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.

P. De Franceschi · L. Dondini (✉)
Department of Agricultural and Food Sciences, University of Bologna, Viale Giuseppe Fanin 44, Bologna, Italy
e-mail: luca.dondini@unibo.it

Present Address:
P. De Franceschi
Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Centro di ricerca Cerealicoltura e Colture industriali (CREA-CI), Via di Corticella 133, Bologna, Italy

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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,

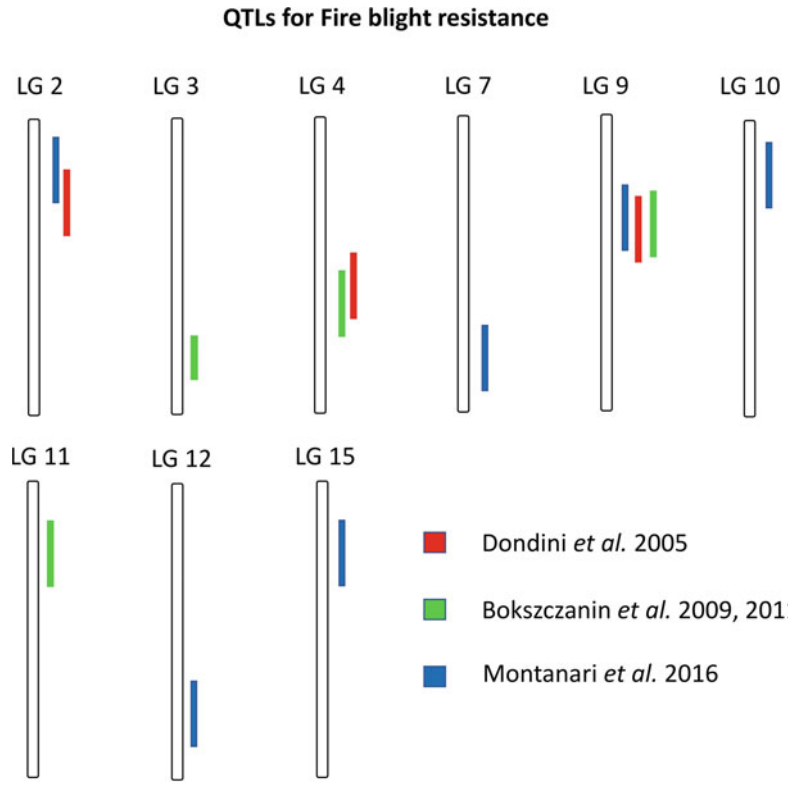

Fig. 6.1 Schematic representation of positions of known QTLs for fire blight resistance. Colors of QTL bars correspond to supporting literature

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

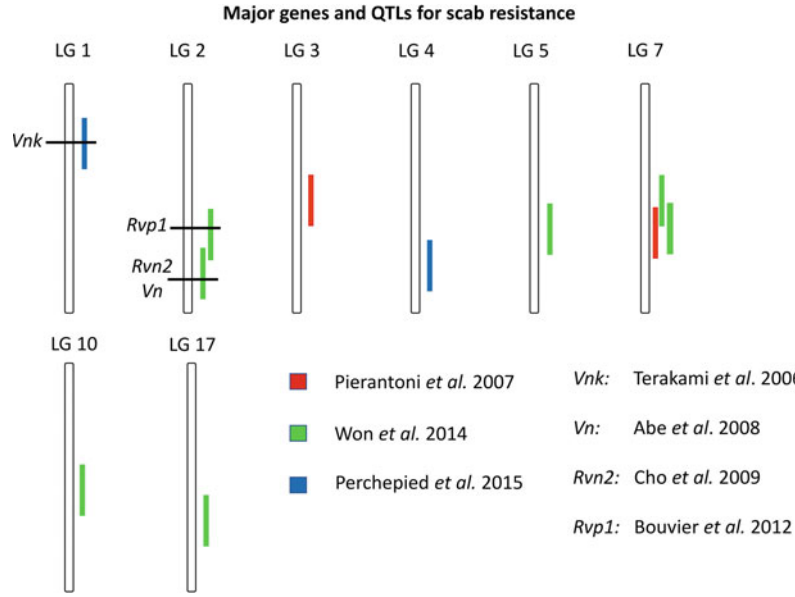

Fig. 6.2 Schematic representation of positions of major genes and QTLs for pear scab resistance

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).

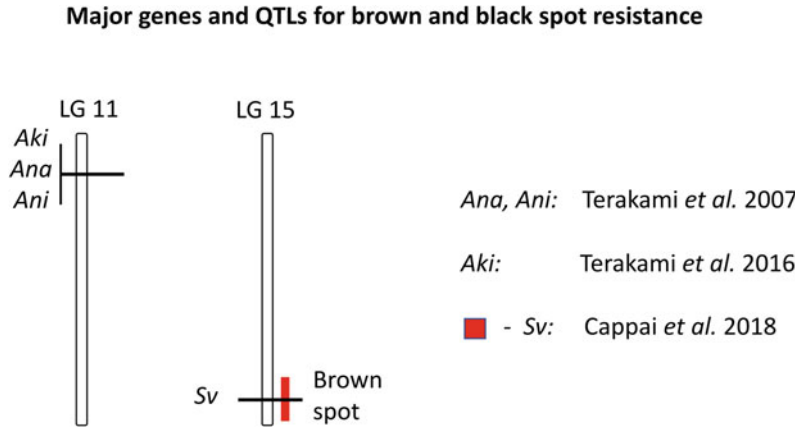

Fig. 6.3 Schematic representation of positions of major genes and QTLs for black and brown spot resistance

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.

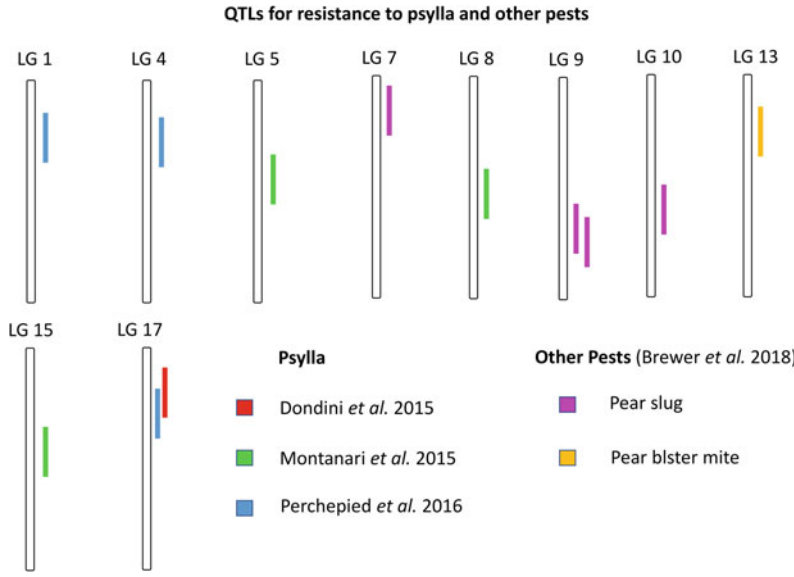

Fig. 6.4 Schematic representation of the positions of QTLs for psylla and other pests resistance

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

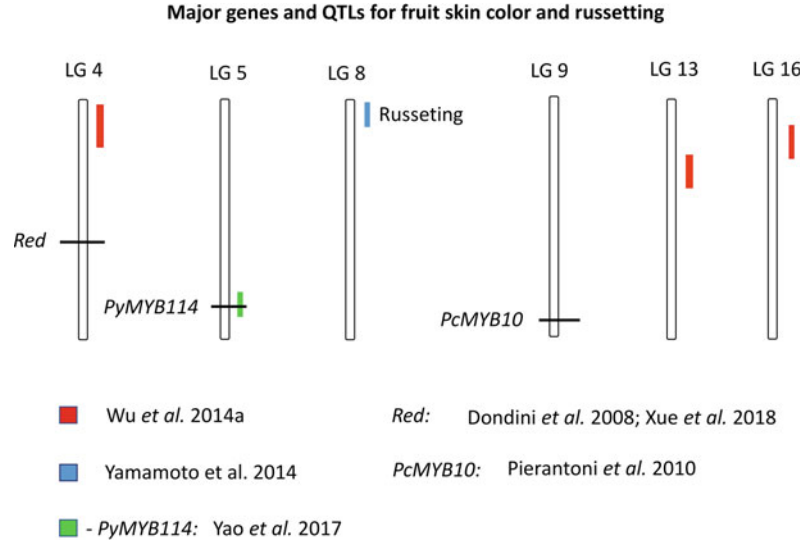

Fig. 6.5 Schematic representation of positions of major genes and QTLs for red skin color and for fruit russeting. QTLs refer mainly to Asian pears, whereas positions of the *Red* locus (from 'Max Red Bartlett') and of the *PcMYB10* gene for European pear are reported herein

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.

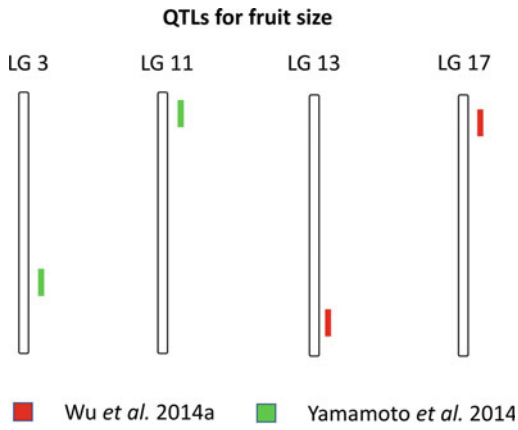

Fig. 6.6 Schematic representation of positions of QTLs for fruit size

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

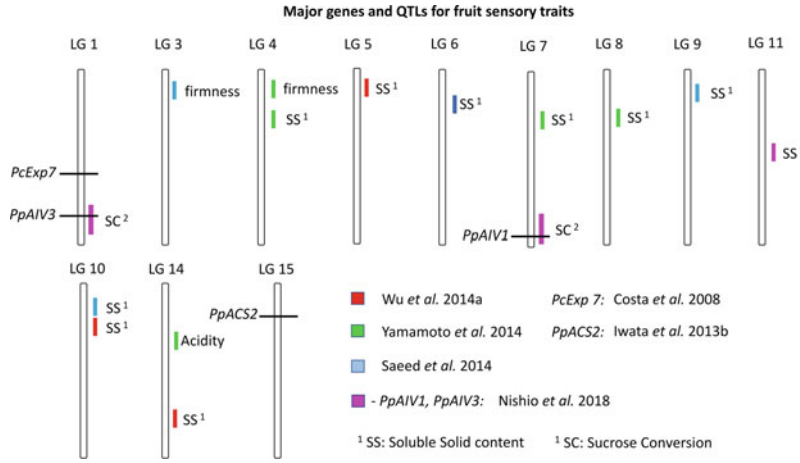

Fig. 6.7 Schematic representation of positions of major genes and QTLs for soluble solid content (SS), firmness, and acidity

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).

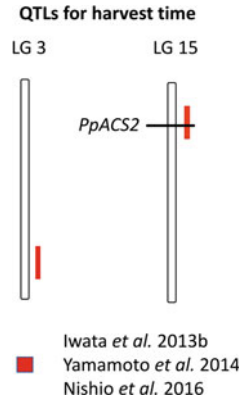

Fig. 6.8 Schematic representation of positions of major genes and QTLs for harvest time

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.

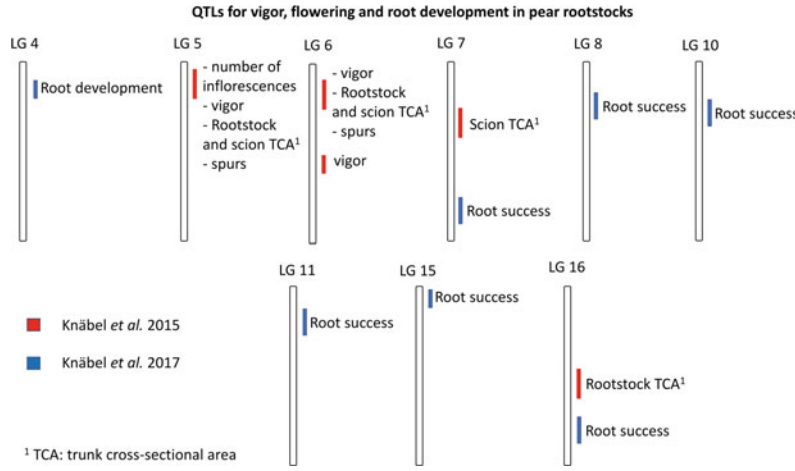

Fig. 6.9 Schematic representation of positions of QTLs for vigor, flowering, and root development in pear rootstocks

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.

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