Chapter 1

Apple Breeding: Present and Future

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Abstract Apple breeding has been extremely successful in providing a highly diverse fruit crop. Recent (>50 millions years ago) genome-wide duplication (GWD) resulted in the 17 chromosomes in the Pyrae (Velasco et al. 2010) and confirmed the origin of cultivated apple on *Malus sieversii* being the same species as *M. × domestica*. *Malus*, as many other species in the family Rosaceae, shows gametophytic self-incompatibility (GSI), which forces outcrossing. GSI at the pistil is regulated by extracellular ribonuclease, S-RNase, which is encoded by S locus. Growers and agronomists have provided multiple cultivars with different colors, shapes, resistances, climatic adaptation or industrial aptitudes. The aim in apple breeding was the combination of different kinds of resistance and good fruit quality to produce dessert cultivars and cultivars for processing. Some of the best of these cultivars display resistance to scab (*Venturia inaequalis*), mildew (*Podosphaera leucotricha*), fire blight (*Erwinia amylovora*), bacterial canker (*Pseudomonas syringae*), red spider mite (*Panonychus ulmi*), winterfrost and good fruit quality. Different scab resistance sources of wild species (Vf, Vr, VA) were combined in the new series of cultivars. Multiple efforts worldwide have conserved most of that variation, the pillar for the traditional and new techniques profiting from the

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analysis of the apple genome, the genome-wide association studies (GWAS), identifying SNPs and genes, the analysis of genes differentially expressed (GDE) identified by qRT-PCR and microarray analysis, and the recent molecular genetic tool CRISPR/Cas9 to edit and correct the genome.

Keywords Germplasm • Diversity • Breeding • Hybridization • Genomics • Assisted selection • Molecular markers • Phenotypic traits

1.1 Introduction

The apple stopped being a semi-luxury a long time ago, and is now a basic foodstuff. After all, at 56 million mt harvested annually, it is in twelfth place in world production of foodstuffs (maize 604 million mt, citrus fruits 103 million mt, bananas 57 million mt) (FAO 1998). The trend is increasing. The reason for this development is the nutritional-physiological value of the fruits as well as their good handling in harvesting, storage, transportation and marketing. The apple has become a product which is traded worldwide.

The physiological value of the apple lies in its high vitamin, antioxidant, and mineral content. The apple contains all important free amino acids in harmonious combination. The vitamin content, it is true, varies substantially between the varieties and years, but one is still able to cover a large part of the daily vitamin requirement with just the regular consumption of apples. The dietary effect of apples stems also from the high content and combination of fruit sugars, in which varieties with increased fructose content are particularly recommended for diabetics. On the basis of these figures, one can probably only have an inkling of the variety of different flavors that are available from apples. But this also explains the interest in maintaining many different varieties, in order to also be able to appraise and enjoy the fine differences (Hartmann 2015). The nostalgia for old varieties is great at present, as a certain degree of simplicity of taste is unmistakable in the few varieties available in supermarkets. But the breeding work is going on worldwide.

Apples have been known as a foodstuff for about 10,000 years. They spread from their center of diversity, an area corresponding roughly to present-day south-west China, Kazakhstan and Kirgizia and the bordering foothills of the Himalayas, via central Asia, from there via the historic Silk Road in the Near East, and from there through the Greeks and Romans to Europe. The wild species *Malus sieversii*, found in Central Asia, is today regarded as the original form. On the way to Europe, natural cross-fertilization with *M. orientalis*, found in the Caucasus, took place. In an easterly direction, it spread via southern China to eastern Asia, where it is likely that cross-fertilization took place, most likely with *M. prunifolia* and *M. baccata*. In a northerly direction, dissemination took place via the Trans-Caucasus to southern Russia. It is

possible that cross-fertilization took place here with the wood-apple native to Europe, *Malus sylvestris praecox*. In the Persian Empire, there was already plantation-like cultivation of fruit in the seventh to fifth centuries before Christ. The dissemination of the apple, which, as everyone knows, is distinguished by a very high genetic variability, was always accompanied by an appropriate selection, so that in the end tasty and large-fruited forms were multiplied, which can be seen as the archetypes of today's varieties. A precondition of this was that one understood how to vegetative multiply fruit coppices, i.e. refine them, in order to be able to keep and propagate the selected best in this manner, again and again. This was possibly deduced by Babylonian gardeners roughly 2,500–3,000 years ago. This is how apples began to be *domesticated*. This took place about 3,000–4,000 years later on the old Silk Road, when goats and sheep were domesticated between the Euphrates and the Tigris, which happened about 7,000–8,000 years earlier (Büttner et al. 2000a; Juniper et al. 1999).

Thanks to Homer, we know from the Odyssey (800 B.C.), that Greeks were already naming different types of apple (Petzold 1990). The Romans also knew many varieties of apples by name, and spread these to the North and West in Europe (Hurt 1916). Stock containers were able to be excavated from sunken Viking ships (1000–500 A.D.), which, among other things, contained apples as an ingredient of preserved food. Today many of the *old* varieties still known, thus came from the seeds of these varieties in the Middle Ages and later. It is clear that the first varieties of apple to reach America came with emigrants from Europe in the late Middle Ages.

Genuine pomology, that is to say the scientific treatment of varieties of fruit, began in the eighteenth century, and the science of fruit cultivation first appeared as a specialist subject within plant cultivation at the end of the nineteenth century. Some important varieties from this time are still being cultivated, such as cvs. Delicious (USA before 1880), Prince Albrecht of Prussia/Prinz Albrecht von Preussen (Germany around 1865), Cox Orange (England around 1850), Boskoop (Holland 1856), Gravenstein (Italy before 1670), McIntosh (Canada 1796), Granny Smith (Australia before 1870) or Goldparmäne (France before 1700) (Fischer 2010; Way et al. 1992). The form taken by the production of fruit 100–200 years ago was the standard tree. Intensive plantation cultivation of fruit with small-crowned spindle-bush trees according to strict economic parameters was only possible after the introduction of dwarfing rootstocks. Its development only began in the middle of the twentieth century – only about 70 years ago!

More and more integrated and biological (*organic*) cultivation methods are becoming increasingly important in all main fruit production areas of the world. The situation concerning the authorization of pesticides is becoming more critical as increased attention is focused on the conservation of the environment. The control of certain pests or diseases with chemicals is already being questioned to some degree. An alternative solution here is the cultivation

of resistant varieties which can be grown with much less pesticides. Unfortunately, however, there is still a very low level of acceptance of resistant varieties in the commercial cultivation of apples.

At present, the essential conditions that must be met before a new variety can be introduced on the market are:

- a) With respect to the varieties:
  - 1. Two colors with attractive shiny red shades as an over color
  - 2. Sweet, aromatic taste
  - 3. Firm, crisp fruit flesh with a long shelf life
- a) With respect to the trade:
  - 4. The willingness to introduce a new variety
  - 5. Substitution of an established variety
  - 6. Considerable advertising expenditure.

In the final analysis, the grower must and will produce what the trade wants, not the other way round, whereby it does not matter to the trade at present how the varieties are produced, as long as they conform to the required quality standards. There will be, however, certain changes with the introduction of trademarks such as Eco Label or Certified Organic labels. The demand for organically-grown products will increase even if the price is higher (Fischer and Fischer 2008; Kellerhals and Gessler 1995).

Producing fruit, especially apples, organically—and to the best quality in the long term—is not very easy and requires experienced specialists. In addition, pest control is still necessary in organic cultivation; the more sensitive the varieties grown, the more protection is needed. All these factors are only of little interest to the consumer, who wants the following criteria:

- a) High-quality fruits, free of pesticide residues (the consumer who is not well informed in this area feels this is more probable in organic cultivation, which explains the increased demand for this)
- b) Continuously, consistently good supply
- c) Diversity in varieties, (whereby the demand here keeps within limits).

The resistance of one variety to specific diseases or pests resulting from the breeding work for decrease in the use of plant protection agents in cultivation is still not a sales argument. This requires more intensive explanation and corresponding advertising (Stehr 2000).

1.1.1 Origin

Recent (>50 million years ago) genome-wide duplication (GWD) resulted in the 17 chromosomes in the Pyreae (Velasco et al. 2010) and confirmed the origin of cultivated apple on *Malus sieversii* being the same species as *M. × domestica*; closely related to *M. orientalis* and to *M. × asiatica* (a Chinese cultivated apple form), and less closely to *M. sylvestris*, *M. baccata*, *M. micromalus* and *M. prunifolia*.

The wild apple *Malus sieversii* (Ldb.) Roem is found in the Tian Shan Mountains in Central Asia, and it is thought to be originated about 4,000–10,000 years ago. Apples were brought to west by the Silk Route where they hybridized with other wild apples, such as *M. baccata* (L.) Borkh. in Siberia, *M. orientalis* Uglitz. in the Caucasus and *M. sylvestris* Mill. in Europe (Cornille et al. 2014).

Grafting an apple produced instant domestication as in other perennial crops, with further hybridizations but without important genetic introgressions from other species as was believed, such as *Malus sylvestris* (Velasco et al. 2010). The specific fruit of the Pyreae, the pome, is formed by the growth of the lower part of the sepals, which was related to MADS-box genes, involved in flower and fruit development and in the metabolism of sorbitol.

Over 7,000 apple cultivars have been cited, but only a few are relevant in the world production. Those cultivars originated in the nineteenth century, such as McIntosh (1800s), Jonathan (1820s), Cox's Orange Pippin (1830s), Granny Smith (1860s), Delicious (1870s) and Golden Delicious (1890s) (Janick et al. 1996; Noiton and Alspach 1996). Most of them are chance seedlings from which clones were selected, or frequently from controlled hybridizations.

Local cultivars, many of them conserved in germplasm banks, were cited so early as the 18th century, such as those in Spain (Sarmiento, 1986), where hybridization, selection of polyploids and introgression were the main origin of variability which was fixed, instant domestication, by grafting (Pereira-Lorenzo et al. 2017). The use of molecular markers determined that clonality in Spanish collections accounted up to 30 %. In some important varietal groups intra-cultivar variability was found, with seedlings derived from a main clone and some polyploids (21 %) derived from diploids as a result of the union of an unreduced 2n-gamete with a normal n-gamete (Einset 1952).

1.2 Development of Molecular Markers and Marker-Assisted Selection

Marker-assisted selection (MAS) is based on the use of genetic markers to select specific traits or quantitative trait loci (QTL) for which they are associated, normally named *marker-trait association* and *marker-locus-trait association*, respectively) (Ru et al. 2015).

Early selection on progenies of tree breeding programs is one of the main

interests of Marker-assisted seedling selection (MASS), which uses DNA markers to perform an early DNA-based evaluation of genetic traits on seedlings obtained from designed biparental crosses to combine selected traits in order to produce a potential novel cultivar (Ru et al. 2015). MASS shortens the period of seedling selection due to juvenility and large plant sizes of most fruit trees. MASS can also have advantages for: i) the identification of individuals with multiple resistance alleles to a disease; ii) when most of the observed phenotypic variance is due to environmental factors; and iii) when there are unexpected genotype×environment interactions, G×E), which involves to replicate seedlings in one or more locations. Even though DNA markers improve the genetic gain of seedling selection, especially for traits with low heritability, no study has been done in rosaceous tree fruit on the genetic gain of MASS.

Some examples of MASS on apples are (Ru et al. 2015) related with resistances: i) screening Rvi6 (Vf) gene for scab apple resistance; and ii) fire blight (FB-F7QTL) in combination with scab (alleles at the Rvi6 and Rvi4 loci), and powdery mildew (alleles at the Pl2 locus); and a marker-locus-trait association between a gene-based SCAR marker, the Md-ACS1-indel marker designed based on the Md-ACS1 gene) and apple fruit postharvest storability.

1.3 Genetic Diversity within Genus *Malus* and *Malus x domestica*

Although *Malus sieversii* has been identified as the main contributor to the genome of the cultivated apple (*M. x domestica*), multiple species have contributed to the genetic makeup of domesticated apples, in particular the wild European crabapple *M. sylvestris* which is genetically more closely related to *M. domestica* than *M. sieversii* (Cornille et al. 2014). In fact, estimated genetic differentiation based on the analysis of the apple genome (Velasco et al. 2010) was considered *moderate* between *M. x domestica* and *M. sieversii* ($F_{st} = 0.14$) and *great* between *M. x domestica* and *M. sylvestris* and between *M. sieversii* and *M. sylvestris* ($F_{st} = 0.17$ and $F_{st}=0.21$, respectively). Chloroplast genome diversity corroborates the close relationship between *M. x domestica* and *M. sylvestris* with the existence of chlorotypes shared by both species (Coart et al. 2006).

Considering domesticated apples *Malus x domestica*, allelic diversity in genetic resources from mainland Portugal were reported as 11.5 alleles per locus (Ferreira et al. 2016), similar to that reported in northwestern Spain (Galicia and Asturias) by Pereira-Lorenzo et al. (2007) (11.4), to local germplasm from Aragon, northeastern Spain (12.4) (Pina et al. 2014) in Iranian landraces (10.7) (Gharghani et al. 2009), and for Sarajevo and eastern Bosnia accessions (13.5) (Gasi et al. 2013), but slightly lower than those

reported for the Portuguese Azores Islands apple germplasm (15.2 alleles/locus) (Foroni et al. 2012), northeastern Spain (Aragon, Navarre, Basque Country, La Rioja and Catalonia regions) local material by Urrestarazu et al. (2012) (16.7), for the local accessions of Italian apple germplasm maintained at Bologna (16.9) (Liang et al. 2015), by Van Treuren et al. (2010) in the Netherlands (18.5) and that found for whole Spain (18.6) (Pereira-Lorenzo et al. 2017), for whole Europe (23.2) (Urrestarazu et al. 2016) and in the American germplasm bank for *Malus x domestica* with 19.1 alleles per locus (Gross et al. 2014).

Heterozygosity in local cultivars was 0.75 in mainland Portugal (Ferreira et al. 2016), about the same level of the American germplasm bank for *Malus x domestica*, 0.73 (Gross et al. 2014), and slightly higher than the level of diversity found in Aragon-Spain, 0.68 (Pina et al. 2014) but slightly lower than in north-eastern and north-western Spain, 0.80 and 0.83 respectively (Pereira-Lorenzo et al. 2007; Urrestarazu et al. 2012) and in Italy, 0.81 (Liang et al. 2015), Portuguese Azores Islands, 0.82, (Foroni et al. 2012), in in French germplasm (0.83) (Lassois et al. 2016) and broad European apple germplasm (0.81) (Urrestarazu et al. 2016).

Both triploids and tetraploid varieties are known to exist for apple (Brown 1998). Van Treuren et al. (2010) reports 21 % genotypes with 3 alleles at single locus or multiple loci, 10% of them known triploids and also 4 % of samples with even 4 alleles per locus. In Portuguese apple germplasm, among 64 unique genotypes, 12 (≈ 19 %) were putative triploids (Ferreira et al. 2016). Higher values, 24 and 29 %, were detected in Spanish germplasm by Urrestarazu et al. (2012) and Ramos-Cabrera et al. (2007), respectively. Recently, Urrestarazu et al. (2016) in germplasm of the whole of Europe reported lower numbers of triploid genotypes, 162 in 2,031 unique genotypes (8 %).

1.4 Genome-Wide Selection

The use of microarrays was useful to identify genes related to fruit development from pollination to ripeness, with a high correlation with the expression of those genes in tomato (Janssen et al. 2008).

An extremely useful tool for further genetic studies was provided by the genome of cultivated apple (Velasco et al. 2010). Domesticated apple showed a high number of putative genetic genes respecting to other crops and their gene sequences are being used for genome-wide functional studies. Many of genes have been related to resistances, flavor and other agronomy characteristics, such as the *MADS-box* genes, involved in flower and development the pome and in the metabolism of sorbitol. 4.4 SNPs per kb were found and they are useful for association studies with phenotypic traits.

Genome selection (GS) is thought to overcome marker-assisted selection

(MAS) and marker-assisted recurrent selection (MARS), but some authors (Jonas and de Koning 2013) pointed out that crops show a great diversity related to breeding cycles which makes GS more complex than in cattle breeding.

A genome-wide association study (GWAS) was already used to differentiate cider and dessert apples and to identify SNPs and genes related to that differentiation (Leforestier et al. 2015). Genetic differentiation between both groups of cultivars was low, explained because of the mixed types used for both cider and dessert. No genes were reported in the regions related to the SNPs found differentiating both groups, perhaps due to the still scarce knowledge about the genetic control of traits such as bitterness on apples, related to a higher content in polyphenols.

Analysis of genes differentially expressed (GDE) were identified by qRT-PCR and microarray analysis in fruited and deflowered cv. Gala adult trees to understand biennial bearing (Guitton et al. 2016). Some of them were differently expressed, such as TEMPRANILLO (TEM), FLORAL TRANSITION AT MERISTEM (FTM1) and SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL), and homologs of SPL and leucine-rich repeat proteins were found in QTLs previously related to biennial bearing.

1.5 Mating Systems in *Malus*

Malus, as many other species in the family Rosaceae, shows gametophytic self-incompatibility (GSI), which forces out-crossing since the pistil blocks closely related pollen (de Nettancourt. 2001). GSI at the pistil is regulated by extracellular ribonuclease, S-RNase, which is encoded by S locus (Sassa et al. 2010). There is another pollen S gene, tightly linked to the *S-RNase*, which determines the specificity of the pollen. Both genetic units are named S haplotype. Pollen S genes are F-box genes called SLF/SFB. The interaction of Pistil S (*S-RNase*) and pollen S (SLF/SFB) proteins determine incompatibility and, therefore, pollen tube growth in the pistil.

The unlikeness of recombination between *S-RNase* and SFB makes to identify both *S-RNase* and SFB alleles for S-genotyping (Yamane and Tao 2009).

1.6 Hybridization between Species

Fruit breeding aspires toward cultivating new varieties, which will have stable resistance and comply with the demands of the wholesale trade and of consumers. None of the resistant varieties which have become known internationally yet meet these stringent requirements sufficiently, and this is why there are still reservations about their large-scale cultivation. However,

resistant varieties have already been accepted for a long time in the self-supply and home garden sector.

It remains the aim of apple breeding to bring together in new varieties improvements in fruit quality + yield + resistance to different pathogens. Another new challenge is establishing a durable resistance in field cultivation, now that observations from different parts of Europe on the breakdown in the monogenic scab resistance sources from the *Malus floribunda* (Baumgartner et al. 2016; Büttner et al. 2000b; Fischer et al. 2000; Kellerhals and Gessler 1995; Lespinasse 2001) have come to light. So the use of old *vital* cultivars for breeding is under discussion (Bannier 2011; Sattler and Bannier 2016). Positive results do not yet exist. The fruit quality of progenies after crossings with cvs. Rote Sternrenette, Früher Victoria, Kardinal Bea or Prinzenapfel was unacceptable and unsuitable for recommendation (C. Fischer 2002 pers comm; Fischer and Fischer 2008; Rueß 2016).

The main breeding trends are dessert apple varieties for fresh consumption. Fruit quality is the first and foremost priority. The strategy of resistance breeding is characterized by its integration into a system of measures for plant protection in the broadest sense. While doing so, the total success is not aspired to, but rather a type of field resistance which allows both organisms — variety (host) and pathogen — to possess a chance of survival, and the host-parasite ecosystem maintains a natural balance. It is assumed that there is the possibility of living with the pathogens, not complete resistance (immunity), but rather the stable limiting of the damage caused by the pathogens to an economically-acceptable level (Fischer 2011; Koller et al. 1995). It is particularly important also to keep a constant low variation of the pathogens and in this way to keep constant as long as possible the environment-related dynamics of the host-pathogen-relationship. As a result, mutations arising among the harmful organisms are respectively prevented or delayed, or develop strains which are able to overcome the resistance in culture varieties. Therefore, variety, environment and harmful organisms must be *managed* in resistance breeding and later on in cultivation. Even for specialists this requires a high degree of knowledge and considerable instinct (Baumgartner et al. 2015; Fischer and Fischer 2006; Kellerhals and Gessler 1995).

Up to now only a few wild *Malus* species have been used as sources of resistance traits. Distantly-related species like *M. hupehensis*, *M. sargentii*, *M. coronaria*, *M. fusca*, *M. florentina* and *M. trilobata* should be considered as new sources for future breeding for resistance. However, using these species brings along problems with fertility and crossability because of polyploidy and apomixis. A number of back-crossings in conventional breeding methods will be necessary to get excellent fruit quality. Biotechnological methods (marker-assisted selection, gene mapping, genetic engineering, work with double haploids (DH) are possibilities for the future to help integrate resistance genes directly into susceptible varieties (Baumgartner et al. 2016; Dunemann 2017; Emeriewen et al. 2014; Flachowsky et al. 2014). However, this work must be

started with creation of a broad base of pyramiding genes for resistance to various diseases. It would be much easier to use the *M. sieversii* germplasm, which was collected from the American expeditions to Kazakhstan and is being now evaluated at the Geneva (USA), Havelock North (New Zealand) and the Dresden-Pillnitz (Germany) fruit gene banks. Resistance to scab and mildew has been found in progenies from different habitats. Up to now, the base of this resistance is unknown (Baumgartner et al. 2015; Büttner et al. 2000a; Geibel et al. 2000; Hatton 1954; Fischer and Fischer 2004, 2007; Fischer et al. 2005).

1.7 Cross-Pollination to Produce Interspecific Hybrids

Malus species and apple cultivars are usually diploid and have good crossability. Triploid forms are rare and not much suited for breeding. Technical problems at the time of crossing can be the blooming time, but it is solvable today.

In the apple breeding the aim is the combination of different kinds of resistance and good fruit quality in two directions: dessert cultivars and cultivars for processing. The best of these cultivars display resistance to scab (*Venturia inaequalis*), mildew (*Podosphaera leucotricha*), fire blight (*Erwinia amylovora*), bacterial canker (*Pseudomonas syringae*), red spider mite (*Panonychus ulmi*), winter frost and good fruit quality. Different scab resistance sources of wild species (V_f , V_r , V_A) were used to combine it in the new series of cultivars (Baumgartner et al. 2016; Büttner et al. 2004; Fischer and Fischer 1996, 2002, 2006; Kellerhals et al. 2009).

By utilization of all acquired evaluation data of *Malus* species and apple varieties, the following were used mostly as donors in cross-combination programs (Fischer 2011):

As sources for high yield and fruit quality. At the first step: cvs. Cox Orange, Oldenburg, Baumann Renette, Hammerstein and others. At the following steps: Alkmene, Auralia, Clivia, Elstar, Golden Delicious, Helios, Jonathan, Pilot, Pinova, Piros, Undine and others.

As sources for scab resistance. At the first step: cv. Antonova kamienna (V_A). At the following steps: *Malus x floribunda* (V_f), *M. x micromalus* (V_m), *M. x atrosanguinea* (V_f ?), *M. pumila* (V_r), and the cvs. James Grieve, Cox Orange, Oldenburg and others.

As sources for mildew resistance. *Malus x zumi* cv. Calocarpa, *M. x robusta* cv. Persicifolia, *M. x floribunda*, *M. x micromalus*, and the cvs. Dölmener Rosen, James Grieve, Helios, Alkmene, Lord Lambourne, Worcester Parmain,

and others.

As sources for fiber blight resistance. *Malus x robusta* cv. Persicifolia *M. x sublobata*, *M. x floribunda*, *M. prunifolia*, *M. fusca* (Emeriewen et al. 2014; Flachowsky et al. 2014), and a resistant clone from the Pillnitz breeding program Pi-A 44,14, and last also the cvs. Remo, Rewena, Reanda, Regia, Rekarda, Recolor and Rebella (Fischer 2011(Fig. 1.1).

Fig. 1.1 Apple cultivars (a) Rewena, (b) Regia

New sources for resistance breeding, especially for pyramiding resistance genes, were found in the Fruit Gene Bank Dresden-Pillnitz in some *Malus* species and old apple cultivars. However, if the V_f -gene is to overcome, new resistance sources and cultivars with two or more different sources of resistance will be required in the future to stabilize healthiness in the field. The evaluation of the *Malus* wild species in the Pillnitz Fruit Gene Bank showed that only 4 % of all accessions had no symptoms of scab and mildew. Only a few of these are used as donors in breeding up to now, like some *Malus domestica* (scab, mildew), *M. baccata* (scab, mildew, winter frost), *M. x robusta* (scab, mildew, fire blight), *M. micromalus* (scab), *M. x zumi* (mildew) and *M. floribunda* (scab, fire blight). But the potential of wild species is much higher, especially by using modern breeding methods. Furthermore the following species are carriers of resistance genes: *M. coronaria* (scab, mildew), *M. florentina* (scab), *M. fusca* (immune to fire blight), *M. hupehensis* (mildew, scab), *M. sargentii* (mildew, scab), *M. sylvestris* (mildew), *M.*

trilobata (mildew, scab), *M. x halliana* (scab), *M. sieversii* (scab, mildew, fire blight in offsprings) (Büttner et al. 2000b).

To find donors of polygenic resistance to both apple scab and mildew, the assortment of apple cultivars in the Fruit Gene Bank remained untreated with fungicides for 2 years. It was found that 3.5 % of 850 cultivars were not infected with scab or mildew. This result is important for the breeding of resistant apple cultivars with polygenic sources of resistance after the first results of overcoming monogenic scab resistance in apple cultivars. The most susceptible cultivars were found to be those grown all round the world, such as Delicious, Golden Delicious, Granny Smith, Gala, Rubinette and others. The group that showed both scab and mildew resistance includes: cvs. Bittenfelder Sämling, Börtlinger Weinapfel, Engelsberger Weinapfel, Gewürzluiken, Hiberna, Jacob Fischer, Kardinal Bea, Kirschweinling, Merton Prolific, Peasgoods Nonsuch, Rote Sternrenette and Spätblühender Taffetapfel. These cultivars can be regarded as carriers of polygenic scab and mildew resistance but crossing experiments are required to evaluate these assumptions. Of the scab resistant cultivars with *V_f*-resistance only Cidor, Juliane and Rebella were found to be without mildew infection (Fischer and Dunemann 2000). The results are listed in Table 1.1 for the cultivars with the best evaluation after 2 years without fungicide use, Table 1.2 presents the results of scab resistant cultivars. For nonresistant, noninfected cultivars a marker analysis gave no indication of the existence of the well-known genes for monogenic resistance to scab or mildew. These old and new cultivars are suitable for use in landscape improvement, for small orchards and home gardens, and as parents for transferring polygenic resistance in fruit breeding (Fischer and Fischer 2007; Kellerhals and Gessler 1995). Last but not least, Table 1.3 presents the results of apple breeding in Dresden-Pillnitz for cultivars with multiple resistances for professional production, landscaping and home gardening.

Table 1.1 Apple cultivars two years without using of fungicides — cultivars with the best evaluation, suitable for using in breeding new *vital* cultivars and for home gardening

Cultivars	Resistance level in two years				
	Mildew	Scab/Leaf		Scab/Fruit	
	1997	1999	1997	1999	1997
Altländer Pfannkuchen	2	5	2	1	1
Bellefleur krasnij	2	2	1.5	2	2
Bittenfelder Sämling	2	1	1	1	1
Börtlinger Weinapfel	2	2.5	1	1	1.5
Cidor	1.5	1	2	1	1
Dayton (V _F)	2	4	1	1	1
Discovery	2	3	2	1	1.5
Engelsberger Weinapfel	2	2	1	1	1
Engelshofer	2	1	1	1	1
Erbachhofer Mostapfel	2	2	1	1	1
Früher Victoria	1.5	3.5	2	1	1
Gewürzluiken	2	2	1	1	1
Golden Resistent	2	2	2	1	1
Hagloe Crab	2	2.5	1	1	1
Hibernal	2	1.7	1	1	1
Ilga	2	2	2	1	2
Jacob Fischer	2	3	2	2	1.5
Juliane	1	1	1.5	1	1
Kardinal Bea	2	2	2	2	1.5
Kirschweinling	2	2	1	1	1
<i>Malus robusta</i> 5 (P11)	1	1	1	1	1
Merton Prolofic	2	2	1	1	1
Peasgoods Nonsuch	1	1	2	4	1
Präsident Decour	2	1	1	1	1
Prinzenapfel	2	2	2	1	1
Rebella (V _F)	1	2	1	1	1
Remo (V _F)	3	3	1	1	1
Reglindis (V _A)	3	5	1	1	1
Rewena (V _D)	2	2	1	1	1
Riesenboiken	2	2	2	2	2
Rote Sternrenette	2	2	2	1	1
Spätblühender Taffetapfel	1.5	1.5	1	1	1
Gala	3.8	5	5	4.8	8.5
Golden Delicious	4.8	5.3	8.8	8.3	8.8
Granny Smith	7.8	6.5	9	7.8	9
Idared	8.7	8	8.2	7.5	9

Evaluation: 1 = without infection, 9 = extreme susceptible

Source: Fruit Genebank, Inst for Plant Gen and Crop Plant Research (IPK), Dorfplatz 2. O1326 Dresden - Pillnitz

Table 1.2 Apple cultivars two years without fungicides - scab resistant cultivars, suitable for organic production

Cultivars	Origin	Resistance level in two years				
		Mildew	Scab/Leaf		Scab/Fruit	
		1997	1997	1999	1997	1999
Gavin	CAN	6	6.3	1	1	1
Nova Easygro	CAN	3	4.5	1	1	1
Angold	CZ	6.3	6	1	1	1
Jolana	CZ	4.5	4	1	1	1
Melodie	CZ	8.5	5.5	1	1	1
Nela	CZ	2.5	1	1	1	1
Otava	CZ	6.5	5	1	1	1
Rayka	CZ	2.5	2.5	1	1	1
Resista	CZ	3.7	5	1	1	1
Rosana	CZ	5.5	5	1	1	1
Rubinola	CZ	2.5	2	1	1	1
Topaz	CZ	2	2	1	1	1
Vanda	CZ	6	6	1	1	1
Ahra	GER /Ahr	6.3	6	1	1	1
Ahrista	GER /Ahr	6	5.7	1	1	1
Reanda	GER /Pill	3	3	1	1	1
Rebella	GER /Pill	1	2	1	1	1
Reglindis	GER /Pill	3	4.7	1	1	1
Reka	GER /Pill	2.5	2	1	1	1
Releika	GER /Pill	4	4	1	1	1
Relinda	GER /Pill	2.5	3	1	1	1
Remo	GER /Pill	3	3	1	1	1
Remura	GER /Pill	3	3.5	1	1	1
Renora	GER /Pill	5	4.5	1	1	1
Resi	GER /Pill	5	7.5	1	1	1
Retina	GER /Pill	5.5	5	1	1	1
Rewena	GER /Pill	2	4	1	1	1
Baujade	FRA	4	4	1	1	1
Cidor	FRA	1.5	1	2	1	1
Florina	FRA	3.3	4	1	1	1
Judaine	FRA	2.5	3	1	1	1
Judeline	FRA	2.5	4		1	1
Juliane	FRA	1	1	1.5	1	1
Antonovka	RUS	5	4	1	1	1
Antonovka kamienna	RUS	3	4	1	1	1.5
Antonovka poltora fundova	RUS	2.5	2	1	1	1
Dayton (Coop 21)	USA	4.5	4	1	1	1
Enterprize (Coop 30)	USA	5.5	5	1	1	1
Freedom	USA	4	5.5	1	1	1
Gold Rush (Coop 38)	USA	9	6	1	1	1
Golden Resistent	USA	2	2	1.5	1	1
Jonafree (Coop 22)	USA	5.5	4	1	1	1

Liberty	USA	5	6.2	1	1	1
Macfree	USA	6	7	1	1	1
Priscilla	USA	5.3	5.3	1	1	1
Redfree (Coop 13)	USA	5	4.5	1	1	1
Sir Prize	USA	6	6.5	1	1	1

Evaluation: 1 = without infection, 9 = extreme susceptible

Source: Fruit Genebank, Inst for Plant Gen and Crop Plant Research (IPK), Dorfplatz 2. O1326
Dresden - Pillnitz

Table 1.3 Multiple resistance in the Pillnitz Re-cultivars® (Fischer 1999; Fischer et al 2000).
Cultivars are suitable for organic production and home gardening, some cultivars very good for processing

Re-Sorte®	Scab	Source of resistance	Mildew	Fire blight	Bacterial cancer	Red spider mite	Frost damages of blossoms	Winter-frost
Reanda	X ¹	V _f	(X)	X	(O)	O	X	(O)
Rebella	(X)	V _f	X	(X)	X	X	X	X
Regine	(X)	V _f	(X)	(X)	(X)	X	X	X
Rekarda	(X)	V _f	(X)	#	X	X	(X)	O
Releika	(X)	V _f	(O)	(X)	X	X	X	O
Relinda	X	V _f	(X)	(O)	X	O	(X)	X
Remo	(X)	V _f	X	(X)	(O)	(O)	X	X
Rene*	(X)	V _f	O	X	(X)	O	X	(O)
Renora	X	V _f	(X)	(O)	(O)	(O)	(X)	(X)
Resi	(X)	V _f	O	(O)	X	O	X	O
Retina	(X)	V _f	(X)	(O)	(O)	(X)	X	O
Rewena	X	V _f	X	X	X	(O)	X	(O)
Recolor	X	V _A + V _f	O	O	X	(X)	O	(X)
Realka*	X	V _r	O	X	(O)	(O)	O	(O)
Regia	X	V _r	X	X	(X)	(O)	(O)	X
Reka	X	V _r	(X)	(O)	X	O	O	(X)
Releta*	X	V _r	O	(O)	X	(O)	(O)	(O)
Remura*	X	V _r	(X)	(O)	(O)	O	(O)	X
Reglindis	X	V _A	(X)	(X)	(O)	X	X	X

¹X=resistent, (X)=schwach resistant, (O)=schwach anfällig, O=anfällig

* no longer recommended

1.8 In Vitro Culture

Tissue culture is most often used to describe all types of aseptic plant culture procedures leading to plant growth. Tissue culture techniques can involve any of: (a) protoplasts, (b) callus, (c) tissues (apical or root meristems or pith segments), (d) organs, including buds or roots, (e) embryos and (f) plantlets (having both roots and stem/leaves) (De Filippis 2014).

Fruit species breeding based on biotechnological methods employs mainly embryo culture, regeneration from protoplasts, somatic hybridization, in vitro mutant selection, genetic transformation and haploid production (Germanà 2006).

These tissue culture protocols have been used in most of fruit trees with different objectives (Fig. 1.2) (Deberegh and Zimmerman 2002; Magyar-Tábori 2010; Sedlak and Papstein 2016; Xu et al. 2008), such as commercial propagation of elite rootstocks and cultivars (Sarwar and Skirvin 1997; Yepes and Aldwinckle 1994) production of stock for propagation of new obtentions from breeding programs; for genetic transformation (Krens et al. 2015); for production of haploids to overcome some problems related with apple long reproductive cycle, a long juvenile phase, and highly heterozygous (Höfer 2005); or eradication of viruses (Lizarraga et al. 2017; Parstein et al. 2008); cryopreservation of genetic resources and development of synthetic seeds (Dobránszki and Teixeira da Silva 2010).

Mass micropropagation of different apple cultivars, mainly rootstocks, has been widespread in order to have healthy, uniform propagation materials in large quantities within a short time (Zimmerman 1991). For micropropagation, explants are surface sterilized, inoculated on culture establishment/initiation medium, multiplied on the medium mostly consisting of cytokinins and are subjected to rooting (Bhatti and Jha 2010). The success of micropropagation depends on various factors: 1) shoot tips or nodal shoot segments are most commonly used explants for shoot culturing in apple (Bhatti and Jha 2010); 2) BAP is the preferred cytokinin for apple-shoot multiplication; 3) other sugars than the most common sucrose can produce better caulogenic response, namely sorbitol in the rootstocks M9 and M26 (Yaseen et al. 2009); 4) rooting medium, mainly auxins, sugar, ethylene inhibitors and antioxidant content (see review of apple genotypes in Dobránszki and Teixeira da Silva 2010).

The interest of fruit breeders in haploids and doubled haploids (DH), lies in the possibility of shortening the time needed to produce homozygous lines compared to conventional breeding. With tree fruit crops, characterized by a long reproductive cycle, a high degree of heterozygosity, large size, and, sometimes, self-incompatibility, there is no way to obtain haploidization through conventional methods (Germanà 2006). Induction of embryogenesis and regeneration of pollen-derived plants from anther culture and induction of gynogenesis through unpollinated ovaries and ovules in *Malus domestica* (L.)

Borkh, has been reported by several authors. Higher embryogenesis has been reported for microspore culture in the apple cvs. Remo, Rene and Realka than in anther culture although not correspondent in the regeneration phase (Höfer 2004, 2005).

Tissue culture is used in all present practical transformation procedures. Therefore, improvement in tissue culture systems is essential to achieve a more efficient gene transfer, selection and growth of selected transformant lines and so develop a more efficient transformation (De Filippis 2014). Moreover, often the ability to obtain fruit tree plants with new traits or mutations by genetic engineering or by new biotechnological tools depends on the existence of a well-established in vitro regeneration protocol, which depends on the genotype and the type of starting plant tissue used (Rai and Shekhawat 2014).

Fig. 1.2 Laboratory for production of plants in vitro

1.9 Rootstock production

Rootstock has been used to graft apple cultivars since Roman times (Tubbs 1973). Dwarf apple trees were firstly referred in the UK as cv. Paradise in

1597, which were a mixture (Ferree and Carlson 1987). Extensive reviews on apple rootstocks can be found in Brown and Maloney (2005, Ferree and Carlson (1987), Masseron (1989), Webster and Wertheim (2003) and Wertheim (1998). Rootstock breeding has traditionally focused on dwarfing, hardiness, resistance to pathogens and pests, and better adaptability to soil conditions (Brown and Maloney 2005). Size control with rootstocks is about 30–40 % with cv. M.27 with respect to the maximum vigour provided by seedlings as rootstocks (Masseron 1989).

Some remarkable attempts to transform apple rootstocks were those on M26 by using *Agrobacterium rhizogenes* A4 (Lambert and Tepfer 1992), using plasmid binary vector pLDB15 (Norelli et al. 1994), and using *A. rhizogenes* to control vigor (Holefors 1999); M6 using *A. tumefaciens* strain c58Sz707 (Norelli and Aldwinckle 1993); M7 using *A. tumefaciens* (Ko et al. 1998); Jork 9 using *A. tumefaciens* EHA101A and C58C1 (Sedira et al. 2001); Alnarp2 integrating the *rolA* gene to reduce vigor shortening the internodes (Zhu et al. 2001); and MM106 using *A. tumefaciens* strain C58C1 (Radchuk and Korkhovoy 2005).

Transgenic plants are limited in some countries by ethical constraints due to the fact that some of the traits introduced into a plant did not occur in the species (wild and domesticated); therefore, cisgenic plants are considered the future in breeding, because in cisgenesis the introduced gene of interest previously existed as a native promoter in the domesticated or wild species for centuries (Jacobsen and Schouten 2008). Moreover, this technique allows inclusion of new traits to already-selected cultivars maintaining their excellent quality (Krens et al. 2015).

1.10 Germplasm Conservation

1.10.1 In Vitro Germplasm Conservation

Conservation of plant genetic resources prevents the loss of plant species with economic and ecological importance, namely vegetatively-propagated plants (Benelli et al. 2013).

In vitro conservation is currently mainly based on cryopreservation in liquid nitrogen (LN -196 °C) which ensures safe and cost-efficient long-term conservation of plant germplasm (Benson 2008; Engelmann 2012; Reed 2008). Apples have the advantage of being perennial woody species amenable to dormant bud cryopreservation techniques, and apple dormant bud methods have been successfully implemented. The National Center for Genetic Resources Preservation (NCGRPP, Fort Collins, USA) has cryopreserved dormant buds of around 1,900 accessions of apple. In the cryopreservation of dormant buds, branches or scions are harvested after mother plants have been

exposed to a sufficient duration of cold temperatures. The optimum dehydration level of dormant buds is very important to achieve high recovery after cryopreservation (Benelli et al. 2013). Dehydration is usually performed at -5 °C for several days and in most cases up to moisture contents around 25–35 % fresh weight. Before immersion in liquid nitrogen, samples are cooled slowly. In apple nodal sections, Towill et al. (2004) used a cooling rate of 1 °C/h from -5 °C to -30 °C.

There are some conditions for which the dormant bud cryopreservation method is not applicable, namely when buds do not reach a sufficient level of dormancy or apple material only exists in tissue culture (Li et al. 2015). Shoot tips excised from apical or axillary buds of in vitro-grown shoot cultures are the organs most commonly used in cryopreservation for vegetatively-propagated species (Lambardi and De Carlo 2003), and a good alternative to dormant buds. Indeed, in fruit tree cultivars shoot apex freeze-preservation in liquid nitrogen is the most suitable method to maintain viability and phenotypic and genetic stability, thereby ensuring long-term germplasm storage (Paul et al. 2000). Shoot tips are differentiated organs composed of organized tissues and thus not prone to somaclonal variation (Panis and Lambardi 2006).

The cryopreservation protocols established and most frequently employed in apple are encapsulation-dehydration, vitrification, encapsulation-vitrification and droplet-vitrification.

In the encapsulation-dehydration procedure shoot tips are coated in alginate gel, pregrown in liquid medium with sugar, partially desiccated in the air (current or laminar flow) or with silica gel before immersion in liquid nitrogen. Preculture on modified MS medium containing 0.75 M sucrose followed by 6 h of dehydration (21 % residual water) led to the highest shoot regrowth of frozen, coated shoot tips of apple cv. Golden Delicious (Paul et al. 2000).

Vitrification involves treatment of shoot tips with cryoprotectants (usually glycerol and sucrose) followed by dehydration with highly concentrated vitrification solutions before cooling. Encapsulation-vitrification combines encapsulation-dehydration and vitrification, where shoot tips are encapsulated in calcium alginate beads and then treated with vitrification solutions. With the droplet-vitrification methodology, shoot tips treated with vitrification solution are placed on aluminum foil in minute droplets of vitrification solution which allows very high cooling rates and the direct contact between samples and the liquid nitrogen. In apple cv. Gala and 6 other genotypes of 4 different *Malus* species, Li et al. (2015) comparing the droplet-vitrification and encapsulation-dehydration procedures (Feng et al., 2013) and found that mean shoot regrowth levels of the 7 genotypes were lower (48 %) in the droplet-vitrification method than those (61 %) in the encapsulation-dehydration procedure.

Several authors designate the encapsulation-dehydration technique for routine cryopreservation of apple germplasm (Li et al. 2015; Wu et al. 1999).

1.10.2 In Vivo Germplasm Conservation

A local cultivar is created at the moment when a farmer selectively propagates using plant material of the greatest interest. A very significant event had to correspond with initial propagation by grafting. In this sense, it is known that the Romans dominated and promoted grafting for the propagation of different varieties (unknown to us) in Roman times. Greeks and Romans spread the practice across Europe. During the Middle Ages, apple culture was promoted around monasteries. By the end of twelfth century, some famous cultivars were already named, such as Pearmain and Costard (Morgan and Richards 1993).

Most cultivars grown by farmers have been collected and conserved in germplasm collections. In Europe there are more than 30,000 accessions (IPGRI 1996), the majority held in ex situ vegetative field collections. Minimal data reported in each bank were collected in a European *Malus* database (Maggioni et al. 1997). Recently, most germplasm banks have been evaluated at the European level by using microsatellites (Ferreira et al. 2016; Lassois et al. 2016; Pereira-Lorenzo et al. 2017; Urrestarazu et al. 2016) in order to clarify the diversity, eliminate duplicates and to refine true-to-type characterization.

Information about apple genetic resources in the USA can be easily accessed (www.ars-grin.gov). A total of 4,179 accessions are maintained in repositories; 1,456 corresponding to *Malus x domestica*.

In Spain, major collections are located in Asturias, Galicia, Navarra, País Vasco y Zaragoza (Dapena, 1996; Itoiz et al. 2003; Pereira-Lorenzo et al. 2003, 2007). In those regions research programs are focused on local selection and breeding of ancient cultivars, which contain important resources for dessert and cider production.

1.11 Mutation Breeding

Fruit trees, including apples, have been considered attractive material from the viewpoint of mutation breeding because conventional breeding: i) is time consuming, ii) requires large space; and iii) results are unpredictable due to their long juvenile phase and high degree of heterozygosity (Broertjes and van Harten 1988; van Harten 1998).

The most important apple-breeding objectives focus on: i) fruit quality (firm, large and juicy fruits with good flavor and attractive colors without traces of russetting and good storage quality); ii) pest and disease resistance; and iii) shortening the juvenile phase and obtaining high yields in early years.

Natural apple mutants of major significance have been those of compact or spur-type growth, fruit color and time-to-fruit maturity (Spielgel-Roy 1990). Several bud sports displaying important characters have been described, for example skin color sports of several widely grown apple cultivars. The cv. Jonagored, for instance, is a spontaneous sport of the well-known cv. Jonagold, with much more intense early-red-colored fruit, which currently is cultivated in dozens of countries (van Harten 1998). Apple ploidy chimeras are also known to occur leading to *giant* sports. Although the majority of cultivated apples are diploid, triploid cultivars arising from unreduced gametes and even tetraploid sports are known.

The increased impact of natural mutants provides incentives for mutation breeding. Only few induced mutants in fruit trees have been commercialized and are being planted on a large scale, contrary to the situation in ornamentals (Spielgel-Roy 1990). Currently, the FAO/IAEA Mutant Varieties Database (www.mvd.iaea.org) comprises 13 apple varieties. In this database a mutant variety is a new plant variety that is bred through: i) direct use of a mutant line that is developed through physical and chemical mutagenesis, or somaclonal variation and activation of endogenous transposable elements; ii) indirect use of a mutant line/lines, which is/are used as a parental variety/varieties in crossbreeding (crosses between mutant lines or with a commercial variety/varieties); and iii) use of wild species' genes translocated into plant genomes through irradiation-facilitated translocations (www.mvd.iaea.org).

The first apple (*Malus pumila* Mill.) mutant variety, Mori-hou-fu 3A, was officially approved in 1963. It was developed in Japan by irradiation of scions with gamma rays (30 Gy) the main improved attribute being the mutant variety resistance to diseases. In 1970, 4 apple mutant varieties were officially approved 3 in France, and in Canada the mutant variety of McIntosh, McIntosh 8F-2-32, with improved skin color, was developed by irradiation of shoots with gamma rays. In 1995 the apple mutant variety, James Grieve Double Red, was approved, obtained by irradiation with gamma rays (62 Gy) and with the main improved attributes a good well-balanced taste, altered acids to sugars ratio, high yield (www.mvd.iaea.org).

From all new cultivars there arise new natural mutations; the difficulty is to identify them in the field. From the Dresden-Pillniz collection's cv. Pinova there exist nearly 12 mutations, 9 of them are in official testing, possibly 2 or 3 will be new protected cultivars in time (Fig. 1.3).

Fig. 1.3 One of the best mutations of the Pillnitz Pinova apple, the cultivar Evelina, presented at the Fruitlogistica in Berlin 2016 (Photo: Fischer)

1.12 Genetic Transformation

The genome-wide sequence information and functional genomics of fruit trees have established the basis for crop genome engineering to improve fruit trees with unprecedented precision, maintaining most of the genetic identity of the main cultivars. Moreover, there is the recent technology CRISPR/Cas9, a molecular genetic tool used for genome edition and correction (Osakabe and Osakabe 2015). This technique is expected to be the method of choice for tree breeding in the near future. In comparison with traditional breeding, this technique can reduce the process, normally affected by the long juvenile period, gametophytic self-incompatibility (GSI), the high heterozygosity and the time consumed in phenotypic evaluation and lack of precision. Some commercial cultivars have been released in papaya and plum tree, genetically engineered for resistance to viral diseases (Scorza et al. 2013; Tripathi et al. 2007). But there are concerns about the higher cost of these new protected cultivars, along with environmental safety considerations such as outcrossing, because of the risk of transferring genetic traits and secondary and non-target adverse effects such as in proteins which are not known to display any toxic or allergenic properties. In apples, the first report using a single gRNA and Cas9 to produce a first-generation transgenic apple (Nishitani et al. 2016) by

modifying an apple phytoene desaturase (PDS), which is required for chlorophyll biosynthesis and, therefore, transformed plants showed an albino phenotype. It was the first reported genome editing in apple and in the Rosaceae. Genome editing was applied to the apple semi-dwarfing rootstock cultivar JM2 (*Malus prunifolia* (Wild.) Borkh. Seishi $\times$ *M. pumila* Mill. var. *paradisiaca* Schneid. M.9).

1.13 Conclusions and Prospects

Apple is an ancient worldwide crop in which new technologies such as genome-wide selection and genetic transformation are providing promising new cultivars combining high fruit quality with resistances to pests and diseases, and adapted to new climate conditions.

The recent molecular genetic tool CRISPR/Cas9 to edit and correct the genome will surely be the method for tree breeding in the near future, to overcome the long juvenile period, gametophytic self-incompatibility (GSI), the high heterozygosity and the time consumed in phenotypic evaluation and lack of precision of traditional breeding techniques. CRISPR/Cas9 is an extremely useful tool for further genetic studies of the genome of cultivated apples (Velasco et al. 2010) as it accelerates genome-wide association studies (GWAS), identifies SNPs and genes (Leforestier et al. 2015), and analyzes those genes which are differentially expressed (GDE) by using qRT-PCR and microarray analysis (Guitton et al. 2016).

All these new techniques will enable full use of the genetic diversity provided by nature; benefit growers and agronomists; and facilitate collecting, conserving and evaluating old selections, all of which will assure that apples continue to be one of the most desirable of fruits.

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Appendix I Research institutes and online resources

Julius-Kühn-Institut für Züchtungsforschung an Obst, D01326 Dresden-Pillnitz, Pillnitzer Platz
3a, Germany

<https://www.julius-kuehn.de/dresden-pillnitz/>

<https://www.bundessortenamt.de>

Appendix II Genetic resources

www.deutsche-genbank-obst.de

<https://www.julius-kuehn.de/zo/obstsorten-des-jki/>

<https://www.genres.de/kultur-und-wildpflanzen/erhaltung/deutsche-genbank-obst>

Index keywords
Amino Acids
Antioxidant
Apple
Assisted selection
Bacterial canker (<i>Pseudomonas syringae</i>)
Breeding
Climatic adaptation
Consumption
Diabetics
Diversity
Extacellular ribonuclease
Fire blight (<i>Erwinia amylovora</i>)
Flavor
Foodstuffs
Fructose
Gametophytic self-incompatibility
Gamma rays
Genetic diversity
Genetic resources
Genomics
Germplasm
Germplasm conservation
Harvesting
Hybridization
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Industrial aptitudes
Irradiation
Marketing
Mildew (<i>Podosphaera leucotricha</i>)
Mineral
Molecular markers
Mutant
Mutant variety
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Phenotypic traits
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Resistance
Resistance to diseases
Scab (<i>Venturia inaequalis</i>)
Sources of resistances
Stability of resistance
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Vitamin Content
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World production