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Poster Communication Abstract – 1.15

DECODING GENE REGULATION: NAC FAMILY INVESTIGATION IN GRAPEVINE

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Transcription factors (TFs), the DNA-binding proteins, wield their influence over gene expression by recognizing specific short motifs within the promoter region and adjacent sites. TFs are crucial in regulatory networks, revealing gene regulation in agricultural crops. The functionality of TFs extends beyond their recognition of DNA sequence motifs; it encompasses the complex interplay of epigenetic mechanisms, cofactors, and synergistic interactions with other TFs within molecular complexes.

Regarding the goal of our work our attention will be mainly focused on the NAC family of TFs in grapevine, named after its founding members: NO APICAL MERISTEM (NAM), Arabidopsis thaliana transcription activator factor 1/2 (ATAF1/2), and CUP-SHAPED COTYLEDON 2 (CUC2).

The grapevine NAC TFs (VvNACs) intricately govern growth, development, stress responses, and defense mechanisms, including the intricate regulation of berry ripening and leaf senescence. Our research endeavor, part of a nationally significant project, sought to explore gene expression regulation in grapevine and dissect the underlying genetic and epigenetic determinants.

To accomplish this, we employed the state-of-the-art technique of DNA affinity purification sequencing (DAP-Seq), a swift and cost-effective

method for identifying TF binding sites. Our method involved cloning TF genes, *in vitro* expression, capturing target sequences from a genomic DNA library, affinity purification of TF-DNA complexes, and subsequent analysis of the captured DNA using next-generation sequencing. This process yielded comprehensive cistrome and epicistrome maps, enabling a profound comprehension of grapevine cistrome/epicistrome dynamics.

Through DAP-Seq, we meticulously delineated the cistrome of all 74 documented VvNAC proteins. Leveraging large DNA-Seq datasets derived from young grapevine leaves of the Cabernet Franc cultivar, we subjected the data to rigorous statistical and bioinformatic analyses, validating the results for 24 NACs. Subsequent analyses focused on analyzing the data obtained to define the distinct biological functions of some of the NACs.

The results obtained were compared with data obtained from the dual luciferase reporter assay (DLRA) and also from chromatin immunoprecipitation sequencing (ChIP-Seq), two powerful *in vivo* techniques. For this purpose, a ChIP-Seq protocol has been optimized in the grapevine to improve the signal-to-noise ratio and resolution of TF binding sites compared to previous results.

This work explains one of the most important families of TFs in grapevine and provides new information showing how these NAC proteins interact in networks to activate target effector genes involved in various pathways that control plant growth, development, stress responses and defense. A better understanding of these processes will allow us to select specific TFs for use in genetic engineering and genome editing to improve the agronomic properties of grapevine.