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HORTICULTURAL REVIEW

SSR-based DNA fingerprinting of fruit crops

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Abstract

The DNA fingerprinting of fruit crops, based on DNA microsatellite markers that are considered to be the key markers for the molecular analysis of germplasm collections, is reviewed. Simple sequence repeats (SSRs) remain the markers of choice for fingerprinting in humans, animals, plants, and other living organisms. This review, that considers 44 fruit species, provides a set of markers that are suitable for profiling accessions and that should make the databases produced in different laboratories more comparable. Every effort has been made to select SSR markers that are robust and easily scorable considering that such analyses are sometimes used in legal cases. The review first describes the basic protocols, procedures, and methods of data analyses; it then describes the fingerprinting of individual species or groups of species, providing a set of SSR markers and appropriate guidance based on the revised literature and on the authors' experience.

1 | INTRODUCTION

The ultimate scope of this review is to provide scientists, who are involved with the molecular analysis of fruit crop genetic resources, with a set of markers to achieve a robust genetic profile of accessions within an individual fruit species. In addition, such information should assist in making the profile databases shared by different laboratories more comparable than is presently possible.

Every effort has been made in this review to select simple sequence repeat (SSR) markers that are reliable and easily scorable. This is critically important considering that many laboratories are required to provide analyses associated with legal proceedings dealing, for instance, with the defense of intellectual property rights (IPRs) of patented plant varieties. Under such circumstances, there is a need to produce consistent results.

An additional challenge relates to the difficulty of ensuring that the analyses carried out in different laboratories with different protocols, chemicals, and equipment are comparable. However, a robust common set of SSR markers, possibly with long core repeats, and the use of home-made ladders or reference cultivars could enable the objective of having a "universal" exchangeable database of molecular profiles of fruit crops to become a reality.

This review is divided in two parts: the first part describes the basic protocols, procedures, and methods of data analyses that are associated with molecular marker research; while the second part uses a wide range of examples that illustrate the use of markers for 44 specific fruit crops. Specific advice is provided for the application of these methods.

Abbreviation: AB, Applied Biosystems; CTAB, cetyltrimethyl ammonium bromide; DNA VNTR, DNA variable number of tandem repeats; EDVs, essentially derived varieties; FAM, 6-carboxy-fluorescine; HEX, hexachloro-6-carboxy-fluorescine; HW, Hardy-Weinberg; IPRs, intellectual property rights; LR, likelihood ratio; PID, probability of identity; ROX, 6-carboxy-Xrhodamine; SNP, single nucleotide polymorphism; SSR, simple sequence repeat; TD, touchdown; UPGMA, unweighted pair group method with arithmetic mean.

2 | BASIC PROTOCOLS, PROCEDURES, AND DATA ANALYSES

2.1 | DNA extraction

There are many DNA extraction protocols, some of which differ very little from one another. They can be found in technical bulletins provided by companies and in scientific reviews (see for instance the recent and updated review from Rana & Bhat, 2017). Rather than providing an exhaustive review on this topic, we comment on the classical cetyltrimethyl ammonium bromide (CTAB)-based method of Doyle and Doyle (1990) (supplementary material) and on some of the more commonly-used commercial kits.

The CTAB method of DNA extraction from fresh plant tissue was suggested by Murray and Thompson (1980) and taken up by Doyle and Doyle (1990). That historical paper, together with similar papers from the same authors, achieved great popularity and received more than 21,000 citations. The original protocol, that has undergone many adjustments, is shown in Table 1.

Young fast-growing leaves, processed fresh or freeze-dried, are the preferred material for analysis. Occasionally, scientists must analyze unusual or uncommon plant species, "dirty" material like plant-derived foodstuffs, and other such samples. For these cases, several commercial kits, three of which are shown below, are available and have proven to be effective:

- the NucleoSpin Plant II kit (Macherey-Nagel). This uses ground lyophilized material and is suitable for a wide range of material. We have successfully tested this kit on kiwifruit and grape;
- the DNeasy 96 Plant Mini Kit of QIAGEN (Qiagen GmbH, Hilden, Germany). This works well with a wide range of different species. Examples of its application include the assessment of olive oil and wine;
- the Qiagen QIAamp DNA stool mini kit. This is suitable for complex tissues which are different from carefully prepared plant material. We have tested it on olive oil and with other plant-based foodstuffs with successful results (Testolin & Lain, 2005).

As an integral part of DNA extraction, tungsten beads can be used to facilitate leaf tissue lysis (Ivanova et al., 2008).

2.2 | The choice of markers

2.2.1 | Some history

In 1984, Alec Jeffreys discovered the technique of genetic fingerprinting based on the DNA variable number of tandem repeats (DNA VNTR) or minisatellites (Jeffreys et al., 1985). From then onwards, forensic scientists working on humans moved to shorter DNA tandem repeats (1–7 bp long) called microsatellites or SSRs (Figure 1) (Litt & Luty, 1989).

Core Ideas

- DNA-based fingerprinting is a modern molecular approach to characterize germplasm resources.
- SSR (simple sequence repeats) or microsatellites are nowadays the molecular markers of election for genotyping.
- This review reports protocols and markers selected for fingerprinting 44 fruit crop species.

Stretches of tandemly repeat nucleotides are found in any living organisms and, indeed, the technique of fingerprinting based on microsatellite DNA was rapidly adopted in the analysis of plants.

Large datasets of DNA microsatellites have been isolated from enriched libraries of plant tissues and, because of the larger frequency of di-nucleotide repeats compared with tri- or longer repeats (Edwards et al., 1996), microsatellites with di-nucleotide repeats, like AC/GT and AG/CT, are by far the most common molecular markers reported in the literature for plant fingerprinting.

Currently, microsatellites are identified by scanning whole genome sequences and the isolation of microsatellites with long core repeats would be easily achieved. However, the huge number of DNA profiles based on 2-nt repeats produced from hundreds of world laboratories (Guichoux et al., 2011) discourage, in a number of different ways, the search for longer repeats. Unfortunately, while highly polymorphic, 2-nt microsatellites together with the less used 1-nt ones are not as easily scored or clearly merged across platforms and laboratories as are long core repeat markers (Flores-Rentería & Krohn, 2013; Nybom & Lācis, 2021).

2.2.2 | Microsatellite or single nucleotide polymorphism markers?

The use of single nucleotide polymorphism (SNP) markers, that are based on variation in a single nucleotide and obtained from large sequencing projects, has recently been reviewed by Nybom and Lācis (2021). SNPs are indeed the markers of choice for massive genotyping and for constructing genetic maps, given their ease of detection and the reduced cost of screening large collections. However, interestingly, they have been discarded for human forensic genotyping for the simple reason that they are less suitable and too expensive for rou-

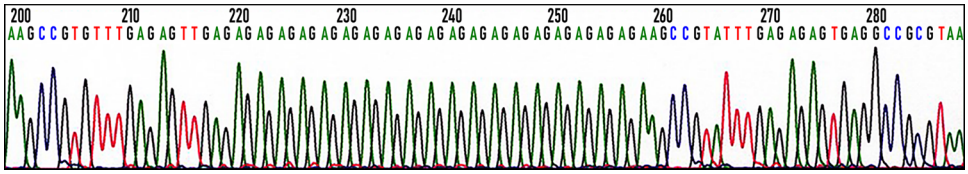


FIGURE 1 SSR (simple sequence repeat), known also as STR (short tandem repeat) or microsatellite, is a short DNA sequence, 1–7 bases long, tandemly repeated several times. The figure shows a microsatellite based on a di-nucleotide GA repeat. Such a sequence, during DNA replication, can undergo mutations that shorten or lengthen the DNA copy thus creating allelic variants among individuals in the population, revealed by PCR and electrophoresis

tine genotyping of low numbers of samples and because they would require the very large databases of SSR-based profiles, that have been accumulated worldwide over many years, to be abandoned.

SSRs are still and will probably remain for the near future the markers of choice because of their low cost, the additional information they provide on the ploidy of the material being studied, and the potential they could offer for comparing the numerous SSR-based datasets created worldwide by genotyping local collections.

For the sake of completeness, we must record those other classes of markers that were developed in the past but are no longer used for fingerprinting. These include random amplified polymorphic DNA (Williams et al., 1990), amplified fragment length polymorphism (Vos et al., 1995), inter simple sequence repeat (Godwin et al., 1997), selective amplification of microsatellite polymorphic loci (Roy et al., 2002), sequence-specific amplification polymorphism (Waugh et al., 1997), diversity arrays technology (Jaccoud et al., 2001) and others that are less known (reviewed in Nybom & Lācis, 2021). The common features of most of these markers is that, as primers are not specific to a particular DNA sequence, their position in the genome cannot be identified; moreover, they are dominant so that the heterozygous genotypes cannot be discriminated from the dominant homozygous one. In addition, they have low reproducibility and poor transferability between distantly related genotypes. None of these markers were ever adopted in human fingerprinting (Testolin & Cipriani, 2010) and, for the same reasons, we consider them unsuitable for use with plants.

2.2.3 | Criteria for SSR marker selection

Following from the above, it is important to consider the criteria for SSR marker selection. The lists provided in the tables below for each fruit crop include some 20 markers for each species or group of species, listed according to their informative content and distributed along the whole genome to reduce the risk of linkage. These lists were selected according to the following criteria:

- tri-nucleotide or longer core repeats, when possible;
- single locus or, at least, two not-overlapping loci;
- high polymorphism;
- low stuttering and consistent reproducibility;
- loci with independent segregation (not linked) that are distributed along the genome.

Twelve or fewer markers, depending on the marker informativeness and the genetic diversity of the crop, have been found to be sufficient for distinguishing accessions derived through sexual reproduction, including selfing. An exception is represented by sport mutations and clonal selections. For such accessions, there is presently no technology available to distinguish among these individuals. This includes genome sequencing with high coverage.

The sets of markers provided are better suited to large studies that are investigating genetic diversity, parentage and kinship. Only germplasm with strong coancestry, like apple, grape, and peach, might require a higher number of markers to solve identity and to discriminate among alternative parentages, but this is really an exception.

It was disappointing to find that for several of even the most popular species, such as apple, only markers with di-nucleotide repeats were available in the literature. Di-nucleotide repeats have been abandoned in human fingerprinting because the resulting stuttering hinders the separation of contiguous alleles. This same problem is observed in plants (see, for instance, Guichoux et al., 2011; Nybom & Lācis, 2021; Testolin & Cipriani, 2010) and, consequently, trinucleotides or longer core repeats need to be adopted.

2.2.4 | The length of the SSR core repeat

In general, the longer the core repeat, the larger the distance between neighbouring alleles. Because of the imprecision in determining the fragment length during the electrophoretic separation of amplicons, large intervals between adjacent alleles, which is typical of microsatellites with long core repeats, greatly help in fragment binning (Chambers et al., 2013).

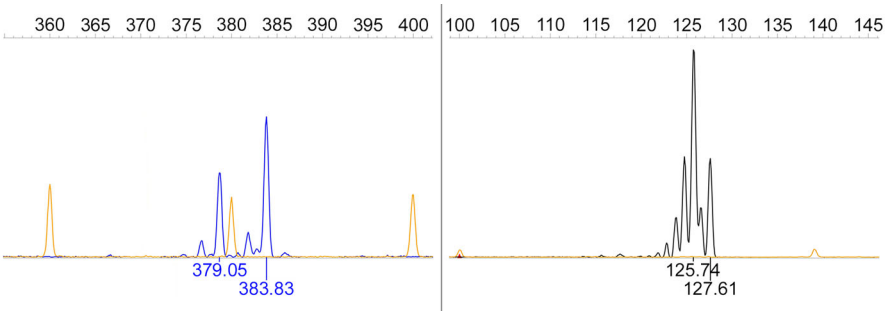


FIGURE 2 A typical kiwifruit DNA profile, showing genotypes with two neighbouring alleles. (Left) the SSR LG15_MK339 with a 4-nt core repeat (TATC); (right) the SSR UDK-414 with a 2-nt core repeat (AG). Note that the vicinity of the two alleles in the 2-nt SSR and the overlapping stuttering, represented by the left "ghost" bands, makes it difficult to identify the true alleles. In both graphs, the orange peaks are the ladder amplicons. The numbers below the peaks of true alleles are base pairs

Di-nucleotide repeats, when regular, have a 2-bp interval between adjacent alleles, and such a short distance makes it hard to correctly define the bins, that is the range of variation in the size of each allele. The standard error in assigning the allele length is ±0.3 to ± 0.8 bp within a single run (Amos et al., 2007; Idury & Cardon, 1997; Weeks et al., 2002). Different protocols, internal standards, matrices, and sequencers that are routinely used for genotyping can generate even larger differences in assigning the length to a given allele. Moreover, di-nucleotide repeats often show a considerable amount of stuttering (a ladder of shorter peaks that appear at intervals of 2 bp), that makes it difficult to identify true adjacent alleles (Figure 2). Weeks et al. (2002) reported that 83% of discrepancies among laboratories in scoring di-nucleotide microsatellites are due to the binning process, when raw allele lengths are converted into allele classes whose average size is then expressed by an integer.

The use of microsatellites with long repeats is the rule in humans, and microsatellites routinely used for forensic purposes have core repeats of four or five bases long (Butler, 2006). Di-nucleotide repeats are banned in human fingerprinting protocols and it is surprising that plant scientists have not followed this same principle. However, di-nucleotide repeats are still in use and a number of practices that could help the binning process are lagging behind in development (Guichoux et al., 2011).

2.3 | PCR amplification

There is a large variability in the PCR mix that is used, and in the thermocycler profiles that are adopted in different studies, and reported in the literature . The procedure that works very well for a range of species has been adopted by the authors (Supplementary Material).

In general, a longer annealing time and a higher and more stringent annealing temperature seem to dramatically improve amplification efficiency of microsatellite loci (Flores-Rentería & Krohn, 2013).

There are a number of issues relating to the use of primers that should be taken into consideration. First, the laser detection system in capillary sequencers requires that one of the primers be labelled with a fluorescent dye, which may be either 6-carboxy-fluorescine (FAM), or hexachloro-6-carboxy-fluorescine (HEX), or 6-carboxy-X-rhodamine (ROX), or tetrachloro-6-carboxy-fluorescine and others. These fluorescent dyes are expensive and a brilliant alternative, for what the inventor called the "poor man's approach to genotyping" (Schuelke, 2000), is to use a FAM-labeled M13 (−21) universal primer: [FAM]-TGT AAA ACG ACG GCC AGT-3′ added to the nonlabeled forward primer. The M13 universal primer anneals well at only 53˚C and the last eight PCR cycles should, therefore, be run with an annealing temperature of 53˚C.

Second, Taq DNA polymerase can catalyze the nontemplate addition of a nucleotide (adenosine) to the 3′ end of PCR-amplified products. This phenomenon is known as the "plus A" addition. Considering that it is easier to drive the plus A reaction to completion than to prevent it, the solution is to add the sequence GTTCTT (or GTTTCTT or similar sequences) to the 5′ end of the unlabeled reverse primer. This is the so-called strategy of "PIG-tailing" (PIG is from the Prostate Investigational Group that set up the solution). There is no need to remove the tail through restriction enzymes as suggested initially. The increased length of amplicons just needs to be taken into consideration.

Thirdly, in the case of bad amplification (extra peaks, etc.), the adoption of the "touchdown" (TD) procedure is recommended. Under normal PCR protocols, primers can produce spurious bands caused by nonspecific binding of the primers.

These spurious bands can increase scoring difficulty and make a locus less useful for genotyping. Don et al. (1991) developed TD-PCR to help eliminate spurious bands and increase the quantity of target DNA produced. For TD-PCR, cycles begin with a very high annealing temperature, well above the melting temperature (2–4˚C). The TD-PCR program is designed to decrease the annealing temperature in small increments (e.g., 0.5–1˚C) every second cycle to the expected annealing temperature (the "touchdown" temperature). Once the reaction reaches the TD temperature, 10 cycles are run at this annealing temperature before final extension. This method reduces the number of spurious bands because less nonspecific annealing occurs at higher temperatures, so only the target region should amplify during early cycles, exponentially increasing the amount of target DNA available in later cycles.

Finally, multiplexing markers with a different fluorescent dye and/or a different range size is strongly recommended in many recent papers as a tool to reduce time and labor (reviewed in Guichoux et al., 2011).

2.4 | Electrophoresis and fragment separation

Fragment separation can be carried out on large acrylamide gels, using equipment that any laboratory can easily obtain with minimal investment. Alternatively, it can be achieved through capillary electrophoresis carried out on automatic sequencers. In human fingerprinting, earlier studies involved detection on silver-stained polyacrylamide gels but, subsequently, that community has embraced fluorescence detection methods involving capillary electrophoresis (Butler, 2006). The use of matrices based on agarose gel is excluded with plant tissue samples because the resolution is not sufficient to clearly separate alleles that differ from each other by only one or two bases. Different capillary sequencers require appropriate sample preparation. The correct sizing and binning of alleles is essential to the robustness of the fingerprinting analysis (Flores-Rentería & Krohn, 2013) and many factors can influence the absolute allele sizing.

2.4.1 | Type and age of capillary matrices

Applied BioSystems (ABI, now Life Technologies Corporation, part of ThermoFischer Scientific) offers, for instance, a series of matrices with different concentrations of the polymer (POP-4 to POP-7). The higher the matrix code the better the resolution of small differences. The results obtained with one matrix are, however, not comparable with those obtained with the other matrices. The recently released sequencer, SeqStudio Genetic Analyzer (ThermoFisher Scientific), offers a unique kind of polymer optimized for both sequencing and genotyping.

2.4.2 | The platform

Different sequencer models, even from the same company, can size the same allele with up to several bases of difference. For instance, Applied Biosystems (AB) declares that sizing with a different platform could produce differences between −2 and +4 bp, with higher differences for longer fragments (source: AB AmpFl STR SGM Plus PCR Amplification Kit, User's Manual, 2006).

2.4.3 | The size standard

The LIZ size standards adopted by the AB are credited with differences of 1–3 bp from each other (AB User Bulletin, 2007). Pawlowski and Maciejewska (2000) compared the results obtained with two different standards, GS500 and FL-CXR, and reported a size deviation in the range of 0.18–0.75 bp for GS500, and a more reduced size deviation (0.06–0.16 bp) for the FL-CXR.

The comparison of commercial size standards dramatically shows their influence in the estimation of the length of alleles (Figure 3).

2.4.4 | Rounding the allele size to the integer

The different base composition of microsatellite amplicons and the ladder fragments result in a different relative mobility of both amplicons and the ladder fragments. Alleles show periodicities that are close to, but not exactly the length of the underlying repeat unit, and the integer to which an allele is rounded does not match the periodicity of the core repeat (Figure 4) (Amos et al., 2007; Idury & Cardon, 1997).

2.5 | Merging data from different runs, platforms, and laboratories

Merging data from different laboratories, from that obtained from different platforms used for capillary fragment separation, and even that from different runs carried out with the same equipment, especially when a project takes months or years to be completed, is challenging.

Describing the strategies that can be used to resolve such issues would require considerable discussion and is out of the scope and objective of this review. However, we present below key points that can assist investigators in merging data from different sources.

2.5.1 | The use of allelic ladders

Chemical companies find it economically convenient to produce ladders only for human DNA microsatellite markers.

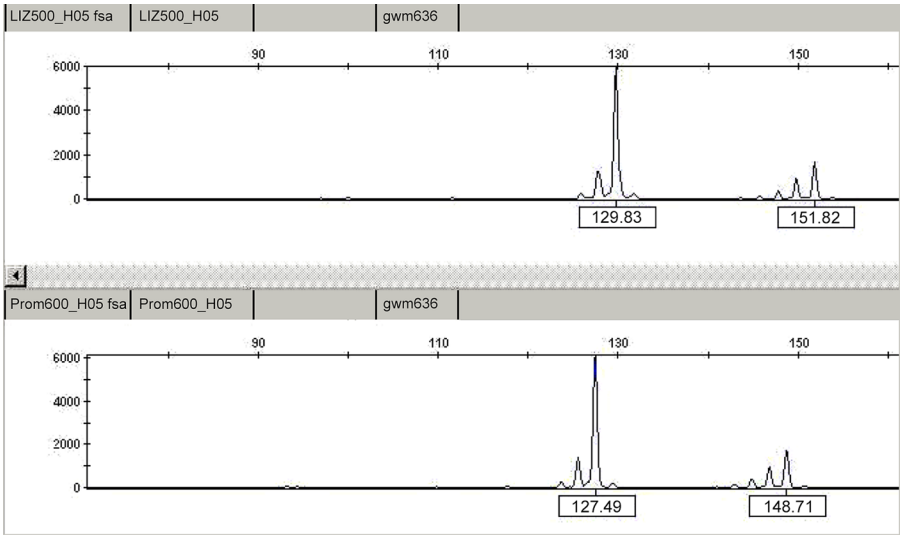
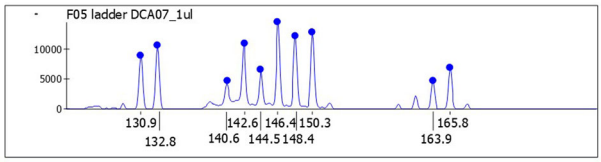


FIGURE 3 Differences in allele sizing of the same genotype at the same SSR marker due to the ladder used: (top) LIZ-500, (bottom) Promega-600. In both profiles, the samples, PCRs, and capillary electrophoresis conditions were the same. The differences in sizing (numbers beneath the SSR peaks) are not constant, and usually increase with the range of alleles, as can be seen in these pherograms, where the difference between the two shorter alleles is 129.83 − 127.49 = 2.34 bp, while the difference between the longer ones is 151.82 − 148.71 = 3.11 bp (from Testolin & Cipriani, 2010)



Sized *	130.9	132.8	140.6	142.6	144.5	146.4	148.4	150.3	163.9	165.8
Rounded	131	133	141	143	144	146	148	150	164	166
True allele **	131	133	141	143	145	147	149	151	165	167

* Size standard ET-ROX 400
** From the sequence

FIGURE 4 Allelic ladders produced for the olive microsatellite DCA7. Peaks representing true alleles are marked with the symbol (•). Values below the peaks are the allele size (bp) estimated by the software, using the ET-ROX 400 size standard. The original size, the value rounded to the integer, and the correct size deduced from sequencing the alleles are reported in the table below the graph. The inconsistency between estimated and true allele sizes is evident. The marker has a regular 2-nt step, but in this case the differences in base composition between the microsatellite and the ET-ROX sizing standard generated an estimate of the average distance between two adjacent alleles of 1.94 bp (165.8 − 130.9 = 34.9 bp instead of 36.0). This underestimated the repeat length and the rounding process generated the wrong allele binning. Other size standards, like GeneScan 600 LIZ of TermoFisher or ILS 600 of Promega perform better

However, homemade allelic ladders can be produced by combining PCR products from different samples that cover the entire, or a large part of, the alleles of a given marker (Figures 4 and 5).

2.5.2 | The use of reference genotypes

If the preparation of an allelic ladder is considered too demanding, a reference genotype can be added into each individual run, selected from among the most well-known cultivars. This method is particularly useful when profiles are merged from different laboratories. The use of reference genotypes is also recommended by The International Union for the Protection of New Varieties of Plants (UPOV, 2021).

2.5.3 | Regular discrepancies

Regular discrepancies might be due to the presence of part of the M13 sequence in the primer, or the presence of the PIG-tail that typically generates differences of 6–7 bp, or the presence of +A added by the Taq polymerase (see Section 2.3 above).

To conclude this section on the protocols used for the analysis of SSR markers, the example provided in Figure 6 shows some of the recent advances in fingerprinting of fruit crops which have applied the protocols currently in use in human fingerprinting. These include the use of microsatellites with long core repeats (3-nt or longer), multiplexing of different markers using different labelling dyes, and fragment separation using a capillary sequencer.

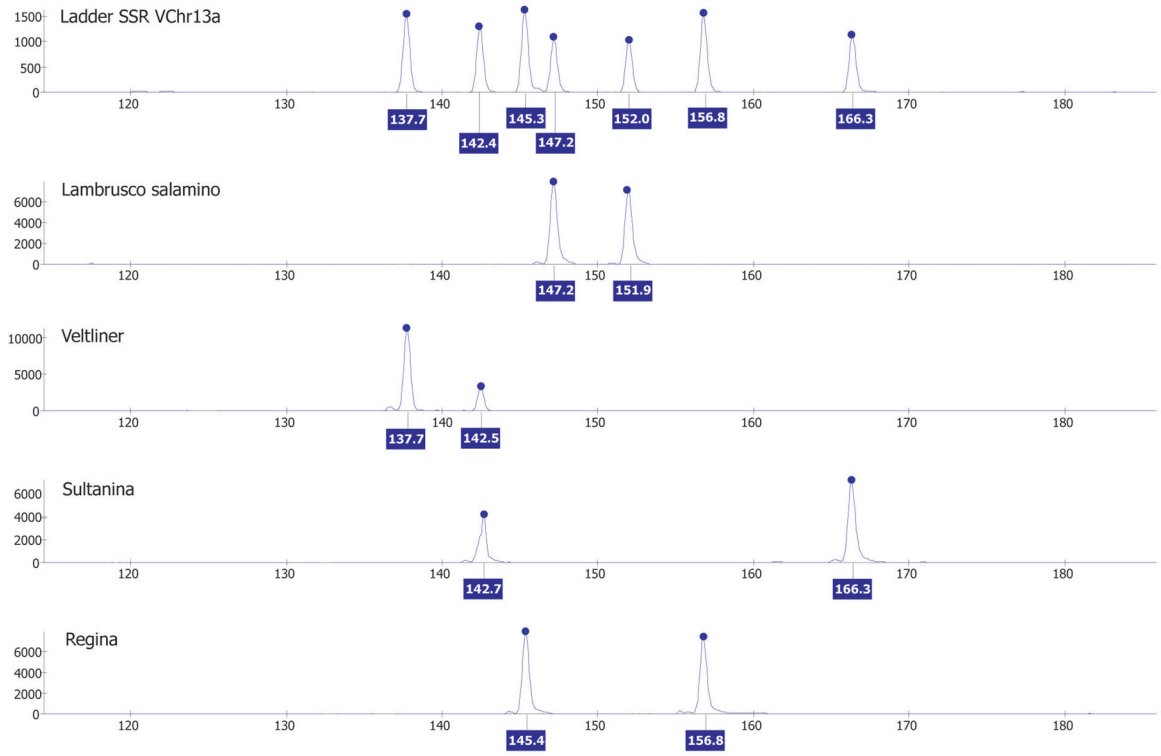


FIGURE 5 Example of an allelic ladder developed for the grape marker VChr13a. The first pherogram reports the ladder produced by mixing the PCR products of four cultivars, whose individual pherograms are reported below. The sizes of the alleles are expressed in bp (from Cipriani et al., 2008)

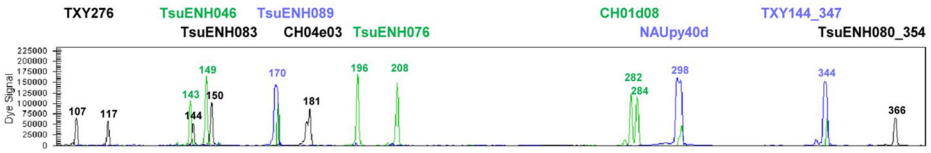


FIGURE 6 Example of a modern fingerprinting, based on 10 SSRs with long repeats, multiplexed using different marker-labelling dyes and markers with different size ranges. PCR products were separated with a capillary electrophoresis sequencer. Numbers above the peaks are the allele size in base pairs. This fingerprinting set was developed for the analysis of the US *Pyrus* Genetic Resources (USPGR) (from Zurn et al., 2020)

2.6 | Data analysis

In the sections that follow, the rationale behind the analyses of identity, parentage, and kinship is described and the techniques of grouping as a function of genetic similarity (clustering) are covered. Several software packages commonly used in the analysis of genetic diversity are detailed and, finally, some of the problems raised specifically by plants (ploidy, chimerism, etc.) are discussed.

2.6.1 | Analysis of identity

Sometimes, two accessions can show an identical DNA profile at all of the markers. The question that arises, therefore, is: do they belong to the same accession or cultivar? This is not a trivial question.

While the presence of differences at a given number of markers in two profiles is a sufficient condition to say that two samples are different from each other, probability is used to determine if identical profiles for two DNA samples belong to the same genotype or not. To resolve this issue, one must compare the probability of a match if the two DNA samples are from the same individual, with the probability of a match if the two samples are from different individuals. In the first case, the probability of a match is 1; in the second case, the match probability (MP) is calculated from the allele frequency in the population.

Consider a single locus (*L*). The probability of the occurrence of a genotype in the population with the profile *La*/*Lb*

depends on the frequency of the alleles *a* and *b*. With an allele frequency of 0.10 and 0.20 for L*a* and L*b* respectively, the probability of occurrence of the *La*/*Lb* genotype is $2ab = 0.04$ (a^2 in case of homozygosity). The product rule (Kirby, 1990) can be applied and the cumulative probability can be calculated as the product of genotype frequencies at each individual locus.

The probability of identity (PID), known also as MP, between two DNA profiles is the ratio between the two probabilities described above, that is

$$\mathrm{PID} = 1/\prod_{i=1}^{n}(g_i)$$

where *g* is the frequency of the genotype *g* at each of the *n* loci.

A minimal percentage of mismatches is allowed by the software programs to take account of genotyping errors and mutations that can occur in vegetatively propagated species.

This formula applies to a population in Hardy–Weinberg (HW) equilibrium, which is not always true for germplasm collections, where inbreeding and coancestry might be frequent and heavily influence the HW equilibrium.

The PID test between pairs of samples requires a large database of profiles from which to extract reliable allele frequencies. Consequently, analyzing an individual sample to see "what it is" is meaningless unless it can be compared with the profiles of different accessions. The creation of this kind of database requires either a significant amount of work in a single laboratory, or the comparison of profiles obtained by different laboratories merged into a single dataset. We again stress that only the adoption of reliable markers, harmonized fingerprinting protocols, and possibly ring tests among laboratories on common samples can allow the merging of data sets from different laboratories.

2.6.2 | Analysis of parentages

In the genetic analysis of a germplasm collection, once the problems of synonymy have been solved, the reconstruction of parent–offspring relationships is the second challenge for the curator.

The procedure consists of two steps. The first one is rather intuitive and involves the exclusion of all candidate parents not compatible with the tested offspring because they do not carry any allele of the offspring at one or more loci.

The second step is based once again on the likelihood ratio between two hypotheses (H1 and H2). The hypothesis H1 is that the candidate parent is the true parent, while the hypothesis H2 is that the candidate parent is an unrelated individual from the same population.

Given that g_o and g_p are the genotypes of the offspring and the alleged parent at a given locus, the likelihood ratio (LR) between the hypotheses H1 and H2 for that locus is

$$\mathrm{LR}(\mathrm{H1},\ \mathrm{H2} \mid \mathrm{g_o}, \mathrm{g_p}) = \mathrm{T}(\mathrm{g_o} \mid \mathrm{g_p}) / \mathrm{P}(\mathrm{g_o})$$

where $T(g_o|g_p)$ is the probability of the offspring's genotype, g_o, given that the alleged parent's genotype is g_p, and $P(g_o)$ is the frequency of the offspring's genotype g_o in the population. One can see in Table 2 of Marshall et al. (1998) the value assumed by the variables $T(g_o|g_p)$ and $P(g_o)$ according to the offspring's and alleged parent's genotypes. The cumulative LR of the inferred paternity is the product of the individual LRs of each locus.

Once one has found the alleged parents, a classical trio analysis can be carried out for all combinations of parent pairs, assuming that either parent of the pair is known (Weir, 1996). If not, paternity can be assigned by comparing likelihood ratios of alternative parent pairs (Thompson & Meagher, 1987).

A statistical pitfall of this blind analysis is the presence of full sibs in the population. The relatedness problem is more serious when either parent is not included in the sampled population, no parent is known "a priori," or the siblings are present in a high percentage and are related to the candidate offspring rather than to the parent (see Marshall et al., 1998 for a more exhaustive discussion).

Several software programs can calculate the first degree parentage, but most of them do not foresee selfing that could be common in plants and/or do not accept missing data and errors in genotyping. CERVUS (www.fieldgenetics.com; Kalinowski et al., 2007; Marshall et al., 1998) is one of the software packages that, although designed for pedigree analysis in animals, meets all of the requirements for pedigree analysis in plants, without any of the restrictions mentioned above.

If the parents are known, seed parent and pollen donor can also be ascertained using polymorphic sequences of cytoplasmic DNA, which is uniparentally inherited. An example can be found, for instance, in determining the origin of the Asian pear "Kosui" (Sawamura et al., 2004).

To conclude we wish to stress once again that a large database of DNA profiles, from which to extract the allele frequencies, is required for any identity and parentage analysis. Large databases can also provide information on more relaxed relationships and kin groups (Blouin, 2003; Butler et al., 2004; Konovalov et al., 2004; Wang, 2004) as well as on the levels of coancestry and inbreeding (Reynolds et al., 1983).

2.6.3 | Analysis of kinship

In forensics, kinship analysis is used as a form of genetic profiling aimed at discovering possible genealogical relationships between individuals based on DNA samples.

Relatedness between individuals and groups can be investigated using DNA markers. A seedling DNA profile is a combination of alleles passed down from the male and female parents. This means that relationships can be investigated between alleged family members. DNA profiling is commonly used to test for potential paternity, parentage, and sibship (whether genotypes are related as sibs) relationships.

For parentage studies, the most basic analysis is based on a simple Mendelian principle: at any given locus, an offspring should possess one allele inherited from its female parent and another allele inherited from its male parent. Therefore, it should be possible to identify the biological parents of an offspring by comparing its genotype to that of potential parents. Plants that do not match the offspring (i.e., do not possess an allele found in the offspring) can be excluded as biological parents, and the combination of two parents (male and female) should explain all of the alleles found in the offspring (although sometimes a single mismatch may be explained by mutation).

To properly analyze parentage using microsatellites (or any other codominant marker), one must understand the probability that an offspring would match an adult by chance. This probability can be high for loci with relatively few alleles and for common alleles at more polymorphic loci. Several methods for estimating this probability have been developed (Jamieson, 1994), and some of these address various problems that may arise in any real study.

Molecular markers, like SSR, can help to investigate not only parentage but also more relaxed relationships, like sibship (brothers or sisters, in human terms) and other familial relationships (uncle–nephew, grandfather–grandson, etc.)

2.6.4 | Clustering

Once a set of accessions has been genotyped, there is sometimes interest in exploring how similar the individuals are to each other; in other words, beside the parental relationships, how are individuals grouped on the basis of their genetic vicinity? Such an explorative analysis can be found regularly in the literature. Although, in our opinion, the analyses of pedigree or kinship appear more appropriate, we present a brief section on clustering. The procedure involved requires the following steps:

- the calculation of matrices of similarity/dissimilarity for each pair of samples;
- the clustering itself and the representation of what is called a similarity tree or dendrogram.

The matrices of similarity/dissimilarity are many depending on the function applied.

Once the matrix of similarity/dissimilarity has been calculated, a hierarchical clustering is achieved through algorithms that reflect the structure present in the set of samples. The two most popular clustering methods are the UPGMA (unweighted pair group method with arithmetic mean) and the "Neighbor joining" method (Saitou & Nei, 1987).

It is worth remembering that the clustering approach described above is a representation of genetic vicinity and has nothing to do with evolution. The correct approach to the estimation of evolutionary distance is based on maximum parsimony and maximum likelihood methods that try to find the minimum number of mutations that have occurred to create the landscape represented by what is called a phylogenetic tree. But that is another story.

2.7 | Software for data analysis

We describe below some of the most common software programs that are used in the analysis of genetic diversity in plants. This is not an exhaustive list and is not intended as an endorsement, but it is an outline of some of the available packages and their uses. They are:

- Arlequin (Schneider et al., 2000; Excoffier & Slatkin, 2005)
- CERVUS (http://www.fieldgenetics.com/)
- GenAlEx (Peakall & Smouse, 2006; Peakall et al., 2012)
- NT-SYSpc (2011; Rholf, 2002)
- MEGA (Kumar et al., 2001)
- COLONY (Jones & Wang, 2009)
- KINGROUP (Goodnight & Queller, 1999; Konovalov et al., 2004)
- STRUCTURE (Pritchard et al., 2000)

There are, of course, many other packages like GENEPOP (Raymond & Rousset, 1995), POPGENE (Yeh et al., 1997), PowerMarker (Liu, 2003), Fstat, Genetix, and PARENTAGE, including multipurpose packages such as R software.

2.7.1 | Arlequin

Arlequin is a powerful genetic analysis package performing a wide variety of tests, including hierarchical analysis of variance. The software is dedicated to the analysis of populations, which can be represented by germplasm collections or by natural populations that can be hierarchically subdivided into groups, populations, and so on. Elementary data can be analyzed in different ways. Among the most common are (i) genotypic data based on molecular markers (SSR, AFLP, RFLP, SNP, etc.); and (ii) haplotypic data based on DNA sequences (mt- or cp-DNA).

2.7.2 | GenAlEx

The GenAlEx package can be downloaded without cost from the website https://biology-assets.anu.edu.au/GenAlEx/Download.html (the instructions should be followed before the content from the zip file is extracted). The version 6.51 is optimized for Excel 2016/Windows 10 and works in Excel as a macro written in Visual Basic for Applications. With GenAlEx being a macro of Excel, all functions of Excel are preserved.

The options for analyses include the determination of genetic and geographic distances, analysis of molecular variance, principal coordinates analysis, and others.

2.7.3 | CERVUS

The program can be downloaded without cost from the website http://www.fieldgenetics.com. It is a suitable software package for the analysis of identity and parentage. Launched in 1998 and repeatedly updated, it combines a robust likelihood-based method with a simple graphical interface. Despite being designed to manage wild animal populations, it applies well to plant populations and plant germplasm. Interesting features, in comparison with other packages, are (i) missing data are accepted; (ii) mis-typing and genotyping errors are allowed; and (iii) selfing is also allowed.

2.7.4 | MEGA

MEGA is a sophisticated software for molecular evolutionary genetic analysis. The website https://www.megasoftware.net/home details the many features that are available in the updated 11th version. Other programs among those that are listed here are, however, more suitable for the basic analyses of identity, paternity, and kinship.

2.7.5 | NT-SYSpc

NT-SYS is classical software for the analysis of clustering and includes the popular UPGMA and Neighbor-joining methods.

NT-SYS accommodates more than two alleles per marker and is suitable for polyploids. The solution adopted by the program is simple: the original fragment size data are transformed into a binary data set, where each allele of the marker is treated as a variable with presence/absence of the given allele scored as 1 (present) or 0 (absent). The reader has to consider that the transformation of the data set in 1/0 must be done by the individual operator, but this should not be a problem.

2.7.6 | KINGROUP

This software calculates expected frequency distribution of relatedness values of specific relationships (full sib, half sib, etc.), and tests the likelihood of a pair of individuals belonging to a specific relationship. Moreover, it estimates an overall likelihood for alternative partitions. The original program of Goodnight and Queller (1999) was largely improved with new features in Konovalov et al. (2004).

2.7.7 | STRUCTURE

STRUCTURE is a program that investigates the population structure of accessions analyzed by DNA fingerprinting. Its uses include inferring the presence of distinct populations and assigning individuals to a population.

Despite plant genetic studies being far from forensic analyses, which are primarily intended for the search of identity and pedigrees, this program is included here for its popularity in the assignment of individuals to populations. The software can also allow proportional assignment to multiple populations: known as the "admixture mode."

2.8 | The tricks of plants: polyploidy, chimerism, and sport mutations

2.8.1 | Polyploidy

A ploidy level greater than 2× is very frequent in horticultural fruit crops. In polyploids, alleles can be present in more than one copy, and allele dosage is difficult to estimate correctly. Attempts to manage polyploids are reported in the literature for sour cherry (Cantini et al., 12001), kiwifruit (Huang et al., 1998), plum (Sehic et al., 2015), rose (Esselink et al., 2004), persimmon (Liang et al., 2015; Wang et al., 2021), and strawberry (Davis et al., 2006; Sargent et al., 2012). The analysis of a small number of samples can be attempted by repeating PCRs and electrophoretic runs several times and applying statistical analyses to the estimate of allele dosages. However, the analysis of large data sets is not easy to achieve. A successful example has been reported in rose (Esselink et al., 2004) where the MAC-PR (microsatellite DNA allele counting - peak ratios) method was adopted to the genotyping of some eighty ornamental accessions. Van Dijk et al. (2012) proposed an improvement on this method: microsatellite allele dose and configuration establishment. A simple, although rough, approach is to treat as present/absent any PCR fragment (Weising et al., 2005). Indeed, we would recommend such an approach, which means scoring and recording the alleles in the case of SSR markers as present/absent,

accepting that information on allele frequency would be lost. In such a way, for instance, AABBCC and AAAABC genotypes are both coded as ABC. This way of codifying the data is known as the "allelic phenotype" approach (Urrestarazu et al., 2018).

2.8.2 | Chimerism

Tri-allelic profiles can occasionally be recorded in diploid genotypes due to the presence of chimerism. Periclinal chimeras are meristem structures in which genetically different cell layers originate from a single cell mutation and coexist in the same tissue. This phenomenon is well known in plants and extensively discussed, for instance, in grape (Hocquigny et al., 2004), but there is evidence that it is not as rare as originally thought even in animal and human tissues. In plants, vegetative propagation can indefinitely maintain a tri-allelic mutant.

The chimeric tri-allelic profiles are not easily accommodated in computer programs that treat codominant markers such as SSRs. One solution is to record the same genotype twice with alternative alleles.

2.8.3 | Essentially derived varieties and clonal variants

In many horticultural and woody ornamental species, sport mutations give rise to clonal variants that are retained by growers when they represent an improvement of the original cultivar. Most fruit crops include these kinds of genotypes in their varietal platforms. Not being derived by sexual reproduction, they are called somatic mutants or clonal variants and, from a legal point of view, they are considered as "essentially derived varieties" (EDVs) (http://www.upov.int).

There is considerable debate with regard to the IPRs of this material. The topic is beyond the scope of this review. However, EDVs can be formally protected under the UPOV convention and the molecular identification of clonal variants is considered valuable proof in disputes relating to IPRs.

Historically, several approaches have been pursued in the attempt to find a method for distinguishing among clonal variants of the same variety, such as (a) high throughput SNP genotyping technologies, (b) genome resequencing based on second generation sequencing technologies, and (c) analysis of copy number variation based on comparative genomic hybridization protocols.

A common rationale of these approaches is to scan a very large part, if not the whole genome. However, the recent advancement of next-generation sequencing technologies has made it most convenient to adopt the whole genome sequencing approach. The result, nonetheless, is not straightforward. For example, we cite the experience of IGA (Applied Genomics Institute of Udine, Italy) who committed in 2010 to distinguish between two clones of the "Sangiovese" wine grape cultivar. The two clones were sequenced at very high coverage (near 100×), which was unusual at that time. Alignment with a good grape reference genome (the PN40024) allowed two single base mutations to be found that differentiated consistently the two clones from each other and from 22 other "Sangiovese" clones (unpublished data). The analysis was very demanding in terms of cost (several hundred thousand Euros at that time) and the time that was required for the sequencing and subsequent analyses. The drawbacks that were experienced were mainly due to the false positives that were produced by sequencing errors and the need to perform hundreds of control PCRs. The project took 2 years to complete. Sequencing technologies are continuously improving along with bioinformatics tools, but currently whole genome sequencing remains largely unfeasible.

3 | MARKERS IN SELECTED SPECIES OF FRUIT CROP

The primers that are reported for each of the selected species in this section were transferred from the original papers through a copy/paste procedure to avoid any mistakes in the reporting. Moreover, primers were checked in the genome assembly of that species, when available, and in a few cases primers were revised and/or reoriented according to the published genome sequence.

3.1 | Almond (*Prunus dulcis* (Mill.) D.A.Webb and related species)

Although there have been tens of di-nucleotide microsatellite markers isolated from almond and reported in the literature (see for instance Dangl et al., 2009; Rigoldi et al., 2015; Testolin et al., 2004), we prefer to suggest the use of microsatellites with longer core repeats (3-nt or more) that have been isolated from peach (see Section 3.30 below) and successfully tested in almond.

Almond is diploid ($2n = 16$) and the researcher might expect one or two alleles per marker according to the heterozygosity of the locus analyzed.

A list of markers and primers, primarily from peach, is reported in Table 1.

3.2 | Apple (*Malus domestica* Borkh and wild relatives and hybrids)

Although the apple genome sequence has been available since 2010 (Velasco et al., 2010), we were unable to find SSRs with long repeats in the literature. We screened some twenty of the

TABLE 1 Recommended markers for almond, apricot, peach, and plum. Touch down (td) protocols, with initial temperatures 2–4˚C above those recommended in this review have also been considered by some authors. Linkage groups (LG) are reported according to the reference genome assembly; the peach genome assembly is GCA_000346465.2.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (˚C)	RG (BP)	Position of primer within the peach genome
RPPG1-017	(AGCTT)5	1	GCTCATCAAAACTCTCAACCA	CCCTTTCTTCAATCCCATC	56	n.a.	2,785,425–2,785,647
RPPG1-041[a]	(ATT)7	1	TGTTGTAATGGATGGTGTCTTC	CTTGGTCTTGGTTTCATTCA	56	n.a.	45,440,855–45,441,082
RPPG1-037[a]	(AGC)7	1	GTCTCTGATCCAAGCCAACT	ACGCTGCCATTGTTTCTATT	53	n.a.	47,629,175–47,629,416
RPPG2-011	(ATTT)5	2	TTTACAGGTGCCTCAACAAA	GTACAGCCGATGGAGAGAAA	53	n.a.	5,473,613–5,473,811
RPPG2-022	(CTGT)6	2	CTGCTGCGTCTGATGATG	ACAGGACAGGACCACTTTCT	53	n.a.	30,174,301–30,174,510
RPPG3-026	(CTGT)6	3	AGAACGCTATTCCCCTGTAA	TCATCCTCTCCAAATGTCAA	53	n.a.	4,012,148–4,012,392
RPPG4-059	(ACTGG)6	4	GACGGCTGTTTATTTGCATT	TGCATTTGTGATCTCGTTTC	56	n.a.	138,756–138,937
RPPG4-077	(AATT)5	4	CCTCGTCTTCAGTCTTTTCTG	CTGTCCCTTCTGTGTTCCTAA	57	n.a.	16,699,641–16,699,799
RPPG4-084	(ATTT)5	4	TCCTCAAAAGTTACCCCAAG	CTTGCTGTGGAAGAAGAACC	58	n.a.	22,535,263–22,535,538
RPPG4-091	(CTTTT)6	4	GGAGGGTAGAGAACAGAGCA	CGGAAGATGTGATTGTGAGA	49	n.a.	23,452,650–23,452,891
RPPG5-018	(ATT)8	5	GCATGAAATTGACCCATACA	TAATTGCTTTGGGGAGGAC	56	n.a.	5,332,381–5,332,568
RPPG5-023	(ATT)7	5	TTGTTTGCACTAGGCTTTGA	TTCTTCTTGCATGTCCTTGA	56	n.a.	16,620,031–16,620,224
RPPG5-030	(AATT)5	5	AAGGCAAGGAATTGGGTAGT	TGGTTTGTCGTAAGAGTCCA	53	n.a.	18,022,091–18,022,256
RPPG6-033	(CTGT)6	6	CATTATCAAACCACGACCAA	AAAGCTCAACAGCGACTTCT	56	n.a.	28,936,519–28,936,634
RPPG6-009[a]	(AAAAC)4	6	GGGCTTGGCTGATAAAATAA	TGGTAAAATAGAAGAGCGAGAAG	53	n.a.	243,925–244,106
RPPG7-026	(ACATT)4	7	TTTGGTGAGTGGGCTCTATT	CTATCGTTCGCTGGTCTTCT	53	n.a.	18,384,554–18,384,719
RPPG7-015[a]	(TAAA)6	7	TCTTGGTGGTGGTGAAGTAA	GAGAGATGGAGGAGGCTGA	58	n.a.	2,301,193–2,301,468
RPPG7-032	(AGG)7	7	AAGGGAGGAGGATTGTGAA	TGGTAGACGGGTAGATGTTG	53	n.a.	21,874,361–21,874,551
RPPG8-028	(AACCC)6	8	AAGGAGCCGACATCAGAAC	TGACCAGAAGCCAAATACATC	58	n.a.	21,154,898–21,155,103
RPPG8-007	(GGT)7	8	ACCACCACCTCTTCCAATC	ACCTCAAAGTGTCCCAGAAA	53	n.a.	86,261–86,468

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

most common SSRs with 2-nt repeats that are reported in the literature (Cmejlova et al., 2021; Lassois et al., 2015; Liang et al., 2015; Patocchi et al., 2009; Urrestarazu et al., 2016) and, following the criteria reported in the sections above, we selected 18 of them (Table 2).

Most of the selected markers show appreciable polymorphism and are consistent with the amplification of a single locus; however, some of these markers deserve further examination. For example, CH02c11 might show a second locus with alleles of the two loci being intermixed. Apple is an ancient polyploid that underwent complex chromosomal rearrangements, leading to the actual chromosome number $x = 17$ (Velasco et al., 2010). At meiosis, apple behaves like a diploid and from that perspective no more than two alleles per marker are expected. However, because of the segmental duplications that previously occurred during the chromosomal rearrangements, extra "alleles" can occasionally be amplified. CH02c11 is such a case.

The markers CH03d07, CH01h10, and CH01f03b show irregular space between adjacent alleles that might make binning difficult.

Most germplasm collections contain triploids which are rather common in several ancient cultivars and their offspring. Triploids are often identified from tri-allelic patterns recorded in the SSR profiles, but the information must be considered with caution because of the occurrence of a tri-allelic pattern in diploid genotypes, due either to the duplication of chromosomal segments or to the amplification of a second locus in homologous chromosomes (Testolin et al., 2019). A flow cytometry analysis would, therefore, be recommended to confirm the ploidy.

As a concluding observation, Cmejlova and coworkers (2021) reported an interesting protocol to carry out a one-tube multiplexed single PCR reaction for 17 of the 18 SSR apple markers that are listed in Table 2.

3.3 | Apricot (*Prunus armeniaca* L., *P. mume* (Siebold) Siebold & Zuccarini, and related species)

Apricot is another species for which there are tens of microsatellite markers that have been isolated from 2-nt enriched genomic libraries and reported in the literature (see for instance Bourguiba et al., 2020; Herrera et al., 2021; Messina et al., 2004). As indicated in Section 2.2.4 above, microsatellites with longer core repeats (3-nt or more) that have been isolated in peach and successfully tested in apricot are preferred.

Apricot is essentially diploid ($2n = 16$) and one or two alleles per marker are expected according to the heterozygosity of the locus analyzed.

The list of markers and primers is in common with that of almond and peach (Table 1).

3.4 | Avocado (*Persea americana* Mill. and related species)

Twenty-two SSR markers, the most informative among those reported in the literature by several authors (Borrone et al., 2007, 2009; Bosa et al., 2018; Sharon et al., 1997), were selected as being the most reliable (Table 3). Those amplifying more than one locus, and those with a high frequency of null alleles were discarded. Nonetheless, we selected unlinked loci, by ranking twice one marker per linkage group.

P. americana is diploid ($2n = 24$) and no more than two alleles per locus are expected. Differential cycles of flower opening, known as complementary synchronous dichogamy, prevent self-pollination and promote out-crossing. Avocado is, therefore, highly heterozygous and a few markers might be sufficient to solve most identities.

3.5 | Banana and plantain (*Musa x paradisiaca* L., *Musa acuminata* Colla, *Musa balbisiana* Colla)

Most edible cultivars of banana and plantain originated from two diploid ($2n = 22$) species, *Musa acuminata* and *Musa balbisiana*, contributing the A and B genome, respectively. Genotypes are classified into six groups (AA, AAA, AB, AAB, ABB, and ABBB) based on their ploidy level and their genomic composition (Hippolyte et al., 2012).

The 22 SSR primer pairs reported in Table 4 are from Irish et al. (2014). These SSR primer pairs were isolated by Crouch et al. (1998), Lagoda et al. (1998) and Hippolyte et al. (2010, 2012). The 22 SSRs were distributed across 10 of the 11 linkage groups (Hippolyte et al., 2010).

Those scientists dealing with *Musa* genotyping must consider the ploidy level of the accessions that they are investigating, as suggested by Perrier et al. (2019) and Brisibe and Ubi (2020).

3.6 | Blueberry (*Vaccinium corymbosum* L. and related species)

The list of 18 SSRs (Table 5) is from the Genetics Laboratory of the US Department of Agriculture, Agricultural Research Service, National Clonal Germplasm Repository in Corvallis, Oregon (Bassil et al., 2020; Bidani et al., 2017). A multiplexed set of 10 SSRs amplified in a single PCR reaction is being used by Bassil et al. (2020).

TABLE 2 Recommended markers for apple (*Malus* spp). The reference genotype (RG) is "Golden Delicious" (from Testolin et al., 2019); the annealing temperature (AT) varies in the literature from 55 to 60°C and touchdown (td) protocols, with initial temperatures 2–4°C above those indicated have also been considered by some authors; linkage groups (LG) are reported according to the reference genome assembly; the apple genome assembly is ASM211411v1.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (BP)	Position of primer within the apple genome
Hi02c07[a]	GA	1	AGAGCTACGGGGATCCAAAT	AAGCATCCCGATTGAAAGG	56	107/113	17,458,609–17,458,713
CH-Vf1	AG	1	ATCACCACCAGCAGCAAAG	CATACAAATCAAAGCACAACCC	56	138/172	28,062,601–28,062,775
CH02c06	GA	2	TGACGAAATCCACTACTAATGCA	GATTGCGCGCTTTTTAACAT	56	234/238	20,116,932–20,117,146
GD12	CT	3	TTGAGGTGTTTCTCCCATTGGA	CTAACGAAGCCGCCATTTCTTT	56	150/191	16,693,021–16,693,151
NZ01a6[b]	GA	4	AGGATTGCTGGAAAAGGAGG	TTAGACGACGCTACTTGTCCT	n.a.	n.a.	29,399,402
CH05f06 [a]	GA	5	TTAGATCCGGTCACTCTCCACT	TGGAGGAAGACGAAGAAGAAAG	56	174/182	22,372,867–22,373,028
CH03d07 [a]	GA	6	CAAATCAATGCAAAACTGTCA	GGCTTCTGGCCATGATTTTA	56	187/205	8,113,428–8,113,594
CH04e05	GA	7	AGGCTAACAGAAATGTGGTTTG	ATGGCTCCTATTGCCATCAT	56	n.a.	17,314,885–17,315,039
CH01h10	GA	8	TGCAAAGATAGGTAGATATATGCCA	AGGAGGGATTGTTTGTGCAC	56	90/108	27,444,005–27,444,078
CH01f03b	GA	9	GAGAAGCAAATGCAAAACCC	CTCCCCGGCTCCTATTCTAC	56	171/179	9,725,612–9,725,764
CH02c11	GA	105[c]	TGAAGGCAATCACTCTGTGC	TTCCGAGAATCCTCTTCGAC	56	219/233	24,261,774–24,261,988 29,455,947–29,456,121
CH02d08	GA	11	TCCAAAATGGCGTACCTCTC	GCAGACACTCACTCACTATCTCTC	56	223/225	9,734,423–9,734,623
CH01f02 [a]	GA	12	ACCACATTAGAGCAGTTGAGG	CTGGTTTGTTTTCCTCCAGC	56	169/179	23,630,173–23,630,323
GD147	AG	13	TCCCGCCATTTCTCTGC	AAACCGCTGCTGCTGAAC	56	139/–	8,149,428–8,149,548
CH04c07	GA	14	GGCCTTCCATGTCTCAGAAG	CCTCATGCCCTCCACTAACA	56	94/112	24,205,467–24,205,561
CH02c09	GA	15	TTATGTACCAACTTTGCTAACCTC	AGAAGCAGCAGAGGAGGATG	56	241/255	50,159,438–50,159,675
CH05e04	GA	16	AAGGAGAAGACCGTGTGAAATC	CATGGATAAGGCATAGTCAGGA	56	n.a.	4,351,622–4,351,767
CH01h01	AG	17	GAAAGACTTGCAGTGGGAGC	GGAGTGGGTTTGAGAAGGTT	56	115/–	6,601,264–6,601,365

AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

[b]No match found in the published genome sequence for one of the two primers.

[c]Potential multiple alignment in the published genome sequence.

TABLE 3 Recommended markers for avocado (*Persea* spp.). The reference genotype is "Simmonds" (from Borrone et al., 2009); touchdown (td) −1.0°C per step for five cycles; for all SSR loci, linkage groups (LG) are reported in accord with Borrone et al. (2009); a genome assembly was not available or was not assembled in chromosomes or linkage groups.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (BP)
AVAG021	(CT)22	1	TGTAAGTTTTAACCCCACAA	AATCACTATTAGAGTTTTCAGTCG	50	178/193
SHRSPa114	(TC)20	2	TGCCAGATGACAGTTTTTCC	ACAGCACATAAGTTCAACTCAGA	60 td	150/–
SHRSPa258	(TC)12(AC)11	2	GCAGGTGCTCATGCTGTAGA	CAAAAATAGAGGTTTGGGGTT	60 td	213/235
SHRSPa305	complex	3	TTGCATTTTCTTTGCTGCAC	CCATGGAGATTGGCATGTTA	60 td	184/–
SHRSPa277	(ATA)5	3	GCTAAAGTCTGGAGATGCCG	TTTGAAAAGAAGGCAAGCGT	60 td	136/–
AVAG03	(TC)17	4	GCACTTCCTAAACTTGCAGGT	CTGAACATCCAATGACAAACATCC	45	103/–
SHRSPa081	(GA)7	4	GGGCTTCAATTCAATCCAATCC	TCTTCAGCACGCCACGAGTCT	60 td	218/–
SHRSPa249	(TA)9	4	CCAGAAGCTGGCAATCTAGC	CCAAACGGGTCCTAATGGTA	60 td	272/276
SHRSPa109	complex	5	TTCCAGCTACTACTCCTCCAGT	AAGGAGGTGAGCCGAATG	60 td	138/164
SHRSPa107	complex	5	CGCAGTCTTCAATGATACCA	CCCCCCTTCACTTCCAA	60 td	165/–
SHRSPa327	(GA)14	5	GAGGAGATGGGTTGGAGTCA	TTGCCAGTAACCCCTTTTATG	60 td	124/–
SHRSPa003	(GA)9	5	ATCAGTCGGTGTGCAGAAC	CACCAGCTCCTGCAAATA	60 td	76/78
AVAG25	(CT)14	6	ATGGTTTTTTCCTGCCCTTT	AACAAGCCCCCTAAAAGAA	50	136/null
AVmix04	complex	6	CCGTTTGCTTCCTGTATC	GTTATCCCTTCCACTTTC	50	172/174
AVAG22	(GA)15	6	GATCATCAAGTCCTCCTTGG	GATCTCATAGTCCAAATAATGC	55	103/116
SHRSPa038	(ATC)8	6	GAAGAAATCCGCCATAATCGT	CACATCAGAGAAAGAAACCTAAACC	60 td	107/117
AVmix03	(TG)16(AG)20	7	GATATTCCTGTTGTCACTGC	AATGTTCCCCATGAAAGTCTCC	50	143/153
SHRSPa082	(CT)7	8	TCCGAAACATCAAACACCAACA	CCGAACAAACACGAATCAGAGA	60 td	224/–
SHRSPa101	(AGA)5	8	GATCTCTTTCGACTCTTTCTCTC	CTCAGCATCCTTCCTTCTAAC	60 td	99/–
SHRSPa157	complex	8	TTCCTCCTTCCGCACTCAAC	TTCTCCAACACTCCTCACGA	60 td	157/233
SHRSPa055	complex	9	TCTCTTCATCAACTCGACTGC	AACGGTATCCAAACGCTAAT	60 td	102/117
SHRSPa057	(CT)16	9	GCAAGGCATTACGATGTCA	CTCTAGTGGACAAAATCGACAA	60 td	194/200

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

Despite of the large genetic diversity of cultivated species of *Vaccinium*, the microsatellites listed in Table 6 are reported to work well in most species. For the expected number of alleles, the investigator needs to consider the different ploidy levels of the *Vaccinium* species and cultivars that are under evaluation.

3.7 | Brazil nut (*Bertholletia excels* Humb. & Bonpl.)

Brazil nut is a species whose fruits are primarily collected from wild trees growing in the Amazon basin. Bolivia is the main producer and exporter, but the species is present also in neighboring countries, like Brazil, Peru, Colombia, Venezuela, and Guyana (Baldoni et al., 2020). The species is allogamous and pollinated by insects (Motta Maués, 2002).

The SSR markers selected (Table 6) are all isolated from an (AG/CT)-enriched library and have been published in two separate papers: markers of the "Bex" series are from Reis et al. (2009), while those of the "Bet" series are from Sujii et al. (2013).

Brazil nut is diploid ($2n = 34$) and one or two alleles are expected in the profile of each SSR marker.

3.8 | Cacao (*Teobroma cacao* L.)

The 15 SSRs shown (Table 7) were selected at the Centre de Coopération Internationale en Recherche Agronomique pour le Dévelopement (CIRAD), Montpellier, France. This list was proposed as the agreed set of SSRs for fingerprinting cacao (Saunders et al., 2004) and has since been adopted by other authors (Everaert et al., 2017; Irish et al., 2010). However, Araújo and coworkers published a new list of SSRs that were isolated from enriched libraries with tetra-, tri- and di-nucleotide repeats (Araújo et al., 2007). The new markers with 3-nt or longer core repeats would solve the problems arising from the use of di-nucleotide repeats (see Section 2.2.4 above). Unfortunately, those new SSRs were tested only on a very limited number of accessions and they have never been adopted by other investigators. For this reason, we have not replaced the set that originated from CIRAD but have added

TABLE 4 Recommended markers for banana and plantain (*Musa x paradisiaca*, *Musa acuminata*, *Musa balbisiana*). The reference genotype is "Cavendish" (AAA) (from Hippolyte et al., 2012); for all SSR loci, the forward primer was designed with a 5′-end M13 extension (5′-CACGACGTTGTAAAACGAC-3′), that enabled the generation of fluorescent amplicons; for all SSR loci with the exception of mMaCIR01 and Ma1-32, the linkage group (LG) is reported in accordance with the reference genome assembly; for mMaCIR01 and Ma1-32, LG is reported in accordance with Hippolyte et al. (2010); the banana genome assembly is ASM31385v2.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (BP)	Position of primer within the banana genome
mMaCIR03[c]	(AG)10	1	TGACCCACGAGAAAAGAAGC	CTCCTCCATAGCCTGACTGC	55	122/124	18,545,498–18,545,586
mMaCIR07[a,c]	(AG)13	1	AACAACTAGGATGGTAATGTGTGGAA	GATCTGAGGATGGTTCTGTTGGAGTG	55	158/170	20,685,220–20,685,339
mMaCIR08[a,c]	Complex	1	ACTTATTCCCCCGCACTCAA	ACTCTCGCCCATCTTCATCC	55	261/265	27,403,582–27,403,807
mMaCIR01[c]	(GA)20	2	TTAAAGGTGGGTTAGCATTAGG	TTTGATGTCACAATGGTGTTCC	55	254/258/264	n.a.
mMaCIR39[c]	Complex	2	AACACCGTACAGGGAGTCAC	GATACATAAGGCAGTCACATTG	55	331/335	16,470,966–16,471,276
mMaCIR13[a,c]	Complex	3	TCCCAACCCCTGCAACCACT	ATGACCTGTCGAACATCCTTT	55	286/–	8,785,096–8,785,352
Ma3-90[c]	(CT)11	3	GCACGAAGAGGCATCAC	GGCCAAATTTGATGGACT	55	150/162/168	9,090,129–9,090,241
mMaCIR152[a,b,c]	Complex	4	CCACCTTTGAGTTCTCTCC	TTTCCCTCTTCGATTCTGT	55	164/–	24,794,917–24,795,040
mMaCIR0164[c,d]	(AC)14	6	AAGACAAGTTCCATTGCTTG	GTTCGGGCTTTCGGT	55	401/407	32,319,150–32,319,518
Ma1-32[c]	Complex	4	n.a	n.a	55	235/245	n.a
mMaCIR27[a,b,d]	(GA)9	6	GGGGAACAGCACGGTCACAT	CCACTCCCCCAACAACACGA	55	235/243/245	178,917–179,131
mMaCIR195[a,c]	Complex	5	GAATCGCCTTAGTCTCACC	TCATGTGCTCCCATCTTT	55	298/–	28,265,759–28,266,015
mMaCIR24[a,c]	(TC)7	6	ATCTTTTCTTATCCTTCTAACG	ATTAGATCACCGAAGAACTC	55	237/247/253	31,295,605–31,298,085
mMaCIR150[c,d]	(CA)10	11	ATGCTGTCATTGCCTTGT	GAATGCTGATACCTCTTTGG	55	257/261	8,697,226–8,697,447
mMaCIR264[a,b,c]	(CT)17	6	AGGAGTGGGAGCCTATTT	CTCCTCGGTCAGTCCTC	55	250/258	32,776,769–32,776,981
mMaCIR0196[c]	(TC)17	7	GCTCCAAACCTCCCTTT	CGATGCCACACTGGAC	55	168/180	26,990,988–26,991,121
mMaCIR0307[a,c]	(CA)6	7	AGACTTGTATCGCTTGGTAAA	ACGCTGCACCAGTCAA	55	162/164	1,492,018–1,492,143
mMaCIR40[c]	(GA)13	8	GGCAGCAACAACATACTACGAC	CATCTTCACCCCCATTCTTTTA	55	176/178/180	11,423,439–11,423,603
mMaCIR260[a,c]	(TG)8	9	GATGTTTGGGCTGTTTCTT	AAGCAGGTCAGATTGTTCC	55	212/–	4,060,079–4,060,252
mMaCIR45[a,c]	(CTCGA)4	10	TGCTGCCTTCATCGCTACTA	ACCGCACCTCCACCTCCTG	55	284/289	27,754,252–27,754,492
mMaCIR231[c]	(TC)10	n.a.	GCAAATAGTCAAGGGAATCA	ACCCAGGTCTATCAGGTCA	55	242/250/276	6,877,432–6,877,640
mMaCIR214[c]	(AC)7	n.a.	CCATTGAGAGATCAACCC	CTATTTGACGTTGGTGGTC	55	119/123	n.a.

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

[b]SNP/s found in the published genome sequence compared with primer/s sequence/s.

[c]SSRs that had M13-tailed.

[d]SSR loci LGs were different in linkage map (Hippolyte et al., 2010).

TABLE 5 Recommended markers for blueberry (*Vaccinium corymbosum* and related *Vaccinium* species). The reference genotype (RG) is "Duke S1" (from Bassil et al., 2020); touch down (td) −1.0°C per step for 10 cycles; for all SSR loci; the linkage group (LG) was reported in accordance with McCallum et al. (2016); a genome assembly was not available or was not assembled in chromosomes or linkage groups.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (bp)
KAN-1007	(CAA)5	2	CAAGCGGTTGAATAACAAAGC	AATAGTTGTGGCCCTTGTGG	62 td	n.a.
KAN-789	(TAA)5	3	TCTAGCCGGCACTCAAGTTT	CATCCATTGAAAGAGCAGCA	62 td	n.a.
NA172[a]	(CAT)5	5	CCTCGTCCTCCTCTTCCTCT	GACTTTGGAGAAGGCGAAG	62 td	301/304
GVC-V24d10b	(TTC)14	5	GGAAACGATGCCGTTTTCTA	CAACCCTTCCAGGTCAAAAA	62 td	n.a.
VCB-C-04624[a]	(CTC)5	7	CATCCCAATGCAGAAGAAG	CCTCTTGTGGGTTAGGGTTTCT	62 td	100/103/106
NA398[a]	(AAAT)5	7	TCCTTGCTCCAGTCCTATGC	CCTTCCACTCCAAGATGC	62 td	227/234
Pr031818819[a]	(GAG)15	8	TCTCTTTCCCTTTTCAAGTGG	ATGATGGAATTCCGAGTTTG	62 td	310/315/321
CA23	(AGA)6	8	GAGAGGGTTTCGAGGAGGAG	GTTTAGAAACGGGACTGTGAGACG	62 td	157/160
GVC-C179	(AGT)5	9	CGTCGTGGAGGCTTAGAAAG	TTCAAAATCACCAGCACCAA	62 td	212/221/230
KAN-262	(CAC)8	n.a.	CGCCCACTCAGTTCATTCTT	ATAGGTGGTGGCTGGTGAGT	62 td	246/260
GVC-C571[a]	(CTT)4	n.a.	TCCCTTCATGTTTTCTCCCA	ATGCGAGTGTGGACTAGGGT	62 td	123/129
GVC-C428	(AAC)4	n.a.	TTGGCCAGAACAACCAAAGT	CGTCGTGTTCCTCTTGTTCA	62 td	251/254/260/281
GVC-V41f03[a]	(CGG)5	n.a.	CGCAATCGCTGCATAGTTTA	TAACGTTGCAACCAATTCCA	62 td	186/–
GVC-V21e04	(CAT)9	n.a.	TTCATGTTGCAATTGTCCTGA	ATAAGATGCTGCTGCTGCG	62 td	n.a.
CA190[b]	(TGC)5	n.a.	TTATGCTTGCCATGGTGGTA	TTGCGAAGGGACCTAGTAGC	62 td	n.a.
KAN-131	(ATC)5	n.a.	AATAATCCGAGTGTCGTCCG	TGTGACGTACGTTCCGATGT	62 td	n.a.
KAN-505[b]	(GAG)5	n.a.	GGAGGCAGTGAGGAGTGAAG	GAATTTTGGAAGGGTCGGAT	62 td	n.a.

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.
[a]SSRs that had M13-tailed.
[b]Multilocus.

TABLE 6 Recommended markers for Brazil nut (*Bertholletia excelsa*). A reference genotype was not available; a genome assembly was not available or was not assembled in chromosomes or linkage groups.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (bp)
Bex01	(AG)22	n.a.	TTCCAGGCATTTTGTTACAG	CAAGAGCGCAGGAGAAGATT	56	n.a.
Bex02	complex	n.a.	GCCATGTTCTCTACAGTCTC	AGTCGGACATCCTTCGTGCT	56	n.a.
Bex03	(AG)13	n.a.	CTACCTACAGGTCCGTGCCA	CGTATTTCGTGTCAAACTCT	54	n.a.
Bex06	(CT)17(CCCT)3	n.a.	TTGATCTTCGCAAGGTCGGT	ACTTCCTCAATCCATCGAGT	56	n.a.
Bex09	(CT)32	n.a.	TATTCCATGGTCCTCCGT	AGTCAATCATCTTCAAGAGT	56	n.a.
Bex12	complex	n.a.	AATTAGCAACAATGCACTGA	ATTCCGTAACATGCTCTTCT	56	n.a.
Bex22	(CT)38	n.a.	GCATTCTCTCATTTTCGCTTG	CCCTAGCAATCGTCGTCTTC	56	n.a.
Bex27	(GA)20	n.a.	ACTGTTCTGATCCGCCATGT	TTTCGACCGTTCAAATACGC	56	n.a.
Bex30	(CT)23(CA)15	n.a.	TGGAACGGTCACTTGAGACA	CCCTCTCTCCTTCGCTTTTT	48	n.a.
Bex32	(TC)21	n.a.	CCCTCCCCCATCTTGAGTAG	CAACCCCTCCTTTTACCATTT	48	n.a.
Bex33	(CT)37	n.a.	CAAGTCTCTGACTCATCGCCTA	ACCAGGTTCAGCAGACGTTC	48	n.a.
Bex37	(CT)19	n.a.	TGCATGCTATGTTTCATTGCT	CACGCAACCTCACAGTCTTG	44	n.a.
Bet01	(GA)6	n.a.	TTTAACTGATGAAAGGCGGACT	TACGCAGAACAGACTCGCTAAA	n.a.	n.a.
Bet05	(TC)14	n.a.	TAATCTCACAACAAATAACG	CTAGCTTGATCCTAGAGAAA	n.a.	n.a.
Bet06	(TC)7	n.a.	CTCTAGGATCAAGCTAGCCCAA	AGGTTATGCTCCAAATAGCAGG	n.a.	n.a.
Bet12	(TC)11	n.a.	ATAAGGACCGCCCATCATC	ATAGCGAGAGCAACCTTTGAAC	n.a.	n.a.
Bet14	(AG)15	n.a.	GTGTACTTCTCTGGTTGGGGC	CCCGAGTTCATTACCCAAACT	n.a.	n.a.
Bet15	(GA)19(AGA)13	n.a.	ACTGCCATCACCAGCATGTAG	GTCCCTTGTGGTCTCTCACAAT	n.a.	n.a.
Bet16	(AG)9	n.a.	TCTTCAAACACTCAAAGGGACA	TGTCTATAAATAGGGGCCTCCC	n.a.	n.a.

Abbreviations: LG, linkage group; AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

TABLE 7 Recommended markers for Cacao (*Teobroma cacao*). U/C marker stands for UENF/CEPLAC; the reference genotype is "IFC 5" where IFC stands for "Institut Francais du Café et Cacao" (from Everaert et al., 2017); linkage groups (LG) are reported according to the reference genome assembly; the cacao genome assembly is GCA_000208745.2.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (bp)	Position of primer within the cacao genome
mTcCIR15[a]	(TC)19	1	CAGCCGCCTCTTGTTAG	TATTTGGGATTCTTGATG	46	236/252	3,690,788–3,691,007
mTcCIR22	Complex	1	ATTCTCGCAAAAACTTAG	GATGGAAGGAGTGTAAATAG	46	289/–	36,352,662–36,352,935
U/C 69	(CAA)10	1	ACGACGACAACAACAAGA	AGGCTGAGGTGGTGCTACA	51	n.a.	24,616,097–24,616,275
U/C 67[b]	(CAA)10	1	ACAGCAACAACGCCGACTA	ATCCTCCAAAGGCAGA	50	n.a.	24,616,079–24,616,293
mTcCIR11[a]	(TC)13	2	TTTGGTGATTATTAGCAG	GATTCGATTTGATGTGAG	46	316/–	40,092,105–40,092,389
mTcCIR60	(CT)7(CA)20	2	CGCTACTAACAAACATCAAA	AGAGCAACCATCACTAATCA	51	196/215	19,251,916–19,252,091
mTcCIR40[b]	(AC)15	3	AATCCGACAGTCTTTAATC	CCTAGGCCAGAGAATTGA	51	282/–	14,462,884–14,463,141
mTcCIR12	Complex	4	TCTGACCCCAAACCTGTA	ATTCCAGTTAAAGCACAT	46	216/254	24,533,112–24,533,282
mTcCIR18	(GA)12	4	GATAGCTAAGGGGATTGAGGA	GGTAATTCAATCATTTGAGGATA	51	346/–	15,301,589–15,301,899
mTcCIR33	(TG)11	4	TGGGTTGAAGATTTGGT	CAACAATGAAAATAGGCA	51	347/–	28,292,991–28,293,268
U/C 10	(GAA)5	5	TGCAGCGGTGGCGGTGGA	TCGAGCCTTCTTCTTCT	50	n.a.	1,010,439–1,010,493
mTcCIR6	(TG)7(GA)13	6	TCCCTCTAAACTACCCTAAAT	TAAAGCAAAGCAATCTAACATA	46	231/–	219,715–220,016
mTcCIR7	(GA)11	7	ATGCGAATGACAACTGGT	GCTTTCAGTCCTTTGCTT	51	154/–	6,182,225–6,182,365
mTcCIR1[a]	(CT)14	8	GCAGGGCAGGCTCAGTGAAGCA	TGGGCAACCAGAAAACGAT	51	n.a.	271,858–271,967
mTcCIR26	Complex	8	GCATTCATCAATACATTC	GCACTCAAAGTTCATACTAC	46	300/–	5,601,110–5,601,394
mTcCIR8[a]	Complex	9	CTACTTTCCCATTTACCA	TCCTCAGCATTTTCTTTC	46	290/–	22,521,863–22,522,150
mTcCIR24[a]	(AG)13	9	TTTGGGGTGATTTCTTCTGA	TCTGTCTCGTCTTTTGGTGA	46	186/–	6,355,181–6,355,360
mTcCIR37[b]	(GT)13	10	CTGGGTGCTGATAGATAA	AATACCCTCCACACAAAT	46	164/–	20,881,692–20,881,981
U/C 33	(GACA)4	n.a.	ATAGAGGAAGCGCAGGTGA	ATGATTACGCCAAGCTCGAA	55	n.a.	n.a.
U/C 122	(CAA)11	n.a.	TCCACCTCCAGACACCAAT	ACAGGAAACAGCTATGACCA	55	n.a.	n.a.
U/C 52	(GATA)12	n.a.	ATAATGTCTCTAGGTCCTCCTGA	ATGACCATGATTACGCCAA	52	n.a.	n.a.
U/C 71	(CAA)12	n.a.	ACAGCAGCAGCAACAACAA	TGACCATGATAAACGCCAA	52	n.a.	n.a.
U/C 97	(CAA)5	n.a.	TTCAGTGGCAGTCACAGGT	TGAAGAAGATGCTGCTGT	51	n.a.	n.a.
U/C 66	(CAA)20	n.a.	ACGACGACAACAACAAGA	ATGGGCGGAATCCATAT	50	n.a.	n.a.
U/C 79	(TTG)12	n.a.	ATGGCTGCTGCTGCA	ACAGCAATTGCAGGGACA	50	n.a.	n.a.

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

[b]SNP/s found in the published genome sequence compared with primer/s sequence/s.

TABLE 8 Recommended markers for cashew (*Anacardium occidentale* L.). A reference genotype was not available. Twelve markers, marked with the superscript letters "a," "b," or "c" were multiplexed based on nonoverlapping allelic size ranges; the fluorescent labels were "a" NED, "b" HEX; and "c" FAM, respectively. A genome assembly was not available or was not assembled in chromosomes or linkage groups.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (bp)
mAoR2	(CA)10(TA)6	n.a.	GGCCATGGGAAACAACAA	GGAAGGGCATTATGGGTAAG	58.2	n.a.
mAoR3[a]	(AC)12(AAAAT)2	n.a.	CAGAACCGTCACTCCACTCC	ATCCAGACGAAGAAGCGATG	60.3	n.a.
mAoR6[c]	(AT)5(GT)12	n.a.	CAAAACTAGCCGGAATCTAGC	CCCCATCAAACCCTTATGAC	58.2	n.a.
mAoR7[b]	(GT)5AT(GT)5	n.a.	AACCTTCACTCCTCTGAAGC	GTGAATCCAAAGCGTGTG	58.2	n.a.
mAoR11[c]	(AT)3(AC)16	n.a.	ATCCAACAGCCACAATCCTC	CTTACAGCCCCAAACTCTCG	60.3	n.a.
mAoR12	(AC)12ATAC(AT)4	n.a.	TCACCAAGATTGTGCTCCTG	AAACTACGTCCGGTCACACA	58.2	n.a.
mAoR16[c]	(GT)8(TA)17(GT)3	n.a.	GGAGAAAGCAGTGGAGTTGC	CAAGTGAGTCCTCTCACTCTCA	60.3	n.a.
mAoR17[b]	(GA)24	n.a.	GCAATGTGCAGACATGGTTC	GGTTTCGCATGGAAGAAGAG	56.1	n.a.
mAoR26	(TA)5CA(TG)6	n.a.	TCCACAAAATCAGCCTCCAC	GAGCGCTCGTGTCCTGTACT	60.3	n.a.
mAoR29[c]	(TG)10	n.a.	GGAGAAGAAAAGTTAGGTTTGAC	CGTCTTCTTCCACATGCTTC	58.2	n.a.
mAoR33	(CT)18(AT)19	n.a.	CATCCTTTTGCCAATTAAAAACA	CACGTGTATTGTGCTCACTCG	56.1	n.a.
mAoR35	(AG)14	n.a.	CTTTCGTTCCAATGCTCCTC	CATGTGACAGTTCGGCTGTT	58.2	n.a.
mAoR41	(GGT)8	n.a.	GCTTAGCCGGCACGATATTA	AGCTCACCTCGTTTCGTTTC	58.2	n.a.
mAoR42[c]	(CAT)9TAT(CTT)7	n.a.	ACTGTCACGTCAATGGCATC	GCGAAGGTCAAAGAGCAGTC	60.3	n.a.
mAoR44	Complex	n.a.	CACGTTCGCATCATCCAA	CGTCAGAGATTACGGCATTG	58.2	n.a.
mAoR46[c]	(ACC)7(AC)3	n.a.	CGGCGTCGTTAAAGCAGT	TCCTCCTCCGTCTCACTTTC	58.2	n.a.
mAoR47	Complex	n.a.	AAGAGCTGCGACCAATGTTT	CTTGAACTTGACACTTCATCCA	58.2	n.a.
mAoR48[a]	(GAA)6(GA)3	n.a.	CAGCGAGTGGCTTACGAAAT	GACCATGGGCTTGATACGTC	58.2	n.a.
mAoR52	(GT)16(TA)2	n.a.	GCTATGACCCTTGGGAACTC	GTGACACAACCAAAACCACA	58.2	n.a.
mAoR55[b]	(AT)6CT(AC)5	n.a.	TGACTTTCAAATGCCACAAC	CTCAAGCTTTCATGGGGATT	58.2	n.a.
mAoR59[b]	(AT)7(GT)14	n.a.	TCCGCCCCTACTCCTATATT	TGGTGTCGACTGCTTCTTGT	51.8	n.a.

Abbreviations: LG, linkage group; AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

10 tri- and tetra-nucleotide markers that were isolated by the Brazilian group (Table 7).

Cacao is diploid ($2n = 20$) and up to two alleles are expected from each SSR marker.

3.9 | Cashew (*Anacardium occidentale* L.)

Cashew is self-fertile, but cross pollination occurs frequently due to flower visits from bees, ants, and butterflies (Freitas & Paxton, 1996). Cashew has a basic chromosome number $n = 21$ ($2n = 42$), although older literature reports several different basic chromosome numbers (Aliyu & Awopetu, 2007). Considering the taxonomic position, cashew is likely an ancient polyploid with a basic chromosome number $x = 7$.

The 21 SSR markers selected (Table 8) are from Croxford et al. (2006). They were tested on a small collection and hence, polymorphism may be low. However, the power of discrimination seems sufficient to easily separate any accession that had been derived from cross pollination.

3.10 | Cherimoya (*Annona cherimola* Mill. and wild species)

We selected 22 SSR markers from the list of 52 described by Escribano et al. (2008). Markers were ranked according to their expected heterozygosity with a few exceptions (Table 9). We removed, for instance, a few informative markers which had an annealing temperature below 50°C.

Cherimoya is diploid ($2n = 14$) but triploid individuals can be found in germplasm collections, especially in interspecific hybrids, like atemoya (*A. cherimola* × *A. squamosa*) (Larranga et al., 2017; Perrone et al., 2022).

3.11 | Cherry (*Prunus avium* L., *P. cerasus* L., and related species)

The SSR markers suggested in Table 10 were selected following the papers of Vaughan and Russel (2004), Clark and Tobutt (2009) and Ordidge et al. (2021). These markers are based on di-nucleotide or more complex core repeats.

TABLE 9 Recommended markers for cherimoya (*Annona chrerimola* and wild species). A reference genotype was not available; a genome assembly was not available or was not assembled in chromosomes or linkage groups.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (bp)
LMCH102	(CT)13	n.a.	GCTAACCATCCATTTACATA	ATAACATTCTTTATCACCATCT	55	n.a.
LMCH103	(GA)19	n.a.	CACAATAATCAGAAAAACATCA	GTGTCTCGTATCCCTCCATA	55	n.a.
LMCH106	(GA)13	n.a.	AACAAATGACAGGAGAGC	ATAATGTATATGACGCTGCT	55	n.a.
LMCH109	(GA)7	n.a.	TATAAAATGGGAAAGCGATCT	CCTCAAAGAGCAATAATCAGC	55	n.a.
LMCH112	(GA)12	n.a.	TAACCCAGGATCTACAATAAT	TTGCATACATTTTCCTATTT	55	n.a.
LMCH115	(GA)13	n.a.	TATAATCCATCAACACAAATAA	TTAGATACACAGAACATACAGC	50	n.a.
LMCH122	(GA)9	n.a.	AGCAAAGATAAAGAGAAGATAA	ATCCAAGCCTATTAACAACT	55	n.a.
LMCH128	(GA)11	n.a.	CTTGTTAAAATGGCTGTTACT	GCATTGAGCTGACATAACTC	55	n.a.
LMCH131	(GA)10	n.a.	AGAAGCACCCAGATAGTCAC	TTGTAGCAATCTCACTTTATCA	55	n.a.
LMCH139	(CT)9	n.a.	CTATCCATCTACGCTTCAAAAT	CTGAGTCGGTTAGACATTGAGA	55	n.a.
LMCH144	(CT)12	n.a.	GTTTGGAAGAGTCGCAGGAT	ACTGTAAAACGCAGACCAAGAT	55	n.a.
LMCH36	(GA)10	n.a.	ATAGAAGATTTACCCAGGAG	GTAAGTAGCTGATTGTTGATCT	50	n.a.
LMCH37	(GA)15	n.a.	TATCGACAACATAGAAAAGTTA	TAGTTAAATCACATCGTATGAC	50	n.a.
LMCH69	(GA)9(GT)3	n.a.	AGCTTTAGCCATGAATTAGA	GAAAGGCTGACGAGATATAA	55	n.a.
LMCH71	(GA)14	n.a.	AGATAACACCCGCCCACTAT	ACAACTTTTCTCCCAACCTATC	55	n.a.
LMCH80	(GA)15	n.a.	AAAACAGAGACTAAAATGAAAT	GAAGATATGCAAGGTATAAATC	55	n.a.
LMCH83	(CT)36	n.a.	CTCTCGTTGACTCGTTTACT	GGTCTCTAGCCTTTACAATC	55	n.a.
LMCH87	(GA)15	n.a.	AGTTAAGACACGAGATGATAAA	CAAGTAAAGACTGAAAGGTTG	55	n.a.
LMCH88	(CT)17	n.a.	GGGAGTTATTAGAGTGTTATTG	AAATTAAGGATTGACTATTTCA	55	n.a.
LMCH89	(CT)11	n.a.	AATACAAATGGAGACGAATA	GTGTCTAATACCATACATACCA	55	n.a.
LMCH91	(CT)8	n.a.	CCTTGAGAAAGTGTCATCTAT	ATAATCCTAGACCATAAAATTC	55	n.a.
LMCH96	(CT)10	n.a.	AGAAGCTGGGAAACAAAACA	ATTCTGGCTTTTAATTGAGGA	55	n.a.

Abbreviations: LG, linkage group; AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

The markers work well with both diploid *P. avium* ($2n = 16$) and tetraploid *P. cerasus* ($2n = 32$) (Cantini et al., 2001; Chenglin et al., 2018; Guarino et al., 2009; Lacis et al., 2009; Mariette et al., 2010) but the investigator must consider the difficulty of scoring up to four different alleles in the latter species. The tentative assignment of allele size to the reference cultivar "Napoleon" is the result of a complex of data collected from several different laboratories. The supplementary material provided by Ordidge et al. (2021) offers a clear example of how difficult it can be to harmonize data from different laboratories, especially when dealing with 2-nt core repeats.

3.12 | Chestnut (*Castanea* Mill. spp)

Chestnut is another species for which mainly SSRs with 2-nt repeats have been retrieved from the literature. However, authors report good scorability either for *C. sativa* or for *C. mollissima* when using the markers included in Table 11 (Inoue et al., 2009; Marinoni et al., 2003; Nie et al., 2021).

CsCAT41 is known to amplify two loci: the CsCAT41a and CsCAT41b (Pereira-Lorenzo et al., 2010, reported in Bouffartigue et al., 2020). Only locus CsCAT41b is usually scored.

Chestnut is diploid ($2n = 24$) and up to two alleles per marker are expected.

3.13 | Citrus (*Citrus* L. spp)

The *Citrus* germplasm and, to some extent, its associated genera *Poncirus, Microcitrus, Fourtunella*, *Eremocitrus*, and in some cases *Papeda* (wild *Citrus*), can be genotyped with the SSR markers shown in Table 12.1. The marker's position in the genome depends on the genome that is used as a reference. Because of such a complexity, the position of each marker in the different reference genomes is reported in the complementary Table 12.2.

These SSR markers are from Kijias et al. (1997), Ahmad et al. (2003), Barkley et al. (2006), Ollitrault et al. (2010), and Hong et al. (2016). The SSR primers are either from the original paper or from the Citrus genome database (https://www.citrusgenomedb.org/). The Citrus Sat database (http://bioinfo.usu.edu/ citSATdb/) was also examined. Markers may be checked in silico but, unfortunately, none of the SSR

TABLE 10 Recommended markers for cherry (*Prunus avium* and *P. cerasus*). The reference genotype is "Napoleon" (harmonized allele sizes from Ordidge et al., 2021); markers are ranked according to Clarke & Tobutt (2009), integrated with information from other authors; touch down (td) −0.5°C per step for 10 cycles, except for the EMPaS series for which the reduction in temperature was 1°C per cycle. For all SSR loci, the linkage group (LG) was reported in accordance with Clarke and Tobutt (2009); a genome assembly was not available or was not assembled in chromosomes or linkage groups.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (bp)
EMPA002	(AG)13	1	TGACAGGTCATCATACCATTTG	CAGGATTAAGCATTGCAAATTA	60 td	105/107
EMPA003	(AC)8	1	AGCCATTCTGAAAAGGTGGA	GCATTCAGCCAACAAAATCA	60 td	n.a.
EMPA011	(AG)16	1	TGTGCTCACTCTCTGCTGCT	TGTGTGGGTTCACAGTCTCC	60 td	n.a.
EMPA005	Complex	1	TGGGTTTGAGCAATATGCAA	CACCAATACACATGCACACG	60 td	n.a.
EMPA001	(AG)4GGGT(AG)26	1	GCTCTGCTGCTTCAACCATT	TTTCCCAACACACTTACCCC	60 td	n.a.
EMPA017	(AG)19	2	ATTTCAATGTGGGGATGAGC	TGAAGTGAGGGAAATGGAGC	60 td	242–
EMPaS12	Complex	3	TGTGCTAATGCCAAAAATACC	ACATGCATTTCAACCCACTC	60 td	139/–
EMPaS02	Complex	3	CTACTTCCATGATTGCCTCAC	AACATCCAGAACATCAACACAC	60 td	142/144
EMPA014	Complex	3	ATTTGCCTATTGGGTTCCTG	TGAATGATCACAGAACATCCAG	60 td	n.a.
PMS3	Complex	4	TGGACTTCACTCATTTCAGAGA	ACTGCAGAGAATTTCACAACCA	55	189/–
EMPaS10	(GA)28	4	GCTAATATCAAATCCCAGCTCTC	GCTAATATCAAATCCCAGCTCTC	60 td	n.a.
EMPaS06	(CT)12	4	AAGCGGAAAGCACAGGTAG	TTGCTAGCATAGAAAAGAATTGTAG	60 td	205/207
BPPCT037	(GA)25	5	CATGGAAGAGGATCAAGTGC	CTTGAAGGTAGTGCCAAAGC	57	138/146
EMPaS14	Complex	5	TCCGCCATATCACAATCAAC	TTCCACACAAAAACCAATCC	60 td	199/211
EMPaS01	(GA)9(GA)11	6	CAAAATCAACAAAATCTAAACC	CAAGAATCTTCTAGCTCAAACC	60 td	n.a.
UDP98-412	(AG)28	6	AGGGAAAGTTTCTGCTGCAC	GCTGAAGACGACGATGATGA	60	n.a.
EMPA004	Complex	6	TACGGTAGGCTTCTGCAAGG	TTGGCAGGTTCTGTTCACAT	60 td	192/194
PS05C03	Complex	7	AGATCTCAAAGAAGCTGA	AGCTTATGCATATACCTG	n.a.	n.a.
EMPA018	(GA)18	8	TCCAAGAACAAAGCCAAAATC	AATTTCAATGCATTCTGGATAG	60 td	99/–
EPDCU5060	(C/GAT)8	n.a	ACCAAATTGGACATGCAACC	CGGTCGAGAAGACTGAGGAG	55	n.a.

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

markers included in that database have been tested in germplasm collections.

Citrus accessions are usually diploid ($2n = 18$), but occasionally tetraploids, triploids and aneuploids have been observed and the ploidy must be taken into account when considering the expected number of alleles (Ahmad et al., 2003). Many *Citrus* cultivars originated by spontaneous mutations and are, therefore, not distinguishable by molecular markers (Ahmad et al., 2003; Barkley et al., 2006).

3.14 | Coconut (*Cocos nucifera* L.)

Coconut, the species that contributes up to 30% of the oil used in human consumption, is a vegetatively propagated monocotyledon. The genotypes are classified as "tall," "dwarf," and "intermediate," and show limited genetic diversity, especially the "dwarf" types which, besides being vegetatively propagated, are also self-pollinating (Teulat et al., 2000). It is only through the recent practice of crossing "tall" by "dwarf" genotypes that genetic diversity has increased (Kamaral et al., 2017).

The SSR markers listed in Table 13 are mainly those reported by Kamaral et al. (2017), who collected three series of SSRs published by Rivera et al. (1999), Perera et al. (2003), and Baudouin et al. (2006). Additionally polymorphic dinucleotide-containing SSR markers reported by Teulat et al. (2000) were added to these markers.

Coconut is diploid ($2n = 2x = 32$), and one or two alleles per SSR marker are expected.

3.15 | Coffee (*Coffea* L. spp)

Coffea canephora (the "robusta" type) and most of the wild coffee species are diploid ($2n = 22$) and show appreciable genetic diversity (Hendre et al., 2014). In comparison, *Coffea arabica* (the "Arabica" type) is tetraploid ($2n = 44$) and shows little genetic diversity, with the most typical monomorphic profile represented by two alleles each one coming from either parent (Combes et al., 2000). The discrimination of "robusta" and wild coffee accessions is therefore easy, while the "Arabica" accessions, because of their ploidy and narrow genetic diversity, are difficult to differentiate from each other even with a relatively large number of markers.

The SSR markers and primers in Table 14.1 are from Combes et al. (2000) and Silvestrini et al. (2007) and have

TABLE 11 Recommended markers for chestnut (*Castanea* spp). The reference genotype is "Marrone fiorentino" (from Pereira-Lorenzo et al., 2017); CsCAT41 marker amplifies in two loci called CsCAT41A and CsCAT41 B (Pereira-Lorenzo et al., 2010); annealing temperature (AT) homogenized for multiplexing from Bouffartigue et al. (2020); for all SSR loci, the linkage group (LG) was reported in accordance with Bouffartigue et al. (2020); a genome assembly was not available or was not assembled in chromosomes or linkage groups.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (bp)
CsCAT6	(AC)24AT(AC)4	1	AGTGCTCGTGGTCAGTGAG	CAACTCTGCATGATAAC	50	159/173
QpZAG36	(AG)19	1	GATCAAAATTTGGAATATTAAGAGAG	ACTGTGGTGGTGAGTCTAACATGTAG	50	217/221
CsCAT17	Complex	2	TTGGCTATACTTGTTCTGCAAG	GCCCCATGTTTTCTTCCATGG	58	149/155
CsCAT14	(CA)22	2	CGAGGTTGTTGTTCATCATTAC	GATCTCAAGTCAAAAGGTGTC	57	133/150
EMCs22	(GA)19	2	GTGCCTCTGTATGCATGGTAAGC	CCAGGTTTAAGAAAGCAAGCATAAC	60	132/134
QrZAG4	(GA)46	3	CGTCTATAAGTTCTTGGGTGA	GTAACTATGATGTGATTCTTACTTCA	48	110/114
EMCs38	(GA)31	4	TTTCCCTATTTCTAGTTTGTGATG	ATGGCGCTTTGGATGAAC	56	240/244
EMCs14	(GAG)7	5	GTGCTTCAGGGACCTTTCTTCTC	GCCGCCGCCTCCTGCTGCTC	68	140/–
CsCAT8	(GT)7(GA)20	6	CTGCAAGACAAGAATTACAC	GAATAACCTGCAGAAGGC	50	203/208
CsCAT16	(TC)20	6	CTCCTTGACTTTGAAGTTGC	CTGATCGAGAGTAATAAAG	50	126/132
EMCs2	(GGC)7	6	GCTGATATGGCAATGCTTTTCCTC	GCCCTCCAGCCTCACCTTCATCAG	55	160/–
QpZAG110	(AG)15	7	GGAGGCTTCCTTCAACCTACT	GATCTCTTGTGTGCTGTATTT	50	210/–
CsCAT41[a,b]	(AG)20	8	AAGTCAGCCAACACCATATGC	CCCACTGTTCATGAGTTTCT	50	228/234
CsCAT15	(TC)12	8	TTCTGCGACCTCGAAACCGA	GCTAGGGTTTTCATTTCTAG	50	124/134
CsCAT1	(TG)5TA(TG)24	8	GAGAATGCCCACTTTTGCA	GCTCCCTTATGGTCTCG	50	215/223
EMCs15	(CAC)9	9	CTCTTAGACTCCTTCGCCAATC	CAGAATCAAAGAAGAGAAAGGTC	55	91/–
CsCAT2[b]	(AG)16	10	GTAACTTGAAGCAGTGTGAAC	CGCATCATAGTGAGTGACAG	55	227/–
QrZAG96	(TC)20	10	CCCAGTCACATCCACTACTGTCC	GGTTGGGAAAAGGAGATCAGA	55	153/155
CsCAT3	(AG)20	12	CACTATTTTATCATGGACGG	CGAATTGAGAGTTCATACTC	50	225/239
CIO[b]	(GA)6	n.a.	TCTGGGGAAACACGAAGC	TATTCCCATTCTGTCCCAAACAT	60	147/150
OCI	(GT)8	n.a.	GGAATAAGTGGGGTGGGTTT	GGGCCAAAGCATCACATTAC	60	146/149
RIC	(GA)6GG(GA)5	n.a.	AAGACAGAGACAGTGGTTTTTGC	TCTGGGGAAACACGAAGC	60	119/123

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

[a]Multilocus.

[b]Locus with null alleles.

been integrated with information from other studies (Benti et al., 2021; Missio et al., 2011; Pruvot-Woehl et al., 2020; Sánchez et al., 2020). The marker's position in the genome depends on the genome that is used as a reference. Because of such a complexity, the position of each marker in the different reference genomes is reported in the complementary Table 14.2.

Several other authors have described large SSR collections isolated from different genomic and EST-based libraries, that is libraries based on expressed sequence tags, but they do not appear to have been used in genotyping coffee collections.

3.16 | Date palm (*Phoenix dactylifera* L. and related species)

Several hundred SSR markers are available in the literature for this species. We identified some 80 that had been tested by different authors (Akkak et al., 2009; Al-Faifi et al., 2016; Chaluvadi et al., 2018; Elmeer & Mattat, 2015; Elmeer et al., 2011; Mathew et al., 2014; Racchi & Camussi, 2018; Shakra et al., 2020) and selected 24 with 3-nt or longer core repeats. These were then ranked for their polymorphic information content, the parameter that was common across the references that were selected (Table 15). The melting temperatures reported by Elmeer et al. (2011) and Elmeer and Mattat (2015) were transformed into annealing temperatures following Shakra et al. (2020), and we also identified the annealing temperature for several markers where the information was missing in the original paper.

Date palm is diploid ($2n = 36$) and up to two alleles per marker are expected. Although there is appreciable synteny between date palm (*P. dactylifera* L.) and oil palm (*Elaeis guineensis* Jacq.), molecular markers isolated in date palm have never been tested in oil palm.

3.17 | Fig (Common fig, *Ficus carica* L.)

There does not appear to be any consensus for a common list of SSRs that can be used in fig genotyping. Consequently,

TABLE 12.1 Recommended markers for citrus (*Citrus* spp). A reference genotype was not available.

Marker	Core repeat	Forward-primer	Reverse-primer	AT (˚C)	RG (bp)
CAG01	AGC	AACACTCGCACCAAATCCTC	TAAATGGCAACCCCAGCTTTG	n.a.	n.a.
CMS19	(TCA)11(TC)14	GGCTTTTGCCCAATGATG	GTTGACCTAAAAGGGGGGAG	55 or 60	n.a.
TAA41	TAA	AGGTCTACATTGGCATTGTC	ACATGCAGTGCTATAATGAATG	55	n.a.
AG14	GA	AAAGGGAAAGCCCTAATCTCA	CTTCCTCTTGCGGAGTGTTC	55	n.a.
CAC15	CAC	TAAATCTCCACTCTGCAAAAGC	GATAGGAAGCGTCGTAGACCC	55	n.a.
TAA3	TAA	AGAGAAGAAACATTTGCGGAGC	GAGATGGGACTTGGTTCATCACG	45	n.a.
CT21	TC	CGAACTCATTAAAAGCCGAAAC	CAACAACCACCACTCTCACG	n.a.	n.a.
CAC23	CAC	ATCACAATTACTAGCAGCGCC	TTGCCATTGTAGCATGTTGG	45	n.a.
ATC09	TCA	TTCCTTATGTAATTGCTCTTTG	TGTGAGTGTTTGTGCGTGTG	n.a.	n.a.
CIBE3397	(TTAT)5	AGGCGGAGATAGAGAAGTAAA	ATCACAACTACGAATACCCAC	55	n.a.
CIBE0447	(TTC)14	CACAAAGAGAGTAACCCACAA	CGTCAAGAAGAGAGAATGATG	55	n.a.
CIBE5866	(AAT)8	ATCTCGCTCACTTCAGAGTT	GGATTATTGTTGTTTCCTCCTC	55	n.a.
CMS4	complex	CCTCAAACCTTCTTCCAATCC	CTGTAAAGTACATGCATGTTGG	55 or 60	n.a.
TAA1	TAA	GACAACATCAACAACAGCAAGAGC	AAGAAGAAGAGCCCCCATTAGC	55	n.a.
TAA33	TAA	GGTACTGATAGTACTGCGGCG	GCTAATCGCTACGTCTTCGC	55	n.a.
CAT01	CAT/CTT	GCTTTCGATCCCTCCACATA	GATCCCTACAATCCTTGGTCC	55	n.a.
CCSME17	(GGC)7	AATGCGTGGGCAATAACTTC	TTCAATATCGGCCCAAACTC	55	n.a.
CCSME50	(GAA)7	GAGTTGGGATTCTGCTGTTGA	GACTGTTGTTCTGATGCCGA	55	n.a.
CIBE0753	(AAT)13	TCTCCTTGCCATTATTTATTT	CAGTTCTCAGTTGCCCGA	57	n.a.
CMS7	(CT)16	CTGTAAAGTACATGCATGTTGG	CTGTAAAGTACATGCATGTTGG	55 or 60	n.a.
TAA15	TAA	GAAAGGGTTACTTGACCAGGC	CTTCCCAGCTGCACAAGC	55	n.a.

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

we compared 10 papers on fingerprinting of fig collections (Akin et al., 2021; Baraket et al., 2011; Boudchicha et al., 2018; Ergül et al., 2021; Ferrara et al., 2016; Knap et al., 2018; Marcotuli et al., 2019; Perez-Jiménez et al., 2012; Poljiula et al., 2021; Rodolfi et al., 2018) and ranked 22 SSRs according to the frequency with which they had been adopted by different authors (Table 16). The top marker among those listed was the one that was most commonly used (8 out of 10 authors) and others were then ranked in descending order. A few markers are usually sufficient to distinguish most cultivars, except mutants and clonal selections. The markers at the top of the list are, therefore, recommended.

The common fig is diploid ($2n = 26$). Triploid cytotypes ($2n = 3x = 39$) can, however, be found in the germplasm (Falistocco, 2009). The alleles per marker can be influenced by this ploidy.

3.18 | Grape (*Vitis* L. spp)

Grape ($2n = 38$) is a polyploid that underwent genome duplications during its evolution. However, it behaves like a diploid and one would expect one or two alleles from a single locus molecular marker. Many papers in the scientific literature are based on a set of microsatellites with 2-nt core repeats, suggested by the European group working in the grape GENRES project (This et al., 2004). We preferred, for the reasons reported in Section 2.2.4 above, to adopt a set of 20 SSR markers with a long core repeat that have been isolated more recently from the grape genome sequence (The French-Italian Public Consortium for Grape Genome Characterization, 2007; Cipriani et al., 2008, 2010).

As few as 12 of the SSR markers within the list shown in Table 17 should be sufficient for the identification of any grape accession derived from sexual reproduction. However, cultivated grapes (*Vitis vinifera*) suffer a strong coancestry because the species is cleistogamous, meaning that the flower self-fertilizes before petals open and offspring from selfing might have been unintentionally and repeatedly selected during domestication. Traces of selfing are evident in the excess of homozygotes that are found at several loci in grape germplasm collections (Cipriani et al., 2010; This et al., 2004). Inbreeding and, in general strong coancestry, could make the analysis of pedigree difficult. Consequently, up to 60 SSR loci, the number depending on the level of polymorphism and the power of discrimination, could be required to discriminate among different parents that are closely related (Vouillamoz & Grando, 2006). For this kind of study, additional SSR markers are easily identified in the literature.

TABLE 12.2 Recommended markers for citrus (*Citrus* spp) in relation to different reference genomes. Linkage groups (LG) are reported according to each reference genome assembly; GBA1—*Citrus maxima* genome assembly: GCA_002006925.1; GBA2—*C. trifoliata* genome assembly: GCA_018350135.1; GBA3—*Citrus sinensis* genome assembly: GCA_000317415.1.

Marker	GBA1	LG	Primer's position within the *Citrus maxima* genome	GBA2	LG	Primer's position within the *Citrus trifoliata* genome	GBA3	LG	Primer's position within the *Citrus sinensis* genome
CAG01	1[a]	1	18,945,500–18,945,601	2	7	8,092,293–8,092,403	3	n.a.	n.a.
CMS19	1	3	25,980,353–25,980,508	2	5	4,319,933–4,320,067	3	3	24,295,111–24,295,273
TAA41	1	2	45,772,930–45,773,034	2	2	29,830,628—29,830,755	3	2	821,458–822,028
AG14	1[b]	2	32,598,191–32,598,307	2[a,b]	2	17,633,342–17,633,459 17,699,470–17,699,587	3	2	17,490,884–17,491,018
CAC15	1[a]	2	7,726,320–7,726,457	2[a]	2	7,098,652–7,098,789	3	2	12,460,587–12,460,725
TAA3	1[a,b,c]	5	596,798–596,917.	2[c]	3	1,265,489–1,265,625	3[b,c]	5	2,447,927–2,448,048
CT21	1[a]	5	43,069,571–43,069,688	2[a,b]	3	37,528,312–37,528,441	3	n.a.	n.a.
CAC23	1	5	2,738,502–2,738,730	2[b]	3	3,345,228–3,345,453	3[b]	5	45,177–45,177
ATC09	1	1	24,944,180–24,944,337	2	5	26,362,440–26,362,588	3	1	9,768,384–9,768,549
CIBE3397	1[a,b]	3	28,269,353–28,269,519	2[a,b]	5	2,121,959–2,122,125	3[a,b]	3	26,639,659–26,639,825
CIBE0447	1	6	14,769,615–14,769,879	2[a]	6	6,412,166–6,412,445	3	n.a.	n.a.
CIBE5866	1[a,b]	6	22,735,785–22,735,984	2[a,b]	6	697,404–697,598	3	6	20,573,992–20,574,193
CMS4	1[a,c]	8	7,174,396–7,174,576	2[a]	8	26,236–26,401	3[a]	8	41,499–41,649
TAA1	1[b]	6	22,861,965–22,862,109	2[b]	6	828,769–828,925	3[a,b]	6	20,444,208–20,444,344
TAA33	1[a,b]	6	4,056,020–4,056,120	2[b]	6	14,548,074–14,548,169	3	6	12,763,549–12,763,644
CAT01	1[c]	1	31,486,290–31,486,424	2[b,c]	7	613,343–613,471	3	n.a.	n.a.
CCSME17	1[b]	8	18,542,595–18,542,798	2[a]	8	5,684,298–5,684,492	3	7	14,116,438–14,116,644
CCSME50	1[a,b]	8	11,277,771–11,277,879	2	n.a.	n.a.	3[a,b]	8	17,217,395–17,218,176
CIBE0753	1[a,b,c]	5	26,060,995–26,061,132	2	n.a.	n.a.	3[a,b,c]	5	16,934,045–16,934,189
CMS7	1	4	3,975,342–3,975,467	2	1	3,571,565–3,571,698	3[a,]	4	18,580,619–18,580,750
TAA15	1[a]	4	28,533,286–28,533,426	2[a]	1	26,632,367–26,632,486	3	4	820,743–820,886

Abbreviation: n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

[b]SNP/s found in the published genome sequence compared with primer/s sequence/s.

[c]One base insertion/deletion found in the published genome sequence compared with primer/s sequence/s.

3.19 | Guava (*Pisidium guajava* L.)

Guava or guajava (*P. guajava* L.) is a fruit crop native to Mexico and Central America and is grown in many countries. It is self-compble but honeybees can contribute from 25 to 41% cross pollination according to observations reported by Purdue University (https://hort.purdue.edu/newcrop/morton/guava.html).

The first set of 13 SSRs shown in Table 18 is from The Aishwath group (Naga Chaithanya et al., 2015, 2016) for the core repeats; the remaining seven are from Kumar et al. (2020). Guava is diploid with a chromosome number of 2n = 22 and one or two alleles per SSR marker are expected. However, from the SSR profiles analyzed in the literature, many cultivars appear homozygous at many markers likely because they have been obtained through selfing.

SSR markers isolated from *P. guajava* were amplified in several related species (Kumar et al., 2020; Risterucci et al., 2005).

3.20 | Hazelnut (*Corylus* L. spp)

The construction of a set of SSR markers for hazelnut fingerprinting was not easy because the markers identified by different research groups varied widely. We have, therefore, suggested a list of 14 SSRs with 3-nt or longer core repeats, which could be multiplexed (see the original papers from Akin et al., 2016 and Freixas-Coutin et al., 2019 for details; N. Bassil 2022, personal communication), to which we added a further 10 historical SSRs with 2-nt core repeats (Table 19). The first series is used mainly in the United States (Bassil et al., 2013; GöKirmak et al., 2009), while the second series

TABLE 13 Recommended markers for coconut (*Cocos nucifera*). A reference genotype was not available; touch down (td) protocols, with initial temperatures 2–4°C above those indicated have also been considered in some papers. Linkage groups (LG) are reported according to the reference genome assembly; the coconut genome assembly is GCA_008124465.1.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (bp)	Position of primer within the coconut genome
CNZ04	(CT)29 TT(CA)10	1	TATATGGGATGCTTTAGTGGA	CAAATCGACAGACATCCTAAA	53	n.a.	5,740,724–5,740,865
CnCir51	n.a.	2	TCTCGTGGATCTCGTC	GCTCTTCCAGTTACGTTT	48	n.a.	54,787,544–54,787,708
CAC06[a]	n.a.	3	TGTACATGTTTTTTGCCCAA	CGATGTAGCTACCTTCCCC	52	n.a.	76,198,911–76,199,053
CNZ21[a]	(CT)30	6	ATGTTTTAGCTTCACCATGAA	TCAAGTTCAAGAAGACCTTTG	54	n.a.	71,832,312–71,832,538
CNZ40	(CT)20	6	CTTGATTGCTATCTCAAATGG	CTGAGACCAAATACCATGTGT	56	n.a.	44,144,038–44,144,162
CNZ44[b]	(GA)15	10	CATCAGTTCCACTCTCATTTC	CAACAAAAGACATAGGTGGTC	52	n.a.	10,758,680–10,758,826
CNZ43[c]	(GA)21	13	TCTTCATTTGATGAGAATGCT	ACCGTATTCACCATTCTAACA	54	n.a.	9,397,088–9,397,240
CnCirH11[a]	n.a.	13	TCATTCAGAGGACAAAAGTT	TAAAAATTCATAAAGGTAAAA	46	n.a.	3,296,706–3,296,868
CNZ46	(CT)24	14	TTGGTTAGTATAGCCATGCAT	AACCATTTGTAGTATACCCCC	54	n.a.	3,155,899–3,155,990
CAC08	n.a.	14	ATCACCCCAATACAAGGACA	AATTCTATGGTCCACCCACA	56	n.a.	9,720,087–9,720,268
CN2A5[b]	(CT)30	16	AAGGTGAAATCTATGAACACA	GGCAGTAACACATTACACATG	54	n.a.	30,005,592–30,005,745
CNZ02	(GA)15	n.a.	CTCTTCCCATCATATACCAGC	ACTGGGGGGATCTTATCTCTG	54	n.a.	n.a.
CN11E6	(CT)21	n.a.	TACTTAGGCAACGTTCCATTC	TAACCAGAAAGCAAAAAGATT	54	n.a.	n.a.
CN1G4	(CT)15	n.a.	GTCGTCCTATACTCATCATCA	GATGCGTATGAGATGTGAGAG	54	n.a.	n.a.
CNZ32	(GA)18	n.a.	TTGATCCCTAAGAGAAGGATC	GAAGAACACCAATGAGGTAAA	54	n.a.	n.a.
CAC04	n.a.	n.a.	CCCCTATAGATCAAAACAAG	CTCAGTGTCCGTCTTTGTCC	58	n.a.	n.a.
CAC65	n.a.	n.a.	GAAAAGGATGTAATAAGCTGG	TTTGTCCCCAAATATAGGTAG	54	n.a.	n.a.
CNZ06	(CT)15	n.a.	ATACTCATCATCATACGACGC	CTCCCACAAAATCATGTTATT	52	n.a.	n.a.
CNZ10	(CT)18(GT)17	n.a.	CCTATTGCACCTAAGCAATTA	AATGATTTTCGAAGAGAGGTC	56	n.a.	n.a.
CNZ12	(CT)15	n.a.	TAGCTTCCTGAGATAAGATGC	GATCATGGAACGAAAACATTA	54	n.a.	n.a.
CnCirC5	n.a.	n.a.	ACCACCAAAGCCAGAGC	GCAGCCACTACCTAAAAAG	50	n.a.	n.a.
CnCirD8	n.a.	n.a.	GCTCTTGATGTGGCTGCT	AGGCGTGTTGAGATTGTGA	54	n.a.	n.a.

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

[b]SNP/s found in the published genome sequence compared with primer/s sequence/s.

[c]One base insertion/deletion found in the published genome sequence compared with primer/s sequence/s.

TABLE 14.1 Recommended primers for coffee (*Coffea* spp). A reference genotype was not available; touch down (td) −0.5°C per step for six cycles.

Marker	Core repeat	Forward-primer	Reverse-primer	AT (°C)	RG (bp)
Sat-235	n.a.	TCGTTCTGTCATTAAATCGTCAA	GCAAATCATGAAAATAGTTGGTG	58	n.a.
Sat-180	n.a.	CATGTGTAATACATTCAACAGTGA	GCAATAGTGGTTGTCATCCTT	60	n.a.
AJ-250253	Complex	CTTGTTTGAGTCTGTCGCTG	TTTCCCTCCCAATGTCTGTA	58	n.a.
I9-3CTG	(TG)21	TGGCCGTGATAATAAACAGC	ATGTGGCAATCTAAAGCCAA	60 td	n.a.
E6-3CTG	(TG)16	CTGGGTTGGTTCTGATTTTG	GGTTCCCAGAGATTCTCTCC	60 td	n.a.
E12-3CTG	(CA + TA)38	TGCTTAGGCACTTGATATAGGA	CACGTGCAAGTCACATACTTTA	60 td	n.a.
AJ-250258	Complex	AACTCTCCATTCCCGCATTC	CTGGGTTTTCTGTGTTCTCG	62	n.a.
C2-2CATC	(ATC)14	CTCTCCCTCAGTCAATTCCA	CTTGGTCTCCCTCCTTTTTC	60 td	n.a.
MR-336	n.a.	GAGTCGTCCACACTGCTTGA	CATCTGCTTTGGTCCCTGAT	60	n.a.
AJ-250254	(CA)15(CG)4CA	GGCTCGAGATATCTGTTTAG	TTTAATGGGCATAGGGTCC	58	n.a.
Sat-237	n.a.	CAAGAGCAGACGATTCTCAATCT	TTGGGGTTAGGAAATCACAAT	58	n.a.
4-1CTG	(TG)8	AAAAAGCTGGTCCATGTCAA	GGGGCGTTCAGTTATAAACA	60 td	n.a.
17-2CTG	Complex	AGGCCTTCATCTCAAAAACC	AGCGTTACTTGAGGCAAAGA	60 td	n.a.
Sat-41	n.a.	AGTGTAACTTTAGTTCTTGC	ATTTAATGGGCATAGGGTC	58	n.a.
E8-3CTG	(CA)14	CACTGGCATTAGAAAGCACC	GGCAAAGTCAATGATGACTC	60 td	n.a.
Sat-171	n.a.	TTCCCCCATCTTTTTCTTTC	TTGTATACGGCTCGTCAGGT	58	n.a.
AJ-250257	(CTCACA)4/(CA)9	GACCATTACATTTCACACAC	GCATTTTGTTGCACACTGTA	58	n.a.
MR-054	n.a.	TGATGTGGAAGGCCATTG	GCCCCTATTATGACCCATGC	62	n.a.
32-2CTG	(CA)12	AAGGGGAGTGGATAAGAAGG	GGCTGGATTTGTGCTTTAAG	60 td	n.a.
AJ-250255	Complex	CCCTCCCTGCCAGAAGAAGC	AACCACCGTCCTTTTCCTCG	58	n.a.
AJ-250260	Complex	TGATGGACAGGAGTTGATGG	TGCCAATCTACCTACCCCTT	58	n.a.
CFGA-465	(AG)18	ACCCTTTACTACTTATTTACTCTC	ACATCCCCTTGCCATTTCTTC	62	n.a.

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

is still popular among European scientists (Boccacci & Botta, 2010; Boccacci et al., 2005, 2006, 2021).

Hazelnut is diploid ($2n = 22$) and self-incompatible. The genome is highly heterozygous, a condition that has generated large genetic diversity among the accessions. A few markers are therefore sufficient to discriminate most germplasm accessions.

3.21 | Kiwifruit (*Actinidia* Lindl. spp)

Kiwifruit is dioecious, with male and female genotypes present in all species. Cross pollination is therefore obligatory and, consequently, the genome is highly heterozygous.

Several different species grown commercially belong to the genus *Actinidia*, including the *A. chinensis/A. deliciosa* complex, *A. arguta*, *A. eriantha* and others that are less popular. The basic chromosome series is high ($x = 29$) as a consequence of several ancestral polyploidizations that occurred during evolution. In addition, commercial genotypes can be either diploid ($2n = 58$), tetraploid ($2n = 116$), or hexaploid ($2n = 174$). The consequences of this complex botanical and biological landscape are that it is difficult to differentiate accessions and difficult to correctly score the true alleles in polyploids (Huang et al., 1998; Zhen et al., 2004).

Included in the list shown in Table 20 is a marker trio capable of differentiating gender. The trio is composed of three markers identified on the *SyGI* gene, the *FrBy* gene and the housekeeping *Ankyrin*. In male genotypes, all three markers produce an amplicon each, while female genotypes produce an amplicon only for *Ankyrin*. Such simple signals of presence/absence discriminate the gender in different species at any ploidy level (De Mori et al., 2022).

3.22 | Lychee (*Litchi chinensis* Sonn.)

We selected 20 SSR markers which were the most polymorphic, according to heterozygosity and the number of alleles per sample, among those that were listed by Viruel et al. (2004) and Arias et al. (2020). Most of these markers have a 3-nt or longer core repeat (Table 21).

TABLE 14.2 Recommended markers for citrus coffee (*Coffea* spp) in relation to different reference genomes. Linkage groups (LG) are reported according to each reference genome assembly; GBA1—*Coffea arabica* genome assembly: GCA_003713225.1; GBA2—*Coffea canefora* genome assembly: GCA_900059795.1; GBA3—*Coffea eugenioides* genome assembly: GCA_003713205.1.

Marker	GBA1	LG	Primer's position within the *Coffea arabica* genome	GBA2	LG	Primer's position within the *Coffea canefora* genome	GBA3	LG	Primer's position within the *Coffea eugenioides* genome
Sat-235	1[a]	1c	36,409,488–36,409,690	2[a]	1	23,700,199–23,700,398	3	n.a.	n.a.
Sat-180	1[a,b]	1e	45,921,029–45,921,127	2[a,b]	1	35,646,397–35,646,527	3[a,b]	1	47,760,418–47,760,519
AJ-250253	1[a]	2c2e	10,609,407–10,609,698 10,466,775–10,467,046	2[a]	2	10,784,021–10,784,806	3[a]	2	12,324,316–12,324,577
I9-3CTG	1[a,b]	2e	14,583,085–14,583,261	2[a,b]	2	15,865,243–15,865,419	3	n.a.	n.a.
E6-3CTG	1[b]	3c3e	5,392,001–5,392,305 4,306,889–4,307,189	2[b]	3	5,391,454–5,391,758	3[b]	3	6,273,750–6,274,050
E12-3CTG	1	3c3e	8,169,877–8,169,988 7,136,657–7,136,807	2	3	8,384,597–8,384,708	3	3	9,337,610–9,337,760
AJ-250258	1	4c4e	8,023,154–8,023,240 10,082,824–10,082,910	2	n.a.	n.a.	3	4	7,588,063–7,588,149
C2-2CATC	1[b]	4c4e	36,376,761–36,376,933 37,913,664–37,913,864	2[b]	4	21,200,996–21,201,168	3[a,b]	4	39,062,088–39,063,319
MR-336	1[a,b]	5c5e	43,841,895–43,842,029 37,255,638–37,255,729	2[a]	5	27,341,976–27,342,110	3[a]	5	49,975,142–49,975,273
AJ-250254	1[b]	6c6e	37,272,554–37,272,701 48,937,255–48,938,532	2[b]	6	34,249,062–34,249,191	3[b]	6	50,300,479–50,300,625
Sat-237	1	6c6e	1,420,786–1,420,909 1,801,670–1,801,785	2	6	1,525,902–1,526,031	3	6	2,616,796–2,616,905
4-1CTG	1[a]	6e	173,580–173,659	2	n.a.	n.a.	3[a]	6	3,682,187–3,682,266
17-2CTG	1[a,b]	6c	29,536,174–29,536,353	2	n.a.	n.a.	3[a,b]	6	42,688,909–42,689,080
Sat-41	1	6c	37,272,572–37,272,702	2	n.a.	n.a.	3	n.a.	n.a.
E8-3CTG	1	6e	3,861,129–3,861,290	2	6	3,7 21,778–3,721,939	3[a]	6	5,477,851–5,478,009
Sat-171	1	10c10e	2,750,163–2,750,299 3,585,945—3,586,081	2[b]	10	3,536,410–3,536,538	3	10	3,904,894–3,905,022
AJ-250257	1[a]	10c10e	44,740,712–44,740,814 39,766,155–39,766,271	2[a]	10	26,576,309–26,576,411	3[a]	10	33,187,798–33,187,896
MR-054	1[a]	10c10e	8,595,840–8,595,976 10,949,856–10,949,998	2[a]	10	10,387,241–10,387,381	3	10	10,387,241–10,387,381
32-2CTG	1[b]	11c11e	27,851,236–27,851,321 35,760,480–35,760,569	2[b]	11	25,129,129–25,129,212	3[b]	11	37,480,190–37,480,279

(Continues)

TABLE 14.2 (Continued)

Marker	GBA1	LG	Primer's position within the *Coffea arabica* genome	GBA2	LG	Primer's position within the *Coffea canefora* genome	GBA3	LG	Primer's position within the *Coffea eugenioides* genome
AJ-250255	1[b]	11c11e	37,489,968–37,490,131 29,404,639–29,404,796	2[b]	11	26,725,114–26,725,271	3	11	39,389,733–39,389,888
AJ-250260	1[a]	11c11e	34,189,403–34,189,503 40,107,790–40,107,922	2[a]	11	31,211,933–31,212,044	3[a]	11	44,891,734–44,891,848
CFGA-465	1[a,c]	11c11e	27,156,010–27,156,170 35,056,368–35,056,528	2	11	n.a.	3	11	n.a.

Abbreviation: n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

[b]SNP/s found in the published genome sequence compared with primer/s sequence/s.

[c]One base insertion/deletion found in the published genome sequence compared with primer/s sequence/s.

Litchi has been reported to possess variable diploid chromosome numbers with $2n = 28$, 30, or 32. This variation in chromosome number is thought to be because the modern species have more than one wild progenitor. However, this may not be a problem in genotyping accessions.

3.23 | Macadamia (*Macadamia integrifolia* Maiden & Betche, *M. tetraphylla* L.A.S.Johnson and cultivated hybrids)

Macadamia is a species that is native to Queensland, Australia, but it was domesticated in Hawaii (USA) at the end of the nineteenth century following the introduction of a small sample of seeds. The genetic base is, therefore, narrow and grafting that is used for propagation of the few preferred cultivars exacerbates this characteristic (Nock et al., 2019).

Among the SSR markers listed in Table 22, 10 SSRs were selected among those reported by Schmidt et al. (2006), while 12 are from Nock et al. (2014).

Macadamia is diploid ($2n = 28$) and up to two alleles per SSR marker are expected.

3.24 | Mango (*Mangifera indica* L. and related species)

Two partial sets of SSR markers are included in Table 23, 12 of which are from Ravishankar et al. (2015b) (see also Ravishankar et al., 2011) and 11 from dos Santos Alves et al. These were selected as being the most informative. Several markers for linkage, according to Yamanaka et al. (2019), were not included in this list.

Mango is diploid ($2n = 40$). Its high number of chromosomes suggests that it has a polyploid genome in its origin. However, genetic markers are inherited in a disomic fashion. Mango may, therefore, be treated as diploid and up to two alleles per locus are expected.

3.25 | Mangosteen (*Garcinia mangostana* L. and *Garcinia cochinchinensis* (Lour.) Choisy)

In Table 24, 22 SSR markers are reported, all with 3-nt or longer core repeats, being the most polymorphic among those listed by Arias et al. (2020).

In a recent paper (Midin et al., 2017), the number of chromosomes and genome size of *G. mangostana* were determined through chromosome counting, flow cytometry and molecular k-mer analyses. The chromosome count revealed that the chromosome number is rather variable, with $2n = 74$ to 110. This instability was attributed to

TABLE 15 Recommended markers for date palm (*Phoenix dactylifera* and related species). A reference genotype was not available; annealing temperature (AT) was not reported for primer pairs in Elmeer et al. (2011, 2015) (original papers), for these primers AT is as reported in Shakra et al. (2020); linkage groups (LG) are reported according to the reference genome assembly; the date palm genome assembly is GCA_009389715.1.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (˚C)	RG (bp)	Position of primer withinthe date palm genome
DP176[a]	(CAA)9	1	GCCATTAACGAAATGGCTTG	GTTTGCACATAGCGCTTCAA	55	n.a.	1,522,699–1,522,877
DP170[a,b]	(AGGG)5	1	TCTTTGGGCTTACGACAACC	GTATGGCCCAAGATGCAGAT	52	n.a.	22,804,531–22,804,712
DP165	(AATA)5	3	AAGCATCCTATGGCTTTGACA	GGGCTGTATGTGATGCATTG	55	n.a.	15,338,071–15,338,274
DP168[a]	(CAT)8	3	GCAGCAAAGCCCTTAGGC	GGTGTTATGTGCAGCCAATG	55	n.a.	13,203,488–13,203,678
mPdCIR015[a,b]	(GA)15	3	AGCTGGCTCCTCCCTTCTTA	GCTCGGTTGGACTTGTTCT	54	n.a.	7,935,017–7,935,134
DP179	(ATTT)5	5	GGTTAGCCATCCAAAAGTGG	TATGTAGCCTCCACCGCATC	55	n.a.	15,120,322–15,120,486
KSU-PDL25[a]	(GGA)4	5	ACGGGAAGCTGGACCTTG	CTACAAACCCAGCAGACATAG	61	n.a.	15,270,055–15,270,193
DP167	(GAAA)6	5	ACATCCAATGGCATCCAAAT	GGGTTTCCAGGTTTTCTTCTC	55	n.a.	6,995,661–6,995,796
DP160[a]	(GAAA)5	9	AAGAGCGACAATCATGACCA	GGAAATTGAAGGGCATCTTG	52	n.a.	10,009,109–10,009,283
DP152	(TAT)8	9	ACGAGTTTTTGGGAGAGCAA	GCAAGTTGCCAACATTCTTGT	54	n.a.	9,546,098–9,546,301
KSU-PDL39	(GTG)4	9	AAACTGGTGACAACAAAACTG	CATGATTACTCCTCATCATCA	55	n.a.	1,770,535–1,770,671
DP177	(AGGC)6	10	TTCCTTGGGCTCACTTCAAC	TAACATGCCAGCAAAGGTGA	55	n.a.	8,218,086–8,218,286
DP174	(CGTG)5	11	CTCTGTCGTACGGAGGAAGG	GTGGCACTATCACGCTCTCA	55	n.a.	23,381,118–23,381,332
mPdCIR010[a]	(GA)22	14	ACCCCGGACGTGAGGTG	CGTCGATCTCCTCCTTTGTCTC	55	n.a.	6,323,834–6,323,979
DP171[a,b]	(TTC)10	16	GTGGGAGTAGCGAGGTATGG	GTCCGGCACTTTAGGAAGTT	56	n.a.	3,914,114–3,914,298
DP153[a]	(TCA)9	17	TCATCACAGGCAATGGCTAA	GCAGATGGCCATTGAACC	52	n.a.	14,557,310–14,557,494
DP172	(AGG)11	n.a.	GGTGTTTGGGCCTATTTCCT	GTCCTCCTCCTCCTCTGTCC	54	n.a.	n.a.
KSU-PDL21	(CTT)6	n.a.	GCTACTCCTTCTTCTTCTCCTT	TGATGATTGGTTGAGATTAAGA	55	n.a.	n.a.
KSU-PDL29	(GTT)11	n.a.	AGCACATGGCAGTTACTCTAC	AACAACAACAATCAGTCCAAA	55	n.a.	n.a.
DP157	(TC)19	n.a.	TGGACAATGACACCCCTTTT	GCCCACACAACAACCTCTCT	55	n.a.	n.a.
DP175	(CA)19	n.a.	ACACACACACACACACACACC	GTGGCTTCTTTTTGGCTGTC	60	n.a.	n.a.
DP169	(AAT)12	n.a.	GCATGGACTTAATGCTGGGTA	GGTTTTCCTGCCAACAACAT	57	n.a.	n.a.

Abbreviations: RG, reference genotype (alleles in base pairs); n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

[b]SNP/s found in the published genome sequence compared with primer/s sequence/s.

TABLE 16 Recommended primers for fig (Common fig, *Ficus carica*). A reference genotype was not available; MFC4 marker amplifies in two loci; touch down (td) −1.0°C per step for five cycles; linkage groups (LG) are reported according to the reference genome assembly; the fig genome assembly is GCA_009761775.1.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (bp)	position of primer within the fig genome
FCUP038-6[a]	Complex	2	CAATGTATCATTTCATCTCACGAA	AGTTCCCATGTTTGGTTACTGA	60 td	n .a.	323,782–323,930
FCUP070-2[a,b]	(AG)15	2	TTCAACTTCAACCTTCACCAA	TTTGTCTAAGGAGGCTTATTGTCA	60 td	n .a.	13,758,503–13,758,647
LMFC31[a]	(GA)15	3	GTAAAATGAAAATTGGAGTATT	TTGAAGATATTGTTGTATGCT	55	n .a.	19,049,634–19,049,855
MFC1[a]	(CT)13	3	ACTAGACTGAAAAAACATTGC	TGAGATTGAAAGGAAACGAG	55	n .a.	15,725,999–15,726,172
LMFC27[a,b]	(TG)17(AG)6	3	ATTTCTTCAACTTTTGTAATGA	CCTTTTGTCTACATATACCTTT	55	n .a.	20,696,709–20,696,871
MFC8	Complex	3	GTGGCGTCGTCTCTAATAAT	TATTCTATGCTGTCTTATGTCA	50	n .a.	19,898,904–19,899,059
FCUP068-1	Complex	3	GGAATTACCGTCCATGGCTA	CGCCACTCTCTCTCTCCACT	60 td	n .a.	9,152,754–9,152,917
LMFC24	(CT)10	5	ACTTCTTCATATTTGGTATAGG	TTCATAAACTGGTCTAAAAGA	55	n .a.	13,081,051–13,081,300
LMFC30	(CT)18(CA)6	6	TTGTCCGTTTCTTATACAAT	TCTTTTTAGGCAGATGTTAG	55	n .a.	6,173,522–6,173,757
LMFC21[a,b]	(TC)9	6	ATGTCAAAACACCAGCTCTA	AAGAATAGAAAACCTGAAAAAG	55	n .a.	14,066,962–14,067,205
MFC2[a,4]	(AC)18(AT)7	7	GCTTCCGATGCTGCTCTTA	TCGGAGACTTTTGTTCAAT	55	n .a.	18,283,891–18,284,038
FCUP066-7[a]	(CA)14	7	CCCTCTCGAAGAAGAAGCA	CTACAGGAAATGGGCCTCAA	60 td	n .a.	7,368,064–7,368,209
LMFC38	(CT)20	7	CTCAACGTCCGTACTAACTA	CTAAGGAATAAAAGGAGAAAA	55	n .a.	18,672,115–18,672,307
LMFC23	(AG20)	9	TTTCGTGTCTAACGATCAAAAA	CTCCCATCTCCAACTCCATC	55	n .a.	4,995,484–4,995,595
MFC4[3]	(AT)4(AC)11	10	CCAAACTTTTAGATACAACTT	TTTCTCAACATATTAACAGG	55	n .a.	16,226,332–16,226,533
LMFC37	(CT)12	11	AAGTACATCTTCACCATTGA	ATTAAACTCTTCATTCATCAGT	55	n .a.	7,219,994–7,220,179
LMFC14[a]	(GA)16	12	CAAAACTCACACCAATAATC	TAATCTGCAAAAAGATGACTA	55	n .a.	14,395,805–14,395,999
LMFC19[a]	(AT)11(AG)12	13	CTTATGAAAACTCGGTAGAAG	AATGAATGGAAATGATCTTG	55	n .a.	1,656,393–1,656,674
MFC3	Complex	n.a.	GATATTTTCATGTTTAGTTTG	GAGGATAGACCAACAACAAC	55	n .a.	n.a.
LMFC18	(GA)9	n.a.	CACATCCACACACCAAAGAG	TACCACAGACTCACCCAATTAT	55	n .a.	n.a.
LMFC26	(GA)15	n.a.	ATGTTATAGTTGAGTGAGGATAA	AAATAGTGGATCTTGCATGT	55	n .a.	n.a.
LMFC25	(TC)11	n.a.	GATTCTGATTAAAGGGTATTT	GCTTTCCAAATCTAAAGTAAC	55	n .a.	n.a.

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

[b]SNP/s found in the published genome sequence compared with primer/s sequence/s.

[c]Multilocus.

TABLE 17 Recommended makers for grape (*Vitis* spp). The reference genotype is "Merlot" (from Cipriani et al., 2010); touch down (td) −0.5°C per step for 10 cycles; linkage groups (LG) are reported according to the reference genome assembly; the grape genome assembly is GCA_000003745.2.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (bp)	Position of primer within the grape genome
VChr1a	ATCC	1	TTCATACCTTGCAGGGAGCTA	TGATTTCCATTCCCAAATTCA	55 td	222–	2,819,297–2,819,495
VChr1b[a]	ATCC	1	AGATGGGTGGCATTAGCAAG	TTATTTCCCTCCCTCGCTGT	55 td	99–111	11,457,785–11,457,877
VChr2a[a]	AGGC	2	GGTCCGCTTTTGAGAAGAAA	CATGTGAACGCGCTAAACAC	55 td	137–146	4,694,585–4,694,711
VChr3a[a]	AAT	3	CAATCATATGAGCAAGGCATGT	GCTTCCTGAAATTTGTGTCCA	55 td	181–199	9,542,014–9,542,191
VChr5a[a]	AGATG	5	ACTTGGCGAGTATTTGTTCTAAA	CCGCTTTGTGAAGGTATCCA	55 td	241–253	14,027,012–14,027,187
VCHr5c	ACAT	5	CCCATCAGTTTGCCTATGAA	TTTGATCTTGTTATTGTGCTGTTAC	55 td	100–	4,080,604–4,080,706
VChr5b	AAAG	5	CTTCTCGGTCATGGTCATTG	CTCCTTCCACCTCTGGTTCA	55 td	183–198	9,474,726–9,474,904
VChr6a[a]	AATC	6	AATGTTGAGCTTTGGGCTTG	CCAATTCTTCCATACCTCAAAA	55 td	180–184	7,188,477–7,188,641
VChr8a	AAT	8	ACCCACTGCCACTCTCTCAT	AAATCTCCGGGATCCTTTTG	55 td	196–206	15,200,499–15,200,651
VChr9a[a]	AAG	9	GCGACAGCATCACTTCAATC	GAATTGCCAAGGACAAGGAG	55 td	90–	6,426,756–6,421,451
VChr11a	AAAG	11	GGGATAAGGTGAAAGCCTCA	ATGCTTGGTATCTGGCAACC	55 td	186–207	7,401,226–7,401,403
VChr12a	AATT	12	GCTTTAAATGTTAGATTAGGGCACTC	TCCATGTTGTTTGCTCTTTCC	55 td	134–141	6,174,660–6,174,775
VChr13a[a]	AAAAG	13	TGGCAGAGCAAATGAATCAA	TTGGATGGATTGGAATGACC	55 td	144–151	7,509,727–7,509,862
VChr14a[a]	AATC	14	AACCTGGGATGCTGAGAATG	TGCATGCATATGGATCTTGT	55 td	188–	18,914,166–18,914,279
VChr15a[a]	ATCC	15	CAATCCCAACAGTTCCATGA	CGTTTTCTCCTTCGGACAAG	55 td	149–	19,977,749–19,977,880
VChr16b[a]	AATT	16	ATAAGGCGCTGACTTTGTGA	CCAGGAGATCAACCACCATT	55 td	165–189	15,921,733–15,921,902
VChr18a[a]	AAGG	18	TTCCCACCCGGTAAATATGA	CATCCAAACATCACGCTGAG	55 td	159–171	8,512,593–8,512,740
VChr18b	AGGC	18	ATACGCAAATGATCACAGCA	CATTTTCTCCATGGCCTCAT	55 td	146–	14,028,762–14,028,897
VChr19a[a]	AAG	19	TGGATTCACCATTGTCCTCA	CGAGGATACCAACAAGAATGAA	55 td	142–146	977,575–977,698
VChr19b[a]	AGAT	19	TTTGTTAGGTGTTGTTACCCGTTA	ATCTTCTGGCCATGTGGTTC	55 td	162–166	8,693,674–8,693,820

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs).

[a]Primer pairs found inverted according to the published genome sequence.

TABLE 18 Recommended markers for guava (*Pisidium guajava* L.). The reference genotype is "Allahabad Safeda," a popular cultivar in India, for the first 13 markers coded as "mPgCIR"; * = the allele size of the "GUV" series is the amplified clone of "Allahabad Safeda" reported by Kumar et al. (2020). Linkage groups (LG) are reported according to the reference genome assembly; the guava genome assembly is GCA_016432845.1.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (bp)	Position of primer within the guava genome
mPgCIR46[a,b]	(GA)36	2	ATAGAACGCCATGTTACCAA	AGGCTTATCTGTTACACCA	55	126–126	9,383,401–9,383,524
mPgCIR192	(GA)23	4	ACGCTAACTATCGAAATGCT	ACTACGCACTTGATGGAGAT	55	154–154	43,546,905–43,547,040
mPgCIR175[a]	(GA)16	4	GCATTATGTGCCAAGCAA	TGCCAAGGTGTAATGTTGTA	55	113–154	10,805,686–10,805,836
mPgCIR108[a]	(GA)13/(GGAG)3	5	GGACCTCACAGAAGTTCAC	CGCTGTTTACACTGTCGTT	55	180–180	38,551,823–38,551,963
mPgCIR179	(GA)16	6	GGGTCTCGACTAAAGAAGGA	CCTCCATTTGCATCAACTTT	55	134–166	33,386,427–33,386,552
mPgCIR48[b]	(GA)20	7	GCAGCTTCTTCAATGTTC	AAAACTTGGCAACGTCAGT	55	124–124	26,715,167–26,715,241
mPgCIR240[a]	(GA)22	7	CGAATGTCCAAGATTCAGTT	CCTTCTTCATCTCAGCCTTT	55	172–172	35,203,939–35,204,096
mPgCIR245	(GA)11(GGTA)3	8	CCAGACAAAATTCCAACG	AAATAGCCTCTCCAATCACA	55	136–136	17,193,182–17,193,368
mPgCIR218[a]	(GA)24	8	CTGTTGCCTCAGATCGTAAT	CAATGCAAAGCCATGATAGT	55	119–119	9,290,981–9,291,102
mPgCIR139[a,b]	(GT)9/(GA)9	9	ATAATCCCCTCCCATAACTA	CCAACTCAACATGAGAAGC	55	225–225	21,341,904–21,342,107
mPgCIR195	(GT)8	9	GCCGTAGACATAAGTTTCAG	GCCCTTATCAAGTCCATGT	55	149–149	26,422,215–26,422,331
mPgCIR180	(GT)9	10	CATGGATTCAACTCTTGTCG	CTACATTGGAAGCAGAATGG	55	151–151	22,493,458–22,493,557
mPgCIR220[a]	(GT)8/(GA)20	10	AGAGCAGTGGTTGCTATTTT	CCATCTCTTACTTTTCTTGTG	55	218–218	9,973,255–9,973,486
GUV32	(AG)6	n.a.	AGCTTCGGATCAGTTAGTCCCT	TCCCAGTCTCTCTCTATCTCGC	43	283*	n.a.
GUV1-37	(CT)8	n.a.	TCCCAGTCTCTCTCTATCTCGC	AGCTTCGGATCAGTTAGTCCCT	40.9	369*	n.a.
GUV4-42-2	(TCAA)3	n.a.	CCCCTTTTGTTTTCACCTAACA	CTTCGGATCGAGAGAGAGAGAG	50.9	287*	n.a.
GUV31[a]	(GA)23	6	GTGTTGGAGAGGTTTTGTGTGA	TTGCGGTACATGGTTTCTTATG	60	274*	13,036,052–13,036,309
GUV4-53	(GCC)6	7	TATCCCTCATCTCCTTCACCTT	ATATTAGAGAGCCTGTGGTCCG	56.9	238*	24,344,133–24,344,349
GUV2-43	(GA)9	8	CCAAGAAGAGAGAAAGAGACGG	ATGTATGGTGAGTGTGTGAGGG	57.6	228*	31,184,020–31,184,226
GUV19	(AG)18	9	AGCGAGGTATTGGTGAGATAGC	GTTTCTGACTTTTCACGTTCCC	63.3	374*	2,660,491–2,660,849

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

[b]SNP/s found in the published genome sequence compared with primer/s sequence/s.

TABLE 19 Recommended primers for hazelnut (*Corylus* spp). A reference genotype was not available; the SSR reported in bold might be organized for multiplexing with an annealing temperature (AT) of 52°C. See this review and the original papers of Akin et al. (2016) and Freixas Coutin et al. (2019) for further information. For all SSR loci, the linkage group (LG) was reported in accordance with the reference genome assembly; the halzenut genome assembly is GCA_901000735.2.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (bp)	Position of primer within the hazelnut genome
BR270[a]	(CTG)6	1	AGCACCTCCTCTGCTTCCTA	TTCCTCCTCTGCTCCAAATG	52	n.a.	28,117,042–28,117,120
BR343[a]	(TGC)6	1	CAACAGATCCCAGGTTAAAAGG	TATGTTTCGGGACTTGGACTTC	52	n.a.	48,632,623–48,632,989
GB395	(CTC)6	2	TGTTATTTTCTCATCTGCGTGG	CTCTGATCGAACCAACCATGT	52	n.a.	18,028,305–18,028,654
CaT-B501[a]	(GA)21	2	GAAATTCAATCACACCAATAAAGCA	CCTCCCTTGTCCTCATCACTG	55	n.a.	10,946,448–10,946,548
CaT-B504	(CT)18	2	CGCCATCTCCATTTCCCAAC	CGGAATGGTTTTCTGCTTCAG	55	n.a.	3,556,903–3,557,054
CAC-C008[a,d]	(AAG)11	3	TTTCCGCAGATAATACAGGG	TCCTTTGCTTTGGACCAG	52	n.a.	14,414,970–14,415,152
BR414[d]	(AAT)6	4	ATCGCATCACGGAAGAGAAG	TGACGAGAACCTAGGGATCTATTT	52	n.a.	31,071,503–31,071,601
CaT-B507[a,d]	complex	4	CTAAGCTCACCAAGAGGAAGTTGAT	GCTTCTGGGTCTCCTGCTCA	55	n.a.	18,975,847–18,976,012
CaT-B508[a,d]	(GA)10	4	GGGTCAAGATTTGATAAAGTGGGA	GCACTCCACTTGTGCGTTTTC	55	n.a.	32,580,267–32,580,408
BR259	(TCA)10	5	GAAGGATGAATGGAAGTTGGAG	AAGATCGGCTTCGAGAATATCA	52	n.a.	32,897,599–32,897,826
GB875[b]	(GGA)9	5	ATGATGATGAGGAGGAGGAGAA	CAAAATCAGGCATACAGAACCA	52	n.a.	8,671,780
GB673F	(TCACCA)5	5	CAACAATGGGAATGTTGCAG	GGGCCAATAGCAAAAGTTCA	52	n.a.	36,035,010–36,035,345
CaT-B503[a,c]	(GA)18	5	CTCAATTCACTCGAACGGATAC	AGCCGATACCAGCCTCTCGC	55	n.a.	23,778,071–23,778,172
GB950[a,d]	(TGG)7	6	GAAGAAGACGAGGAGCACATTT	ACTGAGCATTTCCAACCCATAC	52	n.a.	315,867–316,002
CaC-B020[a,b]	(GA)19	6	GGGAAAATACTCCAAATCGCT	TCACCGAGCCGTATAATC	60	n.a.	24,915,474
BR438	(TCA)8	7	ATCTCTGCCCCTCTCTCTCTCT	AACTAACACCGTTGCTGATCCT	52	n.a.	26,275,821–26,275,994
BR464[a,d]	(ATC)7	8	GTGCAAACAGTCGCTATCATCT	CGAGGACCCATAAGAGAACATC	52	n.a.	20,107,750–20,108,008
CaT-B505[d]	complex	8	AGAGAACGACTTTGTATGACAAAGA	TTGAACCATTAAATACATCATGTGA	55	n.a.	3,532,941–3,533,039
GB949[a,d]	(TGG)7	9	TTTGGAGGGAGACAGTTTGG	GGTTGGCCAAGAATGAGAGA	52	n.a.	17,800,225–17,800,358
CaT-B107[d]	(CT)14	9	GTAGGTGCACTTGATGTGCTTTAC	AACACCATATTGAGTCTTTCAAAGC	55	n.a.	20,561,271–20,561,364
CaT-B502[b,d]	complex	9	CTCATGACTGCCCATTTCTCG	AGGCATGCAGGCTTCACAC	50	n.a.	4,696,751
LG688[a,6]	(TTC)5	10	TTGGAGCAATGAGTCGTGTAAG	TCGTTTATTTGGGGAATCTCTG	52	n.a.	7,876,642–7,876,992
BR322[a,6]	(ACT)7	11	TCTCTTCCTTGCCACCTCAG	AAGATGGGGTTCGAGGAGAC	52	n.a.	279,054–279,142
CaC-B028	(AG)16	n.a.	ATGGACGAGGAATATTTCAGC	CCTGTTTCTCTTTGTTTTCGAG	55	n.a.	n.a.

Abbreviations: RG, reference genotype (alleles in base pairs); n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

[b]No match found in the published genome sequence for one of the two primers.

[c]SNP/s found in the published genome sequence compared with primer/s sequence/s.

[d]SSR loci LGs were different in their previous assignment based on segregation in a mapping population (Gürcan et al., 2010; Peterschmidt, 2013).

TABLE 20 Recommended markers for kiwifruit (*Actinidia* spp). The reference genotype is "Hongyang" (diploid) (unpublished data); markers that are used in combination are in italics for gender analysis: male genotypes amplify fragments in all three markers, female genotypes only in Ank-UD (see text for details); touch down (td) can be applied, with initial temperatures 5°C above the one indicated and −1°C per cycle for five cycles; for sex-markers td −0.5°C per step for 10 cycles; linkage groups (LG) are reported according to the reference genome assembly; the kiwifruit genome assembly is GCA_003024255.1.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (bp)	Position of primer within the kiwifruit genome
Ank-UD	–	1	TTTGCCTAAAGAAAGTAGTAGTCAT	AATTATGAATGGGAGGCTTCTT	55 td	n.a.	18,540,922–18,541,055
UDK-407[a]	(AG)21	3	CGTTCGAGGGTAAGGAGTTG	CCTAACCCGACCGTAGTGTG	55	n.a.	18,345,676–18,345,768
UDK-414[a]	(AG)20	4	GCCATTTCAAGAACATTTTTTG	TGGCTATATTTTGCAAGCCC	55	n.a.	6,948,734–6,948,845
UDK-409[c]	(AG)19	6	ATGACCTATTGCCAAGTGGC	TTGTGTGTACCCACCACCC	55	n.a.	10,953,400–10,953,501
NZK-769[a]	(CTT)8	7	ACAGCTGAGTTGGCAATATG	AGGAATCACCTGTGTTAGTG	55	n.a.	12,607,609–12,607,709
UDK-030[a]	(AG)9(AC)15	8	TCATGTTTGTGGTTGAGTTGTG	AGCAATAAACTCAAGCGCGT	55	104/113	25,249,446–25,249,530
UDK-026	(AC)14	11	CGCTGACCAGATTCTGATGA	TTGAAAATCACTGAGCACAACC	55	n.a.	11,429,289–11,429,413
UDK-037	complex	13	CATTTCAGTGTTAACTCTTTCG	CCCTCTTTCAAAGGTTCTCT	55	73/75	16,259,889–16,259,942
UDK-040[a]	(AC)15(AG)21	13	TCGAGTTACCTAGCTACTCCGC	CAAGGGAAGAAAATGTTGAACC	55	143/183	15,362,425–15,362,546
UDK-448	GA	13	CTTCGCCATCTACGGTAACA	GCCTATGTTCCACCGGTC	55	124/132	7,888,439–7,888,544
UDK-034[b]	(AC)12(AG)17	19	TTATATGGTGCGGCATGCTA	TGAATGCAGAAGGCAATCAG	55	188/194	13,948,118–13,948,288
UDK-413[a]	(AG)21	19	GCACTTTACACCTCTCAATCAA	AAAGATGTTTTTGAACCATGCA	55	n.a.	12,450,772–12,450,875
NZK-766[a]	complex	21	CATCGAAGCATATGAAACAG	CTCCTGAGGTTAAGACGGAG	55	n.a.	17,116,088–17,116,218
UDK-001[a,b]	(AC)10(TC)16	21	GAATCGCGTAATGATTGATGG	GTTTCCCACTCTGCAAAAGC	55	270/–	13,141,818–13,142,069
UDK-035[a]	(AC)17	23	AAGAGCCATAGCTTATTCACCG	AAGTAAAGCCATTGTCATTGCA	55	n.a.	16,931,135–16,931,253
UDK-015[a,b]	(AC)21	24	CCGAGTCATGATCGAGTTGA	GGCTCAACTTGGAGAAGTGG	55	n.a.	12,646,215–12,646,312
S-514T	(GAA)13	25	CTGGATCAGCTTCTGGACT	GGCAAAAGATGAAAAGAGTG	55	212/229	2,128,086–2,128,298
SyGI	–	25	ATACGAAGTTTGAATCGGTGATTCGTC	CTAAGCCCTAGGGAAATATTTTAAAA	55 td	n.a.	n.a.
FrBy-UD	–	25	ATTCCATTCTCATTACTAACAACTC	CCTAAAATAAACCAACCAAGAAAAC	55 td	n.a.	n.a.
UDK-092[a]	AG	26	ATCACATGAATACGTGGATTGC	TGTGTGTTCATTTGTCATCCTT	55	n.a.	10,291,960–10,292,119
UDK-039[a]	(AC)10	27	GGTTTGATCGGTCTTCGAAA	ATAAATGTGTGCCAGTGCGA	55	n.a.	5,071,117–5,071,251
NZK-768[a,b]	(CTT)5	28	GATCCGGGTTTTAAGAAG	ATGGAGTTCCTATGCTTCTG	55	n.a.	28: 847,046–847,239
NZK-767	(CTT)5	n.a.	GATCAGTTGAGGCAAGAAG	GGAGTCATCTATGGAACTTG	55	n.a.	n.a.

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

[b]SNP/s found in the published genome sequence compared with primer/s sequence/s.

[c]One base insertion/deletion found in the published genome sequence compared with primer/s sequence/s.

TABLE 21 Recommended markers for lychee (*Litchi chinensis*). A reference genotype was not available; for annealing temperatures (AT) see Arias et al. (2020); linkage groups (LG) are reported according to the reference genome assembly; the lychee genome assembly is GCA_019925255.1.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (˚C)	RG (bp)	Position of primer within the lychee genome
stv-lic_01347	(TGG)5	1	GAAGCCACAAGAGAAGAGTTGACG	AAACACAACAAACCCATTACCCAC	63 ± 1	n.a.	43,559,860–43,559,985
stv-lic_07043	(GGT)7	1	ATAACGACATCCAAGTGGAGAAGG	ACCTGTCAACAAGAACCCGAATAG	63 ± 1	n.a.	40,788,726–40,788,849
stv-lic_07417[a]	(TCA)8	1	ACCATTTCAGTAAACTATGGGTGGTC	CCACACATCAATTCTAAGAAACATATCG	63 ± 1	n.a.	35,166,367–35,166,485
stv-lic_08181[b]	(TA)6	1	TATAATTTCCACCGTGCTTGTGTG	CTCGTTTAAAGCACAAAGCCTAGC	63 ± 1	n.a.	35,200,684–35,200,795
stv-lic_00878[a]	(AGA)8	2	TATGGACCGAATTCTCCTTCATTG	CCAATCTTCACAACCCAAAATAGC	63 ± 1	n.a.	21,490,379–21,490,508
stv-lic_19633[a,b]	(TTGT)7	2	CCCCATCTTCATTTTATTTATTGTTG	ATGTGGGTATCTTTCTTTTCAGCC	63 ± 1	n.a.	32,542,619–32,542,729
stv-lic_01270	(ACA)5	4	ATCACTCTATGCATCACTTGCAGC	TTCTAACACCATTTCTCTGTCTCAGG	63 ± 1	n.a.	1,807,812–1,807,923
stv-lic_05730[b]	(GA)8	4	TCGTGTTGGGTTCACATAAAGTTG	AGCTTGTAGGAAAATAAGGGTGGG	63 ± 1	n.a.	10,981,326–10,981,423
stv-lic_10896[b]	(AG)7	6	AACCAGAGATGGTGAGGATGAGAG	AGTAAGACACGAACGAGAATTGGG	63 ± 1	n.a.	6,033,435–6,033,535
stv-lic_00456[b]	(CTCA)4	8	GTGTAAAACACAACGACGACGAAG	AAACAGTAAACGAAGCCAAACTGTG	63 ± 1	n.a.	3,303,018–3,303,136
stv-lic_05155[b]	(ATAC)4	8	CGACAAATGCATTCACATACACG	TCTGGTCAACTTTCTTCACAATCG	63 ± 1	n.a.	1,870,175–1,870,265
stv-lic_04717[a,b]	(TTTTG)4	9	GTCAGGTTGGTTCGATGTGTTG	CGCTGCTAGGTCTTTTCTTAGCTG	63 ± 1	n.a.	23,966,831–23,966,902
stv-lic_06125[b]	(CAT)7	9	ATGGAGAATGAATCAGTCGGAGAC	AAACAGGCAAATAATGAGAAAGCG	63 ± 1	n.a.	20,306,236–20,306,329
stv-lic_06873	(TTG)4	10	TGGTTCCATGGAGAATAATAATACGAG	GTAGCGCAATGAAACCAAAGAATC	63 ± 1	n.a.	614,473–614,560
stv-lic_02612[a,b]	(AG)6	10	CGCAGATTGACAGAACAGAGATTG	ACCCAAGTACGCCTTTCCTTTTAG	63 ± 1	n.a.	5,126,978–5,127,074
stv-lic_18234[b,c]	(TACA)6	11	TGAGCTTAAGGCATGATACTTTTCG	CCTTTTAGAGATGCTCAAAGTCTGC	63 ± 1	n.a.	370,123–370,186
stv-lic_06578[b]	(AAC)6	13	GACCAATCCTTCAGAGAAAAAGAAC	TCAGTTGATATGCACCAATTAAAGC	63 ± 1	n.a.	20,475,213–20,475,335
stv-lic_00007	(GA)6	15	TCGTCTTAGGGTTTTCTTCTGCTG	CGAACCACCGTATTATTCCATTTC	63 ± 1	n.a.	6,349,050–6,349,184
stv-lic_16470	(AGT)4	n.a.	CTTCGTCAGTACAAGGAGGAGGAG	AACCACTTCAATGTCCATAGAGCC	63 ± 1	n.a.	n.a.
stv-lic_05167	(TACA)5	n.a.	AACGTTCCAACATGAAACCAAGAC	TAGGGGGCTTTTATAATCAGGACG	63 ± 1	n.a.	n.a.

Abbreviations: RG, reference genotype (alleles in base pairs); n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

[b]SNP/s found in the published genome sequence compared with primer/s sequence/s.

[c]One base insertion/deletion found in the published genome sequence compared with primer/s sequence/s.

TABLE 22 Recommended markers for macadamia (*Macadamia integrifolia*, *M. tetraphylla*, and cultivated hybrids). A reference genotype was not available; linkage groups (LG) are reported according to the reference genome assembly; the macadamia genome assembly is GCA_013358625.1.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (˚C)	RG (bp)	Position of primer within the macadamia genome
MinμS0007[a]	(GA)11	2	CTGATTTATGATGGTAAAGGAC	GGTGAATCAAAGATTAGACAAC	50	n.a.	9,742,771–9,742,862
Mac003[a]	(AT)9	3	TGGACCATTGAGGAGTTGGACTGT	TCCACCGTTTCACTTTCGTCAGCC	60	n.a.	196,058–196,302
Mac007	(CT)11	3	AGGCCTTGGGATGTTCCAGTGTGA	GCAATCAACACAAGCACCTGTGGC	60	n.a.	24,782,250–24,782,598
Mac012[a,b]	(AC)10	3	TATCAGGACCATCAACAATGATTT	GCCTGTTGTAGGTAAAGTGGAGAT	60	n.a.	5,798,944–5,799,231
Mac002[a]	(CT)8	4	CCCAACTGGGTTTGCAAGGACCAA	AGTAGCCGCGAGCTGATCGAAGAT	60	n.a.	18,998,006–18,998,271
Mac004[a]	(AT)11	4	CAAGAGTGTCCAGCGAGGGAATGC	GGGAGACATCATACTTTTGACACATGCC	60	n.a.	1,113,409–1,113,623
Mac006	(AG)11	4	TTTCATCATTGATCATCATAGGTACA	GAGCTAATACTTAACCAGGTGAACA	55	n.a.	16,041,924–16,042,220
Mac010[a,b]	(AG)11	4	GCAACTGGATCAGCACATAAGAAT	TCCGATCATAGTCTTAGCATTTCA	55	n.a.	19,778,855–19,779,100
Mac011	(CT)9	5	AGAGGGCGAGATCCCTGACTCTGA	TGAAATTTGGCGTGGGGAAAGCGT	60	n.a.	12,202,572–12,202,738
MinμS0005[a]	(GA)5(AGA)4	8	GCTTCAAAGACGGACGATCC	GAGGTATGTGTAATTCTCTCC	50	n.a.	34,789,479–34,789,674
Mac008[a]	(AT)10	9	AACGGTTATGTCAAGTGCAACAGGA	TGACTTTAGCCCTCACTTCAAAGCCA	60	n.a.	11,781,049–11,781,414
Mac001	(AT)11	10	GTGACTGGTGGACACCAAAACCCA	GCACTAGGTGTCACCCCCACTTCT	60	n.a.	31,997,714–31,998,104
Mac009[a,b]	(AAG)13	13	CAACTCTCTCTCCCTCAGATTCTC	TAAATCTATGCCACATCACTAGGC	60	n.a.	14,732,668–14,732,883
MinμS0002	(CA)6	n.a.	AGTGGAGAAGTGACTTGCAC	ACAAAGATGGCAATGCGAGG	50	n.a.	n.a.
MinμS0017	(C)13(A)10	n.a.	ACTTAAATGAAGTTTCAGCTAGCCCC	GACTTATACCTCAAAAATAAGAGGTCC	52	n.a.	n.a.
MinμS0020	(CCA)8(CCTCCA)2	n.a.	CACACCACAGACCCCCCA	TCCTCCGATAAGCAAGAGCA	48, 50	n.a.	n.a.
MinμS0029	(GA)27	n.a.	AGTTGCATTCACAGGCTCAC	CGCGTGATGTATATGATCCAG	46, 50	n.a.	n.a.
MinμS0030	(GA)18	n.a.	GCAAGAGCACAATCATCTCATAC	TTCGACTGTCAACCACACCAG	48, 53	n.a.	n.a.
MinμS0047	(GGT)5	n.a.	GGAGAAAGGATGGAGATGTG	TCTGGTTCGGAGAAGTCTAC	48	n.a.	n.a.
MinμS0050	(GA)7	n.a.	GAGCACAATTGCATCAGCATC	TGGAGGGTACAGGTATAGAC	50	n.a.	n.a.
MinμS0052	(CT)4CC(CT)13	n.a.	GAGTGCTTGTCGACGAATTC	CAGGCCCATCTTGTATACTG	48, 50	n.a.	n.a.
Mac005	(AAG)10	n.a.	CATAGCATGAGTTTCAAGGGATAA	ATTACAAACCCACTCTTCGATTT	60	n.a.	n.a.

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

[b]SNP/s found in the published genome sequence compared with primer/s sequence/s.

TABLE 23 Recommended markers for mango (*Mangifera indica* and related species). A reference genotype was not available; linkage groups (LG) are reported according to the reference genome assembly; the mango genome assembly is GCA_011075055.1.

Marker	Core repeat	LG	Forward-Primer	Reverse-primer	AT (˚C)	RG (bp)	Position of primer within the mango genome
MiIIHR26[a]	Complex	1	GCGAAAGAGGAGAGTGCAAG	TCTATAAGTGCCCCCTCACG	55	n.a.	1,969,919–1,970,066
MiIIHR36[b]	(TC)17	1	TCTATAAGTGCCCCCTCACG	ACTGCCACCGTGGAAAGTAG	55	n.a.	1,969,919–1,970,145
MiL20	Complex	1	GGATTGACAAGGAGGGGAAT	ATTTGGCGTTTTGAAACCTG	54	n.a.	27,229,274–27,229,464
MiIIHR15[a]	Complex	2	CTAACCATTCGGCATCCTCT	TCTGTGATAGAATGGCAAAAGAA	55	n.a.	3,088,270–3,088,403
MiL07[b]	(TG)12	2	TCCCACTTGGATAGCATTGA	ATTTGGGTGCTATTCTTGCC	54	n.a.	7,954,357–7,954,652
MiL27	(TG)9	2	AAGACATCATGGCCACTTGAC	CGAAGACCATGGTGGATTA	54	n.a.	10,357,603–10,357,905
MiIIHR17[a]	Complex	3	GCTTGCTTCCAACTGAGACC	GCAAAATGCTCGGAGAAGAC	55	n.a.	11,193,058–11,193,280
MiIIHR24	Complex	3	GCTCAACGAACCCAACTGAT	TCCAGCATTCAATGAAGAAGTT	55	n.a.	1,457,995–1,458,221
MiIIHR31	(GAC)6	3	TTCTGTTAGTGGCGGTGTTG	CACCTCCTCCTCCTCCTCTT	55	n.a.	1,169,213–1,169,422
MiL16[a,b]	(TG)6C(GT)6	3	ATGAGCCGATTGGGCTATTA	TGATCAAATGTGGCTTGCTC	54	n.a.	4,250,604–4,250,815
MiL04	(TG)10	5	TGAGCCCAAATTGAGATTGTC	CTTGCTGTTCTTGCCATCAA	54	n.a.	20,767,232–20,767,397
MiL01[a]	(TG)6	7	TCGCTTATGGCTCCAAGTTT	GGCAATGGTCCTGATGAAGT	54	n.a.	19,755,594–19,755,717
MiIIHR18[a,b]	(GT)12	8	TCTGACGTCACCTCCTTTCA	ATACTCGTGCCTCGTCCTGT	55	n.a.	1,675,561–1,675,709
MiL14	(AC)15	8	TGGTTTACTGTGCACATGCC	CTAGCCCCGAACATGAAGAG	54	n.a.	5,217,368–5,217,579
MiIIHR30	(CT)13	9	AGCTATCGCCACAGCAAATC	GTCTTCTTCTGGCTGCCAAC	55	n.a.	16,833,152–16,833,331
MiIIHR19[a]	(AC)11	9	TGATATTTTCAGGGCCCAAG	AAATGGCACAAGTGGGAAAG	55	n.a.	17,008,579–17,008,748
MiL21[a]	(TAT)4	9	AACCGGAGATGCTGAAATTG	ATTGCAGGAACCATCCTTCA	54	n.a.	17,057,124–17,057,327
MiL29[a]	(ATTCCC)2	13	AATGACAATGGGGGTGAAAA	GTTCGGAGAAAAGTGTGGGA	54	n.a.	2,332,264–2,332,358
MiIIHR23	Complex	18	TCTGACCCAACAAAGAACCA	TCCTCCTCGTCCTCATCATC	55	n.a.	13,125,782–13,125,888
MiL37[a]	Complex	19	TTGGGTATCCTTTGGAGTGC	CAGCCTGAAAATGCAAGAGA	54	n.a.	2,065,280–2,065,589
MiIIHR13[a]	(CCCTTT)3(CTCTTT)6	20	CCCAGTTCCAACATCATCAG	TTCCTCTGGAAGAGGGAAGA	55	n.a.	10,840,807–10,840,971
MiIIHR34	(GGT)9(GAT)5	n.a.	CTGAGTTTGGCAAGGGAGAG	TTGATCCTTCACCACCATCA	55	n.a.	n.a.
MiL08	(ATT)3	n.a.	TCGGTTTCGATTCATAACCTC	AAAGCATCGGTAGTCGGTTG	54	n.a.	n.a.

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

[b]SNP/s found in the published genome sequence compared with primer/s sequence/s.

TABLE 24 Recommended markers for mangosteen (*Garcinia mangostana* and *G. cochinchinensis*). A reference genotype was not available; for the annealing temperature (AT) see Arias et al. (2020); a genome assembly was not available or was not assembled in chromosomes or linkage groups.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (˚C)	RG (bp)
stv-gam00231	(TTG)4	n.a.	GTTGCACTCCTCCGAGGTCAG	TTCTTTTTGATTTCTTGCAGGTGG	63 ± 1	n.a.
stv-gam00278	(GAT)5	n.a.	TTTGGAGTAGCACTTACCAAAGGG	GATTGAATCTTCACCACAACCCTC	63 ± 1	n.a.
stv-gam00546	(CAT)4	n.a.	ATACACCTCATACAACTCCGGCTC	CACAGGGATAGGGATAGGGATAGG	63 ± 1	n.a.
stv-gam00560	(ACT)4	n.a.	GATAAAAGAGGCAATGTGTGAGGG	TGCAACAAAGAAACAACACCACTC	63 ± 1	n.a.
stv-gam00645	(TGT)5	n.a.	AGAAGCTCAAGTCTGTCCTTGGTG	ACTCAGAAGAAGGAATTCCACGC	63 ± 1	n.a.
stv-gam00660	(GAA)4	n.a.	CTAGCCACTCATGGTGGTAAAGTG	TAAAAGCCAGAAAGGAGACTCGAC	63 ± 1	n.a.
stv-gam01553	(CCTCTA)4	n.a.	TGAACCTGCTCTGTCTGCTCTG	TCGAACTGGTGGTAGAGGTAGAGG	63 ± 1	n.a.
stv-gam01788	(TTC)4	n.a.	TCCCCATTTCCATCTCTTAACATC	TTGGATTAATAAAATGGGTGGTCC	63 ± 1	n.a.
stv-gam01820	(TCT)6	n.a.	AGAGAAGACCTGTCGCACATAGG	AGCGACTTGTTAGGGAAAGGC	63 ± 1	n.a.
stv-gam02195	(TTC)4	n.a.	AGAAACAGACCAGAATTGTGAGGG	TTGTGTGATTGCTAGTGTGGATTG	63 ± 1	n.a.
stv-gam02824	(AAT)4	n.a.	CAGTGGTAGCTCGCTCCTAGAATG	ATCTCATCTCTGATCCTCTGGGTG	63 ± 1	n.a.
stv-gam02895	(AAC)10	n.a.	ACAGCCACAATAGTCATCCTCCTC	TTTGGTTGTTTTGATGAGGTTCTG	63 ± 1	n.a.
stv-gam03495	(GGA)6	n.a.	TTCGAGGAAGGATAAGTTGTTTGG	CATAAACCAAACCATCAAAGAACCC	63 ± 1	n.a.
stv-gam03790	(CCA)4	n.a.	CTTCTCAATGATCCCCATGTTTG	AAGGTTTCTTTGCGTTTTGTTTCC	63 ± 1	n.a.
stv-gam03796	(TATG)4	n.a.	GGATGTGAGTGAAGTTAGTGACCG	TATAATCCATCATCACCCATGACG	63 ± 1	n.a.
stv-gam04008	(TTC)4	n.a.	TTCTTTGGTTTCTTGACGCTTAGG	TCATCAACCCCACTAAAACTCCAC	63 ± 1	n.a.
stv-gam04053	(AGA)7	n.a.	TAGACAAGGACAAGTGCAAGTCCC	CTAAGCACTACTTCTGCCAGCCAC	63 ± 1	n.a.
stv-gam04292	(CTC)4	n.a.	ATCATGATCTGCAGCAATATGCC	AGTTACATGAATATGACGACGGGG	63 ± 1	n.a.
stv-gam05115	(ATG)6	n.a.	TTGATGGTAATGTGGGATTTGATG	GAGTCTGTCTTCACATCTGCAACC	63 ± 1	n.a.
stv-gam05474	(TCT)5	n.a.	CAAAGCCACCAACTTACCAAAAAC	TGGTTTTAGAGGATGACGTGTGAG	63 ± 1	n.a.
stv-gam05897	(TCG)4	n.a.	CTCTCACTTCCTCTTCTTGGATGG	ATGATGATGACGATGATGACAATG	63 ± 1	n.a.
stv-gam06047	(ACA)4	n.a.	GAAGGTAGATATGTGGAGCAAGCC	AAATTGAGAGTTTCCCCTTTGAGC	63 ± 1	n.a.

Abbreviations: LG, linkage group; RG, reference genotype (alleles in base pairs); n.a., not available.

mutation and aneuploidy. The genome size was estimated to be $2C = 6.00 \pm 0.17$ pg corresponding to 5.92 Gbp. It is not clear from the literature what the expected number of alleles per marker might be.

3.26 | Oil palm (*E. guineensis* Jacq. and *Elaeis oleifera* (Kunth) Cortés)

The oil palm (*E. guineensis*) is native to west and south-west Africa, occurring between Angola and Gambia. It has been cultivated in several tropical countries outside of Africa, particularly Malaysia and Indonesia, which together produce most of the world's palm oil. The oil is used in food and beauty products and is also used as a biofuel. A second less-important species is *E. oleifera* which is native to Central and South America and more tolerant to disease compared with *E. guineensis* (from the Malaysian Oil Palm Genome Programme (MyOPGP) at the URL http://genomsawit.mpob.gov.my/).

Both oil palm species are diploid ($x = 16$, $2n = 32$) and up to two alleles per SSR marker are expected.

The SSR markers selected (Table 25) have been isolated from *E. oleifera* by different research groups (Sing et al., 2013; Kalyana Babu et al., 2019; Zolkafli et al., 2021) and tested by Chee et al. (2015) on accessions of the same species and hybrids.

3.27 | Olive (*Olea* L. spp)

Olive, although considered diploid, has a high number of chromosomes ($2n = 46$). The genome, which is very large ($1C \approx 1.5$œ1.6 billion bases), could have originated through an interspecific hybridization followed by polyploidization—a hypothesis based on the lack of complementarity among chromosomes of the haploid series. Nonetheless, olive is self-incompatible and, therefore, highly heterozygous.

The primers selected (Table 26) are from the review of Baldoni et al. (2009), to which seven SSRs with 5-

TABLE 25 Recommended markers for oil palm (*Elaeis guineensis*). A reference genotype was not available; linkage groups (LG) are reported according to the reference genome assembly; the oil palm genome assembly is GCA_015461965.1.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (bp)	Position of primer within the oil palm genome
sEg00035	n.a.	1	TTATTGATTGATGCAAGATACAC	TTGATAAAATACAAGAGATAGCA	52	n.a.	20,666,531–20,666,671
mEgCIR03808	n.a.	1	CCGCTAACTTGGTATAC	ATTTCCAGCAGCTAATC	52	n.a.	2,844,229–42,844,442
mEgCIR03376[a]	n.a.	1	CCCTCCCTGCTACCTTCT	TTATGTGAGTGCCTTTGATG	52	n.a.	36,991,420–36,991,614
mEgCIR02595[a]	n.a.	2	TCAAAGAGCCGCACAACAAG	ACTTTGCTGCTTGGTGACTTA	52	n.a.	114,163,709–114,163,938
mEgCIR03428	n.a.	3	GACAGCTCGTGATGTAGA	GTTCTTGGCCGCTATAT	52	n.a.	64,464,144–64,464,286
sMg00042	n.a.	5	CCGAATAGAAGAGGAAAGAATA	AGGTTTGGTGGAGAAGTGTT	52	n.a.	27,529,670–27,529,885
mEgCIR03311[a]	n.a.	5	AATCCAAGTGGCCTACAG	CATGGCTTTGCTCAGTCA	52	n.a.	87,604,070–87,604,678
mEgCIR02492	n.a.	6	CATCAAGCATGACTGCAAGTAA	TTCCGAATTTGGATGAATCC	52	n.a.	75,640,898–75,641,135
sMg00108[a]	n.a.	7	ACGAAACAGAGGCATAGAGACT	ACAATTAAACAGCAACGCTAGA	52	n.a.	60,026,054–60,026,201
mEgCIR00783[b]	n.a.	7	GAATGTGGCTGTAAATGCTGAGTG	AAGCCGCATGGACAACTCTAGTAA	52	n.a.	72,901,704–72,901,983
mEgCIR03389	n.a.	9	GTCCATGTGCATAAGAGAG	CTCTTGGCATTTCAGATAC	52	n.a.	57,049,710–57,049,782
mEgCIR02600	n.a.	9	GGGGATGAGTTTGTTTGTTC	CCTGCTTGGCGAGATGA	52	n.a.	74,353,950–74,354,201
mEgCIR00177	n.a.	10	TGAATGTGTGTGCAATGTGTAT	ATAGTCAATAATCGTAGGAAAATG	52	n.a.	35,768,143–35,768,314
mEgCIR03546	n.a.	11	GCCTATCCCCTGAACTATCT	TGCACATACCAGCAACAGAG	52	n.a.	54,916,197–54,916,458
mEgCIR02332	n.a.	13	GAAGAAGAGCAAAAGAGAAG	GCTAGGTGAAAAATAAAGTT	52	n.a.	23,769,128–23,769,300
mEgCIR00369	n.a.	14	GGGTAGCAAACCTTGTATTA	ACTTCCATTGTCTCATTATTCT	52	n.a.	17,007,774–17,008,031
mEgCIR03298	n.a.	15	GACTACCGTATTGCGTTCAG	GGTTTTGGTTCGTGGAG	52	n.a.	11,717,575–11,717,675
sMg00025	(TC)11	16	GAGGAGGAGGGGAGAAGAGT	AAATACCATTCAGAGAAAGCAC	52	n.a.	4,489,199–4,489,389
mEgCIR03649	n.a.	n.a.	TTTAGAGGACAAGGAGATAAG	CGACCGTGTCAAGAGTG	52	n.a.	n.a.
mEgCIR03544	n.a.	n.a.	AGCAGGGCAAGAGCAATACT	TTCAGCAGCAGGAAACATC	52	n.a.	n.a.
mEgCIR03358	n.a.	n.a.	CCAAGGAACAACATAGA	GTTCCCATCCTATTAGAC	52	n.a.	n.a.
mEgCIR02427	n.a.	n.a.	GAAGGGGCATTGGATTT	TACCTATTACAGCGAGAGTG	52	n.a.	n.a.
mEgCIR00521	n.a.	n.a.	GTGACTTTGGGCTGAAT	ACAGCATCTCCAACTCTATC	52	n.a.	n.a.

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

[b]One base insertion/deletion found in the published genome sequence compared with primer/s sequence/s.

TABLE 26 Recommended markers for olive (*Olea* spp). The reference genotype is "Arbequina" (from De la Rosa et al., 2013) for the Oleagen series, and Baldoni et al. (2009) and http://www.oleadb.it/ for the remainder; for all SSR loci, except for the markers reported in bold (Baldoni et al., 2009), the linkage group (LG) was reported in accordance with the reference genome assembly; the olive genome assembly is GCA_902713445.1.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (bp)	Position of primer within the olive genome
UDO-043	(GT)12	1	TCGGCTTTACAACCCATTTC	TGCCAATTATGGGGCTAACT	52	176/–	n.a.
Oleagen H3[a]	ATGAGG	1	TGGCCATAAGATTGATGATGA	CGATTACCTCGGCCATTCTA	55	197/224	24,175,692–24,175,908
Oleagen-H6	CTCTTC	1	CGGTATCATTTGCAGAGCAC	AGGGGAGTGGGTTGTTGTTA	55	141/168	24,175,768–24,175,931
Oleagen-H2	ACCAGC	2	TCAAAATGATGCAACAACCC	TGCAGGAGCAGAAACATTTG	55	204/216	5,428,123–5,428,312
Oleagen-H4[a,b]	GCTCCG	3	ACACGGAGGATCCAAGTCTG	TGAGCAGGTGGTAGAGGCTT	55	137/154	1,910,950–1,911,072
Oleagen -H21[a,b]	CCTCCA	3	AAGAGTTGTTTCTGCCGCTC	CGCCAAAGCTACACATGAGA	55	289/295	25,257,028–25,257,300
GAPU103 A	(TC)26	4	TGAATTTAACTTTAAACCCACACA	GCATCGCTCGATTTTTATCC	57	150/159	2,778,672–2,778,825
GAPU101[a,c]	Complex	7	CATGAAAGGAGGGGGACATA	GGCACTTGTTGTGCAGATTG	57	182/206	12,205,645–12,205,817
DCA-03[c]	(GA)19	7	CCCAAGCGGAGGTGTATATTGTTAC	TGCTTTTGTCGTGTTTGAGATGTTG	50	232/243	13,734,755–13,734,968
DCA-16[b]	(GT)13(GA)29	8	TTAGGTGGGATTCTGTAGATGGTTG	TTTTAGGTGAGTTCATAGAATTAGC	50	124/146	14,571,828–14,571,927
GAPU71 B	Complex	8	GATCAAAGGAAGAAGGGGATAAA	ACAACAAATCCGTACGCTTG	57	124/144	3,063,375–3,063,479
DCA-14[b,c]	Complex	9	AATTTTTTAATGCACTATAATTTAC	TTGAGGTCTCTATATCTCCCAGGGG	50	191/–	16,733,797–16,733,956
EMO-90[a,b,c]	(CA)10	9	CATCCGGATTTCTTGCTTTT	AGCGAATGTAGCTTTGCATGT	55	188/194	9,994,920–9,995,084
DCA-05	(GA)15	9	AACAAATCCCATACGAACTGCC	CGTGTTGCTGTGAAGAAAATCG	50	202/206	n.a.
DCA-09	(GA)23	11	AATCAAAGTCTTCCTTCTCATTTCG	GATCCTTCCAAAAGTATAACCTCTC	55	184/206	n.a.
Oleagen -H20	GGTGAA	20	TTCAACCAGTCCTCCCAGTC	GTAAGCGAGGGAGAGGCTTT	55	234/246	7,054,977–7,055,204
DCA-18	Complex	n.a.	AAGAAAGAAAAAGGCAGAATTAAGC	GTTTTCGTCTCTCTACATAAGTGAC	50	168/179	n.a.
DCA-17	Complex	n.a.	GATCAAATTCTACCAAAAATATA	TAATTTTTGGCACGTAGTATTGG	50	113/179	n.a.
DCA-07	(AG)19	n.a.	GGACATAAAACATAGAGTGCTGGGG	AGGGTAGTCCAACTGCTAATAGACG	60	147/–	n.a.
DCA-15	(CA)3G(AC)14	n.a.	GATCTTGTCTGTATATCCACAC	TATACCTTTTCCATCTTGACGC	50	246/266	n.a.
DCA-13	(CA)15	n.a.	GATCAGATTAATGAAGATTTGGG	AACTGAACCTGTGTATCTTGCATCC	55	120/124	n.a.
UDO-036	(GT)19(AG)5	n.a.	AACACTGTGCCACCTCAACA	GAACCCAACCCCCATCTTAC	57	n.a.	n.a.

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

[b]SNP/s found in the published genome sequence compared with primer/s sequence/s.

[c]SSR loci LGs were different to those in the linkage map (Baldoni et al., 2009).

nucleotide repeats isolated by the Spanish group of De la Rosa et al. (2013) were added. This selection follows the criterion of preferentially selecting, where possible, SSRs with a long core repeat. At the end of the list, five additional SSR markers, which are among those repeatedly used in the literature (Khadari et al., 2019; Trujillo et al., 2014), were included. Many other published papers support this choice of markers but, for the sake of brevity, are not included.

Markers selected generally amplify a single locus and up to two alleles per locus are expected. However, several subspecies from Madeira Island (*O. europaea* subsp. *cerasiformis*) and North Africa (*O. europaea* subsp. *maroccana*) might be either tetraploid or hexaploid (Besnard et al., 2008).

A new set of SSRs with long core repeats, has recently been published by Li et al. (2020).

3.28 | Papaya (*Carica papaya* L. and wild relatives)

The list of SSR markers selected (Table 27) was extracted from that published by de Oliveira, de Oliveira Amorim et al. (2010) and de Oliveira, dos Santos Silva et al. (2010). Markers were ranked with initial preference being given to markers with a 3-nt core repeat and then by using other criteria such as high expected heterozygosity.

Papaya is diploid ($2n = 18$) (Chávez-Pesqueira & Núñez-Farfán, 2017; Hasibuzzaman et al., 2020) with three flower sex types, that is, males, females, and hermaphrodites, controlled by a single locus with at least three alleles: M1, a dominant allele for male plants; M2, a different dominant allele for hermaphrodite plants; and m, a recessive allele for female plants. All combinations of dominant alleles, that is, MM, MMh, and Mh Mh (where M represents the male and Mh represents the hermaphrodite alleles) are lethal to the zygote (Lee et al., 2018). Several putatively sex-linked genes are under investigation, but associated markers have not yet been published.

3.29 | Passion fruit (*Passiflora edulis* Sims and related species)

The markers selected (Table 28) were chosen for their high expected heterozygosity according to Ortiz et al. (2012) (coded BrPe), Oliveira et al. (2005) (coded PE), and Araya et al. (2017). Priority was given to markers with 3-nt or longer core repeats.

Most passion fruit accessions are diploid ($2n = 18$) and up to two alleles per marker is expected in the analysis.

3.30 | Peach (*Prunus persica* (L.) Batsch and related species)

The authors isolated the first SSR markers in peach (Cipriani et al., 1998) and demonstrated the considerable potential of these markers to profile peach accessions (Testolin et al., 2000). However, those SSRs were isolated from AC- and AG-enriched libraries and suffered the drawbacks of di-nucleotide repeats described in the first part.

The whole sequence of the peach genome was published subsequently (The International Peach Genome Initiative, 2013), providing the opportunity to isolate and test some two hundred SSRs with long core repeats in several stonefruit species and to suggest a new list of SSR markers that would be suitable for fingerprinting those species (Dettori et al., 2015).

A total of 20 markers were selected from the original list which are suggested for peach, almond, apricot, and Japanese plum (Table 1).

Peach, almond, apricot, and Japanese plum are all diploid ($2n = 16$) and up to two alleles for each marker are expected. Peach germplasm has been considerably limited with the introduction, at the beginning of the twentieth century, of Chinese cling peaches from the Shanghai region into the United States of America where they were repeatedly used in breeding as recurrent parents (Scorza et al., 1985). The genetic diversity in western countries is, therefore, narrow (Testolin et al., 2000). However, almost any of the accessions that originated through sexual reproduction (selfing included) that we tested were easily distinguished with the use of as few as 8–10 SSR markers. The 20 markers shown in Table 1 would not be enough for the analysis of pedigree and kinship. In those cases, the literature can offer many other SSR markers to broaden the scope of the analysis.

3.31 | Pear (*Pyrus* L. spp)

The selection of the markers for pear, from among the many hundreds of SSR markers found in the literature, was not easy. After an exhaustive analysis of the literature, we decided to prepare two lists of 12 SSR markers each.

The first list (Table 29) includes 12 SSR markers with long core repeats, the first 10 of which were suggested for a single multiplexed PCR reaction by Zurn et al. (2020). They were demonstrated to distinguish European and Asian pear.

The second part of the list includes 12 markers that are also frequently used in the literature for fingerprinting both European and Asian pears (Baccichet et al., 2020; Bennici et al., 2018; Liu et al., 2015; Montanari et al., 2020; Sehic et al., 2012; Urrestarazu et al., 2015;

TABLE 27 Recommended markers for papaya (*Carica papaya* and wild relatives). A reference genotype was not available; a genome assembly was not available or was not assembled in chromosomes or linkage groups.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (bp)
CP10	Complex	n.a.	AAAAATCACAGCACGTATGGTT	GAAATTACAAATGGGCAAAAAG	58	n.a.
CP53	(AAC)8	n.a.	CAGACACCATGAAGATTTGG	GTGGGTCCTTCTCCTTTGA	56	n.a.
CP03	(AATA)7	n.a.	GAAGGCCCGTGTAAGTGC	TGGTGAAAATTGGAAAGGAG	58	n.a.
CP58	(TTG)7	n.a.	TTCCACGAGACAAACTGTACG	CTCCCACCGCTGTACTTGA	58	n.a.
CP72	(ATAC)7(AT)6	n.a.	CCCAAATCACCTTTTTCTCTC	AACGTGAACTGAGGGTGGA	58	n.a.
CP62	(AT)8(AAG)10	n.a.	ACCTTGGCCCACCACTATT	TTGATTCTGCTTGTGGGAGA	59	n.a.
CP02	(AGG)9	n.a.	AGGCGAAATCGGAAGAGAG	CTGGTAAAACGACGATGACG	59	n.a.
CP61	(AT)12(AC)8	n.a.	GGAGTGATGTAAGTGCCTCAT	CATGAGCCCTACTCCGAAC	57	n.a.
CP94	Complex	n.a.	TCGCAGAGAAAGAGAAAGCA	TCCTCTCACCAGTTATTGGAA	58	n.a.
CP54	(AT)10(ATA)7	n.a.	TTAAGACTATATGGGCCAAGC	TTAGGTCAAGGATGAAACCA	56	n.a.
CP95	Complex	n.a.	CAGTCCTTGCAGGCGATTA	CTCAACCATTTTTCCTCACCA	60	n.a.
CP31	(AT)6(GT)10	n.a.	AAGGGTACGTCATGGAGCA	TCTGTCGCCTTTTATACTCTTG	57	n.a.
CP73	Complex	n.a.	GAAACCGCTCATTTGCATT	TTGATTACTCCCCTCCATCTC	58	n.a.
CP07	Complex	n.a.	CCTAGCATTGCCTTGAGGTC	GCCCACTATTCACATTCACACC	60	n.a.
CP57	(CA)6(TA)7	n.a.	TTGAGTCTTGGTTTCAACTCC	TTCCCACTATCTTCTGTTTGG	56	n.a.
CP100	(GA)11(AG)23	n.a.	TGATCGCTTTCGCTTCACT	GATTTCACTGCCACGGACT	58	n.a.
CP52	(AT)10(AG)12	n.a.	GGAAAGATCATAGAAACAGTGG	TGCTATCTTGGTTGTCTCTCA	55	n.a.
CP16	(AT)13	n.a.	TCAACTATTTCCCCCGCATA	CACCTCCTTGTCCAAAGGTT	60	n.a.
CP71	(CT)14	n.a.	TCCCCAACCTCAAGAAGATAA	TTACACCACCATCGCCATC	59	n.a.
CP51	(AT)16	n.a.	TGAAAAGGACCCACACGTAA	GCAATCGAATCTTTCTTTACCC	59	n.a.
CP35	(TA)12	n.a.	GGACGAAGCTCCACAATCA	GGCAATCAAACCAAATGAGG	59	n.a.
CP49	(AT)12	n.a.	CCTGAAAGCAACCATTTCTA	TCGCTGGAGCTGTAAGAGA	56	n.a.
CP55	(AT)12	n.a.	TCAACCCACTTCGTCTCCA	CAACCCCTTGGGCTATTTG	60	n.a.
CP97	(TA)11	n.a.	TTTCTGTTACCTCTCTCGGATT	GAGATGACAACCATACAGCAA	56	n.a.

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

Velázquez-Barrera et al., 2022). All are included in the list suggested by Evans et al. (2009) and were previously agreed as being suitable by the European Cooperative Programme for Plant Genetic Resources. Most markers in this second set had been isolated in apple and were adopted in pear owing to their confirmed transferability (Liu et al., 2015; Montanari et al., 2020). Pear is mainly diploid ($2n = 34$), but most germplasm collections of European pears contain triploids, which are rather common in ancient cultivars and their offspring.

3.32 | Pecan (*Carya illlinoensis* (Wangenh.) K.Koch)

Pecan, a species of the Juglandaceae, is a large deciduous walnut tree native to North America, which is grown in the United States and in several other countries worldwide.

Pecan is outcrossing (Lovell et al., 2021) and the genome is therefore highly heterozygous. The genome is diploid ($x = 16$, $2n = 32$) (Grauke et al., 2001) and up to two alleles per SSR marker are expected.

The 22 SSR markers reported (Table 30) were selected among those published, giving precedence to those matching the criteria reported above (possibly three nucleotides or longer core repeats, high polymorphism, etc.). The "Ciz" series is from Zhang et al. (2020); the "PM-" series is from Grauke et al. (2003).

Many of the markers on the list were successfully tested by Zhang and coworkers in other pecan nut species, that is, *C. cathayensis, C. dabieshanensis*, and *C. hunanensis* (Zhang et al., 2020).

3.33 | Persimmon (*Diospyros* L. spp)

The first three primers shown in Table 31 are from Guo and Luo (2006, 2008), while the remainder are from Liang et al. (2015) except for the last three that are from Hwang et al. (2010). We would suggest that any screening starts with markers with a long core repeat (>2 nt). All of the primers amplify accessions of *D. kaki* and *D. lotus* very well. Other species of *Diospyros* have only been assayed to a limited extent.

TABLE 28 Recommended markers for passion fruit (*Passiflora edulis* and related species). A reference genotype was not available; markers coded BrPe are from Araya et al. (2017), while those coded PE are from Oliveira et al. (2005). A genome assembly was not available or was not assembled in chromosomes or linkage groups.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (˚C)	RG (bp)
BrPe0002	(AG)12	n.a.	AAAGCCCAGATGAAGTGAA	GGCTCCAATCAGAAGTGT	55	n.a.
BrPe0003	(TC)11	n.a.	CTTTCTCTCCCTATACCC	CCCTCCATAATCACATAAC	55	n.a.
BrPe0006	(TC)10	n.a.	AAGGAAAAGAACAGCCTCA	CGCTCTCAAATCAGTCAAA	55	n.a.
BrPe0010	(TC)9	n.a.	GAAGAAAAAAGGGCTTG	GTTAGGGTTTGGAGGA	55	n.a.
BrPe0014	(AG)7	n.a.	AATATGGCTGGGGAAAAC	TTCCTGTCTTTGGACCTT	57	n.a.
BrPe0021	(TA)7	n.a.	ACTTCCTCATCATTCG	GCTATGCCTCTTTTTG	55	n.a.
BrPe0023	(CT)7	n.a.	AGATACCACACCCAATAG	TTGGAGTTGTTGGGGA	55	n.a.
BrPe0024	(TC)7	n.a.	CCCTACCTTTCTCTGCTT	CATCTCCTCTATCTCCTTC	55	n.a.
BrPe0028	(TA)6	n.a.	CAAAAGGAACAGGGAAGA	GAAAGAGAGAAAGACAGAGA	55	n.a.
BrPe0031	(TA)9	n.a.	AGGTCGGTGGGTGTGTTTAG	CATTCAACTCCCCAAAAGGT	60	n.a.
BrPe0032	(AT)13	n.a.	TTGCACAATGACCAATGTTGT	CTGAGCACCTTGTCAAAATACA	60	n.a.
BrPe0033	(AT)8	n.a.	GCCATGAGAGACTTGGGAGA	CGGTTGCCAAAAAGAAGAGA	60	n.a.
BrPe0036	(TC)6	n.a.	TCGGACCTTAAAACCGAGAA	CAGCACCAAAATTTGACGAG	60	n.a.
BrPe0037	(TG)6	n.a.	TGATAATGCAGCGAAAGAGC	TCACACTCCATTTGCTCTGC	60	n.a.
BrPe0038	(AT)6	n.a.	TTTCAACTTTTCGTGTGTGC	TGTTGTTGCTTGGAAGGATG	60	n.a.
BrPe0042	(AT)8	n.a.	CATGCATTCATTTGTTTTTCTTG	GATGCTGGGAAAAAGAGTGC	60	n.a.
BrPe3011	(TTC)4	n.a.	CCGGTCTTCCTGATTGACTC	CCTCTCTCACCTGGAACTGC	60	n.a.
BrPe3012	(TCT)4	n.a.	CGCCCTTTCTGAAGATAATCC	GCAATGCTAAGAAGGCCAAG	60	n.a.
BrPe3014	(AGA)5	n.a.	CGGAAGCGTGCTCATAAAGT	AAGCCTGTGAGGTTGATTCG	60	n.a.
BrPe3027	(GGT)4	n.a.	CCAAAATGCCCAAAATGTCT	GTCCGTGAGGAGATGTCGAT	60	n.a.
PE01	(GT)7	n.a.	CAGGATAGCAGCAGCAATGA	AGCCAAATGTCAAACTGAAC	54	n.a.
PE07	(AG)22	n.a.	TGCTCATTGATGGTGCTTG	TCGTCTCTTCTCCTCCTTCA	52	n.a.
PE08	(GTTGTG)4	n.a.	TCTAATGAGCGGAGGAAAGC	CCGGATACCCACGCATTA	54	n.a.
PE10	(AG)16 complex	n.a.	AACCTTGATCTCCAGCCTAT	GTTTTCGCCCGCGTATT	57	n.a.

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

D. lotus is diploid ($2n = 30$), *D. virginiana* tetraploid ($2n = 60$), while *D. kaki*, the cultivated persimmon, is mainly hexaploid ($2n = 90$). Therefore, while assaying germplasm of cultivated table persimmon, up to six alleles per marker could be expected (Wang et al., 2021).

3.34 | Pineapple (*Ananas comosus* (L.) Merr. and several botanical varieties)

The 16 SSR markers selected by Nashima et al. (2020), from among the 160 SSR markers isolated by genome sequencing and tested on a panel of 25 pineapple accessions, are shown in Table 32. These markers have 3- to 6-nt long core repeats, while 2-nt markers were excluded by the authors from their use in genotyping for the reasons reported in Section 2.2.4 above. Nashima et al. (2020) also reported several sets of three markers each that were suitable for identifying all accessions within the panel and, because the three SSR markers amplified different ranges of PCR fragments, a single multiplexed PCR reaction was easily carried out. We were unable to search the microsatellite marker database, PineElm_SSRdb (Chaudhary et al., 2016) and did not consider SSRs with short repeats (Rodríguez et al., 2013).

Pineapple has 25 chromosomes, out of the expected 28 that would have originated from the polyploidization of the seven ancestral chromosomes, according to the results of genome sequencing (Ming et al., 2015). The profiles at the 16 markers selected for genotyping always show one or two alleles per marker.

3.35 | Pistachio (*Pistacia* L. spp.)

Among the many hundred SSRs isolated by different techniques and available in the literature (Albaladejo et al., 2008; Khodaeiaminjan et al., 2018; Motalebipour et al., 2016; Pazouki et al., 2010; Topçu et al., 2016), we concentrated on the few series that have tested a significant number of *Pistacia vera* genotypes. This selection was consequently limited

TABLE 29 Recommended markers for pear (*Pyrus* spp). The reference genotype is "William" (from Urrestarazu et al., 2015); the primers in the first list were transcribed either from the GDR database or from Zurn et al. (2020); those in the second list are from the GDR database; *common to both lists; M13 and PIG-tails, if present, were removed; the annealing temperature (AT) varies in the literature from 55 to 60˚C and touch down (td) protocols with an initial temperatures of 62 or 60˚C have been considered by some authors for an AT of 55˚C. A genome assembly was not available or was not assembled in chromosomes or linkage groups (LG). LGs are reported according to the GDR database (Liu et al., 2015; Urrestarazu et al., 2015; Velázquez-Barrera et al., 2022).

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (˚C)	RG (bp)
First list						
Ch01d08 *	(GA)n	3/15	CTCCGCCGCTATAACACTTC	TACTCTGGAGGGTATGTCAAAG	60	245/281
NAUpy26a	(TGTTAT)3	3	GCTAAACAAGATAAGCGACGAG	TCCAAATGTTTCCATCGTGTAAA	55	n.a.
CH04e03 *	(GA)n	5	TTGAAGATGTTTGGCTGTGC	TGCATGTCTGTCTCCTCCAT	60	179/204
TsuENH046	(TTC)5	6	GGTCATCACCCACTTAAAAACCA	GTGCCCTGAAGTAATTGAGATGG	55	n.a.
TsuENH089	(CCACCG)2(CCA)4	7	TTCACTGCCCTTTTTACGTATGC	CCCCGACAATCTGTAGAGAATCA	55	n.a.
TsuENH083	(ACC)6	11	ACTCTCCGCAAAACAATGTCGTA	TGTGAGAGTTTGAGGAGGAGAGC	55	n.a.
TsuENH076	(ACG)6	11	CATTAATACGCTGCTGTTTCTGC	ACTTGAATTGGGGTAGGGATTGT	55	n.a.
NAUpy40d	GATCAT	12	ATCCCAGCATTTCCCAAGATTTT	CCGTCCGTGATACCTCTGTC	55	n.a.
TXY276	(CAGCT)5	n.a.	CCCTACAGAGTCATGCATCC	TTGATGCTGGAGACGAGAAA	58	n.a.
TXY144_347	GAG	n.a.	GTTTCCTGAAACTTGCCAATTGAAAC	TCTGCTACTTTCAACCCCATCT	62 td	n.a.
TsuENH080_354	(CCA)n(CCT)n	n.a.	GCTACCACAACAAGCTCAACAG	TATGTCATCCTGCTGATGTCC	62 td	n.a.
TXY185	(CAG)7	n.a.	GGAGGACCAACAGCAACATT	AGCAAGATCAAGCCAGGTGT	58	n.a.
Second list						
CH02b10	GA	2	CAAGGAAATCATCAAAGATTCAAG	CAAGTGGCTTCGGATAGTTG	58	122/128
Ch01d08 *	GA	3/15	CTCCGCCGCTATAACACTTC	TACTCTGGAGGGTATGTCAAAG	60	245/281
CH03g07	GA	3	AATAAGCATTCAAAGCAATCCG	TTTTTCCAAATCGAGTTTCGTT	60	227/243
CH04e03 *	GA	5	TTGAAGATGTTTGGCTGTGC	TGCATGTCTGTCTCCTCCAT	60	179/204
CH03d12	Complex	6	GCCCAGAAGCAATAAGTAAACC	ATTGCTCCATGCATAAAGGG	60	108/125
EMPc117	(CT)17	7	GTTCTATCTACCAAGCCACGCT	CGTTTGTGTGTTTTACGTGTTG	55	88/115
CH01f07a	CT	10	CCCTACACAGTTTCTCAACCC	CGTTTTTGGAGCGTAGGAAC	55	177/184
EMPc11	(AC)13	11	GCGATTAAAGATCAATAAACCCATA	AAGCAGCTGGTTGGTGAAAT	55	150/–
Ch01d09	GA	12	GCCATCTGAACAGAATGTGC	CCCTTCATTCACATTTCCAG	58	152/160
GD147	(AG)7	13	TCCCGCCATTTCTCTGC	AAACCGCTGCTGCTGAAC	55	125/–
CH05c06	GA	16	ATTGGAACTCTCCGTATTGTGC	ATCAACAGTAGTGGTAGCCGGT	60	88/92
GD96	(TC)22	17	CGGCGGAAAGCAATCACCT	GCCAGCCCTCTATGGTTCCAGA	55	n.a.

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

TABLE 30 Recommended markers for pecan (*Carya illlinoensis*). A reference genotype was not available. For all SSR, the loci linkage group (LG) was reported in accordance with the reference genome assembly; the pecan genome assembly is GCA_018687715.1.

Marker	Core repeat	LG	Forward-primer	Reverse-pPrimer	AT (°C)	RG (bp)	Position of primer within the pecan genome
Ciz074[a]	(TTA)6	1	TGCCGTCATAGGAAGAAAGG	AAAGCCATTTGGCACGTTAG	60	n.a.	46,874,134–46,874,295
PM-GA41	(CT)9	1	TCTTCAGAAAAAACCCTTACCTCTCT	G(A)5TATAAACTCCCATAGTACCCACAT	56	n.a.	12,586,896–12,586,955
Ciz022[a]	(AG)10	1	CGTTAGGTTCTCTCCGCAAG	CGTCAAACGGAGAATCCTGT	60	n.a.	10,657,562–10,657,774
PM-CIN22[a]	(CTT)10	2	TTACTTTTGGATTATTTGTATCATATCTTCT	CCAACAAGGGAAGCCAACTT	54	n.a.	16,657,879–16,657,945
Ciz040[c]	(AGAA)5	2	TACGCATACCCACACACACA	CAGTTCAGTTGCCTATGCTTTG	60	n.a.	2,673,661–2,673,866
Ciz071[b]	(TCT)6	3	CAAAGAGGCGAATCCATGTT	AATAAATAGTCGGCGGGTCC	60	n.a.	3,950,086–3,950,334 3,948,205–3,948,451
PM-CIN13	(GAA)14	3	CCGCAGATGGTTTGAAGAA	ACAAATTCCTCACTCCGGAG	54	n.a.	4,435,251–4,435,348
Ciz003[a,c]	(ATA)8	4	TCTGTAGTGGATGCTAAATGCAA	GCATTGGCATTGGTTTCTCT	60	n.a.	6,806,800–6,806,999
PM-CIN4	(CTT)12	5	GGCATCAGAGAAGGCTCCT	CTCACCCGTCTCTAGGGCTA	57	n.a.	24,681,969–24,682,061
Ciz070[c]	(TA)7	5	TGCTTCCCAGCCAATACTCT	CCGTGGGATTTAGTGAATGG	60	n.a.	46,301,787–46,302,040
Ciz043[a,c]	(TA)10(CA)6	5	GTTGCAAGCATAACTTGTAACCA	TCAAACCAAACCAACAAGCA	60	n.a.	27,540,184–27,540,422
Ciz052	(A)14(AAT)5	6	CCTCATCGAAACAAGCTTCC	TGCAACACTGCATGTCACAC	60	n.a.	27,555,625–27,555,824
Ciz055[c]	(AAT)11	7	TGCTTTAGTGACCAACGGAA	TGCTTCTGCTTCTCCTCCTC	60	n.a.	41,465,459–41,465,700
Ciz072	(GGC)6	7	AATGGGAATGGTTCAAGTGC	GGTGAGCAAGTGGCTGATCT	60	n.a.	4,627,286–4,627,544
PM-GA38	(CT)12	7	AAAAGTTTTAGGGTTGTTTGCTCTCT	GT(A)3GCCTACAACCTACAACAGTCTATG	56	n.a.	40,845,804–40,845,864
Ciz073	(ATT)7	8	GGGATATGCATGTGCTTATGTT	AGATCTCCGATCGAGGTTGA	60	n.a.	9,901,713–9,901,967
Ciz047[c]	(GAG)6(GAA)5	8	TGGGAAGAGGATTGTTGCTC	CCTCATCCGCATCTTCTTCT	60	n.a.	33,954,545–33,954,763
Ciz059	(TA)8	9	AATAAGCGCCTTGTTCCTGA	ATGCAATAATGGATCCCTCG	60	n.a.	7,230,144–7,230,382
PM-CIN27[c]	(CTT)13	10	CCCCAACTCAATTACAACCTCTTC	TGTTCATTCTGCACACACACAA	55	n.a.	292,374–292,418
Ciz039	(TTA)6	11	TGTGTCGTGCAACGTTTGT	TCTTCGTAGAATGACGCTTCC	60	n.a.	36,172,802—36,172,981
Ciz058	(T)10(TTAA)6	15	TCAATCAATGTCGTTTGGGA	AACGAGACCCAATGTCGAAC	60	n.a.	9,754,440–9,754,655
Ciz045	(AC)6(AT)8	n.a.	ATCCATGTCCAGGAAGCAAC	ACGGTGCAGTGGTGTTGTAA	60	n.a.	n.a.

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

[b]Potential primer pairs multiple alignment within the published genome sequence.

[c]SNP/s found in the published genome sequence compared with primer/s sequence/s.

TABLE 31 Recommended markers for persimmon (*Diospyros kaki*, *D. lotus* and wild species). A reference genotype was not available; the DKMPx primers are from Guo and Luo (2006), the markers of the ssedk series are from Soriano et al. (2011), the remaining markers are from Liang et al. (2015); the annealing temperatures (AT) below 55˚C were changed to 55˚C by other authors; touch down (td) protocols, with initial temperatures of 60˚C and −0.5˚C decrease for 10 cycles have also been considered (Hwang et al., 2010). Linkage group (LG) was reported in accordance with the reference genome assembly; the persimmon genome assembly data were released in the form of the PersimmonDB (http://persimmon.kazusa.or.jp).

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (˚C)	RG (bp)	Position of primer within the persimmon genome
8125[a,b]	(GGC)4	1	TTATCCCATCAAAGCAACCCAC	CTGCCAACTTCTTCTCCATCTCC	55	n.a.	28,878,635–28,878,801
5553[b]	(GTAGTG)3	4	CCAGTTGATGGCAATGGGAGGC	GGTGCGATGTTGGAGGGAAGAG	56	n.a.	6,118,647–6,118,848
ssrDK11[a]	(GA)16	4	ATGTTTCAGGGGTTCCATTG	TCACTCGTCTTTGCCTTTCC	60	n.a.	12,984,122–12,984,241
ssrDK29[c]	(CCTTT)8	5	ATCATGAGATCAGAGCCGTC	CACGTTAACGTTACGGAACA	57	n.a.	1,299,159–1,299,237 1,393,316–1,393,394
4379[a,b]	(GAG)9	7	TGACTCTGCTCCACAGGCACTTC	CTCGTCTGGCAATTCTGCTTCG	56	n.a.	3,190,090–3,190,266
1554[a]	(CAT)6	12	CACCGCATCCTCTTCGACATCC	ACGCATCCGTCAAATCACAACA	56	n.a.	10,588,552–10,588,711
9004[a,b]	(GCAGGA)3	12	GCCACAAACTTCACAGAGGACC	AGGCGAGTGCGAGTAAGACGAA	55	n.a.	32,890,280–32,890,513
DKs76[a]	(AGG)7	12	TCGGCTTCACCTATGTTG	CGATTCCTTGGACCTTTG	52	n.a.	28,117,816–28,117,913
DKs91	(AG)7	12	CGGAAGAGGGAGAAATCG	GAATCGGGAAAGCAAGTT	55	n.a.	2,603,242–2,603,399
8917[a,b]	(AT)10	13	ACACGTTCAGTACCAGGAGGGA	AGTACCACAAACCACCAGTGG	55	n.a.	1,804,610–1,804,745
ssrDK12	(GA)14	15	AGATGGAGTGACAGAGACTG	CCCCTTAAGTCTTAGCTAATTAC	53	n.a.	23,325,054–23,325,178
ssrDK25	(CT)15	n.a.	GGGGTAATATGAATTGAATC	CTCAGAGAGGAGAAGAAATAG	50	n.a.	n.a.
ssrDK14	(AG)16	n.a.	GTGAAGGAACCCCATAGAA	CCATCATCAGGTAGGAGAGA	55	n.a.	n.a.
6615	(CTT)7	n.a.	ACACTCCACTCTACCCAAATACC	GACATCATAAGTCAAAGCACGAA	55	n.a.	n.a.
mDp17	(GA)21	n.a.	CCAAATCATTCGAAGCCAAT	CCTTCACCGATGTCCTTTGT	52	n.a.	n.a.
DKMP1	(GCCA)10	n.a.	GGGTATCCTTGCCTGCTC	CGAACTGGTTGGTGACGG	50	n.a.	n.a.
DKMP2	(TCCG)5	n.a.	GGGTAATCTTGCCTGCTC	CTTGCTGACTCTTGGGTGT	50	n.a.	n.a.
DKMP3	(GCCA)5	n.a.	ATGTGCGTTGAAAGTGTCG	GAGGGTTGTGAAATGGAAAG	50	n.a.	n.a.
1430	(GAG)5	n.a.	TCAGTAAAGCTGCGGGCATC	ACGGTTCTCCTGATCCTCACG	56	n.a.	n.a.
6665	(TA)9	n.a.	TGACCAACCCCAAAGTGTGGGAG	AGGTCCCTCTGGTGAGCACATGC	60	n.a.	n.a.
ssrDK16	(GA)12	n.a.	ACTACAACGGCGGTGAGAAC	GTCCTTCACTTCCCGCATT	53	n.a.	n.a.
ssrDK31	(CT)15	n.a.	AGTTCTTGCGATGGGATTTG	GATGAGATGGGCTGATTGCT	60	n.a.	n.a.
ssrDK30	(TG)9(AG)17	n.a.	TGGTGATCGTGGTAGTGGTT	GGCCTAATCTCTGTCCATCC	58	n.a.	n.a.

Abbreviations: RG, reference genotype (alleles in base pairs); n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

[b]SNP/s found in the published genome sequence compared with primer/s sequence/s.

[c]Potential primer pairs multiple alignment within the published genome sequence.

TABLE 32 Recommended markers for pineapple (*Ananas comosus* and wild relatives). The reference genotype is "Seijo Cayenne" (from Nashima et al., 2020); For-primers: 5′-gctacggactgacctcggac fam dye label; Rev-primers 5′-gtttctt PIG-tail; each SSR marker was matched to the corresponding pineapple genome sequences: GCA_001540865.1 (Ming et al., 2015) and GCA_902162155.2 submitted by the Fujian Agriculture and Forestry University in 2019. Linkage groups (LG) and positions matched between the two versions except for the TsuAc284 marker. TsuAc284 primers align on LG1 in the case of GCA_001540865.1 and on LG24 in the case of GCA_902162155.2.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (bp)	position of primer withinthe Pineapple genome
TsuAc284[a]	(AATA)6	1	GGCCCCAGAAATAGTGTTGAT	CCATAAATTGTGGCCTGTGAA	55	178/–	22,857,399–22,857,531
		24					12,330,953–12,331,085
TsuAc244	(TTA)11	3	TATGCCTGAACCAAAATCAGG	ATACCCTCATTCCCCACAAAT	55	202/205	15,806,250–15,806,403
TsuAc336	(CGGTGA)5	4	AAATGTCAAGCGATGCTTCTG	TCTACGCATCCGTATAGCAAA	55	225/–	15,362,079–15,362,255
TsuAc300[a]	(AACCC)6	4	GAATCTCAACTGGGTCGAGG	AGTTTGAAAGCGAGAGAGCG	55	294/302	14,092,919–14,093,175
TsuAc313[a]	(AAATA)5	4	AGGGGAATTAGGGTTTTAGGG	CAATCCCATGCATAGTGAACC	55	176/–	3,790,420–3,790,554
TsuAc335[a]	(CACGCA)6	6	AATTCCCAGTTGAGAAATCGAG	TGTTTACGGTGTGTTTGTTCG	55	235/241	909,173–909,362
TsuAc317[a]	(ATAAA)5	7	GGGGAACTCAAAATGTCCTTG	GCAGCTGCACAACAACTCTC	55	186/–	14,698,116–14,698,256
TsuAc235[a]	(AAT)5	7	GACACCGTAATCCGTTGTAAA	CTACGGCCAATACAAACTCCA	55	230/–	5,364,189–5,364,373
TsuAc341[a]	(GCTCGT)5	10	GGACAACATATTTCGCAATGG	TCTGCGAGACTGGAAGAAGAG	55	179/181	169,459–169,590
TsuAc278	(ATTA)5	10	CTCTTCGATCCCGATCTCTCT	GAGCAACAACCCACAAAGCTA	55	176/191	219,299–219,429
TsuAc342[a]	(GAGAGG)5	15	GAGAAGCGACTGCGATTTAGA	GACGAGGAGGAAGAGGAACC	55	179/192	11,002,607–11,002,739
TsuAc334	(CACTAC)5	15	CTCGCCTTACATGACTGAAGC	TGTCTCGTTCCGATCTCAATC	55	214/–	291,263–291,430
TsuAc269	(TGTA)6	17	CCCTTGTTCTTTGCCACAAT	CAGTCGCGTTCATGATTTGT	55	309/323	4,470,409–4,470,670
TsuAc299[b]	(CTCAT)6	17	TTTGCCATGTGAACCAGTGT	GCATGCATATTAAATGGGGG	55	130/–	3,464,873–3,464,958
TsuAc319	(CATCC)5	18	TACAGGGCCAGAAGAGTTGTC	GCTTCGCATATCTCTCTCGTG	55	168/174	6,494,349–6,494,489
TsuAc346[a]	(CACACG)5	20	TAGAATCCTCACCCCCTCAAT	GTACCAAATTCCACACCGAGA	55	157/182	9,467,768–9,467,900

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

[b]SNP/s found in the published genome sequence compared with primer/s sequence/s.

TABLE 33 Recommended markers for pistachio (*Pistacia vera* and related species). A reference genotype was not available; the markers coded CUPVDxxx are from Topçu et al. (2016), while those coded CUPVSiirtxxxx are from Ziya Motalebipour et al. (2016). A genome assembly was not available or was not assembled in chromosomes or linkage groups.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (bp)
CUPVD408	(GAA)8	n.a.	AGTCCCACCACTTCCTTATC	CACATTCACATTCATCTTCATC	58	n.a.
CUPVD538	(ATCTTC)4	n.a.	AACCAACCCAGTGACAAAAC	GATGAAGAATGGAGATGATGTG	58	n.a.
CUPVD562	(TTC)7	n.a.	TCCTTTGGATGGTAGTTGC	TGATGGTTCTTGTGTTGAATAG	58	n.a.
CUPVD661	(TTC)6	n.a.	TTCGTAACCAATAAGGGTCTG	TATCGGATTAGGAGTGATGATG	58	n.a.
CUPVD544	(AAG)5	n.a.	GTGGAAGAAAGGGTTGTAACG	CCCACTCACTGAGCAATTCT	56	n.a.
CUPVD609b	(AAG)6	n.a.	GAGGCGATAGAGATGTTTCAC	AACCCTTTCTTCTTCATTTACC	56	n.a.
CUPVD659	(AAG)7	n.a.	CAGGAAACTCGGTAATAGCAC	CAGTGGGAAAAAGCACTTATC	58	n.a.
CUPVD501	(TTG)10	n.a.	CCGATTCTGTGAGAGTATTTTC	AACCACTATCATCCGACTACAC	51	n.a.
CUPVSiirt357	(CACATC)5	n.a.	CAGTGCATATTCGGTGGACTT	AGTGAGGCTAGAATGCCCGTA	58	n.a.
CUPVSiirt876	(TTA)12	n.a.	TACCCTGGTAATGCTCGATCT	CAGGAACCAGCAGAAACAAAA	58	n.a.
CUPVSiirt847	(TAT)11	n.a.	CTTGGCTACATCTCCCCTCTT	ACGACCACAAGGAAAGTGGTT	58	n.a.
CUPVSiirt349	(CAGACT)4	n.a.	CAAACCCAGACATTGTGGACT	CATTGGTTGCATGCTTCTTCT	58	n.a.
CUPVSiirt22	(AAG)8	n.a.	CCGTGCCACAATTAATCCTAA	CGCGATGAATAGCAAGATTTC	58	n.a.
CUPVSiirt271	(ACC)9	n.a.	GATTCCCGCAGTTAAAGCAC	CACTTGGCCAGGGACTGTA	58	n.a.
CUPVSiirt95	(ATA)8	n.a.	CACCCTCGTAACCAAATACCC	TCTGAGATTGTTGCCTGTATTC	58	n.a.
CUPVSiirt764	(ATGGAA)6	n.a.	ATCATTCCAAGCGTGCTTATC	GAGAAGGTTGAATAAAGGGTGA	58	n.a.
CUPVSiirt743	(AAAATC)5	n.a.	TAAGGGGTGGCCAAGAATTAC	CGGTTTTTAGGGCTAGGAGGT	58	n.a.
CUPVSiirt1784	(TAA)9	n.a.	TCAAGATCCTCATATTCCTAGCC	CCATTATCAGATGCCCTGTGT	58	n.a.
CUPVSiirt543	(ATA)11	n.a.	CTTTAGTGTTTGGGCCAGCTA	GAATTCGGCAGACATAGACGA	58	n.a.
CUPVSiirt889	(AAT)9	n.a.	CTCATTGCCATGCATTACTGTT	TTGCATAGGACTACGAGAAAGC	58	n.a.
CUPVSiirt37	(TTA)10	n.a.	TCAGGACAGTGGTGAAGGAGT	CTTTGGCTTCTCCAAGATTCA	58	n.a.
CUPVSiirt794	(AAAG)6	n.a.	CGGATGCTCCTAAAAGTCTTGA	TGCTTCATACCGGTTCTTGAT	58	n.a.

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

to a set of 64 SSRs developed and tested on twelve *P. vera* cultivars by Topçu et al. (2016) and a set of 204 SSRs developed and tested on 24 *P. vera* cultivars by Motalebipour et al. (2016).

According to the criteria of SSR selection described above (single locus, core repeats of 3-nt or longer, high polymorphism, etc.), the SSRs from these two studies were ranked according to expected heterozygosity and the effective number of alleles, and we selected the first 22 from the compiled list. All of these SSRs had three to six nucleotide-long motifs (Table 33).

Pistachio is diploid ($2n = 30$) and up to two alleles are expected for each marker. Being mostly dioecious and therefore outcrossing, genetic diversity could be high and genotypes should be easily differentiated from each other.

3.36 | Plum (*Prunus salicina* Lindl. and related diploid species and *Prunus domestica* L.)

The two main cultivated plum species are the Japanese plum (*P. salicina* Lindl), diploid ($2n = 16$) and several related diploid interspecific hybrids and the European plum (*P. domestica* L.), which is hexaploid ($2n = 6x = 48$). Diploid genotypes are easy to genotype (Guerrero et al., 2021; Mnejja et al., 2004)

In Dettori et al. (2015), we found that Japanese plum is easily genotyped with the SSR markers isolated from peach, providing high transferability between the two species. We suggest, therefore, that the panel of markers isolated in peach is suitable for Japanese plum (Table 2).

The European plum, being hexaploid, is more difficult to genotype. However, a set of SSR markers suggested by several authors (Gaši et al., 2020; Nybom et al., 2020; Sehic et al., 2015; Urrestarazu et al., 2018) was tested with success in European plum (Table 34).

In our opinion, when dealing with polyploidy, rather than trying to define the dosage of each individual allele (as suggested by Nybom et al., 2020), recording just the presence/absence of alleles is easy and accurate (as suggested by Urrestarazu et al., 2018). For further insight to this subject, see the discussion on polyploids in Section 2.8.1 above.

Finally, European plum shows limited genetic diversity, with the presence of only one major haplotype, likely because

TABLE 34 Recommended markers for plum (*Prunus domestica*, hexaploid). See Table 2 for diploid plum (*P. salicina* and related interspecific hybrids). The reference genotype is "Stanley" (from Nybom et al., 2020); (a) the REVERSE-PRIMER reported in Nybom et al. (2020) for the marker UDP96-005 is not correct. For all SSR loci, the linkage group (LG) was reported in accordance with the GDR database and Urrestarazu et al., 2018. A genome assembly was not available or was not assembled in chromosomes or linkage groups.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (bp)
UDP96-005	(AC)16 & (CT)11	1	GTAACGCTCGCTACCACAAA	CACCCAGCTCATACACCTCA(a)	56	102/104/111/113/136
CPPCT-029	(CT)24	1	CCAAATTCCAAATCTCCTAACA	TGATCAACTTTGAGATTTGTTGAA	57	n.a.
BPPCT034	(GA)19	2	CTACCTGAAATAAGCAGAGCCAT	CAATGGAGAATGGGGTGC	56	215/223/237
BPPCT-039	(GA)20	3	ATTACGTACCCTAAAGCTTCTGC	GATGTCATGAAGATTGGAGAGG	58	126/132/136/138/152/177
BPPCT007	(AG)22	3	TCATTGCTCGTCATCAGC	CAGATTTCTGAAGTTAGCGGTA	60	123/127/133/139/147
UDP96-008	(CA)23	3	TTGTACACACCCTCAGCCTG	TGCTGAGGTTCAGGTGAGTG	55	n.a.
CPSCT-005	(CT)15	4	CTGCAAGCACTGCGGATCTC	CCCATATTCCCAACCCATTA	62	n.a.
pchgms2	(CT)24	4	GTCAATGAGTTCAGTGTCTACACTC	AATCATAACATCATTCAGCCACTGC	57	n.a.
BPPCT040	(GA)14	4	ATGAGGACGTGTCTGAATGG	AGCCAAACCCCTCTTATACG	58	126/128/146
BPPCT014	(AG)23	5	TTGTCTGCCTCTCATCTTAACC	CATCGCAGAGAACTGAGAGC	58	186/204/222/232/238
BPPCT-025	(GA)29	6	TCCTGCGTAGAAGAAGGTAGC	CGACATAAAGTCCAAATGGC	58	n.a.
UDP98-407	(GA)29	6	AGCGGCAGGCTAAATATCAA	AATCGCCGATCAAAGCAAC	58	190/194/203
UDP98-412	(AG)28	6	AGGGAAAGTTTCTGCTGCAC	GCTGAAGACGACGATGATGA	58	n.a.
CPSCT-026	(CT)16	7	TCTCACACGCTTTCGTCAAC	AAAAAGCCAAAAGGGGTTGT	46	165/182/193/200
CPPCT-033	(CT)16	7	TCAGCAAACTAGAAACAAACC	TTGCAATCTGGTTGATGTT	57	n.a.
UDP97-402	(AG)17	7	TCCCATAACCAAAAAAAACACC	TGGAGAAGGGTGGGTACTTG	57	n.a.
PacA33	(GA)16	8	TCAGTCTCATCCTGCATACG	CATGTGGCTCAAGGATCAAA	58	180/188/198
UDP98-409	(AG)19	8	GCTGATGGGTTTTATGGTTTTC	CGGACTCTTATCCTCTATCAACA	57	n.a.

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

of the limited number of accessions transferred into Europe from Western Asia during the period when plum moved westward (Urrestarazu et al., 2018).

3.37 | Pomegranate (*Punica granatum* L. and wild relatives)

A set of agreed SSR markers does not appear to have been published for pomegranate. Consequently, we collected markers isolated and/or evaluated by Currò et al. (2010), Hasnaoui et al. (2010, 2012), Pirseyedi et al. (2010), Soriano et al. (2011), Parvaresh et al. (2012), Ravishankar et al. (2015), Zarei and Sahraroo (2018), Patil et al. (2020) and Gunnaiah et al. (2021) and those from a few other relevant papers. Among these markers, we selected and ranked 24 SSRs (Table 35) according to the criteria described in the general section above (single locus, polymorphism, long core repeats, etc.).

Pomegranate is diploid ($2n = 16$, occasionally $2n = 18$ in double flowered cultivars) and is self-compatible with a low rate of outcrossing (13%). This would explain the low genetic variability found in germplasm collections worldwide and is the reason for including as many as 24 SSR markers in the list shown (Table 35).

3.38 | Quince (*Cydonia oblonga* Mill.)

Quince has 34 chromosomes, like apple and pear, and like the sister genera behaves like a diploid.

The first nine SSRs within the chosen list (Table 36) are from Bassil et al. (2015); a further nine, of which four were isolated from apple and five from pear by different authors, were assayed in quince by Azad et al. (2013).

According to a number of published studies, additional SSR markers that have been isolated from apple and pear also work with quince (see, for example, Xuan et al., 2013; Yamamoto et al., 2004; Yüksel et al., 2013).

3.39 | Rambutan (*Nephelium lappaceum* Lour.)

A set of 20 highly polymorphic SSR markers, based on heterozygosity and number of alleles per sample, were selected for diploid rambutan ($2n = 32$) from among those listed by Arias et al. (2020). The first markers on the list have 3-nt or longer core repeats (Table 37).

3.40 | Raspberry (*Rubus idaeus*)

The first eight SSRs shown in Table 38 are ranked from Fernández-Fernández et al. (2011) and are organized for multiplexing; the reminder are from Castillo et al. (2010). This set of SSRs was used by Girichev et al. (2017) to genotype 76 raspberry and three blackberry accessions.

Raspberry is diploid ($2n = 14$) and is easy to genotype.

3.41 | Blackberry (*Rubus fruticosus* and aggregated species)

Blackberry is a complex botanical taxon and, although close to raspberry, it was convenient to consider it separately because of the number of aggregated species, the hybridization that has occurred and apomixy. These characteristics make it difficult to genotype blackberry together with raspberry accessions.

The SSRs shown in Table 39 are from Zurn et al. (2018) and the first eight SSRs on the list are organized for multiplexing.

Blackberries range from diploid ($2n = 14$) to dodecaploid ($2n = 12x = 84$) (Meng & Finn, 2002). Genotyping blackberry and its hybrids requires close attention because of their complex genetic origin and the variable level of ploidy (Castillo et al., 2010; Dossett et al., 2012; Zurn et al., 2018).

3.42 | Sapodilla (*Manilkara zapota* (L.) P. Royen)

A set of 22 highly polymorphic SSR markers, based on heterozygosity and number of alleles per sample, were selected for the diploid sapodilla ($2n = 26$) from among those listed by Arias et al. (2020). All but two markers in the list have 3-nt or longer core repeats (Table 40).

3.43 | Strawberry (*Fragaria* L. spp)

The genus *Fragaria* is characterzed by different ploidy levels that vary from $2x$ ($2n = 14$) typical of the woodland *F. vesca*, to $4x$, to $6x$ ($2n = 42$) typical of the musk *F. moschata*, $8x$ ($2n = 56$) typical of the cultivated strawberry (*Fragaria x ananassa*), and $12x$ ($2n = 84$), which is typical of several interspecific hybrids (Bringhurst, 1990).

SSR-based genotyping in the cultivated strawberry and other polyploids is not easy and requires considerable skill in identifying the peaks of true alleles (Davis et al., 2006; Sargent et al., 2006, 2012).

Two sets of markers have been chosen for strawberry: the first set is from Chambers et al. (2013), made up of eight SSRs with long repeats (3 nt or more) and proposed for multiplexing; the second set is from Govan et al. (2008), which is made up of 10 SSRs, unfortunately with 2-nt core repeats, equally proposed for multiplexing (Table 41). No reference profile is reported in Table 41 as no such profile was found for a diploid genotype.

TABLE 35 Recommended markers for pomegranate (*Punica granatum* and wild relatives). A reference genotype is not available; touch down (td) decreased −1°C at each cycle for 10 cycles. Linkage groups (LG) were reported according to the genome assembly; the pomegranate genome assembly is GCA_007655135.2.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (bp)	Position of primer within the pomegranate genome
Pom014[a]	(GA)27	1	CGCATTTGGTTGTAGAAGAC	AGGAGCGTCTGTTTTAATCTT	65 td	n.a.	1,029,964–1,030,150
POM_AAC14[a]	(CA)7	1	CGAGAACCGTTAGTCATGC	AGTGACGGCAGGACAAGAAC	60 td	n.a.	15,806,857–16,449,194?
pg18	(TCA)14	2	TCTAAGGGCAGAATGGCACT	TGGCACTAGATCCGTAAATCTC	59	n.a.	22,631,861–22,632,027
Pom021	(AC)28	2	GACTGGAAGAAGCAGAGACT	GAAAAGGAAGTAGCAGAGCA	65 td	n.a.	42,596,165–42,596,346
ABRII-MP42[a]	(GA)9	2	GAGCAGAGCAATTCAATCTC	AACAATTTCCCATGTTTGAC	60 td	n.a.	39,115,638–39,115,812
ABRII-MP33[a]	(AG)12	2	TCTGTTTATTGCTGAAAGGG	TCTTCTTCTTCTCCACCGTA	60 td	n.a.	20,906,599–20,906,684
Pom013[c]	(CT)19	3	CACACCCTTCATCAAAAGAT	GGACTAACAACCAGCCATAG	65 td	n.a.	32,259,836
Pom046	(GA)21	3	CTTCCTCCTACCGAACTATG	CCCACTTTGACACTTCTACC	65 td	n.a.	33,976,740–33,976,961
Pom055	complex	4	GAGACAATTGGGATCAGAAA	AGTCGACGAACTGTGAAATC	65 td	n.a.	15,933,603–15,933,829
ABRII-MP28[a]	(GAGG)3(GA)19	4	ATCCTCTGTCTTTGTGTTCG	TGAGTAATTCCGGTCAGAAG	60 td	n.a.	13,486,214–13,486,548
ABRII-MP30[a]	(TGAGC)3	4	CCCAGTTTGTAGCAAGGTA	AAGCTGACATTCTTTGAAGC	60 td	n.a.	6,474,481–6,474,637
pg17[a,b]	(TCA)14	4	CATCAGACTACGATGGCACT	GCATAATAGCCTTCAATTTACA	55	n.a.	11,265,972–11,266,089
POM_AGC11[a]	complex	4	CGTCATCCCTTATGTTCTTC	CTGGGGAAGTCGACGAAG	55	n.a.	33,039,408–33,039,567
ABRII-MP12[a]	(CA)11	4	TTGAGTCCCGATCATATCTC	TCAATCTGTCAGGAACAACA	60 td	n.a.	1,667,718–1,667,968
Pom045	(CA)10	5	ATGAATGAGGAAGACGAAAA	GTGCTCCATCCATACAAAAT	65 td	n.a.	11,892,892–11,893,031
ABRII-MP04	(GT)7	5	CAGGTGATTGACTACTTGG	CAGATCTACAATAACATCAC	60 td	n.a.	7,324,780–7,324,961
Pom010[a]	(AG)19	6	CCTCATTGCTGATGAATCTT	ACTCGAGAAGCTCTGTGAAG	65 td	n.a.	22,703,604–22,703,816
Pom006[a]	complex	7	TACTAGGTGGAACCGAACTT	CCTTGACAACCTCATCTCAT	65 td	n.a.	25,470,624–25,470,757
POM_AGC5[a]	(TA)6(TG)6	7	TTCGATATTGTTTATTGTGTCG	CAACGAACTAGACGACACAC	55	n.a.	8,281,452–8,281,533
pg4	complex	8	CTGATGTAATGGCTGAGCAAA	GCACTTGAACAAAGAGAATGC	56	n.a.	18,137,748–18,137,967
ABRII-MP26[a,c]	(AG)25	8	TTTCTCGAAGAATTGGGTAA	CTGAGTAAGCTGAGGCTGAT	60 td	n.a.	1,274,747
ABRII-MP34	(GAA)3	n.a.	GGAAGAAGCAGAGCAATAGA	GTCCTGAGTAACCTGAGCTG	60 td	n.a.	n.a.
POM_AAC1	(CT)9(TA)8	n.a.	GGGTCTTCCTAATTCTCTGG	TACAACTTCGGACTCACTTGC	55	n.a.	n.a.
Pom024	(AG)27	n.a.	GGAGATTTGAATTGGGAAGT	GTGGACTAACTCAAGCAAGG	65 td	n.a.	n.a.

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

[b]SNP/s found in the published genome sequence compared with primer/s sequence/s.

[c]Primer Rev partially aligns within the published genome sequence.

TABLE 36 Recommended markers for quince (*Cydonia oblonga*). The reference genotype is apple "Golden delicious" (from Testolin et al., 2019); linkage groups (LG) from apple except NZ02b1 that is from pear; touch down (td) protocol, with initial temperatures of 62˚C decreased by 1˚C at each cycle for nine cycles, then 28 cycles at 52˚C. A genome assembly was not available or was not assembled in chromosomes or linkage groups.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (˚C)	RG (bp)
CH03d01	(GA)n	2	CGCACCACAAATCCAACTC	AGAGTCAGAAGCACAGCCTC	62 td	n.a.
CH02h11a	(GA)n	4	CGTGGCATGCCTATCATTTG	CTGTTTGAACCGCTTCCTTC	60	n.a.
NH011b	complex	4	GGTTCACATAGAGAGAGAGAG	TTTGCCGTTGGACCGAGC	55	n.a.
CH04e03	(GA)n	5	TTGAAGATGTTTGGCTGTGC	TGCATGTCTGTCTCCTCCAT	62 td	n.a.
CH04e05	(GA)n	7	AGGCTAACAGAAATGTGGTTTG	ATGGCTCCTATTGCCATCAT	62 td	n.a.
CH01h10	(GA)n	8	TGCAAAGATAGGTAGATATATGACCA	AGGAGGGATTGTTTGTGCAC	62 td	90/108
NB103a	(AG)31	10	TTGTAGGGAAAATGATGAAGCCA	GTGTTGATACTCTCTCTCTC	n.a.	n.a.
CH03d02	(GA)n	11	AAACTTTCACTTTCACCCACG	ACTACATTTTTAGATTTGTGCGTC	62 td	n.a.
CH02d08	(GA)n	11	TCCAAAATGGCGTACCTCTC	GCAGACACTCACTCACTATCTCTC	56	223/225
NH030a	(AG)18	11	GCAACAGATAGGAGCAAAGAGGC	TCCAAAGTTCAACACAGATCAAGAG	n.a.	n.a.
CH01f02	(GA)n	12	CTGGTTTGTTTTCCTCCAGC	ACCACATTAGAGCAGTTGAGG	62 td	169/179
CH05d04	(GA)n	12	ACTTGTGAGCCGTGAGAGGT	TCCGAAGGTATGCTTCGATT	60	n.a.
GD147	(AG)7	13	TCCCGCCATTTCTCTGC	AAACCGCTGCTGCTGAAC	62 td	139/-
NZ02b1	(GA)14	15	CCGTGATGACAAAGTGCATGA	ATGAGTTTGATGCCCTTGGA	62 td	n.a.
CH01d08	(GA)n	15	CTCCGCCGCTATAACACTTC	TACTCTGGAGGGTATGTCAAAG	58	n.a.
CH05a04	(GA)n	16	GAAGCGAATTTTGCACGAAT	GCTTTTGTTTCATTGAATCCCC	62 td	n.a.
NH007b	(AG)25	16	TACCTTGATGGGAACTGAAC	AATAGTAGATTGCAATTACTC	55	n.a.
NH015a	(AG)19	17	TTGTGCCCTTTTTCCTACC	CTTTGATGTTACCCCTTGCTG	50-55	n.a.

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

TABLE 37 Recommended markers for rambutan (*Nephelium lappaceum*). A reference genotype was not available; for annealing temperature (AT) see Arias et al. (2020); linkage groups (LG) were reported according to the genome assembly; the rambutan genome assembly is GCA_021234005.1.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (˚C)	RG (bp)	Position of primer within the rambutan genome
stv-nel_00333	(TATG)4	1	ACTCTGCTGTGTTTTGACCCTTTC	CGAGAAAGACACGGTAAGTGTGAC	63 ± 1	n.a.	2,964,788–2,964,899
stv-nel_04772[a]	(CAT)7	5	AGACAGAGAGGTAATGATGGCCC	ATCATCAACAGCAGCAGATTCTTG	63 ± 1	n.a.	19,751,760–19,751,893
stv-nel_05372	(CCTC)4	6	TTTTCGTACGTTTAGTGCCATGTG	GGCTTCCAAGAAACCACCTTTATC	63 ± 1	n.a.	9,321,904–9,322,036
stv-nel_05097[a]	(GAT)6	7	CGTCACCAAAGAATCTCCAATCTC	AAAAGGGGTGTTTTCAGGCTTAAC	63 ± 1	n.a.	20,539,529–20,539,668
stv-nel_05277[a]	(CTG)6	7	CAGCGCCATTTAGAAGCTGACTAC	AATTGCAACAGCATCAGAAACCTC	63 ± 1	n.a.	14,953,007–14,953,121
stv-nel_07181[a,b]	(AC)6	7	AGTTCAAAAAGTTCGGATGTCCTG	GATGATCCCCAAATCGTATTTAGAAG	63 ± 1	n.a.	11,345,970–11,346,102
stv-nel_08453[b]	(AG)7	7	GCCATTTCTGTACGTGTTCACAG	AAATAGTAAACCTCGTTGGGCTCC	63 ± 1	n.a.	14,744,902–14,745,005
stv-nel_08865	(ATGT)10	8	TTTCACAAAACACCTCTACAGTCCAG	GGACATCCTACAAAACCAGTGGAG	63 ± 1	n.a.	16,432,815–16,433,030
stv-nel_05023[a]	(AG)7	9	GAGAATTTGATGAAACTCACCGAG	AACAATTGCTTTGGTTTAAGATGG	63 ± 1	n.a.	12,413,504–12,413,641
stv-nel_05532[a]	(AG)8	9	TTTTCAAAGGGTTTTGTGAAATGG	AGTAGAGCTTTCACCGCATCAAAC	63 ± 1	n.a.	12,256,841–12,256,940
stv-nel_03406	(ATAC)6	10	TTGGTGTTAGCTAGTGAATAAGGATGAG	CAAATTAGCATTATTACTGGTGGGATG	63 ± 1	n.a.	19,532,401–19,532,519
stv-nel_06049[b]	(CAT)6	10	TTGCTTTGATCATCACTCTCATCC	TGATGACAAGGGAGTTTACTGGTG	63 ± 1	n.a.	15,565,676–15,565,797
stv-nel_07884[a]	(TTC)7	11	GGAATGGTCTAAGATTACACCCCC	GGGTTTGTGAAAGTGTGAGTGATG	63 ± 1	n.a.	9,821,217–9,821,314
stv-nel_00221	(GGAGAG)5	11	CTTTCTTCTTGGAATTTGGAGGTG	CTGCATCAAACACGATAAAACCAC	63 ± 1	n.a.	8,928,244–8,928,336
stv-nel_06172[b]	(GA)6	11	CACGTGAAAATGACCATAAGGACC	TTCGATGTGTCGATCTCTGTCTTC	63 ± 1	n.a.	326,287–326,373
stv-nel_05827[a]	(ATTA)6	12	TCAATTGAATGCGGGAAACTAGAG	GCATGCATAACTCTTGTTTTTGTAAGG	63 ± 1	n.a.	17,482,031–17,482,188
stv-nel_03033	(TC)6	12	TTCCAAGTATTTACTGGCTTTGGC	ATAAATCCCCCAAATGCATCTTTC	63 ± 1	n.a.	13,361,550–13,361,677
stv-nel_13493[a,c]	(CTT)4	15	ATCTGCTCGACTTCAGAATGGC	AACGACGACGAAGAAGAAAGAAAG	63 ± 1	n.a.	4,465,694–4,465,824
stv-nel_11760[a]	(TC)6	16	CAACAGAGACCTGAGGATTTCCC	AACCCCACCTCAATCATAGACATC	63 ± 1	n.a.	6,449,861–6,449,984
stv-nel_15792	(AG)8	17	TTCTCTCAGATGTCTTTGGACTTTAGC	TGTATATATGGTGCCTTGGATCCTTC	63 ± 1	n.a.	2,487,778–2,487,858

Abbreviations: RG, reference genotype (alleles in base pairs); n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

[b]One base insertion/deletion found in the published genome sequence compared with primer/s sequence/s.

[c]The published genome sequence differs in three bases compared with primer Rev sequence (AA**GTG**CGACGAAGAAGAAAGAAAG).

TABLE 38 Recommended markers for raspberry (*Rubus idaeus*). The reference genotype is "Tulameen" (from Fernández-Fernández et al., 2011); the SSR markers coded as Rubusxxxx are from Graham at al. (2004), and the remaining are from Castillo et al. (2010); markers reported in bold were organized for multiplexing by Fernández-Fernández et al. (2011); linkage groups (LG) are reported according to Graham et al. (2004); a genome assembly was not available or was not assembled in chromosomes or linkage groups.

Marker	Core repeat	LG	Forward-Primer	Reverse-primer	AT (˚C)	RG (bp)
Rubus270a	(GA)10	2	GCATCAGCCATTGAATTTCC	CCCACCTCCATTACCAACTC	51	156/188
RhM003	(TG)10	2	CCATCTCCAATTCAGTTCTTCC	AGCAGAATCGGTTCTTACAAGC	50	196/214
Rubus223a	complex	3	TCTCTTGCATGTTGAGATTCTATT	TTAAGGCGTCGTGGATAAGG	51	148/–
Rubus285a	(TC)9	3	TCGAGAAGCTTGCTATGCTG	GGATACCTCAATGGCTTTCTTG	52	175/193
RiM017	(TG)6	4	GAAACAGGTGGAAAGAAACCTG	CATTGTGCTTATGATGGTTTCG	59	n.a.
Rubus275a	(AG)27	5	CACAACCAGTCCCGAGAAAT	CATTTCATCCAAATGCAACC	51	142/178
RiM019	(AG)12	5	ATTCAAGAGCTTAACTGTGGGC	CAATATGCCATCCACAGAGAAA	52	169/185
RhM021	(TC)6	5	CAGTCCCTTATAGGATCCAACG	GAACTCCACCATCTCCTCGTAG	50	n.a.
Rubus123a	(AG)8	6	CAGCAGCTAGCATTTTACTGGA	GCACTCTCCACCCATTTCAT	52	142/148
RiM015	(ATC)5	6	CGACACCGATCAGAGCTAATTC	ATAGTTGCATTGGCAGGCTTAT	62	n.a.
RhM011	(TC)18	7	AAAGACAAGGCGTCCACAAC	GGTTATGCTTTGATTAGGCTGG	56	278/286
RhM043	(AC)6	n.a.	GGACACGGTTCTAACTATGGCT	ATTGTCGCTCCAACGAAGATT	56	n.a.
RhM001	(CA)7	n.a.	GGTTCGGATAGTTAATCCTCCC	CCAACTGTTGTAAATGCAGGAA	51	n.a.
RiM036	(TG)7	n.a.	AGCAACCACCACCTCAACTAAT	CTAGCAGAATCACCTGAGGCTT	51	n.a.
RhM023	(CAT)5	n.a.	CGACAACGACAATTCTCACATT	GTTATCAAGCGATCCTGCAGTT	53	n.a.
RiG001	(AT)6	n.a.	TGTCCGATCCTTTTCTTTGG	CGCTTCTTGATCCTTGACTTGT	55	n.a.

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

TABLE 39 Recommended markers for blackberry (*Rubus fruticosus* and aggregated species). Markers reported in bold were organized for multiplexing (Zurm et al., 2020). The SSR markers coded as ERubLRSQ_x are from Woodhead at al. (2008), those coded as RH_Mex are from Lewers et al. (2008), those coded as Rix and Rox are from Dossett et al. (2015), that coded as Rubleafx is from Graham et al. (2004), the remaining are from Castillo et al. (2010). Touchdown (td) protocol, with initial temperatures of 65°C decreased by 1°C at each cycle for 10 cycles, then 30 cycles at 55°C (Zurn et al., 2018). A genome assembly was not available or was not assembled in chromosomes or linkage groups.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (bp)
ERubLRSQ_07-4_D05	n.a.	n.a.	CTTCTTTCCAACCGATTTC	GTTTACGAATTGATTTCATCAACC	65btd	n.a.
RH_MEa0006bG05	n.a.	n.a.	GAAGCAGCAGCAAGACCTTT	GTCTGGCAGTTGGAGCAGTT	65btd	n.a.
RH_MEa0007aG06	n.a.	n.a.	CTTCCCCCTATAAATCCCGA	GTTTGTTCAGGCCAGTCAATGTCA	65btd	n.a.
RH_MEa0011dG03a	n.a.	n.a.	CCCTCACTCTCTCCATGCTC	TTGGCTTCAATTCTCCCATC	65btd	n.a.
RH_MEa0013dA06	n.a.	n.a.	TCCATCTCTATCCCGAAACG	AGCTGTTTTTGTTGGGGTTG	65btd	n.a.
RH_MEa0015cE06	n.a.	n.a.	TTGGGAGTGGAAAGAACAGG	GTGATGACGGTGATGGACAG	65btd	n.a.
RH_MEa0016bC11	n.a.	n.a.	CAGGGAATGAAGCTGGTGTT	TTCAGCTTCTTCTTCTGCTGG	65btd	n.a.
Ro942	n.a.	n.a.	AATCGTCGCCTGCAATATTTAC	GTTTCTGTAATACTAGGCTCCACCGC	65td	n.a.
RH_MEa0003dF05	n.a.	n.a.	TCCCCGGTCTACATATTCCA	GTTTACGAATTGATTTCATCAACC	65 td	n.a.
RH_MEa0008cF01	n.a.	n.a.	AGATGGAATTCCTAGGGCGT	CGTCTCTCTGCAATTCCTCC	65 td	n.a.
RH_MEa0013bC12	n.a.	n.a.	GTTGTGACCAAGCAAGAGCA	CCAATTTCTGCAGGGTTGTT	65 td	n.a.
RH_MEa0016aD11	n.a.	n.a.	TACCCTCATGTCCTCCCAAG	ATTAATGACCGACCCCTTCC	65 td	n.a.
Ri11795	(GAA)8	n.a.	ATCCAACCCTTCATTCTCTGTT	AACCTGATCACCCTCCAATG	65 td	n.a.
Ri5037	(GAA)6	n.a.	CACGAGTAACACTCCCAAATGA	GTTTCTTGGAATTGGGGTTATTCTG	65 td	n.a.
Ro4261	(TTC)9	n.a.	AATAGCATGGAATCCACTCACC	GTTTCTTGGAATTGGGGTTATTCTG	65 td	n.a.
Ro4532	(TTG)6	n.a.	AGTTCATCAATTTGAGGGATGG	TCTCATTCCAGATGGGTTATCA	65 td	n.a.
Ro6594	(TTC)9	n.a.	TTTGAGAGGACGAATGTCGTTA	TCGATGATCATATCATTCCACC	65 td	n.a.
Ro4261	(TTC)9	n.a.	AATAGCATGGAATCCACTCACC	GTTTCTTGGAATTGGGGTTATTCTG	65 td	n.a.
Ro4532	(TTG)6	n.a.	AGTTCATCAATTTGAGGGATGG	TCTCATTCCAGATGGGTTATCA	65 td	n.a.
Ro6594	(TTC)9	n.a.	TTTGAGAGGACGAATGTCGTTA	TCGATGATCATATCATTCCACC	65 td	n.a.
Ro942	(GAA)7	n.a.	AATCGTCGCCTGCAATATTTAC	GTTTCTGTAATACTAGGCTCCACCGC	65 td	n.a.
Rubleaf97	n.a.	n.a.	AACAAAGCTCCTCGACCAGA	CAAATTCGACACCACCTATCAG	65 td	n.a.
RhM011	(TC)18	n.a.	AAAGACAAGGCGTCCACAAC	GTTTCGAGATGGTCAGTCCAACA	65 td	n.a.
RhM003	(TG)10	2	CCATCTCCAATTCAGTTCTTCC	GTTTGGTTATGCTTTGATTAGGCTGG	65 td	n.a.
RiM019	(AG)12	5	ATTCAAGAGCTTAACTGTGGGC	GTTTAGCAGAATCGGTTCTTACAAGC	65 td	n.a.

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

TABLE 40 Recommended markers for sapodilla (*Manilkara zapota*). A reference genotype is not available; marker code: "maz_" stands for "std-maz_"; for annealing temperatures (AT) see Arias et al. (2020); a genome assembly was not available or was not assembled in chromosomes or linkage groups.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (˚C)	RG (bp)
maz_08303	(TTG)4	n.a.	AACCTGTTCAGCTAGGACTTGCAC	AATTCTTTTGAACCCATCTCAGCC	63 ± 1	n.a.
maz_06044	(CTCTTT)4	n.a.	AGCATATCCTGGTCCTCCTCTTTC	AACAAGTGAGTTTTTGCCCTCATC	63 ± 1	n.a.
maz_00161	(CAT)4	n.a.	ATGGTAGTGGTGATGGCGATAGAG	TTTGTGATCGATATTTTGTGTGGC	63 ± 1	n.a.
maz_03945	(AGA)5	n.a.	TTGTTCATTTTTGAGTCTTGCTGC	CATGAAAAATGCCAAAATCCTAGC	63 ± 1	n.a.
maz_04927	(GAC)5	n.a.	CAATATGGAGCTCATGAAAGACCC	CAAACTATGACCATCCCTTTCAGG	63 ± 1	n.a.
maz_06859	(GAA)6	n.a.	TCATTTGGTTCCTTGTGATTTATGG	GGGACCTAATTGCTTACTTCTCTCATC	63 ± 1	n.a.
maz_08151	(AGA)5	n.a.	AAAGCAAGTAATCAGGGTTCCACC	TTCATCGTTTGGGTTCATCTTCTC	63 ± 1	n.a.
maz_10490	(TGCG)4	n.a.	GGGATCTGCATTTTCTCGGTAAG	GTAGAATAACCCACACAACTCCGC	63 ± 1	n.a.
maz_02138	(TCT)4	n.a.	GAAAGCAAAATAGAGCCGGAAAC	TCAATGGTTAGTTCATCGTTTCAATG	63 ± 1	n.a.
maz_03685	(TTC)6	n.a.	ATGGTATTCAGGTGGATGATGACG	CGGACAACAGAGTACACAGCCATAC	63 ± 1	n.a.
maz_05984	(AGA)5	n.a.	TTGCCATCGATTTTTCTCTTCTTC	AGCAAAGAAGTAGGTCGTGGTGAG	63 ± 1	n.a.
maz_06694	(TGT)5	n.a.	TTGAGTGCCGACTCTAGGGTTTAG	CCTGATGATCGCTTTAAGCATTG	63 ± 1	n.a.
maz_06856	(AGAC)4	n.a.	AAGGGAACTGCTTTTTCTTCCTTC	CAGAAATACAAACCAATGGAATCG	63 ± 1	n.a.
maz_06932	(GAA)6	n.a.	GAAATGTGTGAATTGCACGTACC	AGAATCAACATTACCTACAAACCAGG	63 ± 1	n.a.
maz_09208	(GAA)5	n.a.	TCAGTACTCAGAAGTTACTAATGTCCGC	TCATTGGTCCTTAGTAGTGTCCCTG	63 ± 1	n.a.
maz_10106	(CTT)6	n.a.	TCCTCATATCGTTTCACCACACTC	AAAGATTCTGATATTTCCATTGTTGTTG	63 ± 1	n.a.
maz_11051	(ATTT)4	n.a.	AGGATTATGCATTAGGGGAAGTTG	CCAGGGATGTGATACAAGTGATTC	63 ± 1	n.a.
maz_13002	(TTAT)4	n.a.	TTTTCTCCTTTTTACATAGCCCTAGTTG	GGAAACACCAAAGGGTACACAAAC	63 ± 1	n.a.
maz_01644	(GAA)5	n.a.	TGAACAAGCTTAAGAAAACTGCCC	AATTAGCACACAGAACTGGGAACC	63 ± 1	n.a.
maz_02673	(CAAA)4	n.a.	ATATTTATGCATTGATGCGTGGAG	AACTTGCACTGTCGTCTTGTTCAC	63 ± 1	n.a.
maz_08175	(GA)8	n.a.	TTGATGAAGAGGATGAGGAGGAAC	CTTAGCCCTCTCTTGAGCAAACTG	63 ± 1	n.a.
maz_12205	(TC)7	n.a.	AAGCACCCTCATGATTAGAACTGC	GTGCTGCACTATTGCTCATCTCAG	63 ± 1	n.a.

Abbreviations: LG, linkage group; RG, reference genotype (alleles in base pairs); n.a., not available.

TABLE 41 Recommended markers for strawberry (*Fragaria* spp). A reference genotype is not available; markers in the first set are from Chambers et al. (2013), those in the second set are from Govan et al. (2008); see Section 3.41 for details on PCR mix and profile; *for marker UFFa3-D11 we observed an insertion of three nucleotides in the reference sequence in the reverse primer: TACCTTCTTCTGCATTCACCATGAC; linkage groups (LGs) are reported according to the reference genome assembly; the strawberry genome assembly is GCA_000184155.1.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (˚C)	RG (bp)	Position of primer within the strawberry genome
First set							
FG1a	(CTTT)4	1	TGGTTTGCCGGTAGCAAATAGCAGCA	TGACACACACTCTCTCTGTCTGATCCCT	57	n.a.	20,844,532–20,844,616
FG1c	(CTCAGG)6	1	TTGCGGAGATGCAAGCTGAAAGCA	TGTGCGATTCTGAAGCAGCCAGGA	57	n.a.	9,307,428–9,307,907
FG2a	(TATG)4	2	TGAACTGGTCCATCGGTGCTGAAA	TGATCACACAATACGCATTACCAAGCCT	57	n.a.	1,702,689–1,702,993
FG2c[a]	(TTTG)4	2	GGTCACCAACAACACTCACAGATGGT	TCATACTACCCACCACATGGGAGCAA	57	n.a.	30,461,934–30,462,340
UFFa3-D11[a]*	(AGA)3	7	GCCTTGATGTCTCGTTGAGTAG	TACCTTCTGCATTCACCATGAC	57	n.a.	15,978,148–15,978,315
FG7a	(TCAAATAG)8	7	GCAGTGCTACATCGACTCAGGTCCAA	ACCAAGGAAGTGCCGAAGTGGGTTT	57	n.a.	4,669,129–4,669,312
FG7c	(TAGGGA)6	7	AGGTGTCCAAAGAGGGTTGCTGTAGA	TCCCTCTCCCAATAACCCTTTGCTTC	57	n.a.	2,673,035–2,673,335
FG7e[a]	(AGAGAC)6	7	ACGGTGCCGAGATGCCTGATTACT	GCTGATCTCCACTTCCTCTCCTATCACCA	57	n.a.	6,103,403–6,103,919
Second set							
EMFn182	(GT)8	1	GCAACAAAGGAGGTTAGAGTCG	TGGTGAGTGCTCATTGTTCC	56	n.a.	9,483,737–9,483,914
EMFn121[b]	(GT)12(GA)9	2	GGTCCCTAAGTCCATCATGC	GAGTGGATGCAAACATGAGC	56	n.a.	11,319,335–11,319,572
EMFn170	(CT)9	3	CAGTTTGCCCAACAACAAGG	TTGATGGCAACAAATCACG	56	n.a.	13,625,966–13,626,203
EMFvi166[a]	AG	3	ACCGACAGCTGAGTTAGAGGAG	AGTCATAGGACCCCACTTCAAA	50	n.a.	11,606,448–11,606,692
ChFaM-023[a,c]	n.a.	4	AGGAGAAGACCGGCTGTGTA	TGCCTATAGCTGTGGCTGTG	51	n.a.	22,325,610–22,325,740
EMFn111	n.a.	4	GAAGCTCCTCTCACAAAGTTAAGG	CCTTTGTTGATGTTGTTGTTGA	55	n.a.	n.a.
EMFvi136	TC	4	GAGCCTGCTACGCTTTTCTATG	CCTCTGATTCGATGATTTGCT	59	n.a.	16,045,156–16,045,294
EMFn181	(AG)37	5	CCAAATTCAAATTCCTCTTTCC	GCCGAAAAACTCAAACTACCC	60	n.a.	n.a.
EMFv104	AG	6	TGGAAACATTCTTACATAGCCAAA	CAGACGAGTCCTTCATGTGC	59	n.a.	22,794,192–22,794,294
ARSFL11	GA	7	GCGAAGCATAACTGGCAGTATCTG	GCGGGCCTAGGTGATCTTGGA	59	n.a.	5,519,523–5,519,756

Abbreviations: AT, annealing temperature; RG, reference genotype (alleles in base pairs); n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

[b]SNP/s found in the published genome sequence compared with primer/s sequence/s.

[c]One base insertion/deletion found in the published genome sequence compared with primer/s sequence/s.

TABLE 42 Recommended markers for walnut (*Juglans* spp). The reference genotype is "Chandler" (from Vischi et al., 2017); markers are ranked according to their information content (Vischi et al., 2017); the annealing temperatures (AT) are reported according to the literature—Vischi et al. (2017) adopted 57°C for all markers with success; linkage groups (LG) are reported according to the reference genome assembly; the walnut genome assembly is GCA_002916465.2.

Marker	Core repeat	LG	Forward-primer	Reverse-primer	AT (°C)	RG (bp)	Position of primer within the walnut genome
Contig_40[a]	(CTGT)5	1	TGGGCTGAGCTGGATTGCCGT	TCCACCGTCATGGTTTCCACG	59	241–	50,046,014–50,046,210
WGA349[b]	(CT)14	1	GTGGCGAAAGTTTATTTTTTGC	ACAAATGCACAGCAGCAAAC	n.a.	293–	13,933,530–13,933,751
WGA069[b]	complex	2	TTAGTTAGCAAACCCACCCG	AGATGCACAGACCAACCCTC	45–58	180–	2,473,090–2,473,246
WGA089[a]	(GT)13(GA)21	3	ACCCATCTTTCACGTGTGTG	TGCCTAATTAGCAATTTCCA	53–58	235–	40,894,644–40,894,836
Contig_1681[a,c]	(TTC)8	4	AGAGATTTCTCCAGGAAGGCTCC	TCTGGTGGCCAACGATAGCCGA	62	223–229	31,429,801–31,429,988
WGA331[a]	(GA)13	4	TCCCCCTGAAATCTTCTCCT	CGGTGGTGTAAGGCAAATG	53–58	292–294	15,969,011–15,969,177
WGA027	(GA)30	5	AACCCTACAACGCCTTGATG	TGCTCAGGCTCCACTTCC	51–55	225–229	3,448,502–3,448,704
Contig_642	(CAG)7	6	TGAAAGGTTTTGGCCTCCAATGG	TGAGATCATGGGCTGCCTGTAGG	59	281–	8,381,220–8,381,483
Contig_1528[a]	(CCT)7	8	CCGAAGAGATCCTAAGCTCAACC	GAGGTGGAAATGATGGTGGGGTG	59	173–182	11,024,654–11,024,792
WGA225	(AG)14	8	AATCCCTCTCCTGGGCAG	TGTTCCACTGACCACTTCCA	n.a.	homo null?	3,863,994–3,864,174
WGA376[a]	complex	9	GCCCTCAAAGTGATGAACGT	TCATCCATATTTACCCCTTTCG	n.a.	266–274	17,865,073–17,865,271
Contig_156	(TTTG)6	10	TGCAAGAGTGGCGCAGGCACTG	TGGTAGCCTAATCTCATGGCTCG	60	319–	25,489,577–25,489,864
WGA009[a]	(GA)16	10	CATCAAAGCAAGCAATGGG	CCATTGCTCTGTGATTGGG	48–58	250–260	1,170,049–1,170,273
WGA321[b]	(GA)14	10	TCCAATCGAAACTCCAAAGG	GTCCAAAGACGATGATGGA	50–58	260–264	7,157,421–7,157,643
Contig_1692[a]	(CCA)6	12	CAATGGTCAGTTTCCGTCCGATC	CGAGCTCGAATACTTCTCGTCG	58	227–	19,624,621–19,624,807
WGA004[a]	complex	12	TGTTGCATTGACCCACTTGT	TAAGCCAACATGGTATGCCA	50–58	249–	18,385,844–18,386,067
WGA071	(GA)6(G)12	12	ACCCGAGAGATTTCTGGGAT	GGACCCAGCTCCTCTTCTCT	45	226–228	19,005,391–19,005,585
Contig_721	(CTT)8	14	ACCCCTTGGTTTGAACTGCGAC	AGATCCAACTTTCGCGTGGAAC	57	391–	1,713,194–1,713,557
WGA072	(CT)14	14	AAACCACCTAAAACCCTGCA	ACCCATCCATGATCTTCCAA	55–58	160–	5,123,070–5,123,199
WGA118	(GA)18(GT)11	15	TGTGCTCTGATCTGCCTCC	GGGTGGGTGAAAAGTAGCAA	55–62	204–218	32,136,985–32,137,177

Abbreviations:RG, reference genotype (alleles in base pairs); homo null? = lack of amplification; n.a., not available.

[a]Primer pairs found inverted according to the published genome sequence.

[b]SNP/s found in the published genome sequence compared with primer/s sequence/s.

[c]One base insertion/deletion found in the published genome sequence compared with primer/s sequence/s.

Some specific comments can be made about the required PCR conditions for strawberry. The forward primers from within the first set were 6-FAM M13-labeled and amplified in a single PCR reaction. The M13 sequence was 5′-TGTAAAACGACGGCCAGT-3′. The annealing temperature for the first 30 cycles, after denaturation, was set to 57˚C and was followed by eight cycles of PCR with a lower annealing temperature (53˚C) to incorporate the 6-FAM M13 label (Chamber et al., 2013). Unfortunately, the single dye (FAM) used to label all eight primer pairs prevents identification of amplicons corresponding to individual loci or primer pairs. Detailed information about amplification conditions can be found in Chamber et al. (2013) and in Schuelke (2000).

Multiplexing for the second set was obtained by adjusting the forward primer concentrations in three multiplexes as follows: Multiplex 1, 1—EMFn121 6-FAM 0.175 μM, EMFv104 6-FAM 0.3 μM, EMFn181 0.35 μM; Multiplex 2—ChFaM-023 VIC 0.175 μM, EMFn111 6-FAM 0.15 μM, EMFn170 NED 0.175 μM and EMFvi136 6-FAM 0.15 μM; Multiplex 3—ARSFL11 NED 0.175 μM, EMFn182 6-FAM 0.15 μM and EMFvi166 6-FAM 0.15 μM. The PCR temperature profile was "touchdown" with an annealing temperature from 55 to 50 with a −0.5˚C decrease at each cycle for the first 10 cycles.

3.44 | Walnut (*Juglans* L. spp)

We tested most of the microsatellite markers reported in the literature (Dangl et al., 2005; Marrano et al., 2019; Pollegioni et al., 2011; Vischi et al., 2017; Woeste et al., 2002; Zhang et al., 2010), selected 20, and ranked them according to their information content (Table 42). These markers were successfully used with both *J. regia* and *J. nigra*, the two most common walnut species. Unfortunately, we did not find published information on linkage groups.

Walnut is diploid ($2n = 32$) and up to two alleles are expected for each marker.

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AUTHOR CONTRIBUTION

Raffaele Testolin: Conceptualization; data curation; formal analysis; methodology; writing—original draft; **Rachele Messina**: Data curation; methodology; **Guido Cipriani**: Formal analysis; investigation; methodology; writing—review and editing; **Gloria De Mori**: Data curation; formal analysis; methodology; writing—review and editing.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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