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Construction of a reference linkage map of *Vitis amurensis* and genetic mapping of *Rpv8*, a locus conferring resistance to grapevine downy mildew

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Abstract

Downy mildew, caused by the oomycete *Plasmopara viticola*, is one of the major threats to grapevine. All traditional cultivars of grapevine (*Vitis vinifera*) are susceptible to downy mildew, the control of which requires regular application of fungicides. In contrast, many sources of resistance to *P. viticola* have been described in the *Vitis* wild species, among which is *V. amurensis* Rupr. (*Vitaceae*), a species originating from East Asia.

To measure the general level of resistance, the sporulation of *P. viticola* and the necrosis produced in response to infection, five quantitative and semi-quantitative parameters were scored 6 days post-inoculation (dpi) on a *V. amurensis* S1 progeny (Table 1, Fig. 1, Fig. 2).

A genetic linkage map of *V. amurensis*, based on 122 simple sequence repeat and 6 resistance gene analogue markers, was established using the same S1 progeny. This map covers 975 cM on 19 linkage groups, which represents 82% of the physical coverage of the *V. vinifera* reference genetic map (Fig. 3).

A quantitative trait locus (QTL) analysis allowed us to identify on linkage group 14 a major QTL controlling the resistance to downy mildew found in *V. amurensis*, which explained up to 86.3% of the total phenotypic variance. This QTL was named 'Resistance to *Plasmopara viticola* 8' (*Rpv8*).

Analysis of downy mildew resistance traits

| Variable name | Description | Scoring |
|---------------|---|---|
| OIV452 | Symptom-based semi-quantitative scoring of downy mildew resistance adapted from the criteria of the Office International de la Vigne et du Vin (OIV ; Anonymous 2009; http://news.researc-concept.net/images/oiv/client/Code_descripteurs_2ed_FR.pdf) | From 1 (very susceptible) to 9 (totally resistant) (Figure 1): 1 = abundant sporulation densely covering the whole disc area, absence of plant necrosis 3 = abundant sporulation present in large patches, absence of plant necrosis 5 = limited sporulation present in intercostal patches, necrotic flecks or speckles 7 = sparse sporulation, necrotic spots 9 = no sporulation, absence of necrosis or necrotic points |
| SDSC | Percentage of sporulating leaf discs | From 0 to 100% |
| SPNB | Number of sporangia per cm ² of leaf disc measured with a Z2 Coulter Counter (Beckman Coulter). | Quantitative. Number of sporangia per ml of suspension converted to number of sporangia per cm ² of leaf disc |
| NSURF | Visual semi-quantitative scoring of necrotized surface | From 1 (very large) to 9 (very small) (Figure 1) |
| NDSC | Percentage of necrotized discs | From 0 to 100% |

Table 1. Variables scored to assess the resistance level to downy mildew.

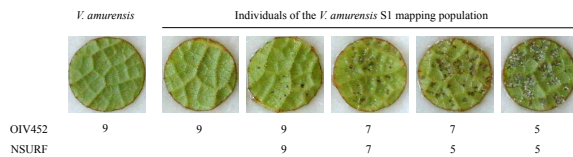


Fig. 1 Range of the segregating phenotypes more commonly observed 6 dpi on 1 cm leaf discs in the *V. amurensis* S1 mapping population. The traits describing the necrotic symptoms produced in response to infection are linked to the resistance level parameters: the higher the resistance, the weaker the necrotic response to infection.

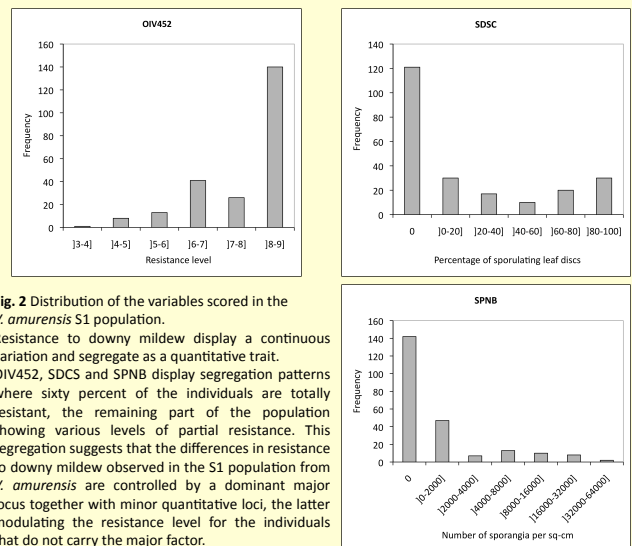


Fig. 2 Distribution of the variables scored in the *V. amurensis* S1 population. Resistance to downy mildew display a continuous variation and segregate as a quantitative trait. OIV452, SDSC and SPNB display segregation patterns where sixty percent of the individuals are totally resistant, the remaining part of the population showing various levels of partial resistance. This segregation suggests that the differences in resistance to downy mildew observed in the S1 population from *V. amurensis* are controlled by a dominant major locus together with minor quantitative loci, the latter modulating the resistance level for the individuals that do not carry the major factor.

First genetic map of an asian *Vitis* species : *V. amurensis*

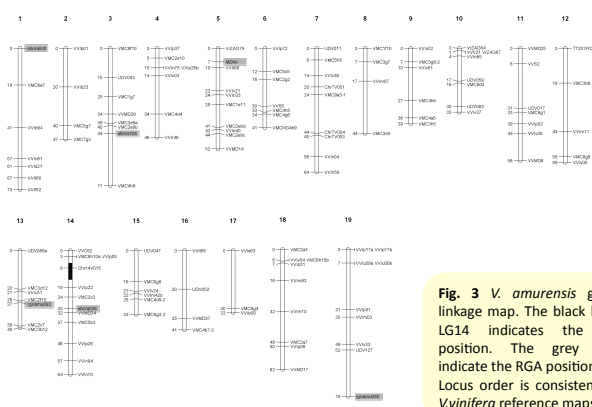


Fig. 3 *V. amurensis* genetic linkage map. The black bar on LG14 indicates the *Rpv8* position. The grey boxes indicate the RGA position. Locus order is consistent with *V. vinifera* reference maps.

QTL detection

A major QTL controlling resistance to downy mildew was detected on LG14 for OIV452, DSCS and SPNB. Analysis based on DSCS gave the highest LOD score (65.45) and explained 86.3% of the total phenotypic variance and thus 100% of the genetic variance, considering the broad sense heritability estimated at 86.2%.

The locus at this position was denoted 'Resistance to *Plasmopara viticola* 8' (*Rpv8*).

The genome interval between the markers VVlp05 and VVlp22 that flanks the *Rpv8* confidence interval covered a physical distance of 15.11 Mb, which encompasses 502 genes, according to the 12X grape genome sequence (<http://www.genoscope.cns.fr/externe/Genome-Browser/Vitis/>).

A minor QTL was detected for OIV452 and located on LG15.

A second minor factor was detected for NSUR on LG17.

Conclusions

This paper reports the first grapevine genetic linkage map based on an intra-specific progeny of a *Vitis* species other than *V. vinifera*.

V. amurensis map and the previously published *V. vinifera* maps share a high level of macro-synteny.

Furthermore, our work allowed us to identify on LG14 a new major QTL controlling resistance to downy mildew in *V. amurensis*, named *Rpv8*. This locus is located on a genomic region never previously described to control downy mildew resistance in grapevine and related *Vitis* species.