Chapter

Quantitative Trait Loci Associated with Agronomical Traits in Strawberry

Harun Karci, Habibullah Tevfik, Nesibe Ebru Kafkas and Salih Kafkas

Abstract

The cultivated strawberry (*Fragaria* x *ananassa*) is derived from *Fragaria chiloensis* and *Fragaria virginiana* species a few centuries ago, and it is one of the most preferred and consumed berries all over the world because of its a good source in terms of many nutritional elements. Strawberry has high genetic variability and adaptation to different environmental conditions due to its highly heterozygous nature. In the last decades, many farmers, breeders, researchers even consumers have started to focus on berry quality traits such as large fruit, uniform shape, high fruit firmness, high fruit sensorial quality (aroma contents), color, gloss, and resistance to pathogens. Thus, the development of novel strawberry cultivars or genotypes with high nutritionally quality traits has become one of the main aims in strawberry breeding programs. Biotechnological tools such as the identification of quantitative trait loci (QTL) and marker-assisted selection (MAS) are the most widely used technologies in fruit breeding programs for shortening the breeding period. Identification of QTLs in agnomical important traits are very valuable tools for early selection in strawberry breeding programs. This chapter is focused on QTL and marker assisted breeding studies in strawberry to date and provides new perspectives on molecular breeding in strawberry breeding.

Keywords: strawberry, QTL, NGS, SNP

1. Introduction

Strawberry is one of the temperate climate fruit species which can be grown almost anywhere in the world from Ecuador to Siberia within wide ecological limits due to its high adaptability. Strawberry is loved by everyone and has a great market advantage in fresh and industrial consumption [1]. Strawberry, which is one of 23 species in the Rosaceae family, including apple, cherry, and rose species, is in the *Fragaria* genus [2]. The most common commercially grown strawberries are octoploid ($2n = 8x = 56$) and haploid chromosome number ($n = 7$) [3] and constitute a large part of the world's production [1]. The estimated genome size of the Camasora strawberry cultivar which belongs to the *Fragaria* x *ananassa* Duchesne species has been reported as 813.4 Mb [4]. Strawberry production in the world is increasing day

by day. Strawberry contains important mineral substances and vitamins for human health and nutrition, such as salicylic acid, calcium, iron, and phosphorus. In addition, strawberry is rich in antioxidants and phenolic compounds for human health. These health benefits play an important role in the rapid increase of its production [5]. Many studies reported that strawberry consumption has positive effects on human health, preventing aging, Alzheimer's, obesity, and cancer diseases, especially heart diseases [6–8].

In the strawberry breeding programs, cultivars should be productive throughout the whole season, tolerant to diseases post harvesting, resistant to long transportation, with high fruit firmness and high fruit quality are the main breeding objectives for many years and many studies have been carried out intensively in European countries, especially in the USA, and Asian countries such as China [9, 10]. However, recently, some breeding programs have been carried out in strawberries covering topics such as adaptation to arid conditions or climate change like GOODBERRY supported by the European Union's Horizon 2020 research and innovation program [10]. Breeding programs should use several strawberry genetic resources or different populations and also focus on the development of new genotypes or lines resistant or tolerant to different abiotic stress conditions as breeding criteria. Selection of promising lines in strawberry breeding will be possible not only with traditional breeding methods but also with biotechnological approaches in strawberry breeding. However, identifying major and minor QTL regions associated with such complex traits that are severely affected by environmental conditions and controlled by multiple genes is a very difficult and time-consuming task in strawberries due to its open pollinated and highly heterozygous nature.

Many agriculturally important traits, such as yield and quality, tolerance to environmental stresses, and resistance to certain diseases, are controlled by polygenes, which complicates the breeding process as phenotypic traits only partially reflect the genetic influence of the individuals. These complex traits are defined as quantitative traits (multifactorial traits or polygenic), and specific regions in the genome of genes associated with a trait are described as quantitative trait loci (QTLs). Detection of QTL region or a gene in the plant genome is a complex process. QTL analysis can simply be expressed as the determination of the relationship between phenotype and genotypic data. All the loci are used to divide the population used in mapping into different genotypic groups based on the presence or absence of a particular locus and to determine whether there are significant differences between groups related to that specific trait [11, 12]. Depending on the marker system and population types, if there is a significant difference between the phenotypic means (2 or 3) of the groups, it indicates that the locus has a QTL controlling the relevant trait [13]. QTL mapping also measures the relative effects of alleles on traits. It also provides the basis for marker-assisted selection (MAS), which not only determines the physical location of QTLs on the genome but also shortens breeding time [14]. QTLs determined from several different locations are called more stable QTL (sQTL) and they are preferred for MAS due to their reliability. A genetically diverse and well-segregated population is required for QTL mapping and determining their position in the genome. QTL analysis is performed by determining the correlation between the phenotypic and genetic data with help of different algorithms [15]. The first necessary condition in QTL mapping is intra-species or interspecies populations acquired F2, recombinant inbred lines (RILs), backcross 1 (BC1), double haploid lines (DHLs), closely isogenic lines (NILs), and full-sib F1 (pseudo-testcross) lines [16].

A set of QTLs determined in the same region or confidence interval (CI) region for more than one trait is defined as a set of QTLs or overlapping QTLs (cQTLs) [17]. cQTLs are potentially important QTLs that control multiple traits. The QTL is classified as a major QTL if it accounts for 10% or more of the total phenotypic variation in the population, and as a minor QTL if it accounts for 10% or less. Many mapping definitions such as linkage, association, NIL, F2, or BC can be used to detect QTLs. Classical biparental linkage mapping involves the crossing of parents with the contrasting phenotype for a trait [18]. QTL analysis uses the linkage between two loci and recombination in biparental populations, while association mapping (AM) uses variations generated by landraces and genotypes found in natural populations. The populations used in association mapping are divided into different groups based on recombination, such as biparental populations. In other words, it describes the correlation of traits and markers in more than one biparental population. Thus, an associated locus in the AM means that it has a similar effect on many individuals in the population [19].

Thanks to the advancements in next-generation sequencing technologies and the reduction of sequencing costs, high-density sequencing can be easily performed and big data can be obtained for the detection of more variants. Higher density linkage maps can be constructed by using the obtained big genotypic data and can increase the chance of detecting rare loci. As a result, these advances greatly contributed to the identification of QTL regions in the genome. High-density genetic maps are constructed using genotyping by sequencing (GBS) data and have been used to successfully identify QTLs related to phenological, biochemical fruit traits, and pathogen resistance or tolerance in many different species. In addition, QTLs that control abiotic stress factors such as cold, heat, salinity, frost, and drought can also be more accurately detected with the help of high-density genetic linkage maps. The purpose of this section is to review the current literature on the identification and evaluation of the genomic positions of QTLs that control phenological, morphological and biochemical fruit quality traits, and disease tolerance traits in strawberries.

2. Linkage mapping in strawberry

The principle of genetic mapping is that genes segregate on chromosome recombination during meiosis [20]. If two genes do not segregate independently, that means they are closely linked to each other on the same chromosome. The genes that are very close to each other or tightly linked will be transferred together from the parent to the progenies. While the two genes are not close to each other on the same chromosome the chance of them segregating separately from each other is increased. Thus, the genetic distance between genes can be calculated accordingly.

The first step of genetic mapping is the selection of two genetically very distant parents that demonstrate obvious genetic differences in many traits of interest. The parents must be far apart genetically for the exhibition of sufficient polymorphism. However, if two parents are too distant genetically, some undesirable events can accrue, such as sterility in progenies and high segregation distortion during the linkage analysis.

The structure of populations consists of two ways; firstly, inbred lines derived from homozygous parents; the second one is the inbreeding lines or cultivars and individuals derived from the crossing of the allogamy species. Thus, two-way

pseudo-testcross, half-sib, and full-sib populations derived from crossings can be used for genetic mapping in outcrossing species. The progenies from the selfing of the F1 generation (F2), backcross (BC), recombinant inbred lines (RILs), double haploids (DHs), and near isogenic lines (NILs) can be preferred for mapping in alloploid species according to the breeding objectives [14, 21, 22]. The selection of population type plays an important role in successfully constructing the linkage maps. Although F2 progenies are obtained from selfing F1 hybrids, BC lines are generated by crossing F1 progenies back with one of the parents. RILs are produced by single-seed selections derived from individuals of the F2 population. The selections should be continued from six to eight generations. Double haploid lines (DHLs) are developed by doubling the gametes of individuals. DHLs regenerate in tissue culture studies after chromosome doubling from pollen grains or haploid embryos derived from crosses.

The first aim of molecular breeding is the construction of linkage groups. The linkage mapping detects genes or markers association with molecular markers and their chromosomal positions and they are valuable tools in order to perform positional cloning of desired/known genes or related regions and to consist of contigs and scaffolds of physical maps at the chromosomal level.

The first linkage mapping report in *Fragaria* was performed using an isozyme marker and a morphological (yellow fruit color) trait, using individuals of a cross between *F. vesca* cultivars Yellow Wonder and Baron Solemacher [23]. Another finding was reported that an isozyme locus (PGI-2) was used in the same population in non-runnering r locus-related morphological traits [24]. The first linkage map in *Fragaria* was constructed similarly in diploid *F. vesca* [25]. The difference between these maps was that they were constructed in the F2 population produced by crossing Baron Solemacher and W6, a non-runnering European cultivar belonging to *Fragaria vesca ssp. vesca*, and *Fragaria vesca ssp. americana* W6, a wild runnering genotype. A total of 75 random amplified polymorphic DNA (RAPD) markers, two isozymes, an STS marker associated with the alcohol dehydrogenase gene, and the runnering locus were used in linkage mapping, and the length of the map in seven linkage groups was calculated as 445 cM.

The genetic linkage map of octoploid strawberries was constructed by using amplified fragment length polymorphism (AFLP) markers [26]. A total of 515 markers and 119 F1 plants of a two-way pseudo-test cross of Capitola (CA75.121–101 × Parker) and CF1116 (Pajaro × (Earliglow × Chandler)) were used in linkage map construction. A total of 28 and 30 linkage groups were constructed in the maternal and paternal linkage maps. The female map covered 1604 cM and the male map length was 1496 cM. The second map of the diploid *Fragaria* population was generated from the F2 population developed from an interspecific hybridization of *F. vesca* 815 and *F. bucharica* 601 [27]. A total of 78 markers were mapped, 68 of them were simple sequence repeats (SSRs), and the map length was computed as 448 cM. This mapping was important because it was the first SSR-based linkage map in strawberry. This map was enriched by different mapping studies using restriction fragment length polymorphism (RFLPs), AFLPs, SSRs, SNPs, expressed sequence tags (ESTs) and gene-specific markers located in seven linkage groups and the total map length was 528.1 cM. This map is considered the reference framework map in the genus *Fragaria* [27–32].

An AFLP-based genetic map was generated by Lerceteau-Köhler et al. [26], and this map was enriched by Rousseau-Gueutin et al. [33]. The researchers explained that a total of 213 genotypes and many AFLP markers, sequence-characterized

amplified regions (SCARs) and SSRs were located in linkage groups. In the female map, 367 loci were mapped into 28 linkage groups, and the map length was 2582 cM, while a total of 440 markers were placed into 26 linkage groups in the male map and the length was 2165 cM. The final map is considered as the first comprehensive reference map for octoploid strawberries. This map helps the construction of the homoeologous of the four *Fragaria* x *ananassa* linkage groups using the *F. vesca* reference map. Another linkage map was also constructed in *F. virginiana*. A total of 319 and 331 SSR-based markers were located in 33 and 32 linkage groups which belong to maternal and paternal maps, respectively [34, 35]. One of the most comprehensive linkage maps in octoploid strawberries was constructed with 490 transferrable SSR or gene-specific markers in individuals obtained from Redgauntlet × Hapil. The authors stated that the constructed linkage map can be used as a framework for future genetic studies and to facilitate the marker-assisted selection in octoploid strawberry breeding. This high-density map was constructed which represents 91% of the *Fragaria* x *ananassa* genome having a map length of 2140.3 cM and consisting of 549 markers [36].

A total of 186 SSR markers were mapped into 28 linkage groups in octoploid strawberry, derived from Holiday × Korona crossing, the estimated map length was 2050 cM [37]. Another linkage map in *Fragaria* x *ananassa* was generated using a total of 4474 SSRs (3746 EST-SSRs in *F. vesca*, 603 EST-derived in *Fragaria* x *ananassa,* and 125 transcriptomic-derived from *Fragaria* x *ananassa*) were used for mapping. All markers were first mapped onto a parent-specific linkage map using three different populations such as 02–19 × Sachinoka, Kaorino × Akihime, and 0212921 × 0212921 inbred lines which were assembled onto one linkage map. The constructed map length was calculated as 2364.1 cM [38]. The backcross progenies of a cross between *F. vesca* and *F. viridis* were utilized in linkage mapping and the map length was computed as 241.6 cM [39, 40]. The construction of linkage groups in octoploid strawberries can be used for comparative studies between the diploid and octoploid maps.

The development of NGS technology and a decrease in unit cost in sequencing increased the improvement of the different sequencing platforms such as ddRAD-seq, DarT-seq, and WGRS (whole genome re-sequencing). Construction of the well-saturated linkage maps is very important for the discrimination of the homoeologous belonging to each chromosome in strawberries. Tennessen et al. [41] used a novel approach called Phylogenetics of Linkage-MapAnchored Polyploid Sub-genomes (POLiMAPS) and understanding of the octoploid strawberry sub-genomes in *F. vesca* subsp. *bracteata*, *Fragaria chiloensis*, and *F. virginiana* populations. The researchers stated that one of these sub-genomes is close to diploid *F. vesca* and the diploid *F. iinumae*, others are close to an unknown ancestor of *F. iinumae*. Meanwhile, the IStraw 90 K Axiom® array was developed by the RosBREED project with international cooperation. The IStraw 90 K Axiom® array was mined from the first draft of the *F. vesca* genome (v1.0). This array contributed to the construction of the map for detection of the homoeologous sub-genomes [42]. It was constructed by using 26 *Fragaria* species, 16 *F.* x *ananassa* and 10 individuals belonging to wild strawberry species. Totally, mined 6594 SNP loci were located on the first SNP linkage map generated by Bassil et al. [42]. The digest restriction-associated DNA sequencing and diversity array technology were used for the construction of high-density linkage maps (ddRAD-Seq, [43]; DarT, [44]). In another study, the IStraw90 SNP array and genotyping by sequencing technology (GBS) were utilized by Mahoney et al. [45] in order to construct a highly saturated

linkage group in diploid strawberry, *F. iinumae*. Sargent et al. [36] consisted of the highly saturated linkage map using many polymorphic loci (8407 SNPs) and the IStraw 90 K Axiom® and this map contributed to the detection of the *F. ananassa* genome structure. The SNP alleles on the linkage map were compared with the corresponding *F. vesca* and *F. iinumae* alleles, and the haploSNPs were categorized in the current study. HaploSNPs, which are sub-genome specific markers that have saturated common markers, can be used to compare QTL regions in strawberries, revealing the ancestral-associated loci [36]. These linkage maps provided a better understanding of the genome structure of the octoploid strawberry and increased the interest in SNP-based linkage mapping studies for practical breeding applications.

In strawberry breeding, the development of linkage maps is very important in order to reveal the genetic structure of complex traits because this complexity can be only solved using the QTL mapping and/or association mapping approaches [46]. Although a few association mapping studies have been reported in strawberries, many genetic mapping studies have been performed using biparental populations that have been generated from different crosses [26, 32, 33, 47–50].

3. QTLs in strawberry

A QTL is defined as the point or part of coding or non-coding chromosomal regions representing a trait quantitatively. QTL mapping is based on biparental or natural populations and aims at the detection of correlation between phenotypic traits and genomic regions. To carry out the QTL mapping, variant detection in genomic data is completed and phenotypic values are evaluated for each individual in the population. Detection of the chromosomal position related to important genes is one of the major aims of QTL studies. In this section, we will review the previous QTL studies about agronomically important traits that have large effects on strawberry breeding.

In the cultivated octoploid strawberry, the detected QTLs were located at homoeologous loci, meaning each locus can be represented up to four times in the genome as homoeologous loci, each presenting two homologous alleles. These QTLs at homoeologous locations can be considered as homoeo-QTLs [32, 51].

4. QTLs related to the morphological and biochemical traits in strawberry

The construction of a high-resolution genetic map is significant because the SNP array development has been for the QTL analyses associated with agronomically important traits in strawberry breeding and cultivation. Researchers from different countries have come together to solve the problems in strawberry breeding around the world. They decided to use the strawberry gene resources and aim to develop new strategies depending on the solution to current problems [10]. Therefore, big data that is generated from these projects needs to be mined fast and effectively because QTL analysis of large-scale data is a problem of software that must be merged large amounts of genotypic and phenotypic data into many individuals of a population.

To date, the detected QTLs have been related to fruit quality and reproductive traits. A number of QTLs have also been identified, which are associated with remontancy, flowering time, runner production, fruit shape, fruit firmness, and

biochemical fruit traits such as anthocyanin, fatty acids, phenolic compounds, chlorophyll content, gamma-decalactone and mesifurane (strawberry aroma compounds), and titratable acidity [51–53].

In QTL mapping related to flowering time and some vegetative traits in the (*F. vesca*) woodland strawberry population, three QTLs were detected in LG IV, LG VII, and LG VI. These novel QTLs in this study were identified for the first time, and they were located within the previously detected FvTFL1 gene. Additionally, a stable QTL was determined using the phenotypic data obtained from different locations experiments and the flowering time governing by gene(s) located in this region can be associated with these loci. The authors stated that these candidate genes associated with flowering time genes can be used for the detection of the major QTL regions in a closely related octoploid strawberry [54].

Rey-Serra et al. [55] genotyped the F1 population and F2 population crosses in order to identify genetic variability within the populations, using IStraw35k and IStraw90k SNP arrays. A total of 14,595 and 7977 SNPs were mined in F1 and F2 populations, respectively. According to QTL analysis, although a total of 33 QTLs were detected in the F1 FC50 × FD54 population for shape traits in LG III, IV, and VI, eight QTL regions were identified in the F2 population. The detected a major QTL linked by fruit shape in LG III has 25% total phenotypic variance. Another QTL associated with fruit firmness was calculated as 26.9% total phenotypic variance in LG VII. However, the researchers reported that two QTLs were mapped in LGIII and LGIV for the neck without achenes regions. On the other hand, Nagamatsu et al. [56] conducted a study in order to identify QTLs associated with fruit shape using a MAGIC population derived from full-sib families. In genome-wide association analysis, QTL regions were detected within chromosomes 6 and 7 in genome-wide association analysis.

The short-day type is a problem in strawberry cultivation due to limiting the whole season's productivity, since most of the strawberry cultivars have the short-day type. Day-neutrality is a good choice for decreasing the intensity of the harvest in particular seasons. Thus, everbearing or day-neutral cultivars were preferred to control cultural cultivation applications easily. Day-neutrality is the desired situation in a strawberry to spread throughout the harvest season. Weebadde et al. [47] studied flower blooming traits under long-day conditions using a biparental population of Honeoye × Tribute for QTL analysis. The present study was carried out in different locations and eight QTLs linked to the everbearing were detected as major QTLs with one of these loci having nearly 36% of the total phenotypic variation. The researchers reported that day-neutrality in a strawberry was a multigenic trait. A QTL study related to runner production of strawberries was also performed on the same strawberry population [57]. They determined a single nucleotide variant, ChFaM148–184 T, with 32.4% of the total phenotypic variation for runner production. They stated that day-neutrality phenotypic traits might be controlled either by a single gene or tightly linked polygenes. Associated with runner production, flowering time, and repeat were studied by Sooriyapathirana et al. [58] using the SSR markers. A major QTL was detected linked to day-neutrality within the LG IV. Several QTLs were identified associated with flower and runner production in the present study on different linkage groups.

A major QTL was detected on LG IV associated with day-neutrality in *F.* x *ananassa* in a population generated from Capitola and CF1116 [59]. This major QTL was also in the same location in a previous study using the *Fragaria virginiana ssp. glauca* [60]. Verma et al. [61] reported that this QTL was intensely utilized in the strawberry

breeding program at UC Davis [62]. The determined QTL had a single dominant allele on one sub-genome governing the transformation from short-day to day-neutrality [59]. Dominant variants like this are quite common in polyploidy species in order to reduce functional day-neutrality [63–65]. Another different major QTL was also detected in the Honeoye and Tribute population on LG IV. This major QTL is very significant to being identified in the same chromosomal region in all of the data from five different locations. The locus ChFaM148–184 T was found highly linked with this QTL and the total phenotypic explained variation was 32.4%. Verma et al. [61] stated that they performed the QTL analysis related to the day-neutrality in the RosBREED strawberry germplasm dataset. The results demonstrated that there is a major QTL in the same linkage group, LG IV [61].

Another comprehensive QTL study was carried out using an F1 population generated from 232 and 1392, selections and a total of 33 QTLs were identified in 17 agronomic and fruit quality traits such as yield or fruit size and fruit quality traits such as soluble solids content (SSC), ascorbic acid, titratable acidity (TA), color and firmness using SSRs, and AFLPs markers [32]. The authors identified a few candidate genes within the linked QTLs. The identified gene, FaGaLUR, related to the d-galacturonic acid pathway in strawberry fruit and is predicted to govern L-ascorbic acid on LG IV. The QTLs related to anthocyanins and acidity were located in LG V. Within only one QTL, FaMYOX gene was identified and joined to myoinositol biosynthesis. Another QTL associated with fruit firmness was located within the same chromosomal region governed by gene FaExp2 in LG VII. The detected QTL expressed an SGR-like gene that was determined to be linked by photosynthetic chlorophyll-protein complexes and this gene was co-located in the same chromosomal position. The authors stated that photosynthetic chlorophyll-protein complexes might be controlled for yield and yield-related traits. Labadie et al. [66] performed QTL analysis using the F1 population of cultivated strawberries (*F.* x *ananassa*). They have phenotyped the individuals in two successive years' fruit quality traits, such as flavonoid, anthocyanin, flavonols, flavan-3-ols, anthocyanin, flavonoids, phenolics, and total antioxidant capacity. A total of 178 QTLs on the female and male linkage maps of 152 flavonoid metabolic and colorimetric traits were detected, and these QTLs overlapped the previously identified QTL regions related to flavonoid and taste-related traits. The colorimetric QTLs were located within the LG III and LG VI by homologs.

Populations	Analyzed Traits	No. of QTL	Phenotypic Variation	References
F. x *ananassa* cv. Capitola *F.* x *ananassa* breeding line CF1116	*Colletotrichum acutatum* and *Phytophthora cactorum* resistance	10	5.8–12.2%	Denoyes-Rothan et al. [67]
F. x *ananassa* cv. Capitola *F.* x *ananassa* breeding line CF1116	34 fruit quality traits	22	6.5–16.0%	Lerceteau-Kohler et al. [68]
F. vesca, *F. bucharica*	6 flowering-related traits	9	10.6–30.3%	Sargent et al. [28]
F. x *ananassa* F1 population derived from Tribute and Honeoye	Flower blooming trait (Day-neutrality)	8	11.5–36%	Weebadde et al. [33]
F. x *ananassa* line 232 *F.* x *ananassa* line 1392	17 agronomical and fruit quality traits	33	9.2–30.5%	Zorrilla-Fontanesi et al. [50]

Populations	Analyzed Traits	No. of QTL	Phenotypic Variation	References
F. x *ananassa* cv. Capitola *F.* x *ananassa* breeding line C141116	19 different fruit-related traits	87	5–17%	Lerceteau-Kohler et al. [32]
F. x *ananassa* line 232, *F.* x *ananassa* line 1392	48 volatile compounds	70	14.2–92.8%	Zorrilla-Fontanesi et al. [51]
F. x *ananassa* cv. Tochiotome *F.* x *ananassa* cv. Itigoiyukanbohonnou2gou (Nou2gou)	Strawberry anthracnose resistance	9	7.7–14.4%	limura et al. [69]
F. x *ananassa* cv. Capitols *F.* x *ananassa* breeding line CF1116	Perpetual flowering and runnering	19	4.0–59.3%	Gaston et al. [59]
F. x *ananassa* F2 cross Dover × Camarosa	Fruit-quality traits	—	—	Molina-Hidalgo et al. [70]
F. x *ananassa* line 232 *F.* x *ananassa* line 1392	Fruit-quality traits	—	—	Sánchez-Sevilla et al. [53]
F. x *ananassa* F1 population derived from Tribute and Honeoye	Flower blooming trait (Day-neutrality) Runner production	2	32.4–63.8%	Castro et al. [57]
F. x *ananassa* cv. Redgauntlet *F.* x *ananassa* cv. Hapil	*Verricillison dahliae* resistance	11	—	Antanaviciute et al. [71]
F. x *ananassa* F1 population derived from Tribute and Honeoye	Flower blooming traits (Day-neutrality) Runner production	major QTLs	10.0–34.4%	Sooriyapathirana et al. [58]
F. vesca, *F. bucharica*	Polyphenolic compounds	76	—	Urrutia et al. [72]
F. x *ananassa* University of Florida breeding population sets	*Xanthomonas fragariae* resistance	a major QTL	—	Roach et al. [73]
F. x *ananassa* F1 cross Delmarvel × Selva	Fruit-quality traits	27	4.8–10.7%	Castro and Lewers [50]
F. x *ananassa* University of Florida breeding population sets	*Phytophthora cactorum* resistance	4	13.7–25.3%	Mangandi et al. [74]
F2 population derived from the cross *F. vesca* *F. semperflorens* cv. Hawaii-4(H4) × *F. vesca* subsp. *vesca* (FV), (denoted H4 × FV)	Flowering time and vegetative growth	3	12–25%	Samad et al. [54]
F. x *ananassa* University of Florida breeding population sets	*Colletotrichum gloeosporioides* resistance	a major QTL	17–29.8%	Anciro et al. [75]
F. x *ananassa* University of Florida breeding population sets	*Colletotrichum acutatum* resistance	a major QTL	50%	Salinas et al. [76]
F. x *ananassa* F1 cross Emily × Fenella	*Phytophthora cactorum* resistance	3	10.3–36.5%	Nellist et al. [77]
F. x *ananassa* cv. Capitola *F.* x *ananassa* breeding line CF1116	Fruit-quality traits (f flavonoid-related traits)	178	18–44%	Labadie et al. [66]

Populations	Analyzed Traits	No. of QTL	Phenotypic Variation	References
F1 population cross between 'FC50' and 'FD54' and an F2 population cross between 'Camarosa' and 'Dover',	Fruit shape, firmness, and other quality traits	33	12.6–36.8%	Rey-Serra et al. [55]
F. x *ananassa* cv. Benihoppe *F.* x *ananassa* 105(14–9) breeding line from cv Sachinoka	Chlorophyll content	7	1.4–26.4%	Siddique et al. [78]
RosBREED strawberry germplasm set	Flower blooming trait (Day-neutrality)	—	—	Verma et al. [61]

Although QTL studies are limited to the last two decades, numerous QTLs related to strawberry fruit quality traits and several reproductive traits have been identified in various strawberry breeding studies around the world in recent years. Lerceteau-Köhler et al. [51] studied the QTLs associated with fruit quality traits such as fruit size, firmness, color, sugars, organic acids, and anthocyanins using an octoploid strawberry F1 population generated from the cross between Capitola and CF116. Totally, 87 QTLs associated with quality traits were identified, and phenotypic variation for all the QTLs ranged from 5% to 17%. The authors reported that QTLs linked to the soluble solids content (SSC) were identified in LG III, V, and VI in the same population. Castro and Lewers [50] identified a total of 27 QTL linked to fruit quality traits and the total phenotypic variation for each QTL was computed, ranging from 4.8% to 10.7%. There are colocations in QTLs associated with anthocyanins and antioxidant capacity or total phenolics while the QTLs were identified in different LGs. These colocations can be very important for indirect selection according to the higher antioxidant capacity of fruits [50]. The authors stated that they identified three QTLs related to SSC that were placed on LG II, LG V, and LG VI and used the van Dijk et al. [37] genetic map to compare the results of two studies; the QTLs for SSC were located on LG VI. Castro and Lewers [50] also performed QTL analysis for SSC/TA rate and titratable acidity (TA), and an association was detected on LG VI. These results demonstrated that these QTLs were associated with each other. The QTL linked by TA was determined in LG IV. The linkage map must be saturated with common markers for comparison of QTL regions obtained from previously described findings, such as the haplo-SNPs developed by Sargent et al. [36]. The highly saturated HK SNP genetic map was used for QTL analysis in fruit quality traits and resistance to pathogens [79]. The detected QTL-linked SSC was located on LG VI close to QTL regions of previous studies. Lerceteau-Köhler et al. [51] and Verma et al. [79] discovered QTLs for sucrose and glucose traits that were very close to the SSC. The detected QTLs in populations with different genetic backgrounds could be located close to each other within the same chromosomal regions because fruit quality traits such as glucose and sucrose have similar missions in metabolism.

Zorrilla-Fontanesi et al. [52] detected a total of 70 QTLs associated with volatile compounds using the 232 × 1392 population and 35 of them were identified in two or three successive years. These QTLs were located on all LGs with the exception of LG II, this distribution can be explained as the pleiotropic effect of the one locus over the volatile organic compounds (VOCs). Because VOCs have a high correlation, the QTLs for different esters and alcohols are also included in a cluster. Therefore, every single

locus was determined to be involved in the biosynthesis or regulation of all the related VOCs. The phenotypic variation percentages were computed, ranging from 14.2% to 92.8%. It can be considered that these major QTLs governing the strawberry fruit aroma were controlled by the several set of loci and had a higher effect than the multiple loci because if this trait had been governed by multiple loci, phenotypic variation percentages would not be higher like this. The two major components related to the VOCs, such as mesifurane and γ-decalactone, whose associated QTLs have ranged from 42% to 67.3% of the total phenotypic variation, and they detected one QTL that has above 90% of the total phenotypic variation. Expression analysis was performed using the parental and F1 progenies and the FaOMT gene was detected in the content of mesifurane in strawberries [52]. This expression was found in progenies, and the expression level of the FaOMT gene was calculated to be higher in the ripening periods. γ-decalactone is a type of lactone and the most abundant lactone in red-ripened fruit. It provides the 'peachy' feel in strawberry [80, 81]. Although this lactone was identified as intensively in the line 1392 parent and was not found in the line 232 parent, this lactone transferred to only half of all progenies in the population. The linked loci were placed in the LG III [52].

Sánchez-Sevilla et al. [53] published a study using genome-wide RNA-seq analysis and bulk segregant analysis, and they detected a novel gene associated with the fatty acid desaturase, FaFAD1, governing γ-decalactone content in strawberry. Similarly, Chambers et al. [82] identified the same gene linked to γ-decalactone using another segregated population as a complement to previous research performed by Sánchez-Sevilla et al. [53]. The developed markers related to the genes, FaOMT and FaFAD1, can be used for the evaluation of these population for prediction of the phenotype with 100% accuracy. Cruz-Rus et al. [83] validated these markers using different and wider strawberry germplasm and the validation percentage was found above 91%. Consequently, the obtained results demonstrated that these genes can be used for future strawberry breeding programs.

In strawberries, phenolic compounds such as anthocyanins, flavonoids, and phenylpropanoids accumulate in ripen strawberries [84, 85]. Phenolic compounds have an important role in protection against abiotic and biotic stress conditions. The transcriptomic analysis of different strawberry genotypes was used to reveal genes' associations with phenolic composition. According to differentially expressed analysis, a candidate gene within the ESTs, FaPRX27, was detected in the ripening period. The researchers mapped the FaPRX27 gene in two segregating populations, FaPRX27 and association QTLs were located in the same chromosomal regions (LG III) and they also mapped a QTL linked to fruit color trait [49].

Molina-Hidalgo et al. [70] characterized the rhamnogalacturonate lyase gene (FaRGlyase1) using an oligonucleotide-based microarray platform in strawberries. The gene FaR-Glyase1 was mapped in a linkage map derived from Dover × Camarosa and with a QTL linked to fruit firmness in LG I.

5. QTLs related to pathogens resistance in strawberry

One of the most important shortcomings in strawberry breeding is the lack of commercially cultivated strawberry cultivars with high resistance to many destructive pathogens. Developing disease-resistant cultivars or improving existing cultivars in breeding programs is a priority objective for many strawberry breeding programs around the world [86]. Diseases based on pathogens are not only a very big issue

limiting strawberry production, but also restricts the breeding programs. Thus, resistance to diseases has become a significant focus point for strawberry breeding due to its large effects. In this regard, many major and minor QTLs linked to resistance to pathogens have been identified in cultivated octoploid strawberries so far.

The first QTL related to disease resistance in a strawberry was performed and several RAPD markers associated with the Rfp1 locus, a gene that confers resistance to *Phytophthora fragariae* var. *Fragariae* have been reported by Haymes et al. [87]. Then, two sequence-characterized amplified region (SCAR) markers closely associated with the Rpf1 gene were found to be linked with resistance to *P. fragariae* [88]. For resistance to *Colletotrichum acutatum* pathogenicity, SCAR markers associated with the Rca2 gene were developed [68]. The fruit quality traits were one of the first QTL analyses among the agronomically important traits in strawberries. The population derived from Capitola and CF1116 had a total of 213 genotypes that had many opposite traits and were used for the creation of the mapping population. QTLs related to 34 fruit quality traits were identified in this population [89]. The phenotypic evaluation was carried out over two consecutive years and a total of 22 QTLs were detected, among them 8 QTLs on the female and 14 on the male map and phenotypic variance (PV) values were calculated ranging from 6.5% to 16% in these traits. Denoyes-Rothan et al. [67] determined QTL regions related to resistances of *Cerastium acutatum* and *Phytophthora cactorum* using the same map developed by Lerceteau-Kohler. Although all progenies were screened for *P. cactorum* reaction levels, selected 185 plants were screened in terms of their resistance and susceptibility to *C. acutatum*, while the entire progeny was screened for reaction to *P. cactorum*. Totally, five QTLs with a PV ranging from 5.8% to 10.2% were identified for the resistance to *C. acutatum*.

A total of 39 full-sib families derived from octoploid strawberries were utilized in order to test for resistance to anthracnose fruit rot (AFR). Additionally, a validation population consisting of 77 advanced selections and 10 cultivars was tested in the second season. The phenotyping of accessions was performed every week, and genotyping data was obtained using the IStraw35 SNP array. A major QTL linked by resistance to *C. acutatum* that is named FaRCa1 QTL was located at 55–56 cM of LG VI and the phenotypic variation of this locus was calculated as 50%. It was identified that this locus has a dominant allelic nature in all trials. In this study, a locus linked by AFR was detected in LG VI, while markers associated with resistance to *C. acutatum* were found in LG VII in the previous finding gene, Rca2 [76].

QTLs linked with resistance to *Verticillium dahlia* were identified in the cultivated octoploid strawberry [71]. Researchers investigated resistance to the crown and root rot diseases caused by *Colletotrichum gloeosporioides*, *P. cactorum,* and leaf spot caused by *Xanthomonas fragariae* in strawberry. The clonal replicates were derived from 139 full-sib families. More than 1100 strawberry plants were monitored for crown and root rot symptoms in two successive seasons [61].

Roach et al. [73] reported that defined QTL associated with resistance to bacterial leaf spot led by *X. fragariae* (FaRXf1) and that this QTL was controlled by a single dominant allele and demonstrated a 1:1 segregation. According to pedigree-based QTL analysis, major and minor QTLs such as Cg1 and Cg2 were determined as linked with resistance to *C. gloeosporioides* and verified in two successive years [74, 75]. Although total phenotypic variation belongs to Cg1, it explained 26% of the total PV, while Cg2 was identified as minor and explained 5% of the total PV. The associated QTLs that resistance to pathogens such as *P. cactorum* or phytophthora, FaRPc2, were identified and tested [74, 79]. It was noticed that this locus represented 35% of total

the phenotypic variation in *P. cactorum*. Three major QTLs for resistance to *P. cactorum*, a water-borne disease, were detected using the segregation population. These QTLs account for 37% phenotypic variation in the population. On the other hand, the researchers reported that several loci associated with crown rot disease caused by *P. cactorum* using a genome-wide association study (GWAS) of 114 individuals were identified in the present study. The loci linked by crown rot were mapped within the LG VI and VII, while the manhattan plot obtained from GWAS demonstrated that associated loci were located on chromosomes 5 and 7 [77].

Gray mold is a pathogenic disease that causes postharvest decay in many different species. This disease, caused by *Botrytis cinerea*, infects different plant tissues and is more destructive, especially in mature fruits and this increases post-harvest economic losses. Petrasch et al. [90] detected the QTLs related to gray mold using the 50 K Axiom SNP array [91] and five full-sib families. A total of nine QTLs were found most significant according to LOD score and these QTLs were located on chromosomes 3, 4, 5, and 7 different sub-genomes. Since the identified QTLs were minor QTLs and distributed among the different sub-genomes of chromosomes, the genes responsible for gray mold can be controlled by polygenes.

6. Marker-assisted selection (MAS) in strawberry

Marker-assisted selection is one of the most important breeding techniques that shorten the breeding period in modern molecular breeding studies. To date, different marker technics such as SSRs and SNPs were used in octoploid strawberry breeding programs for MAS associated with many agronomical traits [44, 52, 92].

Simple sequence repeat markers are preferred in molecular studies due to their high level of polymorphism, abundance in the whole genome, co-dominant nature, and reliability. However, this system is not cost-effective for screening of large breeding populations. Another reason is that it produces more than a pair of alleles due to having sub-genomes of octoploid strawberries. Thus, SSRs are not practical because of their multi-allelic nature for large breeding populations and for cloning of associated genes. Although rapid DNA isolation protocols and next-generation genotyping platforms were recently developed by Noh et al. [93] for strawberry breeding, these applications are very expensive and laborious because of the need to obtain high-quality DNA in species with a high content of phenolic compounds, such as strawberry.

In the last two decades, high-resolution melting (HRM) and TaqMan-based markers have been utilized for MAS in strawberry breeding effectively. On the other hand, probe-based methods such as Kompetitive Allele-Specific PCR (KASP) markers can be designed for any species because they are flexible. However, these markers are not feasible for multiplex genotyping while HRM is a post-PCR analysis and does not require sequence validation of the target region. In this regard, SNP-based DNA markers such as endpoint genotyping and KASP should be continued for use in marker-assisted breeding in strawberries.

There is a desirable strategy in MAS is that detected QTL is governed by one or a few major loci or genes. Especially if loci on major QTLs were located in a single sub-genome and this is a positive situation for effective MAS in the cultivated octoploid strawberry. The validated DNA loci associated with traits have been widely used in numerous breeding programs, and the detected linked DNA loci have recently been verified and will be used in breeding programs [61].

The c-decalactone is a volatile compound and is responsible for 'peach-like' aroma in strawberries. Chambers et al. [82] and Sánchez-Sevilla et al. [53] identified the fatty acid desaturase gene (FaFAD1), which is responsible for the c-decalactone biosynthesis process. Sánchez-Sevilla et al. [53] discovered a desaturase gene (FaFAD1) on LG III and they tested all cultivars. Although the gene responsible for this volatility was amplified in less than half of the cultivars, it did not amplify in the rest of the cultivars. The researchers developed a functional SCAR marker in the upstream region of the strawberry genome. The gene created a 500 bp PCR product if the strawberry cultivars have this gene, if not, it does not generate any PCR product. An SSR marker responsible for this volatile compound was developed on an 11 kb upstream genic region related to FaFAD1. It generated a PCR product with 205 bp.

One of the HRM primer pairs developed in the FaFAD1 genic region, GDHRM5 has been used in the UF breeding program. Developing a codominant marker is desired to distinguish individuals with homozygous and heterozygous loci. Recently, a candidate gene associated with mesifurane, the FaOMT gene, was replaced on LG VII [52]. The authors developed the marker based on agarose gel and they identified the functional PCR amplification in 248 bp and the non-functional locus in 217 bp (after 30 bp deletion). Thus, the scoring of the products was performed according to the absence or presence of amplification of the mesifurane gene.

Remontancy is defined as the unresponsiveness of the day-light and it is an important trait to provide throughout the whole season of production in strawberry cultivation. Gaston et al. [59] detected the QTL linked to the day-neutrality, transition from short-day to remontant blooming, governed by the FaPFRU locus identified in the UC Davis breeding program. Another genetic mapping discovery revealed that an SSR locus detected was mostly associated with FaPFRU [94]. This SSR marker was validated in RosBREED germplasm and about 90% of the accessions that were not producing any bands on 129 bp did not flower under the long day conditions.

The screening of populations using the DNA marker for disease resistance in strawberry become more popular, particularly in the last decades. The resistance to *C. acutatum* pathogenicity test is one of these tests, and the Rca2 locus was found to be associated with this trait [68]. It has a dominant allele that is responsible for resistance, and these AFLP markers were converted into two SCAR markers, which amplified a 240 bp PCR product. Thus, these SCAR markers have been used easily in separation by agarose gel-based electrophoresis. The resistance to root and crown rot caused by *P. cactorum* locus was found as the FaRPc2 on the linkage group VII [74]. The FaRPc2 is detected in the major QTL region, which is responsible for about 35–40% of the phenotypic variation [61]. Van de Weg [95] identified the gene resistance to red stele in strawberries. The Rpf1 gene has been used for MAS as resistance factor R1 to *P. fragariae* var. *fragariae*. Recently, one SSR marker associated with R1 was developed and amplification was performed in 99% of the strawberry samples [61]. The previous study took place using a total of 49 *F.* x *ananassa* individuals to verify the Rfp1 gene that is associated with disease resistance. The results demonstrated that 17 accessions created the PCR fragment while 32 individuals were evaluated as susceptible [96]. The angular leaf spot disease caused by *X. fragariae* is the main problem in strawberries. Recently, a single dominant locus located in linkage group VI was associated with this bacterial disease resistance in octoploid strawberries [73].

The reason for the high interest and appreciation for strawberries is their sweetness. And with the method developed by Schwieterman et al. [97], the sweetness can be predicted in strawberry individuals. The QTL region associated with the soluble solids content (SSC) is an indicator of sweetness in strawberries [98], EMFv006 was

located on LG VI using the 'Capitola' CF1116 population [51]. The QTL analysis was performed using the 609 strawberry accessions, which consisted of worldwide promising lines or selections and cultivars in 2011 and 2012 [96, 99] The detected QTL was determined very closely near to the EMFv006 SSR marker on LG VI [61]. Verma et al. [61] investigated the association of this marker with the SSC trait in different germplasm sets, but there was no association between the trait and marker EMFv006. That means using this marker in MAS studies may not be a good idea.

In this section, we summarized QTLs associated with important agronomical traits and different disease resistance traits, which are very important in strawberry breeding. And these QTLs can be converted into DNA markers that are closely linked to the desired traits. These markers can be used in MAS in strawberry breeding due to their easy scoring, not being complicated, and giving more accurate results. These markers were developed for MAS in many genetic resources as a common idea for solving the problems encountered in strawberry breeding.

7. Future prospects in strawberry breeding

Thanks to the increase of genomic studies in plant breeding, it consists of many areas, such as the development of new approaches and strategies in order to shorten processes, such as the development of promising new cultivars or genotypes with desired characteristics. In this way, advances in strawberry breeding depending on genomic developments are very important processes for octoploid cultural strawberries. Detection of the four sub-genomes of the strawberry genome and different next-generation sequencing methods will provide the opportunity to make a more accurate genetic assembly. The genomic selection in the early period can take place with a more accurate and easier explanation of the functions of the SNPs associated with all traits of the strawberry. The identification and characterization of candidate gene regions linked by economically important traits will facilitate advances in genomic selection. Achieving the targeted objectives in strawberry breeding will enable high-density genetic and physical maps to be obtained and effective marker-assisted selection with the reduction of sequencing unit costs.

In breeding studies, geneticists, breeders, and even farmers want to monitor the process of identifying the underlying biological problems of a phenotypic trait and those associated with genomics. Therefore, many studies have been conducted on the detection of QTLs associated with many traits. However, not only the determination of QTL regions but also the detection of structural and copy number variations will allow the related trait to be examined from a wider perspective. As a result, researching the functions of candidate genes associated with traits and post-transcriptional translation processes, including epigenetic variations, supplies the necessary infrastructure in genomic sequencing. In the future, it will be possible to replace undesirable loci associated with desired traits with methods such as CRISPR-cas9.

Therefore, new loci associated with traits will be discovered in many breeding programs with new technological and genomic approaches in future strawberry breeding.

8. Conclusion

In order to detect more stable QTL regions in plants, it is basically needed to have a high-quality genome assembly. By increasing the quality of the octoploid strawberry

genome, it can be ensured that the four sub-genomes are brought together or differentiated with a good assembly. In addition, better coverage of individuals' genomes will be ensured with next-generation SNP sequences. In this way, it will allow a more accurate description of the genome functions of the relevant QTL regions. It will also allow the discovery of additional chromosomal regions that control economically important fruit traits and the development of functional markers targeted by candidate genes. Their high-resolution mapping along with the reduction of unit cost in sequencing will allow the discovery of rare markers associated with economically important traits.

Recently, a quality genome assembly and the mapping of QTLs associated with economically important traits and their use in MAS have greatly facilitated breeding programs. To date, most of the QTLs detected are environment specific. The number of experiments, the year, and the application and cultivation conditions are very important for QTL studies. In particular, the selection of populations such as F2, F3, and BC to be used for mapping is very important, as are other conditions. The distance of associated QTLs, the impact of major QTLs on the population, environmental factors, population size used in mapping, and based on individual errors affect the accuracy of QTLs. However, increasing the number of individuals in the population allows the estimation and determination of the phenotypic variance value of minor QTLs. On the other hand, the techniques used for the validation of QTLs are very important for the use of linked markers in MAS. Some important loci may be eliminated due to the selection of inadequate verification methods of linked markers. Therefore, the discovery of QTLs associated with economically important traits in strawberries for selection at an early stage is very significant. In this process, this summary can be useful for QTLs linked to strawberry traits.

Author details

Harun Karci, Habibullah Tevfik, Nesibe Ebru Kafkas* and Salih Kafkas
Department of Horticulture, Faculty of Agriculture, Çukurova University, Adana, Turkey

*Address all correspondence to: ebruyasakafkas@gmail.com

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