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Review

Genomics of pear and other Rosaceae fruit trees

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The family Rosaceae includes many economically important fruit trees, such as pear, apple, peach, cherry, quince, apricot, plum, raspberry, and loquat. Over the past few years, whole-genome sequences have been released for Chinese pear, European pear, apple, peach, Japanese apricot, and strawberry. These sequences help us to conduct functional and comparative genomics studies and to develop new cultivars with desirable traits by marker-assisted selection in breeding programs. These genomics resources also allow identification of evolutionary relationships in Rosaceae, development of genome-wide SNP and SSR markers, and construction of reference genetic linkage maps, which are available through the Genome Database for the Rosaceae website. Here, we review the recent advances in genomics studies and their practical applications for Rosaceae fruit trees, particularly pear, apple, peach, and cherry.

Key Words: apple, co-linearity, genome sequence, peach, pear, reference map.

Introduction

The family Rosaceae consists of about 2500 species from 90 genera and includes diverse plants, which are primarily native to temperate regions (Hummer and Janick 2009). This family was traditionally classified into several subfamilies: Amygdaloideae, Maloideae, Rosoideae, Spiraeoideae, and others (Hummer and Janick 2009). In 2007, three subfamilies were suggested: Dryadoideae, Rosoideae and Amygdaloideae (Potter *et al.* 2007); the latter subfamily includes the former Amygdaloideae, Maloideae and Spiraeoideae. Many economically important crops producing edible fruits (e.g., apple, apricot, cherry, loquat, peach, pear, plum, quince, raspberry, and strawberry), nuts (e.g., almond), and ornamentals (e.g., rose) belong to the Rosaceae.

The most economically important members of the Rosaceae are apples (*Malus* × *domestica* Borkh.) and pears (*Pyrus* spp.), both of which belong to the subfamily Amygdaloideae, tribe Pyreae. Annual world fruit production of apples exceeds 80 million tons (FAOSTAT 2013), making them the third most important fruit (after citrus and banana). Pears are the second most important fruit species in Rosaceae, with world production of approximately 25.2 million tons (FAOSTAT 2013). Four important *Pyrus* species are commercially grown for edible fruit: Japanese pear (*P. pyrifolia* Nakai), European pear (*P. communis* L.), and Chinese pears (*P. bretschneideri* Rehd. and *P. ussuriensis* Maxim.) (Bell *et al.* 1996). Loquat (*Eriobotrya japonica* (Thunb.) Lindl.) is also an important fruit tree that belongs to the tribe Pyreae along with pears and apples. Several *Prunus* (“stone fruit”) species are also important fruit trees and include peaches and nectarines (*P. persica* (L.) Batsch), plums (*P. domestica* L., *P. salicina* Lindl.), apricots (*P. armeniaca* L., *P. mume* Siebold et Zucc.), and cherries (*P. avium* L., *P. cerasus* L.), which belong to the subfamily Amygdaloideae, tribe Amygdaleae. Annual world fruit production (in million tons) is as follows: peaches and nectarines, 21.6; plums, 11.5; apricots, 4.1; and cherries 2.3 (FAOSTAT 2013). In Japan, domestic fruit production (in thousand tons) is as follows: apples, 742; pears, 294; peaches, 125; apricots, 124; plums, 21; and cherries, 18 (FAOSTAT 2013).

Basic chromosome number $x = 7$, 8, 9, 15 or 17 was observed for Rosaceae members (Dirlewanger *et al.* 2009b, Evans and Campbell 2002, Potter *et al.* 2007). The subfamily Rosoideae, which contains rose, raspberry, and strawberry, has $x = 7$. The tribe Amygdaleae of subfamily Amygdaloideae, known for almond, apricot, cherry, peach, and plum, has $x = 8$. The former subfamily Spiraeoideae (tribe Spiraeeae of subfamily Amygdaloideae,) has $x = 9$. Basic chromosome number $x = 17$ is observed for the tribe Pyreae of subfamily Amygdaloideae, which contains apple, loquat, pear, and quince. Challice (1974, 1981) suggested that the Pyreae was generated by an allopolyploidization event between “Amygdaleae” ($x = 8$) and “Spiraeeae” ($x = 9$). Recent molecular genetic studies contradicted the allopolyploidization and supported the autopolyploid origin of hybridization between closely related members of Spiraeeae (Evans and Campbell 2002). Velasco *et al.* (2010)

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showed that a relatively recent (ca. 50 million years ago) genome-wide duplication resulted in the transition from nine ancestral chromosomes to 17 chromosomes in apple, based on whole-genome sequencing analysis.

In recent years, international collaborative studies by the Rosaceae research community have hastened progress in developing genetic and genomic resources for representative crops such as apple (*M.* × *domestica*), peach (*P. persica*), and strawberry (*Fragaria* spp.) (Shulaev *et al.* 2008); this strategy was based on a consensus that there are multiple Rosaceae model species (Dirlewanger *et al.* 2009b). These resources, including expressed sequence tags (ESTs), bacterial artificial chromosome (BAC) libraries, physical and genetic maps, and molecular markers and bioinformatics tools, are available through the Genome Database for the Rosaceae (GDR; http://www.rosaceae.org). The availability of this database has rendered various rosaceous crops highly amenable to functional and comparative genomics studies. Here we review recent progress in genomics studies on Rosaceae fruit trees such as apple, pear, peach, and cherry, and we discuss the newly accumulated knowledge and resources for comparative genomics studies on this family.

Genome sequences of Rosaceae fruit crops

Whole-genome sequences have been reported for Chinese pear (Wu *et al.* 2013), European pear (Chagné *et al.* 2014), apple (Velasco *et al.* 2010), peach (Verde *et al.* 2013), Japanese apricot (Zhang *et al.* 2012), wild strawberry (Shulaev *et al.* 2011) and cultivated strawberry (Hirakawa *et al.* 2014) (**Table 1**). The draft genome of the Chinese pear 'Dangshansuli' (*P. bretschneideri*) is now available (Wu *et al.* 2013). A total of 2103 scaffolds span 512.0 Mb (97.1% of the estimated genome size, 527 Mb) and are not anchored to the 17 chromosomes. The Chinese pear genome assembly contains 42,812 protein-coding genes, and about 28.5% of them encode multiple isoforms. The identified repetitive sequences (271.9 Mb in total) account for 53.1% of the genome. The difference in size between the pear and apple genomes is mainly due to the presence of repetitive sequences (predominantly transposable elements), whereas genic regions and protein-coding genes are similar in both species. A draft genome assembly of European pear 'Bartlett' (Chagné *et al.* 2014) contains 142,083 scaffolds and covers a total of 577.3 Mb (96.2% of the estimated genome size, 600 Mb). A total of 43,419 putative genes were predicted, of which 1219 are unique to European pear and are not found in other dicots plant genomes sequenced. Analysis of the expansin gene family and other cell wall-related genes showed their involvement in fruit softening in both European pear and apple. It is expected that pear genome sequences of Chinese and European pears will be assigned to 17 pseudo-chromosomes, which will greatly help us to conduct genetics and genomics studies in pears.

An international consortium has published a draft genome sequence of the domesticated apple 'Golden Delicious', a common founder cultivar in many breeding programs (Velasco *et al.* 2010). The genome assembly of 'Golden Delicious' consists of 122,146 contigs spanning a total of 603.9 Mb (81.3% of the estimated genome, 742.3 Mb). Seventeen pseudo-chromosomes (GDR, *Malus* × *domestica* Genome v1.0p) were obtained from these contigs. A total of 57,386 putative protein-coding genes were predicted. The MADS-box gene family involved in flower and fruit development is expanded in apple to 15 members. The other gene families related with transport and assimilation of sorbitol are also expanded, and are involved in Rosaceae-specific metabolism.

A high-quality draft reference genome sequence, Peach v1.0, of the doubled haploid genotype of the peach cultivar 'Lovell' has been reported (Verde *et al.* 2013). Since 'Lovell' is completely homozygous, its genome assembly has facilitated obtaining a reliable and unbiased reference genome. Using 827 markers from an updated *Prunus* reference map (Howad *et al.* 2005), Verde *et al.* (2013) organized 215.9 Mb of the Peach v1.0 genome into eight pseudomolecules covering 81.5% of the estimated genome (265 Mb). A total of 27,852 protein-coding genes were predicted. Furthermore, comparative analyses showed that the ancestral triplicated blocks in peach are detected, and that putative paleoancestor regions are detectable.

The genome of Japanese apricot or mei (*P. mume*) was one of the first genomes to be sequenced in the subgenus *Prunus* of the genus *Prunus* (Zhang *et al.* 2012). Japanese apricot was domesticated in China more than 3000 years ago as an ornamental plant and fruit tree. A 237-Mb genome assembly was generated from 29,989 scaffolds, 84.6% of which were further anchored to eight chromosomes in a

Table 1. Details of whole-genome sequencing in Rosaceae crops

	Pyrus bretschneideri	*Pyrus communis*	*Malus* × *domestica*	*Prunus persica*	*Prunus mume*	*Fragaria vesca*
Common name	Chinese pear	European pear	apple	peach	Japanese apricot	woodland strawberry
Cultivar name	Dangshansuli	Bartlett	Golden Delicious	Lovell	BJFU1210120008	Hawaii 4 (PI551572)
No. of contigs	25,312	182,196	122,146	–	45,592	–
No. of scaffolds	2103	142,083	–	391	29,989	3263
Genome assembly size (Mb)	512.0	577.3	603.9	215.9	237	209.8
Coverage (%)	97.1	96.2	81.3	81.5	84.6	95
Estimated genome size (Mb)	527	600	742.3	265	280	240
No. of putative genes	42,812	43,419	57,386	27,852	31,390	34,809
No. of pseudo-chromosomes	–	–	17	8	8	7
Reference	Wu *et al.* 2013	Chagné *et al.* 2014	Velasco *et al.* 2010	Verde *et al.* 2013	Zhang *et al.* 2012	Shulaev *et al.* 2011

genetic map constructed by restriction-site-associated DNA sequencing (RADseq); 31,390 protein-coding genes were annotated and integrated using *ab initio* gene prediction methods. By comparison of the *P. mume* genome with the available data, nine ancestral chromosomes of the Rosaceae family were reconstructed (Zhang *et al.* 2012).

Strawberry is one of the most important Rosaceae crops, and genomes were sequenced for wild woodland strawberry (Shulaev *et al.* 2011) and cultivated octoploid strawberry (Hirakawa *et al.* 2014). The woodland strawberry *F. vesca* ($2n = 2x = 14$), a diminutive herbaceous perennial, has a small genome (240 Mb) that shares substantial sequence identity with the genomes of the cultivated strawberry (*F.* × *ananassa*) and other economically important rosaceous plants. A total of 209.8 Mb (>95%) of the genome sequence were included in 272 representative scaffolds out of 3262 scaffolds, which were anchored to seven pseudochromosomes in the genetic linkage map. Gene prediction modeling identified 34,809 putative protein-coding genes. Macrosyntenic relationships between *Fragaria* ($x = 7$) and *Prunus* ($x = 8$) predict a hypothetical ancestral Rosaceae genome that had nine chromosomes. Furthermore, the whole genome sequences of peach, apple and strawberry were analyzed and compared by using 1399 orthologous regions between the three genomes, suggesting the ancestral genome ($x = 9$) to the extant *Fragaria*, *Prunus* and *Malus* genomes (Illa *et al.* 2011, Jung *et al.* 2012).

Genome-wide molecular markers

SSR markers

Simple sequence repeat (SSR) markers, or microsatellites, provide a reliable method for evaluation of genetic diversity and construction of genetic maps because of their co-dominant inheritance and the allelic abundance (Weber and May 1989). More than 1000 SSR markers have been developed in Japanese and European pears from genome sequences (Fernández-Fernández *et al.* 2006, Inoue *et al.* 2007, Sawamura *et al.* 2004, Yamamoto *et al.* 2002a, 2002b, 2002c), ESTs (Nishitani *et al.* 2009, Zhang *et al.* 2014), and next-generation sequencing (NGS) data (Yamamoto *et al.* 2013). Recently, a large number of SSR markers have been developed from the whole-genome sequence of Chinese pear 'Dangshansuli' (Chen *et al.* 2015). SSR markers developed in pear have been often used as anchor loci for reference genetic linkage maps of pear (Chen *et al.* 2015, Yamamoto *et al.* 2007).

In apple, hundreds of SSR markers have been developed (Celton *et al.* 2009, Gianfranceschi *et al.* 1998, Guilford *et al.* 1997, Liebhard *et al.* 2002, 2003, Moriya *et al.* 2012, Silfverberg-Dilworth *et al.* 2006, van Dyk *et al.* 2010) and used to construct high-quality genetic linkage maps with high marker density. Among *Prunus* spp., a large number of SSR markers have been developed for peach and almond (Aranzana *et al.* 2002, 2003, Cipriani *et al.* 1999, Dirlewanger *et al.* 2002, Howad *et al.* 2005, Nishitani *et al.* 2007, Sosinski *et al.* 2000, Testolin *et al.* 2000, Yamamoto *et al.* 2002d, 2003, 2005), cherries (Cantini *et al.* 2001, Downey and Iezzoni 2000, Joobeur *et al.* 2000, Struss *et al.* 2002), and apricot (Lopes *et al.* 2002).

SNP markers

Although at present SSR markers seem to be the best choice for genetics and genomics studies, marker systems with even higher throughput, such as single-nucleotide polymorphisms (SNPs), have been developed based on whole-genome sequencing data. Using NGS technology, Montanari *et al.* (2013) have developed 1096 SNPs from three European pear cultivars. A total of 857 polymorphic SNP markers were validated and mapped using a segregating population of European pear 'Old Home' × 'Louise Bon Jersey' and interspecific breeding families derived from Asian (*P. pyrifolia* and *P. bretschneideri*) and European pear pedigrees. Japanese pear 'Housui' (syn. 'Hosui') has also been used for EST sequencing of 185 Mb and genome sequencing of 529 Mb (Terakami *et al.* 2014). Using the GoldenGate assay, Terakami *et al.* (2014) evaluated 1536 SNPs detected in EST and genome sequences of 'Housui', and mapped 609 SNPs on its linkage map. Using RADseq, Wu *et al.* (2014) have genotyped Chinese pear SNPs by NGS and mapped 3143 SNPs on a linkage map.

The 8K apple Infinium SNP chip has been developed by the USA-based international research program RosBREED (Chagné *et al.* 2012). To discover genome-wide SNPs, 27 apple cultivars were chosen to represent worldwide breeding germplasms and were re-sequenced at low coverage by NGS technology. Of 2,113,120 SNPs detected, 7867 were selected for the apple 8K SNP array; after evaluation in segregating families and a germplasm collection, 5554 were found to be polymorphic (Chagné *et al.* 2012). Despite this progress, the number of robust and evenly distributed SNP markers in the 8K array was not sufficient. Recently, a 20K SNP array has been developed by the European research program FruitBreedomics, which focuses on bridging the gap between breeding and genomics (Bianco *et al.* 2014). This SNP array has been developed to enable high-precision genome-wide association analyses and pedigree-based analysis because of rapid decay of linkage disequilibrium. The SNPs included in this array were predicted from re-sequencing data derived from the genome sequences of 13 apple cultivars and one accession of crab apple (*M. micromalus*).

Using NGS technology, the International Peach SNP Consortium has re-sequenced the whole genomes of 56 peach breeding accessions (Verde *et al.* 2012, 2013) and developed a 9K SNP array (Verde *et al.* 2012). Using the GoldenGate assay, Martínez-García *et al.* (2013) have evaluated a set of 1536 SNPs of peach (*P. persica*) developed from the whole-genome sequences of three cultivars. The RosBREED Consortium has also developed a 6K SNP array for diploid sweet cherry (*P. avium*) and allotetraploid sour cherry (*P. cerasus*) (Peace *et al.* 2012).

Reference genetic linkage maps

High-density reference genetic linkage maps constructed with genome-wide molecular markers are important for many genetic and breeding applications in Rosaceae fruit trees including marker-assisted selection (MAS), mapping of quantitative trait loci (QTLs), identifying DNA markers for fingerprinting, and map-based gene cloning. Because good, comprehensive books and reviews have been produced that describes mendelian traits and QTLs in Rosaceae fruit trees (Dirlewanger *et al.* 2009a, Korban and Tartarini 2009, Salazar *et al.* 2014), it would be impractical to repeat that information. Instead we describe high-density reference genetic linkage maps in pear, apple and *Prunus*.

Pear reference maps

Among *Pyrus* spp., integrated high-density genetic linkage maps are available for the European pear cultivars 'Bartlett' and 'La France' and the Japanese pear cultivar 'Housui'; these maps are based on SSRs from pear, apple, and *Prunus*, amplified fragment length polymorphisms (AFLPs), isozymes, and phenotypic traits (Terakami *et al.* 2009, Yamamoto *et al.* 2002c, 2004a, 2007). The linkage maps of 'Bartlett', 'La France', and 'Housui' consisted of 447, 414, and 335 marker loci, respectively, and covered 17 linkage groups (LGs), which matched the basic chromosome number of pear ($x = 17$). Recently, Terakami *et al.* (2014) established a SNP assay to evaluate 1536 SNPs detected in the EST and genome sequences of 'Housui', and mapped 609 SNPs on a linkage map of 'Housui'. After all available SNP and SSR markers were integrated, the latest version of updated reference genetic linkage map of 'Housui' was reconstructed (**Fig. 1**), which consists of 1033 loci, including 609 SNPs from transcriptome and genome analyses, 61 SNPs from potential intron polymorphism markers (Terakami *et al.* 2013), 202 pear SSRs, 141 apple SSRs, and 20 other markers. Montanari *et al.* (2013) evaluated a set of 1096 European pear SNPs and 7692 apple SNPs, and mapped 857 and 1031 SNPs, respectively, on pear genetic maps. On the basis of whole-genome sequencing of *P. bretschneideri*, Chen *et al.* (2015) constructed a consensus genetic map consisting of 734 SSR loci derived from 1341 newly designed SSRs. Using RADseq, Wu *et al.* (2014) mapped 3143 SNPs on linkage maps of Chinese pear.

Apple reference maps

Several apple reference genetic linkage maps have been published. The first RFLP-based reference maps for 'Prima' and 'Fiesta' were constructed using 152 F_1 individuals and the two maps were aligned using 67 multi-allelic markers (Maliepaard *et al.* 1998). SSR-based integrated genetic linkage maps for 'Fiesta' and 'Discovery' were constructed using 840 molecular markers including 129 SSRs (Liebhard *et al.* 2002, 2003). A new set of 148 apple microsatellite markers has been developed and mapped on the reference linkage maps of 'Fiesta' and 'Discovery' (Silfverberg-Dilworth *et al.* 2006). Recently, the 8K Infinium SNP chip described above was used to construct a high-density genetic linkage map in apple (Chagné *et al.* 2012). In the FruitBreedomics project, 21 full sib families were SNP-genotyped, resulting in the genetic mapping of approximately 15,800 SNP markers (Bianco *et al.* 2014).

Prunus reference maps

The framework *Prunus* mapping population for construction of the reference map was an F_2 population (referred to as the T × E population) produced by crossing almond (*Prunus dulcis*) 'Texas' × peach (*P. persica*) 'Earlygold' and selfing a single F_1 plant (MB 1-73) (Joobeur *et al.* 1998). The T × E map contained 562 marker loci (Dirlewanger *et al.* 2004a). Howad *et al.* (2005) established a *Prunus* reference map using a set of six F_2 plants, one F_1 hybrid, and one parent of the F_1 hybrid, which could jointly define 65 possible different genotypes by the markers mapped on the T × E map. Howad *et al.* (2005) identified and mapped 264 SSR markers from 401 different SSR primer pairs. Recently, Verde *et al.* (2013) have aligned the eight main scaffolds (pseudo-chromosomes) against the updated version of the *Prunus* reference map constructed by Howad *et al.* (2005).

A consensus cherry genetic linkage map has been developed using 94 individuals from an interspecific cross, 'Napoleon' (*P. avium*) × *P. nipponica* accession F1292; this map consisted of 174 loci, including 160 SSR loci and 6 gene-specific markers, and covered 680 cM (Clarke *et al.* 2009). Cabrera *et al.* (2012) developed a sweet cherry (*P. avium*) reference linkage map using Rosaceae Conserved Orthologous Set (RosCOS) markers and SSR markers. RosCOS markers were identified from 3818 rosaceous unigenes comprised of two or more ESTs corresponding to single-copy genes in *Arabidopsis* (Cabrera *et al.* 2009, 2012). Of the 627 RosCOS markers, 81 SNPs representing 68 genome-wide RosCOS were mapped in four F_1 populations and placed on the consensus sweet cherry linkage map that included previously reported SSRs, indel, and *S-RNase* markers and spanned 779.4 cM. Klagges *et al.* (2013) constructed SNP-based high-density genetic maps of sweet cherry using intraspecific progenies from crosses between parental lines 'Black Tartarian' × 'Kordia' (BT × K) and 'Regina' × 'Lapins' (R × L). Of 5696 SNP markers tested, 723 and 687 were mapped onto eight LGs in BT × K and R × L, respectively. The obtained maps spanned 752.9 and 639.9 cM, with an average distance between markers of 1.1 and 0.9 cM, respectively. Very recently, genotyping-by-sequencing (GBS), a new methodology based on high-throughput sequencing, was applied for genome mapping in sweet cherry (Guajardo *et al.* 2015).

Marker-assisted selection in Japanese pear

MAS can accelerate selection and reduce the progeny size and the cost of raising individuals to maturity in the field, especially in fruit trees (Luby and Shaw 2001). In Japanese

Fig. 1. The latest version of integrated reference genetic linkage map of Japanese pear 'Housui' based on SNP and SSR markers. A total of 81 SSR loci including 67 from pear ESTs or 454 genome sequencing analysis and 14 from apple, which were included in the 'Housui' map of Yamamoto *et al.* (2013), were added to the recently published SNP-based map (Terakami *et al.* 2014). Linkage groups are designated as Ho1 to Ho17, HoX1 and HoX2. The number to the left of each marker indicates genetic distance (cM). SSR markers (green, underlined) were developed from pear. SSR markers (red, italicized) were developed from apple. SNP markers developed by transcriptome analysis are denoted by JPsnpHou and SNP markers developed from potential intron polymorphism markers are denoted by TsuSNP. Distorted segregation is indicated by a significant P value of the χ^2 test: *P = 0.05, **P = 0.01, ***P = 0.005.

Fig. 1. (continued)

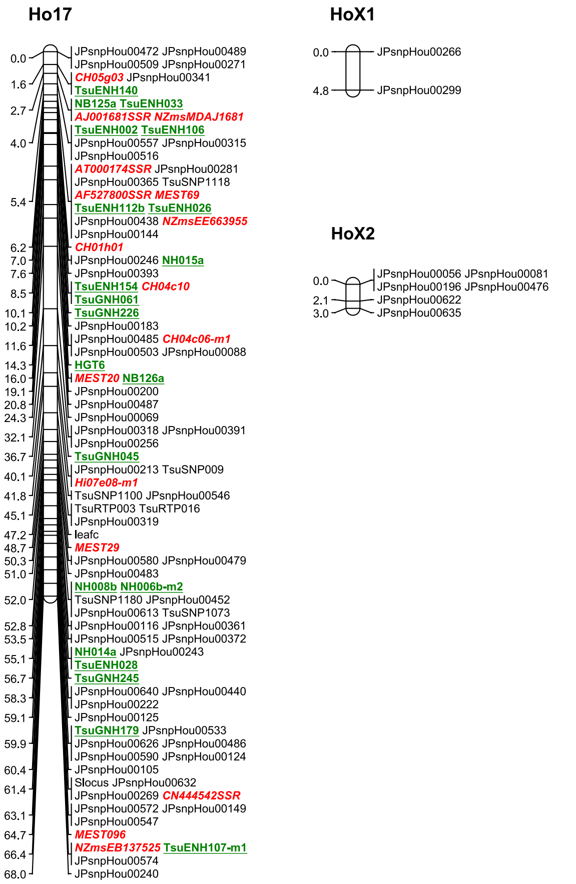

Fig. 1. (continued)

pear, several molecular markers associated with genes of interest traits have been identified and used for MAS in practical breeding programs of the National Agriculture and Food Research Organization (NARO) Institute of Fruit Tree Science, Japan (**Table 2**). Since several characteristics were already analyzed by genome mapping, QTL analysis, or both, the positions of responsible genes (loci) were identified in genetic linkage maps and tightly linked molecular markers were identified; these data are deposited in the public database of the Applied Crop Genomics Research Center (http://www.naro.affrc.go.jp/genome/index.html). DNA markers have been identified that are associated with genes for resistance to scab disease caused by *Venturia nashicola* (Gonai *et al.* 2012, Iketani *et al.* 2001, Terakami *et al.* 2006) and for resistance (or susceptibility) to black spot disease caused by a Japanese pear pathotype of *Alternaria alternata* (Banno *et al.* 1999, Iketani *et al.* 2001, Terakami *et al.* 2007). Self-incompatibility in Japanese pear is controlled by a single multi-allelic *S*-locus, and *S*-genotype identification is important for breeding and selection of pollen donors for fruit production. Several molecular assays for rapid and reliable *S*-genotype determination have been established, such as polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis (Ishimizu *et al.* 1999) and allele-specific PCR amplification (Nashima *et al.* 2015). The S_4^{sm} allele of the self-compatible cultivar 'Osa-Nijisseiki' (a mutant of the self-incompatible cultivar 'Nijisseiki') has been identified and found to lack a 236-kbp genomic region that includes the S_4-*RNase* coding region (Okada *et al.* 2008). Molecular markers associated with the following fruit-related traits were also revealed: fruit storage potential controlled by ethylene production (the 1-aminocyclopropane-1-carboxylate [ACC] synthase gene; Itai *et al.* 2003), fruit skin color (Inoue *et al.* 2006, Yamamoto *et al.* 2014), and harvest time (Yamamoto *et al.* 2014). These markers can be used for MAS in Japanese pear breeding programs.

Synteny in Rosaceae fruit trees

It is expected that comparative genomics in Rosaceae fruit

Table 2. Molecular markers associated with genes of interest in Japanese pear and their positions in genetic linkage maps

Characteristics	Gene symbol	Gene sources	Linkage group nos.	Associsted molecular markers (Accession nos.)	F-primer sequences (5′-3′)	R-primer sequences (5′-3′)[a]	References
Scab resistance to *V. nashicola*	*Vnk*	Kinchaku	1	TsuENH184 (AB621908)	cctccctcagtacccatcaa	GTTTCTTtgaactccttcactcaccttcc	Gonai *et al.* 2012, Terakami *et al.* 2006
				TsuENH101 (AB621905)	tgcctaatggaagggtccta	GTTTCTTcaaggaagagaagaccgacg	
				TsuENH157 (AB621907)	tagcagcagctctcctccac	GTTTCTTgtcagcacccctctgatgtt	
Black spot susceptibility	*A*	Osa Nijisseiki	11	CMNB41/2350	gacagcgtccta		Banno *et al.* 1999
	Ani	Osa Nijisseiki	11	CH04h02	ggaagctgcatgatgagacc	ctcaaggatttcatgcccac	Terakami *et al.* 2007
				CH03d02	aaactttcactttcacccacg	GTTTCTTactacatttttagatttgtgcgtc	
	Ana	Nansui	11	CH04h02	ggaagctgcatgatgagacc	ctcaaggatttcatgcccac	Terakami *et al.* 2007
				CH03d02	aaactttcactttcacccacg	GTTTCTTactacatttttagatttgtgcgtc	
Self-incompatibility	*S*	Japanese pear	17	S-RNase	tttacgcagcaatatcag	acrttcggccaaataatt	Ishimizu *et al.* 1999, Nashima *et al.* 2015
Self-compatibility	S_4^{sm}	Osa Nijisseiki	17	SM	tcgtcttagggatttccaatgc	gccttaagggttcattgggc	Okada *et al.* 2008
Fruit skin color		Niitaka	8	OPH-19-425	ctgaccagcc		Inoue *et al.* 2006
	FruC	Akiakari	8	Mdo.chr8.10	tgcagccctcaaacttttct	caacccaactccagcaattt	Yamamoto *et al.* 2014
				CH04g12	caccgatggtgtcaacttgt	caacaaaatgtgatcgccac	
Fruit storage	*PpACS2*	Japanese pear	15	ACC synthase	gtcacagaatcaacgattga	agtagaacgcgaaaacaaat	Itai *et al.* 2003
Harvest time	HarT-1 (QTL)	Taihaku	3	BGA35 (AB219799)	agagggagaaaggcgatt	GTTTCTTgcttcatcaccgtctgct	Yamamoto *et al.* 2014
	HarT-2 (QTL)	Taihaku	15	PPACS2	ggtatctttgtcggcaatc	gctctcaaggctttcttctctc	Yamamoto *et al.* 2014

[a] GTTTCTT: pig tail sequence for DNA sequencer analysis.

trees will be able to integrate conserved candidate genes, molecular markers associated with interest traits, and QTLs, in order to verify how the genetic and molecular factors control traits like fruit quality and texture across species and genera. Therefore, synteny or comparative genome mapping is an important approach, which determines the homologous genes of related species, as well as the co-linearity (conservation of the gene order) among conserved genomic regions.

Co-linearity between Pyrus and Malus

Yamamoto *et al.* (2001) applied apple SSR markers intergenerically for the characterization of several pear species (*P. pyrifolia*, *P. bretschneideri*, *P. ussuriensis*, *P. communis*, and *P. calleryana*). Nucleotide repeats were detected in the amplified fragments of pear and apple by both sequencing and Southern blot analyses, and the differences in fragment sizes between pear and apple were due mainly to the differences in the number of such repeats. The SSR markers are applicable across genera in the tribe Pyreae, subtribe Pyrinae, which includes apple, pear, quince (*Cydonia oblonga* Mill.), and loquat (Liebhard *et al.* 2002, Soriano *et al.* 2005, Yamamoto *et al.* 2001, 2004a, 2004b). When pear genetic linkage maps ('Bartlett' and 'La France') were compared with the apple reference maps ('Discovery' and 'Fiesta'), 66 apple SSR loci could be positioned onto the homologous LGs of pear (Yamamoto *et al.* 2007). Furthermore, SSR locus positions within LGs were almost identical in pear and apple, indicating good co-linearity in all 17 LGs. Gisbert *et al.* (2009) used SSR markers from apple and pear to construct genetic linkage maps of loquat cultivars 'Algerie' and 'Zaozhong-6'; the loquat maps showed a high synteny with apple maps when anchored SSR markers were used. Fukuda *et al.* (2014) identified almost perfect co-linearity of LG10 among loquat, pear, and apple. These findings suggest that all chromosomes of the genera in the tribe Pyreae show co-linearity despite considerable differences in the genome sizes, which range from 1.11 pg/2C to 1.57 pg/2C (Dickson *et al.* 1992, Dirlewanger *et al.* 2009b).

Co-linearity within Prunus

The marker transferability is extremely high within *Prunus*. For example, among 277 *Prunus* SSRs, including 141 from peach (*P. persica*), 58 from apricot (*P. armeniaca*), 31 from almond (*P. dulcis*), 9 from sweet cherry (*P. avium*), 4 from sour cherry (*P. cerasus*), and 6 from Myrobalan plum (*Prunus cerasifera* Ehrh.), 95.3% showed PCR amplification in Myrobalan plum (Dirlewanger *et al.* 2004a). Furthermore, Mnejja *et al.* (2010) examined *Prunus* SSR markers for transferability across rosaceous crops using nine species, almond (*P. dulcis*), peach (*P. persica*), apricot (*P. armeniaca*), Japanese plum (*Prunus salicina* Lindl.), European plum (*Prunus domestica* L.), sweet cherry (*P. avium*), apple (*M.* × *domestica*), pear (*P. communis*), and strawberry (*F.* × *ananassa*). Of the 145 SSRs derived from *Prunus* species, 83.6% of amplified bands of the expected size range were identified in other *Prunus* species, and the proportion of SSRs showing polymorphism was also high (63.9%) (Mnejja *et al.* 2010). In contrast, only 16.3% of the *Prunus* SSRs were transferable across species of other Rosaceae genera such as apple, pear, and strawberry (Mnejja *et al.* 2010).

SSR markers developed for various *Prunus* species have been intensively used to compare *Prunus* linkage maps (Dirlewanger *et al.* 2004b). Detailed map comparisons were performed using common SSR markers between the reference genetic linkage map T × E (Joobeur *et al.* 2000) and the maps of *P. armeniaca* (Lambert *et al.* 2004), *P. davidiana* (Foulongne *et al.* 2003), and *P. cerasifera* (Dirlewanger *et al.* 2004a). The distribution and order of SSR markers in all *Prunus* species show complete synteny except for a reciprocal translocation between LGs 6 and 8 detected in peach and almond (Dirlewanger *et al.* 2004b, Jáuregui *et al.* 2001). The SNP-based sweet cherry maps displayed high synteny and co-linearity of all eight LGs with the *Prunus* reference map and with the peach genome v1.0 (Klagges *et al.* 2013).

Synteny between Pyrus (Malus) and Prunus

Transferability of SSR markers is very low between tribes, as shown by comparing *Prunus* and *Pyrus* (*Malus*). Cipriani *et al.* (1999) found that only 18% of peach SSRs showed amplified bands in apple. Similarly, Yamamoto *et al.* (2004a) observed that only 10% of the *Prunus* SSRs could be transferred to the genetic linkage maps of *Pyrus* ('Bartlett' and 'Housui'). Only one out of 15 apple SSR markers was transferable to *Prunus* (Liebhard *et al.* 2002). A total of 613 RosCOS markers were successfully amplified and mapped on the *Prunus* T × E reference map. These RosCOS markers will be useful for further investigations of syntenic relationships between *Pyrus* (*Malus*) and *Prunus*. Furthermore, several other reports have showed synteny within Rosaceae plants (Sargent *et al.* 2009, Vilanova *et al.* 2008) and Rosaceae vs. other family (Staton *et al.* 2015).

Conclusion and perspectives

In this manuscript, we describe to focus recent progress on whole-genome sequences, genome-wide SNP and SSR markers, construction of reference genetic linkage maps, and synteny studies in Rosaceae fruit trees, which will help us to develop new cultivars with desirable traits by MAS and new genomic-based strategies in breeding programs.

Genetic improvement of Rosaceae fruit trees is strongly hampered by their large tree size, long generation, an extended juvenile phase for seedling (Luby and Shaw 2001, Rikkerink *et al.* 2007). Therefore, it is considered that MAS and marker-assisted breeding can accelerate selection and reduce the progeny size and the cost of raising individuals to maturity in the field (Luby and Shaw 2001, Rikkerink *et al.* 2007). However, attempts to MAS in fruit tree breeding programs remain limited for a few simply inherited traits, because marker development for MAS via bi-parental QTL mapping is also hindered by the same complications. Newly developed high-throughput genotyping technologies such as SNP chips and genotyping using NGS have enabled new genomic-based strategies such as genome-wide association studies (GWAS), which are an alternative to bi-parental QTL mapping in long-lived perennials. Selection based on genomic predictions of breeding values, i.e., genomic selection (GS, Meuwissen *et al.* 2001) is another alternative for MAS. The robust and evenly distributed genome-wide SNP markers combined with reference genetic linkage maps, help us to use new genomic-based strategies such as GWAS and GS, which are now emerging as powerful tools in pear, apple, and forest tree breeding programs (Grattapaglia and Resende 2011, Iwata *et al.* 2013a, 2013b, Kumar *et al.* 2012, 2013).

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