Phenotyping for grapevine QTL identification. The case of resistance to *Plasmopara viticola* and *Erysiphe necator*. A review.

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1 Introduction

Grapevine species belonging to the genus *Vitis* display susceptibility, partial or total resistance to *Plasmopara viticola* Berl. & De Toni (the causal agent of downy mildew - DM) and to *Erysiphe necator* Sch. (the causal agent of powdery mildew - PM). Due to the impact of DM and PM diseases on *Vitis vinifera* L. cultivation, research has focused for a long time on the identification and introgression of wild grapevine resistance into cultivated varieties.
Since 2000, genetic studies have provided significant progress for grapevine breeding. To date, thirty-one loci of resistance to *P. viticola* (called *Rpv*) and thirteen loci of resistance to *E. necator* (called *Ren* or *Run*) have been identified in American and Asian *Vitis* species but also in some *Vitis vinifera* varieties (Table 1; https://www.vivc.de). Some American *Vitis* species such as *V. aestivalis*, *V. cinerea*, *V. rotundifolia*, *V. riparia*, *V. rupestris* and a few Asian species such as *V. amurensis* or *V. piasezkii* have been studied. Despite their limited level of resistance compared to other *Vitis* species, it is only recently that an interest in *Vitis vinifera* varieties has appeared because new germplasms have been investigated.

Resistance loci have been located in different chromosomes and usually in genomic regions rich in NBS-LRR-like resistance genes, called R genes (Di Gaspero *et al*., 2007, Moroldo *et al*., 2008). Some loci co-localized in grapevine genome but showed different resistance haplotypes, resistance levels and/or specificities toward pathogen isolates.

P. viticola and *E. necator* are both biotrophic pathogens that can infect all grapevine green tissue: leaves, shoots, inflorescences and young berries. The resistance loci identified, induce various levels of resistance. The resistance conferred by most of the loci is partial. The observation of the symptoms, shows that pathogen sporulation is present but in a lower amount than for a susceptible variety. Very few loci, such as those identified in *V. rotundifolia* or *V. piasezkii*, induce a total resistance to *P. viticola* or *E. necator*. No sporulation is observed but hypersensitive response (HR) is frequently produced.

2 QTL characterization

The characterization of quantitative-trait-loci (QTL) involves the association between individual allelic variants and phenotypes. Nowadays, the availability of the grapevine reference genome and the developments in the genetic field (e.g. genotyping by sequencing technologies) allow the discovery of thousands of molecular markers, the unification of grapevine linkage groups and the production of precise and dense genetic maps. For these reasons, the genotyping in QTL studies no longer represents an obstacle to loci discovery and location.

Phenotyping is currently the main bottleneck in resistance QTL mapping. This is due to the several biological components involved and their direct impact on the resistance expression. Moreover, the technical difficulties in managing and standardizing the evaluations limit also the process automation. Finally, the consequential costs and the time associated with the practice contribute to this bottleneck.

QTL	Chr	Origin of resistance	Resistance level
Rpv1	12	*V. rotundifolia*	Partial
Rpv2	18	*V. rotundifolia*	Total
Rpv3	18	*V. rupestris*	Partial
Rpv4	4	American *Vitis spp*	Partial
Rpv5	9	*V. riparia*	Partial
Rpv6	12	*V. riparia*	Partial
Rpv7	7	American *Vitis spp*	Partial
Rpv8	14	*V. amurensis*	Partial
Rpv9	7	*V. riparia*	Partial
Rpv10	9	*V. amurensis*	Partial
Ren1	13	*V. vinifera*	Partial
Ren2	14	*V. cinerea*	Partial
Ren3	15	American *Vitis spp*	Partial
Ren4	18	*V. romanetii*	Partial
Ren5	14	*V. rotundifolia*	Total
Ren6	9	*V. piasezkii*	Total
Ren7	19	*V. piasezkii*	Partial
Ren8	18	American *Vitis spp*	Partial
Ren9	15	American *Vitis spp*	Partial
Ren10	2	American *Vitis spp.*	Partial
Ren11	15	*V. aestivalis*	Partial
Run1	12	*V. rotundifolia*	Total
Run2	18	*V. rotundifolia*	Partial

Table 1: Characteristics of the first ten *Rpv* and of all the *Ren/Run* loci, identified since 2000.

References

Rpv1: Merdinoglu *et al*., 2003. *Rpv2*: Wiedemann-Merdinoglu *et al*., 2006. *Rpv3* and *Rpv7*: Bellin *et al*., 2009. *Rpv4* and *Ren3*: Welter *et al*., 2007. *Rpv5* and *Rpv6* : Marguerit *et al*., 2009. *Rpv8*: Blasi *et al*., 2011. *Rpv9*: Moreira *et al*., 2011. *Rpv10*: Schwander *et al*., 2012. *Ren1*: Hoffmann *et al*., 2008. *Ren2*: Dalbó *et al*., 2001. *Ren4*: Ramming *et al*., 2011; Mahanil *et al*., 2012. *Ren5*: Blanc *et* al., 2012. *Ren6* and *Ren7*: Pap *et al*., 2016. *Ren8*: Zyprian *et al*., 2016. *Ren9*: Zendler *et al*., 2017. *Ren10*: Teh *et al*., 2019. *Ren11*: Karn *et al*., 2021. *Run1*: Pauquet *et al*., 2001. *Run2*: Riaz *et al*., 2011.

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Phenotyping for grapevine resistance may take place in the vineyard, the greenhouse, or the laboratory.

Phenotyping in the vineyard needs several years of observation, depending on favorable climate conditions for disease development. The number of observations per year varies between studies. Leaves and bunches are generally assessed for the presence of disease. Sometimes, the symptoms are also observed on rachis and stems.

Figure 1: In the vineyard, *P. viticola* oil spots on leaves and *E. necator* on bunches

Phenotyping in the greenhouse takes place during one season. The symptoms observed are generally the result of a natural infection by the pathogen. Whole plants or defined leaves are considered. Several assessments are carried out during the season.

Figure 2: In the greenhouse, natural infection by *E. necator*.

Phenotyping in the laboratory uses plants from the. greenhouse. Defined leaves are collected and directly used or leaf discs are excised. The inoculation by the pathogens and the incubation are conducted under controlled conditions using generally standardized methods.

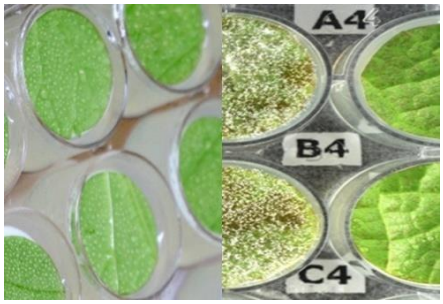

Figure 3: In laboratory conditions, artificial infection of leaf discs with *P. viticola.* Symptoms of susceptibility and of resistance.

The variability in the resistance phenotyping methods used reflects the continuous search by grapevine breeders, geneticists and pathologists for reliable data able to predict the performance of the different genotypes and loci.

This review is based on the phenotyping strategies implemented to map the *Rpv* and *Run/Ren* loci in the last twenty years. The detailed analysis of the methods used, shows that whatever the environment considered: the vineyard, the greenhouse or the laboratory, some elements can be considered as sources of variation. The plant material (experimental design, type and age), the inoculum (natural or controlled) and the assessment method (date, frequency, type of scoring) can influence the reliability of the resistance response.

3 Conclusions

This analysis reveals the strengths and weaknesses of phenotyping methods used for resistance loci identification and mapping. This analysis proposes also to standardize experimental protocols by providing some defined informations.
Biological results emerging from grapevine resistance mapping studies will be discussed in the light of the above phenotyping considerations.

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