

Integrated approaches for the functional characterization of miRNAs in grapevine

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Abstract (250 words)

Micro(mi)RNAs are small non-coding RNAs that regulate several pathways and are widely recognised as key players in plant development, tissue differentiation, and many other important physiological processes, including plant adaptation to biotic and abiotic stresses. The release of plant genomes and the application of high throughput sequencing have considerably extended miRNA discovery across many species, including grapevine (*Vitis* spp.). Despite their relevance in plant development, functional studies in grapevine to clarify the function of miRNAs are not yet available. Through the grapevine genetic improvement platform IMPROVIT at CNR-IPSP (<http://www.ipsp.cnr.it/en/thematics/turin-headquarter-thematics/improvit/>), we developed integrated approaches to discover miRNA function in grapevine. Transgenic plants were generated by either overexpressing or silencing vvi-miR393, a miRNA conserved in different plant species, and vvi_miC137, a grapevine-specific miRNA whose function is unknown despite being transcriptionally regulated in response to biotic and abiotic stresses. A total of 212 transgenic grapevines from two *V. vinifera* cultivars (Chardonnay and Bragat rosa) and the 110R rootstock were characterized. Molecular analyses showed that overexpressing lines increased the expression of the selected miRNAs up to 10-fold, whereas silencing by short tandem target mimic (STTM) approach reduced the expression of mature miRNAs by 70%. Five independent transgenic lines for each genotype and construct were acclimatised in greenhouse. Additionally, vvi_miC137 lines were ecophysiologicaly characterized under well-watered and drought conditions. Preliminary results showed that vvi_miC137 influenced plant development and leaf gas exchanges, its partial silencing improved grapevine growth performance. This miRNA could represent a new potential target for genetic improvement by gene editing.

Keywords: miRNAs, genetic transformation, functional studies, grapevine development, water stress.