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REGULATORY NETWORK BEHIND THE BERRY RIPENING: THE ROLE OF *VITIS VINIFERA* NAC60 TRANSCRIPTION FACTOR

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Grapevine (Vitis vinifera) is one of the most important fruit crop in the world. As winemaking plays a central role in the economy of many developed countries, the regulation of the berry ripening has been studied intensely to underline physiological, biochemical and molecular characteristics that influence fruit and wine quality. Transcriptomic data obtained during berry development and integrated network analyses allowed to identify some members of the VvNAC gene family as candidate genes for the regulation of the onset of berry ripening. The NAC TFs family is functionally involved in a large variety of plant growth and development related programs. VvNAC60, which expression is very low in the vegetative/green tissues, significantly high in the mature/woody organs and shows a high negatively correlation with genes that are down regulated during ripening, was identified as "switch" gene and might represent a master regulator for the transition from vegetative-to-mature growth. With the aim to characterize the function of VvNAC60, the Chromatin Immunoprecipitation Sequencing (ChIP-Seq) approach was selected to identify putative targets of this transcription factor. A preliminary structure prediction of the VvNAC60, obtained using either comparative modeling and de novo structure prediction methods, allowed to design and produce polyclonal antibodies anti-NAC60. Moreover, an efficient protocol for nuclei isolation and chromatin shearing has been optimized and preliminary results are reported. At the same time, in order to set up an Electrophoresis Mobility Shift Assay (EMSA), that allows to detect and characterize protein-nucleic acid complexes, a tagged NAC60 recombinant protein will be expressed and purified. The EMSA technique could be used to check the goodness of hypothetical positive controls in the ChIP-Seq experiments and to further validate the obtained results. It is well known that NAC TFs undergo an intensive post-transcriptional regulation that includes microRNA-mediated cleavage. Vv-miR164 has already been validated as a development-stage specific miRNA regulating VvNAC33 expression, another "switch" gene. Based on this evidence, an artificial-miRNAs design of Vv-miR3626, the putative VvNAC60 regulator, is ongoing and further berry infiltration experiments, aimed at the study of VvNAC60 upstream regulation, will be performed.