

## RECONSTRUCTING THE VVINAC FACTORS INTRA FAMILY REGULATION NETWORK ORCHESTRATING THE GRAPEVINE BERRY RIPENING

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Grapevine is one of the most important fruit crops as it is widely cultivated and the winemaking industry has a huge worldwide economic relevance. Berry ripening is strongly influenced by environmental conditions and the global warming is negatively altering the maturation process. To provide a key in maintaining high quality grapes and wine, uncovering the still largely unknown ripening molecular mechanisms becomes crucial.

In previous studies, integrated network analyses have been applied to the global gene expression atlas and to a berry-specific dataset, allowing the identification of a new category of genes, called switch genes, putatively involved in the regulation of the transcriptional shift that characterized the vegetative-to-mature phase transition in grapevine. Among switches, four NAC (NAM/ATAF/CUC) transcription factors, VviNAC11, VviNAC13, VviNAC33 and VviNAC60 were found. Moreover, another NAC member, the VviNAC17, has been identified as molecular marker of berry ripening. Given the important regulative roles in plant development and stress responses exerted by NAC factors in different species, we decided to improve our knowledge about their involvement in the grapevine development, especially in the berry ripening.

In this work, a total of fourteen selected VviNACs genes, including switches, were analyzed by DNA Affinity Purification and sequencing (DAP-seq) and transient over expression to identify putative targets and transcriptional relationships among them. To define a regulation and co-regulation network between the NAC family members, some candidate VviNACs target genes were tested for their direct NAC-mediated regulation by Dual

Luciferase Reporter Assays (DLRA); indeed, the NACs are reported to regulate their own and each other transcription, forming a complex regulatory web and providing a finely tuned control system. Finally, a hierarchical network of the validated VviNAC-VviNACs regulations was constructed, showing the enormous complexity of the developmental regulatory mechanisms putatively orchestrated by the VviNAC family and clearly indicating that NAC TFs could act as both activators and repressors.