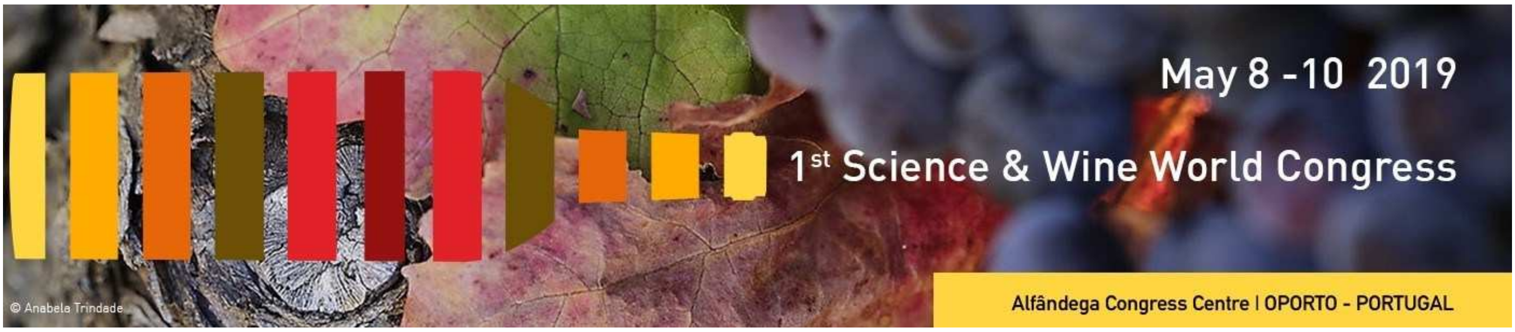
Aiming at grapevines with increased resistance to pathogens, reaching structural genome modifications



By Vera Quecini, Iraci Sinski, Patrícia S. Ritschel, Thor V. M. Fajardo

Resistance to pathogens is an important goal in the development of novel grapevine cultivars throughout the world by conventional and biotechnology-assisted breeding programs. **Fungal and viral diseases cause direct losses in berry production**, but also affect the quality of the final products. Methods for chemical control are available for fungus, but they increase the production costs, decrease fruit quality, and negatively affect culture sustainability. For viral pathogens, the situation is also complex, since it has to rely on the control of vector insects, if that is the way of transmission, or, on the replacement of highly colonized plants by new, clean ones. Thus, **the introduction of genetic resistance is economically and environmentally desirable**. Resistance to fungi and viruses is often found in wild or non-commercial *Vitis* genotypes, which simultaneously carry many undesirable traits to the progeny when used in crosses with elite cultivars. Recently, precision breeding strategies, such as genetic engineering and genome editing, allow the precise introduction of resistance characters in elite cultivars (Figure 1). However, the overall performance of the plants submitted to precision breeding techniques is not always as expected.

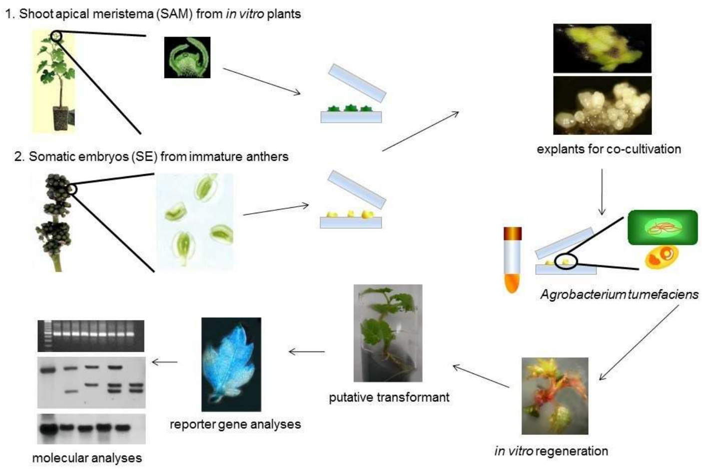


Figure 1. Schematic representation of the techniques used to generate genetically engineered grapevines.

In the current work, we have generated genetically engineered grapevines that express proteins with antimicrobial properties against fungal pathogens or a virus-derived sequence, in a hairpin orientation, which triggers the specific degradation of an RNA essential to the formation of novel infective viral particles. As expected, the genetically engineered grapevines exhibited increased resistance to the pathogens, in comparison to the non-engineered plants, although the infection was still detected in the modified plants (Figure 2). Further investigation demonstrated that the plants carrying larger portions of sequences from viral origin exhibited higher levels of a structural genome modification called methylation, and lower levels of resistance (Figure 3). Methylation consists in the addition of methyl radicals to certain bases of the DNA and is considered an epigenetic modification, since it interferes with the activity of the DNA without modifying the sequence. Taken together, our results demonstrated that **the introduction of sequences of viral origins into grapevine genome is associated to genome structural changes and reduced expression of resistance against pathogens, thus indicating that the effectiveness of resistance strategies employing sequences of viral origin is subject to epigenetic regulation in grapevine.**

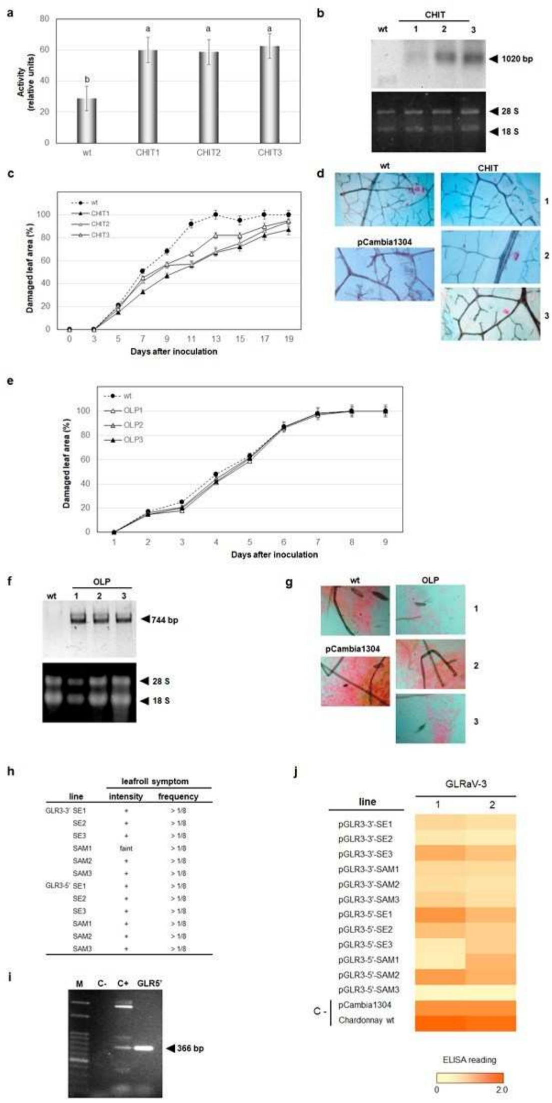


Figure 2. Evaluation of disease responses against fungus (a-g) and virus (h-j) for genetic engineered and wild type plants. a In vitro assay of chitinase activity. Letters represent Tukey's HSD at p < 0.05 (one-way ANOVA). b RNA hybridization employing a gene-specific probe. c Disease progression evaluated by the percentage of damaged leaf area after infection. d Light microscopy analyses of fungal structures in infected leaf discs at day 9. e Disease progression evaluated by the percentage of damaged leaf area after infection. f RNA hybridization employing a gene-specific probe. g Light microscopy analyses of fungal structures in infected leaf discs at day 5. h Frequency and intensity of leafroll symptom in hpGLR3 lines. i RT-PCR amplification of a GLRaV-3 specific fragment in symptomatic

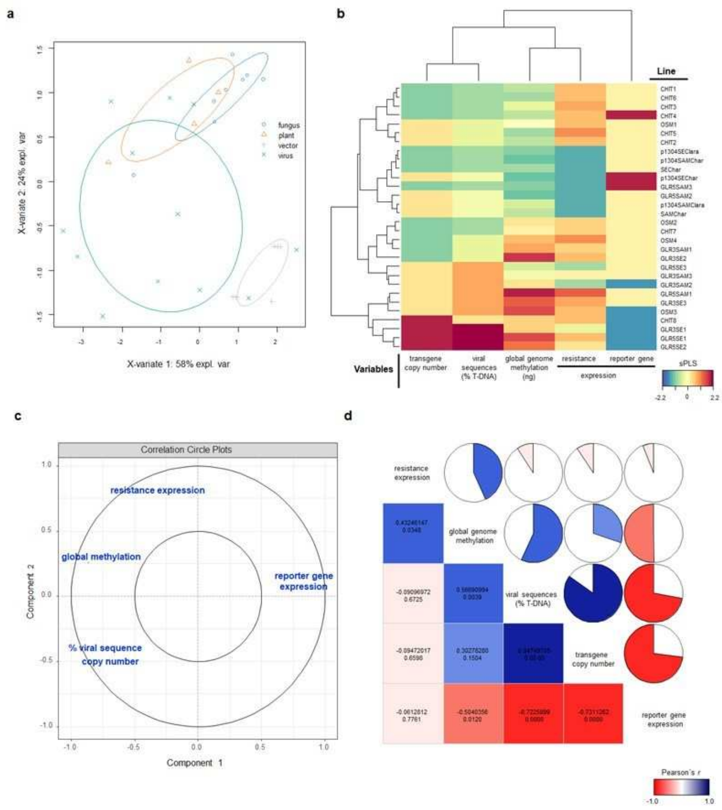


Figure 3. Multivariate analyses of genomic features and trait expression in grapevine genetically engineered lines. a sPLS-DA plot of the individuals using the origin of the gene of interest as discriminant. Ellipses represent 95% confidence levels. b Clustered image map of the similarity matrix obtained by sPLS-DA results. Similarity is represented as heatmap, ranging from − 2.2 (blue) to 2.2 (red), and dendrograms derived from hierarchical clustering of the similarity results are represented for the genetically engineered lines (vertical) and variables (horizontal). c Correlation circle plot of the variables used in sPLS-DA analysis. d Heatmap and pie graph representation of the Pearson correlation between the investigated variables for the genetically engineered grapevine CHIT, OLP and hp-GLR3 lines. Pearson's r is given inside the squares along with its p value.

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