REVIEW

Breeding rootstocks for *Prunus* species: Advances in genetic and genomics of peach and cherry as a model

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Prunus rootstock is an important choice in optimizing productivity of grafted cultivars. Nevertheless, many *Prunus* rootstocks are notoriously intolerant to hypoxia which is caused by waterlogging and/or heavy soils. There is no available information to help select *Prunus* rootstocks that are tolerant to stress conditions such as root hypoxia caused by excess moisture. Information from genetic maps has demonstrated a high level of synteny among *Prunus* species, and this suggests that they all share a similar genomic structure. It should be possible to identify the genetic determinants involved in tolerance to hypoxia and other traits in *Prunus* rootstocks by applying methods to identify regions of the genome involved in the expression of important traits; these have been developed mainly in peach which is the model species for the genus. Molecular markers that are tightly linked to major genes would be useful in marker-assisted selection (MAS) to optimize new rootstock selection. This article provides insight on the advances in the development of molecular markers, genetic maps, and gene identification in *Prunus*, mainly in peach; the aim is to provide a general approach for identifying the genetic determinants of hypoxia stress in rootstocks.

Key words: Hypoxia, linkage map, marker-assisted selection, molecular markers, family *Rosaceae*.

INTRODUCTION

Rosaceae is a numerous and economically important family of angiosperms. It is divided into three subfamilies: *Amygdaloideae*, *Dryadoideae*, and *Rosoideae* (Potter et al., 2007). The genus *Prunus* includes important fruit crops such as peach (*P. persica* (L.) Batsch, 2*n* = 16), sweet cherry (*P. avium* (L.) L., 2*n* = 16), sour cherry (*P. cerasus* L., 2*n* = 4x = 32), apricot (*P. armeniaca* L., 2*n* = 16), almond (*P. dulcis* (Mill.) D.A. Webb, 2*n* = 16), prune (*P. domestica* L., 2*n* = 6x = 48), and plum (*P. salicina* Lindl., 2*n* = 16). Cultivars of these species are normally grafted onto a compatible rootstock to restrict scion vigor, provide better anchorage, or allow better adaptation to biotic and abiotic stresses that are characteristic of certain soils or climates. Most currently available rootstocks come from interspecific crosses and the rootstock/scion combination varies depending on the compatibility among species. For example, plum rootstocks mainly originate from *P. domestica* L., *P. insititia* L., and *P. cerasifera* Ehrh.; they can also be used for peach, plum, apricot, and almond. Interspecific peach × almond hybrids (*P. persica* × *P. dulcis*) are mainly used as rootstock for peach and almond. Cherry rootstocks come from *P. cerasus* and *P. cerasifera*, which are used for sweet and sour cherry (Moreno, 2004).

Much of the available information about the performance of different rootstocks is empirical data from many trials carried out at different locations. Detection and analysis of the underlying genetic variation can lead to the understanding of the molecular bases of the biological phenomena that distinguish them, such as tolerance or susceptibility to a given type of stress. The development of markers to help select individuals with traits that are complex to evaluate should speed up the development of new rootstocks that are resistant or tolerant to multiple diseases or stresses.

The agronomic definition of stress related to waterlogging tolerance is that of maintaining relatively high productivity under flooded as compared with non-flooded conditions (Setter and Waters, 2003). This definition suggests that a waterlogging-tolerant genotype can have a tolerance mechanism associated with an escape response to the anaerobic condition. These escape mechanisms include induced dormancy or reduced growth during the waterlogging period and rapid recovery

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after stress is removed (Setter and Waters, 2003). One of the most economical methods to reduce damage caused by waterlogging, or other conditions that induce hypoxia or anoxia in soils, is to introduce tolerance in existing cultivars through breeding. For this, anoxia-tolerant germplasm is needed, as well as appropriate methods to evaluate individuals of interest. Perhaps it would be even more useful to understand the genetic basis of anoxia tolerance (Zhou, 2010). Identifying genes involved in the expression of a character and the development of associated molecular markers is useful for the accurate and early selection of tolerant individuals in a breeding program. However, molecular markers must fulfill two main requisites to be functional: (i) to have polymorphic sequences within the species and (ii) to have functional polymorphisms that affect the plant phenotype with a high correlation between DNA polymorphism and the character of interest (Polidoros et al., 2009).

The objective of this review was to provide insight on the advances in the development of molecular markers, genetic maps, and gene identification in *Prunus*, mainly peach which is the model species for the genus; also to provide a general approach to identify the genetic determinants of hypoxic stress tolerance because the method that will be used should be similar to methods used for other traits of agronomic interest.

THE GENOMICS OF *Prunus*

Genetic improvement methods in *Prunus* cultivars and rootstocks have changed very little over the last 50 yr (Dirlewanger et al., 2004), but they still exhibit some differences. In contrast to the development of new scion cultivars, in which evaluating each generation can require 2 or 5 yr, evaluation cycles in rootstock programs can require 7 to 10 yr (Beckman and Lang, 2003). However, enhanced methodologies and new technologies have provided important tools for improving evaluation systems and selection of individuals. Marker-assisted selection (MAS) is a promising strategy for improving classical breeding methods (Knapp, 1998). It is based on information from genetic linkage maps that allow the detection of so-called quantitative trait loci (QTLs). Linkage maps also provide an understanding of the genetic bases of economically important traits. Marker-assisted selection allows the pre-selection of trait years before they can be evaluated in the field; this saves time and space in the development of new cultivars and allows selection to be focused on genotypes that carry appropriate alleles that will be passed on to descendants. Most of the research done with MAS in *Prunus* has been focused on developing scion cultivars, mostly in peach. The peach is one of the best genetically characterized species in the family *Rosaceae* (Abbott et al., 2002; Shulaev et al., 2008), not only for its economic importance but because of its small genome size. The first version of its genome sequence was published in 2010 in the Genome Database for *Rosaceae* (GDR) website (www.rosaceae.org) where information can also be obtained on the genetic and genomic resources of the different genera of *Rosaceae* (Jung et al., 2008; 2014). In 2013, the International Peach Genome Initiative published the peach genome sequence (Verde et al., 2013), and version 2.0 (Peach v2.0) of the genome is currently available at Phytozome (http://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Ppersica_er), Istituto di Genomica Applicata (IGA, Udine, Italy; http://services.appliedgenomics.org/fgb2/iga/prunus_persica_v2/gbrowse/prunus_persica_v2/) and GDR (https://www.rosaceae.org/species/prunus_persica/genome_v2.0.a1).

Knowledge about genome sequences of species of the family *Rosaceae* (available on GDR web page) has confirmed the level of synteny among genomes (Zhebentyayeva et al., 2008; Velasco et al., 2010; Shulaev et al., 2011; Verde et al., 2013) of this taxon. A degree of microsynteny had previously been confirmed between the bacterial artificial chromosome (BAC) sequences of *Prunus* and the *Arabidopsis* genome, as well as between *Prunus* BACs and the complete or partial genomes of other model species, such as *Populus* and *Medicago* (Jung et al., 2009). Synteny appears to be greater between *Prunus* and *Populus* than between *Prunus* and *Medicago* even though *Medicago* is evolutionarily closer to *Prunus*. These synteny analyses provide an opportunity to study the relationships between the structure and function of genomes of interest and facilitate map saturation with markers that are shared between species and identify genes in less studied organisms, such as *Prunus*. The first comparative genomic analysis of *Rosaceae* was published in 2011 (Illa et al., 2011b) using information about markers developed in this study and located in the genomes of *Fragaria*, *Malus*, and *Prunus*. Clear syntenic blocks were observed in the family, and an ancestral hypothetical genome was constructed that had nine chromosomes. Taking this into consideration, available information on markers and genes in these species will allow the identification of homologous species in those genomes that have not yet been sequenced; this will allow a more rapid and precise parallel advance in breeding programs by using available detailed information about each one of them.

Molecular markers

There are three main types of markers: (i) morphological, also called classical or visible, which are phenotypic traits, (ii) biochemical, which are mostly isoenzymes, and (iii) molecular (or DNA), which are DNA sequences that have single-nucleotide polymorphisms (SNPs) or insertion/deletion (INDEL) polymorphisms that can be detected or identified by various techniques based on polymerase chain reaction (PCR), sequencing, or hybridization (Winter and Kahl, 1995; Jones et al., 1997; 2009). Molecular

markers are the most widely used, mainly because they are abundant and not affected by environmental factors or the developmental or physiological state of the plant (Winter and Kahl, 1995).

Molecular markers have become a very important tool in plant breeding because of their usefulness in characterizing regions of the genome related to both qualitative and quantitative traits. They have allowed the deduction of the genome structures of a number of species, including fruit such as peach, apple, and strawberry (Zhebentyayeva et al., 2008; Velasco et al., 2010; Shulaev et al., 2011; Verde et al., 2013), as well as the determination of their sequences and genetic map locations.

Microsatellites (Litt and Luty, 1989) or simple sequence repeat (SSR; Tautz et al., 1986) are among the most used markers; they are sequences of 2 to 6 bp (Chambers and MacAvoy, 2000) repeated in tandem that are frequently detected in prokaryote and eukaryote genomes (Kalia et al., 2011; Zane et al., 2002). They are found in both coding and non-coding regions and distributed throughout the genome. The characteristics of microsatellite sequences have made them the molecular marker of choice for many types of studies because of their high polymorphism and ability to identify both alleles in diploid organisms and given that they are co-dominant. They are very useful in identifying individuals propagated sexually or vegetatively since it is very improbable that two randomly selected individuals will have exactly the same alleles if several markers are used (Parida et al., 2009; Kalia et al., 2011). These markers have been widely used for the molecular characterization of scion cultivars of *Prunus* species (Cantini et al., 2001; Dirlewanger et al., 2002; Aranzana et al., 2003a; Struss et al., 2003; Pedersen, 2006; Rojas et al., 2008; Akpinar et al., 2010; Maghuly and Laimer, 2011) and rootstock cultivars (Serrano et al., 2002; Struss et al., 2002; Liu et al., 2007; Turkoglu et al., 2010; Arismendi et al., 2012). Arismendi et al. (2012) analyzed 26 commercial *Prunus* rootstocks used in Chile that belong to the subgenera *Amygdalus* (peaches), *Prunus* (plums), and *Cerasus* (cherries). They found the highest genetic diversity in *Cerasus*, followed by *Prunus* and *Amygdalus*. They also indicated that the 26 studied genotypes could be identified with a minimum of three microsatellite markers (PMS-3, BPPCT-037, and BPPCT-036); this is important information for characterizing germplasm used in a breeding program and confirming the identity of individuals from interspecific crosses.

The highest genetic diversity found among individuals of the subgenus *Cerasus,* described by Arismendi et al. (2012), can be explained by the self-incompatibility system found in some members of the family *Rosaceae*. These species have a gametophytic self-incompatibility system controlled by the multi-allelic locus *S*, which does not allow a plant to be fertilized by its own pollen or by that of closely related individuals (De Nettancourt, 2001). Thus, in these species, plants must be cross-pollinated to produce fruit. The self-incompatibility reaction is triggered when the *S* gene is expressed in the pollen (*SFB* gene) and pistil (*S-RNasa* gene), which stops pollen tube growth. Knowledge of the genetic and molecular bases of the self-incompatible reaction has allowed the application of molecular techniques such as PCR amplification of *S* alleles with consensus or specific primers that amplify the two introns, which are part of the sequence of the *S-RNasa* gene (Tao et al., 1999; Tobutt et al., 2001; Wiersma et al., 2001; Sonneveld et al., 2001; 2003; Wünsch and Hormaza, 2004; 2005; Sonneveld et al., 2006), or amplify regions of the *SFB* gene (Sonneveld et al., 2005). The peach is self-fertile; in contrast, most cultivated *Prunus* species, such as cherry, almond, plums, and apricot, are self-infertile and therefore self-incompatible. Due in part to self-incompatibility, genetic diversity is higher in self-infertile cultivar species than in peach (Mnejja et al., 2010). Knowledge of the *S* genotypes of each cultivar has been useful for selecting appropriate pollen donors in commercial orchards and in breeding programs to ensure fruit production. Identification of the *S* genotype of *Prunus* rootstocks would allow these to be distinguished one from the other, be used as a tool to confirm hybridization in the process of obtaining new interspecific hybrid individuals, and as a complement to analysis performed with microsatellite markers.

Single-nucleotide polymorphisms are the most common type of variation found in DNA (Brookes, 1999), and they are valuable markers for high-resolution genetic mapping, genetic variation studies, and association mapping in plants. A number of methods to identify SNPs have been described (Ganal et al., 2009): by searching expressed sequence tag (EST) databases (Batley et al., 2003), amplicon re-sequencing (Choi et al., 2007), complete sequence of a genome (Velasco et al., 2007), and more recently, high throughput sequencing technology (Barbazuk et al., 2007). Advances in next-generation techniques have reduced the cost of DNA sequencing to the point where it is now feasible to perform genotyping-by-sequencing (GBS) to analyze small- and large-sized genomes with high diversity and allow the identification of thousands of markers for a species (Elshire et al., 2011; Poland et al., 2012; Ward et al., 2013; Guajardo et al., 2015). Future applications of GBS in genetic improvement will allow breeders to perform genomic selection of new germplasm or species without having to first develop a molecular tool, which is needed with other types of molecular markers; it will also allow conservation biologists to determine population structure without needing previous knowledge of the genome or diversity of the species under study (Elshire et al., 2011).

Genetic improvement and commercialization of rootstocks requires precisely identifying all available material since it is very difficult to observe the

morphological traits of rootstocks after they have been grafted. Furthermore, most traits of interest in rootstocks are strongly influenced by the environment and the developmental stage of the plant (Casas et al., 1999). A combination of markers, such as microsatellites and *S* haplotypes, to characterize the material used in a rootstock breeding program will benefit the precision of the program.

Construction of genetic maps

There are two basic map variants designed to assign a physical location to markers and genes in the genome: linkage maps, defined in units of recombination frequency, and physical maps in which the distance between loci is the physical or nucleotide distance. Genetic maps allow the locations of QTLs to be established as well as the eventual position of specific genes related to or responsible for the expression of any character. The possibility of using markers to tag these genes is based on the probability that two loci (markers or genes) are transmitted together from parents to offspring, which in turn, directly depends on the distance between them along the chromosome. For heterozygous species, such as *Prunus* spp., segregating populations are based on parents with phenotypes that are not necessarily contrasting for the trait under study, but which show segregation in the progeny.

The development of linkage maps for annual crops, such as corn and rice, began several years before it started in *Prunus* (Gardiner et al., 1993; Gowda et al., 2003) and other woody species; thus, MAS is already being used in these annual species mainly to detect pathogen resistance (Choudhary et al., 2008). Because fruit trees have a long juvenile period, some species are self-incompatible and trees are large, the development of molecular markers has taken more time. This is true not only for scion cultivars but mainly for rootstocks where marker availability is very scarce. The number of markers required to construct a map varies and depends on the number of chromosomes of the species; more markers are needed for species with larger genomes (Mohan et al., 1997). Molecular markers closely linked to QTLs can potentially be used in MAS in which selection is based more on DNA polymorphisms than on phenotypic variants (Zhang et al., 2010). Most of the information related to the DNA markers that have been used in *Prunus*, generated maps and identified QTLs and genes, is available in the Genome Database for *Rosaceae* (GDR) (www.rosaceae.org).

The genetic map published by Joobeur et al. (1998) is considered as the reference map for the genus *Prunus*. The analysis of 75 F_2 individuals of a cross between almond 'Texas' and peach 'Earlygold' resulted in what is known as the T×E map. It was initially constructed with 246 markers (235 RFLPs and 11 isozymes) and covered a total distance of 491 cM with a mean density of 2 cM per marker. This map was compared with the one published previously for almond (Viruel et al., 1995); it was observed that the two maps were homologous or syntenic and the the markers were distributed in eight linkage groups. The T×E cross provided a highly polymorphic population for linkage studies, allowed the establishment of a common terminology for the linkage groups, and provided a set of markers with known positions that could be transferred to other species of the genus.

Using the information found on the T×E map, other maps were constructed and genetic analyses were performed that were associated with agronomic traits in almond populations (Joobeur et al., 2000; Ballester et al., 2001), almond × peach (Jáuregui et al., 2001; Bliss et al., 2002), apricot (Vilanova et al., 2003; Lambert et al., 2004), and peach (Dettori et al., 2001; Yamamoto et al., 2001; Etienne et al., 2002; Foulongne et al., 2003). However, the use of these maps is limited in *Prunus* because most were constructed with RFLPs that require complex and laborious laboratory procedures. The low genetic variability in species such as peach and to some extent, in apricot (Byrne, 1990) also limited the use of these markers. Given their high polymorphism, co-dominant inheritance, and the simplicity of the methods used to develop them, microsatellites, or simple sequence repeat (SSR), appeared as appropriate markers (Morgante and Olivieri, 1993). Many of these were developed in peach (Cipriani et al., 1999; Sosinski et al., 2000; Testolin et al., 2000; Aranzana et al., 2002; Dirlewanger et al., 2002) and cherry (Downey and Iezzoni, 2000; Cantini et al., 2001). By collecting all the available information, Aranzana et al. (2003b) analyzed 109 SSRs developed in peach and cherry by different research groups and mapped 96 of them on the *Prunus* reference map (Joobeur et al., 1998). This contribution brought the T×E map to a total of 342 markers, 105 of which were SSRs that had a total length of 522 cM.

Dirlewanger et al. (2004) published one of the key studies in the genomics of *Prunus* species. In their work, 220 additional markers were positioned on the T×E map (89 SSRs, 5 ESTs, and 126 RFLPs obtained mainly by using *Arabidopsis thaliana* primers, which were highly conserved compared with rice sequences [Domínguez et al., 2003]); they also compared the species of the genus using anchor markers. The T×E map was thus expanded to 562 markers covering 519 cM with a mean density of 0.92 cM per marker; 87% of the loci corresponded to known DNA sequences and 37% of these were associated with a putative protein. Comparing the positions of the anchor markers of the T×E map (RFLPs, SSRs, and isoenzymes) with those of maps constructed with other *Prunus* populations (Viruel et al., 1995; Joobeur et al., 2000; Ballester et al., 2001; Jáuregui et al., 2001; Dettori et al., 2001; Yamamoto et al., 2001; Etienne et al., 2002; Bliss et al., 2002; Vilanova et al., 2003; Foulongne et al., 2003; Lambert et al., 2004), it was found that the genomes of the diploid ($2n = 16$) species peach, almond, apricot, cherry, *P. davidiana* (Carrière) N.E. Br., *P. cerasifera*,

and *P. ferganensis* (Kostov & Rjabov) Kovalev & Kostov are essentially co-linear; it was therefore concluded that the genus *Prunus* can be treated as a single genetic unit. Given this high degree of molecular marker transferability among members of *Rosaceae*, rootstock breeding programs can also use markers from other *Prunus* species.

In recent years, new and more complete linkage maps have been developed for species in the genus *Prunus*. Saturation of the T×E map has been further increased with different types of markers and the identification of markers closely linked to traits that are important for genetic improvement. Research also continues to identify candidate genes responsible for traits of agronomic interest and to develop more genomic tools, such as physical mapping and identification of the positions of large collections of ESTs on the reference map. The study by Howad et al. (2005) increased the SSR markers in the T×E map from 185 to 449 by a strategy known as bin mapping (Vision et al., 2000). With this strategy, a normal-sized mapping population (60-250 individuals) can be used to construct a saturated map with markers located with high precision; new markers can later be added to the map with less precision using a subgroup of highly informative plants. The study by Howad et al. (2005) used 'Earlygold', the F_1 hybrid, and six trees of the T×E population; it allowed new markers to be located within a chromosome fragment with a mean size of 7.8 cM. After this study, the T×E map contained 826 markers; considering that the total map distance covered is 524 cM (Dirlewanger et al., 2004; Howad et al., 2005), the marker coverage has a mean density of 0.63 cM per marker.

Genetic maps developed in recent years have incorporated SNPs because they have fewer detection and evaluation errors than microsatellites, and it is possible to map QTLs with greater precision than other types of markers (Ball et al., 2010; Yu et al., 2011). An example is the study by Martínez-García et al. (2013) in which maps were constructed for two peach populations. There was a previous map for one population (intraspecific population Pop-DG from a cross between the non-melting flesh 'Dr. Davis' and the fresh consumption peach 'Georgia Belle') (Ogundiwin et al., 2009). In this new study, 1536 SNPs were evaluated with a genotyping assay (GoldenGate, Illumina, San Diego, California, USA) that allowed mapping 738 SNPs in the Pop-DG population and 1037 in the Pop-DF population. A consensus map was constructed with 588 SNPs covering 454 cM and with a mean distance of 0.81 cM between markers. The International Peach SNP Consortium (IPSC) developed a chip for this species consisting of 8144 SNPs, which was validated by 709 peach accessions and identified 6869 polymorphisms (Verde et al., 2012). Pirona et al. (2013) used this chip to analyze the F_2 PI91459 ('NJ Weeping' × 'Bounty') population (W×By, 103 individuals), which led to the identification of a candidate gene that appears to control maturation date in peach, while Eduardo et al. (2013) and Sánchez et al. (2014) used the chip to identify QTLs for fruit volatile organic compounds in peach. A chip is also available for cherry with 5696 SNPs, which was validated by evaluating 269 cherry and 330 sour cherry accessions (Peace et al., 2012). This chip was used by Klagges et al. (2013) to construct maps of two cherry populations, that is, 'Black Tartarian' × 'Kordia' (BT×K) with 89 individuals and 'Regina' × 'Lapins' (R×L) with 121 individuals. Results included 723 and 687 markers mapped in eight linkage groups on the BT×K and R×L maps, respectively. The maps covered 752.9 cM for BT×K and 639.9 cM for R×L with mean distances of 1.1 and 0.9 cM per marker, respectively.

Each genetic map is unique and is the product of the characteristics provided by the parents of the mapping population and the type of markers used. Although the same group of markers is used to construct maps, there is no guarantee that all the markers will be polymorphic in populations with different progenitors. That is why common markers, or anchors, are needed to correlate information between two maps. Because it is essential to know the real location of markers when they are used to locate QTLs in a genetic map, it is important that the positions of discordant markers be confirmed in the construction of new maps to detect possible chromosome rearrangements or the existence of duplicated loci in the genome. It is also necessary to have more well-distributed markers in the linkage groups of each species to ensure marker availability to select polymorphic loci in the study population and construct new maps.

Identification of QTLs and genes

Quantitative trait loci and major genes are based on the association of a particular phenotypic trait with a DNA region (genotype) (Salazar et al., 2014). Many of these traits have quantitative inheritance, which is frequently controlled by multiple genes and/or influenced by the environment. To identify genes responsible for expressing a characteristic by the candidate gene approach (Pflieger et al., 2001), it is necessary to identify and map DNA sequences related to structural or regulatory genes whose biological function is known to affect the character of interest. If the map positions of these sequences co-localize with those of the main genes or QTLs for this character, a cause-effect relationship between DNA sequences and specific phenotypes can be demonstrated, for example, by finer mapping.

As mentioned above, the objective of constructing and saturating genetic maps is to more precisely locate genes and QTLs that could explain the expression of traits of interest. Using information from available maps, a number of groups have investigated QTLs and genes that are responsible for fruit characteristics and stress resistance, but there is still no available information to identify QTLs in *Prunus* rootstocks. Dirlewanger et al. (2006) compiled the information already published for

all SSR markers as well as EST-SSRs and AFLPs; they constructed a new version of the map that this group had published in 1998 ('Ferjalou Jalousia' × 'Fantasia', or J×F map) (Dirlewanger et al., 1998) in which they had identified QTLs associated with fruit quality in peach (Dirlewanger et al., 1999). The number of F_2 progeny was increased to 207 individuals, which were segregated for five traits reported as Mendelian; they also found a new character which they called aborting fruit (*Af*). All the Mendelian agronomic traits were located in the linkage map and SSR or AFLP markers associated with each of them were found. The marker associated with the *Af* trait allowed an early identification of individuals that would have problems at the productive stage, which is one of the objectives of MAS. Boudehri et al. (2009), using the same F_2 population and fine mapping of a locus, subsequently reported the first description of the genes involved in a fruit quality character of a perennial tree. They developed 1024 combinations of AFLP primers tightly linked to the *D* locus that controls fruit acidity. The recording of phenotypes of individuals that showed recombination linked to this locus led to its precise location within a 0.4 cM interval. Prior to this study, very few fine genetic maps using a large number of trees had been published, and only pathogen-resistance genes had been analyzed (Lu et al., 1998; Claverie et al., 2004); thus, the detailed genetic and physical characterization of the *D* locus was described as the first step toward isolating the gene or genes involved in peach fruit acidity.

Sequencing of cDNA libraries, obtained from the mRNA of a tissue, is another common strategy to identify genes being transcribed under a given condition. Le Dantec et al. (2010) used the progeny of the J×F mapping in peach (Dirlewanger et al., 2006) and the diploid set of bin mapping of *Fragaria* (Sargent et al., 2008) to identify candidate genes involved in the organoleptic quality of peach fruit and perform syntenic analysis between two genera, which can be expressed as the conservation or coherence of markers and genes and their order on the chromosomes of different genomes. They constructed two cDNA libraries from fruits of 'Fantasia' at two stages of development (Dirlewanger et al., 1998; Dirlewanger et al., 2006). These libraries were used to generate a set of EST sequences known as PeachESTdb. A total of 1730 peach unigenes were obtained after assembling the raw data along with 59 candidate genes that were selected because they were potentially involved in the sweetness, acidity, or phenolic content of the fruit or in fruit growth and development. Fifty-four pairs of primers designed from the candidate genes and producing PCR products in peach were tested in strawberry; 36 pairs of primers produced amplicons in the latter species, which provided a source of candidate genes that could be used with other species of *Rosaceae*. Eight candidate genes were mapped in peach, 14 in strawberry, and four in both species, confirming the synteny model proposed by other groups using comparative mapping. Various co-localizations between candidate genes and QTLs were mapped, this must be the object of further research to define the possible roles of these genes in the corresponding traits.

Another research group (Eduardo et al., 2010) used two progenies from peach crosses to search for QTLs related to fruit quality characteristics. One population was an F_1 population of 'Bolero' (B) × 'OroA' (O) with 129 individuals. The other population included 169 F_2 individuals from 'Contender' (C) × 'Ambra' (A). The B map consisted of 26 SSRs and a cleaved amplified polymorphic sequence (CAPS) that covered 255.4 cM, or 49.2% of the T×E map coverage; the O map consisted of 16 SSRs and covered 129.9 cM (25% of T×E map). Both populations were analyzed phenotypically for 2 yr to evaluate maturation date, fruit weight, fruit epidermis color, total juice soluble solids, acidity, and pH. Data at flowering time and flower type were only analyzed in B×O for one yr. One or two QTLs were detected per character in each population; most were localized in the same region forming QTL clusters, especially in linkage group 4. This was likely caused by a pleiotropic effect of the maturation date masking the identification of other QTLs for different traits, although it is more probable that it was due to the low map densities obtained. The authors recommended pedigree analysis based on molecular markers to better select the parents included in producing a population to improve the degree of heterozygosity in F_1 populations and ensure a large number of polymorphisms in F_2 populations in peach breeding programs (Eduardo et al., 2010).

In addition to developing maps to identify the genes responsible for characteristics related to fruit quality, there is interest in identifying genes involved in the response to different types of stress. To facilitate the mapping of genes that control chilling injury, which is a physiological disorder appearing at post-harvest stages in peach, Ogundiwin et al. (2008) developed the ChillPeach database consisting of 7862 ESTs and 4468 unigenes obtained from the mesocarp tissue of two progenies with contrasting tolerances to chilling injury. The datasets contained various putative SNPs and 184 unigenes with high-quality SSRs, 42% of which were new for *Prunus*. They used microarrays that contained 4261 ChillPeach unigenes; the analysis and posterior quantitative RT-PCR (qRT-PCR) for 13 selected genes indicated that ChillPeach is rich in genes that are specific to the fruit and induced by cold; this demonstrates the usefulness of this database for transcriptomic analysis in peach. This research group (Ogundiwin et al., 2009) subsequently constructed a linkage map with 211 markers (three morphological markers, 11 candidate genes related to maturation, and 13 cold-response genes), 21 new EST-SSRs from the ChillPeach database (Ogundiwin et al., 2008), and 58 previously reported SSRs, among others; they reported the mapping of genes that were hypothetically related to

texture, pigmentation, taste, and cold response in peach fruit. They used intraspecific progeny called Pop-DG from the cross of a non-melting flesh peach 'Dr. Davis' and the melting flesh 'Georgia Belle'. The Pop-DG map covered 818 cM of the peach genome and had a mean distance of 4 cM between markers; this map was co-linear with the *Prunus* reference map (Joobeur et al., 1998; Dirlewanger et al., 2004), with 39 common SSR markers. Likewise, the bin-mapping strategy was used in the T×E population with DNA from the same individuals found in the study by Howad et al. (2005), who mapped another 159 markers on the reference map: 59 candidate genes related to maturation, 50 cold-response genes, and 50 new EST-SSRs from the ChillPeach database: their locations were deduced from the Pop-DG map by comparative mapping. Various candidate genes and EST-SSRs were co-localized with loci for the main genes and QTLs for cold damage systems in the Pop-DG map. The usefulness of this "genetic map of fruit quality" is the co-localization of QTLs related to fruit quality with candidate genes on the same map, which is very important for understanding the genetic control of important production traits.

It is well known that winter chilling together with adequate heat accumulation determines the flowering date of a given cultivar (Sherman and Beckman, 2003). Fan et al. (2010) used an F_2 population of 378 peach trees from a cross between two genotypes with contrasting chilling requirements to construct a linkage map and chart QTLs. The map included 96 SSRs (36 shared with the T×E map), 30 AFLPs, and one morphological marker in eight linkage groups covering 535 cM, with a mean distance of 4.2 cM between markers. The chilling and heat requirements of each genotype's flower buds were evaluated for 2 yr and flowering dates were recorded for 4 yr. Twenty QTLs with additive effects were identified for three traits, including a main QTL for the chilling requirement and two main QTLs for the flowering date. Most QTLs were co-localized with QTLs for other traits; therefore, either there is close linkage between genes that regulate different traits or genes have pleiotropic effects. This first report on mapping QTLs for the chilling requirement for flowering will facilitate MAS of cultivars that require little chilling and also help to identify and understand the genes that control the chilling requirement. It has been suggested that there may be a single temperature sensor and an action system regulating both the chilling and heat requirement for the flowering date (Fan et al., 2010).

Mnejja et al. (2010) used available information on genetic maps for species of the family *Rosaceae* to study 145 pairs of SSR primers and determine their transferability in eight cultivars of nine *Rosaceae* species (almond, peach, apricot, plum, prune, cherry, apple, pear, and strawberry). Of these markers, 25 came from almond genomic DNA (Mnejja et al., 2005), 22 from almond ESTs, 25 from peach genomic DNA (Dirlewanger et al., 2002; Le Dantec et al., 2010), 25 were ESTs isolated from peach, and 25 were from plum genomic DNA (Mnejja et al., 2004). The remaining 23 markers were from apricot (13 ESTs and 10 genomic) (Hagen et al., 2004). These markers were all polymorphic in their respective species. Most primers (83.6%) amplified segments of the expected size range in other *Prunus* species. Their transferability, which is the proportion of microsatellites that were amplified and polymorphic, was also high in *Prunus* (63.9%). Thirty-one SSRs were amplified and polymorphic in all the studied species of *Prunus*; 12 of these were distributed over the entire genome and proposed as the "universal *Prunus* set" that could be useful for comparative studies and constructing linkage maps with common markers. In contrast, only 16.3% of all the studied SSRs were transferable to species of other *Rosaceae* genera (apple, pear and strawberry), which confirms the necessity or convenience of using other types of markers for genetic studies between genera.

Illa et al. (2011a) selected 273 sequences from EST collections that were candidate genes of metabolic pathways affecting growth and certain fruit traits, such as maturity, texture, sugar and organic acid content, aroma, and color, these were all mapped in the *Prunus* reference map. They used the bin-mapping strategy with the same eight trees used in previous studies (Howad et al., 2005; Ogundiwin et al., 2009). This strategy proved to be very efficient because it allowed mapping 206 candidate genes mainly based on segregating one or more SNPs. These candidate genes were distributed throughout the *Prunus* genome and provided a new resource for genetic analysis in the different species of the genus. The total number of candidate genes localized by bin-mapping in the T×E map was increased to 314 with this study; these genes could determine the genetic base of fruit quality in *Prunus*, which is the key information for breeding these species.

The identification of hypoxia-tolerant genes of *Prunus* rootstocks is being studied at the Centro de Estudios Avanzados en Fruticultura (CEAF) in Chile. Almada et al. (2013) published the identities of hypoxia-tolerant genes, which were class 1 *non-symbiotic Hb*-like (*nsHb*) and class 3 *truncated Hb*-like (*trHb*). Although the putative genes *nsHb* and *trHb* were induced by hypoxia in the roots of all analyzed genotypes of *Prunus*, independently of their tolerance to hypoxia, they observed that expression levels were higher in the tolerant rootstocks. They found that other abiotic stresses, such as salt stress and low temperature stress, are also regulated by these genes. Arismendi et al. (2015) performed transcriptomic sequencing of two *Prunus* rootstocks, 'Mariana 2624' and 'Mazzard F12/1', which are tolerant and sensitive to hypoxia, respectively, to identify candidate genes involved in the response to root hypoxia. They identified a group of differentially expressed genes exclusively upregulated in the tolerant genotype, which are associated with enzymes of posttranslational protein modifications.

Their results represent a valuable source of information for further studies to identify the mechanism and genes that define hypoxia tolerance in *Prunus*.

CONCLUSIONS

The practical purpose of developing markers and genetic maps, as well as sequencing the complete genomes of a number of species, is to help understand gene locations, which is a basic platform for subsequent development to understand their function, regulation, and expression, especially for genes responsible for traits of agronomic interest in breeding programs. Chromosomal positions of disease resistance and fruit quality genes have been identified using available information about markers and genetic maps. Markers associated with these genes have also been identified, which can be used in breeding programs for early selection to produce new cultivars with desirable traits.

The development of higher density maps will provide researchers with a wide variety of tools for QTL mapping and markers for MAS. Unfortunately, available information about some species, such as cherry, and *Prunus* rootstocks is still limited; comparative mapping has become and will continue to be a widely used technique. The gaining of knowledge about gene sequences of species such as peach, apple, and strawberry will promote research of other species of the family *Rosaceae* given the high co-linearity among their genomes.

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