-LDs during plant evolution based on comparison of orthologous sequences, and further reveal that the number and sites of miR-SNP in diverse miRNA families exhibit distinct divergence. Finally, 346 target genes for the new miRNAs were predicted and they include a number of Amur grape stress tolerance genes and many genes regulating anthocyanin synthesis and sugar metabolism.

Deep sequencing of short RNAs from Amur grape flowers and berries identified 72 new potential miRNAs and 34 known but non-conserved miRNAs, indicating that specific miRNAs exist in Amur grape. These results show that a number of regulatory miRNAs exist in Amur grape and play an important role in Amur grape growth, development, and response to abiotic or biotic stress.