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TRANSCRIPTIONAL REPROGRAMMING AND EPIGENOMIC LANDSCAPE DYNAMICS DURING GRAPEVINE SOMATIC EMBRYOGENESIS PROCESS

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Somatic embryogenesis (SE), a morphogenic process that takes advantage of the regenerative potential of plants to replicate whole plants starting from somatic explants, can be a source of variation with potential applications in plant breeding. It is one of the most suitable tools to apply functional genomics studies and genetic improvement in plants. However, beyond a few pioneering works mainly focused on model plants, the molecular characterization of SE mechanisms is still elusive, especially for woody species. In grapevine, this process is affected by many factors such as explant type, culture conditions and, most importantly, genotype. Many cultivars, in fact, have shown recalcitrance to tissue culture and transformation, and very low SE competence. Thus, the understanding of SE competence behind the regenerative aptitude is fundamental to the widespread application of the so-called "next-generation breeding techniques", such as cisgenesis and genome editing, in grapevine.

Here, we explored genetic and epigenetic features of the SE process in grapevine by investigating the behavior of two genotypes showing opposite SE competence. Embryogenic tissues were induced from immature stamens excised from field-collected flower clusters of Sangiovese (highly competent for embryogenesis) and Cabernet Sauvignon (poorly competent for embryogenesis). A multilayered approach was used to profile mRNA, smallRNAs and methylated DNA with high-throughput sequencing technologies in the initial explants, on undifferentiated calli induced after 40 days of culture, and in embryogenic and non-embryogenic calli after 3 months of culture.

A comprehensive comparison of transcriptomes of the different types of calli with the grapevine gene expression atlas revealed that, in grapevine, the dedifferentiation to the callus formation during the embryogenesis process occurs via a 'berry' developmental pathway. This is dissimilar from that shown in Arabidopsis, in which dedifferentiated calli are more similar to the tip of a root meristem. Interestingly, a Gene Ontology (GO) analysis revealed that secondary metabolism and gene expression regulation/epigenetics are the enriched functional categories of genes differentially down- and up-regulated in embryogenic vs non-embryogenic calli, respectively. These results prompted us to define the epigenetic landscape dynamics during SE in grapevine, revealing a significant increase in DNA methylation, especially in intergenic regions, in the embryogenic calli tissues. Finally, we proposed potential key regulators of SE in different genotypes that could represent putative targets of next-generation breeding techniques in grapevine.