# Research in Plain English (RIPE)

## A Novel Grape Downy Mildew Resistance Locus from *Vitis rupestris*

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Summary by Michelle Podolec

#### The Takeaway

- North American grape species are genetically diverse and have adapted to a wide variety of environments and pathogen pressures.
- Grape breeders have introduced some popular disease resistant hybrids and varieties into commercial viticulture, but the biological diversity of the native species remains largely underutilized .
- Researchers crossed V. *rupestris* with V. *riparia* to identify economically relevant genomic loci using a linkage map to identify quantitative trait loci (QTL).
- Researchers identified a major QTL at 12.46 cM on chromosome 10 for the female parent V. *rupestris* in the integrated map for naturally infected plants, and a secondary QTL, called Rpv28.2 (contributing 24.3% phenotypic variance) on leaf discs inoculated with the pathogen.
- Identification of these QTLs will help grape breeders to select stronger parent candidates for downy mildew (DM) resistant hybrid crosses.

#### Background

Cultivation of Vitis *vinifera* L. grapes relies on the use of numerous fungicide applications that are potentially harmful to humans and the environment. Disease resistant grape varieties, could dramatically reduce the amount of fungicides used by growers. Native North American wild grape species that co-evolved with North American pathogens have been a traditional source of grape resistance genes. Their genetic diversity strengthens their resistance to fungal and oomycete (downy mildew is an 'oocyte' pathogen) diseases. Early interspecific grapevine crosses in the late 19<sup>th</sup> and early 20<sup>th</sup> century between V. *vinifera* and North American grape species focused on breeding phylloxera- and lime-tolerant scions (often called 'hybrid direct producers' in Europe) and rootstocks. Several interspecific American hybrid wine varieties and rootstocks used worldwide today are a result of these early resistance breeding efforts.

Current breeding work utilizes some of the disease resistance diversity available in wild Vitis *spp.*, but there is more to be discovered. The high level of variation in wild grape vines in North America suggests more genetic diversity than is commercially utilized in hybrid cultivars today. A recent example is the introduction of Pierce's disease resistance from V. *arizonica* (Riaz et al. 2009) and powdery and downy mildew resistance from *Muscadina rotundifolia* into commercial varieties (Feechan et al., 2013, Agurto et al. 2017).

In this paper, researchers describe how they constructed a linkage map and quantitative trait locus (QTL) in an F1 cross between Vitis *rupestris* and Vitis *riparia*. These parent plants from two Vitis species are adapted to live in very different habitats. V. *rupetris* (female parent) is a sprawling shrub form found on sandy soils, gravel bars, intermittent streams and stony soils, that can tolerate nutrient poor soils. V. *riparia* (male parent) is a widespread, high-climbing liana found on moist alluvial soils with good drainage.

Researchers expected to find a wide diversity of important viticultural traits, and hoped this cross would '... allow mapping of economically relevant genomic loci.' It turns out that the researchers were correct in their suspicions. However, the first trait documented was not related to environmental tolerance, but to disease resistance.

#### Methods

Researchers crossed a female flowered V. *rupestris* and a male flowered V. *riparia*. The F1 progeny were grown out and planted after a few years of growth in the lab and nursery in Brookings, South Dakota; Columbia, Missouri; and Geneva, New York. A total of 357 resulting vines were genotyped using genotyping-by-sequencing to develop the genetic maps.

Seedlings were phenotyped for resistance to downy mildew (DM, caused by *Plasmopora viticola*) while still in the greenhouse by visual ratings the incidence and severity of DM on the leaves 5 d after inoculation. The researchers used a leaf disc disease assay to determine virulence on the F1 progeny, three additional grapevines (V. *riparia* Gloire de Montpelier, *M. rotundifolia* Thomas, and V. *vinifera* F2-35) and the 2 parents of the F1 population (V. *riparia* P1588271 and V. *rupestris* P1588160).



A through E: Degree of resistance in leaf disk assay based on OIV descriptors 452-1. Panels A, B, C, D and E show examples very low, low, medium, high and very high level of resistance, where 50% or more, approximatey 20%, 10%, less than 5% and none of the leaf disk area was covered by sporangiophores, respectively, 7 days post-inoculation. Scores assigned to the levels of resistance depicted in panels A, B, C, D and E were 1, 3, 5, 7 and 9, respectively.

**F through H:** Examples of leaves with various degree of resistance in naturally infected potted plants in greenhouse. Disease resistance was evaluated using a ten-point scale (0 though 9): F, very low-level of resistance (score 0), 50% or more of the leaf blade blighted by the pathogen; G, low-level resistance (score 3), extensive damage of areas of 2 to 5 cm in diameter; H, high-level resistance (score 8), suppressed growth of the pathogen with bligthed spots limited to < 1 cm diameter. Disease coverage was evaluated by the observing the area of abaxial side of the leaf covered by sporangiphores. Photos were taken of the adaxial side of leaf after the disease had run its course and the affected leaf areas were killed by the pathogen.

Finally, a quantitative trait locus (QTL) analysis was performed to detect any significant associations between the phenotypes, traits and genetic markers.

#### Results

The researchers filtered 348,888 genetic GBS markers down to maternal and paternal SNP markers and developed parental genetic maps.

Inoculation assays with an aggressive DM strain, *Plasmopara viticola* strain MO-1, showed that the wild grapevine parents of the F1 progeny had higher resistance to DM infection than that of V. *vinifera* cultivars.

QTL mapping showed that the trait of DM resistance mapped to a major QTL at 12.46 centimorgans (cM, a measure of DNA sequence) on chromosome 10 for the female parent V. *rupestris* for naturally infected vines. A nearby, but different locus, also on chromosome 10, also was identified in leaf disc assays. Together, these QTL explained 66.5% of the variance in disease resistant phenotypes and results in the identification of the new DM resistance locus called "Rpv28".

### **Conclusions and Practical Considerations**

In this study, only the female parent (V. *rupestris*) appears to have contributed resistance genes to this specific strain of downy mildew. By selecting two resistant grapevine parents with resistance at different loci, grape breeders could produce F1 progeny with additional opportunities for resistance. Developing a more effective marker-assisted selection would be helpful in developing additional markers related to the new QTL Rvp28. Surveying existing grapevine germplasm repositories for Rpv28 resistance would help to identify additional breeding material for this trait.

Further exploration of V. *rupestris* and V. *riparia* genetics would be helpful for breeders hoping to mitigate climate change impacts to Vitis crops by exploiting the existing genetic adaptations and species diversity. Wild V. *rupestris* is currently considered as "threatened status" due to habitat loss, highlighting the importance and urgency of conducting more explorations for the future of viticulture.

The researchers suggested that the field would also benefit by having access to a common genetic trait library where researchers and producers could consider the best candidate parents to achieve specific traits in their crosses.

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