

## Cherry breeding in the world: current analysis and future perspectives

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### Nuove tecniche e obiettivi nel miglioramento genetico del ciliegio

**Riassunto.** Il miglioramento genetico del ciliegio dolce è relativamente recente, se confrontato con altre colture di maggiore importanza. Nonostante ciò, negli ultimi 30 anni, sono state rilasciate numerose cultivar, il che ha contribuito ad un aumento significativo della produzione di ciliegie. Il più importante programma di miglioramento genetico pubblico partito nel XX secolo rimane tutt'ora attivo e nuovi programmi si stanno sviluppando in paesi come Cile, Cina o Spagna. Più recentemente, anche il settore privato ha iniziato a giocare un ruolo sempre più importante, in particolare, in California dove si sta conducendo un programma di miglioramento mirato allo sviluppo di cultivar a maturazione precoce e con un basso fabbisogno in freddo. Nonostante questo, la produzione di ciliegie deve affrontare sfide molto significative, sia in relazione alle conseguenze del cambiamento climatico, su scala mondiale (in particolare a causa del riscaldamento globale) che all'emergenza di nuovi parassiti e malattie (ad esempio la mosca della frutta, *Drosophila suzukii*). I breeder devono quindi aggiungere nuovi caratteri all'interno degli schemi di selezione, oltre a quelli tradizionali ed inevitabili come la produttività, il diametro dei frutti, la durezza e la qualità aromatica, ecc. Tuttavia, a causa di caratteristiche specifiche ed alla lunghezza del periodo di giovanilità della pianta, il miglioramento genetico è stato tradizionalmente limitato ad una base genetica piuttosto ristretta. Potrebbe quindi non essere semplice trovare alleli interessanti per caratteri di adattabilità agli stress biotici ed abiotici nel portfolio dei breeder. Per questa ragione, la preservazione e la caratterizzazione delle risorse di germoplasma dovrebbe essere considerata una priorità urgente. Con i recenti sviluppi tecnologici nell'area della biologia molecolare e della bioinformatica, l'uso delle informazioni basate sul DNA, attraverso un approccio di selezione assistita dei marker molecolari, è diventata una realtà per i genetisti che si occupano di ciliegio. Sebbene sforzi importanti nella ricerca siano ancora necessari per sbrogliare la determinazione genetica dei principali caratteri di interesse agronomico, queste metodologie consentono già una riduzione significativa nei costi di miglioramento. In questo articolo sono riportati i princi-

pali obiettivi e le metodologie attualmente utilizzate dai breeder che si occupano di ciliegio; successivamente vengono brevemente affrontate le diverse prospettive riguardanti le nuove direzioni da intraprendere e le presenti necessità.

**Parole chiave:** ciliegio dolce, miglioramento genetico, selezione fenotipica e genotipica.

### Introduction

Sweet cherry (*Prunus avium*) and sour cherry (*Prunus cerasus*) production worldwide reached, during the marketing year 2018-19 (April 2018-March 2019), 3,3 million metric tons (USDA Foreign Agriculture Service, Settembre 2018). Although it is not always easy to differentiate between the two species, it is generally believed that sweet cherry accounts for at least 2,2 million tons. There is a global slight increase in production, which is the result of the arrival to the cherry market of countries such as Chile or China. On the other hand, the production of countries such as France or Germany has largely declined (Bujdosó and Hrotkó, 2017).

In terms of breeding, sweet cherry is a very active species and, according to Sansavini and Lugli (2008), 230 new cultivars were released during the period 1991-2004. Hence, apart from numerous public breeding programs which remain active since several decades, new actors are emerging, in particular from the private sector. The most important active breeding programs were recently described in Quero-García *et al.* (2017a). These authors also presented a selection of cultivars having either global or local importance and/or being promising, derived both from public and private structures.

For these reasons, the situation of the sweet cherry sector could be regarded with optimism. Nevertheless, breeders and scientists should remain particularly alert in face of new threats that could very quickly jeopardize the production of this highly appreciated, but also highly fragile, fruit. Hence, the arrival, ten years ago, of an invasive pest such as *Drosophila suzukii* in the USA or in Europe has already dramatically impacted the production in numerous countries.

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Other diseases such as *Xylella fastidiosa* have not yet become a serious issue in cherry but remain a subject of concern. Moreover, the consequences of climate change are already visible in several growing regions and breeders will have to integrate more and more rapidly this new context.

The focus of this paper will be put on the most important practical and strategical aspects of sweet cherry breeding by covering in details the main objectives and methodologies currently considered by sweet cherry breeders. A final section will open up new perspectives at medium and long term.

### Current objectives in sweet cherry breeding

The main breeding goals for sweet cherry breeders have been previously described (Fogle, 1975; Bargioni, 1996; Brown *et al.*, 1996; Sansavini and Lugli, 2008; Kappel *et al.*, 2012; Quero-García *et al.*, 2017a; Dondini *et al.*, 2018; Quero-García *et al.*, in press). In this section, the most important traits will be discussed by classifying them, in a tentative way, by order of importance.

Undoubtedly, as for any other agricultural crop, yield is a fundamental trait for the economic sustainability of a sweet cherry grower. However, the prize paid per kg will primarily depend on fruit size. Ideally, a modern sweet cherry cultivar should be highly and regularly productive but producing as well large fruit. It is important though to recall that productivity and fruit size will also be dependent on agricultural practices, such as soil fertility, training systems and pruning, use of chemical growth regulators, choice of rootstock, etc. Nevertheless, hybrids producing small fruit less than 8 grams will be systematically discarded in the vast majority of breeding programs. Regularity of production, which is a component of yield over time, is becoming increasingly important in the context of modern intensive orchards, which are equipped with complex protection structures against biotic or abiotic stresses, representing very high initial investments for the growers. One way of minimizing the risks of production irregularity is by planting solely self-fertile cultivars, since the synchronization of blooming between the target cultivar and its pollinizer is not always easy to guarantee (for a review of the flowering biology of sweet cherry, and in particular of its gametophytic self-incompatibility system, see Herrero *et al.*, 2017).

The third most important trait, after yield and fruit size, is fruit firmness. Indeed, it is positively correlated with post-harvest shelf-life and is a key trait for the export-oriented productions. But it is also a highly

appreciated trait by consumers, in particular when associated to other positive attributes such as freshness, crunchiness, juiciness, etc.

Tasting quality is becoming more and more strategic in order to gain differentiation capacity, in a market which is already filled with a high number of cultivars. However, this trait will only be thoroughly evaluated in the last phases of selection. One example of a program which has recently put strong emphasis on tasting quality is the one of the University of Bologna, with its series of cultivars ‘Sweet’ (Lugli *et al.*, 2012).

Among the abiotic stresses, tolerance to rain-induced fruit cracking has been for a long time a fundamental trait for sweet cherry breeders. Today, numerous modern growers, thanks to the advent of a new generation of dwarfing rootstocks, but also because of recent pest outbreaks, such as the arrival of *Drosophila suzukii*, plant new orchards with anti-rain covers, or in confined structures such as tunnels or greenhouses. However, fruit from susceptible cultivars (such as ‘Brooks’) can also crack under these protective structures in the absence of rain, as long as a high relative humidity is present. For this reason, cracking tolerant cultivars are still highly appreciated and needed by growers. As it is a very complex phenomenon (see for review Knoche and Winkler, 2017), no reliable phenotyping protocols exist yet for a precise characterization of cultivars’ susceptibility/tolerance to cracking. Hence, breeders can only evaluate this trait by gathering several years of observations, and of course, in areas where rainfall is frequent at harvest time.

Resistance to winter frost has been traditionally an important breeding trait in Central and Northern European countries, where sweet cherry is cultivated at the margins of the traditional distribution area of this species. Oppositely, tolerance to warm temperatures has been gaining increasing attention in the last years. First, cherry production in several traditional areas is becoming problematic due to the increase of autumn and winter temperatures, which provoke an insufficient fulfillment of the chilling requirements for flowering. Second, there is a growing interest in expanding the areas of production towards regions/countries with warm climates, in the same way of what was accomplished more than one century ago for other Rosaceae crops such as apple and peach. The second most important trait which is directly linked to an increase of spring and summer temperatures is the susceptibility/tolerance to double fruit formation. When flower initiation occurs in newly developing buds, in general after harvest, temperatures

above 30°C will provoke a doubling of the pistil primordia. In the next spring, flowers with two pistils will be pollinated and will form double fruits, which are in many cases of non-commercial value (Wenden *et al.*, 2017).

Traditionally, sweet cherry has been a species much less sensitive to pests and diseases than other Rosaceae crops such as apple, peach or apricot. Nevertheless, with the implementation of high density orchards, one question is whether diseases will be more serious on these somehow artificially ‘weakened’ trees. The second reason which might fuel the interest in searching for new sources of tolerance/resistance to diseases is the growing social concern on the health and environmental risks related to pesticides use. Up to now, only specific programs have systematically worked on the tolerance/resistance to bacterial and fungal diseases, the most important being bacterial canker caused by *Pseudomonas* spp. and blossom blight and brown rot caused by *Monilinia* spp., respectively.

Other important goals for cherry breeders are the extension of the maturity period or the suitability for the mechanical harvest. Regarding the first goal, breeders from Europe and the US have, for long time, looked for cultivars reaching maturity as early or before ‘Burlat’, but showing a better fruit quality in terms of size, firmness and cracking tolerance.

## Methodologies

The same references given for the definition of major sweet cherry breeding goals can be consulted for the description of the methodologies. Once the objectives of a breeding program have been established, two main operations are needed: the creation of new hybrids, which will be developed giving quantitative data on the results of several breeding programs, and the selection of superior hybrids.

### Hybridization

Flowering biology in sweet cherry, as in other fruit trees, is a very complex biological process, highly influenced by numerous genetic and environmental factors. We have recalled the importance of traits related to adaptability to warm temperatures, both during summer (flower initiation stage), and during autumn/winter (endodormancy phase). However, even when optimal environmental conditions are met until the release of ecodormancy, other factors can still dramatically affect fertilization. These are related to stigmatic receptivity, pollen tube growth, pollen-pistil interaction and ovule fertility (Herrero *et al.*, 2017).

Most cherry breeders generate segregating F1 families through hybridization between inter-compatible parents. Self-fertile cultivars are most often used as paternal genitors, as they are considered universal donors and hence they can pollinate any cultivar. However, because these cultivars are often highly productive, it may be more appropriate to use them as maternal genitors. In that case, flowers must be emasculated (through removal of anthers) prior to pollination. When using self-incompatible maternal genitors, only pollinator exclusion through the use of bags or insect-proof nets is necessary.

In order to generate variability, breeders do not only conduct controlled pollinations but open pollinations as well. These allow breeders to test, according to the previous knowledge of the maternal parent’s agronomic attributes, whether a high percentage of superior offspring will be obtained from numerous pollinizers, which is traditionally called in quantitative genetics, ‘general combining ability’ (GCA). When doing controlled crosses, on the opposite, breeders seek to exploit the breeding value of pairwise parental combinations, what is called ‘specific combining ability’ (SCA). Controlled crosses can be achieved by hand-pollination, as explained above, or by the use of insect pollinators. In the second case, two possibilities exist: first, breeders can use confined structures where two parental genitors will be isolated and pollination will be ensured by bumble bees; second, fruit can be harvested from trees of two cultivars that are planted in isolated plots and where pollination is ensured by bees and wild bumble bees. A third possibility that has not yet been much explored in sweet cherry breeding is what is called poly-cross. In this case, breeders chose several cultivars that are inter-compatible and place them in confined structures with bumble-bees. For a particular seedling, a breeder will not know which cultivar is the paternal parent, but he will be sure that this parent can only belong to a certain group of potential genitors. In this context, by using molecular markers, a breeder might manage to assess paternity of all his hybrids and hence, he could end up producing subsets of controlled crosses and estimating SCAs. On the other hand, without the use of molecular markers, the hybrids derived from one maternal parent, will give him a ‘restricted’ vision of the GCA. All these possibilities are summarized in Figures 1 and 2.

One of the main advantages of controlled crosses versus open pollination is that one can cross cultivars that could not be inter-crossed in nature because their flowering periods are not synchronous. In general, when breeders cross early and late-blooming culti-

vars, they collect pollen from the early cultivar and use it to pollinate the late one. This is particularly true if the early-blooming cultivar is also a cultivar with an early maturity date, such as ‘Burlat’. Indeed, this type of cultivar produces in general seed with undeveloped embryos which require the use of rescue in vitro techniques. However, when the later blooming cultivar is less productive (or fertile) than the early one (for example, ‘Regina’ or ‘Fertard’ versus ‘Lapins’ or ‘Belise’), the latter will be used as maternal parent. In that case, pollen from the late-blooming cultivar can be harvested during the previous year, kept in a freezer (at -20°C) and used a year later to pollinate the early-blooming cultivar. When using confined structures and bumble-bees, synchronization of flowering can be achieved in two different ways. If potted trees are used, early-blooming cultivars can be placed in a double wall refrigerated storage to slow down the transition from endodormancy release to flowering. The second option is to speed up the flowering transition of late-bloom cultivars, by placing the potted trees in growth chambers, greenhouses, etc.

The use of any of these different techniques will depend on the breeding objectives and economic resources of breeders. Hand-pollination is cheap and

flexible. When rich collections of cultivars are available, a high level of genetic diversity can be exploited. It requires though skilled personnel for pollen handling, emasculation and pollination tasks. However, the success rate of hand pollination is rather low, unpredictable and random in sweet cherry; hence, a typical situation is that when a breeder performs 20 crosses, for only a few he will succeed in producing large families. For this reason, breeders might need to combine hand pollination with the use of bumble bees or isolated plots when collaborations can be established with growers or experimental stations. These two types of strategies can also be utilized when a breeder has gained information on the value of a specific cross and wants to repeat it but with the aim of producing hundreds of full-sibs. As for open pollinations, they might be a cheap and simple way to select a first set of genitors out of a large collection, based on their GCA.

*Seed germination*

Seed germination in sweet cherry can be as critical as fruit set to ensure the success of a breeding program. Detailed procedures can be found in Iezzoni *et al.* (2017) and numerous references exist concerning protocols for in vitro embryo rescue. Different possibilities for seed germination will be privileged, depending on the objectives and resources of each program. Hence, breeders who focus on open pollinations and manage to generate high amounts of seed might use a non-sterile stratification method to overcome endocarp dormancy, without the need of breaking each stone. When breeders concentrate most of their efforts on controlled crosses and hence produce a lower number of seeds than in an open-pollination context, it is recommended to remove the endocarp manually to accelerate and improve the release of dormancy imposed by the endocarp. Finally, when seeds are collected from very early maturing cultivars, such as ‘Burlat’, ‘Earlise’, ‘Early Star’, etc., in vitro embryo rescue will allow a higher percentage of germination of undeveloped embryos. Nevertheless, the success rate of embryo culture is extremely variable between years and cultivars (Balla and Brozik, 1996).

Quantitative data on the variability of fruit set and seed germination is not abundant in literature but several programs published data. In 2008, Gradinariu *et al.* described the results of breeding activities at Iasi Fruit Research Station, Romania, covering a long period, from 1981 to 2004. Out of 706365 pollinated flowers, 133150 hybrid fruits were harvested, which represents a fruit set rate of 18.8%. However, only 13453 seeds were viable, yielding in the end 6493 seedlings, which represents a global success rate

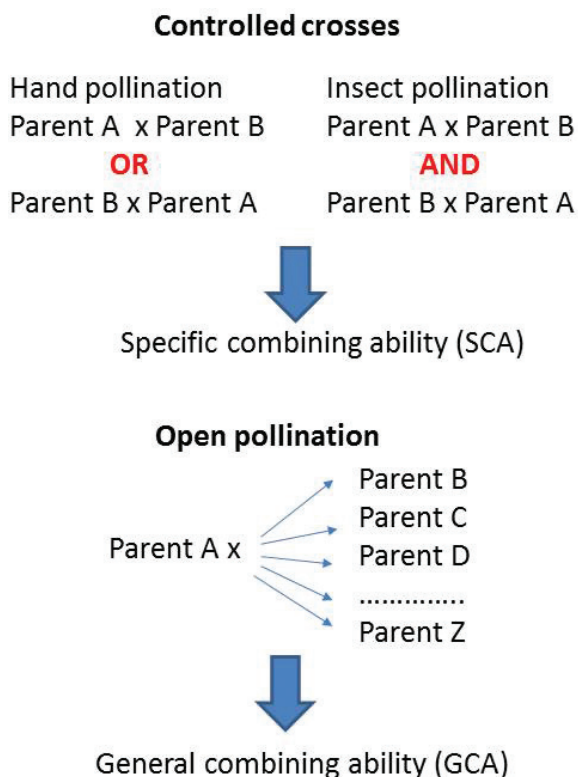


Fig. 1 - Schematic representation of the breeders possibilities in terms of hybridization: controlled crosses and open pollination  
 Fig. 1 - Rappresentazione schematica delle possibilità che hanno i genetisti in termini di ibridizzazione: incroci controllati ed impollinazione aperta.



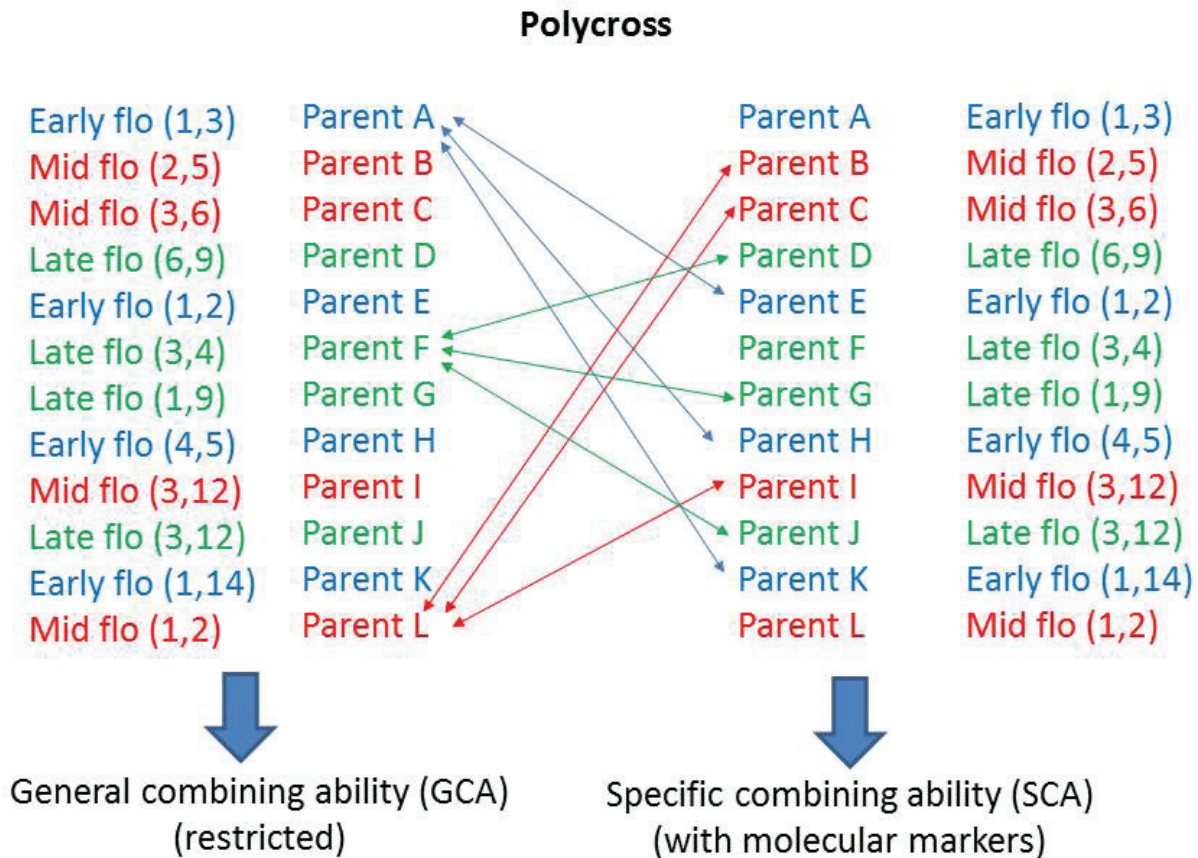


Fig. 2 - Schematic representation of the breeders possibilities in terms of hybridization: polycross. Randomly chosen alleles of the gametophytic self-incompatibility (GSI) system are indicated into parenthesis. Only the possibilities for one parent of each class (early, mid or late flowering) are indicated.

*Fig. 2 - Rappresentazione schematica delle possibilità che hanno i genetisti in termini di ibridizzazione: i poli-incroci. Gli alleli scelti casualmente nel sistema di auto-incompatibilità gametofitica sono indicati tra parentesi. E' indicata una sola possibilità per un genitore di ogni classe (a fioritura precoce, media o tardiva).*

(from flower to seedling) of 0.91%. The variability between years was relatively high for fruit set, with a maximum of 35.7% and a minimum of 5.8%. For the global rate of success, it ranged from 0 to 2.9%. When considering all harvested fruit, average germination rate was of 5%, with a maximum of 25.2 % and a minimum of 0%.

López-Corrales *et al.* (2014) published the results of the breeding program recently initiated in the Jerte Valley, Extremadura, Spain, by differentiating between individual crosses made during the period 2006-2008. Out of 36906 flowers pollinated, 6334 fruits were harvested, which represents an average fruit set of 17.2%. Among all harvested fruit, 90.4% contained viable embryos, and an average germination rate of 31.9% was achieved. The global success rate (from flower to seedling) was of 4.9%. Across years, fruit set ranged between 13.5 and 20.6% whereas germination rate was particularly low in 2006 (1%), as compared to 2007 and 2008 (44.7 and 46.9%, respectively). Authors did not specify whether technical problems were encountered in 2006, putati-

vely explaining such low level of germination. As for the female genitors used, fruit set was highly variable, ranging from 0 to 41.6%, as well as germination rate, ranging from 0 to 62.6%. Interestingly, 98.7% of the seedlings produced came from three cultivars, 'Ambrunés', 'Hudson' and 'Pico Colorado', which were involved in 47% of all combinations. Cultivar 'Hudson' appeared in the Jerte Valley as a very good genitor, with both fruit set and germination rates above 40%. This result might explain the overall difference in global success rate between this program and the one at Iasi, Romania.

At INRA-Bordeaux, data from the last 11 years, corresponding to a new phase of the breeding program, has recently been synthesized (unpublished results). Concerning hand-pollinations, results are presented in Table 1. Percentage of fruit set ranges from 0 to 8.2%, with an average of 4.7%, whereas germination rate shows a much higher variability, with values comprised between 6.3 and 71.9% and a mean value of 21%. Overall, the global success rate (from flower to fruit) is in average almost equal to

1%, ranging from 0.3 to 2.6%. Hence, the program at INRA has a significantly lower average fruit set as compared to those of Romania (Iasi) or Spain (Jerte Valley); however, germination rate is higher than in Romania, both programs having in the end very similar global success rates. Twenty-two maternal genitors were used, showing high variation in terms of fruit set and germination rates, with ranges of 0-19.1% and 0.3-84.9%, respectively. One possible explanation of the lower average fruit set observed at INRA, as compared to the two other programs, might arise from the unequal number of pollinated flowers among maternal genitors. Indeed, cultivars ‘Fertard’, ‘Regina’ and ‘Fermina’ represented 24.3, 21.1 and 14.7% of all the pollinated flowers, and the percentage of fruit set on these cultivars was of 1.96, 4.3 and 1.8%, respectively. This result was not too surprising as it is well known that these cultivars show fertility issues and irregularity of production, partially due to their high chilling requirements for flowering. Luckily, ‘Fertard’ showed a very high percentage of germination, close to 66%, compensating for the observed low level of fruit set. As for the Jerte Valley program, the size of families at INRA-Bordeaux is extremely variable, with only 31% of the families formed by more than 50 seedlings.

The high variability and unpredictability observed in sweet cherry breeding programs, in terms of fruit set and germination rates, is one of the main reasons why most programs produce a relatively modest number of hybrids per year, if compared to other fruit crops such as apple or peach. Among the breeding programs described in Sansavini and Lugli (2008), data on the number of seedlings produced per year was given for 12 of them, ranging from 80 to 1000.

Nevertheless, several breeding programs, both private and public, manage to produce today several thousands of hybrids per year (Courtney, 2016; Quero-García *et al.*, 2017a).

As already mentioned, some crosses yield a much higher number of hybrids than others. On the other hand, several agronomical important traits are only present in few cultivars. This is the case of self-fertility (cultivars ‘Stella’ and its descendants, such as ‘Lapins’ or ‘Sweetheart’) or of cracking tolerance (cultivars ‘Regina’ or ‘Fermina’). Furthermore, breeders quickly realized that numerous combinations yielded a very high percentage of hybrids with undesirable traits, in particular small and soft fruits and hence, restricted their initial ambitions to those crosses yielding an acceptable percentage of good quality hybrids. In recent genetic studies (see section 3-4), the dominance of unfavorable alleles for fruit weight or fruit firmness (Zhang *et al.*, 2010; Cai *et al.*, 2018), was confirmed, explaining this situation. Altogether, these factors account for the narrowness of the genetic base traditionally used by cherry breeders. Anderson (1998) wrote that each breeding program used, on average, four to six main genitors and Choi and Kappel (2004) described the inbreeding and co-ancestry in North American breeding programs. The fact that the genetic base of modern commercial cultivars is narrow does not only arise from the utilization of a reduced number of parental genitors. This can be linked to the weight breeders will put into certain goals. For instance, during the first phase of the INRA breeding program, former breeders highly prioritized the production of hybrids with firm and large fruit and hence, cultivar ‘Fercer’, which was the first INRA cultivar to present these attributes, is the parent of

Tab. 1 - Statistical results deriving from manual pollination crossings made at INRA-Bordeaux from 2008 to 2018.  
Tab. 1 - Risultati statistici derivanti da incroci da impollinazione manuale fatti presso INRA-Bordeaux dal 2008 al 2018.

Year	Nb of crosses	Nb of flowers	Nb of harvested fruits	Nb of seedlings	% Fruit set	% Germination	% Success
2008	29	42346	2019	128	4.8	6.3	0.3
2009	39	55682	2044	157	3.7	7.7	0.28
2010	18	44140	2370	730	5.4	30.8	1.65
2011	15	20299	1129	142	5.6	12.6	0.7
2012	2	5361	0	0	0	-	0
2013	4	8296	182	93	2.2	51.1	1.12
2014	6	11068	89	64	0.8	71.9	0.58
2015	22	16751	1380	441	8.2	32	2.63
2016	12	15382	407	260	2.6	63.9	1.69
2017	10	23894	1232	255	5.2	20.7	1.07
2018	13	22896	1636	355	7.1	21.7	1.55
Total / Means	170	266115	12488	2625	4.7	21	0.99

most of the newest INRA cultivars (Quero-García *et al.*, 2017b).

#### *Phenotypic selection*

Although each breeding program will use its own customized phenotyping protocols, published protocols exist (Chavoshi *et al.*, 2014).

In the first phase of selection, the phenotyping is based on field observations and not on quantitative lab-based measurements. In most programs, the following traits will be taken into account: blooming and maturity periods, yield precocity, productivity, fruit size, firmness, appearance (including the lack of defaults) and eating quality. For certain programs, tolerance or resistance to abiotic or biotic stresses will already be qualitatively assessed at this stage, including tests in controlled conditions. A minimum of two years of observations are needed to conduct the first hybrids' selection although three years are often employed by breeders.

In a second phase, the best performing selections will be grafted on one or several rootstocks to be evaluated at one or multiple locations. A minimum of two replicates per hybrid is necessary, although the higher the number, the most precise will be the phenotypic evaluation. In the same way, choosing well contrasted sites in terms of soil, climate, pathogen pressure, etc, will allow breeders rapidly assessing the phenotypic plasticity, or, what is called in quantitative genetics, the genetic  $\times$  environment (G $\times$ E) interactions, of their selections. At this stage, productivity will be evaluated more precisely in order to produce yield estimates and fruit weight/size and fruit firmness will be quantitatively assessed in the lab, as well as key quality parameters such as sugar and acid content.

Depending on the strategy of each program or on the value of the most promising hybrids selected in the second phase, a second round of multi-site trials can be implemented or selections can be planted directly in pre-commercial trials.

Juvenility will always be an obstacle to achieve rapid gains in sweet cherry breeding. In order to shorten the juvenility phase, breeders have several options. The most straightforward is the use of highly dwarfing rootstocks, able to bear fruit two or three years after being planted in the field. However, this will considerably increase the cost of a breeding program. A more economical way may be to adopt agronomical practices to reduce vigor of hybrids planted on their own roots, by inducing rapid lateral ramification, bending of shoots, etc. Another way of increasing genetic gain is to increase the heritability of the studied traits, that is, to improve the quality of phe-

notyping and hence, reducing the impact of environmental variance. This can be achieved by grafting two instead of one copy of each seedling. If using extra-dwarfing rootstocks, the space allotted to two plants might be equivalent to that of a seedling planted on its own roots. Again, this will however increase the cost as the double of rootstock mother plants will have to be purchased. Finally, a third option to cut down the costs is to reduce the area used for selection. In the first breeding program launched in Chile (E. Gratacos, pers. comm.), up to three hybrids were grafted upon the same rootstock mother plant. This strategy requires high technicity and special care in the labelling of branches. On the other hand, evaluation of each hybrid will only be based on a fraction of a tree, and on a relatively limited number of fruit.

#### *Genotypic selection*

In the last 20 years, molecular assisted selection (MAS) has become a highly promising tool for sweet cherry breeders. The recent revolution brought by the next-generation sequencing (NGS) technologies allowed an extremely rapid decrease in the cost of sequencing. Hence, the genome of sweet cherry has been recently completely sequenced (Shirasawa *et al.*, 2017). Moreover, it is today much easier to generate at a low cost thousands of polymorphic markers, named SNPs (Peace *et al.*, 2012), which are subsequently used to study the regions of the genomes controlling the variation of important agronomic traits of interest.

Nevertheless, the discovery of such regions, as well as the deployment of MAS strategies, is always a laborious process. The most common methodology is the mapping of QTLs ('Quantitative Trait Loci') in segregating families, derived from biparental crosses. First, the genotyping of all progenies allows the construction of parental genetic maps. Second, by phenotyping the same progenies and analyzing jointly genotypic and phenotypic data, QTLs are detected and mapped onto the parental chromosomes, or linkage groups (LG). In general, phenotyping has to be performed during several years in order to validate the stability of a given QTL. Once researchers are confident on the significance and utility of their QTLs, the latter have to be validated in other genetic backgrounds before diagnostic markers can be widely transferred to breeders. The most important parameter in QTL detection is the trait's percentage of phenotypic variation explained by the QTL. However, it is also fundamental that this QTL is detected in a relatively precise way, in terms of statistical confidence interval.



Results from genetic mapping and QTL detection studies have been recently reviewed (Quero-García *et al.*, 2017a; Dondini *et al.*, 2018; Quero-García *et al.*, in press). Only the most relevant studies in terms of QTL discovery and implementation of MAS approaches will be cited. Sweet cherry is characterized by a majority of polygenic traits, with only very few traits of interest controlled by a single locus, in contrast to what is observed in peach. Today, only self-fertility and fruit color have been described as being monogenic and are being used in routine in MAS programs (Haldar *et al.*, 2010; Sooriyapathirana *et al.*, 2010; Sandefur *et al.*, 2016).

Concerning more complex and quantitatively inherited traits, studies have focused primarily on bloom date (including its components chilling and heat requirements) and maturity period (Dirlewanger *et al.*, 2012; Castède *et al.*, 2014), fruit weight (Zhang *et al.*, 2010; Rosayara *et al.*, 2013; de Franceschi *et al.*, 2013), fruit firmness (Campoy *et al.*, 2015; Cai *et al.*, 2018), cracking tolerance (Quero-García *et al.*, 2014; Quero-García *et al.*, 2017b) and fruit acidity (Quero-García *et al.*, in press). The most interesting QTLs are represented in Figure 3. Two chromosomes, or linkage groups (LG), 2 and 4, are highlighted as some of the most significant QTLs, for important agronomical traits, have been detected in these LGs, which are hot-spots of recombination and regions which have been subject to intensive selection (Cai *et al.*, 2017).

Up to date, the most important QTL in terms of MAS is the one located on LG 2, explaining, depending on the family and year considered, up to 30% of the phenotypic variance of fruit weight. Hence, this QTL has been related to domestication and most modern commercial cultivars contain either two favorable alleles (homozygosity) or one favorable and one unfavorable allele (heterozygosity) (Peace, 2011). Although numerous other QTLs influence fruit size, it seems highly likely that a hybrid possessing one unfavorable allele at the QTL on LG2 will not produce fruit extremely large, let's say, larger than 10-12 g. For this reason, it is of utmost interest for breeders to eliminate from the greenhouse stage, the seedlings containing the unfavorable allelic combinations.

A review on the numerous applications of DNA-informed breeding of rosaceous crops was recently published by Peace (2017). Apart from classical MAS approaches, other applications that have been recently implemented in sweet cherry are the determination of genome-wide breeding values of breeding germplasm across numerous traits (Piaskowski *et al.*, 2018) and the prediction of maturity date into new environments (Hardner *et al.*, 2019). Concerning MAS in sweet

cherry, savings of 80000 dollars were estimated by the elimination of almost half of 3000 seedlings tested in 2010 and 2011 in the Washington State University (WSU) program. At WSU, MAS concerns in routine the following traits: fruit color, fruit weight, maturity date and self-fertility. So far, at INRA-Bordeaux, MAS has only been applied for the trait fruit weight. An economic evaluation of the savings generated by MAS activities has not yet been conducted but 51% of seedlings were eliminated from 11 families (only families with over 100 seedlings were considered for MAS), created within the period 2012-2016. Overall, 949 seedlings were culled from a total of 1860, with a selection rate (in this case 'negative selection' or in other words, 'elimination rate'), ranging from 38 to 67%.

## Perspectives

As for all crops which have undergone long lasting efforts in the field of breeding and selection, the importance of genetic resources will become increasingly higher for sweet cherry breeders. Although sweet cherry is a relatively 'rustic' species, there are not enough favorable alleles in today's cultivars for traits of adaptation against biotic and abiotic stresses. For this reason, breeders are paying more attention to the collections of genetic resources held by numerous countries. However, there is much room for improvement in the management of these collections and a much stronger international collaboration is needed to efficiently evaluate them in terms of phenotypic and genotypic diversity, as well as to foster materials exchanges. One of the aims of COST Action FA1104 (<https://www.bordeaux.inra.fr/cherry/>) was to develop this type of collaborations. Hence, this network contributed to the launching of a small ECPGR (European Cooperative Program for Plant Genetic Resources)-funded project, EuCherry, which has allowed analyzing with 14 molecular markers (SSR, or microsatellites) a set of 324 cultivars (either 'modern commercial' or 'local landraces' type cultivars) from 18 countries. Furthermore, three breeders from Germany, Spain and France participated in 2014 to a mission aiming at collecting sweet and sour cherry stones from Azerbaijan, in the supposed area of origin of these species (López-Ortega, 2015). It is however clear for cherry breeders that using old landraces, or even wild materials (mazzards), will entail the need of conducting several rounds of backcrosses in order to get rid of the genetic load from these materials. For these reasons, it might be convenient to organize breeding efforts in a way that prospective



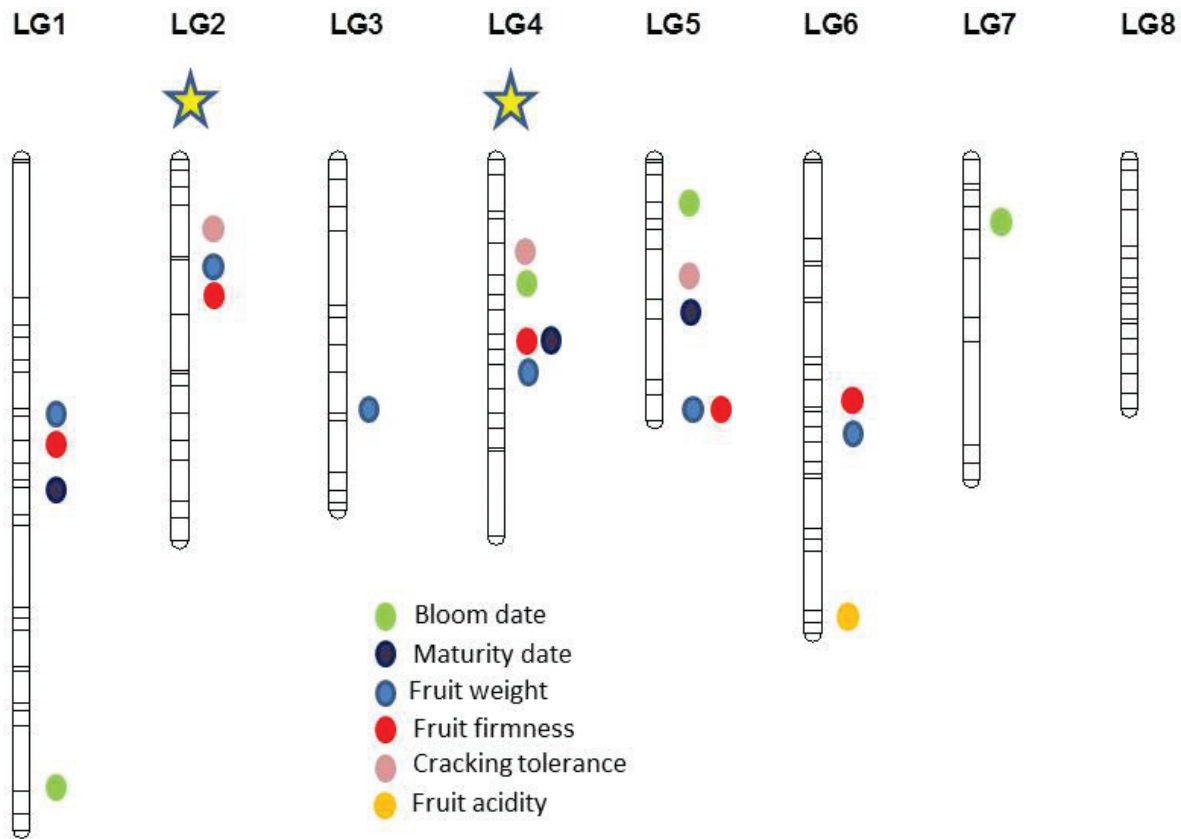


Fig. 3 - Approximate genomic position of the QTLs detected for the most important agronomic traits in sweet cherry (LG: Linkage group).  
 Fig. 3 - Posizioni genomiche approssimative dei QTL rilevati per le più importanti caratteristiche agronomiche in ciliegio dolce (LG: linkage group).

crosses, that is, the so-called, pre-breeding activities, are conducted by public institutions whereas the classic commercial breeding is mostly achieved by private companies. This is the strategy that has been set today at INRA for the fruit tree species with active on-going research programs (apple, pear, peach, apricot, sweet cherry, *Pyrus* and *Prunus* rootstocks), in close collaboration with its major private partners, CEP Innovation and Novadi.

In the field of genomics and MAS approaches, there is still great progress to accomplish in terms of QTL characterization but most importantly, in terms of translational work from scientists to breeders. Nevertheless, this will only be achieved when breeders (either from public or private institutions) will be convinced that the investment in this field is worth it. Hence, research has to be conducted as well in the practical, logistic and economic implementation of such programs. Today, the only program which has seriously adopted MAS, as a major and systematic breeding strategic tool, is the one conducted at WSU. Concerning research activities, several teams focus their efforts in the improvement of the QTL detection and characterization, by a variety of approaches. For example, at INRA-Bordeaux, fine mapping strategies

are being implemented, by working with a family of 1400 full-sibs derived from a cross between cultivars 'Regina' and 'Garnet', in order to clearly separate close QTLs within 'hotspot' regions such as those in LGs 2 or 4.

In the field of phenotypic selection, less research efforts have been made as compared to the area of genomics. Nevertheless, techniques such as NIR (Near-infrared) spectroscopy are being tested in order to facilitate high throughput phenotyping of complex traits such as fruit firmness, sugar and acid content (Quero-García *et al.*, in press). Imaging techniques, whether through the use of cameras or drones have not been yet widely tested. They could be particularly useful for traits related to phenology, disease resistance or cracking tolerance. Another factor that will greatly improve the efficiency of a breeding program is to have, as soon as possible, a sound assessment of the adaptability (through the estimation of G×E interactions) of new selected hybrids. While most breeding programs will evaluate the selected hybrids, after the first evaluation phase, at different sites, it is less common to directly replicate all initial hybrids and plant them in different environments. An alternative, and less expensive option, which has been for

example implemented at INRA for the apple breeding program (François Laurens, personal communication) is to divide one family in equal lots of seedlings and plant them at different locations. Obviously, this is feasible and advisable when families are large; hence, it could be an interesting strategy when working with open-pollinations and seeking at the evaluation of GCA. As this type of replicated trials can be highly expensive, breeders might launch collaborative networks, in which their best selections could be tested in other breeders' plots. In the European context, this would allow testing hybrids in extremely variable conditions. This type of initiative is today under discussion within the framework of the 'Sweet and sour cherry group' of EUFRIN (European Fruit Research Institutes Network) and could soon be implemented.

In conclusion, given all the difficulties and constraints faced by sweet cherry breeders, three goals should be definitely pursued: first, an intensification of collaboration between breeders, between scientists, and between breeders and scientists, within and between countries; second, the strengthening of links between public research institutions and private breeders, but also growers, nurserymen and in general, all actors involved in the cherry sector; third, a higher organization and structuration of the sweet cherry sector, in particular within the European Union.

### Acknowledgements

The research and breeding work conducted at INRA-Bordeaux in sweet cherry is a collective mission accomplished by the A3C team ('Adaptation of Sweet Cherry to Climate Change') within UMR BFP, with the precious help of the personnel from the UEA (Fruit Tree Experimental Unit). Breeding activities are achieved through a bilateral cooperation with the nurserymen consortium, CEP Innovation. Special thanks are also due to numerous breeders and colleagues for fruitful discussions and collaborations with a special mention to Amy Iezzoni, Cameron Peace, Mirko Schuster and Gregorio López-Ortega.

### Abstract

Modern cherry breeding is relatively recent, as compared to other major crops. Nevertheless, in the last 30 years, numerous cultivars have been released, which has contributed to a significant increase in cherry production. The most important public breeding programs launched during the XXth century remain still active and new programs emerge in coun-

tries such as Chile, China or Spain. More recently, private actors are playing an increasingly important role, in particular those located in California, which conduct breeding preferentially for early-maturing and low-chilling cultivars. Nevertheless, cherry production faces significant threats, either related to the consequences of the global climate change (and in particular to the global warming) or to the emergence of new pest and diseases (such as for example the fly *Drosophila suzukii*). Hence, breeder have to incorporate new traits into their selection schemes, on top of the traditional and unavoidable ones, such as productivity, fruit size and firmness, tasting quality, etc. However, because of specific characteristics and of the length of the juvenility period, breeding has been traditionally limited to a rather narrow genetic base. Thus, it might not be straightforward to find interesting alleles in the breeder' portfolios for traits of adaptability to biotic and abiotic stresses. For this reason, the preservation and characterization of germplasm resources should be considered as an urgent priority. With the recent technological developments in the area of molecular biology and bioinformatics, the use of DNA-based information, through molecular marker-assisted selection approaches, has become a reality for cherry breeder. Although important research efforts are still needed in order to disentangle the genetic determinism of the main traits of agronomic interest, these methodologies allow already significant reductions in the breeding costs. In this paper, the major goals and methodologies currently considered by sweet cherry breeder will be reviewed, and perspectives with regards to new directions and needs will be briefly developed.

**Key words:** sweet cherry, breeding, genitor, phenotypic and genotypic selection.

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