EXPERIMENTAL VALIDATION OF COMPUTATIONAL PREDICTIONS OF GRAPEVINE (VITIS VINIFERA L.) microRNA

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MicroRNAs (miRNAs) are key post-transcriptional regulatory elements of eukaryotic gene expression. These RNA molecules are approximately 21 nucleotides (nt) long and are coded by MIR genes, transcribed by RNA polymerase II.

Biogenesis of miRNAs starts with the synthesis of a large primary transcript (pri-miRNA) which is recognized by RNase III enzymes Dicer-like1 that mediates the specific excision of mature miRNAs from the pri-miRNA via the initial generation of imperfect hairpin precursor (pre-miRNA). Once exported to the cytoplasm, mature miRNA molecules become active and can be incorporated into the RNA Induced Silencing Complex (RISC). miRNAs recognize complementary sequences in target mRNAs and guide them to endonucleolytic cleavage or transcriptional arrest.

It has become clear that miRNAs play a crucial role in a wide range of developmental and physiological pathways. Hence, the identification and characterization of their target sequences is essential to understanding the functions of miRNAs.

This work is part of a wider project on grapevine smallRNAs, deriving from the VIGNA (Vitis Genome Analysis) project that annotated 140 conserved MIR genes and described their genomic structure, their transcriptional landscape and putative targets. Here we present the validation of predicted miRNA targets and of pri-miRNA alternative splicing events.

The approach we used to validate candidate target genes was the modified 5’Rapid Amplification of cDNA Ends (RACE) which is to-date the most widely used method to detect in vivo RNA cleavage products. In particular we have focused on target genes that seem to be up- or down-regulated by different MIR genes family such as miR160, miR397, miR398 and miR408 in berry during light and heat stress treatments. Moreover, these MIR genes were highly expressed in root during standard conditions in microarray experiments. These target genes are involved in several physiological pathways such as auxin signal transduction and phenylpropanoid pathway.

Using next-generation transcriptome sequencing data it was possible to describe not only the genomic structure of MIR genes but also to map many constitutive and alternative splicing events. From those analyses it was hypothesized that the maturation of a number of miRNAs are regulated through splicing of their primary transcripts.

To validate the predicted splicing events, and to map transcription starting site and 3’ transcript end, we performed classic 5’ and 3’ RACE experiments on three different pri-miRNA: pri-miR162, pri-miR394b and pri-miR482. Cloning and sequencing of RACE products confirmed bioinformatic predictions, showing different splicing patterns for different pri-miRNAs.
Validation of miRNAs is still in progress and upcoming results will augment our understanding of miRNA gene structure and function in the grapevine genome.