High-Density SNP Linkage Map Construction and QTL Analysis of Firmness in Octoploid Strawberry (*Fragaria* × *ananassa*)[W사1]

JUN-GEOL YEUM

DEPARTMENT OF PLANT SCIENCE
THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

ABSRACT

The allo-octoploid cultivated strawberry (*Fragaria x anananssa Duchesne*) has been produced around the world because of its pleasant flavor and health benefitting properties. There have been many obstacles in molecular breeding of strawberry due to the extremely complex nature of its genome composed of four relatively similar sub-genomic chromosome set. However, with recent advances in sequencing and scaffolding technology, chromosome-scale octoploid genome information is now available. In addition, with the development of various molecular markers, it is possible to construct high-density linkage maps that can be useful for molecular breeding. In this study, we constructed a high-density bin map using IStraw90

Axiom® SNP array and genotyping-by-sequencing (GBS)-based markers, and an F2 populations derived from inbred lines. As a result, a high-density linkage bin map of 3,974.6 cM in length was constructed, consisting of 1,245 bins and 33 linkage groups, covering 87.7% of the total physical length of the octoploid genome. This high-density linkage map was used to improve the quality of the octoploid reference genome, 'Camarosa'. The chromosomes 1-2, 2-1, 6-2, and 6-4 of 'Camarosa', which has scaffolding errors in large scale, were reassembled based on the linkage map constructed in this study. Furthermore, this high-density linkage map was used to analyze QTL for firmness, one of the traits that is important for the quality of strawberry fruits. Among the nine QTL for firmness detected in 'L80' linkage bin map, QTL 'qFaFL7' containing the firmness-related genes on chromosome 6-3b was the major QTL explaining 26% of phenotypic value with LOD value of 6. On the other hand, in the 'T137' linkage bin map, five QTL were detected, but the LOD and phenotypic value was too low to be considered major QTL. In[o2][o3] summary, the high-density linkage maps, 'L80' constructed in this study will be a framework for molecular breeding of agriculturally important traits in strawberry.

Keywords: Allo-octoploid strawberry, IStraw90 Axiom SNP array, genotyping-by-sequencing (GBS), reference genome, high-density linkage map, fruit firmness

Student number: 2018-23834

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LIST OF ABBREVIATIONS

GBS	Genotyping-by-sequencing
SNP	Single nucleotide polymorphism
cM	Centi Morgan (the unit of genetic distance)
LOD	Logarithm of the odds
CTAB	Cetyl trimethy lammonium bromide
BWA	Burrow wheelers alignment
SAM	Sequence alignment/map
GATK	GenomeAnalysisTK
LG	Linkage group
QTL	Quantitative trait loci
SMRT	Single-molecule real-time
RAPD	Random amplified polymorphic DNA
AFLP	Amplified fragment length polymorphisms
SSR	Simple sequence repeat
DArT	Diversity arrays technology
AF	Allele frequency

INTRODUCTION

Strawberries belong to the genus *Fragaria L.* in the family Rosaceae, subfamily Rosoideae (Potter et al., 2007). The genus *Fragaria* has a basic chromosome number of seven (x=7) (Ichijima, 1926). There are various *Fragaria* species with different ploidy levels, ranging from diploid to decaploid (Ahmadi et al. 1992; Hummer et al. 2009). The cultivated strawberries (*F. x anananssa Duchesne*) are octoploid (2n = 8x = 56), which originated from a cross between a few clones of South American *F. chiloensis (L.) Miller* and North American *F. virginiana (L.) Miller* about 250 years ago (Darrow, 1966). Because of its pleasant flavor, nutrient factors and health benefitting properties, strawberry has a large economic and commercial values (Giampieri et al., 2014) which are evidenced by the continuous increase of the strawberry production (http://faostat.fao.org/site/567/).

There have been many obstacles in molecular breeding of strawberry due to the extremely complex nature of its genome, which also leads to difficulty in assembly of octoploid strawberry genome (Folta and Davis, 2006; Hirakawa et al., 2014; Koskela et al., 2016). Strawberry is an allopolyploid composed of four different diploid genomes. The allopolyploidy nature of strawberry make it very difficult to distinguish closely related subgenomes

when assemble the strawberry genome (Ming and Wai, 2015). For this reason, diploid *F. vesca* and incomplete octoploid *F. x ananassa* reference genome have been the basis of genetic studies in octoploid strawberry (Shulaev et al., 2011; Tennessen, 2014; Bassil et al., 2015; Davik et al., 2015; Edger et al., 2017). However, recent long-read sequencing methods including single-molecule real-time (SMRT) and Nanopore sequencing enabled construction of the chromosome-scale reference genome of the octoploid *F. ananassa*. The chromosome-scale reference genome covers approximately 99% of the estimated genome size. These reference genome provide an unprecedented opportunities for molecular breeding in strawberry (Edgar et al., 2019b).

Molecular markers and linkage maps are essential for plant molecular breeding and genetic studies. Various molecular markers have been developed during last several decades including random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP), simple sequence repeat (SSR), diversity arrays technology (DArT), Affymetrix IStraw90 Axiom array, and genotyping-by-sequencing (GBS) in strawberry (Gidoni et al., 1994; Congiu et al., 2000; Degani et al., 2001; Lerceteau-Köhler et al., 2003; Davis et al., 2006; Sánchez-Sevilla et al., 2015; Bassil et al., 2015; Vining et al., 2017). As the markers have been gradually developed, there have been many studies aimed to construct linkage maps in octoploid

strawberry. The first genetic linkage map of *F.* × *ananassa* was constructed using amplified fragment length polymorphism (AFLP) markers. These genetic linkage maps with total linkage distances of 1,604 cM and 1,496cM was constructed using 235 and 280 amplified fragment length polymorphism (AFLP) markers, for female and male maps, respectively (Lerceteau-Köhler et al., 2003). Isobe et al., (2013) reported a linkage map consisting of simple sequence repeat (SSR) with a total linkage distance of 2364.1 cM (Isobe et al., 2013). The Affymetrix IStraw90 Axiom array was the first high-throughput genotyping platform for octoploid strawberry (Bassil et al., 2015). A linkage map spanning 1,820 cM was constructed with the 8,407 SNP markers of Axiom® IStraw90 array (Sargent et al., 2016). In case of Affymetrix IStraw90 Axiom array, it is a highly simple, convenient and cost-effective method compared to other genotyping technologies. In 2017, the first linkage maps of three octoploid strawberry was constructed using genotyping-by-sequencing (GBS). The linkage groups covered 99% of the *F. vesca* reference genome (Vining et al., 2017).

The firmness of strawberry is one of the main factors influencing the consumers' acceptance (Døving and Måge, 2002). It is evidenced that the higher the firmness assures the higher the storage capacity and the better quality of the fruit (Liu, 2016). Furthermore, fruit with low firmness have the

disadvantage of increased pathogen susceptibility (Dotto et al., 2006). Therefore, fruit firmness has been an important target in strawberry breeding programs (Darrow, 1966; Barritt, 1979; Scheerens and Brennemaan, 1991; Khanizadeh et al., 2000; Giampieri et al., 2014; [o4]Hancock et al., 2016; Mora et al., 2019). Fruit firmness is controlled by many factors such as cuticle properties, cell wall structure and turgor (Chaib et al., 2007; Saladie et al., 2007). Therefore, firmness is a very complex and polygenic trait (Brummell and Harpster, 2001). To date, there has been studied on QTL analysis targeting firmness in various fleshy fruits such apple, melon, peach and sweet cherry as well as tomato which is considered a model plant for fleshy fruits (Kenis et al., 2008; Moreno et al., 2008; Cao et al., 2012; Chapman et al., 2012; Balas et al., 2017). Similarly, QTL analysis targeting firmness have been studied in strawberry (Dotto et al., 2006; Zorrilla-Fontanesi et al., 2011; Lerceteau-Köhler et al., 2012; Antanaviciute, 2016; Pott et al., 2018). [W사5] Until now, several QTL regions for firmness have been detected. Among them, according to the most detailed study of firmness as the target trait, eight QTL regions were detected on chromosome 1, 2, 3, 4 and 7 based on SSR-based linkage map. Also, in the Axiom array-based linkage map, by comparing the physical position of markers detected in QTL regions with the expansin position known to affect strawberry fruit firmness, three markers that are

thought to be closely related to firmness were finally selected. However, this result is based on physical position of diploid reference genome, therefore further study based on the new octoploid reference genome is required.

In this study, we constructed a high-density linkage map for the F_2 mapping population which shows differences in firmness using the IStraw90 Axiom® SNP array and GBS markers for identification of subgenome-specific loci. This method can substitute the disadvantage that the whole genome resequencing is expensive, and has the advantages that the density and coverage of the mapping population is excellent as in whole genome resequencing. Firstly, we identified and corrected the scaffolding error of the octoploid 'Camarosa' reference genome using this high-density linkage map (domesticated type) and two previously published linkage map (wild types). Secondly, using our 'L80' linkage bin map, a significant QTL region with an LOD value of 6 and associated with the expansin genes affecting the firmness of strawberry fruit was identified in Chromosome 6-3b. Therefore, the construction of the domesticated type high-density linkage map will be beneficial to further researches not only in genomic studies but also in molecular breeding.

MATERIALS AND METHODS[W사6]

Plant materials, DNA extraction and reference genome

Inbred lines ('Bennihoppe 8-10', 'Sachinoka KJ12 12', 'Bennihoppe 8-9', and 'Chandler KJ13 13') derived from the relatively high firmness variety *F.* × *ananassa* 'Bennihoppe' and two low firmness varieties *F.* × *ananassa* 'Sachinoka' and 'Chandler' were used as parental lines (Rho et al., 2012). [o7]Two F_2 mapping populations 'L80' and 'T137' were used for linkage map construction. 'L80' was derived from a cross between 'Bennihoppe 8-10' and 'Sachinoka KJ12 12' and 'T137' is a cross between 'Bennihoppe 8-9' and 'Chandler KJ13 13'. Total genomic DNA was extracted from young leaves of 149 F_2 plants and their parental lines ('L80') and those of 147 F_2 plants and their parental lines ('T137') using modified cetytrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987).

Two octoploid strawberry reference genomes were used in this study. One is a reference genome named 'Wongyo (unpublished)', which is a highly homozygous inbred line and a high-quality reference genome covering a total of 804 Mb. The other is 'Camarosa', which is the first completed octoploid strawberry reference genome representing ~99% of the estimated genome size (Edgar et al., 2019b).

GBS library preparation and sequencing

The GBS libraries were constructed for the 'L80' 'T137' populations and their parental lines. DNA concentrations were diluted to 80 ng/$\mu\ell$ for library preparation. Genomic DNA was double digested with the restriction enzymes *Eco*RI and *Mse*I as previously described by Han et al., (2018). The adapters of two restriction enzymes were ligated to both enzyme cut-sites of digested gDNA with different barcodes for each sample. After amplification and quality control, the library were sequenced using Hiseq 4000 at Macrogen (Seoul, Republic of Korea).

Sequencing data processing and SNP calling

The CLC genomics Workbench (v8.5.1) was used to check sequencing quality (QC) and trim the sequence reads. Quality trimming value was set to 0.01 (Q=20). For trimming, ambiguous nucleotides were removed to allow maximal 2 nucleotides. Sequence length less than 80 was removed. A pipeline implemented in Python program was used for the processing of trimmed data. This pipeline uses several software tools as Burrow Wheelers Alignment (BWA), Sequence Alignment/Map (SAM) Tools, and Picard (Li and R. Durbin, 2009; Li et al., 2009; Truong et al., 2012). The raw reads were aligned to the reference genome using the Burrow-Wheeler Aligner program v0.7.12 (Li and Durbin, 2009). To group and sort the aligned read, Picard Tools v1.19 and SAMtools v1.1 were used. For SNP calling, the Genome Analysis ToolKit (GATK) UnifiedGenotyper v3.8-0 was used (Depristo et

al., 2011). SNPs were filtered for minimum genotype quality of Q30, allele frequency (AF) and a minimum sequencing depth 3.

GBS SNP dataset filtering and genotyping

Processed GBS reads were aligned against the reference genome of *F.* × *ananassa* 'Wongyo' for SNP calling. The following criteria were used for further SNP filtering: (i) SNP loci with no calling data in both parents was removed; (ii) threshold for removal was set to $P < 0.01$; (iii) based on the fact that one parent is clearly called homozygous, the uncertain genotype of the opposite parent was determined (iiii). In case of one parent was no-call or heterozygous, or both parents were heterozygous, it was adjusted to the genotypes around the SNP loci. Lastly, we performed parent-based genotyping of paternal line to "A" and maternal line to "B". The genotypes were labeled as "A" or "B" for homozygous genotypes, and "H" for heterozygous genotypes. 'Chandler', 'Sachinoka' and individuals following 'Chandler' and 'Sachinoka' genotypes were coded as "A", whereas 'Bennihoppe' and individuals following 'Bennihoppe' genotypes were coded as "B" (Rabbi et al., 2014; Moumouni et al., 2015).

Axiom marker dataset filtering and genotyping

To select F_2 plants for Genotyping using Affymetrix of Axiom® Strawberry Array (IStraw90K), gDNA quality was evaluated using ND-1000 spectrophotometer

(Thermofisher, Waltham, and USA) and Quant-iT™ PicoGreen ® dsDNA Reagent (Thermofisher, Waltham, and USA). Among the 'L80' F_2 plants, 94 individuals having high recombination rates, which were calculated using GBS data, were selected. The Axiom SNP array experiment were performed at DNA Link (Seoul, Korea). The quality of raw data for each individual were determined using the default Dish QC values by Affymetrix Power Tools (APT). After removing DQC, the genotype calling were performed using APT with AxiomGT1 BRLMM-P algorithm. For genotyping analysis, Axiom Analysis Suite v4.0.1 was used.

Bin map and SNP linkage map construction

In this study, we constructed a bin map to solve the genotyping error and missing data. The ratios of SNPs with both parental genotypes was calculated for each window, defined as a window unit of 2.0 Mb and a moving window size of 200 Kb. When more than 70% of SNPs had one parental genotype, the window was called as homozygous; otherwise, the window was called as heterozygous. Adjacent windows with same genotype were combined into a recombination bin. Recombination breakpoints were determined as the position where a different genotype emerged. A linkage map of bins was constructed by using the Carthagene software (De Givry et al., 2005). The criteria used to construct a linkage group were a 3.0 LOD threshold score and limited distance of 50 cM. Among several LGs, the best map order was determined based on LOD scores and recombination frequencies. The Kosambi genetic mapping function was used to calculate the distances between

bin markers. The resulting linkage map was then drawn using the MapChart2.2 software (Voorrips. R, 2002).

Comparison of linkage map to physical map

After construction of the linkage map using bin marker, linkage map and physical map were compared using ALLMAPS software (Haibao Tang., 2015). The physical map information was based on the genome sequence of ‘Wongyo’ reference. In the case of chromosome divided into two linkage groups, the gap connecting the two divided linkage groups was set to 50 cM. [W사8]

Scaffold reassembly in 'Camarosa’ reference genome

The scaffolds of the ‘Camarosa’ reference genome were reassembled of the scaffolding errors using the linkage bin map of the domesticated population, ‘L80’ and linkage maps of the two wild type populations, ‘Del norte’ and ‘PI552277’ (Hardigan et al., 2019). Firstly, in order to identify and correct the scaffolding errors of the ‘Camarosa’ reference genome, the ALLMAPS program (--links=3) was used to identify the collinearity between the ‘Camarosa’ reference genome and three linkage maps. In case of ‘L80’ population, the sequence between -250bp and +250bp based on the SNP position was extracted. Then, the physical position in the Chandler reference genome was detected through the local blastn (Evalue of 3, maximum under of HSPs of 1.0 and maximum target sequence of 10.0) on the Linux server.

Secondly, the gap position was detected in 'Camarosa' reference genome, and among the gaps that matches the region where the chromosomes were twisted, the gap with the most average value was selected. Based on the selected gap, the chromosomes were divided. Finally, using the ALLMAPS program (--links=3), the 'Camarosa' reference genome were reassembled with the 'Camarosa' reference genome divided by the gap position and three linkage maps.

Measurement of firmness

Strawberry firmness was evaluated by modified method of non-destructive way. Fruit firmness was leveled by compressing 80~90% ripe fruit between thumb and forefinger and scored from hard to mushy (Leveled 1 to 10, level 1; very soft; level 10; very firm) (Mathey et al., 2013). [o9]Firmness evaluation was conducted at the National Institute of Horticulture and Herbal Science in 2018.

QTL analysis

Using the two genetic bin maps and firmness phenotypic data, composite interval mapping was performed using Windows QTL Cartographer v2.5 (Wang et al., 2012). The result were screened for functional QTL based on an LOD value 2.5. The closest bin marker to the QTL with the highest LOD was selected to represent QTL in firmness. The proportion of phenotypic variation explained by each QTL was estimated using R^2 (%) value.

RESULTS

Genotyping by sequencing and Axiom array genotyping

Sequencing data of GBS libraries for 'L80' and 'T137' were obtained and aligned to 'Wongyo' reference genome assembly. A total of 61,862 ('L80') and 75,595 ('T137') raw SNPs were obtained from the Illumina sequencing. After quality filtering (minimum 30 phred-score, minimum read depth of 3 and minimum calling rate of 70%, 6,312 and 11,378 SNPs were obtained for 'L80' and for 'T137', respectively. SNPs fitted to a segregation ratio of 1:2:1 (p-value > 0.01) were selected and 855 and 1,091 GBS SNPs were finally obtained for 'L80' and 'T137', respectively (Table 1).[W사10] In case of the Axiom SNPs, only 'L80' population were used for analysis. The scores for the probes were classified into six categories produced by the Affymetrix software: (1) Poly High Resolution (PHR) (4,463; 3.23%); (2) Call Rate Below Threshold (CRBT) (4,714; 3.41%); (3) Other (20,571; 14.9%) (4) Off-Target Variant (OTV) (123; 0.09%); (5) Mono High Resolution (MHR) (100,851; 73.03%); (6) No Minor Homozygote (NMH) (7,377; 5.34%). Along with the first group (PHR) markers suggested by the company, second group (CRBT) and third group (Other) markers were used for blastn to align 'Wongyo' reference genome. As a result, total 4,936 Axiom markers were multiple alignment locus and showed a 1:2:1 segregation ratio (p-value > 0.01) (Table 2).

Table 1. Summary of GBS-based SNP marker sets

Name	Population size	Raw SNPs	QC filtered	Final marker*
L80	149	61,862	6,312	855
T137	147	75,595	11,378	1,091

*Final marker means that the F_2 genotype matched to 1:2:1 segregation ratio selected through the chi square test (p-value > 0.01).

Table 2. Number of markers in six classes for Axiom IStraw90 genotyping array

Name	PHR	CRBT	Other	OTV	MHR	NMH	Filtered SNP	Total
L80	4,463	4,714	20,571	123	100,851	7,377	4,936	138,099

Bin map construction

We used F. × ananassa chromosome information with the reference in this study. High-density genetic maps of populations with high linkage disequilibrium contain many redundant markers that provide no new information, but increase the computational requirements of mapping. Furthermore, a small percentage of genotypes are falsely called due to sequencing error. To complement for missing data and genotyping errors and to construct an ultra-high-density linkage map, a modified sliding window approach was applied (Huang et al., 2009). A slightly modified sliding window approach (Han et al., 2016) was used to investigate recombination breakpoints and construct a bin map. Adjacent SNPs with the same genotype were integrated into a single recombination bin, and the genotype of each bin was determined based on the ratio between SNPs from two parents. This bin was used as a marker for construct a linkage map. A total of 5,791 SNPs (‘L80’) and 1,091 SNPs (‘T137’) were used to construct the bin map. All SNPs were distributed in 1,245 bins (‘L80’) and 463 bins (‘T137’), grouped into 33 and 30 linkage groups, respectively. The average length of bin is 0.65Mb (‘L80’) and 1.74Mb (‘T137’), which means that the length of one bin representing the physical genome region in the ‘L80’ population is shorter than in the ‘T137’ population (Table 3 and Table 4). Most of bin marker length were more than 1Mb, ranging from 100kb to 11.8Mb (‘L80’) and 100kb to 19.1Mb (‘T137’).

Table 3. The total number of SNPs and bin markers mapped to the 'L80' linkage map.

Chr.	No. SNPs	Linkage group	No. bins	Average distance between bin markers (cM)	Average length of bins (Mb)	LG length (cM)	Total physical length (Mb)
1-1	160	1-1	45	2.0	0.57	92.1	25.8
1-2	167	1-2	45	2.7	0.54	119.3	24.5
1-3	153	1-3	43	3.0	0.58	130.5	24.9
1-4	331	1-4	46	2.0	0.50	91.1	23
2-1	94	2-1	34	4.9	0.79	166.9	26.8
2-2	283	2-2	37	4.9	0.72	181.4	26.4
2-3	113	2-3	39	5.0	0.76	195.2	29.4
2-4	119	2-4	33	4.9	0.83	162.6	20.9
3-1	178	3-1	49	2.7	0.60	131.3	26.6
3-2	220	3-2	53	2.3	0.57	120.9	28.1
3-3	267	3-3	56	2.4	0.55	131.8	26.4
3-4	402	3-4	65	2.0	0.48	130.4	28.1
4-1	137	4-1a	12	4.9	0.64	58.7	24.3
		4-1b	26	1.8		47.3	
4-2	118	4-2	38	4.5	0.72	171.8	27.5
4-3	296	4-3	46	3.9	0.59	180	27
4-4	119	4-4	29	4.3	1.07	123.6	31
5-1	469	5-1	69	2.3	0.39	161.7	26.2
5-2	180	5-2	51	2.4	0.49	123.1	24.8
5-3	254	5-3	58	3.0	0.50	173.5	28.3
5-4	200	5-4	46	3.2	0.56	145.9	25.6
6-1	394	6-1a	20	2.3	0.60	46.2	32.2
		6-1b	35	3.1		108.1	
6-2	132	6-2a	14	3.3	0.86	46.8	34.2
		6-2b	27	2.9		78.1	
6-3	162	6-3a	15	3.3	0.79	50.2	37.1
		6-3b	28	4.2		118.8	
6-4	116	6-4a	11	2.8	0.82	30.8	32.9
		6-4b	30	3.0		89.1	
7-1	112	7-1	25	5.0	0.86	126.1	20.8
7-2	335	7-2	49	3.1	0.58	149.9	28
7-3	91	7-3	31	5.7	0.74	175.3	22.6
7-4	189	7-4	40	2.9	0.55	116.1	21.8
Mean	206.8	-	37.7	3.4	0.65	120.4	27.0
Total	5,791	33	1245	-	-	3974.6	719.0

Table 4. The total number of SNPs and bin markers mapped to the 'T137' linkage map.

Chr.	No. SNPs	Linkage group	No. bins	Average distance between bin markers (cM)	Average length of bins (Mb)	LG length (cM)	Total physical length (Mb)
1-1	22	1-1	13	2.5	1.44	32.4	18.7
1-2	12	1-2	6	14.0	3.72	83.8	22.3
1-3	26	1-3	9	1.4	0.72	12.8	6.5
1-4	22	1-4	11	4.4	1.00	48	11
2-1	38	2-1	16	2.3	1.54	36.3	24.7
2-2	102	2-2	38	2.5	0.66	94.1	25.1
2-3	38	2-3	21	3.4	1.29	70.9	27
2-4	36	2-4	13	4.2	1.52	54.6	19.7
3-1	28	3-1a	8	4.8	2.08	38.4	24.9
		3-1b	4	4.2		16.7	
3-2	37	3-2	14	6.0	2.11	84.5	29.5
3-3	52	3-3	21	3.7	1.12	76.8	23.5
3-4	3	-	-	-	-	-	-
4-1	55	4-1	24	2.2	0.87	53.5	20.8
4-2	36	4-2	15	4.3	1.50	64.6	22.5
4-3	24	4-3	10	2.3	1.73	23.2	17.3
4-4	13	4-4	6	10.4	3.32	62.2	19.9
5-1	22	5-1	14	9.8	1.87	137.2	26.2
5-2	64	5-2	23	5.0	0.97	115.5	22.4
5-3	62	5-3	22	3.8	1.23	82.5	27.1
5-4	42	5-4a	5	4.9	1.93	24.4	25.1
		5-4b	8	8.4		67.2	
6-1	76	6-1	34	2.9	0.96	100.1	32.6
6-2	37	6-2	15	9.2	2.11	138	31.7
6-3	67	6-3	32	3.7	1.13	118	36.3
6-4	45	6-4a	14	5.0	1.52	70.5	33.5
		6-4b	8	1.5		11.7	
7-1	31	7-1	18	3.3	1.17	60.2	21.1
7-2	25	7-2	10	5.6	2.40	55.8	24
7-3	56	7-3	22	4.1	0.99	91	21.8
7-4	20	7-4	9	2.3	2.07	20.8	18.6
Mean	39.0	-	15.4	4.7	1.59	64.9	23.5
Total	1,091	30	463	-	-	1945.7	529.3

SNP linkage map construction

Two linkage bin maps have been constructed to improve genome quality and QTL analysis. The linkage maps were constructed with a total of 1,245 (‘L80’) and 463 bins (‘T137’), and Carthagene software was used. As a result, a linkage maps consisting of 33 linkage groups (‘L80’) and 30 linkage groups (‘T137’) corresponding to 28 strawberry chromosomes was constructed. In case of ‘L80’ population, chromosome 4-1, 6-1, 6-2, 6-3, 6-4 were divided into two linkage groups and in case of ‘T137’ population, chromosome 3-1, 5-4, 6-4 were divided into two linkage groups. Also, in the ‘T137’ population, the number of SNP markers in the chromosome 3-4 was only three, so the linkage group could not be developed (Fig. 1 and Fig. 2). The total length of the linkage maps were 3,974.6 cM (‘L80’) and 1,945.7 cM (‘T137’) based on the genetic distance, and 780.9 Mb (‘L80’) and 677.6 Mb (‘T137’) based on the physical distance. In the ‘L80’ population, it was confirmed that the genetic and physical lengths were longer than ‘T137’ population. Also, in ‘L80’ population, length per bin were approximately 3.4 cM and 0.65 Mb. On the other hands, in ‘T137’ population, length per bin were approximately 4.7 cM and 1.74 Mb (Table 3 and Table 4). These results indicate that the linkage bin map constructed using GBS and Axiom markers is high-density rather than the linkage bin map constructed using only GBS markers.

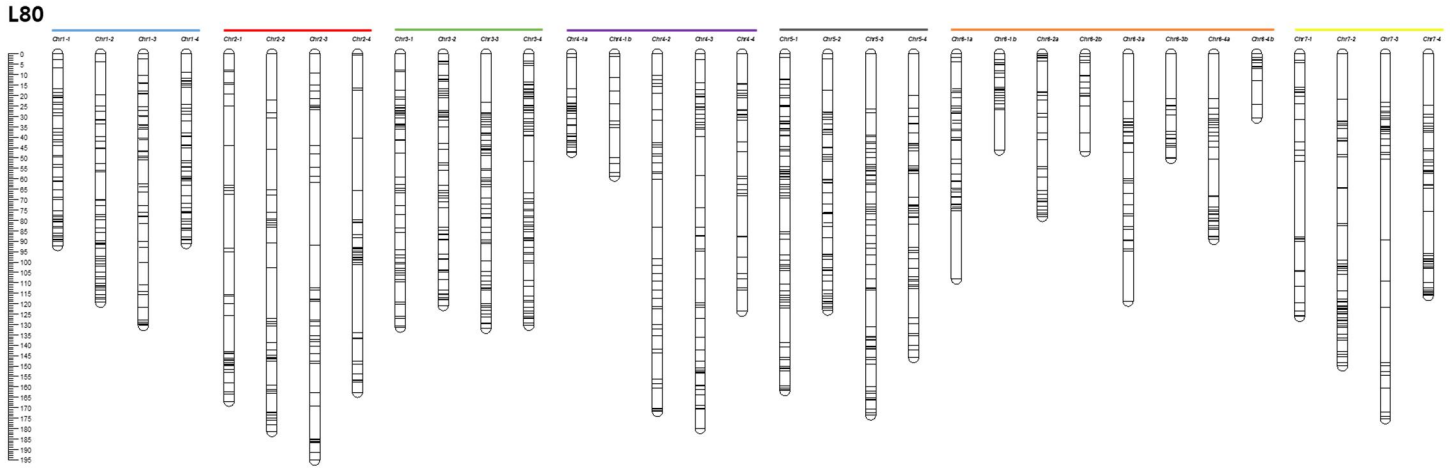


Figure 1. Construction of 'L80' SNP linkage bin map

High-density linkage bin map of the 'L80' population derived from the cross between 'Benihoppe' and 'Doyonoka'. The linkage bin map consists of GBS and Axiom marker sets. Linkage groups are denoted LG1-LG33 corresponding to 28 chromosomes in strawberry. Chr4-1, 6-1, 6-2, 6-3 and 6-4 are divided into two linkage groups. The color of the upper lines, grouped by sub-genome, indicates to seven *F. vesca* chromosomes. The bar on the left is an indicator of the length of the linkage groups.

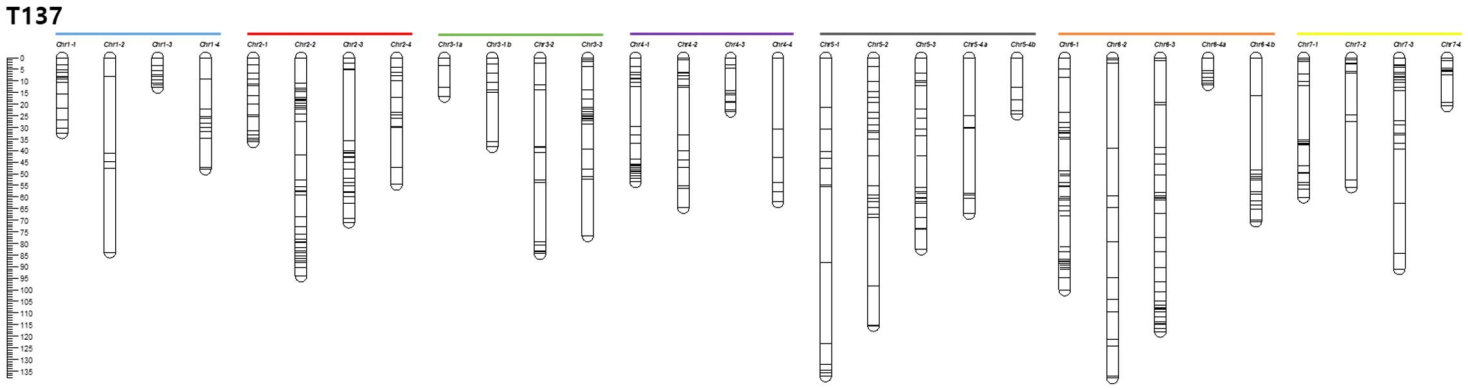


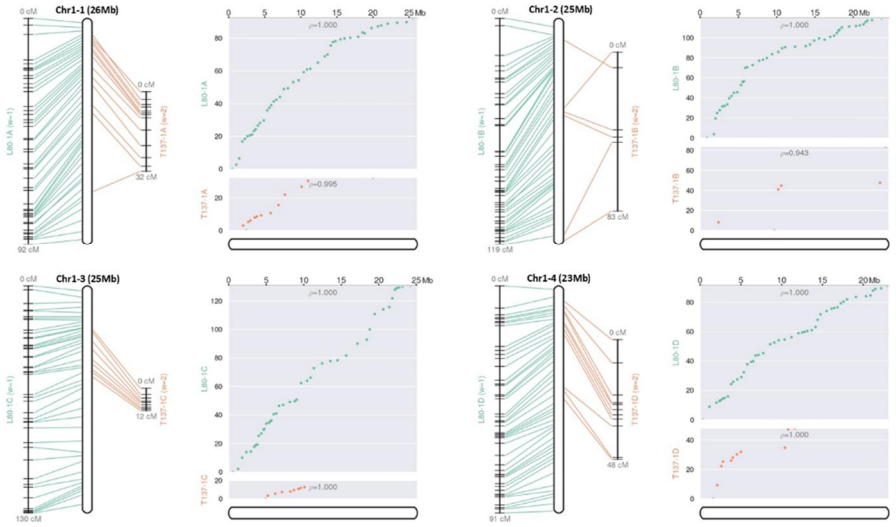
Figure 2. Construction of 'T137' SNP linkage bin map

High-density linkage bin map of the 'T137' population derived from the cross between 'Benihoppe' and 'Sachinoka'. The linkage bin map consists of only GBS markers. Linkage groups are denoted LG1-LG30 corresponding to 27 chromosomes in strawberry. The linkage group corresponding to Chr3-4 has not been constructed. Chr3-1, 5-4 and 6-4 are divided into two linkage groups. The color of the upper lines, grouped by sub-genome, indicates to seven *F. vesca* chromosomes. The bar on the left is an indicator of the length of the linkage groups.

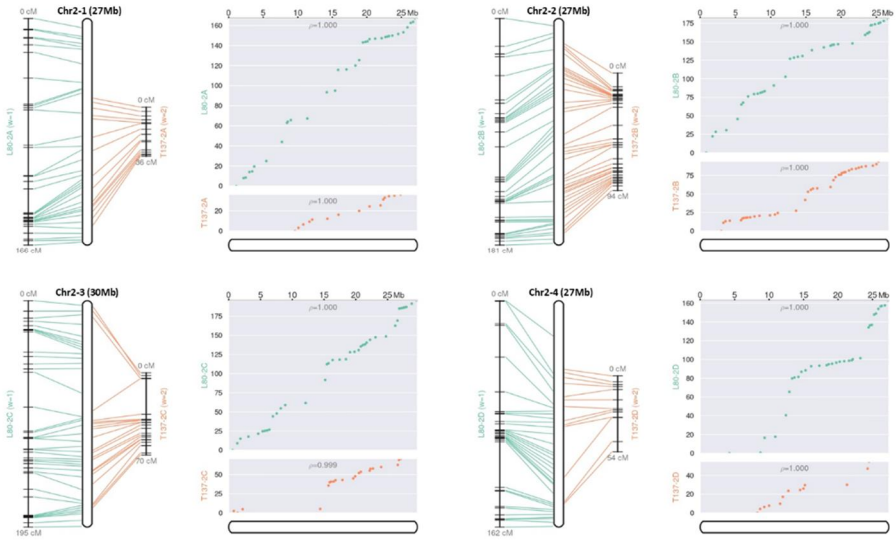
Relationship between linkage map and physical map

To evaluate the linkage maps constructed with 1,245 (‘L80’) and 463 bins (‘T137’), the physical position of ‘Wongyo’ reference genome and genetic position of two linkage groups were compared. It was confirmed that the collinearity was high in most of the bin markers in all 28 chromosomes. In case of two linkage groups are generated in one chromosome, the gap between the genetic position of the last marker of one linkage group and the genetic position of the first marker of the other linkage group was set to 50 cM and connected. The 50 cM is an option value used when developing a linkage group in the Carthagene program. By connecting the two divided linkage groups, it was easier to identify the collinearity. Although the ‘L80’ population has more bin markers than the ‘T137’ population, the Pearson correlation values were generally higher or equal, except for chromosome 5-2 and 7-2. Also, the region covering 28 chromosome genome sequences was 719.0 Mb (87.7%) in the ‘L80’ population and 529.3 Mb (64.6%) in the ‘T137’ population, showing a higher coverage in the ‘L80’ population (Fig 3; Table 3 and Table 4). The reason is that the number of markers in the ‘L80’ population composed of the GBS and Axiom markers is larger than that of the ‘T137’ population composed only GBS markers, and the flanking sequence of the Axiom marker has the characteristics of octoploid that align with up to four duplicated homoeologous octoploid sequences. Compared to linkage maps published in the previous study (Hardigan et al., 2019), it was confirmed that the linkage map of the ‘L80’ population showed higher coverage.

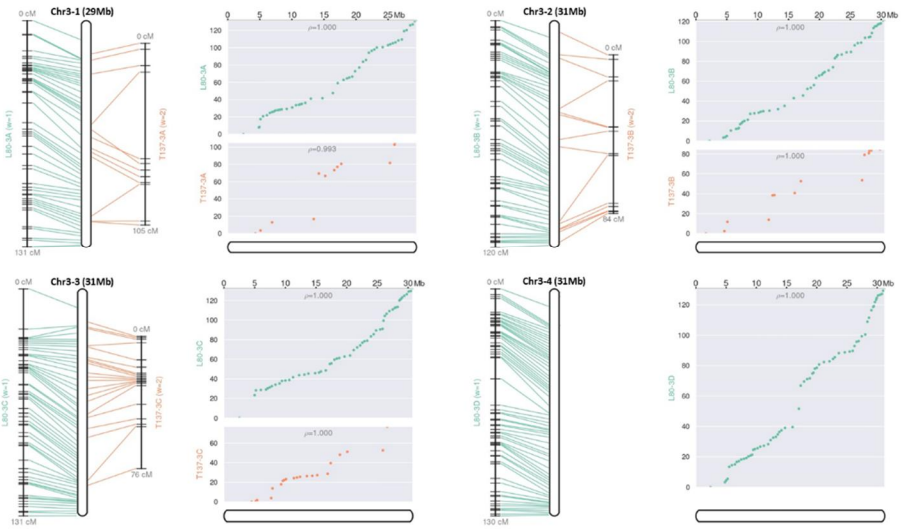
Chr1


Chr1-1 (26Mb)
Chr1-2 (25Mb)
Chr1-3 (25Mb)
Chr1-4 (23Mb)

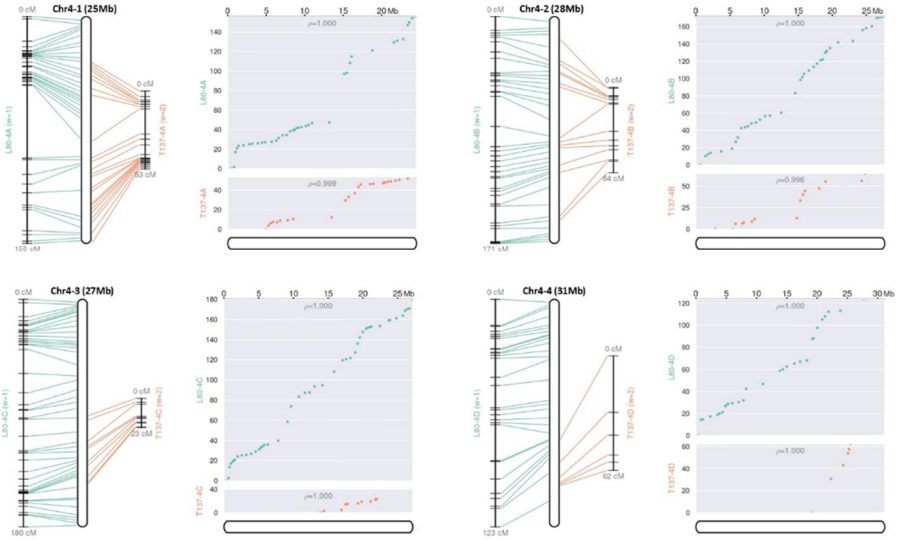
Chr2


Chr2-1 (27Mb)
Chr2-2 (27Mb)
Chr2-3 (30Mb)
Chr2-4 (27Mb)

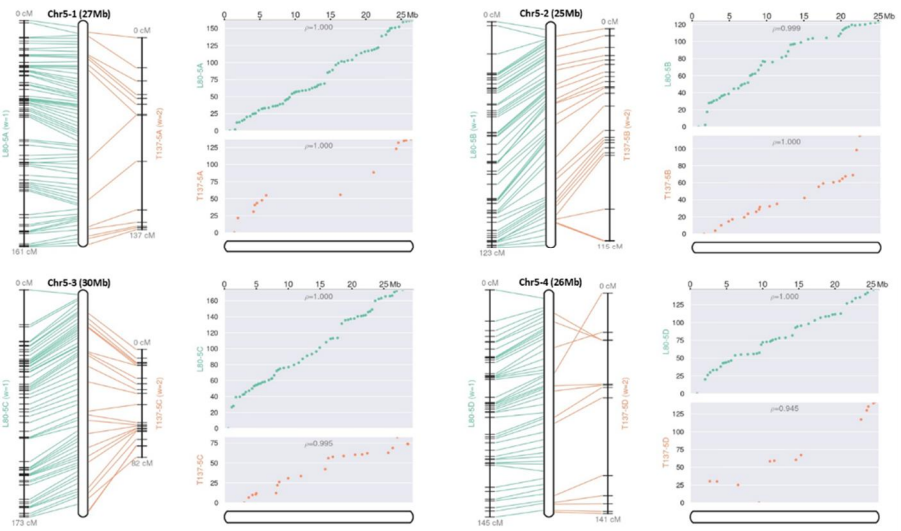
Chr3


Chr3-1 (29Mb)
Chr3-2 (31Mb)
Chr3-3 (31Mb)
Chr3-4 (31Mb)

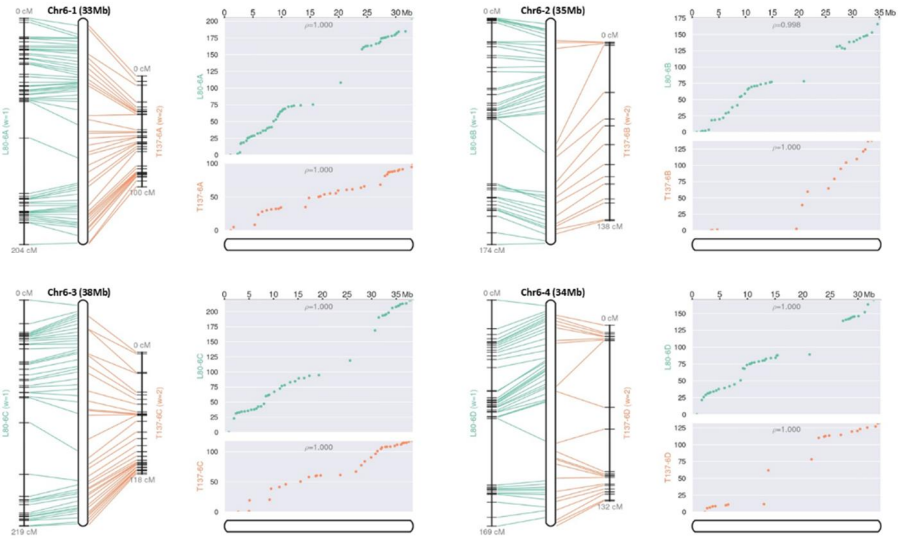
Chr4


Chr4-1 (25Mb)
Chr4-2 (28Mb)
Chr4-3 (27Mb)
Chr4-4 (31Mb)

Chr5


Chr5-1 (27Mb)
Chr5-2 (25Mb)
Chr5-3 (30Mb)
Chr5-4 (26Mb)

Chr6


Chr6-1 (33Mb)
Chr6-2 (35Mb)
Chr6-3 (38Mb)
Chr6-4 (34Mb)

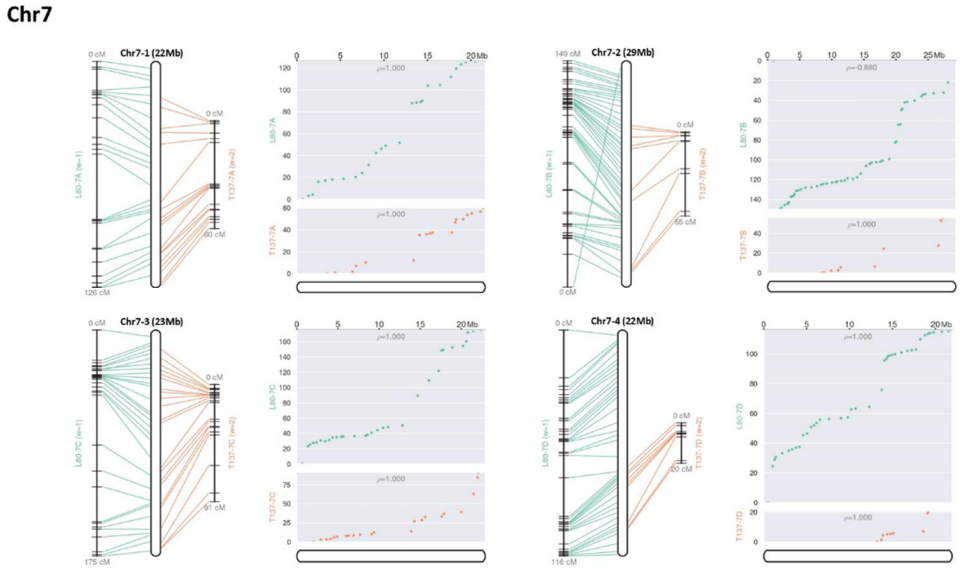


Figure 3. Comparison of the two linkage bin maps with 'Wongyo' reference genome.

Left and right bar shows two different linkage bin map. Left bar is 'L80' population, right bar is 'T137' population. Middle bar represents physical map of 'Wongyo' reference genome. The graph shows the Pearson correlation values and collinearity for genetic position and physical position of 28 chromosomes. The markers corresponding to 'L80' population are indicated by green color lines and dots, and the markers corresponding to 'T137' population are indicated by orange color lines and dots.

Scaffolds reassembly

To correct the 'Camarosa' reference genome, chromosome reassembly of scaffolding errors was processed in ALLMAPS program using our linkage bin map and two (published) linkage maps. As a result of identifying the collinearity by comparing the physical position of the 'Camarosa' reference genome and three linkage maps, a wide range of scaffolding errors could be identified in the four chromosomes 1-2, 2-1, 6-2, 6-4 (Fig 4a). The range of physical positions that generated these uncollinearity was 5,288,841 bp to 5,393,984 bp (Chromosome 1-2), 18,256,718 bp to 18,411,584 bp (Chromosome 2-1), 26,559,923 bp to 27,260,197 bp (Chromosome 6-2) and 1,827,818 bp to 2,786,280 bp (Chromosome 6-4), respectively. After that, as a result of extracting the range of the actual gap position, it was 5,346,824 bp to 5,347,824 bp (Chromosome 1-2), 18,304,402 bp to 18,304,427 bp (Chromosome 2-1), 27,212,991 bp to 27,213,991 bp (Chromosome 6- 2) and 2,204,157 bp to 2,205,157 bp (Chromosome 6-4), respectively. Each uncollinearity chromosomes were divided based on the actual gap, and the divided chromosomes were reassembled using the three linkage maps and ALLMAPS program. We showed four chromosomes completely reassembled (Fig 4b). It was identified that the physical position of the 'Camarosa' reference genome and the genetic position of the three linkage maps became more collinear than 'Camarosa' reference genome before reassembly (Fig 4).

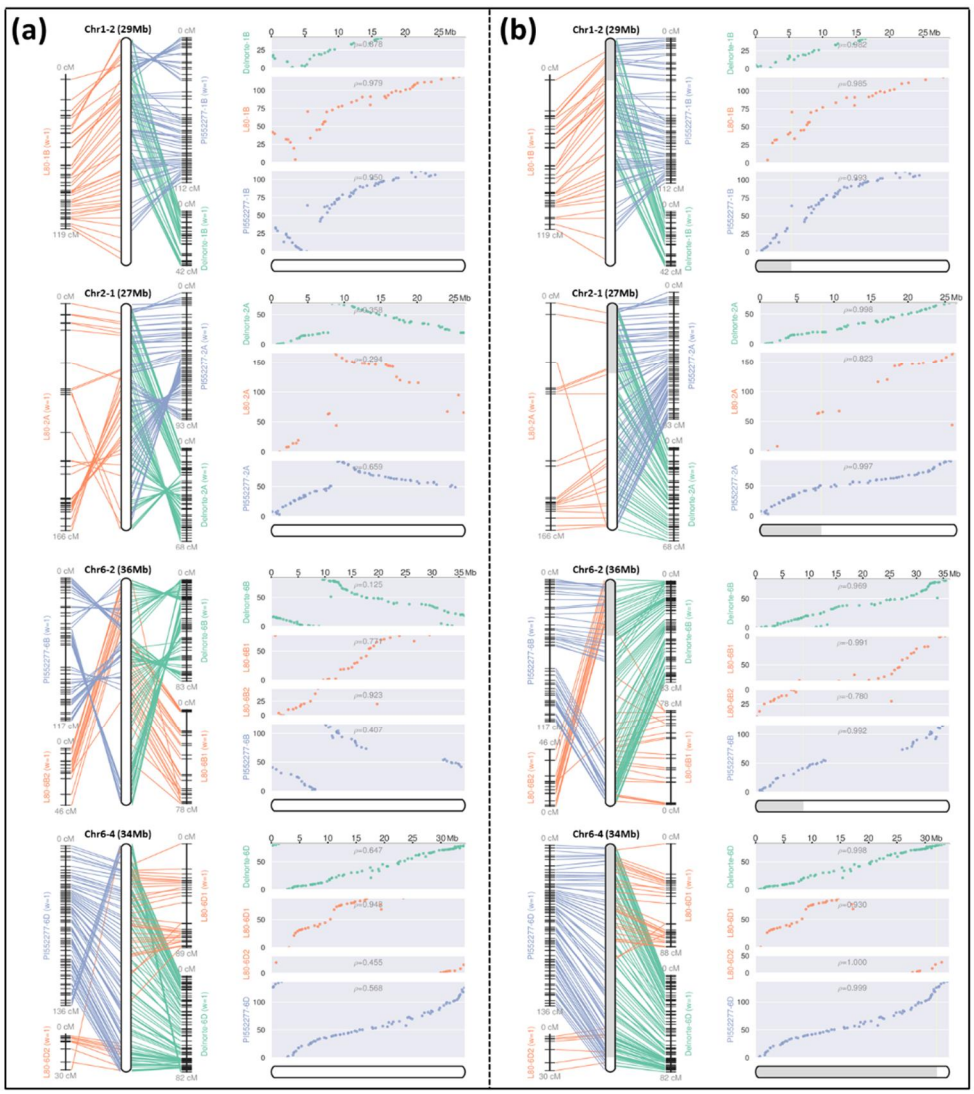


Figure 4. Four chromosome reassembly of 'Camarosa' reference genome.

(a) represents the comparison between physical position of the 'Camarosa' reference genome and genetic position of the three linkage maps. Show discordance on all three linkage maps. (b) represents the comparison between physical position of the 'Camarosa' reference genome, which has been reassembled, and genetic position of the three linkage maps. In the bar, black and white color shows that the chromosomes were reassembled.

QTL analysis for firmness in ripe fruit and identify of association with firmness-related genes

QTL controlling firmness in ripe fruit were detected in two population. Phenotype data for firmness and two linkage bin maps were used to identify QTL. In the 'L80' population, a total of nine QTL were mapped to the linkage bin map constructed in this study. On the other hands, only 5 QTL were detected in the 'T137' population. In case of 'L80' population, QTL were detected on chromosome 1-2, 1-4, 2-2, 3-1, 3-2, 4-1b, 6-3b, 7-2, 7-3. In case of 'T137' population, QTL were detected on chromosome 2-2, 3-3, and 4-1. Chromosome 3-2 showed the highest LOD score and explained 34.6% of total phenotypic variation in the 'L80' population. Chromosome 2-2 and 4-1 showed the highest LOD score and explained 0.1% and 8.8% of total phenotypic variation, respectively in the 'T137' population. Chromosome 4-1 region were the common QTL in the two populations, but the LOD value was relatively low. The 'L80' population detected relatively more QTL regions compared to the 'T137' population, and detected a region showing a higher LOD value because the 'L80' population is a linkage bin map composed of a high-density marker set rather than the 'T137' population (Table 6 and Table 7). As a result, the major QTL are not the regions where QTL are commonly detected in the two linkage groups, but the QTL on chromosome 3-2 and 6-3b region, which has a relatively high LOD value in the 'L80' population, appeared to be major QTL controlling firmness in strawberry ripe fruit (Fig 5). In addition, we investigated whether QTL

positions associated with fruit firmness are overlapped with firmness-related genes position. The physical position of the genes was extracted from annotation data of the octoploid reference genome, 'Wongyo' (Appendix 1 and Appendix 2). The physical positions of the closest bin markers linked to fruit firmness QTL were compared to the firmness-related gene location (Table 7 and Table 8). Since the sliding window size of bin markers was 2Mb, the genes existing between the 1Mb before and 1Mb after the bin marker was extracted. Comparison of the physical positions between the bin markers and firmness-related genes showed that twenty-nine and thirteen bin markers were located close to the firmness-related gene positions in L80 and T137 population, respectively. Among them, there were five markers belonging to the major QTL region 'qFaFL7'. The markers' name are 'Chr6-3_30.70', 'Chr6-3_32.65', 'Chr6-3_32.95', 'Chr6-3_33.45' and 'Chr6-3_34.20' and the markers ranged from 30.7Mb to 34.2Mb. Two firmness-related genes ranging from 30.1 Mb to 33.6 Mb associated with the markers, *EXPA2* and *EXPA3* were identified.

Table 5. Summary of QTL for firmness in 'L80' population[o11].

QTL	Chromosome	Location (cM)	LOD	R^2 (%)	Additive effect	Dominant effect	Location (Mb)
qFaFL1	Chr1-2	90.7-98.2	3.6	16.0	0.1858	0.5497	11.1-16.3
qFaFL2	Chr1-4	84.6-87.9	2.8	28.4	0.6971	0.6545	20.9-21.2
qFaFL3	Chr2-2	0-21.5	2.6	5.6	0.4104	0.3789	8.5-17.5
qFaFL4	Chr3-1	112-118.7	3.3	48.9	0.6007	0.6191	27.2-27.5
qFaFL5	Chr3-2	56.9-66.4	6.6	34.6	0.5354	0.1446	19.1-20.4
qFaFL6	Chr4-1b	0-11.1	3.6	8.4	0.3066	0.0756	15.2-15.9
qFaFL7	Chr6-3b	12.8-35.7	6.5	25.6	0.4746	0.0835	30.7-34.2
qFaFL8	Chr7-2	29.8-49.5	2.6	1.2	0.2053	0.2059	20.9-27.4
qFaFL9	Chr7-3	23.3-48.3	2.5	17.2	0.3809	0.302	1.4-11.1

Table 6. Summary of QTL for firmness in 'T137' population[o12]

QTL	Chromosome	Location (cM)	LOD	R^2 (%)	Additive effect	Dominant effect	Location (Mb)
qFaFT1	Chr2-2	16.5-24.3	2.7	0.4	0.1098	0.4247	6.0-11.0
qFaFT2	Chr2-2	24.3-46.9	3.2	0.1	0.1091	0.5513	11.0-15.3
qFaFT3	Chr3-3	13-21.4	3.0	22.0	0.3489	0.1647	7.9-9.6
qFaFT4	Chr3-3	21.4-39.3	2.6	18.7	0.3368	0.1382	9.6-17.4
qFaFT5	Chr4-1	22.3-44.6	3.2	8.8	0.0495	0.5186	15.3-17.0

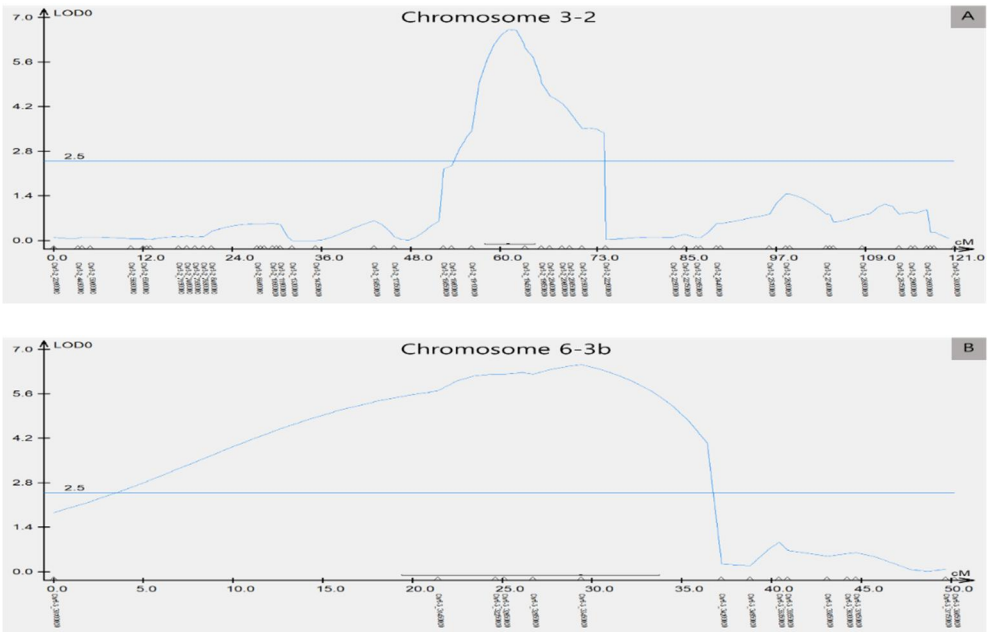


Fig 5. Positions of two major QTL for firmness in 'L80' population.

Two major QTL was identified for firmness in strawberry fruit on chromosome 3-2 (A) and 6-3b. Major QTL which has highest LOD score was located in 61.4 cM (A) and 29.5 cM (B), respectively.

Table 7. Comparison of bin markers most closely linked to firmness QTL and genes[o13] in 'L80' population

QTL	Bin marker (Mb)	Population	QTL region (cM)	Firmness genes		
				Name	Linkage group	Physical position (Mb)
qFaFL1	Chr1-2_12.40	L80	90.7-98.2	*PG-1II*	Chr1-2	12.3
				PG-1II	Chr1-2	12.5
				PG-1II	Chr1-2	12.6
qFaFL1	Chr1-2_13.80	L80	90.7-98.2	*PG-1II*	Chr1-2	14.8
qFaFL1	Chr1-2_14.35	L80	90.7-98.2	*PG-1II*	Chr1-2	14.8
qFaFL1	Chr1-2_14.90	L80	90.7-98.2	*PG-1II*	Chr1-2	14.8
				BGAL3-1II	Chr1-2	15.5
				BGAL6-1II	Chr1-2	15.5
qFaFL1	Chr1-2_16.25	L80	90.7-98.2	*BGAL3-1II*	Chr1-2	15.5
				BGAL6-1II	Chr1-2	15.5
				EXPA7-1II	Chr1-2	16.8
				PG-1II	Chr1-2	16.9
qFaFL3	Chr2-2_0.85	L80	0-21.5	*BGAL3-2II*	Chr2-2	0.7
qFaFL6	Chr4-1_15.15	L80	0-11.1	*PG-4I*	Chr4-1	14.6
				PG-4I	Chr4-1	14.6
qFaFL6	Chr4-1_15.50	L80	0-11.1	*PG-4I*	Chr4-1	14.6
				PG-4I	Chr4-1	14.6
qFaFL7	Chr6-3_30.70	L80	12.8-35.7	*EXPA3-6III*	Chr6-3	30.1
qFaFL7	Chr6-3_32.65	L80	12.8-35.7	*EXPA2-6III*	Chr6-3	33.6
qFaFL7	Chr6-3_32.95	L80	12.8-35.7	*EXPA2-6III*	Chr6-3	33.6
qFaFL7	Chr6-3_33.45	L80	12.8-35.7	*EXPA2-6III*	Chr6-3	33.6
qFaFL7	Chr6-3_34.20	L80	12.8-35.7	*EXPA2-6III*	Chr6-3	33.6
qFaFL8	Chr7-2_21.00	L80	29.8-49.5	*EXPA4-7II*	Chr7-2	20.6
qFaFL8	Chr7-2_21.35	L80	29.8-49.5	*EXPA4-7II*	Chr7-2	20.6
qFaFL8	Chr7-2_21.75	L80	29.8-49.5	*EXPA4-7II*	Chr7-2	22.5
qFaFL8	Chr7-2_22.90	L80	29.8-49.5	*EXPA4-7II*	Chr7-2	22.5
qFaFL8	Chr7-2_23.95	L80	29.8-49.5	*EXPA8-7II*	Chr7-2	24.1
				PG-7II	Chr7-2	24.5
				EXPB2-7II	Chr7-2	24.7
qFaFL8	Chr7-2_24.20	L80	29.8-49.5	*EXPA8-7II*	Chr7-2	24.1
				PG-7II	Chr7-2	24.5
				EXPB2-7II	Chr7-2	24.7
qFaFL8	Chr7-2_24.55	L80	29.8-49.5	*EXPA8-7II*	Chr7-2	24.1
				PG-7II	Chr7-2	24.5
				EXPB2-7II	Chr7-2	24.7
qFaFL9	Chr7-3_1.35	L80	23.3-48.3	*PG-7III*	Chr7-3	1.1
qFaFL9	Chr7-3_1.60	L80	23.3-48.3	*PG-7III*	Chr7-3	1.1
qFaFL9	Chr7-3_2.00	L80	23.3-48.3	*PG-7III*	Chr7-3	1.1
				EXOPG-7III	Chr7-3	2.8
qFaFL9	Chr7-3_2.40	L80	23.3-48.3	*EXOPG-7III*	Chr7-3	2.8
				BGAL-7III	Chr7-3	3.1
qFaFL9	Chr7-3_2.90	L80	23.3-48.3	*EXOPG-7III*	Chr7-3	2.8
				BGAL-7III	Chr7-3	3.1
qFaFL9	Chr7-3_3.45	L80	23.3-48.3	*EXOPG-7III*	Chr7-3	2.8
				BGAL-7III	Chr7-3	3.1
qFaFL9	Chr7-3_3.90	L80	23.3-48.3	*BGAL-7III*	Chr7-3	3.1
qFaFL9	Chr7-3_9.80	L80	23.3-48.3	*EXPB18-7III*	Chr7-3	10.4

qFaFL9	Chr7-3_10.50	L80	23.3-48.3	*EXPB18-7III*	Chr7-3	10.4

Table 8. Comparison of bin markers most closely linked to firmness QTL and genes[o14] in 'T137' population

QTL	Bin marker (Mb)	Population	QTL region (cM)	Firmness genes		
				Name	Linkage group	Physical position (Mb)
qFaFT1	Chr2-2_6.00	T137	16.5-46.9	*BGAL-2II*	Chr2-2	7.0
qFaFT1	Chr2-2_6.20	T137	16.5-46.9	*BGAL-2II*	Chr2-2	7.0
qFaFT1	Chr2-2_6.50	T137	16.5-46.9	*BGAL-2II*	Chr2-2	7.0
qFaFT1	Chr2-2_6.70	T137	16.5-46.9	*BGAL-2II*	Chr2-2	7.0
qFaFT1	Chr2-2_7.10	T137	16.5-46.9	*BGAL-2II*	Chr2-2	7.0
qFaFT1	Chr2-2_7.70	T137	16.5-46.9	*BGAL-2II*	Chr2-2	7.0
qFaFT2	Chr2-2_13.60	T137	16.5-46.9	*EXOPG-2II*	Chr2-2	13.1
				EXPA20-2II	Chr2-2	13.1
qFaFT2	Chr2-2_14.90	T137	16.5-46.9	*PL8-2II*	Chr2-2	14.8
				PG-2II	Chr2-2	15.5
qFaFT3	Chr3-3_7.90	T137	13-39.3	*EXPA13-3III*	Chr3-3	7.7
qFaFT4	Chr3-3_11.40	T137	13-39.3	*EXPA12-3III*	Chr3-3	11.8
qFaFT4	Chr3-3_11.90	T137	13-39.3	*EXPA12-3III*	Chr3-3	11.8
qFaFT4	Chr3-3_12.40	T137	13-39.3	*EXPA12-3III*	Chr3-3	11.8
qFaFT5	Chr4-1_15.30	T137	22.3-44.6	*PG-4I*	Chr4-1	14.6

DISCUSSION[W사15]

In strawberry, the first linkage map of octoploid strawberry (*F. x ananassa*) was constructed using AFLP markers (Lerceteau-Köhler et al., 2003). It had several limitations: (i) the number of markers was too small per linkage map, (ii) marker densities were relatively low, and (iii) the total map size was also relatively small. After construction of this linkage map, various molecular markers have been developed. A high-density linkage map was constructed using the various molecular markers (Isobe et al., 2013; Sargent et al., 2016; Vining et al., 2017). In this study, we used the Affymetrix IStraw90 Axiom array and genotyping-by-sequencing (GBS) to construct high density linkage maps. These methods have several competitive advantages compared to other genotyping methods. The Affymetrix IStraw90 Axiom array has the advantages of high marker density approximately one marker per every 0.5 cM, and no risks of sequencing errors (Bassil et al., 2015). Genotyping-by-sequencing (GBS) has the advantage of being able to identify high-throughput and cost-effective to identify single-nucleotide polymorphisms (SNPs) on a whole-genome scale. In this study, using the advantages of these two techniques, a marker set was selected and used for linkage map construction. In the case of Axiom array markers, special modification method was applied. It was to convert the Axiom marker set based on the diploid reference genome, '*F. vesca*' into Axiom marker set based on the octoploid reference genome, '*F. x ananassa*'. That is, if the flanking

sequence of these '*F. vesca*'-based marker is blasted to the octoploid reference genome, the flanking sequences are multiple-aligned to the subgenome. Using this characteristic, the number of markers was increased and thereby, a linkage map of octoploid strawberry was constructed to cover the entire genome spans as much as whole genome shotgun sequencing based linkage map, 'Del norte' (Hardigan et al., 2019) and also to cover the break of the physical map caused by large homozygous regions.[W사16] Therefore, in this study, the 'L80' linkage bin map constructed by applying the modification method is a suitable for constructing a physical map of the octoploid strawberry reference genome using an inbred line such as 'Wongyo' as well as for covering the large homozygous regions of 'Camarosa'. Our high-density linkage map will contribute to a molecular marker breeding to enable genomic selection and precise mapping of agronomically important genes and have numerous applications in genomic research in octoploid strawberry.

With the development of sequencing and scaffolding technologies, a chromosome-scale reference genome 'Camarosa' was assembled in 2019 (Edger et al., 2019). It provided the physical framework needed to molecular breeding. However, as reported in Micheal A. Hardigan, wild species octoploid map revealed some chromosomal rearrangements on chromosomes 1-2, 1-4, 2-1, 2-3, 6-2, and 6-4, indicating intra-chromosomal scaffolding errors in the physical reference genome. Especially, scaffolding errors occurred large region on chromosome 1-2, 2-1, 6-2, and 6-4. In this study, we identified and corrected the scaffolding error using a high-density map, 'L80', and two previously constructed wild species maps. As some

chromosomes were reassembled, the scaffolding error of 'Camarosa' was corrected and improved, and it will be very useful framework for studies on DNA variation discovery, gene discovery, genetic mapping, QTL analysis and genome wide association study based on this octoploid reference genome.

There are a number of the genes and enzymes associated with fruit firmness: Expansin, Polygalactosidase (PG), Pectate lyase (PL), pectin methylesterase (PME), and B-galactosidase (Harrison et al., 2001; Posé, S et al., 2011; Jiménez-Bermudez, S et al., 2002; Duvetter, T et al., 2005). Among them, the gene associated with the major QTL region in my study was expansin, so we explore this genes, intensively. The genes are present in cell walls and are associated with cell wall expansion, affecting firmness of strawberry. In strawberry, seven expansin genes have been reported to regulate fruit softness (Harrison et al., 2001; Dotto et al., 2006). Of these, three genes (*FaEXP1, FaEXP2*, and *FaEXP5*) showed correlations between fruit-specific firmness and their mRNA expression level (Dotto et al., 2006). In the previous study, QTL for fruit firmness were detected on chromosome 1, 2, 3, 4 and 7. In addition, the physical position of the markers detected in the QTL region was compared with the expansin genes. As a result, three markers were reported to be located close to the expansin gene position (Antanaviciute et al., 2016). (

)In this studies, we used the two linkage bin map developed by GBS and Axiom array to analyze QTL controlling firmness in strawberry fruit. Mapping populations were derived from one relatively high firmness and two relatively low firmness. In addition to the detected chromosome in the previous study, it was additionally

detected in chromosome 6. The similarity between the QTL analysis of the previous study and this study was that QTL is detected in various regions of the chromosome. On the other hand, when comparing the QTL analysis of the previous study with this study, a few differences were identified. On the contrary to the previous study based on the diploid reference genome, QTL analysis was conducted based on the octoploid reference genome in this study. In previous study, the physical positions of the three markers located closely to the position of the expansin genes were located on *F. vesca* chromosome 2 and 7. By contrast, we were able to detect a total of fifteen bin markers located close to the position of the expansin genes. In 'L80' linkage bin map, the physical positions of five bin markers out of fifteen were located on *F. x ananassa* chromosome 1-2, 1-4, 6-3b, and 7-2. In 'T137' linkage bin map, the physical position of ten bin markers out of fifteen are located on *F. x ananassa* chromosome 2-2 and 3-3. In this study, the major QTLs 'qFaFL5' and 'qFaFL7' were observed only in the 'L80' linkage bin map. However, major QTL has not been observed in the 'T137' linkage bin map, and we think it is because of the low density of the marker. Meanwhile, among the major QTL in the 'L80' linkage bin map, only the one bin marker 'Chr6-3_4.8' belonging to QTL 'qFaFL7' was located close to the physical position of the expansin genes. As the result of this study, we could show the potential of using a high-density linkage map for QTL analysis in strawberry and it is expected that the reliable marker of the QTL region observed in the 'L80' linkage bin map will be helpful in the firmness study of octoploid strawberry breeding. However, to be more accurate and reliable, functional study is necessary to identify

whether the gene associated with these markers affects strawberry firmness, and also it will be needed to phenotypic investigations of more diverse environments.

REFERENCES

Ahmadi, Hamid, and Royce S. Bringhurst. (1992) Breeding strawberries at the decaploid level. Journal of the American Society for Horticultural Science, 117(5), 856-862.

Antanaviciute, L. (2016) Genetic mapping and phenotyping plant characteristics, fruit quality and disease resistance traits in octoploid strawberry (Fragaria× ananassa) (Doctoral dissertation, University of Reading).

Balas, F., López-Corrales, M., Serradilla, M. J., Cai, L., Iezzoni, A., & Wünsch, A. (2017, June) Firmness QTL mapping using an'Ambrunés'×'Sweetheart'sweet cherry population. In VIII International Cherry Symposium 1235 (pp. 41-46).

Barritt, B. H., & BH, B. (1979) BREEDING STRAWHERRIES FOR FRUIT FIRMNESS.

Bassil, N. V., Davis, T. M., Zhang, H., Ficklin, S., Mittmann, M., Webster, T., Mahoney, L., Wood, D., Alperin, E. S., Rosyara, U. R., Putten, H. K. V., Monfort, A., Sargent, D. J., Amaya, I., Denoyes, B., Bianco, L., Dijk, T. V., Pirani, A., Amylezzoni, Main, D., Peace, C., Tang, T., Whitaker, V., Verma, S., Bellon, L., Brew, F., Herrera, R., & Weg, E. V. D. (2015) Development and preliminary evaluation of a 90 K Axiom® SNP array for the allo-octoploid cultivated strawberry Fragaria× ananassa. BMC genomics, 16(1), 155.

Brummell, D. A., & Harpster, M. H. (2001) Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. In plant cell walls (pp. 311-340). Springer, Dordrecht.

Cao, K., Wang, L., Zhu, G., Fang, W., Chen, C., & Luo, J. (2012) Genetic diversity, linkage disequilibrium, and association mapping analyses of peach (Prunus persica) landraces in China. Tree genetics & genomes, 8(5), 975-990.

Chaïb, J., Devaux, M. F., Grotte, M. G., Robini, K., Causse, M., Lahaye, M., & Marty, I. (2007) Physiological relationships among physical, sensory, and

morphological attributes of texture in tomato fruits. Journal of Experimental Botany, 58(8), 1915-1925.

Chapman, N. H., Bonnet, J., Grivet, L., Lynn, J., Graham, N., Smith, R., Sun, G., Walley, P. G., Poole, M., Causse, M., King, G. J., Baxter, C., & Seymour, G. B. (2012) High-resolution mapping of a fruit firmness-related quantitative trait locus in tomato reveals epistatic interactions associated with a complex combinatorial locus. Plant Physiology, 159(4), 1644-1657.

Congiu L, Chicca M, Cella R, Rossi R, Bernacchia G. (2000) The use of random amplified polymorphic DNA (RAPD) markers to identify strawberry varieties: a forensic application. Molecular Ecology 9:229–2325

Darrow, G. M. (1966) The strawberry. History, breeding and physiology. The strawberry. History, breeding and physiology.

Davik, J., Sargent, D. J., Brurberg, M. B., Lien, S., Kent, M., & Alsheikh, M. (2015) A ddRAD based linkage map of the cultivated strawberry, *Fragaria x ananassa*. PLoS One, 10(9).

Davis, T. M., DiMeglio, L. M., Yang, R., Styan, S. M., & Lewers, K. S. (2006) Assessment of SSR marker transfer from the cultivated strawberry to diploid strawberry species: functionality, linkage group assignment, and use in diversity analysis. J Am Soc Hortic Sci 131:506–512

De Givry, S., Bouchez, M., Chabrier, P., Milan, D., & Schiex, T. (2005) CARHTA GENE: multipopulation integrated genetic and radiation hybrid mapping. Bioinformatics, 21(8), 1703-1704.

Degani, C., Rowland, L. J., Saunders, J. A., Hokanson, S. C., Ogden, E. L., Golan-Goldhirsh, A., & Galletta, G. J. (2001) A comparison of genetic relationship measures in strawberry (*Fragaria* × *ananassa* Duch.) based on AFLPs, RAPDs, and pedigree data. Euphytica 117:1–12

DePristo, M. A., Banks, E., Poplin, R., Garimella, K. V., Maguire, J. R., Hartl, C., Philippakis, A. A., Angel, G. D., Rivas, M. A., Hanna, M., Mckenna, A., Fennell, T. J., Kernytsky, A. M., Sivachenko, A. Y., Cibulskis, K., Gabriel,

S. B., Altshuler, D., & Daly, M. J. (2011) A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nature Genetics, 43(5), 491.

Dotto, M. C., Martínez, G. A., & Civello, P. M. (2006) Expression of expansin genes in strawberry varieties with contrasting fruit firmness. Plant Physiology and Biochemistry, 44(5-6), 301-307.

Døving, A., & Måge, F. (2002) Methods of testing strawberry fruit firmness. Acta Agriculturae Scandinavica, Section B-Plant Soil Science, 52(1), 43-51.

Duvetter, T., Fraeye, L., Hoang, T. V., Buggenhout, S. V., Verlent, L., Smout, C., Loey, A. V., Hendrickx, M. (2006) Effect of pectinmethylesterase infusion methods and processing techniques on strawberry firmness. Journal of food science, 70(6), s383-s388.

Edger, P. P., Poorten, T. J., VanBuren, R., Hardigan, M. A., Colle, M., McKain, M. R., Smith, R. D., Teresi, S. J., Nelson, A. D. L., Wai, C. M., Alger, E. I., Bird, K. A., Yocca, A. E., Pumplin, N., Ou, S., Ben-Zvi, G., Brodt, A., Baruch, K., Swale, T., Shiue, L., Acharya, C. B., Cole, G. S., Mower, J. P., Childs, K. L., Jiang, N., Lyons, E., Freeling, M., Puzey, J. R., & Knapp, S. J. (2019) Origin and evolution of the octoploid strawberry genome. Nature Genetics, 51(3), 541-547.

Edger, P. P., VanBuren, R., Colle, M., Poorten, T. J., Wai, C. M., Niederhuth, C. E., Callow, P., Mckain, M. R., Shi, J., Collier, C., Xiong, Z., Mower, J. P., Slovin, J. P., Hytönen, T., Jiang, N., Childs, K. L., & Knapp, S. J. (2018) Single-molecule sequencing and optical mapping yields an improved genome of woodland strawberry (*Fragaria vesca*) with chromosome-scale contiguity. Gigascience, 7(2), gix124.

Folta, K. M., & Davis, T. M. (2006) Strawberry genes and genomics. Critical Reviews in Plant Sciences, 25(5), 399-415.

Giampieri, F., Alvarez-Suarez, J. M., & Battino, M. (2014) Strawberry and human health: Effects beyond antioxidant activity. Journal of Agricultural and

Food Chemistry, 62(18), 3867-3876.

Gidoni D, Rom M, Kunik T, Zur M, Izsak E, Izhar S, Firon N. (1994) Strawberry-cultivar Identification using randomly amplified polymorphic DNA (RAPD) markers. Plant Breed 113:339–342

Han, K., Lee, H. Y., Ro, N. Y., Hur, O. S., Lee, J. H., Kwon, J. K., & Kang, B. C. (2018) QTL mapping and GWAS reveal candidate genes controlling capsaicinoid content in Capsicum. Plant Biotechnology Journal, 16(9), 1546-1558.

Hardigan, M. A., Feldmann, M. J., Lorant, A., Famula, R., Acharya, C., Cole, G., Edger, P. P., & Knapp, S. J. (2019) Chromosome evolution of octoploid strawberry. BioRxiv, 861526.

Hirakawa, H., Shirasawa, K., Kosugi, S., Tashiro, K., Nakayama, S., Yamada, M., Kohara, M., Watanabe, A., Kishida, Y., Fujishiro, T., Tsuruoka, H., Minami, C., Sasamoto, S., Kato, M., Nanri, K., Komaki, A., Yanagi, T., Huoxin, Q., Maeda, J., Ishikawa, M., Kuhara, S., Sato, S., Tabata, S., & Isobe, S. N. (2014) Dissection of the octoploid strawberry genome by deep sequencing of the genomes of *Fragaria* species. DNA Research, 21(2), 169-181.

Harrison, E. P., McQueen-Mason, S. J., Manning, K. (2001) Expression of six expansin genes in relation to extension activity in developing strawberry fruit. Journal of Experimental Botany, 52(360), 1437-1446.

Hummer, K. E., & Hancock, J. (2009) Strawberry genomics: botanical history, cultivation, traditional breeding, and new technologies. In Genetics and genomics of Rosaceae (pp. 413-435). Springer, New York, NY.

Ichijima, K. (1926) Cytological and genetic studies on Fragaria. Genetics, 11(6), 590.

Jiang, G. L. (2013) Molecular markers and marker-assisted breeding in plants. Plant breeding from laboratories to fields, 45-83.

Jiménez-Bermúdez, S., Redondo-Nevado, J., Muñoz-Blanco, J., Caballero, J. L.,

López-Aranda, J. M., Valpuesta, V., Pliego-Alfaro, F., Quesada, M. A., Mercado, J. A. (2002) Manipulation of strawberry fruit softening by antisense expression of a pectate lyase gene. Plant physiology, 128(2), 751-759.

Kenis, K., Keulemans, J., & Davey, M. W. (2008) Identification and stability of QTL for fruit quality traits in apple. Tree Genetics & Genomes, 4(4), 647-661.

Khanizadeh, S., DeEll, J., Cousineau, J., & Lagrave, I. (1998, August) Comparison of three methods to evaluate fruit firmness in advanced strawberry selections. In XXV International Horticultural Congress, Part 7: Quality of Horticultural Products 517 (pp. 463-466).

Koskela, E. A., Sønsteby, A., Flachowsky, H., Heide, O. M., Hanke, M. V., Elomaa, P., & Hytönen, T. (2016) TERMINAL FLOWER 1 is a breeding target for a novel everbearing trait and tailored flowering responses in cultivated strawberry (*Fragaria* × *ananassa* Duch.). Plant Biotechnology Journal, 14(9), 1852-1861.

Lerceteau-Köhler E, Guerin G, Laigret F, Denoyes-Rothan B. (2003) Characterization of mixed disomic and polysomic inheritance in the octoploid strawberry (*Fragaria* × *ananassa*) using AFLP mapping. Theoretical and Applied Genetics, 107:619–628

Lerceteau-Köhler, E., Moing, A., Guérin, G., Renaud, C., Petit, A., Rothan, C., & Denoyes, B. (2012) Genetic dissection of fruit quality traits in the octoploid cultivated strawberry highlights the role of homoeo-QTL in their control. Theoretical and Applied Genetics, 124(6), 1059-1077.

Li, H., & Durbin, R. (2009) Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics, 25(14), 1754-1760.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., & Durbin, R. (2009) The sequence alignment/map format and SAMtools. Bioinformatics, 25(16), 2078-2079.

Liu, L., Ji, M. L., Chen, M., Sun, M. Y., Fu, X. L., Li, L., Gao, D. S., & Zhu, C. Y. (2016) The flavor and nutritional characteristic of four strawberry varieties

cultured in soilless system. Food Science & Nutrition, 4(6), 858-868.

Luciano Da Costa, E. S., Wang, S., & Zeng, Z. B. (2012) Composite interval mapping and multiple interval mapping: procedures and guidelines for using Windows QTL Cartographer. In Quantitative trait loci (QTL) (pp. 75-119). Humana Press.

Mathey, M. M. (2013) Phenotyping diverse strawberry (*Fragaria* spp.) germplasm for aid in marker-assisted breeding, and marker-trait association for red stele (Phytophthora fragariae) resistance marker Rpf1.

Ming, R., & Wai, C. M. (2015) Assembling allopolyploid genomes: no longer formidable. Genome Biology, 16(1), 27.

Mora, F., Zúñiga, P. E., & Figueroa, C. R. (2019) Genetic variation and trait correlations for fruit weight, firmness and color parameters in wild accessions of *Fragaria chiloensis*. Agronomy, 9(9), 506.

Moreno, E., Obando, J. M., Dos-Santos, N., Fernández-Trujillo, J. P., Monforte, A. J., & Garcia-Mas, J. (2008) Candidate genes and QTL for fruit ripening and softening in melon. Theoretical and Applied Genetics, 116(4), 589-602.

Moumouni, K. H., Kountche, B. A., Jean, M., Hash, C. T., Vigouroux, Y., Haussmann, B. I. G., & Belzile, F. (2015) Construction of a genetic map for pearl millet, *Pennisetum glaucum* (L.) R. Br., using a genotyping-by-sequencing (GBS) approach. Molecular breeding, 35(1), 5.

Posé, S., García-Gago, J. A., Santiago-Doménech, Nieves., Pliego-Alfaro, F., Quesada, M. A., Mercado, F. A. (2011) Strawberry fruit softening: role of cell wall disassembly and its manipulation in transgenic plants. Genes, Genomes and Genomics, 5(1), 40-48.

Potter, D., Eriksson, T., Evans, R. C., Oh, S., Smedmark, J. E. E., Morgan, D. R., Kerr, M., Robertson, K. R., Arsenault, M., Dickinson, T. A., & Campbell, C. S. (2007) Phylogeny and classification of Rosaceae. Plant Systematics and Evolution 266:5–43

Rabbi, I. Y., Hamblin, M. T., Kumar, P. L., Gedil, M. A., Ikpan, A. S., Jannink,

J. L., & Kulakow, P. A. (2014) High-resolution mapping of resistance to cassava mosaic geminiviruses in cassava using genotyping-by-sequencing and its implications for breeding. Virus Research, 186, 87-96.

Rho, I. R., Woo, J. G., Jeong, H. J., Jeon, H. Y., & Lee, C. H. (2012) Characteristics of F1 hybrids and inbred lines in octoploid strawberry (*Fragaria× ananassa* Duchesne). Plant Breeding, 131(4), 550-554.

Saladié, M., Matas, A. J., Isaacson, T., Jenks, M. A., Goodwin, S. M., Niklas, K. J., Xiaolin, R., Labavitch, J. M., Shackel, K. A., Fernie, A. R., Lytovchenko, A., O'Neill, M. A., Watkins, C. B., & Rose, J. K. C. (2007) A reevaluation of the key factors that influence tomato fruit softening and integrity. Plant Physiology, 144(2), 1012-1028.

Sánchez-Sevilla JF, Horvath A, Botella MA, Gaston A, Folta K, Kilian A, Denoyes B., & Amaya I. (2015) Diversity Arrays Technology (DArT) marker platforms for diversity analysis and linkage mapping in a complex crop, the octoploid cultivated strawberry (*Fragaria × ananassa*). PLoS ONE 10:e0144960

Scheerens, J. C., & Brenneman, G. L. (1991) Fruit quality patterns among strawberry cultivars based on decade of release or area of adaptation. The strawberry into the 21st century. Timber Press, Portland, Ore, 111-114.3

Shulaev, V., Sargent, D. J., Crowhurst, R. N., Mockler, T. C., Folkerts, O., Delcher, A. L., Jaiswal, P., Mockaitis, K., Liston, A., Mane, S. P., Burns, P., Davis, T. M., Slovin, J. P., Bassil, N., Hellens, R. P., Evans, C., Harkins, T., Kodira, C., Desany, B., Crasta, O. R., Jensen, R. V., Allan, A. C., Michael, T. P., Setubal, J. C., Celton, J. M., Rees, D. J. G., Williams, K. P., Holt, S. H., Rojas, J. J. R., Chatterjee, M., Liu, B., Silva, H., Meisel, L., Adato, A., Filichkin, S. A., Troggio, M., Viola, R., Ashman, T. L., Wang, H., Dharmawardhana, P., Elser, J., Raja, R., Priest, H. D., Jr, D. W. B., Fox, S. E., Givan, S. A., Wilhelm, L. J., Naithani, S., Christoffels, A., Salama, D. Y., Carter, J., Girona, E. L., Zdepski, A., Wang, W., Kerstetter, R. A.,

Schwab, W., Korban, S. S., Davik, J., Monfort, A., Denoyes-Rothan, B., Arus, P., Mittler, R. Flinn, B., Aharoni, A., Bennetzen, J. L., Salzberg, S. L., Dickerman, A. W., Velasco, R., Borodovsky, M., Veilleux, R. E., & Folta, K. M. (2011) The genome of woodland strawberry (*Fragaria vesca*). Nature Genetics, 43(2), 109.

Tang, H., Zhang, X., Miao, C., Zhang, J., Ming, R., Schnable, J. C., Schnable, P. S., Lyons, E., & Lu, J. (2015) ALLMAPS: robust scaffold ordering based on multiple maps. Genome Biology, 16(1), 3.

Tennessen, J. A., Govindarajulu, R., Ashman, T. L., & Liston, A. (2014) Evolutionary origins and dynamics of octoploid strawberry subgenomes revealed by dense targeted capture linkage maps. Genome Biology and Evolution, 6(12), 3295-3313.

Truong, H. T., Ramos, A. M., Yalcin, F., de Ruiter, M., van der Poel, H. J., Huvenaars, K. H., Hogers, R. C. J., Enckevort, L. J. G. V., Janssen, A.,Orsouw, N. J. V., & van Eijk, M. J. (2012) Sequence-based genotyping for marker discovery and co-dominant scoring in germplasm and populations. PloS one, 7(5).

Vining, K. J., Salinas, N., Tennessen, J. A., Zurn, J. D., Sargent, D. J., Hancock, J., & Bassil, N. V. (2017) Genotyping-by-sequencing enables linkage mapping in three octoploid cultivated strawberry families. PeerJ 5:e3731.

Voorrips, R. E. (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. Journal of Heredity, 93(1), 77-78.

Zorrilla-Fontanesi, Y., Cabeza, A., Domínguez, P., Medina, J. J., Valpuesta, V., Denoyes-Rothan, B., Sánchez-Sevilla, J. F., & Amaya, I. (2011) Quantitative trait loci and underlying candidate genes controlling agronomical and fruit quality traits in octoploid strawberry (*Fragaria× ananassa*). Theoretical and Applied Genetics, 123(5), 755-778.

ABSTRACT IN KOREAN

The allo-octoploid cultivated strawberry (Fragaria x ananassa Duchesne) 는 좋은 맛, 영양학적인 요소, 그리고 건강에 유익한 속성들로 인해 전세계적으로 생산되어오고 있지만, 4 개의 매우 유사한 sub-genomic 염색체 세트로 구성된 유전체의 매우 복잡한 특성으로 딸기의 분자 육종에 많은 어려움이 있었다. 하지만, 최근 시퀀싱과 스캐폴딩 기술의 발달로 염색체규모의 8 배체 유전체가 구축되어 딸기의 분자 육종을 위한 기반이 다져졌다. 이와 더불어 다양한 분자 마커의 발달로 분자육종뿐만 아니라 유전체 연구에도 도움이 될 수 있는 고밀도의 유전자 지도 구축이 가능하게 되었다. 이 연구에서 우리는 IStraw90 Axiom® SNP array 와 Genotyping-by-sequencing (GBS)기반의 마커를 슬라이딩 윈도우 방법을 이용하여 고밀도의 빈 맵을 구축하였다. 그 결과, 1,245 개의 빈과 3 개의 연관그룹들로 구성되며 8 배체 유전체 전체 물리적 길이의 87.7%를 커버하는 총 3,974.6 cM 길이의 고밀도 연관지도가 구축되었다. 이렇게 구축된 고밀도의 유전자 지도는 첫번째로 8 배체 유전체인 카마로사 유전체의 품질을 향상시키는데 이용되었다. 큰 길이의 스캐폴딩 오류가 나타나는 카마로사 염색체 1-2, 2-1, 6-2 그리고 6-4 번이 이 연구에서 구축된 유전자 지도 및 이전 연구에서 구축된 2 개의 유전자 지도의 순서를 기반으로 재조립되었다.

이 향상된 8 배체 딸기 유전체로 인해 DNA 변이의 발견, 유전자 발견, 유전자 지도 구축, QTL 분석 그리고 전장유전체 연관성 분석과 같은 연구 분야에서 더 정확하고 향상된 분석이 가능하게 되었다. 두번째로 이 고밀도의 유전자 지도는 딸기 과실의 품질에 중요하게 여겨지는 형질 중에 하나인 경도에 대하여 QTL 분석을 하는데 이용되었는데, 이 연구에서는 총 9 개의 QTL 을 찾아 낼 수 있었다. 또한, QTL 이 나타나는 영역에 빈 마커들과 딸기 과실의 경도에 영향을 주는 expansin 유전자의 위치를 비교함으로써 염색체 3-2 에서 가장 유의미한 마커를 도출해 낼 수 있었다. 결론적으로 고밀도, 고품질의 8 배체 유전체 및 유전자 지도 구축을 통해 딸기 분자 육종의 기초가 되는 발판이 만들어졌으며, 딸기 과실의 경도에 영향을 주는 expansin 유전자 위치에 가까이 연관되는 분자 마커를 확인 할 수 있었다. 본 연구 결과를 통하여, 경도에 관련된 연구뿐만 아니라 다른 중요한 형질들에 대한 분자 육종에 기반한 연구가 진행되어 딸기 신품종 육성을 가속화할 것으로 기대된다.

주요어: Allo-octoploid strawberry, IStraw90 Axiom SNP array, genotyping-by-sequencing (GBS), 표준 유전체, 고밀도 유전자 지도, 과실 경도

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Appendix 1. The physical position and description of genes related to fruit firmness in QTL regions of 'L80' population

Gene	Chr.	Start	End	Description (Similar to-)
g00119584	Chr1_2	12304413	12305979	Polygalacturonase (Prunus persica OX%3D3760)
g00119633	Chr1_2	12532414	12533974	Polygalacturonase (Prunus persica OX%3D3760)
g00119634	Chr1_2	12559870	12561066	Polygalacturonase (Prunus persica OX%3D3760)
g00120004	Chr1_2	14775201	14779529	GSVIVT00026920001: Probable polygalacturonase (Vitis vinifera OX%3D29760)
g00120143	Chr1_2	15499044	15508532	Os01g0875500: Beta-galactosidase 3 (Oryza sativa subsp. japonica OX%3D39947)
g00120146	Chr1_2	15528142	15529455	BGAL6: Beta-galactosidase 6 (Arabidopsis thaliana OX%3D3702)
g00120330	Chr1_2	16771309	16772498	EXPA7: Expansin-A7 (Arabidopsis thaliana OX%3D3702)
g00120343	Chr1_2	16858140	16862138	GSVIVT00026920001: Probable polygalacturonase (Vitis vinifera OX%3D29760)
g00088948	Chr2_2	6969685	6976200	BGAL3: Beta-galactosidase 3 (Arabidopsis thaliana OX%3D3702)
g00123344	Chr4_1	14616406	14618375	GSVIVT00026920001: Probable polygalacturonase (Vitis vinifera OX%3D29760)
g00123346	Chr4_1	14621051	14621757	GSVIVT00026920001: Probable polygalacturonase (Vitis vinifera OX%3D29760)
g00001268	Chr6_3	30098678	30099322	EXPA3: Expansin-A3 (Arabidopsis thaliana OX%3D3702)
g00001949	Chr6_3	33620623	33621817	EXPA2: Expansin-A2 (Oryza sativa subsp. japonica OX%3D39947)
g00148152	Chr7_2	20632186	20633500	EXPA4: Expansin-A4 (Arabidopsis thaliana OX%3D3702)
g00145538	Chr7_2	22514418	22515756	EXPA4: Expansin-A4 (Arabidopsis thaliana OX%3D3702)
g00145939	Chr7_2	24147888	24149732	EXPA8: Expansin-A8 (Arabidopsis thaliana OX%3D3702)
g00146038	Chr7_2	24475934	24478370	Polygalacturonase (Prunus persica OX%3D3760)

g00146108	Chr7_2	24741525	24745696	EXPB2: Putative expansin-B2 (Arabidopsis thaliana OX%3D3702)
g00130361	Chr7_3	1065755	1068121	Polygalacturonase (Actinidia deliciosa OX%3D3627)
g00130651	Chr7_3	2756289	2757938	plaa2: Exopolygalacturonase (Fragment) (Platanus acerifolia OX%3D140101)
g00130694	Chr7_3	3053252	3065124	lacZ: Beta-galactosidase (Photobacterium profundum (strain SS9) OX%3D298386)
g00131920	Chr7_3	10356998	10358206	EXPB18: Expansin-B18 (Oryza sativa subsp. japonica OX%3D39947)

Appendix 2. The physical position and description of genes related to fruit firmness in QTL regions of 'T137' population

Gene	Chr.	Start	End	Description (Similar to-)
g00088948	Chr2_2	6969685	6976200	BGAL3: Beta-galactosidase 3 (Arabidopsis thaliana OX%3D3702)
g00090149	Chr2_2	13055686	13058012	plaa2: Exopolygalacturonase (Fragment) (Platanus acerifolia OX%3D140101)
g00090165	Chr2_2	13101184	13102462	EXPA20: Expansin-A20 (Arabidopsis thaliana OX%3D3702)
g00090549	Chr2_2	14801080	14804894	At3g07010: Probable pectate lyase 8 (Arabidopsis thaliana OX%3D3702)
g00090728	Chr2_2	15542817	15545056	Polygalacturonase (Malus domestica OX%3D3750)
g00041214	Chr3_3	7668371	7669882	EXPA13: Expansin-A13 (Arabidopsis thaliana OX%3D3702)
g00042006	Chr3_3	11773161	11774131	EXPA12: Expansin-A12 (Arabidopsis thaliana OX%3D3702)
g00123344	Chr4_1	14616406	14618375	GSVIVT00026920001: Probable polygalacturonase (Vitis vinifera OX%3D29760)
g00123346	Chr4_1	14621051	14621757	GSVIVT00026920001: Probable polygalacturonase (Vitis vinifera OX%3D29760)
g00124150	Chr4_1	18613686	18615890	At1g48100: Polygalacturonase At1g48100 (Arabidopsis thaliana OX%3D3702)