Chapter 13
Strawberries

J.F. Hancock, T.M. Sjulin and G.A. Lobos

Abstract The major cultivated strawberry species, *Fragaria × ananassa*, is a hybrid of two native species, *F. chiloensis* and *F. virginiana*. Strawberry breeders are focused on improving local adaptations, fruit quality, productivity and disease resistance, and many are interested in developing new day-neutral cultivars. Some of the major pathogens worldwide are *Botrytis cinerea, Colletotrichum* spp., *Phytophthora cactorum, Phytophthora fragariae* and *Verticillium albo-atrum*. The genetics of many of the horticulturally important traits have been investigated in strawberry and a number of genes have been characterized and cloned that are highly expressed during fruit ripening and maturation. Marker systems have been developed in strawberry for genetic linkage mapping and QTL have been identified for the day neutrality trait and several other fruit characteristics. Transgenic strawberries have been produced with herbicide and pest resistance and an effective marker-free transformation process has been developed. Two major EST libraries have been generated as genomic resources.

13.1 Introduction

The most popular cultivated strawberry is the dessert strawberry, *Fragaria× ananassa*. Annual world production of this species has steadily grown through the ages, with quantities doubling in the last 20 years to over 2.5 million tones (FAO Production Statistics). Most of the production is located in the northern hemisphere (98%), but there are no genetic or climatic barriers preventing greater expansion into the southern hemisphere. There are two primary types of strawberries now grown commercially, day-neutral and short day plants. Long day ('everbearing') plants are also available, but they are only commercially important in southern California.

J.F. Hancock

Department of Horticulture, 342C Plant and Soil Sciences Building, Michigan State University, East Lansing, Michigan 48824, USA
e-mail: hancock@msu.edu

There are two major production systems utilized in the world – matted rows and hills. The matted row system employs runners as the primary yield component. Both mother and daughter plants are allowed to runner freely, with periodic training into narrow rows. The hill or 'plasticulture system' relies on crowns as the primary yield component, and any runners that form are removed. The hill system is used primarily in areas having warm winters and either hot or moderate summers such as California, Florida, Italy and Spain. Matted rows are used to grow short day cultivars in climates with short summers and cold winters such as continental Europe and northern North America (Hancock 1999). In general, cultivars perform best in one or the other cultural system, although some are more flexible than others.

The most widely planted cultivar in the world is 'Camarosa', released from the University of California (UC) breeding program. It is important in all climates with mild winters (Florida, Southern U.S.A., Australia, Italy, New Zealand, South America, South Africa, Turkey, Mexico and Spain). The newer UC release 'Ventana' appears likely to be the next dominate cultivar in the warmer regions. 'Honeoye' has gained the broadest foothold in world climates with cold winters, followed by 'Earliglow' which dominates eastern U.S.A. crop area and Dutch bred 'Elsanta' which predominates in Europe.

Three other strawberry species are of minor importance in the world, *Fragaria chiloensis*, *F. vesca* and *F. moschata*. *Fragaria chiloensis* is currently grown to a small extent in Chile, but was widely grown there until the late 1800s when it was replaced by *F. ×ananassa*. It was domesticated 1,000 years ago by the indigenous Chilean Mapuches and was spread widely by the Spanish during the colonization period. *Fragaria vesca* was probably cultivated by the ancient Romans and Greeks, and by the 1300s, it was being grown all across Europe (Darrow 1966). *Fragaria vesca*, the alpine strawberry or fraise de bois, had its widest popularity in the 1500s and 1600s in Europe before the introduction of strawberry species from the New World. It is now generally restricted to home gardens where its small, aromatic fruits are considered a delicacy; most of the varieties grown are everbearers. The musky-flavored *F. moschata* (Hautbois or Hautboy) was also planted in gardens by the late 15th century, along with the green strawberry, *F. viridis*. *Fragaria viridis* was used solely as an ornamental all across Europe, while *F. moschata* was utilized for its fruit by the English, Germans and Russians. Neither of these two species is of current commercial importance.

13.2 Evolutionary Biology and Germplasm Resources

The strawberry belongs to genus *Fragaria* in the Rosaceae. Its closest relatives are Duchesnea and Potentilla. There are four basic fertility groups in *Fragaria* that are associated primarily with their ploidy level or chromosome number (Table 13.1). The most common native species, *F. vesca*, has 14 chromosomes and is considered a diploid. The most important cultivated strawberry, *F. ×ananassa*, is an octoploid with 56 chromosomes. It is an accidental hybrid of *F. chiloensis* and *F. virginiana*

Table 13.1 Strawberry species of the world and their important horticultural traits

Species	Ploidy	Location	Important traits
F. vesca L.	2x	Worldwide	Bright red, aromatic, soft fruit; long ovate-variable; cold, heat and drought tolerant; multiple disease resistances; self-compatible
F. viridis Duch.		Europe and Asia	Firm, green-pink fruit; spicy, cinnamon like flavor; self-incompatible
F. nilgerrensis Schlect.		Southeastern Asia	Pink, tasteless to unpleasant fruit; subglobose; immune to aphids and several leaf diseases; self-compatible
F. daltoniana J. Gay		Himalayas	Shiny red, tasteless fruit; ovoid to cylindrical; self-compatible
F. nubicola Lindl.		Himalayas	Fruit resembles *F. vesca*; self-incompatible
F. iinumae Makino		Japan	Spongy, nearly tasteless fruit; elongate; cold tolerant; self-compatible
F. yesoensis Hara.[1]		Japan	Fruit resembles *F. nipponica*; self-compatible
F. mandshurica Staudt		North China	Very acid fruit; subglobose to obovoid; self-incompatible
F. nipponica Makino.[1]		Japan	Unpleasant flavored fruit; cold tolerant; globose to ovoid; self-incompatible
F. gracilisa A. Los.		North China	Elongated and ovate fruit; self-incompatible
F. pentaphylla Losinsk		North China	Bright red, firm fruit with little flavor; ovoid-globose; multiple leaf disease resistances; self-incompatible
F. corymbosa Losinsk		North China	Seeds in deep pits; dioecious
F. orientalis Losinsk	4x	Russian Far East/ China	Soft fruit with slight aroma; obovoid; trioecious
F. moupinensis (French.) Card		North China	Resembles *F. nilgerrensis*; orange red, spongy fruit; nearly tasteless; dioecious
F. ×bringhurstii Staudt	5x	California	Intermediate to *F. vesca* and *F. chiloensis*; dioecious
F. moschata Duch.	6x	Euro-Siberia	Light to dark dull purplish red fruit, soft, irregular to ovoid; musky flavored and aromatic; tolerant to shade, cold and water-logged soil; immune to powdery mildew; trioecious
F. chiloensis (L.) Miller	8x	Western N. America and Chile	Dull red brown, white flesh, mild, firm, round to oblate; very broad range of adaptations and traits; trioecious

396 J.F. Hancock et al.

Table 13.1 (continued)

Species	Ploidy	Location	Important traits
F. virginiana Miller		North America	Soft to deep red or scarlet fruit; white flesh, tart, aromatic; very broad range of adaptations and traits; trioecious
F. iturupensis Staudt		Iturup Island	Spherical, bright red; trioecious
F. ×ananassa Duchesne ex Lamarck		Worldwide	Very large, red, fruit; variable in all traits

[1] According to Staudt (1989), *F. nipponica* and *F. yesoensis* are the same species.
Staudt 1989, 1999, Galletta and Bringhurst 1991, Hummer 1995, Bors and Sullivan 1998

that arose in the mid-1700s when plants of *F. chiloensis* from Chile were planted in France next to *F. virginiana* from the eastern seaboard of the United States (see more details below).

An accurate taxonomy of the native strawberry species is still emerging. Diploid, tetraploid and hexaploid species are found in Europe and Asia (Table 13.1), but octoploids are restricted to the New World and perhaps Iturup Island northeast of Japan (Staudt 1989). Only one diploid species, *F. vesca*, is located in North America. The genomic complement of the octoploids is likely AAA'A'BBB'B' (Bringhurst 1990), with *F. vesca* probably being the A genome donor. The B genome donor has not been clearly elucidated, although molecular evidence is accumulating that Japanese *F. iinumae* may be it (Davis 2004).

The most likely scenario is that the octoploids originated in northeastern Asia when *F. vesca* combined with other unknown diploids, and the polyploid derivatives then migrated across the Bering Strait and dispersed across North America (Hancock 1999). It is possible that *F. chiloensis* and *F. virginiana* are extreme forms of the same biological species, separated during the Pleistocene, which subsequently evolved differential adaptations to coastal and mountain habitats. The two species are completely inter-fertile, carry similar cpDNA restriction fragment mutations (Harrison et al. 1997) and have very similar nuclear internal transcribed spacer (ITS) regions (Potter et al. 2000).

Polyploidy in *Fragaria* probably arose through the unification of 2*n* gametes, as several investigators have noted that unreduced gametes are relatively common in *Fragaria* (Hancock 1999). Staudt (1984) observed restitution in microsporogenesis of a F_1 hybrid of *F. virginiana* × *F. chiloensis*. In a study of native populations of *F. chiloensis* and *F. vesca*, Bringhurst and Senanayake (1966) found frequencies of giant pollen grains to be approximately 1% of the total. Over 10% of the natural hybrids generated between these two species were the result of unreduced gametes.

The inheritance patterns of the octoploids are in dispute. Lerceteau-Köhler et al. (2003) concluded that *F. ×ananassa* has mixed segregation ratios using AFLP markers, as they found the ratio of coupling vs. repulsion markers fell between the fully disomic and polysomic expectations. However, two other studies evaluating isozyme, SSR and RFLP segregation observed predominantly disomic ratios,

indicating that the octoploid strawberry is completely diploidized (Arulsekar and Bringhurst 1981, Ashley et al. 2003).

Most commercial strawberries have been selected to be strict hermaphrodites, but sex is regulated as a single gene trait in *F. vesca*, *F. chiloensis*, *F. virginiana* and *F. ×ananassa* (Ahmadi and Bringhurst 1991). Female (*F*) (pistillate) is dominant to hermaphrodite (*H*), which is dominate to male (*M*) (staminant). Females are heterogametic (*F/H* or *F/M*), while hermaphrodites can be homo- or heterogametic (*H/H* or *H/M*) and males are homogametic (*M/M*). A range in fertility can be found in hermaphrodites ranging from self infertility to complete fruit set (Stahler et al. 1990, 1995, Luby and Stahler 1993). In *F. orientalis* and *F. moschata*, Staudt (1967) found tetrasomic inheritance for sex and he described the alleles for sex as male suppressor *SuM* (*F*) dominant to male inducer *Su+* (*H*) and to the female suppressor *SuF* (*M*). *SuF* was dominant to *Su+*.

While there appear to be some barriers to interfertility among the diploid strawberries, they all can be crossed to some extent, and meiosis in the hybrids is regular, even in cases where the interspecific hybrids are sterile (Federova 1946, Staudt 1959, Fadeeva 1966). There are at least three overlapping groups of diploid species that are inter-fertile (Bors and Sullivan 1998, 2005): (1) *Fragaria vesca*, *F. viridis*, *F. nubicola* and *F. pentaphylla* (2) *F. vesca*, *F. nilgerrensis*, *F. daltoniana* and *F. pentaphyta* (3) *F. pentaphyta*, *F. gracilis* and *F. nipponica*. *Fragaria iinumae* may belong in group 3 or in an additional group, as no fertile seeds have been recovered when it was crossed with either *F. vesca*, *F. viridis* or *F. nubicola*, but it has not been crossed with enough other species to accurately classify it. *Fragaria iinumae* does, however, have a glaucous leaf trait that is unique among the diploids, and its chloroplast RFLPs cluster it with *F. nilgerrensis* in a group that is isolated from the rest (Harrison et al. 1997).

Numerous valuable characteristics exist in the lower ploidy species that could be of value in the cultivated species (Darrow 1966, Hancock 1999). A particularly excellent comparison of the quantitative and qualitative differences between the diploids can be found in Sargent et al. (2004b). *Fragaria iinumae*, *F. vesca* and *F. nipponica* are likely highly cold tolerant as they are located on cold, alpine meadows. *F. vesca* have high tolerance to heat and drought, and high aroma along with resistance to Verticillium wilt (Arulsekar 1979), powdery mildew (Harland and King 1957) and crown rot (*Phytophthora cactorum*) (Gooding et al. 1981). *Fragaria moschata* is found under heavy shade and is immune to powdery mildew (Maas 1998). *Fragaria viridis* tolerates alkaline soils. In a comprehensive study of diploid species in Ontario, Bors and Sullivan (1998) found *F. nilgerrensis* to have immunity to aphids and leaf diseases. *Fragaria iinumae* produced unusual tap roots from runners. *F. moschata* survived a particularly cold winter in water-logged soil and displayed excellent leaf disease resistance. *Fragaria pentaphylla* was extremely vigorous, with unusually bright red, firm fruit and leaf disease immunity.

The incorporation of traits from a number of lower ploid species has been accomplished through pollinations with native unreduced gametes or by artificially doubling chromosome numbers. The utility of this approach has been shown for a wide range of species in *Fragaria* and in the related genus *Potentilla* (Hancock et al. 1996).

Particular success in incorporating lower ploidies into the background of *F.* ×*ananassa* has come through combining lower ploidy species and then doubling to the octoploid level (Sangiacomo and Sullivan 1994, Bors and Sullivan 1998).

Native clones of *F. chiloensis* and *F. virginiana* also offer a rich genetic storehouse and may be more useful in improving *F.* ×*ananassa* than the lower ploidies, as they cross readily with the cultivated types and offer as much if not more genetic diversity. Some of the wild clones have particularly interesting flavors and aromas that have not yet been characterized, and they possess resistance to extreme environments, as well as a number of disease and pest problems. In addition, variability exists in several yield-related physiological traits including: (1) heat and cold tolerance (2) rates and patterns of CO_2 fixation (3) the levels of dry matter allocated to reproduction (4) the number of flowering cycles, and (5) the length of the floral induction period.

In many cases, important components of yield can be combined with known disease resistances (Hancock et al. 2001). Most reports of pest resistance are limited to one disease or insect, but there are some genotypes that have been identified with multiple resistances. Clones of *F. chiloensis* have been described that carry resistance to aphids, 2-spotted spider mites, red stele, leaf spot, powdery mildew and root lesion nematodes. Two clones in particular from California, RCP 37 and CA 11, stand out as they are resistant to most of the pests described above, have very high photosynthetic rates (Hancock et al. 1989), and originated on dry, salty dunes.

Recently an elite group of 38 strawberry accessions was selected to represent the diversity found in *F. chiloensis* and *F. virginiana*, and was evaluated for plant vigor, flower number per inflorescence, flowering date, runner density, fruit set, fruit appearance and foliar disease resistance (Hancock et al. 2001). This collection is available at the National Clonal Germplasm Repository at Corvallis, Oregon, U.S.A. (http://www.ars.usda.gov/main/site_main.htm?modecode = 53581500). Among the individual taxa of the octoploid species, the largest fruit sizes were observed in the cultivated land races of *F. chiloensis* from South America, although the native clones of North American *F. chiloensis* ssp. *pacifica* were much more vigorous (Fig. 13.1). *Fragaria virginiana* ssp. *platypetala* had by far the largest fruit of any *F. virginiana* subspecies, rivaling the native clones of *F. chiloensis*. Day neutrals were found among northern *F. virginiana* ssp. *virginiana* and *F. virginiana* ssp. *glauca* accessions. Northern *F. virginiana* ssp. *virginiana* flowered the earliest and longest of any taxa, and had the most deeply colored fruit. By far the greatest winter hardiness was found in *F. virginiana* ssp. *glauca* and ssp. *virginiana*. Considerable genotype × location interaction was observed for many of the traits measured, indicating that individual site analyses cannot always be used to predict the broad range performance of individual genotypes; however, a few genotypes were impressive at all locations including CFRA 368 (California) with its unusually large, early fruit, and NC 95191 (North Carolina), Frederick 9 (Ontario) and RH 30 (Minnesota), which were very vigorous and had unusually good color.

A much larger sample of 270 genotypes of wild *F. virginiana* and *F. chiloensis* from the National Clonal Germplasm Repository at Corvallis, Ore. has also been compared in a greenhouse at Michigan State University for variation in fourteen
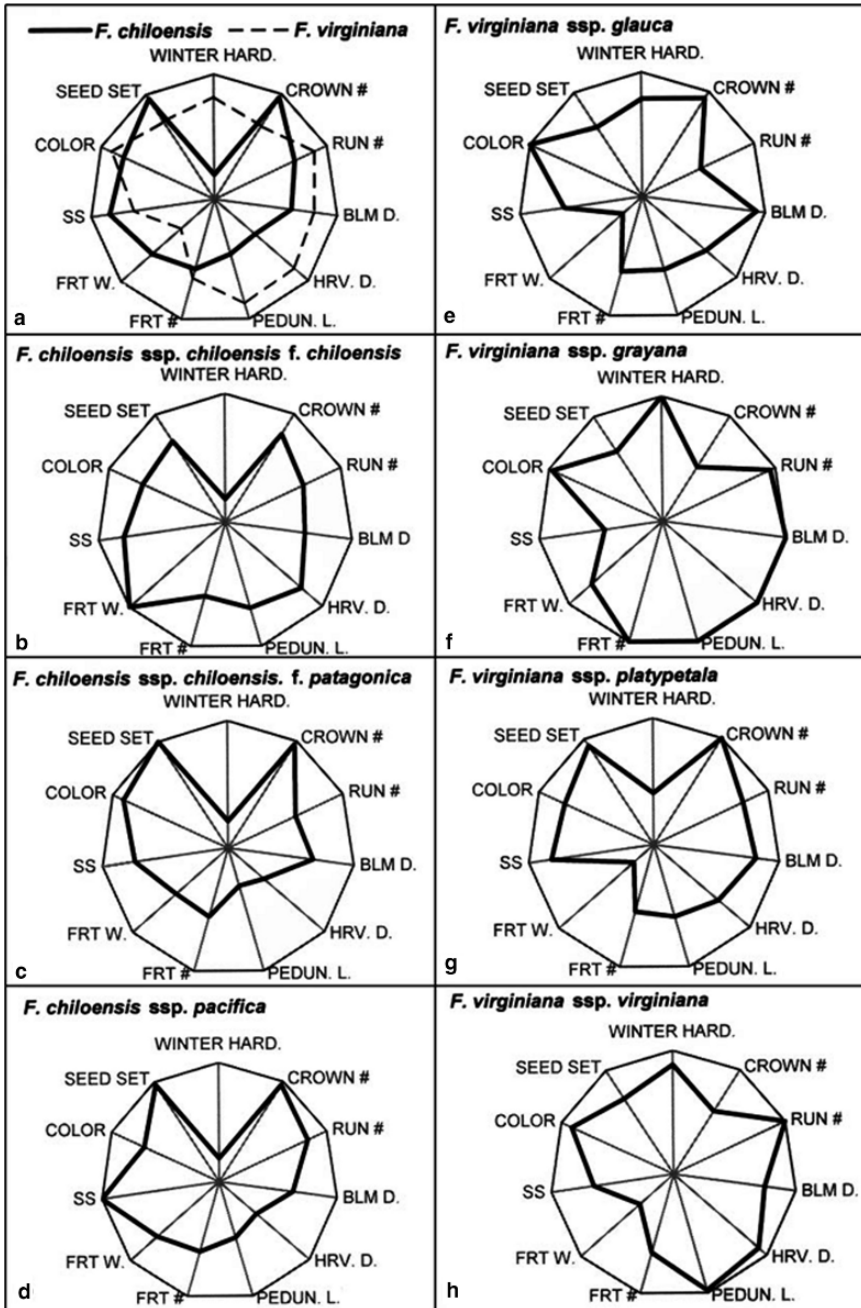
Fig. 13.1 Pictograms contrasting the horticultural traits of *F. virginiana* and *F. chiloensis* (**a**), and the various octoploid subspecies (**b–h**) (Hancock et al. 2003). The outer circumference represents the highest mean value of any of the subspecies. Each axis was normalized by dividing each trait by the highest overall value

horticultural traits (Hancock et al. 2003). Significant levels of variation were found for all but a few of the traits at the species, subspecies, regional and genotypic level, with the highest amount of variation generally being partitioned among genotypes. *Fragaria chiloensis* was superior to *F. virginiana* for crown number, fruit weight, soluble solids and seed set, while *Fragaria virginiana* was superior for runner production, peduncle length, fruit number, fruit color and winter hardiness. *Fragaria chiloensis* ssp. *pacifica* had the highest soluble solids and among the earliest bloom dates, highest crown numbers and highest seed set. *Fragaria chiloensis* ssp. *chiloensis* f. *chiloensis* produced the largest fruit and some of the earliest bloom dates and longest peduncles. *Fragaria chiloensis* ssp. *chiloensis* f. *patagonica* had some of the highest crown numbers and the highest percentage seed set. *Fragaria virginiana* ssp. *platypetala* produced the most crowns and its fruit ripened earliest. *Fragaria virginiana* ssp. *glauca* were the latest flowering, had the darkest fruit color and the most flowering cycles. *Fragaria virginiana* ssp. *virginiana* displayed the most winter dieback, longest peduncles, and the highest flower and runner numbers.

Among the most impressive individual genotypes, CFRA 0024 (*F. chiloensis* ssp. *chiloensis* f. *chiloensis*) possessed unusually high crown numbers, was extremely early blooming and displayed multiple fruiting cycles. CFRA 1121 (*F. chiloensis* ssp. *chiloensis* f. *chiloensis*) had unusually long peduncles and much higher than average values for fruit weight, soluble solids, fruit color and seed set. CFRA 0094 (*F. chiloensis* ssp. *pacifica*) was extremely early flowering and had much darker fruit color than most other *F. chiloensis* genotypes. CFRA 0368 (*F. chiloensis* ssp. *lucida*) flowered unusually early and had among the largest fruit. CFRA 0366 (*F. chiloensis* ssp. *lucida*) possessed unusually long peduncles and the largest fruit of any North American genotype. CFRA 0560 and CFRA 1369 (*F. virginiana* ssp. *glauca*) had an unusual combination of multiple flowering cycles and high runner production. CFRA 1170 and 1171 (*F. virginiana* ssp. *virginiana*) were unusually late fruiting and had high numbers of large fruit on long peduncles. CFRA 1385 and JP 9531 (*F. virginiana* ssp. *virginiana*) had extremely high flower numbers, long peduncles and large fruit.

13.3 History of Improvement

The wood strawberry, *F. vesca*, dominated strawberry cultivation in Europe for centuries, until *F. virginiana* from eastern Canada and Virginia began to replace it in the 1600s. All of the clones that found their way to Europe were wild in origin, as the aboriginal peoples of North America did little gardening with strawberries. The early cultivar development of *F. virginiana* was primarily conducted by growers who found raising seed imported from North America often resulted in horticulturally important variations.

One of the domesticated Chilean clones of *F. chiloensis* found its way into Europe in the 1700s compliments of a French spy, Captain Amédée Frézier (Darrow 1966, Wilhelm and Sagen 1974). Unfortunately, early reports on the

Chilean strawberry were negative, as the plants were largely barren because Frézier had inadvertently brought back staminate plants. French horticulturalists solved the problem when they discovered that the 'Chili' would produce fruit when pollinated by *F. moschata* or *F. virginiana*. The Chilean strawberry reached its highest acclaim in Brittany, and by the mid-1800s, there was probably more *F. chiloensis* cultivated in France than its native land.

Unusual seedlings began to appear in Brittany and other gardens with unique combinations of fruit and morphological characteristics. While the origin of these seedlings was initially clouded, the great French Botanist Antoine Nicholas Duchesne determined in 1766 that they were hybrids of *F. chiloensis* × *F. virginiana* and he named them *Fragaria* × *ananassa* to denote the perfume of the fruit that smelled like pineapple (*Ananas*). It is not clear where the first hybrids of The Pineapple or Pine strawberry appeared, but they must have arisen early in the commercial fields of Brittany and in botanical gardens all across Europe such as the Trianon, the Royal Garden at Versailles where Duchesne studied.

Formal strawberry breeding was initiated in England in 1817 by Thomas A. Knight (Darrow 1966, Wilhelm and Sagen 1974). He was one of the first systematic breeders of any crop, and used clones of both *F. virginiana* and *F. chiloensis* in his crosses. He produced the famous 'Downton' and 'Elton' cultivars, noted for their large fruit, vigor and hardiness. Michael Keen, a market gardener near London, also became interested in strawberry improvement about this time and developed 'Keen's Imperial' whose offspring, 'Keen's Seedling' is in the background of many modern cultivars (Fig. 13.2). This variety dominated strawberry acreage for close to a century.

Thomas Laxton of England was the most active breeder during the later part of the 18th century and released a number of important varieties including 'Noble' and 'Royal Sovereign'. These two varieties were grown on both sides of the Atlantic Ocean, and were popular until the middle of the 20th century. 'Nobel' was known for its earliness, cold hardiness and disease resistance. 'Royal Sovereign' was popular because of its earliness, productivity, flavor, attractiveness and hardiness.

Charles Hovey, of Cambridge, Massachusetts, produced the first important North American cultivar, 'Hovey', by crossing the European pine strawberry, 'Mulberry' with a native clone of *F. virginiana* in 1836. It was the first variety of any fruit to come from an artificial cross in America and for awhile made the strawberry the major pomological product in the country (Hedrick 1925).

Albert Etter of California developed dozens of varieties around the turn of the century utilizing native *F. chiloensis* clones (Wilhelm and Sagen 1974, Fishman 1987). His most successful variety was 'Ettersburg 80' (1910), which was widely grown in California, Europe, New Zealand and Australia. Renamed as 'Huxley', it was still popular in England as late as 1953. Ettersburg 80 was extremely drought resistant, of outstanding dessert and jam quality due to its solid bright red color, and was unusually hardy for a California type. Other outstanding Etter varieties were 'Ettersburg 121', 'Fendalcino' and 'Rose Ettersburg'. While his releases were very successful as cultivars, they may have had their greatest impact as breeding parents. Most California cultivars (and many others) have an Ettersburg variety in their background (Darrow 1937 and 1966, Sjulin and Dale 1987).

Fig. 13.2 The strawberry 'Keen's Seedling', which was a sensation in England in the late 1800s. It is in the pedigree of many modern cultivars

In the middle of the 20th century, a number of particularly active breeding programs emerged in Scotland, England, Germany and Holland. In Scotland, Robert Reid developed a series of red stele resistant varieties utilizing American 'Aberdeen' as a source of resistance. His variety 'Auchincruive Climax' dominated acreage in Great Britain and northern Europe until its demise due to June yellows in the mid 1950s. He then released 'Redgauntlet' (1956) and 'Talisman' (1955), which served as suitable replacements. In England, D. Boyle produced a large series of varieties with the prefix 'Cambridge'; 'Cambridge Favorite' (1953) became the most important of the group and dominated the acreage in Great Britain by the 1960s. In

Germany, R. von Sengbusch's produced a 'Senga' series, of which 'Senga Sengana' (1954) became paramount. 'Senga Sengana' was widely planted for its processing quality and is still important in Poland and other eastern European countries. In Holland, H. Kronenberg and L. Wassenaar's released several cultivars, of which 'Gorella' (1960) made the greatest impact. It was noted for its size, bright red glossy skin and red flesh.

George Darrow came to the USDA in Beltsville, Maryland in the 1920s and began his illustrious career with the release of 'Blakemore' in 1929 and 'Fairfax' in 1933. 'Blakemore' became the major southern U.S. variety in the mid-1930s and 'Fairfax' was widely planted in the middle of this century from southern New England to Maryland and westward to Kansas. These two cultivars were used extensively in breeding, finding their way into the ancestry of a diverse array of cultivars grown in all parts of the U.S. Other important releases from Darrow were 'Pocahontas', 'Albritton', 'Surecrop' and 'Sunrise'. Donald Scott took over the program in the 1950s and released 'Midway', 'Redchief', 'Guardian' and 'Earliglow'. An active USDA breeding program was also conducted at Corvallis, Oregon in the middle of the 20th century by Darrow, G.F. Waldo and F.J. Lawrence. Some of the more important cultivars emerging from this program were 'Narcissa' (1932), 'Brightmore' (1942), 'Hood' (1965) and 'Benton' (1974).

H. Thomas and E. Goldsmith's of the University of California released the important cultivars 'Lassen' and 'Shasta' in 1945. 'Shasta' was widely grown in the central coast of California in the 1950s and 1960s because of its large size, firmness and long season. 'Lassen' was grown extensively in southern California about the same period, prized for its short rest period and high productivity. Thomas and Goldsmith ultimately left the University and founded the highly successful breeding program of Driscoll Associates in Watsonville, California (Sjulin 2006). Royce Bringhurst and Victor Voth took over the Cal-Davis program in the 1950s and generated an amazing succession of internationally important, Mediterranean adapted cultivars including 'Tioga' (1964), 'Tufts' (1972), 'Aiko' (1975), 'Pajaro' (1979), 'Chandler' (1983), 'Selva' (1983), 'Camarosa' (1992) and 'Seascape' (1991).

Several significant breeding programs were conducted by various Agricultural Experiment Stations in the early to mid-1900s. A.N. Brooks in Florida selected 'Florida 90' (1952) from an open pollinated population of 'Missionary'. This variety had excellent flavor, very high yields and found an important seasonal niche in March and early April as other southern production diminished. Miller and Hawthorn released 'Klonmore' (1940), 'Headliner' (1957) and 'Dabreak' (1961) in Louisiana with leaf resistance to leaf spot and scorch, and good shipping quality. A number of important cultivars came out of New Jersey including 'Pathfinder' (1937) and 'Sparkle' (1942) introduced by J. H. Clark and 'Jerseybelle' (1955) developed by F. A. Gilbert. In New York, George Slate released 'Catskill' in 1933 for its large attractive berries and high productivity.

The greatest concentration of breeding activity in the world was centered in the U.S.A. and Europe until the modern period, although the Japanese produced two important varieties: Dr. H. Fukuba's 'Fukuba' (1899), noted for its large size and high flavor (Darrow 1966), and K. Tamari's 'Kogyoku' (1940), respected for its

vigor, earliness and fruit size (Mochizuki 1995). 'Fukuba' was the most important variety in forcing culture until the early 1970s. 'Kogyoku' was one of the leading field grown cultivars after World War II, until it lost importance to the American import 'Donner' in the 1950s (Darrow 1966).

13.4 Current Breeding Efforts

There are numerous public and private breeding programs across the world that focus on improvement of *F*. ×*ananassa*. The largest European efforts are found in France, Italy, the Netherlands, Spain and the U.K. The most active North American programs are located in British Columbia, California, Florida, Maryland, New York, Nova Scotia, Ontario, Oregon and Quebec. In Asia, the largest number of public and private breeding programs is found in Japan. *Fragaria chiloensis* is being bred at the Universidad de Talca in Chile.

The Centre Interrégional de Recherche et d'Expérimentation de la Fraise (CIREF) directed by Philippe Chartier in France is active in developing short-day and day neutral cultivars with superior fruit appearance and flavor as well as soil disease tolerance. The most important recent cultivars from this program are 'Ciflorette' and 'Cirafine'. Two other important French breeding efforts are Darbonne which produced 'Darselect' and Marionnet SARL which released 'Mara des Bois' and the new 'Matis'.

In Italy, there is a national program 'Frutticoltura' funded by the Minister of Agriculture, with a number of institutions being involved. G. Baruzzi and W. Faedi lead this effort. Concentration is being placed on developing new dessert varieties with adaptations to the south, Po Valley and north mountain regions including disease resistance and tolerance to alkaline soils. Significant new cultivars from this program are 'Patty', 'Granda' and 'Queen Elisa'. A private company, Consorzio Italiano Vivaisti (C.I.V.) directed by A. Martinelli is also active in producing dessert varieties for both north and south Italy, and have generated 'Marmolada', 'Clery' and 'Miranda'.

The breeding effort of Fresh Forward, directed by B. Meulenbroek in the Netherlands, is concerned with developing types with broad adaptations, high yields and large fruit size. The most important European cultivar, 'Elsanta' (1981) came from the public progenitor of this program and their newest variety, 'Sonata', is increasing in popularity.

In Spain, a breeding program at the Universidad de Malaga conducted by J.M. Lopez-Aranda and C. Soria is concentrating on producing cultivars for Huelva. Their goals are to breed high yielding, early cultivars for the fresh market with large, high quality fruit. Their most important new cultivars are 'Andana' and 'Carisma'. The private firm, Plantas de Navarra S.A. (PLANASA) directed by D. Sanchez is also very active in searching for highly productive, high quality types for Spain. This program's most recent successes are 'Tudla', 'Cartuno' and 'Candonga'.

David Simpson of East Malling Research in England is concentrating on combining excellent fruit quality with resistance to diseases, particularly *Verticillium dahliae* and *Sphaerotheca macularis*. His most important cultivars are 'Florence', 'Pegasus' and 'Flamenco'. Edward Vinson Limited also has an active program at Kent, with a focus on day-neutrals. Their recent cultivar 'Everest' has proven to be widely adapted.

The largest U.S. breeding programs reside at the University of California at Davis, and the USDA-ARS centered at Beltsville, Maryland and Corvallis, Oregon. Now under the direction of D.V. Shaw and K.D. Larson, the Cal-Davis program is known for its broadly adapted, large-fruited and high yielding cultivars. Some of the most important new releases from this program are 'Aromas', 'Diamante', 'Ventana' and 'Albion'. The USDA-ARS program in Maryland is probably the longest continually maintained program in the world. It was directed by G.J. Galletta through the latter part of the 20th century in collaboration with A.D. Draper, and most recently is under the direction of K.S. Lewers. The most important cultivars from these programs are 'Allstar', 'Tribute', 'Tristar', 'Northeaster', 'Delmarvel' and 'Ovation'. These cultivars are noted for their resistance to soil pathogens, particularly *Phytophthora fragariae*. C.E. Finn now conducts the USDA breeding effort at Corvallis, Oregon. The current emphasis in this program is on developing high quality types for the processed market, although effort is shifting towards developing fresh market cultivars. Recent cultivars include 'Redcrest', 'Redgem', 'Pinnacle' and 'Tillamook'.

State supported programs in the U.S.A. are located in Washington (P. Moore), Michigan (J. Hancock), Minnesota (J. Luby), New Jersey (G. Jelenkovic), New York (C. Weber), North Carolina (J. Ballington), Wisconsin (B. Smith), Maryland (H. Swartz) and Florida (C. Chandler). The current goals of these programs are: (1) Florida – high fresh fruit and shipping quality including resistance to water damage, high late November to mid-March yields, anthracnose resistance (2) Michigan – day neutral types with higher heat tolerance and resistance to soil pathogens, germplasm development using wild octoploids (3) and (4) Minnesota and Wisconsin – winter hardiness, high quality, disease resistance, germplasm development using wild octoploids (5) New Jersey – early cultivars with excellent fruit flavor and size that are adapted to matted row and hill culture (6) New York – fruit quality including size, symmetry; high, steady yields; black root rot resistance (7) and (8) North Carolina and Maryland – superior genotypes that are resistant to anthracnose and are adapted to annual hill plasticulture systems, and (9) Washington – June bearing types with firm, easily harvested fruit for processing and fresh outlets, increased disease and insect resistance (particularly fruit rots and aphid transmitted viruses). Some of the most important cultivars that have come out of these programs over the years are 'Honeoye' and 'Jewel' (New York), 'Mesabi' (Minnesota), 'Raritan' (New Jersey), 'Strawberry Festival' and 'Sweet Charlie' (Florida).

There are several large breeding programs in Canada that are federally funded. At Agriculture and Agri-Food Canada in Kentville (Nova Scotia), A. Jamison is making wide use of European cultivars to produce early season, red stele resistant types. He is building on the previous decades of work of D.L. Craig and L.E. Aalders. Some

of the most significant cultivars to come out of this program are 'Bounty', 'An-napolis', 'Glooscap', 'Kent' and 'Cavendish'. An Ontario program led by A. Dale at the University of Guelph is concentrating on large-fruited, firm types and he is actively exploiting native germplasm. Recent releases from his program are 'Star-tyme', 'Sapphire' and 'Serenity'. S. Khanizadeh at the Horticultural Research and Development Centre in Quebec is searching for large fruited, pale skin colored and firm types with resistance to red stele. Numerous cultivars have been released from this program including the most recent 'Harmonie', 'St-Jean d'Orléans', 'St-Laurent d'Orléans' and 'La Clé de Champs'.

Probably the largest program in Canada is the Agriculture and Agri-Foods Canada program in Agassiz, British Columbia. It was originally run by H. Daubeny, and now C. Kempler. The predominant cultivar grown in the Pacific Northwest, 'Totem', was released from this program in 1971. 'Sumas' and 'Shuswap' were two other important cultivars from this program that has focused on June-bearing types with excellent processing characteristics (intense internal and external color, high soluble solids, high titratable acidy, low pH, and intense flavor).

R. Harrison (Production, Breeding and Research Department) and B. Mowrey (Head Plant Breeder) of the Driscoll Strawberry Associates in Watsonville, California direct the most vigorous private, breeding effort in the U.S. Their primary goals focus on consumer attributes of flavor, appearance and shelf life, coupled with the production attributes of fruit size, timing of harvest and harvestability. Other private efforts are conducted by Plant Sciences Inc (California), New West Fruit Corporation (California) and Well-Pict Inc. (California).

Significant breeding work is also being conducted in Japan. Probably the largest program in Japan is operated by the National Research Institute of Vegetables, Ornamental Plants and Tea with two branches at Kurume and Morioka. Numerous other Prefecture Experiment Stations are actively breeding strawberries including ones at Aichi, Chiba, Hyogo and Saga. Common goals are to produce large, dessert quality berries that are adaptable to forcing culture.

13.5 Genetics of Important Traits

13.5.1 Disease and Pest Resistance

Several soil pathogens damage strawberry roots, resulting in vigor declines and ultimately death (Table 13.2). Two very common problems across the world are red stele or red core caused by *Phytophthora fragariae* Hickman and Verticillium wilt caused by *Verticillium albo-atrum* Reinke & Berth. and *V. dahlia*. Black root rot is also widespread and is caused by a complex of organisms including *Pythium*, *Rhizoctonia* and the root lesion nematode (*Pratylenchus penetrans* Cobb). Fusarium wilt or Fusarium yellows (*Fusarium oxysporum* Schl. f. sp. *fragariae* Winks and Williams) is of major importance in Japan, Korea and Australia.

Table 13.2 Inheritance patterns of disease resistance in strawberries

Disease	Observations	Selected references
Bacteria		
Angular leaf spot – *Xanthomonas fragariae*	Resistant types identified; three or four unlinked loci regulate resistance; isolate × cultivar interactions do not exist; *Fragaria* species vary in their levels of resistance	Lewers et al. 2003, Hildebrand et al. 2005, Xue et al. 2005
Fungi		
Alternaria leaf spot (black leaf spot) – *A. alternate*	Resistant types identified; race variation in pathogen; single dominant locus for susceptibility	Yamamoto et al. 1985, Takahashi 1993
Anthracnose – *Colletotrichum fragariae*	Resistant types identified; race variation in pathogen; types resistant to *C. fragariae* tend to be resistant to *C. acutatum*	Delp and Milholland 1981, Smith and Black 1987, Smith et al. 2007
Anthracnose – *C. acutatum*	Resistant types identified; major and minor genes are associated with resistance; QTL identified for resistance	Gimenez and Ballington 2002, Denoyes-Rothan et al. 2005, Lerceteau-Köhler et al. 2005, Smith et al. 2007
Black root rot – Several Species	Tolerant genotypes identified	Wing et al. 1995, LaMondia 2004, Particka and Hancock 2005
Cactorum crown rot – *Phytophthora cactorum*	Resistant genotypes identified, *F. vesca* may have stronger resistance	van der Scheer 1973, Gooding et al. 1981, Bell et al. 1997
Fusarium wilt – *Fusarium oxysporum*	Resistant genotypes identified	Kim et al. 1982, Cho and Moon 1984, Takahashi 2003, Mori et al. 2005
Grey mold – *Botrytis cinerea*	Resistant genotypes identified; resistance is quantitative and additive; fruit firmness may be related	Maas and Smith 1978, Barritt 1980, Popova et al. 1985
Leaf scorch – *Diplocarpon earliana*	Resistant types identified; race variation in pathogen; parents not useful in predicting resistance of progeny	Nemec 1971
Leaf spot – *Mycosphaerella fragariae*	Resistant types identified; moderate levels of heritability	Nemec 1971, Shaw 1988, Delhomez et al. 1995

Table 13.2 (continued)

Disease	Observations	Selected references
Powdery mildew – *Sphaerotheca macularis*	Resistant types identified; general and specific combining ability is important; resistance is highly heritable; cuticle thickness is associated with resistance	Hsu et al. 1969, Murawski 1968, Simpson 1987, Nelson et al. 1996, Davik and Honne 2005
Red stele root rot – *Phytophthora fragariae*	Resistant genotypes identified; inbreeding can concentrate resistance genes; specific combining ability is very important; data fits a gene for gene model	Daubeny 1964, Melville et al. 1980a, Van de Weg 1997, Maas 1998
Verticillium wilt – *V. albo-atrum* and *V. dahlia*	Resistant genotypes identified; additive variation is important; resistance is partially dominant	Gooding et al. 1975, Maas 1989, Shaw and Gordon 2003
Viruses		
Arabis mosaic	Little resistance identified	Murant and Lister 1987
Clover phyllody	Resistant genotypes identified	Chiykowski and Craig 1975
Raspberry ringspot	Little resistance identified	Murant and Lister 1987
Strawberry latent ringspot	Little resistance identified	Murant and Lister 1987
Tomato black ring	Resistant genotypes identified	Murant and Lister 1987
Tomato ringspot	Resistant genotypes identified	Converse 1987
Misc. virus complexes	Resistant genotypes identified to regional virus complexes including yellows; a high proportion of the genetic variability is additive	Barritt et al. 1982, Graichen et al. 1985, Sjulin et al. 1986

Resistant and/or tolerant genotypes have been found for all four of these major soil pathogens, although the underlying genetics of resistance have only been studied for red stele and Verticillium wilt (Table 13.2). Resistance to Verticillium wilt has been shown to be inherited in an additive fashion with partial dominance. Red stele resistance has been demonstrated in several studies to be regulated primarily through additive interactions, although Van de Weg (1997) has provided evidence that red stele resistance fits a gene for gene model, with five virulence and five resistance genes. Haymes et al. (1997) found molecular markers that were tightly associated with one of the resistance loci (*Rpf1*). No further molecular characterizations have been made on these genes or any other resistance genes in strawberry.

Fumigation has been widely employed to control soil pathogens, but the impending ban on methyl bromide fumigation has stimulated increased interest in developing resistant cultivars. Without fumigation, cultivars yield 50% less fruit on average. Screens of the California breeding population on fumigated and non-fumigated soil have uncovered little general resistance to the total array of soil pathogens normally found in strawberry soils (Larson and Shaw 1995a,b), although a screen of eastern breeding material did uncover some tolerant individuals (Particka and Hancock 2005).

Among the foliar diseases, three are very widespread and can cause serious damage including, leaf blight [*Phomopsis obscurans* (Ell. and Ev.) Suton], Ramularia leaf spot, [*Mycosphaerella fragariae* (Tul.) Lindau] and leaf scorch [*Diplocarpon earliana* (Ell. & Everh.) Wolf]. Alternaria leaf spot or black leaf spot (*Alternaria alternata* (FR.) Keissler) causes serious damage in Europe, New Zealand and Korea. Powdery mildew [*Sphaerotheca macularis* (Wallr. Ex Fr.) Jaez] is also found across most of the strawberry range, although it rarely does economic damage. Angular leaf spot, *Xanthomonas fragariae* Kennedy and King, is a rapidly growing problem in strawberries all across the world (Maas et al. 1998). Moderate to high levels of heritability have been found for resistance to leaf spot, leaf scorch, powdery mildew and ramularia leaf spot (Table 13.2). Black leaf spot resistance has been reported to be controlled at a single locus.

Anthracnose is a common problem in strawberries, causing a wide array of symptoms including fruit rot, crown rot, and lesions of the stolons, petioles and leaves. Anthracnose diseases of strawberry are caused by *Colletotrichum fragariae* A.N. Brooks, *C. acutatum* J.H. Simmonds, and *C. gloeosporioides* (Penz.) Penz. & Sacc. in Penz. *Colletotrichum acutatum* is the primary pathogen causing crown rot in Europe (Denoyes and Baudry 1995), while *C. fragariae* is the most common cause of crown rot in the southeastern U.S.A. (Howard et al. 1992). *C. acutatum* is the primary pathogen in Israel and California. Sources of resistance to anthracnose fruit and crown rots exist in strawberry (Table 13.2); however, the genetic factors conditioning host resistance to crown and fruit infection differ and only a few genotypes are resistant to both fruit and plant infection. Strong environmental × genotype interactions affect the expression of resistance, and multiple isolates of *C. acutatum*, *C. gloeosporioides* and *C. fragariae*, vary in pathogenicity to *Fragaria* genotypes (Smith and Black 1990). Denoyes-Rothan et al. (2005) found both major and minor gene resistance to *C. acutatum*, with the major gene common in the germplasm evaluated. MacKenzie et al. (2006) discovered that resistance to *C. fragariae* and *C. gloeosporoides* was nonspecific, and the major gene resistance described by Denoyes-Rothan et al. (2005) to *C. acutatum* may be effective against these other 2 *Colletotrichum* species.

Phytophthora cactorum (Leb. & Cohn) Schroet also causes widespread incidences of severe crown (Cactorum crown rot) and fruit rots (leather rot), particularly in warm climates. Other important fungal fruit rots are: (1) Botrytis fruit rot or gray mold (*Botrytis cinerea* Pers. ex. Fr.), which is a worldwide problem (2) Mucor fruit rot (*Mucor mucedo* L. ex Fries), sometimes important in the U.S.A. and U.K. (3) Rhizopus leak [*Rhizopus* (spp.)] a particular problem in the U.K. but worldwide

in scope (4) Tan-brown rot, [*Discohainesia oenotherae* (Cook & Ellis)], a major problem in humid strawberry regions (5) *Phomopsis obscurans* (Ellis & Everh.) Sutton in Florida, and (6) Septoria hard rot (*Septoria fragariae*) rarely a problem in the U.S.A. but common in Europe and Australia. Moderate to high levels of heritability have been found to grey mold and leather rot (Table 13.2).

A gene encoding a polygalacturonase-inhibiting protein (PGIP) has been cloned that shows developmental regulation and pathogen-induced expression in strawberry and likely plays a role in defense against fruit rots (Mehli et al. 2004, 2005, Schaart et al. 2005). After inoculation with *Botrytis cinerea*, fruit of five cultivars ('Elsanta', 'Korona', 'Polka', 'Senga Sengana' and 'Tenira') showed a significant induction in PGIP expression and the most resistant one, 'Polka', had the highest constitutive expression. Work is ongoing to produce transgenic strawberries that over-express PGIP sequences and screen them for resistance to *B. cinerea*.

Strawberries across their range are hosts to numerous viruses and phytoplasma. Among the most important are the aphid-borne viruses involved in the yellows complex (mottle, mild yellow-edge, crinkle and vein banding viruses) and the nematode-borne (raspberry ringspot virus, tomato black ring, strawberry latent ringspot virus and arabis mosaic virus) (Maas 1998). There are also two important phytoplasma diseases spread by leaf hoppers, Aster yellows which are caused by a variety of species and green petal or clover phyllody. Resistance has been identified to the regional virus complexes found in the Pacific Northwest, but no formal genetic analyses have been performed (Table 13.2).

The nematodes causing the most widespread problems include the Northern root-knot nematode (*Meloidogyne hapla* Chitwood), root lesion nematode and the needle nematode (*Longidorus elongatus* de Man). Resistance has been described for all these pests, although no genetic studies have been conducted (Table 13.3).

Two aphids are widespread that damage strawberries, the strawberry root aphid, *Aphis forbesi* Weed and the strawberry aphid *Chaetosiphon fragaefolii* Cockerell. The strawberry aphid is found all across the range of cultivation, while the strawberry root aphid is restricted to east of the Rockies in the U.S.A. The latter is most important as a vector of virus disease. Resistant genotypes have been identified for the strawberry aphid, and resistance has been shown to be regulated by more than one locus with partial dominance and additive action.

Other important strawberry pests are plant bugs (*Lygus* spp.), root weevils (*Otiorhynchus* spp.), strawberry weevil (*Anthonomus signatus* Say), two-spotted spider mite (*Tetranychus urticae* Koch.) and the cyclamen mite [*Steneotarsonemus pallidus* (Banks)]. Moderate levels of resistance have been identified to two-spotted spider mite, strawberry aphid and black vine weevil (Table 13.3). Both leaf volatile and essential oil content have been examined as possible inhibitors to two-spotted spider mite attack (Hamilton-Kemp et al. 1988, Khanizadeh and Bélanger 1997).

Breeding for resistant types has been frequently complicated by negative correlations between resistance and horticulturally important traits (Maas and Galletta1989, Hancock et al. 1990). For example, Bringhurst et al. 1967 found Verticillium wilt resistance was negatively correlated with yield. Breeding for disease resistance has been further complicated by the presence of eco-or biotypes of the pathogen. In the

Table 13.3 Inheritance patterns of pest resistance in strawberries

Disease	Observations	Representative studies
Insects		
Black vine weevil – *Otiorhynchus sulcatus*	Tolerant genotypes identified; probable quantitative inheritance; trichome density associated with resistance	Cram 1978, Shanks and Doss 1986, Doss and Shanks 1988
Blossom weevil – *Anthonomus rubi*	Resistance is under independent genetic control from flowering time; additive genetic variance is the most important	Simpson 1997, 2002
Cyclamen mite – *Steneotarsonemus pallidus*	Resistant genotypes identified	Oydvin 1980
Obscure root weevil – *Sciopithes obscurus*	Tolerant genotypes identified	Cram 1978
Root aphid – *Aphis forbesi*	Resistant genotypes identified	Darrow et al. 1933
Strawberry aphid – *Chaetosiphon fragaefolii*	Resistant genotypes identified; regulated by more than one locus, with partial dominance and additive action, but highly resistant types are recoverable in backcross generations	Shanks and Barritt 1974, Barritt 1980, Crock et al. 1982
Strawberry root weevil – *Otiorhynchus* sp.	Tolerant genotypes identified	Cram 1978
Tarnished plant bug – *Lygus lineolaris*	Resistant genotypes identified	Tingey and Pillemer 1977, Schaefers 1980, Handley et al. 1991
Two-spotted Spider mites – *Tetranychus urticae*	Resistant genotypes identified; resistance is biotype specific; strong additive and dominance effects; little genotype × environment interaction	Schuster et al. 1980, Barritt and Shanks 1981, Shanks et al. 1995, Medina et al. 1999
Woods weevil – *Nemocestes incomptus*	Tolerant genotypes identified	Cram 1978
Nematodes		
Root lesion – *Pratylenchus penetrans*	Resistant genotypes identified	Szczygiel 1981c, Potter and Dale 1994
Needle – *Longidorus elongates*	Resistant genotypes identified, segregation patterns suggest high heritability	Szczygiel 1981a
Northern root-knot *Meloidogyne hapla*	Resistant genotypes identified, segregation patterns suggest high heritability	Szczygiel and Danek 1974, Szczygiel 1981b, Edwards et al. 1985

case of red stele root rot, there are over ten known races in the U.S., 12 in the U.K., 6 in Canada and 6 in Japan (Table 13.2). Regional variation in cultivar susceptibility to pathogens has also been documented for Alternaria leaf spot, ramularia leaf spot, leaf scorch, Verticillium wilt and anthracnose (Hancock et al. 1996b).

13.5.2 Environmental Adaptation

Strawberries are grown across a vast environmental zone, but several environmental factors commonly limit their productivity including: (1) heat and drought (2) salinity (3) winter cold (4) spring frosts and (5) insufficient chilling hours.

One of the most important production problems of strawberries is drought, which is often associated with high temperatures. Prior to the general use of irrigation, losses due to drought were very high and less than optimum soil moisture still plagues parts of all non-irrigated production regions. While there are numerous published suggestions that cultivars vary in their resistance to heat and drought, there are few formal genetic studies (Table 13.4). Heat and drought tolerance have also been described in several native genotypes of *F*. *virginiana* and *F*. *chiloensis*.

Excess salt from irrigation water is a major production problem in many arid agricultural regions. Irrigation with water containing more than 100 ppm sodium or chloride ions results in enough salt accumulation to cause yield loss without visible plant injury (Brown and Voth 1955). Some cultivars have been shown to be more 'salt tolerant' than others ('Lassen', 'Festival naya' and 'Fresno'), but few surveys have been made. Perhaps the best source of salt tolerance will come from native genotypes of *F*. *chiloensis* which live alongside the ocean in Chile and California (Hancock and Bringhurst 1979).

Strawberries generally bloom in early spring, when the chance of frost is relatively high. Flower buds, open flowers and young fruit are all injured by frost. Pistils are most sensitive to damage; however, some damage is likely to all flower parts if temperatures fall to –2° C (Darrow 1966). Winter freezing injury to the strawberry crown and inflorescence buds is also a serious limitation to strawberry production throughout the upper half of the Northern Hemisphere. Non-acclimated strawberry plants are usually killed when the crown temperature remains at –3° C for more than 1 or 2 hours (Scott and Lawrence 1975). Acclimated strawberry plants can survive crown temperatures of – 12° C to –15° C, although injury such as decreased vigor is visible at higher temperatures (Zurawicz and Stushnoff 1977, Marini and Boyce 1979).

Cultivars grown in the more northern regions of North America and Europe tend to be more winter hardy and this hardiness is highly heritable (Table 13.4). A wide range in bloom tolerance to frost has also been described, although regional correlations are not always apparent (Ourecky and Reich 1976). To elucidate the molecular basis of cold acclimation in strawberry, NDong et al. (1997) used differential screening to identify genes associated with low temperature acclimation. They identified three transcripts, *Fcor1 –3* (Fragaria Cold-Regulated 1–3), whose levels

Table 13.4 Genetics of adaptation, productivity, plant habit and fruit quality in strawberry

Attribute	Observations	Representative studies
Adaptations		
Chilling requirement	Quantitatively inherited; resistant genotypes identified	Darrow 1966
Concentrated ripening	Quantitatively inherited; negatively correlated with yield	Denisen and Buchele 1967, Moore et al. 1970, Barritt 1974, Moore et al. 1975
Drought and heat	Resistant genotypes identified	Hancock et al. 1990
Flowering date	Quantitatively inherited; earliness partially dominant; bloom and ripening dates closely correlated	Powers 1945, Wilson and Giamalva 1954, Zych 1966, Scott et al. 1972
Frost tolerance	Quantitatively inherited; resistant genotypes identified	Darrow and Scott 1947, Ourecky and Reich 1976
Harvest date	Quantitatively inherited; bloom and ripening dates closely correlated	Wilson and Giamalva 1954, Zych 1966
Photoperiod sensitivity	Numerous models proposed from single dominant gene to quantitative inheritance	Ahmadi et al. 1990, Serce and Hancock 2005, Shaw and Famula 2005
Salinity tolerance	Resistant genotypes identified	Hancock and Bringhurst 1979, Hancock et al. 1990
Winter cold hardiness	Quantitatively inherited; highly heritable; resistant genotypes identified	Powers 1945
Productivity		
Flower number	Quantitatively inherited through several yield components; both additive and epistatic variation is important depending on the population	Morrow et al. 1958, Watkins et al. 1970, Spangelo et al. 1971, Lal and Seth 1981
Fruit size	Quantitatively inherited, with 6 to 8 allelic pairs regulating fruit expansion; both additive and epistatic variation is important depending on the population	Comstock et al. 1958, Sherman et al. 1966, Hansche et al. 1968, Scott et al. 1972
Runner number	Quantitatively inherited; high general combining ability	Simpson and Sharp 1988
Total yield	Quantitatively inherited; both additive and epistatic variation is important depending on the population; often negative interactions between yield components	Hansche et al. 1968, Watkins et al. 1970, Spangelo et al. 1971, Webb 1974, Mason and Rath 1980, Shaw et al. 1989

Table 13.4 (continued)

Attribute	Observations	Representative studies
Fruit quality		
Acidity	Controlled with varying levels of additive and dominance control depending on the population	Duewer and Zych 1967, Lal and Seth 1979, Shaw et al. 1987, Shaw 1988
Firmness	Quantitatively inherited; flesh firmness and skin toughness often correlated positively	Hansche et al. 1968, Ourecky and Bourne 1968, Barritt 1979, Shaw et al. 1987
Ease of calyx removal	Quantitatively inherited; much additive variation; low capping force can be dominant	Brown and Moore 1975, Barritt 1976
Color	Skin and flesh color quantitatively inherited; largely additive with a few major genes; Internal and external color poorly correlated	MacLachlan 1974, Murawski 1968, Lundergan and Moore 1975, Shaw and Sacks 1995
Pedicle length	Quantitatively inherited	MacIntyre and Gooding 1978, Dale et al. 1987
Sugar content	Controlled with varying levels of additive and dominance control depending on the population; individual sugars vary more than total sugars; negative association between soluble solids and yield	Duewer and Zych 1967, Lal and Seth 1979, Wentzel 1980, Shaw et al. 1987, Shaw 1988
Vitamin C	Quantitatively inherited, with partial dominance for high levels	Hansen and Waldo 1944, Anstey and Wilcox 1950, Lundergan and Moore 1975, Lal and Seth 1979

changed dramatically after cold-acclimation. Transcript accumulation for *Fcor3* was the most closely correlated with freezing tolerance, suggesting it may be a useful marker for this trait. *Fcor3* encodes a polypeptide that shows high identity with PSI polypeptides from spinach and barley.

Insufficient chilling can result in reduced yields in many of the regions of the world with moderate winters. Cultivars vary substantially in their chilling requirements, time of bloom and ripening dates; however, few quantitative genetics studies have been performed on these characteristics (Table 13.4). Cultivars that are adapted to warm southern areas, such as the southern U.S., Mediterranean regions and Africa, appear to have the shortest rest periods and these plants are capable of growing and ripening fruit during the short days of summer. Considerable variation in bloom and ripening dates also exist within regions of adaptation, with time of bloom and ripening dates often being closely correlated. Earliness can act as a partially dominant trait (Powers 1945, Scott et al. 1972).

13.5.3 Flowering and Fruiting Habit

There are two types of octoploid plants that can produce more than one crop a year: day-neutrals and long-day; although continuums in growth habit and flowering behavior make rigid classifications difficult (Nicoll and Galletta 1987). Short day cultivars tend to have a limited harvest window, and as a result day-neutral cultivars have increased in importance. The inheritance behavior of multiple cropping in strawberries has been the subject of numerous studies. Hypothesis concerning the inheritance of day-neutrality have ranged from a single recessive gene (Darrow 1937), a single dominant gene (Ahmadi et al. 1990), two dominant complementary genes (Ourecky and Slate 1967), two or more complementary dominant genes of equal potency and at least four recessive genes (Powers 1954). The most recent studies suggest that a large portion of the variance can be explained by a dominant gene (Shaw and Famula 2005), although numerous other loci probably play a role in conditioning day-neutrality (Serce and Hancock 2005). Major QTL have been identified for photoperiod sensitivity in *F*. × *ananassa*, as will be described in the section on genetic mapping, but no attempts have been made to identify the specific gene(s) responsible (Weebadde et al. 2007).

The genetics of multiple cropping in 'alpine' forms of European *F*. *vesca* is much simpler than that of *F*. × *ananassa*, due partly to their diploid instead of octoploid nature. The everbearers 'Baron Solemacher' and 'Bush White' contain a homozygous recessive gene for day neutrality (Brown and Wareing 1965). Day neutrality has not been observed in North American populations of *F*. *vesca*, and when California clones were crossed with alpine forms, at least three genes were identified that controlled photoperiodism (Ahmadi et al. 1990). Molecular markers have been identified that are closely linked to the seasonal flowering locus in *F*. *vesca* (Cekic et al. 2001).

Most day-neutral types of diploids and octoploids produce limited numbers of runners, although Simpson and Sharp (1988) found considerable variation for stolon production and yield in everbearing, octoploid types. General combining ability was the strongest component of fruit yield, but specific combining ability played a more important role in stolon production. They suggested that early fruiting and adequate stolon production could be combined in an everbearing type. Yu and Davis (1995) found a tight genetic linkage between runnering and a phosphoglucoisomerase locus in diploid strawberry.

13.5.4 Fruit Quality

Several factors restrict consumer acceptance of strawberry fruit including size, flavor, nutrition and color. Other important factors are flesh firmness and skin toughness. Size of fruit is inherited quantitatively, with 6 to 8 allelic pairs controlling fruit expansion (Table 13.4). There is a decline in size of fruits from the primary to inferior positions, and the relative decline varies substantially among genotypes. Several genetic studies have shown that a large part of the genetic variance for fruit size is

epistatic, although there is still considerable additive variability, depending on the parents. Much genetic variability has also been identified in breeding populations for firmness. Flesh firmness and skin toughness are often correlated positively and are generally inherited quantitatively.

A number of recent molecular studies have searched for the genes in strawberry that are involved in cell wall modification during ripening and therefore influence fruit-firmness. Harrison et al. (2001) identified and characterized a number of expansin genes (*FaExp2* to *FaExp7*), which likely induce cell wall extension in vitro. Messenger RNA from most of these were present in leaves, roots and fruit, except for *FaExp5*, which showed fruit specific expression. Castillejo et al. 2004 isolated four pectin esterases genes from strawberry (*FaPE1* to *FaPE4*). *FaPE1* was specifically expressed in fruit and was up-regulated by auxin treatment in green fruit and down regulated by exogenous applied ethylene in ripe and senescing fruits. The repression of *FaPE1* may be involved in textural changes during fruit senescence. Blanco-Portales et al. (2004) identified a fruit-specific gene encoding for a HyPRP protein involved in the anchoring of polyphenols to cell membranes. Salentijn et al. (2003) found the expression of two genes associated with lignin metabolism (cinnamoyl CoA reductase and cinnamyl alcohol dehydrogense) to vary dramatically between soft fruited ('Gorella') and firm-fruited ('Holiday') cultivars.

Three full length cDNAs encoding ß-galactosidases (*Faßgal1*, *Faßgal2* and *Faßgal3*) were isolated from a library representing red fruit transcripts by Trainotti et al. (2001). Two of the genes had a C-terminus domain that was structurally related to known animal peptides with sugar-binding ability. Galactose is released during the dismantling of cell walls and the galactosidases are thought to play an important role in the mobilization of galactose. In a study of salt extractable proteins from the cell walls of immature and ripe strawberry, Iannetta et al. (2004) identified seven abundant polypeptides; two of which were thought to be important determinants in the regulation of the sugar:acid balance (mitochondrial malate dehydrogenase and mitochondrial citrase synthase).

Soluble solids and acidity are controlled with varying levels of additive and dominance control (Shaw et al. 1987, Shaw 1988). Shaw (1988) found little difference in the soluble solids and total sugars in his breeding population, although he did observe significant genotypic variation in sucrose, glucose, fructose and acidity levels. Wenzel (1980) found a negative association between soluble solids concentration and yield. Vitamin C content has also been shown to be polygenic, with some parents displaying partial dominance for high levels and some progeny having higher levels than their parents (Hancock et al. 1996b). Several studies have described variation in the flavor of progeny families suggesting additive quantitative control (Darrow 1966), although few formal genetic studies on this character have been conducted. Zubov and Stankevich (1982) found significant seedling variation in fruit consistency, anthocyanin content and vitamin C, but not flavor. GCA was greater than SCA for all the other traits except vitamin C.

An NADPH-dependent D-galacturonic acid reductase gene (*GalUR*) was isolated and characterized from strawberry to determine its role in vitamin C content (Agius et al. 2003). Expression of *GalUR* correlated closely with ascorbic acid levels during

strawberry fruit ripening and GalUR protein levels were found to be associated with ascorbic acid content in four species of *Fragaria* (*F*. ×*ananassa*, *F*. *chiloensis*, *F*. *virginiana* and *F*. *moschata*). The gene was not engineered into strawberry, but overexpression of *GalUR* in Arabidopsis enhanced vitamin C content two-to three-fold.

Skin and flesh color have been shown to be largely under the control of additive variation, although a few genes appear to have much larger effects than others (Table 13.4). Internal and external colors are probably regulated by separate sets of genes as correlations between these two parameters are small. No molecular studies to date have attempted to associate specific genes with segregation for fruit color in *F*. ×*ananassa*, but Wilkinson et al. (1995) has identified a gene for chalcone synthase (CHS) that is highly expressed in ripening strawberry fruit and is likely a key enzymatic step in flavonoid biosynthesis. Aharoni et al. (2001) has also cloned the transcription factor *FaMYB1* from ripening fruit, which plays a key role in the biosynthesis of anthocayanins and flavonols. Deng and Davis (2001) found a polymorphism in the flavone 3-hydroxylase gene to be associated with a yellow fruit color in *F*. *vesca*.

Hoffman et al. (2006) used RNAi-induced silencing to reduce activity of CHS in strawberry fruits. They used a construct containing the partial sense and corresponding antisense sequences of CHS separated by an intron (from a strawberry quinone oxidoreductase gene). An *Agrobacterium* suspension containing the gene was injected into 14-day-old fruit still attached to the plant. Almost white fruit were produced when the injection was repeated three days in a row.

DNA microarrays have been utilized to identify and clone genes associated with strawberry flavor and aroma. Aharoni et al. (2000) found a novel strawberry alcohol acyltransferase (SAAT) in *F*. ×*ananassa* cultivar 'Elsanta' that is critical in flavor biogenesis in ripening fruit. This gene combines acyl-CoA and alcohol to generate the esters, the most important class of volatile compounds in fruit. Aharoni et al. (2004) also cloned the gene, *F*. *ananassa Nerolidol Synthase 1* (*FaNES1*), which was found in all three octoploid species, but not in *F*. *vesca* and *F*. *moschata*. It generates linalool and nerolindol when supplied by geranyl disphosphate or farnesyl diphosphate. They also found *F*. *vesca* to carry an insertion mutation in a terpene synthesase gene that differs from the one in the cultivated strawberry (*F*. ×*ananassa Pinene Synthase*). This insertion limited its expression and further altered aroma by reducing quantities of pinene and myrcene.

In a study of the catalytic properties of AAT in different strawberry species and cultivars, Olías et al. (2002) found that heptanol was the best straight-chain substrate for three European varieties, while hexanol was the prefered alcohol for two American cultivars; a genotype of *F*. *vesca* had the highest activity with pentanol. The cultivars had generally similar patterns of activity on straight chain acyl-CoAs, except for 'Eros' which had much higher activity than the others for pentanoyl-CoA. *F*. *vesca* also showed much lower activities for pentanoyl-CoA than most of the cultivars, and much higher levels of activity for acetyl-and propionyl-CoA.

A number of other genes have been characterized that are highly expressed during fruit ripening and maturation. Manning (1998) generated a cDNA library from

messenger RNA isolated from ripe fruit, and identified a number of genes encoding enzymes of phenylpropenoid metabolism, and genes for cellulase, expansins, cysteine proteinase and acyl carrier protein (Manning 1998). Three mRNAs with fruit specific, ripening-enhanced expression have also been identified in ripening fruit using polymerase chain reaction (PCR) differential display (Wilkinson et al. 1995). When sequenced, they had high homology with known proteins including: (1) an annexin which may play a role in membrane function and cell wall structure (2) chalcone synthase which is a key enzymatic step in flavonoid biosynthesis, and (3) a ribosomal protein, most likely a 40S subunit. In addition, a gene (*njjs4*) has been identified which is associated with the process of seed maturation and fruit ripening, and is related to the class-I LMW heat-shock-protein-like genes (Medina-Escobar et al. 1998). Two auxin-induced and one auxin-repressed mRNAs from unknown genes have been cloned from receptacles of immature green fruit (Reddy et al. 1990, Reddy and Poovaiah 1990). Yubero-Serrano et al. (2003) identified a gene encoding a lipid transfer protein (*Fxaltp*) in strawberry fruit that responds to ABA, wounding and cold stress. Aharoni et al. (2001) cloned the transcription factor *FaMYB1* from ripening fruit, which plays a key role in the biosynthesis of anthocayanins and flavonols.

DNA microarrays have also been used to profile cosmic patterns of gene expression during ripening. Aharoni and O'Connell (2002) found 441 transcripts to differ significantly between the achene and receptacle tissues. The most common transcripts found in achenes were those for signal and regulation cascades associated with achene maturation, and stress tolerance. Representatives included phosphatases, protein kinases, 14-3-3 proteins and transcription factors. Several genes were identified in the receptacle that encode proteins related to stress, the cell wall, DNA/RNA protein and primary metabolism.

13.5.5 Yield

Yield is the product of a combination of characters, such as number and size of fruit, plant vigor, hardiness, and disease resistance of the plant. Crown number per row area is often the factor most strongly associated with yield, although flower number and fruit size are also important components. High crown numbers can be achieved through either high levels of stolon production or branch crown production.

Strong compensatory interactions have often been found between the various yield components (plant density, crowns per plant, trusses per crown, fruit per truss, etc.), indicating that breeding for high fruit numbers or individual fruit size by themselves will not necessarily increase productivity (Hancock et al. 1996b). However, outlier types do exist with both large fruit and high fruit numbers (Hancock and Bringhurst 1988).

Considerable levels of genetic variability have been described for most yield components, although the relative levels of additive and non-additive variation have varied greatly from study to study (Table 13.4). In most studies, sufficient levels of additive variation were considered available for rapid improvement of yield.

Hansche et al. (1968) found extensive levels of genetic associated with fruit size, firmness and yield in the University of California (UC-Davis) breeding program, but not appearance. A significant genetic correlation existed between fruit size and yield, indicating that plants with large berries have a genetic potential for high yield. When the UC-Davis breeding population was evaluated 20 years later, heritability estimates were not significantly different from the ancestral population (Shaw et al. 1989). In a few breeding populations, non-additive gene influences have appeared to be more important than additive ones (Watkins et al. 1970, Spangelo et al. 1971), suggesting that crosses should be designed to exploit all the genetic variance, whether it be additive, dominant, or epistatic.

13.5.6 Adaptability to Mechanical Harvesting

A recurring objective in strawberry breeding has been to produce types adapted to mechanical harvesting, although few cultivars have been developed that produce consistently profitable yields when machine harvested. Paramount are concentrated ripening for once over harvest, long pedicles and either easy calyx removal or long necks for machine decapping. There is considerable variation for concentrated ripening (Denisen and Buchele 1967, Moore et al. 1970, Barritt 1974), although concentrated ripeners are often lower yielding than longer season types (Moore et al. 1975). Ease of calyx removal shows considerable additive genetic action (Barritt 1976), and in some parents, low capping force is dominant (Brown and Moore 1975). Considerable variation in pedicle and fruit neck length has also been reported (MacIntyre and Gooding 1978, Dale et al. 1987). Unfortunately, few cultivars have been released for mechanical harvesting (Daubeny et al. 1980).

13.6 Crossing and Evaluation Techniques

13.6.1 Breeding Systems

The dessert strawberry is an outcrossed crop that is relatively sensitive to inbreeding (Morrow and Darrow 1952, Melville et al. 1980b), and it can be asexually propagated by runners, so most varietal improvement programs have been based on pedigree breeding where elite parents are selected each generation for inter-crossing. If adequate population sizes are maintained, changes in levels of homozygosity across generations appear to be minimal (Shaw and Sacks 1995). Since highly heterozygous genotypes can be propagated as runners, few breeding programs have developed hybrid varieties using inbred lines, although some cultivars have been developed this way.

Selfing has been successfully employed in a number of instances to concentrate genes of interest (Hancock et al. 1996a) and backcrossing has been used occasionally to incorporate specific traits. Barritt and Shanks (1980) moved resistance to the strawberry aphid from native *F. chiloensis* to *F. ×ananassa*. Bringhurst and

Voth (1976, 1984) transferred the day neutrality trait from native *F. virginiana* spp. *glauca* to *F. ×ananassa*. Approximately 3 generations were necessary to restore fruit size and yield to commercial levels.

Numerous studies have been designed to test the effects of temporal, spatial and developmental variation on production traits, with the final intent to develop efficient selection strategies (Gooding et al. 1975, Hortynski 1989). Shaw and coworkers (1987) found that within a single year, the distribution of genetic and environmental variance components for a single trait vary continuously, with heritabilities for yield and fruit size being highest in the middle of the season. They also found that seedling location has a large effect on the expression of genetic variation (Shaw 1989, Shaw et al. 1989). Shaw (1991) observed that nursery treatments induced large interactions for production traits in annual systems, especially those that condition variable levels of plant development and chilling. His studies indicated that crossing among parents chosen for breeding value may be more effective than simple clonal performance in generating superior seedling populations. In fact, the performance of seedlings may be very different when propagated as runner plants or when grown in different environments.

13.6.2 Pollination and Seedling Culture

The blossom of strawberry is composed of many pistils, each with its own style and stigmata, attached to a receptacle that on fertilization of the pistils develops into a fleshy 'fruit'. The true fruits of the strawberry are the achenes which carry one seed and are found on the surface of the swollen receptacle. A single blossom may have 20–400 pistils that develop into seeds, depending on the size of the blossom and its position on the cluster. Primary flowers have the largest number of pistils, with secondary, tertiary and later flowers having progressively fewer (Hancock 1999).

Strawberry flowers are usually emasculated using a scalpel, tweezers or thumb nails, by carefully removing the ring of sepals, petals and anthers surrounding the receptacle. Care is taken not to rupture any anthers. Emasculation is usually done 1 to 3 days before anthesis to prevent selfing; this is frequently done when the first white of the petals begins to show as the sepals separate. Emasculated flowers must be protected from foreign pollen either by bagging or isolation. Cotton gauze is often used to cover individual flowers or clusters. Much of the hybridization work is done using potted plants in the greenhouse as it is easier to prevent contamination and control the environment.

Pollen is collected by removing individual anthers from the blossoms 1 to 2 days before anthesis and placing them in vials to dehisce, or by detaching flowers from the clusters, removing petals and sepals, and placing them overnight in paper lined shallow vessels such as petri plates. Pollen will remain viable for several days if stored at room temperature, and for several years if stored at 4° C under low humidity. Pollen can be transferred to stigmas with a small camel's hair brush, a rubber-tipped rod or a finger tip. Alcohol is commonly used to sterilize the transfer vehicles between crosses.

Fruits ripen 25–30 days after pollination at 18–25°C. Large quantities of fruit can be processed by threshing them in water with a 10 to 15 second spin in a food blender. The pulp floats, while the seeds sink. Smaller quantities of fruit can be mashed on absorbent paper and the seeds scraped off after the residue has dried. For long term storage, seeds are generally placed in coin envelopes and held under low humidity at 1–4°C. Under these conditions seed remain viable for over 20 years, depending on genotype (Scott and Draper 1970).

For germination, seeds should be spread on the surface of the soil and held under light. Without pre-treatment, seedling emergence is irregular, with some seeds germinating within 10 days, while others can take up to 90 days. Time of emergence can be normalized by after-ripening the seed for 2.5–3 months at 1–4°C (Bringhurst and Voth 1957), or scarifying it for 10–15 minutes in concentrated sulfuric acid. Seedlings are generally allowed to grow for six weeks in the seed trays until they have a few true leaves and then they are transplanted into pots, where they are grown for another 6 to 8 weeks before being planted in the field.

13.6.3 Evaluation Techniques

Field plantings of first-test seedlings intended for matted row culture are generally planted in the spring at 45–60 cm spacing and are allowed to form small matted blocks that are about 25 cm wide. Hybrids intended for annual hill systems are planted in the fall in plastic covered ridges at 20 cm spacing. Elite clones are selected from both systems in the summer of the second year and runners are collected in the fall for trial planting in the third year. A randomized block design is then used to evaluate the elite clones in replicates of 5–10 plants maintained at commercial spacing. The hybrids are evaluated for one to two years, and then runners from these are sent to collaborators for further testing in randomized designs. Decisions about release are generally made 6–8 years after the initial crosses.

13.7 Biotechnological Approaches to Genetic Improvement

13.7.1 Genetic Mapping and QTL Analysis

Numerous marker systems have been developed in strawberry for genetic linkage mapping and QTL analysis (Hadonou et al. 2004, Sargent et al. 2004a, Cipriani et al. 2006). These have been shown to be broadly applicable across all strawberry species, although SSRs developed from other Rosaceae species have only limited utility.

Davis and Yu (1997) provided the first diploid map of *F. vesca*, using RAPD markers and isozymes, plus some morphological traits. They crossed the cultivar Baron Solemacher of *F. vesca* f. *semperflorens* and a wild clone of *F. vesca* ssp. *vesca* collected in New Hampshire, and developed an 80-marker map in the

F_2 population that represented all seven linkage groups and was 445 cM long. Unusually high levels of segregation distortion was noted (47%) that was skewed toward the maternal grandparent, 'Baron Solemacher'. Davis and Yu speculated that the segregation distortion was caused by the maternal cytoplasm favoring maternal genes.

Deng and Davis (2001) used a candidate gene approach to determine the molecular basis of the yellow fruit color locus (c) in diploid strawberry. They employed PCR and degenerate primer pairs to examine segregation patterns in intron length polymorphism's of a number of genes involved in the anthocyanin biosynthetic pathway. They studied F_2 progeny populations of a wild clone of northern California *F. vesca* 'Yellow Wonder' × an *F. nubicola* genotype from Pakistan, and were able to place five genes into their previously published map. They found $F3H$, the gene encoding flavanone 3-hydrolase, to be the likely candidate for the yellow fruit color locus.

Most recently, a diploid map of 78 markers was constructed from a hybrid population of *F. vesca* ssp. *vesca* f. *semperflorens* × *F. nubicola* (Sargent et al. 2004a). They authors used a combination of SSRs, SCARs, gene specific markers and morphological markers that came from the GenBank data base and other studies. All seven linkage groups were identified in their map that covered 448 cM. Segregation distortions were noted at 54% of the loci that were skewed toward the paternal parent *F. nubicola*. They speculated that the segregation distortions were due to meiotic irregularities or the self-incompatible nature of *F. nubicola*.

Lerceteau-Köhler et al. (2003) used a total of 727 AFLP markers and 119 individuals to build both a female map and a male map from the cross of 'Capitola' × CF1116 ['Pajaro' × ('Earliglow' × 'Chandler')]. The female map was built with 235 markers and was 1604 cM long, while the male map was 1496 cM long with 280 markers. Only 3.2% of the markers displayed distorted segregation ratios. They detected 30 linkage groups on the female side and 28 on the male side, but did not develop a consensus map of the two parents. The female genome size was estimated to be 2870 cM, while the male genome size was 1861 cM.

Viruel et al. (2002) used 300 SSR and RFLP markers and 86 progeny to build a consensus linkage map with 17 linkage groups and a total distance of 627 cM. 120 markers were unlinked or linked to only one marker, suggesting the need for more markers to build a complete map. Only 10% of the markers showed distorted segregation ratios.

Weebadde et al. (2007) genotyped sixty-seven individuals of the cross 'Tribute' × 'Honeoye' with AFLP markers. Out of 611 polymorphic bands obtained using 52 primer combinations, 410 single dose restriction fragments (SDRFs) were identified and 23 linkage groups. Most of the markers (255/410) remained unlinked, indicating the need for more markers and larger population sizes to build a map with wide genome coverage.

Only a few quantitative trait loci (QTL) analyses have been conducted in strawberry. In the study of Weebadde et al. (2007), two AFLP markers were significantly associated with segregation of the day-neutrality trait at a 0.01% level and five at a 0.1% level (Fig. 13.3). Several of these markers were not linked, indicating
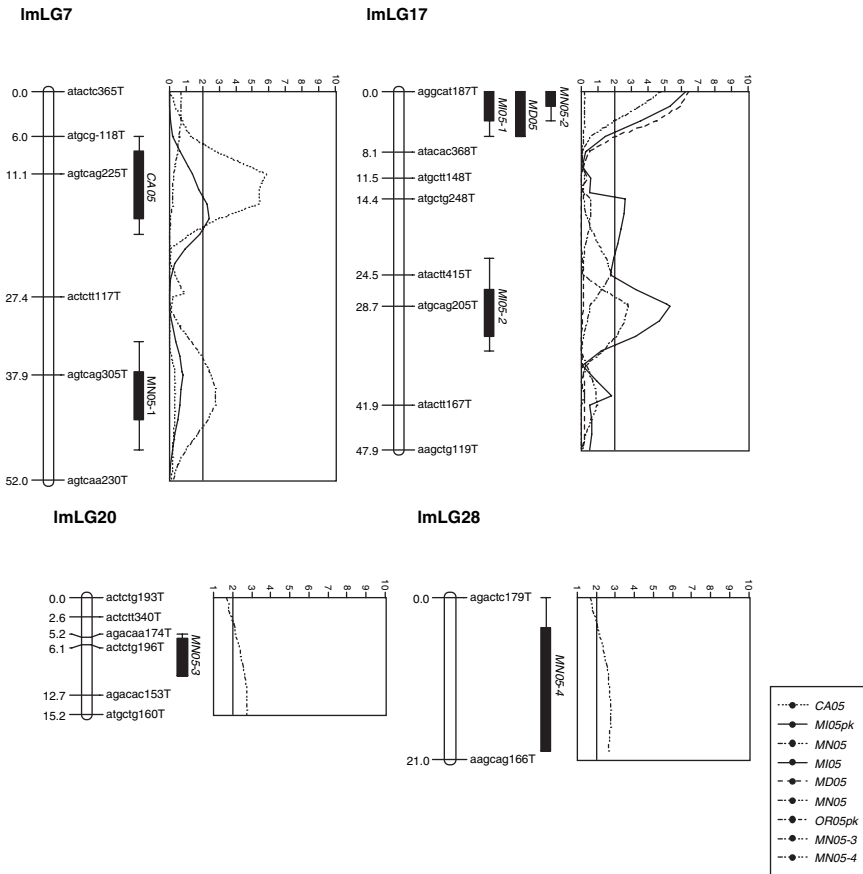
Fig. 13.3 QTL for the day-neutral flowering response detected in a segregating population of 'Tribute' (day-neutral) × 'Honeoye' (short day) strawberries evaluated in Michigan (MI), Minnesota (MN), Maryland (MD), California (CA) and Oregon (OR). All the QTL associated with day-neutrality were derived from the cultivar 'Tribute' (Webbadde et al. 2007)

that day-neutrality is a quantitative trait in the octoploids. Haymes found AFLP markers linked to three red stele resistance genes (Hokanson and Maas 2001). Lerceteau-Köhler et al. (2004) found fourteen QTL associated with seven characters (fruit height, ratio fruit height/diameter, fruit color, firmness, malate content, glucose content and ratio fructose to glucose). The percentages of phenotypic variance explained by the QTL ranged from 12% to 20%.

13.7.2 Regeneration and Transformation

Strawberries were one of the first crops to be routinely proliferated through micropropagation (Zimmerman 1991), and regeneration systems for *F*. ×*ananassa* have been developed with disarmed strains of *Agrobacterium tumefaciens* using

anthers, callus, flower buds, leaf discs, protoplasts, petioles, stems, stipules, roots and runners (Hokanson and Maas 2001, Passey et al. 2003). Callus, petiole sections and leaf discs have also been used in combination with *A. tumefaciens* to regenerate *F. vesca* (Haymes and Davis 1998, Alsheikh et al. 2002). Genotypes vary widely in the success of the various techniques, and some are quite recalcitrant to all techniques. A genetic line of *F. ×ananassa*, LF9, has been developed that produces transformed shoots in as few as 15 days (Folta et al. 2006).

Most of the transgenic strawberries have been generated using *Agrobacterium*-mediated tranformation systems. *Agrobacteriun* strains LBA4404 and EHA105 have been most commonly employed, with pBIN19 derivates as the binary vector (Graham 2005). In most cases, leaf or stem based systems have been utilized in Murashige and Skoog (MS) medium. The hormones have been BA plus 2,4-D or IAA. Regenerates have generally been selected using 25 mg/L kanamycin, and contamination after inoculation has been limited by using cefotaxime, carbenicillim and ticaricillin. Biolistics have been employed in three instances with strawberry. Cordero de Mesa et al. 2000 bombarded leaf discs with *Agrobacterium* coated gold microprojectiles as a means to enhance stable transformation of GUS. Wang et al. (2004) bombarded strawberry calli with tungsten particles coated with the pBY520 plasmid using PDS-1000/He. Agius et al. (2005) used particle bombardment to effect transient transformation of strawberry fruit.

An effective marker-free transformation process has recently been successfully tested in strawberry (Schaart et al. 2004). In it, a vector was constructed in which site-specific recombination left only the 35S promoter of cauliflower mosaic and a GUS encoding sequence. The system incorporated an inducible site-specific recombinase to eliminate the selectable marker. Fully marker-free transgenic 'Calypso' plants were obtained through this procedure.

Two types of herbicide resistance have been engineered into strawberry through *Agrobacterium* mediated transformation. The phosphinothricin acetyl transferase gene (PAT) was incorporated into the 'Selekta' strawberry using *Agrobacterium* mediated gene transfer (du Plessis et al. 1995, 1997). Putatively transformed shoots were rooted and established in the greenhouse and most transgenic plants were found to be resistant to the herbicide glufosinate-ammonium (Ignite®). The CP4.EPSP synthase gene, which confers resistance to glyphosate (Roundup®) was introduced into 'Camarosa' (Morgan et al. 2002), and when 73 independent transformations were sprayed with Roundup in the nursery, a range of responses were noted from complete resistance to death. Expression levels of the *CP4.EPSPS* gene was strongly correlated with phenotype. The best lines were subsequently tested in the field and appeared to produce good quality fruit.

To provide insect resistance, the cowpea protease trypsin inhibitor gene (*CpTi*) (Agricultural Genetics Company, Cambridge, England) was incorporated into strawberry via *Agrobacterium* mediated transformation using the *NPTII* marker (Graham et al. 1995, Graham et al. 1997, 2002). The insertion of *CpTi* into strawberry cultivars 'Melody' and 'Symphony' was found to reduce vine weevil (*Otiorhynchus sulcatus*) damage in both greenhouse and field trials. The transgenic lines showed increased root growth, less larval feeding and fewer pupae. In other work,

strawberries transformed with the lectin *Galanthus nivalis agglutin gene* (*GNA*), did not show any significant reduction in weevil feeding (Graham 2005).

Enhanced resistance to *Verticillium dahliae* was observed in transgenic 'Joliette' strawberry plants expressing a *Lycopersicon chilense* chitinase gene (*pcht28*) under the control of the CAMV 35S promoter (Chalavi et al. 2003). A stipule regeneration system was used with *Agrobacterium*-mediated gene transfer. Constituative expression of the chitinase gene was demonstrated by northern analysis, and in growth chamber studies, transgenic strawberry plants had significantly higher resistance than controls, based on rates of crown infection and leaf wilting symptoms.

The antisense of strawberry pectate lyase was incorporated into 'Chandler' strawberry under control of the 35S promoter to increase fruit firmness (Jiménez-Bermúdez et al. 2002). At full ripening, no differences in color, shape and weight were noted between the transgenic and control plants, but the transgenics were significantly firmer. Pectase lyase activity was 30% lower in ripe transgenic fruit than the control. In another study, Agius et al. (2003) found that expression of an antisense sequence of a strawberry pectate lyase gene reduced ascorbic acid content, presumably through reduced pectin solubilization in cell walls of transgenic plants.

Two endo-β-1,4-glucanase (EG) genes, *cel1* and *cel2*, have been isolated from strawberry that are closely related to tomato genes influencing softening. *Cel1* were expressed specifically in ripening fruit (Manning 1998), while *cel2* mRNA was found primarily in young vegetative tissues and early green fruit (Trainotti et al. 1999). *Cel1* has been cloned into strawberry in the antisense orientation via *Agrobacterium* – mediated transformation using the plant binary vector pBIN-PLUS (Woolley et al. 2001). In the transgenic strawberries, mRNA was strongly suppressed in ripe fruit; however, EG activity and firmness were not affected. The incorporation of *cel1* had no effect on the transcription *cel2*.

The S-adenosylmethionine hydrolase gene (*SAMase*) has been incorporated into strawberry which controls ethylene biosynthesis and presumably effects fruit softening (Mathews et al. 1995). Strawberries are not climacteric fruit, but do have a limited response to ethylene and it is possible that reductions in ethylene biosynthesis during the post harvest period could slow down softening.

De la Fuente et al. (2006) has cloned *FaGAST* from strawberry, which encodes a small protein with 12 cysteine residues conserved in the C-terminal region that is similar to a group of proteins in other plant species that regulate cell division and elongation. Expression of *FaGAST* in transgenic *F*. *vesca*, under the control of CaMV-35S, resulted in delayed fruit growth, reduced fruit size, late flowering and low sensitivity to gibberellin. Apparently, *FaGAST* plays a role in arresting fruit elongation during strawberry fruit ripening.

The acidic dehydrin gene *WCOR410* from wheat was transferred to strawberry in an attempt to improve freezing tolerance (Houde et al. 2004). The WCOR410 protein has been associated with the plasma membrane in wheat and its levels have been correlated with freezing tolerance. After acclimation, transgenic strawberry leaves had a 5° C improvement in freezing tolerance compared to controls. However, there was not a difference in the freezing tolerance of non-acclimated transgenics and controls, suggesting that another factor induces its expression during cold acclimation.

Transgenic strawberries have also been developed that expressed anti-freeze protein gene (*AFP*) isolated from white flounder (Firsov and Dolgov 1998), but no data from freezing trials has been published.

In an earlier attempt to increase the freezing tolerance of strawberries, the transcription factor *CBF1* from Arabadopsis was overexpressed in the strawberry 'Honeoye' (Owens et al. 2002). The *CBF* genes are part of a family of cold and drought inducible transcription factors that bind to promoters containing a C-Repeat/DehydrationResponsive Element (CRT/DRE). This element is found in many cold-induced plant genes. *Agrobacterium*-mediated transformation with a CaMV35S-CBF1 construct was utilized to produce transgenic strawberries. The freezing tolerance of strawberry leaf-discs from non-acclimated plants was significantly increased by 3–5° C. However, the freezing tolerance of floral tissue was not, despite expression of the *CBF1* transgene in receptacles at levels similar to developing leaves. A putative ortholog [*Fragaria* ×*ananassa CBF1* (*FaCBF1*)] was identified with 48% amino acid identity to *CBF1* from Arabidopsis.

The late embryogenesis abundant protein gene (*LEA3*) from barley (*Hordeum vulgare*) was used to transform the 'Toyonaka' strawberry (Wang et al. 2004) in hopes of increasing the resistance of strawberry to salt stress. Calli from anthers were transformed by particle bombardment with plasmid pBY520. In vitro plants of transgenic strawberry had significantly less wilting than controls under 50 mmol (19% vs. 62%) and 100 mmol NaCl (43% vs. 96%).

Mezzetti et al. (2004) developed transgenic strawberries and raspberries carrying the *defH9-iaaM* auxin-synthesizing construct, composed of the regulatory region of the *DefH9* gene from snapdragon and the *iaaM* coding region from *Pseudomonas syringae*. The *defH9-iaaM* gene was found to promote parthenocarpy in emasculated flowers of both strawberry and raspberry, and to increase fruit size, weight and yield.

The *FBP7* promoter (*floral binding protein7*) from Petunia was found to be active in floral and fruit tissues of strawberry, using the ß-glucuronidase gene as a reporter (Schaart et al. 2002). GUS activity was found in floral and fruit tissues, but not vegetative ones, although gus-derived mRNAs were found in roots and petioles. The *35S* promoter was found to be sixfold stronger than the *FBP7* promoter.

Agius et al. (2005) used a transient expression system to conduct a functional analysis of homologous and heterologous promoters in strawberry fruit. The *CaMV 35S* promoter was fused to the *LUC* gene to optimize the transient assay. The *GalUR* promoter from strawberry was found to be active in fruit and under light regulation. Slight activity in fruit was found for the pepper fibrillin promoter, but not for the tomato polygalacturonase promoter.

A MADS box gene from strawberry, *STAG1*, has been cloned and characterized in transgenic plants (Rosin et al. 2003). *STAG1* shares 68–91% sequence homology with *AGAMOUS* from numerous plant species. Analysis of the expression patterns of a GUS marker gene driven by the *STAG1* promoter revealed that *STAG1* was active in stamens, receptacles, petals, central pith and vascular cells during floral development and achenes, pith and cortical cells during fruit ripening.

13.7.3 Genomic Resources

Two major EST libraries of have been generated as genomic resources in strawberry. A cDNA library of over 1,800 ESTs has been produced from whole plants treated with salicyclic acid by Folta et al. (2005). This effort is part of a major Rosaceae genomics project (Jung et al. 2004). Batley et al. (2005) have generated over 23,600 ESTs from a range of tissues, developmental stages and experimental conditions, and identified 11,690 single nucleotide polymorphisms (SNPs) and 4,200 SSRs for mapping. Their focus is on traits such as day neutrality and the fruit characteristics of firmness, flavor, taste, aroma and color. cDNA libraries of *F. vesca* are also being constructed by Davis (2005) and Slovin (person. comm.).

References

Agius F, González-Lamothe R, Caballero JL, Muñoz-Blanco J, Botella MA, Valpuesta V (2003) Engineering increased vitamin C levels in plants by overexpression of a D-galacturonic acid reductase. Nat Biotechnol 21:177–181

Agius F, Amaya I, Botella MA, Valpuesta V (2005) Functional analysis of homologous and heterologous promoters in strawberry fruits using transient expression. J Exp Bot 56:37–46

Aharoni A, De Vos C, Wein M, Sun Z, Greco R, Kroon A, Mol J, O'Connell A (2001) The strawberry *FaMYB1* transcription factor suppresses anthocyanin and flavonol accumulation in transgenic tobacco. Plant J 28:319–332

Aharoni A, Giri A, Verstappen F, Bertea C, Sevenier R, Sun Z, Jongsma M, Schwab W, Bouwmeester H (2004) Gain and loss of fruit flavor compounds produced by wild and cultivated strawberry species. Plant Cell 16:3110–3131

Aharoni A, Keizer L, Bouwmeester H, Sun Z, Alvarez-Huerta M, Verhoeven H, Blaas J, van Houweilingen A, De Vos C, van der Voet H, Jansen R, Guis M, Mol J, Davis R, Schena M, van Tunen A, O'Connell A (2000) Identification of the SAAT gene involved in strawberry flavor biogenesis by use of DNA microarrays. Plant Cell 12:647–661

Aharoni A, O'Connell A (2002) Gene expression analysis of strawberry achene and receptacle maturation using DNA microarrays. J Exp Bot 53:2073–2087

Ahmadi H, Bringhurst RS (1991) Genetics of sex expression in *Fragaria* species. Am J Bot 78:504–514

Ahmadi H, Bringhurst RS, Voth V (1990) Modes of inheritance of photoperiodism in *Fragaria*. J Am Soc Hortic Sci 115:146–152

Alsheikh M, Suso H, Robson M, Battey N (2002) Appropriate choice of antibiotic and Agrobacterium strain improves transformation of antibiotic-sensitive *Fragaria vesca* and *F. v. semperflorens*. Plant Cell Rep 20:1173–1180

Anstey TH, Wilcox AN (1950) The breeding value of selected inbred clones of strawberries with respect to their vitamin C content. Sci Agric 30:367–374

Arulsekar S (1979) *Verticillium* wilt resistance in the cultivated strawberries and preliminary studies on isozymes genetics in *Fragaria*. PhD. University of California, Davis

Arulsekar S, Bringhurst RS (1981) Genetic model for the enzyme marker PGI in diploid California *Fragaria vesca*. J Hered 73:117–120

Ashley M, Wilk J, Styan S, Craft K, Jones K, Feldman K, Lewers K, Ashman T (2003) High variability and disomic segregation of microsatellites in the octoploid *Fragaria virginiana* Mill. (Rosaceae). Theor Appl Genet 107:1201–1207

Barritt BH (1974) Single harvest yields of strawberries in relation to cultivar and time of harvest. J Am Soc Hortic Sci 99:6–8

Barritt BH (1976) Evaluation of strawberry parent clones for easy calyx removal. J Am Soc Hortic Sci 101:590–591

Barritt BH (1979) Breeding strawberries for fruit firmness. J Amer Soc Hort Sci 104:663–665

Barritt BH (1980) Resistance of strawberry clones to Botrytis fruit rot. J Am Soc Hortic Sci 105:160–164

Barritt BH, Daubeny HA (1982) Inheritance of virus tolerance in strawberry. J Am Soc Hortic Sci 107:278–282

Barritt BH, Shanks CH (1980) Breeding strawberries for resistance to the aphids *Chaetosiphon fragaefolii* and *C. thomasi*. HortScience 15:287–288

Barritt BH, Shanks CH (1981) Parent selection in breeding strawberries resistant to two-spotted spider mites. HortScience 16:323–324

Batley J, Keniry A, Hopkins C, Mountford H, Logan E, Gramzow L, Morrison B, Spangenberg G, Edwards D (2005) A new genomics resource for strawberry: Towards molecular genetic markers for day neutrality traits. In: Plant & Animal Genomes XIII Conference, San Diego, CA

Bell JA, Simpson DW, Harris DC (1997) Development of a method for screening strawberry germplasm for resistance to *Phytophthora cactorum*. Acta Hortic 439:175–180

Blanco-Portales R, López-Raéz J, Bellido M, Moyano E, Dorado G, González-Reyes J, Caballero JL, Muñoz-Blanco J (2004) A strawberry fruit-specific and ripening-related gene codes for a HyPRP protein involved in polyphenol anchoring. Plant Mol Biol 55:763–780

Bors B, Sullivan JA (1998) Interspecific crossability of nine diploid *Fragaria* species. HortScience 32:439 (abst.)

Bors B, Sullivan JA (2005) Interspecific hybridization of *Fragaria vesca* subspecies with *F. nilgerrensis*, *F. nubicola*, *F. pentaphylla*, and *F. viridis*. J Am Soc Hortic Sci 130:418–423

Bringhurst R (1990) Cytogenetics and evolution in American *Fragaria*. HortScience 25:879–881

Bringhurst RS, Hansche PE, Voth V (1967) Inheritance of verticillium wilt resistance and the correlation of resistance with performance traits of the strawberry. Proc Am Soc Hortic Sci 92:369–375

Bringhurst RS, Senanayake YDA (1966) The evolutionary significance of natural *Fragaria chiloensis* × *F. vesca* hybrids resulting from unreduced gametes. Am J Bot 53:1000–1006

Bringhurst RS, Voth V (1957) Effect of stratification on strawberry seed germination. Proc Am Soc Hortic Sci 70:144–149

Bringhurst RS, Voth V (1976) Origin and evolutionary potentiality of the day-neutral trait in octoploid *Fragaria*. Genetics 83:s10

Bringhurst RS, Voth V (1984) Breeding octoploid strawberries. Iowa State University J Res 58:371–381

Brown GR, Moore JN (1975) Inheritance of fruit detachment in strawberry. J Am Soc Hortic Sci 100:569–572

Brown JG, Voth V (1955) Salt damage to strawberries. Calif Agric 9:11–12

Brown T, Wareing PF (1965) The genetical control of the everbearing habit and three other characters in varieties of *Fragaria vesca*. Euphytica 14:97–112

Castillejo C, de la Fuente J, Iannetta P, Botella M, Valpuesta V (2004) Pectin esterase gene family in strawberry fruit: study of FaPE1, a ripening-specific isoform. J Exp Bot 55:909–918

Cekic C, Battey J, Wilkinson JQ (2001) The potential of ISSR-PCR primer-pair combinations for genetic linkage analysis using the seasonal flowering locus in *Fragaria* as a model. Theor Appl Genet 103:540–546

Chalavi V, Tabaeizadeh Z, Thibodeau P (2003) Enhanced resistance to *Verticillium dahliae* in transgenic strawberry plants expressing a *Lycopersicon chilense* chitinase gene. J Am Soc Hortic Sci 128:747–753

Chiykowski LN, Craig DL (1975) Reaction of strawberry cultivars to clover phyllody (green petal) agent transmitted by *Aphrodes bicincta*. Can Plant Dis Survey 55:66–68

Cho CT, Moon BJ (1984) Studies on the wilt of strawberry caused by *Fusarium oxysporum* f.sp. *fragariae* in Korea. Korean J Plant Prot 23:74–81

Cipriani G, Pinosa F, Bonoli M, Faedi W (2006) A new set of microsatellite markers for *Fragaria* species and their application in linkage analysis. J Hortic Sci Bio 81:668–675

Comstock RE, Kelleher T, Morrow EB (1958) Genetic variation in an asexual species, the garden strawberry. Genetics 43:634–46

Cordero de Mesa M, Jiménez-Bermúdez S, Pliego-Alfaro F, Quesada MA, Mercado JA (2000) *Agrobacterium* cells as microprojectile coating: a novel approach to enhance stable transformation rates in strawberry. Aust J Plant Physiol 27:1093–1100

Cram WT (1978) The effect of root weevils (Coleoptera: Curculionidae) on yield of five strawberry cultivars in British Columbia. J Entomol Soc B C 75:10–13

Crock JE, Shanks CH Jr, Barritt BH (1982) Resistance in *Fragaria chiloensis* and *F*. ×*ananassa* to the aphids *Chaetosiphon fragaefolii* and *C*. *thomasi*. HortScience 17:959–960

Dale A, Gray VP, Miles NW (1987) Effects of cultural systems and harvesting techniques on the production of strawberries for processing. Can J Plant Sci 67:853–862

Darrow GM (1937) Strawberry improvement. In: Better plants and animals 2. USDA Yearbook of Agriculture, pp 496–533

Darrow GM (1966) The Strawberry. history, breeding and physiology. Holt, Rinehart and Winston, NewYork

Darrow GM, Scott DH (1947) Breeding for cold hardiness of strawberry flowers. Proc Am Soc Hortic Sci 50:239–242

Darrow GM, Waldo GF, Schuster CE (1933) Twelve years of strawberry breeding. A summary of the strawberry breeding work of the United States Department of Agriculture. J Hered 24:391–402

Daubeny HA (1964) Effect of parentage in breeding for red stele resistance of strawberry in British Columbia. Proc Am Soc Hortic Sci 84:289–294

Daubeny HA, Lawrence FJ, Martin LW, Barritt BH (1980) 'Tyee', a new strawberry cultivar suited to machine harvest. Sta Bull, Agric Exp Sta, Oregon State Univ 645:40–42

Davik J, Honne BI (2005) Genetic variance and breeding values for resistance to a wind-borne disease [*Sphaerotheca macularis* (Wallr. ex Fr.)] in strawberry (*Fragaria* ×*ananassa* Duch.) estimated by exploring mixed and spatial models and pedigree information. Theor Appl Genet 111:256–264

Davis R (2005) A diploid platform for strawberry genomics. In: Plant & Animal Genomes, San Diego, CA

Davis TM (2004) Identification of putative diploid genome donors to the octoploid cultivated strawberry, *Fragaria* ×*ananassa*. In: Plant & Animal Genomes XII Conference, San Diego, CA

Davis TM, Yu H (1997) A linkage map of the diploid strawberry, *Fragaria vesca*. J Hered 88:215–221

De la Fuente JI, Amaya I, Castillejo C, Sánchez-Sevilla J, Quesada MA, Botella MA, Valpuesta V (2006) The strawberry gene *FaGAST* affects plant growth through inhibition of cell elongation. J Exp Bot 57:2401–2411

Delhomez N, Carisse O, Lareau M, Khanizadeh S (1995) Susceptibility of strawberry cultivars and advanced selections to leaf spot caused by *Mycosphaerella fragariae*. HortScience 30: 592–595

Delp BR, Milholland RS (1981) Susceptibility of strawberry cultivars and related species to *Colletotrichum fragariae*. Plant Dis 65:421–423

Deng C, Davis TM (2001) Molecular identification of the yellow fruit color (c) locus in diploid strawberry: a candidate gene approach. Theor Appl Genet 103:316–322

Denisen EL, Buchele WF (1967) Mechanical harvesting of strawberries. Proc Am Soc Hortic Sci 91:267–273

Denoyes B, Baudry A (1995) Species identification and pathogenicity study of French *Colletotrichum* strains isolated from strawberry using morphological and cultural characteristics. Phytopathology 85:53–57

Denoyes-Rothan B, Guerin G, Lerceteau-Köhler E, Risser G (2005) Inheritance of resistance to *Colletotrichum acutatum* in *Fragaria ×ananassa*. Phytpathology 95:405–412

Doss RP, Shanks Jr CH (1988) The influence of leaf pubescence on the resistance of selected clones of beach strawberry (*Fragaria chiloensis* (L.) Duchesne) to adult black vine weevils (*Otiorhynchus sulcatus* F.). Sci Hortic 34:47–54

Du Plessis HJ, Brand RJ, Glynn-Woods C, Goedhart MA (1995) Genetic engineering leads to a herbicide-tolerant strawberry. S Afr J Sci 91:218

Du Plessis HJ, Brand RJ, Glynn-Woods C, Goedhart MA (1997) Efficient genetic transformation of strawberry (*Fragaria ×ananassa* Duch.) cultivar Selekta. Acta Hortic 447:289–294

Duewer RG, Zych CC (1967) Heritability of soluble solids and acids in progenies of the cultivated strawberry (*Fragaria ×ananassa* Duch.). Proc Am Soc Hortic Sci 90:153–157

Edwards WH, Jones RK, Schmitt DP (1985) Host suitability and parasitism of selected strawberry cultivars by *Meloidogyne hapla* and *M. incognita*. Plant Dis 69:40–42

Fadeeva TS (1966) Communication 1. Principles of genome analysis (with reference to the genus *Fragaria*). Genetika 1:12–28

Federova NJ (1946) Crossibility and phylogenetic relations in the main European species of *Fragaria*. Compt Rend (Doklady) Acad Sci USSR 1:12–28

Firsov AP, Dolgov SV (1998) Agrobacterial transformation and transfer of the antifreeze protein gene of winter flounder to the strawberry. Acta Hortic 484:581–586

Fishman R (1987) Albert Etter: fruit breeder. Fruit Varieties J 41:40–46

Folta KM, Dhingra A, Howard L, Stewert P, Chandler CK (2006) Characterization of LF9, an octoploid strawberry genotype selected for rapid regeneration and transformation. Planta 224:1058–1067

Folta KM, Staton M, Stewart PJ, Jung S, Bies DH, Jesdurai C, Main D (2005) Expressed sequence tags (ESTs) and simple sequence repeat (SSR) markers from octoploid strawberry (*Fragaria ×ananassa*). BMC Plant Biol 5:12

Galletta GJ, Bringhurst RS (1991) Strawberry management. In: Galletta GJ, Himelrick D (eds) Small fruit crop management. Prentice Hall, Englewood Cliffs, NJ, pp 83–156

Gimenez G, Ballington JR (2002) Inheritance of resistance to *Colletotrichum acutatum* Simmonds on runners of garden strawberry and its backcrosses. HortScience 37:686–690

Gooding HJ, Jennings DL, Topham TP (1975) A genotype-environment experiment on strawberries in Scotland. Heredity 34:105–115

Gooding HJ, McNicol RJ, MacIntyre D (1981) Methods of screening strawberries for resistance to *Sphaerotheca macularis* (Wall ex Frier) and *Phytophthora cactorum* (Leb. and Cohn). J Hortic Sci 56:239–245

Graham J (2005) *Fragaria* strawberries In: Litz R.E. (ed) Biotechnology of fruit and nut crops. CABI Publishing, Wallingford, UK

Graham J, Gordon SC, McNicol RJ (1997) The effect of the *CpTi* gene in strawberry against attack by vine weevil (*Otiorhynchus sulcatus* F. Coleoptera: Curculionidae). Ann Appl Biol 131:133–139

Graham J, Gordon SC, Smith K, McNicol RJ, McNicol JW (2002) The effect of the Cowpea trypsin inhibitor in strawberry on damage by vine weevil under field conditions. J Hortic Sci Biotechnol 77:33–40

Graham J, McNicol RJ, Greig K (1995) Towards genetic based insect resistance in strawberry using the Cowpea trypsin inhibitor gene. Ann Appl Biol 127:163–173

Graichen K, Proll E, Leistner HU, Kegler H (1985) Preliminary results of purification experiments with strawberry viruses. Archiv Phytopathol Pflanzenschutz 21:499–501

Hadonou A, Sargent D, Wilson F, James C, Simpson DW (2004) Development of microsatellite markers in *Fragaria*, their use in genetic diversity analysis, and their potential for genetic linkage mapping. Genome 47:429–438

Hamilton-Kemp TR, Andersen RA, Rodriguez JG, Loughrin JH, Patterson CG (1988) Strawberry foliage headspace vapor components at periods of susceptibility and resistance to *Tetranychus urticae* Koch. J Chem Ecol 14:789–796

Hancock JF (1999) Strawberries. CABI Publishing, Wallingford, UK

Hancock JF, Bringhurst RS (1979) Ecological differentiation in perennial, octoploid species of *Fragaria*. Am J Bot 66:367–375

Hancock JF, Bringhurst RS (1988) Yield component interactions in wild populations of California *Fragaria*. HortScience 23:889–891

Hancock JF, Callow PW, Serçe S, Phan PQ (2003) Variation in the horticultural characteristics of native *Fragaria virginiana* and *F. chiloensis* from North and South America. J Am Soc Hortic Sci 128:201–208

Hancock JF, Finn C, Hokanson S, Luby JJ, Goulart B, Demchak K, Callow P, Serce S, Schilder A, Hummer K (2001) A multistate comparison of native octoploid strawberries from North and South America. J Am Soc Hortic Sci 126:579–586

Hancock JF, Flore JA, Galletta GJ (1989) Variation in leaf photosynthetic rates and yield in strawberries. J Soc Hortic Sci 64:449–454

Hancock JF, Maas JL, Shanks CH, Breen PJ, Luby JJ (1990) Strawberries (*Fragaria* ssp). In: Moore J, Ballington J (eds) Genetic Resources in Temperate Fruit and Nut Crops International Society of Horticultural Sciences, Wageningen, The Netherlands, pp 489–546

Hancock JF, Sakin M, Luby JJ, Dale A, Darnell D (1996) Germplasm resources in octoploid strawberries: Potential sources of genes to increase yield in northern climates. In: Proceedings of the IV North American Strawberry Conference, Gainesville, FL

Hancock JF, Scott DH, Lawrence FJ (1996b) Strawberries. In: Janick J, Moore JN (eds) Fruit breeding. vol II. vine and small fruits. John Wiley and Sons, NewYork, pp 419–470

Handley DT, Dill JF, Pollard JE (1991) Field susceptibility of twenty strawberry cultivars to tarnished plant bug injury. Fruit Varieties J 45:166–169

Hansche PE, Bringhurst RS, Voth V (1968) Estimates of genetic and environmental parameters in the strawberry. Proc Am Soc Hortic Sci 92:338–345

Hansen E, Waldo GF (1944) Ascorbic acid content of small fruits in relation to genetic and environmental factors. Food Res 9:453–461

Harland SC, King E (1957) Inheritance of mildew resistance in *Fragaria* with special reference to cytoplasmic effects. Heredity 11:257

Harrison EP, McQueen-Mason SJ, Manning K (2001) Expression of six expansin genes in relation to extension activity in developing strawberry fruit. J Exp Bot 52:1437–1446

Harrison RE, Luby JJ, Furnier GR (1997) Chloroplast DNA restriction fragment variation among strawberry (*Fragaria* spp.) taxa. J Am Soc Hortic Sci 122:63–68

Haymes K, Davis TM (1998) Agrobacterium–mediated transformation of 'Alpine' *Fragaria vesca*, and transmission of transgenes to R1 progeny. Plt Cell Rep 17:279–283

Haymes KM, Henken B, Davis TM, van de Weg ME (1997) Identification of RAPD markers linked to a *Phytophthora fragariae* resistance gene (*Rpf1*) in the cultivated strawberry. Theor Appl Genet 94:1097–1101

Hedrick UP (1925) The Small Fruits of New York. J.B. Lyon Company, Printers, Albany, NY

Hildebrand PD, Braun PG, Renderos WE, Jamieson AR, McRae KB, Binn M (2005) A quantitative method for inoculating strawberry leaves with *Xanthomonas fragariae*, factors affecting infection, and cultivar reactions. Can J Plant Pathol 27:16–24

Hoffman T, Kalinowski G, Schwab W (2006) RNAi-induced silencing of gene expression in strawberry fruit (*Fragaria ×ananassa*) by agroinfiltration: a rapid assay for gene function analysis. Plant J 48:818–826

Hokanson S, Maas JL (2001) Strawberry biotechnology, vol. 21. John Wiley & Sons, NewYork

Houde M, Dallaire S, N'Dong D, Sarhan F (2004) Overexpression of the acidic dehydrin WCOR410 improves freezing tolerance in transgenic strawberry leaves. Plant Biotechnol J 2:381–387

Hortynski J (1989) Genotype-environmental interaction in strawberry breeding. Acta Hortic 265:175–180

Howard CM, Maas JL, Chandler CK, Albregts EE (1992) Anthracnose of strawberry caused by the *Colletotrichum* complex in Florida. Plant Dis 76:976–981

Hsu CS, Watkins R, Bolton AT, Spangelo LPS (1969) Inheritance of resistance to powdery mildew in the cultivated strawberry. Can J Genet Cytol 11:426–438

Hummer K (1995) What's new in strawberry genetic resources: raw materials for a better berry. In: Pritts MP, Chandler CK, Crocker TE (eds) Proceedings of the IV North American Strawberry Conference, University of Florida, Orlando, pp 79–86

Iannetta P, Escobar N, Roos H, Souleyre E, Hancock R, Witte C, Davis H (2004) Identification, cloning and expression analysis of strawberry (*Fragaria ×ananassa*) mitochondrial citrate synthase and mitochondrial malate dehydrogenase. Physiol Plant 121:15–26

Jiménez-Bermúdez S, Redondo-Nevado J, Muñoz-Blanco J, Caballero JL, López-Aranda JM, Valpuesta V, Pliego-Alfaro F, Quesada MA, Mercado JA (2002) Manipulation of strawberry fruit softening by antisense expression of a pectate lyase gene. Plant Physiol 128: 751–759

Jung S, Jesudurai C, Staton M, Du Z, Ficklin S, Cho I, Abbott A, Tomkins J, Main D (2004) GDR (Genome Database for Rosaceae): Integrated web resources for Rosaceae genomics and genetics research. BMC Bioinformatics 5:130–138

Khanizadeh S, Bélanger A (1997) Classification of 92 strawberry genotypes based on their leaf essential oil composition. Acta Hortic 439:205–210

Kim CH, Seo HD, Cho WD, Kim SB (1982) Studies on varietal resistance and chemical control to the wilt of strawberry caused by *Fusarium oxysporum*. Korean J Plant Prot 21:61–67

Lal D, Seth JN (1979) Studies on genetic variability in strawberry (*Fragaria ×ananassa* Duch.). Progressive Hortic 11: 49–53

Lal D, Seth JN (1981) Studies on combining ability in strawberry (*Fragaria ×ananassa*): 1. Number of inflorescences, number of flowers, days to maturity and number of fruits. Can J Genet Cytol 23: 373–378

LaMondia JA (2004) Field performance of twenty-one strawberry cultivars in a black root rot-infested site. J Am Pom Soc 58:226–232

Larson KD, Shaw DV (1995a) Relative performance of strawberry genotypes on fumigated and nonfumigated soil. J Am Soc Hortic Sci 120:274–277

Larson KD, Shaw DV (1995b) Strawberry nursery soil fumigation and runner plant production. HortScience 30:236–237

Lerceteau-Köhler E, Guerin G, Denoyes-Rothan B (2005) Identification of SCAR markers linked to *Rca2* anthracnose resistance gene and their assessment in strawberry germplasm. Theor Appl Genet 111:862–870

Lerceteau-Köhler E, Guérin G, Laigret F, Denoyes-Rothan B (2003) Characterization of mixed disomic and polysomic inheritance in the octoploid strawberry (*Fragaria ×ananassa*) using AFLP mapping. Theor Appl Genet 107:619–628

Lerceteau-Köhler E, Moing A, Guerin G, Renaud C, Courlit S, Camy D, Praud K, Parisy V, Bellec F, Maucourt M, Rolin D, Roudeillac P, Denoyes-Rothan B (2004) QTL analysis for fruit quality traits in octoploid strawberry (*Fragaria × ananassa*). Acta Hortic 663:331–335

Lewers KS, Maas JL, Hokanson SC, Gouin C, Hartung JS (2003) Inheritance of resistance in strawberry to bacterial angular leafspot disease caused by *Xanthomonas fragariae*. J Am Soc Hortic Sci 128:209–212

Luby JJ, Stahler MM (1993) Collection and evaluation of *Fragaria virginiana* in North America. Acta Hortic 345:49–54

Lundergan CA, Moore JN (1975) Inheritance of ascorbic acid content and color intensity in fruits of strawberry (*Fragaria ×ananassa* Duch.). J Am Soc Hortic Sci 100:633–635

Maas JL (1998) Compendium of stawberry diseases, 2nd edn. APS Press, Beltsville, Maryland

Maas JL, Galletta GJ (1989) Germplasm evaluation for resistance to fungus-incited diseases. Acta Hortic 265:461–472

Maas JL, Galletta GJ, Draper AD (1989) Resistance in strawberry to races of *Phytophthora fragariae* and to isolates of *Verticillium* from North America. Acta Hortic 265:521–526

Maas JL, Smith WL (1978) 'Earliglow', a possible source of resistance to Botrytis fruit rot in strawberry. HortScience 13:275–276

MacIntyre D, Gooding HJ (1978) The assessment of strawberries for decapping by machine. Hortic Res 18:127–137

MacKenzie SJ, Legard DE, Timmer LW, Chandler CK, Peres NA (2006) Resistance of strawberry cultivars to crown rot caused by *Colletrotrichum gloeosporioides* isolates from Florida is nonspecific. Plant Dis 90:1091–1097

MacLachlan JB (1974) The inheritance of colour of fruit and the assessment of plants as sources of colour in the cultivated strawberry. Hortic Res 14:29–39

Manning K (1998) Genes for fruit quality in strawberry. In: Cockshull KE, Gray D, Seymour GB, Thomas B (eds) Genetic and environmental manipulation of horticultural crops, vol. 51–61. CAB International, Wallingford, UK

Marini RP, Boyce BR (1979) Influence of low temperatures during dormancy on growth and development of 'Catskill' strawberry plants. J Am Soc Hortic Sci 104:159–162

Mason DT, Rath N (1980) The relative importance of some yield components in East of Scotland strawberry plantations. Ann Appl Bio 95:399–408

Mathews H, Wagoner W, Kellogg J, Bestwick R (1995) Genetic transformation of strawberry: Stable integration of a gene to control the biosynthesis of ethylene. In Vitro Cell Dev Biol 31:36–43

Medina JL, Moore PP, Shanks CH, Gil FF, Chandler CK (1999) Genotype × environment interaction for resistance to spider mites in *Fragaria*. J Am Soc Hortic Sci 124:353–357

Medina-Escobar N, Cárdenas J, Muñoz-Blanco J, Caballero JL (1998) Cloning and molecular characterization of a strawberry fruit ripening-related cDNA corresponding a mRNA for a low-molecular-weight-heat-shock protein. Plt Mol Biol 36:33–42

Mehli L, Kjellsen TD, Dewey FM, Hietala AM (2005) A case study from the interaction of strawberry and *Botrytis cinerea* highlights the benefits of comonitoring both partners at genomic and mRNA level. New Phyt 168:465–474

Mehli L, Schaart JG, Kjellsen TD, Tran DH, Salentijn EMJ, Schouten HJ, Iversen T-H (2004) A gene encoding a polygalacturonase-inhibiting protein (PGIP) shows developmental regulation and pathogen-induced expression in strawberry. New Phyt 163:99–110

Melville AH, Draper AD, Galletta GJ (1980a) Transmission of red stele resistance by inbred strawberry selections. J Am Soc Hortic Sci 105:608–610

Melville AH, Galletta GJ, Draper AD, Ng TJ (1980b) Seed germination and early seedling vigor in progenies of inbred strawberry selections. HortScience 15:49–750

Mezzetti B, Landi L, Pandolfini T, Spena A (2004) The defH9-iaaM auxin-synthesizing gene increases plant fecundity and fruit production in strawberry and raspberry. BMC Biotechnol 4:1–10

Mochizuki T (1995) Past and present strawberry breeding programs in Japan. Advances in Strawberry Research 14:9–17

Moore JN, Brown GR, Bowen HL (1975) Evaluation of strawberry clones for adaptability to once-over mechanical harvest. HortScience 10:407–408

Moore JN, Brown GR, Brown ED (1970) Comparison of factors influencing fruit size in large-fruited and small-fruited clones of strawberry. J Am Soc Hortic Sci 95:827–831

Morgan A, Baker CM, Chu JSF, Lee K, Crandell BA (2002) Production of herbicide tolerant strawberry through genetic engineering. Acta Hortic 567:113–115

Mori T, Kitamura H, Kuroda K (2005) Varietal differences in Fusarium wilt-resistance in strawberry cultivars and the segregation of this trait in F_1 hybrids. J Jap Soc Hortic Sci 75:57–59

Morrow EB, Comstock RE, Kelleher T (1958) Genetic variances in strawberries. Proc Am Soc Hortic Sci 72:170–85

Morrow EB, Darrow GM (1952) Effects of limited inbreeding in strawberries. Proc Am Soc Hortic Sci 59:269–276

Murant AF, Lister RM (1987) European nepoviruses in strawberry. In: Converse RH (ed) Virus diseases of small fruits. USDA/ARS, Washington, DC

Murawski H (1968) Studies on heritability in strawberry varieties. Height of inflorescences, mildew resistance, fruit colour, flesh colour and the shape of the berries. Arch Gartenb 16:293–318

NDong C, Quellet F, Houde M, Sarhan F (1997) Gene expression during cold acclimation in strawberry. Plt Cell Physiol 38:863–870

Nelson MD, Gubler WD, Shaw DV (1996) Relative resistance of 47 strawberry cultivars to powdery mildew in California greenhouse and field environments. Plant Dis 80:326–328

Nemec S (1971) Studies on resistance of strawberry varieties and selections to *Mycosphaerella fragariae* in southern Illinois. Plant Dis Rep 55:573–576

Nemec S, Blake RC (1971) Reaction of strawberry cultivars and their progenies to leaf scorch in southern Illinois. HortScience 6:497–498

Nicoll MF, Galletta GJ (1987) Variation in growth and flowering habits of Junebearing and everbearing strawberries. J Am Soc Hortic Sci 112:872–880

Olías R, Pérez AG, Sanz C (2002) Catalytic properties of alcohol acyltransferase in different strawberry species and cultivars. Agric Food Chem 50:4031–4036

Ourecky DK, Bourne MC (1968) Breeding and Instron evaluation of strawberry firmness. HortScience 3:92–93

Ourecky DK, Reich JE (1976) Frost tolerance in strawberry cultivars. HortScience 11:413–414

Ourecky DK, Slate GL (1967) Behavior of the everbearing characteristics in strawberries. Proc Am Soc Hortic Sci 91:236–248

Owens CL, Thomashow MF, Hancock JF, Iezzoni AF (2002) CBF1 orthologs in sour cherry and strawberry and the heterologous expression of CBF1 in strawberry. J Am Soc Hortic Sci 127:489–494

Oydvin J (1980) Records of two-spotted spider mite *Tetranychus urticae* Koch, strawberry mite *Steneotarsonemus pallidus* Banks and strawberry mildew *Sphaerotheca macularis* (Wallr.) Magn. in a progeny test of strawberries, 1976–77. Forskning og Forsok i Landbruket 31:1–9

Particka C, Hancock JF (2005) Field evaluation of strawberry genotypes for tolerance to black root rot on fumigated and nonfumigated soil. J Am Soc Hortic Sci 130:688–693

Passey AJ, Barrett KJ, James DJ (2003) Adventitious shoot regeneration from seven commercial strawberry cultivars (*Fragaria ×ananassa* Duch.) using a range of explant types. Plant Cell Rep 21:397–401

Popova IV, Konstantinova AE, Zekalashvili AU, Zhananov BK (1985) Features of breeding strawberries for resistance to berry molds. Sov Agric Sci 3:29–33

Potter JW, Dale A (1994) Wild and cultivated strawberries can tolerate or resist root-lesion nematode. HortScience 29:1074–1077

Potter D, Luby JJ, Harrison RE (2000) Phylogenetic relationships among species of *Fragaria* (Rosaceae) inferred from non-coding nuclear and chloroplast DNA sequences. Syst Bot 25:337–348

Powers L (1945) Strawberry breeding studies involving crosses between the cultivated varieties (*Fragaria ×ananassa*) and the native Rocky Mountain strawberry (*F. ovalis*). J Agric Res 70:95–122

Powers L (1954) Inheritance of period of blooming in progenies of strawberries. Proc Am Soc Hortic Sci 64:293–298

Reddy ASN, Jena PK, Mukherjee SK, Poovaiah BW (1990) Molecular cloning of cDNAs for auxin-induced mRNAs and developmental expression of the auxin-inducible genes. Plant Mol Biol 14:643–653

Reddy ASN, Poovaiah BW (1990) Molecular cloning and sequencing of a cDNA for an auxin-repressed mRNA: correlation between fruit growth and repression of the auxin-regulated gene. Plant Mol Biol 14:127–136

Rosin FM, Aharoni A, Salentijn EMJ, Schaart JG, Boone MJ, Hannapel DJ (2003) Expression patterns of a putative homolog of *AGAMOUS*, *STAG1*, from strawberry. Plant Sci 165:959–968

Salentijn EMJ, Aharoni A, Schaart JG, Boone MJ, Krens FA (2003) Differential gene expression analysis of strawberry cultivars that differ in fruit firmness. Physiol Plant 118:571–578

Sangiacomo MA, Sullivan JA (1994) Introgression of wild species into the cultivated strawberry using synthetic octoploids. Theor Appl Genet 88:349–354

Sargent DJ, Davis TM, Tobutt KR, Wilkinson MJ, Battey NH, Simpson DW (2004a) A genetic linkage map of microsatellite, gene-specific and morphological markers in diploid *Fragaria*. Theor Appl Genet 109:1385–1391

Sargent DJ, Geibel M, Hawkins JA, Wilkinson MJ, Battey NH, Simpson DW (2004b) Quantitative and qualitative differences in morphological traits revealed between diploid *Fragaria* species. Ann Bot 94:787–769

Schaart JG, Krens FA, Pelgrom KTP, Mendes O, Rouwendal JA (2004) Effective production of marker-free transgenic strawberry plants using inducible site-specific recombination and a bifunctional selectable marker gene. Plant Biotechnol 2:233–240

Schaart JG, Mehli L, Schouten HJ (2005) Quantification of allele-specific expression of a gene encoding strawberry polygalacturonase-inhibiting protein (PGIP) using Pyrosequencing. Plant J 41:493–500

Schaart JG, Salentijn EMJ, Krens FA (2002) Tissue-specific expression of the β-glucuronidase reporter gene in transgenic strawberry (*Fragaria ×ananassa*) plants. Plant Cell Rep 21:313–319

Schaefers GA (1980) Yield effects of tarnished plant bug feeding on June-bearing strawberry varieties in New York State. J Econ Entomol 73:721–725

Schuster DJ, Price JF, Martin FG, Howard CM, Albregts EE (1980) Tolerance of strawberry cultivars to twospotted spider mites in Florida. J Econ Entomol 73:52–54

Scott DH, Draper AD (1970) A further note on the longevity of strawberry seed in cold storage. HortScience 5:439

Scott DH, Draper AD, Greeley LW (1972) Interspecific hybridization in octoploid strawberries. HortScience 7:382–384

Scott DH, Lawrence FJ (1975) Strawberries. In: Janick J, Moore JN (eds) Advances in fruit breeding. Purdue University Press, pp 71–79

Serce S, Hancock JF (2005) Inheritance of day-neutrality in octoploid species of *Fragaria*. J Am Soc Hortic Sci 130:580–584

Shanks CH, Barritt BH (1974) *Fragaria chiloensis* clones resistant to the strawberry aphid. HortScience 9:202–203

Shanks CH, Chandler CK, Show ED, Moore PP (1995) *Fragaria* resistance to spider mites at three locations in the United States. HortScience 30:1068–1069

Shanks CH, Moore PP (1995) Resistance to two-spotted spider mite and strawberry aphid in *Fragaria chiloensis*, *F*. *virginiana*, and *F*. *×ananassa* clones. HortScience 30: 596–599

Shaw DV (1988) Genotypic variation and genotypic correlations for sugars and organic acids of strawberries. J Am Soc Hortic Sci 113:770–774

Shaw DV (1989) Variation among heritability estimates for strawberries obtained by offspring-parent regressions with relatives raised in separate environments. Euphytica 44:157–162

Shaw DV (1991) Recent advances in the genetics of strawberries. In: Dale A, Luby JJ (eds) The Strawberry into the 21st Century Timber Press, Portland, Oregon, pp 76–83

Shaw DV, Bringhurst RS, Voth V (1987) Genetic variation for quality traits in an advanced-cycle breeding population of strawberries. J Am Soc Hortic Sci 112:699–702

Shaw DV, Bringhurst RS, Voth V (1988) Quantitative genetic variation for resistance to leaf spot (*Ramularia tulasnei*) in California strawberries. J Am Soc Hortic Sci 113:451–456

Shaw DV, Bringhurst RS, Voth V (1989) Genetic parameters estimated for an advanced-cycle strawberry breeding population at two locations. J Am Soc Hortic Sci 114:823–827

Shaw DV, Famula T (2005) Complex segregation analysis of day-neutrality in domestic strawberry (*Fragaria ×ananassa* Duch.). Euphytica 145:331–338

Shaw DV, Gordon TR (2003) Genetic response for reaction to *Verticillium* wilt in strawberry with two stage family and genotypic selection. HortScience 38:432–434

Shaw DV, Sacks EJ (1995) Response in genotypic and breeding value to a single generation of divergent selection for fresh fruit color in strawberry. J Am Soc Hortic Sci 120:270–273

Sherman WB, Janick J, Erickson HT (1966) Inheritance of fruit size in strawberry. Proc Am Soc Hortic Sci 89:309–17

Simpson DW (1987) The inheritance of mildew resistance in everbearing and day-neutral strawberry seedlings. J Hortic Sci 62:329–334

Simpson DW, Easterbrook MA, Bell JA (2002) The inheritance of resistance to the blossom weevil, *Anthonomus rubi*, in the cultivated strawberry, *Fragaria ×ananassa*. Plant Breed 121: 72–75

Simpson DW, Sharp RS (1988) The inheritance of fruit yield and stolon production in everbearing strawberries. Euphytica 38:65–74

Sjulin TM (2006) Private strawberry breeders in California. HortScience 41:17–19

Sjulin TM, Dale A (1987) Genetic diversity of North American strawberry cultivars. J Am Soc Hortic Sci 112:375–385

Sjulin TM, Robbins J, Barritt BH (1986) Selection for virus tolerance in strawberry. J Am Soc Hortic Sci 111:458–464

Smith BJ, Black LL (1987) Resistance of strawberry plants to *Colletotrichum fragariae* affected by environmental conditions. Plant Dis 71:834–837

Smith BJ, Black LL (1990) Morphological, cultural and pathogenic variation among *Colletotrichum* species isolated from strawberry. Plant Dis 74:69–76

Spangelo LPS, Hsu CS, Fejer SO, Bedard PR, Rouselle GL (1971) Heritability and genetic variance components for 20 fruit and plant characters in the cultivated strawberry. Can J Genet Cytol 13:443–456

Stahler MM, Ascher PD, Luby JJ, Roelfs AP (1995) Sexual composition of populations of *Fragaria virginiana* (Rosaceae) collected from Minnesota and western Wisconsin. Can J Bot 73:1457–1463

Stahler MM, Luby JJ, Ascher PD (1990) Comparative yield of female and hermaphroditic *Fragaria virginiana* germplasm collected in Minnesota and Wisconsin. In: Dale A, Luby JJ (eds) The Strawberry into the 21st Century Timber Press Portland, Oregon pp. 104–105

Staudt G (1959) Cytotaxonomy and phylogenetic relationships in the genus *Fragaria*. IX Int Bot Congr Proc 2:377

Staudt G (1967) The genetics and evolution of heterosis in the genus *Fragaria* II.. Species hybridization of *F. vesca ×F. orientalis* and *F. viridis ×F. orientalis* (in German). Z Pflanzenz 58:309–322

Staudt G (1984) Cytological evidence of double restitution in *Fragaria*. Plant Sys Evol 146: 171–179

Staudt G (1989) The species of *Fragaria*, their taxonomy and geographical distribution. Acta Hortic 265:23–33

Staudt G (1999) Systematics and geographic distribution of the American strawberry species: taxonomic studies in the genus *Fragaria* (Rosaceae: Potentilleae). Univ Calif Publ Bot 81:1–162

Szczygiel A (1981a) Trials on susceptibility of strawberry cultivars to the needle nematode, *Longidorus elongatus*. Fruit Sci Rep 8:127–131

Szczygiel A (1981b) Trials on susceptibility of strawberry cultivars to the northern root-knot nematode, *Meloidogyne hapla*. Fruit Sci Rep 8:115–119

Szczygiel A (1981c) Trials on suceptibility of strawberry cultivars to the root lesion nematode, *Pratylenchus penetrans*. Fruit Sci Rep 8:121–125

Szczygiel A, Danek J (1974) Pathogenicity of three species of root parasitic nematodes to strawberry plants as related to methods of inoculation. Zeszyty Problemowe Postepow Nauk Rolniczych 154:133–149

Takahashi H (1993) Breeding of strawberry cultivars resistant to Alternaria black spot of strawberry (*Alternaria alternata* strawberry pathotype). Bull Akita Prefectural Coll Agric 19: 1–44

Takahashi H, Yoshida Y, Kanda H, Furuya H, Matsumoto T (2003) Breeding of Fusarium wilt-resistant strawberry cultivar suitable for field culture in Northern Japan. Acta Hortic 626:113–118

Tingey WM, Pillemer EA (1977) Lygus bugs: crop resistance and physiological nature of feeding injury. Bull Entomol Soc Am 23:277–287

Trainotti L, Ferrarese L, Vecchia F dalla, Rascio N, Casadoro G (1999) Two different endo-β-1,4-glucanases contribute to the softening of strawberry fruits. J Plant Physiol 154:355–362

Trainotti L, Spinello R, Piovan A, Spolaore S, Casadoro G (2001) beta – Galactosidases with a lectin-like domain are expressed in strawberry. J Exp Bot 52:1635–1645

Van de Weg WE (1997) A gene-for-gene model to explain interactions between cultivars of strawberry and races of *Phytophthora fragariae* var *fragariae*. Theor Appl Genet 94:445–451

Van der Scheer HAT (1973) Susceptibility of strawberry to isolates of *Phytophthora cactorum* and *Phytophthora citricola*. Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent 38:1407–1415

Viruel MA, Sánchez D, Arús P (2002) An SSR and RFLP linkage map for the octoploid strawberry (*Fragaria ×ananassa*) In: Plant & Animal Genome x Conference, San Diego, CA

Wang J, Ge H, Peng S, Zhang H, Chen P, Xu J (2004) Transformation of strawberry (*Fragaria ×ananassa* Duch.) with late embryogenesis abundant protein gene. J Hortic Sci Biotechnol 79:735–738

Watkins R, Spangelo LPS, Bolton AT (1970) Genetic variance components in cultivated strawberry. Can J Genet Cytol 12:52–59

Weebadde C, Wang D, Finn CE, Lewers KS, Luby JJ, Bushakra J, Sjulin TM, Hancock JF (2007) Using a linkage mapping approach to identify QTL for day-neutrality in the octoploid strawberry. Plant Breed (In press)

Wenzel WG (1980) Correlation and selection index components. Canadian Journal of Genetics and Cytology 13, 42–50

Wilhelm S, Sagen JA (1974) A History of the Strawberry. University of California Division of Agriculture Publication 4031, Berkeley

Wilkinson JQ, Lanahan MB, Conner TW, Klee HJ (1995) Identification of mRNAs with enhanced expression in ripening strawberry fruit using polymerase chain reaction differential display. Plant Mol Biol 27:1097–1108

Wilson WF Jr, Giamalva MJ (1954) J. Days from bloom to harvest of Louisiana strawberries. Proc Am Soc Hortic Sci 63:201–204

Wing KB, Pritts MP, Wilcox WF (1995) Field resistance of 20 strawberry cultivars to black root rot. Fruit Varieties J 49:94–98

Woolley LC, James DJ, Manning K (2001) Purification and properties of an *endo*-β-(1,4)-glucanase from strawberry and down-regulation of the corresponding gene, *cell*. Plant 214:11–21

Xue SM, Bors RH, Strelkov SE (2005) Resistance sources to *Xanthomonas fragariae* in non-octoploid strawberry species. HortScience 40:1653–1656

Yamamoto M, Namiki F, Nishimura F, Kohmoto K (1985) Studies on host-specific AF-toxins produced by *Alternaria alternata* strawberry pathotype causing Alternaria black spot of strawberry (3). Use of toxin for determining inheritance of disease reaction in strawberry cultivar Morioka-16. Ann Phytopathol Soc Jpn 51:530–535

Yu H, Davis TM (1995) Genetic linkage between runnering and phosphoglucoisomerase allozymes, and systematic distortion of monogenic segregation ratios in diploid strawberry. J Am Soc Hortic Sci 120:687–690

Yubero-Serrano EM, Moyano E, Medina-Escobar N, Munoz-Blanco J, Caballero JL (2003) Identification of a strawberry gene encoding a non-specific lipid transfer protein that responds to ABA, wounding and cold stress. J Exp Bot 54:1865–1877

Zimmerman RH (1991) Micropropagation of temperate zone fruit and nut crops. In: Debergh PC, Zimmerman RH (eds) Micropropagation: Technology and application. Kluer Academic, Dordrecht, The Netherlands, pp 231–264

Zubov AA, Stankevich KV (1982) Combining ability of a group of strawberry varieties for fruit quality characters (In Russian). Sov Genet 18:984–992

Zurawicz E, Stushnoff C (1977) Influence of nutrition on cold tolerance of 'Redcoat' strawberries. J Am Soc Hortic Sci 102:342–346

Zych CC (1966) Fruit maturation times of strawberry varieties. Fruit Varieties Hortic Dig 20:51–53