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IMPROVING GRAPE RESILIENCE TO DROUGHT EXPLOITING THE CRISPR/CAS TECHNOLOGY: FUNCTIONAL CHARACTERIZATION OF THE TARGET GENE THROUGH SPRAY INDUCED GENE SILENCING (SIGS)

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Climate change has been significantly impacting the food chain production, resulting in decreased quality and yield. In this line, water is one of the major factors limiting the productivity of agricultural ecosystems. Due to the ongoing climate change, drought events are predicted to become more frequent and severe. Fruit crops, in particular, face challenges with traditional breeding methods due to financial constraints, limited land resources, and long generation times. In this context, the utilization of 'New Genomic Techniques' (NGTs) has emerged as a promising avenue for expediting the development of genetically improved cultivars. However, NGTs also suffer from limitations that hinder their implementation. One crucial limitation is the lack of knowledge on gene responsible for specific traits of interest. In this regard, the emerging SIGS technique could represent a useful strategy to elucidate gene function in short time. This is especially important for woody crops like grapevine, which require long time for the application of conventional or NGTs approaches.

SIGS is based on the high-pressure application of double stranded RNAs (dsRNAs) directly on leaf surface. In this study, we produced dsRNAs targeting a specific grapevine endogenous gene sequence in vitro, putatively involved in drought stress tolerance responses. Previous studies demonstrated that the downregulation of a glutathione S-transferase (*GST*) gene in *Arabidopsis* mutants increases endogenous abscisic acid (ABA) levels and triggers the plant anti-oxidant system, enhancing drought resilience in primed plants. Our survey focused on a putative *VvGST* gene inhibited by drought stress in grapevine, and we evaluated the plant ecophysiological and molecular responses of potted 'Chardonnay' vines after high pressure spraying the leaves (dsGST). Leaf gas exchange, leaf water potential (ΨLeaf), and the expression of stress-related and silencing machinery-related genes were monitored in dsGST and dsGFP (the latter used as negative control of the technique) plants either submitted or not to water deprivation. Interestingly, the dsGST-treated plants exhibited increased resilience to severe water deficit conditions, as indicated by the ecophysiological measurements. Molecular analysis using RT-qPCR assay of stress- and ABA -related genes confirmed the priming effect of the treatment. Furthermore, biochemical analysis through HPLC-DAD of ABA levels and resveratrol (considered as antioxidant marker) in leaves was conducted. Results showed how the SIGS approach can represent a powerful technique for functional genomic studies in grapevine. Building upon these findings, we employed a self-designed cisgenic-like construct to transform embryogenic calli of Chardonnay and 110 Richter genotypes aiming to develop knock-out mutants of *VvGST40*.