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ORIGINAL PAPER

Quantitative trait loci and underlying candidate genes controlling agronomical and fruit quality traits in octoploid strawberry (*Fragaria* × *ananassa*)

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Abstract Breeding for fruit quality traits in strawberry (*Fragaria* × *ananassa*, $2n = 8x = 56$) is complex due to the polygenic nature of these traits and the octoploid constitution of this species. In order to improve the efficiency of genotype selection, the identification of quantitative trait loci (QTL) and associated molecular markers will constitute a valuable tool for breeding programs. However, the implementation of these markers in breeding programs depends upon the complexity and stability of QTLs across different environments. In this work, the genetic control of 17 agronomical and fruit quality traits was investigated in strawberry using a F_1 population derived from an intraspecific cross between two contrasting selection lines, '232' and '1392'. QTL analyses were performed over three successive years based on the separate parental linkage maps and a pseudo-testcross strategy. The integrated strawberry genetic map consists of 338 molecular markers covering 37 linkage groups, thus exceeding the 28 chromosomes. 33 QTLs were identified for 14 of the 17 studied traits and approximately 37% of them were stable over time. For each trait, 1–5 QTLs were identified with individual effects ranging between 9.2 and 30.5% of the phenotypic variation, indicating that all analysed traits are complex and quantitatively inherited. Many QTLs controlling correlated traits were co-located in homoeology group V, indicating linkage or pleiotropic effects of loci. Candidate genes for several QTLs controlling yield, anthocyanins, firmness and L-ascorbic acid are proposed based on both their co-localization and predicted function. We also report conserved QTLs among strawberry and other Rosaceae based on their syntenic location.

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Introduction

The cultivated strawberry (*Fragaria* × *ananassa*), which belongs to the Rosaceae family, is the most consumed berry fruit worldwide. Global annual production has reached four million tons in the last few years (FAOSTAT 2009; http://faostat.fao.org), with the major producing area located in the coastal region of California (USA) (Folta and Davis 2006). Spain was the third worldwide producer in 2009, with more than 95% of the fields located in Huelva, at the south-west of the country, where climatic conditions and soil characteristics suit similar cultivars to those grown in California (Medina-Minguez 2008). The strawberry fruit is highly valued for its delicate flavor and nutritional value. However, key attributes contributing to fruit quality have

been reduced or lost because breeding of modern cultivars has mainly focused on agronomical traits such as fruit size and yield. Therefore, improvement of strawberry flavor and nutritional quality has become an important factor in current breeding programs.

Fruit development and ripening is a highly programmed process relying on the synergistic and coordinated action of many genes, leading to a number of biochemical and physiological transformations which determine the nutritional and organoleptic quality traits of strawberry fruit (Fait et al. 2008; Folta and Davis 2006). Among these traits, fruit texture notably affects overall quality and consumer appreciation. Excessive softening limits shelf life and storability, thereby increasing disease susceptibility and economic loss. During ripening, strawberry fruit become less firm due to degradation of the cell wall and several genes involved in this process have been isolated and characterized (Castillejo et al. 2004; Civello et al. 1999; Jiménez-Bermúdez et al. 2002; Redondo-Nevado et al. 2001; Trainotti et al. 1999a, b). In particular, expansins are key endogenous regulators of plant cell wall remodeling (Cosgrove 2000a, b) and their involvement in fruit softening have been demonstrated in various crops, such as tomato (Brummell et al. 1999). During strawberry ripening, there is a sharp increase in anthocyanins, the pigments contributing to strawberry color, and also of other nutraceutical compounds, such as flavonoids and L-ascorbic acid (Fait et al. 2008; Folta and Davis 2006; Hancock 1999). Anthocyanin accumulation is controlled through the coordinated expression of genes encoding the flavonoid biosynthetic pathway enzymes, which appear to be regulated at the transcriptional level by R2R3 MYB and basic helix-loop-helix (bHLH) transcription factors (Allan et al. 2008). Strawberry cultivars exhibit a continuous range in fruit color from whitish yellow fruits of 'Weisse Ananas' or light orange of 'Madame Moutot', to blackish red of 'Rubina' (http://www.upov.int/en/publications/tg-rom/tg022/tg_22_10.pdf). Consumer preferences for strawberry fruit color can change over time, with orange red fruits favoured over dark red fruits nowadays in the majority of markets. Therefore, the genetic control of this trait is also an important goal in strawberry breeding.

A complex balance of aroma compounds, sugars, acids and texture determines the flavor of strawberry fruits. The content of sugars increases rapidly until the fruit is fully ripe. In contrast, total acidity declines during ripening. The sugar/organic acid ratio is the most important parameter of fruit taste, with moderately high levels of both contributing to a better acceptance (Perkins-Veazie 1995). The major soluble sugars in strawberry fruit are glucose, fructose and sucrose and the main organic acids are citrate and malate (Fait et al. 2008; Hancock 1999). Acids also regulate cellular pH and may influence the appearance of fruit pigments within the tissue (Yoshida et al. 2003). Strawberry is particularly rich in ascorbic acid, which concentration increases during fruit ripening (Agius et al. 2003). A number of alternative pathways have been proposed for L-ascorbic acid biosynthesis in plants and many of the genes have been isolated and characterized (Davey et al. 2000; Valpuesta and Botella 2004). It has been reported for other fruits belonging to the Rosaceae, such as apple (*Malus* × *domestica*) or pear (*Pyrus communis*), that increased antioxidant contents, in particular higher levels of L-ascorbic acid, may be associated with improved fruit postharvest quality (Barden and Bramlage 1994; Hancock and Viola 2005; Veltman et al. 2000). Recently, the genetic control of fruit L-ascorbic acid content has been investigated in tomato (Rousseaux et al. 2005; Schauer et al. 2006; Stevens et al. 2007) and apple (Davey et al. 2006), confirming the quantitative nature of this trait.

Identification of the genetic determinants governing both fruit quality and agronomical traits, such as yield, is essential for the sustained improvement of strawberry. However, these important traits are quantitative in nature and controlled by multiple genes or quantitative trait loci (QTLs). The use of linkage maps together with the phenotypic characterization of these traits allows the estimation of the number of loci governing them and the identification of their positions in the genome (Tanksley 1993). The identification of such regions/QTLs and the development of markers closely linked to relevant QTLs will assist breeding through marker-assisted selection (MAS). QTL studies for agronomical and/or fruit quality traits have been carried out in a variety of crops within the Rosaceae. In peach, which constitutes a model species within this family, fruit quality and agronomical traits have been mapped in different populations (Abbott et al. 1998; Dirlewanger et al. 1999; Eduardo et al. 2010; Etienne et al. 2002a; Ogundiwin et al. 2008; Quilot et al. 2004). Recently, progress in dissecting quantitative traits in peach has pointed to an *endopolygalacturonase* and a *leucoanthocyanidin dioxygenase* as candidate genes controlling flesh texture and browning, respectively (Ogundiwin et al. 2008; Peace et al. 2005). Similar progress has been achieved in apple with the identification of several fruit quality and physiological QTLs (Davey et al. 2006; Kenis and Keulemans 2007; Kenis et al. 2008; King et al. 2001; Liebhard et al. 2003). The candidate gene approach has also been used in apple to co-locate an *expansin* (*Md-Exp7*) and an ethylene-dependent *polygalacturonase* (*Md-PG1*) with QTLs controlling fruit texture and/or softening, as well as to identify the R2R3 MYB transcription factor *MdMYB10* in the region of a QTL for red color pigmentation (Costa et al. 2008, 2010; Chagné et al. 2007). In raspberry (*Rubus idaeus*), two *MYB* genes important in the transition from green to red fruit lie within a QTL for fruit

color (McCallum et al. 2010), and in sweet cherry (*Prunus avium* L.), the gene *PavMYB10* (homologous to apple *MdMYB10*) was located within the interval of a major QTL for fruit skin and flesh coloration (Sooriyapathirana et al. 2010).

When compared with other crops, progress in strawberry has been hindered by its complex octoploid genome. *Fragaria* × *ananassa* is an allo-octoploid species ($2n = 8x = 56$) originated from the hybridisation between two wild octoploid species, *F. chiloensis* and *F. virginiana* (Darrow 1966). In addition, this species is considered as highly heterozygous and susceptible to inbreeding depression (Shaw 1997). Two major features have made the genetic and genomic study of polyploids extremely difficult. First, increased allelic and non-allelic combinations due to multiple alleles that result in complex gene actions and interactions for QTL. Second, meiotic configurations in polyploids undergo complex biological processes, including either bivalent or multivalent formation, or both (Doerge and Craig 2000). The first linkage map of cultivated strawberry was constructed using non-transferable AFLP markers (Lerceteau-Köhler et al. 2003). This first study suggested a mixed disomic and polysomic inheritance in octoploid strawberry. In contrast, *Fragaria vesca,* a diploid relative of the cultivated species, has a small genome and has become a model for the genetic characterization of this genus. A saturated reference map has been developed for this species using mainly SSR and gene-specific markers (Sargent et al. 2006, 2007). Recently, a couple of studies have compared the diploid reference map to that of the cultivated species and have shown essentially complete macrosynteny and high levels of colinearity between them, as well as an extensive diploidization of the cultivated strawberry genome (Rousseau-Gueutin et al. 2008; Sargent et al. 2009). The publication of the first draft of the wild strawberry *F. vesca* genome represents a major progress for this genus and will facilitate the identification of candidate genes co-localizing with syntenic regions underlying QTLs in *F.* × *ananassa* (Shulaev et al. 2011). Although much work has been devoted to the physiology and molecular mechanisms of plant development and fruit ripening, the late genetic characterization has hampered the analysis of QTLs in this important crop. Thus, few and preliminary studies have analysed QTLs controlling sugars and organic acids in strawberry fruit or determined the genetic architecture of day-neutrality in this species (Lecerteau-Köhler et al. 2006; Weebadde et al. 2008).

During the last few years, an emphasis has appeared towards the development of 'functional' linkage maps containing molecular markers from transcribed regions of the genome. This allows the evaluation of co-location between genes, and/or genic-markers, and QTLs and, ultimately, it increases the chances of identification of genes responsible for the variation in the investigated trait (Aubert et al. 2006; Ogundiwin et al. 2009; Srinivas et al. 2009). The identification of the genes responsible for the variation is essential for a better understanding of the genetic mechanism governing the trait. The advantage of having a functional marker within the locus of interest as compared to a linked marker is particularly important in complex traits, which are controlled by a variable number of loci, each contributing in a reduced amount to the variation of the character. However, to identify candidate genes in strawberry, the number of gene-based markers needs to be increased in published linkage maps (Rousseau-Gueutin et al. 2008; Sargent et al. 2009). The objective of the present study was to understand the genetic basis controlling fruit quality and agronomical traits in strawberry. To achieve this goal: (1) we have phenotyped a F_1 population derived from the cross between two contrasting lines, '232' and '1392', during three successive years, (2) we have developed 'functional' linkage maps of strawberry in which almost 70% of the markers are derived from transcribed regions of the genome, and (3) we have identified QTLs and candidate genes for yield and related traits as well as for important fruit quality characters such as sugars, L-ascorbic acid, acidity, color and firmness. A number of them contributed to more than 20% of the variation and were stable over time.

Materials and methods

Plant material

The octoploid strawberry mapping population consists of a full-sib family of 95 F_1 individuals derived from an intraspecific cross between two IFAPA selection lines, '232' (Sel. 4-43 × 'Vilanova') and '1392' ('Gaviota' × 'Camarosa'). The two parental lines were chosen because they differ in important agronomical and fruit quality traits. '232' is a very productive strawberry line, whereas '1392' has firm fruits with high levels of sugars, acids, anthocyanins and L-ascorbic acid. Crosses were carried out in the spring of 2004 and during three successive years both parents and F_1 individuals were planted in mid-October in the strawberry-producing area of Huelva (Spain) until the end of May. Six vegetatively reproduced plants of each parental line and four plants of each F_1 line were arranged in a double row in zig-zag on mulched raised beds of 35-cm high and 50-cm wide. The population was grown under macro tunnels of polyethylene following conventional practices with an inter-row distance of 30 cm and a distance between plants of 25 cm.

The diploid *Fragaria* bin mapping population FV × FB consisted of the *F. vesca* 815 parental line, the F1 hybrid

and the 6 bin set seedlings described in Sargent et al. (2008). An additional seedling (31) was also genotyped when a marker was assigned to the redundant bins II:50 and III:53.

DNA extraction

Total genomic DNA was isolated from 130 mg of young leaves from the two parents and the 95 F_1 plants using a modified CTAB method based on that of Doyle and Doyle (1990). DNA was quantified at 260 nm using a NanoDrop spectrophotometer (ND-1000 V3.5, NanoDrop Technologies, Inc.) and its quality was checked by two absorbance ratios, 260/230 and 260/280 nm, and also by agarose gel electrophoresis. DNA was diluted in sterile water to a stock concentration of 1:200 (approximately 10–15 ng/µl) and stored at −20°C for use in PCR analysis.

Molecular marker development and analysis

Microsatellites

Two-hundred-and-two previously reported SSR loci (Gil-Ariza et al. 2006; Keniry et al. 2006; Rousseau-Gueutin et al. 2008; Sargent et al. 2004, 2006, 2007, 2008; Zorrilla-Fontanesi et al. 2011) were analysed for polymorphism in the '232' × '1392' mapping population. Fifty-four of these markers (comprising the majority of the SSRs derived from genomic DNA) were chosen because they were evenly distributed throughout the *Fragaria* diploid reference map (FV × FB; Sargent et al. 2007). They were first tested for polymorphism in the parents and six individuals of the '232' × '1392' population. Subsequently, those showing polymorphic bands were amplified in the entire mapping population. In addition, 14 *Fragaria* SSRs have been newly developed in this investigation (Table S1) and were also tested for polymorphism in the '232' × '1392' mapping population and in the FV × FB population. Polymorphic loci were amplified in the entire octoploid mapping population and/or the bin set of the FV × FB population (Sargent et al. 2008). PCR conditions and product visualization were performed on DNA from both mapping populations as described in Zorrilla-Fontanesi et al. (2011). The novel marker ChFaM149 was amplified using the following optimised PCR conditions: 5 mM $MgCl_2$, 300 µM of each dNTP, 0.4 µM of each specific primer and 50 ng of template DNA.

Amplified fragment length polymorphisms

Six AFLP primer combinations, selected according to their readability and number of polymorphic bands, were used to genotype the entire mapping population. Templates for AFLP reactions were prepared using the protocol of Vos et al. (1995) with some modifications. The restriction digestion–ligation (RL) steps were carried out in 96-well PCR plates using 500 ng of template DNA in 10 µl of sterile water. DNA from each genotype was digested first with *Tru*I for 2 h at 65°C in a final volume of 45 µl. The enzyme *Eco*RI was then added and the reaction incubated for 90 min at 37°C prior to the ligation of adapters. Pre-amplification reactions were carried out using standard *Eco*RI (E) and *Tru*I (T) primers with one selective nucleotide (E+C/T+A) in a total volume of 20 µl with 1× reaction buffer (Biotools; [75 mM Tris HCl (pH 9.0), 50 mM KCl, 20 mM $(NH_4)_2SO_4$]), 0.2 mM of each dNTP, 1.5 ng/µl of each primer, 1.5 mM $MgCl_2$, 1 U of *Taq* DNA polymerase (Biotools) and 5 µl of the RL product diluted fivefold. The adopted PCR profile was as follows: 94°C for 30 s, then 23 cycles of 94°C for 30 s, annealing at 56°C for 30 s and extension at 72°C for 1 min. A final extension was carried out at 72°C for 5 min. The selective amplification steps were performed with various combinations of E and T primers using two additional selective nucleotides (E+3/T+3) in a total volume of 20 µl with 1× reaction buffer (Biotools), 0.2 mM of each dNTP, 0.3 ng/µl of the E primer, 1.5 ng/µl of the T primer, 1.5 mM $MgCl_2$, 1 U of *Taq* DNA polymerase (Biotools) and 5 µl of the pre-amplification product diluted 30-fold. The adopted PCR profile was as follows: 94°C for 30 s, 1 cycle of 94°C for 30 s, annealing at 65°C for 30 s and extension at 72°C for 1 min, then 14 cycles of 94°C for 30 s, annealing at 65°C for 30 s (reduced each cycle by 0.7°C) and extension at 72°C for 1 min. Finally 23 cycles of 94°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 1 min and a final extension at 72°C for 6 min. All the amplification reactions were carried out in a BioRad iCycler. Reaction products were mixed with 13 µl of formamide loading buffer (98% formamide, 10 mM of EDTA and 0.1% xylene cyanol) and were denatured and concentrated at 95°C for 10 min. Finally, 5–6 µl of each sample was separated in denaturing polyacrylamide gels and visualized as described in Zorrilla-Fontanesi et al. (2011).

Single strand conformational polymorphisms markers

Two strawberry anthocyanin biosynthesis genes, *chalcone isomerase* (*CHI*) and *flavanone-3-hydroxilase* (*F3H*) (Deng and Davis 2001), along with *FaTIR1*, the *F.* × *ananassa* homologue of the *Arabidopsis* auxin receptor *TIR1* (F. Csukasi and V. Valpuesta, unpublished), were located in the '232' × '1392' map. Primers used and detection of *CHI* were as described in Deng and Davis (2001). For the *F3H* gene, a novel primer pair was developed to amplify a region covering the first intron (FH3-F: 5′-GCAAAAA GGGTGGCTTCATC-3′ and F3H-R: 5′-GTCACAAT

CTCGCGCCAATC-3′). Primers used for amplification of *FaTIR1* were FaTIR1-F: 5′-GAATTTGAGCTACGCCA TTATC-3′ and FaTIR1-R: 5′-ATCAAGGCCGACATCT TCAA-3′. Since no polymorphism was detected in agarose or denaturing polyacrylamide gels, the single-strand conformation polymorphisms (SSCPs) technique (Orita et al. 1989) was used to map *F3H* and *FaTIR1*. In addition, microsatellites ChFaM062, ChFaM070, ChFaM083 and ChFaM170 (Zorrilla-Fontanesi et al. 2011) were also mapped by SSCPs, as the majority of them were located in ESTs with homology to transcription factor genes and did not show polymorphism in denaturing polyacrylamide gels.

For SSCP analysis, 25 ng of genomic DNA was amplified by PCR in a 25 µl PCR reaction containing 1× PCR buffer (Biotools), 2 mM $MgCl_2$, 200 µM of each dNTP, 0.2 µM of each specific primer and 1 U of DNA polymerase (Biotools). The PCR profile included a denaturing step of 94°C for 3 min, then 35 cycles of 94°C for 30 s, annealing at the corresponding temperature for each primer (61°C for *F3H* and 60°C for *FaTIR1*), and extension at 72°C for 45 s. Finally, an extension step at 72°C for 3 min was added. 10 µl of each reaction was loaded on a 1.2% agarose gel to control PCR amplification. PCR products, which ranged between 167 and 250 bp in length, were then subjected to SSCP electrophoresis. Briefly, 15 µl of amplified DNA was mixed with 10 µl of formamide loading buffer, denatured at 95°C for 5 min and immediately cooled on ice. 4–5 µl was loaded onto a non-denaturing 8% polyacrylamide (37.5:1) gel in 0.5× TBE. Samples were electrophoresed at 4°C in a Sequi-Gen GT Nucleic Acid Electrophoresis Cell (BioRad) of 38 × 30 cm using 0.5× TBE running buffer. SSCP alleles were separated for at least 12 h at 1.5 W, although larger fragments were electrophoresed for proportionally longer periods. Fragments were visualized with silver staining as described in Zorrilla-Fontanesi et al. (2011). At least two different SSCP bands from each gene were picked from the gels and sequenced to verify their identity.

Map construction

Polymorphic fragments were scored by two different researchers. Bands too faint to score were considered as missing data or were rerun. A χ^2 analysis for goodness of fit was performed to test the segregation ratios obtained to those expected for single or multiple dose markers under both disomic or octosomic inheritance (Lerceteau-Köhler et al. 2003). Markers were considered to have significantly skewed ratios at $P \leq 0.05$.

To analyse the mapping population, a double pseudo-testcross strategy was employed (Grattapaglia and Sederoff 1994). In such strategy, linkage analysis is carried out for each parent separately considering that single dose markers heterozygous in one parent (in JoinMap® 4, coded as lm or np for female and male markers, respectively) and homozygous in the other parent (ll or nn, respectively) segregate as in a backcross (1:1 ratio for genotypes ll, lm or nn, np) and markers heterozygous in both parents segregate as in a F_2 cross (segregation type <hkxhk> and 3:1 ratio for genotypes k_ and hh in the progeny). An analysis for co-segregating fragments generated from the same primer pair allowed the identification of allelic pairs (codominant markers) segregating in a 1:1:1:1 (genotypes ac, ad, bc, bd for <abxcd> or ee, eg, ef, fg for <efxeg>) or 1:2:1 (hh, hk, kk) fashion. Initially, we constructed the separated parental maps using MapMaker/Exp v.3.0 (Lander et al. 1987) as described in Rousseau-Gueutin et al. (2008). This step served to check genotyping errors and to establish the best marker order for each map using the maximum likelihood mapping algorithm. Next, we used JoinMap® 4 software (van Ooijen 2006) to construct an integrated map using 1:1, 3:1 and codominant markers and the population coded as CP (cross-pollinated; linkage phases originally unknown). Finally, to generate the two parental maps, 1:1 and codominant markers (including 1:2:1 markers) from each parental line were translated to a BC_1 (backcross) coding using the segregation phases determined in the integrated map. Grouping trees were constructed using independence test LOD score and the default settings in JoinMap. For most of the linkage groups, a minimum LOD threshold of 5.0 was chosen, although for some of them this LOD value was decreased to 4.0 or 3.0 if markers comprising the groups showed linkage in previously published *Fragaria* diploid or octoploid maps. Then, the 'Strongest Cross Link' (SCL) parameter was used to inspect if the assignment of a marker to a group was suspicious, to assign previously ungrouped markers to already established groups or to join different LGs belonging to the same chromosome. To create the maps for all the selected LGs, we applied the Kosambi mapping function and the regression mapping algorithm (Stam 1993) with the following parameters: recombination threshold of 0.35 (integrated map) or 0.40 (parental maps), ripple value of 1, jump threshold of 5.0. Only first or second rounds were trusted. When marker order was contradictory with that determined by MapMaker, the latter order was fixed to generate the linkage maps in JoinMap. Markers showing distorted segregation ratios were included in the map when they did not disrupt previous marker order. Linkage maps were drawn using MapChart 2.2 for Windows (Voorrips 2002).

Nomenclature of markers and linkage groups

SSR marker names are followed by the size of the allele. For codominant markers, the two alleles of each parent are shown separately in the parental maps and together in the

integrated map. This allows the identification of the segregation type, which correlates with the accuracy with which the markers can be mapped (Maliepaard et al. 1997). Linkage phase information is provided as 0 or 1, indicating the grandparental origin of the mapped alleles and allowing the identification of markers linked in coupling or repulsion phase. AFLP marker names consisted of the name of the E+3/T+3 primers followed by the size of the fragment. SSCP markers are labelled with an S followed by the number of the polymorphic allele recovered. The seven homoeology groups (HGs) were named I to VII, as the corresponding LGs in the diploid *Fragaria* reference map (Sargent et al. 2008), followed by an F (for female LGs), an M (for male LGs) or F/M (for integrated LGs). LGs within each HG were numbered arbitrarily although the same number in male and female maps indicates the homologous groups.

Trait evaluation

For each line (parental and F_1 progeny lines), a total of 17 traits, including 6 agronomical and 11 fruit quality traits, were evaluated during three successive years (2007, 2008 and 2009), with the exception of flowering time, which was measured only in 2008 and 2009. Agronomical traits were evaluated in the field while fruit quality traits were measured after harvesting ripe fruits from all plants the same day (to minimize environmental variability). Flowering time (FT) was evaluated by recording the number of days from planting to anthesis for each clone and the average value was used in the analyses; plant height (PHE) was visually rated on a scale of 1–3, where 1 = low, 2 = medium and 3 = high for each plant and then averaged per line; plant width (PWI, in cm) was measured as the transverse diameter of each plant; plant yield (YI, in g) and fruit number (FN) per plant were evaluated after weighing and counting all harvested fruits for each line during each season and dividing by the number of plants. Average fruit weight (FW, in g) was calculated dividing the weight of all harvested fruits for each line by the number of fruits.

Fruit firmness (FIRM, in g) was measured on six fruits per line and in two opposite sides of each fruit using a penetrometer fitted with a 3.5-mm probe (Effegi FDP500). Soluble solids content (SSC, in °Brix) was evaluated in five fruits per line with a refractometer (Atago PR32) by adding a few drops of strawberry juice onto the lens. External fruit color was measured on two fruits per clone (8 fruits per line) using a Minolta chromameter (CR-10, Minolta Corp., Ramsey, NJ) which provided the following CIELAB color space values: a^* (green–red spectrum), b^* (blue–yellow spectrum), L^* (brightness–darkness) and hue angle (h° = arctangent [b^*/a^*]), where 0°= red–purple, 90°= yellow, 180°= bluish-green and 270°= blue (McGuire 1992). Chroma (C^*) was then calculated as $C^* = (a^{*2} + b^{*2})^{1/2}$.

For further chemical analyses, 10–15 fruits per line were cut, frozen in liquid nitrogen, powdered in liquid nitrogen using a coffee grinder and stored at −80°C. For those analyses, three technical repetitions per line were assessed. Titratable acidity (TA) and pH were measured with an automatic titrator fitted with pH meter and autoburete (Titroline easy, Schott North America, Inc.), diluting 1 g of fruit powder in 100 ml of distilled water. Titrations were carried out to an end pH of 8.1 with 0.01 N NaOH and the results were referred to grams of citric acid per 100 g of fresh weight. L-Ascorbic acid (L-AA) was measured by HPLC (Davey et al. 2006). In brief, 0.25 g of fruit powder was homogenized with 1 ml of cold extraction buffer (metaphosphoric acid 2%, EDTA 2 mM) and kept at 0°C for 20 min. Then, samples were centrifuged at 14,000 rpm for 20 min at 4°C and the supernatant was filtered (0.45-μM nylon membranes) and transferred to HPLC vials kept on ice. Finally, 5 μl was injected in a C18 reverse-phase HPLC column (ZORBAX RXC18, Agilent Technologies) and detection was carried out at 254 nm in a photodiode array detector (G1315D, Agilent Technologies). The mobile phase utilized for the elution consisted of a filtered and degassed solution of 0.1 M NaH_2PO_4 0.2 mM EDTA pH 3.1 (Harapanhalli et al. 1993) and a flow rate of 0.7 ml/min. The L-AA content was calculated by comparison with values obtained from a standard curve. The results were expressed as milligram of L-AA per 100 g of fresh weight. Total anthocyanins (ANT) were determined following the protocol of Bustamante et al. (2009) with some modifications. 0.3 g of fruit powder was diluted in 3 ml of 1% (v/v) hydrochloric acid–methanol and kept at 0°C for 30 min. The slurry was centrifuged at 5,000 rpm for 10 min at 4°C, and 1 ml of the resultant supernatant was transferred to a 1.5 ml eppendorf tube. 4 μl of the supernatant was directly measured at 515 nm in a NanoDrop spectrophotometer. The amount of anthocyanins was calculated using $E_{molar} = 36{,}000$ L mol^{-1} cm^{-1} (Woodward 1972). The results were expressed as milligrams of pelargonidin-3-glucoside per 100 g of fresh weight.

Descriptive statistical analysis

Different modules of the STATISTICA 7.0 software package (StatSoft, Inc. 2007) were employed to analyse the phenotypic data. The mean values for both parents and each F_1 line were calculated for each trait and year. To verify the normality of the trait distributions, the Shapiro–Wilk test was applied (Shapiro and Wilk 1965) and global means, ranges, standard deviations (SD), skewness and kurtosis were also calculated for these distributions.

Pearson correlation coefficients among traits and with each trait over the 3 years were obtained using the correlation matrices module. In addition, a *t* test was employed to compare the mean values of the parents and, finally, transgressive lines were identified as those lines with higher or lower trait values than the highest or lowest parent by at least twofold the standard deviation of that parent.

QTL mapping

QTL analyses were performed separately for each year using MapQTL® 5 software package (van Ooijen 2004). To compare with other QTL studies in *Fragaria* (Lecerteau-Köhler et al. 2004; Weebadde et al. 2008) and simplify calculations, QTL detection was carried out on the parental maps generated by JoinMap in backcross configuration, so that only two QTL genotypic classes were assumed and modeled. First, the non-parametric test of Kruskal–Wallis was used to identify significant associations between phenotypic traits and molecular markers. Then, an interval mapping (IM) analysis (Lander and Botstein 1989) was performed to locate preliminary QTL positions. LOD thresholds were estimated with a 1,000-permutation test (Churchill and Doerge 1994) for each trait and year on each map and QTLs with LOD scores greater than the genome-wide threshold at $P \leq 0.05$ were declared significant. A forward selection was carried out to fix significant regions using markers as cofactors so that markers significantly associated with the trait (LOD score greater than the chromosome-wide threshold at $P \leq 0.05$) were selected. Finally, the resultant set of cofactors was used in the multiple QTL method (MQM; Jansen 1993; Jansen and Stam 1994) to control the genetic background for a better location of the QTLs. If the LOD value associated with a cofactor fell below the threshold, the cofactor was removed and the analysis repeated. This process was continued until the cofactor list remained stable. For both IM and MQM, a step size of 1 cM was used. The phenotypic variance explained by a single QTL was calculated as the square of the partial correlation coefficient (R^2) with the observed variable and gene effects estimated as the difference between the mean distributions of the quantitative trait associated with each genotypic class. QTLs with overlapping confidence intervals (LOD score drop of 2 units on either side of the likelihood peak), close likelihood peaks and globally similar allelic effects were considered as co-localizing.

The identified QTLs were named in italics using the trait abbreviation followed by the name of the LG in which the QTL was located. QTL positions and 1-LOD and 2-LOD confidence intervals were drawn using MapChart 2.2 for Windows.

Results

Marker amplification and polymorphism

A total of 216 SSR primer pairs, 202 previously published and 14 newly developed, were analysed in a subset of the '232' × '1392' mapping population (see "Materials and methods"). Among them, 146 (67.6%) were polymorphic and were assessed in the entire population, yielding 323 polymorphic and 319 monomorphic bands. The major source of SSRs was ESTs obtained from *F.* × *ananassa* (73) or *F. vesca* (26), altogether comprising ~68% of the total number of polymorphic SSR loci. The majority of *F.* × *ananassa* ESTs were derived from two different fruit organs, achenes and receptacle, at green and red ripening stages (Bombarely et al. 2010), making the EST-derived markers of great value for the identification of candidate genes associated with fruit-related traits. Two strawberry anthocyanin biosynthesis genes, *chalcone isomerase* (*CHI*) and *flavanone-3-hydroxylase* (*F3H*) (Deng and Davis 2001) and the auxin receptor *FaTIR1* (F. Csukasi and V. Valpuesta, unpublished) were genotyped in the '232' × '1392' population. Four ESTs with homology to regulatory proteins were monomorphic for their SSRs and were mapped using single strand conformational polymorphisms (SSCP; see "Materials and methods"). These 7 primer pairs generated 16 polymorphic fragments. Finally, 84 segregating markers obtained from 6 AFLP primer combinations were used for increasing map saturation. Collectively, 447 polymorphic fragments were obtained. Among them, only 24 (5.4%) fitted multiple dose ratios and therefore, the remaining 423 (94.6%), which fitted single dose ratios, were used to construct the octoploid linkage maps. For SSR and SSCP markers, each primer pair yielded ~2.2 polymorphic bands, which represent 50.3% of total amplified bands, while each AFLP primer combination yielded ~14 segregating bands. In total, 151 (35.7%) fragments segregated 1:1 for '232', 119 (28.1%) segregated 1:1 for '1392' and 153 (36.2%) fragments, which were heterozygously present in both parents, segregated 3:1. A high number of markers were heterozygous in both parents, as expected for a mapping population derived from an intraspecific cross between two lines with similar Californian pedigree. Only 5 (3.3%) markers from the female parent, 20 (16.8%) from the male parent and 16 (10.5%) heterozygous markers showed segregation distortion (at $P \leq 0.05$). After analysing all possible combinations of co-segregating fragments generated from each primer pair, 86 alleles produced a total of 37 codominant (1:1:1:1 or 1:2:1) markers that were used, together with dominant heterozygous markers, to anchor homologous linkage groups.

The majority of the SSR markers used in this report were previously assigned to linkage groups (LG) on the

diploid *Fragaria* reference map (Sargent et al. 2006, 2007, 2008; Zorrilla-Fontanesi et al. 2011). Among the 14 newly developed SSRs, only ChFaM149, which was developed using the *FaCAD1* gene (Blanco-Portales et al. 2002), was mapped in FV × FB (Sargent et al. 2007). Thus, the remaining markers were tested for polymorphism in the bin set of the FV × FB population and nine of them were polymorphic (Table S1). All, but ChFaM262 mapped to previously described bins and could be placed in the diploid reference map (Table S1). The position of marker ChFaM262, which defined a new bin, was determined using the *F. vesca* genome sequence (http://www.strawberrygenome.org/). ChFaM262 was located at the beginning of the LGI, approximately at 2 cM from the top marker (EMFvi072). The gene *FaTIR1* was bin mapped to bin II:47 in the diploid *Fragaria* reference map using the SSCP technique.

Linkage maps of '232' × '1392'

For QTL analyses, one map was constructed for each parental line splitting up markers into separate parental datasets coded as a BC1. The linkage maps of '232' and '1392' contained a total of 154 and 126 markers, including splitted information of codominant heterozygous markers, distributed in 26 and 25 linkage groups (LGs), respectively, and covering 693.5 and 590.5 cM of the octoploid genome (Table 1). The number of markers for each LG varied from 2 to 12, with an average of 5.7 for '232' and 2–11, with an average of 5.0 for '1392'. The length of the LGs in the '232' map ranged from 2.3 to 74.3 cM, with an average length of 25.7 cM and an average marker spacing of 5.5 cM. The length of the LGs in the '1392' map ranged from 1.1 to 50.1 cM, with a mean of 23.6 cM and resulting in an average distance between markers of 5.9 cM. The female map contained 18% more markers than the male map, which is an expected outcome when using a pseudo-testcross strategy, as a consequence of the lower level of heterozygosity in one of the parents (Yin et al. 2001).

To locate dominant heterozygous markers and also for genome comparison purposes, an integrated '232' × '1392' map was created (Table 1; Fig. S1). This map spanned a cumulative length of 1,259.8 cM, covering 57.4% of the most saturated *F.* × *ananassa* map reported to date (Rousseau-Gueutin et al. 2008). The integrated map consisted of a total of 338 molecular markers distributed in 37 LGs, exceeding the expected number of 28 LGs. The number of markers per LG ranged from 2 to 18, with an average of 8.1 markers. The average size of LGs was 30 cM, ranging from 4.4 to 76.1 cM. The average marker spacing was 4.3 cM, with only 4 gaps larger than 20 cM. A few small inversions in marker order and differences in map distances could be detected when compared with the parental maps, but they were considered minor artefacts since they only extended over a few centimorgans. Of the 37 LGs obtained in the integrated map, 26 (70.3%) comprised markers from both parents and were actually integrated. The remaining 11 LGs (29.7%) mainly contained markers from either the female or the male parent and could not be integrated. The level of polymorphism differed across the seven homoeology groups (HGs), with a high number of markers located in HGs II, III, IV, and V whereas HGs I, VI and VII were less polymorphic. LGs in HG VI were ~23 cM long, contained an average of 4.5 markers per LG and most of the female and male LGs could not be integrated. For HG V, the four homoeologous LGs were identified, as each LG resulted from the integration of a female and a male LG (homologous LGs). The majority of markers with distorted segregation clustered in one or two LGs belonging to HGs II, III and IV (Fig. S1). These markers were not discarded because they are usually linked to loci negatively affecting biological processes such as gametogenesis, fertilization or embryogenesis and reveal the occurrence of natural selection (Lyttle 1991). As an example, a skewed region was found at one edge of LG II-F/M.4 suggesting a strong selection against alleles inherited from one of the paternal grandparents.

All LGs in the integrated map included at least one anchor marker mapped in the diploid *Fragaria* reference map (FV × FB), thus allowing the assignment of all recovered fragments to one of the seven diploid *Fragaria*

Table 1 Parental maps (BC_1) and integrated linkage map (CP) obtained with JoinMap 4

Description of the linkage maps	Female map	Male map	Integrated map
SSR markers	125	93	250
AFLP markers	22	30	77
Others	7	3	11
Total number of mapped markers	154	126	338
Total number of linkage groups	26	25	37
Mean no. of markers per linkage group (and SD)	5.7 (±3.0)	5.0 (±2.5)	8.1 (±5.2)
Range of marker no. per linkage group	2–12	2–11	2–18
Average marker spacing (cM)	5.5	5.9	4.3
Mean size per linkage group (cM) (and SD)	25.7 (±20.1)	23.6 (±15.5)	30 (±20.6)
Range size per LG (cM)	2.3–74.3	1.1–50.1	4.4–76.1
Largest gap (cM)	31.9	34.2	33.7
Cumulative Genome Length (cM)	693.5	590.5	1259.8
Genome Coverage (%)	31.6%[a]	26.9%[a]	57.4%[a]

[a] Percentage of genome respect to the most saturated *F.* × *ananassa* map (Rousseau-Gueutin et al. 2008)

LGs and therefore, map comparisons between diploid and octoploid species (Fig. S1). As a result, high levels of macrosynteny and colinearity have been found between the diploid and octoploid genomes as previously reported (Rousseau-Gueutin et al. 2008; Sargent et al. 2009). Nevertheless, the order of some markers was altered, as for example, an inversion found in I-F/M.1 comprising markers CFVCT005B and UFFxa16H07 (Fig. S1). Similar discrepancies in marker order have been reported in other studies (Rousseau-Gueutin et al. 2008; Sargent et al. 2009). In addition, some duplicated loci were recognized in *F.* × *ananassa*. For instance, marker ChFaM120 was mapped to LG II of *F. vesca,* whilst it mapped to HGs II and V in *F.* × *ananassa*. Marker ChFvM140 amplified loci located in LGs III and VI of *F. vesca*, whilst it mapped to HGs III, VI but also HG II in *F.* × *ananassa*. In contrast, other markers were located in two LGs of *F. vesca* and in the corresponding HGs in *F.* × *ananassa*, indicating that these gene duplications have been conserved in *Fragaria*. Examples are marker CFVCT005 which mapped to LGs I and IV of *F. vesca* and to HGs I and IV of *F.* × *ananassa*. Among the 146 SSRs analysed, only marker ChFaM129 was located in a different HG when compared with the diploid *Fragaria* map; a single segregating locus was mapped to LG III-F/M.2 in *F.* × *ananassa* but was located to bin VI:31 in diploid *Fragaria* (Zorrilla-Fontanesi et al. 2011). In a number of cases, more than one fragment generated by the same primer pair mapped to different positions of the same LG, such as *F3H* on HG I, indicating either different recombination frequencies between the male and female parents, polysomic inheritance remnants or duplicated loci.

Eight SSRs that had been reported as genome-specific markers (Denoyes-Rothan unpublished; Zorrilla-Fontanesi et al. 2011) mapped to LGs of all the seven HGs, allowing the identification of individual genomes within the octoploid genome of *F.* × *ananassa* (Fig. S1). These markers will be useful for identification of homologous LG across different *F.* × *ananassa* mapping populations. In addition, three anchor SSRs to the *Prunus* genome have been mapped in '232' × '1392'. Thus, marker BPPCT028 (in *Prunus* PG1) was mapped to *F.* × *ananassa* LG V-F/M.3, ChFaM014 (a SSR in the ortholog of *Prunus MADS5,* which has been mapped to PG1 by Xu et al. 2008) to LG IV-F/M.1 and ChFvM213 (equivalent to M9a from *Prunus* and bin mapped to PG1:34) to LG IV-F/M.6. The three markers are located in syntenic blocks between *Fragaria* and *Prunus* genomes according to Illa et al. (2011).

Phenotypic distribution and relationship between traits

The '232' × '1392' mapping population derives from two strawberry parental lines differing in important agronomical and fruit quality traits. The 17 analysed traits, mean phenotypic values and basic statistical parameters for the two parental lines and the F_1 progeny are shown in Table 2. The female parent '232' was superior to the male parent in important agronomical characters, such as plant width (PWI), yield (YI) or number of fruits produced per plant (FN). The male parent '1392' exhibited superior fruit quality traits, such as firmness (FIRM), soluble solids content (SSC), titratable acidity (TA) or L-ascorbic acid content (L-AA). Traits were analysed in the whole population and the two parental lines during three consecutive years, 2007, 2008 and 2009, with similar mean values and range of variation for each trait over 3 years. Interestingly, considerable phenotypic variation for most of the traits was found in the progeny, even in traits, where no significant differences were found between the parents (Table 2). This is likely caused by the high heterozygosity of the two parents, leading to new allele combinations in the descendants. A number of transgressive lines were detected in one or both directions for most of the traits. Only SSC and green to red spectrum (a^*) did not show any transgressive line in the three tested years (Table 2). The seventeen agronomical and fruit quality traits analysed displayed continuous variation and the majority fitted a normal distribution, indicating that they were under polygenic control. Because similar phenotypic distributions were obtained for the 3 years, only those corresponding to year 2009 are graphically presented (Fig. S2).

Pearson correlation coefficients between different traits are shown in Table 3. Although a number of traits significantly correlated to each other and in general showed similar values in their correlation coefficients and significance levels over the 3 years, other traits only correlated significantly in one or 2 years. Among agronomical traits, the highest significant correlation was found between yield and fruit number ($r \sim 0.83$), indicating that the increase in yield is mainly a consequence of an increase in fruit number rather than to an increase in fruit weight. Plant height (PHE) and width also correlated with yield and, therefore, with fruit number suggesting that these traits also influence yield. No significant correlation was found between fruit weight and plant height or plant width, suggesting that more vigorous genotypes do not necessarily generate larger fruits. Among the fruit quality traits, the highest correlation was found between a^* and chroma (C^*), although all color traits, including anthocyanins, the main pigments contributing to strawberry color, were highly correlated. Since lower values of L^*, h°, a^*, b^* and C^* indicate overall darker fruit color, the correlations between anthocyanins and color readings were negative. Titratable acidity showed some positive correlations with L-AA and total anthocyanins and negative correlations with L^* and h° although, as expected, its strongest negative

Table 2 Phenotypic variation of 17 agronomical and fruit quality traits evaluated in '232', '1392' and the F_1 population during three consecutives years (2007, 2008 and 2009)

Trait	Year	Parents[a]			F1 progeny[b]				
		232	1392	232 versus 1392	Mean (SD)	Range	Skew.	Kurt.	Transgr.
Flowering time (days)	2008	60	50	n.s.	68.50 (±9.8)	35.30–91.00	−0.89	1.23	14
	2009	86	84	n.s.	80.70 (±7.2)	57.20–98.50	−0.30	0.37	27
Plant height	2007	2.25	2.87	*	2.18 (±0.60)	1.00–3.00	−0.01	−0.66	8
	2008	1.38	2.90	***	1.97 (±0.59)	1.00–3.00	0.16	−0.56	0
	2009	1.63	2.13	*	2.00 (±0.54)	1.00–3.00	0.22	−0.33	11
Plant width (cm)	2007	28.38	26.00	**	26.26 (±2.36)	18.83–32.25	−0.15	0.35	9
	2008	22.83	21.61	n.s.	22.95 (±2.90)	14.25–30.06	−0.39	0.35	1
	2009	23.75	20.63	n.s.	22.03 (±2.65)	14.75–30.17	0.04	0.96	3
Yield (g)	2007	1727.25	1161.67	–	1,391.60 (±312.64)	650.00–1,990.00	−0.20	−0.20	–
	2008	1081.00	902.50	–	932.02 (±245.52)	433.33–1,572.50	0.60	−0.12	–
	2009	1136.25	1005.00	–	956.39 (±261.34)	463.33–1,573.33	0.04	−0.67	–
Fruit number	2007	71.50	58.00	–	69.36 (±16.82)	34.67–108.67	0.00	−0.45	–
	2008	52.60	41.75	–	46.15 (±10.99)	25.50–74.75	0.42	−0.59	–
	2009	58.50	51.25	–	53.72 (±15.18)	28.67–94.75	0.37	−0.57	–
Fruit weight (g)	2007	24.16	20.03	–	20.14 (±2.52)	13.92–26.02	−0.08	−0.11	–
	2008	20.55	21.62	–	20.15 (±3.07)	13.16–29.66	0.47	0.67	–
	2009	19.42	19.61	–	18.06 (±2.87)	11.36–24.40	−0.10	−0.40	–
Fruit firmness (g)	2007	288.50	353.50	***	338.16 (±38.53)	248.00–413.12	−0.34	−0.44	5
	2008	299.17	351.67	**	328.42 (±37.86)	227.50–410.00	0.02	−0.27	0
	2009	287.50	444.17	***	339.28 (±34.43)	270.00–435.00	0.24	−0.15	0
Soluble-solids content (°Brix)	2007	5.50	8.24	*	7.07 (±1.25)	4.10–9.43	0.05	−0.73	0
	2008	7.58	11.50	*	8.96 (±1.11)	6.28–11.67	0.14	−0.46	0
	2009	7.52	12.08	*	9.79 (±1.40)	6.56–12.66	−0.33	−0.42	0
Titratable acidity (g/100g FW)	2007	0.43	0.50	***	0.47 (±0.08)	0.27–0.74	0.23	0.02	54
	2008	0.63	0.69	**	0.63 (±0.08)	0.43–0.89	0.34	0.38	44
	2009	0.43	0.69	***	0.57 (±0.07)	0.36–0.79	0.19	0.54	5
pH	2007	4.02	3.95	n.s.	4.06 (±0.14)	3.72–4.23	0.23	0.10	40
	2008	3.52	3.50	n.s.	3.85 (±0.15)	3.44–4.21	0.00	0.14	87
	2009	4.07	3.88	***	3.94 (±0.12)	3.69–4.29	0.25	0.16	18
L-Ascorbic acid (mg/100g FW)	2007	35.76	47.29	***	38.68 (±6.60)	23.23–54.74	0.27	−0.39	25
	2008	43.55	52.95	*	39.19 (±5.32)	26.48–53.48	0.21	−0.36	38
	2009	32.05	44.00	**	37.43 (±4.63)	26.08–51.25	0.34	0.27	4
Anthocyanins (mg/100g FW)	2007	27.39	37.44	***	26.90 (±5.96)	12.09–41.65	0.13	−0.34	41
	2008	22.8	34.06	***	25.35 (±6.17)	12.84–42.60	0.47	−0.10	28
	2009	24.88	30.18	***	27.39 (±6.36)	12.39–45.28	0.43	0.20	49
a^*	2007	46.74	34.72	**	41.18 (±3.26)	34.03–47.90	−0.06	−0.44	0
	2008	46.13	40.55	*	41.57 (±3.20)	34.76–46.93	−0.34	−0.86	0
	2009	44.87	40.02	*	43.09 (±2.62)	36.22–47.20	−0.89	0.49	0
b^*	2007	18.87	13.46	*	16.04 (±3.28)	8.60–22.40	−0.29	−0.39	4
	2008	17.44	13.71	*	15.93 (±2.57)	10.96–20.70	−0.01	−0.85	0
	2009	19.73	15.43	**	17.16 (±2.43)	12.03–23.28	0.27	−0.27	0
L^*	2007	36.80	29.80	***	32.40 (±2.5)	26.60–38.60	−0.09	−0.39	0
	2008	32.90	29.70	*	31.20 (±2.2)	26.70–38.30	0.23	0.01	1
	2009	33.10	28.30	**	30.90 (±2.1)	25.30–36.80	0.14	0.54	0

Table 2 continued

Trait	Year	Parents[a]			F1 progeny[b]				
		232	1392	232 versus 1392	Mean (SD)	Range	Skew.	Kurt.	Transgr.
h°	2007	24.00	20.40	n.s.	21.10 (±3.7)	10.10–28.90	−0.77	0.85	6
	2008	19.70	19.80	n.s.	21.10 (±2.4)	15.20–26.60	0.13	−0.34	0
	2009	23.90	20.40	*	21.70 (±2.6)	17.00–29.2	0.69	0.42	0
C^*	2007	50.71	38.55	**	44.52 (±4.39)	35.62–55.80	0.06	−0.47	0
	2008	49.10	43.23	*	44.82 (±4.15)	34.47–53.33	−0.36	−0.60	0
	2009	49.21	42.92	**	46.56 (±3.50)	32.69–52.30	−1.03	1.69	1

d days, *FW* fresh weight

[a] Average values of the parental lines and significance level of the comparison test: *significant at $P < 0.05$, **significant at $P < 0.01$, ***significant at $P < 0.001$, *n.s.* not significant, − not analysed)

[b] The F_1 population is described by mean, standard deviation (SD), range of variation, skewness (Skew.), kurtosis (Kurt.) and the number of transgressive lines (Trangr.)

correlation was with fruit pH, as also reported in tomato (Saliba-Colombani et al. 2001).

To assess the inter-year variability of traits, we calculated Pearson's correlation coefficients for each trait among the three tested years (Table S2). All the characters showed significant correlations between years, with the only exception of h° in 2008 versus 2009 and flowering time. The highest correlations were found for pH in 2007 versus 2009 ($r = 0.65$, $P \leq 0.001$) and for total anthocyanins in 2008 versus 2009 ($r = 0.63$, $P \leq 0.001$). Conversely, the lowest correlations were found for pH in 2007 versus 2008 ($r = 0.24$, $P \leq 0.05$) and in 2008 versus 2009 ($r = 0.25$, $P \leq 0.05$), and for the blue–yellow spectrum (b^*) in 2008 versus 2009 ($r = 0.23$, $P \leq 0.05$).

Identification of QTLs in '232' × '1392' and stability over years

QTLs were analysed using the average trait values from each year separately and the BC_1 data set for all the quantitative traits. A total of 47 QTLs were detected (listed in Table 4) using MQM for all traits except for flowering time, L^* and h°, with their chromosomal locations shown in Fig. 1. Because QTLs for each trait in different years in the same chromosomal regions (with overlapping confidence intervals) were considered to be the same, the 47 QTLs can be summarized in 33 significant associations (14 in the female map and 19 in the male map). Among them, 12 (36.4%) QTLs were stable over 2 or all 3 years and are indicated in bold in Table 4. QTLs were identified across the seven HGs of the '232' × '1392' maps, ranging from 1 QTL in HG VI to 15 QTLs on HG V. Interestingly, clusters of QTLs were detected on HGs II, III, IV and V. Most of the QTLs (69.7%) displayed gene effects in the same direction as predicted by the phenotype of parental lines while 10 QTLs (30.3%) showed opposite effects. Between one (plant height, a^*, b^* and C^*) and five QTLs (yield and fruit number) have been identified per trait, with the phenotypic variation (R^2) explained by each QTL ranging from 9.2% (for *antV-M.2* in 2008) to 30.5% (for *pHV-M.2* in 2009). Owing to both the limited average size of LGs (24.7 cM) and the backcross configuration of the analysis, the LOD thresholds obtained in the permutation tests (~2.7) were lower than in other QTL studies. We include two QTLs (*antV-M.2* and *aV-M.2*) that fall just below this threshold but were significant in other years.

QTLs for yield and fruit number co-located in LGs II-M.4, III-F.1 and V-M.4, with the latter being stable over 2 years. A QTL for fruit number was also detected in the same region in the homologous female LG V-F.4 in 2009, suggesting that this QTL is heterozygous in both parental lines. In the same region of LG V-M.4, a QTL for plant width was detected in 2009. All of them can be caused by different but tightly linked QTLs although one single QTL controlling the three traits (yield, fruit number and plant width) seems a more plausible explanation. In fact, loci affecting yield through an effect in fruit number have been previously reported (Huang et al. 2009; Shi et al. 2009). Additional QTLs affecting plant width and yield were detected in other linkage groups of the same HG (*pwV-M.1* in 2008 and *yiV-F.2* in 2009). All these QTLs on HG V can be referred to as 'homoeoQTLs' since they map to homoeologous regions of the different *F.* × *ananassa* constitutive genomes. In addition, a QTL controlling plant height over 2 years was detected on LG IV-F.6.

Co-locations among fruit quality QTLs or between fruit quality and agronomical QTLs were detected in HGs III, IV and V. In LG IV-F.2 a QTL for L-AA stable over 2 years co-located with titratable acidity and pH. A different and stable QTL over the 3 years controlling L-AA

Table 3 Phenotypic correlations among 17 agronomical and fruit quality traits measured in the F_1 progeny for three successive years

Traits	Year	FT	PHE	PWI	YI	FN	FW	FIRM	SSC	TA	pH	L-AA	ANT	*a**	*b**	*L**	*h*°
PHE	2007	–															
	2008	0.22*															
	2009	n.s.															
PWI	2007	–	0.43***														
	2008	n.s.	0.36**														
	2009	n.s.	0.30**														
YI	2007	–	0.46***	0.63***													
	2008	n.s.	0.53***	0.63***													
	2009	n.s.	0.64***	0.58***													
FN	2007	–	0.42***	0.56***	0.82***												
	2008	n.s.	0.51***	0.64***	0.84**												
	2009	n.s.	0.53***	0.50***	0.83***												
FW	2007	–	n.s.	n.s.	n.s.	−0.39***											
	2008	n.s.	n.s.	n.s.	0.40***	n.s.											
	2009	n.s.	n.s.	n.s.	0.32**	−0.25*											
FIRM	2007	–	n.s.	−0.27*	−0.27*	−0.23*	n.s.										
	2008	n.s.	n.s.	n.s.	−0.30**	−0.22*	n.s.										
	2009	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.										
SSC	2007	–	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.									
	2008	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.25*									
	2009	n.s.	n.s.	n.s.	−0.39***	−0.27*	n.s.	0.37***									
TA	2007	–	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.								
	2008	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.								
	2009	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.								
pH	2007	–	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	−0.79***							
	2008	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	−0.65***							
	2009	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.21*	−0.73***							
L-AA	2007	–	n.s.	−0.23*	n.s.	−0.23*	n.s.	n.s.	0.27*	0.32**	n.s.						
	2008	n.s.	n.s.	n.s.	−0.26*	n.s.	−0.23*	n.s.	0.27*	n.s.	n.s.						
	2009	n.s.	n.s.	n.s.	−0.30**	−0.25*	n.s.	0.22*	0.39***	0.36**	n.s.						
ANT	2007	–	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.					
	2008	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.					
	2009	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.25*	−0.26*	n.s.					
*a**	2007	–	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.22*	n.s.	−0.24*				
	2008	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	−0.53***				
	2009	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	−0.52***				
*b**	2007	–	−0.23*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.27*	n.s.	n.s.	0.62***			
	2008	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	−0.52***	0.67***			
	2009	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	−0.45***	0.60***			

Table 3 continued

Traits	Year	FT	PHE	PWI	YI	FN	FW	FIRM	SSC	TA	pH	L-AA	ANT	*a**	*b**	*L**	*h*°
*L**	2007	–	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	−0.37**	0.31**	n.s.	n.s.	0.38***	0.52***		
	2008	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	−0.25*	n.s.	n.s.	n.s.	−0.50***	0.64***	0.71***		
	2009	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	−0.29**	−0.26*	−0.26*	n.s.	−0.25*	−0.37***	0.43***	0.73***		
h°	2007	–	−0.23*	n.s.	n.s.	n.s.	n.s.	−0.26*	n.s.	−0.23*	0.22*	n.s.	n.s.	0.39***	0.76***	0.42***	
	2008	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	−0.34**	0.35**	0.78***	0.68***	
	2009	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	−0.23*	n.s.	n.s.	−0.31**	0.27*	0.91***	0.68***	
*C**	2007	–	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.23*	n.s.	n.s.	0.91***	0.79***	0.43***	0.53***
	2008	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	−0.58***	0.97***	0.78***	0.73***	0.51***
	2009	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	−0.54***	0.95***	0.80***	0.58***	0.54***

Only significant correlations are shown (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, *n.s.* non-significant). The sign of the correlation coefficient indicates the direction of correlations, see "Materials and methods" for trait abbreviation

was identified in LG V-M.1, co-localizing with a QTL that reduced plant width, *pwiV-M.1*. Association of QTLs for fruit L-AA and QTLs modifying plant yield has also been identified in tomato (Schauer et al. 2006). The largest cluster of QTLs was found in a region of approximately 35 cM in LG V-M.2, involving QTLs for titratable acidity, pH, anthocyanin content and the color parameters *a**, *b** and *C**. Co-location of QTLs for anthocyanins and color parameters is in agreement with the high correlations found between them, as reported in raspberry (McCallum et al. 2010). Co-location of anthocyanins and acidity QTLs, although apparently unrelated, can be explained by the fact that anthocyanins accumulate in cell vacuoles and their absorption spectrum is pH dependent (Yoshida et al. 2003). A QTL for anthocyanins in LG III-F.1 and a QTL for SSC in LG V-M.4 co-located with yield related QTLs. In addition, another QTL for SSC was detected in LG VI-M.3 over 2 years. Other QTLs for fruit quality traits, as *sscII-F.1*, *firmII-F.6*, *antII-M.6*, *antIII-F.1*, *firmVII-F.1* and *L-AAVII-M.1* were detected only 1 year, suggesting a stronger environmental effect over these loci.

Association of genic-markers with QTLs

Although we expect a coincidental number of associations between genes and QTLs, given the number of genic markers in the map and assuming a random distribution of candidate genes, we have identified some co-locations that might be relevant. ChFaM004 was mapped to LGII-M.6 at the 23.9 cM position, within the confidence interval of QTL *AntII-M.6,* explaining ~12% of the variation in anthocyanin content (Fig. 1; Table 4). The EST from which this marker is derived encodes a putative R2R3 MYB transcription factor highly homologous to the C1 protein, involved in the activation of anthocyanin biosynthetic genes in maize (Sainz et al. 1997). Similarly, marker ARSFL-099, in *Fa-Exp2* was mapped to LG VII-F.1 at the 31.9 cM position, within the confidence interval of QTL *firmVII-F.1,* controlling fruit firmness. *Fa-Exp2* encodes a fruit specific auxin-independent expansin whose expression increases during ripening (Civello et al. 1999; Dotto et al. 2006). Marker ChFaM070, located in an EST with high homology (*e* value $2e^{-34}$) to the senescence-inducible chloroplast stay-green (SGR) protein of *Pisum sativum* (Sato et al. 2007), was linked to QTLs underlying fruit number and yield in LG V-M.4. Marker ChFaM007 (in a putative galactosyl-transferase) mapped to LG II-F.1, close to a QTL for SSC and ChFaM109, located in a putative transcriptional activator, mapped to LG V-M.2 close to the co-localizing QTLs for titratable acidity, pH, anthocyanins and color parameters. Marker ChFaM083 (in a putative zinc-binding transcription factor) mapped to LG I-M.2, in the confidence interval of QTL *fwI-M.2* controlling fruit

Table 4 QTLs detected for 14 agronomical and fruit quality traits in the '232' × '1392' population based on multiple-QTL model mapping (MQM)

Trait	Year	QTL	LOD thr.[a]	LOD max.[b]	Position[c]	Closest marker (position)[d]	R^{2e}	Effect[f]
Plant height (PHE)	2008	***pheIV-F.6***	**2.7**	**2.81**	**12.27**	**ChFvM087-236 (15.20)**	**13.7**	**0.44**
	2009	***pheIV-F.6***	**2.6**	**3.22**	**18.20**	**ChFaM148-164 (19.58)**	**13.0**	**0.40**
Plant width (PWI)	2008	*pwiV-M.1*	2.6	3.71	19.73	ChFaM022-198 (19.77)	16.0	2.33
	2009	*pwiV-M.4*	2.6	3.08	26.16	ChFaM269-445 (30.96)	15.1	2.05
Yield (YI)	2007	*yiI-F.1*	2.7	2.83	34.62	UFFxa16H07-251 (29.62)	15.1	−242.17
		yiIII-F.1	2.7	2.89	6.45	caa/aac-278 (4.45)	14.5	−236.65
		yiV-M.4	**2.7**	**3.67**	**20.16**	**cca/agg-500 (14.17)**	**19.1**	**272.92**
	2009	*yiII-M.4*	2.6	3.05	18.49	ARSFL017-217 (18.52)	12.6	−188.68
		yiV-F.2	2.7	2.70	20.44	ChFaM018-198 (17.44)	12.9	186.55
		yiV-M.4	**2.6**	**3.44**	**4.53**	**EMFn010-223/240**[h]	**22.1**	**559.98**
Fruit number (FN)	2007	***fnV-M.4***	**2.7**	**3.44**	**8.53**	**EMFn010-223/240 (4.53)**	**15.4**	**13.27**
	2008	*fnIII-F.2*	2.7	2.76	29.33	ChFaM088-156[h]	10.7	7.13
	2009	*fnII-M.4*	2.7	3.05	18.49	ARSFL017-217 (18.52)	11.8	−10.78
		fnIII-F.1	2.7	3.91	17.45	BFACT036-159/130 (24.08)	18.5	−12.83
		fnV-F.4	2.7	4.87	3.34	*FaMYB1*-186/207[h]	25.2	−15.21
		fnV-M.4	**2.7**	**4.75**	**4.53**	**EMFn010-223/240**[h]	**23.7**	**14.75**
Fruit weight (FW)	2007	***fwI-M.2***	**2.6**	**2.81**	**9.42**	**ChFaM206-216**[h]	**12.0**	**−1.75**
	2008	***fwI-M.2***	**2.7**	**3.18**	**2.10**	**EMFn182-186 (1.10)**	**12.1**	**−2.14**
		fwV-M.3	2.7	5.49	7.00	UDF034-169 (7.28)	22.4	2.91
Firmness (FIRM)	2009	*firmII-F.6*	2.6	2.60	1.04	Fvi11-282/280[h]	9.6	21.24
		firmVII-F.1	2.6	3.99	39.64	ChFaM160-193[h]	15.2	−27.07
Soluble-solids content (SSC)	2007	*sscV-M.4*	2.6	2.73	30.17	ChFaM269-445 (30.96)	11.6	−0.85
		sscVI-M.3	**2.6**	**2.60**	**0.00**	**cct/aca-146**[h]	**10.7**	**−0.83**
	2009	*sscII-F.1*	2.7	3.61	58.07	Fvi11-302/310 (54.07)	17.6	1.18
		sscVI-M.3	**2.7**	**3.01**	**1.00**	**cct/aca-146 (0.00)**	**12.7**	**−1.01**
Titratable acidity (TA)	2007	***taV-M.2***	**2.6**	**2.65**	**2.00**	**ChFaM106-144 (0.00)**	**12.1**	**−0.06**
	2008	*taIV-F.2*	2.7	2.70	74.29	ChFaM023-153/171[h]	15.5	−0.07
		taV-M.2	**2.6**	**3.32**	**6.10**	**ChFaM044-226**[h]	**14.7**	**−0.06**
	2009	***taV-M.2***	**2.6**	**4.26**	**8.24**	**ChFaM109-150**[h]	**18.1**	**−0.06**
pH	2007	***pHV-M.2***	**2.6**	**3.28**	**6.10**	**ChFaM044-226**[h]	**15.2**	**0.10**
	2008	*pHIV-F.2*	2.7	4.01	70.65	EMFvi136-138 (66.65)	19.4	0.13
	2009	***pHV-M.2***	**2.6**	**6.96**	**24.75**	**ChFaM142-209 (28.76)**	**30.5**	**0.12**
L-AA	2007	***LAAIV-F.2***	**2.7**	**2.97**	**74.29**	**ChFaM023-153/171**[h]	**13.7**	**−4.89**
		LAAV-M.1	**2.7**	**4.28**	**11.64**	**ChFaM108-177 (14.39)**	**18.6**	**−5.68**
		LAAVII-M.1	2.7	2.92	11.05	caa/aac-250[h]	12.0	−4.59
	2008	***LAAV-M.1***	**2.6**	**3.42**	**3.00**	**EMFvi108-188 (0.00)**	**15.1**	**−4.30**
	2009	***LAAIV-F.2***	**2.7**	**2.77**	**74.29**	**ChFaM023-153/171**[h]	**14.1**	**−3.57**
		LAAV-M.1	**2.6**	**4.18**	**28.37**	**BFACT005-173 (33.75)**	**21.9**	**−4.33**
Anthocyanins (ANT)	2008	*antIII-F.1*	2.7	2.85	24.08	BFACT036-159/130[h]	10.3	3.96
		antV-M.2[g]	**2.5**	**2.14**	**8.10**	**ChFaM044-226 (6.11)**	**9.2**	**−3.72**
	2009	*antII-M.6*	2.6	3.04	15.52	CFVCT027-131[h]	11.7	4.55
		antV-M.2	**2.6**	**5.97**	**6.10**	**ChFaM044-226**[h]	**24.8**	**−6.62**
*a**	2007	***aV-M.2***[g]	**2.6**	**2.11**	**2.00**	**ChFaM106-144**[h]	**11.4**	**2.19**
	2008	***aV-M.2***	**2.7**	**4.68**	**1.00**	**ChFaM106-144 (0.00)**	**22.5**	**3.04**

Table 4 continued

Trait	Year	QTL	LOD thr.[a]	LOD max.[b]	Position[c]	Closest marker (position)[d]	R^{2e}	Effect[f]
*b**	2007	***bV-M.2***	**2.7**	**3.86**	**0.00**	**ChFaM106-144**[h]	**17.3**	**2.72**
	2008	***bV-M.2***	**2.7**	**3.86**	**1.00**	**ChFaM106-144 (0.00)**	**18.1**	**2.c26c**
*c**	2008	*cV-M.2*	2.6	3.96	3.00	ChFaM106-144 (0.00)	18.5	3.57

Those QTLs identified in 2 or all 3 years are marked in bold

[a] LOD thresholds determined by permutation test for each trait in each year and each map

[b] The maximum significant LOD detected for each QTL

[c] The most likely position (in cM) of the QTL from the top of the LG

[d] The most closely associated marker locus and its map position

[e] Percentage of the total phenotypic variation explained by the QTL

[f] Estimated additive effect calculated as mu_A–mu_H, where mu_A and mu_H are the estimated mean of the distribution of the quantitative trait associated with the "a" or "h" genotypes, respectively

[g] QTLs detected just below the threshold but significant in other years

[h] Markers located in the LOD peak position

weight in 2007 and 2008. The identification of transcription factors as positional candidate genes is appealing since phenotypic variation in nature is more likely to result from variation in genes encoding transcription factors than from cis-acting regulatory changes (Martin et al. 2010).

Three QTL controlling fruit L-AA content were detected, which together accounted for ~45% of the variation. *FaGalUR* encodes an enzyme of the D-galacturonate pathway for ascorbic acid biosynthesis and over-expression in *Arabidopsis* increased L-AA content two- to threefold (Agius et al. 2003). Polymorphic alleles of this gene were mapped to the bottom of two LGs, IV-F/M.5 and IV-F/M.6, in HG IV using an SSR in the first intron, although additional bands were monomorphic (ChFaM148; Fig. S1). The homologous *F. vesca* gene was also mapped to a homologous region of LGIV in this species as an *aldo–keto reductase* (*AKR*; Sargent et al. 2007). Interestingly, the QTL *LAAIV-F.2* was identified at the bottom of LG IV-F.2, belonging to the same HG (Fig. 1). Therefore, although it will need further investigation, it is possible that another homoeologous copy of *FaGaLUR* in LG IV-F/M.2 may be controlling that L-AA variation. The majority of the enzymes in ascorbic acid biosynthetic pathways and recycling have been characterized and due to the high colinearity between *F.* × *ananassa* and *F. vesca*, we decided to use the publicly available genome sequence of *F. vesca* (Shulaev et al. 2011) to identify the position of key candidate genes (Table 5). Interestingly, we found candidate

Table 5 Locations of selected ascorbic acid biosynthetic genes in *F. vesca* linkage groups (FG). Position (in bp) of markers flanking L-AA QTLs have been included below

Gene	Enzyme	FG	FG accession	Position	FG length	Gene references
FaMYOX	Myo-inositol oxygenase	V	CM001057.1	1,049,986	29,328,693	Cruz-Rus et al. (2011)
FaGME	GDP-mannose-3′,5′-epimerase	VI	CM001058.1	519,518	38,222,195	Cruz-Rus et al. (2011)
FaG1PP	L-Galactose-1-phosphate phosphatase	III	CM001055.1	1,116,722	27,879,571	Cruz-Rus et al. (2011)
FaGLDH	L-Galactono-1,4- lactone dehydrogenase	VI	CM001058.1	23,087,148	38,222,195	do Nascimento et al. (2005)
FvMDHAR	Monodehydroascorbate reductase	VI	CM001058.1	12,867,809	38,222,195	Cruz-Rus et al. (2011)
FvDHAR	Dehydroascorbate reductase	VII	CM001059.1	15,472,191	23,403,891	Cruz-Rus et al. (2011)
FvGMP	GDP-mannose pyrophosphorylase	VII	CM001059.1	17,616,626	23,403,891	http://www.strawberrygenome.org
Marker	QTL			Position		
EMFvi108	*LAAV-M.1*			39,961		
BFACT005				25,765,900		
ChFaM208	*LAAVII-M.1*			22,076,927		
BFACT018				18,720,516		

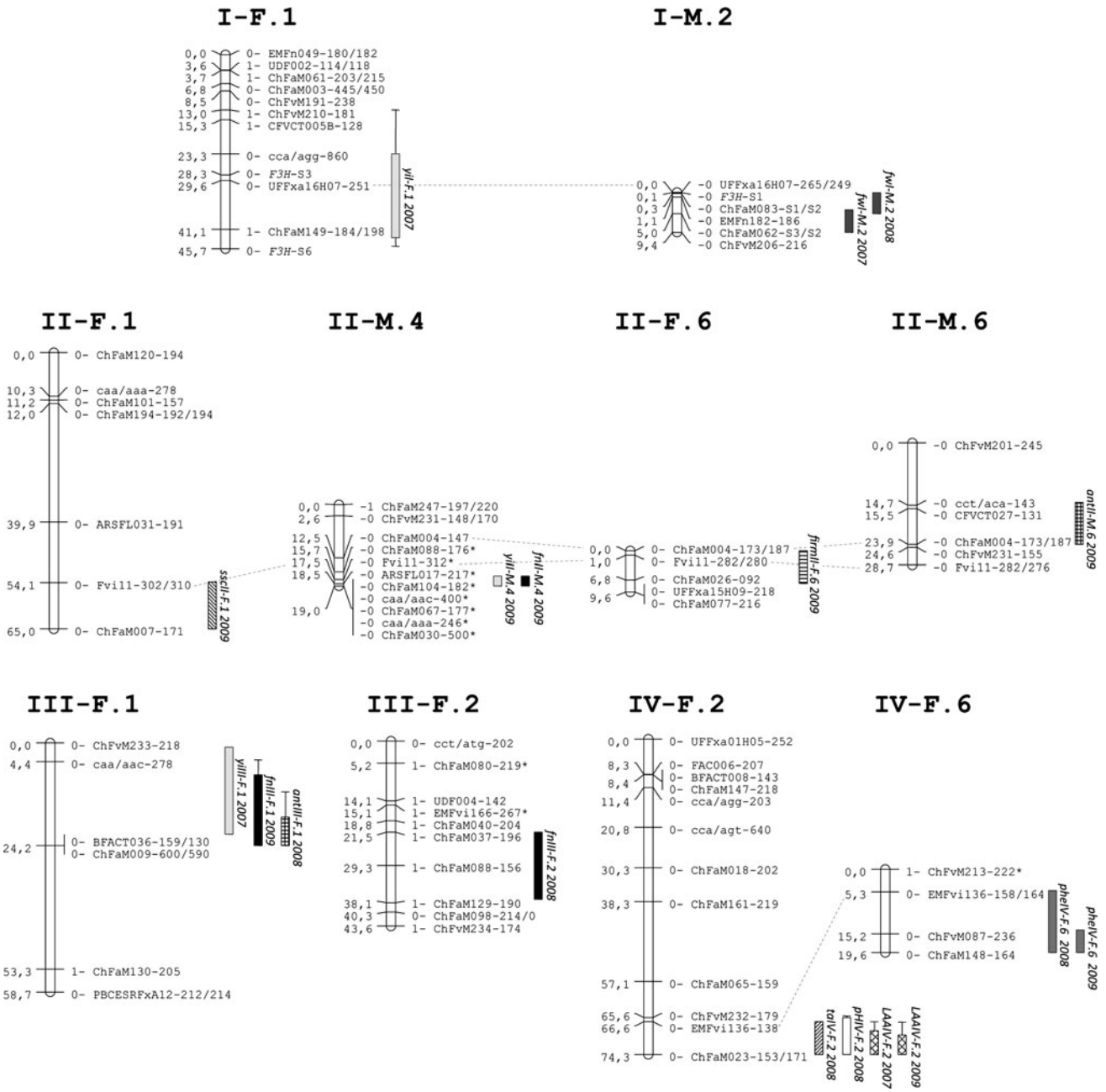

Fig. 1 Location of QTLs controlling agronomical and fruit quality traits analysed in three consecutive years (2007, 2008, 2009) using MQM. Only linkage groups including QTLs are presented. *phe* Plant height, *pwi* plant width, *yi* yield, *fn* fruit number, *fw* fruit weight, *firm* firmness, *ssc* soluble-solids content, *ta* titratable acidity, *LAA* L-ascorbic acid, *ant* anthocyanins, *a* green–red spectrum, *b* blue–yellow spectrum, *C* chroma. *Thick and thin bars mark* 1-LOD and 2-LOD support intervals of each QTL, respectively. ‡QTLs detected just bellow the threshold, but significant in other years

genes in the proximity of markers linked to the two other QTLs for L-AA. The gene *myo-inositol oxygenase* (*FaMYOX*) was located at the top of *F. vesca* LGV, within the region corresponding to QTL *LAAV-M.1*. Also, *dehydroascorbate reductase* (*FvDHAR*) and *GDP-mannose pyrophosphorylase* (*FvGMP*) were both located very close to markers within the confidence interval of *LAAVII-M.1* (Table 5; Figs. 1, S1).

Discussion

Octoploid strawberry genetic map

The *F.* × *ananassa* map here presented is based on a F_1 population derived from the cross between two closely related selection lines, as revealed by the relatively high number of common markers (heterozygous in both parents)

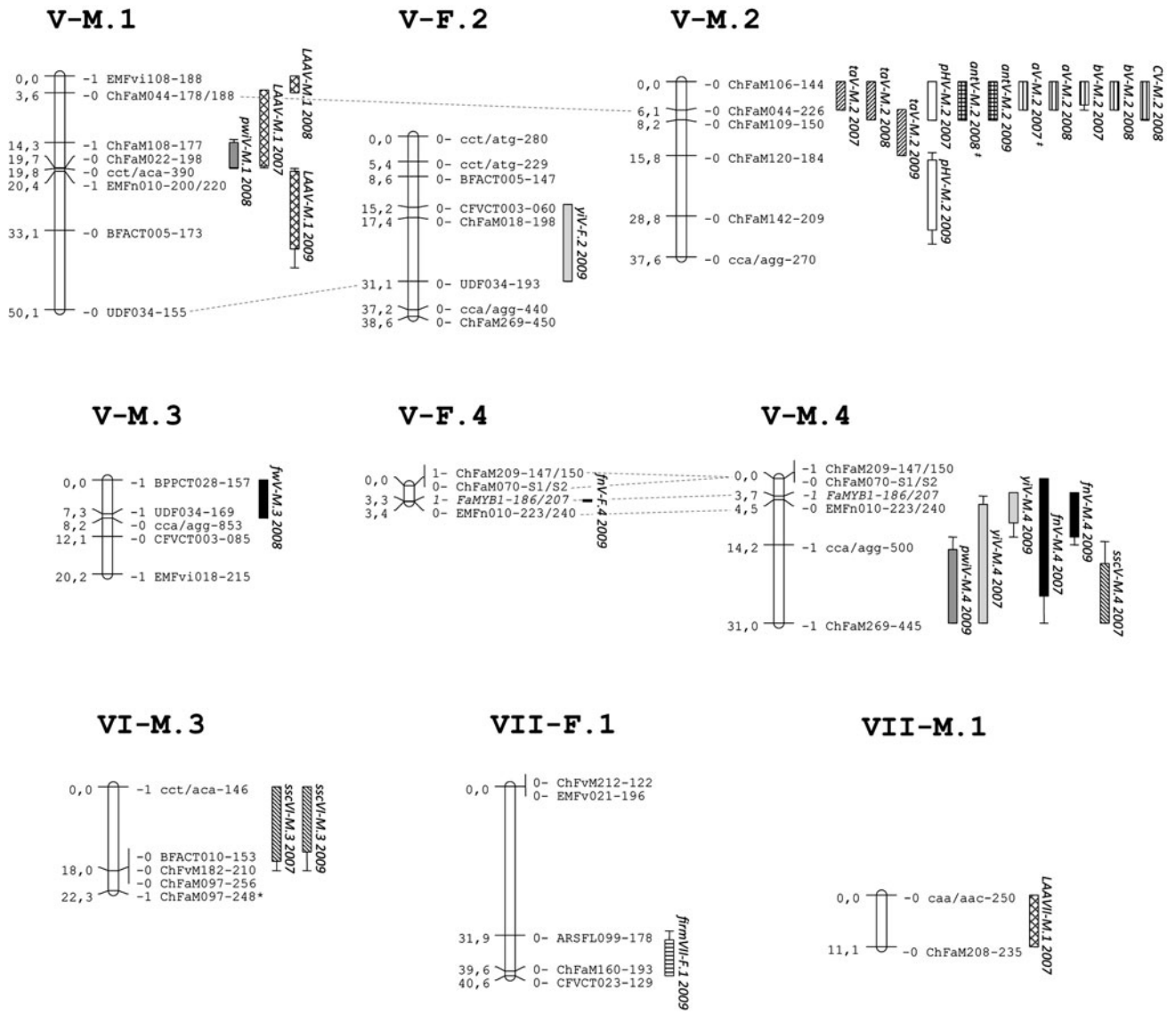

Fig. 1 continued

and the moderately low proportion of segregating bands per locus when compared with other populations (Rousseau-Gueutin et al. 2008; Sargent et al. 2009). The choice of these two selection lines as parents of the mapping population was based on their contrasting phenotypes for a high number of agronomical and fruit quality traits and their adaptation to the Mediterranean and Californian climatic conditions. Thus, the alleles segregating in the progeny are probably common in the current breeding stocks of this area. Other leading strawberry producers also belong to this agro-environment, such as Turkey, Egypt and Morocco (FAOSTAT, 2009; http://faostat.fao.org), adding value to the QTLs detected in this work. Although *F.* × *ananassa* has a high capacity of adaptation to very different agro-environments, from tropical up to nordic areas, each cultivar is adapted to specific cultural and climatic conditions (López-Aranda 2008). Therefore, the comparison of the QTLs here identified with others detected in strawberry populations adapted to higher latitudes would increase our knowledge of the stability of fruit quality QTLs over different genetic backgrounds and environments.

The maps here developed for the octoploid strawberry will assist future comparative QTL mapping studies in *Fragaria* and other species of the Rosaceae, since the core markers employed to develop the map consists of codominant and transferable SSR markers (Zorrilla-Fontanesi et al. 2011). In addition, our linkage maps also include a number of transferable markers with *Prunus*, such as BPPCT028, *MADS5* and M9a but also other conserved markers in Rosaceae (Illa et al. 2011). This transferability also allowed the identification of the homoeologous LGs of *F.* × *ananassa* that correspond to each of the seven LGs of

the wild diploid relative *F. vesca*, considered as one of the ancestors that partially contributed to the genome of the octoploid strawberry (Rousseau-Gueutin et al. 2009). Finally, because most of the mapped SSRs were derived from *F.* × *ananassa* ESTs mainly involved in metabolic and regulatory processes relevant to strawberry fruit ripening (Bombarely et al. 2010), these maps represent a useful tool for the identification of candidate genes which may be functionally associated with QTLs controlling fruit yield and quality traits.

In the integrated map here reported, 338 loci have been positioned across the seven HGs of the octoploid genome, a similar number of markers to that used in Sargent et al. (2009). However, the number of loci and map lengths of the separated parental maps were lower than those of Sargent et al. (2009), as we have not used 3:1 dominant markers to generate these maps (using a BC1 population type). In this way, the pseudo-test cross strategy could be employed and a simple two QTL genotypes modelled for QTL analysis. These markers covered about 57% of the most saturated octoploid *Fragaria* map (Rousseau-Gueutin et al. 2008) and additional gene-specific markers need to be added to cover the gaps. However, this will be greatly facilitated with the recent publication of the *F. vesca* genome and the generation of new markers with the new high throughput sequencing technologies available.

Clusters of markers with distorted segregation on particular genomic regions suggest the presence of sub-lethal genes (Cai et al. 1994; Jarrell et al. 1992; Landry et al. 1991; Prince et al. 1993). These loci are likely the main factors responsible for the "low viability" phenotype since they may act in pre- or post-zygotic selection mechanisms. The negative effect of these alleles can be estimated by the degree of the distortion. In the integrated '232' × '1392' map, genetic factors on LGs III-F/M.1 and IV-F/M.1 appear to have larger effects than those on LGs II-F/M.4 or IV-F/M.6. In some cases, distortions only affected one parent because the low viability locus was inherited from this line (as in LG II-F/M.4) and in others the loci may be present in both parental lines (as in LGs III-F/M.1, IV-F/M.1 and IV-F/M.6). In a different study in strawberry, distorted markers were preferentially distributed in three male groups of HG IV (Rousseau-Gueutin et al. 2008). Assessment of correspondence between these distorted areas with those of LGs IV-F/M.1 and IV-F/M.6 of '232' × '1392' could be further analysed using genome specific markers for each LG of HG IV.

QTLs and candidate genes in '232' × '1392'

The 33 QTLs for the 14 agronomical and fruit quality traits distributed in the 7 HGs of *F.* × *ananassa* were those having larger effects due to the size of our mapping population (Andersson et al. 1994). Approximately, one-third of them were stable in 2 or all 3 years. One QTL for titratable acidity (*taV-M.2*) and another controlling L-AA (*LAAV-M.1*) were the two most stable QTLs, since they were detected over all 3 years. A second QTL for L-AA, *LAAIV-F.2*, was stable over 2 years. The stability of these QTLs adds value to our findings because L-AA, a highly appreciated fruit characteristic, is highly influenced by the environment (Davey et al. 2007). Stable QTLs for L-AA have also been reported in different tomato mapping populations (Stevens et al. 2007). The percentages of phenotypic variation explained by the detected QTLs were generally less than 20%, indicating that all analysed traits are complex and quantitatively inherited in strawberry. In several fruit species, as peach (Dirlewanger et al. 1999; Yoshida 1970), pummelo (Cameron and Soost 1977) or apple (Maliepaard et al. 1998), fruit acidity appears to be controlled by single loci. However, this is not the case of strawberry because each of the two QTLs here reported only explains about 15% of the variation. Similar to our results, in processing (Paterson et al. 1988) as well as in fresh market tomato (Saliba-Colombani et al. 2001), components of fruit acidity are conditioned by several QTLs with relatively small effects.

Three QTLs were detected controlling L-AA content in the ripe fruits, with alleles increasing the concentration inherited from '1392' for *LAAV-M.1* and *LAAVII-M.1*, while for QTL *LAAIV-F.2* positive alleles were provided by '232', which contains less L-AA than '1392'. The genetic control of L-AA has also been studied in apple, also belonging to the Rosaceae. Similar to our results, three significant QTLs for fruit flesh L-AA content with a maximal contribution of 31% per QTL were found in apple under field conditions, although the stability of these QTLs over time was not tested (Davey et al. 2006). *FaGaLUR,* a key gene in the D-galacturonic acid pathway in strawberry fruit (Agius et al. 2003) is located at the bottom of LGs belonging to HG IV, in a homoeologous region where *LAAIV-F.2* was detected. Interestingly, a recent study in tomato has associated an ascorbic acid QTL with the D-galacturonic acid pathway (Di Matteo et al. 2010). Further fine mapping of additional copies of the gene is required in parallel with screening for differences in gene sequence and expression levels to determine whether *FaGaLUR* is the gene underlying *LAAIV-F.2*. Syntenic candidate genes involved in L-AA biosynthesis or recycling were identified through the use of the genome sequence of *F. vesca*. Co-location between genes *FvDHAR* and *FvGMP* and markers within the confidence interval of *LAAVII-M.1* was detected in *F. vesca*. The control of L-AA in tomato fruit has been linked to monodehydroascorbate reductase (MDHAR), an enzyme that together with dehydroascorbate reductase (DHAR) are involved in recycling oxidised monodehydroascorbate and dehydroascorbate to L-AA (Stevens et al.

2008; Stevens et al. 2007). Co-location between a *GMP* and a QTL for L-AA has also been detected in tomato (Stevens et al. 2007). *GMP* (also known as *VTC1*) encodes a key enzyme in the L-galactose pathway for L-AA biosynthesis (Valpuesta and Botella 2004). Finally, *FaMYOX* was identified within the markers EMFvi108 and BFACT005, which flanked *LAAV-M.1*, although the position of *LAAV-M.1* varied along the 3 years and covered a large region. The biosynthetic route of myo-inositol may be relevant for L-AA accumulation in strawberry fruit, as the gene *FaMYOX* is expressed during fruit ripening in parallel to the increase in L-AA content (Cruz-Rus et al. 2011), and overexpression of *MYOX* in *Arabidopsis* increased ascorbic acid levels two- to threefold (Lorence et al. 2004). Mapping of these three candidate genes in '232' × '1392' is required to confirm these co-locations in octoploid strawberry.

Similar to what we found in strawberry, association between R2R3 MYB transcription factors and QTLs controlling fruit color and/or anthocyanins have also been reported in apple, sweet cherry and raspberry (Chagné et al. 2007; McCallum et al. 2010; Sooriyapathirana et al. 2010). The anthocyanin biosynthetic gene *flavanone 3-hydroxylase* (*F3H*) in HG I did not co-located with fruit color or anthocyanin content QTLs despite that an association of this gene with fruit red skin color and yellow fruit color has been reported in peach and *F. vesca*, respectively (Deng and Davis 2001; Illa et al. 2011). Similarly, we did not find any QTL for anthocyanins or fruit color co-localizing with other genes involved in anthocyanin biosynthesis such as *chalcone isomerase* (*CHI*), *flavonol synthase* (ChFaM247), *dihydroflavonol reductase* (ChFvM234) or *FaMYB1* (mapped to HGV), a ripening regulated MYB transcription factor with a role in the down-regulation of anthocyanins and flavonols in strawberry (Aharoni et al. 2001).

A QTL for fruit firmness was located in the same chromosomic region as *Fa-Exp2*, an *expansin* specifically expressed in strawberry fruits (Dotto et al. 2006). Another *expansin* gene associated with fruit softening in apple, *Md-Exp7*, has been located in the region of one major apple QTL for fruit firmness (Costa et al. 2008). However, phylogenetic analysis of *Md-Exp7* with other *expansins* including *Fa-Exp2* suggests that they are not orthologs, at least at sequence level (Costa et al. 2008; Harrison et al. 2001).

Pleiotropic QTLs controlling fruit yield and underlying candidate genes

Yield is the most economically important trait in crops, but also the most complex because it is heavily influenced by an interaction of environment with the majority of growth and developmental processes. Several studies have explored the genetic basis of yield and yield-component traits (such as seed/fruit size, weight or number) allowing the identification of yield-related traits such as biomass or plant architecture (Cong et al. 2008; Frary et al. 2000; Huang et al. 2009; Maccaferri et al. 2008; Shi et al. 2009). Yield in the '232' × '1392' strawberry population was highly correlated with fruit number (correlation coefficients higher than 0.82 for the 3 years; Table 3). Accordingly, QTLs for yield and fruit number co-located in two genomic regions, on the same and on different LGs belonging to HGs II and V. In addition, homoeoQTLs controlling plant width were located in similar regions to yield and fruit number in LG V-M.4 and V-M.1, with male alleles having negative effects on this trait, as occurred with yield. In contrast, '1392' alleles of the yield and fruit number QTLs on LG II-M.4 increased yield and fruit number. Fruit weight was only weakly correlated with yield in two of the analysed years. QTLs for fruit weight were detected for 2 years in LG I-M.2 and 1 year in V-M.3. Interestingly, QTLs for yield were identified in different homoeologous LGs of HG I and V. In summary, homoeologous loci controlling fruit number affect yield but also the same or different loci in other LGs belonging to the same HG may control yield by an effect in fruit weight.

A strawberry *SGR*-like gene was mapped to LG V-M.4, in the same chromosomic region as QTLs for yield and yield related traits. The *SGR* gene is required for the dismantling of photosynthetic chlorophyll–protein complexes during senescence (Hörtensteiner 2009). Because of the potential contribution of the stay-green trait to increased yield and drought tolerance, it has been intensively studied in crops, such as sorghum (Walulu et al. 1994), soybean (Pierce et al. 1984), maize (Gentinetta et al. 1986), durum wheat (Spano et al. 2003) or potato (Schittenhelm et al. 2004). In addition, marker ChFaM209 (a putative homeobox transcription factor) mapped close to *SGR*, indicating that any of these genes could be involved in yield. However, that chromosomic region of LG V-M.4 and V-F.4 contains only heterozygous co-dominant markers that have been transformed using only the maternal and paternal information in the pseudo-testcross maps. In doing so, half of the progeny is not used and the distance between markers is reduced compared to the integrated CP map (Fig. S1). Therefore, detection of these QTLs using a CP analysis would increase mapping resolution in that particular area and determine the extent of co-location between the QTLs and the candidate genes.

Comparison of *F.* × *ananassa* QTLs with QTLs from *Prunus* and *Malus*

A comparative genome analysis anchoring common molecular markers of *Prunus* and *Fragaria* genetic

reference maps to the *Malus* physical map reported high correspondence between marker positions and conserved syntenic blocks were identified among the three genera (Illa et al. 2011). As a preliminary analysis and using the information provided by that study, we have compared the *Fragaria* QTLs here identified with QTLs controlling similar traits in *Prunus* and *Malus* populations.

QTLs for L-AA content of fruit flesh were identified on *Malus* linkage groups (MG) 6, 10 and 11, with an additional QTL controlling dehydroascorbate (and total ascorbic acid) on MG17 (Davey et al. 2006). Linkage group FG5 of the *Fragaria* diploid reference map (HG V of. *F* × *ananassa*) is mostly syntenic with *Malus* MG6, but also shares a syntenic region with MG13 and MG14. Thus, it may be possible that the stable QTL *LAAV-M.1* controlling L-AA in strawberry is homologous to apple QTL on MG6.

While only two QTLs were detected for fruit firmness in strawberry, QTLs controlling this trait have been identified in different apple populations in linkage groups 1, 2, 10, 14 and 16 (Costa et al. 2008, 2010; Kenis et al. 2008; King et al. 2001). A possible correspondence may exist for strawberry QTL *firmVII-F.1*, that co-locates with *Fa-Exp2,* because linkage group FG7 is syntenic to the *Malus* homoeologous chromosomes MG1, MG7 and a block of MG2, were fruit firmness QTLs have been detected. Interestingly, the apple expansin *Md-Exp7* has been associated with the QTL controlling firmness on MG1 (Costa et al. 2010). Based on these results, it is tempting to speculate that fruit firmness in these two distant genera within the phylogeny of the Rosaceae is partly controlled by the expression or activity of *expansins*.

QTLs controlling fruit pH and acidity were identified at the end of LG IV-F.2 (FG4) and at the beginning of LG V-M.2 (FG5) in strawberry. In peach, QTLs for pH and/or titratable acidity have been identified at the end of PG1 (syntenic with FG4) and at the beginning of PG5 (syntenic with FG5) (Dirlewanger et al. 1999; Etienne et al. 2002b). Sweet cherry QTLs for the color parameters *a** and *b** have been mapped to the end of PG6 and PG8 (Sooriyapathirana et al. 2010). These QTLs may correspond to the strawberry QTLs for anthocyanin content *antII-M.6* and *antIII-F.1*, since FG2 is syntenic to PG8 (and PG1) and the upper half of FGIII is syntenic to the end of PG6 (and PG4). Finally, a stable QTL for fruit weight has been detected in sweet cherry PG6 (Zhang et al. 2010) in a region that may be syntenic with the strawberry QTL identified on LG I-M.2 (FG1).

In this study, we report agronomical and fruit quality QTLs in octoploid strawberry and provide candidate genes that, based on their map location and predicted function, could be responsible for the variation of the traits under study. Several strawberry QTLs have syntenic QTLs in *Malus* or *Prunus* indicating that they might be conserved in other Rosaceae and are likely governed by equivalent genetic determinants. Clearly, deeper molecular analysis and further high-resolution mapping of QTLs using conserved markers will be required, but this study can provide a roadmap for a better understanding of key agronomical and fruit quality traits in strawberry and probably in other Rosaceae. Markers linked to these QTLs could be very useful for MAS in breeding programs after they have been validated by determining the target phenotype in additional populations with different genetic backgrounds.

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