Quality oriented fruit breeding: Peach [*Prunus persica* (L.) Batsch]

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Abstract

Promising new tools for peach fruit quality breeding have been revised in this work. These tools included fruit quality evaluation through physical, chemical and sensorial parameters and post-harvest storage evaluation. The development of a feasible method for an early testing of peach selections generated in breeding programs is also well described together with the discussion of the inheritance of the main fruit quality traits in peach. In addition, non-destructive evaluation methods such as near-infrared, electronic nose and non-destructive impact response were revised. Finally several strategies for the development of molecular marker associated to fruit traits were also revised. Methodologies for the analysis of marker-assisted selection include the use of mapping populations segregating for desired characters. To date, twenty five monogenic genes and QTLs have been mapped in different peach genetic linkage maps. Other markers being used included expressed sequences tags (ESTs) cloned gene analogs (CGAs) and single point mutations (single nucleotide polymorphisms, SNPs). More recent efforts are being oriented to the elaboration of physical maps and the complete sequencing of the peach genome.

Key words: Consumers, fruits, genotypes, molecular markers, new cultivars, peach, quality, sensory evaluation, marker assisted selection.

Introduction

Peaches [*Prunus persica* (L.) Batsch] and nectarines [*P. persica* (L.) Batsch, var. *nectarina*] belong to the Rosaceae family and are thought to have originated in China [132]. The relatively short time, as compared to other fruits, between early project steps (e.g., crossing) and obtaining of marketable products, and the consistent interest in new cultivars makes it an interesting model fruit species for describing a quality oriented breeding approach. Breeding practices in peach also face unique challenges resulting from the narrow genetic background of commercial cultivars [64, 135], the large plant size and the differences in trait expression between juvenile and mature trees [136]. Recently, peach breeding programs have also suffered a dramatic cut-off of public economic support which has forced many programs to close [83]. On the other hand, peach consumption remains low and stable at approximately 5.9 pounds per capita per year [38, 39]. The challenge for breeders is to capture the interest of peach consumers, and the proper way to reach this task is offering consistently good quality products [83]. The market for new peach varieties is quite dynamic, and new typologies of fruits have been developed on last years. Non-melting and stony hard flesh, low-acid, saucer shape and deanthocyanic epidermis are the new typologies that breeders are proposing to the fruit industry looking to satisfy consumer expectation and generate product differentiation at the selling point (Fig. 1).

The introduction of new cultivars on the market is vital for fruit industry to get better products and to attract consumers with innovative fruit types. However, in an extremely competitive market as the present the path from field selection to commercial production is becoming always more uncertain and expensive. Furthermore, market has a tendency to split into two classes of produce: high-quality and commodity (low price) [131], and new cultivars should aim to reach the highest level. Breeding and marketing should find closer relationships to allow new released cultivars to maintain long-term premium prices. Ferguson *et al.* [66] propose a "club variety strategy" to control planting and marketing

Figure 1. Acceptability assessment of fresh peach selections developed by a peach breeding program and applied to 40 untrained testers executed at the University of Chile campus in Santiago.

to provide a profitable specialty crop. In the case of fruit such as peach and nectarine that need to be maintained for a long post-harvest period, preconditioning of fruits is an innovative treatment that allows extending storage life and quality [5, 99]. These strategies can be successful if breeding is developed on clear genetic bases aimed to reach excellence in quality. A present-day successful breeding strategy cannot be based anymore, as it was in the past, for the most part on breeder's instinct. In fact, 79% of the breeding programs, only the breeder and/or co-workers are in charge of assessing the fruit quality and only 14% of them use a trained panel within their evaluation and selection procedures [83].

Quality assessment can find new implements thanks to the technological development of destructive and non-destructive instruments. This can support early selection of breeding lines based on quality traits. Sensory techniques can be of help in understanding fruit quality; peach and nectarines appreciation is based on specific eating traits that can be studied to develop standard protocols of sensory evaluation [60]. Crisosto *et al*. [45] suggest that cultivars should be classified in organoleptic groups and promote the development of a minimum quality index within different groups, rather than accepting the commonly used generic minimum quality index based on ripe soluble solids content. In the last decade, many techniques such as molecular markers have become available for peach crop breeding. These methods are now moving from laboratory evaluation to field application [16, 104, 134]. Molecular approach can strengthen any step of a breeding program supporting breeder's experience in decision making with a detailed genetic knowledge [100].

This article offers an overview of the current approaches being developed to optimise breeding for fruit quality in peach. These approaches include fruit quality evaluation trough physical, chemical and sensorial parameters and post-harvest storage evaluation. In addition, non-destructive evaluation methods such as near-infrared, electronic nose and non-destructive impact response were revised together with the discussion of the inheritance of fruit traits. Finally, recent strategies for molecular marker development associated to fruit traits were revised.

Fruit Quality Evaluation

Physicol-chemical parameters: Intrinsic peach quality can be investigated through several physical and chemical parameters. An effective approach for new cultivar development should be based on getting better knowledge of genetic and physiological bases of specific parameters, chosen for their major importance on overall intrinsic fruit value such as healthy substances or consumer perceived quality such as aroma and texture.

Rodríguez *et al*. [130] performed a detailed monitoring of fruits during storage through 21 parameters related to nutritional composition and consumer acceptability. The parameters evaluated were: weight, pH, titratable acidity, soluble solids concentration (SSC), moisture, vitamin C, soluble sugars, protein, dietary fibre (NDF, ADF, peptic substances), ash and mineral contents (macro-elements: Na, K, Ca, Mg and P; microelements: Fe, Cu, Mg, and Zn). Most studies focus on a more limited number of parameters, and it would be of importance to identify those of major importance for quality. On the other hand, generic single quality index have been developed, based on SSC [39, 43, 44, 79, 114]. However, according to Crisosto *et al*. [45] they do not seem able to decrease confusion in the market and do not contribute to provide solutions for consumption problems. In fact, sweetness perception is known to be affected by a number of parameters, in particular acidity, with the overall consumer appreciation related more to the titratable acidity/refractometric index ratio than to the SSC alone [111]. Recent studies propose a model to predict acidity of peach fruits [95].

Substances with positive effect on health are increasing their importance defining fruit quality. Antioxidants such as phenolics, thiols, carotenoids and tocopherols, which may protect against chronic diseases, are present in peach and can be considered as a part of cultivar value [46]. In this sense, the selection of peaches with high concentrations in phenolic compounds and enhanced antioxidant, antimicrobial and colorant properties, would be a first step in the development of new varieties with better functional properties [32]. On the other hand, fruit volatiles production is determined by genetic traits and affected by pre- and post-harvest condition. These substances can be analyzed by several techniques [128]. The formation of aroma compounds is a dynamic process connected to fruit ripening. During this process, concentrations of volatiles change both qualitatively and quantitatively. In peaches and nectarines, volatiles include esters, aldehydes, benzyl alcohol and limonene, benzaldehyde, hexanal, 2-hexanal and alcohols, with gamma and delta lactones [34, 61, 62] acting in association with other volatiles such as C_6 aldehydes, aliphatic alcohols and terpenes [12].

Texture is an essential feature of fruit quality perception; consumers associate it to freshness and wholesomeness [67]. Most fresh market peaches belong to the melting flesh group which shows rapid softening at ripening. Flesh firmness is a basic indicator of peach quality being related to horticultural maturity at harvest and must be kept from harvest to consumption. Fruit firmness is commonly measured on the two opposite cheeks of fruit, after removing the fruit skin, using a firmness tester with an 8 mm diameter cylindrical plunger. However, firmness measurement should be supported by other approaches coupling sensory and instrumental measurements to better appreciate desirable quality traits such as crispness and crunchiness [68]. Particular attention should be paid to detection of defects reducing consumer acceptance and limiting a fruit's potential post-harvest market life such as mealiness (or woolliness), one of the major negative attributes in peaches characterized by the lack of juiciness without variation of the tissue water content and greatly affecting sensory texture [26, 42]. Flesh mealiness is unacceptable for consumers, since it is perceived only tasting fruits before any visual symptoms are expressed [40]. Ortiz *et al*. [116] defined mealiness as a defect that could be quantified by instrumental means and described as lack of crispness, low hardness and low juiciness. Authors have programmed developments of the operative protocol toward a validation of the model through comparisons with responses of trained sensory panel. The development of non-destructive instrumental methods to assess woolliness is also proposed. Peach flesh mealiness has been investigated also by Crisosto and Labavitch [40] through a quantitative method based on percentage free juice/extractable juice. This approach is based on the observation of water in fruit which can be classified as 'free' or 'bound' water. Free water is the water that gives ripe fruit a juicy character; and bound water is that which is associated with polymer or solute hydration spheres and pectin gels in mealy fruit. The percentage of free/extractable juice had a higher

correlation to mealiness measured visually and by a trained panel than the extractable juice method. These authors propose the free juice measurement as an objective and accurate method to evaluate mealiness potential of stone fruit breeding lines.

Non-destructive Evaluation Methods

Quality is not a single, well-defined attribute but comprises many properties or characteristics essentially changeable during fruit maturation. Statistical combination of measurements by several sensors will increase the likelihood of predicting overall quality [3]. Flesh firmness, SSC and titratable acidity measurements are a good way to monitor fruit maturation and to predict potential sensorial quality and bruising damage during peach harvest and post-harvest handling. Ripening protocols traditionally utilize a penetrometer-type fruit firmness measure to monitor softening, a refractometer for SSC and a titrator for titratable acidity, all of them are instruments that need to destroy the sample in order to perform the measure. When destructive measurements are used, the tendency is to use as few samples as possible which often results in increased lot to lot variability in the parameter measured. Thus, the sample variability became a factor to consider during research and/or commercial applications involving destructive firmness measures [146]. Until recently, methods of assessing fruit texture properties non-destructively were not commercially available, but this situation is changing. In peach breeding there is no experience in the use of non-destructive instruments for measuring quality attributes within a progeny. Considering the narrow genomic base of this species, it could be quite interesting to prospect the potential accuracy of non-destructive instruments for evaluating the segregation of fruit quality traits within a peach progeny.

***Electronic nose*:** Among these new instruments, the electronic nose (e-nose) is one of the most promising non-destructive assessments of fruit quality, particularly analyzing volatiles emitted by fruits. E-noses are based on new chemical sensors that utilize differences in the electrochemical properties of volatiles. Compounds released by fruits are adsorbed and subsequently desorbed onto an array of semi-conducting polymer sensors, each characterized by its own degree of reactivity and selectivity [138]. E-nose technology offers the possibility to exploit information on aroma to assess fruit ripening stage, and more generally, fruit quality. Benedetti *et al.* [19] determined that the e-nose was able to separate four peach cultivars into four independent clusters. On other study, this instrument was able to discriminate four peach cultivars even if they shared a common progenitor, indicating the powerfulness of the instrument for genetic studies [85] (Fig. 2). In addition, Moltò *et al*. [107] employed gas chromatography to investigate the main chemical components of peach aroma and relate their concentration with the response of transducers sensitive to delta and gamma decalactones. Sensor responses had good correlations with a classification made by experts on the basis of visual appearance of ripening. The sensors were more sensitive, detecting also skin breakage and showed a good correlation with firmness measurements determined with a penetrometer. E-nose has been tested for evaluating sensorial properties of peaches and nectarines [49]; obtaining results that encourage further investigations about interactions between natural olfaction and e-nose. Since visual aspect and aroma are primary aspects of peach quality, Di Natale *et al*. [50] combined visible spectra and e-nose data as an improvement of overall fruit quality non-destructive determination.

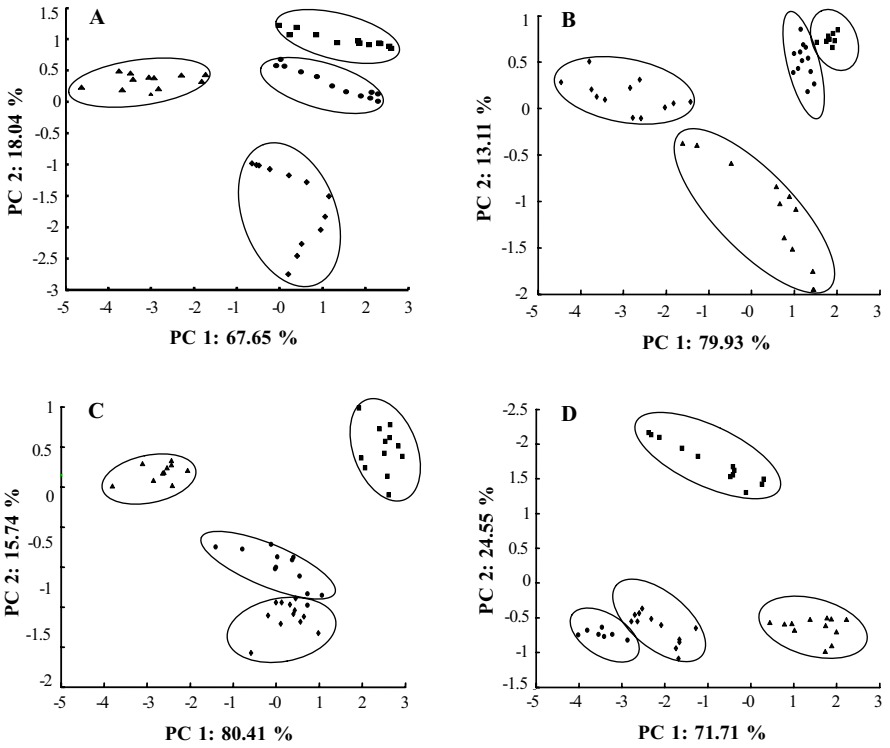

Figure 2. Principal component analysis (PCA) for aroma, determined with the electronic olfactory system EOS 835, for Tardibelle (A), Ryan Sun (B), September Sun (C) and Autumn Red (D) peach cultivars after different cold storage evaluation periods plus a variable period at 21ºC. Storage periods: 0 days 0ºC + 4 days 21ºC (■), 14 days 0ºC + 3 days 21ºC (●), 28 days 0ºC + 2 days 21ºC (▲) and 42 days 0ºC + 2 days 21ºC (♦) (Infante *et al*., in press.).

***Near infrared technology*:** Other important non-destructive instrument for measuring fruit quality is the near infrared technology (NIR). The NIR's functioning is based in the utilization of the light properties, in particular in the NIR zone, through the interaction of this energy with molecules of the sample. This interaction depends on the structure and chemical composition of the fruit tissue. NIR spectroscopy is used for the identification of molecules containing hydrogen atoms, as well as for analyses to determine the quantity of water, alcohol, amines and any other compounds with C-H, N-H or O-H groups [120]. Fruit can be

non-invasively assessed for internal quality attributes, such as SSC through correlation with their NIR spectra. Partial least-squares, multiple linear regression or other multivariate techniques are typically employed in this process [71]. The chances of a successful implementation of NIR systems depend on several factors, of which model robustness is the most important technical one. The accuracy of the NIR calibration models should be based on large datasets, encompassing several orchards, climate conditions, seasons and operational conditions, such as temperature, and optimized towards robustness by incorporating appropriate pre-processing methods [115].

***Impact response*:** There are also notorious advancement on technology based in impact response for measuring non-destructively fruit firmness and for helping to define dynamics of fruit quality during ripening, identifying the optimal stage for eating. A non-destructive firmness sensor prototype for pre-commercial sorting of peaches has been proposed by Gutierrez *et al*. [75]. Basically, fruits can be modelled as viscous-elastic spheres. The reaction force when hitting a rigid surface depends strongly on the Poisson's ratio, the elastic modulus (which is related with firmness), curvature, mass and velocity. Fruits less mature are more elastic, and viscosity grows with level of maturity. The principle of these sensors is that the ''harder" the fruit is, the stronger should be the impact, and the shorter the time of contact with the sensor. This firmness sensor classifies fruit peaches in three categories: very firm, firm and not firm, with a success of over 80%. A non-destructive approach to investigate peach fruit firmness was performed by Valero *et al*. [146] using a bench top version of a commercial online impact firmness measurement system (Sinclair iQTM firmness tester, Sinclair Systems International, LLC, Fresno, CA). The aim was to segregate fruits in three categories such as "ready to eat", "ready to buy" and "mature and immature". However, the device showed the higher accuracy (80-87%) in separating fruits in two groups ("ready to eat" versus "others" or "mature and immature"). Non-destructive firmness has been investigated also in combination with reflectance values, yielding interesting results in predicting consumer acceptance of fruits [131].

Sensory Evaluation

Sensory-based techniques are being used in supporting breeding and testing new cultivars and new storage practices. A fruit quality based only on external appearance and sweetness is obsolete. A more detailed knowledge of genetic bases of substances determining sensorial traits such as sourness, astringency and aroma could be of major importance for breeding. Some programs count on stable trained panel, specialized on stone fruits, which support breeding and evaluation of new selections [82] together with consumer tests (Fig. 1). However, the economic sustainability of such tools normally is assured only if assessors are regularly involved also in sensory studies of other products, in research and services that could generate a financial support for recruitment, training and incentives.

The strength of sensory evaluation is the possibility to provide a complete fruit profile, valid for products comparison, shelf-life monitoring and prediction of consumer acceptance. This allows for weighing single parameters in terms of influence on overall perception. Crisosto *et al*. [45] had assessors evaluating four sensorial traits: sweetness, sourness, aroma and flavour. In addition, Predieri *et al*. [125] performed a more detailed sensory evaluation including traits such as odour, firmness, juiciness, sweetness, acidity, aroma, sourness and astringency. Sweetness is known to be one of the most important peach quality traits and its perception is correlated to both SSC and acidity. Acidity is known to affect peach appreciation, while astringency is not a common parameter in peach evaluation and is generally intended as a negative sensorial trait, indicating not ripen fruits [118]. Robertson *et al*. [129] found astringency to be the parameter determining the low appreciation of peach cultivars Bailey and Boone County. However, astringency is a very complex sensory perception with close interactions with acidity [93, 144]. Predieri *et al*. [125] found the cultivars Big Top and Royal Gem having a high liking/astringency linear correlation. More detailed studies would better define if, at least for some cultivar, astringency will confirm to be perceived as a positive trait for peach appreciation and how it is correlated to ripening and consumer appreciation in different fruit typologies. Cascales *et al*. [31] studied sensory quality of peach 'Caterin' during ripening to assess the best state for consumption and found perceivable differences between peaches of different degrees of maturity. Among the most important sensorial traits, intensity of flavour and sweetness increased significantly from the under-ripe to semi-ripe states and then decreased on reaching ripeness. Authors found that the most suitable time for harvesting and consumption of this peach variety was semi-ripe. The sensory profile of the semi-unripe peach reached optimum intensity scores which recommended harvesting at this stage, since under such conditions the peaches continue to ripen up to the moment of consumption. Colaric *et al*. [36] studied sensory attributes and chemical composition in peach and nectarine fruits of nine different cultivars. Interesting correlation between sensory and biochemical parameters were found. Sweetness was influenced by citric acid, shikimic acid and sugars/organic acids ratio; perceived aroma correlated with total organic acids, sucrose, sorbitol and malic acid; taste was related to malic/citric acid ratio, total sugars, sucrose, sorbitol and malic acid. Sensory evaluation was used as a part of a program to develop minimum quality indexes on 49 cultivars (23 peaches, 26 nectarines), and data were based on a trained panel evaluation and subjected to principal component analysis [45]. This approach could be of help in supporting selection phase in breeding projects. Sensory evaluation was applied for validating the aim of a breeding program oriented to include non-melting flesh (NMF) trait in peaches [24]. The study demonstrated that NMF trait did not compromise fruit flavour in the peach genotypes studied. These authors enphasise the usefulness of descriptive sensory evaluation and principal component analysis in evaluating the sensory quality of new breeding releases. In addition, sensory evaluation has permitted to understand that low malic acid or sub-acid trait (*D/d*) affects the quality performance in long-term post-harvest [108], showing, for example, how 'Maria Dolce', a sub-acid nectarine, presented higher acceptability than 'Venus', a normal acid cultivar [83]. The sub-acid genotypes are interesting because they could be harvested at early stages of maturation, assuring precociously an appropriate balance sugar/organic acid ratio coupled with firmer flesh adequate to support manipulation and transport.

Consumer test: The ultimate objective of the production, handling and distribution of fresh fruits, including peach, is to satisfy the consumer. Consumer science states that it is of major importance to observe quality from the point of view of the consumer [117, 121, 137]. For a favourable purchase decision the appearance of fresh fruit (visible quality) is a primary criterion in making decision [89]. Size, colour and shape correspond to particular cultivar standards, while absence of defects and homogeneity of fruit are common visual quality factors for peaches. Visible quality encourages purchase and support marketing/placement choices (market of destination, price). External appearance is often addressed as "quality" and is the basic aim of a number of peach breeding programs. However, it is not sufficient by itself to guarantee consumer satisfaction and repeated sales, since eating quality is of major importance. Consumer acceptance tests can indicate eating traits assuring minimum quality indexes [43]. Consumer surveys have associated low peach consumption mainly to a lack of both satisfactory sensorial traits and information about how to get the best fruit eating quality [38]. Consumer acceptance was investigated by Crisosto and Crisosto [41] on two low acid and two high acid melting flesh cultivars. Consumer degree of liking and acceptance increased constantly as ripe SSC increased until in both low and high acid cultivars it reached a *plateau*. However, maximum acceptance was attained at different ripe SCC levels depending on the cultivar. This indicates that a proposed minimum quality index should be specific for each cultivar and not simply based on SSC to be reliable and assure consumer satisfaction across all cultivars.

Knowledge of destination market and consumer information: New cultivar development should be based on requirements of well determined markets. There are some markets, as the Asian markets, that prefer the white flesh over the yellow one, even in the canned peaches. Other markets niches prefer the NMF as the principal flesh type; these are normally Mediterranean and some Latin-Americans markets. Some breeding programs are focused on the local market but other look to the global market; in this case the last are exposed to a greater challenge than the first.

The mutability of consumer preferences should aware peach breeders to be prepared to present to the market new genotypes that could fulfil consumer expectations. However, these expectations should be predicted or foreseen 10-12 years in advance, when the breeder is doing the original crosses. Considering that there is not a known scientific base to predict the future behaviour of peach consumers, the best strategy to be prepared to this uncertain scenario is to destine part of the effort in breeding to distinct and new traits (saucer shape, sub-acid flesh, deanthocyanic epidermis, etc.). Finally, an accurate use of sensorial analysis techniques may provide tools for better understanding the role of sensory traits in consumer acceptance and preferences. The knowledge of sensorial characteristics of peaches is also a tool for communicating to the consumers the peculiar traits of the fruit, to orient their choice and to enhance the affection for this fruit species.

Classical Breeding

Breeding strategies and programs: The 20th century may well be recalled as the Golden Age of Peach Breeding. It was an era which saw the development of thousands of novel cultivars, with estimates running as high as 100-130 in certain years, and notable enhancement of such fruit quality traits as increased fruit size, fuller and more extensive blush, better skin ground colour, increased flesh/pit ratio, etc. This unparalleled and, probably, unrepeatable run appears linked to two main factors: (i) a radical extension of seasonality from a harvest calendar of two-three months to one of four-six months, a range that virtually covers the entire year if both hemispheres are taken into account; and (ii) a lowering of the chilling requirement that enabled the industry to expand from its traditional temperate-cold climates into southern districts of Mediterranean and sub-tropical climates [133].

During the decade (1991-2001), peach and nectarine cultivars have been generated through controlled crosses (43-61%), open pollination (15-21%) and bud mutation (4-5%), although for many of these new releases it is impossible to clearly define parents [47]. Peach is a natural self-pollinated species, which outcrossing range varied from 15 to 30% [106]. The most common method for producing new cultivars is still the cross of chosen parents and the selection within the progeny of those genotypes that share the most appropriate combination of traits, which are evaluated superior to the original parents and to the reference cultivar harvested on the same period. Selection of individuals to be continued as candidates for introduction as new cultivars is largely subjective, based in the experience of the breeder and the characteristics of established cultivars [135].

Homozygosity is useful in seed propagated rootstock peach, for example on 'Nemaguard' (*Prunus persica* x *davidiana*), but when breeding is the final purpose, a method that could ensure a high degree of genetic variation is required. Heterozygosity is sustained in two methods: i) crossing unrelated genotypes, some times followed by self-pollination of selected F1s to assure the extreme segregants, and ii) selection for heterozygous polygenic systems that yield desirable forms [135]. Most of peach breeding programs adjust permanently their strategy for reaching their genetic goals. The broad crossing of standard or reference cultivars with new genotypes carrying novel genes is the basic strategy for producing genetic variability over a known basement that could give in short time enriched genotypes, or even potentially new cultivars. When such selected genotypes are individualized, going to the F2 of these individuals permit the emergence of recessive alleles, that helps the breeder to understand the available genetic base.

Shortening breeding cycles: Peach, among top fruit species, could be considered as one of the easiest for reaching breeding goals in rationale time. It does not have a long juvenile phase that could delay the observation of reproductive traits; on the contrary it is possible to induce a seedling to bear fruit after 18 month on the nursery. In terms of breeding efficiency, Infante *et al.* [82] surveying 29 breeding programs showed that they license 0.88 varieties/program per year, evaluating 3,579 seedlings/program per year, and on average they release 3.7 cultivars for every 10,000 seedlings evaluated.

The shortening of breeding cycles would be possible mainly through a proper management of the nursery/orchard and an efficient application of a selection protocol to the progenies. Two or at least three bearing seasons would be enough to select or discard a seedling from a segregating population. From seed germination to first seedling selection it should pass 4-5 years.

Further, these selections would be propagated onto proper rootstocks and be evaluated for other 4-5 years. The nature of these inescapable steps, particularly considering the year to year variability on fruit quality traits, obligates to evaluate crops of selected individuals during consecutive seasons, if robust results are pursued. This consideration imposes a condition that makes difficult to hasten these specific steps of a breeding program. Perhaps the only breeding step that could be shortened is the accomplishment of bearing seedlings. This is possible by forcing the seeds and establishing the plants directly into the orchard, when they have 5-7 leaves, allowing the seedlings crop at the second year [135]. Germination of 'Venus' and 'PR Red' nectarine seeds, harvested in the same season and non-stratified, could be optimized if they are dipped in 100 mg·L^{-1} GA_3 with 100 mg·L^{-1} BA solutions, permitting a rapid obtaining of normal plants [84]. In mild climates and with early genotypes this technique could work reasonably good, because seeds could be forced in mid spring, and they would dispose 3-4 months with high temperatures that permit reaching an adequate plant high before the autumn arrival. If we are working with late genotypes, seeds will be harvested at the end of the summer, so the most of the time the climate conditions do not permit a vigorous seedling growth. In this case it is better to do a normal stratification procedure and wait for the next spring to sown.

When extremely early-maturing genotypes are sought, extremely early-maturing genotypes should be used as female parents. The flesh of these genotypes mature before the embryo is fully developed [135], so *in vitro* embryo culture would be the proper technique to rescue each individual of such crosses. Peach embryos in the proper culture media are held for 3-7 weeks in cold storage (4ºC) where they continue to grow and are prompt for germination [85]. Once the germination occurred *in vitro*, seedlings are transferred to an acclimatized greenhouse, where plants are hardened, and further, these seedlings continue to grow in the field.

Inheritance of main quality fruit traits: In order to develop an adequate analysis, the quality breeding will consider only those traits that affect directly the fruit. However, in a broad analysis it could be considered other traits that affect the tree shape, which could indirectly affect the fruit quality too. For example, the weeping tree shape (normal/weeping) [109] or the brachitic dwarf (normal/brachitic dwarf) [91], both traits could allow a better light exposition of fruits and therefore a better skin coloration and quality.

The genetic control of peach flesh texture is a well known Mendelian trait that was first described by Bailey and French in 1941. These authors determined that the melting flesh type (*M*) was dominant over the no melting type (*m*) [14]. Traditionally these NMF genotypes have been used for the canning industry because this particular flesh could be boiled maintaining the texture integrity. The canning peaches are characterized by an epidermis that lack red colour, not attractive for fresh consumption. However, on last years this situation is changing. There are some programs, as the University of Florida peach breeding program, that have bred new peach genotypes, as 'Gulfcrest', 'Gulfprince' and 'Gulfking' that combined a NMF with red epidermis [65]. In terms of new fresh typologies, Bellini *et al*. [18] have licensed a NMF nectarine called 'Maria Dorata' that could be used for fresh consumption and for canning, avoiding in this last case using caustic soda and other pollutant chemicals for fruit peeling in the canning peach industry.

The pit/flesh adherence had shown to be straight associated to flesh texture, resulting in phenotypes that show clingstone (*f*) NMF (*m*) fruits or freestone (*F*) melting (*M*) peaches. Breeding programs that are aimed to develop peaches for fresh consumption traditionally have favoured the freestone trait because it easily expressed on the progeny and also because this kind of fruit is much easy to eat. However, frequently these genotypes are susceptible to form callus within the cavity between the flesh and the pit, which is an undesirable trait that affects quality. Furthermore the freestone genotypes are more susceptible to water loss during cold storage. On recent years, some European private breeding programs, as Maillard's in France, are proposing new nectarine and peach genotypes that combine the melting flesh trait with the clingstone trait.

In the early 70, a new flesh type, the stony hard, a recessive trait described as melting flesh/stony hard flesh (*Hd/hd*), was reported by Yoshida [157]. The stony hard fruit texture is characterized by the absence of both ethylene production and post-harvest softening in mature fruit. This phenotype is different to the NMF genotype, which could produce the same or even more ethylene than melting fleshed peaches [25]. Stony hard is believed to result from a mutation in ethylene production [76, 77]. Therefore, it is presumed that there are individual plants with melting and non-melting genotypes showing the stony hard phenotype. It is evident that the stony hard trait was inherited independently of the melting flesh/non-melting flesh trait, showing that this gene was epistatic to the *M/m* [76]. Peaches are climacteric fruits, where softening is induced by ethylene [20, 72]. Several Asian peach genotypes, for example 'Yumyeong' and 'Hakuto', have been profusely used for incorporating this trait into new cultivars, and there are some western breeding programs that already have licensed new cultivars with this trait (University of New Yersey, CRA-FRF of Forlì).

Concerning flesh colour, it is well known that the white flesh is a dominant allele over the yellow flesh (*Y/y*) [37], but traditionally breeding programs of western countries have been focused mainly to obtain yellow fleshed varieties because the white flesh was frequently associated to poor handling performance in post-harvest, and susceptibility to flesh browning. Nevertheless, on the present, it is possible to dispose white fleshed cultivars with excellent storage ability. White-fleshed peaches and nectarines show a unique aroma and flavour, clearly distinguishable from the yellow flesh types and are as preferred as the yellow-fleshed by common consumers in Europe [110]. There is also available in the peach germplasm a deep red or blood coloured fleshed typology, which genetic control is recessive to the normal type [135]. Some authors suggest that red-flesh peach varieties have a greater potential health benefit based on antioxidant content and antioxidant activity as compared to the white/yellow-flesh varieties [30].

The low-acidity or sub-acidity is a trait governed by a dominant allele (*D/d*) [108], originally found in Asian germplasm. These genotypes are characterized by their low-acid taste even when they are not completely ripe. There are different classes of low-acid genotypes, varying from those that show a rather poor eating quality with "flat flavour" to those that show a balanced sugar/acid ratio. 'Maria Dolce' is a low-acid nectarine described by the

breeder who released it as a "gusto miele" variety type that means "honey taste" [17].

The fuzz of peach skin generally works as a barrier for enjoying a bite of fresh peach because it interferes with taste and also because there are non-specific lipid transfer protein allergens allocated mainly in the skin [22]. The gene that determines the fuzzy skin phenotype is dominant over the glabrous skin or nectarine phenotype (*G/g*). The nectarine arose from a mutation of peach and is characterized by a smaller size than peach and normally has higher SSC [135]. The post-harvest performance usually is better than in peach and similar to Japanese plums, so they could be handled and kept in cold storage for more than 30 days without affecting their quality [83]. Among peaches, there are differences in fuzz density, there are normal fuzzed peaches and "rough skin" peaches, the normal pubescence being dominant over the last [135].

About the fruit shape, a perfect round form is generally considered as nice looking fruit. There are some climate conditions that affect fruit shape, as the number of chilling units that the plant is exposed to during the autumn - winter time. When the chilling accumulation is not enough, the fruit acquires an elongated shape with a pronounced tip, on the other hand, if the same cultivar is cultivated under the proper chilling unit's accumulation, the shape will be normal [29]. However, there are also fruit shape types that are in higher proportion genetically governed. The saucer shape is an example of this; the flat shape being dominant over the normal shape (*S/s*) [94]; nevertheless the *S/S* is a lethal allele combination [73]. Saucer-shaped fruit is normally associated with aromatic white flesh, genotypes which are very popular in Asian countries. One of the practical problems that arose in flat peaches is an open tip that could be the free entrance of fungal infection and fruit cracking, so this problem should be considered in breeding programs interested on saucer-shaped peaches [86]. At the present, it is possible to dispose new genotypes which combine the saucer shape with yellow flesh (*S/s y/y*) or with glabrous skin (*S/s g/g*) allowing the enrichment of an originally narrow genetic base.

Molecular Breeding

Molecular characterization (isoenzyme, RFLPs, RAPDs, AFLPs and SSRs): The identification and characterization of peach genotypes have been based during long time on morphological and physiological traits. However, such traits are not always available for analysis and can be affected by environmental conditions. Molecular markers offer important advantages over the use of these conventional markers [100, 154].

Isoenzymes were among the first genetic markers to be utilized in peach. Nevertheless, the utilization of these dominant markers is limited by the small number of *loci* that can be analyzed with conventional enzyme staining methods and the low variation in some *loci*. These markers are particularly useful in the characterization of *Prunus* species as almond and plum, because both are outcrossing species with high level of polymorphisms [9, 27], in comparison with peach, a predominantly autogamous species [64, 135] which shows few isoenzyme polymorphisms [4, 9, 28, 59, 113]. On the other hand, restriction fragment length polymorphism (RFLP) markers are based on the differential hybridization of DNA fragments from restriction-enzyme digestion [141]. RFLP markers are co-dominant and can detect a virtually unlimited number of markers. This is particularly important in peach because of the mentioned low level of variation. These markers have been used in the molecular characterization of peach genotypes and the construction of genetic linkage maps[48].

Random amplified polymorphic DNA (RAPD) markers are based on the PCR amplification of random locations in the genome [153]. RAPD techniques have been successfully used in peach for identifying cultivars [96, 88, 125] and construction of maps [48, 152]. In contrast to isoenzymes and RFLPs, they are dominant markers. This feature, as well as their variable degree of repeatability and problems in transferring across populations, limits their utilization to map construction. These difficulties can be overcome by converting RAPDs to sequence-characterized amplified regions or SCARs [119]. SCAR is a PCR-based method that employs specific primers and can potentially be converted into co-dominant markers and is less sensitive to PCR conditions. In addition, amplified restriction fragment length polymorphism (AFLP) technology is a powerful DNA fingerprinting technology based on the selective amplification of a subset of genomic restriction fragments using PCR [149]. AFLP has a number of advantages over the RAPD: more *loci* analyzed and better reproducibility of banding [123]. These markers have been mainly used in peach for genetic mapping and molecular characterization of cultivars [97, 139].

Finally, simple sequence repeat (SSR or microsatellite) markers, also based on PCR technique, are the best suited markers for the assessment of genetic variability within crop species because of their high polymorphism, abundance and co-dominant inheritance [74, 135]. In the case of peach, SSR markers covering the almost whole genome have been obtained [6, 7, 35, 54, 70, 143, 149, 150, 156]. These markers have been mainly used in peach for molecular characterization of cultivars and related species [101, 102] (Fig. 3) and genetic mapping [4, 8, 56, 58, 63, 147] (Fig. 4).

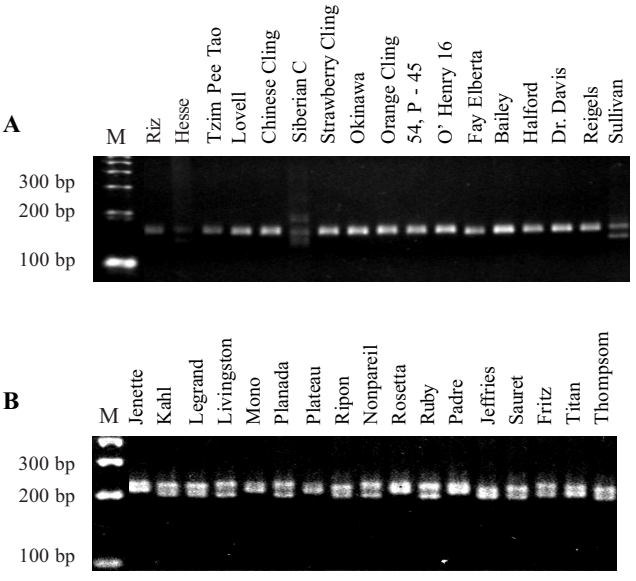

Figure 3. Methaphor® agarose gels showing the allelic segregation of the UDP96-013 microsatellite (SSR) marker in peach (A) and almond (B) cultivars, the high degree of homology for the SSR loci between *Prunus* species and the lesser heterozygosity level observed in peach in relation to almond [100]. M, 1kb Plus DNA Ladder (Invitrogen, Madrid, Spain).

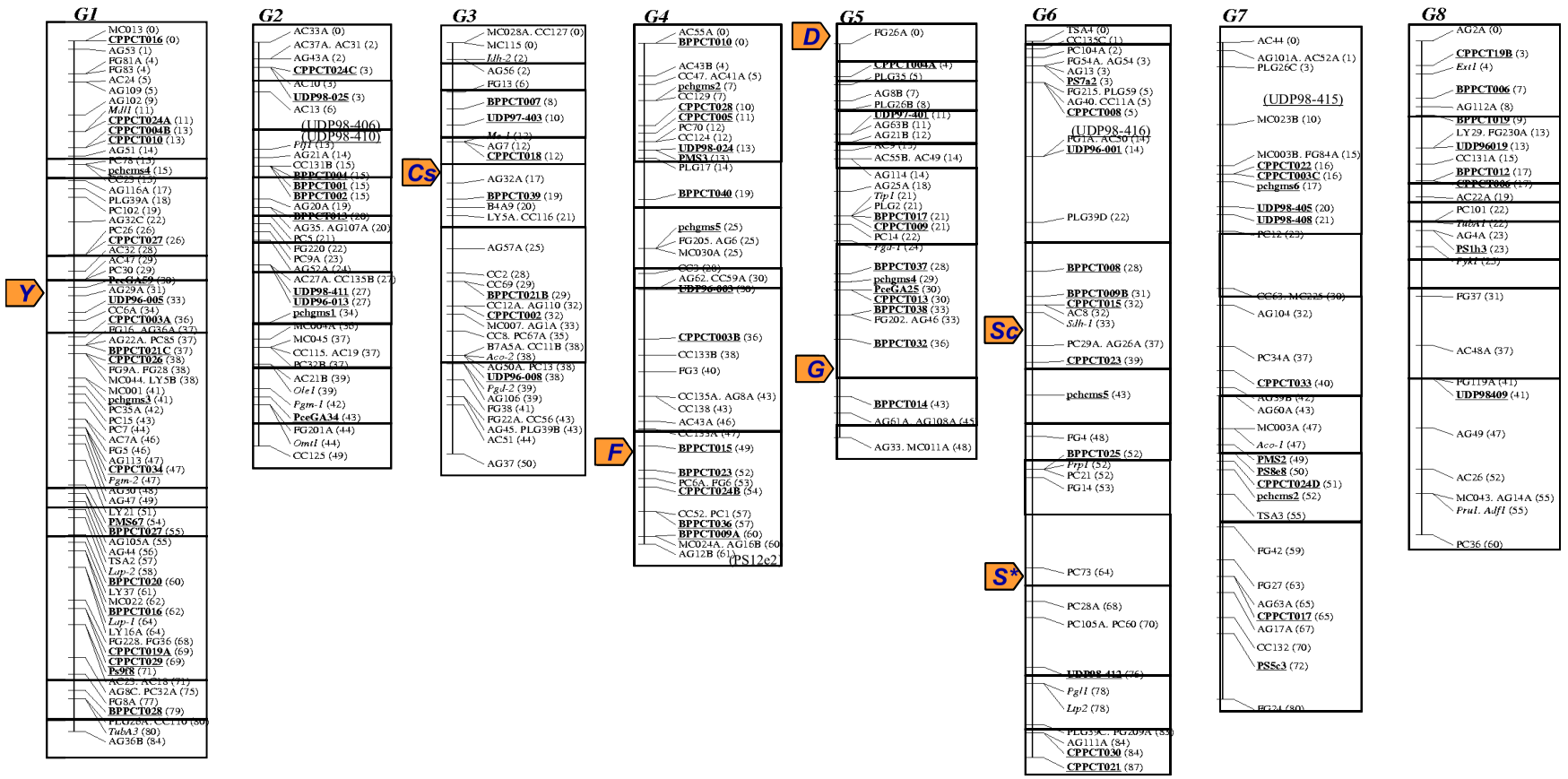

Figure 4. *Prunus* reference map of the 'Texas' (almond) x 'Earlygold' (peach) F2 population obtained using SSR markers, with the approximate location of the main monogenic peach fruit quality traits including flesh color (*Y*), flesh color around the stone (*Cs*), flesh adhesion (*F*), non-acid fruit (*D*), skin hairiness (*G*), skin color (*Sc*), and fruit shape (*S**) genes [8,55]. In addition, the 67 different regions established in the bin mapping [80] are represented.

Genetic linkage analysis (map construction, QTL analysis and candidate gene analysis): Genetic linkage analysis was initially performed in peach using morphological and isoenzyme markers [33, 155]. The development of RFLPs at the beginning of the 1980s provided a virtually unlimited source of high quality markers, located all over the genome, making map construction with markers a feasible endeavour for most plant species. The first map for peach based almost entirely in RFLP markers was constructed by Rajapakse *et al.* [127]. This map detected the eight expected linkage groups and spanned for approximately 400 cM. Another map constructed by Foolad *et al*. [69] with an F2 population of the interspecific cross between a peach selection (54P455) and the almond cultivar 'Padre' (5xP), had a similar marker composition (101 RFLPs and 6 isozymes). Other maps were performed by Dirlewanger and Bodo [51] and Dirlewanger *et al*. [52, 53] using RFLPs and RAPDs. The most recent utilization of PCR-based markers has increased the opportunities for mapping and tagging a wide range of traits. AFLPs allow detection of a higher level of polymorphism in peach than isoenzymes, RFLPs or RAPDs [55]. Finally, SSR markers are currently becoming the markers of choice for genetic mapping in peach [8, 48, 58, 63, 140].

A saturated linkage map for *Prunus* was obtained in an almond (cv. Texas, syn. 'Mission') x peach (cv. Earlygold) F2 progeny (TxE map) including 246 markers (235 RFLPs and 11 isoenzymes), the *Prunus* reference map [11]. All markers studied mapped in the eight linkage groups found with an initial distance of 491 cM [87]. The TxE map has been progressively improved [8] with the addition of more markers of good quality, such as additional RFLPs and simple-sequence repeats (SSRs). The current version includes 562 markers (361 RFLPs, 185 SSRs, 11 isozymes and 5 STSs), which cover a total distance of 534 cM with high density (average density 0.92 cM/marker and largest gap of 7 cM) [56] (Fig. 4). The development of markers that could be obtained with simpler methods than RFLPs, such as RAPDs and SSRs (particularly the latter given their high quality), fostered the improvement of other more saturated maps such as the Px5 ['Padre' (almond) x 54P455 (peach)] map with 161 markers including six morphological genes and eight resistance-gene analogue sequences [21]. The similar order of molecular markers observed in different *Prunus* maps, when compared to the *Prunus* reference map [8], suggests a high level of synteny within the genus [55, 56, 58] and should also facilitate the successful transfer of sets of markers and coding sequence among species.

A recent strategy for the location of new markers in an established genetic linkage map is the "selective" or "bin" mapping approach. This technique allows mapping with the use of a subset of plants of a population from which a map is already available. The advantage of this strategy is that allows mapping with less time and cost and is adequate for simplifying the construction of high density maps. The use of this set of 6 individuals promises to be a very useful resource for peach genetic linkage studies in the future. The reference map has been divided in 67 "bins" or regions (from 8 to 25 cM) to locate the future markers [80] (Fig. 4). These authors have incorporated 151 SSRs to the *Prunus* reference map using only 6 individuals from the TxE ('Texas' x 'Earlygold') *Prunus* reference population of 65 individuals.

Finally, candidate gene approaches have proven to be useful for finding associations between genes involved in relevant metabolic pathways and major genes or QTLs and have been performed in peach [63]. These authors isolated eighteen cDNAs encoding key proteins in soluble sugar and organic acid metabolic pathways. Twelve candidate genes were localized in the genetic linkage map including ripening date, fruit development period, flesh weight, pH, titratable acidity, SSC, malic acid, citric acid, quinic acid, sucrose, glucose and fructose (Table 1).

Use of genome databases (development of ESTs, SNPs and microarrays, physical mapping and gene cloning): More recent markers being used in the development of marker associated to fruit quality traits in peach are those obtained from either cDNA sequences (expressed sequences tags, ESTs) or databases (cloned gene analogs, CGAs) and those based on single point mutations (Single Nucleotide Polymorphisms, SNPs) [142].

The EST analysis has provided a first picture of the numerous peach genes potentially involved in fruit development also providing an extensive reservoir for gene cloning and genetic mapping in peach. A recent collection of ESTs from peach and other *Prunus* based on cDNA libraries has been released to public databases, and more than 83,751 putative unigenes have been detected (http://www.bioinfo.wsu.edu/gdr/), the Genome Database for Rosaceae (GDR). In this data base, a comparison of *Prunus* map is performed together with the development of EST. In addition, current studies about mapping EST, physical mapping and transcriptomic are being developed [1]. This data base is a valuable resource in genomic and genetic research in peach. Single Nucleotide Polymorphisms (SNPs) are the most frequent molecular markers in the genome with a co-dominant inheritance. Unigene contigs have been searched for SNPs in this GDR database with a total of 5,284 SNPs, a frequency of 0.65 SNPs/100 bp [105]. The above mentioned research is complementary to the other works regarding EST development in *Prunus* performed by different research groups in Italy as part of the work of the Italian National Consortium for Peach Genomics (http://www.itb.cnr.it/estree/) [124]. In this database is also presented a collection of 75,404 sequences analyzed from different cDNA libraries obtained in peach. In addition, approximately 200 ESTs were selected for mapping on a physical framework map (in collaboration with A. Abbot from the University of Clemson). In addition, a total of 33,189 SNPs was identified and further analysis concentrated on a subset of different SNPs representing genes putatively involved in important aspects of secondary metabolism. Recently a data management system that facilitates the analysis of large volumes of information in an EST Project Workflow (JUICE) has been developed in integrated functional and genomic project in peach in Chile (http://www.genomavegetal.cl/juice_system/) [92]. In addition, analysis of different ESTs using the National Center for Biotechnology Information (NCBI) databases (http://www.ncbi.nlm.nih.gov) indicated significant similarity to protein coding sequences in the database and open interesting new possibilities in peach genomics.

As part of the effort of these two groups to increase and enrich the genomics resources in different *Prunus* species, it is starting the fabrication of different peach microarrays using unigene sets as probes. A group of nearly 4,600 unique ESTs derived from peach mesocarp have been sequenced to analyze the expression profile of the unigene set during fruit development and the identification of additional genes involved in this process [103]. The development of microarrays has been also described in peach in the study of fruit quality with an important potentiality in the

development of marker associated to these important horticultural characteristics. These microarrays have been also used to investigate transcriptome changes during transition from pre-climacteric to climacteric phase in peach fruit [145].

Using high-information content fingerprinting (HICF) the first physical map has been generated in peach [1]. This map is composed of 2,138 contigs containing 15,655 BAC clones. This physical map also integrates 2,633 markers including peach ESTs, cDNAs and RFLPs. This physical map is anchored to the *Prunus* reference map (TxE) (Fig. 4) using 152 core genetic probes. The anchored contigs span over 44.5 Mb (or 15%) of the peach genome, 309, 189 b [15]. On the other hand, although gene cloning studies in peach fruit quality are very scarce, recently some authors are starting this task. Hayama *et al*. [78] identified a new expansin gene closely associated with peach fruit softening. These authors indicated that while several expansins show a general increase in expression levels during the later stages of fruit development, some isoforms show a greater association with softening than others. In addition, Morgutti *et al*. [112] identified important changes in endopolygalacturonase levels and characterized a putative endo-PG gene during fruit softening in peach genotypes with non-melting and melting flesh fruit phenotypes. In addition, one of the first gene sequences reported in peach fruit quality has been that of acidity obtained from peach fruits [23], to which followed some other genes abundantly expressed during fruit development.

Table 1. Markers associated to the main oligogenic or monogenic and polygenic (QTLs) fruit traits in peach.

Trait	Symbol	Marker	Linkage group	Reference
Oligogenic or monogenic traits				
Flesh colour	*Y*	RAPD	G1	Warburton *et al.* [151]
Flesh colour	*Y*	AFLP	G1	Abbott *et al.* [2]
Flesh colour	*Y*	RFLP	G1	Bliss *et al.* [21]
Flesh adhesion (clingstone/freestone)	*F*	RFLP	G4	Abbott *et al.* [2]
Flesh adhesion	*F*	RFLP	G4	Dettori *et al.* [48]
Flesh adhesion	*F*	RFLP	G4	Quarta *et al.* [126]
Flesh adhesion	*F*	AFLP	G4	Yamamoto *et al.* [156]
Skin hairiness	*G*	AFLP	G5	Dirlewanger *et al.* [56]
Skin hairiness	*G*	RFLP	G5	Bliss *et al.* [21]
Non-acid fruit	*D*	RAPD	G5	Dirlewanger *et al.* [56]
Non-acid fruit	*D*	RFLP	G5	Bliss *et al.* [21]
Skin colour	*Sc*	SSR	G6	Yamamoto *et al.* [156]
Skin colour	*Sc*	SSR	G6	Picañol *et al.* [122]
Fruit shape	*S**	RFLP	G6	Dirlewanger *et al.* [56]
Polygenic traits (QTLS)				
Ripening time	-	RFLP	G4	Quarta *et al.* [126]
Ripening time	-	SSR	G4	Verde *et al.* [147]
Ripening time	-	RFLP	G4	Dirlewanger *et al.* [56]
Maturity time	-	SSR	G4	Etienne *et al.* [63]
Fruit develop cycle	-	RFLP	G4	Abbott *et al.* [2]
Fruit develop cycle	-	SSR	G4	Etienne *et al.* [126]
Soluble solids	-	RFLP	G4	Abbott *et al.* [2]
Soluble solids	-	RFLP	G4	Quarta *et al.* [126]
Soluble solids	-	SSR	G4	Etienne *et al.* [63]
Soluble solids	-	SSR	G4	Verde *et al.* [147]
Soluble solids	-	SSR	G5	Picañol *et al.* [122]
Fructose content	-	AFLP	G4	Abbott *et al.* [2]
Fructose content	-	RFLP	G4	Etienne *et al.* [63]
Glucose content	-	RFLP	G4	Abbott *et al.* [2]
Glucose content	-	RFLP	G4	Dirlewanger *et al.* [56]
Glucose content	-	RFLP	G4	Etienne *et al.* [63]
pH	-	RFLP	G5	Abbott *et al.* [2]
pH	-	SSR	G5	Dirlewanger *et al.* [52]
Titratable acidity	-	RFLP	G5	Dirlewanger *et al.* [56]
Titratable acidity	-	RFLP	G5	Etienne *et al.* [63]
Titratable acidity	-	SSR	G5	Picañol *et al.* [122]
Titratable acidity	-	SSR	G5	Dirlewanger *et al.* [52]
Malic acid content	-	RFLP	G5	Dirlewanger *et al.* [56]
Malic acid content	-	RFLP	G5	Etienne *et al.* [63]
Citric acid content	-	RFLP	G5	Dirlewanger *et al.* [56]
Citric acid content	-	RFLP	G5	Etienne *et al.* [63]
Productivity	-	RFLP	G6	Dirlewanger *et al.* [56]
Sucrose	-	RFLP	G5	Etienne *et al.* [63]
Sucrose	-	RFLP	G5	Etienne *et al.* [63]
Sucrose	-	SSR	G5	Dirlewanger *et al.* [52]
Fruit diameter	-	AFLP	G6	Abbott *et al.* [2]
Fruit weight	-	RFLP	G6	Abbott *et al.* [2]
Fruit weight	-	RFLP	G6	Etienne *et al.* [63]
Fruit weight	-	SSR	G5	Dirlewanger *et al.* [52]
Skin colour	-	RFLP	G6	Quarta *et al.* [126]
Skin colour	-	SSR	G6	Verde *et al.* [147]
Quinic acid	-	RFLP	G8	Etienne *et al.* [63]

Marker-assisted selection: In general, developing new cultivars is a long and tedious process, involving the generation of large population of seedlings from which is selected the best genotype. Whereas the capacity of breeders to generate large populations from crosses is almost unlimited, the management, study and selection of these seedlings are the main limiting factors in the generation of new releases. In this sense, marker-assisted selection (MAS) is emerging as a very promising strategy for increasing selection gains [10, 98].

Early selection with molecular markers allows an accurate screening of seedlings several years before the traits can be evaluated in the field, makes possible the accumulation of different genes/QTLs agronomic traits of interest, or shortens the number of generations to recover the genotype of the cultivated species after a cross with an exotic genotype or wild species [10, 16, 56]. Selection by molecular markers is particularly useful in fruit tree crops with a long juvenile period, and when the expression of the gene is recessive or the evaluation of the character is otherwise difficult, as with resistance to biotic or abiotic stress [98, 134, 142]. If sufficient mapping information is known, MAS can dramatically shorten the number of generations required to "eliminate" the undesired genes of the donor in backcrossing programs. Marker *loci* linked to major genes can be used for selection, which

is sometimes more efficient than direct selection for the target gene [90].

The use of mapping populations segregating for the characters of interest has been the principal approach for the analysis of marker-trait association in peach. The analysis of co-segregation among markers and characters allows establishing the map position of major genes and QTLs responsible for their expression. Several genetic linkage maps have been developed using different molecular markers to identify major genes and QTLs associated to important agronomic traits in peach. Mapping quantitative characters by identifying quantitative trait *loci* (QTL) is becoming an important tool in tree breeding. To date, twenty five monogenic genes and QTLs linked to fruits quality traits have been mapped [2, 16, 21, 52, 53, 57, 63, 81, 122] (Fig. 4 and Table 1). In addition, three F1 populations segregating for key quality factors, flesh firmness, skin colour, SSC, acidity and post-harvest life [122] (Table 1). On the other hand, different SCAR markers (from RAPDs) are being evaluated for MAS in fruit quality breeding in peach, including the identification of the *Ff* (flesh adhesion) gene in peach [88]. An European integrated project, titled ISAFRUIT (http://www.isafruit.org), has been recently dedicated to the study of the genetics of fruit quality and health properties [13]. In this project, bin mapping strategy is currently used in the location of candidate genes involved in fruit quality in peach [81]. In addition, in side this project, 16 important QTLs controlling major fruit quality components have been mapped including acidity, sucrose content, fruit weight, pH and others [57] (Table 1).

Conclusive Remarks and Future Prospects

A key point for peach marketing is to maintain consumer confidence by assuring more than acceptable quality levels. Peach breeding planners should take into account this point from the very early steps a project, considering factors connected to fruit production, storage and distribution and hedonic value of fruit. This means, for example, that beside cultural practices maximizing the production of homogeneous marketable fruit (e.g., size and colour), harvest date, storage modality and duration should be determined considering also the influence on sensorial traits at consumption. Sensory evaluation may provide original data for a comprehensive evaluation of fruit quality as related to its commercial potential. On the other hand, future works regarding marker-assisted selection (MAS) of peach for fruit quality must include the comparative mapping of different progenies. Additional advantages encouraging the utilization of new technologies to peach tree crop improvement include a small genome size, high levels of synteny between genomes and a well-established international network of cooperation among researchers. Genomic methodologies including expressed sequences tags (ESTs) cloned gene analogs (CGAs) and single point mutations (single nucleotide polymorphisms, SNPs) may make it possible to discover genes of interest in fruit quality selection in peach. Finally, more recent efforts are being oriented to the elaboration of physical maps, the development of quick gene sequencing and cloning tools and the complete sequencing of the peach genome to develop efficient molecular markers applicable to assistant selection in peach breeding programs. A modern breeding approach should be aimed to coupling the highest biotechnology with the awareness of consumer preferences and exigencies.

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