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The V_f gene for scab resistance in apple is linked to sub-lethal genes

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Summary

V_f is the most widely used resistance gene in the breeding for scab resistant apple cultivars. Distorted segregation ratios for V_f-resistance have frequently been reported. Here we revealed that sub-lethal genes caused the distorted segregation. The inheritance of V_f was examined in six progenies by testing linked molecular markers. Three progenies showed distorted segregations that could be explained by three sub-lethal genes (*sl1*, *sl2* and *sl3*), of which *sl1*, *sl2* were closely linked to V_f. The *sl1* gene was located at about 14 cM from V_f and expressed itself only in the presence of another independently segregating sub-lethal gene *sl3*. Only the double homozygous recessive genotypes (*sl1sl1 sl3sl3*) were lethal, which occurred at first as dwarf and poor vigour plants during the first three months after germination. The s*l2* gene was also linked to V_f and its lethality was expressed prior to seed germination and also required the homozygous recessive presence of *sl3*. The map position of *sl3* has not yet been identified. The linkage of V_f to sub-lethal genes usually results in a shortage of V_f-resistant progenies. But in some exceptional crosses, it will lead to abundance of resistant seedling.

Introduction

Apple scab (*Venturia inaequalis*) is one of the major diseases in most apple growing areas around the world. At least seven different major genes for scab resistance have been identified (V_f, V_a, V_m, V_r, V_b, V_{bj} *and* V_g) (Crosby et al. 1992; Bénaouf and Parisi, 2000; Durel et al. 2000). V_f is the most widely used gene that originates from '*Malus floribunda* 821' (Williams and Kuc 1969). Most of the newly released scab resistant apple varieties possess this gene (Lespinasse 1989; Crosby et al. 1992). Hough et al. (1953) found the V_f-resistance was inherited monogenically. However, this single gene model seemed to be inconsistent to the later observed skewed segregation ratios for resistance to susceptibility (Kellerhals 1989; Yang and Krüger 1994; Tartarini 1996, 1999; Conner et al. 1997; Bus et al. 1999). To explain these distorted segregations, existences of cumulative minor genes conferring resistance in the absence of V_f and of modifier genes were postulated (Lamb et Hamilton 1969; Rousselle et al.1974; Gessler 1992; Lespinasse 1989).

Many molecular markers for V_f have been reported and mapped (Koller et al. 1994; Gianfranceschi et al. 1996; Yang and Korban 1996; Gardiner et al. 1996; Hemmat et al. 1998; Tartarini et al. 1999; Xu and Korban 2000; Vinatzer et al. 2001). Genome walking and cloning studies revealed that V_f is part of a gene cluster located between the markers M18 and AL07 (Patocchi et al. 1999, Vinatzer et al. 2001; Belfanti et al. 2004). To improve our understanding of the inheritance of V_f, we tested various breeding progenies by molecular markers flanking the V_f locus. We explained that distorted segregation ratios of V_f, were caused by its linkage to two different sub-lethal genes.

Table 1. General data on the six tested progenies

Cross	Classificaiton[a]	Seeds sown	Germination %	Number of seedlings				
				In resistance tests		Not-tested (%[b])		
				#	%[b]	Root rot	Poor Vigour	Death during incubation
1997								
Idared × **S**antana	S × R	200	96.5	189	97.9	2.1		
Ecolette × **S**antana	R × R	200	91.0	118	64.8	8.8	26.4	
Santana × **T**opaz	R × R	200	92.5	182	98.4	1.6		
1998								
Ecolette × **S**antana	R × R	184	92.4	136	80.0		18.8	1.2
Santana × **E**colette	R × R	216	94.9	144	71.7		16.6	11.7
Ecolette × **T**opaz	R × R	167	95.2	159	100.0			

[a]R: Resistant due to the presence of V_f; S: susceptible ($v_f v_f$)
[b]Percentages are calculated on the basis of the germinated seeds

Materials and methods

Plant materials

We examined six progenies segregating for V_f by molecular markers linked to this gene (Table 1). In 1997, three progenies were examined: 'Idared' × 'Santana' (I×S), 'Santana' × 'Topaz' (S×T), and 'Ecolette' × 'Santana' (E×S). In 1998, three progenies were tested: E×S, S×E and E×T. Both seed samples of E×S in 1997 and 1998 were from the same lot. 'Ecolette' and 'Santana' are new scab resistant cultivars of Plant Research International originating from the crosses 'Elstar' × 'Prima' and 'Elstar' × 'Priscilla', respectively.

Molecular markers

The core marker examined was the codominant AL07-SCAR (Tartarini et al. 1999). Seven other markers were tested where applicable: M18-CAPS, U01-SCAR (Gianfranceschi et al. 1996), SCAR-D20 (Gardiner et al. 1996; Yang and Korban 1996), and the RAPDs $OPA15_{900}$, $OPC09_{900}$, (Tartarini et al. 1996; Gardiner et al. 1996), $OPAG12_{800\ and}$, $OPAB19_{1430}$ (Maliepaard et al. 1998). These markers will be abbreviated to AL07, M18, U01, D20, AG12, A15, C09, and AB19. AL07 and M18 are codominant markers while U01, D20, C09 and AG12 are dominant. A sketch linkage map showing the relative order of these markers was given in Figure 1. We converted RAPD marker $OPC09_{900}$ into the SSR marker C09 (Fw 5'-CTCTCCTTTCATCACCCCAC-3', Rev 5'-GTTTGAATTTTCAGATGTTGCTACCT-3') by identification of a variable number of CT repeats after cloning and sequencing of the 900 bands from 'Ecolette' and 'Santana' (GenBank accns: CW916837, CW916838, CW916839). This SSR marker was tested on fifteen cultivars to reveal its diversity: 'Braeburn', 'Elise', 'Elstar', Fiesta', 'Golden Delicious', 'Ingrid Marie', 'Priscilla', 'Santana' and 'Topaz' showed a single band of 155 bp; 'Prima' and 'Ecolette' had two bands of 149 and 155 bp; '*Malus floribunda* 821' of 149 and 153 bp, Jonathan of 155 and 163 bp, and 'Idared' of 155 and 171 bp. The original V_f allele is linked to SSR C09-149.

DNA extraction & amplification conditions

Leaves needed for DNA-extraction were collected shortly after the completion of resistance tests. In 1998 leaves were also collected from seedlings that were excluded from resistance tests because of poor vigour. DNA was extracted from young leaf material (about 0.5 cm^2) following the mini-prep method of Haymes (1996) and dissolved in 20-100 μl TE buffer to a concentration of 40 ng μl^{-1}. M18, AG12, A15, and AB19 were amplified in a Perkin Elmer Cetus DNA 480 Thermal Cycler, while the robust SCAR markers AL07, U01, and D20 were also amplified in a Hybrid Thermal Cycler.

PCR amplification and electrophoresis conditions for AL07, U01, and M18 were the same as reported by Gianfranceschi et al. (1996) and Tartarini et al. (1999), those for D20 were identical to that of U01. Tartarini (1996) was followed for A15, C09, and AB19 markers. For AG12, the reaction mixture was identical to that for A15, but the amplification program was 4 min. at 94 °C,

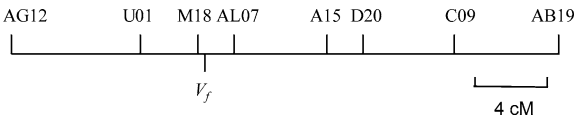

Figure 1. Approximate linkage map of tested markers of the V_f region.

25 cycles of 1 min. at 94°C, 1 min. at 35°C, 2 min. at 72°C, then 40 cycles of 1 min. at 94°C, 1 min. at 40°C, 2 min. at 72°C. For SSR C09, the reaction mixture consisted of 2 μl 10x buffer, 1.2 μl $MgCl_2$ (25 mM), 0.4 μl dNTPs (10 mM), 1μl of each primer (2 μM), 0.06 μl Taq (5 U/μl) and 1μl gDNA 10 (ng/μl) in a total 20 μl volume. After an initial denaturation at 94°C for 2.5 min, the amplification was carried out for 34 cycles at 94°C for 30 s, 60°C for 30 s and 72°C for 1 min, and a final extension at 72°C for 5 min. PCR products were analysed on an ABI 377 (Applied Biosystems).

Statistical tests

Segregation ratios for markers were examined by the chi-square test. All tests were applied at the 95% confidence level unless indicated differently.

Results

Genotype selection Germination rates were high and differed slightly among the six progenies (Table 1), indicating that all progenies were raised similarly at this stage. Initially leaves for DNA extraction were sampled shortly after the disease tests, this procedure allowed selection against certain genotypes. Firstly, some seedlings (about 2–9%, Table 2) were not included in the resistance tests because they died untimely due to root rot. Secondly, the E × S and S × E progenies showed a considerable number of poor vigour seedlings prior to disease assessment because they had no fully developed primary leaf at the time of inoculation. These poor vigour seedlings had green cotyledons, epicotyls, and leaves but showed following symptoms: (1) retarded growth after germination, (2) extremely short internodes, and (3) leaves were smaller than the cotyledons, although the number of leaves could be equal to that of normal seedlings (Figure 2). Most of these poor vigour seedlings died within three months after germination, while some survived for a longer time. These symptoms were similar to the early dwarf described by Alston (1976).

Genotyping of parental cultivars by molecular markers

Genotypes of the parental cultivars are given in Table 2. 'Santana', 'Ecolette', and 'Topaz' are heterozygous

Table 2. Presence (1) or absence (0) of marker-alleles that are linked in coupling phase to the V_f-allele for 9 cultivars and 8 molecular markers.

	Molecular Marker							
Cultivar	$AG12_{800}$	U1-SCAR[d]	M18-CAPS[d]	AL07-SCAR[d]	$A15_{800}$	D20-SCAR[d]	$C09_{900}$e	$AB19_{1430}$
Santana[a]	0	1	1	1	0	0	0	0
Ecolette	1	1	1	1	1	1	1	1
Topaz	1	1	1	1	1	1	0	0
Priscilla	0	**1**	**1**	**1**	**0**	**0**	**0**	0
Prima	**1**[b]	**1**	**1**	**1**	**1**	**1**	**1**	1
Idared	0	0	0	0	0	0	0	0
Braeburn	0	0	0	0	0	0	0	0
Elstar	0	0	0	0	0	0	0	0
M. floribunda 821	**1**	**1**	**1**	1[c]	**1**	**1**	**1**	**1**

[a] Underlined genotypes have the resistance gene V_f.
[b] Data in bold have also been reported by King et al. (1999).
[c] *M. floribunda* 821 probably has the AL07 allele for V_f in homozygous condition
[d] For U01-SCAR, AL07-SCAR, and D20, the sizes of the alleles linked to V_f are 320, 466, and 500 bps, respectively (Gianfranceschi et al. 1996, Gardiner et al. 1996, Tartarini et al. 1999). M18-CAPS always give a 850 bp fragment. Its allele for V_f includes two restriction sites for *Taq*I (Gianfranceschi et al. 1996).
[e] Represented by the markers RAPD $OPC09\text{-}_{900}$ and SSR C09 (see Materials & Methods). The RAPD marker to V_f is actually 893 bp after sequencing.

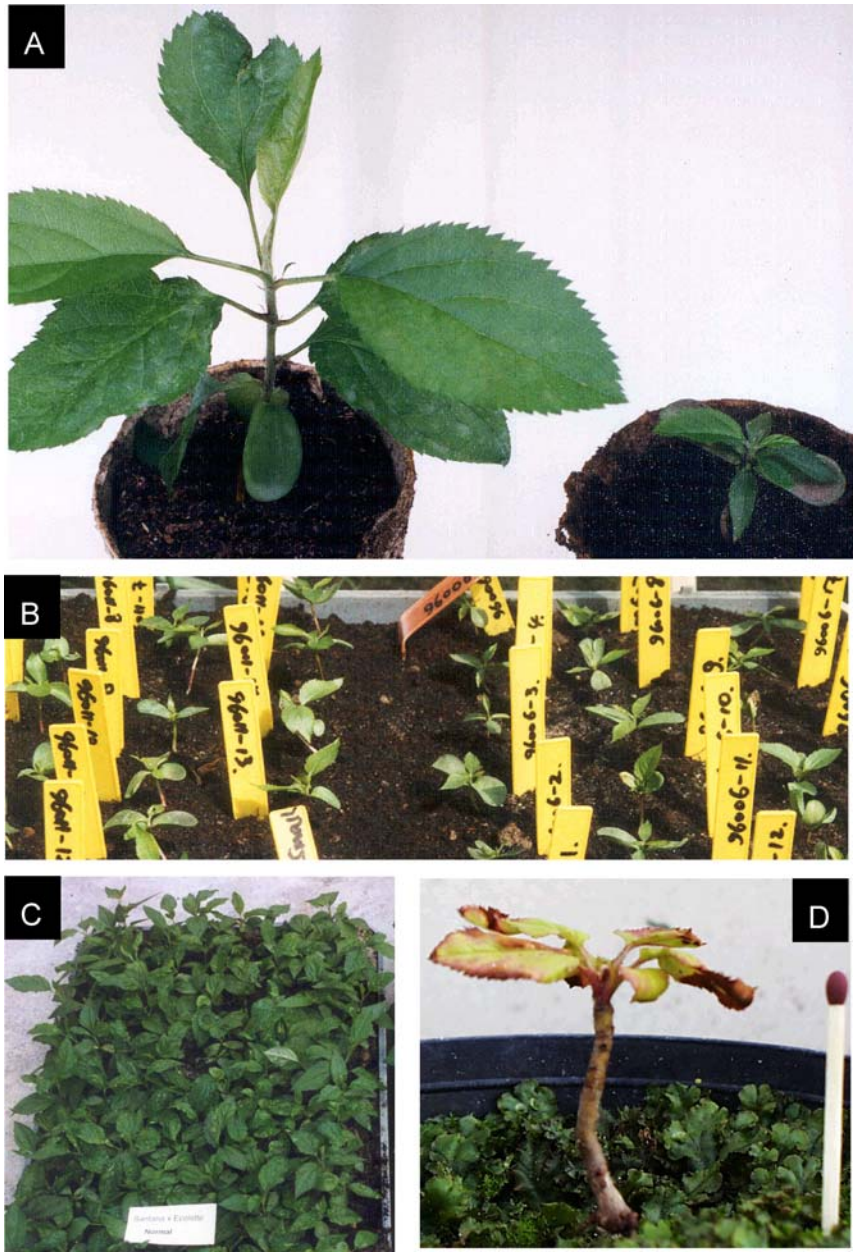

Figure 2. Phenotype presentations of dwarfed, sublethal and of normal seedlings.
A: A normal (left) and a dwarfed (right) seedling with normal green leaves from the cross 'Ecolette' × 'Santana', 16 weeks after germination.
B: Dwarfed seedlings of the crosses 'Ecolette' × 'Santana' (left) and 'Santana' × 'Ecolette' (right) at four weeks after germination, all of which showed normal green leaves and most of which died.
C: Normal seedlings of the cross 'Santana' × 'Ecolette', picture taken on the same day as figure 2B.
D: Surviving dwarfed seedling one year after germination having a wooded stem.

Table 3. Distributions of AL07 marker genotypes for three progenies tested in 1997 and three progenies tested in 1998

Cross		*AL07-V_f*					
		0[a]	1	2	Total	X^2 [b]	*p*-value
1997							
Idared × **S**antana	$v_f \times V_f$	94	95		189	0.005	0.0564
Ecolette × **S**antana	$V_f \times V_f$	17	60	41	118	9.80	**0.9926**
Santana × **T**opaz	$V_f \times V_f$	52	80	50	182	2.48	0.7106
1998							
Ecolette × **S**antana	$V_f \times V_f$	40	71	25	136	3.57	0.8322
Santana × **E**colette	$V_f \times V_f$	nt[c]	nt	nt			
Ecolette × **T**opaz	$V_f \times V_f$	34	83	42	159	0.96	0.3812

[a] numbers indicate that the marker is present in heterozygous (1) or homozygous (2) condition for the 466 bp allele of AL07 that is in coupling phase to the dominant V_f allele (Tartarini et al. 1999), or that this allele is absent (0).
[b] for the expected 1:1 or 1:2:1 ratio for $v_f\ v_f \times V_f\ v_f$ and $V_f\ v_f \times V_f\ v_f$ crosses respectively
[c] Only lethal and dwarfed seedlings were tested (see text).

for V_f according to the codominant markers AL07 and M18. All the scab susceptible cultivars lack the markers for V_f as expected. 'Priscilla' and its descendant 'Santana' have a reduced linkage drag from their common V_f source '*M. floribunda* 821' compared to 'Prima' and its descendant 'Ecolette', as indicated by the lack of the markers AG12, A15, and D20 of *M. floribunda* 821. 'Topaz' lacks SSR C09-149 and AB19. '*M. floribunda* 821' showed to be homozygous for the AL07 allele for V_f, thus confirming the findings of Tartarini et al. (1999). The genotyping of '*M. floribunda* 821', 'Prima', and 'Priscilla' is in agreement with that by King et al. (1999), except that we found 'Priscilla' to lack AG12.

AL07 marker segregation in progenies

AL07 segregated regularly in the crosses I×S and E×T fitting to the expected 1:1 and 1:2:1 ratio (Table 3). However, it segregated irregularly in the crosses: E×S, S×E and S×T (Table 3). The irregularity of the latter cross is not clear from Table 3, but showed up after a more in depth analysis. The irregular segregating crosses will be described in more detail below.

'Ecolette' × 'Santana' (E×S) & 'Santana' × 'Ecolette' (S×E)

Both 'Santana' and 'Ecolette' carry V_f, and the parental origin of their *AL07* allele for V_f can be deduced in their offspring by the D20 marker because it is present in 'Ecolette' and absent in 'Santana' (Table 2). The co-dominant marker AL07 can distinguish V_f homozygotes from V_f heterozygotes. If a seedling is heterozygous and lacks D20, then *AL07-V_f* most likely descends from 'Santana'. In this way we can distinguish four groups of seedlings: (1) *AL07* heterozygous with 'Ecolette' as V_f source, (2) *AL07* heterozygous with 'Santana' as V_f source, (3) *AL07-V_f* homozygous, and (4) *AL07-v_f* homozygous. The observed numbers of seedlings for these four groups were 18, 42, 17, and 39 respectively. Thus *AL07* heterozygotes with 'Ecolette' as V_f source as well as *AL07-V_f* homozygotes were in shortage, whereas the other two groups were present in abundance compared to the 29.5 seedlings expected according to a 1:1:1:1 segregation ratio. The observed segregation shows that 'Santana' passed *AL07* to 50% of its progeny, while 'Ecolette' did so to 29%, about half of the expected 50%. Since we discarded a considerable part (26%) of the original progeny because of poor vigour and lethality (Table 1), *AL07* of 'Ecolette' may be linked to a lethal gene (*a*). A genetic model of two complementary sub-lethal genes is postulated (Table 4) to explain why only 50% of the AL07-*a* genotype is lethal. In this model, only *aacc* genotypes die. Gene *a* explains the high proportion of seedlings that died when having the *AL07-V_f* allele of 'Ecolette'. The simultaneous presence of gene *c* is needed to explain why only part of the seedlings died that had the *AL07-V_f* allele of 'Ecolette'. Under this model, most of the very small seedlings should possess the *AL07-V_f* allele of 'Ecolette' as well as its D20 marker. Since these plants had already been discarded in 1997, we set up a new experiment in 1998 in which new seedlings of this same cross as well as of its reciprocal (S×E) were examined. Again, 66 seedlings (around 20%–28%) of poorly growing and early dying were observed in both crosses (Table 1). Almost all of

them (64) showed D20, while AL07 revealed that the V_f homozygous to heterozygous ratio was 32:33, fitting to the 1:1 ratio expected under the proposed genetic model. This result definitely showed *AL07* of 'Ecolette' to be linked to a sub-lethal gene.

The data do not allow conclusions on the dominance or recessiveness of this gene. However, from a biological point of view recessiveness is most likely. E×S can now be genotyped as ($V_f a / v_f A$, *cc*) × ($V_f a / V_f a$, *Cc*). Ignoring recombination events between *AL07*, V_f and *a*, we expect a $v_f V_f : V_f V_f : V_f V_f$ ratio of 2:3:1 (Table 4), which fits very well to the observed 41:60:17 segregation for AL07 ($\boldsymbol{X}_2^2 = 0.03$, $p = 0.015$).

'Santana' × 'Topaz' (S×T)

AL07 seemed to fit a regular 1:2:1 segregation expected for a codominant marker (Table 3). However, a distorted segregation showed up when we looked deeper into the parental source of V_f. 'Topaz' possesses two markers (AG12, D20) which flank V_f at a somewhat larger distance and which are absent in 'Santana' (Table 2). These markers were used to estimate the parental source of *AL07* for each seedling. This give rise to four groups: (1) *AL07* heterozygotes with 'Santana' as V_f source, (2) *AL07* heterozygotes with 'Topaz' as V_f source, (3) *AL07*-V_f homozygotes, and (4) *AL07*-v_f homozygotes. The observed numbers of seedlings for these four groups were 28, 52, 52, and 50 respectively. These data showed that the heterozygotes with the *AL07*-V_f allele of 'Santana' are in shortage ($\boldsymbol{X}_3^2 = 9.03$, $p = 0.97$). Since no seedlings died shortly after germination (Table 1), the related selection should have occurred prior to germination. To explain this, a genetic model was postulated similar to that for E×S:

Table 4. Genotypes of the gametes of 'Ecolette' and 'Santana' and their offspring in view of V_f and the sub-lethal genes *a* and *c*. Gene *a* is assumed to be linked to V_f.

Gametes of Santana	Gametes of Ecolette ½ $\boldsymbol{V_f}$ *ac*	½ v_f *Ac*
¼ V_f *a C*	⅛ $\boldsymbol{V_f}$ V_f *aa Cc*	⅛ V_f v_f *Aa Cc*
¼ V_f *a c*	⅛ $\boldsymbol{V_f}$ V_f *aa cc* †[a]	⅛ V_f v_f *Aa cc*
¼ v_f *a C*	⅛ $\boldsymbol{V_f}$ v_f *aa Cc*	⅛ v_f v_f *Aa Cc*
¼ v_f *a c*	⅛ $\boldsymbol{V_f}$ v_f *aa cc* †	⅛ v_f v_f *Aa cc*

[a] Lethal genotypes
V_f of 'Santana' is passed to 50% of the surviving part of the progeny (3/6), and V_f of 'Ecolette' to 33% (2/6). The expected $V_f V_f : V_f v_f : v_f v_f$ ratio is 1:3:2, and 2:1 for that of (V_f V_f + V_f v_f): $v_f v_f$.

Table 5. Genotypes of the gametes of 'Santana' and 'Topaz' and their offspring under two genetic models in view of V_f and the sub-lethal genes *b* and *c*. Gene *b* is linked to V_f.

Gametes of Santana	Gametes of Topaz ½ $\boldsymbol{V_f}$ *Bc*	½ V_f *bc*
¼ $\boldsymbol{V_f}$ *bC*	⅛ $\boldsymbol{V_f}$ V_f *Bb Cc*	⅛ $\boldsymbol{V_f}$ v_f *bb Cc*
¼ $\boldsymbol{V_f}$ *bc*	⅛ $\boldsymbol{V_f}$ V_f *Bb cc*	⅛ $\boldsymbol{V_f}$ v_f *bb cc* †
¼ v_f *BC*	⅛ V_f v_f *Bb Cc*	⅛ v_f v_f *Bb Cc*
¼ v_f *Bc*	⅛ V_f v_f *Bb cc*	⅛ v_f v_f *Bb cc*

[a] Lethal genotypes:
Vf from 'Santana' is passed to 43% (3/7) of the surviving part of the progeny, and V_f of 'Topaz' is 57% (4/7). The expected $V_f V_f : V_f v_f : v_f v_f$ ratio is 2:3:2, and the expected (V_f V_f + V_f v_f): $v_f v_f$ ratio is 5:2.

two independently segregating, complementary recessive genes (*b* and *c*) of which one (*b*) is linked to V_f (Table 5). A putative genotype for S×T is ($V_f b / v_f B$, *Cc*) × ($V_f B / v_f b$, *cc*). Most of the aborted offspring will carry the V_f allele of 'Santana'. Its frequency was reduced from 0.5 (= 4/8) under regular segregation to 0.43 (= 3/7) under the current model (Table 5). The expected numbers of seedlings for the four genotype groups are 26, 52, 52, and 52, which fit very well to the observed ones mentioned above ($\boldsymbol{X}_2^3 = 0.34$; p = 0.048). Genes *a* and *b* are not the same because of differential selection against the V_f and v_f alleles of Santana in the crosses E×S and S×T.

'Ecolette' × 'Topaz'

AL07 showed a regular segregation, although both cultivars were involved in irregularities when crossed with Santana. This regular segregation fits with the genotypes of 'Ecolette' and 'Topaz' as deduced from the previous crosses (Tables 4 and 5). 'Ecolette' should be homozygous *for B* since 'E×S' did not segregate while 'Santana' is heterozygous for this gene. Topaz presumably is homozygous *AA* since no young seedlings died untimely in S×T. 'E×T' is therefore genotyped as $V_f V_f$ *AaBBcc* × $V_f V_f$ *AABbcc*. Consequently, no offspring will possesses neither *aacc* nor *bbcc*, and the entire offspring will be viable.

Discussion

We found that the V_f gene for scab resistance is linked to two different sub-lethal genes that can cause skewed segregation ratios for V_f-resistance. These genes are denoted as *sl1* and *sl2* (see below). *sl1* is expressed after

seed germination while *sl2* is expressed prior to seed germination. Due to this different stage of expression, *sl2* will be epistatic over *sl1*. Both genes are expressed only when another sub-lethal gene is simultaneously present in homozygous recessive condition. The two lethal phenotypes are so different that we proposed new pairs of complementary genes at first. However, the second gene of each pair had the same allelic composition over all crosses for each parental genotype, regardless whether *sl1* or *sl2* was expressed (Tables 4 and 5, *c* locus). We therefore propose for the sake of simplicity to combine these two complementary genes into one theoretical gene *sl3*, till new to come data show the contrary.

Distorted segregations

All the distorted ratios presented here were in favour of susceptibility. This phenomenon can thus reduce the efficiency of resistance breeding programs. However, in principle, crosses can also display a surplus of resistant offspring. For instance, the viable part of the cross (V_f*Sl1* v_f*sl1, sl3 sl3*) × (V_f*Sl1* v_f*sl1, sl3 sl3*) will entirely have V_f, thus showing a 1:0 ratio instead of the commonly expected 3:1. Indeed, some of our v_f v_f × V_f v_f breeding populations showed 80% V_f-resistant seedlings where only 50% was expected. This was true for crosses between Elstar and various AL07 heterozygous selections of Priscilla × Prima (Van Heusden and Van Arkel, unpublished).

The observed lethality may be related to a deficiency of or an unbalance in a vital pathway. During the breeding process, the original V_f locus has been introgressed from the wild species *M. floribunda* into cultivated cultivars through recombination events. 'Prima' and its derived cultivar 'Ecolette' still carry the *sl1* allele of this wild species, since both cultivars carry the *sl1* flanking markers D20 and AB19 of '*Malus floribunda* 821' (Table 2). The cultivars 'Santana' and 'Topaz' lack this wild segment. 'Santana' carries nevertheless a recessive *sl1* allele that is linked to the recessive V_f allele (Table 4). This indicates that the sub-lethal gene *sl1* already occurred in the cultivated apple, and is thus not due to the introgression of V_f.

The current findings can explain some of the previously reported skewed segregation ratios for V_f. For instance, Tartarini (1996) observed a significant shortage of resistant plants to be accompanied by a similar shortage of molecular markers for V_f in the cross 'Prima' × 'Golden Delicious'. The similarities between his and our results on E×S are striking. Firstly, similar frequencies were observed for the V_f-allele from 'Prima' ($p = 0.27$) and that for 'Ecolette' ($p = 0.28$). Secondly, 'Golden Delicious' is strongly represented in both crosses, namely by itself (Tartarini 1996) and by its first generation descendants 'Ecolette' and 'Santana'. Thirdly, 'Prima' is involved in both crosses as parent (Tartarini 1996) or as grandparent of 'Ecolette'.

Sub-lethality and phenotypic penetrance

We used the term sub-lethality to indicate that *sl1* on its own is not lethal, but requires partnership with another (postulated) gene. None of the molecular marker linkage maps we know was helpful in the identification of a putative location of *sl3* because they did not show skewed segregations of the V_f (homologous) region (Maliepaard et al. 1998, Liebhard et al. 2003; 'Durello di Forli' × Fiesta', Tartarini personal communication; 'Jonathan' × 'Prima', Van de Weg unpublished), or because no report was made on distorted segregations (Conner et al. 1997 combined with Hemmat et al. 2003; Calenge et al. 2003). Regrettably, our research project did not allow performing a genome-wide scan by molecular markers.

As long as this additional gene has not been identified, it can not be excluded that the survival part of the *sl1sl1* genotypes is due to a partial penetrance of the lethal phenotype of a single locus. However, if that is true, then the level of penetrance would likely to be environment sensitive, in which case it is unlikely that the same proportion of lethal plants would have occurred in different years ('Ecolette' × 'Santana' tested in two successive years) and in different countries: The Netherlands (our research), Italy (Tartarini 1996) and New Zealand (Bus et al. 2002). We therefore found the jury of our current findings in favour of a two-gene model with full penetrance of lethality in *sl1sl1 sl3sl3* genotypes. Indeed, our genetic model allowed Bus et al. (2002) to explain his observed skewed segregations and to confirm the map position of *sl1*.

Gene denotation

In apple, a convention on the denotation of genes is still lacking. Here, we propose to denote the sub-lethal genes *a*, *b,* and *c* as *sl1*, *sl2* and *sl3* respectively following the gene nomenclature of Søgaard and Wettstein-Knowles (1987) used in barley. The symbol *sl* stands for **s**ub-**l**ethal, indicating the phenotype conferred by the genes. The italicised symbol indicates a gene, locus, or allele; the non-italicised symbol indicates the

Table 6. Segregation ratios of a series of molecular markers that map in the V_f region of the crosses 'Ecolette' × 'Santana' and their X^2 and p values for the 1:2:1 or 1:1 ratio

Marker	Segregation	X^2	df	p
$AG12_{800}$	41:77	10.98	1	0.999078
U01	–	–	–	–
M18-CAPS*	39:63:16	9.51	2	0.991391
AL07-SCAR*	41:60:17	9.80	2	0.992553
$A15_{900}$	35:83	19.53	1	0.999990
D20-SCAR	34:84	21.19	1	0.999996
$C09_{900}$	32:86	**24.71**	1	**0.999999**
$AB19_{1430}$	33:85	22.9	1	0.999998

relative phenotype. The first letter is in lower case to indicate recessiveness of the genes.

Gene *sl2* is recessive since in case of dominance also part of the V_f homozygotes of S×T should have died prior to emergence of the seedlings. Our data do not allow conclusions on the dominance or recessiveness of *sl1* and *sl3*. Since all other identified lethal genes in apple were recessive (Klein et al. 1961; Alston et al. 2000), we assumed this gene to be recessive too.

Mapping of sl1

The *sl1* gene could be mapped due to the availability of many markers around the V_f gene (Maliepaard et al. 1998) and well defined distorted progenies (E×S and S×E). The markers showing the strongest distorted segregation are likely to be nearest to the gene for lethality (Cheng et al. 1996). Considering the cross E×S, *sl1* should be near $C09_{900}$ (Table 6), which mapped at 9 cM from AL07 and at 14 cM from V_f in the cross Prima × Fiesta (Maliepaard et al. 1998). The map positions of *sl2* and *sl3* have still to be resolved.

sl1sl3-lethality and pale green lethal, and compactness

Our lethal and dwarfed seedlings had cotyledons, epicotyls and leaves of normal green colour (Fig. 2A and 2B) which were distinct from 'pale green lethal' (PGL) which is associated with chlorophyll deficiency causing PGL seedlings to be yellowish green (Way et al. 1976). Apart from a different appearance, PGL was governed by a single recessive, phenotypically completely penetrating gene *l* (Klein et al. 1961, Way et al. 1976). Similarities in both lethalities are associated with phenotypes (a poor growth of epicotyl and leaves) and the involvement of the same genome segment as *l* had been located at around 9 cM from V_f (Alston 1976). It is not clear yet whether the poor growth of our and PGL seedlings are due to the (sub) lethal genes themselves, or due to the genetic co-localization of *sl1*, *l*, and the recessive gene *n* for compact growth that was identified by Decourtye (1967) (see below). s*l1* and *n* can not be the same gene since a considerable proportion of the compact seedlings remained viable and growing for at least 6 months, thus probably being '*sl1sl1 Sl3. nn' genotypes.* Regrettably, we did not quantify all these different phenotypic classes. Additionally, we observed a single one, year old plant of only 4 cm in height (Figure 2D). Finally, we did not observe any leaves that were chlorophyll deficient.

Map position of the n gene

Decourtye (1967) showed *n* to be linked with *Vf* and all his V_f cultivars as well as 'Golden Delicious' carried *n* in heterozygous condition. Since 'Ecolette' and 'Santana' are V_f cultivars that also have 'Golden Delicious' as a grandparent, it would be no surprise that one or both of them possess the *n* allele too. By being linked to V_f (in coupling phase), *n* is also linked to *sl1*, which explains why most of the lethal seedlings of 'Ecolette' × 'Santana' showed compact growth. Decourtye (1967) based the linkage of V_f and *n* on the overall shortage of susceptible compact seedlings as well as on the overall surplus of resistant normal seedlings of three progenies. However, not all his segregation ratios fitted to a single locus model. His data can be perfectly explained when expression of sub-lethal genes is also taken into account. This extended genetic model indicates that *n* has 11% recombination with V_f instead of the 24% estimated by Decourtye (1967) (Table 7). This result further emphasis the involvement of this genomic region in functional abnormalities. For the sake of completeness it should be mentioned that in all our analysis we assumed full penetrance of both the lethal and compact growth phenotype.

Concluding remarks

Distorted segregation ratios for V_f resistance are often attributed to modifier genes or to specific combinations of minor genes. This report shows that at least some of the observed distorted ratios for V_f-resistance are due to linkage of V_f to sub-lethal genes. This study underlines the power of molecular markers in elucidating

Table 7. Segregation data for normal versus compact growth and V_f-resistance in four apple progenies as observed by Decourtye (1967) and as expected according to our genetic model of wo complementair sub-lethal genes (*sl1*, *c*)

							R		S		
Progeny	Normal	Compact	X^2 3:1	R	S	X^2 1:1	Nor	Com	Nor	Com	X^2
Decourtye											
Golden Delicious selfed	44	19	0.9 ns								
Reinette du Mans × OR32T41	468	124	5.2**[,2]	327	265	6.5**	214	113	254	11	22.2****[,2]
Reinette du Mans × OR38T17	116	29	1.8 ns	65	80	1.6 ns	42	23	74	6	3.4 ns[2]
OR51T86 × Douce de Hollande	186	69	0.5 ns	133	122	0.5 ns	71	62	115	7	9.2*[,2]
Total	**814**	**241**	**2.5 ns**	**525**	**467**	**3.4 ns**					
Expected under the proposed genetic model[3]											
Golden Delicious selfed	50	13	3.3 ns								
Reinette du Mans × OR32T41	469	123	0.0 ns[4]	296	296	6.5**	186	110	282	14	7.6 ns[5]
Reinette du Mans × OR38T17	115	30	0.0 ns	73	73	1.6 ns	46	27	69	3	3.3 ns[4]
OR51T86 × Douce de Hollande	191	64	0.6 ns	128	128	0.5 ns	71	57	121	7	0.7 ns[5]

[1] * $p = 0.05$; ** $= 0.025$; *** $= 0.01$; **** $= 0.0005$

[2] in comparison to the one locus model of Decourtye (1967), 3 df.

[3] 'Golden Delicious' = (*nv$_f$A/Nv$_f$a, Cc)*; 'Reinette du Mans' × 'OR32T41' and 'Reinette du Mans' × 'OR38T17' = (*nv$_f$a/Nv$_f$a, Cc*) × (*nV$_f$a/Nv$_f$A, cc*); 'OR51T86' × 'Douce de Hollande' = (*nV$_f$/Nv$_f$*) × (*nv$_f$/nv$_f$*) with *A* and *C* present in homozygous condition in at least one of the parents. (Note: *a* is here synonymous to *sl1*, but the data can equally well be explained by *sl2* provided that this gene is also at around 14 cM from V_f)

[4] In comparison to the observed data, 1df;

[5] In comparison to the observed data, after joining classes SN and SC because of the low number of plants in class SC.

the actual, unforeseen cause of phenotypic phenomena. As far as the authors are aware, this is the first report in agriculture on linkages between a resistance gene and lethal factors have been established by the use of markers.

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