1 Apple

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1.1 Introduction

1.1.1 Origin of the Domesticated Apple

The genus *Malus* belongs to the Rosaceae family and forms with its closely related fruit (*Pyrus* and *Cydonia*) and ornamental (*Amelanchier, Aronia, Chaenomeles, Cotoneaster, Crateagus, Pyracantha, Sorbus*) genera, the subfamily Maloideae (Challice 1974). This subfamily is believed to be an allopolyploid, that evolved from a hybridization between a Spiraeoidae ($x = 9$) and a Prunoidae ($x = 8$) ancestor resulting in the basic haploid number of $x = 17$ for the Pomoidae (Lespinasse et al. 1999). Most *Malus* species are diploids ($2n = 34$), but a few are triploid (e.g., *M. hupehensis* and *M. coronaria*), or tetraploid (e.g., *M. sargentii*), while some species show variable levels of ploidy (Way et al. 1989). Little information is available on the karyotype of apple. The lengths of the chromosomes in haploid *M. domestica* range from 1.5 to 3.5 µm, with 11 of them being submetacentric, and six being metacentric with respect to the position of the centromere (Bouvier et al. 2000). The longest, and possibly a second chromosome carry a satellite.

With the number ranging from eight to about 122 (Robinson et al. 2001; Harris et al. 2002), there is no agreement among taxonomists as to how many species this genus comprises. The higher estimates may also include many interspecifics, as the species are widely compatible and readily interbreed (Korban 1986). This characteristic has been deployed in apple breeding for the introgression of pest and disease resistance genes. For this reason as well as the assumed interspecific origin of the eating apple in general (Korban 1986; Korban and Chen 1992; Robinson et al. 2001), it seems appropriate to identify the domesticated apple as *M. x domestica* Borkh. However, more recently it has been argued that the correct nomenclature is *M. pumila* Mill. (Korban and Shirvin 1984), and that this species should include the wild apple identified as *M. sieversii* (Lebed.) Roem. (Mabberley et al. 2001). Vavilov (1951) also referred to the wild apple as *M. pumila* when describing the centers of origin of cultivated plant species, which is in complete opposition to the view of another well-known Russian botanist, Ponomarenko, who denied the existence of this species (Way et al. 1989). However, the relatedness of the domesticated and wild apples is strongly supported by the small degree of morphological, biochemical and molecular variation between the two species (Harris et al. 2002). The same could be said of the European wild crabapple *M. sylvestris*. This also belongs, together with *M. sieversii*, to *M. pumila* Mill. (Westwood, in Way et al. 1989), and may have been the result of a separate introduction of the wild apple into Europe. However, the UK research team has not adhered to its own recommendation in later papers and refers to the domesticated apple as *M. domestica* Borkh., while recognizing *M. sieversii* from Central Asia as a separate species (Robinson et al. 2001; Harris et al. 2002). As it suits a purpose of these reviewers, we adhere to the nomenclature according to Way et al. (1989), who identify *M. domestica* and *M. sieversii* as separate species.

The domestication of the apple went hand in hand with the civilization of mankind and has been described extensively by Morgan and Richards (1993). There is evidence of fruit gathering having started as early as the Neolithic times (Juniper et al. 1999). Cultivation increased with propagation through cuttings and also with the discovery of grafting techniques (Morgan and Richards 1993). The fixing of genotypes had a long-lasting effect on apple production, enabling varieties to be grown in orchards and pro-

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viding horticulturalists with the possibility of selecting the best varieties from the many that would have only suited processing because of their bitterness and astringency. Even today, apple production is dominated by cultivars, such as McIntosh (1800s), Jonathan (1820s), Cox's Orange Pippin (1830s), Granny Smith (1860s), Delicious (1870s), Golden Delicious (1890s) and Braeburn (1940s), which were mostly selected from chance seedlings over 100 years ago. By this period, apple had reached all the corners of the world, as emigrants from the Old World introduced them into their new home countries. In Asia, these varieties often replaced the local varieties selected from the native species *M. prunifolia* and its cultivated species *M. asiatica* (Morgan and Richards 1993). It is only recently that bred cultivars developed in the 1930/40s and introduced in the 1960/70s, such as Royal Gala (Kidd's Orange Red × Golden Delicious), Jonagold (Delicious × Jonathan), Fuji (Ralls Janet × Delicious), and Elstar (Ingrid Marie × Golden Delicious), have made major inroads in some countries, even completely replacing existing cultivars. For example, China's enormous growth in apple production is entirely due to the introduction of Fuji.

1.1.2 Apple Production and Exports

With the advent of the new bred cultivars, apple production started to increase rapidly, with several Southern Hemisphere countries, into which apple was introduced, starting to develop major apple industries as they took advantage of the seasons being opposed to those in the Northern Hemisphere (Morgan and Richards 1993). Table 1 shows that in 2004, the world production of apples was an estimated 59 million metric tonnes (MT) produced on 5,280,638 ha of trees (http://faostat.fao.org). After bananas (71 million MT), grapes (65 million MT) and oranges (63 million MT), apples are the fourth biggest fruit crop in the world and production is more than three times that of pears (18 million MT). At 20.5 million MT, China produced over one third of the world production, with the USA being a distant second at 4.3 million MT (Table 1). However, many of the large producers do not export much of their crops, as they have large internal markets, with most of the fruit probably being processed. At 6.2 million MT, about 10% of the world production of apples is exported.

1.1.3 Breeding Strategy

The traditional method of apple improvement by selecting the best phenotypes from seedlings grown from open-pollinated seeds was replaced by deliberate hybridization about 200 years ago. The science of breeding started with the first controlled cross-pollinisation carried out by Thomas Knight early in the nineteenth century (Brown 1975). However, initially little progress was made in improving apple cultivars through controlled crossing, which has been attributed to poor selection of parents (Janick et al. 1996). The success of the relatively recent introductions must be attributed to the selection of parents with good fruit quality. Royal Gala, Fuji, and Jonagold were selected in the first generation from the best commercial cultivars, notably Golden Delicious and Delicious, available at the time of crossing.

Table 1. Estimated apple production (for 2004) and exports (for 2003) (× 1000 metric tonnes) by country (FAOSTAT data)

Country	Production	Export
China	20,503	609
USA	4,290	546
Poland	2,500	349
France	2,400	804
Iran	2,350	109
Turkey	2,300	19
Italy	2,012	708
Russian Federation	1,900	1
Germany	1,600	70
India	1,470	9
Argentina	1,262	200
Chile	1,100	601
Brazil	978	76
Japan	881	17
Ukraine	850	10
Romania	810	0
South Africa	701	326
Hungary	680	8
North Korea	660	0
Spain	614	73
New Zealand	550	323
Mexico	503	0
Uzbekistan	500	4
Egypt	485	0

Apple is self-incompatible and highly heterozygous, which results in very diverse progeny with only a few of them being a major improvement on the parents. As most characters are under polygenic control, low efficiency in genetic improvement of breeding lines together with a long juvenile period make breeding in this crop a slow and expensive process. Hence most apple breeders cannot afford long-term breeding strategies based on recurrent selection achieving incremental gains for a range of characters in each generation (Bringhurst 1983; Oraguzie et al. 2004). Instead, the most common breeding strategy in apple is a limited version of recurrent selection, which is applied to fewer but larger progenies derived from a limited number of parents, selected for a few characters to be improved in a new cultivar (Janick et al. 1996). As breeders cannot afford the time to develop test-crosses to assess the ability of crossing combinations to achieve the breeding goals (Bringhurst 1983), there will be an aspect of chance in the parent selection for a high specific combining ability (SCA) with regard to quantitatively inherited traits. The effect of parents with poor fruit quality is illustrated by the breeding of scab-resistant cultivars carrying the *Vf* gene from *M.* x *floribunda* 821, a crabapple with small fruit of low quality. The first cultivar, Prima (Dayton et al. 1970), is an F_4 descendant of *M.* x *floribunda* and was introduced about 30 years after the Purdue-Rutgers-Illinois (PRI) breeding program started with the specific objective of developing pest and disease resistant cultivars (Crosby et al. 1992). In spite of an "unceasing, single-minded emphasis on moving the *Vf* gene into an adapted type" (Janick et al. 1996), 35 years later there still are no cultivars that have had a considerable impact on pipfruit production by replacing major susceptible cultivars. Breeders have not been able to make the scab-resistant cultivars "catch up" with the eating quality expected of new cultivars today. Nevertheless, the program might have made still less progress if the breeders had been aiming to achieve too many breeding objectives at the same time, which creates inefficiencies as large numbers of seedlings are required to improve the chances of meeting all selection criteria (Brown 1975; Oraguzie et al. 2004).

1.1.4 Breeding Objectives

The principal breeding objective for apple is to increase the marketability of the fruit (Janick et al. 1996). As most breeding programs aim to develop new cultivars for the fresh market, the emphasis is on appearance and eating quality meeting the consumers' expectation of pleasurable fruit consumption, linked with storability to extend the market window. Selection criteria for external quality mostly pertain to skin color, the pattern and amount of fruit covered with color, and the size and shape of the fruit, while internal quality is predominantly determined by flesh texture and flavor (Janick et al. 1996). However, selection criteria may differ in accent, as different breeders aim to develop new cultivars specific to the particular market they target (Laurens 1999) and long-term breeding goals are being increasingly determined by consumer preference research. For example, in reply to an increased consumer interest in the nutritional value of fruit and vegetables, apple is currently being investigated particularly as a source of antioxidants (Davey and Keulemans 2004; Thielen et al. 2004; Lichtenthaler and Marx 2005), which may help prevent diseases and ageing (Raskin and Ripoll 2004; Graziani et al. 2005). On the other hand, a health concern is that apple is a well-known source of allergens. Genetic markers have been identified for genes controlling development of allergens in apple (Gao et al. 2005a, b) (see also Sect. 1.3.2.5.6) and ways are being sought to reduce their negative effect (Hoffman-Sommergruber and the SAFE consortium 2005).

Breeding for pest and disease resistance comes a close second as a major objective (Laurens 1999). Apple is host to a wide range of pests and diseases (Way et al. 1989), many of which need to be controlled in order for commercial production to be profitable. The use of plant resistance is widely regarded as the preferred means of controlling pests and diseases. There are major socio-economic advantages in using resistant cultivars, because they help reduce production costs and diminish the effects on the users and environment because of the reduced requirements for equipment, labour, and fossil fuels (Way et al. 1989; Hogenboom 1993). However, while the potential benefits of resistance breeding are large with regard to the wider impact of pesticide use, the savings to the grower in the direct costs of disease protection are only about 4% of the value of the annual crop (Merwin et al. 1994). The savings also may easily be offset by

market fluctuations and may be reduced by the emergence of other diseases requiring additional control (Merwin et al. 1994). Consumer objection to the use of pesticides was a significant driver for apple breeders to include resistance breeding as a major objective in the development of new cultivars (Laurens 1999), but this to date has not translated into consumers showing a preference for resistant varieties. Although new resistant selections with improved fruit quality are available (Crosby et al. 1992; Fischer et al. 1999), their success in the market place is determined foremost by their ability to differentiate themselves based on appearance and texture in direct competition with the current susceptible cultivars (Murphy and Schertz Willet 1991; Merwin et al. 1994). Therefore, the value of disease resistance to the marketers may prove to be only incremental, until resistant varieties provide an opportunity to rapidly reap the financial benefits of increased demand for fruit produced with reduced chemical inputs, e.g. in organic production systems. These gains will be realised in the long-term only if resistances are durable.

Climatic adaptation is a general breeding objective that ensures trees are productive, bear regularly, and produce fruit with minimal defects, and is achieved by selecting for tree habit, vigor, duration of the juvenile period, and flowering season (Janick et al. 1996). A few breeding programs have more specific objectives to meet the needs of their industries, e.g. adaptation to cold hardiness for climates with severe winters, or low chilling requirements for some subtropical climates. New cultivars often are selected to replace cultivars occupying certain market windows, but in some cases the aim is to extend the marketing period by selecting for very early, or very late maturing cultivars (Laurens 1999).

1.1.5 Molecular Markers and Genetic Maps

Most of the molecular research to date has focused on identifying genetic markers for pest and disease resistance genes, as apple has proved to be a rich source of simply inherited resistance genes with major effects (Table 2). Initially, isoenzymes were used, but they were rapidly superseded by DNA-based markers (see Sect. 1.2). Many different types of markers are available to breeders now, but it has become clear that highly informative markers, such as microsatellite (SSR) and single nucleotide polymorphism (SNP) markers are required to identify resistance genes that are linked or residing in clusters (e.g. Bus et al. 2005b). To date, the primary use of genetic markers in resistance breeding has been in the application of marker-assisted selection (MAS) for pyramided resistance genes in seedling progenies, but they also are an important tool for germplasm screening for sources of resistance (see Sect. 1.5), in host-pathogen interaction research, and map-based cloning of resistance genes (see Sect. 1.6). The mapping of resistance gene loci increasingly shows that they are often linked (Hemmat et al. 2003; Bus et al. 2005a, b), or form part of a gene cluster (Vinatzer et al. 2001; Xu and Korban 2002b). Recent research has also shown that quantitative trait loci (QTL), e.g. for scab resistance, map to the same chromosomal regions as major genes (Durel et al. 2003; Calenge et al. 2004), which suggests that these QTLs probably include residual resistance of "defeated" major effect genes (Pedersen and Leath 1988). The same research has shown that some QTLs are isolate-specific, which suggests that they conform to a gene-for-gene relationship and therefore are subjected to the same risk of resistance "breakdown" as major effect genes (see Sect. 1.4). In apple, gene-for-gene relationships have been demonstrated for *Venturia inaequalis* (Boone and Keitt 1957; Williams and Shay 1957; Bagga and Boone 1968a, b); and apple-cedar rust *Gymnosporangium juniperi-virginianae* (McNew 1938; Niederhauser and Whetzell 1940; Aldwinckle 1975b). The presence of biotypes overcoming major resistance genes suggests that gene-for-gene interactions exist for woolly apple aphid (*Eriosoma lanigerum* Hausm.) (Giliomee et al. 1968; Sandanayaka et al. 2003) and the rosy leaf curling aphid (*Dysaphis devecta* Wlk.) (Alston and Briggs 1968, 1977). Major gene resistances against powdery mildew are also common, while resistance to diseases, such as fire blight and crown rot are predominantly under polygenic control. The same applies to polyphagous insect species, such as leafrollers, although it recently was shown that the resistance to the New Zealand native leafroller species *Ctenopseustis obliquana* Walk. in Prima is controlled by a major gene (Wearing et al. 2003).

QTL mapping is becoming more important in apple breeding as more QTLs are detected not only for pest and disease resistance characters, but increasingly for fruit and tree characters as well (King et al. 2000, 2001; Durel et al. 2003; Liebhard et al. 2003a, c; Calenge et al. 2004; Stankiewicz-Kosyl et al. 2005). Successful mapping of QTL for use by breeders re-

Table 2. Major genes for resistance or susceptibility[z] in apple

Gene	Species	*Malus* source	Reference
Apple scab			
Va	*Venturia inaequalis*	Antonovka PI172623	(Hough et al. 1970)
Vb	*Venturia inaequalis*	Hansen's baccata #2	(Dayton and Williams 1968)
Vc	*Venturia inaequalis*	Cathay	(Korban and Chen 1992)
Vbj	*Venturia inaequalis*	*Malus baccata jackii*	(Dayton and Williams 1968)
Vd	*Venturia inaequalis*	Durello di Forlí	(Tartarini et al. 2004)
Vf	*Venturia inaequalis*	*M. floribunda* 821	(Hough et al. 1953)
Vfh	*Venturia inaequalis*	*M. floribunda* 821	(Bénaouf and Parisi 2000)
Vg	*Venturia inaequalis*	Golden Delicious	(Bénaouf et al. 1997)
Vh8	*Venturia inaequalis*	*M. sieversii* W193B	(Bus et al. 2005a)
Vj	*Venturia inaequalis*	Jonsib	(Korban and Chen 1992)
Vm	*Venturia inaequalis*	*M. micromalus* 245-38	(Dayton et al. 1970a)
Vh2	*Venturia inaequalis*	Russian apple R12740-7A	(Bus et al. 2005b)
Vr2	*Venturia inaequalis*	Russian apple R12740-7A	(Patocchi et al. 2003)
Vh4	*Venturia inaequalis*	Russian apple R12740-7A	(Bus et al. 2005b)
Powdery mildew			
Pl-1	*Podosphaera leucotricha*	*M.* x *robusta* OP 3762	(Knight and Alston 1968)
Pl-2	*Podosphaera leucotricha*	*M.* x *zumi* OP 3752	(Knight and Alston 1968)
Pl-8	*Podosphaera leucotricha*	*M. sargenti* 843	(Korban and Dayton 1983)
Pl-d	*Podosphaera leucotricha*	D12	(Visser and Verhaegh 1980)
Pl-m	*Podosphaera leucotricha*	Mildew Immune Selection	(Dayton 1977)
Pl-w	*Podosphaera leucotricha*	White Angel	(Batlle and Alston 1996)
Aphids			
Er-1	*Eriosoma lanigerum*	Northern Spy	(Knight et al. 1962)
Er-2	*Eriosoma lanigerum*	*M.* x *robusta*	(King et al. 1991)
Er-3	*Eriosoma lanigerum*	Aotea	(Bus et al. 2000)
Sd-1	*Dysaphis devecta*	Cox's Orange Pippin	(Alston and Briggs 1968)
Sd-2	*Dysaphis devecta*	Northern Spy	(Alston and Briggs 1977)
Sd-3	*Dysaphis devecta*	*M.* x *robusta* OP MAL59/9	(Alston and Briggs 1977)
Sm-h	*Dysaphis plantaginea*	*M.* x *robusta* OP MAL59/9	(Alston and Briggs 1970)
Miscellaneous pests and diseases			
Cob-1	*Ctenopseustis obliquana*	Prima	(Wearing et al. 2003)
Gb[z]	*Glomerella cingulata*	Golden Delicious	(Thompson and Taylor 1971)
Gy-a	*Gymnosporangium juniperi-virginianae*	Spartan	(Aldwinckle et al. 1977)
Gy-b	*Gymnosporangium juniperi-virginianae*	Spartan	(Aldwinckle et al. 1977)
Pc	*Phytophthora cactorum*	Northern Spy	(Alston 1970)
Ps-1[z]	*Phyllosticta solitaria*	Jonathan	(Mowry and Dayton 1964)
Ps-2[z]	*Phyllosticta solitaria*	Idared	(Mowry and Dayton 1964)

quires appropriate and rigorous phenotyping techniques, as well as maps saturated with markers that are transportable across genetic backgrounds. The development of the genetic marker maps, e.g. Liebhard et al. (2002, 2003b), perhaps is the easier task, as the meaningful measurement of some quantitatively inherited characters, such as fruit texture (King et al. 2001), is difficult and further complicated by environmental factors (Kearsey and Luo 2003).

In this chapter we describe the advances made in the development and application of molecular techniques in apple breeding to date. We cover the areas of genetic map construction, gene mapping, identification of QTLs, the application of MAS and map-based cloning, following the gene annotation of Alston et al. (2000). Finally, we will discuss the most advanced technologies that are being developed, and future directions of cultivar improvement.

1.2 Construction of Genetic Maps

1.2.1 Brief History of Genetic Mapping in Apple

The earliest genetic maps of apple were developed in the USA and took advantage of the ready availability of Random Amplified Polymorphic DNA (RAPD) markers during the nineties. They also included a small number of isoenzyme markers (Hemmat et al. 1994; Conner et al. 1997). These maps were specific to the genetic background of the mapping parents because of the poor transferability of RAPD markers. For that reason, an international initiative based in Europe developed a genetic map with a number of codominant transportable markers. These were mostly Restriction Fragment Length Polymorphisms (RFLPs) plus a few microsatellite markers (Maliepaard et al. 1998). The most complete map to date is constructed with 129 microsatellites, as well as larger numbers of dominant Amplified Fragment Length Polymorphisms (AFLPs) and RAPDs to assist in filling in gaps (Liebhard et al. 2003b). Such robust polymerase chain reaction (PCR)-based saturated reference maps are essential for whole genome scanning and for understanding complex traits controlled by several Quantitative Trait Loci (QTLs). Several groups worldwide are currently developing transportable genetic maps for apple and a fully saturated consensus map of apple is still required.

1.2.2 First-Generation Maps

Progress in construction of apple genetic maps is summarized in Table 3. The first map (Hemmat et al. 1994) exhibits isoenzyme, RFLP and RAPD markers distributed over 21 and 24 linkage groups, for the cultivars Rome Beauty and White Angel, respectively. Neither of these cultivars was being used in the Cornell University breeding program at the time. However, the second set of maps, for accessions Wijcik McIntosh, NY 75441-67 and NY 75441-58, that were being used in that breeding program, also relied heavily on the contribution of RAPD markers, limiting their usefulness in other progenies. The number of linkage groups (19, 16 and 18 respectively) had been reduced to a number closer to that of the chromosome number of *Malus* ($n = 17$), indicating that these maps were more saturated than previous ones (Conner et al. 1997).

Because of the low transferability of RAPD markers between different cultivars and laboratories, several groups have developed more specific microsatellite markers (also called SSRs or Simple Sequence Repeats). These highly polymorphic and transferable markers proved to be the marker of choice. The first microsatellite markers mapped in apple included some of those identified by Guilford et al. (1997) and Hemmat et al. (1997), as well as four developed by Horticulture Research International (HRI), Wellesbourne, UK. The use of these markers, plus a number of codominantly segregating isoenzymes and RFLPs in a Prima × Fiesta population of 152 seedlings, permitted alignment of the 17 linkage groups and construction of the first integrated apple map (Maliepaard et al. 1998). This initial apple reference map utilized a small number of AFLP markers as well as RAPDs to assist in filling the longer intervals. The cultivars Prima and Fiesta are used in European breeding programs and as such are central to the succession of research programs on genetic mapping in apple situated there: European Apple Genome Mapping Project (EAGMP), Durable Apple Resistance in Europe (DARE) (Lespinasse and Durel 1999) and High-Quality Disease Resistant Apples for a Sustainable Agriculture (HiDRAS). Information from this collaboration, plus that from the mapping of 41 microsatellite markers in the White Angel × Rome Beauty population (Hemmat et al. 2003) enabled cross-referencing of US linkage group numbering with that adopted in Europe. This Prima × Fiesta population has been used to map QTL for apple scab (Durel et al. 2003) and fireblight (Calenge et al. 2005b) (see Sect. 1.4.2).

The genetic map constructed in a Fiesta × Discovery population of 267 individuals (Liebhard et al. 2003b) contains the largest core of robust PCR based markers to date, namely 129 microsatellites, including loci identified by Gianfranceschi et al. (1998) and Liebhard et al. (2002). These markers are supplemented by 710 dominant RAPDs and AFLPs, enabling a good coverage of the 17 linkage groups. The construction of this map was aided by the use of a robotic workstation to set up the large number of PCR reactions required. This reference map has already been used as the framework for mapping QTL (Liebhard et al. 2003a, c; Calenge et al. 2005a, b) – (see Sect. 1.4 below) and Resistance gene analogs (RGAs) that are homologues of nucleotide binding-site (NBS)/leucine-rich repeat resistance genes (LRRs)

Table 3. Genetic maps of apple

Cross	Pop size	Number of markers		Marker Type						Length of map	Reference	Traits
		Female parent	Male parent	Isoen-zyme	RFLPs	RAPD	AFLP	Micro-satellite	Others	cM (female, male)		
Rome Beauty × White Angel	56	156	253	34	8	367	–	–	–	–, 950	Hemmat et al. 1994	*Pl-w*
Wijcik McIntosh × NY 75441-67	114	238	110	6	–	138	–	–	–	1206 (integrated WM), 692	Connor et al. 1997	Skin color, *Vf*, columnar habit, juice pH
Wijcik McIntosh × NY 75441-58	172	181	183	6	–	266	–	–	–	1206, 898	–	
Prima × Fiesta	152	194	163	17	124	133	9	10	SCAR = 1 *Rf*, BC226	842, 984	Maliepaard et al. 1998	*Vf*, *Sd-1*, *Ma*, *SI*
Fiesta × Discovery	112	202	227	–	–	217		118		914, 1015	Liebhard et al. 2002	–
Fiesta × Discovery.	267	439	499	–	–	235	475	129	SCAR = 1 *Rf*, BC226	1144, 1455 (F × D integrated 1371)	Liebhard et al. 2003b	–
	–	–	–	–	–	–	–	–				–
Fiesta × Discovery	44 (subset of 112)	–	–	–	–	–	–	–	18 RGAs (NBS LRR)	Partial map, based on Liebhard et al. 2003b	Baldi et al. 2004	RGAs
Discovery × TN10-8	149	–	–	13	–	–	102	62	22 RGAs (43 bands generated by NBS profiling mapped)	1,219 (integrated map)	Calenge et al. 2004, 2005	*Vg*, scab QTL, RGAS
Telamon × Braeburn	257	259	264	–	–	–	463	20		1039, 1245	Kenis and Keulemans 2005	For QTL analysis growth habit and fruit quality

(Baldi et al. 2004). These RGAs were isolated using a PCR-based strategy based on degenerate primers for conserved sequence motifs in the NBS region and include members of the two major groups of NBS-LRRs described in plants – those possessing a toll-interleukin repeat region, and those lacking it. Eighteen NBS-LRR analogues were mapped as either cleaved amplified polymorphic sequences (CAPS) or single-strand conformation polymorphism (SSCP) markers. These RGAs were distributed widely over the apple genome, covering 12 linkage groups. Potential clustering and association with loci conferring pathogen resistance was noted.

Employment of at least two microsatellite markers per linkage group from this map enabled Calenge et al. (2004, 2005b) to orientate an integrated map constructed in a related Fiesta × Discovery progeny with regard to the two earlier reference maps (Maliepaard et al. 1998; Liebhard et al. 2003b). They then used NBS profiling (Van der Linden et al. 2004) to simultaneously amplify and map 23 polymorphic markers with sequence homology to *Malus* NBS-LRRs, as well as 20 markers with unrelated homologies (Calenge et al. 2005a). Most of the NBS markers were organized in more or less extended clusters, as found in other plant species (Michelmore and Meyers 1998; Young 2000). An extended cluster comprising 13 markers on Linkage Group 2 in a region around *Vr2* is particularly noteworthy (Calenge et al. 2005a). Tight clusters displaying no recombination events were observed on LG 2, LG 10 and LG 17. NBS markers mapped close to major scab and powdery mildew resistances on LG 2, LG 8, LG 10 and LG 12 (*Vr2, Pl-w, Vd* and *Vg* respectively) and to QTLs for resistance to scab and powdery mildew identified previously in this progeny (Calenge et al. 2003, 2004, Calenge and Durel 2006).

The recent construction of a genetic map in a progeny from Telamon × Braeburn has added a further resource for QTL analysis of columnar growth habit and of fruit quality (Kenis and Keulemans 2005) Interestingly, this is the first map in Braeburn, a key cultivar in the New Zealand breeding program, where a population of at least 600 plants of Royal Gala × Braeburn is being developed for mapping of fruit quality attributes (R. Volz, unpublished). The application of the planned QTL analyses from the Telamon × Braeburn progeny to studies of fruit quality in other progenies would be improved by adding more microsatellite markers, as these are low (20) in comparison to the Fiesta × Discovery and Discovery × TN-8 maps (129 and 62) respectively. It has been reported that in Europe a molecular marker map is already under construction for Fuji × Mondial Gala, and that one for Fuji × Braeburn was initiated in 2005 (Costa et al. 2005). These maps in aggregate will provide a valuable resource that will enable rapid progress to be made in establishing the genetics of apple fruit quality.

The recent development of large numbers of Expressed Sequence Tag (EST) sequences for apple (Crowhurst et al. 2005; Korban et al. 2005; Newcomb et al. 2006) has given apple researchers access to a new source of a vast number of potential markers (there are currently nearly 260,000 *Malus* sequences in the public database, GenBank). Polymorphic microsatellite sequences have been identified in great numbers in the EST databases. These EST-microsatellites are being added to those previously developed from both genomic DNA and from the untranslated regions of apple cDNAs. The first apple genetic map utilizing EST-microsatellites is under construction in a cross between Royal Gala and A689-24 (E. Rikkerink et al., work in progress). Single Nucleotide Polymorphism (SNP) markers are also being developed using ESTs. These markers are present throughout the genome and can be used directly to map genes that are hypothesized to be involved in the trait of interest (i.e. are candidate genes). SNPs are already widely used for genetic mapping and association studies in human, animals and plants and represent the markers that will be used in second generation maps in apple.

1.2.3 Genome Organization and Homeology

There are several hypotheses concerning the allotetraploid (or amphidiploid) origin of domestic apple (see Sect. 1.1 and Maliepaard et al. 1998), each of which imply a certain level of duplication within the genome. Maliepaard et al. (1998) were the first to identify the duplication of an entire linkage group (LG 5 and LG 10) by examining the positions of multilocus EST-RFLP markers on a molecular marker linkage map of the cross Prima × Fiesta. An update of this map (Van de Weg et al., in preparation) identified additional duplications (Fig. 1). The amount of homology differed across linkage groups. Some linkage groups seem to be entirely homoeologous to a single other linkage group, such as LG 5 and LG 10, LG 13 and LG 16, and LG 9 and LG 17. Other linkage groups are composite, with several segments, each of which is homoeologous to a segment of a differ-

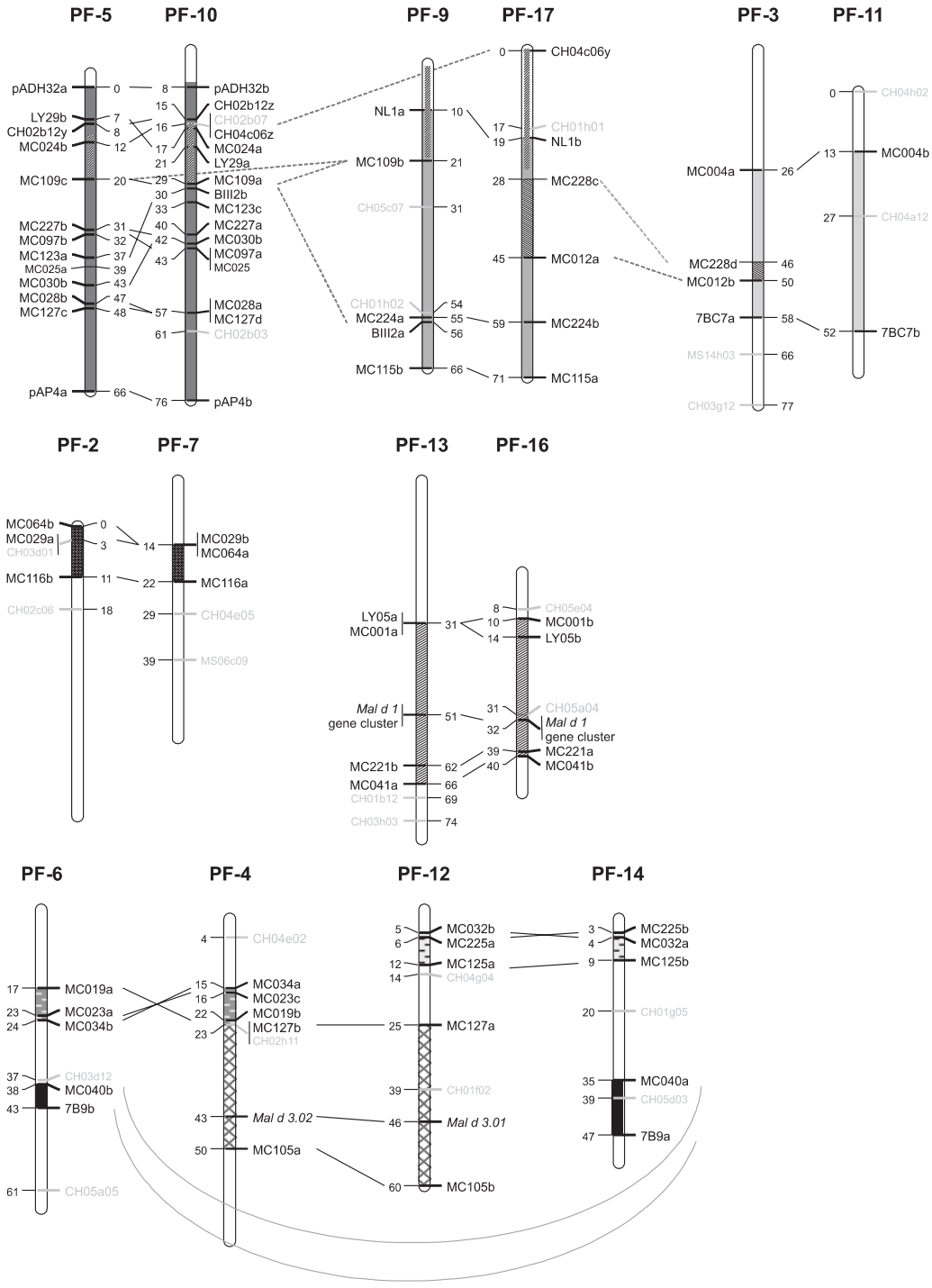

Fig. 1. Map positions of multi-locus markers reveal duplication patterns within the apple genome. Map positions are according to an update of the Prima × Fiesta map of Maliepaard et al. (1998) (van de Weg et al. in prep). The different duplicated (mostly homoeologous) chromosome segments are indicated by different filling patterns of bar segments. Duplicated markers are printed in *black* and are connected by *lines*. Single locus microsatellite-markers (in *grey*) were added as points of reference. Linkage groups are named and orientated according to Maliepaard et al. (1998), except for the orientation of the LG 2, LG 5 and LG 13 that were inverted according to the orientation of their homoeologous linkage groups. Map positions of the *Mal d 1* and *Mal d 3* genes are according to Gao et al. (2005a, b). This figure was kindly supplied by Eric van de Weg and arose in part within the framework of the project EU-DARE (Durable Apple Resistance in Europe, FAIR5, CT97-3898)

ent linkage group. For instance, the proximal part of LG 4 is homoeologous to the proximal part of LG 6, while the more distal part of LG 4 is homoeologous to the distal part of LG 12 (Fig 1). While LG 5 and LG 10 are clearly homoeologous to each other, they also have some markers in common with LG 9 and LG 17 (MC109, MC224, CH04c06). Similarly, LG 3 and LG 17 have two EST-RFLP markers in common, and thus share partial sequences. These shared markers may be due to different genes of a gene family that are dispersed over pairs of homoeologous linkage groups, e.g. *Mal d 1* (Gao et al. 2005b; and Sect. 1.3.2.5.6) where *Mal d 1* represents a gene cluster of seven genes on LG 13 and nine on LG 16.

The order in map position of markers is sometimes slightly different between homoeologous segments. It is not clear whether these differences are real, and arose from genomic rearrangements, or are artefacts due to tension among marker scores during the integration of unbalanced maternal and paternal data sets. For the composite LG 4, LG 6, LG 12 and LG 14, an inversion may have occurred during translocation events, either for the proximal parts of LG 4 and LG 6, or the distal parts of LG 6 and LG 14. Based on these results, Van de Weg et al. (in preparation) proposed to change the Maliepaard (1998) orientation of LG 2, LG 5 and LG 13 to make them consistent with that of their homoeologous linkage groups, thus facilitating comparative analysis within the apple genome.

1.2.4 Comparative Mapping Across Genera

In comparison to other plant systems, for which comparative genome mapping has proven to be a valuable approach both to study genome evolution and to transfer mapping information between genera, only preliminary studies have been carried out to compare the genome maps of apple with others.

1.2.4.1 *Malus* and *Pyrus*

The first example of comparative genome mapping between apple and other members of the Maloideae has been the alignment with the linkage maps of pear. In the course of developing maps for Japanese (Hosui) and European (Bartlett) pears, Yamamoto et al. (2004) located 36 apple microsatellite loci (Liebhard et al. 2002) on the pear map. All pear linkage groups were aligned to the apple consensus map, suggesting conservation of genome organization between apple and pear. This was confirmed in the conservation of the order of loci and the distances between them, which is in agreement with the conserved karyotype between the two genera. An extension of this study to include 69 apple microsatellites and the pear cultivar La France confirmed this finding (Yamamoto et al. 2005). In the same way, apple microsatellite markers from LG 10, 12 and 14 have been mapped in detail on three pear linkage groups by Pierantoni et al. (2004), following the characterization of more than 100 apple microsatellites in four cultivars of pear. For practical purposes, comparative mapping could help to transfer genomic information from apple to the less-studied pear. As a first example, Yamamoto et al. (2005) located the *Vnk* locus for the resistance to pear scab (*Venturia nashicola*) on LG 1 of the Japanese pear cultivar Kinchaku, 33 cM from the map position of CH-Vf2, a close marker for the *Vf* resistance to apple scab in *Malus* (Vinatzer et al. 2004). These studies indicate that further, more detailed map alignment between apple and pear could help pear researchers.

1.2.4.2 *Malus* and *Prunus*

In the first comparison between genomes within the Rosaceae family, 30 loci in the *Prunus* almond × peach (Texas × Earlygold) reference map were found to have homologous counterparts in the Prima × Fiesta apple reference map (Dirlewanger et al. 2004). Generally one linkage group of *Prunus* corresponds to two homeologous apple linkage groups because of the allotetraploid origin of the apple genome, e.g. *Prunus* LG 4 with apple LG 5 and LG 10; half of *Prunus* LG 1 with apple LG 13 and LG 16. As well as these large collinear blocks, major genome rearrangements were identified, e.g. the rearrangement between LG 1 of *Prunus* and LG 8, LG 13 and LG 16 of *Malus*. However the synteny seems conserved between the two species. Nevertheless, considering the high economic importance of *Malus* and *Prunus* species, and in regard to the complementarity of the genomic resources for the two systems (i.e. large characterized EST datasets in apple compared with physical map and small genome in peach), a high-density alignment of the two genomes should be a priority in the next few years, in order to consider the Rosaceae genome as a single system, as has been done for cereals (Keller and Feuillet 2000). Consideration needs to be given to the type of mark-

ers to be used. Dirlewanger et al. (2004) found that microsatellite markers were not as useful as RFLPs and isoenzymes for map comparisons, because only a small proportion of microsatellites mapped had more than one copy.

1.3 Gene Mapping

The task of identification of genetic markers for resistances to the economically significant pests and diseases of apple (i.e. apple scab, powdery mildew and rosy and woolly apple aphid) has been simplified by the large number of resistances to these pathogens that are controlled by major genes (Table 2). A number of major apple resistance genes have now been assigned to linkage groups; *Vf* (Maliepaard et al. 1998) - to LG 1, *Vm* to LG 17 (Patocchi et al. 2005), *Vr2*, *Vh2, Vh4, Vt57, Vbj* and *Vh8* to LG 2 (Bus et al. 2004; Gygax et al. 2004; Patocchi et al. 2004; Bus et al. 2005a,b), *Sd-1* and *Sd-2* to LG 7 (Maliepaard et al. 1998; Cevik and King 2002a), *Pl-w* plus *Er-1* and *Er-3* to LG 8 (Maliepaard et al. 1998; James and Evans 2004; Chagné, Gardiner and Durel, unpublished), *Vd* to LG 10 (Tartarini et al. 2004), *Pl-2* to LG 11 (Seglias and Gessler 1997), *Vg* plus *Pl-d* and *Vb* to LG 12 (Durel et al. 1999; James et al. 2004; Erdin et al. 2006).

1.3.1 Methods Used to Map Major Genes in Apple

For major gene resistances, the relatively speedy process of BSA (Bulked Segregant Analysis) suffices, rather than the time-consuming development of a complete map for the variety in question. This involves identification of markers using pooled DNA from a number of resistant and susceptible plants (Michelmore et al. 1991), in order to develop partial maps around resistance loci. The earliest markers for apple resistance genes were obtained using BSA with RAPDs and the method is still widely utilized by some groups. The efficiency of gene tagging with anonymous markers has been further facilitated by the introduction of automation for DNA extraction from plant tissue, setting up PCR reactions and loading of agarose gels (Cook et al. 2002; Cook and Gardiner 2004). Reproducibility of reactions using RAPD primers is enhanced by automation, and a laboratory throughput of 1,200 samples in a 24-hour period is now possible. RAPD markers are normally converted to more robust sequence specific markers (e.g. SCARs, sequence characterized amplified region), CAPS (cleaved amplified polymorphic sequence) for final map construction and use for MAS.

The publication of the comprehensive microsatellite-based framework map of Liebhard et al. (2003b) has opened up the way to whole genome scanning in apple. Patocchi et al. (2004, 2005 and Erdin et al. 2006) first utilized this approach in their identification of a microsatellite marker linked to *Vr2* and later to locate *Vm* to LG 17 and *Vb* to LG 12. It was successfully modified by James and Evans (2004) in a screen of bulked DNA from a population segregating for the *Pl-w* resistance to enable location of this gene at the top of LG 8. Later, Rusholme (unpublished) confirmed the location of *Pl-2* on LG 11 in a similar approach, utilizing screening of bulked DNA of extreme phenotypes with 3-4 microsatellite markers/linkage group. Patocchi et al. (2005) discuss the significant parameters in setting up a whole genome scanning experiment. These include the interdependent parameters of number of plants and number of microsatellite markers per linkage group, plus the degree of detail in the microsatellite map, and polymorphism in the markers. The recent development of 157 new microsatellite markers brings the total published markers available to over 300 and subset of 86 highly polymorphic microsatellite markers covering 85% of the apple genome with an average density of one marker per 15 cM have been selected as a resource for whole genome scanning (Silfverberg-Dilworth et al.; http://www/hidras.unimi.it). EST databases 2006 are being currently used as a resource for further microsatellite marker development (Gardiner and Korban, unpublished).

A drawback of microsatellites is that they are generally anonymous markers that can be located at large distances from the resistance genes, and therefore they may not be the most suitable for MAS in breeding programs. Another, more recent approach consists of identifying candidates for the gene, or even the polymorphism that is directly responsible for the observed phenotype. This approach is commonly termed the candidate gene approach. Candidate resistance genes identified by searching EST databases with sequence or protein motifs from known resistance genes from model plant systems have proved to be a rich source of genetic markers for resistance genes in apple. Candidates from all known classes of resistance genes

have proved to generate good markers for apple R genes (Gardiner et al. 2003). To date candidate gene markers have been identified for 13 different resistances to apple scab, powdery mildew and woolly apple aphid. Candidate genes are most economically screened initially as RFLPs across mini-populations, and markers mapping as RFLPs close to specific genes are then converted to PCR-based markers such as SNPs or SCARs for mapping in large segregating populations (Gardiner et al. 2003). Other workers have found that NBS-LRR homologues generated by PCR using degenerate primers (Baldi et al. 2004) or from the new methodology of NBS-profiling (Calenge et al. 2005a) generate effective markers. These frequently map in the vicinity of known major resistance loci as well as QTL, accelerating the identification of the genomic regions where functional resistances are located.

1.3.2 Target Traits

1.3.2.1 Apple Scab Resistance Genes

Apple scab resistance genes have received the most attention by genetic mapping groups, because of the significance of the economic impact of apple scab on production. It is also relatively easy to identify markers for major resistances to apple scab in comparison with other resistances. This is because of the relative reliability of phenotypes obtained from glasshouse screening of very young seedlings from mapping populations for response to infection by *Venturia inaequalis*, compared with other pathogens.

1.3.2.1.1 *Vf* The first report of a marker linked to *Vf* was that of the isoenzyme *Pgm-1* (Manganaris et al. 1994) (Table 4). In the same year use of bulked segregant analysis (BSA) enabled speedy identification of a number of RAPD markers linked to this gene (Durham and Korban (1994) (OPA15), Koller et al. (1994) (OPU01, OPM18), Tartarini (1996) (OPC09) and Yang and Kruger (1994) (OPD20)). DNA for BSA was extracted from phenotypic extremes, either from segregating populations, or from varieties. Further markers linked to *Vf* were identified by the same technique and mapped either directly as RAPDs, or after conversion to more robust SCAR or CAPS markers (Gardiner et al. (1996) (OPH01, OPR16); Gianfranceschi et al. (1996) (OPUO1, OPM18); Tartarini (1996) (OPAM19, OPAL07); Yang et al. (1997a) (OPAR4); Yang et al. (1997b) (OPK16); and Hemmat et al. (1998) (S5, B505, S29, P198, B398)). *Vf* was mapped to LG 1 of Prima on the reference map of Maliepaard et al. (1998).

King et al. (1998) and Patocchi et al. (1999a) developed fine maps around *Vf*, locating this resistance in a short interval between OPM18 and OPAL07, thus resolving discrepancies among earlier maps concerning the relationship of these two markers and *Vf*. Later, the colocation of OPAM19 and OPAL07 reported by King et al. (1998) was confirmed by Tartarini et al. (1999). Xu and Korban (2000) constructed a highly saturated AFLP map around *Vf* using a 'narrow down' bulked segregant strategy and converted these markers to SCARs (Xu et al. 2001a). These were later employed to develop a revised higher order fine map around *Vf* (Huaracha et al. 2004). The closest SCAR markers to *Vf* (ACS 3, ACS 7 and ACS 9) are extremely reliable for MAS. The first physical map of the *Vf* region, constructed by Vinatzer et al. (2001), located four homologs of the tomato *Cf* gene family to a 350 kb region around *Vf*. Xu and Korban (2004) have performed detailed pairwise sequence comparisons among these and concluded that the 4 paralogs have arisen by divergent selection on 4 original somatic variations.

Recently, bacterial artificial chromosome (BAC) clones within the contig encompassing *Vf* have been successfully employed as a source of multiallelic microsatellite markers (Vinatzer et al. 2004), termed CHVf-1 and CHVf-2. Analysis of linkage of the *Vf* – coupled alleles, plus *Vf* markers OPM18 and AL07SCARs indicates that a clone of *M. micromalus*, SA573-3, Golden Gem, *M. prunifolia* 19651 and M.A. 16 all carry *Vf*. The use of these markers in combination will enable breeders to predict quickly and economically in germplasm collections, which scab resistant plants carry resistances other than *Vf*.

Gardiner et al. (2003) employed RFLP screening of a mini-population of Royal Gala × A172-2 to identifiy very close linkage to *Vf* of a candidate apple EST (GenBank accession DR033891) derived from a Royal Gala (susceptible) library. This EST possesses homology to the *Hcr-Vf* resistance genes and mapped to the same region on LG 1 of A172-2 as markers derived from the candidate genes of Xu and Korban (2002b). Several other apple candidate ESTs mapped to *Vf* more distantly.

Table 4. Mapping of *Vf* resistance to apple scab

	Progeny	No. sdlgs	Method	Markers (marker/distance) (cM from *Vf*)	Marker class	Reference
A. Initial mapping	Jonathan × A849-7	37	Segregation analysis	*Pgm-1*/8	isoenzyme	Manganaris et al. 1994
	Idared × A679-12	58	–	–	–	–
	Prima × Spartan	63	–	–	–	–
	Liberty × Royal Gala	39	–	–	–	–
	COOP selections, commercial cultivars	15	BSA	$OPA15_{900}$/n.d.	RAPD	Durham and Korban 1994
	M. floribunda 821	7	–	–	–	–
	Idared × *M. floribunda* 821	59	BSA	$OPU01_{400}$/19.7	RAPD	Koller et al. 1994
	–	–		$OPM18_{900}$/10.6	RAPD	–
	Susceptible cultivars plus:	10	BSA	$OPD20_{600}$/n.d.	RAPD	Yang and Kruger 1994
	Prima × A142/5 (resistant sdlgs), *M.floribunda*, Pillnitz (for BSA)	5	–	(25.0, 20.0% recombination frequency)	–	–
	81/19-35 × Margol (mapping)	28	–	–	–	–
	81/19-35 × 87/7-10 (mapping)	158	–	–	–	–
	Granny Smith × A679-2	98	BSA	$OPH01_{1100}$/10	RAPD	Gardiner et al. 1996
	Royal Gala × A172-2	160	–	$OPR16_{400}$/14, 13	–	–
	Florina × Nova Easygro	500	Cloning/sequencing	$OPU01_{400}$/4	RAPD → SCAR	Gianfranceschi et al. 1996
	Florina × Golden Delicious	100	–	$OPM18_{450,230,170}$/1.9	RAPD → CAPS	–
	Prima × Golden Delicious	40	BSA	$OPM19_{2200}$/0.9	RAPD	Tartarini 1996
	–	–	–	$OPAL07_{580}$/0.9	RAPD	–
	–	–	–	$OC09_{900}$/8.8	RAPD	–
	–	–	–	$OPC08_{1100}$/15.5	RAPD	–
	–	–	–	$OPAB19_{1430}$/13.4	RAPD	–
	COOP selections, commercial cultivars	10	BSA	$OPAR4_{1400}$/3.6	RAPD	Yang et al. 1997a
	81/19-35 × 87/7-10	10	–	–	–	–
	–	138	Cloning/sequencing	$OPAR4_{1400}$/3.6	SCAR	
	COOP selections, commercial cultivars	10	BSA	$OPK16_{1300}$	RAPD	Yang et al. 1997b
	81/19-35 × 87/7-10	10	–	–	–	–
	–	138	Cloning/sequencing	$OPK16_{1300}$/4.3	SCAR	–
	Prima × Spartan (for BSA)	38	BSA based on *Pgm-1* genotype	$S5_{2500}$/1.3 $B505_{1700}$/7.8	RAPD RAPD	Hemmat et al. 1998
	Golden Delicious × Prima (mapping)	73	–	P198 $_{750}$/26.8 B398 $_{480}$/10.8	RAPD RAPD	–

Table 4. (continued)

	Progeny	No. sdlgs	Method	Markers (marker/distance) (cM from *Vf*)	Marker class	Reference
B. Fine mapping	Prima × Fiesta	155	Genetic mapping (JoinMap 2.0, Stam and van Ooijen, 1995)	$OPM19_{2200}$/0.7, $OPAL07_{580}$/0.7, $OPM18_{900}$/0	RAPD RAPD RAPD, RFLP	King et al. 1998
	Florina × Nova Easygro	491	Genetic mapping	$OPAL07_{466}$/0.9	SCAR	Tartarini et al. 1999
	5 other seedling progenies	125	(JoinMap 1.4, Stam, 1993)	$OPM19_{526}$/0.9	SCAR	
	Florina × Nova Easygro	521	Genetic mapping	$OPAL07_{466}$/1.1	SCAR	Patocchi et al. 1999a
	Braeburn × FAW 167	279	(JoinMap 1.4, Stam, 1993)	$OPM18_{900}$/0.2	CAPS	
	Fuji × Ariwa	409				
	Co-op selections 1-38	38	Narrow-down BSA	$OPM18_{450}$/0.4	CAPS	Xu and Korban 2000
	Commercial cultivars	10	–	ET9MC3-1/0.4	AFLP	–
	M. floribunda 821	–	–	EA2G11-1, EA12MG16-1,	AFLPs	–
	Resistant Co-op selections	38	Genetic mapping (CRI-MAP v 2.4, Green et al. 1990)	EA11MG4-1, ET2MC8-1, ET3MG10-1, ET8MG1-1, ET8MG7-1/0	–	–
	Co-op 17 × Co-op 16 (resistant seedlings)	203	–	$OPM19_{526}$, $OPAL07_{466}$/0.2 EA9MC15-1, EA4MG1-1	SCARs AFLPs	–
	Jonafree × III. Del.no.1 (resistant seedlings)	227	–	EA16MG2-1, ET4MC14-1, ET8MG16-1, ET3MG10-2, ET10MG8-1/0.2	–	–
	–	–	Physical mapping	*HcrVf1*, *HcrVf2*, *HcrVf3*, *HcrVf4* mapped to 350 kb interval around *Vf*	Gene homologs	Vinatzer et al. 2001
	Resistant Co-op selections	38	Genetic mapping	$OPM18_{450}$/0.4	CAPS	Xu et al. 2001a
	Co-op 17 × Co-op 16 (resistant seedlings)	203	(CRI-MAP v 2.4, Green et al. 1990)	ACS-6/0.4, ACS-3, ACS-7, ACS-9/0	SCAR SCARs	
	Jonafree × III. Del.no.1 (resistant seedlings)	227	–	$OPM19_{526}$, $OPAL07_{466}$/0.2 ACS-1, ACS-2, ACS-4, ACS-5, ACS-8, ACS-10, ACS-11/0.2	SCARs SCARs	–
	–	–	Physical mapping	*Vfa 1*, *Vfa2*, *Vfa3*, *Vfa4* mapped to 200kb interval around *Vf*	Gene paralogs	Xu and Korban 2002b
	Royal Gala × A172-2	160	Screening of ESTs with homology to R genes	2 bands from DR033891 mapped between *Vfa* 1, 2 and *Vfa* 3, 4	RFLP	Gardiner et al. 2003
	16 Crosses	1412	Genetic mapping	ACS-6/0.2 ACS-7, ACS-9/0 ACS-3/0.1	SCAR SCARs SCAR	Huaracha et al. 2004

1.3.2.1.2 *Vm* Bulked segregant analysis with RAPD markers was employed to identify the OPB12 marker for *Vm*, using a population three generations removed from *M.* x *atrosanguinea* 804. OPB12RAPD was converted to a SCAR marker and verified in a second population derived from a selection of *M. x atrosanguinea* 804. Joint segregation analysis on the combined data indicated a distance of 6 cM between *Vm* and OPB12 (Cheng et al. 1998) (Table 5). OPB12 has proved recently to be linked to *Vm* in the breeding parent X2225 derived from *M. micromalus* (Richards, Rikkerink, Bassett and Plummer, unpublished). Recently, Patocchi et al. (2005) performed a whole genome scan with three selected microsatellite markers per linkage group to locate *Vm* at the end of LG 17, the first major resistance gene in this genomic region. A new microsatellite marker that co-segregated with the resistance phenotype (Hi07h02) will be invaluable for MAS.

OPB12 is also linked to apple scab resistances derived from Red Sauce o.p. F91-135 A002-100 and Zelenovka Sotchnaya o.p. F91-184 A003-020 (Rusholme et al. unpublished). The same group used bulked segregant analysis of a Red Sauce o.p. population to identify linkage of OPAY5 to apple scab resistance and this marker was transferable into *M. x atrosanguinea* 804 and Zelenovka Sotchnaya o.p. populations. Microsatellite Hi07h02 has recently been mapped to the resistance in the *M. micromalus*, Zelenovka Sotchnaya o.p and Red Sauce o.p. populations, indicating a close relationship, if not the same gene, between *Vm* and the resistances from Red Sauce o.p. and Zelenovka Sotchnaya o.p. (Chagné et al. unpublished).

M. micromalus has been reported to carry both *Vm* and a 'masked gene' (Shay et al. 1953), that has been demonstrated to be allelic to *Vf* (Dayton and Williams 1968, 1970). This second gene is likely to be that identified by amplification of the *Vf* specific alleles of the microsatellite markers CHVf-1 and CHVf-2 in DNA from *M. micromalus* (Vinatzer et al. 2004).

1.3.2.1.3 Apple Scab Resistances from Differential Hosts 2 and 4 that Map to Linkage Group 2

Several streams of research have contributed to the knowledge of markers linked to apple scab resistances mapping to LG 2 in differential hosts 2 and 4 derived from Russian apple R12740-7A (conditioning stellate necrotic and hypersensitive reactions, respectively).

1.3.2.1.3.1 Vh2 Bulked segregation with RAPDs was used to identify marker OPL19 that mapped close to *Vh2* from differential host 2 (Gardiner et al. 1999a; Bus et al. 2000) (Table 5). The use of its derivative OPL19SCAR for MAS in a second population was reported by Bus et al. (2002). At this stage, the host 2 was mistakenly identified in several publications as accession TSR34T132 from the Purdue-Rutgers-Illinois apple breeding program (Bus et al. 2005b). However, the correct identification number is now known to be TSR34T15 (Lespinasse, personal communication). Hemmat et al. (2002) reported that a gene they identified as *Vr* was closely flanked by a SCAR marker $OPB18_{620bp}$ and a more distant microsatellite CH02b10 reported by (Gianfranceschi et al. 1998). More recent mapping using $CH02b10_{121bp}$, $OPL19_{433bp}$SCAR and $OPZ13_{900bp}$SCAR (Gygax et al. 2004) in the population Royal Gala × TSR34T15 has indicated that *Vr* and *Vh2* are identical and that the gene maps to LG 2 (Bus et al. 2005b). This was confirmed in a second population Sciglo × A68R03T057 derived from a non-differential accession of Russian apple R12740-7A. It was therefore proposed that the gene conditioning stellate necrotic reactions from Russian apple R12740-7A be known as *Vh2*, and that the name *Vr* be reserved for the original race-non specific gene from this accession.

1.3.2.1.3.2 Vt57 *Vt57* was identified in the Sciglo × A68R03T057 population through the use of differential screening of the population with several isolates of *V. inaequalis* (Bus et al. 2005b). It conditions a chlorotic resistance reaction, and maps 3 cM from OPL19SCAR on LG 2 (versus 1 cM for *Vh2*) (Table 5).

1.3.2.1.3.3 Vh4 Similarly, research has been performed in parallel by teams internationally on the host 4 derivative of Russian apple conditioning a hypersensitive response. (Hemmat et al. 2002) reported the identification of a RAPD marker from primer S22 that they converted to S22SCAR and mapped in R12740-7A within 9 cM of *Vx* (Table 5).

In New Zealand, S22SCAR was mapped at a similar distance or closer to *Vh4* in Royal Gala × TSR33T239 populations (Bus et al. 2002; 2005b). This group also reported a distant linkage of *Vh4* to OPB10RAPD (Bus et al. 2000) and OPB10SCAR (Bus et al. 2005b), but had been unable to identify new markers closer to the gene. Boudichevskaia et al. (2004) identified the linkage of $OPAD13_{950bp}$ to a gene they termed *Vr1* that segregated in three Regia progenies. However consideration of pedigree, resistance phenotype and linkage information for all markers, including $OPAD13_{950bp}$ in the Royal Gala × TSR33T239 population (Gardiner et al. unpublished) suggests that this gene is iden-

Table 5. Mapping of major scab resistance genes, apart from *Vf*, in apple

Gene	Progeny	No. sdlgs	Method	Markers marker/distance (cM from gene)	Marker class	Linkage group assignment	Reference
Vm	Empire × NY74828-12 (3 generations from *M.* x *atrosanguinea*)	59	BSA	$OPB12_{687}$/6.0	SCAR	–	Cheng et al. 1998
–	Royal Gala × OR45T132 (selection of *M.* x *atrosanguinea* 804)	184	Verification by genetic mapping	–	–	–	–
Vm	Golden Delicious × Murray	142	Whole genome scan	Hi07h02/0 CH05d08y/3.5	microsatellite microsatellite	LG 17	Patocchi et al. in press
Vh2	Royal Gala × TSR34T15 (F2 of R12740-7A)	192	BSA	$OPL19_{550}$/2.5	RAPD	–	Gardiner et al. 1999a, Bus et al. 2000
Vh2	Golden Delicious × TSR34T15	122	Population screen	$OPL19_{433}$/n.d. (8.2% recombination frequency [r.f.])	SCAR	–	Bus et al. 2002
Vh2	Empire × R12740-7A	315	BSA	$OPB18_{620}$/n.d. (0.8% r.f.)	SCAR	–	Hemmat et al. 2002
	–		Population screen	$CH02b10_{122}$/n.d. (7.8% r.f.)	microsatellite	–	–
	–	–	–	–	(ex Gianfranceschi et al. 1998)	–	–
Vh2	Royal Gala × TSR34T15	192	Comparative mapping (JoinMap v. 3.0, Van Ooijen and Voorrips, 2001)	$CH02b10_{121}$/8.8 $OPZ13_{900}$/5.0 (ex Gygax et al. 2004) $OPL19_{433}$/1.0 $CH05e03_{165}$/10.0	microsatellite SCAR SCAR microsatellite	LG 2	Bus et al. 2005b
Vh2	Sciglo × A68R03T057	111	As above	$CH02b10_{121}$/9.0	microsatellite	–	Bus et al. 2005b
	–		–	$OPL19_{433}$/5.0	SCAR	–	–
	–		–	$CH05e03_{165}$/4.0	microsatellite	–	–
VT57	Sciglo × A68R03T057	111	Single strain inoculation, comparative mapping (JoinMap v. 3.0, Van Ooijen and Voorrips, 2001)	$CH02b10_{126}$/2.0 $OPL19_{433}$/3.0 $CH05e03_{166}$/4.0	microsatellite SCAR microsatellite	LG 2	Bus et al. 2005b
Vh4	Royal Gala × TSR33T239	242	BSA	$OPB10_{>2000}$/22.1	RAPD	–	Bus et al. 2000
Vh4	Empire × R12740-7A	315	BSA	$S22_{1300}$/n.d. (9.8% r.f.) $S6_{800}$/n.d. (23% r.f.)	SCAR RAPD	– –	Hemmat et al. 2002

Table 5. (continued)

Gene	Progeny	No. sdlgs	Method	Markers marker/distance (cM from gene)	Marker class	Linkage group assignment	Reference
Vh4	Royal Gala × TSR33T239	154	Population screen	$S22_{1300}$/n.d. (9.8% r.f.)	SCAR	–	Bus et al. 2002
	Regia × Pingo	191	BSA	$OPAD13_{950}$ n.d. (15% r.f.)	SCAR	–	Boudichevskaia et al. 2004
	Regia × Pinova	188	–	–	–	–	
	Regia × Delbarestivale	97	–	–	–	–	
Vh4	Royal Gala × TSR33T239	242	Genetic mapping	$S22_{1300}$/4.0	SCAR	LG 2	Bus et al. 2005b
	–	–	(JoinMap v. 3.0,	$CH02c02a_{170}$/5.0	Microsatellite	–	–
			Van Ooijen and Voorrips, 2001)	$OPB10_{>2000}$/19.0	SCAR		
	–	–	–	$OPAD13_{950}$/7.0	SCAR	–	Gardiner et al. unpublished
Vr2	GMAL 2473 × Idared	377	BSA	$EA35MA41_{262}$/0	AFLP	LG 2	Patocchi et al. 2004
	–	–	–	$EA37MA39_{188}$/0	AFLP	–	–
	–	–	–	$CH02c02a_{176}$/0	microsatellite	–	–
	–	–	–	$CH02f06_{146}$/6.9	microsatellite	–	–
Vr2	Fiesta × Discovery	44	Comparative mapping	ARGH37/3.5 ARGH17 3.5	RGA RGA	–	Baldi et al. 2004
Vr2	Discovery × TN10-8	149	Whole genome scan	NBS2M9/2.0	NBS marker	–	Calenge et al. 2005
–	–	–	Comparative mapping	NBS2M10/2.0	NBS marker	–	–
			on another framework map	NBS2R9/1.0	NBS marker		
				NBS3M3/1.0	NBS marker		
–	–	–	–	NBS2M4/2.0	NBS marker	–	–
–	–	–	–	NBS3M1b/3.0	NBS marker	–	–
Vbj	A722-7 × Golden Delicious	148	BSA	$OPZ13_{773}$/0	SCAR	LG 2	Gygax et al. 2004
		–	–	$OPT06_{410}$/5.8	SCAR	–	–
–	–	–	–	$OPK08_{743}$/10.2	SCAR	–	–
–	–	–	Comparative mapping	$CH2c06_{248}$/0	microsatellite	–	–
–	–	–	(JoinMap v 2.0,	$CH5e03_{150}$/2.1	microsatellite	–	–
			Stam and van Ooijen, 1995)	$CH3d01_{115}$/8.3	microsatellite		
Vh8	Royal Gala × *M. sieversii* W193B	152	Comparative mapping	$OPL19_{433}$/1.3	SCAR	LG 2	Bus et al. 2005a
			(JoinMap v. 3.0,	$OPB18_{628}$/4.3	SCAR		
			Van Ooijen and Voorrips, 2001)	OPB 18_{799} (*Vh8* SCAR)/5.1	SCAR		
				$CH3d01_{124}$/18.5	microsatellite		

Table 5. (continued)

Gene	Progeny	No. sdlgs	Method	Markers marker/distance (cM from gene)	Marker class	Linkage group assignment	Reference
Va	Fortune × PRI 1841-11 and	120	BSA	$P136_{700}$/n.d. (18% r.f)	RAPD; SCAR primers unpubl.	LG 1?	Hemmat et al. 2003
	NY489 × PRI 1841-11	–	–	$B398_{480}$/n.d. (16% r.f)	RAPD	–	–
				ACS-6/n.d. (24% r.f.)	SCAR	–	–
Vb	Empire × Hansen's baccata #2	140	BSA	$B220_{700}$/n.d. (12% r.f)	RAPD; SCAR primers unpubl.	LG 1?	Hemmat et al. 2003
	–	–	–	$OPAM19_{450}$/n.d. (24% r.f)	SCAR	–	–
–	–	–	–	ACS-1; $OPU01_{400}$/26% r.f.)	SCARs	–	–
Vb	Golden Delicious × Hansen's baccata #2	149	Whole genome scan	Hi02d05/7.8	microsatellite	LG 12,	Erdin et al. 2006
				Hi07f01/9.7	microsatellite	not LG 1	
Vd	Durello di Forlì × Fiesta	146	Genetic mapping	$OPAF07_{880bp}$/2.0	RAPD	LG 10	Tartarini et al. 2004
–	–	–	–	G63Tru91a/2.0	microsatellite	–	
–	Discovery × TN10-8	149	Comparative mapping on another framework map	NBS3M13/2.0	NBS marker	–	Calenge et al. 2004
				NBS2M18/1.0	NBS marker		
				NBS2M12/1.0	NBS marker		
				NBS3M8/3.0	NBS marker		
Vg	Prima × Fiesta	149	Single strain inoculation; mapping	MC105/3.0	RFLP	LG 12	Durel et al. 1999
Vg	Discovery × TN10-8	149	Genetic mapping	CH01d03/0.5	microsatellite	–	Calenge et al. 2004
Vg	Discovery × TN10-8	149	Genetic mapping	NBS2M14/5.0	NBS marker	–	Calenge et al. 2005
				NBS3M11/0.7	NBS marker	–	
Vmis	Splendour × MIS o.p. 93.051 G01-048	155	BSA	$OPAS11_{760}$/<8.0	RAPD	Not determined	Gardiner et al. 2001
				$OPAS07_{690}$/<17.5	RAPD		

tical to *Vh4*, and that since the first naming of the gene takes precedence over later namings, it should be identified as *Vh4*. *Vh4* has been mapped to LG 2 (Bus et al. 2005b). Screening of EST candidate R genes using RFLP analysis, followed by conversion to SNPs has indicated that screening of candidate genes will be a useful route for developing further markers for both *Vh2* and *Vh4* (Gardiner et al. unpublished).

1.3.2.1.4 Other Major Apple Scab Resistances Mapping to Linkage Group 2 Described below are three other major apple scab resistances that have been mapped to LG 2, which possesses the largest number of resistances to apple scab of any linkage group. QTL for scab and mildew resistance have also been identified there – see Sect. 1.4.2, (Calenge et al. 2004; Calenge and Durel, in preparation) as well as numerous RGAs (Baldi et al. 2004; Calenge et al. 2005a) -see below. This high concentration of active and potential resistances makes LG 2 of high priority for an apple genome sequencing initiative.

1.3.2.1.4.1 Vr2 *Vr2* from GMAL 2473 was reported by Patocchi et al. (2004). Four markers were obtained by BSA using both RAPDs and AFLPs. Two of these markers segregated with the resistance (EA35MA41 and EA37MA39), making their future SCAR derivatives excellent tools for MAS (Table 5). A fifth marker, a microsatellite that also co-segregated with the resistance, was identified by a whole genome scan using selected markers from the map of Liebhard et al. (2002). This marker (CH02c02a) enabled the location of *Vr2* on LG 2 at about 43 cM from *Vh2*, which excludes any possibility that these two resistance genes are identical. However, the question of the relationship between *Vr2* and *Vh4* is not yet completely resolved. Uncertainty about the origin of the Russian apple accession used, the low number of seedlings with distinctive HR, the difference in their distances to CH02c02a, and the absence of data for S22SCAR suggest that they are different. On the other hand, since 65% of the seedlings of the GMAL2473 × Idared family were resistant (Patocchi et al. 2004), GMAL2473 may well carry two, possibly linked, scab resistance genes, one of which may be *Vh4*, while the other gene is the true *Vr2* gene. The two-gene hypothesis is supported by the distinct phenotypes of HR (for *Vh4*) and chlorotic/necrotic (for *Vr2*) resistance reaction. Another reason for the segregation of *Vr2* not fitting a clear R:S ratio may be segregation distortions, since LG 2 is well-known for these (Maliepaard et al. 1998; Liebhard et al. 2003b; Bus et al. 2005a).

Baldi et al. (2004) located two RGAs within 3.5 cM of the putative location of *Vr2*, while Calenge et al. (2005a) mapped six markers identified by NBS profiling to a genomic region corresponding to 5 cM around *Vr2* and seven more in the next 3 cM in the direction of *Vh4* confirming that the region around *Vr2* and *Vh4* is extremely rich in potential resistance genes and certainly warrants further analysis.

1.3.2.1.4.2 Vbj RAPD markers for *Vbj* (OPZ13 and OPK08) have been identified by BSA and converted to SCAR markers (Gygax et al. 2004). These were mapped around *Vbj* together with three microsatellite markers (CH02c06, CH05e03 and CH03d01) previously defined as members of LG 2 by (Liebhard et al. 2002) (Table 5).

1.3.2.1.4.3 Vh8 A new scab resistance from *M. sieversii* accession W193B was identified and distinguished from *Vh2* with the aid of a new race of *V. inaequalis*, race 8. Although no distinction between *Vh2* and *Vh8* could be made on the basis of genetic marker studies with the original markers for *Vh2* (OPL19 and $OPB18_{628bp}$), the latter marker produced a second band of 799 bp that was specific to the *Vh8* gene (Bus et al. 2005a). Sequencing of the products of the OB18 PCR reaction from both resistant parents enabled the development of a second marker (*Vh8*SCAR) that could distinguish the two genes as that also exhibited a band in the presence of *Vh8* that was not exhibited by *Vh2*. Microsatellite CH03d01 from LG 2 maps 5 cM from *Vh8* (Table 5). Sequencing of the products of the OPB18 PCR reaction from both resistant parents enabled the development of a second marker (*Vh8*SCAR) that could distinguish the two genes.

Consideration of the mapping data for the *Vh2* and *Vh4* genes from Russian apple R12740-7A, plus the information on markers for *Vh8* and *Vbj* and *Vr2*, enabled Bus et al. (2004) to use the then available markers to delineate a map of LG 2 that locates *Vbj*, *Vh2* and *Vh8* close to each other, and at a distance from *Vr2* and *Vh4* (Fig 2).

1.3.2.1.5 *Va* Hemmat et al. (2003) employed BSA to identify linkage of P136RAPD to the hypersensitive *Va* resistance from Antonovka PI1762623 and then developed a SCAR marker (sequence not published) (Table 5). Screening of their mapping population with a range of markers for *Vf* demonstrated that many markers for this gene map on the opposite side to P136. These markers include those reported to map with *Vf* in a high resolution map (Xu et al. 2001a)

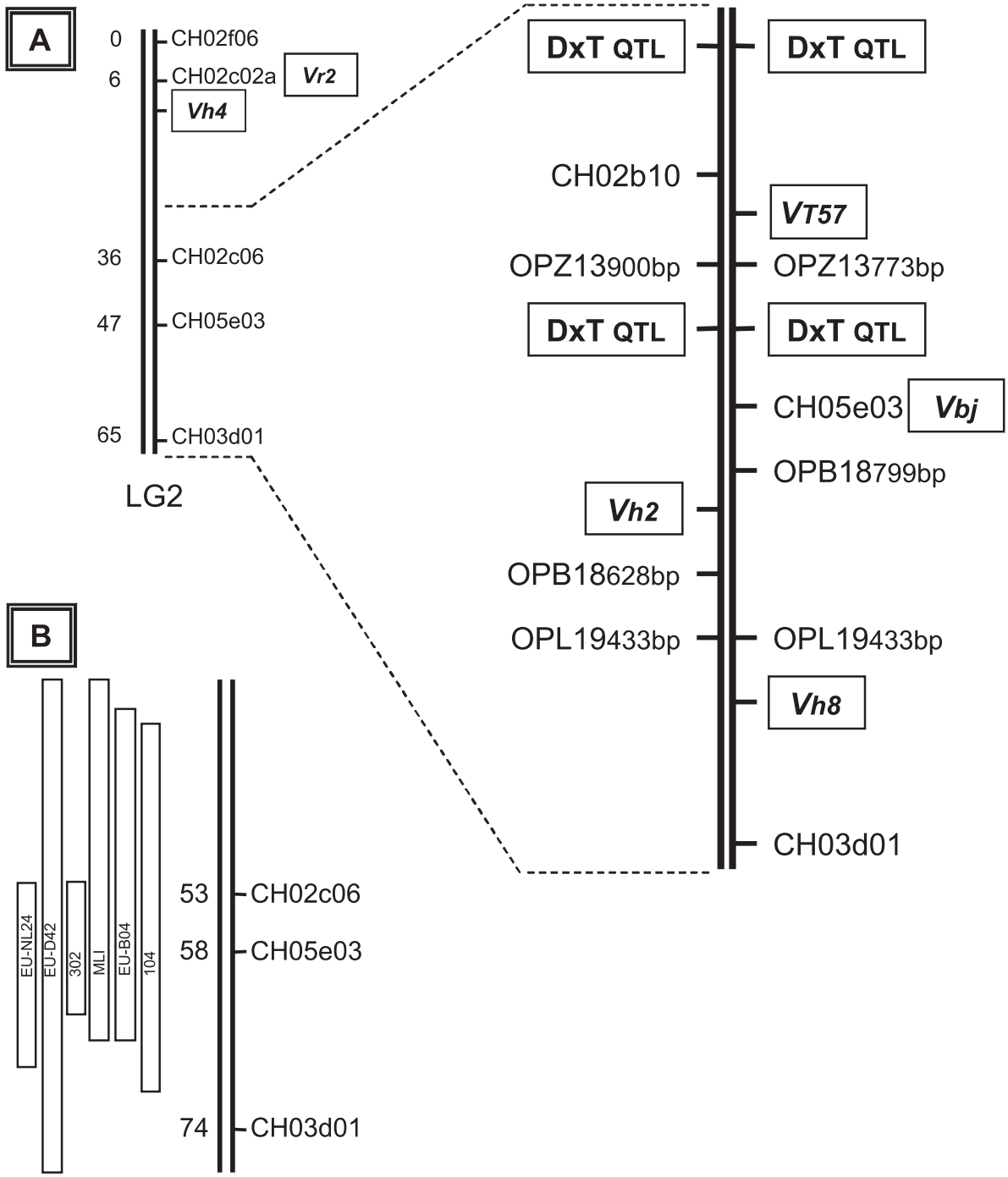

Fig. 2. Delineation of a scab resistance gene cluster on LG 2 of apple based on the genetic maps for the individual major genes (**A**). The diagram on the right is a higher magnification of the diagram on the top left in the area containing several QTL and major genes. The chromosome regions of LG 2 identified as carrying QTLs for scab resistance in a Discovery × TN10-8 family (**B**) have been mapped according to their QTL peaks. (Adapted from Bus et al. 2005b)

but not all *Vf* markers mapped in this population. This indicates that *Va* may be located on LG 1, with a recombination frequency of 27% between the *Va* and *Vf* loci. This agrees with Dayton and Williams (1968), who had earlier concluded that *Va* and *Vf* were not allelic.

1.3.2.1.6 *Vb* In the same paper as their *Va* study, Hemmat et al. (2003) reported the linkage of B220RAPD to *Vb* (chlorotic resistance reaction from Hansen's baccata #2) and that several markers for *Vf* mapped on the opposite side to B220SCAR (primer sequence unpublished), but in a different order from that found around *Vf* (Table 5). Test crosses had indicated that *Vb* and *Vf* were not allelic (Dayton and Williams 1968). B220SCAR mapped in repulsion to resistance phenotype in the *Va* population (above), and also in material not related to Hansen's baccata #2, including *M. floribunda, M. prunifolia, M. zumi* calocarpa, David, Liset, Prairiefire, Carmine Crab, D95-295 Redleaf Crab and AV Redleaf Crab. Hansen's *baccata* #1 amplified fragments with 11 of the 16 *Vf* primers tested. *M. baccata jackii* amplified fragments from four of the primers. Rusholme and Gardiner (unpublished) mapped B220RAPD distantly, at 30 cM, to *Vb* in a population derived from the GMAL2477 accession of Hansen's baccata #2 and identified a new RAPD marker (OPAJ03) mapping inside B220 at 25 cM from *Vb*. They confirmed the finding of M. Hemmat and S. Brown (personal communication) that a marker for *Vbj* ($OPZ13_{773bp}$SCAR) mapped outside B220RAPD. However, the *Vf* markers reported by Hemmat et al. (2003) were not exhibited by Hansen's *baccata* #2 GMAL2477. A recent conference report (Erdin et al. 2006) clarifies the issue. A whole genome scan of plants in a Golden Delicious × Hansen's *baccata* #2 progeny using microsatellite markers demonstrated that *Vb* maps to the distal end of LG 12 and not to LG 1. This result is consistent with the early test cross results (Dayton and Williams 1968), and demonstrates the power of the whole genome scan for mapping of major resistances to linkage group.

1.3.2.1.7 *Vd* The resistance from the old Italian apple cultivar Durello di Forli that has been described as conferring high field tolerance to apple scab (3B type reaction) and a stellate necrotic reaction in glasshouse grown seedlings exposed to the EU-D-42 race 6 reference strain of *Venturia inaequalis* has been mapped to one end of LG 10 (Tartarini et al. 2004) using the microsatellite map developed by this team (Tartarini et al. unpublished). The markers $OPAF07_{880bp}$RAPD and G63Tru91a, that flank *Vd* closely (Table 5), are in repulsion phase to the resistance and will need to be converted to markers linked to the presence of a fragment, before becoming useful for MAS. Four markers identified by NBS profiling mapped to a genomic region corresponding to 5 cM around *Vd* in another apple progeny (Calenge et al. 2005a). The high level of resistance to race 6 conferred by *Vd* would make it a useful reinforcement to the otherwise effective *Vf* resistance that has been broken by race 6 (Bénaouf and Parisi 2000).

1.3.2.1.8 *Vg* *Vg*, the major gene derived from Golden Delicious that confers resistance to apple scab incited by race 7 of *V. inaequalis*, breaker of the *Vf* resistance from *Malus floribunda* 821 was first described by Bénaouf et al. (1997). Screening of the Prima × Fiesta framework mapping population of Maliepaard et al. (1998) with differential strains of *V. inaequalis* enabled Durel et al. (1999) to map *Vg* 3 cM from a new RFLP marker on LG 12 (Table 5). Mapping in a second framework mapping population (Discovery × TN10-8) enabled location of the gene 0.5 cM from the microsatellite CH01d03 (Calenge et al. 2004). NBS profiling identified two more markers close to *Vg*, one 5 cM upstream of *Vg* and the other flanking the resistance at 0.7 cM (Calenge et al. 2005a).

1.3.2.1.9 *Vmis* Scab resistance segregates from an open pollinated seedling (93.051 G01-048) of the mildew immune seedling described by Dayton (1977) as being the product of a pollination of a domestic apple with an unknown crab apple. Initial analysis by bulked segregant analysis resulted in identification of two RAPD markers, OPAS07 and OPAS11 (Gardiner et al. 2001) (Table 5). As the phenotype segregation data suggested the presence of a second gene, this analysis has been carried into the second generation, resulting in the identification of new markers with flanking markers located in a 15 cM span around *Vmis* (Gardiner et al. unpublished).

1.3.2.2 Powdery Mildew Resistance Genes (Table 6)

Mapping of resistances to powdery mildew is much more time consuming than mapping apple scab resistances, because of the need to phenotype seedling populations over several years to ensure that the adult resistance phenotype has been attained and that this

Table 6. Mapping of major genes for powdery mildew resistance in apple

Gene	Progeny	No. sdlgs	Method	Markers marker/distance (cM from gene)	Marker class	Linkage group assignment	Reference
Pl-w	Jester × White Angel Gloster 69 × White Angel	40 80	*LAP* isoenzyme analysis	*Lap-2an*/n.d. (2.6 – 3.1% recombination frequency)	Isoenzyme		Batlle and Aston 1996
Pl-w	Prima × Fiesta	152	Mapping of *LAP-2*			LG 8	Maliepaard et al. 1998
Pl-w	Katja × White Angel	80	BSA	EM M02 /6.4 EM M01/4.6	AFLP → SCAR AFLP → SCAR	–	Evans and James 2003,
	Fiesta × (Gloster 69 × White Angel)	267	Whole genome scan	CH01e12/10 CH05a02y/13	microsatellite microsatellite	–	James and Evans 2004
Pl-d	Fiesta × A871-14	272	Whole genome scan	CH03c02/8.0	microsatellite	LG 12	James et al. 2004
	–	–	BSA	$OPA01_{900}$/4.0 (repulsion)	RAPD	–	
	–	–	–	ETA-CTC/5.0	AFLP	–	
	–	–	–	EM DM01/9.0	AFLP; SCAR	–	–
	–	–	Whole genome scan	Ch01d03/13.0	microsatellite	–	–
Pl-1	85/23-2 × 81/19-35	64	BSA	$OPAT20_{450}$/4.0 $OPD02_{1000}$/5.0	SCAR RAPD	–	Markussen et al. 1995
Pl-1	Idared × 78/18-4	233	Population screen	$OPAT20_{450}$/7.0	SCAR	–	Dunemann
	–	–	BSA	$AU_{?bp}$SCAR/3-4	AFLP; SCAR	–	et al. 2004
	–	–	–	$AU_{<100}$CAPS /3-4	–	–	–
Pl-1	–	150	Whole genome scan	–	–	LG 12	Lesemann and Dunemann 2006
Pl-n	X3191 × Novosibirski Sweet o.p. 91.117 A01-003	200	Screen of 76 phenotype extremes	$OPAT20_{450}$/9 $AU_{<100}$CAPS/9.5 AU_{600}SNP trans/9.5	SCAR CAPS SNP	Not determined	Dunemann et al. 2004
Pl-2	A679-2 × Iduna	358	QTL analysis	$OPN18_{1000}$/ $OPO04_{1800}$/ $OPK15_{1400}$/ $OPAG02_{450}$/	RAPDs spanning a QTL of 28 cM	–	Seglias and Gessler 1997 and Gianfranceschi et al. 1999
Pl-2	Fiesta × SA572-2	61	Population screen	$OPAT20_{900}$/6	RAPD	–	Dunemann et
–	–	–	Genetic map construction	OPAJ13600/11	RAPD	–	al. 1999
Pl-2	Royal Gala × A689-24	190	Population screen	$OPN18_{950}$/7	RAPD	–	Gardiner et al. 1999a
Pl-2	Fiesta × Discovery and A679-2 × Iduna	112	Comparative microsatellite mapping	–	–	LG 11	Liebhard et al. 2002

Table 6. (continued)

Gene	Progeny	No. sdlgs	Method	Markers marker/distance (cM from gene)	Marker class	Linkage group assignment	Reference
Pl-2	Royal Gala × A689-24	190	BSA	$OPU02_{1700}$/8	RAPD → SCAR	–	Gardiner
–	–	–	BSA	$OPAY17AB16a_{1100}$/5.9	RAPD → SCAR	–	et al. 2003
–	–	–	BSA	$OPAY17AB16b_{400}$/7.1	RAPD → SCAR	–	–
–	–	–	EST screening	GenBank DR033891/closest band 0.9	RFLP	–	–
–	–	–	–	GenBank DR033886/closest band 1.1	RFLP	–	–
–	–	–	–	GenBank DR033892 /closest band 2.9	RFLP	–	–
–	–	–	–	GenBank DR033893/4.1	RFLP	–	–
–	–	–	–	GenBank DR033888/5.4	SCAR	–	–
Pl-m	Fuji × MIS o.p. 93.051 G02-054	174	BSA	$OPAC20_{1800}$/14.4	RAPD	Not determined	Gardiner et al. 1999a
Pl-m	Fuji × MIS o.p. 93.051 G02-054	–	Genetic mapping	$OPAC20_{1800}$/0.7	SCAR	–	Gardiner et al. 2003
			(JoinMap v.2.0, Stam and van Ooijen 1995)	$OPN18_{1000}$/13.5	SCAR		
				$OPAY17AB16a_{1100}$/5.5	SCAR		
–	–	–	–	$OPAY17AB16b_{400}$/7.3	SCAR	–	–
–	–	–	–	$OPU02_{2000}$/8.1	SCAR	–	–
–	–	–	EST screening	GenBank DR033892/closest band 1.1	RFLP	–	–
–	–	–	–	GenBank DR033886/closest band 1.6	RFLP	–	–
–	–	–	–	GenBank DR033888 /5.8	SCAR	–	–
Pl-a	M9 × Aotea	277	Marker screening	$OPN18_{1000}$/11.5	SCAR	Not determined	Gardiner et al. 2004

phenotype is stable. It is necessary to expose the seedlings to the inciting organism *Podosphaera leucotricha* in the field, as it cannot be cultured in vitro.

1.3.2.2.1 *Pl-w* The first markers reported to be linked to a major resistance to powdery mildew were isoenzymes linked to the *Pl-w* gene derived from the crab apple White Angel (Manganaris 1989; Manganaris and Alston 1992; Hemmat et al. 1994; Batlle and Alston 1996). The closest was *Lap-2* (Table 6). Batlle and Alston also used segregation analysis to identify a complementary but unlinked gene *Rw* that was required for expression of resistance by *Pl-w*.

A whole genome scan using phenotype bulks demonstrated that the microsatellites CH01e12 (locus 1) and CH05a02y flank *Pl-w*, at positions 10 and 12 cM, respectively from the gene (James and Evans 2004). These microsatellites both map to LG 8 (Liebhard et al. 2002) confirming the assignment of *Pl-w* to this linkage group that was first indicated by the mapping of *Lap-2* to *LG* 8 by (Maliepaard et al. 1998). A study employing bulked segregant analysis using amplified fragment length polymorphisms (AFLPs) enabled the identification of two markers that map inside CH01e12 at 4.6 and 6.4 cM (EM M01 and EM M02, respectively) (Evans and James 2003; James and Evans 2004). Baldi et al. (2004) located an RGA marker 7 cM from the putative position of *Pl-w* in the Fiesta × Discovery population and Calenge et al. (2005a) mapped three NBS-LRR homologs within 2 cM of the putative position of *Pl-w* in the Discovery × TN10-8 population.

1.3.2.2.2 *Pl-d* This strong mildew resistance is derived from an open pollinated crab apple from the South Tyrol, Italy (Visser and Verhaegh 1976). Bulked segregant analysis identified AFLP and RAPD markers mapping to *Pl-d* (James et al. 2004) (Table 6). One of the AFLPs has been converted to a SCAR marker that maps to one side of *Pl-d*. In the course of the same study, two flanking microsatellite markers that were identified in a whole genome scan located *Pl-d* on the bottom of LG 12, a region where other disease resistance genes have been identified, including *Vg* (Durel et al. 1999), and NBS markers (Calenge et al. 2005a).

1.3.2.2.3 *Pl-1* Markussen et al. (1995) first reported $OPAT20_{450}$SCAR as a close marker for the *Pl-1* mildew resistance from *M. robusta* (Table 6). *Pl-1* was later verified to map at a distance of about 7 cM from the gene in a separate population (Dunemann et al. 2004). Two more markers have been identified using BSA with AFLPs. One was converted to a SCAR (AU-SCAR) and also a CAPs marker AU-CAPs (Urbanietz 2002; Dunemann et al. 2004) that maps 3-4 cM from *Pl-1*, making this marker a very valuable tool for selection purposes. *Pl-1* has been mapped to LG 12 in the vicinity of *Vg* and *Pl-d* following a whole genome scan (Lesemann and Dunemann 2006).

1.3.2.2.4 *Pl-n* Screening of a segregating population derived from a cross between the susceptible breeding parent X3191 and mildew resistant Novosibirski Sweet o.p. (91.117 A01- 003) demonstrated clearly that the *Pl-1* marker $AT20_{450bp}$SCAR is linked to resistance to powdery mildew infection derived from Novosibirski Sweet o.p. (Table 6). Development of a new SNP marker for this population from AU-CAPs enabled the mapping of this second *Pl-1* marker to the mildew resistance (Dunemann et al. 2004). The relationship between *Pl-1* and *Pl-n* is currently unclear; *Pl-n* may be a new gene, perhaps allelic to *Pl-1*.

1.3.2.2.5 *Pl-2* The earliest molecular markers for the *Pl-2* resistance from *M. zumi* were RAPDs obtained using a QTL mapping approach (Seglias and Gessler 1997; Gianfranceschi et al. 1999) (Table 6) but the gene clearly segregated as a single gene in a Royal Gala × A689-24 family in New Zealand (Bus et al. 2000). Dunemann et al. (1999) screened the markers that Markussen et al. (1995) had identified for *Pl-1* and found that $AT20_{900bp}$RAPD mapped at a similar distance from *Pl-2* as the 450 bp band had mapped to *Pl-1*. Gardiner et al. (1999a) utilized the OPN18 RAPD marker from the first study to construct DNA bulks on the basis of genotype as well as phenotype and identified a second, flanking marker for *Pl-2* (OPU02). They reported that the markers mapped in absence of phenotype were located less than 12 cM apart, making them useful for MAS. Gardiner et al. (2003) reported two more SCARs mapping inside OPN18SCAR, both derived from BSA with a combination of OPAY17RAPD and OPAB16RAPD primers (OPAY17/OPAB16a and b SCARs). They also mapped OPAC15/OPAZ16SCAR distal to OPU02SCAR and used this map of anonymous markers as a framework to locate EST markers (Gardiner et al. 2003). One hundred and ten ESTs were mined from an EST database of 30,000 unigene sets on the basis of sequence homology to recognized resistance genes from other plants and screened as RFLP probes over mini-populations segregating for a range of resistances. This enabled the detection of putative

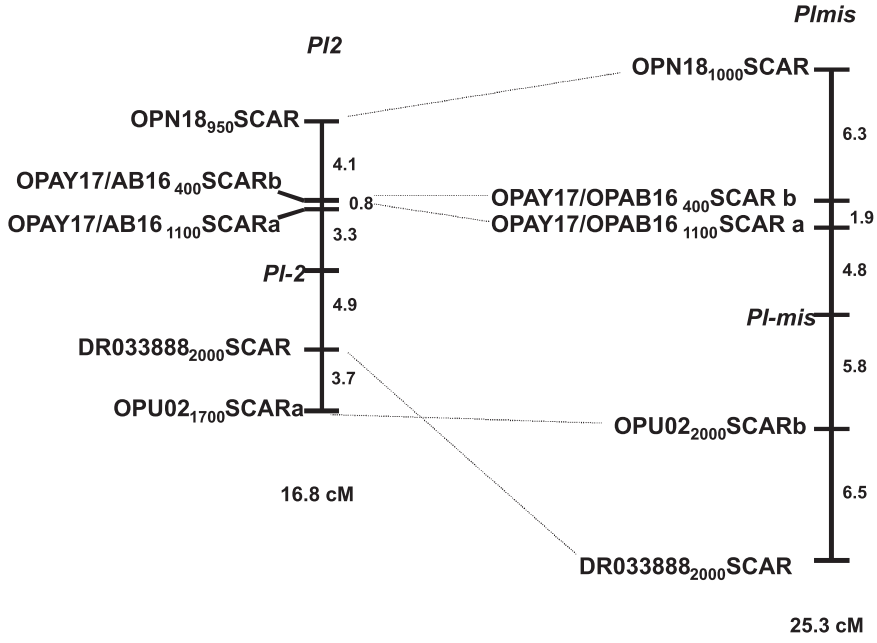

Fig. 3. Comparison of *Pl-2* and *Pl-m* genetic linkage maps using PCR based markers only. The *vertical line* represents the linkage group with marker loci *on the left*, interval sizes (in cM) *on the right* and the total length (in cM) *at the base* of the maps (after Gardiner et al. 2003)

linkages to *Pl-2* and other genes. These were then confirmed in larger subsets of the mapping population.

Loci characterized by ESTs mapped to either side of *Pl-2* in regions where it had previously proved difficult to locate anonymous SCAR markers. Of ESTs mapped as RFLPs within 3 cM of *Pl-2*, a band from GenBank accession DR033891 showing homology to HcrVf2 mapped 0.9 cM from *Pl-2*. The closest bands from GenBank accession DR033886 and GenBank accession DR033892 (no homology to known R gene classes) mapped at 1.1 and 2.9 cM, respectively on the other side of *Pl-2*. A second NBS-LRR homolog (GenBank accession DR033893) mapped just outside of GenBank accession DR033892. An EST exhibiting homology to the NBS-LRR class of R genes (GenBank accession DR033888) was converted to a PCR based marker that mapped at 5.4 cM from *Pl-2*, inside OPUO2SCAR.

It has been noted that GenBank accession DR033891 also maps close to *Vf* (Sect. 1.3.2.1.1). It is interesting to speculate that it might be involved in the scab resistance QTL located close to *Pl-2* that was reported by Liebhard et al. (2003c).

Mapping of a common microsatellite in the A679-2 × Iduna population of (Seglias and Gessler 1997) and the Fiesta × Discovery population (Liebhard et al. 2002) enabled assignment of *Pl-2* to LG 11. This has been confirmed by Rusholme et al. (unpublished) in a whole genome scan of DNA bulks with 74 microsatellite markers, as well as the framework map under construction by Rikkerink et al. (unpublished).

1.3.2.2.6 *Pl-m* This strong mildew resistance segregates as a major gene from an open pollinated seedling (93.051 G07-062) of the mildew-immune seedling described by Dayton (1977) as being the product of a pollination of a domestic apple with an unknown crab apple. The first marker reported for *Pl-m*, OPAC20, was obtained using BSA with RAPDs (Gardiner et al. 2001) (Table 6) and the SCAR derivative mapped very closely to the resistance at 0.7 cM (Gardiner et al. 2003). Four SCAR markers previously mapped around *Pl-2* also mapped to *Pl-m* and the marker order around the resistance phenotype was conserved. Fragment length was conserved for three of these anonymous markers and for the NBS-LRR marker derived from GenBank accession DR033888.

Figure 3 shows simplified maps around *Pl-m* and *Pl-2*, constructed with the PCR-based markers in common only. Two other ESTs with no known sequence homology to R genes (GenBank accessions DR033886 and DR033892) also identified markers for both mildew resistances when mapped as RFLPs. Whole genome scanning with the same 74 microsatellites used to assign *Pl-2* to LG 11 did not enable as-

signment of *Pl-m* to a linkage group, and therefore the possibility that *Pl-2* and *Pl-m* may not map to the same group (Rusholme et al. unpublished) and that the genomic regions around these two genes may be homeologous rather than homologous cannot yet be ruled out. As *Pl-2* and *Pl-m* have already been pyramided in a resistance breeding population (Bus et al. unpublished), test crosses from seedlings with both genes would provide information on this question, as would more intensive microsatellite mapping of *Pl-m*.

1.3.2.2.7 *Pl-a* The rootstock Aotea 1 derived from *M. sieboldii* (Taylor 1981) has been observed to carry resistance to powdery mildew, as well as to apple scab (*Vat*) and woolly apple aphid (*Er-3*). When markers for *Pl-2* and *Pl-m* were screened across a population phenotyped for three years for mildew resistance, it was noted that two of the markers (OPN18SCAR and $OPUO2_{2100bp}$SCARc) mapped to *Pl-a* (Table 6), indicating that *Pl-a* may share a positional relationship with *Pl-2* and *Pl-m* (Gardiner et al. 2004).

1.3.2.3 Rosy Leaf Curling Aphid Resistance Genes

1.3.2.3.1 *Sd-1* Three very close RFLP markers (MC06a, 2B12a and MC029b) mapped within 2 cM from the *Sd-1* gene for resistance to biotypes 1 and 2 of *Dysaphis devecta* from Cox's Orange Pippin (Roche et al. 1997a) in a Prima × Fiesta population (Table 7). Four RAPD markers mapped more distantly. The RFLP 2B12a was later converted into $2b12a_{196bp}$SCAR (Roche et al. 1997b) and its linkage with *Sd-1* was confirmed through pedigree analysis. The original mapping population was later employed to construct the detailed genetic map of Maliepaard et al. (1998), which located *Sd-1* at the top of LG 7. Repeated phenotyping, plus fine mapping in over 700 seedlings from six more families later enabled the co-location of MC064a with *Sd-1* in a 1.3 cM interval between $2b12a_{196bp}$SCAR and microsatellite SdSSRa (Cevik and King 2002a).

1.3.2.3.2 *Sd-2* Co-segregation of $2b12a_{196}$SCAR and SdSSRa in a small population segregating for the *Sd-2* gene from Double Red Northern Spy that confers resistance to *D. devecta* biotype 1 only, indicated that *Sd-2* is tightly linked to *Sd-1*, and is probably allelic to *Sd-1* (Cevik and King 2002a) (Table 7).

1.3.2.4 Woolly Apple Aphid Resistance Genes

1.3.2.4.1 *Er-1* Markers G327 and OPC20RAPD that flanked the *Er-1* resistance from Northern Spy at 12 and 8 cM, respectively were identified by bulked segregant analysis (Gardiner et al. 1999a; Bus et al. 2000) (Table 7) and converted to SCARs for use in MAS. OP05SCAR, a marker close to *Er-3* is linked more distantly to *Er-1* (Gardiner et al. 2004). More recently, mapping of two microsatellite markers (CH01c06 and CH02g09) from Liebhard et al. (2002) made it possible to assign *Er-1* to LG 8. Interestingly, CH01c06 was located only 2 cM from *Er-1* (Chagné, Durel and Gardiner unpublished), which makes it a suitable marker for marker-assisted selection.

1.3.2.4.2 *Er-3* A novel resistance to woolly apple aphid identified in the rootstock Aotea 1 (Taylor 1981) has been studied in a segregating population (M9 × Aotea 1) and named *Er-3* (Gardiner et al. 1997; Bus et al. 2000). The close linkage of $OP05_{1700bp}$ SCAR to this resistance (0.8 cM) (Table 7) indicated that it would be useful for MAS. This was confirmed by the detection of only four recombinants in a 2nd generation population of 121 plants, using the modified marker $OP05_{880}$SCAR constructed to segregate in this particular population (Bus et al. 2000). Candidate genes developed from ESTs that mapped to *Er-3* include two NBS LRR homologs (GenBank accession DR033890 and GenBank accession DR033887), a receptor protein kinase (GenBank accession DR033889) and a leucine rich repeat EST with homology to extensin and tomato *Cf-2* (GenBank accession DR033885). GenBank accessions DR033885 and DR033887 have been converted to PCR based markers and mapped to *Er-1* as well as *Er-3* (Gardiner et al. unpublished). Screening of these two markers in a Discovery × TN10-8 population enabled the assignment of *Er-1* and *Er-3* to LG 8 (Chagné, Durel and Gardiner unpublished). This has been confirmed for *Er-3* by mapping of microsatellite CH02g09 12 cM (Liebhard et al. 2003c) from *Er3* in the M9 × Aotea 1 population (Chagné, Durel and Gardiner unpublished).

1.3.2.4.3 *Er-m* The same pollinated seedling (93.051 G07-062) that is the source of the *Pl-m* resistance (see above) also carries a novel resistance to woolly

Table 7. Mapping of major genes for resistance to aphids in apple

Trait Resistance to	Gene	Progeny	No. sdlgs	Method	Markers (marker/distance) (cM from gene)	Marker class	Linkage group assignment	Reference
1. Rosy leaf curling aphid (*Dysaphis divecta*)	*Sd-1*	Prima × Fiesta	141	Linkage analysis (JoinMap v.2.0, Stam and van Ooijen, 1995)	MC029b/2 MC064a/1 2B12a/1	RFLP RFLP RFLP	–	Roche et al. 1997a
	Sd-1	Prima × Fiesta	77	Marker conversion, pedigree analysis	$DdARM_{196}$	SCAR	–	Roche et al. 1997b
–	*Sd-1*	Prima × Fiesta	152	Mapping	–	–	LG 7	Maliepaard et al. 1998
–	*Sd-1*	Resistant cultivars Susceptible cultivars	9 8	BSA	ETC/MCTT-1/1.4	AFLP → SCAR		Cervik and King 2002a
–	–	6 segregating families	759	Linkage analysis	MC064a/0 *Sd-1* located in 1.3 cM	RFLP	–	–
–	–	–	–	–	interval between $2B12a_{196}$	SCAR	–	–
–		–	–	–	and SdSSRa	AFLP; microsatellite	–	–
–	*Sd-2*	Double Red Northern Spy × Totem	47	Linkage analysis	$2B12a_{196}$/0 SdSSRa/0	SCAR microsatellite	LG 7	Cervik and King 2002a
2. Woolly apple aphid (*Eriosoma lanigerum*)	*Er-1*	Sciglo x Northern Spy	132	BSA	$G327_{1600}$/11.6 $OPC20_{2000}$/7.9	RAPD; SCAR RAPD; SCAR	–	Gardiner et al. 1999a, Bus et al. 2000
	Er-1	Sciglo × Northern Spy	132	Linkage analysis	$OPO05_{1700}$/9.6 $DR033885_{1500}$/23 trans $DR033887_{900}$/23	SCAR SNP SCAR	–	Gardiner et al. 2004
–	*Er-1*	Discovery × TN10-8	149	Marker screening	$DR033885_{1300}$ $DR033887_{750}$	SNP SCAR	LG 8	Chagné et al. unpublished
–	*Er-1*	Sciglo x Northern Spy	94	Marker screening	CH01c06/2	microsatellite	LG 8	Chagné et al. unpublished
–	*Er-3*	M.9 × Aotea	131	BSA	$OPO05_{1700}$/0.8	RAPD; SCAR	–	Gardiner et al. 1997
–					$OPA01_{1250}$/3.3	RAPD (trans)	–	Bus et al. 2000
–	*Er-3*	M.9 × Aotea	277	EST screening	$DR033885_{500}$/9.8; 7.0	RFLP; SNP	–	Gardiner et al. 2004
–	–	–	–	–	DR033890 /closest band 5.7 $DR033887_{750}$/7.8; 7.0 DR033889 /closest band 8.0	RFLP RFLP; SCAR RFLP	–	and Gardiner et al. unpublished
–	*Er3*	M.9 × Aotea	277	Marker screening	CH02g09/12	microsatellite	LG 8	Chagné et al. unpublished
–	*Er-m*	Fuji × MIS o.p. 93.051 G02-054	153	BSA	$OPA04_{950}$/7 $OPZ20_{1200}$/6	RAPD → SCAR RAPD	Not determined	Gardiner et al. unpublished
–	*Er-l*	Prima × Longfield o.p. 93.043 G07-062	144	BSA	$OPAD01_{630}$/13	RAPD; SCAR	Not determined	Gardiner et al. 2001

apple aphid that does not map to markers for *Er-1* and *Er-3*. Flanking markers OPA4SCAR and OPZ20RAPD have been identified by BSA (Table 7) (Gardiner et al. unpublished).

1.3.2.4.4 *Er-l* A single RAPD marker, OPA01, has been identified for a novel woolly apple aphid resistance derived from an open pollinated seedling of Longfield (93.043 G07-062) (Gardiner et al. 2001). Markers identified to date for other woolly apple aphid resistances do not map to *Erl* (Table 7) (Gardiner et al. unpublished).

1.3.2.5 Other Major Gene Traits

1.3.2.5.1 Self-Incompatibility (*SI*) Maliepaard et al. (1998) mapped the self-incompatibility locus as an allele-specific marker (Janssens et al. 1995) in the Prima × Fiesta framework map (Table 8). It is closely associated with AAT-1 isoenzyme and the RFLP MC038b on the lower end of LG 17.

1.3.2.5.2 Rootsuckers (*Rs*) Joint segregation analysis of root sucker formation with RAPD markers segregating from White Angel identified linkage of RAPD P124e with a single locus determining the formation of root suckers (*Rs*) (Weeden et al. 1994) (Table 8). This locus was assigned to a US linkage group that corresponds to LG 17 in the European numbering system (Maliepaard et al. 1998; Hemmat et al. 2003).

1.3.2.5.3 Fruit skin color (*Rf*) Weeden et al. (1994) found that the isoenzyme marker *Idh-2* was closely linked to red skin color in a Rome Beauty × White Angel population. Study of segregation of BC226SCAR (identified by BSA) in the same Rome Beauty × White Angel population indicated that the basis for control of red/yellow skin color was simple (Cheng et al. 1996) (Table 8). Fruit of progeny with the dominant 1160 bp fragment from Rome Beauty or the 1180 bp fragment from White Angel (or both) were red skinned, while the recessive 1230 bp fragment inherited from both parents segregated with yellow skin. In three other progenies, only the 1160 bp fragment segregated with red skin. A second fragment of 1320 bp could be associated with yellow skin, in addition to the 1230 bp fragment. Screening of 56 other cultivars indicated that this marker system could be used to predict skin color in most cases. BC226 mapped to the US LG 3 (Conner et al. 1997) and to European LG 9 between two RFLP markers in the Prima × Fiesta framework map (Maliepaard et al. 1998). Comparative DNA sequencing would have to be carried out to confirm that the BC226 locus in the Rome Beauty × White Angel cross is allelic to the BC226 locus amplified in the Prima × Fiesta framework population, and the putative LG 9 assignment.

1.3.2.5.4 Fruit Juice pH (*Ma*) Acidity in apple is mainly due to malic acid and Nybom (1959) demonstrated that low acid fruit (pH 3.8 and above) was determined by the presence of homozygous recessive alleles for the *Ma* gene, *ma ma*. Mapping of fruit juice pH <3.7 in a Wijcik McIntosh × NY75441-58 population enabled the identification of a RAPD marker for *ma* ($S65_{600}$) (Table 8) (Conner et al. 1997). Acid-fruited progeny were assumed to have at least one copy of *Ma* and both parents (acid fruited) were heterozygous *Ma ma*. Using similar criteria, Maliepaard et al. (1998) located *Ma* on the distal end of LG 16, co-segregating with the RAPD $OPT16_{1000}$.

1.3.2.5.5 Columnar habit (*Co*) The *Co* gene in apple that was identified in a mutant of McIntosh (Fisher 1970) decreases branching, internode length and plant height, while increasing spur formation. The columnar character is believed to be controlled by a dominant allele, but modifiers may be involved (Lapins 1976). Bulked segregant analysis was used to identify a RAPD marker for the columnar habit that contained a repeat of $(GA)_{17}$ (Table 8) (Hemmat et al. 1997). This RAPD fragment was converted to a microsatellite marker (SSR^{CO}), where the null allele was linked to *Co*. Conner et al. (1997) then mapped two further RAPDs, B347 and B318 to a 5 cM interval around *Co* on the US linkage group corresponding to LG 10 in the EU framework map. SSR^{CO} (designated USASSR11) was later mapped directly to LG 10 in Fiesta (Maliepaard et al. 1998).

1.3.2.5.6 Fruit Allergens (*Mal d*) Allergies to fresh apples in Northern and Central European populations sensitized to birch pollen arise from four allergens identified to date: Mal d 1 (a Bet v 1 homologous protein) belonging to a group of pathogenesis related (PR10) proteins, Mal d 2 (apple thaumatin-like protein), Mal d 3 (apple non-specific lipid transfer protein) and Mal d 4 (apple profiling) (see Gao et al. 2005a). Knowledge of the genetics of allergenicity caused by healthy apple fruit will enable breeding for low allergen

Table 8. Mapping of major genes not involved in resistance, in apple

Gene	Progeny	No. sdlgs	Method	Markers marker/distance (cM from gene)	Marker class	Linkage group assignment	Reference
SI	Prima × Fiesta	152	Map construction	AAT-1/<1 MC038b/1	Isoenzyme RFLP	LG 17	Maliepaard et al. 1998
Rs	Rome Beauty × White Angel	56	Joint segregation analysis	P124e/not determined	RAPD	LG 17	Weeden et al. 1994
Rf	Rome Beauty × White Angel	56	Joint segregation analysis	*Idh-2*/not determined	Isoenzyme	–	Weeden et al. 1994
Rf	Rome Beauty × White Angel	72	BSA, mapping	$BC226_{1180}$/<2 *Idh-2*/<2	RAPD; SCAR Isoenzyme	–	Cheng et al. 1996
Rf	Wijcik McIntosh × NY75441-58	172	Linkage analysis	$BC226_{1175}$/0	RAPD (= 1,160 bp SCAR fragment above)	–	Conner et al. 1997
Rf	Prima × Fiesta	152	Mapping of BC226 SCAR			LG 9	Maliepaard et al. 1998
Ma	Wijcik McIntosh × NY75441-58	172	Map construction	$S65_{600}$/7.1	RAPD	–	Conner et al. 1997
Ma	Prima × Fiesta	152	Map construction	$OPT16_{1000}$/0	RAPD	LG 16	Maliepaard et al. 1998
Co	Wijcik McIntosh × NY5441-67	126	BSA	$OPA11_{1000}$/? → SSR^{CO}	RAPD, trans microsatellite	–	Hemmat et al. 1997
–	Wijcik McIntosh × NY75441-58	172	Linkage analysis	SSR^{CO}/6	microsatellite, trans	–	
Co	Wijcik McIntosh × NY75441-58	172	Map construction $B318_{440}$/3.2	$B347_{890}$/1.8 RAPD	RAPD	–	Conner et al. 1997
Co	Prima × Fiesta	152	Mapping of SSR^{co}	–	microsatellite	LG 10	Maliepaard et al. 1998
Mal d 1 gene family	Prima × Fiesta plus Jonathan × Prima	144 196	PCR based cloning; mapping of allele specific markers for each member of family (18 members)	–	–	–	Gao et al. 2005b
Subfamilies I, IV	–	–	–	Gene specific markers	SNP, microsatellite	LG 13, LG 16	–
Subfamilies II, III	–	–	–	Gene specific markers	SNP, microsatellite	LG 16	–
Mal d 1.05	–	–	–	Gene specific marker	SNP, microsatellite	LG 6	–
Mal d 2.01A	Prima × Fiesta	141	PCR based cloning; mapping of allele specific markers	Gene specific marker	SNP	LG 9	van de Weg, personal communication
Mal d 2.01B	–	–	–	Gene specific marker	SNP	–	–
Mal d 3.01	Jonathan × Prima	196	PCR based cloning; mapping of allele specific markers	Mal d 3.0101a-JO/0,	SNP	LG 12	Gao et al. 2005a

Table 8. (continued)

Gene	Progeny	No. sdlgs	Method	Markers marker/distance (cM from gene)	Marker class	Linkage group assignment	Reference
Mal d 3.02	Prima × Fiesta plus Jonathan × Prima	144 196	PCR based cloning; mapping of allele specific markers	Mal d 3.0201c-PM/0	SNP	LG 4	Gao et al. 2005a
Mal d 4.01	Prima × Fiesta	141	PCR based cloning; Gene specific marker		SNP	LG 9	van de Weg, personal communication
-2 copies	plus Jonathan × Prima	175	Mapping of allele specific markers	–	–	–	–
Mal d 4.2A	–	–	–	Gene specific marker	SNP	LG 2	–
Mal d 4.3A	–	–	–	Gene specific marker	SNP, SSR	LG 8	–
Md-ACS1	Prima × Fiesta	144	Mapping of gene specific molecular marker		SCAR	LG 15	Costa et al. 2005
Md-AC01	Prima × Fiesta Fuji × Mondial Gala	144 ?	Mapping of gene specific molecular marker	–	SCAR	LG 10	Costa et al. 2005
sl-1	6 progenies segregating for *Vf*		Mapping with *Vf* markers	–	± 14 cM from *Vf*	LG 1	van de Weg, personal communication
sl-2	6 progenies segregating for *Vf*		Mapping with *Vf* markers	–	1–8 cM from *Vf*	LG 1	van de Weg, personal communication

cultivars for European consumers with this food allergy.

1.3.2.5.6.1 Mal d 1 A number of conserved and specific primers were designed to obtain all possible *Mal d 1* sequences from Prima and Fiesta. PCR cloning of fragments, sequencing and genome walking towards the ends of genes enabled construction of allele-specific SNP and microsatellite primers for 18 *Mal d 1* genes that could be classified into four sub-families according to intron size and presence/absence. These genes were mapped directly in the Prima × Fiesta population, as well as a Jonathan × Prima progeny (Table 8). Seven genes from sub-families I and IV mapped in a 30–35 cM interval between the RFLP markers MC001 and MC041 on LG 13, and nine on the homeologous LG 16. One gene mapped to LG 6 and one has not yet been mapped (Gao et al. 2005b).

1.3.2.5.6.2 Mal d 3 PCR-based cloning and sequencing of DNA from the parents of the Prima × Fiesta framework mapping population of Maliepaard et al. (1998) resulted in the identification of two distinct genes, each with several sequence variants, that encode Mal d 3 proteins. SNP markers were constructed for each of the genes, *Mal d 3*.01 and *Mal d 3.0*, and mapped to homeologous segments of LG 12 and 4 between two RFLP markers shared in common (MC127 and MC105) (Gao et al. 2005a) (Table 8).

1.3.2.5.6.3 Mal d 2 Similar techniques have been employed to map two copies of *Mal d 2* to an identical position on LG 9 (Table 8) (Gao et al. 2005c)

1.3.2.5.6.4 Mal d 4 Two copies of a *Mal d 4* gene mapped to LG 9 and two single copy genes mapped to LG 2 and LG 8 respectively (Table 8) (Gao et al. 2005c).

1.3.2.5.7 Ethylene Production Shelf life in apple is a significant factor in determining the economic value of an apple cultivar, particularly in countries that rely on shipping of product to distant markets. In the course of a study examining the role of enzymes involved in the biosynthesis of ethylene and shelf life of apple fruit stored at room temperature after harvest, two key genes of the ethylene biosynthesis pathway were mapped. Both have proved to be candidates for marker-assisted breeding, as homozygotes for alleles *Md-AC01-1* and *MdACS1-2* yield fruit with lowest ethylene production and superior shelf life (Costa et al. 2005).

1.3.2.5.7.1 Md-ACS1 The *Md-ACS1* marker developed by Harada et al. (2000) mapped to LG 15 in the Prima × Fiesta framework mapping population (Costa et al. 2005) (Table 8). Its location is distant from a known QTL for fruit firmess that was previously identified in this population (King et al. 2000; Maliepaard et al. 2001). *Md-ACS1* exhibited a relatively large effect on ethylene content and apple fruit shelf life.

1.3.2.5.7.2 Md-AC01 A codominant gene specific marker for *Md-AC01* developed from full-length gene sequences derived from apple gDNA and mRNA mapped to LG 10 in two populations (Table 8). The clear, although small effect of *Md-AC01* on ethylene production, coupled with its location at the border of the 5% interval for fruit firmness QTL (King et al. 2000; Maliepaard et al. 2001), indicates that the role of *Md-AC01* in determining shelf life requires further examination (Costa et al. 2005).

1.3.2.5.8 Sub-lethal Genes (*sl*) Distorted segregation ratios have frequently been reported in populations segregating for the *Vf* gene (e.g., Yang and Kruger 1994; Tartarini 1996; Conner et al. 1997; Gardiner et al. 1999b; Tartarini et al. 1999; Bus et al. 2002). Analysis of this phenomenon in six progenies has indicated that these distortions could be explained by three homozygous recessive sub-lethal genes (*sl-1, sl-2, sl-3*). *Sl-1* mapped about 14 cM from *Vf*, and *sl-2* between 1–8 cM from *Vf*. Both genes required the presence of an unlinked gene, *sl-3*, for expression (Van de Weg, personal communication). A good understanding of the role of sub-lethal genes will facilitate strategic choice of parents by breeders to provide progenies with optimal proportion of seedlings with *Vf*.

1.4 QTL Trait Mapping

1.4.1 QTL Identification and Mapping in Apple

'Quantitative trait' describes a character for which the observed variation is due to the segregation of several genes and where, for each gene, the effects of the allelic differences on phenotype are generally small compared with the effects of the environment for each gene (Kearsey and Poon 1996). Genetic mapping of quantitative trait loci (QTL) involves identifying and determining the degree of association between continuous quantitative traits and sets of genetic markers.

The ability to assess complex phenotypes in apple at the seedling stage, such as tree architecture, fruit texture, fruit size and susceptibility to storage disorders using genetic markers would greatly accelerate new variety development. In addition to the selection of advantageous traits, markers linked to complex traits could be used to select against negative characteristics, and could even be used to select the combination of parents that would give rise to progeny with the desired genotype.

An essential requisite for accurate QTL identification in any plant species is a saturated genetic map covering the entire genome. If certain regions of the genome are not adequately represented by genetic markers, then QTLs located in such regions will not be reliably mapped, because it will be difficult to determine if the QTL has a genuinely small phenotypic effect, or is merely weakly linked to flanking markers (Lander and Botstein 1988). Several genetic maps have been constructed for apple using a range of genetic markers, such as random amplified polymorphism (RAPDs), restriction fragment length polymorphisms (RFLPs), amplified length polymorphism (AFLPs) and isozymes (Hemmat et al. 1994; Conner et al. 1997; Seglias and Gessler 1997; Maliepaard et al. 1998) (see Sect. 1.2.2). More recently, codominant microsatellite markers have been employed, and the development of 115 new microsatellite markers, mapped in the Fiesta × Discovery population (Liebhard et al. 2002; Liebhard et al. 2003b) has established a saturated, robust apple map comprising 1,140 cM and 1,450 cM in Fiesta and Discovery, respectively. This genetic map has been aligned with the Iduna × A679/2 map (Gianfranceschi et al. 1998) using common microsatellite loci, demonstrating that existing linkage maps, such as those published by Conner et al. (1997) and Hemmat et al. (1994) could easily be enriched and subsequently aligned and integrated with the Fiesta × Discovery map (Liebhard et al. 2002, 2003b), providing a valuable tool for QTL detection and analysis in apple. Map alignment with a consensus, saturated map will enable the detailed comparison of QTL positions between populations (King et al. 2000; Durel et al. 2003; Liebhard et al. 2003a, b, c; Calenge et al. 2004).

In addition to the classical QTL mapping approaches, there are other resources that can be utilised in the identification of QTLs. For example, genetic markers based on the sequence homology between the NBS domain in plant resistance genes identified loci which co-segregated with apple scab and powdery mildew resistance QTLs previously detected in a Discovery × TN10-8 population (Calenge et al. 2005a). Since such QTLs could sometimes be the result of residual resistance encoded by defeated major resistance genes, this is effectively a candidate gene mapping approach. Genome synteny between related species has also been exploited in QTL detection, with comparative mapping approaches used to identify QTLs conserved between maize and rice (Chardon et al. 2004) maize and sorghum (McIntyre et al. 2004) and maritime and loblolly pines (Chagné et al. 2003), suggesting that QTLs detected in genomes of other species belonging to the Rosaceae family, could be used to aid the identification of QTLs in apple.

1.4.2 Mapping QTLs for Disease Resistance

Most of the disease resistance genes characterized to date in apple are single dominant genes (see Sect. 1.3). Such genes commonly confer resistance to the pathogen in a gene-for-gene manner and are therefore in theory, easily overcome by the pathogen's ability to mutate to virulence (Crute and Pink 1996). In view of the ease with which a pathogen can break down single gene resistances, illustrated by the two recently discovered races of apple scab, able to overcome *Vf* (Parisi et al. 1993; Roberts and Crute 1994), it is likely that durable resistance to apple pathogens will be established through the pyramiding of different resistance genes with different resistance specificities into a single cultivar. Pathogen resistance conferred by QTLs would be a valuable addition to breeding portfolios of major resistance genes, as incorporating QTLs into a single cultivar is likely to be more effective than the combining of major genes alone (Parlevliet and Zadoks 1977). However, it is likely that several QTLs with significant phenotypic effects would be required to achieve a level of resistance comparable to that controlled by major genes (Liebhard et al. 2003c).

1.4.2.1 QTLs for Resistance to Powdery Mildew

Kellerhals et al. (2000) identified two major QTLs for powdery mildew resistance in the accession A 679-2 (Table 9). One of these QTLs originates from *M. zumi*, (*Pl-2* parent) and the other is linked to the *Vf* locus (approximately 16 cM from *Vf*) and is likely to origi-

Table 9. Summary of QTLs for resistance to powdery mildew

Parents		No. indv.[a]		Method of phenotypic assessment	Number of QTL detected	Reference
Female	Male	P/T	G/T		Linkage group (LG or G), LOD score and percentage of variance explained by QTL[b]	
Iduna	A 679-2	450	189	Artificial infection in field Scored: 5 point scale	**A 679-2 map:** LG 3, 5, 16 **Iduna map:** LG 2, 3, 5, 7, 8, 9	Kellerhals et al. 2000[c]
Idared	U 211	98	98	Assessed: 3 years in nursery 2 years in orchard Scored: 5 point scale	**U 211 map:** G 2: LOD 12.0; 72.1% and LOD 12.1; 71.5% G 3: LOD 2.6; 37.5% and LOD 8.9; 72.4% G 4 = LG 12: LOD 9.3; 64.9% and LOD 8.8; 71.9% and LOD 10.8; 72.0% **Idared map:** G 3 = LG 2: LOD 3.3; 40.6% and LOD 2.4; 39.4% G 5 = LG 15: LOD 7.9; 61.0%	Stankiewicz-Kosyl et al. 2005[d]
Discovery	TN10-8	149	149	Natural infection in field Assessed over 5 seasons Scored: 10 point scale	**Discovery × TN10-8 map:** LG 2: LOD 3.0–9.01; 7.4%–22.5% LG 13: LOD 3.74–9.73; 7.5%–27.4% LG 1: LOD 3.0; 7.4% LG 8: LOD 5.27–8.45; 8.9%–19.5% LG 10: LOD 3.99–4.02; 7.9%–8.3% LG 14: LOD 3.45; 5.7% LG 17: LOD 4.36–4.64; 8.8%–10.5%	Calenge and Durel 2006[e]

[a] Number of individuals used in QTL detection, either in phenotyping population (P/T) or in map construction and genetic marker analysis (G/T)

[b] Linkage groups containing QTL are listed with the prefix LG or G. Where possible, the LOD score of the associated QTL and the percentage of phenotypic variance (PVE) it explains are included, with the LOD score first. Where several linkage groups are included, the range of associated LOD score and PVE have been included.

[c] Linkage maps not aligned with adopted consensus map (Liebhard et al. 2003b), thus standard linkage group nomenclature not used.

[d] Where possible linkage groups have been aligned with those of the consensus map (Liebhard et al. 2003b) and are annotated as such.

[e] Linkage maps have been aligned with the consensus map (Liebhard et al. 2003b), and standard nomenclature is used.

nate from *Malus floribunda* 821. Eight other putative QTLs were also identified, six of which were located on the genome of the susceptible parent, Iduna. Mildew infection was assessed in the field using a 5-point classification scale, and the non-parametric Kruskal-Wallis test was used to determine the association of mapped RAPD markers with powdery mildew resistance. Stankiewicz-Kosyl et al. (2005) used MAPMAKER/EXP 3.0 and MAPMAKER/QTL 1.1, to analyse data from a limited number of 98 individuals derived from an Idared × U211 cross and map 10 QTLs involved in powdery mildew resistance (Table 9). Although the genetic maps of Idared and U211 span only five and four linkage groups, respectively, these maps were specifically constructed around genomic regions of interest. Several of the markers used made it possible to align some of these linkage groups with those of Maliepaard et al. (1998).

Stankiewicz-Kosyl et al. (2005) assessed trees over a period of three years in the nursery and two years in the field. Of the 10 QTLs detected five of them were associated with powdery mildew resistance in only one year. QTL U7, mapped on the equivalent of LG 12, was associated with powdery mildew resistance over four years and explained between 47.8% and 72.0% of phenotypic variation. Apple scab resistance genes such as *Vg* (Durel et al. 1999) and *Pl-d* (James et al. 2004) have already been mapped to LG 12, suggesting the possibility of resistance QTL/gene clusters in this region.

Calenge and Durel (2006) also assessed the occurrence of powdery mildew resistance in a population of 149 individuals derived from a Discovery × TN10-8 cross (Calenge et al. 2004) across five seasons over four years (Table 9). Using MapQTL software, two QTLs on LG 2 and LG 13 were consistently identified over all five seasons and explained between 7.5 and 27.4% of the phenotypic variation depending upon the season, making them ideal candidates to select for in marker-assisted breeding programs. Five other QTLs were also identified during either one or more seasons on LG 1, LG 8, LG 10, LG 14 and LG 17 and several of these QTLs were mapped to the same region as previously identified major resistance genes, or resistance gene clusters. Calenge and Durel (2006) hypothesised that the detection of a range of QTLs over the five seasons could be the result of environmental effects, such as climate, tree growth and development, or changes to the *P. leucotricha* local populations. The fluctuation in the presence/absence of these five QTLs over the four seasons indicates that to explain this powdery mildew resistance fully, assessments over more years will be necessary. Indeed, Calenge and Durel (2006) plan to maintain and continue to assess this population beyond this study, to determine a key set of QTL that control resistance to powdery mildew.

1.4.2.2 QTLs for Resistance to Apple Scab

QTLs have been identified for apple scab resistance using the reference genetic maps (Sect. 1.2.2) constructed in the following populations; Prima × Fiesta (Durel et al. 2003), Fiesta × Discovery (Liebhard et al. 2003c), Discovery × TN10-8 (Calenge et al. 2004) (Table 10). A summary of these results plus those from progenies of Discovery × Prima and Durello de Forli x Fiesta is presented in Durel et al. (2004).

Durel et al. (2003) used two monoconidial strains of race 6 to identify QTLs controlling resistance in both Fiesta and Prima. Detailed QTL analysis using both MCQTL (Jourjon et al. 2000) and MapQTL (Van Ooijen 2004) software identified four genomic regions that were significantly involved in partial resistance, characterized by a reduction in sporulation (Table 10). One of these regions was located close to the original *Vf* gene and it is possible that the observed partial resistance was due to a closely linked gene, or a result of a residual effect of the overcome *Vf* gene (Durel et al. 2003). The remaining three additional regions identified on LG 15, 11 and 17 were novel locations for association with scab resistance.

Liebhard et al. (2003c) carried out extensive assessment of field resistance to apple scab over a three-year period involving three different geographical sites. Using MapQTL, eight QTLs were identified that contributed to apple scab resistance; six for leaf scab and two for fruit scab (Table 10). Interestingly, although Discovery demonstrated a greater degree of resistance, most of the identified QTLs were attributed to Fiesta, the more susceptible parent, indicating a high degree of homozygosity at the resistance gene loci in Discovery that prevented their detection in the progeny because of the lack of segregation. The high levels of resistance observed in individuals during the study confirmed that Discovery was a strong resistant parent for breeding (Liebhard et al. 2003c). The strongest scab resistance QTL from Prima × Fiesta mapped to LG 17 (Liebhard et al. 2003c), coinciding with a scab resistance QTL that Durel et al. (2003) identified, and similarly LG 11 was identified in both studies as possessing a region of interest. One of the QTLs detected by Liebhard et al. (2003c) that

Table 10. Summary of QTLs for apple scab resistance

Parents		No. indv.[a]		Method of phenotypic assessment	Number of QTL detected	Reference
Female	Male	P/T	G/T		Linkage group (LG or G), LOD score and percentage of variance explained by QTL[b]	
Prima	Fiesta	143	143	Glasshouse inoculated: Race 6 strains (strain 302, EU-D-42)	**QTLs in four main genomic regions identified:** LG 1 (isolate specific): LOD 8.2–8.9; 16.0–17.8% LG 11: LOD 5.4–9.5; 16.5–22.8% LG 15 (isolate specific): LOD 3.1–3.3; 5.6–6.0% LG 17: LOD 3.6–5.9; 9.4–13.4%	Durel et al. 2003[c]
Fiesta	Discovery	251	251	Infected leaves placed in all locations and artificial inoculations at Wädenswil and Conthey Detached leaf assay and 9 point scale 3–4 times/season Fruit assessed using 4 point scale	**Fiesta × Discovery map:** **Leaf scab:** LG 6, 7, 10, 11, 12, 17: LOD 2.3–13.2; 4.0–23% **Fruit scab:** LG 15, 17: LOD 2.8–4.9; 7.0–9.0%	Liebhard et al. 2003c[c]
Discovery	TN10-8	149	149	Glasshouse tested Scored: Infection on 6 point scale Sporulation on 8 point scale	**Discovery:** LG 2, 5, 12, 13, 15, 17 **TN10-8:** LG 1, 2 **Discovery × TN10-8 map:** 3 major QTL for partial resistance to most isolates: LG 1, 2, 17: LOD 3.16–26.59; 5.1–51.1% QTL for single isolate resistance LG 5: LOD 5.4–12.57; 12.5–20.8%	Calenge et al. 2004[c]

[a] Number of individuals used in QTL detection, either in phenotyping population (P/T) or in map construction and genetic marker analysis (G/T)

[b] Linkage groups containing QTL are listed with the prefix LG. Where possible, the LOD score of the associated QTL and the percentage of phenotypic variance (PVE) it explains are included, with the LOD score first. Where several linkage groups are included, the range of associated LOD score and PVE have been included.

[c] Linkage maps have been aligned with the consensus map (Liebhard et al. 2003b), and standard nomenclature is used.

accounted for 4% of the phenotypic variability was located on LG 12 in a position comparable to *Vg* (Van de Weg unpublished data).

Calenge et al. (2004) used a panel of eight monoconidial isolates to inoculate replicated progeny from a Discovery × TN10-8 cross, resulting in the identification of numerous QTLs across seven linkage groups (with MapQTL), depending upon the isolate used (Table 10). Combining QTLs with overlapping confidence intervals and close likelihood peaks revealed three major QTLs on LG 1, LG 2 and LG 17. The region identified on LG 1 corresponds to the region around *Vf* that Durel et al. (2003) identified as contributing between 16.0% and 17.8% of phenotypic variation, and the QTL identified on LG 17 (Calenge et al. 2004) is also in agreement with a QTL mapped in both Fiesta and Discovery that explained 23% of the observed phenotypic variability. Calenge et al. (2004) also detected additional QTLs on LG 5, 13 and 15 to only one or two isolates and a QTL on LG 2 that appeared to control more broad-spectrum resistance to apple scab. This QTL spans a region around the major scab resistances *Vbj*, *Vh2* and *Vh8* (Calenge et al. 2004; Durel et al. 2004). The identification of isolate-specific QTL indicates that some partial resistance QTL could be involved in a pathogen-mediated recognition response, similar to major genes.

These reports of detecting QTLs contributing to disease resistance highlight the importance of phenotyping segregating populations over several years and in different environments. Different infection pressures in different years, especially years with low disease incidence, can lead to high within-genotype variability (Liebhard et al. 2003c). Location-specific pathogen populations are another possible cause of increased variability, expressed as genotype-location interaction. This occurs particularly with pathogens such as apple scab, where the first wave of infection in a season is often due to ascospores originating from crosses between fungal strains from the previous growing season and where it is likely that particularly effective ascospore-derived progeny can then multiply asexually as the season progresses. An example of this is the QTL detected by Liebhard et al. (2003c) on LG 10 for leaf scab, as this QTL was only associated with data gathered at the Wädenswill location, over three years. Stankiewicz-Kosyl et al. (2005) identified five QTLs for powdery mildew resistance that were only associated with resistance for a single year. A QTL that is detected in a single year is dependent upon the allelic difference at a particular locus, the interaction of the QTL with environmental factors and/or the alteration of the expression of the QTL over time with plant development (Stankiewicz-Kosyl et al. 2005). Assessment of QTL detection over several years is one way of independently verifying the presence of QTL in the same genetic background, which can in turn minimise additional sources of variation.

1.4.2.3 QTL for Resistance to Fire Blight

Although accessions of apple displaying resistance to fire blight have been identified, the genetic control of this resistance is not well understood, and is thought to have a quantitative, polygenic aspect (Lespinasse and Aldwinckle 2000). Using two populations derived from crosses between Prima × Fiesta and Fiesta × Discovery respectively, Calenge et al. (2005b) described the first comprehensive identification of QTL controlling fire blight resistance in apple (Table 11). Several QTL were detected in both progenies, with one QTL on LG 7, derived from the common parent Fiesta explaining 34.3–46.6% of the resistance. The identification of this major QTLs in both populations demonstrated its robustness in two different genetic backgrounds. Four minor QTL were also identified on LG 3 (Prima and Fiesta), LG 12 (Discovery) and LG 13 (Discovery), each explaining 4.4–7.9% of the variation. Using a different strain of fire blight, and a different Fiesta × Discovery progeny, Khan et al. (2006) confirmed a QTL for resistance on LG 7 of Fiesta and demonstrated the stability of this QTL. The minor QTL identified by Calenge et al. (2005b) were not detected in this study.

In addition, Calenge et al. (2005b) utilized a number of microsatellite markers in common between apple and pear to compare the location of the QTLs they detected in apple with QTLs for resistance to fire blight that had been previously mapped in pear (Dondini et al. 2004). In two cases, the microsatellite markers detecting loci close to identified fire blight resistance QTL in apple, also identified loci in pear that mapped close to fire blight resistance QTLs. Further investigation of these potentially homologous fire blight resistance QTLs could aid the identification of potential new resistance QTLs candidates in both crops, and enhance our understanding of the synteny between pear and apple. Calenge et al. (2005b) also compared inter-loci interactions for all possible two-way combinations of markers to identify potential epistatic QTLs. The recurrent involvement of certain genomic

Table 11. Summary of QTLs for resistance to fire blight

Parents		No. indv.[a]		Method of phenotypic assessment	Number of QTL detected	Reference
Female	Male	P/T	G/T		Linkage group (LG or G), LOD score and percentage of variance explained by QTL[b]	
Prima	Fiesta	144	144	Artificial inoculation in glasshouse with strain CFBP 1430 Assessed 7 and 14 dpi using a multipoint scale	**Fiesta × Prima:** **Fiesta map:** LG 7: 7 dpi, LOD 18.43; 43.2% 14 dpi, LOD 19.14; 46.6% **Prima map:** LG 3: 14 dpi, LOD 4.09; 7.5%	Calenge et al. 2005[c]
Fiesta	Discovery	188	188	–	**Fiesta × Discovery:** **Fiesta map:** LG 3: 7 dpi, LOD 3.57; 4.4% LG 7: 7 dpi, LOD 26.82; 42.6% 14 dpi, LOD 13.39, 34.3% **Discovery map:** LG 12: 7 dpi, LOD 3.53; 5.4% LG 13: 17 dpi, LOD 4.87; 7.9%	
Fiesta	Discovery	86	251	Artificial inoculation in glasshouse with strain Ea610 Assessed 6,13,20 and 27 dpi using a multipoint scale	**Fiesta × Discovery:** **Fiesta map:** LG 7; 13, 20, 27 dpi, LOD 7.5–8.1; 37.5–38.6%	Khan et al. 2006[c]
Idared	*M.* x *robusta*	150	150	Artificial inoculation	***M.* x *robusta* map:** LG 5	Peil et al. 2006[c]

[a] Number of individuals used in QTLs detection, either in phenotyping population (P/T) or in map construction and genetic marker analysis (G/T)

[b] Linkage groups containing QTLs are listed with the prefix LG or G. Where possible, the LOD score of the associated QTL and the percentage of phenotypic variance (PVE) it explains are included in parentheses, with the LOD score first. Where several linkage groups are included, the range of associated LOD score and PVE have been included.

[c] Linkage maps have been aligned with the consensus map (Liebhard et al. 2003b), and standard nomenclature is used.

regions indicated putative epistatic QTLs that need to be confirmed in more detailed future analyses utilizing larger progeny sets.

A recent report by Peil et al. (2006) has identified a QTL controlling fireblight resistance on LG 5 of *M.* x *robusta*.

1.4.3 Mapping QTLs for Tree Growth and Development

Many morphological and developmental traits in apple are perceived to be under complex genetic control. When Lawson et al. (1995) compared marker data of Rome Beauty × White Angel progeny with phenotypic data, single loci controlling branching type, reproductive budbreak and root suckering were identified using the Microsoft Excel macro, QUIKMAP (designed by N. F. Weeden and J. Barnard) and the program LINKAGE-1 (Suiter et al. 1983). However, of these three traits only root sucker formation appeared to be under simple Mendelian genetic control (See Sect. 1.3.2.5.2). Branching type and reproductive budbreak gave a range of phenotypes for which the genetic basis was not immediately apparent (Table 12). Using only the phenotypic extremes of these traits, initial genetic analysis enabled loci contributing major portion of the variation to be identified, but it became obvious that other genetic loci were probably involved in the expression of branching and budbreak (Lawson et al. 1995). However, the ability to detect other genes in this study would have been limited, because, although data was available for over 400 markers (isozymes, RAPDs and RFLPs), segregation data was only available from 56 individuals, and thus only major gene effects could have been examined in this population. Limited population size in QTL detection exercises may lead to an underestimation of QTL number, overestimation of QTL effect, and a failure to accurately quantify QTL interactions. Vales et al. (2004) explored the effect of population size in the estimation of barley stripe rust QTLs and showed that as population size increased, so did the number of QTLs detected and that the overestimation of the percentage of variance explained by the QTLs was reduced.

Further work by Conner et al. (1998) used a population derived from a cross between the columnar mutant Wijcik McIntosh and accession (NY 75441-58) to position additional QTLs influencing tree growth and development. Maps were constructed for each parent, consisting of approximately 180 RAPD and isozyme loci. These maps were aligned using markers heterozygous in both parents. The positions of these putative QTLs were established by a range of statistical analyses of marker and phenotype data using MINITAB. One to eight QTLs were identified as involved in the control of height increment, internode number and length, base diameter, branch number and leaf break (Table 12). Most of the regions identified were associated with a specific trait for one year and many of the traits assessed were related to each other, and when mapped appeared to be clustered on linkage groups. The largest cluster was identified on LG 10, close to the position of the *Co* gene (Conner et al. 1997). Conner et al. (1998) suggested that other large clusters of marker trait associations could be the result of single loci with pleiotropic effects. Previously, Lawson et al. (1995) also hypothesised that vegetative budbreak, which correlated with the segregation of the terminal bearing characteristic could be the result of the pleiotropic effect of this gene.

Using the extensive Fiesta × Discovery linkage map, consisting of 804 genetic markers (a significant proportion of these microsatellites) and covering all 17 apple chromosomes, Liebhard et al. (2003a) undertook a more comprehensive analysis of several quantitative physiological traits in apple (Table 12). Both single parent linkage maps and the integrated map were used in MapQTL analysis to identify the contributor of the effective allele, map position and effect. For some traits, data was collected from own rooted seedling populations as well as grafted individuals. Three QTLs were detected for seedling stem diameter and two QTLs for seedling leaf size, but these could not be detected using the grafted plants, where independent QTLs were identified. Liebhard et al. (2003a) also positioned six QTLs for tree height increment. As found by others (Lawson et al. 1995; Conner et al. 1998), there were associations among some of these different growth and development traits. Of the six regions (Liebhard et al. 2003a) identified for height increment, four coincided with QTLs for stem diameter, indicating that these traits are related or clustered in some way. Conner et al. (1998) also reported a correlation between height increment and 'base diameter increment' identifying single markers on two linkage groups associated with this trait. Investigations into blooming traits identified five QTLs associated with blooming characters, located on five different linkage groups (Liebhard et al. 2003a). Similarly one of these QTLs for number of flower bunches was located very

Table 12. Summary of QTLs for tree growth and development

Parents Female	Male	No. indv.[a] P/T	G/T	Method of phenotypic assessment	Number of QTL detected Linkage group (LG or G), LOD score and percentage of variance explained by QTL[b]	Reference
Rome Beauty (RB)	White Angel (WA)	82	56	Branching habit: 3 point scale and with and without spurs Vegetative budbreak: 5 point scale, over 3 years Reproductive budbreak, used phenological categories of Chapman and Catlin (1976), over 2 years Bloom time: 2 dates used	**White Angel:** **Terminal bearing (*Tb*):** LG 6, possible QTL or masked by spurring **Bloom time:** LG 1	Lawson et al. 1995[c]
Wijcik McIntosh	NY75441-58	172	172	Tree vigour assessed by tree height and base diam (3 years plus again in year 9) Branch no. scored after 3rd year of growth Leaf break scored: 5 times at weekly intervals, 6 point scale Columnar form: visual assessment	**Wijcik McIntosh × NY75441-58 map:** **Height increment:** LG 6, 7, 9, 10, 11, 12, 21 (3.9–7.9%) **Internode length:** LG 5, 6, 9, 10 (4.6–23.1%) **Internode no:** LG 1, 5, 7, 10, 12, 21 (4.3–16.8%) **Base diameter increment:** LG 2, 7, 10, 14, 16, 21 (4.0–8.5%) **Base diameter:** LG 7, 9 (5.5–7.5%) **Branch number:** LG 7, 10 (7.1–24.3%) **Leaf break:** LG 3, 6, 7, 9, 11, 12, 15 (3.9–7.3%)	Conner et al. 1998[c]

[a] Number of individuals used in QTL detection, either in phenotyping population (P/T) or in map construction and genetic marker analysis (G/T)

[b] Linkage groups containing QTL are listed with the prefix LG. Where possible, the LOD score of the associated QTL and the percentage of phenotypic variance (PVE) it explains are included in parentheses, with the LOD score first; except for the Wijcik McIntosh × NY75441-58 map where only PVE are listed. Where several linkage groups are included, the range of associated LOD score and PVE have been included.

[c] Linkage maps not aligned with adopted consensus map (Liebhard et al. 2003b), thus standard linkage group nomenclature not used.

Table 12. (continued)

Parents		No. indv.[a]		Method of phenotypic assessment	Number of QTL detected	Reference
Female	Male	P/T	G/T		Linkage group (LG or G), LOD score and percentage of variance explained by QTL[b]	
Fiesta	Discovery	251	251	Leaf area, stem diameter measured on seedlings at one site over 2 years. Stem diam, height increment, blooming, no. measured over next 3 years in all locations	**Fiesta × Discovery map:** **Seedling:** **Stem diam:** LG 2, 15, 17 (LOD 3.1-4.8; 6.0–10.0%) **Leaf size:** LG 9, 17 (LOD 3.0–4.2; 6.0–8.0%) **Tree:** **Height increment:** LG 3, 5, 8, 11, 13, 17 (LOD 2.4-6.2; 5.0–11.0%) **Stem diam:** LG 1, 2, 3, 8, 11, 13, 14, 15, 17 (LOD 1.7-6.5; 4.0–13%) **Blooming time:** LG 7, 10, 17 (LOD 2.5-3.6; 5–13%) **No. bunches:** LG 8, 15 (LOD 3.6-5.1; 7.0–10.0%) **Juvenile phase length:** LG 3, 15 (LOD 3.2-4.0; 6.0–8.0%) **Fruit harv. date:** LG 3 (LOD 4.7; 16.0%)	Liebhard et al. 2003a[d]

[d] Linkage maps have been aligned with the consensus map (Liebhard et al. 2003b), and standard nomenclature is used.

close to another QTL for juvenile phase length on LG 15, but on the alternate linkage group of the Fiesta parent.

QTLs or major genes have been identified for a variety of growth and development characteristics in apple with a view to using marker-assisted selection to develop new varieties with shorter juvenile phase, later blooming to avoid frost injury, and desired branching patterns. Controlling the growth of the grafted apple scion, through the use of a dwarfing rootstock, such as Malling 9 (M.9) permits a higher planting density, which in turn results in an increased yield per hectare. However, the precise physiological or genetic mechanism by which a rootstock induces dwarfing is not well understood. In a similar approach to that of Lawson et al. (1995), Rusholme et al. (2004) used bulked segregant analysis (BSA) of the phenotypic extremes of a segregating population to identify genetic markers flanking a single gene, *Dw-1*, that contributed to the dwarfing effect of M.9. *Dw-1* has been mapped to LG 5 with microsatellite markers (Celton et al. 2006).This single major effect gene did not explain all of the variation observed in the segregating population, indicating that additional genes could be involved in the control of dwarfing. However, this initial genetic analysis was based on individuals that had been assigned to one of four simplified phenotypic classes. QTL analysis with more detailed phenotyping on a larger population is required to determine how many loci are involved in addition to the one identified. Such a whole genome-based approach, in addition to identifying QTL involved in dwarfing, will also enable additional QTL involved in the control of flowering to be identified, and hence the postulated relationship between the dwarfing ability of rootstocks and grafted scion precocity to be determined.

When identifying genetic markers for traits controlled by major, simply inherited genes, it is possible to use straightforward, accelerated approaches that are designed to target specific regions of the genome, such as BSA (Michelmore et al. 1991) or candidate gene screening (Gardiner et al. 2003) as well as more detailed genetic analysis of the whole genome. In addition to identifying markers for major genes, BSA has also been used to identify QTL for increased yield in soybean (Yuan et al. 2002) and drought tolerance in maize (Quarrie et al. 1999) through the analysis of recombinant inbred lines. In more genetically diverse species, such as apple, it is possible that significantly more individuals would be required in each bulked DNA sample, to ensure that each allele is represented in the bulks at the same frequency as in the population, as several marker alleles are likely to be present (Quarrie et al. 1999). Although BSA has been employed as a cost-effective approach to identify genetic markers for a major locus contributing to dwarfing of apple scions by the rootstock M.9, genetic mapping of QTL using whole population analysis is a more precise method, likely to identify additional smaller QTL that also impact on phenotype, enabling full characterization and understanding of the dwarfing trait.

1.4.4 Mapping QTLs for Fruit Quality

In addition to positioning QTLs for a range of growth characteristics, Liebhard et al. (2003a) also assessed a range of traits associated with fruit development and quality. The development of genetic markers linked to key physiological traits in apple would significantly accelerate and improve the efficiency of new cultivar development in apple. Such rapid and non-destructive marker-based assessment of young seedlings for fruit characteristics would greatly reduce the number of generations required for cultivar development, an invaluable benefit in a crop with such a long generation time. Liebhard et al. (2003a) identified a QTL on LG 3 of the variety Discovery that explained 16% of the variability associated with fruit 'harvest date' and eight QTLs controlling 'fruit weight' (Table 13). It could be predicted that some blooming traits and fruit traits would exhibit co-segregation. Indeed one of the three QTLs identified for the 'number of fruit' coincided with a QTLs for 'number of flower bunches', yet poor correlation was detected (Liebhard et al. 2003a) between the phenotypes of these traits, which was attributed to other potential QTL affecting the same traits, or changes in tree behavior with time. It is recognised that as the tree enters different phases of development and growth, the expression of certain traits can change, making accurate phenotypic assessments difficult. Continued assessment of such populations is essential to establish true phenotypes and subsequently identify the genetic loci involved accurately.

Fruit texture is also a complex character and is of key importance in the development of new apple varieties that comply with consumer preference. Several quite different aspects of fruit composition can be assessed to determine fruit texture, such as fruit

Table 13. Summary of QTL for fruit quality

Parents Female	Male	No. indv.[a] P/T	G/T	Method of phenotypic assessment	Number of QTL detected Linkage group (LG or G), LOD score and percentage of variance explained by QTL[b]	Reference
Fiesta	Discovery	251	251	No. fruit, fruit weight, flesh firmness (penetrometer), sugar content, acidity measured over next 3 years in all locations	**Fiesta × Discovery map:** **Tree:** **No. fruit:** LG 5, 15, 16 (LOD 3.2–4.5; 8.0–10.0%) **Fruit weight:** LG 1, 3, 6, 8, 10, 12, 15, 16 (LOD 2.5–17.0; 7.0–31.0%) **Flesh firmness:** LG 6, 11, 12, 14 (LOD 3.6–12.3; 6.0–27.0%) **Sugar content:** LG 3, 6, 8, 9, 14 (LOD 3.1–5.1; 3.6–12%) **Fruit acidity:** LG 8, 16 (LOD 4.7–6.2; 42.0–46.0%) (*Ma* locus on LG 16)	Liebhard et al. 2003a[c]
Prima	Fiesta	152	152	Fruit firmness (2 penetrometer readings) Stiffness by acoustic resonance Sensory descriptors (hardness, crispness, granularity, spongy texture, slow breakdown, juiciness, overall liking) scored on scale of 0–100:	**Prima × Fiesta map:** **Fruit firmness:** LG 1, 8, 10 (LOD 4.7–7.4; 16.0–22.0%) **Resonant freq:** LG 10 (LOD 4.6; 21.0%) **Hardness:** LG 10 **Crispness:** LG 1, 5, 10, 12, 13, 16 (LG 16, LOD 6.0; 24%) **Juiciness:** LG 1, 12, 16 (LG 16, LOD 14.8; 46%) **Granularity:** LG 2 (LOD 5.1; 24%)	King et al. 2000[c]

[a] Number of individuals used in QTL detection, either in phenotyping population (P/T) or in map construction and genetic marker analysis (G/T)

[b] Linkage groups containing QTL are listed with the prefix LG. Where possible, the LOD score of the associated QTL and the percentage of phenotypic variance (PVE) it explains are included in parentheses, with the LOD score first. Where several linkage groups are included, the range of associated LOD score and PVE have been included.

[c] Linkage maps have been aligned with the consensus map (Liebhard et al. 2003b), and standard nomenclature is used.

Table 13. (continued)

Parents		No. indv.[a]		Method of phenotypic assessment	Number of QTL detected Linkage group (LG or G), LOD score and percentage of variance explained by QTL[b]	Reference
Female	Male	P/T	G/T			
Prima	Fiesta	130	152	6 fruit per tree sampled, texture: wedge fracture test, compression test, cells in fruit cortex analysed	**Slow breakdown:** LG 1 **Sponginess:** LG 1, 5, 6, 16 (LG 16, LOD 7.7; 30.0%) **Overall liking:** LG 12, 16 (LG 16, LOD 11.3; 38.0%) **Prima × Fiesta map:** **Compression:** LG 1, 6, 8, 12, 15 (LOD 4.09–8.62; 16.0–27%) **Wedge measures:** LG 1, 7, 15, 16 (LOD 4.51–9.83; 15.0–32.0%) **Specific gravity:** LG 6 (LOD 7.99; 28.0%), 16 (LOD 4.54; 15.0%) **Fruit weight:** LG 4 (LOD 4.53; 25.0%) **Stress at first failure (compression):** LG 13 (LOD 3.51) **Work of fracture (wedge fracture):** LG 7 (LOD 4.51) **Circularity of cells:** LG 3 (LOD 3.3)	King et al. 2001[c]

firmness, crispness and juiciness. These characteristics are in turn determined by several variables such as cell size or cell wall strength. It is therefore particularly complicated to accurately identify QTLs controlling fruit texture, making fruit texture a challenging candidate for marker-assisted breeding.

King et al. (2000, 2001) carried out a detailed genetic dissection of fruit textural attributes using a population derived from the Prima × Fiesta cross that had been used earlier for the construction of the first reference genetic map (Maliepaard et al. 1998) (See Sect. 1.2.2). Fruit was assessed using a range of mechanical measurements and sensory parameters. Using MapQTL, QTLs accounting for differing degrees of variation for firmness, stiffness and a number of sensory attributes were identified on seven linkage groups (Table 13). This research also provided insight into the relationship between some of the mechanical measurements and sensory perceptions (King et al. 2000). Further work (King et al. 2001) extended the range of mechanical measurements to include compression and wedge fracture tests. The wedge fracture tests identified significant QTLs on LG 16 and LG 1. The QTL on LG 16 was located in the same region as QTL identified for certain sensory textural attributes, such as crispness and juiciness. Linkage group 16 has also been shown to contain the *Ma* acidity locus (Maliepaard et al. 1998). King et al. (2001) determined that the apparent association of the *Ma* gene with regions contributing to sensory assessments was unlikely to be the result of 'perceptual interactions' with the *Ma* locus. Four QTLs controlling fruit firmness, measured by penetrometer, were also identified by Liebhard et al. (2003a) in the Fiesta × Discovery reference population. The results of this study can be compared with the previous studies (King et al. 2000, 2001), as both genetic maps were constructed in part using codominant markers and have the Fiesta parent in common. Liebhard et al. (2003a) and King et al. (2000, 2001) identified three QTLs for fruit firmness (using penetrometer readings) on the common linkage groups LG 3, LG 12 and LG 16. However King et al. (2000) also identified additional QTLs for fruit firmness, (measured by penetrometer) across four more linkage groups. Investigations with the related Fiesta × Discovery population did not detect this range of QTLs, indicating that expression of Fiesta alleles contributing to fruit firmness could be different in different genetic backgrounds, or that certain alleles may only be expressed in certain environments (Liebhard et al. 2003a).

1.4.5 Conclusions

There are many favorable complex traits that would be desirable to select for in the development of new apple varieties. The studies detailed in this Section, where many QTLs have been identified for disease resistance, tree architecture and fruit quality traits, are the initial steps that will lead to the unravelling of such complex traits, and the development of molecular markers linked to major QTLs that can be deployed in the marker-assisted selection of parents and progeny in apple breeding programs. These broad-ranging studies emphasise that to develop robust genetic markers that will be useful to apple breeders, it is essential that QTL are accurately positioned on the apple genome. There are many factors that can influence and further enhance reliable QTL identification, such as the use of dense genetic maps constructed with codominant markers and robust, objective phenotyping methods. Estimates of variance for site-to-site and occasion-to-occasion variance can be accurately determined by the inter- and intra-site replication of only a relatively small proportion of the population (Lynn 1998), and maximising the number of recombinant individuals in a population can also enable greater genetic resolution. If marker-assisted selection is to be successfully applied to traits controlled by QTLs, it is important to remember that QTL analysis of a population can detect only differences between the inherited parental alleles (Liebhard et al. 2003a). Therefore, to fully estimate the phenotypic effect of identified QTL alleles, they should also be compared with the equivalent and ineffective allele at the same locus (Liebhard et al. 2003a). Once QTL and associated markers have been identified, their conserved position in different genetic backgrounds, such as other cultivars or genetically more diverse germplasm accessions, should be established to ensure that the genetic markers developed have the widest applicability to new variety development.

The continued development of new QTL mapping models and algorithms designed to extract the maximum amount of information about QTL positions and effects will also aid in more accurate positioning of QTLs on the apple genome. Unfortunately, a précis of methods of QTLs detection is beyond the scope of this review, but of interest is a study by Maliepaard et al. (2001) that recently compared the more traditional simple interval mapping and approximate multiple QTL model mapping with Bayesian multiple QTL

analysis, in the context of experimental data derived from a large full-sib family in apple.

New statistical tools and methods are also currently being assessed in a pedigree-analysis based approach to QTLs mapping in apple (Van de Weg et al. 2004). Pedigree-analysis is an ideal approach to identify QTL in data gathered from more complex and diverse populations, such as those derived from multiple founders or collected from ongoing breeding programs. This method of QTL identification, originally used for detecting QTLs in human populations, has several advantages for plant geneticists, namely: detailed data on QTL variation within relevant breeding populations will be generated, since multiple alleles will be present; the context of identified QTL alleles can be examined; and the cost efficiency of the QTL mapping exercise will be improved as existing selection experiments can be utilised (Bink et al. 2002).

1.5 Marker-Assisted Breeding

The advantages and limitations of conventional versus molecular breeding have recently been discussed in general terms in a review article by Oraguzie et al. (2004). A major advantage of the use of markers is that they increase the breeding efficiency by enabling early selection for adult traits; simultaneous selection for multiple traits, including resistance gene pyramids; and selection for traits that are expensive to phenotype. Strategies can be developed for the efficient marker-assisted introgression of a range of traits into one cultivar (Servin et al. 2004). In this chapter, we will discuss marker-assisted breeding (MAB) using examples of its application, and the potential of whole-genome selection as part of a fast-breeding strategy combined with the reduction of the juvenile phase.

1.5.1 Germplasm Screening

Genetic markers linked to a specific gene are an efficient modern alternative for the screening of germplasm for the distribution of that gene, compared with the traditional cumbersome allelism tests (MacHardy 1996). For example, evaluation of selected germplasm with the OPB12SCAR marker for *Vm* showed that it was present in two other species only, apart from *M. micromalus* and *M.* x *atrosanguinea* which are the two primary allelic sources for this gene (Dayton and Williams 1970), out of 28 species tested (Cheng et al. 1998). However, such marker data needs to be interpreted with caution, as presence of a marker does not necessarily mean that the gene is present, or *vice versa*, since even if the marker and gene are in linkage disequilibrium, rare recombination events can uncouple the association between the particular alleles of the marker and gene of interest. Furthermore, as resistance loci in plants are frequently located in clusters (Michelmore and Meyers 1998), a marker may well be expected to be linked to more than one gene. This was demonstrated with the OPL19SCAR marker, which could not distinguish the *Vh2* and *Vh8* genes for scab resistance (Bus et al. 2005a). As microsatellite markers are very polymorphic, there is more opportunity for a specific allele to be linked to a resistance gene than for SCAR markers. While there are many germplasm sources with resistances allelic to *Vf* (Williams et al. 1966; Williams and Dayton 1968; Dayton and Williams 1970; Dayton et al. 1970), only *M. micromalus*, *M. prunifolia* 19651, and M.A.16 have been suggested to carry this gene, because all three amplify the same allele for the two very closely linked CHVf-1 and CHVf-2 microsatellite markers (Vinatzer et al. 2004). In the same study, it was shown that the F_2 selections 26829-2-2 and 26830-2 are not descendants of the original cross between *M. floribunda* and Rome Beauty (Hough et al. 1953), because a CHVf-1 allele of 137 bp amplified from both accessions was not present in either of the parents of this original cross.

1.5.2 Marker-Assisted Selection

Genetic markers enable the selection of combinations of both specific genes and QTLs, which cannot be identified through phenotypic selection, as epistatic effects are usually involved. Using the example of resistance breeding, in the past such combinations would have been eroded during the breeding process and eventually led to a loss of the quantitative resistances, which in turn put the major gene resistances under pressure. This sometimes had disastrous consequences, as was shown with the 'Vertifolia effect' of *P. infestans* on potato (*Solanum tuberosum*) in spite of this cultivar carrying two major genes for late blight resistance (Vanderplank 1984). Hence, the monetary value of genetic markers to a breeding program goes

well beyond the replacement cost of traditional phenotypic selection techniques (Luby and Shaw 2001) and should include an estimation of their contribution to realizing the potential value of durable resistances in food production. The cost of marker assisted selection (MAS) can be reduced in cases where an initial phenotypic selection can be performed prior to MAS. An example can be seen in a glasshouse screen for scab resistance in a family segregating for genes conditioning distinctly different resistance reactions, e.g. Liberty (*Vf* conditioning chlorotic resistance reactions) × TSR33T239 (*Vh4* conditioning HR resistance reactions epistatic to *Vf*). Since we know now from marker analysis that one of these resistance reactions is epistatic to the other, the number of marker analyses could be halved by discarding the susceptible seedlings and the seedlings showing chlorosis (Bus et al. 2000). Further cost efficiency of MAS will be achieved through the development of (semi-) automated DNA extraction and marker analysis systems. Robotic systems and the use of marker multiplexes (Cook and Gardiner 2004; Frey et al. 2004) will reduce the costs of both labour and consumables.

The success of MAS with one marker is determined mostly by the linkage distance of the marker to the gene of interest. If one assumes a recombination rate of 5% between a marker and a gene, a rate not met in many breeding families (Bus et al. 2000), MAS would result in 14.3% of the selected seedlings not carrying the desired combination of resistances in the case of three pyramided genes, and 26.5% in the case of six genes. A recombination rate of 1% for each marker would see the levels of inaccurate selection drop to 3.0% and 5.9%, respectively. However, when using flanking markers, the distance of the markers becomes much less of an issue, as the rate of inaccurate selection declines to less than 1%, even if the recombination rate is 5% for both markers for each of the three genes, and selection is carried out for one or both markers for each gene. With recombination rates of 1%, inaccurate selection becomes negligible. One issue for some types of markers, such as SCARs, is that their transportability may be limited, hence it is prudent to check linkages in a new parent prior to developing progenies intended for MAS. Once reliable markers have been developed, a strategy can be developed to efficiently pyramid the resistance genes from a number of breeding parents (Servin et al. 2004). One can also utilise more transportable markers from previously published apple maps if the position of the gene of interest in the genome (or failing that the SCAR marker) has been confirmed. On the other hand these transportable markers (mainly microsatellites and RFLPs) are more labour intensive than SCARs.

The application of MAS has been shown to be successful in several examples involving epistatic interactions between resistance genes (Bus et al. 2000, 2002). In the case of the A163-42 × TSR34T15 family, where *Vf* (conditioning chlorotic resistance reactions) and *Vh2* (conditioning stellate necrotic resistance reactions) were combined, a number of seedlings showed an unexpected hypersensitive response. Marker analysis with the OPL19SCAR and AL07SCAR revealed that in 91% of the cases these pin-point lesions were the result of a synergistic effect between the two genes (Bus et al. 2002) (Table 14). A similar effect was shown for *Vf* with relatively more seedlings showing no or Class 2 symptoms (*sensu* Chevalier et al. 1991) when carrying the gene in homozygous state, than those carrying it in heterozygous state (Tartarini et al. 2000).

Genetic markers are also an important tool in understanding the segregation of traits involving segregation distortions. For example, the naming of the *Er-3* gene for woolly apple aphid resistance consistent with a single gene was based on the R:S = 1:1 segregation in one M.9 × Aotea family (Bus et al. 2000). When a progeny of this family was crossed with Royal Gala, only 17% percent of the seedlings were resistant, which suggested that the single gene model was not correct. However, MAS with the OPO05SCAR developed for *Er-3* confirmed that there had been a segregation distortion (Table 15) as the marker was also not segregating 1:1 as expected. In contrast, the consistent discrepancies between the AT20SCAR marker segregating 1:1, while the phenotypes for the *Pl-1* powdery mildew resistance gene do not (Dunemann et al. 2004), is consistent with the two-gene hypothesis proposed for this resistance (Alston 1977).

1.5.3 Marker-Aided Introgression

The term MAS usually refers to the introgression of a gene, or a limited number of genes, e.g. in the case of pyramiding resistance genes. A step up from MAS is the use of genetic markers to select for a wide range of traits within one breeding cycle, or for "whole genome selection" (Pradhan et al. 2003), i.e. the selection of the genome resembling one of the parents. For example, the number of progeny of a backcross between a crabapple selected for a specific resistance gene and

Table 14. The segregation data for the *Vf* and *Vh2* apple scab resistance genes and AL07SCAR and OPL19SCAR markers in an A163-42 × TSR34T15 family. Adapted from Bus et al. (2002)

Marker		Phenotype[z]					Dead	Total
L19	AL07	HR	SN	3A	3B	S		
+	+	20	162	13	45	29	7	276
+	–	1	156	0	5	60	37	259
–	+	1	11	3	48	15	4	82
–	–	0	3	1	1	37	22	64
		22	332	17	99	141	70	681

[z] *HR* = hypersensitive response, *SN* = stellate necrosis, *3A* and *3B* = chlorosis with sporulation, *S* = susceptible (scale adapted from (Chevalier et al. 1991))

Table 15. The segregation data for the *Er3* woolly apple aphid resistance gene and PO05SCAR marker in a Royal Gala × S26-E290 family. Adapted from Bus et al. (2000)

O05	Woolly apple aphid phenotype						Segregation[z]		Total
Marker	0	1	2	3	4	5	R	S	
+	18	2	0	0	0	1	18	3	21
–	2	0	0	0	13	84	2	97	99
	20	2	0	0	13	85	20	100	120

[z] *R* = immune (Class 0); *S* = susceptible (Classes 1–5)

a high quality apple cultivar can not only be reduced to the resistant progeny, but to the number of seedlings carrying genomes most resembling that of the quality grandparent(s) as well as the desired resistance gene. In a crop with a long juvenile period, such as apple, considerable cost savings in the breeding program may be achieved by not having to grow seedlings until fruiting and to perform fruit evaluations on them. Obviously, the larger the number of markers used in the selection, the more effective this approach will be. However, the optimum number will be determined by balancing the extent of selection achieved with the cost of achieving it. As with MAS, the economics of this technology is determined for a large part of the market value of the character(s) of interest (Moreau et al. 2000).

At HortResearch, the whole genome selection approach is being investigated in combination with a technique of reducing the juvenile period in order to develop "fast breeding". It has been shown that the juvenile period in apple can be reduced from on average five years from seed germination, to about one to one and a half year by growing the seedlings continuously in the glasshouse (Zimmerman 1971; Aldwinckle 1975a). The aim of the HortResearch fast breeding program is to reduce the breeding cycle from cross to cross from the current six years on average, to two years. In initial studies involving growing the seedlings under optimal conditions in a phytotron, inducing flower bud formation, providing sufficient chilling, and forcing the seedlings to flower, have to date shown a success rate of only 15% within 10 months from germination (Bus et al. 2001) compared with over 68% for glasshouse grown seedlings (Aldwinckle 1975a). Further research is being carried out to increase the efficiency of this breeding strategy.

1.6 Map-Based Cloning

In the 1990s several disease resistance genes (Martin et al. 1993; Bent et al. 1994; Jones et al. 1994; Lawrence et al. 1995) and a few pest resistance genes (Milligan et al. 1998; Rossi et al. 1998) were cloned from tomato, tobacco, *Arabidopsis* and flax. Because of their dominant nature and major effect, resistance genes have become the most common plant genes to be targeted by map-based cloning techniques in recent years and

apple is no exception to this rule. In fact, all known apple map-based cloning projects embarked upon to date have been for disease or pest resistance genes. By the late 1990s, as detailed in Sect. 1.3, several partial maps had been constructed around disease resistance genes in apple with the most detailed maps being around the *Vf* locus conferring resistance to apple scab (Sect. 1.3.2.1 and Table 4). Map-based cloning projects to isolate the *Vf* gene were initiated in Europe by a Swiss/Italian consortium and later in the USA by the University of Illinois (see references below).

1.6.1 *Vf*

Considerable progress has been made in the last few years in identifying the *Vf* gene, the first apple disease resistance gene to be cloned. Apple BAC libraries have been developed from at least four different sources containing *Vf*. Vinatzer et al.(1998) developed the first BAC library of almost 37,000 clones from Florina, a cultivar containing the *Vf* locus. It has an average insert size of 120 kb and is expected to cover about five haploid genomes. Xu et al. (2001b) developed a 31,000 clone library from *Malus floribunda* clone 821, the original source of the *Vf* locus. This library has an average insert size of 125 kb and is also expected to cover about five haploid genomes. Subsequently Xu and Korban (2002a) constructed a 35,000 clone BAC library with an average insert size of 110 kb from the *Vf* containing cultivar GoldRush. This again represents approximately five haploid genome equivalents. The present authors have developed a 56,000 clone BAC library and a 168,000 clone cosmid library from an apple breeding parent containing both the *Vf* apple scab resistance locus and the powdery mildew resistance locus *Pl-2* (Rikkerink et al. unpublished). These two libraries are each expected to cover nearly seven haploid genome equivalents.

In reality, the map-based cloning projects that utilise these BAC resources have relied on a mixture of pure map-based techniques and candidate gene approaches made possible by information provided by other plant systems. Patocchi et al. (1999b) identified a 550 kb minimal BAC tiling path containing the *Vf* locus, based on detailed analysis of markers around the gene and new markers generated from the BAC clones in the region. A similar but somewhat smaller contig of 290 kb around the gene was later developed by Xu and Korban (2002b), assisted by previous saturation mapping with AFLPs (Xu et al. 2000, 2001a). Vinatzer et al. (2001) continued the work of Vinatzer et al. (1998) and Patocchi et al. (1999a), using BAC inserts to probe a large cDNA library combined with partial cDNA sequencing to identify three putative genes in the *Vf* region that showed homology to the cloned *Cladosporium fulvum* (*Cf*) resistance genes from tomato. These cDNA clones were then used to identify the portions of the BACs that needed to be sequenced to derive the sequence of the entire open reading frame corresponding to each of these cDNAs. They also derived partial sequence data from a fourth candidate. These candidates were named *HcrVf1 to HcrVf4*. Xu and Korban (2002b) used a slightly different approach, based on screening BAC subclones with labelled total cDNA. The BAC subclones containing transcribed regions were then partially sequenced and a full gene sequence was obtained by a combination of RACE (rapid amplification of complementary DNA ends) and further sequencing of clones containing resistance-like sequences. The first candidates identified were also used to develop additional PCR-based screens for further (similar) genes. This yielded an almost identical set of four candidate genes to that of Vinatzer et al. (2001) near *Vf* that they labelled *Vfa1* to *Vfa4*. Based on available sequence data *Vfa1* = *HcrVf1*, *Vfa2* = *HcrVf2* and *Vfa4* = *HcrVf3*. It is reasonable to assume, in the absence of full sequence data, that *Vfa3* may be the same as *HcrVf4*, although the relative location of these four genes does not quite agree between the two contigs with *Vfa4* (i.e. *HcrVf3*) being the furthest clone from *Vfa1* (i.e. *HcrVf1*) in one contig, but *HcrVf4* (i.e. presumably *Vfa3*) being the furthest in the other contig. This could also be explained if there are in fact five candidates, and *Vfa3* is not the same as *HcrVf4*. Recently Belfanti et al. (2004) expressed one of their candidate genes (*HcrVf2*) in a susceptible apple (Gala) under a 35S promoter and demonstrated that this construct confers resistance against apple scab. This result may indicate that this candidate is the *Vf* gene. However, the interpretation of this result could be complicated by the substitution of the native promoter with the 35S promoter that (presumably) drives higher expression. The same group reported recently (Silfverberg-Dilworth et al. 2005) that the resistance in these *HcrVf2* transformants could be overcome by a scab race that specifically overcomes the *Vf* resistance. This result strengthens the conclusion that *HcrVf2* is actually the *Vf* gene. A contribution to *Vf* resistance by another gene in the cluster cannot be formally ruled out without similar transformation

data for all the candidates and indeed there is now transgenic evidence to suggest that in fact two of the candidates at the *Vf* locus can each (independently) confer a degree of resistance to scab (Malnoy et al. 2006).

1.6.2 *Sd-1*

Cevik and King (2002a) developed a high-resolution map around the *Sd-1* aphid *Dysaphis devecta* resistance locus. Subsequently, Cevik and King (2002b) used the abovementioned 'Florina' library to develop a BAC contig around the *Sd-1* locus. They identified several putative NBS-LRR resistance-like gene sequences within a BAC in this contig (Genbank AM167520), suggesting the presence of a cluster of these genes. This library is however not expected to contain the gene encoding this resistance as the *Sd-1* resistance gene is derived from Cox's Orange Pippin, which does not feature in the ancestry of Florina. Cevik and King (2002b) also confirmed that Florina is susceptible to the aphid. More work using libraries from an aphid resistant host will therefore be required to identify the gene(s) responsible for conferring the resistance present at this locus.

1.7 Advanced Work

1.7.1 Tools Developed: Transformation, ESTs, Microarrays and Functional Genomics

1.7.1.1 Transformation

Agrobacterium-based apple transformation was first demonstrated by James et al. (1989) using a disarmed Ti-binary vector and has since been demonstrated in a number of laboratories around the world using several different apple cultivars (Sriskandarajah et al. 1994; Yao et al. 1995; Puite and Schaart 1998). These transformation events can be stably maintained (James et al. 1995) in the plant. Initially transformation was used to introduce various heterologous (trans)genes largely aimed at providing pathogen or pest protection, including the attacin family (Norelli et al. 1994) and T4 lysozyme (Ko et al. 2002) lytic proteins, chitinases (Bolar et al. 2000, 2001) and avidin or streptavidin (Markwick et al. 2003). More recently, it has been used to deliver endogenous genes in order to identify their function or effect on pathogen resistance. These studies have included *Vf* gene candidate *HcrVf2* (Belfanti et al. 2004) mentioned above, introducing an apple homologue of the *Arabidopsis* regulatory gene *NPR1* as well as apple proteins that are known to interact with the *E. amylovora* secreted type III effector protein DspE (Aldwinckle et al. 2003). Another strategy for functional analysis attempts to turn off the genes in order to either identify function (Dandekar et al. 2004), or modify the plant's development to create a novel phenotype or mimic a useful phenotype such as dwarfing (Bulley et al. 2005).

Methods for gene knock-down using RNA interference technology (Wesley et al. 2001) have also begun to be applied in apple (Gilissen et al. 2005). There is significant scope to extend this list to further genes, such as other disease resistance genes that are likely to be identified in the near future, as well as genes that may play a role in the various defense pathways that can now be identified in apple EST databases (see below). For example, we have also started investigating the function of members of several key protein families identified in the EST sequencing effort (Crowhurst et al. 2005, Newcomb et al. 2006).

Introducing genes by transformation is not only a useful analytical tool, but also is a way to circumvent the difficulty of introgressing useful single genes into new varieties by pseudo-backcrossing. This effectively becomes equivalent to the true backcrossing-based introgression that can be performed in selfing species to recreate existing varieties with new characteristics. The introgression of useful simply inherited characters such as disease resistance from wild germplasm into commercial varieties is a relatively standard breeding strategy for autogamous crops. In such crops, a variety that is very similar to the original but containing the introgressed character can be recreated by repeated backcrossing. Introgression is not as simple in non-autogamous crops like apple. The re-creation of a variety with a single introgressed gene (or more correctly a small region in linkage disequilibrium with this gene) in non-autogamous crops such as apple by traditional backcrossing is rendered impractical by the low success rate with traditional backcrossing, because the resultant apple progeny are either unviable or much less vigorous. The combination of various gene cloning/identification methods and gene transformation now make this possible in apple. Precautions are required, because of the possi-

bility of somaclonal variation in plant tissue culture (Courtial et al. 2001), but the essential character of the existing variety can be maintained and the new variety could be marketed essentially as a new sport of the variety, with an added advantage (e.g. a scab resistant Gala). In the case of apple, characterization of transformants has only been carried out in containment. It would be interesting to see whether most transformed apples perform true to type when they are grown under field conditions for extensive periods of time.

1.7.1.2 EST and Candidate Gene Sequencing

In the last five years a considerable effort has gone into developing more advanced genomics resources for apple, in addition to the large insert library resources discussed in Sect. 1.6. An extensive public domain EST resource now exists for apple. This is largely derived from cDNA based single pass sequencing carried out by HortResearch (Newcomb et al. 2006) and more recently, from a program initiated by a US consortium (Korban et al. 2005). Over 250,000 apple ESTs are in the public domain at the time of writing and these probably represent a substantial proportion of the expressed genes in apple, since they form well over 30,000 non-redundant clusters of sequence. A bioinformatic analysis of the combined sequencing efforts is still needed to get a more accurate measure of the number of non-redundant sequences that these ESTs represent. Since they are largely based on single pass sequencing from one end, it is likely a number will end up falling into the same non-redundant contig once complete cDNA sequence becomes available. There has also been considerable progress in identifying candidate genes of particular classes by PCR based approaches. We have recently used this approach to sequence parts of over 350 candidate disease resistance genes (Rikkerink et al. submitted manuscript) and build on the smaller datasets of these genes already available from apple (Lee et al. 2003, Baldi et al. 2004).

1.7.1.3 Microarrays

Microarrays are now becoming an important tool in the global characterization of gene expression in plants (e.g., Liu 2005). They consist of high density arrays on glass slides using either PCR-amplified cDNAs, or long oligonucleotides complementary to the transcribed part of genes. The expression of a large number of genes can be simultaneously assayed by hybridizing these slides with labeled RNA prepared from plants subjected to different treatments or from different tissue types, and looking for hybridization patterns that suggest a significant change in expression. HortResearch has also gone on to develop a 5,000 oligonucleotide pilot and a 16,000 oligonucleotide microarray from their EST data (Crowhurst et al. 2005). These arrays are based on oligonucleotides of approximately 50 bases in length with a Tm near 74 °C and have started yielding information on RNA expression profiles of the corresponding ESTs (Janssen and Schaffer, personal communication)

1.7.1.4 Functional Genomics

Some of the other tools required for any comprehensive functional analysis have also been developed in apple. These include RNA interference, expression in apple cell lines, the use of model plant species such as *Arabidopsis thaliana* and micro-organisms such as *E. coli* or yeasts to express apple genes in order to develop assays for their biochemical function. Expression of candidate genes in *Arabidopsis thaliana* has helped narrow down candidates for SNP marker development and subsequent genetic mapping.

1.7.2 Third-Generation Maps: Physical

Currently the only physical maps of apple that exist are around specific resistance genes that have been targets for map-based cloning (see Sect. 1.6). Other novel technologies such as radiation hybrid mapping have not been developed or applied to accelerate map development. Given that the apple genome is modest in size and that the price of whole genome sequencing efforts are decreasing, it is likely that genome sequencing (see below) will overtake such strategies and make them more or less obsolete.

1.8 Future Scope of Work

1.8.1 Association Mapping and Other Ways to Link Genotype to Phenotype

A major goal of research in the future will consist of developing faster and better methods to link genotype information to both desirable and undesirable phenotype information. Association mapping, which

utilises the phenomenon of linkage drag (disequilibrium) to identify candidate genome regions (and at its extreme, candidate genes) that show statistically significant associations between phenotypes and markers, is likely to be one of these methods. To improve the chance of identifying candidate genes for any given phenotype by whole genome scans will require methodology that can generate very dense genetic maps – since the region that stays in linkage disequilibrium (LD) with the phenotype is expected to rapidly decay because of recombination. Microarray technology in partnership with SNPs could potentially generate enough markers and the methodology to simultaneously assay a large number of SNPs in a single genotype. The recently developed EST databases can be used to identify many of these SNPs. In order to be able to effectively utilise this tool and to identify the most appropriate germplasm, we will also need to develop knowledge about the rate of LD decay in various apple populations and how uniform (or non-uniform) this decay is across the genome. Other methodologies such as targeting local lesions in genomes (TILLING) can now utilise EST sequence data and could potentially rapidly generate much greater variation in plant phenotypes than exists in the wild (Slade et al. 2005).

1.8.2 Structural Genomics and Whole Genome Sequencing

When compared with our understanding of the genome of the model plant species *Arabidopsis* and rice, it is clear that apple still lags a long way behind these plant genomes. Although a certain degree of lag with respect to these crops is inevitable, the range of resources identified above indicate that this gap could be closed significantly during the next decade. More detailed maps, or the construction of proper whole chromosome physical maps will be required before the apple community can realistically contemplate whole genome sequencing in apple. It is likely that the existing sequencing effort in the related genus *Prunus* will yield both an interesting start point for comparative analysis of these two important Rosaceae genera, and some actual leads to help construct physical maps in apple. As might be reasonably expected, there is already some indication of significant levels of synteny between apple and other Rosaceae genera (particularly with *Pyrus*, another member of the Maloideae) (see Sect. 1.2.4).

Even without a whole genome analysis, a pilot comparative study sequencing BACs around one or a few homologous loci in several members of the Rosaceae would be informative in terms of the level of microsynteny. This sequencing would probably also yield other useful information, such as the nature of transposable elements, the identity of transposon families present in several of the Rosaceae, and the gene density in these species. Information about transcribed transposons could also be deduced from the EST sequencing efforts. HortResearch has already identified ESTs in its database that appear to be interrupted by transposon-like sequences (E. Rikkerink unpublished), but a more comprehensive analysis might identify many more of the active transposons in apple. Comparative BAC sequencing might also begin to cast some additional light on the origin of the Maloideae as a subfamily within the Rosaceae (see also Sect. 1.1.1). While genome resources for apple have come a long way in the last decade, much remains to be done.

There is a commitment now within the Rosaceae research community to support the complete genome sequencing of peach as the first crop in the family. However, the world-wide economic importance of apple means that it should follow reasonably quickly as the next logical Rosaceae member to sequence in full. This would generate very significant amounts of comparative data in regions outside the immediate (transcribed) gene-space, which could play important regulatory roles. The haploid genome size of members of the Rosaceae is not unreasonably large (262–743 Mb, Table 16) when compared with the genome size of other plants like *Arabidopsis* (145 Mb), rice (420 Mb) and poplar (550 Mb). Moreover, even if little is known about the structure of the apple genome, its relatively small size compared with the complex and highly-repeated sequence-rich genomes of maize and pine, and the fact that it is probably an ancient polyploid suggests that it may have a true haploid size close to that of peach and be of relatively low complexity. The average physical/genetic ratio for apple (estimated at 0.51 Mb/cM using the most complete maps available) is lower than that observed in tomato (0.77 Mb/cM). Since positional cloning of QTL has been successfully carried out in tomato, this suggests that map-based chromosome walking is feasible in apple. Therefore, whole genome sequencing of many of the major Rosaceae genomes is well within the realms of possibility in the next decade. Indeed, the technical limitations that were encountered by pioneer whole

Table 16. Genome size and physical/genetic correlation of distance in several plant species

Species	Common name	Physical size (Mb/C)	Maximum genetic length (cM)	Ratio (Mb/cM)[1]	Status of genome sequencing
Arabidopsis thaliana	*Arabidopsis*	145	675	0.21	Completed
Populus deltoides	Poplar	550	2,300	0.24	Completed
Oryza sativa	Rice	420	1,490	0.28	Completed
Lycopersicum esculentum	Tomato	980	1,280	0.77	In progress[2]
Zea mays	Maize	2,300	1,860	1.24	In progress
Pinus pinaster	Maritime pine	25,700	1,850	13.89	Still impractical
Rubus ideaus	Raspberry	280	789	0.35	–
Prunus persica	Peach	262	712	0.37	Physical mapping in progress[3]
Fragaria spp	Strawberry	392	445	0.88	Limited BAC resources
Pyrus communis	Pear	496	949	0.52	–
Malus x *domestica*	Apple	743	1,454	0.51	BAC and EST resources[4]

[1] Average physical/genetic ratio based on most complete genetic maps
[2] Gene space sequencing
[3] Physical mapping partially completed and several thousand ESTs also exist
[4] see above for details

genome sequencing projects in the past (e.g. human and *Arabidopsis* genomes) can be easily circumvented now by the progress made in terms of sequencing methods and bioinformatics analysis, as well as the availability of large sequencing facilities. One striking example is the sequencing of the poplar genome in less than two years (Brunner et al. 2004).

The alternative (and complementary) way for accessing genomic information relies on the use of cDNA libraries instead of BAC genomic libraries. As detailed above, this approach has been extensively used in apple where over a quarter of a million of ESTs have been produced recently. However, as EST data can only partially compensate for complete genome data, whole genome sequencing is therefore still required. ESTs are proving to be a good source for the microsatellite markers and SNPs (Newcomb et al. 2006) that should help to generate more detailed maps. Even though most of the ESTs developed by HortResearch are derived from a single variety (Royal Gala) SNPs can still be identified at a reasonable frequency (presumably because of the highly heterozygous nature of apple). These more detailed maps can then act as a springboard for developing whole chromosome physical maps. Alternatively, this could be done by a random strategy relying on BAC fingerprinting to develop contigs, using new methods such as overlapping oligonucleotide (OVERGO) probes (Wesley et al. 2001) or a combination of these approaches.

If the cost of sequencing goes down significantly, then the option of assembly from deep sequencing using a whole genome shotgun (WGS) library approach, as opposed to a hierarchical shotgun sequencing approach, becomes feasible for apple. Another alternative strategy could be to concentrate initial sequencing efforts on transcribed regions of the genome. In some species such as tomato there is evidence of reasonably sharp demarcation lines between transcriptionally active and more silent portions of the genome, the euchromatic regions making up the former perhaps constituting less than one-quarter of the total genome (Van der Hoeven et al. 2002). Van der Hoeven et al. (2002) based these deductions on sequence data from a set of BACs biased by being selected because they contained transcribed genes.

There is anecdotal evidence for a higher gene density on BACs than might be expected by chance based on the number of times random BAC sequencing identifies ESTs in the HortResearch database (Rikkerink et al. unpublished). Sequence analysis of several complete BACs would indicate if concentrating on euchromatic regions is a viable strategy for sequencing the "more important" parts of the apple genome. Of course this strategy also suffers from the major drawback that it assumes the non-euchromatic regions are

less important. It is possible that this assumption is based largely on inability to make sense of the function of DNA in these regions. This is particularly pertinent to remember, now that there is increasing evidence that short transcribed RNAs in fact may sometimes contain very significant regulatory information and tie in with endogenous RNA interference-based methods of gene control. While there are many disadvantages to apple lagging behind the model crops in terms of genomics efforts, some of the advantages include learning important lessons from these model systems about paying closer attention to the less-well characterized parts of plant genomes. In these may well lie the secret to many of the interesting and useful properties of our own favorite plant system.

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