Crossing *Plasmopara viticola* strains in controlled conditions to uncover the genomic bases of downy mildew resistance breakdown in grapevine

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Abstract. The biotrophic oomycete *Plasmopara viticola* is the causal agent of downy mildew, one of the major grapevine diseases. We report here a successful strategy to cross compatible strains of this pathogen and obtain a large viable progeny. We used this method to study the offspring between two *P. viticola* strains able to overcome two major grapevine resistance factors (*Rpv3*, *Rpv10*). Thanks to the genomic resources now available, we will genotype this progeny to build an unprecedented linkage map and uncover the genomic bases of grapevine resistance breakdown displayed by *P. viticola* virulent strains.

1 Introduction

Annual sexual reproduction is a central part of the *P. viticola* life cycle. Under autumnal conditions, gamete- producing organs, called antheridia and oogonia, are formed inside the host tissue. The mating between sexually compatible strains leads to the production of thick-walled oospores that are typical of oomycetes. They overwinter in fallen plant residues before starting primary infections in the next spring (Viennot-Bourgin, 1949).

The possibility to control and re-enact the complete life cycle of the pathogen in laboratory can improve our understanding of the key steps of its sexual phase. Thus, the controlled production of oospores is of special interest to address the environmental factors that affect oospore formation, maturation, viability and germination.

However, the study of *P. viticola*'s sexual reproduction in controlled conditions is particularly challenging because of its obligatory biotrophic lifestyle. The long maturation period needed for oospores to be able to germinate is also an important hindrance to the phenotypic characterization of strains obtained from these sexual spores.

Nevertheless, several studies have succeeded in inducing the sexual reproduction of *P. viticola* strains in laboratory. They aimed to study the sexual phase of the pathogen (Ronzon-Tran Manh Sung & Clerjeau, 1988) or the inheritance of traits of interest in the offspring such as fungicide tolerance (Harms *et al.* 2000, Gisi *et al.*, 2007).

Notably, Wong et al. (2001) demonstrated that *P. viticola* is a heterothallic species with two self-incompatible mating types, P1 and P2. This means that for fecundation to occur, one strain must meet a second one with the opposite mating type. Recently, our team produced a high quality reference genome (Dussert *et al.*, 2019) that later allowed to unravel the genetic bases of the mating system using a genome-wide association approach. Dussert et al. (2020) identified the *P. viticola* mating-type (MAT) locus in a 570-kb non-recombining, repeat-rich region of the genome encompassing 40 genes. The region is heterozygous (MAT-a/MAT-b) in the P1 mating type and homozygous (MAT-a/MAT-a) in the P2 mating type, indicating dominance of the MAT-b allele.

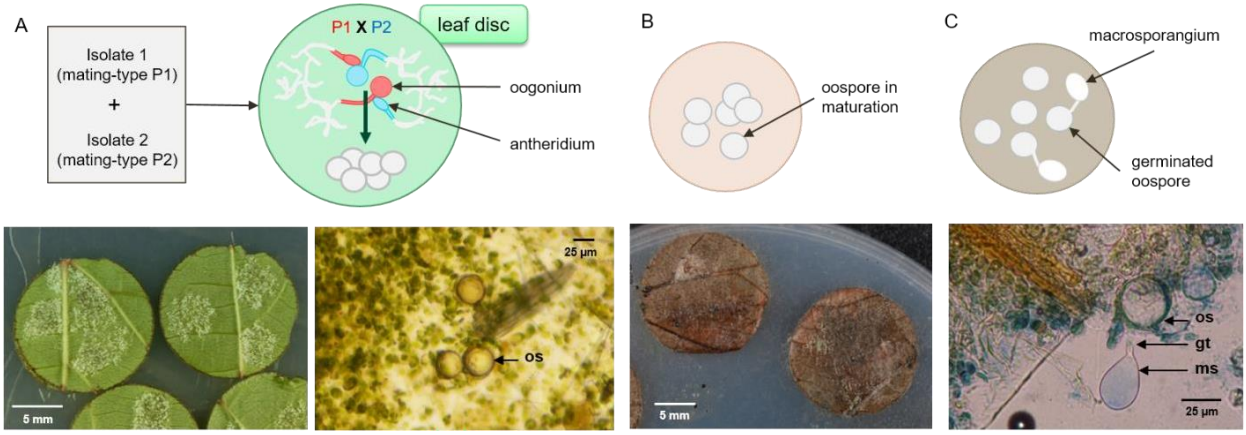

Figure 1: Crossing of *P. viticola* isolates and production of viable oospores. **A.** Fecundation and oospore formation. Sporulating leaf discs 8 days after co-inoculation and oospores observed inside a leaf disc 3 weeks after co-inoculation (os: oospore) **B.** Maturing oospores. Leaf discs 3 months after co-inoculation. **C.** Germinated oospores with macrosporangia ready to be harvested and individually inoculated on fresh leaf discs. (gt: germ tube, ms: macrosporangia). Pictures: INRAE, I. Mazet.

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2 Handling the sexual cycle of the grapevine downy mildewpathogen in controlled conditions

The ability to identify compatible genotypes and cross them to obtain oospores opens the possibility to reliably study the inheritance and the determinism of phenotypic traits of strains of interest.

We took advantage of this to perform a cross between two compatible *P. viticola* strains. We co-inoculated grapevine leaf discs with the two selected parents and we observed oospores after three weeks at T = 10 °C (Fig. 1A). Then, these were put under maturation conditions (T = 4 °C) for an extended period of up to 8 months (Fig. 1B), before placing them in germinating conditions (one week at T = 22 °C) for macrosporangia to form (Fig. 1C). We recovered germinated oospores individually, and 44% successfully initiated infection on leaf discs. In total, we generated and isolated more than 200 viable full-sib offspring.

Here, we use this approach to lean into *P. viticola* genomics and uncover the bases of the overcoming of grapevine resistance factors.

3 Application to the study of grapevine resistance breakdown by *P. viticola*

The creation and deployment of grapevine cultivars genetically resistant to downy mildew is a major management option to control the disease. Breeding efforts are based on the introgression of *Resistance to Plasmopara Viticola* (*Rpv*) loci from wild *Vitis* species into cultivated grapevine (*Vitis vinifera*) (Merdinoglu *et al.*, 2018). Some of them show high efficiency, but these genetic factors typically do not provide complete resistance to the pathogen. Consequently, although host susceptibility is determined by major genes, the interaction is phenotypically quantitative. Unfortunately, a number of *P. viticola* isolates able to overcome the resistance conferred by different *Rpv* genes have been already reported in the past decade (Peressotti *et al*., 2010, Delmotte *et al.*, 2014, Wingerter *et al.*, 2021, Paineau *et al.*, 2022).

The risk of rapid breakdown of the resistance of new cultivars makes it urgent to assess *P. viticola*'s capacity of adaptation and its origins. While *Rpv* loci in grapevine are increasingly well characterized (Merdinoglu *et al.*, 2018), the genetic determinism of pathogen virulence is still unknown.

Thus, we aimed to uncover the genomic bases of *P. viticola* adaptation to grapevine resistance by taking advantage of (i) the identification of virulent strains isolated from resistant cultivars ; (ii) the genomic data now available for this species ; (iii) the possibility to cross compatible strains and retrieve a progeny large enough to carry out a Quantitative Trait Loci (QTL) mapping study. This approach is particularly promising because of the phenotypically quantitative interaction observed in this pathosystem.

We selected two parental strains of interest, respectively INRAE-Pv1419 (P1) and INRAE-PV412 (P2). They both overcome *Rpv3*-mediated resistance, and INRAE-Pv1419 also overcome *Rpv10*-mediated resistance (Paineau *et al.*, 2022). They were crossed and their progeny was recovered as described in Figure 1. In addition, we subsequently performed monospore isolation.

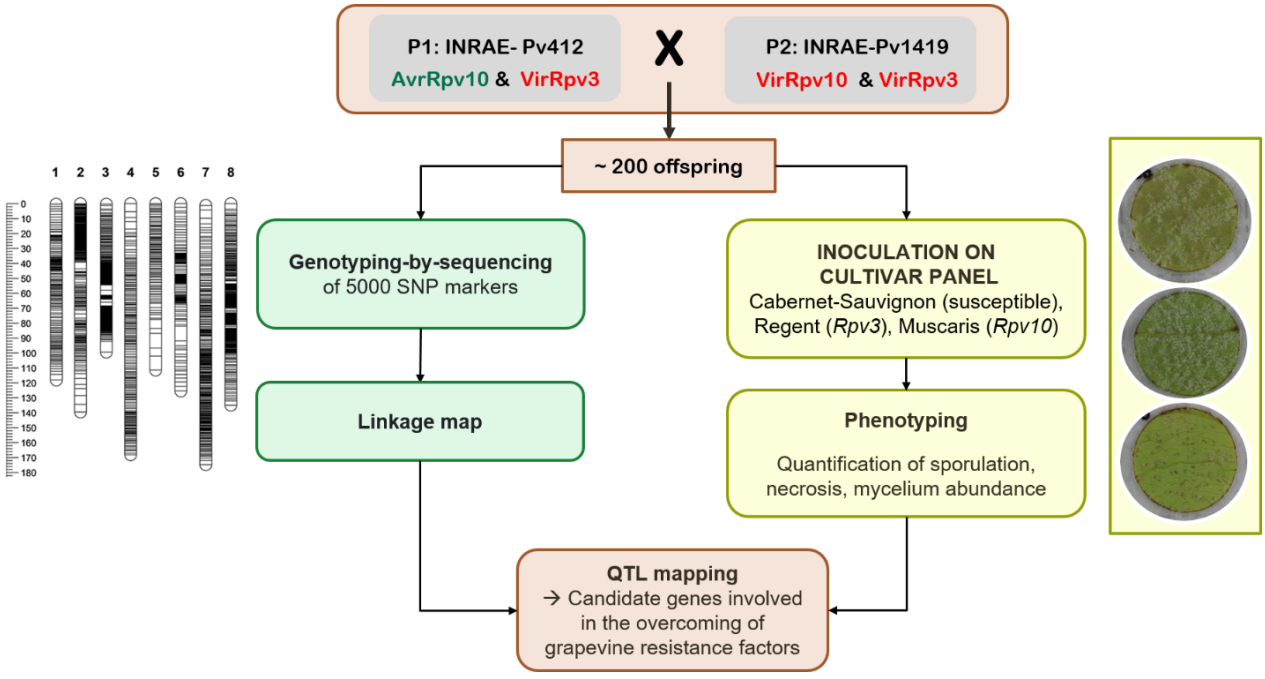

Figure 2: QTL mapping strategy to identify loci in the pathogen genome that are involved in resistance breakdown. On the right, response of three grapevine cultivars to *P. viticola* inoculation (from top to bottom: cv. Cabernet-Sauvignon, cv. Regent, cv. Fleurtai). Necrosis spots and low sporulation indicate efficient immune response.

Each offspring (n = 203) was cross-inoculated on leaf discs from a panel of grapevine cultivars carrying *Rpv3*, *Rpv10* or none (Fig. 2). The phenotyping of pathogenicity-related traits (sporulation, necrosis, mycelium abundance) in the progeny informs on the mode of inheritance of the resistance breakdowns. The segregation of the virulence trait on *Rpv10* plants is of special interest for us, given that it is one of the two *Rpv* genes present in the second generation of INRAE ResDur varieties (Schneider *et al.*, 2019).

Based on the genome long read sequencing of the parental strains, we designed a set of 5000 single nucleotide polymorphisms (SNPs) as genetic markers that will be used to genotype the offspring using an innovative approach of targeted genotyping-by-sequencing (GBS) (Scaglione *et al.*, 2019). This will allow to perform a linkage analysis and build the first genetic map of *P. viticola* (Fig. 2).

The combination of phenotyping and genotyping information will allow us to statistically associate genetic markers to the ability to overcome resistance. This QTL mapping approach will provide a set of underlying genes whose functional role in virulence could be investigated. In particular, we will be able to assess whether resistance breakdown is coming from the mutation of a single major locus or rather from the accumulation of multiple adaptations. Moreover, the identification of genetic markers linked to resistance breakdown will open the possibility to monitor *P. viticola* strains in vineyards without the need to regularly conduct large phenotyping experiment.

4 Conclusions

On the long run, with the efforts of the French National Observatory for the Deployment of Resistant Grapevine Varieties (OSCAR) (Guimier *et al.*, 2019), we will be able to monitor the evolution of the virulence of *P. viticola* populations as resistant cultivars are deployed in the vineyards.

Overall, this project will contribute to an efficient management of the durability of resistance to downy mildew in cultivated grapevine.

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