# Identification and Mapping of a New Apple Scab Resistance Gene

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**Abstract**

**Here we report the identification of a new resistance gene (*Vd3*) against apple scab (*Venturia inaequalis*) from the apple selection 1980-015-25 of the breeding program at Plant Research International. This accession also contains the *Vf* gene. We mapped *Vd3*, using SSR and DArT markers, on linkage group 1, at a distance of 6 cM from *Vf* gene, but in repulsion phase to *Vf*. Based on pedigree analysis and resistance tests, it could be deduced that 1980-015-25 had inherited *Vd3* from the founder D3. This gene provides resistance to the highly virulent EU-NL-24 strain of the race 7 of *V. inaequalis*. This strain has overcome the resistance from both *Vf* and *Vg*. However, *Vd3* has been not effective against the majority of other *V. inaequalis* strains we used in our disease tests.**

## INTRODUCTION

Apple scab, caused by the fungal pathogen *Venturia inaequalis* (Cooke) G. Wint., is one of the most devastating diseases for the apple (*Malus* × *domestica* Borkh) growing in temperate zones with humid springs and summers. Most of the commercial apple cultivars are susceptible to the disease, and therefore growers have to spray 20-30 times with fungicides in a season. The use of resistant cultivars could reduce the cost to the growers and also contribute to a safer environment and produce healthier apples for consumers.

But the introgression of traits from wild germplasm into pip fruit cultivars by means of classical breeding is painstakingly slow. Introgression of the apple scab resistance gene *Vf* from *Malus floribunda* 821 into the marketable top quality apple cultivar Santana took more than 50 years. Studies indicated that some *V. inaequalis* strains have been detected that are able to overcome *Vf* resistance (Parisi et al., 1993). These strains are especially present in North-western Europe and consequently spread around. Recent studies showed that for durable resistance in apples, several resistance genes should be accumulated (pyramiding). Fortunately, many loci which include both major genes and QTLs that confer resistance to apple scab have been discovered in *Malus* (Calenge et al., 2004; Gessler et al., 2006). 11 major apple scab resistance genes have been mapped (Gessler et al., 2006). Molecular markers linked to these genes are available and in this context, we show here the results within marker assisted selection (MAS) that aim to identify and to select the parents for conducting a breeding program. In this work we report the identification and mapping of a new apple scab resistance gene (*Vd3*) against apple scab that provides resistance to the highly virulent EU-NL-24 strain of the race 7 of *V. inaequalis* capable of overcoming the resistance from *Vf* and *Vg*.

## MATERIAL AND METHODS

### Plant Material and DNA Extraction

The apple population used in this study for the evaluation of the scab resistance and the mapping of *Vd3*, is named population 2000-012C. It is derived from the cross between the scab resistant selection 1980-015-025 and the susceptible selection 1973-001-041.

**Evaluation of Scab Resistance**

Scab resistance was evaluated with mist evaporation under tunnel, where 10 replicates of the progeny seedlings were randomly sprayed with a monoconidial suspension of the race EU-NL-24 of *V. inaequalis* ($10^5$ conidia/ml). This pathogen is capable of overcoming the *Vf* gene (Parisi et al., 2004). Plants were incubated for 48h at 20°C and 100% relative humidity, and then transferred to a greenhouse with a relative humidity of 85-90%. Scab inoculation was performed in the four youngest leaves. Disease symptoms were assessed macroscopically after 14-17 days and rated in eight classes indicating the amount of sporulation and using as threshold for resistance the class 0 (no sporulation). The resistance test shows that this R-gene induces a hypersensitivity pit-type and chlorotic reaction (data not shown).

**DarT Markers**

DArT markers were carried out in Diversity Arrays Technology LTD (Yarralumla, Australia) as described in Wittenberg et al. (2005).

**SSR Markers**

All of the SSRs in linkage group (LG) 1 available at the HiDRAS (High-quality Disease Resistant Apples for a Sustainable Agriculture) database (http://www.hidras.unimi.it/) were screened in the population 2000-012C. SSR amplifications were performed in a final volume of 20 µl, containing 75 mM Tris-HCl, pH 8.8; 20 mM $(NH_4)_2SO_4$; 1.5 mM $MgCl_2$; 0.2 mM of each dNTP; 0.5 µM of fluorescent dye-labelled forward primer; 0.5 µM of reverse primer; 20 ng of genomic DNA; and 1 U of SuperTaq DNA polymerase using the following temperature profile: 94°C for 2 min 30 s, then 34 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min, finishing with 72°C for 5 min. Samples were analyzed on an ABI PRISM 3700 DNA Analyzer and scored with GENOTYPER version 3.6. The SSR CH-*Vf1* were developed by (Vinatzer et al., 2004) from a BAC clone containing the *HcrVf1* gene.

**Testing of Specific Markers Linked to Other Scab R-Genes in LG1**

The *Va*-linked RAPD marker P-136 reported by Hemmat et al. (2003) and the *Vf2ARD* marker developed by Boudichevskaia et al. (2008) were also analyzed in the population 2000-012C to test its association with *Vd3*.

**Linkage Analysis**

The linkage analysis was carried out using JoinMap 3.0 software (Van Ooijen and Voorrips, 2001). Linkage groups were established using as threshold a minimum logarithm of odds (LOD) of 3.0 and a recombination frequency lower than 0.4. The *Vd3* gene was mapped as a dominant gene based on the phenotypic data.

**RESULTS AND DISCUSSION**

**Evaluation of Scab Resistance**

Inoculation of 92 $F_1$ plants from the segregating progeny 2001-012 with the *Vf*-virulent strain EU-NL-24 yielded two groups: 51 plants appeared to be susceptible (sporulating leaf area from 1 to 100%) and 41 plants were classified as resistant (no sporulating area). This segregation fitted the ratio of 1:1 based on the Chi-square test ($X^2$ = 1.08) indicating a monogenic inheritance.

**Linkage Map**

A new apple scab resistanc gene, called *Vd3*, could be mapped on LG1, closely to *Vf*, but on the homologous chromosome, so in repulsion to the CH-*Vf* marker. We have named this gene *Vd3*. For further mapping of *Vd3* SSRs in LG1, seven out of nine SSRs screened were polymorphic in the mapping population and 6 of them were incorporated to the map. In the case of the DArT markers, from a total of 105 polymorphic markers 5

were mapped on LG1. The marker *Vf2ARD* developed by Boudichevskaia et al. (2008) based on the sequence of *HcrVf2* in the apple accessions 'Antonovka', 'Realka' and 'Discovery' was mapped in a similar location to that one reported by these authors but at 4.4 cM from *Vf*. *Vd3* was mapped on LG1 as a dominant gene at a distance of 6.1 cM of the *Vf* gene (Fig. 1). This position does not correspond with previously mapped resistance genes against *V. inaequalis* in LG1 (Gessler et al., 2006; Boudichevskaia et al., 2008). The genetic position of *Vd3* permitted us to discard this gene as *Va* because of the position reported to it. There are two different locations for the *Va* gene based on the map reported by Gessler et al. (2006). In both locations the *Va* gene is outside the interval between the SSRs Hi12c02 and CH05g08 and in both cases at a distance of about 25 cM from *Vf*. This distance is in agreement to that one reported previously by Hemmat et al. (2003) using as the resistance donor Antonovka PI 172633. Conversely, *Vd3* is close to *Vf* (6.1 cM) and is in the region flanked by those SSRs. Moreover, *Vd3* lead to a chlorotic symptoms, in contrast to the *Va* gene (Dayton and Williams, 1968). In the case of *Vf2ARD*, both genes are different. The first one is coming from the different genetic distance (Fig. 1) and the second one is the presence of the *Vf2ARD* marker in the susceptible parent of our population (data not shown).

## CONCLUSIONS

We have shown in these studies that a new apple scab resistance gene providing resistance to the highly virulent EU-NL-24 strain of the race 7 of *V. inaequalis* has been identified. *Vd3* is a novel resistance gene, about 6 cM below *Vf*. However, *Vd3* has been not effective against the majority of other *V. inaequalis* strains, as we showed in our disease tests (104, EU-B04, 302, EU-D42, 1066). In targeting a durable resistance, we want to demonstrate in these future years that several resistance genes would be included in the same cultivar. To expand these studies, the development and use of molecular markers linked to these genes will allow to select any cultivars of interest that can be used as progenitors in any breeding programs.

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## Figures

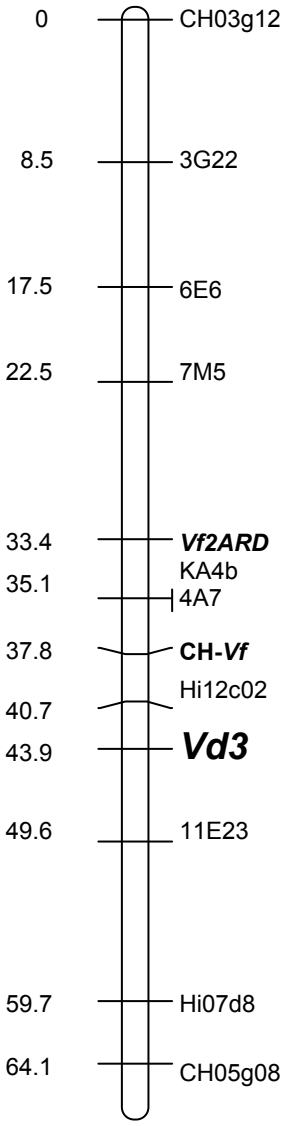


Fig. 1. Linkage Group 1 of population 2000-012C carrying *Vf* and *Vd3*.