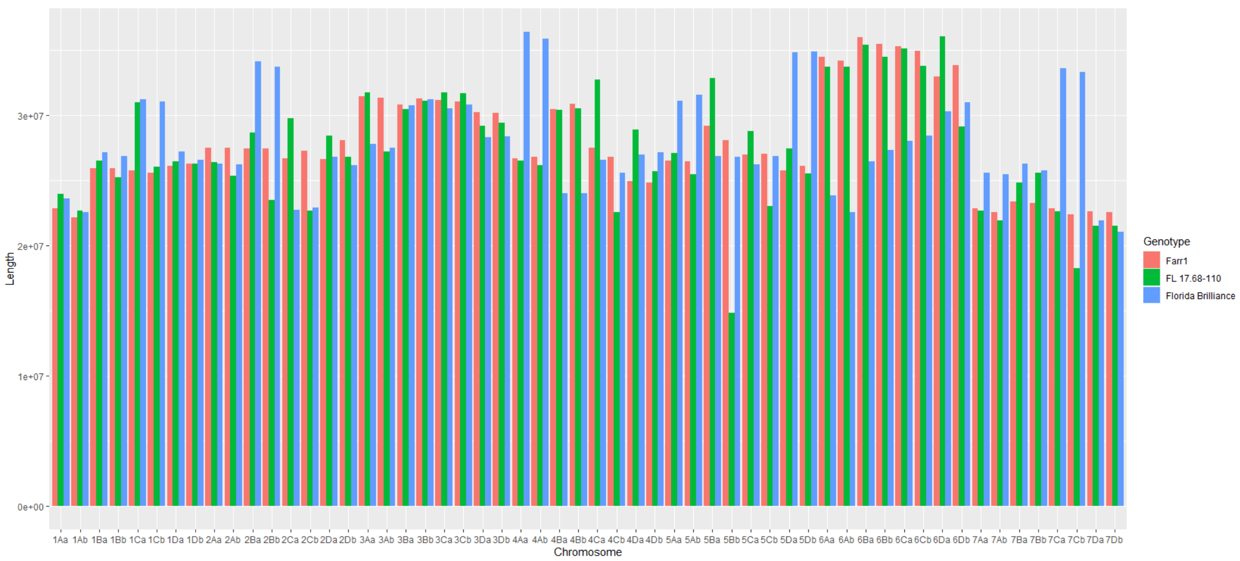
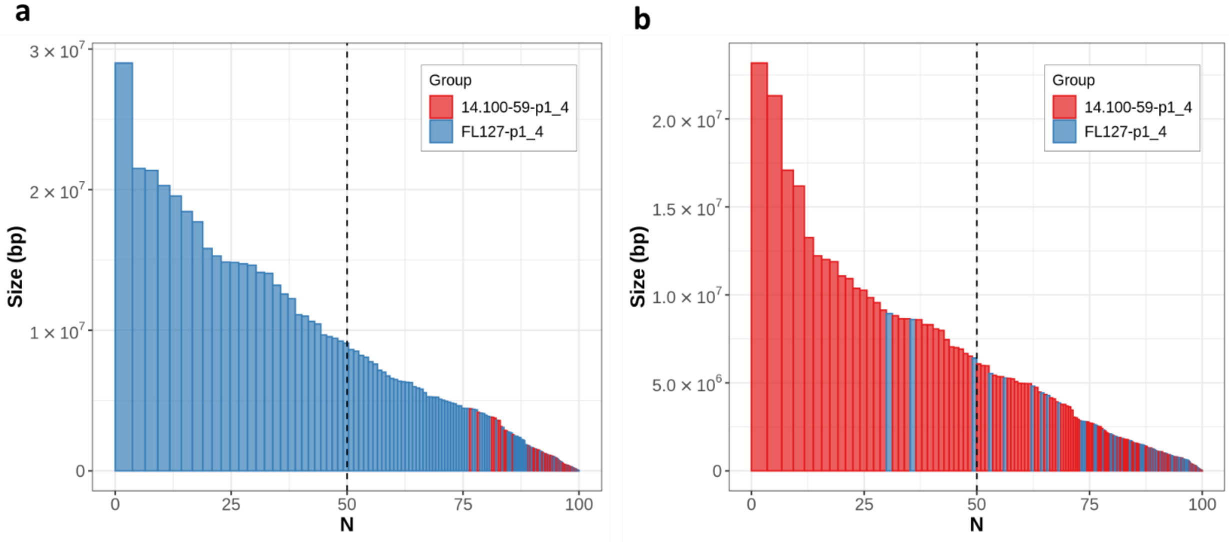


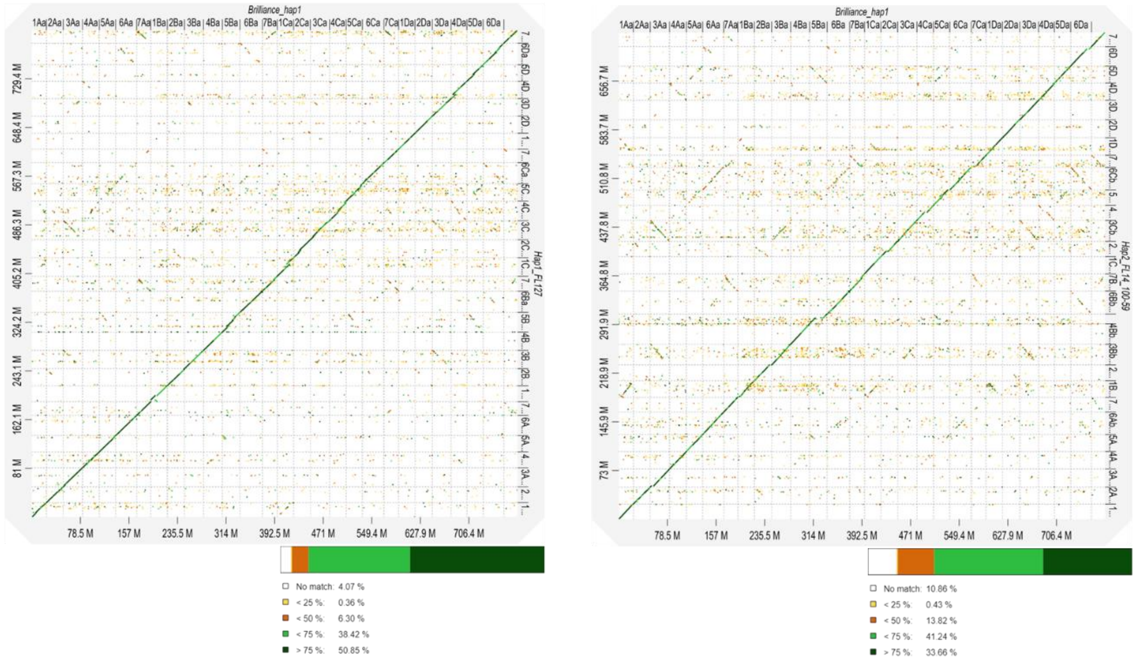
Supplementary Figure S1. Length of chromosome among three genomes; Royal Royce (Farr1), FL17.68-110 and ‘Florida Brilliance’ (FaFB1).



Supplementary Figure S2. Phase block plots for two haploids of FL17.68-110, 'Florida127' (FL127, **a**) and FL14.100-59 **(b)**, using Merqury. Most haplotype blocks are matched with the k-mer group from the corresponding haplotype, and blocks from incorrect haplotypes are found as small entities.

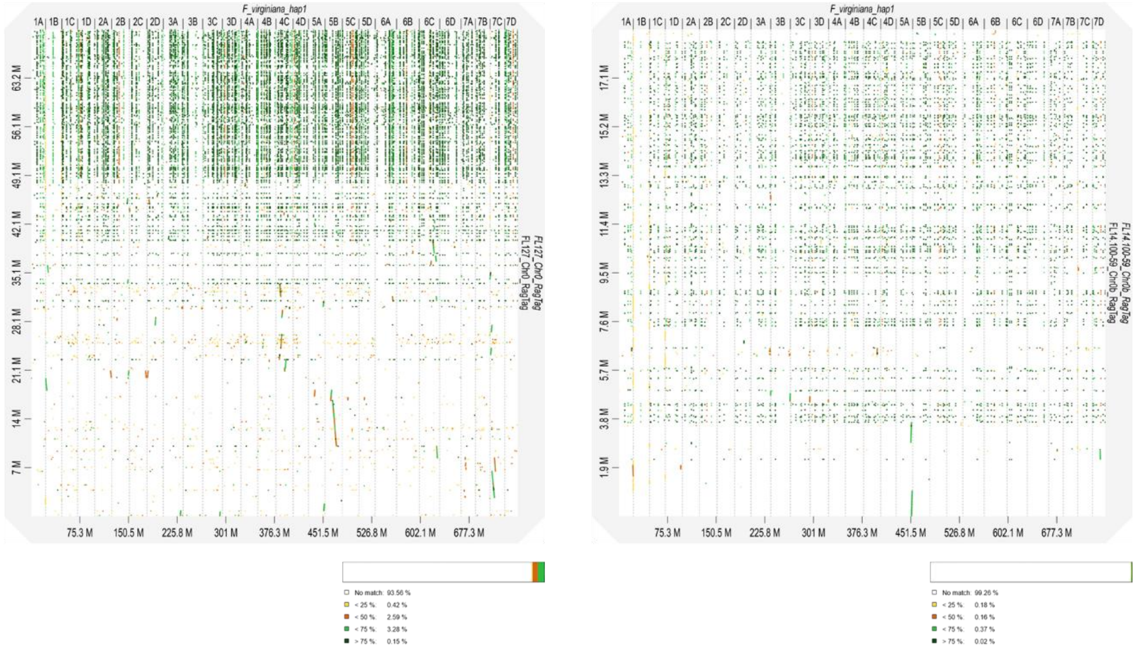
a. Collinearity between FaFB1 and FL127

b. Collinearity between FaFB1 and FL14.100-59

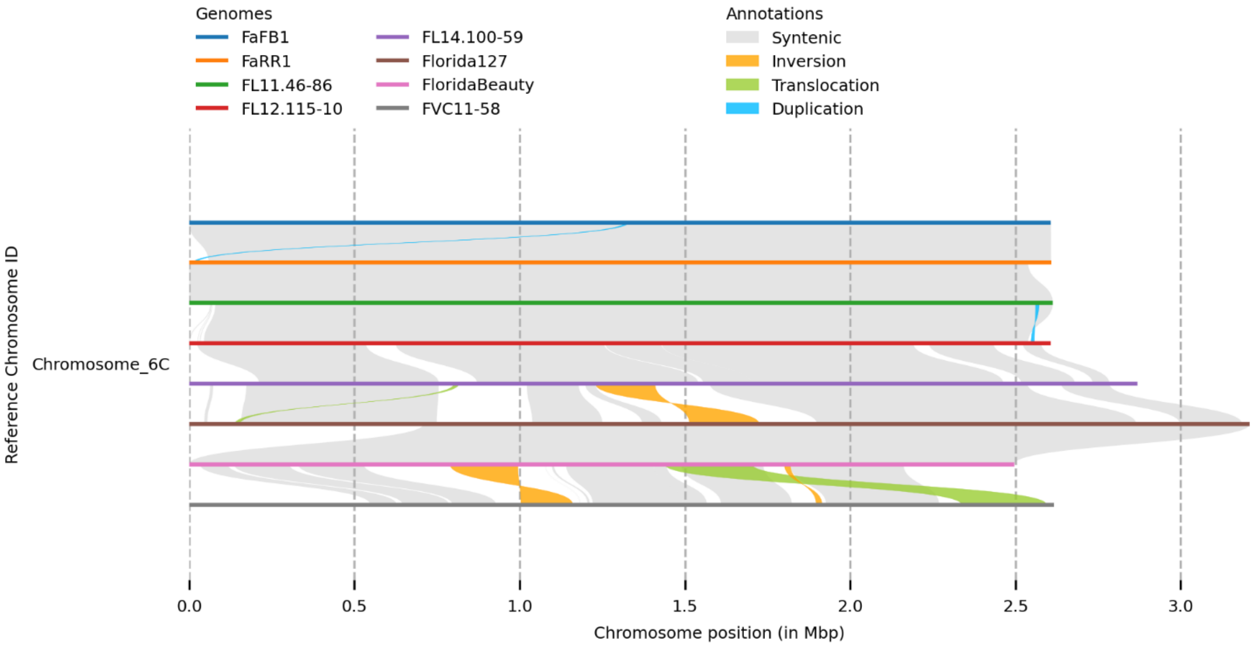


c. Collinearity between *F. virginiana* and unscaffolded contigs from FL127

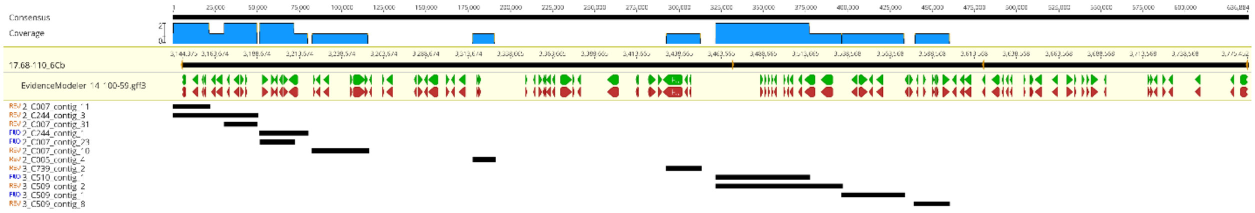
d. Collinearity between between *F. virginiana* and unscaffolded contigs from FL14.100-59



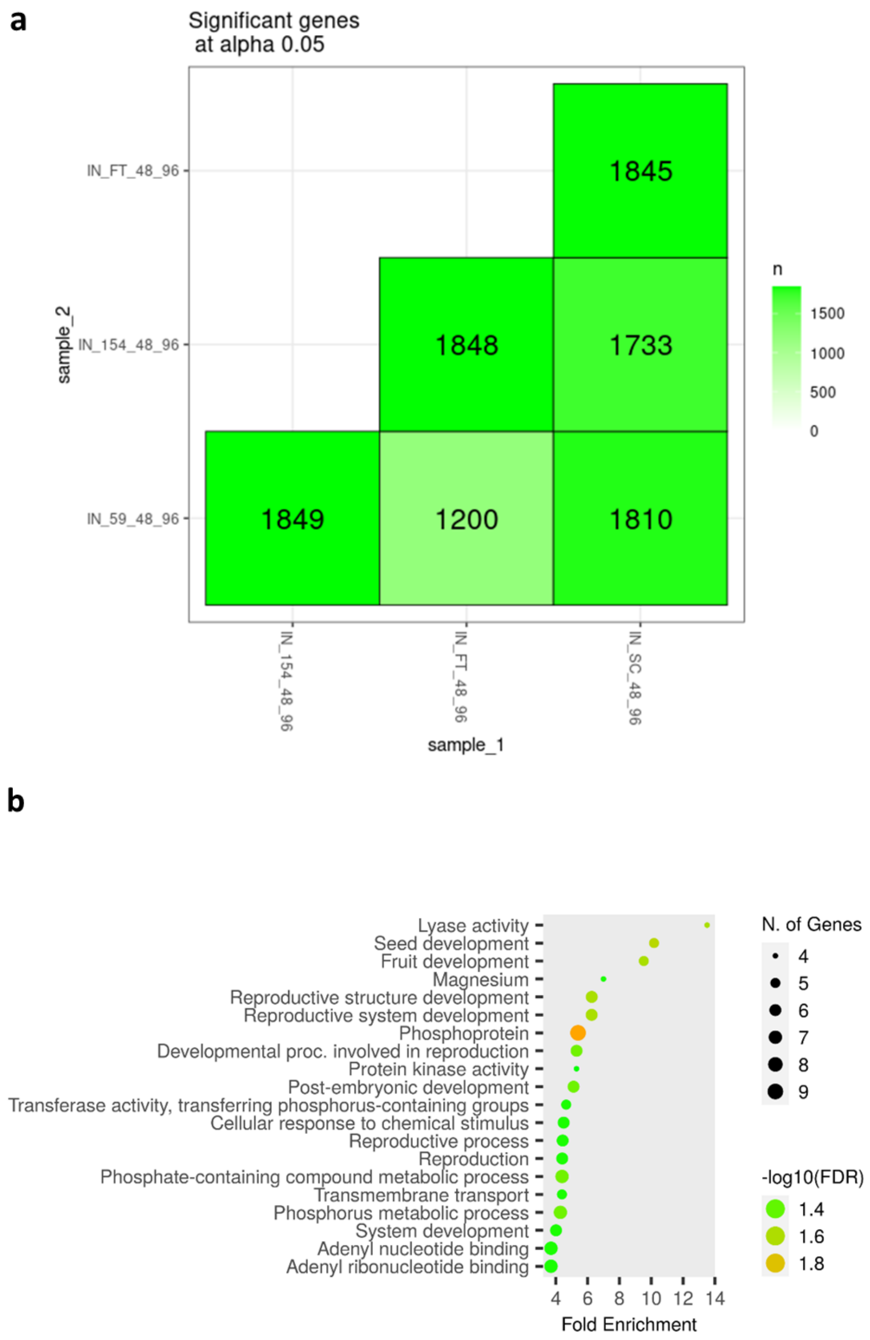
Supplementary Figure S3. Dot plots generated comparing two haploids of FL17.68-110 to *Fragaria ananassa* and *F. virginiana*. Brilliance (FaFB1) was compared with Haploid 1 **(FL127, a)** and Haploid 2 **(FL14.100-59, b)**. Additionally, dot plots illustrate the comparison of *F. virginiana* with unscaffolded contigs from FL127 (**c**) and FL14.100-59 (**d**), respectively.



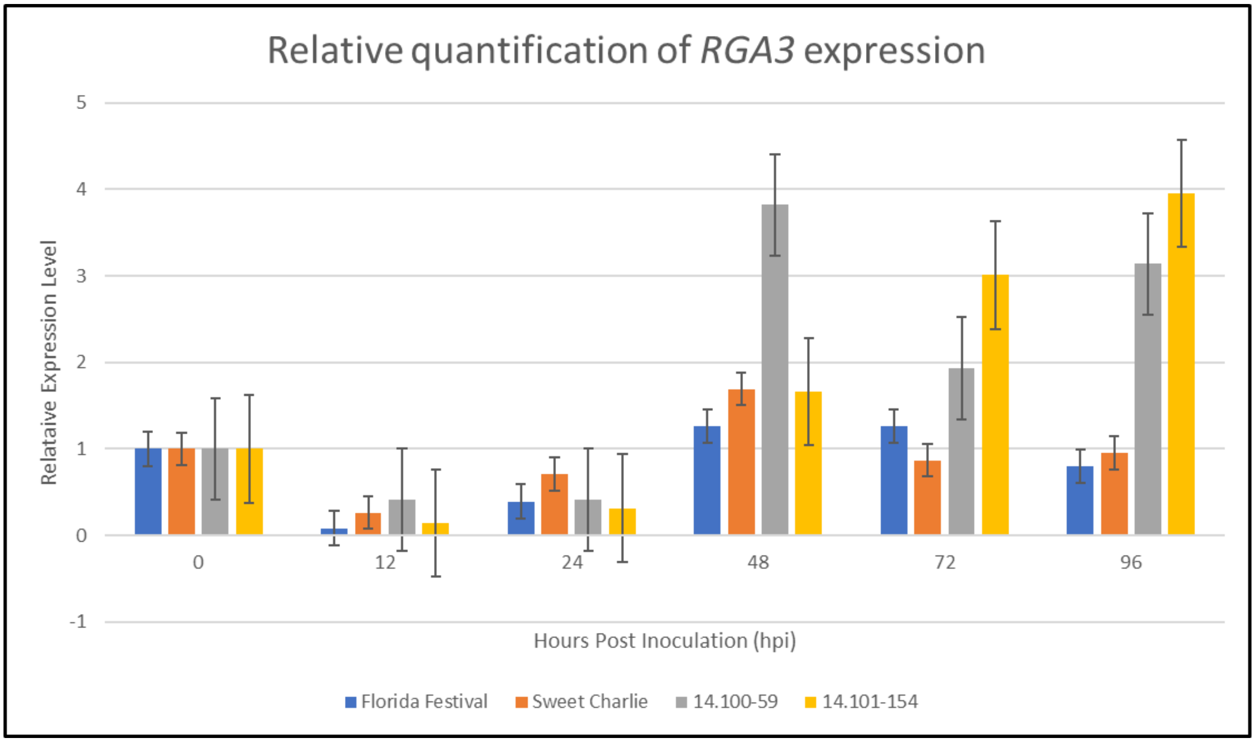
Supplementary Figure S4. Comparative genomics of eight octoploid strawberry reference genomes; ‘Florida Brilliance’ (FaFB1), Royal Royce (FaRR1), FL11.46-86, FL12.115-10, FL14.100-59, Florida127, Florida Beauty and FVC11-58.



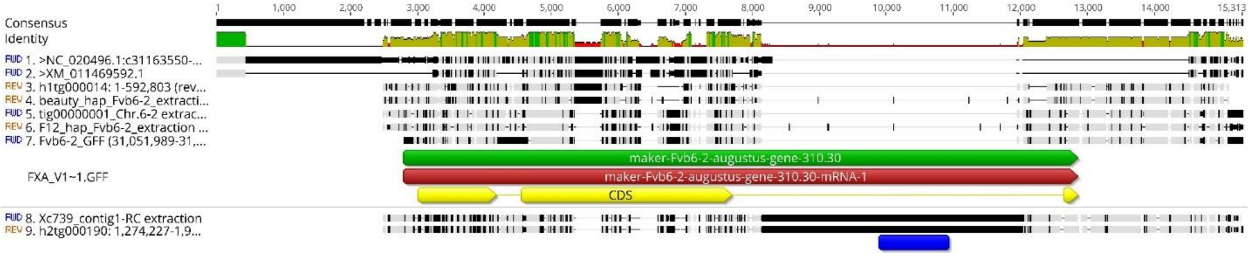
Supplementary Figure S5. Result of mapping bacterial artificial chromosome (BAC) clone contigs to a 600-kb *RXf1* in the FL14.100-59 genome.



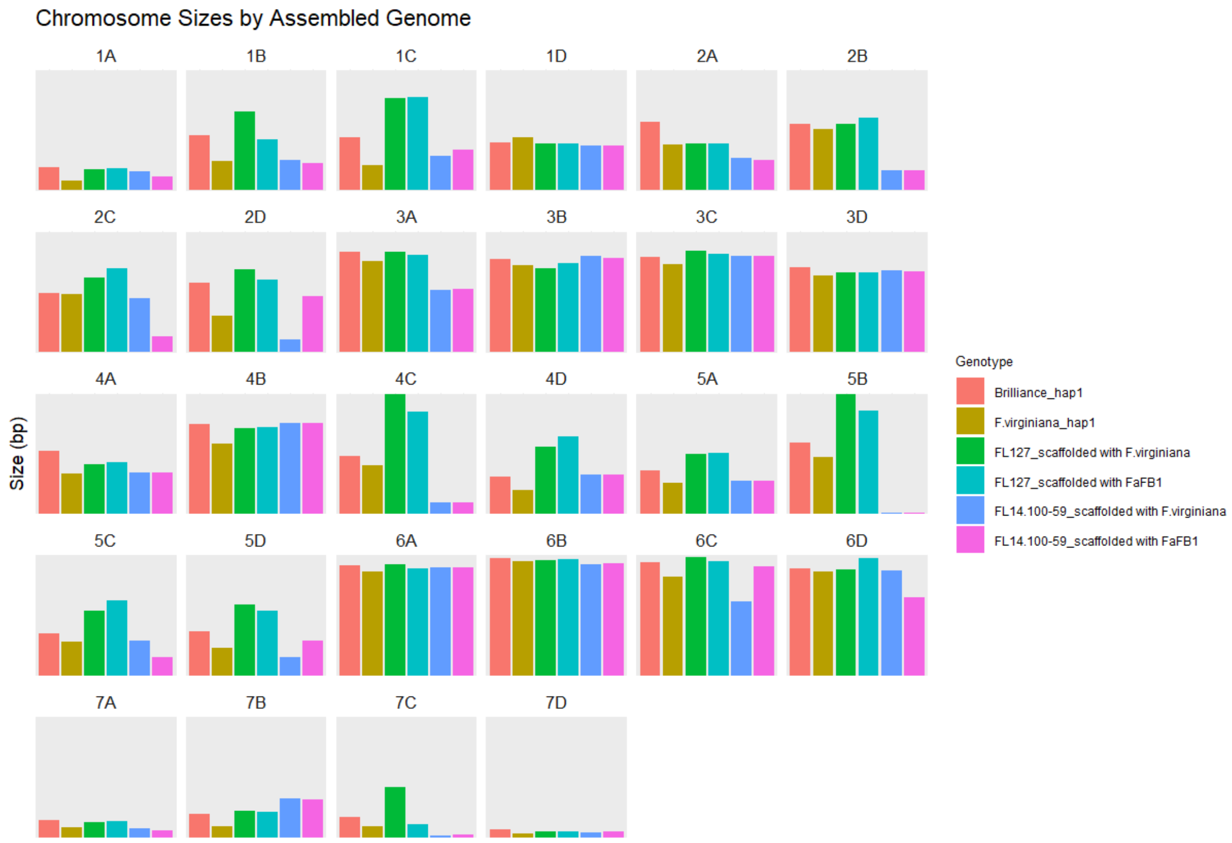
Supplementary Figure S6. **(a)** The number of significant genes from multi-directions comparison (FT: Florida Festival, 154: FL14.101-154, 59: FL14.100-59, and SC: Sweet Charlie). The label ‘IN’ indicates samples from *Xanthomonas fragariae* inoculation, and ‘48_96’ indicates samples from 48 and 96 hours after inoculation combined into a group. **(b)** Dot plot of GO (Gene Ontology) of differentially expressed genes (DEGs) showing the top 20 enriched pathways.



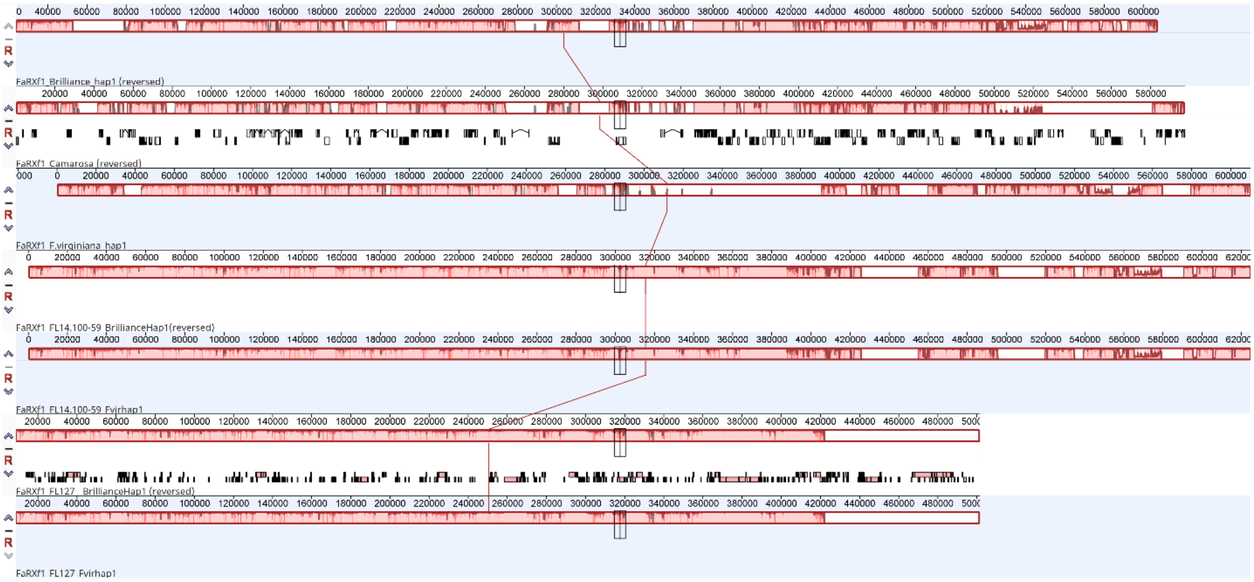
Supplementary Figure S7. Relative quantification of candidate gene *RGA3* expression in four cultivars at six different time points. Reverse transcript quantitative PCR (RT-qPCR) was conducted with the housekeeping gene *FaPDH* as a reference.



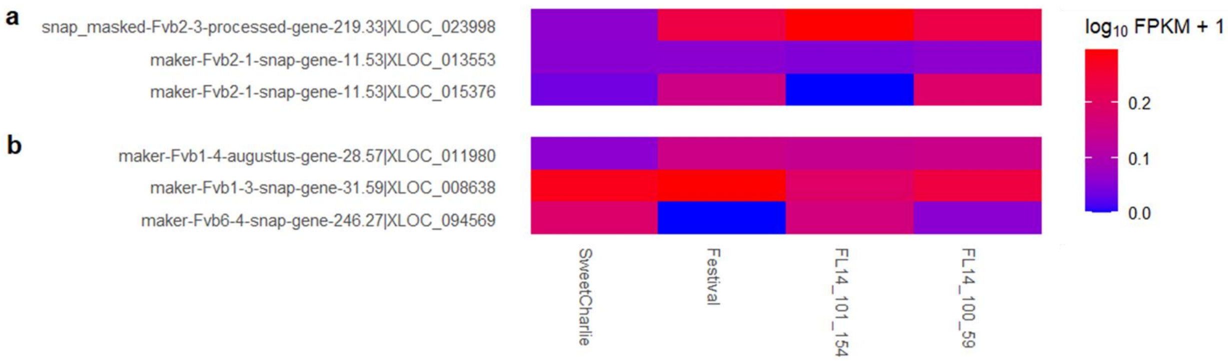
Supplementary Figure S8. Genomic sequence alignment of nine genomic sources for *RGA3* (1: genomic sequence from *Fragaria vesca*, 2: mRNA sequence from *F. vesca*, 3: genomic sequence from Florida127, 4: genomic sequence from Florida Beauty, 5: genomic sequence from FaFB1, 6: genomic sequence from FL12.115-10, 7: genomic sequence from 'Camarosa', 8: genomic sequence from FL14.101-225, 9: genomic sequence from FL14.100-59). Lane 8 and 9 are the ALS resistant genotypes. The blue annotation indicates the 3,820-bp insertion.



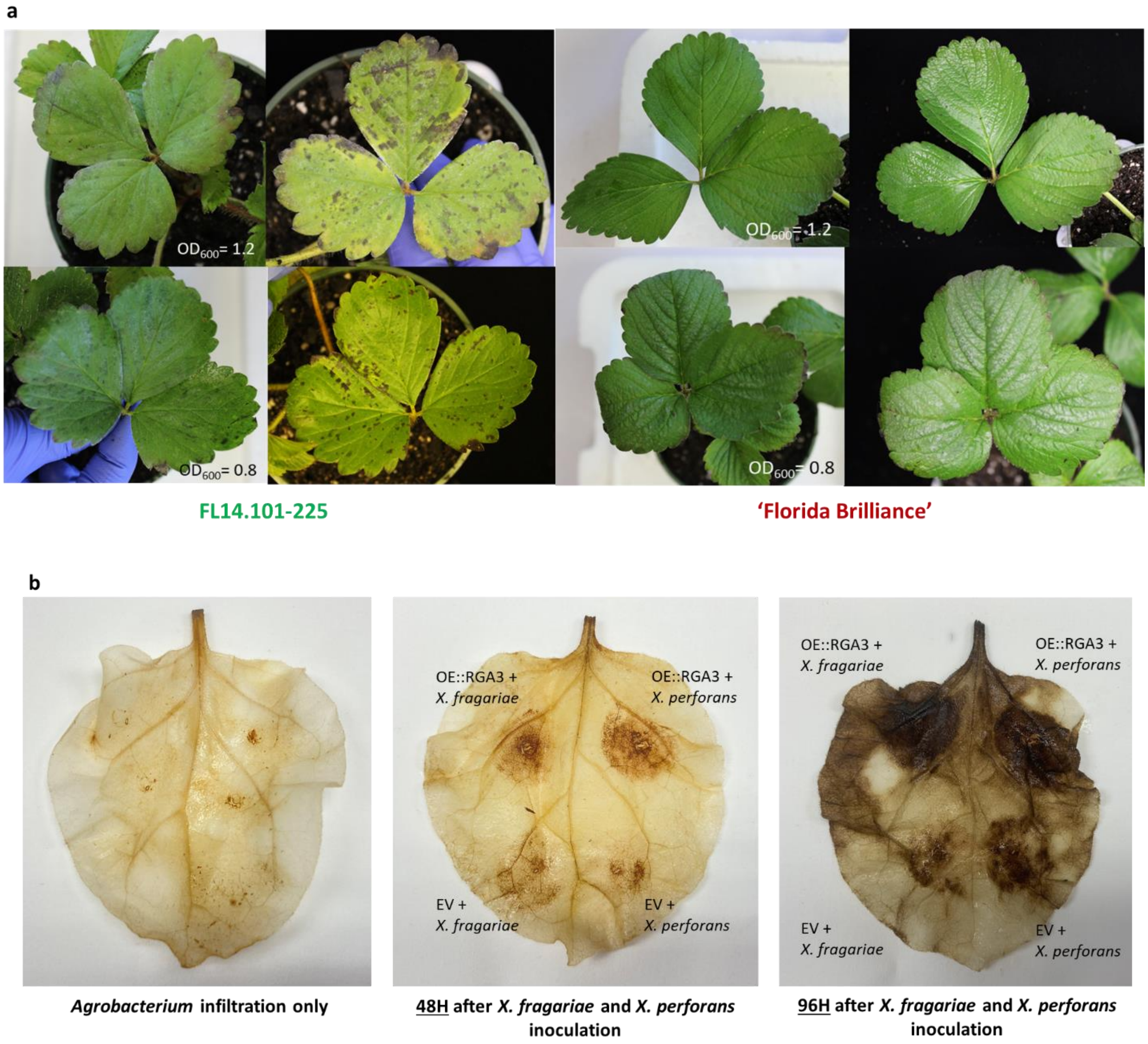
Supplementary Figure S9. Comparison of chromosome sizes between genomes. The first two blocks represent the chromosome sizes of the octoploid strawberry reference genomes, ‘Florida Brilliance’ (FaFB1) and *Fragaria virginiana*, respectively. The subsequent two blocks correspond to the assembled genomes of haplotype 1 (‘FL127’) from FL17.68-110, scaffolded using the *F. virginiana* and FaFB1 reference genomes. The final two blocks represent haplotype 2 (FL14.100-59) from FL17.68-110, scaffolded using the *F. virginiana* and FaFB1 reference genomes.



Supplementary Figure S10. Comparison of the genomic structure of *RXf1* across seven genomes. From the top lane downward, each lane represents the *RXf1* region derived from: ‘Florida Brilliance’ (FaFB1), ‘Camarosa’, *Fragaria virginiana*, FL14.100-59 scaffolded using FaFB1, FL14.100-59 scaffolded using *F. virginiana*, ‘Florida127’ scaffolded using FaFB1, and ‘Florida127’ scaffolded using *F. virginiana*.



Supplementary Figure S11. The expression of *PR1* (*pathogenesis-Related 1*, a) and *FRK1* (*Flagellin Receptor Kinase 1*, b) in four cultivars, Sweet Charlie, Festival, FL14.101-154, and FL14.100-59.



FL14.101-225

'Florida Brilliance'

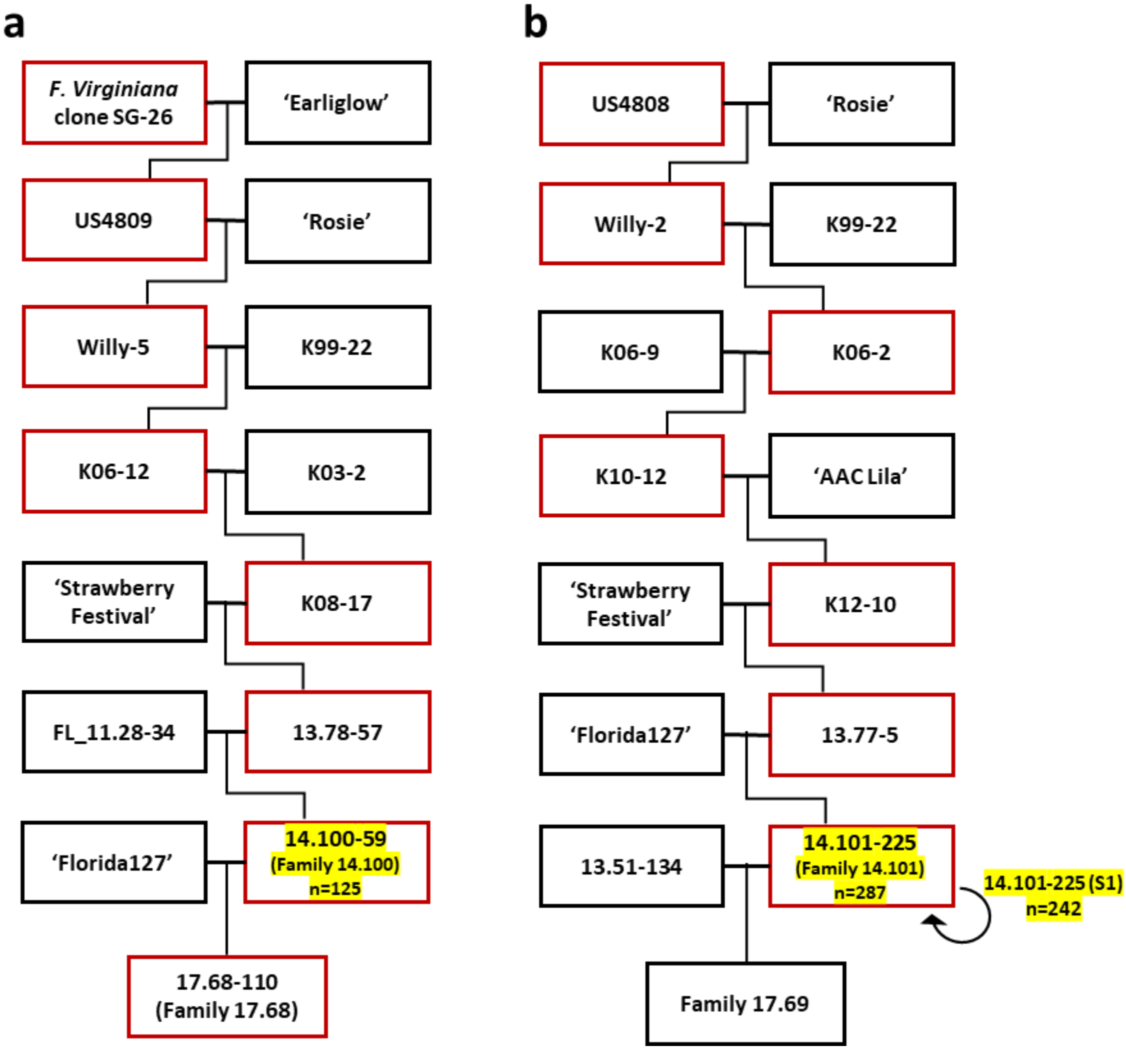
***Agrobacterium* infiltration only**

48H after *X. fragariae* and *X. perforans* inoculation

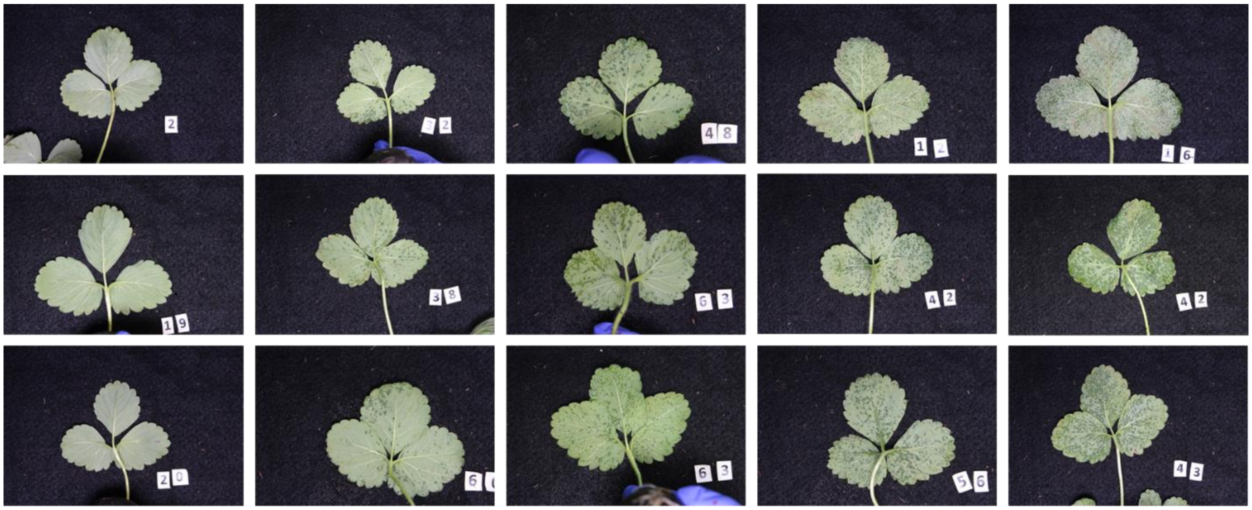
96H after *X. fragariae* and *X. perforans* inoculation

Supplementary Figure S12. Detection of hypersensitive reactions (HR) in octoploid strawberry cultivars and *Nicotiana tabacum*. **(a)** Two different concentrations of *Xanthomonas fragariae* inoculum, OD$_{600}$ 1.2 and 0.8, was vacuum-infiltrated into two strawberry cultivars, ALS-resistant FL14.101-225 and -susceptible 'Florida Brilliance'. The HR was observed at 24 and 96 hours after inoculation. **(b)** Transient overexpression of *RGA3* with inoculation of *X. fragariae* and *X. perforans* (bacteria causing bacterial spot in tomato and pepper) was performed in *Nicotiana tabacum*. Reactive oxygen species (ROS) was observed by diaminobenzidine tetrahydrochloride (DAB) staining.

Supplementary Figure S13. Genomic sequence alignment of three genomic sources for *RGA3*.



Supplementary Figure S14. Pedigree map of full-sib families used and generated in this study. (**a**) Family 14.100. (**b**) families 14.101 and selfing population 14.101-225. Red boxes indicate resistant individuals or families segregating for resistance. This figure is adapted and modified from Roach et al., 2016.



1
(0-20 %)

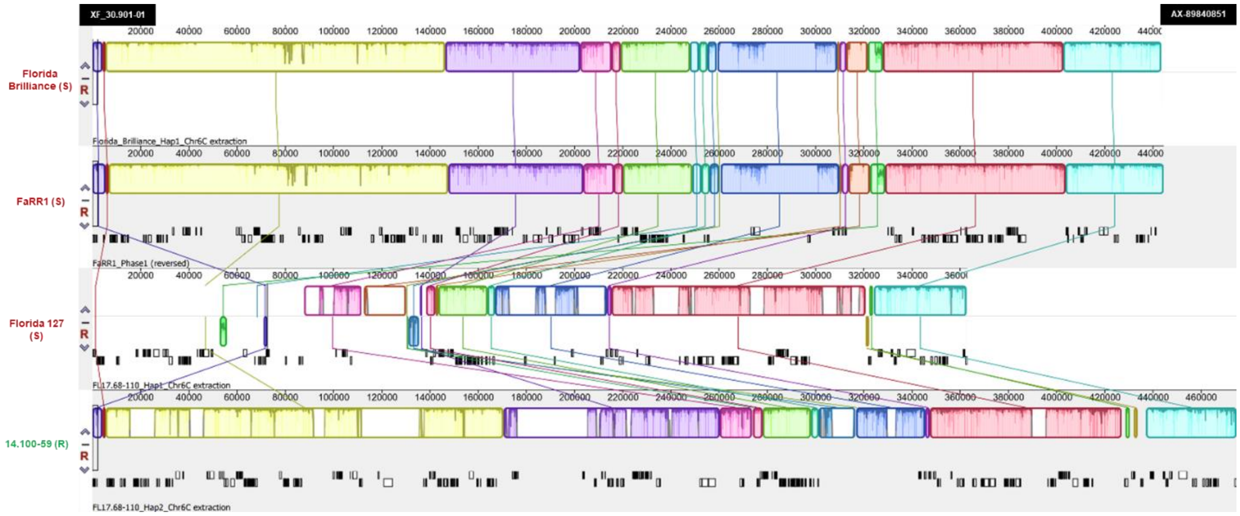
2
(20-40 %)

3
(40-60 %)

4
(60-80 %)

5
(80-100 %)

Supplementary Figure S15. Disease index for angular leaf spots in octoploid strawberry. Individual plants were scored for percent diseased leaf area on five scales from 0 to 100% with 20% increment.



Supplementary Figure S16. Genomic structure of *FaRXf1*. The structural variations and rearrangements between four sequences, ‘Florida Brilliance’ (FaFB1), Royal Royce (FaRR1), ‘Florida127’ and FL14.100-59, were diverse in the interval region between marker XF_30.901-01 and AX-89840851.