

ISOLATION OF SSR MARKERS TIGHTLY ASSOCIATED TO THE VM APPLE SCAB RESISTANCE GENE

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The hypersensitive reaction is a resistance mechanism in which after cellular contact between pathogen and the host, a plant reaction can be observed, usually leading to the fast death of the pathogen's underlying cells. Hypersensitivity-based resistance is well studied in many model systems: basically, the defence cascade, which often ends macroscopically with hypersensitivity and biochemically with the synthesis of phytoalexins, is activated through host recognition of the pathogen. In *Malus* this is the case of *Vm* resistance. Historically, Dayton and Williams (1970) used *Vm* to denote the resistance gene conditioning the pit-type HR reaction carried by *M. micromalus* 24538 and *M. atrosanguinea* 804. The interest around this gene derives not only from its evident, typical and fast hypersensitive response, but also from the fact that this is a monogenic resistance, relatively easy to be introgressed in commercial apples.

Two molecular markers—OPB12SCAR (Cheng *et al.*, 1998) and SSR Hi07h02—tightly linked to *Vm* gene have already been reported (Patocchi *et al.*, 2005). The SSR marker allowed the mapping of *Vm* on linkage group 17 in a region where also scab resistance QTL have been reported (Durel *et al.*, 2004).

Considering all these knowledge, a program to create the conditions for a rapid isolation of the apple scab resistance gene *Vm* was started.

Large *Vm*-segregating populations have been created, phenotyped in greenhouse after pathogen inoculation and genotyped using molecular markers associated with the *Vm* resistance gene. A Florina BAC library from Vinatzer *et al.* (1998) has been pooled to perform an 'heterologous' chromosome walking aimed at identifying new markers tightly linked to the *Vm* gene. The BAC library screening with the Hi07h02 marker resulted in the identification of three BAC clones carrying the marker alleles of Florina. The BAC-ends of these clones were sequenced and both used to develop new SSRs tightly linked to the *Vm* gene and to perform a second chromosome walking step. Two new microsatellites showing a high level of polymorphism in different populations (Fiesta x Discovery, Golden Delicious x Murray, Galaxy x Murray) were developed and mapped in the distal part of the LG 17. One of these new SSR markers (*Vm*1SSR) showed no recombinants among 1260 tested seedlings and the other (*Vm*2SSR) showed only 6 recombinants. Genetic distances from *Vm* were 0 and 0.47 cM, respectively therefore the resistance gene has been located between *Vm*2SSR and the SSR Hi07h02 (this marker showed 7 recombinants).

A new BAC library from cv. Murray DNA (carrying the *Vm* resistance gene) was constructed and pooled in order to identify clones containing the genomic region containing the *Vm* gene. Two BAC clones amplifying the resistance allele in coupling with the *Vm* gene were isolated and the sequencing is in progress to identify candidate resistance genes.