Characterization of the *Rpv12* locus in a haplotype-separated grapevine genome sequence

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Abstract

Grapevine downy mildew, caused by *Plasmopara viticola*, imposes a major challenge on viticulture since the 19th century. Attempts to counteract the disease by crossing the noble European *Vitis vinifera* with resistant American *Vitis* wild species in the early 20th century remained discouraging due to transmission of undesirable characteristics. The recent development of genetic marker analyses allowed to identify resistance mediating genomic regions from extra-European *Vitis* species and to follow them in cascaded back crosses to *V. vinifera*. More than 31 genetic loci are currently known to contribute to resistance to *P. viticola*. One of these is *Rpv12* (resistance to *P. viticola* 12), initially identified in the Asian species *V. amurensis*. This locus was identified in 2013 (Venuti *et al.*) and it was compared to the American locus *Rpv3* in transcriptomic and metabolomic studies (Chitarrini *et al.*, 2020). However, these investigations were not yet able to identify candidate resistance genes. Thus, to delimit the locus and to reveal its possible resistance genes, the *Rpv12* carrying genotype Gf.99-03 (Geilweilerhof 2014-099-0003, *V*IVC: 27131) was sequenced in combination with its parental genotypes 65-153-18 (*V*IVC: 41129) and Gf.43-21 (Geilweilerhof 2904-043-0021, *V*IVC: 27130). Long read data were computed into a high quality haplotype separated genome assembly of Gf.99-03. Gene annotation of the newly assembled genome sequence was supported by RNA-Seq analyses from various tissues such as leaves, stems, tendrils and roots. Also, comprehensive differential gene expression analysis of experimentally inoculated leaf discs at different time points after inoculation with *P. viticola* was performed. Approximately 600 differentially expressed genes were identified.

The *Rpv12* locus is delimited by the simple sequence repeat (SSR)-markers UDV-014 and UDV-370. Differentially expressed genes in the resistance carrying haplotype of Gf.99-03 were identified and checked for uniqueness. In addition, the locus was searched for typical resistance gene analogs like *NLR*s (nucleotide binding site leucine rich repeats). A cluster of putative resistance mediating genes including *CNL*s was identified in this region.

Keywords: downy mildew**,** fully-phased genome assembly, infection experiment, leaf disc assay, *Plasmopara viticola*, resistance, *Rpv12,* TrioBinning, *Vitis spec*.