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Genetic analysis of the resistance to foliar diseases in *Muscadinia rotundifolia*

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Introduction

Viticulture is an important component of French agriculture, both in economical and cultural terms. Nevertheless, a wide range of pathogens threatens French viticulture. The current strategy to control most grapevine diseases totally relies on the use of fungicides. This practice is not only expensive but also dangerous for the environment and human health. A cost-effective and environmentally friendly alternative to chemicals is the use of resistant varieties. However, all cultivated European grapevine varieties are susceptible to most of the pathogens, thus the resistance needs to be introduced from other *Vitaceae* through breeding programs. Grapevine downy mildew caused by the Oomycete *Plasmopara viticola* and grapevine powdery mildew caused by the ascomycete *Erysiphe necator* are two of the major diseases affecting *Vitis* spp, causing important losses in yield. All the cultivated European grapevine varieties are susceptible to *P. viticola* and *E. necator*, but various sources of resistance are described in the American and Asian *Vitis* species, and also in *Muscadinia rotundifolia* which is closely related to the true *Vitis*. Our main goal is thus to create new grapevine varieties with durable resistance to cryptogamic diseases such as downy and powdery mildew, pyramiding genes derived from various sources of resistance.

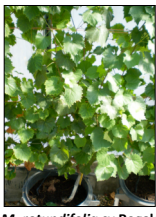


Plasmopara viticola (downy mildew) on the abaxial side of a leaf

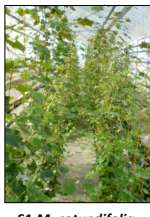


Erysiphe necator (powdery mildew) on the adaxial side of a leaf

Muscadinia rotundifolia is a source of disease resistance



M. rotundifolia cv Regale



S1 *M. rotundifolia*

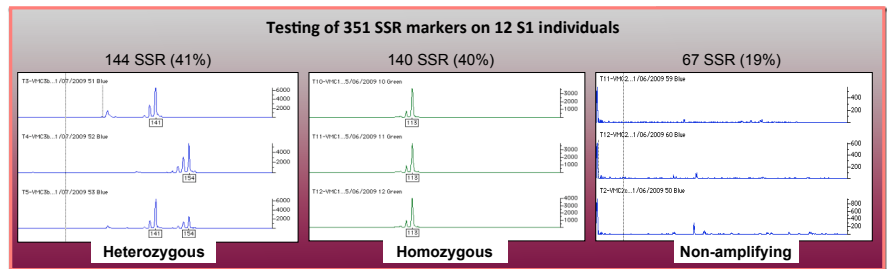
M. rotundifolia (2n=40) is a species closely related to the true *Vitis* (2n=38). It is native to the southeastern United States, and has been cultivated for over 400 years. In these regions, Muscadine cultivars are suitable for juice, jelly, wine production, or fresh market.

M. rotundifolia shows a high level of resistance to a wide spectrum of grapevine diseases. Two resistance QTLs (Quantitative Trait Loci) against downy mildew derived from *M. rotundifolia*, named *Rpv1* and *Rpv2* (1), and a resistance gene to powdery mildew originating from *M. rotundifolia*, named *Run1* (2), have been identified from crosses between *M. rotundifolia* and *Vitis vinifera*. In order to analyse the resistance factors in a pure muscadine background, we created a 200 individual S1 population of *M. rotundifolia* cv Regale.

Genetic mapping of *M. rotundifolia*

To accelerate the functional characterization of resistance to pathogens, we are developing a genetic map from *M. rotundifolia*, using SSR markers the S1 population. This map will also allow us to analyse the level of synteny between *V. vinifera* and *M. rotundifolia*.

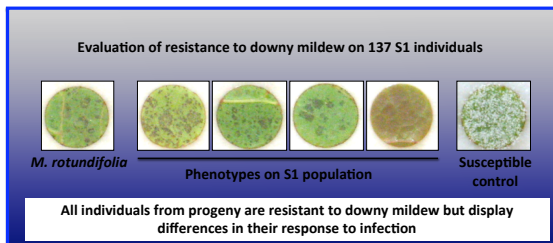
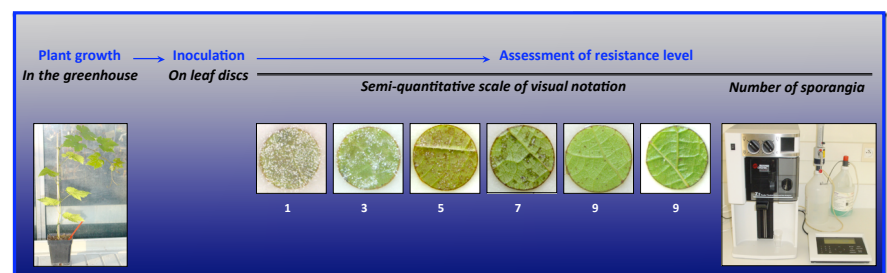
Until now, 85 out of the 144 SSR markers segregating in a sample of the S1 population have been tested on the whole mapping population and 77 have been mapped on 19 chromosomes. Preliminary results indicate a well preserved macrosynteny between *M. rotundifolia* map and the genetic map of *V. vinifera*.



Assessment of resistance to downy mildew in *M. rotundifolia*

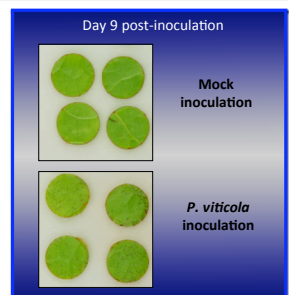
In order to study resistance to downy mildew in standardized conditions, we used a bio-assay previously developed in our lab. Young plants are produced from green cuttings in the greenhouse. Leaf discs are sampled from these plants and then are artificially inoculated by spraying in a Petri dish with a quantified suspension of sporangia. Seven days after inoculation, leaf discs are rated according to a scale of notation based on the OIV 452 descriptor, ranging from 1, which means very susceptible, to 9, which means highly resistant. Resistance is also assessed with other parameters like the presence of necrosis and the number of sporangia produced per unit of leaf area.

All individuals of the *M. rotundifolia* S1 population showed resistance to *P. viticola* but we could observe different levels of necrosis.



To assess if observed necrosis are caused by the infection with *P. viticola* or some other physical issue, we inoculated 19 genotypes in parallel with *P. viticola*, and with sterile water. The inoculation was this time realised by putting the discs in flotation on a quantified suspension of sporangia. Moreover, in order to correlate necrosis to the development of the pathogen, we took samples at different times post inoculation for further histological observations.

Preliminary observations at macroscopic level indicate that necrosis is correlated to pathogen infection, since no necrosis was observed on water-inoculated discs. Nevertheless, quantitative necrosis response of the leaves regarding the infection level of the pathogen remains to be clarified.



Conclusion and future prospects

The genetic map of *M. rotundifolia* will be completed with up to 150 SSR markers, and a final map of *M. rotundifolia* will be a powerful tool for future functional analysis of its resistance to pathogens such as *E. necator* (powdery mildew agent). It will also give a glimpse into the genome organization of *M. rotundifolia*, by comparison with *V. vinifera*. The *M. rotundifolia* S1 population doesn't seem to segregate for resistance to downy mildew, but further histological observations will be made to clarify the significance of necrosis regarding the development of *P. viticola* in muscadine leaves. In a near future, the *M. rotundifolia* S1 population will be screened for its resistance to *E. necator*.

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