Genetic and biochemical properties of apples that affect storability and nutritional value

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Summary

Apple is a highly appreciated fruit in many temperate parts of the world, and is presently grown in many countries with a total world production of more than 71 million tonnes. Economically, apple is the fourth most important fruit crop after citrus, grapes and banana. Apples are consumed fresh, directly after harvest or after a storage period for up to 6 months or even longer. Apples can also be processed to produce, e.g., juice, sauce, slices, vinegar and cider. Most of the cultivated apples belong to the species *Malus × domestica* Borkh. in the Rosaceae family. More than 7500 apple cultivars have been described from different countries. However, only a few of them have sufficient quality and productivity. Many cultivars are limited by different diseases that reduce the apple quality and market acceptability. Research attempts have recently been focused specifically on some traits which are economically very important, e.g. disease tolerance, fruit texture and quality. This introductory paper forms part of a PhD study that aims to quantify the storage disease tolerance of some apple cultivars by performing inoculation tests with fungal spores on harvested fruits. Using DNA analysis, attempts will subsequently be made to develop tools for molecular identification and characterization of genes involved in storage disease in co-operation with INRA Angers, using microarray technique. Other factors related to fruit quality and nutritional value that may be connected to the level of fungal disease tolerance will also be investigated by pomological characterization, firmness testing, and chemical analyses.

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1. Introduction

Apple (*Malus × domestica* Borkh.) is the fourth most important fruit crop after citrus, grapes and banana, and one of the commercially most important horticultural crops grown in temperate parts of the world (Ferree and Warrington, 2003). Apple belongs to the Rosaceae family which includes many well-known genera with economically important fruits, particularly edible, temperate-zone fruits and berries such as apple, pear, almond, apricot, cherries, peach, plums, strawberries and raspberries. Among these, apple with a world production of more than 71 million tonnes, cultivated in many countries in the world, can be considered as one of the most important horticultural plants (FAO, 2009).

Apple fruit has multiple uses and this fact makes it popular in the entire world, also in areas where it is more difficult to grow. In most cases, apples are consumed fresh or after storage for up to 6 months or even longer (usually requiring ultra-low oxygen storage facilities). Apples can also be processed into juice, sauce, slices, vinegar and cider (Folta and Gardiner, 2009). Apple has been considered as a symbol for the healthy fruit which eliminates the need for a doctor: “an apple a day keeps the doctor away”.

Since a long time back, the apple tree has been cultivated and used to feed humans and animals. Cultivation of apples has been known for 3000 years in Greece and Persia. The Old Silk Road from eastern China to the Black Sea is claimed to have played an important role in the evolution of cultivated apples (Juniper et al., 1999). Apples can be grown under different climatic conditions, ranging from temperate climates such as southern Siberia or the Mediterranean to subtropical climates such as Brazil or South Africa. Nowadays, it has become increasingly popular to cultivate apples also in subtropical and tropical (high altitudes) areas since they fetch a comparatively high price on the market.

2. Taxonomy of apple

Most of the cultivated apples belong to *Malus × domestica* (also known as *M. pumila)* in Rosaceae family. The commercial apple is a hybrid species with a complex history of inter- and intraspecific hybridization. The scientific name of domestic apple is therefore often written with a '×' between the genus and species (Korban and Skirvin, 1984). Rosaceae family includes many well-known and appreciated genera with economically important edible temperate-zone fruits. Rosaceae is subdivided into several subfamilies including Maloideae. This subfamily includes approximately 1000 species in 30 genera characterized by the distinctive fruit, the pome, and a base chromosome number; x = 17 (Evans and Campbell, 2002; Luby, 2003; Folta and Gardiner, 2009). Maloideae contains many of the commercially most valuable fruits like apples and pears, some ornamentals and also invasive plants. Different studies including cytology and morphology, flavone analysis and isozyme analysis, have suggested that Maloideae subfamily originates from hybridization between a Spiraeoideae ancestor and a Prunoideae ancestor, followed by fusion of unreduced gametes to form a fertile organism (Currie, 2000).

The genus *Malus* consists of five sections (*Malus*, *Sorbomalus*, *Chloromeles*, *Eriolobus* and *Docyniopsis*) based on morphological traits and flavonoid similarities. Section *Malus* consists of series *Malus,* including many European and Asian species (including *M. sieversii* and *M. × domestica*) and series *Baccatae*. Section *Sorbomalus* includes series *Sieboldianae* (native to Japan), *Florentinae* (from south-east Europe), *Kansuenses* and *Yunnanenses*. Section *Chromeles* consists exclusively of North American species. Section *Eriolobus* consists of only one species from eastern Mediterranean and finally, section *Docyniopsis* includes some species originally from Japan, Taiwan and South-East Asia (Phipps et al., 1990).

The total number of species in the genus *Malus* varies between different studies and according to the different views on taxonomy. The minimum number of species was

reported as eight by Likhonos (1974) while Ponomarenko et al. (1986) reported as many as 78 species within the genus. Harris et al. (2002) recognized 55 species while Zhou (1999) recognized only 30–35 species. According to Robinson et al. (2001), the genus *Malus* comprises 25–47 species, depending on the rank given to several taxa and the acceptance of new species and putative hybrids. The difficulty in species delimitation has been reported to stem from the high genetic diversity, hybridization potential, polyploidy occurrence and presence of apomixis (Campbell et al., 1991). Morphological studies (Phipps et al., 1990; Robinson et al., 2001) along with biochemical analysis (William, 1982) and molecular techniques have been conducted in order to characterize and classify different species in the Maloideae subfamily.

The majority of apple cultivars are diploid with 2n = 34 and a genome of moderate size (1C = 2.25 pg which corresponds to approximately 1.5×10^9 bp) (Janssen et al., 2008) whereas some cultivars are triploid with 2n = 3x = 51 (Pereira-Lorenzo, 2009). Possibly, the Maloideae subfamily has resulted from an ancient autopolyploidization of a 9 chromosome progenitor to 18 chromosomes. Then it was followed by a chromosome loss resulting in current 17-chromosome apple cultivars whereas other subfamilies are x = 7, 8 or 9 (Folta and Gardiner, 2009; Giovannoni, 2010).

3. Origin of apple

Apple species are distributed throughout very large regions of the world including West Asia, Himalayan, Central Asia, India, Western provinces of China, Europe and some parts of America and Africa (Juniper et al., 1999). Historical studies have shown that apple seed transfer by human or animals have probably helped in its distribution from the center of origin (the region where the species originated) to other parts of the world.

Central Asia has been reported to contain the greatest diversity of *Malus*, and this area also appears to be the center of origin of the domesticated apple (Janick et al., 1996). This is in accordance with Vavilov's hypothesis about the wild apples in central Asia and their close relatives being the progenitors of the domesticated apple (Harris et al., 2002). Nowadays, *M. sieversii* which grows wild in Kazakhstan and Kyrgyzstan, is thought to be the main progenitor species (Pereira-Lorenzo, 2009). *Malus sieversii* has very high similarity with *M. × domestica* in morphology and fruit flavor. According to observations made on extensive collection tours, *M. sieversii* has been claimed to incorporate all the fruit qualities which are present in the domesticated apples (Forsline, 1995).

Relationships among apple species have been evaluated by morphological and molecular DNA analysis, and have confirmed that *M. sieversii* is the species from which apple domestication started (Forte et al., 2002). This species may have hybridized with *M. prunifolia*, *M. baccata* and *M. sieboldi* to the East and with *M. turkmenorum* and *M. sylvestris* to the West. Subsequently, well-established apple cultivars were selected and introduced into Europe and especially the Mediterranean regions by the Romans (Juniper et al. 1999). Other *Malus* species are occasionally used for introgression into modern cultivars but this usually requires several generations of back-crossing to reach acceptable fruit size and quality.

4. Apple cultivars

Malus × domestica contains over 7500 cultivars that have originated from different countries in the world. Many cultivars have desirable characteristics which make them suitable for cultivation under specific conditions, but only a few dozen of these are grown commercially on a worldwide scale (Moore et al., 1991).

Development of new apple cultivars is a time- and money-consuming process since a cultivar must be very good in several characteristics, e.g., uniform and consistent yield, commercial fruit quality, good post-harvest storability and shipping quality, high consumer demand and finally resistance against diseases, pests and storage disorders (Ferree and Warrington, 2003). Recently, research attempts have become more focused on those traits which are demanded from costumers. According to Pereira-Lorenzo (2009), important traits to consider are fruit size, shape, color, acidity, sweetness, flavour, resistance to diseases and abiotic stress, harvest time, storability and shelf-life. In the early and middle parts of the last century, many small apple growers grew a few cultivars for their own use and for the local markets. Nowadays, almost all commercially grown apples are stored for some times before being sold, and therefore cultivars need to have good storage ability (Ferguson and Boyd 2002). Most (old) apple cultivars have been selected in or around established orchards as chance seedlings whereas more recent cultivars generally are derived from breeding programs or have been selected as sports (mutants) from other cultivars (Janick et al., 1996; Brown et al., 2005). Although transgenic plants have been produced for a number of apple cultivars (Seong et al., 2005; Chevreau et al., 2011: Wu et al., 2011), GMO fruits have not yet been released on the market.

Some of the major important cultivars are listed in Table 1 (Ferree and Warrington, 2003; Hampson et al., 2003). About twenty years ago, ‘Golden Delicious’ was the most widely grown cultivar in the world, followed by ‘Delicious’, ‘Cox’s Orange Pippin’, ‘Rome Beauty’, ‘Belle de Boskoop’, ‘Granny Smith’, ‘Jonathan’ and ‘McIntosh’ (Moore et al., 1991).

Since then cultivars like 'Elstar', 'Fuji', 'Gala' and 'Jonagold' have become very popular while especially culinary apples like 'Rome Beauty' and 'Belle de Boskoop' have decreased in popularity.

Table 1. Some important apple cultivars, country of origin and storage ability.

Cultivar	**Origin**	**Storage ability**
'Golden Delicious'	USA	Resistant to storage disease*
'Delicious'	USA	Medium resistant to storage disease
'Cox's Orange Pippin'	England	Susceptible to bitter rot, not suitable for long storing
'Granny Smith'	Australia	Long-keeping apple with low ethylene production
'Jonathan'	USA	Resistant to storage disease
'McIntosh'	Canada	Medium susceptible to storage disease
'Jonagold'	USA	Long storability if harvested at optimal time
'Braeburn'	New Zealand	Susceptible to bitter rot and other calcium-related disorders
'Elstar'	Netherland	Slightly susceptible to storage disease
'Fuji'	Japan	Long shelf life, resistance to bitter rot
'Gala'	New Zealand	No significant storage disease
'Aroma'	Sweden	Susceptible to fungal decay and bruising
'Ingrid Marie'	Denmark	Susceptible to fungal decay, cracks, and bruising

Sources: Moore et al., 1991; Andersen and Crocker, 2000; Ferree and Warrington, 2003; Hampson et al., 2003; Tahir, 2006; Ahmadi-Afzadi et al.; 2011.

* Refers especially to two major storage diseases; blue mould and bitter rot (unpublished data; Ahmadi-Afzadi et al.).

5. Biology of apple

The apple tree has hermaphroditic flowers with a gametophytic type of self-incompatibility controlled by a single multiallelic locus (Pereira-Lorenzo, 2009). Therefore, at least two different cultivars must be interplanted in the orchard to ensure high levels of cross-pollination in order to achieve adequate fruit development. Alternatively, trees of undomesticated *Malus* species can be interplanted among (or top-worked onto) some of the trees in the row. Blooming and ripening time of different cultivars vary considerably and form different categories, e.g. early, middle and late blooming or ripening cultivars.

Flowering in apple is the result of several physiological changes from vegetative to reproductive phase. Like in many other fruit crops, newly initiated apple buds become dormant in late summer or early autumn. Winter chilling (defined as a certain number of hours at or below 7.2 °C) is necessary to break bud dormancy. If chilling is not sufficient, both flower buds and vegetative buds (producing leaves only) are delayed. Flowering time of the different cultivars must overlap to a large extent for pollination to be successful. Number of days from pollination to fruit ripening varies considerably due to inherent differences between cultivars and to environmental effects (e.g., weather conditions). During this period, physiological processes like cell division and expansion, starch accumulation, ethylene production, and color changes take place (Janssen et al., 2008).

6. Apple production and geographical distribution

The global apple production remained stable for a large part of the previous century until China began to expand its apple production in the 1990s. Currently, China is the largest apple producer in the world with a production of more than 31 million tonnes, which is several times higher than the production of the four countries in the closest positions, e.g. USA, Turkey, Poland and Iran. China is currently responsible for approximately half of the world apple productions. Some of the main apple-producing countries and their production volumes are listed in Table 2 (FAO, 2009).

Most temperate-zone woody deciduous trees, including apple, require a certain amount of chilling accumulation during the wintertime to break bud endodormancy before active shoot growth in the spring and for normal growth (O'Rourke, 2003). In general, apples are therefore suitable for growing mainly in areas with a temperate climate. However apples can also be grown in other climates, like subtropical and even tropical areas at high altitudes, where sometimes two crops can be produced per year (Pereira-Lorenzo, 2009). Apple production has thus been reported from countries like India, Mexico, Brazil, Egypt, South Africa, Kenya, Ethiopia, Uganda and Zimbabwe (Wamocho and Ombwara, 2001; Ashebir et al., 2010). In the subtropical and tropical areas of Asia, India appears to be the largest apple producer. Many different apple cultivars are grown in the northern, mountainous parts of the country, especially in the provinces of Jamma and Kashmir (Verma et al., 2010). In the subtropical areas of the America, apples are grown, e.g., in the highlands of the northern regions of Mexico and also in large subtropical areas of Brazil (Leite, 2008; Hauagge, 2010). In Africa, the most important apple-growing country is South Africa, where roughly 20,000 ha of apple are cultivated (Cook, 2010).

Table 2. List of main apple producing countries in the world (FAO, 2009)

Country	**Production (MT*)**	**Country**	**Production (MT)**
China	31684445	Syria	360978
USA	4514880	New Zealand	357000
Turkey	2782370	Belgium	310000
Poland	2626270	Australia	291134
Iran	2431990	Serbia	281868
Italy	2313600	Portugal	280078
France	1953600	Algeria	267496
India	1795200	Switzerland	252086
Russia	1596000	United Kingdom	243000
Brazil	1222890	Greece	235000
Chile	1090000	Moldova	210000
Germany	1070680	Azerbaijan	204237
Argentina	1027090	Czech Republic	170400
Japan	845600	Tajikistan	148000
Ukraine	853400	Kyrgyzstan	146000
North Korea	719682	Peru	137044
South Africa	702284	Lebanon	126500
Uzbekistan	635000	Israel	114378
Spain	594800	Kazakhstan	112000
Hungary	575368	Armenia	110000
Egypt	550000	Tunisia	110000
Mexico	525000	Macedonia	106356
Romania	517491	Slovenia	95662
Austria	485609	Georgia	80700
South Korea	480000	Afghanistan	72765
Belarus	431573	Bosnia and Herzegovina	71507
Canada	413096	Turkmenistan	64000
Netherlands	407000	Uruguay	58775
Morocco	400000	Lithuania	53259
Pakistan	366360	**World production**	**71286632**

* million tonnes

7. Apple diseases

Apples are subjected to a variety of diseases with several causal agents e.g. fungi, bacteria, viruses, mycoplasmas and nematodes but there are also disorders with unknown causal agents. Most disorders result in the loss of total yield. The economic losses caused by different diseases can be exceedingly variable according to the pathogen vigor, i.e., some are able to kill whole tree, others can infect fruits and make them unmarketable whereas others may cause only minor symptoms. Disease control is a major annual expense for growers in most apple-producing areas. The grower needs to control early-season diseases like apple scab as well as summer diseases and also some storage diseases. A well-integrated approach is usually needed to achieve successful disease management, e.g., application of fungicides, pesticides and bactericides (the latter usually not allowed in Europe), selection of resistant or tolerant rootstocks and scion varieties, biological disease control and selection of a suitable site for the orchard (Jönsson, 2007; Dewasish and Amal, 2010). Some of the most important apple diseases are described below.

7.1. Fungal diseases

Fungal diseases are the main problem for commercial apple production in humid regions. It has been reported that apple is host to over 70 infectious diseases which most of them are caused by pathogenic fungi. They cause root rots, leaf spots, leaf blights, blossom blights, fruit decay, fruit spots, canker and post-harvest decay. **Apple scab** (*Venturia inaequalis*) is usually the main apple fungal disease in commercial apple production in temperate and humid regions. Scab mainly attacks the leaves and fruits (Sandskar, 2003). Apple cultivars differ greatly in regard to their resistance level to scab. In Europe and New Zealand, over 50 scab-resistant cultivars have been introduced based on apple breeding programs, e.g. 'Prima', 'Redfree' and 'Liberty' (Benaouf and Parisi, 2000; Bowen et al., 2011).

Powdery mildew (*Podosphaera leucotricha*) can be a serious disease wherever apples are cultivated. It usually infects leaves, flowers and even fruits with masses of fungal mycelia and spores spread over the surface. Powdery mildew distribution and epidemic is strongly dependent on environmental conditions, e.g., relative humidity, hourly ambient temperature and total daily duration of rainfall. In order to control powdery mildew, application of fungicides is recommended (Moore et al., 1991; Ferree and Warrington, 2003).

Brown-rot caused by *Monilinia fructicola* is another apple disease which causes blossom wilt, spur dieback, cankering and fruit rot. This disease is usually more problematic around harvest time because commercial losses due to fruit decay increase gradually up to harvest time and it is associated to numbers of injured fruits (Grove et al., 2003). Black rot caused by *Botryosphaeris obtusa*, Sooty blotch caused by *Peltaster fructicola*, Brooks fruit spot caused by *Mycosphaerella pomi*, Crown and root rot caused by *Phytophthora* spp., and European canker caused by *Nectria galligena* are also important apple diseases (Xu and Robinson, 2010).

7.1.1. Fungal diseases affect apple storage ability and fruit quality

Blue mould caused by *Penicillium expansum* is the most common post-harvest disease of apple fruits. It mainly attacks injured and physically damaged fruits and produces soft, malodorous lesions with a dark brown color. The fungus then proceeds to produce green to blue conidia on the fruit surface. The quick spread of the disease during storage causes much infection and subsequent severe fruit loss in commercial apple production (Rosenberger, 1990; McCallum et al., 2002; Pianzzola et al., 2004).

Meanwhile, *Penicillium* produces the carcinogenic mycotoxin patulin in decayed fruits. This mycotoxin is a major health hazard for people who consume high quantities of fruit juices (Brause et al., 1996; Beretta et al., 2000). In order to eliminate the damaging effect of *P. expansum* during storage and to avoid health problems, some efforts have been

made to identify and introduce tolerant cultivars (Pianzzola et al., 2004; Moake et al., 2005).

Bitter rot is known as one of the most destructive and difficult to control apple diseases when an epidemic has occurred. It is caused by *Colletotrichum gloeosporioides* and *Colletotrichum acutatum*. This disease usually begins by release of conidia and infection of the fruits in late spring when temperatures become higher. The lesions on the fruits are small, circular, light tan to brown spots in the beginning, and then become larger and more brown. This disease can also be considered as a problematic post-harvest disease in many commercial apple orchards (Peres et al., 2005; Jönsson, 2007).

Another important storage disease is **Bull's eye rot** caused by *Pezicula malicorticis*. Infection can occur at any time during fruit development until the harvest, but usually does not become visible on the fruits until later when exposed to cold-storage temperature, during transport and in the shops. The lesions are most often brown with a pale center that looks like a bull's eye (Tahir, 2006; Valdebenito et al., 2010).

Moldy-core and **core rot** are also other important apple fruits diseases which cause production losses during fruit ripening and storage. Moldy core decay is predominantly caused by *Alternaria alternate* and wet core rot caused by *Penicillium* spp is typically found after harvest when fruits are in storage (Truchek, 2004).

7.2. Other diseases caused by bacteria and viruses

Fire blight, caused by *Erwinia amylovora*, also known as fruit blight, pear blight and spur blight, is a very serious bacterial disease which affects tree trunks and branches, and can kill a whole orchard in only a few years. It has been reported on more than 200 species of plants whereas the main host species are in Rosaceae family. It is a common disease in warm and temperate regions. Apple cultivars are widely different in their resistance to fire blight. 'Rome Beauty', 'Jonathan' and 'Granny Smith' are susceptible whereas 'Delicious', 'McIntosh' and 'Golden Delicious' are resistant (Moore et al., 1991; Khan et al., 2006;

Nybom et al., in press). Viruses, viroids, phytoplasma and other virus-like organisms produce over 50 identified diseases in apple. They are widely different in their destructiveness. Some of the important diseases are apple mosaic, flat limb, tomato ring spot and chlorotic leaf spot with symptoms on several parts of the tree (Ram and Bhardwaj, 2004).

8. Apple fruit quality and disease resistance

8.1. Apple contents involved in fruit quality

Fruit quality is defined as degree of excellence of fresh fruits and it is a combination of different characteristics or properties. These characteristics are usually attractive to consumers in terms of market acceptability or human health improvement (Kader, 1999). It has been reported that consumers generally prefer apple fruits that are juicy, crisp and sweet. Meanwhile, there are several other factors that determine the fruit quality and some of these may be associated to disease resistance, e.g., hardness, acidity, ethylene production level, flesh texture, antioxidant content, phenol content, harvest time and fruit maturity (Jenks and Bebeli, 2011; Nybom et al., in press). Variation in phytochemical content is caused by many factors, such as heritable traits of the cultivars, harvest and storage procedures, and processing of the apples (Boyer and Liu, 2004).

8.2. Apple chemical contents and disease resistance

As already mentioned, different compounds in the fruit can probably play a role in resistance to fungal diseases, especially storage diseases. It has been reported that total phenol content is one of the factors affecting the apple storability and disease resistance. A large number of volatile compounds are important in disease resistance of apple like alcohols, aldehydes, carboxylic esters and ketones. In resistant cultivars, phenolic components accumulate at a higher rate than in susceptible cultivars (Dixon and Hewett, 2000; Usenik et al., 2004; Treutter, 2005; Lattanzio et al., 2006). Among phenolic compounds, the flavonoid quercetin has been considered the most important agent. Sanzani et al. (2009a) has recently investigated the role of quercetin as an alternative strategy to control blue mould and patulin accumulation in 'Golden Delicious'. By exogenous application of different phenolic compounds, they found that quercetin is effective in controlling blue mould and patulin accumulation. Subsequent studies have demonstrated that this control is achieved through an increased transcription of genes

involved in the quercetin biosynthetic pathway (Sanzani et al., 2009b; Sanzani et al., 2010).

In a wide variety of plants, organic acids and nutritional compounds such as vitamin C and glutathione are associated with fruit taste and quality. These compounds are apparently related also to the level of disease resistance (Ferguson & Boyd, 2002). The relationship between harvest day and vitamin C content of apple fruits has been investigated by Davey et al. (2007). Low pH can enhance *P. expansum* colonization, which means that cultivars with a lower pH in their fruits are more susceptible to fungal attack (Prusky et al., 2004).

8.3. Impact of apple peel in disease resistance

It has been hypothesized that the main defense mechanism against fungal infection involves the fruit peel. According to many authors, most of the apple phytochemicals such as ascorbic acid, glutathione, antioxidative enzymes, phenols and cuticular waxes like ursolic acid, are mainly localized in the peel. Ursolic acid is a ubiquitous triterpenoid and the main cuticular waxes present in apple peel that can be considered as a post harvesting parameter in order to reduce shelf life diseases (McGhie et al., 2005; Lata, 2007, Frighetto, et al., 2008). An environmental impact has also been shown. Thus, the sun-exposed side of the apple contains a higher level of antioxidants that are involved in resistance to decay caused by fungi (Ma and Cheng, 2003).

8.4. Fruit texture, harvest time and disease resistance

The association between fruit quality (firmness and softening), harvest date and level of resistance to post harvest diseases has been investigated (Ahmadi-Afzadi et al., 2011; Kellerhals et al., in press). A significant difference was noted between investigated cultivars regarding the size of disease symptoms resulting from inoculations. Late-ripening cultivars with high levels of firmness and little softening were, as expected, the least affected by blue mould. From a genetic point of view, the most interesting cultivars are, however, those that had relatively small symptoms in spite of being early-ripening and/or only medium firm (Ahmadi-Afzadi et al., 2011).

9. Breeding and biotechnology of apple

Apple production in the world, presently around 71.7 million tonnes (FAO), suffers great losses every year due to different diseases during growth season, during harvest and also during post-harvest processing. To reduce the production losses, improvement of disease resistance is one of the most important steps. Presently, breeders have focused more on resistance when developing new cultivars. Breeding of disease resistant cultivars can also reduce disease control costs and meet consumer demands concerning the avoidance of pesticide residues in the fruits. Identification and breeding of such cultivars will increase the level of disease tolerance in the field. A main step in breeding is to gain better knowledge about genetic resources that are suitable in breeding programs, and this will also help to conserve biological diversity.

9.1. Traditional apple breeding

Records of human use of apples originate from the beginning of civilization when agriculture and apple growing was initiated. The earliest application of apple breeding took place when humans simply selected nice apples from different trees. Selection based on desirable traits can thus be seen as the first step of breeding. The apple breeding process was also influenced by the invention of grafting. Morgan et al. (1993) reported that grafting genotypes would have increased the quality of apple orchards because only the best cultivars would have been propagated rather than a random collection of their offspring.

By introduction of controlled pollination systems and development of new crossing techniques, breeders focused more on breeding based on crossing to produce seeds with a known pedigree. The first controlled pollination apple breeding program was done when Thomas A. Knight (1806) crossed different apple genotypes and then selected superior phenotypes. This is still the way in which breeders conduct the breeding process; mating parents with suitable traits in order to transfer a desirable trait in the pollen parent to a recipient seed parent with a superior phenotype. It is the most effective way to increase the

frequency of the desirable alleles due to the relatively high additive variance in most of the traits (Janick et al., 1996; Folta and Gardiner, 2009). Another commonly used strategy is mass selection. In this strategy, apple breeders select parents from commercial cultivars with favorable characteristics, cross them and then select progeny to test on rootstock for commercial release (Janick et al., 1996).

9.2. Application of molecular markers in apple

Traditional breeding of new valuable apple cultivars takes a long time and is very costly in most cases. Therefore, the efficiency of apple breeding can be enhanced by use of more informative and precise techniques such as molecular markers. Molecular markers can be used for different purposes; some are just used for generating genotype-specific DNA profiles while others are used to tag genes and thus help to, e.g., select desirable seedlings.

Reliable and reproducible markers linked to desirable traits can be applied in breeding programs. Molecular markers can help the breeder to choose better parents for the crosses and reduce the time needed for making selections among the seedling offspring, and consequently increase the breeding efficiency.

Molecular markers are classified into different categories, e.g., biochemical i.e. isoenzyme and DNA markers. They can be biomolecules related to a genetic trait, or just a difference in the sequence of a piece of DNA.

9.2.1. Isoenzyme markers

Isoenzymes are different forms of an enzyme that vary in size or conformation. Isoenzyme markers have been used for clonal identification of apple (Gardiner et al., 1996), and for developing markers for important genes (Hemmat et al., 1994; Chevreau et al., 1999; Pereira-Lorenzo et al., 2003). Presently their role has, however, been overtaken by DNA-based markers.

9.2.2. DNA markers

Because of the low level of polymorphism in isoenzymes, other groups of molecular markers, i.e. DNA markers, were developed that are able to detect more polymorphism. DNA markers are classified into a wide range of different discriminative techniques that reveal the genetic diversity between or within different species or cultivars. DNA markers are widely used for various purposes like studies of genetic diversity and phylogenetic analyses (Coart et al., 2003), constructing linkage maps (Liebhard et al., 2002), QTL analysis (Liebhard et al., 2003) and marker assisted selection (Costa et al., 2004).

(a) DNA markers and apple diversity

Different types of DNA markers have been used to evaluate the genetic diversity of apple cultivars. Goulao and Oliveira (2001) evaluated the degree of similarity between 41 commercial cultivars of apple with 13 SSRs (simple sequence repeats) and seven ISSRs (inter-simple sequence repeats) markers. Genetic similarity of 41 apple cultivars was assessed by RAPD (random amplified polymorphic DNA) and AFLP (amplified fragment length polymorphism) markers by Goulao et al. (2001). Oraguzie et al. (2001) used RAPD to evaluate the genetic relationships among four subsets of apple germplasm (including 155 genotypes; modern and old cultivars) in New Zealand. In other studies, genetic diversity of apple genotypes has been evaluated with different markers like RFLP (restriction fragment length polymorphism) (Gardiner et al., 1996), AFLP (Tignon et al., 2000, 2001), SSRs (Oraguzie et al., 2005; Pereira-Lorenzo et al., 2007) and RAPDs (Royo and Itoiz, 2004).

(b) DNA markers and gene tagging

Presently, there is much research on investigation of molecular markers linked to genes controlling apple traits. Several molecular markers associated with resistance to apple scab have been identified. Among the major identified genes, *Rvi6* (*Vf*) gene was the first attractive scab resistance gene used in breeding programs around the world (Koller et al., 1994; Manganaris et al., 1994; Hemmat et al., 1995; Gardiner et al., 1996; Tartarini,

1996; Yang and Korban, 1996). However, some races of *V. inaequalis* have been able to overcome the *Rvi6* (*Vf*) resistance and started to attack formerly resistant apple cultivars in the 1990s. A promising way to reach a more durable form of resistance can be achieved by pyramiding genes (incorporation of two or more resistance (*R*) genes in the same cultivar) (Xu and Korban, 2000; MacHardy et al., 2001). This method can delay or even prevent the breakdown of the *R* genes and create cultivars with durable resistance to apple scab.

DNA based markers have been identified also for some other important genes, including, e.g., genes related to ethylene biosynthesis and firmness of the fruits like *Md*-ACS1 (Costa et al., 2005; Oraguzie et al., 2007; Li and Yuan, 2008; Nybom et al., 2008b; Zhu and Baritt, 2008), *Md-ACO1* (Costa et al., 2005), *Md-PG1* (Wakasa et al., 2006) and *Md-Exp7* (Costa et al., 2008), genes related to chilling requirement (*Chr*) (Lawson et al., 2005), genes related with apple fertility (*MADS-box*) (Yao et al., 1999), fruit color (Cheng et al., 1996), resistance to fire blight (Malnoy et al., 2004) and powdery mildew (Markussen et al., 1995).

(c) Marker Assisted Selection (MAS) and QTL mapping

Marker Assisted Selection (MAS) is selection based on molecular markers that are linked to a favorable trait. The target trait can either be a qualitatively inherited trait (regulated by a monogene or major gene) or a quantitatively inherited gene (minor gene or QTL: Qualitative Trait Locus). Because many apple traits of agronomic importance are qualitative with clear and easily interpreted inheritance, most researches into markers have focused on qualitative traits. As already mentioned, many DNA markers linked to genes have already been identified. These markers can be used in different ways such as: early selection of traits (traits can be screened during the juvenile phase), use of traits as markers to identify the transformed plant after transformation (marker traits) and selection of traits which are too expensive or difficult to measure directly (Currie, 2000).

Several complete or partial genetic maps based on linkage analysis have already been developed for the apple genome. A saturated apple genome map was constructed by

Liebhard et al., (2003) with different types of DNA markers e.g. AFLP, SSRs, RAPD and SCAR. Two other genetic maps have also been developed by Silfverberg-Dilworth et al., (2006) and Fernandez-Fernandez et al. (2008) based on just one single population. Recently, an integrated consensus genetic map was constructed by N'Diaye et al. (2008). This consensus map was constructed based on segregation data from four genetically connected crosses (Discovery × TN10-8, Fiesta × Discovery, Discovery × Prima, Durello di Forli × Fiesta) with a total of 676 individuals.

(d) Identifying candidate genes

Beside the molecular markers, other techniques are now available to analyze the pattern of gene expression in plants. One of these techniques is the microarray technology that can be used to identify interaction between expressed genes and a specific trait. The DNA microarray technique is a high throughput technology by which the expression of the whole genome is studied in a single experiment. Recently, identification of putative candidate genes controlling fruit quality was investigated and the results showed that a microarray could be used to identify candidate genes potentially correlated to fruit quality QTLs (Soglio et al., 2009). Map positioning and functional allelic diversity of a new putative expansin gene (*Md-Exp7*) associated with fruit softening was analyzed by Costa et al. (2008). In another study, heterologous comparative genomics of apple was conducted to identify candidate genes involved in fruit quality (Costa et al., 2009).

10. Towards a better understanding and control of fungal storage diseases in apple

10.1. Breeding for disease resistance

Postharvest decay caused by fungal diseases is the major factor limiting the storage life of apples. Although control of these pathogens can be achieved by the application of chemical fungicides, environmental concerns and increasing public concern about the impact of chemicals on human health requires the development of new approaches. As mentioned above, new high-quality apple cultivars with resistance to postharvest pathogens can be developed via breeding methods. For some diseases like apple scab, several sources of resistance are known (Jönsson and Tahir, 2005; Tahir and Jönsson, 2005; Nybom et al., 2008a). No major genes have as yet been described for resistance against the common storage diseases but quantitatively inherited resistance probably exists. Possibly, level of tolerance against storage diseases is also related to some other traits that have already been evaluated, such as the contents of ethylene and polyphenolics (Nybom et al., 2008b; Blazek et al., 2007) and the acidity of apple tissues (Prusky et al., 2004). Other properties are also apparently involved in the resistance to fungal diseases in apple, e.g., fruit firmness as well as some physiological characteristics, e.g., fruit maturity and harvest time.

10.2. Bio-control of disease resistance

Much concern has recently been raised about patulin produced by *P. expansum*. Patulin is a mycotoxin, which is harmful to human health. The application of synthetic chemicals such as fungicides is a primary method for prevention of postharvest decay of apple fruits in order to eliminate patulin production. However, restrictions are being made on the use of fungicides because of the public concerns regarding human health and also because of the environmental risks of these chemicals. Therefore, alternatives to the conventional fungicides are needed to reduce losses from postharvest decay. Different bio-

control strategies can be applied to control decay caused by fungi, such as the use of antagonistic microorganisms or natural biocides, and the increase of natural defense mechanisms involving some plant components, such as phenolics, e.g., quercetin (Sanzani et al., 2009a) or ARS (Dey and Mikhailopuloa, 2009).

Phenolic lipids alkylresorcinols (ARs) are secondary metabolites synthesized mainly by plants and by a few fungi or bacteria. The synthesis can take place during normal development and/or in response to stressful conditions such as infection, wounds, and UV radiation. ARS have shown an inhibitory effect on bacteria, fungi and insects while they do not show any obvious negative effect on animals or humans. Therefore, these compounds can be applied as inhibitory agents against fungi (García et al., 1997; Hassan et al., 2007; Dey and Mikhailopuloa, 2009).

11. Aims of this PhD study

This PhD study aims to quantify the storage disease tolerance of some apple cultivars by performing inoculation tests with fungal spores on harvested fruits. Some factors that may be connected to the level of fungal disease tolerance will also be investigated: by pomological characterization (e.g. ripening rate), mechanical testings (e.g. fruit texture), chemical testings (contents of chemical compounds in the fruit flesh, some of which may also have health-promoting actions) and DNA marker screenings (e.g. ethylene-affecting genes). Bio-control of storage disease will also be investigated by spraying of ARS-containing solutions on *P. expansum*-inoculated fruit of different apple cultivars. The inhibitory effect of these ARS-containing solutions will then be evaluated by measuring the amount of the fungal symptoms. Finally this project will also include molecular identification and characterization of genes involved in storage disease. This will be carried out in co-operation with INRA Angers, using apple microarrays to identify candidate genes involved in fungal resistance and possibly also other traits like fruit softening and ethylene production which are related to fruit resistance. Application of these markers will make it possible to screen and classify apple cultivars regarding their disease tolerance.

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