Chapter 10
Apple

Susan Brown

Abstract The cultivated apple, *Malus xdomestica* Borkh., is a interspecific hybrid complex of allopolyploid origin. The progenitor species is thought to be *M. sieversii* (Lodeb.) Roem., which hybridized with both European and Asian species throughout its domestication. Modern breeding continues to employ relatively few of the 25–30 species of *Malus* from throughout the northern hemisphere for both scion and rootstock development. The apple is the most produced temperate tree crop and is widely grown throughout the temperate zone and recently it has been expanding into subtropical and tropical zones. Major goals of scion breeding programs include fruit quality, disease resistance (scab, fire blight, powdery mildew), nutritional components and excellent postharvest traits to allow long storage and use as a fresh-cut product. Rootstock breeding efforts emphasize resistance to abiotic and biotic stress as well as plant vigor control. Much progress has been seen in the integration of biotechnology with the development of transformation systems, multiple maps, a large number of markers, extensive EST libraries and, most recently, with the whole genome sequencing of apple. Research has identified marker–traits associations for various disease resistance, plant architecture, postharvest, and flavor traits. International collaborative efforts are actively working to exploit the biotechnological approaches to understand the genetic basis of a range of commercially important traits to improve the efficiency of breeding programs.

Keywords *Malus x domestica* • Pome fruit • Pip fruit • Allopolyploid • Origin • Apple scab • *Venturia* • Powdery mildew • *Podosphaera* • Fire blight • Rootstock • Dwarfing • Allergenicity • Fresh-cut • Domestication • Post harvest • Antioxidants • Marker traits association • Incompatibility

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1 Introduction

1.1 Economic Importance

Apple production has increased by more than 50% over the last 20 years with the bulk of this growth in China (Table 10.1). Currently, China is the largest producer of apples, the USA second, followed by apple producers in the European Union (with Poland, Italy, and France being the largest producers). In the USA, apple production is valued at more than $2.5 billion dollars annually. The apple is the third most valuable fruit crop in the USA, following grapes and oranges. More than 60% of apple production is marketed as fresh fruit.

Apples are used for fresh consumption or processed into a number of different products such as apple sauce, apple slices, baby food, juice, cider, brandy, and distilled spirits. The markets for fresh-cut and organic apple are also increasing and expanding the availability of fresh apples in the fast food industry and for school lunch programs.

1.2 Taxonomy, Basic Botany and Description of the Crop

Luby (2003) provided an excellent review of the taxonomy of apple. Apple is an interspecific hybrid complex that usually is designated as *Malus* x*domestica* Borkh. or *Malus domestica* Borkh. Apple is a member of the subfamily Maloideae of the Rosaceae family. The haploid chromosome number is x = 17. Apple is an allopolyploid, but behaves like a diploid. Gametophytic self-incompatibility and inbreeding depression encourages outcrossing in nature and in breeding programs.

While diploids are frequent, triploids can occur spontaneously in crosses between diploids. Such triploids have larger leaves and fruit than their diploid relatives but are pollen sterile and cannot supply pollen for fertilization. Many popular cultivars ('Jonagold,' 'Mutsu') are triploids and prized for their quality and fruit size. Some breeders have tried to use triploids in breeding with mixed results (Sato et al. 2007).

There are 25–30 species of apple reported (Table 10.2). The four species native to North America formed distinct groups on the basis of simple sequence repeats (SSRs).

Table 10.1 Apple production (1,000 MT) in the world (data from http://faostat.fao.org)

	1986–1990	1996–2000	2005–2008
Americas	7,434	8,988	9,257
Asia	11,451	28,912	38,259
Europe	21,088	17,097	15,456
World	41,451	57,408	65,631

Table 10.2 Major species of *Malus* found in the northern hemisphere (Way et al. 1990)

Region	Common species found	
North America	*M. angustifolia*	*M. fusca*
	M. coronaria	*M. ioensis*
Europe	*M. florentina*	*M. sylvestris*
	M. pumila	
Asia Minor	*M. pumila*	*M. trilobata*
Himalaya	*M. sikkimensis*	
SW China	*M. prattii*	*M. yunnanesis*
SE China	*M. micromalus*	
Central China	*M. honanensis*	*M. hupehensis*
NW China	*M. kansuensis*	*M. sieversii*
N & NE China	*M. asiatica*	*M. prunifolia*
	M. baccata	
Taiwan	*M. doumen*	
Japan	*M. baccata*	*M. sieboldii*
	M. halliana	*M. tschonoskii*
	M. sargentii	
Korea	*M. asiatica*	*M. prunifolia*
	M. baccata	*M. sieboldii*
	M. micromalus	

Many *Malus* species have been used and continue to be used in breeding, with the increased recognition of the value of diversity and a means to study genes present in these relatives of cultivated apple.

1.3 Adaptation

Apples are grown in most temperate climates and they require a period of cold (temperatures below 45°F/7°C) to bloom and grow normally. For standard cultivars chill units of 500–1,000 are needed, while low chill cultivars require 400–600 h. Heat units are also needed.

Since several apple-producing areas require cultivars with low chilling hour requirements, research on low chilling has expanded (Labuschagne et al. 2001). Broad sense heritability values of 30% were calculated for total variation in number of buds sprouting and 62% for time of bud sprouting (Labuschagne et al. 2002, 2003). In areas of adequate winter chilling, cold hardiness is often a concern as is later blooming to avoid spring frosts. The issue of climate change, although controversial, is offering new challenges to apple producers worldwide, with more erratic climatic conditions, new pathogens and in some areas an increased frequency of hail. Heat tolerance and sunburn susceptibility are being investigated as major issues as is drought susceptibility.

2 Origin and Domestication of Scion Cultivars

2.1 Center of Origin

Although Malus species are found throughout the northern hemisphere, the center of origin of apple includes Asia Minor, the Caucasus, central Asia, Himalayan India and Pakistan and western China, areas where at least 25 native species of *Malus* occur. The Old Silk Road crossing from the Black Sea region to western China was important in the evolution of cultivated apple (Juniper et al. 1998; Zhou 1999; Luby et al. 2001) (Table 10.2).

Malus sieversii (Lodeb.) Roem. is thought by many to be the progenitor species of apple hybridizing with *M. prunifolia* Borkh., *M. baccata* Borkh., and *M. sieboldii* (eastern species) and with *M. turkmenorum* and *M. sylvestris* Mill. (western species). Selected cultivars from such random hybridizations were established and disseminated through grafting. There are reports of apples from 4,000 BC and later Roman authors documented apple culture. Apple cultivars being grown in Western Europe were cut off from their parental origins and evolved in relative isolation (Luby 2003).

However, research on chloroplast diversity raised interesting questions about the relationship of European wild apple *Malus sylvestris* and domesticated apple (Coart et al. 2006). A close relationship between the two was established by the existence of natural hybrids between the wild and cultivated forms and at the cytoplasmic level with the detection of eight shared chloroplast haplotypes.

North America became a "melting pot" not only for settlers but also for apple genetic diversity. Settlers brought apple seeds or grafts when they arrived, but many of these apples were not well adapted to the "new world." Settlers quickly established apple orchards for a source of apple cider, as the safety of drinking water was a concern. Settlers soon learned to propagate the seedlings best suited to the new climate. Meanwhile, individuals such as John Chapman 'Johnny Apple seed' disseminated apple seeds as new territories expanded to the west. Thousands of new cultivars were established and named (Luby 2003).

2.2 Domestication of Crop

Andrew Knight was the first documented apple breeder. The establishment of apple breeding programs worldwide often coincided with the establishment of research stations in growing regions that often partnered with universities. The history of apple is very rich and is detailed in both popular press and in the scientific literature.

Overviews of breeding and cultivar releases over time are found in an overview of the Brown and Maloney (2003), Laurens' (1999) review of breeding programs and objectives and in Knight et al. (2005) survey of breeding methodology and accomplishments.

3 Genetic Resources

The importance of conservation and characterization of germplasm is recognized worldwide (Büttner et al. 2004). Information from the large center of origin of apple increasingly is being published and studied. Zhou (1999) detailed apple genetic resources in China as Forsline et al. (2003) reviewed the collection, maintenance, characterization, and utilization of wild apples of central Asia.

3.1 Scion

The European Cooperative Program for Plant Genetic Resources has a Malus/Pyrus working group (http://www.wcpgr.cgiar.org/workgroups/malus_pyrus/malus-pyrus.html), and many of its members are in charge of their country's germplasm collection. The group represents a total of over 20,000 accessions in 13 countries. Büttner et al. (2004) evaluated the use of *Malus* germplasm in Germany, while Fischer and Dunemann (2000) used the collection to search for scab and mildew resistance. In Spain, Pereira-Lorenzo et al. (2008) evaluated local Spanish cultivars and used simple sequence repeat (SSR) markers for discrimination and to eliminate duplicates from the collection.

The US Department of Agriculture's Agricultural Research Service is responsible for the Clonal repository of Apples, Grapes and Tart Cherry in Geneva, NY. Researchers at this unit have been very active in the acquisition of materials from the center of origin (Central Asia) and in making material, especially *Malus sieversii*, available for study by researchers worldwide (Forsline et al. 2003). Volk and Richards (2008) detailed the availability of information on apple germplasm, including genotypic information, via the use of GRIN (Genetic Resources Information Network) database. In addition, for ex situ conservation of apple, a seed-based core collection for *Malus sieversii* has been established (Volk et al. 2005).

3.2 Rootstock

Rootstock germplasm includes many of the same *Malus* species used as sources of resistance in scion breeding. Some novel objectives include the use of apomictic species for seed production of clonal stocks, the selection for resistance/tolerance to specific environmental rigors of the root such as drought, water logging, salinity, nutrient deficiency, and other challenges.

3.3 Germplasm Diversity

Genetic diversity and population structure has been examined in *Malus sieversii*, a wild progenitor species of domesticated apple (Richards et al. 2008). Examination of

almost 950 individuals from 88 half-sib families from eight *M. sieversii* populations from Kazakhstan revealed that differentiation was mostly congruent with geographical location. Among the eight collection sites there were two narrow and two broadly distributed clusters, with the southwestern collection sites more admixed and more diverse than the northern sites.

Korban's (1986) review of interspecific hybridizations documented early studies in this area and researchers continue to try to exploit genes from apple's wild relatives for everything from genes for resistance to studying drought, winter hardiness (Luby et al. 1999) and nutrient uptake. Many researchers are concentrating on genetic studies of *Malus sieversii* as the probable progenitor species of apple (Coart et al. 2003, 2006). *Malus orientalis* Uglitzk. Ex Juz. from Turkey and southern Russia is also under investigation (Volk et al. 2008).

The inbreeding concerns expressed by Noiton and Alspach (1996) remained the same or may be worse. The common progenitors of the past ('Golden Delicious,' 'Delicious' 'McIntosh,' 'Cox's Orange Pippin,' 'Jonathan') have declined in use as parents, only to be replaced by cultivars only one generation removed. Most related species of apples have been used in resistance breeding, with *Malus floribunda* prevalent in scab resistant material.

3.4 Major Traits and Sources for Traits

Sources of some traits are listed briefly, with more detailed information in the section on breeding and markers.

3.4.1 Quality

Improving quality is an objective for breeders worldwide, yet defining quality, quantifying quality and its components and minimizing environmental influence is a huge challenge. Apple juiciness, firmness, crispness, and aroma are crucial to quality but are complex characteristics that are affected by many environmental factors, making their study and improvement difficult. Contrasting instrumental tests with sensory perception is important, but equally complex and expensive. Trained taste panels are best, however most breeding programs find this extra cost prohibitive and rely on staff within the research program. Yet as advances are made in quality, new techniques are being developed and used to dissect components of these traits and identify significant marker–trait associations.

3.4.2 Apple Scab (*Venturia inaequalis*)

In breeding for resistance to apple scab x, breeders mainly focused on the V_f gene from *Malus floribunda* 821. Recently other sources of resistance are being targeted

such as Russian seedling R1270-4A and *Malus sieversii*, especially for their prospects for pyramiding resistance and for understandings of race specificity especially susceptibility to races 2 and 4 of scab. The V_m from *Malus micromalus* Makino and *M. atrosanguinea* 804 (Spaeth) C. Schneider confers resistance to all but race 5 of scab. V_b from Hansen's baccata # 2 and V_{bj} from *Malus baccata jackii* Rehder have not been used extensively in cultivar development, but their resistance to other diseases is generating interest in their use. Numerous reports of additional sources of scab resistance provide many leads for future study (Gessler et al. 2006).

3.4.3 Powdery Mildew (*Podosphaera leucotricha*)

Sources of mildew resistance include Pl_1 from *Malus* × *robusta* Carr Rehd. and Pl_2 from *M. zumi* (Matsum.) Rehder (Knight and Alston 1968), *Plw* from White Angel' (Gallot et al. 1985; Batlle and Alston 1996), *Pld* from D12 a selection from open pollinated crabapple seed (Visser and Verhaegh 1976), and Pl_m, which has been eroded (Dayton 1977). Other sources of resistance have been identified (Fischer and Dunemann 2000; Schuster 2000), including quantitative resistance from U211 (Stankiewicz-Kosyl et al. 2005).

3.4.4 Fire Blight

Malus robusta 5 is the major source of resistance used in rootstock and scion breeding. Commercial cultivars with fairly good resistance include 'Delicious' and the scab resistant variety 'Liberty.'

3.4.5 Vitamin C and Antioxidants

Improving and documenting the nutritional components of apple cultivars is important yet also very complex. There are many publications on individual cultivars and several germplasm screens done in apple including Stushnoff et al. (2003) and Nybom et al. (2008a, b, c) that provide evidence of the wide range of variation for these compounds.

3.4.6 Red Pigmentation for Ornamentals and for Enhancing Antioxidants

Red pigmentation in apple flesh and foliage is derived primarily from *Malus pumila* var. *niedzwetzkyana* and its derivatives. Although a dominant gene for anthocyanin production was proposed, a deficiency of red plants is often noted. Highly pigmented cultivars, especially with red flesh, are of interest due to their ornamental

and nutraceutical properties, with researchers in New Zealand emphasizing this as one of their breeding goals. The genetics, genomics and complexity of apple skin and flesh color is detailed more extensively in the section on genomics.

4 Major Breeding Achievements

4.1 Scion

Reviews of breeding and program objectives and achievements include Laurens (1999), Knight et al. (2005), Brown and Maloney (2003, 2004) and Gardiner et al. (2007), but a literature review of apple breeding or a scan of recent cultivar releases or commercialization is evidence of the ultimate accomplishment: new and improved varieties and knowledge to be used in the improvement process.

In reviewing the literature, advances in our knowledge of quality and how to measure it, the factors affecting of flesh browning and the complexity and intrigue of enhancing total or specific antioxidants is evident.

Despite the challenges, disease resistance breeding has widened in scope and added to our knowledge of sources of resistance, their location in the genome and their interactions. Interest in insect resistance has experienced a revival and this research area will advance our ability to produce fruits suitable to the organic market.

Food safety has become important and apple allergens are one aspect of this topic. Apple allergens have been identified and their mode of action and type have been confirmed, the effect of processing on the different allergens has been studied, and the location of these allergens have been documented. This knowledge has largely been collected over the last 10 years and it promises to aid breeders in the choice of parents and breeding strategies in developing low allergen apples.

4.2 Rootstock

Reviews of apple rootstocks and their breeding include those by Webster and Wertheim (2003), Cummins and Aldwinckle (1983), and Ferree and Carlson (1987). Dwarfing, induction of precocity, disease resistance (*Phytophthora,* fire blight, scab), and climatic adaptation remain important goals. Breeding programs and releases include Ag-Canada in Quebec (Khanizadeh et al. 2000), Japan (Soejima et al. 2000), the ‘Supporter’ series of rootstocks from Pillnitz, Germany (Fischer 2001), and rootstocks developed in Poland (Jakubowski and Zagaja 2000).

Among the best-known rootstock breeding programs, the East Malling program in England has continued the tradition of releasing rootstocks with some recent releases that have been patented in the USA. Attributes of the joint Cornell/USDA apple breeding program are detailed in Robinson et al. (2003) and Fazio et al. (2006b).

5 Current Goals and Challenges of Breeding

One of the biggest challenges for breeding programs is to find funding to maintain active programs of sufficient capacity to develop and maintain large populations for varietal development. Funding to conduct sufficient phenotyping and genotyping to develop robust markers for marker-assisted breeding is also needed.

5.1 Disease Resistance Breeding

European researchers have taken a lead in the testing of organic apple production and all the complexities involved. Learning more about larger scale organic production will help in devising strategies to produce apple varieties with suitable resistance and fruit quality so that reduced sprays are a reality (Weibel and Haseli 2003).

5.2 Low Allergenicity Apples

Tremendous progress has occurred in advancing our knowledge of allergens in apple. Four major allergens in apple have been identified, researched and mapped: Mal d 1, Mal d 2, Mal d 3, and Mal d 4 (Gao et al. 2005a; 2005b; 2005c). Lipid transfer proteins (LTP) have been implicated in fruit allergies. When over 80 cultivars from two countries were evaluated for LTP there was about a 100-fold difference in LTP among cultivars (Sancho et al. 2008). Cultivars with low levels of Mal d 1, previously designated as low allergenic, did not always have low levels of LTP. LTPs need to be tested and confirmed using oral challenges before discussions of allergenicity can be made.

5.3 Processing and Fresh-Cut Markets

Apple cultivars suited to the fresh-cut market are needed; not just nonbrowning apples, but fruit that maintain firmness, are not prone to microbial growth and has no flavor change with time. The fresh-cut industry offers convenience to consumers,

but the producers have food safety concerns. Toivonen and Brummell (2008) reviewed the biochemical bases of appearance and texture changes in fresh-cut fruits and vegetables.

5.4 Rootstocks

Resistances to biotic and abiotic stresses continue to be a primary goal of apple rootstock breeding. Rootstock induced reduction of plant vigor not only is important culturally but also holds promise of elucidating scion-stock interactions. Replant disease remains a complex but real problem, with several new stocks performing well in old orchard sites with this disorder. Many traits related to plant propagation or orchard performance are objectives, but some can be negatively correlated. One example is that while breeders are selecting against tendency for burr knot production, reduction or elimination of this trait can make propagation and rooting of liners more difficult. The development of rootstock maps should allow marker-assisted breeding to become a reality in rootstocks.

6 Breeding Methods and Techniques

6.1 Major Traits and Selection Techniques

Future challenges associated with adaptation involves climate change, with the resulting introduction of diseases and insects associated with warmer climates, changing bloom times and the chance for frost with earlier blooming. The incidence of hail is also increasing; making the use of hail nets a prospect for regions that have never needed protected cultivation.

Research on quantifying bud break, prolonged dormancy and chilling requirement is critical to understanding and producing apple trees for low-chill regions. Progress in this area is evident (Labuschagne et al. 2001, 2002, 2003).

6.1.1 Disease Resistance

Disease resistance ideally encompasses more than one resistance, as a scab resistant apple will still be prone to powdery mildew and fire blight. However, as the number of resistances increases, so does the challenge of obtaining commercially acceptable fruit quality. With climate change, comes the introduction of new pathogens in regions formerly inhospitable to their spread. Partial resistances or field resistance to diseases are also being targeted to provide producers with a less intense spray strategy. However, several groups are still investigating how to screen for partial

resistance and what this will mean under commercial production. Fischer and Fischer (2008) provided an excellent overview of the challenges of trying to incorporate multiple resistance yet obtain quality. Despite these challenges there are many recent scab resistant apples in the industry that are well received by consumers.

6.1.2 Apple Scab (*Venturia inaequalis*)

An excellent review of apple scab and genes for resistance was published by Gessler et al. (2006). The most widely used gene V_f, from *Malus floribunda* 821 is the most likely to erode. Thus it is important to use other genes such as genes in the Russian Seedling R12740-7A (Hemmat et al. 2002; Bus et al. 2005a, b) and the V_m from *Malus micromalus* and *M. atrosanguinea* 804 which confers resistance to all but race 5 of scab. V_b from Hansen's baccata # 2 and V_{bj} from *Malus baccata jackii* have not been used extensively in cultivar development, but their resistance to other diseases is generating interest in their use. Recently Soufflet-Freslon et al. (2008) reported on a new gene and QTLs from 'Dulmener Rosenapfel' that confer resistance to scab.

6.1.3 Fire Blight

Sources of resistance include Robusta 5 and wild *Malus* species including *Malus sieversii* (Fazio et al. 2006a). Unfortunately many of the new popular cultivars are very susceptible. Breeding for resistance to fire blight is more challenging than for other pathogens as there is differential resistance which is hard to measure as the environment and the growth status of the plant can impact the screening procedures. Furthermore since different strains are being used by different researchers, the results are difficult to compare. There are both shoot and blossom infection in scions and scion infection can travel to the rootstock.

6.1.4 Powdery Mildew (*Podosphaera leucotricha*)

Pl_1 from *Malus*×*robusta* Carr Rehd. and Pl_2 from *M. zumi* (Knight and Alston 1968), *Plw* from White Angel' (Gallot et al. 1985; Batlle and Alston 1996), *Pld* from D12 a selection from open pollinated crabapple seed (Visser and Verhaegh 1976), and Pl_m (Dayton 1977) have been used in breeding. Other sources of resistance have been identified (Fischer and Dunemann 2000; Schuster 2000), including quantitative resistance from U211 (Stankiewicz-Kosyl et al. 2005). Bus (2006) used a partial diallel design used to study resistance in six apple progeny and found that parental performance was not a good indication of the performance of its progeny.

There is a concern about the potential erosion of major genes for resistance due to the breakdown of Pl_2 observed in France (Caffier and Parisi 2007; Caffier and Laurens 2005). Sources of resistance other than Pl-2 [*M. hupehensis* (Pampan.) Rehder,

M. mandshurica (Maxim) V. Komarov, *M. robusta* Rehder, *M. sargentii* Rehder, *M. sieboldii* (Regel) Rehder, D12, Mildew Immune Selection, and 'White Angel' remained resistant to the virulent population. The combination of Pl-2 with quantitative resistance genes resulted in a high level of resistance (Caffier and Parisi 2007).

6.1.5 Valsa Canker (*Valsa ceratosperma*)

An inoculation protocol developed by Abe et al. (2007) was effective in screening for resistance to Valsa canker and this research identified *M. sieboldii* as having a high level of resistance that was effective against several different isolates.

6.1.6 Alternaria

Resistant cultivars are homozygous for the recessive gene *alt alt*. Apple cultivars reported to have resistance include: 'Indo,' 'Red Gold,' 'Raritan,' 'Delicious,' 'Fuji,' 'Golden Delicious,' 'Ralls,' 'Toko,' 'Tsugaru,' 'Mutsu,' 'Jonagold,' and 'Jonathan' (Sawamura 1990). Another source of resistance is a Korean cultivar *M. bacatta* cv. Jeongsean (Heo et al. 2006).

6.1.7 National Variety and Rootstock Testing

In the USA, regional projects such as the NE183 "Multidisciplinary evaluation of apple varieties," similar to the NC 140 apple rootstock trials, have added to our knowledge of rootstocks, rootstock/scion interactions and scion varieties (Miller et al. 2005). The NE-183 trials also had separate plantings for insect and diseases studies. There have been studies on cultivar susceptibilities to some of the less studied diseases, such as *Colletotrichum acutatum* (Biggs and Miller 2001). The EUFRIN (European Fruit Research Institutes Network) has an extensive network of multisite tests to trial new scion cultivars and clones (Stehr 2009).

6.1.8 Insect Resistance

Aphids: Stoeckli et al. (2008) identified molecular markers associated with QTLs for resistance to the rosy apple aphid (*Dysaphis plantaginea* Passerini) and the leaf curling aphid (*Dysaphis* cf. devecta) and confirmed the presence of resistance alleles in cultivars like 'Wagener' and 'Cox's Orange Pippin' that have been reported to confer resistance to their progeny.

Miñaro and Depena (2008) evaluated tolerance of some scab-resistant apple cultivars to the rosy apple aphid (RAA), *Dysaphis plantaginea*, a major apple pest.

The use of tolerant cultivars would contribute to nonchemical crop protection. The susceptibility of nine scab-resistant apple cultivars to RAA was evaluated in greenhouse trials and field observations conducted over 2 years. Significant differences were observed among cultivars in aphid abundance and damage level 21 days after an infestation in the greenhouse. 'GoldRush' and 'Galarina' were considered tolerant, and 'Jonafree' and 'Redfree' were highly susceptible.

Woolly apple aphid (*Eriosoma lanigerum* Hausm.) resistance is an important breeding objective, especially in rootstocks. The *Er1* and *Er2* genes derived from 'Northern Spy' and 'Robusta 5,' respectively, are the two major sources used. The gene *Er3*, from 'Aotea 1' (an accession classified as *Malus sieboldii*), is a new major gene for WAA resistance. Genetic markers linked to the *Er1* and *Er3* genes were identified by screening RAPD markers across resistant and susceptible DNA bulks. The closest RAPD markers were converted into sequence-characterized amplified region (SCAR) markers and Er1 and Er3 were assigned to LG 08 of 'Discovery,' while the *Er2* gene was mapped on LG 17 of 'Robusta 5.' Markers for each gene were validated for their utility for marker-assisted selection in separate populations (Bus et al. 2007).

Germplasm screens for resistance to plum curculio, were not promising for genetic solutions to this problem (Meyers et al. 2007), yet tests for resistance to apple maggot (Meyers et al. 2008) have yielded some interesting sources of resistance to investigate further.

6.1.9 Plant Architecture

Groups of researchers are collaborating to advance research on plant architecture and modeling. This has resulted in more in-depth studies of branching and genotypic/phenotypic difference (Costes et al. 2006; Lauri et al. 2008; Kenis and Keulemans 2007; Segura et al. 2007). An emphasis has been placed on the geometry of plant architecture but also the topography. QTL analysis for complex architectural traits was conducted using progeny of 'Starkrimson' × 'Granny Smith' (Segura et al. 2007). This research across disciplines and germplasm promises to advance our understanding of plant architecture and its manipulation.

6.1.10 Columnar

Combining columnar habit with resistance to apple scab has been a goal of several programs and has resulted in the release of several scab resistant columnar apples in Romania (Braniste et al. 2008) and in Latvia (Ikase and Dumbras 2004). The release of nonresistant columnar releases has also accelerated.

6.1.11 Genetic Parameters

Genetic studies have also increased in apple. Tancred et al. (1995) studied the inheritance patterns and heritability of ripening date and suggested that determining the mean harvest of the two parents was a good way to predict offspring harvest date, while fruit shape was found to have a narrow sense heritability of 0.79 (Currie et al. 2000). The effects of a recurrent selection program was examined by Oraguzie et al. (2001), who also looked at the heritability of fruit quality in open-pollinated families and found that heritability estimates of harvest date and fruit weight were high (>0.70) but sensory traits were moderate; with russet 0.34–0.54 and firmness 0.26–0.59 (Alspach and Oraguzie 2002). Softening has been the subject of recent studies by Iwanami et al. (2005; 2008), who suggest that at least two harvest dates are needed in studying apple softness.

The presence of an open calyx in disease resistant apples is a large concern due to secondary pathogens entering the core early in fruit formation. The incidence of core rot may be low (1–3%), but is enough for fruit to be rejected due to contamination concerns.

Sensory and consumer testing have helped provide a better understanding of what consumers want (Harker et al. 2008). New instruments will aid our ability to quantify important components of apple quality. Collaborative research efforts on an international scale will also further progress.

6.1.12 Harvest Determination

A generic starch iodine chart is a simple way to assess the stage of maturity (Blanpied and Silsby 1992). Although not all cultivars have harvest stages that correspond to the iodine staining, it provides breeders with a quick and low-cost measure of relative stage of maturity or staining. Several harvest dates should be assessed to best judge the recommended harvest maturity and quality at those dates. As selections advance through trials more detailed measurements of maturity, involving ethylene production would be helpful.

Fruit softening and its challenges: Fruit softening is important to breeders, producers and consumers, but it seems the more we learn, the more questions we have and the more clues we obtain on this complex phenomenon. Knowledge of ACS (1-aminocyclopropane 1-carboxylate synthase gene) and ACO (1-aminocyclopropane 1-carboxylic acid oxidase gene) have added to our understanding of the importance of ethylene and the many steps involved in its production and perception, but more genes are being implicated as important components of softening. Transgenic studies, functional analyses and the testing of markers associated with ACS and ACO across a wider range of populations will enhance our understanding of genotypic differences.

1-methylcyclopropene (1-MCP), marketed as Smartfresh, blocks the effect of ethylene on apples. Its use is adding to our knowledge of cultivar variation in response to this treatment as well as the effect of ethylene on volatiles that contribute to perception of flavor.

6.1.13 Storage Disorders

Our ability to understand genetic susceptibility to storage disorders will be aided by advances in genomics and by the use of well-characterized populations. The use of parents susceptible to specific disorders, such as 'Honeycrisp' and its susceptibility to bitter pit, soft or ribbon scald and rots will add to our knowledge of the inheritance of such disorders. Resistance to superficial scald continues to be the focus of several groups, especially in relation to alpha-farnescene.

A better understanding of various rots and chilling disorders is also needed.

Fruit mineral nutrition: In the mineral nutrition of apples, the site is important for evaluation of some nutrients (Volz et al. 2006). It is best to select across sites and seasons to assess susceptibility to bitter pit. While fruit calcium might be a useful means of indirect selection for bitter pit susceptibility, this worked within, but not among, families (Volz et al. 2006). Korban and Swiader (1994) suggested that two dominant genes were responsible for resistance to bitter pit, but this finding needs additional confirmation.

Sensory testing and understanding consumer preferences and satisfaction: The importance of quality, sensory testing, and obtaining a better understanding of consumer perceptions and preferences has expanded greatly over the last few decades. Means of quantifying components of fruit quality, such as firmness (Harker et al. 1996), texture (Harker et al. 2002a), and sweetness and acidity (Harker et al. 2002b) have been researched extensively. Such studies have contrasted trained sensory panelists and objective measurements of Brix and acid. Titratable acidity was the best predictor of acidity and values need to differ by 0.08% titratable acidity to be perceived. For Brix, sensory panels were only able to detect a difference with a change of more than 1°Brix. Sensory panels were recommended for differentiation of sweetness and flavor. Harker et al. (2008) determined that increasing firmness usually was associated with increased preference although some people prefer soft apples. Higher Brix and acidity can improve preference for apples that are firm, but not if they are soft. Improved protocols for quality evaluations and sensory testing are needed, although great progress has been made in this area.

It is best to evaluate fruit softening after at least two harvest dates for obtaining a genotypic mean for softening (Iwanami et al. 2005). Iwanami et al. (2008) obtained narrow sense heritability for postharvest fruit softening. Softening rate as measured by parent–offspring regression was high ($h^2 = 0.93$), but as estimated by sib analysis it was only moderately high ($h^2 = 0.55$).

6.1.14 Physiological Studies Coupled with Genetic Investigations

Physiology is a focus as researchers examine some of the challenges in apple production. Fruit thinning, fruit set, and abscission are being evaluated. Apple germplasm with different rates of abscission varied for internal ethylene concentration by three orders of magnitude (Sun et al. 2009). A genomic study of shade-induced apple abscission revealed 66 unique genes involved and suggested that better methods of thinning might be a future outcome (Zhou et al. 2008).

6.1.15 Storage and Storage Disorders

Blazek et al. (2007) studied cultivars and selections over a 3-year period as to characteristics related to good storage and freedom from storage diseases. An ideotype was proposed and important characteristics identified. Thresholds were established for some of the parameters; higher skin toughness and thickness, low ethylene production, naturally high calcium content, high total phenolics and antioxidants, high flesh firmness, and high fruit acidity as expressed by pH. Inoculation with one of the bitter rot pathogens, *Pezicula alba* Guthrie was suggested as a final screening on selections with the ideal ideotype.

6.1.16 Health and Antioxidant Research

Reviews by Boyer and Liu (2004) and Biedrzycka and Amarowicz (2008) illustrated the importance and complexity of antioxidants in apple. Assessing apple germplasm collections for antioxidants were the focus of studies by Stushnoff et al. (2003) and Nybom et al. (2008a, b, c). The complexity of antioxidants in apple has generated controversy over which phenolics are most bioavailable or the best to target for improvement (Lee et al. 2003). Eberhardt et al. (2000) suggested that certain apple phenolics had antioxidants equivalent to 1,500 mg of vitamin C and were more important to target for improvement. Lata (2008) stressed the importance of site and season on efforts to quantifying antioxidants in apple. Khanizadeh et al. (2007) reported polyphenol composition and total antioxidant capacity of selected apple genotypes for processing. Davey and Keulemans (2004) and Davey et al. (2006) has concentrated on the importance of vitamin C, including QTL studies. Planchon et al. (2004) explained that some of the variability in vitamin C content was due to sampling.

6.2 Breeding methodology

Methodology in apple breeding has been reviewed in different chapters of Moore and Janick's "Methods in Fruit Breeding" (1983), in Janick et al. (1996) and breeding programs worldwide were reviewed in Brown and Maloney (2003). Unfortunately, very few of the studies each breeder routinely makes within their program are published and those that are published may be hard to find as they published in many different journals.

6.2.1 Rootstock Propagation

While propagation of seedlings onto rootstocks provides an estimation of performance on clonal stocks, the added cost and record-keeping has some of the largest apple breeding programs preferring to plant seedlings on their own roots and fast-tracking promising selections by doing rapid propagation of promising selections

after one or a few years of fruiting. This reduces the costs substantially. However programs that have partnered with nurseries that provide propagation have an advantage and lower costs. The Cornell program has found that optimizing seedling growth in the first several years enables us to have fruit in many progenies 4 years after planting without the added expense of rootstocks.

6.2.2 Parental Selection

In planning crosses, attention must be paid to the parents carrying recessive genes for pale green lethal, genetic dwarfs and also sublethals (Alston et al. 2000; Gao and van de Weg 2006), as these will greatly reduce the number of usable progeny obtained. Crosses of heterozygotes for these traits will reduce populations by 25% for each trait.

Although there are still gaps in our knowledge of S-alleles in apple, fully compatible or semi-compatible matches should be targeted when possible (Broothaerts 2003; Matsumoto et al. 2007; Nybom et al. 2008a). The use of markers, especially S-alleles, to verify proposed parentage, has resulted in the discovery of quite a few faulty pedigrees. Surprisingly, even the seed parent of crosses has been in error.

6.2.3 Pollen Collection, Emasculation, Pollination and Fruit Set

There are cultivars or selections that are very sensitive to emasculation, perhaps causing abscission from the wounding that occurs. Other selections/cultivars may have pistils with curled styles that are injured during the emasculation process. No more than two flowers per cluster are recommended for pollination as greater numbers usually result in some of them abscising. To avoid contamination between crosses, 70% alcohol should be used to kill pollen on any surfaces used in pollination such as fingers or brushes.

Screening methods have been established for many of the more problematic pathogens (scab, mildew, rusts, fire blight and for the rootstock pathogens such as Phytophthora species). Breeders must ensure that they know the races they are using in inoculations.

The ability to apply preselection to breeding populations has long been a goal, but screening for disease resistance has been the primary application. Slowly molecular markers are starting to be used, but while markers are being developed they still need to be tested for their validity and their robustness. Markers must be tested in different genetic backgrounds and the populations where they are used should be maintained to test for any juvenile/adult interactions.

6.2.4 Testing and Replication

Many programs have their own systems of replication number and testing strategies. A study on replication in the initial selection trials of clonally propagated crops suggests that any increase in trial area for initial selection is best used for increasing

the number of genotypes tested and growing just one plant per genotype (Aikman and Langton 1983). How many at each stage often is a function of funding. Some programs have research stations willing to act as test sites, providing valuable performance information to the breeder without substantial costs. Other programs rely on testing with cooperative growers, but this system is not without its risks of lost data. There are programs that offer advanced testing for a fee.

6.2.5 Record Keeping

There are almost as many record keeping systems, as there are breeding programs. Although a universal system would be highly desirable, each breeder has different priorities and systems of evaluation. While effective phenotyping is important in breeding and in genomic research, the reality of many breeding programs is that efficiency in breeding often requires few detailed records on individual seedlings, but more detailed records and phenotyping on promising individuals in that cross. Genetic studies can be detailed in records, but if each population was thoroughly characterized, breeders could not go through the large number of seedlings that need to be evaluated to discern the few desirable segregants.

6.2.6 Statistics

The use of unbalanced designs common to most fruit breeding programs was addressed by (Durel et al. 1998). Genetic parameters (narrow-sense heritabilities and genetic correlations) were estimated for major traits in apple using large unbalanced data sets, aided by the use of wide-pedigree information. The software REML VCE took into account the complex pedigrees of the apple-breeding populations, by combining the restricted maximum likelihood procedure with the construction of the entire relationship matrix between hybrids planted in the field and their ancestors. Narrow-sense heritability estimates ranged from 0.34 to 0.68 for traits exhibiting a normal distribution. Heritability values (~0.35–0.40) were obtained for fruit size, texture, flavor, juice content, attractiveness, and russeting. Higher values of heritability were obtained for vigor, as assessed by trunk circumference (0.51) and powdery mildew resistance (0.68). Additive genetic correlations between traits were estimated and showed a very high relationship between fruit-quality traits.

6.2.7 Ploidy Manipulation

A comparison among reciprocal diploid × triploid crosses suggested that 2x × 3x (but not 3x × 2x) can be used in apple breeding, with trunk circumference index (circumference relative to average circumference of diploid progeny of the same age) used as an early indicator of whether the seedlings will flower and bear fruits (Sato et al. 2007).

In any breeding program, the establishment of clear objectives per cross and and culling thresholds and/or agreement on limiting factors for discarding selections need to be made.

6.2.8 Propagation and Release of Varieties

With the advent of the 'Pink Lady' model of exclusive licensing and trademarking to ensure quality and control demand, the apple industry entered into the era of controlled management of new varieties. The following are some of the cultivars marketed under some type of exclusive or controlled management system, often with production royalties: 'Pink Lady,' 'Pacific Rose.' 'Jazz,' 'Delblush,' 'Ambrosia,' 'Sonya,' 'Cameo,' and 'SweeTango' (MN 1914). The scab resistant cultivars 'Ariane' and 'Juliet' are also controlled and trademarked.

Innovative partnerships among breeding program and nurseries have resulted in multisite testing, providing important information on genotype by environment interactions. One example is the company Novadi in France that partners with breeding programs and nurseries in the pursuit and testing of new cultivars (Laurens and Pitiot 2003).

7 Integration of New Biotechnologies

7.1 State of the Map(s)

Tremendous progress has been made in map construction since the first maps of apple ('White Angel' × 'Rome Beauty') were published (Hemmat et al. 1994). Currently maps of differing marker density are available for at least 50 scion cultivars: including 'Prima' × 'Fiesta' (Maliepaard et al. 1998; Liebhard et al. 2003b).

A linkage map of the columnar, reduced branching mutation, 'Wijcik McIntosh' was constructed along with maps of two scab resistant selections by Conner et al. (1998), followed by maps of 'Braeburn' × 'Telamon,' a columnar genotype (Kenis and Keulemans 2005), and 'Fiesta' × 'Totem' (a columnar genotype) (Fernandez-Fernandez et al. 2008).

Maps of 'Delicious' and 'Ralls Janet' were constructed from progeny of each parent crossed with 'Mitsubakaido' (*Malus sieboldii*) as the pollen parent for each. (Igarashi et al. 2008). N'Diaye et al. (2008) developed a consensus map using four different populations ('Discovery' × TN 10-8, 'Fiesta' × 'Discovery,' 'Discovery' × 'Prima,' and 'Durello di Forli' × 'Prima'). Additional mapping populations are being developed and used for fine scale mapping, synteny studies, and in attempts to locate and clone genes of interest. Populations include 'Royal Gala' × A689-24 in New Zealand, several for physical map construction and in Italy, eight populations are being used.

Maps have also been constructed for three rootstock cultivars/selections (Malling 9, Robusta 5, Ottawa 3) (Celton et al. 2009; Fazio, personal communication).

7.1.1 Marker Development

Evolution of marker use in apple mirrors that in many plants, starting with isozymes, then RAPDS (random amplified polymorphic markers), RFLPs (restriction fragment length polymorphic markers), AFLPs, SCARs (sequence characterized amplified region) and progressing to SSRs (simple sequence repeats) and SNPs (single nucleotide polymorphisms).

7.1.2 Simple Sequence Repeats

Over 300 simple sequence repeats (SSRs) have been developed and tested in apple (Liebhard et al. 2002; Silfverberg-Dilworth et al. 2006) and a set of recommended SSRS to be used in PCR multiplex was recently released (Patocchi et al. 2008).

7.1.3 Universal Primers in the Rosaceae

In an effort to develop more "universal" markers across the Rosaceae, Sargent et al. (2008) contrasted *Malus* cDNA sequences with homologous *Arabidopsis* sequences to identify putative intron–exon junctions and conserved flanking exon sequences. Primer pairs were designed from the conserved exon sequences flanking predicted intron–exon junctions. Eleven loci polymorphisms in 'Fiesta' × 'Totem' mapped to seven LGs. 38% of these genes were successfully mapped in *Fragaria* and *Prunus* revealing some patterns of synteny across genera. Similarly, Gasic et al. (2009) developed markers from apple ESTs that were then tested on 50 individual members of the Rosaceae, representing 3 genera and 14 species). They found that transferability ranged from 25% in apricot to 59% in the more closely related pear.

After analyzing over 350,000 EST sequences in apple, a set of 93 new markers was mapped in apple that coded for 210 single nucleotide polymorphisms (SNP) (Chagne et al. 2008). This demonstrates the potential for SNP discovery and utilization in apple. Several research groups in the USA and Italy are developing additional SNPs and pursuing pedigree based association studies (Oraguzie et al. 2007a).

7.2 Traits Marked with Molecular Markers

7.2.1 Scab Resistance Genes

Many groups have developed markers for V_f, (reviewed in Gardiner et al. 2007). Markers for V_r, V_x, V_{h2}, $V_{h4,}$ (Bus et al. 2005b; Hemmat et al. 2002), Vh_8 (Bus et al. 2005a),V_m from *Malus micromalus* and *M. atrosanguinea* 804 (Cheng et al. 1998; Patocchi et al. 2005), V_b (Erdin et al. 2006), V_{bj} from *Malus baccata* jackii (Gygax et al. 2004), V_a from 'Antonovka' (Hemmat et al. 2003) have also been developed.

Some clones of 'Antonovka' possess the V_a gene, but some clones only transmit polygenic inheritance (Quamme et al. 2003). QTLs for scab resistance were reported by Liebhard et al. (2003c), Calenge et al. (2004) and Schouten and Jacobsen (2008).

7.2.2 Powdery Mildew (*Podosphaera leucotricha*)

Markers exist for *Plw* from 'White Angel' (Evans and James 2003), *Pld* from an open pollinated crabapple selection (James et al. 2004), Pl_1 from *Malus robusta* (Markussen et al. 1995; Dunemann et al. 2007), Pl_2 from *Malus zumi* (Dunemann et al. 1999), and quantitative resistance from clone U211 (Stankiewicz-Kosyl et al. 2005). Field studies conducted over 4 years have identified some stable and unstable QTLs for mildew resistance (Calenge and Durel 2006).

7.2.3 Fire Blight

The marker CHO3E03 was useful for resistance from *Malus robusta* 5 (Peil et al. 2007a) but it needs to be tested on populations with other resistance donors to assess its utility. Peil et al. (2007b) established strong evidence for this fire blight resistance gene from *Malus robusta* location on linkage group 3. A major QTL for resistance was identified on LG 7 of 'Fiesta' in two progenies and four minor QTLs were also found on LG2 3, 12 and 13 (Calenge et al. 2005). Several significant digenic interactions were also identified, suggesting putative epistatic QTLs. Two distinct major QTL for fire blight were found to colocalize on linkage group 12 in apple genotypes 'Evereste' and *Malus floribunda* clone 821, carrying distinct QTL alleles at that genomic position (Durel et al. 2009).

7.2.4 Alternaria

Markers linked to Alternaria blotch resistance (Soejima et al. 2000) and susceptibility (Heo et al. 2006) have been reported. More testing of these markers is required.

7.2.5 Columnar

Columnar or reduced branching apples provided breeders with a means to study the genetics of plant form. The columnar trait is dominant, but there is usually a deficiency of columnar types in the progenies studied. Hemmat et al. (1997) found a DNA marker for columnar growth habit that contained a simple sequence repeat. Conner et al. (1997) developed a genetic linkage map for the source of columnar, 'Wijcik McIntosh,' and conducted a QTL study on its effect (Conner et al. 1998).

A population of standard 'Fuji' by the columnar genotype 'Tuscan' was used to identify RAPD markers linked to the columnar gene. From the closest RAPD marker, a SCAR (sequence characterized amplified region) marker was developed. This marker produces a 670 bp product in columnar material that is absent in non-columnar plants (Kim et al. 2003). Next, a population of 'Spur Fuji' × 'Telamon' allowed Tian et al. (2005) to map the *Co* gene between the SSR markers CH03d 11 and COL on linkage group 10. The region around the *Co* gene was constructed using nine new markers and three markers developed earlier. Inter-simple sequence repeat (ISSR) markers were used by Zhu et al. (2007) in an effort to find markers closer to the Co gene, but although additional markers were mapped the closest was 10 cM away.

QTL studies have also targeted some populations with one columnar parent in an effort to learn more about the effect of the Co gene on branching and other components of plant architecture (Kenis and Keulemans 2007; 2008).

Increasingly, derivatives from 'Wijcik McIntosh,' such as 'Telamon' and others are being used in genetic studies of architecture and branching. There is a great degree of variation in columnar form in different clones heterozygous for the columnar gene.

7.2.6 Dwarfing Genes

Pilcher et al. (2008) used Celton et al.'s (2009) mapping population of 'Malling 9' × 'Robusta 5' to map markers associated with dwarfing genes. Markers need to be tested on other rootstock populations and also on scion material to see how the markers perform in populations with different genetic backgrounds.

7.2.7 S-Incompatibility

This area of marker research is readily applicable and has aided breeders in their design of crosses and has also indicated where parentage is not as documented. While more *S* alleles need to be resolved due to high homology with existing *S*-alleles, the progress in this area has been excellent (Broothaerts 2003; Matsumoto et al. 2007). When Nybom et al. (2008a) evaluated a collection of cultivars in Sweden they found that five alleles, *S1–S3*, *S5*, and *S7*, had frequencies ranging from 11 to 18%, whereas the remaining 9 alleles were below 6%. Additional studies have revealed the need for studies on S alleles outside of *Malus domestica*.

7.2.8 Softening (ACS, ACO, and Ethylene)

Research on ACS (1-aminocyclopropane-1carboxylate synthase gene) has evolved rapidly. In 2000, Harada et al. identified an allele associated with low ethylene production in apple cultivars. Later, an allelotype of a ripening-specific

1-aminocyclopropane-1-carboxylate synthase gene was found to define the rate of fruit drop in apple (Sato et al. 2004). Oraguzie et al. (2007b) studied the influence of Md-ACS1 allelotype and harvest season within an apple germplasm collection on fruit softening during cold air storage, finding that Md-ACS1-2/2 allelotypes had a slower rate of softening than the other genotypes. In another study, the amount of MdACO transcripts in seeds was found to be a good indicator of abscission following benzylaminopurine application (Dal Cin et al. 2007).

Genotyping of Md-ACS1 and Md-ACO1 for parents and their suitability for marker-assisted selection was assessed by Zhu and Barritt (2008) who found only 8 of 95 cultivars homozygous for ACS-2 or AC0-1. Such homozygotes had firmer flesh at harvest and after 1 month storage at 0°C. The eight homozygotes included four breeding selections and 'Delblush,' 'Fuji,' 'Pacific Beauty,' and 'Sabina.' Later, characterization of cultivar differences in alcohol acyltransferase and 1-aminocyclopropane-1carboxylate synthase gene expression and volatile compound emission during apple fruit maturation and ripening was evaluated (Zhu et al. 2008a).

Nybom et al. (2008b) determined that modern apple breeding is associated with a significant change in the allelic ratio of ethylene production gene Md-ACS1 with a shift towards the allele associated with less ethylene production.

7.2.9 Flavor (Volatiles)

Improvement of apple flavor by breeding or biotechnology is a complex problem and has many challenges (Brown 2008). Research on QTL mapping of aroma compounds (Dunemann et al. 2009) is an important first step, as is the discovery via genomics that showed that aroma production in apple is controlled by ethylene primarily at the final step in each biosynthetic pathway (Schaffer et al. 2007). Rowan et al. (2009) also examined volatiles in a 'Royal Gala' × 'Granny Smith' cross and used principal component analysis to discriminate progeny as to level and type of esters. Dunemann et al. (2011) found that functional diversity of the alcohol acyl-transferase gene (MdAAT1) was associated with fruit ester volatile content in apple cultivars.

7.3 MAS (Seedling and Parental Selection)

A challenge to developing effective molecular markers for marker-assisted breeding is accurate phenotyping and the funding to conduct such phenotyping on sufficient individuals and populations. While many molecular markers have been developed and identified, few programs use them extensively due to cost, lack of funds, or inadequate knowledge about the robustness of these markers. Apple breeders need to have markers that are easy to use and inexpensive. There is a clear need for better, high throughput DNA extractions and multiplexing would be an advantage (Patocchi et al. 2008).

Breeders must assess if the use of markers is both effective in breeding and cost effective (Luby and Shaw 2001). Pyramiding several genes for resistance is one example where markers would be very useful and the objective would very difficult to achieve readily without markers. The ability to differentiate sports by use of markers and to identify the mechanisms responsible for their activation would also be invaluable to nurseries interested in intellectual property right protection and to breeders in assessing how to manipulate key traits.

QTL studies in disease resistance have been highlighted in the section on disease resistance, but QTL studies in other areas have also progressed. Conner et al. (1998) study of QTL of tree growth and development has been followed by QTL studies of fruit texture and firmness (King et al. 2000; 2001), physiological attributes (Liebhard et al. 2003a), QTLS for plant form and fruit quality (Kenis and Keulemans 2007; 2008), aphid resistance (Stoeckli et al. 2008), vitamin C (Davey et al. 2006), and plant architecture (Segura et al. 2007).

Association mapping and linkage disequilibrium are both challenges and advances in our approach to understanding the apple genome (reviewed in Oraguzie et al. 2007a). Pedigree assisted breeding is starting to be utilized across several programs which will help understand its use in breeding programs and genetic studies (van de Weg et al. 2004).

7.4 Genomics

Gardiner et al. (2007) reviewed genomics research in apple. The database availability of expressed sequences has accelerated genomic (Newcomb et al. 2006; Park et al. 2006; Wisniewski et al. 2008), microarray (Lee et al. 2007; Pichler et al. 2007), and functional genomics studies (Janssen et al. 2008) in apple.

Over 150,000 expressed sequence tags in apple collected from 43 different cDNA libraries, representing 34 different tissues and treatments, were analyzed by Newcomb et al. (2006). Clustering of these sequences resulted in a set of 42,938 nonredundant sequences (17,460 tentative contigs and 25,478 singletons), representing about one-half the expressed genes from apple. Park et al. (2006) used a more targeted approach to large-scale statistical analysis of expressed sequence tags by targeting biochemical pathways for precursors to volatile ester production to identify genes with potential roles in apple fruit development and biochemistry.

Lee et al. (2007) used a microarray from young and mature fruits of 'Fuji' and determined that many of the genes involved in early fruit development were also active in other organs. When global gene expression analysis of apple fruit development from the floral bud to ripe fruit (eight time points) was examined using a 13,000 gene microarray and compared with a microarray on tomato, 16 genes were identified in both apple and tomato that may have important roles in ripening (Janssen et al. 2008).

Gleave et al. (2008) examined over 120,000 ESTs to find 10 sequences that could be classified into seven plant miRNAs (microribonucleic acids). These small, non-coding RNAs play important regulatory roles.

Wisniewski et al. (2008) studied the response of 'Royal Gala' apple to low temperature and water deficit using expressed sequence tag analysis. This study provided more detailed information based on different source tissue (bark versus xylem, leaf versus root) and two different stresses, one short term (24 h cold) and one chronic (2 weeks of drought).

The use of cDNA suppression subtractive hybridization analysis revealed rapid transcriptional response of apple to fire blight disease (Norelli et al. 2009).

The proteomic analysis of the major soluble components in 'Annurca' apple flesh by Guarino et al. (2007) is the first of many such analyses. Increasingly, metabolomic research is being conducted, including examination of metabolomic changes that precede the development of apple superficial scald (Rudell et al. 2009).

7.4.1 Color as an Example of Progress in Genetics and Genomics

While fruit color was always discussed as a qualitative trait, breeders realized that red versus yellow was only one attribute of color, given that color intensity, pattern and the percent surface covered were also variables. Cheng (1996) identified a RAPD marker linked to red color in 1996. Advances in genomics in apples resulted in numerous groups reporting progress in this area at nearly the same time. Takos et al. (2006) reported that light induced expression of a MYB gene was what regulated anthocyanin biosynthesis in red apples. Chagne et al. (2007) mapped a candidate gene (MDMYB10) for red flesh and foliage color in apple and Espley et al. (2007) reported that red coloration in apple fruit was due to the activity of the MYB transcription factor, MdMYB10. Then, Ban et al. (2007) isolated and conducted functional analysis of this MYB transcription factor gene. In silencing anthocyanidin synthase in apple, Szankowski et al. (2009a) found a shift in polyphenol profile and a sublethal phenotype, emphasizing the importance of anthocyanin in apple.

7.4.2 Development of Research Communities and Databases

The AppleBreed Database was envisioned as an easily accessible way to link molecular and phenotypic data from multiple, pedigree-verified populations including crosses, breeding selections and cultivars (Antofie et al. 2007). This database was developed as part of the European HIDRAS project, but has applications beyond that project. The HIDRAS: High Quality Disease Resistant Apples for a Sustainable Agriculture (users.unimi.it/hidras/) was reviewed by Gianfranceschi and Soglio (2004). HIDRAS was preceded by the collaborative project DARE (Durable resistance to scab and mildew in apple) (Evans et al. 2000) and followed by ISAFRUIT, a European project involving over 200 researchers and 60 Research units, that is

aimed at quality fruit from "the seed to consumption" (http://www.isafruit.org). Recently a large project called 'Fruitbreedomics' has been funded that combines breeding in collaboration with genomics across many institutions and countries.

In the USA, the GDR (Genome Database for Rosaceae) is an integrated Web database for Rosaceae genomics and genetics data (Jung et al. 2007) at http://www.bioinfo.wsu.edu/gdr/.

Shulaev et al. (2008) reviewed multiple models of genomics in the Rosaceae and shows the community building evident among researchers in this family. There is a Rosaceae white paper at http://www.Rosaceaewhitepaper.com, which represents efforts of the community to document issues across the Rosaceae. These activities resulted in the RosBREED project (http://www.rosbreed.org) that has as a goal enabling marker-assisted selection in the Rosaceae (Iezzoni et al. 2010).

7.5 Transgenics

Reviews of transgenics in apple include Brown and Maloney (2004), Sansavini et al. 2005; Gardiner et al. (2007) and Gessler and Patocchi (2007). Transgenics with fire blight resistance were discussed by Malnoy and Aldwinckle (2007) and transgenic rootstocks were reviewed by Dolgov and Hanke (2006).

Numerous scion cultivars have been transformed, starting with the transformation of 'Greensleeves' apple in 1989. Many of the top commercial varieties and some of their sports ('Cox's Orange Pippin,' 'Elstar,' 'Fuji,' 'Gala,' 'Greensleeves,' 'Jonagold,' 'McIntosh,' 'Orin') and a scab resistant variety ('Florina') have been transformed. Many transgenes have been targeted, with a priority on imparting resistance to disease and insects.

7.5.1 Resistance to Apple Scab

The synergistic activity of endochitinase and exochitinase from *Trichoderma harzianum* against the pathogenic fungus (*Venturia inaequalis*) in transgenic apple plants revealed that expression of some genes had a fitness cost, plants could be resistance but of extremely low vigor (Bolar et al. 2001). However overexpression of the apple MpNPR1 gene conferred increased disease resistance without a loss of vigor (Malnoy et al. 2007).

The transformation of apple with the cloned scab resistance gene (HcrVf2) from *Malus floribunda* provided the first functional confirmation of a cloned apple gene (Belfanti et al. 2004). This research has progressed to expression profiling in HcrVf-2-transformed apple plants in response to *Venturia inaequalis,* with 523 unigenes identified (Paris et al. 2009). Recently, Malnoy et al. (2008) demonstrated that two receptor-like genes, *Vfa*1 and *Vfa*2, conferred resistance to apple scab. Szankowski et al. (2009b) found that varying the length of the native promoter of HcrVF2 influenced the degree of resistance expressed.

7.5.2 Resistance to Fire Blight

Rapid transcriptional response of apple to fire blight disease was revealed by cDNA suppression subtractive hybridization analysis (Norelli et al. (2009). Malnoy and Aldwinckle (2007) provide an overview of the many transgenic approaches to conferring resistance to this pathogen.

7.5.3 Fungal Resistance

Szankowski et al. (2003) transformed 'Holsteiner Cox' and 'Elstar' with the stilbene synthase gene from grapevine (*Vitis vinifera* L.) and a PGIP gene from kiwi (*Actinidia deliciosa*) to try to impart resistance to fungal diseases.

7.5.4 Modification of Plant Growth or Architecture (Rootstock and Scion)

Zhu et al. (2000) found that integration of the rolA gene into the genome of the vigorous apple rootstock A2 reduced plant height and shortened internodes. In 2005, Bulley et al. reported that the modification of gibberellin biosynthesis in the grafted apple scion allowed the control of tree height independent of the rootstock. Overexpression of the *Arabidopsis gai* (gibberellin-insensitive gene) in apple also significantly reduced plant size (Zhu et al. 2008b).

7.5.5 Flowering Genes

Kotoda et al. (2006) reported that antisense expression of the terminal flowering gene (MdTFL1) reduced the juvenile phase in apple. Overexpression of an FT-homologous gene of apple induced early flowering in *Arabidopsis*, poplar and apple (Tränkner et al. 2010).

7.5.6 Rootstock Transformation

Rootstocks have been transformed with the *rol* genes from *Agrobacterium* (Zhu et al. 2000) and with genes to impart resistance to fire blight (reviewed in Malnoy and Aldwinckle 2007). The rootstocks transformed include A2, M.7, M.26, M.9, 'Marubukaido,' and *Malus micromalus* Makino.

7.5.7 Anti-sense or Silencing

Transgenic approaches to silencing genes often reveal important information about the interaction of genes. Dandekar et al. (2004) found that down regulation of

ethylene had a strong effect on the apple fruit flavor complex. Silencing leaf sorbitol synthesis altered long-distance partitioning and apple fruit quality (Teo et al. 2006).

7.5.8 Cisgenesis

Currently cisgenesis, defined as genetic modification of plants inserting genes from the plant itself or from crossable relatives, is a focus of several apple projects (Schouten and Jacobsen 2008). Such programs often also target "markerless technology."

The whole genome sequencing of a clone of 'Golden Delicious' has been completed at the Istituto Agrario San Michele all'Adige (IASMA) in Italy (http://www.ismaa.it) (Velasco et al. 2010) and researchers at Washington State University and South Africa are now partnering with these researchers and others in France in the sequencing of a doubled haploid of 'Golden Delicious.' Enhanced collaboration among breeding programs and genomic groups is providing the integration crucial to future success and application. Genomics has opened up new opportunities for improving apple and for learning some of the issues involved in the many complex traits being targeted. Enhanced collaboration on an international scale is an excellent way for us to further our breeding goals and to add to the database of phenotypic and genotypic information.

References

Abe, K., Kotoda, N., Kato, H. and Soejima, J. (2007) Resistance sources to Valsa canker (*Valsa ceratosperma*) in a germplasm collection of diverse *Malus* species. Plant Breeding 126, 449–453.

Aikman, D.P. and Langton, F.A. (1983) Replication in initial selection trials of clonally propagated crops. Euphytica 32, 821–829.

Alspach, P.A. and Oraguzie, N.C. (2002)Estimation of genetic parameters of apple (*Malus domestica*) fruit quality from open pollinated families. New Zealand J. Crop Hort. Sci. 30, 219–228.

Alston, F.H., Philipps, K.L. and Evans, K.M. (2000) A *Malus* gene list. Acta Hort. 538, 561–570.

Antofie, A., Lateur, M., Oger, R., Patocchi, A., Durel, C.E. and van de Weg, W.E. (2007) A new versatile database created for geneticist and breeders to link molecular and phenotypic data in perennial crops: the AppleBreed DataBase. Bioinformatics 23, 882–891.

Ban, Y., Honda, C., Hatsuyama, Y., Igarashi, M., Bessho, H. and Moriguchi, T. (2007) Isolation and functional analysis of a myb transcription factor gene that is a key regulator for the development of red coloration in apple skin. Plant Cell Physiol. 48, 958–970.

Batlle, I. and Alston, F. H. (1996) Genes determining leucine aminopeptidase and mildew resistance from ornamental apple, 'White Angel'. Theor. Applied Genetics 93, 179–182.

Belfanti, E., Silfverberg-Dilworth, E., Tartarini, S., Patocchi, A., Barbieri, M., Zhu, J., Vinatzer, B.A., Gianfranceschi, L., Gessler, C. and Sansavini, S. (2004) The HcrVf2 gene from a wild apple confers scab resistance to a transgenic cultivated variety. Proc. Natl. Acad. Sci. USA 101, 886–890.

Biedrzycka, E. and Amarowicz, R. (2008) Diet and Health: Apple polyphenols as antioxidants. Food Reviews Internat. 24, 235–251.

Biggs, A.R. and Miller, S.S. (2001) Relative susceptibility of selected apple cultivars to *Colletotrichum acutatum.* Plant Disease 85, 657–660.
Blanpied, D. and Silsby, K. (1992) Predicting harvest date windows for apples. Cornell Cooperative Extension Information Bulletin 221.
Blazek, J., Opatova, H., Golias, J. and Homutova, I. (2007) Ideotype of apples with resistance to storage disorders. Hort. Sci. (Prague) 24, 107–113.
Bolar, J.P., Norelli, J., Harman, G.E., Brown, S.K., and Aldwinckle, H.S. (2001) Synergistic activity of endochitinase and exochitinase from *Trichoderma harzianum* against the pathogenic fungus (*Venturia inaequalis*) in transgenic plants. Transgenic Research 10, 533–543.
Boyer, J. and Liu, R.H. (2004) Apple phytochemicals and their health benefits. Nutrition J. 3:5 (www.nutritionj.com/content/3/1/5).
Braniste, N., Militaru, M. and Budan, S. (2008). Two scab resistant columnar apple cultivars. Acta Hort. 767, 351–354.
Broothaerts, W. (2003) New findings in apple S-genotype analysis resolve previous confusion and request the re-numbering of some S-alleles. Theor. Appl. Genet. 106, 703–714.
Brown, S.K. (2008) Breeding and biotechnology for flavor development in apple (*Malus* x *domestica* Borkh.) pp. 147–156. In: Havkin-Frenkel, D. and Belanger, F.C. (eds.): *Biotechnology in flavor production*. Blackwell Publishing.
Brown, S.K. and Maloney, K. E. (2004) *Malus* x *domestica* Apple. pp. 475–511. In: R. Litz (ed.) *Biotechnology of Fruit and Nut Crops.* CAB International, Oxon, United Kingdom.
Brown, S.K. and Maloney, K.E. (2003) Genetic improvement of apple: Breeding, markers, mapping and biotechnology. pp. 31–59. In: Ferree, D. and Warrington, I. (eds.) *Apples: Botany, Production and Uses*. CAB International, Cambridge, MA, USA.
Bulley, S.M., F.M. Wilson, P. Hidden, A.L. Phillips, S.J. Croker and D.J. James. (2005) Modification of gibberellin biosynthesis in the grafted apple scion allows control of tree height independent of the rootstock. Plant Biotech. J. 3, 215–223.
Bus, V. G. M., Chagne, D., Bassett, C.M., Bowatte, D., Calenge, F., Celton, J.M., Durel, C.E., Malone, M.T., Patocchi, A., Ranatunga, A.C., Rikkerink, E.H.A., Tustin, D.S., Zhou, J. and Gardiner, S.E. (2007) Genome mapping of three major resistance genes to woolly apple aphid (*Eriosoma lanigerum* Hausm.). Tree Genetics Genomics 4, 223–236.
Bus V.G.M., Laurens F.N.D., van de Weg W.E., Rusholme R.L., Rikkerink E.H.A., Gardiner S.E., Bassett H.C.M., Kodde L.P. and Plummer K.M. (2005a) The *Vh8* locus of a new gene-for-gene interaction between *Venturia inaequalis* and the wild apple *Malus sieversii* is closely linked to the *Vh2* locus in *M. pumila* R12740-7A. New Phytologist 166,1035–1049.
Bus V.G.M., Rikkerink E.H.A., van de Weg E.W., Gardiner S.E., Bassett H.C.M., Kodde L.P., Parisi L., Laurens F.N.D., Rusholme R., Meulenbroek B. and Plummer, K.M. (2005b) The Vr and Vx scab resistance genes in two differential hosts derived from Russian apple R12740-7A map to the same linkage group of apple. Mol. Breeding 15, 103–116.
Bus, V. G. M. (2006) A partial diallel study of powdery mildew resistance in six apple cultivars under three growing conditions with different disease pressures. Euphytica 148, 235–242.
Büttner, R., Fischer, M., Forsline, P.L., Geibel, M. and Ponomarenko, V.V. (2004) Gene banks for the preservation of wild apple genetic resources. J. Fruit Ornamental Plant Research 12, 99–104.
Caffier, V. and Laurens, F. (2005) Breakdown of *Pl*2, a major gene of resistance to apple powdery mildew, in a French experimental orchard. Plant Path. 54, 116–124.
Caffier, V. and Parisi, L. (2007) Development of apple powdery mildew on sources of resistance to *Podosphaera leucotricha*, exposed to an inoculum virulent against the major resistance gene Pl-2. Plant Breeding 126, 319–322.
Calenge, F., Drouet, D., Denance, C., van de Weg, W.E., Brisset, M.-N., Paulin, J.P. and Durel, C.-E. (2005) Identification of a major QTL together with several minor additive or epistatic QTLs for resistance to fire blight in apple in two related progenies. Theor. Appl. Genet. 111,128–135.
Calenge, F. and Durel, C.-E. (2006) Both stable and unstable QTLs for resistance to powdery mildew are detected in apple after four years of field assessments. Mol. Breeding 17, 329–339.

Calenge F., Faure A., Goerre M., Gebhardt C., Van De Weg W.E., Parisi L., Durel C-E. (2004) Quantitative Trait Loci (QTL) analysis reveals both broad-spectrum and isolate-specific QTL for scab resistance in an apple progeny challenged with eight isolates of *Venturia inaequalis*. Phytopath. 94, 370–379.

Celton, J.M., Tustin, S., Chagne, D. and Gardiner, S. (2009) Construction of a dense genetic linkage map for apple rootstocks using SSRs developed from *Malus* ESTs and *Pyrus* genomic sequencing. Tree Genetics and Genomes 5, 93–107.

Chagne, D., Carlisle, C.M., Blond, C., Volz, R.K., Whitworth, C.J., Oraguzie, N.C., Crowhurst, R.N., Allan, A.C., Espley, R.V., Hellens, R.P. and Gardiner, S.E. (2007) Mapping a candidate gene (MDMYB10) for red flesh and foliage color in apple. BMC Genomics 8, 212.

Chagne, D., Gasic, K., Crowhurst, R.N., Han, Y., Bassett, H.C., Bowatte, D.R., Lawrence, T.J., Rikkerink, E.H.A., Gardiner, S.E. and Korban, S.S. (2008) Development of a set of SNP markers present in expressed genes of the apple. Genomics 92, 353–358.

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

Cheng, F.S., Weeden, N.F. and Brown, S.K. (1996) Identification of co-dominant RAPD markers tightly linked to fruit skin color in apple. Theor. Appl. Genet. 93, 222–227.

Coart, E., Vekemans, X., Smulders, M.J.M., Wagner, I., Van Huylenbroek, J., Van Bockstaele, E. and Roldan-Ruiz, I. (2003) Genetic variation in the endangered wild apple (*Malus sylvestris* (L.) Mill.) in Belgium as revealed by amplified fragment length polymorphism and microsatellite markers. Mol. Ecology 12, 845–857.

Coart, E.L.S., Van Glabeke, S., DeLoose, M., Larsen, A.S. and Roldan-Ruiz, I. (2006) Chloroplast diversity in the genus *Malus*: new insights into the relationship between the European wild apple (*Malus sylvestris* (L.) Mill.) and the domesticated apple (*Malus domestica* Borkh.). Mol. Ecology 15, 2171–2182.

Conner, P.J., Brown, S.K. and Weeden, N.F. (1997) Randomly amplified polymorphic DNA-based genetic linkage maps of three apple cultivars. J. Amer. Soc. Hort. Sci. 122, 350–359.

Conner, P.J., Brown, S.K. and Weeden, N.F. (1998) Molecular-marker analysis of quantitative traits for growth and development in juvenile apple trees. Theor. Appl. Genet. 96, 1027–1035.

Costes, E., Lauri, P.E. and Regnard, J.L. (2006) Analyzing fruit tree architecture, implication for tree management and fruit production. Hort. Rev. 32, 1–61.

Cummins, J.N. and Aldwinckle, H.S. (1983) Breeding apple rootstocks. Plant Breeding Rev. **x**, 294–394.

Currie, A. J., Ganeshanandam, S., Noiton, D. A., Garrick, D., Shelbourne, C. J. A. and Orgaguzie, N. (2000) Quantitative evaluation of apple fruit shape (*Malus* × *domestica* Borkh.) by principal component analysis of Fourier descriptors. Euphytica 11, 221–227.

Dal Cin, V., Boschetti, A., Dorigoni, A. and Ramina, A. (2007) Benzylaminopurine application on two different apple cultivars (*Malus domestica*) displays new and unexpected fruit abscission features. Ann. Bot. 99, 1195–1202.

Dandekar, A.M., Teo, G., Defillippi, B.G., Uratsu, S.L., Passey, A.J., Kader, A.A., Stow, J.R., Colgan, R.J. and James, D.J. (2004) Effect of down-regulation of ethylene biosynthesis on fruit flavor complex in apple fruit. Transgenic Research 13, 373–384.

Davey, M.W., Kenis, K. and Keulemans, J. (2006) Genetic control of fruit vitamin C contents. Plant Physiol. 142, 343–351.

Davey, M.W. and Keulemans, J. (2004) Determining the potential to breed for enhanced antioxidant status in *Malus*: Mean inter- and intra-varietal fruit vitamin C and glutathione contents at harvest and their evolution during storage. J. Agric. Food Chem. 52, 8031–8038.

Dayton, D .F. (1977) Genetic immunity to apple mildew incited by *Podosphaera leucotricha*. Hortscience 12, 225–226.

Dolgov, S.V. and Hanke, V. (2006) Transgenic temperate fruit tree rootstocks. p. 335–350. In; Fladung, M. and Ewald, E. (eds.). Tree Transgenesis. Recent Developments. Springer-Verlag.

Dunemann, F., Bracker, G., Markussen, T. and Roche, P. (1999) Identification of molecular markers for the major mildew resistance gene Pl2 in apple. Acta Hort. 484, 411–416.

Dunemann, F., Peil, A., Urbanietz, A., and Garcia-Libreros, T. (2007) Mapping of the powdery mildew resistance gene *Pl*1 and its genetic association with an NBS-LRR candidate resistance gene. Plant Breeding 126, 476–481.

Dunemann, F., Ulrich, D., Boudichevskaia,A.. Grafe, C. and Weber, W.E. (2009) QTL mapping of aroma compounds analysed by headspace solid-phase microextraction gas chromatography in the apple progeny 'Discovery' x 'Prima'. Mol. Breeding 23, 501–521.

Dunemann, F., Ulrich, D., Malysheva-Otto, L., Weber, W.E., Longhi, S. Velasco, R. and Costa, F. (2011) Functional allelic diversity of the apple alcohol acyl-transferase gene mdAAT1 associated with fruit ester volatile contents in apple cultivars. Mol. Breeding (on-line early).

Durel, C.-E., Denance, C. and Brisset, M.-N. (2009) Two distinct major QTL for fire blight co-localize on linkage group 12 in apple genotypes 'Evereste' and *Malus floribunda* clone 821. Genome 52, 139–147.

Durel, C.E., Laurens, F., Fouillet, A. and Lespinasse, Y. (1998) Utilization of pedigree information to estimate genetic parameters from large unbalanced data sets in apple. Theor. Appl. Genet. 91, 1077–1085.

Eberhardt, M.V., Lee, C.Y. and Liu, R.H. (2000) Antioxidant activity of fresh apples. Nature 405, 903–904.

Erdin, N., Tartarini, S., Broggini, G.A.L., Gennari, F., Sansavini, S., Gessler, C., and Patocchi, A. (2006) Mapping of the apple scab-resistance gene *Vb*. Genome 49, 1238–1245.

Espley, R.V., Hellens, R.P., Putterill, J., Stevenson, D.E., Kutty-Amma, S., and Allan, A.C. (2007) Red coloration in apple fruit is due to the activity of the MYB transcription factor. MdMYB10. The Plant Journal 49, 414–427.

Evans, K.M. and James, C.M. (2003) Identification of SCAR markers linked to *Pl-w* mildew resistance in apple. Theor. Appl. Genet. 106, 1178–1183.

Evans, K., Lespinasse, Y. and Durel, C. (2000) Durable resistance to scab and mildew in apple- A European project. Pesticide Outlook, 84–87.

Fazio, G., Aldwinckle, H., Mcquinn, R., Robinson, T. (2006a) Differential susceptibility to fire blight in commercial and experimental apple rootstock cultivars. Acta Hort. 704, 527–530.

Fazio, G., Robinson, T., Aldwinckle, H., Mazzola, M., Leinfelder, M., Parra, R. (2006b) Traits of the next wave of Geneva apple rootstocks. Compact Fruit Tree 38, 7–11.

Fernandez-Fernandez, F., Evans, K.M., Clarke, J.B., Govan, C.L., James, C.M., Maric, S. and Tobutt, K.R. (2008) Development of an STS map of an interspecific progeny of *Malus*. Tree Genetics & Genomes 4, 469–479.

Ferree, D. C. and Carlson, R.F. (1987) Apple Rootstocks. pp. 107–143. In: Rom, R.C. and Carlson, R.F. (eds.). *Rootstocks for Fruit Crops*. John Wiley & Sons. US.

Fischer, M. (2001) New dwarfing and semi-dwarfing apple and pear rootstocks. Acta Hort. 557, 55–62.

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

Fischer, M. And Fischer, C. (2008) The Pillnitz Re-series of apple cultivars-Do they hold promise? -80 years of professional German fruit breeding. Erwerbs-Obstbau 50, 63–67.

Forsline, P.L., Aldwinckle, H.S., Dickson, E.E., Luby, J.J. and Hokanson, S.C. (2003) Collection, maintenance, characterization and utilization of wild apples of central Asia. Hort. Rev. 29, 1–62.

Gallot J.C., Lamb, R.C. and Aldwinckle, H.S. (1985). Resistance to powdery mildew from some small-fruited *Malus* cultivars. Hortscience 20, 1085–1087.

Gao, Z.S. and van de Weg, W. E. (2006) The Vf gene for scab resistance is linked to sub-lethal genes. Euphytica 151, 123–132.

Gao, Z.S., van de Weg, W.E., Schaart, J.G., Schouten, H.J., Tran, D.H., Kodde, L.P., van der Meer, I.M., van der Geest, A.H.M., Kodde, J., Breiteneder, H., Hoffmann-Sommergruber, K., Bosch, D. and Gilissen, L.J.W.J. (2005a) Genomic cloning and linkage mapping of the *Mal d1 (PR-10)* gene family in apple (*Malus domestica*). Theor. Appl. Genet. 111, 171–183.

Gao, Z.S., van de Weg, W.E., Schaart, J.G., van der Meer, I.M., Kodde, L.P., Laimier, M., Breiteneder, H., Hoffmann-Sommergruber, K., and Gilissen, L.J.W.J. (2005b) Linkage map positions and allelic diversity of two Mal d 3 (non-specific lipid transfer protein) genes in the cultivated apple (*Malus domestica*) Theor. Appl. Genet. 110, 479–491.

Gao, Z.S., van de Weg, W.E., Schaart, van Arkel, G., Breiteneder, H., Hoffmann-Sommergruber, K., and Gilissen, L.J.W.J. (2005c) Genomic characterization and linkage mapping of the apple allergen genes *Mal d 2* (thaumatin-like protein) and *Mal d 4* (profilin). Theor. Appl. Genet. 111, 1087–1097.

Gardiner, S.E., Bus, V.G.N., Rusholme, R.L., Chagne, D., and Rikkerink, E. (2007) Apples: pp.1-62. In: Kole, C. (ed.) *Genome Mapping and Molecular Breeding in Plants: Fruits and Nuts.* Springer, NY.

Gasic, K., Han, Y., Kertbundit, S., Shulaev, V., Iezzoni, A.F., Stover, E.W., Bell, R.L., Wisniewski, M.E., and Korban, S.S. (2009) Characterization and transferability of new apple EST-derived SSRs to other Rosaceae species. Mol. Breeding (on-line early).

Gessler, C., Patocchi, A., Sansavini, S., Tartarini, S. and Gianfranceschi. L. (2006) *Venturia inaequalis* resistance in apple. Critical Reviews Plant Sci. 25, 473–503.

Gessler, C. and Patocchi, A. (2007) Recombinant DNA technology in apple. Adv. Biochem. Engin./Biotechnology 107, 113–132.

Gianfranceschi, L. and Soglio, V. (2004). The European project HIDRAS: innovative multidisciplinary approaches to breeding high quality disease resistant apples. Acta Hort. 663, 327–330.

Gleave, A.P., Ampomah-Dwamena, C., Berthold, S., Dejnoprat, S., Karunairetnam, S., Nain, B., Wang, Y.Y., Crowhurst, R.N. and MacDiarmid, R.M. (2008) Identification and characterisation of primary microRNAs from apple (*Malus domestica* cv. Royal Gala) expressed sequence tags. Tree Genetics & Genomes 4, 343–358.

Guarino, C., Arena, S., De Simopne, L., D'Ambrosia, C., Sanotora, Simona, Rocco, M., Scaloni, A. and Marra, M. (2007) Proteomic analysis of the major soluble components in Annurca apple flesh. Mol. Nutr. Food Res. 51, 255–262.

Gygax, M., Gianfranceshi, L., Liebhard, R., Kellerhals, M., Gessler, C. and Patocchi, A. (2004) Molecular markers linked to the apple scab resistance gene V_{bj} derived from *Malus baccata jackii*. Theor. Appl. Genet. 109, 1702–1709.

Harada, T., Sunako, T., Wakasa, Y., Soejima, J., Satoh, T. and Niizeki, I. (2000) An allele of the 1-aminocyclopropane-1-carboxylate synthase gene (Md-ACS1) accounts for the low level of ethylene production in climacteric fruits of some apple cultivars. Theor. Appl. Genet. 101, 742–746.

Harker F.R., Kupferman, E.M., Marin, A.B., Gunsun, F.A., and Triggs, C.M. (2008) Eating quality standards for apples based on consumer preferences. Postharvest Biol. Technol. 50, 70–78.

Harker, F.R., Maindonald, J.H. and Jackson, P.J. (1996) Penetrometer measurement of apple and kiwi firmness: operator and instrument differences. J. Amer. Soc. Hort. Sci. 121, 927–936.

Harker, F.R., Maindonald, J.H., Murray, S.H., Gunson, F.A., Hallet, I.C. and Walker, S.B. (2002a) Sensory interpretation of instrumental measurements 1: texture of apple fruit. Postharvest Biol. Technol. 24, 225–239.

Harker, F.R., Marsh, K.B., Young, H., Murray, S.H., Gunson, F.A. and Walker, S.B. (2002b) Sensory interpretation of instrumental measurements 2: sweet and acid taste of apple fruit. Postharvest Biol. Technol. 24, 241–250.

Hemmat, M., Brown, S.K., Aldwinckle, H.S., Mehlenbacher, S.A. and Weeden, N.F. (2003) Identification and mapping of markers for resistance to apple scab from 'Antonovka' and 'Hansen's baccata #2'. Acta Hort. 622, 153–162.

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

Hemmat, M., Weeden, N.F., Conner, P.J. and Brown, S.K. (1997) A DNA marker for columnar growth habit in apple contains a simple sequence repeat J. Amer. Soc. Hort. Sci. 122, 347–349.

Hemmat, M., Weeden, N.F., Manganaris, A.G. and Lawson, D.M. (1994) Molecular marker linkage map for apple. J. Heredity 85, 4–11.

Heo, S., Kim, D., Yun, H.R., Hwang, J.H., Lee, H.J. and Shin, Y.U. (2006) Development of AFLP markers linked to resistance against Alternaria blotch in apple (*Malus domestica*). Hort. Environ. Biotech. 47, 324–328.

Iezzoni, A., Weebadde, C., Luby, J., Chengyan, Y., Van de Weg, E., Fazio, G., Mann, D., Peace, C.P., Bassil, N.V. and McFerson, J. (2010) RosBREED: Enabling marker assisted breeding in Rosaceae. Acta Hort. 859, 389–394.

Igarashi, M., Abe, Y., Hatsuyama, Y., Ueda, T., Fukasawa-Akada, T., Kon, T., Kudo, T., Sato, T. and Suziki, M. (2008) Linkage maps of the apple *(Malus* x *domestica* Borkh.) cvs. 'Ralls Janet' and 'Delicious' include newly developed EST markers. Mol. Breeding 22, 95–118.

Ikase, L and Dumbras, R. (2004) Breeding of columnar apple trees in Latvia. Biologia 2, 8–10.

Iwanami, H., Ishiguro, M., Kotoda, N., Takahashi, S. and Soejima, J. (2005) Optimal sampling strategies for evaluating fruit softening after harvest in apple breeding. *Euphytica* 144, 169–175.

Iwanami, H., Moriya, S., Kotoda, N., Takahashi, S. and Abe, K. (2008) Estimations of heritability and breeding value for postharvest fruit softening in apple. J. Amer. Soc. Hort. Sci. 133, 92–99.

Jakubowski, T. and Zagaja, S.W. (2000) 45 years of apple rootstock breeding in Poland. Acta Hort. 538, 723–727.

James, C.M., Clarke, J.B. and Evans, K.M. (2004) Identification of molecular markers linked to the mildew resistance gene *Pl-d* in apple. Theor. Appl. Genet. 110, 175–181.

Janick, J., Cummins, J.N., Brown, S.K., Hemmat, M. (1996) Apples. pp 1–77. In: Janick J., Moore J.N. (eds). *Fruit Breeding Volume 1. Tree and Tropical Fruits*. John Wiley, New York, NY.

Janssen, B.J., Thoday, K., Schaffer, T.J., Alba, R., Balakrishan, L., Bishop, R., Bowen, J.H., Crowhurst, R.N., Gleave, A.P., Ledger, S., McArtney, S., Pichler, F.B., Snowden, K.C. and Ward, S. (2008) Global gene expression analysis of apple fruit development from the floral bud to ripe fruit. BMC Plant Biology 8, 16.

Jung, S., Staton, M., Le, T., Blenda, A., Svancara, R., Abbott, A. and Main, D. (2007) GDR (Genome Database for Rosaceae): integrated web database for Rosaceae genomics and genetics data. Nucleic Acids Research 1–7.

Juniper, B.E., Watkins, R. and Harris, S.A. (1998) The origins of apple. Acta Hort. 484, 27–33.

Kenis, K. and Keulemans, J. (2005) Genetic linkage maps of two apple cultivars (*Malus domestica* Borkh.) based on AFLP and microsatellite markers. Mol. Breeding 15, 205–219.

Kenis, K. and Keulemans, J. (2007) Study of tree architecture of apple (*Malus* x *domestica* Borkh.) by QTL analysis of growth traits. Mol. Breeding 19, 193–208.

Kenis, K. and Keulemans, J. (2008) Identification and stability of QTLs for fruit quality traits in apple. Tree Genetics & Genomes 4, 647–661.

Khanizadeh, S., Groleau, Y., Granger, R., Cousineau, J. and Rousselle, G.L. (2000) New hardy rootstocks from the Quebec apple breeding program. Acta Hort. 538, 719–721.

Khanizadeh, S., Tsao, R., Rekika, D., Yang, R., Charles, M.T. and Vasantha Rupasinghe, H.P. (2007) Polyphenol composition and total antioxidant capacity of selected apple genotypes for processing. J. Food Comp. Analysis 21, 396–401.

Kim, M.Y., Song, K.J., Hwang, J.-H., Shin, Y.U. and Lee, H.J. (2003) Development of RAPD and SCAR markers linked to the Co gene conferring columnar growth habit in apple (*Malus pumilla* Mill.). J. Hort.Sci. Biotech 78, 512–517.

King, G.J., Lynn, J.R., Dover, C.J., Evans, K.M. and Seymore, G.B. (2001) Resolution of quantitative trait loci for mechanical measures accounting for genetic variation in fruit texture of apple (*Malus pumila* Mill.). Theor. Appl Genet. 102, 1227–1235.

King, G.J., Maliepaard, C., Lynn, J.R., Alston, F.H., Durel, C.E., Evans, K.M., Griffon, B., Laurens, F., Manganaris, A.G., Schrevens, E., Tartarini, S. and Verhaegh, J. (2000) Quantitative genetic analysis and comparison of physical and sensory descriptors relating to fruit flesh firmness in apple (*Malus pumila* Mill.). Theor. Appl. Genet. 100, 1074–1084.

Knight, R. L. and Alston, F.H. (1968) Sources of field immunity to mildew (*Podosphaera leucotricha*) in apple. Can. J. Genet. Cytol. 10, 294–298.

Knight, V.H., Evans, K.M., Simpson, D.W. & Tobutt, K.R. (2005) Report on a desktop study to investigate the current world resources in Rosaceous fruit breeding programmes. Submitted to Defra, August 2005.

Korban, S.S. (1986) Interspecific hybridization in *Malus*. HortScience 21, 41–48.

Korban, S.S. and Swiader, J.M. (1994) Genetic and nutritional status in bitter pit resistant and – susceptible apple seedlings. J. Amer. Soc. Hort. Sci. 109, 428–432.

Kotoda, N., Iwanami, H., Takahashi, S., and Abe, K. (2006) Antisense expression of MdTFL1-like gene, reduces the juvenile phase in apple. J. Amer. Soc. Hort. Sci. 131, 74–81.
Labuschagne, I. F., Louw, J. H., Schmidt, K., Sadie, A. (2003) Budbreak number in apple seedlings as selection criterion for improved adaptability to mild winter climates. HortScience 386, 1186–1190.
Labuschagne, I.F., Louw, J.H., Schmidt, K. and Sadie, A. (2001) Genotypic variation in prolonged dormancy symptoms in apple families. HortScience 37, 157–163.
Labuschagne, I.F., Louw, J.H., Schmidt, K. and Sadie, A. (2002) Genetic variation in chilling requirement in apple progenies. J. Amer. Soc. Hort. Sci. 127, 663–672.
Lauri, P.E., Bourdel, G., Trottier, C. and Cochard, H. (2008) Apple shoot architecture: evidence for strong variability for bud size and composition and hydraulics within a branching zone. New Phytologist 178, 798–807.
Laurens, F. (1999) Review of the current apple breeding programs in the world: Objectives for scion cultivar improvement. Acta Hort. 484, 163–170.
Laurens, F. and Pitiot, C. (2003) French apple breeding program: A new partnership between INRA and the nurserymen of Novadi. Acta Hort. 622, 575–582.
Lata, B. (2008) Apple peel antioxidant status in relation to genotype, storage type and time. Scientia Hort. 117, 45–52.
Lee, K.W., Kim, Y.J., Kim, D-O, Lee, H.J. and Lee, C.Y. (2003) Major phenolics and their contribution to the total antioxidant capacity. J. Agric. Food Chem. 51, 6516–6520.
Lee, Y.-P., Yu, G.-H., Seo, Y.S., Han, S.E., Choi, Y-O, Kim, D., Mok, I-G., Kim, W.T. and Sung, S.-K. (2007) Microarray analysis of apple gene expression engaged in early fruit development. Cell Biology Morphogenesis 26, 917–926.
Liebhard, R., Gianfranceschi, L., Koller, B., Ryder, C.D., Tarchini, R., Van de Weg, E. and Gessler, C. (2002) Development and characterization of 140 new microsatellites in apple (*Malus* x *domestica* Borkh.). Mol. Breeding 10, 217–241.
Liebhard, R., Kellerhals, M., Pfammatter, W., Jertmini, M. and Gessler, C. (2003a) Mapping quantitative physiological traits in apple (*Malus* x *domestica* Borkh.). Plant Mol. Biology 52, 511–526.
Liebhard, R., Koller, B., Gianfranceschi, L. and Gessler, C. (2003b) Creating a saturated reference map for the apple (*Malus* x *domestica* Borkh.) genome. Theor. Appl. Genet. 106, 1497–1508.
Liebhard, R., Koller, B., Patocchi, A., Kellerhals, M., Pfammatter, W., Jermini, M. and Gessler, C. (2003c) Mapping quantitative field resistance against apple scab in a 'Fiesta' x 'Discovery' progeny. Phytopath. 93, 493–501.
Luby, J. (2003) Taxonomic classification and brief history. pp. 1–14. *Apples. Botany, Production and Use.* In: Ferree, D.C. and Warrington, I.J. (eds.) CABI Publishing, Cambridge, MA.
Luby, J., Forsline, P., Aldwinckle, H., Bus, V. and Geibel, M. (2001) Silk-road apples – Collection, evaluation and utilization of *Malus sieversii* from central Asia. HortScience 36, 225–231.
Luby, J., Hoover, E., Paterson, M., Larson, D., and Bedford, D. (1999) Cold hardiness in the USDA *Malus* core germplasm collection. Acta Hort. 484, 109–114.
Luby, J.J. and Shaw, D.V. (2001) Does marker-assisted selection make dollars and sense in a fruit breeding program? HortScience 36, 872–879.
Maliepaard, C., Alston, F., van Arkel, G., Brown, L.M., Chevreau, E., Dunemann, F., Evans, K.M., Gardiner, S., Guilford, P., van Heusden, A.W., Janse, J., Laurens, F., Lynn, J.R., Manganaris, A.G., den Nijs, A.P.M., Periam, N., Rikkerink, E., Roche, P., Ryder, C., Sansavini, S., Schmidt, H., Tartarini, S., Verhaegh, J.J., Vrielink-van Ginkel, M. and King, G.J. (1998) Aligning male and female linkage maps of apple (*Malus pumila* Mill.) using multi-allelic markers. Theor. Appl. Genet. 97, 60–73.
Malnoy, M. and Aldwinckle, H.S. (2007) Development of fire blight resistance by recombinant DNA technology. Plant Breeding Rev. 26, 315–358.
Malnoy, M., Jin, Q., Borejsza-Wysocka, E. E. and Aldwinckle, H. S. (2007) Overexpression of the apple MpNPR1 gene confers increased disease resistance in *Malus* x *domestica*. Molecular Plant Microbe Interactions 20, 1568–1580.

Malnoy, M., Xu, M., Borejsza-Wysocka, E. E., Korban, S. and Aldwinckle, H. S. (2008) Two receptor-like genes, *Vfa*1 and *Vfa*2, confer resistance to the fungal pathogen *Venturia inaequalis* inciting apple scab disease. Mol. Plant-Microbe Interactions 21, 448–458.

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

Matsumoto, S., Eguchi, T., Bessho, H. and Abe, K. (2007) Determination and confirmation of S-RNase genotypes of apple pollinators and cultivars. J. Hort. Sci. Biotech. 82, 323–329.

Meyers, C.T., Leskey, T.C. and Forsline, P.T. (2007) Susceptibility of fruit from diverse apple and crabapple germplasm to attack by plum curculio (Coleoptera: Curculionidae). J. Econ. Entomol. 100, 1663–1671.

Meyers, C.T., Reissig, W., Forsline, P.L. (2008) Susceptibility of fruit from diverse apple and crabapple germplasm to attack from apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae). J. Econ. Entomol. 101, 206–215.

Miller, S., C. Hampson, R. McNew, L. Berkett, S. Brown, J. Clements, R. Crassweller, E. Garcia, D. Greene and G. Greene. (2005) Performance of apple cultivars in the 1995 NE-183 Regional project planting: 111. Fruit sensory characteristics. J. Amer. Pomol. Soc. 59, 28–43.

Miñaro, and Depena, E. (2008) Tolerance of some scab-resistant apple cultivars to the rosy apple aphid, *Dysaphis plantaginea* Crop Protection 27, 391–395.

Moore, J.N. and Janick, J. (1983) Methods *in Fruit Breeding*. Purdue University Press.

N'Diaye, A., Van de Weg, E., Kodde, L.P., Koller, B., Dunemann, F., Thiermann, M., Tartarini, S, Gennari, F. and Durel, C.E. (2008) Construction of an integrated consensus map of the apple genome based on four mapping populations. Tree Genetics & Genomes 4, 727–743.

Newcomb, R.D., Crowhurst, R.N., Gleave, A.P., Rikkerink, E.H.A., Allan, A.C., Beuning, L.L., Bowen, J.H., Gera, E., Jamieson, K.R., Janssen, B.J., Laing, W.A., McArtney, S., Nain, B., Ross, G.S., Snowden, K.C., Souleyre, E.J.F., Walton, E.F., and Yauk, Y.-K. (2006) Analyses of expressed sequence tags from apple. Plant Physiol. 141,147–166.

Noiton, D.A.M. and Alspach, P.A. (1996) Founding clones, inbreeding, coancestry, and status number of modern apple cultivars. J. Amer. Soc. Hort. Sci. 121, 773–782.

Norelli, J.L., Farrell, R.E., Bassett, C.L., Baldo, A.M., Lalli, D.A., Aldwinckle, H.A., and Wisniewski, M. E. (2009) Rapid transcriptional response of apple to fire blight disease revealed by cDNA suppression subtractive hybridization analysis. Tree Genetics & Genomes 5, 27–41.

Nybom, H., Rumpunen, K., Persson Hovmalm, H., Marttila, S., Rur, M., Garkava-Gustavsson, L. and Ollsson, M. (2008) Towards a healthier apple – Chemical characterization of an apple gene bank. Acta Hort. 765, 157–164.

Nybom, H., Sehic, J. and Garkava-Gustavsson, L. (2008a) Self-incompatibility alleles of 104 apple cultivars grown in northern Europe. J. Hort. Sci. Biotech. 83, 339–344.

Nybom, H., Sehic, J. and Garkava-Gustavsson, L. (2008b) Modern apple breeding is associated with a significant change in the allelic ratio of ethylene production gene Md-ACS1. J. Hort. Sci. Biotech. 83, 673–677.

Oraguzie, N.C., Hofstee, M.E., Brewer, L.R. and Howard, C. (2001) Estimation of genetic parameters in a recurrent selection program in apple. Euphytica 118, 29–37.

Oraguzie, N.C., Rikkerink, E.H.A., Gardner, S.E. and De Slva, H.N. (2007a) Association Mapping in Plants. Springer, New York, NY.

Oraguzie, N.C., Volz, R.K., Whitworth, C.J., Bassett, H.C.M., Hall, A.J. and Gardiner, S.E. (2007b) Influence of Md-ACS1 allelotype and harvest season within an apple germplasm collection on fruit softening during cold air storage. Postharvest Biol. Technol. 44, 212–219.

Paris, R., Cova, V., Pagliani, G, Tartarini, S., Komjanc, M. and Sansavini, S. (2009) Expression profiling in Hcr-Vf-2-transformed apple plants with in response to *Venturia inaequalis*. Tree Genetics and Genome 5, 81–91.

Park, S., Sugimoto, N., Larson, M.D., Beaudry, R. and van Nocker, S. (2006) Identification of genes with potential roles in apple fruit development and biochemistry through large-scale statistical analysis of expressed sequence tags. Plant Physiol. 141, 811–824.

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

Patocchi, A., Fernandez-Fernandez, F., Evans, K., Gobbin, D., Rezzonico, F., Boudichevskaia, A., Dunemann, F., Stankiewicz-Kosyl, M., Mathis-Jeanneteau. F., Durel, C.E., Gianfranceschi, L., Costa, F., Toller, C., Cova, V., Mott, D., Komjanc, M., Barbaro, E., Kodde, L., Rikkerink, E., Gessler, C. and van de Weg, W.E. (2008) Development and testing of 21 multiplexed PCRs composed of SSRs spanning most of the apple genome. Tree Genetics & Genomics 5, 211–223.

Peil, A., Garcia-Libreros, T., Richter, K., Trognitz, B., Hanke, M.V. and Flachowsky, H. (2007a) Strong evidence for a fire blight resistance gene of *Malus robusta* located on linkage group 3. Plant Breeding 126, 470–475.

Peil, A., Hanke, M.V., Flachowsky, H., Richter, K., Garcia, T. and Trognitz, B. (2007b) Developing molecular markers for marker assisted selection of fire blight resistant apple seedlings. Acta Hort. 763, 117–122.

Pereira-Lorenzo, S., Ramos-Cabrer, A.M., Gonzalez-Diaz, A.J. and Diaz-Hernandez, M.B. (2008) Genetic assessment of local apple cultivars from La Palma, Spain, using simple sequence repeats (SSRs). Scientia Hort. 117, 160–166.

Pichler, F.B., Walton, E.F., Davy, M., Triggs, C., Janssen, B., Wunsche, J.N., Putterill, J. and Schaffer, R. J. (2007) Relative developmental, environmental, and tree-to-tree variability in buds from field-grown apple trees. Tree Genetics & Genomes 3, 329–339.

Pilcher, R.L., Celton, J.-M., Gardiner, S.E. and Tustin, D.S. (2008) Genetic markers linked to the dwarfing trait of apple rootstock 'Malling 9'. J. Amer. Soc. Hort. Sci. 133, 100–106.

Planchon, V., Lateur, M., Dupont, P. and Lognay, G. (2004) Ascorbic acid level of Belgian apple genetic resources. Scientia Hort. 100, 51–61.

Quamme, H.A. Hampson, C.R., Hall, J.W., Sholberg, P.L, Bedford, K.E. and Randall P. (2003) Inheritance of apple scab resistance from polygenic sources based on greenhouse and field evaluation. Acta Hort. 622, 317–321.

Richards CM, Volk GM, Reilley AA, Henk AD, Lockwood D, Reeves PA, Forsline PL. (2008) Genetic diversity and population structure in *Malus sieversii*, a wild progenitor species of domesticated apple. Tree Genetics & Genomics (on-line early).

Robinson, T.L, Aldwinckle, H.S., Fazio, G. and Holleran, T. (2003) The Geneva series of apple rootstocks from Cornell: Performance, disease resistance, and commercialization. Acta Hort. 622, 513–520.

Rowan, D.D., Hunt, M.B., Dimourot, A., Alspach, P.A., Weskett, R., Volz, R.K., Gardiner, S.E. and Chagne, D. (2009) Profiling fruit volatiles in the progeny of a 'Royal Gala' x 'Granny Smith' apple (*Malus* x *domestica*) cross. J. Agric. Food Chem. 57, 7953–7961.

Rudell, D., Mattheis, J.P., Maarten, L.A. and Hertog, T.M. (2009) Metabolomic change precedes apple superficial scald symptoms. J. Agric. Food Chem. 57, 8459–8466.

Sancho, A.I., van Ree, R., van Leeuwen, A., Meulenbroek, B.J., van de Weg, E., Gilissen, L.J.W.J., Puehringer, H., Laimer, M., Martinelli, A., Zaccharini, M., Vazquez-Cortes, S., Fernandez-Rivas, M., Hoffmann-Sommergruber, K., Mills, E.N.C., and Zuidmeer, L. (2008) Measurement of lipid transfer protein in 88 apple cultivars. Allergy Immunology 146, 19–26.

Sansavini, S., Belfanti, E., Costa, F. and Donati, F. (2005) European apple breeding programs turn to biotechnology. Chronica Hort. 45, 16–19.

Sargent, D.J., Marchese, A., Simpson, D.W., Howad, W., Fernandez-Fernandez, F., Monfort, A., Arus, P., Evans, K.M. and Tobutt, K.R. (2008) Development of "universal" gene-specific markers from *Malus* spp. cDNA sequences, their mapping and use in synteny within Rosaceae. Tree Genetics & Genomes (on-line early).

Sato, T., Harada, T., Niizeki, M., Kudo, T., Akada, T., and Wakasa, Y. (2004) Allelotype of a ripening-specific 1-aminocyclopropane-1-carboxylate synthase gene defines the rate of fruit drop in apple. J. Amer. Soc. Hort. Sci. 129, 32–36.

Sato, M., Nyui, T., Takahashi, H. and Kanda, H. (2007) Comparison of flowering and fruiting of seedlings from reciprocal crosses between diploid and triploid apple cultivars. J. Japan Soc. Hort Sci. 76, 97–102.

Sawamura K, 1990. Alternaria blotch. In: Jones AL, Aldwinckle HS, eds. *Compendium of Apple and Pear Diseases*. St. Paul, Minnesota, USA: APS Press, 24–25.

Schaffer, R.J., Friel, E.N., Souleyre, E.J.F., Bolitho, K., Thodey, K., Ledger, S., Bowen, J. -H., Ma, J.H., Nain, B., Cohen D., Gleave, A.P., Crowhurst, R.N., Janssen, B.J., Yao, J.L. and Newcomb, R.D. (2007) A genomics approach reveals that aroma production in apple is controlled by ethylene predominantly at the final step in each biosynthetic pathway. Plant Physiology 144, 1899–1912.

Schouten, H.J. and Jacobsen, E. (2008) Cisgenesis and intragenesis, sisters in innovative plant breeding. Letters. Trends Plant Science 13, 260–261.

Schuster, M. (2000) Genetics of powdery mildew resistance in *Malus* species. Acta Hort. 583, 593–595.

Sedov, E.N., Salina, E.S., Levgerova, N.S., Serova, C.M. (2007) Breeding apple varieties for orchards producing raw materials. Russian Agric. Sci. 33, 89–91.

Segura, V., Denance, C., Durel, C.-E. and Costes, E. (2007) Wide range QTL analysis for complex architectural trait in a 1-year-old apple progeny. Genome 50, 159–171.

Shulaev, V., Korban, S.S., Sosinski, B., Abbott, A.G., Aldwinckle, H.S., Folta, K.M., Iezzoni, A., Main, D., Arús, P., Dandekar, A.M., Lewers, K., Brown, S.K., Davis, T.M., Gardiner, S.E., Potter, D. and Veilleux, R.E. (2008) Multiple models for Rosaceae genomics. Plant Physiology 147, 985–1003.

Silfverberg-Dilworth, E., Matasci, C.L., Van de Weg, W.E., Van Kaauwen, M.P.W., Walser, M., Kodde, L.P., Soglio, V., Gianfranceschi, L., Durel, C.E., Costa, F., Yamamoto, T., Koller, B., Gessler, C. and Patocchi, A. (2006) Microsatellite markers spanning the apple (*Malus* x *domestica* Borkh.) genome. Tree Genetics & Genome 2, 202–224.

Soejima, J., Abe, K., Kotoda, N. and Kato, H. (2000). Recent progress of apple breeding at the apple research center in Morioka. Acta Hort. 538, 211–214.

Soufflet-Freslon, V., Gianfranceschi, L., Patocchi, A., and Durel, C. -E. (2008) Inheritance studies of apple scab resistance and identification of Rvi14, a new major gene that acts together with other broad-spectrum QTL. Genome 51, 657–667.

Stankiewicz-Kosyl, M., Pitera, E. and Gawronski, S.W. (2005) Mapping QTL involved in powdery mildew resistance of the apple clone U 211. Plant Breeding 124, 63–66.

Stehr, R. (2009) Standard testing agreement for plant material developed by EUFRIN working group. Acta Hort. 814, 333–336.

Stoeckli, S., Mody, K., Gessler, C., Patocchi, A., Jermini, M. and Dorn, S. (2008) QTL analysis of aphid resistance and growth traits in apple. Tree Genetics & Genome 4, 833–847.

Stushnoff, C., McSay, A.E., Forsline, P.L., and Luby, J. (2003) Diversity of phenolic antioxidant content and radical scavenging capacity in the USDA apple germplasm core collection. Acta Hort. 623, 305–311.

Sun, L., Bukovac, M.J., Forsline, P.L., van Nocker, S. (2009) Natural variation in fruit abscission related traits in apple (*Malus*). Euphytica 165, 55–67.

Szankowski, I., Briviba, K., Fleschhut, J., Schonherr, J., Jacobsen, H.J. and Kiesecher, H. (2003) Transformation of apple (*Malus domestica* Borkh.) with the stilbene synthase gene from grapevine (*Vitis vinifera* L.) and a PGIP gene from kiwi (*Actinidia deliciosa*). Plant Cell Report 22, 141–149.

Szankowski, I., Flachowsky, H., Li, H., Halwirth, H., Treutter, D., Regos, I., Hanke, M.-V., Stich, K. and Fischer, T.C. (2009a) Shift in polyphenol profile and sublethal phenotype caused by silencing of anthocyanidin synthase in apple (*Malus* sp.). Planta 229, 681–692.

Szankowski, I., Waldmann, S., Degenhardt, J., Patocchi, A., Paris, R., Silfverberg-Dillworth, E., Broggini, G. and Gessler, C. (2009b) Highly scab-resistant transgenic apple lines achieved by introgression of HcrVF2 controlled by different native promoter lengths. Tree Genetics & Genomes 5, 349–358.

Tancred, S.J., Zeppa, A.G., Cooper, M. and Stringer, J.K. (1995). Heritability and patterns of inheritance of the ripening date of apples. HortScience 30, 325–328.

Takos, A.M., Jaffee, F.W., Jacob, S.R., Bogs, J., Robinson, S.P. and Walker, A.R. (2006) Light induced expression of a MYB gene regulates anthocyanin biosynthesis in red apples. Plant Physiol. 142, 1216–1232.

Teo G., Suzuki Y., Uratsu S.L, Lampinen B., Ormonde N., Hu W.K., DeJong T.M. and Dandekar A.M. (2006) Silencing leaf sorbitol synthesis alters long-distance partitioning and apple fruit quality. Proc. Natl. Acad. Sci. USA 103, 18842–18847.

Tian, Y.-K., Wang, C.-H., Zhang, J.-S., James, C. and Dai, H.-Y. (2005) Mapping Co, a gene controlling the columnar phenotype of apple, with molecular markers. Euphytica 145, 181–188.

Toivonen, P.AQ.A. and Brummell, P.A. (2008) Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. Postharvest Biology Technol. 48, 1–14.

Tränkner, C., Lehmann, S., Hoenicka,H., Hanke, M.V., Fladung, M., Lenhardt, D., Dunemann, F., Gau, A., Schlangen, K., Malnoy, M. and Flachowsky, H. (2010) Over-expression of an FT-homologous gene of apple induces early flowering in annual and perennial plants. *Planta* 232, 1309–1324.

van de Weg, W.E., Voorrips, R.E., Finkers, R., Kodde, L.P., Jansen, J. and Bink, M.C.A.M. (2004) Pedigree genotyping: A new pedigree based approach of QTL identification and allele mining. Acta Hort. 663, 45–50.

Velasco, R., Zharkikh, A., Affourtit, J., Dhingra, A., Cestaro, A., Kalyanaraman, A., Fontana, P., Bhatnagar, S. K., Troggio, M., Pruss, D., Salvi, S., Pindo, M., Baldi, P., Castelletti, S., Cavaiuolo, M., Coppola, G., Costa, F., Cova, V., Dal Ri, A., Goremykin, V., Komjanc, M., Longhi, S., Magnago, P., Malacarne, G., Malnoy, M., Micheletti, D., Moretto, M., Perazzolli, M., Si-Ammour, A., Vezzulli, S., Zini, E., Eldredge, G.,Fitzgerald, L. M., Gutin, N., Lanchbury, J., Macalma, T., Mitchell, J. T., Reid, J., Wardell, B., Kodira, C., Chen, Z., Desany, B., Niazi, F., Palmer, M., Koepke, T., Jiwan, D., Schaeffer, S., Krishnan, V., Wu, C., Chu, V. T., King, S. T., Vick, J., Tao, Q., Mraz, A., Stormo, A., Stormo, K., Bogden, R., Ederle, D., Stella, A., Vecchietti, A., Kater, M. M., Masiero, S., Lasserre, P., Lespinasse, Y., Allan, A. C., Bus, V., Chagné, D., Crowhurst, R. N., Gleave, A. P., Lavezzo, E., Fawcett, J. A., Proost, S., Rouzé, P., Sterck, L., Toppo, S., Lazzari, B., Hellens, R. P., Durel, C. E, Gutin, A., Bumgarner, R. E., Gardiner, S. E., Skolnick, M., Egholm, M., Van de Peer, Y., Salamini, F., and Viola, R. (2010) The genome of the domesticated apple (*Malus* x*domestica* Borkh.). Nature Genetics 42, 833–839.

Visser, T.and Verhaegh, J.J. (1976) Review of tree fruit breeding carried out at the Institute for Horticultural Plant Breeding at Wageningen from 1951 to 1976. Proc. Eucarpia tree fruit breeding, Wageningen. pp. 113–132.

Volk, G.M. and Richards, C.M. (2008) Availability of genotypic data for USDA-ARS National Plant Germplasm System accessions using the Genetic Resources Information Network (GRIN) database. HortScience 43, 1365–1366.

Volk, G. M., Richards, C. M., Reilley, A. A., Henk, A. D., Forsline, P. L., Aldwinckle, H. S. (2005) Ex situ conservation of vegetatively propagated species: development of a seed-based core collection for *Malus sieversii*. J. Amer. Soc. Hort. Sci. 130, 203–210.

Volk, G.M., Richards, C.M., Reilley, A., Henk, A.D., Reeves, P.A., Forsline, P.L., Aldwinckle, H. (2008) Genetic diversity and disease resistance of wild *Malus orientalis* from Turkey and southern Russia. J. Amer. Soc. Hort. Sci. 133, 383–389.

Volz, R.K., Alspach, P.A., Fletcher, D.J. and Ferguson, I.B. (2006) Genetic variation in bitter pit and fruit calcium concentrations within a diverse germplasm collection. Euphytica 149, 1–10.

Way, R. D., Aldwinckle, H.S., Lamb, R. C., Rejman, A., Sansavini, S., Shen, S., Watkins, R., Westwood, M.N. and Yoshida, Y. (1990) Apples. (*Malus*). In: J. N. Moore, and J. R. Ballington Jr (eds), Genetic Resources of Temperate Fruit and Nut Crops, 3—62. ISHS, Leuven, Belgium. Acta Hort 290.

Webster, A.D. and Wertheim, S.J. (2003) Apple rootstocks. pp. 91–124. In: Ferree, D. and Warrington, I (eds.): *Apples: Botany, Production and Uses*. CABI Publishing, Cambridge, MA.

Weibel, F. and Haseli, A. (2003) Organic apple production – with emphasis on European experiences. pp, 551–583. *Apples. Botany, Production and Use.* In: Ferree, D.C. and Warrington, I.J. (eds.) CABI Publishing, Cambridge, MA.

Wisniewski, M., Bassett, K., Norelli, J., Macarisin, D., Artlip, T., Gasic, K., and Korban, S. (2008) Expressed sequence tag analysis of the response of apple (*Malus* x *domestica* 'Royal Gala') to low temperature and water deficit. Physiologia Plantarum 133, 298–317.

Zhou, C., Lakso, A., Robinson, T. and Gan, S. (2008) Isolation and characterization of genes associated with shade-induced apple abscission. Mol. Genetics Genomics 280, 83–92.

Zhou, Z.-Q. (1999) The apple genetic resources in China: The wild species and their distributions, informative characteristics and utilization. Genetic Resources Crop Evolution 46, 599–609.

Zhu, L.H., Ahlman, A., Li, X.Y. and Welander, M. (2000) Integration of the rolA gene into the genome of the vigorous apple rootstock A2 reduced plant height and shortened internodes. *J. Hort. Sci. Biotech.* 76, 758–763.

Zhu, Y. and Barritt, B.H. (2008) Md-ACS1 and Md-ACO1 genotyping of apple (*Malus* x *domestica* Borkh.) breeding parents and suitability for marker assisted selection. Tree Genetics & Genomes 4, 555–562.

Zhu, Y., Rudell, D and Mattheis, J. (2008a) Characterization of cultivar differences in alcohol acyltransferase and 1-aminocyclopropane-1carboxylate synthase gene expression and volatile compound emission during apple fruit maturation and ripening. Postharvest Biol. Technol. 49, 330–339.

Zhu, L.H., Li, X.Y. and Welander, M. (2008b) Overexpression of the *Arabidopsis gai* gene in apple significantly reduces plant size. Plant Cell Rep. 27, 289–296.

Zhu, Y.D., Zhang, W., Li, G.C. and Wang, T. (2007) Evaluation of inter-simple sequence repeat analysis for mapping the Co gene in apple (*Malus pumila* Mill.). J. Hort. Sci. & Biotech. 82, 371–376.