MicroRNAs Behaved Differently in Drought-Tolerant and Sensitive Grape Genotypes Responsive to Drought Stress

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Background  Drought is a major environmental factor limiting grapevine production and quality. The roles of microRNAs of different grapetypes at post-transcriptional level upon drought stress were largely unknown. In this study, we investigated miRNAs in drought-sensitive grapevine (Cabernet Sauvignon, CS) and drought-tolerant grapevine (110R) in response to drought stress.

Methods  Total RNA was extracted from tissue culture seedlings of D 0, D 5 and D15 from CS and 110R after drought treatment, using TRIzol reagent (Invitrogen, United States). Concentration, RIN value and integrity of total RNA were measured Agilent Bioanalyzer 2100 System (Agilent Technologies, CA, USA). Total RNA samples were sent to Allwegene Technologies (Beijing, China) for the small RNA (sRNA) library construction using TruSeq Small RNA Sample Prep Kits (Illumina, San Diego, USA). A total of 18 libraries were constructed, including three replicates of three stages of CS and 110R samples. Illumina Hise-Q2000/2500 platform (Illumina, CA, USA) was used for single-end sequencing (1 × 50 bp).

Results  A total of 488 known miRNAs and 892 predicted novel miRNAs belonging to 48 miRNA families were identified from CS and 110R under drought conditions. The abundances of 11 and 21 conservative miRNAs and 22 and 48 novel miRNAs (MFEI > 1.6) in CS and 110R showed significantly differences in response to drought stress, respectively. Different accumulation trends of miRNAs upon drought observed in different genotype, were confirmed by RT-qPCR. Gene Ontology analysis demonstrated that predicted target genes of miRNAs were predominately associated with biological process, cellular component, and molecular function. Finally, the GUS reporter-aided analysis of the promoter activities revealed drought induced VvmiR169d and VvmiR398a down-regulation in grapevine. Further, the root length were also shorter and responded differently to drought stress in VvmiR156b transgenic lines.
Fig. 1 Expression levels of miRNAs of CS and 110R under drought.

Fig. 2 VmiRNA156b-overexpression lines in response to drought stress under 150 mM mannitol. (A and B) Root length of the WT and transgenic lines were measured after 9 d. (C and D) Hypocotyl length and fresh weight were measured after 9 d. All the dates with three technical repeats and error bars with mean values ± SD (n = 15). Letters ‘a’, ‘b’ and ‘c’ indicate significant differences (p < 0.05, Duncan’s Multiple Range Test).

Conclusion In summary, a total of 488 known miRNAs and 892 predicted novel miRNAs belonging to 48 miRNA families were identified from CS and 110R under
drought conditions. The abundances of 11 and 21 conservative miRNAs and 22 and 48 novel miRNAs (MFEI > 1.6) in CS and 110R showed significantly differences in response to drought stress, respectively. Gene Ontology analysis demonstrated that predicted target genes were predominately associated with biological process, cellular component, and molecular function. Our results reveal new perspectives in understanding miRNAs involved in the response of grapevines to drought stress, thus providing a foundation for further developing a comprehensive understanding of the molecular networks involving miRNAs and their targets in response to drought stress in grape.

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**References**