

EXPLORING GENETIC VARIABILITY OF SUSCEPTIBILITY GENES IN GRAPEVINE: A RECENT FRONTIER TO DISSECT DISEASE RESISTANCE

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Grapevine is one of the most important and studied tree crops worldwide. Obtaining resistance to pathogens as *Plasmopara viticola* (the causal agent of downy mildew, DM) has always been a main goal in Europe since spreading of these pathogens at the end of the 19th century. In the last decades, due to the need to reduce environmentally-impacting fungicides, breeders focused on crossing Eurasian *Vitis vinifera* with wild American and Asian species to obtain resistant individuals. Unfortunately, dominantly inherited gene-based resistance has shown to be race-specific in some cases, to confer partial immunity and to be potentially overcome within a few years from the introduction of the resistance trait. Recently, the identification of susceptibility genes in herbaceous and tree crops, as factors required by the pathogen to infect the host-tissue, has opened up a chance for their exploitation as an alternative to breed for resistant plants. On the footprint of the research conducted on Arabidopsis, genes associated with DM susceptibility have been discovered also in the grapevine genome.

Four susceptibility genes were re-sequenced (Illumina, 1,000X depth) in 190 grapevine accessions including 23 wild, 28 *vinifera* and 139 hybrid individuals to discover new sources of broad-spectrum recessively inherited resistance against *P. viticola*. The scouted genes were *VvDMR6-1*, *VvDMR6-2*, *VvDLO1*, *VvDLO2* involved in susceptibility to DM. These genes were mapped on the reference genome and analysed to identify polymorphisms and haplotypes using dedicated software to study the mutation effect. Regarding those mutations with putative impact on gene function, within the 190 accessions we observed ~14% accessions mutated in *VvDMR6-1* and ~18% mutated in *VvDMR6-2*, only a handful of accessions that were mutated in both genes. ~21% and ~16% accessions showed mutations in *VvDLO1* and *VvDLO2* respectively, and again only a few accessions were mutated in both genes. 86% of the total impacting mutations were SNPs (in the strict sense) while 14% were substitutions. Out of the 129 accessions carrying selected mutations ~83% were hybrids whereas 7% and 10% were respectively wild species and *vinifera* varieties. When taking into account haplotype frequencies, highly shared haplotypes (in ~40% of the mutation-carrying accessions) were observed for *VvDMR6-2* and *VvDLO1*, whereas for *VvDMR6-1* and *VvDLO2* almost every accession showed a specific haplotype.

Moreover, a validation of Illumina results was carried out with a Sanger sequencing on 25 selected accessions as informative for interesting non-synonymous or synonymous substitutions in one or more of the genes under investigation. Basing on these findings, the *VvDMR6-1* and *VvDLO1* protein model based on confirmed mutations-carrying haplotypes was drafted with the aim to investigate the impact of amino acids substitution on protein folding and function.

These results will inform grapevine genetics and corroborate genomic-assisted breeding programs for resistance to biotic stresses.