Challenges in breeding high quality apples with durable disease resistance

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Abstract

*The appearance of scab (*Venturia inaequalis*) strains that are able to overcome the broadly used Vf (Rvi6) scab resistance as well as the spread of new diseases such as* Marssonina coronaria*, highly relevant for organic apple growing, require adapted strategies for the breeding of new varieties. Approaches for breeding new high quality apple varieties with diverse resistance to several diseases considering phenotypic and molecular selection techniques and results achieved up to now in the Agroscope apple breeding programme will be shown and discussed.*

Keywords: apple breeding, molecular markers, *Venturia inaequalis, Erwinia amylovora, Podospharea leucotricha*

Introduction

Breeding for durable disease resistance combined with high fruit quality and good and regular yields is a major objective in many apple breeding programmes throughout Europe and in most areas of apple growing worldwide. The major apple diseases are apple scab (*Venturia inaequalis*), powdery mildew (*Podosphaera leucotricha*) and fire blight (*Erwinia amylovora*). However, storage diseases are also of importance, especially in organic growing, and some new diseases such as the leaf disease *Marssonina coronaria* require attention. Apple breeding aims developing high quality varieties with durable disease and pest resistance. MAB (marker-assisted breeding) is an extremely useful approach to achieve this goal (Kellerhals *et al*., 2013).

Apple scab

Apple scab is the most devastating fungal disease in apple growing in most temperate growing conditions (Gessler *et al*., 2006). Breeding for scab resistance has a long history going back to the beginning of the 20st century and getting more intensive after the Second World War. A large range of scab resistant cultivars was released in the last decades. However, most of them go back to the resistance derived from *Malus floribunda* 821, called *Vf* and later renamed *Rvi6* (Bus *et al*. 2009). Since about 20 years there is more frequent resistance breakdown observed in some cultivars carrying *Rvi6* resistance (Parisi *et al*., 1993). However, some other carriers of *Rvi6* are still resistant under comparable disease pressure and showing no scab symptoms at all. It is anticipated that this resistance may be due to strong QTLs present in these cultivars. Within the Vinquest initiative (www.vinquest.ch), information about the emergence and distribution of virulent *V. inaequalis* pathotypes is collected. This project aims to introduce the monitoring of *V. inaequalis* virulences on a regular basis (Patocchi *et al*., 2009). A set of differential hosts has been identified for the monitoring of *V. inaequalis* populations in specific orchards established in locations spread over many apple growing areas mainly in Europe. The set will be extended as new gene-for-gene relationships are defined and appropriate hosts identified. The findings of this research assist breeders in determining promising combinations of scab resistance genes for breeding strategies based on gene pyramiding.

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Pyramiding of scab resistance genes has become easier as molecular tools for the detection of the presence of those genes are available (Patocchi *et a*l., 2009). The Agroscope apple breeding program considers pyramiding of different scab resistance sources:

- Major genes such as *Rvi6*, *Rvi2*, *Rvi4*, *Rvi11*, *Rvi12* and *Rvi15*.
- Partial resistance originating from parents that are known to carry this characteristic.

The objective is to consider a broad range of genetic background in order to enlarge the genetic base in apple breeding as suggested also by Bannier (2011).

Fire Blight

The development of fire blight tolerant and/or resistant cultivars is of great importance for sustainable fire blight management. Currently only a few tolerant cultivars are supposed to fulfill market requirements, e.g. 'Ladina', developed by Agroscope in Wädenswil (Leumann *et al*., 2013). Genotypes with low fire blight susceptibility have been detected among advanced selections in breeding programmes, fruit genetic resources and commercial cultivars (Szobiczewski *et al*., 2011). Strong fire blight resistance is present in different wild *Malus* species, e.g. *M.* × *robusta* 5 (MR5) (Peil *et al*, 2008), *M. baccata* (Norelli *et al*., 1986, Peil *et al*., 2009), *M. fusca*, *M. baccata and* 'Evereste' of unknown origin (Durel *et al.,* 2009). However, fruit of wild resistance genitors is of small size and low eating quality. The introduction of wild species resistance into commercial fruit quality is time-consuming. It requires repeated pseudo-backcrossing with high quality parents followed by selection for the desired traits within the progeny (Baumgartner *et al*., 2011). The undesired share of wild apple genome is reduced on average by 50 % with each introgression cycle. However, with traditional breeding, the juvenile period of apple lasts four to five years, slowing down the introduction of new traits. Fast-track breeding approaches are used to shorten the generation time from usually four or five years to about two years (Volz *et al*., 2009, Baumgartner *et al*., 2011). With a set of molecular markers covering the whole genome, individuals might be identified with a high proportion of genome inherited from the elite parents (Volz *et al*., 2009). Selection for fire blight resistance can be based on molecular markers or phenotypic inoculation tests. A strong fire blight resistance QTL (FB_MR5) was identified on linkage group 3 in *M.* × *robusta* 5 (Peil *et al*., 2008). SSR (simple sequence repeat, microsatellite) markers are available for marker assisted selection (Calenge *et al*., 2005, Khan *et al*., 2007, Peil *et al*., 2008, LeRoux *et al*., 2010, Fahrentrapp *et al*., 2013). Furthermore, phenotypic evaluation of tree and fruit characteristics is included in the selection of resistant plants for further introgression cycles.

Material and Methods

Apple scab

Based on marker-assisted parent selection, apple crosses were performed in spring 2012 with the aim to pyramid different scab resistances (*Rvi6*, *Rvi2*, *Rvi4*). Seeds were extracted in autumn and subsequently stratified for 8 weeks in humid sand at 2°C to allow for regular germination in seed trays at the end of January 2013. Seedlings were grown in the glasshouse at 18 to 20°C and artificially inoculated at the 4 to 6 leaf stage using a suspension of conidia dispersed in water at a concentration of 350'000 conidia per ml and high relative humidity. The inoculum was originally collected from scabbed leaves originating from apple trees not treated with fungicides and subsequently used for the glasshouse scab screening where leaves with strong scab symptoms were collected and frozen at -20°C for further inoculations. The inoculum should not contain isolates vir2, vir4

or vir6. Around 14 DPI (days post infection) scoring of leaf symptoms was performed using basically the scale of Chevalier *et al*. (1991), added with the symptoms induced by the scab resistance gene *Rvi2* (SN, stellate necrosis) and *Rvi4* (PPP, pin point pits) according to Bus *et al*. (2005). Molecular analysis was performed in collaboration with the company Ecogenics, Schlieren, Switzerland (www.ecogenics.ch) following the protocol of Frey *et al*. (2004) using multiplex PCR's with fluorescently labelled primers. SSR and SCAR (sequence characterized amplified regions) markers linked to the scab resistance genes *Rvi*2, *Rvi*4, *Rvi*6 and the powdery mildew resistance locus *Pl*1 were used according to Table 1.

Table 1: Molecular markers used for the analysis of the progenies

Resistance gene	*Marker used*	*Marker type*
Rvi2	CH05E03, OPL19	SSR, SCAR
Rvi4	CH02C02a, Hi22d06	SSR, SSR
Rvi6	CHVf1	SSR
Pl1	AT20	SCAR

Fire blight

Fire blight artificial glasshouse screening was performed according to Baumgartner *et al*., (2012). Lesion length was measured 7, 14 and 21 DAI (days after inoculation) and transformed in percent of total shoot length. The cultivars 'Enterprise' and 'Gala Galaxy' were used as resistant and susceptible control, respectively.

Results and Discussion

At Agroscope, we systematically test the parents of crosses for the presence of resistance loci towards apple scab, powdery mildew and, if suitable and available, also towards fire blight prior performing crosses. In Fig. 1 the presence of scab resistance genes based on the analysis of markers closely linked to them, is indicated for the parents. The segregation in different resistance classes and susceptibility (class 4) is displayed. Depending on the genetic background introduced from the respective parents, the segregation in resistance classes and susceptibility is variable. *Rvi6* induces symptoms as described by Chevalier *et al.* (1991) with classes 0, 1, 2, 3a, 3b and 4. However, we usually do not observe pin point pits (class 1, PPP) when incorporating only *Rvi6*. When *Rvi4* or *Rvi15* is present in a parent, PPP should be observed in a share of the progeny plants. Fig. 1 clearly shows that this was the case for progeny 1 (one parent with *Rvi15*) and for progeny 2 (one parent with *Rvi4*) with almost 50% class 1 (PPP). Stellate necrosis (SN) is a symptom related to *Rvi2*. The progeny 'Golden Delicious. x Gala' was used as control for the quality of the scab inoculation. In this progeny, as expected, almost 100% of the progeny plants were susceptible (class 4).

Molecular markers were applied to detect the presence of the different scab resistance genes. This allowed comparing the phenotypic observation (Fig. 1) with the molecular results. This comparison is shown in Figures 2 and 3. In general, the correspondence of phenotypic and molecular results is good. The presence of at least one of the scab resistance markers for *Rvi6*, *Rvi2* and *Rvi4* should lead to a reaction class of 0-3b according to Chevalier *et al*. (1991) and/or SN or pin point pits (PPP). Only in one single plant SN was observed phenotypically without the presence of the markers used for Rvi2. Fourteen plants (7%, expected 6.25%) showed class 4 susceptibility reaction and no marker was present in these genotypes.

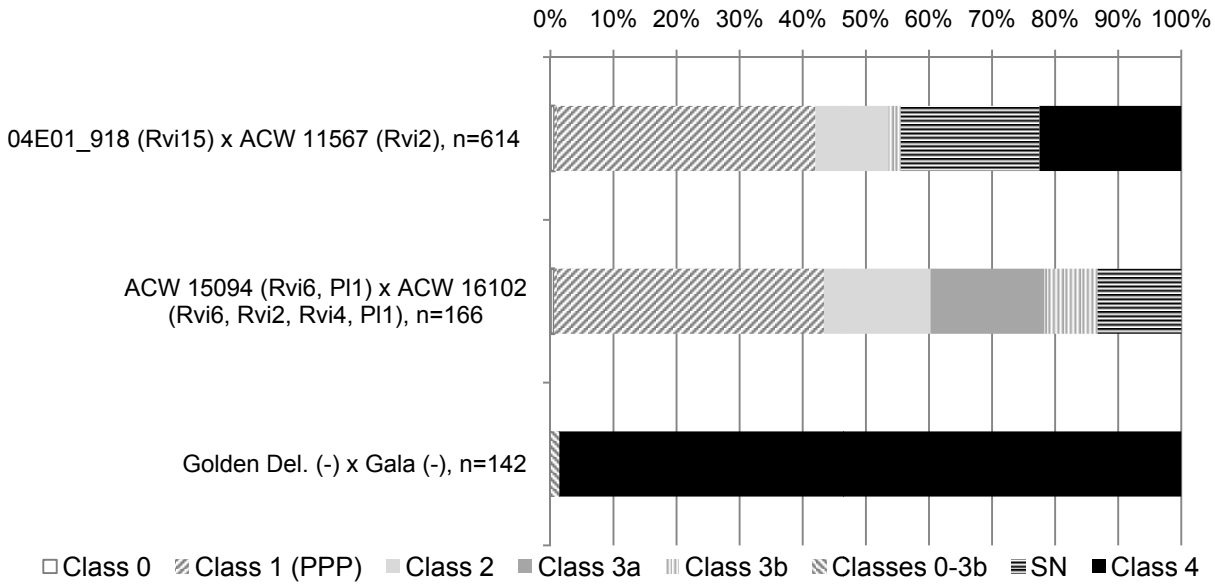

Figure 1: Segregation of progenies for different scab resistance and susceptibility classes in the glasshouse seedling inoculation test from crosses integrating a range of different scab resistance genes.

Seven plants showed a class 4 reaction but also at least one marker for a scab resistance locus. Such deviations might be due to false interpretation of phenotypic symptoms or possible recombination between the resistance gene and the corresponding SSR marker. A total of 40 plants (20.3 %) showed *Rvi6* in homozygous status. As both parents carry *Rvi6* in a heterozygous situation a share of 25 % homozygous progeny plants was expected.

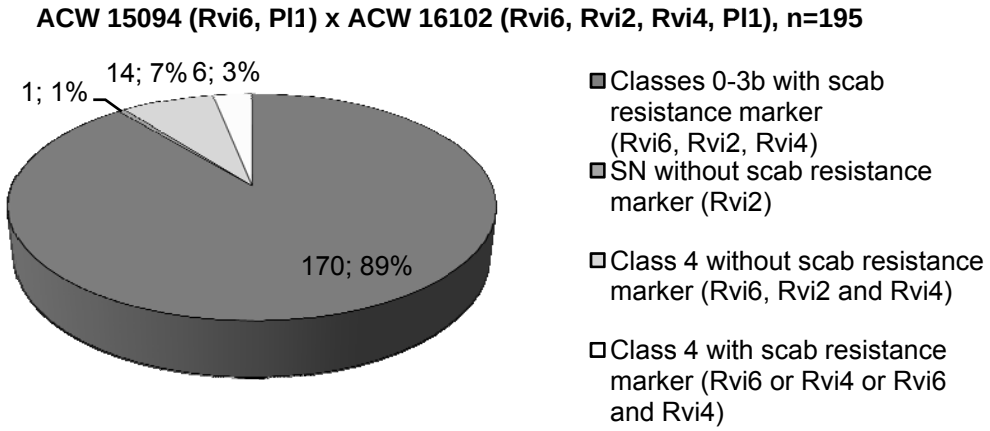

Figure 2: Comparison of phenotypic and molecular analysis of the progeny ACW 15094 x ACW 16102 for the scab resistance genes *Rvi6*, *Rvi2* and *Rvi4.*

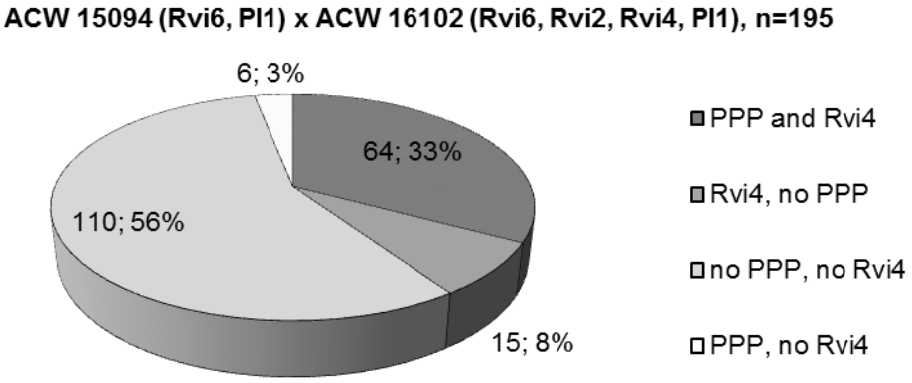

Figure 3: Comparison of phenotypic and molecular analysis of the progeny ACW 15094 x ACW 16102 for the scab resistance genes *Rvi4* only.

Figure 3 displays the analysis of the same progeny but focused on the *Rvi4* resistance derived from the parent 'ACW 16102'. 33 % of the progeny plants carried the marker for *Rvi4* and phenotypically expressed PPP. In 8 % of the plants the marker for *Rvi4* was present but phenotypically no PPP were visible. 56 % of the plants showed no PPP and did not carry the marker. 3 % showed PPP symptoms but did not carry the marker. In general, a segregation of 50:50 in carrying and not carrying *Rvi*4 should be expected. This ratio was almost obtained and the deviation from what has been expected might be due to the fact that the PPP symptoms might have been masked by symptoms caused by other genes present (*Rvi6* and or *Rvi2*).

Agroscope has developed a range of advanced selections carrying pyramided scab resistance. Some of these selections with pyramided scab resistance were planted in orchards located in Germany in collaboration with FOEKO, where resistance breakdown occurred in *Rvi6*-cultivars. First year observations have shown no scab on selections with pyramided scab resistance. However it is too early to draw conclusions.

Fire blight

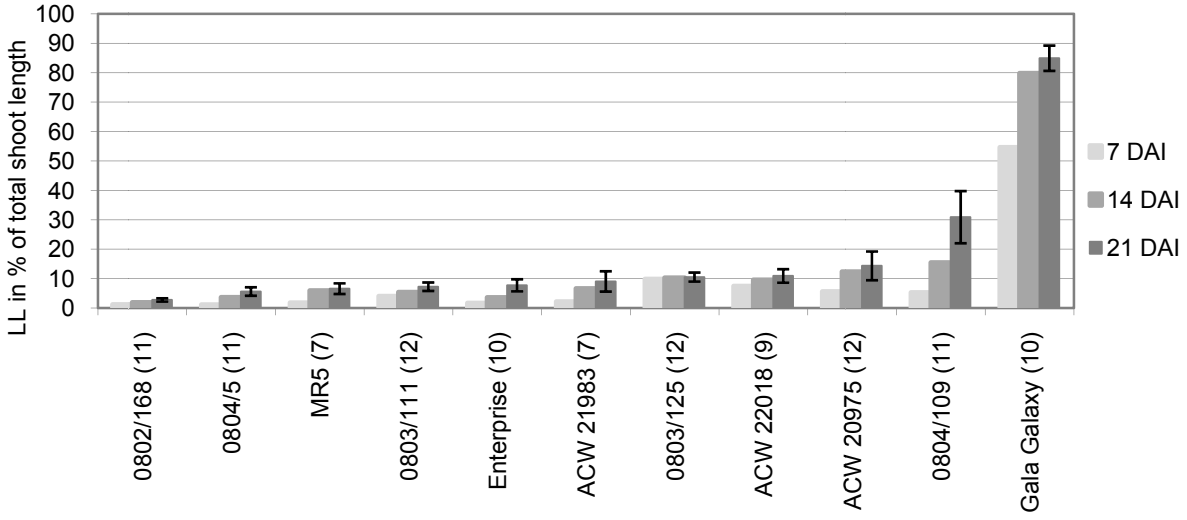

Figure 4: Mean lesion length (LL) in percent of total shoot length (with standard error) for 'MR5' and eight F2 MR5 progeny plants compared to 'Enterprise' and 'Gala Galaxy' 7, 14 and 21 DAI with *E. amylovora* (in brackets number of inoculated plants).

Results of artificial shoot inoculation tests in the glasshouse are shown in Figure 4 for '*M. x robusta* 5' and eight F2 genotypes carrying MR5 resistance. 'Enterprise' (highly tolerant) and 'Gala Galaxy' (susceptible) were used as controls. All F2 progeny genotypes tested display a high level of fire blight tolerance, with two genotypes that showed even less symptoms than '*M. x robusta* 5'. Vogt *et al*. (2013) have detected a gene-for gene relationship in the host-pathogen system *M. x robusta* 5 – *E. amylovora.* The breeding of highly fire blight resistant cultivars is under way and F3 generations from the original wild ancestor were obtained. However, although applying fast-track breeding approaches without genetic modification, it will take another 10 to 15 years to release a cultivar carrying FB_MR 5 fire blight resistance having commercial fruit quality.

Conclusion

The development of apple cultivars with commercial fruit quality and carrying pyramided resistance towards scab, powdery mildew and fire blight is under way. Molecular selection is a valuable tool to achieve this goal. However, there is need to broaden the genetic basis and to develop tools for more frequent integration and detection of partial resistances known to be durable and present in modern selections but also in old varieties (Kellerhals *et al*., 2012).

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References

Bannier H.-J. (2011): Moderne Apfelzüchtung: Genetische Verarmung und Tendenzen zur Inzucht. *Erwerbs-Obstbau* **52**: 85-110

Baumgartner, I.O, Patocchi, A., Franck, L. & Kellerhals, M. (2011). Fire blight resistance from 'Evereste' and Malus sieversii used in breeding for new high quality apple cultivars: strategies and results. *Acta Hort*. (ISHS) **895**: 391-397.

Baumgartner, I.O., Leumann L.R., Frey J.E., Joos M., Voegele R.T. & Kellerhals M. (2012). Breeding apples to withstand infection pressure by fire blight and other diseases. *Proceedings of the 15th Ecofruit Conference 2012, Stuttgart-Hohenheim*. 14-21.

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 Vh2 and Vh4 scab resistance genes in two differential hosts derived from Russian apple R12740-7A map to the same linkage group of apple. *Mol. Breeding* **15**: 103-116

Bus, V., Rikkerink, E., Aldwinckle, H.S., Caffier, V., Durel, C.E., Gardiner, S., Gessler, C., Groenwold, R., Laurens, F., Le Cam, B., Luby, J., Meulenbroek, B., Kellerhals, M., Parisi, L., Patocchi, A., Plummer, K., Schouten, H.J., Tartarini, S. & van de Weg, W.E. (2009). A Proposal for the Nomenclature of *Venturia inaequalis* Races. *Acta Hort*. *814*: 739-746

Calenge, F., Drouet, D., Denancée´, C., Van de Weg, W.E., Brisset, M.-N., Paulin, J.P. & Durel, C.-E. (2005). Identification of a major QTL together with several minor additive or epistatic QTLs for resistance to fire blight in apple in two related progenies. *Theor. Appl. Genet*. **111**(1): 128–-135. doi:10.1007/s00122-005-2002-z. PMID:15856158.

Chevalier M., Lespinasse Y. & Renaudin S. (1991): A microscopic study of the different classes of symptoms coded by the Vf-gene in apple for resistance to scab (*Venturia inaequalis*). *Plant Pathology* **40**: 249-256

Durel, C-E., Denancé, C. & Brisset, M.-N. (2009). Two distinct major QTL for resistance to fire blight co-localize on linkage group 12 in apple genotypes 'Evereste' and Malus floribunda clone 821. *Genome* **52**: 1-9.

Fahrentrapp, J, Broggini, G.A.L., Gessler, C., Peil, A., Kellerhals, M., Malnoy, M., & Richter, K. (2013). Fine Mapping of the Fire Blight Resistance Locus in *Malus x robusta* 5 on Linkage Group 3. *Acta Hort*. **976**: 499-500.

Frey J.E., Frey B., Sauer C., Kellerhals M. (2004). Efficient low-cost DNA extraction and multiplex fluorescent PCR method for marker-assisted selection in breeding. *Plant Breed*.**123**: 554-557

Gessler C., Patocchi A., Sansavini S., Tartarini S. & Gianfranceschi L. (2006): *Venturia inaequalis* resistance in apple. *Critical reviews in plant sciences* **25**: 473-503

Kellerhals, M., Szalatnay, D., Hunziker, K., Duffy, B., Nybom, H., Ahmadi-Afzadi, M., Höfer, M., Richter, K. & Lateur M. (2012). Diversity in European pome fruit genetic resources evaluated for disease resistance. *Trees* **26**: 179-–189. DOI: 10.1007/s00468 – 011 – 0660 – 9.

Kellerhals, M., Baumgartner, I.O., Leumann, L., Frey, J.E. & Patocchi, A. (2013). Progress in Pyramiding Disease Resistances in Apple Breeding. *Acta Hort*. **976**: 487-491.

Khan, M.A., Durel, C.-E., Duffy, B., Drouet, D., Kellerhals, M., Gessler, C. & Patocchi, A. (2007). Development of molecular markers linked to the 'Fiesta' linkage group 7 major QTL for fire blight resistance and their application for marker-assisted selection. *Genome*, **50**(6): 568–577. doi:10.1139/G07-033. PMID: 17632578.

Le Roux, P.-M.F., Khan, M.A., Broggini, G.A.L. Duffy, B., Gessler, C. & Patocchi, A. (2010). Mapping of quantitative trait loci for fire blight resistance in the apple cultivars 'Florina' and 'Nova Easygro'. *Genome* **53**: 710-722.

Leumann L., Baumgartner I.O., Lussi L., Frey L., Nölly M., Kellerhals M. & Weber M., (2013). Ladina, die neue feuerbrandrobuste Apfelsorte. *Schweiz. Z. Obst- und Weinbau*, **1**, 10-13.

Markussen T, Krüger J, Schmidt H, & Dunemann F (1995). Identification of PCR-based markers linked to the powdery mildew resistance gene Pl1 from Malus robusta in cultivated apple. *Plant Breeding* **114**:530-534.

Norelli, J.L., Aldwinckle, H.S. & Beer, S.V. (1986). Differential susceptibility of *Malus* spp. cultivars Robusta 5, Novole, and Ottawa 523 to *Erwinia amylovora*. *Plant Disease* **70**: 1017-1019.

Patocchi A, Frei A., Frey J. E., & Kellerhals M. (2009). Towards improvement of marker assisted selection of apple scab resistant cultivars: *Venturia inaequalis* virulence surveys and standardization of molecular marker alleles associated with resistance genes. *Molecular Breeding* **24**: 337-347

Parisi L., Lespinasse Y., Guillaumes J. & Krüger J. (1993): A new race of Venturia inaequalis virulent to apples with resistance due to the Vf gene. *Phytopathology* **83**: 533-537.

Peil, A., Hanke, M.-V., Flachowsky, H., Garcia-Libreros, T., Horner, M. & Bus, V. (2008). Confirmation of the fire blight QTL of Malus robusta 5 on linkage group 3. *Acta Hort*. **793**: 297-–303.

Peil, A., Bus, V.G.M., Geider, K., Richter, K., Flachowsky, H. & Hanke, M.-V. (2009). Improvement of Fire Blight Resistance in Apple and Pear. *Intl. J. Plant Breed.* **3**:1-27.

Szobiczewski P., Mikiciński A., Lewandowski M., Żurawicz E., Peil A., Richter K. & Kellerhals M. (2011). Selection for fire blight resistance of apple genotypes originating from European genetic resources and breeding programs. *Acta Hort*. **896**: 399-405.

Vogt, I., Wöhner, Th., Richter, K., Sundin, G.W., Wensing, A., Savory, E.A., Geider, K., Day B., Hanke, M.-V. & Peil, A. (2013). Gene-for gene relationship in the host-pathogen system Malus x robusta 5 – Erwinia amylovora. *New Phytologist* **197**: 1262-1275.

Volz, R.K., Rikkerink, E., Austin, P., Lawrence, T. & Bus, V.G.M. (2009). "Fast-breeding" in apple: a strategy to accelerate introgression of new traits into elite germplasm. *Acta Hort*. (ISHS) **814**: 163-168.