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Application of DNA markers for detection of scab resistant apple cultivars and selections

Mattisson H. and Nybom H.

Balsgård-Department of Crop Science, Swedish University of Agricultural Sciences, Fjälkestadsvägen 459, 29194 Kristianstad, Sweden, email: Hilde.Nybom@vv.slu.se

Summary: A DNA marker-based study was undertaken to identify the occurrence of major scab resistance genes in some apple cultivars and selections of importance for apple breeding. Unfortunately none of the RAPD-based markers previously reported to detect the V_a, V_b, V_r and V_x genes produced unambiguous results. By contrast, the CAPS marker M18 produced the expected three bands in all cultivars and selections already known or suspected to have the V_f gene, as well as in the Russian cultivar 'Antonovka Polotora Funtovaja' suspected to have V_a resistance which however may be allelic to V_f. V_f-carrying selections and newly named cultivars 'Frida' and 'Fredrik' are grown successfully in Sweden without fungicides, suggesting that the V_f-resistance breaking scab races 6 and 7 have not yet become a problem. The SCAR marker B12 detected the V_m gene in 'Prairifire', 'Rouville', clones 'OR45T132' and 'OR48T70', and selection '16-36-193'. The SSR locus CH02b10 detected one band at 118 bp in 'Reka'. This is presumed to be identical to the V_r gene marker previously reported.

Key words: *Malus* x *domestica*, *Venturia inaequalis*, apple, DNA marker, resistance, scab

Introduction

Apple scab, caused by the fungus *Venturia inaequalis* Cke., is the most detrimental disease in commercial apple orchards. Ten to twenty yearly applications with fungicides are usually required in orchards with scab susceptible cultivars (*MacHardy*, 1996; *Holb* et al., 2005), therefore, resistance against apple scab is one of the major goals in apple breeding. Both dominantly inherited resistance, caused by a number of major genes, and polygenically controlled so-called field resistance is available in the *Malus* germplasm. The most commonly used of the major genes is V_f obtained from *Malus floribunda* 821 and occasionally also from *M. atrosanguinea* 804 (*Crosby* et al., 1992) and *M. micromalus* and *M. prunifolia* 19651 (*Vinatzer* et al., 2004). The V_f gene has been mapped to linkage group 1 on the European linkage map (*Maliepaard* et al., 1998), in close proximity to two other less well-known genes, V_a and V_b. V_a is reported to occur in e.g. the selection 'PI 172623' derived from the Russian cultivar 'Antonovka' and V_b in e.g. 'Hansen's *Malus baccata* no. 2' (*Hemmat* et al., 2003).

The V_m gene occurring in both *M. micromalus* and *M. atrosanguinea* 804 has also been used in some breeding programs (*Crosby* et al., 1992) and a DNA marker has been developed (*Cheng* et al., 1998). Yet another source of resistance has been obtained from an American selection, known as 'Russian seedling R12740-7A' since it was obtained from seed originating in Russia (*Bus* et al., 2005). This seedling appears to have carried at least two and perhaps three different major genes, see reviews in *Bus* et al. (2004, 2005). The corresponding genes are known as V_r (most likely synonymous with V_{h2}, and possibly synonymous or allelic with V_{bj} from *M. baccata* 'jackii' and V_{h8}) and V_x (most likely synonymous with V_{r1}, V_{r2} and V_{h4}), all of which have been mapped to linkage group 2.

Recently, genes providing resistance against some less common scab races have also been identified and mapped, like V_g (found in 'Golden Delicious' and some of its offspring) in linkage group 12 and V_d (found in 'Durello di Forli') in linkage group 10, and also some quantitative trait loci (QTLs) (*Durel* et al., 2004).

Problems with accurate early scoring of scab resistance versus susceptibility can now be solved by DNA marker screening of the seedlings. Even more important is the ability to correctly identify the different resistance genes in the parental material and the seedlings, thus enabling successful gene pyramiding. The present study was undertaken to identify the occurrence of major scab resistance genes in some apple cultivars and selections of importance for apple breeding.

Material and methods

Plant material

A total of 36 clones, cultivars and selections, grown at Balsgård or the field station in Kivik in the South of Sweden, were analysed (*Table 1*). The material included 5 historically

important clones with differential resistance to scab obtained from Purdue University, U.S.A.; clone 'OR42T173' is a derivative (2 generations removed) of 'Russian seedling 12740-7A' and acts as a differentiator for scab race 2, clone 'Geneva SR' was obtained by open pollination of *M. niedzwetzkyana* and acts as a differentiator for scab races 2 and 3, clone 'TSR18T13' is a derivate (four generations removed) of 'Russian seedling' and acts as a differentiator for scab race 4, clone OR48T70 is derived (2 generations removed) from *M. micromalus* and clone 'OR45T132' is derived (2 generations removed) from *M. atrosanguinea* 804, both of these act as indicators of scab race 5. The remaining material consisted of 16 named cultivars, one selection from the U.S.A. ('Co-op 28'), two selections from All-Russia Research Institute of Horticultural Breeding in Orel ('16-36-193' and '18-13-27'), and 12 selections from the breeding program at Balsgård ('K:0963'–K:1430').

Young leaves from the 36 apple genotypes were collected in the summer 2004 and then stored at –80 °C until DNA extraction.

DNA extraction

Total genomic DNA was extracted using the Qiagen DNeasy™ Plant Mini Kit. The following changes were made to the manufacturer's protocol; 100 mg of leaf tissue was ground using a disposable plastic pestle in 200 µl of Buffer AP1 in 1.5 ml eppendorf tubes. An additional 200 µl of Buffer AP1 and 4 µl of RNase A (100 mg ml^{-1}) was added to the ground leaf tissue. In the last step the DNA was eluted two times with 50 µl Buffer AE.

PCR amplification

We screened 36 DNA samples using a total of 8 previously described DNA markers (*Gianfranceschi* et al., 1996, 1998; *Cheng* et al., 1998; *Hemmat* et al., 2002, 2003). These markers were obtained using single RAPD (Random Amplified Fragment Polymorphism) primers or especially designed primer pairs for CAPS (Cleaved Amplified Polymorphic Sequences), SCAR (Sequence Characterized Amplified Regions) or SSR (Simple Sequence Repeat) markers. Each utilized primer or primer pair had already been reported to act as a marker for a gene conferring resistance against a specified race of apple scab. The primers were obtained from MWG-Biotech, Germany except for OPB18 which was obtained from Operon Biotechnologies, Inc. All reactions were performed in a Px2 Thermal Cycler (Thermo Hybaid) programmed according to the original protocols (see references above). The amplification products were separated by gel electrophoresis on 1.5% agarose gels except for the SSR markers (see below). The amplification conditions for each primer/primer pair, if different from described are as follows.

Four RAPD primers were used in our attempts to detect the V_a, V_b, V_r and V_x genes. Reactions with the primers OPB18 (V_r) and S22 (V_x) (*Hemmat* et al., 2002), and with P136 (V_a) and B220 (V_b) (*Hemmat* et al., 2003) contained 1 unit of *Taq* polymerase (ABgene), 2.5 µl of the supplied buffer, 3 mM $MgCl_2$, 0.2 mM dNTP, 0.6 µM primer and 5 ng DNA in a total volume of 25 µl.

The CAPS marker M18 (*Gianfranceschi* et al., 1996) was used to detect the V_f gene. The PCR reactions contained 1 unit of *Taq* polymerase, 1.5 µl of the supplied buffer, 1.5 mM $MgCl_2$, 0.1 mM dNTP, 0.2 µM of each primer and 5 ng DNA in 15 µl total volumes. The digestion reactions contained 2 units of *Taq*I (Sigma-Aldrich, Inc.), 1.5 µl of the supplied buffer and 2–5 µl PCR product in a total volume of 15 µl.

The SCAR marker B12, designed to detect the V_m gene, was used with the New Zealand amplification conditions (*Cheng* et al., 1998) except that the total volume of the reactions was 15 µl instead of 12.5 µl. For detection of the V_x gene, a SCAR marker was also used in addition to the above-mentioned RAPD marker S22: namely S22-STS, which was developed from S22 (*Hemmat* et al., 2002). The PCR reactions contained 0.5 unit of *Taq* polymerase, 1.5 µl of the supplied buffer, 1.5 mM $MgCl_2$, 0.12 mM dNTP, 0.4 µM of each primer and 5 ng DNA in a total volume of 25 µl.

Finally, one SSR primer pair was used, CH02B10 originally developed by *Gianfranceschi* et al. (1998) and later used for detection of V_r by *Hemmat* et al. (2002). The PCR reactions contained 1 unit of *Taq* polymerase, 1.5 µl of the supplied buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.2 µM of each primer and 5 ng DNA in a total volume of 15 µl. The forward primer was marked with HEX at the 5' end. The PCR products were run on an 3730 DNA Analyzer (Applied Biosystems), and the results were interpreted with GeneMapper® Software v. 3.0 (Applied Biosystems).

Results and Discussion

Detection of V_a and V_b

Two RAPD primers described by *Hemmat* et al. (2003) for detection of V_a and V_b were used in our study. The difficulties in producing valid results with RAPR primers are well-known and have prompted the conversion of RAPD markers to more unambiguous marker types like SCAR and CAPS but in some cases such conversion has proved difficult or impossible. As yet, there is only the original RAPD marker (a 700 bp fragment amplified by primer P136) for the V_a gene detected in 'Antonovka PI 172633' and some of its offspring (*Hemmat* et al., 2003). In addition, *Hemmat* et al. (2003) screened four other 'Antonovka' types but none of these produced the V_a marker. Instead two of the 'Antonovka' types amplified markers for the V_b gene, which occurs on the same linkage group.

Among our samples were several different types of 'Antonovka' as well as two 'Antonovka'-derived cultivars, 'Reglindis' and 'Angold'. 'Reglindis' has been reported to carry V_a resistance (*Hemmat* et al. 2003) but the resistance is only partial in both 'Reglindis' and 'Angold' and appears to be polygenic, and is therefore usually instead denoted as V_A (*Fischer* et al., 2003; *Vejl* et al., 2003). Unfortunately the

RAPD primer P136 produced very complex band patterns in our analyses. Despite manipulations with the amplification conditions, we were not able to obtain patterns that could be unambiguously interpreted across the entire set of DNA samples and these results were therefore discarded from further analysis. Interestingly, one of the possible V_a-candidates, 'Antonovka Polotora Funtovaja', instead appears to have the V_f gene (see below), which has been mapped to the same region as V_a (*Hemmat* et al., 2003). The original study reporting V_a and V_f to be separate genes (*Dayton & Williams*, 1968) was based on a small set of test crosses. Possibly the analysis of a larger set of crosses, involving several other scab-resistant 'Antonovka' clones, would instead prove these genes to be allelic (*Hemmat* et al., 2003).

The V_b gene has also been mapped to the same region as V_a and V_f, and may therefore be allelic to these (*Hemmat* et al., 2003; *Durel* et al., 2004). The RAPD marker for V_b (a 700 bp fragment amplified with primer B220) has been reported in several cultivars and selections including 'Hansen's *Malus baccata* no. 2' and its derivatives but also in e.g. *M. floribunda*, 'Antonovka Kamenichka' and 'Prairifire' (*Hemmat* et al., 2003). The latter two were included among our samples. Again, the RAPD primer produced band patterns that were too complex for accurate identification of the linked marker. We did, however, find that 'Prairifire' appears to carry the V_m gene (see below).

Detection of V_f

The CAPS marker M18 produced three bands of 170, 230 and 450 bp (obtained by cutting the originally amplified fragment with the restriction enzyme *Taq*I) in all the cultivars and selections that were already known to have the V_f gene (*Figure 1; Table 1*). The previously established homozygosity for selection 'Co-op 28' (V_fV_f) was corroborated in our study, since it had only the three marker bands whereas heterozygous genotypes also have one undigested band, resulting in a four-band profile.

All the analysed Balsgård selections resulted from crosses with a parent alleged to have V_f resistance, and they all had the V_f marker as expected. These selections, as well as the newly named 'Frida' and 'Fredrik' from the Balsgård breeding program, have been grown in S Sweden for about ten years with no fungicide treatments. So far, we have not seen any symptoms of scab on leaves or fruits, suggesting that scab races 6 and 7, which have broken the V_f resistance, have not yet become a problem in Sweden.

Interestingly, we found that one cultivar from our gene bank, 'Antonovka Polotora Funtovaja', also had the V_f

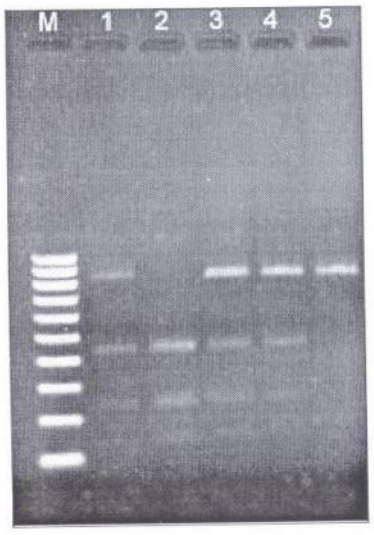

Figure 1 Band patterns obtained from the CAPS marker M18 after digestion with *Taq*I, showing one band for the v_f allele and three bands for the V_f allele. M is a 100-bp ladder ranging from 100-1000 bp. Lane no. 1 'Prima' (V_fv_f), 2 'Co-op 28' (V_fV_f), 3 'K:1016' (V_fv_f), 4 'K:1430' (V_fv_f) and 5 'Antonovka Polotora Funtovaja' (v_fv_f).

Table 1 Tested apple genotypes, previously reported type of scab resistance (question mark means that resistance source has been described as likely but to our knowledge not yet evidenced by DNA analysis), and the results from screening for three different DNA-based markers with marker presence indicated by + for the two first markers, and with size (bp) for alleles detected with the third marker.

Genotype	Resistance	M18, V_f	B12, V_m	CH02B10, V_x
Cultivars				
Angold	V_A?	–	–	123.2, 142.4
Antonovka Kamenichka	V_b	–	–	129.6, 155.1
Antonovka Pamtorutka		–	–	110.8, 129.4
Antonovka Polotora Funtovaja		+	–	110.9, 129.5
Dolgo Crab (*Malus baccata*)		–	–	115.1, 155.7
Florina	V_f	+	–	129.3, 131.4
Fredrik	V_f?	+	–	135.6, 155.5
Freedom	V_A?, V_f	+	–	119.0, 155.7
Frida	V_f?	+	–	118.9, 135.6
Prairifire	V_b	–	+	112.1
Prima	V_f, V_g	+	–	119.0, 123.1
Reglindis	V_A? V_f*	–	–	142.3, 155.6
Reka	V_r?	–	–	**118.0**, 129.4
Rubinola	V_f	+	–	119.1, 127.4
Rouville	V_m	–	+	129.5, 142.3
Zhigulevskoe		–	–	119.3, 121.2
Clones				
Geneva SR (*M. niedzwetskyana*)		–	–	127.3, 133.6
OR42T173	V_r?	–	–	127.4, 131.5
OR45T132	V_m	–	+	139.9, 142.3
OR48T70	V_m?	–	+	113.0, 155.5
TSR18T13		–	–	119.2
Selections				
Co-op 28	V_fV_f	++	–	123.2
K:0963		+	–	119.1, 142.3
K:1002		+	–	119.1, 135.7
K:1016		+	–	118.9, 135.6, 142.3
K:1058		+	–	135.6, 155.5
K:1107		+	–	129.5, 135.7
K:1160		+	–	131.4, 144.5
K:1181		+	–	131.5, 144.6
K:1210		+	–	127.4, 144.6
K:1301		+	–	131.6, 155.5
K:1325		+	–	129.4, 131.5
K:1343		+	–	117.1, 129.6
K:1430		+	–	129.4, 131.3
16-36-193	V_m	–	+	129.4, 142.4
18-13-27	V_f	+	–	155.8

*'Reglindis' has been shown to carry other V_f markers previously but is still not thought to actually carry the monogenic V_f-resistance (*Fischer & Dunemann*, 2000).

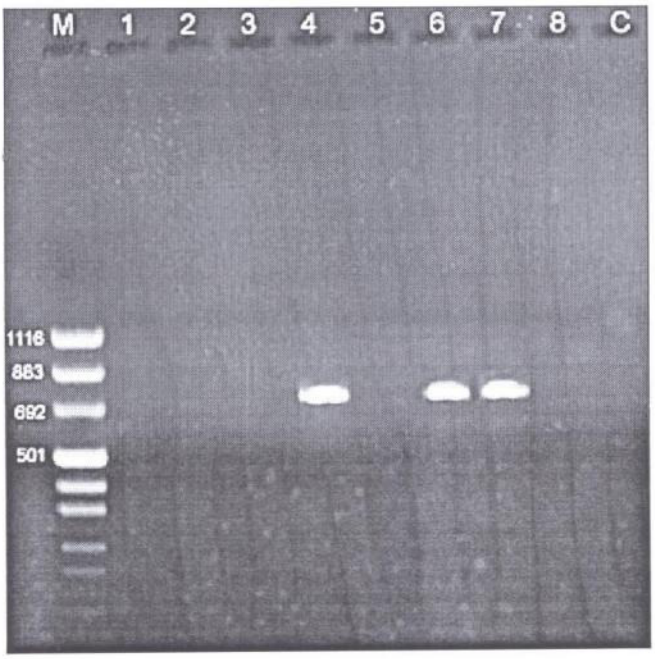

Figure 2 Band patterns obtained with the SCAR marker B12, which detects a band in samples with the V_m gene. Lane no. 1 'OR42T173', 2 'Geneva SR', 3 'TSR18T13', 4 'OR48T70' (positive), 5 'Freedom', 6 'Rouville' (positive), 7 'OR45T132' (positive) and 8 'Zhigulevskoe'. C is a blank control (no DNA).

marker. This tree was planted at Balsgård in 1947 but we have unfortunately no record of its origination. The genes one would expect in 'Antonovka' types, V_a and V_b (according to *Hemmat* et al., 2003), are closely linked to V_f and may even be allelic to this gene.

Detection of V_m

The SCAR marker B12 detected the V_m gene in 'Rouville', clone 'OR45T132' and selection '16-36-193', all of which are already known to have this gene (*Figure 2*). Another two samples also tested positive for this marker, 'OR48T70' and 'Prairifire' (*Table 1*). Clone 'OR48T70' is derived from *M. micromalus* and like 'OR45T132' it acts as an indicator of scab race 5, which is resistant against the V_m gene (*Cheng* et al. 1998). 'Prairifire' is an ornamental small-fruited cultivar, which has previously been reported to carry the V_b gene (*Hemmat* et al., 2003). Our results therefore suggest that it may represent a successful example of gene pyramiding.

Detection of V_r and V_x

There has been considerable confusion about the group of resistance genes mapped to linkage group 2, including V_r (most likely synonymous with V_{h2} and possibly allelic with V_{bj} and V_{h8}) and V_x (most likely synonymous with V_{r1}, V_{r2} and V_{h4}) (*Bus* et al., 2004, 2005). Looking at the samples included in our analysed material, V_r resistance has been reported for 'Reka' (*Fischer* et al., 2003). A possible V_x candidate is the scab indicator clone 'TSR18T13' which should carry V_r or perhaps more likely V_x since it has inherited race-specific resistance from 'Russian seedling R12740-7A', and this resistance appears to be different from the V_r-resistance carried by 'OR42T173'. In addition, *Bus* et al. (2005) reported V_r resistance in clone 'TSR34T15' and wrote that it was synonymous with the scab indicator 'OR42T173' included in our samples. However, the difference in microsatellite markers reported for 'TSR34T15' by *Bus* et al. (2005) and for our sample of 'OR42T173' (reported below) shows that these two cannot be identical.

Two different RAPD primers (OPB18 and S22) have been reported to be useful in the detection of V_r and V_x, treated as separate, unlinked genes (*Hemmat* et al., 2002). Both OPB18 and S22 primers were used in the present study but the band patterns produced were too complex for unambiguous identification of a marker band at 620 bp or at 1300 bp, respectively. A more user-friendly SCAR marker, S22-STS, has been developed from the RAPD marker S22 for identification of cultivars that carry the V_x gene (*Hemmat* et al., 2002). The S22-STS fragment was not detected in any of our samples but this may simply have been due to lack of material with the V_x gene. *Boudichevskaia* et al. (2004) report that e.g. 'Regia' and 'Remura' from the same breeding program as 'Reka' have a newly developed SCAR-marker for V_x, but they did not find this marker in 'Reka'.

The SSR locus CH02b10 was originally developed and used in a study of diversity among apple cultivars (*Gianfranceschi* et al., 1998; *Liebhard* et al., 2002). We found a total of 17 apparently different bands (110.8–110.9, 112.1, 113.0, 115.1, 117.1, 118.0, 118.9–119.3, 121.2, 123.1–123.2, 127.3–127.4, 129.3–129.6, 131.3–131.6, 133.6, 135.6–135.7, 142.3–142.4, 144.5–144.6, 155.1–155.8) (*Table 1*). Four samples showed only one band each and are presumably homozygous for an allele in this locus. Selection 'K:1016' instead showed three bands and thus appears to be triploid which is in accordance with the large size of both the tree itself and its fruit.

One fragment amplified by the primers for this locus has been reported to co-segregate with V_r resistance (*Hemmat* et al., 2002). This fragment had a size of 122 bp when analysed on agarose gels (*Hemmat* et al., 2002) but according to *Bus* et al. (2005), it was only 121 bp in an automatic sequencer-based analysis when calibrated to the allele sizes reported by *Liebhard* et al. (2002). Comparison of allele sizes between studies can be somehwat problematic due to differences between amplification conditions and technical equipment as discussed by *Bus* et al. (2005). Thus one of the samples analysed in our study, 'Florina', had two alleles at 129.3 and 131.5 bp whereas *Gianfranceschi* et al. (1998) instead report of alleles at 131 and 133 bp, and *Liebhard* et al. (2002) report of alleles at 133 and 135 bp for this cultivar. Similarly, we found two alleles at 119.0 and 123.1 in 'Prima' whereas *Liebhard* et al. (2002) report 123 and 127 for the same cultivar.

Taking the between-laboratory size differences into consideration, we can expect that a V_r resistance marker

would appear at around 117–118 bp in our analysis. A band at 117.1 bp was found in the selection 'K:1343' derived from open pollination of the American selection 'Co-op 14' (carrying V_f resistance) whereas nine samples had a band at 119.1 bp (118.9–119.2). Interestingly, 'Reka', alleged to have V_r resistance (*Fischer* et al., 2003), had a band in between of these two, namely at 117.95 bp. This band is most likely identical to the marker for V_r-resistance.

The CH02b10 locus has been reported to contain perfect (GA) repeats (*Gianfranceschi* et al., 1998). The band that we found in 'Reka' does, however, differ with only one bp from the two closest bands (at 117.1 and 119.1 bp, respectively) found in other samples, suggesting that it results from a restructured allele. Also the crabapple 'Prairifire' has one allele, 112.1 bp, which does not fit into a 2 bp-step series of alleles. By contrast, the other band in 'Reka' was 129.4, which is close to the average size of 129.5 bp for a band found in 10 other samples (129.3–129.6).

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References

Boudichevskaia, A., Flachowsky, H., Fischer, C., Hanke, V. & Dunemann, F. (2004): Development of molecular markers for V_{r1}, a scab resistance factor from R12740-7A apple. Acta Hort. 663: 171–175.

Bus, V., van de Weg, W. E., Durel, C. E., Gessler, C., Calenge, F., Parisi, L., Rikkerink, E., Gardiner, S., Patocchi, A., Meulenbroek, M., Schouten, H. & Laurens, F. (2004): Delineation of a scab resistance gene cluster on linkage group 2 of apple. Acta Hort. 663: 57–62.

Bus, V. G. M., Rikkerink, E. H. A., van de Weg, W. E., Rusholme, R. L., Gardiner, S. E., Bassett, H. C. M., Kodde, L. P., Parisi, L., Laurens, F. N. D., Meulenbroek, E. J. & Plummer, K. M. (2005): The V_{h2} and V_{h4} scab resistance genes in two differential hosts drived from Russian apple R12740-7A map to the same linkage group in apple. Molecular Breeding 15: 103–116.

Cheng, F. S., Weeden, N. F., Brown, S. K., Aldwinckle, H. S., Gardiner, S. E. & Bus, V. G. (1998): Development of a DNA marker for V_m, a gene conferring resistance to apple scab. Genome 41: 208–214.

Crosby, J. A. , Janick, J., Pecknold, P. C., Korban, S. S., O'Connor, P. A., Ries, S. M., Goffreda, J. & Voordeckers, A. (1992): Breeding apples for scab resistance: 1945–1990. Fruit Var. J. 46:145–166.

Dayton, D. F. & Williams, E. B. (1968): Independent genes in *Malus* for resistance to *Venturia inaequalis*. Proc. Amer. Soc. Hort. Sci. 92: 89–93.

Durel, C. E., Calenge, F., Parisi, L., van de Weg, W. E., Kodde, L. P., Liebhard, R., Gessler, C., Thiermann, M., Dunemann, F., Gennari, F., Tartarini, S. & Lespinasse, Y. (2004): An overview of the position and robustness of scab resistance QTLs and major genes by aligning genetic maps of five apple progenies. Acta Hort. 663: 135–140.

Fischer, M. & Dunemann, F. (2000): Search for polygenic scab and mildew resistance in apple varieties cultivated at the fruit genebank Dresden-Pillnitz. Acta Hort. 538: 71–77.

Fischer, M., Geibel, M. & Fischer, C. (2003): The future of disease-resistant apples. Acta Hort. 622: 329–333.

Gianfranceschi, L., Koller, B., Seglias, N., Kellerhals, M. & Gessler, C. (1996): Molecular selection in apple for resistance to scab caused by *Venturia inaequalis*. Theor. Appl. Genet. 93: 199–203.

Gianfranceschi, L., Seglias, N., Tarchini, R., Komjanc, M. & Gessler, C. (1998): Simple sequence repeats for the genetic analysis of apple. Theor. Appl. Genet. 96: 1069–1076.

Hemmat, M., Brown, S. K. & Weeden, N. F. (2002): Tagging and mapping scab resistance genes from R12740-7A apple. J. Amer. Soc. Hort. Sci. 127: 365–370.

Hemmat, M., Brown, S. K., Mehlenbacher, S. A. & Weeden, N. F (2003): Identification and mapping of markers for resistance to apple scab from 'Antonovka' and 'Hansen's baccata #2'. Acta Hort. 622: 153–161.

Holb, I. J., Heijne, B., Withagen, J. C. M., Gáll, J. M. & Jeger, M. J. (2005): Analysis of summer epidemic progress of apple scab in different apple production systems in the Netherlands and Hungary. Phytopathology 95: 1001–1020.

Liebhard, R., Gianfranceschi, L., Koller, B., Ryder, C. D., Tarchini, R., Van de Weg, E. & Gessler, C. (2002): Development and characterisation of 140 new microsatellites in apple (*Malus* x *domestica* Borkh.). Mol. Breeding 10: 217–241.

MacHardy, W. E. (1996): Apple Scab, Biology, Epidemiology and Management, APS Press, Minnesota, USA. 545 pp.

Maliepaard, C., Alston, F. H., van Arkel, G., Brown, L. M., Chevreau, E., Dunemann, F., Evans, K. M., Gardiner, S., Guilford, P., van Heusden, A. W., Janse, J., Laurens, F., Lynn, J. R., Manganaris, A. G., den Nijs, A. P. M., Periam, N., Rikkerink, E., Roche, P., Ryder, C., Sansavini, S., Schmidt, H., Tartarini, S., Verhaegh, J. J., Vrielink-van Ginkel, M. & King, G. J. (1998): Aligning male and female linkage maps of apple (*Malus pumila* Mill.) using multi-allelic markers. Theor. Appl. Genet. 97: 60–73.

Vejl, P., Skupinová, S., Blazek, J., Sedlák, P., Bardová, M., Drahsová, H., Blazková, H. & Milec, Z. (2003): PCR markers of apple resistance to scab (*Venturia inaequalis* Cke.) controlled by V_f gene in Czech apple breeding. Plant Soil Environ. 49: 427–432.

Vinatzer, B. A., Patocchi, A., Tartarini, S., Gianfranceschi, L., Sansavini, S. & Gessler, C. (2004): Isolation of two microsatellite markers from BAC clones of the V_f scab resistance region and molecular characterization of scab-resistant accessions in *Malus* germplasm. Plant Breeding 123: 321–326.