

THE ROLE OF VVNAC61 BEHIND GRAPE BERRY AGING AND STRESS RESPONSES

AMATO A.*, FORESTI C.*, ORDUÑA L.***, MATUS J. T.***, VANDELLE E.*, DANZI D.*
, BELLON O.*, TORNIELLI G. B.*, ZENONI S.*

*) Department of Biotechnology, University of Verona, Verona, Italy

**) Institute for Integrative Systems Biology (I2SysBio), Universitat de València-CSIC, Valencia, Spain

NAC61, grapevine, late/post-ripening, abiotic/biotic stress, stilbenoid metabolism

Fruit ripening is a complex process involving physiological and biochemical changes, maximizing the fruit quality traits. The grapevine berry is a typical non-climacteric fruit, and to reach its final composition, it undergoes a developmental process comprising vegetative and ripening growth phases. The metabolisms featured in each developmental stage have been described, however the molecular mechanisms controlling late- and post-ripening stages are still poorly understood.

In this work, we report the role of NAC61, a grapevine NAC transcription factor, in regulating metabolic processes occurring from the onset of ripening, known as veraison, and particularly active during the following stages.

As first, we show that *NAC61* is upregulated in post-veraison stages and its expression correlates with the sugar content increase due to the osmotic stress characterizing ripening and post-ripening grape berries.

We demonstrate that the ectopic expression of *NAC61* in *Nicotiana benthamiana* leaves induces water-soaking-like phenotype and programmed cell death.

We narrow down a list of NAC61 high confidence targets (HCTs) by combining transcriptomic analysis of grapevine leaves transiently overexpressing *NAC61*, and DNA affinity purification and sequencing (DAP-seq) analyses. We reveal that NAC61 HCTs are mainly represented by genes acting in stilbene biosynthesis and regulation, and in osmotic and oxidative/biotic stress responses, that are biological processes inherent in late- and post-

ripening development phases. The direct regulation of the stilbene synthase regulator *MYB14*, the osmotic stress-related gene *DHN1b*, and the *Botrytis cinerea* susceptibility gene *WRKY52*, was validated.

We also inspect the *NAC61* upstream regulation, demonstrating its own activation and that it is a target of *NAC60*, a proposed master regulator of grapevine organ maturation. Moreover, *NAC61* interacts with *NAC60* triggering common targets activation. Additionally, we demonstrate that *NAC61* expression is enhanced by high temperature and *Botrytis cinerea* infection during berry post-harvest dehydration.

We believe this work would be of interest because it describes a master regulator of ripening progression in a tree crop species, which could prove a useful target towards maintaining necessary crop performance and fruit-quality characteristics.