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Strawberry

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ABSTRACT

The cultivated strawberry *Fragaria* × *ananassa* Duch. is the most important berry fruit crop worldwide. Approximately 4.1 Million metric tonnes are produced worldwide each year. Strawberries are cultivated in more than 70 countries worldwide and the standards required for success in a new strawberry cultivar have changed substantially during the past 50 years. Market requirements and cultural practices have shifted the relative importance of different traits and the main objectives of international breeding programs vary according to the environmental conditions of cultivation, the production systems in this area, and the utilization of fruit. In addition to yield capacity and fruit quality resistance to important pests, pathogens and abiotic stress conditions became more and more important. Particular attention is paid to the improvement of resistance to *Verticillium* wilt, black root rot disease, red stele, anthracnose and grey mould disease for example. However, classical breeding is still hampered by the lack of effective selection strategies which allow a screening of thousands of seedlings within a few weeks or months. Molecular markers which can help to overcome this problem are only available for a handful of traits. Nevertheless, the number of molecular studies is increasing and the first genome sequence of the diploid wild strawberry *Fragaria vesca* is available. This and the high number of favourable features have lead to the fact that *F. vesca* has emerged as an attractive model system for structural and functional genomics within the *Rosaceous* crops. With the publication of the first linkage studies in octoploid strawberry a milestone has been reached to take classical strawberry breeding to a new level. The availability of DNA markers linked to QTLs as well as cloning of individual genes which significantly contribute to complex traits will be very helpful for breeders to select for a specific introgression of interest.

Keywords: breeding, genetic resources, *in vitro* culture, molecular genetics, origin

CONTENTS

ORIGIN AND HISTORY OF THE CROP

At least since the end of the last Ice Age strawberries are native to forests in areas which belong nowadays to the European, Asian and American continents. As typical representatives of the holarctic flora, species of the genus *Fragaria* had probably the same geographical distribution almost before that time. The bi-hemisperic distribution

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Invited Review

(Euro-Asia and America) of the phylogenetic old species *Fragaria vesca* L. indicates the affiliation of the genus to the arcto-tertiary flora (Staudt 1961). Strawberries, fruits of the native strawberry *F. vesca* L., have always been gathered and consumed from the wild. Fossil records of strawberry achenes from the Neolithicum in South-West Germany demonstrated that strawberries were collected already at that time (Bertsch and Bertsch 1947). Historical writings of Romans and Greeks described the strawberry. Ovid and Virgil wrote of strawberry in their poems, and Pliny (AD 23-79) listed its fruit 'Fraga' as a natural product of Italy (Hancock 1999). Because native strawberries were available in the forests in big amounts their cultivation was not necessary. Even Charlemagne (AD 742-814) did not list strawberry in his 'Capitulare de villis et cortis imperialibus'. The first references to strawberry cultivation in Europe appear in the French literature of the 14th century. King Charles V planted over 1,000 strawberries in the Royal Gardens of the Louvre in Paris (Staudt 1961). It is known that *F. vesca* L., the wood strawberry, was cultivated in European gardens since the 15th century. The first colour illustration of the strawberry was published in 1485 by Schöffer in his German book 'Herbarius zu Teutsch or Gart der Gesundheit'. At that time the strawberry was grown widely in apothecary gardens all over Europe. And all parts of the plants were used in medical teas, syrups, tinctures and ointments (Hancock 1999).

Several different forms of *Fragaria vesca* L. were identified by botanists in the 1500s, including albino types and everbearing ones from the Alps (*F. vesca* f. *semperflorens* Duchesne). While the common wood strawberry flourishes only once, the everbearing flourishes and fruits from April until October. This has been and still is the reason for planting *F. vesca* f. *semperflorens* in gardens all over the world in spite of its fairly small fruits.

The musky-flavoured *Fragaria moschata* Weston was also planted in European gardens by the late 15th century, together with the green strawberry *Fragaria viridis* Weston. *F. viridis* was used solely as an ornamental, while *F. moschata* was utilized for its fruit by the English, Germans and Russians (Hancock 1999). The wood strawberry, *F. vesca* L., dominated strawberry cultivation in Europe for centuries, until *F. virginiana* Miller from Canada and Virginia began to replace it.

American octoploids were used before Europeans settled in America. North American Indians used fruits of *F. virginiana* to flavour bread and beverages (Jones 1994). Jacques Cartier, who discovered the St. Lawrence River in 1534, was probably the first to bring *F. virginiana* to Europe (Hancock 1999). *F. virginiana* grows in open spaces of the forests and on alpine meadows. Today it is distributed in North America from the East coast to the Rocky Mountains and from New Mexico to Alaska. Seeds were taken to Europe in 1556, but the first certain record of *F. virginiana* in a garden catalogue is from 1624 (Jones 1994). Because of its deep red colour it became known as the Scarlet strawberry. For nearly 200 years cultivars of the Scarlet strawberry were a favoured garden fruit because of their significant flavour and the early ripening time. Fruits ripen approximately three to four weeks earlier than the native *F. vesca.* In 1820 approximately 30 cultivars of *F. virginiana* were in cultivation.

The discovery of the strawberry is not only connected with North America. Also at the Pacific coast of South America a new species was found. The cultivated strawberry of South America, *Fragaria chiloensis* (L.) Miller has a long and rich history. It was utilized well over 1,000 years ago by the indigenous tribes. Strawberry fruits were used by the native Chileans fresh, dried, as a fermented juice or as medicine (Hancock 1999). During the period of Spanish exploration and conquest, *F. chiloensis* was spread throughout north-western South America. Strawberry cultivation by the native people was mostly limited to garden plots. The Spaniards established larger commercial plantings along the coastal area. There were probably 500-700 ha from the late 1700s until 1970 (Hancock *et al.* 1997). Today *F. chiloensis* is distributed discontinuously along the Pacific coast, usually on sand dunes, from the Aleutian Islands to California. The distribution achieves an altitude of 1,600 meters in the Cordilleras. The first introduction of *F. chiloensis* to Europe was in 1714 by a French Captain Amedee Frezier (Darrow 1966). Frezier brought plants rather than seeds and five plants survived the journey to France. After their arrival in Europe the first reports were negative: the plants were barren because Frezier had brought back pistillate plants and the need of the cross pollination was recognized (Hancock 1999). French gardeners solved the problem when they discovered that the Chilean plants would produce fruit when pollinated by *F. moschata* or *F. virginiana*. Unusual seedlings with unique combinations of fruit and morphological characters began to appear in the gardens of Brittany. In 1766, Antoine Nicolas Duchesne, a young French botanist determined that these seedlings were hybrids of *F. chiloensis* and *F. virginiana*. Recognizing the perfume of the fruit as smelling like pineapple (*Ananas*) he named them *Fragaria* × *ananassa* as a new species (Darrow 1966). The two monographs by Duchesne of 1766 and 1788 should be considered as landmarks in research on strawberries, especially their chapters on systematics and taxonomy (Staudt 2003). Molecular studies investigating cpDNA verified that *F.* × *ananassa* is an interspecific hybrid between *F. chiloensis* and *F. virginiana* and from the analysis of two non-coding regions three haplotypes (V, C, and X) in *F.* × *ananassa* were found (Honjo *et al.* 2009).

In the 19th century the formal strawberry breeding was initiated in England by the work of Michael Keen and Thomas A. Knight (Darrow 1966). Their famous cultivars became the background of many European modern cultivars. Keen, a market gardener near London, developed 'Keen's Imperial' and its offspring 'Keen's Seedling'. At that time the berries were sensationally big, of a deep red colour with a particularly good flavour (Hancock 1999). In Germany, the first selections were initiated 1870 by G. Göschke and later by his son (Thiele and Knauth 1953). Some cultivars like 'Königin Luise', 'Amazone' or 'Zarathustra' are still famous among the garden enthusiasts. Some of the most popular releases in the early 20th century were: 'Madame Moutot' (France 1910), 'Deutsch Evern' (by Soltwedel, Germany 1902) or 'Oberschlesien' and 'Mieze Schindler' (by Schindler, Germany 1919 and 1925). 'Madame Moutot' was popular in France and other European countries until the late 1960s due to the size and productivity (Darrow 1966). 'Deutsch Evern' was for many decades famous as the earliest cultivar not only in Europe but also in America and Africa (Thiele and Knauth 1953). Today the dessert strawberry, *Fragaria* × *ananassa*, dominates strawberry cultivation in the arable regions of the world (Hummer 2008).

BOTANICAL DESCRIPTION AND GENETIC RESOURCES

The strawberry belongs to the genus *Fragaria* in the *Rosaceae* family. Its closest relatives are *Duchesnea* Smith and *Potentilla* L. Botanically, a strawberry is an aggregated fruit, originating from receptacular tissue. A number of ovaries occur on a common receptacle which later form one-seeded fruits, or achenes. The enlarged receptacle with achenes is considered the berry but is often termed a 'fruit' in horticultural sense (Perkins-Veazie 1995). The genus *Fragaria* consists of approximately 22 species (Hummer 2008) depending upon the rank given to several taxa and the acceptance of putative hybrids. The European and American species of *Fragaria* have been defined by Staudt (1989; 1999) and the key to the Himalayan species of *Fragaria*, *F. bucharica* Losinsk, *F. daltoniana* J. Gay and *F. nubicola* (Hook. F.) Lindl ex Lacaita, was given by Staudt in a newer report (2006). Chinese species are under study but require further collection and examination in light of global taxonomy. An accurate synonymy and phylogeny of the strawberry species is under development (Hummer 2008). Polyploidy in *Fra-*

garia probably arose through the unification of 2n gametes, several investigations have demonstrated that unreduced gametes are relatively common in *Fragaria* (Hancock 1999). The origin of the octoploids is discussed in northern Asia, when *Fragaria vesca* L. hybridized with other unknown diploids, and the polyploids derivates migrated then across the Bering Strait and dispersed across North America (Hancock *et al.* 2008b). It is suggested that *Fragaria iturupensis* Staudt distributed on Mount Atsunupuri, Iturup Island, of the Kurile Islands, Sakhalin Territory, in the Russian Federation, should be an important connector of the phylogenetic development of American octoploid species (Hummer *et al.* 2009).

The *Fragaria* species can be organized according to their geographic range. *F. vesca* has the largest native range, encompassing most of Europe, Asia, and the Americas. All other species are more restricted in ecogeography, being clustered primarily in Euro-Siberia, northern China and Manchuria, Indo-South China, Japan, and the Americas. Japan is particularly rich in species, with at least four endemic species radiating across its islands (Hancock 1999).

In view of the large number of cultivars released in the comparable short history of the dessert strawberry *Fragaria* × *ananassa*, it has been become clear that the preservation of cultivars could not be ensured by horticultural production and marketing; the maintenance of cultivars in genebanks is an important option. On the other hand there is a growing interest in utilization of native germplasm in breeding programmes. Numerous valuable characteristics exist in lower ploidy species that could be of value in the cultivated species (Darrow 1966; Hancock 1999; Hancock *et al.* 2008b). Strawberry was chosen to be on the Annex 1 list of the International Treaty on Plant Genetic Resources for Food and Agriculture. A strategy for coordinating the Global conservation of strawberry genetic resources was developed (Hummer 2008). The Global Crop Diversity Trust provided support for this initiative and considers this strategy document to be an important framework for conservation of strawberry genetic resources. This strategy based on a strawberry genebank questionnaire assembled by 37 responders from 27 countries. Altogether 12,121 total numbers were registered including wild species accessions, landraces, and cultivars and advanced breeding lines. The biggest collections were represented by the USDA ARS National Clonal Germplasm Respository in Corvallis, Oregon (1,924 accessions), by the Agriculture and Agri-Food Canada, Harrow, Ontario collection (1,782 accessions), by the collection of the All-Russian Research Institute of Plant Industry in Petersburg, Russian Federation (1,210 accessions), by the Fruit Genebank of the Julius Kühn-Institut, Institute for Breeding Research on Horticultural and Fruit Crops in Dresden, Germany (660 accessions, **Fig. 1**) and by the collection of the CIFA Malaga, Spain (660 accessions). The primary collections consist of living plants, protected in containers in greenhouses or screenhouses, or in the field. Conservation of vegetatively propagated material is more complicated and expensive than that of crops maintained as seeds. Therefore, secondary backup collections are of great importance established *in vitro* under refrigerated temperatures. Today cryopreservation is valuable as a secondary backup for the long-term storage of clonal germplasm (Reed 2008). A strategy paper suggests the development of two genebanks supported by the Trust in China and Chile to collect and preserve the critical endangered native wild and landrace genetic resources which exist in those regions. In addition, a granting system for improved health of strawberry in genebanks should be supported, and training of genebank staff in standard protocols is needed. Regarding characterization and evaluation, a coordination of data and a web accessible database listing strawberry genetic resources should also be supported (Hummer 2008).

In the frame of the COST (European Cooperation in the field of Scientific and Technical Research) Action 836, the regional, inter-country network for genetic resources in strawberry in Europe was established and the first European

Fig. 1 Strawberry collection of the Fruit Genebank of the Julius Kühn-Institut, Institute for Breeding Research on Horticultural and Fruit Crops in Dresden, Germany. Plants are maintained in a box system under automatic irrigation in the field. In the front of the table the cultivar 'Antara' can bee seen.

Fig. 2 Recovery of the *Fragaria* cultivar 'Aprikose' 6 weeks after cryopreservation using the 'Plant Vitrification Solution 2' method. For recovery the cultures were incubated on multiplication medium in Petri dishes (Höfer 2010).

inventory was developed. On the base of the results, a new Action 863 EUROBERRY was initiated focused on selected topics of major importance for the European berry production system and quality control. An improvement of the European cooperation regarding the genebank will be realized with the EU project AGRI GEN RES 036 'European small berries genetic resources'. The results of a common evaluation and utilization of 20 microsatellite (SSR) markers will lead to the construction of an European core collection of strawberries, suitable for *ex situ* conservation of genetic resources and for assessing the potential genetic diversity of disease resistances and/or healthy compounds, which is not exploited in strawberry breeding until now.

The Fruit Genebank of the Julius Kühn-Institut in Dresden, Germany, representing one of the biggest collections in Europe, is partner of both projects. On the basis of a comprehensive evaluation and data analysis, 18 primary descriptors describing plant, leaf, flower and fruit characteristics independently from cultivation were selected for a large screening. Twelve further descriptors were suggested for resistance and inner fruit quality traits as secondary descriptors. The same descriptors were proposed as appropriate primary and secondary descriptors to be used in the European GENBERRY project for 88 varieties of the cultivated octoploid strawberry (*Fragaria* × *ananassa* Duch.). The

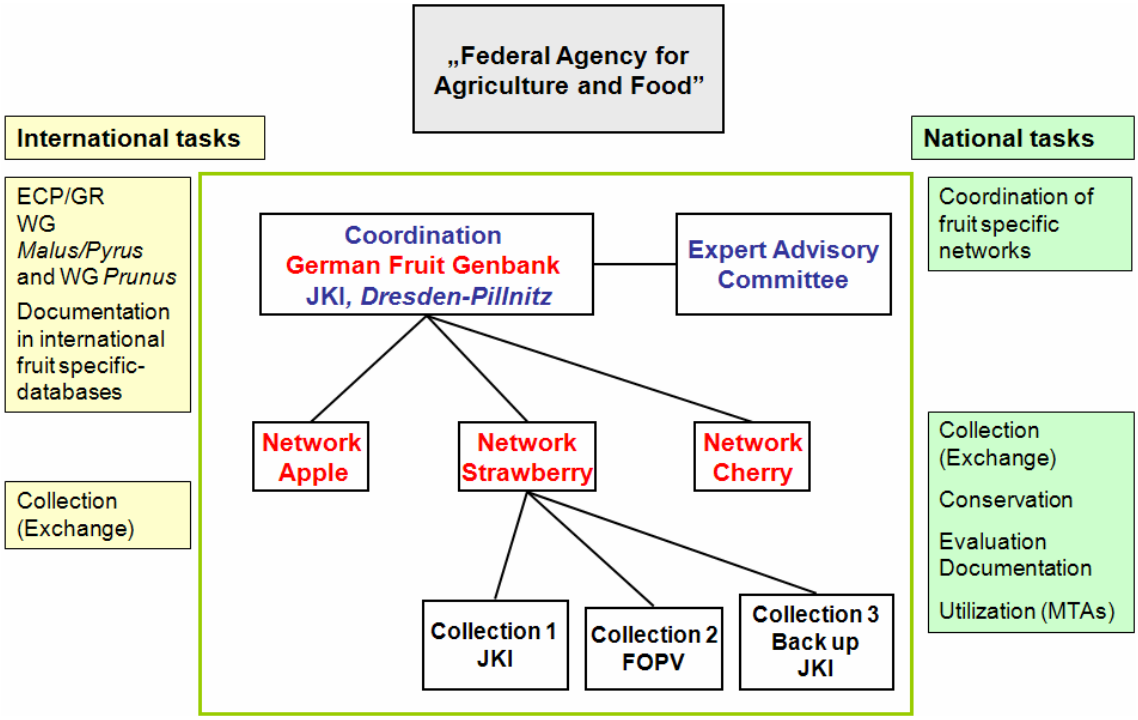

Fig. 3 Organization of the German Fruit Genbank.

GENBERRY project is also focused on effective handling of *ex situ* collections and development of methods to build back-ups. An effective method for *in vitro* cold storage was developed and adapted. The average storage duration at 4°C for strawberry cultivars was 22 months and for wild species accessions nine months using five-chamber-bags as storage containers, and a combined medium without plant growth regulators or vitamins (Höfer and Reed 2011). However, first results on cryopreservation of strawberry microshoots suggest that this method is more cost effective for long-term storage (**Fig. 2**).

In addition to the Fruit Genebank of the Julius Kühn-Institut in Dresden (Germany), which is one of the largest collections of fruit genetic resources in Europe, there are a multitude of activities in Germany which are aimed to preserve fruit crop species in public and private germplasm collections. Regrettably, such a form of preservation of genetic resources appears not to be safe and sustainable. The loss of individual cultivars cannot be excluded in the case that public collections will be closed or private collectors die. To minimize the risk of losing local fruit genetic resources 'The German Fruit Genbank', a decentral network, which is aimed on the coordination of different germplasm collections, has recently been established. The German Fruit Genbank is coordinated by the Institute for Breeding Research on Horticultural and Fruit Crops in Dresden-Pillnitz which is guided by the Federal Agency for Agriculture and Food and advised by an expert advisory committee. The expert advisory committee consists of pomologists and experts from fruit research institutions, nurseries, nature conservation organisations, the Federal Office for Plant Varieties and the German Fruit-growing Association. The whole network of the German Fruit Genbank is divided into different fruit species specific sub-networks (e.g. apple network, strawberry network etc.). Each sub-network consists of different selected collections (**Fig. 3**). The sub-networks are working on the development of specific preservation guidelines for the respective fruit species. A total of three networks (strawberry, cherries, and apple) have taken up their activities until today and the establishment of networks for further fruit crops has been scheduled. The strawberry network consists of 389 strawberry cultivars which include German varieties, varieties with socio-cultural, local or historical importance or cultivars which are known to have important traits (e.g. resistances). These cultivars are preserved in two field collections (at the JKI in Dresden and at the Federal Office for Plant Varieties in Wurzen) and one cryopreservation collection (at the JKI in Dresden) as a secondary backup for the long-term storage (Flachowsky and Höfer 2010).

ECONOMIC IMPORTANCE, USES AND AREAS OF PRODUCTION

The genus *Fragaria* consists of approximately 22 species. The majority of these species are diploid. Commercially important is the octoploid species *Fragaria* × *ananassa* Duch. The cultivated strawberry *F.* × *ananassa* is the most important berry fruit crop in the world. In 2008, strawberries ranged with approximately 4.1 million metric tonnes produced on 255,366 hectares of land on position 19 of the most important fruit crops (http://faostat.fao.org). Strawberries are cultivated in more than 70 countries worldwide with U.S.A. (1.2 million metric tonnes), Spain (264,000 metric tonnes) and Turkey (261,000 metric tonnes) as the leading countries. In Europe strawberries are cultivated in 36 countries with Russia, Spain, Poland and Germany as the main producers. The attractive strawberry fruits are favoured for their excellent taste and are health promoting due to their richness in vitamins, minerals and antioxidative compounds (Hannum 2004). In plant science, the strawberry fruit is an excellent model for studies on non-climacteric fruits, where fruit ripening is not ethylene dependent.

BREEDING OBJECTIVES

The standards required for success in a new strawberry cultivar have changed substantially during the past 50 years. This results from the fact that standards of productivity and quality have increased dramatically. In addition, both market requirements and cultural practices have shifted the relative importance of specific traits. The main objectives of a breeding program vary according to the environmental conditions of cultivation, the production systems in this area, and the utilization of fruit for fresh market or processing. However all characters the selection is focused on can be summarized into three main areas: yield capacity, fruit quality (fruit appearance, size, color, shipping quality, shelf life, and flavor) and resistance or tolerance to important pests, pathogens and abiotic stress conditions.

Current breeding effort in numerous public and private breeding programs across the world was reviewed by Hancock *et al.* (2008b). The main objectives in breeding and selection were also described. This paper will focus only on the latest reports concerning this issue.

Disease and pest resistance

Verticillium wilt, caused by *Verticillium* sp., is considered among the most important diseases affecting strawberry production worldwide and breeding for resistance is in the focus of a range of breeding programs. Recently, Shaw *et al.* (2010) reported on a marked improvement of genetic resistance to Verticillium wilt in the University of California strawberry breeding program since 1994 based on the identification of resistant germplasm and generating of highly resistant lines, and backcrossing. Breeding for resistance has yet to resolve the problem completely, but the results suggest that the multiple-trait selection strategy employed has generated substantial improvement in a commercial strawberry cultivar.

Black root rot is a widespread disease of strawberry that causes the death of feeder roots and the degradation of structural roots resulting in an overall decrease in productivity. Black root rot is caused by a disease complex between fungi (*Rhizoctonia fragariae*, *Pythium* sp.) and nematodes (*Pratylenchus penetrans*). Most researchers believe that this disease has almost replaced red stele (*Phytophthora fragariae*) as the most serious root disease (Pritts and Wilcox 1990). Varying levels of tolerance in strawberry cultivars were detected and progeny populations were created to determine the amount of genetic variability for black root rot tolerance. From the results it was concluded that the genetic improvement in tolerance to this disease will be difficult to achieve, except perhaps under very heavy pathogen pressure (Particka and Hancock 2005, 2008).

Phytophthora cactorum also causes severe crown and fruit rots, particularly in warm climates. Genetic studies of inheritance of resistance to this disease revealed that most of the selection response obtained through genotypic selection would be transferred to segregating offspring (Shaw *et al.* 2008).

Three *Colletotrichum* species are major pathogens of strawberry. Strawberry anthracnose crown rot is caused by *C. fragariae* and *C. gloeosporioides.* The anthracnose fruit rot is caused by *C. acutatum*. Since the 1980s, increased losses due to anthracnose fruit and crown rots in the United States may be related to changes in cultivars and to widespread use of annual plasticulture production rather than the matted-row production system. Anthracnose-resistant cultivars are a major objective of most strawberry breeding programs in the southern United States (Smith 2008). A breeding strategy for improving resistance to strawberry black spot (*Colletotrichum acutatum*) was also developed in the United Kingdom, Spain and other countries (Refoyo *et al.* 2009; Simpson and Hammond 2009).

Botrytis cinerea, a pathogen economically important on soft fruits and other crops, produces severe agricultural losses. The Chilean strawberry, *F. chiloensis* is one of the wild parents of commercial strawberry and has the capacity to tolerate the infection from the pathogenic fungus. A study was performed to make a genetic analysis of wild and cultivated individuals collected from different regions of Central South Chile and compare the *Botrytis* infection performance. The results indicate that the samples of *F. chiloensis* are more genetically homogeneous and can be classified as tolerant to the infection by *Botrytis cinerea* (González *et al.* 2009a).

Fruit quality

Berries have a high nutritional value as they contain powerful antioxidants and other bioactive compounds. Genetic and environmental factors affect production and storage of such compounds. For this reason breeding and biotechnological approaches are currently used to control or to increase the content of specific health-related compounds in fruits. The main bioactive compounds determining the nutritional quality of berries, the major factors affecting their content and activity, and the genetic options currently available to achieve new genotypes able to provide, under controlled cultivation conditions, berries with the proper balance of bioactive compounds for improving consumer health are reviewed by Battino *et al*. (2009). The current knowledge on the potential impact of strawberry on human health, with particular attention on compounds and indirect mechanisms of action not exhaustively considered, was updated by Tulipani (2009).

In the last years the nutritional value of strawberry fruits has been widely studied and requested by the consumers, especially for the general health benefits. These benefits can mainly be ascribed to the total antioxidant capacity (TAC) of fruit which is determined by a complex combination of bioactive compounds. Both quality parameters, like firmness, color, soluble solids content and titratable acidity, and antioxidant attributes, like TAC and total phenolic content, are good tools to describe the nutritional quality of the fruit. Germplasm (cultivars, selections and wild species) were screened concerning these parameters. Results indicate that the effect of the genotype on strawberry nutritional quality is stronger than that of the cultivation conditions. However, commercial cultivation did not show a high range of variation of fruit nutritional quality, particularly for the nutritional parameters. The study of offspring originating from different cross combinations showed that fruit nutritional quality can be considered an inheritable trait and that the variability of fruit nutritional quality among commercial cultivars can be improved by breeding. *F. virginiana* spp. *glauca* seems to be an important genetic source of the fruit nutritional quality (Capocasa *et al.* 2008a, 2008b, 2009).

In the last years special attempt was related to understand the flavor components of eating quality in strawberry. There is a range of reports on sensorial evaluation of cultivars grown in different environmental conditions. Genotypes with low flavoring rating are often judged as “not sweet enough”, thus linking flavor to sweetness. Instrumental analysis confirmed that typically these selections have low soluble solids content and/or high titratable acidity, thus explaining their lack of sweetness (Jouquand *et al.* 2008). Volatile compounds that varied only quantitatively did not seem to influence the flavor rating. A new method for quantification of fruit-to-fruit variation in strawberries by both sensory and instrumental analyses was recently developed and applied (Gunness *et al.* 2009). This study suggests that fruit-to-fruit variation is substantial in soluble solids content, titratable acidity and fruit firmness and sensory characteristics, such as 'fruit odour', 'sweet flavour' and 'flavour aftertaste', whereas other characteristics show similar variation among panelists for both individual fruit and bulk puree analyses.

One of the important characteristics of fruit ripening is volatile aroma production. Recent developments in fruit aroma research were reviewed by Song and Forney (2008). Both sensory studies and instrumental analysis confirm the importance of volatile production in fruit and its contribution to eating quality. Sensory analysis should further define the contribution of individual volatile compounds to total flavor quality. Volatile biosynthesis and its contribution to fruit eating quality is very complex, and is influenced by many factors, such as genome, harvest maturity, and postharvest handling and storage.

Day-neutral strawberries

Day-neutral (DN) strawberry cultivars of *F.* × *ananassa* now play an important commercial role in Mediterranean areas, but there is also little success in continental climate. DN strawberries together with alternative cultural systems can be used for either season extension and/or off-season production. A number of breeding programs around the

world are focused on day-neutral strawberry types (Faedi *et al.* 2009) and research is aimed on the evaluation of DN types for further genetic improvement, i.e antioxidant activity (Khanizadeh *et al.* 2008). Day-neutrality in the commercial strawberry is also in the focus of molecular research to understand the inheritance and the genetic background. The knowledge about genetics of day-neutrality, the expression of day-neutrality under different environments, the source of the day-neutral trait in breeding was recently updated by Hancock *et al.* (2008a).

Consequences from climatic changes

The temperate regions in the world are projected to get hotter, rainfall patterns are expected to change and the weather will become more variable over the next century. These more extreme weather conditions will have an important impact on breeding objectives. New cultivars will need to be able to withstand more hotter days and nights, more rapid fluctuations from hot to cold, heavy intermittent rainfall, and longer droughts. They have to be able to grow with little chilling, and to initiate flower buds in most environmental conditions (Dale 2009). Dale proposed the need to breed cultivars adapted to more than one contrasting environment. Recently, a program was developed to select day-neutral strawberry cultivars that would be adapted throughout eastern North America. The hypothesis is that by breeding in two contrasting environments cultivars can be produced that are widely adapted and highly heterozygous like an outbred population.

Climate change will also influence local pests by increasing the reproductive period, especially in spring. Strawberry may be the most vulnerable berry crop when climate change proceeds, as it is a favorite host for several emerging pests and also for new invading pests (Tuovinen 2009).

CLASSICAL BREEDING

The main compendium on history of strawberry domestication and breeding was published by Darrow (1966), later detailed description were given by Hancock (1999) and Hancock *et al.* (2008b). Perhaps the most important early selection of a garden strawberry cultivar was made by Michael Keens (1806), who created the cultivar 'Keen's Imperial' by selecting one plant from a number of hybrids. Thomas A. Knight began the systematic breeding of strawberries in England in 1817. By the early 1800's a number of gardeners on both sides of the Atlantic, mainly in England, later in Holland and Germany, as well as in North America, realized that larger, sweeter strawberries could be made by breeding, growing out seedlings, and selecting the offspring for improved traits. At the beginning of the 20th century, when plant breeding started to develop as an agricultural science, a number of scientific institutions in Europe and North America were founded and strawberry breeding programs appeared. Since that time strawberry breeders have cooperated and developed lots of new cultivars. During the 20th century, the area of strawberry production increased worldwide and strawberries were grown in various environmental conditions. The fruit quality was drastically improved with the development of superior production and management systems. There was a special demand for cultivars adapted to these environments. In this context, the breeders have to focus research on more efficient breeding and selection strategies.

The commercial strawberry has a narrow germplasm base, even though its progenitor species have an extensive geographical range (Hancock *et al.* 2001). North-American as well as European genetic improvement began with a restricted group of European *F.* × *ananassa* cultivars, *F. virginiana* and *F. chiloensis* accessions. The cultivars originating from this background played the predominant role in most public and private breeding programs for the next 100 years. The majority of the genes in modern North American cultivars still comes from only seven nuclear and 10 cytoplasmic sources, even though at least eight native clones have been incorporated into cultivars in the last half century. These include: two unnamed clones of *F. chiloensis* from the Pacific Northwest, two unnamed clones of *F. virginiana* from Oregon and Alaska, two selections of *F. viginiana* from the Rocky Mountains, the Huachi Grande clone of *F. chiloensis* from Ecuador, and the Del Norte clone of *F. chiloensis* from northern California (for references see Hancock *et al.* 2001).

The utilization of narrow germplasm in breeding, if continued, could cause deleterious effects of inbreeding and genetic vulnerability to diseases, pests, and environmental stresses. Exploration of *F. virginiana* germplasm has been intensified in North America since the 1990th (Luby *et al.* 1992). Most of the successful incorporations of wild-derived traits have come through backcrossing, most notably is the movement of day-neutrality from *F. virginiana* into the cultivated strawberry by R.S. Bringhurst. Hancock *et al.* (1993) suggested reconstituting *F.* × *ananassa* by intercrossing of elite clones of the two species. This approach will increase genetic diversity in the *F.* × *ananassa* gene pool, produce higher levels of heterozygosity and generate unique coadapted complexes. Results obtained from crosses between elite selections of *F. virginiana* and *F. chiloensis* indicate that substantial breeding progress can be made by reconstructing *F.* × *ananassa* if care is taken to select elite, complementary genotypes of *F. virginiana* and *F. chiloensis* (Luby *et al.* 2008).

The Corvallis Repository, which has the strawberry genebank for the US National Plant Germplasm System, is cooperating with genebanks and many scientists around the world to increase the availability of wild strawberry species for crop improvement. Evaluations of the germplasm indicate that breeders will find considerable variation for fruit quality traits, disease and pest reactions, reproductive efficiency, and fruiting habit. Evidence of ecological and geographical differentiation suggests that local populations may be sources of genes for specific regional adaptation.

Recently, Shaw and Larson (2008) evaluated the genetic improvement of cultivars released from the University of California breeding program from 1945-1966 and between 1993 and 2004 using growing systems with important features of the horticultural practices common during the period of release for each set of cultivars. Values for fruit yield, fruit size, commercial fruit appearance, and fruit firmness, averaged over both horticultural systems, were 47-140% greater for modern cultivars than for the early-generation of cultivars, with the largest increase observed for fruit yield. Comparisons between early-generation and modern cultivars suggest changes for individual traits of 1-3% per year. These results suggest that the majority of the genetic change obtained by selection for these traits was stable to the most important alterations in horticultural practices over the period evaluated, and provide no indication of any specific adaptation of recent strawberry cultivars to modern horticultural system.

Improved strawberry cultivars have resulted from pedigree selection, since strawberry is highly heterozygous due to its polyploid origin. Each cycle starts with controlled crosses among selected parents. It is primarily the emphasis on differing traits between parents at this stage that determines the relative levels of improvement later in the breeding cycle, and parental selection offers the greatest opportunity for efficient incorporation of novel breeding characteristics. Large genetic variability among seedlings obtained from crosses is the major factor for the selection of desirable genotypes. Initial evaluations are performed on the basis of seedling performance. The retained seedlings from the primary population are kept for subsequent evaluation based on plots of runner plants. After a few years of subsequent screening on desirable characters, selection and runner propagation of elite seedlings, the selected genotypes are evaluated in multi-location trials under commercial conditions. The breeding cycle starts with a cross

and ends with the release of a new cultivar which is superior to its parents and adapted to production systems. This process takes between 8 and 12 years. The duration of the cycle also depends on additional controlled tests required to analyze some characters in the breeding program, like disease resistance tests in greenhouse.

KARYOTYPING

The chromosome number is used in systematics and taxonomy of strawberries for a long time as it can be used clearly to separate species. The strawberry genus, *Fragaria* (*Rosaceae*), has a basic chromosome number of x = 7. The ploidy level of species is relevant to explain phylogenetic relationships; lower chromosome numbers are in most cases ancestral to species with higher numbers. Cultivated strawberries (*F.* × *ananassa*) are octoploid (2n = 8x = 56) and first hybridized from *F. chiloensis* and *F. virginiana*. Europe has no known native octoploid species, and only one Asian octoploid species has been reported: *F. iturupensis*, from Iturup Island (Hummer *et al.* 2009). Thirteen diploid (2n = 2x = 14) species are known in *Fragaria* in two centres of diversity namely Central Asia and Far East, four tetraploid species (2n = 4x = 28) which are confirmed to East and South East Asia, one hexaploid (2n = 6x = 42) species native to Europe, and three octoploid species (2n = 8x = 56) species (Staudt 1989; Staudt 2009). Artificial triploid (2n = 3x = 21), tetraploid, pentaploid (2n = 5x = 35), octoploid, decaploid (2n = 10x = 70), 16-ploid, and 32-ploid plants have been constructed and cultivated. Surprisingly, chromosome counts and flow cytometry revealed that *F. iturupensis* includes natural decaploid genotypes (Hummer *et al.* 2009).

In order to clarify phylogenetic relationships among taxa and to elucidate the origin of the polyploid species, phylogenetic utility of two nuclear genes was explored in genus *Fragaria*. The results supported the presence of three main diploid genomic pools in the genus and demonstrated the occurrence of independent events of polyploidisation. In addition, the data provided evidence supporting an allopolyploid origin of the hexaploid *F. moschata*, and the octoploids *F. chiloensis*, *F iturupensis* and *F. virginiana*. Accordingly, a new pattern summarizing our present knowledge on the *Fragaria* evolutionary history is proposed (Rousseau-Gueutin *et al.* 2009).

MOLECULAR TECHNOLOGIES

Fingerprinting-technologies in strawberry

Different DNA fingerprinting-technologies and strategies have been reported in strawberry. A number of studies were based on the use of randomly amplified polymorphic DNA (RAPD) markers. One of the earliest reports was published by Degani *et al.* (1998). They used a set of 10 RAPD primers for the characterization of 41 major strawberry (*Fragaria* × *ananassa*) cultivars. These primers produced 15 polymorphic fragments. Ten of the markers derived from seven primers were absolutely required for distinguishing the cultivars. RAPD markers were also used by Harrison *et al*. (2000). A total of 24 morphological traits and 36 RAPD markers were assessed among 318 wild octoploid strawberry (*Fragaria* spp.) genotypes from diverse habitats across the northern USA. RAPD marker frequencies and certain morphological traits were significantly different between the *F. chiloensis-platypetala* and *F. virginiana-glauca* species complexes. Based on their results the authors concluded that the patterns of diversity for morphological traits must be considered, along with more selectively-neutral molecular markers, to formulate effective sampling strategies and to properly estimate the quantity and apportionment of diversity within germplasm (Harrison *et al.* 2000). Degani and co-workers also used amplified fragment-length polymorphism (AFLP) markers and RAPD markers for evaluation of 19 major strawberry cultivars grown in the United States and Canada. They demonstrated the usefulness of AFLP markers for cultivar identification in strawberry (Degani *et al.* 2001). AFLP markers were also tested by Tyrka *et al.* (2002). Six cultivars and 13 salinity tolerant clones were evaluated using simplified *Pst*I based AFLP procedure. The authors suggested this simplified method as a powerful tool for effective identification of selected strawberry genotypes. Furthermore, the method was suggested for assessing the level of genetic diversity in strawberry cultivars and breeding lines. Korbin *et al.* (2002) characterized a Polish fruit plant germplasm using RAPD and inter simple sequence repeat (ISSR)-PCR markers. They estimated the genetic distance between genotypes and their relatedness. In many cases, both RAPD- and ISSR-based genetic similarity confirmed relatedness connected with biological origin and with the place where the cultivar was developed. However, some diversity connected with the technique used for molecular marker generation was observed. A parallel study using two data sets (RAPD and ISSR) was performed to reduce the number of potential mistakes connected with one or the other method (Kuras *et al.* 2004). ISSR markers were also used for cultivar identification and for studies on diversity of Chilean strawberry (*F. chiloensis*) by other authors (Arnau *et al.* 2003; Carrasco *et al.* 2007; Debnath *et al.* 2008; Gonzalez *et al.* 2009a).

Kunishisa and co-workers (2003, 2005, 2009a, 2009b) suggested cleavage amplified polymorphic sequence (CAPS) markers for identifying mislabelled or patent-infringing strawberry cultivars in the marketplace. They developed six CAPS markers and used them to distinguish among 14 commercial strawberry cultivars. The results obtained by Kunishisa *et al.* (2003) were highly reproducible among different DNA extraction methods, different organs and different researchers. However, in octoploid strawberry CAPS markers produce often pattern, which are not as easy to distinguish (Kunihisa *et al.* 2003). Multiple homologous sites are the reason for simultaneous amplification of non-selective PCR products. To overcome this problem, the authors used "cluster-specific amplification" based on the nucleotide sequences of PCR products. They were able to improve the band clarity of different CAPS markers and confirmed their ability to distinguish among 64 strawberry cultivars. In a recently published study Kunihisa *et al.* (2009b) showed that 16 CAPS markers are enough to identify 117 cultivars with a probability of at P>99.91%.

Simple sequence repeat (SSR) markers were used by Shimomura and Hirashima (2006) and Hokanson *et al.* (2006). Whereas Hokanson *et al.* (2006) used SSR markers to assess diversity among all of the proposed taxa of *F. virginiana* and *F. chiloensis*, Shimomura and Hirashima (2006) used this marker type for identification of octoploid strawberry cultivars for the protection of breeders' rights. Govan *et al.* (2008) identified a set of multiplexed SSR markers for genotyping of strawberry cultivars and their octoploid progenitors. They tested more than 100 SSR markers on two *F.* × *ananassa* genotypes. A total of 32 markers were selected for genotyping. These markers were tested on 16 strawberry cultivars and ten out of them were selected for a genotyping set. Subsequently, the set has successfully been used to distinguish a selection of 56 cultivated strawberry, and four octoploid *Fragaria* species accessions (Hokanson *et al.* 2006). So far so good, all the methods tested produced more or less reproducible results and were absolutely sufficient to answer the respective question. Comparative studies with data sets obtained in different studies are only possible in a few cases. To overcome this problem, scientific experts cooperating within the *Malus*, *Pyrus* and *Prunus* working groups of the European Cooperative Programme for Plant Genetic Resources (ECPGR) compiled a defined set of SSR markers and decided that this set should be used for all future studies. Such an international agreement for genotyping of strawberry is still missing.

Table 1 Major genes mapped in different strawberry species.

Gene	Trait	Marker system	Marker	Species	x =	Reference
c	Yellow fruit locus	RAPD	B191A	*F. vesca*	2	Deng and Davis 2001
		STS	F3H			
SFL	Seasonal flowering	ISSR	835$(AG)_8$YC x 841 $(GA)_8$YC	*F. vesca*	2	Cekic *et al.* 2001
		SCAR	SCAR1, SCAR2, SCAR3	*F. vesca*	2	Albani *et al.* 2004
RNase A/S	Self-incompatibility	STS, RFLP	F3'H, TSA3	*F. vesca*	2	Bošković *et al.* 2010
RNase B/T	Self-incompatibility	SSR	EMFn228, ARSFL-007	*F. vesca*	2	Bošković *et al.* 2010
r	Non-runnering	SSR	EMFv022, EMFvi146	*F. vesca* f. *semp.* × *F. nubicola*	2	Sargent *et al.* 2004
s	Perpetual flowering	SSR	EMFvi025, EMFn017	*F. vesca* f. *semp.* × *F. nubicola*	2	Sargent *et al.* 2004
pg	Pale-green leaf	SSR	Fvi20, EMFv006	*F. vesca* f. *semp.* × *F. nubicola*	2	Sargent *et al.* 2004
Rpf1	Resistance to *P. fragariae*	RAPD	OPO-16A-C, OPO-8F, OPO-8A,	*F.* × *ananassa*	8	Haymes *et al.* 1997;
		SCAR	SCAR-R1, RPF1NF1/RPF1NR1			Hokanson and Maas 2001; Rugienius *et al.* 2006; Gelvonauskiene *et al.* 2007
Rpf3	Resistance to *P. fragariae*	AFLP	E36M59H, E40M61E, E39M51H	*F.* × *ananassa*	8	Hokanson and Maas 2001
Rpf6	Resistance to *P. fragariae*	AFLP	E39M51B, E44M59E	*F.* × *ananassa*	8	Hokanson and Maas 2001
Rca2	Resistance to *C. acutatum*	AFLP	aga/cac_488, aga/cac_280, atc/caa_175, aga/cag_320	*F.* × *ananassa*	8	Lerceteau-Köhler *et al.* 2005
		SCAR	STS-Rca2_417, STS-Rca2_240			
EV	Everbearing	RAPD	OPE07-1, OPB05-1	*F.* × *ananassa*	8	Sugimoto *et al.* 2005

Markers linked to major genes

Genome mapping and molecular breeding in strawberry was recently reviewed by Davis *et al.* (2007). The number of markers linked to genes encoding for agronomical important traits in wild and cultivated strawberry is low. Several examples are given in **Table 1**. In the wild, diploid strawberry *F. vesca* the yellow fruit locus *c* was recently mapped by Deng and Davis (2001) using a candidate gene mapping approach. Intron-containing segments of different structural genes of the flavonoid biosynthetic pathway coding for chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR) and anthocyanidin synthase (ANS) were cloned, sequenced and mapped. Furthermore, the *RAN* (regulation of anthocyanin biosynthetic pathway) factor, a *Del*-like regulatory gene, was also mapped. An association was found between the *c* locus ad the *F3H* gene, which is located at the bottom of linkage group I (Deng and Davis 2001). The gene *SEASONAL FLOWERING LOCUS* (*SFL*) was recently mapped in *F. vesca* by Cekic *et al.* (2001) and Albani *et al.* (2004). Based on results observed by physiological observations it was suggested that the *SFL* gene seems to be a floral repressor which is inactivated under short day conditions and cool temperatures in the autumn, but reactivated by winter cold (Battey *et al.* 1998; Albani *et al.* 2004). The *SFL* gene controls the restriction of the flowering period from late April until early June. Consequently, the *SFL* gene is responsible for the seasonal switch from generative to vegetative growth, which is one of the most important differences between annual and perennial plants. A candidate gene based cloning strategy using information available from annual model plants like *Arabidopsis thaliana* appears to be difficult (Albani *et al.* 2004). However, the *FLOWERING LOCUS C* gene of *Arabidopsis* is one, but not the only one, possible candidate. Three major genes (*r*, *s* and *pg*) coding for the morphological traits non-runnering, perpetual flowering and pale-green leaf were recently mapped by Sargent *et al.* (2004) in a cross between *F. vesca* f. *semp.* and the diploid species *F. nubicola*. Flanking SSR markers are available now for each of the three genes (**Table 1**).

In *F.* × *ananassa*, tightly linked RAPD or AFLP markers for the red stele resistance genes *Rpf1*, *Rpf3* and *Rpf6* have been identified by Haymes *et al.* (1997) and Hokanson and Maas (2001). SCAR markers linked to *Rpf1* were also described (Hokanson and Maas 2001; Rugienius *et al.* 2006). These SCAR markers have successfully been used for selection of crossbred parents in practical strawberry breeding programs by Rugienius *et al.* (2006) and Gelvonauskiene *et al.* (2007).

The everbearing locus, which seems to be regulated by a dominant major gene in a Japanese *F.* × *ananassa* breeding population (Ahmadi *et al.* 1990; Monma *et al.* 1990; Igarashi *et al.* 1994), was recently mapped by Sugimoto *et al.* (2005). The two RAPD markers OPE07-1 and OPB05-1 are the closest linked markers found which flank the everbearing locus with a distance of 11.8 and 15.8 cM (Sugimoto *et al.* 2005).

Linkage mapping in diploid strawberry

First linkage analyses in strawberry have been performed in diploid strawberry. Isozyme markers were linked to morphological traits like the yellow fruit color of the *F. vesca* cultivar 'Yellow Wonder' (Williamson *et al.* 1995) or non-runnering (Yu and Davis 1995). The first linkage map was also performed in *F. vesca* (Davis and Yu 1997). This map consisted of 75 RAPD and 5 isozyme markers. The map spanned a map distance of 445 cM with a total of seven linkage groups. A second map was constructed by Sargent *et al.* (2004) using an interspecific cross *F. vesca* x *F. nubicola*. This map was the first strawberry map which is based on SSR markers. The map was 448 cM in length and nearly identical to the map described by Davis and Yu (1997). Sargent *et al.* (2006b) added new SSR markers and expanded this map to a reference map with a total of 182 markers.

Linkage mapping in octoploid strawberry

The identification of RAPD markers linked to the *Rfp1* gene for resistance to *Phytophthora fragariae* by Haymes *et al.* (1997) was the first report on linkage in octoploid strawberry. Two out of these RAPD markers were subsequently converted to SCAR markers and shown to be closely linked to *Rpf1* (Haymes *et al.* 2000). The first map for the octoploid *F.* × *ananassa* was recently published by Lerceteau-Köhler *et al.* (2003). A total of 113 full-sib progeny from a cross between the variety 'Capitola' and the clone CF1116 were screened with AFLP markers. Based on these markers two parental maps were separately constructed with a total length of 1,604 and 1,496 cM, respectively. The female map consisted of 235 markers distributed on 30 linkage groups. The male map consisted of 280 markers located on 28 linkage groups (Lerceteau-Köhler *et al.* 2003). Another map was published by Weebadde *et al.* (2008). This map was generated with a population of 127 lines originated from a cross between the day-neutral cultivar 'Tribute' and the short-day cultivar 'Honeoye'. The population was screened

with AFLP markers and 429 single dose restriction fragments (SDRF) were placed on a consensus map of 1,541 cM and 43 linkage groups. A total of eight QTL were found for day-neutrality. None of these QTL explained more than 36% of the phenotypic data. Based on the results obtained it was concluded that day-neutrality is likely a polygenic trait (Weebadde *et al.* 2008).

Genetic mapping of sex determination in the octoploid, subdioecious wild strawberry, *Fragaria virginiana*, was recently published by Spigler *et al.* (2008, 2010). This SSR marker based map has a total length of 2,373 cM and includes 212 markers on 42 linkage groups. Results obtained in this study supported the model of gender determination in subdioecious *F. virginiana* with at least two linked loci with major effects. Both sex expression traits (female fertility and male sterility) were mapped on linkage group 41 (LG 41, VI.C-m) with a genetic distance of about 6 cM (Spigler *et al.* 2008, 2010). Spigler and co-workers adduced evidence for recombination between the sex determining loci and concluded that *F. virginiana* is an example for the youngest sex chromosomes (proto-sex chromosomes) in plants.

Sargent *et al*. (2009) recently published a linkage map developed from 174 F1 seedlings derived from a cross between the *F.* × *ananassa* cultivars 'Redgaunlet' and 'Hapil'. This map spans a total length of 3,116 cM with 69 linkage group fragments and consists of 218 SSR markers, 11 gene specific markers and 86 AFLP and RAPD markers. The female map covers 1,675.3 cM with 32 linkage groups. The male map has a total length of 1,440.7 cM with 37 linkage groups. Based on transferable SSR or gene-specific markers all linkage groups could be identified as homologous to one of the seven diploid linkage groups of *Fragaria* (Sargent *et al.* 2009). When marker order was compared to the diploid reference map, complete colinearity was observed. Seven homoeologous linkage groups for *F.* × *ananassa* were also identified by Rousseau-Gueutin *et al*. (2008). The strawberry comparative map revealed also a high level of colinearity between diploid and octoploid *Fragaria* species. The extensive genome conservation between diploid and octoploid strawberry supports the use of diploid *Fragaria* as a model system for studying genomics and molecular dissection of the octoploid species (Rousseau-Gueutin *et al.* 2008). Furthermore, the authors demonstrated that disomic behaviour is predominant in *F.* × *ananassa*, which makes genetic linkage mapping and gene isolation easier as expected for an octoploid genotype.

Synteny to other Rosaceous crops

Synteny between the genomes of *Fragaria* (berry fruits) and *Prunus* (stone fruits) was studied by Vilanova *et al.* (2008). The synteny found in this study seems to be sufficient to allow the transfer of information on marker or gene or QTL position from one of these species to the other. All the information which is now available in peach for example can be used to improve the knowledge about strawberry genetics. On the other side, the diploid model *F. vesca* with its small genome size, the rapid life cycle, the high number of seeds per cross and the small plants may become a very efficient organism for reverse genetics and other genomics applications that may provide useful information for other *Rosaceous* crops (Shulaev *et al.* 2008; Vilanova *et al.* 2008).

Mapping of quantitative traits

Only a few QTL studies have been published to date in strawberry (Davis *et al.* 2007). In diploid strawberry QTLs for different vegetative and reproductive traits have been detected (Sargent *et al.* 2006a). In octoploid strawberry QTLs for fruit quality, for sugar and organic acids, for resistance to *P. cactorum* and *P. acutatum* and day-neutrality were described (Denoyes-Rothan *et al.* 2004; Lerceteau-Köhler *et al.* 2004, 2006; Weebadde *et al.* 2008).

Fruit flavor

The cultivated strawberry *F.* × *ananassa* is one of the most favoured fruit crops because of its attractive fruits and its excellent taste. Intensive research has been made to identify substances contributing to the flavour. Beside sugars and acids more than 360 volatile compounds have been reported, and 15 to 20 out of them were found to be important for sensory perception.

However, the sensory quality of economical important strawberry cultivars is often criticized by consumers. Especially the typical “woodstrawberry-like flavour”, which is preferred by consumers, is missing in traded cultivars. This flavour is typical for the diploid wood strawberry *F. vesca*, but normally not present in octoploid cultivars of the cultivated strawberry *F.* × *ananassa*. Only a few cultivars, like the old German cultivar 'Mieze Schindler', have a comparable flavour. On this account much research has been done to identify key substances which contribute significantly to a better taste. Zabetakis and Holden (1997) compared the spectrum of volatiles in fruits of the wood strawberry *F. vesca* and *F.* × *ananassa* cv. 'Elsanta'. The authors found reduced levels of 2,5-dimethyl-4-hydroxy-2H-furan-3-one (DMHF) and 2,5-dimethyl-4-methoxy-2H-furan-3-one (mesifuran) in 'Elsanta' whereas the total amount of these two furanones was much higher in *F. vesca*. Furthermore, they showed that the ratio of DMHF (total)/ mesifuran is much smaller in the cultivated strawberry. Zabetakis and Holden (1997) concluded that the absolute amount of and the ratio between these two furanones may be responsible for the perception of wild strawberry flavour as more “strawberry-like”. Pyysalo *et al.* (1979) identified five esters (3-methyl-2-butenyl acetate, carveyl acetate, methyl nicotinate, methyl anthranilate and methyl *N*-formylanthranilate) in *F. vesca* which were not described in strawberry before. These esters were present in *F. vesca*, but not in *F.* × *ananassa* cv. 'Senga Sengana', except for 3-methyl-2-butenyl acetate. Methylanthranilate (MA) has been subsequently identified by Ulrich *et al*. (1997) as the semivolatile compound that is responsible for the “woodstrawberry-like flavour” in *F. vesca*. Ulrich and co-workers examined 27 European strawberry cultivars and 20 wild species. Based on their results the authors suggested that strawberries can be divided into three different aroma types (**Fig. 4**). The first type is the “MA-containing type”. The MA-free strawberry genotypes can be divided into sensorial pleasant “ester-types” and the less pleasant “DHF (2,5-dimethyl-4-hydroxy-3(2*H*)-furanone)-types”. Regrettably, high levels of DHF were also found in MA-containing types (Urruty *et al.* 2002). Based on this fact it is assumed that only the presence of a single substance like MA cannot be the key to a better taste. It seems rather that the “woodstrawberry-like flavour” is a mixture of different compounds in a well balanced ratio. This fact and the fact that MA is only inherited to one-third of the offspring (Olbricht *et al.* 2008) make breeding of tasty strawberry cultivars much difficult.

Recently, a candidate gene has been identified which obviously may have a significant role in ester production of *F. chiloensis* fruit. Gonzalez *et al.* (2009a) studied the volatile compounds during development and fruit ripening in F. *chiloensis*, which is known to have great aroma and flavour properties. They found that the esters, butyl acetate, ethyl acetate, ethyl butanoate and ethyl hexanoate, are the most abundant volatiles in fully ripe fruit. Esters are known to be synthesized by alcohol acyltransferases (AATs) and Gonzales *et al*. (2009b) were able to isolate a full-length cDNA (*FcAAT1*) from *F. chiloensis* fruit which displayed the three motifs characteristic of most alcohol acyltransferases (AATs). The authors showed that the level of *FcAAT1* transcripts increased during fruit ripening and they found a good correlation between AAT activity and the total content of esters. Based on their results it was concluded that the *FcAAT1* gene may have a significant role in ester production of *F. chiloensis* fruit.

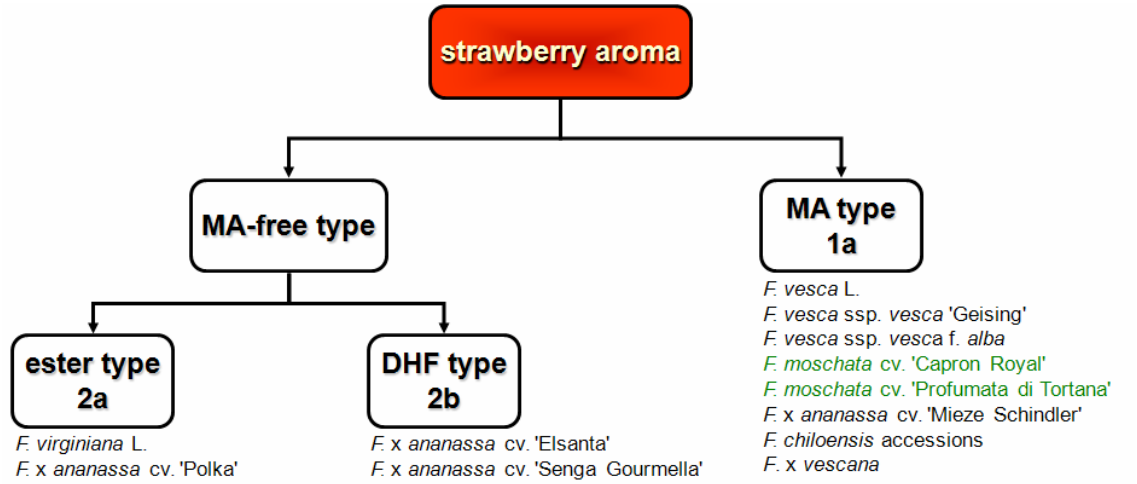

Fig. 4 Aroma types of strawberry according to Ulrich *et al.* (1997). Different examples published by Urruty *et al.* (2002) and Olbricht *et al.* (2008) were added. MA methylanthranilate, DHF 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone; Cultivars with high levels of MA and DHF are written in green.

Genes affecting vegetative growth and flowering time

Differentiation of long (runners) and short shoots (crown branches) is an important developmental trait in strawberry. Recently, it has been suggested by Hytönen *et al.* (2009) that gibberellin (GA) may play a role in regulation of shoot differentiation. Based on their results Hytönen *et al.* (2009) concluded that GA is needed for runner initiation in strawberry. Furthermore, they found that inhibition of GA biosynthesis leads to the formation of crown branches. The authors were able to show that GA plays a role also in the photoperiod-regulated differentiation of axillary buds. Beyond that GA seems to delay the growth of strawberry plants.

Recently, the *FaGAST* gene which affects plant growth has been isolated and identified by de la Fuente *et al.* (2006). This gene was classified because of its high homology to the tomato *GAST1* (*GA Stimulated Transcript 1*) gene. *FaGAST* encodes a small protein with 12 cysteine residues conserved in the C-terminal region similar to a group of proteins identified in other species with diverse assigned functions such as cell division, elongation, or elongation arrest. The *FaGAST* gene was found to be differentially expressed in the receptacle of strawberry fruits and in roots. Its expression in fruit increases at two ripening stages in which fruit growth slows down. Constitutive overexpression in transgenic plants of *F. vesca* and *Arabidopsis* supported its role in cell elongation arrest (de la Fuente *et al.* 2006). Transgenic plants of both species were retarded in their growth. Beside the morphological changes such as smaller leaves *FaGAST* also affected flowering time. In contrast to non-transformed control plants most transgenic lines of *F. vesca* failed to flower during the first season. Furthermore, the size of transgenic strawberry fruits was reduced. Overexpression of *FaGAST* in *F. vesca* reduced the size of strawberry fruits. The expression profile, the regulation of *FaGAST* by gibberellin, and the results obtained by transgenic overexpression in *F. vesca* and *Arabidopsis* suggest a role of *FaGAST* in arresting cell elongation during strawberry fruit ripening. How this protein is able to delay the growth of strawberry plants and how it may interfere with the GA response is not clear at the moment. Therefore further research is needed.

Genetic regulation of flowering time in strawberry is poorly understood. In seasonal flowering genotypes of the diploid wild strawberry *F. vesca* flowering is controlled by short-days (SD) and low temperatures (Sønsteby and Heide 2008). Flowering induction occurs under SD conditions and low temperatures, whereas long-day conditions (LD) and high temperatures inhibit flower development. The same is true for seasonal flowering types of the octoploid *F.* × *ananassa*. Flowering induction is controlled by SD and low temperature. However, flowering of several strawberry genotypes occurs at low temperatures and independently of photoperiod (Heide 1977; Konsin *et al.* 2001; Sønsteby and Heide 2006, 2007). In everbearing genotypes, the regulation of flowering is not well understood. They are mostly classified as day-neutrals (Darrow 1966; Guttridge 1985). A quantitative LD response of flowering that increased in strength with increasing temperature was recently found for the everbearing diploid *F. vesca* ssp. *semperflorens* cultivars 'Rügen' and 'Baron Solemacher' (Sønsteby and Heide 2008). A comparison with the everbearing *F.* × *ananassa* cultivar 'Elan' demonstrated an identical reaction to LD conditions and increasing temperatures. Based on these facts it was concluded that a similar flowering control system is present in diploid *F. vesca* and octoploid *F.* × *ananassa* strawberry. In a recent study a number of strawberry genes homologous to flowering genes of *Arabidopsis* have been identified by Mouhu *et al.* (2009). A total of 66 genes with sequence homology to known genes of all flowering pathways have been identified by EST sequencing and bioinformatics. The expression of 25 selected genes was analyzed. The selected genes are representatives of various flowering pathways. No significant differences in expression of these genes were found between everbearing and short-day strawberry genotypes. The everbearing cultivar 'Baron Solemacher' has recessive alleles of an unknown repressor designated as *SEASONAL FLOWERING LOCUS* (*SFL*). The recessive alleles of *SFL* are responsible for continuous flowering habit.

First insights into regulation of fruit color

Strawberry is a strongly colored fruit and fruit color is one of the most important fruit quality parameters. The fruit color is exclusively caused by one individual group of flavonoids, the anthocyanidins. A schematic application of the strawberry flavonoid biosynthesis is given in **Fig. 5**. The main pigments of strawberry (*F.* × *ananassa*) fruits are derivatives of the anthocyanidins pelargonidin and cyanidin (Goiffon *et al.* 1999; Nyman and Kumpulainen 2001; Andersen *et al.* 2004). Pelargonidin derivatives are bright red colored. They are the predominant pigments. Cyanidin derivatives are minor pigments which are dark red colored. Additionally, flavonols (derivatives of kaempferol and quercetin) are formed, which certainly serve as co-pigments (Henning 1981; Hertog *et al.* 1992; Häkkinen and Auriola 1998; Häkkinen and Törrönen 2000; Olsson *et al.* 2004).

Interestingly, strawberry fruits produce another prevalent group of flavonoids during early stages of fruit development. In unripe fruits, large amounts of the 3',4'-hydroxylated flavan 3-ols catechin, epicatechin and the derived proanthocyanidins are accumulated (Macheix *et al.* 1990;

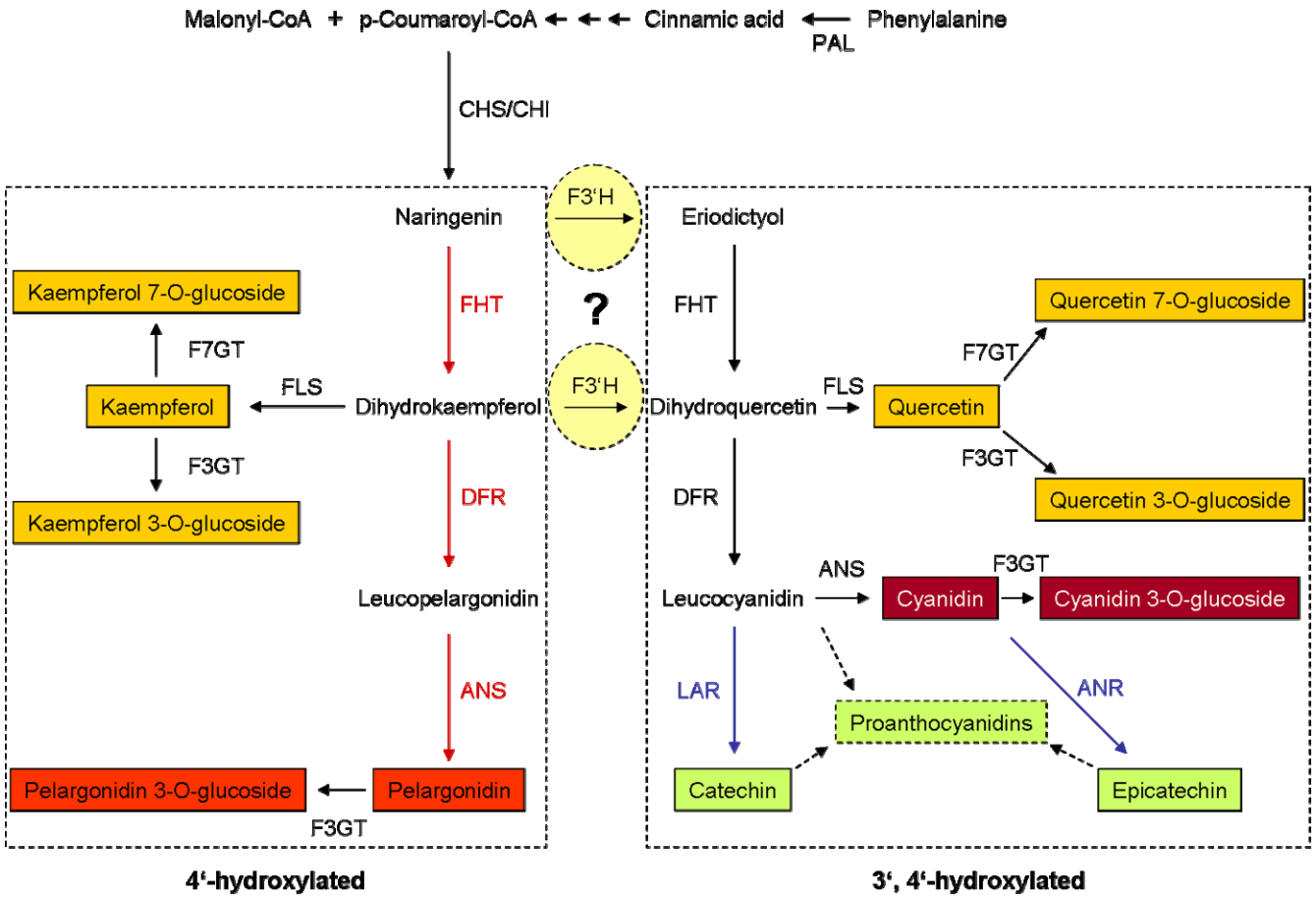

Fig. 5 Simplified flavonoid pathway in strawberries. PAL phenyalanine ammonia lyase, CHS chalcone synthase, CHI chalcone isomerase, F3'H flavonoid 3' hydroxylase, FHT flavonone 3-hydroxylase, FLS flavonol synthase, DFR dihydroflavonol 4-reductase, ANS anthocyanidin synthase, F3GT flavonoid 3-*O*-glucosyltransferase, F7GT flavonoid 7-O-glucosyltransferase, LAR leucocyanidin reductase, ANR anthocyanidin reductase, dark red - minor pigments in ripe fruits, bright red – prevalent pigments in ripe fruits, dark yellow – co-pigments in ripe fruits, green – prevalent flavonoids during early stages of fruit development, red arrows – enzymes with preference for 4'-hydroxylated flavonoids, blue arrows – enzymes with preference for 3',4'-hydroxylated flavonoids.

Halbwirth *et al.* 2006). They are supposed to serve in the restriction of plant pathogen development, like gray mold (*Botrytis cinerea*), which infects the strawberry flower but keeps quiescent until fruit ripening (Schlösser 1994). Furthermore, astringent flavonoids in pale unripe fruits vs. attractive colored ripe fruits with low astringency and high sugar content refer to an evolutionary process for enhanced seed-dispersal by animals. Unripe fruits with immature seeds are not intended to be eaten, whereas mature seeds should be dispersed.

The different flavonoid classes formed during different stages of fruit development seem to fulfill different important functions. A two-phase and dual mode flavonoid biosynthesis in strawberry is therefore assumed (Halbwirth *et al.* 2006). First indications found by Cheng *et al.* (1991) and Moyano *et al.* (1998) were recently confirmed by Halbwirth *et al.* (2006) who showed that nearly all main enzymes of the polyphenol pathway show two peaks during strawberry fruit development. The chalcone synthase/chalcone isomerase (CHS/CHI) showed the highest activity at stage 1 (small-sized 0.7 cm green fruits). Subsequently, the activity decreased. A second increase was found at stage 6 (full-ripe red fruits with 2.5 cm fruit size). Two activity peaks at stages 2 (middle-sized 1.5 cm green fruits) and 6 were found for phenyalanine ammonia lyase (PAL), flavonone 3-hydroxylase (FHT), dihydroflavonol 4-reductase (DFR), flavonoid 3-*O*-glucosyltransferase (F3GT) and flavonoid 7-*O*-glucosyltransferase (F7GT). In contrast, no flavonol synthase (FLS) activity could be detected in the early stages of fruit development. The FLS reached its maximum at stage 5 (turning-stage fruits with 2.5 cm fruit size).

Such a specialized flavonoid biosynthesis has to be tightly regulated during fruit development. Early biosynthetic steps catalyzed by CHS, CHI, FHT and DFR are common to the formation of 4'-hydroxylated and 3',4'-hydroxylated flavonoids, but in some strawberry cultivars FHT and DFR were shown to prefer 4'-hydroxylated substrates (Almeida *et al.* 2007). Subsequent biosynthetic pathways are mostly divergent. It may be assumed that this dual-mode of flavonoid biosynthesis is regulated by different types of transcription factors (TFs) as in other plants (Winkel-Shirley 2001) where it was shown that anthocyanin and flavonol biosynthesis are regulated by a specific subgroup of R2R3-MYB type TFs. Whereas biosynthesis of flavonols can be activated by a R2R3-MYB TF without any co-activator, the synthesis of anthocyanins and proanthocyanins requires the physical interaction of the R2R3-MYB regulator with a basic helix-loop-helix (bHLH) co-activator. In addition to the R2R3-MYB and bHLH regulators a subgroup of WDR (WD-repeat) proteins participates also in the regulation of anthocyanin genes by blocking negative regulators of the bHLH coactivator (**Fig. 6**).

For strawberry, the MYB-type TF *FaMyb1* was identified and its expression increases in developing fruits in parallel to anthocyanidin synthesis (Almeida *et al.* 2007). In transgenic tobacco *FaMYB1* acts as a repressor of pigmentation (Aharoni *et al.* 2001). Recent results obtained in the white-fruited Chilean strawberry *Fragaria chiloensis* argue also for the presence of a repressive transcription factor, which inhibits several structural genes of the flavonoid biosynthesis (Saud *et al.* 2009). Therefore, it is assumed that *FaMyb1* could serve as a repressor of pigmentation in strawberry fruits. Recently, the R2R3-MYB type TF *FaMYB10* (EU155162) has been identified. The *FaMYB10*

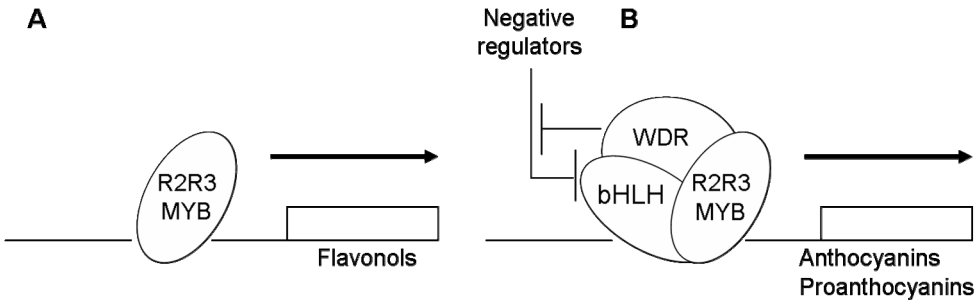

Fig. 6 Simplified model of the flavonol (**A**) and anthocyanin/proanthocyanin biosynthesis (**B**) in accordance to Quattrocchio *et al.* (2006).

transcription factor seems to be homolog to *MdMYB10* transcription factor of apple. The *MdMYB10* transcription factor is one of three nearly identical R2R3-MYB TFs which were published to be involved in the regulation of red coloration in apple (Takos *et al.* 2006; Ban *et al.* 2007; Espley *et al.* 2007). Unfortunately, nothing is known about the function and the expression of *FaMYB10* in strawberry.

Recently, it was found that allergens could also have a regulatory function in pigment formation. *Fra a* 1, the homologous gene to the birch pollen allergen *Bet v* 1, was shown to be down-regulated in four colorless (white) strawberry mutants (Hjerno *et al.* 2006). Different enzymes of the flavonoid biosynthetic pathway, such as chalcone synthase, dihydroflavonol reductase, flavanone 3-hydroxylase, and methyltransferase were also down-regulated in one and four out of the colorless mutants, respectively. The transient RNAi-mediated silencing of the *Fra a* gene in strawberry fruits of the red-fruited cultivar 'Elsanta' with an ihpRNA construct resulted in a reduced production of anthocyanins and upstream metabolites. This effect is consistent with the parallel down-regulation of the phenylalanine ammonia lyase (*FaPAL*) and to a lesser extent of the chalcone synthase (*FaCHS*) transcript levels in these fruits (Muñoz *et al.* 2010). The results obtained by Muñoz *et al.* (2010) demonstrated that *Fra a* expression is directly linked to flavonoid biosynthesis and argue for an essential biological function of the *Fra a* allergen(s) in pigment formation in strawberry fruit.

It is to note that the phenolic composition of native strawberry species differs significantly from that of *F.* × *ananassa*. Simirgiotis *et al.* (2009) compared the phenolic composition of forms *chiloensis* and *patagonica* of *F. chiloensis* with that of the *F.* × *ananassa* cv. 'Chandler'. Proanthocyanidins, hydrolysable tannins, anthocyanins, and flavonol glycosides were found to be the main phenolic constituents in all three species. The main flavonol glycoside of the two native species was quercetin 3-*O*-glucuronide. The minor anthocyanins identified were cyanidin-malonyl-glucoside and pelargonidin-malonyl-glucoside. The highest amount of anthocyanin was found in the *F.* × *ananassa* cultivar whereas the main phenolic in the native white strawberry was ellagic acid.

Fragaria vesca - an ideal model for functional genomics in *Rosaceous* plants

F. × *ananassa* is an accidental hybrid of *F. chiloensis* and *F. virginiana* that arose in mid-1700s. The cultivated strawberry has 56 chromosomes and the genomic complement is likely AAA'A'BBB'B' (Bringhurst 1990). The diploid woodland strawberry (*Fragaria vesca*) is probably being the A genome donor (Hancock *et al.* 2008b). In recent time *F. vesca* has emerged as an attractive system for structural and functional genomics due to its many favourable features (Folta and Davis 2006; Slovin *et al.* 2009). Plants of *F. vesca* are very small, but compact enough to be grown in a small scale in a laboratory. About 100 plants can be grown per square meter. Furthermore, *F. vesca* plants are easy to propagate; their flowers are self-compatible and produce more than 2,500 seeds per plant (Darrow 1966). Very attractive for genetic studies is the short life cycle, which is approximately 10-16 weeks and allows the production of several seed generations per year (Shulaev *et al.* 2008). The genome of *F. vesca* is with 206 Mbp one of the smallest plant genomes and a first genomic sequence is available (Shulaev *et al.* 2008; Pontaroli *et al.* 2009; Shulaev *et al.* 2011). The establishment of different molecular and biotechnological tools such as PCR and marker techniques (Hancock *et al.* 2008b; Shulaev *et al.* 2008), transient assays for reverse genetics (Agius *et al.* 2005; Hoffmann *et al.* 2006; Griesser *et al.* 2008) and protocols for the production of stable transformed plants (Folta and Davis 2006; Quesada *et al.* 2007; Qin *et al.* 2008) led to the fact that strawberries have emerged to a model species within the *Rosaceae* family. In a recently published paper Slovin and co-workers (2009) suggested the use of inbred lines of *F. vesca* f. *semperflorens* var. 'Yellow Wonder' for downstream genetic analyses or assessment of gene function. Seeds designated as 'Yellow Wonder' are commercially available from several commercial sources and listed by the United States National Clonal Germplasm Repository. Plants of 'Yellow Wonder' are day neutral, do not runner, and have yellow fruit colour. These three traits have been analyzed genetically in *F. vesca* and shown to be encoded by recessive genes (Brown and Wareing 1965; Williamson *et al.* 1995; Battey *et al.* 1998), and efforts were being made to clone the responsible genes.

IN VITRO CULTURE AND GENETIC ENGINEERING

Initial developments in strawberry biotechnology were reviewed by Hokanson and Maas (2001). The latest and very comprehensive review on strawberry culture *in vitro* was published by Debnath and Teixeira da Silva (2007).

Micropropagation

Micropropagation in strawberry was introduced about 35 years ago by Boxus (1974). Strawberry was one of the first horticultural species which was available for micropropagation in tissue culture labs, research stations, and nurseries. The advantages of *in vitro* propagation in strawberry are: pathogen-free propagation of plants independent from field conditions and damage by soil fungi or viruses; high number of propagules from a definite mother plant; plants are valuable for storage under cold conditions (ideal for germplasm preservation); more vigorous growth of tissue-culture derived plants, producing more crowns and runners, yield and number of inflorescences per crown. Tissue culture plants in strawberry are derived from pre-existing axillary buds through shoot proliferation following shoot morphogenesis to retain the genetic composition of the mother plant. Single meristems can be also used as shown by Morel (1960) for meristem culture in orchids aimed on virus elimination. Since that time meristem culture alone or in combination with heat treatment is widely used to obtain virus and fungus free strawberry plants. Most common is to use the meristem from the runner tips. A detailed description of the methodology of meristem culture, virus elimination, and shoot proliferation using different type of explants is given

by Debnath and Teixeira da Silva (2007). Strawberry micropropagation is widely used in commercial laboratories worldwide.

In the last years attempts were made to use large-scale liquid cultures in a bioreactor system as potential to resolve the manual handling of the various stages of micropropagation and to increase shoot multiplication *in vitro* (Debnath 2008, 2009).

Adventitious shoot regeneration and somatic embryogenesis

A necessary precondition for the genetic manipulation in strawberry is an efficient regeneration system that would provide the means for recovering genetically modified plants following *Agrobacterium*-mediated or other methods of gene transfer. In general, there are two ways of plant regeneration: through somatic embryogenesis or through organogenesis by adventitious shoot regeneration. The development of *in vitro* regeneration systems based on adventitious shoot regeneration using a range of explants, and culture conditions was summarized by Mercado *et al.* (2007b). Adventitious shoot regeneration in strawberry is very efficient and has been demonstrated using leaves, petioles, stipules, stem tissue, runner tissue, the peduncular base of flower buds, roots, runners and immature embryos. A detailed review on methodology is given by Debnath and Teixeira da Silva (2007). However, to carry out transformation experiments, efficient levels of regeneration are necessary for a range of commercial strawberry cultivars. A comparison of the genetic background in seven strawberry cultivars suggested a strong genetic component amongst the different cultivars determining their regeneration capacity (Passey *et al.* 2003). Recently, Husaini and Abdin (2007) reported on shoot regeneration in the strawberry cv 'Chandler' which has been achieved simultaneously through both somatic embryogenesis and shoot bud formation. There was a synergistic effect of photoperiod, dark, and chilling treatments on somatic embryogenesis, whereas chilling treatment had an inhibitory effect on shoot organogenesis. Utilization of somatic embryogenesis in strawberry is in a preliminary stage.

Other tissue culture systems

There is a range of tissue culture systems in strawberry which were developed in the last three decades aimed on regeneration of plants for true-to-type propagation of mother genotypes or for breeding purposes.

There are a few reports on the development of protoplast culture system in strawberry aimed on direct gene transfer of foreign DNA, on somatic hybridization based on fusion of two different strawberry genotypes not-crossable in nature, and/or on exploitation of somaclonal variation which could occur in a population of protoplasts. A reproducible and efficient protoplast to plant-system has been developed by Nyman and Wallin (1992) using leaf- and petiole-derived protoplasts. The regenerated plants were octoploid, hexadecaploid, diplodecaploid, and mixoploid. However, to our knowledge the protoplast system in strawberry was not really introduced into pre-breeding possibly due to its fragility concerning isolation and regeneration methods and low efficiency of plants regeneration. The only report on protoplast fusion between *F.* × *ananassa* and *F. vesca* is given by Wallin (1997).

The production of haploid plants by anther or microspore culture is widely used for breeding strategies in other plant species, i.e. cereals. However, the application of anther culture to strawberry was less successful. Plant regeneration was obtained by Niemirowicz-Szczytt *et al.* (1983) and Owen and Miller (1996), but this technique was also never used in the pre-breeding process.

The occurrence variation in plants regenerated from *in vitro* cultures is named 'somaclonal variation'. Somaclonal variation is not desirable for commercial micropropagation, however it could be a valuable tool in plant breeding to obtain genotypes with altered traits. There are a few publications in strawberry where such type of genetic changes were obtained, as modified white colour of fruit flesh, less susceptibility to soil borne fungi, earliness, modified calyx separation, less mildew susceptibility, chlorophyll mutants, dwarfism, and ploidy level changes (reviewed in Debnath and Teixeira da Silva 2007).

Cold storage and cryopreservation

Shoot-proliferating cultures of strawberry can be stored *in vitro* for months under cold conditions. The first report on this technique was published in 1976 (Mullin and Schlegel 1976). Later the technique was improved by adaption of culture media, storage bags and vessels, and pre-culture and culture conditions (Reed 1992, 2002). The low-temperature storage is now a powerful tool in germplasm preservation and it is used especially in laboratories of national genebanks to build up a secondary backup.

In the last years cryopreservation methods were developed also for strawberry. Cryopreservation has the following advantages compared to low temperature storage: the storage can be extended in duration from months to years and is theoretically unlimited which saves time and labour for handling the cultures; the space for maintaining the cultures is low and a higher number of genotypes can be stored. The first report was on cryopreservation of alginate-coated meristems from in vitro-grown strawberry following dehydration by a vitrification solution (Hirai *et al.* 1998). Later the technique was improved. Successful cryopreservation protocols for strawberry are reported using PVS2 vitrification (Niino *et al.* 2003; Pinker *et al.* 2009), encapsulation technique (Hirai *et al.* 1998; Clavero-Ramirez *et al.* 2005) and control rate cooling (Reed and Hummer 1995). Average recoveries of 61% for 19 cultivars and of 30% for six wild species were determined by Höfer and Reed (2011) using PVS2 vitrification. The experiments showed that the cryopreservation approach is applicable for building a long term back up collection for a genebank.

Genetic transformation

A summary on genetic engineering in strawberry was recently published by Hanke and Flachowsky (2010). The cultivated strawberry is due to its facile vegetative and generative propagation an ideal subject for genetic transformation. Although traditional breeding methods have achieved steady improvement in agronomic traits, the lack of useful economic characters still remains a major challenge. The state of the art in strawberry transformation was reviewed by several authors (Graham 2005; Folta and Dhingra 2006; Folta and Davis 2006; Quesada *et al.* 2007; Debnath and Teixeira da Silva 2007; Qin *et al.* 2008). Enormous advances have been made in strawberry genetic transformation since the first transgenic plants were obtained in 1990 by two independent groups (Nehra *et al.* 1990; James *et al.* 1990). Besides *F.* × *ananassa*, transformation systems were also developed for related species, like *F. vesca* (El-Mansouri *et al.* 1996; Haymes and Davis 1998; Alsheikh *et al.* 2002; Zhao *et al.* 2004; Oosumi *et al.* 2006) and *F. moschata* (Mezzetti *et al.* 2002).

The most important approach in strawberry relies on *Agrobacterium tumefaciens*-mediated leaf disk transformation (Mathews *et al.* 1995; Puite and Schaart 1996; Martinelli *et al.* 1996; du Plessis and Brand 1997; Barcelo *et al.* 1998; Ricardo *et al.* 2000; Mezzetti 2003; Gruchala *et al.* 2004). Direct gene delivery into protoplast by electroporation was also reported (Nyman and Wallin 1988). A combined *Agrobacterium*-biolistic method was described later (Cordero de Mesa *et al.* 2000). A new methodology to produce transgenic strawberries was developed using a temporary immersion bioreactor system (Hanhineva and Karenlampi 2007). Regardless of the sufficient regeneration levels achieved from leaf explants, the regeneration of trans-

formed strawberry plants remains difficult and seems to be strongly genotype dependent. Since the 1990's, reliable protocols using *Agrobacterium tumefaciens*-mediated transformation were established for several commercial cultivars. A detailed survey of literature is given by Mezzetti (2003) and Mezzetti and Constantini (2006). Recently, a high efficiency protocol for *Agrobacterium*-mediated transformation was published by Husaini (2010). The effective production of marker-free transgenic strawberry plants using inducible site-specific recombination and a bifunctional marker gene was described by Schaart (2004). There are several promoter studies in strawberry. A tissue specific expression using the floral binding protein 7 promoter from *Petunia* was used by Schaart *et al*. (2002). Transgene expression driven by a heterologous phloem-specific promoter was published by Zhao *et al.* (2004). Agius *et al.* (2005) used a transient expression system to conduct a functional analysis of homologous and heterologous promoters in fruit. A near root-specific promoter was described recently (Vaughan *et al.* 2006).

Transformation studies in strawberry are focused on modification of several traits, like insect resistance (Graham *et al.* 2002), virus resistance (Finstad and Martin 1995), fungal resistance (Chalavi *et al.* 2003; Schestibratov and Dolgov 2005; Vellicce *et al.* 2006; Mercado *et al.* 2007a), herbicide resistance (du Plessis and Brand 1997; Morgan *et al.* 2002), abiotic stress (Owens *et al.* 2002; Wang *et al.* 2004; Houde *et al.* 2004; Owens 2005; Khammuang *et al.* 2005; Husaini and Abdin 2008), fruit quality and development (Mathews *et al.* 1995; Jiménez-Bermúdez *et al.* 2002; Mezzetti *et al.* 2002, 2004; de la Fuente *et al.* 2006; Lunkenbein *et al.* 2006; Palomer *et al.* 2006; Park *et al.* 2006; Santiago-Domenech *et al.* 2008), and plant morphology (Wawrzynczak *et al.* 2005) (reviewed in Hanke and Flachowsky 2010). There are some newer reports on following topics: Recently, the plant secondary metabolism was changed using the gene encoding stilbene synthase from grape. The changed metabolite profile suggested that chalcone synthase was down-regulated by genetic modification. Changes in the levels of phenolic compounds led to increased susceptibility of the transgenic strawberry to grey mould fungus (Hanhineva *et al.* 2009). Further studies were focused on fruit softening during ripening. The ripening-related polygalacturonase genes *FaPG1* which plays an important role during ripening was up-regulated. Most of the transgenic lines showed an increase in soluble solid content and yielded fruits significantly firmer than did the control. These results indicate that *FaPG1* plays a central role in strawberry softening (Quesada *et al.* 2009). Youssef *et al*. (2009) reported on transgenic strawberry lines a fruit specific strawberry pectate lyase gene (*FaplC*) to evaluate the role of this gene on fruit softening. Ripen fruits showed a significant down-regulation of *FaplC.* The agronomic behaviour of transgenic plants was evaluated during two conescutive years. Fruit set increased in the two transgenic lines when compared with control plants. Firmness of full ripen fruits was significantly higher than control fruits, while color and soluble solids were not affected. The increase of firmness was maintained when ripe fruits were stored for 3 days at 25°C. Histological analysis of ripe fruits showed lower intercellular spaces and a higher degree of cell to cell contact area in transgenic fruits. Altogether, these results suggest a direct relationship between pectate lyase gene expression and strawberry fruit softening. There are also other reports on postharvest behavior of transgenic strawberry with polygalacturonase or pectatlyase genes silenced which confirmed that the inhibition of both genes improved firmness and decreased fungal infection (Garcia-Gago *et al.* 2009). The introduction of a cold- inducible transcription factor *CBF1* from *Arabidopsis* into strawberry increased the resistance to cold conditions (Jin *et al.* 2009). Strawberry anthocyanin level was engineered by transformation with late flavonoid pathway genes, like DFR and ANS (Montironi *et al.* 2009).

There are a few studies related to environmental risk assessment of transgenic plants in strawberry. The formation of chimeras during transformation has been reported in strawberry by several authors (Mathews *et al.* 1998; Monticelli *et al.* 2002) and is considered to be one of the major problems for strawberry transformation. Abdal-Aziz *et al.* (2006) described high frequencies of non-T-DNA sequence integrations in transgenic strawberry plants obtained through *Agrobacterium* transformation. An environmental risk evaluation of transgenic strawberry expressing a rice chitinase gene was performed in greenhouse, semi-greenhouse and field and revealed no effect on other plants, microflora, morphological characteristics and yield (Asao *et al.* 2003). The sexual transmission of transgenes to R1 generation progeny was reported for *F.* × *ananassa* (James *et al.* 1995) and for *F. vesca* (Haymes and Davis 1998). Cordero de Mesa *et al.* (2004) studied the activity of *CaMV 35S* promoter in floral organs and pollen of transgenic strawberry in respect of the environmental spread of the transgene through pollen dispersal.

The fact that the garden strawberry *Fragaria* × *ananassa* contains an octoploid genome made it difficult to use this species as a model for molecular studies and the interpretation of the transformation events. The wild strawberry *F. vesca* that contains a diploid genome represents an ideal model for functional genomics research in *Rosaceae* (Oosumi *et al.* 2006). However, recently transformation protocols were developed for a rapid-cycling genotype LF9 of *F.* × *ananassa* which allows high-throughput studies of gene function in the octoploid genetic background (Folta *et al.* 2006).

FUTURE WORK AND PERSPECTIVES

The development of molecular markers, the performance of linkage analyses, the isolation and functional characterization of individual genes as well as the introduction of automated sequencing strategies have lead to an substantial increase of our knowledge about strawberry during the last ten years. Whereas the last decade was characterized by the introduction of new technologies which produce hundreds of thousands of data within a little while the next ten years will be more focused on the development of bioinformatics tools which allow us to handle this flood of information. The next step will be the transfer of knowledge from science to practice. It is expected that the phenotype based breeding will be more and more replaced by a more directed selection which is based on information about the genotype and independent from environmental conditions. The increasing number of cloned genes and the understanding of their contribution to complex biological systems will give us new opportunities for creative synthesis of new varieties (McCouch 2004).

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