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Pyramiding resistance genes and widening the genetic base of the apple (*Malus* × *domestica* Borkh.) crop

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Abstract: Apple breeding is active worldwide and yet the apple crop is in a precarious state as it relies on few dominant cultivars and only the *Rvi6* (formerly *Vf*) gene, that confers resistance to scab, has been extensively exploited in the cultivars entered the market in recent years. However, there are some 20 disease resistance genes described in apple and the apple germplasm includes thousands of accessions in the repositories. In this paper, a breeding programme is described, whereby 36 genotypes, including ancient and contemporary apple cultivars, were crossed to produce a new set of selections that combine extensive genetic resources with pyramided resistance genes to several apple diseases, such as scab and powdery mildew. The 110 cross combinations carried out successfully, of the 260 initially planned, produced 7,876 offsprings, reduced to 2,969 after screening with molecular markers associated with five resistance genes. Selections with three or two resistance genes and good agronomic characteristics were kept for further field observations with the aims of creating new cultivars for the market and new parents for future breeding projects.

Keywords: Fruit crop; fruit genetics; fruit breeding; pathogen-resistant genes; marker-assisted breeding; sustainable agriculture; horticulture.

1. Introduction

Apple breeding is well developed worldwide. Reviews of reports and websites as well as visits to experimental stations worldwide indicate two key features of the approaches to apple improvement: (1) emphasis on the introduction of resistance to disease in new selections and, on the other hand, (2) recurrent and frequent use of superior genotypes that are in many cases the most popular cultivars in the market.

The first feature is certainly positive and the inclusion of resistance to diseases and, to a more limited extent, pests in the mating designs responds to the need for making the apple crop more sustainable, as is recommended, for instance, by the European Union (https://ec.europa.eu/info/food-farming-fisheries/sustainability/environmental-sustainability_en).

In apple, resistance genes to scab (*Venturia inaequalis*), powdery mildew (*Podosphaera leucotricha*), fire blight (*Erwinia amylovora*), apple canker (*Nectria galligena*) and woolly aphid (*Eriosma lanigerum*) have been identified, mapped in most cases, and markers for marker-assisted breeding (MAB) have been developed for some of them (Patocchi et al., 2009; Bus et al., 2011; Laurens et al., 2011; Baumgartner et al., 2015; Khajuria et al., 2018; Papp et al., 2020). Pyramiding resistance genes has become a common practice in apple breeding (Baumgartner et al., 2015) and from this point of view the most recent achievements are valuable (Patocchi et al., 2020).

The second feature is the restricted genetic diversity of the crop owing to the recurrent use of superior genotypes (Janick et al., 1996; Noiton and Alspach 1996). In spite of the high number of apple cultivars described in the many world pomologies, and estimated 7,000 (Way et al., 1990) to 30,000 (http://www.agri-benchmark.org/), the process of selection focused on aesthetic standards, cold storage and shelf life imposed by the market has led to the recurrent use of a small number of parental genotypes and the consequent narrowing of the genetic base (Janick et al., 1996). A pedigree analysis carried out on 439 apple cultivars revealed that a few founding clones, namely 'Cox's Orange Pippin', 'Golden Delicious', 'Red Delicious', 'Jonathan' and 'McIntosh' were included in the pedigree of 64% of the cultivars investigated (Noiton and Alspach 1996). It is interesting but, among a group of 27 cultivars carrying the *Vf* gene for scab resistance analysed in the same work, the coefficients of coancestry with the five founding clones were of the same order of magnitude.

The recent history of the crop does not show great changes. For instance, in 2007 only 13 apple cultivars covered nearly 72% of the entire European apple acreage according to the agribenchmark foundation (http://www.agribenchmark.org/). A similar situation is reported for the United States, where nine top cultivars with large coancestry dominate the market, with a cohort of dozens of first-degree relatives, so that authors state that "Americans are eating apples largely from a single family tree" (Migicovsky et al., 2021). Pedigrees of many recent cultivars show that the use of few popular cultivars of the market as 'good' parents is still common amongst breeders (Muranty et al., 2020; Migicovsky et al., 2021), while the scab-resistant cultivars of the market still rely mainly on the *Rvi6* (formerly *Vf*) gene from *Malus floribunda 821*, a monogenic resistance whose exploitation in breeding dates back to 1945 (Hough et al., 1953, Patocchi et al., 2009).

Some twenty scab resistance genes have been described in recent years (Bus et al., 2011, Papp et al., 2020) and some of these have entered national breeding programs in Brazil, the Czech Republic, France, Poland, Romania, Russia, The Netherlands, the United Kingdom, and the United States of America (Crosby et al., 1992), and more recently in Belgium, Germany, Hungary, Italy, Latvia, Lithuania, New Zealand, Norway, Serbia and Switzerland (Sansavini et al., 2004; Patocchi et al., 2020), and possibly other countries not mentioned in the cited papers.

It is still hard to predict the value of the progeny derived from controlled crosses since the apple is self-incompatible (Way et al., 1990; Janick et al., 1996). The most long-lasting apple PRI (Purdue University, Rutgers University and the University of Illinois) program jointly managed by the three Institutions of the acronym concluded that some 380,000 screened seedlings yielded as few as 9 released selections in 45 years of activity (Crosby et al., 1992).

Marker-assisted selection (MAS) and genomic selection based on the genome-wide estimation of breeding value of individuals are promising approaches to apple breeding but are still far from being implemented (Kumar et al 2012).

We provide in the present paper a contribution to the apple breeding effort by illustrating the results of a breeding program initiated in 1998 at the University of Udine, Italy, inspired by a paper from Noiton and Shelbourne (1992), with the aims of widening the genetic diversity of the cross populations and combining multiple resistances to several pathogens, such as scab, powdery mildew and others.

2. Materials and Methods

2.1. The mating design

The crossing plan was based on a North Carolina mating design II (NC II), where half-sib families are produced which have either the same father but different mothers or vice versa (Acquaah 2012). The seed parents were initially represented by 26 commercial cultivars, including ancient and contemporary cultivars with minimal coancestry according to the known pedigrees and showing large genetic diversity in phenotypic traits, such as ripening time, fruit size and shape, skin colour and fruit taste and storage (Table 1).

Table 1. Cultivars used mainly as seed parents in the apple mating design. Information on the genetic and geographic origins is from several well-known pomological books such as 'The apples of New York' (Beach et al., 1903), the 'fruitID' website (https://www.fruitid.com/), the paper from Way et al. (1990), as well as the recent papers of Muranty et al. (2020) and Migicovsky et al. (2021), based on molecular kinship analysis.

Cultivar	Genetic origin	Geographic origin
Ancient cultivars		
'Annurca rossa'	Unknown	Campania, Italy
'Api Etoilée' *	Unknown	France
'Appia'	Unknown	Lazio, Italy
'Astrakhan white'	Unknown	Baltic Sea States
'Astrakhan red'	Unknown	Baltic Sea States
'Belle de Boskoop' *	Chance seedling or 'Reinette de Monfort' mutant	The Netherlands
'Calville blanc d'hiver'	'Calville Rouge' × 'Reinette Franche'	Possibly France
'Calville rouge'	Unknown	Possibly France
'Decio'	Unknown	Veneto, Italy
'Gelata'	Unknown	Centre-Southern Italy
'Limoncella'	Unknown	Italy
'McIntosh'	Chance seedling	Ontario, Canada
'Permain dorée'	Unknown	Europe
'Rosa mantovana'	Unknown	Lombardy, Italy
Modern cultivars		
'Delicious'	syn. 'Red Delicious', possibly 'Yellow Bellflower' × unknown	U.S.A.
'Di Corone'	syn. 'Prima', Co-op 2, PRI 14-510 × N.J. 123249	U.S.A.
'Fuji'	'Ralls Janet' × Delicious'	Japan
'Golden Delicious'	'Grimes Golden' × unknown	U.S.A.
'Granny Smith'	possibly French crab × unknown	Australia
'Jonagold' *	'Golden Delicious' × Jonathan'	U.S.A.
'M014.05' *	Chance seedling	Kazakhstan
'M015.01' *	Chance seedling	Kazakhstan
'M018.04'	Chance seedling	Kazakhstan
'Red Chief '	Belongs to the Starking's mutant family	U.S.A.
'Rome Beauty'	Chance seedling	U.S.A.
'Royal Gala'	'Gala' clone = 'Kidd's Orange Red' × 'Golden Delicious'	New Zealand
'Stark Splendour'	Uncertain pedigree	New Zealand

* Several crosses derived from these parents were abandoned for different reasons, but mainly because of limited seed yield.

Pollen donors were initially represented by ten selections carrying multiple genes of resistance to several apple diseases, such as scab, powdery mildew and fire blight. Resistance to woolly aphid was also included. The pollen donors are listed in Table 2 (see also Figure 1).

The 260 initial cross combinations were reduced during the implementation of the program, mainly because markers associated to several resistant loci were not retrieved from the literature and because of the poor fruit set and seed yield of several cross combinations. The direction of the crosses is merely indicative and reciprocal crosses could be carried out as required by the different flowering times.

Table 2. Parents carrying resistance genes/QTLs used in the mating design. These resistant parents were used mainly as pollen donors.

Selection (*)	Pedigree	Genes/QTLs of resistance to scab (b)	Further resistance genes (c)
'Antonovka'DCA* (a)	unknown	*Va*	
'Ariwa'	Golden Delicious × A 849-5	*Rvi1, Rvi6*	*Pl1, FB*
'DD59'*	Durello × Discovery	*Rvi13, QTL* (Discovery)	
'DF120'*	Durello × Fiesta	*Rvi13, QTLs* (Fiesta)	
'Florina'*	BC ×, Rome beauty, M. floribunda	*Rvi1, Rvi6*	*WA*
'GK13'	Goldrush × Realka	*Rvi1, Rvi2,Rvi4, Rvi6,*	
'GM37'	Goldrush × Murray	*Rvi1, Rvi5, Rvi6*	
'Golden Orange'	Ed Gould Golden × PRI 1956-6	*Rvi6*	PM_{QTL}
'GR40'*	Goldrush × Russian seedling R12	*Rvi2, Rvi4*	
'HM100'	Harmony × Murray	*Rvi5, Rvi6*	

* These selections were used in a reduced number of cross combinations during the implementation of the programme, either because we were not able to find in time the markers associated to R genes or QTLs and because of the limited seed yield (see results for details)
'Antonovka' DCA = an 'Antonovka' genotype introduced from the University of Bologna collection and whose resistance locus is still under investigation (Bus et al., 2012);
Va = resistance gene/QTL carried by 'Antonovka' not yet identified (Bus et al., 2012), *Rvix* = scab resistance genes coded according to Bus et al., 2011 (see Table 3 for the correspondence with the former names of genes, QTL = Quantitative Trait Loci
Pl-1 = gene of resistance to powdery mildew; *FB* = resistant to fire blight, PM_{QTL} = possible QTL of resistance to powdery mildew, *WA* = gene of resistance to woolly aphid.

2.2. Controlled pollination

Pollen was extracted from flowers collected at the balloon stage and stored at 4°C until use. Cymes in seed parents were bagged before flowers opened. Flowers were hand pollinated without demasculation, since self-incompatibility makes self-pollination very rare (Janick et al., 1996). After fruit set, the paper bags were removed and replaced with net bags until fruit harvest. Fruit were collected overripe, seeds were extracted, dried at room temperature for two days and stored in paper bags at 4°C up to mid-January, when seeds were moistened overnight and sown.

2.3. Seed sowing and seedling growth

Seeds were sown in 96-hole trays filled with the commercial soil mix Brill3 (65% blonde peat, 35% black peat) covered with a thin layer of fine vermiculite and moistened. Trays were stacked, covered with polyethylene and stored for 45 days against a shaded north-facing wall to allow the day-night alternating temperatures overcome seed dormancy. Finally, trays were transferred to a cold greenhouse (up to 28 °C by day and down to 10 °C at night) for germination. Young seedlings with two leaves were transplanted to 35-hole trays and subsequently into 1-litre pots and grown in a heated greenhouse.

2.4. Phenotyping

A preliminary phenotype screening was carried out for three years, by observing the incidence of scab and discarding any susceptible seedlings. This helped reduce the number of molecular analyses (see below).

Phenotypic observations were carried out the first year on potted seedlings by spraying a suspen-

sion of 4.5×10^5 conidia/ml, counted in a Thoma chamber. The sprayed seedlings were then held for two days in a greenhouse at 18-20 °C and c. 95% RH maintained with a humidifier. The second and third years, observations were carried out in the orchard to which seedlings had been transplanted and infection was induced by hanging on the row wires scab-diseased apple shoots collected from commercial orchards.

Figure 1. Six apple selections of those listed in Table 2 and carrying resistances to diseases such as scab, powdery mildew, fire blight, and aphids and used as R parents to transfer pyramided resistances to a large array of susceptible seed parents.

2.5. Molecular screening

Two 5 mm-diameter discs were cut with a paper punch from young leaves of the remaining seedlings, placed in 96-well plates and stored at -80 °C until DNA isolation. Genomic DNA was extracted by incubating the discs for 10 min at 95 °C in 100 µL of extraction solution (E7526, Sigma-Aldrich, St. Louis, MO, USA). Subsequently, an equal volume of dilution solution (D5688, Sigma-Aldrich, St. Louis, MO, USA) was added to neutralize the extract. Then 1 µL of the extract was resuspended in 100 µl of dilution solution and two µl of diluted extract were used as template for PCR.

SSRs associated with resistance genes were selected from the literature and from information from colleagues and are reported in Table 3. Because markers associated with the Rvi2 gene were far apart, two markers were analysed, one (CH02b10) 8 cM upstream and one (CH05e03) 10 cM downstream of the mapped locus, following the suggestion of Tanksley (1983) to minimise recombination to crossover interference. Only progeny carrying alleles of both markers coupled with the R locus were considered to carry the resistant haplotype.

Forward primers were labelled at the 5' end with 6-FAM or HEX dyes (Sigma-Aldrich, St. Louis, MO, USA). PCRs were multiplexed and carried out by using the Type-It Microsatellite PCR kit (Qiagen) according to the manufacturer's instructions. Reactions were carried out on 10 µl. The PCR steps consisted of 5 min initial activation at 95 °C, followed by 28 to 30 3-step cycles of: 30 s denaturation at 95 °C, 90 s annealing at 60 °C, 30 s extension at 72°C, and 30 min of final extension at 60 °C.

Table 3. Markers associated with the different resistance loci used for the molecular screening of seedlings. Information on markers, alleles and location in the linkage group were retrieved at the time when the molecular screening was carried out.

Gene (a)	Linkage group (b)	Marker (c)	Allele in coupling (bp)	Distance from the locus (cM)	Reference
Rvi2 (Vr,Vh2)	2	CH02b10	118	8 (up)	Bus et al 2005
		CH05e03	166	1.6	Patocchi 2008 pers comm
Rvi4 (Vr,Vh4)	2	CH02c02a	176	5	Bus et al 2005
Rvi5 (Vm)	17	Hi07h02	225	0	Patocchi et al 2005
Rvi6 (Vf)	1	CH-Vf1	158	0	Vinatzer et al 2004
Pl1	12	Pl-1-AT20	450	4.5	Markussen et al 1995

Former name of the gene in brackets
The linkage groups follow the nomenclature of N'Diaye et al., (2008) cited in Bus et al., (2011) except for Pl-1 for which the map location is from Dunemann et al., (2007)
Primers were downloaded from the Hidras website (https://sites.unimi.it/camelot/hidras/); primers are available also at the Rosaceae web site (https://www.rosaceae.org/search/markers).

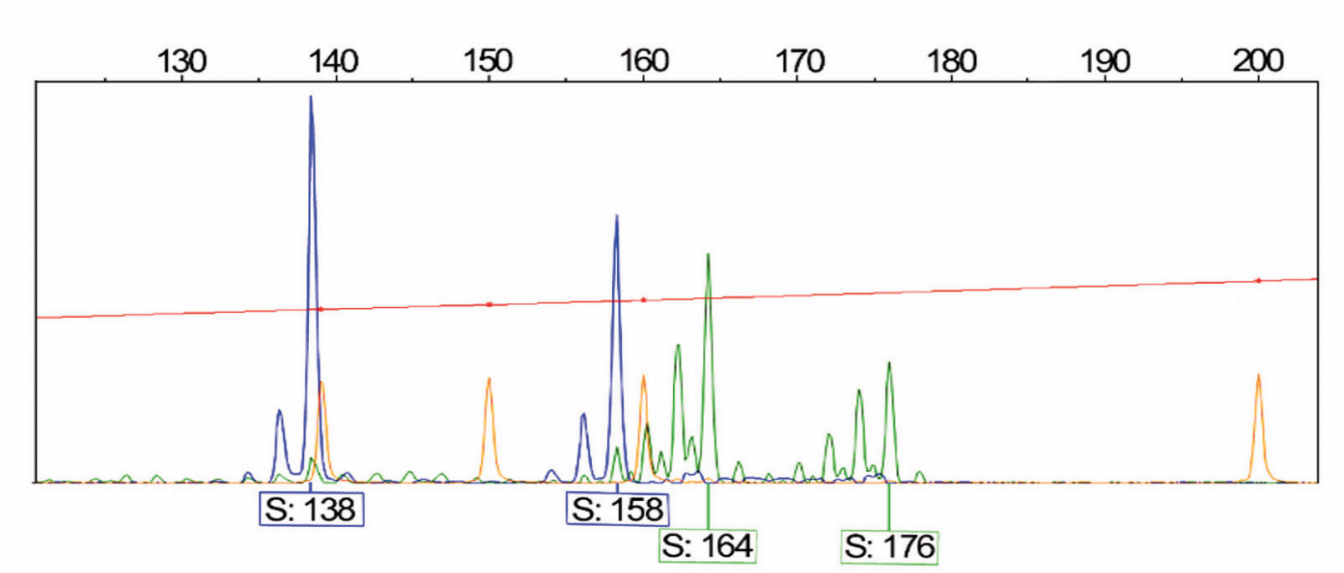

Figure 2. Pherogram of a seedling carrying markers associated with Rvi6 and Rvi4 scab resistance loci as an example of the marker-assisted selection (MAS). The marker CH-Vf1 (blue peaks) carries the fragment 158 associated to the Rvi6 (formerly Vf) scab resistance locus. The marker CH02c02a (green peaks) carries the fragment 176 associated to the Rvi4 (formerly Vr) scab resistance locus. Stuttering peaks were not labelled. In orange the ladder peaks. Red line is the calibration curve for the fragment size (in bp).

PCR products were diluted 1:80 with dH2O and 2 µl of the dilution were mixed with 7.98 µl of formamide and 0.02 µl of GeneScan 500 LIZ standard (Life Technologies, Grand Island, NY, USA). The mixture was denatured at 95 °C for 2 min, kept on ice for 5 min, and then run on an ABI3730 DNA analyser (Applied Biosystems, Foster City, CA, USA). Allelic fragments were analysed with the Peak Scanner™ v1.0 software (Applied Biosystems, Foster City, CA, USA) (Figure 2).

2.6. Grafting, transplanting and progeny evaluation

Screened progeny were grafted onto M9 clonal rootstocks using buds collected from the apical shoot of the original seedlings kept unpruned to reduce the juvenile period, as described in Janick et al., (1996) and following the advice of colleagues of Agroscope Changin-Wädensvil, Switzerland (M. Kellerhals, pers. comm.). At the end of the second season following summer grafting, plants were transplanted to a new orchard, spaced 3.5 m between rows and 0.40 m along the rows. They were observed for 2 or 3 productive cycles.

Observations were carried out on plant productivity, fruit appearance, shape and size, phenotypic resistance to scab and powdery mildew (data not shown). At the end of this first selection cycle, 3 replicates of each of the selected 75 progeny were propagated on M9 clonal rootstocks and grown for a second cycle of observation.

3. Results and Discussion

3.1. Results from the cross plan

The crossing design was completed in three years and, in spite of the care taken and considerable time spent, several parents were abandoned for the low seed yield ('Api Etoilée', 'Belle de Boskoop', 'Jonagold'). The resistant selections abandoned were 'Antonovka DCA', 'DD59', 'DF120' and 'GR40' because we were not able to find in time the markers associated with the resistance loci (hereinafter called R loci) for the molecular screening. 'Florina' because both *Rvi1* and *Rvi6* genes were present in other parents and because complete aphid resistance is not a priority for the Italian apple industry.

As a result, the cross combinations kept for the subsequent screening were reduced from 260 to 110. The number of progeny obtained from the individual cross combinations is reported in the Supplementary Table 1. In total there were 7,876 seedlings, but with large differences in progeny size among cross combinations.

3.2. Phenotypic and marker-assisted selection

The phenotypic screening of resistant progeny carried out either in the greenhouse or in the open field meant that the number of progeny to be analysed with markers associated to R loci could be reduced to 2,969 (Supplementary Table 2). The results for each individual cross combination are reported in the Supplementary Tables 1 to 7.

The presence of scab susceptible progeny among the progeny analysed with molecular markers was relatively high (633, to which 55 individuals from the Ariwa progeny carrying *Rvi6-/Pl1+*, for a total of 688 out of 2,969, 23.2%), in spite of the preliminary phenotypic screening carried out on the original 7,876 seedlings. This was not surprising, since visual assessment is prone to errors (Rousseau et al., 2013) and in our case it was exacerbated by the inhomogeneity of plants derived from different cross combinations and the different phenotypic reactions to scab of the resistance genes studied (Gessler et al., 2006).

In Tables S1-3 to S1-7, we report the analysis of segregation of scab and powdery mildew R loci and, because of the bias introduced with the preliminary phenotypic screening, the class of susceptible progeny, i.e. those that did not carry any R allele, was not considered in the chi-square analysis. Most segregations were in agreement with the model of independent segregation of the R loci and this was to be expected with the R loci being located on different chromosomes. In several cases, progeny carrying the *Rvi6* gene were below the expected frequencies. This could be due either to the presence of sub-lethal genes linked to *Rvi6* (Gao and van de Weg 2006) and/or to the presence of a number of weak plants in the progeny showing chlorosis and high sporulation as reported long time ago by Chevalier and co-workers, working on the Vf gene (1991).

As a result of the molecular screening (Figure 2), 43 progeny were found carrying three genes of resistance to scab (*Rvi2, Rvi4, Rvi6*); 657 carried two genes of resistance to scab in one of the following combinations: *Rvi2 + Rvi4* (41 progeny), *Rvi2 + Rvi6* (28 progeny), *Rvi4 + Rvi6* (27 progeny), while 108 progeny were resistant to both scab and powdery mildew, as they carried the *Rvi6 + Pl-1* gene combination (Table 4).

Table 4. Number of selections carrying different resistance loci as a result of the screening with molecular markers. R genes are coded according to Bus et al., 2011.

Combinations of R genes (a)	No. of selected progeny
Three genes	
Rvi2 + Rvi4 + Rvi6	43
Two genes	
Rvi2 + Rvi4	41
Rvi2 + Rvi6	28
Rvi4 + Rvi6	27
Rvi5 + Rvi6	561
Rvi6 + Pl-1	108
One of the following genes:	
Rvi2, Rvi4, Rvi5, Rvi6, Pl-1	1.528
TOTAL	2.336

Further genes (*FB* = resistance to fire blight, PM_{QTL} = possible QTL of resistance to powdery mildew, *WA* = gene of resistance to woolly aphid) not screened with associated markers could be present among the selected progeny.

3.3. Field observations and selection of superior genotypes

After phenotypic and molecular screening, only 2,336 seedlings of the 7,876 produced, carrying at least one resistance locus, were kept and grafted onto M9 rootstock. Lack of space in the field meant that no more than 12 seedlings from each cross family could be selected for agronomic observations; moreover, for several families the number of seedlings was less than 12. The total number of seedlings observed in the field was therefore 1,993.

Observations of plant productivity, fruit appearance, shape and size, phenotypic resistance to scab and powdery mildew (data not shown) allowed selection of 75 progeny (Figures 3 and 4), all carrying at least two resistance genes, confirmed by marker-assisted analysis.

They were propagated by grafting onto M9 clonal rootstocks and three replicates of each selection were grown for the second cycle of observations currently underway.

4. Conclusions

This paper reports a breeding design aiming to enlarge the genetic base of apple crop and to produce a new set of potential parents carrying multiple resistances to the main apple diseases. Coping with a large number of cross combinations was challenging. It took three years to complete the mating design and, despite this, we were forced to abandon more than half of the planned cross combinations for the reasons reported as above. We obtained progeny from 110 out of the 260 original crosses planned and, after screening for resistance and agronomic evaluation, the final result was 75 selections, all with two or more resistances to scab and, in some cases, other apple diseases, and all derived from parents characterized by large genetic diversity.

Phenotypic screening together with marker-assisted selection of cross families allowed rapid selection of resistant seedlings. Unfortunately, at the time we initiated the breeding programme, markers for assisted selection were not available for all R loci we were studying. We were confident of eventually

getting these markers but this was not to be, and we were not able to follow the segregation of several R loci (the *Va* locus from 'Antonovka', several QTLs of resistance to scab and powdery mildew, and a resistance to fire blight). We are confident that some of the 75 selections have inherited further resistances not yet analysed. Yet, we know that a strong QTL of resistance to fire blight has been identified in genotypes, like '(Starking) Delicious' (Khan et al., 2007) that are in the pedigree of our cross parents. The integration by chance of these resistances in our selections should add them value as they are and when they are used as parents in future breeding programmes.

Figure 3. Selection from the cross 'Golden Delicious' × HM100. The selection, which resembles the 'Golden Delicious' parent, carries two genes for resistance to scab: *Rvi5* (formerly *Vm*) and *Rvi6* (formerly *Vf*).

Figure 4. Selection from the cross Granny Smith × HM100. The selection has inherited the red color of the skin (improved compared to the parent HM100 likely for the presence of minor genes) and carries two genes of resistance to scab: *Rvi5* (formerly *Vm*) and *Rvi6* (formerly *Vf*).

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References

Acquaah, G. (2012) Principles of plant genetics and breeding. 2nd ed. Wiley-Blackwell, Oxford.

Baumgartner, I.O., Patocchi, A., Frey, J., Peil, A. and Kellerhals, M. (2015) 'Breeding Elite Lines of Apple Carrying Pyramided Homozygous Resistance Genes Against Apple Scab and Resistance Against Powdery Mildew and Fire Blight', *Plant Mol Biol Rep* 33, pp. 1573-1583. doi: 10.1007/s11105-015-0858-x

Beach, S.A., Booth, N.O. and Taylor, G.M. (1903) *The Apples of New York*. Voll I and II. J.B. Lyon Company, Albany.

Bus, V., Rikkerink, E., 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. and 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, pp. 103-116.

Bus, V.G.M., Rikkerink, E.H.A., Caffier, V., Durel, C.-E. and Plummer, K.M. (2011) 'Revision of the Nomenclature of the Differential Host Pathogen Interactions of Venturia inaequalis and Malus', *Ann Rev Phytopathology*, 49, pp. 391-413. doi: 10.1146/annurev-phyto-072910-095339

Bus, V.G.M., van de Weg, W.E., Peil, A., Dunemann, F., Zini, E., Laurens, F.N.D., Blažek, J., Hanke, V. and Forsline, F.L. (2012) 'The role of Schmidt 'Antonovka' in apple scab resistance breeding', *Tree Genet Genomes*, 8, pp. 627-642. doi: 10.1007/s11295-012-0470-2

Chevalier, M., Lespinasse, Y. and 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 Pathol*, 40, pp. 249-256.

Crosby, J.A., Janick, J., Pecknold, P.C., Korban, S.S., O'Connon, R.S.M., Goffreda, J. and Voordeckers, A. (1992) 'Breeding Apples for Scab Resistance: 1945-1990', *Fruit Var J*, 46(3), pp. 145-166.

Dunemann, F., Peil, A., Urbanietz, A. and Garcia-Libreros, T. (2007) 'Mapping of the apple powdery mildew resistance gene Pl1 and its genetic association with an NBS-LRR candidate resistance gene', *Plant Breed*, 126, pp. 476-481. doi: 10.1111/j.1439-0523.2007.01415.x

Gao, Z. and van de Weg, W. (2006) 'The Vf gene for scab resistance in apple is linked to sub-lethal genes', *Euphytica*, 151, pp. 123-33. doi: 10.1007/s10681-005-9082-3

Gessler, C., Patocchi, A., Sansavini, S., Tartarini, S. and Gianfranceschi, L. (2006) 'Venturia inaequalis resistance in apple', *Crit. Rev Plant Sci*, 25, pp. 473-503. doi: 10.1080/07352680601015975

Hough, L. F., Shay, J. R., and Dayton, D. F. (1953) 'Apple scab resistance from Malus floribunda Sieb', *Am Soc Hort Sci*, 62, pp. 341-347.

https://www.fruitid.com/ (Accessed: 20 January 2021)

Khajuria, Y.P., Kaul, S., Wani, A.A. and Dhar, M.K. (2018) 'Genetics of resistance in apple against

Venturia inaequalis (Wint.) Cke', *Tree Genet Genomes*, pp. 14-16. doi: 10.1007/s11295-018-1226-4

Khan, M.A., Durel, C.-E., Duffy, B., Drouet, D., Kellerhals, M., Gesler, C. and 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, pp. 568-577. doi: 10.1139/g07-033

Kumar, S., Bink, M.C.A.M., Volz, R.K., Bus, V.G.M. and Chagné, D. (2012) 'Towards genomic selection in apple (Malus × domestica Borkh.) breeding programmes: Prospects, challenges and strategies', *Tree Genet Genomes*, 8, pp. 1-14. doi: 10.1007/s11295-011-0425-z

Janick, J., Cummins, J.N., Brown, S.K. and Hemmat, M. (1996) 'Apples', In Janick, J., Moore, J.N. (eds) *Fruit Breeding*, *Volume I: Tree and Tropical Fruits*. John Wiley & Sons, New York, USA.

Laurens, F., Durel, C.E., Patocchi, A., Peil, A., Salvi, S., Tartarini, S., Velasco, R. and van de Weg, E. (2011) 'Review on apple genetics and breeding programmes and presentation of a new European initiative to increase fruit breeding efficiency', *J Fruit Sci*, 27, pp. 102-107.

Markussen, T., Krüger, J., Schmidt, H. and 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 (6), pp. 530-534. doi: 10.1111/j.1439-0523.1995.tb00850.x

Migicovsky, Z., Gardner, K.M., Richards, C., Chao, C.T., Schwaninger, H.R., Fazio, G., Zhong, G.-Y. and Myles, S. (2021) 'Genomic consequences of apple improvement', *Hortic Res* 8: 9. doi: 10.1038/s41438-020-00441-7

Muranty, H., Denancé, C., Feugey, L., Crépin, J.-L., Barbier, Y., Tartarini, S., Ordidge, M., Troggio, M., Lateur, M., Nybom, H., Paprstein, F., Laurens, F. and Durel, C.-E. (2020) 'Using whole-genome SNP data to reconstruct a large multi-generation pedigree in apple germplasm', *BMC Plant Biology*, 20: 2. doi: 10.1186/s12870-019-2171-6

N'Diaye, A., van de Weg, W.E., Kodde, L.P., Koller, B., Dunemann, F., Thiermann, M., Tartarini, S., Gennari, F. and Durel, C.E. (2008) 'Construction of an integrated consensus map of the apple genome based on four mapping populations', *Tree Genet Genomes*, 4, pp. 727-743. doi: 10.1007/s11295-008-0146-0

Noiton, D. and Shelbourne, C.J.A. (1992) 'Quantitative genetics in an apple breeding strategy', *Euphytica*, 60, pp. 213-219. doi: 10.1007/BF00039401

Noiton, D.A.M. and Alspach, P.A. (1996) 'Founding clones, inbreeding, coancestry, and status number of modern apple cultivars', *J Amer Soc Hort Sci*, 121, pp. 773-782. doi: 10.21273/JASHS.121.5.773

Papp, D., Gao, L., Thapa, R., Dan Olmstead, D. and Khan, A. (2020) 'Field apple scab susceptibility of a diverse Malus germplasm collection identifies potential sources of resistance for apple breeding', *CABI Agric Biosci,* 1, 16. doi: 10.1186/s43170-020-00017-4

Patocchi, A., Walser, M., Tartarini, S., Broggini, G.A.L., Gennari, F., Sansavini, S. and Gessler, C. (2005) 'Identification by genome scanning approach (GSA) of a microsatellite tightly associated with the apple scab resistance gene Vm', *Genome*, 48, pp. 630-636. doi: 10.1139/G05-036

Patocchi, A., Frei, A., Frey, J.E. and 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', *Mol Breeding*, 24, pp. 337-347. doi: 10.1007/s11032-009-9295-6

Patocchi, A., Wehrli, A., Dubuis, P.-H., Auwerkerken, A., Leida, C., Cipriani, G., Passey, T., Staples,M., Didelot, F., Philion, V., Peil, A., Laszakovits, H., Rühmer, T., Boeck, K., Baniulis, D., Strasser, K., Vávra, R., Guerra, W., Masny, S., Ruess, F., Le Berre, F., Nybom, H., Tartarini, S., Spornberger, A., Pikunova, A. and Bus, V. (2020) 'Ten years of VIN-QUEST: first insight for breeding new apple cultivars with durable apple scab resistance', *Plant Dis*, 104(8), pp. 2074-2081. doi: 10.1094/PDIS-11-19-2473-SR

Rousseau, C., Belin, E., Bove, E., Rousseau, D., Fabre, F., Berruyer, R., Guillaumès, J., Manceau, C., Jacques, M.-A. and Boureau, T. (2013) 'High throughput quantitative phenotyping of plant resis-

tance using chlorophyll fluorescence image analysis', *Plant Methods*, 9, 17. doi: 10.1186/1746-4811-9-17

Sakurai, K., Brown, S.K. and Weeden, N. (2000) 'Self-incompatibility Alleles of Apple Cultivars and Advanced Selections', *HortSci*, 35 (1), pp. 116-119.

Sansavini, S., Donati, F., Costa, F. and Tartarini, S. (2004) 'Advances in apple breeding for enhanced fruit quality and resistance to biotic stresses: new varieties for the European market', *J Fruit Ornam Plant Res*, Special edition, 12, pp. 13-52.

Tanksley, S.D. (1983) 'Molecular markers in plant breeding', *Plant Mol Biol Rep*, 1, pp. 3-8.

Vinatzer, B.A., Patocchi, A., Tartarini, S., Gianfraneschi, L., Sansavini, S. and Gessler, C. (2004) 'Isolation of two microsatellite markers from BAC clones of the Vf scab resistance region and molecular characterization of scab-resistant accessions in *Malus* germplasm', *Plant Breeding*, 123(4), pp. 321-326.

Way, R.D., Aldwinkle, H.S., Lamb, R.C., Rejman, A., Sansavini, S., Shen, T., Watkins, R., Westwood, M.N. and Yoshida, Y. (1990) 'Apple (Malus)', In Moore, J.N., Ballington Jr, J.R. (eds). *Genetic Resources of Temperate Fruit and Nut Crops*. Vol I. ISHS, Wageningen, Holland, pp. 1-62.