UNIVERSITÀ DEGLI STUDI DI PADOVA

DAFNAE

Department of Agronomy Food Natural resources Animals and Environment

Vine-SCROLL

An innovative biotechnological approach for the in Situ Crispr-affinity puRification Of reguLatory eLements in grapevine

Cis-regulatory elements (CREs) such as transcriptional enhancers, insulators or silencers regulate condition-specific gene expression by recruiting transacting factors and chromatincomplexes and interacting with target genes by long-range DNA looping. In plants, Identifying the molecular composition ofa specific CRE in situ could provide unprecedented insight into the mechanisms regulating its activity in physiological orpathological conditions and on the most efficient way to intervene altering the expression of key genes related to important traits such as quality, productivity, or resistance to biotic/abiotic stresses. Indeed, it is now widely accepted that future application of NewBreeding Techniques is likely to be more focused on non-coding rather than coding DNA regions. Unfortunately, in contrast to protein-coding genes, our understanding of cis-regulatory DNA is very limited albeit the availability of a number of innovative approaches which greatly simplified the study of the chromatin conformation and of the trans/cis regulatoryelements interactions. The limit in any approach aimed at deciphering the organization and structure of a specific target gene CRE lies in the fact that purifying asmall chromatin segment from the nucleus remains a significant challenge. This project is aimed at deepening the knowledge on the regulatory complexes of key genes involved inthe response to both biotic and abiotic stress, but also in grapevine berry quality through an innovative biotechnological approach based on the selective immunoprecipitation of DNA-protein complexes mediated by a mutated Cas9 followed byprotein/nucleotide sequencing for the simultaneous identification of locus-specific chromatin proteins and long-range DNA interactions. Grapevine protoplasts will be transformed with a mutated Cas9 protein (dCas9) devoid of endonuclease activity but capable of binding DNA in a specific way thanks to the aid of sgRNAs designed on the promoters of target genes or on specific CREs. Grapevine protoplasts will be isolated from different grapevinetissues/organs, including leaves, and green or mature berries to promote the modulation of gene expression and trigger condition-specific regulatory complexes. Target DNA-protein complexes will be isolated using an innovative approach based on chromatinfixation/fragmentation, purification of the protein complexes by dCas9-specific immunoprecipitation or by affinity, reverse crosslinking for the release of DNA and chromatin

proteins and proteomic and genomic analysis based on mass spectrometry (MS) and NGS sequencing for the identification of regulatory genomic proteins of target loci and long-range DNA interactions.

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