Project title:	Genotyping of apple rootstock breeding pipeline for pest and disease resistance markers to streamline breeding programme renewal
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Key staff:	Suzanne Litthauer
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[The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.]

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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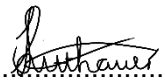
Signature  Date 26 November 2020

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GROWER SUMMARY

Headline

NIAB EMR continues to breed and select improved rootstocks for apple and pear, and this project encompasses a continuation of work covered in former project TF224 (2015-2020). The agronomic performance of the rootstock selections from the East Malling programme is considered excellent, but a stronger focus on pest and disease resistance at the early stages of selection is required for the programme to remain internationally competitive. Marker assisted selection (MAS) for fire blight resistance and dwarfing was performed in trial selections, pre-selections and seedling families, and resistance *Phytophthora cactorum,* fire blight and woolly apple aphid confirmed in a range of material through direct inoculations. MAS reduced the number of individual trees maintained in the rootstock breeding programme, enhancing efficiency and allowing the redeployment of staff time and field resources to better characterise the remaining material.

Background

Improved rootstocks are essential for profitable and sustainable production in tree-fruit crops. Factors important to growers include dwarfing (to reduce the cost of pruning and picking), induction of precocious and reliable cropping, freedom from suckers, good anchorage and resistance to pests and diseases. Ease of propagation and good scion-stock compatibility are also important in the nursery.

In 2008, EMR (now NIAB EMR), the HDC (now AHDB Horticulture) and the International New Varieties Network (INN) launched a Rootstock Club (EMRC) to breed, develop, distribute and commercialise new rootstock breeding material from East Malling (EM), world-wide.

For UK growers, the AHDB involvement in the development of new rootstocks from NIAB EMR's programme will ensure material will be available to UK levy payers. The AHDB helps to 'steer' breeding objectives to meet the specific requirements of UK growers and ensures that appropriate newly selected rootstocks are trialled further before release to the UK industry.

INN has members in the USA, Chile, South Africa, Australia, New Zealand and throughout Europe. In each country, members can produce virus-free (VF) certified rootstocks and premium quality VF certified finished trees. INN members will arrange, evaluate and select from their own trials to identify those rootstocks best suited to each country's specific growing conditions.

The EMRC aims to develop a range of apple, pear and quince rootstocks to suit different growing conditions. Breeding objectives include:

- new dwarfing and semi-dwarfing stocks for apple and pear
- improved scion-graft compatibility, in particular for pear
- increased precocity and productivity
- increased fire-blight and/or woolly apple aphid resistance
- enhanced tolerance to replant disease

Summary

DNA markers for disease resistance and dwarfing were employed to screen key selections of the East Malling apple rootstock pipeline, in order to reduce the running cost of the breeding programme beyond 2020 and lay the ground for routine marker assisted selection in future. DNA-based methods were combined with direct inoculations to confirm resistance to pest and disease, and to further limit the number of individuals maintained in the breeding programme:

- Selections and pre-selections were screened for markers linked to fire blight (FB) resistance, a small number of which were also included in direct inoculations with FB.
- A number of selections and pre-selections were screened for woolly apple aphid (WAA) resistance through direct inoculations, and any individuals susceptible to both FB and WAA were discarded
- All selections and pre-selections that were retained following pest and disease screening were screened for markers linked to dwarfing.
- Seedlings germinated in 2019 that were susceptible to *P. cactorum* were culled in a destructive direct inoculation screen. The remaining resistant seedlings, along with seedlings germinated in 2018, subsequently underwent indirect inoculations with WAA and/or MAS for FB markers: families expected to segregate for FB resistance underwent MAS and seedlings were deselected if FB resistance loci were absent; families expected to segregate for WAA were inoculated with WAA and susceptible seedlings were deselected.

Financial Benefits

Although rootstock breeding is a very long-term project, there are major financial advantages to the development and selection of rootstocks with improved agronomic performance including reduced pest and disease susceptibility. AHDB support to this and previous projects ensures that UK growers will have access to new UK-bred rootstocks.

Feedback from INN indicates that slimming down the existing pipeline (> 7,000 individuals in 2019) of material to identify the most promising breeding lines at an earlier stage would be highly desirable. Targeted culling would reduce the germplasm that NIAB EMR needs to

maintain in the field and in glasshouse collections, leading to significant saving in field, glasshouse and staff time.

Better characterisation of breeding lines would also allow breeders to make better crosses much sooner, speeding up the breeding cycle.

Action Points

None at this point.

SCIENCE SECTION

Introduction

The development of effective breeding approaches to incorporate host resistance is a key component in strategies towards sustainable and financially viable fruit production (Kellerhals et al., 2017). Although infections such as fire blight and woolly apple aphid usually enter the tree through the scion, rootstock resistance is very important for orchard management as it can prevent loss of an entire tree from an infected scion (Kellerhals et al., 2017). Traditional rootstock breeding in apple is a very long process, with commercial release often taking in excess of 30 years. Marker-assisted selection (MAS), in combination with speed-breeding techniques, have the potential to significantly reduce both seedling selection timescales and generation intervals. It is in the interest of the EMRC and commercial partners to be stricter in early selection for pest and disease resistance of material in the breeding pipeline. To achieve this, MAS will be routinely employed to improve our ability to make better crosses, fast-track interesting material into parental lines or trials, and eliminate susceptible individuals from the seedling populations at an early stage. In relation to this project, focus was on selecting individuals that are resistant to *P. cactorum*¸ fire blight and/or woolly apple aphid, and carry loci linked to dwarfing.

Fire blight (FB), caused by the Gram-negative enterobacterium *Erwinia amylovora*, is a destructive, commercially significant bacterial disease affecting apple and pear (Emeriewen et al., 2019). A number of commercial cultivars of apple, including rootstock M.9, are susceptible to FB, with the pathogen able to destroy entire orchards in a single growing season. Current disease control methods are not fully effective and options for chemical treatment are very limited, with antibiotic–resistant *E. amylovora* strains already reported (Sobiczewski et al., 2011; Kellerhals et al.; 2017; Emeriewen et al., 2019). Robust and reliable SSR (microsatellite) markers are available for FB resistance. Major QTLs linked to FB resistance have been mapped in families derived from the wild apple genotypes *Malus* × *robusta* 5 (Peil et al., 2007; Gardiner et al., 2012); *Malus floribunda* 821 (Durel et al., 2009), and the ornamental cultivar ‘Evereste’ (Durel et al., 2009). These three resistance sources have been introduced into material in the EM rootstock breeding programme with the aim of breeding for FB resistance. In this project, published SSR markers were used to assess the presence of the FB_MR5 resistance locus from *M. robusta* 5 (linkage group 3; Fahrentrapp et al., 2013), and of the Fb_E resistance locus from ‘Evereste’ (linkage group 12; Parravicini et al., 2011). SSR markers linked to FB_E were also used to assess the resistance locus from *M. floribunda* 182 (FB_flo), as the marker set currently used cannot distinguish between these two sources of FB resistance. Indeed, it is not yet known whether the genetic control of FB

resistance of *M. floribunda* 821 is the same as the FB_E locus, unrelated, or allelic. Using these two sets of published SSR markers, MAS was performed on a range of selections, pre-selections, and seedlings. In addition, FB resistance was confirmed in a limited number of selections, pre-selections, and parental genotypes though direct inoculation experiments.

The woolly apple aphid (WAA; *Eriosoma lanigerum*) is an important aphid pest of apple rootstocks. The aphids colonise susceptible apple rootstocks and can eventually fell a tree if left unchecked. The EM rootstock breeding programme has incorporated different WAA resistance sources, including from 'Northern Spy', *M. robusta* 5, and *M. floribunda* 182. Molecular markers have been published for resistance genes *Er1* (linkage group 8) from 'Northern Spy' and *Er2* (linkage group 17) from *M. robusta* 5 (Bus et al., 2000; Bus et al., 2007). As reported in 2019 (report for EMRC (TF224) 2018-2019), a set of 13 preliminary SSR markers were identified in the regions where WAA resistance genes have been previously mapped. This preliminary set was tested on a small number of genotypes to determine if the set could potentially be used in MAS in the apple rootstock program. It was determined that, while partially informative, more work is needed to identify more informative markers, including screening of segregating populations. Identification of SSR (and SNP) markers linked to WAA resistance will be continued, particularly as part of the CTP-FCR funded PhD project '*Resistance and susceptibility in interactions between apple and woolly aphids*'. Since reliable molecular markers for WAA have not yet been optimised, in this project resistance to WAA in selections, pre-selections, and seedlings was determined through direct inoculations.

Similarly, as no molecular marker linked to *P. cactorum* resistance are currently available, resistance to *P. cactorum* was assessed in this project by means of a destructive direct inoculation of newly-germinated seedlings.

Dwarf fruit trees have played a vital role in horticultural applications throughout history, with cultivation of low-growing dwarf apple trees dating back to ancient Greece (Mudge et al., 2009). While dwarfing is one of the most important selection criteria in rootstocks, the genetic mechanisms and root-scion interactions involved in size control are not yet fully understood (Mudge et al., 2009; Goldschmidt, 2014). A three-locus model for rootstock-induced dwarfing was proposed following analysis in the M432 mapping population (M.116 x M.27; Antanaviciute et al., 2012, Fernández-Fernández et al., 2012, Harrison et al., 2016). By utilizing a previously-identified association between a high root bark percentage (a high proportion of the whole root area consisting of root cortical cells) and rootstock-induced dwarfing, three QTLs (present in linkage groups 5, 11, and 13) were identified that control root bark percentage and which are also associated with dwarfing. Through further analysis of recombinant genotypes in the M432 population, Ms Magdalena Cobo Medina (as part of

her work for the CTP-FCR funded PhD project '*Combining root architecture, root function and soil management to improve production efficiency and quality of apples*') was able to narrow down the regions in LG5 and LG11 where the dwarfing QTLs *Rb1* and *Rb2* are situated, respectively. Combined, *Rb1* and *Rb2* have the most significant effect on dwarfing. Ms Cobo Medina subsequently developed a preliminary set of SSR markers linked to these two loci that can be used to identify the dwarfing haplotypes in rootstock populations. The marker set was used in this project to screen a number of selections and pre-selections and identify individuals that carry both dwarfing loci *Rb1* and *Rb2*.

Materials and methods

Genotyping

Genomic DNA preparation

For each individual included in genotyping analysis, 6-8 discs of healthy, green leaf tissue was collected and dried on silica gel. Two leaf discs were used for genomic DNA extractions, while the remainder were stored as backup. Genomic DNA extractions were performed in a 96-well format using the silica bead method (Edge-Garza et al., 2014). Genomic DNA preparations were quantified using the NanoDrop™ (Thermo Scientific) spectrophotometer and working dilutions of 5 ng/µL DNA prepared using sterile water.

Marker-assisted selection (MAS) for fire blight (FB) resistance

In the first round of genotyping, material was screened for SSR markers linked to FB resistance loci FB_R5 (from *M. robusta* 5), FB_E (from 'Evereste'), and FB_flo (from *M. floribunda* 182). The following material was included in this first round:

- Selections, which are the most agronomically interesting individuals from families currently in evaluation
- Pre-selections, which are currently in the propagation phase prior to grafting for preliminary trials or use in further breeding
- Seedlings from family M606 which were selected for woolly apple aphid resistance in 2017 and which are expected to segregate for both resistance sources
- Seedlings raised by the breeding programme in 2018 which are expected to segregate for one or both resistance sources
- Seedlings germinated in 2019, which were pre-selected for *P. cactorum* resistance through direct inoculation of seedlings in May 2019.

Three LG3 SSR markers (FEM14, FEM47 and FEM19) were used to assess the presence of the FB_R5 resistance locus from *M. robusta* 5 (Fahrentrapp et al., 2013). Four SSR markers

from linkage group 12 (LG12) namely, ChFbE01, ChFbE02, ChFbE06 and ChFbE09 were used to assess the presence of the Fb_E resistance locus from 'Evereste' (Parravicini et al., 2011). The four SSR markers for FB_E were also used to assess the resistance locus from *M. floribunda* 182 (FB_flo). Primers were labelled with HEX, FAM, PET, or NED, and multiplex PCR reactions were performed using the 'Type-It' microsatellite kit (Qiagen Ltd., Crawley, UK) according to the manufacturer's instructions. Amplification was performed using a Qiagen touchdown PCR protocol with annealing temperature set at 58˚C to 53˚C. Capillary electrophoresis was performed in an Applied Biosystems 3130xl Genetic Analyzer with GeneScan 500 LIZ™ size standard, and fragment analysis completed using GENESCAN and GENOTYPER software (Applied Biosystems). Genomic DNA for 'Evereste', *M. robusta* 5, and *M. floribunda* 182 were included as positive controls in all plates, with DNA from M.M.106 included as negative control. Individuals were only regarded as containing the appropriate FB resistance source if all resistance-linked alleles were detected, i.e. three resistance-linked alleles for FB_R5; four resistance-linked alleles for FB_E and FB_flo (Schlathölter et al. 2018).

Marker-assisted selection (MAS) for dwarfing

In the second round of genotyping, MAS for dwarfing was done for selections and pre-selections that still remained following FB resistance screens (MAS and direct inoculations) and WAA inoculation. Markers used were a preliminary set for dwarfing developed by Ms Magdalena Cobo Medina as part of her work for the CTP-FCR funded PhD project '*Combining root architecture, root function and soil management to improve production efficiency and quality of apples*'. Four SSR markers from LG5 (MD5001, MD5002, MD5003, and MD5004), and three markers from LG11 (MD11001, MD11002, and MD11003) were used to assess the presence of two loci linked to dwarfing (Harrison et al 2016). All seven markers were combined in a single multiplex. Primers were labelled with HEX, FAM, PET, or NED, and multiplex PCR reactions were performed using the 'Type-It' microsatellite kit (Qiagen Ltd., Crawley, UK) according to the manufacturer's instructions. Amplification was performed using a Qiagen touchdown PCR protocol with annealing temperature set at 57˚C to 52˚C. Capillary electrophoresis was performed in an Applied Biosystems 3130xl Genetic Analyzer with GeneScan 500 LIZ™ size standard, and fragment analysis completed using GENESCAN and GENOTYPER software (Applied Biosystems). Genomic DNA for M.9 and M.27 were included as positive controls in all plates, along with DNA from all parental genotypes. Individuals were only regarded as containing the appropriate dwarfing source if the correct haplotype is present, i.e. linked alleles were detected in all markers for both LG5 and LG11 (*Rb1* and *Rb2*, respectively).

Pest and disease resistance screening

Phytophthora cactorum direct inoculation of seedlings

Apple seedlings germinated in 2019 that were predicted to segregate for resistance to *P. cactorum* were included in a destructive seedling screen in May-August 2019 as follows:

Following sowing, stratification and germination as normal, seedlings were moved to a temperature-controlled (20°C) glasshouse compartment in mid-May. The number of seedlings in each tray were counted and recorded. Seedlings were inoculated with a $2x10^{-4}$ zoospore suspension consisting of a mixture of zoospores from three *P. cactorum* isolates (P295, 62471, and R36/14; ratio 1:1:1). Inoculations were carried out by evenly pipetting 15 mL of inoculum per tray onto the base of stems. Scoring was done by counting and discarding all dead or symptomatic seedlings two weeks after inoculation, and was repeated three weeks after inoculation. Surviving seedlings were potted up in the fourth week following inoculation, during which any dead or symptomatic seedlings were again counted and discarded. All surviving seedlings were given an individual seedling number. Final scoring and discarding of susceptible seedlings were carried out 12 weeks after inoculation. Segregation ratios for each seedling family were calculated by using the total number of seedlings germinated, the final number of resistant (surviving and asymptomatic) seedlings counted in week 12, and the total number of susceptible seedlings counted across all scoring session.

Woolly apple aphid (WAA) direct inoculation

Seedlings germinated in 2018 and 2019, as well as a number of selections, were inoculated with WAA between late spring and early autumn to determine resistance. Colonies of *Eriosoma lanigenum* (WAA) collected from the field/glasshouses or from plant material maintained over winter in the cold store were used to inoculate seedlings by applying aphids to each tree. Trees were pre-scored prior to inoculation and final scoring carried out three to four weeks following inoculation. Scoring was done using a 0-5 scale (0 indicating no established colonies, and 5 indicating multiple established colonies). Individuals were classified as susceptible (score 3-5), moderately susceptible (score 2), fairly resistant (1), or resistant (0). Results were deemed inconclusive if the pre-score was higher than the final score.

Fire blight (FB) direct inoculation

In 2019 and 2020, direct inoculations of apple and pear material were performed according to the protocol employed at Agroscope (Khan et al., 2007) with slight modifications. In brief, the procedure was as follows:

For each genotype of apple (and pear) assessed at NIAB EMR, nine inoculated replicates and one mock-inoculated replicate per genotype were included in the direct inoculation. Replicates were grafted onto M.9 T337 rootstocks (apple) or QC rootstocks (pear) in February and successful grafts grown in the glasshouse for approximately 10 weeks. M.9 was included as a susceptible control for apple, with 'Comice' included as susceptible control for pear. Inoculations were performed on shoots of minimum length 10cm in a containment glasshouse setting in May. Inoculum of *Erwinia amylovora* strain ACW610 Rif (concentration 109 cfu.mL^{-1}) was injected into the shoot tip just above the first unfolded leaf, using a syringe. Length of shoots and of necrotic lesions were measured 14-, 21-, and 28-days following inoculation. Scoring on days 14 and 21 was done by measuring length of the outside visible lesion, while the final scoring involved scraping into the stem with a scalpel blade to measure the length of the internal lesion. The length of the internal lesion was averaged across replicates for each genotype and expressed as a percentage lesion length, relative to total shoot length. The average percentage lesion length for each genotype was subsequently normalised to the average percentage lesion length of the susceptible control. Level of tolerance to FB was classified according to the normalised percentage lesion length as follows: resistant (< 25%), partially tolerant (25%-60%), susceptible (60%-100%), and highly susceptible (> 100%).

Results

Phytophthora cactorum inoculation of seedlings

Seedlings from 11 families germinated in 2019 were subjected to a destructive screen for resistance to *P. cactorum* (Table 1). No seedlings germinated in family M619 (AR86-1-20 x C.G.11). Only one seedling from family M624 (*M. floribunda* 821 x M.M.106) germinated and was therefore excluded from the destructive screen. All surviving seedlings were potted up and individually labelled. Segregation ratios were calculated, although these ratios are not informative in families with a low number of seedlings.

Table 1. Seedlings germinated in 2019 subjected to a destructive screen for *P. cactorum* resistance, showing family code, parentage, and category. Total number of seedlings germinated, as well as number of resistant and number of susceptible seedlings are shown. Segregation ratios (%) are shown in parentheses.

			***P. cactorum* destructive seedling screen**		
Family	**Parentage**	**Category**	**Germinated**	**Resistant**	**Susceptible**
M618	AR295-6 x C.G.202	Commercial breeding	72	44 (61%)	28 (39%)
M619	AR86-1-20 x C.G.11	Commercial breeding	0	-	-
M620	Bud.9 x 'Evereste'	Pre-breeding	8	7 (87%)	1 (13%)

M621	C.G.11 x AR295-6	Pre-breeding	9	7 (78%)	2 (22%)
M622	C.G.202 x M547-41	Commercial breeding	110	62 (56%)	48 (44%)
M623	C.G.202 x 'Evereste'	Commercial breeding	6	2 (33%)	4 (67%)
M624	*M. floribunda* 821 x M.M.106	Pre-breeding	1	-	-
M626	*M. robusta* 5 x AR295-6	Commercial breeding	74	58 (77%)	17 (23%)
M627	M.116 x C.G.935	Commercial breeding	310	124 (40%)	186 (60%)
M628	M.116 x C.G.11	Commercial breeding	136	56 (41%)	80 (59%)
M629	M.116 x AR295-6	Pre-breeding	18	12 (67%)	6 (33%)
M630	M.116 x *M. robusta* 5	Commercial breeding	103	54 (52%)	49 (48%)
M631	'Northern Spy' x 'Evereste'	Commercial breeding	683	215 (31%)	468 (69%)

MAS for fire blight (FB) resistance

Leaf material was collected and DNA extracted for 19 selections (Table 2), of which 3 selections (M547-001, M547-008, and M547-041) were included in MAS for FB resistance. The remaining 16 selections were excluded from the MAS as these either had susceptible pedigrees, or pedigrees for which no markers linked to FB resistance are available. All 3 selections included in MAS were found to carry the FB resistance locus FB_flo (as determined using markers for resistance locus FB_E).

Prior to commencement of this project individuals were pre-selected from 28 families that had been evaluated by breeders up to October 2018, as well as from 8 families planted in 2015 that underwent a shortened evaluation in the field. Leaf material was collected and DNA extracted for all these pre-selections alive in spring 2019 (Table 3). Pre-selections from families M550, M557, M585, and M590 were excluded from MAS due to FB-susceptible pedigrees, while no markers linked to FB resistance were available for families M563, M563a, M565, M568, or M594. MAS was subsequently performed for the remaining 27 families, 21 of which were derived from the Geneva series of rootstocks (Robinson et al., 2003) that carry FB_R5, five which were derived from 'Evereste', and one family derived from 'Evereste' x C.G.30. Families M556, M556a ('Ottawa' 3 x o.p.) were included in the MAS, as resistance locus FB_E, FB_R5 or FB_flo could potentially be contributed by the pollen donor. Selections were regarded as 'resistant' if the appropriate FB-resistance loci were present.

At this stage of MAS, all selections and pre-selections were retained to screen for WAA resistance.

In addition to selections and pre-selections, MAS was also performed for seedlings from family M606 ('Evereste' x C.G.202) which have not yet been evaluated in the field, but which underwent pest screening in a glasshouse environment. Seedlings from family M606 were

germinated in 2017, and the 280 individually-labelled seedlings were potted up and inoculated with WAA. Forty-two seedlings scored as ‘resistant’ or ‘fairly resistant’ and were selected, while the remaining seedlings were discarded. To identify seedlings in which multiple resistances are pyramided, leaf samples had been collected from the 42 selected M606 seedlings in 2017, and DNA was extracted and MAS for FB resistance was performed on these seedlings in 2019 (Table 4). Two seedlings (M606-224 and M606-226) were found to be resistant to WAA and carry both FB_R5 and FB_E resistance loci, while an additional two seedlings (M606-227 and M606-230) were fairly resistant to WAA and also contained both FB-resistance loci. At this stage in the selection process, M606 seedlings were kept if resistant to WAA and containing either FB resistance locus.

Table 2. Selections forming part of the apple rootstock breeding programme, showing parentage, stage of selection, and results from MAS[1] for FB[2] resistance.

Selection	Parentage	Stage of selection	MAS[1] for FB[2] resistance	
			Prediction	Result
M430-249	M.I.S. x M.27	Under testing	None available	None available
M432-203	M.27 x M.116	SC211 Trial Guard	Susceptible (pedigree)	Susceptible (pedigree)
M432-217	M.27 x M.116	SC211 Trial Guard	Susceptible (pedigree)	Susceptible (pedigree)
M480-003	M.9 x M.116	SC211 Trial Guard	Susceptible (pedigree)	Susceptible (pedigree)
M482-013	M.9 x M.116/G.202	SC211 Trial Guard	Susceptible (pedigree)	Susceptible (pedigree)
M508-001	M.13 x JM.7	SC211 Trial	None available	None available
M508-049	M.13 x JM.7	SC211 Trial	None available	None available
M509-022	Unknown	SC211 Trial Guard	None available	None available
M546-009	M.9 x JM.7	Under testing	None available	None available
M546-022	M.9 x JM.7	2018 Trial (died)	None available	None available
M546-110	M.9 x JM.7	SC211 Trial	None available	None available
M547-001	M.9 x *M. floribunda* 821	2018 Trial (died)	Family segregates for FB_flo	FB_flo present
M547-008	M.9 x *M. floribunda* 821	SC211 Trial	Family segregates for FB_flo	FB_flo present
M547-041	M.9 x *M. floribunda* 821	SC211 Trial	Family segregates for FB_flo	FB_flo present
M549-059	M.13 x JM.7	Under testing	None available	None available
M549-083	M.13 x JM.7	SC211 Trial	None available	None available
M549-094	M.13 x JM.7	Under testing	None available	None available
M549-122	M.13 x JM.7	Under testing	None available	None available
M549-146	M.13 x JM.7	Under testing	None available	None available

[1] MAS; marker-assisted selection
[2] FB; fire blight

Table 3. Families of pre-selections forming part of the apple rootstock breeding programme showing parentage, category, and number of individuals planted and pre-selected. Number of pre-selections included in MAS[1] for FB[2] resistance are shown. 'Resistant' refers to the number of individuals in which the appropriate FB-resistance locus/loci are present; 'Susceptible' shows the number of pre-selections in which the FB-resistance loci are absent.

Family	Parentage	Category	Year planted	Year budded	Number planted	Pre-selected	MAS[1] for FB[2] resistance			
							Prediction	Screened	Resistant	Susceptible
M550	AR86-1-20 x M.9	Pre-breeding	2009	2010	83	5	Susceptible pedigree	0	-	-
M553	AR86-1-20 x C.G.202	Commercial breeding	2010	2011	140	11	Segregate for FB_R5	9	7	2
M554	M.M.106 x C.G.30	Commercial breeding	2010	2011	367	10	Segregate for FB_R5	10	5	5
M555	C.G.30 x o.p.	Pre-breeding	2010	2011	307	8	Segregate for FB_R5	8	5	3
M556	'Ottawa 3' x o.p.	Pre-breeding	2010	2011	242	6	Unknown	7	0	7
M557	M.116 x M.9a	Pre-breeding	2011	2012	93	3	Susceptible pedigree	0	-	-
M558	C.G.30 x M.116	Commercial breeding	2011	2012	114	1	Segregate for FB_R5	1	0	1
M560	AR86-1-20 x C.G.11	Commercial breeding	2011	2012	242	6	Segregate for FB_R5	6	3	3
M562	M.M.106 x C.G.202	Commercial breeding	2011	2012	228	4	Segregate for FB_R5	4	3	1
M563	M.M.106 x Bud.9	Pre-breeding	2011	2012	127	6	None available	0	-	-
M555a	C.G.30 o.p.	Commercial breeding	2012	2013	123	9	Segregate for FB_R5	9	4	5
M556a	'Ottawa 3' o.p.	Commercial breeding	2012	2013	85	9	Unknown	9	0	9
M560a	AR86-1-20 x C.G.11	Commercial breeding	2012	2013	184	16	Segregate for FB_R5	16	4	12
M562a	M.M.106 x C.G.202	Commercial breeding	2012	2013	212	17	Segregate for FB_R5	17	5	12
M563a	M.M.106 x Bud.9	Pre-breeding	2012	2013	83	4	None available	0	-	-
M564	C.G.202 x AR295-6	Commercial breeding	2012	2013	10	1	Segregate for FB_R5	1	1	0
M565	Bud.9 x M.116	Pre-breeding	2012	2013	8	4	None available	0	-	-
M566	Bud.9 x 'Evereste'	Pre-breeding	2013	2014	20	3	Segregate for FB_E	3	0	3
M567	M.27 x C.G.11	Pre-breeding	2013	2014	11	2	Segregate for FB_R5	2	0	2
M568	'Torstein' x M.27	Pre-breeding	2013	2014	4	1	None available	0	-	-
M570	C.G.202 o.p.	Commercial breeding	2013	2014	86	9	Segregate for FB_R5	7	3	4
M571	C.G.11 o.p.	Pre-breeding	2013	2014	76	5	Segregate for FB_R5	5	2	3
M574	'Evereste' x M.9	Pre-breeding	2014	2015	303	17	Segregate for FB_E	17	8	9
M578	C.G.11 x AR295-6	Pre-breeding	2014	2015	52	3	Segregate for FB_R5	3	2	1
M580	C.G.30 x AR295-6	Pre-breeding	2014	2015	148	5	Segregate for FB_R5	5	3	2
M581	M.27 x C.G.11	Pre-breeding	2014	2015	41	4	Segregate for FB_R5	4	0	4
M582	M.M.106 x C.G.30	Commercial breeding	2014	2015	35	5	Segregate for FB_R5	5	3	2

Family	Parentage	Category	Year planted	Year budded	Number planted	Pre-selected	MAS[1] for FB[2] resistance			
							Prediction	Screened	Resistant	Susceptible
M585	M.9 EMLA x 'Sally'	Pre-breeding	2014	2 015	9	4	Susceptible pedigree	0	-	-
M587	C.G.202 x AR295-6	Commercial breeding	2015	2016	64	7	Segregate for FB_R5	7	4	3
M588	AR295-6 x C.G.202	Commercial breeding	2015	2016	64	10	Segregate for FB_R5	10	4	6
M589	'Evereste' x C.G.30	Pre-breeding	2015	2016	745	132	Segregate for FB_R5 and FB_E	131	32	99
M590	M.13 x M.116	Pre-breeding	2015	2016	14	4	Susceptible pedigree	0	-	-
M591	M.M.106 x C.G.30	Commercial breeding	2015	2016	95	27	Segregate for FB_R5	27	8	19
M592	C.G.30 x M.27	Pre-breeding	2015	2016	248	51	Segregate for FB_R5	51	19	32
M593	Bud.9 x 'Evereste'	Pre-breeding	2015	2016	69	11	Segregate for FB_E	11	0	11
M594	'Novole' x M.116	Commercial breeding	2015	2016	46	8	None available	0	-	-

[1] MAS; marker-assisted selection
[2] FB; fire blight

Table 4. Results for screening of seedlings from family M606 ('Evereste' x C.G.202) which were selected following WAA[1] inoculation and subsequently included in MAS[2] for FB[3] resistance in 2019. Seedlings are presented sorted in approximate decreasing order of resistance.

Seedling	WAA[1] resistance	MAS[2] for FB[3] resistance
M606-224	Resistant	FB_R5 and FB_E present
M606-226	Resistant	FB_R5 and FB_E present
M606-133	Resistant	FB_E present; FB_R5 absent
M606-136	Resistant	FB_E present; FB_R5 absent
M606-228	Resistant	FB_E present; FB_R5 absent
M606-234	Resistant	FB_E present; FB_R5 absent
M606-240	Resistant	FB_E present; FB_R5 absent
M606-248	Resistant	FB_E present; FB_R5 absent
M606-256	Resistant	FB_E present; FB_R5 absent
M606-259	Resistant	FB_E present; FB_R5 absent
M606-192	Resistant	FB_R5 present; FB_E absent
M606-199	Resistant	FB_R5 present; FB_E absent
M606-209	Resistant	FB_R5 present; FB_E absent
M606-265	Resistant	FB_R5 present; FB_E absent
M606-254	Resistant	FB_R5 and FB_E absent
M606-270	Resistant	FB_R5 and FB_E absent
M606-271	Resistant	FB_R5 and FB_E absent
M606-227	Fairly resistant	FB_R5 and FB_E present
M606-230	Fairly resistant	FB_R5 and FB_E present
M606-003	Fairly resistant	FB_E present; FB_R5 absent
M606-145	Fairly resistant	FB_E present; FB_R5 absent
M606-147	Fairly resistant	FB_E present; FB_R5 absent
M606-150	Fairly resistant	FB_E present; FB_R5 absent
M606-231	Fairly resistant	FB_E present; FB_R5 absent
M606-261	Fairly resistant	FB_E present; FB_R5 absent
M606-263	Fairly resistant	FB_E present; FB_R5 absent
M606-268	Fairly resistant	FB_E present; FB_R5 absent
M606-276	Fairly resistant	FB_E present; FB_R5 absent
M606-278	Fairly resistant	FB_E present; FB_R5 absent
M606-280	Fairly resistant	FB_E present; FB_R5 absent
M606-134	Fairly resistant	FB_R5 present; FB_E absent
M606-200	Fairly resistant	FB_R5 present; FB_E absent
M606-220	Fairly resistant	FB_R5 present; FB_E absent
M606-244	Fairly resistant	FB_R5 present; FB_E absent
M606-266	Fairly resistant	FB_R5 present; FB_E absent
M606-141	Fairly resistant	FB_R5 and FB_E absent
M606-210	Fairly resistant	FB_R5 and FB_E absent
M606-215	Fairly resistant	FB_R5 and FB_E absent
M606-221	Fairly resistant	FB_R5 and FB_E absent
M606-223	Fairly resistant	FB_R5 and FB_E absent
M606-235	Fairly resistant	FB_R5 and FB_E absent
M606-279	Fairly resistant	FB_R5 and FB_E absent

[1]WAA; Woolly apple aphid

[2]MAS; Marker assisted selection

[3]FB; Fire blight

A variety of seedlings raised in 2018 and 2019 were included in MAS for FB resistance. In spring 2019, leaf material was collected from all individually-labelled seedlings raised in 2018 (Table 5). MAS was performed only for seedlings from three families (M612, M614, and M615) that were expected to segregate for FB resistance loci FB_R5 or FB_E. In families expected to segregate for only one source of FB resistance, all seedling in which the appropriate locus was present were selected, while the rest were discarded. In family M615 ('Evereste' x C.G.30) expected to segregate for two resistance sources (FB_R5 and FB_E), only seedlings carrying both resistance loci were selected, while the rest were discarded. In early autumn 2019, leaf material was collected from all individually-labelled seedlings germinated in 2019 that survived the destructive *P. cactorum* resistance screen (Table 5). MAS for FB resistance was carried out on seedlings from all families but one (M629; M.116 x AR295-6), for which markers linked to FB-resistance are not available. In most families expected to segregate for only one source of FB resistance, all seedling in which the appropriate locus was present were selected, while the rest were discarded. However, all seedlings for families M627 (M.116 x C.G.935) and M631 ('Northern Spy' x 'Evereste') were kept, with those containing FB-resistance loci labelled. Families expected to segregate for the FB-resistance locus FB_flo were screened with markers for locus FB_E as previously described. In families expected to segregate for more than one resistance source (M622, C.G.202 x M547-41; M623; C.G.202 x 'Evereste') seedlings were selected if carrying one or both sources of resistance. Segregation ratios were calculated for all families screened, although this ratio is less informative in families with a low number of seedlings.

Table 5. Seedlings raised in 2018 and 2019, showing family code, year germinated, parentage, category, total number of seedlings per family, and results of MAS[1] for FB[2] resistance. Total number of seedlings screened for each family, as well as number of seedlings selected and deselected are shown. Segregation ratios (%) are shown in parentheses.

Family	Year	Parentage	Category	Number of seedlings	MAS[1] for FB[2] resistance			
					Prediction	Screened	Selected[3,4]	De-selected[4]
M612	2018	C.G.11 x AR295-6	Pre-breeding	10	Segregate for FB_R5 (~50%)	10	1 (10% R)	9 (9% S)
M613	2018	M.116 x AR295-6	Pre-breeding	85	None available	0	-	-
M614	2018	C.G.30 x AR440-1	Pre-breeding	60	Segregate for FB_R5 (~50%)	50	28 (56% R)	22 (44% S)
M615	2018	'Evereste' x C.G.30	Pre-breeding	625	Segregate for FB_R5 and FB_E (~25% both)	562	108 (19% R)	454 (81% S)
M616	2018	'Novole' x M.9	Pre-breeding	147	None available	0	-	-
M617	2018	*M. koreana* x M.9	Pre-breeding	150	None available	0	-	-
M618	2019	AR295-6 x C.G.202	Commercial breeding	39	Segregate for FB_R5 (~50%)	39	2 (5% R)	37 (95% S)
M620	2019	Bud.9 x 'Evereste'	Pre-breeding	7	Segregate for FB_R5 (~50%)	7	3 (43% R)	4 (57% S)
M621	2019	C.G.11 x AR295-6	Pre-breeding	7	Segregate for FB_R5 (~50%)	7	2 (29% R)	5 (71% S)
M622	2019	C.G.202 x M547-41	Commercial breeding	57	Segregate for FB_R5 (~50%) and FB_flo (~25%)	57	43 (75% R)	14 (25% S)
M623	2019	C.G.202 x 'Evereste'	Commercial breeding	2	Segregate for FB_R5 and FB_E (~25% both)	2	1 (50% R)	1 (50% S)
M624	2019	*M. floribunda* 821 x M.M.106	Pre-breeding	1	Segregate for FB_flo (~50%)	1	1 (100% R)	0 (0% S)
M626	2019	*M. robusta* 5 x AR295-6	Commercial breeding	52	Segregate for FB_R5 (~50%)	52	1 (2% R)	51 (98% S)
M627	2019	M.116 x C.G.935	Commercial breeding	126	Segregate for FB_R5 (~50%)	126	126 (46% R; 54% S)	0
M628	2019	M.116 x C.G.11	Commercial breeding	55	Segregate for FB_R5 (~50%)	55	30 (55% R)	25 (45% S)

M629	2019	M.116 x AR295-6	Pre-breeding	10	None available	0	-	-
M630	2019	M.116 x *M. robusta* 5	Commercial breeding	51	Segregate for FB_R5 (~50%)	51	26 (51% R)	25 (49% S)
M631	2019	'Northern Spy' x 'Evereste'	Commercial breeding	193	Segregate for FB_E (~50%)	194	194 (38% R; 62% S)	0

[1] MAS; marker-assisted selection
[2] FB; fire blight
[3] R; 'resistant', relevant FB-resistant locus/loci present
[4] S; 'susceptible', relevant FB-resistant locus/loci absent

Fire blight (FB) inoculations for resistance screening

Between 2015 and 2017 FB direct inoculations for apple and pear were carried out at Agroscope (CH), always introducing common control cultivars for harmonization of results. Following discontinuation of this service in 2017 FB inoculations were performed at NIAB EMR for the first time in May 2019, including 10 selections and a number of parental genotypes incorporated in the rootstock breeding programme. In 2020, FB inoculations were expanded to 26 apple genotypes including: eight selections that had been screened in 2019; five selections and six pre-selections not screened in 2019; and two parental genotypes ('Novole' and *M. koreana*) for which level of FB resistance is not known. Susceptible control M.9 and resistant control C.G.935 (Norelli et al.,2003; Russo et al., 2007) were included in both screens.

Clear disease symptoms were observed in all replicates of susceptible control M.9, and resistant control C.G.935 was 'resistant' in both sets of screening (Table 6). No lesions were observed in mock-inoculated replicates of any genotype in either year. Disease symptoms were more severe in 2019 than in 2020. AR837-19 was 'susceptible' in 2019 and 'partially tolerant' in 2020; AR839-9 was 'highly susceptible' in 2019 and 'partially tolerant' in 2020. Both AR837-19 and AR839-9 had been categorised as 'partially tolerant' in screenings done by Agroscope. R80 was 'susceptible' in 2019 and 'resistant' in 2020. These discrepancies could be due to the high level of infection observed in the 2019 screen, possibly as a result of inoculation and scoring being done comparatively later in the year.

Despite the higher disease severity in 2019, results were reproducible for the remaining genotypes screened in both years. AR835-11 was 'partially tolerant' in both 2019 and 2020, and this corresponds with previous reports from Agroscope. FB marker data has shown that the FB_R5 locus is absent in AR295-6 (*M. robusta* 5 x 'Ottawa' 3), and it was previously classified as 'partially tolerant' in screening done at Agroscope. However, AR295-6 was 'susceptible' in both the 2019 and 2020 inoculation screens, which corresponds with the absence of the FB_R5 locus indicated by markers. Selections M547-1 and M547-41 (M.9 x *M. floribunda* 821) both contain the FB_flo resistance locus and were partially tolerant and resistant, respectively. Six selections from family M553 (AR86-1-20 x C.G.202) which contain the FB_R5 locus were included in the 2020 screen, and were either partially tolerant or resistant.

'JM.7' (*M. prunifolia* 'Seishi' x M9), *M. prunifolia* 'Novole', and *M. koreana* were resistant to FB inoculation, suggesting that these genotypes could be utilised as yet-uncharacterised sources of resistance to FB. Indeed, selections M549-94 (M.13 x JM.7), M508-49 (M.13 x JM.7), and M546-110 (M.9 x JM.7) were resistant to FB inoculation. JM.7 has been reported

as partially tolerant to FB (Soejima et al., 2010) but was scored as resistant in our screen, which could be attributed to differences in the scoring metrics employed. These data demonstrate the need to repeat screening of genotypes over multiple years with sufficient replicates, with the inclusion of appropriate controls for harmonisation of data.

FB inoculations were also performed on 26 pear selections and parental genotypes in 2020 (Table 7). ‘Comice’ was included as a susceptible control, and was ‘highly susceptible’ in the direct inoculation screen. Genotypes PQ5-13 (C84 o.p.) and PQ34-1 (QR517-9 x QR708-12) have previously been reported as susceptible and were susceptible in the 2020 screen. ‘Old Home’, OHxF87 ('Old Home' x 'Farmingdale 87'), and P298-18 (‘Williams’ x US309) have been reported as resistant, and were resistant in the direct inoculation. OHxF333 ('Old Home' x 'Farmingdale 333') and P155-3 (‘Comice’ x 19B29) were partially tolerant, despite being previously reported as resistant. All other genotypes derived from ‘Old Home’ were either partially tolerant or resistant.

Table 6. Apple selections and parental material included in FB[1] direct inoculation screen for resistance. Genotype, parentage, and presence of any FB[1] loci are shown. Tolerance to FB[1] was assessed in direct inoculations in 2019 and 2020.

Genotype	Parentage	FB[1] markers	Tolerance to FB[1]
AR295-6	*M. robusta 5* x 'Ottawa 3'	FB_R5 absent	Susceptible[2,3]
AR682-6	M.26 x M.I.793	none available	Highly susceptible[2]
AR835-11	M.I.793 x M.9	none available	Partially tolerant[2,3]
AR837-19	M.3 x M.1	none available	Susceptible[2]; Partially tolerant[3]
AR839-9	M.7 x M.27	none available	Highly susceptible[2]; Partially tolerant[3]
AR852-3	AR362-16 o.p.	none available	Susceptible[2]
B24	AR10-2-5 x AR86-1-22	none available	Highly susceptible[2]
Bud.9	M.9 x 'Krasni Standard'	none available	Susceptible[2]
C.G.935	'Ottawa 3' X *M. robusta* 5	FB_R5 present	Resistant[2,3]
JM.7	*M. prunifolia* 'Seishi' X M.9	none available	Resistant[2]
M. koreana	-	none available	Resistant[3]
M.9 (FPM)	unknown	none available	Highly susceptible[2,3]
M508-001	M.13 x JM.7	none available	Susceptible[3]
M508-049	M.13 x JM.7	none available	Resistant[2,3]
M509-022	unknown	none available	Highly susceptible[2]
M546-022	M.9 x JM.7	none available	Susceptible[2]
M546-110	M.9 x JM.7	none available	Resistant[2,3]
M547-001	M.9 x *M. floribunda* 821	FB_flo present	Partially tolerant[2,3]
M547-041	M.9 x *M. floribunda* 821	FB_flo present	Resistant[2,3]
M549-122	M.13 x JM.7	none available	Highly susceptible[3]
M549-146	M.13 x JM.7	none available	Susceptible[3]
M549-059	M.13 x JM.7	none available	Susceptible[2]
M549-083	M.13 x JM.7	none available	Partially tolerant[3]
M549-094	M.13 x JM.7	none available	Resistant[3]
M553-002	AR86-1-20 x C.G.202	FB_R5 present	Resistant[3]
M553-028	AR86-1-20 x C.G.202	FB_R5 present	Resistant[3]
M553-032	AR86-1-20 x C.G.202	FB_R5 present	Resistant[3]
M553-064	AR86-1-20 x C.G.202	FB_R5 present	Resistant[3]
M553-085	AR86-1-20 x C.G.202	FB_R5 present	Partially tolerant[3]
M553-112	AR86-1-20 x C.G.202	FB_R5 present	Partially tolerant[3]
'Novole'	*M. prunifolia*	none available	Resistant[3]
Pajam2 (M.9)	unknown	none available	Highly susceptible[2]
R59	AR134-31 x AR86-1-23	none available	Partially tolerant[3]
R80 (HDC)	AR134-31 x AR86-1-22	none available	Susceptible[2]; Resistant[3]
'Torstein'	*M. sylvestris*	none available	Highly susceptible[3]

[1]FB; Fire blight
[2]Direct inoculation in 2019
[3]Direct inoculation in 2020

Table 7. Pear selections and parental material included in FB[1] direct inoculation screen for resistance, assessed in 2020. Genotype and parentage are shown, as well as any tolerance to FB[1] previously reported, and tolerance observed in the 2020 screen.

		Tolerance to FB[1]	
Genotype	**Parentage**	**Previously reported**	**Observed**
BP1	unknown	-	Highly susceptible
'Comice'	unknown	Highly susceptible	Highly susceptible
OHxF333	'Old Home' x Farmingdale 333	Resistant	Partially tolerant
OHxF34	'Old Home' x Farmingdale 34	-	Partially tolerant
OHxF51	'Old Home' x Farmingdale 51	-	Resistant
OHxF87	'Old Home' x Farmingdale 87	Resistant	Resistant
'Old Home'	unknown	Resistant	Resistant
P155-3	'Comice' x 19B29	Resistant	Partially tolerant
P298-18	'Williams' x US309	Resistant	Resistant
PQ5-13	C84 o.p.	-	Partially tolerant
PQ5-8	C84 o.p.	Susceptible	Susceptible
PQ34-1	QR517-9 x QR708-12	Susceptible	Susceptible
PQ34-3	QR517-9 x QR708-12	-	Resistant
PQ34-6	QR517-9 x QR708-12	-	Resistant
PQ35-2	QR708-36 x QR708-12	-	Partially tolerant
PQ35-3	QR708-36 x QR708-12	-	Partially tolerant
PQ37-2	OHxF87 x B627	-	Resistant
PQ37-3	OHxF87 x B627	-	Resistant
PQ37-5	OHxF87 x B627	-	Resistant
PQ37-7	OHxF87 x B627	-	Resistant
PQ37-8	OHxF87 x B627	-	Resistant
PQ38-2	QR708-36 o.p.	-	Partially tolerant
PQ39-1	QR517-9 o.p.	-	Resistant
PQ39-3	QR517-9 o.p.	-	Resistant
PQ39-4	QR517-9 o.p.	-	Partially tolerant
PQ39-5	QR517-9 o.p.	-	Resistant

[1]FB; Fire blight

Woolly apple aphid (WAA) inoculation for resistance screening

The breeding programme mainly incorporated WAA resistance genes *Er1* from 'Northern Spy' and *Er2* from *M. robusta* 5 (Bus et al., 2007). *M. floribunda* 182 was also used in crosses to introduce WAA resistance through a yet-uncharacterised resistance source (named 'ER_flo' for the purpose of this work).

Seedlings germinated in 2018 from the three families that were excluded from MAS for FB resistance, and which were expected to segregate for WAA resistance (or where resistance is unknown), were included in a WAA resistance screen in May 2019 (Table 8). Seedlings scored either 'resistant' or 'fairly resistant' were selected, while seedlings scored 'moderately susceptible' or 'susceptible' were deselected and discarded. Family M613 (M.116 x AR295-6) was predicted to segregate for WAA resistance (~50:50), and this was reflected in the results. Of the 77 individuals of M613 screened, 30 susceptible and moderately susceptible individuals were discarded, while 27 individuals were selected as resistant. 11 individuals classified as 'fairly resistant', and 10 with 'inconclusive' results, were kept for be re-evaluated through inoculation in 2020. Family M616 ('Novole' x M.9) had unknown predicted tolerance as it was not known whether 'Novole' carries WAA resistance. Family M616 was revealed to be largely susceptible and was therefore deselected and discarded. Predictions on the tolerance of M617 (*M. koreana* x M.9) could also not be made, as the tolerance of *M. koreana* to WAA was not known. Interestingly, ~50:50 segregation was observed for family M617. Since the source of WAA resistance from *M. koreana* is currently uncharacterised, family M617 can be utilised to map and further study this source of resistance. As a result, all surviving individuals of M617 were kept for mapping *M. koreana* WAA resistance. All 'resistant', 'fairly resistant', and 'moderately susceptible' seedlings (99 in total), as well as 6 'inconclusive' seedlings, were re-evaluated through WAA inoculation in 2020 to confirm the phenotype.

Between late spring and autumn 2020, WAA inoculation was performed for seedlings germinated in 2019 which had been retained after MAS for FB resistance and were still alive at the time of WAA inoculation. In addition, WAA inoculations were done on seedlings germinated in 2018 for which screening was to be repeated (as described above). The majority of WAA inoculations and scoring were done by Ms Cindayniah Godfrey as part of her work and training for the CTP-FCR funded PhD project '*Resistance and susceptibility in interactions between apple and woolly aphids*'.

The combined results for WAA screening for all seedling populations screened in 2019 and 2020 are shown in Table 8. A number of seedlings from families M613 and M617 died between 2019 and 2020 and were excluded from the analysis. Segregation ratios were

calculated to express the percentage of selected (resistant and fairly resistant) seedlings and the percentage of deselected (susceptible and moderately susceptible) seedlings in each family, with any scored ‘inconclusive’ excluded from the calculations. Segregation ratios were only calculated for families with more than 40 seedlings screened. Since a higher-than-expected number of seedlings were scored as ‘resistant’ or ‘fairly resistant’ in families expected to segregate for WAA resistance, these seedlings, along with any ‘inconclusive’ seedlings, will be re-inoculated with WAA in future.

WAA direct inoculations were also performed for a number of selections and pre-selections in 2019, with three to five replicates of each genotype pre-scored and inoculated with WAA in June. However, no increase in established colonies was observed two weeks after inoculation, and trees were re-inoculated with WAA in August 2019. Despite re-inoculation, results for all selections remained inconclusive and it was decided to repeat the WAA inoculation in 2020. WAA direct inoculations were repeated for selections and pre-selections between summer and autumn 2020 (Table 9), by which time FB marker data was available (Table 2). In total, 62 selections/pre-selections (three to five replicates of each) were included in the WAA direct inoculations. Any genotypes for which WAA colonies were observed in the field or glasshouse without direct inoculation (‘self-inoculation’) were categorised as ‘susceptible’ and not included in the direct inoculation. Selections/pre-selections that were both FB susceptible (according to FB marker data, direct inoculation, or pedigree) and WAA susceptible were deselected and discarded. WAA direct inoculations will be repeated in future for any genotypes scored as ‘inconclusive’, ‘resistant’, or ‘fairly resistant’, along with 54 genotypes that have not yet been screened.

Table 8. Combined results of WAA[1] screening of seedlings performed in 2019 and 2020, showing family code, year seedlings were germinated, parentage, and prediction for segregation. Total number of seedlings inoculated with WAA are given, as well as the number of seedlings scored as resistant/fairly resistant, susceptible/moderately susceptible, and for which results were inconclusive. Segregation ratios (%) are shown in parenthesis where calculated.

Family	Year germinated	Parentage	Prediction	Total number tested	Resistant/ Fairly resistant	Susceptible/ Moderately susceptible	Inconclusive
M613	2018	M.116 x AR295-6	Segregate for *Er1* (~50%)	76	34 (48%)	36 (51%)	6
M616	2018	'Novole' x M.9	Resistance unknown	146	18 (12%)	128 (88%)	-
M617	2018	*M. koreana* x M.9	Resistance unknown	148	73 (53%)	69 (47%)	-
M618	2019	AR295-6 x C.G.202	Segregate for *Er2* (likely <50%)	3	2	1	-
M620	2019	Bud.9 x 'Evereste'	Susceptible pedigree	3	0	3	-
M621	2019	C.G.11 x AR295-6	Susceptible pedigree	2	0	2	-
M622	2019	C.G.202 x M547-41	Segregate for *Er2* (~50%) and for Er_flo	43	19 (59%)	13 (41%)	11
M623	2019	C.G.202 x 'Evereste'	Segregate for *Er2* (~50%)	1	0	1	-
M624	2019	*M. floribunda* 821 x M.M.106	Segregate for *Er1* and for Er_flo (~50%)	1	-	-	1
M626	2019	*M. robusta* 5 x AR295-6	Segregate for *Er2* (~50%)	1	1	0	-
M627	2019	M.116 x C.G.935	Segregate for *Er1* (~50%)	108	89 (93%)	7 (7%)	12
M628	2019	M.116 x C.G.11	Segregate for *Er1* (~50%)	29	19	0	10
M629	2019	M.116 x AR295-6	Segregate for *Er1* (~50%)	10	4	0	6
M630	2019	M.116 x *M. robusta* 5	Segregate for *Er1* and *Er2* (~50%)	23	15	4	4
M631	2019	'Northern Spy' x 'Evereste'	Segregate for *Er1* (~50%)	175	86 (59%)	61 (41%)	28

[1]WAA; woolly apple aphid

Table 9. Combined results of WAA[1] screening of seedlings performed in 2019 and 2020, showing selection number, parentage, and tolerance to WAA following direct inoculation

Genotype	Parentage	Tolerance to WAA[1]
M432-203	M.27 x M.116	Fairly resistant
M432-217	M.27 x M.116	Susceptible (self-inoculation)
M480-003	M.9 x M.116	Fairly resistant
M482-013	M.9 x M.116/C.G.202	Susceptible (self-inoculation)
M508-001	M.13 x JM.7	Resistant
M509-022	Unknown	Inconclusive
M546-009	M.9 x JM.7	Inconclusive
M546-110	M.9 x JM.7	Resistant
M547-008	M.9 x *M. floribunda* 821	Fairly resistant
M549-059	M.13 x JM.7	Susceptible (self-inoculation)
M549-146	M.13 x JM.7	Resistant
M550-012	AR86-1-20 x M.9	Resistant
M553-002	AR86-1-20 x C.G.202	Resistant
M553-028	AR86-1-20 x C.G.202	Resistant
M553-032	AR86-1-20 x C.G.202	Resistant
M553-064	AR86-1-20 x C.G.202	Fairly resistant
M553-085	AR86-1-20 x C.G.202	Resistant
M553-112	AR86-1-20 x C.G.202	Fairly resistant
M553-124	AR86-1-20 x C.G.202	Susceptible
M554-040	M.M.106 x C.G.30	Susceptible (self-inoculation)
M554-072	M.M.106 x C.G.30	Susceptible (self-inoculation)
M554-092	M.M.106 x C.G.30	Susceptible (self-inoculation)
M554-209	M.M.106 x C.G.30	Resistant
M554-343	M.M.106 x C.G.30	Susceptible
M555-030	C.G.30 o.p.	Susceptible (self-inoculation)
M555-136	C.G.30 o.p.	Resistant
M555-189	C.G.30 o.p.	Susceptible (self-inoculation)
M555-282	C.G.30 o.p.	Susceptible
M555a-087	C.G.30 o.p.	Moderately susceptible
M555a-118	C.G.30 o.p.	Resistant
M556-036	'Ottawa 3' o.p.	Susceptible (self-inoculation)
M556-052	'Ottawa 3' o.p.	Susceptible (self-inoculation)
M556a-054	'Ottawa 3' o.p.	Susceptible
M557-006	M.116 x M.9	Resistant
M557-007	M.116 x M.9	Resistant
M557-064	M.116 x M.9	Resistant
M560-003	AR86-1-20 x C.G.30	Resistant
M560-087	AR86-1-20 x C.G.30	Moderately susceptible
M560-094	AR86-1-20 x C.G.30	Resistant
M560-167	AR86-1-20 x C.G.30	Fairly resistant
M560-214	AR86-1-20 x C.G.30	Resistant
M560a-002	AR86-1-20 x C.G.11	Resistant
M560a-003	AR86-1-20 x C.G.11	Fairly resistant

Genotype	Parentage	Tolerance to WAA[1]
M560a-018	AR86-1-20 x C.G.11	Resistant
M560a-020	AR86-1-20 x C.G.11	Fairly resistant
M560a-046	AR86-1-20 x C.G.11	Inconclusive
M560a-097	AR86-1-20 x C.G.11	Fairly resistant
M560a-123	AR86-1-20 x C.G.11	Inconclusive
M560a-127	AR86-1-20 x C.G.11	Resistant
M560a-135	AR86-1-20 x C.G.11	Fairly resistant
M560a-148	AR86-1-20 x C.G.11	Inconclusive
M562-108	M.M.106 x C.G.202	Fairly resistant
M562-139	M.M.106 x C.G.202	Fairly resistant
M562a-002	M.M.106 x C.G.202	Resistant
M562a-010	M.M.106 x C.G.202	Moderately susceptible
M562a-058	M.M.106 x C.G.202	Resistant
M562a-085	M.M.106 x C.G.202	Susceptible
M562a-149	M.M.106 x C.G.202	Fairly resistant
M562a-182	M.M.106 x C.G.202	Moderately susceptible
M562a-185	M.M.106 x C.G.202	Susceptible
M563-045	M.M.106 x Bud.9	Resistant
M563-054	M.M.106 x Bud.9	Resistant
M563-065	M.M.106 x Bud.9	Fairly resistant
M563-090	M.M.106 x Bud.9	Resistant
M563-109	M.M.106 x Bud.9	Susceptible (self-inoculation)
M563a-014	M.M.106 x Bud.9	Susceptible
M563a-016	M.M.106 x Bud.9	Moderately susceptible
M563a-048	M.M.106 x Bud.9	Resistant
M563a-068	M.M.106 x Bud.9	Resistant
M564-003	C.G.202 x AR295-6	Fairly resistant
M565-001	Bud.9 x M.116	Fairly resistant
M571-066	C.G.11 o.p.	Resistant
M582-025	M.M.106 x C.G.30	Fairly resistant

[1]WAA: Woolly apple aphid

MAS for dwarfing

In autumn 2020, selections and pre-selections that had been retained following FB resistance screening (MAS and direct inoculations) and WAA resistance screenings, and which were still alive in August 2020, were included in MAS for dwarfing. In total, 15 selections and 165 pre-selections (from 30 different families), as well as all parental genotypes, were screened for dwarfing markers. Since these selections and pre-selections had been planted in the field and budded in previous years, records on observed vigour in the field were available and compared with results from the marker analysis.

Table 10 provides the results for the dwarfing marker analysis and observed vigour for selections, as well as dwarfing marker results and reported vigour for relevant parental genotypes. The majority of selections contained only one of the two (LG5 or LG11) dwarfing loci. Two selections (M432-203 and M480-003) contain no dwarfing loci due to recombination. Selection M546-022 (derived from a cross between two dwarfing genotypes, M.9 and JM.7) contains both dwarfing loci from M.9 and was observed as being 'medium dwarf' in the field. JM.7 (M.9 x *M. prunifolia*) is reported as dwarfing, while containing only the LG5 dwarfing locus from M.9. The majority of selections from family M549 (derived from a cross between dwarfing JM.7 and vigorous M.13) were observed as being 'dwarf' to 'medium' in the field, even when LG5 locus from vigorous M.13 is present. Interestingly, the only selection from this family that is 'medium vigorous' (M549-146) contains the LG5 dwarfing locus from M.9. All three selections from family M547 (M.9 x *M. floribunda* 821) contained both dwarfing loci from M.9, but exhibit vigour ranging from 'dwarf' to 'medium vigour' in the field.

Table 11 provides results of dwarfing marker screening for pre-selections from 30 families and their parental genotypes. Generation of dwarfing progeny was largely achieved through the introduction of dwarfing loci either via crosses with M-series rootstocks directly, or by crossing with the semi-dwarfing Geneva (C.G.) rootstocks (Robinson et al., 2003) or dwarfing rootstock Bud.9 (Budagovsky, 1974). The majority of pre-selections screened either had no dwarfing loci, or only one of the two dwarfing loci (LG5 or LG11). In eight families derived from rootstock C.G.30 (M.9 x *M. robusta* 5; carrying both M.9 dwarfing loci), a number of pre-selections (27 individuals in total) were found to carry both M.9 dwarfing loci. In family M592 (C.G.30 x M.27), seven out of 19 pre-selections carried the two dwarfing loci from either M.9 or M.27, while no individual carried both M.9 and M.27 sources together. In families M560a and M571 derived from C.G.11 (*M. robusta* 5 x M.26), 3 individuals carry the two M.26 loci.

Seven families were derived from crosses with C.G.202 (M.27 x *M. robusta* 5), yet the dwarfing loci from M.27 were absent in all individuals. Dwarfing marker allele profiles for the C.G.202 parental genotype, along with allele profiles from a set of twelve fingerprinting SSR

markers, revealed that C.G.202 is not derived from M.27. Allele profiles confirmed, however, that C.G.202 is derived from *M. robusta 5*. Indeed, FB marker analysis of pre-selections and seedlings (Table 3 and Table 5) confirmed the introduction of the FB_R5 locus from C.G.202 to progeny. Taken together, these data suggest that either C.G.202 in the NIAB EMR germplasm collection is not true to type, or that C.G.202 is not derived from M.27 as reported (Robinson et al., 2003). Similar discrepancies were observed in three families derived from Bud.9 (M.9 x 'Krasni Standard'), in which no individuals carry all alleles linked to the M.9 dwarfing loci. Analysis of allele profiles for Bud.9 and M.9 confirmed that the parental genotype Bud.9 used in the crossing programme was not derived from M.9, suggesting that Bud.9 in the NIAB EMR genebank is either not true to type, or that Bud.9 is not derived from M.9 as reported (Budagovsky, 1974).

Parental genotype 'Ottawa 3' (M.9 x 'Robin Crabapple') is a dwarfing rootstock, which carries only the LG5 dwarfing locus from M.9 (Table 11). This LG5 locus from M.9 is present in two of the four individuals of family M556a ('Ottawa 3' o.p.). It is unclear whether this haplotype will result in dwarfing characteristics similar to 'Ottawa 3' as a result. Pres-selections that do carry the single dwarfing locus were observed as 'very dwarfing to dwarfing' in the field, compared to 'medium dwarfing' observed in the two pre-selections in which no dwarfing loci are present. Similarly, families M590 (M.13 x M.116) and M594 ('Novole' x M.116) were derived from M.116 (M.27 x M.M.106), a semi-invigorating rootstock which carries only the LG11 dwarfing locus of M.27 (Table 11). Four individuals from M590 and one from M594 carry this LG11 dwarfing locus of M.27 and observed vigour for these pre-selections range from 'dwarfing to medium dwarfing'.

No selections or pre-selections were discarded as a result of the dwarfing marker screening, but it was noted which individuals contain both dwarfing loci (LG5 and LG11) which could be passed to progeny.

Table 10. Results from dwarfing marker screening of selections and parental genotypes, showing genotype, parentage, category, presence of any dwarfing loci, and reported or observed vigour.

Genotype	Parentage	Category	Dwarfing locus present[1]	Vigour[2]
M.9	Unknown	Parent	M.9 loci	dwarfing[3]
M.13	Unknown	Parent	none	vigorous[3]
M.27	M.9 x M.13	Parent	M.27 loci	very dwarfing[3]
M.116	M.27 x M.M.106	Parent	M.27 LG11 locus	semi-invigorating[3]
M.M.106	'Northern Spy' x M.1	Parent	none	vigorous[3]
JM.7	M.9 x *M. prunifolia*	Parent	M.9 LG5 locus	dwarfing[3]
M.I.S	Unknown	Parent	-	unknown[3]
M. floribunda 821	-	Parent	-	unknown[3]
M430-249	M.I.S. x M.27	Selection	M.27 LG5 locus	dwarf to medium dwarf[4]
M432-203	M.27 x M.116	Selection	Recombinations	medium dwarf to medium[4]
M480-003	M.9 x M.116	Selection	Recombinations	dwarf to medium dwarf[4]
M508-001	M.13 x JM.7	Selection	M.9 LG5 locus	medium[4]
M508-049	M.13 x JM.7	Selection	M.13 LG5 locus	medium dwarf[4]
M509-022	Unknown	Selection	Unknown	dwarf to medium dwarf[4]
M546-022	M.9 x JM.7	Selection	M.9 loci	medium dwarf[4]
M546-110	M.9 x JM.7	Selection	M.9 LG5 locus	medium[4]
M547-001	M.9 x *M. floribunda* 821	Selection	M.9 loci	medium to medium vigorous[4]
M547-008	M.9 x *M. floribunda* 821	Selection	M.9 loci	medium dwarf to medium[4]
M547-041	M.9 x *M. floribunda* 821	Selection	M.9 loci	dwarf to medium[4]
M549-083	M.13 x JM.7	Selection	M.9 LG5 locus	medium[4]
M549-094	M.13 x JM.7	Selection	M.13 LG5 locus	dwarf to medium dwarf[4]
M549-122	M.13 x JM.7	Selection	M.13 LG5 locus and M.9 LG5 locus	medium dwarf[4]
M549-146	M.13 x JM.7	Selection	M.9 LG5 locus	medium to medium vigorous[4]

[1]LG; linkage group

[2]In ascending order of vigour: very dwarf; dwarf; medium dwarf; medium; medium vigorous; vigorous; very vigorous

[3]Vigour for parental genotypes as per breeding records and literature

[4]Vigour for selections as observed in budded trees in the field

Table 11. Parental genotypes and pre-selections included in MAS[1] for dwarfing, showing genotype or family code, parentage, category, dwarfing loci present (parental genotypes) or expected (pre-selections), reported vigour (parental genotypes), and results of MAS[1] for dwarfing. Total number of individuals screened for each family, as well as number of individuals containing all loci, only one dwarfing locus, or no dwarfing loci, are shown.

Genotype/family	Parentage	Category	Dwarfing locus[1]	Reported vigour	MAS[2] for dwarfing			
					Total screened	All dwarfing loci	One dwarfing locus	No dwarfing loci
AR 86-1-20	M.27 x M.M.106	Parent	M.27 LG11 locus[3]	semi-invigorating	-	-	-	-
AR295-6	'Ottawa 3' x *M. robusta* 5	Parent	M.9 LG5 locus[3]	dwarfing	-	-	-	-
Bud.9	M.9 x 'Krasni Standard'	Parent	Unknown (should be M.9 loci) [3]	dwarfing	-	-	-	-
'Evereste'	PRI-187-11 o.p.	Parent	-	unknown	-	-	-	-
C.G.11	*M. robusta* 5 x M.26	Parent	M.26 loci[3]	semi-dwarfing (M26)	-	-	-	-
C.G.202	M.27 x *M. robusta* 5	Parent	Unknown (should be M.27 loci) [3]	semi-dwarfing	-	-	-	-
C.G.30	M.9 x *M. robusta* 5	Parent	M.9 loci[3]	semi-dwarfing	-	-	-	-
JM.7	M.9 x *M. prunifolia*	Parent	M.9 LG5 locus[3]	dwarfing	-	-	-	-
M. floribunda 821	-	Parent	-	unknown	-	-	-	-
M.116	M.27 x M.M.106	Parent	M.27 LG11 locus[3]	semi-invigorating	-	-	-	-
M.13	Unknown	Parent	none[3]	vigorous	-	-	-	-
M.27	M.9 x M.13	Parent	M.27 loci[3]	very dwarfing	-	-	-	-
M.9	Unknown	Parent	M.9 loci[3]	dwarfing	-	-	-	-
M.I.S	Unknown	Parent	-	-	-	-	-	-
M.M.106	Northern Spy' x M.1	Parent	None[3]	vigorous	-	-	-	-
'Novole'	*M. prunifolia*	Parent	-	unknown	-	-	-	-
'Ottawa 3'	M.9 x 'Robin Crabapple'	Parent	M.9 LG5 locus[3]	dwarfing (>M9; <M26)	-	-	-	-
Lubera 'Sally'	-	Parent	-	very dwarf	-	-	-	-
M550	AR86-1-20 x M.9	Pre-selection	M.27 loci[4]	-	1	0	0	1
M553	AR86-1-20 x C.G.202	Pre-selection	M.9 loci[4]	-	3	0	0	3
M554	M.M.106 x C.G.30	Pre-selection	M.9 loci[4]	-	2	1	1	0
M555	C.G.30 x o.p.	Pre-selection	M.9 loci[4]	-	3	2	1	0
M555a	C.G.30 o.p.	Pre-selection	M.9 loci[4]	-	6	1	3	2
M556a	'Ottawa 3' o.p.	Pre-selection	M.9 LG5 locus[4]	-	4	0	2	2
M557	M.116 x M.9	Pre-selection	M.9 loci[4]	-	1	0	1	0
M560	AR86-1-20 x C.G.30	Pre-selection	M.9 loci[4]	-	5	4	1	0
M560a	AR86-1-20 x C.G.11	Pre-selection	M.9 loci[4]	-	14	2	9	3
M562	M.M.106 x C.G.202	Pre-selection	M.9 loci[4]	-	2	0	0	2

Genotype/family	Parentage	Category	Dwarfing locus[1]	Reported vigour	MAS[2] for dwarfing			
					Total screened	All dwarfing loci	One dwarfing locus	No dwarfing loci
M562a	M.M.106 x C.G.202	Pre-selection	M.9 loci[4]	-	10	0	0	10
M563	M.M.106 x Bud.9	Pre-selection	M.9 loci[4]	-	4	0	0	4
M563a	M.M.106 x Bud.9	Pre-selection	M.9 loci[4]	-	4	0	0	4
M564	C.G.202 x AR295-6	Pre-selection	M.9 loci[4]	-	1	0	0	1
M565	Bud.9 x M.116	Pre-selection	M.9 loci[4]	-	1	0	0	1
M568	'Torstein' x M.27	Pre-selection	M.27 loci[4]	-	1	0	0	1
M570	C.G.202 o.p.	Pre-selection	M.9 loci[4]	-	5	0	0	5
M571	C.G.11 o.p.	Pre-selection	M.9 loci[4]	-	2	1	1	0
M574	'Evereste' x M.9	Pre-selection	M.9 loci[4]	-	8	3	2	3
M578	C.G.11 x AR295-6	Pre-selection	M.9 loci[4]	-	2	0	2	0
M580	C.G.30 x AR295-6	Pre-selection	M.9 loci[4]	-	3	2	0	1
M582	M.M.106 x C.G.30	Pre-selection	M.9 loci[4]	-	3	1	1	1
M585	M.9 x Lubera 'Sally'	Pre-selection	M.9 loci[4]	-	4	2	1	1
M587	C.G.202 x AR295-6	Pre-selection	M.9 loci[4]	-	4	0	3	1
M588	AR295-6 x C.G.202	Pre-selection	M.9 loci[4]	-	4	0	0	0
M589	'Evereste' x C.G.30	Pre-selection	M.9 loci[4]	-	33	8	13	12
M590	M.13 x M.116	Pre-selection	M.9 loci[4]	-	4	0	4	0
M591	M.M.106 x C.G.30	Pre-selection	M.9 loci[4]	-	4	3	0	1
M592	C.G.30 x M.27	Pre-selection	M.9 loci and/or M.27 loci[4]	-	19	7	11	1
M594	'Novole' x M.116	Pre-selection	M.27 LG11 locus[4]	-	8	0	1	6

[1]LG; linkage group

[2]MAS; marker-assisted selection

[3]Dwarfing loci confirmed present in parental material

[4]Dwarfing loci desired in pre-selections

Discussion

In the first round of MAS, selections and pre-selections were screened for markers linked to FB resistance. No selections were deselected as a result of the FB marker screen, and the marker sets were irrelevant to the majority of selections due to their pedigree: 16 selections are derived from susceptible genotypes or from genotypes for which no FB resistance markers are available. This collection of material partly reflects the change in priorities in breeding objectives over the years (selections were planted before 2009). Only three selections were derived from a genotype for which FB markers are available (*M. floribunda* 821), and were all determined to contain the FB_flo resistance locus. MAS for FB resistance was more effective among the pre-selections since the majority of families were expected to segregate for FB_R5 and/or FB_E, with only 10 families derived from susceptible or unrelated genotypes. In total, 385 pre-selections from 27 families were subjected to MAS for FB resistance, with 125 (32%) identified as carrying the appropriate FB resistance source. While no pre-selections or selections were deselected at this stage, the results greatly limit the number of genotypes that must be screened through direct inoculation with FB. While direct inoculations with FB are important to confirm resistance, the process is expensive, labour-intensive, and time-consuming, and the number of genotypes that can be screened is limited by the need for grafted trees and secure glasshouse facilities. Indeed, across the two inoculation experiments in 2019 and 2020, only 35 different apple genotypes (including one positive control, one negative control, and 18 selections and pre-selections) could be screened for FB resistance. Selections and pre-selections for which the presence of FB resistance loci had been confirmed were shown to be either partially tolerant or resistant to FB direct inoculation. Among the nine individuals derived from FB resistant genotype JM.7 (for which no markers are available), only four were partially tolerant or resistant. It could be argued that, had markers been available for the resistance source of JM.7, the five susceptible genotypes would not have been included in the direct inoculation, leaving space for other individuals where FB resistance is suggested by marker data and must be confirmed. This problem is exacerbated in pear rootstock breeding, where no markers are available for the FB resistance sources introduced in the breeding programme.

MAS for FB resistance was most effective among seedling populations raised in 2018 and 2019. Of the six families of seedlings germinated in 2018, three families were expected to segregate for FB resistance (but not for WAA resistance) and were therefore screened with FB markers: 622 seedlings were screened of which only 137 seedlings (22%) were selected. Early-stage deselection was even more effective in seedlings germinated in 2019 due to combining a destructive *P. cactorum* resistance screen with MAS for FB: in total, 1530

seedlings from 13 families were germinated, with only 492 (32%) seedlings selected after combined *P. cactorum* and FB marker screens.

Currently, resistance to woolly apple aphid (WAA) can only be determined through direct inoculations or through observations of 'self-inoculation' in the field or glasshouse. While more time-consuming and labour-intensive than MAS, the direct inoculations nonetheless resulted in deselection of a number of individuals, particularly in seedling populations raised in 2018 and 2019. The three families germinated in 2018 which did not undergo MAS for FB were screened for WAA resistance by direct inoculation. Across the three families, 370 seedlings were screened and only 107 WAA resistant seedlings (in two families) retained as part of the breeding programme. Deselection through direct WAA inoculation was slightly less effective among 2019 seedlings, due to a larger than expected number of seedlings showing resistance: only ~41% of seedlings were deselected for being susceptible to WAA.

A similar problem was observed when screening selections and pre-selections for WAA resistance. Of the 73 pre-selections screened, only 23 were scored as WAA susceptible, with 5 inconclusive. WAA direct inoculations must be repeated for all selections, pre-selections and seedlings for which WAA resistance was inconclusive, along with 58 pre-selections that have not yet been tested. It is clear that the efficiency of deselection of WAA-susceptible material will be greatly improved by the availability of markers linked to WAA resistance. While preliminary markers for WAA have been identified, these markers are not sufficiently robust and informative for reliable predictions. Phenotypic data and populations are available for completing this work, and it is hoped that the current CTP-funded PhD project on WAA would make significant progress towards identifying a marker set appropriate for MAS for WAA resistance.

Despite the challenging nature of WAA and FB direct inoculations, the combination of MAS and resistance screening resulted in deselection of a number of selections and pre-selections. Pre-selections and selections shown to be both WAA and FB susceptible were discarded. Of the 19 selections and 420 pre-selections present in the collection at the start of this project, upon completion of the combined deselection processes only 15 selections and 165 pre-selections were retained. These individuals were subsequently screened for dwarfing loci using the preliminary set of markers linked to the dwarfing loci *Rb1* and *Rb2*. While the marker set was effective in identifying individuals in which both complete dwarfing loci were present, it was clear that predicting vigour of an individual based only on the presence or absence of these two loci was difficult. This was particularly apparent in individuals with intermediate vigour, and where only one of the two dwarfing loci was present. While the combination of *Rb1* and *Rb2* have the greatest effect on dwarfing (Harrison et al., 2016), the effect of the third dwarfing QTL on LG13 cannot be discounted and may play a more important role in

semi-invigorating/medium dwarfing individuals, or in individuals where the full *Rb1* and *Rb2* loci are not present. Furthermore, predictions on dwarfing can be even more difficult in genotypes derived from yet-uncharacterised dwarfing sources, such as JM.7. The discrepancies between the presence or absence of dwarfing loci and the observed vigour could be due to numerous factors. Firstly, the vigour observed in selections and pre-selections are based on only one budded individual present in the field. Replicated trials are required for proper assessment of vigour in rootstocks. While control genotypes such as M.9 are present in the plots at EM, the observations are made by eye and it is not always clear how well these compare to standardised (if available) vigour characteristics published for released rootstocks. Lastly, observed vigour can be affected by factors such as the presence of pest or disease or by precocity, with more precocious rootstocks appearing dwarf due to targeting of resources to fruiting rather than vegetative growth. Despite these challenges, and for the purposes of this project, the dwarfing marker set currently in use can serve to select only those individuals where both *Rb1* and *Rb2* dwarfing loci are present. No selections or pre-selections were discarded as a result of the dwarfing marker screening, but it was noted which individuals contain both dwarfing loci, which could in turn be passed to progeny. This information will be particularly valuable when selecting material for pre-breeding in the rootstock breeding programme.

Conclusions

MAS is an effective tool that facilitates early deselection of material in the rootstock breeding programme. MAS is particularly effective when combined with an early seedling-stage destructive screen, limiting the number of grafted and rooted individuals that must be screened through time-consuming and resource-heavy direct inoculation methods. Taken together, these methods enhance efficiency by reducing the number of individual trees maintained in the rootstock breeding programme.

Knowledge and Technology Transfer

EMRC management committee meeting – East Malling, March 2020

Glossary

DNA; Deoxyribonucleic acid

EM; East Malling

EMRC; East Malling Rootstock Club

FB; Fire blight

LG; Linkage group

MAS; Marker assisted selection

PCR; Polymerase chain reaction

SSRs; Simple-sequence repeats

WAA; Woolly apple aphid

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