

4. Introduction to Apple (*Malus* × *domestica*)

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1 Origin and History

Apple is the most important temperate fruit crop and has been cultivated in Asia and Europe from antiquity (Janick et al., 1996). The genus *Malus* has, according to most authorities, 25–30 species and several subspecies of so-called crabapples. The cultivated apple is supposed to be the result of interspecific hybridization. The denomination *Malus* × *domestica* has been generally accepted as the appropriate scientific name (Korban and Skirvin, 1984). The main progenitor of the domestic apple is considered to be *Malus sieversii* which grows wild in the Heavenly Mountains (Tien Shan) at the boundary between western China and the former Soviet Union to the edge of the Caspian sea (Morgan and Richard, 1993; Forsline and Aldwinckle, 2004). Central Asia is the area of greatest diversity and the center of origin. The apple was carried by humans to the Middle East, Europe and eventually to North America. Several collection trips to Central Asia have shown that *M. sieversii* is very diverse and has all qualities present in *M. × domestica*. *Malus sieversii* is endemic to the Republic of Kazakhstan (Harris et al., 2002; Hokanson et al., 1998; Luby et al., 2001). In his book ‘The Story of the Apple’ from 2006, B.E. Juniper refers to both the domestic apple and the wild Central Asian apple as *Malus pumila*. The USDA has also adopted this convention. However, Coart et al. (2006) calls into question this hypothesis and declares *Malus sieversii* to be the correct denomination for the wild Central Asian apple, and *Malus* × *domestica* for the domestic apple.

Way et al. (1990) stated that while the genus *Malus* is very diverse genetically, cultivated varieties of apple have a rather narrow genetic base, the two cultivars ‘Delicious’ and ‘Golden Delicious’ being predominant. The world’s most important commercially produced apple cultivars belong to the species *Malus* × *domestica*

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Borkh. Some other species also have significance in commercial apple production and almost all scab resistant cultivars commercially available have *M. × floribunda* Siebold ex Van Houtte in their ancestry. Genes for disease resistance have also been obtained from a wide spectrum of other *Malus* species such as *M. micromalus* Makino, *M. × atrosanguinea* (Spaeth) C. Schneider, *M. baccata jackii* Rehder and *M. sargentii* Rehder (Korban and Skirvin, 1984).

The history of apple is documented in many civilisations. Each country has fostered its own range of cultivars adapted to its growing conditions and to the requirements of their people. The discovery of ‘McIntosh’ (1796, USA), ‘Jonathan’ (1826, USA), ‘Rome Beauty’ (1848, USA), ‘Cox Orange’ (1850, UK), ‘Granny Smith’ (1868, Australia), ‘Red Delicious’ (1880, USA) and ‘Golden Delicious’ (1890, USA) were of historic importance. Moreover, ‘Red Delicious’ and ‘Golden Delicious’ are still among the most important apple cultivars worldwide.

2 Apple Production Worldwide

More than 50 million tons of apples are produced worldwide annually (www.faostat.fao.org). Apples are adaptable to various climates, but best adapted to the cool temperate zone from about 35–50° latitude. They have a more northern range than many other tree fruits, due to relatively late blooming and cold hardiness. World apple production has slightly increased in recent years and is supposed to further increase (Table 1). However, experts estimate a significant increase in production and consumption in the coming years, mainly in Asia. For North America and Europe the production is predicted to remain stable (World Apple Report, 2006). In China, the main varieties are ‘Fuji’ (60.4%, ‘Red Delicious’ (9.7%), ‘Golden Delicious’ (6.2%), ‘Jonagold’ 3.0%, ‘Gala’ 2.8% and others 1.9%. (Youngbin, 2006). The primary destination markets for China are Southeast Asia and Russia. As the emerging economies surrounding China and economic developments in China itself contribute to a considerable increase of apple consumption in Asia, it is not likely that large quantities of apples from China will be exported to Europe or North America in the near future.

According to the World Apple Report (Desmond O. Rourke) China is the major apple supplier to Asia and much of the growth in the apple trade in Asia is from imports from China. Fresh apples imported from China into Asian countries in 2005

Table 1 Trends in world apple production (× 1.000 tons)

Area	2000	2005	2010*	2015*
Europe	14.104	12.394	13.739	14.245
North America	5.671	5.187	5.487	5.521
Asia	23.638	28.383	29.788	32.718
Southern Hemisphere	4.301	4.978	5.795	6.330
World	59.199	63.489	68.441	72.820

Source: World Apple Report, 2006.

* forecast.

showed that China supplied 68.2% of all apples in SE Asia and 54.1% in South Asia. Apple imports from China for selected countries included India (19.8%), Hong Kong (30.3%), Bangladesh (44.6%), Singapore (56.9%), Malaysia (59.6%), Thailand (63%), Indonesia (69.2%) and the Philippines (92.9%).

3 Breeding

The first controlled apple crosses are attributed to Thomas A. Knight in 1806. According to Alston and Spiegel-Roy (1985), for most tree fruit crops the selection of superior phenotypes and their subsequent random mating, followed by mass selection, is the most effective means of increasing the number of favorable alleles, because there is a relatively high additive variance governing the inheritance of most traits. Fruit breeders usually select superior genotypes in the F1 progenies. Modern breeding goals in apple are determined by market and consumer requirements. The markets are becoming more and more global, leading to the worldwide cultivation of apples such as ‘Golden Delicious’, ‘Delicious’, ‘Gala’, ‘Fuji’ and ‘Braeburn’ in addition to cultivars that are locally produced and desired by consumers. As part of sustainable cultivation systems, cultivars also need to satisfy economical and ecological demands. Towards addressing ecological concerns, there is an intensive effort to develop disease resistant, high quality cultivars. In many apple production areas of the world the major fungal diseases are apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*). The bacterial disease fire blight (*Erwinia amylovora*) is a considerable threat to apple production and is difficult to control. Disease resistant varieties allow a significant reduction of orchard pesticide inputs in temperate climates, where a greater number of treatments are typically needed in comparison to other crops. The production of apples in adapted and sustainable systems could improve the image of apples as a healthy food and contribute to increased food safety. However, none of the disease resistant cultivars has yet achieved a breakthrough in the marketplace comparable to varieties such as ‘Golden Delicious’ or ‘Gala’. However the success of disease resistant varieties at the point of sale is not related to their disease resistance attributes, as the successful introduction of a new cultivar is primarily determined by wholesalers and retailers who perceive fruit quality as the principal consumer driver and design their marketing strategy accordingly. A recent consumer test performed by Agroscope Changins-Wädenswil, Switzerland, in a local supermarket revealed fruit quality criteria to be more important to consumers than the attribute ‘from organic production’ (Table 2). On the production side, disease resistant cultivars need to be durable with respect to resistance attributes.

3.1 Breeding Strategies for Durable Disease Resistance

The challenge of breeding varieties durably resistant to disease can be approached in different ways, including marker assisted selection. Gessler et al. (2006) performed a comprehensive review on *Venturia inaequalis* resistance in apple, including the

Table 2 Importance of different quality and production criteria to consumers in a survey in Switzerland, June 2004, 200 consumers, in percent

	Firmness	Crispness	Flavor	Organic production
Not important	10	6	5	57
Important	51	43	47	25
Very important	39	51	48	18

development of molecular markers for these resistances. The availability of molecular markers and genetic linkage maps enables the detection and the analysis of major resistance genes, as well as of quantitative trait loci (QTL) contributing to the resistance of a genotype (Liebhard et al., 2003b). A promising route to reduction of the risk of resistance breakdown is the combination of several functionally different resistances in a cultivar. Examples of molecular selection of genotypes with such pyramided genetic resistance against scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*) are promising. Molecular markers are available nowadays that allow the detection of the major scab resistances *Vf*, *Vr* and *Vbj* (Tartarini et al., 1999; Hemmat et al., 2002; Gygax et al., 2004) and the *Pl₁*, *Pl₂*, *Pl_d* and *Pl_w* mildew resistances (Markussen et al., 1995; Seglias and Gessler, 1997; James and Evans, 2004). At Agroscope Changins-Wädenswil, progress has been achieved in establishing a system for marker assisted selection (Frey et al., 2004) whereby up to eight markers are analysed in a single multiplex reaction, substantially reducing costs. Systems have been established that allow a microsatellite based screening on an automated fragment analyzer.

In the framework of the European DARE project (Durable Apple Resistance in Europe), local European cultivars were examined as sources for durable scab resistance in apple (Laurens et al., 2004). It is known that the *Vf* scab resistance widely used in apple breeding programs can be overcome by specific races or strains of the fungus (Parisi et al. 2002). During this research very diverse and complex resistance behaviors were found: the cultivars which showed the widest range of resistances were mostly local cultivars as well as some newly selected hybrids that combine major genes for resistance with partial resistances.

To achieve a comparable level of resistance to that conferred by major genes, several quantitative resistance loci would have to be combined. Liebhard et al. (2003a) performed a QTL analysis based on a genetic linkage map that was constructed by using a segregating population of the cross between the apple cultivars 'Fiesta' and 'Discovery'. The progeny was observed for three years at three different sites in Switzerland and field resistance against apple scab was assessed. The QTL analysis revealed 8 genomic regions whereby six conferred resistance against leaf scab and two resistance against fruit scab. However, the effectiveness of these QTLs has to be confirmed at higher disease levels, and in other genetic backgrounds.

There is also scope for breeding fire blight resistant apple cultivars by exploiting genetic variation in germplasm and by developing QTL markers. Fire blight, caused by the bacterium *Erwinia amylovora*, is the most serious bacterial disease of pipfruit. Forsline and Aldwinckle (2002) screened the USDA Apple Collection at

Geneva N.Y., including apple germplasm from Asia and Europe, for natural occurrence of fire blight and found no major resistance genes to this disease. However, QTLs for resistance to fire blight have been found in the cultivated apple *Malus × domestica* (cultivars ‘Fiesta’ and ‘Nova Easygro’; Khan et al., 2006, Khan et al., 2007) as well as in wild *Malus* species (*Malus robusta* 5, Peil et al., 2007).

3.2 Pest Resistance

Evidence for a genetic basis of pest resistance in apple has been given for several herbivore species, for example for the woolly apple aphid (*Eriosoma lanigerum*) (Knight et al., 1962), and the brownheaded leafroller (*Ctenopseustis obliquana*) (Wearing et al., 2003). An indication of the genetic regions associated with pest resistance has been provided for *Dysaphis devectora* (Roche et al., 1997) and *Eriosma lanigerum* (Bus et al., 2007).

3.3 Cisgenics in Fruit Trees

Introgression of desired traits from wild germplasm and specific donors into top quality apple cultivars is a challenge that can be approached efficiently by the new approach of cisgenesis (Schouten et al., 2006), whereby only the allele of interest is inserted. Cisgenesis is defined as the genetic modification of a plant, inserting genes of the same plant species or from other crossable relatives. A cisgenic plant does not contain genes from outside the gene pool of the classical breeder.

3.4 Recurrent Breeding Strategies

The Horticulture and Food Research Institute of New Zealand Ltd (Plant and Food Research) has established an Apple Genetics Population to maintain biodiversity for cultivar development (Noiton and Alspach, 1996) and to provide genetic information on important apple characteristics (Oraguzie et al., 2000, 2001). The population is anticipated to provide novel fruit characteristics for application in a long-term breeding strategy based on recurrent selection. Families were derived from open-pollinated seed from a wide range of apple cultivars, as well as crab apples (*Malus spp.*), contributed from repositories from different countries (Noiton et al., 1999). Adapted recurrent selection strategies such as those applied in New Zealand can be considered amongst the most promising strategies to keep and increase genetic variability.

3.5 Genetics of Apple

The majority of apples are diploids ($2n = 34$). However, some triploids and tetraploids exist. Genomics is now being used to gain a better understanding of

the genetic control of, as well as the interactions among traits. Whole genome sequences are being currently determined for *Malus* (see Chapter “Rosaceous Genome Sequencing: Perspectives and Progress”).

Genetic linkage maps allow the identification of quantitative trait loci (QTL), which can identify chromosomal regions controlling phenotypic traits (Collard et al., 2005). Such a linkage map should be densely covered with molecular markers, in order to obtain the maximum probability of identifying a QTL (Liebhard et al., 2003a). The saturation of linkage maps with molecular markers (AFLP, RAPD, SSR, SCAR markers) has been considerably improved during recent years (Liebhard et al., 2003a; Maliepaard et al., 1998; Silfverberg-Dilworth et al., 2006; Celton et al., 2008; Fernández-Fernández et al., 2008).

3.6 Sources for Increased Genetic Diversity

Some years ago, efforts were made to enlarge the genetic basis in apple by collecting material in the centers of origin. An expedition to Kazakhstan and Kyrgyzstan by Forsline (1995) was successful and *Malus* collections included 65 accessions (18,000 seeds) representing 3 species endemic to that area. Collection of cuttings of elite materials was kept to a minimum due to restrictions in the USA quarantine facilities. Some unique germplasm from areas that had not been previously explored was collected. Seven ecosystems were explored in 12 expeditions. In the meantime, this genetic material is being screened for a wide range of fruit and tree characters. The Fruit Genebank at Dresden-Pillnitz (GER) collected about 7000 seeds from 55 accessions, as well as scions from 28 accession of *Malus hupehensis*, *Malus kansuensis*, *Malus prattii*, *Malus sieboldii*, *Malus transitoria* and *Malus toringoides* in 2001 during an expedition to 6 sites in the Chinese provinces Sichuan and Chongqing (Geibel and Hohlfeld, 2003).

A national inventory of top and small fruit genetic resources in Switzerland completed in spring 2005 (Kellerhals and Egger, 2004) is the basis for a complete and secure conservation of fruit genetic resources in Switzerland. In the course of the inventory, a fundamental project of the national plan of action, information was collected regarding the origin, abundance and frequency of accessions.

Inventorying, collecting, characterizing, evaluating and utilizing the fruit genetic resources e.g. in a breeding program are of great public relevance. It enables retention and utilization of a heritage for future generations, broadening the genetic basis in breeding programs and the meeting of consumers' evolving demands for healthy new and innovative products. The inventory of the national collection has demonstrated the rich genetic fruit diversity in Switzerland. We have used classical pomological knowledge to verify and determine varietal trueness-to-type. However, many samples remained undetermined, probably being unnamed chance seedlings or unknown varieties. It might be worth considering modern molecular techniques to determine the correct varieties and to highlight synonyms and homonyms (King et al., 1998).

4 Genetic Resources

The United Nations Conference on Environment and Development was held in Rio de Janeiro in 1992. A key agreement adopted was the Convention on Biological Diversity (CBD). The convention has fostered international activities for the conservation and sustainable use of plant genetic resources for food and agriculture. Three main goals are followed: the conservation of biological diversity, its sustainable use, and the sharing of benefits from their use. The conservation of plant genetic resources (PGR) has gained significantly in importance and is now accepted as an essential responsibility of national governments (Engels, 2002). This situation is demonstrated by the impressive number of nations which have ratified the CBD, endorsed the International Undertaking on Plant Genetic Resources, or both. Bioversity is the world's largest international research organization dedicated solely to the conservation and use of agricultural biodiversity and is non-profit and independently operated. In 2006, IPGRI (International Plant Genetic Resources Institute) and the International Network for the Improvement of Banana and Plantain (INIBAP) became a single organization. IPGRI and INIBAP changed their name to Bioversity International. The new name reflects an expanded vision of its role in the area of biodiversity research for development, seeking to advance the conservation and use of plant genetic diversity for the well-being of present and future generations. The European Cooperative Program for Plant Genetic Resources (ECPGR) is a collaborative program including most European countries and is aimed at facilitating the long-term conservation and increased utilization of plant genetic resources in Europe. It was founded in 1980 on the basis of the recommendations of the United Nations Development Program (UNDP), the Food and Agriculture Organization of the United Nations (FAO) and the Genebank Committee of the European Association for Research on Plant Breeding (EUCARPIA). The program is entirely financed by the member countries and coordinated by IPGRI. The ECPGR Documentation and Information Network, with the creation of crop specific and multi crop databases has contributed to making information available on ex situ conserved germplasm. As a result of an EU-funded project and ECPGR support, the EURISCO catalogue (<http://eurisco.ecpgr.org/>) provides on-line passport information on accessions conserved in European collections. The ECPGR network on fruit comprises working groups on *Malus/Pyrus*, *Prunus* and *Vitis*. Special central databases for each species are established and currently a European *Malus/Pyrus* collection is being established. As in many crops, the genetic diversity in fruit species has considerably decreased in the last decades and a few fruit species and cultivars grown worldwide have become predominant. ECPGR decided to establish an effective, efficient and rational European conservation system, with an initial focus on existing ex situ genebank collections in European countries. The goal is to create **A European Genebank Integrated System (AEGIS)** for plant genetic resources for food and agriculture, aimed at conserving the genetically unique and important accessions for Europe and making them available for breeding and research. Such material will be safely conserved under conditions that ensure genetic integrity and viability in the long term.

The United States Department of Agriculture (USDA) Agricultural Research Service (ARS) maintains a collection of apple germplasm in Geneva NY that includes over 8500 accessions representing at least 50 species. Of these, some 2600 accessions are clonally propagated cultivars, 3100 are seedlings mostly representing species collections, 1600 are in the form of seed, and 1250 are wild by elite hybrids that were generated specifically for genetic studies (Simon et al., 2008). The core mission of this collection includes the acquisition, maintenance, characterization and distribution of the diversity of *Malus*. The collection was primarily characterized with 154 descriptors including pomological, pathological, anatomical and physiological characteristics which are recorded in the Germplasm Resources Information Network (GRIN: www.ars-grin.gov). A set of microsatellite markers was recently included and the data are publically available.

4.1 Cryoconservation

The long-term storage of germplasm under cryogenic conditions is an efficient approach (Towill et al., 2004). The USDA – ARS, Plant Genetic Resources Unit at Geneva, NY (USA) has processed 1915 accessions of *Malus* representing 48 species using a winter vegetative bud method for cryopreservation. Overall the method has been successful with 91% of the lines tested having viability after cryo-exposure of 40% or more. Genotypic differences in survival were observed and the success varied from year to year. For *M. × domestica* 95% of the accessions tested have been cryopreserved. For species other than *M. × domestica*, 83% met the criterion. As an internal control, eight lines have been collected, cryopreserved and recovered through grafting each year. While genomics and bioinformatics are essential tools to understand and exploit variation of genes, genomes and genepools, cryopreservation with its minimal requirements of space and maintenance continues to gain importance as a means of long term physical storage of genetic materials (Forsline et al., 2003).

Breeding strategies most often consider the most advanced selections and cultivars and thus lead to a narrowing of the genetic base. The introgression of traditional varieties and accessions into the genepool is often feared due to undesirable characteristics that might be incorporated. However, there is scope for considering a wider genetic basis in apple breeding for sustainable fruit production. The directed use of genetic resources in genetic apple improvement programs could address many components of sustainable apple growing systems.

4.2 Nutritional Value and Health Issues

Regular consumption of fruits and vegetables in general are considered to decrease the risk of chronic diseases such as cancer and cardiovascular diseases. Apples are low in energy, but good sources of vitamins, minerals, pectin as well as secondary plant metabolites. Based on epidemiological studies, it seems that apples may play

a role in maintaining a healthy lifestyle in general. Apple consumption may be associated with a reduced risk of cancer, heart disease and Type II diabetes as well as an increased weight loss when compared to other fruits and vegetables (Boyer and Rui 2004). Pectin, as a soluble fibre has a positive impact on satiety and may attenuate blood glucose and blood lipid levels when ingested in high amounts.

Apples contribute importantly to the flavanoid intake in humans. Thus they act as major antioxidants that may scavenge and neutralize free radicals, which in turn play a role in the onset of degenerative diseases (Biedrzycka and Amarowicz 2008). However, their contents vary considerably with the variety, storage conditions and other pre- and postharvest conditions. Stushnoff et al. (2003) examined juice and fruit tissue from 321 *Malus* species, selections and cultivars from the USDA Plant genetic Resources Unit at Geneva N.Y. A huge diversity in total phenolics and antioxidant capacity was detected with the aim of supplying breeders with data on antioxidant composition as a guide to parental selection. Information is being gathered on phenols and flavonols to benefit postharvest physiology studies. Data on the compositional diversity of apple germplasm is retained to aid medical epidemiological and dietary intervention studies, as well as identify bioextracts for supplemental application. Fruit were collected from the core collection at Geneva, NY, from wild apples collected in Central Asia, and from apple trees in the University of Minnesota germplasm collection.

Approximately 2% of the West-European population has an apple allergy. The use of molecular markers for allergy research is new. Apple allergy is dominated by IgE antibodies against Mal d 1 in areas where birch pollen is endemic. Apples with significantly decreased levels of Mal d 1 would allow most patients in these areas to eat apples without allergic reactions (Gilissen et al. 2005). Mal d 1 expression was successfully reduced by RNA interference. This translated into significantly reduced *in vivo* allergenicity. These observations support the feasibility of the production by gene silencing of apples hypoallergenic for Mal d 1 (Gilissen et al. 2005).

5 Looking Forward

Apple's unique cultivation history and genetic resources have led to the production of outstanding cultivars through many breeding programs. Today's present day challenges will require implementation of these same resources in new and clever ways. The increasing genomics tools in the species will accelerate genomics-enabled breeding strategies, ensuring a continued supply of apples that contribute to their sustained cultivation as well as human health and nutrition.

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