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RESEARCH

# Identification of quantitative trait loci (QTL) and development of markers associated with fruit quality traits in strawberry (*Fragaria* × *ananassa* Duch.)

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**Abstract** Significant breeding targets in *Fragaria* species are improvement of the fruit quality traits that the bright red color of these fruits, attributed to anthocyanins, due to its appeal and nutritional value. To reveal the inheritance of the fruit quality traits, we performed quantitative trait loci (QTL) analysis using segregating populations derived from female 'Fortuna' and male 'Rubygem' cultivars. Entire plants with parents were evaluated for nine phenotypic features in the 2021 and 2022 production years. A total of 28 QTLs were detected for nine (9) quality traits. QTLs controlling regions corresponding to Fana_SNP and iStraw35 arrays were recorded for whole associated loci in each trait. The highest associated locus with phenotypic variation was detected as 26.1% and 10.50 LOD score in LG16 corresponding to Fvb6-1 around 13.3 Mb in the chroma value of the fruit. Linked loci were validated using designed HRM primers, and confirmative results were exhibited from extreme phenotypic plants. Ax-184476583 marker has a major QTL with a 5.24 LOD score and 13.3% phenotypic variance (PV) for xylose, it was located near the 5.2 Mb of Fvb2-3. A marker associated with the lightness value of fruit flesh color was produced in a range of 5.6 and 25.3% PV. Ax-184139018 marker for chroma value of fruit flesh color separated accessions according to phenotype. Markers were pointed on LG2, LG5, LG16 and LG18 corresponding to chr1, chr3, chr6 and chr7, respectively. These markers have been validated for the first time in strawberry breeding, and they could be used to be evaluated in marker-assisted selection for future strawberry breeding programs.

**Keywords** *Fragaria* × *ananassa* · Strawberry · QTL · Development · HRM

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## Introduction

Strawberry (*Fragaria* × *ananassa*), which belongs to the Rosaceae family, is one of the most widely cultivated species all over the world (Hancock 1999). The cultivated strawberry cultivars are octoploid (2n = 8x = 56), and the haploid chromosome number is n = 7 (Sargent et al. 2009; Hummer

et al. 2011; Rho et al. 2012). The strawberry has a complex genomic nature due to its sub-genomes that are derived from interspecies hybridization (Tennessen et al. 2014).

The production of strawberries in the world has increased in the last decade, and approximately 4.5 Mt increases in production were detected until today. The most producer countries of 2021 all over the world were China (3,389,620 t), the USA (1,211,090 t), and Turkey (669,195 t), respectively (Faostat 2023). The strawberry has high genetic variability and a high environmental adaptation ability and is cultivated in wide ecological regions from the poles to the Equator (Karci et al. 2023). Strawberry, which has a high commercial value, attracts the attention of growers because its life cycle is quite short, and it reaches harvest maturity in approximately one to two months after flowering. Therefore, the product can be grown throughout the year thanks to day-neutral, everybearing, and June-bearing cultivars. And, there is a serious variation in sugar contents and flavors among strawberry varieties (Sharma and Yamdagni 1999).

The main purposes of strawberry breeding programs are to increase the size of the fruits, yield per unit area, and resistance to diseases, and pests with higher flavored strawberry fruits. The main fruit quality traits of strawberries include visual properties (color homogeneity and redness of color), organoleptic properties (taste, aroma, smell, and texture), and sensory properties (hardness) (Cardello 1995). The sugar and acid balance in the content of the fruit is the main component that makes up the taste criteria. The sense of sweetness in fresh fruits is highly correlated with the main sugar contents, which are glucose, fructose, sucrose and xylose compounds (Rey-Serra et al. 2021). The consumer preferences for strawberry fruit color may change over time. However, consumers prefer attractive fruits by thinking that dark red strawberries have a higher aroma (Schwieterman et al. 2014; Li et al. 2021). On the other hand, dark red colored fruits are riper fruits and have a relatively shorter shelf life. Therefore, the selection of promising novel genotypes with remarkable and high sugar and aroma, a better understanding of their genetic mechanisms, and the development of molecular markers associated with these biochemical fruit quality traits are quite significant and important breeding objectives (Vallarino et al. 2019; Fan et al. 2021).

The color of the strawberry fruit is a significant quality characteristic that varies widely in the genus *Fragaria* and affects the distribution of anthocyanins, from completely white fruits to deep red (Hancock 1999). The differences in the color variation of strawberry fruits determine the anthocyanin degree, which is an important biochemical component of the fruit (Ngo et al. 2007), and anthocyanin pigments of fruits also protect against various biotic and abiotic stresses in plants (Zhang et al. 2014). The increase of anthocyanins, flavonoids such as quercetin and kaempferol, and carotenoids such as lutein, zeaxanthin, and β-carotene pigments encourages strawberry color, as well as other compounds such as L ascorbic acid (Hancock 1999; Folta and Davis 2006; Fait et al. 2008; Amaya et al. 2024). Colorimetric measures the color properties of fruits, providing quantitative data on their appearance, which is critical for assessing ripeness, quality, and consumer appeal (Ngo et al. 2007; Fernández-Lara et al. 2015; Zitouni et al. 2020). The colorimetry is used in breeding programs, quality control, and post-harvest monitoring to optimize strawberry production and marketability (Adelina et al. 2023). In strawberries, colorimetry often employs the CIE L*a*b* color space to quantify color changes due to processing, storage, or ripening. In colorimetry, chroma and hue values are key components of color perception, providing insight into a color's saturation. These parameters are particularly useful when analyzing the quality and visual attributes of fruits in strawberries (Fernández-Lara et al. 2015; Zitouni et al. 2020; Adelina et al. 2023). The genomic selection and identification of genes or genomic regions linked to fruit color and its colorimetric properties will supply the possibility of marker-assisted selection (MAS), and accelerate the breeding process and the stage of developing novel promising accessions in strawberries.

Marker-assisted selection (MAS) is an efficient tool to eliminate undesired plants or develop novel promising lines or genotypes in plant breeding programs to shorten breeding process with selection in the early stage. Quantitative trait loci (QTLs) is a specific region of the genome that correlates with variation in a quantitative trait, and QTLs were typically identified through the analysis of genetic markers and phenotypic data to detect the marker-trait association for MAS. To date, several papers have been published related to the identification of QTLs linked by fruit

quality traits using different molecular markers (Castro and Lewers 2016; Lerceteau-Kohler et al. 2006; Lerceteau-Köhler et al. 2012; Verma et al. 2017).

Here, this study aims to (i) characterize the Fortuna × Rubygem F1 strawberry population in terms of fruit quality characters such as sugar compounds and colorimetric features, (ii) identify QTLs associated with sucrose, xylose, and fruit color and its parameters in the strawberry genome and (iii) validate the markers linked by traits.

## Material and methods

### Plant material and DNA extraction

A total of 164 intraspecific F1 accessions derived from *Fragaria* × *ananassa* Duch. cvs. 'Fortuna' and 'Rubygem' were used for QTL analysis in the present research. The leaf samples belonging to 164 F1 hybrids were collected for DNA isolation from Yaltir Agricultural Products INC. located in the province of Adana, Turkiye. Ten plants from each genotype were cultivated in high tunnel greenhouses in March-June of 2021 and 2022 seasons.

Genomic DNA from the gathered samples was extracted utilizing the CTAB (cetyltrimethylammonium bromide) technique (Doyle and Doyle 1987) with minor alterations (Kafkas et al. 2005). The quality of the extracted genomic DNAs was controlled by 0.8% agarose gel. The measurement of concentration was performed using both the gel electrophoresis method and Qubit Fluorimeter (Invitrogen) by following the standard procedures.

### Phenotyping of sugar contents of the strawberry fruits

The harvest of the both parents and F1 accessions were carried out 37 and 38 days after anthesis in full red phase for biochemical and monitoring analyses. After harvest, the concentrations of the sugar components such as glucose, fructose, sucrose and xylose from homogenized strawberry juice were determined using the HPLC technique according to the method developed by Crisosto et al. (1997). A total of 140 F1 individuals were used for sugar profile analysis in 2021 and 160 F1 plants were evaluated in 2022, since enough fruits were not harvest for sugar analysis. The five fruits were randomly harvested from 10 plants (repetitions) when they had attained homogenous red colorations. And, sugar analysis was performed in HPLC using three replications of previously homogenized samples (Shimadzu, Prominence LC-20A) RID (Refractive Index) detector and Coregel-87C (7.8 × 300 mm) column. Separations were performed at 70 °C at a flow rate of 0.6 ml $min^{-1}$. Elution was isocratic ultrapure water. The individual sugars were calculated according to their standards and expressed as percent fresh weight (FW). The calibration curves of the references were created, and the content was measured according to these created calibration curves.

### Characterization of strawberry individuals with fruit color parameters

The flesh and core color observation and measurements were performed in 155 and 160 F1 plants in 2021 and 2022, respectively. Analysis was carried out using 20 fruits from ten plants belonging to each genotype at harvest maturity (full red ripe fruits) with three replications. Fruit color of flesh (excluding core) and fruit color of core observation were carried out according to International Union for the Protection and New Varieties of Plants variety database (UPOV, 2012). Fruit color of flesh (excluding core) was classified as whitish (1), light pink (2), orange red (3), light red (4), medium red (5) and dark red (6). And, fruit color of core was evaluated as white (1), light red (2), medium red (3).

The skin and flesh color measurements were recorded in values of lightness (L) of the hunter scale using by Colorimeter Hunter Lab (Miniscan EZ 4500L, Virgian, USA), and Chroma (C) and hue (h°) values were calculated according to the CIE L*a*b* Standards. Lightness (L) represents the lightness of the fruit, with values ranging from 0 (black) to 100 (white). Chroma refers to the degree of color saturation, indicating how vivid or dull it appears. Hue defines the type of color (e.g., red, yellow, blue) and is represented as an angle in the CIE L*a*b* color space. Hue helps differentiate between ripeness stages. The hue angle (in degrees) ranges from 0° to 360° (0° = Red, 90° = Yellow, 180° = Green, 270° = Blue). As strawberries ripen, the hue angle shifts closer to red (lower degrees). $C = sqrt(a^2 + b^2)$

and h = arctan(b/a)*(180/π) formulas were used to calculated chroma and hue values.

Therefore, fruit color of flesh (excluding core) (FCF), fruit color of core (FCC), lightness value of fruit (LVF), chroma value of fruit (CVF), hue value of fruit (HVF), lightness value of flesh color (LVFC), hue value of flesh color (HVFC), chroma value of flesh color (CVFC) properties were calculated using these methods. The strawberry fruit pictures belonging to both parents and extreme F1 accessions in terms of color visual in fruits were given in Fig. 1.

## Comparative analysis of the traits

The sugar contents such as glucose (G), fructose (F), sucrose (S), xylose (X) and the observations with measurements of fruit color parameters such as fruit color of flesh (excluding core) (FCF), fruit color of core (FCC), lightness value of fruit (LVF), chroma value of fruit (CVF), hue value of fruit (HVF), lightness value of flesh color (LVFC), hue value of flesh color (HVFC), chroma value of flesh color (CVFC) of the population were evaluated for detection of the correlations. All of data belonging to each feature were given in Supplementary Table 1. Correlation coefficients and their levels of significance were calculated using Pearson method. Statistic significance degree was set up using $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. All phenotypic properties performed in both growing seasons were used to draw correlation among the traits and trait names were illustrated with an abbreviation for years 21 and 22 at the end of the names, respectively (2021 and 2022). The heatmap of the correlations was generated by R packages 'gplots' and 'RColorBrewer' in RStudio. The correlation coefficients were shared in Supplementary Table 2.

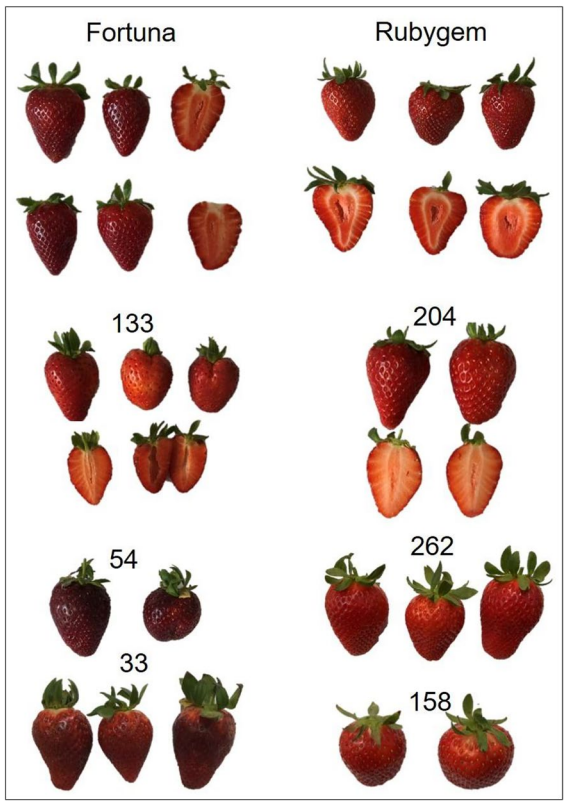



**Fig. 1** The flesh and core colors of fruits from parents of the 'Fortuna × Rubygem' population and visually selected F1 plants with extreme phenotypes

## QTL mapping

The genetic maps of the 'Fortuna × Rubygem' F1 strawberry population constructed by Ayvaz Sönmez et al. (2024) were used for the detection of the quantitative trait loci associated with several agronomic traits. To detect QTLs, the entire genome scanning was conducted for each of the quantitative traits using a general interval mapping approach (Lander and Botstein 1989; Korol et al. 1995). The analysis was based on mixture-model using ML-algorithms of the MultiQTL software (Korol et al. 2001). First, for each trait-chromosome combination, a single-QTL model was fitted and tested for significance. Then, the experimental-wise significance was evaluated for all effects using the concept of false discovery rate (Benjamini and Hochberg 1995). The detected QTL effects were symbolized following the nomenclature described by McIntosh et al. (1998).

The linked markers distributed to genetic linkage maps were mined according to genomic locations to identify the distribution to reference genome sequences. Strawberry genome has four sub genomes as homoeologous for each chromosome. Therefore, homoeologous genomes (HG) of the associated markers were detected. That is, the distribution of linked markers in different linkage groups (LGs) in the strawberry reference homoeologous genomes (HG) was evaluated for each trait. In addition, Fana_SNP and iStraw35 panels refer to *F.* × *ananassa* cv. Camarosa reference genome (Edger et al. 2019), and *F. vesca* cv. Hawaii4 reference genome (Tennessen et al.

2014), respectively. Chromosomes for both reference genomes were indicated as 'Fvb'. The first number next to it refers to the chromosome number; the number after the hyphen refers to the subgenome of the *F.* × *ananassa* cv. Camarosa reference genome. If there were no subgenomes, it belongs to *F. vesca* cv. Hawaii4 reference genome.

### Development of the associated molecular markers

The most highly associated with xylose, the lightness value of fruit flesh color, and the chroma value of fruit flesh color were tested with High Resolution Melting (HRM) analysis using the five (5) individuals with extreme phenotyping (five minimum and five maximum plants) each trait. HRM primers were designed using 300 bases from the right and left of the associated markers using Camarosa cv. reference genome sequences (Edger et al. 2019) and fasta files were recorded for each marker. HRM primers were designed to have a maximum PCR product size of around 150 bp (Untergasser et al. 2012). A total of three HRM primer pairs were designed using the online software Batchprimer 3.0 with the standard parameters (You et al. 2008). Forward and reverse sequences of HRM primers were shared in Supplementary Table 3. HRM analysis and amplification reactions were performed according to Kafkas et al. (2015). HRM was carried out in two steps in a total volume of 12.5 µL containing 20 ng DNA, 75 mM Tris–HCl (pH 8.8); 20 mM $(NH_4)_2SO_4$; 2.0 mM $MgCl_2$; 0.01% Tween 20; 200 mM each dNTP and 0.6 µL hot-start Taq DNA polymerase. In the first step of PCR, conditions were set as follows: initial denaturation at 94 °C for 3 min, followed by 10 cycles at 94 °C for 30 s, 58 °C for 45 s, and 72 °C for 60 s. The second step contains 30 cycles at 94 °C for 30 s, 58 °C for 45 s, and 72 °C for 60 s, and a final extension at 72 °C for 10 min. After the PCR was completed, the reactions were subjected to illustration for agarose gel electrophoresis with 3% concentration.

The associated markers were evaluated according to their homozygous reference (AA), homozygous alterative (BB) and heterozygous alternative alleles (AB) and box plots of the distribution to population with allele numbers were drawn using the phenotypic data of the both years with R package "ggplot2" in R Studio.

## Results

### Evaluation of the phenotypes of traits

#### *Glucose*

The glucose contents of strawberry fruits from the Fortuna × Rubygem F1 population were detected in 2021 and 2022 growing seasons. In 2021, the glucose contents of the Fortuna and Rubygem varieties were identified as 1.34 ± 0.083% and 1.80 ± 0.050%, respectively. And, this content in Fortuna and Rubygem varieties in 2022 were 1.62 ± 0.018% and 1.80 ± 0.065%, respectively. On the other hand, the glucose levels in F1 progenies ranged from 0.74 ± 0.007% to 2.15 ± 0.268% with an average 1.44 ± 0.035% in 2021. In 2022, the glucose contents of the hybrids were recorded between 0.64 ± 0.002% and 2.31 ± 0.241% with an average 1.34 ± 0.040%. A nominal distribution curve was drawn using both production values in glucose contents (Fig. 2).

#### *Fructose*

The contents of the fructose in Fortuna and Rubygem female and male parents were detected as 1.67 ± 0.081% and 2.14 ± 0.048% in first growing season (2021). The fructose components of F1 accessions varied from 1.08 ± 0.008% to 2.74 ± 0.269% with an average 1.84 ± 0.041% in 2021, however, this value ranged from 0.88 ± 0.001% and 2.72 ± 0.301% in F1 progenies with an average 1.72 ± 0.045% in 2022. In addition, Fortuna and Rubygem cultivars exhibited the 2.00 ± 0.232% and 2.180 ± 0.080% in 2022. Fructose values were distributed normally and distribution curve was printed in two growing seasons (Fig. 2).

#### *Sucrose*

The values of sucrose of Fortuna parent were measured as 0.98 ± 0.212% and 0.56 ± 0.094%, in both seasons (2021 and 2022), respectively, while the average sucrose values of Rubygem in 2021 and 2022 were calculated as 0.99 ± 0.059% and 0.50 ± 0.047, respectively. Similarly, the average value of F1 accessions in the first production phase was identified as 0.38 ± 0.019%, and the sucrose contents of the individuals varied between

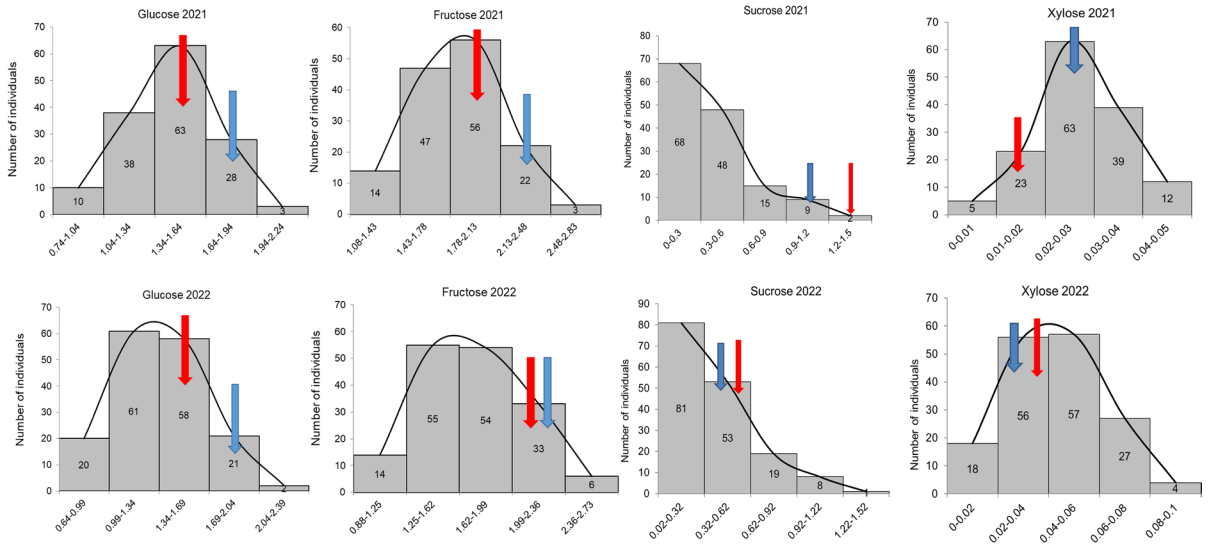



**Fig. 2** Distribution curves of the glucose, fructose, sucrose and xylose, and fruit color and its parameters in the strawberry population two successive years

0.009 ± 0.003% and 1.25 ± 0.135%. On the other hand, this value varied between 0.020 ± 0.00% and 1.29 ± 0.328%, and this value's mean was detected as 0.38 ± 0.021% in 2022. In the first year of the study, the sucrose values of the parents were identified more than the second-year contents. Although these contents belonging to parents were located as ranging from 0.9 to 1.5% in 2021, they calculated between 0.32 and 0.62% in 2022 according to the distribution of the bar plot (Fig. 2).

*Xylose*

The xylose content of Fortuna as a mean was 0.02 ± 0.001% in 2021 and 0.04 ± 0.003% in 2022, while the average contents of Rubygem cultivar were recognized as 0.02% and 0.04% in both years, respectively. The concentration of the xylose of F1 individuals demonstrated the normal distribution, and the range of them was identified among 0.001 ± 0.00% and 0.04 ± 0.004%, respectively. The xylose contents of F1 accessions varied between 0.003 ± 0.00% and 0.05 ± 0.016% in the first growing season, and they reached up to 0.08 ± 0.014% in the second product stage. Using these values, the distribution curve and bar plots were drawn for xylose (Fig. 2).

Fruit color of flesh (excluding core)

The monitoring of the fruit color of flesh (excluding core) was evaluated according to UPOV (2012), which is a strawberry descriptor. Previously prepared classifications of fruit color were observationally recorded as 1: whitish, 2: light pink, 3: orange-red, 4: light red, 5: medium red, 6: dark red. Many individuals were identified, in the ranges of 5–6 classes. And, the parents of the population were found in the same group (Fig. 3).

Fruit color of core

This visual observation of the accessions of the fruit color of the core Fortuna × Rubygem F1 population was scored according to UPOV criteria (2012). The classification of the fruit color of the core varied as 1: white, 2: light red, 3: medium red. The Fortuna variety was determined as medium red in both years, while the Rubygem variety was determined as light red. In both years, the average fruit core color of F1 individuals was determined as light red, and it was also determined that the core colors of the individuals varied from white to medium red (Fig. 3).

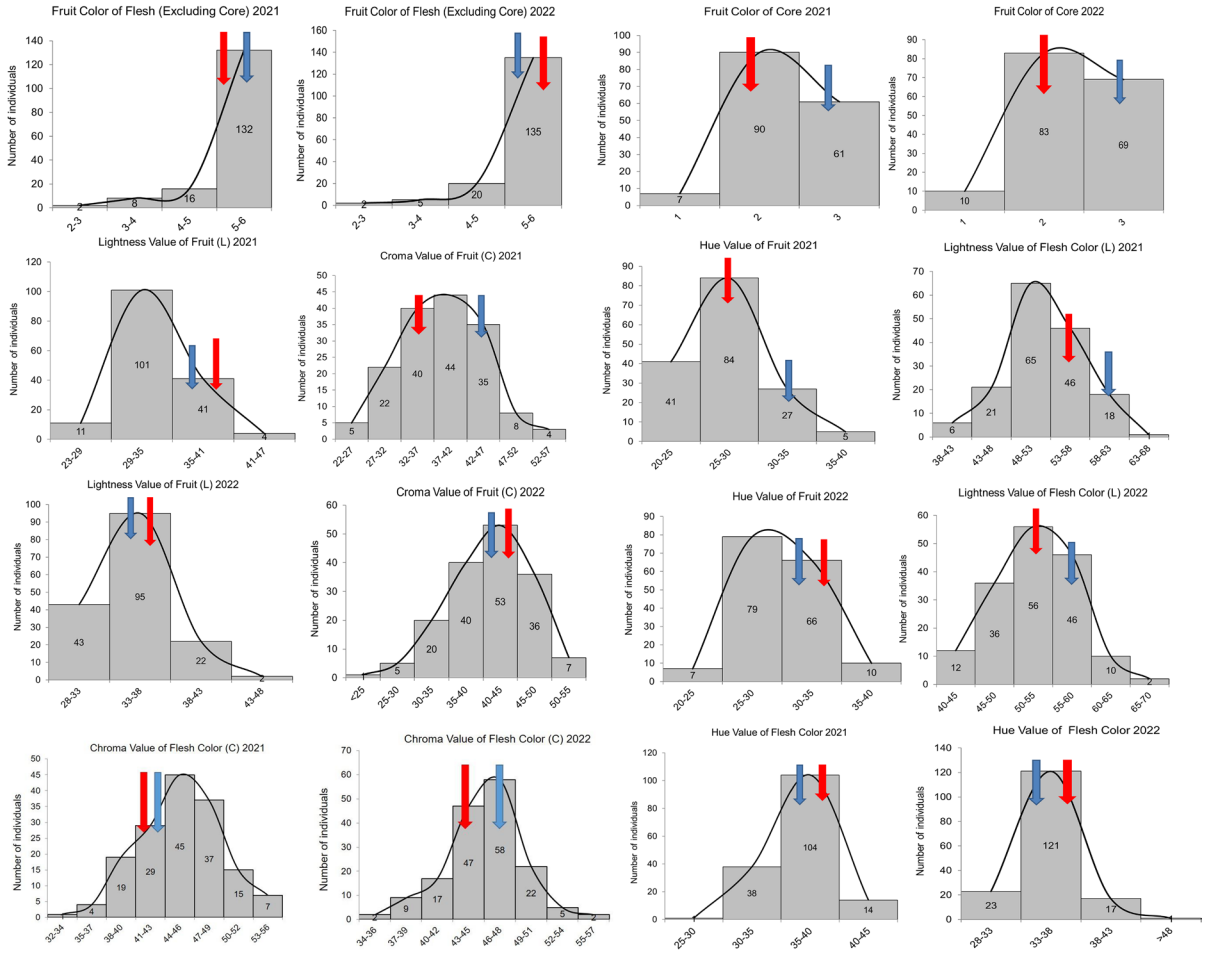



**Fig. 3** Bar plots with distribution curves of the fruit colorimetry traits in the strawberry population for two successive years. The blue arrow refers to 'Fortuna' and the red arrow refers to 'Rubygem' cultivars

## Lightness value of fruit

The lightness value of the fruit of the Fortuna was 35.78 ± 1.90 and 33.74 ± 1.15 in 2021 and 2022. Rubygem's values were 35.16 ± 1.08 and 39.78 ± 1.14, respectively. In the first year, this value for F1 individuals varied between 25.67 ± 0.38 and 41.25 ± 3.45, while the average was 32.20 ± 1.48. In the second year, the lightness value between F1's varied between 28.03 ± 0.35 and 43.69 ± 5.07, and the average value was 35.03 ± 1.34 (Fig. 3).

## Chroma value of fruit

In both years' values (2021 and 2022), the average values of the chroma values of the Fortuna were determined as 44.93 ± 1.21 and 40.46 ± 2.31, while the chroma values of the Rubygem were determined as 35.55 ± 2.10 and 40.52 ± 1.21, respectively. Between the two observations, a slight decrease in chroma value was observed in the Fortuna, while a slight increase was observed in Rubygem. The average value in the F1 population was 38.18 ± 2.74 and 41.04 ± 2.20, respectively. Its distribution among F1 accessions varied between 22.33 and 56.22 in 2021 and varied between 24.97 and 54.21 in 2022 according to Fig. 3.

## Hue value of fruit

The hue value of the Fortuna was determined as 30.26 ± 0.82 in 2021 and 31.65 ± 1.42 in 2022

and Rubygem was determined as 29.79 ± 2.78 and 39.00 ± 1.32, in both years, respectively. It has been observed that the average values in the F1 population in 2021 and 2022 were 28.72 ± 2.01 and 31.28 ± 1.83, respectively, and its distribution within the population according to distribution curve is 20.67–37.96 in the first year and 21.28–38.32 in the second year (Fig. 3).

Lightness value of flesh color

The average values of the lightness of flesh color were examined for 2021 and 2022 values, Fortuna had values of 58.68 ± 2.44 and 56.31 ± 3.46, and Rubygem had values of 56.13 ± 1.59 and 57.85 ± 1.68, in both years respectively. The averages of F1 accessions were observed as 52.01 ± 2.20 and 52.94 ± 1.51, respectively, and the ranges within the population in bar plot curve were observed between 38.09 and 63.20 and between 40.73 and 65.55 in both years, respectively (Fig. 3).

Hue value of flesh color

The hue value of the flesh color of the Fortuna variety was determined as 37.13 ± 0.76 in the first season and 38.15 ± 4.31 in the second season. At the same time, measurements of Rubygem for consecutive years exhibited 36.81 ± 1.06 and 35.47 ± 1.24. The averages of this value among F1 individuals of both seasons were 35.87 ± 1.44 and 36.20 ± 1.90, respectively (Fig. 3).

Chroma value of flesh color

The chroma value of the flesh color of the Fortuna and Rubygem parents were calculated as 43.26 ± 2.58 and 41.34 ± 2.59 in the first season, while this values were 42.56 ± 1.96 and 49.45 ± 0.51 in 2022. The mean value of both years in F1 plants generated as 45.09 ± 2.52 and 45.53 ± 1.67 in successive years. The number of plants of the closest mean in 2021 were produced from 45 accessions. And, 58 individuals comprised the most contributed to mean value in the second season. (Fig. 3).

Correlation analysis

The correlation analysis was carried out using the glucose (G), fructose (F), sucrose (S), xylose (X) sugar contents and the fruit color of flesh (excluding core) (FCF), fruit color of core (FCC), lightness value of fruit (LVF), chroma value of fruit (CVF), hue value of fruit (HVF), lightness value of flesh color (LVFC), hue value of flesh color (HVFC), chroma value of flesh color (CVFC) fruit color parameters. The positive and negative correlation coefficients were recorded in Supplementary Table 2. The highest correlation was positively released as $r = 0.800^{***}$ between LVF and CVF colorimetric properties generated from 2022 measurements. The lowest correlation was exhibited as $r = -0.152^{*}$ from pair traits of HVFC_21 and FCF_22 negatively. On the other hand, the correlation was performed among the sugar components and glucose, fructose and xylose have significant correlation each other in both year measurement values. In addition, sucrose has only glucose and fructose values of 2022. The strongest correlation was detected among the glucose and fructose in 2022 with $r = 0.981^{***}$ while lowest correlation was identified as $r = -0.152^{*}$ between xylose and glucose in 2021. In addition, observations for fruits were based on flesh and core of the fruits. In general, these observations were negatively set up a correlation with measurements of lightness, chroma and hue values of fruits. Positive correlation was identified between chroma value of the flesh color and fruit color of flesh (excluding core) and fruit color of core (Fig. 4).

Identification of the QTLs

*Sucrose*

In the 'Fortuna × Rubygem' population, several linked loci associated with sugar compounds such as sucrose and xylose contents were identified in Rubygem and Fortuna linkage maps. The major QTL related to sucrose content was detected on LG31 in 2021 and 2022 consecutive years in only Rubygem male linkage group. The generated linked locus Ax-123359583 was detected in both years on LG31 ranging from 13.02 to 16.68 cM and the explained phenotypic variance (PV) value was calculated as 14.1% and 18.7% in 2021 and 2022, respectively. And, LOD score of this locus was identified as 4.60 and 7.16 in both years. Furthermore, the closest locus Ax-184233903 overlapped with Ax-123359583 on 13.02 and 14.24 cM in 2022. It has 17.6% explained phenotypic variance (PV), and the LOD value was

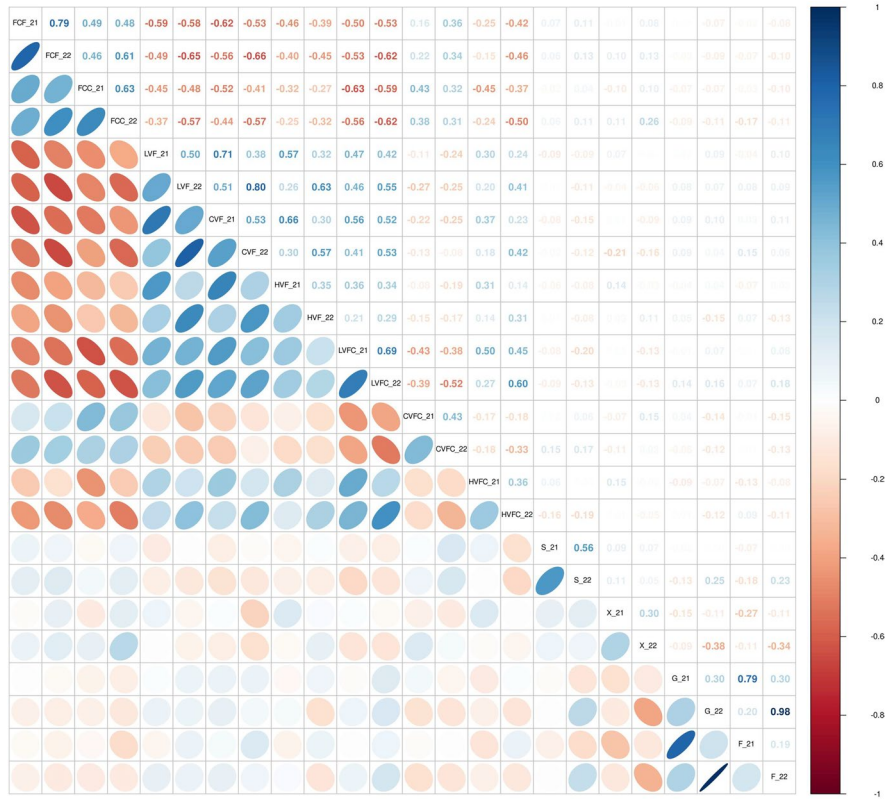



**Fig. 4** Correlation heatmap of the sugar components and colorimetric traits detected in 2021 and 2022. Abbreviations of traits were given as the glucose (G), fructose (F), sucrose (S), xylose (X) sugar contents and the fruit color of flesh (excluding core) (FCF), fruit color of core (FCC), lightness value of fruit (LVF), chroma value of fruit (CVF), hue value of fruit (HVF), lightness value of flesh color (LVFC), hue value of flesh color (HVFC), chroma value of flesh color (CVFC) fruit color parameters with identification years (21 and 22)

6.00. The significance of all linked loci ranged from < 0.001 and 0.009 (*p*) (Fig. 5, Table 1).

*Xylose*

The associated loci with xylose were pointed at the beginning of the linkage group 20 varied of 3.11 and 5.52 cM in the Fortuna genetic map. A similar position of the LG20 was found for the Rubygem linkage group, and the upper tip of the LG20 was associated with xylose around 15.03 cM. The Ax-184476583 was the highest linked locus in both seasons for xylose content in Fortuna and Rubygem maps (Fig. 5, Table 1).

Fruit color of flesh (excluding core)

The QTLs related to fruit flesh color were detected in LG5 and LG16 in the Fortuna genetic map, while the linked genomic position of the Rubygem map was in LG16. In 2022, the second year of growth, the PV value of the Ax-184320629 locus was identified as 19.7% in LG 16, and the LOD score was 6.4 in both parents. Another major controlling genomic fragment in LG16 was placed on the Rubygem map with a PV value of 25.4% and an LOD score of 10.1 which was the Ax-166507450 locus located at 38.4 cM (Fig. 5, Table 2).

Fruit color of core

The main correlated mutations into QTL regions related to fruit color of core were found in both parent maps, and LG16 represented both growing seasons. In the Fortuna genetic map, the Ax-184320629 was the closest marker in two consecutive years. The responsible position ranges were calculated from the beginning of LG16 to 1.23 cM. In Rubygem, the Ax-184320629 was also found as the closest marker in both productive seasons. The length of the controlling genomic intervals was defined between 41.39 and 45.57 cM. Furthermore, the other associated loci of both years were Ax-166509944 and Ax-166524983, respectively. Their phenotyping variances represented 17.8% and 16.4% of the total phenotypic variance

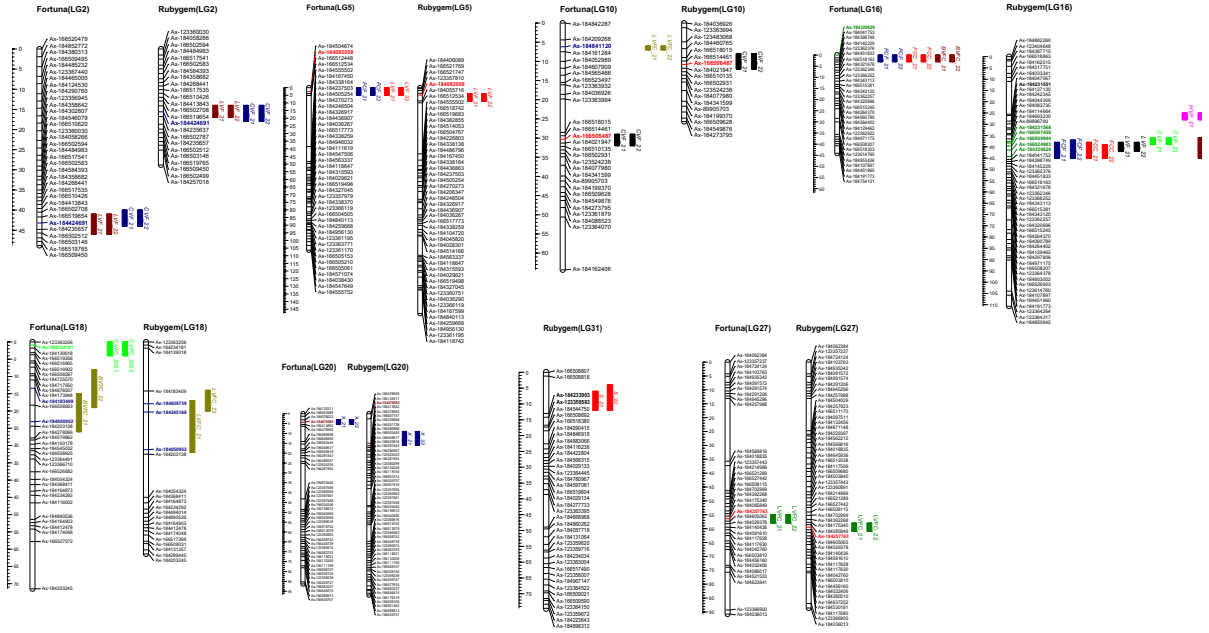



**Fig. 5** QTL regions onto linkage groups for each trait, bold and colored marker names refer to the highest linked loci and their positions in linkage groups

**Table 1** List of the highest linked QTLs and loci into for sucrose and xylose traits belonging to 2021 and 2022 production years

| Trait | Years | LGs | QTL range(cM) | LOD | PV (%) | Loci | Position (cM) | Significance (p) | Chrs | Positions |
|---|---|---|---|---|---|---|---|---|---|---|
| Sucrose | Rubygem | | | | | | | | | |
| | 2021 | 31 | 13.02–16.68 | 4.60 | 14.1 | Ax-123359583 | 14.24 | < 0.001 | Fvb7 | 21,148,506 |
| | 2022 | 31 | 1.22–14.24 | 6.00 | 17.6 | Ax-184233903 | 13.02 | < 0.001 | Fvb7-3 | 3,683,105 |
| | 2022 | 31 | 13.02–16.68 | 7.16 | 18.7 | Ax-123359583 | 14.24 | < 0.001 | Fvb7 | 21,148,506 |
| Xylose | Fortuna | | | | | | | | | |
| | 2021 | 20 | 3.11–5.52 | 2.20 | 7.1 | Ax-184476583 | 4.28 | 0.009 | Fvb2-3 | 5,196,664 |
| | 2022 | 20 | 3.11–5.52 | 5.24 | 13.3 | Ax-184476583 | 4.28 | < 0.001 | Fvb2-3 | 5,196,664 |
| | Rubygem | | | | | | | | | |
| | 2021 | 20 | 2.63–16.26 | 2.20 | 7.1 | Ax-184476583 | 15.03 | 0.009 | Fvb2-3 | 5,196,664 |
| | 2022 | 20 | 2.63–16.26 | 5.24 | 13.3 | Ax-184476583 | 15.03 | < 0.001 | Fvb2-3 | 5,196,664 |

(PV) and LOD scores were 6.65 and 6.20 in the 2021 and 2022 seasons, respectively (Fig. 5, Table 2).

## Lightness value of fruit

The lightness value of the fruit is one of the scales of the expression on the surface of the fruit. The highest association related to the lightness value of fruit was calculated in LG2, and it's the phenotypic variance (PV) value of the Ax-1844424691 was 12.9%, and the LOD was 4.2 in both parental maps. This locus, which was observed at 42.23 cM in the Fortuna maternal map, was found at 15.64 cM in the Rubygem paternal map. Another associated (Ax-184082058) locus was observed in LG5 in both maps, and the rates of the PV of the population in this trait were 4.9% and 9.1% in 2021 and 2022, respectively. In the Rubygem, one more linked marker, Ax-166509944, was detected in

**Table 2** List of the highest associated loci into QTL regions for fruit color of flesh (excluding core) and fruit color of core traits belonging to 2021 and 2022 production years

| Trait | Years | LGs | QTL range(cM) | LOD | PV (%) | Loci | Position (cM) | Significance (p) | Chrs | Positions |
|---|---|---|---|---|---|---|---|---|---|---|
| Fruit color of flesh (excluding core) | Fortuna | | | | | | | | | |
| | 2021 | 5 | 0–3.88 | 2.60 | 7.5 | Ax-184082058 | 2.63 | 0.001 | Fvb3-3 | 27,841,572 |
| | 2022 | 5 | 0–3.88 | 2.00 | 5.6 | Ax-184082058 | 2.63 | 0.006 | Fvb3-3 | 27,841,572 |
| | 2021 | 16 | 0–1.23 | 3.20 | 11.1 | Ax-184320629 | 0 | 0.001 | Fvb6-1 | 18,898,605 |
| | 2022 | 16 | 0–1.23 | 6.40 | 19.7 | Ax-184320629 | 0 | < 0.001 | Fvb6-1 | 18,898,605 |
| | Rubygem | | | | | | | | | |
| | 2021 | 16 | 38.34–41.39 | 5.70 | 15.5 | Ax-166509944 | 39.56 | < 0.001 | Fvb6 | 23,594,387 |
| | 2021 | 16 | 37.73–39.56 | 6.00 | 16.3 | Ax-166507450 | 38.34 | < 0.001 | Fvb6 | 24,255,999 |
| | 2021 | 16 | 41.39–45.57 | 3.20 | 11.1 | Ax-184320629 | 44.34 | 0.001 | Fvb6-1 | 18,898,605 |
| | 2022 | 16 | 37.73–39.56 | 10.10 | 25.4 | Ax-166507450 | 38.34 | < 0.001 | Fvb6 | 24,255,999 |
| | 2022 | 16 | 41.39–45.57 | 6.40 | 19.7 | Ax-184320629 | 44.34 | < 0.001 | Fvb6-1 | 18,898,605 |
| Fruit color of core | Fortuna | | | | | | | | | |
| | 2021 | 16 | 0–1.23 | 2.33 | 8.1 | Ax-184320629 | 0 | 0.005 | Fvb6-1 | 18,898,605 |
| | 2022 | 16 | 0–1.23 | 2.00 | 6.9 | Ax-184320629 | 0 | 0.008 | Fvb6-1 | 18,898,605 |
| | Rubygem | | | | | | | | | |
| | 2021 | 16 | 41.39–45.57 | 2.33 | 8.1 | Ax-184320629 | 44.34 | 0.005 | Fvb6-1 | 18,898,605 |
| | 2021 | 16 | 38.34–41.39 | 6.65 | 17.8 | Ax-166509944 | 39.56 | < 0.001 | Fvb6 | 23,594,387 |
| | 2022 | 16 | 39.56–44.34 | 6.20 | 16.4 | Ax-166524983 | 41.39 | < 0.001 | Fvb6 | 23,019,961 |
| | 2022 | 16 | 41.39–45.57 | 2.00 | 6.9 | Ax-184320629 | 44.34 | 0.008 | Fvb6-1 | 18,898,605 |

LG16, and the corresponding genomic region was defined in the range of 38.34 and 41.39 cM (Fig. 5, Table 3).

Chroma value of fruit

All the F1 progenies were characterized to reveal genetic variability of the 'Fortuna x Rubygem' population for the chroma degrees. The chroma refers to the saturation of the color of strawberry fruit skin. And, the responsible fragments of the strawberry genome in QTL analysis were pointed out in LG2 and LG10 of the Fortuna linkage map. The identified locus in LG2 in both growing seasons was Ax-184424691 which was positioned at 42.23 cM in Fortuna, while this marker was located at 15.64 cM in Rubygem. Ax-166506487 locus was detected at LG10 on both maps, whereas these markers were placed at the different positions of the LG10 in the Fortuna and Rubygem linkage groups. Additionally, a few linked markers were found in only LG16 of the Rubygem map, and detected QTL in LG16 had a major effect due to its higher phenotypic variance of value (26.1%). The LOD value of Ax-184231568 was 10.5, and the range of the responsibility was between 36.51 to 38.34 cM (Fig. 5, Table 3).

Hue value of fruit

QTL was detected in LG16 only Rubygem map, and the closest marker was associated with Ax-184231551 in both trial seasons. The range of the linkage group was calculated between 25.33 and 28.58 cM. The phenotypic variance (PV) value of Ax-184231551 was explained as 11.4% and 16.6%, and the LOD values of this marker were 4.10 and 6.30 in 2021 and 2022, respectively (Fig. 5, Table 3).

Lightness value of flesh color

QTL regions were determined in LG16, LG18, and LG27 in Fortuna and Rubygem genetic maps. The responsible position of the lightness value of flesh color in both linkage groups was overlapped genomic fragments in LG18 and LG27. In Fortuna, the highest LOD score in linked all linkage groups was observed as 3.52 in LG27 with a 10% PV value. The major QTL ranging from 36.51 to 45.57 cM was detected in LG16 of Rubygem map, although the majority in LG16 was calculated at 1.23 cM. The closest marker, Ax-166507450, explained the phenotypic variance (PV), and the LOD score in LG16 of Rubygem map was 25.3% and 9.7, respectively (Fig. 5, Table 4).

Hue value of flesh color

In the 2021 and 2022 growing seasons, the two closest loci related to the hue value of fruit flesh color were observed in LG16, in only Rubygem. The phenotypic variance value of Ax-166507450 was explained with 7.0%, while its LOD score was 2.4, and its position on the map was determined at 38.34 cM. The other linked locus was Ax-166509944 which had a 10.1% phenotypic variance value. Additionally, it produced a 3.7 LOD score and was positioned at 39.56 cM on the Rubgem map (Fig. 5, Table 4).

Chroma value of flesh color

This is one of the color parameters, the chroma value of flesh color (C) was associated with at the beginning of the LG18 corresponding to HG VII and the fourth homoeologous linkage. The highest LOD score belonging to Ax-184234181 was recognized as 4.4 and its phenotypic explained variation (%) was noted as 11.3 (%) as a major QTL region linked to this trait in strawberry (Fig. 5, Table 4).

Distribution of the homoeologous QTLs

The highest associated markers in whole properties were classified according to Fortuna and Rubygem parents with common markers. For instance; a marker linked to both traits or more was listed for each parent in detail in Table 5. The density of the markers for color-related markers was carried out in HG I, III, VI, VII. However, two more linkage groups in Fortuna than Rubygem were identified that supply the inheritance from Fortuna such as LG5 and LG18 associated with lightness and chroma values in fruit and flesh color, respectively. The common controlling genomic regions linked to color traits were mainly gathered in LG2 and LG16, corresponding to HG I and HG VI in Fana_SNP and iStraw35 arrays in both parents.

**Table 3** The highest associated loci into QTL regions for lightness value of fruit (L), chroma value of fruit (C) and hue° value of fruit in 2021 and 2022 years

| Trait | Years | LGs | QTL range (cM) | LOD | PV (%) | Loci | Position (cM) | Significance (p) | Chrs | Positions |
|---|---|---|---|---|---|---|---|---|---|---|
| Lightness value of fruit (L) | Fortuna | | | | | | | | | |
| | 2021 | 2 | 41.70–44.77 | 4.20 | 12.9 | Ax-184424691 | 43.23 | < 0.001 | Fvb1-4 | 16,449,267 |
| | 2022 | 2 | 41.70–44.77 | 3.40 | 10.4 | Ax-184424691 | 43.23 | 0.001 | Fvb1-4 | 16,449,267 |
| | 2021 | 5 | 0–3.88 | 1.70 | 4.9 | Ax-184082058 | 2.63 | 0.008 | Fvb3-3 | 27,841,572 |
| | 2022 | 5 | 0–3.88 | 3.30 | 9.1 | Ax-184082058 | 2.63 | < 0.001 | Fvb3-3 | 27,841,572 |
| | Rubygem | | | | | | | | | |
| | 2021 | 2 | 14.10–16.84 | 4.20 | 12.9 | Ax-184424691 | 15.64 | < 0.001 | Fvb1-4 | 16,449,267 |
| | 2022 | 2 | 14.10–16.84 | 3.40 | 10.4 | Ax-184424691 | 15.64 | 0.001 | Fvb1-4 | 16,449,267 |
| | 2021 | 5 | 3.66–8.67 | 1.70 | 4.9 | Ax-184082058 | 4.91 | 0.008 | Fvb3-3 | 27,841,572 |
| | 2022 | 5 | 3.66–8.67 | 3.30 | 9.1 | Ax-184082058 | 4.91 | < 0.001 | Fvb3-3 | 27,841,572 |
| | 2021 | 16 | 38.34–41.39 | 2.80 | 8.0 | Ax-166509944 | 39.56 | 0.006 | Fvb6 | 23,594,387 |
| | 2022 | 16 | 38.34–41.39 | 4.40 | 12.3 | Ax-166509944 | 39.56 | < 0.001 | Fvb6 | 23,594,387 |
| Chroma value of fruit (C) | Fortuna | | | | | | | | | |
| | 2021 | 2 | 41.70–44.77 | 3.20 | 10.1 | Ax-184424691 | 43.23 | 0.001 | Fvb1-4 | 16,449,267 |
| | 2022 | 2 | 41.70–44.77 | 2.90 | 9.1 | Ax-184424691 | 43.23 | 0.001 | Fvb1-4 | 16,449,267 |
| | 2021 | 10 | 29.41–31.25 | 2.14 | 5.9 | Ax-166506487 | 30.33 | 0.009 | Fvb5 | 19,945,982 |
| | 2022 | 10 | 29.41–31.25 | 2.20 | 5.8 | Ax-166506487 | 30.33 | 0.009 | Fvb5 | 19,945,982 |
| | Rubygem | | | | | | | | | |
| | 2021 | 2 | 14.10–16.84 | 3.20 | 10.1 | Ax-184424691 | 15.64 | 0.001 | Fvb1-4 | 16,449,267 |
| | 2022 | 2 | 14.10–16.84 | 2.90 | 9.1 | Ax-184424691 | 15.64 | 0.001 | Fvb1-4 | 16,449,267 |
| | 2021 | 10 | 9.92–11.76 | 2.14 | 5.9 | Ax-166506487 | 10.84 | 0.009 | Fvb5 | 19,945,982 |
| | 2022 | 10 | 9.92–11.76 | 2.20 | 5.8 | Ax-166506487 | 10.84 | 0.009 | Fvb5 | 19,945,982 |
| | 2021 | 16 | 36.51–38.34 | 5.10 | 14.2 | Ax-184231568 | 37.73 | < 0.001 | Fvb6-1 | 14,317,608 |
| | 2021 | 16 | 37.73–39.56 | 8.00 | 21.1 | Ax-166507450 | 38.34 | < 0.001 | Fvb6 | 24,255,999 |
| | 2022 | 16 | 36.51–38.34 | 5.90 | 15.6 | Ax-184231568 | 37.73 | < 0.001 | Fvb6-1 | 14,317,608 |
| | 2022 | 16 | 36.51–38.34 | 10.50 | 26.1 | Ax-184231568 | 37.73 | < 0.001 | Fvb6-1 | 14,317,608 |
| Hue° value of fruit | Rubygem | | | | | | | | | |
| | 2021 | 16 | 25.53–28.58 | 4.10 | 11.4 | Ax-184231551 | 26.14 | 0.001 | Fvb6-1 | 13,290,467 |
| | 2022 | 16 | 25.53–28.58 | 6.30 | 16.6 | Ax-184231551 | 26.14 | < 0.001 | Fvb6-1 | 13,290,467 |

**Table 4** QTL regions with the highest linked loci for lightness value of flesh color (L), hue° value of flesh color and chroma value of flesh color (C) in both production seasons

| Trait | Years | LGs | QTL range (cM) | LOD | PV (%) | Loci | Position (cM) | Significance (p) | Chrs | Positions |
|---|---|---|---|---|---|---|---|---|---|---|
| Lightness value of flesh color (L) | Fortuna | | | | | | | | | |
| | 2021 | 10 | 4.39–7.48 | 2.00 | 5.9 | Ax-184841120 | 6.24 | 0.013 | Fvb5-1 | 12,411,623 |
| | 2022 | 10 | 4.39–7.48 | 2.90 | 8.1 | Ax-184841120 | 6.24 | 0.002 | Fvb5-1 | 12,411,623 |
| | 2021 | 16 | 0–1.23 | 2.30 | 8.8 | Ax-184320629 | 0 | 0.007 | Fvb6-1 | 18,898,605 |
| | 2022 | 16 | 0–1.23 | 3.03 | 10.0 | Ax-184320629 | 0 | 0.002 | Fvb6-1 | 18,898,605 |
| | 2021 | 18 | 18.34–24.35 | 2.40 | 7.1 | Ax-184050952 | 23.11 | 0.005 | Fvb7-4 | 8,963,592 |
| | 2022 | 18 | 11.00–18.34 | 2.16 | 6.4 | Ax-184183409 | 13.44 | 0.01 | Fvb7-4 | 5,931,753 |
| | 2021 | 27 | 55.47–57.32 | 3.52 | 10.0 | Ax-184257763 | 56.39 | < 0.001 | Fvb2-1 | 1,325,436 |
| | 2022 | 27 | 55.47–57.32 | 3.31 | 9.7 | Ax-184257763 | 56.39 | < 0.001 | Fvb2-1 | 1,325,436 |
| | Rubygem | | | | | | | | | |
| | 2021 | 16 | 36.51–38.34 | 6.30 | 17.2 | Ax-184231568 | 37.73 | < 0.001 | Fvb6-1 | 14,317,608 |
| | 2021 | 16 | 37.73–39.56 | 9.70 | 25.3 | Ax-166507450 | 38.34 | < 0.001 | Fvb6 | 24,255,999 |
| | 2021 | 16 | 41.39–45.57 | 2.30 | 8.8 | Ax-184320629 | 44.34 | 0.007 | Fvb6-1 | 18,898,605 |
| | 2022 | 16 | 36.51–38.34 | 4.40 | 12.0 | Ax-184231568 | 37.73 | < 0.001 | Fvb6-1 | 14,317,608 |
| | 2022 | 16 | 38.34–41.39 | 9.50 | 23.8 | Ax-166509944 | 39.56 | < 0.001 | Fvb6 | 23,594,387 |
| | 2022 | 16 | 41.39–45.57 | 3.03 | 10.0 | Ax-184320629 | 44.34 | 0.002 | Fvb6-1 | 18,898,605 |
| | 2021 | 18 | 17.90–31.22 | 2.35 | 6.7 | Ax-184245168 | 20.34 | 0.004 | Fvb7-4 | 7,488,759 |
| | 2021 | 18 | 20.34–32.46 | 2.40 | 7.1 | Ax-184050952 | 31.22 | 0.005 | Fvb7-4 | 8,963,592 |
| | 2022 | 18 | 14.23–20.34 | 2.00 | 5.6 | Ax-184639739 | 17.9 | 0.014 | Fvb7-4 | 7,384,453 |
| | 2021 | 27 | 58.58–60.43 | 3.52 | 10.0 | Ax-184257763 | 59.5 | < 0.001 | Fvb2-1 | 1,325,436 |
| | 2022 | 27 | 58.58–60.43 | 3.31 | 9.7 | Ax-184257763 | 59.5 | < 0.001 | Fvb2-1 | 1,325,436 |
| Hue° value of flesh color | Rubygem | | | | | | | | | |
| | 2021 | 16 | 37.73–39.56 | 2.40 | 7.0 | Ax-166507450 | 38.34 | 0.013 | Fvb6 | 24,255,999 |
| | 2022 | 16 | 38.34–41.39 | 3.70 | 10.1 | Ax-166509944 | 39.56 | < 0.001 | Fvb6 | 23,594,387 |
| Chroma value of flesh color (C) | Fortuna | | | | | | | | | |
| | 2021 | 18 | 0–1.84 | 4.40 | 11.3 | Ax-184234181 | 0.92 | < 0.001 | Fvb7-4 | 2,990,829 |
| | 2022 | 18 | 1.84–3.68 | 1.92 | 5.5 | Ax-166519356 | 3.07 | 0.018 | Fvb7 | 19,919,443 |
| | 2022 | 18 | 0.92–3.07 | 1.80 | 4.9 | Ax-184139018 | 1.84 | 0.003 | Fvb7-4 | 3,230,802 |

**Table 5** The summary of association between traits and linked loci in all traits of '*Fortuna* × *Rubygem*' F1 population

| Traits | LGs | Loci | Chrs | Positions | Panels |
|---|---|---|---|---|---|
| Fortuna | | | | | |
| Lightness value of fruit (L) | 2 | Ax-184424691 | Fvb1-4 | 16,449,267 | Fana_SNP |
| Chroma value of fruit (C) | | | | | |
| Fruit color of flesh (excluding core) | 5 | Ax-184082058 | Fvb3-3 | 27,841,572 | |
| Lightness value of fruit (L) | | | | | |
| Fruit color of flesh (excluding core) | 16 | Ax-184320629 | Fvb6-1 | 18,898,605 | |
| Fruit color of core | | | | | |
| Lightness value of flesh color (L) | | | | | |
| Lightness value of flesh color (L) | 18 | Ax-184183409 | Fvb7-4 | 5,931,753 | |
| Chroma value of flesh color (C) | | | | | |
| Rubygem | | | | | |
| Lightness value of fruit (L) | 2 | Ax-184424691 | Fvb1-4 | 16,449,267 | Fana_SNP |
| Chroma value of fruit (C) | | | | | |
| Fruit color of flesh (excluding core) | 16 | Ax-166507450 | Fvb6 | 24,255,999 | iStraw35 |
| Lightness value of flesh color (L) | | | | | |
| Hue° value of flesh color (H) | | | | | |
| Chroma value of fruit (C) | | | | | |
| Fruit color of flesh (excluding core) | 16 | Ax-166509944 | Fvb6 | 23,594,387 | |
| Fruit color of core | | | | | |
| Lightness value of fruit (L) | | | | | |
| Lightness value of flesh color (L) | | | | | |
| Hue° value of flesh color (H) | | | | | |

Development of the linked markers

The markers that can be used for marker-assisted selection (MAS) for future strawberry breeding programs were tested in extreme phenotypic individuals by HRM analysis. The promising three markers for MAS were identified in xylose, lightness value of flesh color, and chroma value of flesh color (Fig. 6A). The putative marker for xylose was placed in the third homoeologous of HG II, while the other superior markers linked to lightness and chroma values of flesh color were mined from HG V-a and HG VII-d, respectively. These markers have the highest potential for MAS, however, they should be tested in distinct segregated populations and domestic or commercial genotypes for these traits.

Genetic effects of the single nucleotide polymorphisms (SNPs) over the highly associated traits were identified to reveal the responsible allele types. Box plots of the heterozygous and homozygous genotypes with average phenotypes were drawn and given Fig. 6B. For average xylose values in 2021 and 2022, genetic effect of Ax-184476583 SNP explained 7.1% and 13.3% of the total phenotypic variance when considering heritability. This polymorphism that created by major QTL demonstrated an underdominance effect where the homozygous genotype (AA) had higher xylose content than either of the heterozygous (AG) and homozygous (GG) genotypes. The strongest linked marker for lightness value of flesh color (Ax-184841120) exhibited 5.9% and 8.1% of the total phenotypic variance, and heterozygous genotypes (CT) had higher LVFC values. The lowest chroma value of flesh color was inherited from homozygous (CC) genotypes. This polymorphism was detected in Ax-184139018 which has 4.9% phenotypic variance of total phenotypic variance.

## Discussion

In this paper, the recent aim of the strawberry breeding programs is to improve the taste of maintaining current yields. The key target traits are sugar content and fruit acidity, assessed through SSC (soluble solids content) and TA (titratable acidity) in breeding

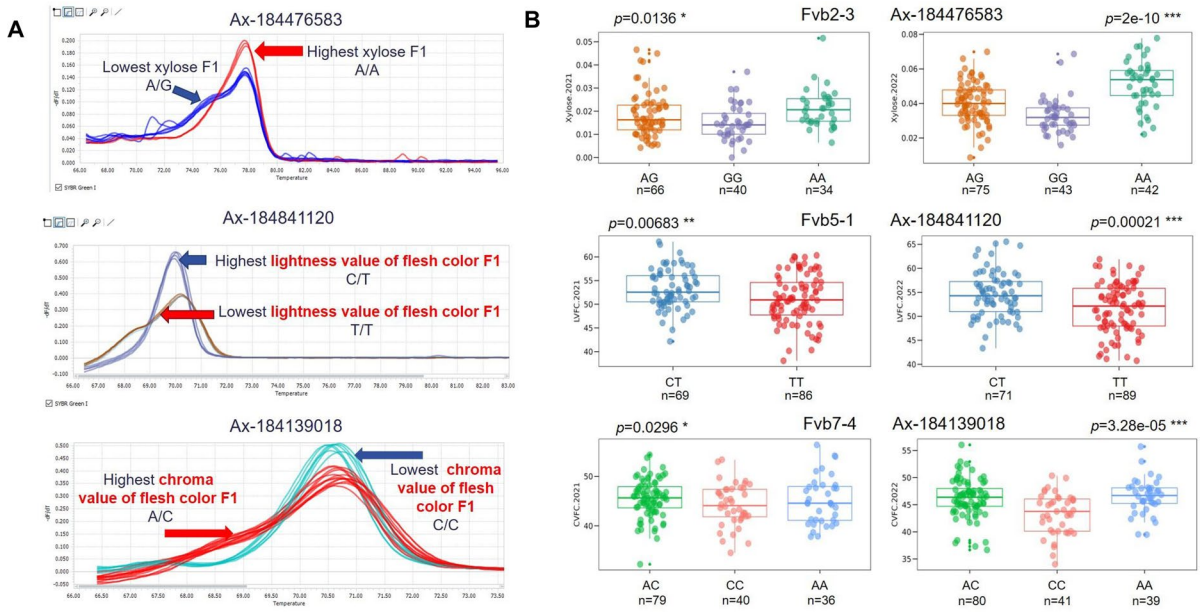



**Fig. 6** **A** HRM curves of the highest linked loci for xylose, lightness value of flesh color (L) and chroma value of flesh color (C), **B** Genetic effects of the SNPs with segregation alleles and the number of homozygous/ heterozygous genotypes (n) in xylose, lightness value of flesh color and chroma value of flesh color in 2021 and 2022 phenotyping $*p<0.05$, $**p<0.01$, $***p<0.001$. 'Fvb' and first number next to it refers to chromosome number, the number after hyphen refers to subgenome of related chromosome

programs. These traits exhibit variable heritability, are influenced by environmental factors, and involve genotype-by-environment interactions across various fruit crops, complicating efforts to enhance taste. To address these challenges, the study emphasizes the importance of understanding the genetic control of fruit quality traits. This knowledge is crucial for identifying specific compounds that influence sugar content and some quality traits, aiding in the selection of markers for breeding novel high-quality strawberry cultivars. The research identifies many QTLs associated with sucrose and quality traits, such as colors in ripe strawberry fruit. Overall, these findings contribute to the understanding of the genetic basis of strawberry taste and offer insights into potential markers for developing improved strawberry cultivars.

Sweetness perception in fresh fruits is highly correlated to the major sugar content being glucose, fructose, and sucrose compounds. Metabolic traits have been studied using this approach, including the identification of QTL for volatile organic compounds, glucose, fructose, sucrose, malic, citric, and L-ascorbic acids (Zorrilla-Fontanesi et al. 2011; Lerceteau-Köhler et al. 2012; Vallarino et al. 2019). Most of the attempts were carried out using previously developed SSRs, ALFP, and SSCP markers, and the association was supplied with polymorphic markers (Lerceteau-Köhler et al. 2003; Shulaev et al. 2011). A major QTL for sucrose with 22–30% of the phenotypic variation was identified in both consecutive years on the fourth homoeologous of LG5 using the EST-SSR markers (Wada et al. 2020). The highest significant association with sucrose was identified using the MAGIC population by genome-wide association studies (GWAS). In total, four QTLs were linked to sucrose content which has LODs ranging from 4.63 to 7.64 in strawberry, no association was recorded in glucose and fructose (Wada et al. 2020). Lerceteau-Köhler et al. (2012) have detected metabolite QTLs linked by glucose, sucrose, and fructose which are located to homoeologous linkage groups of the octoploid population using Capitola cultivar and CF1116 breeding line derived from the Pajaro x (Earlyglow x Chandler) map previously developed by Rousseau-Gueutin et al. (2008). One of the compounds of the sugars, sucrose-related loci was

recognized as the first homoeologous of the LG6 (only one year) and LG7 (in both years) in CF1116 and Capitola maps, respectively. Vallarino et al. (2019) identified the QTL regions linked to primary metabolites, L-ascorbic acid (L-AA) and other related traits such as soluble solid content (SSC), titratable acidity (TA), pH, raffinose, sucrose, xylose, succinic acid, and L-AA using bi-parental population, 232 (Selection 4–43 × Vilanova) and 1392 (Gaviota x Camarosa). The majority of the sugar-related genomic positions were HG V-d in successive years, however, minor effects on the other HG were observed in the sucrose and xylose compounds. In the present report, we dissected the QTLs controlling sucrose and xylose compounds using genetic variability of the Fortuna and Rubygem strawberry cultivars. The regions of the QTLs pointed within LG31 corresponding to HG VII in *F. vesca* and HG VII-c in *F.* x *ananassa* linked to sucrose were identified, and QTLs for xylose were significantly correlated to the tip of HG II-c in successive years. Thus, the differentiation of the controlling fragments of the sucrose and xylose compounds has been expressed that they have different genetic backgrounds according to metabolite traits, although they have originated from similar locations in the United States. For instance; the plant material of the current paper, Fortuna was recognized from the US in 1961, while Rubygem has derived from Earlibrite (Chandler et al. 2000) and Carlsbad (Voth et al. 1994) cvs., Earlibrite consisted of a dozen crosses from Earlibelle cv.. On the other hand, Capitola cv. used by Lerceteau-Köhler et al. (2012) improved from teams of the CF1116 breeding line derived from Pajaro x (Earlyglow x Chandler), these have developed in the US in 1979, 1975 and 1983, respectively (Zurn et al. 2022). In similar, 232 (Selection 4–43 × Vilanova) and 1392 (Gaviota x Camarosa) breeding lines, plant materials of Vallarino et al. (2019), pedigrees were from the US (Gaviota and Camarosa were released in California) and Spain (Vilanova was developed by I.V.I.A in 1990). That's why, the responses across QTL studies carried out over the families derived from different parents sampled from closely common locations have distinct metabolite degrees. And, the simple expression of why chosen of those cultivars as parents was that they have extreme concentrations or phenotypes such as fruit size, high sugar, acid, anthocyanins, and L-ascorbic properties (Lerceteau-Köhler et al. 2012; Castro and Lewers 2016; Verma et al. 2017; Vallarino et al. 2019). Genetic dissection of the US national strawberry germplasm originated from mainly the US, Europe, Asia, and Africa, a total of 539 plants including the mentioned cultivars were separated from each other clearly in genetic structure analysis due to the heterozygous nature of the strawberry, with cultivars used in QTL findings (Zurn et al. 2022).

Attempts to understand the mechanisms linked to fruit color were carried out, however, there were no directly responsible genes for color parameters in strawberries. These parameters L, a, and b values were utilized for the identification of the QTLs with genomic locations performed by Lerceteau-Köhler et al. (2012), Wada et al. (2020) and Alarfaj et al. (2021). QTLs associated with the lightness of the fruit surface and flesh color were mostly distributed to only one of the four homoeologous linkage groups (Lerceteau-Köhler et al. 2012). However, the majority of the homoeo-QTLs linked to fruit color-related traits were on the HG I, III, and VI. On the other hand, mapping of the QTLs for parameter L consisted of the five genomic regions corresponding to HG III-b,-c, HG VI-c, and HG VII-a,-b (Alarfaj et al. 2021). Thus, QTLs for the lightness value of fruit (L) were mainly correlated with HG I-d, HG III-c HG VI, although QTLs for the lightness value of flesh color (L) were mapped to both parents located on HG II-a, HG VI, VI-a and HG VII-d. The common controlling intervals over the similar homoeologous groups were detected in both reports, therefore, the major QTLs covered the matched linkage groups.

A crucial key point of the fruit color is a main messenger for anthocyanin and nutritional value and that assumes a role related to attractive and fruit quality features nutritionally for strawberries (Denoyes et al. 2023). The story of the anthocyanin synthesis is expressed with the correlation between intensity, hue, and chroma evaluations on the strawberry fruits. The main and most abundant anthocyanin element is pelargonidin 3-glucoside, and the minor one is cyanidin 3-glucoside (Aaby et al. 2012). Anthocyanin and its relative compounds are colored pigments, that affect fruit color. Derived from anthocyanin, pelargonidin supplies the lightness by carrying out during synthesis (Fischer et al. 2014). The responsible anthocyanin gene MYB has joined to form flavonoid biosynthesis, and it plays an important role in the stage of the regulation of the anthocyanin in strawberry (Schaart et al. 2013). In addition, the controlling regions of the MYB genes

in *F.* x *ananassa* genome 'Wongyo 3115' and 'Camarosa' reference genome positions in Chr1 (Manivannan et al. 2021) were quite close fragments according to our QTL results about lightness and chroma value of fruits. Thus, there may be a strong linkage between associated markers and around the MYB genes within Chr1 that the synthesis of the anthocyanin in strawberries.

Our results revealed the first QTL intervals associated with the hue and chroma values of strawberry fruits. The main controlling areas of QTLs were mainly focused on the HG VI with HG I-d homoeologous genomes. The validated three highest linked SNP markers associated with xylose, the lightness value of flesh color, and chroma value of flesh color have quite potential to assess in the breeding programs for marker-assisted selection in strawberries.

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**Author contributions** EK and SK conceived and designed the experiments. DAS carried out crossing to consist of the population. IÜ and HT extracted the DNAs of the population and its parents. IÜ, HK, ŞHA, AEG and BO performed most of the experiments and HK analyzed the data and carried out mapping. IÜ and HK wrote the draft manuscript. The authors read and approved the final manuscript.

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**Data availability** No datasets were generated or analysed during the current study.

**Declarations**

**Conflict of interest** The authors declare no competing interests.

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