

NACS INTRA-FAMILY HIERARCHICAL REGULATORY NETWORK ORCHESTRATING GRAPE BERRY RIPENING

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Fruit ripening is a complex and irreversible process genetically and epigenetically regulated and affected by many endogenous and exogenous factors. In grape, the undergoing global warming is changing berry ripening timing and progression, with consequences on the harvest date and therefore negative impact on wine quality. Uncovering the molecular mechanisms and identifying key regulators governing berry ripening could then provide important tools in maintaining high quality grapes and wine. NAC (NAM/ATAF/CUC) transcription factors represent an interesting family due to their key role in the developmental processes control, such as fruit-ripening-associated genes expression, and in the regulation of multiple stress responses. In grapevine 74 NAC family members have been identified and 13 of them have been selected as putative regulators of berry ripening: *NAC01*, *NAC03*, *NAC05*, *NAC11*, *NAC13*, *NAC17*, *NAC18*, *NAC26*, *NAC33*, *NAC37*, *NAC39*, *NAC60* and *NAC61*. Genome wide analyses (DAP-seq and ChIP-seq), transcriptomics (microarray) and functional assays (dual luciferase and BiFC) permitted to reconstruct a hierarchical intra-family regulatory network. Most of the selected NACs resulted as transcriptional activators of other NACs, but a few did not; this may be due to their need to interact with other transcriptional partners. Indeed, many NACs interacted with each other to regulate the expression of other NACs. Auto- and cross-regulation within the family members provide a finely tuned control system, showing the enormous complexity of the ripening regulatory mechanisms putatively orchestrated by the NAC family. *NAC33* and *NAC60* resulted among the NAC-NAC network key regulators and functional studies demonstrated that *NAC33* is a promoter of organ de-greening and a repressor of the vegetative organ growth during the vegetative-to-mature phase transition, and that *NAC60* is

a regulator of whole plant senescence- and ripening-related processes. Among the NACs-highly-regulated NAC genes we found *NAC61*, which was recently identified as a late- and post-ripening processes regulator, and *NAC34*, *NAC05*, *NAC20*, *NAC37* and *NAC08*, which functions have not yet been investigated. To in-depth validate and in investigate gene networks orchestrating berry ripening, we are going to profile gene expression and identify open chromatin regions at individual cells level throughout berry development. Thanks to this fundamental information we will be able to define cell-type specific regulatory elements, developmental trajectories and transcriptional relationships among NAC transcription factors in different berry cell types.