6 Pear

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6.1 Introduction

Pears are among the oldest of the world's fruit crops. Cultivar development has been continuous since early days and now pears are grown in all temperate zones.

6.1.1 Origin and Early Development

Pear species belong to the genus *Pyrus*, the subfamily *Maloideae* (*Pomoideae*) in the family *Rosaceae*. There are 22 widely recognized primary species (Table 1), which are distributed to Europe, temperate Asia and mountainous areas of northern Africa (Bell et al. 1996). All species of *Pyrus* are intercrossable and there are no major incompatibility barriers to interspecific hybridization, in spite of the wide geographic distribution of this genus (Westwood and Bjornstad 1971). So, classification is often difficult, giving similar taxa designated as different species by some authorities. This genus is considered to originate in the mountainous area of western and southwestern China during the Tertiary periods (65–55 million years ago) and to spread into the east and west. Two sub-centers (Central Asia, and Eastern China) of diversity for the genus have been identified (Vavilov 1951). Dispersal is believed to have followed the mountain chains both east and west (Bell et al. 1996). Speciation probably involved geographic isolation and adaptation to colder and drier environments (Rubzov 1944). Kikuchi (1946) classified *Pyrus* species into three groups, small fruited species with two carpels, large fruited species with five carpels, and their hybrids with 3–4 carpels. Small fruited species, called as Asian pea pears, are used for ornamental purpose or rootstocks. Of large fruited species with five carpels, there are three major species, *P. communis* L. (pear or European pear), *P. bretschneideri* Rehd. or *P. ussuriensis* Maxim. (Chinese pear) and *P. pyrifolia* Nakai (Japanese pear: Nashi), which are commercially cultivated in temperate zone. *P. communis* is native to Europe, and is the main commercial species in Europe, North America, South America, Africa and Australia. *P. bretschneideri* is the main species in northern and central China. *P. pyrifolia* is the main species in Japan, southern and central China and Korea. *P. nivalis* Jacq., the snow pear is cultivated in Europe for making perry. *P. pashia* P. Don. is cultivated in northern India, Nepal and southern China. Asian pears are thought to have been domesticated in prehistoric times and to have been cultivated in China for at least 3,000 years (Lombard and Westwood 1987). European pears are thought to have been in Europe since as early as 1000 BC. Homer referred to a large orchard with pears in the Odyssey written in between 900 and 800 BC. The earliest written records of Japanese pears date back to the ancient manuscript of the Emperor Jito in AD 693 (Kajiura 1994).

6.1.2 Evolution of *Pyrus*

Maloideae includes the genus *Malus* (apple). The basic chromosome number of the *Maloideae* ($x = 17$) is high compared to other *Rosaceae* subfamilies ($x = 7$ to 9), indicating a polyploid origin. Classical biochemical studies on leaf phenolic compounds, isozyme studies and botanical data support the hypothesis of an allopolyploid origin (Chevreau et al. 1997). It has been suggested that the *Maloideae* arose as an amphidiploid of two primitive forms of *Rosaceae,* crossing a basic chromosome number of 8 and 9 (Sax 1931; Zielinski and Thompson 1967). These were possibly primitive members of the *Prunoideae* ($x = 8$) and *Spiraeodeae* ($x = 9$). A recent molecular study of the chloroplast gene *rbc*L suggests that *Spiraeodeae* is the maternal ancestor of *Maloideae* (Morgan et al. 1994). The majority of cultivated pears are functional diploids ($2n = 34$). A few polyploid (triploids and

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Table 1. *Pyrus* species. Adapted from Bell et al. (1996)

Species	Distribution
Asian pea pears	
P. calleryana Decne.	Central and South China
P. koehnei Schneid.	South China, Taiwan
P. fauriei Schneid.	Korea
P. dimorphophylla Makino	Japan
P. betulaefolia Bunge	North-East China
Asian large fruited pears	
P. pyrifolia Nakai	Japan, Korea, Central China
P. pashia P. Don.	Nepal, Pakistan, India, West China
P. hondoensis Nakai et Kikuchi	Japan
P. ussuriensis Maxim.	North-East China, Korea, Siberia
P. kawakamii Hayata	Taiwan, South-East China
West Asia	
P. amygdaliformis Vill.	Mediterranean Europe
P. elaeagrifolia Pall.	Turkey, Russia, South-East Europe
P. salicifolia Pall.	Iran, Russia
P. syriaca Boiss.	Lebanon, Israel, Iran
P. regelii Rehd.	Afghanistan, Russia
P. globra Boiss.	Iran
North Africa	
P. gharbiana Trab.	Morocco
P. longipes Coss. et Dur.	Algeria
P. mamorensis Trab.	Morocco
Europe	
P. communis L.	Europe, Turkey
P. nivalis Jacq.	Central, South, West Europe
P. cordata Desv.	South Europe

tetraploids) cultivars of *P. communis* and *P. bretschneideri* exist. Speciation in *Pyrus* has proceeded without a change in chromosome number (Zielinski and Thompson 1967). The genome size of *P. communis* has been estimated by using flow cytometry (Arumuganathan and Earle 1991). According to their report, DNA content of *P. communis* is a 1.03 pg/2C, compared to 0.54 pg/2C in peach and the genome size is approximately 496 Mbp/haploid nucleus.

6.1.3 Morphology and Growth Habitat

The flowers of *Pyrus* usually have 5 sepals, 5 petals, many stamens and 2 to 5 pistils. Asian pea pears (*P. calleryana, P. betulaefolia, P. dimorphophylla* etc.) have 2 pistils, while the major edible species (*P. communis, P. bretschneideri* and *P. pyrifolia*) have 5 pistils. The number of pistils equals the number of carpels. The *Pyrus* have mixed flower buds with leaf and flower initials, an ovary and 2–5 carpels. Carpels are united with each other at the receptacles and each locule has 2 ovules giving a rise to a maximum seed number of 10. The Pear fruits have a core with a fleshy pith and a cortex of flesh. The European pear (*P. communis*) combines a buttery juicy texture with good flavor and aroma. Asian pears are characterized by a crispy texture and unique flavor. Pears are usually grown as a compound genetic system, consisting of a fruiting scion grafted on a rootstock. The rootstock is used for the control of scion vigor and an adaptation to some environmental factors such as alkaline soil, flooding, drought cold hardiness and so on. In some cases of *P. communis* cultivation, trees consist of three components: the scion, the rootstock and an interstock. The

interstock is used where the scion and the rootstock will be incompatible, but will be united each other with the interstock.

6.1.4 Production and Economic Importance

Pear is the third important temperate fruit species after grape and apple, with a world production of 17.2 million metric tons (FAO: Food and Agriculture Organization of the United Nations 2003). Asia produced the most (11 million t), followed by Europe (3.1 million t), North and Central America (894,000), and South America (813,000). The European pear (*P. communis*) production is concentrated in five production area: Europe, North America, South America, South Africa and Oceania, while Asian pears (*P. bretschneideri* or *P. pyrifolia*) production is concentrated in Asia only. Different area produces different pear species. Among countries, China produced the most (9.4 million t), followed by United States (837,000 t), Italy (822,000 t), Spain (682,000 t), Argentina (560,000 t), South Korea (386,000 t), Germany (374,000 t), Japan (366,000 t), Turkey (360,000), and South Africa (320,000). Increased pear production in recent 10 years mainly reflects larger crops in China, and the production in China exceeds 50% of the world production. Production in China increased by 218% between 1990 and 1999. On the basis of the new plantations of pear trees, extension of world pear production is estimated (Segre 2002). A strong extension in pear growing is expected in South America and China. In many countries, pear production is concentrated in areas favorable to its cultivation which results in a good fruit quality. For example, in Italy, more than 70% of output of pears comes from the lowlands of Emilio Romagna and Veneto (Sansavini 1990) and in United States, production is concentrated in the states of California, Washington, and Oregon (Bell et al. 1996). The world yield average is stable but declining a little (Segre 2002). Yields in Asia and Europe are below the world average while other larger productive area is high. Pears figure prominently in international trade. Pear exports in 1998/1999 were 1.5 million metric tons increasing from 1 million metric tons in 1990 (Segre 2002). Main suppliers in Northern Hemisphere were Italy, Belgium, China and United States, and Argentina and Chile were leading exporters in Southern Hemisphere. Pears have many uses: fresh fruits, fruit juice, perry, syrup, cubes for fruit salads, canned products and dry fruits. But pears are grown mainly for the fresh market and for canning industry (Jackson 2003). About 80% of the total production is destined for fresh consumption.

6.1.5 Nutritional Composition

On the basis of 100 grams of the edible portion, European pears (*P. communis*) provide 54 calories of food energy (Standard Tables of Food Composition, released by Ministry of Education, Culture, Sports, Science and Technology, Japan 2003). They consist of 84.9% water, 0.3% protein, 0.1% fat, 14.4% carbohydrate, and 1.9% fiber. Of the major mineral nutrients, there are 140 mg of potassium, 5 mg of calcium, 4 mg of magnesium, 13 mg of phosphorus, 0.1 mg of iron. Of vitamin contents, there are 3 mg of ascorbic acid, and trace amounts of other vitamins. While Japanese pears (*P. pyrifolia*) provide 43 calories of food energy. They consist of 88.0% water, 0.3% protein, 0.1% fat, 11.3% carbohydrate, and 0.9% fiber. There are 140 mg of potassium, 2 mg of calcium, 5 mg of magnesium, 11 mg of phosphorus, and 3 mg of ascorbic acid. Asian pears are usually rich in water with less content of sugars and starch. Asian pears are characterized as a dietary or healthy fruit. The sugar content in fruit depends on the metabolism of unloaded sugars. Pears belong to the *Rosaceae*, in which the main translocating sugar is sorbitol. Sorbitol is converted into glucose, fructose, and sucrose in fruit. The composition of these four sugars plays a key role in sweetness of pear fruits. The differences in sugar composition within species are reported (Kajiura et al. 1979). According to their report, Japanese pears tend to have high sucrose content, on the other hand Chinese pears tend to have low content of sucrose, and European pears tend to have high fructose and starch contents.

6.1.6 Breeding Objective

6.1.6.1 Breeding of New Pear Varieties

In pear breeding programs, improvement of fruit quality is a main objective. A few reports on the inheritance of fruit characters have dealt with pears (Abe et al. 1993; Crane and Lewis 1949; Machida and

Kozaki 1975, 1976). It is very important, for increasing breeding efficiency, to elucidate the mode of the main characters which influences fruit quality. These characters are the fruit weight, flesh firmness, soluble solid content, organic acid content, ripening time and storage potential. However, the attributes that constitute good quality in one species may be different in others. This is the case with European pears and Asian pears. Attributes that constitute good quality among European pears are such as soft, buttery texture, but those among Asian pears are such as juicy, crisp, cracking flesh. The most distinctive characters of Asian pears are their maturation on the tree, no requirement of ripening treatment like off tree, and smooth round shape. Ideal fruit for Japanese market should be large (about 10 cm in diameter), regular and round (Kajiura 1994), although some old cultivars bear fruits with various types of shape, ranging from fusiform and pyriform to oblate. Asian pears are quite distinct from European pears. In European pears, fruit size is important and should exceed 7 cm in length and 6 cm in diameter (Bell et al. 1996). A pyriform shape is preferable. Some important commercial characters of pear breeding are given below.

Fruit Appearance Pears are mainly served as fresh marketing and must have an attractive appearance. The fruit color is the most important factor for the fruit appearance. There are wide variations in skin colors. Yellow, green and red pears attract Chinese consumers (Wei and Gao 2002). In Japan, yellow-green and brown russet pears are preferred. In European pears, the skin should be free of russet and should resist bruising from handling during harvest, grading, storing and ripening. The skin color should be golden yellow and bright with or without a red blush, although green or greenish-yellow type is also acceptable (Bell et al. 1996).

Disease Resistance Disease resistance has become a major concern in the development of new pear varieties. Pears are susceptible to a number of diseases, mostly caused by fungi. In European pears, resistance against fungal disease such as scab (*Venturia pirina*), powdery mildew (*Podosphaera leucotricha*), brown spot (*Stemphylium vesicarium*) and fire blight (*Erwinia amylovova*) is the important breeding objective. Especially, in North America as well as many regions of Europe, the fire blight is widespread in occurrence and devastating effect. In Europe, there is a reduction in pear growing area, mainly due to the fire blight (Deckers and Schoofs 2002). The prime objective of breeding programs in these regions is improved resistance to fire blight in these affected area. While in Asian pears, resistance against fungal disease such as scab (*Venturia nasicola*), rust (*Gymnosporangium asaticum)* and black spot (*Alternaria alternata* Japanese pear pathotype) is receiving attention. Rust (*Gymnosporangium*) and scab (*Venturia*) are differentiated into different species between European and Asian pears. Resistance to the black spot disease has been a major breeding objective in Japan and Korea.

Resistance to Insects Pear psylla, *Cacopsylla pyricola*, can be a limiting factor in European pear production. It is a native species that produces abundant honeydew, which allows a sooty fungus to grow on the fruit surface. The result can be severe tree injury. Codling moth (*Cydia pomonella* L.) is also single most important pest of pears. Resistance to Pear psylla and codling moth is a major breeding objective in Europe and North America.

Ripening Period The earlier harvesting often has a higher commercial value and the orchard scale can be increased owing to the dispersion of labor. The harvest season ranges from July to November in Japan. Early cultivars make growers more profit in Japan. So there is a trend for breeding earlier maturing cultivars in Japan. This is also the case with China. However, pear germplasm resources preserved at present are rich in mid season and late maturing cultivars while there are insufficient early maturing cultivars (Cao et al. 2000). Uniformity of maturity and uniform ripening are important in European pears.

Storage Quality For the world market, it would be useful to have new pear varieties with long storage ability, which would allow continuous marketing during the whole year. In Japan, before the development of the railway or road network growing regions were limited to the suburbs of big cities such as Tokyo and Osaka (Kajiura 1994). In addition, early-maturing cultivars were limited because of their short shelf lives. Even though refrigerating systems has been developed, late- or mid-maturing cultivars are predominant. So new pear varieties with long storage ability are needed especially for an international trade.

Growth Habit Most pears are upright and vigorous, although some variability occurs in breeding populations. But compact columnar habit is not observed in

pears, but found in apples. The recent trend in fruit production is oriented to dwarf and compact trees, which are easier to prune, chemical spray and harvest. A size-controlling rootstock can be a useful tool to produce trees of reduced height. However, a scion mutant with changes in growth habit is found. 'Conference Light', a mutant of 'Conference' shows a reduction of 20% in vegetative growth in comparison with standard 'Conference'. 'Abate Light' also shows a reduction of 40% in vegetative growth in comparison with standard 'Abate Fetel' (Deckers and Schoofs 2002).

Self-Compatibility Most pears show self-incompatibility and do not have a parthenocarpic ability. This self-incompatibility is not a preferable trait for growers because for good cross pollination and fruit set growers must plant cultivars together that are mutually compatible and that flower at the same time. In commercial production of pears, factors such as climate conditions at the time of bloom, effective periods of pollination, pollination methods are important and these need to be considered every year because pollination affects the stable pear production. In Asian countries such as Japan and Korea, artificial pollination is used for stable production and making perfect round shaped fruits. It would be very valuable if new pear varieties have the ability to self-pollinate. This major obstacle to achieve this objective is a shortage of germplasm resources with self-compatibility. A Japanese pear 'Osanijisseiki' and a Chinese pear 'Jinzhuili', self-comaptibility mutants of 'Nijisseiki' and 'Yali' respectively, are available for making new cultivars with self-fertile.

6.1.6.2 Breeding for New Pear Rootstocks

Pear cultivars are still almost exclusively propagated through budding or grafting onto rootstocks. Success in pear culture depends on the use of appropriate rootstocks. The development of improved rootstocks is an important phase of pear breeding. Currently, in vitro propagation of pear is easy, but the trees on their own roots often perform less well than those propagated on good rootstocks (Wertheim 2002). In European pear production, pear trees should not be too vigorous, as they must be suitable for high-density plantings. So the most important breeding objective in production of European pears is to develop rootstocks that induce smaller size and precocity. Clonal quince (*Cydonia oblonga* L.) rootstocks, which can induce different degrees of dwarfing relevant to high-density planting, are used in areas with not too cold winters. When pear is grafted on quince the tree may be reduced by 30 to 60% of the standard size. But, they are usually sensitive to fire blight and to lime-induced chlorosis with less iron absorption. Among quince rootstocks, 'Quince A' ('EMA'), 'B' ('EMB'), and 'C' ('EMC'), were released from East Malling, UK, have been the leading pear rootstocks planted in Europe and other regions. Of these three quince rootstocks, 'EMC' is dwarfing as a result of the heavy and precocious cropping that it induces. Recently, promising rootstocks are the selections 'QR 193-16' of the breeding program of East Malling, 'Pyrodwarf' from Germany, 'Pyriam' from France and Fox series from Italy (Deckers and Schoofs 2002). It may be better to develop dwarfing *Pyrus* rootstocks than to improve the quince rootstocks, because more grafting incompatibility is encountered between intergenic than intragenic grafts. However, the frequency developing dwarf *Pyrus* rootstocks is low and none of the present *Pyrus* clonal selections are easy to propagate. *P. communis* seedlings, especially of the main commercial cultivars 'D'Anjou', 'Winter Nelis' and 'Bartlett', are still widely used although trees on them can be too vigorous and they are susceptible to fire blight and pear decline. However, they have very good winter hardiness, good graft compatibility with scion cultivars and low susceptibility to lime induced iron chlorosis.

While in Asian pear production, a dwarf rootstock has not been developed. Japanese pear trees generally have their vigor controlled by being trained on a horizontal trellis and therefore developing a dwarf rootstock has not been a priority. Most widely used rootstocks are open-pollinated seedlings of semi wild *P. pyrifolia* and pea pear, *P. dimorphophylla* in Japan. Seedlings of *P. betulaefolia* strains originating in northern China should be used where stony pear (Yuzuhada: physiological disorder causing rough skin) is a problem. Strains of *P. calleryana* tolerant to water logging have been selected in Japan. The rootstocks used widely for Asian pears in China are seedlings of *P. bretschneideri, P. pyrifolia, P. betulaefolia* and *P. calleryana* (Wei and Gao 2002). Since rootstocks used for pear in Japan and China are all seedlings, which may be from interspecific or intraspecific hybridization and cannot provide uniform growth. This sometimes makes pear management complicated. In the future, uniform vegetative propagated rootstocks and methods for careful selections are needed.

6.2 Construction of Genetic Maps

Linkage maps and molecular markers would be useful in traditional crossbreeding programs for perennial crops such as fruit tree species. However, genetic studies in pear, as in many fruit trees, have been rare. There is little information on genetic linkage maps, and development of molecular markers on pears despite many researches on apple mapping and molecular markers. The long juvenile periods, the space necessary to manage large number of progenies and the high level of heterozygosity due to a gametophytic incompatibility have limited inheritance studies to a few morphological characters (Chevreau et al. 1997).

6.2.1 Development of Molecular Markers

6.2.1.1 Isozymes

The first report on the use of isozymes in pears was in 1980 by Santamour and Demuth to identify six ornamental cultivars of *P. calleryana* by peroxidase patterns. Peroxidase diversity has also been studied on several species of *Pyrus* (Menendez and Daley 1986) and on 172 cultivars of *P. pyrifolia* (Jang et al. 1991, 1992). Isozymes' variability of several enzymes in pollen was reported by Cerezo and Socias y Company (1989). However, these approaches are used for cultivar identification and in a desire to differentiate genetic sports. Chevreau et al. (1997) examined the inheritance and linkage of isozyme loci in *P. communis* varieties. They analyzed the polymorphisms of 11 enzymes (AAT: Aspartate aminitransferase, ENP: Endopeptidase, EST: esterase, LAP: Leucine aminopeptidase, PRX: Peroxidase, SOD: Superoxide dismutase, ADH: Alcohol dehydrogenase, DIA: Diaphorase, PGD: 6-Phosphogluconate dehydrogenase, PGI: Phosphoglucoisomerase, PGM: Phosphoglucomutase) in 11 progenies from controlled crosses. According to their report, 22 loci were identified and segregation was scored for 20 loci. Three pairs of duplicated loci forming intergenic hybrid bands were detected and these were found to correspond to equivalent duplicated genes in apple. They identified 49 active alleles and one null allele and revealed three linkage groups, which could all be related to existing groups on the apple map. Conservation of isozyme patterns, duplicated genes and linkage groups indicates a high degree of synteny between apple and pear. But no linkage map for pears was constructed based on the information of isozyme analysis.

6.2.1.2 DNA-Based Markers

RAPD RAPD has been widely used on pear genetic studies because RAPD has the advantages of being readily employed, requiring small amounts of genomic DNA. RAPD markers have been successfully used for identification and genetic relationships of pear. Oliveira et al. (1999) investigated molecular characterization and phenetic similarities between several cultivars of *P. communis* and *P. pyrifolia* and several wild species by RAPD markers. A total of 118 *Pyrus* spp. and cultivars native mainly to east Asia were analyzed by RAPD markers to evaluate genetic variation and relationships among the accessions (Teng et al. 2001, 2002). According to their reports, RAPD markers specific to species were identified, and the grouping of the species and cultivars by RAPD largely agrees with morphological taxonomy. RAPD markers have also been used to identify parentage (Banno et al. 2000). Banno et al. (1999) also identified an RAPD marker linked to the gene conferring susceptibility to black spot disease (*Alternaria alternata* Japanese pear pathotype).

AFLP AFLP technology is a powerful tool that combines DNA restriction and PCR amplification. AFLP has several advantages over the RAPD technique, like a higher number of loci analyzed and a higher reproducibility of banding patterns. Monte-Corvo et al. (2000) investigated the genetic relationships among 39 cultivars including 35 *P. communis* and 4 *P. pyrifolia* cultivars using AFLP and RAPD markers. They confirmed that AFLP markers were five times more efficient in detecting polymorphism per reaction. Although some differences can be noticed between the dendrograms resulting from AFLP and RAPD analyses, both techniques produced similar results. Yamamoto et al. (2002b) also made 184 and 115 polymorphic AFLP fragments using 40 primer combinations in the F_1 population originating from 'Bartlett' and 'Hosui', respectively. They reported that the average number of polymorphic fragments per primer combination was 4.6 in 'Bartelett' and 2.9 in 'Hosui'.

SSR SSRs are excellent sources of polymorphisms in eukaryotic genomes. The development of SSRs is labor intensive. However, SSRs have been very useful in

studying diversity in *Pyrus*. Yamamoto et al. (2002a) constructed a genome library enriched with (AG/TC) sequences from 'Hosui' Japanese pear using the magnetic beads method. They obtained 85 independent sequences containing 8–36 microsatellite repeats. Out of the 85 sequences, 59 contained complete (AG/TC) repeats. Thirteen primer pairs could successfully amplify the target fragments, and showed a high degree of polymorphisms in the Japanese pear. Kimura et al. (2002) identified 58 Asian pear accessions from six *Pyrus* species using these nine SSR markers with a total of 133 putative alleles. They obtained a phenogram based on the SSR genotypes, showing three major groups corresponding to the Japanese, Chinese and European groups. Moreover, nine apple SSRs were intergenetically applied to the characterization of 36 pear accessions (Yamamoto et al. 2001). All of the tested SSR primers derived from apple produced discrete amplified fragments in all pear species and accessions. The differences in fragment size are mostly due to the differences in repeat number. A total of 79 alleles were detected from seven SSR loci and thus pear and apple varieties could be differentiated. This data show that *Pyrus* family has a close genetic relationship with *Malus* family.

RFLP and Others RFLPs have been used to identify Japanese pears, including the parentage of 10 cultivars, with two minisatellite probes from human myoglobin DNA (Teramoto et al. 1994). Similar attempts have been made to distinguish *Pyrus* species with RFLPs of chloroplast DNA (Iketani et al. 1998; Katayama and Uematsu 2003). However, these markers were used for cultivar identification and investigating genetic relationships among *Pyrus* species. Itai et al. (1999) have identified RFLP markers linked to the locus that determine the rate of ethylene evolution in ripening fruit of the Japanese pear by using two probes of 1-aminocyclopropane-1-carboxylate (ACC) synthase genes in ethylene biosynthetic pathway. SCARs were developed from RAPDs to evaluate and identify *P. communis* and *P. pyrifolia* cultivars (Lee et al. 2004). ISSR markers also have been used for cultivar identification and taxonomic relationships in pears (Monte-Corvo et al. 2001). Another unique markers, copia-like retrotransposons have been identified in pears (Shi et al. 2002). They suggest that the transposition of retrotransposons take place during evolution leading to diversification. However, no data on the inheritance of these markers has yet been reported.

6.2.2 Constructing Linkage Maps

First linkage maps in *Pyrus* species were developed for 'Kinchaku' and 'Kosui' Japanese pears using RAPD markers (Iketani et al. 2001). Black spot and pear scab are the most severe diseases of Japanese pear. Only a few cultivars are susceptible to black spot, on the other hand most cultivars of Japanese pear are susceptible to pear scab. A survey of *P. pyrifolia* germplasm has identified 'Kinchaku' as the only cultivar having resistance. They used the pseudo-testcross method (Grattapaglia and Sederoff 1994) and constructed two separate maps from segregation data of 82 F_1 individuals. The reason for using the pseudo-testcross method is that it is very difficult to make F_2 or backcross populations because pears take long periods to progressing generation and don't have a self-pollination nature. The linkage map for 'Kinchaku' consisted of 120 loci in 18 linkage groups (LG) spanning 768 cM, while that for 'Kosui' contained 78 loci in 22 linkage groups extending over 508 cM. This is the first report of a linkage map of pear species. The resistance allele of pear scab (*Vn*) and the susceptibility allele of black spot were mapped in different linkage groups in 'Kinchaku'. However, in both maps the number of linkage groups do not converge into a basic chromosome number ($x = 17$). Therefore, the total map length is still not sufficient for covering the complete genome. The length of the apple genome was reported to be 1,200 cM or a little more (Conner et al. 1997). Pear has the same basic chromosome number as apple. In addition, the nuclear DNA content of pear species is estimated 3/4th or 4/5th of that of apple (Dickson et al. 1992). These two maps are estimated to cover at least about a half of the total pear genome (Iketani et al. 2001).

The second linkage maps were reported using 63 F_1 individuals obtained from an interspecific cross between the European pear 'Bartlett' and the Japanese pear 'Hosui' by Yamamoto et al. (2002b, 2004). They constructed maps based on AFLPs, SSRs (from pear, apple and *Prunus*), isozymes, and phenotypic traits (leaf color and S-genotype). The map of 'Bartlett' consisted of 256 loci including 178 AFLPs, 76 SSRs (32 pear, 39 apple, 5 *Prunus*), one isozyme and a self-incompatibility locus on 19 linkage groups over a total length of 1,020 cM (Fig. 1). The average distance between each pair of loci is 4.0 cM. The size of linkage groups ranges from 88 cM (LG 4) to 11 cM (LG 18). The segregation of many markers on LG 14 is largely

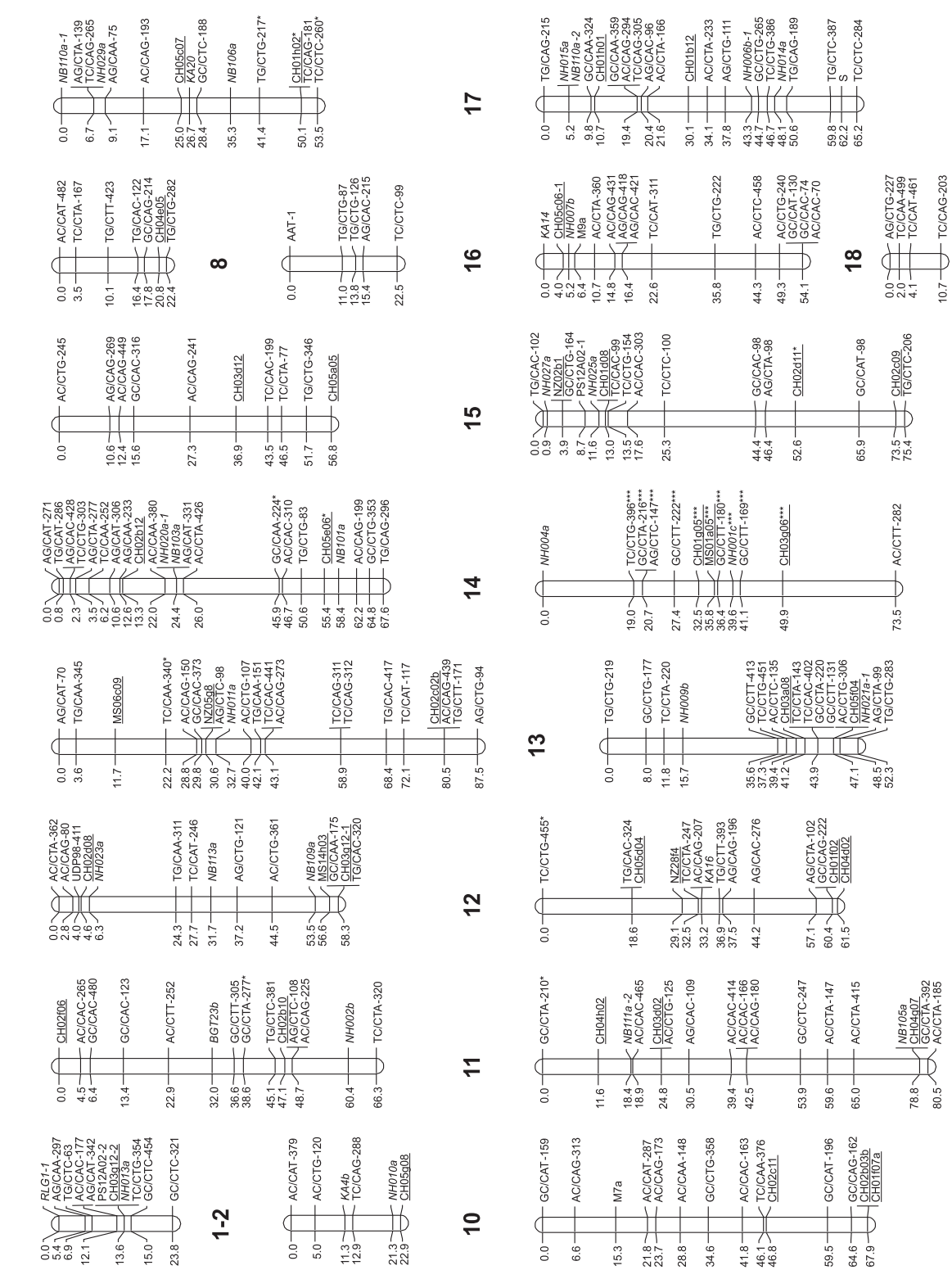

Fig. 1. A genetic linkage map of the European pear 'Bartlett' (Yamamoto et al. 2004). The designation of AFLP markers is based on primer combination and size. SSR loci from apple and pear are *underlined* and in *italics*, respectively. The self-incompatibility locus is denoted by *S*. *Asterisks* indicate distorted segregations of markers according to the chi-square test. Distortion at the 5%, 1% and 0.1% level are indicated as *, ** and ***, respectively

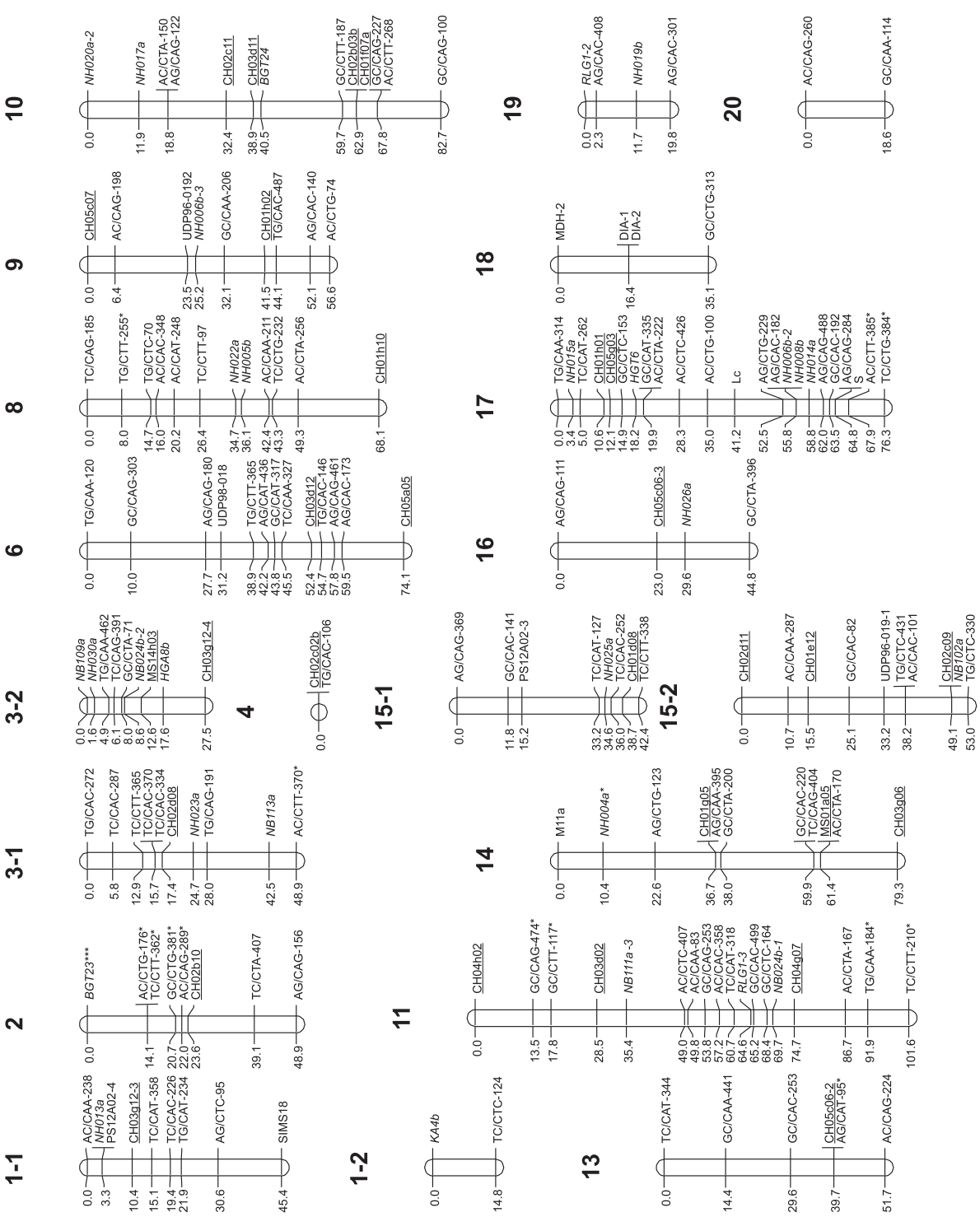

Fig. 2. A genetic linkage map of the Japanese pear 'Hosui' (Yamamoto et al, 2004). The *symbols* and *asterisks* are the same as Fig. 1. Young leaf color is denoted by *Lc*

distorted. The self-incompatibility locus (S-locus) is in the bottom of LG17. While the map of 'Hosui' contained 180 loci including 110 AFLPs, 64 SSRs (29 pear, 29 apple, 6 *Prunus*), two phenotypic traits and four other markers on 20 linkage groups encompassing a genetic distance of 995 cM (Fig. 2). Genetic linkage maps of these cultivars are aligned using 37 codominant markers that show segregating alleles in both cultivars (Yamamoto et al. 2002b, 2004). They found that of tested 80 SSRs obtained from apple, more than four-fifth could produce discrete PCR bands in pear. Similar findings were observed in European pears by another reaserch group (Pierantoni et al. 2004). Yamamoto et al. (2004) reported that 38 apple SSR markers showed 39 segregating loci on the linkage map of 'Bartlett', and that 27 SSRs produced 29 loci on that of 'Hosui'. Moreover, they considered synteny between pear and apple linkage maps. Total 36 SSRs originating from apple were mapped on the genetic linkage maps of 'Bartlett' and apple. Only two SSR loci were aligned to different linkage groups between pear and apple. Other 34 apple SSR loci were positioned in presumably homologous linkage groups of pear. All pear linkage groups were successfully aligned to the apple consensus map by at least one apple SSR, indicating that positions and linkages of SSR loci were well conserved between pear and apple. Their trials were the first major effort in comparing maps of apple and pear. Next, more SSRs and molecular markers for agronomically important characters could be developed to construct fine linkage maps resulting in useful for marker assisted selection.

Other maps were developed for two European pear cultivars 'Passe Crassane' and 'Harrow Sweet' using SSRs, MFLPs, AFLPs, RGAs and AFLP-RGAs markers in 99 F_1 individuals (Dondini et al. 2004). The existence of different levels of susceptibility to fire blight, one of the most terrible diseases, was distributed in European pear cultivars. This suggests that it is possible to identify quantitative trait loci (QTL) related to resistance in pear germplasm. 'Passe Crassane' is a susceptible cultivar and 'Hallow Sweet' is resistant. The 'Passe Crassane' map consists of 155 loci including 98 AFLPs, 37 SSrs, 6 MFLPs, 4 RGAs, and 10 AFLPs-RGA for a total length of 912 cM organized in 18 linkage groups. The average distance between each pair of loci is 5.8 cM. The size of each linkage group ranges from 7.0 to 92.9 cM. The 'Hallow Sweet' map consists of 156 loci including 101 AFLPs, 35 SSRs, 3 MFLPs, 3 RGAs and 14 AFLPs-RGA for a total length of 930 cM organized in 19 linkage groups. The sizes of these maps are comparable with the report by Yamamoto et al. (2002b).

6.3 Gene Mapping and QTL Detection

So far, there is only a report on the QTL analysis in *Pyrus* (Dondini et al. 2004). The only and the first QTL mapping involved the fire blight resistance in European pears. Fire blight continues to spread throughout western, central and southern Europe despite quarantine measures treated (Jock et al. 2002). The existence of different levels of susceptibility to fire blight is distributed in European pear cultivars. Fire blight resistance in pear is known as a quantitative trait (Dondini et al. 2002). Dondini et al. (2004) constructed two genetic linkage maps of the parental lines 'Passe Crassane' (susceptible) and 'Hallow Sweet' (resistant) using SSRs, MFLPs, AFLPs, RGAs and AFLP-RGAs markers and conducted QTL analysis of fire blight resistance. QTL analysis identified four regions of 'Hallow Sweet' associated with fire blight resistance, while no QTLs related to resistance were found in susceptible 'Passe Crassane'. This represents a first step of marker-assisted selection (MAS) approach in pear breeding programs designed to select new fire blight resistant genotypes. Moreover, the presence of each putative QTL of SSR markers makes it possible to transfer map information to different pear cross populations.

6.4 Marker-Assisted Breeding

A long juvenile period and high level of heterozygosity due to a strict gametophytic incompatibility have limited the parental combinations in pear breeding programs. Marker-assisted selection (MAS) is considered to be a powerful tool for increasing selection efficiency by identifying favorable genetic combinations in fruit trees as well as other crops. The major advantage of MAS is the ability to evaluate many traits at the seedling stage in fruit trees that have a long juvenile phase. Especially, MAS in pear breeding programs can be particularly important for traits that are difficult to evaluate. However, available markers for MAS are limited to some extent in *Pyrus*. Banno et al. (1999) tested 250 RAPD primers to screen

a pair of bulked DNA samples derived from open-pollinated progenies of Japanese pear 'Osa Nijisseiki' to identify markers linked to the susceptible *A* gene of black spot disease, caused by *Alternaria Alternata* Japanese pear pathotype. The CMNB41 primer generates a 2,350 bp fragment, which is present in the susceptible bulk, but not in the resistant one. This RAPD marker, CMNB41/2350, is at a distance of about 3.1 cM from the susceptible *A* gene. They found that the frequency of occurrence of the CMNB41/2350 marker was 96% in susceptible cultivars and progenies of 'Osa Nijisseiki' × 'Oharabeni'.

Ethylene production by cultivated Japanese pear fruits varies from 0.1 nl g^{-1} h^{-1} to 300 nl g^{-1} h^{-1} during fruit ripening, suggesting there are both climacteric and non-climacteric cultivars. Climacteric-type fruits exhibit a rapid increase in ethylene production and have a low storage potential, while non-climacteric fruits show no detectable ethylene production and fruit quality maintained for over a month in storage. Fruit storage potential is closely related to the maximum level of ethylene production in Japanese pear. Itai et al. (1999, 2003b) have cloned three ACC (1-aminocyclopropane-1-carboxylate) synthase genes (*PPACS1, 2, 3*), and studied their expression during fruit ripening. *PPACS1* was specifically expressed in cultivars of high ethylene production, while *PPACS2* was specifically expressed in cultivars of moderate ethylene production. Moreover, they have identified RFLP markers linked to the ethylene evolution rate of ripening fruit using RFLP analysis with two ACC synthase genes (*PPACS1* and *PPACS2*). RFLPs were designated as A (2.8 kb of *PPACS1*) linked to high levels of ethylene (> 10 nl g^{-1} h^{-1}) and B (0.8 kb of *PPACS2*), linked to moderate levels of ethylene (0.5 nl g^{-1} h^{-1}–10 nl g^{-1} h^{-1}), when the total DNA was digested by *Hin*dIII. These markers (A and B) are useful for the selection of Japanese pear cultivars with enhanced post-harvest keeping ability. These markers were converted to more convenient and easier PCR-based CAPS markers (Itai et al. 2003a). Furthermore, linkage analysis of these two markers were conducted in the F_2 populations derived from self-pollinated 'OT16', a F_1 of 'Osa Nijisseiki' (a self-compatible mutant of 'Nijisseiki') × 'Cili', which revealed that the recombination frequency between the two markers was 20.8±3.6%. F_2 populations in *Pyrus* have not been reported so far because of a strict gametophytic incompatibility. These are the first populations of self-pollinated F_2 in *Pyrus* species.

Most pear cultivars have been classified as self-incompatible. Therefore, the proposition of pollinizers inter-planted in the orchard is a requirement to get an economic crop from most of the cultivars (Sanzol and Herrreo 2002). The progression of our understanding of incompatibility in *Pyrus* has accelerated greatly since the mid-1990s. In *Pyrus*, gametophytic self-incompatibility is controlled by a single polymorphic gene locus, the S-locus. The S-locus harbors a multi-allelic gene, which encodes for S-RNase that blocks incompatible-tube growth through the style (Ushijima et al. 1998). In Japanese pear, cDNAs encoding S_1- to S_9-RNase have been isolated and sequenced (Sassa et al. 1997; Ishimizu et al. 1998; Takasaki et al. 2004). Based on the nucleotide sequences, Ishimizu et al. (1999) established a PCR-RFLP (S_1- to S_7-) system for S-genotype assignments in Japanese pear. Takasaki et al. (2004) modified this system and finally established the system for discriminating S_1- to S_9-allele in Japanese pear. Both S-alleleic constitution and cross-incompatibility groups have been for Japanese pear, although the situation contrasts with the scarce information available in European pear. In Asian countries, artificial pollination is often used for stable production, therefore knowing S-genotype of commercial cultivars is very important thing, in comparison with open-pollination in Europe. Recently, molecular techniques have started to be used for the identification of S-genotypes in European pears (Sanzol and Herreo 2002; Zuccherelli et al. 2002; Zisovich et al. 2004). Six *S*-allele (Sa- to Sh-) was identified using 10 cultivars by Zuccherelli et al. (2002), four S-allele (S1- to S4-) was identified using seven cultivars by Sanzol and Herreo (2002), and seven S-allele (Si- to So) was identified by Zisovich et al. (2004). Both the methods and the determination of S-gentypes will facilitate the stable production.

6.5 Future Scope of Works

Breeding pears is complicated by their long juvenile phase and complex genetic structure. Pears present a high level of heterozygosity, therefore a great deal of segregation must be taken into account for in breeding populations. Moreover, the lack of morphological markers in pears has been the obstacle to limit the improvement of selection techniques. However, approaches to the improvement of pears

by breeding have changed markedly in recent years, due to our expansion of knowledge and techniques on genes and gene function. This trend will be accelerated by the development of biochemical and molecular markers linked to important horticultural traits. Pear breeding will be entering a new era of knowledge acquisition with the start of large scale genomics programs. We expect pear genomics research programs to deliver a vast amount of data which will lead to a better understanding of this crop in terms of its relationship with the environment and its metabolic pathways. Finally, the increased knowledge provided by genomic studies will bring new tools to assist the creation of cultivars more adapted to the future request for stable pear production.

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