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GENOMIC ANALYSIS OF CONSERVED MICRORNAS GENES IN GRAPEVINE

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miRNAs are small non coding RNA molecules, whose mature form is 20-21 nt long and is involved in post transcriptional gene regulation events. The primary transcript (pri-miRNA) is recognized by RNase III enzymes (Dicer like) and yields the typical stem-loop secondary structures of the precursor miRNA. The precursor (pre-miRNA) is in turn processed and gives rise to the mature miRNA product that, once exported in the cell cytoplasm, becomes active and can be loaded in the RNA Induced Silencing Complex (RISC) that mediates the silencing of target genes.

Because of their roles in many crucial biological processes, such as plant development and morphogenensis, defense against biotic and abiotic stress and the regulation of metabolism, structural and functional characterization of any genome must include studies of miRNA gene families. In this work we present an integrated approach towards the structural characterization of miRNA genes in the genome of the grapevine (*Vitis vinifera* L.). Since miRNAs are often conserved across species at both the sequence and functional levels, we used bioinformatic tools to identify putative conserved miRNA genes and their putative targets. Using comparative approaches, we identified 140 putative pre-miRNAs organized in 31 gene families. Two thirds of these families are present in arabidopsis, poplar and rice, whereas others that were previously restricted to arabidopsis have now been shown to be conserved in grapevine. Evolutionary analyses provide detailed hypotheses as to the mechanisms of expansion of gene families that have undergone an expansion in grapevine. Oligo arrays and cDNA deep sequencing analyses were performed to validate the predicted miRNAs. In particular CombiMatrix platform was used to produce a 12K microarray comprising probes specific for 81 different mature miRNAs, which was hybridized with small RNA from six different grapevine tissues. Statistical analyses confirmed expression for most of the predicted genes showing various expression profiles. Deep sequencing of cDNA tags derived

from polyA$^+$ RNA of different tissues allow validation of pri-microRNA expression and tentative pri-microRNA mapping. Prediction and validation of non conserved miRNAs is still in progress and upcoming results will augment our understanding of miRNA gene structure and function in the grapevine genome.