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RNA interference in the Vineyard: a potentially transformative tool?

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The mechanism of gene silencing, also called RNA interference (RNAi), involves the production of small RNA molecules named small-interfering RNA or siRNA. These stretches of double-strand RNA (dsRNA) are 21 to 24 nucleotides in length. Their production will trigger a series of molecular reactions that will either repress gene expression (Transcriptional Gene Silencing or TGS) or protein synthesis (Post-Transcriptional Gene Silencing [PTGS]). It is a conserved mechanism in animals and plants that has been extensively studied for the past thirty years since its discovery. Scientists have engineered this mechanism to develop new technology tools to understand gene-to-trait associations (Carbonell et al. 2014, de Felippes et al. 2012). Yet, when a silenced gene in plants can confer an advantageous trait such as better yield or resistance to a fungus, it usually is through genetic engineering with the production of Genetically Modified Organisms.

Therefore, there is a need for developing innovative and transformative tools with better acceptance of scientific novelty and a trustful appreciation of how basic knowledge can lead to applications in vineyards. RNAi-based plant protection against fungal pathogens, viruses, and other pests is regarded as a promising lab-to-field application. Based on RNAi's principle, it delivers dsRNA-based molecules that trigger an RNA interference mechanism in plants (Sherman et al. 2015). The nucleic sequence of the ectopically applied RNA molecules will determine the targeted genes(s) deriving from a fungus, a virus, a bacterium, or the plant itself.

One significant advantage of the technology is the potential avenue to overcome the excessive use of chemical pesticides. The versatility to easily change the nucleic acid sequences can also prevent possible pathogen resistance over time. The RNA molecules can be designed to silence the activity of several genes at a time, which can be very important with multiple genes agronomy traits. The availability of robust *in silico* tools for designing RNA sequences with high predicted silencing efficiency, the generation of numerous genomic resources in grapevine, and the relatively low cost to produce RNA molecules nowadays make unique the opportunity to develop RNAi-based molecules applications in a vineyard (Dalakouras et al. 2020). Another advantage of the molecule is that it can be rapidly degraded, limiting long-term persistence in the environment. Besides, due to the non-transgenic nature of the methodology, it should have a relatively better approval by the public. There are growing examples of scientific studies in many crops, including in grapevine, that validated the approach in a lab setting. Yet, several factors must be considered to validate such a tool in vineyards. Here is a non-exhaustive list of these factors that can be related to the plant itself or to the RNA molecule itself, 1) the penetration and stability of the RNA molecules at the leaf surface, 2*)* the systemic movement of the silencing within the plants, and finally, 3) the length of the dsRNA and its magnitude of the silencing.

Different methods to optimize the delivery can be envisioned, too (foliar spray, nanoparticles, trunk injection). Due to its ease in a vineyard setting, foliar application is a promising avenue, but several physical barriers will limit the uptake of RNA-based molecules through the plant leaves. The cuticle is the first barrier limiting the absorption of exogenous hydrophilic and polar molecules like nucleic acids (Schreiber 2005). Cuticle limitation can be overcome somewhat by high-pressure spraying, but the damage to the integrity of the leaves' structures will need to be assessed (Dalakouras et al. 2016). The cellular uptake also remains a significant hurdle for the delivery of RNA molecules with physical barriers like plant cell walls that have pore size exclusion limits, up to 50 nm in grapevine, for example (Palocci et al. 2017). In practice, the diameter of double-stranded RNA molecules (~2.4 nanometers) would fit most of the pores. However, depending on the RNA molecules' length, they can bend and twist, increasing thus their diameters (Lipfert et al. 2014). To overcome this limitation, nanoparticles and other nanostructures designed to carry the RNA molecules or favor their stiffness can help maintain their diameter to its lowest value (Demirer et al. 2019, Zhang et al. 2022). Besides, the nanoparticles can help prevent the degradation of dsRNA at the leaf surface and thereby improve the cellular uptake (Mitter et al. 2017, Schwartz et al. 2020).

The choice in length and sequence of the dsRNA molecules applied to silence the target genes is critical. The best results of efficient silencing were observed with short sequences of 21-22 nt of siRNA that complies with several RNA-interference criteria (Ahmed et al. 2020, Bennett et al. 2020, Dalakouras et al. 2018). In this context, the development of robust *in-silico* tools to predict efficient siRNA molecules and the increasing genomic resources via genome sequencing will contribute to identifying for each candidate gene the most efficient siRNA species (21-24 nt) for systemic silencing and extended protection of RNAi-based ectopic application in vineyards.

Overall, most advances in dsRNA delivery have focused on methods and nanomaterials to aid the penetration of leaf structure and the protection of dsRNA in lab conditions. RNA production was also a concern, but large-scale manufacturing is on its way to producing large quantities at a negligible cost like $2 per gram of RNA (Zotti et al. 2018). Yet, the processability and scalability of the ectopic application of RNA molecules to a vineyard remain validated. Additionally, to make the technology even more attractive, the urgent need is to develop properly *in silico* prediction tools to enable the identification of the proper siRNA sequences that predict the most significant silencing of a given gene with a systemic effect on the entire plant for an extended time during the growing season.

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