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José Quero-García, Camille Branchereau, Teresa Barreneche, Elisabeth Dirlewanger

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Review

DNA-informed breeding in sweet cherry: current advances and perspectives

José Quero-García[1*], Camille Branchereau[1], Teresa Barreneche[1] and Elisabeth Dirlewanger[1]

[1] INRAE, Univ. Bordeaux, UMR Biologie du Fruit et Pathologie, 33882 Villenave d'Ornon, France; camille.branchereau@inrae.fr; teresa.barreneche@inrae.fr; elisabeth.dirlewanger@inrae.fr
[*] Corresponding author: jose.quero-garcia@inrae.fr; Tel.: +33-557122437

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Abstract: Sweet cherry (*Prunus avium* L.) world production has been steadily increasing in the last decades. Accordingly, breeders are releasing new cultivars at an unprecedented pace, in order to meet growers and consumers demands. However, new challenges are faced in sweet cherry cultivation, in particular regarding abiotic and biotic stresses related to climate change and to invasive pests or emerging diseases, respectively. There is a growing demand for new cultivars which combine high fruit quality and adaptation to these environmental constraints. As sweet cherry breeding is a long process, modern scientific tools and methodologies are needed to accelerate the progress and optimize selection schemes. A promising field of research and application was opened a few decades ago with the advent of molecular biology techniques which enabled the exploration of variability at the DNA, or genomic level. The generation of genomic tags, called molecular markers, allowed the characterization of genetic resources and the study of the genetic determinism of the main agronomic traits of interest. Since then, strategies to use this information in breeding have been developed, called marker-assisted breeding or, more generally, DNA-informed breeding. The objectives of this review are: (i) to recall the main scientific achievements in the fields of genetics in sweet cherry that could be directly beneficial for breeders, (ii) to summarize the practical steps needed to implement DNA-informed breeding and to illustrate several strategies adopted by sweet cherry breeders and (iii) to open new perspectives for a more efficient integration of these methodologies in breeding programs.

Keywords: *Prunus avium*; molecular marker; quantitative trait locus (QTL); DNA-informed breeding; marker-assisted parent selection (MAPS); marker-assisted seedling selection (MASS)

1. Introduction

Sweet cherry (*Prunus avium* L.) is a popular and highly appreciated temperate fruit. World production has been steadily increasing during the last decades to reach approximately 2,6 million tons in 2020 (FAO, 2020). Nevertheless, this increase has been mostly driven by a few large producers and in 2019, the first ten sweet cherry growing countries (Turkey, USA, Chile, Uzbekistan, Iran, Spain, Italy, Greece, Ukraine and Syria) represented 75% of the world production.

The boost in sweet cherry production has been related to an increase in the yield per ha due to the release of new cultivars characterized by a high and regular productivity, as well as large fruit size, an extended production season, and an intensification of the production systems. The latter was achieved thanks to the release of a new generation of dwarfing and semi-dwarfing rootstocks (Hrotko and Rozpara, 2017). Despite the fact that sweet cherry has been cultivated for at least 2000 years, modern breeding is relatively recent in comparison to other fruit species. Hence, it would have been initiated around 1800, according to Hedrick et al. (1915). During the first half of the XXth century, modern breeding programs were implemented simultaneously by public institutions and private breeders in the USA, Canada, United Kingdom, Russia and many other European countries. Since the 1970's, the history,

objectives and methodologies characterizing sweet cherry breeding have been described (Bargioni, 1996; Brown et al., 1996; Dondini et al., 2018; Fogle, 1975; Iezzoni et al., 2017; Kappel, 2008; Kappel et al., 2012; Quero-García et al., 2017a; Quero-García et al., 2019; Sansavini and Lugli, 2008).

Sweet cherry breeding has been slower than in other fruit trees, such as apple or peach, for several reasons other than the economic weight of each crop. First, sweet cherry is characterized by a gametophytic system of incompatibility (GSI), which is also common to many other species from the *Rosaceae* family (Crane and Lawrence, 1929). Hence, breeders can only make crosses between compatible cultivars. Second, flowering in sweet cherry is highly dependent on climatic conditions (reviewed in Herrero et al., 2017). Not only breeders must avoid frost damages, as in other *Prunus* species with early flowering (peach, apricot, almond), but pollination, both in terms of pollen tube growth and ovule viability, rely on optimal temperature conditions. Third, once the pollination has taken place, the rate of germination of embryos is highly variable, combination and year-dependent (reviewed in Iezzoni et al., 2017). In the end, it is highly challenging for cherry breeders to produce numerous hybrids for many different cross combinations (Quero-García, 2019). Fourth, sweet cherry juvenility period is particularly long, as compared to other *Prunus* species, with an average of five years, in particular when seedlings are observed by planting them on their own roots, without grafting. Despite these hurdles, numerous breeding programs have released successful cultivars (Quero-García et al., 2017).

Nonetheless, sweet cherry growers, and hence breeders as well, still face numerous threats. First, sweet cherry is a species highly impacted by climate change and in particular global warming (Luedeling, 2012). The increase in temperatures observed during the dormancy period (both endo and eco-dormancy) before flowering, and after fruit harvest, will put at risk cherry production in three different manners: (i) warm winters will provoke an insufficient fulfilment of chilling requirements, a poor fruit set and a loss of production; (ii) warm springs will cause an advance in flowering time and hence an increased risk of frost damage and (iii) high temperatures after harvest (in June-July) will entail the formation of double pistils and consequently, of double fruits, in the subsequent harvest season (Wenden et al., 2017). Second, very few desirable alleles have been found for traits related to the tolerance or resistance to abiotic or biotic stresses. For instance, a very small number of cultivars or landraces are low-chilling (e.g., 'Cristobalina') or tolerant to rain-induced fruit cracking (e.g., 'Regina' or 'Fermina'). Concerning tolerance to sweet cherry pests and diseases, although these had not been traditionally considered as key targets by breeders, the situation is rapidly evolving due to a growing social demand for environment-friendly agricultural practices. Sweet cherry breeders have traditionally worked with a narrow genetic base (Choi and Kappel., 2004; Quero-García et al., 2017b). However, in order to seek for new sources of genetic variability, they will need to characterize and integrate new germplasm into their breeding portfolio, whether it is from the pool of modern cultivars, local landraces or even wild accessions.

To help sweet cherry breeders cope with all these challenges and constraints, new tools and methodologies need to be developed and implemented. One possibility to optimize and facilitate the breeders' strategies and activities is to utilize molecular biology technologies. In particular, the use of molecular markers is progressively gaining interest among sweet cherry breeders, as recently reviewed by Dondini et al. (2018), Iezzoni et al. (2017, 2020), and Quero-García et al. (2019). In this review, the latest scientific results will be updated before focusing on the practical possibilities offered to breeders by these technologies and concluding with a section on perspectives and future needs.

2. Results from genetic studies

The deployment of molecular biology techniques after the discovery of the 'Polymerase Chain Reaction' (PCR) in 1986 allowed the exploration of plant genomes. A diversity of molecular markers was tested and the first studies on sweet cherry which relied on these technologies were published in the 1990's (Dirlewanger et al., 2009). Ten years later, a technical revolution in the field of genome sequenc-

ing brought the so-called 'Next-Generation Sequencing' (NGS) technologies. The first *Prunus* species for which the genome was fully sequenced was peach (Verde et al., 2013) and more recently, four sweet cherry cultivars have also been sequenced: 'Satonishiki' (Shirasawa et al., 2017), 'Tieton' (Wang et al., 2020), 'Big Star' (Pinosio et al., 2020) and 'Regina' (Le Dantec et al., 2020). These resources are extremely valuable for the study and characterization of genes controlling the variation of agronomic traits of interest.

2.1. Characterization of the gametophytic self-incompatibility system (GSI)

The first applications of biotechnology in the field of sweet cherry breeding consisted in the study of cultivars or other genetic resources with biochemical and later, DNA-based markers. A very active area of research was the characterisation of the GSI system, which is genetically determined by the *S*-locus, mapped on linkage group (LG) 6. This locus, with multiple alleles, encodes a ribonuclease (S-RNase) expressed in the style and a pollen-expressed protein, with an F-box domain, called S-locus F-box protein (SFB) (Herrero et al., 2017). While the variants of the S-RNase and SFB genes are known as alleles, the variants of the *S*-locus (the two genes considered together) are termed S-haplotypes (Tao and Iezzoni, 2001). The SI reaction is the result of an interaction between the style and pollen S products, S-RNase and SFB, in an allele-specific manner. Hence, the growth of the pollen tube will be inhibited when the pollen and the style express the same allele, and fertilization can only occur when the *SFB* allele expressed by the haploid pollen is different from the two S-*RNase* alleles expressed in the style (diploid tissue).

For this reason, growers must know the genetic cross-compatibility of different cultivars, as well as their relative flowering time, to ensure fruit set. Cultivars that are cross-incompatible are said to belong to the same incompatibility group (IG). Before the advent of molecular markers, the assignment to these IG groups was carried out by controlled pollinations and/or observation of the pollen tube growth (Crane and Brown, 1937; Matthews and Dow, 1969). After cloning and sequence characterization of the S-RNase, Tao et al. (1999) designed consensus PCR primers in the conserved regions of the S-RNase sequences. The differentiation between S-RNase alleles was made by detecting intron size polymorphism, revealed in a high-throughput manner with an automatic sequencer (Sonneveld et al., 2006). The S-locus pollen factor (SFB) was characterized later (Yamane et al., 2003) and additional genotyping methods were proposed, also based on intro size variation (Vaughan et al., 2006). Combining the genotyping of both S-locus genes, allowed the reliable discrimination of a large number of S-haplotypes (Vaughan et al., 2008). Several compilations of S-genotyping results have been published (Tobutt et al., 2004; Schuster, 2012) and today, data is available for 1483 accessions (Schuster, 2020).

Similar molecular approaches were developed to characterize the sources of self-compatibility (or self-fertility) in sweet cherry. The most popular source of self-compatibility was derived from accession 'JI2420', which was obtained from pollination of the early selection 'Emperor Francis' with pollen of cultivar 'Napoleon', previously mutated by X-irradiation (Lewis and Crowe, 1954). This accession was considered as a pollen mutant, with non-functional pollen during the SI reaction, the resulting self-compatibility being linked to the S_4 S-haplotype, which was named S_4' (Boskovic et al., 2000). Accession 'JI2420' was further crossed with cultivar 'Lambert' to give rise to 'Stella', the first self-compatible commercially released cultivar (Lapins, 1975). Specific molecular DNA markers based on PCR were designed to detect the S_4' mutation (Ikeda et al., 2004; Sonneveld et al., 2005; Zhu et al., 2004;). Two other sources of self-compatibility have been discovered and characterized, both coming from natural mutations: cultivars 'Cristobalina' and 'Talegal Ahin' from Spain (Cachi and Wünsch, 2014a; Wünsch and Hormaza, 2004;) and cultivar 'Kronio', from Italy (Calabrese et al., 1984; Marchese et al., 2007). In both cases, DNA tests based on linked Simple-Sequence Repeat (SSR) markers, have been designed to detect the mutations conferring self-compatibility (Cachi and Wünsch, 2014b; Marchese et al., 2007).

2.2. Genetic diversity studies

The characterization of sweet cherry germplasm diversity, whether from the wild or the cultivated pool, in particular with SSR and S-allele genotyping, has been previously documented (Iezzoni et al., 2017). Several studies consistently showed an important reduction in genetic diversity from wild to landrace to modern cultivars (Campoy et al., 2016; Mariette et al., 2010). Nevertheless, most of these studies included material from one region or country, with sometimes a subset of modern accessions from different countries. Although several scientists used common markers, after a standard set of 16 SSR loci and eight reference genotypes were recommended (Clarke and Tobutt, 2009), it is not easy to compare results from different studies since data variability between laboratories has been well documented (Jones et al., 1997; Rajput et al., 2006). Small differences, in the order of one base pair, have been observed in the size estimation of alleles, as well as in the number of stutter bands (Jones et al., 1997). A recent study aimed at implementing an international sweet cherry SSR database through the alignment of SSR marker data collected in numerous European collections (Ordidge et al., 2021). Groups of previously unknown matching accessions, that is, genetically indistinguishable, were identified and a number of errors in prior datasets were resolved. In parallel, a wide study of genetic diversity was conducted by working with 314 accessions coming from 19 European countries and 14 SSR loci (Barreneche et al., 2021). Despite the identification of numerous duplicates, a higher genetic diversity as compared to previous studies was revealed, which could be structured in four ancestral populations (Barreneche et al., 2021).

SSR markers are recommended for genetic diversity studies because they are highly polymorphic and with less than 20 well-selected loci, an accurate picture of the genetic relationships between the accessions of a given germplasm set can be produced (Clarke and Tobutt, 2009). Nevertheless, with the advent of the NGS technologies, new sources of molecular markers became available. More precisely, Single-Nucleotide Polymorphism (SNP) markers, which are extremely abundant within plant genomes, started to be used for genetic studies (Vanderzande et al., 2019). In sweet cherry, a 6K SNP chip was designed within the USA RosBREED project (Peace et al., 2012) and more recently, a new 6+9 K SNP chip has been released (Vanderzande et al., 2020). The first chip was used by Campoy et al. (2016) for a genetic diversity study of the germplasm collection held by INRAE. These authors confirmed a distinction between two groups of accessions, modern cultivars and landraces, as initially reported with SSR markers by Mariette et al. (2010). However, the high number of SNPs used allowed a further sub-division in nine subgroups, several of which corresponded to different eco-geographic regions of landraces distribution. The same type of markers was used to ascertain the paternity of important cultivars in breeding programs, such as ‘Bing’, ‘Emperor Francis’, ‘Sweetheart’, ‘Van’ and ‘Windsor’ (Howard et al., 2021; Iezzoni et al., 2020).

2.3. Genetic maps, QTL detection and validation

Despite the importance and utility of germplasm characterization for breeding purposes, the most exciting promises that were made to breeders were the possibility to select their parental cultivars and seedlings based on markers linked to traits of interest. This was initially called marker-assisted selection (MAS), which could be further sub-divided into different specific operations, as it will be detailed in the section 3. For this revolution to take place, it was first necessary to identify molecular markers within the plant genome which would be genetically linked to genes controlling a significant part of the phenotypic variance of the considered agronomic traits.

The first and simplest implemented methodology was the detection and mapping of Mendelian Trait Loci (MTL) or Quantitative Trait Loci (QTL). This approach is based on the construction of genetic maps, which are represented by groups of molecular markers genetically linked within each chromosome or LG. These maps are constructed by genotyping the offspring derived from a particular cross and by estimating the genetic distance, expressed in a unit called centimorgan, or cM, between each pair of loci. Genetic distance is a function of the proportion of recombinant gametes (recombination, or cross-overs, occur during meiosis) between each pair of loci. Conceptually, we can consider that the higher the distance between too loci, the higher the frequency of crossing-over between these too loci.

The second step is the phenotypic characterization of the same offspring in order to correlate, with appropriate statistical procedures, the genotypic and phenotypic information produced for each individual genotype. In sweet cherry, as in all allogamous and highly heterozygous species, the simplest approach is to work with a population derived from the cross of two distinct cultivars, and to apply the pseudo-test cross strategy proposed by Grattapaglia and Sederof (1994).

The main characteristics of published genetic maps in sweet cherry are recalled in Table 1.

Table 1. Summary of the main genetic maps developed in sweet cherry.

Reference	Population(s)			N° individuals	Type of marker
	Cross(es)	Origin	Type		
Boskovic and Tobutt, 1998	'Napoleon' × *P. nipponica* 'Napoleon' × *P. incisa*	Inter-specific	F_1	63, 44	Isoenzymes
Dirlewanger et al., 2004	'Regina' × 'Lapins'	Intra-specific	F_1	122	SSR
Olmstead et al., 2008	'Emperor Francis' × 'NY 54' (and the reciprocal)	Intra-specific	F_1	190	SSR, CAPS
Clarke et al., 2009	'Napoleon' × *P. nipponica*	Inter-specific	F_1	94	SSR, isoenzymes
Cabrera et al., 2012	'NY 54' × 'Emperor Francis' 'Regina' × 'Lapins' 'Namati' × 'Summit' 'Namati' × 'Kurpnoplodnaya'	Intra-specific	F_1	113, 100, 77, 80	SSR, SNP (RosCOS)
Klagges et al., 2013	'Black Tartarian' × 'Kordia' 'Regina' × 'Lapins'	Intra-specific	F_1	89, 121	SNP (6K)
Castède et al., 2014	'Regina' × 'Garnet'	Intra-specific	F_1	117	SNP (6K)
Skipper et al., 2014	'Colney' × 'C210-7' ('Lapins' × 'Sweetheart')	Intra-specific	F_1	138	SNP (6K)
Wang et al., 2015	'Wanhongzhu' × 'Lapins'	Intra-specific	F_1	100	SNP (SLAF)
Guajardo et al., 2015	'Rainier' × 'Rivedel'	Intra-specific	F_1	166	SNP (GBS)
Calle et al., 2018	'Vic' × 'Cristobalina' 'Cristobalina' × 'Cristobalina' ('Brooks' × 'Cristobalina') × ('Brooks' × 'Cristobalina')	Intra-specific	F_1, F_2	161, 97, 67	SNP (6K)
Cai et al., 2019	'Fercer' × 'X'	Intra-specific	F_1	67	SNP (6K)
Isuzugawa et al., 2019	'Beniyutaka' × 'Benikirari'	Intra-specific	F_1	93	SNP (RAD-Seq)
Calle and Wünsch, 2020	'Ambrunés' × 'Sweetheart'	Intra-specific	F_1	140	SNP (6K)
Calle et al., 2021	'Vic' × 'Cristobalina'	Intra-specific	F_1	161	SNP (6+9K)

SSR: Simple Sequence Repeat; CAPS: Cleaved Amplified Polymorphism Sequence; SNP: Single Nucleotide Polymorphism; RosCOS: *Rosaceae* Conserved Orthologous Set; SLAF: Specific-locus Amplified Fragment Polymorphism; GBS: Genotype-By-Sequencing; RAD-Seq: Restriction-site Associated DNA Sequencing.

Not all these maps were subsequently used for QTL detection studies, as will be detailed hereafter. Biochemical markers such as isoenzymes were only used to build inter-specific maps (Boskovic and Tobutt, 1998; Clarke et al., 2009). Taking advantage of the high synteny observed between *Prunus* species (Dirlewanger et al., 2004), numerous SSR markers were tested on sweet cherry. However, due to the lack of transferability between species for certain markers, and to the absence of polymorphism between the parental cultivars used to create the mapping populations, scientists searched for SNP markers, in order to saturate (that is, to map a high number of markers able to cover the genomic regions of interest) more rapidly and efficiently the existing maps.

Concerning the main published QTL studies, a synthesis is presented in Table 2, by categorizing these studies according to the type of trait studied.

Table 2. Summary of all QTLs identified on sweet cherry for different traits of agronomic interest. For each reference, the linkage groups where the most significant QTLs were detected are marked in bold.

Trait	Reference	Main QTLs	
		Linkage group	PVE max*
Fruit weight (size)	Zhang et al., 2010	**2**, 6	54 (2)
	Rosyara et al., 2013	1,**2,3**,6	-
	Campoy et al., 2015	1,**2**,3,**5,6**,8	37 (1)
	Calle and Wüsnch, 2020	1,**2,5**	45 (2)
	Calle et al., 2020a	**1**,**3**	17 (1)
Fruit firmness	Campoy et al., 2015	1,**2**,3,**5,6**,8	37 (2)
	Cai et al., 2019	**4**	85
	Calle and Wüsnch, 2020	**4**	64
	Calle et al., 2020a	**1**,**6**	23
Fruit and skin color	Sooriyapathirana et al., 2010	**3**,6,8	88
	Calle et al., 2021a	**3**	35
Tolerance to rain-induced fruit cracking	Quero-García et al., 2021	1,**2**,3,**4,5**,6,7	26 (5)
Sugar content (SSC)	Zhao et al., 2014a	**2,4**	-
	Quero-García et al., 2019	**3**	21
	Calle and Wüsnch, 2020	3,**4**	34 (4)
Titratable acidity, pH	Zhao et al., 2014a	2, 4, 6	
	Quero-García et al., 2019	**1**,3,5,**6**	30
	Calle and Wüsnch, 2020	**6**	22
Phenolic compounds	Calle et al., 2021	**1**,2,**3**,4,7	78 (1)
Flowering date or bloom time	Dirlewanger et al., 2012	**1,4**,5	46 (4)
	Castède et al., 2014	**1,4**,5,**6**,7	46 (4)
	Calle et al., 2020b	**1,2**	60 (1)
Chilling and heat requirements	Castède et al., 2014	**1,4**,6	33 (4)
Maturity date	Dirlewanger et al., 2012	1,**4**,5	22 (4)
	Quero-García et al., 2019	**4**	50
	Isuzugawa et al., 2019	**4**	48
	Calle and Wüsnch, 2020	1,2,3,**4**	52 (4)
Crop load	Quero-García et al., 2019	**1,6**	18 (6)
Powdery mildew and bacterial canker resistance	Zhao et al., 2014b	1,3,**5**,6	-

PVE max: maximum percentage of phenotypic variance explained; *the number in parentheses, when present, indicates the LG with the highest PVE value. Some references (-) do not provide PVE values; SSC: soluble solids content.

2.3.1. Fruit size and weight

The first QTL detection studies concerned the traits fruit size and weight (Zhang et al., 2010). These traits are the most critical for a sweet cherry breeder since today, a majority of growers will refuse planting a cultivar which produces fruit under 8 g. Furthermore, empirical breeders' observations suggested that when crossing two cultivars, a large proportion of seedlings had a fruit weight inferior to both parents' average fruit weight. Hence, being able to eliminate these hybrids with undesired fruit weight prior to field planting would represent a tremendous gain for breeders. Zhang et al. (2010) conducted a thorough phenotypic study by measuring all fruit dimensions (size and weight of both sweet cherry flesh and pit) and by counting the number of mesocarp cells. Two major fruit weight QTLs were identified, on LG2 and LG6, the first one co-localized with a QTL associated to the number of cells whereas the second one co-localized with a QTL associated to pit size. Thus, from a breeder's point of view, QTL on LG2, flanked by SSR markers CSPSCT038 and BPPCT034, appeared as the most promising. As the population used in this study was derived from the cross between an early selection, 'Emperor Francis', and a wild cherry (syn. mazzard), 'NY54', these QTLs were associated to the domestication process. Furthermore, the allele responsible for the phenotype 'small fruit' of the LG2 QTL was dominant over the 'large fruit' allele, confirming previous empirical observations and highlighting the importance of getting rid of these undesirable alleles from breeder's seedlings.

Based on these promising results, and by integrating another population, derived from the cross between cultivars 'Regina' and 'Lapins', as well as four other sour cherry populations, De Franceschi et al. (2013) demonstrated that two genes from the 'cell number regulator' (*CNR*) gene family, previously reported to be involved in the control of fruit weight in the model species tomato, were mapped in the genomic region of QTLs in LGs 2 and 6. A third study, based on the same sweet cherry populations, two additional derived from crosses 'Namati' × 'Summit' and 'Namati' × 'Krupnoplodnaya', as well as 23 founders (cultivars involved in the pedigree of the major parental genitors used in breeding programs), was conducted with FlexQTL software (Rosyara et al., 2013). FlexQTL follows a Bayesian approach and analyses simultaneously data from various-sized pedigreed populations comprising multiple generations and connected by common ancestry (Bink et al., 2008). In this way, it allows the simultaneous QTL discovery and validation in multiple genetic backgrounds. In their study, Rosyara et al. (2013) reported six QTLs: three on LG2 and one each on LGs 1, 3 and 6. By combining different populations, the genetic control of fruit weight appeared to be much more complex than initially reported by Zhang et al. (2010). Nevertheless, out of the three potential QTLs detected on LG2, the first one, corresponding to the QTL reported by Zhang et al. (2010), explained the largest part of the phenotypic variance; it was named FW_G2a.

A known strategy to increase power detection of QTLs, when relatively large populations are created and evaluated, is to cumulate numerous years of phenotypic data. Campoy et al. (2015) implemented this strategy, by using the software MultiQTL, and studying two populations, 'Regina' × 'Lapins' and 'Regina' × 'Garnet', during 7 and 4 years, respectively. These authors detected QTLs in all the LGs reported by Rosyara et al. (2013) but also one high-effect QTL on LG5. More recently, Calle and Wünsch (2020) studied several fruit-quality related traits, including fruit size, through a pedigree-based approach with FlexQTL. They used six populations of variables sizes, derived from crosses between local Spanish cultivars, 'Ambrunés' and 'Cristobalina', and modern cultivars ('Brooks', 'Lambert' and 'Vic') as well as from self-pollinations derived from self-compatible cultivar 'Cristobalina'. Three genomic regions previously identified for fruit weight on LGs 1, 2 and 5 were reported, with the highest PVE values observed for QTLs on LGs 2 and 5, depending on the studied year. Lastly, Calle *et al*. (2020a) studied fruit weight in a population derived from the cross between 'Ambrunés' and 'Sweetheart' and detected QTLs on LGs 1 and 3.

Despite this high number of potentially useful QTLs for breeding, the main efforts for validating and using fruit weight QTLs have been restricted (see section 3) to the one on LG2 initially discovered

by Zhang et al. (2010). According to Rosyara et al. (2013), seven and four different alleles were identified for markers CPSCT038 and BPPCT034, respectively, providing various possible QTL haplotypes. Peace (2011) summarized this haplotype information for 44 cultivars and selections from the Washington State University (WSU) breeding program. More recently, Szilagyi et al. (2022) reported the evaluation of 11 cultivars from the Hungarian breeding program with these SSR markers, covering a range of fruit weight from 3.9 to 10.3 g.

Although SSR markers are currently widely adopted, SNP markers are becoming more efficient because per-sample costs are dropping faster than is occurring for SSR markers. Moreover, with the increasing use of genetic maps built with SNP markers, it is more straightforward to directly validate SNP markers linked to the QTL under study than to identify in a second step, SSR markers present in this genomic region. For instance, one low-cost genotyping platform called 'Kompetitive Allele Specific PCR' (KASP) offers high throughput genotyping suited to screening thousands of individuals for a few SNP markers linked to the QTLs of interest. Dirlewanger et al. (2020) applied this technique for a preliminary study aimed at validating the FW_G2a QTL by testing the trait predictiveness of one KASP marker on a subset of 'Regina' × 'Lapins' and 'Regina' × 'Garnet' populations (32 individuals for each cross), as well as 31 accessions from the INRAE germplasm collection. This marker was able to discriminate genotypic groups significantly associated with fruit weight across the three validating sets.

2.3.2. Fruit firmness

Another highly important trait for sweet cherry breeders is fruit firmness. Campoy et al. (2015) found numerous co-localizations between fruit weight and fruit firmness QTLs, which was coherent with the significant and negative correlations observed between both traits. As for fruit weight, the largest firmness QTLs were located on LGs 2, 5 and 6.

By working on different genetic backgrounds, a major firmness QTL was detected on LG4 (Cai et al., 2019; Calle and Wünsch, 2020). In the study of Cai et al. (2019), it was identified on three different types of populations, which included wild cherries and hybrids derived from crosses between cultivated and wild materials. As for the trait fruit weight, it was found that alleles responsible for the non-desired phenotype, that is, 'soft fruit', were dominant over alleles associated to the 'firm fruit' phenotype. Furthermore, only wild accessions were homozygous for the 'soft fruit' alleles and most of the improved modern cultivars were homozygous for the 'firm fruit' alleles, suggesting that this locus represents a selection signature, that is, it has been involved in the domestication process. The reason why Campoy et al. (2015) could not detect this major QTL on cultivars 'Regina', 'Lapins' and 'Garnet' was because they all had fixed the favourable firmness alleles at this locus.

In the study of Calle et al. (2020a), a new major QTL for fruit firmness was detected on LG1 on cultivar 'Ambrunés', which is a traditional Spanish cultivar characterized by a late maturity period and a very good shelf-life. As in the study by Campoy et al. (2015), fruit weight and fruit firmness were significantly correlated but in this case the correlation was positive. This result has important implications for breeders and highlights the potential value of 'Ambrunés' as breeding parent.

Dirlewanger et al. (2020) tested the predictiveness of a KASP marker linked to the firmness QTL on LG5 reported by Campoy et al. (2015). In this case, this marker could only distinguish firm or soft fruit individuals within 'Regina' × 'Lapins' and 'Regina' × 'Garnet' populations but not among the accessions of the germplasm collection. It is highly likely that these accessions segregate for the large-effect QTL of LG4, hence shading the effect of the LG5 QTL.

2.3.3. Other fruit-quality related traits

By working on the same mapping population as Zhang et al. (2010), Sooriyapathirana et al. (2010) discovered a major QTL on LG3 controlling skin and flesh colour, which behaved as a Mendelian trait. A candidate gene was identified, *PavMYB10*, it was a homolog of apple *MdMYB10 gene*, responsible

for the red fruit flesh and foliage. In sweet cherry, the allele associated to red or mahogany colour (such as that of 'Bing' or 'Burlat' fruit) is dominant to the allele associated to blush colour (such as that of 'Rainier' or 'Napoleon' fruit). Sooriyapathirana et al. (2010) found two other minor QTLs on LGs 6 and 8 and more recently, Calle et al. (2021a) confirmed the presence of a major QTL on LG3, although with a PVE significantly inferior to the one detected in the first study. Sandefur et al. (2016) developed a DNA test using an SSR marker, named *Rf*, which was developed from SNP array haplotypes. Three genotypic classes were characterized within offspring: *rfrf* (blush skin colour and yellow-white flesh), *Rfrf* (red to mahogany skin and flesh colour) and *RfRf* (usually dark mahogany skin and flesh colour).

One of the most serious problems for sweet cherry growers is rain-induced fruit cracking, which is a multi-factorial and highly complex phenomenon (Knoche and Winkler, 2017). Although cultivar differences exist in terms of tolerance to fruit cracking, no resistant cultivar has yet been identified (Quero-García et al., 2017). In a preliminary study, Quero-García et al. (2014) characterized the population 'Regina' × 'Lapins' with three different methodologies: by evaluating the number of cracked fruits at maturity from trees planted in the field; by a lab-controlled test, based on the immersion of intact fruit in distilled water (Christensen test) and by working with potted trees under tunnels with canopy irrigation. QTL results were relatively comparable between the three methodologies and in subsequent years, the first methodology was favoured, since it allowed evaluating a higher number of progenies, although cracking was dependant on meteorological conditions. Subsequently, Quero-García *et al*. (2021) reported the first stable cracking-tolerance QTLs by working with three populations and combining multiple years of data. Furthermore, the integration into simple linear models of variables accounting for the amount of rainfall that each genotype received before maturity allowed an increase in the precision of the QTL detection. Three main genomic regions were identified, each one associated with a different type of cracking, on LGs 2, 4 and 5, controlling fruit side, stem end, and pistillar end fruit cracking, respectively.

The genetic determinism of traits related to fruit tasting quality has been recently studied. Zhao et al. (2014a) reported two major QTLs for flesh sugar content on LGs 2 and 4 by characterizing for three years a total of 601 pedigree-linked individuals; however, their results were not highly consistent across years. These authors reported also three QTLs for fruit titratable acidity (TA), on LGs 2, 4 and 6. Quero-García et al. (2019) detected more QTLs for fruit TA as compared to sugar content, they were also more stable and significant, in particular the one at the bottom of LG6. On the opposite, Calle and Wünsch found a strong QTL on LG4 for sugar content but detected as well as a QTL for TA on LG6, at a collinear position to the one reported by Quero-García et al. (2019).

Finally, Calle et al. (2021a) conducted the first study on sweet cherry dealing with the genetic determinism of phenolic compounds (phenolic acids and flavonoids) by working with the population 'Vic' × 'Cristobalina'. A major QTL associated to hydroxycinnamic acid content was mapped on a narrow region of LG1 and a promising candidate gene (CG), *ρ-coumarate 3-hydroxylase* (*C3H*), was found within the QTL interval.

2.3.4. Phenology-related traits

A special focus has been put on traits flowering (or bloom) and maturity dates, for which a high level of synteny has been observed within the *Prunus* genus (Dirlewanger et al., 2012). These were reported to be highly polygenic but for certain QTLs high PVE values were observed.

In sweet cherry, the most significant flowering date QTLs were mapped on LGs 1 and 4. The first one was detected on the parental maps of early-flowering cultivars, such as 'Lapins' or 'Garnet' (Castède et al. 2014) and, more recently, on the extra-early-flowering and low-chilling Spanish cultivar 'Cristobalina' (Calle et al., 2020a), reaching for the latter 60% of the PVE. On all the genetic maps considered, within the confidence interval of this QTL were mapped CGs called '*DORMANCY-ASSOCIATED MADS-box'* or *DAM* genes. These genes, six in number (DAM1-6), were initially characterized in a

non-dormant peach mutant, 'evergrowing' (evg), which showed a deletion in this region and did not cease growth to enter dormancy even in dormancy-inducing conditions (Bielenberg et al., 2008). Calle et al. (2021b) characterized these genes on cultivar 'Cristobalina', by comparing their genomic sequences with those of a panel of cultivars with contrasted chilling requirements, and several mutations in regulatory regions were proposed to be correlated with the low-chilling and early-flowering phenotype. On a more applied basis, these authors designed PCR markers which could be useful for MAS of early blooming seedlings derived from 'Cristobalina'.

The flowering date QTL on LG4 was detected on late-blooming cultivar Regina, by working both with the 'Regina' × 'Lapins' and the 'Regina' × 'Garnet' populations (Castède et al., 2014; Dirlewanger et al., 2012). By combining multiple years of phenotyping, and due to the high heritability observed for the trait flowering date, the confidence interval of this QTL was relatively narrow and allowed Castède et al. (2015) to search for positional CGs, among which, the most promising were involved in the biosynthesis pathway of gibberellins and in the temperature pathway. Flowering in fruit perennials depends on a correct fulfillment of chilling (or cold) requirements (CR) during the endodormancy period and of heat requirements (HR) during the ecodormancy period (Lang et al., 1987). In order to dissect flowering time into CR and HR, Castède et al. (2014) evaluated these traits during three years on population 'Regina' × 'Garnet'. As previously demonstrated on peach (Fan et al., 2010), a significant number of co-localizations between QTLs for CR and HR and flowering date QTLs were identified, in particular on LGs 1 and 4. Nevertheless, confidence intervals of flowering date QTLs were smaller than those of CR or HR QTLs, demonstrating the complexity and difficulty for the precise phenotyping of CR and HR.

With the aim of further narrowing down the flowering date LG4 QTL confidence interval, Branchereau et al. (2022) worked with a new mapping population, derived from the reciprocal crosses 'Regina' × 'Garnet' and 'Garnet' × 'Regina', and composed of almost 1400 individuals. Through a fine mapping approach, the genomic region of the flowering date QTL was narrowed down from more than 2 million base pairs (bp) to 80000 bp, a region containing no more than 15 CGs. Based on this approach, two KASP markers were designed within this new reduced confidence interval and were validated on three different populations, the mapping population 'Regina' × 'Lapins', a set of 105 cultivars from the INRAE germplasm collection and a set of 51 modern cultivars.

Maturity date is also a phenology-related trait characterized within *Prunus* species by its high heritability and polygenic control, with at least 5 different QTLs identified by several authors (see Table 2). The most important QTL is the one present on LG4 at a sufficient genetic distance from the one controlling flowering date, excluding the possibility of a pleiotropic genetic control of both traits by a single locus. On the opposite, this maturity date QTL was mapped by Quero-García et al. (2019), by working with a small mapping population of 67 individuals, derived from the cross between cultivar 'Fercer' and an unknown parent (hence called 'X'), on a very close region to the fruit firmness QTL reported by Cai et al. (2019) on the same population. Whether in sweet cherry these two major loci controlling fruit firmness and fruit maturity date on LG4 correspond to the same pleiotropic gene or to two close linked genes still remains to be elucidated. As highlighted by Dirlewanger et al. (2012), the genomic region controlling maturity date on LG4 shows high synteny between *Prunus* species. In peach, Pirona et al. (2013) proposed a CG from the family of the NAC transcription factors. In sweet cherry, Isuzugawa et al. (2019) characterized two homologous genes of this gene family in the same QTL region.

2.3.5. Other agronomic traits

Zhao et al. (2014b) reported a major QTL of resistance to powdery mildew (*Podosphaera clandestina*) and bacterial canker (*Pseudomonas syringae* spp.) on LG5, by working with the same population as Zhao et al. (2014a). The first one is a fungal disease particularly serious in the north-western coast of the United States whereas bacterial canker is the major bacterial disease in sweet cherry (Børve et al., 2017; Puławska et al., 2017).

A preliminary study focused on fruit yield reported two stable QTLs on LGs 1 and 6 mapped on the 'Regina' cultivar (Quero-García et al., 2019). A relatively simple phenotyping protocol was used, by evaluating qualitatively the amount of fruit, as a proxy of crop load, with a visual scale ranging from 0 (no fruit) to 9 (branches fully covered by fruit).

Overall, all these studies clearly demonstrated that certain LGs are particularly interesting from a breeder's point of view, in terms of number of detected QTLs and contribution to the amount of phenotypic variance explained. It is the case of LGs 2 and 4, which have been specifically studied in terms of genetic structure via a pedigree-based haploblocking approach (Cai et al., 2017; Calle and Wünsch, 2020). By combining the information on genetic diversity, ancestry, recombination patterns and QTL results from different traits, these authors concluded that these so-called 'QTL hotspots' were under positive selection in breeding. Nevertheless, by considering the diversity of traits studied so far, key QTL regions appear to be harbored as well by LGs 1, 3, 5 and 6.

2.4. New approaches

QTL detection through classical mapping strategies may be complemented by 'Genome-Wide Association Studies' (GWAS), in which there is no need to create populations and the correlation between genotype and phenotype is searched within collections of germplasm resources, with different levels of genetic diversity. This approach has been used by Cai et al. (2019) to confirm the existence of a major fruit firmness QTL on LG4 by working with INRAE sweet cherry germplasm collection.

In the last 20 years, a promising methodology has been proposed to optimize selection through the massive use of molecular markers: it is the so-called 'Genomic Selection' (GS) (Meuwissen et al., 2001). The concept behind this approach is that all polymorphisms present in one organism's genome are potentially linked to a trait of interest. Hence, when genotyping individuals with thousands of markers, all the information generated by these markers can be used by breeders, not only the one provided by markers linked to previously identified major genes or QTLs. The implementation of GS is carried out in two main steps. First, a reference population is used to estimate the effects on the trait of interest of all markers used to genotype this population; hence, a prediction equation is established. Second, genomic estimated breeding values are predicted for individuals which do not belong to the reference population. The advantage of this methodology is that once the reference population has been well characterized for different traits, subsequent individuals do not need to be phenotyped and their breeding values can be assessed solely with the use of markers.

Dirlewanger et al. (2020) published results from a preliminary study working with the simplest possible type of reference population: the offspring from the 'Regina' × 'Garnet' mapping population already used for QTL detection studies (Campoy et al., 2015; Castède et al., 2014). In this case, part of the population is used to build the model and the rest of individuals to validate it. A first analysis for trait flowering date showed a promising and high prediction accuracy of breeding values based on molecular information.

Once breeders have selected promising advanced lines, these must be evaluated in multiple environments to characterize their phenotypic plasticity, but this type of trials is quite expensive. For this reason, genomic prediction can also be used to inform elite selection advancement and cultivar deployment across multiple environments. Hardner et al. (2019) tested this approach on sweet cherry to predict maturity date of elite selection or modern cultivars in environments where they had not been evaluated. Limited genotype × environment (G×E) interactions were highlighted for this trait indicating that phenotypes of individuals were stable across similar environments.

Finally, genomic information can also be used to estimate genomic breeding values within breeding parents and unselected offspring. Piaskowski et al. (2018) conducted such type of study by analyzing 505 individuals from the WSU breeding program which had been phenotyped for 32 phenological, disease response and fruit quality traits over three years.

3. DNA-informed breeding

3.1. General considerations

Although MAS has been the main utilization of molecular markers that has been considered by breeders, many other possibilities exist. For this reason, several authors (Moose and Mumm, 2008; Peace, 2017) started to apply a wider terminology, such as marker-assisted breeding (MAB) or DNA-informed breeding. In the specific area of *Rosaceae* crops, several recent reviews have recalled the possibilities offered by molecular markers to increase the efficiency of breeding programs (Aranzana et al., 2019; Iezzoni et al., 2020; Laurens et al., 2018; Peace, 2017; Ru et al., 2015). For instance, Peace (2017) described in a highly detailed manner all the applications offered by DNA information, by considering the following possible operations: 'marker-assisted parent selection' (MAPS), 'marker-assisted seedling selection' (MASS), 'marker-assisted introgression' (MAI) and 'genomic selection' (GS). In the next sub-section, we will mainly give examples of MAPS and MASS implemented in sweet cherry breeding programs.

One of the objectives of recent European and USA large genomics research projects, such as FruitBreedomics or RosBREED, was to bridge what was called 'chasm' between genomics research and applied breeding. In order to establish the conditions under which MASS can be cost-efficient, several authors have modelled the costs of breeding operations over time (Edge-Garza et al., 2015; Luby and Shaw, 2001). Ru et al. (2016) studied genetic gain efficiency by conducting computer simulations and comparing several types of MASS to traditional seedling selection. Key parameters were broad-sense heritability (H) and broad-sense predictiveness (P) of the trait under study. Hence, when P>H, the DNA test captures most of the genetic effects and breeders can rely heavily upon this test, by conducting positive selection and keeping the class of seedlings with the best genotypic score. On the opposite, where P<H, the reliance on the DNA test is not so high, and in this case, breeders may just conduct negative selection by discarding the worst seedlings according to genotypic information.

3.2. Implementation in sweet cherry

The first and easiest utilization of DNA information by sweet cherry breeders is the genetic characterization of their breeding germplasm, in particular the most important breeding parents (IBP). Checking identity and the S-alleles carried by each IBP is compulsory in order to avoid creating hybrids from unwanted combinations or even worse, to avoid complete failure of a particular cross, if both parents share the same S-alleles. In the last years, several breeders from USA, France, Germany, Hungary, Spain and most probably other countries, have started to systematically characterize their IBPs for the FW_G2a QTL flanked by SSR markers CPSCT038 and BPPCT034. The combinations of alleles for each marker allow the establishment of two haplotypes for each cultivar studied. For many studied cultivars, the haplotypes correspond to those described by Rosyara et al. (2013) and hence, they can be associated to a positive or negative effect on trait fruit weight. However, as SSR markers are highly polymorphic, new alleles can be found, in which case the effect on the trait is considered unknown. Peace (2011) established four categories for fruit weight haplotypes: 'large fruit', 'small fruit', 'presumably small fruit' and 'unknown effect', by considering the information provided by Rosyara et al. (2013) on haplotypes and the available fruit size data for a panel of cultivars and selections.

At INRAE, following this terminology, 27 cultivars were characterized, including 10 INRAE released cultivars (see Table 3).

Table 3. Haplotype description of 27 cultivars used in the INRAE breeding program for the fruit weight QTL on linkage group 2 (FW_G2a) according to the allelic composition of the flanking SSR markers CPSCT038 and BPPCT034 (length in bp of the PCR amplification product for the corresponding primers is given). Green color corresponds to a positive 'large fruit' effect, red color corresponds to a negative 'small fruit' effect, yellow color corresponds to a negative 'presumed small fruit' effect and when no color is present the haplotype effect is 'unknown'. Cultivars were ordered according to the presence of favorable haplotypes.

Cultivar name	Haplotype 1		Haplotype 2	
	CPSCT038	BPPCT034	CPSCT038	BPPCT034
Ferdiva	190	255	190	255
Fermina	190	255	190	255
Feroni	190	255	190	237
Fertard	190	255	190	255
Fertille	190	255	190	255
Firmred (Late Garnet™)	190	255	190	255
Folfer	190	237	190	255
Garnet	190	255	190	255
Kordia	190	255	190	255
Vanda	190	255	190	255
Fercer (Arcina™)	190	255	190	253
Ferdouce	190	255	204	255
Grace Star	190	255	204	223
Regina	190	255	204	223
Stacatto	190	255	204	255
Sweetheart	190	255	204	255
Bedel (Belise™)	190	255	190	223
Burlat	190	237	190	223
Maraly (Earlired ™)	190	255	190	235
Belge	190	255	190	257
Noire de Meched	190	255	190	257
Ferprime	190	255	192	223
Rainier	190	255	204	235
Samba	190	255	204	235
Viva	190	255	204	235
Ferobri	190	223	190	223
Lapins	204	235	204	255

This information allows the establishment of crosses for which breeders will have the guarantee of obtaining seedlings with favorable haplotypes for this QTL. However, FW_G2a QTL explains, according to the genetic background and year considered, between 20 and 50% of the phenotyping variance. This means that the other fruit weight QTLs will still play an important role in the new seedlings' fruit weight. Breeders will not be able to use this DNA test to perform MASS when crossing cultivars which both share favorable haplotypes, as for instance, cultivars 'Folfer' and 'Firmred'. However, if a breeder chooses to cross 'Folfer' with 'Lapins' or 'Grace Star', in order to introgress self-fertility of the latter into 'Folfer', he may use the FW_G2a DNA test and eliminate all seedlings which will have inherited the unfavorable haplotype from 'Lapins', '204/235', or the haplotype with unknown effect from 'Grace Star': '204/223'.

A third DNA test which can be very useful to plan crosses is the one related to the major QTL for fruit color on LG3 (Sandefur et al., 2016). Indeed, if breeders want to make sure that all seedlings produce red (or mahogany) fruit, since the allele associated to 'red' phenotype is dominant versus the one linked to 'blush' phenotype, they will have to choose at least one cultivar which is homozygous for the 'red color' allele. Otherwise, by crossing two heterozygous cultivars, such as 'Regina' and 'Lapins', a quarter of the offspring will produce blush fruit (Quero-García, comm. pers.). Alternatively, if one breeder aims at creating new blush cultivars, by crossing a blush-type cultivar, such as the highly popular cultivar 'Rainier', with a red-fruit type cultivar, he will have to make sure that the latter is heterozygous for the fruit color QTL. Then, at least 50% of the resulting offspring will produce blush fruit.

Today, the sweet cherry breeding program which has implemented MASS in a more systematic way is the one carried out at WSU, as described in Quero-García et al. (2019). The molecular markers used are SSRs and the program proceeds in a multilayered way. A first set of DNA tests is applied to characterize genitors and carefully plan crosses. Apart from self-fertility (Haldar et al., 2010) and fruit weight, a non-published DNA test is also performed to characterize the fruit maturity major QTL located on LG4. The second round of tests is performed on seedlings for self-fertility and fruit weight (when families segregate for these traits), by eliminating those non-self-fertile and those carrying unfavorable fruit weight alleles (or haplotypes). On the remaining seedlings, DNA tests are performed for fruit color and maturity date and allow breeders to sort seedlings into predicted market-class categories, such as early season mahogany, early season blush, mid-to-late-season mahogany and mid-to-late-season blush. Finally, a fourth DNA test can be performed on specific market classes. For example, a DNA test is performed only on the mid-to-late-season category for powdery mildew resistance. A last DNA test is performed on certain combinations to identify seedlings that are tolerant to rain-induced fruit cracking, based on markers flanking the pistillar end tolerance QTL identified on LG5 (Quero-García et al., 2021).

In order to help breeders to adopt DNA-informed breeding approaches, it is necessary to quantify the economic gain that can be generated, or, symmetrically, the resource savings. The program of WSU estimated to provide resource savings of 80K dollars for the periods 2010-2011 and 2013-2014, with 50 and 80% of 3000 and 3400 seedlings discarded in each period, respectively. At INRAE-Bordeaux, estimations do not rely on a methodology as precise as the one reported by Edge-Garza et al. (2015). However, empirical calculations were made for the period 2012-2016, during which MASS was applied on 1860 seedlings, out of which 949, representing 51%, were culled. When considering only direct costs for the breeding program, the savings reached approximately 5K dollars; however, when taking into consideration public salaries (most of the work devoted to the raising and field characterization of seedlings is performed by public servants), the savings exceeded 50K dollars (Quero-García, comm. pers.).

4. Perspectives

In terms of QTL detection and validation, research must still be conducted in order to better characterize these important genomic areas. First, QTL confidence intervals are still too large, in particular for

highly complex traits such as for example rain-induced fruit cracking tolerance. Second, most of studied traits appear as highly complex, the case of fruit weight with up to three potential QTLs on LG2 being one of the most significant. Fine mapping strategies would help disentangling the situation and proposing diagnostic markers for each of the considered regions. For FW_G2a QTL, for example, SSR markers CPSCT038 and BPPCT034 are approximately 16 cM apart (Klagges et al., 2013), meaning that approximately one-sixth of the seedlings will inherit a recombinant genotype between these two markers, which complicates the interpretation of the genotypic results. Third, QTL hotspots have been identified, in particular in LGs 2 and 4, and applying simultaneously MASS for several QTLs controlling different traits within these genomic regions will be challenging. Developing additional DNA markers that span these regions at smaller cM intervals to track recombination remains an important goal.

At INRAE, researchers have started the phenotyping of the large 'Regina' × 'Garnet' / 'Garnet' × 'Regina' population for other traits than flowering date (Branchereau et al., 2022). Preliminary unpublished results have already allowed to reduce the confidence intervals of several QTLs or to confirm intervals previously detected on the original 'Regina' × 'Garnet' population through multi-year analyses. A synthesis of numerous analyses has allowed the selection of stable QTLs with relatively narrow confidence intervals for traits maturity date, crop load, fruit weight, fruit firmness and tolerance to rain-induced fruit cracking. SNP markers present in these genomic regions have been transformed into KASP markers and are being tested for validation on the same materials as those studied by Branchereau et al. (2022).

As conceptually described by Peace et al. (2014) and further illustrated in sweet cherry by Rosyara et al. (2013) or Calle and Wünsch (2020), QTL discovery and validation through pedigree-based analyses is highly powerful. Nevertheless, it requires the creation of several inter-connected populations, out of which at least a few must have a relatively important size (Iezzoni, comm. pers.), as well as the genotyping and phenotyping of all individuals. Further, collecting this information for a maximum number of genitors involved in the pedigree of these populations will significantly enhance the accuracy of results. One of the limitations of numerous sweet cherry breeding programs is that often cultivars and breeding parents are of unknown origin or are derived from open pollinations, that is, only the maternal genitor is known. Another strategy for QTL discovery and/or validation is the above-mentioned GWAS performed on germplasm collections. Here, experience on other fruit crops such as apple suggests that a large genetic diversity must be used as well as a high number of molecular markers to precisely cover all genomic regions of interest (Durel, comm. pers).

Genomic selection is a promising tool that sweet cherry breeders might consider. Here again, the difficulty is the generation of a sufficiently representative training population, on which intensive phenotypic and genotypic data will allow the establishment of accurate predictive models. Contrary to what has been described in certain species, preliminary results of Dirlewanger et al. (2020) suggest that breeders would not need an extremely high number of molecular markers to genotype the seedlings under study. However, despite the decrease in the cost of genotyping, when the DNA extraction is added, the cost of the whole operation is still relatively high. In addition to DNA polymorphisms, other sources of molecular variation, such as transcripts or metabolites, have been proposed to be used for trait prediction. However, it is highly challenging to obtain large amounts of 'omics' data on the typically large breeding populations. For this reason, Rincent et al. (2018) proposed an alternative methodology based on near-infrared spectroscopy (NIRS) as a high-throughput, low cost and non-destructive tool to indirectly access the phenotypic variation. By using the spectra generated by NIRS technology to compute relationship matrices, these authors developed models for predicting complex traits in wheat and poplar, and coined this approach 'phenomic selection' (PS). Nsibi (2021) has recently compared this new strategy to GS on apricot, and results were highly promising.

In addition to new scientific approaches and methodological tools, sweet cherry breeders need to gain knowledge on a wider range of traits, in particular those dealing with the adaptation to biotic and abiotic stresses. Nevertheless, sources of allelic variation for several of these traits are yet unknown, and

a wider genetic diversity will have to be explored. Furthermore, these traits may be highly complex to evaluate on large numbers of seedlings and great expectations rely on new high throughput phenotyping platforms, such as those related to proximal or remote sensing, in combination with the burst of edge-computing and artificial intelligence technologies. Once sweet cherry breeders will start having access to DNA tests associated to a wider range of agronomic traits, new methodologies will have to be implemented to develop multi-trait or multi-QTL (in a context of resistance genes pyramiding, for example) DNA-informed breeding strategies. Finally, from a more technical and economic side, new platforms and private companies are expected to provide more efficient and cheaper services so that breeding can fully benefit from all these scientific advances.

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