Breeding Apple (*Malus* × *Domestica* Borkh)

S. Pereira-Lorenzo, A.M. Ramos-Cabrer, and M. Fischer

1 Introduction

The apple tree is a hybrid originating from a combination of wild species (*Malus sieversii* is supposed to be the main contributor). Growers at first selected the best specimens by seedlings, but when grafting was discovered as a mean of vegetative propagation, improvement in fruit quality became faster. Apple is cultivated in most of the temperate regions due to the fruit's quality, its easiness to propagate, and its natural aptitude to bear. Apples are considered a healthy fruit, as the saying goes 'an apple a day keeps the doctor away'. An apple tree can reach up to 10 m height above its own roots, having a globose canopy and the longevity between 60 and 100 years. Depending on the rootstock and the age of the tree, the roots can occupy between 2 and 104 m^2, although most frequently they range between 10 and 30 m^2 (Atkinson 1980).

1.1 Reproductive Biology

The apple tree is a monoecious species with hermaphroditic flowers. Three to six flowers in cymes (the first flower is the most advanced) appear in mixed buds (Dennis 1986, 2003). It produces rose epigynous flowers, sometimes white, with five sepals, petals, and pistils and up to 20 stamens. The development of a multicarpellate inferior ovary (forming the core) and the accessory tissue after fecundation becomes in the fruit known as pome (Ryugo 1988).

Apples are self-incompatible though some cultivars are partially self-compatible (Lespinasse 1992). Most of the apple cultivars are diploid ($2n = 34$ chromosomes) and some of the main cultivars are triploid ($2n = 3x = 51$), e.g., 'Boskoop', 'Kaiser

S. Pereira-Lorenzo
Escola Politécnica Superior, Departamento Producción Vegetal, Universidad de Santiago de Compostela, Campus de Lugo, 27002
e-mail: santiago.pereira.lorenzo@usc.es

S.M. Jain, P.M. Priyadarshan (eds.), *Breeding Plantation Tree Crops: Temperate Species*, DOI: 10.1007/978-0-387-71203-1_2,

Wilhelm', 'Gravensteiner', 'Jonagold', and mutants, 'Kanadarenette' and others. Triploids are not suitable as pollinators. Mostly wild species are diploid, and a few are triploid and tetraploid. Pereira-Lorenzo et al. (2007) and Ramos-Cabrer et al. (2007) found that 29% of the local cultivars in northern Spain were triploids, producing an average of 15% heavier apples.

Parthenocarpic apple cultivars exist but they are not relevant in commercial production (Dennis 1986). As most cultivars are self-unfruitful, cross-pollination, mainly by insects, is necessary. Knowledge of possible combinations is needed for the best success of apple production (Table 1), since only 10–30% of the flowers develop into fruit.

Self-incompatibility in apple is of gametophytic type and is controlled by a single multiallelic locus named the S-locus (Broothaerts et al. 2004). Pollen

Table 1 Cross-pollination between new apple cultivars (adapted from Fischer 2000)

Pollinator Mother cultivar	Pia	Piflora	Pikant	Pilot	Pingo	Pinova	Pirol	Piros	Reanda	Rebella	Reka	Relinda
Pia	–	+		+	+	+	+	+	+	+	+	
Piflora	+	–		+	+	+	+	+	+	+	+	
Pikant		+	–	+	+	+		+	+	+	+	+
Pikkolo			+	+		+		+				
Pilot	+	+	+	–		+			+	+	+	
Pingo	+	**o**		+	–	+	+		+	+		
Pinova	+	+	+	+		–	+	+	+	+	+	
Pirol	+	+			+	+	–	+	**o**	+		
Piros			+			+		–	+		+	
Reanda		+	+	+		+	**o**	+	–	+	+	+
Rebella	+	+	+	+	+	+	+	+		–	+	+
Regine	+	+	+	+	+	+	+	+	+	+	+	+
Reglindis		**o**	+			+			+	+	+	+
Reka			+	+		+		+	+		–	+
Releika				+	+	+	+	+	+	+	+	
Relinda									+			–
Remo	+		+	+		+	+	+	+		+	+
Rene	+		+								+	+
Renora	+	+	+	+	+	+	+		+	+	+	+
Resi						+	**o**	+		+		+
Retina	+	+	**O**				**o**	+	+	+	+	+
Rewena	+	+	+	+		+		+	+	+	+	+
Elstar			+	+		+		+		+	+	+
Golden Delicious	+	**o**	+	+	+	+	+	+	+	+	+	+
Idared	+	+	+	+	+	+	+		+	+		
Jonagold	+	**o**	**O**	**o**			+			+	+	**o**
Prima			**O**				**o**				+	
Shampion			+	+		+						

Table 1 (continued))

Remo	Renorn	Resi	Retina	Rewena	Elstar	Golden Delicious	Idared	James Grieve	Jonagold	Prima	Shampion
	+	+	+	+	+	+	+	+	−		
		+	+			o					
+	+	+		+	+	+	+	+	−	+	+
+	+					+	+	+	−		+
+				+	+	+	+	+	−		o
		+	+		+		+				
+		+	+	+	+	+	+	+	−		+
		+	+		−	+	+	+			
+	+		+			+	+	+	−	o	+
+			+	+		+	+	+	−	+	
+	+	+	+	+		+	+	+	−		
+	+	+	+	+	+	+	+	+	−		
+			+				+	+	−	+	
+	+	−	+	+		+	+	+	−	+	
+	+	+	+	+			+	+			
+			+	+			+	+		+	
−	+	+	+	+	+	+	+	+	−	+	
+			+	+			+	+			
+	−	+	+	+			+	+	−	+	
+		−	+	+			+	+	−	+	
+			−	+		+	+	+	−	+	
+			+	−		+	+	+	−	+	
+			+	+	−	+		+	−	+	−
+		+	+	+	+	−	+	+	−		
+		+		+		+	−	+			+
			o	−	o	−	+	+	−		−
+			+					+		−	
					o	o	+	+	−		−

tubes elongate through the styles. As they grow, they are attacked by cytotoxic proteins. Expression of specific inhibitors avoids a lethal attack. Style toxic proteins are the product of the S-gene (S-RNases). Pollen tube growth is inhibited when the pollen shares the same S-allele with the pistil on which the pollen germinates. Eighteen different S-alleles have been differentiated; only three of them are the most frequent (*S2, S3*, and *S9*).

1.2 Main Species

Apple, pear, plum, and peach trees belong to the Rosaceae family. Apple and pear, as other genera, have been classified inside Maloideae family because they produce pome type fruits (Bretaudeau and Faure 1991; Janick et al. 1996).

Scientific nomenclature for apples has changed since Linnaeus denominated *Pyrus malus*. Other denominations in the past have been *M. communis*, *M. sylvestris*, *M. pumila*, and *M. domestica* (Ryugo 1988; Harris et al. 2002). The domesticated apple is the result of an interspecific hybridization named *Malus* × *domestica* Borkh (Janick et al. 1996). This name has been substituted to the previous *M. pumila* (Forsline et al. 2003). The cultivated apple is a functional diploid ($2n = 34$) (Ryugo 1988) although it is frequently present as triploids (Pereira-Lorenzo et al. 2007).

The number of species in the genus *Malus* is uncertain and still under controversy. Robinson et al. (2001) explained that the number of species in genus *Malus* depends upon the rank given to several taxa, species being subspecies and putative hybrids, and the nomenclature of the taxa is complex. Harris et al. (2002) pointed out about 55 species (between 8 and 79 have been recognized) according to the classification of Phipps et al. (1990). Zhou (1999) classified 30–35 species. Only 17 are recorded in the USDA, NRCS (2006) Plants Database (www.plants.usda.gov) (Table 2) (Fig. 1).

Way et al. (1990a, b) gave details of 33 main species. Forsline et al. (2003) referred to 27 primary species, 5 secondary ones, and 11 *Malus* species hybrids. Janick et al. (1996) counted 37 species and 16 secondary ones (*Malus* species hybrids). Way et al. (1990a, b) and Janick et al. (1996) agreed in nine species in series Pumilae in which they also included *M. domestica* and *M. sieversii*. Forsline et al. (2003), however, denominated that series as Sieversinae, including *M. sieversii* but not *M. domestica* as it has now been considered a natural hybrid, *M. x domestica*. The classification of 27 primary apple species according to Forsline et al. (2003) is included in Table 2, with indications of the origin and use when these are known. A total of 22 of 27 species (82%) are from Asia (11 located mainly in China), 4 in North America, 2 in Europe, and 1 in Japan. Six species are used for fruit—*M. sieversii*, *M. sylvestris, M. angustifolia, M. ioensis, M. coronaria*, and *M. hupehensis*. Five out of 27 are recognized as ornamental and 12 as possible rootstocks.

1.3 Climatic and Environmental Requirements

The apple tree adapts well to different climates. Apple is cultivated from northern Europe down to the tropics where two crops can be obtained at high altitudes. It has been introduced in South America, South-Africa, New Zealand, and Australia. Most of the old cultivars require a long rest period, but new selections with less requirements allow them to be cultivated in subtropical areas. Petropoulou (1985) classified apple cultivars attending to chilling requirements in six classes and related a shorter rest period with lower growth (Table 3). Some cultivars are very resistant to low temperatures (−35°C). Some were selected for very short seasons, 3 months from blooming, while others require up to 6 months.

Table 2 Species in *Malus* genus (adapted from Forsline et al. 2003; USDA, NRCS 2006)

Sections	Series	Primary species	Commom name	Origin	Uses
Malus Langenf.	*Sieversinae* Langenf.	*M. sieversii* (Lodeb.) Roem.		Asia	Rootstock, fruit
Malus Langenf.	*Sieversinae* Langenf.	M. orientalis	Caucasian apple	Asia	
Malus Langenf.	*Sieversinae* Langenf.	*M. sylvestris* (L.) Mill.	European crabapple	Europe	Fruit, roostock
Baccatus Jiang	*Baccatae* (Rehd.) Rehd.	*M. baccata* (L.) Borkh.	Siberian crabapple	Asia	Rootstock, ornamental
Baccatus Jiang	*Hupehenses* Langenf.	*M. hupehensis* (Pampan.) Rehd.	Chinese crab apple, tea crab apple	China, Taiwan	Rootstock, ornamental, fruit
Baccatus Jiang	*Hupehenses* Langenf.	*M. halliana* (Anon.) Koehne	Hall crab apple	China	Rootstock, ornamental
Baccatus Jiang	*Sikkimenses* Jing	*M. sikkimensis* (Wenzig) Koehne		Asia	Rootstock
Sorbomalus Zabel.	*Sieboldiane* (Rehd.)	*M. sieboldii* (Regel) Rehd.	Toringa crab apple	Asia	Rootstock, ornamental
Sorbomalus Zabel.	*Kansuenses* (Rehd.) Rehd.	*M. kansuensis* (Batal.) Schneid.		China	
Sorbomalus Zabel.	*Kansuenses* (Rehd.) Rehd.	*M. transitoria* (Batal.) Schneid.		China	Rootstock
Sorbomalus Zabel.	*Kansuenses* (Rehd.) Rehd.	*M. toringoides* (Rehd.) Hughes	Cutleaf crab apple	China	Rootstock
Sorbomalus Zabel.	*Kansuenses* (Rehd.) Rehd.	*M. komarovii* (Sarg.) Rehd.		China, North Korea	

Table 2 (continued)

Sections	Series	Primary species	Commom name	Origin	Uses
Sorbomalus Zabel.	*Kansuenses* (Rehd.) Rehd.	*M. xiaojinensis* Cheng et Jiang		China	
Sorbomalus Zabel.	*Kansuenses* (Rehd.) Rehd.	*M. fusca* (Raf.) Schneid.	Oregon crabapple	Northern America	
Sorbomalus Zabel.	*Yunnanenses* Rehd.	*M. yunnanensis* (French) Schneid.		Asia	Rootstock, ornamental
Sorbomalus Zabel.	*Yunnanenses* Rehd.	*M. prattii* (Hemsl.) Schneid.		China	
Sorbomalus Zabel.	*Yunnanenses* Rehd.	*M. honanensis* Rehd.		China	
Sorbomalus Zabel.	*Yunnanenses* Rehd.	*M. ombrophilla* Hand.-Mazz.		China	
Sorbomalus Zabel.	*Florentinae* Rehd.	*M. florentina* (Zuccagni) Schneid. (= *M. crataegifolia* (Savi) Koehne)	Florentine crab apple, hawthorn-leaf crab apple	Europe	
Chloromeles (Decne.) Rehd.		*M. ioensis* (Wood.) Brit.	Prairie crabapple, western crab apple	Northern America	Ornamental, fruit
Chloromeles (Decne.) Rehd.		*M. coronaria* (L.) Mill.	sweet crabapple	Northern America	Ornamental, fruit
Chloromeles (Decne.) Rehd.		*M. angustifolia* (Ait.) Michx.	Southern crabapple	North America	Fruit, ornamental
Docyniopsis Schneid.		*M. doumeri* (Bois.) Chev.		Asia	Rootstock
Docyniopsis Schneid.		*M. melliana* (Hand.-Mazz.) Rehd.		China	

Table 2 (continued)

Sections	Series	Primary species	Commom name	Origin	Uses
Docyniopsis Schneid.		*M. tschonoskii* (Maxim.) Schneid.	Pillar apple	Japan	
Docyniopsis Schneid.		*M. laosensis* Chev.		Asia	Rootstock
Eriolobus (D.C.) Schneid.		*M. trilobata* (Poiret) Schneid.		Asia, Europa	
Cultivated species *Malus* and secondary species including hybrids					
M. × a*rnoldiana* (Rehd.) Sarg. (*baccata* × *floribunda*)					
M. × *atrosanguinea* (Spaeth) Schneid. (*halliana* × *sieboldii*)					
M. × *domestica* Borkh.			Cultivated apple		Fruit, rootstock
M. × *hartwigii* Koehne (*halliana* × *baccata*)					
M. × *micromalus* Mak. (*baccata* × *spectabilis*)					
M. pumila Miller					
M. × *purpurea* (Barbier) Rehd. (*neidzwetzkyana* × *atrosanguinea*)					
M. × *soulardii* (Bailey) Brit. (*ioensis* × *domestica*)					
M. × *sublobata* (Dipp.) Rehd. (*prunifolia* × *sieboldii*)					
M. × *asiatica* Nakai					
M. × *dawsoniana* Rehd. (*fusca* × *domestica*)					
M. floribunda Siebold					
M. × *magdeburgensis* Schoch. (*spectabilis* × *domestica*)					
M. × *platycarpa* Rehd. (*coronaria* × *domestica*)					
M. prunifolia (Willd.) Borkh.					
M. × *robusta* (Carr.) Rehd. (*baccata* × *prunifolia*)					
M. spectabilis (Ait.) Borkh.					
M. zumi (Mats.) Rehd. (*mandshurica* × *sieboldii*)					

Fig. 1 Species in *Malus* genus (adapted from Forsline et al. 2003; USDA, NRCS 2006) (With credits to USDA, ARS, Plant Genetic Resources Unit, Geneva) (*See* Color Insert)

Fig. 1 (continued)

Table 3 Chilling requirements of cultivars and rootstocks apple (adapted from Petropoulou 1985)

Cultivar	Chilling requirements	Days to blooming
Rome Beauty	2700–3100	201
Ingrid Marie	2300–2700	152
Keswick Codlin	2300–2700	136
Antonouka	1900–2300	129
Kidd's Orange Red	1900–2300	115
Early Victoria	1450–1900	106
Cox	1000–1450	99
Winter Banana	1000–1450	98
Falstaff	300–1000	77
Starkspur Golden Delicious	300–1000	77
Greensleeves	300–1000	74
Rootstocks		
M16	2300–2700	139
M25	1800–2300	103
M7	1350–1800	65
M27	950–1350	55
M9	950–1350	44

2 History

The origin of the cultivated apple, *Malus x domestica*, is the genepool of *Malus sieversii* in Middle Asia. Vavilov located the center of origin for *Malus communis* (*M. sieversii*) in Turkistan, Central Asia (Vavilov 1951). The use of molecular markers could confirm that the wild apple located in Central Asia could be the major maternal contributor to the domesticated apple (Harris et al. 2002). Wild fruits were selected and propagated by indigenous populations before 6500 B.C. Cultivation and domestication moved westward along the Silk Road and possibly along a second northern way across Central Russia. Introgression of *Malus orientalis* and *M. sylvestris* var. praecox and *M. sylvestris* var. sylvestris was reduced in *Malus x domestica*. The first mention of cultivated apples in ancient Greece dates from the 9th century B.C. Later on, apples were introduced into the Mediterranean regions and Central Europe by the Romans. Columela wrote about grafting and the most preferred apple cultivars in the year 42 A.D. Greeks and Romans spread the culture across Europe. In East Asia, crossings between *M. sieversii* and *M. baccata* developed the hybridogenic species *Malus x asiatica*, which has been used as local fruit crop since ancient times (Tian shan) (Büttner et al. 2000). In the middle ages, apple culture was promoted greatly around monasteries. By the end of the 12th century, some famous cultivars were known, such as 'Pearmain' and 'Costard' (Morgan and Richards 1993). In the 16th century, dwarf rootstocks were recommended to graft selected cultivars (Tubbs 1973). By the beginning of the 20th century, the main objective of

breeding was to transfer the high-quality traits of the fruit along with resistance to three economically important apple diseases: fire blight, scab, and powdery mildew. A review can be found further on.

2.1 Cider History

Orton (1995) explained how the first apple beverage could have been made with crab apples (wild apples). Hebrews called cider 'Shêkar', the Greeks 'Sikera' (a drink obtained by cooking apples with fermented juice), and a beverage, 'Phitarra', was obtained by boiling pieces of apples in water with honey in the Basque country (www.applejournal.com/fr05.htm). By the end of the 4th century, the Latin word 'Sicera' was introduced, becoming Cider in English, Sidre and Cidre in French, and Sidra in Spanish. In France and Spain, apple trees for cider production were planted abundantly from the 10th and 11th century on (Boré and Fleckinger 1997; Rivas 2004). In the 15th century, fruit growing specialists recommended the use of sour-sweet apples to improve taste and the addition of a few acid apples to avoid blackening. With the spreading of phylloxera among the vineyards, cider began to replace wine.

Cultivars for cider production were essential in the development of the cider industry. The first apple description for cider production (65 cultivars) was published in France in 1589 (Boré and Fleckinger 1997). The first selections for cider production also began in France in 1883 with a detailed study for each region. During 1949 and 1970, more than 1000 cultivars were collected and identified, 70 of them being recommended for cider production. Since 1953, five cultivars have been selected for juice production in France ('Judor', 'Jurella', 'Judeline', 'Judaine', and 'Juliana') and one for cider ('Cidor'). In Germany, some of the multiresistant Re-cultivars® have been recommended for processing since 1990 ('Remo', 'Rewena', 'Relinda', and 'Rene') (Fischer et al. 2001a).

Cider apple production dropped considerably between 1968 (2 millions t) and 1990 (650,000 t) (Boré and Fleckinger 1997). Although new plantations are being established, cider production is based mainly on traditional orchards with high vigor and scattered apple trees, over 10 million in 1990. A similar situation can be found in Spain where regular plantations for cider production only account for about 8000 ha in the northern regions (MAPA 1990).

3 Socioeconomic Importance

3.1 Area and Production

Apples are cultivated mainly in temperate zones, and they adapt very well to different climates. The cultivated area in 2004 was 51.6 million ha with a total production of 61.6 million t (Table 4). The main area of apple production is

Table 4 Apple area (ha) and production (t) in 2004

Country	Area (ha)	Production (t)	Country	Area (ha)	Production (t)
Albania	2300	120,000	Australia	30,000	484,096
Belarus	68,000	200,000	New Zealand	11,000	500,000
Belgium	8272	323,800	Oceania	41,000	984,096
Czech Republic	12,700	280,781			
France	58,180	2,216,940			
Germany	70,000	1,592,000	Morocco	26,100	393,140
Greece	15,500	288,000	Egypt	29,000	490,000
Hungary	36,000	680,000	Algeria	30,000	1,262,444
Italy	61,469	2,069,243	South Africa	31,000	762,558
Moldova, Republic of	70,000	338,000	Tunisia	32,000	121,000
Netherlands	10,000	436,000	Africa	148,100	3,029,142
Poland	160,000	2,500,000			
Portugal	21,600	287,600	Israel	6000	125,000
Romania	120,235	1,097,837	Armenia	8000	300,000
Russian Federation	386,000	16,000	Lebanon	9400	140,000
Serbia and Montenegro	27,000	183,571	Turkmenistan	12,000	40,000
Slovenia	3293	230,000	Kyrgyzstan	25,000	123,000
Spain	40,000	603,000	Korea, Republic of	26,000	350,000
Switzerland	5190	230,000	Georgia	28,000	60,000
Turkey	108,900	2,300,000	Tajikistan	40,000	93,000
Ukraine	150,000	850,000	Kazakhstan	41,000	140,000
UK	9000	125,000	Japan	41,300	881,100
USSR		2,030,000	Pakistan	48,000	380,000
Others	89,439	446,450	Syrian Arab Republic	48,000	215,000
Europe	1,533,078	19,444,222	Azerbaijan, Republic of	50,000	220,000
			Korea, Dem People's Rep	71,000	660,000
Peru	9900	146,083	Uzbekistan	94,000	500,000
Canada	20,813	370,338	Iran, Islamic Rep of	150,000	2,400,000
Brazil	32981	977,863	India	250,000	1,470,000
Chile	39,000	1,250,000	China	2,100,550	22,163,000
Argentina	40,000	56,000	Others	10,148	105,636
Mexico	62,000	503,000	Asia	3,058,398	30,365,736
USA	162,500	4571,440	World	**5,160,190**	**61,823,546**
Others	12,420	125,626			
America	379,614	8,000,350			

Source: www.fao.org

located in Asia, a nucleus that accounts for nearly double in terms of area and production in comparison with Europe. The main producers are China, Poland, Turkey, France, USA, and Algeria.

3.2 Market Uses

Apples are produced mainly for the fresh market (Way and McLellan 1989). In the USA, apples are processed into five basic products, viz., juice, canned puree, canned slices, dried apples, and frozen slices. Apple juice and canned sauce are the dominant products (one-half and one-third, respectively). Apples are also processed into vinegar, jelly, apple butter, mincemeat, and fresh slices. Small quantities are also made into apple wine, apple essence, baked whole apples, apple rings, and apple nectar. All these products represent between 44% and 46% of the apple production in the USA (Way and McLellan 1989). Another important product is cider, mainly in France, the UK, and Spain, although it is gaining popularity in the USA. Smock and Neubert (1950) consider that the most important product prepared from apples is pure fermented apple juice or cider, except in the USA and Canada.

Clarified apple juice is the main product and its preparation involves pressing, clarification treatment, filtration, and packaging (Bump 1989). Natural apple juice comes from the press and the addition of ascorbic acid or heating makes it flocculate and form unstable compounds. Pulpy (crushed) apple juice has a light color and a high pulp content. To produce pulpy apple juice, washed apples are coarsely grinned and the mash processed in a pulper with a fine screen. The pulped juice is passed through a vacuum chamber for deaeration to minimize oxidation and preserve its light color. Frozen apple juice concentrate production is based on a concentrate of 43° Brix to 70–74° Brix that is reconstituted in clarified or natural types by adding water. Apple juice and concentrates are used as base for blended fruit juices and drinks.

In the USA, 'apple wine' is distinguished from cider by its higher content of alcohol due to the adding of sugar during fermentation or by adding alcohol after fermentation or both (Smock and Neubert 1950). 'Apple brandy', a distilled cider product, can be used directly for consumption or for fortifying apple wine. The meaning of the term cider can vary depending upon the region of the world (Downing 1989). In England, it is known as 'fermented juice', 'hard cider' in the USA, 'cidre' in France, 'sidre' in Italy, 'sidra' in Spain, and 'Apfelwein' or 'Apfelmost' in Germany and Switzerland. There are several types of cider depending upon the preparation method (Smock and Neubert 1950; Downing 1989). 'Sparkling sweet cider' is produced by fermenting apple juice just enough to give it some effervescence and it contains less than 1% alcohol by volume. Fermentation and further steps are carried out in a closed system to retain the natural carbon dioxide that forms. 'Sparkling cider' also

retains gas produced during fermentation, but it has a low sugar and high alcohol content (3.5%). 'Sweet cider' is a noneffervescent cider produced by partial fermentation of apple juice in an open tank or by adding sugar to a completely fermented juice. 'Dry cider' is a completely fermented apple juice, commonly called 'hard cider', with an alcohol content of 6–7%. 'Carbonated cider' refers to any cider charged with commercial carbon dioxide to produce effervescence. 'Champagne-type cider' is produced in a similar way to champagne, effervescence being produced in the final product by a secondary fermentation of the dry cider in bottles. Sugar and champagne yeast are added before bottling.

Sugar is responsible for the softness in apple juice, whereas acid (normally measured as malic acid) gives it the tartness. Tannins support astringency, referring to the bite, the body, or the pungency (Downing 1989). Levels of sugar and acid are normally measured by chemical tests, while astringency is judged best by taste. Juice is preferred to make the body of soft cider not too sweet or too heavy (Downing 1989). Astringency is less significant than a correct sugar–acid ratio and the juice should not have more than 0.1% tannin. Downing (1989) pointed out that juice used for fully fermented and sparkling cider should be high in sugar, of moderate acidity, and fairly astringent.

4 Genetic Resources

4.1 Centers of Origin

In 1930, Vavilov suggested that Turkistan was the area where *M. sieversii* and *M. domestica* could have originated (Robinson et al. 2001). These wild species produce apples quite similar to domestic ones. This area offers a great variety in apples; therefore many authors agree that Central Asia is the center of origin of *M. domestica* (Janick et al. 1996). Zhou (1999) referred China as the origin place since about 80% of all species of the genus can be found in this country. Büttner et al. (2000) suggested that some *Malus* species with large fruits developed between Middle Asia to Central Europe. They consider that out of that genepool in Middle Asia, *M. sieversii* contributed the most to the origin of *M. x domestica*. The 'Silk Road' could have brought about introgressions of *M. orientalis* and *M. sylvestris* var. praecox from Caucasia and southeastern Russia. According to these authors, the indigenous species in Central Europe, *M. sylvestris* var. sylvestris, were not involved in the domestication of the apple. However, Boré and Fleckinger (1997) and Luby (2003) pointed out that hybridization could have contributed to diversify local apple cultivars. Janick et al. (1996) and Forte et al. (2002) consider that *M. sieversii* could have hybridized with other species such as *M. orientalis, M. sylvestris, M. baccata, M. mandshurica*, and *M. prunifolia*. With the support of the nuclear ribosomal internal transcribed

spacer (ITS), Harris et al. (2002) explained that the Central Asian wild apple and the domesticated apple can be grouped with *M. asiatica*, *M. orientalis*, *M. niedzwetzkyana*, and *M. prunifolia*. Apple selections could have been introduced directly from wild species in Western Europe and later on, hybridizations could have been important in bringing about new cultivars with specific characteristics (Harris et al. 2002).

4.2 Germplasm Banks Worldwide

An extensive review on European *Malus* germplasm has been made available (IPGRI 1996). More than 30,000 accessions are conserved *ex situ*. Most of those accessions were characterized and evaluated using IBPGR (1982) and UPOV (1974) descriptors. However, these efforts were not enough to compare the complete variability found in Europe. A minimum number of data (Passport data) was collected in European *Malus* database (Maggioni et al. 1997) without success.

In the USA, a total of 4179 accessions are maintained in repositories, of which 1456 corresponded to *Malus x domestica* (www.ars-grin.gov). The exact number of accessions is still unknown.

One of the most important European *Malus* gene banks is located in Germany (Dresden-Pillnitz) with more than 300 accessions of *Malus* species and hybrids, and nearly 1000 apple cultivars from around the world (Fischer and Fischer 2000; Fischer et al. 2003).

In Spain, the main resources are located in Asturias, Galicia, Navarra, País Vasco (Basque country), and Zaragoza (Dapena 1996; Itoiz and Royo 2003; Pereira-Lorenzo et al. 2003; Pereira-Lorenzo et al. 2007). Research programs are focusing on the development of apple cultivars for dessert and cider production.

High costs and damage risks from pests and diseases or the environment encouraged the development of cryopreserved, dormant apple buds for cultivars (Forsline et al. 1998; Forsline 2000). On populations, Volk et al. (2005) evaluated the minimum number of seedlings needed to capture more than 90% of the genetic diversity of the original populations and stated that a total of 35 trees within each population should be used as parents in crosses in order to obtain seeds for long-term *ex situ* conservation of *M. sieversii*.

5 Breeding Objectives and Tools

5.1 Cultivars

Until the mid 20th century, most apple cultivars were selected from seedlings (Janick et al. 1996). Apple diversity is very high due to polymorphism (Pereira-

Lorenzo et al. 2003, 2007), but commercial types depend on a reduced number of cultivars. Noiton and Alspach (1996) determined that 64% of 439 selections had their origin in among five cultivars: 'McIntosh' (101 cultivars), 'Golden Delicious' (87 cultivars), 'Jonathan' (74 cultivars), 'Cox's Orange Pippin' (59 cultivars), and 'Red Delicious' (56 cultivars). Among them, 96 cultivars had two or more as parents. Other cultivars used frequently in crosses were 'James Grieve', 'Rome Beauty', and 'Wealthy'.

Estimations have shown that in the last 5 years, 43% of the registered cultivars in France were mutations from commercial cultivars in use at the time and six of them cannot be differentiated clearly from the originals (Le Lezec et al. 1996).

This reduced number of cultivars used in breeding programs can be explained by the lack of information of the germplasm banks, which reduces their possible use (Noiton and Alspach 1996). The main problem when using a reduced number of cultivars is the inbreeding among future generations, in comparison with other fruit trees as peach, raspberry, or chestnut. Nowadays, new approaches can be afforded to increase genetic variability in commercial releases such as collecting seedling from the supposed original species *M. sieversii* (Forsline and Aldwinckle 2004) or using old cultivars (Lateur and Populer 1996a, b). But in breeding, inbreeding problems are not yet visible in seeding populations due to the elevated heterozygocity of the genus *Malus*.

The first cultivar obtained by crossing was attributed to Thomas Andrew Knight (1759–1838). Another method to obtain new cultivars consisted in the selection of mutations and chimeras (Janick et al. 1996); these develop shoots with a stable variation when they are propagated vegetatively. The crossing of two parents is now, as it always has been, the main method in apple breeding (combination breeding).

Genetic transformation has the advantage that it maintains cultivar identity since (Brown and Maloney 2005). Although progress is being made, there are problems with the field-testing of transgenic apples as quality traits are too complex to be improved by this biotechnology. The expression of transformed genes is still uncertain and needs more methodical and practical research. On the other hand, the general acceptance of genetic modified organism (GMOs) is not very good and it requires more information for the public. Maybe it would be better if it were possible to transfer species-owned genes instead of foreign genes, like fire blight resistance (Krens et al. 2004).

Currently, the main characteristics of cultivated apples are (1) size over 100 g or 70 mm as a minimum for the market; (2) colors: yellow, green, red, bicolor, and brown in susceptible apples to russeting; (3) acidity: sweet apples when malic acid is lower than 4.5 g/L and bitter when it is over that limit; (4) tannins: sharp apples are those with more than 2 g/L of tannic acid; (5) sweetness: most of the cultivars contain between 12° and 18° Brix; (6) harvesting period from August to December; and (7) resistance to diseases and abiotic stress.

The eating quality is difficult to measure objectively (Hampson et al. 2000). Contribution of crispness accounts for about 90% of the variation in texture liking. Juiciness, aroma, sweetness, and sourness change their relative importance from year to year. They account for about 60% of variation in flavor liking. Sweetness and sourness are better predictors of liking than analytical measurements of soluble solids and titratable acidity. Formal sensory evaluation is a reliable way for screening breeding selections (Hampson et al. 2000). Some researchers have found poor correlation between soluble solids (% SS), titratable acidity (TA), and firmness with sensory perceptions of sweetness, sourness, and texture (Bourne 1979a, b, c; Watada et al. 1981).

The main cultivars used for cider are differentiated on the basis of their acidity and tannin levels. Four groups of apples can be classified considering acidity and tannin contents (Downing 1989; Lea 1990): bittersweet apples contain more than 0.2% (w/v) tannins and less than 0.45% (w/v) acidity (calculated as malic acid). Sharp apples have less than 0.2% (w/v) tannins and more than 0.45% (w/v) acidity. A subgroup of this classification, bittersharps, has the same range of acidity but a tannin content over 0.2% (w/v). Sweet apples have less than 0.2% (w/v) tannins and 0.45% (w/v) acid.

Different types of apples should be mixed to obtain a good cider (Downing 1989). Low-acid cultivars for the basic juice and high acid levels add tartness to the cider. Aromatic cultivars as 'Cox's Orange' add flavor and bouquet to a cider. Astringent apples can improve body and flavor. As a rule, no more than 10% of astringent cider should be added to an acidic cider and no more than 20% should be added to any blend. Apples should be mature and free from starch. Blending with fermented stock is preferred since the fermentation of fresh juice cannot always be predicted. Cider apples have a higher tannin and sugar content than culinary apples but are lower in acid (Downing 1989). Dessert and culinary apples lose more body and flavor due to fermentation than cider apples.

The ideal cider apple is slightly riper than the fresh market one (Downing 1989). As apples mature, the starch turns into sugar, increasing sweetness and flavor. Unripe apples produce juice with a 'starchy' or 'green apple' flavor. Acidity and astringency also decrease after harvest, both with a pronounced effect on the flavor of the juice.

If we compare commercial cultivars' characteristics (Iglesias et al. 2000) with some of the most frequently used cider apple cultivars in Spain (Table 5), we can say that the acidity of various groups, such as 'Elstar' and 'Reinetas,' is equivalent to some cider cultivars, such as 'Raxao'. Cultivars producing high levels of tannins are rarer, such as 'Teórica' or 'Collaos'.

Some special characteristics can be important in the use of specific cultivars, as (1) sensibility to *russeting,* which produces a brown aspect that is specific in some cultivars such as 'Reineta Gris de Canada', 'Boskoop'; (2) growing habit, spur types, and weeping; (3) late blooming; (4) high cold hardiness; (5) resistance

Table 5 Main characteristics from main commercial cultivars and local ones

Cultivar	Blooming	Harvest	Color	Caliber (mm)	Brix	Tanins	Acidity	Firmness (kg)	Origin
Commercial cultivar Gala Group*	5 Apr	10–25 Aug	Bicolor	73–82	12–14		2.6–4.9	7–9	New Zealand
Elstar Group*	10 Apr	10–30 Aug	Bicolor	74–78	13–15		7.7–9.9	6–7	Golden Delicious × Ingrid Marie
Delicious Group*	5 Apr	1–15 Sept	Red	75–91	11–15		2.5–3.4	7–8	Seedling, USA
Golden Group*	20 Apr	15–25 Sept	Yellow-Green	69–90	13–17		3.6–9.0	6–10	Goden Reineta × Grime Golden, USA
Reinetas Group*	20 Apr	5–15 Sept	Yellow-Brown	74–85	12–18		11–13	8–10	Ancient cultivars, origin unknown
Jonagold Group*	10 Apr	1–15 Sept	Bicolor	80–92	14–17		5–6	5–8	Delicious × Jonathan
Braeburn Group*	1 Apr	25 Oct to 5 Nov	Bicolor	74–84	12–14		5–7	8–9	Seedling, New Zealand
Granny Smith Group*	14 Apr	1–25 Nov	Green	78–90	12–13		9	8	Seedling from French Crab, Australia
Fuji Group*	10 Apr	5–25 Nov	Bicolor	70–84	13–17		3.1–4.2	7–9	Seedling from Ralls Janet × Red Delicious, Japan
Local cultivars									
Blanquina	9 May	32 Oct	Yellow-Green	50–70	12	0.9	4.4	11	Spain
Collaos			Red			4.9	2.1		Spain
Cristalina	13 May	23 Oct	Red	56–65	12	0.9	5.7	11–12	Spain
De la Riega			Bicolor			1.4	5.2		
Marialena	5–11 May	22 Sept	Bicolor	53–59	13	1.4	4.8	8–9	Spain
Raxao	20 May	23 Oct	Bicolor	56–71	12	1.5	7.7	10–12	Spain
Teórica	12 May	22 Oct	Red	47–58	14	4.0	14.5	11–13	Spain
Mingán		25 Oct	Bicolor	60–66	12–15	2	3	10–13	Spain
Tres en Cunca	4 Apr	20 Sept	Yellow	53–72	13	1.2	7.2	10	Spain

*Data adapted from Iglesias et al. (2000)

to pests and diseases; and (6) local cultivars that need less treatment in comparison with commercial ones and are desired in their area.

5.1.1 Genes and Effects

Main characteristic genes have been localized in different cultivars and are used in breeding programs (Table 6). Genes related with ethylene biosynthesis (ACS, ACO, and ACC) regulate conservation and fruit softening. Albinism (*al* gene), pale green lethal seedlings (*l* gene), and color due to anthocyanin genes, as well as greasy skin (*Gr* gene), have been studied by several researchers. Genes affecting petals have been found controlling apetaly (*ape*) or double petals (*Pd*). Tobutt (1994) related apetaly with the ability to produce parthenocarpy fruits. Some genes have been found affecting fruit quality, such as aroma (*Ar*), malic acid (*Ma*), or bitter pit (*Bp-1* and *Bp-2*). Sensibility to russeting is attributed to the *Ru* gene (Alston and Watkins 1975). Genes related to chilling requirement (*Chr*) and early budbreak (*Ebb*) have been discussed by Decourtye and Brian (1967) and Lawson et al. (1995), respectively. Studies in growth regulation have provided deep knowledge in apple dwarfing (genes *st-1* and *st-2* for sturdy dwarf; genes *dw-1, dw-3*, and *dw-4* for early dwarf; and *cr* for crinkle dwarf), regrowth promoter (*G*), Gibberellin gene, Knotted 1-like homoeobox expressed during growth and development, gene *MdPIP1* controlling fruit expansion and in plants under osmotic stress, and *DAD1* as inhibitor of programmed cell death. Genes related with apple fertility are *MADS-box* genes associated with the development of floral meristems and organ identity, *MDH1* (apple homoeobox gene) involved in the control of plant fertility, pollen lethal (*P-1, P-2, P-3, P-4*, and *P-5*), and pollen incompatibility (S-alleles).

Also, different pest- and disease-resisting genes have been localized (Table 6), such as genes for curling aphids resistance (*Dysaphis devecta* Wlk.) (genes *Sd-1* to *Sd-3* and the precursor *Pr-Sd*), for WAA resistance (*Eriosoma lanigerum*) (*Er-1*), for yellow mottle (*ym-1, ym-2, ym-3*), for hypersensitivity to *D. plantaginea* (*Sm-h*), for fire blight resistance (Alston and Briggs 1970), for *Glomerella cingulata* susceptibility (*Gb*), for *Gymnosporangium* resistance (*Gy-a* and *Gy-b*), for *Phyllosticta solitaria* susceptibility (*Ps-1* and *Ps-2*), for *Phytophthora cactorum* resistance (*Pc*), for *P. leucotricha* resistance (*Pl-1*, *Pl-2, Pl-w*, and *Pl-d*) by and the precursor *Pr-Pl-1*, and for scab resistance (*Venturia inaequalis*) (*Va, Vb, Vbj, Vf, Vfn, Vm, Vr, Vr2*).

Quantitative traits loci have been studied for branching habit, vegetative bud break, reproductive bud break, bloom time, and root suckering, using molecular markers (Lawson et al. 1995), in combination with random amplified polymorphic DNAs (RAPDs) to study juvenile tree growth (Conner et al. 1998). Quantitative trait loci (QTLs) for stem diameter, plant height increment, leaf size, bloom traits, juvenile phase, and fruit characteristics have been evaluated by Liebhard et al. (2003), fruit quality by King et al. (2000), scab resistance by Calenge et al. (2004), and powdery mildew resistance by Stankiewicz-Kosyl et al. (2005).

Table 6 Apple genes for different characteristics

Gene denomination or abbreviation	Gene effect	References
1-aminocyclopropane-1-carboxylate synthase (ACS) and 1-aminocyclopropane-1-carboxylate oxydase (ACO).	Ethylene biosynthesis during ripenning	Dong et al. (1991); Dong et al. (1992); Castiglione et al. (1999); Harada et al. (2000); Costa et al. (2005)
1-aminocyclopropane-1-carboxylic acid (ACC) synthase, 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase)		
1-aminocyclopropane-1-carboxylic acid synthase (ACS)	Fruit softening	Oraguzie et al. (2004)
E-1, *E-2*	Early ethene production	Batlle (1993)
Polygalacturonase (PG)	Hydrolyze pectins that softens the fruit	Dong et al. (1998)
Al	Albinism	Crane and Lawrence (1933)
l	Pale green lethal seedlings	Klein et al. (1961)
Anthocyanin biosynthesis genes	Skin color	Kim et al. (2003)
UDP glucose-flavonoid 3-O-glucosyltransferase (pUFGluT)	Anthocyanin expression in apple skin	Kondo et al. (2002)
Rf	Anthocyanin in fruit skin	Wilcox and Angelo (1936); Wakasa et al. (2003).
Rt	Anthocyanin in all tissues	Sampson and Cameron (1965)
Gr	Greasy skin	Alston and Watkins (1975)
Ru	Russetted fruit skin	Alston and Watkins (1975)
Ape	Apetaly	Tobutt (1994)
Atc	Atrophied corolla	Decourtye (1967)
Ca-a, *Ca-b*	Deciduous calyx	Henning (1947)
Pd	Double petals	Sampson and Cameron (1965)
Ar	Aromatic fruit flavor	Alston and Watkins (1975)
Ma	Malic acid	Nybom (1959)
Bp-1, *Bp-2*	Bitter pit resistance	Korban and Swiader (1984)
Yfl	Yellow/cream flesh	Crane and Lawrence (1933)
Chr	Chilling requirement	Decourtye and Brian (1967)
Ebb	Early budbreak	Lawson et al. (1995)
Co	Columnar habit	Lapins and Watkins (1973)
Sp-1, *Sp-2*, *Sp-3*	Spur type habit	Decourtye and Lantin (1969)
dw-2	Compact habit	Decourtye (1967)
W	Weeping habit	Sampson and Cameron (1965)

Table 6 (continued)

Gene denomination or abbreviation	Gene effect	References
Tb	Terminal bearing	Lawson et al. (1995)
st-1, *st-2*	Sturdy dwarf	Alston (1976)
dw-1, *dw-3*, *dw-4*	Early dwarf	Alston (1976)
Cr	Crinkle dwarf	Alston (1976)
G	Regrowth promoter	Alston (1976)
Gibberellin 20-oxidase gene	Hormone	Kusaba et al. (2000)
Knotted 1-like homoeobox	Expressed during growth and development	Watillon et al. (1997)
MdPIP1	Fruit expansion and in plants under osmotic stress	Hu et al. (2003)
DAD1	Inhibitor of programmed cell death	Dong et al. (1998)
MADS-box genes	Development of floral meristems and organ identity	Sung and An (1997); Sung et al. (1999); Sung et al. (2000); Yao et al. (1999); Van der Linden et al. (2002)
MDH1, apple homoeobox gene	Involved in control of plant fertility	Watillon et al. (1997)
P-1, *P-2*, *P-3*, *P-4*, *P-5*	Pollen lethal	Heilborn (1935)
S	Pollen incompatibility	Kobel et al. (1939)
S-alleles	Pollen incompatibility	Boškovi· and Tobutt (1999); Broothaerts et al. (1995); Broothaerts et al. (2003); Kitahara and Matsumoto (2002a, b); Kobel et al (1939); Matityahu et al. (2005)
Sd-1 to *Sd-3*, precursor *Pr-Sd*	Curling aphids resistance	Alston and Briggs (1968, 1977); Roche et al. (1997)
Er-1	Woolly apple aphid (WAA) resistance	Knight et al. (1962); Sandanayaka et al. (2003)
ym-1, *ym-2*, *ym-3*	Yellow mottle	Sadamori et al. (1964)
Sm-h	*Dysaphis plantaginea* hypersensitivity	Alston and Briggs (1970)
Gb	*Glomerella cingulata* susceptibility	Thompson and Taylor (1971)
Gy-a and *Gy-b*	*Gymnosporangium* resistance	Aldwinckle et al. (1977)
Ps-1 and *Ps-2*	*Phyllosticta solitaria* susceptibility	Mowry and Dayton (1964)
Pc	*Phytophthora cactorum* resistance	Alston (1970)

Table 6 (continued)

Gene denomination or abbreviation	Gene effect	References
Pl-1, *Pl-2*, *Pl-w*, and *Pl-d*; precursor *Pr-Pl-1*	*Podosphaera leucotricha* resistance	Knight and Alston (1968); Dunemann et al. (1999); Markussen et al. (1995); Alston et al. (2000); Batlle and Alston (1996); Batlle (1993); Dayton (1977); Korban and Dayton (1983)
Va, *Vb*, *Vbj*, *Vf*, *Vfn*, *Vm*, *Vr*, *Vr2*		Dayton and Williams (1968); Barbieri et al. (2003); Patocchi et al. (1999a); Patocchi et al. (1999b);

5.1.2 Local Cultivars

Normally, apple production, as with other crops, focuses on regular plantations established with a few highly productive genotypes of extraordinary quality. However, great quantities of apples are produced in small orchards, generally established with local cultivars that form reservoirs of the main origins of variability. These cultivars have been selected locally and rusticity is normally one of their main values. But they also satisfy the acceptance of local consumers, having more flavor, likely due to their aptitude to be cultivated with less sprays in order to achieve a more ecological production.

If we take into account some of the main characteristics that define an apple cultivar (Table 5), local cultivars present similar characteristics to those broadly spread. Possibly, the old fashion look of the local varieties is their most outstanding characteristic. Standardization and globalization in marketing apples have hardly reduced the number of varieties cultivated. Brown and Maloney (2005) have pointed out the importance of name recognition in marketing apples. In Spain, most of the local cultivars have nearly disappeared from commercial orchards. However, the situation can change in the future with the revalorization of local products and Denominations of Origin, as it has happened previously with winery grapes. Local cultivars contribute greatly in cider production, no doubt due to the lower price of cider apples, but this has also tended to reduce interest in their breeding. Presently, the knowledge in local apple cultivars is increasing, a situation which can serve to diversify the apple market (Pereira-Lorenzo et al. 2003; Díaz-Hernández et al. 2003; Itoiz and Royo 2003; Pereira-Lorenzo et al. 2007).

Some of the resistant local varieties could be used in breeding in order to transfer polygenic resistance, a very important fact considering the first breakdown of the monogenic scab resistance of *M. floribunda* (Kemp et al. 2004; Fischer et al. 2001b).

5.1.3 Growth Habit

Until now, vigor and growth habit were controlled by dwarfing rootstocks and growth regulators with the aim to establish high-density orchards. A new approach focuses on the genes involved in tree architecture, such as columnar, *Co*, which does not allow the growth of lateral branches and the fruit appear in spurs over the main axe (Tobutt 1985, 1994; Quinlan and Tobutt, 1990). Although some new cultivars with this gene have been released ('Maypole', 'Tuscan', 'Trajano', 'Telamon'), it has been recognized that still a lot of work must be done in order to achieve a similar gustative quality among the present cultivars.

Four fruiting types have been proposed by Lespinasse (1992) based on the vegetative growth and fruiting habit:

1. Columnar. An axe is covered with spurs. It is controlled by a dominant gene and was previously discovered in 'Wijcik McIntosh'. It hardly needs pruning and tends to bear biannually.
2. Spur. It is characterized by short shoots in the scaffold limbs. Trees tend to be upright and numerous spurs appear close to the trunk. It tends to hold a biannual production.
3. Spindle. It is presented by ('standard') 'Golden Delicious'. Varieties tend to be spreading with wide crotches and frequent branching. They bear on spurs and shoots that are generally 1–3 years old. The fruiting zone tends to move away from the trunk to the outer sides of the tree (IBPGR 1982).
4. Tip bearer, characterized by 'Granny Smith'. Varieties tend to have upright main scaffold limbs with narrow crotches and frequent branching (IBPGR 1982). They bear a large part of the crop upon the ends of the previous year's shoots. This kind of cultivar has a shorter production time and a more regular bearing pattern than type 1 or 2 (Lauri and Costes 2004).

5.1.4 Stylar Incompatibility and Molecular Markers

Apple varieties exhibit a self-incompatibility mechanism, preventing fertilization following self-pollination (reviewed by De Nettancourt 2001). Pollination studies based on microscopic evaluation of pollen-tube growth through the pistil allowed to discriminate 11 different S-alleles in apples (*S1–S11*) Kobel et al (1939) and 26 cultivars were classified. Using IEF and NEPHGE followed by RNase activity staining, Bošković and Tobutt (1999) identified the gene product for *S1–S11* and they added 14 more S-alleles (numbered *S12–S25*). To resolve the discrepancies in S-allele assignment, Broothaerts (2003) reexamined the identity of S-alleles known from domestic apple cultivars, designing allele-specific primer pairs to selectively amplify a single S-allele per reaction. Highly similar S-alleles that were coamplified with the same primer pair were discriminated through their distinct restrictive digestion pattern. In most cases,

Broothaerts results (2003) coincided with those obtained through phenotypic and S-RNase analysis.

5.2 Rootstocks

Rootstocks have been used at least from Roman times as they were used to graft selected cultivars onto seedlings (Tubbs 1973). The first reference in the UK of 'Paradise' as a dwarf apple tree was in 1597. A 1629 reference describes how this tree was used as rootstock to develop small trees. Dwarfing rootstocks known as 'Paradise' or 'Doucin' in 18th century Europe were a mixture (Ferree and Carlson 1987). Fourteen different types were mentioned in 1870.

Extensive reviews on apple rootstocks can be found in Ferree and Carlson (1987), Masseron (1989), Webster and Wertheim (2003), Wertheim (1998), and Brown and Maloney (2005). Rootstock breeding has focused on size control (dwarfing), tolerance to low temperatures (hardiness), tolerance to pathogens and pests, and on adaptability to different soil conditions (Brown and Maloney 2005). Dwarfing was very effective respecting the seedlings (14 m high) to 30–40% of 'M.27' (Masseron 1989).

There is no easy explanation about the size control by the rootstock. Some hypotheses include graft union anatomy (Soumelidou et al. 1994), the ABA:IAA ratio in dwarf rootstocks (Kamboj et al. 1999), and hydraulic conductivity and dwarfing (Atkinson et al. 2003). Atkinson et al. (2003) found a lower hydraulic conductivity in dwarfing rootstocks compared with semivigorous rootstocks. These observations were consistent with lower xylem-to-phloem ratios and changes in xylem vessel anatomy in dwarf rootstocks, which might explain their influence in shoot behavior when used on grafted plants. Soumelidou et al. (1994) suggested that the failure of auxin in cross-graft union with dwarf rootstocks reduces rootstock xylem production, with poor water and mineral supply to the scion. Kamboj et al. (1998) measured a higher ratio of ABA:IAA in dwarf rootstocks.

The main diseases that affect rootstocks are crown and root rot (*Phytophthora* spp.), fire blight (*Erwinia amylovora* Burrill Winslow et al.), and canker (*Nectria*). WAA (*E. lanigerum* Hausmann) has been considered the main pest. Other problems affecting rootstocks are burknots, genes *bu-1, bu-2* (Decourtye 1967), and root suckering, *Rs*, (Lawson et al. 1995).

Although seedlings from wild or cultivated apples were and are the main origin of rootstocks, clonal rootstock 'MI.793' (hybrid between Northen Spy and M.2) appeared in 1989 in nurseries (Masseron 1989).

The most frequently used rootstocks in the world were selected in the UK during the 20th century. 'M.2', 'M.7', and 'M.9' belong to the serial East Malling (EM) obtained between 1912 and 1913 by Hatton and now denominated 'M1' to 'M16' (Masseron 1989). They were selected from populations

used in different countries. 'M.9' is the most used rootstock in Europe and comes from the population 'Paradis Jaune' from Metz, and some clonal and sanitary selections were recently obtained: (1) United Kingdom, 'M9 EMLA'; (2) The Netherlands, 'M9 NAKB'; and (3) France, 'PAJAM 1' ('Lancep') and 'PAJAM 2' ('Cepiland').

'M.106' and 'M.111' belong to the serial Malling Merton with 15 types, from 'M.101' to 'M.115', resistant to *E. lanigerum* and medium to strong vigor (Masseron 1989). They were selected in 1952 from crosses between 'Northen Spy' and various selections of the serial EM. 'M.106' are some of the most interesting rootstocks for cider production (Díaz-Hernández et al. 2003) because they provide the minimum vigor avoiding trellis. Díaz-Hernández et al. (2003) compared the two most interesting rootstocks, 'M106' and 'M111', with some important cider apple cultivars in northern Spain. As reported previously (Masseron 1989), 'M.106' showed less vigor, although not significantly different and induced a considerably higher productivity than 'M.111' for 'Reineta Encarnada' and 'Teórica' (Díaz-Hernández et al. 2003). 'M.25', 'M.26', and 'M.27' belong to the serial Malling (M). They were selected in 1960 and are not resistant to *E. lanigerum*.

Breeding programs initiating in 1953 in the USA have been reviewed by Brown and Maloney (2005). Several selections of the Cornell Geneva series (CG) are fire blight resistant and are under study. One of the most well-known American rootstocks is 'Michigan Apple Clone 9', 'MAC 9', which preformed poorly in hot dry soils (Webster and Wertheim 2003).

Al-Hinai and Roper (2004) established a trial to check if different rootstocks influence the growth and quality of 'Gala' fruits. They used four rootstocks, 'M.26', 'Ottawa 3', 'M.9 Pajam 1', and 'Vineland (V)-605'. In conclusion, rootstocks had no effect on fruit growth, final size, or yield. Apple fruit size was influenced by the crop load. When Marini et al. (2002) adjusted the effect of apple rootstocks on the weight of 'Gala' fruits for crop load, they found differences between rootstocks but agreed that longer period of study would be necessary.

Rootstock has effect on gene expression patterns and, therefore, over grafted scions. Jensen et al. (2003) discussed the different influence of 'M.7' rootstocks (with reduced susceptibility to fire blight) and 'M.9 NAKB T337' ('M.9 T337') rootstocks (highly susceptible to fire blight). They found differences in the expression of a number of photosynthesis-related, transcription/translation-related, cell division related genes and stress-related gene expression; therefore, expressed genes might influence the tree stature, stress tolerance, photosynthetic activity, and fire blight resistance.

New challenges on apple dwarfing rootstocks are being considered as seen in selections from Russia ('B.146' and 'B.491'), Sweden ('BM 427'), USA ('G.65' and other CG- and G-rootstocks), Japan ('JM.1', 'JM.5', and 'J.M.8'), Czech Republic ('J-TE-G'), UK ('M.20'), Poland ('P.22', 'P.59', 'P.61', 'P.66'), Canada ('V.3'), Germany ('Supporter' 1 to 4), and Romania ('Voinesti 2') (Webster and Wertheim 2003).

5.3 Molecular Markers

Traditional methods for identification and classification of cultivars are based on morphological and agronomical characters, being the only methods that are legally recognized at present (Bailey 1983; REGLAMENTO (CE) N° 2100/94 DEL CONSEJO DE 27 de julio de 1994, D.O.C.O. 1.9.94). As with morphology, molecular markers were first used to focus on cultivar identification due to the relevance in breeding in order to have sharp differentiations between cultivars that have not been disturbed by environmental influence. The first studies in isoenzymes for clonal identification were done by Chyi and Weeden (1984), Menendez et al. (1986a, b), Weeden and Lamb (1985), and Manganaris (1989).

Nowadays, multiple molecular markers exist as isoenzymes, restriction fragment length polymorphisms (RFLP), RAPD, microsatellites, amplified fragment length polymorphism (AFLP), SCAR, or ISSR that allow differentiating varieties (Karp and Edwards 1998). The molecular markers are biomolecules that can be related with a genetic characteristic. There are two general types of molecular markers: proteins and DNA. The first markers, developed at the end of the 1970s, were based on the identification of proteins and isoenzymes. Isoenzymes constitute a system of multiple molecular forms of enzymes in which heterogeneity is partly due to genetic factors and partly to posttranslational modifications (Moss 1982).

Identification techniques of proteins and isozymes are based on electrophoresis analysis in starch gel (Smithies 1955; Torres 1989) and in the visualization of enzymatic products by histochemical methods (Hunter and Markert 1957). This technique is considered to be a magnificent tool to evaluate genetic resources (Karp et al. 1997), and it continues to be one of the most frequently used markers among investigations in genetic diversity of forest trees (Wagner et al. 2004). Its main limitation is the relatively low level of polymorphism detected in comparison to molecular markers based on DNA.

RFLP is a technique based on hybridization of complementary strands. RAPD , AFLP, and SSR (simple sequence repeats) or microsatellites were developed using polymerase chain reaction (PCR) technique that amplifies specific areas of DNA.

SSR markers offer greater advantages in respect to another molecular markers, because they are found abundantly in the genomes and are normally uniformly distributed, as well as very variable and codominant. Each locus is defined by a pair of primers; therefore, the information can be easily interchanged between laboratories. The SSR markers were also found to be useful for cultivar identification and phenetic relationship assessment, revealing advantages due to higher reproducibility over other commonly employed PCR-based methods, namely RAPD and AFLP (Goulão and Oliveira 2001).

Microsatellites are the variations or mutations of very short DNA sequences. Differences between individuals consist in variations in the number of

repetitions of the same sequence. The origin of such polymorphism can be due to sliding in the DNA replication (Zane et al. 2002). Other possible causes of polymorphism generation consist in different types of mutations as deletions and insertions that will also change the size of the microsatellite. The biggest problem is that it needs hi-resolution gels to obtain all the information contained, plus the great initial effort that is required to clone and sequence the primers. The use of microsatellites for genotyping can occasionally be complicated by the preferential amplification of some alleles if the optimal temperature is not used (Fernández-Fernández et al. 2004). In addition, if there are mutations in the matching zones of the primers, the result could be null alleles. This circumstance has already been pointed out by Callen et al. (1993) as a possible problem associated with the use of microsatellite markers. If undetected, a null allele would merely result in that individual being scored as a homozygot, therefore resulting in a loss of information (Marinoni et al. 2003).

A European project formed by 11 European groups, named HiDRAS (*hi*gh-quality *di*sease *r*esistant *a*pples for a *s*ustainable agriculture), is aimed at the identification of the genetic factors that control fruit quality. They are looking for molecular markers linked to fruit quality and pathogen resistance to improve 'marker-assisted selection' (Gianfranceschi and Soglio 2004).

5.3.1 Isoenzymes

The first studies in isoenzymes for clonal identification were done by Chyi and Weeden (1984), Menendez et al. (1986a, b), and Weeden and Lamb (1985), contributing later to the first genetic maps (Lawson et al. 1995). Gardiner et al. (1996) used isoenzymes, RAPD's, and RFLP's to find out the parents of cv. Braeburn, very common in New Zeeland.

Heritability of seven isoenzymes was studied by Chevreau et al. (1985); later on, Weeden and Lamb (1987) published the genetics and linkage between 19 isoenzymes loci and Manganaris (1989) 13 isoenzymes. Got was suggested to be linked to the incompatibility gene *S* (Manganaris and Alston 1987) and proposed its use as a marker. Manganaris and Alston (1988a) found a linkage between acid phosphatase with the gene *ENP-1* (endopeptidase) and the lethal gene in apple *GENE 1*. Genetics of Lap isoenzyme and its variations between main apple cultivars were shown by Manganaris and Alston (1992a). The highly polymorphic peroxidase was used for cultivar identification (Manganaris and Alston 1992b). Locus Pgm-1 was closely linked to the *Vf* scab-resistance gene (Manganaris et al. 1994). Heretability and linkage of Got with other isoenzymes was published by Manganaris and Alston (1988b) and its use for cultivar and rootstock identifications in 1989. Lawson et al. (1995) found that blooming was correlated with Prx-c. Linkage to woolly aphid resistance was studied using stylar ribonucleases and Got-1 (Tobutt et al. 2000).

The following isoenzymes have been defined: Aconitase (Aco-1, Aco-2, Aco-3, Aco-4) by Hemmat et al. (1994) and Chevreau et al. (1999); acid phosphatase

(Acp-1, Acp-2 Ap, Acp-3, Acp-4, Acp-5) by Manganaris and Alston (1988b), Chevreau and Laurens (1987), and Hemmat et al. (1994); alcohol dehydrogenase (Adh-2) by Manganaris (1989); Catechol oxidase (Ctx-1 Co-1, Ctx-2 Co-2) by Chevreau et al. (1999); diaphorase (Dia-1, Dia-2, Dia-5, Dia-6) by Chevreau et al. (1999) and Weeden and Lamb (1987); endopeptidase (Enp-1 Enp) by Chevreau and Laurens (1987); esterase, esterase cathodic (Est-1, Est-2, Est-3, Est-4, Est-c) by Manganaris and Alston (1992a), Chevreau et al. (1985), Pereira-Lorenzo et al. (2003); formate dehydrogenase (Fdh-1 Fdh, Fdh-2) by Hemmat et al. (1994) and Chevreau et al. (1999); glutamate oxaloacetate (Got-1, Got-2, Got-4, Got-5 Aat-5) by Manganaris and Alston (1987, 1988a) and Chevreau et al. (1999); glucosephosphate isomerase (cytosolic and plastid) (Gpi-cl, Gpi-p) by Weeden and Lamb (1987); isocitrate dehydrogenase (Idh-1, Idh-2, Idh-3) by Chevreau (1984), Weeden and Lamb (1987) and Manganaris (1989); leucine aminopeptidase (Lap-1, Lap-2, Lap-3, Lap-4) by Manganaris and Alston (1992b); malate dehydrogenase (Mdh-1, Mdh-2, Mdh-3, Mdh-4) by Manganaris (1989) and Weeden and Lamb (1987); malic enzyme (Me-1) by Weeden and Lamb (1987); 6-phosphogluconate dehydrogenase (Pgd-1 Pgd-c1, Pgd-2 Pgd-c2, Pgd-3 PGD-3) by Weeden and Lamb (1987) and Manganaris (1989); phosphoglucose isomerase (Pgi-3 PGI-3) by Manganaris (1989) and Pereira-Lorenzo et al. (2003); phosphoglucomutase (Pgm1 Pgm-p1, Pgm-2, Pgm-3, Pgm-4, Pgm-5) by Weeden and Lamb (1987), Manganaris (1989) and Pereira-Lorenzo et al. (2003); peroxidase (Prx-1, Prx-2, Prx-3, Prx-4, Prx-7, Prx-C1, Prx-C2) by Manganaris and Alston (1992c); shikimate dehydrogenase (Skd Skdh) by Hemmat et al. (1994); superoxide dismutase (Sod-1, Sod-2, Sod-3, Sod-4, Sod-5) by Manganaris and Alston (1987) and Chevreau et al. (1999); and triosephosphate isomerase (Tpi-1 Tpi-pl, Tpi-3, Tpi-3, Tpi-5 Tpi-c2) by Weeden and Lamb (1987).

Batlle and Alston (1994) pointed out the interest of using isozymes for tracing the transference of the resistance to mildew (*P. leucotricha* (Ell. et Ev.) Salm.) between *M. hupehensis* and cultivated apples. James and Evans (2004) used a set of microsatellites, AFLP and RAPD primers, to identify markers linked to mildew resistance. In recent years, genetic markers have been developed for a number of resistance genes, such as for apple scab (*V. inaequalis*). Bus et al. (2004) who work with microsatellites presented the discovery of a new scab-resistance gene (*Vh8*) that maps to linkage group 2. RAPD markers, located in a chromosomal region that confers scab resistance to apples, were used to screen *Malus* germplasm accessions. The following results were discussed in relation to the introgression of resistance loci together with marker-assisted selection (King et al. 1999).

Durel et al. (2004) studied five mapping populations looking for partial scab resistance against several races of *V. inaequalis*. They worked with SSR and AFLP to test each population, and the genetic maps for both parents of each population were constructed. The occurrence of new virulent races that are able to overcome the *Vf* resistance (Benaouf and Parisi 2000) has initiated the search for new resistance sources, as well as further genetic and molecular

characterization of the already known strong resistances. So, Boudichevskaia et al. in 2004 developed molecular markers for *Vr1*, a scab-resistance factor. A selection attending to adverse factors of the apple tree using molecular markers was emphasized by Tartarini et al. (1997). Screening of seedlings for peroxidase allozyme variation was found to be a reliable method to preselect apple dwarf types (Tang and Zhang 1992). The availability of molecular markers and genetic linkage maps enables the detection and the analysis of major resistance genes as well as of QTL contributing to the resistance of a genotype (Liebhard et al. 2003).

Allozyme analysis indicated that the genetic integrity of native populations of *Malus* was effectively protected against gene flow from cultivated apple (Dickson et al. 1991). Recommendations for the efficient sampling of genetic diversity from natural populations of *M. sieversii* were formulated based on an analysis of population structure using allozyme markers (Lamboy et al. 1996). Isoenzymes were also used to study hybridization and species differentiation by Dickson et al. (1991). In 2002, the morphologic and isoenzymatic characterization of the collection of native apple tree cultivars gathered in the 'Centro de Investigaciones Agrarias de Mabegondo' (CIAM) was published (Pereira-Lorenzo et al. 2002). In this work, 408 accessions of apple tree were studied and compared with 32 nonnative commercial varieties. The same study allowed obtaining results with respect to the genetic variability in the collection of the CIAM (Pereira-Lorenzo et al. 2003). The variability level found has been elevated, since 86% of the introductions are original, which are maintained in the Germplasm Bank. The rest of the accessions turned out to be repetitions of others and even of nonnative commercial and extensively cultivated varieties, such as 'Reina de Reinetas' and 'Reineta Blanca'.

Also a recent study has been published about the isoenzymatic variability of the germplasm of native apple tree cultivars that has been established in the last years by the Public University of Navarre using seven isoenzymatic systems (Itoiz and Royo 2003).

Isoenzymes were employed to determine the genetic structure of 202 trees representing *M. sylvestris* from different regions in western Germany, and the results were compared to similar data on 321 old and new cultivars of *M x domestica* (Wagner et al. 2004). The results of this study indicate that gene flow in either direction has been minimal.

5.3.2 Microsatellites

The first study with microsatellites in apple tree was published by Guilford et al. (1997), who described the first three SSRs in apple tree. Gianfranceschi et al. (1998) extended it to 17. Later, Liebhard et al. (2002) elevated the number of SSR to 140 and used them to propose a map of global linkage in apple tree.

Costa et al. (2004) developed an SSR marker associated with fruit firmness. Kenis and Keulemans (2004) worked with RFLP and microsatellites in order to study the genetic control of tree architecture in apple.

Hokanson et al. (1998) screened 66 *Malus x domestica* Borkh accessions from the USDA-ARS Plant Genetic Resources Unit core collection with a set of eight SSR. Later, they made the characterization of another 142 accessions, which represents an extensive range of *Malus* species and derived hybrids (Hokanson et al. 2001).

The genetic variation within and between wild apple samples and cultivated apple trees was investigated with AFLP and SSR to develop a genetics conservation program for the endangered wild apple (*M. sylvestris*) in Belgium (Coart et al. 2003). One hundred and forty-two French local cultivars were screened with nine SSR markers to get a characterization of the apple genetic resources in France (Lawrens et al. 2004).

SSRs were used in genetic identification by Guilford et al. (1997), Hokanson et al. (1998, 2001), Liebhard et al. (2002), Kitahara et al. (2005), Pereira-Lorenzo et al. (2007), Cabe et al. (2005), and Oraguzie et al. (2005). SSRs were used in the study of the genetic variation in wild apple by Coart et al. (2003). They were also used for the study of haploids by Hofer et al. (2002) and the tea crabapple *M. hupehensis* by Benson et al. (2001).

SSRs allowed the development of genetic maps by Liebhard et al. (2002), Hemmat et al. (2003), and Gianfranceschi et al. (1998). They were also used to study *Vf* scab-resistance region by Vinatzer et al. (2004). Vinatzer et al. (2004) localized two microsatellites markers for the *Vf* gene, and they were also used to verify the genealogical tree of the *Vf* cultivar 'Florina'. Hemmat et al. (2002) provided SSR markers for *Vr* and *Vx*, mapping those genes in R12740-7A accession, and Patocchi et al. (2004) for *Vr2*.

5.3.3 Other Markers

Segregation patterns of AFLP markers have been studied by Li et al. (2004). AFLPs have been used for the construction of genetic maps including the *Vf* gene for scab resistance (Xu and Korban 2000, 2002) and for cultivar identification (Tignon et al. 2000; Tignon et al. 2001a; Tignon et al. 2001b).

AFLPs have been combined with other PCR-based molecular markers and FISH for mapping resistance to aphids (*Sd1*) by Cevik and King (2002a,b). In addition, AFLPs, RAPDs, SSRs, and SCAR were used to set up a saturated reference map by Liebhard et al. (2002).

Other combined markers used to define genetic maps have been isoenzymes and RAPDs (Hemmat et al. 1994), RFLPs, RAPDs, isozymes, SSRs, and SCARs (Maliepaard et al. 1998) with the location of the scab-resistance gene (*Vf*), resistance to rosy apple aphid (*Sd1*), self-incompatibility (*S*), and fruit acidity (*Ma*). Combination of SSRs and ISSRs were used for identification by Goulão and Oliveira (2001).

Unspecific markers as RAPDs have been profusely used to localize molecular markers for fruit skin color (Cheng et al. 1996), molecular markers for powdery mildew resistance (Markussen et al. 1995; Dunemann et al. 1999), *Vf*, *Vm* genes (Cheng et al. 1998; Hemmat et al. 1998), to verify apomictic seedlings (Ur-Rahman et al. 1997), and to build linkage maps (Conner et al. 1997).

SCARs have been defined for scab genes *Vm* and *Vf* (Cheng et al. 1998; Shupert et al. 2004), powdery mildew gene *Pl1* (*P. leucotricha* (Ell. & Ev.) E.S. Salmon) (Evans and James 2003), and columnar *Co* gene (Kim et al. 2003).

Combinations of AFLPs, RAPDs, and SSRs provided scab markers for *Vr2* gene (Patocchi et al. 2004); RAPDs and SSRs were used to study columnar *Co* gene (Hemmat et al. 1997); RAPDs and SSRs defined markers for scab *Vr* and *Vx* genes (Hemmat et al. 2002); RAPDs and SCARs were used to study *Vf* gene (Tartarini et al. 1999); and RAPDs, SCARs, and SSRs for scab-resistance *Vbj* gene (Gygax 2004). Different markers, such as RAPDs, isoenzymes in combination with morphology, were used in cultivar identification by Royo and Itoiz (2004) and SSRs and ISSRs by Goulão and Oliveira (2001).

Gene tagging with DNA markers has been used to follow the inheritance of individual genes, such as those conferring scab resistance, *Vm* (Cheng et al. 1998).

5.3.4 Cultivar Classification by Biotechnological Methods

Cultivar classification was one of the main aspects in breeding since it allowed differentiating cultivars for different purposes.

Before 20th century, agronomists tried to classify varieties by painting them in very fine detail, as can be seen in many 17th and 18th century canvasses. During the last century, most apple classification studies focused on giving detailed descriptions, which were based mainly on fruit morphology and agronomic characteristics. Descriptions were made by pomologists who carefully detailed the main characteristics that defined each cultivar, adding along frequently a precise handmade picture illustration (Guinea 1957).

It was not until the second half of the 20th century that apple cultivars began to be classified attending systematic guidelines as those set by IBPGR (1982) or UPOV (1974). IPGRI is focused in the conservation of genetic resources and includes more details on the origin of the cultivars (passport data), as well as relevant cytological information and isoenzymes. UPOV (1974) is mainly focused on cultivar protection and, therefore, can be used as a guideline to distinguish cultivars with the purpose of obtaining new patents. Both guidelines provide main and secondary characteristics with different variation levels according to the total variability found previously between apple cultivars. An update including more molecular markers is needed, such as microsatellites that are very accurate in distinguishing cultivars, although it is understood that morphology is required to define them.

Systematics allowed applying statistics to the evaluation in order to get strong evidence on such variations between cultivars. Statistics applied to classifications are mainly based on means, variance, ANOVA, principal component analysis, and cluster analyses (Pereira-Lorenzo et al. 2003; Royo and Itoiz 2004). Within these guidelines, we can understand how difficult differentiation is when a high number of accessions are involved, and molecular markers are excellent instruments in differentiating these (Oraguzie et al. 2005). New classifications complemented with the use of molecular markers enable to identify genetic variations avoiding environmental influence. An extensive study was developed in a Spanish collection of local cultivars (408 accessions) in order to know the main origins of variability, find out duplications, and classify them. Cultivar description is fundamental for the management of germplasm banks, and in the Spanish collection it allowed to remove 53 duplications (Pereira-Lorenzo et al. 2002, 2003). Spanish cultivars were studied during 3 years for phenology, fruit, leave, and flower based on UPOV (1974) and IBPGR (1982) descriptors. A total of 89 characteristics were evaluated and split into 279 variables. The code used in the descriptor lists is indicated in brackets. Three steps were defined for morphology: (1) variability description; (2) variance analysis; and (3) multivariate analysis. To increase the capacity of discrimination, high-discriminant isoenzyme systems were developed.

Rootstocks have been classified by different authors and we can find excellent descriptions by Masseron (1989) and Webster and Wertheim (2003). Some very detailed descriptions of cultivars from different countries have been made, like from France (Boré and Fleckinger 1997), Spain (Guinea 1957; Coque et al. 1996; Pereira-Lorenzo et al. 2002, 2003), the UK (Morgan and Richards 1993), and the USA (Beach et al. 1905).

Different molecular markers have been used for rootstock and cultivar identification: (1) isoenzymes (Manganaris 1989; Weeden and Lamb 1985; Pereira-Lorenzo et al. 2003); (2) AFLPs (Tignon et al. 2000, 2001a); (3) SSRs (Oraguzie et al. 2005; Pereira-Lorenzo et al. 2007); (4) ISSRs (Goulão and Oliveira 2001); and (5) RAPDs (Royo and Itoiz 2004).

QTLs have been studied for branching habit, vegetative bud break, reproductive bud break, bloom time, and root suckering using molecular markers (Lawson et al. 1995), in combination with RAPDs to study juvenile tree growth (Conner et al. 1998). QTLs for stem diameter, plant height increment, leaf size, bloom traits, juvenile phase, and fruit characteristics have been evaluated by Liebhard et al. (2003), fruit quality by King et al. (2000), scab resistance by Calenge et al. (2004), and powdery mildew resistance by Stankiewicz-Kosyl et al. (2005).

5.4 Resistance to Pests and Diseases

Complete reviews have been made by Grove et al. (2003) and Beers et al. (2003).

Resistance to fire blight has been one of the main objectives in the Geneva breeding program (Norelli et al. 2003a; Norelli et al. 2003b). Susceptibility of

'M9' and 'M26' rootstocks has encouraged the selection of resistant rootstocks, such as 'G.16', 'G.30', or 'G.65' (Grove et al. 2003).

Scab (*V. inaequalis*) is one of the main high-cost diseases for growers. As the result of breeding programs developed during the last 50 years, several cultivars including resistance from *M. floribunda* 821 have been released. However, growers do not incorporate them to the new orchards due to their inferior quality. It is not clear if resistance is due to a cluster of genes or to a major *Vf* gene (Barbieri et al. 2003). A total of eight genes for scab resistance have been described—*Va, Vb, Vbj, Vf, Vfn, Vm, Vr,* and *Vr2* (Dayton and Williams 1968; Barbieri et al. 2003; Patocchi et al. 1999a, b, 2004; Xu and Korban 2002). *Vm* is a resistant gene to scab derived from *Malus x atrosanguinea* 804 and *Malus micromalus* 245-38 (Cheng et al. 1998). A selection identified in the USA from an open pollination seed obtained in Russia denominated as R12740-7A was identified by Hemmat et al. (2002) as carrying *Vr* and *Vx* genes. GMAL 2473 is an apple scab-resistant selection thought to carry the resistance gene *Vr2* (Patocchi et al. 2004).

New cultivars including durable resistance are needed for organic growing and integrated fruit production (IFP). Some cultivars with known resistance to scab are being evaluated now (Sandskar and Gustafsson 2004). Some of them are (resistance gene in brackets) as follows: (1) from Canada, 'MacFree' (*Vf*), 'Novaspy'® (*Vf*), 'Nova Easygro' (*Vr*), and 'Richelieu' (*Vf*); (2) from the Czech Republic, 'Selena'® (*Vf*), 'Topaz'® and 'Vanda' (*Vf*); (3) from France, 'Baujade' (*Vf*), 'Florina'® or 'Priam' (*Vf*), 'Judaine' (*Vf*), and 'Judeline' (*Vf*); (4) from Germany, 'Reglindis'® (*Va*), 'Reka'® (*Vr*), 'Regia'® (*Vr*), 'Rewena'®, 'Rebella'®, 'Retina'®, 'Resi'®, 'Releika'®, 'Renora'®, 'Remo'® (*Vf*), 'Ahrista'® (*Vf*), 'Gerlinde'® (*Vf*), and others; (5) from the USA, 'Liberty'® (*Vf*), 'Prima'® (*Vf*), 'Freedom'® (*Vf* + *Vr*), and 'Priscilla' (*Vf*); (6) from Holland 'Santana'® (*Vf*); (7) from Switzerland 'Ariwa'® (*Vf*); and (8) from Russia, 'Antonovka kamienna' (*Va*), 'Imrus' (*Vf*), and 'Antonovka Pamtorotuka' (Va).

Powdery mildew (*P. leucotricha* (Ell. & Ev.) E.S. Salmon) reduces tree photosynthesis and transpiration and may produce partial defoliation (Grove et al. 2003). Between cultivars and rootstocks, we can find different susceptibility, with 'Golden Delicious' being less susceptible than 'Gala' or 'Granny Smith'. 'Malling-Merton' rootstocks are very susceptible (Janick et al. 1996). *Pl-1, Pl-2, Pl-w*, and *Pl-d* genes for resistance have been described by Knight and Alston (1968), Dunemann et al. (1999), Markussen et al. (1995), Alston et al. (2000), Batlle and Alston (1996), Batlle (1993), Dayton (1977), Korban and Dayton (1983), and the precursors *Pr-Pl-1* by Batlle (1993) and Batlle and Alston (1996).

WAA resistance of rootstocks has been reviewed by Sandanayaka et al. (2003). Three major WAA resistant genes have been identified—*Er1* (Knight et al. 1962; King et al. 1991), *Er2* (King et al. 1991), and *Er3* (Sandanayaka et al. 2003), which are carried by the apple cultivars 'Northern Spy', 'Robusta 5', and 'Aotea', respectively. *Er1* and *Er2* each had a higher level of resistance and these resistance factors appeared to be in the phloem tissue (Sandanayaka et al. 2003).

As we explained about rootstocks, WAAs encouraged an important breeding program in 1952 that produced two frequently used rootstocks 'M.106' and 'M.111', resistant to *E. lanigerum* (Masseron, 1989). They were selected in 1952 from crosses between 'Northen Spy' and some selections of the serial EM.

The rosy leaf-curling aphid (*Dysaphis devecta* Wlk.) causes severe leaf curl with conspicuous red galls. Alston and Briggs (1968, 1970 and 1977) and Roche et al. (1997) described three aphid biotypes and four resistance genes providing resistance to these biotypes: (1) *Sd-1* gene for biotypes 1 and 2 from 'Cox's Orange Pippin'; (2) *Sd-2* gene for biotype 1 derived from 'Northern Spy'; and (3) *Sd-3* gene for biotype 3 derived from *M. robusta* and *M. zumi*. Cevik and King (2002a,b) showed that Sdh-1 and Sdh-2 loci are tightly linked. *Pr-Sd* has been described as precursor of *Sd* genes by Alston and Briggs (1977).

Breeding programs to combine different resistances with good fruit quality and with high and regular yield are very important. An excellent model for such a complex breeding program is the German apple breeding work at Dresden-Pillnitz. The first aim of this program was to breed both good fruit quality and high yield. All clones with high susceptibility to scab and mildew were eliminated in field evaluations. The program developed the 'Pi-series' of apple cultivars ('Pi' = Pillnitz), which included 'Pinova'®, 'Pilot'®, 'Piros'®, and others. 'Pinova'® and its red mutation 'Evelina' are two of the most interesting cultivars of the future.

In scab-resistance breeding, the cultivar 'Antonovka kamienna' was used at first as a polygenic scab-resistant source (Schmidt 1938), and later the *M. floribunda* and other wild species with different resistance sources (*Vf, Vm, VA*) were also involved. The resistance breeding program was extended in Pillnitz for mildew, fire blight, bacterial canker, red spider mite, and abiotic damage, such as winter frost and spring frosts. The results are the cultivars of the 'Re-series' ('Re' = Resistance) including 'Remo'®, 'Rebella'®, 'Rewena'®, 'Regia'®, 'Reglindis'®, and others. These cultivars have good fruit quality, early and high cropping, and show resistance to scab and to some extent to other fungal and bacterial diseases (Fischer and Fischer 1996; Fischer 1994, 2000).

One of the most important results of the Pillnitz apple resistance breeding program was the selection of a number of cultivars with resistance to economically important diseases using conventional recombinant breeding methods. The advanced Pillnitz resistant cultivars have been tested under a wide range of environmental conditions. They demonstrated their ability to maintain their resistance and provide fruit suitable either for fresh market and/or processing. With their resistance properties, they are suitable for organic fruit production and IFP. Triple and multiple resistant cultivars are selected with resistance to scab, mildew, and fire blight within the Re-cultivars™ 'Remo'®, 'Rewena'®, 'Regia'®, and 'Rebella'®. 'Rebella'® was found to have resistance not only to fungi and fire blight but also to bacterial canker, red spider mite, apple aphids, and abiotic damages. All other cultivars have a different level of multiple resistances (Table 7). These multiple resistances can be transmitted to the offspring by classical recombination breeding and requires no genetic engineering.

Table 7 Multiple resistances in the Pillnitz Re-cultivars™

Re-cultivar™	Scab	Source of resistance	Mildew	Fire blight	Bacterial canker	Red spider mite	Spring frost	Winter freeze
Reanda	R[1]	Vf	LR	R	LS	S	R	LS
Rebella	LR	Vf	R	LR	R	R	R	R
Regine	LR	Vf	LR	R	LR	R	R	R
Releika	LR	Vf	LS	LR	R	R	R	S
Relinda	LR	Vf	LR	LS	R	S	LR	R
Remo	LR	Vf	R	LR	LS	LS	R	R
Rene	LR	Vf	S	R	LR	S	R	LS
Renora	R	Vf	LR	LS	LS	LS	LR	LR
Resi	LR	Vf	LS	LR	R	S	R	S
Retina	LR	Vf	LR	LS	LS	LR	R	S
Rewena	R	Vf	R	R	R	LS	R	LS
Realka	R	Vr	S	R	LS	LS	S	LS
Regia	R	Vr	R	R	LR	LS	LS	R
Reka	R	Vr	LR	LS	R	S	S	LR
Releta	R	Vr	S	LS	R	LS	LS	LS
Remura	R	Vr	LR	LS	LS	S	LS	R
Reglindis	LR	V_A	LR	LR	LS	R	R	R

[1] R: resistant; LR: low resistance; LS: low susceptibility; S: susceptible

5.4.1 Overcoming of the *Vf* Scab Resistance

The *Vf* scab resistance is overcome under different wet conditions especially in northern Europe. Scab develops and results in consecutive infections during the summer, if no fungicides have been applied. Scab on *Vf* resistant cultivars has been observed at Ahrensburg, North Germany, since 1984 (Krüger 1999). At different degrees of intensity, some *Vf* Re-cultivars™ carried weak infections, sometimes with defensive reactions. At another location in northern Germany, the infection occurred as a primary infection at an early stage before blossom opening and caused severe symptoms on peduncles, calyx, and, somewhat later, on petals. Foliar infections spread from 'Gerlinde'® (*Vf*) to the neighboring 'Ecolette'® (*Vf*), 'Topaz'® (*Vf*), and 'Rebella'® (*Vf*). At another location near the Baltic Sea, scab was observed in 2000 only on 'Prima' and 'Ecolette'® and in 2001 on all tested resistant cultivars (Höhne 2001; Fischer et al. 2005). In spite of the lability of the *Vf* scab resistance, these multiple-resistant cultivars are now of interest because of their stable fire blight resistance (Fischer 1994, Fischer and Richter 1999). One year with scab infection is not synonymous with regular yearly infection.

At other locations in the middle and south of Germany, resistant cultivars remained free of infection till now. The resistant cultivars produced defensive reactions, with the exception of 'Reglindis'® (V^A), considered field resistant, with very light sporulation lesions. This reaction is typical for polygenic V^A resistance. The ones remaining free of scab under all conditions were 'Reka' (*Vr*), 'Recolor'® (V^A + *Vf*), and 'Regia'® (*Vr*).

Apparently, the entire genetic background of the resistant cultivars is the cause of differences in resistance stability. Probably, not only one *Vf* gene exists but also three closely related genes. If one or two genes are absent, the resistance is instable (Benaouf and Parisi 2000; Lespinasse 2001). The results indicate that a number of resistant cultivars remained healthy in their respective locations, which allows a rather stable resistance to be assumed. This group includes 'Reglindis'® (V^A), 'Reka' (*Vr*), 'Regia'® (*Vr*), 'Renora'® (*Vf*), 'Relinda'® (*Vf*), 'Reanda'® (*Vf*), and 'Rewena'® (*Vf*). However, the future needs new cultivars with two or three different resistance sources in order to stabilize healthiness in the fields, if the *Vf* gene is overcome and does not work any longer.

What we can do? For durability of scab resistance in the field, we recommend (1) no 'monoculture' with *Vf* cultivars; (2) tolerance of a slight leaf infection on polygenic/oligogenic resistant cultivars to preserve the stability of the host–pathogen system (using V^A- or *Vr* cultivars like 'Reglindis'® or 'Reka' in change with *Vf* cultivars); and (3) three fungicide sprays in early spring would be enough to control infections. In the following seasons, some cultural measures were employed successfully, such as using urea sprays during and after leaf drop in autumn in order to promote leaf rotting or collecting mechanically infected leafs by means of large vacuum cleaners (Triloff 2006).

After these treatments, no primary scab infections were observed in the following spring. Results so far show that a very significant reduction of fungicide spray applications, up to 80%, can be achieved without significant scab and mildew infections in orchards (Fischer and Fischer 2002; Fischer et al. 2005).

In apple breeding, there is still an aim to bring together improvements in fruit quality + yield + resistance to different pathogens in new cultivars. Another new challenge is to establish a lasting resistance in field cultivation, based on observations carried on in different parts of Europe on the breakdown in monogenic scab-resistance sources from *M. floribunda* (Weibel et al. 1997; Fischer et al. 1998; Fischer and Dunemann, 2000; Fischer and Fischer 2002). The stabilization of the *Vf* resistance in the field by breeding needs (1) promoting the breeding of cultivars with two or more resistance sources by pyramiding different resistance genes; and (2) using more cultivars with polygenic scab resistance in combination breeding programs (Lespinasse 2001).

6 Conclusions

Earnest efforts have been made by different researchers in order to understand the apple tree: (1) the botanical aspects; (2) breeding and selection of cultivars and rootstocks; (3) variability and genetic resources; (4) knowledge of pests and diseases; (5) breeding for resistance; (6) technical and genetic aspects in the improvement of the crop; and (7) development of powerful techniques such as molecular markers to assist breeding programs.

However, some questions remain and they should be addressed in the future: (1) the convenience of a more variety of cultivars that combine high-quality, good postharvest conservation and resistance to main pests and diseases; (2) dwarf or semidwarf rootstocks with good resistance to cold winters, excellent compatibility, and enough vigor to avoid trellis (columnar habit in new cultivars should help to it); (3) a phylogeny review, including new tools such as molecular markers, should be afforded in order to understand better this complex genus; and (4) a complete world evaluation of apple genetic resources that would allow to maintain a high variability avoiding an elevated number of repetitions.

Acknowledgments We would like to thank USDA, ARS, Plant Genetic Resources Unit (PGRU) for the pictures the author made in the apple collection at PGRU.

References

Aldwinckle, H.S., Lamb, R.C., and Gustafson, H.L. (1977). Nature and inheritance of resistance to *Gymnosporangium jumperi-virginianae* in apple cultivars. *Phytopathology*. 67: 259–266.

Al-Hinai, Y.K., and Roper, T.R. (2004). Rootstock effects on growth, cell number, and cell size of 'Gala' apples. *J. Amer. Soc. Hort. Sci.* 129(1): 37–41.

Alston, F.H. (1970). Resistance to collar rot *Phytophthora cactorum* (Leb and Cohn) Schroet in apple. *Report, East Malling Research Station for* 1969: 143–145.

Alston, F.H. (1976). Dwarfing and lethal genes in apple progenies. *Euphytica*. 25: 505–514.

Alston, F.H., and Briggs, J.B. (1968). Resistance to *Sappaphis devecta* (WIk.) in apple. *Euphytica*. 17: 468–472.

Alston, F.H., and Briggs, J.B. (1970). Inheritance of hypersensitivity to rosy apple aphid *Dysaphis plantaginea* in apple. *Can. J. Genet. Cytolo*. 12: 257–258.

Alston, F.H., and Briggs, J.B. (1977). Resistance genes in apple and biotypes of *Dysaphis devecta*. *Ann. Appl. Biol*. 97: 75–81.

Alston, F.H., and Watkins, R. (1975). Apple breeding at East Malling Proceedings EUCARPIA Symposium on Tree Fruit Breeding 1973, Canterbury, pp. 14–29.

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

Atkinson, D. (1980). The distribution and effectiveness of the roots of tree crops. *Hort. Rev*. 2: 425–490.

Atkinson, C.J., Else, M.A., Taylor, L., and Dover, C.J. (2003). Root and stem hydraulic conductivity as determinants of growth potential in grafted trees of apple (*Malus pumila* Mill.). *J. Exp. Bot*. 54: 1221–1229.

Barbieri, M., Belfanti, E., Tartarini, S., Vinatzer, B.A., Sansavini, S., Dilworth, E., Gianfranceschi, L., Hermann, D., Patocchi, A., and Gessler, C. (2003). Progress of the map based cloning of the Vf-resistance gene and functional verification: preliminary results from expression studies in transformed apple. *HortScience*. 38: 1–3.

Batlle, I. (1993). Linkage of isoenzymic genes with agronomic characters in apple. PhD thesis, University of London, 181p.

Batlle, I., and Alston, F.H. (1994). Isoenzyme aided selection in the transfer of mildew (*Podosphaera leucotricha*) resistance from *Malus hupehensis* to the cultivated apple. *Euphytica*. 77: 11–14.

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

Beach, S.A., Booth, N.O., and Taylor, O.M. (1905). The Apples of New York, Vol I. J.B. Lyon Company Printers, Albany, New York.

Beers, E.H., Suckling, D.M., Prokopy, R.J., and Avilla, J. (2003). Ecology and management of apple arthropod pests. In: D.C. Ferree and I.J. Warrington (eds.), Apples: Botany, Production and Uses. CAB International, Wallington, Oxford, UK, pp. 489–519.

Benaouf, G., and Parisi, L. (2000). Genetics of host-pathogen relationship between *Venturia inaequalis* races 6 and 7 and Malus species. *Phytopathology*. 90: 236–242.

Benson, L.L., Lamboy, W.F., and Zimmerman, R.H. (2001). Molecular identification of *Malus hupehensis* (Tea crabapple) accessions using simple sequence repeats. *HortScience*. 36(5): 961–966.

Boré, J.M., and Fleckinger, J. (1997). Pommiers à cidre, variétés de France. INRA, Paris, 771 pp.

Bošković, R., and Tobutt, K.R. (1999). Correlation of stylar ribonuclease isoenzymes with incompatibility alleles in apple. *Euphytica*. 107: 29–43.

Boudichevskaia, A., Flachowsky, H., Fischer, C., Hanke, V., and Dunemann, F. (2004). Development of molecular markers for Vr1, a scab resistance factor from R12740-7[a] apple. XIth Eucarpia Symposium on Fruit Breed & Genetics. F. Laurens and K. Evans (eds.). *Acta Hort*. 663, ISHS 2004. Vol I, 171–175.

Bourne, M.C. (1979a). Rupture tests vs. small-strain tests in predicting consumer response to texture. *Food Technol*. 10: 67–70.

Bourne, M.C. (1979b). Fruit texture-an overview of trends and problems. *J. Texture Stud*. 10: 83–94.

Bourne, M.C. (1979c). Texture of temperate fruits. *J. Texture Stud*. 10: 25–44.

Bretaudeau, J., and Faure, Y. (1991). Atlas d'Arboriculture Fruitière. Lavoisier, 2, 207 pp.

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.

Broothaerts, W., Janssens, G.A., Proost, P., and Broekaert, W.F. (1995). cDNA cloning and molecular analysis of two self-incompatibility alleles from apple. *Plant Mol. Biol*. 27: 499–511.

Broothaerts, W., Van Nerum, I., Boscovic, R. and Tobutt, K. (2003). Apple selfincompatibility genotypes: An overview. *Acta Hort*. 622: 379–387.

Broothaerts, W., Van Nerum, I, and Keulemans, J. (2004). Update on and review of the incompatibility (S-) genotypes of apple cultivars. *HortScience*. 39(5): 943–947.

Brown, S.K., and Maloney, K.E. (2005). *Malus x domestica* apple. In: R.E. Litz (ed.), Biotechnology of Fruit and Nut Crops. Cambridge, MA: CABI Publishing, pp. 475–511.

Bump, V.L. (1989). Apple pressing and juice extraction. In: D.L. Downing (ed.), Processed Apple Products. New York: Van Nostrand Reinhold Publishing Co. pp. 53–82.

Bus, V.G.M., van de Weg, W.E., Durel, C.E., Gessler, C., Parisi, L., Rikkerink, E.H.A., Gardiner, S.E., Meulenbroek, E.J., Calenge, F., Patocchi, A., and Laurens, F.N.D. (2004). Delineation of a scab resistance gene cluster on linkage group 2 of apple. *Acta Hort*. 663: 57–62.

Büttner, R., Fischer, M., Forsline, P.L., Geibel, M., and Ponomarenko, V.V. (2000). Genebank work for preservation of the genetic diversity of apple. *Acta Hort*. 538: 39–42.

Cabe, P.R., Baumgarten, A., Onan, K., Luby, J.J., and Bedford, D.S. (2005). Using microsatellite analysis to verify breeding records: a study of 'Honeycrisp' and other cold-hardy apple cultivars. *HortScience*. 40(1): 15–17.

Calenge, F., Faure, A., Goerre, M., Gebhardt, C., van de Weg, W.E., Parisi, L., and 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*. *Phytopathology*. 94(4): 370–379.

Callen, D.F., Thompson, A.D., Shen, Y., Philips, H.A., Richards, R.I., and Mulley, J.C. (1993). Incidence and origin of "null" alleles in the $(AC)^n$ microsatellite markers. *Am. J. Hum. Genet*. 52: 922–927.

Castiglione, S., Pirola, B., Sala, F., Ventura, M., Pancaldi, M., and Sansavini, S. (1999). Molecular studies of ACC synthase and acc oxidase genes in apple. *Acta Hort*. 484: 305–310.

Cevik, V., and King, G.J. (2002a). High-resolution genetic analysis of the Sd-1 aphid resistance locus in *Malus* spp. *Theor. Appl. Genet*. 105: 346–354.

Cevik, V., and King, G.J. (2002b). Resolving the aphid resistance locus Sd-1 on a BAC contig within a sub-telomeric region of Malus linkage group 7. *Genome* 45: 939–945.

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

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.

Chevreau, E. (1984). Contributions a l'etude de la genetique du Pommier: apport de l'analyse enzymatique. These de Doctor Ingenieur, Orsay, 101 pp.

Chevreau, E., and Laurens, F. (1987). The pattern of inheritance in apple *(Malus* x d*omestica* Borkh): further results from leaf isozyme analysis. *Theor. Appl. Genet*. 71: 268–277.

Chevreau, E., Lespinasse, Y., and Gallet, M. (1985). Inheritance of pollen enzymes and polyploid origin of apple (Malus x domestica Borkh). *Theor. Appl. Genet*. 71: 268–277.

Chevreau, E., Manganaris A.G., and Gallet, M. (1999). Isozyme segregation in five apple progenies and potential use for map construction. *Theor. Appl. Genet*. 98: 329–336.

Chyi, Y.S., and Weeden, N.F. (1984). Relative isozyme band intensities permit the identification of the 2n gamete parent of triploid apple cultivars. *HortScience*. 19(6): 818–819.

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

Conner, P.J., Brown S.K., and Weeden, N.F. (1997). Randomly amplified polymorphic DNA-based genetic linkage maps of three apple cultivars. *J. Am. 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.

Coque, M., Díaz, M.B., and García, J.C. (1996). El cultivo del manzano en Asturias. Servicio de Publicaciones del Principado de Asturias, Asturias, 223 pp.

Costa, F., Stella, S., Magnani, R., and Sansavini, S. (2004). Characterization of apple expansion sequences for the development of ssr markers associated with fruit firmness. *Acta Hort*. 663: 341–344.

Costa, F., Stella, S., Weg, W., Guerra, W., Cecchinel, M., Dallavia, J., Koller, B., and Sansavini, S. (2005). Role of the genes Md-ACO1 and Md-ACS1 in ethylene production and shelf life of apple (*Malus domestica* Borkh). *Euphytica*. 141: 181–190.

Crane, M.B., and Lawrence, W.J.C. (1933). Genetical studies in cultivated apples. *J. Genet*. 28: 265–296.

Dapena, E. (1996). Review of Spanish collections. In: H.J. Case (ed.) European *Malus* Germplasm. Proceedings, 21–24 June 1995, Wye College, University of London, IPGRI, 42.

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

Dayton, D.F., and Williams, E.B. (1968). Independent genes in Malus for resistance to *Venturia inaequalis*. *Proc. Am. Soc. Hort. Sci*. 92: 89–94.

Decourtye, L. (1967). Étude de quelques caractères a controle génétique simple chez le pommier (*Malus* sp) et le poirier (*Pyrus communis*). *Les Annales de l'Amélioration des Plantes*. 17: 243–266.

Decourtye, L., and Brian, C. (1967). Détermination des desoins en froid de pépins pomacées interprétation des courbes de germination. *Les Annales de l'Amélioration des Plantes*. 17: 375–391.

Decourtye, L., and Lantin, B. (1969). Contribution a la connaissance de mutants spurs de pommier; hérédité du caractère. *Les Annales de l'Amélioration des Plantes*. 19: 227–238.

De Nettancourt, D. (2001). Incompatibility and Incongruity in Wild and Cultivated Plants. Springer-Verlag, Berlin.

Dennis, F.G. (1986). Apple. In: S.P. Monseline (ed.), Handbook of Fruit Set and Development. CRC Press, Boca Raton, FL, pp. 1–45.

Dennis, F.J. (2003). Flower, pollination and fruit set and development. In: D.C. Ferree and I.J. Warrington (eds.), Apples: Botany, Production and Uses. London, UK: CAB International, pp. 153–166.

Díaz-Hernández, B., Ciordia-Ara, M., Coque-Fuertes, M., and Pereira-Lorenzo, S. (2003). Agronomic behaviour of six Asturian apple (*Malus x domestica*) cultivars for cider production over two rootstocks. *J. Am. Pomol. Soc*. 57(3): 121–127.

Dickson, E.E., Kresovich, S., and Weeden, N.F. (1991). Isozymes in North American Malus (Rosaceae): hybridization and species differentiation. *Syst. Bot*. 16: 363–375.

Dong, J.G., Kim, W.T., Yip, W.K., Thompson, G.A., Li, L., Bennett, A.B., and Yang, S.F. (1991). Cloning of a cDNA encoding 1-aminocyclopropane-1-carboxylate synthase and expression of its mRNA in ripening apple fruit. *Planta*. 185: 38–45.

Dong, J.G., Olson, D., Silverstone, A, and Yang, S.F. (1992). Sequence of a cDNA coding for a 1-arninocyclopropane-1-carboxylate oxidase homolog from apple- fruit. *Plant Physiol*. 98: 1530–1531.

Dong, Y.-H., Kvarnheden, A., Yao, J.-L., Sutherland, P.W., Atkinson, R.G., Morris, B.A., and Gardner, R.C. (1998). Identification of pollination-induced genes from the ovary of apple (*Malus domestica*). *Sex. Plant Reprod*. 11: 277–283.

Downing, D.L. (1989). Apple cider. In: D.L. Downing (ed.), Processed Apple Products. Avi Book, New York, pp. 169–187.

Dunemann, F., Bräcker, G., Markussen, T., and Roche, P. (1999). Identification of molecular markers for the major mildew resistance gene Pl^2 in apple. *Acta Hort*. 484: 411–416.

Durel, C.E., Calenge, F., Parisi, L., van de Weg, W.E., Kodde, L.P., Liebhard, R., Gessler, C., Thiermann, M., Dunemann, F., Gennari, F., Tartarini, S., and Lespinasse, Y. (2004). An overview of the position and robustness of scab resistance QTLs and major genes by aligning genetic maps of five apple progenies. Eucarpia Symposium in fruit breeding and genetics, 1–5 Sept. 2003, Angers, France. ISHS. *Acta Hort*. 663: 135–140.

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.

Fernández-Fernández, F., Clarke, J.B., and Tobutt, K.R. (2004). Preferential amplification of microsatellite alleles: an example in apple. XIth Eucarpia Symposium on Fruit Breed & Genetics. F. Laurens and K. Evans (eds.). ISHS. *Acta Hort*. 663: 87–89.

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

Fischer, C. (1994). Breeding apple cultivars with multiple resistance. In: M. Kellerhals and H. Schmidt (eds.), Progress in Fruit Breeding. Kluwer Academic Publishers, Dordrecht, pp. 45–50.

Fischer, C. (2000). Multiple resistant apple cultivars and consequences for the apple breeding in the future. *Acta Hort*. 538: 229–234.

Fischer, C., and Fischer, M. (1996). Results in apple breeding at Dresden-Pillnitz – Review. *Gartenbauwissenschaft*. 61: 139–146.

Fischer, C., and Richter, K. (1999). Results in fire blight resistance in the Pillnitz apple breeding program. *Acta Hort*. 489: 279–285.

Fischer, C., Schreiber, H., Büttner, R., and Fischer, M. (1998). Testing of scab resistance stability of new resistant cultivars within the apple breeding program. *Acta Hort*. 484: 449–454.

Fischer, C., Fischer, M., and Dierend, W. (2001b). Stability of scab resistance – Evaluation, problems and chances of durability. *Eucarpia Fruit Breeding Section Newsletter*. 5: 11–12.

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

Fischer, M., and Fischer, C. (2000). Evaluation of the species and cultivars of Malus at the Fruit Genebank Dresden-Pillnitz and using the results in apple resistance breeding. Proc. 11th Fruit Congress in Yugoslavia, Nov. 2000, pp. 29–30.

Fischer, M., and Fischer, C. (2002). The Dresden-Pillnitz long-term apple breeding program and its results. *Compact Fruit Tree*. 35: 21–25.

Fischer, M., Fischer, C., and Dierend, W. (2005). Evaluation of the stability of scab resistance in apple: a co-operation between gene bank curator, breeder and fruit grower. *Plant Genetic Resources Newsletter*. 142: 36–42.

Fischer, M., Geibel, M., and Büttner, R. (2003). Zwischen 'Anacuta' und 'Pinova' – Bilanz 10 jähriger Genbankarbeit für Obst in Dresden-Pillnitz. *Vorträge Pflanzenzüchtung*. 57: 25–36.

Fischer, M., Schüler, W., Fischer, C., and Gerber, H.-J. (2001a). Eignung Pillnitzer Apfelsorten-Neuzüchtungen für die Herstellung von Verarbeitungsprodukten aus biologisch orientiertem Anbau. *Flüssiges Obst*. 68(H.1): 20–24.

Forsline, P.L. (2000). Procedures for collection, conservation, evaluation and documentation of germplasm using Malus as an example. *Acta Hort*. 522: 223–234.

Forsline, P.L., and Aldwinckle, H.S. (2004). Evaluation of Malus sieversii seedling populations for disease resistance and horticultural traits. *Acta Hort*. 663: 529–534.

Forsline, P.L., Aldwinckle, H.S., Dickson, E.E., Luby, J.J., and Hokanson, S. (2003). Chap. 1: Collection, maintenance, characterization and utilization of wild apples of Central Asia A. p. 1–61. In: J. Janick, P. Forsline, E. Dickscon, R. Way and M. Thompson (eds.), Horticultural Reviews, vol. 29. Wild Apple and Fruit Trees of Central Asia.

Forsline, P.L, McFerson, J.R., Lamboy, W.F., and Towill, L.E. (1998). Development of base and active collection of Malus germplasm with cryopreserved dormant buds. *Acta Hort*. 484: 75–78.

Forte, A.V., Ignatov, A.N., Ponomarenko, V.V., Dorokhov, D.B., and Savelyev, N.I. (2002). Phylogeny of the *Malus* (Apple Tree) species, inferred from the morphological traits and molecular DNA analysis. *Russ. J. Genet*. 38(10): 1150–1161.

Gardiner, S.E., Bassett, H.C.M., and Madie, C. (1996). Isozyme, randomly amplified polymorphic DNA (RAPD), and restriction fragment-length polymorphism (RFLP) markers used to deduce a putative parent for the 'Braeburn' apple. *J. Amer. Soc. Hort. Sci.* 121(6): 996–1001.

Gianfranceschi, L., and Soglio, V. (2004). The European 589 project HiDRAS: innovative multidisciplinary approaches to breeding high quality disease resistant apples. *Acta Hort*. 663: 327–330.

Gianfranceschi, L., Seglias, N., Tarchini, R., Komjanc, M., and Gessler, C. (1998). Simple sequence repeats for the genetic analysis of apple. *Theor. Appl. Genet*. 96: 1069–1076.

Goulão, L., and Oliveira, C.M. (2001). Molecular characterisation of cultivars of apple (*Malus domestica* Borkh.) using microsatellite (ISSR and SSR) markers. *Euphytica*. 122: 81–89.

Grove, G.G., Eastwell, K.C., Jones, A.L., and Sutton, T.B. (2003). Diseases of apple. In: D.C. Ferree, and I.J. Warrington (eds.), Apples: Botany, Production and Uses. CAB International, Wallington, Oxford, UK, pp. 459–488.

Guilford, P., Prakash, S., Zhu, J.M., Rikkerink, E., Gardiner, S., Bassett, H., and Forster, R. (1997). Microsatellites in *Malusx domestica* (apple) abundance, polymorphism and cultivar identification. *Theor. Appl. Genet*. 94: 249–254.

Guinea, E. (1957). Manzanas de España (Pomografía hispánica). Instituto Nacional de Investigaciones Agronómicas. Ministerio de Agricultura. Madrid, 248 pp.

Gygax, M. (2004). The use of different PCR-based technologies to analyse the genetics of apple scab resistance conferred by the resistance genes Vbj and Vf. Dissertation 15384, Swiss Federal Institute of Technology, Zurich, Switzerland.

Hampson, C.R., Quamme, H.A., Hall, J.W., MacDonald, R.A., King, M.C., and Cliff, M.A. (2000). Sensory evaluation as a selection tool in apple breeding. *Euphytica*. 111: 79–90.

Harada, T., Sunako, T., Wakasa, Y., Soejima, J., Satoh, T., and Niizeki, M. (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*. 102: 742–746.

Harris, S.A., Robinson, J.P., and Juniper, B.E. (2002). Genetic clues to the origin of the apple. *Trends Genet*. 18: 416–430.

Heilborn, O. (1935). Reductive division pollen lethality. *Acta Hort*. 11: 129–184.

Hemmat, M., Brown, S.K., and Weeden, N.F. (2002). Tagging and mapping scab resistance genes from R1270-7A apple. *J. Am. Soc. Hort. Sci.* 127: 365–370.

Hemmat, M., Weeden, N.F., Aldwinckle, H.S., and Brown, S.K. (1998). Molecular markers for the scab resistance (V^f) region in apple. *J. Am. Soc. Hort. Sci.* 123: 992–996.

Hemmat, M., Weeden, N.F., and Brown, S.K. (2003). Mapping and evaluation of *Malus X domestica* microsatellites in apple and pear. *J. Am. Soc. Hort. Sci.* 128: 515–520.

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. Am. Soc. Hort. Sci.* 122: 347–349.

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

Henning, W. (1947). Morphologisch, systematische und genetische Untersuchungen an Arten und Artbastarden der Gattung Malus. *Der Züchter*. 17: 289–349.

Hofer, M., Gomez, A., Aguiriano, E., Manzanera, J.A., and Bueno, M.A. (2002). Analysis of simple sequence repeat markers in homozygous lines of apple. *Plant Breed.* 121: 159–162.

Höhne, F. (2001). Schorfresistenz bei Apfel am Standort Rostock durchbrochen. *Obstbau*. 26: 458–459.

Hokanson, S.C., Lamboy, W.F., Szewc-McFadden, A.K., and McFerson, J.R. (2001). Microsatellite (SSR) variation in a collection of *Malus* (apple) species and hybrids. *Euphytica*. 118: 281–294.

Hokanson, S.C., Szewc-McFadden, A.K., Lamboy, W.F., and McFerson, J.R. (1998). Microsatellite (SSR) markers reveal genetic identities, genetic diversity, and relationships in a *Malus x domestica* Borkh. core subset collection. *Theor. Appl. Genet*. 97: 671–683.

Hu, C.G., Hao, Y.J., Honda, C., Kita, M., and Moriguchi, T. (2003). Putative PIP1 genes isolated from apple: expression analyses during fruit development and under osmotic stress. *J . Exp. Bot*. 54: 2193–2194.

Hunter, R.L., and Markert, C.L. (1957). Histochemical demonstration of enzymes separated by zone electrophoresis in starch gels. *Science*. 125: 1294–1295.

IBPGR. (1982). Descriptor list for apple (*Malus*). In: R. Watkins, and R.A., Smith, (eds.), International Board for Plant Genetic Resources, Rome (Italy); Commission of the European Communities, Brussels (Belgium). Committee on Disease Resistance Breeding and Use of Genebanks *Division*: AGP *Publ.place:* Brussels (Belgium) *Publisher:* CEC; IBPGR *Publ.date:* Nov 1982, 46p.

Iglesias, I., Carbó, J., Bonany, J., Dalmau, R., Guanter, G., Montserrat, R., Moreno, A., and Pagès, J. (2000). Manzano, las variedades de más interés. Institut de Recerca i Tecnologia Agroalimentàries, Barcelona, 240 pp.

IPGRI. (1996). European *Malus* germplasm. In: H.J. Case (ed.), Proceedings of a Workshop, 21–24 June 1995. Wye College, University of London.

Itoiz, R., and Royo, B. (2003). Isoenzymatic variability in an apple germplasm bank. *Genet. Resour. Crop. Ev.* 50: 391–400.

Janick, J., Cummins, J.N., Brown, S.K., and Hemmat, M. (1996). Apples. In: J. Janick and J.N. Moore (eds.), Fruit Breeding, Tree and Tropical Fruits. John Wiley & Sons, Inc., Vol I pp. 1–77.

James, C.M., and Evans, K.M. (2004). Identification of molecular markers linked to the mildew resistance genes Pl-d and Pl-w in apple. *Acta Hort.* 663: 123–127.

Jensen, P.J., Rytter, J., Detwiler, E.A., Travis, J.W., and McNellis, T.W. (2003). Rootstock effects on gene expression patterns in apple tree scions. *Plant Mol. Biol.* 493: 493–511.

Kamboj, J.S., Browning, G., Blake, P.S., Quinlan, J.D., and Baker, D.A. (1999). GC-MS-SIM analysis of abscisic acid and indole-3-acetic acid in shoot bark of apple rootstocks. *Plant Growth Regul.* 28(1): 21–27.

Kamboj, J.S., Quinlan, J.D., Guardiola, J.L., and Garcia Martinez, J.L. (1998). The apple rootstock and its influence on endogenous hormones. *Acta Hort.* 463: 143–152.

Karp, A., and Edwards, K.J. (1998). DNA markers: a global overview. In: G. Caetano-Anollés and P.M. Gresshoff (eds.), DNA Markers: Protocols, Aplications and Overviews., Wiley, New York, pp. 1–13.

Karp, A., Kresovich, S., Bhat, K.V., Ayad, W.G., and Hodgkin, T. (1997). Molecular tools in plant genetic resources conservation: a guide to the technologies. IPGRI Technical Bulletin N.2, Rome, 27 pp.

Kemp, H., Van der Maas, M.P., Voorrips, R.E., Groenwold, R., and van de Weg, W.E. (2004). An assessment of the durability and susceptibility of scab resistance in apple cultivars. *Acta Hort.* 663: 221–224.

Kenis, K., and Keulemans, J. (2004). QTL analysis of growth characteristics in apple. XIth Eucarpia Symposium on Fruit Breed & Genetics. *Acta Hort.* 663(1): 369–374.

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 pumila* Mill). *J. Hort. Sci. Biotechnol.* 78: 512–517.

King, G.J., Alston, F.H., Batlle, I., Chevreau, E., Gessler, C., Janse, J., Lindhout, P., Manganaris, A.G., Sansavini, S., Schmidt, H., and Tobutt, K. (1991). The 'European Apple Genome Mapping Project' – developing a strategy for mapping genes coding for agronomic characters in tree species. *Euphytica.* 56: 89–94.

King, G., Tartarini, S., Brown, L., Gennari, F., and Sansavini, S. (1999). Introgression of the Vf source of scab resistance and distribution of linked marker alleles within the Malus gene pool. *Theor. Appl. Genet.* 99: 1039–1046.

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., and Tartarini, S. (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.

Kitahara, K., and Matsumoto, S. (2002a). Sequence of the S10 cDNA from "McIntosh" apple and a PCR-digestion identification method. *HortScience.* 37: 187–190.

Kitahara, K., and Matsumoto, S. (2002b). Cloning of the S25 cDNA from 'McIntosh' apple and an S25-allele identification method. *J. Hort. Sci. Biotechnol.* 77: 724–728.

Kitahara, K., Matsumoto, S., Yamamoto, T., Soejima, J., Kimura, T., Komatsu, H., and Abe, K. (2005). Parent identification of eight apple cultivars by S-RNase analysis and simple sequence repeat markers. *HortScience.* 40: 314–317.

Klein, L.G., Way, R.D., and Lamb, R.C. (1961). The inheritance of a lethal factor in apples. *Proc. Am. Soc. Hort. Sci.* 77: 50–53.

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, R.L., Briggs, J.B., Massee, A.M., and Tydeman, H.M. (1962). The inheritance of resistance to woolly aphid. *Eriosoma lanigerum* (Hausmm.) in the apple. *J. HortScience*. 37: 207–218.

Kobel, F., Steinegger, P., and Anliker, J. (1939). Weitere Untersuchungen über die Befruchtungsverhältnisse der Apfel und Birnsorten. *Landwirtschaftliches Jahrbuch der Schweiz*. 53: 160–191.

Kondo, S., Hiraoka, K., Kobayashi, S., Honda, C., and Terahara, N. (2002). Changes in the expression of anthocyanin biosynthetic genes during apple development, *J. Am. Soc. Hort. Sci.* 127: 971–976.

Korban, S.S., and Dayton, D.F. (1983). Evaluation of Malus germplasm for sources of resistance to powdery mildew. *HortScience*. 18: 219–220.

Korban, S.S., and Swiader, J.M. (1984). Genetic and nutritional status in bitter pit resistant and susceptible seedlings. *J. Amer. Soc. Hort. Sci.* 109: 428–432.

Krens, F.A., Pelgrom, K.T.B., Schaart, J.G., Den Nijs, A.P.M., and Rouwendsal, G.J.A. (2004). Clean vector technology for marker-free transgenic fruit crops. *Acta Hort*. 663: 431–435.

Krüger, J. (1999). Vorkommen der Schorfrassen 1 bis 6 auf den Ahrensburger Apfelfeldern. *Erwerbsobstbau*. 41: 129–130.

Kusaba, S., Honda, C., and Kano-Murakami, Y. (2000). Characterization of gibberellin 20-oxidase gene in apple. *Acta Hort*. 538: 605–608.

Lamboy, W.F., Yu, J., Forsline, P.L., and Weeden, N.F. (1996). Partitioning of allozyme diversity in wild populations of *Malus sieversii* L. and implications for germplasm collection. *Am. Soc. Hort. Sci.* 121: 982–987.

Lapins, K.O., and Watkins, R. (1973). Genetics of compact growth. *Report of East Malling Research Station for 1972*, p. 136.

Lateur, M., and Populer, C., (1996a). Les variétés de pommier RGF (Ressources Génétiques Frutiéres). *Le Fruit Belge*. 459: 25–29.

Lateur, M., and Populer, C., (1996b). Les variétés de pommier RGF diffusees pour la Station de Phytopathologie de Gembloux. *Le Fruit Belge*. 460: 57–60.

Lauri, P.É., and Costes, E. 2004. Progress in whole-tree architectural studies for apple cultiva characterization at INRA, France – Contribution to the idotype approach. *Acta Hort*. 663, 357–362.

Lawrens, F., Durel, C.E., and Lascostes, M. (2004). Molecular characterization of French local apple cultivars using SSRs. XIth Eucarpia Symposium on Fruit Breed & Genetics. F. Laurens and K. Evans (eds.). *Acta Hort*. 663: 639–642.

Lawson, D.M., Hemmat, M., and Weeden, N.F. (1995). The use of molecular markers to analyze the inheritance of morphological and developmental traits in apple. *J. Am. Soc. Hort. Sci.* 120: 532–537.

Le Lezec, M., Babin, J., Belouin, A. (1996). Variétés inscrites an catalogue. L'Arboriculture Fruitiére, 496: 25–32.

Lea, A. (1990). Amarganess and astringency: the procyanidins of fermented apple ciders. In: R. Rouseff (ed.), Amarganess in Foods and Beverages. Elsevier, Amsterdam, pp.123–143.

Lespinasse, Y. (1992). Le Pommier In: A. Gallais and H. Bannerot (eds.), Amélioration des espèces végétales cultivées-objectifs et critères de sélection. INRA, Paris, pp. 579–594.

Lespinasse, Y. (2001). D.A.R.E. Newsletter No. 4, INRA, Angers.

Li, Y.H., Han, Z.H., and Xu, X. (2004). Segregation patterns of AFLP markers in F1 hybrids of a cross between tetraploid and diploid species in the genus Malus. *Plant Breed*. 123: 316–320.

Liebhard, R., Gianfranceschi, L., Koller, B., Ryder, C.D., Tarchini, R., van De Weg, E., and Gessler, C. (2002). Development and characterisation of 140 new microsatellites in apple (*Malus* × *domestica* Borkh.). *Mol. Breed.* 10: 217–241.

Liebhard, R., Koller, B., Patocchi, A., Kellerhals, M., Pfammatter, W., Jermini, M., and Gessler, C. (2003). Mapping quantitative field resistance against apple scab in a 'Fiesta' × 'Discovery' progeny. *Am. Phytopathol. Soc*. 93(4): 493–501.

Luby, J.J. (2003). Taxonomic classification and brief history. In: D.C. Ferree and I.J. Warrington (eds.), Apples: Botany, Production and Uses. CAB International, Wallington, Oxford, UK, pp. 1–14.

Maggioni, L., Janes, R., Hayes, A., Swinburne, T., and Lipman, E. (1997). Report of a working group on Malus/Pyrus. First meeting, 15–17 May. Dublin, Ireland. IPGRI, Roma.

Maliepaard, C., Alston, F.H., 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.

Manganaris, A.G. (1989). Isoenzymes as genetic markers in apple breeding. PhD Thesis, University of London, 430 pp.

Manganaris, A.G., and Alston, F.H. (1987). Inheritance and linkage relationships of glutamate oxaloacetate transaminase isoenzymes in apple. I. The gene *Got-l*, a marker for the S incompatibility locus. *Theor. Appl. Genet*. 74: 154–161.

Manganaris, A.G., and Alston, F.H. (1988a). Inheritance and linkage relationships of glutamate oxaloacetate transaminase in apple 2. The genes *GOT-2* and *GOT*-4. *Theor. Appl. Genet*. 76: 449–454.

Manganaris, A.G., and Alston, F.H. (1988b). The acid phosphatase gene *ACP-1* and its linkage with the endopeptidase gene *ENP-1* and the pale green lethal gene *l* in apple. *Acta Hort*. 224: 177–184.

Manganaris, A.G., and Alston, F.H. (1992a). Genetics of esterase isoenzymes in *Malu*s. *Theor. Appl. Genet*. 83: 467–475.

Manganaris, A.G., and Alston, F.H. (1992b). Genetics of leucine aminopeptidase in apple. *Theor. Appl. Genet*. 83: 345–352.

Manganaris, A.G., and Alston, F.H. (1992c). Inheritance and linkage relationships of peroxidase isoenzymes in apple. *Theor. Appl. Genet*. 83: 392–399.

Manganaris, A.G., Alston, F.H., Weeden, N.F., Aldwinckle, H.S., Gustafson, H.L., and Brown, S.K. (1994). Isozyme Locus Pgm-1 is tightly linked to a gene (Vf) for scab resistance in apple. *J. Am. Soc. Hort. Sci*. 119(6): 1286–1288.

MAPA. (1990). Anuario de estadística agraria. Ministerio de Agricultura, Pesca y Alimentación, 678p. ISBN: 84-7479-274-6.

Marini, R.P., Barden, J.A., Cline, J.A., Perry, R.L., and Robinson, T. (2002). Effect of apple rootstocks on average 'Gala' fruit weight at four locations after adjusting for crop load. *J. Am. Soc. Hort. Sci*. 127: 749–753.

Marinoni, D., Akkak, A., Bounous, G., Edwards, K.J., and Botta, R. (2003). Development and characterization of microsatellite markers in *Castanea sativa* (Mill.). *Mol. Breed*. 11:127–136.

Markussen, T., Kruger, J., Schmidt, H., and Dunemann, F. (1995). Identification of PCR-based markers linked to the powdery mildew resistance gene Pl^1 from *Malus robusta* in cultivated apple. *Plant Breed*. 114: 530–534.

Masseron, A. (1989). Les porte-greffe pommier, poirier et nashi. CTIFL, pp. 112–174.

Matityahu, A., Stern, R.A., Schjneider, D., and Goldway, M. (2005). Molecular identification of a New apple S-RNase–S29–cloned from "Anna", a low-chilling-requirement cultivar. *Hortscience*. 40(3): 850–851.

Menendez, R.A., Larsen, F.E., and Fritts, R. (1986a). Fingerprinting apple cultivars by electrophoretic isozyme banding patterns. *J. Environ. Hort*. 4(3): 101–107.

Menendez, R.A., Larsen, F.E., and Fritts, R. (1986b). Identification of apple rootstock cultivars by isozyme analysis. *J. Am. Soc. Hort. Sci.* 111(6): 933–937.
Morgan, J., and Richards, A. (1993). The Book of Apples. Ebury Press, London, 304 pp. ISBN: 0-09-177759-3.
Moss, D.W. (1982). Alkaline phosphatase isoenzymes. *Clin. Chem.* 28: 2007–2016.
Mowry, J.B., and Dayton, D.F. (1964). Inheritance of susceptibility to apple blotch. *J. Hered.* 55: 129–132.
Noiton, D.A.M., and Alspach, P.A. (1996). Founding clones, inbreeding, coancestry, and status number of modern apple cultivars. *J. Am. Soc. Hort. Sci.* 121(5): 773–782.
Norelli, J.L., Holleran, H., Johnson, W., and Robinson, T. (2003a). Resistance pf Geneva and other apple rootstocks to *Erwinia amylovora*. *Plant Dis.* 87: 26–32.
Norelli, J.L., Jones, A.L., and Aldwinckle, H.S. (2003b). Fire blight management in the 21st century: using new technologies that enhance host resistance in apple. *Plant Dis.* 87: 756–765.
Nybom, N. (1959). On the inheritance of acidity in cultivated apples. *Hereditas*. 45: 332–350.
Oraguzie, N.C., Iwanami, H., Soejima, J., Harada, T., and Hall, A. (2004). Inheritance of the Md-ACS1gene and its relationship to fruit softening in apple (*Malus x domestica* Borkh.). *Theor. Appl. Genet*. 108: 1526–1533.
Oraguzie, N.C., Yamamoto, T., Soejima, J., Suzuki, T., and De Silva, H.N. (2005). DNA fingerprinting of apple (*Malus* spp.) rootstocks using Simple Sequence Repeats. *Plant Breed.* 124: 197–202.
Orton, V. (1995). The American Cider Book. North Point Press edition, New York, 136 pp.
Patocchi, A., Bigler, B., Koller, B., Kellerhals, M., and Gessler, C. (2004). Vr2: a new apple scab resistance gene. *Theor. Appl. Genet*. 109: 1087–1092.
Patocchi, A., Gianfranceschi, L., and Gessler, C. (1999a). Towards the mapbased cloning of V^f: fine and physical mapping of the V^f region. *Theor. Appl. Genet*. 99: 1012–1017.
Patocchi, A., Vinatzer, B.A., Gianfranceschi, L., Tartarini, S., Zhang, H.B., Sansavini, S., and Gessler, C. (1999b). Construction of a 550 kb BAC contig spanning the genomic region containing the apple resistance gene Vf. *Mol. Genet. Genomics*. 262: 884–891.
Pereira-Lorenzo, S., Ascasíbar-Errasti, J., Ramos-Cabrer, A.M., and Piñeiro-Andión, J. (2002). Colección de cultivares autóctonos gallegos de manzano (*Malus xdomestica*) del Banco de Germoplasma de Mabegondo. Monografía INIA-Serie Agricultura.
Pereira-Lorenzo, S., Ramos-Cabrer, A.M., Ascasíbar-Errasti, J., and Piñeiro-Andión, J. (2003). Analysis of apple germplasm in Northwestern Spain. *J. Am. Soc. Hort. Sci.* 128(1): 67–84.
Pereira-Lorenzo, S., Ramos-Cabrer, A.M., and Díaz-Hernández, M.B. (2007). Evaluation of genetic identity and variation of local apple cultivars (*Malus x domestica*) from Spain using microsatellite markers. *Genet. Resour. Crop Ev.* 54: 405–420.
Petropoulou, S.P. (1985). Temperature related factors as selection criteria in Apple breeding. PhD Thesis, University of London.
Phipps, J.B., Robertson, K.R., Smith, P.G., and Rohrer, J.R. (1990). A checklist of the subfamily Maloideae (Rosaceae). *Can. J. Bot.* 68: 2209–2269.
Quinlan, J.D., and Tobutt, K.R. (1990). Manipulating fruit tree structure chemically and genetically for improved performance. *HortScience*. 25(1): 60–64.
Ramos-Cabrer, A.M., Díaz-Hernández, M.B., and Pereira-Lorenzo, S. (2007). Use of microsatellites in the management of genetic resources of Spanish apple cultivars. *J. Hort. Sci. Biotechnol.* 82(2): 257–265.
Rivas, D.M. (2004). La sidra asturiana. Picu Urriellu, Xixón, 221 pp.
Robinson, J., Harris, S.A., and Juniper, B.J. (2001). Taxonomy of the genus *Malus* Mill. (Rosaceae) with emphasis on the cultivated apple, *Malus domestica* Borkh. *Plant Syst. Evol.* 226: 35–58.

Roche, P., Van Arkel, G., and Van Heusden, A.W. (1997). A specific PCRassay based on an RFLP marker closely linked to the Sd1 gene for resistance to biotypes 1 and 2 of the rosy leaf curling aphid in apple. *Plant Breed.* 116: 567–572.

Royo, B.J., and Itoiz, R. (2004). Evaluation of the discriminance capacity of RAPD, isoenzymes and morphologic markers in apple (*Malus x domestica* Borkh.) and the congruence among classifications. *Genet. Resour. Crop Ev.* 51: 153–160.

Ryugo, K. (1988). Fruit Culture. John Wiley & Sons, New York, 344p.

Sadamori, S., Haniuda, T., Tsuchiya, S., and Yoshida, Y. (1964). Studies on aberrant leaf in apple, 1: observations on the frequency of aberrant leaf in apples and their seedlings. *Bulletin of the Horticultural Research Station, Morioka, Japan, Series C,* No. 2, pp. 1–7.

Sampson, D.R., and Cameron, R.F. (1965). Inheritance of bronze foliage, extra petals and pendulous habit in ornamental crab apples. *Proc. Am. Soc. Hort. Sci.* 96: 717–722.

Sandanayaka, W.R.M., Bus, V.G.M, Connolly, P., and Newman, R. (2003). Characteristics associated with wooly apple aphid resistance, *Eriosoma lanigerum*, of three apple rootstocks. *Entomologia Experimentalis et Applicata.* 109: 63–72.

Sandskar, B., and Gustafsson, M. (2004). Classification of apple scab resistance in two assortment orchards. *Genet. Resour. Crop Ev.* 51: 197–203.

Schmidt, M. (1938). *Venturia inaequalis* (Cooke) Aderh. VIII. Weitere Untersuchungen zur Züchtung schorfwiderstandsfähiger Apfelsorten. *Züchter* 10: 280–291.

Shupert, D., Smith, A.P., Janick, J., Goldsbrough, P.B., and Hirst, P.M. (2004). Segregation of scab resistance in three apple populations: molecular marker and phenotypic. *HortScience.* 39(6): 1183–1184.

Smithies, O. (1955). Zone electrophoresis in starchgels: group variation in the serum proteins of normal human adults. *Biochem. J.* 61: 629–641.

Smock, R.M., and Neubert, A.M. (1950). Apples and Apple Products. Interscience Publishers, INC, New York.

Soumelidou, K., Battey, N.H., John, P., and Barnett, J.R. (1994). The anatomy of the developing bud union and its relationship to dwarfing in apple. *Ann Bot.* 74: 605–611.

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

Sung, S.K., and An, G. (1997). Molecular cloning and characterization of a MADS-box cDNA clone of the Fuji apple. *Plant Cell Physiol.* 38: 484–489.

Sung, S.K., Yu, G.H., and An, G.H. (1999). Characterization of MdMADS2, a member of the SQUAMOSA subfamily of genes, in apple. *Plant Physiol.* 120: 969–978.

Sung, S.K., Yu, G.H., Nam, J., Jeong, D.H., and An, G.H. (2000). Developmentally regulated expression of two MADS-box genes, MdMADS3 and MdMADS4, in the morphogenesis of flower buds and fruits in apple. *Planta.* 210: 519–528.

Tang, X., and Zhang, W. (1992). Studies on pre-selection of dwarf apple seedlings by starch gel electrophoresis. *Acta Hort.* 317: 29–34.

Tartarini, S., Gianfranceschi, L., Sansavini, S., and Gessler, C. (1999). Development of reliable PCR markers for the selection of the Vf gene conferring scab resistance in apple. *Plant Breed.* 118(2): 183–186.

Tartarini, S., Negri, P., and Sansavini, S. (1997). Il miglioramento genetico per la resistenza alle aversità biotiche del melo: il ruolo dei marcatori nella selezione assistita. *Riv. Frutticoltura.* 1: 63–66.

Thompson, J.M., and Taylor, L. (1971). Genetic susceptibility to *Glomerella* leaf blotch in apple. *J. Hered.* 62: 303–306.

Tignon, M., Kettmann, R., and Watillon, B. (2000). AFLP: use for the identification of apple cultivars and mutants. *Acta Hort.* 521: 219–226.

Tignon, M., Lateur, M., Kettmann, R., and Watillon, B. (2001a). Distinction between closely related apple cultivars of the belle-fleur family using RFLP and AFLP markers. *Acta Hort*. 546: 509–513.

Tignon, M., Watillon, B., and Kettmann, R. (2001b). Identification of copia-like retrotransportable element by apple. *Acta Hort*. 546: 515–520.

Tobutt, K.R. (1985). Breeding columnar apples at East Malling. *Acta Hort*. 159: 63–68.

Tobutt, K.R. (1994). Combining apetalous parthenocarpy with columnar growth habit in apple. *Euphytica*. 77: 51–54.

Tobutt, K.R., Boskovic, R., and Roche, P. (2000). Incompatibility and resistance to woolly apple aphid in apple. *Plant Breed*. 119: 65–69.

Torres, A.M. (1989). Isozyme analysis of tree fruits. In: D.E. Soltis and P.S. Soltis (ed.), Isozymes in Plant Biology. Chapman and Hall, London, pp. 192–205.

Triloff, P. (2006). Das Laub-Saugen: ein neues Werkzeug in der modernen Schorfbekämpfung? *Obstbau*. 30: 69–73.

Tubbs, F.R. (1973). Research fields in the interaction of rootstocks and scions in woody perennials. *Hort. Abst*. 43: 247–253, 325–335.

UPOV. (1974). Draft guidelines for the conduct of test for distinctness, homogeneity and stability (APPLE). International Union for the Protection of new varieties of plants (UPOV), 23 pp.

Ur-Rahman, H., James, D.J., Hadonou, A.M., and Caligari, P.D.S. (1997). The use of RAPD for verifying the apomictic status of seedlings of *Malus* species. *Theor. Appl. Genet*. 95: 1080–1083.

USDA, NRCS. (2006). The plants database National Plant Data Center, Baton Rouge, LA 70874-4490 USA [en línea]. Disponible en http://plants.usda.gov [consulta 9 enero 2006].

Van der Linden, C.G., Vosman, B., and Smulders, M.J.M. (2002). Cloning and characterization of four apple MADS box genes isolated from vegetative tissue. *J. Exp. Bot*. 53: 1025–1036.

Vavilov, N. (1951). Estudios sobre el origen de las plantas cultivadas. ACME Agency, Buenos Aires, 147 pp.

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

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

Wagner, I., Schmitt, H.P., Maurer, W, and Tabel, U. (2004). Isoenzyme polymorphism and genetic structure of *Malus sylvestris* (L.) Mill. Native in western areas of Germany with respect to Malus xdomestica Borkh. XIth Eucarpia Symposium on Fruit Breed & Genetics. F. Laurens and K. Evans (eds.). *Acta Hort*. 663: 545–550.

Wakasa, Y., Ishikawa, R., Niizeki, M., Harada, T., Jin, S., Senda, M., and Akada, S. (2003). Majin: A Miniature DNA element associated with the genomes of pome fruit trees. *HortScience*. 38: 17–20.

Watada, A.E., Abbott, J.A., Hardenburg, R.E., and Lusby, W. (1981). Relationships of apple sensory attributes to headspace volatiles, soluble solids and titratable acids. *J. Amer. Soc. Hort. Sci*. 106: 130–132.

Watillon, B., Kettmann, R., Boxus, P., and Burny, A. (1997). Knotted1-like homeobox genes are expressed during apple trees (*Malus domestica* [L.] Borkh) growth and development. *Plant Mol. Biol*. 33: 757–763.

Way, R.D., Aldwinckle, H.S., Lamb, R.C., Rejman, A., Sansavini, S., Shen, T., Watkins, R., Westwood, M.N., and Toshida, Y. (1990a). Apples (*Malus*). In: Genetic Resources of Temperate Fruits and Nut Crops I. Netherlands, ISHS. 488p.

Way, R.D., Aldwinckle, H.S., Lamb, R.C., Rejman, A., Sansavini, S., Shen, T., Watkins, R., Westwood, M.N., and Toshida, Y. (1990b). Apples (*Malus*). *Acta Hort*. 290: 3–62.
Way, R.D., and McLellan, M.R. (1989). Apple cultivars for processing. In: D.L. Downing (ed.), Processed Apple Products. Avi, Van Nostrand Reinhold, New York, pp. 1–29.
Webster, A.D., and Wertheim, S.J. (2003). Apple rootstocks. In: D. Ferree and I. Warrington (eds.), Apples: Botany, Production and Uses. CAB International, Wallingford, UK, pp. 91–124.
Weeden, N.F., and Lamb, R.C. (1985). Identification of apple cultivars by isozyme phenotypes. *J. Amer. Soc. Hort. Sci*. 110(4): 509–515.
Weeden, N.F., and Lamb, R.C. (1987). Genetics and linkage analysis of 19 isozyme loci in apple. *J. Am. Soc. Hort. Sci*. 112: 865–872.
Weibel, F., Tamm, L. and Kellerhals, M. (1997). Resistenzeinbrüche bei schorfresistenten Apfelsorten. *Bio Aktuell*. 8: 6
Wertheim, S.J. (1998). Apple rootstocks. In: S.J. Wertheim (ed.), Rootstock Guide. Fruit Research Station, Wilhelminadorp, pp. 19–60.
Wilcox, A.N., and Angelo, E. (1936). Apple breeding studies, 1: fruit colour. *Proc. Am. Soc. Hort. Sci*. 33: 108–113.
Xu, M.L., and Korban, S.S. (2000). Saturation mapping of the apple scab resistance gene V^f using AFLP markers. *Theor. Appl. Gen*. 101: 844–851.
Xu, M., and Korban, S.S. (2002). AFLP-derived SCARs facilitate construction of a sequence-ready BAC contig of a 1.1 Mb segment that spans the *Vf* locus in the apple genome. *Plant Mol. Biol*. 50: 803–818.
Yao, J.-L., Dong,Y.-H., Kvarnheden, A., and Morris, B. (1999). Seven MADS-box genes in apple are expressed in different parts of the fruit. *J. Am. Soc. Hort. Sci*. 124: 8–13.
Zane, L., Bargelloni, L., and Patarnello, T. (2002) Strategies for microsatellite isolation: a review. *Mol. Ecol*. 11: 1–16.
Zhou, Z.Q. (1999). The apple genetic resources in China: the wild species and their distributions, informative characteristics and utilisation. *Genet. Resour. Crop Ev*. 46: 599–609.