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Exploring the impact of NPR3 gene silencing on the interaction between grapevine and mycorrhizal fungi through genome editing

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Abstract

One of the main plant defence mechanisms is the Systemic Acquired Resistance (SAR) mediated by Salicylic Acid (SA). This is a heightened and broad-spectrum immune response initiated by the exposure to pathogens, inducing resistance not only in the infected site, but also throughout the entire plant. It was demonstrated that plant immune system can be regulated by two classes of SA receptors: NONEXPRESSOR OF PR GENES 1 (NPR1) and NPR1-LIKE PROTEIN 3 and 4 (NPR3/NPR4). While NPR1 is required for SA-induction followed by the expression of pathogenesis-related (PR) protein and resistance against pathogens, NPR3/NPR4 serve as transcriptional co-repressors of SA-responsive genes. The aim of this work was to trigger SAR by suppressing NPR3, and to investigate how plant response affects its ability to recruit beneficial microorganisms, specifically arbuscular mycorrhizal fungi (AMF). To this aim, embryogenic calli were obtained from anther and ovaries of grapevine (cultivar Chardonnay) and NPR3 knock out lines were achieved using CRISPR/Cas9 technique. Three regenerated lines, along with a backbone and a wild-type lines, were inoculated in axenic condition with the AMF *Rhizophagus irregularis* to test their recruitment ability. After the acclimatation, plants were transferred in the greenhouse and forty-five days later both roots and leaves were collected. Root colonization was evaluated using the Trouvelot method and significant differences in colonization level were observed among plants. Consequently, root DNA and RNA were extracted for metabarcoding and RNAseq analysis. Additionally, metabolomic analysis targeting metabolites involved in plant-microorganism interactions are ongoing.

Keywords: CRISPR/Cas9, grapevine, arbuscular mycorrhizal fungi (AMF), Systemic Acquired Resistance (SAR), multi-omics