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MOLECULAR MARKERS USAGES IN CULTIVATED FRUIT TREES FROM *ROSACEAE* FAMILY

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Abstract

Most fruit tree species cultivated in Romania, such as apple, pear, plum, peach, apricot, cherry, as well as some berry species such as blackberry, raspberry and strawberry belong to the Rosaceae family. As most of these species are woody perennials, the traditional methods of creating new varieties are taking much longer time than in the case of annual species. New methods and tools based on the recent discoveries in molecular genetics are being developed in order to shorten the time needed to create new commercial varieties. Molecular markers, specific DNA regions linked to genes responsible for various traits (color, shape, taste, resistance/tolerance to biotic and abiotic stresses, etc.) are some of the tools used in genotype-assisted breeding programs. This review sums up the main results of studies on molecular markers regarding cultivated fruit tree species of Rosaceae family with commercial importance.

Key words*: molecular markers, Rosaceae, Malus, Prunus, molecular breeding.*

INTRODUCTION

Rosaceae family consists of 91 genera and 2,950 species (Christenhusz and Byng, 2016). Most cultivated fruit species from Romania belong to this family. These species are important not only in alimentation, but also as ornamentals.

Recent sequencing techniques and genomes sequencing brought up new data that can be used for a variety of purposes, such as identification and characterization of genes responsible for agronomically important traits, genotyping by sequencing, marker assisted selection, identification of molecular markers linked to the traits of interest (Soundararajan et al., 2019). Molecular markers such as Single Nucleotide Polymorphism (SNP), Random Amplification of Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR), Inter Simple Sequence Repeats (ISSR), Cleaved Amplified Polymorphic Sequence (CAPS), Sequence-Related Amplified Polymorphism (SRAP), Sequence Characterized Amplified Region (SCAR), can be used as tools for genetic and physical mapping of genomes, the identification of genes controlling various processes and phenotypes (trait association), genetic diversity and evolutionary analyses, and in marker-assisted breeding for crop improvement.

Single Sequence Repeats (SSRs) are molecular markers that have been used extensively for genotyping commercial crops, as they are multiallelic, highly polymorphic, they have high discriminatory potential and good reproducibility, and can detect polyploidy (Nybom and Lācis, 2021). Within the *Rosaceae* family, multiple SSR markers have been developed for most cultivated species. To improve comparison among different studies, European Cooperative Programme for Plant Genetic Resources (ECPGR; www.ecpgr.cgiar.org) published recommended SSR loci sets for apple, pear, cherry and plum (Nybom and Lācis, 2021). SSR markers may be used to discriminate between different genotypes of the same species and frequently those of closely related species, so they are used frequently in variability studies, fingerprinting and map construction. Mnejja et al. (2010) studied 145 *Rosaceae* SSR primer

pairs (genomic and EST-derived) for transferability in nine *Rosaceae* species (almond, peach, apricot, Japanese plum, European plum, cherry, apple, pear, and strawberry), ending up with 32 polymorphic SSRs for all Prunus species studied and proposed a set of 12 SSRs as an "universal *Prunus* set". Fan et al. (2013) developed a set of SSR markers from pear that could be used in other Rosaceae species, their transferability varying from 12% (cherry) to 58% (apple). Heterologous markers designed from a species may be used in PCR amplification to amplify SSRs in a second related species phenomenon known ask transferability of SSR information.

Single Nucleotide Polymorphisms (SNPs) are the most abundant molecular markers present in a genome, some of their advantageous features being that they are relatively stable during evolution and they have a low mutation rate (Patel et al., 2015). SNPs in Rosaceae species were identified in various projects involving Sanger sequencing to sequence EST collections, direct sequencing of PCR products amplified from genomic regions of interest, through the utilization of the cleaved amplified polymorphic site approach where suitable restriction enzymes were available, high-throughput deep sequencing of both genomic DNA and mRNA, and genotyping by sequencing (Longhi et al., 2014).

In traditional breeding programs, the length of juvenile phase (length of time for seedling to produce the first flowers) affects the breeding cycle, specifically in respect to fruit traits evaluation, and consequently the selection of the best hybrids and cultivars. For instance, in *Prunus* species, the juvenile phase lasts 1-2 years for peach, 2-3 years for Japanese plums, and 3-5 years for sweet cherries (Carrasco et al., 2013). Plant domestication began roughly around 11,050 BC. Early farmers aimed for improvement of morphological traits, such as seed or fruit size and plant structure, or of physiological ones, such as the timing of germination and fruit ripening. However, domestication came with unintended consequences, such as more deleterious mutations as compared to wild types and lack of genetic diversity (Hunter, 2018). Study of plant domestication in the context of the new discoveries in the fields of genetics and genomics could lead toward new approaches in plant breeding. Molecular breeding adds genetic and genomic analysis to assist traditional breeding, not only reducing the breeding time, but also aiding in the selection of plants possessing traits of interest linked to specific molecular markers.

The present study aims to review the use of molecular markers in the past two decades in connection with the main species of fruit trees from *Rosaceae* family: apple, pear, quince, plum, almond, apricot, peach and cherry.

Apple

Cultivated apple, *Malus x domestica* Borkh., is an interspecific hybrid, with the main progenitors being *Malus sieversii* M. Roem. and/or *Malus sylvestris* L. (Brown et al., 2012). RFLP markers have been employed to study the chloroplast DNA variation in *M. sylvestris, M. sieversii* and *M. domestica* with the purpose of determining the phylogenetic relationship between the two species (Coart et al., 2006), the results of the study showing a closer relationship between *M. domestica* and *M. sylvestris* as opposed to *M. sievestrii* and *M. domestica*. In the same study, nuclear SSRs have been used to discriminate among wild, cultivated and hybridized genotypes. Koopman et al. (2007) used multilocus microsatellite haplotype-sharing as a tool to study introgression for cultivated (*M. domestica*) into wild apple (*M. sylvestris*) as well as gene flow among the remnant populations of *M. sylvestris*, demonstrating the usefulness of the method in obtaining general genetic information on population structure and population differentiation. Later, Cornille et al. (2012) used SSR markers to study the contribution of various *Malus* species to the genome of cultivated apple, *M. domestica* and determined that the two major contributors to domestic apple genome are *M. sylvestris* and *M. sieversii*.

Several economically important traits in apple have been studied using molecular markers, such as resistances to apple scab resistance, powdery mildew, and to the fire blight, red foliage, red fruit color, russeting, columnar growth.

Venturia inequalis is the most important fungal pathogen that affects cultivated apple. Consequently, multiple studies have been

published, trying to unravel the genetics behind apple scab resistance. Patocchi et al. (2004) identified a qualitative apple scab resistant gene, *Vr2*, in the accession GMAL 2473, and developed three AFLP and one RAPD molecular markers associated to this gene. Gigax et al. (2004) developed RAPD, SCAR and SSR molecular markers associated to apple scab resistance gene *Vbj* from crab apple *Malus baccata* Jackii. Huaracha et al. (2004) used AFLP derived SCARs to narrow down the region of *Vf* locus for scab resistance. Another scab resistence gene is *Vh8*, discovered in *M. sieversii* by Bus et al. (2005). SCAR and SSR markers helped in demonstrating that *Vh8* locus differs but it is linked with the locus containing the *Vh2* gene from the F2 descendant of *Malus pumila* progenitor R12740-7A. Scab-resistance gene *Vb* was mapped on the linkage group 12 using SSR markers (Erdin et al., 2006). Lastly, Broggini et al. (2009) identified six *HcrVf2* paralogs in *M. domestica.* SSR markers were developed from BAC clones and used to map the six *Vf2* paralogs.

Podosphaera leucotricha is another fungal pathogen that affects apple, causing powdery mildew disease. Stankiewicz et al. (2002) used SCAR markers linked to the apple powdery mildew resistant gene *Pl2* in apple breeding for disease resistance. James et al. (2004) identified additional molecular markers (AFLP, RAPD and SSR) linked to a different mildew resistance gene, *Pl-d*.

Fire blight is a disease affecting apple, pears and several other *Rosaceae* species, caused by the bacterium *Erwinia amylovora*. Several types of molecular markers linked to fire blight have been developed to be used in future breeding programs. Khan et al. (2007) developed RAPD, SCAR and SSR markers linked to the QTL for fire blight resistance present in linkage group 7. Gardiner et al. (2012) mapped both SSR and SNP markers associated to QTLs for fire blight resistance in *Malus x robusta*. Recently, Emeriewen et al. (2020) used SNPs and SSRs to construct a dense genetic map of a *Malus fusca* C.K.Schneid. accession with resistance to fire blight disease.

Russeting, manifested by cuticle cracking followed by the replacement of the epidermis by a corky layer that protects the fruit surface from water loss and pathogens in apple is a disorder controlled genetically. Falginella et al. (2015), mapped the *Ru_RGT* locus from linkage group 12, using SNP and SSR markers, as a putative gene that controls this disorder.

Fresh apples can cause food allergies related to birch pollen. Gao et al (2005) used SNAP and SSR markers to map *Mal d 1 (P R10)* gene family, that encode an apple allergen, in *M. domestica*. From the eighteen genes belonging to this family, sixteen were found to be grouped into two clusters, one cluster with seven genes located on linkage group 13, and the second cluster with nine gene located on linkage group 16. One additional gene was mapped on linkage group 6, whereas another gene remained unmapped.

Color is one of the most studied traits in plant species. Chagne et al. (2007) reported that *MdMYB10* present in the *Rni* locus is encoding a transcription factor, a putative gene controlling red foliage and red coloration of apple fruit core. The authors located both SNPs and SSRs within the anthocyanin biosynthetic and regulatory genes, which may be responsible for red flesh phenotypes. Zhang et al. (2014) used 2 allele-specific DNA markers, (*A* and *MdMYB1* alleles) in order to discriminate between cultivars with different skin colors (red vs non-red skin color). El-Sharkawy et al. (2015) used SSRs to perform genetic fingerprinting in order to determine the relationship between red-colored parent 'Kidd's D-8' (KID) and the 'sport' apple mutant Blondee (BLO) (somatic mutation), a rare anthocyanin-deficient yellow-skin variety.

Columnar growth of apple trees is a useful trait for varieties used in high density planting. Yi-Ke et al. (2004) identified a RAPD marker in apple closely linked to *Co* gene responsible for columnar/non columnar trait.

Kumar et al. (2013) used genome wide SNP arrays to evaluate systematically the relative contribution of various genomic regions to several quality-related traits (fruit firmness, titratable acidity, red-flesh coverage) as well as contributions to some physiological disorders (internal flesh browning, bitter pit, fruit spitting).

Recent advances in sequencing techniques decreased both the price and time needed for whole genomes to be sequenced. Apple

genome was the tenth sequenced whole genome after *Arabidopsis*, rice, poplar, grape, papaya, sorghum, cucumber, maize, and soybean. To date, there are three apple whole genomes published (Peace et al., 2019). Once enough of a genome is sequenced, a plethora of data is open to be used for a wide array of studies. Among these, fishing for putative molecular markers to be used for a variety of purposes is a priority for many researchers. Chagne et al. (2008) identified over 70,000 putative SNPs using *in silico* data, and 93 molecular markers containing 210 coding SNPs were mapped and could be used in further research. In a study made on ESTs from domestic apple cultivars Royal Gala, Pinkie, Pacific Rose, and dwarfing rootstock M9, Newcomb et al. (2006) identified multiple SSR and SNP markers. AG repeats where most common (88% of dinucleotides repeats), followed by AT repeats (7.6%), and AC repeats (4%). GC repeats were exceedingly rare (0.05%). Among sequences containing a dinucleotide repeat with more than 100 bp of flanking DNA, 83% contained SSRs in the putative 5' UTR, 2% in the putative coding region, and 15% in the putative 3' UTR. In addition, more than 18,000 biallelic SNPs were identified. A high-density genetic map was constructed from a Jonathan and Golden Delicious cross to be used for QTL analysis and SNP marker development (Sun et al, 2015). Liu et al (2016) used SSR and SRAP markers to construct a molecular genetic linkage map of *M. sieversii*.

SSR markers have been used to study the eupolyploidization and aneuploidization in *Malus* seedlings following diploid crosses (Considine et al., 2012), the results of the study indicating that aneuploidy exceeded eupolyploidy. Following the study, the authors also proposed a protocol for accelerating apple triploid breeding program using co-dominant markers. Han et al. (2011) developed and used SSR markers to construct an apple SSR-based genetic linkage map, which was used to demonstrate the presence of segmental and genome-wide duplications in apple genome, providing new insights in the complex polyploidy origin of apple.

Molecular markers could also be used for the identification of duplicates in collections as well as for correctly naming misidentified plants, thus reducing the size of collections without reducing the genetic variation (Harris et al., 2002). RAPD markers have been used to differentiate between cultivars, to analyze the maternal and paternal contribution to pedigrees, or to assess the variation between varieties, and SSR markers appears to be valuable for varietal genotyping and pedigree analysis, as well as anchor markers in genome mapping (Harris et al., 2002). Iannaccone et al. (2007) used a combination of RAPD and SSR markers analysis and flow cytometry to discriminate between all the clones of a valuable apple cultivar analyzed (Annurca), and to offer a method for efficient management for valuable germplasm preservation. Liu et al. (2014) used SSR markers to develop cultivar identification diagram (CID) strategy to identify apple cultivars and varieties easily with several pairs of SSR primers. Muranty et al. (2020) used whole genome SNP data to identify the relationships between more than 1400 old apple cultivars and reconstruct pedigrees, bringing new understanding off empirical selection and providing data for future breeding and selection.

Molecular markers are particularly useful in determining the genetic diversity of a certain population/collection. They have been employed worldwide for this purpose in multiple studies. Zhang et al. (2007) used SSR markers to analyze the genetic structure of *M. sieversii* population from Xinjiang, China. Omasheva et al. (2015) analyzed the genetic diversity of five populations of wild apple (*M. sieversii*) from Zailiysky Alatau using seven SSR markers. Yun et al. (2015) used 14 SSR markers to assess the genetic diversity within an apple collection (South Korea). Pérez-Romero et al. (2015) analyzed 29 domestic Apple accessions from Andalusia (Southern Spain) using 12 SSR markers. Öz et al. (2020) used 16 SSR markers to analyze 94 Eastern Anatolian apple accessions and found them different genetically from Anatolian apple accessions.

Finally, molecular markers can bring to light information of the far past of humankind. Genotyping with SSR primers specific for *Malus* confirmed that the mummified seeds found in amphorae in the cellar of a 1st century

BC Roman villa on Elba Island are apple seeds, and comparison with wild and modern domesticated seeds revealed that most archaeological seeds (from three amphorae) showed only 17% correlation with contemporary *Malus* accession. One amphora however contained seeds with genetic and morphological correlations with living *Malus sylvestris*. The results of this study brought new data on Roman economy and culture on Elba Island from the 2nd to the 1st century BC (Milanesi et al., 2016).

Pear

In pear (*Pyrus* sp.), molecular markers have been used to map various traits: fruit storage potential with CAPS markers (Itai et al., 2003), pear scab resistance with RAPD and AFLP markers (Terakami et al., 2006), black spot disease with SSRs (Terakami et al., 2007), self-incompatibility with CAPS markers (Moriya et al., 2007), length of pedicel, single fruit weight, soluble solid content, transverse diameter, vertical diameter, calyx status, flesh colour, juice content, number of seeds, skin colour, and skin smoothness with SNPs and SSRs (Wu et al., 2014), rootstock-induced dwarfing and precocity with SSRs (Knäbel et al., 2015), postzygotic hybrid necrosis with SSRs (Montanari et al., 2016), fruit quality traits (firmness, crispness, juiciness, sweetness, sourness, flavour intensity, fruit scuffing, shape, russet, fruit weight) with SNPs (Kumar et al., 2019).

Study of genetic variability is another objective for which molecular markers are being used. Genetic variability in South Korean cultivars (*Pyrus pyrifolia* Nak. and *Pyrus communis* L.) was assessed with RAPD and SCAR markers (Lee et al., 2004). Chinese cultivars assessed with RAPD (Lin et al., 2011) and SSRs (Xue et al., 2018). Belarus pear cultivars (Urbanovich et al., 2011) and Russian cultivars belonging to *Pyrus ussuriensis* Maxim., *Pyrus bretschneideri* Rehder., *Pyrus pyraster* L., *Pyrus elaegrifolia* Pall. and *P. communis* were assessed with SSRs (Yakovin et al., 2011). Jiang et al. (2015) developed 24 RBIP markers to assess the genetic variability in more than 100 *Pyrus* accessions from Eurasia. Li et al. (2015) investigated fruit transcriptome through massively parallel sequencing, identifying more than 30,000 SNPs and more than 7,000 putative SSR markers with the potential to be used in linkage map construction and marker-assisted breeding programs.

Quince

Not many studies are done to date regarding quinces (*Cydonia* sp.). However, ISSR markers associated with fruit traits: fire blight susceptibility, yield, mean fruit weight, citric acid content, soluble solid content, and fruit drop, have been developed by Ganopoulos et al. (2011), to be used for breeding purposes. Also, genetic diversity of Iranian quinces (Azad et al., 2013) and that of Turkish cultivars (Yüksel et al., 2013) were assessed with SSRs.

Prunus sp.

Genus *Prunus* consists of over 200 species of tree and shrubs, and it consists of five subgenera: *Amygdalus* (peaches and almonds), *Cerasus* (cherries), *Prunus* (plums), *Laurocerasus* (evergreen laurel-cherries), and *Padus* (deciduous bird-cherries), according to Rehder (1940).

AFLP markers were used to assess the genetic structure and differentiation among *Prunus* germplasm accessions belonging to seven cultivated species from subgenera *Prunus, Amygdalus* and *Cerasus* (Aradhya et al., 2004). Data acquired from the study could be used for genetic resources conservation and management.

European plum, *Prunus domestica* L., is a hexaploid species (2n = 48). A study of worldwide plum germplasm using sequence-based SNP markers confirmed that *P. domestica* originated as an interspecific hybrid of the diploid species *Prunus cerasifera* Ehrh. (cherry plum) and the tetraploid species *Prunus spinosa* L. (sloe), which in turn may be an interspecific hybrid of *P. cerasifera* and an unknown Eurasian plum species (Zhebentyayeva et al., 2019).

Genetic diversity studies on plums were performed in different reagions of the world. RAPD and ISSR markers were used to assess the genetic diversity and relationships among cultivated and wilt Tunisian plums (Ben Tamarzizt et al., 2015). In Romania, plum accessions from Râmnicu-Vâlcea Fruit Research Station, belonging to *P. domestica* and *Prunus insititia* L. were analyzed using nine SSR markers in order to categorize and genetically characterize them (Pop et al., 2018). In a study with the purpose of characterizing

phenotypically and genotypically plum accessions from Tunisia, SSR markers in combination with *S*-allele intron markers were used to discriminate between eighteen diploid accessions, belonging to *Prunus salicina* Lindl. and *P. cerasifera*, and five polyploid accessions, belonging to *P. spinosa* and *P. insititia* (Baraket et al., 2019). ECPGR recommends the use of nine SSR loci as a standard set for genotyping European plum accessions, as the polymorphism in the loci is enough to differentiate between plum accessions in spite of problems caused by hexaploidy in both *P. domestica* and *P. insititia* (Nybom et al., 2020). The nine SSR loci were used to differentiate between 165 plum accessions, demonstrating a major dichotomy between *P. insititia*- and *P. domestica*-related cultivars.

As fruit set and yield depend largely on pollination, Meland et al. (2020) used SSR markers to determine the plum pollinizers' success rate in Norwegian orchards and identify pollen donors for each embryo, in order to reveal the level of self-pollination of the self-fertile cultivars and to assess individual pollinizer effectiveness, and lastly offering a valuable guideline to plum producers.

Almond

Domestication is one of the turning points in the establishments of human societies in Neolithic age (Delplancke et al., 2013). Nuclear and chloroplast SSRs were used to study the origin and dissemination of cultivated almond, *Prunus dulcis* Mill. (Zeinalabedini et al., 2010), proving that cultivated almond was disseminated from Asia to the Eastern Mediterranean basin and then to the Western Mediterranean basin, and subsequently to the New World. Delplancke et al. (2012) used a combination of nuclear and chloroplast SSR molecular markers to study the gene flow among wild (*Prunus orientalis* Mill.) and domesticated almond (*P. dulcis*) species, and they detected a high genetic diversity in both cultivated and wild almond. Almond tree domestication in the Mediterranean basin was studied using the same combination of SSR molecular markers, and both types of markers detected a single domestication event in the Eastern side of the Mediterranean basin (Delplancke et al., 2013).

ISSR and RAPD markers were employed to demonstrate the genetic stability of the almond plantlets obtained by micropropagation (Martins et al., 2004).

Genetic diversity of almonds was assessed in multiple studies. Portuguese almond cultivars and several wild almond (*Prunus webbii* Vierh.) plants was evaluated using ISSR and RAPD markers (Martins et al., 2003). Xu et al. (2004) used EST-SSRs to study genomic diversity of Chinese and Mediterranean region almond cultivars. Xie et al. (2006) used EST-SSR and genomic SSR markers to study the genetic diversity of Chinese almond cultivars. Gouta et al (2010) studied the genetic diversity of Tunisian almond varieties in comparison to European and American varieties using SSR markers, bringing new data that could be used in future breeding programs, as well as delivering genetic tools in the management of the genetic resources.

Going beyond genetic diversity studies, Wu et al. (2009) identified twelve SNPs-anchored genes using high resolution melting (HRM) technique, including abiotic stress-responsive genes, allergy and detoxification-related genes. SSR markers were used to construct a linkage map and identify QTLs associated with kernel chemical composition to be used in creating varieties with increased kernel quality (i Forcada et al., 2012). Lastly, Goonetilleke et al. (2018) used genotyping by sequencing technique to discover and map SNPs in two almond cultivars, developing more than 300 map pairs, with the potential to be used in the almond genetic analysis and genetic improvement.

Apricot

Apricot domestication and diffusion in the Mediterranean basin were studied by Bourguiba et al. (2012) using SSR markers. Following the study, more than 200 apricot accessions were grouped into 3 genetic clusters: Irano-Caucasian, North Mediterranean and South Mediterranean. In addition, two main routes of apricot diffusion from the Irano-Caucasian gene pool into the Mediterranean basin were detected: North Mediterranean and Southwest Mediterranean. Using SSR markers on 271 cultivated samples and 306 wild apricots across Eurasia, Liu et al. (2019) studied the apricot domestication, gene flow and species divergence. Apparently at least

three domestication events gave rise to European, Southern Central Asian and Chinese cultivated apricots.

Genetic diversity of apricot was assessed in Turkey using SSR markers (Akpinar et al., 2010), in Tunisia with AFLP markers (Krichen et al., 2012), in China with SSR and ISSR markers (Liu et al., 2015), and fluorescent AFLP markers (Yuan et al., 2007), in Italy using AFLP markers (Ricciardi et al., 2002), and in Siberian apricots (*Prunus sibirica* L.) using nuclear SSRs (Wang et al., 2014). European, Irano-Caucasian, Chinese and Central Asian apricots were assessed with SSR molecular markers (Zhebentyayeva et al., 2003).

Regarding the linkage of markers to important traits, Olukolu et al. (2009) reported the construction of male and female high-density maps, as well as 12 putative chilling requirement (CR) QTLs using SSR and AFLP markers. Soriano et al (2005) developed AFLP-RGA (resistance gene analogues) markers as a means for marker-assisted selection and map-based cloning of R-genes in apricot.

Following de novo transcriptome analysis of Siberian apricots (*P. sibirica*), Dong et al. (2014) identified more than 7000 putative SSRs, most of them being dinucleotides (66%) and trinucleotides (31%), that will be extremely useful for future studies of breeding, genetic diversity and gene excavation about this species.

Peach

Genetic diversity of peach, *Prunus persica* Batsch, was assessed in Chinese peach cultivars using SSRs (Li et al., 2008).

Molecular markers have been used to map various traits in peach: flat fruit shape with SSR markers (Picanol et al, 2013; Lopez-Girona et al., 2017), fruit quality and chilling injury with SNPs (Martinez-Garcia et al., 2013), fruit acidity with SSRs and SNPs (Eduardo et al., 2014), slow ripening fruit with SSRs (Eduardo et al., 2015), fruit flesh color, fruit skin pubescence, fruit shape, sub-acid fruit, stone adhesion-flesh texture, resistance to green peach aphid using SNPs (Lambert et al, 2016), brachytic dwarfing with SSRs and SNPs (Cantin et al., 2018), stony hard with SSRs (Cirilli et al., 2018) and SNPs (Jiao et al., 2015), fruit maturity with SNPs (Elsadr et al., 2019), ever-growing with AFLP markers (Wang et al., 2002). Chou et al. (2020) developed a set of SNP markers linked to CR trait using the HRM technique. Shi et al. (2020) constructed a high-density SNP linkage map detecting 40 QTLs linked to 10 fruit-related traits, including fruit weight, fruit diameter, percentage of red skin color, eating quality, fruit flavor, red in flesh, red around pit, adherence to pit, fruit development period and fruit fiber content.

Yu et al. (2012) and Han et al. (2014) developed cultivar identification diagrams (CIDs) based on RAPD markers for cultivar identification of Chinese peach cultivars for fruit consumption and for the identification of ornamental peach cultivars.

Cherry

Genetic diversity in cultivated (samples from different regions in Europe as well as from different breeding programs worldwide) and wild sweet cherry (samples from France) was assessed with SSR primers to determine the effect of domestication and breeding on the genomic diversity (Mariette et al., 2010). It appears that the domestication in the case of cherry led to a breeding-related genetic bottleneck that in turn resulted in a narrow genetic diversity. Koepke et al. (2012) used a 3'UTR sequencing to develop SNP markers to detect genetic variability in closely related sweet cherry genotypes. Hewit et al. (2016) used a combination of approaches (a gel-based approach, a reduced representation sequencing, a 6k cherry SNParray, and whole genome sequencing) to identify genome wide polymorphisms (SNPs) in closely related genotypes of sweet cherry.

Genetic diversity in cherries was assessed with SSR markers in German wild and cultivated sweet cherry (Schueler et al., 2003), Turkish wild sweet cherry (*Prunus avium* L.) (Ercisli et al., 2011; Oz et al., 2013), and Italian (Campania) cultivars (Muccillo et al., 2019). Ukrainian sweet cherry cultivars were assessed using ISSR markers (Ivanovych et al., 2017).

CONCLUSIONS

Molecular markers are several of the tools used in genotype-assisted breeding programs. This review strives to bring together how molecular markers have been employed in fruit tree

species of *Rosaceae* family with commercial importance.

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