Proceedings of the LXVI SIGA Annual Congress
Bari, 5/8 September, 2023
ISBN: **978-88-944843-4-2**

Poster Communication Abstract – 6.27

THE DAP-SEQ AS A TOOL TO EXPLORE GRAPEVINE FLORAL CISTROME

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MYB108A, DAP-seq, NGS, flower, anther

In the post-genomics Era, the enormous amount of data generated by omics technologies being stored in public databases considerably exceeds the analytical capacities of humans, making it imperative to use increasingly powerful computational resources to process, analyse and store this information. Nowadays, several studies aim at specifically addressing this issue in grapevine (Vitis vinifera L.), a plant which is quickly establishing itself as an appealing 'model' species for studying non-climacteric fleshy fruits. In addition to genomics and transcriptomics, the recent development of regulatory genomics, also known as "regulomics", has garnered growing interest in crop species as transcriptional regulation of genes plays a critical role in any biological process, including phenotypic plasticity and fruit development. DNA-Affinity Purification Sequencing (DAP-Seq) is a high-throughput experimental procedure that allows to discover transcription factors binding sites (TFBS) interrogating genomic DNA with in vitro-expressed TFs. DAP-Seq is the ultimate approach for TFBS discovery and provides a scalable alternative for non-conventional model species where genetic transformation is difficult, to rapidly and inexpensively investigate large number of TFs. The idea behind this assay is to combine next-generation sequencing (NGS) of a genomic DNA library with in vitro expression of affinity-purified TFs to generate cistrome maps for a wide

range of species. In this study, we took advantage of DAP-Seq to investigate the cistrome landscape of a representative subset of grapevine TFs. These TFs have been previously identified by means of an RNA-Seq approach performed on ten different grapevine floral whorls): calyx, calyptra, anther, filament, stigma, ovary and embryo, in both pre- and post-anthesis, and their expression was proven to be highly specific for a given whorl. Taking advantage of some systems biology in silico analysis, for each TF it was isolated a subset of target genes featured by a similar expression pattern and by a high specificity degree for the related floral organ. These genes were designed as “high confidence targets” (HCTs) and were used to generate tissue-specific regulatory networks to infer the function of a given TF based on the identification of its target genes. Subsequently, the putative role of the TFs was empowered by a cis regulatory elements enrichment analysis, finding in this way the most probable binding site of a TF basing on its own cistrome. Among all the TFs, the attention was finally focused on VvMYB108A, a transcriptional regulator which was observed to be highly specific for anther development. The analysis of the related HCTs regulative network and the de novo motif discovery strongly support the putative role of this VvMYB108A in male fertility specific molecular mechanisms.