

## Review Article

# Pear genetics: Recent advances, new prospects, and a roadmap for the future

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## Abstract

Pear, belonging to the genus *Pyrus*, is one of the most economically important temperate fruit crops. *Pyrus* is an important genus of the Rosaceae family, subfamily Maloideae, and has at least 22 different species with over 5000 accessions maintained or identified worldwide. With the release of draft whole-genome sequences for *Pyrus*, opportunities for pursuing studies on the evolution, domestication, and molecular breeding of pear, as well as for conducting comparative genomics analyses within the Rosaceae family, have been greatly expanded. In this review, we highlight key advances in pear genetics, genomics, and breeding driven by the availability of whole-genome sequences, including whole-genome resequencing efforts, pear domestication, and evolution. We cover updates on new resources for undertaking gene identification and molecular breeding, as well as for pursuing functional validation of genes associated with desirable economic traits. We also explore future directions for “pear-omics”.

## Introduction

As a member of the Rosaceae family and the subfamily Maloideae [1], pear has a wide range of germplasm resources and an ancient cultivation history. There are at least 22 known *Pyrus* species, with over 5000 accessions that are either cataloged or maintained around the world. These accessions have wide morphological and physiological variability and different ecological adaptations. Among these species, *P. communis* is mostly cultivated in Western countries, while *P. pyrifolia*, *P. bretschneideri*, *P. ussuriensis*, and *P. × sinkiangensis* are primarily cultivated in Asian countries. China is a major producer of pear, accounting for 71.40% of world pear production, and supplies approximately 17.60% of the export pear market (<http://www.fao.org/faostat/en/#home>, 2019). However, the current export price of Chinese pears is lower than that of the international average market price. This is because fruits of major Asian pear cultivars have higher stone cell contents, often lack an overall attractive appearance, particularly compared with those of red-colored European pears,

and have relatively bland flavors [2–4]. Traditional pear breeding efforts are mainly dependent on sexual crosses between parental types and the selection of promising or opportunistic seedlings, mutation breeding and selection of improved mutants, and the selection of desirable spontaneous bud mutants. Overall, controlled sexual crosses are most often used in pear breeding.

Pears are often self-incompatible and have long juvenile periods (5–7 years), often leading to the independent segregation of traits of interest in the resultant hybrids from sexual crosses. Overall, traditional pear breeding is difficult, time-consuming, and costly, as it requires the long-term commitment of labor, materials, and land-space resources. Therefore, the availability and use of molecular markers and genomic selection can significantly contribute to overcoming some of these constraints of traditional breeding.

Prior to the release of the pear genome sequence, only a few genes and markers associated with important economic traits were identified. However, once genomic tools and new genetic approaches became available, a

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**Table 1.** Comparisons among different sequenced pear genomes

Contigs	'Dangshansuli'	Bartlett1.0	BartlettDHv2.0	'Shanxi Duli'	'Zhongai 1'
Number of contigs	25,312	182,196	620	595	1241
Total size of contigs (Mb)	501.3	507.7	501	497	510.6
N50 contig length (kb)	35.7	6.6	5300	1571.5	1277.3
Longest contig (Mb)	0.3	0.1	/	/	6.5
<b>Scaffolds</b>					
Number of scaffolds	2,103	142083	592	139	784
Total size of scaffolds (Mb)	512	577.3	496.9	532.7	510.6
N50 scaffold length (kb)	540.8	88.1	6500	28122.4	23450
Longest scaffold (Mb)	4.1	1.2	/	45.5	31.9
Anchored size to the chromosome (Mb)	386.7	171.3	445.1	500	506.3
Anchored rate to the chromosome (%)	75.5	29.7	84.2	94	99.2

wide variety of valuable technologies and outcomes have been pursued and achieved in pear, including genetic transformation, genome sequencing, molecular markers, genetic and physical mapping, and comparative genomic analyses. Furthermore, the availability of tools and data resources has offered new opportunities for the efficient and robust discovery of genes controlling desirable fruit quality traits, fruit productivity traits, and postharvest storage life, as well as those that greatly shorten the breeding cycle in pear.

In 2013, the first genome sequence of Asian pear was released [5], thereby allowing delineation of chromosome evolution, genomic structure, and patterns of genetic variations. With the availability of a high-quality pear genome sequence, several gene families involved in controlling desirable and economic traits have now been identified [6–13]; the development of reliable and robust molecular markers [14], new genetic mapping initiatives [15], and analysis of genome evolution [5] have also occurred. This new knowledge and these resources will continue to have significant impacts on efforts for the genetic improvement of pears.

In this review, we will provide a summary of pear genome sequences and highlight the applications of high-throughput technologies that have contributed new knowledge about the biology of pear. These advances have provided new knowledge on the origins of pear and new insights into our understanding of the independent domestication history of Asian and European pears. Furthermore, the construction of high-density genetic linkage maps and the pursuit of genome-wide association studies (GWAS) have contributed to the identification of genomic loci for key genes regulating various economic and desirable agronomic traits. In addition, this review covers developments of multiple omics resources useful for the identification of candidate genes and evaluates the impacts of these findings on future efforts for pear breeding. This roadmap for the pear genome will serve as a useful guide for pursuing genetic improvement efforts toward developing new high-quality and well-adapted pear cultivars. This roadmap is also critical for addressing issues of the pear

response to climate change, as well as of ever-changing consumer demands and preferences.

### Overview of Asian and European pear genome sequences

Pear has a basic chromosome number of 17 ( $2n = 34$ ). Different pear species have distinctly different genome sizes, ranging from 500 to 650 Mb, and possess high numbers of repeats and transposable elements (TEs), as well as high levels of heterozygosity.

The first draft genome sequence of the pear cv. 'Dangshansuli' (*P. bretschneideri* Rehd.), also known as Chinese white pear (Asian pear), was assembled based on a bacterial artificial chromosome (BAC)-by-BAC strategy, thereby alleviating issues of high rates of heterozygosity and the complexity of repeated reads [5]. A total of 2,103 scaffolds were assembled for this pear genome with an N50 of ~0.54 Mb, representing 97.1% (512.0 Mb) of the estimated genome size (527.0 Mb) with 194× genome coverage (Table 1). Based on a high-density genetic map, a total of 386.7 Mb sequences, corresponding to ~75.5% of the assembled genome, were anchored to all 17 chromosomes of the pear genome. Repetitive sequences accounted for 53.1% (271.9 Mb) of the assembled genome. A high long-terminal repeat (LTR) expansion rate suggested that the pear genome was in continuous expansion. Compared with the apple (*Malus × domestica* Borkh.) genome, another member of the subfamily Maloideae, it was proposed that the presence of large numbers of repeat sequences primarily contributed to the size differences between the pear and apple genomes [16]. Importantly, genes involved in the synthesis of stone cells, sugars, and volatile compounds, as well as those involved in disease resistance and self-incompatibility, were identified in the pear genome. However, haplotype features and allele-specific expression were difficult to determine owing to the high rates of heterozygosity, as well as the lack of available accessions with haplotype-derived homologous chromosomes in Asian pears. Subsequently, a haplotype-resolved genome for pear (*P. bretschneideri*) was developed

using a new approach for protoplast isolation from pollen combined with single-cell DNA sequencing of 12 pollen cells and 'barcode' phasing of 38,304 BAC sequences [17]. Thus, the assembled genome sizes of the haploid genomes A and B were 546 Mb and 536 Mb, respectively. The haploid genome assembly also revealed that 8.12% of the genes identified in the first pear reference genome featured mosaic assemblies [17].

European pear, *P. communis* L., also an economically important species that is widely cultivated in Western countries, has distinct phenotypic and fruit quality characteristics that differ from those of Asian pear, including fruit shape, taste, lignin content, and aroma [2–4, 18, 19]. The whole genome sequence and annotation of European pear have aided in pursuing comparative genomic studies with Asian pear [20].

A draft genome sequence of the European pear 'Bartlett' version 1.0 was assembled and released using next-generation sequencing (NGS) technology (Roche 454) [20]. A total of 142,083 scaffolds were assembled, corresponding to 577.3 Mb, and represented 96.2% of the expected 600 Mb of the European pear genome (Table 1) [20]. The number of predicted genes in European pear was higher than that reported for most other plant species, but this number was similar to that identified in Asian pear. This result might be expected due to the incidence of whole genome duplication (WGD) events in members of the Maloideae subfamily [16]. In addition, the predicted coding region length (1,209 bp), exon length, and gene density in the European pear genome were found to be similar to those detected in Asian pear.

Recently, an updated version of the European pear genome using a double-haploid 'Bartlett' cultivar was published [21]. The quality and completeness of the European pear draft genome, designated BartlettDHv2.0, were greatly enhanced by integrating multiple technologies [21]. In this updated draft genome, a total of 496.9 Mb sequences were assembled, and 445.1 Mb were anchored and oriented across all 17 chromosomes of the pear genome using both Hi-C data and a high-density genetic map (Table 1). A total of 50% of the sequences (~247 Mb) in the European pear genome were found to be repetitive sequences, and 37,445 protein-coding genes were annotated, corresponding to a 13% reduction in predicted protein-coding genes from the previous two draft genomes of both *P. communis* and *P. bretschneideri*.

In addition to these three genome sequences of cultivated pears, genomes of the wild pear *P. betulaefolia* Bunge [22] and 'Zhongai 1' (*P. ussuriensis* × *P. communis*), a dwarfing hybrid rootstock [23], were recently sequenced and assembled in ongoing efforts to expand the pool of sequenced pear genomes. Most of the pear genome sequences and annotation-related datasets have been stored in the Genome Database of Rosaceae (GDR: <https://www.rosaceae.org/tools/jbrowse>) [24].

With the development of NGS, many resequencing studies are now underway. These expanded sequencing datasets have been widely used to explore the origin,

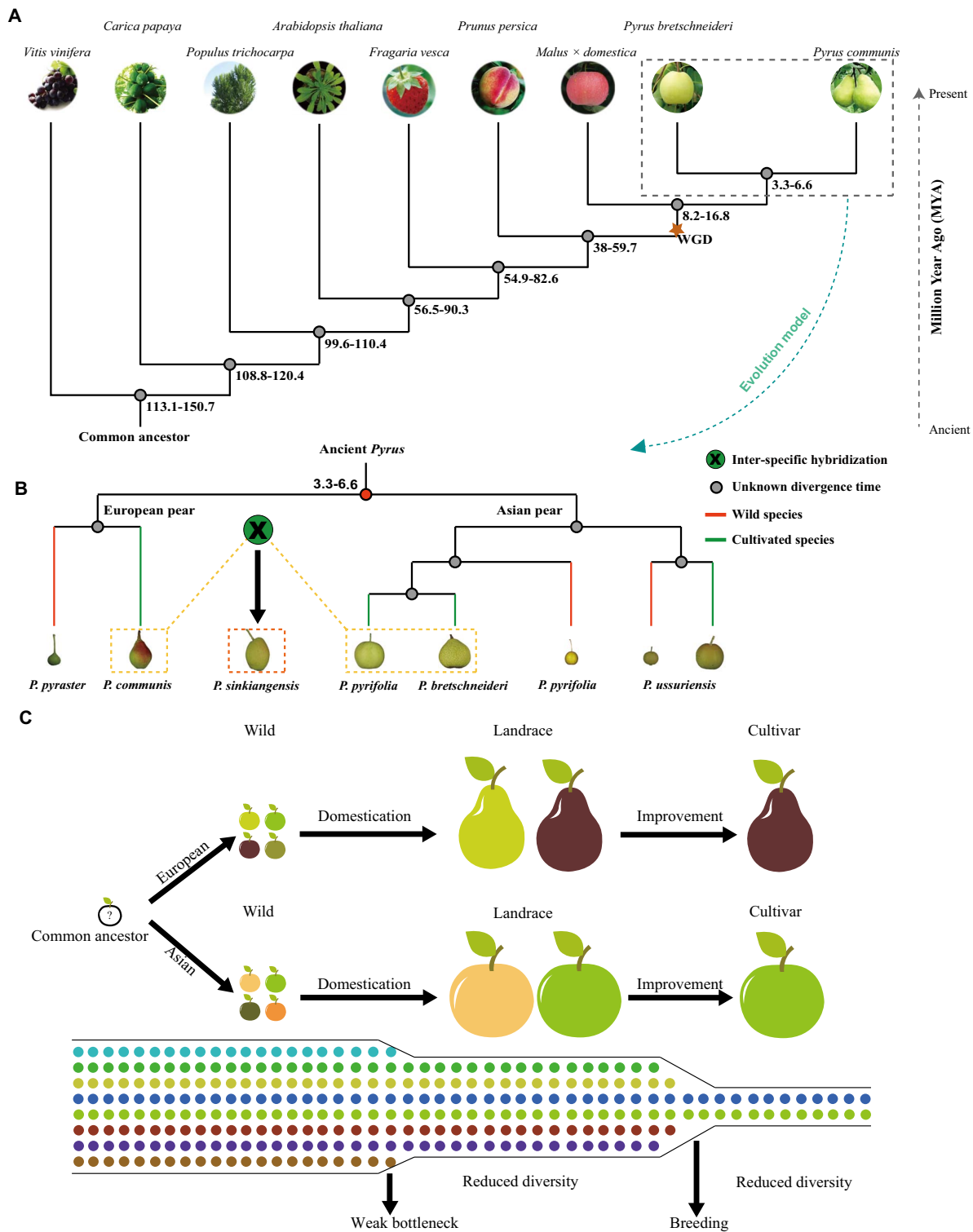
domestication history, and evolutionary processes of various fruit crops, including those for peach [25], grape [26], and apple [27].

Recently, 113 representative *Pyrus* accessions worldwide were resequenced [28]. This study led to the proposal of a new paradigm for pear germplasm dispersion and domestication. In this paradigm, it was suggested that current Asian and European pears originated from the southeast region of China, spread over to central Asia, and were then subsequently dispersed across Asia and Europe. Thus, each of the cultivated Asian and European pears independently underwent domestication in local regions [28]. Due to such independent domestication events, Asian and European pears were subjected to different selection pressures to meet the fruit appearance and taste preferences of different human populations. Generally, Asian pear has a round-shaped fruit with notable features, including crisp flesh, high stone cell content, low acidity, minimal aroma, high sugar, and mild flavor, whereas European pear is characterized by a typical pyriform-shaped fruit with smooth flesh, few stone cells, soft flesh, strong aroma, and strong flavor [2–4, 29]. At the genomic level, a total of 9.29 Mb of the genome, containing 857 genes, was identified to carry selective signatures in Asian pears, whereas a total of 5.35 Mb of genomic regions, containing 248 genes, was identified to carry selective signatures regions in European pears. However, only 47 genes were found to be common between the two pear types, suggesting that these genes were selected upon during the domestication of both Asian and European pears. These findings supported the hypothesis that Asian pears and European pears were independently domesticated in different regions.

## Pear domestication and improvement

Understanding the domestication and genetic improvement of a crop at the whole genome level can aid in future efforts to improve crop yield and other traits of interest.

By using resequencing and population genetic analysis of four different populations of pear (Asian wild, Asian cultivated, European wild, and European cultivated accessions), it was revealed that a weak domestication event occurred in pear [28]. The divergence time of Asian pears from European pears appeared to occur from 3.3 to 6.6 million years ago (MYA) compared to other plant species, including grape (*Vitis vinifera*), apple (*M. × domestica*), peach (*Prunus persica*), woodland strawberry (*Fragaria vesca*), poplar (*Populus trichocarpa*), papaya (*Carica papaya*), and the model plant *Arabidopsis* (*Arabidopsis thaliana*) (Fig. 1A). Furthermore, relationships between five domesticated pear populations and their corresponding wild relatives were delineated, with findings supporting an evolutionary model based on independent domestication of European and Asian pears (Fig. 1B). Specifically, it was demonstrated that *S-RNase* genes underwent rapid



**Figure 1.** A schematic diagram of divergence time and an evolutionary model of multiple plant species, including *Pyrus*, as well as a model of the domestication of Asian and European pears. (A) Divergence time of nine species, including *Vitis vinifera*, *Malus × domestica*, *Pyrus communis*, *Pyrus bretschneideri*, *Prunus persica*, *Fragaria vesca*, *Populus trichocarpa*, *Carica papaya*, and *Arabidopsis thaliana*. The estimated divergence times (MYA) are inferred based on single-copy orthologous groups and shown at each node. WGD, whole genome duplication. (B) An evolutionary model of wild and cultivated pear species [28]. (C) A schematic representation of the independent domestication process of Asian and European pears along with subsequent modern breeding efforts for cultivar improvement. The domestication process of pear has experienced a weak bottleneck resulting in slightly reduced diversity, while the improvement process shows significantly reduced diversity; thus, new efforts for restoring diversity are extremely urgent in the genetic improvement of pear cultivars.

evolution and balancing selection. Furthermore, the relatively low level of overlap in signatures of selection between Asian and European pears suggested that the

targets of selection differed between the two pear types. Moreover, the independent domestication model of Asian and European pears was also supported by the incidence



of separate groups in the phylogenetic tree, which further demonstrated that the divergence time of Asian and European pears occurred much earlier and prior to any possible human intervention. Notably, genes associated with fruit size, sugars, organic acids, stone cells, and volatile compounds were present in regions with selective sweep signatures (Fig. 1C). To further explore genetic changes at the RNA level that occurred during domestication and improvement, Li et al. [30] used a transcriptome dataset of 41 pear (*P. pyrifolia*) genotypes, consisting of 14 wild, 12 landrace, and 15 improved genotypes, to explore the genetic changes related to the domestication and improvement of pear. It was found that 11.13 Mb of genome sequence carried selective signatures of pear domestication, while 4.04 Mb of genomic regions carried selective signatures of pear improvement (Fig. 1C). Of particular note, several genes related to sugar content, stone cell content and fruit size were located in these selected regions, with some of these genes mapping to previously reported QTLs [30].

### Genome-wide variations

A high degree of diversity can be expected at the genomic level in self-incompatible perennial plants, such as that reported for *Pyrus* [28]. Often, NGS and long-read sequencing analyses detect numerous types of DNA sequence variants, including SNPs, InDels, and structural variations (SVs).

Genome resequencing efforts offer new knowledge pertaining to taxonomic classifications, phylogenetic relationships, evolutionary history, domestication, and genetic resources for pursuing innovative molecular-based breeding efforts. A recent genome-wide variation study involving 113 pear accessions representing all known *Pyrus* species (cultivated and wild, collected from 26 countries) confirmed the delineation of Asian and European pears [28]. This study revealed that *P. × sinkiangensis* was derived from a hybridization event between a cultivated Asian pear and a cultivated European pear.

Although RNA-seq is often used to assess levels of genome-wide gene expression, it also allows for the identification of genomic variants within transcribed coding gene regions. RNA-seq was used for SNP mining of the fruit of five different pear cultivars, including ‘Hosui’, ‘Yali’, ‘Nanguoli’, ‘Kuerlexiangli’, and ‘Starkrimson’ [31]. Subsequently, fruits at the enlarged fruit stage were collected from a group of 41 accessions of *P. pyrifolia* consisting of 15 enhanced genotypes, 12 landraces, and 14 wild accessions and subjected to RNA-seq. This transcriptome analysis led to the identification of 875,319 high-quality SNPs [30]. Based on these SNP data, landrace and wild pears were found to be closely related to each other, while a low level of genetic diversity was observed in the improved cultivar group [30]. Moreover, using nucleotide diversity ( $\pi$ ) and  $F_{ST}$  values, it was found that different selective sweeps occurred during both domestication and improvement. Moreover, the differential expression of

selected genes showed a 20.89% decrease from the wild group to the landrace group, while a 23.13% increase was observed from the landrace group to the enhanced genotype group. This result indicated that diversifying selection might play an important role during the improvement of pear [30].

A reduced-representation genotyping-by-sequencing (GBS) strategy targeting regions flanking restriction enzyme (RE) sites was developed by Elshire et al. [32]. This genotyping approach focuses on DNA sequence polymorphisms around methylation-sensitive RE sites and yields genome-wide polymorphism data across numerous samples. Unlike SNP arrays, GBS involves the simultaneous detection and scoring of SNPs in a population of interest; thus, this approach is free from ascertainment bias. The availability of reference genome sequences for both Asian [5] and European [20] pears has facilitated the implementation of GBS in pear genetic studies. Kumar et al. [33] conducted a GBS study using a single RE (*Bam*HI) to assess genetic diversity in Asian and European pears. Moreover, GBS was found to be useful for constructing a high-density linkage map, fine-mapping QTLs for red skin color [34], and pursuing genomic selection (GS) in interspecific populations of pear [35]. The restriction enzyme *Ape*KI was used to construct high-density linkage maps for Asian [36] and European pears [37]. Recently, a two-enzyme (*Eco*RI and *Nla*III)-based GBS was used to construct a linkage map for Asian pear [38]. Regardless of the enzyme system, GBS poses technical uncertainties that can result in the uneven sequencing of samples, the nonuniform distribution of SNPs, and missing genotype data [39].

With the release of pear reference genome sequences along with the availability of large numbers of SNPs, it has become feasible to design high-density SNP arrays for pear. Recently, a high-density 200K SNP genotyping array was constructed [40]. This array was used to improve the genome assembly, genetic mapping, and GWAS in pear [40]. A 70K Axiom array was also released [41]. Owing to its efficiency, flexibility, high throughput, and low cost, such a SNP array will serve as an important reference tool for GWAS and will be highly useful in pursuing further germplasm improvement and breeding efforts.

### Genetic linkage maps: Construction and mapping of trait-linked loci

Genetic linkage maps are powerful tools for use in investigating and understanding how agronomic traits are inherited from their parents. Prior to the assembly of the first Asian pear genome, studies focused on developing frameworks and encryption of linkage mapping [42–45]. It was not until Yamamoto et al. [46] used an F1 population of two European pear cultivars, ‘Bartlett’ and ‘La France’, that two independent maps for pear consisting of 17 linkage groups and corresponding to the basic chromosome number ( $n=17$ ) were constructed. Subsequently,

with the rapid development of pear genome sequences [5, 20], genome-wide DNA markers, including SNPs and simple sequence repeats (SSRs), were identified and developed. Wu et al. [15] used 3,143 SNPs from restriction-associated DNA sequencing (RAD-seq) to construct the first SNP-based high-density genetic map for pear and then used 98 SSR markers to anchor the corresponding linkage groups. The resulting map consisted of 3,241 markers, spanning 2,243.4 cM with an average distance of 0.70 cM between markers. This map has enhanced the development and analysis of pear genetic maps, along with the identification of QTLs for traits of interest that are useful in marker-assisted breeding (MAB) efforts [15].

Subsequently, linkage maps of different genetic backgrounds were constructed. However, these linkage maps lacked common markers, rendering it difficult to conduct comparative studies and to pursue analysis across different pear populations (Table 2). Li et al. [14] collected all genetic linkage maps of European pears and used common SSR markers across these different maps to merge them into a single integrated consensus map. This map allowed for anchoring a total of 291.5 Mb of the 'Bartlett' v1.0 sequence, exploring genetic structure patterns, conducting comparative studies among different maps, and identifying QTLs [14].

Genetic linkage maps can also be used to localize positions or genetic regions controlling target agronomic traits. Several important pear fruit traits have been located on particular chromosomes. As most traits assessed in linkage mapping populations to date are quantitative in nature, QTLs related to these traits are distributed along several linkage groups (LGs) (Table 2) [15, 47]. For example, QTLs for single fruit weight were identified on LGs 2, 3, 7, 8, 10, 11, 13, and 17. Moreover, a QTL for fruit firmness was identified on LG4, while those for harvest time were identified on LG3 and LG15 in Japanese pear. Another important economic trait is fruit skin color, in particular red-color pigmentation, a QTL for which was first located on LG4 using an F1 population of 'Max Red Bartlett', a red color mutant of 'Bartlett' (Table 2) [48]. Subsequently, map-based cloning was used to identify a red skin-related QTL on the tail end of LG5 associated with the *PyMYB114* gene, which regulates fruit anthocyanin biosynthesis [49]. Furthermore, QTLs for various disease resistance-related traits, such as fire blight [45], pear scab [50], and black spot [42], were identified along different linkage groups (Table 2) [51–65].

### Genome-wide association studies (GWAS) in pear

Genome-wide association studies (GWAS) serve as effective approaches for exploring genome-level genetic architecture(s) and have been widely used to identify genetic variants associated with human-related diseases. These approaches are adopted for use in plant population studies to identify candidate loci associated with complex

traits. Thus, GWAS provides opportunities for identifying candidate genetic loci associated with complex traits in pear and for developing robust molecular markers useful in pursuing MAB to enhance the rapid and accurate development of new cultivars.

The development of GWAS models varies with demands for degrees of accuracy, speed, and population size. A generalized linear model (GLM) can handle multiple variables and takes into account the population structure in the form of a covariance structure defined by the index *Q* [66]. Population structure can result in false-positive genotype-phenotype associations, and PCA is commonly used to quantify and correct for population structure in GWAS. Moreover, a mixed linear model (MLM) is also available for use in GWAS. An efficient mixed-model association (EMMA) [67] and a genome-wide efficient mixed-model association (GEMMA) [68] can serve as variance components and are exact methods, both belonging to MLM. EMMA eXpedited (EMMAX) [69, 70] and genome-wide rapid association using mixed model and regression (GRAMMAR) are approximate methods that allow for fast calculation of GWAS. However, GRAMMAR tends to underestimate associations [71]. Thus, a comprehensive overview of various factors must be taken into consideration, such as target species, quantitative and qualitative traits, population size, accuracy and speed, when different GWAS models are to be used.

For GWAS in perennial plants, such as fruit trees, natural germplasm populations are more readily available than controlled-cross (or hybridized) populations. GWAS has been conducted on various fruit trees, including apricots [72], peaches [73], and apples [27]. Recently, GWAS was conducted using 312 sand pear (*P. pyrifolia*) accessions and identified five loci associated with three fruit phenological traits, as well as 37 loci associated with eight fruit quality traits. Furthermore, a new gene, *PbrSTONE*, controlling stone cell conformation, was identified within these association loci [74].

Therefore, GWAS is a viable and useful approach for identifying QTLs/genes for various critical fruit quality traits and other desirable traits, as well as for contributing to the development of robust tools and resources for pursuing MAB in pear and other long-lived woody perennial trees, including self-incompatible fruit trees.

### Marker-assisted selection (MAS), marker-assisted breeding (MAB), and genomic selection (GS) in pear

As pear has a long generation time (5 to 7 years), traditional pear breeding programs are expensive and time-consuming [75]. Marker-assisted selection (MAS) and marker-assisted breeding (MAB) are deemed viable approaches for the molecular mapping of genes, as well as for pursuing genetic improvement efforts, particularly of long-lived perennial fruit trees, as most economic traits are complex and controlled by QTLs [76].

**Table 2.** QTL mapping of different agronomic traits in pear

Population	Population size	Marker type	Number of markers		Map length (cM)		Number of linkage groups (LGs)		Interval (cM)		Traits	LGs	Reference
			Female	Male	Female	Male	Female	Male	Female	Male			
'Kinchaku' × 'Kosui'	82	RAPD	120	78	768	508	18	22	4.2	4.2	Black spot	11	42
'Bartlett' × 'Hosui'	63	AFLP, SSR	226	54	949	926	18	17	4.9	4.9	Self-incompatibility	17	43
'Passe Crassane' × 'Harrow Sweet'	99	SSR, AFLP, MFLP, AFLP-RGA, RGA	155	156	912	930	18	19	5.8	5.8	Fire blight	2a, 2b, 4, 9	45
'Bartlett' × 'Hosui'	63	AFLP, SSR	256	180	1020	995	19	20	4	4	Self-incompatibility	17	44
'Bartlett' × 'Hosui'	63	AFLP, SSR	447	1000	1000	1000	17	17	2.3	2.3	-	-	46
'Hosui' × 'La France'	55	AFLP, SSR	414	1156	1156	1156	17	17	2.8	2.8	-	-	46
'Abbé Fénel' × 'Max Red Bartlett'	95	MFLP, SSR	123	110	908.1	879.8	18	19	7.4	7.4	Pear scab	3, 7	50
'Abbé Fénel' × 'Max Red Bartlett'	95	MFLP, SSR	123	110	908.1	879.8	18	19	7.4	7.4	Skin color	4	48
'Hosui' × 'La France'	-	AFLP, SSR	484	1204	1204	1204	17	17	2.49	2.49	Pear scab	2, 11, 14	52
'Bartlett' × 'Hosui'	-	AFLP, SSR	504	356	1080	1180	17	16	2.1	2.1	Self-incompatibility	17	53
'Bartlett' × 'Hosui'	63	AFLP, SSR	335	1174	1174	1174	17	17	3.5	3.5	-	-	53
'Yali' × 'Jingbaili'	145	AFLP, SSR	402	18	18	18	1395.9	1395.9	3.8	3.8	Leaf width	10, 15	54
'Abbé Fénel' × 'Max Red Bartlett'	-	SSR	-	-	-	-	-	-	-	-	Red skin color	9	55
'Nittaka' × 'Suyangri'	94	RAPD, AFLP, SSR	106	122	1006	1168	19	19	9.5	9.5	Fruit weight	2, 7, 8, 10	56
'Bayuehong' × 'Dangshansuli'	97	AFLP, SRAP, and SSR	214	122	1352.7	1044.3	17	17	6.3	6.3	Fruit diameter	10, 15	47
'Red Bartlett' × 'Nanguo pear'	74	SRAP	103	105	602.2	650	20	20	4.9	4.9	Fruit length	7 (two years), 8	57
'Bartlett' × 'Hosui'	63	SSR, SNP	485	965	965	965	17	17	2	2	Fruit shape index	1, 2 (two years), 7, 8	58
'Hosui' × 'La France'	55	SSR, SNP	370	415	1160	1177	17	20	3.1	3.1	SSC	2, 5, 6	58
PEAR1 × PEAR2	143	SSR, SNP	250	314	1132.3	1136.8	17	17	4.53	4.53	Fruit maturity date	8 (two years)	59

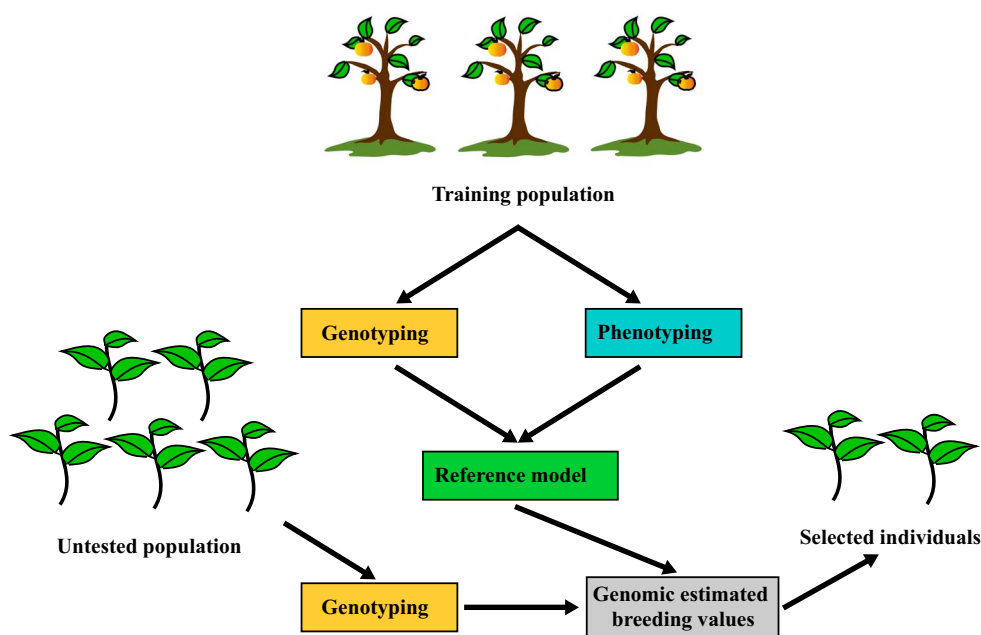
(Continued)

Table 2. Continued

Population	Population size	Marker type	Number of markers		Map length (cM)		Number of linkage groups (LGs)		Interval (cM)		Traits	LGs	Reference
			Female	Male	Female	Male	Female	Male	Female	Male			
'Akiakari' × 'Taihaku'	93	SSR, EST-SSR	208	275	799.1	1039.1	17	17	3.84	3.78	Fruit skin color Fruit weight Total soluble solids content	8 (two years) 3, 11 4, 8	60
'Bayuehong' × 'Dangshansuli'	56	SSR	734		1661.4		17		2.3		Firmness Preharvest fruit drop Harvest time	4 (two years) 1, 15 (two years)	61
'Aihuali' × 'Chili'	215	SSR, SLAF	12				1				Dwarf growth habit	16	62
'Red Clapp's Favorite' × 'Mansoo'	161	SSR, SLAF	4797		2703.6		17		0.56				63
Nine published maps		SSR, SNP	5085		3266		17		0.6				14
'Spadona' × 'Harrow Sweet'	162	SNP	2036		1433		17		0.70		Vegetative budbreak time	5, 8, 9, 13, 15, 17	37
'Mantianhong' × 'Hongxiangsu'	345	SNP	2606		1847		17		0.71				64
'Bayuehong' × 'Dangshansuli'	102	SSR, SNP	3241		2243.4		17		0.7		Skin color Single fruit weight Transverse diameter Vertical diameter Soluble solid content Flesh color	4, 13, 16 (two years) 13, 17 3, 11, 17 11, 17 (two years) 5, 10, 14	15
'Yuluxiang' × 'Mantianhong'	162	SNP	2489		1668		17		0.67		Skin smooth Length of pedicel Calyx status Juice content Number of seeds	2, 17 2, 14, 17 6 (two years) 1, 5 5 (two years), 9, 14, 17 (two years)	64
'E1 Dorado' × 'Potomac'	81	SNP	27052		1034.97		17		0.82		Fire blight	2	65
'Old Home' × 'Bartlett'	100	SNP	29703		1147.22		17		0.63		Fire blight	2	65
'NJA2R59T6' × 'Bartlett'	82	SNP	37182		1080.5		17		0.59		Fire blight	2	65

Note: AFLP, amplified fragment length polymorphism; cM, centi-Morgan; EST, expressed sequenced tag; LGs, linkage group; MFLP, microsatellite-anchored fragment length polymorphism; RAPD, random amplified polymorphic DNA; RGA, resistance gene analog; SLAF, specific locus amplified fragment; SNP, single nucleotide polymorphism; SRAP, sequence-related amplified polymorphism; and SSR, simple sequence repeat.





**Figure 2.** A pipeline for genomic selection

Early use of molecular marker systems included SSRs, amplified-fragment length polymorphisms (AFLPs), sequence-characterized amplified regions (SCARs), random amplified polymorphic DNAs (RAPDs), and cleaved amplified polymorphic sequences (CAPS) [15, 77–79]. All these molecular markers relied on the use of gel electrophoresis methods for screening individuals, seedlings, selections, and germplasm accessions. The release of whole genome sequences and the availability of robust SNP markers have contributed to accelerated progress in breeding programs. For example, high-quality and high-density linkage maps combined with molecular markers have allowed for fine-mapping of the region around a gene for susceptibility to black spot disease, as well as for identifying QTLs for 11 pear fruit-related traits [80].

High-throughput and accurate SNP-based markers, SNP arrays, and GBS can assist in pursuing early trait-predictive assays [37, 81]. DNA markers flanking major causal loci, such as those for red pear fruit skin color, can be used for selection, but a traditional MAS scheme is not well suited for complex traits that are controlled by several loci. GS is a form of MAS that utilizes thousands of genome-wide markers simultaneously to calculate their associations with trait phenotypes in a training population and to predict the genomic estimated breeding values (GEBVs) of individuals in a tested population [82]. The GEBVs of selection candidates are estimated solely based on SNP genotypes and estimated SNP effects. Hence, outstanding candidates can be identified at very early stages of development prior to phenotyping, thereby reducing generation intervals and increasing breeding efficiency (Fig 2). GS is best suited for polygenic traits, and high-density genotyping is essential for the application of GS to ensure that all QTLs are in population-wide linkage disequilibrium with SNP markers.

In a preliminary study to assess the utility of GS in a sand pear (*P. pyrifolia*) breeding program, Iwata et al. [83] screened 76 cultivars using 162 genome-wide markers and found that genome-wide predictions for GS were highly accurate (0.75) for harvest time and moderately accurate (0.38–0.61) for resistance to black spot, firmness of flesh, fruit shape (longitudinal section), fruit size, acid content, and number of spurs. Minamikawa et al. [84] used an Illumina Golden Gate genotyping assay comprising 1536 SNPs to conduct genomic predictions of fruit phenotypes in a *P. pyrifolia* population consisting of 86 cultivars and 765 seedlings. They found that the accuracy of genomic prediction was further enhanced when full-sib family data of a target family were available and suggested that phenotypic data collected in a breeding program were useful for pursuing GWAS and GS when these methods were combined with genome-wide marker data. Kumar et al. [35] evaluated the genetic architecture of 10 fruit phenotypes (including sensory traits) and used GBS to assess the potential of GS in 550 hybrid seedlings.

The above studies have demonstrated that the accuracy of genomic predictions in pear is moderate, particularly as most fruit quality traits are complex and polygenically controlled. Therefore, additional studies must be conducted to develop larger genotype-phenotype datasets to further improve the prediction accuracy of the fruit phenotypes of untested seedlings.

### Multiple omics: Identifying genes related to important traits

#### Candidate gene mining at the whole-genome level

As pear genomes have become available, the ability to identify gene families associated with desirable traits or

traits of interest has improved. Recently, studies have focused on identifying genes associated with fruit quality traits, such as those controlling skin color, sugar content, stone cells, and aromatic compounds. Members of the MADS-box gene family, MYB gene family, and lateral organ boundary domain (LBD) gene family were identified as candidate genes associated with anthocyanin biosynthesis in pear [8, 10, 85]. Sugar transporters, a SWEET gene family, the hexokinase gene family, and the phosphofructokinase gene family were identified as candidate genes associated with sugar biosynthesis [6, 9, 86, 87]. For the stone cell trait, MYB gene family, BZR gene family, and KNOX gene family were reported to be involved in stone cell conformation and related lignin biosynthesis in pear [8, 88, 89]. Flavor is a more complicated trait, and some candidate pear genes were identified as candidate genes contributing to aromatic compounds present in pear fruit [90, 91].

### Transcriptomes

A transcriptome, usually generated by RNA-seq, corresponds to all transcripts within a single cell or a population of cells at either a particular developmental stage or a physiological state [92].

The release of large-scale transcriptomic data for pear, including those for different cultivated species, developmental stages, tissues, and treatments for specific trait investigations, has facilitated the exploration of functional genes. Transcriptomes of ‘Dangshansuli’, ‘Nanguoli’, ‘Yali’, ‘Hosui’, ‘Kuerlexiangli’, ‘Starkrimson’, and *P. pyrifolia* accessions at key developmental stages were released, and these provided insights into biological processes underlying fruit quality traits, including stone cell contents, sugars, and volatiles [31]. In addition, gene expression during four developmental stages of pear pollen provided opportunities for studying the growth and cessation of pear pollen tubes [93]. Furthermore, transcriptome data from seven tissues were exploited to track the expression patterns of gene pairs between two pear subgenomes, thus providing critical evidence for unbiased subgenome evolution following palaeopolyploidization in pear and serving as valuable resources for investigating tissue-specific gene expression in pear [94].

In pursuit of specific pear traits, transcriptome analysis studies have focused on color pigment development, dormancy, and biotic and abiotic stresses. Transcriptomes of ‘Starkrimson’ pear versus its green color mutant, bagged versus unbagged ‘Pingguoli’, color-fading ‘Red Bartlett’ versus non-color-fading ‘Starkrimson’, russet- versus green-pericarp individuals of the ‘Qingxiang’ × ‘Cuiguan’ F1 group, and the color mutant ‘Red Zaosu’ versus ‘Zaosu’ were compared. As a result, several key candidate genes, including *LAR*, *ANR*, *Myb4-like1*, *myb4-like2*, *CCR*, *CAD*, and *PpBBX24*, were identified as candidates for color pigmentation [95–99]. Bud dormancy is a key developmental process for perennial plants to survive under adverse environmental conditions. When the transcriptomes of endodormant and ecodormant

Japanese pear (‘Kosui’) flower buds were subjected to RNA-seq, it was found that phytohormones, such as ethylene, were involved in endodormancy release [100]. Several studies were conducted to investigate cold, drought, salt stress, and black spot disease in pear, yielding critical datasets that would facilitate the identification and functional analysis of candidate genes [101–106].

### Proteomics

Proteomics is a tool for investigating proteins associated with gene expression and for delineating biochemical networks. In pear, a total of 1810 proteins were identified to be involved during the three stages of pear fruit development [107]. Moreover, 35 differentially expressed proteins related to fruit quality were identified, including three proteins related to sugar formation, seven proteins related to aroma synthesis, and 16 proteins related to lignin formation [107]. In another study, a total of 2841 proteins were identified during seven different pear fruit development stages, and it was suggested that invertases do not play a major role in sugar conversion in developing pear fruit; rather, it was likely that sucrose was broken down by sucrose synthases [108]. Furthermore, several putative sugar transporters from diverse gene families demonstrated developmental regulation [108].

To delineate the mechanism of fruit maturity of an early-maturing bud sport of ‘Zaosu’ pear, 75 differentially expressed protein spots were identified between an early-maturing bud sport and its original cultivar ‘Zaosu’ using a combination of 2-DE and MALDI-TOF MS technology [109]. Most of these differentially expressed proteins were closely associated with maturation, thus elucidating the maturation process in these pear genotypes [109].

### Metabolomics

Metabolomics deals with the analysis of large numbers of metabolites within a single cell, tissue, or organ in response to various conditions or treatments. In pear fruit, small molecule metabolites play important roles, such as those involved in basic metabolism; however, there are currently limited metabolomics studies on pear.

In early studies, the levels of glucose, fructose, sorbitol, and sucrose were determined in 10 pear accessions, while the levels of organic acids such as citric acid and malic acid were analyzed in 98 pear accessions using HPLC [3]. More than 100 volatile compounds could be identified in pear fruit [18, 19]. Recently, profiles of volatile compounds related to aroma were analyzed and compared in both ‘Dangshansuli’ and ‘Nanguo’ [110]. It was reported that fatty acid-related aroma volatiles were largely derived from metabolic precursors [110]. As wax is another important postharvest trait for pear, the metabolic profiles of wax compounds of 35 pear accessions from five pear species were evaluated. A total of 146 wax compounds were detected, including those

composed of fatty acids, esters, primary alcohols, and terpenoids [111].

### Small RNAs

MicroRNAs (miRNAs) are a class of 21- to 24-nucleotide noncoding RNAs that play important roles in growth and development [112]. As a result of NGS and high-throughput sequencing (HTS), many miRNAs associated with economic traits were detected in pear [113–115]. A particular focus was on pear fruit development and its regulation by miRNAs. Wu et al. [113] conducted deep sequencing of small RNAs from different developmental stages of ‘Dangshansuli’ fruit and found that miR160 acted as a regulator of an auxin response factor during fruit development [113]. Ma et al. [114] conducted an integrated analysis of RNA-seq and small RNA sequencing dataset from tissues of calyx abscission zones of ‘Korla fragrant pear’ and found that miRNAs miR858b and miR160a-3p were involved in calyx abscission [114]. In addition, miRNAs are also likely linked to pear fruit quality development. Using an integrated analysis of small RNAs and degradome sequencing of fruit skin tissues from ‘Meirensu’ pear combined with biochemical analysis, miR156 and its targeted *SQUAMOSA-promoter binding like (SPL)* gene family were found to participate in pear anthocyanin biosynthesis [115]. In another study, Wu et al. [113] identified nine sugar and acid metabolism-associated miRNAs (including miR1132, miR5077, and miR396b) and 11 lignin biosynthesis-related miRNAs (including miR397a, miR395a, and miR408b\*) following small RNA sequencing of pear fruit collected at different developmental stages [113]. In a subsequent study, it was demonstrated that *PbrmiR397a* reduced the lignin content by silencing three *PbrLAC* genes [116]. Other miRNAs (*pyr-miR1809* and *pyr-miR144-3p*) targeted *LAC* genes [117]. Several studies identified miRNAs associated with flavor and aroma in both ‘Suli’ and ‘Nanguoli’ pears, such as miR172, miR395, miR1132, and miR5077 [113, 118, 119]. Furthermore, biotic/abiotic stress-associated miRNAs were identified in pear through HTS, such as those involved in Apple Stem Grooving Virus (ASGV) defense, among other stress responses, including *pbr-miR164*, *pbr-miR399*, *pbr-miR168*, and *pbr-miR171* [118, 120, 121]. Interestingly, a pear-specific miR6390 that might promote dormancy release by degrading DAM (dormancy-associated MADS-box) was identified using small RNA and degradome sequencing [122].

### DNA methylation

DNA methylation is an important epigenetic modification that participates in diverse biological processes. Whole-genome bisulfite sequencing (WGBS or BS-Seq) or integrated analyses with RNA-seq are exploited in Rosaceae plants, and a Methylation Database for Rosaceae (MDR, <http://mdr.xieslab.org/>) has been established. As a result, various desirable traits associated with differentially methylated regions (DMRs) and DMR-regulated genes have been identified [123, 124].

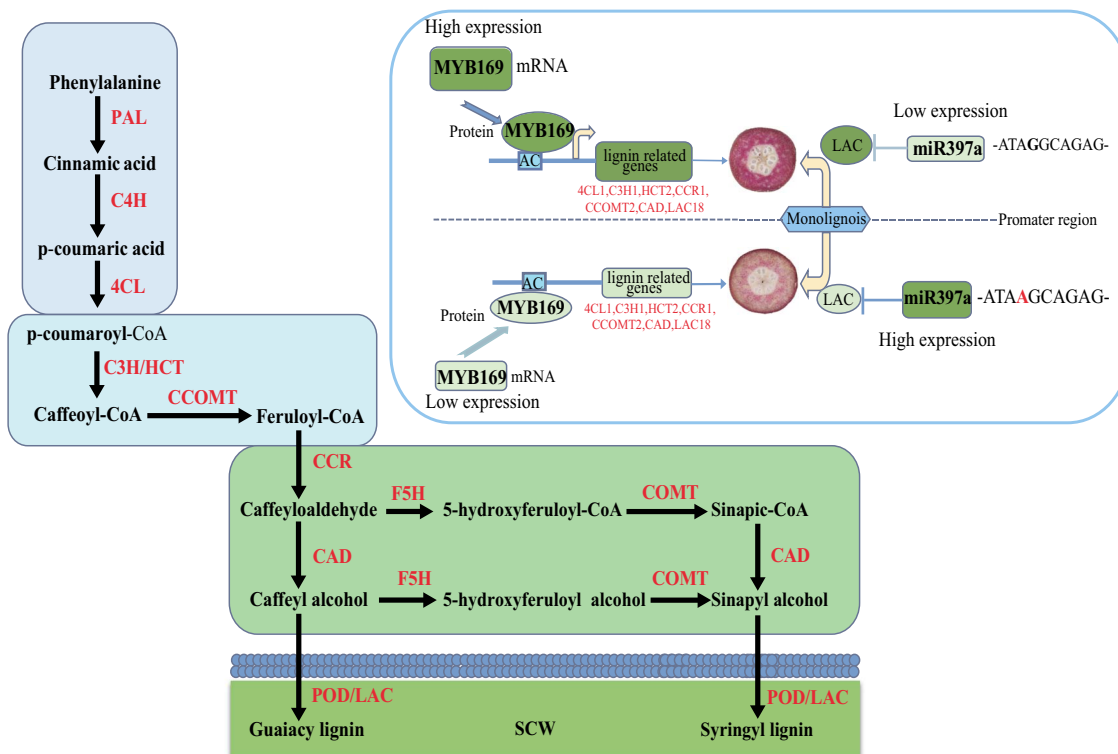
DNA methylation plays essential roles in fruit size development and fruit ripening [125, 126]. Additionally, methylation plays an important role in regulating anthocyanin accumulation. It is well known that MYB TFs play important roles in anthocyanin biosynthesis in Rosaceae plants [127, 128] and that methylation levels of MYB promoters also influence anthocyanin biosynthesis. For example, methylation levels of the *PcMYB10* promoter were associated with the green-skin sport of the pear cv. Max Red Bartlett, where it was observed that higher methylation of the *PcMYB10* promoter resulted in lower expression levels of *PcMYB10* and *PcUFGT* [128]. On the other hand, it was observed that the promoter *PyMYB10* had lower levels of methylation in anthocyanin-rich tissues in the red sport of ‘Zaosu’ pear and its red-stripped pigmentation patterns [127].

### Stone cells

Stone cells are sclerenchyma cells that develop following cessation of cell expansion in pear fruit, wherein secondary cell walls are deposited along primary walls. These stone cells can result in a gritty texture and poor fruit taste and have a negative impact on consumer satisfaction. Moreover, lignin and cellulose are major components of the secondary cell walls of stone cells [129]. Lignin biosynthesis and deposition are highly correlated with the formation of stone cells within the flesh of pear fruit [130, 131]. The component of lignin in pear stone cells consists primarily of guaiacyl-lignin (G-lignin), along with small amounts of syringyl-lignin (S-lignin) and p-hydroxyphenyl lignin (H-lignin) [132, 133]. It is important to point out that the dominant monolignols are mainly bonded with  $\beta$ -O-4 linkages [133].

Currently, stone cell contents in pears are most often evaluated using a frozen-HCl protocol. This protocol was used to determine the stone cell contents of 236 accessions of sand pear (*P. pyrifolia*) at 50 days after bloom [134]. However, this method is limited due to the incomplete separation of stone cells from the flesh of pear fruits and it is both awkward and time-consuming to perform. Recently, a PearProcess software program was developed based on an imaging protocol using computer vision to digitize images, and image processing algorithms were applied [135].

In the lignin pathway (Fig. 3), a series of enzymes are involved in the synthesis of three monolignols (G-, S-, and H-type lignin) from phenylalanine, such as phenylalanine ammonia-lyase (PAL) and cinnamate-4-hydroxylase (C4H) [136, 137]. These monolignols are polymerized into lignin, which is catalyzed by either peroxidase (PRX) or laccase (LAC) enzymes [138]. Among these enzymes, members of the 4CL, CCR, CAD, OMT, PRX, and LAC gene families have been identified [5, 139–143]. The expression levels of *Pb4CL1*, *PbCCR1*, *PbCCR2*, *PbCCR3*, *PbCAD2*, *PbCCOMT1*, *PbCCOMT3*, five *PbPRXs*, and five *PbLACs* were found to be consistent with changes in the lignin content during pear fruit development [139–143]. Several transcription factors are predicted as



**Figure 3.** A proposed mechanism of stone cell development in pear fruit 4CL: 4-coumarate CoA ligase; C3H: p-coumaroyl shikimate 3'-hydroxylase; C4H: cinnamate 4-hydroxylase; CAD: cinnamyl alcohol dehydrogenase; CCOMT: caffeoyl CoA O-methyltransferase; CCR: cinnamoyl CoA reductase; COMT: caffeic acid O-methyltransferase; F5H: ferulic 5-hydroxylase; HCT: hydroxycinnamoyl transferase; LAC: laccase; PAL: phenylalanine ammonia lyase; POD: peroxidase; SCW: second cell wall.

potential regulators of the lignification of stone cells, such as *PbWLM1a*, *PbWLM1b*, *PbKNOX1*, *PbIDD3*, *PbIDD5*, *PbMC1a/1b*, *PbMYB25*, *PbrMYB169*, and *PbMYB52* [89, 144–148]. *PbrMYB169* was found to positively regulate lignification of stone cell formation via regulation of the expression of 4CL1, 4CL2, C3H1, HCT2, CCOMT2, CCR1, CAD, and LAC18 (Fig. 3) [144]. Furthermore, *PbMC1a/1b*, a member of the metacaspase gene family, was identified as an important gene that plays a key role in the lignification of the cell wall, probably by interacting with *PbRD21* to regulate lignin synthesis-associated genes [148]. *PbrmiR397a* was found to be abundantly expressed during the early stages of pear fruit development [113]. It was revealed that *PbrmiR397a* was a key regulator of lignin synthesis in stone cells during fruit development by inhibiting three LAC genes coding for key enzymes in the lignin synthesis pathway [116]. Following analysis of the genome sequences of 60 pear cultivars, a SNP associated with low amounts of lignin in fruits was identified in the promoter of *PbrmiR397a* (Fig. 3) [116]. Thus, several SNP markers were developed for MAS to screen for pear stone cell contents.

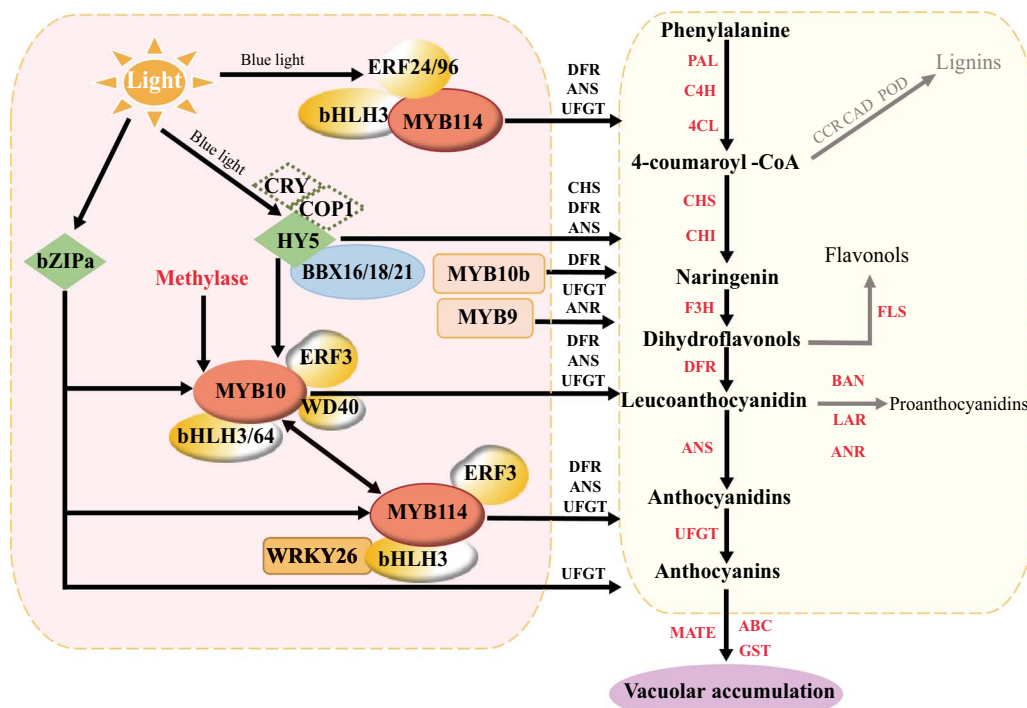
### Fruit color

Anthocyanins are secondary metabolites that play an important role in the color development of both flowers and fruits. Most red-skinned Asian pear fruits acquire anthocyanin-related coloration at the near-ripe stage, whereas the coloration of some European pears is

observed at the beginning of fruit development; these fruits then lose this coloration but later regain red coloration upon approaching maturity.

In European pears, the red coloration gene was mapped to LG4 [48]. However, *PyMYB10*, a transcription factor (TF) controlling pear anthocyanin content, was located on LG9; thus, it was not directly responsible for red- versus yellow-colored pear cultivars [55]. Subsequently, QTLs for red skin color were detected on LG5 in a population of the Chinese white pear (*P. bretschneideri*) using linkage mapping [49]. There were only minor correlations between the expression patterns of anthocyanin biosynthetic genes and the MYB-bHLH-WD40 (MBW) regulatory complex (*PyMYB10*, *PybHLH3*, and *PyWD40*), suggesting that the regulatory pathway of anthocyanin biosynthesis in red pears was complex [149]. Furthermore, *PyMYB10* was reported to regulate anthocyanin biosynthesis in some pear cultivars [150–152], and methylation levels of the *PyMYB10* promoter were associated with the development of green-skinned sports in 'Max Red Bartlett' European pear [128]. Moreover, *PbMYB10b* and *PbMYB9* were found to positively regulate flavonoid biosynthesis in pear fruit [153]. Subsequently, a map-based cloning strategy was used to identify a novel TF, *PyMYB114*, which was found to regulate fruit anthocyanin biosynthesis in pears [49]. In addition, *PybHLH3*, *PyWRKY26*, *PyZIPa*, and *PyMADS18* were also found to be involved in anthocyanin biosynthesis in red pears [154–156].





**Figure 4.** A proposed mechanism for fruit skin color development in pear ANS: anthocyanidin synthase; bZIP: basic region/leucine zipper; 4CL: 4-coumarate coenzyme A ligase; ABC: ATP-binding cassette transporter; ANR: anthocyanidin reductase; AP2/ERF: ethylene response factor/pathogenesis-related transcription factor; BAN: BANYULS; BBX: B-Box; bHLH: basic-helix-loop-helix; C4H: cinnamate 4-hydroxylase; CAD: cinnamyl alcohol dehydrogenase; CCR: cinnamoyl Co A reductase; CHI: chalcone isomerase; CHS: chalcone synthase; COP1: CONSTITUTIVE PHOTOMORPHOGENIC 1; CRY: cryptochrome; DFR: dihydroflavonol 4-reductase; F3H: flavanone 3-hydroxylase; FLS: flavonol synthase; GST: glutathione S- transferase; HY5: ELONGATED HYPOCOTYL 5; LAR: leucoanthocyanidin reductase; MATE: multidrug and toxic compound extrusion; MYB: v-myb avian myeloblastosis viral oncogene homolog; PAL: phenylalanine ammonialyase; POD: peroxidase; UFGT: UDP-glucose; flavonoid-3-O-glucosyltransferase; WD40: WD repeat protein; and WRKY: WRKYGQK domain.

PyMYB114 regulated anthocyanin biosynthesis by forming a complex with PybHLH3 and PyERF3 in Chinese red pears (Fig. 4) [49]. In 'Hongzaosu' pear, both PpERF24 and PpERF96 interacted with PpMYB114 to regulate blue light-modulated anthocyanin biosynthesis (Fig. 4) [157]. In addition, PyWRKY26 interacted with PybHLH3 to cotarget the promoter of PyMYB114 to regulate anthocyanin biosynthesis (Fig. 4) [156]. As light is essential for anthocyanin biosynthesis, Tao et al. [158] found that a blue-light signal transduction module, CRY-COP1-HY5, regulated anthocyanin biosynthesis in red pear [158]. Later, it was found that PybZIPa responded to light and promoted anthocyanin accumulation by activating PyUFGT in Asian pears [154]. Moreover, PyBBX16, PpBBX18 and PpBBX21 antagonistically regulated anthocyanin accumulation through competitive interactions with PpHY5 (Fig. 4) [159, 160].

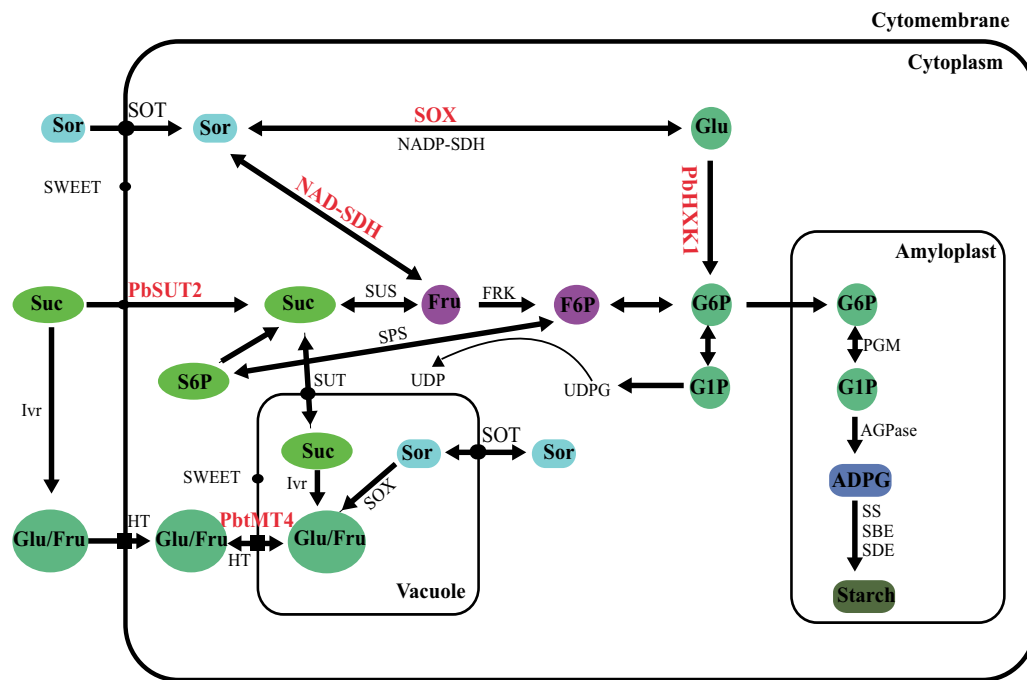
### Sugar and acid contents

Among the various factors influencing fruit quality, sugar is key in determining flavor. The major soluble sugars in ripe pear fruit are glucose, fructose, sucrose, and sorbitol [3]. Sorbitol, a major photosynthetic product in pear, is the predominant carbohydrate that is translocated in the phloem [161] and unloaded into the fruit via the apoplasm [162]. Monosaccharide transporters mediate the transport of a variety of

sugars. Thus far, a total of 18 hexose transporters (STPs), six tonoplast monosaccharide transporters (TMTs or TSTs), six plastidic glucose transporters (*pGlcTs*), and six inositol transporters (*INTs*) have been detected in pear [6]. However, only *PbTMT4* was experimentally demonstrated to participate in sugar accumulation in vacuoles (Fig. 5) [163].

Six sucrose transporters (*SUTs*) were identified in the pear genome [6]. Among these, *PbSUT2* was found to influence the sucrose content in sinks, as well as the overall sugar content during flowering (Fig. 5) [164]. Oura et al. [165] reported that the rate of fruit growth depended on both the capability of the fruit to accumulate sorbitol and the rate of sorbitol conversion [165]. Sorbitol metabolic enzymes include NAD-sorbitol dehydrogenase (NAD-SDH), sorbitol-6-phosphate dehydrogenase (S6PDH), and sorbitol oxidase (SOX). Proteins and full-length cDNAs of both SD6PH and NAD-SDH in pears were purified and determined, respectively (Fig. 5) [165, 166].

Sucrose metabolic enzymes belong to the following two classes: sucrose phosphate synthase (SPS) and sucrose synthase (SS). It was previously reported that SPS and SS were involved in sucrose synthesis in pear, and with sucrose accumulation, the activities of both SPS and SS were found to increase [167]. Moreover, hexokinase (HXK) is a rate-limiting enzyme in glycolysis and controls



**Figure 5.** A proposed mechanism of sugar accumulation in pear fruit ADPase: ADP-glucose pyrophosphorylase; ADPG: ADP-glucose; FRK: fructokinase; Fru: fructose; G1P: glucose-1-phosphate; G6P: glucose-6-phosphate; Glu: glucose; HXK: hexokinase; HT: hexose transporter; Ivr: invertase; PGM: phosphoglucomutase; SBE: starch branching enzyme; SDE: starch debranching enzyme; Sor: sorbitol; SOT: sorbitol transporter; SOX: sorbitol oxidase; SPS: sucrose phosphate synthase; SUS: sucrose synthase; SS: starch synthase; Suc: sucrose; SUT: sucrose transporter; tMT: tonoplast monosaccharide transporter; UDP: uridine diphosphate; and UDPG: UDP-glucose.

cell survival by promoting metabolism and/or repressing apoptosis. HXK plays roles in sugar signaling and acts as a sugar sensor [168, 169]. Thus far, a total of 10 HXK protein sequences have been characterized in pear [87]. *PbHXK1* was found to regulate the sugar content in pear fruit (Fig. 5) [170]. Furthermore, *PbPFP1* was found to increase both fructose and sorbitol levels in pear fruit [9].

There are many different organic acids found in pear fruit that contribute to the unique flavors of different pear cultivars. The main organic acids are malic acid [171]. It was reported that cytosolic aconitase (ACO) and NADP-dependent isocitrate dehydrogenase (NADP-IDH) were involved in citric acid catabolism [172–174], while mitochondrial citrate synthase (CS) was involved in citric acid synthesis in various fruits [175]. The vacuolar H<sup>+</sup>-inorganic pyrophosphatase (V-PPase) [176] and V-ATPase [177] were purified and characterized, and expression of genes coding for these proteins during fruit development were investigated [178–180]. Additionally, a *Py:vVpp* gene encoding the V-PPase subunit, which is likely to be involved in the high citric acid content found in ‘Yandangxueli’, was characterized [181].

## Aroma

Over 200 volatile compounds, primarily esters, alcohols, and aldehydes, have been identified in pear [110, 182, 183]. The fatty acid pathway is the main contributor of pear aroma components [110]. Major enzymes in this pathway include lipoxygenase (LOX), alcohol acyltransferase (AAT), alcohol dehydrogenase (ADH), and hydrogen peroxide lyase (HPL) [184, 185]. Based on

RNA-seq, candidate genes for AAT, LOX, ADH, and HPL were identified [186–188]. In ‘Nanguo’ pear, *PuADH3*, *PuLOX3*, and *PuAAT* were found to be upregulated with increased amounts of aroma esters; thus, suggesting that these genes might regulate the synthesis of aroma [189]. Although ADH, AAT, LOX, and HPL are known to play important roles in both the formation and release of aroma in other species [190–192], TFs involved in aroma formation have not yet been reported in pear. Recently, several miRNAs were found to negatively regulate the expression of key aroma genes during cold storage of ‘Nanguo’ pear fruit, and they were also reported to play important roles in aroma formation [119].

## Fruit maturity

It is likely that differential gene expression can either promote or delay fruit ripening. Thus, efforts are underway to delineate the mechanism of pear fruit maturity [193]. Some key genes regulating fruit maturity in pear have been investigated. Lelievre et al. [194] found that low-temperature treatment has a strong stimulating effect on 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase activity, but it has a weak effect on ACC synthase activity, thus indicating that the expression of ACC synthase genes is regulated by low temperature and thereby contributes to fruit ripening [194].

The plant phytohormone indole acetic acid (IAA) is important in plant growth and development. IAA content can be modulated by conjugating IAA to amino acids using acyl acid amido synthetases of the Gretchen Hagen 3 (GH3) protein family. GH3 proteins can bind to IAA and



IAA amide during pear fruit ripening; moreover, GH3.1 expression is activated by ERFs, thereby reducing the IAA content. Additionally, an actin-related protein, ARP4, is involved in ethylene-mediated fruit ripening, and it may work with pectin methylesterase 1 (PME1) to regulate the pear fruit ripening process [195]. Fruit softening serves as an important index for pear fruit ripening, with polygalacturonase (PG)1 and PG2 playing critical roles in the rapid softening of ‘Starkrimson’ pear fruits [196].

In ‘La France’ pear, PcPG3, PcPME1, PcPME2, PcPME3, PcGAL1, and PcCel2 were proposed to be involved in fruit softening during storage [197, 198]. Moreover, after treatment of ‘Nanguo’ pear fruit with the chemical compound 1-methylcyclopropene (1-MCP), an ethylene inhibitor, ethylene biosynthesis (ACS1, ACS4, and ACO), reception (ERS1a), and response (PG1 and PG2) genes were inhibited by 1-MCP treatment. Furthermore, the -expansin-encoding genes PcExp2, PcExp3, PcExp5, and PcExp6 were also upregulated during ‘La France’ pear fruit ripening, and their expression patterns were consistent with the rates of softening [199].

### Biotic and abiotic stress resistance

Biotic stress is one of the critical factors responsible for decreased fruit quality, yield gaps, tree losses, and increased production costs in pear. The major modes of mitigation for these biological stressors include chemical treatments, pruning, planting disease/pest-resistant cultivars, and physical barriers, such as netting. Often, pests and diseases that attack pear have a wide range of infection strategies and virulence mechanisms that are constantly evolving to adapt to their host. This renders effective and long-term control measures rather challenging to develop. The introduction of R genes, major resistance genes, into commercial cultivars is an effective strategy, but these genes can be overcome by the rapid adaptation of a pathogenic strain due to mutations in individual effector genes [200]. Nevertheless, polygenic disease resistance consists of many additive quantitative loci that can confer durable resistance against rapidly evolving pathogen populations. Moreover, pyramiding of such quantitatively controlled disease and pest resistance loci into susceptible cultivated backgrounds can lead to the development of cultivars with wide-spectrum and durable resistance [201]. QTLs linked to disease and pest resistance have been identified in pear, and molecular markers are becoming widely available for use in breeding programs [202].

As mentioned above, genome sequences of both Asian and European pears provided opportunities to develop high-throughput genotyping platforms to construct dense genetic maps for QTL mapping, identify genes/QTLs for resistance genes, and identify robust molecular markers useful for early screening and genotyping of young seedlings. Due to their economic importance, the genetic basis for fire blight [45, 203, 204], pear scab [80, 205, 206], black spot [80], and pear psylla [201] resistance was of particular interest, and

molecular markers linked to genetic resistance to some of these different biotic stressors were identified and became available for use in pear breeding programs.

Fire blight, caused by *Erwinia amylovora*, is a major threat to production in most of the countries where pears are grown. Different levels of susceptibility to fire blight were reported in European and Asian pear cultivars. Although no monogenic sources of fire blight resistance were reported, approximately 13 resistance loci of major and minor contributions to trait expression were identified for fire blight on LG2, LG4, LG9, LG10, LG11, and LG15 [45, 203, 204]. The genetic sources of these loci were derived from both European and Asian species, mainly *P. communis* and *P. ussuriensis*, respectively. Four QTLs linked to fire blight resistance were mapped onto LGs 2, 4, and 9 in the resistant European pear cultivar ‘Harrow Sweet’, and these QTLs had moderate/minor effects. A suite of AFLP-resistant gene analog (RGA) markers tightly linked to these QTLs were developed for use in MAB programs [45].

Abiotic stress is another important factor in the growth, development, productivity, and geographic adaptation of pear, including tolerance to drought, cold, and salt stresses. In recent years, a few key transcription factors associated with abiotic stress have been identified in pear, such as *PbrWRKY53* [207], *PbrMYB21* [208], *PbrMYB5* [209], *PbrbHLH1* [210], *PbeNAC1* [211], and *PbrNHX2* [212]. Among these, *PbrWRKY53* was found to play a positive role in drought tolerance by binding to the W-box element in the promoter region of *PbrNCED1* [207]. Moreover, transgenic tobacco expressing *PbrMYB21* exhibited higher levels of arginine decarboxylase expression along with a higher accumulation of polyamine, thus contributing to enhanced tolerance to both dehydration and drought stresses [208]. Furthermore, a novel R2R3-type MYB transcription factor, designated *PbrMYB5*, could bind to the promoter of *PbrDNAR2* and contribute to enhanced tolerance to chilling stress in pear [209].

### Dwarf growth habit

The dwarf growth habit allows for the establishment of high-density plantings for pear production. Utilizing suitable dwarf rootstocks or dwarf growth habit cultivars is an important approach to achieve this goal. The French cultivar ‘Nain Vert’, a seedling mutant of *P. communis* exhibiting a dwarf growth habit architecture with distinctive features of a compact crown, short internodes, and short stature, is observed to carry a single dominant gene *PcDw* [62]. However, the molecular mechanism of this dwarf architecture trait remains unknown.

Based on available pear and apple genetic maps along with draft genome sequences [16, 20], several DNA molecular markers, including SSRs and SNPs, tightly linked to the dwarf growth habit trait of ‘Nain Vert’ were developed, and these could serve as useful tools for MAS [213]. To uncover the sequence information, structure, and functional regulation of *PcDw*, the region containing this locus was narrowed down by fine-mapping using

these tightly linked DNA markers, and several candidate genes were inferred [213]. Recently, through comparative transcriptome analysis, the two most likely candidates, an arabinogalactan protein 7-like gene and a protein WVD2-like 7 gene, for the *PcDw* locus were identified, and a systemic overview of the complex regulatory network of the dwarf phenotype was provided [214].

As dwarf rootstocks are used for grafting scion cultivars to reduce the overall vigor of grafted trees, pear breeding efforts are underway to develop dwarf rootstocks that are graft-compatible with various scion cultivars, easily propagated, and highly resistant to both biotic and abiotic stresses. To accelerate the breeding scheme, it is necessary to elucidate the molecular mechanism and identify genetic determinants involved in the control of vigor in pear trees induced by rootstocks.

Two major QTLs, *Dw1* and *Dw2*, were identified from the apple rootstock 'M9' ('Malling 9'), accounting for most of the dwarfing effect conferred to the scion [215]. Recently, using an F<sub>1</sub> segregating population of 'Old Home' × 'Louise Bonne de Jersey' (OH×LB), a QTL influencing scion vigor in pear was mapped to the syntenic position in the apple 'M.9' *Dw1* locus, which is located at the upper end of LG5 [216]. Based on the high degree of synteny between apple and pear [217], it will be worthwhile to conduct comparative genome analysis to explore and delineate the genetic and molecular mechanisms of rootstock-induced dwarfing in pear.

### Genome editing

Sequencing of the pear genome is important for identifying genes and desired traits using genome editing. Recently, CRISPR–Cas9 has been used for genome editing in diverse plant systems [218]. Owing to its high efficiency, simplicity, and versatility of multiplexing, Cas9, a Class 2 type II CRISPR system, was used extensively to manipulate different plant traits, such as disease resistance, crop yield, and quality [219–221]. Subsequently, Cas12a (formerly known as Cpf1), a Class 2 type V endonuclease, was used for editing several plant genomes, such as those for rice [222], tobacco [223], maize [224], and *Arabidopsis* [224], at high efficiencies.

Recently, genome editing studies have been undertaken in a number of horticultural crops. Among these, certain vegetable crops were subjected to more genome editing efforts (72% of all genome editing reports in horticultural crops) than other crops, particularly tomatoes (42% of all genome editing reports in horticultural crops) [225]. Fruit crops are very important commercial horticultural crops. Due to their time-consuming breeding cycle and complex genetic backgrounds, CRISPR–Cas systems have been successfully used in only a few fruit crops. Early efforts focused on using a mutation of the phytoene desaturase (*PDS*) gene that yielded an albino phenotype. This was demonstrated in banana [226], kiwifruit [227], grape [228], citrus [229], apple [230], and strawberry [231, 232]. Strawberry was used as a model fruit crop system for pursuing functional studies of

particular genes of interest, such as the anthocyanin biosynthesis TF R2R3 MYB10, whereby CRISPR–Cas9-mediated gene editing was found to reduce fruit color [231]. In addition, the CRISPR–Cas9 system was designed to target the *DIPM1*, 2, and 4 genes encoding disease-specific (Dsp)A/E-interacting proteins and pathogenicity effectors of *E. amylovora* in apple to enhance genetic resistance against fire blight disease [233].

Efforts have been undertaken to generate compact plants that develop rapid terminal flowers and fruits to accelerate the breeding cycle in tree fruit crops by gene editing of floral repressor genes, such as *CENTRO-RADIALIS* (*CEN*)-like genes [234]. Recently, the CRISPR–Cas9 system was used for the first time in pear by targeting the *TERMINAL FLOWER 1* (*TFL1*) gene, and early flowering phenotypes were observed in 9% of transgenic pear plants targeted for the *PcTFL1.1* gene and 93% of transgenic apple lines targeted for the *MdTFL1.1* gene [235].

Ongoing gene editing efforts are being pursued using the CRISPR–Cas9 system along with other novel gene editing systems, as these tools will be useful for the functional analysis of target genes and enhancing various desirable economic traits in pear.

### Future research prospects

The first pear genome, reported in 2013, has served as a highly valuable resource for gaining sequence information, gene discovery, fine mapping, tool development, and variant detection. Over the last decade, WGS efforts for pear have had significant impacts on our knowledge of pear biology, gene function, inheritance, and trait physiology, leading to the availability of valuable resources and tools that will contribute to the genetic improvement of this highly valuable fruit crop. These resources will also support efforts to shorten the breeding cycle and increase breeding efficiency. For future pear research and breeding strategies, it is highly advisable to integrate different omics technologies into the discovery process of genes controlling traits of interest, the elucidation of gene function mechanisms, support for genotyping and phenotyping, and the acceleration of the breeding cycle. Additionally, advanced NGS methods and development, approaches to shorten the juvenility period, gene editing technology, and gene transfer technologies will all contribute to efforts to breed new pear cultivars with highly desirable economic characteristics.

Below are some specific areas of research interest that should be taken into consideration in future efforts.

### Genome development

Although whole genome sequences of pear are available, each single reference genome does not represent the wide diversity within a species due to the presence of high levels of genomic variation. Recently, a pangenome concept has been proposed that involves comparing the genomes of multiple related individuals,

wherein core genome segments are present in all individuals, while dispensable genome sequences are absent in some individuals. As pear has experienced independent domestication across Asian and European pears, major differences have been observed in different groups of wild and cultivated Asian and European pear accessions. Thus, pangenome sequencing will help us better characterize those subspecies of pear genomes and identify any unique genes that might be present in these genomes. Furthermore, it is necessary to construct a pear pangenome assembly to capture the wide genetic diversity present in *Pyrus*, as this will further support evolutionary studies and aid in breeding efforts in pear. Due to the high level of heterozygosity and large numbers of pear cultivars, short-read sequences and the uncertainty of the physical locations of genes will undoubtedly raise new challenges in constructing such a pear pangenome sequence assembly and sequence mapping.

It is also important to point out that the obtaining the haplotype genome of pear serves as another bottleneck due to the self-incompatibility and long generation time of pear fruit trees. However, long-read sequencing technology could help identify strand-specific sequences and overcome some of these problems.

### Resequencing and phylogenetic studies

As NGS has become less expensive, long-read sequencing has become more popular. Earlier pear resequencing data reached tenfold coverage for NGS [28]; however, this is not sufficient for identifying structural variations (SVs), other than for SNP calling. Such SVs include deletions, insertions, duplications, inversions, and translocations, which can then be identified using NGS data and long-read sequencing combined with multiple algorithms, such as splitting reads, read pairs, and assembly methods, to discover and characterize SVs in the pear genome.

Whole-genome resequencing will also allow for a better understanding of the phylogenetics, domestication, improvement, and evolutionary history of a crop at the whole-genome level. This will provide unprecedented amounts of genomic datasets that will almost certainly allow for advances in modern pear molecular breeding programs.

### Whole-genome association studies

For pear trait association studies, GWAS is an effective approach in natural populations, overcoming the long juvenile period and self-incompatibility issues in *Pyrus*. However, it is important to standardize parameters and units of measurements of various phenotypic data from multiple years and sites to uncover reliable trait associations in such GWAS. With the enhanced reliability and accuracy of trait associations, generated datasets will allow for investigating SVs across multiple genomes associated with target traits. Thus, in addition to SNPs and small InDels, these expanded datasets will allow for

investigating genomes for copy number variants (CNVs), as these SVs confer larger effects on plant phenotypes. In recent years, several SV-GWAS have been undertaken in various plant species [236–238]; however, such studies have not yet been conducted in pear.

### Integrated omics technologies

Currently, omics, which includes genetics, transcriptomics, epigenomics, proteomics, metabolomics, phenomics, and bioinformatics, offers various promising approaches that will accelerate breeding efforts. Thus, pursuing integrated omics efforts will allow for generating new knowledge of yet unknown genes, proteins, and metabolites. Such new knowledge will aid in genetic improvement and breeding efforts in pear. For future pear research, it is critical to exploit integrated omics approaches to enhance our knowledge not only of fruit development and quality traits but also of biotic and abiotic stresses and subsequently translate this knowledge into pear breeding efforts.

### Gene editing

At present, gene editing offers a promising approach to improve new cultivars for traits of interest. Compared with other approaches, gene editing offers advantages in versatility, efficiency, and specificity. Currently, CRISPR-based gene editing can result in many types of mutations in target sequences, including precise base substitutions, small deletions/insertions, large fragment deletions, and gene replacement. Moreover, gene-edited plants can be free of exogenous DNA sequences either through genetic segregation or if CRISPR reagents are delivered as ribonucleoproteins to yield nontransgenic plants. This method may be a quick approach to developing new cultivars or improving existing cultivars.

As genetic transformation and genome editing technologies are limited for pear, it is important to undertake efforts to develop new protocols. This will be critical for pursuing the functional analysis of genes and for moving ahead with robust and efficient breeding of new and improved cultivars.

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## Author Contributions

J.W. and S.S.K. contributed to the manuscript outline and led editing of all sections of the manuscript; J.L. M.Z. and X.L. finalized the organization of the outline. The authors' contributions to the different sections of the manuscript are as follows: "Overview of Asian and European pear genome sequences" and "Pear domestication and improvement" (X.L. S.Z.); "Genome-wide variations" (M.Z., R.W.); "Genetic linkage maps: Construction and trait linked loci mapping" (M.Q., and M.S.); "Marker-assisted selection, marker-assisted breeding, and genomic selection in pear" (A.C.A., K.L.W., and R.V.E.); "Multiple omics: identifying genes related to important traits" (J.L., M.Z., X.L., A.K., C.W., R.W., C.X., G.Y., R.T., H.L., W.W., M.M., K.Z., B.B., J.N., and J.A.); and "Future research prospects" (J.L., M.Z. and X.L.). All authors reviewed the manuscript in the draft and final forms.

## Conflict of interest statement

The authors declare that they have no conflicts of interest.

## Supplementary data

Supplementary data is available at Horticulture Research online.

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