

**Results obtained in intergeneric hybridation**  
**(*Vitis x Muscadinia*)**  
**for breeding disease resistant varieties**  
**and their complementation through genetic engineering**

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In viticulture, the respect of environment and the reduction of inputs have recently become priority aims. Before this sudden awareness, INRA developed since 1974 breeding programmes for disease resistant varieties, that have concerned firstly winegrapes, with powdery and downy mildews as main targets, then rootstocks with infectious degeneration (the so-called “ court-noué ” disease) as target, and more recently tablegrapes. All these programmes are based on the use of genetic resistances issued from the american species *Vitis (Muscadinia) rotundifolia*.

Classically, *Muscadinia* is considered as a section of the genus *Vitis*, differing from the other species (section *Euvitis*) in many anatomical, morphological and caryological characteristics. The chromosomal number is  $2n=40$  instead of  $2n=38$ . Due to the genetic distance, the crosses between *Euvitis* and *Muscadinia* are very difficult and incomplete chromosomal pairing at meiosis results in high sterility of  $F_1$  and  $BC_1$  hybrids that hinders genetic introgression. Nowadays, *Muscadinia* is generally considered as a genus distinct from *Vitis*.

With regard to the resistance to downy and powdery mildews, this breeding strategy, innovative in Europa, but experienced without success in United States since more than 130 years, standed apart from the strategies developed in the same time in Germany and other viticultural countries from central and eastern Europa, and based

on the improvement of the French-American hybrids selected after the great plague of Phylloxera and (or) the use of the asian species *Vitis amurensis*.

The programme was initiated in 1974 at the INRA viticultural station of Bordeaux, then transferred to the viticultural stations of Montpellier in 1984 and Colmar in 1998. After 30 years, we can draw up a balance sheet, that appears clearly positive. A complete resistance to powdery mildew on leaves, stems and bunches, was introduced in *Vitis vinifera* genotypes by six successive back-crosses à partir d'un hybride F<sub>1</sub> *V. vinifera* x *M. rotundifolia*. Resistance was conferred by a single dominant gene named *Run1* (for Resistant to *Uncinula necator*). Resistant plants have been field-evaluated for their cultural aptitudes and the quality of their wines. In 2004, ten selected genotypes, corresponding to 4th and 5th back-crosses to *Vitis vinifera*, will be planted in large field trials with a view to the registration of the best of them on French variety list, within 8 to 10 years. But even if authorized in France, the culture of these varieties will probably not be possible in the AOC vineyards. Faced with this situation, it was decided to introduce directly the *Run1* gene in traditional varieties by genetic engineering, respecting as far as possible their ampelographic characteristics but above all the typicity of their wines. With this aim in view, the map cloning of the gene was initiated in 1998 in the framework of a collaborative project between INRA and CSIRO, with a preliminary fine genetic mapping of the chromosomal fragment bearing the *Run1* gene using AFLP, SSR or RGA markers. Physical mapping is in course, and a contig was built across the *Run1* gene, using a BAC library made from genomic DNA of a BC<sub>5</sub> resistant genotype. Chromosome walking and BAC sequencing are programmed for 2004. First attempts of functional testing to validate genetic sequences corresponding potentially to the *Run1* gene could start in 2005.

But the genotypes bearing the *Run1* gene were found exhibiting also a high level of resistance to downy mildew. Precise phenotypic characterization and use of molecular markers have shown that the resistance was due to several genes. One of them, named *Rpv1* (for Resistant to *Plasmopara viticola*) explains more than 80% of the genetic variability of the character and is tightly linked to the *Run1* gene. According the results of the map cloning of the *Run1* gene, the same strategy will be

used in the next future for the direct transfer of *Rpv1* gene in traditional varieties of *Vitis vinifera*.

One of the main uncertainties connected to the development of new varieties obtained from *Vitis* x *Muscadinia* hybridization concerns the durability of monogenic resistances. The strategy now developed consists in associating the genes *Run1* and *Rpv1* with complementary disease resistance factors. In the case of conventional breeding, these could be issued from the German resistant varieties (mainly the cultivar Regent), since they have been identified as QTLs and located by molecular markers on a genetic map, recently published. With this aim, a collaborative project between INRA and the Institute for Grapevine Breeding Geweilerhof started in the year 2000.

In the case of direct transfer of the *Run1* and *Rpv1* genes by genetic engineering, it could be possible to associate genes encoding PR (Pathogenesis-Related) proteins like enzymes (glucanases, chitinases...) able to destroy some of the mycelium compounds, or genes (*npr1*, *mlo*) inducing non specific defense reactions of the plant against biotic stresses.

With regard to the selection of rootstock varieties resistant to the so-called “court-noué” disease, the strategy developed since 1981 is also based on the use of *Muscadinia rotundifolia*, whose roots exhibit complete resistances not only to Phylloxera but above all to the nematode *Xiphinema index*, vector of the grape fanleaf virus (GFLV) that is the main component of the viral complex causing the disease. Infectious nematodes are unable to transmit the disease to healthy plants, though *Muscadinia rotundifolia* is highly susceptible to the virus. However, the species is not usable directly as rootstock because of redhibitory defects, especially graft-incompatibility with *V. vinifera*. The resistance to the nematode has been introduced in F<sub>1</sub> hybrids *V. vinifera* x *M. rotundifolia*, that got some of the cultural defects of *M. rotundifolia*. Despite their high sterility, few resistant F<sub>1</sub> hybrids have been back-crossed with rootstock varieties, and after a strong selection, a genotype highly resistant to the nematode and virus transmission has been planted in large field trial in

1999. According the results of the experimentation, registration of this new rootstock on the French variety list could be asked as early as 1997.

In a complementary way, a biotechnological approach was developed since 1990 by the INRA plant pathology station of Colmar, the Institute of Plant Molecular Biology (CNRS) of Strasbourg and the laboratories of Moët & Chandon. It was based on the concept of “pathogen-derived resistance” with the aim of introducing in the genome of commercial rootstock varieties the gene encoding the capsidial protein of GFLV. The field experimentation of the first transgenic plants, planted in 1997, has been suddenly stopped in 1999 without decisive results on the efficiency of this strategy. New trials could start again in 2004 in the INRA research center of Colmar.

Actually, the strategy developed by INRA for rootstock breeding aims to :

(\*) firstly, continue the selection in genotypes issued by a second backcross generation from the resistant genotype actually in field trial; as far as possible, resistances to Phylloxera and *Xiphinema index* will be associated with high lime chlorosis tolerance and low conferred vigor.

(\*\*) secondly, associate, either by direct genetic transformation or by hybridation with transgenic plants, the nematode resistance issued from *M. rotundifolia* with a pathogen-derived virus resistance, for increasing the efficiency and the durability of the disease resistance, but also for biosecurity reasons.