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DISSECTION OF THE CONTROL OF THE ONSET OF BERRY RIPENING IN GRAPEVINE: THE ROLE OF NAC TRASCRIPTION FACTORS

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The challenge of this study is an attempt to dissect the regulatory network controlling the transcriptomic reprogramming which takes place along grape berry development. Thanks to an original integrated-networking method, based on the study of the grapevine global gene expression atlas and from a large berry transcriptomic dataset, some grapevine NAC (NAM/ATAF/CUC) members, i.e. *VvNAC33* and *VvNAC60*, have been indicated as putative master regulators able to promote the onset of berry ripening during the annual plant cycle. The NAC family is one of the largest classes of transcription factors in plant kingdom with important functions as components of the regulation of various biological processes. We obtained grapevine plants stably transformed with *VvNAC33* and *VvNAC60* showing phenotypes reflected typical behaviors of plants undergoing ripening and senescence: *VvNAC33* overexpressing leaves showed a yellowing effect due to a chlorophyll breakdown*,* whereas *VvNAC60* overexpressing plant was slightly affected by a reduced dimension in comparison to the control plant. Microarray analysis were performed on stable ectopic expressing leaves and a wide range of cellular processes was affected. Some downstream NAC targets were selected and validated by Dual-Luciferase reporter assay in transfected *Nicotiana benthamiana* leaves; among them, *VvMYBA1*, a known grapevine regulator of the anthocyanin biosynthetic pathway, resulted to act downstream to *VvNAC60*. Moreover, since grapevine plant takes around many years to produce the first fruits, we used the heterologous system tomato to have some results in a shorter time. A functional complementation analysis on *nor* mutant tomato (*Solanum lycopersicum* cv. Ailsa Craig) overexpressing *VvNAC33* and *VvNAC60* were carried out. An additional NAC member, *VvNAC03*, was included in the analysis as a close homologue of tomato *NOR* (non-ripening), known for its crucial role in tomato fruit ripening regulation. Among *VvNAC33* transgenic fruits some of them showed a slight pericarp pigmentation after breaker, whereas all *VvNAC03* and *VvNAC60* transgenic fruits presented pericarps with different degrees of yellowness at the same stage, in comparison to the same-stage *nor/nor* fruits. Moreover, the production of ripening-related ethylene was measured and the expression level of known ripening-related regulator genes were performed on the overexpressing fruits. The results suggested a partial complementation of the *nor* mutation and, thus, the involvement of these *VvNACs* in the organ phase transition to mature growth.

Further analysis will have to be conducted to fully elucidate the regulatory network controlling the transcriptomic shift that occurs in grapevine when plant moves into the developmental mature phase, but this work has begun to shed light on the roles of the members of NAC transcription factor family in the molecular machinery of this complex system.