Compendium of Plant Genomes
Series Editor: Chittaranjan Kole

Schuyler S. Korban *Editor*

The Pear Genome

Springer

Compendium of Plant Genomes

Series Editor

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Whole-genome sequencing is at the cutting edge of life sciences in the new millennium. Since the first genome sequencing of the model plant Arabidopsis thaliana in 2000, whole genomes of about 100 plant species have been sequenced and genome sequences of several other plants are in the pipeline. Research publications on these genome initiatives are scattered on dedicated web sites and in journals with all too brief descriptions. The individual volumes elucidate the background history of the national and international genome initiatives; public and private partners involved; strategies and genomic resources and tools utilized; enumeration on the sequences and their assembly; repetitive sequences; gene annotation and genome duplication. In addition, synteny with other sequences, comparison of gene families and most importantly potential of the genome sequence information for gene pool characterization and genetic improvement of crop plants are described.

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This book series is dedicated to my wife Phullara,
and our children Sourav, and Devleena

Chittaranjan Kole

Preface to the Series

Genome sequencing has emerged as the leading discipline in the plant sciences coinciding with the start of the new century. For much of the twentieth century, plant geneticists were only successful in delineating putative chromosomal location, function, and changes in genes indirectly through the use of a number of "markers" physically linked to them. These included visible or morphological, cytological, protein, and molecular or DNA markers. Among them, the first DNA marker, the RFLPs, introduced a revolutionary change in plant genetics and breeding in the mid-1980s, mainly because of their infinite number and thus potential to cover maximum chromosomal regions, phenotypic neutrality, absence of epistasis, and codominant nature. An array of other hybridization-based markers, PCR-based markers, and markers based on both facilitated construction of genetic linkage maps, mapping of genes controlling simply inherited traits, and even gene clusters (QTLs) controlling polygenic traits in a large number of model and crop plants. During this period, a number of new mapping populations beyond F_2 were utilized and a number of computer programs were developed for map construction, mapping of genes, and for mapping of polygenic clusters or QTLs. Molecular markers were also used in the studies of evolution and phylogenetic relationship, genetic diversity, DNA fingerprinting, and map-based cloning. Markers tightly linked to the genes were used in crop improvement employing the so-called marker-assisted selection. These strategies of molecular genetic mapping and molecular breeding made a spectacular impact during the last one and a half decades of the twentieth century. But still, they remained "indirect" approaches for elucidation and utilization of plant genomes since much of the chromosomes remained unknown and the complete chemical depiction of them was yet to be unraveled.

Physical mapping of genomes was the obvious consequence that facilitated the development of the "genomic resources" including BAC and YAC libraries to develop physical maps in some plant genomes. Subsequently, integrated genetic–physical maps were also developed in many plants. This led to the concept of structural genomics. Later on, the emphasis was laid on EST and transcriptome analysis to decipher the function of the active gene sequences leading to another concept defined as functional genomics. The advent of techniques of bacteriophage gene and DNA sequencing in the 1970s was extended to facilitate sequencing of these genomic resources in the last decade of the twentieth century.

As expected, sequencing of chromosomal regions would have led to too much data to store, characterize, and utilize with the-then available computer software could handle. But the development of information technology made the life of biologists easier by leading to a swift and sweet marriage of biology and informatics, and a new subject was born—bioinformatics.

Thus, the evolution of the concepts, strategies, and tools of sequencing and bioinformatics reinforced the subject of genomics—structural and functional. Today, genome sequencing has travelled much beyond biology and involves biophysics, biochemistry, and bioinformatics!

Thanks to the efforts of both public and private agencies, genome sequencing strategies are evolving very fast, leading to cheaper, quicker, and automated techniques right from clone-by-clone and whole-genome shotgun approaches to a succession of second-generation sequencing methods. The development of software of different generations facilitated this genome sequencing. At the same time, newer concepts and strategies were emerging to handle sequencing of the complex genomes, particularly the polyploids.

It became a reality to chemically—and so directly—define plant genomes, popularly called whole-genome sequencing or simply genome sequencing.

The history of plant genome sequencing will always cite the sequencing of the genome of the model plant Arabidopsis thaliana in 2000 that was followed by sequencing the genome of the crop and model plant rice in 2002. Since then, the number of sequenced genomes of higher plants has been increasing exponentially, mainly due to the development of cheaper and quicker genomic techniques and, most importantly, the development of collaborative platforms such as national and international consortia involving partners from public and/or private agencies.

As I write this preface for the first volume of the new series "Compendium of Plant Genomes," a net search tells me that complete or nearly complete whole-genome sequencing of 45 crop plants, 8 crop and model plants, 8 model plants, 15 crop progenitors and relatives, and 3 basal plants is accomplished, the majority of which are in the public domain. This means that we nowadays know many of our model and crop plants chemically, i.e., directly, and we may depict them and utilize them precisely better than ever. Genome sequencing has covered all groups of crop plants. Hence, information on the precise depiction of plant genomes and the scope of their utilization are growing rapidly every day. However, the information is scattered in research articles and review papers in journals and dedicated Web pages of the consortia and databases. There is no compilation of plant genomes and the opportunity of using the information in sequence-assisted breeding or further genomic studies. This is the underlying rationale for starting this book series, with each volume dedicated to a particular plant.

Plant genome science has emerged as an important subject in academia, and the present compendium of plant genomes will be highly useful to both students and teaching faculties. Most importantly, research scientists involved in genomics research will have access to systematic deliberations on the plant genomes of their interest. Elucidation of plant genomes is of interest not only for the geneticists and breeders, but also for practitioners of an array of plant science disciplines, such as taxonomy, evolution, cytology, physiology,

pathology, entomology, nematology, crop production, biochemistry, and obviously bioinformatics. It must be mentioned that information regarding each plant genome is ever-growing. The contents of the volumes of this compendium are, therefore, focusing on the basic aspects of the genomes and their utility. They include information on the academic and/or economic importance of the plants, description of their genomes from a molecular genetic and cytogenetic point of view, and the genomic resources developed. Detailed deliberations focus on the background history of the national and international genome initiatives, public and private partners involved, strategies and genomic resources and tools utilized, enumeration on the sequences and their assembly, repetitive sequences, gene annotation, and genome duplication. In addition, synteny with other sequences, comparison of gene families, and, most importantly, the potential of the genome sequence information for gene pool characterization through genotyping by sequencing (GBS) and genetic improvement of crop plants have been described. As expected, there is a lot of variation of these topics in the volumes based on the information available on the crop, model, or reference plants.

I must confess that as the series editor, it has been a daunting task for me to work on such a huge and broad knowledge base that spans so many diverse plant species. However, pioneering scientists with a lifetime experience and expertise on the particular crops did excellent jobs editing the respective volumes. I myself have been a small science worker on plant genomes since the mid-1980s and that provided me the opportunity to personally know several stalwarts of plant genomics from all over the globe. Most, if not all, of the volume editors are my longtime friends and colleagues. It has been highly comfortable and enriching for me to work with them on this book series. To be honest, while working on this series, I have been and will remain a student first, a science worker second, and a series editor last. And I must express my gratitude to the volume editors and the chapter authors for providing me the opportunity to work with them on this compendium.

I also wish to mention here my thanks and gratitude to the Springer staff, Dr. Christina Eckey and Dr. Jutta Lindenborn in particular, for all their constant and cordial support right from the inception of the idea.

I always had to set aside additional hours to edit books beside my professional and personal commitments—hours I could and should have given to my wife, Phullara, and our kids, Sourav, and Devleena. I must mention that they not only allowed me the freedom to take away those hours from them but also offered their support in the editing job itself. I am really not sure whether my dedication of this compendium to them will suffice to do justice to their sacrifices for the interest of science and the science community.

Kalyani, India Chittaranjan Kole

Preface

The pear, belonging to the *Pyrus* genus and subtribe Malinae of the Amygdaloideae subfamily within Rosaceae, is the third most important temperate fruit tree crop, with an annual worldwide production of ~18 million tons (2014 FAOSTAT). The genus *Pyrus* includes at least 22 known species with over 5000 accessions maintained worldwide. These accessions display wide variations in morphological and physiological traits along with broad adaptation to wide agroecological environments. It is reported that the ancient *Pyrus* likely arose during the Tertiary period, between 55 and 65 million years ago (Mya), in the mountainous regions of southwestern China. From there, it has been dispersed across mountainous ranges, both toward east and west regions, resulting in the evolution of two distinct major groups, commonly referred to as European and Asian pears. Asian pears have been cultivated for about 3300 years ago, while European pears have been cultivated for more than 2000 years.

While the cultivated European pears predominantly belong to *P. communis*, the cultivated Asian pears belong to several major species, including *P. pyrifolia*, *P.* × *bretschneideri*, *P.* × *sinkiangensis*, and *P. ussuriensis*. Fruit of European pears is characterized by their typical pyriform shape (bulbous bottoms and tapering tops), although there are some with oblate or globose shapes, with soft and fine-grained flesh, few stone or lignified cells, along with a strong aroma and flavor. Fruit of Asian pears is predominantly round in shape, although there are some with pyriform shapes, firm, with a crispy flesh, high sugar, and low acid contents, along with faint aroma and mild flavor.

The pear tree is cross-pollinated, self-incompatible, and with a long juvenility period of 5–7 years. However, there are little barriers to interspecific hybridization in pear despite its wide geographic distribution. Although genetic studies are limited, it is well documented that there is a wide genetic variability in pear. Most commercially grown cultivars have been selected as chance seedlings and then subsequently maintained through vegetative propagation, although there are few cultivars that have been developed from breeding programs via sexual hybridization. There are few releases of new pear cultivars that have been derived from various breeding programs from around the world. As with other tree fruit breeding programs, classical pear breeding is a long-term and expensive effort. Thus, recent advances in pear genomics are paving the way for a new and promising path for pear genetic improvement initiatives and efforts.

In recent years, modern genetic and genomic tools have resulted in the development of a wide variety of valuable resources, including molecular markers, genetic mapping, genetic transformation, structural and functional genomics resources, genome sequencing, and genome-wide association studies, as well as comparative genomic studies. These tools and resources offer unparalleled opportunities to pursue genetic improvement efforts to combine fruit quality, high productivity, precocious fruit-bearing, long postharvest storage life, along with elevated levels of resistance to various major diseases and insect pests of pear. Furthermore, these new genetic tools and genomic resources provide unprecedented opportunities to explore and understand genetic variation, evolution, and domestication of pear, as well as to better establish population-level relationships among different pear species. In the past few years, completion of whole-genome assemblies of "Dangshansuli", an Asian pear, and "Bartlett", a European pear, has enabled new discoveries in pear, including those of genomic structure, chromosome evolution, and patterns of genetic variation. All this wealth of new resources will have a major impact on our knowledge of the pear genome and its expanding resources. In turn, these resources and knowledge will have significant impacts on efforts for genetic improvement of pears.

The Pear Genome book will cover our current knowledge of botanical and taxonomic classifications; origin, distribution, and early documented distribution of pear; germplasm resources; genetic studies and genetic improvement efforts; genetic linkage maps; molecular genetic and QTL analysis, along with genomic analysis; whole-genome sequencing strategies and outcomes; repetitive and regulatory sequences; self-incompatibility; stone cell development; vegetative budbreak analysis; fire blight genetics and genomics; functional genomic analysis; whole-genome duplication in pear and its comparisons to apple; and potential opportunities and challenges for future genetic improvement efforts of pears.

All 16 chapters included in this volume will provide a wealth of information and comprehensive overview of the status of early and ongoing efforts to discern the genetics, breeding, and genomics of the pear. This book will offer ideas, opportunities, and pathways that will support future research and discovery efforts that will not only contribute to our expanded knowledge of various traits of this important fruit crop, as well as our understanding of the pear genome as a whole, but these will also contribute to overall advances in genetic enhancement efforts of the pear.

Urbana, USA Schuyler S. Korban

Contents

Abbreviations

4CL	4-coumarate: coenzyme A ligase
AFLP(s)	Amplified fragment length polymorphism(s)
CAD	Cinnamyl alcohol dehydrogenase
CCR	Cinnamoyl-CoA reductase
CR(s)	Chilling requirement(s)
CRISPR/cas9	Clusters of regularly interspaced short palindromic repeats/cas9 associated protein
CU(s)	Chilling unit(s)
DIR	Dirigent
G × E	Genotype × environment
GE	Genetic engineering
GMO	Genetically modified organism
GRF	Growth-regulating factor
GS	Genomic selection
GSI	Gametophytic self-incompatibility
GWAS	Genome-wide association study(ies)
HCT	Hydroxycinnamoyl-CoA: shikimate/quinate hydroxycinnamoyltransferase
HSF(s)	Heat shock transcription factor(s)
IRAP	Inter-retrotransposon amplified polymorphism
LD	Linkage disequilibrium
LG(s)	Linkage group(s)
LM	Linkage mapping
MAB	Marker-assisted breeding
MAS	Marker-assisted selection
NGS	New gene sequencing
NPBT	New plant breeding techniques
OA	Organic agriculture
OMT	O-methyltransferase
POD	Peroxidase
QTL(s)	Quantitative trait locus/loci
RAPD(s)	Random amplified fragment length polymorphism(s)
RBIP	Retrotransposon-based insertion polymorphism
RNAi	RNA interference
SBP	SQUAMOSA promoter binding protein

SI	Self-incompatibility
siRNAs	Small interfering RNAs
SNP(s)	Single nucleotide polymorphism(s)
SSAP	Sequence-specific amplification polymorphism
SSN	Sequence-specific nuclease technology
SSR(s)	Simple sequence repeat(s)
TE(s)	Transposon(s)/able element(s)
TF(s)	Transcription(al) factor(s)
VB	Vegetative budbreak
VIGS	Virus-induced gene silencing
WGD(s)	Whole-genome duplication(s)
ZHD	Zinc finger homeodomain

Botany and Taxonomy of Pear

1

Muriel Quinet and Jean-Pierre Wesel

Abstract

Pear belongs to the Rosaceae family as most of the cultivated fruit trees. It is the second fruit tree crop in terms of production after apple. Its production has increased these last decades to reach a world production of more than 27 megatons for almost 1,600,000 ha. Pears have been cultivated in Europe and in Asia for more than 5000 years. Of all known and reported pear species and interspecific hybrids, five are mainly cultivated. These include the European pear, *Pyrus communis*, and the Asian pears *P. pyrifolia*, *P.* × *bretschneideri*, *P. ussuriensis*, and *P. sinkiangensis*. Fruits of European pears are elongated and have a full-bodied texture, while those of Asian pears are round and have a sandy texture. The *Pyrus* genus belongs to the Amygdaloideae subfamily and the Malinae tribe and consists of about 75–80 species and interspecific hybrid species. As several hybridizations are observed among *Pyrus* species, this renders the distinction among some pear species rather difficult. The origin of the *Pyrus* genus dates back to the Oligocene epoch, about 33.35–25.23 Mya. It is a genus of mainly deciduous trees and shrubs spread throughout temperate Eurasia, reaching the Atlas Mountains in North Africa and extending to Japan and South China. *Pyrus* species produce generally simple leaves alternately arranged. Leaves are glossy green on some species, densely silvery hairy in some others. *Pyrus* flowers are white, borne in corymbs on short spurs or lateral branchlets and are composed of five sepals, five petals, numerous stamens, and usually a five-locular ovary with free styles. The *Pyrus* fruit is a pseudo-fruit composed of the receptacle or the calyx tube, greatly dilated, enclosing the true fruit, and consisting of five cartilaginous carpels, known as the core. Morphological characters of the leaf, fruit, and calyx are commonly used to differentiate among *Pyrus* species. There are thousands of pear cultivars over the world with wide diversity for fruit shape, taste, and texture. In this chapter, we have focused on the description of cultivated *Pyrus* species and on some of the main cultivated cultivars.

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1.1 Introduction

Two of the main pear species that are cultivated include *Pyrus communis* L. and *P. pyrifolia* (Burm.f.) Nakai (Hedrick et al. 1921). *P. communis* is native to central and Eastern

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Europe and to southwest Asia, and it is known as European pear or common pear. It is one of the most important fruits of temperate regions, and it is the pear of common cultivation in Europe, America, Oceania, and Africa (Hedrick et al. 1921; Bassil and Postman 2010). The cultivation of *P. communis* makes up about one-third of the total pear production (Chagné et al. 2014). While *P. pyrifolia* is native to East Asia, and it is mainly cultivated in Asia, it is currently also cultivated in America, Oceania, and Europe (Bretaudeau and Fauré 1991; White 2002; Faoro and Orth 2014). Other *Pyrus* species are also commonly grown in Asia, including *P.* × *bretschneideri*, *P. ussuriensis,* and *P. sinkiangensis* (Wu et al. 2013). *P. pyrifolia* is known by many names including Asian pear, Chinese pear, Korean pear, Japanese pear, Taiwanese pear, nashi, and sand pear (Hedrick et al. 1921; Bailey and Bailey 1976; Petri and Herter 2002; Lee et al. 2012). Some of these vernacular names include other pear species, as some cultivars of *P.* × *bretschneideri* and *P. ussuriensis* are also called nashi pears, or *P.* × *bretschneideri* is also known as Chinese white pear (Chagné et al. 2014). For the sake of clarity, all these will be collectively grouped and referred to as Asian pears. While fruits of European pears are elongated and have full-bodied textures, fruits of Asian pears are round and have sandy textures (Silva et al. 2014). All these *Pyrus* species are botanically referred to as pome fruits and belong to the Rosaceae family, as many other fruit tree species including other pome fruits, apple and quince, and stone fruits, such as cherry, almond, peach, apricot, plum, and nectarine.

The first landmarks of pear as a cultivated tree in Europe were found in ancient Greece (Hedrick et al. 1921). Pear is currently cultivated worldwide, and its production has increased over the last decades to reach a world production of more than 27 megatons for almost 1,600,000 ha in 2016 (Fig. 1.1a, b) (FAO 2018). China is the largest producer of pear fruits worldwide, producing about 20 times more pears than all other main producers (Fig. 1.1c) (FAO 2018). In 2016, Asia contributed for 79% of pear production, Europe for 10%, America for 7%, Africa for 3%, and Oceania for less than 1% (FAO 2018). The pear tree is the second Rosaceous fruit tree crop grown in terms of production and the fifth in terms of harvested area (Fig. 1.2). Overall, the main cultivated fruit tree is apple, and pear production is about 30% of apple production. Pear and apple yields average 168,000 hg/ha over the last years, and are the best yields among Rosaceous fruit trees (FAO 2018).

1.2 Origin and Cultivation of Pear

1.2.1 Origin of Pear

The exact origin of the cultivated European pear tree is not known (Hedrick et al. 1921). According to Debuigne and Couplan (2006), it may result from the hybridization of several wild pear species from Europe and Minor Asia, including *P. communis* subsp. *pyraster* (L.) Ehrh. The wild pear tree of *P. communis* subsp. *pyraster* has likely originated from the mountains of Minor Asia or from Europe (Opoix 1896; Pesson and Louveaux 1984; Paris 1996). It could be deemed as a relic of warm oak forests and would be indigenous of the medio-European flora (Aas 1999). It most probably migrated to central and Western Europe 7500 to 4500 years ago during the warm post-glacial period (Aas 1999). The natural range of the species has not been precisely identified as it is difficult to distinguish wild from cultivated *P. communis* (Aas 1999). Currently, the species could be found in large areas of temperate regions of Europe, Asia, and America at altitudes of up to 800 m (Pesson and Louveaux 1984).

In contrast, domestication of Asian pears, including their centre(s) of origin along with time periods, is clearly documented (Silva et al. 2014). As reported in written Chinese (Shijing) and in other books, the major Asian species, cultivated for at least 1500 years, are *P. pyrifolia* and *P. ussuriensis* (Silva et al. 2014). In Japan, pear seeds dating back to the first century ACN have been found during excavations of the Toro Ruins in the Shizuoka prefecture (Saito 2016).

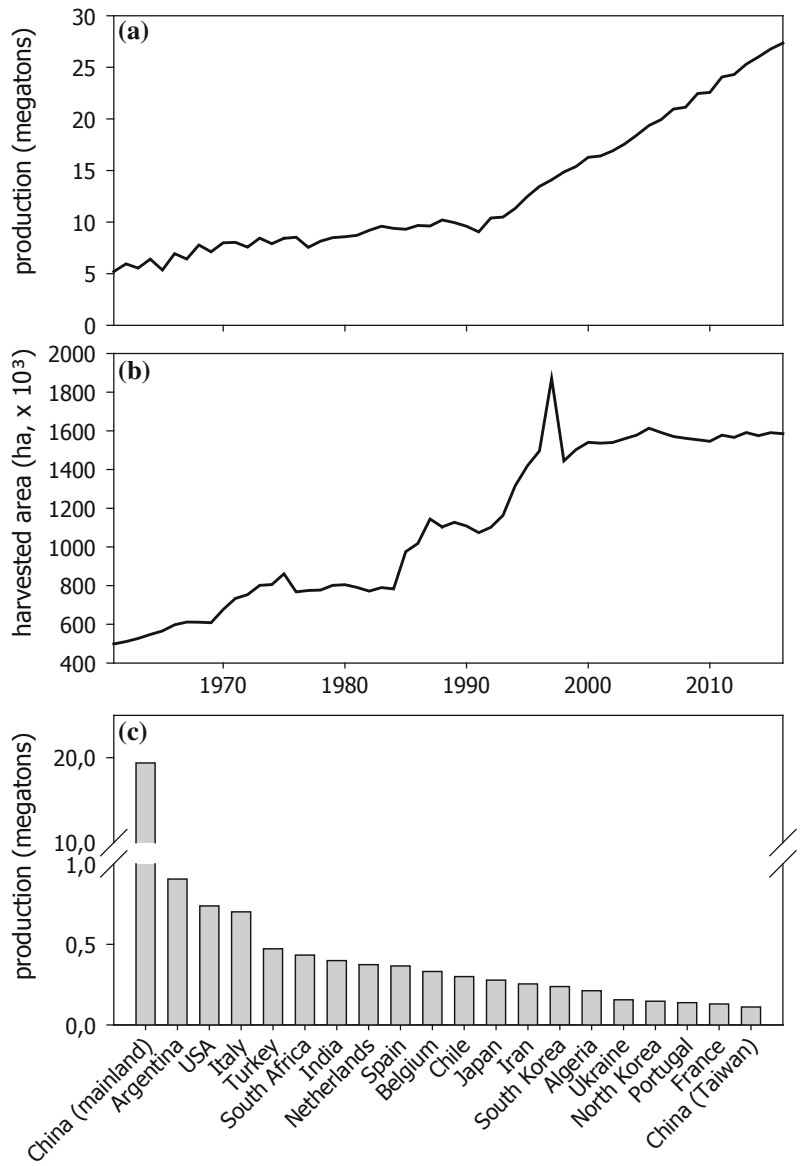

Fig. 1.1 Evolution of worldwide pear cultivation. **a** Pear production and **b** pear harvested areas between 1961 and 2016. **c** Main countries producing pears in 2016. Based on FAOSTAT database (FAO 2018)

1.2.2 History of Pear Cultivation

In comparison to other fruit tree species, pear cultivation has occurred rather late, and this is mainly due to the small fruit size of primitive pears (De Vilmorin and Clebant 1996). Pear domestication has taken place independently in the Far East (China) and in the Caucasus region (Ferradini et al. 2017). Pear has been cultivated in ancient Greece under the name of 'Achras' around 2800 ACN (Hedrick et al. 1921; Bretaudeau and Fauré 1991). By this time, pear has also been cultivated in both ancient Egypt and ancient Rome; however, its cultivation in China would have to go back to 4000 ACN (Bretaudeau and Fauré 1991).

In Homer's Odyssey is the first mention of pear cultivation in Greek literature (Royer 1853; Hedrick et al. 1921); however, the first definitive records of pear cultivation are found in the writings of Theophrastus in 370–286 ACN (Leroy 1867; Hedrick et al. 1921). Theophrastus

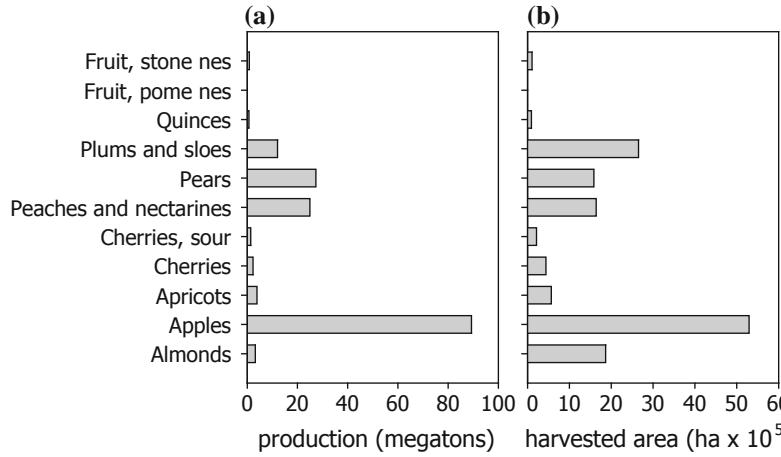

Fig. 1.2 Rosaceous fruit tree production (**a**) and harvested area (**b**) in 2016. Based on FAOSTAT database (FAO 2018)

distinguishes between wild and cultivated pears, and he makes reference to four pear cultivars, including 'Myrrha', 'Nardinon', 'Onychinon', and 'Talentiaion' (Leroy 1867; Hedrick et al. 1921). He writes about the propagation of pears from seeds, roots, and cuttings, as well as recognizes the necessity for cross-pollination though he does not offer reasons for this practice (Hedrick et al. 1921). In 178 ACN in Italy, Cato wrote the first book, written in Latin, on agriculture, and described six pear cultivars (Hedrick et al. 1921). Cato describes almost every method of propagating, grafting, caring for, and keeping fruits known to twentieth-century fruit growers (Hedrick et al. 1921). Following two centuries, Pliny described 41 pear cultivars in *Historia naturalis* (Leroy 1867). From Pliny, we know that the Romans valued pears for medicinal purposes, as well as for food (Hedrick et al. 1921). Subsequently and for a period of 1500 years, there are a few new facts that have been offered regarding the evolution of the pear (Hedrick et al. 1921). Many Roman writers mentioned pear, but they have all copied Theophrastus, Cato, and Pliny (Hedrick et al. 1921). In Japan, the first evidence of pear cultivation is found in the Chronicles of Japan (720 ACN), which mention that cultivation of fruits and nuts has been promoted during the Jito Tenno era (686–696 ACN) to fight famine (Saito 2016).

In Europe, there is no mention of new pear cultivars during the early Middle Ages, but in the eleventh century, Charlemagne has recommended planting fruit trees, including pear trees, in *Capitulare de Villis* (Leroy 1867). Therefore, the credit for establishing the first notable landmark in the history of the pear in France is due to Charlemagne (Hedrick et al. 1921). In fact, he has commanded his orchardists to plant pears of distinct kinds for distinct purposes and has cited the following three cultivars: 'Dulciores' for fresh fruit, 'Cocciore' for cooking, and 'Serotina', a late maturing variety (Leroy 1867). Following Charlemagne, there are no records on agricultural activities for the next five centuries (Hedrick et al. 1921). Undoubtedly, fruit tree farming must have been preserved in abbeys; however, there are no records of names of the pear cultivars cultivated in Western Europe during this period until the end of the fourteenth century (Leroy 1867).

During the fifteenth century, the printing press was by then developed, and books about horticulture were written and printed (Leroy 1867). The *Seminarium* of Charles Estienne, printed in 1540, offered brief descriptions of 16 pear cultivars that are still known to this day (Leroy 1867). From *Le Théâtre d'Agriculture,* written by De Serres and published in 1608, we know that many pears of diverse shapes, colours, flavours, and perfumes existed in the year 1600 in France

(Hedrick et al. 1921). Enthusiasm for pears rapidly increased due to the interest of a French royal prosecutor, Le Lectier (Leroy 1867). Le Lectier collected all available fruits of his time and in his country (Hedrick et al. 1921). In *Catalogue des arbres cultivés* published in 1628, he classified 260 pear cultivars based on their maturation. The French King Louis XIV (1638–1715) promoted pear cultivation, and during his reign, new cultivars were developed (Leroy 1867). Hitherto, the development of new cultivars was done through picking and transplantation of trees encountered in nature or in cultivated gardens. Although it has been a common practice since ancient Rome, cultivar selection of *P. communis* was mainly developed during the eighteenth century in Europe (Pesson and Louveaux 1984). In Japan, the concept of cultivars and cultural techniques were developed during the middle of the Edo era (1603–1867). 'Shokokusanbutsuchou' was the first recorded Japanese pear cultivar in 1735, and it was mentioned along with over 100 pear cultivars (Saito 2016).

During the eighteenth century in Europe, knowledge and understanding of plant sexuality have prompted the pursuit of plant breeding (Leroy 1867). Growers have made crosses and sowed seeds in order to develop new cultivars (Table 1.1) with improved pear fruit flavour, texture, size, and colour (Hedrick et al. 1921). Most of these new cultivars have been developed in Belgium, and several of these cultivars are cultivated to this day (Leroy 1867).

Pear improvement efforts in Belgium within a single century surpass all other previous efforts (Hedrick et al. 1921). Belgian pear growers and well-suited soil and climate conditions must be given credit for the development of the modern pear (Hedrick et al. 1921). The first and most famous Belgian to sow pear seeds in order to obtain new cultivars was Abbot Nicolas Hardenpont (1705–1774), and a dozen or more new pears have been credited to him (Hedrick et al. 1921). Hardenpont's best cultivars have been known since 1758, including the popular 'Passe-Colmar' (1758), 'Beurré d'Hardenpont' (1759), 'Délice d'Hardenpont', 'Beurré Rance', and 'Délice du Panisel' (1760–62). 'Beurré d'Harpendont' could still be found in tree nurseries worldwide, although it is now known as 'Glou Morceau' in Anglo-Saxon countries and as 'Beurré d'Arenberg' in France. Jean-Baptiste Van Mons has followed Hardenpont's lead by developing about 500 new pear cultivars among thousands found in Belgium between 1758 and 1900. Among these, 'Beurré d'Anjou' (syn. 'Nec plus Meuris') has been exported to America where it is still cultivated. It is important to point out that the designation of 'Anjou' or 'd'Anjou' has been erroneously used for this variety when first introduced to both America and England. Nevertheless, almost 40 pear cultivars developed by Van Mons have remained under cultivation at the beginning of the twentieth century (Hedrick et al. 1921). In fact, it is Van Mons' work that has promoted fruit-growing in Europe and America, and pomologists are in general agreement that until his time, no man has exerted such profound influence on the field of pomology (Hedrick et al. 1921). Again, it is Belgian breeders from *Pomone tournaisienne* who have developed 160 pear cultivars, including 'Beurré de Naghin' (Wesel 1996). In the Belgian city of Mechelen, Pierre Joseph Esperen developed 70 cultivars, such as 'Bergamotte Esperen', while in another Belgian city Jodoigne, 13 breeders developed about 200 new pear cultivars (Wesel 1996). Among the latter group of cultivars, and of particular note, are 'Triomphe de Jodoigne', developed by the brothers Bouvier, 'Alexandrina', developed by Alexandre Bivort, and 'Madame Grégoire', developed by Xavier Grégoire (Wesel 1996).

As new cultivars have been developed in Belgium, similar efforts have been undertaken in France, leading to such present-day cultivars as 'Beurré-Hardy', 'Bonne Louise d'Avranches', 'Doyenné du Comice', and 'Triomphe de Vienne', in the UK, resulting in 'William's (Bon Chrétien)', 'William's Duchess', and 'Conference', and in the USA, notably 'Clapp's Favourite'. Although central and western Europe have contributed some efforts for the development of pear cultivars, somewhat similar to those efforts undertaken in Italy, France, Belgium, and

Table 1.1 Major cultivars of European pear (*Pyrus communis*) identified during the eighteenth and nineteenth centuries

Cultivar	Synonyms	Breeder(s)	Year	Country
Beurré d'Hardenpont	Beurré d'Arenbert Glou Morceau	N. Hardenpont	1759	Belgium
William's	Bartlett Bon Chrétien Williams	Stair/William	1770	UK
Légipont	Fondante de Charneux Miel de Waterloo Köstliche von Charneux	M. Légipont	1805	Belgium
Durondeau	Poire de Tongres Beurré Durondeau	Ch.-L. Durondeau	1811	Belgium
Beurré d'Anjou	Nec plus Meuris Anjou	J. B. Van Mons	1822	Belgium
Joséphine de Malines		J. Esperen	1830	Belgium
Beurré Hardy		Ernest Bonnet	1830	France
Rocha		P. A. Rocha	1836	Portugal
Doyenné du Comice	Vereinsdechants birne Decana del Comicio	Jardin du Comice	1849	France
Beurré de Naghin		N. de Naghin	1858	Belgium
Madame Grégoire		X. Grégoire	1860	Belgium
Clapp's favourite	Clapps Liebling	T. Clapp	1860	USA
Abbé Fetel	Abate Fetel	Abbé Fetel	1869	France
Triomphe de Vienne		J. Colaud alias (Côte)	1870	France
Conference		Firme Rivers	1890	UK
Packhams Triumph		C. H. Packham	1896	Australia
Forelle			>1670	Germany

England, it is Germany that is most noted for providing valuable literature in the field of pomology (Hedrick et al. 1921).

In Japan, commercial pear production has substantially increased around the same period of time as in Europe due to successive discoveries of two chance pear seedlings, 'Nijisseiki' and 'Chojuro', around the year of 1890 (Saito 2016). During the Edo period in Japan (1603–1868), over 150 cultivars have been documented (Silva et al. 2014). Whereas cultivars of European pears have come to the New World almost entirely from the countries of Belgium and France, along with three or four major cultivars of English origin that have been most commonly grown in North America in the twentieth century (Hedrick et al. 1921). Most, if not all of the cultivars that have originated in USA, until the middle of the nineteenth century, have come from imports due to French, Dutch, and English settlements (Hedrick et al. 1921). Moreover and of particular impact on the US pear industry is the introduction of oriental (Asian) pears and their hybrids (Hedrick et al. 1921). Asian pear cultivation has intensified in the USA around 1938 (Bretaudeau and Fauré 1991), and has since spread worldwide (Bretaudeau and Fauré 1991). It is reported that the oriental, Chinese, or sand pear came into America from Asia by way of Europe through the Royal Horticultural Society of London (Hedrick et al. 1921). Hybridizations with the European pear gave rise to 'Le Conte' (1846), 'Kieffer' (1873) or 'Garber' (1880) (Hedrick et al. 1921). It is important to point out that

cultivation of *P. pyrifolia* dates back to 693 ACN in Japan (Bretaudeau and Fauré 1991).

During the twentieth century, private and national research stations in Europe, North America, and Asia established fruit breeding programs to develop new commercial cultivars. Overall, the number of newly developed and released cultivars of pear has been a lot less than those for apple (Brewer and Palmer 2011). Among the limited number of pear cultivar releases developed from pear breeding programs is 'Concorde', developed at East Malling (UK) in 1977 and derived from a cross between 'Conference' and 'Doyenné du Comice'. However, efforts undertaken by Japanese and Chinese breeding programs during the twentieth- and twenty-first centuries resulted in the release of various new Asian pear cultivars (Jun and Hongsheng 2002; Teng 2011; Saito 2016).

Overall, several pear breeding programs have focused their efforts on pest and disease resistance, fruit quality and appearance, duration of harvest season, self-fertility, yield, and growth habit (Jun and Hongsheng 2002; Brewer and Palmer 2011; Dondini and Sansavini 2012). It is only in the last 15–20 years that nearly 300 novel cultivars, including about 200 European pear and 100 Asian pear cultivars, have been released (Dondini and Sansavini 2012). Nowadays, there are several thousands of pear cultivars that are available worldwide. Among these, approximately ten cultivars account for 90% of the world production of pears (Pesson and Louveaux 1984; Miranda et al. 2010). However, due to cultivar history and propagation methods, some cultivars are known under different names in different regions or that different cultivars are grown/promoted as being the same; thus clearly indicating that pear cultivars are not as well characterized as previously reported (Evans et al. 2015). Therefore, genetic molecular markers are currently being used to screen accessions of different germplasm collections, and considerable efforts are needed to verify and confirm accurate identities of accessions in worldwide national collections (Evans et al. 2015).

1.3 Taxonomy and Phylogeny of Pears

1.3.1 The *Pyrus* Genus Within Rosaceae

Both European and Asian pears belong to the genus *Pyrus* of the family Rosaceae within the Order Rosales, belonging to the Rosids subclass, and within the Eudicot core (Chase et al. 2016). The Rosaceae family is monophyletic with a moderately large angiosperm lineage containing 90 genera and between 2500 and 2900 species (Stevens 2017). Rosaceae is a heterogeneous family that is divided into the following three subfamilies, according to APG IV, Dryadoideae, Rosoideae, and Amygdaloideae (Stevens 2017). Previously, largely based on fruit and other morphological characteristics, Rosaceae was divided into four subfamilies, including Rosoideae, Maloideae, Amygdaloideae, and Spiraeaoideae (Xiang et al. 2017). However, recent molecular analyses support the separation of the former Rosoideae (s.l.) into Rosoideae (s.s.) and Dryadoideae, and in combining the previous Maloideae, Amygdaloideae (s.s.), and Spiraeaoideae into the current Amygdaloideae (s.l.) (Stevens 2017; Xiang et al. 2017). The species richness of Rosaceae could be partly related to polyploidization and to species radiation in the family's history (Xiang et al. 2017). Relationships among Rosaceae tribes and genera remain unclear, in part because of polyploidy events and rapid separation/diversification among some clades (Xiang et al. 2017). Phylogenetic studies of Xiang et al. (2017) suggest that Dryadoideae is the basal clade of Rosaceae, and it is the sister of the combined clade of Rosoideae and Amygdaloideae. The age of the crown Rosaceae is about 101.6 Mya with the separation of Dryadoideae, followed by an immediate divergence of the two largest subfamilies Rosoideae and Amygdaloideae at 100.7 Mya (Xiang et al. 2017).

The subfamily Amygdaloideae contains about 1000 species (Xiang et al. 2017), and it is divided

into 11 tribes, including the Malinae (Stevens 2017). All, but two of the tribes of Amygdaloideae, must have diverged between 96 and 88 Mya., with no further activity for the next 20 Mya (Xiang et al. 2017). The Malinae may represent a rapid but ancient radiation (Campbell et al. 2007; Stevens 2017; Xiang et al. 2017). This is perhaps associated with whole genome duplication in the stem lineage, and accompanied with climatic changes that must have occurred at the end of the Palaeocene and all through towards the beginning of the Oligocene (Xiang et al. 2017). The stem group Malinae is dated back to the late Palaeocene, with subsequent divergence in the Eocene and Oligocene epoques (Lo and Donoghue 2012).

Despite efforts to elucidate relationships within the Malinae, relationships among the major sublineages, generic limits, and divergence times have remained uncertain (Campbell et al. 2007; Lo and Donoghue 2012). Most probably, hybridization has played a part in the Malinae evolutionary history, as hybridization is unusually common among genera in this tribe (Campbell et al. 2007). Comparisons of genetic linkage maps within Malinae have suggested that all chromosomes of the genera in this tribe show co-linearity despite considerable differences in genome sizes (Yamamoto and Terakami 2016). The Malinae contains 1000 species organized within 30 genera (Stevens 2017). However, Malinae is also known as Cydoniaceae, Malaceae, Mespilaceae, Pyraceae, or Sorbaceae (Stevens 2017). Furthermore, Malinae is characterized by a north temperate distribution, production of leaves with deciduous stipules, flowers with a gynoecium that is at least half-way inferior, and a fleshy hypanthium 'pome' fruit (Stevens 2017). Several important edible fruits are members of this tribe, such as apple (*Malus*), pear (*Pyrus*), quince (*Cydonia*), loquat (*Eriobotrya*), chokeberry (*Aronia*), and serviceberry (*Amelanchier*) (Campbell et al. 2007). In addition, the Malinae tribe includes valued ornamentals, such as some cotoneasters (*Cotoneaster*), hawthorns (*Crataegus*), Japanese quinces (*Chaenomeles*), firethorns (*Pyracantha*), and mountain ashes (*Sorbus*) (Campbell et al. 2007).

1.3.2 Phylogeny of *Pyrus*

The genus *Pyrus* is characterized by a high genetic variability, and it consists of around 75 species and interspecific hybrid species, along with thousands of cultivars (Ferradini et al. 2017; Stevens 2017). Estimates of *Pyrus* diversity vary between 50 and 80 species, according to various publications (Table 1.2), and the numbers of accepted species differ as a consequence of poorly understood species limits (Korotkova et al. 2014). Indeed, up to 900 *Pyrus* species names have been recorded (Zheng et al. 2014). However, the number of primary (i.e., not of hybrid origin) species has been relatively consistent, and approximately 20 putative primary species are widely recognized (Zheng et al. 2014). Estimation of genetic diversity among *Pyrus* spp. has been difficult due to low morphological diversity, lack of differentiating characters among species, and widespread cross-ability (Yao et al. 2010). Although they are interspecies compatible, *Pyrus* species are typically self-incompatible (Yue et al. 2014).

The *Pyrus* origin dates back to the Oligocene epoque, about 33.35–25.23 Mya (Korotkova et al. 2018). It is a genus of deciduous trees and shrubs occurring throughout temperate Eurasia, reaching the Atlas Mountains in North Africa, and extending to both Japan and South China (Korotkova et al. 2018). Assessing species diversity in *Pyrus* is challenging due to high morphological plasticity and frequent hybridizations within the genus (Korotkova et al. 2018). Thus, this genus is characterized by very low genetic distances between taxa (Korotkova et al. 2014). Currently, the genus is subdivided into the following four sections: *Pyrus* sect. *Pyrus*, *Pyrus* sect. *Xeropyrenia* Fed., *Pyrus* sect. *Argyromalon* Fed., and *Pyrus* sect. *Pashia Koehne* (Korotkova et al. 2018). However, phylogenetic analyses

Table 1.2 List and origin of *Pyrus* species (Asanidze et al. 2011; Silva et al. 2014)

Species	Country or region of origin
P. alnifolia (S. and Z.) Franch. and Sav.	Russian Far East, China, Japan, Korea, Taiwan
P. americana DC	Greenland, USA, Canada
P. angustifolia Aiton	USA, Canada
P. arbutifolia (L.) L.f.	USA
P. aria (L.) Ehrh.	USA, Canary Islands, North Africa, All of Europe
P. armeniacifolia T.T. Yu	China
P. aucuparia var. dulcis (K.) A. and G.	All Europe
P. aucuparia var. randaiensis Hayata	Taiwan
P. baccata L.	Russia, Mongolia, China, Korea
P. baccata var. aurantiaca Regel	Russia, Mongolia, China, Korea
P. baccata var. himalaica Maxim.	China, Bhutan, India, Nepal
P. baccata var. mandshurica Maxim.	Russia, China, Japan, Korea
P. betulifolia Bunge	China, Laos
P. boissieriana Buhse	Azerbaijan, Turkmenistan, Iran
P. bulgarica Kuth. and Sachokia (*P.* × *nivalis* Jacq.)	Western Europe, Central Eastern and Southern
P. calleryana Decne.	China, Korea, Taiwan, Vietnam
P. calleryana var. dimorphophylla (Makino) Koidz.	Japan
P. calleryana var. fauriei (C. K. Schneid.) Rehder	Korea
P. calleryana var. koehnei (C. K. Schneid.) T. T. Yu	China
P. cathayensis Hemsl.	China
P. caucasica Fed.	Eastern Europe and Central Greece
P. chamaemespilus (L.) Ehrh.	Western Europe, Central Eastern and Southern
P. communis L.	All Europe
P. communis subsp. gharbiana (T.) Maire	Algeria, Morocco
P. communis subsp. *P. marmorensis* (Trab.) Maire	Morocco
P. communis subsp. *P. pyraster* (L.) Ehrh.	Western Europe, Central Eastern, and Southern
P. communis var. cordata (Desv.) H.f.	UK, Portugal, Spain, France
P. coronaria L.	Canada, USA
P. coronaria var. ioensis Alph. Wood	USA
P. cossonii Rehder	Algeria
P. crataegifolia Savi	Turkey, Albania, Serbia, Greece, Italy, Macedonia
P. cuneifolia Guss.	Central Eastern Europe, South and Central
P. cydonia L.	Iran, Armenia, Azerbaijan, Russia, Turkmenistan
P. decipiens Bechst.	All Europe and North Africa
P. delavayi Franch.	China

(continued)

Table 1.2 (continued)

Species	Country or region of origin
P. demetrii Kuth	Georgia
P. discolor Maxim.	China
P. diversifolia Bong.	USA, Canada
P. domestica (L.) Sm.	Algeria, Cyprus, Eastern Europe Central, West and Meridional
P. doumeri Bois	Vietnam
P. elaeagrifolia Pall.	Turkey, Ukraine, Albania, Bulgaria, Greece, Romania
P. elaeagrifolia subsp. kotschyana	Turkey
P. floribunda Lindl.	USA, Canada
P. folgner (C. K. Schneid.) Bean	China
P. foliolosa Wall.	Burma, Bhutan, India, Nepal, China
P. fusca (Raf.) C. K. Schneid.	USA, Canada
P. georgica Kuth	Georgia
P. germanica (L.) Hook. f.	Middle East, Eastern Europe, Central, Southern and Northern Asia
P. gharbiana Trab.	Morocco
P. glabra Boiss.	Iran
P. gracilis Siebold and Zucc.	Japan
P. harrowiana Balf. f. and W. W. Sm.	China, India, Nepal, Burma
P. heterophylla Regel and Schmalh.	Kyrgyzstan, Tajikistan, China
P. hondoensis Nakai and Kikuchi	Japan
P. hupehensis Pamp.	China, Taiwan
P. indica Wall.	South Asia and Far East Asia
P. intermedia Ehrh.	All Europe
P. japonica Thunb.	Japan
P. kansuensis Batalin	China
P. keissleri (C. K. Schneid.) H. Lev.	China, Myanmar
P. ketzkhovelii Kuth	Georgia
P. korshinskyi Litv.	Afghanistan, Tajikistan, Uzbekistan
P. korshinskyi Litv. subsp. bucharica (Litv.) B. K	Former Soviet Union
P. kumaoni Decne.	Middle East, Far East and South Asia
P. lanata D. Don	Afghanistan, India, Nepal, Pakistan
P. malus subsp. paradisiaca (L.)	Western, Eastern, and Central Europe and Greece
P. matsumurana Makino	Japan
P. minima Ley	UK
P. nebrodensis Guss.	Italy - Sicily
P. nussia Buch.-Ham. ex D. Don	Far East, South Asia
P. pinnatifida Ehrh.	All Europe
P. pohuashanensis Hance	Russia, China, Korea
P. praemorsa Guss	South of Italy, France
P. prattii Hemsl.	China
P. prunifolia Willd.	China

(continued)

Table 1.2 (continued)

Species	Country or region of origin
P. pseudopashia T.T. Yu	China
P. pyrifolia var. pyrifolia	China, Laos, Vietnam
P. ringo var. kaido Wenz	China
P. ringo Wenz.	China, Korea
P. sachokiana Kuth.	Georgia
P. salicifolia Pall.	Iran, Armenia, Turkey, Azeebaijan
P. sanguinea Pursh	Canada, USA
P. scabrifolia Franch.	China
P. scalaris (Koehne) Bean	China
P. sieboldii Regel	China, Japan
P. sikkimensis Hook. f.	China, Bhutan, India
P. sinensis var. maximowicziana H. Lev.	Korea
P. spectabilis Aiton	China
P. spinosa Forssk.	Central Eastern Europe, South, and Central
P. sudetica Tausch	Western Europe, Central Eastern, and Southern
P. syriaca Boiss.	Caucasus and Middle East Region
P. taiwanensis Iketani and H. Ohashi	Taiwan
P. torminalis (L.) Ehrh.	North Africa, Middle East, South Caucasus, whole Europe
P. trilobata (Poir.) DC.	Israel, Lebanon, Turkey, Bulgaria, Greece
P. trilobata (Poir.) DC.	Turkey, Bulgaria, Greece, Israel, Lebanon
P. tschonoskii Maxim.	Japan
P. turkestanica Franch.	Kyrgyzstan, Tajikistan, Turkmenistan, Afghanistan
P. ussuriensis Maxim.	Russia, China, Japan, Korea, Brazil
P. vestita Wall. ex G. Don	China, Bhutan, India, Nepal, Myanmar
P. vilmorinii (C. K. Schneid.) Asch. and Graebn.	China
P. xerophila T. T. Yu	China
P. yunnanensis Franch.	China, Myanmar
P. zahlbruckneri (C. K. Schneid.) Cardot	China
P. × *bretschneideri* Rehder	China
P. × *complexa* Rubtzov	Former Soviet Union
P. × *hopeiensis* T. T. Yu	China
P. × *phaeocarpa* Rehder	China
P. × *serrulata* Rehder	China
P. × *sinkiangensis* T. T. Yu	China
P. × *uyematsuana* Makino	Japan, Korea

have supported that *Pyrus* is a monophyletic group containing two major clades that diverged far prior to any possible human intervention (Kim et al. 2015; Zheng et al. 2014; Korotkova et al. 2018; Wu et al. 2018). The first is an eastern Asian clade with a crown group age of 15.7 Mya, and the second is a western Eurasian clade that comprises species from Europe,

Southwest Asia, and the Caucasus region, displaying a crown group of 12.38 Mya (Korotkova et al. 2018). The separation of these two clades may be related to the recession of the Turgai Strait, a Mesozoic epicontinental seaway that has separated Europe from Asia until the late Oligocene (Korotkova et al. 2018). However, Wu et al. (2018) have estimated that both clades diverged between 6.6 and 3.3 Mya. Their hypothetical common ancestor seems to have originated in China before dissemination through central Asia and then eventually on to western Asia and Europe (Wu et al. 2018). Within the western Eurasian clade, a major period of diversification has likely occurred in the Middle to Late Miocene when Caucasian and Southwest Asian lineages have diversified (Korotkova et al. 2018). Most of the extant diversity of *Pyrus* in western Eurasia appears to have originated in the Pliocene and the Pleistocene (Korotkova et al. 2018). *Pyrus* species diversity is concentrated in western Eurasia to eastern Asia, and particularly in China (Silva et al. 2014). Speciation in *Pyrus* is complex, and several currently accepted *Pyrus* species have not been recovered as monophyletic, thus indicating that current species limits require re-evaluation (Zheng et al. 2014; Korotkova et al. 2018).

Within the *Pyrus* genus, there are only a few species that have been domesticated for commercial production (Bao et al. 2007; Wu et al. 2013). Most cultivated *Pyrus* species include *P. communis* (European pear), and the Asian pear species of *P. ussuriensis* Maxim., *P. pyrifolia*, *P.* × *bretschneideri* Rehd., and *P. sinkiangensis* Yü (Wu et al. 2013; Ferradini et al. 2017). These have been domesticated from the following wild species, *P. communis* is derived from the wild European species *P. pyraster*, while the cultivated *P. ussuriensis* is derived from the wild *P. ussuriensis*, whereas *P. pyrifolia* and *P.* × *bretschneideri* are derived from the wild *P. pyrifolia* and finally *P. sinkiangensis* is derived from hybridization between the cultivated *P. communis* and either the cultivated *P. pyrifolia* or *P.* × *bretschneideri* (Wu et al. 2018). Although the majority of cultivated pears are diploid ($2n = 2x = 34$), a few cultivars of *P. communis* and *P.* × *bretschneideri* are known to be polyploids (Ferradini et al. 2017).

Currently, there are several studies aiming to estimate genetic distances among different pear cultivars/genotypes present in gene banks and in various breeding programs (Bao et al. 2007; Bassil and Postman 2010; Silva et al. 2014; Chang et al. 2017; Ferradini et al. 2017; Wu et al. 2018). Pear cultivars can be subdivided into two major groups, the occidental (European) and the oriental (Asian) pears, as confirmed by molecular data (Bao et al. 2007; Bassil and Postman 2010; Yue et al. 2014; Ferradini et al. 2017). European cultivars belong to *P. communis* and are most likely derived from one or two wild species, *P. pyraster* (L.) Burgsd. and/or *P. caucasica* Fed. (Ferradini et al. 2017). Therefore, European pear cultivars have a narrow genetic base (Miranda et al. 2010); whereas, cultivated pears native to East Asia belong to the following five groups, including the Ussurian pear (*P. ussuriensis*), Chinese white pear (*P.* × *bretschneideri*), Chinese sand pear (*P. pyrifolia*), Xinjiang pear (*P. sinkiangensis*), and the Japanese pear (*P. pyrifolia*) (Bao et al. 2007; Katayama et al. 2016). Phylogenetic studies of *Pyrus* cultivars native to East Asia have revealed contradictory results; thus, additional studies are required to resolve issues of origin and evolution of Asian pear cultivars (Bao et al. 2007; Bassil and Postman 2010; Iketani et al. 2012; Chang et al. 2017; Wu et al. 2018). However, Chang et al. (2017) have explored the evolution routes of *Pyrus* in China and highlighted the spread of pears from the Shanxi province to other regions of northern China. From China, pears were then disseminated throughout central Asia before they were spread over to western Asia and then on to Europe (Wu et al. 2018).

1.3.2.1 *Pyrus* Species in Western Eurasia

In general, occidental pears are distributed in Europe, northern Africa, Asia Minor, Iran, Central Asia, and Afghanistan (Zheng et al. 2014). They have been geographically divided into the following three subgroups: West Asian species, European species, and North African species

(Zheng et al. 2014; Zamani et al. 2017). It is reported that there are 12 primary species present in western Eurasia, including five European species (*P. communis*, *P. caucasica*, *P. pyraster*, *P. nivalis* Jacq., and *P. cordata* Desv.), five West Asian species (*P. elaeagrifolia* Pall, *P. spinosa* Forssk syn. *P. amygdaliformis* Vill., *P. regelii* Rehd., *P. salicifolia* Pall., and *P. syriaca* Boiss.), and three North African species (*P. cossonii* Rehd. syn. *P. longipes* Balansa ex Coss. & Durieu, *P. gharbiana* Trab., and *P. mamorensis* Trab.), while the remaining species are putative interspecific hybrids (Zheng et al. 2014). Further phylogeny studies have been conducted to characterize relationships among occidental primary species (Zheng et al. 2014). It is revealed that European species may be the latest derived occidental species and displaying lower levels of genetic diversity compared to West Asian species (Zheng et al. 2014). Moreover, European pears are most likely independently derived from West Asian species and North African species, as *P. nivalis* and *P. cordata* are more related to West Asian species, primarily to *P. spinosa;* whereas, *P. caucasica*, *P. pyraster,* and *P. communis* are more closely related to the North African species (Zheng et al. 2014). Among West Asian species, *P. regelii* is an early diverging and isolated species (Zheng et al. 2014), while the three African species are well differentiated with *P. gharbiana* and *P. mamorensis* and are more related to European species (Zheng et al. 2014).

It has been reported that wild occidental pears primarily inhabit two types of habitats, mesophytic forests and xerophytic open woodlands (Zamani et al. 2017; Korotkova et al. 2018). Xerophytic woodlands constitute a vegetation-type characteristic for arid and semi-arid regions of Southwest Asia, including the Caucasus ecoregion (Korotkova et al. 2018). Xerophytic woodlands likely play an important role in the diversification of *Pyrus* as these habitats comprise a considerable number of *Pyrus* species. The Caucasus ecoregion contains approximately 25 endemic species (Korotkova et al. 2018). Moreover, the majority of Caucasian pears inhabit xerophytic open woodlands and display morphological adaptations such as narrow leaves (Korotkova et al. 2018). The other remaining species mainly inhabit mesophytic forests and display broad leaves (Korotkova et al. 2018). Thus, wild pear species have diverged into numerous local ecogeographical races and species that are interfertile with the cultivated pear (Asanidze et al. 2011). It is important to point out that the country of Iran is also rich in *Pyrus* species, with about 23 taxa, and also has both xerophytic and mesophytic species (Zamani et al. 2017). These species occur throughout the north-east region through northern hyrcanian forests to the north-west (Azerbaijan province) and all the way to the southwest region in the Fars Province (Zamani et al. 2017).

The cross-compatibility among various *Pyrus* species raises questions on the taxonomy of *Pyrus* species (Zamani et al. 2017). For example, *P. caucasica*, an endemic species of the Caucasus, has been classified initially as a European pear, *P. communis*, but has been subsequently deemed as a separate species based on morphological differences of leaf margins (Asanidze et al. 2011). Although earlier studies have deemed *P. caucasica* as a completely independent species because of its morphological differences and its separate geographical distribution, it is now considered as a wild subspecies of *P. communis* (Asanidze et al. 2011). Furthermore, another wild ancestor of the cultivated European pear, *P. pyraster*, native to Eastern and Central European countries, including the Balkan Peninsula and Turkey, has also been considered either as a species or a subspecies of *P. communis* by different reports (Asanidze et al. 2011; Korotkova et al. 2018). Similar conflicting findings have been reported for other species, such as *P. balansae* Decne., *P. boissieriana* Buhse, *P. salicifolia*, *P. syriaca*, *P. georgica* Kuth., *P. demetrii* Kuth., *P. ketzkhovelii* S. Kuthath, and *P. sachokiana* Kuth. (Asanidze et al. 2011). Recently, Aydin and Dönmez (2015) have revised species taxonomy, present in Turkey, and have proposed species modifications. They have proposed that *P. pseudosyriaca* should be treated as a new botanical variety of *P. syriaca*, while *P. serikensis* and

P. boissieriana are reduced to synonyms of *P. cordata*, and *P. elaeagrifolia* Pall., respectively. In addition, subsp. *kotschyana* (Boiss.) Browicz is reassessed as *P. kotschyana* Boiss. ex Decne (Aydin and Dönmez 2015), while Zamani et al. (2017) have assessed the usefulness of biological markers to evaluate the taxonomic significance of Iranian pear taxa.

Pear improvement efforts undertaken in Europe have depended on *P. communis* and *P. nivalis*. Although *P. communis* is widely cultivated worldwide, its origin is not well understood. It is likely that *P. communis* may have other species in its genetic background, including *P. pyraster*, *P. caucasica*, *P. eleagrifolia*, *P. spinosa*, *P. nivalis*, and *P. syriaca* (Silva et al. 2014; Korotkova et al. 2018). On the other hand, *P. nivalis* is used in wine making and has been of great importance in both Britain and France for over 400 years (Silva et al. 2014).

1.3.2.2 *Pyrus* Species in East Asia

Oriental pears are distributed from the Tian Shan region and the Hindu Kush Mountains in Central Asia eastward to Japan (Zheng et al. 2014). There are nine proposed primary *Pyrus* species in East Asia, five have originated from China (*P. pyrifolia*, *P. ussuriensis*, *P. pashia* D. Don, *P. calleryana* Dcne, and *P. betulifolia* Bge), two from Japan (*P. dimorphophylla* Makino and *P. hondoensis* Yu), one from the Korean Peninsula (*P. fauriei* Schneid.), and one from Taiwan Island (*P. koehnei* Schneid.) (Zheng et al. 2014). The remaining species are most likely interspecific hybrids although their parentages remain uncertain (Zheng et al. 2014). In China, pear trees have originated in the mountainous regions of Southwestern China, and have spread both westward and eastward (Chang et al. 2017). A total of 69 *Pyrus* species are found in China. Of these, 13 have originated in China, including species with commercial cultivars, such as the Chinese white pear (*P.* × *bretschneideri*), Chinese sand pear and Japanese pear (*P. pyrifolia*), Sinkiang pear (*P. sinkiangensis*), and the Ussurian pear (*P. ussuriensis*) (Kell et al. 2015; Chang et al. 2017).

The Ussurian pear is mainly cultivated in North China, especially in Northeast China (Teng et al. 2015). The Chinese white pear is cultivated in North China and occupies the most important position in commercial pear production (Teng et al. 2015). The Chinese sand pear is naturally distributed in south China and owns plentiful cultivar resources (Teng et al. 2015). The Japanese pear refers to pears located in Japan, and has fruit traits similar to those of the Chinese sand pear (Teng et al. 2015). Wild *P. ussuriensis* is widely distributed in north-eastern China, eastern Russia, the Korean Peninsula, and central and northern Honshū in Japan (Iketani 2016). In Japan, two botanical varieties of *P. ussuriensis*, var. *aromatica* and var. *hondoensis,* are native to the northern area and the central area of the main island, respectively (Iketani 2016; Katayama et al. 2016). At least two native Japanese and one native Chinese *Pyrus* species, namely *P. ussuriensis, P. calleryana*, and *P. pseudopashia* T.T. Yu, are included in the National Red List (Kell et al. 2015; Iketani 2016). Early on, the Japanese pear is suspected to have originated from native plants in Japan; however, it is subsequently reported that *P. pyrifolia* is most likely introduced to Japan during prehistoric times (Iketani 2016).

Phylogeny studies have revealed incidence of close relationships among Asian *Pyrus* species. For example, Yue et al. (2014) have reported that the oriental pear cluster can be divided into two subgroups. One subgroup consists of three *P. betulifolia* accessions, while the other subgroup consists of all other cultivars and species, namely *P. pyrifolia*, *P. ussuriensis*, *P. pashia*, *P. dimorphophylla*, *P. fauriei*, *P. serrulata*, *P. hopeiensis*, *P. phaeocarpa*, *P. xerophila*, and *P. hondoensis*. Likewise, Zheng et al. (2014) have supported the existence of subclades for *P. ussuriensis* and *P. pashia,* but they have not resolve relationships among the remaining haplotypes. According to Wu et al. (2018), Asian pear accessions are clustered into the following four groups: a first large group that includes accessions of both *P.* × *bretchneideri* and *P. pyrifolia*; a second group that includes wild

accessions of China, Japan, and Korea; a third group that clusters wild and cultivated accessions of *P. ussuriensis*; and a fourth group that includes all cultivated accessions of *P. sinkiangensis.*

Although genetic differentiation between groups of native populations and those of cultivars was usually high, cultivars were not well differentiated from each other (Iketani et al. 2012). The classification of cultivated pears could indeed be problematic due to cross-compatibility and introgression between species (Iketani 2016; Katayama et al. 2016). As for cultivated Asian pears, Bao et al. (2007) demonstrated that Chinese sand pears and Chinese white pears were clustered together, and that Japanese cultivars had sandy pears as parents, while Ussurian pears clustered separately (Bao et al. 2007). However, Bassil and Postman (2010) grouped Ussurian pear and Chinese white pear cultivars in the same clusters. According to Yao et al. (2010), some cultivars of Ussurian pear clustered with some Chinese white pears, while other Chinese white pears generally clustered with Chinese sand pear and Japanese pears. More recently, Chang et al. (2017) showed that Japanese sand pear and Chinese sand pear cultivars shared similar genetic backgrounds and exhibited a high degree of kinship. Earlier, Iketani et al. (2012) reported that Japanese pear cultivars had a simple genetic structure, while Chinese and Korean pear cultivars were admixtures of Japanese pear and native *P. ussuriensis*. Subsequently, Teng et al. (2015) showed that there were no real genetic differences detected among Chinese sand pear, Chinese white pear, and Japanese pear.

Globally, Asian pear cultivars have been deemed to be genetically continuous, and have a very narrow genetic diversity compared with that of wild species (Iketani et al. 2012). In this context, Iketani et al. (2012) have proposed that Asian pear cultivars should be regarded as a single group, although this may not be accepted by horticulturists. An alternative strategy is to divide Asian pears into four cultivar groups instead of species, namely *Pyrus* Ussurian pear group, *Pyrus* Chinese white pear group, *Pyrus* Chinese sand pear group, and the *Pyrus* Japanese pear group (Iketani et al. 2012).

1.4 Botanical Description of Pear

All *Pyrus* species are tree-like woody plants (Hedrick et al. 1921). They are medium-sized trees often with a tall, narrow crown, but with only a few species that are shrubby. Leaves are alternately arranged, simple, 2–12 cm in length, glossy green in some species, or densely silvery hairy in some others (Hedrick et al. 1921). Most pears are deciduous, but one or two species in Southeast Asia are evergreen. Flowers are usually white, borne in corymbs on short spurs, or on lateral branchlets (Hedrick et al. 1921). Flowers are about 2–4 cm in diameter, and have five sepals, five petals, numerous stamens, and five-locular ovary with usually free styles. The fruit is a pome, measuring 1–4 cm in diameter in wild species, and up to 18 cm in length and 8 cm in width in some cultivated forms (Hedrick et al. 1921). The form of the fruit varies in most species from oblate, or globose, to pyriform (Hedrick et al. 1921). The fruit is a pseudo-fruit composed of the receptacle, or a calyx tube that is greatly dilated and enclosing the true fruit, which consists of five cartilaginous carpels, known as the core (Hedrick et al. 1921). The flesh usually bears grit cells (sclereids) when ripened on the tree (Hedrick et al. 1921). Leaf and fruit traits are commonly used to distinguish among *Pyrus* species (Asanidze et al. 2011; Zamani et al. 2017). European pears are elongated and have full-bodied textures, while Asian pears are round in shape and have sandy textures (Silva et al. 2014).

Pear trees are self-incompatible, exhibiting typical gametophytic self-incompatibility, as with other Rosaceous species (Sassa et al. 2009; Franceschi et al. 2012). Gametophytic self-incompatibility is controlled by a single multi-allelic locus, the so-called *S*-locus. In Pyrinae, the *S*-locus contains the single pistil-side *S* determinant, the S-RNase, which is expressed

in the pistil, and multiple pollen-expressed *S*-locus *F*-box genes, designated as *SFBB* (for S-locus F-box brothers), that are expressed in the pollen (Franceschi et al. 2012). *Pyrus* species are pollinated by insects, and flowers produce nectar to attract these insects (Pesson and Louveaux 1984; Mayer et al. 1990; Quinet et al. 2016). The sugar content of pear nectar is usually lower (often <10–15%) compared to that detected in other fruit tree species (Farkas et al. 2002; Faoro and Orth 2011; Quinet et al. 2016). Although intra-specific self-incompatibility is present, interspecies hybridization is common in *Pyrus* (Hedrick et al. 1921; Iketani 2016; Katayama et al. 2016; Zamani et al. 2017).

Due to self-incompatibility, pear cultivars are vegetatively propagated by grafting. The European and Asian pears readily intergraft with other pears (Hedrick et al. 1921). The main rootstocks used for European pear are *P. communis*, *P. betaefolia*, or quince (*Cydonia oblonga*), while the main rootstocks used for Asian pear are *P. pyrifolia*, *P. communis*, *P. pashia*, *P. calleryana*, *P. ussuriensis*, or *P. belulaefolia* (Bretaudeau and Fauré 1991).

The next sections will focus on detailed descriptions of the main cultivated pear species *P. communis* for the European pear, and on *P. pyrifolia*, *P. ussuriensis*, *P.* × *bretschneideri*, *and P. sinkiangensis* for the Asian pears. Distinct phenotypic traits have been selected during domestication of European and Asian pears (Wu et al. 2018).

1.4.1 European Pear

1.4.1.1 Description

P. communis is a medium-sized tree, reaching 20 m tall and a diameter of 90 cm. Annual growth of wild *P. communis* is 0.5–1.5 m (Aas 1999). European pear trees bear fruit after 4–8 years of growth, and their life spans could reach up to 200 years, depending on the rootstock used (Hedrick et al. 1921; Hessayon 1990). For cultivation, pear trees are pruned to facilitate harvest and to allow for light incidence into the canopy of the tree to promote flowering and for good fruit development (Bretaudeau and Fauré 1991). Most pear cultivars are grafted onto clonal quince rootstocks (Hessayon 1990). The most popular is quince A, producing trees which grow about 3–6 m in height (Hessayon 1990). Pears can be trained and grown as bushes, dwarf pyramids, cordons, espaliers, or fans (Fig. 1.3) (Hessayon 1990). Pear trees favour sunny areas and do not tolerate shadowing (Hessayon 1990). Furthermore, pear trees have good tolerance to a wide variety of soil conditions, including those of soil texture and pH. However, they are exigent for soil freshness, and are not well suited for either dry soils nor for flooded soils (Hedrick et al. 1921; Hessayon 1990).

Although the wild European pear produces fruits regularly, it rarely reproduces by seeds (Hedrick et al. 1921). Suckering seems to be the dominant form of proliferation of these wild forms, thereby allowing these wild types to maintain their favourable biotopes.

European pear trees have an upright, oblong, or pyramidal, and compact top (Hedrick et al. 1921). Branches are greyish brown or dark reddish brown. Branchlets are glossy, smooth, glabrous, with more or less conspicuous lenticels (Hedrick et al. 1921). Leaf buds are prominent, plump, obtuse or pointed, mostly free, while flower buds are larger and plumper than leaf buds (Hedrick et al. 1921). Leaves are glossy dark green, ovate to elliptic with crenate to serrate margins, and measure 7–9 cm (Rameau et al. 1989). The petiole is as long as the blade, and when young, they are both pubescent (Coste and Flahault 1903). Foliage turns into shades of red and yellow in the fall season. Flowering occurs in early spring and lasts between 6 and 20 days, depending on the cultivar (Bretaudeau and Fauré 1991). Inflorescences are corymbs of 5–15 flowers, with centripetal flowering (Fig. 1.4a) (Rameau et al. 1989; Bretaudeau and Fauré 1991). Flowers are hermaphroditic and creamy white (occasionally flushed with pale pink), and have a diameter of 2.5–3.5 cm (Fig. 1.4b) (Coste and Flahault 1903). They are composed of five triangular-lanceolate sepals of 5–9 × 3–4 mm, five obovate (13–15 × 10–13 mm) white petals, about 20 stamens, with purple anthers and free

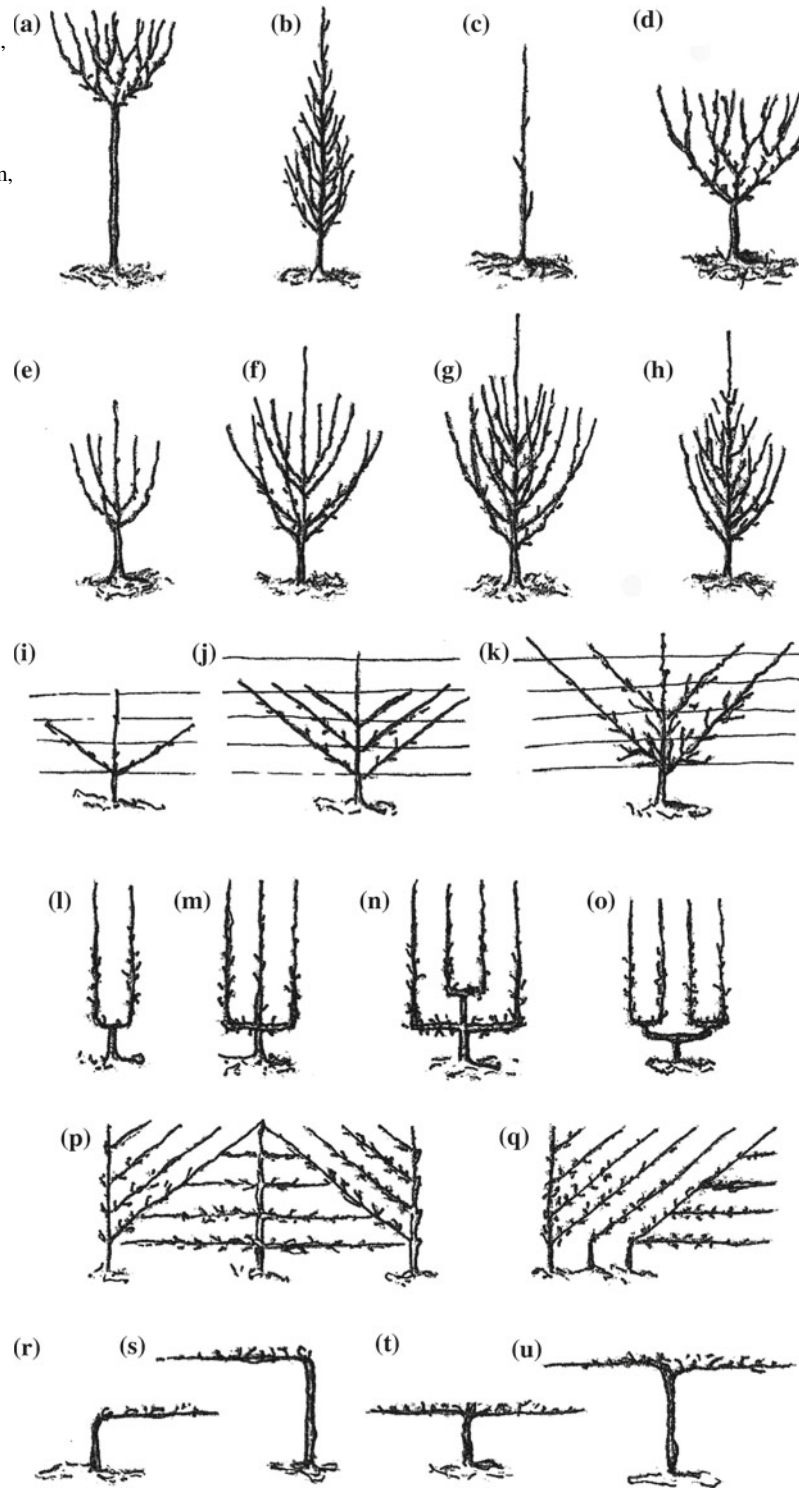

Fig. 1.3 Description of training systems. **a** Standard, **b** spindle, **c** vertical cordon, **d** vase, **e–h** pyramid, **i–k** palmette, **l** U-shaped, **m,n** palmette verrier, **o** double U-shaped, **p** Cossonet system, **q** oblique cordon, and **r–u** horizontal cordon

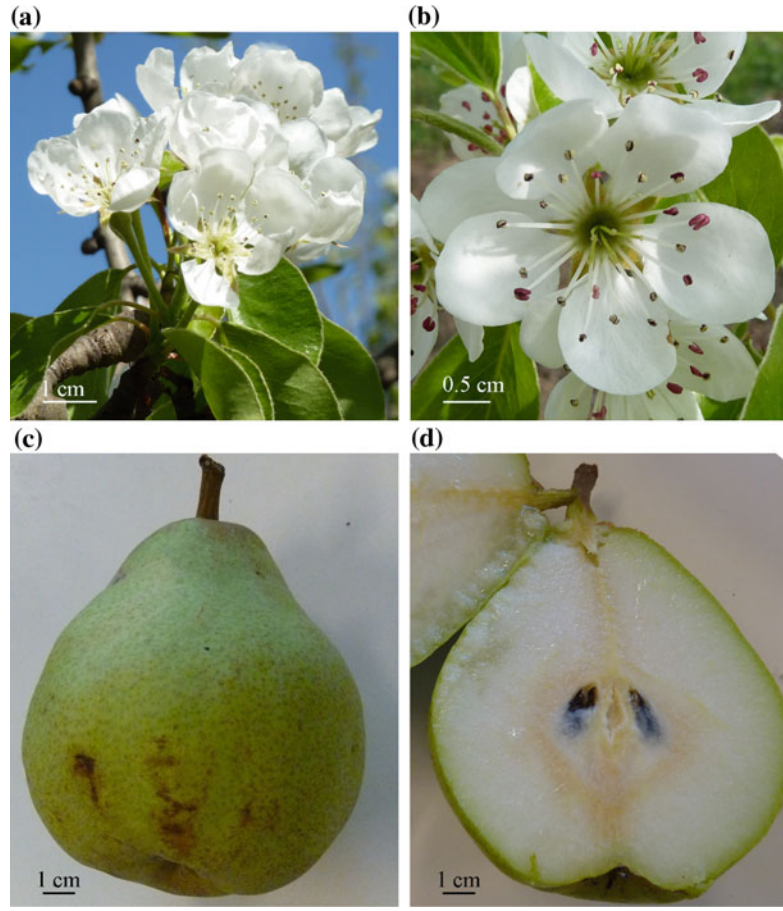

Fig. 1.4 Inflorescence (**a**), flower (**b**), and fruit (**c**, **d**) of *Pyrus communis*. **c** Whole fruit and **d** longitudinal section

filaments, and a single gynoecium composed of usually five carpels (Bretaudeau and Fauré 1991). The styles are free, and the ovary is five-locular with two ovules per locule (Pesson and Louveaux 1984; Bretaudeau and Fauré 1991). Anthers have a longitudinal dehiscence (Paris 1996). Pedicels are 2–3.5 cm long, pubescent, or glabrate.

Upon pollination and/or fertilization, flowers produce edible pear-shaped fruits that ripen from mid-summer to fall, depending on cultivar (Hedrick et al. 1921; Bretaudeau and Fauré 1991). Pome fruits are green, yellowish or reddish green, globose, subglobose, ovoid, or pyriform, 30–160 × 15–120 mm in size (Fig. 1.4c, d, Fig. 1.5). Sepals are persistent. Flesh is white, yellowish, sometimes pink or wine-red, rarely salmon-coloured; it is firm, melting, or buttery and when ripening on the tree with few or many grit cells (Hedrick et al. 1921). Seeds are large, brown, or brownish, often tufted at the tips, sometimes abortive or wanting (Hedrick et al. 1921). Parthenocarpy is present in several European pear cultivars, and it is characterized by the development of fruit without pollination and fertilization of the egg, resulting in seedless fruit (Nyéki et al. 1998; Moriya et al. 2005; Quinet and Jacquemart 2015). However, parthenocarpic fruits are generally smaller than fertilized fruits (Moriya et al. 2005; Quinet and Jacquemart 2015).

1.4.1.2 European Pear Cultivars

Over 3000 cultivars of the European pear are known (Table 1.3) (Hedrick et al. 1921). They flower in early spring when temperatures reach 10 °C (Bretaudeau and Fauré 1991). Different cultivars flower for periods lasting between 6 and

Fig. 1.5 Fruits of European pear cultivars. **a** 'Beurré d'Anjou', **b** 'Clapp's Favourite', **c** 'Concorde', **d** 'Conference', **e** 'Doyenné du Comice', and **f** 'Triomphe de Vienne'

20 days (Bretaudeau and Fauré 1991). Although the flowering period is usually short, fruit maturation takes place between the months of July, for early maturing cultivars, and January to March, for winter maturing cultivars (Bretaudeau and Fauré 1991). European pears are usually harvested at green stage, and are allowed to ripen at room temperature. Some of the most popular cultivated European pear cultivars include the following listing. In Europe, eight cultivars represent 80% of the production, and these include 'Conference', 'William', 'Abbé Fétel', 'Spadona', 'Doyenne du Comice', 'Kaiser', 'Dr. Jules Guyot', and 'Coscia' (Dondini and Sansavini 2012). In north America, 'Beurré d'Anjou', 'Williams', 'Doyenné du Comice', 'Bosc', 'Concorde', and 'Forelle' are largely grown (USA Pears 2018). Below is a detailed description of some of the most popular pear cultivars.

'Abbé Fétel' is a French cultivar, identified in 1866 (Dondini and Sansavini 2012). It produces large-sized and elongated fruits of medium quality (Dondini and Sansavini 2012). The fruit is pyriform, golden yellow, and at times it may develop a red blush (Dondini and Sansavini 2012). This cultivar could also produce

Table 1.3 List of some European pear and hybrid cultivars (Flores 1999; Drudze 2004; Jain and Priyadarshan 2009; Bassil and Postman 2010; Dondini and Sansavini 2012)

Cultivar	Synonyms	Country	Mode of selection[a]
Abbé Fetel	Abate Fetel	France	Chance seedling
Alexander Lucas	Beurré Alexandre Lucas	France	
Ambrosia		USA	US 571 × 'Honeysweet'
Arganche	Klementinka, Mustafabey, Zaharoasa de Vara	Yugoslavia	
Ayers		USA	*P. communis* × *P. pyrifolia* hybrid
Bambinella		Malta	
Bella di Giugno		Italy	
Belle Lucrative		Belgium	
Black Worcester		UK	
Blake's Pride		USA	
Blanquilla	pera de agua, blanquilla de Aranjuez	Spain	
Bosc	Calebasse Bosc, Beurré Bosc, Carafon de Bosc, Beurré d'Apremont	Belgium	
Beurré Clairgeau		France	O.P. Duchess D'Angouleme
Beurré Hardy	Gellert's butterbirne, French butter pear	France	Seedling
Beurré d'Anjou	Nec plus Meuris Anjou	Belgium	Chance seedling
Beurré Superfin		France	Chance seedling
Butirra Precoce Morettini		Italy	'Coscia' × 'Bartlett'
Butirra Rosata Morettini		Italy	'Coscia' × 'Beurre Clairgeau'
Carmen		Italy	'Guyot' × 'Bella di Giugno'
Cascade		Oregon	'Max Red Bartlett' × 'Comice'
Catillac	Cadillac, Gros monarque, Chartreuse	France	
Churchland	Church	USA	Seedling
Clairgeau	Beurré Clairgeau, Clairgeau de Nantes	France	
Clapp's Favourite		USA	'Flemish Beauty' × 'Bartlett'
Clara Frijs		Denmark	
Coloree de Juillet		France	
Concorde		UK	'Conference' × 'Doyenné du Comice'
Conference		UK	
Corella		Australia	
Coscia		Italy	
Delbard Premiere		France	'Akca' × 'Dr. Jules Guyot'
Don Guindo		Spain	
Doyenne de Juillet	Doyenne d'été	Belgium	
Doyenné du Comice		France	O.P. seedling

(continued)

Table 1.3 (continued)

Cultivar	Synonyms	Country	Mode of selection[a]
Dr. Jules Guyot	Limonera	France	
Duchesse d'Angouleme		France	Chance seedling
Earlibrite	Clapp Favourite x Russet Bartlett	Canada	
Eletta Morettini		Italy	'Beurré Hardy' × 'Passe Crassane'
Farmingdale		USA	Chance seedling—O. P. Anjou?
Flemish Beauty	Fondante des Bois, Gros quessois d'été, Gros Davy, Poire de Persil	Belgium	Chance seedling
Forelle	Trout pear	Germany	
Gaspard		USA	
General Leclerc		France	
Gerburg		Germany	
Giffard	Beurré Giffard	France	
Glou Morceau	Beurré d'Hardenpont	Belgium	
Gorham		USA	'Bartlett' × 'Josephine de Malines'
Harobig		Canada	
Harovin Sundown		Canada	'Bartlett' × US56112-146
Harrow Crisp		Canada	'Bartlett' × US56112-146
Harrow Delight		Canada	
Harrow Gold		Canada	'Harvest Queen' × 'Harrow Delight'
Harrow Red		Canada	
Harrow Sweet		Canada	'Bartlett' × 'Purdue 80-51'
Harvest Queen		Canada	
Highland		USA	'Bartlett' × 'Comice'
Hortensia		Germany	'Nordhäuser Winterforelle' × 'Clapp's Liebling'
Huntington		USA	seedling
Jeanne d'Arc		France	'Beurré Diel' × 'Doyenne du Comice'
Joséphine de Malines		Belgium	
Jubileer D'Ar		Bulgaria	'Clapp's Favourite' × 'Klementina'
Junsko Zlato		Yugoslavia	'Precoce de Trevoux' × 'Doyenne de Juillet'
Kieffer		USA	*P. communis* × *P. pyrifolia* hybrid
Latgale		Latvia	'Kurzemes Sviesta' × 'Clapp's Favourite'
Laxtons Superb		UK	'Marie Louise' × 'Bartlett'
Le Conte		USA	*Pyrus* hybrid × *P. lecontei* *P. communis* × *P. pyrifolia*

(continued)

Table 1.3 (continued)

Cultivar	Synonyms	Country	Mode of selection[a]
Louise Bonne	Bonne Louise d'Avranches, Louise Bonne de Jersey	France	
Luscious		USA	
Merton Pride (England, 1941)		UK	
Moonglow		USA	
Orcas		USA	Seedling
Orient		USA	*P. communis* × *P. pyrifolia* hybrid
Packhams Triumph	Packham	Australia	'Uvedale St. Germain' × 'Bartlett'
Passe Crassane		France	Seedling selection
Pineapple		USA	*P. communis* × *P. pyrifolia* hybrid
Rocha		Portugal	Chance seedling
Rosemarie		South Africa	
Seckel	Honey pear, Sugar pear	USA	
Starkrimson	Red Clapps	USA	Mutation of 'Clapp's Favourite'
Stinking Bishop	Moorcroft, Malvern Hills, Malvern Pear, Choke Pear, Choker	UK	
Summercrisp		USA	Unknown
Taylors Gold		New Zealand	A mutant clone of 'Comice'
Tosca		Italy	'Cossia' × 'Williams'
Turandot		Italy	'Dr. J. Guyot' × 'Bella di Giugno'
Uta		Germany	'Madame Verte' × 'Beurré Bosc'
Vicar of Winkfield	de Curé, Belle de Berry, Belle Eloïse, Bon Papa	France	
Virgouleuse	Virgoulette, Paradis d'Hiver, Chambrette, etc.	France	
Williams	Bartlett, Williams bon chrétien	UK	Chance seedling
Winter Nelis	Bonne de Malines, Colmar Nélis	Belgium	

[a]For those cultivars with blanks denotes unknown mode of selection/identification; O.P.: open pollination

parthenocarpic fruits (Dondini and Sansavini 2012). It has been brought back into commercial orchards for its original elongated shape and good fruit taste. In addition, it excels in southern European orchards due to its recent market claims (Dondini and Sansavini 2012).

'Beurré d'Anjou' is also known as 'Anjou', 'Winter Meuris', and 'Nec Plus Meuris' (Hedrick et al. 1921). It is a Belgian cultivar developed/identified by Van Mons in 1823. It produces medium-sized fruits of good quality that ripen in October–November (Bretaudeau and

Fauré 1991). The fruit is doliform, yellow, blushed heavily with red russet, and borne on a very short thick stems (Fig. 1.5a) (Hedrick et al. 1921; Dondini and Sansavini 2012). Fruit flesh is yellowish white in colour, luscious, buttery, slightly tart, and very sweet (Hedrick et al. 1921). This cultivar is losing favour in Europe due to difficulties in its management, size, or productivity, while it is widely grown in North America, South America, and South Africa (Dondini and Sansavini 2012).

'Concorde', derived from a cross between 'Conference' and 'Doyenné du Comice', and developed at the East Malling Research Station in England (Hessayon 1990). It produces medium-sized fruits of excellent quality that ripen in October (Hessayon 1990). The fruit is golden green, oftentimes with a golden yellow russetted spots, and has a vanilla sweet flavour and a firm texture (Fig. 1.5c) (Hessayon 1990). It is known for its tall, elongated neck, along with its firm and dense flesh. 'Concorde' is a late flowering cultivar (Hessayon 1990).

'Conference' is an English cultivar and developed/selected at the end of the nineteenth century (Dondini and Sansavini 2012). It produces medium-sized fruits of good quality that ripen in October (Bretaudeau and Fauré 1991). The fruit is long, pyriform, green, and prone to smooth russet on the skin, and it is sweet and juicy when fully ripe (Fig. 1.5d) (Hessayon 1990). 'Conference' fruit has a long shelf life (Dondini and Sansavini 2012). It is a mid-season flowering cultivar (Hessayon 1990). 'Conference' is reliable under less than perfect growing conditions (Hessayon 1990). It develops parthenocarpic fruits although pollination ensures a better crop (Hessayon 1990; Quinet et al. 2014). It is the European pear *par excellence* and accounts for ~32% of European pear production (Dondini and Sansavini 2012).

'Coscia' is an Italian cultivar, developed/identified in the 1800s, also known under the name of 'Ercolini' (Dondini and Sansavini 2012). It produces medium-sized fruits of good quality that ripen either in July or early August (Bretaudeau and Fauré 1991). The fruit is short, pyriform, and light green turning yellow in colour when ripe, along with a red blush on light-exposed side (Dondini and Sansavini 2012). Flesh is cream-white, with a granular texture, slightly scented, juicy, and sugary. Its cropping is variable, and it is slightly susceptible to internal breakdown (Dondini and Sansavini 2012).

'Doyenné du Comice', also known as 'Comice' and 'Fondante du Comice', is a French cultivar selected in 1849 (Dondini and Sansavini 2012). It produces large-sized fruits of excellent quality that ripen in October (Bretaudeau and Fauré 1991). The quality is so good that the fruits of this cultivar are called by many as the best of all pears (Hedrick et al. 1921). It is mainly cultivated as espalier trees. The fruit is turbinate and has a pale green-brownish colour that turn lighter in colour when approaching full ripeness (Fig. 1.5e), very sweet, creamy-coloured flesh, along with a juicy and somewhat buttery texture (Hedrick et al. 1921; Hessayon 1990; Dondini and Sansavini 2012). It is a late flowering cultivar (Hessayon 1990). 'Doyenné du Comice' is not very reliable under less than perfect growing conditions and requires warm temperatures, as well as shelter from strong winds (Hessayon 1990). Unfortunately, it is losing favour in Europe due to difficulties in management of these trees (Dondini and Sansavini 2012).

'Forelle' is a German cultivar, dating back to the end of the seventeenth century, although its origin is unknown (Hedrick et al. 1921; Dondini and Sansavini 2012). It produces small- to medium-sized fruits of medium quality that ripen in the winter (Dondini and Sansavini 2012). The fruit is ovoid and has a greenish skin which turns bright yellow, along with flecks of crimson-coloured spots when fully ripe. The flesh is crisp, firm yet juicy, with bright and candy sweet flavours. 'Forelle' is distinguished among other pear fruits of its kind by its trout-like specklings from which comes the name Forelle, the German word for trout (Hedrick et al. 1921). This cultivar has recently found renewed interest as its fruit is pleasingly different from other melting flesh types of traditional pear cultivars (Dondini and Sansavini 2012).

'Packham's Triumph' originated in Australia at the end of the nineteenth century, and it is

mainly cultivated in the southern hemisphere (Bretaudeau and Fauré 1991; Dondini and Sansavini 2012). It produces medium to large fruits that ripen in October (Bretaudeau and Fauré 1991). The fruit is pyriform, has a bumpy green skin, and a sweet juicy flavour (Hessayon 1990; Bretaudeau and Fauré 1991; Dondini and Sansavini 2012). 'Packham's Triumph' is an early flowering cultivar. This cultivar is also losing favour in Europe due to difficulty in its management, size, or productivity. Nevertheless, it is widely grown in North America, South America, and South Africa (Dondini and Sansavini 2012).

'Rocha' is a Portuguese cultivar from 1840 and accounts for 90% of the production of pears in Portugal (Dondini and Sansavini 2012). It produces very large fruits of excellent quality that ripen in the fall (Dondini and Sansavini 2012). The fruit is turbinate and greenish yellow in colour with some russet (Dondini and Sansavini 2012). It is sweet and fragrant with white-yellow flesh, and it can be eaten either while it is crisp or as it softens. This cultivar could also produce parthenocarpic fruits (Dondini and Sansavini 2012).

'Spadona', also known as 'Blanquilla', is a very old cultivar of unknown origin (Dondini and Sansavini 2012). It produces small- to medium-sized fruits of medium quality that ripen in August (Dondini and Sansavini 2012). The fruit is pyriform, with a smooth, pale green colour, and sometimes red-tinged when exposed to sunlight. Its pulp is white, with a fine to medium-fine texture, and a sugary taste. The fruit does not have a good shelf life. It is mainly cultivated in Southern Europe (Dondini and Sansavini 2012).

'Williams' is an English cultivar, first discovered in 1765 by a schoolmaster, Mr. Stair (Dondini and Sansavini 2012). It is also known as 'William Bon Chrétien' and 'Bartlett'. It produces large fruits of very good quality that ripen towards the end of August or early September (Bretaudeau and Fauré 1991). The fruit is pyriform to roundish, pale green to yellow in colour, shapely, along with a sweet and juicy flesh (Hessayon 1990; Dondini and Sansavini 2012). However, its storability is rather poor (Hessayon 1990). 'Williams' is a mid-season flowering cultivar (Hessayon 1990). This cultivar accounts for ~13% of the European production (Dondini and Sansavini 2012). It is still unsurpassed as the best summer pear cultivar in both Europe and the Americas (Dondini and Sansavini 2012). It is also the only cultivar used by the canning industry for juice making and for fresh-cut slices, either alone or in fruit salads (Dondini and Sansavini 2012).

1.4.2 Asian Pear

Asian pears constitute a group quite distinct in aspects of tree and fruit as compared to European pear. However, not all characters absent in occidental species are found in all species of the oriental group (Hedrick et al. 1921). Among Asian pears, most common differences, besides region of origin, are found in leaves and calyces (Hedrick et al. 1921). The leaves in most species are markedly acuminate, and their margins are sharp-serrate or setose-serrate (Hedrick et al. 1921). Persistent calyx is observed in *P. ussuriensis,* and few persistent calyces are present in *P. pyrifolia* and *P.* × *bretschneideri* (Iketani et al. 2012). The main cultivated species are *P. pyrifolia*, *P. ussuriensis*, *P.* × *bretschneideri*, and *P. sinkiangensis.* Flowering date depends on cultivars, and fruit maturation ranges between July and October (Bretaudeau and Fauré 1991). Asian pears reach optimum quality when allowed to ripen on the trees, similar to apples and peaches, but not to European pears.

1.4.2.1 Description of the Cultivated Species

Pyrus pyrifolia

P. pyrifolia is a vigorous, upright, and 7–15-m-tall tree (Hedrick et al. 1921; eFloras 2008). Branchlets are slender, purplish brown or dark brown when old, terete, tawny villous, or tawny tomentose when young, soon glabrescent, glabrous when old, and sparsely lenticellate (Hedrick et al. 1921; eFloras 2008). Leaf buds

are sharply pointed, plump, and thick at the base, with scales tomentose at margin and apex (Hedrick et al. 1921; eFloras 2008). Stipules are 1–1.5 cm long, caducous, linear-lanceolate, membranous with villous and entire margins, and an acuminate apex (eFloras 2008). Leaves measure 7–12 × 4–6.5 cm, are ovate-oblong, sometimes ovate, glabrous, or brown lanate when young; the leaf base is rounded or subcordate, rarely broadly cuneate; the leaf apex is acute, and the leaf margin spinulose-serrate (Bretaudeau and Fauré 1991; eFloras 2008). Flower buds are thick, short, conical, plump, free, and arranged singly on very short spurs (Hedrick et al. 1921). *P. pyrifolia* flowers in April (eFloras 2008). Inflorescences are umbellate-racemose clusters of 6–9 white flowers with caduceus bracts (Hedrick et al. 1921). Flowers measure 2.5–3.5 cm, are composed of 5 sepals, 5 petals, 20 stamens, and 4–5 carpels, and are borne on slender pedicels of 3–5 cm (Hedrick et al. 1921; Bretaudeau and Fauré 1991). Hypanthium is cupular and abaxially glabrous (eFloras 2008). Sepals are 0.6–1.2 cm long, triangular-ovate, and long-acuminate with an acuminate apex, and glandular denticulate margins (Hedrick et al. 1921; eFloras 2008). The abaxial side of sepals is glabrous, and the adaxial side is brown tomentose (Hedrick et al. 1921; eFloras 2008). Petals measure about 2 cm, oval, and entire, with a short clawed base and a rounded apex (Hedrick et al. 1921; eFloras 2008). Stamens are half as long as petals (eFloras 2008). Gynoecium is composed of a 4–5-loculed ovary, with two ovules per locule, usually five glabrous styles (rarely four), nearly as long as stamens (eFloras 2008). In August, *P. pyrifolia* produce round, slightly pyriform fruits, with a diameter of 2–2.5 cm and a brownish colour, with pale dots and caduceus sepals (eFloras 2008). Fruiting pedicel is 3.5–5.5 cm long (Hedrick et al. 1921; Iketani et al. 2012). Cultivated fruits are larger with a 5–6 cm diameter (Hedrick et al. 1921). Sand pears are commonly apple-shaped (Hedrick et al. 1921), and in China and Japan, there are a number of pomological cultivars, which, however, differ from each other, but less than cultivars of the European pear (Hedrick et al. 1921). *P. pyrifolia* hybridizes freely with *P. communis,* and several of these hybrids are important commercial cultivars in North America (Hedrick et al. 1921). Hybrid pears are more pyriform, and are of much better flavour than those of their oriental parents, and their calyces are either persistent or deciduous (Hedrick et al. 1921).

Pyrus ussuriensis

Trees of *P. ussuriensis* are 15 m tall (eFloras 2008). Branchlets are yellowish grey to purplish brown when young, yellowish grey, or yellowish brown when old (Hedrick et al. 1921; eFloras 2008). Branches are also glabrous or sparsely pubescent, and sparsely lenticellate (eFloras 2008). Buds are ovoid with an obtuse apex, and scales are sparsely pubescent or subglabrous at margins (eFloras 2008). Stipules are caducous, linear-lanceolate, 0.8–1.3 cm long, membranous with a glandular denticulate margin and acuminate apex (eFloras 2008). Leaves are ovate to broadly ovate, glabrous or tomentose when young, soon glabrescent, with a rounded or subcordate base, long spinulose-serrate margin, and a shortly acuminate or caudate-acuminate apex. The leaf blade measures 5–10 × 4–6 cm, and the petiole measures 2–5 cm (eFloras 2008). *P. ussuriensis* flowers in May, and produces white flowers with a diameter of 3–3.5 cm (eFloras 2008). Flowers are grouped by 5–7 in densely corymb with caducous, membranous, and linear-lanceolate bracts of 1.2–1.8 cm (Hedrick et al. 1921; eFloras 2008). Inflorescence peduncle and flower pedicel are tomentose when young and soon glabrescent; flower pedicel is 2–5 cm long (eFloras 2008). Flowers are composed of 5 sepals, 5 petals, 20 stamens, and 5 carpels. The hypanthium is campanulate, abaxially glabrous, or slightly tomentose (eFloras 2008). Sepals are triangular-lanceolate, 5–8 mm long, abaxially glabrous, and adaxially tomentose with margins that are initially glandular denticulate and with an acuminate apex (eFloras 2008). Petals are obovate or broadly ovate, glabrous, and measure 1.8 × 1.2 cm (eFloras 2008). Stamens are shorter than petals, and are nearly as long as styles (eFloras 2008). The

gynoecium is composed of a five-loculed ovary with two ovules per locule and five styles that are sparsely pubescent. Between August and October, *P. ussiriensis* produces yellow, subglobose fruits, of 2–6 cm in diameter, with persistent sepals, and a pedicel of 1–3 cm (Hedrick et al. 1921; eFloras 2008; Iketani et al. 2012). *P. ussuriensis* fruits require a ripening period in order to be edible (Teng et al. 2015). Cultivated fruits are much larger than wild fruits, although they are usually small and are not the tastiest of pears to humans (Iketani et al. 2012; Teng et al. 2015).

Pyrus × *bretschneideri*

P. × *bretschneideri* is a small-sized tree, reaching 5–8 m tall (eFloras 2008). Branchlets are purplish brown when old, terete, robust, densely pubescent when young, glabrous when old, and sparsely lenticellate (eFloras 2008). Buds are dark purple, ovoid with an obtuse apex, and pubescent scales at margin and apex (eFloras 2008). Stipules are caducous, linear or linear-lanceolate, 1–1.3 cm long, membranous, pubescent with glandular denticulate margin and acuminate apex (eFloras 2008). Leaves are ovate or elliptic-ovate, densely tomentose when young, soon glabrescent with a broadly cuneate base, spinulose-serrate margin, and acuminate apex (eFloras 2008). The leaf blade is 5–11 × 3.5–6 cm, and the petiole is 2.5–7 cm (eFloras 2008). *P.* ×*bretschneideri* flowers in April, and produce umbel-like racemes with 7–10 white flowers, and caduceus linear bracts of 1.5–3 cm (eFloras 2008). Inflorescence peduncle is tomentose when young, soon glabrescent, and flower pedicel is pubescent and 1.5–3 cm long (eFloras 2008). Flowers are 2–3.5 cm in diameter, and are composed of 5 sepals, 5 petals, 20 stamens, and 4–5 carpels (eFloras 2008). Hypanthium is cupular, slightly pubescent when young. Sepals are triangular, 3.5–5 mm long, abaxially glabrous, and adaxially brown tomentose with a glandular denticulate margin and acuminate apex (eFloras 2008). Petals are ovate with a shortly clawed base and rounded apex; sepals measure 1.2–1.4 × 1–1.2 cm (eFloras 2008). Stamens are half as long as petals and as long as styles (eFloras 2008). Gynoecium is composed of 4–5-loculed ovary, with two ovules per locule, and 4–5 glabrous styles (eFloras 2008). Between August and September, *P.* × *bretschneideri* produces ovoid or subglobose fruits that are yellow with fine dots, and have a diameter of 2–2.5 cm (eFloras 2008). Sepals are caduceus, the fruiting pedicel is glabrous, and 1.5–3 cm long (eFloras 2008; Iketani et al. 2012). Fruits are much larger under cultivation, are very juicy, and are shaped more like the European pear (Iketani et al. 2012).

Pyrus sinkiangensis

Trees of *P. sinkiangensis* are up to 6–9 m tall (eFloras 2008). Branchlets are purplish brown or greyish brown, terete, glabrous, and white lenticellate (eFloras 2008). Buds are ovoid, with an acute apex and pubescent scales at margins (eFloras 2008). Stipules are 8–10 mm long, caduceus, linear-lanceolate, membranous, white tomentose, with an acuminate apex and sparsely glandular and denticulate margins (eFloras 2008). Leaves are ovate, elliptic, or broadly ovate, either glabrous or white tomentose when young (eFloras 2008). Leaf petiole measures 3–5 cm, and leaf blade measures 6–8 cm × 3.5–5 cm (eFloras 2008). Leaf base is rounded, leaf margin is crenate or subentire basally and serrulate apically, and leaf apex is shortly acuminate (eFloras 2008). *P. sinkiangensis* flowers during the month of April, producing white flowers of 1.5–2.5 cm in diameter, and these are organized in umbel-like racemes of 4–7 flowers (eFloras 2008). The inflorescence peduncle and flower pedicel are tomentose, when young, and glabrescent; the flower pedicel is 1.5–4 cm in length. Bracts are 1–1.3 cm long, caducous, linear-lanceolate, membranous with long tomentose margins, sparsely glandular, denticulate, and with an acuminate apex (eFloras 2008). Flowers are composed of 5 sepals, 5 petals, 20 stamens, and 5 carpels. The hypanthium is cupular and abaxially glabrous (eFloras 2008). Sepals are triangular-ovate, 6–7 mm long, abaxially brown, tomentose, with an acuminate apex,

and glandular denticulate margins (eFloras 2008). Petals measure 1.2–1.5 × 0.8–1 cm, obovate, shortly clawed at base, and obtusely rounded at the apex (eFloras 2008). Stamens are, at a maximum, half as long as petals (eFloras 2008). The gynoecium contains a five-loculed ovary with two ovules per locule and five styles, but not exceeding the number of stamens (eFloras 2008). *P. sinkiangensis* produces fruits in August and September (eFloras 2008). Wild fruits are yellowish green, either ovoid or obovoid, with persistent sepals, and measure 2.5–5 cm in diameter (eFloras 2008). Fruiting pedicel is 4–5 cm long, thickened distally, and glabrescent (eFloras 2008). Cultivated fruits vary considerably, and combine characteristics of both *P. communis* and *P.* ×*bretschneideri* (Jun and Hongsheng 2002). Generally, the fruit shape is more similar to *P. communis,* but with a long pedicel. Some cultivars bear fruit with a persistent calyx, have a strong aroma, and require a ripening period before they are edible, similar to *P. communis,* while others are juicy and crisp, and do not require ripening as that for *P.* × *bretschneideri*.

1.4.2.2 Asian Pear Cultivars

There are several species and cultivars that are cultivated as Asian pears (Table 1.4). The Japanese cultivars tend to be more round in shape, while Chinese cultivars are more oval or pyriform (pear-shaped). China accounts for most of the world's Asian pear production with *P.* × *bretschneideri* cultivars 'Dong Shan Su Li', 'Ya Li', and 'Huang Hua Li' dominating production (Bassil and Postman 2010). *Pyrus pyrifolia* cultivars 'Kosui' and 'Hosui' make up to 65% of the production area in Japan, followed by 'Nijisseiki' and 'Niitaka', while 'Niitaka' is the primary cultivar in Korea (Bassil and Postman 2010). Some of these cultivars are described below.

'Chojuro' is a Japanese cultivar of *P. pyrifolia* (NSW 2017). It has an early to mid-flowering season, and it is partially self-fertile. It produces oblate fruits of medium size that ripen 135–150 days after full bloom. The fruit is golden brown, fully russetted, and has a poor to moderate eating quality, and a tough and gritty texture. It has a high sugar content and a medium low acid content. Fruits could be stored up to five months (NSW 2017).

'Hosui' is a Japanese cultivar of *P. pyrifolia* that resulted from a cross between 'Kosui' and 'Hiratsuka 1', although it has been previously reported as a progeny of hybridization of 'Ri-14' and 'Yakumo' (Saito 2016; NSW 2017). It is also known as 'Housui' (Saito 2016). This cultivar produces round, medium- to large-sized fruits that ripen early to mid-September, 135–145 days after full bloom (Saito 2016; NSW 2017). The fruit is golden brown, russetted, along with conspicuous white lenticels. It has an excellent eating quality with high sugar and acid contents and a fine-grained texture (NSW 2017). The flesh is crisp and juicy (Saito 2016). Fruit has a good keeping quality and can be stored for 3–4 months. 'Hosui' is a mid-season flowering pear (NSW 2017).

'Huang Hua Li' is a Chinese cultivar of *P. pyrifolia* (Jun and Hongsheng 2002). It produces medium to large-sized round fruits that ripen in mid-August (Jun and Hongsheng 2002). Fruits have a smooth and yellow-brown skin colour.

'Kikusui' is a Japanese cultivar of *P. pyrifolia,* developed in 1927 from a cross between 'Taihaku' and 'Nijisseiki' (NSW 2017). It is also known as the 'twenty-first century'. It produces oblate medium-sized fruits of good quality that tend to be lopsided. The fruit is yellowish green in colour, tender, but cracks following a heavy rain (NSW 2017). It has a high sugar and acid contents. Fruits ripen mid-season, 135–145 days after full bloom, and can be stored up to five months (NSW 2017). 'Kikusui' flowers mid- to late season, and it is partially self-fertile (NSW 2017).

'Kosui', also known as 'Kousui', is a Japanese cultivar of *P. pyrifolia* that has originated from a cross between 'Kikusui' and 'Wasekozo' (Saito 2016). It produces orbicular to oblate fruits that ripen near middle to late August (Saito 2016). The fruit is orange in colour, over a greenish yellow background, along with a partially russetted skin. Fruit flesh is soft, juicy, and

Table 1.4 List of some Asian pear cultivars (Flores 1999; Bassil and Postman 2010; Yue et al. 2014; Saito 2016; Chang et al. 2017; NSW 2017)

Cultivar	Species	Country	Mode of selection*
Akizuki	*P. pyrifolia*	Japan	Niitaka × 'Hosui × Kosui
Arirang	*P. pyrifolia*	Korea	
Atago	*P. pyrifolia*	Japan	
Autumn Sweet			
Ba Li Xiang [Ba Li Hsiang]	*P.* × *bretschneideri*	China	
Bong Ri	*P. pyrifolia*, *x P.* × *bretschneideri*	Korea	*P. pyrifolia*, Nijisseiki × *P.* × *bretschneideri*
Cheih Li	*P.* × *bretschneideri*	China	
Chien Li	*P.* × *bretschneideri*	China	
Chien Pa Li	*P. ussuriensis*	China	
Chinfon Li	*P.* × *bretschneideri*	China	
Choju	*P. pyrifolia*	Japan	Asahi × Kimizukawase
Chojuro (Choujuurou)	*P. pyrifolia*	Japan	Chance seedling
Cili	*P. pyrifolia*	China	
Daisui Li			
Dan Bae	*P. pyrifolia x P. ussuriensis*	Korea	*P. pyrifolia* Chojuro × *P. ussuriensis*
Dangshan Suli	*P.* × *bretschneideri*	China	
Dasui Li			U.C. hybrids
Gold Nijisseiki	*P. pyrifolia*	Japan	
Haeng Soo	*P. pyrifolia*	Korea	*P. pyrifolia*, Kikuchi × Joseng Henjang
Hansen Siberian Pear	*P. ussuriensis*	China	
Hakko	*P. pyrifolia*	Japan	Yakumo × Kosui.
Harbin	*P. ussuriensis*	China	
Hosui	*P. pyrifolia*	Japan	Kosui × Hiratsuka 1
Huagai	*P. ussuriensis*	China	
Hung Li	*P.* × *bretschneideri*	China	
Huiyangqingli	*P. pyrifolia*	China	
Huiyangsuanli	*P. pyrifolia*	China	
Imamuraaki	*P. pyrifolia*	Japan	
Jianbali	*P. ussuriensis*	China	
Jinchuanxueli	*P. pyrifolia*	Japan	
Kikusui	*P. pyrifolia*	Japan	Taihaku × Nijisseiki
Kosui (Kousui)	*P. pyrifolia*	Japan	Kikusui × Wasekozo
Manyuanxiang	*P. ussuriensis*	China	
Meigetsu	*P. pyrifolia*	Japan	Chance seedling
Nanguoli	*P. ussuriensis*	China	
Nansui	*P. pyrifolia*	Japan	
Niitaka	*P. pyrifolia*	Japan	Amanogawa × Imamuraaki

(continued)

Table 1.4 (continued)

Cultivar	Species	Country	Mode of selection*
Nijisseiki (twentieth centyry)	*P. pyrifolia*	Japan	Chance seedling
Nijisseki (twentieth century)	*P. pyrifolia*	Japan	Chance seedling
Okusankichi	*P. pyrifolia*	Japan	Old variety
Olympic			
Pa Li	*P. ussuriensis*	China	
Pai Li (Beijing white pear)	*P.* × *bretschneideri*	China	Old selection from Beijing region
Ping Guo Li (Pingo Li)	*P.* × *bretschneideri*	China	Old selection from Jilin Province
Seigyoku	*P. pyrifolia*	Japan	Nijiseiki × Chojuro
Seuri Li	*P. pyrifolia*	China	
Shen Li		China	
Shin Go	*P. pyrifolia*	Korea	Cheonjichon x Imamuraaki
Shin Li			U.C. hybrids
Shinko	*P. pyrifolia*	Japan	Nijisseiki × Amanogawa
Shin-Soo	*P. pyrifolia*	Korea	Kikuchi × Kimizukawase
Shinseiki	*P. pyrifolia*	Japan	Nijiesiki × Chojuro
Shinsei	*P. pyrifolia*	Japan	Suisei x Shinko
Shinsui	*P. pyrifolia*	Japan	Kikusui × Kimizukawase
Singo	*P. pyrifolia*	Korea (Japan)	
Tang Li	*P. ussuriensis*	China	
Tse Li	*P.* × *bretschneideri*	China	
Tsu Li	*P.* × *bretschneideri*	China	Probably *P. ussuriensis* and *P.* × *bretschneideri*
Xiangshui Li (Hsiang Sui Li)	*P.* × *bretschneideri*	China	
Xuehuali	*P.* × *bretschneideri*	China	
Ya Li	*P.* × *bretschneideri*	China	Old variety
Yakumo	*P. pyrifolia*	Japan	Nijisseiki × Akaho

*For those cultivars with blanks denotes unknown mode of selection/identification

sweet, along with a very fine texture (Saito 2016). 'Kosui' is the principal cultivar in Japan (Saito 2016).

'Niitaka' is a Japanese cultivar of *P. pyrifolia*, resulting from a cross between 'Amanogawa' and 'Chojuro' (Saito 2016). It produces large-sized fruits of long shelf life (Saito 2016). The fruit is orbicular, orange-brown in colour, along with brown russeting, and it ripens beginning at the end of September to early October (Saito 2016). The off-white flesh is sweet and juicy, but a bit coarser than other Asian pears (Saito 2016).

'Shinseiki' is a Japanese cultivar of *P. pyrifolia,* developed from a cross between 'Nijiseiki' and 'Chojuro' (NSW 2017). It produces flat-round fruits of medium size that ripen 125 days after full bloom. The fruit is yellow-green in colour, very smooth, tender, and bruises rather easily (NSW 2017). The flesh is juicy, mild-flavoured, along

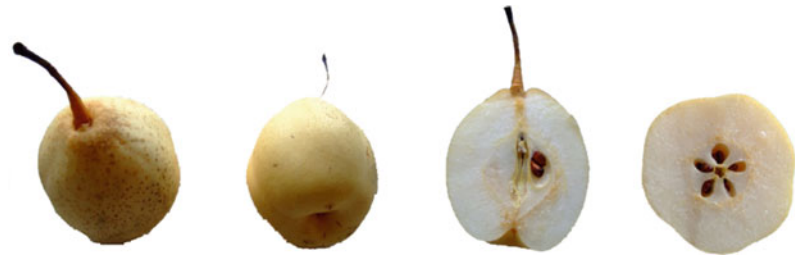

Fig. 1.6 Fruits of the Asian pear cultivar 'Ya-Li'

with a medium sugar and high acid contents (NSW 2017). Fruit storage is short, not exceeding two months. 'Chojuro' flowers late, and it is partially self-fertile (NSW 2017).

'Tsu-Li' is an old Chinese cultivar that most likely has resulted from a cross between *P. ussuriensis and P.* × *bretschneideri* (NSW 2017). It produces ovate pyriform fruits of medium to large size that ripen late, about 176–189 days after full bloom (NSW 2017). The fruit is light green to yellow-green in colour and may have ugly lenticel spotting. It has a good eating quality and contains some stone cells (NSW 2017). It has a sweet taste with a trace of tartness and has a high sugar content along with a moderate acid content. Fruits can be stored for up to six months at 0–1 °C (NSW 2017).

'Ya-Li' is an old Chinese cultivar of *P.* × *bretschneideri* (NSW 2017). It produces turbinate to globular, acute, pyriform fruits of medium to large size and that ripen 175–190 days after full bloom. The fruit is pale yellowish green, shiny, and has a good to excellent eating quality with medium sugar and acid contents and mildly sweet taste (Fig. 1.6) (NSW 2017). 'Ya-Li' flowers very early. In China, 'Ya-Li' is one of the dominant cultivars for export (Jun and Hongsheng 2002).

1.5 Conclusions

Pear is one of the most important fruits grown worldwide, and it is cultivated in all temperate regions. The *Pyrus* genus has about 75–80 species, and several hybridizations have been observed among these species which renders it difficult to distinguish among available pear species. Further investigations are required to better understand the complex evolutionary histories and relationships among species of *Pyrus*. Pear species could be divided into an eastern Asian clade and a western Eurasian clade. In both clades, there are some species that are cultivated, including the European pear *P. communis* and the Asian pears *P. pyrifolia*, *P. ussuriensis*, *P.* × *bretschneideri*, *and P. sinkiangensis.* There are thousands of pear cultivars that are available all over the world, with diverse fruit shape, taste, and texture. However, only a few of these cultivars contribute to most of the world production of pears nowadays. Several pear breeding programs have been involved in developing new commercial cultivars. Undoubtedly, sequencing and annotation of the pear genome, of both European and Asian pears, will help scientists and breeders in better understanding the genetics of pear and in making advances to develop improved genotypes with high fruit nutritional quality and tolerance to biotic and abiotic stresses.

References

Aas G (1999) Die Wildbirne aus systematisch-botanischer Sicht. Berichte Aus Bayer Landesan-Stalt Für Wald Forstwirtsch 23:2–6

Asanidze Z, Akhalkatsi M, Gvritishvili M (2011) Comparative morphometric study and relationships between the Caucasian species of wild pear (*Pyrus* spp.) and local cultivars in Georgia. Flora-Morphol Distrib Funct Ecol Plants 206:974–986

Aydin ZU, Dönmez AA (2015) Taxonomic and nomenclatural contributions to *Pyrus* L. (Rosaceae) from Turkey. Turk J Bot 39:841–849

Bailey LH, Bailey EZ (1976) Hortus third: a concise dictionary of plants cultivated in the United States and Canada, 1st edn. Macmillan, New York, USA, p 1290

Bao L, Chen K, Zhang D, Cao Y, Yamamoto T, Teng Y (2007) Genetic diversity and similarity of pear (*Pyrus* L.) cultivars native to East Asia revealed by SSR (simple sequence repeat) markers. Genet Resour Crop Evol 54:959. https://doi.org/10.1007/s10722-006-9152-y

Bassil N, Postman JD (2010) Identification of European and Asian pears using EST-SSRs from *Pyrus*. Genet Resour Crop Evol 57:357–370

Bretaudeau J, Fauré Y (1991) Atlas d'arboriculture fruitière. Ed. Lavoisier technique et documentation, Paris, France, 289p

Brewer LR, Palmer JW (2011) Global pear breeding programs: goals, trends and progress for new cultivars and new rootstocks. Acta Hortic, 105–119. https://doi.org/10.17660/actahortic.2011.909.10

Campbell CS, Evans RC, Morgan DR, Dickinson TA, Arsenault MP (2007) Phylogeny of subtribe *Pyrinae* (formerly the Maloideae, Rosaceae): limited resolution of a complex evolutionary history. Plant Syst Evol 266:119–145. https://doi.org/10.1007/s00606-007-0545-y

Chagné D, Crowhurst RN, Pindo M, Thrimawithana A, Deng C, Ireland H, Fiers M, Dzierzon H, Cestaro A, Fontana P, Bianco L, Lu A, Storey R, Knäbel M, Saeed M, Montanari S, Kim YK, Nicolini D, Larger S, Stefani E, Allan AC, Bowen J, Harvey I, Johnston J, Malnoy M, Troggio M, Perchepied L, Sawyer G, Wiedow C, Won K, Viola R, Hellens RP, Brewer L, Bus VGM, Schaffer RJ, Gardiner SE, Velasco R (2014) The draft genome sequence of European Pear (*Pyrus communis* L. 'Bartlett'). PLoS ONE 9:e92644. https://doi.org/10.1371/journal.pone.0092644

Chang Y-J, Cao Y-F, Zhang J-M, Tian L-M, Dong X-G, Zhang Y, Qi D, Zhang X (2017) Study on chloroplast DNA diversity of cultivated and wild pears (*Pyrus* L.) in Northern China. Tree Genet Genomes 13:44

Chase MW, Christenhusz MJM, Fay MF, Byng JW, Judd WS, Soltis DE, Mabberley DJ, Sennikov AN, Soltis PS, Stevens PF (2016) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Bot J Linn Soc 181:1–20

Coste H, Flahault C (1903) Flore descriptive et illustrée de la France, de la Corse, et des contrées limitrophes.tome 2. Ed. P. Klincksieck, Paris, France, 627 p

De Vilmorin J-B, Clebant M (1996) Le Jardin des Hommes: l'histoire des plantes cultivées. France, Paris, p 304

Debuigne G, Couplan F (2006) Petit Larousse des Plantes qui Guérissent: 500 plantes. Ed. Larousse, Paris, France, 892 p

Dondini L, Sansavini S (2012) European pear. Fruit breeding. Springer, Boston, MA, pp 369–413

Drudze I (2004) New apple and pear selections from hybrid material of "Iedzeni" in Latvia. Acta Hortic, 895–898. https://doi.org/10.17660/actahortic.2004.663.163

eFloras (2008) eFloras.org Home. http://efloras.org/index.aspx. Accessed 21 Feb 2018. Missouri Botanical Garden, St. Louis, MO & Harvard University Herbaria, Cambridge, MA

Evans KM, Fernández-Fernández F, Bassil N, Nyberg AM, Postman JD (2015) Comparison of accessions from the UK and US national pear germplasm collections with a standardized set of microsatellite markers. Acta Hortic 1094:41–46

FAO (2018) FAOSTAT. http://www.fao.org/faostat/en/#home. Accessed 6 Feb 2018

Faoro ID, Orth AI (2014) Flower visiting insects during the bloom period of Japanese pear orchards in Brazil. XII Int Pear Symp 1094:275–279

Faoro ID, Orth AI (2011) Nectar production and quality in Japanese pear cultivars in south Brazil. Acta Hortic 909:409–414. https://doi.org/10.17660/ActaHortic.2011.909.46

Farkas A, Szabo LG, Orosz-Kovacs Z (2002) Nectar composition in some pear cultivars. Acta Hortic 596:761–765. https://doi.org/10.17660/ActaHortic.2002.596.131

Ferradini N, Lancioni H, Torricelli R, Russi L, Dalla Ragione I, Cardinali I, Marconi G, Gramaccia M, Concezzi L, Achilli A, Veronesi F, Albertini E (2017) Characterization and phylogenetic analysis of ancient Italian landraces of pear. Front Plant Sci 8. https://doi.org/10.3389/fpls.2017.00751

Flores B (1999) The Great book of pears. Ten Speed Press, Berkeley, Toronto, Canada, p 163

Franceschi PD, Dondini L, Sanzol J (2012) Molecular bases and evolutionary dynamics of self-incompatibility in the Pyrinae (Rosaceae). J Exp Bot 63:4015–4032. https://doi.org/10.1093/jxb/ers108

Hedrick UP, Howe GH, George H, Taylor OM, Tukey HB (1921) The pears of New York. J.B. Lyon Co., Albany, USA, 636 p

Hessayon DG (1990) The Fruit Expert. Ed. Expert Books, Norwich, UK, 128 p

Iketani H (2016) Native fruit tree genetic resources in Japan. Breed Sci 66:82–89

Iketani H, Katayama H, Uematsu C, Mase N, Sato Y, Yamamoto T (2012) Genetic structure of East Asian cultivated pears (*Pyrus* spp.) and their reclassification in accordance with the nomenclature of cultivated plants. Plant Syst Evol 298:1689–1700. https://doi.org/10.1007/s00606-012-0670-0

Jain SM, Priyadarshan PM (2009) Breeding plantation tree crops: temperate species. Springer Science & Business Media, New York, USA, p 294

Jun W, Hongsheng G (2002) The production of Asian pears in China. Acta Hortic, 71–80. https://doi.org/10.17660/actahortic.2002.587.4

Katayama H, Amo H, Wuyun T, Uematsu C, Iketani H (2016) Genetic structure and diversity of the wild Ussurian pear in East Asia. Breed Sci 66:90–99

Kell S, Qin H, Chen B, Ford-Lloyd B, Wei W, Kang D, Maxted N (2015) China's crop wild relatives: diversity for agriculture and food security. Agric Ecosyst Environ 209:138–154

Kim YK, Won KH, Lee UY, Yim SH, Shin IS, Kang SS, Han JD, Lee HC (2015) Genetic diversity of Asian

and European pear using simple sequenced repeats markers analysis. Acta Hortic 1094:67–73

Korotkova N, Nauheimer L, Ter-Voskanyan H, Allgaier M, Borsch T (2014) Variability among the most rapidly evolving plastid genomic regions is lineage-specific: implications of pairwise genome comparisons in *Pyrus* (Rosaceae) and other angiosperms for marker choice. PLoS ONE 9:1–16. https://doi.org/10.1371/journal.pone.0112998

Korotkova N, Parolly G, Khachatryan A, Ghulikyan L, Sargsyan H, Akopian J, Borsch T, Gruenstaeudl M (2018) Towards resolving the evolutionary history of Caucasian pears (*Pyrus*, Rosaceae) -Phylogenetic relationships, divergence times and leaf trait evolution. J Syst Evol 56:35–47. https://doi.org/10.1111/jse.12276

Lee H-S, Isse T, Kawamoto T, Woo H-S, Kim AK, Park JY, Yang M (2012) Effects and action mechanisms of Korean pear (*Pyrus pyrifolia* cv. Shingo) on alcohol detoxification. Phytother Res 26:1753–1758. https://doi.org/10.1002/ptr.4630

Leroy A (1867) Dictionnaire de Pomologie, tome 1. Angers, France, p 615

Lo EYY, Donoghue MJ (2012) Expanded phylogenetic and dating analyses of the apples and their relatives (Pyreae, Rosaceae). Mol Phylogenet Evol 63:230–243. https://doi.org/10.1016/j.ympev.2011.10.005

Mayer DF, Miliczky ER, Lunden JD (1990) Pollination of pears. In: Pear production in the Pacific Northwest. University of California Press, Davis, CA, USA

Miranda C, Urrestarazu J, Santesteban LG, Royo JB, Urbina V (2010) Genetic diversity and structure in a collection of ancient Spanish pear cultivars assessed by microsatellite markers. J Am Soc Hort Sci 135:428–437

Moriya Y, Takai Y, Okada K, Ito D, Shiozaki Y, Nakanishi T, Takasaki T (2005) Parthenocarpy and self- and cross-incompatibility in ten European pear cultivars. J Jpn Soc Hortic Sci 74:424–430. https://doi.org/10.2503/jjshs.74.424

NSW (2017) Apples, pears and other pome fruits. https://www.dpi.nsw.gov.au/agriculture/horticulture/pomes. Accessed 23 Feb 2018

Nyéki A, Porpaczy A, Soltész M, Szabo Z, Ivancsics J (1998) Self fertility of pear varieties conditioned by natural self pollination (autogamy). Acta Hortic 475:433–434. https://doi.org/10.17660/ActaHortic.1998.475.53

Opoix O (1896) La Culture du Poirier. Bibliothéque d'Horticulture, Paris, France, p 275

Paris N (1996) Pollinisation du poirier. Bull Apic Tech 23:195–200

Pesson P, Louveaux J (1984) Pollinisation et Productions Végétales. Ed. Quae, France, 704 p

Petri JL, Herter F (2002) Nashi pear (*Pyrus pyrifolia*) dormancy under mild temperature clomate conditions. Acta Hortic, 353–361. https://doi.org/10.17660/actahortic.2002.587.47

Quinet M, Jacquemart A-L (2015) Difference between pollination and parthenocarpy in the "Conférence" pear production. Acta Hortic 1094:359–366. https://doi.org/10.17660/ActaHortic.2015.1094.45

Quinet M, Kelecom S, Jacquemart A-L (2014) S-genotype characterization of 13 North-Western European pear (*Pyrus communis*) cultivars. Sci Hortic 165:1–4. https://doi.org/10.1016/j.scienta.2013.10.023

Quinet M, Warzée M, Vanderplanck M, Michez D, Lognay G, Jacquemart A-L (2016) Do floral resources influence pollination rates and subsequent fruit set in pear (*Pyrus communis* L.) and apple (*Malus* × *domestica* Borkh.) cultivars? Eur J Agron 77:59–69

Rameau JC, Mansion D, Dumé G, Timbal J, Lecointe A, Dupont P, Keller R (1989) Flore forestière française, guide écologique illustré. 1. Plaines et collines. Institut pour le développement forestier, Paris, France, 1785 p

Royer A (1853) Les Annales de Pomologie Belge et Étrangère. Commission royale de Pomologie, Bruxelles, Belgique, p 880

Saito T (2016) Advances in Japanese pear breeding in Japan. Breed Sci 66:46–59

Sassa H, Kakui H, Minamikawa M (2009) Pollen-expressed F-box gene family and mechanism of S-RNase-based gametophytic self-incompatibility (GSI) in Rosaceae. Sex Plant Reprod 23:39–43. https://doi.org/10.1007/s00497-009-0111-6

Silva GJ, Souza TM, Barbieri RL, Costa de Oliveira A (2014) Origin, domestication, and dispersing of pear (*Pyrus* spp.). Adv Agric 2014:1–8. https://doi.org/10.1155/2014/541097

Stevens PF (2017) Angiosperm Phylogeny Website. http://www.mobot.org/MOBOT/research/APweb/. Accessed 10 Feb 2018

Teng Y (2011) The pear industry and research in China. Acta Hortic 909:161–170

Teng Y, Yue X, Zheng X, Cai D (2015) Genetic clue to the origin of cultivated Asian pears inferred from cpDNA haplotypes. Acta Hortic, 31–39

USA pears (2018) List of ten pear varieties—USA pears. http://usapears.org/pear-varieties/. Accessed 22 Feb 2018

Wesel J-P (1996) Pomone jodoignoise. Ed. Jodoigne Passé Présent, Jodoigne, Belgique, 133 p

White A (2002) Asian pear production and research trends in New Zealand and Australia. Acta Hortic 587:107–111

Wu J, Wang Z, Shi Z, Zhang S, Ming R, Zhu S, Khan MA, Tao S, Korban SS, Wang H, Chen NJ, Nishio T, Xu X, Cong L, Qi K, Huang X, Wang Y, Zhao X, Wu J, Deng C, Gou C, Zhou W, Yin H, Qin G, Sha Y, Tao Y, Chen H, Yang Y, Song Y, Zhan D, Wang J, Li L, Dai M, Gu C, Wang Y, Shi D, Wang X, Zhang H, Zeng L, Zheng D, Wang C, Chen M, Wang G, Xie L, Sovero V, Sha S, Huang W, Zhang S, Zhang M, Sun J, Xu L, Li Y, Liu X, Li Q, Shen J, Wang J, Paull RE, Bennetzen JL, Wang J, Zhang S (2013) The genome of the pear (*Pyrus bretschneideri* Rehd.). Genome Res 23:396–408. https://doi.org/10.1101/gr.144311.112

Wu J, Wang Y, Xu J, Korban SS, Fei Z, Tao S, Ming R, Tai S, Khan MA, Postman JD, Gu C, Yin H, Zheng D, Qi K, Li Y, Wang R, Deng CH, Kumar S, Chagné D, Li X, Wu J, Huang X, Zhang H, Xie Z, Li X, Zhang M,

Li Y, Yue Z, Fang X, Li J, Li L, Jin C, Qin M, Zhang J, Wu X, Ke Y, Wang J, Yang H, Zhang S (2018) Diversification and independent domestication of Asian and European pears. Genome Biol 19:77. https://doi.org/10.1186/s13059-018-1452-y

Xiang Y, Huang C-H, Hu Y, Wen J, Li S, Yi T, Chen H, Xiang J, Ma H (2017) Evolution of Rosaceae fruit types based on nuclear phylogeny in the context of geological times and genome duplication. Mol Biol Evol 34:262–281. https://doi.org/10.1093/molbev/msw242

Yamamoto T, Terakami S (2016) Genomics of pear and other Rosaceae fruit trees. Breed Sci 66:148–159

Yao L, Zheng X, Cai D, Gao Y, Wang K, Cao Y, Teng Y (2010) Exploitation of *Malus* EST-SSRs and the utility in evaluation of genetic diversity in *Malus* and *Pyrus*. Genet Resour Crop Evol 57:841–851

Yue X, Liu G, Zong Y, Teng Y, Cai D (2014) Development of genic SSR markers from transcriptome sequencing of pear buds. J Zhejiang Univ Sci B 15:303–312

Zamani A, Attar F, Civeyrel L (2017) Leaf epidermis characters of Iranian *Pyrus* L. (Rosaceae) and their taxonomic implications. Genet Resour Crop Evol 64:159–176

Zheng X, Cai D, Potter D, Postman J, Liu J, Teng Y (2014) Phylogeny and evolutionary histories of *Pyrus* L. revealed by phylogenetic trees and networks based on data from multiple DNA sequences. Mol Phylogenet Evol 80:54–65. https://doi.org/10.1016/j.ympev.2014.07.009

Pear Germplasm Needs and Conservation

2

Joseph Postman

Abstract

Pear (*Pyrus*) species are sources of food, drink, landscape trees, and rootstocks. Different *Pyrus* species possess varied genetic traits that render them useful for diverse purposes. Pear genebanks preserve cultivars, or unique genotypes, as grafted trees. They also store seedlots and seedling populations that may represent pear wild relative species. Seed and seedling collections usually represent species populations from distinct geographic locations rather than unique genotypes. In the USA, the USDA Agricultural Research Service's National Plant Germplasm System maintains a genebank in Corvallis, Oregon, representing world diversity for *Pyrus* that includes more than 2500 unique clones or seedlots. Other pear genebanks around the world tend to be more specialized, focusing on accessions native to the region or in support of focused breeding programs. Molecular techniques and genetic markers have become valuable tools for pear genebank management. Various types of molecular markers can be used to assess genetic diversity, identify gaps in germplasm collections, and help detect redundancy and confirm synonymy. Microsatellite, or simple sequence repeat (SSR), markers, and chloroplast-derived markers are commonly used to accomplish these tasks. Markers can also be used for pedigree analysis, which may either confirm or detect anomalies in pedigrees of genebank accessions. Advances in breeding, developing genetic markers, and identifying major genes in pear cannot be accomplished without access to diverse living collections of *Pyrus* germplasm.

2.1 Commercial Uses of Pears

Pears are produced commercially in mid-latitude temperate regions throughout the world, despite the fact that there are no *Pyrus* species native to North America or from anywhere in the southern hemisphere. Top pear producing countries, with >400,000 metric tons harvested in 2016, are China, Argentina, USA, Italy, Turkey, and South Africa. An additional 16 countries produced >100,000 metric tons (Table 2.1; FAO 2018).

Two distinct centers of origin or centers of wild diversity are recognized for the genus *Pyrus*, the Caucasus Mountains and China. European pear species belong to section '*Pyrus*' of the genus *Pyrus* (Table 2.2; USDA-ARS 2018a). These originated in regions around the Caucasus Mountains between the Black Sea and the Caspian Sea. The taxa *Pyrus communis* ssp. *caucasica* (Fed.) Browicz, *P. communis*

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Table 2.1 Pear yield in metric tons (MT) from 2010 to 2016 in countries producing >100,000 MT in 2016 (FAO 2018)

	2010	2011	2012	2013	2014	2015	2016
China (mainland)	15,057,000	15,795,000	17,073,000	17,300,752	17,964,400	18,699,000	19,388,063
Argentina	670,000	812,633	825,115	890,000	840,000	869,000	905,605
USA	738,085	876,087	778,583	795,692	754,415	744,345	738,770
Italy	736,646	926,542	645,540	743,029	701,558	753,667	701,928
Turkey	380,003	386,382	442,646	461,826	462,336	463,623	472,250
South Africa	368,495	350,527	338,584	364,854	404,260	394,450	433,105
India	336,000	335,000	340,000	325,000	316,700	303,000	399,000
Netherlands	274,000	336,000	199,000	327,000	349,000	349,000	374,000
Spain	476,686	502,434	407,428	425,700	429,548	355,410	366,131
Belgium	307,270	284,827	236,400	305,000	374,300	374,630	331,550
Chile	181,387	196,743	199,247	226,189	240,399	280,870	299,432
Japan	284,900	312,800	299,000	294,400	295,100	276,500	278,100
Iran	121,012	126,115	129,317	140,090	279,580	285,000	254,599
Korea (South)	307,820	290,494	172,599	282,212	302,731	260,975	238,014
Algeria	234,274	233,147	211,191	240,709	228,114	255,344	211,943
Ukraine	141,700	153,100	157,500	169,400	157,690	170,610	156,000
Korea (North)	137,971	143,000	147,000	145,000	144,569	145,963	146,601
Portugal	176,764	230,447	116,287	202,483	210,009	141,186	137,805
France	146,552	162,905	117,262	142,923	132,588	140,833	129,627
Taiwan	174,858	150,013	137,911	109,105	134,549	127,016	111,424
Australia	95,111	123,267	119,274	109,206	98,035	105,243	104,928
Uzbekistan	72,700	68,796	74,000	80,000	87,000	95,000	100,948

ssp. *pyraster* (L.) Ehrh., and *P.*× *nivalis Jacq.* are the primary ancestors of large-fruited European pears (*P. communis* L.) (Fig. 2.1).

Asian pears belong to section '*Pashia*' of the genus. Asian pear species have a more ancient center of diversity in the region around Zhejiang Province in China (Tenga et al. 2015). Large-fruited Asian pears are primarily derived from *P. pyrifolia* (Burm. f.) Nakai, *P.* × *bretschneideri* Rehd., and *P. ussuriensis* Maxim., as well as complex hybrids with other species. In the Indian subcontinent, large-fruited pears are derived from hybrids between *P. pashia* Buch.-Ham. ex D. Don and both European and East Asian pears (Table 2.2; Fig. 2.1; USDA-ARS 2018a). In far-west China where the range of European and Asian pear wild relatives overlap, ancient natural hybrids between *P. communis* and *P. pyrifolia,* known as *P.* × *sinkiangensis* T.T. Yu, have been selected for their large fruit. A commercial industry in the Xinjiang region produces fruit marketed as the 'Chinese Fragrant Pear' or the 'Korla' pear.

For a detailed review of the ancient geographic origins and ancestral relationships of *Pyrus* taxa, please refer to Chap. 4 of this volume by Volk and Cornille.

2.1.1 Pears for Food

Pear cultivars commercially grown for their fruit are valued for traits related to fruit quality and tree architecture that are amenable to efficient

Table 2.2 *Pyrus* species recognized by the USDA National Plant Germplasm System (USDA-ARS 2018a)

Taxon	Genebank accessions	Pyrus section	Native origin
Pyrus armeniacifolia T. T. Yu	0	Pashia	
Pyrus betulifolia Bunge	65	Pashia	China, Laos
Pyrus boissieriana Buhse	0	Pyrus	Azerbaijan, Turkmenistan, Iran
Pyrus × *bretschneideri* Rehder (2)	24		China
Pyrus calleryana Decne.	111	Pashia	China, Korea, Taiwan, Vietnam, naturalized in North America
Pyrus × *canescens* Spach (3)	1	Pyrus	
Pyrus communis L.	1011 (a)	Pyrus	Caucasus, Middle Asia, Western Asia, Europe, widely naturalized
Pyrus communis subsp. *caucasica* (Fed.) Browicz	77	Pyrus	Caucasus, Turkey, Ukraine
Pyrus communis subsp. *pyraster* (L.) Ehrh.	83	Pyrus	Turkey, Europe
Pyrus × *complexa* Rubtzov	2	Pyrus	Armenia, Azerbaijan
Pyrus cordata Desv.	22	Pyrus	UK, France, Portugal, Spain
Pyrus cossonii Rehder	4	Pashia	Algeria
Pyrus dimorphophylla Makino	19	Pashia	Japan
Pyrus elaeagrifolia Pall.	31	Pyrus	Turkey, Ukraine, Southeastern Europe
Pyrus fauriei C. K. Schneid.	34	Pashia	South Korea
Pyrus gharbiana Trab.	8	Pyrus	Algeria, Morocco
Pyrus glabra Boiss.	1	Pyrus	Iran
Pyrus hondoensis Nakai and Kikuchi	41	Pashia	Japan
Pyrus × *hopeiensis* T. T. Yu	0	Pashia	China
Pyrus hybrid	216		
Pyrus koehnei C. K. Schneid.	16	Pashia	China
Pyrus korshinskyi Litv.	5	Pyrus	Kyrgyzstan, Tajikistan, Uzbekistan, Afghanistan
Pyrus × *lecontei* Rehder	0		
Pyrus mamorensis Trab.	15	Pyrus	Morocco
Pyrus × *michauxii* Bosc ex Poir.	0		
Pyrus × *neoserrulata* I. M. Turner	0		China
Pyrus × *nivalis* Jacq. (4)	20		Turkey, Europe
Pyrus pashia Buch.-Ham. ex D. Don	41	Pashia	China, Afghanistan, Iran, Indian subcontinent, Indo-China
Pyrus × *phaeocarpa* Rehder (5)	2		China
Pyrus pseudopashia T. T. Yu	2	Pashia	China
Pyrus pyrifolia (Burm. f.) Nakai	147 (b)	Pashia	China, Laos, Vietnam, Naturalized in Japan
Pyrus regelii Rehder	12	Pyrus	China, Kyrgyzstan, Tajikistan
Pyrus sachokiana Kuth.	2	Pyrus	Georgia

(continued)

Table 2.2 (continued)

Taxon	Genebank accessions	Pyrus section	Native origin
Pyrus salicifolia Pall.	36	Pyrus	Armenia, Azerbaijan, Iran, Turkey
Pyrus × *sinkiangensis* T. T. Yu (6)	7		China
Pyrus spp. [accessions unidentified to species]	88		
Pyrus spinosa Forssk.	57	Pyrus	Turkey, Southeastern Europe, France, Spain
Pyrus syriaca Boiss.	14	Pyrus	Armenia, Western Asia
Pyrus taiwanensis Iketani and H. Ohashi	0	Pashia	Taiwan
Pyrus trilocularis D. K. Zang and P. C. Huang	0	Pashia	China
Pyrus turcomanica Maleev	0	Pyrus	Iran, Kyrgyzstan, Tajikistan, Turkmenistan
Pyrus ussuriensis Maxim.	94 (c)	Pashia	China, Japan, Russian Federation
Pyrus × *uyematsuana* Makino (7)	1		Japan, Korea
Pyrus xerophila T. T. Yu	2	Pashia	China

Number of USDA genebank accessions, taxonomic section, and countries of native origin (USDA-ARS 2018a)
(1) Subtaxa not included, except for *P. communis*
(2) *P.* × *bretschneideri* = cultivated Chinese white pear is a complex hybrid, predominantly of *P. pyrifolia*
(3) *P.* × *canescens* = *P.* × *nivalis* × *P. salicifolia*
(4) *P.* × *nivalis* = *P. communis* × *P. elaeagrifolia*
(5) *P.* × *phaeocarpa* probably = *P. betulifolia* × *P. ussuriensis*
(6) *P.* × *sinkiangensis* is a complex hybrid involving *P. communis*, *P. armeniacifolia*, and *P. pyrifolia*
(7) *P.* × *uyematsuana* probably = *P. dimorphophylla* × *P. ussuriensis*
(a) Includes 985 European pear cultivars
(b) Includes 89 Asian pear cultivars
(c) Includes 49 Asian pear cultivars

Fig. 2.1 Diversity of *Pyrus* germplasm

production. Breeders seek genetic traits to increase fruit quality, size, and productivity, as well as disease and insect resistance. Furthermore, precocity, appropriate flowering and fruiting seasons, and maintaining quality during storage are also important. Resistance to the insect pear psylla (*Cacopsylla pyricola* (Forster)) and to diseases fire blight (*Erwinia amylovora* (Burrill) Winslow et al.), Fabraea (Entomosporium) leaf spot (*Entomosporium mespili* (DC.) Sacc.), and pear scab (*Venturia pirina* Aderh.) is particularly important for improving pear production. Breeding for these traits has long been major objectives of the USDA pear breeding program at Kearneysville, West Virginia, as well as at other pear improvement programs worldwide (Brewer and Palmer 2011; *Pyrus* CGC 2004).

2.1.2 Pears for Drink

Fermented pear cider, perry, is rapidly increasing in popularity in the USA and abroad. Hard cider was popular during colonial times in the USA, and much earlier in Europe. In recent years, there has been a major revival in locally crafted beer, cider, and perry. Many new accessions of perry pear cultivars have been introduced into the USA from Europe in recent years, especially from England, to meet this increased demand. Genetic traits required for perry pears are somewhat different from those traits selected for fruit pear consumption. Cultivars of both groups should have high fruit production, but, like a good wine grape, the fruit of a perry pear must contain high levels of acids and tannins, combined with good flavor that is retained throughout the fermentation process. In contrast, fruit with high tannin content is deemed undesirable for fresh consumption. Fruits produced for the fresh market must also be attractive; whereas perry pears are pulverized and pressed for their juice, thus fruit appearance is not critical. Most perry pears are selections from the species *P.* × *nivalis* Jacq., although many other pear wild relatives have fruit with high tannins along with a range of interesting flavors that have not yet been tapped for perry production. The presence of hard stone cells in fruits of many pear species limits their use in breeding fruit for eating, but has no impact on fermented juice products.

2.1.3 Pears for Ornament

A third use of *Pyrus* germplasm is as ornamental trees. While fruit of pear species grown for food must be large and flavorful, species with small, obscure, and unpalatable fruit are valued in the urban landscape. Numerous cultivars and selections of the Callery pear (*P. calleryana* Decne.) have been introduced to the nursery trade as flowering street trees, many originating from germplasm collected in China by USDA plant explorer Frank Meyer at the start of the twentieth century (Meyer 1922). Although cultivars of *P. calleryana* with profuse early spring displays of white flowers and stunning fall colors are some of the most widely planted flowering trees in North America, in recent years, profuse reseeding of these cultivars has rendered them undesirable in some locations (Culley 2017).

Selections of the willow-leaf pear (*P. salicifolia* Pall.) are appreciated in the landscape for their fine texture, gray, pubescent foliage, and sometimes weeping growth habit (Dirr 1997). Other pear species, including *P. betulifolia* Bunge, *P. dimorphophylla* Makino, *P. elaeagrifolia* Pall., *P. regelii* Rehder, and *P. syriaca* Boiss., have striking foliage, unusual flowers, or unique environmental adaptations. These species should be evaluated for landscape use. Although wood of various *Pyrus* species is used as material for furniture, musical instruments, and kitchen implements, there have been no deliberate efforts to select varieties for genetic traits desirable for these purposes.

2.1.4 Pears for Rootstocks

In the USA, commercial pear production has declined in recent decades. Between 2011 and 2016, pear production dropped from 875,000 to 739,000 metric tons in the USA (FAO 2018;

Table 2.1). The US pear industry attributes lower production to declining consumption, higher production cost relative to other tree fruits, and competition from imported fruit. An important factor in higher production costs is lack of rootstock options (Elkins et al. 2012).

As with most fruit trees, pear cultivars are vegetatively propagated by grafting. The above ground portion of a tree (fruiting cultivar) produces fruit, while the below ground portion (rootstock) anchors the tree and takes up water and nutrients. Often, rootstocks have very different genetic traits than fruiting cultivars. Except in cases of a few naturally compact genotypes, the overall size of a mature grafted pear tree is highly influenced by the rootstock. Unlike apples, which have many choices of size-controlling rootstocks, ranging from very dwarf to very vigorous, pears have limited rootstock options. Currently, seedlings of *P. communis* and occasionally *P. betulifolia* are the most common pear rootstocks used in the USA. Moreover, seedlings of *P. calleryana* are also used as rootstocks in warmer regions. Clonal rootstocks derived from crosses between fire blight-resistant pear cultivars Old Home (OH) and Farmingdale (F) are becoming popular in the USA, with selections OH × F 87 and OH × F 97 being the most widely used. 'Pyrodwarf' and 'Pyro 2-33' from Germany, also derived from crosses with 'Old Home,' are available in the USA as well. These two selections are more dwarfing than OH × F selections, but may have other shortcomings that will limit their use.

Most pear production areas in Western Europe depend on high-density plantings, thereby requiring dwarfing rootstocks, as various OH F rootstock clones are too vigorous. Quince (*Cydonia oblonga*) rootstock cultivars are the only options for adequate vigor control in this case. However, some pear cultivars are incompatible when grafted directly onto quince; therefore, a compatible interstem is required. Research is ongoing in several European countries to develop better, productive, and dwarfing pear rootstocks from *Pyrus* species; however, except for 'Pyrodwarf,' none are in wide use. Currently, rootstocks in production in Europe include quince clones BA29, East Malling A (EMA), EMC, EMH, and Sydo (Elkins et al 2012; Wertheim 2002). Unfortunately, there is lack of suitable dwarfing rootstocks available in Asian pear production areas. Seedlings of *P. betulifolia*, *P. ussuriensis,* and sometimes *P. pyrifolia* are used as rootstocks for Asian pears in northern China. Seedlings of *P. calleryana* and *P. pyrifolia* are the primary rootstocks in southern China. The use of these rootstocks for high-density plantings results in excessive vigor and contributes to high maintenance costs and poor yield (Teng 2011). In Japan, where seedlings of *P. betulifolia* and *P. calleryana* are the primary rootstocks, vigor control of Asian pears is also a challenge. Research is underway in Japan to develop rootstocks that combine dwarfing, ease of propagation, and adaptation to local environmental conditions, but none are yet available for commercial use (Tamura 2012).

One of the greatest needs of the US pear industry is a greater diversity of stress-resistant rootstocks that will promote dwarfing, precocity, and productivity of fruiting cultivars (Elkins et al. 2012). Every pear species is potentially graft compatible with every other pear species, and some originate from regions with very diverse climates, soils, and biotic or abiotic stresses. The wide range of adaptation to various soil types, temperature, moisture, pH, and nutrients as well as to soil-born insects, nematodes, and diseases of *Pyrus* species suggests that there are many unexplored opportunities to identify improved pear rootstocks (Lombard and Westwood 1987).

It is necessary to preserve pear genetic resources not only for their potential to develop improved cultivars for fresh fruit and perry production, but also for unique uses in the landscape and for improved rootstocks.

2.2 Pear Germplasm Conservation

Advances in basic taxonomy research, breeding new cultivars, developing genetic markers, and identifying major genes in pear cannot be accomplished without access to diverse living collections of *Pyrus* germplasm. Accessing germplasm for breeding or tissue for genetic

analysis directly from wild populations or dispersed production areas is very expensive and time-consuming. Fortunately, ex situ germplasm collections are available to provide ready access to needed genetic diversity with 'one-stop-shopping' convenience. In North America, a large pear collection is maintained in the USA by the USDA Agricultural Research Service (ARS) as part of the National Plant Germplasm System (NPGS) and represents worldwide *Pyrus* diversity (Postman et al. 2006). The Canadian Clonal Genebank in Harrow, Ontario, maintains about 100 pear accessions of interest to that country (AAFC 2018).

Large pear germplasm collections in Western Europe are located at the National Fruit Collection at Brogdale Farm in Kent, England; Centre Wallon de Recherches Agronomiques in Gembloux, Belgium; Le Centre INRA Angers-Nantes in France; Federal Research Centre for Cultivated Plants in Dresden-Pillnitz, Germany; and University of Bologna in Bologna, Italy (Morgan 2015).

In the Czech Republic, *Pyrus* genetic resources are maintained at the Research Breeding Institute of Pomology, Holovousy; in Greece at the NAGREF Pomology Institute, Naoussa; in Hungary at the Research and Extension Centre for Fruit Growing, Újfehértó; in Denmark at the Royal Veterinary and Agricultural University, Copenhagen; in Finland at Agrifood Research; in Norway at Planteforsk-Njos; in Sweden at SLU Balsgård; in Poland at the Research Institute of Pomology, Skierniewice; in Portugal at Chaves; in Slovenia at Ljubljana; in Spain at Servicio de Investigación Agroalimentaria, Saragossa; in Yugoslavia at the Center for Fruit Growing, Čačak and Faculty of Agriculture, Belgrade.

In Asia, *Pyrus* genetic resources are maintained at the Zhengzhou Fruit Research Institute in Henan Province, China, and NARO Institute of Fruit Tree Science in Tsukuba, Japan (Morgan 2015). In South Korea, the National Institute of Horticultural Science in Naju maintains large collections of mostly Asian pears (NIHHS 2016). In the Russian Federation, there are important *Pyrus* collections maintained at the Vavilov Research Institutes in St. Petersburg and Maikop, along with smaller collections of local pear varieties maintained at Vladivostok, Volgograd, and Pavlovsk (Maggioni et al 2004). Many non-government organizations throughout the world also maintain significant pear germplasm collections.

2.2.1 USDA-NPGS 'Clonal' Repositories

Prior to 1980, fruit and nut germplasm collections in the USA were largely assembled and maintained by individual plant breeders at universities or research institutes and were often lost when a faculty member or a scientist retired, changed their research focus, or encountered funding shortfalls. In the 1970s, a national plan was proposed to establish a series of US germplasm repositories with perpetual federal funding to provide security and stability for collections of horticultural crops (Brooks and Barton 1977) which would augment the existing germplasm collections maintained primarily as seeds. These collections of fruit and nut crops have been traditionally maintained as 'clonal' collections, as cultivars are propagated by clonal techniques, such as grafting, runners, or cuttings, and maintained as living trees, not as seed. The 'clonal' genebanks often maintain collections of seeds too, representing populations of wild relative species. The first of what was to become a network of eight National Clonal Repositories was established in Corvallis, Oregon, in 1980 to house collections of 26 genera of specialty fruit and nut crops, including *Pyrus* (Jahn and Westwood 1982; Postman et al. 2006; Westwood 1982).

The National Clonal Germplasm Repository (NCGR) in Corvallis is part of the National Plant Germplasm System (NPGS). The mission of the NPGS is to support agriculture by collecting, conserving, characterizing, documenting, and distributing crop plant germplasm (USDA-ARS 2018b).

When the NCGR was first established in the 1980s, several large pear germplasm collections from around the USA were consolidated at the Oregon site (Postman et al. 2010). Collections of

Pyrus species assembled in support of pear rootstock research, collections of heirloom pear cultivars, along with sources of fire blight resistance from the USDA pear breeding program served as the foundations of this collection (Westwood 1982).

2.2.2 NPGS *Pyrus* Collection

The most genetically diverse collection of world pear germplasm is very likely the NPGS pear collection at the NCGR in Corvallis (Postman 2008). This location has an ideal climate for a living pear genebank with mild winters and dry summers resulting in low incidence of diseases, including fire blight. The NCGR maintains approximately 2200 clonal accessions of pear, as well as 400 seedlots representing 36 *Pyrus* taxa (Table 2.2) originating from 55 countries (Table 2.3). Pear wild relatives are more efficiently and economically maintained either as seed or as small populations of seedlings. About 20% of the clonal collection is backed-up onsite,

Table 2.3 USDA *Pyrus* germplasm accessions by country of origin (USDA-ARS 2018c)

Country	Count	Country	Count
Afghanistan	2	Montenegro	3
Albania	32	Morocco	22
Armenia	45	Nepal	15
Australia	21	Netherlands	9
Azerbaijan	2	New Zealand	2
Belgium	51	Pakistan	49
Bulgaria	9	Poland	27
Canada	41	Portugal	4
China	127	Romania	31
Czech Republic	15	Russian Federation	70
Denmark	4	Serbia	19
Estonia	2	South Africa	9
France	180	Spain	2
Georgia	36	Sweden	7
Germany	20	Switzerland	5
Greece	2	Syria	4
Hungary	8	Taiwan	4
India	32	Tajikistan	1
Iran	3	Thailand	1
Israel	7	Tunisia	11
Italy	61	Turkey	44
Japan	85	Turkmenistan	15
Kazakhstan	15	Ukraine	10
Korea, South	37	UK	78
Kyrgyzstan	4	USA	786
Macedonia	38	Uzbekistan	16
Mexico	1	Vietnam	1
Moldova	3		

either as in vitro shoot cultures or as small potted greenhouse trees. Accessions at higher risk of loss due to either lack of cold hardiness or susceptibility to disease are prioritized for backup. Field collections are grown on 10 hectares of orchard plots with a single tree per accession. Cultivars are grafted onto a standard clonal rootstock, and wild species are grown from seeds on their own roots. The NCGR orchards include 850 wild relative species trees and 1350 cultivars. This collection consists of representatives of over 1000 European cultivars, 185 Asian cultivars, and 125 hybrid cultivars. Fruiting cultivars or selections with desirable traits represent a unique arrangement of genes and must be managed as living trees to preserve unique named genotypes.

Nearly all of the primary species of *Pyrus* are represented in the NCGR collection, with much larger numbers of accessions representing species from which large-fruited European and Asian cultivars have developed from (Table 2.2; Fig. 2.1). Exchanges of plant materials with foreign genebanks along with USDA supported expeditions to collect pear wild relatives near centers of wild diversity around the Caucasus Mountains and in Asia have filled taxonomic and geographic gaps in this collection and have expanded the overall size of this holding (Postman et al. 2012). The wild germplasm is maintained as seed, but sometimes, it is supplemented by a small population of seedlings. As limited field space, staff, and budget resources restrict the number of seedlings that can be established long-term as living trees, a seedlot is often represented by three to five seedlings grown in the orchard. A larger number of seedlings may be grown for rare taxa, to represent germplasm likely possessing valuable genetic traits, or for taxa from an under-represented region.

Taxonomic gaps in the NCGR collection include species native to North Africa (*P. gharbiana* and *P. mamorensis*) and species native to central and western Asia (*P. armeniacifolia*, *P. korshinskyi*, *P. syriaca*, and *P. xerophila*) as indicated by the accession counts in Table 2.2. There are also geographic gaps in the collection for species that may be represented elsewhere from their native range. For example, plant materials from Greece, the Balkan region, several countries in the Middle East, Central and Southeast Asia are under-represented (Table 2.3).

2.2.3 Documentation

Genebank accessions are only as valuable as the information associated with them. Passport or provenance data detailing a wild collection site can be associated with climate (e.g., high rainfall and extreme temperatures) or soil data (e.g., tolerance to low pH soils) and suggest adaptive traits that these plants may possess. Field observation data collected from permanent living collections provide information on important phenotypic traits such as flower and fruit phenology, resistance to locally prevalent diseases or insects, or morphologic traits that have agronomic value. All germplasm housed at NPGS genebanks is documented in a public database, the Germplasm Resources Information Network or GRIN (Postman et al. 2010; USDA-ARS 2015). To search GRIN, please visit https://npgsweb.ars-grin.gov/gringlobal/search.aspx?

2.2.4 Distribution

Propagation materials and tissues for germplasm characterization are freely available for research and education purposes from NPGS genebanks. Each year, NCGR fills hundreds of orders for pear germplasm, averaging about ten accessions per order. Between 2010 and 2016, approximately 1500 pear accessions have been distributed annually (USDA-NCGR 2017). Of all distributed materials between the years 1980 through 2018, 25 of the most requested pear accessions are listed in Table 2.4. Named cultivars of *P. communis* tend to be the most requested, with perry (cider) pears being especially popular, a good indication of the importance of the rapidly expanding craft cider market. Red flesh pears, such as 'Summer Blood Birne,' have also been in high demand, thereby demonstrating an interest in developing pears with this unique trait.

Table 2.4 Top 25 most requested USDA *Pyrus* accessions from 1980 to 2018; rank and number of samples shipped (USDA-ARS 2018c)

Cultivar	Accession	Taxon	Rank (shipped)
Seckel	PI 541262	*P. communis*	1 (202)
Yellow Huffcap	PI 541287	*P. communis*	2 (165)
Red pear	PI 541317	*P. communis*	3 (160)
Bartlett	PI 300693	*P. communis*	4 (159)
Thorn	PI 541273	*P. communis*	5 (156)
Taynton Squash	PI 541271	*P. communis*	6 (155)
Barland	PI 541123	*P. communis*	7 (154)
Gin	PI 541195	*P. communis*	8 (146)
Butt	PI 541156	*P. communis*	9 (142)
Summer Blood Birne	PI 312507	*P. communis*	10 (141)
Beurre Superfin	PI 541150	*P. communis*	11 (136)
Blakeney Red	PI 541151	*P. communis*	12 (131)
Joey's Red Flesh Pear	PI 617584	*P. communis*	13 (130)
Hendre Huffcap	PI 541205	*P. communis*	14 (128)
Beurre Bosc	PI 541387	*P. communis*	15 (125)
Warren	PI 541448	*P. communis*	16 (123)
Winnals Longdon	PI 541486	*P. communis*	17 (123)
Ya Li	PI 506362	*P.* × *bretschneideri*	18 (121)
Aurora	PI 541119	*P. communis*	19 (121)
Rousselet de Reims	PI 541256	*P. communis*	20 (119)
Brandy	PI 541305	*P. communis*	21 (118)
Abbe Fetel	PI 260195	*P. communis*	22 (116)
Harrow Delight	PI 541431	*P. communis*	23 (112)
Magness	PI 541299	*P. communis*	24 (111)
Doyenne du Comice	PI 271658	*P. communis*	25 (110)

2.2.5 Clonal Genebank Challenges

Since a clonal genebank accession may be represented by a single tree without replication, some observation data may be difficult to interpret. Data collected over multiple years can sometimes provide a measure of confidence in these observations. Accessions may originate from a distant country or a climate much different from that present at the genebank repository. It can be a challenge to maintain living trees of low-chill and non-hardy genotypes, or trees that are very susceptible to local diseases. Subtropical species such as *P. koehnei* or *P. pashia* may require additional protection against winter weather conditions or necessitate maintaining a backup tree in a greenhouse for security. It is also critical, yet expensive, to ensure that collections are backed-up and secured, so that they are not lost in the event of physical, environmental, or biological disasters.

2.3 Genetic Tools for Genebank Management

Confirmation of fruiting cultivar identities in genebank collections requires detailed comparisons of tree and fruit characteristics to published descriptions, old nursery catalogs, photographs,

and other artwork. 'The Pears of New York' volume, published by the New York State Agricultural Research Station (Hedrick 1921), is one of the most important references for pear identification in the USA. This publication has 80 full-page lithographs and multi-page descriptions of the most promising pear cultivars of the early 1900s, along with thousands of brief descriptions of less important and obscure cultivars. A more recent book details over 500 cultivars and includes more modern cultivars (Morgan 2015). Many other domestic and foreign pomology references also document fruit cultivars of different periods.

Prior to the widespread use of color photography, USDA has employed professional artists to paint detailed, actual-size watercolor paintings of fruit cultivars entering the country, or growing domestically. From 1886 to 1942, thousands of small watercolor paintings, lithographs, and line drawings have been produced, and 7500 are preserved at the USDA National Agriculture Library (NAL) in Maryland. Many are available online, including almost 300 pear images (Fig. 2.2; USDA-NAL 2018a). Collections of historic nursery catalogs are also maintained at NAL (USDA-NAL 2018b) and elsewhere. Conventional references including books, paintings, catalogs, as well as other living collections are needed to verify identities of pear cultivars before they can be used as standards for molecular identification protocols.

Genetic fingerprinting techniques facilitate confirmation of collection materials with those from other, often distant, sources. Genetic signatures are consistent across locations even though phenotypes may vary across growth environments. Identities of trees representing crop wild relatives must likewise be properly identified to their correct species upon receipt into a collection.

2.3.1 Intentional and Unintentional Redundancy

Intentional redundancy, or maintaining duplicate trees, is an important management strategy for insuring security of germplasm collections through onsite backups. Likewise, maintaining identical accessions at different genebanks or genebank locations also contributes to germplasm security.

The use of SSR markers has become a standard tool for DNA fingerprinting to confirm genetic identities of trees and whether or not any two presumed duplicate trees are indeed a match. A comparison of 61 pear accessions received from the Brogdale National Fruit Collection in the UK to accessions of the same name at the NPGS pear collection has demonstrated that 44 accessions have identical allele sizes at 12 SSR loci (Evans et al. 2015); whereas, 12 accessions have distinctly different SSR profiles at six or more loci. Therefore, phenotypic observations or additional SSR comparisons are required to determine which of these accessions are true to type (Evans et al. 2015). For example, the Japanese cultivar Hosui in the Brogdale collection is not a match to a 'Hosui' accession found in the USDA collection. Following phenotypic comparisons and verification, it has been determined that the Brogdale tree has been incorrectly named. In another example, the cultivar Arabitka has exhibited different SSR profiles in these two collections. Following comparisons with a large set of SSR markers, the tree at NCGR is found to be a mislabeled 'Vicar of Winkfield.' Similar efforts with apple accessions obtained from different European collections have revealed that incorrect labels and propagation errors are more common than collection curators would like to see (Evans et al. 2011).

In other instances, a misidentified accession may arise when a graft union fails and the rootstock grows over, or it is inadvertently planted to represent another genotype. The NCGR pear collection is grafted onto a standard clonal rootstock, 'OH × F 333,' and the SSR fingerprint of this rootstock has, on occasion, been detected from a tree that should represent a different genotype. Valuable information confirming synonymy of accessions having different common names can also be gleaned from the use of SSR markers. For example, the following cultivars have been found to be synonyms: 'Bella di

Fig. 2.2 An 'Onondaga' pear fruit harvested by G.W. Soudder in Rowayton, Connecticut, on September 20, 1913, and painted by USDA artist Amanda A. Newton on October 7, 1913

Giugno' = 'Mirandino Rosso,' 'Forelle' = 'Helmershus Roda,' 'Jubileer D'ar' = 'Pautalia,' and 'Flemish Beauty' = 'Lesnaia Krasavitza' (Bassil and Postman 2009). Confirmation of synonymy can help a curator justify removal of a redundant accession, thus freeing up space for another unique accession. When pear genebank collections have been fully genotyped and cultivar identities validated, a database can be established to serve as a resource for identifying trees of historic significance or with unknown identities.

2.3.2 Assess Diversity and Identify Collection Gaps

Analysis of amplified fragment length polymorphisms (AFLPs) has provided useful information about genetic relationships between different groups of pear cultivars and species (Bao et al. 2008). AFLP results have been validated and refined by more recent genetic analyses using SSRs, chlorophyll and genomic sequences, single-nucleotide polymorphisms (SNPs), and other novel techniques (Jiang et al. 2016; Kumar et al. 2017; Volk et al. 2006, 2019; Wuyun et al. 2015). The use of these tools to investigate species relationships and the history of *Pyrus* domestication is reviewed in greater detail in Chap. 4 of this volume.

The development of molecular tools for diversity analysis cannot be accomplished without access to diverse living collections of *Pyrus* germplasm correctly identified to a species or a cultivar. A common and sometimes unanticipated outcome of applying genetic analysis to diversity assessment is to sort out those genotypes that do not group with other samples of the same species. Following closer examination of the phenotypic profile of a tree that is an outlier on a genotypic dendrogram, an accession will often be deemed either as misidentified or as a hybrid species (Volk et al. 2006). These types of assessments are particularly important for genebank collections as scientists rely on these collections to provide true-to-type germplasm for use in their research and breeding programs.

2.3.3 Identify or Confirm Pedigrees

Some pear cultivars are the result of deliberate crosses, and others were chance seedlings of unknown parentage identified as desirable trees. In the case of OH × F pear rootstocks, two fire blight-resistant cultivars have been used as parents in an effort to develop easy-to-propagate, blight-resistant, clonal pear rootstocks. Seeds have been collected in 1952 from an isolated 'Old Home' tree planted next to several 'Farmingdale' pollenizers in British Columbia (Canada) and grown out at an Oregon nursery. Over the next few decades, hundreds of OH × F seedlings have been evaluated for ease of rooting, dwarfing potential, and resistance to important pear diseases including fire blight. A dozen or so selections have been introduced to the nursery trade, and more than 40 numbered OH F selections are deposited at the USDA pear genebank for preservation. Some of these clonal rootstocks have become widely used in propagating fruiting cultivars by commercial nurseries and grown worldwide for pear fruit production.

In a recent study, cultivars 'Old Home' and 'Farmingdale,' along with six OH × F clonal selections were included in an SSR fingerprinting assessment. 'Old Home' was found to share an allele with all of the OH × F selections at all 12 loci, but there was no alignment between 'Farmingdale' and any of the OH × F selections at several loci. Pedigree analysis showed that 'Bartlett' was actually the pollen parent for all six OH × F selections evaluated (Fig. 2.3; Postman et al. 2013). Thus, it was proposed that 'Farmingdale' was not the pollen parent for any of the OH × F rootstocks.

It is not uncommon for marker analysis to reveal anomalies in published cultivar pedigrees; however, in the case of OH × F rootstocks, new generations of rootstock candidates have been developed using OH × F selections as parents, with the intention of obtaining fire blight resistance from 'Farmingdale.' Resistance is highly heritable when 'Farmingdale' is used as either a male or a female parent (Reimer 1950); however, 'Bartlett' is not considered to be a good source of fire blight resistance. The case of OH × F highlights the importance to breeders of having accurate genetic identity and paternity information.

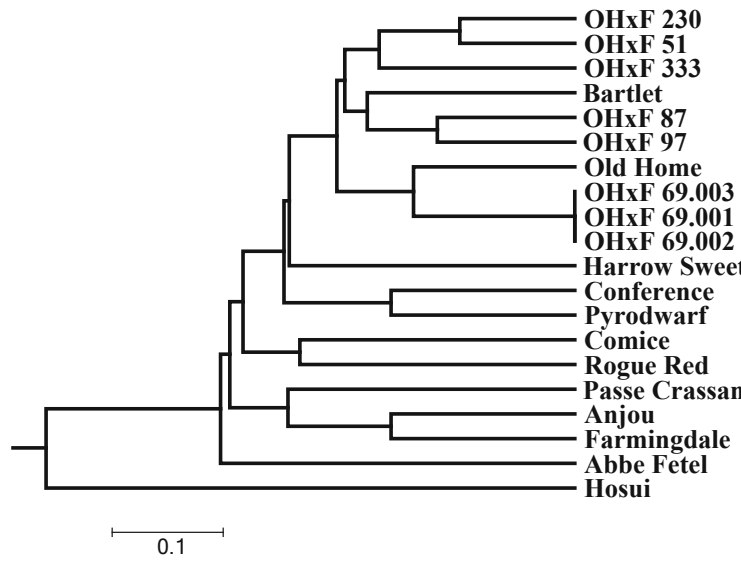

Fig. 2.3 Unweighted pair group method with arithmetic mean (UPGMA) cluster analysis of 20 pear accessions based on 12 simple sequence repeat (SSR) loci (Fig. 4 from Postman et al. 2013)

2.4 Conclusions

Pear collections provide a diverse source of species and cultivars essential to the success of research and breeding programs. Access to such materials is necessary to develop improved cultivars for fresh fruit production, for perry, and as novel ornamental trees. Pear rootstock breeding programs will particularly benefit from access to a wide diversity of *Pyrus* species that may not be desirable for their fruit, but are useful genetic sources for disease resistance and abiotic stress tolerance. Phenotypic observations and genetic tools aid in genebank management to assure that materials are true to type. Genetic markers yet to be identified will allow for rapid detection of genes for valuable traits. Access to correctly identified and diverse living collections of *Pyrus* germplasm will assure that advances, such as those reported in this volume, will continue to be made in breeding and genetic research efforts.

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References

AAFC (2018) Plant gene resources of Canada. Agriculture and Agri-Food Canada (AAFC). http://pgrc3.agr.gc.ca/nodes-noeuds_e.html, 8 Aug 2018

Bao L, Chen K, Zhang D, Li X, Teng Y (2008) An assessment of genetic variability and relationships within Asian pears based on AFLP markers. Scientia Hort 116:374–380

Bassil NV, Postman JD (2009) Identification of European and Asian pears using EST-SSRs from *Pyrus*. Genet Resour Crop Evol 57:357–370

Brewer LR, Palmer JW (2011) Global pear breeding programmes: Goals, trends and progress for new cultivars and new rootstocks. Acta Hortic 909:105–120

Brooks HJ, Barton DW (1977) A plan for national fruit and nut germplasm repositories. HortScience 12:298–300

Culley TM (2017) The rise and fall of the ornamental callery pear tree. Arnoldia 74:1–11

Dirr MA (1997) Dirr's hardy trees and shrubs. Timber Press, Portland, OR

Elkins R, Bell R, Einhorn T (2012) Needs assessment for future US pear rootstock research directions based on the current state of pear production and rootstock research. J Am Pom Soc 66:153–163

Evans KM, Patocchi A, Rezzonico R, Mathis F, Durel C-E, Fernández-Fernández F, Boudichevskaia A, Dunemann F, Stankiewicz-Kosyl M, Gianfranceschi L, Komjanc M, Lateur M, Madduri M, Noordijk Y, van

de Weg WE (2011) Genotyping of pedigreed apple breeding material with a genome-covering set of SSRs: trueness-to-type of cultivars and their parentages. Mol Breed 28:535–547

Evans KM, Fernández-Fernández F, Bassil N, Nyberg A, Postman J (2015) Comparison of accessions from the UK and US national pear germplasm collections with a standardized set of microsatellite markers. Acta Hortic 1094:41–46

FAO (2018) World pear production. Food and Agriculture Organization of the United Nations Crop Statistics. http://www.fao.org/faostat/en/#data, 2 Aug 2018

Hedrick UP (1921) The pears of New York. New York Agricultural Experiment Station. J.B. Lyon Company, Lyon, France

Jahn OL, Westwood MN (1982) Maintenance of clonal plant germplasm. HortScience 17(2):122

Jiang S, Zheng X, Yu P, Yue X, Ahmed M, Cai D, Teng Y (2016) Primitive genepools of Asian pears and their complex hybrid origins inferred from fluorescent sequence-specific amplification polymorphism (SSAP) markers based on LTR retrotransposons. PLoSOne https://doi.org/10.1371/journal.pone.0149192

Kumar S, Kirk C, Wiedow C, Knaebel M, Brewer L (2017) Genotyping-by-sequencing of pear (*Pyrus* spp.) accessions unravels novel patterns of genetic diversity and selection footprints. Hort Res 4:17015

Lombard PB, Westwood MN (1987) Pear rootstocks. In: Rom RC, Carlson RF (eds) Rootstocks for fruit crops. Wiley, New York, pp 145–183

Maggioni L, Fischer M, Lateur M, Lamont EJ, Lipman E (2004) Report of a Working Group on Malus/Pyrus. Second Meeting, 2–4 May 2002, Dresden-Pillnitz, Germany. IPGRI Rome, Italy

Morgan J (2015) The book of pears—the definitive history and guide to over 500 varieties. Chelsea Green Publishing, Vermont

Meyer FN (1922) Agricultural explorations in the fruit and nut orchards of China. USDA Bureau of Plant Industry Bulletin No. 204

NIHHS (2016) National Institute of Horticultural and Herbal Science Locations. http://www.nihhs.go.kr/eng/about/nihhsLocation.do, 8 Aug 2016

Postman J, Hummer K, Stover E, Krueger R, Forsline P, Grauke LJ, Zee F, Ayala-Silva T, Irish B (2006) Fruit and nut genebanks in the US national plant germplasm system. HortScience 41(5):1188–1194

Postman J (2008) World *Pyrus* collection at USDA genebank in Corvallis, Oregon. Acta Hortic 800:527–533

Postman J, Hummer K, Bretting P, Kinard G, Bohning M, Emberland G, Sinnot Q, Weaver B, Ayala-Silva T, Franco T, Mackay M, Guarino L (2010) GRIN-Global: an international project to develop a global plant genebank information management system. Acta Hortic 859:49–55

Postman JD, Aradhya MK, Williams KA, Stover E, Meyer PW (2012) Recent NPGS coordinated expeditions in the Trans-Caucasus region to collect wild relatives of temperate fruit and nut crops. Acta Hortic 948:191–198

Postman JD, Kim D, Bassil N (2013) OH × F paternity perplexes pear producers. J Am Pom Soc 67(3):157–167

Pyrus CGC (2004) Report and genetic vulnerability statement. https://www.ars-grin.gov/npgs/cgc_reports/PyrusCGCReport2004.pdf, 11 March 2018

Reimer FC (1950) Development of blight resistant pear stocks. Oregon Agricultural Exp. Sta. Bulletin 485

Tamara F (2012) Recent advances in research on Japanese pear rootstocks. J Jpn Soc Hortic Sci 81:1–10

Teng Y (2011) The pear industry in China. Chronica Hortic 51:23–27

Teng Y, Yue X, Zheng X, Cai D (2015) Genetic clue to the origin of cultivated Asian pears inferred from cpDNA haplotypes. Acta Hortic 1094:31–39

USDA-ARS (2015) Germplasm resources information network. https://www.ars-grin.gov/, 7 Jan 2018

USDA-ARS (2018a) US National Plant Germplasm System GRIN Taxonomy. https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomyquery.aspx, 19 Mar 2018

USDA-ARS (2018b) The National Plant Germplasm system. https://www.ars-grin.gov/npgs/index.html, 7 Jan 2018

USDA-ARS (2018c) GRIN germplasm collection statistics. https://npgsweb.ars-grin.gov/gringlobal/query/query.aspx, 2 Aug 2018

USDA-NCGR (2017) Annual reports for the National Clonal Germplasm Repository—Corvallis. https://www.ars.usda.gov/pacific-west-area/corvallis-or/national-clonal-germplasm-repository/docs/ncgr-corvallis-annual-reports/, 19 Mar 2018

USDA-NAL (2018a) US Department of Agriculture Pomological Watercolor Collection. https://usdawatercolors.nal.usda.gov/pom/home.xhtml, 10 Mar 2018

USDA-NAL (2018b) Henry G. Gilbert Nursery and Seed Trade Catalog Collection. https://archive.org/details/usda-nurseryandseedcatalog, 10 Mar 2018

Volk GM, Richards CM, Henk AD, Reilley AA, Bassil NV, Postman JD (2006) Diversity of wild *Pyrus communis* based on microsatellite analyses. J Am Soc Hort Sci 131:408–417

Volk GM, Henk AD, Richards CM, Bassil NV, Postman J (2019) Chloroplast sequence data differentiate Maleae, and specifically *Pyrus*, species in the USDA-ARS National Plant Germplasm System. Genet Resour Crop Evol 66(1):5–15

Wertheim SJ (2002) Rootstocks for European Pear: a review. Acta Hortic 596:299–309

Westwood MN (1982) Pear germplasm of the new National Clonal Repository: its evaluation and uses. Acta Hortic 124:57–66

Wuyun T, Amo H, Xu J, Ma T, Uematsu C, Katayama H (2015) Population structure of and conservation strategies for wild *Pyrus ussuriensis* Maxim. in China. PLOS ONE https://doi.org/10.1371/journal.pone.0133686

Genetic Diversity and Domestication History in *Pyrus*

3

Gayle M. Volk and Amandine Cornille

Abstract

The cultivated pear is a major fruit crop in Eurasia that underpins many local economies. However, its origin and domestication history, as well as the diversity of wild pears in natural ecosystems, are at the early stages of exploration. In this chapter, we provide an overview of the described diversity and genetic relationships among wild and cultivated *Pyrus* species. Non-discriminatory morphological characters, poor diagnostic genetic tools, and lack of access to samples scattered throughout worldwide genebank collections make it difficult to definitively elucidate relationships of pear species and more generally *Pyrus* diversification and domestication. High-throughput sequencing is providing advancements in our understanding of the domestication process of the pear, and of biogeography, taxonomy, and ecology of wild pears. This knowledge will be crucial for future breeding programs focused on improving quality and production traits.

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3.1 Introduction: Assessing *Pyrus* Diversity

Cultivated pears are produced throughout temperate regions on both a commercial scale and for local household use; however, their origin and domestication history are at the early stages of exploration. Over the past 4000 years, pear cultivation has led to the identification and/or development of a vast number of landraces and recent cultivars through natural and artificial hybridization. Vegetative propagation by grafting has allowed interesting and/or desirable phenotypes to be maintained and spread (Zohary and Spiegel-Roy 1975). As a result, cultivated pears exhibit a wide range of desirable traits, including fruit attractiveness, flavor, size, and shape. Numerous molecular studies, primarily based mostly on a few marker loci, have been used to characterize the diversity of pear cultivars and the origin of this diversity in wild species. However, the genetics underlying key agronomic traits are just beginning to be understood.

Assessments of pear species diversity and distribution are usually determined using regional inventory and census counts. These records are often not collected using standardized techniques and have gaps with respect to coverage. In addition, recurrent hybridizations and resulting introgressions among species have made it difficult to differentiate species. Consequently, it is difficult to identify the geographical range of wild *Pyrus* species.

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Wild relatives of cultivated pears offer novel allelic diversity and allelic combinations that can provide sources of resistance and tolerance to abiotic and biotic stresses for pear breeding programs. *Pyrus* wild species such as *P. communis* spp. *pyraster*, *P. calleryana*, *P. ussuriensis, P. pyrifolia, P. fauriei, P. dimorphoylla, P. betulifolia*, and *P.* × *nivalis* have desirable levels of disease resistance to various pathogens, including pear leaf spot (*Entomosporium mespili* (DC.) Sacc.), fire blight (*Erwinia amylovora* (Burr.) Winslow et al.), and pear psylla (*Cacopsylla pyricola* (Foerster)) (van der Zwet et al. 1983; Bell 1992; Bell and Itai 2011). These species can be used as parents in breeding programs, as providers of specific alleles for introgression, or as rootstocks. Many wild pear species, including *P. pashia*, *P. korshinskyi, P. syriaca, P.* × *hopiensis*, *P. gharbiana*, *P. betulifolia, P. calleryana, P. cossonii. P. dimorphophylla, P. fauriei, P. pyrifolia, P. ussuriensis*, and *P. xerophila,* are recognized for their desirable rootstock traits, providing tolerance to extreme heat, humidity, and cold, as well as disease resistance (Ercisli 2004; Bao et al. 2008; Zong et al. 2014b; U.S. Department of Agriculture 2017).

This chapter focuses on the measured diversity of wild *Pyrus* species and described relationships between wild species and cultivated forms. The life history traits of pears, with long lifespans and high levels of gene flow among populations and species, combined with their ancient origin, render *Pyrus* as a valuable model for studying fruit tree species diversification. Expanded knowledge of pear genetic diversity and evolution will also assist in pinpointing sources of allelic variation in the wild useful for future breeding programs. Such studies are particularly timely, as wild gene pools may be sources of alleles for resistance to biotic and abiotic stresses (van der Zwet et al. 1983; Bell 1992; Bell and Itai 2011), and these are currently under threat of fragmentation in their centers of origin.

3.2 Diversification of Wild Pears

The genus *Pyrus* is presumed to have originated during the Tertiary Period (65–55 million years ago [Mya]) (Silva et al. 2014), or in particular in the Oligocene Epoque, 33–25 Mya (Korotkova et al. 2018) in the mountainous regions of Western China or Asia Minor. Microsatellite or simple sequence repeat (SSR) markers, as well as genomic studies, have revealed strong genetic differentiation between two main genetic groups, an Occidental (European/Central Asian) and an Asian (East Asia), which diverged between 6.6 and 3.3 Mya (Fig. 3.1) (Liu et al. 2015; Volk et al. 2019; Wu et al. 2018). Two non-coding regions of the cpDNA and one low copy nuclear gene have also demonstrated the differentiation between wild Asian and Occidental pear groups (Zheng et al. 2014). Altogether, this suggests spatial dispersal events to eastern and northern Eurasia, whereby Asian wild species have diversified, and to western Eurasia, whereby Occidental wild species have diversified (Figs. 3.2 and 3.3).

The use of classical microsatellite genetic markers has shed light on the genetic diversity of some wild pear species. Nuclear microsatellite data demonstrated that the genetic variation of wild populations of *P. calleryana, P. communis* subsp. *pyraster*, *P. pashia*, and *P. ussuriensis* is higher within (ranging from 80 to 96%) than among populations (ranging from 4 to 20%) (Liu et al. 2012; Wolko et al. 2015; Zong et al. 2014a; Wuyun et al. 2015) (Table 3.1). This observed wide range across wild *Pyrus* species may be in part due to physical sampling methods used; e.g., distances between sites and familial relationships among individuals. The heterozygosity of these populations ranges from 0.48 for *P. ussuriensis* (Wuyun et al. 2015) to 0.76 for *P. communis* subsp. *caucasica* and *P. communis* subsp. *pyraster* (Table 3.2; Asanidze et al. 2014; Wolko et al. 2015). Hereafter, we review the literature on specific diversity and evolution of Asian (pea pear and large-fruited) and Occidental pears.

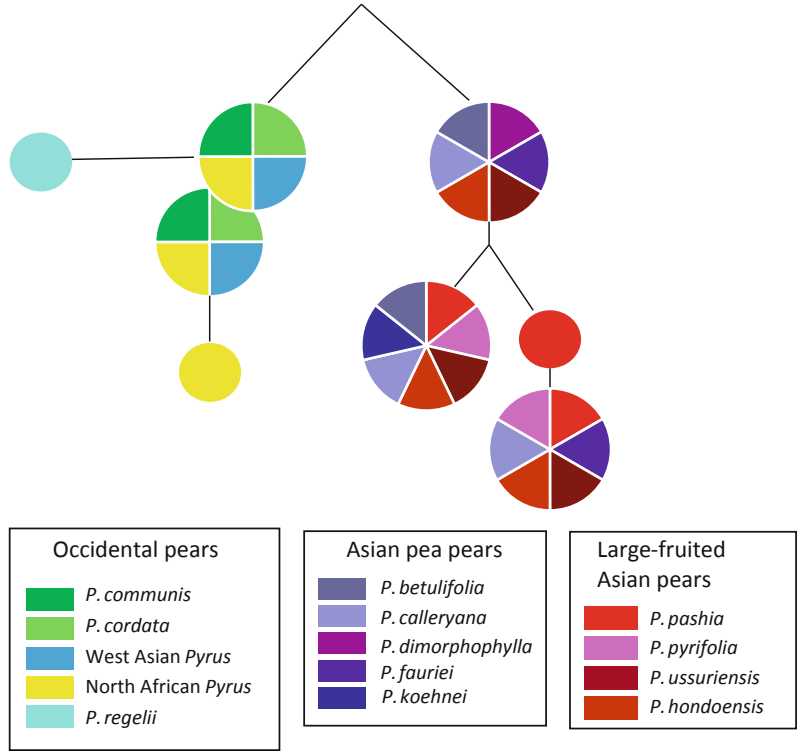

Fig. 3.1 Generalized diagram of network relationships and shared haplotypes of *Pyrus* species, from Volk et al. (2019). North African *Pyrus* species include *P. cossonii*, *P. gharbiana*, and *P. mamorensis,* while West Asian *Pyrus* species include *P. elaegrifolia, P. glabra P. korshinskyi, P. sachokiana, P. salicifolia, P. spinosa*, and *P. syriaca*

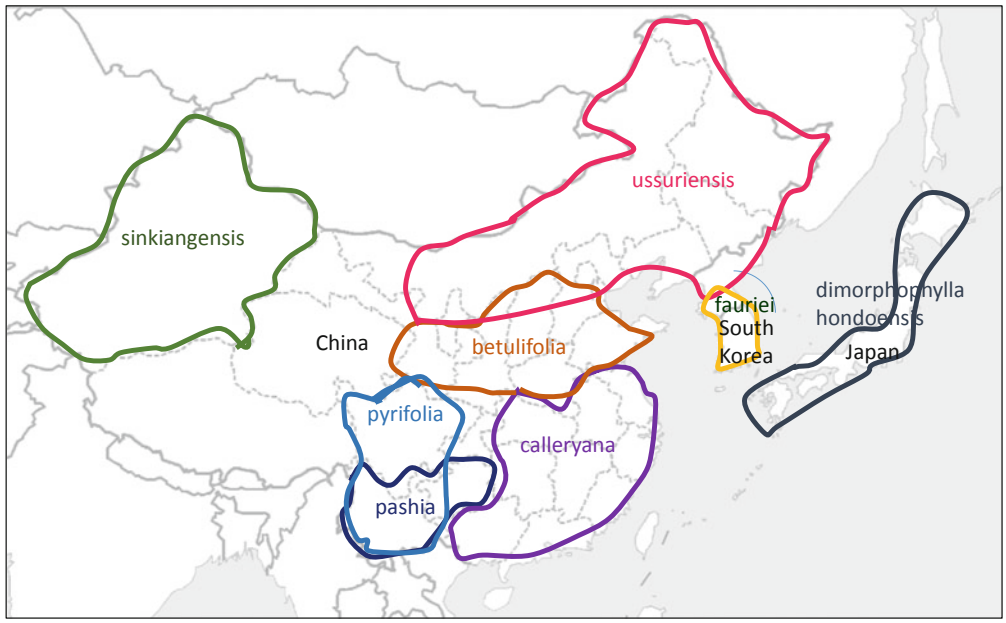

Fig. 3.2 General overview of the geographic distribution of native East Asian wild *Pyrus* species

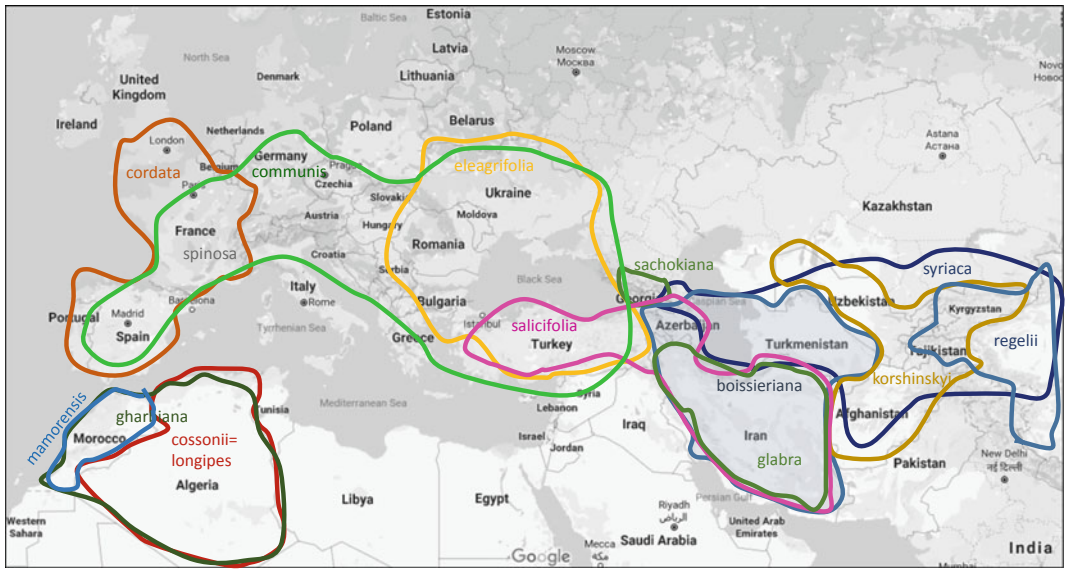

Fig. 3.3 General overview of the geographic distribution of native Occidental wild *Pyrus* species

Table 3.1 Microsatellite marker genetic diversities assessed within and among populations of *Pyrus* species

Taxon	Site location	Number of populations (no.)	Total number of individuals	Among population genetic variation (%)	Within-population genetic variation (%)	Citation
P. calleryana	Zhejiang Province, China	8	77	9	91	Liu et al. (2012)
P. communis ssp. *pyraster*	Poland	6	379	4	96	Wolko et al. (2015)
P. pashia	Yunnan Province, China	4	470	11	89	Zong et al. (2014a)
P. ussuriensis	Heilongjiang, Jilin, Inner Mongolia	13	153	20	80	Wuyun et al. (2015)
Malus sieversii	Kazakhstan	8	949	5	95	Richards et al. (2009)

3.2.1 Genetic Diversity of Asian Wild Pears

Asian wild pears are often described as belonging to either the "pea pear" or the "large-fruited pear" groups. Pea pears, including *P. betulifolia, P. calleryana, P. dimorphophylla, P. fauriei,* and *P. koehnei,* produce fruits that are less than 1 cm in diameter with two carpels (Jiang et al. 2016). In contrast, large-fruited Asian pear species include, among others, *P. pashia, P. pyrifolia, P. ussuriensis, P. xerophyla*, and *P. hondoensis* (Challice and Westwood 1973). It has been difficult to genetically differentiate between "pea" and "large-fruited" pears (Jiang et al. 2016; Zheng et al. 2014). Genetic diversity assessments

Table 3.2 Diversity assessments using microsatellite markers of wild populations of *Pyrus* and *Malus* species, including number of individuals sampled (*n*), number of SSRs used to assess diversity (SSRs), number of effective alleles per locus, expected heterozygosity (He), observed heterozygosity (Ho), and inbreeding coefficient (Fis)

Taxon	Source	*n*	SSRs (no. markers)	Effective alleles/locus (no.)	He	Ho	Fis	Citation
P. betulaefolia	Northern China (Gansu, Shaanxi, Henan, Hebei, Shandong)	326	13	4.11	0.70	0.69	0.009	Zong et al. (2017)
P. calleryana	Zhejiang Province, China	77	14	3.74	0.64	0.57	0.170	Liu et al. (2012)
P. communis ssp. *caucasica*	Georgia	112	11	17.00	0.76		0.135	Asandize et al. (2014)
P. communis ssp. *pyraster*	Poland	192	17	5.66	0.76	0.75	0.018	Wolko et al. (2015)
P. ussuriensis	Heilongjiang, Jilin, Inner Mongolia	12	20	2.44	0.48	0.34	0.220	Wuyun et al. (2015)
P. ussuriensis	China	12	20	2.63	0.56	0.39	0.233	Katayama et al. (2016)
P. ussuriensis	Japan	20	20	4.31	0.74	0.71	0.030	Katayama et al. (2016)
P. ussuriensis	Tibet	8	28	3.22	0.67	0.59	0.070	Xue et al. (2017)
Malus sieversii	Kazakhstan	949	7	14.70	0.75	0.69	0.052	Richards et al. (2009)

of Asian wild pears have focused primarily on differences/relatedness of either within species or between wild species and cultivated forms.

Molecular genetic markers have facilitated identification of basal species and hybrids in the Asian wild *Pyrus* group. Sequence-specific amplification polymorphism (SSAP) suggest that *P. betulifolia*, *P. pashia*, *P. pyrifolia*, and *P. ussuriensis* are primitive genepools of wild Asian species (Jiang et al. 2016). Other original wild Asian species include *P. koehnei* and *P. fauriei* (Zheng et al. 2014). *Pyrus* species of ambiguous identities or origins include *P. dimorphophylla* (sometimes classified as a variety of *P. calleryana*), *P. calleryana* (with leaf shape similar to *P. pashia* and fruit similar to *P. betulifolia*), and *P.* × *bretschneideri* (genetically similar to *P. ussuriensis*). Asian wild pear species resulting from hybridizations between wild pear species include *P. xerophila* (*P. pashia*, × *P. ussuriensis* × Occidental), *P. sinkiangensis* (*P. pyrifolia* × Occidental), *P. phaeocarpa* (*P. betulifolia* × *P. ussuriensis* × *P. pyrifolia*), *P. hondoensis* (*P. dimophophylla* × *P. ussuriensis*), *P. neoserrulata* and *P. serrulata* (*P. calleryana* × *P. pyrifolia*), and *P. hopeiensis* (*P. ussuriensis* × [*P.* × *phaeocarpa* or *P. betulifolia*]) (Jiang et al. 2016; U.S. Department of Agriculture 2017).

3.2.1.1 Genetic Diversity Within the Asian Pea Pear Species

The following *Pyrus* pea pear taxa, *P. betulifolia*, *P. calleryana*, *P. dimorphophylla*, *P. fauriei*, and *P. koehnei* are native to China, Japan, and the Korean peninsula (Fig. 3.2). *Pyrus betulifolia* is described as an ancient pear species that shares some traits with both Asian and Occidental pear types (Zong et al. 2014b, 2017). Diversity of this species, as measured using chloroplast intergenic fragments and microsatellite genetic markers (SSRs), has revealed that the Taihang Mountains are natural genetic barriers, and that range

expansion and contraction events must have occurred during and between glacial periods (Zong et al. 2014b, 2017). Furthermore, populations within *P. betulifolia* are more easily distinguishable using chloroplast markers rather than nuclear SSRs as pollen-mediated gene flow has likely homogenized genetic diversity at the nuclear level (Zong et al. 2017). Future work using additional markers, such as single nucleotide polymorphisms (SNPs), will provide more insights into the population structure of *P. betulifolia.*

On the other hand, *P. calleryana,* native to southern China, Japan, and the Korean Peninsula, is classified as a wild pea pear that shares some similarities with both *P. pashia* and *P. betulifolia* (Jiang et al. 2016). In Southern China, the range of native species of *P. calleryana, P. pashia,* and *P. betulifolia* is found to overlap (Liu et al. 2012; Jiang et al. 2016). Using both nuclear microsatellite and chloroplast sequence markers, two genepools are identified in eight populations of *P. calleryana* in the Zhejiang Province in China (Liu et al. 2012). These genepools correspond to two geographic regions, with one located in the northeast and the other located in the southwest.

3.2.1.2 Genetic Diversity Within the Large-Fruited Asian Pear Species

The wild large-fruited Asian pear species include *P. pashia, P. pyrifolia, P. ussuriensis, P. xerophyla*, and *P. hondoensis. Pyrus ussuriensis* is native to northeastern and north-central Chinese provinces, as well as to Japan (Fig. 3.2; Katayama et al. 2016). Each of chloroplast sequences, SSAPs, and SSRs has been used to assess genetic variations among and within *P. ussuriensis* populations throughout its native range. These genetic studies have revealed existence of a spatial genetic structure across sampling regions. Furthermore, within-population diversity is found to be high, likely due to self-incompatibility, while between-population differentiation is weak, except for those genetically distant populations from Inner Mongolia (Wuyun et al. 2015). It is reported that Inner Mongolian populations may have experienced some bottleneck effects due to their demographic decline (Wuyun et al. 2015).

As *P. pashia* is another ancient species, it may be intermediate between Asian and Occidental pear groups. Whereas, *P. pashia* is native to Southwest China and to the Himalayan region (Fig. 3.2; Zong et al. 2014a). Due to high levels of within-site diversity, based on SSR profiles, Zong et al. (2014a) have proposed that some of the sampled populations may have likely served as sources for range expansions during interglacial periods. Liu et al. (2013) have used chloroplast sequence data to assess the diversity of individuals within 22 populations. As with other wild pear species, a high level of genetic variation is detected within populations. Range expansions may explain lack of correlations between genetic and geographic distances across the range of *P. pashia* (Liu et al. 2013).

3.2.2 Genetic Diversity in the Occidental Pear Species

Occidental pear species are likely to have radiated westward from China and currently occupy overlapping ranges (Fig. 3.3). Chloroplast and nuclear genes have been used to reconstruct the phylogeny of Occidental *Pyrus* species using 50 accessions representing the following 11 species: *P. communis, P. nivalis, P. cordata, P. eleagrifolia, P. spinosa, P. regelii, P. salacifolia, P. syriaca, P. cossonii, P. gharbiana*, and *P. mamorensis* (Zheng et al. 2014). It is found that all Occidental species, except for *P. regelii* and *P. gharbiana,* have shared haplotypes. Moreover, it appears that *P. regelii,* the most easterly West Asian species, must have diversified early, becoming isolated, and it is the only west Asian species *P. regelii* that is monophyletic (Fig. 3.1; Zheng et al. 2014; Volk et al. 2019). In addition, *P. regelii* has an ancestral phenotype with dissected adult leaves and ovaries with few locules (Zheng et al. 2014).

Based on a phylogenetic dendrogram, accessions of some Occidental species, including *P. spinosa, P. cossonii, P. regelii, P. gharbiana,* and *P. mamorensis,* are located on distinct branches (Zheng et al. 2014). In contrast, *P. eleagrifolia, P. nivalis*, and *P. salicifolia* are spread throughout the phylogenetic dendrogram (Zheng et al. 2014). Recently, Volk and co-authors (2019) have observed lower levels of differentiation among Occidental species using chloroplast sequence data (Fig. 3.1). Furthermore, *P. spinosa*, native to Turkey, Southeastern Europe, France, and Spain, has primitive characters, suggesting that it may be yet another ancient species; whereas, *P. salicifolia* and *P. nivalis* have overlapping phenotypes with regard to leaf shape (lanceolate or elliptical leaves) and level of hairiness (Zheng et al. 2014; Paganová 2003). Wild *P. communis* subsp. *pyraster* in Poland and Germany have high levels of diversity within populations, as well as weak correlations between genetic and geographical distance (Wolko et al. 2015; Reim et al. 2017). Recent genomic sequencing data reveal that many pear accessions assigned to Occidental species may be highly admixed (Wu et al. 2018).

3.3 Domestication

Pyrus communis subsp. *communis* is a European pear known for its soft and juicy flesh, and includes cultivars such as 'Bartlett' and 'Anjou'. In contrast, *P. pyrifolia*, the Asian pear, has a crisp and juicy texture. Asian pears include a number of types of cultivated pears, including Chinese white pear cultivars (such as 'Ya Li' and 'Tse Li') and Japanese pears (such as 'Kosui', 'Hosui', and 'Nijisseki'). Genetic markers have been developed and used to reconstruct the domestication process that has resulted in the evolution of European, Chinese white, and Japanese pear cultivars, as well as various Asian landraces that include Chinese sand pears, Ussurian pears, and Xinjiang pears. Recently, SNP data have elucidated this dichotomy between Occidental and Asian cultivated pears (Kumar et al. 2017). These two pear types, from Europe and Asia, respectively, originated from different wild pear relatives specific to their regions of origin (Fig. 3.4). This suggests two independent domestication events, one in Europe and one in Asia from distinct wild species, which was recently confirmed by fully sequenced genomes of a large collection of wild and

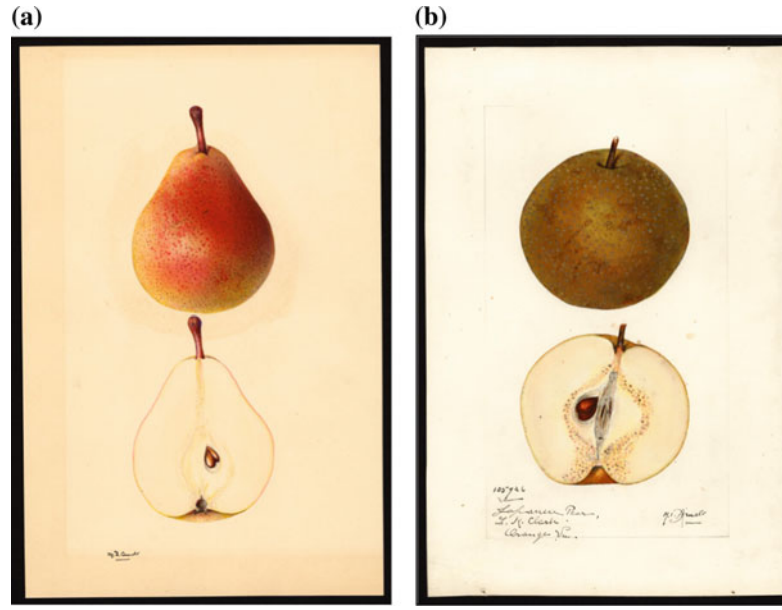

Fig. 3.4 **a** Edible European pear (*P. communis*); **b** Edible Asian pear (*P. pyrifollia*) by Mary Daisy Arnold, U.S. Department of Agriculture Pomological Watercolor Collection. Rare and Special Collections, National Agricultural Library, Beltsville, MD 20705

cultivated pears (Wu et al. 2018). Specifically, *P. communis* subsp. *communis* is derived from *P. pyraster,* and *P. pyrifolia* is derived from the wild *P. pyrifolia* (Wu et al. 2018).

3.3.1 The Chinese White, Japanese, and Chinese Sand Pear Cultivar Groups

The cultivated Chinese white pears, Japanese pears, and Chinese sand pears share a common ancestor, *P. pyrifolia* (Fig. 3.5a; Bao et al. 2007; Jiang et al. 2016).

The Chinese white pear is the most commonly grown pear in northern China, and it is found at the intersection of the native species ranges of *P. ussuriensis* and *P. pyrifolia* (Bao et al. 2007). The Chinese white pears, grown in northern China, may have originated from a gene pool whereby *P. ussuriensis* has hybridized with *P. pyrifolia* (Jiang et al. 2016). *Pyrus* × *bretschneideri* is a hybrid species (sometimes considered to be *P. pyrifolia*) between *P. ussuriensis* and *P. pyrifolia*. This hybrid species, *P.* × *bretschneideri,* is considered as the source species for Chinese white pears (Liu et al. 2015).

The Japanese pear is the most commonly grown commercial pear in Japan. Nishio and co-authors (2016) have used microsatellite markers to assess the genetic diversity and ancestry of modern Japanese pear cultivars. These cultivars are genetically similar to local cultivars from the Kanto region of Japan. Iketani et al. (2010) have found that these local Japanese cultivars are more similar to *P. pyrifolia* of China than *P. ussuriensis* of Japan.

Chinese sand pears are primarily local cultivars grown in Sichuan Province, along the Yangtze River, and in southern regions of China (Song et al. 2014). Chinese white and Japanese pears have fewer numbers of haplotypes than those of Chinese sand pears, suggesting that Chinese sand pears have higher levels of diversity, and are likely to be more basal than other cultivars derived from *P. pyrifolia* (Teng et al. 2015). Although Chinese sand pears may have been derived primarily from *P. pyrifolia* (Jiang et al. 2009), there is some SSAP marker evidence suggesting that Chinese sand and Japanese pears may have resulted from introgressive hybridizations between *P. pyrifolia* and *P. pashia* in Southern China (Jiang et al. 2016).

Zangli pears are yet another Asian pear landrace, native to Eastern Tibet, Western Sichuan, and Northwestern Yunnan provinces. Cultivars

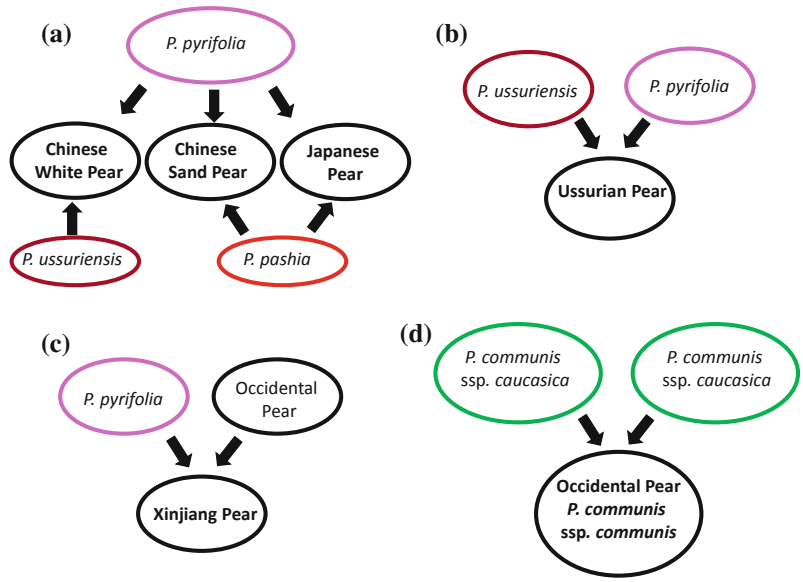

Fig. 3.5 Relationships between cultivated pears (bold) and their progenitor species for **a** Chinese white pear, Chinese sand pear, and Japanese pear; **b** Ussurian cultivated pear; **c** Xinjiang pear; and **d** Occidental pears, *P. communis* subsp. *communis*

of Zangli pears are resistant to bitter cold, dry air, and high winds (Xue et al. 2017). Microsatellite markers have revealed that Zangli pears are genetically similar to Chinese sand pears and that they may have been introduced north from Yunnan and west from Sichuan (Xue et al. 2017).

3.3.2 The Ussurian Cultivated Pear

Ussurian pear cultivars are native to the southern area of northeastern China, as well as to Hebei, Shanxi, and Gansu provinces (Fig. 3.2). Domesticated Ussurian pears are genetically and phenotypically distinct from wild *P. ussuriensis* (Wuyun et al. 2015). Cultivated Ussurian pears are known for their strong cold resistance, and they can endure up to −52 °C (Katayama et al. 2016). The domesticated Ussurian pears have lineages from the following two species, *P. ussuriensis* and *P. pyrifolia* (Fig. 3.5b; Jiang et al. 2016; Yu et al. 2016). It is likely that *P. ussuriensis* and *P. pyrifolia* have also hybridized in the northern part of Japan, where the two species overlap (Katayama et al. 2016). Recently, full-sequencing genome data have revealed that cultivated *P. ussuriensis* is derived from the wild *P. ussuriensis* (Wu et al. 2018). Various samples selected for genomic and genetic analyses may have affected conclusions obtained from the different studies.

3.3.3 The Xinjiang Pear

Xinjiang pear cultivars are derived from hybridizations between *P. pyrifolia* (possibly Chinese white pears) and Occidentals (Fig. 3.5c; Jiang et al. 2016). It is presumed that Occidental pears have been introduced from abroad in the Xinjiang Province in China (Chang et al. 2017). It has been reported that the 'Korla' pear, the most famous Xinjiang pear cultivar, shares chloroplast haplotypes with Chinese white pears, as well as with other Xinjiang cultivated pear accessions (Chang et al. 2017).

3.3.4 The Cultivated European Pear

The European pear, *P. communis* subsp. *communis,* is commercially grown, and is thought to have originated from smaller fruited *P. communis* subsp. *pyraster*, a subspecies native to Eastern Europe, and *P. communis* subsp. *caucasica*, a subspecies native to the Caucasus Mountains of Russia, Crimea, Armenia, and Georgia (Fig. 3.5d; Volk et al. 2006). Microsatellite markers have successfully differentiated *P. communis* subsp. *pyraster* and *P. communis* subsp. *caucasica,* from *P. communis* cultivars (Volk et al. 2006). In a later study, Asanidze and co-authors (2014) have compared local Georgian pear cultivars to wild species of *P. communis* subsp. *caucasica, P. balansae, P. salicifolia, P demetrii, P. syriaca, P. ketzkhovelii*, and *P. sachokiana* found in Georgia. Based on microsatellite marker relationships and morphological similarities, it is likely that *P. communis* subsp. *caucasica* and *P. balansae* (sometimes considered to be *P. communis*; U.S. Department of Agriculture 2017) are progenitors of local Georgian pear cultivars (Asanidze et al. 2011, 2014).

3.4 Conclusions

Altogether, studies based on genetic data, mainly of SSRs and chloroplast sequences, provide a first glimpse of the genetic diversity and evolution of the *Pyrus* genus. Population genetic studies have revealed that within-population variation and gene flow among populations of *Pyrus* species are high, as well as between-species hybridizations recurrent. This adds to the taxonomic complexity of differentiating *Pyrus* species, either based on morphological or genetic traits. Yet, many of the current findings are based on relatively few numbers of markers—nuclear or chloroplast microsatellite or sequence data. The use of genome-wide SNP data using high-throughput sequencing technologies holds promise in reducing costs per marker and per sample (see Kumar et al. 2017; Wu et al. 2018). This research will be limited,

however, based on the availability of true-to-type reference materials and access to wild populations of *Pyrus* species within the native range.

Genebanks currently offer reference materials and some collections of wild species material with detailed passport information (collection site, georeferencing, and half-sib relationships, among others) that can serve as sources of such population genomic studies. Efforts to identify markers that are associated with traits of physiological and agronomic significance will facilitate measurement of "useful" variation within species, thus opening the door to exploring effects of specific allelic diversity within breeding programs.

3.5 Future Directions

Future efforts that unify taxonomic descriptions, based on morphological and genetic characters, of *Pyrus* genetic resources within worldwide genebanks will facilitate access to and use of genebank materials. In addition, further work is required to unravel the large-scale evolutionary history of the *Pyrus* genus, and in particular the origin of edible pears. We must re-assess pear diversity in terms of species and genetic diversity in Europe, Central Asia, and Eastern Asia using genomic tools such as genotyping-by-sequencing, whole-genome sequencing, or SNP arrays (Montanari et al. 2013; Kumar et al. 2017; Xue et al. 2017). The recent release of reference genomes for *P.* × *bretschneideri* (Wu et al. 2013) and for the European pear *P. communis* (Chagné et al. 2014), together with new population-level genetic frameworks designed to search for molecular signatures of evolutionary processes and to infer complex demographic histories (Beichman et al. 2018; Csilléry et al. 2010; Gutenkunst et al. 2010), has rendered studies of genomic consequences of pear domestication timely. Recent resequencing of both wild and cultivated pears has revealed demographic history and genomic signatures of adaptation during pear domestication (Wu et al. 2018). The combination of these genomic approaches is providing us with a more precise picture of the genomic diversity and evolution of the *Pyrus* genus and, more generally, of processes of adaptation in perennials.

References

Asanidze Z, Akhalkatsi M, Gvritishvili M (2011) Comparative morphometric study and relationships between the Caucasian species of wild pear (*Pyrus* spp.) and local cultivars in Georgia. Flora 206:974–986

Asanidze Z, Akhalkatsi M, Henk AD, Richards CM, Volk GM (2014) Genetic relationships between wild progenitor pear (*Pyrus* L.) species and local cultivars native to Georgia, South Caucasus. Flora 209:504–512

Bao L, Chen K, Zhang D, Cao Y, Yamamoto Y, Teng Y (2007) Genetic diversity and similarity of pear (*Pyrus* L.) cultivars native to East Asia revealed by SSR (simple sequence repeat) markers. Genet Resour Crop Evol 54:959–971

Bao L, Chen K, Zhang D, Li X, Teng Y (2008) An assessment of genetic variability and relationships within Asian pears based on AFLP (amplified fragment length polymorphism) markers. Scient Hort 116:374–380

Beichman AC, Huerta-Sanchez E, Lohmueller KE (2018) Using genomic data to infer historic population dynamics of nonmodel organisms. Ann Rev Ecol Evol System 49. https://www.annualreviews.org/doi/abs/10.1146/annurev-ecolsys-110617–62431

Bell RL (1992) Additional East European *Pyrus* germplasm with resistance to pear psylla nymphal feeding. HortScience 27:412–413

Bell RL, Itai A (2011) Pyrus. In: Kole C (ed) Wild crop relatives: genomic and breeding resources, temperate fruits. Springer, Berlin, pp 147–176

Chagné D, Crowhurst RN, Pindo M, Thrimawithana A, Deng C et al (2014) The draft genome sequence of European pear (*Pyrus communis* L. 'Bartlett'). PLoS ONE 9(4):e92644

Challice JS, Westwood MN (1973) Numerical taxonomic studies of the genus *Pyrus* using both chemical and botanical characters. Bot J Linn Soc 67:121–148

Chang Y-J, Cao Y-F, Zhang J-M, Tian L-M, Dong X-G, Zhang Y, Qi D, X-s Zhang (2017) Study on chloroplast DNA diversity of cultivated and wild pears (*Pyrus* L.) in Northern China. Tree Genet Genomes 13:44. https://doi.org/10.1007/s11295-017-1126-z

Csilléry K, Blum MG, Gaggiotti OE, François O (2010) Approximate Bayesian computation (ABC) in practice. Trends Ecol Evol 25(7):410–418

Ercisli S (2004) A short review of the fruit germplasm resources of Turkey. Genet Resour Crop Evol 51:419–435

Gutenkunst RN, Hernandez RD, Williamson SH, Bustamante CD (2010) Diffusion approximations for demographic inference: DaDi. Nature Preced hdl:10101/npre.2010.4594.1

Iketani H, Yamamoto T, Katayama H, Uematsu C, Mase N, Sato Y (2010) Introgression between native and prehistorically naturalized (archaeophytic) wild pear (*Pyrus* spp.) populations in Northern Tohoku, Northeast Japan. Conserv Genet 11:115–126

Jiang Z, Tang F, Huang H, Hu H, Chen Q (2009) Assessment of genetic diversity of Chinese sand pear landraces (*Pyrus pyrifolia* Nakai) using simple sequence repeat markers. HortScience 44:619–626

Jiang S, Zheng X, Yu P, Yue X, Ahmed M, Cai D, Teng Y (2016) Primitive genepools of Asian pears and their complex hybrid origins inferred from fluorescent sequence-specific amplification polymorphism (SSAP) markers based on LTR retrotransposons. PLoS ONE 11(2):e0149192. https://doi.org/10.1371/journal.pone.0149192

Katayama H, Amo H, Wuyun T, Uematsu C, Iketani H (2016) Genetic structure and diversity of the wild Ussurian pear in East Asia. Breed Sci 66:90–99

Korotkova N, Parolly G, Khachatryan A, Ghulikyan L, Sargsyan H, Akopian J, Borsch T, Gruenstaeudl M (2018) Towards resolving the evolutionary history of Caucasian pear Pyrus, Rosaceae)—Phylogenetic relationships, divergence times and leaf trait evolution. J Systemat Evol 56:35–47

Kumar S, Kirk C, Wiedow C, Knaebel M, Brewer L (2017) Genotyping-by-sequencing of pear (*Pyrus* spp.) accessions unravels novel patterns of genetic diversity and selection footprints. Hort Res 4:17015

Liu J, Zheng X, Potter D, Hu C, Teng Y (2012) Genetic diversity and population structure of *Pyrus calleryana* (Rosaceae) in Zhejiang province, China. Biochem System Ecol 45:69–78

Liu J, Sun P, Zheng X, Potter D, Li K, Hu C, Teng Y (2013) Genetic structure and phylogeography of *Pyrus pashia* L. (Rosaceae) in Yunnan Province, China, revealed by chloroplast DNA analyses. Tree Genet Genomes 9:433–441

Liu Q, Song Y, Liu L, Zhang M, Sun J, Zhang S, Wu J (2015) Genetic diversity and population structure of pear (*Pyrus* spp.) collections revealed by a set of core genome-wide SSR markers. Tree Genet Genomes 11:128. https://doi.org/10.1007/211295-015-0953-z

Montanari S, Saeed M, Knäbel M, Kim YK, Troggio M, Malnoy M, Velasco R, Fontana P, Won KH, Durel C-E, Perchepied L, Schaffer R, Wiedow C, Bus V, Brewer L, Gardiner SE, Crowhurst RN, Chagné D (2013) Identification of *Pyrus* single nucleotide polymorphisms (SNPs) and evaluation for genetic mapping in European pear and interspecific hybrids. PLoS ONE 8(10):e77022

Nishio S, Takada N, Saito T, Yamamoto T, Iketana H (2016) Estimation of loss of genetic diversity in modern Japanese cultivars by comparison of diverse genetic resources in Asian pear (*Pyrus* spp.). BMC Genet 17:81. https://doi.org/10.1186/212863-016-0380-7

Paganová V (2003) Taxonomic reliability of leaf and fruit morphological characteristics of the *Pyrus* L. taxa in Slovakia. Hort Sci (Prague) 30:98–107

Reim S, Lochschmidt F, Proft A, Wolf H, Wolf H (2017) Species delimitation, genetic diversity and structure of the European indigenous wild pear (*Pyrus pyraster*) in Saxony, Germany. Genet Resour Crop Evol 64:1075–1085

Richards CM, Volk GM, Reilley AA, Henk AD, Lockwood DR, Reeves PA, Forsline PL (2009) Genetic diversity and population structure in *Malus sieversii*, a wild progenitor species of the domesticated apple. Tree Genet Genomes 5(2):339–347

Silva, GJ, Medeiros Souza T, Lía Barbieri R, Costa de Oliveira A (2014) Origin, domestication, and dispersing of pear (*Pyrus* spp.). Adv Agric 2014:541097

Song Y, Fan L, Chen H, Zhang M, Ma Q, Zhang S, Wu J (2014) Identifying genetic diversity and a preliminary core collection of *Pyrus pyrifolia* cultivars by a genome-wide set of SSR markers. Scientia Hort 167:5–16

Teng Y, Yue X, Zheng X, Cai D (2015) Genetic clue to the origin of cultivated Asian pears inferred from cpDNA haplotypes. Acta Hort 1094:31–39

U.S. Department of Agriculture (2017) Germplasm Resources Information Network (GRIN-Global). GRIN Taxonomy. https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomyquery.aspx. Accessed 7 Dec 2017

van der Zwet T, Stankovic D, Cociu V (1983) Collecting *Pyrus* germplasm in Eastern Europe and its significance to the USDA pear breeding program. Acta Hortic 140:43–45

Volk GM, Richards CM, Henk AD, Reilley AA, Bassil NV, Postman JD (2006) Diversity of wild *Pyrus communis* based on microsatellite analyses. J Amer Soc Hort Sci 131:408–417

Volk GM, Henk AD, Richards CM, Bassil NV, Postman J (2019) Chloroplast sequence data differentiate Maleae, and specifically *Pyrus*, species in the USDA-ARS National Plant Germplasm System. Genet Resour Crop Evol 66(1):5–15

Wolko Ł, Bocianowski J, Antkowiak W, Słomski R (2015) Genetic diversity and population structure of wild pear (*Pyrus pyraster* (L.) Burgsd.) in Poland. Open Life Sci 10:19–29

Wu J, Wang Z, Shi Z, Zhang S, Ming R, Zhu S, Khan MA, Tao S, Korban SS, Wang H, Chen NJ, Nishio T, Xu X, Cong L, Qi K, Huang X, Wang Y, Zhao X, Wu J, Deng C, Gou C, Zhou W, Yin H, Qin G, Sha Y, Tao Y, Chen H, Yang Y, Song Y, Zhan D, Wang J, Li L, Dai M, Gu C, Wang Y, Shi D, Wang X, Zhang H, Zeng L, Zheng D, Wang C, Chen M, Wang G, Xie L, Sovero V, Sha S, Huang W, Zhang S, Zhang M, Sun J, Xu L, Li Y, Liu X, Li Q, Shen J, Wang J, Paull RE, Bennetzen JL, Wang J, Zhang S (2013) The genome of the pear (*Pyrus bretschneideri* Rehd.). Genome Res 23(2):396–408

Wu J, Wang Y, Xu J, Korban SS, Fei Z, Tao S, Ming R, Tai S, Khan MA, Postman JD, Gu C, Yin H, Zheng D, Qi K, Li Y, Wang R, Deng CH, Kumar S, Chagné D, Li X, Wu J, Huang X, Zhang H, Xie Z, Li X, Zhang M, Li Y, Yue Z, Fang X, Li J, Li L, Jin C,

Qin M, Zhang J, Wu X, Ke Y, Wang J, Yang H, Zhang S (2018) Diversification and independent domestication of Asian and European pears. Genome Biol 19:77. https://doi.org/10.1186/s13059-018-1452-y

Wuyun T, Amo H, Xu J, Ma T, Uematsu C, Katayama H (2015) Population structure of and conservation strategies for wild *Pyrus ussuriensis* Maxim. in China. PLoSOne 10(8):e013368. https://doi.org/10.1371/journal.pone.0133686

Xue L, Liu Q, Qin M, Zhang M, Wu X, Wu J (2017) Genetic variation and population structure of "Zangli" pear landraces in Tibet revealed by SSR markers. Tree Genet Genomes 13:26. https://doi.org/10.1007/s11295-017-1110-7

Yu P, Jiang S, Wang X, Bai S, Teng Y (2016) Retrotransposon-based sequence-specific amplification polymorphism markers reveal that cultivated *Pyrus ussuriensis* originated from an interspecific hybridization. Eur J Hort Sci 81:264–272

Zheng X, Cai D, Potter D, Postman J, Liu J, Teng Y (2014) Phylogeny and evolutionary histories of *Pyrus* L. revealed by phylogenetic trees and networks based on data from multiple DNA sequences. Mol Phylogen Evol 80:54–65

Zohary D, Spiegel-Roy P (1975) Beginnings of fruit growing in the old world. Science 187(4174):319–327

Zong Y, Sun P, Liu J, Yue Z, Li K, Teng Y (2014a) Genetic diversity and population structure of seedling populations of *Pyrus pashia*. Plant Mol Biol Rep 32:644–651

Zong Y, Sun P, Liu J, Yue X, Niu Q, Teng Y (2014b) Chloroplast DNA-based genetic diversity and phylogeography of *Pyrus betulaefolia* (Rosaceae) in Northern China. Tree Genet Genomes 10:739–749

Zong Y, Sun P, Yue X, Niu Q, Teng Y (2017) Variation in microsatellite loci reveals a natural boundary of genetic differentiation among *Pyrus betulaefolia* populations in Northern China. J Am Soc Hort Sci 142:319–329

Genetics and Breeding of Pear

4

Lester Brewer and Richard Volz

Abstract

Although *Pyrus* consists of 22 primary species, nearly all scion breeding is focused on three species, including *Pyrus communis* (European pear), *Pyrus pyrifolia* (sand pear), *and Pyrus* × *bretschneideri* (white pear). Most scion breeding programs around the world are in one of two camps: those breeding for European (*P. communis*) soft- or firm-textured pears, and those breeding for crisp-textured Asian pears (*P. pyrifolia* and *P.* × *bretschneideri*). Intercrossing among species is typically limited, except in New Zealand where it is a core aspect of the breeding program. The lack of effective control of pests and diseases in pear combined with increased consumer preferences for fruits grown with low chemical inputs and low environmental impacts is driving breeding programs to incorporate plant resistance to major pests and diseases. On the other hand, the range of vigor-controlling rootstocks for pear production is limited. Quince (*Cydonia oblonga*) rootstocks are preferred in Europe, as they offer vigor control, precocity, and ease of propagation. To date, utilization of quince rootstocks in North America has been restricted due to their lack of cold tolerance. Identification and testing of cold hardy quince selections could change this. *Pyrus* rootstocks are currently preferred in North America and in Asia because of their cold hardiness; however, they are more vigorous than quince, yet their yield efficiency is lower. Thus, vigor control is among breeding targets for *Pyrus* rootstocks. Hybrids between *Pyrus* species are now being used to overcome some of these deficiencies and to include adaption to highly alkaline soils. In addition, other species, such as *Amelanchier*, are being tested for their potentials to confer dwarfing, excellent cold tolerance, potential non-host resistance to pear decline, resistance to fire blight, and good yield efficiency. Recent identification of genetic markers for scion vigor control and precocity is a positive step for future breeding of enhanced *Pyrus* rootstocks. Overall, the development of cultivars and rootstocks with new or improved characters would be facilitated by the availability of molecular markers for traits of interest. However, pear breeding programs lag behind those of apple in application of marker-assisted selection and genomic selection to speed-up cultivar/rootstock development, and to ensure programs are more effective and efficient in

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their utilization of available resources. As current genetic markers are validated in more populations, and the pear reference genome sequence undergoes further refinement, these technologies will play a larger role in pear breeding programs.

4.1 Introduction

Pear is assumed to be an ancient allopolyploid that behaves as a diploid ($2n = 2x = 34$) (Crane and Lewis 1940). There are three important centers of origin for the genus *Pyrus.* The first is in the mountainous regions of Western China, while the second is in Western Asia, comprising Afghanistan, India, Tajikistan, Uzbekistan, and western Tian-Shan, and the third is in the Caucasus Mountains. *Pyrus*, belonging to the family Rosaceae and subfamily Pomoideae, is a diverse genus that includes 22 primary species ranging from the mostly soft-textured European pear, *Pyrus communis* L., to the crisp-textured Asian sand pear, *Pyrus pyrifolia* (Burm.) Nak., and the Chinese white pear, *Pyrus* × *bretschneideri* Rehd. (Bell 1991).

In 2015, world production of pears has been estimated to be 26.6 million metric tonnes, with approximately 20 million metric tonnes of those being crisp-textured Asian-style pears (Belrose 2016). Breeding programs typically fall into one of two groups, those selecting new types of soft-textured European pears, mainly in Europe and North America, and those selecting crisp-textured pears, generally concentrated in South Korea, Japan, China, and New Zealand. Breeders of European pears tend to target such fruit characters, as harvest season extension, red skin color, good fruit size, flavor, improved textural attributes, storage ability, as well as growth habit, and resistance to various diseases and pests, especially against pear scab (*Venturia pirina* Aderh.), fire blight (*Erwinia amylovora* (Burrill) Winslow et al.), and pear psylla (Psyllidae: Psyllinae: *Cacopsylla* spp.) (Dondini and Sansavini 2012). In China, breeding program objectives include high fruit quality, early ripening, long shelf-life, large fruit size, resistance to both scab (*V. nashicola* Tanaka et Yamamoto) and black spot (*Alternaria alternata* (Fr.) Keissler pv. *kikuchiana*), and environmental adaptation through the use of a range of species, including *P.* × *bretschneideri, P. pyrifolia, P. ussuriensis* Maxim*., and P.* × *sinkiangensis* (Teng 2011). Furthermore, breeding programs in Japan focus on genetic improvement of *P. pyrifolia* cultivars, with breeding objectives targeting superior fruit quality, early ripening, self-compatibility, and multiple disease resistance for pear scab *(V. nashicola*) and black spot *(A. alternata*) (Saito 2016). As in Japan, Korean breeding programs focus predominantly on enhancement of *P. pyrifolia* cultivars. Breeding targets include season extension, storage ability, large fruit size, and high aroma, as well as pest and disease resistance, especially for leaf rot *(A. kikuchiana*) and pear scab (*V. nashicola*) (Shin et al. 2002); whereas, the New Zealand breeding program utilizes interspecific hybrids with major breeding objectives of producing convenient (not messy to eat) fruit with high levels of flavor that can be eaten either readily from the tree or after storage, but with a minimum storage life of three months. Furthermore, additional important breeding goals for the New Zealand program include increased fruit precocity and yield, high fruit quality free of internal disorders, variations in red skin colors, a range of fruit flavors and shapes, fruit skin that will not scuff, and disease resistance, especially to both fire blight and pear scab (*V. pirina*). The primary species used to generate interspecific pear hybrids in New Zealand include *P.* × *bretschneideri*, *P. pyrifolia,* and *P. communis*.

It is important to point out that the North American and European pear markets are dominated by a small number of old *P. communis* cultivars, such as 'Williams' Bon Chrétien,' also known as 'Bartlett', 'Conference', 'Abaté Fetel', and D'Anjou' that have been selected before 1900. Pear fruit consumption rates in these regions are generally either static or dropping (Belrose 2016). New cultivars have struggled to get a foothold in these markets. This may be

attributed, in part, to the dominance of a small number of supermarket chains, strong competition with other fruits in the marketplace, and changing Western consumer food demands (Brewer and Palmer 2011). Over the last few decades, consumers desire more convenient fruit and snack foods that are ready to eat, and with consistent quality. Developing products with these attributes would have a positive influence on the economic returns for producers (Brewer and Palmer 2011). Furthermore, new pear cultivars incorporating improved resistances, especially for pests and diseases that have the largest effects on profitable pear production, such as fire blight, pear psylla, and pear scab, are needed to achieve an additional goal of growing pears with low chemical inputs.

The minimal impact of new pear cultivars in European and North American markets contrasts with the situation in China, wherein traditional *P. ussuriensis* and *P.* × *bretschneideri* cultivars maturing in mid- to late-season (i.e., mid-August to September), such as 'Dangshan Suli', 'Yali', 'Nanguoli', and 'Xuehauli' comprise about 40% of all commercially grown cultivars (Cao et al. 2014). Over the past few decades, a substantial increase in Chinese pear production has been attributed, in part, to nearly 100 new cultivars, released to the pear industry over the last 50 years from government and university breeding programs (Belrose 2016). Several of these new cultivars, such as 'Cuiguan' mature very early to early (July to early August), thus extending the season for fresh-eating pear fruit.

Interestingly, despite decline in total pear production in Japan over the past 40 years by over 40%, there has been a reasonable uptake of new cultivars (≈14% in 2012) (Saito 2016). Old cultivars, such as 'Nijisseiki' and 'Chojuro' have been superseded by cultivars released from breeding programs, including 'Kosui' 'Hosui', and more recently 'Akizuki' and 'Nansui.' The success of these new cultivars has been attributed to traits, such as resistance to black spot and improved eating quality.

In comparison with other perennial fruit crops, traditional pear breeding is an expensive and lengthy process as seedling trees take longer to come into fruiting, and juvenile trees have many spines, rendering harvest and management difficult. Furthermore, interstocks are required when quince rootstocks are used for seedling growth, which adds time and expense to the process. Availability of adapted, compatible, and dwarfing precocious *Pyrus* rootstocks would be of great benefit to pear breeding programs and to the pear industry as a whole.

New genomic technologies would offer opportunities for accelerating development and increasing efficiency and effectiveness of breeding programs for developing new pear cultivars, as well as new and improved pear rootstocks.

This review focuses on modern pear breeding approaches, as well as genetics of key selection traits that are important for today's pear breeders. It summarizes recent genomic-related research aimed at improving efficiencies of pear breeding.

4.2 Breeding Systems

Pear has a gametophytic self-incompatibility (GSI) system that ensures pollen fertilization of ovules in flowers and subsequent seed production via outcrossing with other compatible pears. As many of the important horticultural traits in pear are likely controlled by multiple genes, this GSI system ensures that pear progenies are highly heterogeneous, with a wide diversity of possible phenotypes. Nevertheless, the three most important components of any pear breeding program are the following: (1) hybridization of parents, carrying traits of interest, to generate seedling populations expressing new and improved characters, (2) identification of desirable selections carrying those traits of interest among seedling populations, and (3) evaluation and testing of the best-performing selections.

4.2.1 Hybridization

4.2.1.1 Compatibility

GSI is a mechanism triggered by proteins coded by a single locus on linkage group (LG) 17 with multiple *S*-alleles that determine inhibition of

self-incompatible pollen tube growth without damaging self-compatible tubes (Dondini and Sansavini 2012). Genotypes possessing one *S*-allele in common are partially compatible, and under certain conditions may produce progeny that exhibit reduced fruit set and seed production, while those possessing the same two *S*-alleles are fully incompatible (Wang et al. 2017). To date, a large number of unique *S*-alleles have been identified, 28 in *P. communis* (Gharehaghaji et al. 2014; Goldway et al. 2009) and at least 48 across five Asian pear species (Wang et al. 2017). The repeated use of closely related parents in a breeding program may over time result in deleterious concentration of a few *S*-alleles in breeding material of potential value as parents. Of 133 *P. communis* cultivars assessed for their *S*-haplotypes, 75 are found to carry the *S101* allele, probably reflecting the extensive use of 'Bartlett' (*S101*/*S102*) as a parent (Goldway et al. 2009). An understanding of compatible and incompatible mating combinations is therefore critically important to a pear breeder, and this can be derived either from knowledge of the *S*-haplotype(s) of parent candidates, or through past knowledge of cross-performance.

There are no major incompatibility barriers to interspecific hybridization in *Pyrus,* and at least six naturally occurring hybrid taxa have been reported (Bell 1991). Zielinski and Thompson (1967) have found little evidence for hybrid sterility from interspecific hybridizations. However, post-zygotic gene flow barriers may exist between different *Pyrus* species. In New Zealand's pear breeding program, some progeny from crosses between Asian- and European pear-derived parents have shed either little or no pollen when anthers are dried (White and Brewer 2002). Hybrid necrosis (HN) of young pear seedlings has also been observed in some interspecific populations, but this has not been observed in intraspecific crosses. Two distinct HN phenotypic classes have been identified in a genomic mapping study of an interspecific ('PremP003' × 'Moonglow') pear population. These include the following: (i) seedlings that cease growing soon after germination, initially with chlorotic and necrotic leaf regions, then often dying within one month of germination ('Type 1'); and (ii) seedlings that initially develop normally, followed by termination of growth within three months after germination, with leaves beginning to cup downward and progressively becoming chlorotic and necrotic ('Type 2'). For those seedlings that grow normally, these have been classified as 'Type 3.' Interestingly, no significant differences in seed weight or radicle length among these 'Types' are observed in the above pear population at planting (Montanari et al. 2016a). Furthermore, 'Type 1': 'Type 2' + 'Type 3' ratios are consistent with a 3:13 segregation ratio, while Type 2:Type 3 ratios fit a 1:1 segregation ratio, thus indicating possible presence of major genes controlling this interspecific (sub)/lethality trait. In addition, at least a single two-gene epistatic interaction, between loci on LG1 and LG5, originating from Asian and European species, respectively, is attributed to incidence of Type 1 HN, with at least one other locus on LG2 implicated in regulating this phenotype. Molecular markers linked to both lethal phenotypes have been identified for these loci, and these will be useful in selecting parents lacking 'sublethal' alleles in order to maximize progeny numbers from interspecific crosses (Montanari et al. 2016a).

Self-compatibility

Incompatibility has been overcome following identification of a self-compatible natural mutant of 'Nijisseiki', referred to as 'Osanijisseiki' (Saito 2016). Crossing experiments have indicated that this self-compatibility is due to a mutation in the pistil *S* locus, resulting in deletion of the S-ribonuclease allele 4 (S4-RNase) in styles rather than in pollen. 'Osanijisseiki' has been used to develop a number of new self-compatible *P. pyrifolia* cultivars. In another approach, pollen from a heavily gamma-irradiated 'Kosui' tree has been used to pollinate 'Kosui' flowers. This has resulted in identifying a selection with a partial pollen mutation causing loss of pollen incompatibility function,

but retaining its stylar self-incompatibility (Sawamura et al. 2013; Saito 2016).

4.2.1.2 Pollen Collection and Storage

To ensure a full range of parents with different flowering times are available for intercrossing, pollen collection is best completed in advance of the crossing season (Bell et al. 1996). Pollen can be stored either from the previous year, or shoots of up to 1.2 m long, with their base cut along a 25° angle, can be collected at the tight-cluster flower stage before flowering begins, and kept in a greenhouse at 20–25 °C until flowers are fully open to collect anthers, and then extract pollen before dehiscence (van der Zwet et al. 1977). In addition, flowers at the balloon stage can also be collected from the orchard approximately 2 days before they are required (Visser and Oost 1981). Pollen can be extracted using a number of methods, including rubbing anthers over a wire mesh grid (1.5 mm^2) onto paper sheets (Bell et al. 1996), or combed from flowers using fine combs onto foil trays to maximize pollen recovery. Following extraction, pollen should be allowed to dry at approximately 23 °C for 24–48 h, either on a laboratory bench or in an incubator. While pear pollen remains viable at room temperature for 2–3 weeks, it is best refrigerated at approximately 3–5 °C in plastic or glass vials, and placed inside closed containers or stored in a desiccator with indicating silica gel over anhydrous $CaSO_4$ to remove moisture and maximize viability.

Pollen can be stored for 2 years at 2–4 °C and 10% relative humidity (Bell et al. 1996). Pollen can also be frozen at −20 to −120 °C for 2–3 years (Bhat et al. 2012). When pollen is required for use in the orchard, it is best that it is transported in a cooler bin or bag with frozen pads or similar receptacles to keep it chilled. Prior to use, pollen viability can be checked using acetocarmine or other stains following standard procedures (Bell et al. 1996). Pollen can also be germinated in a liquid medium containing 10% sucrose solution and 50 ppm boric acid, and germination rate recorded after 2 h at 23 °C (Visser and Oost 1981).

4.2.1.3 Emasculation, Pollination, and Seed Culture

Flowers are emasculated when the majority reaches balloon stage, at which point any open or excess flowers are removed. A variety of methods can be used for emasculation, including notched scissors, fine combs, finger nails, scalpels, or tweezers (van der Zwet et al. 1977; Bell and Janick 1990). Branches with emasculated flowers can be bagged or whole trees covered with insect proof nets and plastic tents to prevent insect visitation. However, many breeders do not think that this is necessary, as long as the calyx, corolla, and stamens are removed before flowers are open (Bell and Janick 1990). Pollination is ideally completed within 24–48 h following emasculation.

Although many cultivars have a stigma-receptive period of up to 6 to 11 days, some have a shorter receptive period that can cause a significant reduction in fruit set after 48 h from the start of anthesis; e.g., ‘Doyenné du Comice’ (‘Comice’) (Sanzol et al. 2003). In such cases, pollen can be applied to stigmas using a variety of tools, including the stopper of a pollen vial, glass rod, camel hair brush, strip cut eraser, and a fingertip (Bell et al. 1996; van der Zwet et al. 1977). In addition to the type of cultivar, temperature also strongly influences stigmatic receptivity, pollen tube growth, and/or ovule development for successful pollination. For example, ‘Comice’ has a shortened stigma receptivity period and reduced ovule longevity at 17 compared to 13 °C (Tromp and Borsboom 1994). Cool spring conditions decrease pollen tube growth, delay ovule degeneration, and can reduce the overall period for successful pollination (Sanzol et al. 2003).

Pear seeds extracted from fruit produced in crosses require a chilling period or stratification while in a moistened state to break dormancy and initiate germination (Bell et al. 1996). During stratification, seeds will absorb enough water to increase their weight by between 100 and 150% (Brewer, unpublished). Species originating from warm winter climates require a shorter stratification period, and the optimum temperature for

this process is higher (typically 7–10 °C) than for those from cold winter climates where the ideal stratification temperature is 3–5 °C for 60–90 days. Sowing media used by breeders to germinate seeds include a seedbed with a well-aerated medium, such as sand or vermiculite, finely ground peat moss (Bell et al. 1996), or dampened filter paper in petri plates or other closed containers (Montanari et al. 2016a). When grown on filter paper, any fungal development can be quickly identified and treated with a suitable fungicide before germinated seeds are planted (Montanari et al. 2016a). Once seeds have begun to germinate, warm periods of either one or more than 24 h at 20 °C can help stimulate consistent germination.

4.2.1.4 Seedling Growing Methods

Traditionally, pear seedlings grown on their own roots have long juvenility periods. In fact, generation cycles of up to 10 years have been reported for European pears (Brewer and Palmer 2011). Seedlings from Asian species are more precocious; i.e., they have significantly shorter generation cycles (Brewer et al. 2008a). Reduction of the generation cycle is a focus of many breeding programs, as this has the largest influence on the time taken for new products to reach the market (Brewer and Palmer 2011). Breeding systems have been developed to reduce the time taken for seedlings to come into bearing fruit. In New Zealand, seedlings are grown in the greenhouse to accelerate growth rate and increase internode numbers before planting them in an orchard or a nursery. In the orchard, tree top bending is applied when seedlings have produced at least 60 internodes. This bending has a number of benefits, such as reducing terminal growth while enhancing spur formation and flowering on mature wood. After bending the top of the tree, a full trunk girdle is completed, usually in the middle of summer (Brewer et al. 2008a). Fruit on seedling trees with bent tops are generally harvested from the ground, meaning ladders or other harvest devices are not required for the first 3 years of fruiting. Seedlings grown on their own roots are vigorous, and production of excess vegetative growth along with juvenile spines makes fruit thinning and harvest operations difficult. In New Zealand, seedlings are now managed by using rootstocks; wherein, seedlings are grown as fast as possible in a greenhouse (or temporarily in the orchard, if required) before budding or grafting onto elite Quince C rootstocks interstocked with elite 'Beurre Hardy.' The main benefit associated with utilizing rootstocks is improved ease of management, including crop regulation and harvest. Also, the outcome is more representative of what might be expected in commercial production of any future pear cultivar.

4.2.2 Polyploidy

Naturally occurring polyploidy has been identified in both European and Asian cultivars, including that of 'Sha 01', a tetraploid ($2n = 4x = 68$) bud mutant of 'Korla Pear' (Cao et al. 2002, 2014), a tetraploid 'Bartlett', and a triploid ($2n = 3x = 51$) 'Beurré Diel' (Moffett 1933), and 'Anli', a *P. ussuriensis* cultivar (Cao et al. 2002). Triploids have been developed by crossing naturally occurring or induced tetraploids (following colchicine treatment) with diploid parents. Even though there is a range of available polyploids, pear breeding programs rarely use these as to develop new cultivars. In crosses undertaken between Asian species, a range of tetraploid, triploid, and diploid combinations have been generated. For example, crosses between two tetraploids have yielded progeny of which 97% are tetraploid and 3% are aneuploid. Crosses of tetraploids with triploids, and reciprocal crosses, have yielded progenies with more or less equal numbers of triploids (34%), aneuploids (33%), and diploids (26%), while crosses between tetraploids and diploids have mostly produced diploids (61%) and triploids (36%) (Cao et al. 2002). Although there is little documented information on fruit traits in such polyploids, the wide range of phenotypic variations observed in leaf traits suggest there may be unexplored potential for variations in fruit traits among such polyploids (Sun et al. 2011).

4.2.3 Mutation Breeding

Mutagenic agents can be used to increase frequencies of mutations that would otherwise occur naturally at very low rates. Irradiation (X-rays) is the most common method used to modify well-adapted cultivars, typically to improve them for either one or two traits. However, many of these mutations are unstable, and only those that have proved to be stable have found a place in commercial production (Bell et al. 1996). The Food and Agriculture Organization of the United Nations (FAO)/International Atomic Energy Agency (IAED) Database (2000) records five European and two Japanese pear cultivars registered as new mutant cultivars (Ahloowalia et al. 2004). Most commercially available European pear cultivar mutations, whether naturally occurring or induced, involve enhancement of red fruit skin color. Such stable red skin color sports have been used in various pear breeding programs for developing new red-colored fruit skin cultivars.

Other mutations influencing disease resistance and responses to environment have been identified. For example, the most important mutations of Japanese pear include self-compatibility and resistance to black spot disease of 'Nijisseiki' and 'Shinsui'. These have now been used within the Japanese breeding program (Ahloowalia et al. 2004). Natural and induced mutations have also been identified for bloom time, blossom color, ripening period, and growth habit (Hancock and Lobos 2008).

4.3 Target Traits for Selection

A good knowledge of the genetics controlling a target trait of interest is critical in optimizing breeding strategies to maximize genetic gain and develop new cultivars carrying the desired trait. For those complex traits controlled by many genes, estimates of heritability and combining ability provide information of the relative importance of heredity compared with that of environment in determining an individual's phenotype. Narrow-sense heritability (h^2) estimates the extent to which a phenotype is determined by parental genes that are largely additive in their effects. While general combining ability (GCA) measures the average performance of a parent based on the performance of its progeny, specific combining ability (SCA) measures the additional genetic value due to interactions between particular parent genotypes.

In this section, key desirable traits targeted for selection in pear programs will be discussed in detail, including how the trait is measured and what is currently known regarding its genetics.

4.3.1 Fruit Quality

Improved fruit quality is the cornerstone of every pear breeding program. Fruit quality is a complex trait, being a culmination of all external and internal characters of the fruit deemed of commercial importance. Contributing characters to fruit quality include the following: texture; flavor; sweetness; sourness; skin scuffing; skin russet; physiological disorders; levels of bitterness; astringency; absence of grit cells within flesh, skin, and around core tissues; skin color; general appearance; post-harvest performance; and shelf-life. Breeders in different geographic regions place different emphasis on each of these traits in selecting cultivars that perform best for their specific breeding objectives under their climatic conditions.

Breeders often rate overall fruit quality using a composite score, determined from an amalgamation of phenotypic scores of many of the individual traits listed above. This is predominantly a hedonic score, and thus its narrow-sense heritability is often very low (Bell et al. 1996). It has been suggested that eating quality in European pear is governed by non-additive gene effects (i.e., through dominance and/or epistasis), while narrow-sense heritability is completely absent for this trait (Bell et al. 1996). Furthermore, specific combining ability (SCA) is much more important, thus suggesting that effective genetic gain for eating quality could be made by selecting for individuals within families with high SCA (Bell et al. 1996). In other studies,

heritability for overall fruit quality of either European pear or for mixed European and Asian pear families is low (h^2 = 0.09–0.1) (White et al. 2000b). The heritability of a selection index for overall fruit quality weighted each trait in terms of importance before summing individual scores is also low (h^2 = 0.17) (Bell and Janick 1990).

Environmental factors and developmental (maturation and ripening) stages can have considerable influences on many aspects of pear fruit quality (Bell and Janick 1990). Although their interactions with genotypes have not been formally documented, they must either be controlled or accounted for in order to accurately estimate genetic effects on fruit quality within a pear population. Pears of Asian parentages can be harvested either near or at full eating ripeness when fruit starch has been converted into sugar. In fact, tasting of the fruit may help determine stage of maturity. For those genotypes wherein skin color changes during maturation, background color changes from green to yellowish-green which can signal optimum maturity. Changes in flesh firmness (as measured hedonically or with a penetrometer) can also be a useful measure of maturity. Furthermore, likely commercial handling of fruit should also be taken into consideration; i.e., fruit harvested at an earlier stage of maturation for storage versus fruit that will be consumed immediately after picking.

In contrast to Asian pears, fruit of most European pears usually requires storage at cold temperatures to induce proper ripening (Sugar et al. 2009). Lengths of chill induction periods required for European pear vary among different cultivars. Summer maturing pears require a much shorter induction or no induction period (Bower et al. 2003) compared with later maturing pears, such as 'Comice' and 'Beurré D'Anjou' ('Anjou'), which require 4 and 6 weeks of cold storage, respectively; however, this is also dependent upon harvest time (Sugar et al. 2009). If fruits are left on trees to ripen, internal browning and other physiological disorders can often develop during storage or during shelf-life. Therefore, fruits are harvested well before ripening when skin background color is still green, and the flesh is hard and dry. For these fruit types, firmness and initiation of starch hydrolysis (using a starch pattern index) may serve as useful indicators to determine optimum harvest time. Ideally, several samples should be harvested from each seedling as fruit matures to ensure that fruit from at least one of these fruit samples has been collected at optimum harvest time.

4.3.1.1 Texture

Texture is a term used for the overall feel of food in the mouth and comprises properties that can be evaluated by touch. It can include biochemical components, such as particle size and shape, moisture content, lipid content, and cell wall composition, as well as mechanical factors (Sams 1999). Breeding programs often measure pear texture using a hedonic scale, which summarizes influences of fruit firmness, hardness, juiciness, flesh coarseness, grittiness, chewiness, crispness, fruit fiber, skin chewiness, and oral sensory response. This collective 'eating experience' has a very important influence on consumer acceptability of new products (Sams 1999). Although the genetics of pear texture is still poorly understood, seedling populations tend to reveal continuous segregation for this trait, with a general likelihood for polygenic control (Bell and Janick 1990). Bell (1991) has suggested that moderate genetic gain could be achieved through mass selection for texture as relatively large ratios of GCA to SCA variance along with moderate narrow-sense heritability (h^2 = 0.30) have been observed.

Firmness of ripe pear fruit varies considerably among species. European pears are generally eaten when soft, whereas Asian pear types are eaten firm. Most breeding programs concentrate on one or the other, thereby attending to local consumer demand for pear fruit that they are accustomed to.

In most European pear breeding programs, soft, melting, or buttery, and juicy textures are most commonly selected for (Bell et al. 1996), although occasionally either firm (Batlle et al. 2008) or 'almost' crisp textures, similar to 'Abaté Fétel', are also selected. In a study involving 10 European pear seedling populations, wherein

fruit are stored for 70 days at 0.5 °C followed by 7 days at 20 °C, White et al. (2000a, b) have found firmness heritability to be low (h_2 = 0.06). This may reflect the low genetic variation observed for fruit firmness among parents used in the study, and that ripening–inducing conditions have been adequate for this population.

In contrast, heritability for fruit firmness estimated for either Asian or interspecific hybrid pear seedling populations tends to be moderate to high. For example, heritability estimates have ranged from 0.14 to 0.56 for *P. pyrifolia* in a Japanese breeding program (Saito 2016; Abe et al. 1995), while estimates of 0.70 have been reported in *P. pyrifolia*, *P. ussuriensis,* and *P.* × *bretschneideri* seedling populations in a Korean breeding program (Shin et al. 2008). In New Zealand, heritability estimates for seedling populations with Asian, European, and interspecific hybrid parentages (White et al. 2000b) or of pear germplasm, including accessions of the same pear species, as well as those of interspecific hybrids, are high (h^2 = 0.62–0.67) (Kumar et al. 2017). Good genetic progress can be expected to be made in breeding for firm (or soft) textures from such seedling populations where a wide range of fruit firmness is present.

Juiciness is an important component of fruit quality in both European and Asian pears. In European pear, this trait is under both polygenic and monogenic controls (Hancock and Lobos 2008; Zielinski et al. 1965). Using a hedonic method of evaluation for juiciness along a 0–9 scale, White et al. (2000b) have reported that there is a low heritability for juiciness (h^2 = 0.04) in European seedling populations, thereby indicating there is little variation present in parents used. Moreover, when Asian and interspecific seedling populations are incorporated in the analysis, a slightly higher value (h^2 = 0.21) is observed.

Finally, for breeding programs of both European and Asian pears, there is strong selection against presence of grit or stone cells in flesh (h^2 = 0.57), skin, and to a lesser extent around the core, as well as toward fine (rather than coarse) texture (Bell and Janick 1990).

4.3.1.2 Flesh Color

Although white and cream are the most common flesh colors present in pear, green, yellow, pink, and red colors are also known to naturally occur. Segregation for white- and green-colored fruit flesh is controlled by a single gene, with white color being dominant, while green or cream colors serving as alternative alleles (Bell et al. 1996). Furthermore, segregation of progeny from crosses between the red-fleshed 'Sanquinole' and the white-fleshed 'Conference' has revealed that red flesh is dominant over white flesh (Bell et al. 1996).

4.3.1.3 Flavor

Flavor is an important attribute of any pear cultivar. It encompasses a combination of sweetness, sourness, bitterness, and astringency of oral sensory characters of pear fruits, along with volatile components sensed in the nose and throat (Brewer et al. 2008b; Dondini and Sansavini 2012; Bell et al. 1996). An important aspect of flavor is the sugar–acid balance, which is enhanced by the presence of volatiles, particularly in European pears (Eccher Zerbini 2002). As the presence of volatiles in Asian pear is less important, breeders have placed greater emphasis on high sugar levels when selecting genotypes for commercialization. Heritability estimates for overall flavor, from subjective scores, vary from low (h^2 = 0.06) in *P. communis* seedling populations to high (h^2 = 0.54) in interspecific hybrid seedling populations (Bell and Janick 1990).

4.3.1.4 Fruit Sweetness

High fruit sweetness is important for market acceptance of any pear cultivar (Jaeger et al. 2003). Sweetness, scored subjectively on a hedonic scale or assessed as soluble solids concentration, is a quantitative trait (Hancock and Lobos 2008). In an early study by White et al. (2000b), heritability of sweetness in European pear seedling populations and in hybrid European–Asian pear seedling populations is found to be low, h^2 = 0.05 and h^2 = 0.07, respectively, and similar to that (h^2 = 0.05) reported by Shin et al. (1983). These seedling populations have

been developed from crosses among parents selected for 'ideal' levels of sugar.

In contrast, Abe et al. (1995) have reported much higher heritability values (h^2 = 0.37–0.5) using randomly selected combinations of hybrid seedlings from the Japanese pear breeding program at the National Agriculture and Food Research Organization (NIFTS). Progress in breeding for higher sweetness in pear fruit could be achieved by selecting for genotypes with high flesh fructose concentrations. On a mole-to-mole basis, fructose has a perceived sweetness that is ~1.4–2-fold higher than other storage sugars present in pear fruit, including sucrose, sorbitol, and glucose (Harker et al. 2002; Saito 2016). Storage sugars in pear fruit consist of fructose, glucose, sorbitol, and sucrose (Saito 2016; Viera et al. 2013). In a New Zealand study on seedling populations of interspecific hybrids with different proportions of European, Japanese, and Chinese (*P.* × *bretschneideri*) parentages, average sugar levels are found to consist of 59% fructose, 13% glucose, 20% sorbitol, and 8% sucrose (Viera et al. 2013). In a Japanese study including 79 Asian cultivars from Japan, Korea, and China, it is reported that average percentage concentrations of these sugars are found to consist of 36.7% fructose, 15.2% glucose, 23.8% sorbitol, and 24.4% sucrose. In the New Zealand study, individual sugar levels of glucose, fructose, and sucrose contributed to higher genetic variance relative to total phenotypic variance (0.54–0.86) compared with that for total sugars (0.31). Interestingly, sorbitol levels have negative genetic correlation (rG = −0.65) with fructose, a relationship that warrants further investigation. Thus far, genetic markers associated with soluble solids concentration have been identified on LG10, LG5, and LG14, in an F1 population of 'Bayuehong' × 'Dangshansuli', but these have not been detected in all tested years (Wu et al. 2014).

4.3.1.5 Fruit Acidity

Organic acids are yet another significant component of pear fruit flavor serving to balance sweetness. For European pears, a range of acidity between pH 2.4 and 5.4 can be acceptable in commercial cultivars (Bell et al. 1996; Hancock and Lobos 2008). Levels of total organic acid vary within *Pyrus* taxa, wherein an average of 5.98 mg g^{-1} total organic acids has been reported for *P. ussuriensis*, 3.07 mg g^{-1} for *P.* × *bretschneideri*, 2.66 mg g^{-1} for *P. pyrifolia*, and 2.42 mg g^{-1} for *P. communis* (Sha et al. 2011). Moreover, relative and absolute acid levels can also be influenced by the environment (Hudina and Štampar 2004; Sha 2012; Sha et al. 2011). Thus, levels of individual organic acids present in both European and Asian pears can also vary. While malic and citric acids typically dominate, quinic, oxalic, shikimic, fumaric, tartaric, succinic, acetic, and lactic acids are also present (Liu et al. 2016; Sha et al. 2011). Fruit of *P. communis* is found to have higher acetic acid levels, while fruit of *P. ussuriensis* has higher quinic acid levels than those of other *Pyrus* species (Sha et al. 2011). Furthermore, malic and citric acids exhibit significant positive phenotypic correlations with quinic acid; whereas, significant negative correlations are observed between acetic and lactic acid and between quinic and tartaric acids (Sha et al. 2011).

In New Zealand, heritability of acidity evaluated on a hedonic scale was low in both European seedling populations alone, and when Asian and interspecific seedling populations were included, h^2 = 0.07 and 0.09, respectively (White et al. 2000b). Low heritability (h^2 = 0.17) for titratable acid was also identified through a genome-wide association study (GWAS) that included European, Asian, and interspecific hybrids (Kumar et al. 2017). However, Liu et al. (2016) reported high heritability of individual acids, including oxalic (h^2 = 0.88, 0.57), quinic (h^2 = 0.71, 0.58), malic (h^2 = 0.83, 0.77), shikimic (h^2 = 0.82, 0.50), and citric (h^2 = 0.75–0.80), when these were measured in consecutive years in progeny of a reciprocal cross of 'Dangshansuli' × 'Hosui.' It has been suggested that there was a maternal influence for inheritance of these acids. Thus, when breeding for lower acid levels, a parent with the lowest levels of oxalic, quinic, malic, and shikimic acids should be used as the female parent.

Single-nucleotide polymorphisms (SNPs) linked to titratable acidity have been identified on LG2 in a biparental cross between European and Asian species, and also in a genotyping-by-sequencing (GBS) study including European, Asian, and interspecific hybrids (Liu et al. 2011). A SNP associated with titratable acidity was also identified on LG7 in a New Zealand GBS study (Kumar et al. 2017).

4.3.1.6 Fruit Volatiles

Aromatic volatiles complement the sugar/acid balance in fruit and provide a cultivar's distinctive flavor. This is important for European pear cultivars, as they have a wide range of flavors, from the subtle 'Comice' (Eccher Zerbini 2002) to the strong distinctive flavor of 'Bartlett'. A total of 77 volatile compounds have been identified in fruit of 'Bartlett' (Bell et al. 1996), with decadienoate esters contributing the most to its characteristic flavor (Eccher Zerbini 2002). Fruits of other cultivars and selections, developed in breeding programs, with high levels of decadienoate esters are also deemed to possess a 'Bartlett' flavor.

Fruits of Asian pear cultivars are not typically known for their strong aromas, particularly those of Japanese pear, *P. pyrifolia*. However, fruits of some cultivars of *P. ussuriensis* have strong aromas, and these differ in their volatile compound compositions from those found in *P. communis* (Kang 2010). In addition, fruits of *P. ussuriensis* cultivars exhibit a very wide range of olefins, esters, alkanes, aldehydes, phenols, and ketones, and these cultivars serve as valuable breeding material for these aromatic compounds. Li et al. (2004) have identified variations in complex levels of volatile compounds in fruits of cultivars of *P. ussuriensis*, *P. communis*, *P.* × *bretschneideri,* and *P. pyrifolia*. Therefore, it is suggested that inheritance of these compounds is quantitative, and controlled by multiple genes. Analysis of 16 different volatile compounds from two families of *P.* × *bretschneideri* × *P. ussuriensis* has demonstrated high heritabilities for acetone, ethanol, propyl alcohol, and aldehyde, moderate heritabilities for ethylene, isopropanol, propionate ethyl, isovalerate, and low heritabilities for isopentanol and hexanol acetone (Li et al. 2004).

Breeding for flavors complemented by aromatic compounds is an important objective for the New Zealand Institute for Plant and Food Research Ltd (PFR) pear breeding program. Crosses among *P. communis*, *P. pyrifolia,* and *P.* × *bretschneideri* have generated interspecific hybrids bearing fruit with a wide range of different flavors (Brewer et al. 2008b). Adverse flavors, such as alcoholic, grassy, and high acid, are selected against. Interestingly, it has also been possible to select for pears with novel flavors that can develop when fruit are either on the tree and/or at any time during storage. Some individual selections bear fruit that do not seem to produce perceivable volatile flavors (Brewer et al. 2008b), while others bear fruit requiring chill induction before volatile flavors develop. Clearly, there is much for pear breeders to learn in developing cultivars carrying fruit with specific flavors (Xue et al. 2017b).

It is important to point out that those favorable flavors detected in fruit flesh are rarely identified in the skin. This may indicate that flavor development is differentially regulated in these tissues. Although bitterness, grassiness, and astringency can often be present in fruit skin, these are not perceived in fruit flesh (Brewer et al. 2008b).

4.3.1.7 Astringency and Bitterness

While all breeding programs for fresh consumption pears actively select against astringency and bitterness in fruit flesh, often little attention is paid to fruit skin or areas around the core. Breeding for cultivars destined for perry production is an exception, where both bitterness and astringency are desired (Bell et al. 1996). Low levels of astringency and bitterness can be acceptable for fresh consumption when this enhances the overall flavor perception. Bitterness and astringency are associated with presence of phenolic and polyphenolic compounds, including tannins and leucoanthocyanins (Bell et al. 1996). High levels of fruit astringency can be present when wild germplasm is used as parents in crosses for introgression of other desirable traits. In the New Zealand PFR breeding program,

bitterness is often detected in the skin of fruit of seedlings, but not as much in flesh of this fruit. Population-level improvements in decreasing bitterness and astringency have been reported, as both traits have virtually disappeared by the third generation (Brewer et al. 2008b), even though early research has indicated that there is a low heritability ($h^2 = 0.01$) for astringency (White et al. 2000b).

4.3.1.8 Fruit Size

Fruits of various pear species exhibit wide ranges for fruit size, as this is influenced by genetics, environment, and management factors, such as water availability, fruit set, fruit thinning, and overall crop load. *P. calleryana* and *P. betulaefolia*, commonly used as rootstocks, can bear fruit as small as 1 cm in diameter (Hancock and Lobos 2008). These species would require several generations of improvement for fruit to reach a suitable commercial size and eating quality. Cultivars of European, Japanese, and Chinese white pear, such as 'Uvedales Saint Germaine', 'Dongguanli', and 'Xuehuali', respectively, can produce very large fruit (Cao 2014). Pear fruit size is under polygenic control, but a range of heritability values, depending on the population used (Hancock and Lobos 2008). For example, in the NIFTS program in Japan, heritability values of $h^2 = 0.57–0.82$ have been reported for *P. pyrifolia* (Saito 2016), and in the Korean breeding program, heritability values ranging between $h^2 = 0.09$ and $h^2 = 0.85$ have been reported for interspecific hybrid populations among *P. pyrifolia*, *P. ussuriensis,* and *P.* × *bretschneideri* (Shin et al. 2008). In this latter study, heritability variations are dependent on the parental cultivar used in these crosses. For example, 'Whangkeumbae' and 'Gamcheonbae' are found to have high heritabilities, $h^2 = 0.76–0.85$ and $h^2 = 0.47–0.84$, respectively, for fruit size, while 'Niitaka' has a low heritability ($h^2 = 0.11–0.29$).

Quantitative trait loci (QTL) were identified for fruit weight in progeny of 'Bayuehong' × 'Dangshansuli' population, with a marker located at 16.3 cM from a QTL identified on LG17 of 'Dangshansuli'. In the second year of this study, marker Pyb13_250, associated with fruit size, was identified at 99.3 cM on LG13 of 'Bayuehong.' Additional research should be conducted to validate these markers.

4.3.1.9 Functional Compounds

To date, breeding programs have put very little effort into improving health attributes of pear fruit by increasing levels of bioactive compounds. However, consumer preferences are increasingly focused on health-promoting qualities of fruits and vegetables, and consumers can make purchasing decisions based on phytonutrient levels present in these foods (Patil et al. 2016). Researchers have quantified some bioactive compounds present in pear cultivars and germplasm (Abaci et al. 2016; Kolniak-Ostek 2016; Galvis Sánchez et al. 2003; Tanrıöven and Ekşi 2005; Yim and Nam 2015). Fortunately, presence of significant differences in contents of these bioactive compounds among pear cultivars offers opportunities for improvement in future breeding efforts, as does higher concentrations of anthocyanins in red skin and flesh of pear (Abaci et al. 2016; Yim and Nam 2015). Promotion of cultivars with research-supported health benefits is already underway (Sarkar et al. 2015; Stephenson 2015; Barbosa et al. 2013).

4.3.1.10 Storage Period and Shelf-life

Maintaining fruit in good condition during cool storage and until the point of sale is an important attribute of any new cultivar, and it is an important goal in many breeding programs (Bell et al. 1996). The PFR interspecific pear breeding program selection is strongly directed toward fruit that retains high-quality texture attributes following a minimum cold storage period of two months at 0.5–3 °C (Brewer et al. 2008b). Results from segregating seedling populations indicate that fruit storage potential is under polygenic control (Bell et al. 1996). Thus, there are several reasons why fruit may fail storage testing. The most common of these are

post-harvest disorders, such as internal browning, chilling injuries, and flesh spot decay (Brewer et al. 2008b).

Fruit ethylene production at harvest has been negatively associated with storage life in *P. pyrifolia*. Ethylene production in pear is controlled by two *1-amino-cyclopropane-1-carboxylic acid* (*ACC*) synthase genes, *pPPACS1* and *pPPACS2*, with dominant alleles associated with high and moderate ethylene levels, respectively. *PPACS2* has been mapped along the top of LG15 in *P. pyrifolia* (Itai et al. 1999). Many older Japanese pear cultivars carry the dominant *pPPACS1* allele, while newer cultivars tend to possess both recessive alleles. This finding reflects selection for material with longer storage/shelf-life and lower ethylene production in modern Japanese pear breeding programs (Itai and Fujita 2008). Restriction fragment length polymorphism (RFLP) markers for these two genes have been developed to predict low ethylene production in pear material in breeding programs (Itai and Fujita 2008). Interestingly, regulation of genes controlling ethylene production in *P.* × *bretschneideri* cultivars that are either climacteric ('Yali') or non-climacteric '(Hongli') is suggested to be similar to that observed in *P. pyrifolia* (Yamane et al. 2007). However, *P. communis* cultivars do not carry these *pPPACS* haplotypes (Oraguzie et al. 2010), thus suggesting presence of a separate system of ethylene control.

A long shelf-life for fruit following cold storage is also important for any newly released cultivar. Therefore, many breeding programs target a set shelf-life period following cold storage. At PFR, a period of seven days at 20 °C is a minimum standard used to simulate a typical time period for purchase and consumption of fruit (Brewer et al. 2008b). Taking advantage of the extended shelf-life inherent in many old Chinese pear cultivars, the New Zealand program maintains fruit from the best seedlings on a shelf at 20 °C until they either rot, turn internally brown, or shrivel. This approach has allowed for identification of advanced selections for up to 30 days of shelf-life following cold storage (Brewer and Palmer 2011).

4.3.2 Fruit Attractiveness

4.3.2.1 Fruit Shape

Pear fruit shape is under polygenic control with round and ovate shapes observed more frequently than pyriform and turbinate shapes in Asian, European, and interspecific hybrid seedling populations (White and Alspach 1996). A high heritability ($h^2 = 0.55$) for fruit length:-maximum width ratio suggests a relatively rapid progress can be made in breeding for fruit shape (White et al. 2000a). For European pear, acceptable genetic advances could be made for pyriform curvature ($h^2 \sim 0.5$), whereas the location of the point of maximum curvature has a low heritability ($h^2 = 0.01$) (White et al. 2000a). Therefore, identification of fruit shapes that are different from the typical pyriform fruit can be made, especially when pyriform-fruited parents are crossed with parents with either round- or ovate-shaped fruit.

4.3.3 Fruit Skin Ground Color

Background color of pear fruit skin is dependent on the relative concentrations of green (chlorophyll) and yellow (carotenoid) pigments present in the skin epidermis. During the ripening process in most pear cultivars, background color changes from green to either yellow-green or yellow following increase of carotenoids and/or breakdown of chlorophyll; however, the timing of this color change can vary considerably (Bell et al. 1996). In some cultivars, such as 'Conference' the skin remains fully green, but only turns yellow when the fruit is fully ripe, while for other cultivars, this change occurs at the onset of ripening; e.g., 'Packham's Triumph'. Genetic studies in European pear indicate that background skin color is controlled by a major gene, with yellow being dominant over green (Hancock and Lobos 2008). Inoue et al. (2006) have used a bulk segregant analysis of two F1 Japanese pear progenies to identify a 425-bp random amplification of polymorphic DNA (RAPD) marker associated with green skin color exhibiting a recombination rate of 7.3%. This RAPD

marker has been converted into a RAPD sequence-tagged site (STS) marker to identify a QTL at the top of LG8 at 2.2 cM (Yamamoto et al. 2014; Inoue et al. 2006).

4.3.4 Fruit Skin Over-Color

Red fruit over-color is an important breeding target for many programs around the world, as it can greatly enhance attractiveness of fruit (Brewer and Palmer 2011). Currently, red-skinned pears are sold at higher prices in international markets (Steyn et al. 2005). This is due to the low volume of these cultivars, but they also have high eating and storage qualities. Red color pigmentation is the result of accumulation of anthocyanins, specifically of cyanidin3-glactoside and cyanidin3-arabinoside, which are secondary metabolites synthesized, via the flavonoid biosynthetic pathway, in hyperdermal layers of the skin (Steyn et al. 2005; Thomson et al. 2018). Genetic expression of these anthocyanins is highly heritable, and hence can be readily exploited in breeding programs. However, anthocyanin levels are not always consistent, as these can change during fruit development, and may also vary under different environmental conditions, although they can also be enhanced by various cultural production practices (Thomson et al. 2018; Steyn et al. 2005).

In most flowering plants, fruit red skin color levels tend to develop most strongly during ripening (Thomson et al. 2018). Some pear cultivars, such as 'Bon Rouge' (a mutant of 'Bartlett'), 'Flamingo', and 'Rosemarie' appear to deviate from this pattern as they attain their maximum anthocyanin levels midway between anthesis and harvest. From then on, anthocyanin synthesis decreases slowly until harvest time in response to light, temperature, solar radiation, and competition for assimilates (Steyn et al. 2005; Thomson et al. 2018). Color development in pears either requires or is enhanced by light intensity, and wavelength (Thomson et al. 2018).

Dramatic drops in temperature as well as low temperatures promote increases in transcript levels of five anthocyanin biosynthetic genes involved in the anthocyanin biosynthesis pathway, and thereby inducing red skin color development (Ubi et al. 2006). On the other hand, high temperatures reduce anthocyanin biosynthesis through down-regulation of regulatory gene transcription factors for anthocyanin production, including those of *MYB*, *bHLH,* and *WD40* (Steyn et al. 2005; Thomson et al. 2018) which can also reduce the stability of existing anthocyanins (Mori et al. 2007). Anthocyanin degradation and color loss are reported to increase linearly between 10 and 30 °C (Steyn et al. 2005), more so in 'Rosemarie' because of its lower capacity to synthesise anthocyanin (Steyn et al. 2004). Higher concentrations of anthocyanin provide a buffer for color loss before high temperatures visibly affect red coloration of fruit skin (Steyn et al. 2004). Conversely, high-colored cultivars, such as 'Bon Rouge' and 'Flamingo', do not respond to low temperatures for anthocyanin synthesis, while 'Rosemarie' does.

It has been reported that in *P. communis*, high red fruit skin color pigmentation is attributed to spontaneous bud mutations of green-skinned cultivars, including 'Bartlett', 'Comice', and 'Beurré D'Anjou', wherein not only the fruit skin is red, but also those of leaves, especially of new shoot growth (Booi et al. 2005). Often, these mutations are not stable, and some tissues of a tree, such as leaves and fruit, can revert back to the original phenotype (Booi et al. 2005). Nevertheless, stable mutants of these cultivars have been commercialized, such as 'Max Red Bartlett', 'Bonne Rouge', and 'Sensation', all sports of 'Bartlett'. However, many red mutants released commercially, including 'Crimson Gem', a red 'Comice', have had limited success because of poor tree vigor and cropping (Dondini and Sansavini 2012). Furthermore, mutagenesis has also been used to develop commercial cultivars of 'Bartlett' with red skin pigmentation, such as 'Homored' (Dondini and Sansavini 2012). The red tissue color induced by such mutations is controlled by a major dominant gene with a simple 1:1 segregation ratio for red:green seedlings, for both leaf and fruit phenotypes, thus

indicating Mendelian inheritance for this trait (Booi et al. 2005). Subsequently, this red color has been mapped to LG4 using a simple sequence repeat (SSR)-enriched map of an 'Abbé Fétel' × 'Max Red Bartlett' seedling population (Pierantoni et al. 2004; Dondini et al. 2008).

Pierantoni et al. (2010) have mapped *PcMYB10*, which encodes an R2R3-MYB transcription factor involved in the control of the anthocyanin biosynthetic pathway, onto LG9 of both 'Abbé Fétel' and 'Max Red Bartlett'. This corresponds to the same location as *MdMYBa* and *MdMYB10* that control red color pigmentation in fruit skin of apple (Espley et al. 2007). The pear transcription factor *PyMYB10* gene, a likely ortholog of *MdMYB10,* has been positively associated with anthocyanin biosynthesis in ripening fruit of red-skinned pear, and its function has been confirmed (Feng et al. 2010; Yao et al. 2017). Yet, another transcription factor, *PyMYB114,* has been identified on LG5 of Chinese pear (*P.* × *bretschneideri*), and its abundance, correlated with *PyMYB10* in enhancing anthocyanin biosynthesis, is confirmed when co-transformed in both tobacco and strawberry (Yao et al. 2017). Kumar et al. (2017) have also identified a SNP associated with red skin phenotype on LG9, but it is unclear whether or not it is associated with *PcMYB10*. Recently, Ntladi et al. (2018) have mapped a major QTL near the telomeric region on LG9 of 'Abbé Fétel' that is associated with genes MYB21 and MYB39, which is found to be responsive to environmental changes, and varies between years.

Breeding programs have used a range of red-skinned bud sports, such as 'Max Red Bartlett', 'Red Sensation', and 'Rosired', as parents to transfer the red color pigmentation to new cultivars (Dondini and Sansavini 2012). Earlier, it has been reported that phenotypic selection for red leaf color is possible in segregating seedlings of young nursery plants (Booi et al. 2005), and that it is easy for breeders to identify seedlings carrying the dominant gene for red color without using marker-assisted selection (MAS). However, seedlings carrying a gene for red skin color, developed from red-skinned sports, develop leaves and fruit with varying intensities of red color pigmentation (Volz et al. 2008). Some mutants, such as 'Starkrimson', derived from 'Clapp's Favorite', are not capable of transferring red fruit skin coloration to their progeny as the mutation is only present in the epidermis, i.e., the germ layer does not carry the mutation (Bell et al. 1996).

Some genetic sources for red fruit skin color in both Asian and European cultivars are totally dependent on solar radiation and light to induce red blush development on fruit (Zhang 2012; Steyn et al. 2005). Therefore, presence of a gene (s) controlling red skin color from these sources cannot be inferred from red leaf color of seedlings. In a New Zealand study, segregation ratios of 5(non-blush):3(red blush) for fruit blush, derived from *P. pyrifolia* cv. Huobali, are observed in four seedling populations; whereas, segregation ratios of 3(non-blush):1(red blush) are obtained in three other seedling populations. Furthermore, when both parents are descendants of 'Huobali', segregation ratios of 3(non-blush):5 (red blush) in four seedling populations and 7 (non-blush):9(red blush) in three other seedling populations have been observed. These segregation ratios indicate that a complementary two-dominant gene control mechanism is present, wherein both genes are required for color development. A similar segregation pattern for red blush color fruit may also be observed for seedling populations involving *P. communis* cv. Louis Bonne de Jersey, an old French cultivar with red blush fruit (Volz et al. 2008). However, different segregation ratios have been observed at the Zhengzhou Fruit Research Institute (ZFRI) in China in crosses wherein both parents, 'Mantianhong', derived from 'Huobali', and 'Hongxiangsu', derived from 'Korla Pear', have red skin color fruit. Segregation ratios of 3(non-blush): 2 (red blush) and 9(non-blush):8(blush) in seedling populations of 'Mantianhong' × 'Hongxiangsu' and 'Yuluxiang' × 'Mantianhong', respectively, have suggested that the red skin color trait is controlled by a single dominant gene that tends toward green-skinned segregation (Xue et al. 2017a).

In the above Zhengzhou studies, red skin coloration mapped to a 111.9–177.1 cM QTL interval on LG5 (Xue et al. 2017a). This is a different chromosomal location to the dominant gene derived from the European pear 'Bartlett' which is mapped to LG4 (Dondini et al. 2008). Recently, Ntladi et al. (2018) have also identified two SSR markers, NB101a and SamsCo865954, that are closely associated with a major QTL for skin blush on LG5 in 'Flamingo'. These markers are present in approximately 90% of seedlings that scored a high blush level. Thereby, two candidate genes, MYB86 and UDP-glucosyltransferase, have been identified. Earlier, in an F1 population of 102 individuals from a cross of 'Bayuehong' ('Clapp's Favourite' (red sport) and 'Zaosu') × 'Dangshansuli', QTLs for control of red skin color have been mapped to LGs 4, 13, and 16 (Wu et al. 2014). Interestingly, the QTL on LG4 is located at 4.8 cM (Wu et al. 2014), differing from that mapped for 'Bartlett' at 64 cM (Dondini et al. 2008), while QTLs for red blush are located on LG13 or LG16, and are deemed to be novel. Collectively, these results suggest that additional research to elucidate these different loci controlling red color in pear along with their interactions must be conducted.

It is critical to point out that breeding for either full-red or blushed fruiting pear cultivars for hot climate regions is challenging, as fruit skin color loss, close to harvest time, can be high. Therefore, it is important to choose cultivars with the highest anthocyanin levels and fruit blush as parents in breeding programs to minimize the likelihood of anthocyanin degradation due to hot temperatures and intense light exposures in these environments (Steyn et al. 2004). In the joint Spanish Institut de Recerca i Tecnologia Agralimentàries (IRTA)/PFR breeding program, selection of parents with high levels of red color and carrying more than one source of red color genes have been successful in developing pear cultivars that retain high levels of red color at harvest time under Spanish growing conditions (Batlle et al. 2008).

It has been recently reported that very good breeding progress can be made by using parents that both carry more than one source of red color gene(s), as heritability is then found to be high ($h^2 = 0.86$) for red color fruit (Kumar et al. 2017). Once crosses are made using parents carrying multiple sources of red skin fruit color, MAS would be beneficial in identifying seedlings carrying specific sources of red color.

4.3.5 Fruit Russet

Unlike many other fruits, the presence of russet on fruit is acceptable for fresh market pears, as long as russet is smooth, and ideally, fully covering the skin (Bell et al. 1996). Russeting of the fruit pericarp is attributed to accumulation of a cork layer resulting from suppressed biosynthesis of suberin, cutin, and wax, and this layer can be either green or brown in color (Wang et al. 2014). Inoue et al. (2006) have obtained a 3:1 segregation ratio for russet:non-russet and partial russet fruit in an F1 seedling population where both parents have russeted fruit skin, and a 1:1 ratio in an F1 seedling population derived from fully russeted and partially russeted parents. White et al. (2000b) have calculated a low heritability ($h^2 = 0.16$) for russet in ten European pear seedling populations; however, when five Asian and interspecific crosses are included, the heritability is found to increase ($h^2 = 0.55$). This finding is similar to heritability values reported earlier (Bell and Janick 1990), as well as in a GBS study of European, Asian, and interspecific germplasm (Kumar et al. 2017).

Early on, Kikuchi (1924, 1930) has proposed that pear fruit russet is controlled by two loci, *R* and *I*. More recently, it is hypothesized that the *R* locus has a dominant effect on cork layer development, and the modifier locus I has a dominant effect on russet suppression (Saito 2016). In this proposed model, *RR* genotypes are completely russeted, *Rrii* are partially russeted, and *RrI* are partially russeted when environmental conditions are ideal (Hancock and Lobos 2008). A major QTL for russet has been identified on LG8 (Yamamoto et al. 2014; Kumar et al. 2017; Inoue et al. 2006).

4.3.6 Fruit Skin Friction Discoloration (Scuffing)

Marking of fruit skin (scuffing) during post-harvest handling operations and in the supermarket following cold storage is a serious problem for many commercial pear cultivars, as this downgrades fruit quality and discourages purchase (Brewer et al. 2011; Saeed et al. 2014). The mechanism causing scuffing involves a combination of physical stress and biochemical reactions, in particular enzymatic oxidation of polyphenols by polyphenol oxidase (PPO) (Saeed et al. 2014). Harvest maturity can influence scuffing susceptibility, although this trait is genotype dependent (Saeed et al. 2014).

Analysis of interspecific seedling populations derived from European and Asian pedigrees has revealed that scuffing has a high narrow-sense heritability of $h^2 = 0.72$ with a high correlation between years (Brewer et al. 2011). Using germplasm accessions of similar, but wider genetic backgrounds, a subsequent GBS study has confirmed this observed high heritability ($h^2 = 0.61$) and year-to-year repeatability (Kumar et al. 2017). It has been reported that susceptibility to low-scuffing is derived from Asian pear (Brewer et al. 2011), and this is supported by a finding that the largest effect SNP allele associated with scuffing is present in Asian but absent in European pear accessions (Kumar et al. 2017). Scuffing is a complex polygenic trait as highlighted by the identification of 105 QTLs associated with 22 relevant fruit traits, including those of average scuffing score, fruit firmness, polyphenoloxidase (PPO) activity, ascorbic acid concentration, and production of 17 polyphenolic compounds (Saeed et al. 2014). With this many small-effect QTLs distributed over 11 chromosomal regions (LGs 2, 3, 4, 7, 9, 10, 11, 13, 14, 15, and 16), it is suggested that genomic selection is better suited in identifying scuffing-resistant individuals early in the breeding cycle. In a GBS study, Kumar et al. (2017) have identified a SNP for scuffing on LG15.

4.4 Tree Production

Cultivars that produce many branches; i.e., 'feathering', naturally facilitate clonal propagation of trees by nurserymen, especially for those trees that will be planted in traditional orchard systems, wherein within-row planting distances are wider than those of closely planted systems. European pear cultivars 'Conference' and 'Abeté Fetel' produce high numbers of feathers in contrast to 'Passe Crassane' (Dondini and Sansavini 2012) and to Asian cultivars. Some Asian cultivars and interspecific hybrids develop few branches along with very upright-growing shoots. This suggests that heading of young trees planted in a nursery or an orchard, along with use of plant growth regulator treatments may be required to induce feathers. Currently, an understanding of the genetic factors controlling feather/shoot production is lacking.

4.4.1 Precocity

As perennial fruit trees have long juvenile periods, reducing this juvenility period is very important for all these breeding programs (Brewer and Palmer 2011). Pears grown commercially in countries like New Zealand must be competitive with apples in terms of speed to production (Brewer and Palmer 2011). Progress can be made in breeding for a reduced juvenile period in pears as this trait is under additive genetic control (Bell et al. 1996), and there is a positive correlation between length of the juvenility period and precocity of selections propagated onto rootstocks.

In general, seedlings of *P. pyrifolia* are more precocious than those of *P.* × *bretschneideri* and *P. communis* (Bell et al. 1996). Selection of parents for reduced juvenile period and increased precocity over several generations in the New Zealand program has enabled development of seedlings that can come into fruiting within three years following crossing in some interspecific hybrid progenies.

4.4.2 Harvest Season

Extending the harvest season will maximize use of grower and packing house resources and will support efforts in meeting market needs (Dondini and Sansavini 2012; Bell et al. 1996; Brewer and Palmer 2011; Saito et al. 2015). Although there is a high demand for the first fruit of the new season, many early season pear cultivars have poor fruit quality, small fruit size, uneven ripening, and short storability due to internal breakdown (Bell et al. 1996; Dondini and Sansavini 2012; Saito 2016). In an Asian pear seedling population, Abe et al. (1993) have observed a high positive correlation between mid-season ripening parents and fruit weight. Furthermore, the presence of a strong link between high ethylene production and early maturity in Japanese pear cultivars explains their observed poor storability (Itai et al. 2003).

It has been reported that fruit harvest date is a polygenic trait, with low environmental influence (Abe et al. 1993). A high heritability for ripening date, h^2 values of 0.80–0.95, has been reported in seedling populations of Asian heritage (Nishio et al. 2011; Abe et al. 1993). This has been further confirmed in a recent study wherein heritability of $h^2 = 0.83$ has been reported (Hae-Sung et al. 2015). On the other hand, moderate heritability ($h^2 = 0.49$) for ripening date has been reported in seedlings of late ripening parents of European pear heritage (Bell et al. 1996).

QTLs controlling harvest date have been identified at the bottom of LG3 (nearest marker: *BGA35*) and at the top of LG15 (nearest marker: *PPACS2*) of 'Taihaku' (Yamamoto et al. 2014). The *PPACS2* probe for an ACC synthase coding gene, identified in a DNA band of 0.8 kb in length, is found to be specific to *P. pyrifolia* cultivars producing moderate ethylene levels during ripening and storage (Saito 2016; Itai et al. 1999). Recently, Ntladi et al. (2018) have detected a QTL on LG9 of 'Flamingo' explaining more than 30% of the phenotypic variance, with 88% accuracy, for seedlings flowering earlier than either parent in a progeny of 'Flamingo' 'Abate Fetel'.

Given the moderate to high heritability for fruit ripening date reported above, choice of parents in breeding for early or late fruit ripening is important. If both parents are early season cultivars, a greater proportion of their progeny will have this desired trait (Bell et al. 1996). Similarly, if both parents are late-season cultivars, a larger proportion of their progeny will mature later in the season, as compared with progeny from one early- and one late-season parent (Bell et al. 1996). Newly improved early season European pear cultivars that have been released from Italian breeding programs include 'Etrusca', 'Sabina' (Bellini and Nin 2002), 'Tosca', 'Norma', and 'Carmen' (Rivalta et al. 2002), while in Japan, 'Hatsumaru' with fruit quality equivalent to 'Kosui' has recently been released (Saito 2016).

4.4.3 Parthenocarpy

Parthenocarpy, development of fruit without fertilization of ovules and rendering fruit seedless, is a useful commercial trait of European pear. This is especially important in some pear-growing regions in Europe whereby early spring frosts and adverse conditions can prevent effective pollination. It is reported that in some growing environments wherein pear cultivars are capable of developing parthenocarpic fruit, pollinators are not deemed necessary (Bell et al. 1996; Nishitani et al. 2012).

In a study investigating parthenocarpy in 31 accessions of several pear species, including *P.* × *bretschneideri*, *P. ussuriensis*, *P. pyrifolia*, *P. communis,* and interspecific hybrids, it is found that five tested European pear cultivars have consistently set fruit, and the fruit has enlarged size in the absence of pollination (Nishitani et al. 2012). Some Chinese and European cultivars, such as 'Mili', 'Wowoli', 'Alexandrine Douillard', 'Bartlett', and 'La France' are found to have partial compatibility when self-pollinated. Moreover, it is observed that Chinese and Japanese cultivars do not demonstrate consistent and stable fruit set without fertilization when compared to European

cultivars. Among these cultivars, 'La France' is deemed the best-performing cultivar, as non-fertilized fruit weighed only slightly less than pollinated fruit. Furthermore, it is observed that fruit weight and size of non-fertilized fruit are inherited, thus it should be possible to transfer this parthenocarpy trait from the European pear cultivar La France to Japanese or Chinese pears (Nishitani et al. 2012).

It has been reported that three phenylpropanoid pathway-related genes are found to be either up- or down-regulated in highly parthenocarpic pear cultivars (Nishitani et al. 2012). Therefore, breeding for parthenocarpy may be accelerated by using molecular markers for these three genes once these markers are developed and validated across species (Nishitani et al. 2012). However, parthenocarpy is a low priority in most Asian pear breeding programs (Nishitani et al. 2012), as absence of seeds in parthenocarpic fruits is associated with lower fruit flavor and lower soluble solid concentrations (Bell et al. 1996).

4.5 Adaptation to Abiotic and Biotic Stresses

4.5.1 Low-Chill Requirement

Temperate zone cultivars are not well adapted for regions with subtropical climates, wherein chill requirement, necessary to achieve adequate flowering, is often unmet. Breeding for adaptation for low-chill requirement, i.e., flowering after fewer chilling hours, is one approach to develop cultivars with satisfactory yields and acceptable fruit quality in regions with warmer climates. As time of bud break is not a good indicator of chilling requirement, it is preferable to screen seedling trees for number of buds breaking (Rumayor et al. 2005).

Japanese pear cultivars (*P. pyrifolia*) require approximately 800 chill hours to break dormancy (Yamamoto et al. 2010); whereas, the estimated minimum chill hour requirement at 3 ± 1 °C for some European pears such as 'Rocha', 'Packham's Triumph', and 'Forelle' is 750 h, while for others, such as 'Winter Bartlett', 'Red Bartlett', and 'Max Red Bartlett', approximately 1050 h of chilling is required (Kretzschmar et al. 2011). The majority of pear cultivars adapted to subtropical growing conditions belong to *P. pyrifolia.* While most European pear cultivars are not well adapted to these growing conditions, there are a few exceptions. These exceptions include 'Hood' and 'Flordahome' (requiring 250 chill hours between 3–5°C), both are hybrids between *P. communis* and *P. pyrifolia*. 'Flordahome' has been developed and released from the University of Florida breeding program in 1982 (Sherman and Lyrene 2003).

Interspecific hybridizations between *P. pyrifolia* and *P. communis* have been used to develop low-chill European pears; however, fruit quality of low-chill *P. communis* cultivars, such as 'Kieffer' (550 chill hours between 3 and 5 °C), 'Le Conte' (450 chill hours between 3 and 5 °C), and 'Garber', is low (Hauagge and Cummins 2013; Abd El-Zaher et al. 2015). Interestingly, F1 seedling populations in a Mexican pear breeding program have resulted in seedlings with chill requirements ranging from 0 to 500 chill hours (Rumayor et al. 2005). Moreover, evergreen types have been identified from an open-pollinated seed population of 'Hood', as these seedlings do not require low temperatures to break dormancy (Rumayor et al. 2005). Finally, breeders in Egypt have used 'Hood', 'LeConte', and 'Yali' in crosses, and have selected a range of seedlings requiring fewer than 200 chilling hours at 7 °C (Abd El-Zaher et al. 2015; Stephenson 2015; Barbosa et al. 2013).

4.5.2 Cold Hardiness

Pears are grown in many parts of the world where temperatures can drop low enough to cause cold injury to shoots, spurs, trunks, and roots that may result in tree death. Plant cold hardiness is a complex trait, as it is influenced by temperature, day length, and plant physiological status (Palonen and Buszard 1997). Thermal analysis can be used for measuring cold hardiness for some pear tissues (Quamme 1991).

However, breeding for cold adaptation is best undertaken under actual growing environments, which may include Northern regions of the USA, Canada, Europe, Russia, and Mongolia. Although genetic progress has been made, and pear cultivars have been developed that can withstand winter temperatures as low as −30 to −40 °C, fruit quality is not deemed as satisfactory as those commercial cultivars grown in major pear-growing regions (Bell 1991).

Low spring temperatures, particularly early spring frosts, often cause flower damage and crop loss. As flower buds do not supercool, the earlier a cultivar flowers, the greater the risk of spring frost damage (Bell et al. 1996; Palonen and Buszard 1997). Breeding for late flowering to avoid frost or to promote parthenocarpy is an option, as bloom date is highly heritable, but noting that late flowering is not linked to late fruiting (Quamme 1991; Palonen and Buszard 1997).

Although inheritance of cold hardiness has not been investigated in pear, it has been reported that cold hardiness in apple is under polygenic control with additive effects, and with little evidence for incidence of epistasis and dominance (Bell et al. 1996). A range of pear cultivars have been classified for their vulnerabilities to winter injury based on cold damage to xylem and frost injury to buds. In general, it has been reported that pear xylem and flower bud hardiness are not highly correlated (Bell 1991; Bell and Itai 2011).

4.5.3 Disease and Pest Resistance

The genus *Pyrus* is susceptible to damage from various numbers of diseases and pests (Bell et al. 1996). The importance of a specific pest or disease in any particular region will be dictated by the cost of control management as well as the detrimental economic impact on the crop, particularly whereby control is less than fully effective. In some cases, susceptible cultivars are excluded from certain regions due to devastating effects of a pathogen or pest on tree productivity and fruit quality.

Screening for genetic resistance to a pest or disease in a germplasm collection to develop new cultivars with either higher tolerance, or ideally, resistance is an attractive proposition for any pear breeding program. The long-term efficacy of resistance should be carefully considered, as breakdown of resistance by different strains of the pathogen or pest can occur (Bus et al. 2011). Therefore, breeding for durable resistance using multiple resistance genes should be a long-term goal for pear breeding programs, as it is already the case for apple (Bus et al. 2011).

Fruit quality breeding objectives, mentioned in earlier sections, should not be ignored while breeding for disease/pest resistance as no matter how strong and effective the resistance of a cultivar, consumer's interest is mainly focused on fruit attractiveness and eating quality. The genetic background conferring resistance/s should also be taken into consideration. For pear, the breeding cycle is at least 5 years, and evaluation before cultivar release can take in excess of 15 years. Thus, introgression of resistance genes carried by large-fruited eating cultivars and land races of *P. communis*, *P. pyrifolia*, *P.* × *bretschneideri,* and *P. ussuriensis* into new cultivars would yield high fruit quality more readily than introgression of resistance genes from small-fruited *Pyrus* species of poor fruit quality. More specifically, in breeding of European pears, introduction of resistance genes from other European pears is highly desirable, and equally, introduction of resistance genes from Asian species is more suitable in breeding for Asian pears.

This section of the review concentrates on current status of breeding for resistance to the three major diseases of pear, including fire blight, pear scab, and black spot, as well as for the important economic pest of pear psylla (Psylla).

4.5.3.1 Fire Blight Resistance

Fire blight, caused by the bacterium *E. amylovora* (Burrill) Winslow et al., is a serious disease of pear, and indeed of various other Rosaceae species (Van der Zwet et al. 2012). This disease originated in the USA, and has been first reported in 1718 in the Hudson Valley, New York. Since

then, it has spread throughout every region of the USA, as well as throughout Europe, Middle East, Oceania (New Zealand), and has recently been detected in Kurdistan and South Korea (Park et al. 2017). The most common commercial cultivars grown today in North America and in Europe are known to be either susceptible, such as 'Bartlett', 'Abate Fetel', 'Beurré D'Anjou', 'Beurré Bosc', 'Comice', or only moderately resistant such as 'Conference,' to fire blight. These cultivars are grown in regions where climates are not very conducive for fire blight disease development, so growers are able to manage the disease somewhat satisfactorily.

Over the last 40 years, efforts have been undertaken to evaluate and assess fire blight resistance status of *Pyrus* germplasm (Bell et al. 1996; Bell and Itai 2011; Peil et al. 2009; Van der Zwet et al. 2012). While total immunity to fire blight has not been observed, high levels of resistance have been identified in some pear species. The proportion of resistant material in European, circum-Mediterranean, and Central Asian species tends to be lower than that found in East Asian species. However, Van der Zwet et al. (2012) have scored 14 of 75 'popular commercial' European pear cultivars and 24 of 76 Asian/Oriental pear as 'most resistant.' Since the year 2000, several new *P. communis* cultivars have been released that are reported to have high levels of fire blight resistance (Dondini and Sansavini 2012; Hunter and Layne 2004).

Screening methods used to determine fire blight resistance of cultivars, breeding selections, and hybrid seedlings have been reviewed extensively (Bell et al. 1996; Peil et al. 2009). Long-term field assessments are required to confirm a genotype's fire blight status, and a standardized scoring system for rating fire blight infection of trees has been developed. However, there can be substantial non-genetic variability in these assessments; hence, breeders have endeavored to control the timing and entry point of fire blight inoculum to improve assessment of inherent resistance. Artificial plant inoculations and/or use of greenhouse/plastic tent facilities to optimize environmental conditions are now commonplace in breeding programs. Where clonal replicates of a genotype are screened, frequency and severity of infection can be determined and are combined to yield a calculated index of fire blight susceptibility.

*M*ost strains of *E. amylovora* isolated from apple are capable of infecting pear and vice versa (Momol and Aldwinckle 2000). While this bacterium is a relatively genetically homogenous species (Khan et al. 2012), there is a diversity in pathogenicity among different *E. amylovora* strains (Cabrefiga and Montesinos 2005; Wang et al. 2010; Smits et al. 2017). However, unlike in apple (Norelli et al. 1984), to date there is no evidence that differential responses of the pathogen to different resistant pear genotypes exist. Pear genotypes with varying degrees of resistance to fire blight have been inoculated with several different strains of the pathogen, including some that have been previously shown to be differentially virulent on apple. While differences in host resistance and strain virulence have been confirmed, no interactions between host and strain have been observed (Quamme and Bonn 1981; Bell et al. 1990; Bell and Van der Zwet 1996). This has led to the conclusion that differentially virulent strains do not need to be considered in breeding for fire blight resistance in pear, at least in the USA (Bell and Van der Zwet 1987). Nevertheless, given that differentially virulent *E. amylovora* strains have developed against fire blight-resistant apple cultivars, it seems advisable to aim for durable fire blight resistance in pear by incorporating multiple disease resistance genes into pear breeding programs.

The genetics of fire blight resistance first received attention in the USA in the 1960s, when segregation for resistance in breeding progenies, mainly of interspecific Asian × European hybrids, derived from parents of known resistance were observed. No immunity was detected in any pear genotype, and segregation of seedlings for necrotic lesions of shoots following inoculation generally followed a continuous pattern. This suggested that inheritance for resistance was quantitative with presence of several resistance genes, and there was no pattern of inheritance specific to a certain pear species

(Layne et al. 1968; Van der Zwet et al. 1974). Further studies reinforced the hypothesis that additive gene action was the main mechanism by which fire blight resistance was inherited in pear in the USA (Bell et al. 1977), Canada (Quamme et al. 1990), Italy (Bagnara et al. 1996), and France (Durel et al. 2004). At least 18 small-to-moderate-effect QTLs, some of which may be the same, have been identified for control of fire blight resistance in European and Asian pedigrees in three genetic mapping populations (Boksccczanin et al. 2009; Bell 2018; Montanari et al. 2016b; Dondini et al. 2004). This further confirmed earlier findings that fire blight resistance is polygenically controlled.

As considerable parent-to-parent variability in capacity to transmit resistance to progeny has been observed, fire blight resistance cannot be entirely explained by the parent's own phenotypic resistance. This supports hypotheses proposing that non-additive genetic effects may also contribute to fire blight inheritance, although major dominant resistance (Drain 1943; Thompson et al. 1962) or sensitivity (susceptibility) genes in *P. communis* (Thompson et al. 1975) are also likely involved. In genetic mapping studies, minor-effect QTLs controlling resistance have been detected in susceptible parents (Boksccczanin et al. 2009; Montanari et al. 2016b). This may explain recovery of resistant genotypes that are sometimes developed from susceptible parents (Van der Zwet 1977; Bagnara et al. 1993).

4.5.3.2 Resistance to Pear Scab

Pear can be infected by two species of *Venturia,* inciting pear scab disease. *V. pirina* Aderh. infects *P. communis,* while *V. nashicola* (Tanaka and Yamamoto 1964) infects all cultivated species of Asian pear. Each fungal species is specific to its host pear species (Abe et al. 2008; Tanaka and Yamamoto 1964), thus the economic significance of each fungal species is tightly linked to the geographic distribution of the cultivated host species. *V. pirina* occurs worldwide except for East Asia, while *V. nashicola* is restricted to China, Japan, and Korea (González-Domínguez et al. 2017).

Venturia Nashicola

Some wild species of *Pyrus* are fully resistant to *V. nashicola* (Ishii et al. 1992), but of more interest to breeders is the discovery that several commercial pear cultivars are immune to this fungal pathogen, including the Japanese pear cultivar 'Kinchaku' and the Chinese pears 'Hongli', 'Mili', and 'Cangxili'. Furthermore, it has been demonstrated that progeny generated from crosses between either 'Kinchaku' (Abe and Kotobuki 1998a) or genotypes derived from 'Kinchaku' (Terakami et al. 2006) with susceptible cultivars segregate into seedlings either with no symptoms (resistant) or with abundant sporulation (susceptible) (Abe and Kotobuki 1998a). The 'Kinchaku' resistance has been used extensively in Japanese breeding programs, and a scab-resistant cultivar, 'Hoshiakari', carrying the 'Kinchaku' resistance has been named and released (Saito 2016).

A dominant major gene (*Vnk*) controlling this scab resistance has been mapped to LG1, with one SSR marker and five STS markers found to be tightly linked to this gene (Terakami et al. 2006). Two flanking markers, used together, have accurately predicted resistant seedlings in segregating progenies derived from 'Kinchaku' (Gonai et al. 2009). These markers are currently being used in MAS for scab resistance in Asian pear breeding programs in Japan (Yamamoto and Terakami 2016).

Immunity to *V. nashicola* in European pear cultivars, including 'Bartlett' and 'La France', has been reported to be transmitted to their progeny and purported to be controlled by single dominant genes (Abe et al. 2000). Subsequent genetic studies have indicated that a QTL for resistance from 'La France' (Yamamoto et al. 2009) and a major dominant gene conferring resistance from 'Bartlett' (*Rvn2*) (Cho et al. 2009; Bouvier et al. 2012) are likely to be the same, as both mapped to the bottom of LG2. However, the scope of resistance to *V. nashicola* may not be exactly the same for each cultivar, as Yamamoto et al. (2009) have mapped a second QTL for resistance, derived from 'La France', to LG14. Furthermore, two cleaved amplified

polymorphic sequence (CAPS) markers tightly linked to *RVn2* have been developed for likely use in MAS (Cho et al. 2009).

It has been reported that non-host resistance to *V. nashicola* derived from European pears may provide broader spectrum resistance than host resistance derived from Asian pears, as they are effective against all races of the pathogen (Gill et al. 2015). Often, host resistance is race-specific, involving gene-for-gene relationships, and may be less durable. Indeed, five races of *V. nashicola,* collected from various regions in Asia, have shown differential reactions to different hosts (Zhao et al. 2012). However, use of non-host resistance from *P. communis* in Asian pear breeding may be disadvantageous, as it may incorporate less desirable alleles from European pear. Nevertheless, Kim et al. (2016) have introgressed resistance from 'Bartlett' into *P. pyrifolia* 'Whangkeumbae' to develop a new Korean cultivar, 'Greensis'.

Partial resistance to *V. nashicola* has been observed in several Asian pear cultivars and their progeny, as well as in progeny derived from European pear. Abe et al. (2000) speculated that this resistance reaction was under polygenic control. Differences in incidence of necrotic leaf tissues have been observed among commercial Korean cultivars in replicated field trials (Won et al. 2011). Four major gene loci were involved in varying necrotic resistance reactions observed in leaf inoculation studies using a segregating progeny, derived from two resistant seedlings of 'Yali' x 'Jingbaili' that have been backcrossed to their parents, susceptible cultivars 'Yali' (*P.* × *bretschneideri*) and 'Jingbaili' (*P. ussuriensis*) (Zhang et al. 2012).

Venturia pirina

Most *P. communis* cultivars have demonstrated a range of susceptibility to *V. pirina* in the field, although results have not always been consistent (Vondracek 1982; Postman et al. 2005). Hence, most scab resistance in *P. communis* is presumed to be polygenic, and recent genetic mapping in several partially resistant cultivars has confirmed this finding. For instance, resistance in 'Abè Fétel' is proposed to be controlled by two independent major QTLs on LGs 3 and 7, and collectively explaining ~88% of the observed variation in susceptibility in progeny of 'Abè Fétel' × 'Max Red Bartlett', a scab-susceptible cultivar (Pierantoni et al. 2007). A locus on LG1 confers resistance derived from 'Wilder' with a major QTL (67%) co-localized with the major gene *Vnk* on the pear genome (Perchepied et al. 2015). Recently, a major resistance gene (*Rvp1*), derived from 'Navara', has been identified on LG2 (Bouvier et al. 2012), indicating that such genes are present in *P. communis* germplasm. The SSR marker CH02b10 is mapped close to this gene. As is the case for *V. nashicola*, *V. pirina* also shows strain heterogeneity in pathogenicity to different resistance reactions present in *P. communis* (Chevalier et al. 2004). The breeding strategy in *P. communis* should aim to bring together a number of resistance QTL and major genes in order to achieve resistance durability in new cultivars.

Asian pear cultivars are generally resistant to *V. pirina* (Postman et al. 2005) and may serve as useful sources of non-host resistance in European pear breeding. However, these sources of resistance are less well understood. A major QTL is identified on LG4 from a breeding selection, likely derived from *P. pyrifolia* (Perchepied et al. 2015). Moreover, seven QTL controlling resistance (two each on LG7 and LG2, as well as one each on LG5, LG10, and LG17) have been identified in a complex interspecific hybrid family derived from *P. communis*, *P. pyrifolia,* and *P. ussuriensis* (Won et al. 2014). Furthermore, all of these QTLs have exhibited differential responses to discrete *V. pirina* isolates, except for the QTL on LG17 which is effective against all strains. However, the host/non-host nature of the QTL has not been established in this study, as not all accessions in the pedigree have been available for marker analysis.

While resistance to *V. nashicola* in leaf tissues extends to the fruit (Abe et al. 2008), this is not always the case for resistance to *V. pirina*. Some Asian and European pear cultivars (Postman et al. 2005), as well as interspecific hybrids derived from Asian and European pears (Brewer et al. 2009), have exhibited leaf resistance

reactions to *V. pirina,* but have shown some scab on fruit, thus indicating presence of a differential resistance reaction depending on tissue. Hence, reliance on leaf resistance symptoms as an indicator of total plant resistance may not always be appropriate. Further studies are warranted to develop a better understanding of resistance response of pear fruit to *V. pirina.*

4.5.3.3 Pear psylla

Pyrus hosts several species of the pear psylla (Psyllidae: Psyllinae: *Cacopsylla* spp.), but only three are of economic importance (Hodkinson 2009; Ouvrard 2017). *Cacopsylla pyricola* Foerster is the most widespread, and it is presently found in Europe, the Middle East, North and South America, Argentina, Russia, South Korea, and Japan (Ouvrard 2017). *Cacopsylla pyri* Linné dominates in Europe, but has also been reported in the Middle East and Central Asia, including China. *Cacopsylla bidens* Šulc is present in France, Italy, Greece, central Asia, including India, as well as South America (Valle et al. 2017).

The control of pear psylla in commercial pear orchards is handled by using selective pesticides along with a range of active natural predators (Trapman and Blommers 1992). However, the psylla reproduces prolifically, with multiple generations per year, and readily develops resistance to many pesticides (Civolani 2012).

All of the major commercial cultivars of *P. communis* are susceptible to pear psylla. Therefore, incorporation of resistance to this pest into new cultivars has been an important objective for several European pear breeding programs. Fortunately, partially resistant *P. communis* cultivars, originating mainly in Eastern Europe, have been identified (Bell and Stuart 1990; Sestras et al. 2009; Benedek et al. 2010; Bell 1992, 2013a), and used in some breeding programs (Branişte et al. 2008). However, transmission of resistance to progenies has often been poor (Bell 2013b). For example, the old Italian cultivar 'Spina Carpi' is resistant, but it does not transmit this resistance to its progeny (Rivalta et al. 2002). This may reflect the inherent low narrow-sense heritability of this resistance (Bell 2013b).

Immunity to pear psylla within *Pyrus* has not been documented (Quarta and Puggioni 1985; Briolini et al. 1988). However, there is a wide variation in resistance responses to *C. pyricola* among *Pyrus* species, first documented in North America by Westigard et al. (1970) and Quamme (1984), and well summarized by Bell and Itai (2011). East Asian pear species are generally resistant, whereas mid-Asian, Mediterranean, and European species exhibit a wide range of response, from susceptible to resistant.

Introgression of psylla resistance from Asian pear species into high-quality *P. communis* cultivars was initiated in the USA, back in the 1960s. It was reported that large-fruited *P. ussuriensis* material crossed with *P. communis* cv.Bartlett transferred its psylla resistance to a majority of the progeny (Harris and Lamb 1973). Subsequently, a backcrossing strategy to 'Bartlett', as well as to other *P. communis* cultivars was followed in the USA (Harris and Lamb 1973), as well as in both Italy and France (Lespinasse et al. 2008; Nin et al. 2012). Two second-generation cousin hybrids, NY10353 and NY10355, with improved fruit quality performance and resistance to *Psylla,* have been extensively used in breeding programs in the USA, Italy, and France (Pasqualini et al. 2006; Nin et al. 2012; Dondini and Sansavini 2012).

One of the major hurdles in introgressing psylla resistance into new pear cultivars has been the poor fruit quality of resistant progenitors and the seemingly difficult task of improving fruit quality in subsequent generations. Harris and Lamb (1973) have suggested that the *P. ussuriensis* source of resistance avoided some undesirable fruit quality attributes, such as small size and flesh grittiness. However, psylla-resistant selections originating from this source, as well as those derived from Eastern European-resistant *P. communis* cultivars, have not exhibited the quality required of a modern new pear cultivar (Bell 2013b). Thus far, no cultivar has yet been released from these breeding efforts.

It has been reported that psylla resistance from *P. ussuriensis* seems to be under polygenic control (Lespinasse et al. 2008). A major QTL for control of pear psylla located on LG17 of a pear selection, NY10355 (Bouvier et al. 2011), has been confirmed along with two additional QTLs located on LG1 and LG4. A strong epistatic interaction has been observed between the latter QTLs and that on LG17 (Perchepied et al. 2016). Nearly all of the genetic variation in psylla nymph infestation is explained by these QTLs. The major resistance QTL on LG17 has also been identified in segregating progeny of NY10353 (Dondini et al. 2015). The SSR markers CH05G03 (Dondini et al. 2015) and NB126a-2 (Perchepied et al. 2016), closely linked to the QTL controlling resistance on LG17, have been identified from NY10353 and NY10355, respectively, and provide a first step in developing promising resources for MAS.

In other efforts, a *P.* × *bretschneideri* × *P. communis* hybrid that is partially resistant to *C. pyri* is reported to transmit psylla resistance to its progeny when crossed with the *P. communis* cultivar 'Moonglow' (Montanari et al. 2015). This resistance, most likely to be derived from 'Xuehauli', is different from those of other *P. ussuriensis* lines as a QTL for resistance is located on LG8, but not on LG17. This QTL explains up to 30 to 39% of the observed phenotypic variation in total numbers of psylla nymphs. Further, this QTL is found to be stable over two years of testing, along with an SSR marker, CH05a02, that is closely associated with this QTL. Several other minor QTLs for resistance, located on LG5, 11, and 15 (from 'Moonglow'), have also been identified, but these are not stable over years of testing, and their significance is inconclusive. Some interspecific hybrids of susceptible *P. communis* × resistant *P. pyrifolia* have also shown resistance to psylla, but the genetic mechanisms of these resistances are yet unknown (Robert and Raimbault 2005; Pasqualini et al. 2006).

It is unknown if different biotypes of pear psylla exist that can overcome any of the above reported resistances. Puterka (1997) has found that *C. pyricola* collected from five regions in the USA has demonstrated similar responses to both susceptible and resistant pear germplasm from different sources. Interestingly, the *P. ussuriensis*-derived resistance line developed in the USA for *C. pyricola* is also resistant to *C. pyri* in Europe (Robert and Raimbault 2005; Pasqualini et al. 2006), as well as to *C. bidens* in Israel (Shaltiel-Harpaz et al. 2014). These reports suggest presence of a relatively broad-spectrum resistance for pear psylla. Nevertheless, given the rapid development of pesticide-resistant strains of pear psylla over the last few decades (Civolani 2012), breeding should aim for resistance that is durable through pyramiding of different QTLs for resistance (Corwin and Kliebenstein 2017).

The modes of host resistance to pear psylla have been studied extensively for several resistance sources (Bell and Puterka 2004). Both nymphal feeding antixenosis (unpalatability) and nymph antibiosis (mortality) are deemed important, but ovipositional antixenosis is less important for tested resistant selections derived from both *P. ussuriensis* and *P. communis*. In contrast, *P.* × *bretschneideri* resistance, derived from 'Xuehauli', is attributed to both antibiosis and ovipositional antixenosis (Montanari et al. 2015). To date, mapping studies have not yet conclusively revealed the presence of specific QTLs associated with each of these different modes of resistance (Montanari et al. 2015). Further investigation is needed to better understand the genetic mechanism of these different components of *Pyrus* resistance to pear psylla in order to identify better resources for developing psylla-resistant cultivars.

In summary, there is a reasonable understanding of the genetics of the major scab resistance gene *Vnk* for *V. nashicola,* and molecular markers linked to this resistance are being used in some Japanese pear breeding programs. Furthermore, numerous sources of resistance to *V. pirina*, fire blight, and pear psylla have been identified, and these have been used in various pear breeding programs. However, in contrast to *V. nashicola* resistance, these sources of resistance have more complex genetics that is not well documented. Efficient and effective

incorporation of these various genes for resistance to these different diseases and pest into future pear cultivars can only be enhanced following thorough understanding of their genetics involved in these traits, as well as subsequent development and application of their associated molecular markers.

4.6 Rootstock Breeding

Pear growers have a limited number and range of clonal rootstocks to choose from when designing a new orchard, compared with their apple counterparts. This range is even more limited if a vigor-controlling rootstock is required, as dwarfing rootstocks equivalent to the precocious flowering and high-yielding apple rootstock 'Malling 9' are lacking (Knäbel et al. 2015; Brewer and Palmer 2011). Rootstock options for pear growers include several *Pyrus* species and alternatives from other species, such as *Cydonia oblonga* (quince), *Amelanchier alnifolia* (serviceberry), *Actaea spicata (baneberry)*, *Amelanchier canadensis (juneberry), Amelanchier lamarckii (juneberry)*, *Sorbus aucuparia* (mountain ash), *Sorbus alnifolia* (alder-leafed whitebeam), and *Pyronia veitchii* (*C. oblonga* × *P. communis*) (Elkins et al. 2012; Postman 1994).

4.6.1 Quince—*Cydonia oblonga*

Quince rootstocks are preferred in Europe because of their strong vigor control and precocity of the pear scion, as well as ease of propagation (Brewer and Palmer 2011; Necas et al. 2016). However, these have several limitations to more widespread use, including lack of cold hardiness, limited fire blight resistance, scion incompatibility, and susceptibility to iron chlorosis (Elkins et al. 2012). There has been limited breeding of quince rootstocks to address these issues (Brewer and Palmer 2011).

Scion vigor-controlling rootstocks include the semi-dwarfing 'BA29' (60% of tree size compared to that of *P. betulaefolia* seedling rootstock) (Elkins et al. 2012), developed at the French National Institute of Agricultural Research (INRA) and released in 1967 (Simard et al. 2004), the dwarfing 'Quince A' (QA), and the dwarfing 'Quince EMC' (QC) rootstocks, both released from East Malling Research Station in the United Kingdom in the 1920s (Anon.). Graft compatibility testing of pear cultivars on Quince rootstocks have suggested that 'Beurré D'Anjou', 'Comice', 'Old Home', 'Beurré Hardy', 'Flemish Beauty', 'Abbé Fetel', 'Passe Crassane', and 'Maxine' are compatible, but 'Bartlett', 'Beurré Bosc', 'Winter Nelis', 'Clapp's Favourite', and 'Forelle' are not (Lombard and Westwood 1987). Since the release of 'BA29', QA, and QC, the Quince Eline® rootstock has been released by Boomkwekerij Fleuren in Belgium. Quince Eline®, originated from a Romanian breeding program, has been developed for increased frost resistance. This rootstock is comparable to QC for scion vigor and fruit size, and it is reported to have good graft compatibility with most pear cultivars, along with frost resistance to temperatures of about −25 °C (Anon.; Brewer and Palmer 2011). In 2001, East Malling has released 'QR193/16' (EMH), originally claimed to control scion vigor similar to that of QC; however, further research has indicated that vigor control ranges between that of QC and QA (Webster et al. 2000). Although EMH contributes to good fruit size development and has good stool bed performance, it shows poor precocity relative to QC, and it is susceptible to fire blight (Brewer and Palmer 2011). EMH has been selected from seed presumed to have originated from Transcaucasia. Research efforts at the University of Pisa in Italy on breeding rootstocks tolerant to calcareous soils have led to the release of the selection 'Ct.S 212'; however, this is not resistant to fire blight, and more recently has demonstrated some inconsistency in fruit production of grafted scion cultivars (Brewer and Palmer 2011).

In a quest for developing more dwarfing quince rootstocks that have cold resistance, a large number of accessions have been selected

from the National Clonal Germplasm Repository at Corvallis (Oregon, USA) and have been screened for cold hardiness. A total of 22 quince selections have been found to be as hardy, or hardier, than standard commercial *Pyrus* rootstocks, including 'Old Home' × 'Farmingdale 87' and 'Old Home' × 'Farmingdale 97', surviving temperatures as low as −30 °C. Among these, the ten best-performing selections are currently being evaluated in research programs in Wenatchee (Washington State) and Hood River (Oregon) in the USA (Warner 2015). The best-performing rootstocks for cold tolerance have originated from Armenia, Turkmenistan, Russia, Uzbekistan, the Russian Federation, Georgia, and France, with the most cold resistant being *C. oblonga*-Arakseni, 'Avia' from Gebeseud, and 'Akhtubinskaya', an open-pollinated seedling 4 (Einhorn et al. 2017; Anon.).

4.6.2 *Pyrus*

Pyrus rootstocks are the preferred choice in North America, Asia, and Australia. A wide range of species have been used in breeding programs or in commercial orchards, including *P. communis*, *P. betulaefolia* Bge., *P. calleryana* Dcne., *P. pashia* D. Don, *P. xerophila* Yu, *P. ussuriensis* Maxim, *P. heterofolia*, *P. nivalis*, *P. longipes*, and *P. pyrifolia* Nakai (Brewer and Palmer 2011; Tamura 2012; Teng 2011; Simard et al. 2004). *Pyrus* rootstocks have good graft compatibility, a satisfactory range of cold adaptation, and can grow well in low to high pH soils. However, they have limited vigor control and precocity induction of the scion, varying levels of tolerance to *Candidatus* Phytoplasma pyri (inciting pear decline), and are generally difficult to propagate (Brewer and Palmer 2011). A continuing challenge for pear rootstock breeders is to combine vigor control and precocity of the scion, that can be obtained from Quince rootstock options, with other important traits required for a successful rootstock. This may require use of more than a single species to combine all of these required traits.

4.6.2.1 *P. communis*

P. communis is the species most widely used as a rootstock in North America, with seedlings of 'Winter Nelis' and 'Bartlett' being the main rootstocks currently used commercially (Elkins et al. 2012). However, grafted pear trees are mostly vigorous, yet they are adapted to a range of climates and soil types (Hancock and Lobos 2008). Although fire blight susceptibility is common in *P. communis,* seedling populations have been established to develop rootstocks with fire blight resistance along with some tree size reduction or dwarfing (Hancock and Lobos 2008). Globally, there are limited numbers of *P. communis* rootstocks that offer significant grafted tree size reduction. Research efforts in the USA have demonstrated that size of a grafted pear tree on 'Pyrodwarf®' is similar to that grafted on Quince 'BA29' (Brewer and Palmer 2011), and only 61–70% of that grafted on *P. betulaefolia* seedling rootstocks (Elkins et al. 2012). However, tree performance has varied depending on planting site, scion cultivar, and management practices (Elkins et al. 2012). Furthermore, yield efficiency has been poor compared to that obtained with QC, QA, and many *Amelanchier* rootstocks (Einhorn et al. 2017). In 1996, the University of Bologna in Italy has released *P. communis rootstocks* 'Fox 11' and 'Fox 16', and in 2008 has released 'Fox 9'. However, all three rootstocks are more vigorous than quince BA29 (Brewer and Palmer 2011).

From a rootstock breeding perspective, it is important to identify individuals carrying traits required as soon as possible, especially for the scion dwarfing trait. QTLs influencing expression of scion vigor and precocity have been located on LG5 and LG6 of 'Old Home' in an 'Old Home' × 'Louise Bonne de Jersey' seedling population. It is reported that the QTL on LG5 maps to a position that is syntenic to the apple 'Malling 9' *Dw1* locus located at the top end of LG5 (Knäbel et al. 2015). This QTL for rootstock control of numbers of branches produced by a grafted scion cultivar is detected in three successive years, and it is co-located with the flowering trait for total number of

inflorescences on a tree. The microsatellite marker Hi01c04, located within the QTL region on LG5, is heterozygous in both 'Old Home' and 'Louise Bonne de Jersey', and its trait association is found to be consistent over a number of years. A small-effect QTL for root suckering is also detected on LG5 within the same genomic region as that QTL for tree architecture (Knäbel et al. 2015). In the same population, QTLs have been identified on LG7 controlling development of adventitious roots on hardwood cuttings of both 'Old Home' and 'Louise Bonne de Jersey' (Knabel et al. 2017). Both of these discoveries will support efforts in developing genetic markers useful in future breeding efforts of desirable *Pyrus* rootstocks.

4.6.2.2 *P. longipes*

Rootstocks of *P. longipes* offer very good tree root anchorage, graft compatibility, and high tolerance to the bacterial canker *Pseudomonas syringae*, but provide only moderate precocity and yield efficiency, susceptibility to fire blight, and limited tolerance to pear decline (Lombard and Westwood 1987). Breeding efforts at Dresden-Pillnitz in Germany have used *P. longipes* to target improved propagation ability, dwarfing, resistance to biotic and abiotic stress, superior tree anchorage, yield, and fruit quality, as well as reduced suckering and burr knot development (Fischer 2007). A wide range of interspecific crosses have been made, and seven new *Pyrus* rootstocks have been selected, ranging from 'very dwarfing' to 'medium strong'. One of these selections, 'Pi-BU 3', has been reported to confer vigor that is 40–60% of that of *P. betulaefolia* seedling rootstocks (Elkins et al. 2012). Tree losses have been reported in German trials which may indicate that some levels of graft incompatibility must have occurred, and 'Pi-Bu 3' has not matched quince rootstocks for yield or yield efficiency (Brewer and Palmer 2011).

4.6.2.3 *P. nivalis*

Used as a rootstock, perry pear (*P. nivalis*) displays satisfactory tree anchorage, good graft compatibility, limited root suckering, adequate adaption to winter cold temperatures, and high tolerance to pear decline, but only moderate yield precocity and performance, as well as moderate tolerance to bacterial canker (Lombard and Westwood 1987). The Brossier series, developed in France in 1962, have utilized five open-pollinated seedling populations of *P. nivalis* to generate selections having a range of rootstock vigor. Furthermore, seedlings have displayed good graft compatibility, low vigor, and a range of tolerance to fire blight; however, they have also displayed poor to very poor ability for clonal propagation, ranging from 1 to 54% for semi-hardwood cuttings. The best genotype selected in this series, G28-120, confers similar tree vigor to that of 'BA29', it is graft compatible with 'Bartlett,' induces regular cropping and good fruit size, but it is susceptible to fire blight, has low ability for clonal propagation (31% by hardwood cuttings), and does not transplant well (Simard et al. 2004).

4.6.2.4 *P. calleryana*

As a seedling rootstock, *P. calleryana* exhibits very good tree anchorage and graft compatibility, moderate yield efficiency and precocity, moderate susceptibility to fruit cork spot, and resistance to black end of fruit (a physiological disorder of fruit). Grafted trees on this rootstock display high tolerance to various diseases and pests, including fire blight, *Podosphaera leucotricha* Salm. (inciting powdery mildew), *Agrobacterium tumefaciens* Conn. (inciting crown gall), *Phytophthora cactorum* Schroet (causing collar rot), *Eriosoma Pyricola* (woolly pear aphid), and *Pratylenchus vulnus* (root lesion nematode) (Lombard and Westwood 1987). Overall, *P. calleryana* has a superior adaption to most environmental conditions compared with that of *P. pyrifolia,* but it is susceptible to lime-induced chlorosis, and it is only moderately tolerant to pear decline (Tamura 2012; Teng 2011; Bell 1991).

Rootstocks of *P. calleryana* are commonly grown as seedlings in Japan, and in both North and South China. Studies have been conducted to

identify and propagate superior strains using clonal propagation (Teng 2011; Tamura 2012; Banno et al. 1988). Some strains display good rooting ability as softwood cuttings, while others exhibit growth control of grafted scion cultivars (Brewer and Palmer 2011) with marked dwarfing when grafted with Japanese cultivars (Tamura 2012). A particular clone, *P. calleryana* D6, is considered to be superior in Australia, where it is the most commonly used pear rootstock. D6 is a clonal stock selected from seed supplied by Nanjing University (China) in 1929. The rootstock is vigorous, producing a large tree when used for grafting scions, but it is compatible with most cultivars (Anon. 2014). Currently, clonal reselection rather than breeding is being conducted. Therefore, additional research efforts are required before a reliable dwarfing *P. calleryana* rootstock is developed.

4.6.2.5 *P. betulaefolia*

Rootstocks of *P. betulaefolia* have very good soil anchorage and graft compatibility, produce vigorous trees with moderate precocity and yield efficiency in scions, along with fruit that does not display black end, but with low tolerance to cork spot. *P. betulaefolia* has high tolerance to pear decline, bacterial canker, leaf spot, powdery mildew, crown gall, collar rot, woolly aphid, and root lesion nematode (Lombard and Westwood 1987). Similar to *P. calleryana*, it exhibits superior adaption to various environmental conditions, especially to hot humid conditions, and it is used widely throughout Asia (Tamura 2012). In the USA, *P. betulaefolia is* used as a rootstock on heavy clay soils and used as a standard for high vigor (Elkins et al. 2012). Although high vigor is a disadvantage, *P. betulaefolia* rootstocks are very drought and salt tolerant, can withstand temperatures down to −45 °C if cold hardened, but have low tolerance to alkaline soils (Tamura 2012). The use of *P. betulaefolia* rootstocks is also effective for avoiding black end in European pears or 'Yuzuhada' in Japanese pears. Similar to *P. calleryana*, some selections have displayed good rooting, as well as size control of scion cultivars (Tamura 2012).

4.6.2.6 *P. heterofolia*

At INRA, open-pollinated populations of *P. heterofolia* (closely related to *P. betulaefolia*) have been evaluated to select for agronomic traits, particularly for fire blight tolerance and ability for clonal propagation. Seedlings have also been screened for erect nursery habits, without branching, and for iron chlorosis tolerance (Simard et al. 2004). Scion growth grafted onto selection 'P2532' is similar to that on Quince 'BA29', but 'P2532' induces more vigorous growth of scions, similar to that of 'Old Home' × 'Farmingdale 333', and produces fruit of good size, but it is susceptible to fire blight (Simard et al. 2004).

4.6.2.7 *P. xerophila*

Rootstocks of *P. xerophila* may serve as good options in semi-arid regions, as this species is very drought tolerant. The cultivar 'Mu-Li' has displayed superior root growth in highly alkaline soils and can sustain growth in soils up to pH 8.0 (Tamura 2012).

4.6.2.8 *P. pyrifolia* Nakai

Although *P. pyrifolia* has been used as a rootstock in southern areas of China, it is not the rootstock of choice in most countries. It is not cold hardy, can be damaged under conditions of low temperatures (Yu-Lin 1996), displays poor tolerance to drought, but with flood and salt tolerance, yet it grows poorly on alkaline soils, it is susceptible to pear decline, and adapts poorly to clay soils (Bell 1991; Tamura 2012; Elkins et al. 2012). It does not produce root suckers, exhibits good tree anchorage, graft compatibility, good yield efficiency, shows moderate precocity, and has moderate tolerance to fire blight, bacterial canker, and powdery mildew, but can induce black end of in the scion (Lombard and Westwood 1987).

4.6.2.9 *P. ussuriensis* Maxim

Rootstocks of *P. ussuriensis* are the most cold hardy of the *Pyrus* species (down to −50 °C) (Teng 2011) and deemed most suitable for North Eastern China (Yu-Lin 1996). Seedlings have a

low tendency to produce root suckers, although trees have good soil root anchorage, graft compatibility, good yield efficiency, but fruit of scions is susceptible to black end. Furthermore, *P. ussuriensis* is susceptible to pear decline and root lesion nematode, but it is highly tolerant to fire blight, powdery mildew, and woolly aphid (Elkins et al. 2012; Lombard and Westwood 1987).

4.6.2.10 *P. pashia*

Nepal pear (*P. pashia*) is commonly used as a rootstock for Japanese pears in East Asia (Tamura 2012). It is also used as a rootstock in the Yunnan province of China (Yu-Lin 1996). In China, there are wide variations in morphology and vigor within seedling populations, thus providing opportunities for selecting dwarfing types (Teng 2011). *P. pashia* is not cold tolerant, and stems can be damaged at temperatures of −16 °C and below. This species tolerates low pH soils, but not high pH, and can grow on either sandy or clay soils (Bell 1991). Trees have good root anchorage and graft compatibility, but confer only moderate precocity and yield efficiency. *P. Pashia* has high tolerance to pear decline and bacterial canker, moderate tolerance to powdery mildew, collar rot, and woolly aphid, but low tolerance to fire blight, leaf spot, and root lesion nematode (Lombard and Westwood 1987).

4.6.3 *Amelanchier* Species

Dwarfing rootstocks for pear have been selected from *Amelanchier* seedlings at the Bavarian Centre of Pomology and Fruit Breeding in Germany (Brewer and Palmer 2011). This species is considered to possess moderate to high tolerance to fire blight, excellent cold hardiness, fair to good graft compatibility with *Pyrus* (high for 'Comice' and 'Beurré Hardy'), low production of root suckers, and it is potentially a non-host for pear decline, but trees can have poor root anchorage (Einhorn et al. 2017; Lombard and Westwood 1987).

Most evaluated selections offer a higher yield efficiency than 'Pyrodwarf'®, and many are either equivalent to or better than QA, and have either equal or significantly higher levels of cold hardiness than commercial *P. communis* rootstocks. Some selections look very promising as dwarfing rootstock options for US growers (Einhorn et al. 2017).

4.6.4 *Sorbus* Species

Sorbus (mountain ash) is being assessed as a potential pear rootstock that can provide scion dwarfing for intensive production. Although scion dwarfing of less than 40% of the size of *P. betulaefolia* seedling rootstocks has been reported, graft compatibility with *Pyrus* is considered poor to good (Elkins et al. 2012). The dwarfing ability of *Sorbus* along with its high tolerance to several pests and diseases are its best attributes as these trees have only moderate anchorage to the soil, and grafted scions exhibit low precocity and yield efficiency (Lombard and Westwood 1987).

4.6.5 Interspecific and Intergeneric Hybrids

Researchers at INRA have used the best selections from several different species to develop rootstocks adapted to Northern European conditions, and that are dwarfing, tolerant to fire blight, exhibit good productivity, and are easily propagated (Simard et al. 2004). Interspecific hybrids have also been used in collaboration with IRTA in Spain to develop rootstocks adapted to Mediterranean conditions. Crosses between 'Pyriam' (*P. communis*) and four Mediterranean species have been used to combine additional necessary traits of iron tolerance, drought tolerance, and propagation ability (Simard et al. 2004).

Materials of *Pyronia* (*Pyrus* × *Cydonia*) and *Sorbopyrus* (*P. communis* × *Sorbus*) are at early stages of evaluation as potential pear rootstocks. *Pyronia* is considered to have good graft compatibility with pear cultivars (Elkins et al. 2012).

In summary, various *Pyrus* and non-*Pyrus* germplasm are being used as pear rootstocks around the world. However, there have been little focused breeding efforts using this wide germplasm over a sustained period to develop rootstocks that fulfill the requirements of a modern pear orchard. A better understanding is needed of the genetics of important rootstock traits, including dwarfing, precocity, compatibility, and adaptation to a range of abiotic and biotic stresses. This is in stark contrast to our more sophisticated genetic knowledge of many of the fruit and tree characters of the scion itself.

4.7 Genomics-Assisted Breeding

Compared with other rosaceous fruit crop species, genomics-assisted breeding in pear is still in its infancy. Over the last 20 years, new genomic tools have been developed and applied to improve the efficiency and effectiveness of breeding in apple, peach, strawberry, and sweet cherry (Peace 2017; Laurens et al. 2018; van Nocker and Gardiner 2014). Applications range from a better understanding of trait genetics, through confirmation of parentage and pedigree, calculation of relatedness among potential parents, to either single-locus (MAS) or whole-genome-wide marker-assisted (genomic selection [GS]) seedling and parental selection.

The development of genomic resources specific to pear is now progressing quickly and will enable genomic-assisted breeding to proceed. The recently published draft genomes of the Chinese pear 'Dangshansuli' (Wu et al. 2013) and European pear 'Bartlett' (Chagne et al. 2014) have facilitated development of new and lower cost genotyping methods, such as GBS, to produce high-density molecular markers on pear genetic maps (Kumar et al. 2017).

As we have described, the genetics of self-compatibility, scab resistance, and harvest time have been reasonably well studied in Japanese pear, with each controlled by either one or two major genes, or by major-effect QTLs. Markers linked to these traits are being used for MAS in Japanese pear breeding (Saito 2016), thereby reducing progeny size and cost of growing seedlings to maturity in the field (Luby and Shaw 2001). However, for nearly all other selection traits that are important in pear, relationships between phenotype and genotype are less clear. Knowledge is lacking as to how many loci, and which loci, are important in consistently explaining genetic variations observed in specific traits. Further linkage analyses using biparental genetic mapping families and GWAS across less-related individuals in pear breeding germplasm sets of interest will be required to determine these large-effect marker–trait relationships, and how MAS might be best implemented in particular pear breeding programs.

GS offers the potential of utilizing large numbers of molecular markers distributed across the genome, some of which may be linked to small-, as well as to large-effect loci to explain and predict genetic variations in either one or more traits simultaneously, and without necessarily understanding the function(s) of causative loci involved (Kumar et al. 2012; Desta and Ortiz 2014). The advantage of this in fruit tree species, such as that of pear with a 4- to 10-year juvenile period, is that selections can be evaluated as potential cultivars or as breeding parents well prior to fruiting. This can significantly reduce the time frame from crossing to commercial cultivar release and increase the genetic gain per unit time.

In Japanese pear, GS has been conducted using only 162 genome-wide molecular markers in a set of 76 cultivars for nine traits having reasonably high linkage disequilibrium (Iwata et al. 2013a). These predictions have showed mostly moderate correlations with observed values (using leave-one-out cross-validations), indicating the potential of GS technology for use in this breeding germplasm, despite of the relatively low number of markers utilized. Furthermore, it has been demonstrated that GS can also predict segregation of traits in a Japanese pear progeny with reasonable accuracy, based on the whole-genome molecular marker profile of the two parents (Iwata et al. 2013b). Further studies exploring the potential uses of GS in pear breeding are warranted.

In many parts of the world, the genetic makeup of pear fruit available to consumers has not changed over the last 100 years. Efforts to develop enhanced rootstocks for pear have advanced only slightly, and pear production is often limited by the relatively poor performance of the rootstock of choice, particularly when compared with the status for apple. This provides enormous market opportunities for pear breeders to provide novel types of pear fruit and new rootstocks, by taking advantage of the wide and relatively untapped diversity among *Pyrus,* and across other genera for developing new rootstocks.

The biology of pear, as of many perennial tree fruit crops, dictates that classical breeding, which relies solely on phenotype and pedigree to produce new cultivars, will be a relatively slow and costly process in today's world. With appropriate research and cost–benefit analyses, new genomic technologies offer a potential to substantially improve pear scion and rootstock breeding efforts, thereby accelerating development of a range of new pear cultivars that will excite the future consumer, and that can be profitably grown by producers.

References

Abaci ZT, Sevinindik E, Ayvaz M (2016) Comparative study of bioactive components in pear genotypes from Ardahan/Turkey. Biotech Biotechnol Equip 30(1):36–43

Abd El-Zaher MH, Essa MA, Khahil BM, El-Bassel EH (2015) Selection of low chilling requirements of F1 pear hybrid seedlings. J Hort Sci Orn Plants 7:1–6

Abe K, Kotobuki K (1998a) Inheritance of high resistance to *Venturia nashicola* Tanaka et Yamamoto in Japanese pear (*Pyrus pyrofolia* Nakai) and Chinese pear (*P. ussuriensis* Maxim.). J Jpn Soc Hort Sci 67(5):677–680

Abe K, Sato Y, Saito T, Kurihara A, Kotobuki K (1993) Inheritance of ripening time of fruit of Japanese pear (*Pryus pyrifolia* Nakai). Jpn J Breed 43(2):289–298. https://doi.org/10.1270/jsbbs1951.43.289

Abe K, Saito Y, Kurihara A, Kotobuki K (1995) Narrow-sense heritability of fruit characters in Japanese pear (*Pyrus pyrifolia* Nakai). Breed Sci 45:1–5

Abe K, Kotobuki K, Saito T, Terai O (2000) Inheritance of resistance to pear scab from European pears to Asian pears. J Jpn Soc Hort Sci 69:1–8. https://doi.org/10.2503/jjshs.69.1

Abe K, Saito T, Terai O, Sato Y, Kotobuki K (2008) Genotypic difference for the susceptibility of Japanese, Chinese and European pears to *Venturia nashicola*, the cause of scab on Asian pears. Plant Breed 127 (4):407–412

Ahloowalia BS, Maluszynski M, Nichterlein K (2004) Global impact of mutation-derived varieties. Euphytica 135(2):187–204

Anon (2014) Rootstocks. Apple and Pear Australia, 23/5/2018

Anon About Q-Eline. http://www.q-elinenet/about-q-eline/, 25/5/2018

Anon Rootstock research at East Malling: a history. http://www.emr.ac.uk/projects/rootstock-research-east-malling-history/, 25/5/2018

Anon Cydonia Catalog NCGR-Corvallis. https://www.arsusdagov/ARSUserFiles/20721500/catalogs/cydcoldhtml, 25/5/2018

Bagnara GL, Rivalta L, Laghi M, Quarta R, Lecomte P (1993) Cross combinations for fire blight resistance in pear. Acta Hort 338:369–374

Bagnara GL, Rivalta L, Laghi M, Quarta R (1996) Evaluation of fire blight resistance in pear: Combining ability and breeding strategy. Acta Hortic 441:383–392

Banno K, Hayashi T, Tanabe K, Tokuzumi A (1988) In vitro propagation of Japanese pear rootstocks. Plant Tiss Cult Lett 5(2):87–89

Barbosa ACL, Sarkar D, Pinto MDS, Ankolekar C, Greene D, Shetty K (2013) Type 2 diabetes relevant bioactive potential of freshly harvested and long-term stored pears using in vitro assay models. J Food Biochem 37(6):677–686

Batlle I, Lozano L, Iglesias I, Carbó J, Bonany J, White AG, Volz RK, Brewer LR (2008) The IRTA-HR pear scion breeding programme: aiming for high fruit quality under warm growing conditions. Acta Hortic 800:455–460

Bell RL (1991) Pears (*Pyrus*). In: Moore JN, Ballington JR (eds) Genetic resources of temperate fruit and nut crops. Acta Hortic, vol 290, pp 657–700

Bell RL (1992) Additional East European *Pyrus* germplasm with resistances to pear psylla nymphal feeding. HortScience 27(5):412–413

Bell RL (2013a) Host resistance to pear pyslla of breeding program selections and cultivars. HortScience 48:143–145

Bell RL (2013b) Inheritance of resistance to pear psylla nymphal feeding in pear (*Pyrus communis* L.) of European origin. HortScience 48(4):425–427

Bell RL (2019) Genetics, genomics, and breeding for fire blight resistance in pear. In: Korban SS (ed) The pear genome. Intl Springer Publ.

Bell RL, Itai A (2011) *Pyrus*. In: Kole C (ed) Wild Crop Relatives: genomic and breeding resources: temperate fruits. Springer, Berlin, pp 147–177

Bell RL, Janick J (1990) Quantitative genetic analysis of fruit quality in pear. J Am Soc Hort Sci 115(5):829–834

Bell RL, Puterka GL (2004) Modes of host plant resistance to pear psylla: a review. Acta Hortic 663:183–188

Bell RL, Stuart LC (1990) Resistance to eastern European Pyrus germplasm to pear psylla nymphal feeding. HortScience 25(7):789–791

Bell RL, Van der Zwet T (1987) Virulence of *Erwinia amylovora* isolates on *Pyrus* host clones. HortScience 22:1058

Bell RL, Van der Zwet T (1996) Stability of host resistance of pear to fire blight. Acta Hortic 411:413–414

Bell RL, Janick J, Zimmerman RH, Van der Zwet T (1977) Estimation of heritability and combining ability for fire blight resistance in pear. J Am Soc Hort Sci 102(2):133–138

Bell RL, van der Zwet T, Thibault B, Bonn WG, Lecomte P (1990) Environmental and strain effects on screening for fire blight resistance. Acta Hortic 237:343–350

Bell RL, Quamme HA, Layne REC, Skirven RM (1996) Pears. In: Jannick J, Moore JN (eds) Fruit breeding. Tree and tropical fruits. Wiley, NY, pp 441–514

Bellini E, Nin S (2002) Breeding for new traits in pear. Acta Hortic 596:217–224

Belrose I (2016) World Pear Review 2016. www.e-belrose.com

Benedek P, Szabó T, Nyéki J, Soltész M, Szabó Z, Konrád-Németh C (2010) Susceptibility of European pear genotypes in a gene bank to pear psylla damage and possible exploitation of resistant varieties in organic farming. Intl J Hort Sci 16(3):95–101

Bhat ZA, Dhillon WS, S Shafi RH, Rather JA, Mir AH, Shafi W, Rashid R, Bhat JA, Rather TR, Wani TA (2012) Influence of storage temperature on viability and in vitro germination capacity of pear (*Pyrus* spp.) pollen. J Agric Sci 4 (11):128

Boksccczanin K, Dondini L, Przybyla AA (2009) First report on the presence of fire blight resistance in linkage group 11 of *Pyrus ussuerensis* Maxim. J Appl Genet 50(2):99–104

Booi S, Dyk MMv, Preez MGd, Rees DJG, Labuschagne I (2005) Molecular typing of red and green phenotypes of 'Bon Rouge' pear trees, with the use of microsatellites. Acta Hortic 671: 293–297

Bouvier L, Bourcy M, Boulay M, Tellier M, Guérif P, Denancé C, Durel C-E, Lespinasse Y (2011) European pear cultivar resistance to bio-pests: Scab (*Venturia pirini*) and Pyslla (*Cacopyslla pyri*). Acta Hortic 909:459–470

Bouvier L, Bourcy M, Boulay M, Tellier M, Guerif P, Denance C, Durel CE, Lespinasse Y (2012) A new pear scab resistance gene *Rvp1* from the European pear cultivar 'Navara' maps in a genomic region syntenic to an apple scab resistance gene cluster on linkage group 2. Tree Genet Genomes 8(1):53–60

Bower JH, Biasi WV, Mitcham E (2003) Effect of ethylene in the storage environment on quality of 'Bartlett pears'. Postharv Biol Tech 28:371–379

Branişte N, Andrieş N, Ghidra V (2008) Pear genetic breeding to improve Romanian varieties. Acta Hortic 800:491–496

Brewer LR, Palmer JW (2011) Global pear breeding programmes: goals, trends and progress for new cultivars and new rootstocks. Acta Hortic 909:105–120

Brewer LR, Alspach P, Morgan C (2008a) Manipulation of pear seedlings to reduce juvenility. Acta Hortic 800:289–296

Brewer LR, Morgan C, Alspach PA, Volz RK, White AG (2008b) Interspecific pear breeding for flavour and texture. Acta Hortic 800:461–468

Brewer LR, Alspach PA, Morgan C, Bus VGM (2009) Resistance to scab caused by *Venturia pirina* in interspecific pear (*Pyrus* spp.) hybrids. NZ J Crop Hort Sci 37(3):211–218

Brewer LR, Morgan CGT, Alspach PA, Volz RK (2011) Heritability and parental breeding value estimates of abrasion-induced skin discolouration on pear fruit. Acta Hortic 909:127–136

Briolini G, Cappeli A, Rivalta L, Rosati P (1988) Observations on *Pyrus communis* resistance to *Psylla pyri*. Acta Hortic 224:211–222

Bus VGM, Rikkerink EHA, Caffier V, Durel C-E, Plummer KM (2011) Revision of the nomenclature of the differential host-pathogen interactions of *Venturia inaequalis* and *Malus*. Ann Rev Phytopathol 49 (1):391–413. https://doi.org/10.1146/annurev-phyto-072910-095339

Cabrefiga J, Montesinos E (2005) Analysis of aggressiveness of *Erwinia amylovora* using disease-dose and time relationships. Phytopathol 95(12):1430–1437

Cao Ye (2014) Pear varieties in China. China Agricultural Press

Cao Y, Huang L, Li S, Yang Y (2002) Genetics of ploidy and hybridized combination types for polyploid breeding in pear. Acta Hortic 587:207–210

Cao Y, Chang YH, Chen Q, Dai M, Dong X, Hu H, Liu J, Qi D, Shi Z, Sun J, Tian L, Wang Y, Wang W, Zhang YY, Zhang J (2014) Pear varieties in China. China Agricultural Press, Beijing

Chagne D, Crowhurst RN, Pindo M, Thrimawithana A, Deng C, Ireland H, Fiers M, Dzierzon H, Cestaro A, Fontana P, Bianco L, Lu A, Storey R, Knaebel M, Saeed M, Montanari S, Kim YK, Nicolini D, Larger S, Stefani E, Allan AC, Bowen J, Harvey I, Johnston J, Malnoy M, Troggio M, Perchepied L, Sawyer G, Wiedow C, Won K, Viola R, Hellens RP, Brewer L, Bus VGM, Schaffer RJ, Gardiner SE, Velasco R (2014) The draft genome sequence of European pear (*Pyrus communis* L. 'Bartlett'). Plos One 9(4). https://doi.org/10.1371/journal.pone.0092644

Chevalier M, Bernard C, Tellier M, Lespinasse Y, Filmond R, Le Lezec M (2004) Variability in the reaction of several pear (*Pyrus communis*) cultivars to different inocula of *Venturia pirini*. Acta Hortic 663:177–182

Cho KH, Shin IS, Kim KT, Suh EJ, Hong SS, Lee HJ (2009) Development of AFLP and CAPS markers

linked to the scab resistance gene, *Rvn2*, in an inter-specific hybrid pear (*Pyrus* spp.). J Hort Sci Biotech 84(6):619–624
Civolani S (2012) The past and present of pear protections against the pear psylla, *Cacopsylla pyri* L. In: Perveen F (ed) Insecticides—pest engineering. InTech, Rijeka, pp 385–408
Corwin JA, Kliebenstein DJ (2017) Quantitaitve resistance: more than just perception of a pathogen. Plant Cell 29:655–665
Crane MB, Lewis D (1940) Genetical studies in pears. II. A classification of cultivated varieties. J Pomol 18:52–60
Desta ZA, Ortiz R (2014) Genomic selection: genome-wide prediction in plant improvement. Trends Plant Sci 19(9):592–601
Dondini L, Sansavini S (2012) European pear. In: Badenes J, Byrne DH (eds) Fruit breeding. Handbook of plant breeding, vol 8. Springer Science+Business Media, 369–413
Dondini L, Costa F, Pierantoni L, Gaiotti F, Chiodini R, Tartarini S, Sansavini S (2004) The RGA family. Promising gene analog related to fireblight and Sharka resistance in pear and apricot. Acta Hortic 663:161–165
Dondini L, Pierantoni L, Ancarani V, D'Angelo M, Cho KH, Shin IS, Musacchi S, Kang SJ, Sansavini S (2008) The inheritance of the red colour character in European pear (*Pyrus communis*) and its map position in the mutated cultivar 'Max Red Bartlett'. Plant Breed 127(5):524–526
Dondini L, De Franceschi P, Ancarani V, Civolani S, Fano EA, Musacchi S (2015) Identification of a QTL for psylla resistance in pear via genome scanning approach. Sci Hort 197:568–572
Drain BD (1943) Southern pear breeding. Proc Am Soc Hort Sci 42:301–304
Durel CE, Guerif P, Belouin A, Le Lezec M (2004) Estimation of fire blight resistance heritability in the French pear breeding programme using a pedigree-based approach. Acta Hortic 663:251–255
Eccher Zerbini P (2002) The quality of pear fruit. Acta Hortic 596:805–810
Einhorn T, Postman J, Dittrich F, Treutter D, Neumüller M (2017) Development of cold-hardy Quince and Amelanchier rootstocks for dwarfing, precocity, and high productivity of pear. http://interperaweebly.com/uploads/1/7/0/4/17040934/einhorn_presentationpdf
Elkins R, Bell R, Einhorn T (2012) Needs assessment for future US pear rootstock research directions based on the current state of pear production and rootstock research. J Am Soc Hort Sci 66:153–163
Espley R, Hellens RP, Putterill J, Stevenson DE, Kutty-Amma S, Allan AC (2007) Red colouration in apple fruit is due to the activity of the MYB transcription factor, *MdMYB10*. Plant J 49(3):414–427
Feng S, Wang Y, Yang S, Xu Y, Chen X (2010) Anthocyanin biosynthesis in pears is regulated by a R2R3-MYB transcription factor PyMYB10. Planta 232(1):245–255
Fischer M (2007) New pear rootstocks from Dresden-Pillnitz. Acta Hort 732:239–245
Galvis Sánchez AC, Gil-Izquierdo A, Gil MI (2003) Comparative study of six pear cultivars in terms of their phenolic and vitamin C contents and antioxidant capacity. J Sci Food Agric 83(10):995–1003
Gharehaghaji AN, Arzani K, Abdollahi H, Shojaeiyan A, Dondini L, Franceschi Pd (2014) Genomic characterization of self-incompatibility ribonucleases in the Central Asian pear germplasm and introgression of new alleles from other species of the genus *Pyrus*. Tree Genet Genomes 10(2):411–428
Gill US, Lee S, Mysore KS (2015) Host versus nonhost resistance: distinct wars with similar arsenals. Phytopathol 105(5):580–587
Goldway M, Takasaki-Yasuda T, Sanzol J, Mota M, Zisovich A, Stern RA, Sansavini S (2009) Renumbering the S-RNase alleles of European pears (*Pyrus communis* L.) and cloning the *S109 RNase* allele. Sci Hort 119(4):417–422
Gonai T, Terakami S, Nishitani C, Yamamoto T, Kasumi M (2009) The validity of marker-assisted selection using DNA markers linked to a pear scab resistance gene (*Vnk*) in two populations. J Jpn Soc Hort Sci 78(1):49–54
González-Domínguez E, Armengol J, Rossi V (2017) Biology and epidemiology of *Venturia* species affecting fruit crops: a review. Front Plant Sci 8:1496. https://doi.org/10.3389/fpls.2017.01496
Hae-Sung H, Jae-Kyun B, Whee-Cheon K, ll-Sheob S (2015) Inheritance of fruit ripening time in oriental pear (*Pyrus pyrifolia* var. *culta* Nakai). Hort Sci Tech 33:712–721
Hancock JF, Lobos GA (2008) Pears. In: Hancock JF (ed) Temperate fruit crop breeding. Germplasm to genomics. Springer, USA, pp 299–336
Harker FR, Marsh KB, Young H, Murray SH, Gunson FA, Walker SB (2002) Sensory interpretation of instrumental measurements 2: sweet and acid taste of apple fruit. Postharv Biol Tech 24(3):241–250
Harris MK, Lamb RC (1973) Resistance to the Pear Psylla in Pears with *Pyrus ussuriensis* Lineage. J Am Soc Hort Sci 98:378–381
Hauagge R, Cummins JN (2013) Pear breeding for low chilling. In: Erez A (ed) Temperate fruit crops in warm climates. Springer Science and Business Media, B.V., pp 288–303
Hodkinson ID (2009) Life cycle variation and adaptation in jumping plant lice (Insecta: Hemiptera: Psylloidea): a global synthesis. J Nat Hist 43(1–2):65–179
Hudina M, Štampar F (2004) Effect of climatic and soil conditions on sugars and organic acids content of pear fruits (*Pyrus communis* l.) cvs. 'Williams' and 'Conference'. Acta Hortic 636:527–531
Hunter DM, Layne REC (2004) Introductions from the AAFC-Harrow tree fruit breeding programs. Acta Hortic 663:907–910. https://doi.org/10.17660/ActaHortic.2004.663.166
Inoue E, Kasumi M, Sakuma F, Anzai H, Amano K, Hara H (2006) Identification of RAPD marker linked

to fruit skin color in Japanese pear (*Pyrus pyrifolia* Nakai). Sci Hort 107(3):254–258

Ishii H, Udagawa H, Nishimoto S, Tsuda T, Nakashima H (1992) Scab resistance in pear species and cultivars. Acta Phytopathol Entomol Hungar 27:293–298

Itai A, Fujita N (2008) Identification of climacteric and nonclimacteric phenotypes of Asian pear cultivars by CAPS analysis of 1-aminocyclopropane-1-carboxylate synthase genes. HortScience 43(1):119–121

Itai A, Kawata T, Tanabe K, Tamura F, Uchiyama M, Tomomitsu M, Shiraiwa N (1999) Identification of 1-aminocyclopropane-1-carboxylic acid synthase genes controlling the ethylene level of ripening fruit in Japanese pear (*Pyrus pyrifolia* Nakai). Mol Gen Genet 261(1):42–49

Itai A, Kotaki T, Tanabe K, Tamura F, Kawaguchi D, Fukuda M (2003) Rapid identification of 1-aminocyclopropane-1-carboxylate (ACC) synthase genotypes in cultivars of Japanese pear (*Pyrus pyrifolia* Nakai) using CAPS markers. Theor Appl Genet 106(7):1266–1272

Iwata H, Hayashi T, Terakami S, Takada N, Saito T, Yamamoto T (2013a) Genomic prediction of trait segregation in a progeny population: a case study of Japanese pear (*Pyrus pyrifolia*). BMC Genet 14:81

Iwata H, Hayashi T, Terakami S, Takada N, Sawamura Y, Yamamoto T (2013b) Potential assessment of genome-wide association study and genomic selection in Japanese pear *Pyrus pyrifolia*. Breed Sci 63(1):125–140

Jaeger SR, Lund CM, Lau K, Harker FR (2003) In search of the "ideal" pear (*Pyrus* spp.): results of a multidisciplinary exploration. J Food Sci 68(3):1108–1117. https://doi.org/10.1111/j.1365-2621.2003.tb08296.x

Kang X (2010) The research on polymorphism of the flavor components of ripe fruit of Chinese *Pyrus ussuriensis* Maxim local varieties. Central South University Forestry and Technology, China

Khan MA, Zhao Y, Korban SS (2012) Molecular mechanisms of pathogenesis and resistance to the bacterial pathogen *Erwinia amylovora*, causal agent of fire blight disease in Rosaceae. Plant Mol Biol Rep 30 (2):247–260. https://doi.org/10.1007/s11105-011-0334-1

Kikuchi A (1924) On the origin of Japanese pear and inheritance of the skin colours of their fruits. Jpn J Genet 3:1–27

Kikuchi A (1930) On skin color of the Japanese pear, and its inheritance. Contr Inst Plant Ind 8:1–50

Kim Y-K, Kang S-S, Won K-H, Shin I-S, Cho K-S, Ma K-B, Kim M-S, Choi J-J, Choi J-H (2016) Breeding of the scab-resistant pear cultivar 'Greensis'. Korean J Hort Sci Tech 34(4):655–661

Knabel M, Friend AP, Palmer JW, Diack R, Gardiner SE, Tustin S, Schaffer R, Foster T, Chagne D (2017) Quantitative trait loci controlling vegetative propagation traits mapped in European pear (*Pyrus communis* L.). Tree Genet Genomes 13(3). https://doi.org/10.1007/s11295-017-1141-0

Knäbel M, Friend AP, Palmer JW, Diack R, Wiedow C, Alspach P, Deng C, Gardiner SE, Tustin DS, Schaffer R, Foster T, Chagné D (2015) Genetic control of pear rootstock-induced dwarfing and precocity is linked to a chromosomal region syntenic to the apple *Dw1* loci. BMC Plant Biol 15:230. https://doi.org/10.1186/s12870-015-0620-4

Kolniak-Ostek J (2016) Content of bioactive compounds and antioxidant capacity in skin tissues of pear. J Funct Foods 23(Supplement C):40–51. https://doi.org/10.1016/j.jff.2016.02.022

Kretzschmar AA, Brighenti LM, Rufato L, Pelizza TR, Silveira FN, Miquelutti DJ, Faoro ID (2011) Chilling requirement for dormancy bud break in European pear. Acta Hortic 909:85–88. https://doi.org/10.17660/ActaHortic.2011.909.7

Kumar S, Chagne D, Bink MCAM, Volz RK, Whitworth CJ, Carlisle C (2012) Genomic selection for fruit quality traits in apple (*Malus* × *domestica* Borkh.). PLoS ONE 7(5):e36674. https://doi.org/10.1371/journal.pone.0036674

Kumar S, Kirk C, Deng C, Wiedow C, Knaebel M, Brewer L (2017) Genotyping-by-sequencing of pear (*Pyrus* spp.) accessions unravels novel patterns of genetic diversity and selection footprints. Hort Res 4:17015. https://doi.org/10.1038/hortres.2017.15

Laurens F, Aranzana MJ, Arus P, Bassi D, Bink M, Bonany J, Caprera A, Corelli-Grappadelli L, Costes E, Durel C-E, Mauroux J-P, Muranty H, Nazzicari N, Pascal T, Patocchi A, Peil A, Quilot-Turion B, Rossini L, Stella A, Troggio M, Velasco R, van de Weg E (2018) An integrated approach for increasing breeding efficiency in apple and peach in Europe. Hort Res 5:1–14

Layne REC, Bailey CH, Hough LF (1968) Efficacy of transmission of fire blight resistance in *Pyrus*. Can J Plant Sci 48(3):231–243

Lespinasse Y, Chevalier M, Durel CE, Guerif P, Tellier M, Denance C, Belouin A, Robert P (2008) Pear breeding for scab and psylla resistance. Acta Hortic 800:475–481

Li JC, Yi K, Liu C, Sui HT, Wang JZ, Zhang QJ (2004) Studies on the inheritance of volatiles in pear fruit. Acta Hortic 663:345–348

Liu J, Cui H, Wang L, Wang X, Yang J, Zhang Z, Li X, Qiao Y (2011) Analysis of pear fruit acid/low-acid trait by SSR marker. J Fruit Sci 28(3):389–393

Liu L, Chen C-X, Zhu Y-F, Xue L, Liu Q-W, Qi K-J, Zhang S-L, Wu J (2016) Maternal inheritance has impact on organic acid content in progeny of pear (*Pyrus* spp.) fruit. Euphytica 209(2):305–321

Lombard PB, Westwood MN (1987) Pear rootstocks. In: Rom RC, Carlson RF (eds) Rootstocks for fruit crops. Wiley, New York, pp 145–183

Luby JJ, Shaw DV (2001) Does marker-assisted selection make dollars and sense in a fruit breeding program? HortScience 36:872–879

Moffett AA (1933) Cytological studies in cultivated pears. Genetica 15:511–518

Momol MT, Aldwinckle HS (2000) Genetic diversity and host range of *Erwinia amylovora*. In: Vanneste JL (ed) Fire blight. The disease and its causative agent, *Erwinia amylovora*. CABI Publish, Wallingford, pp 55–72

Montanari S, Guérif P, Ravon E, Denancé C, Muranty H, Velasco R, Chagné D, Bus VGM, Robert P, Perchepied L, Durel C-E (2015) Genetic mapping of Cacopsylla pyri resistance in an interspecific pear (*Pyrus* spp.) population. Tree Genet Genomes 11(4):74. https://doi.org/10.1007/s11295-015-0901-y

Montanari S, Brewer L, Lamberts R, Velasco R, Malnoy M, Perchepied L, Guerif P, Durel CE, Bus VGM, Gardiner SE, Chagne D (2016a) Genome mapping of postzygotic hybrid necrosis in an interspecific pear population. Hort Res 3:15064. https://doi.org/10.1038/hortres.2015.64

Montanari S, Perchepied L, Renault D, Frijters L, Velasco R, Horner M, Gardiner SE, Chagne D, Bus VGM, Durel CE, Malnoy M (2016b) A QTL detected in an interspecific pear population confers stable fire blight resistance across different environments and genetic backgrounds. Mol Breed 36(47):1–16

Mori K, Goto-Yamamoto N, Kitayama M, Hashizume K (2007) Loss of anthocyanins in red-wine grape under high temperature. J Exp Bot 58(8):1935–1945

Necas T, Lañar L, Ondrášek I, Náměstek J, Láčík J, Kosina J (2016) Propagation of selected pear and quince rootstocks by hardwood cuttings. Acta Univ Agric Silvic Mendelianae Brun 64(4):1211–1217

Nin S, Ferri A, Sacchetti P, Giordani E (2012) Pear resistance to psylla (*Cacopsylla pyri* L.): a review. Adv Hort Sci 26(2):59–74

Nishio S, Yamada M, Sawamura Y, Takada N, Saito T (2011) Environmental variance components of fruit ripening date as used in both phenotypic and marker-assisted selection in Japanese pear breeding. HortScience 46(11):1540–1544

Nishitani C, Yamaguchi-Nakamura A, Hosaka F, Terakami S, Shimizu T, Yano K, Itai A, Saito T, Yamamoto T (2012) Parthenocarpic genetic resources and gene expression related to parthenocarpy among four species in pear (*Pyrus* spp.). Sci Hort 136:101–109. https://doi.org/10.1016/j.scienta.2011.12.029

Norelli JL, Aldwinckle HS, Beer SV (1984) Differential host × pathogen interaction among cultivars of apple and strains of *Erwinia amylovora*. Phytopathology 74 (2):136–139

Ntladi SM, Human JP, Bester C, Vervalle J, Roodt-Wilding R, Tobutt KR (2018) Quantitative trait loci (QTL) mapping of blush skin and flowering time in a European pear (*Pyrus communis*) progeny of 'Flamingo' × 'Abate Fetel'. Tree Genet Genomes 14 (5):70. https://doi.org/10.1007/s11295-018-1280-y

Oraguzie NC, Whitworth CJ, Brewer L, Hall A, Volz RK, Bassett H, Gardiner SE (2010) Relationships of *PpACS1* and *PpACS2* genotypes, internal ethylene concentration and fruit softening in European (*Pyrus communis*) and Japanese (*Pyrus pyrifolia*) pears during cold air storage. Plant Breed 129(2):219–226

Ouvrard D (2017) *Psyl'list-the world Psylloidea database*. http://www.catalogueoflife.org/annual-checklist/2017/details/database/id/54

Palonen P, Buszard D (1997) Current state of cold hardiness research on fruit crops. Can J Plant Sci 77:399–420

Park DH, Lee Y-G, Cha J-S, Oh C-S (2017) Current status of fire blight caused by *Erwinia amylovra* and action for its management in Korea. J Plant Pathol 99:59–63

Pasqualini E, Civolani S, Musacchi S, Ancarini V, Dondini L (2006) *Cacopsylla pyri* behaviour on new pear selections for host resistance programs. Bull Insect 59(1):27–37

Patil BS, Uckoo RM, Jayaprakasha GK, Palma MA (2016) Consumers' changing perceptions of quality: revisiting the science of fruit and vegetable cultivation for improved health benefits. Acta Hortic 1120:459–468

Peace CP (2017) DNA-informed breeding of rosaceous crops: Promises, progress and prospects. Hort Res 4:17006

Peil A, Bus VGM, Geider K, Richter K, Flachowsky H, Hanke MV (2009) Improvment of fire blight resistance in apple and pear. Intl J Plant Breed 3(1):1–27

Perchepied L, Leforestier D, Ravon E, Guerif P, Denance C, Tellier M, Terakami S, Yamamoto T, Chevalier M, Lespinasse Y, Durel CE (2015) Genetic mapping and pyramiding of two new pear scab resistance QTLs. Mol Breed 35(10). https://doi.org/10.1007/s11032-015-0391-5

Perchepied L, Guerif P, Ravon E, Denance C, Laurens F, Robert P, Bouvier L, Lespinasse Y, Durel CE (2016) Polygenic inheritance of resistance to *Cacopsylla pyri* in a *Pyrus communis* × *P. ussuriensis* progeny is explained by three QTLs involving an epistatic interaction. Tree Genet Genomes 12(6):1–10

Pierantoni L, Cho KH, Shin IS, Chiodini R, Tartarini S, Dondini L, Kang SJ, Sansavini S (2004) Characterisation and transferability of apple SSRs to two European pear F-1 populations. Theor Appl Genet 109(7):1519–1524

Pierantoni L, Dondini L, Cho KH, Shin IS, Gennari F, Chiodini R, Tartarini S, Kang SJ, Sansavini S (2007) Pear scab resistance QTLs via a European pear (*Pyrus communis*) linkage map. *Tree Genet Genomes* 3 (4):311. https://doi.org/10.1007/s11295-11006-10070-11290

Pierantoni L, Dondini L, Franceschi Pd, Musacchi S, Winkel BSJ, Sansavini S (2010) Mapping of an anthocyanin-regulating MYB transcription factor and its expression in red and green pear, Pyrus communis. Plant Physiol Biochem 48(12):1020–1026

Postman JD (1994) Graft compatibility of pear with related genera. Acta Hortic 367:380

Postman JD, Spotts RA, Calabro J (2005) Scab resistance in *Pyrus* germplasm. Acta Hortic 671:601–608

Puterka GJ (1997) Intraspecific variation in pear psylla (Psyllidae: Homoptera) nymphal survival and development on resistant and susceptible pear. Env Entomol 26(3):552–558

Quamme HA (1984) Observations of *Psylla* resistance among several pear cultivars and species. Fruit Var J Agric Food Chem 38(2):34–36

Quamme HA (1991) Application of thermal analysis to breeding fruit crops for increased cold hardiness. HortScience 26(5):513–517

Quamme HA, Bonn WG (1981) Virulence of *Erwinia amylovora* and its influence on the determination of fire blight resistance of pear cultivars and seedlings. Can J Plant Pathol 3(4):187–190

Quamme HA, Kappel F, Hall JW (1990) Efficacy of early selection for fire blight resistance and the analysis of combining ability for the fire blight resistance in several pear progenies. Can J Plant Sci 70:905–913

Quarta R, Puggioni D (1985) Survey on the variety susceptibility to pear psylla. Acta Hortic 159:77–86

Rivalta L, Dradi M, Rosati C (2002) Thirty years of pear breeding activity at ISF Forlì, Italy: a review. Acta Hortic 596:233–238

Robert P, Raimbault T (2005) Resistance of some *Pyrus communis* cultivars and *Pyrus* hybrids to the pear psylla *Cacopsylla pyri* (Homoptera, psyllidae). Acta Hortic 671:571–575

Rumayor FIA, Martínez CA, Vázquez R (2005) Breeding pears for warm climates in Mexico. Acta Hortic 671:31. https://doi.org/10.17660/ActaHort

Saeed M, Brewer L, Johnston J, McGhie TK, Gardiner SE, Heyes JA, Chagné D (2014) Genetic, metabolite and developmental determinism of fruit friction discolouration in pear. BMC Plant Biol 14(1):241. https://doi.org/10.1186/s12870-014-0241-3

Saito T (2016) Advances in Japanese pear breeding in Japan. Breed Sci 66(1):46–59

Saito T, Kotobuki K, Sato Y, Abe K, Machida Y, Kurihara A, Kajiura I, Terai O, Shoda M, Sawamura Y, Ogata T, Masuda R, Nishibata T, Kashimura Y, Kosono T, Fukuda H, Kihara T, Suzuki K (2015) New Japanese pear cultivar 'Nashi chuukanbohon nou 1 gou', with the homozygote of haplotype for self-compatibility (*Pyrus pyrifolia* Nakai). Bull NARO Inst Fruit Tree Sci 20

Sams CE (1999) Preharvest factors affecting postharvest texture. Postharv Biol Technol 15(3):249–254

Sanzol J, Rallo P, Herrero M (2003) Stigmatic receptivity limits the effective pollination period in 'Agua de Aranjuez' pear. J Am Soc Hort Sci 128(4):458–462

Sarkar D, Ankolekar C, Pinto M, Shetty K (2015) Dietary functional benefits of Bartlett and Starkrimson pears for potential management of hyperglycemia, hypertension and ulcer bacteria *Helicobacter pylori* while supporting beneficial probiotic bacterial response. Food Res Intl 69(Supplement C):80–90. https://doi.org/10.1016/j.foodres.2014.12.014

Sawamura Y, Mase N, Takada N, Sato A, Nishitani C, Abe K, Masuda T, Yamamoto T, Saito T, Kotobuki K (2013) A self-compatible pollen-part mutant of Japanese pear produced by crossing 'Kosui' with pollen from gamma-irradiated 'Kosui'. J Jpn Soc Hort Sci 82(3):222–226

Sestras R, Botez C, Ardelean M, Oltean I, Sestras (2009) Response of pear genotypes to psylla sp. attack in central Transylvania, Romania. Acta Hortic 814:845–850

Sha S (2012) Pear organic acid components, content changes and genetic identification. Nanjing Agricultural University, Nanjing

Sha S, Li J, Wu J, Zhang S (2011) Characteristics of organic acids in the fruit of different pear species. African J Agric Res 6:2403–2410

Shaltiel-Harpaz L, Soroker V, Kedoshim R, Hason R, Sokalsky T, Hatib K, Bar-Ya'akov I, Holland D (2014) Two pear accessions evaluated for susceptibility to pear psylla *Cacopsylla bidens* (Šulc) in Israel. Pest Manag Sci 70(2):234–239

Sherman WB, Lyrene PM (2003) Low chill breeding of deciduous fruits at the university of Florida. Acta Hortic 622:599–605

Shin YU, Yim YJ, Cho HM, Yae BW, Kim MS, Kim YK (1983) Studies on the inheritance of fruit characteristics of Oriental pear, *Pyrus serotina* Rehder var. *culta* (in Korean). Res Rep Office Rural Dev (Hort) 25:108–117

Shin IS, Kim WC, Hwang HS, Shin YU (2002) Achievements of pear breeding in Korea. Acta Hortic 596:247–250

Shin IS, Shin YU, Hwang HS (2008) Heritability of fruit characters of interspecific hybrids between *Pyrus pyrifolia* and *P. ussuriensis* or *P. breschneideri*. Acta Hortic 800:535–540

Simard MH, Michelesi JC, Masseron (2004) Pear rootstock breeding in France. Acta Hortic 658:535–540

Smits THM, Duffy B, Sundin GW, Zhao YF, Rezzonico F (2017) *Erwinia amylovora* in the genomics era: from genomes to pathogen virulence, regulation, and disease control strategies. J Plant Pathol 99:7–23

Stephenson K (2015) Northwest pear industry continues nutritional research investment. http://usapears.org/wp-content/uploads/2015/01/Bartlett-Bin.jpg

Steyn WJ, Holcroft DM, Wand SJE, Jacobs G (2004) Anthocyanin degradation in detached pome fruit with reference to preharvest red color loss and pigmentation patterns of blushed and fully red pears. J Am Soc Hort Sci 129(1):13–19

Steyn WJ, Wand SJE, Holcroft DM, Jacobs G (2005) Red colour development and loss in pears. Acta Hortic 671:79–85

Sugar D, Mitcham EJ, Kupferman E (2009) Re-thinking the chill requirement for pear ripening. Postharvest Information Network, December. http://postharvest.tfrec.wsu.edu/REP2009B.pdf

Sun Q, Sun H, Bell R, Li H, Xin L (2011) Variation of phenotype, ploidy level, and organogenic potential of in vitro regenerated polyploids of *Pyrus communis*. Plant Cell Tiss Org Cult 107:131–140

Tamura F (2012) Recent advances in research on Japanese pear rootstocks. J Jpn Soc Hort Sci 81 (1):1–10

Tanaka S, Yamamoto S (1964) Studies on pear scab. II. Taxonomy of the causal fungus of Japanese pear scab. Ann Phytopathol Soc Jap 29:128–136

Tanrıöven D, Ekşi A (2005) Phenolic compounds in pear juice from different cultivars. Food Chem 93(1):89–93

Teng Y (2011) The pear industry and research in China. Acta Hortic 909:161–170

Terakami S, Shoda M, Adachi Y, Gonai T, Kasumi M, Sawamura Y, Iketani H, Kotobuki K, Patocchi A, Gessler C, Hayashi T, Yamamoto T (2006) Genetic mapping of the pear scab resistance gene *Vnk* of Japanese pear cultivar Kinchaku. Theor Appl Genet 113(4):743–752

Thompson SS, Janick J, Williams EB (1962) Evaluation of the resistance to fireblight of pear. Proc Am Soc Hort Sci 80 (105–113)

Thompson JM, Zimmerman RH, Van der Zwet T (1975) Inheritance of fire blight resistance in *Pyrus*. I. A dominant gene, *Se*, causing sensitivity. J Hered 66:259–264

Thomson GE, Turpin S, Goodwin I (2018) A review of preharvest anthocyanin development in full red and blush cultivars of European pear. NZ J Crop Hort Sci 46(2):81–100

Trapman M, Blommers L (1992) An attempt to pear sucker management in the Netherlands. J Appl Entomol 114(1–5):38–51

Tromp J, Borsboom O (1994) The effect of autumn and spring temperature on fruit set and on the effective pollination period in apple and pear. Sci Hort 60 (1):23–30. https://doi.org/10.1016/0304-4238(94)90059-0

Ubi BE, Honda C, Bessho H, Kondo S, Wada M, Kobayashi S, Moriguchi T (2006) Expression analysis of anthocyanin biosynthetic genes in apple skin: Effect of UV-B and temperature. Plant Sci 170(3):571–578

Valle D, Burckhardt D, Mujica V, Zoppolo R, Morelli E (2017) The occurrence of the pear psyllid, *Cacopsylla bidens* (Šulc, 1907) (Insecta: Hemiptera: Psyllidae), in Uruguay. Check List 13(2):1–4. https://doi.org/10.15560/13.2.2088

Van der Zwet T (1977) Possibility of combining low levels of fire blight resistane in pear. Acta Hortic 69:97–103

Van der Zwet T, Oitto WA, Westwood MN (1974) Variability in degree of fire blight resistance within and between *Pyrus* species, interspecific hybrids, and seedling progenies. Euphytica 23:295–304

van der Zwet T, Zook WR, Blake RC (1977) The USDA pear breeding program I. Emasculation and pollination. Fruit Var J Agr Food Chem 31:78–82

Van der Zwet T, Orolaza-Halbrendt N, Zeller W (2012) Fire blight history, biology, and management. APS Press, St. Paul. https://doi.org/10.1094/9780890544839.fm

van Nocker S, Gardiner SE (2014) Breeding better cultivars, faster: applications of new technologies for the rapid deployment of superior horticultural tree crops. Hort Res 1:14022. https://doi.org/10.1038/hortres.2014.22

Viera W, Alspach P, Brewer L, Jhonston J, Winefield C (2013) Genetic parameters for sugar content in an interspecific pear polulation. Euro J Hort Sci 78:56–66

Visser T, Oost EH (1981) Pollen and pollination experiments. III. The viability of apple and pear pollen as affected by irradiation and storage. Euphytica 30 (1):65–70

Volz RK, White AG, Brewer LR (2008) Breeding for red skin colour in interspecific pears. Acta Hortic 800:469–474

Vondracek J (1982) Pear cultivars resistant to pear scab. In: van der Zwet T, Childers NF (eds) The Pear: cultivars to marketing. Horticultural Publ., Gainsville, pp 420–424

Wang D, Korban SS, Zhao Y (2010) Molecular signature of differential virulence in natural isolates of *Erwinia amylovora*. Phytopathology 100(2):192–198

Wang Y-Z, Dai M-S, Zhang S-J, Shi Z-B (2014) Exploring candidate genes for pericarp russet pigmentation of Sand pear (*Pyrus pyrifolia*) via RNA-Seq data in two genotypes contrasting for pericarp color. PLoS ONE 9(1):e83675. https://doi.org/10.1371/journal.pone.0083675

Wang G-M, Gu C, Qiao X, Zhao B-Y, Ke Y-Q, Guo B-B, Hao P-P, Qi K-J, Zhang S-L (2017) Characteristic of pollen tube that grew into self style in pear cultivar and parent assignment for cross-pollination. Sci Hort 216:226–233

Warner G (2015) Promising pear rootstocks. Good Fruit Grower, Apr 16. New Developments//Pears//Research//Varieties

Webster AD, Tobutt KR, Evans KM (2000) Breeding and evaluation of new rootstocks for apple, pear and sweet cherry. Comp Fruit Tree 33(4):100–104

Westigard PH, Westwood MN, Lombard PB (1970) Host preference and resistance and resistance of *Pyrus* species to the pear psylla, *Pyslla pyricola* Foester. J Am Soc Hort Sci 95:34–36

White AG, Alspach PA (1996) Variation in fruit shape in three pear hybrid progenies. NZ J Crop Hort Sci 24 (4):409–413

White AG, Brewer LR (2002) The New Zealand pear breeding project. Acta Hortic 596:239–242

White AG, Alspach PA, Weskett RH, Brewer LR (2000a) Heritability of fruit shape in pears. Euphytica 112 (1):1–7. https://doi.org/10.1023/a:1003761118890

White AG, Brewer LR, Alspach PA (2000b) Heritability of fruit characteristics in pears. Acta Hortic 538:331–337

Won K, Kim Y, Kang S, Song J, Hwang H (2011) Introduction of Korean pear cultivars with high resistance to the scab for organic pear orchard. In*: Organic is life—knowledge for tomorrow, vol 1—organic crop production proceedings of the third scientific conference of the International Society of Organic Agriculture Research (ISOFAR)*, held at the 17th IFOAM Organic World Congress in cooperation

with the International Federation of Organic Agriculture Movements (IFOAM) and the Korean Organizing Committee (KOC), 28 September–1 October 2011 in Namyangju, Korea Republic

Won K, Bastiaanse H, Kim YK, Song JH, Kang SS, Lee HC, Cho KH, Brewer L, Singla G, Gardiner SE, Chagné D, Bus VGM (2014) Genetic mapping of polygenic scab (*Venturia pirina*) resistance in an interspecific pear family. Mol Breed 34(4):2179–2189

Wu J, Wang Z, Shi Z, Zhang S, Ming R, Zhu S, Khan MA, Tao S, Korban SS, Wang H, Chen NJ, Nishio T, Xu X, Cong L, Qi K, Huang X, Wang Y, Zhao X, Wu J, Deng C, Gou C, Zhou W, Yin H, Qin G, Sha Y, Tao Y, Chen H, Yang Y, Song Y, Zhan D, Wang J, Li L, Dai M, Gu C, Wang Y, Shi D, Wang X, Zhang H, Zeng L, Zheng D, Wang C, Chen M, Wang G, Xie L, Sovero V, Sha S, Huang W, Zhang S, Zhang M, Sun J, Xu L, Li Y, Liu X, Li Q, Shen J, Wang J, Paull RE, Bennetzen JL, Wang J, Zhang S (2013) The genome of the pear (*Pyrus bretschneideri* Rehd.). Genome Res 23(2):396–408

Wu J, Li L-T, Li M, Khan MA, Li X-G, Chen H, Yin H, Zhang S-L (2014) High-density genetic linkage map construction and identification of fruit-related QTLs in pear using SNP and SSR markers. J Exp Bot 65 (20):5771–5781

Xue H, Shi T, Wang F, Zhou H, Yang J, Wang L, Wang S, Su Y, Zhang Z, Qiao Y, Li X (2017a) Interval mapping for red/green skin color in Asian pears using a modified QTL-seq method. Hort Res 4:17053. https://doi.org/10.1038/hortres.2017.53

Xue L, Liu Q, Qin M, Zhang M, Wu X, Wu J (2017b) Genetic variation and population structure of "Zangli" pear landraces in Tibet revealed by SSR markers. Tree Genet Genomes 13(1):26. https://doi.org/10.1007/s11295-017-1110-7

Yamamoto T, Terakami S (2016) Genomics of pear and other Rosaceae fruit trees. Breed Sci 66(1):148–159

Yamamoto T, Terakami S, Kimura T, Sawamura Y, Takada N, Hirabayashi T, Imai T, Nishitani C (2009) Reference genetic linkage maps of European and Japanese pears. Acta Hortic 814:599–602. https://doi.org/10.17660/ActaHortic.2009.814.101

Yamamoto RR, Sekozawa Y, Sugaya S, Gemma H (2010) Influence of chilling accumulation time on "Flower Bud Abortion" occurrence in Japanese pear grown under mild winter conditions. Acta Hortic 872(6):69–76

Yamamoto T, Terakami S, Takada N, Nishio S, Onoue N, Nishitani C, Kunihisa M, Inoue E, Iwata H, Hayashi T, Itai A, Saito T (2014) Identification of QTLs controlling harvest time and fruit skin color in Japanese pear (*Pyrus pyrifolia* Nakai). Breed Sci 64 (4):351–361

Yamane M, Abe D, Yasui S, Yokotani N, Kimata W, Ushijima K, Nakano R, Kubo Y, Inaba A (2007) Differential expression of ethylene biosynthetic genes in climacteric and non-climacteric Chinese pear fruit. Postharv Biol Tech 44(3):220–227

Yao G, Ming M, Allan AC, Gu C, Li L, Wu X, Wang R, Chang Y, Qi K, Zhang S, Wu J (2017) Map-based cloning of the pear gene *MYB114* identifies an interaction with other transcription factors to coordinately regulate fruit anthocyanin biosynthesis. Plant J 92(3):437–451. https://doi.org/10.1111/tpj.13666

Yim SH, Nam SH (2015) Antioxidant and whitening activities of five unripe pear cultivars. J Appl Bot Food Quality 88. https://doi.org/10.5073/jabfq.2015.088.026

Yu-Lin W (1996) Chinese pears. China Agricultural Scientech Press, China

Zhang D (2012) Molecular physiological mechanism of coloration induced and regulation of red Chinese sand pears (*Pyrus pyrifolia* Nakai). Zhejiang University, China

Zhang HE, Yue WQ, Wu YQ, Yi W, Han ZH, Zhang XZ (2012) Selection and evaluation of interspecific hybrids of pear highly resistant to *Venturia nashicola*. J Phytopathol 160(7–8):346–352. https://doi.org/10.1111/j.1439-0434.2012.01912.x

Zhao P, Kakishima M, Uzuhashi S, Ishii H (2012) Multigene phylogenetic analysis of inter- and intraspecific relationships in *Venturia nashicola* and *V. pirina*. Eur J Plant Pathol 132(2):245–258

Zielinski QB, Thompson MM (1967) Speciation in *Pyrus*: chromosome number and meiotic behavior. Bot Gazette 128(2):109–112. https://doi.org/10.1086/336386

Zielinski QB, Reimer FC, Quackenbush VL (1965) Breeding behavior of fruit characteristics in pears, *Pyrus communis* L. Proc Am Soc Hort Sci 86(81):87

Linkage Mapping in Pear

5

Jun Wu and Mengfan Qin

Abstract

The past three decades have witnessed the development of genetic linkage maps and use of DNA markers in mapping agronomic traits in many crops. In comparison with other plants, linkage mapping in pear has been initiated rather late, not until the year 2001. Pear is characterized by a typical self-incompatibility, and it has a long generation cycle. Therefore, genetic maps have been constructed using F_1 populations. This may lead to the development of linkage maps of lower resolutions due to the lack of sufficient genetic variations. Fortunately, the development of next-generation sequencing technology has allowed for detection of high-quality genome-wide DNA markers, such as simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs), in larger size populations, thus greatly improving the quality of genetic linkage maps. Overall, linkage maps are highly useful for dissecting complex agronomic traits, and for identifying either quantitative trait loci (QTL) or key genes regulating a target trait of interest. Furthermore, they contribute to efforts to pursue marker-assisted breeding (MAB) in pear.

5.1 What Is a Linkage Map?

A linkage map, also known as a genetic map, is an alignment of molecular markers or known genes, and their positions relative to each other in terms of recombination frequencies rather than their specific positions along each of the chromosomes of a genome. More specifically, a linkage map is based on the recombination frequency between markers during chromosomal crossovers that may occur during meiosis. Therefore, a higher frequency of recombination suggests that there is a wider physical distance between markers, while a lower frequency of recombination suggests a narrower physical distance between markers. The unit used to measure distances among markers along a genetic map is a centimorgan (cM), as this corresponds to a recombination frequency of 1%. A linkage map is a useful tool in pursuing research studies and in breeding efforts as it serves to identify and/or locate new markers, or genes, linked to known markers by testing for genetic linkages among them.

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5.2 What Is a Mapping Population?

To construct a genetic linkage map for a target plant species, the first and most important effort is to establish a proper segregating population for this species. For cross-pollinated species, such as pear, wherein self-incompatibility and inbreeding depression render it impossible to develop mapping populations, such as F_2, backcross (BC), or elite lines, such as recombinant inbred lines (RILs) or near-isogenic lines (NILs), whereby self-breeding is required for several generations. Similar to other fruit tree species, the pear also has a long period of juvenility prior to reaching the reproductive phase, whereby a trait is deemed stable. On average, it takes about 5–8 years to establish a simple F_1 pear population. Fortunately, after thousands of years of distant hybridizations, the genome of the pear is highly heterozygous, and progenies of these hybrids have high levels of segregation for performance of agronomic characters (Wu et al. 2013). For these reasons, an F1 population is usually used in genetic linkage studies for pears.

5.3 Genetic Linkage Maps for Pear

In pear, the history of genetic map construction had gone through three stages. The first stage involved the construction of initial maps using low polymorphism DNA markers. Thereby, the number of linkage groups was not equal to the number of chromosomes of the pear genome, and it was difficult to determine which linkage group corresponded to which chromosome. The second stage involved the construction of reference (or frame) maps. Prior to the release of the reference genome of pear, most of these maps consisted of 17 linkage groups, which was also consistent with that of the apple genome as pear and apple belong to the same subfamily Pomoideae, by using co-dominant simple sequence repeat (SSR) markers. The third stage involved complete sequencing of genomes of the Asian pear 'Dangshansuli' (*Pyrus* × *bretschneideri*) (Wu et al. 2013) and of the European pear 'Bartlett' (*Pyrus communis* L.) (Chagné et al. 2014), thereby allowing for identification and development of large numbers of genome-wide DNA markers. These robust markers have allowed for the development of high-quality genetic maps.

5.3.1 Initial Maps

The first genetic linkage map for pear was constructed using random amplified polymorphic DNA (RAPD) markers developed using an F1 mapping population of 82 individuals of the Japanese pear (*P. pyrifolia* Nakai) cultivars Kinchaku and Kosui, and consisted of two separate maps (Iketani et al. 2001). The linkage map for 'Kinchaku' had 120 markers, distributed over 18 linkage groups, and spanning 768 cM; whereas, the map for 'Kosui' had 78 loci distributed across 22 linkage groups, and spanned 508 cM. Both linkage maps had more than the expected 17 linkage groups, the actual number of chromosomes of the pear genome. In addition, the low numbers of markers coupled with the disadvantages of RAPD markers, such as poor reproducibility and inability to distinguish between homozygote and heterozygous alleles, rendered this genetic map of limited genetic information.

Subsequently, Yamamoto et al. (2002) used an F1 mapping population of 63 individuals derived from the hybridization of the European pear (*P. communis*) cultivar Bartlett and the Japanese pear (*P. pyrifolia* Nakai) cultivar Housu. They constructed two parental maps using amplified fragment length polymorphism (AFLP) and SSR markers developed from pear, apple, peach, and cherry. The map of 'Bartlett' consisted of 18 linkage groups with 226 markers, including 175 AFLPs and 47 SSRs, a single isozyme, and a single *S* locus, spanning 949 cM with an average interval of 4.2 cM. The map for 'Housui' consisted of 17 linkage groups with 154 loci, including 106 AFLPs, 42 SSRs, three isozymes, and two phenotypic traits (self-incompatibility and leaf color), spanning 926 cM with an average distance of 6.0 cM. By identifying common SSR markers shared by the

two parental maps, a group of ten linkage groups was then connected together.

During later efforts, Dondini et al. (2004), Yamamoto et al. (2004), and Pierantoni et al. (2007) constructed yet another three sets of pear genetic maps using AFLPs, SSRs, and other marker types, such microsatellite-anchored fragment length polymorphisms (MFLPs) and resistance gene analogs (RGAs), among others. These maps consisted of linkage groups that were higher in number than the basic chromosome number (n = 17) for pear. Thus, these additional maps could not represent the complete genome of pear.

5.3.2 Frame/Reference Maps

Yamamoto et al. (2007) constructed two genetic maps of the European pear (*P. communis*) cultivars Bartlett and La France using two independent F1 populations. The population of 'Bartlett' (*P. communis*) × 'Housui' (*P. pyrifolia*) was used to construct a map for 'Bartlett,' while the population of 'Shinsei' (*P. pyrifolia*) × '282-12' (a Japanese pear selection derived from 'Housui' × 'La France') was used to construct a map for 'La France'. These two maps relied on AFLPs and SSRs developed from both pear and apple. These two maps consisted of 17 linkage groups that were well aligned together, and corresponded to the basic chromosome number (n = 17) of the pear genome. Incidentally, those SSR markers developed from apple and used in constructing these pear maps showed co-linearity with a saturated reference map for apple. The map length for "Bartlett" was 1016.1 cM, with an average distance of 2.3 cM between markers, while the map length for "La

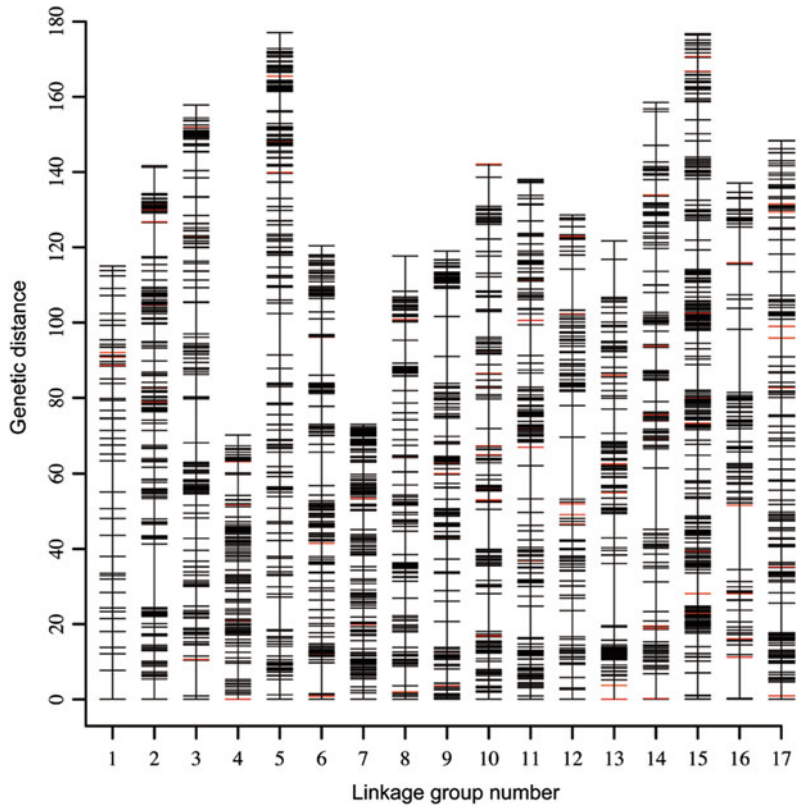

Fig. 5.1 Distribution of SNP and SSR markers on 17 linkage groups of the first high-density genetic map of pear. A black bar indicates a SNP marker, and a red bar indicates an SSR marker. Linkage group number is shown on the x-axis, while the genetic disease is shown on the y-axis (cM)

France" was 1156.7 cM, with an average interval distance of 2.8 cM. Due to their high map lengths and marker densities along with their good co-linearities with an apple reference genetic map, these were deemed as reliable reference linkage maps for pear.

5.3.3 High-Density Linkage Maps

With the development of next-generation sequencing technologies and the release of whole reference genome sequences for pear (Wu et al. 2013; Chagné et al. 2014), massive numbers of SSR and single nucleotide polymorphism (SNP) markers can be identified directly from the pear itself with genome-width coverage. These genomic resources have promoted efforts for constructing high-density genetic linkage maps for pear.

The first SNP-based high-density genetic linkage map for pear has been constructed by Wu et al. (2014). This map was constructed using SNPs integrated along with SSR markers developed by restriction-associated DNA sequencing (RAD-seq). This map consisted of 3143 SNPs and 98 SSRs (3241 markers in total), spanning 2243.4 cM, with an average marker distance of 0.70 cM (Fig. 5.1, Wu et al. 2014). These SSR markers were capable of anchoring all 17 linkage groups to their corresponding chromosomes. Another high-density genetic linkage map for pear has been constructed by Wang et al. (2017) using a hybrid population of 'Red Clapp's Favorite' (*P. communis*) × 'Mansoo' (*P. pyrifolia*), containing 4797 SNP markers and spanning 2703.6 cM, with an average distance of 0.56 cM between adjacent markers.

Genetic maps constructed by different hybrid populations usually vary a lot from each other, and none of them could include the whole genetic information of pear. Yet, lack of common markers making it difficult to do comparison analysis among them. Thus, Li et al. (2017) collected nine published maps and merged them into a single integrated high-density consensus genetic map using common SSR markers, of at least three common SSR markers within the same group, presented in individual maps as bridging markers. The integrated genetic map (I-PCG), using MergeMap (Wu et al. 2011), consisted of 5085 markers, including 1232 SSRs and 3853 SNPs, spanning 3266.0 cM, with a mean interval distance between adjacent markers of 0.64 cM.

The above-mentioned high-density SNP-based linkage maps have greatly improved the quality and resolution of genetic linkage maps for pear. In turn, these maps are very helpful in pursuing fine-mapping of target genes, map-based cloning of qualitative trait loci (QTL) for traits of interest, and promoting the progress of pear-breeding efforts. The details of all published genetic linkage maps are listed (Table 5.1).

5.4 Applications of Genetic Linkage Maps

Genetic linkage maps could be applied in many fields. For example, gene mapping, QTL mapping, map-based cloning, marker-assisted selection (MAS) breeding, comparative mapping, and auxiliary genome assembly, among others.

5.4.1 Gene Mapping

Known genes of traits of interest could be used to construct linkage maps by transferring them into DNA or morphological markers to further investigate their inherited characters. For example, Iketani et al. (2001) mapped two alleles for resistance to pear scab and an allele for susceptibility for black spot on a linkage map for the Japanese pear cv. Kinchaku, and found that these alleles were mapped onto different linkage groups. Yamamoto et al. (2002) mapped an *S* locus (for self-incompatibility) on linkage groups Ba1 and Ho1 of the maps for the European pear cv. Bartlett and the Japanese pear cv. Housui, respectively, and corresponding to the apple linkage group 17 (LG17). Later, Yamamoto et al. (2004) used additional markers to reconstruct linkage maps for 'Bartlett' and 'Housui', and following comparative mapping with apple, they found that the *S* locus mapped onto LG17 of both Japanese and European pears, as well as that of apple. Following the

Table 5.1 Summary of published genetic linkage maps for pear

Population	Size	Marker type	No. markers		Map length (cM)		No. LGs		Interval (cM)	
M	F	M								Reference(s)
F	M	F	M	F						
'Kinchaku' × 'Kosui'	82	RAPD	120	78	768	508	18	22	4.20	Iketani et al. (2001)
'Bartlett' × 'Hosui'	63	AFLP, SSR	226	54	949	926	18	17	4.90	Yamamoto et al. (2002)
'Passe Crassane' × 'Harrow Sweet'	99	SSR, AFLP, MFLP, AFLP-RGA, RGA	155	156	912	930	18	19	5.80	6.00 Dondini et al. (2004)
'Bartlett' × 'Hosui'	63	AFLP, SSR	256	180	1020	995	19	20	4.00	5.50 Yamamoto et al. (2004)
'Bartlett' × 'Hosui'	63	AFLP, SSR	447		1000		17		2.30	Yamamoto et al. (2007)
'Housui' × 'La France'	55	AFLP, SSR	414		1156		17		2.80	Yamamoto et al. (2007)
'Abbè Fétel' (AF) × 'Max Red Bartlett' (MRB)	95	MFLP, SSR	123	110	908.1	879.8	18	19	7.40	8.00 Pierantoni et al. (2007)
'Bartlett' × 'Hosui'	63	AFLP, SSR	335		1174		17		3.50	Terakami et al. (2009)
'Yali' × 'Jingbaili'	145	AFLP, SSR	402		18		1395.9		3.80	Sun et al. (2009)
'Housui' × 'La France'		SSR, SNP	370	415	1160	1177	17	20	3.14	2.84 Yamamoto et al. (2009)
'Niitaka' × 'Suhyangri'	94	RAPD, AFLP, SSR	106	122	1006	1168	19	19	9.49	9.57 Junkyu et al. (2010)
'Bayuehong' × 'Dangshansuli'	97	AFLP, SRAP, SSR	214	122	1352.7	1044.3	17	17	6.32	8.56 Zhang et al. (2013)
'Red Bartlett' × 'Nanguo pear'	74	SRAP	103	105	602.2	650	20	20	4.89	5.20 Zhao et al., (2013)
'Bartlett' × 'Hosui'	63	SSR, SNP	485		965		17		1.99	Yamamoto et al. (2013)
'Bartlett' × 'Hosui'	63	SSR, SNP	951		1341.9		22		1.41	Terakami et al. (2014)
'Bayuehong' × 'Dangshansuli'	102	SSR, SNP	3241		2243.4		17		0.70	Wu et al. (2014)
'Akiakari' × 'Taihaku'	93	SSR, EST-SSR	208	275	799.1	1039.1	17	17	3.84	3.78 Yamamoto et al. (2014)
'Bayuehong' × 'Dangshansuli'	56	SSR	734		1661.4		17		2.26	Chen et al. (2015)
'Red Clapp's Favorite' × 'Mansoo'	161	SSR, SLAF	4797		2703.6		17		0.56	Wang et al. (2017)
Nine published maps	–	SSR, SNP	5085		3266		17		0.64	Li et al. (2017)

development of reference linkage maps for both European and Japanese pears, the precise linkage groups for pear scab resistance gene, located along the middle region of LG1, and black spot response gene, located along the top of LG11, were determined (Yamamoto et al. 2009). Furthermore, the self-incompatibility locus *S* was found to be located along the bottom of LG17 (Yamamoto et al. 2009).

As the red-fruit color for pears is a popular trait for consumers, Dondini et al. (2008) have used a morphological marker for "red color" and mapped it onto LG4 of 'Max Red Bartlett'. Previously, the gene encoding this trait, *MdMyb10*, has been mapped on LG9 in apple. Subsequently, Pierantoni et al. (2010) have cloned *PcMyb10*, found to have 96% amino acid sequence identity with that of *MdMyb10*, from both 'Max Red Bartlett' and 'Williams'. The gene *PcMyb10* is found to map on LG 9 of 'Max Red Bartlett', thereby indicating that this gene is in fact directly responsible for the red skin color of 'Max Red Bartlett'.

The dwarfing trait is another important agronomic character, as it highly impacts efforts for pursuing high-density fruit tree production. In the 1930s, a pear mutant seedling with a significant dwarfing characteristic has been identified in France (Fideghelli et al. 2003). Studies have revealed that the dwarfing trait is controlled by a single dominant gene (Rivalta et al. 2002). The dwarfing trait of pear has been reported to be controlled by the *PcDw* gene in cv. Aihuali (Wang et al. 2011, 2016). Using bulked segregant analysis (BSA) with 500 RAPD and 51 SSR markers from both pear and apple, four markers co-segregating with the dwarf character have been identified (Wang et al. 2011, 2016). The *PcDw* gene is mapped on LG16 of 'Bartlett', and located within very close distances, of 0.4 and 0.8 cM, from markers CN993875 and QauSSR36 (Fig. 5.2, Wang et al. 2016). These latter two molecular markers have been deemed valuable in fine mapping and cloning of the *PcDw* gene.

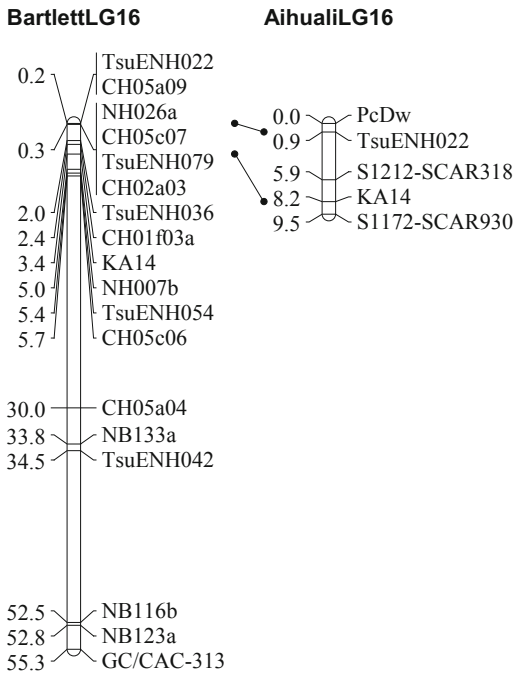

Fig. 5.2 Mapping of a dwarfing trait gene, *PcDw* on LG16, of pear cultivars Aihuali (Wang et al. 2016) and Bartlett (Celton et al. 2009)

5.4.2 QTL Mapping

Marker-assisted QTL-map-based genetic linkage maps are powerful in dissecting the genetic basis of traits in many plant species, including pear. Thus far, about 20 QTLs have been detected in pear. Most of these QTLs are related to fruit traits, while some are related to disease resistance, harvest time, as well as length and width of leaves, among others (Table 5.2).

The first attempt to pursue QTL mapping in pear focused on fire-blight disease resistance (Dondini et al. 2004). In an earlier study, fire-blight resistance in pear has been confirmed to be a quantitative trait (Dondini et al. 2002). Subsequently, interval mapping was conducted using a segregating F_1 population (99 seedlings) of 'Passe Crassane' × 'Harrow Sweet', and identified four putative QTLs on LG2a, LG2b, LG4, and LG9 in the 'Harrow Sweet' map (Dondini et al. 2004). In another effort, QTLs for pear scab disease resistance, reported to be a polygenic trait (Chevalier et al. 2004), have been identified. Pierantoni et al. (2007) conducted interval mapping using a segregating F1 population of 'Abbé Fétel' × 'Max Red Bartlett', and detected two QTLs for pear scab resistance on LG3 and LG7 that were different from those mapped on LG1 (Iketani et al. 2001; Yamamoto et al. 2009). Later, Won et al. (2014) performed a Kruskal–Willis analysis using an interspecific pear progeny, PEAR1 × PEAR2, derived from

Table 5.2 Summary of QTLs of agronomic traits in pear

Trait	Linkage groups	Population	Reference(s)
Fire blight	2a, 2b, 4, 9	'Passe Crassane' × 'Harrow Sweet'	Dondini et al. (2004)
Pear scab	3, 7	'Abbè Fétel' (AF) × 'Max Red Bartlett' (MRB)	Pierantoni et al. (2007)
	1	'Kinchaku' × 'Kosui'	Iketani et al. (2001)
	1	'Housui' × 'La France'	Yamamoto et al. (2009)
	2, 5, 7, 10, 17	PEAR1 × PEAR2	Won et al. (2014)
	1	'Housui' × 'La France'	Yamamoto et al. (2009)
Black spot		'Kinchaku' × 'Kosui'	Iketani et al. (2001)
	11	'Housui' × 'La France'	Yamamoto et al. (2009)
Self-incompatibility	17	'Bartlett' × 'Housui'	Yamamoto et al. (2002, 2004, 2009)
Skin color	4	'Abbè Fétel' (AF) × 'Max Red Bartlett' (MRB)	Dondini et al. (2008)
	9	'Abbè Fétel' (AF) × 'Max Red Bartlett' (MRB)	Pierantoni et al. (2010)
	8 (two year)	'Akiakari' × 'Taihaku'	Yamamoto et al. (2014)
	4, 13, 16 (two year)	'Bayuehong' × 'Dangshansuli'	Wu et al. (2014)
Drawf	16	'Aihuali' × 'Chili'	Wang et al. (2016)
Fruit weight	2, 7, 8, 10	'Bayuehong' × 'Dangshansuli'	Zhang et al. (2013)
	3, 11	'Akiakari' × 'Taihaku'	Yamamoto et al. (2014)
	13, 17	'Bayuehong' × 'Dangshansuli'	Wu et al. (2014)
Fruit diameter	10, 15	'Bayuehong' × 'Dangshansuli'	Zhang et al. (2013)
	3, 11, 17	'Bayuehong' × 'Dangshansuli'	Wu et al. (2014)
Fruit length	7 (two year), 8	'Bayuehong' × 'Dangshansuli'	Zhang et al. (2013)
	11, 17 (two year)	'Bayuehong' × 'Dangshansuli'	Wu et al. (2014)
Fruit shape index	1, 2 (two year), 7, 8	'Bayuehong' × 'Dangshansuli'	Zhang et al. (2013)
SSC	2, 5, 6	'Bayuehong' × 'Dangshansuli'	Zhang et al. (2013)
	4, 8	'Akiakari' × 'Taihaku'	Yamamoto et al. (2014)
	5, 10, 14	'Bayuehong' × 'Dangshansuli'	Wu et al. (2014)
Flesh color	9 (two year)	'Bayuehong' × 'Dangshansuli'	Wu et al. (2014)
Firmness	4 (two year)	'Akiakari' × 'Taihaku'	Yamamoto et al. (2014)
Skin smooth	2, 17	'Bayuehong' × 'Dangshansuli'	Wu et al. (2014)
Length of pedicel	2, 14, 17	'Bayuehong' × 'Dangshansuli'	Wu et al. (2014)
Calyx status	6 (two year)	'Bayuehong' × 'Dangshansuli'	Wu et al. (2014)
Juice content	1, 5	'Bayuehong' × 'Dangshansuli'	Wu et al. (2014)
Number of seeds	5 (two year), 9, 14, 17 (two year)	'Bayuehong' × 'Dangshansuli'	Wu et al. (2014)
Preharvest fruit drop	1, 15 (two year)	'Akiakari' × 'Taihaku'	Yamamoto et al. (2014)
Harvest time	3 (two year), 15 (two year)	'Akiakari' × 'Taihaku'	Yamamoto et al. (2014)

(continued)

Table 5.2 (continued)

Trait	Linkage groups	Population	Reference(s)
	8 (two year)	'Bayuehong' × 'Dangshansuli'	Zhang et al. (2013)
Leaf length	8, 15, 16	'Yali' × 'Jingbaili'	Sun et al. (2009)
Leaf width	10, 15	'Yali' × 'Jingbaili'	Sun et al. (2009)
Leaf length/width	5	'Yali' × 'Jingbaili'	Sun et al. (2009)
Petiole length of leaf	4, 15	'Yali' × 'Jingbaili'	Sun et al. (2009)

European (*P. communis*) and Asian (*P. pyrifolia* and *P. ussuriensis*) pears, and identified seven potential QTLs for pear scab resistance. Among these, two QTLs localized on LG2 of PEAR2, one QTL identified on LG5 of PEAR2, two QTLs detected on LG7, along with both PEAR1 and PEAR2 maps, one QTL localized on LG10 of PEAR1, and one QTL detected on LG17 of PEAR1, were identified.

In another effort, 11 QTLs for four leaf traits, including leaf length, leaf width, leaf length/width, and petiole length were identified using interval mapping (Sun et al. 2009). Among these, four QTLs were associated with leaf length, and localized on LG8, LG15, and LG16. In addition, two QTLs were associated with leaf width, localized on LG10 and LG15, two QTLs were associated with leaf length/width, both localized on LG5, and three QTLs were associated with petiole length, localized on LG4 and LG15. The observed phenotypic variation explained (% Expl) by those QTLs ranged from 7.9 to 48.5%.

Several studies have pursued QTL mapping of various pear fruit traits as most fruit-related traits are polygenic and are controlled by quantitative loci. Some of these loci are localized either within the same or in adjacent regions of a genetic linkage map, depending on years of testing or populations used. It is noteworthy to point out that QTLs of correlated fruit quality traits often tend to map to the same chromosomal region (Zhang et al. 2013; Yamamoto et al. 2014; Wu et al. 2014). For example, fruit weight (or fruit size), fruit diameter (transverse diameter), and fruit length (vertical diameter) are highly correlated during various stages of fruit development. Zhang et al. (2013) have identified four QTLs for fruit weight using an F1 population of 'Bayuehong' × 'Dangshansuli', and these are distributed along LG2, LG7, LG8, and LG10, but without repeatability between years. However, when Wu et al. (2014) have used the same population to construct an SNP-based genetic linkage map, they have identified two QTLs for fruit weight on LG13 and LG17. Interestingly, Yamamoto et al. (2014) have detected yet another two QTLs for fruit weight in Japanese pear cultivars Akiakari and Taihaku.

5.5 Software Resources

Currently, there are limited numbers of software available for constructing linkage maps using F1 populations. The most widely used software is JoinMap (Stam 1993). The JoinMap is a commercial software that runs on an MS Windows platform, providing a user-friendly interface. According to the "double pseudo-test cross" hypothesis (Hemmat et al. 1994), JoinMap relies on the "CP" model to construct genetic linkage maps for F1 populations. In general, markers for a heterozygous male parent and a homozygous female parent are used for paternal mapping; whereas, markers for a heterozygous female parent and a homozygous male parent are used for maternal mapping. Furthermore, markers for two heterozygous parents can be used to identify homozygous linkage groups in these parents.

As presented in the JoinMap manual, markers can be divided into the following five different types: <abxcd>, <efxeg>, <lmxll>, <nnxnp>, and <hkxhk>. However, only <lmxll>, <nnxnp>,

and <hkxhk> can be used for genetic linkage map construction. JoinMap offers several mapping parameters to choose from, depending on the user's preference and requirements. However, a major limitation of the JoinMap software is its computing capacity. With the availability of new generation sequencing (NGS), millions of high-quality markers can be identified, yet this is far beyond the capacity of JoinMap to deal with. Recently, JoinMap version 5 has been released. This latest version is a 64-bit MS windows application that allows for the use of a larger memory computer and parallel computation to process higher numbers of loci in a relatively shorter computing time. This software is available at https://www.kyazma.nl/index.php/JoinMap.

Another software that is available for genetic linkage map construction is HighMap, a proprietary software, which has been developed by Biomarker Technologies Corporation (Liu et al. 2014). HighMap has been particularly designed to handle NGS data. It employs an iterative ordering and error-correction strategy based on a *k*-nearest neighbor algorithm and a Monte Carlo multipoint maximum likelihood algorithm. Compared with JoinMap v4.1, HighMap uses the same data format as JoinMap, but as the numbers of markers increase, marker order accuracy and map distance stability are better than those of JoinMap v4.1, along with a higher computational efficiency for map construction. This software is available at http://highmap.biomarker.com.cn/.

Finally, there is R/qtl, a package of the R project (Broman et al. 2003). The R/qtl software is designed for mapping QTLs in experimental populations, and it can also be used to construct genetic linkage maps using the command *est. map*. Strictly speaking, R/qtl cannot directly recognize the "CP" model. Instead, R/qtl first converts the "CP" model as a "four-way cross" model, and then will allow for data analysis to move forward, such as linkage map construction and linkage mapping. In comparing R/qtl to both JoinMap and HighMap softwares, R/qtl is available completely for free, and it does not have limitations for numbers of markers. However, R/qtl does not provide many optional parameters for mapping algorithms, such as regression mapping or maximum likelihood mapping, as with JoinMap v4.1, which may help improve map quality. The package of R/qtl is available at http://www.rqtl.org.

References

Broman KW, Wu H, Sen S, Churchill GA (2003) R/qtl: QTL mapping in experimental crosses. Bioinformatics 19:889–890

Celton J-M, Chagné D, Tustin SD, Terakami S, Nishitani C, Yamamoto T, Gardiner SE (2009) Update on comparative genome mapping between *Malus* and *Pyrus*. BMC Res Notes 2:182. https://doi.org/10.1186/1756-0500-2-182

Chagné D, Crowhurst RN, Pindo M, Thrimawithana A, Deng C, Ireland H, Fiers M, Dzierzon H, Cestaro A, Fontana P (2014) The draft genome sequence of European pear (*Pyrus communis* L. 'Bartlett'). PLoS ONE 9:e92644

Chen H, Song Y, Li LT, Khan MA, Li XG, Korban SS, Wu J, Zhang SL (2015) Construction of a high-density simple sequence repeat consensus genetic map for pear (*Pyrus* spp.). Plant Mol Biol Rep 33:1–10

Chevalier M, Bernard C, Tellier M, Lespinasse Y, Filmond R, Lezec ML (2004) Variability in the reaction of several pear (*Pyrus communis*) cultivars to different inocula of *Venturia pirina*. Acta Hortic 663:177–182

Dondini L, Tartarini S, Sansavini S, Malaguti S, Bazzi C (2002) Reactivity of European pear (*Pyrus communis*) progenies to fire blight (*Erwinia amylovora*). Acta Hortic 596:211–214

Dondini L, Pierantoni L, Gaiotti F, Chiodini R, Tartarini S, Bazzi C, Sansavini S (2004) Identifying QTLs for fire-blight resistance via a European pear (*Pyrus communis* L.) genetic linkage map. Mol Breed 14:407–418

Dondini L, Pierantoni L, Ancarani V, D'Angelo M, Cho KH, Shin IS, Musacchi S, Kang SJ, Sansavini S (2008) The inheritance of the red colour character in European pear (*Pyrus communis*) and its map position in the mutated cultivar 'Max Red Bartlett'. Plant Breed 127:524–526

Fideghelli C, Sartori A, Grassi F (2003) Fruit tree size and architecture. Acta Hortic 622:279–293

Hemmat M, Weeden NF, Manganaris AG, Lawson DM (1994) Molecular marker linkage map for apple. J Hered 85:4–11

Iketani H, Abe K, Yamamoto T, Kotobuki K, Sato Y, Saito T, Terai O, Matsuta N, Hayashi T (2001) Mapping of disease-related genes in Japanese pear using a molecular linkage map with RAPD markers. Breed Sci 51:179–184

Junkyu C, Na DY, Daeil K, Ilsheob S, Moonyoung K, Heejae L (2010) Genetic linkage mapping using

interspecific hybrid population between Korean wild pear (*Pyrus ussuriensis*) and Japanese pear (P. *pyrifolia*). Hortic Environ Biotech 51:319–325

Li L, Deng CH, Knäbel M, Chagné D, Kumar S, Sun J, Zhang S, Wu J (2017) Integrated high-density consensus genetic map of *Pyrus* and anchoring of the 'Bartlett'v1.0 (*Pyrus communis*) genome. DNA Res 24. https://doi.org/10.1093/dnares/dsw063

Liu D, Ma C, Hong W, Huang L, Liu M, Liu H, Zeng H, Deng D, Xin H, Song J (2014) Construction and analysis of high-density linkage map using high-throughput sequencing data. PLoS ONE 9:e98855

Pierantoni L, Dondini L, Cho KH, Shin IS, Gennari F, Chiodini R, Tartarini S, Kang SJ, Sansavini S (2007) Pear scab resistance QTLs via a European pear (*Pyrus communis*) linkage map. Tree Genet Genomes 3:311–317

Pierantoni L, Dondini L, Franceschi PD, Musacchi S, Winkel BSJ, Sansavini S (2010) Mapping of an anthocyanin-regulating MYB transcription factor and its expression in red and green pear, *Pyrus communis*. Plant Physiol Biochem 48:1020–1026

Rivalta L, Dradi M, Rosati C (2002) Thirty years of pear breeding activity at ISF FORLÌ, Italy. Acta Hortic 233–238

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: Join Map. Plant J 3:739–744

Sun W, Zhang Y, Wenquan LE, Zhang H (2009) Construction of a genetic linkage map and QTL analysis for some leaf traits in pear (*Pyrus* L.). Front Agric China 3:67–74

Terakami S, Kimura T, Nishitani C, Sawamura Y, Saito T, Hirabayashi T, Yamamoto T (2009) Genetic linkage map of the Japanese pear 'Housui' identifying three homozygous genomic regions. J Jpn Soc Hortic Sci 78:417–424

Terakami S, Nishitani C, Kunihisa M, Shirasawa K, Sato S, Tabata S, Kurita K, Kanamori H, Katayose Y, Takada N (2014) Transcriptome-based single nucleotide polymorphism markers for genome mapping in Japanese pear (*Pyrus pyrifolia* Nakai). Tree Genet Genomes 10:853–863

Wang C, Tian Y, Buck EJ, Gardiner SE, Dai H, Jia Y (2011) Genetic mapping of *PcDw* determining pear dwarf trait. J Am Soc Hortic Sci 136:48–53

Wang CH, Li W, Tian YK, Hou DL, Bai MD (2016) Development of molecular markers for genetic and physical mapping of the *PcDw* locus in pear (*Pyrus communis* L.). J Pom Hortic Sci 91:299–307

Wang L, Li X, Wang L, Xue H, Wu J, Yin H, Zhang S (2017) Construction of a high-density genetic linkage map in pear (*Pyrus communis* × *Pyrus pyrifolia* Nakai) using SSRs and SNPs developed by SLAF-seq. Sci Hortic 218:198–204

Won K, Bastiaanse H, Kim YK, Song JH, Kang SS, Han CL, Kang HC, Brewer L, Singla G, Gardiner SE (2014) Genetic mapping of polygenic scab (*Venturia pirina*) resistance in an interspecific pear family. Mol Breed 34:2179–2189

Wu Y, Close TJ, Lonardi S (2011) Accurate construction of consensus genetic maps via integer linear programming. IEEE/ACM Trans Comput Biol Bioinf 8 (2):381–394

Wu J, Wang Z, Shi Z, Zhang S, Ming R, Zhu S, Khan MA, Tao S, Korban SS, Wang H, Chen NJ, Nishio T, Xu X, Cong L, Qi K, Huang X, Wang Y, Zhao X, Wu J, Deng C, Gou C, Zhou W, Yin H, Qin G, Sha Y, Tao Y, Chen H, Yang Y, Song Y, Zhan D, Wang J, Li L, Dai M, Gu C, Wang Y, Shi D, Wang X, Zhang H, Zeng L, Zheng D, Wang C, Chen M, Wang G, Xie L, Sovero V, Sha S, Huang W, Zhang S, Zhang M, Sun J, Xu L, Li Y, Liu X, Li Q, Shen J, Wang J, Paull RE, Bennetzen JL, Wang J, Zhang S (2013) The genome of the pear (*Pyrus bretschneideri* Rehd.). Genome Res 23(2):396–408

Wu J, Li LT, Li M, Khan MA, Li XG, Chen H, Yin H, Zhang SL (2014) High-density genetic linkage map construction and identification of fruit-related QTLs in pear using SNP and SSR markers. J Exp Bot 65:5771–5781

Yamamoto T, Kimura T, Shoda M, Imai T, Saito T, Sawamura Y, Kotobuki K, Hayashi T, Matsuta N (2002) Genetic linkage maps constructed by using an interspecific cross between Japanese and European pears. Theor Appl Genet 106:9–18

Yamamoto T, Kimura T, Saito T, Kotobuki K, Matsuta N, Liebhard R, Gessler C, Weg WEVD, Hayashi T (2004) Genetic linkage maps of Japanese and European pears aligned to the apple consensus map. Acta Hortic 663:51–56

Yamamoto T, Kimura T, Terakami S, Nishitani C, Sawamura Y, Saito T, Kotobuki K, Hayashi T (2007) Integrated reference genetic linkage maps of pear based on SSR and AFLP markers. Breed Sci 57:321–329

Yamamoto T, Terakami S, Kimura T, Sawamura Y, Takada N, Hirabayashi T, Imai T, Nishitani C, Socias ICR, Aspiau MT (2009) Reference genetic linkage maps of European and Japanese pears. Acta Hortic 814:599–602

Yamamoto T, Terakami S, Moriya S, Hosaka F, Kurita K, Kanamori H, Katayose Y, Saito T, Nishitani C (2013) DNA markers developed from genome sequencing analysis in Japanese pear (*Pyrus pyrifolia*). Acta Hortic 976:477–483

Yamamoto T, Terakami S, Takada N, Nishio S, Onoue N, Nishitani C, Kunihisa M, Inoue E, Iwata H, Hayashi T (2014) Identification of QTLs controlling harvest time and fruit skin color in Japanese pear (*Pyrus pyrifolia* Nakai). Breed Sci 64:351–361

Zhao Y, Lin H, Guo Y, Liu Z, Guo X, Li K (2013) Genetic linkage maps of pear based on SRAP markers. Pak J Bot 45:1265–1271

Zhang RP, Wu J, Li XG, Khan MA, Chen H, Korban SS, Zhang SL (2013) An AFLP, SRAP, and SSR genetic linkage map and identification of QTLs for fruit traits in pear (*Pyrus* L.). Plant Mol Biol Rep 31:678–687

Molecular Mapping of Major Genes and QTLs in Pear

6

Paolo De Franceschi and Luca Dondini

Abstract

Pear breeding programs are mainly focused on resistance to biotic stress and fruit quality traits. In the last two decades, major efforts have been undertaken toward identification of major genes and quantitative trait loci (QTLs) linked to both biotic resistance and fruit quality traits, along with their associated molecular markers in order to enable marker-assisted selection and breeding. This chapter will cover most relevant results reported so far pertaining to markers and QTLs linked to resistance to pathogens and pests (such as fire blight, scab, brown and black spot, pear psylla, pear sludge, and blister mite), fruit quality (fruit size, firmness, skin overcolor, russeting, fruit sweetness, and fruit acidity), and other traits (such as tree habit, chilling requirement, and harvest time). Furthermore, summaries of findings of studies conducted before and after the beginning of the genomics era will be provided. In addition, all progenies and selected parental lines capable of conferring traits of interest to their progenies are described herein. The aim is to provide breeders with tools to identify pear ideotypes in which several traits can be combined into a single individual. Furthermore, knowledge of genes and their related functions should serve as the basis for pursuing new plant breeding technologies, such as cisgenesis or DNA editing. These unprecedented advances in genomics and breeding strategies promise to enable dramatic improvements in breeding efficiencies, even for pears, that will also reduce time and costs incurred in today's traditional genetic improvement efforts.

6.1 Introduction

Among the critical objectives of primary importance in pear breeding programs are resistance to biotic stresses, ability to adapt to environmental changes, and desirable fruit quality traits. In the past 20 years, major efforts have been undertaken to identify disease resistance genes and to develop molecular tools that will support breeding programs in overcoming these adversities. In recent years, various studies have also aimed at identifying genes responsible for fruit quality traits whose activities result in high levels of phenotypic variability observed in pears.

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Collectively, these studies have revealed that in most cases disease resistance traits are rather complex; moreover, most fruit quality-related traits are also highly polygenic, in which many loci with minor phenotypic effects are involved rather than a few major genes with major effects.

The synteny between the genomes of apple and pear, as well as transferability of molecular markers between these two species (Pierantoni et al. 2004), has aided in the development of the first genetic maps for pear, in which a number of qualitative trait loci (QTL) linked mostly to disease and pest resistance traits have been identified (see Chap. 5 on linkage maps, and literature cited in this chapter).

Earlier efforts in using molecular approaches have proved to be very useful in studying monogenic and polygenic traits related not only to resistance to various pathogens, inciting fire blight, scab, black and brown spot, and pests, such as pear psylla, but also to fruit quality traits, such as fruit color and size, firmness, as well as acid and sugar contents in pear. As most of these traits of pear are of polygenic nature, several QTLs have been identified.

The first genetic maps for pear have been mainly based on microsatellite or simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers (Yamamoto et al. 2002; Dondini et al. 2004; Pierantoni et al. 2004). However, nowadays the availability of a single nucleotide polymorphism (SNP) chip for genotyping in pear (Montanari et al. 2013) allows for the construction of new generations of high-density maps, using classical segregating populations, thereby dramatically promoting discovery of numbers of new loci, while reducing time and effort involved. In turn, this has greatly facilitated efforts to identify and localize QTLs for disease/pest resistance and those for fruit quality, as well as identify genes responsible for these QTLs, and develop molecular markers for assisted selection and breeding.

With the advent of the genomic revolution, in particular the availability of whole genome sequence approaches and technologies, complete draft sequences for several genomes of various fruit tree species have been published, including those for *Pyrus* × *bretschneideri*, Chinese white pear (Wu et al. 2013b), and for *P. communis*, European pear (Chagné et al. 2014). In particular, availability and utilization of next-generation sequencing (NGS) techniques, in most cases, for analysis of whole transcriptomes, have greatly facilitated identification of those genes, and their related allelic variants, underlying expression of agronomic traits, and in some cases, these have also allowed development of markers for use in marker-assisted selection/breeding (MAS/MAB).

Identifying major genes, their sequences, and functions has allowed efforts to pursue new plant breeding technologies (NPBT), such as the development of cisgenic cultivars, as well as the introduction of specific mutations using CRISPR-Cas9 gene editing (Schaart et al. 2016). Therefore, this chapter aims to provide a review of genes and QTLs identified in *Pyrus* species that will support future breeding efforts.

6.2 Major Genes and QTLs for Resistance Against Pathogens and Pests

Often, plant breeders have very ambitious programs aimed at developing disease- and pest-resistant pear cultivars. Unfortunately, these efforts have been limited in the past due to the scarce knowledge of sources of genetic resistance to various important diseases and pests. However, with recent advances in new genetic and genomic technologies along with the availability of worldwide germplasm, collections of *Pyrus* have allowed for the accumulation of new knowledge of genetic and genomic resources for pear. Currently, a few monogenic sources, as well as QTLs for disease and pest resistance, have been identified. Furthermore, a number of molecular markers have been developed that are potentially useful for MAS.

6.2.1 Resistance to Fire Blight

Few pathogens are as devastating as the bacterial pathogen *Erwinia amylovora* (Burrill) Winslow

et al. that incites fire blight disease in pears, as well as in apples. Despite the presence of quarantine measures in several countries, fire blight disease continues to spread throughout the world and contributing to severe yield losses.

The bacterium takes advantage of either natural openings (flowers) or wounds (caused by hail or pruning cuts, among others) to infect plants; moreover, insects can also serve as carriers. When the bacterium infects plant tissues, it spreads along young shoots producing a characteristic symptom known as 'shepherd's crook' (Dondini and Sansavini 2012). Lack of completely effective control measures has accentuated the importance of the availability of fire blight-resistant cultivars with durable resistance as a promising tool for an effective management strategy for this disease (Dondini and Sansavini 2012; Montanari et al. 2016). Fire blight resistance is known to be a polygenic trait (Le Lézec et al. 1997). Several sources of fire blight resistance are known to be available in the pear germplasm, such as 'Old Home', 'Seckel', 'US309', and 'Michigan 437', *P. ussuriensis,* and *P. pyrifolia*, among others, and these have been used to develop and release a number of resistant cultivars, such as 'Harrow Sweet' and 'Moonglow' (Dondini and Sansavini 2012; Montanari et al. 2016). These plant materials have been used to investigate the genetic basis of resistance and to identify a number of QTLs linked to resistance (Fig. 6.1).

Overall, three QTLs have been identified in linkage groups (LGs) 2, 4, and 9 of the European pear 'Harrow Sweet' (Dondini et al. 2004; Le Roux et al. 2012), while two additional QTLs were identified on LGs 9 and 11 of a resistant accession of *P. ussuriensis* (Bokszczanin et al. 2009, 2011), and a major QTL was found on LG 2 of 'Moonglow' (Montanari et al. 2016). Interestingly, some QTLs have also been identified in susceptible accessions, including those found on LGs 3 and 4 of 'Doyenne du Comice' (Bokszczanin et al. 2009, 2011), as well as those located on LGs 7, 9, 10, 12,

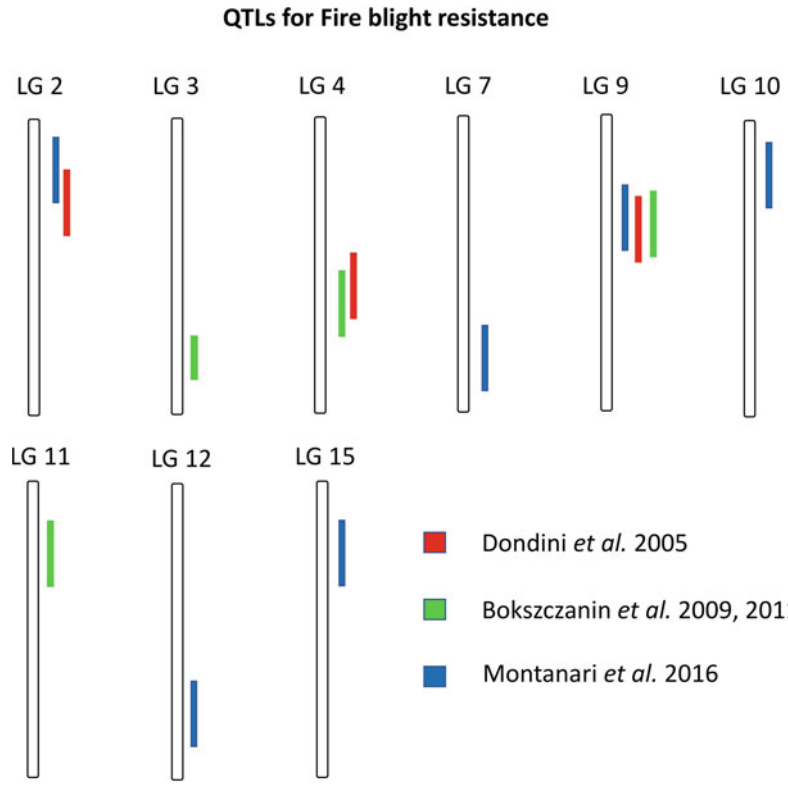

Fig. 6.1 Schematic representation of positions of known QTLs for fire blight resistance. Colors of QTL bars correspond to supporting literature

and 15 of PEAR3, an interspecific hybrid between *P.* × *bretschneideri* and *P. communis* (Montanari et al. 2016). The high numbers of QTLs identified in this latter study were attributed to the use of a high-density map for QTL analysis, wherein an apple and pear Infinium H II 9K SNP array was used for genotyping (Montanari et al. 2013), as well as for phenotyping conducted under different environmental conditions, in both France and New Zealand.

It is important to point out that the two major QTLs identified in 'Harrow Sweet' and 'Moonglow' co-localize around SSR marker TsuENH017, in spite of the fact that the two LOD curves in the two cultivars do not perfectly overlap. The same consideration can be taken into account for QTLs identified on LG 4 of 'Harrow Sweet' and 'Doyenne du Comice', around SSR marker CH02C02, and those found on LG 9 of 'Harrow Sweet' and *P. ussuriensis* in a region around SSR marker CH05C07.

Unfortunately, monogenic sources for fire blight resistance have not yet been identified. However, there is a strong indication of the presence of several major resistance genes in specific regions of the pear genome that could be transferred into new pear cultivars with durable fire blight resistance.

6.2.2 Resistance to Pear Scab

Scab is one of the most serious fungal diseases affecting the European pear, and it is incited by the fungal pathogens *Venturia pirina* Aderh. and *V. nashicola* Tanaka et Yamamoto. Most commonly grown European pear cultivars are susceptible to scab, and unfortunately, there are no commercial cultivars with high levels of resistance to scab. Furthermore, the severity of disease symptoms is also influenced by environmental conditions, as well as by the variability of *V. pirina* biotypes (Chevalier et al. 2004). On the other hand, European pear cultivars seem to serve as sources of resistance to *V. nashicola* (Abe et al. 2008; Cho et al. 2009; Bouvier et al. 2012).

In contrast to fire blight, there are a few monogenic sources for resistance to pear scab that have been identified in both European and Japanese pear cultivars (Fig. 6.2; Abe et al. 2008; Cho et al. 2009; Bouvier et al. 2012). Using interspecific pear hybrids, a single dominant gene, designated as *Vn*, has been identified to confer resistance to *V. nashicola* and proposed to be present in European pears 'La France' and 'Bartlett' (Abe et al. 2008). Subsequently, two additional *V. nashicola* resistance genes have

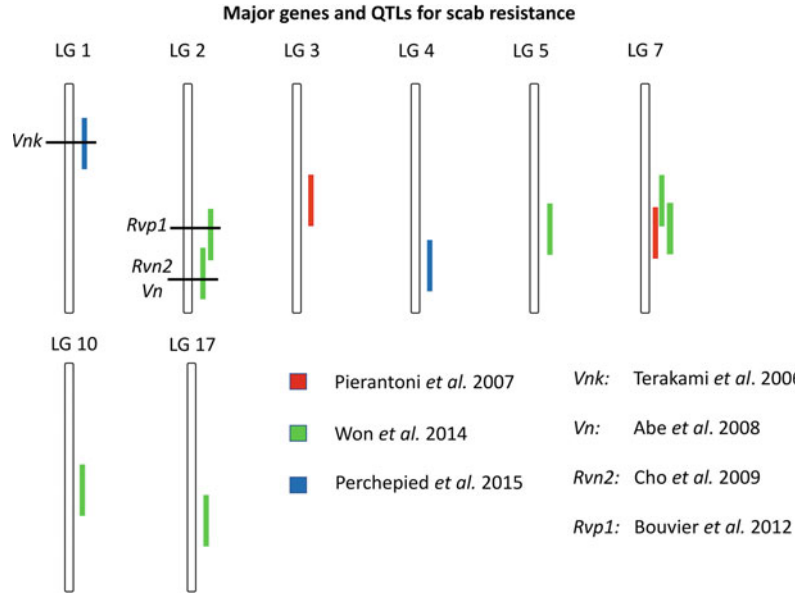

Fig. 6.2 Schematic representation of positions of major genes and QTLs for pear scab resistance

been identified, *Vnk,* mapped on LG 1 of 'Kinchaku'(Terakami et al. 2006), and *Rvn2,* putatively derived from 'Bartlett' (Cho et al. 2009). This latter gene has been mapped to LG 2; however, it is proposed that *Vn* and *Rvn2* could be indeed the same gene (Bouvier et al. 2012). Furthermore, Bouvier et al. (2012) have reported on the presence of yet another monogenic source of resistance to *V. pirina*, the *Rvp1* gene, located on LG 2 of the European pear 'Navara'.

In addition to these monogenic sources of resistance, several QTLs for pear scab resistance have also been identified in recent years (Fig. 6.2) (Pierantoni et al. 2007; Won et al. 2014; Perchepied et al. 2015). Among these, two QTLs have been identified on LG 3 and LG 7 of 'Abbé Fétel' following analysis of a progeny derived from a cross of 'Abbé Fétel' × 'Max Red Bartlett' (a 'Bartlett' red sport); however, no associations have been identified on LG 2 (Pierantoni et al. 2007), wherein the previously described *Rvn2* gene derived from 'Bartlett' was mapped (Cho et al. 2009).

Progeny from the interspecific cross PEAR1 PEAR2, derived from European (*P. communis*) and Asian (*P. pyrifolia and P. ussuriensis*) pears, was inoculated with three single-spore isolates of *V. pirina* and used to develop a high-density linkage map (Won et al. 2014). Using this linkage map, QTLs were identified on LGs 7, 10, and 17 of PEAR1 and on LGs 2, 5, and 7 of PEAR2. Furthermore, the QTL on LG 17 of PEAR1 was found to be effective against all *V. pirina* isolates, while the QTL on LG 7 of PEAR2 was effective against two isolates of *V. pirina* (Won et al. 2014). In addition, the QTLs on LG 7 of PEAR1 and 'Abbé Fétel' seem to map in the same position, while the QTLs of PEAR2 on LG 2 seem to co-localize with *Rvp1* and *Rvn2* genes (Cho et al. 2009; Bouvier et al. 2012). Interestingly, this region has been deemed to be syntenic to an apple scab resistance gene cluster on LG 2 (Bouvier et al. 2012).

Using yet another high-density linkage map, Perchepied et al. (2015) have identified two new QTLs for pear scab resistance against *V. pirina* in P3480, a hybrid with resistance derived from 'Wilder', and in 'Euras'. One locus, designated as qrvp-1, is mapped both as a major gene and as a QTL on LG 1 (within the same region of the *Vnk* gene for resistance against *V. nashicola*), while the second locus, designated as qrvp-o4, is mapped as a QTL on LG 4. Using the cross 'Euras' × P3480, it has been possible to pyramid these two sources of scab resistance into single genotypes (Perchepied et al. 2015). All these findings are summarized in Fig. 6.2.

Overall, the availability of several known sources of pear scab resistance has enabled pursuit of new breeding efforts aimed at selecting new pear genotypes with durable resistance to pear scab.

6.2.3 Resistance and Susceptibility to *Stemphylium vesicarium* and to *Alternaria alternata*

Among the various fungal threats to pears, *Alternaria alternata* (Fries) Keissler and *Stemphylium vesicarium* (Wallr.) E. Simmons, causal agents of black and brown spot, respectively, are among the most widespread diseases. Interestingly, genetic resistance to black spot has been primarily investigated in Japanese pears, while that of brown spot has been investigated more so in European pears.

Early efforts have focused on inducing resistance to *A. alternata* in black spot-susceptible cultivars of apple and pear using gamma-ray irradiation, and have suggested the presence of susceptibility genes that are inactivated by mutagenesis (Sanada et al. 1988; Saito et al. 2001). Subsequently, these susceptibility genes, including *Aki, Ana, and Ani*, have been identified in different Japanese pear cultivars and then mapped to LG 11 of *P. pyrifolia* (Fig. 6.3). These genes are proposed to be involved in and/or responsible for observed necrotic activities of fungal toxins (Iketani et al. 2001; Terakami et al. 2007, 2016). The locus for black spot susceptibility on LG 11 of *P. pyrifolia* has also been confirmed using a genome-wide association study (GWAS) approach (Iwata et al. 2013b).

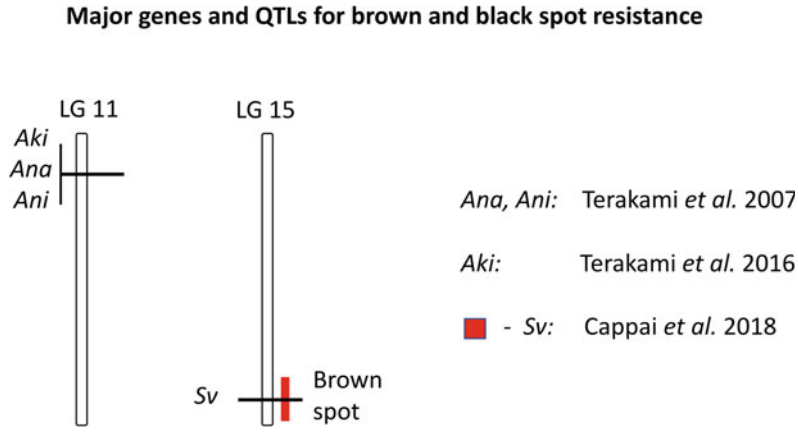

Fig. 6.3 Schematic representation of positions of major genes and QTLs for black and brown spot resistance

On the other hand, most pear cultivars are highly susceptible to brown spot disease, with the important exception of 'Bartlett' and its mutant sports, such as 'Max Red Bartlett' (Llorente and Montesinos 2006). Susceptibility to *S. vesicarium* has been identified, wherein a major QTL for susceptibility is located on LG 15 of 'Abbé Fétel', and the putative position of a susceptibility gene, designated as *Sv*, is estimated to be located at the lower end of the linkage group (Fig. 6.3; Cappai et al. 2018).

Identification of genes controlling susceptibility to black and brown spot diseases will aid in pursuing new plant breeding technologies, such as CRISPR-Cas9 systems, to efficiently develop new pear genotypes with resistance to these fungal pathogens using targeted gene inactivation approaches (Cappai et al. 2018).

6.2.4 Resistance to Pear Psylla and Other Pests

Pear psylla (*Cacopsylla pyri* L.) is a serious pest for pear-growing areas due to the high susceptibility of almost all marketed pear cultivars. Therefore, breeding efforts have focused on identifying sources of tolerance or resistance to pear psylla.

Pyrus fauriei, *P. calleryana,* and *P. ussuriensis* have been identified as sources of psylla resistance (Dondini and Sansavini 2012). The genetic control for resistance to pear psylla is reported to be polygenic; however, only limited studies have been conducted thus far (Bellini and Nin 2002). Nevertheless, resistance to psylla has been introduced from *P. ussuriensis* genotype 'Illinois 65' into a number of pear selections, including 'NY10352', 'NY10353', and 'NY10355' (Westigard et al. 1970; Harris 1973). The latter two selections have been used to characterize resistance responses following pear psylla attack. For example, Pasqualini et al. (2006) have investigated behavior of psyllids on pear selections derived from 'NY10353', while Salvianti et al. (2008) have analyzed differential gene expression in 'NY10355' following challenge with psyllids. In addition, Civolani et al. (2013) have monitored the feeding activity of adults and nymph psyllids on 'NY10353', and have concluded that resistance factors are located in the phloem sap of this selection.

A major QTL for psylla resistance is located on LG 17 of pear selection 'NY10353' (Fig. 6.4; Dondini et al. 2015). This QTL, linked to the nymphal vitality, is first identified using gene scanning, and then subsequently validated following analysis of seedlings of a whole progeny derived from the cross 'NY10353' × 'Doyenne du Comice' (Dondini et al. 2015). In addition, this QTL is also confirmed to be present in 'NY10355' following analysis of a progeny of 'NY10355' ×'Angelys', wherein 'Angelys' is used as a psylla-susceptible parent (Fig. 6.4; Perchepied et al. 2016). Furthermore, Perchepied et al. (2016) have identified four QTLs on LG 1, wherein these QTLs on LG 1 have strong epistatic effects on the QTL on LG 17.

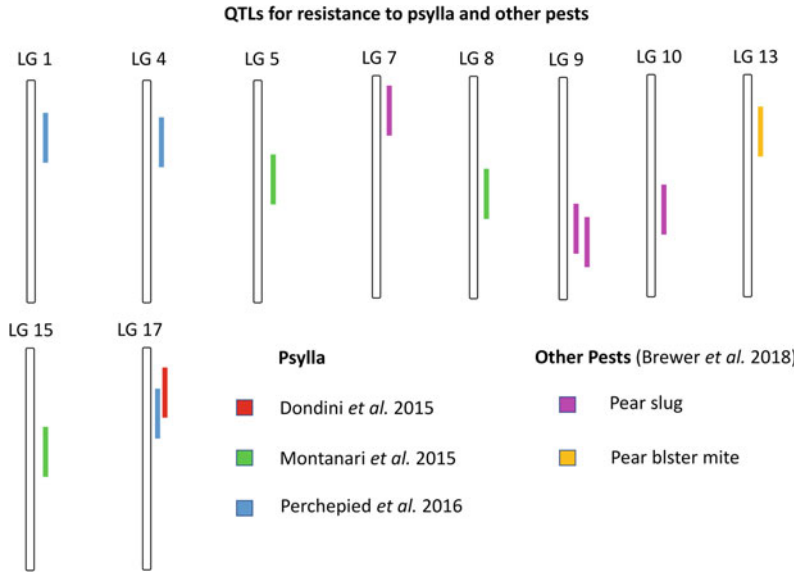

Fig. 6.4 Schematic representation of the positions of QTLs for psylla and other pests resistance

Yet, another source of resistance to pear psylla has been identified, derived from the Chinese white pear *P.* × *bretschneideri*. QTLs for resistance to pear psylla have been identified on LGs 5 and 8 of the hybrid 'PEAR3' ['Xuehuali' (*P.* × *bretschneideri*) × 'Max Red Bartlett' (*P. communis*)], as well as on LG 15 of 'Moonglow', the other parent of the 'PEAR3' × 'Moonglow' progeny used in this study (Fig. 6.4; Montanari et al. 2015).

Very recently, QTLs for resistance to pear slug (the larvae of the sawfly *Caliroa cerasi* L.) and pear blister mite (*Eriophyes pyri* Pagenstecher) have been identified (Brewer et al. 2018) using progeny derived from the cross 'PremP003' × 'Moonglow'. Specifically, a major QTL for resistance to pear blister mite was located on LG 13 of 'PremP003'. For pear slug, three QTLs for oviposition were mapped on LG 7 and LG 9 of 'Moonglow' and on LG 10 of 'PremP003', while another QTL for leaf damage was located on LG 9 of 'Moonglow', just below the oviposition QTL (Fig. 6.4; Brewer et al. 2018).

All the above findings are critical in setting up molecular protocols and MAS breeding strategies aimed at selecting and developing new pear cultivars with combined resistances to different pathogens and pests.

6.3 Major Genes and QTLs for Fruit Quality Traits

As most pear fruit quality traits are under highly polygenic control, with rare exceptions such as the red skin fruit color in European pear, this has hampered identification of major genes. However, with the advent of functional genomics, transcriptomics, and proteomics, many candidate genes or gene families controlling important biosynthetic pathways involved in pear fruit quality have been and are currently under investigation (Lu et al. 2011; Nashima et al. 2013; Li et al. 2014a, 2014b, 2014c, 2014d; Wu et al. 2014b; Dai et al. 2015; Li et al. 2015; Xu et al. 2015; Reuscher et al. 2016; Song et al. 2016; Wei et al. 2016; Zhang et al. 2016; Shen et al. 2017). For further detailed review of functional genomics studies, please refer to Chap. 14.

6.3.1 Fruit Color

Although most common pear cultivars have either yellow or green fruit color, there is an increasing interest and appreciation for cultivars with red skin fruit color. In addition to increased fruit appeal for consumers, red skin color is deemed as a desirable nutritional trait due to the antioxidant activity of anthocyanins, as these flavonoid compounds determine red color pigmentation.

Red skin fruit color in European pears is considered to be a monogenic dominant trait, as confirmed following analysis of seven segregating progenies having one of the following cultivars, 'Max Red Bartlett', 'Cascade', or 'California', as their red-skinned fruit parental line (Dondini et al. 2008). Moreover, this trait is mapped onto LG 4 in 'Max Red Bartlett', a spontaneous red mutant of 'Williams', syn. 'Bartlett' (Fig. 6.5; Dondini et al. 2008).

In Rosaceae, as in most other plant taxa, anthocyanin accumulation is regulated mainly at the transcriptional level, with transcription factors belonging to the Myb family playing a key role (Lin-Wang et al. 2010). The pear transcription factor from European pear (*P. communis*) *PcMYB10*, an ortholog of the apple *MdMYB10* (Espley et al. 2007), is reported to be expressed at much higher levels in 'Max Red Bartlett' than in 'Williams', and it is positively correlated with anthocyanin accumulation during fruit development (Pierantoni et al. 2010). Furthermore, methylation of the *PcMYB10* promoter and its transcriptional silencing are associated with regression to the green color fruit skin phenotype of the same cultivar (Wang et al. 2013). Interestingly, expression of *PcMYB10* in the interspecific hybrid 'Wujiuxiang' ('Ya Li' × 'Bartlett') is positively correlated with anthocyanin accumulation in response to both developmental and cold-temperature induction (Li et al. 2012). These findings clearly point to the role of *PcMYB10* in regulating the anthocyanin biosynthesis pathway during fruit development. Furthermore, it is proposed that *PcMYB10* acts along with a complex containing two other proteins, bHLH (basic helix–loop–helix 33) and WD40 (tryptophan-aspartic acid repeat protein) transcription factors, that bind to promoters of genes for key enzymes of anthocyanin biosynthesis, among which is the gene encoding for UDP-glucose: flavonoid-3-O-glucosyltransferase, *UFGT* (Pierantoni et al. 2010; Wang et al. 2013). This hypothesis is also supported by expression analysis of other European pear cultivars (Li et al. 2012; Wu et al. 2013c; Yang et al. 2013; 2015). Nevertheless, *PcMYB10* is mapped on LG 9 of 'Max Red Bartlett' (Fig. 6.5; Pierantoni et al. 2010). Therefore, it is independent from the 'Red' locus, which maps on LG 4 of 'Max

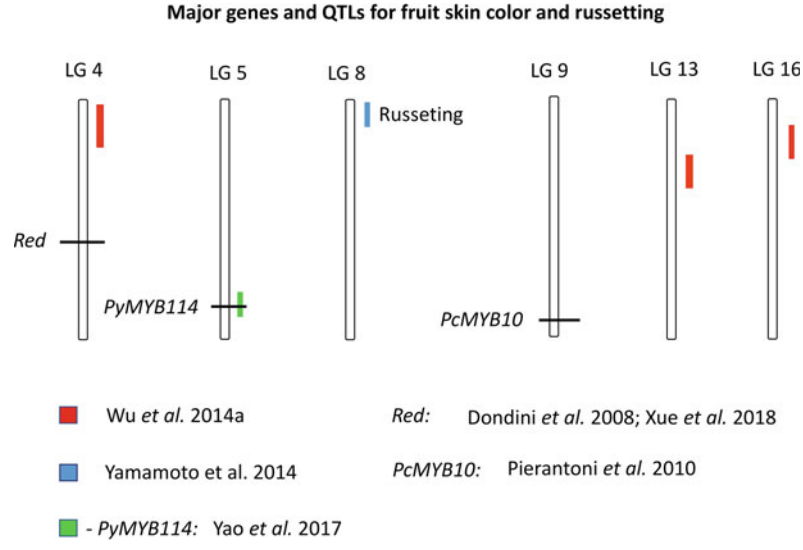

Fig. 6.5 Schematic representation of positions of major genes and QTLs for red skin color and for fruit russeting. QTLs refer mainly to Asian pears, whereas positions of the *Red* locus (from 'Max Red Bartlett') and of the *PcMYB10* gene for European pear are reported herein

Red Bartlett' (Dondini et al. 2008). However, the gene underlying this phenotypic change is yet to be identified, although it must indeed act somehow upstream of *PcMYB10* in the regulation of gene expression.

The red skin fruit color in Asian pears is less frequently observed, and its genetic basis is under investigation. In addition to overall lower accumulation, patterns of anthocyanin synthesis in *P. pyrifolia*, *P. ussuriensis*, and *P.* × *bretschneideri* are different from that observed in *P. communis,* albeit it still correlates with expression of common genes, mainly driven by PcMYB10 orthologs (Feng et al. 2010; Zhang et al. 2011b; Yu et al. 2012; Yang et al. 2014). Expression analysis studies in Chinese pear further support the presence of a common pathway for anthocyanin regulation, involving two Myb transcription factors, PbMYB10b and PbMYB9, promoting expression of *UFGT* and of other genes (Zhai et al. 2016). However, when the genetic control of anthocyanin accumulation has been investigated, discordant results have been obtained. In particular, three QTLs are detected for fruit skin red color in a progeny having 'Bayuehong', a hybrid between the European pear 'Clapp's Favorite' and the Chinese pear 'Zaosuli', as the red-skinned parent (Wu et al. 2014a). One of these QTLs is mapped onto LG 4, but its position (4.8 cM) seems to be incompatible with that of the 'Red' locus (64 cM) found in 'Max Red Bartlett' (Dondini et al. 2008). The other two QTLs have been located on LGs 13 and 16. However, subsequent analysis of the same population has led to the identification of a new QTL located on the bottom of LG 5, and an additional Myb transcription factor, *PyMYB114*, has been identified within this QTL region (Yao et al. 2017). Expression of the *PyMYB114* is positively correlated with red skin coloration, as genetic transformation experiments have confirmed ability of *PyMYB114* to induce anthocyanin biosynthesis, confirming that there are transcription factors, other than the ortholog of *PcMYB10,* that are also involved in expression of this trait.

Xue et al. (2017) have adopted a modified QTL-seq method to compare two DNA pools of red-skinned and green-skinned pears derived from a cross between *P. pyrifolia* cultivars 'Mantianhong' and 'Hongxiangsu', both having red fruits. This analysis has highlighted a 582.5-kb region in chromosome 5 as the main responsible region for red/green fruit color development. This region is compatible with the map position of *PyMYB114* and confirms its position at the bottom of LG 5 as a region controlling this trait in Asian pears. Moreover, unlike in European pear, this study has suggested that the green color is dominant over the red skin color. Therefore, despite the presence of a common biosynthetic pathway for anthocyanin biosynthesis along with a likely conserved role for Myb transcription factors, the genetic control of red skin fruit color appears to be different in Asian and European pears. However, recent analysis of the Chinese pear cultivar 'Red Zaosu', a bud mutant of 'Zaosuli', with red fruits and foliage, has revealed the dominance of red over green phenotypes (Xue et al. 2018). Furthermore, this trait is mapped to the corresponding locus on LG 4 (Xue et al. 2018), at a position that matches with that of the 'Red' locus of 'Max Red Bartlett' (Dondini et al. 2008). On the other hand, a QTL for fruit skin blush is mapped on the bottom of LG 5 in a European pear progeny of 'Flamingo' × 'Abbé Fétel' (Ntladi et al. 2018) and corresponding to the main QTL previously characterized in Asian pear (Yao et al. 2017). These findings reinforce the hypothesis that the same genes regulate anthocyanin biosynthesis and accumulation in European and Asian pears. However, the different genomic positions to which this trait has been associated with reflect its complex genetic control, with many loci playing a role and with the red phenotype arising independently from mutations of various genes.

It should also be noted that an important component of the skin color depends upon suberification of peridermal cells (russeting), conferring a brown color, that is unrelated to the

presence of anthocyanins, which is more likely to occur in Asian rather than in European pears. In fact, a major QTL for this trait has been detected near the top of LG 8 in Japanese pear 'Akiakari' (Fig. 6.5; Yamamoto et al. 2014).

6.3.2 Fruit Size

In pears, like in most cultivated fruit species, fruit size is probably one of the traits that have changed most dramatically during the domestication process. Although the actual fruit size always depends on the interaction between environmental and genetic factors, potential fruit size is genetically determined and varies significantly among different cultivars (Zhang et al. 2006).

Fruit size behaves as a typical quantitative trait, with many loci contributing to its expression. QTL analyses aimed at identifying genomic regions controlling fruit size have been performed mainly in Asian pears (Fig. 6.6). Using progeny of 'Bayuehong' and 'Zaosuli' (*P.* × *bretschneideri*), two QTLs for fruit size were identified on LGs 17 and 13, with the position of QTL 17 found to be compatible with two additional QTLs for transverse and vertical fruit diameter (Wu et al. 2014a). Although this progeny was previously analyzed, resulting in the identification of several QTLs (Zhang et al. 2013), unfortunately, the generated map was based mainly on AFLP and SRAP markers. Thus, these QTLs could not be reliably anchored to reference maps of pear and apple and rendering it difficult to compare positions of these QTLs with those detected in other studies. In yet another study, QTLs for fruit size in Japanese pears were found on LG 11 of 'Akiakari' and LG 3 of 'Taihaku' (Yamamoto et al. 2014), thus once again highlighting how segregation of this trait in different genetic backgrounds might depend on different loci.

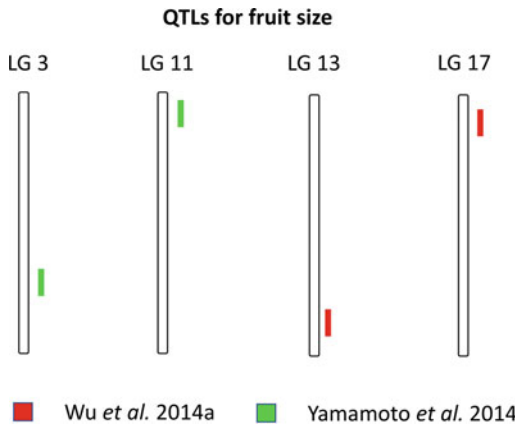

Fig. 6.6 Schematic representation of positions of QTLs for fruit size

Given the complexity of this trait, it is not easy to identify candidate genes for pursuing gene expression studies. 'Da Nanguoli' is a spontaneous large-fruited mutant cultivar of 'Nanguoli' (*P. ussuriensis*), and it has served as a useful tool for studying the genetic mechanism of fruit size. A comparative study of transcript profiling between 'Da Nanguoli' and 'Nanguoli' has revealed the presence of a large pool of genes whose expression is differentially modulated during the development of large-sized and small-sized fruits (Zhang et al. 2011a). While this finding suggests the importance of the role of transcription factors in regulating cellular processes that determine fruit size, the causal mutation has yet to be identified.

Analysis of cytological events involved in fruit development has revealed that fruit size is ultimately determined by the number and size of mesocarp cells, and therefore may vary in response to variations in both cell division and expansion. Larger cell size is responsible for the production of larger fruits in 'Giant La France', a mutant of the European pear 'La France', and it is found to be associated with variations in ploidy of mesocarp cells rather than a result of a genetic mutation (Isuzugawa et al. 2014). Interestingly, polyploidization only impacts fruit flesh, leaving other reproductive tissues diploid, thus suggesting presence of factors determining occurrence and persistence of DNA reduplication in receptacles of 'Giant La France'. Subsequently, two candidate genes, *PcWEE1,* a cell cycle-associated protein kinase, and *PcCCS52A,* an anaphase-promoting complex activator, have been isolated, based on homology with tomato

genes known to play similar roles, and are found to be up-regulated in receptacles of 'Giant La France' (Hanada et al. 2015). This has suggested that differences in expression levels of these two genes may induce DNA reduplication and consequent increase in size of mesocarp cells (Hanada et al. 2015).

When comparing common diploid pear cultivars, variations in fruit size are normally associated with variations in cell number rather than in cell size (Zhang et al. 2006). Homologs of *fw2.2*, a gene controlling fruit size by regulating cell division in tomato (Frary et al. 2000), are proposed to be involved in the same process in different plant species including fruit trees. In cherry trees, some of these *fw2.2* homologs are co-localized with known QTLs for fruit size (De Franceschi et al. 2013). Two genes belonging to this family, *PbFWL1* and *PbFWL2*, have been characterized in Chinese pear and are found to be expressed at higher levels in small-fruited cultivars, consistent with the negative regulatory role of *fw2.2* in cell division (Tian et al. 2016). Therefore, these two genes are good candidates for control of fruit size in pear. However, additional studies are required to study functionality of these genes.

6.3.3 Fruit Sensory Qualities

Fruit taste is determined by many different biochemical factors, such as accumulation of sugars and acids, flesh firmness and texture, and emission of volatile compounds (aroma). However, limited information is available regarding genetic regions controlling these traits in segregating pear progenies, although QTLs for soluble solid content, fruit acidity, and firmness have been identified (Fig. 6.7).

Soluble solid content of pear fruits is essentially determined by sugars and organic acids. The amounts and ratios between these different compounds are critical factors in determining fruit taste and therefore deemed as key components of fruit quality. As sugars and organic acids are primary metabolites, many factors can impact their synthesis and accumulation in fruits. Not surprisingly, QTLs for soluble solid content have been detected in different genomic regions of *P. pyrifolia,* LGs 4 and 8 (Yamamoto et al. 2014), *P.* × *bretschneideri,* LGs 5, 10, and 14 (Wu et al. 2014a), and an interspecific hybrid population of Asian and European pear, LGs 9 and 10 (Saeed et al. 2014). Unfortunately, it is not possible to determine whether or not the two QTLs for soluble solid content in LG 10 (Wu et al. 2014a; Saeed et al. 2014) overlap, although they seem to be located in the same chromosomic region. A recent analysis conducted on a Japanese pear population derived from the cross 'Akizuki' × '373-55', besides a QTL for total sugar content on LG 11, has detected two QTLs associated with the conversion of sucrose to fructose and glucose on LGs 1 and 7 (Nishio et al. 2018). Moreover, two acid invertase (AIV) genes are found in close proximity of both QTLs, thus serving as interesting candidates for control of sugar conversion in pear fruits. On the other hand, a single QTL for fruit acidity, located on LG 14, is reported (Yamamoto et al. 2014). It is noteworthy to point out that the organic acid content can also be significantly influenced by maternal inheritance, suggesting that non-nuclear genes may play important roles as well (Liu et al. 2016).

Fruit firmness is determined by cell wall components, which are degraded by several hydrolases during ripening and leading to fruit softening. QTLs for this trait have been identified on LG 4 (Yamamoto et al. 2014) and LG 3 (Saeed et al. 2014). The latter linkage group, LG 3, has effects on other ripening-related traits, such as fruit friction discoloration, polyphenol oxidase (PPO) activity, and polyphenol content. Furthermore, QTLs associated with PPO activity have been identified on LGs 2 and 3, as well as a number of QTLs associated with contents of 17 polyphenolic compounds have also been identified (Saeed et al. 2014).

In addition to the different enzymes that catalyze cell wall degradation, expansins are proposed to play a role in fruit softening as they disrupt hydrogen bonds between cellulose microfibrils and matrix polysaccharides, thereby rendering substrates available to hydrolases. An

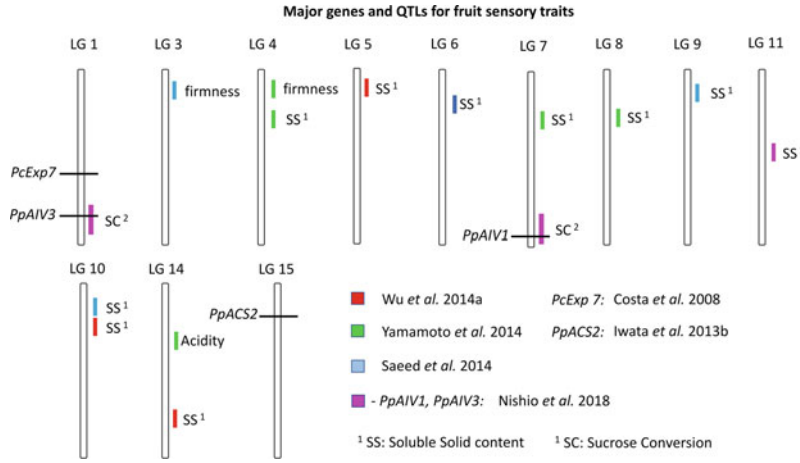

Fig. 6.7 Schematic representation of positions of major genes and QTLs for soluble solid content (SS), firmness, and acidity

expansin gene, *PcExp7*, from *P. communis*, has been mapped on LG 1 in a region in which a firmness QTL has been detected in apple (Costa et al. 2008). The presence of a member of the gene family coding for 1-aminocyclopropane-1-carboxylate synthase, which plays a role in determining harvest time, may also be involved in pear fruit softening (Iwata et al. 2013b; Yamamoto et al. 2014). However, further studies are required to ascertain whether or not such a candidate gene co-localizes with QTLs for firmness in pear.

6.4 Major Genes and QTLs for Other Traits

Most efforts for developing molecular markers for marker-assisted selection (MAS) have focused on traits for resistance to pathogens and pests, as well as on fruit quality traits. However, there are limited efforts in developing molecular markers linked to other traits.

Using a progeny derived from a cross between 'Spadona' (with a low chilling requirement) and 'Harrow Sweet' (with a high chilling requirement) along with a comparative analogy to an apple linkage map, QTLs for bud break (following release from dormancy) have been found on LG 8, corresponding to SSR NAUpy98n, and LG 9, between SSRs NH029 and CH01f03b (Gabay et al. 2017). The same population was analyzed more in depth by developing a high-resolution SNP map, using a genotyping by sequencing (GBS) approach, detecting three additional QTLs on LGs 5, 13, and 15 (Gabay et al. 2018), and confirming the presence of QTLs on LGs 8 and 9. The latter was further confirmed in a different progeny of European pear (Ntladi et al. 2018). For further information on bud break, please look up Chap. 12 of this volume.

Using a genome-wide association study (GWAS) analysis of 76 cultivars of *P. pyrifolia,* QTLs for harvest time have been mapped on LGs 3 (corresponding to SSR marker BGA35) and 15 (identified by the CAPS marker PPACS2) (Fig. 6.8; Iwata et al. 2013b). Incidentally, the marker PPACS2 identifies the position of a member of the 1-aminocyclopropane-1-carboxylate synthase gene family (Iwata et al. 2013b; Yamamoto et al. 2014). In addition, both QTLs have been identified by analyzing a segregating progeny derived from the cross 'Akiakari' × 'Taihaku' (Yamamoto et al. 2014). Furthermore, both markers BGA35 and PPACS2 have been validated by analyzing segregation data in six F1 progenies of *P. pyrifolia*, demonstrating that alleles of 263 bp of PPACS2 and 136 bp of BGA35 are in linkage to the early ripening fruit trait (Nishio et al. 2016). This QTL, together with another QTL found on LG 15, has been identified in the parent 'Taihaku'. Interestingly, results of findings on LG 3 of pear have also been confirmed in a subsequent GWAS in apple in which a major association for ripening time is found on chromosome 3 (Urrestarazu et al. 2017).

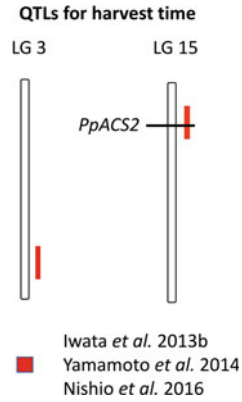

Fig. 6.8 Schematic representation of positions of major genes and QTLs for harvest time

Although other traits such as plant vigor have been phenotyped in 76 cultivars of *P. pyrifolia*, no associations could be found (Iwata et al. 2013b); whereas, associations for plant vigor and early flowering have been detected in pear rootstock breeding studies (Knäbel et al. 2015, 2017). By genotyping a very large progeny derived from the cross 'Old Home' × 'Louise Bonne de Jersey', wherein all seedlings are used for grafting the pear scion cultivar 'Doyenne du Comice', high-density linkage maps have been developed. Using these linkage maps, QTLs have been identified on the top of LG 5 of 'Old Home' for tree architecture, tree vigor, and various precocity traits, including number of branches per tree, tree height, number of inflorescences, number of spurs per tree, trunk cross-sectional areas (TCA) of the rootstock and of the scion around the graft zone, and root suckering (Knäbel et al. 2015). Furthermore, except for a number of inflorescences, additional QTLs have been identified for all other mentioned traits on the top of LG 6 of 'Old Home' and in the middle of LG 6 of 'Louise Bonne de Jersey' (Knäbel et al. 2015). Other minor QTLs, for trunk cross-sectional areas of the scion and of the rootstock, are found on LGs 7 and 16 of 'Louise Bonne de Jersey', respectively (Fig. 6.9; Knäbel et al. 2015). In a different study on apples, a major QTL, controlling most of the dwarfing effects conferred to a scion, has been identified on LG 5 of the apple rootstock 'M9' (Foster et al. 2015). It is proposed that the SSR marker flanking the *Dw1* locus in apple (Hi01c04) also segregates for dwarfing and precocity in pear with an allele of 116 bp in size associated with these traits (Knäbel et al. 2015). The synteny between the apple and pear genomes is very important in identifying candidate genes for controlling various traits, including these reported herein.

Using the same progeny described above, QTLs controlling the development of adventitious roots on hardwood cuttings have been identified on LGs 7, 8, 10, and 11 of 'Old Home' and on LGs 7, 15, and 16 of 'Louise Bonne de Jersey'. In addition, a single QTL associated with callus and root development has been found on LG 4 of 'Louise Bonne de Jersey' (Knäbel et al. 2017). Furthermore, favorable alleles of markers in QTL peaks of LG 7 (ss527788659 in 'Old Home' and ss527789100 in 'Louise Bonne de Jersey') have demonstrated male and female additive and dominance effects for all years (Knäbel et al. 2017). Therefore, the availability of molecular markers will support breeding efforts aimed at selecting new pear rootstocks that are easily propagated along with other desirable traits such as vigor and early flowering of known dwarfing rootstocks available for apples.

Finally, an important trait for consideration pertains to the S-RNase-based gametophytic self-incompatibility (GSI), previously reviewed by De Franceschi et al. (2012) and Wu et al. (2013a). In addition to determining cross-compatibility of cultivars, GSI may also influence transmission of genes in proximity of the *S* locus. The *S-RNase* gene has been mapped on the bottom of LG 17 in both Japanese and European pears (Yamamoto et al. 2002) and consistent with the position of the *S* locus in apple (Maliepaard et al. 1998). Subsequently, identification and mapping of *S*-locus *F*-box brother genes, the male counterpart of S-RNase (Sassa et al. 2007), confirmed their linkage to *S-RNase* (De Franceschi et al. 2011). A detailed information and review of self-incompatibility of pear are provided in Chap. 10 of this volume.

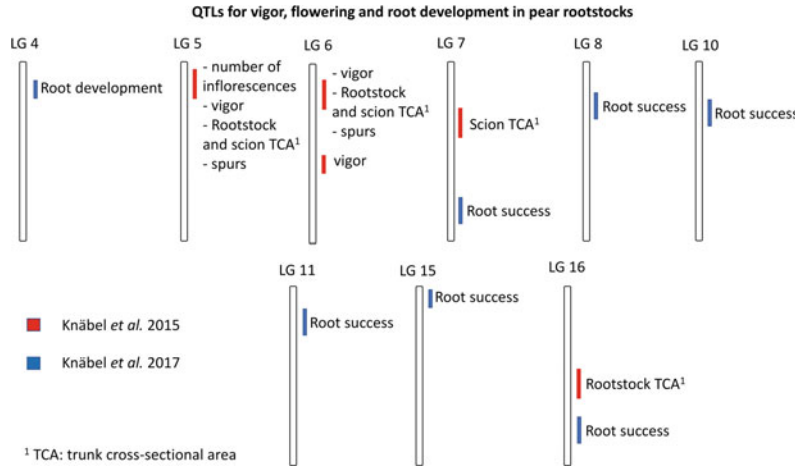

Fig. 6.9 Schematic representation of positions of QTLs for vigor, flowering, and root development in pear rootstocks

6.5 Conclusions

Identification of major genes and QTLs linked to disease and pest resistance, fruit quality, and other tree-related traits in *Pyrus* will certainly contribute to advances in MAS and in other applications offered by the tools of genomics. In particular, identification of QTLs will also assist in identification of additional genes, and possibly of related allelic variants, underlying observed phenotypic effects. These findings will in turn enable design of new additional markers for use in MAS. The release of the genome sequences for the Asian and European pears, along with the availability of high-throughput genotyping techniques, which allows for simultaneous analysis of thousands of markers, will offer opportunities for more targeted and efficient selection of desirable genotypes in a pear breeding population.

The availability of tools for large-scale genotyping will also assist in pursuing GWAS approaches of pear germplasm collections, and enhance efforts in identifying genes and alleles responsible for traits of interest. Unfortunately, the time required for phenotyping remains the greatest bottleneck in pursuing these approaches. Nevertheless, genes controlling various traits can be identified via transcriptomic approaches that next-generation sequencing technologies have made possible. For more information on functional genomics studies in pear, please read Chap. 14.

For those genes with strong effects on phenotypic variability, such as transcription factors, and for major QTLs, molecular marker selection offers serious advantages. Unfortunately, a number of QTLs with minor effects on a phenotype have been presented in this current review. For these cases, the utility of linked markers for MAS is likely to be less effective in supporting pear breeding programs. This is particularly true in instances wherein the cost for genotyping seedlings must be justified when compared to conventional phenotypic selection methods. Nevertheless, novel approaches such as genomic selection are becoming more feasible and offer promise in making significant great advances in this arena (Iwata et al. 2013a; Minamikawa et al. 2018).

Finally, it is important to conclude that once genes and their related functions become known, a critical consideration must be taken into account. Whether, we should choose to use new plant breeding technologies, such as cisgenesis or DNA editing, in inserting mutations and altering gene functions (Schaart et al. 2016), and how best to exploit breeding advantages offered via use of modified genes, either gene mutations or gene editing, with significant reduction in time and costs in developing and releasing improved

pear genotypes with enhanced and desirable traits compared to earlier traditional pear breeding efforts.

References

Abe K, Saito T, Terai O, Sato Y, Kotobuki K (2008) Genotypic difference for the susceptibility of Japanese, Chinese and European pears to *Venturia nashicola*, the cause of scab on Asian pears. Plant Breed 127:407–412. https://doi.org/10.1111/j.1439-0523.2007.01482.x

Bellini E, Nin S (2002) Breeding for new traits in pear. Acta Hortic 217–224. https://doi.org/10.17660/actahortic.2002.596.31

Bokszczanin K, Dondini L, Przybyla AA (2009) First report on the presence of fire blight resistance in linkage group 11 of *Pyrus ussuriensis* Maxim. J Appl Genet 50:99–103. https://doi.org/10.1007/bf03195660

Bokszczanin KL, Przybyla AA, Dondini L, Palucha A (2011) QTLs for fire blight (*Erwinia amylovora*) resistance in *Pyrus ussuriensis*. Acta Hortic 371–373. https://doi.org/10.17660/actahortic.2011.896.52

Bouvier L, Bourcy M, Boulay M, Tellier M, Guérif P, Denancé C, Durel CE, Lespinasse Y (2012) A new pear scab resistance gene *Rvp1* from the European pear cultivar "Navara" maps in a genomic region syntenic to an apple scab resistance gene cluster on linkage group 2. Tree Genet Genomes 8:53–60. https://doi.org/10.1007/s11295-011-0419-x

Brewer L, Shaw P, Wallis R, Alspach P, Aldworth M, Orellana-torrejon C, Chagné D, Bus VGM, Brewer L (2018) Genetic mapping of pear sawfly (*Caliroa cerasi*) and pear blister mite (*Eriophyes pyri*) resistance in an interspecific pear family. Tree Genet Genomes 14:38. https://doi.org/10.1007/s11295-018-1254-0

Cappai F, De Franceschi P, Ciriani A, Collina M, Dondini L (2018) QTLs for susceptibility to *Stemphylium vesicarium* in pear. Mol Breed 38:24. https://doi.org/10.1007/s11032-018-0785-2

Chagné D, Crowhurst RN, Pindo M, Thrimawithana A, Deng C, Ireland H, Fiers M, Dzierzon H, Cestaro A, Fontana P, Bianco L, Lu A, Storey R, Knäbel M, Saeed M, Montanari S, Kim YK, Nicolini D, Larger S, Stefani E, Allan AC, Bowen J, Harvey I, Johnston J, Malnoy M, Troggio M, Perchepied L, Sawyer G, Wiedow C, Won K, Viola R, Hellens RP, Brewer L, Bus VGM, Schaffer RJ, Gardiner SE, Velasco R (2014) The draft genome sequence of European pear (*Pyrus communis* L. 'Bartlett'). PLoS ONE 9:e92644. https://doi.org/10.1371/journal.pone.0092644

Chevalier M, Bernard C, Tellier M, Lespinasse Y, Filmond R, Le Lezec M (2004) Variability in the reaction of several pear (*Pyrus communis*) cultivars to differentinocula of *Venturia pirina*. Acta Hortic 177–182. https://doi.org/10.17660/actahortic.2004.663.25

Cho KH, Shin S, Kim KT, Suh EJ, Hong SS, Lee HJ (2009) Development of AFLP and CAPS markers linked to the scab resistance gene, *Rvn2*, in an inter-specific hybrid pear (*Pyrus* spp.). J Hortic Sci Biotechnol 84:619–624. https://doi.org/10.1080/14620316.2009.11512576

Civolani S, Grandi G, Chicca M, Pasqualini E, Fano EA, Musacchi S (2013) Probing behaviour of Cacopsylla pyri on a resistant pear selection. J Appl Entomol 137:365–375. https://doi.org/10.1111/jen.12003

Costa F, Van De Weg WE, Stella S, Dondini L, Pratesi D, Musacchi S, Sansavini S (2008) Map position and functional allelic diversity of *Md-Exp7*, a new putative expansin gene associated with fruit softening in apple (*Malus* × *domestica* Borkh.) and pear (*Pyrus communis*). Tree Genet Genomes 4:575–586. https://doi.org/10.1007/s11295-008-0133-5

Dai M, Shi Z, Xu C (2015) Genome-wide analysis of sorbitol dehydrogenase (*SDH*) genes and their differential expression in two sand pear (*Pyrus pyrifolia*) fruits. Int J Mol Sci 16:13065–13083. https://doi.org/10.3390/ijms160613065

De Franceschi P, Pierantoni L, Dondini L, Grandi M, Sanzol J, Sansavini S (2011) Cloning and mapping multiple *S*-locus *F*-box genes in European pear (*Pyrus communis* L.). Tree Genet Genomes 7:231–240. https://doi.org/10.1007/s11295-010-0327-5

De Franceschi P, Dondini L, Sanzol J (2012) Molecular bases and evolutionary dynamics of self-incompatibility in the Pyrinae (Rosaceae). J Exp Bot 63:4015–4032. https://doi.org/10.1093/jxb/ers108

De Franceschi P, Stegmeir T, Cabrera A, van der Knaap E, Rosyara UR, Sebolt AM, Dondini L, Dirlewanger E, Quero-Garcia J, Campoy JA, Iezzoni AF (2013) Cell number regulator genes in *Prunus* provide candidate genes for the control of fruit size in sweet and sour cherry. Mol Breed 32:311–326. https://doi.org/10.1007/s11032-013-9872-6

Dondini L, Sansavini S (2012) European pear. In: Badenes ML, Byrne DH (eds) Fruit breeding. Springer Science + Business Media, Boston, MA, pp 369–413

Dondini L, Pierantoni L, Gaiotti F, Chiodini R, Tartarini S, Bazzi C, Sansavini S (2004) Identifying QTLs for fire-blight resistance via a European pear (*Pyrus communis* L.) genetic linkage map. Mol Breed 14:407–418. https://doi.org/10.1007/s11032-005-0505-6

Dondini L, Pierantoni L, Ancarani V, D'Angelo M, Cho KH, Shin IS, Musacchi S, Kang SJ, Sansavini S (2008) The inheritance of the red colour character in European pear (*Pyrus communis*) and its map position in the mutated cultivar "Max Red Bartlett". Plant Breed 127:524–526. https://doi.org/10.1111/j.1439-0523.2008.01500.x

Dondini L, De Franceschi P, Ancarani V, Civolani S, Fano EA, Musacchi S (2015) Identification of a QTL for psylla resistance in pear via genome scanning approach. Sci Hortic 197:568–572. https://doi.org/10.1016/j.scienta.2015.10.018

Espley RV, Hellens RP, Putterill J, Stevenson DE, Kutty-Amma S, Allan AC (2007) Red colouration in apple fruit is due to the activity of the MYB transcription factor, *MdMYB10*. Plant J 49:414–427. https://doi.org/10.1111/j.1365-313X.2006.02964.x

Feng S, Wang Y, Yang S, Xu Y, Chen X (2010) Anthocyanin biosynthesis in pears is regulated by a R2R3-MYB transcription factor PyMYB10. Planta 232:245–255. https://doi.org/10.1007/s00425-010-1170-5

Foster TM, Celton JM, Chagne D, Stuart Tustin D, Gardiner SE (2015) Two quantitative trait loci, *Dw1* and *Dw2*, are primarily responsible for rootstock-induced dwarfing in apple. Hortic Res 2:15001. https://doi.org/10.1038/hortres.2015.1

Frary A, Nesbitt TC, Frary A, Grandillo S, Van Der Knaap E, Cong B, Liu J, Meller J, Elber R, Alpert KB, Tanksley SD (2000) *fw2.2*: a quantitative trait locus key to the evolution of tomato fruit size. Science 289:85–88. https://doi.org/10.1126/science.289.5476.85

Gabay G, Dahan Y, Izhaki Y, Isaacson T, Elkind Y, Ben-Ari G, Flaishman MA (2017) Identification of QTLs associated with spring vegetative budbreak time after dormancy release in pear (*Pyrus communis* L.). Plant Breed 136:749–758. https://doi.org/10.1111/pbr.12499

Gabay G, Dahan Y, Izhaki Y, Faigenboim A, Ben-ari G, Elkind Y, Flaishman MA (2018) High-resolution genetic linkage map of European pear (*Pyrus communis*) and QTL fine-mapping of vegetative budbreak time. BMC Plant Biol 18:1–13. https://doi.org/10.1186/s12870-018-1386-2

Hanada T, Nashima K, Kato M, Takashina T, Ikeda K, Sakamoto Y, Takahashi H, Nakazono M, Oikawa A, Shiratake K, Isuzugawa K (2015) Molecular cloning and expression analysis of the *WEE1* and *CCS52A* genes in European pear (*Pyrus communis* L.) and their possible roles in a giant fruit mutant. J Hortic Sci Biotech 90:511–517. https://doi.org/10.1080/14620316.2015.11668707

Harris MK (1973) Host resistance to the pear psylla in a *Pyrus communis* × *P. ussuriensis* hybrid. Environ Entomol 2:883–887. https://doi.org/10.1093/ee/2.5.883

Iketani H, Abe K, Yamamoto T, Kotobuki K, Sato Y, Saito T, Terai O, Matsuta N, Hayashi T (2001) Mapping of disease-related genes in Japanese pear using a molecular linkage map with RAPD markers. Breed Sci 51:179–184. https://doi.org/10.1270/jsbbs.51.179

Isuzugawa K, Murayama H, Nishio T (2014) Characterization of a giant-fruit mutant exhibiting fruit-limited polyploidization in pear (*Pyrus communis* L.). Sci Hort 170:196–202. https://doi.org/10.1016/j.scienta.2014.03.009

Iwata H, Hayashi T, Terakami S, Takada N, Saito T, Yamamoto T (2013a) Genomic prediction of trait segregation in a progeny population: a case study of Japanese pear (*Pyrus pyrifolia*). BMC Genet 14:81. https://doi.org/10.1186/1471-2156-14-81

Iwata H, Hayashi T, Terakami S, Takada N, Sawamura Y, Yamamoto T (2013b) Potential assessment of genome-wide association study and genomic selection in Japanese pear *Pyrus pyrifolia*. Breed Sci 63:125–140. https://doi.org/10.1270/jsbbs.63.125

Knäbel M, Friend AP, Palmer JW, Diack R, Wiedow C, Alspach P, Deng C, Gardiner SE, Tustin DS, Schaffer R, Foster T, Chagné D (2015) Genetic control of pear rootstock-induced dwarfing and precocity is linked to a chromosomal region syntenic to the apple *Dw1* loci. BMC Plant Biol 15:230. https://doi.org/10.1186/s12870-015-0620-4

Knäbel M, Friend AP, Palmer JW, Diack R, Gardiner SE, Tustin S, Schaffer R, Foster T, Chagné D (2017) Quantitative trait loci controlling vegetative propagation traits mapped in European pear (*Pyrus communis* L.). Tree Genet Genomes 13:55. https://doi.org/10.1007/s11295-017-1141-0

Le Lézec M, Lecomte P, Laurens F, Michelesi JC (1997) Sensibilité variétale au feu bactérien. L'Arboriculture Fruitière 503:57–62

Le Roux PMF, Christen D, Duffy B, Tartarini S, Dondini L, Yamamoto T, Nishitani C, Terakami S, Lespinasse Y, Kellerhals M, Patocchi A (2012) Redefinition of the map position and validation of a major quantitative trait locus for fire blight resistance of the pear cultivar "Harrow Sweet" (*Pyrus communis* L.). Plant Breed 131:656–664. https://doi.org/10.1111/j.1439-0523.2012.02000.x

Li L, Ban ZJ, Li XH, Wu MY, Wang AL, Jiang YQ, Jiang YH (2012) Differential expression of anthocyanin biosynthetic genes and transcription factor *PcMYB10* in Pears (*Pyrus communis* L.). PLoS ONE 7:e46070. https://doi.org/10.1371/journal.pone.0046070

Li G, Jia H, Li J, Wang Q, Zhang M, Teng Y (2014a) Emission of volatile esters and transcription of ethylene- and aroma-related genes during ripening of "Pingxiangli" pear fruit (*Pyrus ussuriensis* Maxim). Sci Hortic 170:17–23. https://doi.org/10.1016/j.scienta.2014.03.004

Li JM, Zheng DM, Li LT, Qiao X, Wei SW, Bai B, Zhang SL, Wu J (2014b) Genome-wide function, evolutionary characterization and expression analysis of sugar transporter family genes in pear (*Pyrus bretschneideri* Rehd). Plant Cell Physiol 56:1721–1737. https://doi.org/10.1093/pcp/pcv090

Li M, Li L, Dunwell JM, Qiao X, Liu X, Zhang S (2014c) Characterization of the lipoxygenase (*LOX*) gene family in the Chinese white pear (*Pyrus bretschneideri*) and comparison with other members of the Rosaceae. BMC Genom 15:1–12. https://doi.org/10.1186/1471-2164-15-444

Li T, Li X, Tan D, Jiang Z, Wei Y, Li J, Du G, Wang A (2014d) Distinct expression profiles of ripening related genes in the "Nanguo" pear (*Pyrus ussuriensis*) fruits. Sci Hortic 171:78–82. https://doi.org/10.1016/j.scienta.2014.03.054

Li JM, Huang XS, Li LT, Zheng DM, Xue C, Zhang SL, Wu J (2015) Proteome analysis of pear reveals key

genes associated with fruit development and quality. Planta 241:1363–1379. https://doi.org/10.1007/s00425-015-2263-y

Lin-Wang K, Bolitho K, Grafton K, Kortstee A, Karunairetnam S, McGhie TK, Espley RV, Hellens RP, Allan AC (2010) An *R2R3* MYB transcription factor associated with regulation of the anthocyanin biosynthetic pathway in Rosaceae. BMC Plant Biol 10:50. https://doi.org/10.1186/1471-2229-10-50

Liu L, Chen CX, Zhu YF, Xue L, Liu QW, Qi KJ, Zhang SL, Wu J (2016) Maternal inheritance has impact on organic acid content in progeny of pear (*Pyrus* spp.) fruit. Euphytica 209:305–321. https://doi.org/10.1007/s10681-015-1627-5

Llorente I, Montesinos E (2006) Brown spot of pear: an emerging disease of economic importance in Europe. Plant Dis 90:1368–1375. https://doi.org/10.1094/PD-90-1368

Lu XP, Liu YZ, Zhou GF, Wei QJ, Hu HJ, Peng SA (2011) Identification of organic acid-related genes and their expression profiles in two pear (*Pyrus pyrifolia*) cultivars with difference in predominant acid type at fruit ripening stage. Sci Hortic 129:680–687. https://doi.org/10.1016/j.scienta.2011.05.014

Maliepaard C, Alston FH, Van Arkel G, Brown LM, Chevreau E, Dunemann F, Evans KM, Gardiner S, Guilford P, Van Heusden AW, Janse J, Laurens F, Lynn JR, Manganaris AG, Den Nijs APM, Periam N, Rikkerink E, Roche P, Ryder C, Sansavini S, Schmidt H, Tartarini S, Verhaegh JJ, Vrielink-van Ginkel M, King GJ (1998) Aligning male and female linkage maps of apple (*Malus pumila* Mill.) using multi-allelic markers. Theor Appl Genet 97:60–73. https://doi.org/10.1007/s001220050867

Minamikawa MF, Takada N, Terakami S, Saito T, Onogi A, Kajiya-Kanegae H, Hayashi T, Yamamoto T, Iwata H (2018) Genome-wide association study and genomic prediction using parental and breeding populations of Japanese pear (*Pyrus pyrifolia* Nakai). Sci Rep 8:11994. https://doi.org/10.1038/s41598-018-30154-w

Montanari S, Saeed M, Knäbel M, Kim YK, Troggio M, Malnoy M, Velasco R, Fontana P, Won KH, Durel CE, Perchepied L, Schaffer R, Wiedow C, Bus V, Brewer L, Gardiner SE, Crowhurst RN, Chagné D (2013) Identification of *Pyrus* single nucleotide polymorphisms (SNPs) and evaluation for genetic mapping in European pear and interspecific *Pyrus* hybrids. PLoS ONE 8:1–11. https://doi.org/10.1371/journal.pone.0077022

Montanari S, Guérif P, Ravon E, Denancé C, Muranty H, Velasco R, Chagné D, Bus VGM, Robert P, Perchepied L, Durel CE (2015) Genetic mapping of *Cacopsylla pyri* resistance in an interspecific pear (*Pyrus* spp.) population. Tree Genet Genomes 11. https://doi.org/10.1007/s11295-015-0901-y

Montanari S, Perchepied L, Renault D, Frijters L, Velasco R, Horner M, Gardiner SE, Chagné D, Bus VGM, Durel CE, Malnoy M (2016) A QTL detected in an interspecific pear population confers stable fire blight resistance across different environments and genetic backgrounds. Mol Breed 36:47. https://doi.org/10.1007/s11032-016-0473-z

Nashima K, Shimizu T, Nishitani C, Yamamoto T, Takahashi H, Nakazono M, Itai A, Isuzugawa K, Hanada T, Takashina T, Matsumoto S, Otagaki S, Oikawa A, Shiratake K (2013) Microarray analysis of gene expression patterns during fruit development in European pear (*Pyrus communis*). Sci Hortic 164:466–473. https://doi.org/10.1016/j.scienta.2013.09.054

Nishio S, Hayashi T, Yamamoto T, Yamada M, Takada N, Kato H, Nishitani C, Saito T (2016) Validation of molecular markers associated with fruit ripening day of Japanese pear (*Pyrus pyrifolia* Nakai) using variance components. Sci Hortic 199:9–14. https://doi.org/10.1016/j.scienta.2015.12.032

Nishio S, Saito T, Terakami S, Takada N, Kato H, Itai A (2018) Identification of QTLs Associated with conversion of sucrose to hexose in mature fruit of Japanese pear. Plant Mol Biol Rep 36(4):643–652. https://doi.org/10.1007/s11105-018-1106-y

Ntladi SM, Human JP, Bester C, Vervalle J, Roodt-Wilding R, Tobutt KR (2018) Quantitative trait loci (QTL) mapping of blush skin and flowering time in a European pear (*Pyrus communis*) progeny of 'Flamingo' × 'Abate Fetel'. Tree Genet Genomes 14:70. https://doi.org/10.1007/s11295-018-1280-y

Pasqualini E, Civolani S, Musacchi S, Ancarani V, Dondini L, Robert P, Baronio P (2006) Cacopsylla pyri behaviour on new pear selections for host resistance programs. BullInsectology 59:27–37

Perchepied L, Leforestier D, Ravon E, Guérif P, Denancé C, Tellier M, Terakami S, Yamamoto T, Chevalier M, Lespinasse Y, Durel CE (2015) Genetic mapping and pyramiding of two new pear scab resistance QTLs. Mol Breed 35:197. https://doi.org/10.1007/s11032-015-0391-5

Perchepied L, Guérif P, Ravon E, Denancé C, Laurens F, Robert P, Bouvier L, Lespinasse Y, Durel CE (2016) Polygenic inheritance of resistance to *Cacopsylla pyri* in a *Pyrus communis* × *P. ussuriensis* progeny is explained by three QTLs involving an epistatic interaction. Tree Genet Genomes 12:108. https://doi.org/10.1007/s11295-016-1072-1

Pierantoni L, Cho KH, Shin LS, Chiodini R, Tartarini S, Dondini L, Kang SJ, Sansavini S (2004) Characterisation and transferability of apple SSRs to two European pear F1 populations. Theor Appl Genet 109:1519–1524. https://doi.org/10.1007/s00122-004-1775-9

Pierantoni L, Dondini L, Cho KH, Shin IS, Gennari F, Chiodini R, Tartarini S, Kang SJ, Sansavini S (2007) Pear scab resistance QTLs via a European pear (*Pyrus communis*) linkage map. Tree Genet Genomes 3:311–317. https://doi.org/10.1007/s11295-006-0070-0

Pierantoni L, Dondini L, De Franceschi P, Musacchi S, Winkel BSJ, Sansavini S (2010) Mapping of an anthocyanin-regulating MYB transcription factor and

its expression in red and green pear, *Pyrus communis*. Plant Physiol Biochem 48:1020–1026. https://doi.org/10.1016/j.plaphy.2010.09.002

Reuscher S, Fukao Y, Morimoto R, Otagaki S, Oikawa A, Isuzugawa K, Shiratake K (2016) Quantitative proteomics-based reconstruction and identification of metabolic pathways and membrane transport proteins related to sugar accumulation in developing fruits of pear (*Pyrus communis*). Plant Cell Physiol 57:505–518. https://doi.org/10.1093/pcp/pcw004

Saeed M, Brewer L, Johnston J, McGhie TK, Gardiner SE, Heyes JA, Chagné D (2014) Genetic, metabolite and developmental determinism of fruit friction discolouration in pear. BMC Plant Biol 14:241. https://doi.org/10.1186/s12870-014-0241-3

Saito A, Nakazawa N, Suzuki M (2001) Selection of mutants resistant to alternaria blotch from in vitro-cultured apple shoots irradiated with X- and γ-rays. J Plant Physiol 158:391–400. https://doi.org/10.1078/0176-1617-00235

Salvianti F, Bettini PP, Giordani E, Sacchetti P, Bellini E, Buiatti M (2008) Identification by suppression subtractive hybridization of genes expressed in pear (*Pyrus spp.*) upon infestation with *Cacopsylla pyri* (Homoptera:Psyllidae). J Plant Physiol 165:1808–1816. https://doi.org/10.1016/j.jplph.2007.12.010

Sanada T, Nishida T, Ikeda F (1988) Resistant mutant to black spot disease of Japanese pear "Nijisseiki" induced by gamma rays. J Jpn Soc Hortic 57:159–166. https://doi.org/10.2503/jjshs.57.159

Sassa H, Kakui H, Miyamoto M, Suzuki Y, Hanada T, Ushijima K, Kusaba M, Hirano H, Koba T (2007) *S* locus *F*-box brothers: Multiple and pollen-specific *F*-box genes with *S* haplotype-specific polymorphisms in apple and Japanese pear. Genetics 175:1869–1881. https://doi.org/10.1534/genetics.106.068858

Schaart JG, van de Wiel CCM, Lotz LAP, Smulders MJM (2016) Opportunities for products of new plant breeding techniques. Trends Plant Sci 21:438–449. https://doi.org/10.1016/j.tplants.2015.11.006

Shen C, Wang J, Jin X, Liu N, Fan X, Dong C, Shen Q, Xu Y (2017) Potassium enhances the sugar assimilation in leaves and fruit by regulating the expression of key genes involved in sugar metabolism of Asian pears. Plant Growth Regul 83:287–300. https://doi.org/10.1007/s10725-017-0294-z

Song L, Wang Z, Wang Z, Meng G, Zhai R, Cai M, Ma F, Xu L (2016) Screening of cell wall-related genes that are expressed differentially during ripening of pears with different softening characteristics. Postharvest Biol Technol 115:1–8. https://doi.org/10.1016/j.postharvbio.2015.12.012

Terakami S, Shoda M, Adachi Y, Gonai T, Kasumi M, Sawamura Y, Iketani H, Kotobuki K, Patocchi A, Gessler C, Hayashi T, Yamamoto T (2006) Genetic mapping of the pear scab resistance gene *Vnk* of Japanese pear cultivar Kinchaku. Theor Appl Genet 113:743–752. https://doi.org/10.1007/s00122-006-0344-9

Terakami S, Adachi Y, Iketani H, Sato Y, Sawamura Y, Takada N, Nishitani C, Yamamoto T (2007) Genetic mapping of genes for susceptibility to black spot disease in Japanese pears. Genome 50:735–741. https://doi.org/10.1139/G07-053

Terakami S, Moriya S, Adachi Y, Kunihisa M, Nishitani C, Saito T, Abe K, Yamamoto T (2016) Fine mapping of the gene for susceptibility to black spot disease in Japanese pear (*Pyrus pyrifolia* Nakai). Breed Sci 66:271–280. https://doi.org/10.1270/jsbbs.66.271

Tian J, Zeng B, Luo SP, Li XG, Wu B, Li J (2016) Cloning, localization and expression analysis of two *fw2.2*-like genes in small- and large-fruited pear species. J Integr Agric 15:282–294. https://doi.org/10.1016/S2095-3119(15)61075-9

Urrestarazu J, Muranty H, Denancé C, Leforestier D, Ravon E, Guyader A, Guisnel R, Feugey L, Aubourg S, Celton J-M, Daccord N, Dondini L, Gregori R, Lateur M, Houben P, Ordidge M, Paprstein F, Sedlak J, Nybom H, Garkava-Gustavsson L, Troggio M, Bianco L, Velasco R, Poncet C, Théron A, Moriya S, Bink MCAM, Laurens F, Tartarini S, Durel C-E (2017) Genome-wide association mapping of flowering and ripening periods in apple. Front Plant Sci 8:1923. https://doi.org/10.3389/fpls.2017.01923

Wang Z, Meng D, Wang A, Li T, Jiang S, Cong P, Li T (2013) The methylation of the *PcMYB10* promoter is associated with green-skinned sport in Max Red Bartlett pear. Plant Physiol 162:885–896. https://doi.org/10.1104/pp.113.214700

Wei S, Tao S, Qin G, Wang S, Tao J, Wu J, Wu J, Zhang S (2016) Transcriptome profiling reveals the candidate genes associated with aroma metabolites and emission of pear (*Pyrus ussuriensis* cv.). Sci Hortic 206:33–42. https://doi.org/10.1016/j.scienta.2016.04.019

Westigard PH, Westwood MN, Lombard PB (1970) Host preference and resistance of *Pyrus* species to the pear psylla, *Psylla pyricola* Foerster. J Am Soc Hortic Sci 95:34–36

Won K, Bastiaanse H, Kim YK, Song JH, Kang SS, Lee HC, Cho KH, Brewer L, Singla G, Gardiner SE, Chagné D, Bus VGM (2014) Genetic mapping of polygenic scab (*Venturia pirina*) resistance in an interspecific pear family. Mol Breed 34:2179–2189. https://doi.org/10.1007/s11032-014-0172-6

Wu J, Gu C, Khan MA, Wu J, Gao Y, Wang C, Korban SS, Zhang S (2013a) Molecular determinants and mechanisms of gametophytic self-incompatibility in fruit trees of Rosaceae. Crit Rev Plant Sci 32:53–68. https://doi.org/10.1080/07352689.2012.715986

Wu J, Wang Z, Shi Z, Zhang S, Ming R, Zhu S, Khan MA, Tao S, Korban SS, Wang H, Chen NJ, Nishio T, Xu X, Cong L, Qi K, Huang X, Wang Y, Zhao X, Wu J, Deng C, Gou C, Zhou W, Yin H, Qin G, Sha Y, Tao Y, Chen H, Yang Y, Song Y, Zhan D, Wang J, Li L, Dai M, Gu C, Wang Y, Shi D,

Wang X, Zhang H, Zeng L, Zheng D, Wang C, Chen M, Wang G, Xie L, Sovero V, Sha S, Huang W, Zhang S, Zhang M, Sun J, Xu L, Li Y, Liu X, Li Q, Shen J, Wang J, Paull RE, Bennetzen JL, Zhang S (2013b) The genome of the pear (*Pyrus bretschneideri* Rehd.). Genome Res 23:396–408. https://doi.org/10.1101/gr.144311.112

Wu J, Zhao G, Yang Y-N, Le W-Q, Khan MA, Zhang S-L, Gu C, Huang W-J (2013c) Identification of differentially expressed genes related to coloration in red/green mutant pear (*Pyrus communis* L.). Tree Genet Genomes 9:75–83. https://doi.org/10.1007/s11295-012-0534-3

Wu J, Li LT, Li M, Khan MA, Li XG, Chen H, Yin H, Zhang SL (2014a) High-density genetic linkage map construction and identification of fruit-related QTLs in pear using SNP and SSR markers. J Exp Bot 65:5771–5781. https://doi.org/10.1093/jxb/eru311

Wu J, Wang D, Liu Y, Wang L, Qiao X, Zhang S (2014b) Identification of miRNAs involved in pear fruit development and quality. BMC Genom 15:1–19. https://doi.org/10.1186/1471-2164-15-953

Xu Y, Li X, Lin J, Wang Z, Yang Q, Chang Y (2015) Transcriptome sequencing and analysis of major genes involved in calcium signaling pathways in pear plants (*Pyrus calleryana* Decne.). BMC Genom 16:1–13. https://doi.org/10.1186/s12864-015-1887-4

Xue H, Shi T, Wang F, Zhou H, Yang J, Wang L, Wang S, Su Y, Zhang Z, Qiao Y, Li X (2017) Interval mapping for red/green skin color in Asian pears using a modified QTL-seq method. Hortic Res 4:17053. https://doi.org/10.1038/hortres.2017.53

Xue H, Wang S, Yao J-L, Zhang X, Yang J, Wang L, Su Y, Chen L, Zhang H, Li X (2018) The genetic locus underlying red foliage and fruit skin traits is mapped to the same location in the two pear bud mutants 'Red Zaosu' and 'Max Red Bartlett'. Hereditas 155:25. https://doi.org/10.1186/s41065-018-0063-7

Yamamoto T, Kimura T, Shoda M, Imai T, Saito T, Sawamura Y, Kotobuki K, Hayashi T, Matsuta N (2002) Genetic linkage maps constructed by using an interspecific cross between Japanese and European pears. Theor Appl Genet 106:9–18. https://doi.org/10.1007/s00122-002-0966-5

Yamamoto T, Terakami S, Takada N, Nishio S, Onoue N, Nishitani C, Kunihisa M, Inoue E, Iwata H, Hayashi T, Itai A, Saito T (2014) Identification of QTLs controlling harvest time and fruit skin color in Japanese pear (*Pyrus pyrifolia* Nakai). Breed Sci 64:351–361. https://doi.org/10.1270/jsbbs.64.351

Yang YN, Zhao G, Yue WQ, Zhang SL, Gu C, Wu J (2013) Molecular cloning and gene expression differences of the anthocyanin biosynthesis-related genes in the red/green skin color mutant of pear (*Pyrus communis* L.). Tree Genet Genomes 9:1351–1360. https://doi.org/10.1007/s11295-013-0644-6

Yang YN, Yao GF, Zheng D, Zhang SL, Wang C, Zhang MY, Wu J (2014) Expression differences of anthocyanin biosynthesis genes reveal regulation patterns for red pear coloration. Plant Cell Rep 34:189–198. https://doi.org/10.1007/s00299-014-1698-0

Yang Y, Yao G, Yue W, Zhang S, Wu J (2015) Transcriptome profiling reveals differential gene expression in proanthocyanidin biosynthesis associated with red/green skin color mutant of pear (*Pyrus communis* L.). Front Plant Sci 6:795. https://doi.org/10.3389/fpls.2015.00795

Yao G, Ming M, Allan AC, Gu C, Li L, Wu X, Wang R, Chang Y, Qi K, Zhang S, Wu J (2017) Map-based cloning of the pear gene *MYB114* identifies an interaction with other transcription factors to coordinately regulate fruit anthocyanin biosynthesis. Plant J 92:437–451. https://doi.org/10.1111/tpj.13666

Yu B, Zhang D, Huang C, Qian M, Zheng X, Teng Y, Su J, Shu Q (2012) Isolation of anthocyanin biosynthetic genes in red Chinese sand pear (*Pyrus pyrifolia* Nakai) and their expression as affected by organ/tissue, cultivar, bagging and fruit side. Sci Hortic 136:29–37. https://doi.org/10.1016/j.scienta.2011.12.026

Zhai R, Wang Z, Zhang S, Meng G, Song L, Wang Z, Li P, Ma F, Xu L (2016) Two MYB transcription factors regulate flavonoid biosynthesis in pear fruit (*Pyrus bretschneideri* Rehd.). J Exp Bot 67:1275–1284. https://doi.org/10.1093/jxb/erv524

Zhang C, Tanabe K, Wang S, Tamura F, Yoshida A, Matsumoto K (2006) The impact of cell division and cell enlargement on the evolution of fruit size in *Pyrus pyrifolia*. Ann Bot 98:537–543. https://doi.org/10.1093/aob/mcl144

Zhang SJ, Wu J, Chen H, Gu C, Tao ST, Wu JY, Zhang SL (2011a) Identification of differentially expressed genes in a spontaneous mutant of "Nanguoli" pear (*Pyrus ussuriensis* Maxim) with large fruit. J Hortic Sci Biotech 86:595–602. https://doi.org/10.1080/14620316.2011.11512809

Zhang X, C Allan A, Yi Q, Chen L, Li K, Shu Q, Su J (2011b) Differential gene expression analysis of Yunnan red pear, *Pyrus pyrifolia*, during fruit skin coloration. Plant Mol Biol Rep 29:305–314. https://doi.org/10.1007/s11105-010-0231-z

Zhang R, Wu J, Li X, Khan MA, Chen H, Korban SS, Zhang S (2013) An AFLP, SRAP, and SSR genetic linkage map and identification of QTLs for fruit traits in pear (*Pyrus* L.). Plant Mol Biol Rep 31:678–687. https://doi.org/10.1007/s11105-012-0544-1

Zhang MY, Xue C, Xu L, Sun H, Qin MF, Zhang S, Wu J (2016) Distinct transcriptome profiles reveal gene expression patterns during fruit development and maturation in five main cultivated species of pear (*Pyrus* L.). Sci Rep 6:1–12. https://doi.org/10.1038/srep28130

The Genome of Pear

7

Jun Wu, Shaoling Zhang and Xiaolong Li

Abstract

As the third most important temperate tree fruit species, the pear occupies an indispensable position of high commercial importance and of beneficial nutritional value. Since the release of genome sequences of the Asian pear and then of the European pear, comprehensive 'big data' explorations have been extensively carried out at the whole-genome level, including those of gene families, functional genomics, and evolution analysis, among others. With the innovation of technology and reduced costs of sequencing, increasing numbers of genome resequencing and transcriptome sequencing projects have also been undertaken based on the reference genome sequence of pear. These efforts will provide credible data to support further functional analyses and valuable guidance in pursuing germplasm improvement and breeding of pear. Herein, research advances in pear genome sequencing and its downstream analyses are summarized and discussed, along with future perspectives.

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7.1 Genome of the Asian Pear

The pear, belonging to the sub-family *Maloideae* in the Rosaceae family, is a diploid, shares a basic chromosome number of $x = 17$ ($2n = 34$), and possesses a highly heterozygous genome. Studies have revealed that there is a high level of heterozygosity in the pear genome and a 1–2% sequence divergence among alleles (Wu et al. 2013).

Using a combination of bacterial artificial chromosome (BAC)-by-BAC and next-generation sequencing, a high-quality draft genome sequence of the Asian pear *Pyrus* × *bretschneideri* cv. Dangshansuli has been released (Wu et al. 2013) (Fig. 7.1). The assembled pear genome consists of 2103 scaffolds with N50 at 540.8 Kb (Table 7.1). This corresponds to 97.1% of the estimated genome size (527.0 Mb), and a total of 512.0 Mb sequence is assembled with 194× coverage. High-density genetic maps constructed using 2005 SNP markers have anchored 796 scaffolds, a total of 386.7 Mb, and representing ~75.5% of the assembled genome. In the pear genome, 42,812 protein-coding genes have been predicted with transcript lengths of 2776 bp and coding lengths of 1172 bp (Wu et al. 2013). This indicates that, on average, there are 4.7 exons per gene. In addition, Illumina RNA-Seq sequences have provided strong support for these predictions. Moreover, 297 miRNAs, 1148 tRNAs, 697 rRNAs, and 395 snRNAs have been identified. Furthermore, 53.1% (271.9 Mb) of the length of the assembled genome

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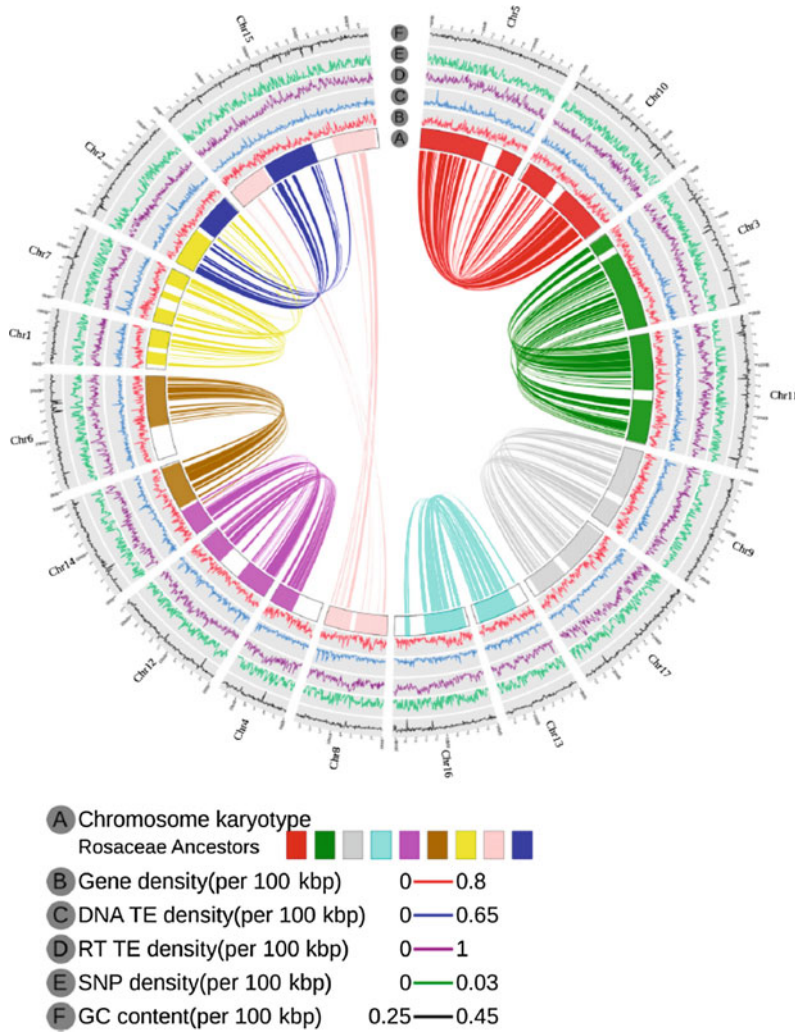

Fig. 7.1 A schematic diagram demonstrating the distribution of basic genomic elements in the Asian pear genome. (A) Chromosome karyotype, wherein colored segments are presented in accordance with the ancestor of Rosacea. (B) Gene density, wherein the rate of sites within a gene region per 100 kbp ranges from a minimum of 0 to a maximum of 0.8 as illustrated by red-colored lines. (C) DNA transposon element (TE) density, wherein the rate of sites within the DNA TE region per 100 kb ranges from 0 to 0.65 as illustrated by blue-colored lines. (D) Retrotransposon element (RT TE) density, wherein the rate of sites within the RT TE regions ranges from 0 to 1, and this is illustrated by purple-colored lines. (E) SNP density, wherein the rate of SNPs per 100 kb ranges from 0 to 0.03, and this is illustrated by green-colored lines. (F) GC content, wherein the rate of GC content ranges from 0.25 to 0.45, and this is illustrated by black-colored. This figure is taken from Wu et al. (2013)

is observed to consist of repetitive sequences. A high long terminal repeat (LTR) expansion rate suggests that the pear genome is in continuous expansion. Compared to the apple genome, it is proposed that the presence of large numbers of repeat sequences in the pear is mainly contributing to genome size differences between the apple and the pear. Further, genes associated with disease resistance, stone cells, sugar, and volatiles, as well as self-incompatibility have been identified in the genome of the Asian pear.

Table 7.1 Comparisons of the Asian pear genome of 'Dangshansuli' and the European pear genome of 'Bartlett'

	'Bartlett'	'Dangshansuli'
Contigs		
Number of contigs	182,196	25,312
Total size of contigs (Mb)	507.7	501.3
N50 contig length (Kb)	6.6	35.7
Longest contig (Mb)	0.1	0.3
Scaffolds		
Number of scaffolds	142,083	2103
Total size of scaffolds (bp)	577	512
N50 scaffold length (Kb)	88.1	540.8
Longest scaffold (Mb) Anchored size to the chromosome (Mb) Anchored rate to the chromosome (%)	1.2 171.3 29.7	4.1 386.7 75.5

7.2 Genome of the European Pear

The European pear, *Pyrus communis*, has a different phenotype and fruit quality characteristics than those of the Asian pear, including fruit shape, fruit taste, lignin content, and aroma, among others. Therefore, sequencing and annotating of the genome of the European pear are as equally important as that of the Asian pear.

Employing next-generation sequencing technology (Roche 454), a draft genome sequence of the European pear cv. Bartlett has been recently assembled and released (Chagné et al. 2014). A total of 142,083 scaffolds have been assembled, corresponding to 577.3 MB with an average of 11.4× genome coverage and representing 96.2% of the expected 600 MB of the European pear genome (Table 7.1). Furthermore, a genetic map consisting of a total of 2279 single-nucleotide polymorphic (SNP) markers, including 1391 and 888 apple and pear SNPs, respectively, is constructed to anchor 171.3 Mb of the assembled genome. Using a combined *ab initio* prediction and homology search approach, a total of 43,419 putative genes are identified (Chagné et al. 2014). The number of predicted genes is higher than those of most other plant species, but it is similar to that identified in the Asian pear. This may be expected due to whole-genome duplication of members of *Maloideae* (Velasco et al. 2010). In addition, the average predicted coding region length (1209 bp), exon length, and gene density in the European pear genome is found to be similar to that detected in the Asian pear. Moreover, a total of 60,820 and 51,425 SNPs have been identified and located within 1000 bases upstream and downstream, respectively, of a predicted gene.

Comparative proteome analysis of 13 different plant species has revealed that the European pear has a close relationship with genomes of the Asian pear and apple and that higher numbers of protein clusters are shared between European and Asian pears (Fig. 7.2). It is observed that the European pear genome has a total of 199.4 Mb of repeated elements; moreover, most common repeated elements are LTRs. These results are consistent with those observed in the Asian pear genome (Wu et al. 2013). In addition, a total of 41 predicted genes are identified as members of the expansin gene family. Furthermore, it is observed that there are more similarities between apple and pear orthologs than that between homologues of the same species, thereby confirming that speciation must have occurred following the genome duplication event (Chagné et al. 2014).

It is important to point out that current efforts are underway in resequencing the European pear genome which will yield a higher-quality sequence draft of this genome. Thereby, it is expected that comparative findings between Asian and European pear genomes are yet to be fully delineated and finalized.

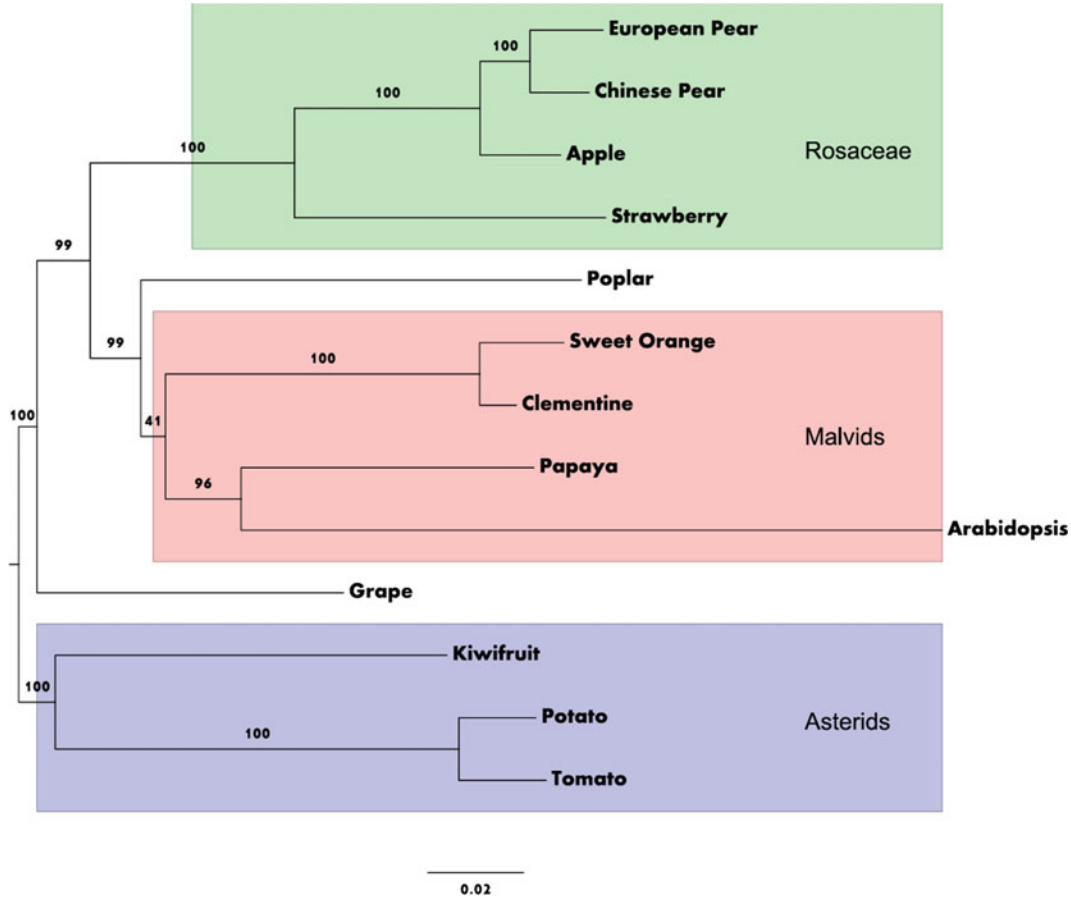

Fig. 7.2 A phylogenetic tree of six rosids, four malvids, and three asterids constructed using 83 euKaryote Orthologous Genes (KOGs). Bootstrap values are listed along each of the branches. Nodes represent speciation events, and branch lengths represent degrees of evolutionary changes over time. The unit for the scale bar at the bottom of the figure corresponds to nucleotide substitutions per site. The high bootstrap values strongly support that species in Rosaceae cluster together to the exclusion of any other and that the separation event of the European pear from the Chinese pear must have occurred following apple speciation (Chagné et al. 2014)

7.3 Evolution of Pear Species: Duplication Events and Chromosome Evolution

Similar to findings in apple, the pear genome has undergone two whole-genome duplication (WGD) events, based on the estimation of four-fold degenerate site transversion (4dTv) values of 13,372 pairs of paralogous genes (Fig. 7.3a). Meanwhile, its distribution supports the fact that this recent WGD event must have occurred first and then followed by the divergence of the pear from the apple. Furthermore, investigation of the expansin gene family has also suggested that divergence event of the European pear from the Chinese pear must have taken place following apple speciation (Chagné et al. 2014).

Substitutions per synonymous site (Ks) values of 16,335 paralogous gene pairs suggest that the recent WGD event in pear must have occurred at 30–45 million years ago (Mya), and have also supported that a paleohexaploidization event must have also occurred in an ancient WGD that took place ~140 Mya (Fawcett et al. 2009). As it is known, the pear and apple share the same

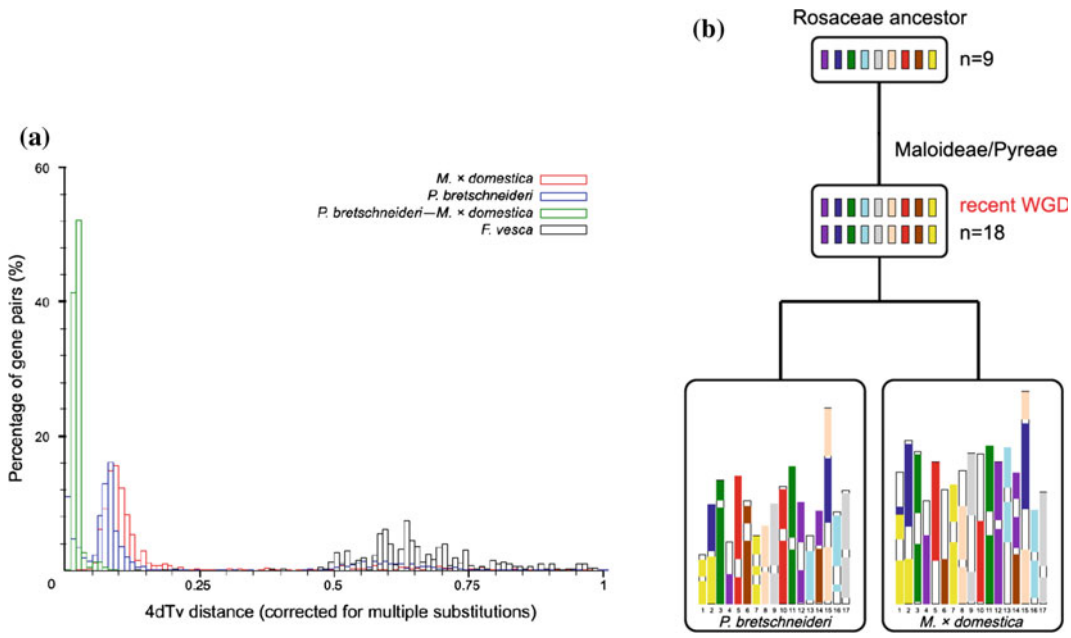

Fig. 7.3 Summary of the duplication events and chromosome evolution in pear. **a** The distribution of fourfold degenerate site (4dTv) distances of duplicate gene pairs in pear, apple, and strawberry. **b** The evolutionary scenario of nine chromosomes of the Rosaceae ancestor (Wu et al. 2013)

numbers of chromosomes, as well as similar chromosome structures. It is proposed that both apple and pear must have been derived from a recent WGD of nine chromosomes of a Rosaceae ancestor, while triplication of seven ancestral chromosomes of eudicots may have undergone additional rearrangements, thereby yielding nine ancestral chromosomes of the Rosaceae (Fig. 7.3b) (Wu et al. 2013).

7.4 Gene Families: Identification and Functional Divergence

A gene family is a set of several similar genes derived from a single ancestor. This family is formed following duplication of such a single original ancestral gene, and members of this family generally have similar biochemical functions. Based on conserved domains of proteins, predicted genes can be grouped into many different families of a genome, with each family possessing similar functions. In large gene families, gene function among members of such families must have diverged, as these must have underwent multiple duplication events resulting in expansion of the numbers of family members. Identification of a gene family can provide abundant knowledge of gene functions, expansion ways, and expression patterns, thereby providing a solid foundation for pursuing research studies related to gene function.

To date, with the release of pear genome sequences, many gene families have been identified and explored at the whole-genome level. Particularly with large gene families, such transcription factors as MYB, ERF, and MADS-box families, among others, have been extensively explored, including identification, evolution, and function prediction (Li et al. 2016, 2018; Wang et al. 2017). In addition, structural genes such as *SWEET* transporters (Li et al. 2017), *F-box* genes (Wang et al. 2016), *B-box* genes (Cao et al. 2017), hexokinase encoding (HK) genes (Yu et al. 2017), and hydroxycinnamoyl transferase encoding (HCT) genes (Ma et al. 2017) have also been globally identified in pear. Further functional verification has been carried out based on

these gene function predictions. For example, genes *MYB169* and *MYB114*, which were identified from the MYB gene family and predicted as regulators controlling lignin synthesis and anthocyanin biosynthesis, respectively, have been verified in pear (Xue et al. 2019; Yao et al. 2017).

7.5 Multiple OMICS: Identifying Genes Related to Important Traits

Transcriptomics, proteomics, and metabolomics provide wide overviews of plant traits at the mRNA, protein, and metabolite levels, respectively (Palma et al. 2011). With the release of the pear genome sequence, various OMICS studies, particularly for comparative OMICS analyses, have been carried out to investigate biological phenomena in pear. Moreover, joint analysis of multiple OMICS will aid in dissecting complex traits and thus will attract more attention with the expansion of genomics data in the future.

Transcriptome analysis is a powerful tool in assessing expression levels of genes in specific tissues and at various stages of development. Thus, it is feasible to predict gene function(s) and investigate regulation mechanism(s). In general, a transcriptome can be classified as either a reference or a non-reference (de novo assembly) transcriptome. For example, transcriptomes of endodormant and ecodormant flower buds of Japanese pear have been analyzed to identify key genes involved in regulation pathways during the release of endodormancy (Bai et al. 2013). In Chinese pear, comparative transcriptome analyses of pre- and post-ripening fruits have been carried out to identify candidate genes associated with fruit ripening (Hao et al. 2018; Huang et al. 2014). As fruit aroma is an important component of fruit quality in pears, candidate genes highly related to aroma biosynthesis during fruit ripening have been identified using unripe fruit of poor aroma and ripe fruit with strong aroma of 'Nanguoli' (*Pyrus ussuriensis*) as tissues for transcriptome analysis (Wei et al. 2016). Furthermore, as pear fruit, storability is an important postharvest trait, and as the yeast *Meyerozyma guilliermondii* inhibits natural decay of stored pear fruit and induces resistance to blue mold decay caused by *Penicillium expansum*, expression of several defense-related genes, such those coding for PAL, POD, and GLU have been found to be significantly modified following transcriptome analysis of fruit treated with *M. guilliermondii* versus untreated pear fruit (Yan et al. 2018). Recently, Li et al. (2019b) have performed a comparative transcriptomic analysis revealing a distinctly different pattern of variation between expression and sequence diversity, and identifying candidate selected genes associated with important fruit quality traits during domestication and improvement in pear (Li et al. 2019b).

Integrating proteomics with transcriptomics is a powerful approach for exploring functional correlations between phenotype and genotype, and in establishing regulation networks (Palma et al. 2011). Studies have been completed using developing fruits of several fruit crops, including strawberry (Bianco et al. 2009), grape (Giribaldi et al. 2010), and papaya (Nogueira et al. 2012). Using proteomics and transcriptomics, Li et al. (2015) have identified a total of 35 important differentially expressed proteins related to fruit quality in pear, including three genes related to sugar synthesis, a single gene related to aroma formation, and 16 genes related to stone cells' content (Li et al. 2015).

Metabolomics is yet another novel approach, building on genomics and proteomics, which performs quantitative analysis for all metabolites in an organism, and traces correlations between metabolites and a phenotype. In pear, metabolomics studies have been carried out to characterize complex phenotypes. Recently, comparative metabolomics analysis of flower buds during endodormancy has identified and characterized metabolic changes induced by chilling temperatures, as well as simulated mild winter and/or climate change scenarios during thermal fluctuation in Japanese pear (Horikoshi et al. 2018). A total of 91 metabolites have been detected and classified into eight groups, including organic acids, fatty acids and sterols, amino acids, amino acid derivatives, phenol lipids, phenylpropanoids, sugars and polyols, and other compounds. This

study has contributed new knowledge on the biological mechanism of dormancy during temperature changes and has elucidated metabolic changes during mild winters and future climate change scenarios (Horikoshi et al. 2018).

7.6 Whole-Genome Resequencing

Resequencing of a genome is an approach that aids in determining nucleotide order of a given DNA fragment for different individuals or populations based on a reference genome and comparative analysis. Following the alignment of sequences of individuals or populations, large numbers of SNPs, insertion/deletions (InDels), structure variations (SVs), and copy-number variations (CNVs) could be identified from different individuals and populations and used to perform downstream analyses. For example, resequencing of wild plants and cultivated types will allow for comparative analysis to reveal the origin of a species and its domestication during evolution, as well as provide valuable genetic resources and important references for plant breeding programs.

Recently, genome resequencing of 113 pear accessions from worldwide collections, representing four different populations, including Asian wild, Asian cultivated, European wild, and European cultivated accessions, was performed (Wu et al. 2018). A total of 18.3 Mb SNPs were identified in this study, and a weak domestication has been observed based on analyses of population structure, diversity, and linkage disequilibrium (LD) (Fig. 7.4). This comprehensive study also clarified the process of divergence of Asian from European pears, as well as dissemination and independent domestication of Asian and European pears. The divergence time of Asian and European pears, 3.3–6.6 Mya, was first reported in this study (Fig. 7.5). Furthermore, evidence for rapid evolution and balance selection for *S-RNase* genes contributing to the maintenance of self-incompatibility in pear has been found. Meanwhile, selective sweep signatures for a total of 9.29 Mb of the genome sequence, containing 857 putative genes, were detected in Asian pears, while 5.35 Mb of the genome sequence, containing 248 putative genes, was identified as selective sweeps in European pears. Notably, there was only 515 kbp of overlap for regions with selective sweep signatures between Asian and European pears, indicating that different genome regions must have undergone selection in Asian and European pear genomes during domestication. Genes associated with fruit size, sugar, organic acid, stone cell, and volatile compounds were identified from these regions with selective sweep signatures.

Therefore, it is possible to conduct additional analyses that will further reveal new knowledge regarding other biological issues based on genomic data released from resequencing various genotypes of pear.

7.7 SNP Arrays

A SNP array is a DNA microarray used to detect polymorphisms within a population. In plants, SNP arrays are useful tools for studying slight variations among whole genomes, as well as for conducting genome-wide association studies (GWAS). Breeding efforts for a number of plant species have been revolutionized following the emergence of SNP arrays.

With the release of the pear genome sequence, along with the availability of large numbers of SNP data, designing high-density SNP arrays is now possible for pear. However, as of yet, the development of pear SNP array has lag behind other plants, such as apple; therefore, efforts are underway to develop such an array. Due to its efficiency, flexibility, high throughput, and low cost, such a SNP array will likely be an important reference tool for GWAS, and highly useful in further germplasm enhancement and breeding efforts in pear. Recently, an integrated 200 K SNP genotyping array has been developed for pear by Dr. Jun Wu's group at Nanjing Agricultural University, and used for genetic mapping construction, genome assembly improvement, and GWAS in pear (Li et al. 2019a). Additionally, a 70 k Axiom® array has been developed by Dr. Sara

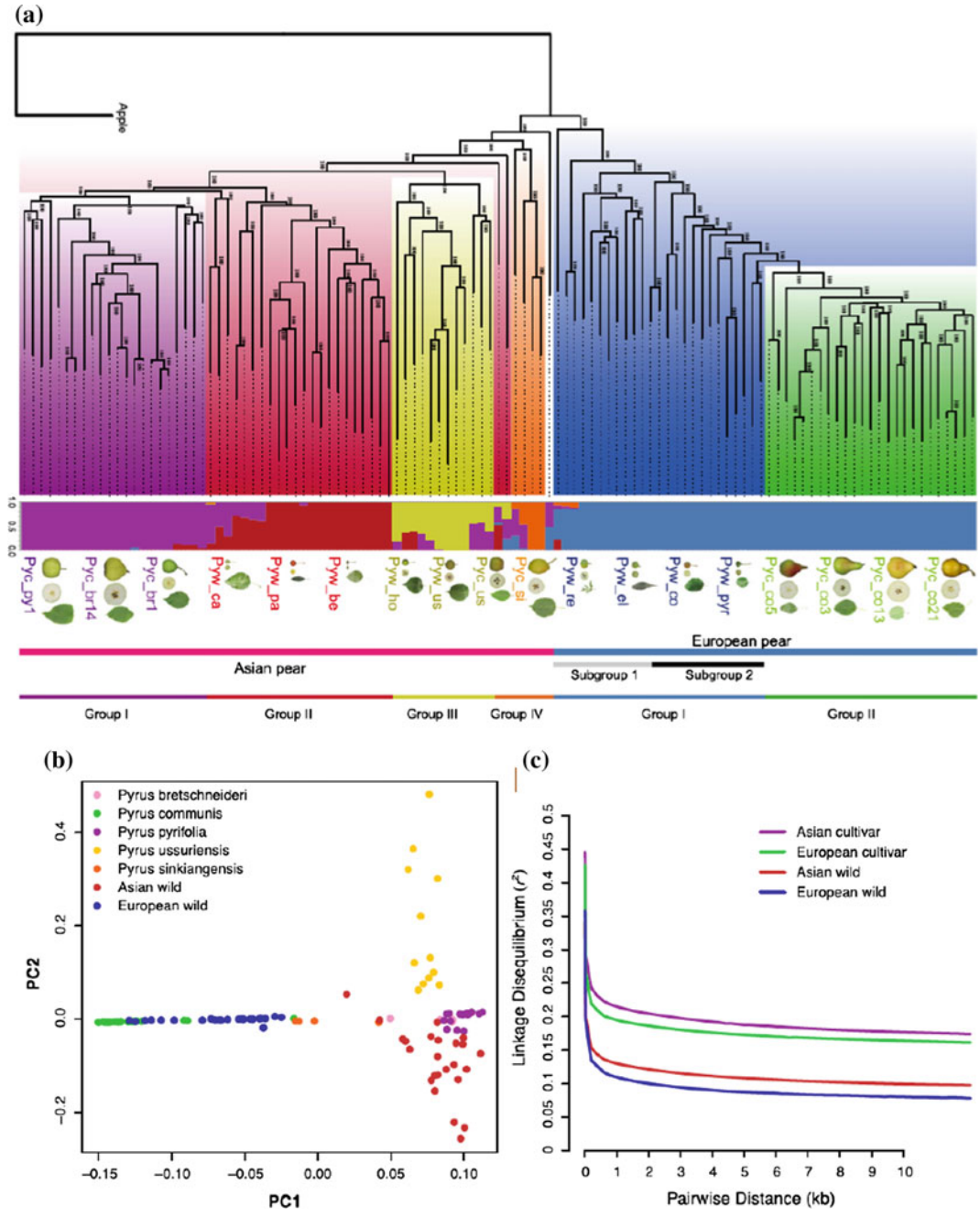

Fig. 7.4 Phylogenetic tree, PCA, and LD analysis of 113 cultivated and wild pear accessions based on whole-genome SNPs. **a** Phylogenetic tree and population structure ($K = 5$) for all 113 pear accessions inferred from whole-genome SNPs, with apple (*Malus* × *domestica*) used as an outgroup. Each color corresponds to a single population as noted. In population structure, each accession is represented by a bar. *Pyw* and *Pyc* correspond to wild and cultivated accessions, respectively, while other codes correspond to abbreviated names of pear species. **b** PCA plots of the first two eigenvectors for all 113 pear accessions. **c** LD decay determined by the correlation of allele frequencies (r^2) against distance (kbp) among polymorphic SNP sites in different pear groups, including cultivated Asian (red), cultivated European (light blue), wild Asian (blue), and wild European (green) (Wu et al. 2018)

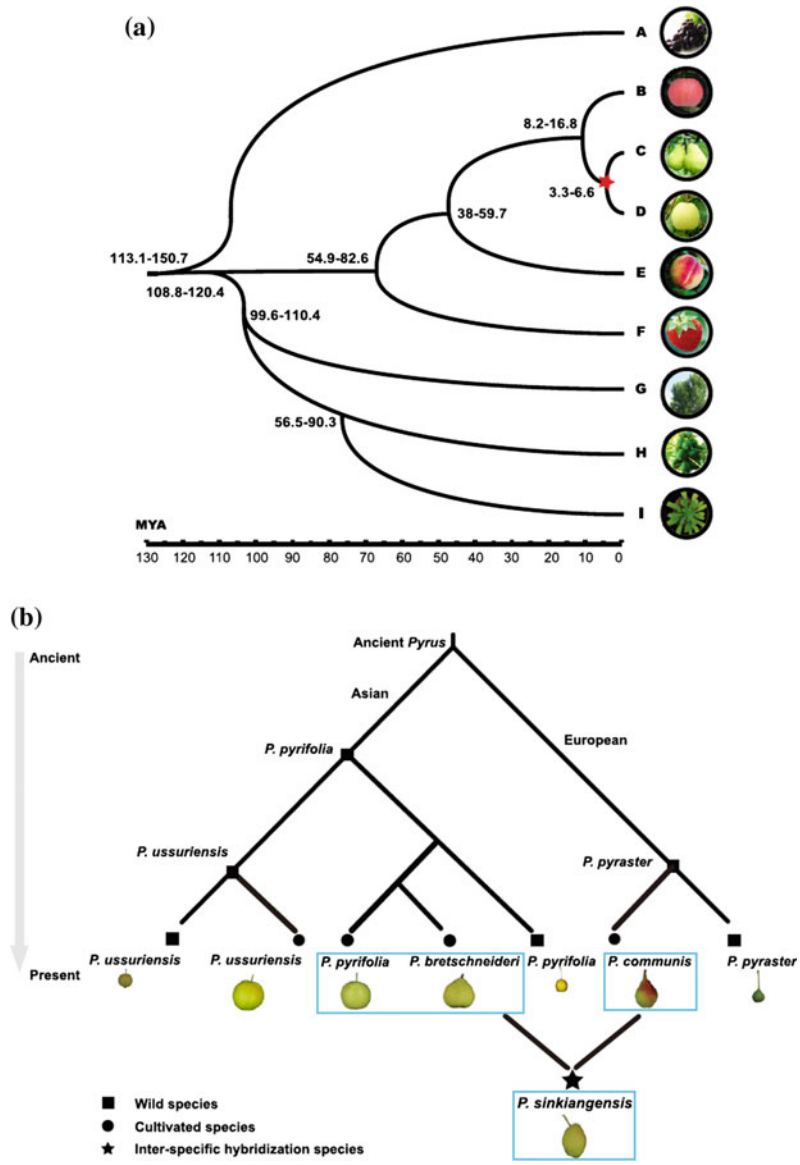

Fig. 7.5 Genetic relationships and divergence times of pear species. **a** Genetic relationships of wild and cultivated pear species. **b** Divergence time of Asian and European pears. A. *Vitis vinifera*; B. *Malus* × *domestica*; C. *Pyrus communis*; D. *Pyrus* × *bretschneideri*; E. *Prunus persica*; F. *Fragaria vesca*; G. *Populus trichocarpa*; H. *Carica papaya*; and I. *Arabidopsis thaliana*. This figure is taken from Wu et al. (2018)

Montanari and co-workers, and has been presented at the RGC9, Nanjing, China, June 26–30, 2018 (http://rgc9.org/ep3_3.php).

7.8 A Genome-Wide Association Study (GWAS)

A genome-wide association study, GWAS, is an observational study of a genome-wide set of genetic variants in different individuals to determine whether or not any variant is associated with a particular trait. As mentioned above, a SNP is the most popular variant used for GWAS.

Thus far, GWAS has been extensively carried out in apple. For example, Mcclure et al. (2018) have conducted a GWAS using 172 apple accessions, linking approximately 55,000 SNPs with 10 phenotypes collected over two years, and have identified loci associated with skin color, harvest date, firmness, and apple scab resistance.

In another study, an Axiom® Apple 480 K SNP array along with a total of 1168 different apple genotypes has been used to investigate candidate genes responsible for flowering and ripening periods in apple (Urrestarazu et al. 2017). Recently, a limited GWAS has been conducted in pear wherein 214 pear accessions have been used in marker-trait associations for several fruit color, fruit shape, and fruit quality traits using genotyping by sequencing (GBS) (Kumar et al. 2017). However, with the release of pear genome sequences along with resequencing data, a large amount of SNP data sets have become available; thus, GWAS efforts in pear will be undertaken in the near future.

References

Bai S, Saito T, Sakamoto D, Ito A, Fujii H, Moriguchi T (2013) Transcriptome analysis of Japanese pear (*Pyrus pyrifolia* Nakai) flower buds transitioning through endodormancy. Plant Cell Physiol 54:1132–1151

Bianco L, Lopez L, Scalone AG, Di CM, Desiderio A, Benvenuto E, Perrotta G (2009) Strawberry proteome characterization and its regulation during fruit ripening and in different genotypes. J Proteomics 72:586–607

Cao Y, Han Y, Meng D, Li D, Jiao C, Jin Q, Lin Y, Cai Y (2017) B-BOX genes: genome-wide identification, evolution and their contribution to pollen growth in pear (*Pyrus bretschneider*i Rehd.). BMC Plant Biol 17:156

Chagné D, Crowhurst RN, Pindo M, Thrimawithana A, Deng C, Ireland H, Fiers M, Dzierzon H, Cestaro A, Fontana P, Bianco L, Lu A, Storey R, Knäbel M, Saeed M, Montanari S, Kim YK, Nicolini D, Larger S, Stefani E, Allan AC, Bowen J, Harvey I, Johnston J, Malnoy M, Troggio M, Perchepied L, Sawyer G, Wiedow C, Won K, Viola R, Hellens RP, Brewer L, Bus VG, Schaffer RJ, Gardiner SE, Velasco R (2014) The draft genome sequence of European pear (*Pyrus communis* L. 'Bartlett'). PloS One 9:e92644

Fawcett JA, Maere S, Peer YVD, Montagu MCEV (2009) Plants with double genomes might have had a better chance to survive the cretaceous-tertiary extinction event. Proc Natl Acad Sci U S A 106:5737–5742

Giribaldi M, Perugini I, Sauvage FX, Schubert A (2010) Analysis of protein changes during grape berry ripening by 2-DE and MALDI-TOF. Proteomics 7:3154–3170

Hao PP, Wang GM, Cheng HY, Ke YQ, Qi KJ, Gu C, Zhang SL (2018) Transcriptome analysis unravels an ethylene response factor involved in regulating fruit ripening in pear. Physiol Plant 163:124–135

Horikoshi HM, Sekozawa Y, Kobayashi M, Saito K, Kusano M, Sugaya S (2018) Metabolomics analysis of 'Housui' Japanese pear flower buds during endodormancy reveals metabolic suppression by thermal fluctuation. Plant Physiol Biochem 126:134–141

Huang G, Li T, Li X, Tan D, Jiang Z, Wei Y, Li J, Wang A (2014) Comparative transcriptome analysis of climacteric fruit of Chinese pear (*Pyrus ussuriensis*) reveals new insights into fruit ripening. PLoS ONE 9: e107562

Kumar S, Kirk C, Deng C, Wiedow C, Knaebel M, Brewer L (2017) Genotyping-by-sequencing of pear (*Pyrus* spp.) accessions unravels novel patterns of genetic diversity and selection footprints. Hort Res 4:17015

Li J, Qin M, Qiao X, Cheng Y, Li X, Zhang H, Wu J (2017) A new insight into the evolution and functional divergence of SWEET transporters in Chinese white pear (*Pyrus bretschneideri*). Plant Cell Physiol 58:839–850

Li JM, Huang XS, Li LT, Zheng DM, Xue C, Zhang SL, Wu J (2015) Proteome analysis of pear reveals key genes associated with fruit development and quality. Planta 241:1363–1379

Li X, Liu L, Ming M, Hu H, Zhang M, Fan J, Song B, Zhang S, Wu J (2019a) Comparative transcriptomic analysis provides insight into the domestication and improvement of pear (P. pyrifolia) fruit. Plant physiol 01322.2018

Li X, Singh J, Qin M, Li S, Zhang X, Zhang M, Khan A, Zhang S, Wu J (2019b) Development of an integrated 200K SNP genotyping array and application for genetic mapping, genome assembly improvement and genome wide association studies in pear (Pyrus). Plant Biotechnol J

Li X, Xue C, Li J, Qiao X, Li L, Yu L, Huang Y, Wu J (2016) Genome-wide identification, evolution and functional divergence of MYB transcription factors in Chinese white pear (*Pyrus bretschneideri*). Plant Cell Physiol 57:824–847

Li X, Tao S, Wei S, Ming M, Huang X, Zhang S, Wu J (2018) The mining and evolutionary investigation of *AP2/ERF* genes in pear (*Pyrus*). BMC Plant Biol 18:46

Ma C, Zhang HP, Li JM, Tao ST, Qiao X, Korban SS, Zhang SL, Wu J (2017) Genome-wide analysis and characterization of molecular evolution of the HCT gene family in pear (*Pyrus bretschneideri*). Plant Syst Evol 303:71–90

McClure KA, Gardner KM, Douglas GM, Song J, Forney CF, DeLong J, Fan L, Du L, Toivonen PMA, Somers DJ, Rajcan I, Myles S (2018) A Genome-wide association study of apple quality and scab resistance. Plant Genome 11(1):170075. https://doi.org/10.3835/plantgenome2017.08.0075

Nogueira SB, Labate CA, Gozzo FC, Pilau EJ, Lajolo FM, do Nascimento JRO (2012) Proteomic analysis of papaya fruit ripening using 2DE-DIGE. J Proteomics 75:1428–1439

Palma JM, Corpas FJ, del Río LA (2011) Proteomics as an approach to the understanding of the molecular physiology of fruit development and ripening. J Proteomics 74:1230–1243

Urrestarazu J, Muranty H, Denancé C, Leforestier D, Ravon E, Guyader A, Guisnel R, Feugey L, Aubourg S, Celton JM, Daccord N, Dondini L, Gregori R, Lateur M, Houben P, Ordidge M, Paprstein F, Sedlak J, Nybom H, Garkava-Gustavsson L, Troggio M, Bianco L, Velasco R, Poncet C, Théron A, Moriya S, Bink MCAM, Laurens F, Tartarini S, Durel CE (2017) Genome-wide association mapping of flowering and ripening periods in apple. Front Plant Sci 8:1923

Velasco R, Zharkikh A, Affourtit J, Dhingra A, Cestaro A, Kalyanaraman A, Fontana P, Bhatnagar SK, Troggio M, Pruss D, Salvi S, Pindo M, Baldi P, Castelletti S, Cavaiuolo M, Coppola G, Costa F, Cova V, Dal Ri A, Goremykin V, Komjanc M, Longhi S, Magnago P, Malacarne G, Malnoy M, Micheletti D, Moretto M, Perazzolli M, Si-Ammour A, Vezzulli S, Zini E, Eldredge G, Fitzgerald LM, Gutin N, Lanchbury J, Macalma T, Mitchell JT, Reid J, Wardell B, Kodira C, Chen Z, Desany B, Niazi F, Palmer M, Koepke T, Jiwan D, Schaeffer S, Krishnan V, Wu C, Chu VT, King ST, Vick J, Tao Q, Mraz A, Stormo A, Stormo K, Bogden R, Ederle D, Stella A, Vecchietti A, Kater MM, Masiero S, Lasserre P, Lespinasse Y, Allan AC, Bus V, Chagné D, Crowhurst RN, Gleave AP, Lavezzo E, Fawcett JA, Proost S, Rouzé P, Sterck L, Toppo S, Lazzari B, Hellens RP, Durel CE, Gutin A, Bumgarner RE, Gardiner SE, Skolnick M, Egholm M, Van de Peer Y, Salamini F, Viola R (2010) The genome of the domesticated apple (*Malus* × *domestica* Borkh.). Nat Genet 42:833–839

Wang GM, Yin H, Qiao X, Tan X, Gu C, Wang BH, Cheng R, Wang YZ, Zhang SL (2016) F-box genes: genome-wide expansion, evolution and their contribution to pollen growth in pear (*Pyrus bretschneideri*). Plant Sci 253:164–175

Wang R, Ming M, Li J, Shi D, Qiao X, Li L, Zhang S, Wu J (2017) Genome-wide identification of the MADS-box transcription factor family in pear (*Pyrus bretschneideri*) reveals evolution and functional divergence. PeerJ 5:e3776

Wei SW, Tao ST, Qin GH, Wang SM, Tao JH, Wu J, Wu JY, Zhang SL (2016) Transcriptome profiling reveals the candidate genes associated with aroma metabolites and emission of pear (*Pyrus ussuriensis* cv.). Scient Hort 206:33–42

Wu J, Wang Y, Xu J, Korban SS, Fei Z, Tao S, Ming R, Tai S, Khan AM, Postman JD, Gu C, Yin H, Zheng D, Qi K, Li Y, Wang R, Deng CH, Kumar S, Chagné D, Li X, Wu J, Huang X, Zhang H, Xie Z, Li X, Zhang M, Li Y, Yue Z, Fang X, Li J, Li L, Jin C, Qin M, Zhang J, Wu X, Ke Y, Wang J, Yang H, Zhang S (2018) Diversification and independent domestication of Asian and European pears. Genome Biol 19:77

Wu J, Wang Z, Shi Z, Zhang S, Ming R, Zhu S, Khan MA, Tao S, Korban SS, Wang H, Chen NJ, Nishio T, Xu X, Cong L, Qi K, Huang X, Wang Y, Zhao X, Wu J, Deng C, Gou C, Zhou W, Yin H, Qin G, Sha Y, Tao Y, Chen H, Yang Y, Song Y, Zhan D, Wang J, Li L, Dai M, Gu C, Wang Y, Shi D, Wang X, Zhang H, Zeng L, Zheng D, Wang C, Chen M, Wang G, Xie L, Sovero V, Sha S, Huang W, Zhang S, Zhang M, Sun J, Xu L, Li Y, Liu X, Li Q, Shen J, Wang J, Paull RE, Bennetzen JL, Wang J, Zhang S (2013) The genome of the pear (*Pyrus bretschneideri* Rehd.). Genome Res 23:396–408

Xue C, Yao JL, Qin MF, Zhang MY, Allan AC, Wang DF, Wu J (2019) *PbrmiR397a* regulates lignification during stone cell development in pear fruit. Plant Biotechnol J 17(1):103–117. https://doi.org/10.1111/pbi.12950

Yan Y, Zheng X, Apaliya MT, Yang H, Zhang H (2018) Transcriptome characterization and expression profile of defense-related genes in pear induced by *Meyerozyma guilliermondii*. Postharvest Biol Technol 141:63–70

Yao G, Ming M, Allan AC, Gu C, Li L, Wu X, Wang R, Chang Y, Qi K, Zhang S, Wu J (2017) Map-based cloning of the pear gene *MYB114* identifies an interaction with other transcription factors to coordinately regulate fruit anthocyanin biosynthesis. Plant J Cell Mol Biol 92:437–451

Yu LA, Li JM, Li LT, Huang YH, Li XL, Qiao X, Liu X, Wu J (2017) Characterisation of the whole-genome wide hexokinase gene family unravels the functional divergence in pear (*Pyrus bretschneideri* Rehd.). J Hort Sci Biotech 93:244–254

Repetitive Sequences in Pear

8

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Abstract

Repetitive sequences account for a large proportion of the pear genome, suggesting that they play critical roles in the evolution of *Pyrus*. One form of repetitive sequences is transposable elements, which have been predominantly investigated thus far, including DNA transposons and retrotransposons. Approximately 22.5% of the 'Bartlett' genome (*P. communis*) and 42.4% of the 'Suli' genome (*P. pyrifolia*) are reported to be Long Terminal Repeat (LTR)-retrotransposons (LTR-RTs). Thus, investigation of transposable elements will offer new insights of the evolutionary history of *Pyrus*. LTR-RTs exhibit high heterogeneity and their copy numbers vary with the *Pyrus* species. The dynamics of LTR-RTs are an important source of genetic variation in *Pyrus* species. As of now, the function and development mechanism of transposable elements have not yet been fully understood. In this chapter, advances of transposable elements in *Pyrus* are presented and discussed.

8.1 Introduction

Repetitive sequences are highly diverse in their organization, abundance, chromosome localization, and variations in sequences within and between chromosomes, and account for a high percent of a plant genome. Among diverse groups of structural and functional repetitive sequences, transposable elements have been widely identified and investigated (Kumar and Bennetzen 1999; Wicker et al. 2007) (Fig. 8.1). Based on mode of transposition, there are two groups of transposable elements. One group, retrotransposons, transposes via RNA using a 'copy and paste' mechanism; whereas, the second group, transposons, transposes via DNA using a 'cut and paste' mechanism (Wicker et al. 2007).

Long Terminal Repeat (LTR)-retrotransposon (LTR-RT) is one form of retrotransposons (Fig. 8.2), as LTR-RTs are flanked by two LTRs, and undergo replicative transposition. These elements have been found in all plant species investigated thus far (SanMiguel et al. 1996; Sabot and Schulman 2006; Wicker et al. 2007). In higher plants, the transposon of LTR-RTs may increase their copy numbers, and increase

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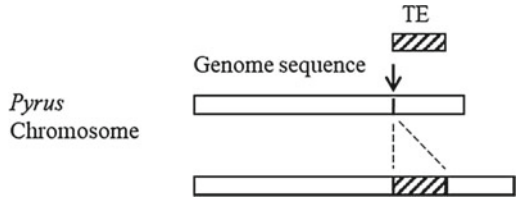

Fig. 8.1 Insertion of transposable elements

genome size (SanMiguel et al. 1998; Peterson et al. 2002; Havecker et al. 2004). For example, more than 50% of the maize and wheat genomes are made-up of retrotransposons (Meyers et al. 2001; Daron et al. 2014). In a wild rice species, *Oryza australiensis*, it is reported that a rapid twofold increase in genome size is likely attributed to transposition of retrotransposons that must have occurred over the last 3 million years (Piegu et al. 2006). This has suggested that retrotransposons may play an important role in expansion of a genome. Interestingly, retrotransposons isolated from plants appear to be rather young in age (El Baidouri and Panaud 2013). Therefore, removal of retrotransposons must also occur in a plant genome. For example, the rice genome has been reported to have lost a large number of retrotransposons, which, in turn, has resulted in rapid reduction of genome size (Ma et al. 2004).

Retrotransposons act by inserting themselves either within or near transcriptionally active regions of a chromosome, thereby resulting either in mutations by disrupting genes, altering gene expression levels, or by driving genomic rearrangements (Feschotte et al. 2002; Shapiro 2005). Kobayashi et al. (2004) have reported that a retrotransposon inserted into a *myb*-related gene is associated with pigmentation loss in grape. While Butelli et al. (2012) have found that insertion of a retrotransposon upstream of an anthocyanin biosynthesis-related gene results in cold-dependent fruit color development in blood orange. Furthermore, it is proposed that environmental stress and demethylation activate retrotransposons and induce duplication events in a genome (Hirochika et al. 2000; De Felice et al. 2009; Tsukahara et al. 2009).

It has been reported that retrotransposons display extreme sequence diversity, and more than thousands of retrotransposon families in plants have been isolated (Havecker et al. 2004; Wicker et al. 2007). An intact retrotransposon is composed of two nearly LTR sequences flanked by target site duplications of usually 4–6 bp in length (Kumar and Bennetzen 1999). The internal domain usually consists of two open reading frames (ORFs) required for transposition. In particular, this internal domain contains a primer-binding site (PBS), a polypurine tract (PPT), and two functional genes, *gag* and *pol*. The *pol* gene encodes three enzymatic regions of a protease, reverse transcriptase, and integrase, while the *gag* gene encodes structural proteins involved in the maturation and packaging of retrotransposon RNA. Some other conserved sequence motifs of the primer-binding site and

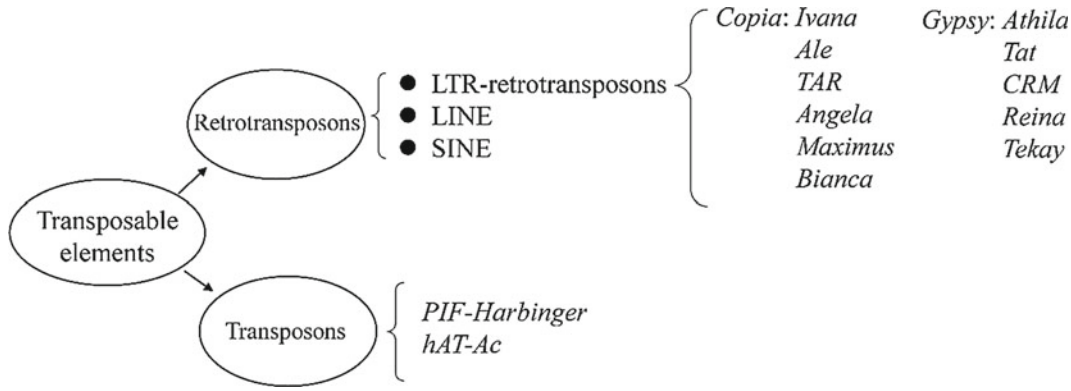

Fig. 8.2 Classification of transposable elements

the PPT are also essential for retrotransposon replication. LTR-retrotransposons can be subdivided into the Ty1-*copia* and the Ty3-*gypsy* groups (Wicker et al. 2007). Within the *pol* gene, the order of reverse transcriptase in the Ty1-*copia* group is in front of integrase, while that in the Ty3-*gypsy* group, it is the integrase that is in front.

The pear, *Pyrus* species, is proposed to have originated in the mountainous regions of western and southwestern China (Rubstov 1944). Pears are geographically classified into occidental and oriental pear groups (Bailey 1917). Major species of oriental pears are native to China (Teng and Tanabe 2004). The oriental pear group consists of wild pea pears and cultivated species with large fruit, thus demonstrating their complex evolutionary history (Zheng et al. 2014). Retrotransposons have been identified in pears and represent a large proportion (43%) of the *Pyrus* genome; therefore, they will provide new insights into the evolutionary history of pears (Wu et al. 2013).

8.2 Retrotransposons and Transposons in Two *Pyrus* Genomes

Recently, whole genomes of the Chinese white pear 'Dangshansuli' or 'Suli' (Wu et al. 2013) and the European pear 'Bartlett' (Chagne et al. 2014) were sequenced. The assembled 'Suli' genome consists of 2103 scaffolds with an N50 of 540.8 kb, totaling 512.0 Mb with 194× coverage. The 'Bartlett' genome is not as well assembled, but it consists of 142,083 scaffolds with an N50 of 6569 bp, totaling 577.0 Mb with 11.4× coverage. The assembled scaffolds have revealed that much of the *Pyrus* genome is retrotransposon-derived (Wu et al. 2013). The LTR-RTs, long-interspersed elements (LINEs), and short-interspersed elements (SINEs) are classified into the retrotransposon group. However, LINEs and SINEs only account for a little proportion of the *Pyrus* genome. A total of 42.4% of the 'Suli' genome and 22.5% of the 'Bartlett' genome are reported to be LTR-RTs (Table 8.1). This high-copy number of retrotransposons is also found in other genomes of members of the Rosaceae family, such as that of *Malus* (37.6%) and *Prunus* (18.6%) (Velasco et al. 2010; Verde et al. 2013). Furthermore, retrotransposons of the *Pyrus* genome have complex structures (Yin et al. 2014), and some are reported to be inserted in many loci in genomes of cultivated *Pyrus* species, but only in a few loci in genomes of wild *Pyrus* species (Jiang et al. 2015). Frequent recombination events followed by transposition of retrotransposons may have played critical roles in the evolution of *Pyrus* genomes.

More than one-thousand LTR-RTs have been isolated in the *Pyrus* genome, and it has been found that retrotransposons are of high heterogeneity, thus contributing to difficulties in LTR-RT classification (Yin et al. 2014; Jiang et al. 2016a). Two methods have been used to classify distinct families of LTR-RTs. The first method is based on coverage and sequence identities, wherein similar LTR-RTs made-up a single family (Du et al. 2010). Whereas, in the second method, families of LTR-RTs are classified based on mapping of these elements to an existing database, such as Repbase. Using this approach, a total of 148 families have been identified in the assembled 'Suli' genome (Yin et al. 2015). Recently, some new LTR-RT families, such as *TGTT* and *AACA* families, have been found in the 'Suli' genome, containing the palindromic dinucleotide 5′-'TG'-'TT'-3′ and the 5′-'AA'-'CA'-3′ motif at the start and at the end of an LTR sequence (Yin et al. 2017).

Transposons move within a genome through a 'cut and paste' strategy, and are characterized by their terminal inverted repeats (TIRs) of variable lengths. Currently, transposons are predicted through a homology search. In *Pyrus*, 7.77% of the 'Suli' genome and 8.04% of the 'Bartlett' genome are reported to be transposons (Wu et al. 2013; Chagne et al. 2014). The *PIF-Harbinger* is the largest family in these two pear genomes. This family carries terminal inverted repeats, and produces a 3 bp target site duplication upon insertion. These TEs contain two ORFs, one encoding a DNA binding protein, while the other encoding a DDE/DDD transposase. The second largest transposon family in

Table 8.1 Distribution of LTR-RTs in two *Pyrus* genomes

	% in 'Suli' genome	% in 'Bartlett' genome
Repeated sequences	53.1	34.14
LTR/*Ty1-copia*	16.88	7.66
LTR/*Ty3-gypsy*	25.48	14.79
DNA transposons	12.12	7.28

Pyrus is *hAT-Ac*, which has been firstly reported from *Zea mays* as an activator or an *Ac* element. Common features of *hAT* transposons include sizes of 2.5–5 kb with short terminal inverted repeats along with short flanking target site duplications generated during the transposition process. The *PIF–Harbinger* and *hAT-Ac* families account for half of the total size of transposons in the *Pyrus* genome (Wu et al. 2013; Chagne et al. 2014). The functions of transposons and their effects on trait performance are not yet well-understood in *Pyrus*.

8.3 High-Copy Number Retrotransposon Families

As it is difficult to analyze each of the LTR-RT families, analysis of copy numbers of these families has been pursued instead. As it is expected, high-copy numbers of LTR-RT families are more representative than low-copy numbers of LTR-RT families. The BLASTN has been used to search assemble genome data based on numbers of LTR-RTs in order to identify which families yield high-copy numbers of LTR-RTs. Using this approach, a total of ten high-copy number of LTR-RT families have been identified in the 'Suli' genome (Jiang et al. 2015). However, it is important to point out that this finding is influenced by numbers of incomplete LTR-RTs, as well as by the method of genome assembly. Incomplete LTR-RTs are often lost in such a prediction; moreover, in the current method of genome assembly, overlapping reads are often ignored during the process of assembly. This would lead to recovery of some high-copy numbers of LTR-RTs, wherein highly similar members are assembled into either a single or a few sequences. Obviously, this problem may be resolved by increasing sequencing depth. Overall, based on whole-genome resequencing, a total of 14 high-copy number LTR-RT families have been identified in Asian pears (Table 8.2) (Jiang et al. unpublished data). Of these families, nine are *copia*-type and five are *gypsy*-type retrotransposons. Interestingly, some of these retrotransposons have also been found in *Malus* and *Prunus* genomes.

8.4 Insertion of Retrotransposons and Marker Development

LTR sequences of LTR-RT flank coding regions at the 5′ and 3′ ends (Bergman and Quesneville 2007). Therefore, they are deemed well suited for developing new molecular markers (Fig. 8.3), as they are ubiquitously distributed, with abundant copy numbers, along with their insertion polymorphisms (Flavell et al. 1992). Thus far, four types of retrotransposon-based markers have been reported. For one type, retrotransposon-based insertion polymorphism (RBIP) markers amplify the junction of LTR and flanking genomes (Kalendar et al. 2011). For another type, inter-retrotransposon amplified polymorphism (IRAP) markers of specific length have been developed by amplifying the intermediate section of two nearby LTRs (Kalendar and Schulman 2006). For a third type, retrotransposon-microsatellite amplified polymorphism (REMAP) markers amplify specific lengths of LTRs to develop simple sequence repeats (SSRs) (Kalendar and Schulman 2006). Finally, sequence-specific amplification polymorphism (SSAP) markers can be developed, as they are similar to amplified fragment length polymorphisms (AFLPs). Although both SSAP and AFLP makers

Table 8.2 High-copy number of LTR-retrotransposon families in *Pyrus*

ID	Name in Repbase database
1	Gypsy-4_PX
2	Copia-100_Mad
3	Copia-24_PX
4	Copia-2_PX
5	Copia-90_Mad
6	Gypsy-3_PX
7	Copia-106_Mad
8	Copia-16_PX
9	Gypsy-46_Mad
10	Copia-13_PX
11	Gypsy-2_PX
12	Gypsy-5_PX
13	Copia-49_Mad
14	Copia-61_Mad

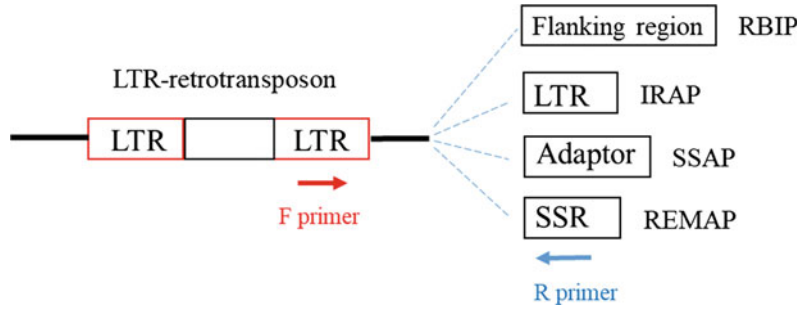

Fig. 8.3 Marker development based on retrotransposons

correspond to restriction size variations of a whole genome, SSAP markers also identify polymorphisms produced by retrotransposon insertions (Waugh et al. 1997). These various types of markers have already been developed in a variety of plant species, and are widely used in studies of genetic diversity, phylogeny, genetic mapping, and cultivar identification.

Kim et al. (2012) have isolated retrotransposons from a bacterial artificial chromosome (BAC) library of *Pyrus*. Subsequently, these retrotransposons have been used to develop 22 RBIP makers, based on the *copia*-like retrotransposon Ppcrt4, and these markers have allowed for the differentiation of 61 of 64 Japanese pear cultivars (Kim et al. 2012). However, a BAC library is too limited in identifying retrotransposons in a pear genome. Furthermore, sequence homology analysis by BLASTN could not identify all retrotransposons, particularly those retrotransposons that are specific to pear. Therefore, Jing et al. (2015) have developed 196 RBIP primers based on whole genome sequence data of 'Suli'. They have developed 24 pairs of primers, of the Ppcr1 subfamily of *copia* retrotransposons, *Ppcr1,* and have used them to investigate genetic diversity among 110 *Pyrus* accessions, including oriental and occidental pears. The *Ppcr1* is found to be inserted in many loci in genomes of cultivated *Pyrus* species, but only in a few loci in genomes of wild *Pyrus* species (Jiang et al. 2015). In another study, eight polymorphic IRAP markers have been developed, and a total of 76 alleles are amplified in 62 pear cultivars (Sun et al. 2015). Overall, it is reported

that both RBIP and IRAP markers have provided only a few information sites. Therefore, SSAP markers have been developed, as they do overcome this observed limitation. Jiang et al. (2016b) have developed 12 SSAP primers in *Pyrus*. Following a population structure analysis, nearly all Asian pear species and cultivar groups have been found to undergo hybridization and must have originated from five primitive genepools (Jiang et al. 2016b). Therefore, LTR-RT-based markers can serve as important tools for pursuing evolutionary analysis studies of *Pyrus*.

8.5 Concluding Remarks and Future Prospects

Overall, retrotransposons account for a high percentage of the pear genome. Their function and influence on characteristics of individual genotypes should be further explored. Until now, LTR-RTs have been successfully used to develop DNA-based markers in some plant species (Smykal et al. 2011; Palhares et al. 2012; Kuhn et al. 2014).

For the future, the following areas of study should be pursued: (1) investigating activity and function of LTR-RTs in the *Pyrus* genome. As most LTR-RTs are silent, environmental stresses and demethylation are reported to activate retrotransposons, and piRNAs in the germline could silence elements, such as retrotransposons. Thus, identifying details of LTR-RT activation mechanisms would be highly informative, as well as determining how LTR-RTs participate in the metabolism process. (2) Studying how variations in traits of the *Pyrus* genome are influenced by insertions of LTR-RTs. As it is known that homology and specific insertion sites of LTR-RTs are found in both oriental and occidental pears, homology sites have indicated that these insertion sites must have existed prior to the divergence of oriental pear and occidental pear. Furthermore, some specific insertion sites are detected in regions around functional genes. Therefore, investigating variations in traits of the *Pyrus* genome that are caused by these insertions of LTR-RTs should be quite informative. (3) Construction of a mutant library of the *Pyrus* genome using LTR-RTs. As DNA transposons are widely used in construction of mutant libraries; e.g., the Ac/Ds transposon tagging method, use of LTR-RTs, which have transposition functions, could also serve as a valuable tool in constructing mutant libraries of the *Pyrus* genome for pursuing functional gene analysis studies.

References

Bailey L (1917) Standard cyclopedia of horticulture. Macmillan Press, New York, USA

Bergman CM, Quesneville H (2007) Discovering and detecting transposable elements in genome sequences. Brief Bioinform 8(6):382–392

Butelli E, Licciardello C, Zhang Y, Liu J, Mackay S, Bailey P, Reforgiato-Recupero G, Martin C (2012) Retrotransposons control fruit-specific, cold-dependent accumulation of anthocyanins in blood oranges. Plant Cell 24(3):1242–1255

Chagne D, Crowhurst RN, Pindo M, Thrimawithana A, Deng C, Ireland H, Fiers M, Dzierzon H, Cestaro A, Fontana P, Bianco L, Lu A, Storey R, Knabel M, Saeed M, Montanari S, Kim YK, Nicolini D, Larger S, Stefani E, Allan AC, Bowen J, Harvey I, Johnston J, Malnoy M, Troggio M, Perchepied L, Sawyer G, Wiedow C, Won K, Viola R, Hellens RP, Brewer L, Bus VG, Schaffer RJ, Gardiner SE, Velasco R (2014) The draft genome sequence of European pear (*Pyrus communis* L. 'Bartlett'). Plos One 9(4):e92644

Daron J, Glover N, Pingault L, Theil S, Jamilloux V, Paux E, Barbe V, Mangenot S, Alberti A, Wincker P, Quesneville H, Feuillet C, Choulet F (2014) Organization and evolution of transposable elements along the bread wheat chromosome 3B. Genome Biol 15 (12):546

De Felice B, Wilson RR, Argenziano C, Kafantaris I, Conicella C (2009) A transcriptionally active *copia*-like retroelement in *Citrus limon*. Cell Mol Biol Lett 14(2):289–304

Du J, Tian Z, Hans CS, Laten HM, Cannon SB, Jackson SA, Shoemaker RC, Ma J (2010) Evolutionary conservation, diversity and specificity of LTR-retrotransposons in flowering plants: insights from genome-wide analysis and multi-specific comparison. Plant J 63(4):584–598

El Baidouri M, Panaud O (2013) Comparative genomic paleontology across plant kingdom reveals the dynamics of TE-driven genome evolution. Genome Biol Evol 5(5):954–965

Feschotte C, Jiang N, Wessler SR (2002) Plant transposable elements: where genetics meets genomics. Nat Rev Genet 3(5):329–341

Flavell AJ, Dunbar E, Anderson R, Pearce SR, Hartley R, Kumar A (1992) Ty1-*copia* group retrotransposons are ubiquitous and heterogeneous in higher plants. Nucleic Acids Res 20(14):3639–3644

Havecker ER, Gao X, Voytas DF (2004) The diversity of LTR retrotransposons. Genome Biol 5(6):225

Hirochika H, Okamoto H, Kakutani T (2000) Silencing of retrotransposons in *arabidopsis* and reactivation by the *ddm1* mutation. Plant cell 12(3):357–369

Jiang S, Cai D, Sun Y, Teng Y (2016a) Isolation and characterization of putative functional long terminal repeat retrotransposons in the *Pyrus* genome. Mob DNA 7:1

Jiang S, Zheng X, Yu P, Yue X, Ahmed M, Cai D, Teng Y (2016b) Primitive genepools of asian pears and their complex hybrid origins inferred from fluorescent sequence-specific amplification polymorphism (SSAP) markers based on LTR retrotransposons. Plos One 11(2):e0149192

Jiang S, Zong Y, Yue X, Postman J, Teng Y, Cai D (2015) Prediction of retrotransposons and assessment of genetic variability based on developed retrotransposon-based insertion polymorphism (RBIP) markers in *Pyrus* L. Mol Genet Genomic 290(1):225–237

Kalendar R, Flavell AJ, Ellis TH, Sjakste T, Moisy C, Schulman AH (2011) Analysis of plant diversity with retrotransposon-based molecular markers. Heredity 106(4):520–530

Kalendar R, Schulman AH (2006) IRAP and REMAP for retrotransposon-based genotyping and fingerprinting. Nat Protoc 1(5):2478–2484

Kim H, Terakami S, Nishitani C, Kurita K, Kanamori H, Katayose Y, Sawamura Y, Saito T, Yamamoto T (2012) Development of cultivar-specific DNA markers based on retrotransposon-based insertional polymorphism in Japanese pear. Breed Sci 62(1):53–62

Kobayashi S, Goto-Yamamoto N, Hirochika H (2004) Retrotransposon-induced mutations in grape skin color. Science 304(5673):982

Kuhn BC, Lopez-Ribera I, da Silva Machad MDEF, Vicient CM (2014) Genetic diversity of maize germplasm assessed by retrotransposon-based markers. Electrophoresis 35(12–13):1921–1927

Kumar A, Bennetzen JL (1999) Plant retrotransposons. Annu Rev Genet 33(1):479–532

Ma J, Devos KM, Bennetzen JL (2004) Analyses of LTR-retrotransposon structures reveal recent and rapid genomic DNA loss in rice. Genome Res 14(5):860–869

Meyers BC, Tingley SV, Morgante M (2001) Abundance, distribution, and transcriptional activity of repetitive elements in the maize genome. Genome Res 11 (10):1660–1676

Palhares AC, Rodrigues-Morais TB, Van Sluys MA, Domingues DS, Maccheroni W Jr, Jordao H Jr, Souza AP, Marconi TG, Mollinari M, Gazaffi R, Garcia AA, Vieira ML (2012) A novel linkage map of sugarcane with evidence for clustering of retrotransposon-based markers. BMC Genet 13:51

Peterson DG, Schulze SR, Sciara EB, Lee SA, Bowers JE, Nagel A, Jiang N, Tibbitts DC, Wessler SR, Paterson AH (2002) Integration of cot analysis, DNA cloning, and high-throughput sequencing facilitates genome characterization and gene discovery. Genome Res 12(5):795–807

Piegu B, Guyot R, Picault N, Roulin A, Saniyal A, Kim H, Collura K, Brar DS, Jackson S, Wing RA, Panaud O (2006) Doubling genome size without polyploidization: dynamics of retrotransposition-driven genomic expansions in *Oryza australiensis*, a wild relative of rice. Genome Res 16(10):1262–1269

Rubstov GA (1944) Geographical distribution of the genus *Pyrus* and trends and factors in its evolution. Am Nat 78:358–366

Sabot F, Schulman AH (2006) Parasitism and the retrotransposon life cycle in plants: a hitchhiker's guide to the genome. Heredity 97(6):381–388

SanMiguel P, Gaut BS, Tikhonov A, Nakajima Y, Bennetzen JL (1998) The paleontology of intergene retrotransposons of *maize*. Nat Genet 20(1):43–45

SanMiguel P, Tikhonov A, Jin YK, Motchoulskaia N, Zakharov D, Melake-Berhan A, Springer PS, Edwards KJ, Lee M, Avramova Z, Bennetzen JL (1996) Nested retrotransposons in the intergenic regions of the *maize* genome. Science 274 (5288):765–768

Shapiro JA (2005) Retrotransposons and regulatory suites. BioEssays 27(2):122–125

Smykal P, Bacova-Kerteszova N, Kalendar R, Corander J, Schulman AH, Pavelek M (2011) Genetic diversity of cultivated flax (*Linum usitatissimum* L.) germplasm assessed by retrotransposon-based markers. Theor Appl Genet 122(7):1385–1397

Sun J, Hao Y, Li L, Song Y, Fan L, Zhang S, Wu J (2015) Evaluation of new irap markers of pear and their potential application in differentiating bud sports and other rosaceae species. Tree Genet Genomes 11(2):1–13

Teng Y, Tanabe K (2004) Reconsideration on the origin of cultivated pears native to East Asia. Acta Hortic 634:175–182

Tsukahara S, Kobayashi A, Kawabe A, Mathieu O, Miura A, Kakutani T (2009) Bursts of retrotransposition reproduced in *Arabidopsis*. Nature 461 (7262):423–426

Velasco R, Zharkikh A, Affourtit J, Dhingra A, Cestaro A, Kalyanaraman A, Fontana P, Bhatnagar SK, Troggio M, Pruss D, Salvi S, Pindo M, Baldi P, Castelletti S, Cavaiuolo M, Coppola G, Costa F, Cova V, Dal Ri A, Goremykin V, Komjanc M, Longhi S, Magnago P, Malacarne G, Malnoy M, Micheletti D, Moretto M, Perazzolli M, Si-Ammour A, Vezzulli S, Zini E, Eldredge G, Fitzgerald LM, Gutin N, Lanchbury J, Macalma T, Mitchell JT, Reid J, Wardell B, Kodira C, Chen Z, Desany B, Niazi F, Palmer M,

Koepke T, Jiwan D, Schaeffer S, Krishnan V, Wu C, Chu VT, King ST, Vick J, Tao Q, Mraz A, Stormo A, Stormo K, Bogden R, Ederle D, Stella A, Vecchietti A, Kater MM, Masiero S, Lasserre P, Lespinasse Y, Allan AC, Bus V, Chagne D, Crowhurst RN, Gleave AP, Lavezzo E, Fawcett JA, Proost S, Rouze P, Sterck L, Toppo S, Lazzari B, Hellens RP, Durel CE, Gutin A, Bumgarner RE, Gardiner SE, Skolnick M, Egholm M, Van de Peer Y, Salamini F, Viola R (2010) The genome of the domesticated apple (*Malus* × *domestica* Borkh.). Nat Genet 42(10):833–839

Verde I, Abbott AG, Scalabrin S, Jung S, Shu SQ, Marroni F, Zhebentyayeva T, Dettori MT, Grimwood J, Cattonaro F, Zuccolo A, Rossini L, Jenkins J, Vendramin E, Meisel LA, Decroocq V, Sosinski B, Prochnik S, Mitros T, Policriti A, Cipriani G, Dondini L, Ficklin S, Goodstein DM, Xuan PF, Del Fabbro C, Aramini V, Copetti D, Gonzalez S, Horner DS, Falchi R, Lucas S, Mica E, Maldonado J, Lazzari B, Bielenberg D, Pirona R, Miculan M, Barakat A, Testolin R, Stella A, Tartarini S, Tonutti P, Arus P, Orellana A, Wells C, Main D, Vizzotto G, Silva H, Salamini F, Schmutz J, Morgante M, Rokhsar DS, Initiative IPG (2013) The high-quality draft genome of peach (*Prunus persica*) identifies unique patterns of genetic diversity, domestication and genome evolution. Nat Genet 45(5):487-U447

Waugh R, McLean K, Flavell AJ, Pearce SR, Kumar A, Thomas BB, Powell W (1997) Genetic distribution of *Bare*-1-like retrotransposable elements in the barley genome revealed by sequence-specific amplification polymorphisms (S-SAP). Mol Gen Genet 253(6):687–694

Wicker T, Sabot F, Hua-Van A, Bennetzen JL, Capy P, Chalhoub B, Flavell A, Leroy P, Morgante M, Panaud O, Paux E, SanMiguel P, Schulman AH (2007) A unified classification system for eukaryotic transposable elements. Nat Rev Genet 8(12):973–982

Wu J, Wang Z, Shi Z, Zhang S, Ming R, Zhu S, Khan MA, Tao S, Korban SS, Wang H, Chen NJ, Nishio T, Xu X, Cong L, Qi K, Huang X, Wang Y, Zhao X, Wu J, Deng C, Gou C, Zhou W, Yin H, Qin G, Sha Y, Tao Y, Chen H, Yang Y, Song Y, Zhan D, Wang J, Li L, Dai M, Gu C, Wang Y, Shi D, Wang X, Zhang H, Zeng L, Zheng D, Wang C, Chen M, Wang G, Xie L, Sovero V, Sha S, Huang W, Zhang S, Zhang M, Sun J, Xu L, Li Y, Liu X, Li Q, Shen J, Wang J, Paull RE, Bennetzen JL, Wang J, Zhang S (2013) The genome of the pear (*Pyrus bretschneideri* Rehd.). Genome Res 23(2):396–408

Yin H, Du J, Wu J, Wei S, Xu Y, Tao S, Wu J, Zhang S (2015) Genome-wide annotation and comparative analysis of long terminal repeat retrotransposons between pear species of *P. bretschneideri* and *P. communis*. Sci Rep 5:17644

Yin H, Du JC, Li LT, Jin C, Fan L, Li M, Wu J, Zhang SL (2014) Comparative genomic analysis reveals multiple long terminal repeats, lineage-specific amplification, and frequent interelement recombination for *Cassandra* retrotransposon in pear (*Pyrus bretschneideri* Rehd.). Genome Biol Evol 6(6):1423–1436

Yin H, Wu X, Shi D, Chen Y, Qi K, Ma Z, Zhang S (2017) TGTT and AACA: two transcriptionally active LTR retrotransposon subfamilies with a specific LTR structure and horizontal transfer in four Rosaceae species. Mob DNA 8:14

Zheng X, Cai D, Potter D, Postmand J, Liu J, Teng Y (2014) Phylogeny and evolutionary histories of *Pyrus* L. revealed by phylogenetic trees and networks based on data from multiple DNA sequences. Mol Phylogenet Evol 80:54–65

Regulatory Sequences of Pear

9

Yongping Cai, Muhammad Abdullah and Xi Cheng

Abstract

Pear (*Pyrus*) is one of the leading and oldest cultivated fruit trees of temperate regions that is grown around the world. Compared to other Rosaceae species, pear research studies have lagged behind other members of the Rosaceae, such as strawberry, peach, and apple. However, the recent completion of whole-genome sequencing projects for pear offers ideal opportunities for pursuing regulatory sequence research studies. A regulatory sequence is a segment of nucleic acids capable of either increasing or decreasing; i.e., regulation of expression of a structural gene. Furthermore, the regulation of gene expression can be undertaken in different ways, such as during transcription, mRNA processing, translation, and via protein stability. It is commonly proposed that the regulation of gene expression occurs primarily at the transcriptional level. A plant transcriptional mechanism consists of two complimentary regulatory modules, *cis*-acting and *trans*-acting elements. *Cis*-acting elements are DNA sequences present in either coding or non-coding regions of the genome. *Cis*-acting elements can also be covered by epigenetic information. On the other hand, *trans*-acting factors are transcription factors (TFs) or other DNA-binding proteins that bind to specific sequences in *cis*-acting elements to either increase or suppress transcription of a given gene. In this instance, chromatin remodeling involves dynamic modification of histones or the DNA sequence itself to allow access of accessible regions within the DNA for *trans*-acting elements to regulate transcription. Furthermore, TFs may influence transcription of multiple genes, and they can function either in a complex manner or combinatorially to bind *cis*-regulatory components at multiple transcription factor binding sites to regulate a unique trait in a controlled pattern of gene expression.

9.1 Introduction

The genus *Pyrus* belongs to the family Rosaceae. It is characterized by a wide genetic diversity with several species and more than 4000 cultivars that can be divided into two major groups, the occidental (European) and oriental (Asiatic) pears. The pear is one of the oldest fruit crops (over 3000 years) grown in the world, and has at least 22 well-recognized species, including *P.* × *bretschneideri*, *P. ussuriensis*, *P. pyrifolia*, *P. sinkiangensis*, and *P. communis*. The pear

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S. S. Korban (ed.), *The Pear Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-030-11048-2_9

genome contains all of the coding and non-coding DNA sequences controlling all functions within all cell types of the pear. *Pyrus* species are functionally diploid ($x = 17$, $2n = 34$), and they are highly heterozygous due to self-incompatibility. Although genome sizes of all *Pyrus* species are not yet available, the nuclear content of *P. communis* (European pear) is 1.03 pg/2C (Chagné et al. 2014). It is estimated that the *P. communis* genome is approximately 577 Mbp per haploid genome equivalents, while that of *P.* × *bretschneideri* (Chinese pear) is 527 Mbp (Wu et al. 2013). In addition, the total number of genes is estimated to be approximately 43,000 (https://www.rosaceae.org/organism/Pyrus/all-species).

In general, DNA sequences consist of coding and non-coding regions. Coding regions consist of genes that encode proteins controlling various biological processes, as well as ribosomal RNAs and proteins. Non-coding regions include maintenance elements, such as telomeres, centromeres, and origins of replication controlling DNA replication. Furthermore, these non-coding regions consist of elements, such as promoters/repressors, insulators, and regulatory RNAs that regulate the spatial and temporal expression of coding genes. These latter regulatory sequences are capable of either increasing or decreasing expression of specific genes. Generally, regulation of gene expression occurs at the level of RNA biosynthesis, and this is accomplished through sequence-specific binding of proteins or transcription factors. Interestingly, transcriptional factors (TFs) may act as either activators/repressors or both. Repressors often act by preventing RNA polymerase from forming a productive complex with the transcriptional initiation (promoter) region, while activators facilitate the formation of a productive complex.

It is noteworthy to point out that DNA sequence motifs (or motifs) aid in predicting epigenomic modifications, thus signifying that TFs play a vital role in regulating the epigenome. Regulatory sequences are commonly linked with messenger RNA molecules that control mRNA biogenesis or translation (Adcock and Caramori 2009). In general, conserved non-coding sequences also have regulatory regions. Thus, these sequences are often the subject of analysis, such as those of the CAAT box, CCAAT box, A-box, and Z-box, among others.

It is commonly known that expression of genes is a tightly regulated process. Specifically, expression must occur in the correct cell type to an appropriate level and at the correct time during cell differentiation and development in response to internal and external signals. Failure of the regulation process of gene expression leads to serious consequences in genetic disease (Barnes 2006). In the post-sequencing genomics era, with advances in both computational methods and genome-wide experimental approaches, it is important to study how different regulatory sequences and proteins interact to control gene expression. Such control must occur not only at a single gene locus, but also globally across the genome within complex biological and transcriptional programs. Changes in gene transcription are mainly controlled by the transcription factor protein that binds DNA to DNA and modulates the transcriptional apparatus. TFs are essential for regulating expression of many genes, and they may play important roles in plant physiological processes, such as development, biotic stress, abiotic stress, as well as structural and functional divergence. Many transcriptional factors have now been identified. In fact, there is a paucity of data related to the regulation of transcriptional factors in the pear genome. TF proteins that bind to DNA-regulatory sequences, usually localized in the 5-upstream region of target genes, modulate the rate of gene transcription. This may result in either increased or decreased gene transcription, protein synthesis, and subsequent altered cellular function. Many transcriptional factors have now been identified, and a large proportion of the pear genome appears to code for these proteins. This is a review of the regulatory sequences of pear, and will specifically focus on *trans*-acting factors and physiological function of TFs in normal cell development and in plant physiological processes.

9.2 Transcription Factor Families in Pear

Transcription factors are classified into different families based on their DNA-binding domains (DBDs) (Riechmann et al. 2000). The general characteristics of pear transcriptional factors are similar to those of other plants and eukaryotes. Pear TF families play important roles in transcriptional regulation of different processes, thus rendering the study of TFs essential for understanding the functions of genes at the molecular level.

Early on, it has been reported that the *Arabidopsis* genome contains 1500 transcriptional factors (Riechmann et al. 2000); however, subsequent analyses reported that the *Arabidopsis* genome has in fact 2000 TFs. Thus, the *Arabidopsis* TF database has been used as a model/basis for identifying and characterizing TFs from pear, and from all other plant species. Currently, the four descriptive databases for *Arabidopsis* TFs include the following: AGRIS (http://agris-knowledgebase.org/AtTFDB/) (Davuluri et al. 2003), PTFDB (http://planttfdb.cbi.pku.edu.cn/) (Riaño-Pachón et al. 2007), RARTAF (http://rarge.gsc.riken.jp/rartf/) (Iida et al. 2005), and DATF (http://atrm.cbi.pku.edu.cn/) (Guo et al. 2005). Each database has utilized different algorithms, and offers different classification criteria for TFs as the number of loci of each set does not overlap fully (Table 9.1). Plant transcriptional factors are characterized by a large number of genes and by a variety of transcriptional factor families when compared with those of either *Caenorhabditis elegans* or *Drosophila melanogaster*. It is important to note that the number of transcription factor genes is not dependent on genome size (Abdullah et al. 2018a; Chen et al. 2018; Su et al. 2017). For example, although the size of *Arabidopsis* is only ~135 Mbp, it contains 2000 TFs, which is a significantly large number of TFs when compared to other similar size genomes (Riechmann et al. 2000). In addition, the ratio of number of transcription factors to the total number of genes in the *Arabidopsis* genome is 5–10%, which is higher than ratios calculated in human (6.0%), *C. elegans* (3.6%), and *D. melanogaster* (4.7%) genomes. Furthermore, this high ratio of TFs detected in *Arabidopsis* is also accompanied by a high diversity of DNA-binding specification when compared to those found in *C. elegans* and *D. melanogaster*. These collective findings suggest that plant transcriptional regulation may be more complex and more diverse than that found in mammalian systems. In fact, many specific transcription factors identified in pear, *Arabidopsis*, and in other plants possess DNA-binding domains found only in plants. For example, WRKY, EIL, NAC, AP2-ERF, Dof, GARP, SBP, TCP, LFY, YABBY, TCP, and AB13-VP1 (B3) are plant-specific transcription factor families (Cheng et al. 2018). Many plant transcriptional factors belonging to large families also share similar DNA-binding domain structures. For example, each of NAC and AP2-ERF families contains >100 members. Furthermore, although MADS-box, bZIP, basic helix-loop-helix (bHLH), HB, and MYB are not plant-specific, they are also large families in plant species. These TF families play critical roles in plant growth and development, and against environmental changes.

9.2.1 MYB TF Family

MYB proteins correspond to a superfamily of transcription factors, known to be one of the largest transcription factor families in the plant kingdom. The genome of *P.* × *bretschneideri* (Chinese white pear) contains 231 non-redundant MYB genes, including 35 R1-*MYBs*, 185 R2R3-*MYBs*, 10 R1R2R3-*MYBs*, and one 4R-like MYB protein (4R-MYB) (Li et al. 2016). MYB domain repeats play a key role in pear and in other plants regulatory networks controlling plant development, metabolism, cell differentiation, plant defense mechanisms, and responses to multiple stresses (Cao et al. 2016b). Members of the MYB family are widely distributed in plants, animals, as well as in other higher eukaryotes. This family has been first identified in the avian myeloblastosis virus as an oncogene, *v-MYB*, where its role is found to regulate the cell cycle (Ito et al.

Table 9.1 A listing of transcription factor families identified in *Arabidopsis*, their corresponding annotation, along with the identified number of gene family members, as reported in different *Arabidopsis* TF databases

TF family	InterPro or GenBank	Riechmann	RARTF	AGRIS	DATF	PlnTFDB
SBP	CAB56581	16	17	16	16	16
WRKY	S72443	72	72	72	72	72
ARF	AAC49751	23	71	22	23	23
AS2		0	0	0	42	0
ARID	IPR001606	4	6	7	10	10
AB13/VP1*	CAA48241	14	51	11	60	56
ALFIN-like	AAA20093	7	47	7	7	7
AP2/EREBP	IPR001471	144	93	136	146	146
TUB	IPR000007	11	11	10	0	10
AUX/IAA	AAC39440	26	21	0	29	27
Bhlh	IPR001606	139	157	162	127	134
Bzip		81	56	73	72	71
AS2		0	0	0	42	0
C2C2(Zn)-BBX	A56133	33	51	30	37	17
C2C2(Zn)-Dof	CAA66600	37	33	36	36	36
C2C2(Zn)-GATA	IPR000679	28	37	30	26	29
C2C2(Zn)-YABBY	AAD30526	6	5	6	5	6
C2H2(Zn)	IPR000822	105	177	211	134	96
C3H-TYPE(Zn)	IPR000232	33	47	165	59	67
CCAAT	A26771/P13434/Q02526	36	37	35	36	43
CPP(Zn)	CAA09028	8	8	8	8	8
E2F/DP	O00716/Q64163	8	8	8	8	7
EIL	AAC49750	6	6	6	6	6
GARP	AAD55941/BAA74528	56	51	55	53	52
GRAS	AAB06318	32	32	31	33	33
HB	IPR001356	89	97	91	87	91
HMG-box	IPR000910	10	11	0	11	11
HSF	IPR000232	26	27	21	23	23
JUMONJI	T30254	9	13	5	17	17
LFY	AAA32826	1	3	1	1	1
MADS	IPR002100	82	106	109	104	102
MYB	IPR001005/IPR000818	190	189	197	199	209
NAC	BAB10725	109	106	94	107	101
Nin-like	CAB61243	15	14	0	14	0
PCG		4	35	0	34	0
TCP	AAC26786	25	24	26	23	24
Trihelix	S39484	28	31	29	26	23
Others		20	215	127	231	375
Totals		1533	1965	1837	1922	1949

2001). Subsequently, the *v-MYB* gene is found to consist of three members, namely *C-MYB*, *A-MYB*, and *B-MYB*. Further studies have led to the identification of the first plant *MYB* gene, *C1*, which is involved in anthocyanin biosynthesis in kernels of *Zea mays* (Paz-Ares et al. 1987). Since then, numerous members of the *MYB* gene family have been identified in genomes of *Arabidopsis*, rice, maize, and soybean, among others, and are reported to be involved in regulating several cellular processes including cell cycle, cell morphogenesis, and responses to both biotic and abiotic stresses.

The MYB protein family is comprised of three domains, including the N-terminal conserved DNA-binding domain, a central transcriptional activation domain, and a C-terminal domain that functions in transcriptional repression (Vargova et al. 2011). The C-terminus is diverse, involved in modulating protein regulatory activity, and responsible for versatile regulatory roles of MYBs. The DNA-binding domain is highly conserved and contains up to four imperfect repeats, each of which consists of about 51–53 amino acids forming three α–helices. Depending on the number of repeats present in the MYB domain, the MYB family found in *P.* × *bretschneideri* is generally classified into four subgroups, namely 1R (R1/2, R3-MYB), 2R (R2R3-MYB), 3R (R1R2R3-MYB), and 4R (harboring four R1/R2-like repeats) (Li et al. 2016). Although four MYB classes are detected in plants, it is R2R3-type MYB proteins that are the most common in plants, including those found in *P.* × *bretschneideri*. In fact, a total of 185 R2R3-*MYBs* is detected in *P.* × *bretschneideri* (Li et al. 2016; Cao et al. 2016b), while the 4R-MYB class is the smallest and harbors four R1/R2-like repeats. In several plant genomes, a single 4R-MYB gene is encoded, but little is known about this MYB protein group in plants.

The R1R2R3-type MYB proteins detected in higher plants are usually encoded by five genes, and they play significant roles in control of the cell cycle. Yet, another heterogeneous class includes proteins having either single or partial MYB repeats, collectively designated as "MYB-related," and it is divided into various subclasses. The R3-type MYB-related genes have evolved from R2R3 to MYB, and they control cell morphogenesis. The R1/R2-type MYB-related genes encode proteins for core components of the central circadian oscillator. Those MYB proteins class with two repeats, R2R3, are widely found in plants, and these must have evolved by loss of the R1 repeat from the R1R2R3-MYB gene ancestor, followed by subgroup expansion during plant evolution (Rosinski and Atchley 1998). While 3R encoded genes of R1R2 MYB encoded genes have evolved by gaining R1 repeats through an ancient intragenic duplication (Jiang et al. 2004). It is the R2R3-type MYB protein that has been categorized into 23 subgroups depending upon the conservation of the DNA-binding domain and amino acid motifs present at the C-terminal region (Dubos et al. 2010). Based on phylogenetic analysis, 185 *PbMYB* genes of *P.* × *bretschneideri* have been divided into 317 subgroups, and these are well supported by additional intron/exon structures and conserved motifs (Li et al. 2016).

In a detailed study of this MYB class in plants, it is revealed that this class of MYB proteins participates in plant tolerance to several biotic and abiotic stresses, hormone signaling, phenylpropanoid biosynthesis, secondary metabolism, cell shape determination, and cell cycle regulation (Table 9.2). Additionally, specific clusters of orthologous and paralogous genes have also been identified that will facilitate the characterization of each subgroup in the *R2R3-MYB* gene family of pears (Table 9.2). Specifically, the *R2R3-MYB* gene family is involved in both positive and negative regulation of many stress-responsive pathways. So far, large numbers of R2R3 MYB proteins have been reported in various plant species, including 177 in sweet orange (Hou et al. 2014), 198 in *Arabidopsis* (Yanhui et al. 2006), 183 in rice (Yanhui et al. 2006), and 209 in foxtail millet (Muthamilarasan et al. 2014). The R2R3-MYB-type subfamily proteins have been reported to be involved in responses to environmental stresses in several plant species, including *Arabidopsis*, wheat, rice,

Table 9.2 A listing of R2R3-MYB transcription factors in pear, their counterparts in *Arabidopsis* and a few other plant species, along with their likely functions

Pyrus × *bretschneideri*	Corresponding counterparts in *Arabidopsis thaliana*	Function(s)	Reference(s)
Pbr016839.1	AtMYB60	Response to environmental stress	Seo and Park (2010), Raffaele et al. (2008)
Pbr002014.1	AtMYB94		
Pbr032528.1	AtMYB96		
Pbr019262.1	AtMYB30		
Pbr011441.1	AtMYB31		
Pbr009823.1			
Pbr033618.1	AtMYB10	Lignin biosynthesis	Zhou et al. (2009), Zhong et al. (2014)
Pbr041094.1	AtMYB58		
	AtMYB63		
	AtMYB72		
Pbr028725.1	AtMYB3	Anthocyanin biosynthesis	Vimolmangkang et al. (2013)
Pbr013413.1	AtMYB7		
Pbr014381.1	AtMYB32		
Pbr038870.1	AtMYB4		
Pbr020365.1	MdMYB3		
Pbr038869.1			
Pbr000876.1			
Pbr020726.1			
Pbr020733.1			
Pbr011095.1	AtMYB15	Involved in cold stress	Reyes and Chua (2007), Agarwal et al. (2006)
Pbr025360.1	AtMYB13		
Pbr030553.1	AtMYB14		
Pbr031687.1			
Pbr024975.1			
Pbr031684.1			
Pbr019908.1			
Pbr028561.1	AtMYB113	Anthocyanin biosynthesis	Li et al. (2012), Uematsu et al. (2014)
Pbr016663.1	AtMYB114		
Pbr016661.1	AtMYB75		
Pbr042132.1	AtMYB90		
	PcMYB10		
	PpMYB10		
	MdMYB10		
	MdMYB1		

(continued)

Table 9.2 (continued)

Pyrus × *bretschneideri*	Corresponding counterparts in *Arabidopsis thaliana*	Function(s)	Reference(s)
Pbr030848.1	AtMYB11	Flavonol biosynthesis	Stracke et al. (2007)
Pbr008630.1	AtMYB12		
Pbr011980.1	AtMYB111		
Pbr001148.1			
Pbr023487.1	AtMYB42	Lignin biosynthesis	Zhao and Dixon (2011), Patzlaff et al. (2003)
Pbr023482.1	AtMYB85		
Pbr041889.1	AtMYB43		
Pbr024975.1	AtMYB20		
Pbr015763.1	AtMYB99		
Pbr012750.1	AtMYB40		
Pbr012624.1	PtMYB1		
Pbr016625.1			
Pbr014479.1			
Pbr029909.1	AtMYB16	Epidermal cells	Jakoby et al. (2008)
Pbr030136.1	AtMYB106		
Pbr007283.1			
Pbr030135.1			
Pbr038434.1			
Pbr019293.1			
Pbr040860.1	AtMYB39	Trichome development	Scoville et al. (2011)
Pbr031409.1	AtMYB9		
Pbr013860.1	AtMYB107		
Pbr021178.1	AtMYB74	Response to biotic stress	Li et al. (2009), Cominelli et al. (2008)
Pbr021193.1	AtMYB102		
Pbr011268.1	AtMYB41		
Pbr018024.1	AtMYB49		
Pbr000268.1			
Pbr001520.1			
	AtMYB28	Glucosinolate biosynthesis	Gonzalez et al. (2009)
	AtMYB29		
	AtMYB76		
	AtMYB34		
	AtMYB51		
	AtMYB122		
	AtMYB47		
	AtMYB95		

(continued)

Table 9.2 (continued)

Pyrus × *bretschneideri*	Corresponding counterparts in *Arabidopsis thaliana*	Function(s)	Reference(s)
Pbr026080.1	AtMYB6	Lignin deposition and stomatal aperture	Liang et al. (2005)
Pbr006685.1	AtMYB61		
Pbr005982.2	AtMYB50		
Pbr039864.1	AtMYB55		
Pbr001888.1	AtMYB86		
Pbr001950.1	AtMYB23	Epidermal cells	Jakoby et al. (2008)
Pbr001952.1	AtMYB66		
Pbr034884.1	AtMYB82		
Pbr034885.1			
Pbr003136.1			
Pbr028978.1	AtMYB121	Root development	Zhou et al. (2009)
Pbr033988.1	AtMYB71		
Pbr020777.1	AtMYB79		
Pbr035306.1	AtMYB48		
Pbr023547.1	AtMYB59		
Pbr016603.1	AtMYB27		
Pbr033457.1			
Pbr033089.1			
Pbr018111.1	AtMYB101	Involved in anther/pollen development	Allen et al. (2007)
Pbr033089.1	AtMYB97		
	AtMYB120		
	AtMYB65		
	AtMYB33		
	AtMYB104		
	AtMYB8		
	AtMYB81		
Pbr017966.1	AtMYB24	Anther development	Mandaokar and Browse (2008)
Pbr027035.1	AtMYB21		
Pbr024420.1	AtMYB57		
Pbr028812.1	AtMYB62	Stress responses	Devaiah et al. (2009)
Pbr013315.1	AtMYB116		
Pbr038922.1			
Pbr020295.1			
Pbr001709.1	AtMYB78	Stress responses	Devaiah et al. (2009)
Pbr008630.1	AtMYB108		
Pbr014994.1	AtMYB112		

(continued)

Table 9.2 (continued)

Pyrus × *bretschneideri*	Corresponding counterparts in *Arabidopsis thaliana*	Function(s)	Reference(s)
	AtMYB2		
Pbr028319.1	AtMYB52	Lignin, xylan, and cellulose biosynthesis	Lee et al. (2009)
Pbr010042.1	AtMYB54		
Pbr039365.1	AtMYB56		
Pbr016851.1	AtMYB69		
Pbr002006.1	AtMYB117		
Pbr035515.1	AtMYB105		
Pbr039075.1	AtMYB89		
	AtMYB110		
Pbr012310.1	AtMYB44	Abiotic stresses	Jung et al. (2007)
Pbr008748.1	AtMYB77		
Pbr025199.1	AtMYB70		
Pbr015309.1	AtMYB73		
Pbr022028.1			
Pbr019687.1			
Pbr035927.1	AtMYB1	Abiotic stresses	Sun et al. (2014)
Pbr041921.1	AtMYB25		
	AtMYB109		
Pbr028904.1	AtMYB53	Root development	Gibbs et al. (2014)
Pbr001638.1	AtMYB92		
	AtMYB93		
Pbr001932.1	AtMYB98	Embryogenesis	Wang et al. (2009)
Pbr017972.1	AtMYB64		
Pbr027028.1	AtMYB119		
Pbr042296.1	AtMYB118		
Pbr006264.1	AtMYB115		
Pbr039284.1	AtMYB22		
	AtMYB100		

soybean, maize, sorghum, and sugarcane. For example, MYB/MYC regulons, such as AtMYC2 and AtMYB2 proteins, respond to drought stress through abscisic acid (ABA)-dependent signaling systems (Abe et al. 2003). Moreover, *AtMYB102* is found to assimilate signaling pathways following wounding and osmotic stress signals in *Arabidopsis* (Denekamp and Smeekens 2003). Lippold et al. (2009) have reported that *AtMYB41* regulates short-term transcriptional responses to water stress. In another study, wheat MYB TF genes including *TaMYB30-B*, *TaPIMP1*, and *TaMYB3R1* have been reported to regulate expression of drought stress-responsive genes (Zhang et al. 2012). Using microarray analysis, plants subjected to water deficit have revealed up-regulation of several defenses and stress-related genes, *TLP4*, *RD22*, and *PR1a*, by the *TaPIMP1* MYB transcription factor, and expression level of this

transcription factor is found to be positively associated with drought tolerance (Zhang et al. 2012). Subsequently, it is reported that enhanced expression of dehydration-responsive genes is observed in both ABA-dependent (*ABF3*, *RD29A*, and *RD29B*) and ABA-independent (*ADH*, *CBF4*, and *COR15A*) signaling pathways in transgenic *Arabidopsis* plants overexpressing *TaMYB3R1* TF (Cai et al. 2015). Apparently, MYB proteins, such as AtMYB61, AtMYB60, AtMYB96, and AtMYB44, control stomatal aperture regulation in *Arabidopsis* under water deficit conditions (Jung et al. 2007; Liang et al. 2005).

Salinity impacts plants in numerous ways by causing metabolic imbalance, ion toxicity, membrane disorganization, osmotic stress, and cellular dehydration, thus, in turn resulting in inhibition of plant growth and development. Plants maintain salt tolerance through induction of ABA and salt over sensitive (SOS) signaling pathways. SOS is also an important signaling and regulatory pathway activated in response to salt stress in an ABA-independent manner. A negative regulator of SOS induction in *Arabidopsis*, AtMYB73, has been identified, and it is specifically activated under salt stress, thereby enhancing tolerance to high salt by modulating expression of *SOS1* and *SOS3* genes (Lee et al. 2014). Similarly, *OsMYB91* from rice has been reported to increase salinity tolerance (Zhu et al. 2015).

Cold acclimation is an important process through which plants increase their tolerance against low temperature with the help of several transcription factors. In this regard, MYB transcription factors have played significant roles. For example, *MYB3* and *MYB61* TFs have enhanced cold tolerance in *Medicago truncatula* by positively regulating expression of the cold acclimation TF gene *MtCAS15* (Zhang et al. 2016). In another study, a single R2R3-MYB encoding gene, *FtMYB12*, from Tartary buckwheat has been identified to mediate *COR15A* gene expression in order to improve cold tolerance (Zhou et al. 2015). Recently, it has been reported that miR159-targeted SlGAMYB genes are essential for fruit ovule development, thus suggesting a dynamic regulation of the miR159/GAMYB module during early stages of fruit development. Specifically, SlGAMYB1/2 silencing in SlMIR159 overexpressing plants results in misregulation of pathways related to ovule and female gametophyte development, leading to earlier fruit initiation and parthenocarpy (Silva et al. 2017). Orthologous genes commonly share similar functions and are clustered within the same clades and subclades; whereas, paralogous genes have generally different functional roles. This suggests that closely related MYB transcription factors can recognize similar target genes and possess functional redundancy (Ogata et al. 1999). Therefore, it is critical that functional analysis studies, via genetic transformation, should be conducted to further delineate the functionality of *PbMYBs* genes that have been thus far identified in pear.

9.2.2 Heat Shock TF (HSFs)

Heat shock TFs (HSFs) play a central role in controlling expression of heat responsive genes by mediating rapid accumulation of heat shock proteins in response to heat stress and to other chemical stressors (Mehta et al. 2010; von Koskull-Döring et al. 2007). Thus far, a total of 29 *HSFs* genes have been identified in Chinese pear (*P.* × *bretschneideri*) (Qiao et al. 2015). Plant *HSFs* gene families contain various numbers of genes, ranging between 20 and 52 members, higher than in any given species (Pirkkala et al. 2001). HSFs are not only involved in protection against stress damage, but they also play roles in degradation of proteins, including intracellular distribution and folding (Wang et al. 2004). Furthermore, HSFs are also involved in plant growth and development, as well as in responses to other abiotic stresses such as drought, cold, and salt (Shim et al. 2009). For example, *HsfA9* is involved in seed maturation and embryogenesis in both *Arabidopsis* and sunflower (Kotak et al. 2007), as well as in tomato (*Solanum lycopersicum*), while *HsfA1a* plays a central role in the regulation of heat stress response in tomato (Mishra et al. 2002), and

HsfA4a acts in controlling tolerance to cadmium in rice (*Oryza sativa*) (Shim et al. 2009).

HSFs have a modular structure with an N-terminal DNA-binding domain (DBD) and an oligomerization domain (OD). The N-terminal DNA-binding domain (DBD) is connected to oligomerization (or HR-A/B region) by a flexible linker of adaptable length (15–80 amino acid residues). However, some HSFs also possess a well-defined domain consisting of a nuclear localization signal (NLS) domain necessary for a nuclear export signal (NES) domain and an activator motif (AHA motif). In general, plant HSFs can be divided into three classes, including A, B, and C, based on structural characteristics of the HR-A/B domain and their phylogenetic analysis. HSF encoding genes belonging to class A and C have an HR-A/B region with insertions of either 21 (class A) or 7 (class C) amino acid residues present within the A and B segments; whereas, HSF encoding genes belonging to class B have no insertions, and are comparatively compact.

Plants have more than 20 *HSF* genes encoding heat shock proteins (HSPs), more than other eukaryotes that contain only one to three such genes. For example, *Arabidopsis* contains 21 *HSFs* genes (15/A, 5/B, and 1/C), while vertebrates contain four *HSF* genes, and *Drosophila* contains only a single *HSF* gene (Guo et al. 2008b; Scharf et al. 2012). HSF encoding genes have been widely studied in the model plant *Arabidopsis*, as well as in other plants, such as maize (*Z. mays*), rice (*O. sativa*), apple (*Malus* × *domestica*), and poplar (*Populus trichocarpa*), among others (Table 9.3). Following complete sequencing of the genome of the Chinese pear (*P.* × *bretschneideri*), this has allowed for an opportunity to conduct an extensive study of HSF encoding genes in pear. It is found that the following HSF encoding genes in pear, including *PbHsfA6a*, *PbHsfA4b*, *PbHsfA3a*, and *PbHsfA4d*, are upregulated under high temperature conditions, thus suggesting that these genes play critical roles in response to heat stress (Qiao et al. 2015). However, it is important to add that unexpectedly some *PbHsf* genes are down-regulated under high temperatures, thus suggesting that these genes may be involved in some other signal transduction pathways in the complex regulatory network of plant stress (Qiao et al. 2015).

9.2.3 WRKY TFs

The WRKY family is among the largest group of plant transcription factors and comprises of 103 members in the pear genome (Huang et al. 2015; Rushton et al. 2010). The WRKY protein family consists of either one or two conserved WRKY domains containing a 60 amino acid sequence, comprising a short peptide, WRKYGQK, and followed by either a C_2H_2 or a C_2HC zinc finger motif structure. These two motifs are essential for binding to the consensus *cis*-acting element, termed the W-box (TTGACT/C). The WRKY family can be classified based on the number of WRKY domains and the feature of the zinc finger motif. Furthermore, WRKY proteins can be divided into three subfamily types. Type I proteins (the WRKY I subgroup) possess two WRKY domains, while type II proteins contain a WRKY domain and a C_2H_2 zinc finger motif, and type III WRKY proteins have a WRKY domain (WRKYGQK) and a C_2HC zinc finger motif.

Since cloning of the first *WRKY* gene, *SPF1* from sweet potato (*Ipomoea batatas*), a large number of *WRKY* genes have been experimentally identified in various plant species, such as sweet kumquat (*Fortunella crassifolia*), rice (*O. sativa*), soybean (*G. max*), poplar (*P. trichocarpa*), and *Arabidopsis* (*A. thaliana*). Additionally, large-scale systematic analyses of the *WRKY* gene family have been undertaken for *A. thaliana*, *O. sativa*, *P. trichocarpa*, *P.* × *bretschneideri*, and *Cucumis sativus* as WRKY TFs are important participants in plant signaling networks for various biotic stress responses and abiotic stress responses (Chen et al. 2012). WRKY TFs are involved in several developmental and physiological processes such as embryogenesis, seed coat development, trichome development, anthocyanin biosynthesis, and hormone signaling. Transgenic *Arabidopsis*

Table 9.3 Classification of *Hsf* transcription factors along with numbers of gene families in six Rosaceae species, including *Pyrus* × *bretschnederi* (Chinese white pear), *Malus* × *domestica* (apple), *Prunus persica* (peach), *Fragaria vesca* (strawberry), *Prunus mume* (Chinese plum), and *Pyrus communis* (European pear) (Qiao et al. 2015)

HSFs	Chinese pear (29)	Apple (25)	Peach (17)	Strawberry (16)	Chinese plum (17)	European pear (33)
HsfA1a	Pbr025227.1	MdP0000517644	Ppa004782m	gene13904	Pm023178	PcP005520.1
b	Pbr041026.1	MdP0000156337	Ppa004559m	gene10474	Pm011227	PcP027354.1
c	Pbr031411.1	MdP0000232623				PCP027124.1
d		MdP0000259645				PcP011761.1
HsfA2a	Pbr019856.1	MdP0000489886	Ppa007300m	gene02705	Pm005519	PcP044449.1
b		MdP0000243895				PcP016141.1
c						PcP034937.1
HsfA3a	Pbr005496.1	MdP0000131346	Ppa015602m	gene30146	Pm026236	PcP016675.1
b	Pbr016805.1	MdP0000606400				PcP026047.1
c		MdP0000174161				
HsfA4a	Pbr000538.1	MdP0000155849	Ppa006534m	gene23802	Pm010169	PcP025026.1
b	Pbr016090.1		Ppa015468m	gene15872	Pm013905	PcP026169.1
c	Pbr022463.1					PcP024177.1
d	Pbr005379.1					PcP015400.1
HsfA5a	Pbr016487.1	MdP0000301101		gene06570	Pm007815	PcP002437.1
b		MdP0000613011				
HsfA6a	Pbr036788.1		Ppa1027143m	gene29004	Pm009237	PcP030606.1
b	Pbr014670.1					PCP018714.1
c	Pbr018847.1					
HsfA7a	Pbr009953.1		Ppa010224m	gene20347	Pm020253	PcP019575.1
b	Pbr012908.1					PcP022776.1
HsfA8a	Pbr012136.1	MdP0000191541	Ppa006514m		Pm005887	PcP006787.1
b		MdP0000172376				PcP031284.1
HsfA9a	Pbr041474.1	MdP0000194672	Ppa016533m	gene12667	Pm027197	PcP005035.1
b	Pbr015630.1	MdP0000319456				PcP027517.1
HsfB1a	Pbr025141.1	MdP0000527802	Ppa009274m	gene24036	Pm026366	PcP024136.1
b	Pbr030422.1	MdP0000578396				PcP030007.1
HsfB2a	Pbr013953.1	MdP0000155667	Ppa009180m	gene13301	Pm019357	PcP030684.1
b			Ppa008441m	gene32416	Pm023788	PcP033244.1
c						PcP007662.1
HsfB3a	Pbr002020.1	MdP0000622590	Ppa014675m	gene02464		PcP029678.1
b	Pbr030436.1	MdP0000202716				PcP024839.1
c	Pbr002038.1					
HsfB4a	Pbr019653.1	MdP0000209135	Ppa026635m		Pm005297	
b		MdP0000129357				
HsfB5a	Pbr016270.1		Ppa011804m	gene02408	Pm010031	PcP044895.1
b						PcP016888.1
HsfC1a	Pbr014107.1	MdP0000230456	Ppa008830m	gene30881	Pm027421	PcP000545.1
b	Pbr016948.1	MdP0000320827		gene		PcP022060.1

plants carrying AtWRKY52/RRS1, a type III member containing WRKY and TIR-NBS-LRR (TNL), have exhibited resistance to the bacterial pathogen *Ralstonia solanacearum* through nuclear interaction with the type III bacterial effector PopP2 (Deslandes et al. 2003). The AtWRKY52 TF also interacts with the RPS4 protein for dual resistance against both fungal and bacterial pathogens. While, WRKY proteins belonging to type II contain a calmodulin (CaM)-binding domain, the C-motif (DxxVxKFKxVISLxxxR), thus suggesting possible regulation by CaM and Ca2+ fluxes (Park et al. 2005). At this time, pear *WRKY* genes, showing extensive autoregulation and cross-regulation, are yet to be investigated for their functional roles, yet it appears that these TFs facilitate transcriptional reprogramming in a dynamic web with built-in redundancy.

9.2.4 SQUAMOSA Promoter Binding Protein (SBP)-Box Genes

The SQUAMOSA promoter binding protein (SBP)-box gene family is a group of plant-specific TFs that play significant roles in many biological processes, such as microsporogenesis, megasporogenesis, trichome development on sepals, ripening of fruit, stamen filament elongation, and homeostasis. SBP-box family genes are present in all photosynthetic organisms, from green algae to multicellular trees, except for animals, prokaryotes, and fungi. The SBP domain is comprised of ~79 amino acids along with 10 conserved cysteine and histidine residues that are interrelated in nuclear localization and DNA-binding (Cardon et al. 1999; Zhang et al. 2015). SBP-box encoding genes cover two zinc-binding sites (Cys-Cys-Cys-His and Cys-Cys-His-Cys), in which most have a three-stranded antiparallel beta-sheet (Yamasaki et al. 2004; Pan et al. 2017; Abdullah et al. 2018b).

Based on specific interactions with a promoter sequence of the SQUAMOSA identity gene, *AmSBP1* and *AmSBP2* are the first reported genes identified in *Antirrhinum majus* (snapdragon) (Klein et al. 1996; Cardon et al. 1999; Pan et al. 2017). Cardon et al. (1997) have identified the first SBP-box gene, *SPL3*, in *Arabidopsis*, and have observed its potential role in regulating flowering under a duration of a long photoperiod. Subsequently, the *SBP* gene family has been identified and thoroughly investigated in many model plants including *Betula pendula* (silver birch) (Lännenpää et al. 2004), *Gossypium hirsutum* (cotton) (Zhang et al. 2014), *Oryza sativa* (Xie 2006), green algae (Kropat et al. 2005), *Solanum lycopersicum* (Salinas et al. 2012), *Cucumis melon* (Ma et al. 2015), and the moss *Physcomitrella patens* (Riese et al. 2007). In *Arabidopsis*, 16 *SBP-box* genes have been identified, and their critical roles have been observed, and investigated in leaf development (Guo et al. 2008a; Usami et al. 2009), leaf primordia formation (Wang et al. 2008), early flowering (Gandikota et al. 2007), gibberellic acid (GA) responses (Zhang et al. 2007), copper homeostasis (Yamasaki et al. 2009), along with nutritional changes and reproductive stage development (Jung et al. 2011). Furthermore, *SPL8* mutants of *Arabidopsis* exhibit differences in pollen sac development, contributing to reduced fertility, and regulating differential patterning of gynoecium development. Furthermore, overexpression of *SPL8* alters plant fertility through crosstalk signaling of gibberellic acid (GA) (Zhang et al. 2007). Likewise, *AtSPL11*, *AtSPL10*, and *AtSPL2* contribute to morphological changes in addition to reproductive phase and shoot maturation (Shikata et al. 2009). In rice, overexpression of *OsSPL14* regulates the reproductive stage of plant development, contributing to significant increases in grain yield and in panicle branching (Miura et al. 2010). Furthermore, *SBP-box* genes play potential roles in the modification of plant architecture and yield traits through the initiation of lateral primordia. In particular, *SBP-box* genes take on an interphase role between phase change and homeostasis. This role of the *SBP-box* gene family should be investigated further in diverse plant systems. As of to date, studies are underway to link *SBP-box* genes with identified flowering pathways. These studies will investigate whether or not homeostatic responses and transitions in

plant growth are common in different plant systems, as well as determine whether or not *SBP-box* gene expression resembles the two sides of a coin. A genome-wide investigation has been conducted in our laboratory wherein 32 *SBP* genes have been identified and isolated from *P.* × *bretschneideri* (Abdullah et al. 2018b). Based on phylogenetic analysis, PbSBP proteins have been classified into seven groups (Abdullah et al. 2018b). This latter study on SBP-box genes in pear will provide additional and detailed information on the role of these genes in fruit crops.

9.2.5 GROWTH-REGULATING FACTOR (GRF) TFs

The growth-regulating factor (GRF) family of plant-specific transcription factors (TFs) serves as positive regulators of growth and development in flowering plants. Although *GRFs* have been primarily studied in leaf tissues, *GRFs* play roles in both vegetative and reproductive shoot apical meristems (SAMs), as well as during various phases of reproductive growth in flowering plants. For example, in rice (*O. sativa*), OsGRFs regulate stem growth induced by the phytohormone gibberellic acid (GA) (van der Knaap et al. 2000).

GRF gene families have been investigated and identified in various plant species, including *A. thaliana*, *O. sativa*, *Z. mays*, *P.* × *bretschneideri*, *Brachypodium distachyon*, and *Brassica rapa* (Cao et al. 2016a). It has been reported that deduced protein products of *GRF* genes contain two conserved domains in the N-terminal regions, the QLC and WRC domains (Cao et al. 2016a). Furthermore, SW12/SNF2 proteins containing the QLC domain, a protein–protein interaction domain that regulates interaction of these proteins with homologs of SNF11 from *Saccharomyces cerevisiae*, form a complex that is involved in chromatin remodeling; whereas, the QLQ domain of GRFs facilitates interaction with GRF-interacting factors (GIFs) (Choi et al. 2004). Moreover, GRF and GIF (GRF-interacting factors) transcriptional complexes have biological roles in the development of gynoecia and anthers (Lee et al. 2018). Furthermore, the GRF-GIF complex is also critical for meristematicity (meristematic competence) and pluripotency of carpel margin meristems (CMMs) and for archesporial differentiation (Lee et al. 2018). The WRC domain plays a functional role in transcriptional control and DNA-binding, and it contains two distinctive features, a nuclear localization signal (NLS) and a zinc finger motif composed of three Cys and one His residues (C3H motif) (Sauer et al. 2004). A barley transcriptional repressor (HRT) has a C3H motif, and it is proposed to bind to a GA response element (GARE), while the WRC domain is likely to act as a DNA-binding domain (Noguero et al. 2013).

Most *GRFs* are strongly expressed in actively growing and developing tissues, such as flower buds, shoot tips, and growing leaves, as revealed by quantitative RT-PCR analysis and RNA gel-blots (Kim et al. 2003). Additionally, *GRFs* are more highly expressed in reproductive organs than in vegetative organs. It has been reported that the rice OsGRF10 and OsGRF3 interact and repress the promoters of *KNOTTED1*-like homeobox (*KNOX)* family genes, which control meristem development, thereby regulating meristem development and restricting cell differentiation in apical meristems of shoots (Ma et al. 2017). Kim et al. (2003) have also reported that overexpressing *GRF* genes in transgenic *Arabidopsis* plants result in larger leaves than those of wild-type plants; whereas, an *Atgrf1/2/3* triple mutant has smaller leaves than wild-type *Arabidopsis*. Moreover, overexpression of *AtGRF5* results in early leaf development, delay of the cell proliferation phase, extensive division of chloroplasts, along with a simultaneous suspension in the onset of the cell expansion phase (Ma et al. 2017).

Various regulatory networks have been involved in establishment and maintenance of meristems and in promoting cell proliferation of developing organ primordia, including the *GRF* family of transcription factors (TFs). *GRFs* may also function in defense signaling and in stress responses; for example, overexpression of *DREB2A* increases plant tolerance to heat stress, osmotic stress, and other abiotic stresses, but it

also results in growth retardation and reduced reproduction (Jin et al. 2014).

9.2.6 Zinc Finger Homeodomain TFs

Zinc finger homeodomain (ZHD) transcription factors are major regulators of specification of higher plants, and they are especially involved in plant development (fiber development) and stress responses (Wang et al. 2015; Khatun et al. 2017). Early on, homeobox genes have been first identified in the fruit fly, but subsequently, these genes have been found and isolated in several organisms, including fungi, plants, nematodes, and humans (Bhattacharjee et al. 2015). As mentioned above, TFs can activate/repress target genes by direct binding to gene motifs or elements, and many TF families have evolved through unique DNA-binding domains that advise their binding activity. One of the well-characterized domains is the homeodomain (HD) which is encoded by 60 conserved amino acids (Wang et al. 2014; Mukherjee et al. 2009).

In plant and animal genomes, homeobox genes are characterized by large gene families, and based on the number, nature, and spacing pattern, they can be also categorized into different groups. Initially, the zinc finger has been classified into the following groups, KNOX, ZM-HOX, BELL, AT-HB8, HAT, and GAL2 (Bharathan et al. 1997; Bhattacharjee et al. 2015). Subsequently, homeobox genes of rice have been classified into 10 subclasses, including HD-Zip I, HD-Zip II, HD-Zip III, HD-Zip IV, KNOX I, KNOX II, BLH, WOX, PHD, and ZF-HD (Bhattacharjee et al. 2015). In a comprehensive study, homeobox genes have been grouped into 14 subclasses, and by adding some new classes, such as DDT, NDX, PHD, SAWADEE, LD, and PINTOX (Mukherjee et al. 2009).

In pear, the HD-Zip has been extensively studied, and the zinc finger is categorized into 14 subgroups (Wang et al. 2015). Genome-wide analysis has identified 52 genes encoding HD-Zip TFs within the pear genome (Wang et al. 2015). It is important to point out that the zinc finger (C_2H_2, C_2C_2, and C_3H) interacts with a single zinc ion, but with new approaches, it has been found that the plant RING finger and the animal Lin-11/Is1-1/Mec-3 (LIM) domain interact with two zinc ions (Yanagisawa 2004; Wang et al. 2014).

A cluster of novel zinc finger homeodomain (ZHD) proteins have been first isolated from *Flaveria* as potential regulators of the gene encoding C4 phosphoenolpyruvate carboxylase (PEPCase), wherein the ZHD domain is capable of binding to DNA, predominantly to the regulatory region of the C_4 PEPCase encoding gene (Windhovel et al. 2001). Furthermore, it is reported that the zinc finger domain is not involved in DNA-binding, although it can boost the protein–DNA interaction facilitated by the HD domain (Windhovel et al. 2001). ZHD proteins have been identified and characterized in various plants, such as *A. thaliana* (Tan and Irish 2006), *G. max* (Deng et al. 2002), *O. sativa* (Jørgensen et al. 1999), and *Triticum aestivum* (Bhattacharjee and Jain 2013).

Expression patterns of HD-Zip genes identified in pear have suggested that these genes are widely involved in salt stress, drought stress, and pathogen infection (Wang et al. 2015). Under conditions of drought stress, expression levels of 15 *PbHB* genes are found to be upregulated, while five other *PbHB* genes are down-regulated (Wang et al. 2015). Specifically, it is reported that *PbHB2* is detected only at 6 h following PEG6000 treatment, while *PbHB1* and *PbHB20* are activated at 12 h, and *PbHB25* and *PbHB4* are upregulated only at 24 h (Wang et al. 2015).

Overall, several members of the ZHD class of proteins are critical components in regulating blue light signaling, vascular development, outer cell layer of a plant organ, response to stress, and control of anthocyanin processing (Khatun et al. 2017). Early on, it has been reported that the gene encoding for the ZHD protein is involved in the regulation of floral development, but subsequently it is found that the *Arabidopsis* protein encoding gene, *AtZHD1*, binds to the promoter

of *EARLY RESPONSE TO DEHYDRATION STRESS 1* (*ERD1*) (Tran et al. 2007). Interestingly, the expression pattern of *AtZHD1* is inducible by salt stress, abscisic acid, and dehydration (Tran et al. 2007; Wang et al. 2014). In addition, as the ZHD protein can interact with some NAC proteins, it has been found that simultaneous overexpression of ZHD and NAC genes contributes to increased drought tolerance in *Arabidopsis* (Tran et al. 2007; Hu et al. 2008). Thus far, 14 *ZHD* genes have been identified in *Arabidopsis*, and their functions have been characterized (Tan and Irish 2006). Recently, ZHD coding genes have been identified in other plant systems, and their functions have been elucidated. For example, four rice *ZHD* genes have been associated with gene regulation, while two soybean proteins, GmZHD1 and GmZHD2, have been identified to bind to the promoter of the gene encoding for calmodulin isoform 4 (GmCaM4), thereby increasing expression levels of these proteins following pathogen induction (Park et al. 2007; Hu et al. 2008; Wang et al. 2014). Furthermore, Hu et al. (2008) have reported that the mini zinc finger (MIF) gene family, possessing the zinc finger, interacts with ZHD, and that their overexpression interferes with the normal functions of ZHD proteins. If this is indeed the case, then ZHD proteins may play important roles in regulating plant physiology and development.

9.2.7 MADS-Box TFs

MADS-domain transcription factors play important roles in both development and evolutionary diversity, such as fruit development, floral organ conformation, and flowering time. MADS-box transcription factors are widespread in animals, plants, and fungi, as they initiate target gene transcription by binding to the CArG-box domain in the *cis*-acting element of the target gene (Riechmann et al. 1996). Based on phylogenetic analysis, *MADS-box* genes have been classified into two large groups, type I (SRF) and type II (MEF2). Type I is divided into Mα, Mβ, and Mr, while type II is divided into $MIKC^C$ type and MIKC* type, and furthermore, $MIKC^C$ can be classified into 12 subfamilies (Becker and Theißen 2003).

In the Chinese pear (*P.* × *bretschneideri*) genome, a total of 95 *MADS-box* genes have been identified and categorized (Wang et al. 2017). Pear type I *MADS-box* genes have been classified into three subfamilies, and type II *MADS-box* genes are divided into 14 subfamilies (Wang et al. 2017). Remarkably, except for a highly conservative MADS (M) domain possessing about 60 amino acid sequences of the N-terminal regions, type II genes also contain an Intervening (I), a C-terminal (C), and a Keratin (K) domain (Kaufmann et al. 2005). Compared with type II, type I genes are relatively simple and lack the K domain, wherein a coding gene usually contains 1–2 exons (Parenicova 2003). It has been reported that $MIKC^C$-group genes are likely to be involved in developmental processes of flowering plants. For example, *Arabidopsis* flowering time genes, including *AGAMOUS-LIKE24* (*AGL24*) and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*), are involved in transition of vegetative to reproductive stages of plant development (Ferrario et al. 2004; Khan et al. 2014). As *MADS-box* genes play critical roles in development and in signal transduction of various organs, such as development and maturation of fruits (Ma and dePamphilis 2000), it has been postulated earlier that the characteristics of plant floral organ development can be explained by the ABC model (Weigel and Meyerowitz 1994).

Based on the ABC model for floral organ development, class A genes specifically regulate occurrence and development of the calyx, while classes A and B genes together control formation of petals, and classes B and C genes together determine the occurrence of stamens, while class C genes regulate the development of carpels. Based on subsequent reverse genetics studies, it has been demonstrated that classes D and E genes also play vital roles in regulating flower morphogenesis. Among these, class D genes mainly regulate the development of ovules (Colombo et al. 1995), while class E genes are mainly involved in regulating the formation and

development of all floral organs (Pelaz et al. 2000). The *MADS-box* gene family has been extensively studied in angiosperms, particularly in the model plant *A. thaliana* (Ma and dePamphilis 2000). In *Arabidopsis*, class A genes are represented by *APETALA1 (AP1)* and *APETALA2 (AP2)* (Mandel et al. 1992), class B genes include *APETALA3 (AP3)* and *PISTILLATA (PI)* (Goto and Meyerwortiz 1994), class C genes are represented by *AGAMOUS (AG)*, class D genes are represented by *SEEDSTICK (STK/AGL11)*, *SHATTERPROOF1 (SHP1/AGL1)*, and *SHP2 (AGL5)*, and class E genes include *SEPALLATA1,2,3,4 (SEP1/2/3/4 and AGL2/4/9/3)* (Mandel and Yanofsky 1998). Among these genes, besides *AP2*, all class A, B, C, D, and E homologous genes belong to the $MIKC^C$-type MADS-box genes. These studies have demonstrated that type II *MADS-box* genes are mainly related to plant floral organ development. On the contrary, the function of type I *MADS-box* genes has seldom been reported. In limited studies, it has been reported that type I *MADS-box* genes are mainly involved in the development of female gametophytes, endosperms, or seeds (Köhler et al. 2003).

In early studies in pear, it has been shown that MADS-box TFs play a vital role in fruit development and maturation (De Folter et al. 2004). More recently, it has been demonstrated that the *PbMADS12* gene together with *PbMYB10* and *PbbHLH3*, all from *P.* × *bretschneideri*, regulate the anthocyanin pathway through activation of the promoters of *PbUFGT1* and *PbDFR1* (Wang et al. 2017). Furthermore, *PbMADS11* and *PbMADS12* seem to serve as master regulators of anthocyanin biosynthesis in response to temperature and light (Wang et al. 2017). The induction of the productive meristem identity MADS-box gene *AP1* following repression of the pear TERMINAL FLOWER1, *PpTFL1s*, is proposed to play a primary role of the *PpTFL1* in mediating floral induction in *P. pyrifolia* Nakai (Bai et al. 2017).

9.2.8 B-Box TFs

The B-box (BBX) family of plant TFs is a functionally diverse subclass of zinc finger TFs containing an N-terminal B-box domain, either alone or sometimes in combination with a CCT [TIMING OF CAB EXPRESSION 1 (TOC1), CONSTANS (CO), and CO-LIKE (COL)] domain (Gangappa and Botto 2014). The BBX domain is a short protein sharing a 40 amino acid residue in length. B-box proteins can be divided into two types, B-box1 and B-box2, based on their consensus sequence and the spacing of zinc-binding residues (Crocco and Botto 2013). BBX proteins play critical roles in regulatory networks controlling seedling photomorphogenesis, shade avoidance, photoperiodic regulation of flowering, and responses to biotic and abiotic stresses. Conserved residues in CONSTANS B-box motifs are known to be involved in mediating protein-protein interactions (Datta et al. 2008). For example, the CONSTANS B-box directly interacts with proteins containing a coiled-coil domain for the SUPPRESSOR OF PHYA1 (SPA1). The CCT domain, a basic motif of 42–43 amino acids, performs a critical role in nuclear protein transport and in transcriptional regulation of BBX proteins. For example, the CCT domain of CO plays a functional role in mediating gene expression by binding the promoter of the FLOWERING LOCUS T (FT) (Griffiths et al. 2003). BBX proteins sequence alignment has revealed that the CCT domain also contains highly conserved sequences. In *Arabidopsis*, of a total of 32 BBX proteins, 17 (BBX1-17) proteins contain a CCT domain (Griffiths et al. 2003).

The CONSTANS-LIKE 3 (COL3) is a critical protein-binding partner for B-BOX32 (BBX32) activity in *Arabidopsis*. The discovery of the interaction of B-BOX32 with COL3 can be used in combination with BBX32 for increased productivity. This regulatory pathway could be applied as an efficient strategy for genetic

manipulation in crops for increased agricultural productivity (Tripathi et al. 2017). In general, *Arabidopsis* BBX proteins are divided into five subfamilies based on their type and number of BBX motif and the presence/absence of a CCT domain (Gangappa and Botto 2014). Subfamily I (BBX1-6) and subfamily II (BBX7-13) possess two B-boxes and a CCT domain, while subfamily III has a single B-box and a CCT domain. Subfamily IV possesses two tandem repeats of B-box motifs, also referred to as the double B-box (DBB); however, this subfamily lacks a CCT domain. Subfamily V (BBX27-32) contains only a short N-terminal B-box domain with either one or two B-box motifs (Gangappa and Botto 2014).

A genome-wide survey of the B-box gene family has been conducted in pear, *P.* × *bretschneideri*. Of 25 BBX encoded genes, seven contained two B-BOX domains along with a conserved CCT domain, while four and five *PbBBXs* were found to contain a single B-BOX and either a conserved CCT domain or only a single CCT domain, respectively, while the remaining nine *PbBBXs* had two B-BOX domains (Cao et al. 2017). The pear BBX encoded genes showed wide variations in molecular lengths, ranging from 142 to 859 amino acids. Additionally, pear *BBX* genes showed highly similar structures within the same clade. For example, eight *PbBBXs* belonging to clades I and III had two exons, while *PbBBXs* belonging to clade IV had three exons, and *PbBBXs* belonging to clade V contained only a single exon, except for *PbBBX24* and *PbBBX25*. These findings suggested that exon gain or loss occurred during evolution of the pear *PbBBXs* gene family, resulting in functional divergence among closely related *PbBBXs* (Cao et al. 2017). Moreover, 52% of *PbBBXs*, 13 genes, were not expressed during the different development stages of pear pollen development, thereby suggesting that these genes might be expressed in other tissues, such as stems, leaves, or roots, or under special conditions. Of the remaining 48% of *PbBBXs*, 12 genes, that were expressed during development-dependent pattern of pollen development in pear, five genes, including *PbBBX6*, *7*, *9*, *11*, and *12*, were expressed at the P1 stage of pollen development (mature pollen grains), while two genes, *PbBBX8* and *PbBBX10*, were expressed at the P2 stage of pollen development (hydrated pollen grain) in pear (Cao et al. 2017). These findings suggested that *PbBBXs* genes were important for signaling processes during pollen growth in pear. Expression profiles of *PbBBXs* genes in different tissues or organs were confirmed using qRT-PCR revealing that *PbBBX6*, *8*, *9*, *11*, and *19* were not expressed in all tested tissues or organs, while *PbBBX1*, *2*, *3*, *4*, *7*, *10*, *14*, *16*, *18*, *20*, *21*, *22*, and *24* were expressed in leaves, and *PbBBX13* and *17* were highly expressed in roots (Cao et al. 2017).

9.3 Concluding Remarks

In general, TFs are a group of regulatory proteins that control gene expression by binding to DNA, and in doing so, they either activate or repress mRNA transcription. Plant TF gene specificity is less obvious than that for animals. Thus far, there are only a few cases that have been reported on plant TF gene specificity, wherein NAC controls development process and stress response, MADS regulates flowering tissue differentiation, B3 controls auxin responses, and AP2/EREBP control plant hormone responses, including those for ethylene, jasmonic acid, auxin, brassinosteroids, and gibberellin, among others.

Pear (*Pyrus*) TFs are characterized by a large number of genes, and by a diverse group of TF gene families. Furthermore, various cellular responses are regulated by highly divergent TFs, such as bHLH, MYB, homeodomain, and zinc ring finger, among others. For each of the hormones involved in plant and growth development, it is likely that there are specific modes of signal transduction, from the receptor to the TFs involved in these processes. Studies of TFs in the model plant *A. thaliana* have provided important insights on the roles of TFs in a variety of plant-specific cellular responses, such as development process, environmental stresses, such as cold, responses to light, drought, high salinity, and plant defense to pathogen infection. Genetic

and molecular studies of TFs in pear have elucidated the roles of different families of TFs in protein-protein interactions and their combinatorial regulation of gene expression. However, an important question that remains unclear, and deserves attention, as to whether or not plant nonfunctional transcription factor binding is indeed nonfunctional? It is also important to continue to investigate how plants have evolved new structures and modified DNA-binding domains that respond to disease resistance, hormonal, and developmental signal transductions.

References

Abdullah M, Cao Y, Cheng X, Meng D, Chen Y, Shakoor A, Gao J, Cai Y (2018a) The sucrose synthase gene family in Chinese pear (*Pyrus bretschneideri* Rehd.): structure, expression, and evolution. Molecules 23:1–16. https://doi.org/10.3390/molecules23051144

Abdullah M, Cao Y, Cheng X, Shakoor A, Su X, Gao J, Cai Y (2018b) Genome-wide analysis characterization and evolution of SBP genes in *Fragaria vesca*, *Pyrus bretschneideri*, *Prunus persica* and *Prunus mume*. Front Genet 9:1–12. https://doi.org/10.3389/fgene.2018.00064

Abe H, Urao T, Ito T, Seki M, Shinozaki K (2003) Transcriptional activators in abscisic acid signaling. Plant Cell 15:63–78. https://doi.org/10.1105/tpc.006130.salt

Adcock IM, Caramori G (2009) Transcription factors. In: Brazen PJ, Drazen JM, Rennard SI, Thomson NC (eds) Asthma and COPD. Elsevier Ltd., The Netherlands, pp 373–380. https://doi.org/10.1016/b978-0-12-374001-4.00031-6

Agarwal M, Hao Y, Kapoor A, Dong CH, Fujii H, Zheng X, Zhu JK (2006) A R2R3 type MYB transcription factor is involved in the cold regulation of CBF genes and in acquired freezing tolerance. J Biol Chem 281:37636–37645. https://doi.org/10.1074/jbc.M605895200

Allen RS, Li J, Stahle MI, Dubroué A, Gubler F, Millar AA (2007) Genetic analysis reveals functional redundancy and the major target genes of the *Arabidopsis* miR159 family. Proc Natl Acad Sci USA 104:16371–16376. https://doi.org/10.1073/pnas.0707653104

Bai S, Tuan PA, Saito T, Ito A, Ubi BE, Ban Y, Morighuchi T, Wilson Z (2017) Repression of TERMINAL FLOWER1 primarily mediates floral induction in pear (*Pyrus pyrifolia* Nakai) concomitant with change in gene expression of plant hormone-related genes and transcription factors. J Exp Bot 68:4899–4914. https://doi.org/10.1093/jxb/erx296

Barnes PJ (2006) Transcription factors in airway diseases. Lab Investig 86:867–872. https://doi.org/10.1038/labinvest.3700456

Becker A, Theißen G (2003) The major clades of MADS-box genes and their role in the development and evolution of flowering plants. Mol Phylogenet Evol 29:464–489. https://doi.org/10.1016/S1055-7903(03)00207-0

Bharathan G, Janssen B-J, Kellogg EA, Freeling M (1997) Did homeodomain proteins duplicate before the origin of angiosperms, fungi, and metazoa? Evolution 94:13749–13753. https://doi.org/10.1073/pnas.94.25.13749

Bhattacharjee A, Jain M (2013) Homeobox genes as potential candidates for crop improvement under abiotic stress. In: Tuteja N, Singh Gill S (eds) Plant acclimation to environmental stress. Springer, New York, NY, pp 163–176. https://doi.org/10.1007/978-1-4614-5001-6_7

Bhattacharjee A, Ghangal R, Garg R, Jain M (2015) Genome-wide analysis of homeobox gene family in legumes: identification, gene duplication and expression profiling. PLoS One 10:1–22. https://doi.org/10.1371/journal.pone.0119198

Cai H, Tian S, Dong H, Guo C (2015) Pleiotropic effects of TaMYB3R1 on plant development and response to osmotic stress in transgenic *Arabidopsis*. Gene 558:227–234. https://doi.org/10.1016/j.gene.2014.12.066

Cao Y, Han Y, Jin Q, Lin Y, Cai Y (2016a) Comparative genomic analysis of the *GRF* genes in Chinese pear (*Pyrus bretschneideri* Rehd.), poplar (*Populus*), grape (*Vitis vinifera*), *Arabidopsis* and rice (*Oryza sativa*). Front Plant Sci 7:1–14. https://doi.org/10.3389/fpls.2016.01750

Cao Y, Han Y, Li D, Lin Y, Cai Y (2016b) MYB transcription factors in Chinese pear (*Pyrus bretschneideri* Rehd.): genome-wide identification, classification, and expression profiling during fruit development. Front Plant Sci 7:1–14. https://doi.org/10.3389/fpls.2016.00577

Cao Y, Han Y, Meng D, Li D, Jiao C, Jin Q, Lin Y, Cai Y (2017) B-BOX genes: genome-wide identification, evolution and their contribution to pollen growth in pear (*Pyrus bretschneideri* Rehd.). BMC Plant Biol 17:1–12. https://doi.org/10.1186/s12870-017-1105-4

Cardon G, Höhmann S, Klein J, Nettesheim K, Saedler H, Huijser P (1999) Molecular characterisation of the *Arabidopsis* SBP-box genes. Gene 237:91–104. https://doi.org/10.1016/S0378-1119(99)00308-X

Chagné D, Crowhurst RN, Pindo M, Thrimawithana A, Deng C, Ireland H, Fiers M, Dzierzon H, Cestaro A, Fontana P, Bianco L, Lu A, Storey R, Knäbel M, Saeed M, Montanari S, Kim YK, Nicolini D, Larger S, Stefani E, Allan AC, Bowen J, Harvey I, Johnston J,

Malnoy M, Troggio M, Perchepied L, Sawyer G, Wiedow C, Won K, Viola R, Hellens RP, Brewer L, Bus VG, Schaffer RJ, Gardiner SE, Velasco R (2014) The draft genome sequence of European pear (*Pyrus communis* L. 'Bartlett'). PloS One 9:e92644

Chen L, Song Y, Li S, Zhang L, Zou C, Yu D (2012) The role of WRKY transcription factors in plant abiotic stresses. Biochim Biophys Acta 1819:120–128. https://doi.org/10.1016/j.bbagrm.2011.09.002

Chen W, Si GY, Zhao G, Abdullah M, Guo N, Li D-H, Sun X, Cai Y-P, Lin Y, Gao J-S (2018) Genomic comparison of the P-ATPase gene family in four cotton species and their expression patterns in *Gossypium hirsutum*. Molecules 23(5):1092. https://doi.org/10.3390/molecules23051092

Cheng X, Su X, Muhammad A, Li M, Zhang J, Sun Y, Li G, Jin Q, Cai Y, Lin Y (2018) Molecular characterization, evolution, and expression profiling of the *Dirigent* (*DIR*) family genes in Chinese white pear (*Pyrus bretschneideri*). Front Genet 9:1–15. https://doi.org/10.3389/fgene.2018.00136

Choi D, Kim JH, Kende H (2004) Whole genome analysis of the *OsGRF* gene family encoding plant-specific putative transcription activators in rice (*Oryza sativa* L.). Plant Cell Physiol 45:897–904

Colombo L, Franken J, Koetje E, van Went J, Dons HJ, Angenent GC, van Tunen AJ (1995) The petunia MADS box gene *FBP11* determines ovule identity. Plant Cell 7:1859–1868. https://doi.org/10.1105/tpc.7.11.1859

Cominelli E, Sala T, Calvi D, Gusmaroli G, Tonelli C (2008) Over-expression of the Arabidopsis *AtMYB41* gene alters cell expansion and leaf surface permeability. Plant J 53:53–64. https://doi.org/10.1111/j.1365-313X.2007.03310.x

Crocco CD, Botto JF (2013) BBX proteins in green plants: insights into their evolution, structure, feature and functional diversification. Gene 531:44–52. https://doi.org/10.1016/j.gene.2013.08.037

Datta S, Johansson H, Hettiarachchi C, Irigoyen ML, Desai M, Rubio V, Holm M (2008) LZF1/SALT TOLERANCE HOMOLOG3, an Arabidopsis B-box protein involved in light-dependent development and gene expression, undergoes COP1-mediated ubiquitination. Plant Cell 20:2324–2338. https://doi.org/10.1105/tpc.108.061747

Davuluri RV, Sun H, Palaniswamy SK, Matthews N, Molina C, Kurtz M, Grotewold E (2003) AGRIS: Arabidopsis gene regulatory information server, an information resource of Arabidopsis *cis*-regulatory elements and transcription factors. BMC Bioinformatics 4:1–11. https://doi.org/10.1186/1471-2105-4-25

De Folter S, Busscher J, Colombo L, Losa A, Angenent GC (2004) Transcript profiling of transcription factor genes during silique development in *Arabidopsis*. Plant Mol Biol 56:351–366. https://doi.org/10.1007/s11103-004-3473-z

Denekamp M, Smeekens SC (2003) Integration of wounding and osmotic stress signals determines the expression of the *AtMYB102* transcription factor gene. Plant Physiol 132:1415–1423. https://doi.org/10.1104/pp.102.019273

Deng X, Phillips J, Meijer AH, Salamini F, Bartels D (2002) Characterization of five novel dehydration-responsive homeodomain leucine zipper genes from the resurrection plant *Craterostigma plantagineum*. Plant Mol Biol 49:601–610. https://doi.org/10.1023/A:1015501205303

Deslandes L, Olivier J, Peeters N, Feng DX, Khounlotham M, Boucher C, Somssich I, Genin S, Marco Y (2003) Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. Proc Natl Acad Sci USA 100:8024–8029. https://doi.org/10.1073/pnas.1230660100

Devaiah BN, Madhuvanthi R, Karthikeyan AS, Raghothama KG (2009) Phosphate starvation responses and gibberellic acid biosynthesis are regulated by the MYB62 transcription factor in *Arabidopsis*. Mol Plant 2:43–58. https://doi.org/10.1093/mp/ssn081

Dubos C, Stracke R, Grotewold E, Weisshaar B, Martin C, Lepiniec L (2010) MYB transcription factors in *Arabidopsis*. Trends Plant Sci 15:573–581. https://doi.org/10.1016/j.tplants.2010.06.005

Ferrario S, Immink RGH, Angenent GC (2004) Conservation and diversity in flower land. Curr Opin Plant Biol 7:84–91. https://doi.org/10.1016/j.pbi.2003.11.003

Gandikota M, Birkenbihl RP, Höhmann S, Cardon GH, Saedler H, Huijser P (2007) The miRNA156/157 recognition element in the 3′ UTR of the Arabidopsis SBP box gene *SPL3* prevents early flowering by translational inhibition in seedlings. Plant J 49:683–693. https://doi.org/10.1111/j.1365-313X.2006.02983.x

Gangappa SN, Botto JF (2014) The BBX family of plant transcription factors. Trends Plant Sci 19:460–471. https://doi.org/10.1016/j.tplants.2014.01.010

Gibbs DJ, Voß U, Harding SA, Fannon J, Moody LA, Yamada E, Swarup K, Nibau C, Bassel GW, Choudhary A, Lavenus J, Bradshaw SJ, Stekel DJ, Bennett MJ, Coates JC (2014) AtMYB93 is a novel negative regulator of lateral root development in Arabidopsis. New Phytol 203:1194–1207. https://doi.org/10.1111/nph.12879

Gonzalez A, Mendenhall J, Huo Y, Lloyd A (2009) TTG1 complex MYBs, MYB5 and TT2, control outer seed coat differentiation. Dev Biol 325:412–421. https://doi.org/10.1016/j.ydbio.2008.10.005

Goto K, Meyerwortiz EM (1994) Function and regulation of the *Arabidopsis* floral hometic gene *PISTILLATA*. Genes Dev 8(13):1548–1560

Griffiths S, Dunford RP, Coupland G, Laurie D (2003) The evolution of *CONSTANS*-like gene families in barley, rice, and Arabidopsis. Plant Physiol 131:1855–1867. https://doi.org/10.1104/pp.102.016188.localization

Guo A, He K, Liu D, Bai S, Gu X, Wei L, Luo J (2005) DATF: a database of *Arabidopsis* transcription factors.

Bioinformatics 21:2568–2569. https://doi.org/10.1093/bioinformatics/bti334

Guo A-Y, Zhu Q-H, Gu X, Ge S, Yang J, Luo J (2008a) Genome-wide identification and evolutionary analysis of the plant specific SBP-box transcription factor family. Gene 418:1–8. https://doi.org/10.1016/j.gene.2008.03.016

Guo J, Wu J, Ji Q, Wang C, Luo L, Yuan Y, Wang Y, Wang J (2008b) Genome-wide analysis of heat shock transcription factor families in rice and *Arabidopsis*. J Genet Genomics 35:105–118. https://doi.org/10.1016/S1673-8527(08)60016-8

Hou XJ, Li SB, Liu SR, Hu CG, Zhang JZ (2014) Genome-wide classification and evolutionary and expression analyses of citrus MYB transcription factor families in sweet orange. PLoS One 9:15–17. https://doi.org/10.1371/journal.pone.0112375

Hu W, Depamphilis CW, Ma H (2008) Phylogenetic analysis of the plant-specific zinc finger-homeobox and mini zinc finger gene families. J Integr Plant Biol 50:1031–1045. https://doi.org/10.1111/j.1744-7909.2008.00681.x

Huang X, Li K, Xu X, Yao Z, Jin C, Zhang S (2015) Genome-wide analysis of WRKY transcription factors in white pear (*Pyrus bretschneideri*) reveals evolution and patterns under drought stress. BMC Genom 16:1–14. https://doi.org/10.1186/s12864-015-2233-6

Iida K, Seki M, Sakurai T, Satou M, Akiyama K, Toyoda T, Konogaya A, Shinozaki K (2005) RARTF: database and tools for complete sets of *Arabidopsis* transcription factors. DNA Res 12:247–256. https://doi.org/10.1093/dnares/dsi011

Ito M, Araki S, Matsunaga S (2001) G2/M-phase-specific transcription during the plant cell cycle is mediated by c-Myb-like transcription factors. Plant Cell 13:1891–1905

Jakoby MJ, Falkenhan D, Mader MT, Brininstool G, Wischnitzki E, Platz N, Hudson A, Hülskamp M, Larkin J, Schnittger A (2008) Transcriptional profiling of mature Arabidopsis trichomes reveals that NOECK encodes the MIXTA-like transcriptional regulator MYB106. Plant Physiol 148:1583–1602. https://doi.org/10.1104/pp.108.126979

Jiang C, Gu J, Chopra S, Gu X, Peterson T (2004) Ordered origin of the typical two- and three-repeat *Myb* genes. Gene 326:13–22. https://doi.org/10.1016/j.gene.2003.09.049

Jin J, Zhang H, Kong L, Gao G, Luo J (2014) PlantTFDB 3.0: a portal for the functional and evolutionary study of plant transcription factors. Nucl Acids Res 42:1182–1187. https://doi.org/10.1093/nar/gkt1016

Jørgensen JE, Grønlund M, Pallisgaard N, Larsen K, Marcker KA, Jensen EO (1999) A new class of plant homeobox genes is expressed in specific regions of determinate symbiotic root nodules. Plant Mol Biol 40:65–77

Jung C, Seo JS, Han SW, Koo YJ, Kim CH, Song SI, Nahm BH, Choi YD, Cheong JJ (2007) Overexpression of *AtMYB44* enhances stomatal closure to confer abiotic stress tolerance in transgenic *Arabidopsis*. Plant Physiol 146:623–635. https://doi.org/10.1104/pp.107.110981

Jung JH, Seo PJ, Kang SK, Park CM (2011) miR172 signals are incorporated into the miR156 signaling pathway at the *SPL3/4/5* genes in *Arabidopsis* developmental transitions. Plant Mol Biol 76:35–45. https://doi.org/10.1007/s11103-011-9759-z

Kaufmann K, Melzer R, Theißen G (2005) MIKC-type MADS-domain proteins: structural modularity, protein interactions and network evolution in land plants. Gene 347:183–198. https://doi.org/10.1016/j.gene.2004.12.014

Khan MRG, Ai XY, Zhang JZ (2014) Genetic regulation of flowering time in annual and perennial plants. Wiley Interdiscip Rev RNA 5:347–359. https://doi.org/10.1002/wrna.1215

Khatun K, Nath UK, Robin AHK, Park J-I, Lee D-J, Kim M-B, Kim CK, Lim KB, Nou IS, Chung MY (2017) Genome-wide analysis and expression profiling of zinc finger homeodomain (ZHD) family genes reveal likely roles in organ development and stress responses in tomato. BMC Genomics 18:695. https://doi.org/10.1186/s12864-017-4082-y

Kim JH, Choi D, Kende H (2003) The AtGRF family of putative transcription factors is involved in leaf and cotyledon growth in *Arabidopsis*. Plant J 36:94–104. https://doi.org/10.1046/j.1365-313X.2003.01862.x

Klein J, Saedler H, Huijser P (1996) A new family of DNA binding proteins includes putative transcriptional regulators of the *Antirrhinum majus* floral meristem identity gene SQUAMOSA. Mol Gen Genet 250:7–16. https://doi.org/10.1007/s004380050046

Köhler C, Hennig L, Spillane C, Pien S, Gruissem W, Grossniklaus U (2003) The polycomb-group protein MEDEA regulates seed development by controlling expression of the MADS-box gene *PHERES1*. Genes Dev 17:1540–1553. https://doi.org/10.1101/gad.257403

Kotak S, Vierling E, Baumlein H, Koskull-Doring PV (2007) A novel transcriptional cascade regulating expression of heat stress proteins during seed development of *Arabidopsis*. Plant Cell 19:182–195. https://doi.org/10.1105/tpc.106.048165

Kropat J, Tottey S, Birkenbihl RP, Depege N, Huijser P, Merchant S (2005) A regulator of nutritional copper signaling in *Chlamydomonas* is an SBP domain protein that recognizes the GTAC core of copper response element. Proc Natl Acad Sci USA 102:18730–18735. https://doi.org/10.1073/pnas.0507693102

Lännenpää M, Jänönen I, Hölttä-Vuori M, Gardemeister M, Porali I, Sopanen T (2004) A new SBP-box gene *BpSPL1* in silver birch (*Betula pendula*). Physiol Plant 120:491–500. https://doi.org/10.1111/j.0031-9317.2004.00254.x

Lee D-K, Geisler M, Springer PS (2009) *LATERAL ORGAN FUSION1* and *LATERAL ORGAN FUSION2* function in lateral organ separation and axillary meristem formation in *Arabidopsis*. Development 136:2423–2432. https://doi.org/10.1242/dev.031971

Lee SB, Kim H, Kim RJ, Suh MC (2014) Overexpression of Arabidopsis MYB96 confers drought resistance in *Camelina sativa* via cuticular wax accumulation. Plant Cell Rep 33:1535–1546. https://doi.org/10.1007/s00299-014-1636-1

Lee S-J, Lee BH, Jung J-H, Park SK, Song JT, Kim JH (2018) GROWTH-REGULATING FACTOR and GRF-INTERACTING FACTOR specify meristematic cells of gynoecia and anthers. Plant Physiol 176 (1):717–729. https://doi.org/10.1104/pp.17.00960

Li L, Yu X, Thompson A, Guo M, Yoshida S, Asami T, Chory J, Yin Y (2009) Arabidopsis MYB30 is a direct target of BES1 and cooperates with BES1 to regulate brassinosteroid-induced gene expression. Plant J 58:275–286. https://doi.org/10.1111/j.1365-313X.2008.03778.x

Li L, Ban ZJ, Li XH, Wu MY, Wang AL, Jiang YQ, Jiang YH (2012) Differential expression of anthocyanin biosynthetic genes and transcription factor *PcMYB10* in pears (*Pyrus communis* L.). PLoS One 7 (9):e46070. https://doi.org/10.1371/journal.pone.0046070

Li X, Xue C, Li J, Qiao X, Li L, Yu L, Huang Y, Wu J (2016) Genome-wide identification, evolution and functional divergence of MYB transcription factors in Chinese white pear (*Pyrus bretschneideri*). Plant Cell Physiol 57:824–847. https://doi.org/10.1093/pcp/pcw029

Liang YK, Dubos C, Dodd IC, Holroyd GH, Hetherington AM, Campbell MM (2005) *AtMYB61*, an R2R3-MYB transcription factor controlling stomatal aperture in *Arabidopsis thaliana*. Curr Biol 15:1201–1206. https://doi.org/10.1016/j.cub.2005.06.041

Lippold F, Sanchez DH, Musialak M, Schlereth A, Scheible W-R, Hincha DK, Udvardi MK (2009) AtMyb41 regulates transcriptional and metabolic responses to osmotic stress in *Arabidopsis*. Plant Physiol 149:1761–1772. https://doi.org/10.1104/pp.108.134874

Ma H, dePamphilis C (2000) The ABCs of floral evolution. Cell 101:5–8. https://doi.org/10.1016/S0092-8674(00)80618-2

Ma Y, Zhang F, Bade R, Daxibater A, Men Z, Hasi A (2015) Genome-wide identification and phylogenetic analysis of the *ERF* gene family in melon. J Plant Growth Regul 34:66–77. https://doi.org/10.1007/s00344-014-9443-z

Ma JQ, Jian HJ, Yang B, Lu K, Zhang AX, Liu P, Li JN (2017) Genome-wide analysis and expression profiling of the *GRF* gene family in oilseed rape (*Brassica napus* L.). Gene 620:36–45. https://doi.org/10.1016/j.gene.2017.03.030

Mandaokar A, Browse J (2008) MYB108 acts together with MYB24 to regulate jasmonate-mediated stamen maturation in Arabidopsis. Plant Physiol 149:851–862. https://doi.org/10.1104/pp.108.132597

Mandel MA, Yanofsky MF (1998) The *Arabidopsis* ALG9 MADS-box gene is expressed in young flower primordia. Sex Plant Reprod 11:22–28

Mandel AM, Gustafson-Brown C, Savidge B, Yanofsky MF (1992) Molecular characterization of the *Arabidopsis* floral homeotic gene *APETALA1*. Nature 360:273–277. https://doi.org/10.1038/360273a0

Mehta TS, Monzur F, Zhao J (2010) Determination of nuclear localization signal sequences for Krüppel-like factor 8. In: Higgins PJ (ed) Transcription factors: methods and protocols. Humana Press, New York, NY, pp 171–186. https://doi.org/10.1007/978-1-60761-738-9

Mishra SK, Tripp J, Winkelhaus S, Tschiersch B, Theres K, Nover L, Scharf KD (2002) In the complex family of heat stress transcription factors, HsfA1 has a unique role as master regulator of thermotolerance in tomato. Genes Dev 16:1555–1567. https://doi.org/10.1101/gad.228802

Miura K, Ikeda M, Matsubara A, Song X-J, Ito M, Asano K, Matsuoka M, Kitano H, Ashikari M (2010) *OsSPL14* promotes panicle branching and higher grain productivity in rice. Nat Genet 42:545–549. https://doi.org/10.1038/ng.592

Mukherjee K, Brocchieri L, Bürglin TR (2009) A comprehensive classification and evolutionary analysis of plant homeobox genes. Mol Biol Evol 26:2775–2794. https://doi.org/10.1093/molbev/msp201

Muthamilarasan M, Khandelwal R, Yadav CB, Bonthala VS, Khan Y, Prasad M (2014) Identification and molecular characterization of MYB transcription factor superfamily in C4 model plant foxtail millet (*Setaria italica* L.). PLoS One 9:e109920. https://doi.org/10.1371/journal.pone.0109920

Noguero M, Atif RM, Ochatt S, Thompson RD (2013) The role of the DNA-binding One Zinc Finger (DOF) transcription factor family in plants. Plant Sci 209:32–45. https://doi.org/10.1016/j.plantsci.2013.03.016

Ogata H, Goto S, Sato K, Fujibuchi W, Bono H, Kanehisa M (1999) KEGG: Kyoto encyclopedia of genes and genomes. Nucl Acids Res 27:29–34. https://doi.org/10.1093/nar/27.1.29

Pan F, Wang Y, Liu H, Wu M, Chu W, Chen D, Xiang Y (2017) Genome-wide identification and expression analysis of SBP-like transcription factor genes in Moso Bamboo (*Phyllostachys edulis*). BMC Genom 18:486. https://doi.org/10.1186/s12864-017-3882-4

Parenicova L (2003) Molecular and phylogenetic analyses of the complete MADS-box transcription factor family in Arabidopsis: new openings to the MADS world. Plant Cell 15:1538–1551. https://doi.org/10.1105/tpc.011544

Park CY, Lee JH, Yoo JH, Moon BC, Choi MS, Kang YH, Chung WS, Lim CO, Cho MJ (2005) WRKY group IId transcription factors interact with calmodulin. FEBS Lett 579:1545–1550. https://doi.org/10.1016/j.febslet.2005.01.057

Park HC, Kim ML, Lee SM, Bahk JD, Yun DJ, Lim CO, Hong JC, Lee SY, Cho MJ, Chung WS (2007) Pathogen-induced binding of the soybean zinc finger homeodomain proteins GmZF-HD1 and GmZF-HD2

to two repeats of ATTA homeodomain binding site in the calmodulin isoform 4 (*GmCaM4*) promoter. Nucl Acids Res 35:3612–3623. https://doi.org/10.1093/nar/gkm273

Patzlaff A, Newman LJ, Dubos C, Whetten RW, Smith C, McInnis S, Bevan MW, Sederoff RR, Campbell MM (2003) Characterisation of *PtMYB1*, an R2R3-MYB from pine xylem. Plant Mol Biol 53:597–608. https://doi.org/10.1023/B:PLAN.0000019066.07933.d6

Paz-Ares J, Ghosal D, Wienand U, Petersont A, Saedler H (1987) Products and with structural similarities. EMBO J 6:3553–3558

Pelaz S, Ditta GS, Baumann E, Wisman E, Yanofsky MF (2000) B and C floral organ identity functions require *SEPALLATA* MADS-box genes. Nature 405:200–203. https://doi.org/10.1038/35012103

Pirkkala L, Nykänen P, Sistonen L (2001) Roles of the heat shock transcription factors in regulation of the heat shock response and beyond. FASEB J 15:1118–1131. https://doi.org/10.1096/fj00-0294rev

Qiao X, Li M, Li L, Yin H, Wu J, Zhang S (2015) Genome-wide identification and comparative analysis of the heat shock transcription factor family in Chinese white pear (*Pyrus bretschneideri*) and five other Rosaceae species. BMC Plant Biol 15:1–16. https://doi.org/10.1186/s12870-014-0401-5

Raffaele S, Vailleau F, Leger A, Joubes J, Miersch O, Huard C, Blée E, Mongrand S, Domergue F, Roby D (2008) A MYB transcription factor regulates very-long-chain fatty acid biosynthesis for activation of the hypersensitive cell death response in *Arabidopsis*. Plant Cell 20:752–767. https://doi.org/10.1105/tpc.107.054858

Reyes JL, Chua NH (2007) ABA induction of miR159 controls transcript levels of two MYB factors during *Arabidopsis* seed germination. Plant J 49:592–606. https://doi.org/10.1111/j.1365-313X.2006.02980.x

Riaño-Pachón DM, Ruzicic S, Dreyer I, Mueller-Roeber B (2007) PlnTFDB: an integrative plant transcription factor database. BMC Bioinformatics 8:1–10. https://doi.org/10.1186/1471-2105-8-42

Riechmann JL, Krizek BA, Meyerowitz EM (1996) Dimerization specifity of Arabidopsis MADS domain homeotic proteins APETALA1, APETALA3, PISTILLATA and AGAMOUS. Proc Natl Acad Sci USA 93:4793–4798

Riechmann J, Martin G, Reuber L, Jiang C, Keddie J, Adam L, Pineda O, Ratcliffe OJ, Samaha RR, Creelman R, Pilgrim M, Broun P, Zhang JZ, Ghandehari D, Sherman BK, Yu G (2000) *Arabidopsis* transcription factors: genome-wide comparative analysis among eukariotes. Science 290:2105–2109

Riese M, Höhmann S, Saedler H, Münster T, Huijser P (2007) Comparative analysis of the SBP-box gene families in *P. patens* and seed plants. Gene 401:28–37. https://doi.org/10.1016/j.gene.2007.06.018

Rosinski JA, Atchley WR (1998) Molecular evolution of the Myb family of transcription factors: evidence for polyphyletic origin. J Mol Evol 46:74–83. https://doi.org/10.1007/PL00006285

Rushton PJ, Somssich IE, Ringler P, Shen QJ (2010) WRKY transcription factors. Trends Plant Sci 15:247–258. https://doi.org/10.1016/j.tplants.2010.02.006

Salinas M, Xing S, Höhmann S, Berndtgen R, Huijser P (2012) Genomic organization, phylogenetic comparison and differential expression of the SBP-box family of transcription factors in tomato. Planta 235:1171–1184. https://doi.org/10.1007/s00425-011-1565-y

Sauer N, Ludwig A, Knoblauch A, Rothe P, Gahrtz M, Klebl F (2004) *AtSUC8* and *AtSUC9* encode functional sucrose transporters, but the closely related *AtSUC6* and *AtSUC7* genes encode aberrant proteins in different *Arabidopsis* ecotypes. Plant J 40:120–130. https://doi.org/10.1111/j.1365-313X.2004.02196.x

Scharf KD, Berberich T, Ebersberger I, Nover L (2012) The plant heat stress transcription factor (Hsf) family: structure, function and evolution. Biochim Biophys Acta 1819:104–119. https://doi.org/10.1016/j.bbagrm.2011.10.002

Scoville AG, Barnett LL, Bodbyl-Roels S, Kelly JK, Hileman LC (2011) Differential regulation of a MYB transcription factor is correlated with transgenerational epigenetic inheritance of trichome density in *Mimulus guttatus*. New Phytol 191:251–263. https://doi.org/10.1111/j.1469-8137.2011.03656.x

Seo PJ, Park CM (2010) MYB96-mediated abscisic acid signals induce pathogen resistance response by promoting salicylic acid biosynthesis in *Arabidopsis*. New Phytol 186:471–483. https://doi.org/10.1111/j.1469-8137.2010.03183.x

Shikata M, Koyama T, Mitsuda N, Ohme-Takagi M (2009) Arabidopsis SBP-box genes *SPL10*, *SPL11* and *SPL2* control morphological change in association with shoot maturation in the reproductive phase. Plant Cell Physiol 50:2133–2145. https://doi.org/10.1093/pcp/pcp148

Shim D, Hwang J-U, Lee J, Lee S, Choi Y, An G, Maritonia E, Lee Y (2009) Orthologs of the class A4 heat shock transcription factor HsfA4a confer cadmium tolerance in wheat and rice. Plant Cell 21:4031–4043. https://doi.org/10.1105/tpc.109.066902

Silva EM, Silva GF, Bidoia DB, Silva Azevedo M, Jesus FA, Pino LE, Peres LE, Carrera E, Lópe-Díaz I, Nogueira FT (2017) microRNA159-targeted *SlGA-MYB* transcription factors are required for fruit set in tomato. Plant J 92:95–109. https://doi.org/10.1111/tpj.13637

Stracke R, Ishihara H, Huep G, Barsch A, Mehrtens F, Niehaus K, Weisshaar B (2007) Differential regulation of closely related R2R3-MYB transcription factors controls flavonol accumulation in different parts of the *Arabidopsis thaliana* seedling. Plant J 50:660–677. https://doi.org/10.1111/j.1365-313X.2007.03078.x

Su X, Sun X, Cheng X, Wang Y, Abdullah M, Li M, Li D, Gao J, Cai Y, Lin Y (2017) Comparative genomic analysis of the *PKS* genes in five species and expression analysis in upland cotton. PeerJ 5:e3974. https://doi.org/10.7717/peerj.3974

Sun P, Zhu X, Huang X, Liu JH (2014) Overexpression of a stress-responsive MYB transcription factor of

Poncirus trifoliata confers enhanced dehydration tolerance and increases polyamine biosynthesis. Plant Physiol Biochem 78:71–79. https://doi.org/10.1016/j.plaphy.2014.02.022

Tan QK-G, Irish VF (2006) The Arabidopsis zinc finger-homeodomain genes encode proteins with unique biochemical properties that are coordinately expressed during floral development. Plant Physiol 140:1095–1108. https://doi.org/10.1104/pp.105.070565

Tran LSP, Nakashima K, Sakuma Y, Osakabe Y, Qin F, Simpson SD, Maruyama K, Fujita Y, Shinozaki K, Yamaguchi-Shinozaki K (2007) Co-expression of the stress-inducible zinc finger homeodomain ZFHD1 and NAC transcription factors enhances expression of the *ERD1* gene in Arabidopsis. Plant J 49:46–63. https://doi.org/10.1111/j.1365-313X.2006.02932.x

Tripathi P, Carvallo M, Hamilton EE, Preuss S, Kay SA (2017) *Arabidopsis* B-BOX32 interacts with CONSTANS-LIKE3 to regulate flowering. Proc Natl Acad Sci USA 114:172–177. https://doi.org/10.1073/pnas.1616459114

Uematsu C, Katayama H, Makino I, Inagaki A, Arakawa O, Martin C (2014) Peace, a MYB-like transcription factor, regulates petal pigmentation in flowering peach "Genpei" bearing variegated and fully pigmented flowers. J Exp Bot 65:1081–1094. https://doi.org/10.1093/jxb/ert456

Usami T, Horiguchi G, Yano S, Tsukaya H (2009) The more and smaller cells mutants of *Arabidopsis thaliana* identify novel roles for *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* genes in the control of heteroblasty. Development 136:955–964. https://doi.org/10.1242/dev.028613

van der Knaap E, Kim JH, Kende H (2000) A novel gibberellin-induced gene from rice and its potential regulatory role in stem growth. Plant Physiol 122:695–704

Vargova K, Curik N, Burda P, Basova P, Kulvait V, Pospisil V, Savvulidi F, Kokavec J, Necas E, Berkova A, Obrtlikova P, Karban J, Mraz M, Pospisilova S, Mayer J, Trneny M, Zavadil J, Stopka T (2011) MYB transcriptionally regulates the miR-155 host gene in chronic lymphocytic leukemia. Blood 117:3816–3825. https://doi.org/10.1182/blood-2010-05-285064

Vimolmangkang S, Han Y, Wei G, Korban SS (2013) An apple MYB transcription factor, MdMYB3, is involved in regulation of anthocyanin biosynthesis and flower development. BMC Plant Biol 13:1–13. https://doi.org/10.1186/1471-2229-13-176

von Koskull-Döring P, Scharf KD, Nover L (2007) The diversity of plant heat stress transcription factors. Trends Plant Sci 12:452–457. https://doi.org/10.1016/j.tplants.2007.08.014

Wang W, Vinocur B, Shoseyov O, Altman A (2004) Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends Plant Sci 9:244–252. https://doi.org/10.1016/j.tplants.2004.03.006

Wang J-W, Schwab R, Czech B, Mica E, Weigel D (2008) Dual effects of miR156-targeted *SPL* genes and *CYP78A5/KLUH* on plastochron length and organ size in *Arabidopsis thaliana*. Plant Cell 20:1231–1243. https://doi.org/10.1105/tpc.108.058180

Wang X, Niu QW, Teng C, Li C, Mu J, Chua NH, Zuo J (2009) Overexpression of *PGA37/MYB118* and *MYB115* promotes vegetative-to-embryonic transition in *Arabidopsis*. Cell Res 19:224–235. https://doi.org/10.1038/cr.2008.276

Wang H, Yin X, Li X, Wang L, Zheng Y, Xu X, Zhang Y, Wang X (2014) Genome-wide identification, evolution and expression analysis of the grape (*Vitis vinifera* L.) zinc finger-homeodomain gene family. Int J Mol Sci 15:5730–5748. https://doi.org/10.3390/ijms15045730

Wang H, Lin J, Li XG, Chang Y (2015) Genome-wide identification of pear HD-Zip gene family and expression patterns under stress induced by drought, salinity, and pathogen. Acta Physiol Plant 37:1–19. https://doi.org/10.1007/s11738-015-1933-5

Wang R, Ming M, Li J, Shi D, Qiao X, Li L, Zhang S, Wu J (2017) Genome-wide identification of the *MADS-box* transcription factor family in pear (*Pyrus bretschneideri*) reveals evolution and functional divergence. PeerJ 5:e3776. https://doi.org/10.7717/peerj.3776

Weigel D, Meyerowitz EM (1994) The ABCs of floral homeotic genes. Cell 78:203–209. https://doi.org/10.1016/0092-8674(94)90291-7

Windhovel A, Hein I, Dabrowa R, Stockhaus J (2001) Characterization of a novel class of plant homeodomain proteins that bind to the C4 phosphoenolpyruvate carboxylase gene of *Flaveria trinervia*. Plant Mol Biol 45:201–214. https://doi.org/10.1023/A:1006450005648

Wu J, Wang Z, Shi Z, Zhang S, Ming R, Zhu S, Khan MA, Tao S, Korban SS, Wang H, Chen NJ, Nishio T, Xu X, Cong L, Qi K, Huang X, Wang Y, Zhao X, Wu J, Deng C, Gou C, Zhou W, Yin H, Qin G, Sha Y, Tao Y, Chen H, Yang Y, Song Y, Zhan D, Wang J, Li L, Dai M, Gu C, Wang Y, Shi D, Wang X, Zhang H, Zeng L, Zheng D, Wang C, Chen M, Wang G, Xie L, Sovero V, Sha S, Huang W, Zhang S, Zhang M, Sun J, Xu L, Li Y, Liu X, Li Q, Shen J, Wang J, Paull RE, Bennetzen JL, Wang J, Zhang S (2013) The genome of the pear (*Pyrus bretschneideri* Rehd.). Genome Res 23(2):396–408

Xie K (2006) Genomic organization, differential expression, and interaction of SQUAMOSA promoter-binding-like transcription factors and microRNA156 in rice. Plant Physiol 142:280–293. https://doi.org/10.1104/pp.106.084475

Yamasaki K, Kigawa T, Inoue M, Tateno M, Yamasaki T, Yabuki T, Aoki M, Seki E, Matsuda T, Nunokawa E, Ishizuka Y, Terada T, Shirouzu M, Osanai T, Tanaka A, Seki M, Shinozaki K, Yokoyama S (2004) A novel zinc-binding motif revealed by solution structures of DNA-binding domains of

Arabidopsis SBP-family transcription factors. J Mol Biol 337:49–63. https://doi.org/10.1016/j.jmb.2004.01.015

Yamasaki H, Hayashi M, Fukazawa M, Kobayashi Y, Shikanai T (2009) SQUAMOSA promoter binding protein-like7 is a central regulator for copper homeostasis in *Arabidopsis*. Plant Cell 21:347–361. https://doi.org/10.1105/tpc.108.060137

Yanagisawa S (2004) Dof domain proteins: plant-specific transcription factors associated with diverse phenomena unique to plants. Plant Cell Physiol 45:386–391

Yanhui C, Xiaoyuan Y, Kun H, Meihua L, Jigang L, Zhaofeng G, Zhiqiang L, Yunfei Z, Xiaoxiao W, Xiaoming Q, Yunping S, Li Z, Xiaohui D, Jingchu L, Xing-Wang D, Zhangliang C, Hongya G, Li-Jia Q (2006) The MYB transcription factor superfamily of Arabidopsis: expression analysis and phylogenetic comparison with the rice MYB family. Plant Mol Biol 60:107–124. https://doi.org/10.1007/s11103-005-2910-y

Zhang Y, Schwarz S, Saedler H, Huijser P (2007) SPL8, a local regulator in a subset of gibberellin-mediated developmental processes in *Arabidopsis*. Plant Mol Biol 63:429–439. https://doi.org/10.1007/s11103-006-9099-6

Zhang Z, Liu X, Wang X, Zhou M, Zhou X, Ye X, Wei X (2012) An R2R3 MYB transcription factor in wheat, TaPIMP1, mediates host resistance to *Bipolaris sorokiniana* and drought stresses through regulation of defense- and stress-related genes. New Phytol 196:1155–1170. https://doi.org/10.1111/j.1469-8137.2012.04353.x

Zhang X, Dou L, Pang C, Song M, Wei H, Fan S, Wang C, Yu S (2014) Genomic organization, differential expression, and functional analysis of the *SPL* gene family in *Gossypium hirsutum*. Mol Genet Genomics 290:115–126. https://doi.org/10.1007/s00438-014-0901-x

Zhang S-D, Ling L-Z, Yi T-S (2015) Evolution and divergence of SBP-box genes in land plants. BMC Genom 16:787. https://doi.org/10.1186/s12864-015-1998-y

Zhang Z, Hu X, Zhang Y, Miao Z, Xie C, Meng X, Deng J, Wen J, Mysore KS, Frugier F, Wang T, Dong J (2016) Opposing control by transcription factors MYB61 and MYB3 increases freezing tolerance by relieving C-repeat binding factor suppression. Plant Physiol 172(2):1306–1323. https://doi.org/10.1104/pp.16.00051

Zhao Q, Dixon RA (2011) Transcriptional networks for lignin biosynthesis: more complex than we thought? Trends Plant Sci 16:227–233. https://doi.org/10.1016/j.tplants.2010.12.005

Zhong R, Lee C, Zhou J, Mccarthy RL, Ye Z (2014) A battery of transcription factors involved in the regulation of secondary cell wall biosynthesis in Arabidopsis. Plant Cell 20:2763–2782. https://doi.org/10.1105/tpc.ondary

Zhou J, Lee C, Zhong R, Ye Z-H (2009) MYB58 and MYB63 are transcriptional activators of the lignin biosynthetic pathway during secondary cell wall formation in *Arabidopsis*. Plant Cell 21:248–266. https://doi.org/10.1105/tpc.108.063321

Zhou M, Wang C, Qi L, Yang X, Sun Z, Tang Y, Tang Y, Shao J, Wu Y (2015) Ectopic expression of *Fagopyrum tataricum* FtMYB12 improves cold tolerance in *Arabidopsis thaliana*. J Plant Growth Regul 34:362–371. https://doi.org/10.1007/s00344-014-9472-7

Zhu N, Cheng S, Liu X, Du H, Dai M, Zhou DX, Yang W, Zhao Y (2015) The R2R3-type MYB gene *OsMYB91* has a function in coordinating plant growth and salt stress tolerance in rice. Plant Sci 236:146–156. https://doi.org/10.1016/j.plantsci.2015.03.023

Self-incompatibility in Pear

10

Shaoling Zhang and Chao Gu

Abstract

Self-incompatibility (SI) has been widely investigated at both molecular and cellular levels in pear. This trait is controlled by a single multi-allelic locus encoding at least two components from the pollen and the pistil. The stylar-*S* determinant is an S-glycoprotein (S-RNase) that can inhibit pollen tube growth in a self-pistil, and induces a series of changes in reactive oxygen species (ROS), calcium (Ca^{2+}), actin cytoskeleton, and phosphatidic acid, leading to programmed cell death in incompatible pollen tubes. At present, a total of 67 *S-RNase* genes have been identified and have served in selecting appropriate pollinators in pear orchards. The pollen-*S* determinant has also been investigated in pear. Although a group of *F-box* genes have been identified in the *S*-locus, it remains unclear as to which gene(s) are involved in self-incompatibility reactions. In pear, only a few cultivars have experienced loss of self-incompatibility, due to either stylar or pollen mutations, or due to polyploidy. Except for the deletion of S_4-*RNase* in cultivar Osa-Nijisseiki, other stylar-tissue mutations, including abnormal expression and post-transcript modification, are difficult to study, and are yet to be explained at the molecular level. Similarly, the mechanism of pollen tissue mutation and polyploidy require further investigations in future studies.

10.1 Introduction

Self-incompatibility (SI) is a common genetic mechanism found in plants as it prevents inbreeding by rejecting self-pollen, thereby promoting outcrossing, and maintaining prior evolution of a species (De Nettancourt 2001). Many flowering plants exhibit a wide range of SI, from 60 to 90 families (Brewbaker 1954), including those of Cruciferae, Solanaceae, Rosaceae, Papaveraceae, and Amaryllidaceae, among others (Lewis 1976). Self-incompatibility is controlled by multiple alleles in a single locus, designated as the *S*-locus. Noteworthy, the genetic mechanism of SI is not identical in different plants. For example, in Cruciferae, SI is determined by the dominant *S*-allele in spores, and it is referred to as sporophytic self-incompatibility (SSI); whereas, in Solanaceae, Rosaceae, and Scrophulariaceae, SI is determined by a single *S*-allele in gametes, and it is referred to as gametophytic self-incompatibility (GSI).

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The pear is a typical gametophytic self-incompatible plant. In self-compatible pear cultivars, the pollen can grow into the ovule through the self-stigma and the self-style. However, in self-incompatible cultivars, following pollen germination on the self-stigma, pollen tubes could pass through the stigma and into the self-style, and then simply stop during the expected period of pollen tube growth toward the ovule. These inhibited pollen tubes have abnormal morphologies along with swelled tips. If the style is cut from the top 1/3 or 1/2 section, the ratio of pollen growth through the cut section is found to increase, but so is the inhibition of pollen growth between 1/3 and 1/2 sections (Zhang et al. 2000). These findings indicate that there is an inhibitor present in self-styles preventing pollen tube growth.

10.2 The Physiological Mechanism of SI

The inhibitor that prevents pollen tube growth has been first unveiled by comparing expressed proteins in styles of the self-incompatible Asian pear cultivar Nijisseiki and its mutant, the self-compatible cultivar Osa-Nijisseiki (Sassa et al. 1992). It is found that this inhibitor is an S-glycoprotein, which is identical to an S-RNase, and it is specifically expressed in styles, and not in leaves, pollen, or germinated pollen grains (Sassa et al. 1993). This S-glycoprotein can be detected in styles approximately 8 days before flowering, and its levels continue to increase during flower development (Hiratsuka et al. 1999). Interestingly, levels of expression of S-glycoprotein in styles vary in different pear cultivars, but they are higher in self-incompatible cultivars than those in self-compatible cultivars (Zhang et al. 2000).

Based on both in vivo and in vitro studies, lengths of pollen tubes are negatively correlated to levels of S-RNase (Hiratsuka et al. 1999, 2001). Furthermore, morphological differences are also noted, wherein pollen tubes are curved in shape with swollen tips prior to arrest of pollen tube growth in self-incompatible styles; whereas, no such morphological observations are noted in non-self-styles (Hiratsuka et al. 1985). These findings suggest that S-RNase induces a series of structural changes in pollen tubes during an SI reaction. Using transmission electron microscopy, similar structures are detected in incompatible and compatible pollen tubes during early stages of pollen growth. However, after 24 h, incompatible pollen tubes are filled with cytoplasm and various organelles, while low amounts of cytoplasm are observed in tips of pollen tubes, along with damaged organelles and thickened cell walls (Gao et al. 2015). These findings suggest that inhibition of pollen germination and pollen tube growth are influenced by levels of S-RNase in pollen tubes.

Moreover, analysis of Ca^{2+} concentrations in cytoplasm of pollen tubes treated by exogenous and endogenous RNase reveals that the effects of stylar S-RNase treatments on Ca^{2+} concentrations are different in self-compatible and self-incompatible pollen tubes. In fact, prior to germination, a cytosolic Ca^{2+} gradient is detected around the germinal aperture in pollen tubes (Jiang et al. 2014; Qu et al. 2007). Although the cause of this observed Ca^{2+} gradient is unclear, there is increasing evidence that the Ca^{2+} channel in the plasma membrane of pollen tubes plays an important role in this observed Ca^{2+} gradient detected in pear (Qu et al. 2007). Interestingly, a similar phenotype is also observed in the flowering poppy weed plant *Papaver rhoeas,* wherein Ca^{2+} induces microfilament depolymerization and programmed cell death in self-incompatible pollen tubes (Wu et al. 2011). Coincidently, the stylar S-RNase in pear could interact directly with the actin protein PbrActin1 in an *S*-haplotype-independent manner, resulting in depolymerization of the actin cytoskeleton, and in turn promoting programmed cell death in self-incompatible pollen tubes. The P156 of PbrS-RNase is essential for PbrS-RNase–PbrActin1 interactions, while the actin cytoskeleton-depolymerizing function of PbrS-RNase does not require an RNase activity (Liu et al, 2007; Chen

et al. 2018). The induced actin cytoskeleton-depolymerization results in programmed cell death in self-incompatible pollen tubes (Wang et al. 2009).

In general, overlapping phenotypes are observed in a GSI reaction. Recently, the reactive oxygen species (ROS) gradient has been investigated in tips of pollen tubes. It is found that this ROS gradient is disrupted by the stylar S-RNase in pears, which in turn leads to Ca^{2+} channel closure and microfilament depolymerization, thereby stimulating degradation of nuclei. These findings suggest that ROS is an upstream regulator for Ca^{2+} to mediate pollen tube growth in a GSI reaction (Wang et al. 2010). Moreover, phosphatidic acid (PA) mitigates S-RNase signaling in pollen by stabilizing the actin, as it has been recently observed. However, expression of phospholipase D (PbrPLDδ1) is enhanced by PbrS-RNase cytotoxicity, resulting in increased PA levels in incompatible pollen tubes. Thus, PbrPLDδ1-derived PA initially prevents depolymerization of the actin cytoskeleton elicited by PbrS-RNase, and delays SI signaling which leads to pollen tube death (Chen et al. 2018). These results provide further insights into the orchestration of the S-RNase-based SI response, in which increased PA levels initially play a protective role in incompatible pollen, until sustained PbrS-RNase activity reaches the point of no return, and pollen tube growth ceases.

10.3 Self-incompatibility Determinants

The *S*-locus in pear should contain at least two genes that are, respectively, stylar-*S* and pollen-*S* determinants. If the single *S*-locus in pollen is identical to one of the two *S*-loci in the style, the pollen presents an SI reaction. Thus, two pear cultivars with identical *S*-genotypes are deemed cross-incompatible (Fig. 10.1a), while two cultivars with overlapping *S*-loci are semi-compatible (Fig. 10.1b), and two cultivars without any overlapping *S*-loci are deemed cross-compatible (Fig. 10.1c).

10.3.1 Stylar-*S* Determinants

10.3.1.1 Identification of Stylar-*S* Determinants

S-RNases have been isolated from styles of pear flowers using a two-dimensional gel electrophoresis (Sassa et al. 1993), and they are found to belong to the T2/S ribonuclease superfamily (Sassa et al. 1996). With the development of molecular technologies, additional numbers of *S-RNase* alleles have been identified from pear cultivars. Until now, 68 and 24 *S-RNase* alleles have been individually isolated from Asian and European pear cultivars, respectively, and *S*-genotypes have been determined in at least 462

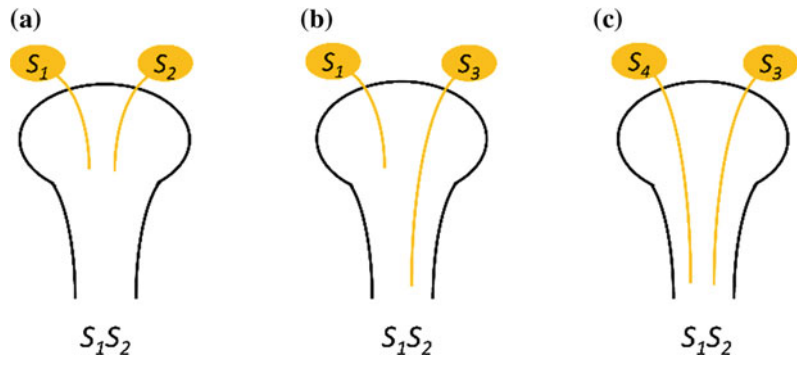

Fig. 10.1 Schematic diagram of self-incompatibility reactions

pear cultivars (Table 10.1). A detailed description of how these various S-genotypes have been identified is presented as follows:

(a) **Cross-pollination test in the field**: Based on the principle of GSI, it is expected that there are three different phenotypes that could be observed following cross-pollination. First, if the two cultivars are cross-incompatible, this indicates there are two identical *S*-loci shared between these two cultivars. Second, if two cultivars are cross-compatible, but half of the progeny is backcross-incompatible with the male parent, then this indicates that there is a single *S*-locus that is shared between these two cultivars. Thirdly, if two cultivars are cross-compatible and all progeny are backcross-compatible with the male parent, this indicates that there is no common *S*-locus present in these two cultivars.

(b) **In vitro culture of pollinated styles**: As pollen tubes are arrested in a style wherein identical *S*-loci are present in these tissues, an in vitro culture of pollinated styles, grown on an agar medium, is used to discern the identity of *S*-loci present in each of the style and the pollen tube (Zhang et al. 2003). Following in vitro culture, if no pollen tubes could pass through the style, this indicates that *S*-loci of the pollen tubes are identical to those of the style. However, if few pollen tubes are capable of passing through the style, this indicates that there is a single *S*-locus in the pollen tubes that is different from that present in the style. While, if large numbers of pollen tubes are capable of passing through the style, then this indicates that at least two *S*-loci present in pollen tubes are different from those present in the style.

(c) **Anatomical observations of pollen tube growth in the style**: At 96 h following pollination, pollinated styles are fixed in an FAA solution (formalin:acetic acid:70% ethanol at a ratio of 5:5:90 by volume) for about 24 h and then transferred to 100% ethanol. Fixed styles are washed with water to remove ethanol, softened in NaOH, and stained with an aniline blue dye. Stained styles are rinsed with water, squashed on a glass slide, and observed under an ultraviolet fluorescent microscope (Wang et al. 2009). If either the majority, some, or none of the pollen tubes could grow through to the bottom section of a style, then this suggests that either two, one, or no *S*-loci in pollen tubes, respectively, overlap with the two *S*-loci of the style.

(d) **S-glycoprotein electrophoresis**: As *S-RNases* are specifically expressed in the style, producing S-glycoproteins, then *S-RNase* alleles could be determined by identifying S-glycoprotein products. In brief, soluble proteins are extracted from styles at pre-bloom stage, and then these are subjected to isoelectric focusing-PAGE (Heng et al. 2015). Following silver staining, different S-glycoproteins will be readily identified, corresponding to the different S-RNase alleles present in the style.

(e) **PCR amplification**: Based on the polymorphism of the length of introns present in *S-RNase* alleles, allele-specific primer pairs are designed from conserved regions to identify different *S-RNase* alleles (Ishimizu et al. 1999). This PCR-based method has been widely used to identify *S-RNase* alleles and *S*-genotypes in pear cultivars. At present, a total of 92 *S-RNase* alleles have been isolated from over 400 pear cultivars that have been successfully *S*-genotyped.

The *S-RNase* alleles isolated from Asian pear cultivars are numbered with Arabic numerals, while those isolated from European pear cultivars are initially numbered with lowercase letters and then re-numbered with Arabic numerals (Goldway et al. 2009). Unfortunately, the numbered *S-RNase* alleles in Asian pears are out of order. For example, the two *S-RNase* alleles isolated from *P.* × *bretschneideri*, S_{20}- and S_{29}-*RNases,* share identical sequences, while the S_7-*RNase* in *P. pyrifolia* shares identical sequences to S_{27}-*RNase* in *P.* × *bretschneideri*. Although similar allele pairs have been recently merged and integrated (Table 10.2; Wang et al. 2017), identities of *S-RNase* alleles are still difficult to discern. In

Table 10.1 Identified *S*-genotypes in cultivated and wild pears

Species	Cultivar	*S*-genotype	Cultivar	*S*-genotype	Cultivar	*S*-genotype	Cultivar	*S*-genotype
P. × *brestschneideri*	Baipisu	$S_{34}S_n$	Dongguoli	$S_{12}S_{35}$	Jinhua no.4	$S_{13}S_{18}$	Wuzihuang	$S_{16}S_{28}$
	Banjinsu	S_5S_{21}	Donghuang	$S_{20}S_{34}$	Jinli	S_1S_1	Xiangchi	S_1S_{21}
	Baoshansu	S_1S_{21}	Dongningwu	S_1S_{17}	Jinzhui	$S_{21}S_{34}$	Xiangchun	S_3S_{19}
	Bayuesu	S_3S_{16}	Eli	$S_{13}S_{34}$	Jiuquanmaili	S_6S_{17}	Xinli no.1	S_8S_{22}
	Bingtang	$S_{16}S_{19}$	Enli	S_1S_{19}	Liuban	$S_{17}S_{19}$	Xuehua	S_4S_{16}
	Boshanchi	$S_{19}S_{27}$	Esu	$S_{15}S_{38}$	Liuleng	$S_{16}S_{19}$	Yali	S_1S_{21}
	Chili	S_1S_{19}	Gaopingdaihuang	S_1S_2	Lixianxinbapan	$SeSx$	Yanguangli	S_1S_{17}
	Daaoao	$S_{12}S_{12}$	Guanyangxueli	$S_{18}S_{27}$	Mili	$S_{19}S_{29}$	Yaoxianyinli	$S_{21}S_x$
	Daaotu	$S_{11}S_{22}$	Haitangsu	$S_1S_{12}S_{19}$	Pingguoli	S_1S_{17}	Yingzhiqing	$S_{12}S_{12}$
	Dacili	$S_{19}S_{27}$	Hongpisu	$S_{12}S_{26}$	Qingli	$S_{19}S_{19}$	Youli	$S_{16}S_{19}$
	Dahebai	$S_{16}S_{19}$	Hongtaiyang	S_8S_{35}	Qingpisu	$S_{34}S_n$	Yunnanbaozhu	$S_{22}S_X$
	Dalijitui	$S_{17}S_{19}$	Huangxiang	S_4S_{27}	Shuidonggua	$S_{15}S_{45}$	Zhaoxiandayali	$S_1S_1S_{21}S_{21}$
	Damianhuang	S_1S_{19}	Jinanxiaohuangli	$S_1S_{12}S_{19}$	Shuihongxiao	$S_{16}S_{19}$	Zhuzuisu	$S_{19}S_{22}$
	Dangshansuli	S_7S_{34}	Jinbangtou	S_mS_{12}	Taihuangli	S_2S_{14}	Zisu	$S_{19}S_{34}$
	Daqingpi	$S_{19}S_{34}$	Jinchuizi	$S_{16}S_{19}$	Tianshengfu	$S_{12}S_{29}$		
	Dashuihe	S_7S_{19}	Jinfeng	$S_{17}S_{19}$	Tianyali	S_1S_{21}		
	Dayali	$S_1S_1S_{21}S_{21}$	Jinhua	S_3S_{18}	Wushantangli	S_8S_{19}		
P. pyrifolia	Aikansui	S_4S_5	Huagao	S_3S_9	Mazili	S_1S_{29}	Wandaxingao	S_3S_9
	Amanogawa	S_1S_9	Huali No.1	S_1S_3	Meigetsu	S_8S_9	Waseaka	S_4S_5
	Atago	S_2S_5	Huali No.2	S_3S_4	Meirensu	S_4S_{12}	Weiningdaihuangli	S_3S_{37}
	Baozhuli	$S_{22}S_{42}$	Huanghua	S_1S_2	Mianli	$S_{19}S_{41}$	Wenshanhongxueli	$S_{31}S_{36}$
	Baxing	S_4S_5	Whangkeumbae	S_3S_4	Mimauraaki	S_1S_6	Whasam	S_3S_5
	Bayun	S_1S_4	Huangli	$S_{22}S_{34}$	Minibae	S_3S_{31}	Xianhuang	S_3S_5
	Cangxixueli	S_5S_{15}	Huangmi	S_1S_6	Niitaka	S_3S_9	Xinhang	S_1S_3
	Chihuali	$S_{26}S_{15}$	Huangpishui	$S_{16}S_{42}$	Nijisseiki	S_2S_4	Xinxue	S_5S_6

(continued)

Table 10.1 (continued)

Species	Cultivar	*S*-genotype	Cultivar	*S*-genotype	Cultivar	*S*-genotype	Cultivar	*S*-genotype
	Chisui	S_1S_2	Huiyanghongli	$S_{46}S_{47}$	Okusankichi	S_5S_7	Xishui	S_4S_5
	Chixianfeng	S_5aS_{18}	Huoba	$S_{26}S_{36}$	Osa-Nijisseiki	S_2S_{4SM}	Xiuyu	S_4S_5
	Choju	S_1S_5	Hangqing	S_1S_4	Pre-Kisui	S_3S_4	Xizilü	S_1S_4
	Chojuro	S_2S_3	Huobali	$S_{26}S_{36}$	Qingchangshilang	S_2S_3	Yanbianmingyueli	S_3Se
	Chubixiang	S_1S_{15}	Jiangdao	S_5S_8	Qingcheng	S_4S_5	Yaoxianhong	S_1S_{21}
	Chulüxia	S_3S_4	Jinchuanyeshengli	S_5S_{13}	Qinggoushageda	$S_{36}S_d$	Yewang	S_3S_9
	Cuiguan	S_3S_5	Jincunxia	S_1S_6	Qingkui	S_1S_3	Yuanxiang	$S_{15}S_{16}$
	Cuilü	S_3S_4	Jingyu	S_4S_{24}	Qingxiang	S_4S_7	Yunnanhuangpishui	$S_{16}S_{19}$
	Cuixing	S_1S_4	Jinqiuli	S_3S_9	Qingyu	S_3S_4	Yunnanmali no.1	S_9S_{42}
	Cuyu	S_3S_4	Jinshui No.1	S_3S_{29}	Qiubai	$S_{19}S_{34}$	Yunnanmali no.2	$S_{42}S_x$
	Daqingli	S_1S_3	Jinshui No.1	S_5S_{29}	Sanhua	S_2S_7	Yunnanwumingli	$S_{22}S_{29}$
	Deshengxiang	S_3S_{29}	Jinzhuguoli	S_3S_{19}	Shinkou	S_4S_9	Yushui	S_3S_4
	Duyi	S_1S_2	kikusui	S_2S_4	Shinsei	S_8S_9	Zaoli18	S_4S_{28}
	Fuyuanhuang	$S_{16}S_{33}$	Kimizukawase	S_1S_5	Shinseiki	S_3S_4	Zaomi	$S_{19}S_{29}$
	Guiguan	S_2S_{16}	Kisui	S_4S_5	Shinshui	S_4S_5	Zaomixingao	S_3S_9
	Hakutasei	$S_{22}S_{34}$	Kosui	S_4S_5	Shisho	S_3S_9	Zaoshengchangshilang	S_2S_4
	Hangqing	S_1S_4	Lijiangbaili	$S_{22}S_{42}$	Shounan	S_1S_3	Zaoshenghuangjin	S_3S_4
	Hongli	S_4S_{36}	Longquansu	S_3S_{22}	shusui	S_1S_5	Zaoyu	S_1S_2
	Hongsucui	S_4S_{12}	Lüyun	S_3S_{29}	Suisho	S_3S_9	Zhaori	S_4S_5
	Hongxiao	$S_{16}S_{19}$	Mandingxue	S_4S_{15}	Suomei	$S_{36}S_{37}$	Zhenghedaxueli	$S_{13}S_{43}$
	Housui	S_3S_5	Mantianhong	S_4S_{12}	Taiwanmili	$S_{11}S_{22}$		
	Huafeng	S_3S_9	Maogongli	$S_{12}S_{13}$	Tianchengzi	S_7S_{12}		

(continued)

Table 10.1 (continued)

Species	Cultivar	*S*-genotype	Cultivar	*S*-genotype	Cultivar	*S*-genotype	Cultivar	*S*-genotype
P. ussuriensis	Anshan No.1	$S_{13}S_{31}$	Jiaihuatubianti	S_1S_{17}	Pitai	$S_{22}S_{43}$	Tangli	$S_{27}S_{30}$
	Baibalixiang	$S_{19}S_{31}$	Jianbali	$S_{12}S_{30}$	Qinglong	S_2S_3	Xiangshui	$S_{17}S_{31}$
	Balixiang	$S_{19}S_{30}$	Jianbazi	$S_{27}S_h$	Ruanbazi	$S_{16}S_{36}$	Xiaoxiangshui	$S_{29}S_{34}$
	Dananguo	$S_{13}S_{34}$	Jiazhibazi	S_5S_{19}	Ruan'erli	$S_{17}S_{31}$	Xiaoxiangshuiyabian	S_eS_x
	Fuanjianba	$S_{16}S_{22}$	Jinbaili	$S_{16}S_{30}$	Saozhoumiaozi	$S_{15}S_{26}$	Xiehuatian	$S_{29}S_{34}$
	Ganguxiangshui	S_eS_x	Lanzhouruan'erli	S_dS_{12}	Shanli	$S_{13}S_{34}$	Xingchengxiehuatian	$S_{17}S_{31}$
	Hanhong	$S_{27}S_{34}$	Liaoyangdaxiangshui	$S_{16}S_{12}$	Shanli no.2	S_8S_{27}	Yaguangli	$S_{19}S_{30}$
	Hanxiang	$S_{12}S_{31}$	Longxiang	$S_{16}S_{42}$	Shanli no.3	S_bS_{41}	Yanbiandaxiangshui	$S_{12}S_{16}$
	Honghuagai	$S_{19}S_{32}$	Maili	$S_{31}S_{40}$	Shanli no.4	ScS_{42}	Yanbianxiehuatian	$S_{17}S_{31}$
	Huagai	$S_{34}S_d$	Matihuang	$S_{16}S_{19}$	Shanli no.5	S_4S_{42}	Yeshengleixingshanli1	S_8S_{27}
	Huagaiwang	$S_{31}S_{31}S_{34}S_{34}$	Nanguoli	$S_{11}S_{17}$	Shanyali	$S_{30}S_{36}$	Youhong	$S_{13}S_{34}$
	Huangjinduima	$S_{19}S_{29}$	Neimenggushanli	$S_{29}S_{41}$	Suandali	S_3S_{29}	Zaobai	$S_{19}S_{42}$
	Hululi	S_aS_b	Pingxiangli	$S_{31}S_d$	Suanliguozi	$S_{19}S_{41}$		
P. sinkiangensis	Ganguheili	$S_{16}S_{54}$	Huangmian	S_1S_{12}	Lanzhouhuachangba	$S_{19}S_{22}$	Qipanxiangli	$S_{22}S_{28}$
	Naixiteamuti	$S_{19}S_{28}$	Jiuquanchangbali	S_6S_{22}	Linxiadadiaodan	$S_{26}S_x$	Seerkefu	$S_{22}S_{28}$
	Aolian	SpS_{32}	Jiuquanmaili	S_6S_{17}	Linxiahuangma	S_3S_e	Sha-01 Xiangli	$S_{22}S_{28}$
	Ganguhongxia	$S_{16}S_x$	Kanglebaiguo	S_bS_i	Linxiasala	$S_{16}S_e$	Sierkefuli	$S_{22}S_{28}$
	Guidechangba	$S_{19}S_{22}$	Kangleganchangba	$S_{22}S_d$	Linxiaxiangba	$S_{12}S_{21}$	Wudoutianli	$S_{26}S_i$
	Hezhengganchangba	$S_{22}S_d$	Kanglesumuli	S_1S_h	Linyaomatianli	$S_{22}S_d$	Xinjianghuangli	$S_{22}S_{28}$
	Hongnahe	$S_{22}S_{28}S_{40}$	Kuerle	$S_{22}S_{34}$	Lǜjuju	$S_{22}S_{28}$	Yilihongjuju	$S_{22}S_{28}$
	Huachangba	$S_{19}S_{22}$	Kuerlexiangli	$S_{22}S_{28}$	Manluwowoguo	$S_{12}S_{21}$	Zaoshujuju	$S_{22}S_{28}$
	Huangjuju	$S_{22}S_{34}$	Kuikejuju	$S_{22}S_{28}$	Moli	$S_{26}S_b$	Zhangyechangba	$S_{19}S_{22}$
	Huangma	$S_{31}S_{40}$	Kunqieke	$S_{19}S_{28}$	Qingmian	S_1S_{18}		

(continued)

Table 10.1 (continued)

Species	Cultivar	*S*-genotype	Cultivar	*S*-genotype	Cultivar	*S*-genotype	Cultivar	*S*-genotype
P. communis	Abbé Fétel	$S_{104}S_{105}$	Conference	$S_{108}S_{119}$	Idaho	$S_{101}S_{119}$	Rosired Bartlett	$S_{101}S_{102}$
	Akça	$S_{102}S_{109}$	Coscia	$S_{103}S_{104}$	Jeanne d'Arque	$S_{101}S_{104}$	Rosmarie	$S_{101}S_{116}$
	Alexandrine Douillard	$S_{103}S_{104}$	Covert	$S_{101}S_{118}$	Joséphine de Malines	$S_{102}S_{104}$	Royal Red	$S_{108}S_{114}$
	Angélys	$S_{105}S_{119}$	Dagan	$S_{101}S_{104}$	Kaiser	$S_{107}S_{114}$	Saint Mathieu	$S_{114}S_{116}$
	Ankara	$S_{103}S_{119}$	Dana's Hovay	$S_{101}S_{111}$	Kieffer	$S_{102}S_{119}$	Santa Maria	$S_{102}S_{103}$
	Aurora	$S_{101}S_{105}$	Delbard première	$S_{101}S_{109}$	Kalle	$S_{101}S_{108}$	Seckel	$S_{101}S_{102}$
	Ayers	$S_{101}S_{102}$	Delfrap	$S_{101}S_{109}$	Koonce	$S_{102}S_{105}$	Seigneur d'Espéren	$S_{101}S_{102}$
	Ballad	$S_{101}S_{119}$	Délices d'Hardenpont	$S_{101}S_{102}$	Koshisayaka	$S_{102}S_{119}$	Serenade	$S_{101}S_{108}$
	Bartlett	$S_{101}S_{102}$	Devoe	$S_{108}S_{118}$	La France	$S_{101}S_{119}$	Sierra	$S_{101}S_{108}$
	Bautomne	$S_{101}S_{108}$	Docteur Jules Guyot	$S_{101}S_{105}$	Lawson	$S_{115}S_{117}$	Silver Bell	$S_{110}S_{119}$
	Besi de Saint-Waast	$S_{101}S_{118}$	Doyenné d'hiver	$S_{101}S_{119}$	Le Lectier	$S_{104}S_{118}$	Red Jewell	$S_{101}S_{102}$
	Beurré Bosc	$S_{107}S_{114}$	Doyenné du Comice	$S_{104}S_{105}$	Limonera	$S_{101}S_{105}$	Red Clapp's	$S_{101}S_{108}$
	Beurré Clairgeau	$S_{105}S_{118}$	Doyenné Gris	$S_{102}S_{108}$	Louise Bonne d'Avranches	$S_{101}S_{102}$	Red Hardy	$S_{108}S_{114}$
	Beurré d'Anjou	$S_{101}S_{114}$	Duchesse d'Angouleme	$S_{101}S_{105}$	Magness	$S_{101}S_{105}$	Reimer Red	$S_{104}S_{114}$
	Beurré de l'Assomption	$S_{102}S_{106}$	El Dorado	$S_{101}S_{107}$	Marguerite Marillat	$S_{102}S_{105}$	Sirrine	$S_{101}S_{107}$
	Beurré Giffard	$S_{101}S_{106}$	Eletta Morettini	$S_{105}S_{114}$	Max Red Bartlett	$S_{101}S_{102}$	Spadona	$S_{101}S_{103}$
	Beurré Hardy	$S_{108}S_{114}$	Emile d'Heyst	$S_{102}S_{119}$	Maxine	$S_{101}S_{113}$	Spadona estiva	$S_{101}S_{103}$
	Beurré Jean Van Geert	$S_{102}S_{104}$	Ercolini	$S_{103}S_{104}$	Michaelmas Nelis	$S_{102}S_{107}$	Spadoncina	$S_{102}S_{103}$
	Beurré Lubrum	$S_{101}S_{104}$	Espadona	$S_{101}S_{110}$	Moonglow	$S_{101}S_{114}$	Star	$S_{101}S_{108}$

(continued)

Table 10.1 (continued)

Species	Cultivar	*S*-genotype	Cultivar	*S*-genotype	Cultivar	*S*-genotype	Cultivar	*S*-genotype
	Beurré Precoce Morettini	$S_{101}S_{103}$	Ewart	$S_{102}S_{114}$	Napoleon	$S_{101}S_{102}$	Starking Delicious	$S_{101}S_{113}$
	Beurré Superfin	$S_{101}S_{110}$	Fertility	$S_{107}S_{118}$	Norma	$S_{101}S_{104}$	Starkrimson	$S_{101}S_{108}$
	Blickling	$S_{102}S_{110}$	Flemish Beauty	$S_{101}S_{108}$	Nouveau Poiteau	$S_{107}S_{114}$	Summer Doyenne	$S_{101}S_{106}$
	Bon Rouge	$S_{101}S_{102}$	Fondante Thirriot	$S_{101}S_{103}$	Old Home	$S_{101}S_{113}$	Sweet Blush	$S_{101}S_{119}$
	Blanquilla	$S_{101}S_{103}$	Forelle	$S_{101}S_{116}$	Olivier de Serres	$S_{101}S_{110}$	Tosca	$S_{102}S_{104}$
	Bon-Chrétien d'Hiver	$S_{101}S_{118}$	French Bartlett	$S_{101}S_{105}$	Onwards	$S_{101}S_{104}$	Triomphede Vienne	$S_{105}S_{110}$
	Bristol Cross	$S_{102}S_{119}$	Garbar	$S_{107}S_{115}$	Orient	$S_{101}S_{102}$	Turnbull Giant	$S_{104}S_{113}$
	California	$S_{101}S_{104}$	General Leclerc	$S_{102}S_{118}$	Ovid	$S_{102}S_{118}$	Tyson	$S_{101}S_{105}$
	Canal Red	$S_{102}S_{104}$	Gentile	$S_{101}S_{106}$	Packham's Triumph	$S_{101}S_{103}$	Urbaniste	$S_{104}S_{119}$
	Cascade	$S_{101}S_{104}$	Glou Morceau	$S_{104}S_{110}$	Passe Crassane	$S_{110}S_{119}$	Verdi	$S_{101}S_{119}$
	Chapin	$S_{102}S_{115}$	Grand Champion	$S_{101}S_{104}$	Pera d'Agua	$S_{101}S_{102}$	William Precoce	$S_{101}S_{105}$
	Charles Ernest	$S_{105}S_{110}$	Harrow Crisp	$S_{101}S_{105}$	Pierre Cornelle	$S_{101}S_{118}$	William's	$S_{101}S_{102}$
	Clapp's Favorite	$S_{101}S_{108}$	Harrow Delight	$S_{101}S_{105}$	Pierre Tourasse	$S_{102}S_{105}$	William's Bon-Chrétien	$S_{101}S_{102}$
	Clapp's Rouge	$S_{101}S_{108}$	Harrow Sweet	$S_{102}S_{105}$	Precoce di Fiorano	$S_{101}S_{103}$	Washington	$S_{101}S_{103}$
	Colorée de Juillet	$S_{101}S_{115}$	Hartman	$S_{101}S_{104}$	Precoce du Trevoux	$S_{101}S_{102}$	Wilder	$S_{101}S_{111}$
	Comte de Flandre	$S_{102}S_{111}$	Harvest Queen	$S_{101}S_{102}$	President Héron	$S_{110}S_{118}$	Winter Cole	$S_{101}S_{107}$
	Comte de Lambertye	$S_{102}S_{110}$	Highland	$S_{101}S_{104}$	Rocha	$S_{101}S_{105}$		
	Concorde	$S_{104}S_{108}$	Honey Sweet	$S_{102}S_{104}$	Red Anjou	$S_{101}S_{114}$		
	Condo	$S_{104}S_{119}$	Howell	$S_{101}S_{104}$	Rogue Red	$S_{105}S_{114}$		

(continued)

Table 10.1 (continued)

Species	Cultivar	*S*-genotype	Cultivar	*S*-genotype	Cultivar	*S*-genotype	Cultivar	*S*-genotype
Interspecific hybridization	11-11	S_2S_{26}	Huasu	S_5S_d	Qiyuesu	S_4S_d	Yaqing	S_4S_{17}
	2-15	S_4S_{12}	Jimi	S_1S_{16}	Shenbuzhi	S_5S_d	Zaoguan	S_4S_{17}
	Beifeng	S_4S_a	Jinmili	$S_{21}S_{28}$	Xingcheng2-23	S_1S_8	Zaomeisu	S_3S_{35}
	Bishan no.2	S_4S_{16}	Jinshuisu	S_4S_{21}	Xinli	$S_{28}S_d$	Zaosu	$S_{22}S_{35}$
	Chaoxianyangli	SeS_3	Jinxiang	$S_{34}S_{37}$	Xinya	S_4S_{34}	Zaosuwei	S_1S_d
	Chikusui	S_3S_4	Jinxiangshui	S_1S_i	Xuefang	S_4S_{16}	Zaoxiangshui	$S_{26}S_{42}$
	Dongmi	S_1S_{42}	Ningmenghuang	$S_{31}S_{32}$	Xuefen	S_3S_x	Zhongai No.1	S_1S_{17}
	Hongxiu no.2	S_1S_{12}	Nongjiaxingao	S_3S_9	Xuefeng	S_4S_{16}	Zhongai No.2	$S_{19}S_{34}$
	Huajin	S_4S_4	Pingboxiang	S_1S_8	Xueqing	S_3S_{16}	Zhongli No.1	S_4S_{35}
	Huangguan	S_4S_{16}	Qinghua	S_1S_4	Xueying	S_3S_{16}	Zhongli no.2	S_4S_{31}
Landrace	Danze	S_3S_5	Hongjujuli	$S_{22}S_{28}$	S7	S_1S_{17}	Wucang	S_2S_3
	Diaodan	S_dS_e	Kuitian	S_dS_e	Shageda	$S_{36}S_d$	Xumo	S_2S_5
	Douli	$S_{30}S_{31}$	Lingwuduli	$S_{27}S_{36}$	Shunxiang	S_dS_e	Yucui	S_2S_4
	Heli	$S_{19}S_{29}$	S5	$S_{17}S_{31}$	Xingyeli	$S_{22}S_c$		

Table 10.2 Renumbering and integration of *S-RNase* alleles in Asian *Pyrus* species

New allelic designation	Former allelic designation	*Pyrus* species	Genbank accession no.
S_6	S_6	*P. pyrifolia*	AB002142.1
	S_{33}	*P. ussuriensis*	DQ138081.1
S_7	S_7	*P. pyrifolia*	AB002143.1
	S_{27}	*P.* × *bretschneideri*	EF643640.1
S_8	S_8	*P. pyrifolia*	AB104908.1
	S_{28}	*P.* × *bretschneideri*	EU375364.1, AY562394.1
	S_{28}	*P. sinkiangensis*	EF566872.1
	S_{34}	*P. pyrifolia*	DQ224345.1
S_{12}	S_{12}	*P. pyrifolia*	EU117115.1, AB426604.1, AY249427.2, HM047239.1
	S_{12}	*P.* × *bretschneideri*	EU081889.1
	S_{36}	*P. pyrifolia*	DQ417607.1
$S_{13.1}$	S_{13}	*P.* × *bretschneideri*	DQ414812.1
$S_{13.2}$	S_{13}	*P. pyrifolia*	AY249428.2, HM047240.1
S_{15}	S_{15}	*P. pyrifolia*	EF643630.1, AY249430.2
	S_{38}	*P. pyrifolia*	DQ666956.1
S_{16}	S_{16}	*P. pyrifolia*	AY249431.2
	S_{16}	*P.* × *bretschneideri*	DQ991388.1, EF643635.1
	S_{31}	*P. pyrifolia*	DQ072113.1
S_{17}	S_{17}	*P.* × *bretschneideri*	EU101466.1, AY249432.3
	S_{34}	*P. pyrifolia*	DQ269500.1
	S_{34}	*P.* × *bretschneideri*	DQ414813.1, DQ494676.1
S_{18}	S_{18}	*P.* × *bretschneideri*	EF643636.1, AY249433.2
	S_{39}	*P. pyrifolia*	DQ666957.1
S_{20}	S_{20}	*P.* × *bretschneideri*	EU360894.1, AY250988.2
	S_{29}	*P.* × *bretschneideri*	EU101462.1, AY601098.1
S_{31}	S_{31}	*P.* × *bretschneideri*	DQ124366.1
$S_{32.1}$	S_{32}	*P. pyrifolia*	DQ072114.1
$S_{32.2}$	S_{32}	*P. ussuriensis*	EU336979.1, DQ124367.1
S_{38}	S_{38}	*P.* × *bretschneideri*	EF643631.2, DQ839239.1
S_{39}	S_{39}	*P.* × *bretschneideri*	EU336980.1, DQ995285.1
S_{42}	S_{33}	Inter-specific hybridization	DQ082897.1
	S_{42}	*P. ussuriensis*	EF689006.1, EF088497.1, EF643637.1
	S_{42}	*P.* × *bretschneideri*	EF689007.1

fact, re-identification of these alleles should be performed in the same pear cultivars, as it is necessary. Moreover, several *S-RNase* alleles of exceptionally high identities should also be tested using cross-pollination of pear cultivars to determine functions of these alleles, such as

those of S_1-*RNase* in *P. pyrifolia* and S_{111}-*RNase* in *P. communis*, which have yielded three different residues.

10.3.1.2 Structural Features of *S-RNase* Alleles

Pyrus S-RNase alleles have five conserved regions, including C1, C2, C3, RC4, and C5, along with a relative hypervariable (RHV) region. Furthermore, amino acid sequences of these alleles contain cysteine and histidine residues that play important roles in S-RNase functions (Fig. 10.2). The only RHV located between the conserved C2 and C3 regions in *Pyrus* is different from the two hypervariable regions, HVa and HVb, found in Solanaceae and Plantaginaceae, although they have similar functions in enriching indels and substitutes. Similar to RNase T2 and RNase Rh, RHV sequences have lower identities among different *S-RNase* alleles, as well as catalytic histidine residues. Besides RHVs, other variable regions have also been detected between the conserved C1 and C2 regions and the upstream region of the conserved C5 region, which are likely associated with *S-RNase* allele-specificity.

Pyrus S-RNase alleles contain only a single intron, while *Prunus S-RNase* alleles have two introns. This intron is inserted into the RHV region and exhibits strong length and sequence polymorphisms. Depending on the size of this intron, ranging from 99 to 1,709 bp, a PCR-based analysis could be used to distinguish different *S-RNase* alleles on either agarose or polyacrylamide gels. This intron is subject to mutations, thus allowing *S-RNase* alleles to effectively maintain their GSI functions.

10.3.2 Pollen-*S* Determinants

Following Northern and Southern blot analyses of pear styles, it has been observed that the S_4-RNase in self-compatible pear cultivars is absent, but this absence does not influence functionality of pollen (Sassa et al. 1997). Thus, the likelihood that S-RNase controls pollen SI is dismissed. Therefore, what is the gene(s) controlling pollen SI? A series of propositions have been made. First, this gene(s) should be specifically expressed in the pollen, and not in other tissues, including the style. Second, this gene(s) should be tightly linked with an *S-RNase* gene and will not undergo recombination, as SI is well maintained in gametophytic species. Third, this gene (s) must have high levels of sequence polymorphisms so that it could be specifically recognized by stylar S-RNases. Based on these propositions, flanking sequences around *S-RNase* alleles have been analyzed using genome sequencing. An *F-box* gene is detected within the *S*-locus, and it has been proposed as a good candidate gene controlling pollen SI in *Prunus* species (Entani et al. 2003; Ushijima et al. 2003).

In pear, the pollen-*S* determinant has been investigated using homologous cloning based on conserved regions of *F-box* genes present in the *S*-locus of *Malus* (apple) and *Prunus* species. A series of two *S*-locus *F-box* genes have been detected that are specifically expressed in the pollen and designated as *S*-locus *F-box* brother (*SFBB*) genes (Sassa et al. 2007). Initially, three *SFBB* genes, $SFBB_{4\text{-}\alpha}$, $SFBB_{4\text{-}\beta}$, and $SFBB_{4\text{-}\gamma}$, and an additional three genes, $SFBB_{5\text{-}\alpha}$, $SFBB_{5\text{-}\beta}$, and $SFBB_{5\text{-}\gamma}$, are found to co-segregate with S_4-*RNase* and S_5-*RNase*, respectively (Sassa et al.

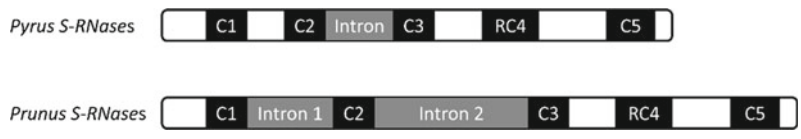

Fig. 10.2 Structures of *S-RNase* alleles in *Pyrus* and *Prunus*

2007). Of these six *SFBB* genes, $SFBB_{4\text{-}\beta}$ has 89.4% amino acid sequence identity to $SFBB_{5\text{-}\beta}$, while high amino acid identities are detected between each of $SFBB_{4\text{-}\alpha}$ and $SFBB_{5\text{-}\alpha}$ (96.4%), and of $SFBB_{4\text{-}\gamma}$ and $SFBB_{5\text{-}\gamma}$ (99.0%). This finding suggests that $SFBB_{\text{-}\beta}$ is more likely to be a pollen-*S* determinant than either $SFBB_{\text{-}\alpha}$ or $SFBB_{\text{-}\gamma}$.

To test for polymorphism of these *SFBB* genes, $SFBB_{\text{-}\gamma}$ genes have been isolated from different *S*-loci. Alignment of amino acid sequences showed that these $SFBB_{\text{-}\gamma}$ genes have lower sequence polymorphisms, with sequence identities ranging from 97.5 to 99.7% (Kakui et al. 2007). Moreover, phylogenetic analysis of *SFBB* genes isolated from pear and apple has revealed that all $SFBB_{\text{-}\gamma}$ genes are clustered together, while $SFBB_{\text{-}\alpha}$ and $SFBB_{\text{-}\beta}$ genes are distributed in different groups (Okada et al. 2011). Thus, the $SFBB_{\text{-}\gamma}$ gene is not deemed as the pollen-*S* determinant.

In other efforts to identify the pollen-*S* determinant, two pear bacterial artificial chromosome (BAC) libraries have been constructed containing S_4-*RNase* and S_4^{sm}-*RNase* alleles. It is found that the S_4^{sm}-locus has a 236 kb deletion, along with 34 open reading frames (ORFs), when compared to that of the S_4-locus (Okada et al. 2008). As the S_4-haplotype of the pear cultivar Osa-Nijisseiki lacks a pistil function, but retains a pollen function (Sato 1993), all 34 ORFs do not serve as pollen-*S* determinants. Within the S_4-locus, a total of six *SFBB* genes have been detected within a 649 Kb region around the S_4-*RNase*. Upstream *SFBB* genes are designated as $SFBB^{4-u1}$, $SFBB^{4-u2}$, $SFBB^{4-u3}$, and $SFBB^{4-u4}$, while downstream genes are designated as $SFBB^{4-d1}$ and $SFBB^{4-d2}$ (Okada et al. 2011). In contrast, 10 *SFBB* genes are detected within a 378 Kb region around the S_2-*RNase*, and these are designated as $SFBB^{2-u1}$, $SFBB^{2-u2}$, $SFBB^{2-u3}$, $SFBB^{2-u4}$, $SFBB^{2-u5}$, $SFBB^{2-d1}$, $SFBB^{2-d2}$, $SFBB^{2-d3}$, $SFBB^{2-d4}$, and $SFBB^{2-d5}$ (Okada et al. 2011). Furthermore, amino acid identities among these *SFBB* genes range between 67.1 and 93.1%, thus revealing high sequence polymorphisms. It is noteworthy to point out that it is puzzling as to which and how many *SFBB* genes are in fact involved in the SI reaction (Fig. 10.3).

In *Prunus*, a gene controlling the pollen-*S* determinant has been identified, wherein an *SFB* allele is stably and tightly linked to the *S-RNase* allele in any *S*-locus (Ushijima et al. 2003). Therefore, the stability of *SFBB* genes in several *Pyrus S*-loci has been investigated. Based on the classification of *SFBB* genes around *S-RNase*, a series of primer sets have been designed for each class of *SFBB* genes (Kakui et al. 2011). As expected, a large number of newly *SFBB* genes have been isolated from different *S*-loci. A phylogenetic analysis has revealed that genes $SFBB^{2-d1}$, $SFBB^{2-d2}$, $SFBB^{2-d4}$, and $SFBB^{2-d5}$ are only present in the S_2-locus, while $SFBB^{4-d2}$ and $SFBB^{4-u4}$ are present in the S_4-locus, and no orthologous genes have been found in other *S*-loci (Fig. 10.3). Therefore, none of these genes is the pollen-*S* determinant. Furthermore, additional detected *SFBB* genes have been classified into eight types, and designated as *SFBB1* to *SFBB8* (Kakui et al. 2011). Of these, *SFBB8* is coded by an $SFBB_{\text{-}\gamma}$ protein, while SFBB1 contains an S4F-box 0/$SFBB^{4-d1}$ that is lacking in the S_4^{sm}-haplotype, along with a truncated SFBB protein found in the S_5-haplotype. Due to these detected S-haplotypes of the pollen, which have normal functions in SI reactions, it is proposed that genes coded by SFBB1, SFBB4, and SFBB8 are not likely to be involved in the pollen-*S* determinant. Thus, the candidate gene(s) controlling the pollen SI is likely to be present in at least one of the SFBB2, SFBB3, SFBB5, SFBB6, and SFBB7

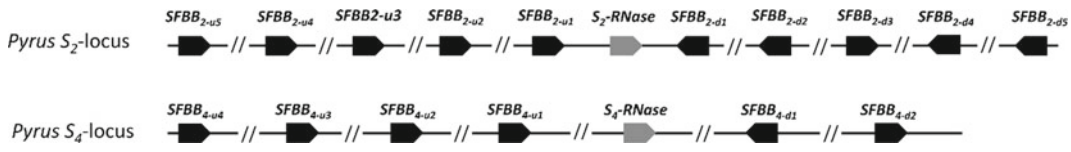

Fig. 10.3 Pear *SFBB* genes around *S-RNase* within S_2 and S_4-loci

types. With the development of gene editing and genetic transformation in pear, it is anticipated that the gene(s) related to SI will be resolved in the near future, and that the pollen-*S* determinant (s) will then be confirmed.

10.4 The Breakdown of Self-incompatibility

10.4.1 Stylar Mutants

10.4.1.1 Absence of the *S_4-RNase* Allele in Cultivar Osa-Nijisseiki

The pear cultivar Osa-Nijisseiki (*P. pyrifolia*) is a bud mutant of the self-incompatible pear cultivar Nijisseiki. Reciprocal crosses have demonstrated that the pollen of cv. Osa-Nijissiki is self-compatible. Furthermore, it is found that pollen of cv. Osa-Nijisseiki is cross-incompatible with pistils of cv. Nijisseiki, while the reciprocal cross has demonstrated that the pollen of cv. Nijisseiki is compatible with pistils of cv. Osa-Nijisseiki (Hirata 1989). This finding suggests that the breakdown of SI in cv. Osa-Nijisseiki has resulted from a stylar mutation.

To better understand the nature of the mutation in pistils of cv. Osa-Nijisseiki, an IEF/SDS-PAGE electrophoresis has been conducted to detect S-RNase expression. It was found that S_2-RNase levels in cvs. Osa-Nijisseiki and Nijisseiki were similar; whereas, S_4-RNase was weakly expressed in cv. Osa-Nijisseiki compared to that in cv. Nijisseiki (Sassa et al. 1993). These findings were further confirmed by later studies conducted by Wu et al. (2007) and Zhang et al. (2000). Subsequently, expression of *S*-proteins was analyzed in pear flowers at different stages of development (Zhang et al. 2000). It was found that S_4-RNase was detectable in pistils of cv. Nijisseiki at 8 days before anthesis (DBA), and it was continuously synthesized until 2 days after anthesis (DAA), with about 4.7-fold increase in levels during these 10 days. In contrast, S_4-RNase was not detected in pistils of cv. Osa-Nijisseiki earlier than 6 DBA, and only low levels were detected at 4 DBA. This was followed by gradual increases in these levels concomitant with flower development. These findings indicated that S_4-RNase had similar, but time-lagged expression patterns in cv. Osa-Nijisseiki compared to those observed in cv. Nijisseiki. Moreover, coded protein levels in cv. Osa-Nijisseiki at 2 DAA corresponded to those detected in cv. Nijisseiki earlier than 4 DBA (Hiratsuka et al. 1999). Thus, it has been proposed that the breakdown of SI in cv. Osa-Nijisseiki was likely attributed to lower S_4-RNase levels present in its pistils.

Subsequently, nucleotide sequences of S_2- and *S_4-RNase* alleles were determined in stylar cDNAs of cv. Nijisseiki (Norioka et al. 1995), but the *S_4-RNase* allele could not be amplified from stylar cDNAs of cv. Osa-Nijisseiki. It was proposed that the *S_4-RNase* allele was unsuccessfully transcribed in styles of cv. Osa-Nijisseiki (Norioka et al. 1996). This finding was further supported by Northern blot analysis of *S-RNases* revealing that *S_2-RNase* could be detected in both cvs. Osa-Nijisseiki and Nijisseiki; whereas, *S_4-RNase* was only detectable in cv. Nijisseiki (Sassa et al. 2007). To assess whether or not *S_4-RNase* was absent from the genome of cv. Osa-Nijisseiki, probes for S_2- and *S_4-RNase* alleles were used in conducting Southern blot analyses. Surprisingly, no hybridization signal was detected for the *S_4-RNase* probe in cv. Osa-Nijisseiki, while a signal was detected for the *S_4-RNase* probe in cv. Nijisseiki. In contrast, hybridization signals for the *S_2-RNase* probe were detected in both cvs. Osa-Nijisseiki and Nijisseiki (Sassa et al. 1997). Therefore, the *S_4-RNase* was likely absent from the genome of cv. Osa-Nijisseiki.

To further confirm the absence of *S_4-RNase* in cv. Osa-Nijisseiki, BAC libraries were constructed for genomes of cvs. Osa-Nijisseiki and Nijisseiki. Following identification of BAC contigs around the *S_4-RNase* gene, chromosome-walking was conducted to assemble these overlapping BAC contigs and then used these for sequencing. Results of sequencing these BAC contigs revealed that a 236 kb region was deleted from the genome of the spontaneous mutant cv. Osa-Nijisseiki when compared to that

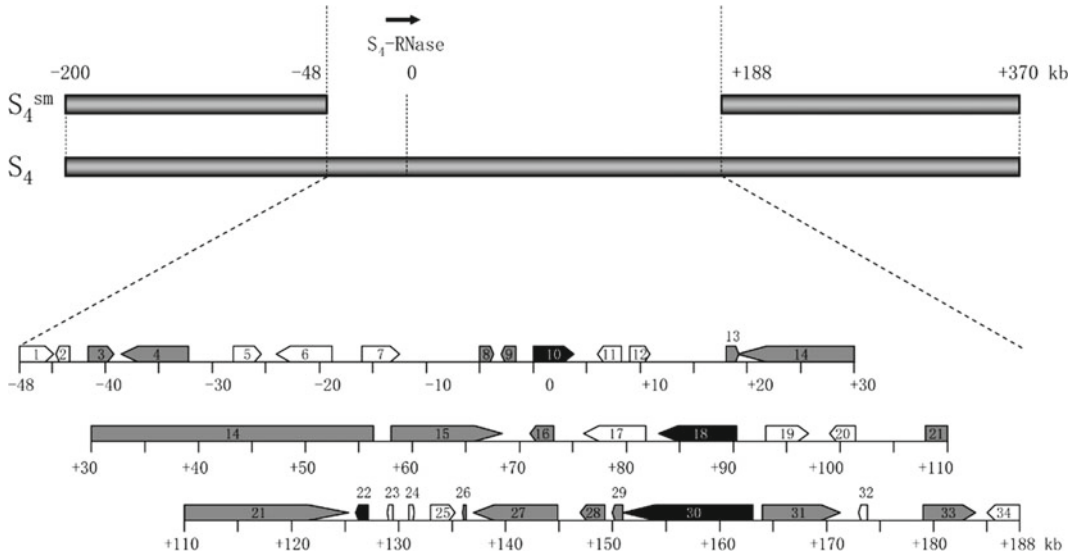

Fig. 10.4 Nucleotide sequence analysis of a deletion junction and a deleted region in S_4-haplotypes of cvs. Osa-Nijisseiki and Nijisseiki

of its original self-incompatible cv. Nijisseiki (Okada et al. 2008). More importantly, this deleted region would have likely contained the *S_4-RNase* allele (Fig. 10.4). Thus, it has been further confirmed that *S_4-RNase* was absent in cv. Osa-Nijisseiki, thereby resulting in an S_4-haplotype of a pistil that was functionally abnormal.

10.4.1.2 A Likely Low Level of Expression of S_{21}-RNase in Cultivar Yanzhuang

The pear cultivar Yanzhuang is a spontaneous mutant of the self-incompatible cultivar Yali (*P.* × *bretschneideri*). Almost 72.0% of 'Yanzhuang' fruit set is a result of self-fertilization, and it displays a strong self-compatibility (SC). To determine which of the reproductive tissues, either the pistil or pollen, have undergone mutation, reciprocal crosses have been made between cvs. Yanzhuang and Yali (Li et al. 2009). It is observed the cross of 'Yali' × 'Yanzhuang' has only an 8.5% fruit set, indicating that the pollen of 'Yanzhuang' is cross-incompatible with pistils of 'Yali'. On the other hand, the reciprocal cross of 'Yanzhuang' × 'Yali' has yielded a 78.0% fruit set, thereby indicating that the pollen of 'Yali' is compatible with pistils of 'Yanzhuang' (Li et al. 2009). Based on these findings, it has been determined that the pistil and pollen of 'Yanzhuang' are functionally abnormal and normal, respectively.

The *S*-genotype of 'Yali' has been initially identified as $S_{21}S_{34}$, as the nucleotide sequence of the *S_{34}-RNase* allele is identical to that of the *S_{17}-RNase* allele (Wang et al. 2017). Therefore, *S*-genotypes of 'Yali' and 'Yanzhuang' have been revised as $S_{17}S_{21}$. Genetic analysis has revealed that individuals in a self-pollinated progeny are genotyped as $S_{21}S_{21}$ and $S_{17}S_{21}$ with a 1:1 ratio ($\chi^2 = 0.02 < 0.05$). This has indicated that the S_{21}-haplotype can be inherited in a self-pollinated progeny. Therefore, the S_{21}-haplotype of the pistil in 'Yanzhuang' is deemed functionally abnormal.

To further explore the underlying reason(s) for these findings, expression levels of the *S_{21}-RNase* allele have been tested in pistils of both 'Yali' and 'Yanzhuang'. Unfortunately, the *S_{21}-RNase* allele is expressed at almost identical levels in pistils of 'Yali' and 'Yanzhuang', as well as those of the *S_{17}-RNase* allele. Thus, the

S_{21}-RNase allele is normally expressed in pistils of 'Yanzhuang'.

SDS-PAGE and protein profiles have also been used to assess levels of S_{21}-RNase expression in pistils of 'Yali' and 'Yanzhuang'. It has been found that SDS-PAGE could not detect any differences in protein profiles of S_{21}-RNase between these two cultivars. However, protein profiles of styles of 'Yanzhuang' have revealed presence of a single faint band, while those of pistils of 'Yali' have revealed presence of two different bands. These findings suggest that the S_{21}-RNase protein is expressed at lower levels in 'Yanzhuang' than in 'Yali', thus contributing to the breakdown of SI in 'Yanzhuang'.

This further begs the question as to what is the reason for the observed low levels of expression of S_{21}-RNase in pistils of 'Yanzhuang'? Alignments of nucleotide and amino acid sequences of *S_{21}-RNase* allele in 'Yanzhuang' and 'Yali' have detected non-synonymous substitution(s) located within the conserved C2 region (Wang et al. 2017). In this scenario, does this non-synonymous substitute(s) changes the function of the *S_{21}-RNase* allele? These questions deserve further attention in future studies.

10.4.1.3 Post-Transcript Modification of *S_{17}-RNase* (*S_{34}-RNase*) in Cultivar Zaoguan

The pear cultivar Zaoguan (*P.* × *bretschneideri*), derived from a cross between cvs. Yali and Qingyu, has an 86.0% fruit set following self-pollination, thus demonstrating a strong self-compatibility trait (Qi et al. 2011a, 2011b). The current assigned *S*-genotype of cv. Zaoguan is *S_4S_{17}*, while the previous *S*-genotype designation has been *S_4S_{34}*. To determine which of the reproductive tissues, either the pistil or pollen, must have undergone a mutation, the two self-incompatible cultivars Xinya and Yaqing have been selected from the progeny of 'Yali' 'Qingyu'. Both 'Yali' and 'Qingyu' have the same S-genotypes as that of 'Zaoguan', and have been used in crosses with 'Zaoguan'. Interestingly, it is observed that the pollen of 'Zaoguan' is cross-incompatible with the pistils of 'Xinya' and 'Yaqing', while the reciprocal crosses are found to be compatible with 'Zaoguan' (Qi et al. 2011a, 2011b). Therefore, the pistil and the pollen of 'Zaoguan' are deemed functionally abnormal and normal, respectively.

To determine which of the *S-RNase* alleles has experienced a loss of function in an SI reaction, self- and cross-pollinated progenies of 'Zaoguan', 'Xinya', and 'Yaqing' are *S*-genotyped by polymerase chain reaction (PCR) using allele-specific primers. Genetic analysis has revealed that individuals in self-pollinated progenies of 'Zaoguan' are *S*-genotyped as *S_4S_{17}* and *$S_{17}S_{17}$*, with a 1:1 segregation ratio ($\chi^2_{0.05,1} = 2.03 < 3.84$). Likewise, individuals in two cross-pollinated progenies of 'Zaoguan' × 'Xinya' and 'Zaoguan' × 'Yaqing' have also been genotyped as *S_4S_{17}* and *$S_{17}S_{17}$*, and yielding 1:1 segregation ratios of $\chi^2_{0.05} = 0.41$ and $\chi^2_{0.01} = 0.87 < 3.84$, respectively. These findings suggest that the *S_{17}*-haplotype of pistils of 'Zaoguan' is functionally abnormal. However, the S_{17}-RNase of 'Zaoguan' has identical amino acid sequences to those of 'Xinya' and 'Yaqing', thus indicating that the *S_{17}-RNase* of 'Zaoguan' has a complete gene structure. Therefore, what is the reason for the observed SI breakdown? Is it an issue of transcript levels? To address these questions, quantitative reverse transcription (qRT)-PCR has been conducted in styles of 'Zaoguan', 'Xinya', and 'Yaqing'. It is observed that each of *S_{17}-RNase* and *S_4-RNase* have similar levels of expression among these three pear cultivars. Thus, the *S_{17}-RNase* is deemed to be normally transcribed in these cultivars. Therefore, this begs the question as to whether or not the function of the *S_{17}-RNase* is blocked at the translational level? To address this question, S-RNase proteins are extracted from pistils of 'Zaoguan', 'Xinya', and 'Yaqing', and then subjected to two-dimensional gel electrophoresis (2D-PAGE). It is found that both S_4-RNase and S_{17}-RNase proteins are detected in both 'Xinya' and 'Yaqing', while S_{17}-RNase is not detected in the pistils of 'Zaoguan' (Fig. 10.5). Therefore, this indicates that *S_{17}-RNase* (*S_{34}-RNase*) is unsuccessfully translated to its corresponding S-glycoprotein in 'Zaoguan'. Taken altogether, it is proposed that the breakdown of SI in 'Zaoguan' is attributed to post-transcript modification of S_{17}-RNase.

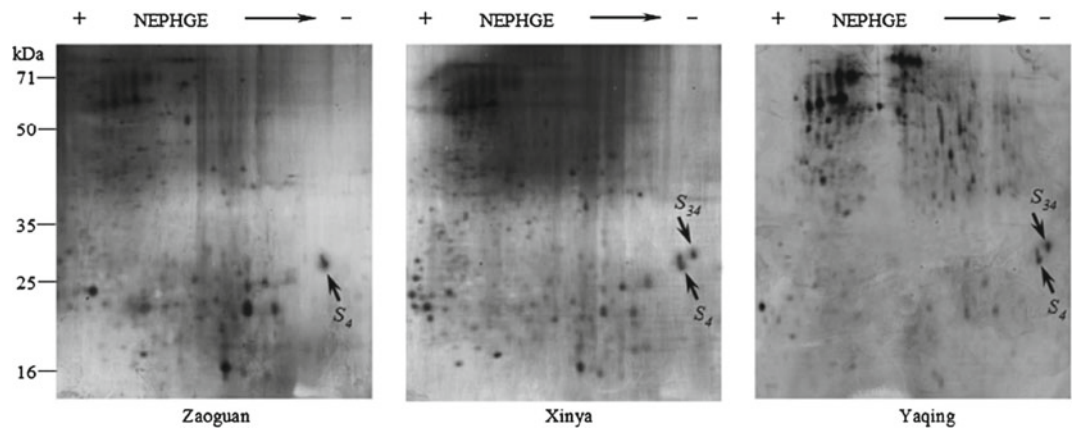

Fig. 10.5 2D-PAGE profiles of style extracts of cvs. Zaoguan, Xinya, and Yaqing

10.4.1.4 Transcript Modification of S_{21}-*RNase* in European Cultivars Abugo and Ceremeño

The following European pear cultivars, Abugo and Ceremeño (*P. communis*), exhibit 74.1 and 65.4% fruit set due to self-pollination, respectively, thus displaying strong self-compatibility (Sanzol 2009). The assigned *S*-genotypes of 'Abugo' and 'Ceremeño' are $S_{10}S_{21}$ and $S_{21}S_{25}$, respectively.

To determine which of the reproductive tissues, either the pistil or pollen, must have undergone a mutation, the following two self-incompatible cultivars, 'Williams' (S_1S_2) and 'Passe Crassane' ($S_{10}S_{21}$), were used in cross-hybridizations (Sanzol 2009). When 'Abugo' was used as a pollinator and crossed with 'Williams', a 29.1% fruit set was obtained, thus demonstrating cross-compatibility, and indicating that the pollen of 'Abugo' was normally functional for successful sexual fertilization. When 'Passe Crassane' was used as a pollinator and crossed with 'Abugo', only 18.5% fruit set was obtained, thus demonstrating cross-incompatibility. These findings suggested that pistils of 'Abugo' were functionally abnormal and contributing to the observed GSI reaction.

To assess the functional abnormality of the *S-RNase* allele, self- and cross-pollinated progenies of 'Williams', 'Abugo', and 'Delbard Esquise' are *S*-genotyped by PCR using allele-specific primers (Sanzol 2009). Genetic analyses have revealed that individuals in the cross-pollinated progeny of 'Williams' × 'Abugo' are assigned S_1S_{10}, S_1S_{21}, S_2S_{10}, and S_2S_{21} genotypes, thus elucidating that S_{10}- and S_{21}-haplotypes are inherited in this progeny. Furthermore, individuals in the self-pollinated progeny of 'Abugo' are genotyped as $S_{10}S_{21}$ and $S_{21}S_{21}$, with a 1:1 observed segregation ratio ($\chi^2 = 0$) (Sanzol 2009); whereas, individuals in the cross-pollinated progeny of 'Abugo' × 'Delbard Esquise' (S_4S_{21}) are genotyped as S_4S_{10}, $S_{10}S_{21}$, S_4S_{21}, and $S_{21}S_{21}$, with a 10:14:6:8 segregation ratio ($\chi^2_{0.05} = 2.4$). These findings suggest that the S_{21}-haplotype of the pistil in 'Abugo' can accept pollen of the same *S*-genotype. Taken together, it is proposed that the S_{21}-haplotype of the pistil of 'Abugo' is functionally abnormal.

However, does this imply that the S_{21}-haplotype is also disordered in 'Ceremeño'? To assess this, several cross- and self-hybridizations have been made (Sanzol 2009). Genetic analyses have revealed that individuals in the self-pollinated progeny of 'Ceremeño' are assigned $S_{21}S_{21}$ and $S_{21}S_{25}$ genotypes, thus indicating that the S_{21}-haplotype of the pollen is self-compatible. Genetic analyses of S-genotypes in cross-pollinated progenies have revealed that individuals in the progeny of 'Williams' × 'Ceremeño' are genotyped as S_1S_{21}, S_1S_{25}, S_2S_{21}, and S_2S_{25}, thus indicating that S_{21}-

and S_{25}-haplotypes are inherited in this progeny (Sanzol 2009). On the other hand, individuals in the cross-pollinated progeny of 'Passe Crassane' × 'Ceremeño' are genotyped as $S_{10}S_{25}$ and $S_{21}S_{25}$, thus indicating that the S_{21}-haplotype of the pollen in 'Ceremeño' has a normal function; whereas, individuals in the cross-pollinated progeny of 'Ceremeño' × 'Passe Crassane' are genotyped as $S_{10}S_{21}$, $S_{21}S_{21}$, $S_{10}S_{25}$, and $S_{21}S_{25}$, thus revealing that pistils of 'Ceremeño' are compatible with the S_{21}-haplotype of pollen in the self-compatible 'Passe Crassane' (Sanzol 2009). Therefore, the S_{21}-haplotype of pistils of 'Ceremeño' is functionally abnormal.

Expression of *S_{21}-RNase* in pistils of 'Abugo', 'Ceremeño', and 'Passe Crassane' was determined using PCR analysis (Sanzol 2009). It was found that *S_{21}-RNase* could not be detected in the pistils of both 'Abugo' and 'Ceremeño', but it was detected in the pistils of 'Passe Crassane'. This finding suggested that the breakdown of SI was attributed to abnormal expression of *S_{21}-RNase* in the pistils of both 'Abugo' and 'Ceremeño'. Alignments of nucleotide sequences between normal and abnormal expressed *S_{21}-RNases* identified three non-synonymous substitutes in coding sequences, a retrotransposon inserted within an intron, along with several point mutations and indels found within the 3'UTR (Sanzol 2009). Thus, it has been proposed that the functionally abnormal *S^{o}_{21}-RNase* was attributed to these observed mutations.

10.4.2 Pollen Mutants

The pear cultivar Jinzhui is a spontaneous mutant of the self-incompatible cultivar Yali, with a 72.0% fruit set, thus demonstrating strong self-compatibility. Similar to 'Yali' and 'Yanzhuang', the *S*-genotype of 'Jinzhui' is $S_{17}S_{21}$, although it has been previously assigned an $S_{21}S_{34}$ genotype (Zhang et al. 2007). To assess the mechanism of SI breakdown in 'Jinzhui', the pear cultivar Yali has been used in crosses with 'Jinzhui'. It is observed that the pollen of 'Yali' is cross-incompatible with the pistils of 'Jinzhui'; whereas, in the reciprocal cross, 78.0% fruit set is obtained, thus displaying strong cross-compatibility (Li et al. 2009). This indicates that the pollen of 'Jinzhui' is functionally abnormal.

To further study the functionally abnormal *S*-haplotype(s) in the pollen of 'Jinzhui', *S*-genotypes have been identified in self- and cross-pollinated progenies (Li et al. 2009). Genetic analyses have identified that 29 individuals in a self-pollinated progeny of 'Jinzhui' are genotyped as $S_{17}S_{17}$ and $S_{17}S_{21}$, thus suggesting that it is only the S_{17}-haplotype that is functionally abnormal (Li et al. 2009). However, when the number of individuals is expanded to include a population of 94, these individuals are genotyped as $S_{17}S_{17}$, $S_{17}S_{21}$, and $S_{21}S_{21}$, which is similar to genotyping results obtained for individuals in the cross-pollinated progeny of 'Yali' × 'Jinzhui'. This outcome suggests that both S_{17}- and S_{21}-haplotypes are functionally abnormal (Wu et al. 2013).

Subsequently, pollen grains and pollen tubes of 'Yali' and 'Jinzhui' have been grown in vitro to further elucidate the viability of these tissues, and it is found that some pollen grains of 'Jinzhui' are aborted (Wu et al. 2013). However, this observation is not sufficient to explain the self-compatibility of 'Jinzhui'. As it is highly unlikely that two *S*-haplotypes of pollen must have undergone simultaneous mutations, it is proposed that it is more likely that a mutated modifier, located outside of the *S*-locus, is the one that takes part in such an SI reaction. In a similar study involving apricot cultivars, it has been found that the breakdown of SI may be caused by an *M*-locus, which is different from the *S*-locus (Wu et al. 2011; Zuriaga et al. 2012).

10.5 Polyploidy

The pear cultivar Sha01 is a spontaneous mutant of the self-incompatible cultivar Kuerlexiangli (*P. sinkiangensis*), genotyped as an $S_{22}S_{28}$, with an 84.0% fruit set, and displaying a strong self-compatibility (Heng et al. 2011). To test for

the breakdown of SI, 'Kuerlexiangli' is used in crosses with 'Sha01'. When 'Kuerlexiangli' is used as a pollinator, fruit set is 4.0%, thus demonstrating cross-incompatibility; however, in the reciprocal cross, fruit set is 84.0%, thereby demonstrating strong cross-compatibility (Qi et al. 2011a, 2011b). These findings reveal that the pistil and the pollen of 'Sha01' are functionally normal and abnormal, respectively.

To assess whether or not *S-RNases* are differentially expressed in pistils of 'Kuerlexiangli' and 'Sha01', qRT-PCR has been conducted. It is found that expression levels of both S_{22}-*RNase* and S_{28}-*RNase* in 'Kuerlexiangli' are almost identical to those detected in 'Sha01'. Moreover, alignments of nucleotide sequences of S_{22}-*RNase* and S_{28}-*RNase* in 'Sha01' and 'Kuerlexiangli' have revealed no differences in these two alleles. These findings have suggested that the two *S*-haplotypes in the pistils of 'Sha01' have the same allelic sequences and almost identical levels of expression to those of 'Kuerlexiangli' in a GSI reaction. Thus, this excludes the possibility that the observed cross-incompatibility between 'Kuerlexiangli' and 'Sha01' results from low levels of expression of *S-RNase* alleles in the pistils of 'Kuerlexiangli'.

Therefore, it is important to identify the reason(s) for the breakdown of SI in 'Sha01'. Allele-specific PCR has only identified two *S-RNase* alleles, S_{22}-*RNase* and S_{28}-*RNase*, in 'Sha 01', as well as in each of the individuals in a self-pollinated progeny. Using genomic DNA as template, semi-quantitative PCR has revealed that densities of amplification products of S_{22}-*RNase* and S_{28}-*RNase* alleles are different in the self-pollinated progeny, with observed segregation ratios of 1:3, 2:2, and 3:1 in all tested

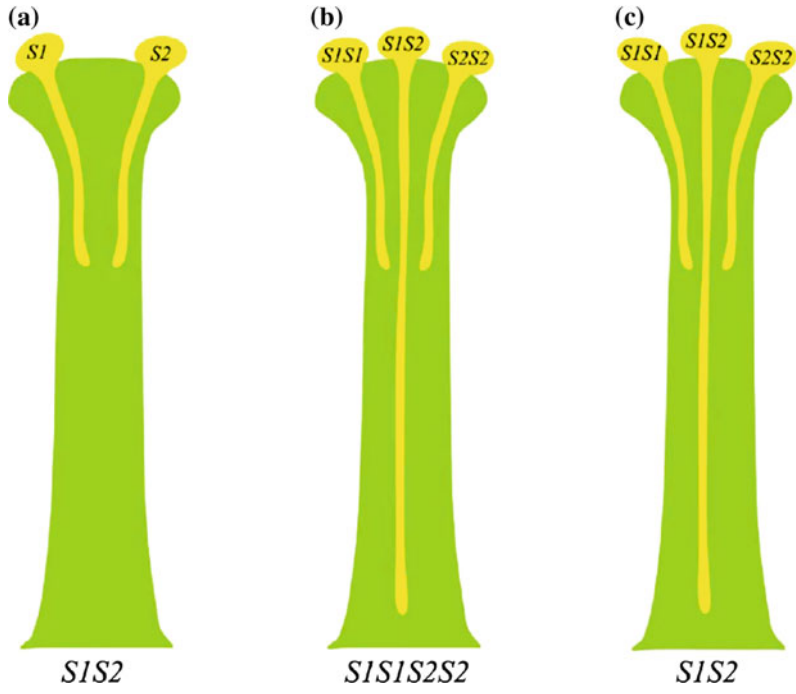

Fig. 10.6 A proposed scheme of how a hetero-diploid pollen grain leads to the breakdown of self-incompatibility in tetraploid plants. **a** During pollination of the S_1S_2 pistil with self-pollen, the S_1 pollen and the S_2 pollen will be rejected by the S_1S_2 pistil. **b** During pollination of an $S_1S_1S_2S_2$ pistil with self-pollen, both the S_1S_1 pollen and the S_2S_2 pollen will be rejected by the pistil. However, the S_1S_2 pollen is compatible with the $S_1S_1S_2S_2$ pistil because of competitive interactions. **c** During pollination of an S_1S_2 pistil with pollen from an $S_1S_1S_2S_2$ plant, the S_1S_1 pollen and the S_2S_2 pollen will be rejected by the pistil. However, the S_1S_2 pollen is compatible with the $S_1S_1S_2S_2$ pistil because of competitive interactions

individuals. This indicates that the numbers of *S_{22}-RNase* and *S_{28}-RNase* alleles are likely different in these individuals. Considering that these different numbers have probably arose as a result of polyploidy, the numbers of chromosomes and nuclear DNA contents are measured in both pear cultivars, 'Sha01' and 'Kuerlexiangli', and in several individuals in the self-pollinated progeny of 'Sha 01'. As expected, it is determined that the number of chromosomes and nuclear DNA contents in 'Sha01' and in individuals of the self-pollinated progeny are almost twofold than those determined in 'Kuerlexiangli'. Genetic analyses have revealed that the observed ratio of individuals genotyped as $S_{22}S_{22}S_{22}S_{28}$: $S_{22}S_{22}S_{28}S_{28}$: $S_{22}S_{28}S_{28}S_{28}$ is approximately 1:4:1 ($\chi^2_{0.05,\ 2} = 1.64 < 6.19$) which is quite different from the expected ratio ($\chi_{0.05,\ 2}{}^2 = 14.07 < 9.49$). This indicates that 'Sha01' is a tetraploid, and its heteroallelic diploid pollen could only achieve self-fertilization. The molecular mechanism of heteroallelic diploid pollen contributing to the breakdown of SI could result from competitive interactions between the two *S*-haplotypes in the pollen.

Likewise, the pear cultivar Daguohuanghua is a spontaneous mutant of the self-incompatible cultivar Huanghua (*P. pyrifolia*). These two cultivars are genotyped by *S_1-RNase* and *S_2-RNase* (Wu et al. 2007). However, fruit set as a result of self-fertilization is over 60.0% in 'Daguohuanghua', thus demonstrating strong self-compatibility. Using 'Huanghua' as a pollinator to hybridize with 'Daguohuanghua', it is observed that fruit set is only 1.0%, thus demonstrating cross-incompatibility; whereas, fruit set of the reciprocal cross is over 70.0%, thus displaying cross-compatibility. These findings suggest that the pistil and the pollen of 'Daguohuanghua' are functionally normal and abnormal, respectively. As sizes of leaves, fruits, and anthers are larger in 'Daguohuanghua' than in 'Huanghua', it is speculated that 'Daguohuanghua' is a tetraploid mutant. This proposed hypothesis has been confirmed using cytological analysis, wherein the number of chromosomes in 'Daguohuanghua' is found to be twofold that of 'Huanghua'. Thus, the breakdown of SI is probably attributed to competitive interactions in hetero-diploid pollen (Fig. 10.6). Similar results have been reported in the hetero-tetraploid Chinese cherry (Huang et al. 2008; Gu et al. 2010, 2013, 2014).

References

Brewbaker JL (1954) Incompatibility in autotetraploid *Trifolium repens* L. I. Competition and self-compatibility. Genetics 39:307–316

De Nettancourt D (2001) Incompatibility and incongruity in wild and cultivated plants. Springer, Berlin, p 322p

Entani T, Iwano M, Shiba H, Che FS, Isogai A, Takayama S (2003) Comparative analysis of the self-incompatibility (S-) locus region of *Prunus mume*: identification of a pollen-expressed *F-box* gene with allelic diversity. Genes Cells 8:203–213

Goldway M, Takasaki-Yasuda T, Sanzol J, Mota M, Zisovich A, Stern RA, Sansavini S (2009) Renumbering the S-RNase alleles of European pears (*Pyrus communis* L.) and cloning the S109 RNase allele. Sci Hortic 119:417–422

Chen J, Wang P, de Graaf BHJ, Zhang H, Jiao H, Tang C, Zhang S, Wu J (2018) Phosphatidic acid counteracts S-RNase signaling in pollen by stabilizing the actin cytoskeleton. Plant Cell 30:1023–1039

Gao YB, Zhou HS, Chen JQ, Jiang XT, Tao ST, Wu JY, Zhang SL (2015) Mitochondrial dysfunction mediated by cytoplasmic acidification results in pollen tube growth cessation in *Pyrus pyrifolia*. Physiol Plant 153:603–615

Gu C, Zhang SL, Huang SX, HengW Liu QZ, Wu HQ, Wu J (2010) Identification of S-genotypes in Chinese cherry cultivars (*Prunus pseudocerasus*). Tree Genet Genomes 6:579–590

Gu C, Liu QZ, Yang YN, Zhang SJ, Khan MA, Wu J, Zhang SL (2013) Inheritance of hetero-diploid pollen S-haplotype in self-compatible tetraploid Chinese cherry (*Prunus pseudocerasus* Lindl). PLoS ONE 8: e61219

Gu C, Liu QZ, Khan MA, Wu J, Zhang SL (2014) Hetero-diploid pollen grains that represent self-compatibility are incompatible with non-self receptors in tetraploid Chinese cherry (*Prunus pseudocerasus* Lindl). Tree Genet Genomes 10:619–625

Heng W, Wu J, Wu H, Cao Y, Nishio T, Zhang SL (2011) Recognition specificity of self-incompatibility in *Pyrus* and *Malus*. Mol Breed 28:549–557

Heng W, Wu HQ, Huang SX, Zhang SJ, Wu J, Fang CQ, Zhang SL (2015) Identification of genotypes and novel RNases in native Chinese pear. J Hortic Sci Biotechnol 83(5):629–634

Hirata N (1989) Self-compatible mutant in Japanese pear. Gamma Field Symp 28:71–80

Hiratsuka S, Nakashima M, Kamasaki K, Kubo T, Kawai Y (1999) Comparison of an S-protein

expression between self-compatible and self-incompatible Japanese pear cultivars. Sex Plant Reprod 12:88–93

Hiratsuka S, Hirota M, Takahashi E, Hirata N (1985) Seasonal changes in the self-incompatibility and pollen tube growth in Japanese pears (*Pyrus serotine Rehd.*). J Japan Soc Hortic Sci 53:377–382

Hiratsuka S, Zhang SL, Nakagawa E, Kawai Y (2001) Selective inhibition of the growth of incompatible pollen tubes by S-protein in the Japanese pear. Sex Plant Reprod 13:209–215

Huang SX, Wu HQ, Li YR, Wu J, Zhang SJ, Heng W, Zhang SL (2008) Competitive interaction between two functional S-haplotypes confer self-compatibility on tetraploid Chinese cherry (*Prunus pseudocerasus* Lindl. cv. Nanjing Chuisi). Plant Cell Rep 27:1075–1085

Ishimizu T, Inoue K, Shimonaka M, Saito T, Terai O, Norioka S (1999) PCR-based method for identifying the S-genotypes of Japanese pear cultivars. Theor Appl Genet 98:961–967

Jiang XT, Gao YB, Zhou HS, Chen JQ, Wu JY, Zhang SL (2014) Apoplastic calmodulin promotes self-incompatibility pollen tube growth by enhancing calcium influx and reactive oxygen species concentration in Pyrus pyrifolia. Plant Cell Rep 33:255–263

Kakui H, Tsuzuki T, Koba T, Sassa H (2007) Polymorphism of *SFBB*-gamma and its use for S genotyping in Japanese pear (*Pyrus pyrifolia*). Plant Cell Rep 26:1619–1625

Kakui H, Kato M, Ushijima K, Kitaguchi M, Kato S, Sassa H (2011) Sequence divergence and loss-of-function phenotypes of S locus F-box brothers genes are consistent with non-self recognition by multiple pollen determinants in self-incompatibility of Japanese pear (*Pyrus pyrifolia*). Plant J 68:1028–1038

Lewis D (1976) Incompatibility in flowering plants. Recept Recognit Ser A2:167–198

Li MF, Li XF, Han ZhH, Shu HR, Li T (2009) Molecular analysis of two Chinese pear (*Pyrus bretschneideri* Rehd.) spontaneous self-compatible mutants, Yan Zhuang and Jin Zhui. Plant Biol 11:774–783

Liu ZQ, Xu GH, Zhang SL (2007) *Pyrus pyrifolia* stylar S-RNase induces alterations in the actin cytoskeleton in self-pollen and tubes in vitro. Protoplasma 232:61–67

Norioka N, Ohnishi Y, Norioka S, Ishimizu T, Nakanishi T, Sakiyama F (1995) Nucleotide sequences of cDNAs encoding S2- and S4-RNases (D49527 and D49528 for EMBL) from Japanese pear (*Pyrus pyrifolia* Nakai) (PGR95-020). Plant Physiol 108:1343

Norioka N, Norioka S, Ohnishi Y, Ishimizu T, Oneyama C, Nakanishi T, Sakiyama F (1996) Molecular cloning and nucleotide sequences of cDNAs encoding *S*-allele specific stylar RNases in a self-incompatible cultivar and its self-compatible mutant of Japanese pear, *Pyrus pyrifolia* Nakai. J Biochem 120:335

Okada K, Tonaka N, Moriya Y, Norioka N, Sawamura Y, Matsumoto T, Nakanishi T, Takasaki-Yasuda T (2008) Deletion of a 236 kb region around *S_4-RNase* in a stylar-part mutant *S_{4sm}*-haplotype of Japanese pear. Plant Mol Biol 66:389–400

Okada K, Tonaka N, Taguchi T, Ichikawa T, Sawamura Y, Nakanishi T, Takasaki-Yasuda T (2011) Related polymorphic F-box protein genes between haplotypes clustering in the BAC contig sequences around the S-RNase of Japanese pear. J Exp Bot 62:1887–1902

Qi YJ, Wang YT, Han YX, Qiang S, Wu J, Tao ST, Zhang SL, Wu HQ (2011a) Self-compatibility of 'Zaoguan' (*Pyrus bretschneideri* Rehd.) is associated with style-part mutations. Genetica 139:1149–1158

Qi YJ, Wu HQ, Cao YF, Wu J, Tao ST, Zhang SL (2011b) Heteroallelic diploid pollen led to self-compatibility in tetraploid cultivar 'Sha 01' (*Pyrus sinkiangensis* Yü). Tree Genet Genomes 7:685–695

Qu HY, Shang ZL, Zhang SL, Liu LM, Wu JY (2007) Identification of hyperpolarization-activated calcium channels in apical pollen tubes of *Pyrus pyrifolia*. New Phytol 174:524–536

Sassa H, Hirano H, Ikehashi H (1992) Self-incompatibility-related RNases in styles of Japanese pear (*Pyrus serotina* Rehd.). Plant Cell Physiol 33:811–814

Sassa H, Hirano H, Ikehashi H (1993) Identification and characterization of stylar glycoproteins associated with self-incompatibility genes of Japanese pear, *Pyrus serotina* Rehd. Mol Gen Genet 241:17–25

Sassa H, Nishio T, Kowyama Y, Hirano H, Koba T, Ikehashi H (1996) Self-incompatibility (S) alleles of the Rosaceae encode members of a distinct class of the T2/S ribonuclease superfamily. Mol Gen Genet 250:547–557

Sassa H, Hirano H, Nishio T, Koba T (1997) Pistil-specific self-compatible mutation caused by deletion of the *S-RNase* gene in Japanese pear (*Prunus serotina*). Plant J 12:223–227

Sassa H, Kakui H, Miyamoto M, Suzuki Y, Hanada T, Ushijima K, Kusaba M, Hirano H, Koba T (2007) *S* locus *F-box* brothers: multiple and pollen-specific *F-box* genes with *S* haplotype-specific polymorphisms in apple and Japanese pear. Genetics 175:1869–1881

Sato Y (1993) Breeding of self-compatible Japanese pear. In: Hayashi T, Omura M, Scott NS (eds) Techniques on gene diagnosis and breeding in fruit trees. FTRS (Fruit Tree Research Station), Tsukuba, Japan, pp 241–247

Sanzol J (2009) Pistil-function breakdown in a new *S*-allele of European pear, S^{o}_{21}, confers self-compatibility. Plant Cell Rep 28:457–467

Ushijima K, Sassa H, Dandekar AM, Gradziel TM, Tao R, Hirano H (2003) Structural and transcriptional analysis of the self-incompatibility locus of almond: identification of a pollen-expressed *F-box* gene with haplotype-specific polymorphism. Plant Cell 15:771–781

Wang CL, Xu GH, Jiang XT, Chen G, Wu J, Wu HQ, Zhang SL (2009) S-RNase triggers mitochondrial alteration and DNA degradation in the incompatible pollen tube of *Pyrus pyrifolia* in vitro. Plant J 57:220–229

Wang CL, Wu J, Xu GH, Gao YB, Chen G, Wu JY, Wu HQ, Zhang SL (2010) S-RNase disrupts tip-localized reactive oxygen species and induces nuclear DNA degradation in incompatible pollen tubes of *Pyrus pyrifolia*. J Cell Sci 123:4301–4309

Wang GM, Gu C, Qiao X, Zhao BY, Ke YQ, Guo BB, Hao PP, Qi KJ, Zhang SL (2017) Characteristic of pollen tube that grew into self-style in pear cultivar and parent assignment for cross-pollination. Scient Hort 216:226–233

Wu HQ, Zhang SL, Qu HY (2007) Molecular and genetic analyses of S-4(SM) RNase allele in Japanese pear 'Osa-Nijisseiki' (*Pyrus pyrifolia* Nakai). Plant Breed 126:77–82

Wu J, Gu C, Du YH, Wu HQ, Liu WS, Liu N, Lu J, Zhang SL (2011) Self-compatibility of 'Katy'apricot (*Prunus armeniaca* L.) is associated with pollen-part mutations. Sex Plant Reprod 24:23–35

Wu J, Li M, Li T (2013) Genetic features of the spontaneous self-compatible mutant, 'Jin Zhui' (*Pyrus bretschneideri* Rehd.). PLoS ONE 8:e76509

Zhang SL, Zhou JT, Xu YL, Chen DX, Xu GH, Wu GF (2003) Semi vitro culture of pear style and identification of the genotypes of pear self-incompatibility. Acta Hortic Sinica 30:703–706 (in Chinese)

Zhang YY, Zhang SL, Wu J, Zhang RP, Li XG (2007) Identification of S-genotypes in 14 pear cultivars. J Fruit Sci 24:135–139 (in Chinese)

Zhang SL, Hiralsuka S (2000) Cultivar and developmental differences in S-protein cincenlralion and self-incompatibility in Japanese pear. HortSci 35:917–920

Zuriaga E, Molina L, Badenes ML, Romero C (2012) Physical mapping of a pollen modifier locus controlling self-incompatibility in apricot and synteny analysis within the Rosaceae. Plant Mol Biol 79:229–242

Stone Cell Development in Pear

11

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Abstract

Pear (*Pyrus* spp.) is one of the most important deciduous fruit trees grown in the world. The genus *Pyrus* belongs to the subfamily Pomoideae of the family Rosaceae. Stone cells (sclereids), heavily lignified cells present in fruit flesh, serve as a distinctive trait of pear fruits. Stone cells are characterized by thickening and lignified cell walls, and their development is closely associated with lignin metabolism. The content and size of stone cell clusters are among the key factors in determining the internal quality of pear fruits. Not only are stone cells critically involved in fruit texture, but they are also closely associated with the overall flavor of pear fruits. Therefore, regulation of the size and content of stone cell clusters is key for improving fruit quality, and in promoting expansion of the pear industry. In this review, effects of stone cells on fruit quality, including texture, flavor, and response to disease, as well as the mechanism of stone cell development in pear fruits, including morphological characteristics, distribution, development, components, formation, and regulation mechanism, will be presented. Moreover, molecular mechanisms of pear lignin metabolism, including pear lignin monomers type, biosynthesis pathway, and identification of key gene families will be also summarized. Finally, we will share some ideas relevant to future research directions pertaining to stone cells in pear.

11.1 Introduction

Stone cells, also known as sclereids, are sclerenchyma cells that serve as a group of lignification cells found in plants that also include tracheary elements, endodermal cells, seed coat cells, and siliques cells (Cai et al. 2010; Barros et al. 2015). Based on their morphologies, sclereids can be divided into short sclereids, macrosclereids, osteosclereids, astrosclereids, and trichosclereids, among others. Stone cells are present in different plant tissues, including stems, leaves, fruits, and seeds, as they play roles in structural support and protection functions, such as in resistance against biotic and abiotic stresses (Zhao and Zhu 2014; Whitehill et al. 2016a, b). In *Pyrus* spp., stone cells are quite abundant in fruit flesh, thus serving a characteristic structural feature of pear fruits (Wu et al. 2013).

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Stone cells in pear fruits are short sclereids, and their development is mainly determined by the pear genotype, but they are also regulated by external environment factors (Tao et al. 2009; Brahem et al. 2017). Such stone cells present in fruit flesh of pears tend to form clusters, and aggregation of multiple stone cells is referred to as stone cell clusters (SCCs) (Nii et al. 2008; Zhang et al. 2017). Various studies have reported that stone cells are key factors in determining pear fruit quality (Li et al. 2019; Xue et al. 2019). Large size and content of SCCs in fruits will lead to decline of internal quality characteristics of pear fruits, which in turn, influence eating quality, as well as processing quality and overall economic value of pear fruits (Choi et al. 2007; Wu et al. 2013; Brahem et al. 2017; Cheng et al. 2017a; Zhang et al. 2017). Therefore, knowledge of the mechanism of development of stone cells, as well as that of regulatory controls of stone cell development, will facilitate cultivation and development of pear cultivars with low content and small size SCCs. This will in turn enhance market competitiveness of pear cultivars and promote sustainable development of the pear industry.

This chapter will cover current knowledge and advances in our understanding of the development mechanism of stone cells in pear fruits and will offer insights into future research studies in this field.

11.2 Stone Cells and Cultivated Pear Species

Pears are mainly divided into two groups, European and Asian pears (Wu et al. 2018). The major cultivated species in Europe and America is *Pyrus communis*, European pears. Whereas the major cultivated species in East Asia include *P. ussuriensis*, *P.* × *bretschneideri*, *P. pyrifolia*, and *P. sinkiangensis* (Xinjiang pear), all known as Asian pears (Lu et al. 2007; Wu et al. 2013, 2018). As content and size of SCCs vary in different pear genotypes, this contributes to differences in fruit quality among different cultivated pear species. Therefore, it is critical to determine the content and size of SCCs in cultivated pear species, including those of *P. communis*, *P. ussuriensis*, *P.* × *bretschneideri*, *P. pyrifolia,* and *P. sinkiangensis*.

11.2.1 SCC Contents in Different Pear Cultivars

Cao et al. (2010) analyzed and determined fruit flesh SCC contents of 265 pear cultivars. These consisted of 117 cultivars of *P.* × *bretschneideri*, 89 cultivars of *P. pyrifolia*, 35 cultivars of *P. ussuriensis*, eight cultivars of *P. sinkiangensis,* and 16 cultivars of *P. communis*. Mean values of SCC contents in fruit flesh (SCC content per 100 g fresh weight) in these different cultivars, belonging to these various cultivated pear species, were as follows: *P. ussuriensis* (1.887 g) > *P. sinkiangensis* (0.939 g) > *P. pyrifolia* (0.552 g) > *P. communis* (0.524 g) > *P.* × *bretschneideri* (0.462 g). These findings were highly valuable in pursuing other studies to characterize genotypic differences among these various cultivated species of pear.

11.2.2 Sizes of SCCs in Different Pear Cultivars

Sizes of SCCs (diameter of clusters) were subsequently determined in 287 pear cultivars. These included 118 cultivars of *P.* × *bretschneideri*, 96 cultivars of *P. pyrifolia*, 44 cultivars of *P. ussuriensis*, 12 cultivars of *P. sinkiangensis*, and 17 cultivars of *P. communis*. It was found that average proportions of SCCs with diameters larger than 300 μm were as follows: *P. ussuriensis* (59.92%) > *P.* × *bretschneideri* (58.27%) > *P. pyrifolia* (50.07%) > *P. sinkiangensis* (47.76%) > *P. communis* (41.02%). Furthermore, average proportions of SCCs with diameters larger than 250 μm were as follows: *P.* × *bretschneideri* (81.78%) > *P. ussuriensis* (81.36%) > *P. pyrifolia* (73.84%) > *P. sinkiangensis* (73.68%) > *P. communis* (69.32%) (Table 11.1) (Tian et al. 2011). Overall, it was reported that in different pear cultivated species, the average

Table 11.1 The average proportion (%) of SCCs, of different diameters, present in fruit flesh of five cultivated pear species

Pyrus species	Diameter of stone cell clusters (μm)					
	>500 (%)	300–500 (%)	250–300 (%)	200–250 (%)	150–200 (%)	<150 (%)
P. ussuriensis	9.85	50.07	21.44	11.13	5.85	1.66
P. pyrifolia	9.78	40.29	23.77	14.68	8.84	2.64
P. communis	2.97	38.05	28.30	18.59	10.20	1.89
P. sinkiangensis	6.21	41.55	25.92	15.64	8.66	2.02
P. × *bretschneideri*	10.98	47.29	23.51	11.36	5.49	1.37

proportion of SCCs with diameters of 300–500 μm was the highest (Table 11.1).

11.3 Stone Cells and Pear Fruit Quality Traits

Stone cells, including content, size, and degree of polymerization, have a significant impact on the internal quality of pear fruits (Choi et al. 2007; Cao et al. 2010; Tian et al. 2011; Yan et al. 2014). In addition, stone cells are also associated with incidence of hard-end disorder, durability, and juice composition of pear fruits (Konarska 2013; Lu et al. 2015; Brahem et al. 2017). Therefore, relationships between stone cells and fruit quality traits will be discussed in further detail.

11.3.1 Flesh Texture

Flesh texture is an important criterion for judging the quality of pear fruits for fresh consumption. It has been reported that the content of SCCs is positively correlated with adhesiveness and chewiness of pear fruits, as well as with fruit firmness (Choi et al. 2007). Moreover, the high content of SCCs in flesh tissues will lead to a gritty texture and a coarse taste; however, it is not significantly associated with fruit gumminess, springiness, and cohesiveness (Li et al. 2004; Choi et al. 2007; Konarska 2013).

The content of SCCs not only influences fruit flesh texture and taste, but its degree of polymerization also has a significant impact on textural characteristics (Kim and Choi 2004a; Yan et al. 2014). For example, although fruits of *P. ussuriensis* cv. Beijing have a higher content of SCCs than those of *P.* × *bretschneideri* cv. Dangshan Su, they have a softer flesh texture than those of 'Dangshan Su.' This is attributed to a lower degree of polymerization of SCCs detected in 'Beijing.' In another example, fruits of *P. pyrifolia* cv. Wonhwang are found to have low SCC content and low degree of polymerization, thus resulting in a highly soft fruit texture. Therefore, a high degree of polymerization of SCCs contributes to formation of gritty-textured fruit flesh (Yan et al. 2014).

It is worth noting that although total amounts of SCCs of fruits of some pear cultivars are similar, flesh textures of fruits of these cultivars can be different. Some cultivars have higher SCC contents, but if diameters of these SCCs are smaller than others, then their flesh textures will be relatively soft. On the other hand, while some cultivars have relatively low SCC contents, wherein diameters of their SCCs are larger than those of other cultivars, then their fruit flesh textures are coarser (Cao et al. 2010; Tian et al. 2011). For example, the content of large diameter SCCs (with a diameter between 80 and 260 μm) of fruits of 'Dangshan Su' are significantly higher than those of *P. pyrifolia*, *P. communis*, and hybrid *P.* × *bretschneideri* × *P. communis*, and it is this high content of large diameter SCCs that leads to a coarse texture of flesh detected in 'Dangshan Su' fruits (Li et al. 2017).

Studies have shown that if the diameter of SCCs is less than 150 μm, then the flesh texture will be highly soft and with no grittiness;

whereas, if the diameter of SCCs is between 150 and 250 μm, then flesh texture will be soft, but with a slight gritty taste. On the other hand, if the diameter of SCCs is more than 250 μm, then the flesh texture will be coarse and gritty.

Taking into account the influences of both content and size of SCCs on flesh texture, it has been reported that SCC content, of diameters >250 μm in size, in fruit flesh per 100 g is an important indicator of texture of flesh of pear fruits (Li et al. 2004, 2017; Tian et al. 2011).

11.3.2 Sugar Content

Sugar is one of the main components of soluble solids. In turn, soluble solid content is an important indicator of the internal quality of pear fruits, as it impacts fruit flavor and consumer appreciation (Choi et al. 2007; Gao et al. 2016; Han et al. 2017).

It is reported that SCC content in pear fruits has little effect on the contents of fructose, sorbitol, and glucose; however, there is a significant negative correlation between SCC content and sucrose content in pear fruit (Kim et al. 2004a; Choi et al. 2007). Therefore, presence of high contents of SCCs may lead to reduced levels of sugar, thereby contributing to an inferior flavor in pear fruit.

11.3.3 Hard End Disorder

Hard end, or black end, is a physiological disorder of pear fruits (Wang et al. 2018). This physiological disorder appears as a result of delayed development of tissues, first resulting in protrusion of the calyx and/or enlargement of the calyx opening when the fruit is half-way through its growth. At first, epidermal tissues of affected areas turn shiny;however, as the disease progresses, tissues harden, and the surrounding mature calyx turns dry, prominent, and black in coloration. Hard end fruit loses its crisp and juicy taste, and this will also have an adverse effect on both internal and external qualities of affected pear fruits (Nii et al. 2008; Lu et al. 2015; Wang et al. 2017a, b).

In comparison with normal healthy fruit, the content and diameter of SCCs of the calyx end of hard end fruit are significantly higher than those of normal fruit at each of the stages of development (Lu et al. 2015; Wang et al. 2018). Therefore, massive accumulation of SCCs promotes hardening of fruit flesh, which is one of the factors triggering hard-end disorder.

11.4 Structural Components of Stone Cells in Pear Fruits

The basic components of plant cell walls generally include polysaccharides (cellulose, hemicellulose, and pectin polysaccharide), lignin, proteins, and mineral compounds, among others (Anderson et al. 2015; Zhong and Ye 2015). The proportion of cellulose in cell wall components is 40.6–51.2%, while hemicellulose is 28.5–37.2%, and lignin is 13.6–28.1% (Pauly and Keegstra 2008). Disruption of biosynthesis of these components will have an impact on cell wall development and even lead to cell wall deformity (Anderson et al. 2015; Zhong and Ye 2015). Therefore, it is important to have a good understanding of structural components of stone cells for future efforts in regulating development of stone cells of pear fruits.

Stone cells of pear fruits are characterized by thickened and heavily lignified secondary cell walls (Tao et al. 2009; Jin et al. 2013). Plant secondary cell walls are mainly composed of lignin, cellulose, and hemicelluloses (xylan and glucomannan). Among these three components, cellulose microfibrils and hemicelluloses form the skeleton structure of secondary cell walls, affording cell walls a degree of mechanical strength, while deposition of lignin enhances mechanical strength of these cell walls, and contributing to their rigidity (Doblin et al. 2010; Keegstra 2010).

Following analysis of cell wall components of pear stone cells, they are found to contain large amounts of lignin, cellulose, and hemicellulose

(xylans). In addition, these stone cells contain certain amounts of procyanidins. On the other hand, parenchyma cells of pear fruits contain high levels of pectin (uronic acids and arabinose) and low levels of lignin. As a result, stone cells are deemed harder and stiffer than parenchyma cells (Brahem et al. 2017).

In other studies, it has been reported that autofluorescence analysis indicated presence of high lignin content, as well as positive phloroglucinol-HCl (Wiesner) staining in stone cells of pear fruits (Tao et al. 2009; Cheng et al. 2017a). Although early studies have reported that the lignin content of stone cells is about 20–30% (Lu et al. 2011; Yang et al. 2014), subsequent analysis has determined that the lignin content in stone cells of different pear cultivars vary from 34.25 to 39.46% (Tian et al. 2017). In addition, there is a significant positive correlation between SCC content and lignin content in flesh of pear fruits. The lignin content of SCCs is also positively correlated with the lignin content in the flesh (Tao et al. 2015; Tian et al. 2017; Zhang et al. 2017). Therefore, lignin is deemed as one of the key components of stone cells.

11.5 Developmental Patterns and Distribution of Stone Cells in Pear Fruit

The content and size of stone cells in pear fruits are variable, as well as their distribution in different tissues of the fruit. Thus, it is important to understand the origin of these stone cells. In particular, it is critical to address the following questions: Where do these stone cells come from? What is the process of their formation? And how are they distributed within the fruit?

11.5.1 Development and Morphology of Stone Cells

Stone cells are initiated 7–15 days after flowering (DAF), and they form and develop between 23 and 67 DAF (Cai et al. 2010; Zhao et al. 2013; Li et al. 2017). Furthermore, the content of pear stone cells exhibits an increase/drop pattern during fruit development (Kim et al. 2004c; Cai et al. 2010; Tao et al. 2015). Moreover, the initial period of stone cell formation and the peak period of stone cell content vary in different pear genotypes and under different growing conditions (Li et al. 2017).

The process of stone cell formation involves secondary thickening and lignification of cell walls of parenchyma cells of fruit flesh tissues, as observed in microscopic anatomical studies (Figs. 11.1 and 11.2) (Nii et al. 2008; Nie et al. 2009; Jin et al. 2013; Zhao et al. 2013). Based on morphological characteristics of stone cells, the developmental process of stone cell formation can be divided into four stages. These stages are designated as follows: prophase, metaphase, anaphase, and telophase, corresponding to Stage I (precursor occurrence), Stage II (cytoplasm gathering), Stage III (protoplast shrinking and secondary wall thickening), and Stage IV (formation of stone cells), respectively (Nie et al. 2009; Zhao et al. 2013). Other studies have divided this process slightly differently into three stages, based on the process of fruit enlargement as follows: Stage I, extensive cell division; Stage II, reduced rate of fruit enlargement; and Stage III, rapid increase in fruit size until harvest (Nii et al. 2008).

During bloom, the receptacle (later developing into a fruit) is composed entirely of parenchyma cells. At this time, cell walls are very thin and cannot be stained with phloroglucinol-HCl (Fig. 11.2a), thus suggesting that lignin accumulation has not yet begun (Zhao et al. 2013). Subsequently, it is found that cell walls of some parenchyma cells present in fruit flesh (mostly those adjacent to fruit vascular bundles) begin to thicken unevenly, wherein inclusions gradually disappear to form thick-walled hollow cells. These cell walls of parenchyma cells will continue to thicken until entire cell walls are thickened, forming sclereid primordium cells (Fig. 11.1a and 11.2b). Parenchyma cells surrounding sclereid primordium cells will also undergo secondary cell wall thickening and lignification (Fig. 11.1b). The

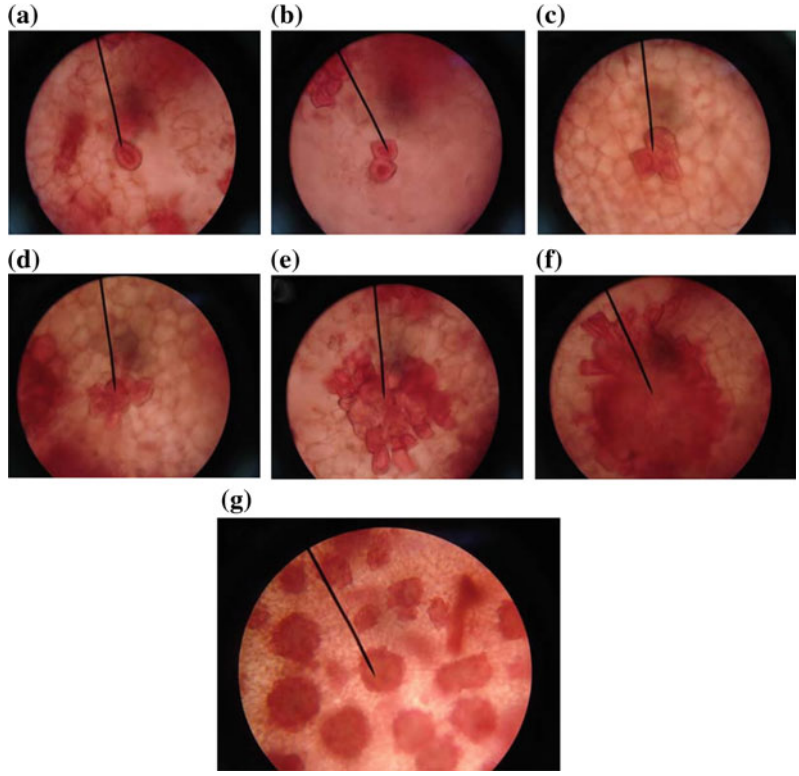

Fig. 11.1 Microscopic analysis of stone cell clusters (SCCs) development in pear fruit (the black pointer shows a stone cell or a SCC). Stone cells were stained with 0.1% Safranin. **a–f** The process of formation of SCCs in *Pyrus* × *bretschneideri* (15–67 days after flowering) and **g** The shape of SCCs of pear fruit of *P.* × *bretschneideri*

Fig. 11.2 Phloroglucinol-HCl staining and microscopic observation of parenchyma cells (**a**), sclereid primordium cells (**b**), and stone cells (**c**) development in pear fruit

newly formed stone cells will gather around sclereid primordium cells, and stone cell aggregation will take place (Fig. 11.1c, d). When stained with phloroglucinol-HCl, cell walls at this stage of development are dyed light purple in color (Fig. 11.2b), thus indicating that lignification has begun (Zhao et al. 2013).

As the fruit continues to develop, the volume of stone cells and the scope of aggregation continue to expand. While cell walls continue to

thicken, the cell lumen is continuously being filled until it turns into a typical stone cell cluster made up of a number of solid stone cells (Fig. 11.1e–g). At this point, all stone cells could turn purplish red in coloration following phloroglucinol-HCl (Fig. 11.2c), thus indicating that stone cells are fully lignified (Zhao et al. 2013).

Around 67 DAF, development of the majority of stone cell clusters is stabilized, and parenchyma cells will no longer differentiate into stone cells. Following this period, parenchyma cells begin to expand, and the fruit volume will rapidly increase. This will contribute to expanded spaces among stone cell clusters, along with a drop in relative contents of these clusters (Nii et al. 2008; Cai et al. 2010; Choi and Lee 2013). By about 100 DAF, the content of stone cell clusters is completely stabilized (Nii et al. 2008; Li et al. 2017).

The process of stone cell formation is also a plant programmed cell death (PCD) process (Zhao et al. 2013). During the period of parenchyma cell differentiation into stone cells, autophagy is observed (Fig. 11.3). For those parenchyma cells that do not differentiate into stone cells, they contain larger nuclei along with small intracellular vacuoles within the dense cytoplasm. However, during the process of differentiation of parenchyma cells into stone cells,

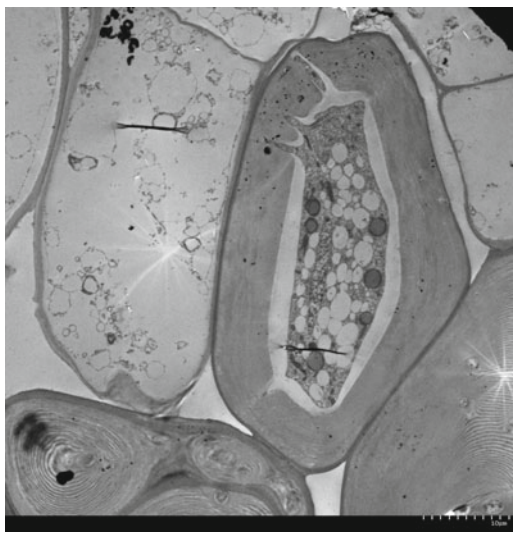

Fig. 11.3 Ultrastructural observation of autophagic vacuoles in stone cells

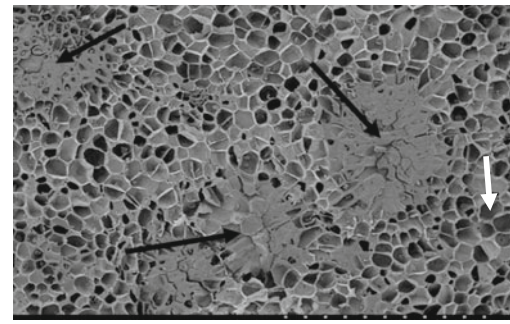

Fig. 11.4 Scanning electron microscopic observation of stone cell clusters (black arrows) and parenchyma cells (white arrow) within the flesh of a pear fruit

small vacuoles gradually merge into a large central vacuole, after which the cytoplasm becomes dispersedly granular, and cellular contents gradually shrink into the center of the cell. At the same time, this process is accompanied by the appearance of autophagic vacuoles (Cheng et al. 2019c). Eventually, both the vacuole and cytoplasm disappear, and the hollow cell lumen is filled entirely by thickened secondary cell walls (Jin et al. 2013; Zhao et al. 2013). Using electron microscopy, it can be noted that the degree of polymerization of stone cell clusters formed by multiple stone cells is higher than that of parenchyma cells (Fig. 11.4). Indeed, stone cell clusters are surrounded by parenchyma cells, and cell walls of parenchyma cells are thinner than those of stone cells (Yan et al. 2014; Brahem et al. 2017).

11.5.2 Secondary Cell Wall Construction and Lignin Deposition in Stone Cells

Cell walls of stone cells are composed of a middle lamella (ML), a primary cell wall (PCW), and a secondary cell wall (SCW). Furthermore, SCWs are generally subdivided into a secondary wall outer layer (S_1), a secondary wall middle layer (S_2), and a secondary wall inner layer (S_3). Interestingly, the ML between two stone cells and PCW of stone cells are relatively thin, and the combination of these two structures is tight. Therefore, these structures form the composite

middle lamella (CML) which consists of ML and PCW. In addition, pits are also present along cell walls of mature stone cells (Figs. 11.5 and 11.6) (Tao et al. 2009; Jin et al. 2013; Zhao et al. 2013; Cheng et al. 2019c).

During development of secondary cell walls of stone cells, large numbers of vesicles and endoplasmic reticulum can be observed adjacent to cell walls (Figs. 11.5 and 11.6), thus indicating that the transport of intracellular material is active in these stone cells (Jin et al. 2013; Zhao et al. 2013). In addition, lignin is generally unevenly deposited, at first, along corner regions of the primary cell wall, and then this expands to other regions of CMLs, as well as to various layers of SCWs. Lignin and cellulose microfibrils

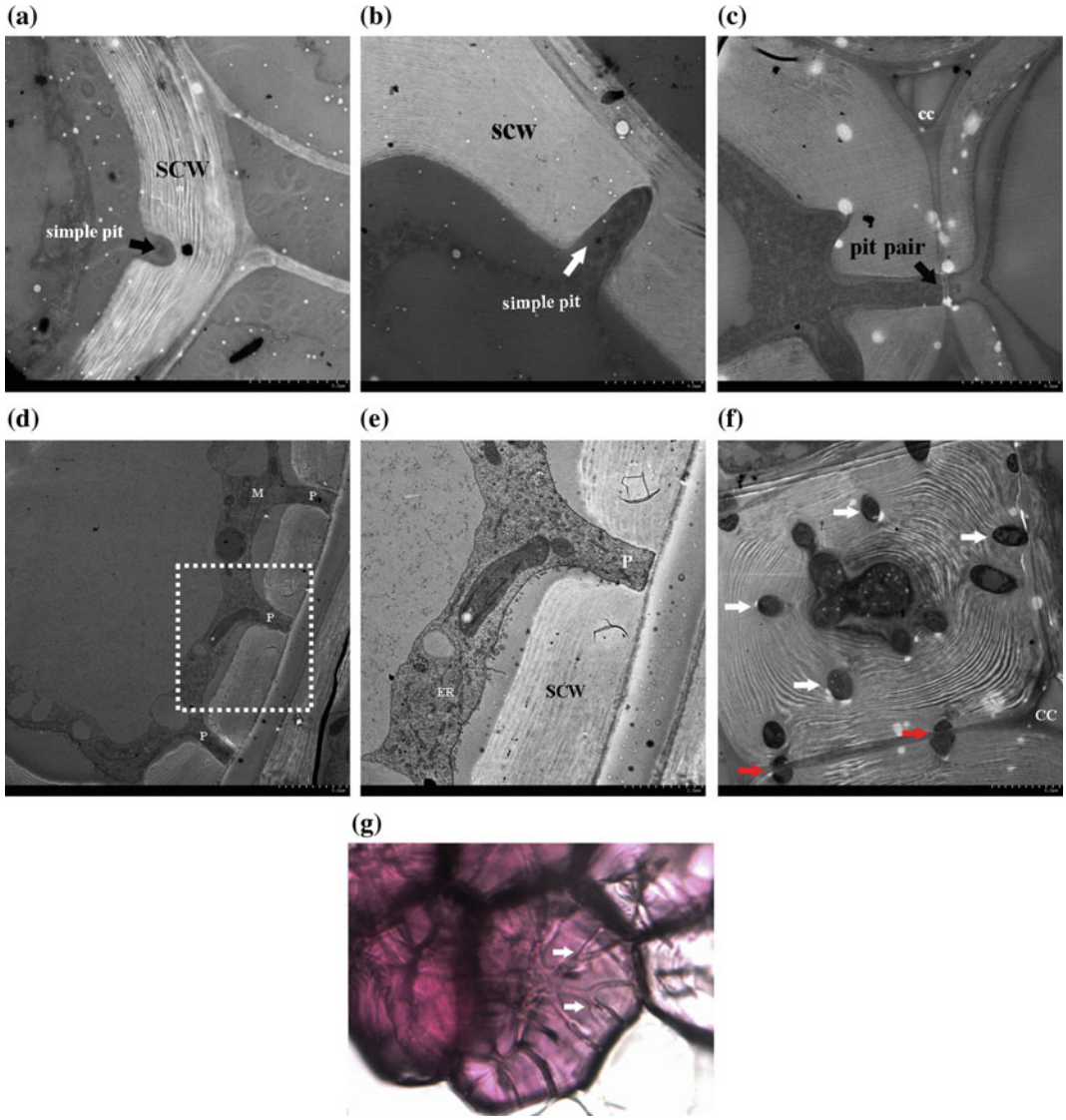

Fig. 11.5 Microscopic and ultramicroscopic observation of pits (P) (white arrows) in pear stone cells. **a**–**b** Observations of the simple pit (longitudinal-section); **c** Observations of the pit pair (longitudinal-section); **d** Ultrastructure of the pit cavity; **e** Shows a magnified version of (**d**); **f** Cross-section of pits on the stone cell; **g** Microscopic observation of pits in pear stone cells; ER: endoplasmic reticulum; M: mitochondria; SCW: secondary cell wall; The red arrow indicates the pit pairs formed between adjacent cells

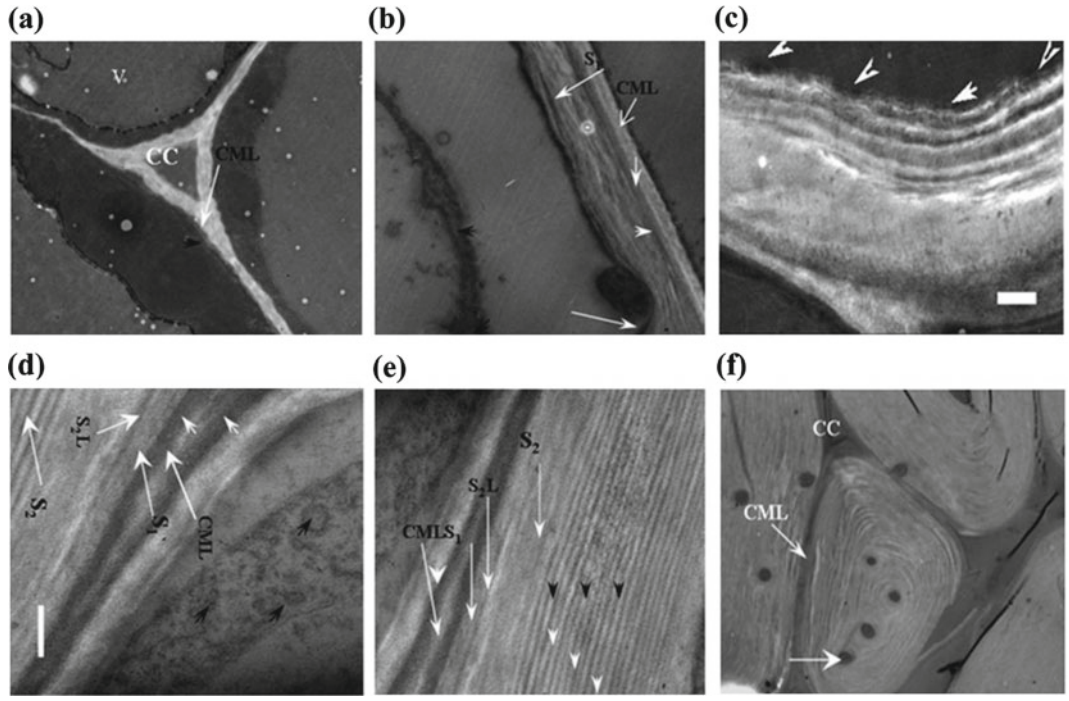

Fig. 11.6 Ultramicroscopy of lignin deposition during stone cell development in pear fruit (Jin et al. 2013). **a** Some cells within the pulp begin to exhibit uneven thickening of the cell wall (short black arrow); **b** Deposition of lignin is initiated from the inner region of the S_1 layer (short white arrows); **c** Lignin particles are deposited unevenly along the inner regions of each microfibril in every S_2 layer (short white arrows); **d** Many secretory vesicles contain high electron-dense material (short black arrows); **e** Lignin deposition (black stripes) within gaps between cellulose microfibrils (white stripes); and **f** Some pits along stone cell walls, cross-section; CC: cell corner; CML: composite middle lamella; S_1: S_1 layer of the secondary wall; S_2L: layer between S_1 and S_2; and S_2: S_2 layer of the secondary wall

alternately arrange their depositions to build up the secondary cell wall (Jin et al. 2013) (Fig. 11.6). However, there are differences in the degrees of lignification of different secondary cell wall layers. In fact, the degree of lignification of the S_2L layer is the highest; moreover, the degree of lignification of cell corner (CC) and CML is higher than that of S_1. It is worth noting that the highest degree of lignification of the S_2L layer is also a characteristic of severe compression wood (Tao et al. 2009; Jin et al. 2013).

11.5.3 Distribution of Stone Cells in Pear Fruits

It is reported that distribution of stone cells within flesh tissues of pear fruit is uneven, and it changes dramatically over the growing season (Choi and Lee 2013). Specifically, it is observed that from 15 to 55 DAF, the distribution of stone cells in 'Dangshan Su' fruit gradually increases, particularly during the period of 39–55 DAF, wherein the density of stone cells is high in the tissue located between the core and the peel. Subsequently, during the period of 63 DAF to maturity, the density of stone cells within the fruit gradually drops, and it is primarily distributed near the core. This may be attributed to a faster rate of stone cell formation compared to the rate of fruit enlargement prior to 63 DAF; thereby, a higher density of stone cells is observed within the pulp at this stage (Cheng et al. 2017a). As stone cells are completely developed after 63 DAF, the volume of parenchyma cells increases rapidly, and the rate of

Fig. 11.7 Stone cell staining of 'Dangshan Su' pear fruits at different stages of development (Cheng et al. 2017a). Transverse sections of fruits at eight stages of development were stained using the Wiesner method (phloroglucinol-HCl). DAF, days after flowering

fruit enlargement also increases, thus contributing to reduction in distribution of stone cells within the flesh (Fig. 11.7) (Nii et al. 2008; Cai et al. 2010; Choi and Lee 2013; Cheng et al. 2017a; Li et al. 2017).

During determination of stone cell content in different tissues of the fruit, including the pulp, peel, and core, it is generally observed that there is a higher stone cell content near the peel and the core of the fruit (Kim et al. 2004c; Choi and Lee 2013; Li et al. 2017). This high content of stone cells present near the peel likely renders this tissue difficult for birds to feed on (Li et al. 2017). Depending on the pear cultivar, the middle pulp has a relatively lower stone cell content than that of other tissues (Li et al. 2017). In general, the size of stone cell clusters in the core of the fruit is higher than that detected in other tissues of the fruit (Tao et al. 2009; Li et al. 2017).

11.6 Stone Cells and Lignin Metabolism

Lignin is a polyphenolic polymer that is directly deposited within plant cell walls. Lignin polymer consists of either a single or five structural units, including a *p*-hydroxyphenyl unit (H-unit), a guaiacyl unit (G-unit), a syringyl units (S-unit), a caffeyl unit (C-unit), and a 5-hydroxy-guaiacyl unit (5H-unit) (Chen et al. 2013; Barros et al. 2015). These five structural units are formed by five monolignols, including *p*-coumaryl alcohol, coniferyl alcohol, sinapyl alcohol, caffeol alcohol, and 5-hydroxyconiferyl alcohol, respectively. Various structural units are mainly connected by ester bonds (C–O–C′) (including linkage bonds, such as *β–O–4*, *α–O–4*, *4–O–5*, and *α–O–γ*) and carbon–carbon bonds (C=C) (including linkage bonds, such as *5–5*, *β–5*, *β–1*, and *β–β*) to form lignin polymers (Vanholme et al. 2010; Eudes et al. 2014).

Synthesis of monolignol must have originated from synthesis of phenylalanine in plastids, and it is subsequently converted into 4-hydroxyphenylpropene alcohols by a series of enzymatic reactions in the cytoplasm. There are various branches of lignin metabolism in different plants; thus, lignin metabolism is a very complex metabolic network (Liu 2012; Barros et al. 2015).

As mentioned above, stone cells are lignification cells, and that lignin is essential for their development. Thus, lignin monomer composition and lignin synthesis pathway within the fruit are critical in defining the mechanism of formation of stone cells. Therefore, we will next focus on pear lignin structure, biosynthetic pathways, and related structural genes.

11.6.1 Composition and Structure of Lignin in Pear

Lignin monomer composition is known to vary in different plant species, tissues, cell types, and cell wall layers. The lignin of gymnosperms is almost predominantly composed of G-units, while the lignin of angiosperms is predominantly composed of G–S-units (Campbell and Sederoff 1996).

The lignin polymer in pear, an angiosperm, is primarily composed of G-units and S-units, with no evidence of presence of either H-units, C-units, or 5H-units (Cai et al. 2010; Jin et al. 2013). Certainly, the content and ratio of G-units and S-units (G/S) of lignin in different pear cultivars are different. Moreover, even within the same pear cultivar, the content of lignin monomers is different at different stages of development (Yan et al. 2014). Based on recent studies of lignin contents in pear fruits, levels of G-units are generally higher than those of S-units, thereby resulting in a G/S ratio that is greater than 1.0 (Cai et al. 2010; Jin et al. 2013). However, for some pear cultivars, such as 'Dangshan Su,' the level of G-units is more than twofold higher than that of S-units (Yan et al. 2014). As an S-unit has methoxy groups at both C_3 and C_5 positions, while a G-unit has only a single methoxy group at the C_3 position (Fig. 11.8), a G-unit can easily form stable, and not easily degraded, C=C bonds at the C_5 position. Therefore, the higher the G-unit content, the more difficult it is to degrade lignin polymers. In addition, presence of a higher G/S lignin ratio in a pear fruit, the more stable are those formed lignin polymers, the more difficult it is to degrade

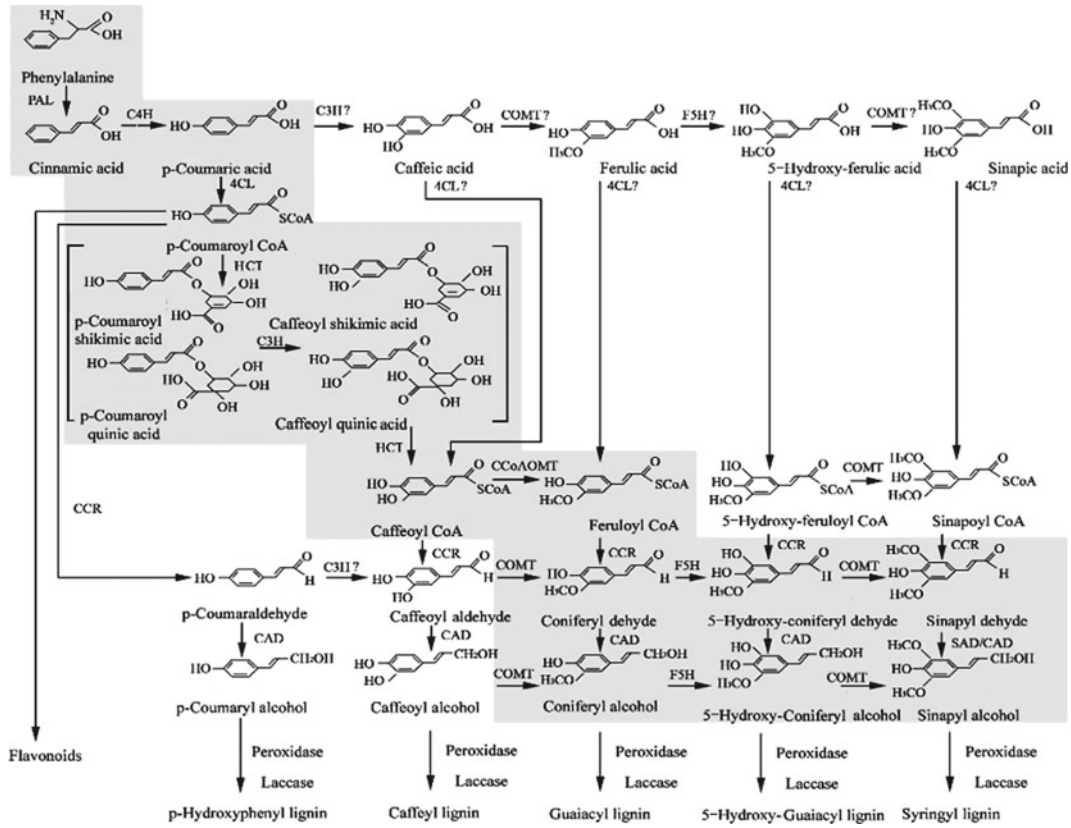

Fig. 11.8 Metabolic pathways of lignin in plants. The shaded sections pertain to the main lignin biosynthesis pathway in pear fruits

such polymers, thus leading to formation of high density and of high degree of polymerization of SSCs (Yan et al. 2014).

Organic elemental analytical results have demonstrated that the lignin of pear fruits mainly contains carbon (C), hydrogen (H), and oxygen (O) elements, but it also contains a small amount of nitrogen (N) elements. Furthermore, the structure of the lignin polymer of a pear fruit has more side chains, along with more hydroxyl groups and less phenolic hydroxyl groups. The linkage bond of a lignin structural unit is generally divided into the following four types, *β–O–4*, *β–1*, *β–5*, and *β–β*.

Of course, it is important to point out that structural properties of lignin of different pear cultivars will have some variations that may influence stability of lignin polymers to a certain extent and ultimately influence formation of stone cells.

11.6.2 Analysis of the Monolignols Metabolic Pathway

The monolignols metabolic pathway can be subdivided into three components. An upstream pathway of monolignols metabolism is the general phenylpropanoid pathway, which subsequently enters into an ester intermediary pathway, and finally into synthesis of various monolignols via a monolignol-specific biosynthesis pathway (Fig. 11.8) (Barros et al. 2015; Pascual et al. 2016). Following transcriptomic, proteomic, metabolomic, gas chromatography–mass spectrometry (GC–MS), ultra-high-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS), and high-performance liquid chromatography (HPLC) analyses, the lignin biosynthesis pathway in pear fruits has been well investigated. This has led to the unraveling of the subsets of pathways involved in the monolignols metabolic pathway (Cai et al. 2010; Wu et al. 2013; Li et al. 2015, 2018a, b; Zhang et al. 2017).

11.6.2.1 The General Phenylpropanoid Pathway

The general phenylpropanoid pathway mainly converts L-phenylalanine into a hydroxycinnamic acid and an acyl-CoA ester. The enzymes responsible for this segment of the metabolic pathway are phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H), and 4-coumarate: coenzyme A ligase (4CL) (Barros et al. 2015).

The metabolites of PAL and C4H, cinnamic acid and *p*-coumaric acid, were detected at different stages of development of pear fruits. The contents of cinnamic acid and *p*-coumaric acid were found to be higher during the vigorous period of stone cell formation and lignin biosynthesis, thus suggesting that they are lignin-synthesizing key precursors (Cai et al. 2010; Wang et al. 2013). In addition, multiple *PAL*s, *C4H*s, and *4CL*s genes, along with their encoded enzymes, were detected by transcriptomic and proteomic analyses, and expression trends were found to be consistent with contents of stone cells and lignin in pear fruit (Li et al. 2015; Zhang et al. 2016, 2017). Thus, it has been proposed that the general phenylpropanoid pathway was closely related to the synthesis of monolignols in pear fruits.

11.6.2.2 The Ester Intermediary Pathway

The ester intermediary pathway mainly synthesizes various hydroxycinnamic acids and coenzyme A thioesters (Pascual et al. 2016). Enzymes involved in this pathway include hydroxycinnamoyl-CoA: shikimate/quinate hydroxycinnamoyltransferase (HCT), coumarate 3-hydroxylase (C3H), and caffeoyl-CoA O-methyltransferase (CCoAOMT). In a study of pear fruit transcriptomes and proteomes, genes encoding these three enzymes have been detected (Wu et al. 2013; Li et al. 2015; Zhang et al. 2016, 2017). This has confirmed existence of this metabolic pathway in pear fruits.

High levels of expression of *HCT*, *C3H,* and *CCoAOMT* have been detected in pear fruits at early stages of development, therein promoting conversion of *p*-coumaroyl-CoA into caffeoyl-CoA, then to feruloyl-CoA, and ultimately leading to accumulation of S-units and G-units. In addition, the conversion reaction of caffeoyl-CoA to feruloyl-CoA is a rate-limiting step in lignin biosynthesis in pear fruits (Wu et al. 2013; Zhang et al. 2016).

11.6.2.3 The Monolignol-Specific Biosynthesis Pathway

Cinnamoyl-CoA reductase (CCR), cinnamyl alcohol dehydrogenase (CAD), and sinapyl alcohol dehydrogenase (SAD) in the monolignol-specific biosynthesis pathway are responsible for converting hydroxycinnamoyl-CoAs to monolignols. Ferulate 5-hydroxylas (F5H) and caffeic acid 3-O-methyltransferase (COMT) can then catalyze formation of precursors of S-units from precursors of G-units (Barros et al. 2015).

During the peak period of stone cell development and lignin content accumulation in a pear fruit, transcription of multiple *CCR*s, *CAD*s, *SAD*s, and *F5H*s has been detected by transcriptome and proteome analyses (Li et al. 2015; Zhang et al. 2016, 2017). Interestingly, Wu et al. (2013) have not detected significant *COMT* transcript levels. Therefore, this begs the following question: Does *COMT* require very low levels of expression to meet the needs of catalytic reactions or is COMT responsible for catalytic steps that do not exist in pear fruits? This important question is yet to be investigated.

11.6.2.4 Monolignols Polymerization

Following synthesis of the lignin monomer, which is catalyzed by peroxidase (POD, EC 1.11.1.7) and laccase (LAC, EC 1.10.3.2), it is coupled with polymerize into a growing lignin polymer (Barros et al. 2015). During the peak period of stone cells development and lignin content accumulation, multiple *POD* genes are upregulated, thus suggesting that these genes may playkey roles in monolignol polymerization (Cao et al. 2016a; Zhang et al. 2017).

Furthermore, several microRNAs, such as miR397a, are downregulated during the period of vigorous lignification of pear fruits, and expression patterns of miR397a during different stages of fruit development are in contrast to those of multiple target genes such as *LAC* (Xue et al. 2018). It is proposed that miR397a can influence lignin biosynthesis by regulating expression of 27 *LAC* genes; thus, *LAC* is critical in lignin synthesis in pear fruits (Wu et al. 2014).

Xue et al. (2018) used 30 pear cultivars of high-stone cell content (average stone cell content ranged between 10.53 and 20.11%) and 30 pear cultivars of low-stone cell content (average stone cell content ranged between 3.71 and 6.78%). Single nucleotide polymorphism (SNP) mutations in a 3000 bp promoter region of the *PbrmiR397a* precursor of these 60 cultivars were compared using whole-genome resequencing. It was observed that the TCA-element, a salicylic acid response element, in the *PbrmiR397a* precursor promoter had a single base mutation in high-stone cell content pears. This directly contributed to less effective salicylic acid induction of *PbrmiR397a* transcription, thereby resulting in upregulation of expression of the target gene, *PbLAC*s, of *PbrmiR397a* in fruits. Thereby, this contributed to accumulation of lignin and development of stone cells (Fig. 11.9). In addition, dual-luciferase reporter assays and genetic transformation also demonstrated that *PbrmiR397a* could affect plant lignin content and cell wall development by regulating *LAC* transcription (Xue et al. 2018).

11.6.3 A Low-Stone Cell Content Bud Sport

A mutation in meristematic cells of the growth point of a bud on a shoot of a fruit tree can lead to the development of a bud sport mutant. When mutant buds grow into shoots and branches, they can develop flowers and fruits that are likely to be different from the original cultivar in morphology, physiology, biochemistry, or even genetics. This trait can be either maintained

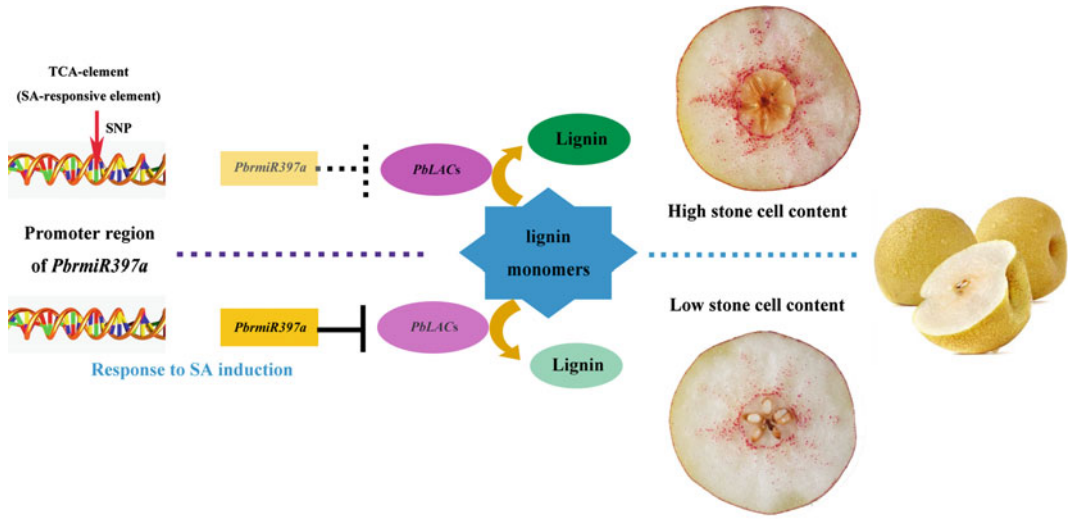

Fig. 11.9 The miR397a-*LAC*s module regulates stone cell formation in pear fruit

through vegetative, or asexual, propagation, or it can be even inherited by the offspring, if the mutation occurs in germ cells (Foster and Aranzana 2018).

Pyrus × *bretschneideri* cvs. Lianglizaosu and Dangshanxinsu are new pear cultivars originating as natural bud sports of 'Dangshan Su' (Wang et al. 2012; Xu et al. 2016; Zhang et al. 2017). The contents of stone cells in fruits of these two pear cultivars, 'Lianglizaosu' and 'Dangshanxinsu,' are significantly lower than those of 'Dangshan Su,' and thus, these are designated as low-stone cell content bud sports. Following several years of observations and comparative studies of these two bud sports with 'Dangshan Su,' it is reported that the low-stone cell content trait is stable. Not only do these two bud sports have the original desirable traits of 'Dangshan Su,' they also set fruit containing significantly lower size and content of SCCs (Wang et al. 2012; Xu et al. 2016; Zhang et al. 2017; Cheng et al. 2019b). Therefore, discovery of these low-stone cell content bud sports provides ideal materials for studying the molecular mechanism of stone cell development.

Using comparative transcriptome analyses between 'Lianglizaosu' and 'Dangshan Su', Zhang et al. (2017) have reported that in addition to identifying structural genes related to lignin monomer synthesis and polymerization, they have observed differences in transcription of genes related to carbon metabolism and to some hypothetical regulatory genes that are likely responsible for observed differences in both content and size of stone cell clusters in pear fruits (Fig. 11.10).

11.6.4 Gene Families Related to Lignin Synthesis in Pear

Analysis of fruit transcriptome and proteome has revealed that most of the lignin synthesis-related genes have multiple members, and together, these members play roles in fruit development. With completion of sequencing of the pear genome, screening and identification of members of the gene family related to lignin metabolism have been successively conducted. The following sections provide an overview of members of the lignin gene family in pear.

11.6.4.1 The 4-Coumarate: Coenzyme A Ligase (4CL) Gene Family

The phenylpropanoid enzyme 4-coumarate: coenzyme A ligase (4CL) acts on the last step of

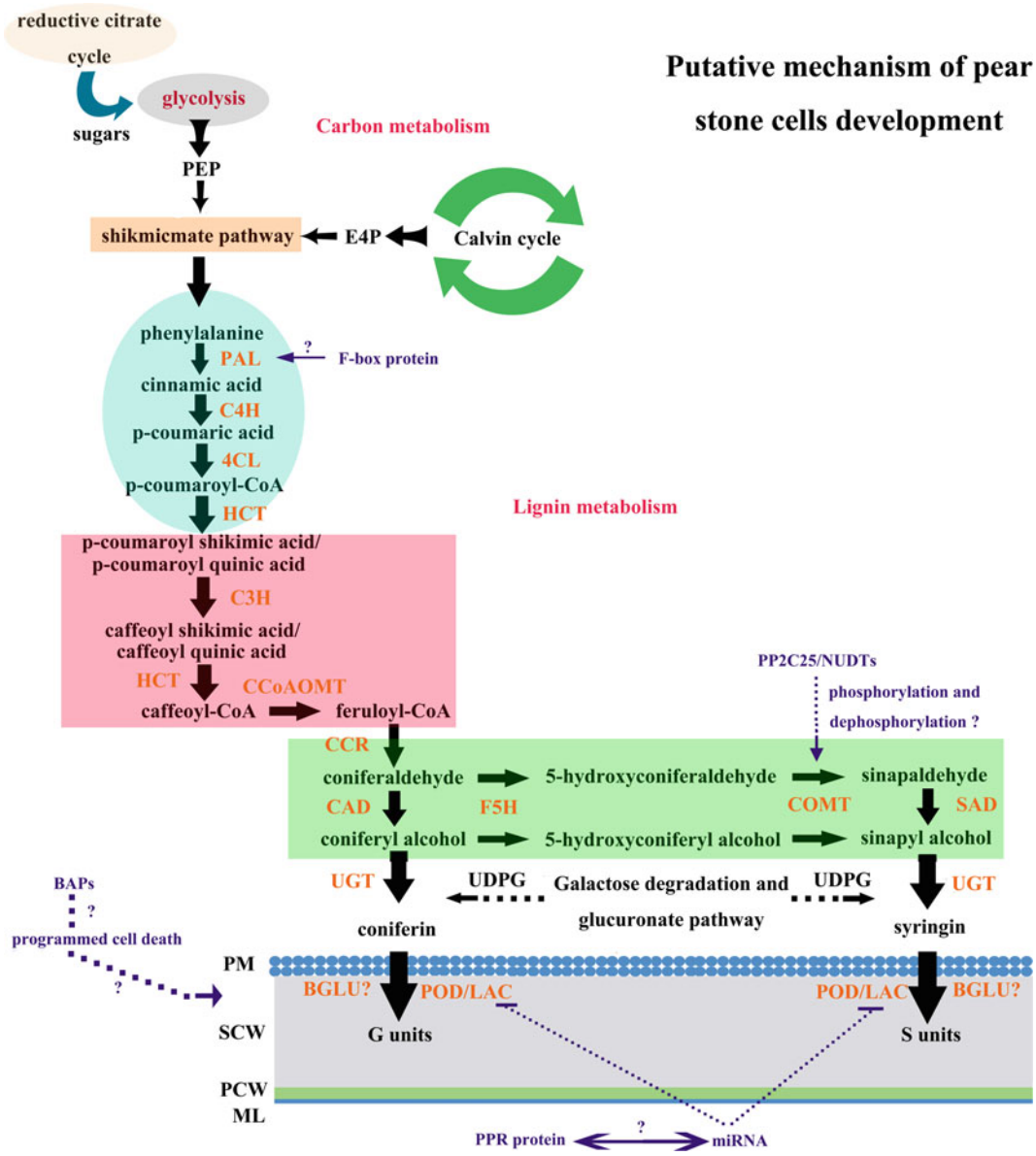

Fig. 11.10 A model illustrating the putative mechanism of pear stone cell development. PEP: Phosphoenolpyruvate; E4P: Erythrose-4-phosphate; UGT: UDP-glucuronosyltransferase; BGLU: β-Glucosidase; UDPG: Uridine diphosphate glucose; BAP: BON1-associated protein; NUDT: Nudix hydrolase; PPR: Pentatricopeptide repeat protein; PP2C25: Probable protein phosphatase 2C 25; PM: Plasma membrane; SCW: Secondary cell wall; PCW: Primary cell wall; and ML: Middle lamella

the general phenylpropanoid pathway, with *p*-coumaric acid, caffeic acid, ferulic acid, 5-hydroxy ferulic acid, and sinapic acid serving as substrates in generating corresponding coenzyme A thioesters. These resultant thioesters, at the branch point of the phenylpropane metabolic pathway, along with the synthesis of various secondary metabolites, are the precursors of lignin, flavonoids, and chlorogenic acid (Barros et al. 2015; Cao et al. 2015, 2016b).

The *4CL* gene family can be divided into two major subfamilies, Class I and Class II. In particular, members related to lignin synthesis are classified as either Class I in phylogenetic trees or as Class II for members related to flavonoid metabolism. A total of 29 members of the *4CL* gene family have been identified and screened in the pear genome, of which 16 members belong to Class I and 13 members belong to Class II. Based on analysis of expression patterns, it is proposed that *Pb4CL1* plays a major role in lignin metabolism, while *Pb4CL2* and *Pb4CL4* are likely to participate in flavonoid metabolism in pear fruits (Cao et al. 2015, 2016b).

11.6.4.2 The Hydroxycinnamoyl-CoA: Shikimate/Quinate Hydroxycinnamoyl transferase (HCT) Gene Family

The hydroxycinnamoyl-CoA: shikimate/quinate hydroxycinnamoyltransferase (HCT) enzymes belong to the plant BAHD acyltransferase superfamily, and have dual activities of shikimate hydroxycinnamoyltransferase (CST) and quinate hydroxycinnamoyltransferase (CQT). HCT can catalyze formation of the coumaroylquinate 3-monooxygenase (C3H) substrate coumaroyl shikimic acid/coumaroyl quinic acid, and at the same time, it can also catalyze C3H production of the caffeoyl shikimic/quinic acid, which is further converted into caffeoyl-CoA.

A total of 82 *PbHCT*s have been identified in the pear genome of *P.* × *bretschneideri*, all of which contain the conserved domains HXXXD and DFGWG. Approximately 25% of the members contain MYB transcription factor binding sites. Transcriptome and qRT-PCR analysis have revealed that expression trends of *PbHCT2*, *PbHCT17*, *PbHCT18*, *PbHCT49,* and *PbHCT50,* at different stages of fruit development, are consistent with changes of lignin contents in pear. Furthermore, there is a high correlation between expression levels of these five genes and contents of stone cells in pear fruits. Therefore, these five *PbHCT*s are proposed to play key roles in lignin synthesis and stone cell development in pear fruits (Ma et al. 2017).

11.6.4.3 The O-Methyltransferase (OMT) Gene Family

The O-methyltransferase (OMT) is a key enzyme in the phenylpropanoid metabolic pathway, which is responsible for the catalytic methylation of lignin precursors, flavonoids, and a series of secondary metabolites. The OMT family can be subdivided into two types, Class I and Class II. Class I is caffeoyl-CoA O-methyltransferase (CCoAOMT), which is mainly involved in monolignol biosynthesis, and Class II is caffeic acid 3-O-methyltransferase (COMT), which not only participates in lignin metabolism, but also catalyzes the synthesis of flavonoids (Cheng et al. 2016).

There are 26 *OMT*s present in the pear genome, including 19 *COMT*s and seven *CCoAOMT*s. Based on phylogenetic tree clustering and expression pattern analysis, *PbCCoAOMT1* and *PbCCoAOMT3* are reported to play major roles in lignin metabolism and stone cell development in pear fruits (Cheng et al. 2016).

11.6.4.4 The Cinnamoyl-CoA Reductase (CCR) Gene Family

The cinnamoyl-CoA reductase (CCR) enzyme belongs to the short-chain dehydrogenase/reductase (SDR) family, which catalyzes the first reaction of lignin-specific synthetic pathways. The CCR substrates have been identified as the following five hydroxycinnamoyl-CoAs, including *p*-coumaryl-CoA, caffeoyl-CoA, feruloyl-CoA, 5-hydroxyferuloyl-CoA, and sinapoyl-CoA, and the product of which is the corresponding hydroxyl cinnamaldehyde (Pan et al. 2014; Cheng et al. 2017a).

A total of 31 *CCR*s genes have been identified in the pear genome, and among these, there are 28 CCR-like clade members and three PbCCR members belonging to a bona fide CCR clade. Furthermore, PbCCR1, 2, and 3 have high genetic relationships with bona fide CCRs of other species and share a characteristic conserved motif, KNWYCYGK, whose spatial three-dimensional structure may be involved in the recognition of CoA. Following analysis of temporal-spatial expression patterns of these three members, it is determined that PbCCR1 and

PbCCR2 play major roles, while PbCCR3 may play a minor role in lignin synthesis in pear fruits (Cheng et al. 2017a).

11.6.4.5 The Cinnamyl Alcohol Dehydrogenase (CAD) Gene Family

The cinnamyl alcohol dehydrogenase (CAD) enzyme belongs to a medium short-chain dehydrogenase/reductase (MDR) family, which is responsible for the reduction of hydroxycinnamaldehydes into hydroxycinnamic alcohols (lignin monomers) (Pan et al. 2014). It has been reported that within the *CAD* gene family, there is a class member, referred to as sinapyl alcohol dehydrogenase (SAD), which is responsible for the conversion of sinapaldehyde into sinapyl alcohol (Li et al. 2001; Cheng et al. 2017a). However, in recent years, other studies have suggested that the so-called SAD and its orthologous gene cannot be exclusively responsible for the synthesis of S-units, and their expression cannot be changed by modifying their S-unit contents (Barakate et al. 2011). Therefore, it is proposed that SADs may only act as alternative or compensating CADs for bona fide CADs in a plant's response to stress.

A total of 26 members of the *CAD* gene family have been identified in the pear genome. These members are characterized by containing either an ADH_zinc_N domain or an ADH_N domain (Cheng et al. 2017a). Following analysis of tissue specificity and temporal expression patterns in developing pear fruits, it has been reported that *PbCAD2* is upregulated, while *PbCAD3* is downregulated during peak periods of fruit stone cell development and lignin content accumulation. Combined with the tertiary structure of proteins, comparative key catalytic sites, sequence similarities, and identity analysis, it has been confirmed that PbCAD2, in a bona fide CAD clade (Class I), is related to lignin synthesis in pear fruits (Cheng et al. 2017a).

11.6.4.6 The Peroxidase (POD) Gene Family

Based on sequence differences and catalytic properties, peroxidases (PODs) can be subdivided into three classes. Class I is present in bacteria, while Class II is present in fungi, and Class III is present in plants. Class III peroxidases play important roles in plant lignin polymerization, cell wall development, and in resistance to stress (Barros et al. 2015; Cao et al. 2016a).

In pear, Class III peroxidase gene family consists of 94 members belonging to 19 subfamilies. Based on quantitative reverse transcriptase (qRT)—polymerase chain reaction (PCR) analysis, it is revealed that five *PbPOD*s, including *PbPRX2*, *PbPRX22*, *PbPRX34*, *PbPRX64*, and *PbPRX75*, belonging to subgroup C, may be involved in regulation of lignin synthesis in pear fruits (Cao et al. 2016a).

11.6.4.7 The Laccase (LAC) Gene Family

Laccase (LAC) is the largest component of multi-copper oxidases (MCOs), and it is the key enzyme responsible for polymerization of monolignols. Xue et al. (2018) have identified 38 *PbLAC*s in the pear genome, and have reported that *PbLAC1*, *2*, *3*, *15*, *18,* and *20* have higher abundance of transcripts in early fruit development using transcriptome analysis. As stone cells are formed in large numbers during the early stages of pear fruit development, it has been proposed that six *PbLAC*s are involved in polymerization of lignin monomers during development of stone cells. In particular, subcellular localization analysis has indicated that PbLAC1, 2, and 18 are all localized in cell walls, and that simultaneous inhibition of expression of these three genes in pear fruit significantly reduces stone cell formation (Xue et al. 2018). Recently, Cheng et al. (2019a) have also demonstrated that *PbLAC* (Pbr003857.1) is associated with lignin synthesis and secondary cell wall development.

11.6.4.8 The Dirigent (DIR) Gene Family

Dirigent proteins (DIRs) are closely related to lignification of plant cells, and they play important roles in secondary cell wall formation (Burlat et al. 2001; Paniagua et al. 2017; Cheng et al. 2018). The dirigent protein model hypothesis suggests that formation of lignin oligomers

is carried out under the strict regulation of DIRs, which controls formation of specific chemical bonds during the monolignol polymerization process to form lignin polymers (Barros et al. 2015; Paniagua et al. 2017).

Cheng et al. (2018) have classified 35 members of the *PbDIR* family into the following four subfamilies: DIR-a, DIR-b/d, DIR-e, and DIR-g subfamilies. Through systematic bioinformatics and qRT-PCR analysis, it is suggested that PbDIR4 belongs to a (+)pinoresinol-forming DIR protein, and it is involved in formation of lignin oligomers during development of stone cells.

11.7 Developmental Regulation of Stone Cells

In addition to genetic factors influencing development of stone cells in pear fruits, the environment plays a regulatory role in formation of these stone cells, including light, water, mineral elements, and hormones. At present, there are some available effective measures for controlling the content of stone cells.

The roles of various factors involved in regulation of stone cells, as well as likely regulation mechanisms of these stone cells in pear fruits will be herein presented.

11.7.1 Pollination

As pear has a gametophytic self-incompatibility (GSI) mechanism, the percentage of self-pollinated fruit set in an orchard is rather low. Therefore, pear trees require cross-pollination to insure adequate fruit set. Moreover, due to presence of the xenia phenomenon in pear, fruit quality, including contents of stone cells in fruits, is influenced to a great extent by pollen from different pear cultivars.

At present, the mechanism of pollination affecting development of stone cells in pear is not yet clearly understood. In cross-pollination of 'Dangshan Su' pear, it is observed that pollination of different pear cultivars can change the contents of stone cells and lignin in the fruit (Cheng et al. 2017b; Li et al. 2018a, b). It is suggested that different male parental pollen can influence expression of miRNA, such as pyr-miR1809 and pyr-novel-miR-144-3p, within the fruit. In turn, this will regulate expression of structural genes, such as *laccase*, in the lignin synthesis pathway, eventually affecting development of stone cells in pear fruits (Cheng et al. 2017b) (Fig. 11.11).

11.7.2 Bagging of Fruit

In pear production practices, bagging of fruits is a common cultivation management measure. Preharvest bagging can change the microenvironment around the fruit, thus affecting fruit quality. Incidentally, effects of the type of bag used on fruit quality may also vary, and in some instances may even contribute to negative outcomes (Wang et al. 2013, 2017a, b; Tao et al. 2015).

As an example, fruits of 'Dangshan Su' were bagged using double-layered paper bags, with brown-colored outer layers and black-colored inner layers, along with two gas-exchange holes present at bottom ends of these bags. It was observed that there were no significant differences in contents of stone cells between bagged fruits and unbagged fruits. Moreover, patterns of accumulation of stone cells and lignin contents in bagged fruits and unbagged fruits were essentially the same during fruit development. However, the activity of cinnamate-4-hydroxylase (C4H) in bagged fruits was lower than that in unbagged fruits during all stages of fruit development (Tao et al. 2015).

In another study, effects of polyethylene (PE)-bagged and white non-woven polypropylene fabric bags on lignin content and metabolism in 'Chili' (*P.* × *bretschneideri*) fruits were investigated (Wang et al. 2017a, b). It was revealed that PE-bagged fruits had the highest lignin contents, followed by unbagged fruits, and finally non-woven fabric-bagged fruits, with the lowest lignin contents. Moreover, white non-woven polypropylene fabric bags contributed to down-regulation of expression of *Pb4CL*, *PbCAD*, and

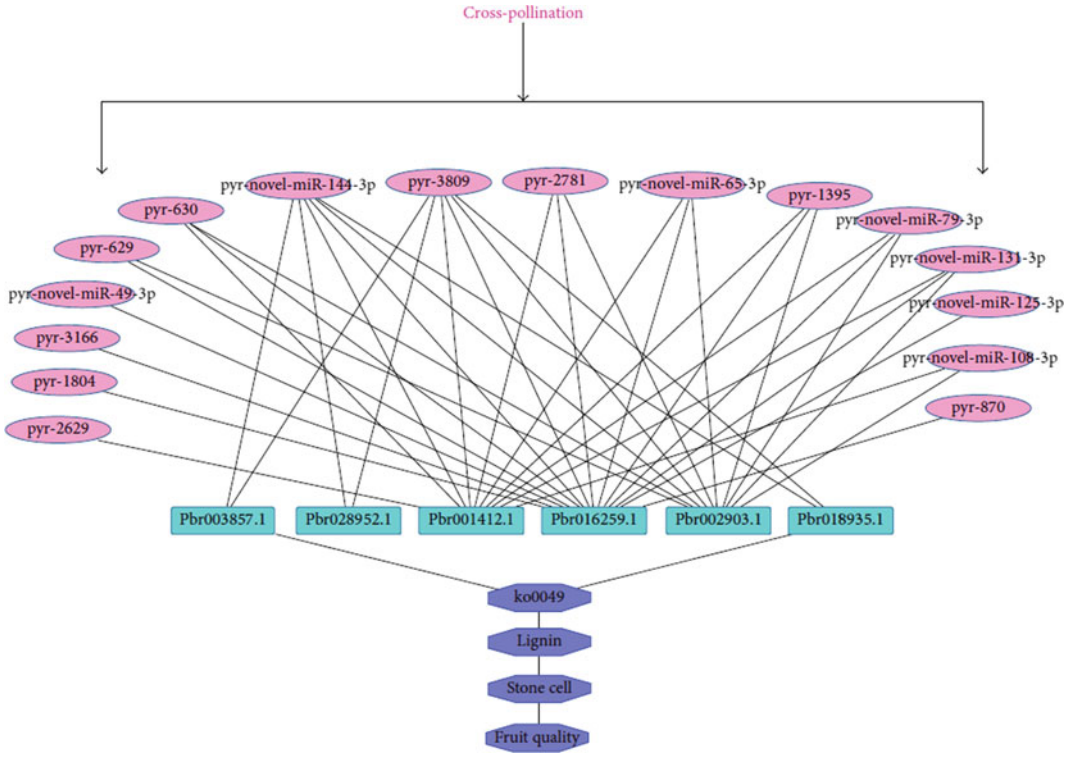

Fig. 11.11 Pathway analysis of microRNA regulation of fruit quality traits in pear

PbPOD genes of the phenylpropanoid metabolic pathway, and leading to lower levels of lignin synthesis; whereas, all these three genes were upregulated in PE-bagged fruits, and contributing to higher levels of lignin synthesis (Wang et al. 2017a, b).

It is critical to ask the question as to why does bagging affect lignin metabolism and stone cell development of pear fruits? Thus far, it has been speculated that expression of key genes in the lignin synthesis pathway may be modified due to the effects of bagging on light intensity and light quality on fruit development.

11.7.3 Water Stress

It has been reported that water stress has a significant effect on stone cell content in pear fruits (Kim et al. 2004b). Lee et al. (2006) investigated the influence of water stress on flowering and fruit development in *P. pyrifolia* cv. Niitaka. They found that water stress at full-bloom and soon after full-bloom would lead to an increase in stone cell content until the fruit reaches maturity, when compared to control (non-stressed) fruits. However, water stress treatment prior to full-bloom did not have any significant effects on stone cell contents of mature fruits. It has been proposed that water stress at full-bloom and after full-bloom contributed to lower levels of calcium (Ca) along with higher POD activities in leaves and in fruit pulp, but had no effects on contents of magnesium (Mg), N, phosphorous (P), and potassium (K) in these tissues (Lee et al. 2006). However, none of these changes were observed in fruits of trees subjected to water stress prior to full-bloom.

The above findings suggest that the mechanism of water stress leading to higher stone cell contents in pear fruits is as follows. Water stress contributes to lower levels of calcium

accumulation in fruits, which leads to increased POD activity, thereby resulting in accumulation of lignin in cell walls. In turn, this will ultimately promote formation of stone cells in fruit pulp tissues. Therefore, water stress during early stages of fruit development influences stone cell formation and development in pear fruits (Lee et al. 2006).

11.7.4 Exogenous Mineral Elements

In general, mineral elements are essential for plant growth and development. It is known that Ca^{2+} is the second signaling messenger in plants. As mentioned above (Sect. 11.5.3), calcium accumulation in leaf and fruit tissues is related to development of stone cells in pear fruits (Kim et al. 2004b).

In recent studies, pear trees were treated with different concentrations of $CaCl_2$ (0.3, 0.5, and 1.0%), and it was found that $CaCl_2$ treatments at 0.5 and 1.0% reduced stone cell contents in fruits compared to those of control (non-treated fruit). However, findings were not as clear for trees treated with 0.3% $CaCl_2$. Nevertheless, $CaCl_2$ treatments at all three levels were found to reduce stone cell size, particularly that of the ratio of the area greater than 200 μm^2. In addition, 0.5% $CaCl_2$ treatment could also reduce cell wall bound and soluble peroxidase enzyme activities (Kim et al. 2004b; Lee et al. 2007).

In another study, pear fruits, at 80 days after flowering, were soaked with 0.5% $CaCl_2$, and then fruits were harvested at maturity and stored (Lu et al. 2015). During storage, not only contents of lignin in $CaCl_2$-treated fruits were significantly lower than those of control fruits, but also activities of PAL, 4CL, CAD, guaiacol peroxidase (G-POD), and syringaldazine peroxidase (S-POD) were significantly lower. In addition, expression levels of *CAD*s genes in $CaCl_2$-treated fruits were also significantly lower compared to those of control (non-treated) fruits (Lu et al. 2015).

In summary, $CaCl_2$ treatment can significantly reduce lignin content, stone cell content, and stone cell size in pear fruits. It is suggested that exogenous sprays of $CaCl_2$ can increase calcium content in pear leaves and fruits (peel and flesh), thus influencing POD activity, which ultimately regulates lignin synthesis and stone cell development. It can be noted that POD activity is regulated by the content of calcium ions in pear fruits. However, the effects of different treatments of $CaCl_2$ and treatment times are different, and the specific mechanism involved in these responses is yet to be explored and elucidated (Kim et al. 2004b; Lee et al. 2007; Lu et al. 2015).

Although there are various studies on the effects of boron and zinc ion sprays on control of stone cell contents in pear fruits undertaken by Chinese researchers, unfortunately none of these have studies been published.

11.7.5 Exogenous Hormones

Plant hormones can regulate multiple metabolic pathways in plants, in which gibberellin (GA) can promote growth and development of crops, early maturity, improve quality, and increase production.

It has been reported that GA applications at the carpopodium stage of development can regulate metabolism of lignin in pear fruits (Yang et al. 2014). During the rapid growth period of the fruit, the content of lignin in GA-treated fruit is lower than that of the control (non-treated) (Yang et al. 2014). Moreover, enzyme activities of CAD and POD in GA-treated fruit are lower than those in control fruit, while PAL activity at early stages of fruit development is significantly lower than that in control fruit. In addition, expression levels of *PpPAL1*, *PpPAL2*, *Pp4CL1*, *Pp4CL2*, and *PpPOD1* in GA-treated fruits are lower than those in control fruits. Furthermore, expression levels of *PpCAD2* in GA-treated fruits are significantly lower than those in control fruits during early stages of development. These findings suggest that GA may affect fruit lignin synthesis and stone cell development by regulating activities of key enzymes and expression of genes involved in lignin synthesis (Yang et al. 2014).

In addition to GA, salicylic acid (SA) has also been reported to regulate the development of stone cells in pear fruits (Zhang et al. 2002; Xue et al. 2018). Sprays of 0.02% SA on trees of *P. sinkiangensis* and *P.* × *bretschneideri* cv. Yali found that these exogenous sprays can inhibit POD activity in the fruit, as well as reduce content and size of stone cell clusters. As an important member of a plant's disease resistance signaling pathway, the role of SA has not yet been elucidated for its specific mechanism of inhibiting the development of stone cells (Zhang et al. 2002).

11.8 Concluding Remarks and Future Prospects

The effects of stone cells (or stone cell clusters), both content and size, on fruit quality, as well as of components of stone cells (biosynthesis pathway and metabolic mechanism of lignin), development process, and distribution of stone cells, and regulation measures of stone cell development have been investigated. However, it is still a long way to unravel the mystery of the mechanism of stone cell formation in pear fruits. Therefore, we would like to propose that the following studies should be undertaken.

11.8.1 Lignification Patterns of Parenchyma Cells in Pear Fruits

As stone cells are lignified parenchyma cells in pear fruits, microscopic observations during early lignification have shown that there are large numbers of Golgi organelles and transport vesicles present in secondary cell walls, thereby indicating that there is an extensive material transport that is being undertaken during this period (Jin et al. 2013; Zhao et al. 2013). Although cellular contents are dissipated during latter periods of fruit growth and lose their abilities to synthesize lignin, secondary cell wall development and lignin deposition do not stop at these latter stages of fruit development. Therefore, this begs the question as to why does lignin accumulate following cell death?

In recent years, many experimental studies have shown that lignin may accumulate from the beginning of cell growth until cell death, and accumulation of lignin continues following cell death (Voxeur et al. 2015). It is assumed that neighboring cells may transport monolignol polymerization-associated enzymes and monolignols to these dead lignified cells (Barros et al. 2015).

Stone cell lignification may undergo a similar process and pattern of development. Pits along cell walls of stone cells may serve as material transport channels to neighboring cells, thereby transporting active oxygen, polymerases, and monolignols (Fig. 11.5) (Jin et al. 2013; Zhao et al. 2013; Barros et al. 2015; Cheng et al. 2019c). Currently, plant cell lignification patterns are mainly classified into three types, including cooperative lignification, partial cooperative lignification, and autonomous lignification (Barros et al. 2015). We speculate that formation of stone cells may belong to either cooperative lignification or partial cooperative lignification. However, at this time, there is a lack of relevant evidence, and therefore, future studies should be undertaken to provide such evidence.

11.8.2 The Branch Pathway of Monolignol Biosynthesis in Pear

Although there is some level of understanding of lignin biosynthetic pathways in pear fruits, pathways of lignin metabolism are complex, with several branches. Thus, these should be explored further. Numerous studies have demonstrated that either promotion or inhibition of a branch of lignin metabolism may yield different results, and perhaps lead to a novel lignin structure. Therefore, it is of great importance to further investigate and clarify branch pathways of lignin metabolism, particularly those involved in

regulating lignin synthesis and stone cell formation. The following are some suggestions for further study:

(a) The newly discovered caffeoyl shikimate esterase (CSE) catalyzes the caffeoyl shikimic/quinic acid and converting it into caffeic acid (Vanholme et al. 2013). At present, this enzyme and its coding gene have not yet been identified in pear and should be investigated. Whether or not this pathway exists in pear is still unknown.
(b) In addition to cinnamic acid and coumaric acid, caffeic acid, ferulic acid, and sinapic acid have also been detected during pear fruit development (Wang et al. 2013). However, 4CL, which catalyzes three hydroxycinnamic acids, has not yet been identified in pear. Therefore, whether or not 4CL is present in the lignin synthesis pathway without HCT and C3H in pear fruit is not yet clear and should be delineated.
(c) The H-units have not been detected in pear lignin, and there are no reports of presence of C-units in lignin of pear fruits. It is postulated that there are two likely scenarios. One is lack of relative polymerases in pear that catalyze both of these lignin monomers, while the other proposes that levels of these enzymes are inadequate in pear to synthesize H-units and C-units precursors, or only have the lowest catalytic activities. Thus, synthetic metabolic pathways of coumaryl alcohol and caffeyl alcohol in pear fruit are yet to be further explored.
(d) Monolignol ferulate transferase (FMT) has been identified in the dicot plant *Angelica sinensis*. This enzyme can catalyze the reaction of feruloyl-CoA with monolignols (coniferyl alcohol and sinapyl alcohol) to form monolignol ferulate conjugates. The latter can be incorporated into lignin polymers (Wilkerson et al. 2014). However, it is not clear whether or not there is a gene encoding FMT in the pear genome.

References

Anderson NA, Tobimatsu Y, Ciesielski PN, Ximenes E, Ralph J, Donohoe BS, Ladisch M, Chapple C (2015) Manipulation of guaiacyl and syringyl monomer biosynthesis in an Arabidopsis cinnamyl alcohol dehydrogenase mutant results in atypical lignin biosynthesis and modified cell wall structure. Plant Cell 27(8):2195–2209

Barakate A, Stephens J, Goldie A, Hunter WN, Marshall D, Hancock RD, Lapierre C, Morreel K, Boerjan W, Halpin C (2011) Syringyl lignin is unaltered by severe sinapyl alcohol dehydrogenase suppression in tobacco. Plant Cell 23(12):4492–4506

Barros J, Serk H, Granlund I, Pesquet E (2015) The cell biology of lignification in higher plants. Ann Bot 115 (7):1053–1074

Brahem M, Renard CM, Gouble B, Bureau S, Le BC (2017) Characterization of tissue specific differences in cell wall polysaccharides of ripe and overripe pear fruit. Carbohydr Polym 156:152–164

Burlat V, Kwon M, Davin LB, Lewis NG (2001) Dirigent proteins and dirigent sites in lignifying tissues. Phytochemistry 57(6):883–897

Cai YP, Li GQ, Nie JQ, Lin Y, Nie F, Zhang JY, Xu YL (2010) Study of the structure and biosynthetic pathway of lignin in stone cells of pear. Sci Hortic 125 (3):374–379

Campbell MM, Sederoff RR (1996) Variation in lignin content and composition mechanisms of control and implications for the genetic improvement of plants. Plant Physiol 110(1):3–13

Cao YF, Tian LM, Li LL, Gao Y (2010) Comparison studies on the stone cell content in flesh of pear cultivars. Acta Hortic Sinica 22(3):417–433

Cao YP, Fang Z, Li SS, Yan CC, Ding QQ, Cheng X, Lin Y, Guo N, Cai YP (2015) Genome-wide identification and analyses of *4CL* gene families in *Pyrus bretschneideri* Rehd. Hereditas 37(7):711–719

Cao YP, Han YH, Li DH, Lin Y, Cai YP (2016a) Systematic analysis of the 4-coumarate: coenzyme A ligase (4CL) related genes and expression profiling during fruit development in the Chinese pear. Genes 7 (10):89

Cao YP, Han YH, Meng DD, Li DH, Jin Q, Lin Y, Cai YP (2016b) Structural, evolutionary, and functional analysis of the class III peroxidase gene family in Chinese pear (*Pyrus bretschneideri*). Front Plant Sci 7:1874

Chen F, Tobimatsu Y, Jackson L, Nakashima J, Ralph J, Dixon RA (2013) Novel seed coat lignins in the *Cactaceae*: structure, distribution and implications for the evolution of lignin diversity. Plant J 73(2):201–211

Cheng X, Xiong Y, Li DH, Cheng J, Cao YP, Yan CC, Jin Q, Sun N, Cai YP, Lin Y (2016) Bioinformatic and

expression analysis of the *OMT* gene family in *Pyrus bretschneideri* cv. Dangshan Su. Genet Mol Res 15 (3). https://doi.org/10.4238/gmr.15038664

Cheng X, Li M, Li D, Zhang J, Jin Q, Sheng L, Cai YP, Lin Y (2017a) Characterization and analysis of *CCR* and *CAD* gene families at the whole-genome level for lignin synthesis of stone cells in pear (*Pyrus bretschneideri*) fruit. Biol Open 6(11):1602–1613

Cheng X, Li G, Ma C, Abdullah M, Zhang J, Zhao H, Jin Q, Cai Y, Lin Y (2019a) Comprehensive genome-wide analysis of the pear (*Pyrus bretschneideri*) laccase gene (*PbLAC*) family and functional identification of *PbLAC1* involved in lignin biosynthesis. PLOS ONE 14(2):e0210892

Cheng X, Li G, Muhammad A, Zhang J, Jiang T, Jin Q, Zhao H, Cai Y, Lin Y (2019b) Molecular identification, phylogenomic characterization and expression patterns analysis of the *LIM* (LIN-11, Isl1 and MEC-3 domains) gene family in pear (*Pyrus bretschneideri*) reveal its potential role in lignin metabolism. Gene 686:237–249

Cheng X, Muhammad A, Li G, Zhang J, Cheng J, Qiu J, Jiang T, Jin Q, Cai Y, Lin Y (2019c) Family-1 UDP glycosyltransferases in pear (*Pyrus bretschneideri*): Molecular identification, phylogenomic characterization and expression profiling during stone cell formation. Mol Biol Rep 46(2):2153–2175

Cheng X, Yan CC, Zhang J, Jin Q, Lin Y, Cai YP (2017b) The effect of different pollination on the expression of Dangshan Su pear microRNA. Biomed Res Int 2:2794040

Cheng X, Su XQ, Muhammad A, Li ML, Zhang JY, Sun YM, Li GH, Jin Q, Cai YP, Lin Y (2018) Molecular characterization, evolution, and expression profiling of the *dirigent* (*DIR*) family genes in Chinese white pear (*Pyrus bretschneideri*). Front Genet 9:136

Choi JH, Choi JJ, Hong KH, Kim WS, Lee SH (2007) Cultivar differences of stone cells in pear flesh and their effects on fruit quality. Hortic Environ Biotechnol 48(1):27–31

Choi JH, Lee SH (2013) Distribution of stone cell in Asian, Chinese, and European pear fruit and its morphological changes. J Appl Bot Food Qual 86:185–189

Doblin MS, Pettolino F, Bacic A (2010) Evans review: plant cell walls: the skeleton of the plant world. Funct Plant Biol 37(5):357–381

Eudes A, Liang Y, Mitra P, Loqué D (2014) Lignin bioengineering. Curr Opin Biotechnol 26(4):189–198

Foster TM, Aranzana MJ (2018) Attention sports fans! The far-reaching contributions of bud sport mutants to horticulture and plant biology. Hortic Res 5:44

Gao Z, Zhang C, Luo M, Wu Y, Duan S, Li J, Wang L, Song S, Xu W, Wang S, Zhang C, Mac C (2016) Proteomic analysis of pear (*Pyrus pyrifolia*) ripening process provides new evidence for the sugar/acid metabolism difference between core and mesocarp. Proteomics 16(23):3025–3041

Han WY, Meng YH, Hu CY, Dong GR, Qu YL, Deng H, Guo YR (2017) Mathematical model of Ca^{2+} concentration, pH, pectin concentration and soluble solids (sucrose) on the gelation of low methoxyl pectin. Food Hydrocolloids 66:37–48

Jin Q, Yan CC, Qiu JX, Zhang N, Lin Y, Cai YP (2013) Structural characterization and deposition of stone cell lignin in Dangshan Su pear. Sci Hortic 155(155):123–130

Keegstra K (2010) Plant cell walls. Plant Physiol 154 (2):483

Kim WS, Choi JH (2004a) Morphological characteristics of stone cells in fruit of pears. HortScience 39(4):829–830

Kim WS, Choi JH (2004b) Stone cells in fruit of pears influenced by water stress and calcium. HortScience 39(4):761

Kim WS, Choi JH (2004c) Morphological characteristics of stone cells in fruit of Asian, European, and Chinese pears. HortScience 39(4):815–816

Konarska A (2013) The relationship between the morphology and structure and the quality of fruits of two pear cultivars (*Pyrus communis* L.) during their development and maturation. Sci World J **2013**:846796

Lee SH, Choi JH, Kim WS, Han TH, Park YS, Gemma H (2006) Effect of soil water stress on the development of stone cells in pear (*Pyrus pyrifolia* cv. 'Niitaka') flesh. Sci Hortic 110(3):247–253

Lee SH, Choi JH, Kim WS, Park YS, Gemma H (2007) Effects of calcium chloride spray on peroxidase activity and stone cell development in pear fruit (*Pyrus pyrifolia* 'Niitaka'). J Jpn Soc Hortic Sci 76 (3):191–196

Li L, Cheng XF, Leshkevich J, Umezawa T, Harding SA, Chiang VL (2001) The last step of syringyl monolignol biosynthesis in angiosperms is regulated by a novel gene encoding sinapyl alcohol dehydrogenase. Plant Cell 13(7):1567–1585

Li XG, Jin L, Jiang ZC, Teng NJ, Sheng BL (2004) The relationship between the content of pear's stone cells and pulp quality. HortScience 39(4):850

Li JM, Huang XS, Li LT, Zheng DM, Xue C, Zhang SL, Wu J (2015) Proteome analysis of pear reveals key genes associated with fruit development and quality. Planta 241(6):1363–1379

Li X, Liu L, Ming M, Hu H, Zhang M, Fan J, Song B, Zhang SL, Wu J (2019) Comparative transcriptomic analysis provides insight into the domestication and improvement of pear (*P. pyrifolia*) fruit. Plant Physiol 180:435–452. https://doi.org/10.1104/pp.18.01322

Li N, Ma Y, Song Y, Tian C, Zhang L, Li L (2017) Anatomical studies of stone cells in fruits of four different pear cultivars. Int J Agric Biol 19(4):610–614

Li SM, Su XQ, Abdullah M, Sun YM, Li GH, Cheng X, Lin Y, Cai YP, Jin Q (2108a) Effects of different pollens on primary metabolism and lignin biosynthesis in pear. Int J Mol Sci 19(8):2273

Li SM, Su XQ, Jin Q, Li GH, Sun Y, Abdullah M, Cai YP, Lin Y (2018b) iTRAQ-based identification of proteins related to lignin synthesis in the pear pollinated with pollen from different varieties. Molecules 23(3):548

Liu CJ (2012) Deciphering the enigma of lignification: precursor transport, oxidation, and the topochemistry of lignin assembly. Mol Plant 5(2):304–317

Lu B, Chen KS, Dong Z, Cao YF, Toshiya Y, Teng YW (2007) Genetic diversity and similarity of pear (*Pyrus* L.) cultivars native to East Asia revealed by SSR (simple sequence repeat) markers. Genet Resour Crop Evol 54(5):959–971

Lu XP, Liu YZ, An JC, Hu HJ, Peng SA (2011) Isolation of a cinnamoyl CoA reductase gene involved in formation of stone cells in pear (*Pyrus pyrifolia*). Acta Physiol Plant 33(2):585–591

Lu GL, Li ZJ, Zhang XF, Wang R, Yang SL (2015) Expression analysis of lignin-associated genes in hard end pear (*Pyrus pyrifolia* Whangkeumbae) and its response to calcium chloride treatment conditions. J Plant Growth Regul 34(2):251–262

Ma C, Zhang HP, Li JM, Tao ST, Qiao X, Korban SS, Wu J (2017) Genome-wide analysis and characterization of molecular evolution of the *HCT* gene family in pear (*Pyrus bretschneideri*). Plant Syst Evol 303 (1):71–90

Nie JQ, Cai YP, Zhang SH, Lin Y, Xu YL, Zhang JY (2009) The anatomic study on relationship of stone cells and parenchyma cells during fruit development of *Pyrus bretschneideri*. Acta Hortic Sinica 36 (8):1209–1214

Nii N, Kawahara T, Nakao Y (2008) The development of stone cells in Japanese pear fruit. J Hortic Sci Biotech 83(2):148–153

Pan HY, Zhou R, Louie GV, Mühlemann JK, Bomati EK, Bowman ME, Dudareva N, Dixon RA, Noel JP, Wang XQ (2014) Structural studies of cinnamoyl-CoA reductase and cinnamyl-alcohol dehydrogenase, key enzymes of monolignol biosynthesis. Plant Cell 26(9):3709–3727

Paniagua C, Bilkova A, Jackson P, Dabravolski S, Riber W, Didi V, Houser J, Gigli-Bisceglia N, Wimmerova M, Budínská E, Hamann T, Hejatko J (2017) Dirigent proteins in plants: modulating cell wall metabolism during abiotic and biotic stress exposure. J Exp Bot 68(13):3287–3301

Pascual MB, El-Azaz J, Torre FNDL, Cañas RA, Avila C, Cánovas FM (2016) Biosynthesis and metabolic fate of phenylalanine in conifers. Front Plant Sci 7:1030

Pauly M, Keegstra K (2008) Cell-wall carbohydrates and their modification as a resource for biofuels. Plant J 54 (4):559–568

Tao ST, Khanizadeh S, Zhang H, Zhang SL (2009) Anatomy, ultrastructure and lignin distribution of stone cells in two *Pyrus* species. Plant Sci 176 (3):413–419

Tao ST, Wang DY, Jin C, Sun W, Liu X, Zhang SL, Gao FY, Khanizadeh S (2015) Cinnamate-4-hydroxylase gene is involved in the step of lignin biosynthesis in Chinese white pear. J Am Soc Hortic Sci 140(6):573–579

Tian LM, Cao YF, Gao Y, Dong XG (2011) Effect of stone cells size and flesh texture in pear cultivars. Acta Hortic Sinica 38(7):1225–1234

Tian LM, Dong XG, Cao YF, Zang Y, Qi D (2017) Correlation of flesh in *Pyrus* fruit with its stone cells lignin. Southwest China J Agric Sci 30(9):2091–2096

Vanholme R, Demedts B, Morreel K, Ralph J, Boerjan W (2010) Lignin biosynthesis and structure. Plant Physiol 153(3):895–905

Vanholme R, Cesarino I, Rataj K, Xiao YG, Sundin L, Goeminne G, Kim H, Cross J, Morreel K, Araujo P, Welsh L, Haustraete J, McClellan C, Vanholme B, Ralph J, Simpson GG, Halpin C, Boerjan W (2013) Caffeoyl shikimate esterase (CSE) is an enzyme in the lignin biosynthetic pathway in *Arabidopsis*. Science 341(6150):1103–1106

Voxeur A, Wang Y, Sibout R (2015) Lignification: different mechanisms for a versatile polymer. Curr Opin Plant Biol 23:83–90

Wang YZ, Dai MS, Zhang SJ, Shi ZB (2012) A review on pear bud sport breeding and research progress in mutant mechanisms. J Fruit Sci 29(4):676–682

Wang B, Zhang N, Yan CC, Jin Q, Lin Y, Cai YP, Zhang JY (2013) Bagging for the development of stone cell and metabolism of lignin in *Pyrus bretschneideri* 'Dangshansuli'. Acta Hortic Sinica 40 (3):531–539

Wang Y, Zhang X, Yang S, Wang C, Lu G, Wang R, Yang Y, Li D (2017a) Heterogenous expression of *Pyrus pyrifolia PpCAD2* and *PpEXP2* in tobacco impacts lignin accumulation in transgenic plants. Gene 637:181–189

Wang YL, Zhang XF, Wang R, Bai YX, Liu CL, Yuan YB, Yang YJ, Yang SL (2017b) Differential gene expression analysis of 'chili' (*Pyrus bretschneideri*) fruit pericarp with two types of bagging treatments. Hortic Res 4:17005

Wang Y, Zhang X, Wang Y, Yang S, Qu H (2018) The changes of intracellular calcium concentration and distribution in the hard end pear (*Pyrus pyrifolia* cv. 'Whangkeumbae') fruit. Cell Calcium 71:15–23

Whitehill JG, Henderson H, Schuetz M, Skyba O, Yuen MMS, King J, Samuels AL, Mansfield SD, Bohlmann J (2016a) Histology and cell wall biochemistry of stone cells in the physical defence of conifers against insects. Plant Cell Environ 39(8):1646–1661

Whitehill JG, Henderson H, Strong W, Jaquish B, Bohlmann J (2016b) Function of sitka spruce stone cells as a physical defense against white pine weevil. Plant Cell Environ 39(11):2545–2556

Wilkerson CG, Mansfield SD, Lu F, Withers S, Park JY, Karlen SD, Gonzales-Vigil E, Padmakshan D, Unda F, Rencoret J, Ralph J (2014) Monolignol ferulate transferase introduces chemically labile linkages into the lignin backbone. Science 344(6179):90–93

Wu J, Wang ZW, Shi ZB, Zhang S, Ming R, Zhu SL, Khan MA, Tao S, Korban SS, Wang H, Chen NJ, Nishio T, Xu X, Cong L, Qi KJ, Huang XS,

Wang YT, Zhao X, Wu JY, Deng C, Gou CY, Zhou WL, Yin H, Qin GH, Sha YH, Tao Y, Chen H, Yang YA, Song Y, Zhan DL, Wang J, Li LT, Dai MS, Gu C, Wang YZ, Shi DH, Wang XW, Zhang HP, Zeng L, Zheng DM, Wang CL, Chen MS, Wang GB, Xie L, Sovero V, Sha SF, Huang WJ, Zhang SJ, Zhang MY, Sun JM, Xu LL, Li Y, Liu X, Li QS, Shen JH, Wang JY, Paull RE, Bennetzen JL, Wang J, Zhang SL (2013) The genome of the pear (*Pyrus bretschneideri* Rehd.). Genome Res 23(2):396–408

Wu J, Wang DF, Liu YF, Wang L, Qiao X, Zhang SL (2014) Identification of miRNAs involved in pear fruit development and quality. BMC Genom 15(1):953

Wu J, Wang YT, Xu JB, Korban SS, Fei ZJ, Tao ST, Ming R, Tai SS, Khan AM, Postman JD, Gu C, Yin H, Zheng DM, Qi KJ, Li, Wang RZ, Deng CH, Kumar S, Chagné D, Li XL, Wu JY, Huang XS, Zhang HP, Xie ZH, Li X, Zhang MY, Li YH, Yue Z, Fang XD, Li JM, Li LT, Jin C, Qin MF, Zhang JY, Wu X, Ke YQ, Wang J, Yang HM, Zhang SL (2018) Diversification and independent domestication of Asian and European pears. Genome Biol 19:1–16

Xu YL, Pan HF, Gao ZH, Yi XK, Qin GH, Qi YJ, Zhang JY (2016) Research on germplasm of 'Dangshansuli' pear. J Fruit Sci 33:34–42

Xue C, Yao JL, Qin MF, Zhang MY, Allan AC, Wang DF, Wu J (2018) *PbrmiR397a* regulates lignification during stone cell development in pear fruit. Plant Biotechnol J 2018:1–15

Xue C, Yao JL, Xue Y-S, Su G-Q, Wang L, Lin L-K, Allan A, Zhang S-L, Wu J (2019) PbrMYB169 positively regulates lignification of stone cells in pear fruit. J Exp Bot 70(6):1801–1814

Yan CC, Yin M, Zhang N, Jin Q, Fang Z, Lin Y, Cai YP (2014) Stone cell distribution and lignin structure in various pear varieties. Sci Hortic 174(1):142–150

Yang SL, Zhang XN, Lu GL, Wang CR, Wang R (2014) Regulation of gibberellin on gene expressions related with the lignin biosynthesis in 'Wangkumbae' pear (*Pyrus pyrifolia* Nakai) fruit. Plant Growth Regul 76 (2):1–8

Zhang YX, Tian ZX, Xi RT, Gao HM (2002) Effect of SA on phemolics metabolization of Ya pear growing fruits. J Agric Univ Hebei 25(3):33–36

Zhang MY, Xue C, Xu L, Sun H, Qin MF, Zhang, S, Wu J (2016) Distinct transcriptome profiles reveal gene expression patterns during fruit development and maturation in five main cultivated species of pear (*Pyrus* L.). Sci Rep 6:28130

Zhang J, Cheng X, Jin Q, Su XQ, Li ML, Yan CC, Jiao XY, Li DH, Lin Y, Cai YP (2017) Comparison of the transcriptomic analysis between two chinese white pear (*Pyrus bretschneideri* Rehd.) genotypes of different stone cells contents. Plos One 12(10): e0187114

Zhao M, Zhu HM (2014) Development and morphology of stone cells in phloem of *Toxicodendron vernicifluum*. Trees 28(5):1553–1558

Zhao SG, Zhang JG, Zhao YP, Zhang YX (2013) New discoveries of stone cell differentiation in fruitlets of 'Yali' pears (*Pyrus bretschneideri* Rehd.). Int J Food Agric Environ 11(3):937–942

Zhong R, Ye ZH (2015) Secondary cell walls: biosynthesis, patterned deposition and transcriptional regulation. Plant Cell Physiol 56(2):195–214

12 Genetic and Genomic Analyses of Vegetative Budbreak in Response to Chilling Units in European Pear (*Pyrus Communis* L.)

Gilad Gabay and Moshe A. Flaishman

Abstract

Dormancy is critical for the normal yearly cycle of fruit trees in temperate zones due to their requirements of exposure to certain numbers of chilling hours. Once the chilling requirement is fulfilled, vegetative budbreak can occur when climatic conditions are favorable. Exposure to insufficient chilling units can lead to delayed vegetative budbreak. Bud dormancy has been studied in perennial fruit trees within the context of the effects of climate change. The recent rise in temperatures worldwide has led to a reduction in chilling units accumulation. Pear cultivars are highly influenced by the number of chilling units accumulated during the winter. However, fruit of most low-chilling cultivars is considered to be of low quality. Study of the genetic mechanism underlying chilling requirements would greatly accelerate adaptation of new pear cultivars to warm climates. As vegetative budbreak date shows high heritability, the potential for breeding a low-chilling requirement pear cultivar is high. However, chilling requirements are subject to a complex genetic mechanism which is probably determined by, or partially derived from, multiple genes. Genetic factors affecting dormancy have been identified for the first time in peach, wherein MADS-box genes associated with dormancy regulation have been reported. Six *DORMANCY-ASSOCIATED MADS-BOX* (*DAM*) genes, and a genomic region, designated as the *evergrowing* (*evg*) locus, have been identified. To date, three *DAM* genes, including *PpDAM1*, *PpDAM2,* and *PpDAM3*, have been identified in Asian pear (*Pyrus* spp.). In previous genetic studies in apple, which has a high level of synteny with pear, quantitative trait loci (QTLs) for chilling requirements have been identified. A QTL common to all families has been located on linkage group 9, suggesting stability of this QTL over different families, climate regions, and years. However, in European pear, a major QTL has been detected on linkage group 8, and an additional QTL on linkage group 9 has also been confirmed. Differentially expressed genes in these regions include *PcDAM1 and PcDAM2,* putative orthologs of *PpDAM1* and *PpDAM2*. Due to a significant genotype × environment (G × E) effect, QTLs associated with G × E vegetative budbreak date have been detected. It has long been known that content levels of metabolites are highly correlated with dormancy phase

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transitions. Metabolites, such as phospholipids, sugars, and fatty acids, including alpha-linolenic acid, play major roles in dormancy regulation in pear. Several pear genes, such as *12-oxophytodienoate reductase 2-like* (alpha-linolenic acid pathway), have been found to be linked to dormancy regulation. A proposed model for pear selection of traits under a changing climate will be discussed.

12.1 Introduction

Perennial plants have developed a dormancy mechanism in temperate regions to overcome severe cold temperature conditions and frost. Dormancy is broadly characterized by three states, including the following: (1) paradormancy, when factors exterior to the bud regulate and maintain the dormancy state—this is usually related to apical dominance; (2) endodormancy, when factors related to chilling accumulation within buds regulate transition between different dormancy states—this is the main state analyzed and discussed in this chapter due to its relevance, which will be further explained; and (3) ecodormancy, when environmental factors, which are mainly related to temperature, signal to the bud, after sufficient chilling accumulation, to be released from dormancy.

Most Rosaceae fruit tree species, including the European pear (*Pyrus communis*), enter a state of dormancy in response to decreasing temperatures. On the other hand, endodormancy release depends on the number of chilling hours (chilling units = CUs) that deciduous trees are exposed to during the winter season (Anderson et al. 1986; Heide and Prestrud 2005). Chilling requirements (CRs) vary among cultivars and among plant species, and these correspond to the number of CUs required for budbreak during the spring. Once trees are exposed to favorable environmental conditions, such as rising temperatures, represented by heat requirement (HR), dormant buds will break, and trees will resume growth (Erez and Lavee 1971; Wigge 2013). The CR is generally characterized by the number of CUs needed for a given cultivar to achieve 50% budbreak under favorable conditions. It is important to point out that CR and HR are synchronized, and well-correlated. Hence, insufficient CUs result in an extended HR period for budbreak; whereas, overexposure to CUs that extend CR results in a shorter period of favorable conditions for budbreak induction (Ruiz et al. 2007). Therefore, when CR is not fulfilled, vegetative budbreak (VB) date is delayed. Hence, VB date can indicate the CR of a particular genotype.

European pear (*P. communis*) cultivars are mostly bred in climate regions wherein winter temperatures are low enough and sustained for the duration of the winter season to satisfy CRs, which in some cases differ from climates of other growing regions, such as those of the Mediterranean region. Therefore, it is unlikely that pear tree performance for traits, such as flower development and fruit set, will be similar over different environments (Labuschagné et al. 2002).

There are various models to estimate the number of CUs. In the Mediterranean region, characterized by low CU accumulation, endodormancy release is more strongly affected by recent climate changes than in areas with higher numbers of CUs. In warm climate regions, a commonly used model to evaluate CU accumulation is a dynamic model developed to evaluate CRs in warm regions, such as those of the Mediterranean and California, as it accounts for negative effects of high temperatures during winter on CU accumulation (Erez et al. 1988).

Recent global warming conditions are expected to result in reductions of CU accumulation, based on model climate predictions (Campoy et al. 2011). As CU accumulation leads to delayed budbreak, which is essential for normal flower and fruit development (Takemura et al. 2015), consequences of reduced CUs for warm areas, such as the Mediterranean, may lead to severe disorders in deciduous fruit tree growth

habits, such as reduced yield and abnormal fruit set. Currently, a standard practice for inducing VB on required dates, for normal fruit growth and for filling gaps between CR and actual CU accumulation in warmer areas, is to spray chemical compounds. However, due to increasing awareness of the environmental effects of this practice, there is a growing demand for fruit trees with low CRs (Celton et al. 2011; Ubi et al. 2010; van Dyk et al. 2010). Therefore, a better understanding of the genetics and of key factors governing dormancy phase transitions is much needed.

12.2 Vegetative Budbreak Date Variations and Chilling Requirements in European Pears

Cultivars of European pear (*P. communis*) are highly influenced by the number of CUs accumulated during the winter. Based on modeling and phenological data, the numbers of CUs required for adequate budbreak can vary from 300 (cv. Spadona) to 1500 (cv. Bartlett). Therefore, most commercial pear cultivars are grown in temperate regions, classified as having intermediate to high CUs, and are poorly adapted to mild climates (Flaishman et al. 2001; Zohary 1997). Recent global climate changes, along with increasing demands for growing pear trees in warm regions, have highlighted the importance of developing low-CR fruit tree cultivars (Busov et al. 2016; Li et al. 2018).

It has been reported that VB date shows high heritably in apple, *Malus* × *domestica* (Labuschagné et al. 2002). Thus, there is a good potential for breeding low-CR pear cultivars adapted to increasing temperatures (Allard et al. 2016). However, CRs corresponding to VB dates are subject to a complex genetic mechanism in deciduous trees, which is probably determined by, or partially controlled by multiple genes (Howe et al. 2000).

12.2.1 Main Effects of Heritability and Variance of Vegetative Budbreak Date

It has been recently reported that VB date variance is controlled by main effects in European pear (Gabay et al. 2017). VB date phenotyping, which indicates chilling requirements, has been conducted over two consecutive years (2014–2015) in two locations in Israel, Bet Dagan (BD) and Tzuba (TZU), that highly differ in their yearly average accumulated CUs (Fig. 12.1). The pear material consists of an F1 population, derived from a cross between ‘Spadona’ (low CR) and ‘Harrow Sweet’ (high CR), as well as commercial pear cultivars and accessions differing in their CRs (Fig. 12.1). Replications of these genotypes have been exposed during the winter to different CUs. Subsequently, trees have been transferred to the same region and exposed to similar heat conditions to induce VB. This has been conducted to determine the genetic component of CR, and to distinguish it from the genetic component for HR.

As estimation of broad-sense heritability (H^2) is reliable for specific environmental conditions and populations (Souza et al. 1998), it is revealed that H^2 estimations in this study are higher within locations, and specific to location. Furthermore, the determined overall mean broad-sense heritability ($H^2 = 0.46$) for pear is lower than those reported in other studies for apple, with estimated values of 0.87 (Allard et al. 2016), 0.88–0.92 (Celton et al. 2011), and 0.62–0.92 (van Dyk et al. 2010). However, specific H^2 values per specific year and location for pear are similar to those obtained in apple, ranging between 0.84 and 0.94.

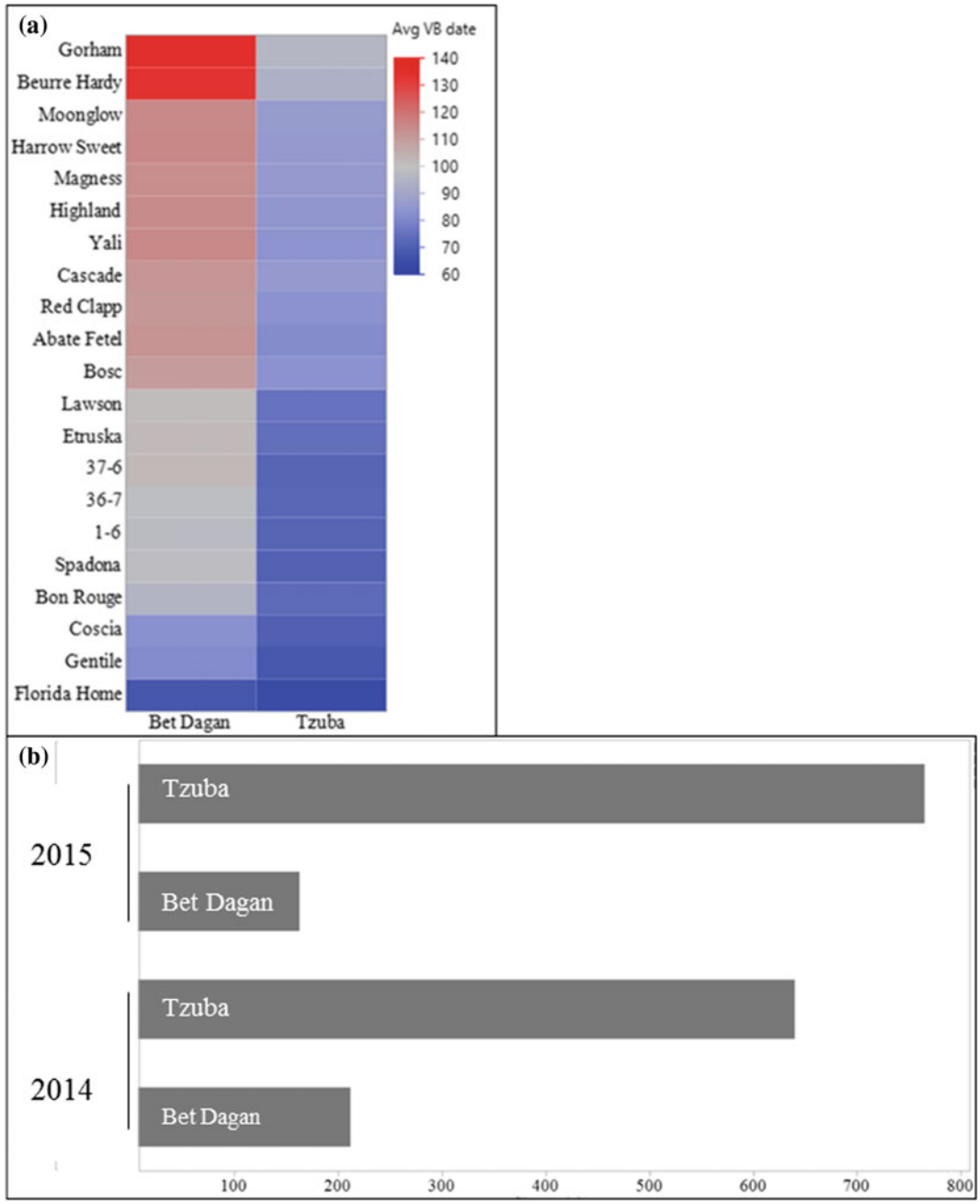

Fig. 12.1 **a** Average days for vegetative budbreak in pear cultivars and accessions in two locations in Israel over two consecutive years (2014–2015) (day 0 = 1st of January). **b** Accumulation of chilling units in two locations over two consecutive years (2014–2015). The *X*-axis corresponds to number of accumulated CUs. The *Y*-axis corresponds to year and location. Tzuba = High--chilling unit accumulation, in the Jerusalem mountains (720 m a.s.l); and Bet Dagan = Low-chilling unit accumulation, in a coastal area (50 m a.s.l)

12.2.2 Breeding Potential of Pears for Low-Chilling Requirements

A large genetic effect is observed for CR and its heritability, thus indicating that there is a high breeding potential for this trait (Celton et al. 2011; Trainin et al. 2013; van Dyk et al. 2010). However, a significant genotype × environment (G × E) interaction is a major factor for VB date, and accounts for 35% of the observed phenotypic variance in pear (Gabay et al. 2017; Labuschagné et al. 2002). Furthermore, a significant effect of genotype on VB date has been observed and accounting for 35.8% of the phenotypic variance for VB date. Although genotypic effects influence the time of VB in pear, genotype × year and genotype × location interactions should be also taken into account when low-CR cultivars are being selected for. These interactions highlight the importance of selecting a particular genotype for a targeted climatic region. Hence, genotypes have different responses to number of CUs and to other climatic components. This renders selection for such a trait complicated under instances of changing climates as genotypes may act differently in upcoming years with predicted increases in yearly average temperatures (Dirlewanger et al. 2012).

Cultivar selection in the targeted climate region does not ensure the cultivar's adaptation to that region, as CU accumulation can sharply decrease within a given climate region. Phenotypic plasticity, a genotype's ability to perform stably across different years and climate regions, plays an important role in cultivar selection. This is particularly critical for such traits as CR, wherein CR is highly influenced by recent increases in worldwide temperatures. Therefore, CR trait stability across environments should serve as an important criterion during breeding. In addition, deciphering genetic and physiological mechanisms of CR interactions with environment will further enhance pear breeding for low CR.

12.3 Quantitative Trait Loci (QTL) Mapping for Vegetative Budbreak

Genetic factors influencing CR were identified for the first time in peach (*Prunus*), a member of Rosaceae (Bielenberg et al. 2008). MADS-box genes associated with dormancy regulation were identified, including six *DORMANCY-ASSOCIATED MADS-BOX* (*DAM*) genes along with a genomic region, designated as the *evergrowing* (*evg*) locus. These genes were proposed to play regulatory functions in bud set, vegetative growth, and cessation of growth (Jiménez et al. 2010). In a later study, quantitative trait loci (QTL) analysis was conducted using a large peach population, and a QTL associated with CR was identified in the same genomic region as that of *evg* (Fan et al. 2010). QTLs associated with CR and dormancy regulation have already been identified in other Rosaceae members. In *Prunus*, the same QTLs have been identified for both CR and bloom date, thereby confirming presence of a strong correlation between these two traits (Dirlewanger et al. 2012).

In previous genetic studies using full-sib families in apple, which shares a high level of synteny with pear (Celton et al. 2009), QTLs for CR have also been identified (Allard et al. 2016; Celton et al. 2011; van Dyk et al. 2010). However, the only QTL common to all families is located on LG9, thus confirming stability of this QTL over different families, climate regions, and years (Allard et al. 2016; van Dyk et al. 2010).

The first QTL analysis for a pear population segregating for VB date (Gabay et al. 2017) has confirmed QTL synteny between apple and pear using data obtained from 'selective genotyping'; i.e., tail analysis. This method can be used to determine linkages between a genetic marker and a target trait at relatively low cost, and with a relatively small number of genotyped individuals (Darvasi and Soller 1992). Furthermore, two QTLs have been detected within the same genomic regions as those found in apple, LG9 and LG8, and

these are determined to be stable over locations and years under diverse climatic conditions. However, recent advances in genotyping methods have enabled more accurate detection of such QTLs.

12.3.1 Fine QTL Mapping Using a High-Resolution Genetic Map

In an earlier study, QTLs associated with CRs using a high-resolution genetic map in closely related species, including apple, have been identified (Allard et al. 2016). Although, pear and apple show high levels of synteny (Celton et al. 2009; Chagné et al. 2014), differences have been observed between genomic regions of apple (LG9) and pear (LG8) associated with chilling requirements. In apple, the most stable and significant QTL has been detected on LG9 in various studies conducted in different locations and years (Allard et al. 2016; Celton et al. 2011; van Dyk et al. 2010). However, the most significant QTL in pear is detected on LG8 (LOD score = 11.49), explaining 28% of the phenotypic variance of VB date (Gabay et al. 2018). This represents the first QTL detection in pear using a reliable genetic map constructed using genotyping-by-sequencing (GBS) data for 162 F1 offsprings (Gabay et al. 2018). Additional QTLs for VB date have been detected on LGs 5 and 15 (Fig. 12.2), and these have also been previously identified in apple (Allard et al. 2016). However, a new QTL associated with VB date has been detected on LG13 in pear (Fig. 12.2). To the best of our knowledge, this QTL has never been identified before in either pear or apple. Synteny within the subfamily Amygdaloideae, which includes European pear (*P. communis)* and apple (*M.* × *domestica)*, has been reported for QTLs associated with traits such as scab resistance (Bouvier et al. 2012), fire blight resistance (Le Roux et al. 2012), and fruit softening (Costa et al. 2008). In addition, simple sequence repeat (SSR) markers have been found to be highly transferable between apple and pear (Celton et al. 2009; Yamamoto and Terakami 2016). Interestingly, locations of QTLs found in pear have been detected within the same regions as those found in apple, but at different levels of significance and phenotypic variances explaining these QTLs (Gabay et al. 2018). Furthermore, QTL mapping using high-resolution genetic maps has enabled accurate detection and confirmation of QTL analysis results reported in both pear (Gabay et al. 2017) and apple (Allard et al. 2016; Celton et al. 2011; van Dyk et al. 2010). The above findings highlight the importance of conducting independent genetic studies in pear, as well as in construction of high-resolution genetic maps to accurately identify genomic regions associated with complex target traits, as well as to accurately determine variance values explained by identified QTLs for these complex traits.

The reliability of a QTL for a trait of interest and its usefulness in pursuing efficient and effective marker-assisted selection strategies are highly dependent on stability of this QTL under different environmental conditions/years, locations, and genetic backgrounds (Allard et al. 2016). Therefore, in a recent study, we have identified 21 European pear cultivars with either very low or high CR (i.e., 'selective genotyping'). These cultivars have been subjected to phenotyping and genotyping-by-sequencing (GBS) analysis to evaluate those QTLs detected in an F1 population of 'Spadona' × 'Harrow Sweet' in diverse genetic backgrounds (Fig. 12.1a). As numbers of accessions used to evaluate identified QTLs have not been sufficient for pursuing genome-wide association studies (GWAS), observed differences may result from other genetic variances that are not associated with CR (Zhu et al. 2008), and therefore our results are deemed preliminary (Gabay et al. 2018). It has been observed that significant molecular markers associated with VB date have been detected in all LGs for which a QTL has been detected in our F1 pear population. However, not all of these markers are located within the highest peak of QTL intervals. Nevertheless, markers found significant in the highest peak of a major QTL are detected on LGs 8 and 9 (Gabay et al. 2018). Therefore, it is assumed that these regions control VB date in diverse genotypes of European pear. In addition, genetic-relatedness analysis of pear cultivars included in this study

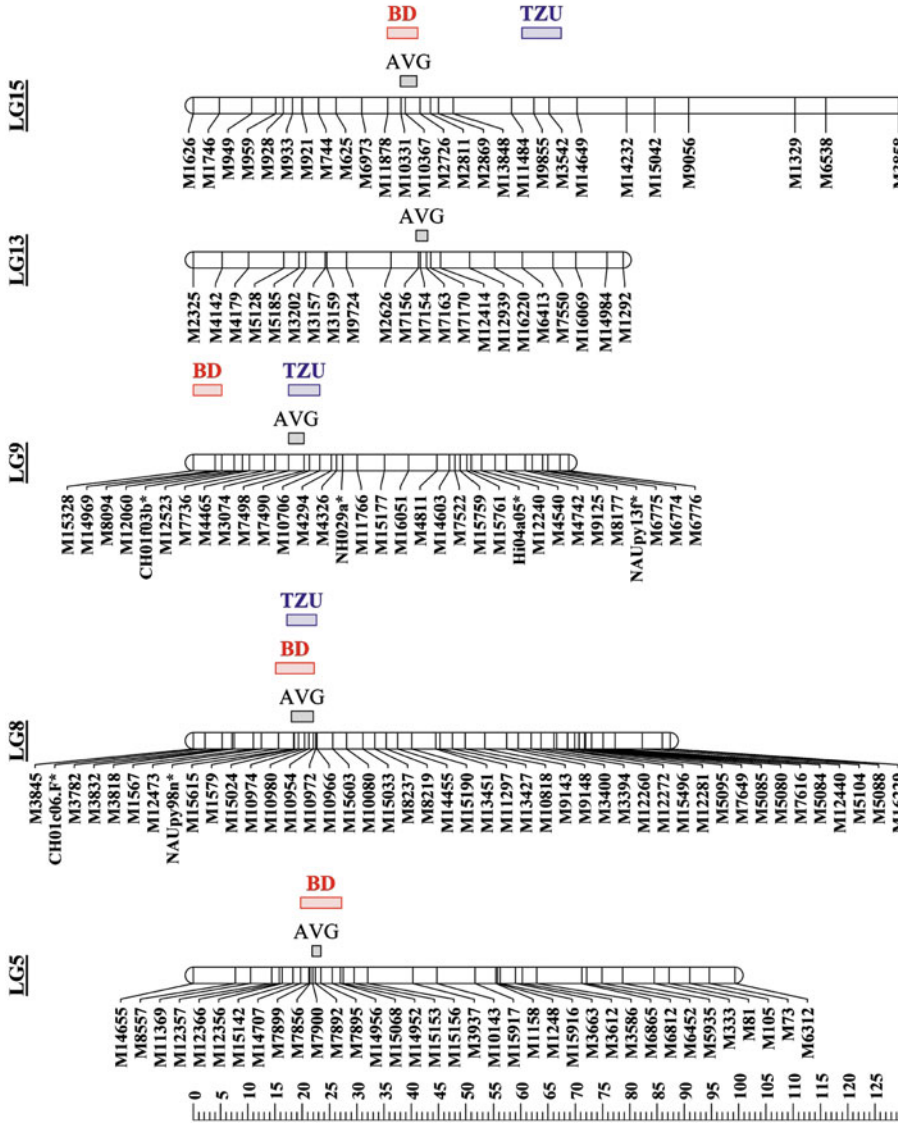

Fig. 12.2 Positions of QTLs for VB date using a high-resolution consensus map of SPD × HS (Gabay et al. 2018). For each QTL, one-LOD support intervals are shown. Black boxes correspond to QTLs for overall mean VB date (AVG). Blue boxes correspond to QTLs for Tzuba (TZU) for high CU accumulation. Red boxes correspond to QTLs for Bet Dagan (BD) for low CU accumulation

has revealed presence of two groups corresponding to CR, thus suggesting that these cultivars share the same genetic mechanism governing the CR trait. However, a notable exception detected among the low-CR group is pear cv. Florida Home (Fig. 12.1a). Although this cultivar is known to be an extremely low-CR cultivar, due to its very early VB date, it is observed that it is not grouped close to other low-CR cultivars. This finding may be attributed to the fact that this cultivar, derived from a cross between European and Asian pears (Villalta et al. 2005), has a significantly different genetic background for CR determination (Gabay et al. 2018). The major QTL, on LG8, associated with VB date has been confirmed across years and locations, having experienced large differences in climatic conditions. Therefore, markers located within the identified QTL interval on LG8 for VB date can be used for future marker-assisted selection in pear breeding programs.

12.3.2 G × E QTLs Associated with Vegetative Budbreak

As there is a significant G × E interaction for vegetative budbreak (Gabay et al. 2017), it has prompted efforts to identify QTL(s) associated with CR, isolated from other environmental effects (Fig. 12.2). The most significant G × E QTLs are detected on LG9 and LG5, and subsequently additional QTLs have been identified on LG8 and LG17 (Gabay et al. 2018). All these QTLs suggest availability of pear genotypes, carrying useful genes/alleles, with differences in mean VB dates between two locations/different climatic conditions. Hence, these QTLs could be useful in predicting genotypic stability across diverse environments. This is important not only to predict cultivar performance over climatic changes, but also for matching CRs of pear cultivars to appropriate growing regions. Furthermore, it is important to point out that adequate VB date trait is relevant for both warm and cold regions for low-CR pear cultivars, due to frost susceptibility (Olukolu et al. 2009).

As low-CR cultivars may fulfill their CRs by midwinter, unexpected warm temperatures during this period may lead to early budbreak induction, but with likely subsequent drop in temperatures, this can result in frost damage. Therefore, it is essential to select high- and low-CR pear cultivars with sufficient phenotypic plasticity and capable of withstanding such changes in weather conditions, thus demonstrating stable performance within the same location, as climatic conditions may vary over the years.

12.4 Key Regulators During Dormancy Phase Transitions

12.4.1 Gene Expression, Gene Annotation, and Pathway Enrichment of Gene Expression for Bud Dormancy

Previous studies have reported on the importance of *DAM* genes, along with other important genes, in regulating gene expression during bud dormancy phase transitions. In apple, which has a high level of synteny with pear (Celton et al. 2009), four *DAM*-like genes have been characterized, including *MdDAMa*, *MdDAMb*, *MdDAMc*, and *MdDAMd*. Expression levels of *MdDAMa* (on LG16) and *MdDAMc* (on LG8) have been observed to differ over time, thus suggesting that these genes play roles in apple tree dormancy (Mimida et al. 2015). To date, three *DAM* genes, including *PpDAM1* (previously *PpMADS13-1*), *PpDAM2* (previously *PpMADS13-2*), and *PpDAM3* (previously *PpMADS13-3*), have been identified in pear (*Pyrus* spp.) (Tuan et al. 2017). Furthermore, it has been observed that expression levels of *PpMADS13-1*, a *DAM* homolog identified in *P. pyrifolia* (Japanese pear), are lower prior to release of endodormancy (Saito et al. 2015). In addition, expression levels of *PpMADS13-2* and *PpMADS13-3* have been found to correlate with different phases of dormancy (Saito et al. 2013). In European pear, *PcDAM1* and *PcDAM2*,

putative orthologs of *PpDAM1* and *PpDAM2*, are reportedly differentially expressed between dormancy phases, thus confirming their roles in dormancy phase transitions in European pear, *P. communis* (Gabay et al. 2019). Other notable genes involved in dormancy break and CR determination include *ParSOC1,* an *Arabidopsis MADS-box* gene homolog identified in apricot (Trainin et al. 2013), and *EARLY BUD-BREAK1* (*EBB1*), first identified in poplar, and more recently an apple homolog (*MdEBB1*) has also been identified (Busov et al. 2016).

Transcriptome profiles during different phases of pear dormancy, as well as those during annual growth cycles have been investigated (Bai et al. 2013; Gabay et al. 2019; Liu et al. 2012). Annotation of gene profiles has been conducted using GO terms, and KEGG pathway assignment has been described. It has been observed that the 'Metabolic Pathways' category is the most enriched. This has suggested the importance of gene regulation of metabolic processes during transitions between different phases of dormancy in both Asian pear (Bai et al. 2013; Liu et al. 2012) and European pear (Gabay et al. 2019). Plant metabolic pathways are usually controlled by diverse groups of genes and characterized by complex gene regulation mechanisms (Xiao et al. 2015). It has been observed that high numbers of differentially expressed (DE) genes, belonging to diverse gene families in the most enriched KEGG pathway, are detected in all comparative dormancy phase transitions, thereby confirming the proposal that metabolic processes are regulated by a complex genetic mechanism (Allard et al. 2016; Celton et al. 2011; Heide and Prestrud 2005; Howe et al. 2000; Leida et al. 2010). Comparisons between pear cultivars differing in CRs have revealed that many of the biological processes of the GO analysis are detected earlier in the low-CR cultivar Spadona compared to those of the high-CR cultivar Harrow Sweet (Gabay et al. 2019). Hence, 'Spadona' must respond earlier than 'Harrow Sweet' to drops in temperature. Biological processes, such as 'Metabolic Process' and 'Biosynthetic Process' must also be active earlier in 'Spadona' than in 'Harrow Sweet' (Gabay et al. 2019).

12.4.2 Major Changes in Metabolite Content Levels During Dormancy and Their Proposed Regulatory Roles

Dormancy is often correlated with sharp changes in metabolite content and composition (Del Cueto et al. 2017; Ionescu et al. 2017; Izadyar and Wang 1999; Wang and Faust 1990). However, to date, metabolite profiling of pear, and specifically of dormancy in European pears, has not been well described. Along with genes associated with dormancy regulation, several metabolites and proteins, such as dehydrins, sugars, fatty acids, polar lipids, and protein kinases have been reported to be involved in dormancy (Eremina et al. 2016; Maruyama et al. 2009). Lipids are proposed to play major roles in establishment of dormancy by modifying the metabolite composition in plants for buds to deal with cold temperatures. Changes in bud membrane metabolites, dominated by fatty acids and lipids, during dormancy will offer optimal physiological conditions for budbreak response in the spring (Wang and Faust 1990). Sugar accumulation (raffinose) has been detected during establishment of dormancy in apple, suggesting that sugar accumulation may protect dormant buds against draught during dormancy (Falavigna et al. 2018).

Previously, it has been reported that accumulation of major groups of metabolites in various groups of fruit crops is found to be correlated with chilling accumulation and dormancy break, such as those of unsaturated fatty acids in peach (Erez et al. 1997), sugars in apple (Falavigna et al. 2018), and phospholipids in blackberry (Izadyar and Wang 1999). Recently, significant changes in more than 50 metabolites are detected between dormancy phase transitions in pear (Gabay et al. 2019). Specifically, three main groups of metabolites, including fatty acids, sugars, and phospholipids, have been found to cluster together with similar patterns of changes during dormancy, thereby suggesting their potential roles in regulation of dormancy. Transcriptome analysis of 'Spadona' (low CR) and 'Harrow Sweet' (high CR) has revealed that 22 DE genes related to the

alpha-linolenic acid pathway, based on the KEGG analysis, are detected. In particular, it has been observed that there is a significant and sharp increase in alpha-linolenic acid content toward the end of dormancy in both pear cultivars. Furthermore, fatty acid profiles in both cultivars are found to be low during all phases of dormancy, but then this is followed by a sharp increase toward a break in dormancy. Previously, it has been reported that fatty acid content is directly correlated with chilling accumulation (Erez et al. 1997). In our recent study, although both pear cultivars have been exposed to the same number of CUs, they have exhibited different fatty acid profiles during dormancy (Gabay et al. 2019). Moreover, in 'Spadona' (low CR), six additional unsaturated fatty acids have been detected, including linoleic acid, which has significantly changed during dormancy. Therefore, it is proposed that changes in fatty acids, such as alpha-linolenic acid, lauric acid, linoleic acid, margaric acid, non-adecylic acid, palmitic acid, and stearic acid contribute to changes in membrane metabolite composition that allow for budbreak. Moreover, it is also important to point out that accumulation of these fatty acids differs between low- and high-CR pear cultivars. Hence, fatty acid profile in low-CR pear changes earlier than that in high-CR pear.

Furthermore, significant changes in contents of 11 sugars are also observed in pear. In both pear cultivars used in our study, changes in patterns of raffinose contents are found to be similar, thereby also confirming recent findings observed during dormancy in apple (Falavigna et al. 2018). It has been suggested that raffinose protects apple buds against drought (Falavigna et al. 2018). This has been supported in our pear study, as raffinose accumulation is observed toward budbreak (Gabay et al. 2019). However, other sugars, such as sucrose, undergo similar patterns of changes during dormancy. As it is reported, sugars are necessary for regulation of bud regrowth regulation (Roitsch and González 2004), and that budbreak in the spring is highly influenced by availability of sugar (Tixier et al. 2017). Therefore, it is assumed that the mechanism by which buds are signaled involves accumulation of sugars or some other factors that can sense sufficient sugar accumulation. Moreover, when chilling is deemed sufficient, these sugars, or other factors, alter the status of buds, from dormancy to active growth, in the spring, as recently reported in grape (Khalil-Ur-Rehman et al. 2017).

In addition, increases in phospholipid content toward dormancy establishment have been observed in pear (Gabay et al. 2019). This has been previously observed in peach bud dormancy, and accompanied by chilling accumulation (Erez et al. 1997). However, this pattern is observed only in a high-CR pear cultivar. Therefore, additional studies should be conducted to confirm this finding with additional groups of pear cultivars.

It has been reported that large numbers of DE transcripts ($n > 4000$) are correlated to metabolites, along with significantly modified contents at different sampling dates during dormancy, and are likely controlled by multiple genes involved in regulating metabolic processes (Xiao et al. 2015). Genetic regulation of dormancy is complex, and it is governed by multiple genes (Allard et al. 2016; Celton et al. 2011; Heide and Prestrud 2005; Leida et al. 2010). This hypothesis is further confirmed by metabolite profiles of pear transcriptomes and their correlations to gene expression profiles during various phases of dormancy (Gabay et al. 2019).

12.5 Integrated System Biology Approaches to Decipher the Regulation Mechanism of Bud Dormancy

12.5.1 Co-localization of Differentially Expressed Genes, During Dormancy Phase Transition, to QTLs Associated with Chilling Requirements and Budbreak Date

Although genes associated with pear dormancy and VB may be located outside QTL intervals, QTL detection can lead to identification of

candidate genes underlying the QTL region, as previously described in tomato (Frary et al. 2000) and rice (Sallaud et al. 2003). Earlier, it has been reported that *DAM* genes are located within the same genomic region of an identified QTL associated with CR in peach (Fan et al. 2010). In another study, wherein alleles of *ParSOC1,* an apricot MADS-box gene, are screened in 48 apricot cultivars differing in CRs, a significant correlation is detected between allele segregation and CR (Trainin et al. 2013). In addition, a homolog of an *AGAMOUS-LIKE24* (*AGL24*) gene in *Arabidopsis thaliana,* regulating flowering and is induced by vernalization, is located close to the QTL on LG9, thus suggesting that the same genetic factors determine CRs in both perennial and annual plants (Allard et al. 2016).

Genes underlying five QTLs associated with vegetative budbreak in pear, identified on LGs 5, 8, 9, 13, and 15, have been identified and characterized based on their levels of expression, as well as their correlations to metabolites. These genes, including *PcDAM1*, *PcDAM2,* and *12-oxophytodienoate reductase 2-like,* involved in the alpha-linolenic acid pathway, have demonstrated significant changes in expression during vegetative budbreak in pear (Gabay et al. 2019).

12.5.2 Key Regulators of Dormancy

Using an integrated systems biology approach, a model for dormancy regulation involving those most significant genomic regions associated with VB identified on LG8 (R^2 = 28%) and LG9 (R^2 = 9.8%) in pear is proposed by Gabay et al. (2019). This model also takes into consideration metabolite contents during transition phases of dormancy. Furthermore, as transcription factors, such as *DAM* genes, are mostly expressed at the beginning of and in mid-dormancy, these putative candidate genes signal trees to enter into dormancy when the temperature begins to drop (Table 12.1). Transcription factors can activate genes related to metabolic pathways, which are mostly at their highest levels of expression during later phases of dormancy. In turn, these genes may play roles in regulating metabolite synthesis, which is essential for buds during dormancy and then for budbreak in the spring (Gabay et al. 2019). It is suggested that metabolites play important roles in dormancy phase transitions based on their profiles during dormancy (Khalil-Ur-Rehman et al. 2017; Erez et al. 1997). At the beginning of dormancy, phospholipids accumulate along with CUs and may be needed for either sugar biosynthesis or to protect buds from drops in temperature, and then followed by sugar accumulation. Sugars may play a role in signaling sufficient CU accumulation, allowing for budbreak as soon as the temperature rises, as previously reported in grape (Khalil-Ur-Rehman et al. 2017). Increases in fatty acids during the last phase of dormancy, toward dormancy break, may lead to membrane changes in buds, due to different metabolite composition, thus yielding optimal conditions for budbreak (Gabay et al. 2019).

12.5.3 Putative Candidate Genes Associated with Regulation of Dormancy

Among those genes demonstrating significant differential expression and underlying QTLs associated with VB date in pear include *PcDAM1* and *PcDAM2* (Gabay et al. 2019). Identification of these genes represents a 'proof of concept' for pursuing an integrated approach, as their roles in dormancy regulation have been previously described in both Japanese pear (Saito et al. 2013, 2015) and apple (Mimida et al. 2015). Using this approach, other additional putative genes, that may play roles in the genetic mechanism governing dormancy, have been detected. These include eight genes related to metabolic pathways, and specifically to alpha-linolenic pathway (*12-oxophytodienoate reductase 2-like*), and four genes encoded transcription factors. In addition, using this integrated approach, six new putative candidate genes, currently uncharacterized, are presumed to play major roles in pear bud dormancy (Gabay et al. 2019).

Table 12.1 Putative pear candidate genes associated with dormancy regulation

Gene	Gene symbol	Gene type[1]	Chr[2]
FT-interacting protein 1-like	LOC103967842	TF	8
PcDAM1-MADS-box protein AGL24-like	LOC103964948	TF	8
PcDAM2-MADS-box protein AGL24-like	LOC103964950	TF	8
MADS-box protein AGL24-like	LOC103964952	TF	8
3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ-like	LOC103967963	MP	8
Chlorophyll a-b binding protein CP24 10A, chloroplastic	LOC103967973	MP	8
12-oxophytodienoate reductase 2-like	LOC103967564	MP	8
Cytochrome b6-f complex iron-sulfur subunit, chloroplastic-like	LOC103944475	MP	9
Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase, chloroplastic-like	LOC103954983	MP	9
Thymidine kinase a	LOC103955051	MP	9
Chlorophyll a-b binding protein 151, chloroplastic-like	LOC103955064	MP	9
Protein phosphatase 2C 56-like	LOC103943902	MP	15
Uncharacterized	LOC103964940	UF	8
Uncharacterized	LOC103944526	UF	9
Uncharacterized	LOC103944497	UF	9
Uncharacterized	LOC103954139	UF	13
Uncharacterized	LOC103943904	UF	15
Uncharacterized	LOC103943918	UF	15

[1]Gene type; TF = transcription factor, MP = metabolic pathways, UC = uncharacterized function
[2]Chr = chromosome number of the pear genome

12.6 Conclusions and Future Research Directions

12.6.1 A Marker-assisted Selection Strategy Taking into Consideration G × E Effects

As pear breeding efforts are lengthy and time-consuming, availability of tools that can facilitate and accelerate the breeding cycle is highly desired. In efforts to develop pear cultivars with adaptability to changing climate conditions and warm growing regions, it is proposed that QTLs associated with VB date and identified on LGs 8 and 9, for main G × E effects, can be used for marker-assisted selection (MAS). These QTLs have been identified in different pear cultivars of diverse genetic backgrounds, thus confirming stability of these QTLs across these backgrounds. Furthermore, these have also been previously identified in apple (Allard et al. 2016; Celton et al. 2011; van Dyk et al. 2010). These identified VB QTLs associated with G × E interaction should also be taken into consideration when selecting pear genotypes under continuous conditions of climate change.

A proposed selection strategy should take into consideration such significant G × E effects (Fig. 12.3a). This model is developed based on data reported herein (Gabay et al. 2019) (Fig. 12.3b). Genotypes with G × E values equal to or near zero (i.e., category II; Fig. 12.3a) are deemed to be more stable across different environmental conditions. In addition, phenotypic values should also be taken into consideration based on the target location. Hence, a low-CR pear cultivar should be selected for warm regions (i.e., Group I; Fig. 12.3a), while a high-CR pear cultivar should be selected for cold regions (i.e., Group III; Fig. 12.3a). For instance,

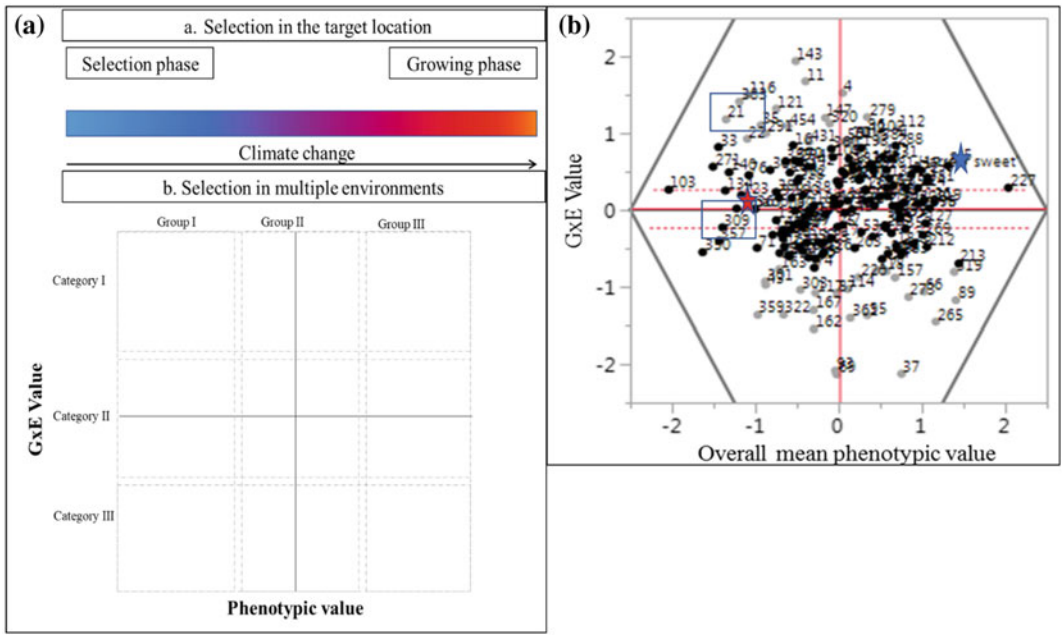

Fig. 12.3 Proposed model for pear selection in a breeding program under conditions of climate change. **a** 1. Selection in a target location may result in an unsuitable cultivar in subsequent years due to climate change. 2. Selection in multiple environments to evaluate phenotypic plasticity of selected genotypes. Group I—genotypes with low phenotypic performance. Group II—genotypes with average phenotypic performance. Group III—genotypes with high phenotypic performance. Category I refers to genotypes with large differences in performance (location a > location b). Category II refers to genotypes with phenotypic stability across different environmental conditions (location a = location b). Category III refers to genotypes with large differences in performance (location a < location b). **b** G × E values versus overall mean of an F1 SPD × HS population. Genotypic differences under normalized scores for vegetative budbreak date between high-chilling unit location (TZ) and low-chill unit location (BD), and their means. The red star denotes cv. Spadona (low-CR cultivar), and blue star denotes cv. Harrow Sweet (high-CR cultivar). Blue frames correspond to genotypes with similar means for normalized vegetative budbreak date with high stability across environments (genotype 309) and with low stability across environments (genotype 21) (Source: adapted from Gabay et al. 2018)

low-chill cultivars should be selected from genotypes that demonstrate stability across environments (genotype 309), while genotypes with low stability across environments (genotype 21) should be discarded, although they have similar means of normalized VB date (Fig. 12.3 b). Selection of new cultivars must be carried out in locations that can simulate climate conditions of the target location in which the cultivar will be grown. Hence, a breeder should consider using the model of climate prediction to choose a location that currently has the same climate conditions as the target location at the predicted date for release.

12.6.2 Further Research

Further research should focus on detected QTL regions and putative candidate genes in European pear (Table 12.1), Asian pear (Saito et al. 2015, 2013), and apple (Allard et al. 2016; Mimida et al. 2015). These QTLs and gene expression profiles should be further assessed in families and cultivars of different genetic backgrounds and under different climatic regions. As it is assumed that CR has a great impact on flower development, fruit quality, and yield (Allard et al. 2016; Bielenberg et al. 2008; Busov et al. 2016; Khalil-Ur-Rehman et al. 2017; Lang et al.

1987), associations between these traits with chilling requirements and vegetative budbreak date should be investigated.

A low-chill apple cultivar, 'Anna,' has been selected under warm temperature conditions and is considered a low-chilling cultivar. However, it has inferior fruit quality and poor storability (Trainin et al. 2016). As most pear breeding efforts are conducted in cold regions (Zohary 1997), it is difficult to determine whether or not fruit quality is associated with CR or that high fruit quality cultivars are better adapted for cold regions. Currently, we are pursuing pear breeding efforts under warm climate conditions in Israel, and selecting low-chill pear of high fruit quality.

References

Allard A, Bink MCAM, Martinez S, Kelner JJ, Legave JM, Di Guardo M, Di Pierro EA, Laurens F, Van De Weg EW, Costes E (2016) Detecting QTLs and putative candidate genes involved in budbreak and flowering time in an apple multiparental population. J Exp Bot 67:2875–2888. https://doi.org/10.1093/jxb/erw130

Anderson JL, Richardson EA, Kesner CD (1986) Validation of chill unit and flower bud phenology models for "Montmorency" sour cherry. Acta Hortic 184:71–78

Bai S, Saito T, Sakamoto D, Ito A, Fujii H, Moriguchi T (2013) Transcriptome analysis of Japanese pear (*Pyrus pyrifolia* Nakai) flower buds transitioning through endodormancy. Plant Cell Physiol 54:1132–1151. https://doi.org/10.1093/pcp/pct067

Bielenberg DG, Wang Y, Li Z, Zhebentyayeva T, Fan S, Reighard GL, Scorza R, Abbott AG (2008) Sequencing and annotation of the evergrowing locus in peach [*Prunus persica* (L.) Batsch.] reveals a cluster of six MADS-box transcription factors as candidate genes for regulation of terminal bud formation. Tree Genet Genomes 4:495–507. https://doi.org/10.1007/s11295-007-0126-9

Bouvier L, Bourcy M, Boulay M, Tellier M, Guérif P, Denancé C, Durel CE, Lespinasse Y (2012) A new pear scab resistance gene *Rvp1* from the European pear cultivar "Navara" maps in a genomic region syntenic to an apple scab resistance gene cluster on linkage group 2. Tree Genet Genomes 8:53–60. https://doi.org/10.1007/s11295-011-0419-x

Busov V, Carneros E, Yakovlev I (2016) *EARLY BUD-BREAK1 (EBB1)* defines a conserved mechanism for control of bud-break in woody perennials. Plant Signal Behav 11:e1073873. https://doi.org/10.1080/15592324.2015.1073873

Campoy JA, Ruiz D, Egea J (2011) Dormancy in temperate fruit trees in a global warming context: a review. Sci Hortic 130:357–372

Celton J-M, Chagné D, Tustin SD, Terakami S, Nishitani C, Yamamoto T, Gardiner SE (2009) Update on comparative genome mapping between *Malus* and *Pyrus*. BMC Res Notes 2:182. https://doi.org/10.1186/1756-0500-2-182

Celton JM, Martinez S, Jammes MJ, Bechti A, Salvi S, Legave JM, Costes E (2011) Deciphering the genetic determinism of bud phenology in apple progenies: a new insight into chilling and heat requirement effects on flowering dates and positional candidate genes. New Phytol 192:378–392. https://doi.org/10.1111/j.1469-8137.2011.03823.x

Chagné D, Crowhurst RN, Pindo M, Thrimawithana A, Deng C, Ireland H, Fiers M, Dzierzon H, Cestaro A, Fontana P, Bianco L, Lu A, Storey R, Knäbel M, Saeed M, Montanari S, Kim YK, Nicolini D, Larger S, Stefani E, Allan AC, Bowen J, Harvey I, Johnston J, Malnoy M, Troggio M, Perchepied L, Sawyer G, Wiedow C, Won K, Viola R, Hellens RP, Brewer L, Bus VGM, Schaffer RJ, Gardiner SE, Velasco R (2014) The draft genome sequence of European pear (*Pyrus communis* L. 'Bartlett'). PLoS ONE 9:1–12. https://doi.org/10.1371/journal.pone.0092644

Costa F, Van De Weg WE, Stella S, Dondini L, Pratesi D, Musacchi S, Sansavini S (2008) Map position and functional allelic diversity of *Md-Exp7*, a new putative expansin gene associated with fruit softening in apple (*Malus* × *domestica* Borkh.) and pear (*Pyrus communis*). Tree Genet Genomes 4:575–586. https://doi.org/10.1007/s11295-008-0133-5

da Falavigna VS, Porto DD, Miotto YE, dos Santos HP, de Oliveira PRD, Margis-Pinheiro M, Pasquali G, Revers LF (2018) Evolutionary diversification of galactinol synthases in Rosaceae: adaptive roles of galactinol and raffinose during apple bud dormancy. J Exp Bot 69:1247–1259. https://doi.org/10.1093/jxb/erx451

Darvasi A, Soller M (1992) Selective genotyping for determination of linkage between a marker locus and a quantitative trait locus. Theor Appl Genet 85:353–359. https://doi.org/10.1007/BF00222881

de Souza VAB, Byrne DH, Taylor JF, De S V (1998) Heritability, genetic and phenotypic correlations, and predicted selection response of quantitative traits in peach: II. An analysis of several fruit traits. J Am Soc Hortic Sci 123:598–603

Del Cueto J, Ionescu IA, Pičmanová M, Gericke O, Motawia MS, Olsen CE, Campoy JA, Dicenta F, Møller BL, Sánchez-Pérez R (2017) Cyanogenic glucosides and derivatives in almond and sweet cherry flower buds from dormancy to flowering. Front Plant Sci 8:1–16. https://doi.org/10.3389/fpls.2017.00800

Dirlewanger E, Quero-García J, Le Dantec L, Lambert P, Ruiz D, Dondini L, Illa E, Quilot-Turion B, Audergon JM, Tartarini S, Letourmy P, Arús P (2012) Comparison of the genetic determinism of two key phenological traits, flowering and maturity dates, in

three *Prunus* species: peach, apricot and sweet cherry. Heredity 109:280–292. https://doi.org/10.1038/hdy.2012.38

Eremina M, Rozhon W, Poppenberger B (2016) Hormonal control of cold stress responses in plants. Cell Mol Life Sci 73:797–810. https://doi.org/10.1007/s00018-015-2089-6

Erez A, Lavee S (1971) Effect of climatic conditions on dormancy development of peach buds. I. Temperature. J Am Soc Hortic Sci J 96:711–714

Erez A, Fishman S, Gat Z, Couvillon GA (1988) Evaluation of winter climate for breaking bud rest using the dynamic model. Acta Hortic 232:76–89

Erez A, Wang SY, Faust M (1997) Lipids in peach buds during dormancy, a possible involvement in dormancy control. Adv Hortic Sci 11:128–132

Fan S, Bielenberg DG, Zhebentyayeva TN, Reighard GL, Okie WR, Holland D, Abbott AG (2010) Mapping quantitative trait loci associated with chilling requirement, heat requirement and bloom date in peach (*Prunus persica*). New Phytol 185:917–930. https://doi.org/10.1111/j.1469-8137.2009.03119.x

Flaishman M, Amihai Shargal AS, Raphael S (2001) The synthetic cytokinin CPPU increases fruit size and yield of 'Spadona' and 'Costia' pear (*Pyrus communis* L.). J Hortic Sci Biotechnol 76:145–149. https://doi.org/10.1080/14620316.2001.11511341

Frary A, Nesbitt TC, Frary A, Grandillo S, Van Der Knaap E, Cong B, Liu J, Meller J, Elber R, Alpert KB (2000) *fw2. 2*: a quantitative trait locus key to the evolution of tomato fruit size. Science 289:85–88

Gabay G, Dahan Y, Izhaki Y, Isaacson T, Elkind Y, Ben-Ari G, Flaishman MA (2017) Identification of QTLs associated with spring vegetative budbreak time after dormancy release in pear (*Pyrus communis* L.). Plant Breed 136(5):749–758 https://doi.org/10.1111/pbr.12499

Gabay G, Dahan Y, Izhaki Y, Faigenboim A, Ben-Ari G, Elkind Y, Flaishman MA (2018) High-resolution genetic linkage map of European pear (*Pyrus communis*) and QTL fine-mapping of vegetative budbreak time. BMC Plant Biol 18(1):175. https://doi.org/10.1186/s12870-018-1386-2

Gabay G, Faigenboim A, Dahan Y, Izhaki Y, Itkin M, Malitsky S, Elkind Y, Flaishman MA (2019) Transcriptome analysis and metabolic profiling reveal the key role of α-linolenic acid in dormancy regulation of European pear. J Exp Bot 70(3):1017–1031. https://doi.org/10.1093/jxb/ery405

Heide OM, Prestrud AK (2005) Low temperature, but not photoperiod, controls growth cessation and dormancy induction and release in apple and pear. Tree Physiol 25:109–114. https://doi.org/10.1093/treephys/25.1.109

Howe GT, Saruul P, Davis J, Chen THH (2000) Quantitative genetics of bud phenology, frost damage, and winter survival in an F2 family of hybrid poplars. Theor Appl Genet 101:632–642. https://doi.org/10.1007/s001220051525

Ionescu IA, López-Ortega G, Burow M, Bayo-Canha A, Junge A, Gericke O, Møller BL, Sánchez-Pérez R (2017) Transcriptome and metabolite changes during hydrogen cyanamide-induced floral bud break in sweet cherry. Front Plant Sci 8:1–17. https://doi.org/10.3389/fpls.2017.01233

Izadyar AB, Wang SY (1999) Changes of lipid components during dormancy in "Hull Thornless" and "Triple Crown Thornless" blackberry cultivars. Sci Hortic 82:243–254. https://doi.org/10.1016/S0304-4238(99)00051-5

Jiménez S, Reighard GL, Bielenberg DG (2010) Gene expression of *DAM5* and *DAM6* is suppressed by chilling temperatures and inversely correlated with bud break rate. Plant Mol Biol 73:157–167. https://doi.org/10.1007/s11103-010-9608-5

Khalil-Ur-Rehman M, Wang W, Xu Y-S, Haider MS, Li C-X, Tao J-M (2017) Comparative study on reagents involved in grape bud break and their effects on different metabolites and related gene expression during winter. Front Plant Sci 8:1–10. https://doi.org/10.3389/fpls.2017.01340

Labuschagné IF, Louw JH, Schmidt K, Sadie A (2002) Genetic Variation in chilling requirement in apple progeny. J Am Soc Hortic Sci 127:663–672

Lang GA, Early JD, Darnell RL (1987) Endo-, para-, and ecodormancy: physiological terminology and classification for dormancy research. Hortic Sci 22:371–377

Le Roux PMF, Christen D, Duffy B, Tartarini S, Dondini L, Yamamoto T, Nishitani C, Terakami S, Lespinasse Y, Kellerhals M, Patocchi A (2012) Redefinition of the map position and validation of a major quantitative trait locus for fire blight resistance of the pear cultivar "Harrow Sweet" (*Pyrus communis* L.). Plant Breed 131:656–664. https://doi.org/10.1111/j.1439-0523.2012.02000.x

Leida C, Terol J, Martí G, Agustí M, Llácer G, Badenes ML, Ríos G (2010) Identification of genes associated with bud dormancy release in *Prunus persica* by suppression subtractive hybridization. Tree Physiol 30:655–666. https://doi.org/10.1093/treephys/tpq008

Li J, Xu Y, Niu Q, He L, Teng Y, Bai S (2018) Abscisic acid (ABA) promotes the induction and maintenance of pear (*Pyrus pyrifolia* white pear group) flower bud endodormancy. Int J Mol Sci 19(1):310. https://doi.org/10.3390/ijms19010310

Liu G, Li W, Zheng P, Xu T, Chen L, Liu D, Hussain S, Teng Y (2012) Transcriptomic analysis of 'Suli' pear (*Pyrus pyrifolia* white pear group) buds during the dormancy by RNA-Seq. BMC Genom 13:700. https://doi.org/10.1186/1471-2164-13-700

Maruyama K, Takeda M, Kidokoro S, Yamada K, Sakuma Y, Urano K, Fujita M, Yoshiwara K, Matsukura S, Morishita Y, Sasaki R, Suzuki H, Saito K, Shibata D, Shinozaki K, Yamaguchi-Shinozaki K (2009) Metabolic pathways involved in cold acclimation identified by integrated analysis of metabolites and transcripts regulated by *DREB1A* and *DREB2A*.

Plant Physiol 150:1972–1980. https://doi.org/10.1104/pp.109.135327

Mimida N, Saito T, Moriguchi T, Suzuki A, Komori S, Wada M (2015) Expression of *DORMANCY-ASSOCIATED MADS-BOX* (*DAM*)-like genes in apple. Biol Plant 59:237–244. https://doi.org/10.1007/s10535-015-0503-4

Olukolu BA, Trainin T, Fan S, Kole C, Bielenberg DG, Reighard GL, Abbott AG, Holland D (2009) Genetic linkage mapping for molecular dissection of chilling requirement and budbreak in apricot (*Prunus armeniaca* L.). Genome 52:819–828. https://doi.org/10.1139/G09-050

Roitsch T, González MC (2004) Function and regulation of plant invertases: sweet sensations. Trends Plant Sci 9:606–613. https://doi.org/10.1016/j.tplants.2004.10.009

Ruiz D, Campoy JA, Egea J (2007) Chilling and heat requirements of apricot cultivars for flowering. Env Exp Bot 61:254–263. https://doi.org/10.1016/j.envexpbot.2007.06.008

Saito T, Bai S, Ito A, Sakamoto D, Saito T, Ubi BE, Imai T, Moriguchi T (2013) Expression and genomic structure of the dormancy-associated *MADS box* genes *MADS13* in Japanese pears (*Pyrus pyrifolia* Nakai) that differ in their chilling requirement for endodormancy release. Tree Physiol 33:654–667. https://doi.org/10.1093/treephys/tpt037

Saito T, Bai S, Imai T, Ito A, Nakajima I, Moriguchi T (2015) Histone modification and signalling cascade of the dormancy-associated *MADS-box* gene, *PpMADS13-1*, in Japanese pear (*Pyrus pyrifolia*) during endodormancy. Plant Cell Env 38:1157–1166. https://doi.org/10.1111/pce.12469

Sallaud C, Lorieux M, Roumen E, Tharreau D, Berruyer R, Svestasrani P, Garsmeur O, Ghesquière A, Notteghem J-L (2003) Identification of five new blast resistance genes in the highly blast-resistant rice variety IR64 using a QTL mapping strategy. Theor Appl Genet 106:794–803

Takemura Y, Kuroki K, Jiang M, Matsumoto K, Tamura F (2015) Identification of the expressed protein and the impact of change in ascorbate peroxidase activity related to endodormancy breaking in *Pyrus pyrifolia*. Plant Physiol Biochem 86:121–129. https://doi.org/10.1016/j.plaphy.2014.11.016

Tixier A, Sperling O, Orozco J, Lampinen B, Roxas AA, Saa S, Earles JM, Zwieniecki MA (2017) Spring bud growth depends on sugar delivery by xylem and water recirculation by phloem Münch flow in *Juglans regia*. Planta 246(3):495–508. https://doi.org/10.1007/s00425-017-2707-7

Trainin T, Bar-Ya'akov I, Holland D (2013) *ParSOC1*, a *MADS-box* gene closely related to Arabidopsis *AGL20/SOC1*, is expressed in apricot leaves in a diurnal manner and is linked with chilling requirements for dormancy break. Tree Genet Genomes 9:753–766. https://doi.org/10.1007/s11295-012-0590-8

Trainin T, Zohar M, Shimoni-Shor E, Doron-Faigenboim A, Bar-Ya'akov I, Hatib K, Sela N, Holland D, Isaacson T (2016) A Unique haplotype found in apple accessions exhibiting early bud-break could serve as a marker for breeding apples with low chilling requirements. Mol Breed 36:158

Tuan PA, Bai S, Saito T, Ito A, Moriguchi T (2017) *Dormancy-Associated MADS-Box* (*DAM*) and the abscisic acid pathway regulate pear endodormancy through a feedback mechanism. Plant Cell Physiol 58:1378–1390. https://doi.org/10.1093/pcp/pcx074

Ubi BE, Sakamoto D, Ban Y, Shimada T, Ito A, Nakajima I, Takemura Y, Tamura F, Saito T, Moriguchi T (2010) Molecular cloning of *dormancy-associated MADS-box* gene homologs and their characterization during seasonal endodormancy transitional phases of Japanese pear. J Am Soc Hortic Sci 135:174–182

van Dyk MM, Soeker MK, Labuschagne IF, Rees DJG (2010) Identification of a major QTL for time of initial vegetative budbreak in apple (*Malus* × *domestica* Borkh.). Tree Genet Genomes 6:489–502. https://doi.org/10.1007/s11295-009-0266-1

Villalta ON, Washington WS, McGregor GR, Richards SM, Liu SM (2005) Resistance to pear scab in European and Asian pear cultivars in Australia. Acta Hortic 694:129–132

Wang SY, Faust M (1990) Changes of membrane lipids in apple buds during dormancy and budbreak. J Am Soc Hortic Sci 115:803–808

Wigge PA (2013) Ambient temperature signalling in plants. Curr Opin Plant Biol 16:661–666

Xiao Y, Ji Q, Gao S, Tan H, Chen R, Li Q, Chen J, Yang Y, Zhang L, Wang Z, Chen W, Hu Z (2015) Combined transcriptome and metabolite profiling reveals that *IiPLR1* plays an important role in lariciresinol accumulation in Isatis indigotica. J Exp Bot 66:6259–6271. https://doi.org/10.1093/jxb/erv333

Yamamoto T, Terakami S (2016) Genomics of pear and other Rosaceae fruit trees. Breed Sci 66:148–159. https://doi.org/10.1270/jsbbs.66.148

Zhu C, Gore M, Buckler ES, Yu J (2008) Status and prospects of association mapping in plants. Plant Genome J 1:5. https://doi.org/10.3835/plantgenome2008.02.0089

Zohary D (1997) Wild apples and wild pears. Bocconea 7:409–416

Genetics, Genomics, and Breeding for Fire Blight Resistance in Pear

13

Richard L. Bell

Abstract

Fire blight, caused by the bacterium *Erwinia amylovora* (Burrill) Winslow et al., is the most serious disease affecting the European pear, *Pyrus communis* L., in North America, Europe, and the Middle East. Control of fire blight is difficult, thus rendering the development of resistant cultivars and rootstocks a high priority. The inheritance pattern of resistance is quantitative, and genetic control is polygenic with additive effects, along with an estimated narrow-sense heritability, from various populations, of 0.40–0.50. There is some evidence for major gene inheritance for resistance. There have been five published studies on presence of genetic markers linked to quantitative trait loci (QTL). Microsatellite or simple sequence repeats (SSR) markers have been the most used marker type, but amplified fragment length polymorphisms (AFLPs) and single nucleotide polymorphisms (SNPs) have also been used. In the first study of the progeny 'Passe Crassane' × 'Harrow Sweet', four putative QTLs have been identified, all detected in 'Harrow Sweet'. A QTL is located on linkage group (LG) HS2a, a second on HS2b, a third on HS4, and a fourth on HS9. In a follow-up study with additional markers that merged HS2a and HS2b, a single QTL is identified controlling disease incidence, severity, and the incidence severity (ISV) index. In addition, three putative QTLs have been identified for disease incidence, severity, and ISV on HS04. In a study of the progeny of 'Doyenné du Comice' × *Pyrus ussuriensis* No. 18, putative QTLs have been identified on LG 11 of the *P. ussuriensis* parent. Another QTL identified on LG 4 of 'Doyenné du Comice' has suggested that resistance genes could be present in susceptible parents, as observed in conventional segregation studies. A follow-up study has identified a QTL on LG 9 of the resistant parent, and additional QTLs on LG 11, as well as on three other linkage groups, have been also found. Furthermore, four additional QTLs have been identified in 'Doyenné du Comice'. In an interspecific seedling population of 'PremP003' (*P.* × *bretschneideri* × *P. communis*) × 'Moonglow' (*P. communis*), a major QTL is mapped to LG 2 of 'Moonglow', which co-locates with a LG 2 QTL found in 'Harrow Sweet'. Three minor QTL have been identified on LGs 9, 10, and 15 of 'PremP003'. The history of pear breeding for fire blight resistance and notable cultivar releases will be also discussed.

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13.1 Introduction

Fire blight, caused by the bacterium *Erwinia amylovora* (Burrill) Winslow et al., is the most serious disease affecting European pear (*Pyrus communis* L.). Originating from North America, fire blight has spread over to England, and in spite of quarantine and control measures, it has continued to spread throughout Western, Central, and Eastern Europe, over to the Middle East, and then to New Zealand (van der Zwet 2002). The disease has influenced pear production more than any other single factor. Its prevalence has largely limited large-scale production in North America to mild and dry valleys of the Pacific coastal region of the province of British Columbia in Canada, and to US states of Washington, Oregon, and California in the USA (Andersen 1956; van der Zwet and Keil 1979). The disease is a constant threat, even in climatically favorable production regions.

The disease has been observed first in the state of New York, as early as 1780 (Denning 1794). The pathogen infects nectarthodes of blossoms, serving as the primary infection court, and actively growing shoots and immature fruits, but can also infect mature branches and trunks through wounds. Infection of shoots typically produces a necrotic 'shepherd's crook' symptom. Rootstocks can also become infected through either infection of root suckers or transmission from an infected trunk.

Control usually involves pre-bloom application of copper compounds, and subsequently with antibiotics or various biocontrols during bloom. Despite these control measures, the disease is often devastating. Once infection occurs, even drastic pruning of infected tissues during the growing season cannot always stop disease progression. All of the major scion cultivars of European pear currently in production and most rootstocks are susceptible to fire blight, thus rendering the development of fire blight-resistant cultivars a high priority.

Genetic resources for fire blight resistance and other traits have been previously reviewed by Westwood (1982), Bell and Itai (2011), and Bell and Leitão (2011). Moreover, breeding methods and strategies along with evaluation/selection techniques have also been previously reviewed by Bell et al. (1996a), Lespinasse and Aldwinckle (2000), Hancock and Lobos (2008), Fischer (2009), Lespinasse et al. (2011), Dondini and Sansavini (2012), and Kellerhals et al. (2017); whereas, goals and progress have been reviewed by Bellini and Nin (1997) and Brewer and Palmer (2011).

13.2 Breeding

13.2.1 History of Breeding Scion Cultivars

The history of selection and breeding of pear for fire blight resistance has been reviewed by Magness (1937), van der Zwet and Keil (1979), Bell et al. (1996a), Bellini and Nin (1997, 2002), Lespinasse and Aldwinckle (2000), Brewer and Palmer (2011), and Dondini and Sansavini (2012). The first fire blight-resistant pear cultivar grown in the USA is 'Seckel', which originated as a chance seedling in an area close to Philadelphia, Pennsylvania. Purposeful selection for fire blight resistance in pears was initiated in the mid- to late 1800s following the introduction of Chinese sand pears [*P. pyrifolia* (Burm.) Nakai], probably via Europe (Hedrick et al. 1921). The first fire blight-resistant interspecific hybrids introduced to the nursery trade have included 'Le Conte', 'Kieffer' (a chance seedling of 'Bartlett') and 'Garber'. These are all chance seedlings, not bred cultivars, grown because of their fire blight resistance; however, they are lacking in fruit quality. The first large-scale evaluation and selection effort involved the introduction of pear species and species hybrids from Asia, and evaluating these materials along with European pear cultivars for their resistance to fire blight (Reimer 1925). A total of 85 European pear (*P. communis*) cultivars or hybrids were artificially inoculated, and data from natural field infection of an additional 500 cultivars/hybrids have been recorded. The goals of these evaluations targeted the development of fire blight-resistant rootstocks and scion cultivars.

Seedlings of *P. ussuriensis* cv. Ba Li Hsiang were found to be highly resistant, but have proven unsatisfactory as rootstocks for *P. communis* scion cultivars. Hybridizations with major cultivars of *P. communis* have resulted in seedlings bearing fruit of poor quality.

In 1915, Reimer discovered a fire blight-resistant cultivar in Illinois, 'Farmingdale', assumed to be a seedling of 'Beurré d'Anjou'. Other early breeding programs for fire blight resistance in the USA have been carried out at the Georgia Experiment Station, releasing 'Pineapple' (van der Zwet and Keil 1979). Furthermore, the University of Tennessee has released eight interspecific hybrids of *P. communis* and *P. pyrifolia*, including 'Ayers', 'Dabney', 'Hoskins', and 'Mooers' (Drain and Shuey 1954); 'Carrick' and 'Morgan' (Drain and Safley 1958); as well as 'Orient' and 'Tenn'. While 'Orient' is a seedling of an interspecific cross of *P. pyrifolia* × *P. communis* made by Walter Van Fleet of Chico, California, who apparently provided it to the Tennessee Agricultural Experiment Station and to the United States Department of Agriculture (USDA), 'Tenn' is a selection from the Tennessee breeding program. None of these cultivars have been widely planted commercially, as they are of mediocre fruit quality, but they are grown mainly by amateur backyard orchardists.

The University of Maryland, in 1905, launched a program mainly for developing hybrids of 'Kieffer' with common European cultivars; however, no cultivars have been released. The University of Minnesota began a limited breeding program in 1908 to develop cold hardy, fire blight-resistant cultivars, using mainly Manchurian *P. ussuriensis* germplasm hybridized to European cultivars. Cornell University launched a pear breeding program at their experiment station in Geneva in 1892, and although the initial focus was on high fruit quality cultivars, the program was expanded to include fire blight resistance as an objective using sources of fire blight resistance from *P. communis* including 'Seckel' and 'Worden Seckel'. A putative *P. ussuriensis* × *P. pyrifolia* hybrid, Illinois 65 (syn. *P. ussuriensis* 65), was also initially introduced into the program as a source of fire blight resistance, but it was found to be also a source of resistance to the insect pest pear psylla, *Cacopsylla pyricola* Förster (Harris 1973; Harris and Lamb 1973).

The USDA pear breeding program was initially operated from 1916 to 1919 at Michigan State University's South Haven Horticultural Experiment Station (Magness 1937). The program developed a number of fire blight-resistant selections, including Michigan-US 437, which served as a progenitor of many of the selections and cultivars produced by this program. The program was continued at a low level at the USDA's Arlington Farm, and then beginning in 1960, a major expansion began at the Beltsville Agricultural Experiment Station in Maryland (Brooks et al. 1967), from which 'Magness', 'Moonglow', and 'Dawn' were released. Later on, it was found that 'Dawn' was moderately susceptible to fire blight, while 'Moonglow' was resistant and 'Magness' was highly resistant, except when infected via trunk wounds, such as those caused by limb spreaders used for tree training. In 1979, the program was transferred to the Appalachian Fruit Research Station in West Virginia, from which 'Potomac' (Bell et al. 1996b), 'Blake's Pride' (Bell et al. 2002), 'Shenandoah' (Bell and van der Zwet 2008), 'Sunrise' (Bell and van der Zwet 2011), and 'Gem' (Bell et al. 2014) were released. Furthermore, the USDA pear breeding program was also the likely source of the fire blight-resistant 'Warren' pear, as most likely it was a sister-seedling of 'Magness'. The original seedling population of these two cultivars, 'Warren' and 'Magness', had been split and planted at two locations, the Arlington Farm and the USDA research station in Meridian, Mississippi. The USDA station might have either shared seedlings with or propagated selections at the Mississippi State University research station. The identity of these two cultivars was confirmed using isozyme analysis, wherein isozyme profiles of these two cultivars were found to be almost identical, as well as following morphological observations, wherein their fruits were also found to be almost identical.

Other breeding programs in the USA included a short-lived program at the University of Illinois (Hough 1944), producing several *P. communis* selections, as well as selections Illinois 65 and Illinois 76, both were deemed as putative *P. ussuriensis* × *P. pyrifolia* hybrids. These selections were subsequently used as sources for resistance to pear psylla, *C. pyricola*, as well as to fire blight by breeding programs at Cornell University, Rutgers University, and USDA. The Rutgers University program introduced 'Mac', 'Star', and 'Lee' (Hough and Bailey 1968), and developed many selections derived from hybridizations between *P. communis* cultivars with selections of either *P. ussuriensis* or *P. pyrifolia*. Purdue University released 'Honeysweet' (Janick 1977), 'P448-2' ('Green Jade'™) (Janick 2004), and 'H2-169' ('Ambrosia'™) (Janick 2006). The University of California at Davis released 'Elliot', a seedling of 'Elliot 4' × 'Vermont Beauty', which gained favor in Europe, but marketed as 'Selena' (Ryugo 1982).

The pear breeding program of Agriculture Canada began at Harrow, Ontario in 1962, and was then transferred to Vineland, Ontario, from 1996 to 2000 (Hunter 2016). This program released 'Harvest Queen', a cultivar with moderate resistance to fire blight, as well as several fire blight-resistant cultivars, including 'Harrow Delight' (Quamme and Spearman 1983), 'Harrow Sweet' (Hunter et al. 1992), 'AC Harrow Gold' (Hunter et al. 2002a), 'AC Harrow Crisp' (Hunter et al. 2002b), 'Harrow Sundown' ('Cold Snap'™) (Hunter et al. 2009), 'AC Harrow Delicious', and 'Harrow Bliss'. The latter two cultivars have been marketed only in Europe. Additional fire blight-resistant selections, including HW 602, HW 623, and HW 624, will also be named and commercialized.

Following spread of fire blight disease to Europe, scion cultivar breeding programs added fire blight resistance to their objectives in several countries. The Institut National de la Recherche Agronomique (INRA) pear breeding program, located at Angers, France, initially used a half-diallel of four resistant selections crossed to three susceptible European pear cultivars (Thibault 1981), but eventually approximately 60,000 seedlings were generated from 55 parents crossed in 200 combinations. This program's objectives pursued development of fire blight resistance by focusing on lack of secondary bloom, found to be a heritable trait (Thibault et al. 1983), as well as reduction of shoot blight. The program released a few cultivars, among them 'Angelys'. While only moderately susceptible to fire blight, it produced no secondary bloom (Le Lézec et al. 2002). Another cultivar, 'Cepuna', matured in early September, was only moderately resistant. Various aspects of this program were reviewed by Le Lézec et al. (1991).

Pear breeding at the Instituto Sperimentale per la Frutticoltura in Forli, Italy commenced in 1968 (Rivalta et al. 2002). Breeding for fire blight resistance, in cooperation with the INRA pear breeding program, was carried out from 1980 to 1995, during which time field inoculations were carried out at the INRA station at Dax, whereby fire blight was endemic. Resistant selections were propagated onto rootstocks, underwent greenhouse bacterial inoculation tests at Angers, France, and pomological field evaluations were conducted in Italy. Susceptible cultivars, such as 'Max Red Bartlett', 'Bella di Guigno', 'Coscia', and 'Starking Delicious', a cultivar with low to moderate resistance, were found to produce seedlings with suitable resistance to fire blight, while some resistant cultivars, such as 'Morgan', 'Dr. Molon', 'Sirrine', and US309 produced progenies of low resistance to fire blight (Lespinasse and Aldwinckle 2000). Selection of parents from commercially acceptable germplasm was a more effective method of developing cultivars of commercial quality combined with acceptable levels of fire blight resistance (Bagnara et al. 1996). Two fire blight tolerant cultivars were developed and released. 'Bohéme' (ISF-FO 80-57-83), was selected from a seedling population of 'Conference' × 'Dr. Jules Guyot', and 'Aida' (ISF-FO 80-104-72), was selected from a cross of 'Coscia' × 'Dr. Jules Guyot'. An additional selection, ISF-FO 80-51-72, also a seedling of 'Coscia' × 'Dr. Jules Guyot', has been undergoing evaluation.

The pear breeding program at the University of Bologna in Italy, launched in 1978 (Musacchi et al. 2005), with resistance to fire blight becoming one of its major goals. As of 2005, three selections, either fire blight resistant or tolerant, including DCA 92050701-14, DCA 91050701-41, and DCA 91050701-39, have been identified from a seedling population of US 309 (resistant) × 'Abbé Fetel' (susceptible). This program has also been investigating molecular markers linked to resistance to fire blight and to pear psylla (Musacchi et al. 2006).

Although fire blight resistance has not been a major goal of the German pear breeding program at Dresden-Pillnitz, two released cultivars, 'Isolda' and 'Uta', have some levels of tolerance to fire blight (Fischer and Mildenberger 2004). Other resistant or tolerant released cultivars, including 'David', 'Hortensia', and 'Manon', have also been released (Dondini and Sansavini 2012).

Using *P. pyrifolia* selections as sources of resistance, the Romanian pear breeding program at the Fruit Tree Institute in Pitesti-Maracineni has released 'Getica' (Sestras et al. 2007; Braniste et al. 2008). The Fruit Research Station in Voinesti has released 'Corina' and 'Euras', and the Fruit Tree Research Station in Cluj has released 'Haydeea'. The Fruit Tree Institute has also released 'Monica', a seedling of 'Santa Maria' × 'Principessa Gonzaga', both parents belonging to *P. communis* (Dondini and Sansavini 2012).

The New Zealand pear scion breeding program has used selections derived from several *P. communis* ('Duchesse d'Angouleme', 'Moonglow', 'Harrow Crisp', 'Harrow Delight', 'Patrick Barry', 'Seckel', and 'Winter Cole'), *P. pyrifolia* ('Nijisseiki', 'Okusankichi', and NJ1), *P. ussuriensis* ('Ping Guo Li'), *P.* × *bretschneideri* ('Ya Li' and 'Xue Hua Li'), and *P. pyrifolia* × *P. communis* hybrid cultivars ('Carrick') as sources of resistance or high fruit quality (Brewer and Palmer 2011; White and Brewer 2002a, b). Selections from resultant progenies are undergoing further evaluations. A major goal of this program is to combine the fine and crisp fruit flesh texture of Asian cultivars with the more aromatic fruit flavor of *P. communis* cultivars. Transfer of disease (e.g., fire blight) and insect (e.g., pear psylla) resistance from Asian cultivars is yet another goal of this program. In 1999, 'Crispie' and 'Maxie', derived from hybridization of *P. pyrifolia* cv. 'Nijisseiki' with *P. communis* cv. Max Red Bartlett, have been released. A third-generation hybrid, 'PIQA Boo' (a numbered selection of PremP009), a complex hybrid of *P. communis*, *P. pyrifolia*, and *P.* × *bretschneideri*, has been recently released. However, fire blight resistance ratings have not yet been published.

13.2.2 History of Breeding Rootstocks

Fruiting-bearing, or scion, pear cultivars are clonally propagated, either by budding or by grafting onto either seedling or clonal rootstocks. These rootstocks have been selected for, based on either availability of seed, as is the case with, for example, 'Bartlett' and 'Winter Nelis' seedling rootstocks, or their ability to positively influence production or various other traits, such as precocity of bearing, tree size control, adaptation to high pH soils, cold hardiness, ease of propagation from cuttings, and resistance to soil pathogens, woolly pear aphids, *Armillaria* root rot, pear decline phytoplasm, or fire blight (Lombard and Westwood 1987). Selection and breeding for pear rootstocks have been previously reviewed (Lombard and Westwood 1987; Bell et al. 1996a; Wertheim 2002; Webster 2003; Hancock and Lobos 2008; Fischer 2009; Lespinasse 2009; Brewer and Palmer 2011; Dondini and Sansavini 2012; Elkins et al. 2012). Elkins et al. (2012) have also provided a listing of 36 rootstock breeding programs throughout the world.

Reimer (1925) has investigated fire blight resistance of *Pyrus* species and cultivars at Oregon State University. These data are based on observations of natural infections rather than controlled inoculations. Lombard and Westwood (1987) have also summarized general reactions of clones or seedlings of 19 *Pyrus* species in this

collection for resistance to fire blight. Moreover, they have also summarized performance of various species deemed suitable as either seedling rootstocks or clonal rootstocks for pear cultivars. In addition, they have also included *Cydonia oblonga* Mill., quince, as it is an important source of dwarfing rootstocks, along with other genera within Rosaceae, for potential use as rootstocks for pear. Unfortunately, all evaluated common clones of *Cydonia* have been found to be susceptible to fire blight, whereas Asian pear species have been found to be more resistant to fire blight, with some variabilities, but with *P. ussuriensis* and *P. calleryana* deemed the most consistently resistant.

Within *P. communis*, the first fire blight-resistant rootstocks have been the 'Old Home' × 'Farmingdale' (OH × F) numbered series (Brooks 1984). However, the correct parentage of these rootstocks has been recently determined to be 'Old Home' × 'Bartlett' using simple sequence repeat (SSR) molecular marker analysis (Postman et al. 2013). 'OH × F 87' is the most commonly used rootstock of this series in North America, as it is fire blight resistant, graft-compatible with all tested scion cultivars, induces a semi-dwarf tree size, promotes precocious fruit bearing, and produces better yield efficiency than other rootstocks of this series. However, it is more difficult to propagate by conventional cuttings and layering (Dondini and Sansavini 2012). Another selection in this series, 'OH × F 40', is also fire blight resistant, graft-compatible, promotes good yield, and good fruit size. However, it induces higher vigor than Quince BA29, but it is less productive than Quince MC, and has lower yield efficiency. Yet another selection, 'OH × F 69', has performed well in trials in California and in Europe (Elkins et al. 2008, 2011; Dondini and Sansavini 2012). However, its yield efficiency is lower than those of both quince and pear seedling rootstocks. In most trials, 'OH × F 69' has demonstrated to be as vigor-inducing as that of seedling rootstocks, but it is winter hardy and has resistance to both fire blight and pear decline.

The French INRA pear rootstock breeding program has been one of the largest and most diverse (Simard et al. 2004). It has developed and released an open-pollinated 'Old Home' selection OH11 as 'Pyriam' in 1997 (Simard and Michelesi 2002). This rootstock has been selected for its ability to reduce scion vigor, and for promoting production, fruit size, graft-compatibility, nursery habit, and propagation from softwood cuttings. Evaluations at several locations throughout France have shown good adaptability to calcareous soils, i.e., tolerance to high pH-induced iron chlorosis.

The Institute for Research and Technology in Food and Agriculture (IRTA), an agricultural research organization of the government of Catalonia, Spain, and the French INRA have initiated a joint pear rootstock breeding program in 1998 to develop pear rootstocks adapted to Mediterranean growing conditions, specifically tolerance to high pH soils; i.e., iron chlorosis, and to water scarcity (Asin et al. 2011). The program involves crosses between the French rootstock 'Pyriam' and four Mediterranean *Pyrus* taxa, including *P. amygdaliformis* Vill., *P. amygdaliformis* Vill. var. *persica* Bornm., a hybrid of *P. communis* var. *cordata* Desv. Hook f. (syn. *P. cordata* Desv.), and *P. elaeagrifolia*. Pall. Seedlings resulting from these crosses were split into two sets, with one set being evaluated in France for rooting ability, upright growth habit, and graft-compatibility with 'Bartlett' (syn. 'Williams Bon Chretien'), used as the scion cultivar, while the second set being evaluated in Spain for tolerance to iron chlorosis, vigor, and graft-compatibility with 'Conference' used as the scion cultivar. Open-pollinated 'Bartlett' seedlings have also been evaluated. Iron chlorosis is measured using a visual rating scale (Sanz and Montañes 1997), while nitrogen is determined using a SPAD meter. It has been determined that seedlings of *P. amygdaliformis*, *P. elaeagrifolia*, and the *P. communis* var. *cordata* hybrid are found to be more resistant to iron chlorosis. Moreover, open-pollinated seedlings of 'Bartlett' are reported to have lower vigor than other tested *Pyrus* materials. Furthermore, interspecific hybrids and open-pollinated seedlings of 'Bartlett' are reported to have similar percentages (18%) of seedlings with no observed chlorosis

and with reduced vigor, less than 50% of that of the *Cydonia* rootstock BA29. Overall, seedlings of the hybrid *P. communis* var. *cordata*, *P. amygdaliformis* var. *persica* clone, and of the open-pollinated 'Bartlett' have yielded the highest percentages of desirable selections.

'Pyrodwarf' (Rhenus 1) and 'BU 2/33' (Rhenus 3) have been developed at the Geisenheim Research Institute and Applied University in Germany from progeny of the resistant 'Old Home' × the susceptible 'Bonne Louise d'Avranches' (Jacob 2002). 'Pyrodwarf' is reported to induce low scion vigor, high fruit-bearing precocity, high fruit yield efficiency, as well as uniform and good fruit size. Furthermore, it has graft-compatibility with major scion cultivars, good anchorage, winter cold hardiness, lacks sucker development, and does not exhibit high soil pH-induced iron chlorosis. Unfortunately, this rootstock has not performed as well in the USA. On the other hand, 'BU 2/33' produces a semi-dwarf to vigorous scion, but induces good production and yield efficiency.

Although *Cydonia* is generally susceptible to fire blight, studies at the Agricultural University in Plovdiv, Bulgaria have found that two edible quince cultivars, 'Hemus' and 'Triumph', are resistant to fire blight, while a third cultivar, 'Du Portugal', is moderately resistant to fire blight (Bobev and Deckers 1999). A number of selections, such as IV-40, from subsequent breeding progenies, generated by crossing these three quince cultivars with susceptible cultivars 'Asenitza' and 'Tzargradska' and by open-pollination, are reported to be resistant to fire blight (Bobev et al. 2011).

In other efforts, the edible quince breeding program at the Pomology Institute, NAGREF, in Naoussa, Greece, and the Technological Education Institute of Larissa, also in Greece, evaluated 49 genotypes, and found eight genotypes that were resistant to fire blight (Papachatzis et al. 2011).

Additional cultivars and germplasm accessions have been found to be moderately resistant to fire blight (Bell, unpublished data). Although most of the genotypes discussed herein were of the edible types, these must be evaluated for their potential as reliable and useful rootstocks for pear scion cultivars.

13.2.3 Disease Resistance Evaluation Methods

As assessment of fire blight disease resistance is rather difficult, several methods have been developed to evaluate disease reactions in pear. Methods of determining levels of host plant resistance/susceptibility have consisted of short-term (disease severity in one year) and long-term (cumulative disease severity over a period of years) observations of infections caused by natural epiphytotics, as well as short-term data, collected based on artificial inoculations of actively growing shoots of established seedlings and propagated trees under greenhouse and field conditions.

A number of host, pathogen, and environmental factors influence expression and phenotypic disease resistance. These include the following: (1) tree age, vigor, and infected tissue; (2) virulence of isolates; (3) inoculum concentration; (4) inoculation method; and (5) temperature and humidity conditions during pre- and post-inoculation periods (Bell et al. 1996a). Thus, fire blight disease resistance findings reported in various studies are highly influenced by differences in any of these factors (van der Zwet and Keil 1979). Young and vigorously growing shoots tend to be more susceptible to fire blight. Furthermore, blossoms are almost always more susceptible than either shoots, older branches, or trunks, even when compared to shoots of fire blight-resistant pear genotypes. In one study, most genotypes resistant to shoot infection are reported to be moderately to highly susceptible to blossom infections (Le Lézec et al. 1985), but with some exceptions, wherein both shoot and blossom resistance have been observed, such as those observed for pear genotypes HW 601 and 'Potomac', among other USDA selections. Interestingly, 'Magness' is essentially immune to blossom infection, due to its underdeveloped nectarthodes, an important infection court. Furthermore, while screening of young seedlings in a

greenhouse, it has been observed that actively growing seedlings, with either 18–24 nodes or are 6–7 months old, are deemed best for distinguishing among levels of resistance/susceptibility to fire blight (Carpenter and Shay 1953; Thompson et al. 1962; Layne et al. 1968).

With the development of the tools of biotechnology, specifically of identifying mutants derived from in vitro mutagenesis, screening for somaclonal variants, and selection of genetic transformants, has led to the development of in vitro methods for plant production. Shoot proliferation can be used to produce plant materials that can be screened for disease resistance at early stages of development, thus decreasing numbers of plants that must be rooted, acclimated to greenhouse conditions, and then evaluated. Proliferating shoot cultures can be inoculated in vitro, and those clones demonstrating low frequencies of necrosis are then selected for propagation, and subjected to further evaluations (Viseur and Tapia y Figeuroa 1987; Hanke and Geider 2002; Paprstein et al. 2014).

It is critical to point out that the strain of *E. amylovora* can affect the severity of infection, due to differences in general virulence (Shaffer and Goodman 1962) and differential virulence of the bacterium; i.e., interactions between bacterial strain and host genotype, as some bacterial strains would infect otherwise disease resistant host genotypes. There is at least one such case reported in apple (Norelli et al. 1984, 1986), but most bacterial strains are not differentially virulent, as it is apparently the case in pear (Quamme and Bonn 1981). In this latter study, only nine bacterial strains have been investigated and assessed. It is noteworthy to point out that differences in general bacterial virulence may influence ratings or measures of resistance, thereby potentially influencing fire blight-resistance findings. Moreover, it may be also important that while screening seedlings of breeding materials is to either use a mixture of non-differential bacterial strains or screen individually against different bacterial strains, sometime during the breeding process. Additionally, to insure durable resistance of new cultivars, it may be important to combine different sources of resistance. If either individual genes or quantitative traits loci (QTLs), along with their markers, can be identified, then they should be combined or 'pyramided'. Furthermore, inoculum concentrations can influence frequency and severity of infections, with lower concentrations resulting in less reliable results. Therefore, a concentration of at least 1×10^7 cfu·ml^{-1} is recommended, and should be used.

For both outdoor and greenhouse inoculations, sustained temperatures of less than 30 °C (86 °F) should be prevalent. Moreover, high relative humidity (85–100% RH) conditions must be maintained before, during, and after inoculation, as it has been demonstrated that high humidity increases the likelihood of success of artificial inoculations (van der Zwet and Keil 1979). Such high humidity conditions can be maintained under greenhouse and nursery environments by constructing a plastic tent and placing a humidifier inside the tent for a period of several days.

Various shoot inoculation methods have been used, including the use of wounding with carborundum, needles, hypodermic syringes, pin-cushion equipped clamps (van der Zwet and Keil 1979), and more recently and widely, scissors. Using scissors involves dipping blades into the inoculum, and then cutting the top two expanding leaves of an actively growing shoot through the midrib. This method has been demonstrated to consistently yield high frequencies of infections.

On the other hand, blossom inoculation studies usually involve use of a uniform number of newly opened blossoms per cluster. These blossoms are inoculated individually with a small drop of inoculum using a repeating pipetter. Alternatively, whole clusters are inoculated using a sprayer, such as a DeVilbiss atomizer.

For evaluation of epiphytotic infections, Mowry (1964) has devised a rating index as follows: (number of infected shoots × 5) + (age of infected wood × 20). Yet another widely used evaluation scheme, a 10-point scale based on a modified Horsfall-Barratt scale (Horsfall and

Barratt 1945), has been later developed for trees that are at least 3 years old (van der Zwet et al. 1970). This latter scheme is based on a visual estimation of percentage of a tree that is infected, age of the oldest infected wood, and relative estimate of the proportion of shoots infected. This scheme is intended for use in an orchard to rapidly assign disease severity scores. Citing various statistical deficiencies of the Horsfall-Barratt disease evaluation system, Bock et al. (2009, 2010) have recommended the use of nearest percent estimates for citrus canker infections of leaves that may be also of use for fire blight disease evaluations. Other systems for fire blight disease categorical scales have relied on use of regularly spaced intervals, usually five intervals, of 20% per interval.

In order to conduct artificial inoculations, actively growing shoots are often used. Measurements of lesion lengths are converted to percentages of total shoot length (Lamb 1960). This type of data has been referred to as percentage lesion length (PLL). Sometimes, classes for disease severity, based on percent ranges, have also been used (Thompson et al. 1962). In some instances, data from unsuccessfully inoculated shoots are excluded, as these are assumed not to be representative of a true resistant reaction. In another approach, it is assumed that lack of observed shoot infection represents a true resistant reaction. To account for these uninfected shoots, an Index of Varietal Susceptibility (IVS), based on both frequency of successful inoculations (F, 0–1) and severity (S, 0–100), has been devised (Thibault et al. 1987). This has been extensively used by the INRA program in France (Le Lézec et al. 1997), among many other breeding programs. The foregoing indices of resistance are based on either maximum lesion length or lesion length after a set period of time following inoculation.

As shoot lesions may develop at different rates, depending on the host genotype or even individual replicate shoots, the area under the disease progress curve (AUDPC) index has been developed to account for these differences and may reflect differences in resistance response (Shaner and Finney 1977; Jeger and Viljanen-Rollinson 2001). This method involves periodic measurements over a period of time, and this has been used in fire blight disease evaluations (Momol et al. 1996). However, various modifications and improvements have been made, including the development of yet another index, termed the area under the disease progress stairs (AUDPS) (Simko and Piepho 2012). This index, along with its associated standardized (sAUDPS) and relative (rAUDPS) variants, is recommended for use for quantification of fire blight disease reactions.

Scoring of blossom infections following artificial inoculations involves determining frequencies of infections of blossoms or clusters, or of both frequency and severity, with the latter based on a scale of symptom progression through either an individual blossom and stalk into the bourse and spur, or to some other woody tissue. Again, various scales have been used to assess blossom disease severity (Bell et al. 2002; Bell and van der Zwet 2008, 2011; Kellerhals et al. 2017).

In vitro-cultured shoots have also been used to evaluate fire blight resistance (Duron et al. 1987; Brisset et al. 1988; Pinet-Leblay et al. 1996; Abdollahi et al. 2004; Paprstein et al. 2014). In general, shoot necrosis is correlated with known susceptibility of a cultivar. Moreover, when mesophyll protoplasts of the fire blight-resistant ‘Old Home’, the susceptible ‘Williams Bon Chretien’ (syn. ‘Bartlett’), and the highly susceptible ‘Passe Crassane’ are co-cultured with *E. amylovora*, protoplast viability, time to division, and time to 10-cell colony stage development have correlated with known resistance/susceptibility of these cultivars (Brisset et al. 1990). Therefore, these alternative systems for fire blight disease evaluations have been deemed useful for instances wherein use of the pathogen in a greenhouse or outdoors is prohibited due to quarantine regulations, or for purposes of studying various aspects of host-pathogen interactions.

13.2.4 Germplasm

There are at least 29 *Pyrus* taxa that are widely accepted as species, and nine naturally occurring interspecific hybrids (Bell et al.1996a; USDA, ARS 2018). One of the major species cultivated for edible fruit is the West European pear, *P. communis*, which is the primary focus of this chapter. Other edible *Pyrus* species of use in breeding of fruit cultivars include *P. pyrifolia* (Burm. f.) Nakai, *P. ussuriensis* Maxim., and the naturally occurring interspecific hybrid, *P.* × *bretschneideri* Rehd. The latter species is at times classified as a subspecies, *P. pyrifolia* spp. *sinensis* T.T. Yu. In South Asia, *P. pseudopashia* T.T. Yu is also cultivated. Large numbers of cultivars, breeding selections, and wild germplasm of several species have been evaluated for their resistance/susceptibility to fire blight. General ratings of resistance/susceptibility reactions at the species level have been presented by Westwood (1982), Lombard and Westwood (1987), Bell (1991), and Bell and Leitão (2011).

Commonly used ancestral sources of fire blight resistance have included the resistant selections US309 and Michigan-US 437, and the moderately resistant cultivars of 'Seckel' and 'Roi Charles de Wurtemberg'. In a 2-year study of epiphytotic fire blight in a collection of mature trees of more than 500 cultivars and selections comprising primarily of *P. communis* cultivars, but also including Asian and Asian × European pear hybrids, approximately 90% of evaluated genotypes are deemed to be susceptible (Oitto et al. 1970). Even among those moderately resistant to resistant germplasm, some infections have continued to progress for a few additional years (van der Zwet and Oitto 1972; van der Zwet et al. 1974a). In a summary of published studies of nearly 400 *P. communis* and interspecific hybrids, approximately 41% of these genotypes have been deemed as either susceptible or variable, 33% as moderately resistant, and only 19% as resistant. Moreover, among 48 *P. ussuriensis* and *P. pyrifolia* cultivars, 31% are deemed as resistant, 10% as moderately resistant, 40% as susceptible, and 19% as variable (van der Zwet and Keil 1979). Although some moderately resistant and resistant cultivars of *P. communis* have been identified, overall this species has been generally deemed as susceptible (Zeller 1978, 1990; van der Zwet and Keil 1979; Thibault et al. 1989). Moreover, although most of *P. pyrifolia* cultivars are susceptible, some moderately resistant germplasm has been identified (Zeller 1978; van der Zwet and Keil 1979; Lespinasse and Aldwinckle 2000). Furthermore, although *P. betulifolia* Bunge is generally deemed as susceptible, a few resistant clones have been identified (van der Zwet et al. 1974a), such as Reimer's resistant selection, which has been used as a seedling rootstock.

On the other hand, the ornamental species *P. calleryana* Decne. has been observed to have a high proportion of fire blight-resistant clones, including 'Bradford', 'Capital', and 'Whitehouse'; whereas, 'Aristocrat', 'Autumn Blaze', and to some degree 'Redspire' have been deemed more susceptible (van der Zwet et al. 1974b; Fare et al. 1991). Nevertheless, ratings for 'Bradford' have been variable (Bell et al. 2004). Generally, clones of the native species *P. ussuriensis* are quite resistant, with 64% being moderately resistant to resistant (Hartman 1957; van der Zwet et al. 1974b; van der Zwet and Keil 1979). However, domestic cultivars of *P. ussuriensis* are more susceptible, perhaps due to interspecific hybridizations with other species, such as with *P. pyrifolia*. Moreover, the interspecific hybrid, *P.* × *bretschneideri*, is deemed to be variable.

The primary source for fire blight resistance in rootstock breeding programs has been an old American *P. communis* cultivar, 'Old Home' (Brooks 1984; Jacob 2002). Due to variabilities in resistance reactions within each *Pyrus* species, it is difficult to assign a consistent resistance rating to a particular species, although there are general trends (van der Zwet et al. 1974a). Overall, among cultivated *Pyrus* species, for either fruit or rootstock, *P. ussuriensis* is deemed the most resistant, followed by *P. calleryana*, *P. betulifolia*, *P.* × *bretschneideri*, *P. pyrifolia*, and *P. communis*.

13.2.5 Biotechnological Approaches for Genetic Improvement

Selections of somaclonal variants and of mutation breeding have been used to develop methods to isolate clones of fire blight susceptible pear cultivars with improved resistance to fire blight. These new clones can be generated using in vitro micropropagation, callus cultures, and adventitious shoot regeneration protocols. In vitro disease resistance, evaluation methods can also be used for early screening of clones for enhanced resistance to fire blight. Plantlets of 'Durondeau' have been regenerated from callus cultures, initiated from root tissues (Viseur 1990). Two somaclonal variants with reduced susceptibility to fire blight have been isolated and determined to be tetraploids. Gamma and ultraviolet irradiation of in vitro-grown leaf explants, followed by adventitious regeneration of plantlets, have been assessed using four commercially important pear cultivars (Pinet-Leblay et al. 1992). The effects of irradiation on adventitious shoot regeneration from leaf tissues have been evaluated, and LD_{50} levels established for both irradiation methods. The LD_{50} for gamma irradiation is reported to be genotype-dependent. Subsequently, compatible and hypersensitive fire blight-resistant reactions could be differentiated in an assay of detached leaves of in vitro-derived pear plantlets of the susceptible 'Doyenné du Comice' and the resistant 'Old Home' (Pinet-Leblay et al. 1996). This assay involves infiltration of leaf tissues using a virulent strain of *E. amylovora*, a *dsp* mutant, and a heterologous pathogen, *Pseudomonas syringae* pv. *tabaci*, followed by observations of differential reactions. This assay is based on findings that the *dsp* mutant is known to be avirulent in compatible (i.e., susceptible) host-pathogen interactions, but will result in necrosis in incompatible (i.e., resistant) interactions. Findings from infiltration of 'Old Home' leaf tissues have suggested that fire blight resistance of 'Old Home' is characterized by hypersensitivity due to observed necrosis in these tissues. Pinet-Leblay et al. (1996) have proposed that this assay can be used as a primary screen for hypersensitive resistance reactions in mutation breeding efforts.

Genetic transformation efforts undertaken to enhance fire blight resistance in pears have been previously reviewed by Hancock and Lobos (2008) and by Dondini and Sansavini (2012). The gene *attacin E*, a lytic peptide gene derived from the silk moth, *Hyalophora cercropia* L., has been introduced into the highly susceptible *P. communis* cultivar 'Passe Crassane' using *Agrobacterium*-mediated transformation, and transgenic pear lines with reduced levels of susceptibility to fire blight have been obtained (Reynoird et al. 1999). In another effort, the harpin gene, *HrpN*, a bacterial inducer of systemic host resistance, is introduced into 'Passe Crassane', and transgenic lines with reduced susceptibility to *E. amylovora* have been obtained (Malnoy et al. 2005a). Furthermore, a gene encoding a depolymerase derived from the phage ΦEa1h, which degrades the capsular exopolysaccharide (EPS) of *E. amylovora*, has also been introduced into 'Passe Crassane', and transgenic lines with significantly decreased susceptibility to fire blight have been observed (Malnoy et al. 2005b). Moreover, a plant defensin gene, *Rs*-AFP2, from radish has been transferred into 'Burakovka' for the purpose of enhancing microbial disease resistance; however, results of fire blight disease reactions of transgenic lines have not yet been published (Lebedev et al. 2002).

13.3 Genetics of Resistance

13.3.1 Inheritance of Resistance and Susceptibility

The inheritance of resistance to fire blight is quantitative, as it is polygenic or controlled by multiple genes acting with additive effects. However, there is some evidence for presence of gene(s) with major effects. In a study involving crosses among *P. communis*, *P. ussuriensis*, and *P. pyrifolia* parents, segregation for resistance of young seedling progenies artificially inoculated

with *E. amylovora* is found to be continuous, and in many cases with normal distribution, regardless of the parental phenotype or species source for resistance (Layne et al. 1968). Therefore, it has been concluded that resistance is primarily polygenically inherited, with either moderate or high heritability, and that either the same or similar genes for resistance may be present in each of these *Pyrus* species. Moreover, the parental phenotype, and to a lesser extent the species source for resistance, significantly influences the proportion of seedlings obtained in each resistance class. In other words, there is variability for transmitting resistance to their progenies in this species. In a few seedling progenies, a skewed segregation pattern has been observed, thus suggesting presence of major genes for resistance, with resistance being dominant. Some distributions could be due to monogenic inheritance with low heritability or expressivity. Furthermore, U-shaped distributions are attributed to monogenic inheritance, with moderate heritability and dominance of resistance. In other interspecific crosses, it has been noted that there is quite a bit of variability in transmission of fire blight resistance (van der Zwet et al. 1974a). Quamme and Bonn (1981) have concluded that general combining ability is greater than specific combining ability, and therefore inheritance of fire blight resistance is polygenic with a high additive genetic variance. Dondini et al. (2002b) have also concluded that based on continuous distribution of infection in young seedlings, resistance from 'Harrow Sweet' and US309 is polygenically inherited. Earlier, Decourtye (1967) has also proposed presence of major genes derived from parents used in his populations. Similarly, Thompson et al. (1962) have concluded that resistance is inherited in a polygenic fashion, but with evidence for major gene inheritance from *P. ussuriensis*. Likewise, Bokszczanin et al. (2012) have found evidence for monogenic resistance from two *P. ussuriensis* parents used in hybridizations with 'Doyenné du Comice', a susceptible *P. communis* cultivar. A continuous, but skewed distribution of disease reactions in progeny of 'Doyenné du Comice' × *P. ussuriensis* var. *ovoidae* 8 is similar to that predicted by Allard (1960) for monogenic inheritance with narrow-sense heritability of 50%. Furthermore, crosses with a *P. calleryana* parent and two *P. pyrifolia* parents have similarly provided evidence for presence of monogenic resistance. Interestingly, a dominant gene for susceptibility has also been proposed (Thompson et al. 1975). However, this finding is based on classifying disease ratings of seedlings for field resistance into two discrete classes, but no bimodal distribution of all ratings has been demonstrated.

Subsequent studies revealed that narrow-sense heritability, estimated from parent-offspring regression, was 0.52 for epiphytotic fire blight of mature seedling trees and of their parents (Bell et al. 1977). Moreover, there were small differences between estimates within crosses of species involving parents of *P. ussuriensis* and *P. pyrifolia* ancestries. In this study, general combining ability was highly significant, while specific combining ability was less significant. In a later study by Quamme et al. (1990), it was reported that general combining ability was significant, but specific combining ability was non-significant. Bagnara et al. (1993) also found that heritability was 50%, with observed differences between crosses accounting for the highest amount of variance. Therefore, they suggested increasing the number of crosses used in such studies. Due to the high environmental variance and non-additive effects, they also concluded that parents should be selected based on their breeding values. However, they also noted that susceptible parents such as 'Bartlett', 'Max Red Bartlett', 'Coscia', and 'Bella di Guigno' could also yield some resistant seedlings. In a later study with some different parents, Bagnara et al. (1996) found that the narrow-sense heritability was approximately 50%, and that both general combining ability and specific combining ability were significant. Similar to their previous study, they also observed that susceptible parents could yield resistant offspring, but also that some resistant parents could also produce susceptible offspring. Subsequently, Durel et al. (2004) analyzed data from the French INRA pear breeding program at Angers, wherein a

population consisting of more than 17,000 seedlings generated from 173 progenies, produced over 10 years by crossing 23 resistant parents with 23 susceptible parents, was evaluated for fire blight resistance. Phenotypic data consisted of five semi-quantitative classes of disease progression. This analysis used a maximum-likelihood (ML) procedure combined with a pedigree matrix to compute heritability and best linear unbiased predictors (BLUP) for parents and ancestors. This method was used in part to compensate for inbreeding due to the recurrent use of some parents, such as 'Williams' (syn. 'Bartlett') in these crosses. The distribution was found to be skewed, with a large proportion of seedlings scored as highly susceptible. Narrow-sense heritability was estimated to be 0.40 ± 0.04, which was slightly lower than those estimates of Bell et al. (1977) and Bagnara et al. (1993, 1996). This was perhaps due to the different parental structure of the population, environmental effects, differences in resistance scoring methods, non-normal distribution of data, and/or the pedigree-based methodology, which was not used in these earlier studies. In any case, BLUP values ranged from 1.91 for 'Campas' to 5.42 for 'Baurotard' (Durel et al. 2004). Therefore, it was proposed that the use of the pedigree method should provide more accurate estimations of heritability and parental breeding values.

13.3.2 Breeding Strategy

Parental selection for fire blight resistance is of the utmost importance. The range of resistance within a species and the polygenic nature of inheritance renders accurate determination of the fire blight resistance phenotype of each prospective parent important. In addition, evaluation methods must be capable of detecting small differences, as well as either minimizing or quantifying environmental variance (Lespinasse and Aldwinckle 2000). This is particularly important for identifying QTLs linked to resistance. As there are differences in transmission of resistance among individual clones within a *Pyrus* species (Thompson et al. 1962; Layne et al. 1968; van der Zwet et al. 1974b), it is suggested that conducting progeny tests may serve as a useful step prior to committing resources for growing and evaluating large progenies, in spite of the moderately high narrow-sense heritability and significant general combining ability (Bell et al. 1977). It is proposed that either test crosses or sub-cross generations between each backcross generation, particularly in an interspecific scheme, are recommended for recovering desirable recessive alleles in homozygous genotypes, and to identify heterozygous parents or to accumulate polygenes in individuals (Lespinasse and Aldwinckle 2000). However, it can also be argued that because of the moderately high narrow-sense heritability that genetic advances can be made when selection is based on phenotypic values (Quamme et al. 1990; Lespinasse and Aldwinckle 2000).

It has been proposed that population size should be determined by considering the heritability of each trait of interest, genetic and environmental variances, and phenotypic and/or genetic correlations among traits of interest. Variances within and between families should be computed from an appropriate genetically diverse population. For example, large negative correlations between fire blight resistance and fruit quality traits would have detrimental effects on simultaneous selection for such target traits. Undesirable fruit traits such as grittiness, poor flavor, and small fruit size are often associated with *P. pyrifolia* or *P. ussuriensis*. In a study of large numbers of parents and seedling populations, derived from *P. communis* progenies and interspecific progenies involving *P. communis* crossed with either *P. ussuriensis*, *P. pyrifolia*, or *P. calleryana*, it is observed that phenotypic (Bell et al. 1976) and genetic (Bell, unpublished data) correlations between seven fruit quality traits and fire blight resistance, while generally negative, are small and usually statistically non-significant. When using these Asian pear species and *P.* × *bretschneideri* as sources of fire blight resistance, larger population sizes are required to increase the likelihood of identifying

selections carrying all desirable traits. It has been suggested that individual seedling population sizes of at least 100 seedlings are required.

For European pear markets, the melting texture of the fruit is a highly desired ideotype for pear cultivars. For breeding for this type of fruit, it is preferable that hybridization is conducted among *P. communis* germplasm, especially as cultivars and selections transmitting high levels of fire blight resistance have also been identified. This is particularly true as the probability of combining fire blight resistance with high fruit quality is greater than that observed in an interspecific *Pyrus* hybridization program that may require several backcross generations. However, this is not necessarily the case in the New Zealand pear breeding program, as it is desirable to combine the aromatic flavor of *P. communis* along with the fine and juicy texture of the best Asian pear germplasm (White and Brewer 2002b).

Interspecific hybridization schemes have been evaluated for transferring fire blight resistance from Asian species into a *P. communis* genetic background (Layne et al. 1968; Layne, unpublished, as cited in Bell et al. 1996a). However, no single crossing scheme has generated a clearly superior proportion of fire blight-resistant seedlings. Nevertheless, crosses between two moderately resistant parents have transmitted resistance to a higher proportion of seedlings than crosses between either moderately resistant parents with susceptible parents or those between susceptible parents. Thus, specific parental combinations are likely to be more important than the *Pyrus* species used as a source of resistance to fire blight.

It is important to note that multistage selection is recommended to increase frequencies of accumulations of desirable alleles into a single genotype, thus requiring a large number of crosses (Lespinasse and Aldwinckle 2000). Therefore, simultaneous multi-trait selection should be also conducted (Bagnara et al. 1996).

13.4 Genomics

13.4.1 Mapping of Quantitative Trait Loci

One of the most important advances in genetics is the development of genetic linkage maps utilizing DNA sequence-based markers, such as microsatellites or SSRs, among other marker types. Studies of linkages of these markers to QTLs have been used to investigate the genetic architecture controlling fire blight host resistance.

In the first study of pear, a seedling population of 99 individuals, derived from a cross between two *P. communis* cultivars, 'Passe Crassane' (susceptible) and 'Harrow Sweet' (resistant), has been used (Dondini et al. 2004). Various markers, including SSRs, microsatellite-anchored fragment length polymorphisms (MFLPs), amplified fragment length polymorphisms (AFLPs), resistance gene analogs (RGAs), and AFLP-RGAs have been used to build linkage maps for the two parents, and have identified four loci linked to fire blight resistance from 'Harrow Sweet' (Dondini et al. 2004). Furthermore, it has been found that 'Harrow Sweet' linkage group (LG) 2, HS2, is divided into two sections, HS2a and HS2b, by 32 centiMorgans (cM) due to incomplete marker coverage. Thus, disease incidence, severity (measured as percent lesion length), and ISV, a weighted mean index based on both incidence and severity (Le Lézec et al. 1985), have been calculated for experiments repeated in three years. It is worth pointing out that disease resistance reactions have been classified into five resistance classes. It is observed that analysis of the distribution of these phenotypic disease resistance reactions has indicated that fire blight resistance is under polygenic control. Furthermore, interval mapping has identified four regions of 'Harrow Sweet' (HS) that are significantly associated with fire blight resistance, while no associations have been detected for 'Passe Crassane'. Interestingly, the most significant association is detected on LG HS2a with SSR

marker CH03H03-1 and AFLP marker M59P38-3. Moreover, the percent phenotypic variance explained by the AFLP marker is 24.6 for incidence, 16.6 for severity, and 16.4 for ISV. Whereas, it is observed that on LG HS2b, the markers AFLP-RGA B3M55-5 and SSR CH03D10 are significantly linked to fire blight resistance, with AFLP-RGA B3M55-5 accounting for 11.8, 9.9, and 9.6% of variances for incidence, severity, and ISV, respectively. In addition, AFLP-RGA T2E32-1 and SSR CH01F02 on HS4 are found to be linked to fire blight resistance, wherein AFLP-RGA T2E32-1 accounts for 9.5, 8.7, and 12.0% of variances for incidence, severity, and ISV, respectively. Finally, it has been found that SSR CH05A03 on HS9 accounts for 6.9, 8.4, and 8.5% of the variance for incidence, severity, and ISV, respectively. Interestingly, it has been reported that detection of the two AFLP-RGAs may indicate presence of major genes for resistance (Dondini et al. 2004).

Subsequently, Le Roux et al. (2012) repeated the analysis of the 'Passe Crassane' × 'Harrow Sweet' cross using additional SSR markers and were able to combine HS2a and HS2b from the previous study (Dondini et al. 2004) into a single contiguous linkage group. A single major QTL that was significantly ($p = 0.0001$) linked to the SSR TsuENH001 and located at 30.1 cM was identified by interval mapping. This SSR marker, flanked by TsuENH017 at 17.0 cM and NH033b at 36.7 cM, accounted for 32.3, 28.9, and 28.1% of phenotypic variances for incidence, severity, and ISV, respectively. Furthermore, on HS04 linkage group, markers SSR CH01d07, located at 23.4 cM, and AT000420-SSR, located at 41.7 cM, were found to be associated with fire blight disease frequency, but only at the 0.005 level of significance. In addition, as reported previously (Dondini et al. 2004), the AFLP-RGA T2E32-1, mapped close to AT000420-SSR and located at 45.2 cM, was found to be associated with disease severity and IVS, but only at the 0.005 level of significance. Thus, it was concluded that associations at the 0.005 level represented only putative QTLs. Interestingly, an analysis of 'Bartlett' and 'Old Home', the only available ancestors in the pedigree of 'Harrow Sweet', did not reveal presence of any of the favorable alleles on HS2. Therefore, it was hypothesized that the favorable allele could be traced back to 'Early Sweet', the pollen parent of Purdue 80-51, the seed parent of 'Harrow Sweet'. However, the favorable allele of AT000420-SSR on HS4 was detected in 'Bartlett', the pollen parent of 'Harrow Sweet'. Fortunately, an analysis of fire blight resistance in the progeny of 'Angelys' × 'Harrow Sweet' validated presence of the HS2 QTL.

In another study, a seedling population of 155 individuals, derived from a cross between *P. communis* 'Doyenné du Comice' (susceptible) and *P. ussuriensis* Maxim. No. 18 (resistant), was evaluated for fire blight disease resistance (Bokszczanin et al. 2009). In this study, disease severity was calculated as percentage lesion length of total shoot length, and seedlings were classified into five disease resistance classes, with each class of 20% in size. Transgressive segregation for fire blight resistance was observed in this population. A putative QTL on LG 11 of the *P. ussuriensis* parent linked to SSR RLG1, located at 0 cM, was found. Moreover, SSR CH03d02a, located at 22 cM, was also significantly associated with resistance, and another QTL linked to SSR CH02c02b on LG 4 of 'Doyenné du Comice' was also identified. These findings suggested that resistance genes could also be found in susceptible germplasm.

In a subsequent analysis of the above population, wherein AFLP markers were included, a QTL on LG 9 of *P. ussuriensis* No. 18, accounting for 61.9% of the phenotypic variance, was found (Bokszczanin et al. 2011). Furthermore, additional QTLs on LGs U11, U_a, U_e, and U_g, accounting for a total of 31.5% of the phenotypic variance, were discovered. The QTLs of LGs U_e and U_g were found to be linked to AFLP-RGA markers, thus confirming presence of resistance genes in these linkage groups. In addition, four QTLs identified on LGs K3, K4, K11, and K_a of 'Doyenné du Comice', collectively accounting for 25.6% of the phenotypic variance, were also discovered. This finding further confirmed earlier conclusions of Bokszczanin et al. (2009) as the susceptible pear cultivar 'Doyenné du Comice' contributed QTLs of small effects for resistance to fire blight.

An interspecific seedling population of PremP003 (*P.* × *bretschneideri* Rehd. × *P. communis* L.) × 'Moonglow' (*P. communis*) was artificially inoculated with *E. amylovora* in France in 2013, and in New Zealand in both 2013 and 2014 (Montanari et al. 2016). A total of 85 seedlings were evaluated in France in 2013, while 90 seedlings were evaluated in New Zealand in 2013, and 105 seedlings in 2014, with 85 seedlings common to both years. Disease progress was measured weekly for four weeks. Infection length as a percentage of shoot length (PLL) and area under disease progress curve (AUDPC) were computed. Analysis of phenotypic distributions detected some transgressive segregation, consistent with polygenic control of fire blight resistance. Furthermore, QTL mapping was conducted, utilizing PLL at 28 dpi and AUDPC, using data for each location, and pooled for all years and locations. Previously, genetic marker maps using single nucleotide polymorphisms (SNPs) and SSRs for the two parents have been developed (Montanari et al. 2013); therefore, these genetic maps were used in this study. A major QTL associated with both PLL and AUDPC was located on LG 2 of 'Moonglow', accounting for 12.9–34.4% of the phenotypic variance, and found to be stable between the two environments. In addition, associated SNP markers were identified, including the "C" allele of ss527789653, located at 15 cM, for data collected in France, and the "G" allele of ss52779655, located at 17 cM, for data collected in New Zealand. However, when data from both environments were pooled, it was observed that the "C" allele of ss527789653 accounted for 66.3 and 66.5% of the *global*R^2 for PLL and AUDPC, respectively (Montanari et al. 2016). Previously, a QTL for fire blight resistance was discovered in 'Harrow Sweet' (Dondini et al. 2004). Therefore, it was suggested that the high effect of this QTL indicated presence of major genes located in this region. In fact, it was noted that chromosome 2 of *P.* × *bretschneideri* was rich in resistance gene paralogues (Wu et al. 2013), and that *P. communis* might also possess such genes.

Based on data collected in France, a QTL peak, co-located with ss475879846, was detected on LG 9 of PremP003, and resistance was associated with the C allele. This QTL accounted for 14.8 and 13.9% of the observed phenotypic variance for disease severity and AUDPC, respectively (Montanari et al. 2016). Comparisons with the QTL located on LG 9 of 'Harrow Sweet' (Dondini et al. 2004) were conducted using a map generated by Celton et al. (2009a, b). It was found that the QTL of 'Harrow Sweet' was linked to SSR CH05a03, and although it was closely mapped to SSRs CH05c07 and NB130b of PremP003, it was located on a different region of LG 9. However, the QTL on LG 9 in the New Zealand experiment mapped close to SSR CH03a03. Therefore, it was concluded that this latter QTL could not be verified as to whether or not it was the same QTL detected in the French experiment (Montanari et al. 2016).

Interestingly, three QTLs for fire blight resistance, mapped to LGs 7, 12, and 15, were only discovered in the New Zealand experiment. These QTLs might be strain-specific, as they were not detected in inoculation experiments in France where a different strain of *E. amylovora* was used. Furthermore, when fire blight inoculation data from both French and New Zealand experiments were combined, a minor QTL linked to the "C" allele of ss475876971 was located on LG 10 of PremP003, and it was found to be epistatic with the locus on LG 2 (Montanari et al. 2016). However, as phenotypic segregation of seedlings at the two locations was different, it was proposed that this QTL required further verification. Yet, another minor QTL linked to the "T" allele of ss47589592 was located on LG 15 of PremP003. Overall, these minor QTLs accounted for 8.1–14.8% of the observed phenotypic variance. In addition, there was a good correlation between QTL results for severity and for AUDPC. However, no homologies could be detected between these minor QTLs and QTLs detected in other pear populations reviewed herein.

It has been reported that the QTL on LG 2 of 'Moonglow', associated with a 176 bp allele of CH02f06 and a 179 bp allele of TsuENH017, was inherited from its pollen parent, 'Roi Charles de Wurtemburg'. This QTL, along with a QTL

on LG 2 of 'Harrow Sweet', mapped by Le Roux et al. (2012), co-located with TsuENH017, thus indicating that it was stable in different genetic backgrounds. However, fire blight resistance was associated with different alleles of TsuENH017. Noting that the allelic profile of this SSR in 'Moonglow' was the same as that identified for the fire blight-resistant 'Old Home' (179:189), reported by Le Roux et al. (2012), it was hypothesized that part of the 'Old Home' fire blight resistance was linked to a 179-bp allele. In addition, there was colinearity between these regions, and that the two pear cultivars had the same haplotypes, with one haplotype associated with resistance in 'Moonglow', while the other haplotype associated with susceptibility in 'Old Home'. It was hypothesized that the two pear cultivars must carry the same QTL, and that pending further validation in other genetic backgrounds, this QTL was a good candidate for marker-assisted breeding (MAB) (Montanari et al. 2016).

Although the minor QTL on LG 9 of PremP003 was associated with a 141 bp allele of CH05c07 and a 90 bp allele of NB130b, both alleles inherited from 'Xue Hua Li', neither of these favorable alleles were found on LG 9 of the fire blight-resistant 'Harrow Sweet' (Dondini et al. 2004; Le Roux et al. 2012). The origins of QTLs mapped onto LGs 7, 12, and 15 could not be determined.

Overall, it is concluded that these data support the hypothesis of polygenic control for fire blight resistance. Furthermore, it is also concluded that a high broad-sense heritability supported the reliability of these detected QTLs. However, it is reported that the $globalR^2$ is less than that of H^2, and this is due either to small population sizes or to presence of additional QTLs in regions of these maps that are not covered by markers. Therefore, it is proposed that pre- and post-zygotic incompatibilities may have prevented saturation of the parental genetic maps due to linkages to a lethal gene.

All results of the above-mentioned QTL studies are summarized in Table 13.1.

A new project, entitled 'RosBREED2: Combining disease resistance and horticultural quality in new rosaceous cultivars', has been initiated in the USA, and involving various international collaborators (Iezzoni et al. 2017). The major goal for pear is to discover and/or validate QTLs in three populations segregating for fire blight resistance.

The genomics of host resistance to fire blight in pear genomics has been reviewed by Yamamoto and Chevreau (2009). Additionally, aspects of genomics of *Malus and Pyrus,* as well as those of the bacterial pathogen *E. amylovora* have also been reviewed by Malnoy et al. (2012).

13.4.2 Resistance Gene Analogues

It has been reported that disease resistance genes from different plant species conferring resistance against various pathogens have conserved regions involved in pathogen recognition and defense response (Staskawicz et al. 1995). Primers can be designed for these regions, and used in PCRs to amplify similar fragments, known as resistance gene analogues (RGAs), in other plant species. RGAs have been identified in fire blight-resistant pear genotypes, including 'Harrow Sweet', 'Old Home', and US309 (Dondini et al. 2002a). In fact, primers have been designed for the P-loop and for GLPL motifs, and then used to generate PCR products. All these primers have amplified a major 500 bp band in all pear genotypes. This band must have resulted from co-migration of more than 80 fragments, which have been subsequently cloned, and grouped by cluster analysis. After sequencing of 15 colonies, followed by FASTA analysis, it has been shown that these sequences have 58–65% homology to known resistance genes or RGA sequences. Alignments among pear RGAs have revealed a high degree of sequence variability, but most of these sequences are found to belong to the TIR-NBS-LRR family. Therefore, it has been proposed that these RGA sequences can serve as genetic markers to search for polymorphisms between fire blight-resistant and susceptible parents, and to establish linkages with fire blight resistance. An analysis of the phylogeny of RGAs in Rosaceae species, including 34 from

Table 13.1 Quantitative trait loci for fire blight resistance in pear

Mapping population	n	Trait	Linkage group	Marker type	Marker	Marker position (cM)	Percent σ^2	Reference(s)
'Passe Crassane' × 'Harrow Sweet'	99	ISV, 28	HS2a	AFLP	M59P38-3	9.0	16.4	Dondini et al. (2004)
			HS2b	AFLP-RGA	B3M55-5	10.7	9.6	
			HS4	AFLP-RGA	T2E32-1	9.8	12.0	
			HS9	SSR	CH05A03	21.9	8.5	
		ISV, 28	HS2	SSR	TsuENH001	30.1	28.1	Le Roux et al. (2012)
			HS4	AFLP-RGA	T2E32-1	45.2	13.3	
'Doyennédu Comice' × *P. ussuriensis* No. 18	155	PLL, 28	DC4	SSR	CH02c02b	56.0	-	Bokszczanin et al. (2009)
			Pu11	SSR	CH03d02a	22.0	-	
PremP003 × 'Moonglow'	85	AUDPC, 28	M2	SNP	ss527789563	15.0	34.4	Montanari et al. (2016)
			P9	SNP	ss475879846	35.0	13.9	
	90, 105		M2	SNP	ss527789655	17.0	17.7	
			P7	SNP	ss475876829	48.0	7.2	
			P12	SNP	ss475880537	48.0	10.3	
			P13	SNP	ss527788568	23.0	10.0	

Trait: *ISV* = Index of varietal susceptibility (Thibault et al. 1987), 28 = days after inoculation, *PLL* = percent lesion length, *AUDPC* = area under the disease progress curve; Linkage group: *HS* = 'Harrow Sweet', *DC* = 'Doyenné du Comice', *Pu* = *P. ussuriensis* No. 18, *M* = 'Moonglow', *P* = PremP003

Pyrus, has found that three clades contain RGAs of *Pyrus*, *Malus*, and *Prunus*, thus indicating a monophyletic origin and conservation of these RGAs in these three genera of Rosaceae (Perazzolli et al. 2014).

References

Abdollahi H, Rugini E, Ruzzi M, Muleo R (2004) *In vitro* system for studying the interaction between *Erwinia amylovora* and genotypes of pear. Plant Cell Tiss Org Cult 79:203–212

Allard RW (1960) Principles of plant breeding. Wiley, New York, USA, p 254

Andersen HW (1956) Diseases of fruit crops. McGraw Hill, New York, USA, p 501

Asin L, Iglesias I, Dolcet-Sanjuan R, Claveria E, Vilardell P, Bonany J, Simard MH (2011) INRA-IRTA pear rootstock breeding program: aiming for tolerance to iron-chlorosis. Acta Hortic 903:207–213

Bagnara GL, Rivalta L, Laghi M, Quarta R, Lecomte P (1993) Cross combinations for fire blight resistance in pear. Acta Hortic 338:369–374

Bagnara GL, Rivalta L, Laghi M, Quarta R (1996) Evaluation of fire blight resistance in pear: combining ability and breeding strategy. Acta Hortic 411:383–392

Bell RL (1991) Pears (*Pyrus*). Acta Hortic 290:657–700. https://doi.org/10.17660/ActaHortic.1991.290.15

Bell RL, Itai A (2011) *Pyrus*. In: Kole C (ed) Wild crop relatives: genomic and genetic resources, temperate fruits. Springer-Verlag, Berlin, Germany, pp 147–177

Bell RL, Leitão JM (2011) *Cydonia*. In: Kole C (ed) Wild crop relatives: genomic and genetic resources, temperate fruits. Springer-Verlag, Berlin, Germany, pp 1–16

Bell RL, van der Zwet T (2008) 'Shenandoah' pear. HortScience 43:2219–2221

Bell RL, van der Zwet T (2011) 'Sunrise' pear. HortScience 46:118–120

Bell RL, Janick J, Zimmerman RH, van der Zwet T (1976) Relationship between fire blight resistance and fruit quality in pear. HortScience 11:500–502

Bell RL, Janick J, Zimmerman RH, van der Zwet T (1977) Estimation of heritability and combining ability for fire blight resistance in pear. J Am Soc Hort Sci 102:133–138

Bell RL, Quamme HA, Layne REC, Skirvin RM (1996a) Pear. In: Janick J, Moore JN (eds) Fruit breeding, volume I: tree and tropical fruits. Wiley, New York, USA, pp 441–514

Bell RL, van der Zwet T, Blake RC, Chandler CK, Scheerens JC (1996b) 'Potomac' pear. HortScience 31:884–886

Bell RL, van der Zwet T, Blake RC (2002) 'Blake's Pride' pear. HortScience 37:711–713

Bell RL, Ranney TG, Eaker TGA, Sutton TB (2004) Resistance to fire blight among flowering pears and quince. HortScience 40:413–415

Bell RL, van der Zwet T, Castagnoli S, Einhorn T, Turner JD, Spotts R, Moulton GA, Reighard GL, Shane WW (2014) 'Gem' pear. HortScience 49:361–363

Bellini E, Nin S (1997) The breeding of pear tree worldwide (*Pyrus communis*). Riv Fruttic 59:19–30

Bellini E, Nin S (2002) Breeding for new traits in pear. Acta Hortic 596:217–224

Bobev S, Deckers T (1999) Field susceptibility to fire blight of pome fruits in Bulgaria. Acta Hortic 489:221–224

Bobev SG, Angelov LT, Govedarov GI, Postman JD (2011) Quince (*Cydonia oblonga*) emerges from the ashes of fire blight. Acta Hortic 918:911–915

Bock CH, Gottwald TR, Parker PE, Cook AZ, Ferrandino F, Parnell S, van den Bosch F (2009) The Horsfall-Barratt scale and severity estimates of citrus canker. Eur J Plant Pathol 125:23–38

Bock CH, Gottwald TR, Parker PE, Cook AZ, Ferrandino F, Welham S, van den Bosch F, Parnell S (2010) Some consequences of using the Horsfall-Barratt scale for hypothesis testing. Phytopathology 100:1030–1041

Bokszczanin K, Dondini L, Przybyla AA (2009) First report on the presence of fire blight resistance in linkage group 11 of *Pyrus ussuriensis* Maxim. J Appl Genet 50(2):99–104

Bokszczanin K, Dondini L, Przybyla AA, Palucha A (2011) QTLs for fire blight (*Erwinia amylovora*) resistance in *Pyrus ussuriensis*. Acta Hortic 896:371–373

Bokszczanin KL, Przybyla AA, Schollenberger M, Gozdowski D, Madry W, Odziemkowski S (2012) Inheritance of fire blight resistance in Asian *Pyrus* species. Open J Genet 2:109–120

Braniste N, Andries N, Ghidra V (2008) Pear genetic breeding to improve the Romanian varieties. Acta Hortic 800:491–496

Brewer LR, Palmer JW (2011) Global pear breeding programmes: goals, trends and progress for new cultivars and new rootstocks. Acta Hortic 909: 105–119

Brisset MN, Paulin JP, Duron M (1988) Feasibility of rating fire blight susceptibility of pear cultivars (*Pyrus communis*) on *in vitro* cuttings. Agronomie 8:707–710

Brisset MN, Ochatt SJ, Paulin JP (1990) Evidence for quantitative responses during co-culture of *Pyrus communis* protoplasts and *Erwinia amylovora*. Plant Cell Rep 9:272–275

Brooks L (1984) History of the Old Home × Farmingdale pear rootstocks. Fruit Var J 38:126–128

Brooks HJ, van der Zwet T, Oitto WA (1967) The pear breeding program of the United States Department of Agriculture. Chron Hort 7:34–35

Carpenter TR, Shay JR (1953) The differentiation of fire blight resistant seedlings within progenies of interspecific crosses of pear. Phytopathology 43:156–162

Celton J-M, Chagné D, Tustin SD, Terakami S, Nishitani C, Yamamoto T (2009a) Update on comparative genome mapping between *Malus* and *Pyrus*. BMC Res Notes 2:182. https://doi.org/10.1186/1756-0500-2-182

Celton J-M, Tustin DS, Chagné D, Gardiner SE (2009b) Construction of a dense genetic linkage map for apple rootstocks using SSRs developed from *Malus* ESTs and *Pyrus* genomic sequences. Tree Genet Genomes 5:93–107

Decourtye L (1967) Etude de quelque caracteres a controle genetique simple chez le pommier (*Malus* sp.) et le poirier (*Pyrus communis* L.). Ann Amel Plantes 17:243–265

Denning W (1794) On the decay of apple trees. New York Soc Prom Agric Arts Manuf Trans 2:219–222

Dondini L, Sansavini S (2012) European pear. In: Badenes ML, Byrne DH (eds) Fruit breeding. Springer, New York, USA, pp 369–413

Dondini L, Tartarini S, Nocelli E, Sansavini S (2002a) Cloning and characterization of resistance gene analogues (RGA) in European pear (*Pyrus communis*). Acta Hortic 596:207–210

Dondini L, Tartarini S, Sansavini S, Malaguti S, Bazzi C (2002b) Reactivity of European pear (*Pyrus communis*) progenies to fire blight (*Erwinia amylovora*). Acta Hortic 596:211–214

Dondini L, Pierantoni L, Gaiotti F, Tartarini S, Bassi C, Sansavini S (2004) Identifying QTLs for fire-blight resistance via a European pear (*Pyrus communis* L.) genetic linkage map. Mol Breed 14:407–418

Drain BD, Shuey GA (1954) Breeding and testing fire blight-resistant pears. Tenn Agric Exp Stn Bull 236. 20 p.

Drain BD, Safley LM (1958) Morgan and Carrick - Two blight-resistant pears. Tenn Agric Expt Sta Bull 263. 10 p.

Durel CE, Guérif P, Belouin A, Le Lezec M (2004) Estimation of fire blight resistance heritability in the French pear breeding program using a pedigree-based approach. Acta Hortic 663:251–265

Duron M, Paulin JP, Brisset MN (1987) Use of *in vitro* propagated plant material for rating fire blight susceptibility. Acta Hortic 217:317–324

Elkins R, Klonsky K, DeMoura R, DeJong TM (2008) Economic evaluation of high density versus standard orchard configurations: case study using performance data for 'Golden Russet Bosc' pears. Acta Hortic 800:739–746

Elkins R, Castagnoli S, Embree C, Parra-Quezada R, Robinson T, Smith T, Ingels C (2011) Evaluation of potential rootstocks to improve tree precocity and productivity. Acta Hortic 909:184–194

Elkins R, Bell RL, Einhorn T (2012) Needs assessment for future US pear rootstock research directions based on the current state of pear production and rootstock research. J Am Pomol Soc 66:153–163

Fare DC, Gilliam CH, Ponder HG (1991) Fire blight susceptibility, growth and other characteristics in ornamental pears in Alabama. J Arboricult 17:257–260

Fischer M (2009) Pear breeding. In: Jain SM, Priyadarhan PM (eds) Breeding plantation tree crops: temperate species. Springer Science, New York, USA, pp 135–159

Fischer M, Mildenberger G (2004) New pear cultivars from Dresden-Pillnitz. Acta Hortic 663:899–901

Hancock JF, Lobos GA (2008) Pears. In: Hancock JF (ed) Temperate fruit crop breeding. Springer, New York, USA, pp 299–335

Hanke V, Geider K (2002) A new approach to evaluate fire blight resistance in vitro. Acta Hortic 590:397–399

Harris MK (1973) Host resistance to the pear psylla in a *P. ussuriensis* × *P. communis* hybrid. Environ Entomol 2:833–887

Harris MK, Lamb RC (1973) Resistance to the pear psylla in pears with *Pyrus ussuriensis* lineage. J Am Soc Hort Sci 98:378–381

Hartman H (1957) Catalogue and evaluation of the pear collection at the Oregon Agricultural Experiment Station. Oregon Agric Exp Stn Tech Bull 41

Hedrick UP, Howe GH, Taylor OM, Francis EH, Tukey HB (1921) The pears of New York. New York Dept Agric 29th Annu Rep vol 2, part 2

Horsfall JG, Barratt RW (1945) An improved grading system for measuring plant diseases. Phytopathology 35:655

Hough LF (1944) The new pear breeding project. Ill State Hort Soc Trans 78:106–113

Hough LF, Bailey CH (1968) Star, Lee and Mac - three blight resistant fresh market pears from New Jersey. Fruit Var Hort Digest 22:43–45

Hunter DM (2016) Fifty years of pear breeding: an overview of the Harrow (Ontario, Canada) pear breeding program. Meyve Bilini (Fruit Sci) 3:1–7

Hunter DM, Pinsonneault P, Kappel F, Quamme HA, Bonn WG, Layne REC (1992) 'Harrow Sweet' pear. HortScience 27:1331–1334

Hunter DM, Kappel F, Quamme HA, Bonn WG (2002a) 'AC Harrow Gold' pear. HortScience 37:224–226

Hunter DM, Kappel F, Quamme HA, Bonn WG (2002b) 'AC Harrow Crisp' pear. HortScience 37:227–229

Hunter DM, Kappel F, Quamme HA, Bonn WG, Slingerland KC (2009) 'Harovin Sundown' pear. HortScience 44:1461–1463

Iezzoni A, Peace C, Main D, Bassil N, Coe M, Finn C, Gasic K, Luby J, Hokanson S, McFerson J, Norelli J, Olmstead M, Whitaker V, Yue C (2017) RosBREED2: progress and future plans to enable DNA-informed breeding in the *Rosaceae*. Acta Hortic 1172:115–118

Jacob HB (2002) New pear rootstocks from Geisenheim, Germany. Acta Hortic 596:337–344

Janick J (1977) The 'Honeysweet' pear. HortScience 12:357

Janick J (2004) 'P448-2' (Green Jade™) pear. HortScience 39:454–455

Janick J (2006) 'H2-169' (Ambrosia™) pear. HortScience 41:467

Jeger MJ, Viljanen-Rollinson SLH (2001) The use of the area under the disease-progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars. Theor Appl Genet 102:32–40

Kellerhals M, Schütz S, Patocchi A (2017) Breeding for resistance to fire blight. J Plant Pathol 99(Special issue):37–43

Lamb RC (1960) Resistance to fire blight of pear varieties. Proc Am Soc Hort Sci 75:85–88

Layne REC, Bailey CH, Hough LF (1968) Efficacy of transmission of fire blight resistance in *Pyrus*. Can J Plant Sci 48:231–243

Le Lézec M, Thibault B, Balavoine P, Paulin JP (1985) Sensibilité varietale du pommier et du poirier au feu bactrien. Phytoma 365:37–44

Le Lézec M, Bautrais P, Belouin A (1991) L'amélioration du poirier pour la résistance au feu bacterien. Arboricult Fruitère 440:29–37

Le Lézec M, Lecomte P, Laurens F, Michelesi JC (1997) Sensibilité variétale au feu bacterien. Partie II. Poirier. Arboricult Fruitière 504:33–37

Le Lézec M, Belouin A, Guérif P, Lespinasse Y (2002) "Angelys", a new winter pear to replace "Passe Crassane". Acta Hortic 596:265–269

Le Roux P-MF, Christen D, Duffy B, Tartarini S, Dondini L, Yamamoto T, Nishitani C, Terakami S, Lespinasse Y, Kellerhals M, Patocchi A (2012) Redefinition of the map position and validation of a major quantitative trait locus for fire blight resistance of the pear cultivar 'Harrow Sweet' (*Pyrus communis* L.). Plant Breed 131:656–664

Lebedev VG, Dolgov SV, Lavrova SV, Lunin VG, Narodikski BS (2002) Plant-defensin genes introduction for improvement of pear phytopathogen resistance. Acta Hortic 596:167–172

Lespinasse Y (2009) Review of pome fruit breeding in Europe: which strategies for the near future? Acta Hortic 814:865–872

Lespinasse Y, Aldwinckle HS (2000) Breeding for resistance to fire blight. In: Vanneste JL (ed) Fire blight: the disease and its causative agent, *Erwinia amylovora*. CABI Publishing, Wallingford, UK, pp 253–273

Lespinasse Y, Guérif P, Durel CE (2011) Strategies for fire blight resistance breeding in pear (*Pyrus communis*): 30 years of experience. Acta Hortic 909:51–58

Lombard PB, Westwood MN (1987) Pear rootstocks. In: Rom RC, Carlson RF (eds) Rootstocks for fruit crops. Wiley, New York, USA, pp 145–183

Magness JR (1937) Progress in pear improvement. In: USDA yearbook of agriculture. USDA, Washington, DC, USA, pp 615–630

Malnoy M, Venisse JS, Chevreau E (2005a) Expression of a bacterial effector, harpin N, causes increased resistance to fire blight in *Pyrus communis*. Tree Genet Genomes 1:41–49

Malnoy M, Faize M, Venisse JS, Geider K, Chevreau E (2005b) Expression of viral EPS-depolymerase reduces fire blight susceptibility in transgenic pear. Plant Cell Rep 23:632–638

Malnoy M, Martens S, Norelli JL, Barny M-A, Sundin GW, Smits THM, Duffy B (2012) Fire blight: applied genomic insights of the pathogen and host. Ann Rev Phytopathol 50:475–494

Momol MT, Norelli JL, Aldwinckle HS, Zeller W (1996) Use of the area under the disease progress curve for quantification of resistance of apple and pear varieties and rootstocks to *Erwinia amylovora*. Acta Hortic 411:373

Montanari S, Saeed M, Knäbel M, Kim Y-K, Troggio M, Malnoy M, Velasco R, Fontana P, Won K-H, Durel C-E, Perchepied L, Schaffer R, Wiedow C, Bus V, Brewer L, Gardiner SE, Crowhurst RN, Chagné D (2013) Identification of *Pyrus* single nucleotide polymorphisms (SNPs) and evaluation for genetic mapping in European pear and interspecific *Pyrus* hybrids. PLoS One 8:e77022

Montanari S, Perchepied L, Renault D, Frijters L, Velasco R, Horner M, Gardiner SE, Chagné Bus VGM, Durel C-E, Malnoy M (2016) A QTL detected in an interspecific pear population confers stable fire blight resistance across different environment and genetic backgrounds. Mol Breed 36:47

Mowry JB (1964) Maximum orchard susceptibility of pear and apple varieties to fire blight. Plant Dis Rptr 48:272–276

Musacchi S, Ancarani V, Gamberini A, Giatti B, Sansavini S (2005) Progress in pear breeding at the University of Bologna. Acta Hortic 671:141–144

Musacchi S, Dondini L, Pierantoni L, Gaiotti F, Ancarani V, Sansavini S (2006) Pear breeding and development of molecular markers linked to fire blight and psylla. Italus Hortus 13:60–63

Norelli JL, Aldwinckle HS, Beer SV (1984) Differential host × pathogen interactions among cultivars of apple and strains of *Erwinia amylovora*. Phytopathology 74:136–139

Norelli JL, Aldwinckle HS, Beer SV (1986) Differential susceptibility of *Malus* spp. Robusta 5, Novole, and Ottawa 523 to infection by *Erwinia amylovora*. Plant Dis 70:1017–1019

Oitto WA, van der Zwet T, Brooks HJ (1970) Rating of pear cultivars for resistance to fire blight. HortScience 5:474–476

Papachatzis A, Kalorizou H, Vagelas I, Sotiropoulos T, Tsipouridis K (2011) Screening quince cultivars and hybrids for resistance to fire blight (*Erwinia amylovora*). Acta Hortic 918:933–936

Paprstein F, Sedlak J, Sillerova J, Korba J (2014) In vitro evaluation of cultivar resistance to fire blight. Acta Hortic 1056:259–262

Perazzolli M, Malacarne G, Baldo A, Righetti L, Bailey A, Fontana P, Velasco R, Malnoy M (2014) Characterization of resistance gene analogues (RGAs) in apple (Malus x domestic Borkh.) and their evolutionary history of the Rosacea family. PLoS ONE 9(2): e83844. https://doi.org/10.1371/journal.pone.0083844

Pinet-Leblay C, Turpin FX, Chevreau E (1992) Effect of gamma and ultraviolet irradiation on adventitious regeneration from *in vitro* cultured pear leaves. Euphytica 62:225–233

Pinet-Leblay C, Brisset MN, Chevreau E, Paulin JP (1996) *In vitro* screening for resistance to fire blight using a pathogenicity mutant of *Erwinia amylovora*. Acta Hortic 411:415–416

Postman J, Kim D, Bassil N (2013) OH × F paternity perplexes pear producers. J Am Pomol Soc 67:157–167

Quamme HA, Bonn WG (1981) Virulence of *Erwinia amylovora* and its influence on the determination of fire blight resistance of pear cultivars and seedlings. Can J Plant Pathol 3:187–190

Quamme HA, Spearman GA (1983) 'Harvet Queen' and 'Harrow Delight' pear. HortScience 18:770–772

Quamme HA, Kappel F, Hall JW (1990) Efficacy of early selection for fire blight resistance and the analysis of combining ability for fire blight resistance in several pear progenies. Can J Plant Sci 70:905–913

Reimer FC (1925) Blight resistant pears and characteristics of pear species and stocks. Oregon Agric Exp Stn Bull 214

Reynoird JP, Mourgue F, Norelli J, Aldwinckle HS, Brisset MN, Chevreau E (1999) First evidence for improved resistance to for blight in transgenic pear expressing the attacin E gene from *Hyalophora cercropia*. Plant Sci 1459:23–31

Rivalta L, Dradi M, Rosati C (2002) Thirty years of pear breeding activity at ISF Forli, Italy. Acta Hortic 596:233–238

Ryugo K (1982) Breeding resistance to fire-blight bacteria, *Erwinia amylovora*, in pears. Acta Hortic 124:33–36

Sanz ML, Montañes L (1997) Diagnóstico visual de la clorosis férrica. Int Téc Econ Agraria 93:7–22

Sestras R, Sestras A, Barbos A (2007) The response of pear cultivars to *Erwinia amylovora* attack in Central Transylvania conditions, Romania. In: 18th EUCARPIA genetic resources section meeting, Piešt'any, Slovak Republic, pp 138–139

Shaffer WH, Goodman RN (1962) Progression *in vivo*, rate of growth *in vitro*, and resistance to streptomycin, as indices of virulence of *Erwinia amylovora*. Phytopathology 52:1201–1207

Shaner G, Finney RE (1977) The effect of nitrogen fertilization on the expression of slow-mildewing in Knox wheat. Phytopathology 67:1051–1056
Simard MH, Michelesi JC (2002) 'Pyriam': a new pear rootstock. Acta Hortic 596:351–355
Simard MH, Michelesi JC, Masseron A (2004) Pear rootstock breeding in France. Acta Hortic 658:535–540
Simko I, Piepho H-P (2012) The area under the disease progress stairs: calculation, advantage, and application. Phytopathology 102:381–389
Staskawicz BJ, Ausbel FM, Baker BJ, Ellis JG, Jones JDG (1995) Molecular genetics of plant disease resistance. Science 268:661–667
Thibault B (1981) Pear breeding for fire blight resistance program and first studies in France. Acta Hortic 117:61–69
Thibault B, Welcker C, Lespinasse Y (1983) Heritability of the character 'secondary blooming' on pear (*Pyrus communis*). Acta Hortic 139:181–193
Thibault B, Lecomte P, Hermann L, Belouin A (1987) Comparison between two methods of selection for resistance to *Erwinia amylovora* in young seedlings. Acta Hortic 217:265–272
Thibault B, Belouin A, Lecomte P (1989) Sensibilité variétale du Poirier au feu bacterien. Arboricult Fruitière 421:139–148
Thompson SS, Janick J, Williams EB (1962) Evaluation of fire blight resistance of pear. Proc Am Soc Hort Sci 80:105–113
Thompson JM, Zimmerman RH, van der Zwet T (1975) Inheritance of fire blight resistance in pear. I: a dominant gene, *Se*, causing sensitivity. J Hered 66:259–264
USDA, ARS (2018) GRIN-global. HTTP: ars-grin.gov. Last accessed 10 Mar 2018
van der Zwet T (2002) Present worldwide distribution of fire blight. Acta Hortic 590:33–34
van der Zwet T, Oitto WA (1972) Further evaluation of the reaction of "resistant" pear cultivars to fire bight. HortScience 7:395–397
van der Zwet T, Keil HL (1979) Fire blight: a bacterial disease of rosaceous plants. In: Agriculture handbook, 510. USDA, Washington, DC
van der Zwet T, Oitto WA, Brooks HJ (1970) Scoring system for rating the severity of fire blight in pear. Plant Dis Rep 54:835–839
van der Zwet T, Oitto WA, Blake RC (1974a) Fire blight resistance in pear cultivars. HortScience 9:340–342
van der Zwet T, Oitto WA, Westwood MN (1974b) Variability in degree of fire blight resistance within and between *Pyrus* species, interspecific hybrids, and seedling populations. Euphytica 23:295–304
Viseur J (1990) Evaluation of fire blight resistance of somaclonal variants obtained from the pear cultivar 'Durondeau'. Acta Hortic 273:275–284
Viseur J, Tapia y Figeuroa M (1987) In vitro co-culture as a tool for the evaluation of fire blight resistance in pears and apples. Acta Hortic 217:273–281
Webster AD (2003) Breeding and selection of apple and pear rootstocks. Acta Hortic 622:499–512
Wertheim SJ (2002) Rootstocks for European pear: a review. Acta Hortic 596:299–309
Westwood MN (1982) Pear germplasm of the new national clonal repository: its evaluation and uses. Acta Hortic 124:57–65
White AG, Brewer LR (2002a) Pear breeding in New Zealand. Acta Hortic 587:175–178
White AG, Brewer LR (2002b) The New Zealand pear breeding project. Acta Hortic 596:239–242
Wu J, Wang Z, Shi Z, Zhang S, Ming R, Zhu S, Khan MA, Tao S, Korban SS, Wang H, Chen NJ, Nishio T, Xu X, Cong L, Qi K, Huang X, Wang Y, Zhao X, Wu J, Deng C, Gou C, Zhou W, Yin H, Qin G, Sha Y, Tao Y, Chen H, Yang Y, Song Y, Zhan D, Wang J, Li L, Dai M, Gu C, Wang Y, Shi D, Wang X, Zhang H, Zeng L, Zheng D, Wang C, Chen M, Wang G, Xie L, Sovero V, Sha S, Huang W, Zhang S, Zhang M, Sun J, Xu L, Li Y, Liu X, Li Q, Shen J, Wang J, Paull RE, Bennetzen JL, Wang J, Zhang S (2013) The genome of the pear (*Pyrus bretschneideri* Rehd.). Genome Res 23:396–408
Yamamoto T, Chevreau E (2009) Pear genomics. In: Folta KM, Gardiner SE (eds) Genetics and genomics of rosaceae, plant genetics and genomics: crops and models, vol 6. Springer Science + Business Media, New York, USA, pp 163–186
Zeller W (1978) Field trials on the resistance of pear and apple varieties to fire blight (*Erwinia amylovora*) (natural and artificial infection). Acta Hortic 86:15–23
Zeller W (1990) Test of pome fruit susceptibility to fire blight in the Federal Republic of Germany. In: ECSC-EEC-EAEC. Fire blight of Pomoidae (*Erwinia amylovora* Burrill, Winslow et al.), applied research in Europe. Brussels, Luxembourg, pp 110–115

Functional Genomics

14

Songling Bai and Yuanwen Teng

Abstract

Several closely related species of commercial importance in the genus *Pyrus* are cultivated throughout the world. In eastern Asia, specifically in China, Japan, and Korea, the East Asian pear, including the Chinese white pear (*P. pyrifolia* white pear group, also referred to as *P.* × *bretschneideri*), the Chinese sand pear (*P. pyrifolia*), the Japanese pear (*P. pyrifolia*), the Ussurian pear (*P. ussuriensis*), and the Xingiang pear (*P. sinkiangensis*), is cultivated, while in the rest of the world, the European pear (*P. communis*) is more commonly grown. Whole-genome sequences have been released for both *P.* × *bretschneideri* cv. Dangshansuli (also known to belong to *P. pyrifolia* white pear group) and *P. communis* cv. Bartlett. As a result of these draft pear genome sequences, major advances have been made in pursuing functional genomics studies in pear.

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14.1 Introduction

With the release of draft genome sequences for the Asian pear, *Pyrus* × *bretschneideri* cv. Dangshansuli (Wu et al. 2013a), and the European pear, *P. communis* cv. Bartlett (Chagné et al. 2014), increased efforts have been undertaken to pursue functional genomics studies in pear. Many large-scale studies have identified numerous candidate genes related to various traits of horticultural importance associated with tree growth and development, as well as with various flowering, fruiting, and fruit quality characters (Nashima et al. 2013b; Xie et al. 2013; Wang et al. 2014; Nham et al. 2015; Yang et al. 2015; Reuscher et al. 2016; Zhang et al. 2016; Shi et al. 2017; Wang et al. 2017; Zhang et al. 2017). These studies were followed by more in-depth studies to investigate functions of some of these important genes using various functional genetic analysis approaches (Huang et al. 2015; Jin et al. 2016; Niu et al. 2016; Tuan et al. 2016; Li et al. 2017a).

In this chapter, we will cover genomic databases and tools that have been developed for the pear genome that are critical in pursuing functional genomics studies. This will be followed by a review of recent advances in our knowledge of gene functions related to important horticultural traits of the pear, such as vegetative/reproductive phase transition, grafting, fruit coloration, and development of stone cells, among others.

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https://doi.org/10.1007/978-3-030-11048-2_14

14.2 Databases for Genomic Resources

14.2.1 The Expressed Sequence Tag (EST) Database for Pear

The Genome Database for Rosaceae (GDR, https://www.rosaceae.org) is a resource for expressed sequence tags (ESTs), genome sequences, and data mining tools for various members of the Rosaceae family (Jung et al. 2014). Prior to completion of draft whole-genome sequences for the pear genome, the GDR has included a small set of pear ESTs. The latest version (v5) of this database includes 1760 EST reads, yielding 259 assembled contigs and 964 singlets. The use of this EST database has been limited following the release of whole-genome draft sequences for *Pyrus*.

14.2.1.1 A Whole-Genome Sequence Database for the Chinese White Pear

Wu et al. (2013a) published the first draft genome sequence of the Chinese white pear cv. Dangshansuli (*P.* × *bretschneideri,* also reported to belong to the white pear group of *P. pyrifolia*). The draft genome size of this Asian pear is estimated to be 512 Mb and corresponding to 97.1% of the estimated genome size. This genome sequence database is hosted at the Nanjing Agriculture University (http://peargenome.njau.edu.cn). At present, this database provides genome sequences of this Asian pear, as well as that of the European pear, *P. communis* cv. Bartlett (Chagné et al. 2014). In this database, genome sequences for the Asian pear are assembled into 2103 scaffolds with a total of 42,812 gene loci identified.

Wu et al. (2013a) also published pseudomolecule sequences in $(GIGA)^{n}DB$ (http://gigadb.org/dataset/100083) and assigned predicted gene loci into pseudomolecules. The NCBI database has also presented pear genome data based on publicly available raw sequence data (https://www.ncbi.nlm.nih.gov/genome/12793?genome_assembly_id=40827). This draft genome sequence consists of 2192 scaffolds with 47,086 predicted proteins.

14.2.1.2 A Whole-Genome Sequence Database for the European Pear

Genome sequences of the European pear cv. Bartlett (*P. communis*), published by Chagné et al. (2014), have been submitted to the GDR, which also has several useful applications, such as BLAST and GBrowser, among others (https://www.rosaceae.org/organism/Pyrus/communis). Recently, Li et al. (2017b) have reassembled these sequences, and this dataset has been deposited in yet another database, referred to as 'Bartlett V1.1' (http://peargenome.njau.edu.cn).

14.2.1.3 The KEGG Database

The KEGG database (http://www.kegg.jp/ or http://www.genome.jp/kegg/) covers an encyclopedia of genes and genomes. The primary objective of the KEGG database project is to assign functions to genes and genomes, both at the molecular and at the higher structural/organismal levels. Molecular-level functions are stored in the KO (KEGG orthology) database, wherein each KO is defined as a functional orthologue of genes and proteins (Kanehisa et al. 2017). The KEGG orthology of the Chinese white pear is available based on predicted genes/proteins in NCBI (http://www.kegg.jp/dbget-bin/www_bget?gn:T03446).

14.3 Functional Genomics Studies in Pear

14.3.1 Phase Transition of Annual Growth

Similar to other woody perennial trees, the life cycle of pear is different from that of annual plants. Pear trees have long juvenility periods, and it takes 8–10 years for European pear seedling trees to reach reproductive maturity (Layne and Quamme 1975). Once reproductive maturity

is reached, flower buds are formed on lateral buds, on 2-year-old wood of European pears and on 1-year-old wood of some Asian pears, on an annual basis. Floral bud formation and differentiation for the following growing season are initiated soon after completion of shoot elongation in late spring or early summer (Ito et al. 1999). During the fall season, leaves wilt and drop, and pear trees enter endodormancy during which formed buds are repressed by internal cues and are not capable of sprouting, even under suitable growth conditions until the chilling requirement is fulfilled (Lang et al. 1987). After fulfillment of the chilling requirement, these buds can potentially begin to sprout and grow, but low temperatures during the winter will hinder bud growth (Faust et al. 1997). With elevated temperatures in early spring, buds will expand, proceed to sprout, and bloom; this is followed by development of new leaves, shoot elongation, along with early fruit development (Saito et al. 2015b). Thus, vegetative and reproductive growth proceeds simultaneously within the same year; thereby, annual growth is highly regulated and coordinated and involves many complex regulatory pathways.

14.3.1.1 Induction of Flower Bud Initiation

Floral bud induction is an important event, signaling the beginning of a new reproductive cycle. Although pear is a long-day plant, the detailed floral bud induction pathway, by environmental cues, has not yet been well characterized. It is reported that pear *Flowering Locus T* homologues, *PpFT1a* and *PpFT2a*, are involved in the induction of flower bud initiation, but are not the determinants of flowering (Bai et al. 2017b). Instead, the transcriptional drop in expression of *Terminal Flower Like 1* (*TFL1*) homologues, *PpTFL1-1a* and *PpTFL1-2a*, prior to flower bud initiation, is in fact the primary trigger for flower bud initiation. Furthermore, several hormone-related transcription factors are potentially involved in *PpTFL1*-mediated floral induction (Bai et al. 2017b).

14.3.1.2 The Regulation of Endodormancy

It has been reported that dormancy-associated MADS-box (*DAM*) genes encode members of MADS-box transcription factors that have been implicated to play important roles in dormancy in a mutant peach (*Prunus persica*) genotype (Bielenberg et al. 2008). Subsequently, two research groups have independently identified three pear *DAM* genes (Saito et al. 2013; Niu et al. 2016). Although these two groups have identified the same set of *DAM* genes from two different pear cultivars, they have used different nomenclatures for these genes. As a result, this has created some level of confusion. For example, the *DAM1* gene identified by Niu et al. (2016) is a homologue of *PpMADS13-2,* from *P. pyrifolia,* previously identified by Saito et al. (2013).

DAM genes belong to the flower regulator group of genes that include the *SHORT VEGETATIVE PHASE* and *AGMOUS-LIKE 24* with an *EAR* motif, functioning as transcriptional repressors. Some reports have proposed that pear *DAM* genes repress growth by targeting one of the two *FLOWERING LOCUS T* (*FT)* homologues, specifically that of *PpFT2a* (Fig. 14.1). However, this proposed hypothesis lacks critical evidence; in particular, there is no CArG motif identified in the promoter region of *PpFT2*. Therefore, it cannot yet be excluded that PpDAMs bind to other related motifs to repress transcription of *PpFT2*.

Several lines of evidence have supported the proposal that C-repeated binding factor (CBF) proteins from *P. pyrifolia,* specifically PpCBF2 proteins, directly induce expression of *PpDAM*s by binding to CRT/DRE motifs (Fig. 14.1) (Saito et al. 2015a; Niu et al. 2016). However, expression patterns of *PpCBF2* and *PpDAM* are found to be inconsistent, thereby suggesting that other members of the CBF group or other transcriptional factors (TFs) are potentially involved in the regulation of *DAM* genes (Saito et al. 2015a; Niu et al. 2016).

It has long been known that abscisic acid (ABA) content in plant tissues is significantly

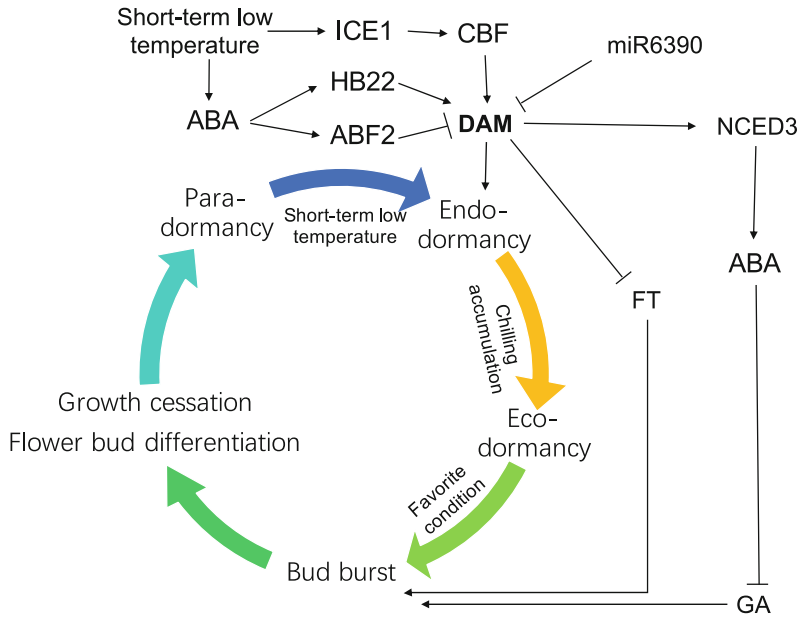

Fig. 14.1 An illustration of the regulation of pear bud dormancy

correlated with endodormancy establishment and release. The expression pattern of *PpCYP707A3,* encoding for cytochrome P450, in *P. pyrifolia* is highly associated with chilling accumulation (Li et al. 2018), and that *PpDAM1* directly upregulates expression of PpNCED3, coding for the enzyme 9-*cis*-epoxycarotenoid dioxygenase (Tuan et al. 2017). Simultaneously, the ABA response element (ABRE)-binding transcription factor, PpAREB1 (=PpABF2), which binds to three ABRE motifs in the promoter region of *PpDAM1*, negatively regulates its activity. In turn, this forms a feedback regulation mechanism between PpDAMs and each of the ABA metabolism and the signaling pathway during endodormancy in pear (Tuan et al. 2017).

Based on degradome sequence data, it is reported that miR6390 targets *PpDAM* genes (Niu et al. 2016). Furthermore, miR6390 and *PpDAM* have shown contrasting expression patterns, thus indicating that miR6390 might play a critical role in dormancy release via degradation of *PpDAM* transcripts (Niu et al. 2016). However, additional studies are required to verify the role of miRNAs in regulating pear tree dormancy.

14.3.2 Fruit Development

As fruit growth and development are of particular interest, there have been increasing functional genomics studies to understand the functional roles of genes involved in fruit development, as well as of various fruit quality traits. In pear, there are several cultivated pear species, including *P. pyrifolia*, *P.* × *bretschneideri, P. sinkiangensis, P. ussuriensis,* and *P. communis,* that produce fruits of commercial importance with varying fruit development and fruit quality traits. For example, *P. ussuriensis* and *P. communis* bear climacteric fruits requiring post-harvest ripening, while fruits of other pear species are readily edible at maturity following harvest. In addition, fruits of *P. communis* pears are mostly gourd-shaped, have soft and smooth flesh with few stone cells, high sugar and acid contents, along with a strong aroma. Likewise, fruits of *P. ussuriensis* usually have good aroma and strong flavor. In contrast, fruits of Asian pears are mostly round in shape, have crisp flesh, high stone cell contents, low aroma and flavor, and with some species having high sugar and low acid contents.

To better understand fruit development characteristics in different pear species, Zhang et al. (2016) compared transcriptomes of developing fruits of five different pear species and identified differentially expressed genes related to fruit quality and development. In addition, several ethylene synthesis genes and polyphenol oxidase-related genes were identified as co-expressed genes, thus suggesting their potential functions during fruit ripening.

Stone cells are peculiar cells in pear fruits. During the development of pear fruits, stone cells are mainly formed following rapid cell division. Fruits of some pear cultivars have high stone cell contents, which significantly influence their quality. Stone cells are particular types of parenchyma cells that differentiate into cells with thickened secondary cell walls that are highly lignified, and referred to as sclerenchyma cells. Zhang et al. (2016) have identified several genes, such as *4CL* (encoding 4-coumarate CoA ligase), *C3H* (encoding *p*-coumarate 3-hydroxylase), *CA5H* (encoding coniferyl aldehyde 5-hydroxylase), and *CAD* (encoding cinnamyl alcohol dehydrogenase) with relatively high levels of expression at early stages of fruit development for all tested pear cultivars. Furthermore, genes regulating hydroxycinnamoyl transferases (HCT), which reduce the H-lignin content, have also been identified and found to be expressed at early stages of fruit development. Specifically, caffeoyl-CoA *o*-methyltransferase (CCOMT)-related genes are specifically expressed in *P. ussuriensis*, and they are likely related to high contents of stone cells in flesh tissues of these fruits. Similarly, Zhang et al. (2017) have reported that by comparing transcriptomes of fruits of two pear cultivars with different stone cell contents, more than 7000 differentially expressed genes have been identified, including many lignin biosynthesis-related genes. These include genes coding for coumaroylquinate 3-monooxygenase (C3H), shikimate O-hydroxycinnamoyl transferase (HCT), ferulate 5-hydroxylase (F5H), cinnamyl alcohol dehydrogenase (CAD), and peroxidase (POD), as well as genes related to carbon metabolism, such as those coding for sorbitol dehydrogenase-like (SDH-like) and ATP-dependent 6-phosphofructokinase (ATP-PFK). Although the detailed regulatory pathway for stone cell formation has not yet been characterized, these large-scale transcriptome data provide solid basis for further studies. For further detailed information on stone cell development, please see Chap. 11 in this volume.

Some physiological and molecular mechanism studies have been conducted to investigate different fruit development characteristics, as well as fruit quality traits. For example, it has been observed that fruit texture is influenced by *ACO* (coding for 1-aminocyclopropane-1-carboxylate oxidase) and then by *XTH* (coding for xyloglucan endotransglucosylase/hydrolase)-related genes, thereby contributing to cell wall disassembly and loosening (Zhang et al. 2016). In another example, it has been found that fruit ripening of the European pear 'Bartlett,' while still hanging on the tree, can be enhanced by spraying trees with ethylene, as this contributes to fruit softening (Murayama et al. 2006). It has since been discovered that endo-*PG* genes play various important roles in many different fruit maturation characteristics (Hiwasa et al. 2004; Murayama et al. 2006). A microarray analysis study has revealed that a cupin family protein gene and two unannotated genes in *P. communis*, but absent in Japanese pear (*P. pyrifolia*), may be involved in the ripening process specific to *P. communis* (Nashima et al. 2013a).

14.3.3 Red Coloration of Fruit

Red pears are attractive, deemed to have better nutritional value, and have gained more consumer preference. To date, red-colored pear cultivars (or sports) have been identified in both Asian pears and European pears.

Development of red coloration depends on accumulation of anthocyanins in peels of pear fruits. Anthocyanin is synthesized in the cytosol and then transported to the vacuole by a glutathione S-transferase (GST) (Tanaka et al. 2008). The biosynthesis of anthocyanin involves several well-characterized enzymes, including

chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), flavonoid 3′-hydroxylase (F3′H), dihydroflavonol 4-reductase (DFR), anthocyanidin synthase (ANS), and UDP-glucose: flavonoid 3-O-glucosyltransferase (UFGT) (Fig. 14.2). Spatial and temporal expression of genes coding for these enzymes are regulated at the transcriptional level by various TFs, particularly those of the well-studied MYB-bHLH-WD40 (MBW) complex, which is composed of MYB, basic Helix-loop-Helix (bHLH), and WD40 (Broun 2005; Hichri et al. 2011). In many horticultural crops, R2R3 MYB proteins have been reported as important TFs for activation of anthocyanin biosynthesis genes (Kobayashi et al. 2004; Takos et al. 2006; Espley et al. 2009; Medina-Puche et al. 2014). Similarly, a set of pear MYB genes, namely *PcMYB10, PyMYB10*, and *PyMYB114*, contribute to the anthocyanin biosynthesis in the fruit peel (Fig. 14.2 and Table 14.1).

Although cultivars with red-colored fruits have been discovered in both European and Asian pears, their genetic regulation of the red coloration is different. For European pear 'Max Red Bartlett,' which is a somatic mutant of 'Bartlett,' the red coloration of fruit peel depends on active transcription of the *PcMYB10* gene, although it is not quite clear as to why *PcMYB10* is the one that is transcribed (Pierantoni et al. 2010). Another study has mapped the red locus to linkage group (LG) 4, a locus different from that of *PcMYB10* (Dondini et al. 2008), thus suggesting an unknown upstream regulator of PcMYB10 corresponds to the main regulator of red coloration of 'Red Bartlett.' In addition, DNA methylation levels of the promoter of *PcMYB10* are correlated with red coloration of 'Max Red Bartlett' (Wang et al. 2013).

For some red European pear cultivars, such as 'Max Red Bartlett,' red coloration peaks during early stages of fruit development, and then fades to red-green at maturity. This reduces the commercial value of these cultivars. Wang et al. (2017) have identified 947 differentially expressed genes by comparing transcriptomes of fruit peels of 'Red Bartlett' and 'Starkrimson.' It has been found that during the red color fading phase of 'Red Bartlett,' the structural gene *LDOX* and six *GST* family genes are downregulated, while *FLS*, *LAC*, *POD*, and five light-responding genes are significantly upregulated. Additionally, 45 genes encoding transcription factors *MYB*, *bHLH*, *WRKY*, *NAC*, *ERF*, and zinc finger have been identified among 947 DEGs. Based on this wealth of information, a detailed regulatory pathway is emerging and under current development.

Traditional Asian pear fruits usually have smooth green (or yellow) and brown-russet skin colors, but in recent years, development of red-colored Asian pear is rapidly increasing. Several genes involved in the regulation of anthocyanin biosynthesis have already been identified (Table 14.1). The red pear cultivar 'Bayuehong' is a progeny of European pear 'Clapp's favorite' and 'Zaosu' pear, and the latter cultivar is a hybrid of 'Pingguoli' (*P. pyrifolia*) and 'Mishirazu' (*P. communis*). 'Bayuehong' develops red color on the sunny side of the fruit peel. Based on genetic analysis, an R2R3 MYB transcription factor, *PpMYB114*, is found to be responsible for regulating red coloration of 'Bayuehong' (Yao et al. 2017). It is reported that PpMYB114 interacts with an ERF transcription factor, PpERF3, and PpbHLH3 to co-regulate anthocyanin biosynthesis (Yao et al. 2017). In another pear cultivar, 'Red Zaosu', a red-colored somatic mutant of 'Zaosu', *PbMYB10b* (=*PpMYB114*) is identified as an activator of the anthocyanin and proanthocyanin pathways, and PbMYB9 is found to be an activator of proanthocyanin, anthocyanin, and flavanol pathways (Zhai et al. 2016). As red color developmental patterns of Asian pears differ from those of European pears (Qian et al. 2013), germplasm resources are deemed highly useful for studying the regulatory mechanism(s) of pear fruit coloration.

There are various approaches for studying functions of genes in pears. For one, transient expression of pear genes can aid in studying functions of these genes. Interestingly, this is widely used for the study of anthocyanin production. In fact, it has been observed that

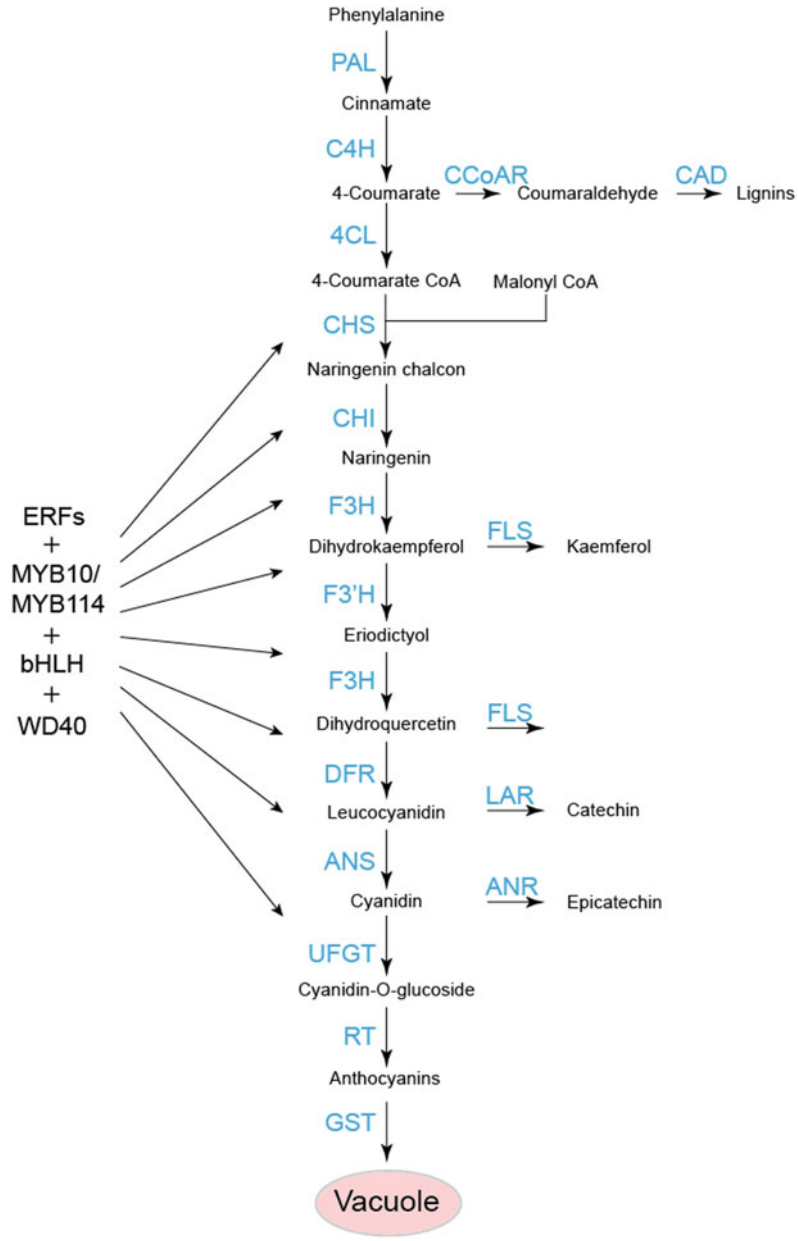

Fig. 14.2 Genes involved in the anthocyanin biosynthesis of pear fruits

overexpression of the *PpMYB114/bHLH/ERF3* complex in tobacco leaves and in strawberry can significantly induce synthesis of anthocyanin. This confirms the important roles of *PpMYB114* and *PpERF3* in the biosynthesis of anthocyanin (Yao et al. 2017). Moreover, transient overexpression of some other genes, including *PbMYB10b*, *PbMYB9*, and *PbMYB3*, several *EFR* genes, and *BBX* family genes in pear fruit alter anthocyanin accumulation in fruit peel (Zhai et al. 2016 and Ni et al. 2019; Bai 2019). Therefore, such transient assays serve as good preliminary tests prior to pursuing development of stable transgenic plants for further testing.

In another approach, virus-induced gene silencing (VIGS) assays have been used for studying gene functions during anthocyanin

Table 14.1 Genes involved in the regulation of anthocyanin biosynthesis in pear

Gene name	Asian/European pear	Gene family	Function(s)	Reference(s)
MYB10	Both	MYB	Directly activates structural genes	Feng et al. (2010), Pierantoni et al. (2010), Wang et al. (2013)
WD40	Asian pear	WD40	Forms the MWB complex	Qian et al. (2017)
bHLH3/33	Asian pear	bHLH	Forms the MWB complex	Qian et al. (2017)
ERF3	Asian pear	AP2/ERF	Interacts with MYB114	Yao et al. (2017)
HY5	Asian pear	bZIP	Directly activates structural genes and MYB10	Tao et al. (2018)
SPL	Asian pear	SPL	Interacts with MYB10 to destabilize the MBW complex	Qian et al. (2017)
miR156	Asian pear	miRNA	Contributes to SPL degradation	Qian et al. (2017)
COP1	Asian pear	F-box	Destabilizes HY5 and MYB10	Tao et al. (2018)
CRY1	Asian pear	Cryptochrome	Destabilizes COP1	Tao et al. (2018)
CRY2	Asian pear	Cryptochrome	Destabilizes COP1	Tao et al. (2018)
MYB114/MYB10b	Asian pear	MYB	Directly activates structural genes	Zhai et al. (2016), Yao et al. (2017)
MYB9	Asian pear	MYB	Directly activates structural genes	Zhai et al. (2016)
PyMADS18	European pear	MADS	Not yet clarified	Wu et al. (2013b)

accumulation. Although the host efficiency of the tobacco rattle virus (TRV) has not yet been well characterized in pear and in other rosaceous plants, the TRV-based VIGS system has been used in many studies. For example, using VIGS, it has been reported that silencing of *PpMYB114*, *PpbHLH*, and *PpERF3* inhibits biosynthesis of anthocyanin in 'Red Zaosu' (Yao et al. 2017).

Bagging of fruit is an efficient and common method used to improve color development in many fruit crops. However, it has been reported that light reactions of Asian and European red pears are quite different. It has been observed that removing bags prior to maturation efficiently induces anthocyanin accumulation in Asian red pear, but not in European pear fruits, thus suggesting presence of different signal transduction pathways in response to light (Qian et al. 2013). RNA-Seq analysis of peels of bagged red pear fruits of *P. pyrifolia* has identified a total of 8870 non-redundant differentially expressed genes, including *HY5*, *CRY-DASH,* and a CO-like transcription factor. This has indicated that other light-responsive transcriptional factors are also involved in anthocyanin accumulation in red Asian pears (Bai et al. 2017a).

14.3.4 Fruit Russet

Fruit russeting is a unique feature of some important commercial pear cultivars, and so this trait is of particular interest. Fruit russeting is characterized by a corky and netlike texture of

the fruit peel. It is known that the peel is made up of cuticle lamellae, epidermal cell layers, and cork cambium, wherein the cork cambium forms a thick-walled cell layer; i.e., cork layer, in a mature pear fruit. In Asian sand pears, *P. pyrifolia*, there are variations in peel colors, including russet, green, and mixtures of russet and green. The russet peel of sand pear is attributed to accumulation of a cork layer. This is an important horticultural trait as the cork layer can protect fruit from external stresses caused by diseases, insects, unfavorable weather conditions, and shipping hazards. Wang et al. (2014) have compared transcriptomes of peel russet formation in two pear genotypes of contrasting peel colors, and have identified candidate genes for suberin, cutin, and wax biosynthesis in russet peels. They have proposed that genes encoding putative cinnamoyl-CoA reductase (CCR), cinnamyl alcohol dehydrogenase (CAD), and peroxidase (POD) are involved in lignin biosynthesis and in pigmentation of russet peels of sand pears.

14.4 Scion–Rootstock Interactions

As with other fruit trees, pear trees are propagated by grafting. Grafting is a practice involving fusion of tissues, vascular tissues, from two genetic systems, the scion and the rootstock. The newly established communication between the rootstock and the scion can induce alterations in traits of the two fused genetic partner systems, including stress tolerance, dwarfing, fruit development, and other phenotypic changes. To date, the detailed mechanism for regulation of one grafting partner by the other partner is not well characterized. A well-accepted hypothesis is that matter exchanges through the vascular bundle play important roles. In the past two decades, macromolecules, including proteins, mRNAs, and siRNAs, have been identified in the phloem sap, shedding light on the study of the mechanism of the mutual effects on the graft system. Among these macromolecules, mRNAs have been the main focus of study thus far.

It has been found that some pear endogenous mRNAs are capable of transport through the phloem, including *NAM/ATAF1/2/CUC2 PROTEIN*, *GA IINSENSITIVE*, *WUSHEL RELATED HOMEOBOX T1,* and *KNOTTED1* (Zhang et al. 2012, 2013; Duan et al. 2015, 2016). This transport involves the movement protein binding protein 2C (Duan et al. 2015) and the polypyrimidine tract binding protein (Duan et al. 2016), which directly bind to mRNAs to assist in the movement. Although the detailed mechanism for the mutual regulation in the graft system, these advances have helped researchers in using transportable mRNAs in pursuing the development of new pear breeding efforts.

14.5 Abiotic Stresses

Abiotic stresses affect growth, development, productivity, as well as various economic traits of pears. To cope with abiotic stress, pears have evolved sophisticated mechanisms to respond to such stresses, ranging from perception of stress signals to modification of physiological and biochemical responses. Unlike model species, there are only a few studies focusing on the function of a particular gene on stress response; this is partially due to lack of reliable approaches for investigating abiotic stress in pear. However, several exciting and conclusive studies have used heterologous ectopic expression approaches, and have obtained some interesting findings, although such approaches may potentially lead to false positive results. For example, ectopic expression of *PubHLH1,* from *P. ussuriensis,* in transgenic tobacco has conferred enhanced tolerance to cold stress (Jin et al. 2016). While, overexpression of *PbrMYB21*, from *P. betulaefolia,* in tobacco has conferred enhanced dehydration and drought tolerance (Li et al. 2017a). Furthermore, using a VIGS assay, it has been further confirmed that *PbrMYB21* positively regulates drought stress (Li et al. 2017a). In addition, ectopic expression of a novel NAC transcription factor, *PbeNAC1,* in tobacco leads to enhanced cold and drought tolerance.

ICE1 is an important gene in the cold-responsive pathway. In a recent study, Huang et al. (2015) have reported that *PuICE1* of *P. ussuriensis* can be upregulated by various abiotic stresses, such as cold and dehydration. Using transgenic tomato plants overexpressing *PuICE1,* it has been demonstrated that this gene confers enhanced tolerance to cold. In fact, the PuHHP1 protein physically interacts with PuICE1 and regulates the transcriptional activity of *PbDREBa*, which further confers tolerance to multiple other stresses (Huang et al. 2015).

All the above reports provide new knowledge of the underlying mechanism(s) of abiotic responses and expand our understanding of the complex signaling network involved in abiotic stress responses.

14.6 Other Traits

Besides the traits introduced above, genes involved in some other important traits have also been studied. For example, the functions of S-RNase and SFBB genes in self-incompatibility reactions have been well characterized in pear. For detailed information on these genes as well as other traits, please look at Chap. 10, as well as other chapters in this volume.

14.7 Conclusions

Functional genomics studies require enriched gene resources and information, as well as advanced technologies. Advances in large-scale technology, such as next-generation sequencing, proteome analysis, and metabolism analysis, have all significantly expanded availability of applicable tools for functional genomics studies in pear. These tools, along with the release of genome sequences of Asian and European pears, have been critical in identifying many candidate genes potentially involved in various traits of interests. However, compared to apple and citrus, pear functional genomics studies are still lagging behind, partly due to lack of reliable approaches to further characterize and analyze gene functions. In recent years, some important genes have been identified by using genetic analysis along with a heterologous transgenic system. Such a strategy will be more likely used in future pear functional genomics studies. On the other hand, there has been success in using a homologous transgenic system in European pear (Freiman et al. 2012). The expanded use of these systems will significantly accelerate our functional genomics studies in pear in the future.

References

Bai S, Sun Y, Qian M, Yang F, Ni J, Tao R, Li L, Shu Q, Zhang D, Teng Y (2017a) Transcriptome analysis of bagging-treated red Chinese sand pear peels reveals light-responsive pathway functions in anthocyanin accumulation. Sci Rep 7(1):63

Bai S, Tao R, Tang Y, Yin L, Ma Y, Ni J, Yan X, Yang Q, Wu Z, Zeng Y, Teng Y (2019) BBX16, a B-box protein, positively regulates light-induced anthocyanin accumulation by activating MYB10 in red pear. Plant Biotechnol J. https://doi.org/10.1111/pbi.13114

Bai S, Tuan PA, Saito T, Ito A, Ubi BE, Ban Y, Moriguchi T (2017b) Repression of *TERMINAL FLOWER1* primarily mediates floral induction in pear (*Pyrus pyrifolia Nakai*) concomitant with change in gene expression of plant hormone-related genes and transcription factors. J Exp Bot 68(17):4899–4914

Bielenberg DG, Wang YE, Li Z, Zhebentyayeva T, Fan S, Reighard GL, Scorza R, Abbott AG (2008) Sequencing and annotation of the evergrowing locus in peach [*Prunus persica* (L.) Batsch] reveals a cluster of six MADS-box transcription factors as candidate genes for regulation of terminal bud formation. Tree Genet Genomes 4(3):495–507

Broun P (2005) Transcriptional control of flavonoid biosynthesis: a complex network of conserved regulators involved in multiple aspects of differentiation in *Arabidopsis*. Curr Opin Plant Biol 8(3):272–279

Chagné D, Crowhurst RN, Pindo M, Thrimawithana A, Deng C, Ireland H, Fiers M, Dzierzon H, Cestaro A, Fontana P, Bianco L, Lu A, Storey R, Knabel M, Saeed M, Montanari S, Kim YK, Nicolini D, Larger S, Stefani E, Allan AC, Bowen J, Harvey I, Johnston J, Malnoy M, Troggio M, Perchepied L, Sawyer G, Wiedow C, Won K, Viola R, Hellens RP, Brewer L, Bus VG, Schaffer RJ, Gardiner SE, Velasco R (2014) The draft genome sequence of European pear (*Pyrus communis* L. 'Bartlett'). PLoS ONE 9(4):e92644

Dondini L, Pierantoni L, Ancarani V, D'Angelo M, Cho KH, Shin IS, Musacchi S, Kang SJ, Sansavini S (2008) The inheritance of the red colour character in European pear (*Pyrus communis*) and its map position

in the mutated cultivar 'Max Red Bartlett'. Plant Breed 127(5):524–526

Duan XW, Zhang WN, Huang J, Hao L, Wang SN, Wang AD, Meng D, Zhang QL, Chen QJ, Li TZ (2016) PbWoxT1 mRNA from pear (*Pyrus betulaefolia*) undergoes long-distance transport assisted by a polypyrimidine tract binding protein. New Phytol 210 (2):511–524

Duan XW, Zhang WN, Huang J, Zhao LM, Ma C, Hao L, Yuan H, Harada T, Li TZ (2015) KNOTTED1 mRNA undergoes long-distance transport and interacts with movement protein binding protein 2C in pear (*Pyrus betulaefolia*). Plant Cell Tiss Org Cult 121(1):109–119

Espley RV, Brendolise C, Chagne D, Kutty-Amma S, Green S, Volz RK, Putterill J, Schouten HJ, Gardiner SE, Hellens RP, Allan AC (2009) Multiple repeats of a promoter segment causes transcription factor autoregulation in red apples. Plant Cell 21 (1):168–183

Faust M, Erez A, Rowland LJ, Wang SY, Norman HA (1997) Bud dormancy in perennial fruit trees: physiological basis for dormancy induction, maintenance, and release. HortScience 32(4):623–629

Feng S, Wang Y, Yang S, Xu Y, Chen X (2010) Anthocyanin biosynthesis in pears is regulated by a R2R3-MYB transcription factor PyMYB10. Planta 232(1):245–255

Freiman A, Shlizerman L, Golobovitch S, Yablovitz Z, Korchinsky R, Cohen Y, Samach A, Chevreau E, Le Roux P-M, Patocchi A, Flaishman MA (2012) Development of a transgenic early flowering pear (*Pyrus communis* L.) genotype by RNAi silencing of PcTFL1-1 and PcTFL1-2. Planta 235(6):1239–1251

Hichri I, Barrieu F, Bogs J, Kappel C, Delrot S, Lauvergeat V (2011) Recent advances in the transcriptional regulation of the flavonoid biosynthetic pathway. J Exp Bot 62(8):2465–2483

Hiwasa K, Nakano R, Hashimoto A, Matsuzaki M, Murayama H, Inaba A, Kubo Y (2004) European, Chinese and Japanese pear fruits exhibit differential softening characteristics during ripening. J Exp Bot 55 (406):2281–2290

Huang X, Li K, Jin C, Zhang S (2015) ICE1 of *Pyrus ussuriensis* functions in cold tolerance by enhancing PuDREBa transcriptional levels through interacting with PuHHP1. Sci Rep 5:17620. https://doi.org/10.1038/srep17620

Ito A, Yaegaki E, Hayama H, Kusaba S, Yamaguchi I, Yoshioka H (1999) Bending shoots stimulates flowering and influences hormone levels in lateral buds of Japanese pear. Hortscience 34(7):1224–1228

Jin C, Huang XS, Li KQ, Yin H, Li LT, Yao ZH, Zhang SL (2016) Overexpression of a bHLH1 transcription factor of *Pyrus ussuriensis* confers enhanced cold tolerance and increases expression of stress-responsive genes. Front Plant Sci 7:441

Jung S, Ficklin SP, Lee T, Cheng CH, Blenda A, Zheng P, Yu J, Bombarely A, Cho I, Ru S, Evans K, Peace C, Abbott AG, Mueller LA, Olmstead MA, Main D (2014) The genome database for Rosaceae (GDR): year 10 update. Nucl Acids Res 42(Database issue):D1237–1244

Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K (2017) KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucl Acids Res 45(D1):D353–D361

Kobayashi S, Goto-Yamamoto N, Hirochika H (2004) Retrotransposon-induced mutations in grape skin color. Science 304(5673):982

Lang GA, Early JD, Martin GC, Darnell RL (1987) Endodormancy, paradormancy, and ecodormancy—physiological terminology and classification for dormancy research. HortScience 22(3):371–377

Layne REC, Quamme HA (1975) Pears. In: Janik J, Moore JN (eds) Advances in fruit breeding. Purdue University Press, West Lafayette, IN, pp 38–70

Li J, Xu Y, Niu Q, He L, Teng Y, Bai S (2018) Abscisic acid (ABA) promotes the induction and maintenance of pear (*Pyrus pyrifolia* white pear group) flower bud endodormancy. Int J Mol Sci 19(1):310. https://doi.org/10.3390/ijms19010310

Li K, Xing C, Yao Z, Huang X (2017a) PbrMYB21, a novel MYB protein of *Pyrus betulaefolia*, functions in drought tolerance and modulates polyamine levels by regulating arginine decarboxylase gene. Plant Biotechnol J 15(9):1186–1203

Li L, Deng CH, Knabel M, Chagne D, Kumar S, Sun J, Zhang S, Wu J (2017b) Integrated high-density consensus genetic map of *Pyrus* and anchoring of the 'Bartlett' v1.0 (*Pyrus communis*) genome. DNA Res 24(3):289–301

Medina-Puche L, Cumplido-Laso G, Amil-Ruiz F, Hoffmann T, Ring L, Rodriguez-Franco A, Luis Caballero J, Schwab W, Munoz-Blanco J, Blanco-Portales R (2014) MYB10 plays a major role in the regulation of flavonoid/phenylpropanoid metabolism during ripening of *Fragaria ananassa* fruits. J Exp Bot 65(2):401–417

Murayama H, Sekine D, Yamauchi Y, Gao M, Mitsuhashi W, Toyomasu T (2006) Effect of girdling above the abscission zone of fruit on 'Bartlett' pear ripening on the tree. J Exp Bot 57(14):3679–3686

Nashima K, Shimizu T, Nishitani C, Yamamoto T, Takahashi H, Nakazono M, Itai A, Isuzugawa K, Hanada T, Takashina T (2013a) Microarray analysis of gene expression patterns during fruit development in European pear (*Pyrus communis*). Sci Hortic 164:466–473

Nashima K, Takahashi H, Nakazono M, Shimizu T, Nishitani C, Yamamoto T, Itai A, Isuzugawa K, Hanada T, Takashina T, Kato M, Matsumoto S, Oikawa A, Shiratake K (2013b) Transcriptome analysis of giant pear fruit with fruit-specific DNA reduplication on a mutant branch. J Jpn Soc Hortic Sci 82(4):301–311

Nham NT, de Freitas ST, Macnish AJ, Carr KM, Kietikul T, Guilatco AJ, Jiang CZ, Zakharov F, Mitcham EJ (2015) A transcriptome approach towards understanding the development of ripening capacity in

'Bartlett' pears (*Pyrus communis* L.). BMC Genomics 16:762

Ni J, Bai S, Zhao Y, Qian M, Tao R, Yin L, Gao L and Teng Y (2019) Ethylene response factors Pp4ER-F24and Pp12ERF96 regulate blue light-induced anthocyanin biosynthesis in 'Red Zaosu' pear fruits byinteracting with MYB114. Plant Mol Biol 99:67–78

Niu Q, Li J, Cai D, Qian M, Jia H, Bai S, Hussain S, Liu G, Teng Y, Zheng X (2016) *Dormancy-associated MADS-box* genes and microRNAs jointly control dormancy transition in pear (*Pyrus pyrifolia* white pear group) flower bud. J Exp Bot 67(1):239–257

Pierantoni L, Dondini L, De Franceschi P, Musacchi S, Winkel BSJ, Sansavini S (2010) Mapping of an anthocyanin-regulating MYB transcription factor and its expression in red and green pear, *Pyrus communis*. Plant Physiol Biochem 48(12):1020–1026

Qian M, Zhang D, Yue X, Wang S, Li X, Teng Y (2013) Analysis of different pigmentation patterns in 'Mantianhong' (*Pyrus pyrifolia* Nakai) and 'Cascade' (*Pyrus communis* L.) under bagging treatment and postharvest UV-B/visible irradiation conditions. Scient Hort 151(2):75–82

Qian M, Ni J, Niu Q, Bai S, Bao L, Li J, Sun Y, Zhang D, Teng Y (2017) Response of miR156-SPL module during the red peel coloration of bagging-treated Chinese sand pear (*Pyrus pyrifolia* Nakai). Front Physiol 8:550

Reuscher S, Fukao Y, Morimoto R, Otagaki S, Oikawa A, Isuzugawa K, Shiratake K (2016) Quantitative proteomics-based reconstruction and identification of metabolic pathways and membrane transport proteins related to sugar accumulation in developing fruits of pear (*Pyrus communis*). Plant Cell Physiol 57(3): 505–518

Saito T, Bai S, Imai T, Ito A, Nakajima I, Moriguchi T (2015a) Histone modification and signaling cascade of the *dormancy-associated MADS-box* gene, *PpMADS13-1*, in Japanese pear (*Pyrus pyrifolia*) during endodormancy. Plant Cell Environ 38 (6):1157–1166

Saito T, Bai S, Ito A, Sakamoto D, Saito T, Ubi BE, Imai T, Moriguchi T (2013) Expression and genomic structure of the dormancy-associated MADS box genes *MADS13* in Japanese pears (*Pyrus pyrifolia* Nakai) that differ in their chilling requirement for endodormancy release. Tree Physiol 33(6):654–667

Saito T, Tuan PA, Katsumi-Horigane A, Bai SL, Ito A, Sekiyama Y, Ono H, Moriguchi T (2015b) Development of flower buds in the Japanese pear (*Pyrus pyrifolia*) from late autumn to early spring. Tree Physiol 35(6):653–662

Shi DQ, Tang C, Wang RZ, Gu C, Wu X, Hu S, Jiao J, Zhang SL (2017) Transcriptome and phytohormone analysis reveals a comprehensive phytohormone and pathogen defence response in pear self-/cross-pollination. Plant Cell Rep 36(11):1785–1799

Takos AM, Jaffé FW, Jacob SR, Bogs J, Robinson SP, Walker AR (2006) Light-induced expression of a *MYB* gene regulates anthocyanin biosynthesis in red apples. Plant Physiol 142(3):1216–1232

Tanaka Y, Sasaki N, Ohmiya A (2008) Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. Plant J 54(4):733–749

Tao R, Bai S, Ni J, Yang Q, Zhao Y, Teng Y (2018) The blue light signal transduction pathway is involved in anthocyanin accumulation in 'Red Zaosu' pear. Planta 248(1):37–48

Tuan PA, Bai S, Saito T, Ito A, Moriguchi T (2017) *Dormancy-associated MADS-box* (*DAM*) and the abscisic acid pathway regulate pear endodormancy through a feedback mechanism. Plant Cell Physiol 58 (8):1378–1390

Tuan PA, Bai SL, Saito T, Imai T, Ito A, Moriguchi T (2016) Involvement of *EARLY BUD-BREAK*, an AP2/ERF transcription factor gene, in bud break in Japanese pear (*Pyrus pyrifolia* Nakai) lateral flower buds: expression, histone modifications and possible target genes. Plant Cell Physiol 57(5):1038–1047

Wang YZ, Dai MS, Zhang SJ, Shi ZB (2014) Exploring candidate genes for pericarp russet pigmentation of sand pear (*Pyrus pyrifolia*) via RNA-seq data in two genotypes contrasting for pericarp color. PLoS ONE 9 (1):e83675

Wang Z, Meng D, Wang A, Li T, Jiang S, Cong P, Li T (2013) The methylation of the *PcMYB10* promoter is associated with green-skinned sport in max red bartlett pear. Plant Physiol 162(2):885–896

Wang ZG, Du H, Zhai R, Song LY, Ma FW, Xu LF (2017) Transcriptome analysis reveals candidate genes related to color fading of 'Red Bartlett' (*Pyrus communis* L.). Front Plant Sci 8:455

Wu J, Wang Z, Shi Z, Zhang S, Ming R, Zhu S, Khan M, Tao S, Korban S, Wang H, Chen N, Nishio T, Xu X, Cong L, Qi K, Huang X, Wang Y, Zhao X, Wu J, Deng C, Gou C, Zhou W, Yin H, Qin G, Sha Y, Tao Y, Chen H, Yang Y, Song Y, Zhan D, Wang J, Li L, Dai M, Gu C, Wang Y, Shi D, Wang X, Zhang H, Zeng L, Zheng D, Wang C, Chen M, Wang G, Xie L, Sovero V, Sha S, Huang W, Zhang S, Zhang M, Sun J, Xu L, Li Y, Liu X, Li Q, Shen J, Wang J, Paull R, Bennetzen J, Zhang S (2013a) The genome of the pear (*Pyrus bretschneideri* Rehd.). Genome Res 23(2):396–408

Wu J, Zhao G, Yang YN, Le WQ, Khan MA, Zhang SL, Gu C, Huang WJ (2013b) Identification of differentially expressed genes related to coloration in red/green mutant pear (*Pyrus communis* L.). Tree Genet Genomes 9(1):75–83

Xie M, Huang Y, Zhang YP, Wang X, Yang H, Yu O, Dai WH, Fang CB (2013) Transcriptome profiling of fruit development and maturation in Chinese white pear (*Pyrus bretschneideri* Rehd). BMC Genom 14:823

Yang YN, Yao GF, Yue WQ, Zhang SL, Wu J (2015) Transcriptome profiling reveals differential gene expression in proanthocyanidin biosynthesis associated with red/green skin color mutant of pear (*Pyrus communis* L.). Front Plant Sci 6:795

Yao G, Ming M, Allan AC, Gu C, Li L, Wu X, Wang R, Chang Y, Qi K, Zhang S, Wu J (2017) Map-based cloning of the pear gene MYB114 identifies an interaction with other transcription factors to coordinately regulate fruit anthocyanin biosynthesis. Plant J 92(3):437–451

Zhai R, Wang ZM, Zhang SW, Meng G, Song LY, Wang ZG, Li PM, Ma FW, Xu LF (2016) Two MYB transcription factors regulate flavonoid biosynthesis in pear fruit (*Pyrus bretschneideri* Rehd.). J Exp Bot 67(5):1275–1284

Zhang JY, Cheng X, Jin Q, Su XQ, Li ML, Yan CC, Jiao XY, Li DH, Lin Y, Cai YP (2017) Comparison of the transcriptomic analysis between two Chinese white pear (*Pyrus bretschneideri* Rehd.) genotypes of different stone cells contents. PLoS ONE 12(10):e0187114. https://doi.org/10.1371/journal.pone.0187114

Zhang MY, Xue C, Xu LL, Sun HH, Qin MF, Zhang SL, Wu J (2016) Distinct transcriptome profiles reveal gene expression patterns during fruit development and maturation in five main cultivated species of pear (*Pyrus* L.). Sci Rep 6:28130. https://doi.org/10.1038/srep28130

Zhang WN, Duan XW, Ma C, Harada T, Li TZ (2013) Transport of mRNA molecules coding NAC domain protein in grafted pear and transgenic tobacco. Biol Plantarum 57(2):224–230

Zhang WN, Gong L, Ma C, Xu HY, Hu JF, Harada T, Li TZ (2012) Gibberellic acid-insensitive mRNA transport in *Pyrus*. Plant Mol Biol Rep 30(3):614–623

Whole-Genome Duplications in Pear and Apple

15

Hao Li, Chien-Hsun Huang and Hong Ma

Abstract

Whole-genome duplications (WGDs) are widespread in angiosperms, and are proposed to have contributed to angiosperm diversification. Pear (*Pyrus*) and apple (*Malus*) belong to the large and diverse Maleae tribe, and their genome sequences have extensive syntenic blocks covering much of the chromosomes, thus providing strong support for WGDs. Comparative analyses further indicate that at least a single WGD is shared by both pear and apple, and it has likely occurred following pear/apple lineage split from that of strawberry (*Fragaria*). Furthermore, phylogenomic analysis of thousands of nuclear genes, from public genome datasets and from over 120 transcriptomic datasets, has uncovered strong evidence of presence of thousands of gene duplicates for a WGD in the ancestor of pear, apple, and of other fleshy-fruit-producing genera of the subtribe Malinae, following divergence of dry-fruit-bearing lineages of Maleae. Moreover, over 1000 gene duplicates from the Malinae WGD have been mapped to syntenic blocks in the apple genome, thus supporting the hypothesis that syntenic blocks found in apple (and pear) have been generated by the Malinae WGD, dated in late Eocene (~38–42 million years ago). Further, nearly two-thirds of gene duplicates, initially retained following the Malinae WGD, have been lost in the apple genome, with relatively rapid losses in early Oligocene. Finally, the Malinae-WGD-generated duplicates are enriched in GO categories for transcriptional regulation, including members of the MADS-box gene family, possibly contributing to the evolution of fleshy fruits in Malinae. There is also supporting evidence for this finding provided by functional analysis of several apple MADS-box genes.

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15.1 Introduction

Pear is one of the oldest and most widespread fruits of the world, and it has been cultivated for more than 3000 years, with thousands of cultivars that are available nowadays (Lombard and Westwood 1987). Fruits are the defining characteristics of angiosperms, and contribute to

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angiosperm evolutionary success by protecting and dispersing seeds. Moreover, fruits are also economically and ecologically important by providing foods and nutrition to humans and to animals. Fruits have a wide variety of morphological types and often exhibit important features that distinguish one species from another (Seymour et al. 2013).

The pear belongs to the angiosperm family Rosaceae, which is a moderately large family with three subfamilies, 16 tribes, ~100 genera, and ~3000 species (Hummer and Janick 2009; Phipps 2014). In many angiosperm families, there are generally only one or few types of morphologically similar fruits. For example, Brassicaceae species (cabbage, radish, and their relatives) produce silique or silicle types of dehiscent dry fruits. Also, members of Fabaceae (such as soybean and peanut), Poaceae (such as rice and corn), and Vitaceae (grape) produce legumes (bean pods), caryopsis (grain), and berries, respectively. On the other hand, Rosaceae species have highly distinctive types of fruits, including fleshy pomes (with a relatively soft core and multiple seeds such as pear and apple), drupes (with a hard central shell and a single seed such as peach, cherry, and plum), dry achene (with a thin wall and a single seed, such as strawberry), and aggregate fruits (such as raspberry and blackberry). Many of the fleshy fruit-bearing species have been domesticated and produce economically important fruits (Potter et al. 2007).

Within Rosaceae, pear and apple belong to a large tribe, known as Maleae, which corresponds to the subfamily Maloideae, as described in early classifications using morphological characters. Maleae consists of more than 30 genera, including *Pyrus* (pear), *Malus* (apple and crabapple), *Docynia*, *Eriolobus*, *Sorbus* (rowan and mountain-ash), *Cydonia* (quince), *Chaenomeles*, *Photinia*, *Rhaphiolepis*, *Eriobotrya* (loquat), *Crataegus* (hawthorn), *Mespilus*, *Amelanchier* (serviceberry), *Vauquelinia*, and *Kageneckia*, among others, and at least 500 species (Schulze-Menz 1964; Xiang et al. 2017) (Fig. 15.1).

As *Pyrus* and *Malus*, among other genera of Maleae, have a basic chromosome number of $x = 17$, it has been proposed, based on morphological characters, that the ancestor of Maleae is derived from allopolyploidization between ancestors of two other subfamilies, the Spiraeoideae ($x = 9$) and the Amygdaloideae ($x = 8$) (Evans and Campbell 2002). However, as of yet, there is no molecular supporting evidence for this proposed hypothesis. Furthermore, all Maleae genera bearing pome-like fleshy fruits form a monophyletic group and are members of the subtribe Malinae. Whereas, the genera of *Vauquelinia*, *Kageneckia*, and *Lindleya* produce dry dehiscent fruits and form early divergent lineages (Potter et al. 2007; Xiang et al. 2017) (Fig. 15.1).

As of to date, whole-genome sequences of at least 11 Rosaceae species have been published, including those of *Pyrus* × *bretschneideri*, *Pyrus communis*, *Malus* × *domestica*, *Prunus avium*, *Prunus mume*, *Prunus persica*, *Fragaria vesca*, *Rosa roxburghii*, *Rosa multiflora*, *Rosa chinensis*, and *Rubus occidentalis* (Velasco et al. 2010; Shulaev et al. 2011; Zhang et al. 2012; Wu et al. 2013; Chagné et al. 2014; Lu et al. 2016; VanBuren et al. 2016; Daccord et al. 2017; Shirasawa et al. 2017; Verde et al. 2017; Nakamura et al. 2018; Raymond et al. 2018). Among these, the Asian pear (*P.* × *bretschneideri*) and apple (*M.* × *domestica*), hereafter referred to as pear and apple, respectively, unless otherwise noted, have been extensively investigated, along molecular and genomic evolution levels, as well as in breeding and cultivation efforts. Genome-wide analyses have provided strong evidence supporting the hypothesis that several whole-genome duplication (WGD) events have occurred during the evolution of Rosaceae, thus facilitating their adaptive radiation (Rousseau-Gueutin et al. 2009; Lo et al. 2010; Considine et al. 2012; Burgess et al. 2014; Fougere-Danezan et al. 2015).

Events of WGDs contribute to the recovery of duplicates of all genes at the same time, thereby resulting in initial doubling of chromosome numbers; however, over time, they are often followed by chromosomal rearrangements and

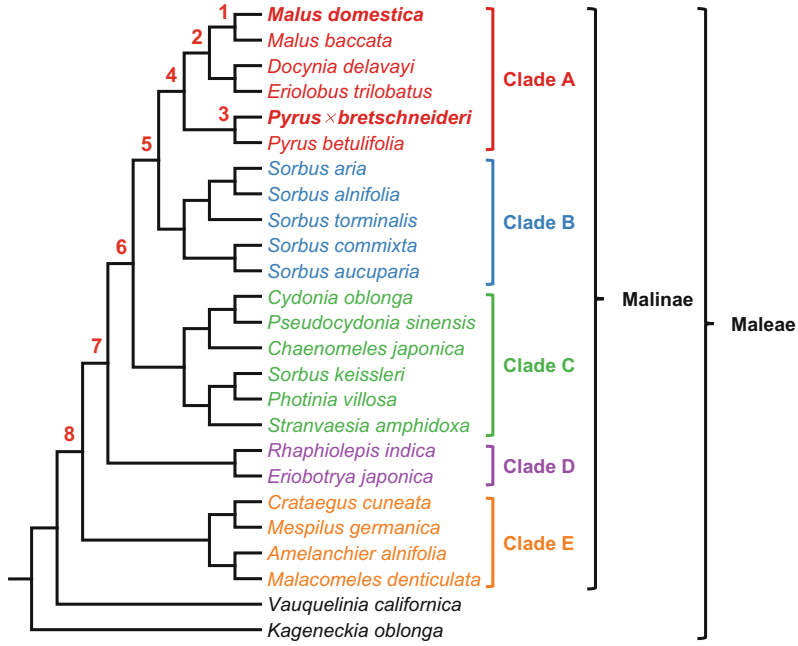

Fig. 15.1 Phylogenetic relationships of 25 Maleae species. The phylogeny is based on a recently published phylogenetic tree of the Rosaceae family using hundreds of nuclear genes from over 120 species (Xiang et al. 2017). The 23 Malinae species used are divided into five clades, Clades A to E, and represented in five different colors. Red colored numbers at nodes indicate ancestor nodes of apple and pear, and progressively with additional genera within Malinae

loss of many duplicate copies (Pontes et al. 2004; Madlung et al. 2005; Albalat and Canestro 2016). Importantly, those retained duplicates provide abundant genetic materials for functional gene evolution, such as subfunctionalization, involving division of original functions into two duplicates (Cusack and Wolfe 2007), neofunctionalization, involving acquisition of a new function in a duplicate copy (Blanc and Wolfe 2004), and gene conservation induced by dosage effects, contributing to increased production of a beneficial gene product (Freeling 2009; Bekaert et al. 2011; Hudson et al. 2011). These various processes contribute to genomic novelty, organismal complexity, speciation, and adaptive radiation (Stebbins 1940; Levin 1983; Soltis et al. 2003; Rieseberg and Willis 2007; Maere and Van de Peer 2010; Mayrose et al. 2011; Arrigo and Barker 2012). Consequently, such changes may allow organisms to benefit from either new ecological opportunities or to respond new environmental challenges (Ohno 1970; Hahn 2009; Maere and Van de Peer 2010; Schranz et al. 2012; Fawcett et al. 2013).

In this chapter, the syntenic evidence for WGDs in published pear and apple genomes will be presented (Wu et al. 2013; Daccord et al. 2017), and this WGD will be linked to one of two WGDs that have likely occurred in the common ancestor of pear, apple, and other members of Malinae (Xiang et al. 2017), hereafter referred to as the Malinae WGD. Interestingly, comparisons of these genomic and phylogenomic/phylotranscriptomic studies have allowed for analysis of the origin of the WGD, as revealed by pear and apple genome sequences. Furthermore, this has also provided valuable information on chromosomal distribution of duplicates in the apple. Subsequently, detailed analyses of gene duplicates from the Malinae WGD have provided new knowledge of patterns of gene retention and losses, as well as of rates of such losses during the evolutionary history of Malinae. Furthermore, comparative

analyses allowed for GO annotation of duplicates in apple and helped in unraveling the evolutionary history of Malinae MADS-box genes that potentially contribute to the development of pome fruits.

15.2 Genome Sequences of Pear and Apple Reveal Extensive Syntenic Evidence for WGD

Previous analyses of chromosome numbers and genome sequences of Rosaceae species have supported suggestions that members of this family must have undergone either one or more WGDs (Dickinson et al. 2007; Velasco et al. 2010; Wu et al. 2013; Chin et al. 2014; Zhao et al. 2016). In particular, it has been proposed, based on chromosome numbers, that there is a WGD event that is shared by Maleae members (Vamosi and Dickinson 2006; Dickinson et al. 2007). Furthermore, analysis of the pear genome has revealed numerous duplicate genes (paralogs), which are aligned in 870 collinear regions of different lengths (Wu et al. 2013), and forming large syntenic blocks covering major portions of chromosomes (Table 15.1). Specifically, $\geq 90\%$ in lengths of each of the following four chromosome pairs are covered by syntenic blocks: Chr03 and Chr11, Chr05 and Chr10, Chr09 and Chr17, as well as Chr13 and Chr16. Moreover, large fragments of chromosomes or chromosomal arms in seven additional pairs are syntenic, including Chr01 and Chr07 (upper region), Chr02 (upper) and Chr15 (middle upper), Chr02 (lower) and Chr07 (upper), Chr04 (lower) and Chr12 (lower), Chr06 (lower) and Chr14 (lower), Chr08 and Chr15 (upper and lower), and Chr12 (upper) and Chr14 (upper) (Table 15.1). Furthermore, a recent high-quality apple genome sequence has also provided strong and convincing support for WGD, with syntenic blocks covering most regions of all 17 chromosomes (Velasco et al. 2010; Daccord et al. 2017), and along with strikingly similar patterns to those detected in the pear genome (Table 15.1).

Wu et al. (2013) performed comparative analysis of pear and apple genome sequences (Velasco et al. 2010) and found that WGD events in pear and apple, supported by extensive syntenic blocks described above, must have occurred in their common ancestor (Wu et al. 2013). Besides this WGD, they also proposed an earlier WGD in pear and apple, which might correspond to a well-known paleohexaploidization event that took place about ~ 140 million years ago (Mya), although it has much less support in pear and apple genomes (Wu et al. 2013). In addition, the strawberry genome seems to lack large-scale within-genome duplication (Shulaev et al. 2011). Furthermore, an analysis of syntenic blocks between pear ($x = 17$) and strawberry ($x = 7$) revealed that there is generally a two-to-one correspondence between chromosomes of pear to those of strawberry (Wu et al. 2013; Chagné et al. 2014; Li et al. 2017). In other words, the proposed older WGD in pear and apple, which could also have been shared by strawberry, is not obvious in the comparative analysis between pear and strawberry genomes, consistent with the relatively weak evidence for this event (Wu et al. 2013). Moreover, an observed ancestral chromosome reconstruction for Rosaceae suggests that the ancestor for this family has nine chromosomes (Wu et al. 2013).

In summary, the above findings in genomes of the pear and apple indicate that these two fruit crop species have similar and extensive duplicated genomic regions that have likely resulted from the same WGD event that has occurred prior to the divergence of pear and apple. Therefore, these resources offer opportunities for unraveling the roles that duplication events of genomes and genes might have played in the evolution of these two tree fruit species. However, these studies have not yet provided a more precise timing of the WGD that is shared by the pear and the apple.

Table 15.1 Summary of synteny blocks between chromosome pairs in apple and pear[a]

Pear Chr. A	Pear Chr. B	Apple Chr. A	Apple Chr. B
Chr01	Chr07 (lower)	Chr01 (upper)	Chr15 (middle lower)
		Chr01 (lower)	Chr07 (lower)
Chr02 (upper)	Chr15 (middle upper)	Chr02 (upper)	Chr15 (middle upper)
Chr02 (lower)	Chr07 (upper)	Chr02 (lower)	Chr07 (upper)
Chr03	Chr11	Chr03	Chr11
Chr04 (upper)	NA	Chr04 (upper)	Chr13 (lower)
		Chr04 (middle)	Chr06 (middle)
Chr04 (lower)	Chr12 (lower)	Chr04 (lower)	Chr12 (lower)
Chr05	Chr10	Chr05	Chr10
Chr06 (upper)	NA	Chr06 (upper)	Chr16 (lower)
Chr06 (lower)	Chr14 (lower)	Chr06 (lower)	Chr14 (lower)
Chr08	Chr15 (upper & lower)	Chr08	Chr15 (upper & lower)
Chr09	Chr17	Chr09	Chr17
Chr12 (upper)	Chr14 (upper)	Chr12 (upper)	Chr14 (upper)
Chr13	Chr16 (upper)	Chr13 (upper)	Chr16 (upper)

[a]Synteny blocks were summarized from previous studies (Wu et al. 2013; Daccord et al. 2017). Blocks cover $\geq$ 90% of chromosome length in both of chromosomes are in red. White and blue backgrounds are used only for indicating different Chromosomes. Chr., chromosome; upper, the upper region of a chromosome; lower, the lower region of a chromosome; middle, the middle region of a chromosome not including either end of the chromosome; middle upper, the middle region of a chromosome adjacent to the upper region of the chromosome; middle lower, the middle region of a chromosome adjacent to the lower region of the chromosome; upper and lower, both of the upper and lower regions of a chromosome, but not including the middle region of the chromosome

15.3 Phylogenomic Analyses of Multiple Species Place Two WGD Events Close to the Origin of Maleae

As mentioned in the previous section, presence of extensive syntenic chromosomal blocks in pear and apple genomes supports incidence of a WGD, which has likely occurred prior to the divergence of these two fruit tree species, but following their split from strawberry (Wu et al. 2013). In order to accurately place the WGD event in the evolutionary history of these two fruit tree species, it is necessary to establish a well-resolved phylogeny of Rosaceae and to analyze sequences of many more members of Rosaceae. Recently, a well-resolved Rosaceae phylogeny has been reconstructed, with highly supported clades for each of the subfamilies and tribes, as well as well-resolved relationships among subfamilies and tribes using hundreds of nuclear genes from 125 transcriptomics and genomic datasets (Xiang et al. 2017). A portion of this newly established phylogeny is presented herein for tribe Maleae and its large subtribe Malinae (Fig. 15.1). In this phylogenetic tree, Malinae is divided into five clades, designated herein as Clades A to E, with pear and apple belonging to Clade A (Fig. 15.1).

Using the new Rosaceae phylogeny as a reference (Xiang et al. 2017), WGD can be detected using phylogenomic analysis of thousands of gene families obtained from many species with available transcriptome datasets (Xiang et al. 2017) (see Fig. 15.2a for the two WGDs detected in Maleae, denoted with circles 1 and 2, and see below for additional description). This phylogenomic approach has been effectively used to detect strong support for incidence of WGDs in common

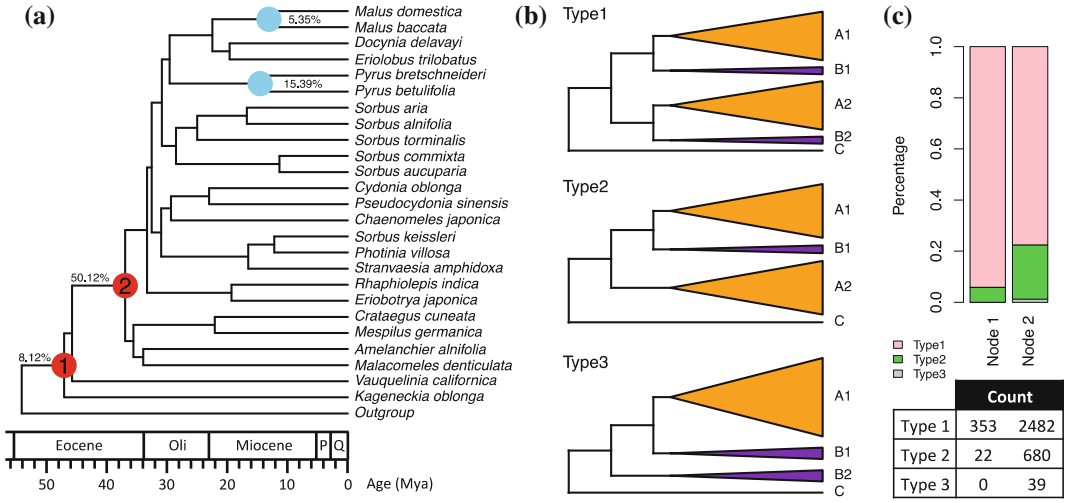

Fig. 15.2 WGDs supported by multiple gene duplication events shared by Maleae members determined by phylogenomic methods. **a** Two WGD events on the backbone of the Maleae phylogeny are marked by red circles, numbered 1 and 2, while two other possible WGD events shared by either pear (*Pyrus*) or apple (*Malus*) genera are noted with blue circles. Notations written below the phylogenetic tree refer to geological ages (million years) and correspond to geological periods estimated by molecular clock analysis (Xiang et al. 2017). **b** Three possible topologies for each of duplicated gene trees are illustrated. **c** By mapping of duplication in gene trees with respect to species trees, numbers of gene duplication events at each node, with strong bootstrap (>50 bp), are determined. The number of counts is then divided into three types for additional detailed information. Both percentages and actual gene pair numbers, of each type at nodes marked by numbers 1 or 2, are shown. These results are obtained from a recent study (Xiang et al. 2017)

ancestors of angiosperms (Jiao et al. 2011), in Asteraceae (Huang et al. 2016), and in other groups (Jiao et al. 2012; Cannon et al. 2015; Li et al. 2015; Yang et al. 2015). It is important to point out that this approach has been deemed reliable (Kellogg 2016). One of the advantages of this phylogenomic approach is the ability to place occurrence of WGD events relative to the species phylogeny and in between nodes of species divergence. In addition, it is possible to estimate the timing of WGD events along a geological time scale, particularly when the species phylogeny corresponds to molecular clock estimates of divergence times. Such information about WGD events can help demonstrate and explain likely effects of WGDs on species and gene function evolution within the context of geological ages.

The basic approach is to construct thousands of gene trees using sequences from whole genomes or transcriptomes, and then to compare topologies of these gene trees with that of the reference species tree, thereby mapping gene duplications present in each gene tree in between nodes on the species tree. When large numbers of gene duplication events are detected before a specific node on a species tree, it is proposed that a WGD event is responsible for incidence of such gene duplications at nearly the same time. To assess the strength of support for such a WGD, topologies of the gene tree adjacent to the node of duplication can be further assessed (Fig. 15.2b). For example, presence of a node with three or more species in the reference species tree allows for classification of observed topologies into three types of gene retention in each of duplicated subclades following the node of interest. These would include the following types, wherein type I retains both gene copies in both large and small subclades; whereas, types II and III lack gene duplicates for whole small or large subclades, respectively (Fig. 15.2b). Among these, type I topology provides the strongest evidence among the three types due to the presence of more genes to infer an accurate phylogeny.

In the phylogenomic analysis of Rosaceae species, a total of 9482 gene family trees with greater than 85% taxon coverage were used to detect gene duplications (Xiang et al. 2017). When a node was found with >50 bootstrap support values along with the same two species found in each of its duplicated subclades, a gene duplication was mapped and counted to the corresponding position of the reference species tree. These findings provided evidence for a duplication event (Fig. 15.2a, circle numbered 1) shared by all Maleae members with 8.12% (375 pairs) of gene families showing duplication, and among these, 7.64% (353 pairs) had strong support (type I) (Fig. 15.2c). Strikingly, a stronger signal was detected for a WGD event (circle numbered 2) shared by members of Malinae (all Maleae members, except for the early divergent *Vauquelinia* and *Kageneckia*), as supported by 50.12% (3201 pairs) of gene families that were duplicated at this node, with 38.86% having a type I topology.

As described in the previous section, the common ancestor of pear and apple must have experienced a WGD event following divergence from strawberry (Wu et al. 2013). Based on analysis of the apple genome, this WGD has been dated 30–45 Mya (Velasco et al. 2010). With more than 120 genomic and transcriptomic datasets, phylogenomic findings support incidence of two WGD events that must have occurred successively near the origin of Maleae, around 38–42 Mya and 48–55 Mya, respectively (Fig. 15.2a). In addition, evidence for incidence of polyploids has also been previously reported to occur within Maleae for members of the genera *Sorbus*, *Crataegus*, and *Amelanchier* (Vamosi and Dickinson 2006; Dickinson et al. 2007). All these members are included in the subtribe Malinae and are represented by the clade marked with number 2 (Fig. 15.2a). Moreover, it is proposed that a recent WGD may have occurred within *Pyrus*, with 15.39% (585 pairs) gene families duplicated before speciation of *P.* × *bretschneideri* and *P. betulifolia* (Xiang et al. 2017); however, further analysis using genomic datasets is needed to confirm occurrence of this event.

15.4 Possible Effects of the Two WGD Events Near the Origin of Maleae on Evolution of Fruit Tree Species

The two WGD events shared by Maleae/Malinae might have facilitated the evolutionary process of these species and contributed to multiple morphological variations of members of Maleae. Recent molecular phylogenetic analyses of Rosaceae have expanded the subfamily Amygdaloideae to include Maleae and others, in addition to peach and plum. The ancestral fruit type of the expanded Amygdaloideae was proposed to be a follicetum with several to many carpels (Xiang et al. 2017). This ancestral fruit type further evolved into one with five carpels for the common ancestor of Maleae and its sister tribe Gillenieae. Subsequently within Maleae, following divergence of dry-fruit producing lineages (i.e., *Kageneckia*), additional changes have likely led to the evolution of fleshy pome fruits. These likely changes in fruit structure include partial 'sinking' of the ovary into the hypanthium and their fusion (Xiang et al. 2017), as well as transformation of the fruit type from one with thin and non-fleshy hypanthium/pericarp to that with fleshy tissues. In Maleae, five carpels were fused together as a coccetum (such as that found in *Vauquelinia*), while the hypanthium became urceolate (cup-like) and further closed-up with carpels, evolving into either partially inferior, such as that of *Crataegus*, or fully inferior ovaries, such as those of pear (*Pyrus*) and apple (*Malus*).

Molecular clock analysis, using nuclear gene sequences with the newly established phylogeny as a reference (Xiang et al. 2017), supports the proposal that the timing of fruit character transitions is correlated with those of WGDs and climate events. Molecular clock estimates indicate that the tribe Maleae has split from Gillenieae ~54 Mya, just after the Paleocene–Eocene boundary, with further incidents of divergence within Maleae beginning soon afterward. The earlier WGD (Fig. 15.2a, circle numbered 1) shared by all Maleae members is

estimated to have occurred in early Eocene, which has been the hottest period since the Cenozoic Era, including both the Paleocene–Eocene Thermal Maximum (PETM) and the Early Eocene Climate Optimum (EECO) (Zachos et al. 2008).

Within Maleae, after the separation of *Kageneckia* (with a follicetum fruit type), the ancestor of *Vauquelinia* and other genera have likely produced the coccetum fruit type, with a short lag period from the early WGD event. Whereas, the second WGD is shared by the fleshy-fruited genera of Maleae (all in Malinae) (Fig. 15.2a, circle numbered 2), and it is estimated to have occurred in late Eocene when the Earth experienced a continuous drop in temperature and humidity. This has been closely followed by a short glaciation period with many extinctions in Europe (Zachos et al. 2001; Hooker et al. 2004). The extremely high percentage (50.12%) of gene pairs retained after the WGD and the rapid taxon separation/diversification after the WGD strongly suggest that duplicate genes have contributed to diversification of Maleae genera. Therefore, it is likely that the new gene copies from the two Maleae WGDs have allowed Maleae members to evolve into species producing new fruit types under selective forces of both dramatic climate changes and interactions with animals/insects feeding on Maleae fleshy fruits. See below for additional discussion of genes affected by WGDs.

15.5 Chromosome Distribution of Malinae-WGD-Derived Duplicated Genes in Apple

Phylogenomic analysis of thousands of gene trees with sequences from pear, apple, and multiple other members of Maleae has provided strong support for presence of a WGD event shared by members of Malinae (Xiang et al. 2017) (Fig. 15.2a). However, is this WGD the same as the one revealed by extensive syntenic blocks in pear and apple genomes? To address this question, chromosomal distribution of 2985 gene families has been evaluated for duplication at the node for Malinae (Xiang et al. 2017). For this analysis, a gene family is defined as a group of homologous genes derived from the same ancestral gene after divergence of Malinae from its sister lineage, *Vauquelinia*. These gene families have a duplication detected just before the node of Malinae, thus supporting incidence of a Malinae WGD (Fig. 15.2a, circle numbered 2).

To detect chromosome distribution of the Malinae-WGD-derived gene duplicates in apple, we have analyzed 1043 gene families, each with an apple gene in each of two duplicated clades. It is revealed that longest synteny chromosomal blocks, described above in Sect. 15.2, also contain the most duplicated gene pairs derived from the Malinae WGD (Fig. 15.3). For example, 120 gene families contain syntenic gene pairs on Chr05 and Chr10, 102 on Chr09 and Chr17, 90 on Chr03 and Chr11, and 90 on Chr13 and Chr16. Generally, the more the synteny blocks cover a chromosome, the more duplicated gene pairs are detected in these blocks, as illustrated by the above-mentioned four chromosome pairs. In contrast, chromosome pairs with lower coverage by synteny blocks also contain fewer pairs of duplicates from the Malinae WGD. Most duplicated genes, a total of 812 pairs, are located within synteny blocks between two different chromosomes. However, some duplicated gene pairs are located within the same chromosome, e.g., Chr05 and Chr05. This latter finding could be attributed to either genome rearrangement or some other events that may have occurred following the Malinae WGD event. This deserves further analysis to achieve a better understanding of this observed phenomenon.

Furthermore, analysis of a recent apple genome sequence (Daccord et al. 2017) has detected gene numbers and duplicated gene pairs in synteny blocks between different chromosome pairs of apple, as presented in Table 15.2. These results reveal that, despite chromosome rearrangements and additional gene duplications, WGD-derived duplicated gene pairs identified in each synteny block account for about 30–50% of all genes within the same synteny block (Table 15.2). For example, Chr03 has 2529 genes and Chr11 has 2728 genes, while 1180

Table 15.2 Gene numbers and duplicated gene pair numbers in synteny blocks in apple[a]

Apple Chr. A	Gene # in Chr. A	Apple Chr. B	Gene # in Chr. B	Gene pair #
Chr01 (upper)	325	Chr15 (middle lower)	301	128
Chr01 (lower)	1478	Chr07 (lower)	1509	781
Chr02 (upper)	1710	Chr15 (middle upper)	1421	849
Chr02 (lower)	1060	Chr07 (upper)	1084	383
Chr03	2529	Chr11	2728	1180
Chr04 (upper)	401	Chr13 (lower)	88	35
Chr04 (middle)	292	Chr06 (middle)	254	94
Chr04 (lower)	1320	Chr12 (lower)	1363	701
Chr05	3166	Chr10	2961	1461
Chr06 (upper)	177	Chr16 (lower)	156	63
Chr06 (lower)	1433	Chr14 (lower)	1314	773
Chr08	2162	Chr15 (upper & lower)	2074	1078
Chr09	2515	Chr17	2444	1116
Chr12 (upper)	933	Chr14 (upper)	867	394
Chr13 (upper)	2308	Chr16 (upper)	2285	1322

[a]Synteny blocks were summarized from a previous study (Daccord et al. 2017). The second column (Gene # in Chr. A) indicates the total gene number in the synteny block shown in the first column. The fourth column (Gene # in Chr. B) indicates the total gene number in the synteny block shown in the third column. The fifth column (Gene pair #) indicates the duplicated gene pair number in the synteny block shown in the row. Synteny blocks and the corresponding duplicated gene pair numbers were identified by MCScanX (Wang et al. 2012). Other notes are same as Table 15.1

gene pairs support synteny between these two chromosomes, they account for 46.7% of genes present on Chr03 and for 43.3% of those on Chr11. Chromosome pairs with higher synteny block coverage have most of the duplicated gene pairs, such as Chr05 and Chr10 (1461), Chr13 and Chr16 (1322), Chr03 and Chr11 (1180), and Chr09 and Chr17 (1116). The number of gene pairs detected between syntenic blocks in the apple genome is much larger than the 2985 gene families with duplication from the Malinae WGD, as revealed by phylogenomic analysis of multiple species in the Maleae tribe. The reason for this observed difference is likely due to an incomplete transcriptome sequencing used to obtain gene sequences for most of the species included in the analysis, and criteria used for at least 85% species coverage of gene families, as well as other requirements limiting the number of genes used in this analysis. Nevertheless, the wide distribution of most duplicates in apple, from the Malinae WGD, detected in apple syntenic regions (Fig. 15.3a) suggests that syntenic regions are the result of the Malinae WGD. To test this hypothesis, the average Ks value (ratio of observed synonymous changes to possible synonymous changes used as a measure of evolutionary age) between paralogs is estimated. For 90 pairs of apple genes on Chr03 and Chr11, detected by phylogenomic analysis and due to the Malinae WGD, the Ks value is estimated to be 0.28, which is very close to the Ks value of 0.24 for all apple paralogs (1180 pairs) found between Chr03 and Chr11. This supports the hypothesis that these two types of paralogs are probably generated by the same WGD event.

As mentioned above, among 2985 gene families with two duplicated Malinae clades, 1043 gene families have two duplicates in the apple genome, but the remaining 1942 gene families, with an ancestral Malinae duplication, must have undergone loss of at least one duplicate in apple. Furthermore, 947 gene families have retained one duplicate in the apple genome. Thus, their

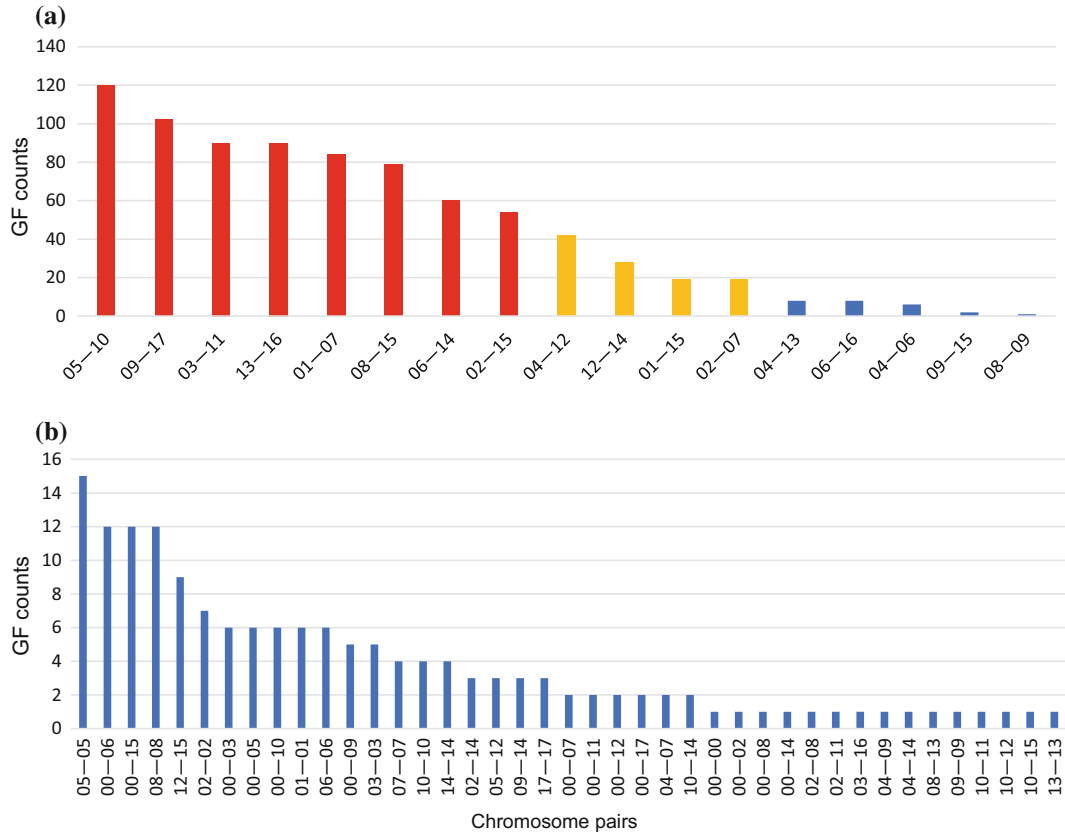

Fig. 15.3 Counts of gene families of Malinae-ancestor-derived duplicated apple gene pairs in corresponding chromosome pairs. Data of chromosome synteny blocks are derived from a recently published apple genome (Daccord et al. 2017). A total of 58 chromosome pairs are shown. **a** 17 chromosome pairs that are supported by synteny analyses in the previous study, and **b** 41 chromosome pairs that are not supported by synteny analyses in the previous study. Red color, synteny blocks cover $\geq$ 90% of chromosome length in both chromosomes (see Table 15.1); yellow color, synteny blocks cover <90%, but $\geq$30% of chromosome length in both chromosomes; blue color, within one chromosome or synteny blocks cover <30% of chromosome length in both chromosomes. GF, gene family

chromosome distribution is investigated. If the gene loss rate is proportional to chromosome size, longer chromosomes with more genes should have more of these 947 single-copy genes. Based on the physical map of the apple, Chr15 is the longest among all 17 chromosomes (Daccord et al. 2017), and it is found to carry most of the single-copy genes (Fig. 15.4). However, other relatively long chromosomes, such as Chr05 and Chr13, are found to have similar numbers of single-copy genes, when compared to those found on shorter chromosomes, such as Chr10 and Chr07 (Fig. 15.4). Therefore, loss of duplicates derived from the Malinae WGD must have been uneven among different chromosomes of the apple genome.

15.6 Retention of Duplicates and Their Loss Rates During the Evolution of Pear and Apple

The pear and apple are closely related, belonging to the same small clade, when compared with other genera in Malinae (Fig. 15.1) (Xiang et al.

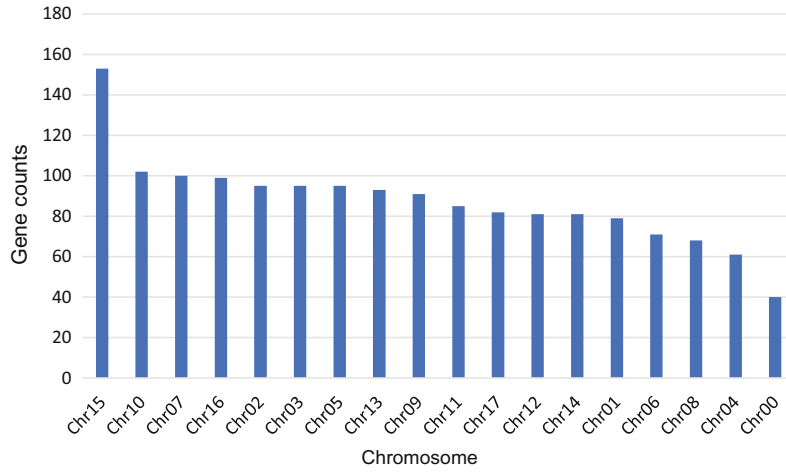

Fig. 15.4 Chromosome distribution of retained genes in 947 gene families with one duplicate retained in apple. Every gene in each orthogroup is accounted for. Gene location data are derived from a recently published apple genome (Daccord et al. 2017)

2017). Therefore, distribution of duplicates on chromosomes of pear, which has undergone the same Malinae WGD as that of apple, is probably similar to that found in apple. As almost two-thirds (1942) of the total (2985) detected gene duplicates in Malinae have lost at least one copy in apple, it would be of interest to determine the evolutionary timeline of these losses in pear and apple. Using sequence datasets generated for many genera in Malinae (Xiang et al. 2017), we have investigated the retention/loss number and loss rate of duplicates during different periods of evolution. Those duplicates found in apple and pear, as well as those detected at eight ancestral nodes, Nodes 1 to 8 (Fig. 15.1), wherein Node 8 represents the most recent common ancestor of Malinae, are presented in Tables 15.3 and 15.4. For any specific node, and if any descendant lineage contains a duplicate, then it is assumed that the node would also have this gene. On the other hand, if none of descendant lineages of a node has a specific duplicate, then it is assumed that the node lacks a copy. These findings have revealed that losses are distributed into eight successive periods along the backbone, from the Malinae ancestor to the extant pear and apple (Tables 15.3 and 15.4).

Among 2985 gene families with two duplicates in the Malinae ancestor (Node 8 in Fig. 15.1), 886 gene families have two detected duplicates in pear and 1043 in apple, accounting for 29.7 and 34.9% of the total, respectively. Following analysis of gene families having 2, 1, or 0 duplicate(s) in pear and/or apple, it is determined that 2106 gene families (70.6% of the total) have at least one detected duplicate in pear and/or apple, while only 215 gene families have no detected duplicate in both pear and apple (Fig. 15.5). This suggests that the vast majority of such genes have important functions in pear and/or apple. Furthermore, as about two-thirds of all 2985 families have likely experienced gene loss during the evolution of pear and apple, 70.3% in pear and 65.1% in apple, a single gene copy might be sufficient for undertaking their functions in pear and apple. It is likely that, as domesticated species, pear and apple might have experienced relaxed selection pressure under human cultivation and might have lost some of those genes retained in wild relatives in other Malinae genera.

We have further analyzed the rate of duplicate gene loss over time during the period of evolution from the Malinae ancestor (Fig. 15.1; Node 8) to extant pear and apple. Those gene families with losses have been divided into two types. In one type, 'one-duplicate loss' refers to events wherein a duplicate number has changed between two adjacent nodes from either 2 to 1 or from 1 to 0; and a second type, 'two-duplicate loss' refers to events wherein a duplicate number has changed from 2 to 0 between two adjacent nodes (Fig. 15.6a). The average loss rates of 'one-duplicate loss' and 'two-duplicate loss'

Table 15.3 Summary of gene families with duplicates lost in any of the 6 nodes during evolution of pear[a]

Rest # of duplicates	Node 8	Node 7	Node 6	Node 5	Node 4	Node 3	Pear	GFs #
No duplicate lost	2	2	2	2	2	2	2	886
Lost after Node 3	2	2	2	2	2	2	1	490
	2	2	2	2	2	2	0	53
Lost after Node 4	2	2	2	2	2	1	1	603
	2	2	2	2	2	1	0	161
	2	2	2	2	2	0	0	95
Lost after Node 5	2	2	2	2	1	1	1	309
	2	2	2	2	1	1	0	67
	2	2	2	2	1	0	0	70
	2	2	2	2	0	0	0	27
Lost after Node 6	2	2	2	1	1	1	1	89
	2	2	2	1	1	1	0	26
	2	2	2	1	1	0	0	15
	2	2	2	1	0	0	0	10
	2	2	2	0	0	0	0	10
Lost after Node 7	2	2	1	1	1	1	1	26
	2	2	1	1	1	1	0	3
	2	2	1	1	1	0	0	5
	2	2	1	1	0	0	0	1
	2	2	1	0	0	0	0	0
	2	2	0	0	0	0	0	0
Lost after Node 8	2	1	1	1	1	1	1	21
	2	1	1	1	1	1	0	10
	2	1	1	1	1	0	0	1
	2	1	1	1	0	0	0	5
	2	1	1	0	0	0	0	1
	2	1	0	0	0	0	0	1
	2	0	0	0	0	0	0	0
								Total: 2985

[a]Data here are derived from the previous WGD results (Xiang et al. 2017). Different background colors are used only for indicating different loss time. Blue, gene families retained 2 duplicates in the ancestor at this node; green, gene families retained 1 duplicate in the ancestor at this node; red, gene families retained 0 duplicate in the ancestor at this node. GFs, gene families

events are 114.5 and 9.3 per million years, respectively. Furthermore, both 'one-duplicate loss' and 'two-duplicate loss' events have higher average rates in the period between Nodes 5 and 4 than those in other periods (Fig. 15.6b; orange bars). The timing of these duplicate loss events between Nodes 5 and 4 is estimated to be about 30–31 Mya, corresponding to early Oligocene (Xiang et al. 2017). Geological studies have indicated that during this time period, global temperature and humidity have become steady following the dramatic drop in late Eocene, as mentioned in a previous section. In addition, this period coincides with the expansion of angiosperms (Zachos et al. 2001; Hooker et al. 2004), which is consistent with evolution of highly diverse Malinae genera.

Previous analyses have also revealed that highly redundant gene pairs must have undergone either relatively less negative selection or neutral selection, and are usually lost more rapidly than more divergent gene pairs during the early period following duplication (Ohno 1970; Lynch and Force 2000; Conant and Wolfe 2008; Li et al. 2016). Taken together, these findings suggest that many duplicated gene pairs might have experienced limited diversification following duplication, thereby retaining two partially redundant copies in the Malinae ancestor of pear and apple (Fig. 15.1; Node 8). Subsequently, many such duplicates must have been lost quickly between Nodes 5 and 4, but less rapidly during other periods, thereby facilitating likely adaptation of different genera to various new environments.

Table 15.4 Summary of gene families with duplicates lost in any of the 7 nodes during evolution of apple[a]

Rest # of duplicates	Node 8	Node 7	Node 6	Node 5	Node 4	Node 2	Node 1	Apple	GFs* #
No duplicate lost	2	2	2	2	2	2	2	2	1043
Lost after Node 1	2	2	2	2	2	2	2	1	357
	2	2	2	2	2	2	2	0	53
Lost after Node 2	2	2	2	2	2	2	1	1	377
	2	2	2	2	2	2	1	0	93
	2	2	2	2	2	2	0	0	45
Lost after Node 4	2	2	2	2	2	1	1	1	213
	2	2	2	2	2	1	1	0	63
	2	2	2	2	2	1	0	0	35
	2	2	2	2	2	0	0	0	9
Lost after Node 5	2	2	2	2	1	1	1	1	324
	2	2	2	2	1	1	1	0	61
	2	2	2	2	1	1	0	0	39
	2	2	2	2	1	0	0	0	22
	2	2	2	2	0	0	0	0	27
Lost after Node 6	2	2	2	1	1	1	1	1	96
	2	2	2	1	1	1	1	0	18
	2	2	2	1	1	1	0	0	10
	2	2	2	1	1	0	0	0	6
	2	2	2	1	0	0	0	0	10
	2	2	2	0	0	0	0	0	10
Lost after Node 7	2	2	1	1	1	1	1	1	22
	2	2	1	1	1	1	1	0	5
	2	2	1	1	1	1	0	0	6
	2	2	1	1	1	0	0	0	1
	2	2	1	1	0	0	0	0	1
	2	2	1	0	0	0	0	0	0
	2	2	0	0	0	0	0	0	0
Lost after Node 8	2	1	1	1	1	1	1	1	20
	2	1	1	1	1	1	1	0	5
	2	1	1	1	1	1	0	0	6
	2	1	1	1	1	0	0	0	1
	2	1	1	1	0	0	0	0	5
	2	1	1	0	0	0	0	0	1
	2	1	0	0	0	0	0	0	1
	2	0	0	0	0	0	0	0	0
									Total: 2985

[a]Data here are derived from the previous WGD results (Xiang et al. 2017). Different background colors are used only for indicating different loss time. Blue, gene families retained 2 duplicates in the ancestor at this node; green, gene families retained 1 duplicate in the ancestor at this node; red, gene families retained 0 duplicate in the ancestor at this node. GFs, gene families

15.7 Gene Ontology Annotations of Duplicated Genes and Evolutionary History of MADS-Box Genes Related to Fruit Development in Pear and Apple

To gain a better understanding of functions of duplicated genes from the Malinae WGD, we have further assessed Gene Ontology (GO) annotations of 2452 gene families retaining at least one duplicate in apple, with one representative apple gene from each family. Among these gene families, 1294 have annotation information in agriGO v2 (Tian et al. 2017). Interestingly, among 'molecular function' categories, five significant GO terms have been detected. These are related to catalytic activity (GO:0003824) and binding (GO:0005488), particularly phosphatase activity (GO:0016791), transcription factor activity, and RNA binding (GO:0008135) (Fig. 15.7). Furthermore, among 'biological process' categories, 23 GO significant terms have been detected, and most are related to cellular process (GO:0009987) and metabolic process (GO:0008152) (Fig. 15.8). However, no significant term has been detected within the 'cellular

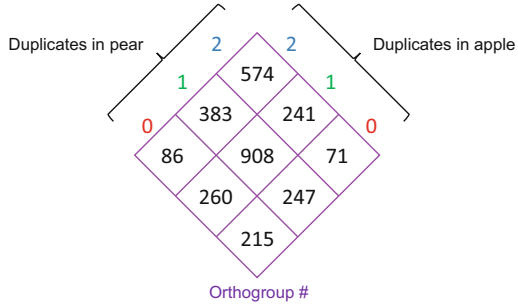

Fig. 15.5 Counts of gene families with either 2, 1, or 0 duplicate(s) present in genomes of pear and apple. Numbers written outside of the purple box represent retained duplicate(s) in either pear or apple. Whereas, numbers written within the purple box represent number of gene families with corresponding duplicates present in pear and apple, as indicated by numbers written outside of the box. The colors used to indicate either 2, 1, or 0 number of duplicates are the same as those used in Tables 15.3 and 15.4

component' category. These findings suggest that gene families supporting the Malinae WGD include many genes homologous to those known to be involved in various transcription regulatory networks, cellular processes, as well as metabolic processes, and potentially play important roles in the adaptation of Malinae species. Duplicates from the Malinae WGD could have diverged sufficiently to either have different expression patterns or even gain new functions. In turn, this has allowed different lineages represented by pear, apple, and other genera to adapt to new environments during their evolution.

Anatomical structures of pome fruits of pear, apple, and of other Malinae genera are derived from fusion of the ovary with a floral tube (hypanthium) consisting of lower portions of sepals, petals, and stamens (Pratt 1988). In several plant

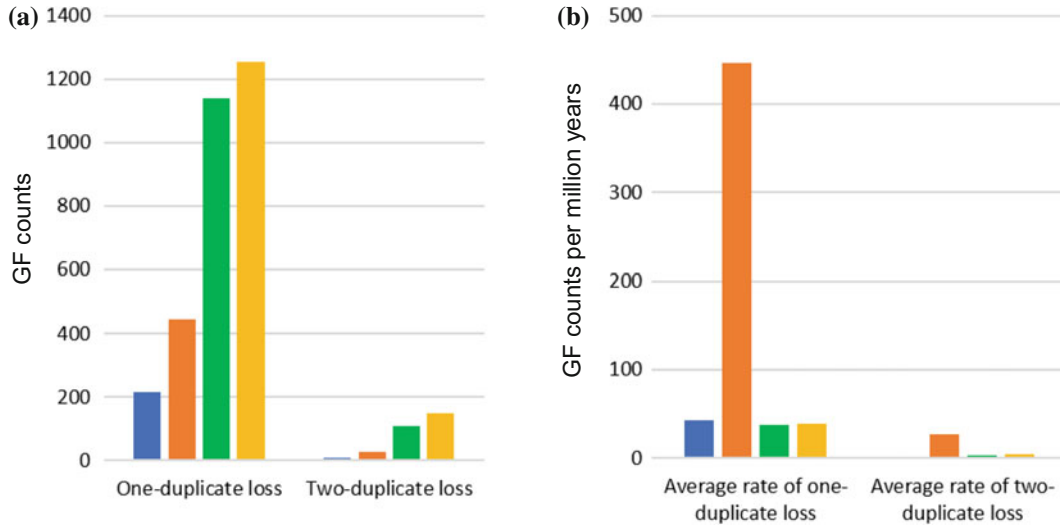

Fig. 15.6 Counts of gene families with either one or two apple/pear duplicate(s) lost between two adjacent ancestral nodes (**a**) and rates of their loss per million years (**b**). A 'one-duplicate loss' refers to an event wherein duplicate numbers have changed either from 2 to 1 or from 1 to 0 between two adjacent nodes. A 'two-duplicate loss' refers to an event wherein a duplicate number changed from 2 to 0 between two adjacent nodes. Blue color, gene loss events that must have occurred between Nodes 8 and 5, as shown in Fig. 15.1, including orthogroups with duplicate loss during transition from either Node 8 to Node 7 (36–34 Mya), Node 7 to Node 6 (34–33 Mya), or Node 6 to Node 5 (33–31 Mya). Orange color, gene loss events occuring between Nodes 5 and 4 (31–30 Mya). Green color, gene loss events occurring between Node 4 and apple, including orthogroups with duplicate loss during transition from either Node 4 to Node 2 (30–22 Mya), Node 2 to Node 1 (22–12 Mya), or Node 1 to apple (12 Mya). Yellow color, gene loss events occurring between Node 4 and pear, including orthogroups with duplicate loss during transition from Node 4 to Node 3 (30–14 Mya) and from Node 3 to pear (14 Mya). Ages of each node are derived from an estimation presented in our previous study (Xiang et al. 2017). GF, gene family

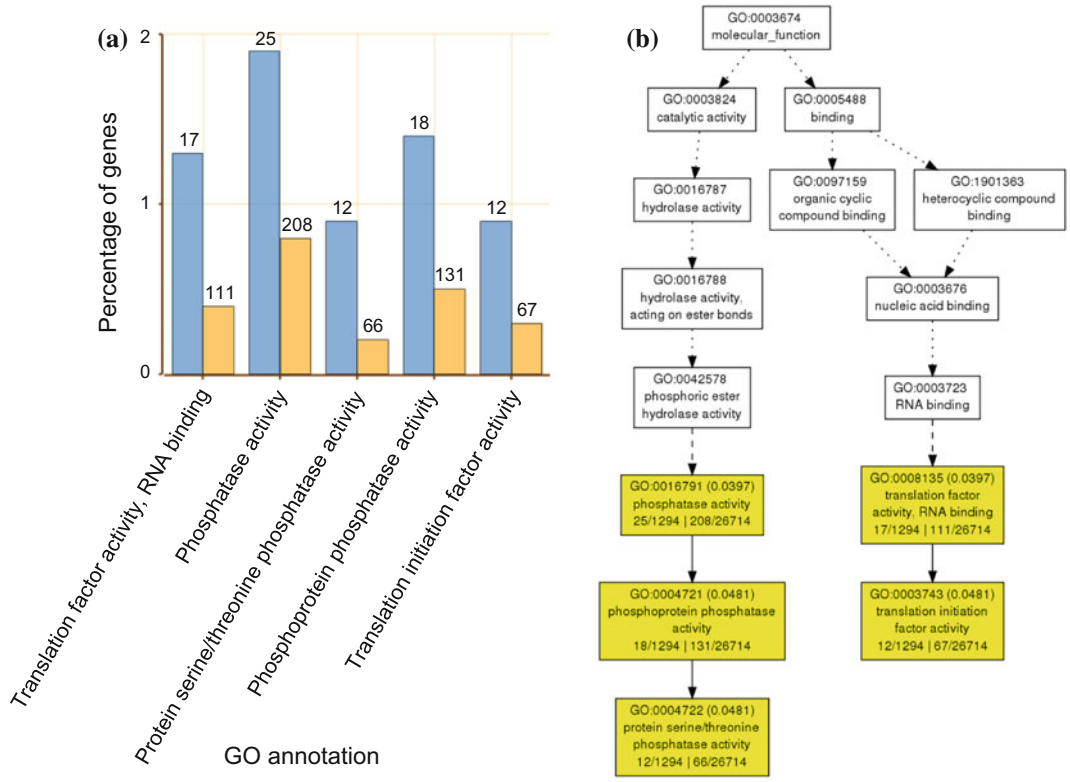

Fig. 15.7 GO analysis of 1294 apple genes from 2452 gene families among 'molecular function' categories. GO annotation results and the hieratical graph are derived from agriGO v2 (Tian et al. 2017). **a** A GO abundance chart of all GO significant terms. Blue color, percentage of genes in 1294 apple genes; orange color, percentage of genes in all apple genes. Numbers written above each bar represent gene number in either 1294 input apple genes or in all 26,714 apple genes in agriGO v2. **b** A hieratical graph of GO annotations. Yellow-green colored boxes indicate five GO significant terms shown in (**a**)

species, including *Arabidopsis thaliana* and *Antirrhinum majus*, among others, flower and fruit development are controlled by members of the MADS-box gene family. Specifically, *AGAMOUS* (*AG*), *SHATTERPROOF1/2* (*SHP1/2*), and *FRUITFULL* (*FUL*) [related to *APETALA1* (*AP1*)] genes are important for ovary and fruit development in several plant species (Seymour et al. 2013). Molecular studies have revealed that these genes are also important for fruit development in apple. First, apple genes closely related to *AG* and *FUL* are differentially expressed during development of the pome fruit (Yao et al. 1999). Moreover, genes related to *SHORT VAGETATIVE PHASE* (*SVP*), contributing to enlarged sepals when overexpressed, are expressed in the apple fruit (Masiero et al. 2004). To determine whether or not the Malinae WGD has influenced the copy number of these MADS-box genes during evolution of apple and pear, we have reconstructed phylogenetic relationships of *FUL*, *AP1*, *AG*, *SHP*, and *SVP* genes. Sequences of these genes have been obtained from 28 Rosaceae species, including 25 members of Maleae (Fig. 15.1), *Prunus mume*, *Prunus persica*, and *Fragaria vesca*, as well as four other eudicots, used as outgroups, including *Glycine max*, *Medicago truncatula*, *Arabidopsis thaliana*, and *Brassica rapa*. Phylogenetic analyses of these genes have indicated that duplicates of *FUL*, *AP1*, *AG*, *SHP*, and *SVP* genes, due to the Malinae WGD, are often retained in apple and/or pear, as well as in other Malinae species (Fig. 15.9). This has suggested that the

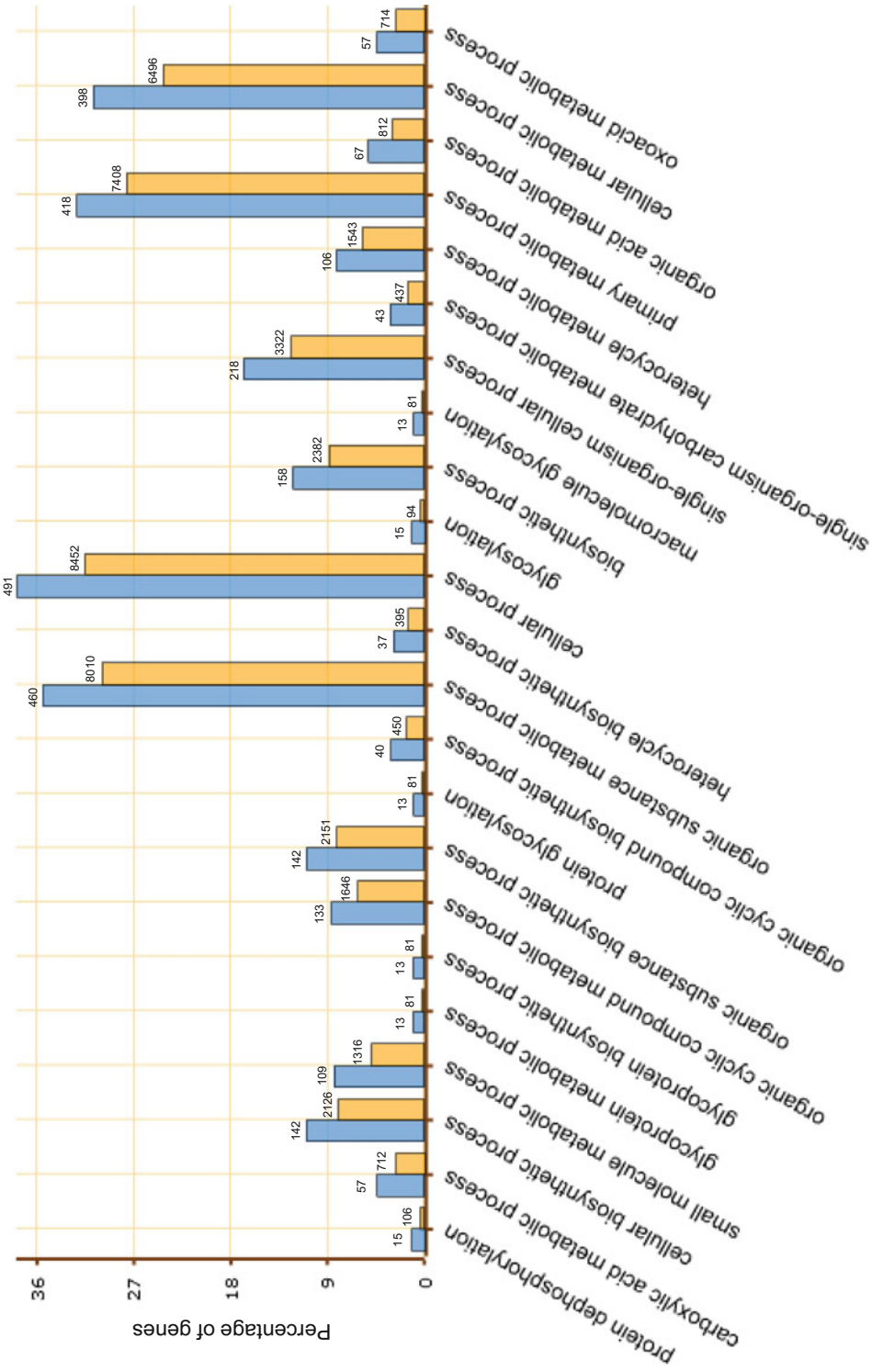

Fig. 15.8 GO abundance chart of all significant terms of 1294 apple genes among 'biological process' categories. GO annotation results and a hieratical graph are derived from agriGO v2 (Tian et al. 2017). Blue color, percentage of genes in 1294 apple genes; orange color, percentage of genes in all apple genes. Numbers above each of the bars refer to gene numbers in either 1294 input apple genes or in all 26,714 apple genes in agriGO v2

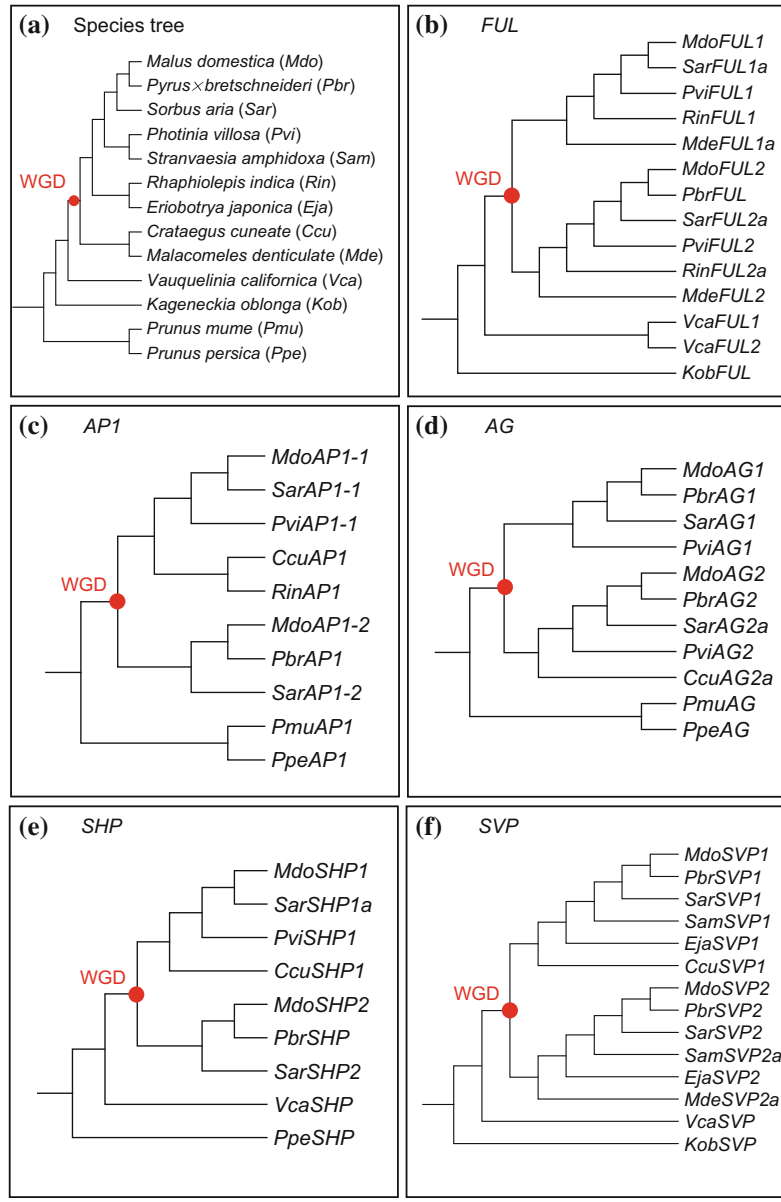

Fig. 15.9 Evolutionary history of *FUL*, *AP1*, *AG*, *SHP*, and *SVP* MADS-box genes in Maleae. The phylogeny is based on a recent study (Xiang et al. 2017). **a** Species tree of 13 Rosaceae species, including 11 Maleae species. **b–f** Gene trees of *FUL*, *AP1*, *AG*, *SHP*, and *SVP*, respectively. Red circle, Malinae WGD

Malinae WGD must have contributed to expansion of these genes, and that these MADS-box gene duplicates may have potentially contributed to the evolution of pome fruits in Malinae.

Overall, recently published genome sequences of pear and apple in Maleae, along with phylogenomics analyses of thousands of genes from multiple Maleae species and others, while using a well-resolved phylogeny of Rosaceae as a reference, have provided valuable information on WGD and gene duplication in these species. Both extensive syntenic chromosome blocks in pear and apple along with thousands of gene duplicates support existence of a WGD event that must have

occurred in the ancestor of Malinae. Subsequently, about 30% of duplicated gene pairs from this WGD are retained, and these can be mostly detected in synteny blocks in both pear and apple genomes. These duplicated genes are involved in transcription regulatory networks and in other cellular or metabolic processes. In addition to these retained duplicates, about two-thirds of duplicates resulting from the WGD event that must have occurred in the Malinae ancestor have been lost during subsequent evolution, with the highest rate of loss occurring about 30–31 Mya. These losses may correspond to differential adaptation of various genera to new environments, including the divergence of apple and pear.

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References

Albalat R, Canestro C (2016) Evolution by gene loss. Nat Rev Genet 17:379–391

Arrigo N, Barker MS (2012) Rarely successful polyploids and their legacy in plant genomes. Curr Opin Plant Biol 15:140–146

Bekaert M, Edger PP, Pires JC, Conant GC (2011) Two-phase resolution of polyploidy in the *Arabidopsis* metabolic network gives rise to relative and absolute dosage constraints. Plant Cell 23:1719–1728

Blanc G, Wolfe KH (2004) Widespread paleopolyploidy in model plant species inferred from age distributions of duplicate genes. Plant Cell 16:1667–1678

Burgess MB, Cushman KR, Doucette ET, Talent N, Frye CT, Campbell CS (2014) Effects of apomixis and polyploidy on diversification and geographic distribution in *Amelanchier* (Rosaceae). Am J Bot 101:1375–1387

Cannon SB, McKain MR, Harkess A, Nelson MN, Dash S, Deyholos MK, Peng Y, Joyce B, Stewart CN, Rolf M, Kutchan T, Tan X, Chen C, Zhang Y, Carpenter E, Wong GK-S, Doyle JJ, Leebens-Mack J (2015) Multiple polyploidy events in the early radiation of nodulating and nonnodulating legumes. Mol Biol Evol 32:193–210

Chagné D, Crowhurst RN, Pindo M, Thrimawithana A, Deng C, Ireland H, Fiers M, Dzierzon H, Cestaro A, Fontana P, Bianco L, Lu A, Storey R, Knabel M, Saeed M, Montanari S, Kim YK, Nicolini D, Larger S, Stefani E, Allan AC, Bowen J, Harvey I, Johnston J, Malnoy M, Troggio M, Perchepied L, Sawyer G, Wiedow C, Won K, Viola R, Hellens RP, Brewer L, Bus VGM, Schaffer RJ, Gardiner SE, Velasco R (2014) The draft genome sequence of European pear (*Pyrus communis* L. 'Bartlett'). PLoS ONE 9: e92644

Chin S-W, Shaw J, Haberle R, Wen J, Potter D (2014) Diversification of almonds, peaches, plums and cherries—molecular systematics and biogeographic history of *Prunus* (Rosaceae). Mol Phylogenet Evol 76:34–48

Conant GC, Wolfe KH (2008) Turning a hobby into a job: how duplicated genes find new functions. Nat Rev Genet 9:938–950

Considine MJ, Wan YZ, D'Antuono MF, Zhou Q, Han MY, Gao H, Wang M (2012) Molecular genetic features of polyploidization and aneuploidization reveal unique patterns for genome duplication in diploid *Malus*. PLoS ONE 7:57–65

Cusack BP, Wolfe KH (2007) When gene marriages don't work out: divorce by subfunctionalization. Trends Genet 23:270–272

Daccord N, Celton JM, Linsmith G, Becker C, Choisne N, Schijlen E, van de Geest H, Bianco L, Micheletti D, Velasco R, Di Pierro EA, Gouzy J, Rees DJG, Guerif P, Muranty H, Durel CE, Laurens F, Lespinasse Y, Gaillard S, Aubourg S, Quesneville H, Weigel D, van de Weg E, Troggio M, Bucher E (2017) High-quality *de novo* assembly of the apple genome and methylome dynamics of early fruit development. Nat Genet 49:1099–1106

Dickinson TA, Lo E, Talent N (2007) Polyploidy, reproductive biology, and Rosaceae: understanding evolution and making classifications. Plant Syst Evol 266:59–78

Evans RC, Campbell CS (2002) The origin of the apple subfamily (Maloideae; Rosaceae) is clarified by DNA sequence data from duplicated GBSSI genes. Amer J Bot 89:1478–1484

Fawcett JA, Van de Peer Y, Maere S (2013) Significance and biological consequences of polyploidization in land plant evolution. In: Leitch IJ (ed) Plant genome diversity. Springer, Vienna, pp 277–294

Fougere-Danezan M, Joly S, Bruneau A, Gao XF, Zhang LB (2015) Phylogeny and biogeography of wild roses with specific attention to polyploids. Ann Bot 115:275–291

Freeling M (2009) Bias in plant gene content following different sorts of duplication: tandem, whole-genome, segmental, or by transposition. Annu Rev Plant Biol 60:433–453

Hahn MW (2009) Distinguishing among evolutionary models for the maintenance of gene duplicates. J Hered 100:605–617

Hooker JJ, Collinson ME, Sille NP (2004) Eocene-Oligocene mammalian faunal turnover in the Hampshire Basin, UK: calibration to the global time scale and the major cooling event. J Geol Soc London 161:161–172

Huang CH, Zhang CF, Liu M, Hu Y, Gao TG, Qi J, Ma H (2016) Multiple polyploidization events across Asteraceae with two nested events in the early history revealed by nuclear phylogenomics. Mol Biol Evol 33:2820–2835

Hudson CM, Puckett EE, Bekaert M, Pires JC, Conant GC (2011) Selection for higher gene copy number after different types of plant gene duplications. Genome Biol Evol 3:1369–1380

Hummer KE, Janick J (2009) Rosaceae: taxonomy, economic importance, genomics. In: Folta KM, Gardiner SE (eds) Genetics and genomics of Rosaceae. Springer, New York, USA, pp 1–17

Jiao Y, Leebens-Mack J, Ayyampalayam S, Bowers JE, McKain MR, McNeal J, Rolf M, Ruzicka DR, Wafula E, Wickett NJ, Wu X, Zhang Y, Wang J, Zhang Y, Carpenter EJ, Deyholos MK, Kutchan TM, Chanderbali AS, Soltis PS, Stevenson DW, McCombie R, Pires JC, Wong GK-S, Soltis DE, dePamphilis CW (2012) A genome triplication associated with early diversification of the core eudicots. Genome Biol 13:R3

Jiao Y, Wickett NJ, Ayyampalayam S, Chanderbali AS, Landherr L, Ralph PE, Tomsho LP, Hu Y, Liang H, Soltis PS, Soltis DE, Clifton SW, Schlarbaum SE, Schuster SC, Ma H, Leebens-Mack J, dePamphilis CW (2011) Ancestral polyploidy in seed plants and angiosperms. Nature 473:97–100

Kellogg EA (2016) Has the connection between polyploidy and diversification actually been tested? Curr Opin Plant Biol 30:25–32

Levin DA (1983) Polyploidy and novelty in flowering plants. Am Nat 122:1–25

Li H, Shi Q, Zhang ZB, Zeng LP, Qi J, Ma H (2016) Evolution of the leucine-rich repeat receptor-like protein kinase gene family: ancestral copy number and functional divergence of *BAM1* and *BAM2* in Brassicaceae. J Syst Evol 54:204–218

Li LT, Deng CH, Knabel M, Chagne D, Kumar S, Sun JM, Zhang SL, Wu J (2017) Integrated high-density consensus genetic map of *Pyrus* and anchoring of the 'Bartlett' v1.0 (*Pyrus communis*) genome. DNA Res 24:289–301

Li Z, Baniaga AE, Sessa EB, Scascitelli M, Graham SW, Rieseberg LH, Barker MS (2015) Early genome duplications in conifers and other seed plants. Sci Adv 1:e1501084

Lo EYY, Stefanovic S, Dickinson TA (2010) Reconstructing reticulation history in a phylogenetic framework and the potential of allopatric speciation driven by polyploidy in an agamic complex in *Crataegus* (Rosaceae). Evolution 64:3593–3608

Lombard PB, Westwood MN (1987) Pear rootstocks. In: Rom RC, Carlson RF (eds) Rootstocks for Fruit crops. Wiley, New York, pp 145–183

Lu M, An HM, Li LL (2016) Genome survey sequencing for the characterization of the genetic background of *Rosa roxburghii* Tratt and leaf ascorbate metabolism genes. PLoS ONE 11:e0147530

Lynch M, Force A (2000) The probability of duplicate gene preservation by subfunctionalization. Genetics 154:459–473

Madlung A, Tyagi AP, Watson B, Jiang H, Kagochi T, Doerge RW, Martienssen R, Comai L (2005) Genomic changes in synthetic *Arabidopsis* polyploids. Plant J 41:221–230

Maere S, Van de Peer Y (2010) Duplicate retention after small- and large-scale duplications. In: Dittmar K, Liberles D (eds) Evolution after gene duplication. Wiley, Hoboken, New Jersey, pp 31–56

Masiero S, Li MA, Will I, Hartmann U, Saedler H, Huijser P, Schwarz-Sommer Z, Sommer H (2004) *INCOMPOSITA*: a MADS-box gene controlling prophyll development and floral meristem identity in *Antirrhinum*. Development 131:5981–5990

Mayrose I, Zhan SH, Rothfels CJ, Magnuson-Ford K, Barker MS, Rieseberg LH, Otto SP (2011) Recently formed polyploid plants diversify at lower rates. Science 333:1257

Nakamura N, Hirakawa H, Sato S, Otagaki S, Matsumoto S, Tabata S, Tanaka Y (2018) Genome structure of *Rosa multiflora*, a wild ancestor of cultivated roses. DNA Res 25:113–121

Ohno S (1970) Evolution by gene duplication. Springer, Berlin

Phipps JB (2014) Flora of North America North of Mexico, Vol. 9, Magnoliophyta: Picramniaceae to Rosaceae. Oxford University Press, New York and Oxford

Pontes O, Neves N, Silva M, Lewis MS, Madlung A, Comai L, Viegas W, Pikaard CS (2004) Chromosomal locus rearrangements are a rapid response to formation of the allotetraploid *Arabidopsis suecica* genome. Proc Natl Acad Sci USA 101:18240–18245

Potter D, Eriksson T, Evans RC, Oh S, Smedmark JEE, Morgan DR, Kerr M, Robertson KR, Arsenault M, Dickinson TA, Campbell CS (2007) Phylogeny and classification of Rosaceae. Plant Syst Evol 266:5–43

Pratt C (1988) Apple flower and fruit: morphology and anatomy. Hort Rev 10:273–307

Raymond O, Gouzy J, Just J, Badouin H, Verdenaud M, Lemainque A, Vergne P, Moja S, Choisne N, Pont C, Carrere S, Caissard JC, Couloux A, Cottret L, Aury JM, Szecsi J, Latrasse D, Madoui MA, Francois L, Fu XP, Yang SH, Dubois A, Piola F, Larrieu A, Perez M, Labadie K, Perrier L, Govetto B, Labrousse Y, Villand P, Bardoux C, Boltz V, Lopez-Roques C, Heitzler P, Vernoux T, Vandenbussche M, Quesneville H, Boualem A, Bendahmane A, Liu C, Le Bris M, Salse J, Baudino S, Benhamed M, Wincker P, Bendahmane M (2018) The Rosa genome provides new insights into the domestication of modern roses. Nat Genet 50:772–777

Rieseberg LH, Willis JH (2007) Plant speciation. Science 317:910–914

Rousseau-Gueutin M, Gaston A, Ainouche A, Ainouche ML, Olbricht K, Staudt G, Richard L, Denoyes-Rothan B (2009) Tracking the evolutionary history of polyploidy in *Fragaria* L. (strawberry): new insights from phylogenetic analyses of low-copy nuclear genes. Mol Phylogenet Evol 51:515–530

Schranz EM, Mohammadin S, Edger PP (2012) Ancient whole genome duplications, novelty and diversification: the WGD radiation lag-time model. Curr Op Plant Biol 15:147–153

Schulze-Menz GK (1964) Rosaceae. In: Melchior H (ed) Engler's Syllabus der Pflanzenfamilien. Gebrüder Borntraeger, Berlin, pp 209–218

Seymour GB, Ostergaard L, Chapman NH, Knapp S, Martin C (2013) Fruit development and ripening. Annu Rev Plant Biol 64:219–241

Shirasawa K, Isuzugawa K, Ikenaga M, Saito Y, Yamamoto T, Hirakawa H, Isobe S (2017) The genome sequence of sweet cherry (*Prunus avium*) for use in genomics-assisted breeding. DNA Res 24:499–508

Shulaev V, Sargent DJ, Crowhurst RN, Mockler TC, Folkerts O, Delcher AL, Jaiswal P, Mockaitis K, Liston A, Mane SP, Burns P, Davis TM, Slovin JP, Bassil N, Hellens RP, Evans C, Harkins T, Kodira C, Desany B, Crasta OR, Jensen RV, Allan AC, Michael TP, Setubal JC, Celton JM, Rees DJG, Williams KP, Holt SH, Rojas JJR, Chatterjee M, Liu B, Silva H, Meisel L, Adato A, Filichkin SA, Troggio M, Viola R, Ashman TL, Wang H, Dharmawardhana P, Elser J, Raja R, Priest HD, Bryant DW, Fox SE, Givan SA, Wilhelm LJ, Naithani S, Christoffels A, Salama DY, Carter J, Girona EL, Zdepski A, Wang WQ, Kerstetter RA, Schwab W, Korban SS, Davik J, Monfort A, Denoyes-Rothan B, Arus P, Mittler R, Flinn B, Aharoni A, Bennetzen JL, Salzberg SL, Dickerman AW, Velasco R, Borodovsky M, Veilleux RE, Folta KM (2011) The genome of woodland strawberry (*Fragaria vesca*). Nat Genet 43:109–116

Soltis DE, Soltis PS, Tate JA (2003) Advances in the study of polyploidy since plant speciation. New Phytol 161:173–191

Stebbins GL (1940) The significance of polyploidy in plant evolution. Am Natur 74:54–66

Tian T, Liu Y, Yan HY, You Q, Yi X, Du Z, Xu WY, Su Z (2017) agriGO v2.0: a GO analysis toolkit for the agricultural community, 2017 update. Nucl Acids Res 45:W122–W129

Vamosi JC, Dickinson TA (2006) Polyploidy and diversification: a phylogenetic investigation in Rosaceae. Int J Plant Sci 167:349–358

VanBuren R, Bryant D, Bushakra JM, Vining KJ, Edger PP, Rowley ER, Priest HD, Michael TP, Lyons E, Filichkin SA, Dossett M, Finn CE, Bassil NV, Mockler TC (2016) The genome of black raspberry (*Rubus occidentalis*). Plant J 87:535–547

Velasco R, Zharkikh A, Affourtit J, Dhingra A, Cestaro A, Kalyanaraman A, Fontana P, Bhatnagar SK, Troggio M, Pruss D, Salvi S, Pindo M, Baldi P, Castelletti S, Cavaiuolo M, Coppola G, Costa F, Cova V, Dal Ri A, Goremykin V, Komjanc M, Longhi S, Magnago P, Malacarne G, Malnoy M, Micheletti D, Moretto M, Perazzolli M, Si-Ammour A, Vezzulli S, Zini E, Eldredge G, Fitzgerald LM, Gutin N, Lanchbury J, Macalma T, Mitchell JT, Reid J, Wardell B, Kodira C, Chen Z, Desany B, Niazi F, Palmer M, Koepke T, Jiwan D, Schaeffer S, Krishnan V, Wu C, Chu VT, King ST, Vick J, Tao Q, Mraz A, Stormo A, Stormo K, Bogden R, Ederle D, Stella A, Vecchietti A, Kater MM, Masiero S, Lasserre P, Lespinasse Y, Allan AC, Bus V, Chagne D, Crowhurst RN, Gleave AP, Lavezzo E, Fawcett JA, Proost S, Rouze P, Sterck L, Toppo S, Lazzari B, Hellens RP, Durel C-E, Gutin A, Bumgarner RE, Gardiner SE, Skolnick M, Egholm M, Van de Peer Y, Salamini F, Viola R (2010) The genome of the domesticated apple (*Malus* × *domestica* Borkh.). Nat Genet 42:833–839

Verde I, Jenkins J, Dondini L, Micali S, Pagliarani G, Vendramin E, Paris R, Aramini V, Gazza L, Rossini L, Bassi D, Troggio M, Shu SQ, Grimwood J, Tartarini S, Dettori MT, Schmutz J (2017) The peach v2.0 release: high-resolution linkage mapping and deep resequencing improve chromosome-scale assembly and contiguity. BMC Genomics 18:225

Wang Y, Tang H, Debarry JD, Tan X, Li J, Wang X, Lee TH, Jin H, Marler B, Guo H, Kissinger JC, Paterson AH (2012) MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. Nucleic Acids Res 40:e49

Wu J, Wang Z, Shi Z, Zhang S, Ming R, Zhu S, Khan MA, Tao S, Korban SS, Wang H, Chen NJ, Nishio T, Xu X, Cong L, Qi K, Huang X, Wang Y, Zhao X, Wu J, Deng C, Gou C, Zhou W, Yin H, Qin G, Sha Y, Tao Y, Chen H, Yang Y, Song Y, Zhan D, Wang J, Li L, Dai M, Gu C, Wang Y, Shi D, Wang X, Zhang H, Zeng L, Zheng D, Wang C, Chen M, Wang G, Xie L, Sovero V, Sha S, Huang W, Zhang S, Zhang M, Sun J, Xu L, Li Y, Liu X, Li Q, Shen J, Wang J, Paull RE, Bennetzen JL, Wang J, Zhang S (2013) The genome of the pear (*Pyrus bretschneideri* Rehd.). Genome Res 23:396–408

Xiang Y, Huang C-H, Hu Y, Wen J, Li S, Yi T, Chen H, Xiang J, Ma H (2017) Evolution of Rosaceae fruit types based on nuclear phylogeny in the context of geological times and genome duplication. Mol Biol Evol 34:262–281

Yang Y, Moore MJ, Brockington SF, Soltis DE, Wong GK-S, Carpenter EJ, Zhang Y, Chen L, Yan Z, Xie Y, Sage RF, Covshoff S, Hibberd JM, Nelson MN, Smith SA (2015) Dissecting molecular evolution in the highly diverse plant clade Caryophyllales using transcriptome sequencing. Mol Biol Evol 32:2001–2014

Yao J, Dong Y, Kvarnheden A, Morris B (1999) Seven MADS-box genes in apple are expressed in different parts of the fruit. J Am Soc Hort Sci 124:8–13

Zachos J, Pagani M, Sloan L, Thomas E, Billups K (2001) Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292:686–693

Zachos JC, Dickens GR, Zeebe RE (2008) An early Cenozoic perspective on greenhouse warming and carbon-cycle dynamics. Nature 451:279–283

Zhang QX, Chen WB, Sun LD, Zhao FY, Huang BQ, Yang WR, Tao Y, Wang J, Yuan ZQ, Fan GY, Xing Z, Han CL, Pan HT, Zhong X, Shi WF, Liang XM, Du DL, Sun FM, Xu ZD, Hao RJ, Lv T, Lv YM, Zheng ZQ, Sun M, Luo L, Cai M, Gao YK, Wang JY, Yin Y, Xu X, Cheng TR, Wang J (2012) The genome of *Prunus mume*. Nat Commun 3:1318

Zhao L, Li X, Zhang N, Zhang S-D, Yi T-S, Ma H, Guo Z-H, Li D-Z (2016) Phylogenomic analyses of large-scale nuclear genes provide new insights into the evolutionary relationships within the rosids. Mol Phylogenet Evol 105:166–176

Future Breeding Strategies

16

Kamila Łucja Bokszczanin

Abstract

Pear breeding is considered as one of the most important sectors of temperate fruit breeding. While this follows breeding efforts for apple, new technologies and approaches are awaiting pear breeders on the horizon. New plant breeding techniques, tested for their efficacy in other fruit trees, as well as conventional methods will be presented in this chapter. Moreover, the potential combination of these approaches toward development of 'smart' pear cultivars will be also described. Furthermore, as there is an observed trend of elevated consciousness of the health benefits of organically grown crops among consumers worldwide, the issue of organic pear breeding strategies pear will also be discussed. Based on the principles of organic plant breeding, any breeding technique is evaluated against four mandatory criteria, and must meet genome- and cell-level integrity, capability for propagation, as well as preservation against crossing barriers. Thus, the use of molecular markers as diagnostic tools is not excluded in organic breeding. For future pear breeding strategies, the merger of different 'omics' technologies will provide holistic approaches for discovery of gene function, elucidate mechanisms of gene function, support genotyping, and accelerate the breeding cycle. Furthermore, nanotechnologies utilized in gene transfer, phenotyping, detection of pathogens, and sequencing will also contribute to faster, more precise and specific high-quality monitoring, and consequently breeding of cultivars resistant to biotic and abiotic stresses.

16.1 Directions and Strategies for Pursuing Pear Breeding

In order to fulfill consumer needs and render cultivars successful in markets, it is crucial to set specific directions and conceptualize strategies for breeding. This involves deep knowledge of the global pear fruit industry, including those of extended networks of fruit growers, breeders, and pear marketers, as well as an understanding of fruit industry preferences, in particular for characteristics of new cultivars. Most pear breeding programs worldwide have focused on efforts to combine superior pear fruit quality, high productivity, precocious fruit bearing, long postharvest storage life, along with multiple disease resistance (e.g., resistance to pear scab and black spot diseases) and pest resistance, as well as of self-compatibility. Developing pear cultivars with early ripening or attractive fruit

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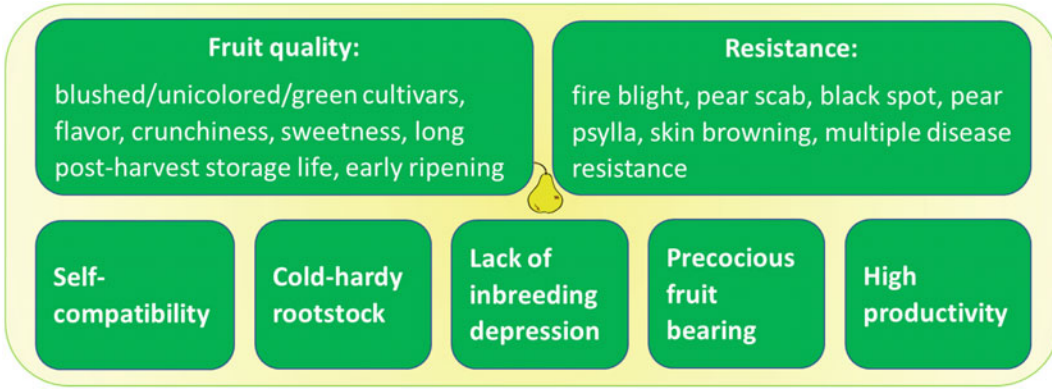

Fig. 16.1 Directions for pear breeding

appearance is also important. Moreover, avoiding inbreeding depression is essential for future breeding, and therefore expanding the pool of genetic resources used in pear breeding programs is critical.

Overall, the following traits of interest for pear breeding have emerged in recent years. These traits include blushed-fruit cultivars, solid colored fruit cultivars, in particular green-colored fruit cultivars, as well as a distinctive flavor profile of fruits, crunchiness and sweetness of fruits, along with resistance to various diseases and pests, particularly for fire blight, pear psylla, fruit skin browning, along with early fruit ripening, as well as cold-hardy dwarfing rootstocks (Fig. 16.1). These breeding goals could be achieved using various spectra of methods, individually or in combination, including advanced biotechnology techniques, hybridization, and mutagenesis. These breeding strategies will greatly benefit from the use of 'omics' technologies and nanotechnology for phenotyping, as well as for selection of superior lines during early stages of development. These innovation-driven newest developments, among others in plant biotechnology, will allow for pursuing advanced 'smart' breeding efforts for pear. However, there are some limitations for genetic engineering (GE) in certain regions of the world, especially in the European Union whereby cultivation of genetically modified organisms (GMOs) or GMO crops is restricted solely to MON810 maize. Nevertheless, some of these new genetic technologies, including GE, are receiving acceptance in other parts of the world, such as in North America. More recently, the powerful CRISPR technology, referring to 'clusters of regularly interspaced short palindromic repeats (CRISPR)/cas9 associated protein,' for gene editing will also have a significant impact on genetic enhancement efforts in various crops, including pears (Malnoy et al. 2016).

However, it is also important to take into consideration the growing importance and interest in organic cultivation of fruit trees, including

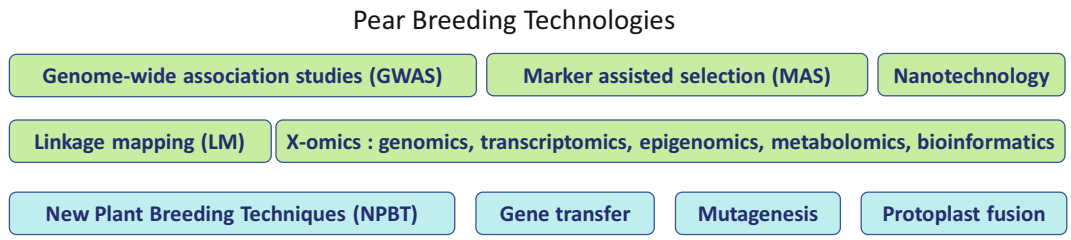

Fig. 16.2 Available technologies for future pear breeding strategies. Technologies allowed in organic breeding are in green boxes

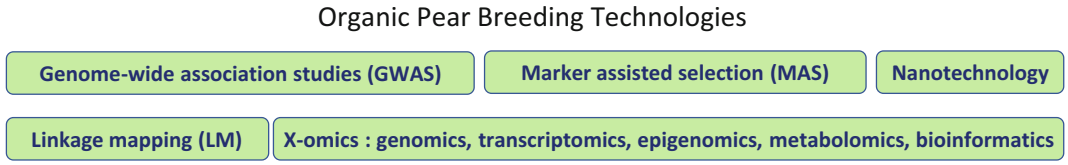

Fig. 16.3 Technologies for future pear organic breeding strategies

pear, and related organic pear breeding strategies. Although GE has opened up new pathways for genetic improvement, worldwide standards for organic agriculture (OA) do not allow for GE or any products derived from GE. Instead, alternative breeding approaches are pursued based on norms and standards for OA, not only at the technical level, but also at social and organizational levels, by including other value-chain players and consumers (Nuijten et al. 2016). All available pear breeding technologies are presented in Fig. 16.2, while those specific for organic breeding are presented in Fig. 16.3.

16.2 Conventional Pear Breeding

16.2.1 Marker-Assisted Selection (MAS)

Although marker-assisted selection (MAS) has been conceptualized three decades ago (Smith and Simpson 1986), it remains an important tool for fruit breeding. As the fields of molecular genetics and genomics have advanced, they have become valuable tools for improving breeding efficiency by allowing for early screening and selection of progenies and/or seedlings possessing traits of interest at the seed or seedling stage. The benefits of MAS for a plant breeder are greatest when the targeted species has a long generation cycle, and it is expensive to grow and maintain. Thus, MAS holds particular promise for fruit trees, such as pears, as they have generation cycles of 5–7 years to reach maturity, and they are costly to establish and grow in the field.

Often, well-characterized perennial tree germplasm beneficial to breeders is limited in its genetic diversity. A narrow genetic base in fruit breeding programs can certainly contribute to serious vulnerability to diseases, pests, and climatic changes. It is quite common that wild relatives have not been largely exploited as sources of desirable traits, including disease resistance, fruit quality, and rootstock characteristics. It is important to expand the genetic base, and to have access to large and wide collections of diverse germplasm to avoid such vulnerabilities.

There have been successful examples of using MAS in tree fruit breeding programs (Migicovsky et al. 2016). In order to establish genotype-phenotype relationships and advance MAS in apple, over 24,000 phenotype scores were extracted from the USDA-Germplasm Resources Information Network (GRIN) database, and these were linked to over 8000 single nucleotide polymorphisms (SNPs) from 689 apple accessions obtained from the USDA apple germplasm clonal collections maintained at Geneva, NY (Migicovsky et al. 2016).

16.2.2 Genome-Wide Association Studies (GWAS) and Genomic Selection (GS)

High-throughput genotyping technologies, such as DNA chips (Gupta et al. 2008), and genotyping using next generation sequencing (NGS) (Davey et al. 2011) have enabled new genomic-based strategies, such as genome-wide association studies (GWAS). This is an approach for detecting target genes or quantitative trait loci (QTL) based on associations between genome-wide markers and phenotypes caused by linkage disequilibrium (LD) between molecular markers and either causal genes or QTLs. The GWAS approach is an alternative to bi-parental QTL mapping in long-lived perennials. It does not require establishment of

segregating populations, which is time-consuming and costly. Moreover, the high mapping resolution offered by GWAS is amplified in many perennials due to a relatively rapid LD decay in highly diverse perennial crops. The correlation between a molecular marker and a causal variant is related to the level of LD between these two, wherein the higher the LD, the more likely the marker will serve as an indicator for presence of the causal variant. While rapid LD decay results in high mapping resolution, it also means that a very high density of markers is required for effective GWAS, as correlations among markers surrounding the causal variant decay very quickly. In some cases, generating sufficient coverage for GWAS by saturating the genome with molecular markers may be prohibitively expensive due to rapid LD decay. The costs of marker discovery and genotyping are likely to continue to decrease, thus rendering GWAS more affordable in the future.

The rapid decay of LD observed in apple suggests that millions of SNPs may be required for pursuing a well-powered GWAS (Migicovsky et al. 2016). However, rapid LD decay also promises to enable extremely high-resolution mapping of causal variants, which holds great promise for advancing MAS. A GWAS of 36 apple phenotypes has confirmed presence of an association between fruit color and an *MYB1* locus, as well as between the transcription factor NAC18.1 and harvest date and fruit firmness (Migicovsky et al. 2016). As a result, harvest time and fruit size have been predicted with relatively high accuracies ($r > 0.46$) using genomic prediction (Migicovsky et al. 2016). In turn, a high LD has been attributed to genetic bottlenecks during domestication and breeding of Japanese pear (Iwata et al. 2013). A genetic bottleneck increases the extent of LD by eliminating recombinant lineages (Iwata et al. 2013). Even when loci remain polymorphic during bottlenecks, the numbers of allelic combinations across loci can be greatly reduced, thereby leading to extensive haplotype structure (Hamblin et al. 2011). In a pear GWAS program, 76 Japanese pear cultivars have been genotyped for 162 DNA markers resulting in significant associations for harvest time, black spot resistance, and numbers of spurs (Iwata et al. 2013).

It is noteworthy to point out that inclusion of a large number of unrelated individuals in GWAS anticipates that a large number of recombination events must have occurred in the history of the target genetic material under study. Whereas, in linkage mapping (LM), it is only those recombination events captured through the development of a bi-parental cross that are exploited, thus resulting in recovery of a relatively large proportion of DNA of shared co-ancestry among individuals. One of the main advantages of GWAS over traditional LM is its superior mapping resolution. Markers detected in GWAS are deemed to be closely linked to causal genes and major QTL controlling important agronomic traits. In some cases, the likely causal genetic variant itself can be identified through GWAS (Migicovsky et al. 2016). However, in LM, large genomic intervals, often spanning millions of nucleotides, are identified, thus rendering it difficult or unlikely to identify the causal genetic variant.

In instances wherein a trait of interest co-segregates well with, for example, a wild relative species, yet it is completely absent in the cultivated germplasm, then a different breeding approach than that of GWAS is needed. Specifically, when phenotypes are well-segregated for a trait of interest, GWAS is of no use. Instead, a bi-parental cross between wild and cultivated individuals must be made to genetically map the trait of interest. LM in the resulting bi-parental population allows for such co-segregating traits to be genetically mapped. However, it has been observed that in fruit crops, such as apple, pear, and grape, wild and domesticated germplasm share segregating polymorphisms, and these are not readily or easily differentiated. In such instances, confounding effects of co-ancestry may not be strong enough, and GWAS may be the genetic mapping approach of choice. Additionally, when a trait of interest does not

co-segregate well with its ancestry, but rather it is differentially expressed in two populations, it may be possible to perform GWAS using wild and domesticated plant materials.

Although a simple distinction between GWAS and LM is useful, unfortunately, experimental designs blur this distinction, and they tend to exploit the benefits of both approaches, thus uncovering numerous genotype-phenotype associations. For example, a Multi-parent Advanced Generation InterCross (MAGIC) population is generated by intercrossing multiple parental lines rather than a single bi-parental cross. In another strategy to increase recombination frequency in a progeny for enhanced mapping is to utilize inbred offsprings (Cavanagh et al. 2008). However, development of inbred lines in perennial fruit trees is rather not feasible, thereby necessitating implementation of other mating designs. For example, a factorial mating design consisting of four female parents and two pollen parents has been used in an apple study (Kumar et al. 2012). This family-based design has allowed for identification of molecular markers linked to several fruit quality traits, including fruit firmness, internal browning, and titratable acidity, that are useful in MAS (Kumar et al. 2013). Therefore, alternative mating designs serve as promising tools for enhancing mapping resolution when performing LM between wild and domesticated crops.

In another alternative strategy for MAS, selection of either elite or desirable lines is based on genomic predictions of breeding values, and this is referred to as genomic selection (GS). GS allows for selection of superior genotypes based on genomic estimated breeding values (GEBV), as it derives information based on genome-wide markers. Thus, GS is more effective than MAS, particularly for traits controlled by large numbers of genes. Furthermore, GS is similar to GWAS as it utilizes LD between markers on one hand and causal genes and QTL on the other. However, unlike GWAS, GS is designed to detect genes and QTL and aims to predict the genetic potential; e.g., breeding values, of breeding lines without locating genes and QTL (Iwata et al. 2013). In fact, GS can avoid issues of uncertainty in QTL identification and effect estimation, which can be problematic in MAS, by simultaneously estimating effects of all marker loci. This simultaneous estimation of genomic effects provides further benefits as effects that are too small to be declared 'statistically significant' can be captured by markers. Due to these features, GS is proposed as efficient, even for low-heritability polygenic traits (Lorenz et al. 2011); whereas, MAS is deemed unsuitable for improvement of such traits (Iwata et al. 2013). In Japanese pear, *Pyrus pyrifolia*, genome-wide predictions for GS have been determined to be accurate at high probability levels ($p = 0.75$) for harvest time, at medium probability levels ($p = 0.38$–0.61) for resistance to black spot (incited by *Alternaria gaisen* Nagano), firmness of flesh, fruit shape in longitudinal section, fruit size, fruit acid content, and numbers of spurs, and at low levels ($p < 0.2$) for all soluble solids content and for tree vigor (Iwata et al. 2013).

It has been proposed that both GWAS and GS will be useful in accelerating genetic improvement of Japanese pear (Iwata et al. 2013). In fact, significant associations have been detected for harvest time, black spot resistance, and numbers of spurs (Iwata et al. 2013). However, accumulating large data sets sufficient for conducting such analyses is rather difficult for fruit trees due to their long juvenility periods, large plant sizes, and at times difficulties in phenotyping. Therefore, collecting and maintaining a genetically diverse pear collection, including wild relatives, are a valuable resource for developing new and enhanced pear cultivars. For instance, several Asian pear species are known to serve as candidates for fire blight resistance (incited by *Erwinia amylovora* [Burrill] Winslow et al.), carrying both polygenic and presumably monogenic resistance, depending on the genotype (Bokszczanin et al. 2012). Moreover, transgressive segregation for fire blight resistance has been observed within progenies of crosses among fire blight susceptible, moderately susceptible, and resistant pear parents (Bokszczanin et al. 2012).

16.2.3 Mutagenesis

Based on EU definition of genetically modified organisms (GMOs), mutagenesis is not regarded as a process that results in the development of GMOs. Thus, mutagenesis is deemed as an alternative strategy for introducing genetic variability in cultivars or in parental germplasm used in cross-hybridizations. In fact, mutagenesis has been successfully implemented in pear breeding programs, either for directly enhancing cultivars for specific traits or for yielding valuable mutants that can be used either in cross-hybridizations or for pursuing biotechnology studies for genetic enhancement (Fujimaki 1996; van Harten 1998).

Most often, irradiation treatments have been used to induce mutations in fruit trees. Among traits affected by mutagenesis, plant size, ripening time, fruit color, and self-fertility have been reported (Spiegel-Roy 1990). Moreover, irradiation was used to obtain dwarfing rootstocks of apple (Przybyla 1988). Several forms of mutations have been induced in European pear (*P. communis*), including variations in bloom time, blossom color, ripening time, fruit color, and compact growth habit (Predieri and Zimmerman 2001). As an alternate strategy, mutation breeding for Japanese pear was initiated by the Institute of Radiation Breeding using gamma irradiation. Since the 1980s, several induced mutants with some levels of resistance to black spot disease have been selected from 'Nijisseiki', 'Osanijisseiki', 'Shinsui', and 'Kisui' using chronic or acute gamma irradiation (Masuda et al. 1997). Among these selected mutants, four cultivars were named and released, including 'Gold Nijisseiki' (Kotobuki et al. 1992), 'Osa Gold' (Masuda et al. 1998), 'Kotobuki Shinsui' (Kitagawa et al. 1999), and 'Shizukisui' (Sawano et al. 2011). 'Gold Nijisseiki' demonstrated levels of resistance to black spot that were intermediate between those known for 'Chojuro' and 'Nijisseiki.' Moreover, this resistance was found to be inherited by offsprings, as well as detection of incomplete recessive mutations that were induced in L-II histogenic cell layers (Sanada et al. 1994).

One of the main problems of mutagenesis is the induction of chimeral mutants. The risk of incidence of such chimeral mutants can be reduced by irradiating in vitro-grown buds (Decourtye 1982; Broertjes 1982; Lacey and Campbell 1982). Predieri and Zimmerman (2001) have irradiated in vitro-grown shoots of six European pear cultivars using gamma rays (3.5 Gy). Subsequently, mutant trees have been selected for improved characters related to reproductive growth, such as early bearing and consistent annual productivity. Furthermore, variations in overall fruit characters, such as amounts of russeting, fruit shape, and fruit size, have also been observed in these mutants (Predieri and Zimmerman 2001).

16.3 'Smart' Breeding

Several new plant breeding techniques (NPBTs), representing significant advances toward crop improvement, are currently being implemented in breeding programs. Although NPBTs make use of genetic modification technology, the resulting end-products do not contain any foreign genes. Consequently, NPBT products are genetically similar to or may be even indistinguishable from conventionally bred plants. These strategies include cisgenesis and intragenesis, as well as gene editing techniques. Products from NPBTs may be grouped into three classes as follows: (1) plants that carry a new DNA fragment, often a new gene and/or regulatory element; (2) plants that do not carry a new DNA fragment, but carry a mutation or a native DNA modification; and (3) plants that do not carry a new DNA fragment or any native DNA modification.

16.3.1 Techniques to Shorten the Juvenility Period

Induced early flowering has been applied to fruit trees to accelerate breeding efforts. Fruit species, such as pear and apple, have a long generation cycle (5–7 years). Thus, fruit breeding is a

long-term endeavor, particularly when novel traits from related wild species are introgressed into a domesticated cultivar, as multiple breeding cycles are required to remove genetically linked undesirable traits, derived from wild species.

A member of the *APETALA1/FRUITFULL* group of *MADS-box* genes, isolated and cloned from silver birch (*Betula pendula*), designated as *BpMADS4*, has been found to drastically reduce the juvenility period when introduced into apple, thereby promoting flower induction in seedlings within the first year of growth (Flachowsky et al. 2011). An early flowering transgenic apple line expressing the *BpMADS4* gene has been developed, thereby affording future efforts opportunities to exploit this technology in combination with MAS to pyramid disease resistance genes for apple scab, powdery mildew, and fire blight (Flachowsky et al. 2011). Schlathölter et al. (2018) have already been successful in obtaining null apple segregants carrying both heterozygous resistance to fire blight (caused by *E. amylovora*) and homozygous resistance to the *Rvi6* gene for scab (incited by *Venturia inaequalis*). They have also used a rapid crop cycle breeding approach, based on overexpression of the birch *MADS4* transcription factor, in apple (Schlathölter et al. 2018). While transgenic lines expressing this *BpMADS4* gene are helpful in drastically reducing the generation time in fruit breeding efforts, it is often desirable to develop a cultivar that does not contain a transgene, so as it is not deemed a GMO crop. Such a desired outcome can be facilitated by using a transgene that is dominant and heterozygous, thus yielding only 50% offspring carrying the desired gene in each generation. Therefore, once the rapid cycling of generations is completed, a non-GMO tree possessing desirable traits from wild relatives, but not the transgene, can then be easily selected (Flachowsky et al. 2011). Nevertheless, 'Arctic' apple, a genetically engineered apple for non-browning of fruit flesh, has been approved for commercial production in the USA, and it is currently being grown in Midwest orchards.

An alternative to developing transgenic fruit trees expressing such a MADS-box gene from birch, virus-induced gene silencing (VIGS) can be used to shorten the juvenility period in fruit trees, among other plants. VIGS involves the use of a viral vector to infect a plant with a particular gene, resulting in an RNA-mediated defense response to silence expression of a target gene within a plant (Lu et al. 2003). It has been reported that the *apple latent spherical virus* (ALSV) does not induce disease symptoms in an infected plant, and can be used as a vector for VIGS (Igarashi et al. 2009). When ALSV has been used to express an *Arabidopsis thaliana* florigen while also silencing expression of a *Malus* × *domestica TERMINAL FLOWER* (*TFL*) gene, *MdTFL1-1,* in apple or a *P. communis TFL* gene, *PcTFL1-1,* in pear, flowering of these regenerated fruit trees can be reduced down to 3 months or less. In a test orchard, it has been reported that neither transmission via an insect vector nor horizontal transmission via pollen has been detected (Nakamura et al. 2011). In another study, Kishigami et al. (2014) have reported that approximately 99% of seedlings from ALSV-infected trees can be deemed virus-free. Finally, ALSV can be eliminated from an infected tree by using high temperature, allowing for vegetative propagation of such a tree, thus resulting in fruit deemed exempt from restrictions on GMOs (Yamagishi et al. 2016). Therefore, VIGS is a promising method for reducing the juvenile phase period of fruit trees, such as pear, allowing for a shorter generation time, and facilitating backcrossing, when deemed necessary, for breeding elite selections with wild relatives (Migicovsky and Myles 2017).

16.3.2 Grafting of Scion Cultivars onto a Genetically Modified (GM) Rootstock

There are several available approaches wherein GM rootstocks can be useful for improving performance of non-genetically modified (GM) scion cultivars. Using genetic modification technologies, characteristics of a rootstock, such as rooting ability, adaptation to heavy soils, or resistance to soil-borne diseases and pests, can be

improved. This would, in turn, enhance performance of a non-GM scion cultivar.

In another application of GM technology, rootstocks can be used as target materials for gene silencing through RNA interference (RNAi) (Kalantidis 2004). Small interfering RNAs (siRNAs) are natural silencing signals in plants; thus, siRNAs can be generated in transgenic plants using RNAi-expression vectors. The efficacy of RNAi to confer virus resistance in wild-type sweet cherry (*Prunus avium*) has been demonstrated in scions grafted onto a GM rootstock (Zhao and Song 2014). For this, a *Prunus* necrotic ringspot virus (PNRSV)-resistant transgenic cherry rootstock has been developed by introducing an RNAi vector expressing siRNAs against the PNRSV coat protein (Song et al. 2013). Subsequently, a non-GM sweet cherry scion cultivar has been grafted onto this transgenic rootstock. The transfer of PNRSV-targeting siRNA signal molecules from the rootstock to the non-transgenic scion has been confirmed, and enhanced PNRSV resistance of grafted sweet cherry scions has been observed. These findings have demonstrated, for the first time, transfer of transgene-derived siRNAs from a GM rootstock to a non-GM scion in grafted trees, and that these transferred siRNAs could enhance virus resistance of these grafted scions (Schaart et al. 2016).

Therefore, this approach could be explored in pear to develop GM pear rootstocks with resistance to fire blight, among other diseases, as well as with adaptation to cold temperatures for enhanced cold hardiness, or for dwarfing. Then, these rootstocks could be used for grafting of non-GM scion cultivars.

16.3.3 Cisgenesis

Cisgenesis refers to the development of plants via genetic modification strategies using only those genes derived from either the species itself or from a species that can intercross with this species using conventional methods. It is important to note that conventional methods may include such technologies as embryo rescue to overcome hybridization barriers. For example, in instances of either wide crosses or interspecific hybridizations, wherein distantly related parents belonging to different species or even genera, post-zygotic barriers, such as endosperm abortion, can be overcome by using embryo rescue. In fact, rescue of hybrid embryos from intra- and inter-specific crosses, commonly used in apple breeding programs, is aimed at increasing seed germination efficiency, as well as recovery of higher numbers of individuals obtained via sexual hybridization.

Genes used in cisgenesis strategies are introduced either as extra copies of the desired gene or as natural dominant variants of the desired gene with improved characteristics to confer resistance or enhanced resistance to a particular disease or some other desired trait. For example, cisgenic apple lines have been developed with enhanced resistance to fire blight disease using the cisgene *FB_MR5* from the wild apple *M.* × *robusta* 5, and introducing it into the fire blight susceptible cultivar Gala Galaxy (Kost et al. 2015). By the way, fire blight disease is one of the most serious diseases of pear, and therefore, such a strategy should be explored to introduce fire blight resistance into susceptible pear cultivars.

16.3.4 Intragenesis

Intragenesis is similar to cisgenesis, as all elements introduced via genetic modification are derived either from within the species of interest or from a cross-compatible species. However, intragenesis differs from cisgenesis by allowing use of new gene combinations generated by in vitro rearrangements of functional genetic elements. These new combinations of functional elements, such as regulatory elements or transposable elements, will offer new opportunities for genetic enhancement. For example, such opportunities may deal with temporal and spatial activation of a desirable gene of interest in a target tissue, or organ, of a plant.

Efforts to either enhance or regulate gene expression by introducing a stronger promoter

will drive gene expression to enhance expression of a trait of interest, such as plant disease resistance or fruit color pigmentation, among others. It is important to point out that intragenesis cannot be achieved through conventional breeding, as new combinations are unlikely to arise in such a breeding scheme (Holme et al. 2013). Therefore, pear breeding efforts can be certainly advanced further via the use of intragenesis for genetic enhancement of disease resistance or of fruit quality traits.

16.3.5 Gene Editing

Gene editing, also known as genome editing, involves a group of technologies that allow for targeted DNA insertion, deletion, or alteration of a particular gene or segment of a genome. Several approaches for genome editing have been developed, using a sequence-specific nuclease technology (SSN). These nucleases are synthetic proteins that bind to a specific DNA target sequence and induce a break in the DNA (a 'lesion'). Such a DNA break is subsequently repaired by the plant's native DNA repair machinery. There are three types of SSN applications, including SSN-1 which results in gene knockout, SSN-2 which results in a targeted mutation, and SSN-3 which results in gene replacement. Interestingly, accurate native DNA machinery leads to either a single base substitution (SSN-2) or introduction of a new DNA fragment (SSN-3); whereas, non-accurate repair machinery results in a deletion (SSN-1).

Gene editing techniques include zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN), clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9), and oligonucleotide-directed mutagenesis (ODM), also known as the rapid trait development system (RTDS). Recently, it has been demonstrated that CRISPR/Cas9 can be used in apple to modify the genome (Nishitani et al. 2016). The CRISPR-Cas9 system has generated a lot of excitement in the scientific community, as this technology is faster, cheaper, more accurate, and more efficient than other existing genome-editing methods. Mutations resulting from ODM can be also obtained using traditional mutagenesis; however, the advantage of ODM over traditional mutagenesis is that it does not produce thousands of other mutations (Limera et al. 2017).

The use of such gene editing technologies in pear is currently ongoing, and will have a significant impact in pursuing genetic improvement efforts to address various important traits that will enhance pear genotypes for such traits.

16.3.6 RNA-Dependent DNA Methylation (RdDM)

An RNA-dependent DNA methylation (RdDM) approach involves design of recombinant genes that produce RNA molecules matching either the target gene or its promoter region, and their subsequent introduction into plant cells. Such RNA molecules are recognized by the RNA-induced silencing complex (RISC), thereby resulting in methylation of the corresponding DNA, which in turn blocks expression of the target gene.

This approach has been recently used in devising strategies to accelerate apple breeding. It is reported that significant changes in 24 nucleotide (nt) sRNAs, that are the hallmarks of the RdDM pathway, are suggestive of a correlation between epigenetic modifications and floral transition (Guo et al. 2017). Therefore, differentially expressed miRNAs and siRNAs between vegetative and floral buds have been identified following small RNA (sRNA) sequencing data analysis. Bioinformatics analysis of these sRNAs has shed new light of our understanding of floral transition in woody plants (Guo et al. (2017). This is quite helpful in pursuing similar studies in pear.

Elucidation of the mechanism regulating floral transition is critical for both pear and apple breeding, as well as for their cultivation (Bangerth 2009). Furthermore, as for other crops, pear improvement and breeding strategies can benefit from the use of epi-marks of promoter regions of

a gene(s) for 'fine-tuning' gene expression in pear cultivars (Gallusci et al. 2016).

16.4 Organic Breeding

Nowadays, the importance of organic farming has gained more attention. Worldwide standards for OA do not allow GE or any products derived from GE. As organic certification is based on the farming process rather than on end-products as such, this may also impact breeding as an activity within the agricultural industry, as the breeding activity will be evaluated for compliance with organic rules and values (Van Bueren et al. 2003, 2010; Nuijten et al. 2016). A notable difference between EU and US regulations is that in EU legislation of GE, both the process and the product of GE are taken into consideration, while in the USA, it is only the final product that is evaluated (Araki and Ishii 2015). However, in the USA, the National Organic Standards Board has decided to update organic standards to exclude cultivars and derived organic products developed via new generations of GE and gene editing techniques (Nuijten et al. 2016). In Europe, a position paper of the International Federation of Organic Agriculture Movements (IFOAM) EU GROUP (Nuijten et al. 2016) has urged that cultivars derived from NPBT that engineer living organisms in cells and/or nuclei through technical, chemical, or biotechnological intervention should be designated as GE. Thus, these cultivars are subject to risk assessment, and if authorized for release, these are subject to mandatory traceability and labeling requirements that apply to other GE techniques.

Based on the principles for organic plant breeding, as described by the European Consortium for Organic Plant Breeding (ECO-PB) (2012), and in the IFOAM Norms for organic production and processing in 2014 (IFOAM 2014), any breeding technique is evaluated against four mandatory criteria that must be met. These criteria include the following: (i) genome-level integrity, (ii) cell-level integrity,

Table 16.1 Criteria for evaluation of breeding technologies along with principles for organic plant breeding according to the European Consortium for Organic Plant Breeding (ECO-PB) and the International Federation for Organic Agriculture Movements (IFOAM) Norms of 2014

Breeding technology	Genome-level integrity	Cell-level integrity	Ability for propagation	Preservation of crossing barriers	Breeder's privilege is affected	Farmers rights on farmer-sown seeds are affected
Chemical mutagenesis, irradiation	No	No	Yes	Yes	No	No
Cisgenetics	No	No	Yes	Yes	Yes (patent)	Yes (patent)
Cytoplast fusion	Yes	No	Case-specific	No	Possibly	Possibly
Marker-assisted selection	Yes	Yes	Yes	Yes	No	No (patent?)
Minichromosomes	No	No	Yes	No	Yes (patent)	Yes (patent)
Oligo directed mutagenesis	No	No	Yes	Yes	No	No
Reverse breeding	No	No	Yes	Yes	No	No
RNA Interference (RNAi)	No	No	Yes	Yes	Yes (patent)	Yes (patent)
Transgenetics	No	No	Possibly	No	Yes (patent)	Yes (patent)
Zinkfinger Nuclease III	No	No	Yes	Possibly	Yes (patent)	Yes (patent)
Zinkfinger Nucleases I and II	No	No	Yes	Yes	Yes (patent)	Yes (patent)

(iii) ability for propagation, and (iv) preservation of crossing barriers. Farm-saved seed is preferred, but it is not an exclusive criterion. Table 16.1 summarizes such techniques and assesses their validity for use in organic pear breeding.

16.5 Advanced NGS Methods and Nanodiagnostics to Accelerate Pear Breeding

16.5.1 NGS-Based Methods

Current advances in genomics, including DNA sequencing, are the most important tools in plant breeding and biotechnology. For the first time, important genes for a trait can be accurately identified and at low cost in almost any organism. Rapid developments in NGS technologies over the last decade have opened up many new opportunities for discovery of relationships between genotypes and phenotypes.

Third generation systems (TGS) will quickly become more common in plant research, as additional breeding materials are sequenced. The transition of high-throughput-sequencing data into useful information for breeders is one of the main goals, and it has been documented in many successful collaborations. Currently, TGS are being introduced to streamline sequencing protocols. Several platforms such as Helicos Heliscope® (Thompson and Steinmann 2010), Complete Genomics® (Drmanac et al. 2010), Nanopore® (Greninger et al. 2015), and Pacific Biosciences SMRT® (Eid et al. 2009) have incorporated new modifications. First, polymerase chain reaction (PCR) is no longer required before sequencing, and secondly, the signal is captured in real time. This indicates that the signal is either a fluorescent signal (Pacbio) or an electric current (Nanopore), and it is monitored during the enzymatic reaction of adding nucleotides to the complementary strand. Additionally, all of these platforms process millions of sequence reads in parallel with very long reads, and in some cases, up to 10 kb in length (English et al. 2012).

Nanopore-based DNA sequencing protocols allow for single molecule electrical detection of a DNA sequence, and have potentials for low sample preparation work, high-speed, and low-cost (Branton et al. 2008). These advances offer dramatic forward steps in improving this inexpensive and potentially more rapid alternative to NGS technologies (Khiyami et al. 2014). Recently, the development of the newest Oxford™ nanopore technology has provided novel improvements in molecular sensing, such as real-time data streaming, improved simplicity, efficiency and scalability of workflows, as well as direct analysis of the molecule of interest. These platforms, along with new bioinformatic tools, have provided complete annotated sequences.

16.5.2 Nanotechnology

It is common knowledge that conventional or traditional plant breeding methods are time-consuming. Nanodiagnostic tools, including microfluidics, nanofluidics, nanomaterials, and bioanalytical nanosensors, among others, offer opportunities for advancing and enhancing plant breeding programs. These tools can potentially overcome problems in dealing with issues, such as biotic and abiotic resistance, production, and prevention protocols, and are likely to be used in field-based assays for transgene expression assays, among others (Stewart 2005). Nanodiagnostic methods, among other nanotechnology tools, enable higher precision breeding as they offer new opportunities for selecting and transferring genes, while reducing the time required to remove redundant genes, and also allowing a breeder to access useful genes from distant plants (Abd-Elsalam and Alghuthaymi 2015). It has been demonstrated that a honeycomb mesoporous silica nanoparticle (MSN) system with 3-nm pores can transport DNA and chemicals into isolated plant cells and intact leaves (Torney et al. 2007). Nanofluidics, such as the Open Array or the Fluidigm Dynamic Array technologies supply automated PCR mixes for mega-molecular breeding assays. Moreover, nanotechnology can specifically target specific

plant pathology problems in agriculture, such as in plant-pathogen interactions, and provide new strategies for crop disease control (Khiyami et al. 2014).

Nanoparticles and quantum dots (QDs) have emerged as essential tools for fast detection of particular biological markers with high accuracies. Biosensors, QDs, nanostructured platforms, nanoimaging, and nanopore DNA sequencing tools offer opportunities for improving sensitivity, specificity, and speed of pathogen detection, facilitating high-throughput analysis, and high-quality monitoring, and crop protection. This is of particular benefit for all crops, but in particular for long-lived tree fruit crops, such as pears. Furthermore, nanodiagnostic kits can easily and readily detect potential serious plant pathogens, thus allowing experts to help farmers in averting disease epidemics. In addition, using nanotools or nanoparticles for gene transfer in plant cells may lead to advances in developing new disease-resistant pear cultivars, as this will minimize expenses for use of agrochemicals required for plant disease control, and in alleviating environmental concerns (Taylor et al. 2005; Sekhon 2014).

16.5.3 Omics Technologies

Nowadays, X-omics approaches accelerate the breeding process, as they complement research efforts of targeted studies, yielding knowledge of, thus far, unrecognized genes, proteins, and metabolites. The collection of such new knowledge will provide significant support for improvement of breeding programs and facilitate the development of new better cultivars. Within this context, there is a special role for '*-omics'* technologies in dissecting genetic mechanisms that underpin the systemic functionality at the organismic level. Combinations of cell biological and molecular strategies with '*omics*' technologies, such as genomics, transcriptomics, epigenomics, proteomics, metabolomics, and bioinformatics can provide valuable information for breeding programs (Langridge and Fleury 2011). Current examples of transcriptomics technologies include RNA-Seq, massive analysis of cDNA ends (MACE), miRNA-Seq (smallRNA-Seq). Whereas, examples of genomics technologies include exome sequencing, whole genome sequencing, de novo-sequencing, and target enrichment. Examples of epigenomics technologies include methyl-Seq and bisulfite-seq.

Undoubtedly, there will be new technologies that will become available in the near future as well. Deep sequencing of transcriptomes is also a powerful tool for analysis of precise levels of expression for each gene in a sample. It consists in quantifying short cDNA reads, obtained by NGS technologies, in order to compare whole transcriptomes among genotypes grown under different environmental conditions. Whereas, miRNAs are non-coding short RNAs involved in the regulation of different physiological processes, which can be identified by high-throughput sequencing of RNA libraries obtained by reverse transcription of purified short RNAs, and by in silico comparisons with known miRNAs of other plant species.

Altogether, NGS techniques and their applications have increased the resources available for plant breeding efforts of pear trees, among other tree species, thereby closing earlier gaps of genetic tools available for perennial trees in comparison with annual plant species. The usefulness of X-omics platforms in Solonaceae has been demonstrated by one such example of elucidating the pollen thermotolerance mechanism (Bokszczanin et al. 2013). Thus, X-omics will have similar impacts in efforts to expand knowledge of critical traits of pear and corresponding genetic improvement efforts of this important economic tree fruit crop.

References

Abd-Elsalam KA, Alghuthaymi MA (2015) Nanodiagnostic tools in plant breeding. J Nanotech Mater Sci 2 (2):1–2

Araki M, Ishii T (2015) Towards social acceptance of plant breeding by genome editing. Trends Plant Sci 20:145–149

Bangerth KF (2009) Flower induction in mature, perennial angiosperm fruit trees: similarities and discrepancies with annual/biennial plants and the involvement of plant hormones. Sci Hortic 122:153–163. https://doi.org/10.1016/j.scienta.2009.06.014

Bokszczanin K, Przybyla A, Schollenberger M, Gozdowski D, Madry W, Odziemkowski S (2012) Inheritance of fire blight resistance in Asian *Pyrus* species. Open J Genet 2:109–120. https://doi.org/10.4236/ojgen.2012.22016

Bokszczanin KL, Solanaceae Pollen Thermotolerance Initial Training Network (SPOT-ITN) Consortium, Fragkostefanakis S (2013) Perspectives on deciphering mechanisms underlying plant heat stress response and thermotolerance. Front Plant Sci 4:315. https://doi.org/10.3389/fpls.2013.00315

Branton D, Deamer DW, Marziali A, Bayley H, Benner SA, Butler T, Di Ventra M, Garaj S, Hibbs A, Huang X, Jovanovich SB, Krstic PS, Lindsay S, Ling XS, Mastrangelo CH, Meller A, Oliver JS, Pershin YV, Ramsey JM, Riehn R, Soni GV, Tabard-Cossa V, Wanunu M, Wiggin M, Schloss JA (2008) The potential and challenges of nanopore sequencing. Nat Biotechnol 26(10):1146–1153

Broertjes C (1982) Significance of in vitro adventitious bud techniques for mutation breeding of vegetatively propagated crops. In: Induced mutations in vegetatively propagated plants, II. International Atomic Energy Agency, Wien, pp 1–9

Cavanagh C, Morell M, Mackay I, Powell W (2008) From mutations to MAGIC: resources for gene discovery, validation and delivery in crop plants. Curr Opin Plant Biol 11:215–221. https://doi.org/10.1016/j.pbi.2008.01.002

Davey JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM, Blaxter ML (2011) Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nat Rev Genet 12:499–510

Decourtye L (1982) Bilancio di 20 anni di miglioramento delle specie legnose da frutto mediante la mutagenesi e prospettive attuali. In: Atti della giornata di studio sull'uso di tecniche nucleari per il miglioramento genetico dei fruttiferi, Roma, pp 21–40

Drmanac R, Sparks AB, Callow MJ, Halpern AL, Burns NL, Kermani BG, Carnevali P, Nazarenko I, Nilsen GB, Yeung G, Dahl F, Fernandez A, Staker B, Pant KP, Baccash J, Borcherding AP, Brownley A, Cedeno R, Chen L, Chernikoff D, Cheung A, Chirita R, Curson B, Ebert JC, Hacker CR, Hartlage R, Hauser B, Huang S, Jiang Y, Karpinchyk V, Koenig M, Kong C, Landers T, Le C, Liu J, McBride CE, Morenzoni M, Morey RE, Mutch K, Perazich H, Perry K, Peters BA, Peterson J, Pethiyagoda CL, Pothuraju K, Richter C, Rosenbaum AM, Roy S, Shafto J, Sharanhovich U, Shannon KW, Sheppy CG, Sun M, Thakuria JV, Tran A, Vu D, Zaranek AW, Wu X, Drmanac S, Oliphant AR, Banyai WC, Martin B, Ballinger DG, Church GM, Reid CA (2010) Human genome sequencing using unchained base reads on self-assembling DNA nanoarrays. Science 327:78–81. https://doi.org/10.1126/science.1181498

Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, Peluso P, Rank D, Baybayan P, Bettman B, Bibillo A, Bjornson K, Chaudhuri B, Christians F, Cicero R, Clark S, Dalal R, Dewinter A, Dixon J, Foquet M, Gaertner A, Hardenbol P, Heiner C, Hester K, Holden D, Kearns G, Kong X, Kuse R, Lacroix Y, Lin S, Lundquist P, Ma C, Marks P, Maxham M, Murphy D, Park I, Pham T, Phillips M, Roy J, Sebra R, Shen G, Sorenson J, Tomaney A, Travers K, Trulson M, Vieceli J, Wegener J, Wu D, Yang A, Zaccarin D, Zhao P, Zhong F, Korlach J, Turner S (2009) Real-time DNA sequencing from single polymerase molecules. Science 323:133–138. https://doi.org/10.1126/science.1162986

English AC, Richards S, Han Y, Wang M, Vee V, Qu J, Qin X, Muzny DM, Reid JG, Worley KC, Gibbs RA (2012) Mind the gap: upgrading genomes with Pacific Biosciences RS long-read sequencing technology. PLoS ONE 7:e47768. https://doi.org/10.1371/journal.pone.0047768

European Consortium for Organic Plant Breeding (ECO-PB) (2012) Position paper on organic plant breeding. ECO-PB, Frankfurt, Germany

Flachowsky H, Le Roux PM, Peil A, Patocchi A, Richter K, Hanke MV (2011) Application of a high-speed breeding technology to apple (*Malus* × *domestica*) based on transgenic early flowering plants and marker-assisted selection. New Phytol 192:364–377. https://doi.org/10.1111/j.1469-8137.2011.03813.x

Fujimaki H (1996) Tangency of artificial mutation to recombinant-DNA in plant breeding. In: Gamma field symposia: the tangency of mutation induction and genetic engineering in plant breeding, vol 35, pp 1–4. Institute of Radiation Breeding NIAR MAFF, Ibaraki, Japan

Gallusci P, Hodgman C, Teyssier E, Seymour GB (2016) DNA methylation and chromatin regulation during fleshy fruit development and ripening. Front Plant Sci 7:807. https://doi.org/10.3389/fpls.2016.00807

Greninger AL, Naccache SN, Federman S, Yu G, Mbala P, Bres V, Stryke D, Bouquet J, Somasekar S, Linnen JM, Dodd R, Mulembakani P, Schneider BS, Muyembe-Tamfum JJ, Stramer SL, Chiu CY (2015) Rapid metagenomic identification of viral pathogens in clinical samples by real-time nanopore sequencing analysis. Genome Med 7:99. https://doi.org/10.1186/s13073-015-0220-9

Guo X, Ma Z, Zhang Z, Cheng L, Zhang X, Li T (2017) Small RNA-sequencing links physiological changes and RdDM process to vegetative-to-floral transition in apple. Front Plant Sci 8:873. https://doi.org/10.3389/fpls.2017.00873

Gupta PK, Rustgi S, Mir RR (2008) Array-based high-throughput DNA markers for crop improvement. Heredity 101:5–18

Hamblin MT, Buckler ES, Jannink JL (2011) Population genetics of genomics-based crop improvement methods. Trends Genet 27:98–106

Holme IB, Wendt T, Holm PB (2013) Intragenesis and cisgenesis as alternatives to transgenic crop development. Plant Biotechnol J 11(4):395–407. https://doi.org/10.1111/pbi.12055

Igarashi A, Yamagata K, Sugai T, Takahashi Y, Sugawara E, Tamura A, Yaegashi H, Yamagishi N, Takahashi T, Isogai M, Takahashi H, Yoshikawa N (2009) Apple latent spherical virus vectors for reliable and effective virus-induced gene silencing among a broad range of plants including tobacco, tomato, *Arabidopsis thaliana*, cucurbits, and legumes. Virology 386:407–416. https://doi.org/10.1016/j.virol.2009.01.039

International Federation of Organic Agriculture Movements (IFOAM) (2014) The IFOAM Norms. IFOAM, Bonn, Germany

Iwata H, Hayashi T, Terakami S, Takada N, Sawamura Y, Yamamoto T (2013) Potential assessment of genome-wide association study and genomic selection in Japanese pear *Pyrus pyrifolia*. Breed Sci 63:125–140. https://doi.org/10.1270/jsbbs.63.125

Kalantidis K (2004) Grafting the way to the systemic silencing signal in plants. PLoS Biol 2:1059–1061

Khiyami MA, Almoammar H, Awad YM, Alghuthaymi MA, Abd-Elsalam KA (2014) Plant pathogen nanodiagnostic techniques: forthcoming changes? Biotechnol Biotechnol Equip 28:775–785

Kishigami R, Yamagishi N, Ito T, Yoshikawa N (2014) Detection of apple latent spherical virus in seeds PCR and seedlings from infected apple trees by reverse transcription quantitative sequencing deep: evidence for lack of transmission of the virus to most progeny seedlings. J Gen Plant Pathol 80:490–498. https://doi.org/10.1007/s10327-014-0541-3

Kitagawa K, Nagara M, Uchida M, Inoue K, Murata K, Masuda T, Yoshioka T, Kotobuki K (1999) A new Japanese pear cultivar 'Kotobuki Shinsui'. Bull Tottori Hortic Exp Stn 3:1–13

Kost TD, Gessler C, Jänsch M, Flachowsky H, Patocchi A, Broggini GAL (2015) Development of the first cisgenic apple with increased resistance to fire blight. PLoS ONE 10(12):e0143980. https://doi.org/10.1371/journal.pone.0143980

Kotobuki K, Sanada T, Nishida T, Fujita H, Ikeda F (1992) 'Gold Nijisseiki', a new Japanese pear cultivar resistant to black spot disease induced by chronic irradiation of gamma-rays. Bull Nat Inst Agrobiol Resour 7:105–120

Kumar S, Chagne D, Bink MC, Volz RK, Whitworth C, Carlisle C (2012) Genomic selection for fruit quality traits in apple (*Malus* × *domestica* Borkh.). PLoS ONE 7:e36674 https://doi.org/10.1371/journal.pone.0036674

Kumar S, Garrick D, Bink M, Whitworth C, Chagne D, Volz R (2013) Novel genomic approaches unravel genetic architecture of complex traits in apple. BMC Genom 14:393. https://doi.org/10.1186/1471-2164-14-393

Lacey CND, Campbell IA (1982) Progress in mutation breeding of apples (*Malus pumila* Mill.) at Long Ashton Research Station, Bristol, United Kingdom. In: Induced mutations in vegetatively propagated plants, II. International Atomic Energy Agency, Wien, pp 11–28

Langridge P, Fleury D (2011) Making the most of 'omics' for crop breeding. Trends Biotechnol 29:33–40. https://doi.org/10.1016/j.tibtech.2010.09.006

Limera C, Sabbadini S, Sweet JB, Mezzetti B (2017) New biotechnological tools for the genetic improvement of major woody fruit species. Front Plant Sci 8:1418. https://doi.org/10.3389/fpls.2017.01418

Lorenz AJ, Chao S, Asoro FG, Heffner EL, Hayashi T, Iwata H, Smith KP, Sorrells ME, Jannink JL (2011) Genomic selection in plant breeding: Knowledge and prospects. Adv Agron 110:77–123

Lu R, Martin-Hernandez AM, Peart JR, Malcuit I, Baulcombe DC (2003) Virus-induced gene silencing in plants. Methods 30:296–303. https://doi.org/10.1016/S1046-2023(03)00037-9

Malnoy M, Viola R, Jung MH, Koo OJ, Kim S, Kim JS, Velasco R, Nagamangala Kanchiswamy C (2016) DNA-free genetically edited grapevine and apple protoplast using CRISPR/Cas9 ribonucleoproteins. Front Plant Sci 7:1904 [PMC free article] [PubMed]

Masuda T, Yoshioka T, Inoue K, Murata K, Kitagawa K, Tabira H, Yoshida A, Kotobuki K, Sanada T (1997) Selection of mutants resistant to black spot disease by chronic irradiation of gamma-rays in Japanese pear 'Osanijisseiki'. J Jpn Soc Hortic Sci 66:85–92

Masuda T, Yoshioka T, Sanada T, Kotobuki K, Nagara M, Uchida M, Inoue K, Murata K, Kitagawa K, Yoshida A (1998) A new Japanese pear cultivar 'Osa Gold', resistant mutant to the black spot disease of Japanese pear (*Pyrus pyrifolia* Nakai) induced by chronic irradiation of gamma-rays. Bull Natl Inst Agrobiol Resour 12:1–11

Migicovsky Z, Myles S (2017) Exploiting wild relatives for genomics-assisted breeding of perennial crops. Front Plant Sci 8:460. https://doi.org/10.3389/fpls.2017.00460

Migicovsky Z, Gardner KM, Money D, Sawler J, Bloom JS, Moffett P, Chao CT, Schwaninger H, Fazio G, Zhong G, Myles S (2016) Genome to phenome mapping in apple using historical data. Plant Genome 9. https://doi.org/10.3835/plantgenome2015.11.0113

Nakamura K, Yamagishi N, Isogai M, Komori S, Ito T, Yoshikawa N (2011) Seed and pollen transmission of Apple latent spherical virus in apple. J Gen Plant Pathol 77:48–53. https://doi.org/10.1007/s10327-010-0275-9

Nishitani C, Hirai N, Komori S, Wada M, Okada K, Osakabe K, Yamamoto T, Osakabe Y (2016) Efficient

genome editing in apple using a CRISPR/Cas9 system. Sci Rep 6(1)

Nuijten E, Messmer MM, Lammerts van Bueren ET (2016) Concepts and strategies of organic plant breeding in light of novel breeding techniques. Sustainability 9:18. https://doi.org/10.3390/su9010018

Predieri S, Zimmerman RH (2001) Pear mutagenesis: In vitro treatment with gamma-rays and field selection for productivity and fruit traits. Euphytica 117:217. https://doi.org/10.1023/A:1026594103277

Przybyla A (1988) Selection of dwarf mutants of apple vegetative rootstocks obtained by gamma irradiation. Hodowla Roślin, Aklimatyzacja i Nasiennictwo 32 (1/2):255–260

Sanada T, Sagisaka K, Soejima J, Moriguchi T, Teramoto S, Kotobuki K (1994) Inheritance of intermediate resistance to black spot disease in an induced Japanese pear mutant, 'Gold Nijisseiki'. J Jpn Soc Hortic Sci 62:689–693

Sawano I, Suzuki K, Kamata N, Nakajima T, Kuroyanagi E, Taneishi M, Hisada H (2011) Breeding Japanese pear 'Shizukisui' with resistance to black spot disease. Bull Shizuoka Res Inst Agr Forest 4:45–49

Schaart JG, van de Wiel CCM, Lotz LAP, Smulders MJM (2016) Opportunities for products of new plant breeding techniques. Trends Plant Sci 21:5. https://doi.org/10.1016/j.tplants.2015.11.006

Schlathölter I, Jänsch M, Flachowsky H, Broggini GAL, Hanke MV, Patocchi A (2018) Generation of advanced fire blight-resistant apple (*Malus* × *domestica*) selections of the fifth generation within 7 years of applying the early flowering approach. Planta 247 (6):1475–1488

Sekhon BS (2014) Nanotechnology in agri-food production: an overview. Nanotechnol Sci Appl 7:31–53

Smith C, Simpson SP (1986) The use of genetic polymorphisms in livestock improvement. J Anim Breed Genet 103:205–217. https://doi.org/10.1111/j.1439-0388.1986.tb00083.x

Song G, Sink KC, Walworth AE, Cook MA, Allison RF, Lang GA (2013) Engineering cherry rootstocks with resis-tance to Prunus necrotic ring spot virus through RNAi-mediated silencing. Plant Biotechnol J 11:702–708

Spiegel-Roy P (1990) Economic and agricultural impact of mutation breeding in fruit trees. Mutat Breed Rev 5:1–26

Stewart CN Jr (2005) Monitoring the presence and expression of transgenes in living plants. Trends Plant Sci 10(8):390

Taylor TM, Davidson PM, Bruce BD, Weiss J (2005) Liposomal nanocapsules in food science and agriculture. Crit Rev Food Sci Nutr 45(7–8):587–605

Thompson JF, Steinmann KE (2010) Single molecule sequencing with a HeliScope genetic analysis system. Curr Protoc Mol Biol 7(7):10. https://doi.org/10.1002/0471142727.mb0710s92

Torney F, Braine GT, Victor SY, Kan W (2007) Mesoporous silica nanoparticles deliver DNA and chemicals into plants. Nat Biotech 2:295–300

Van Bueren EL, Struik PC, Tiemens-Hulscher M, Jacobsen E (2003) The concepts of intrinsic value and integrity of plants in organic plant breeding and propagation. Crop Sci 43:1922–1929

Van Bueren EL, Østergård H, De Vriend H, Backes G (2010) Role of molecular markers and marker assisted selection in breeding for organic and low-input agriculture. Euphytica 175:51–64

Van Harten AM (1998) Mutation breeding, theory and practical applications. Cambridge University Press. ISBN: 0-521-47074-9

Yamagishi N, Li C, Yoshikawa N (2016) Promotion of flowering by apple latent spherical virus vector and virus elimination at high temperature allow accelerated breeding of apple and pear. Front Plant Sci 7:171. https://doi.org/10.3389/fpls.2016.00171

Zhao DY, Song GQ (2014) Rootstock-to-scion transfer of transgene-derived small interfering RNAs and their effect on virus resistance in nontransgenic sweet cherry. Plant Biotechnol J 12:1319–1328