Genome re-annotation of the wild strawberry

Fragaria vesca using extensive Illumina- and SMRT-based RNA-seq datasets

Yongping Li¹, Wei Wei¹, Jia Feng¹, Huifeng Luo¹, Mengting Pi¹, Zhongchi Liu¹,², and Chunying Kang¹,*

¹Key Laboratory of Horticultural Plant Biology (Ministry of Education), College of Horticulture and Forestry Sciences, Huazhong Agricultural University, Wuhan 430070, China, and ²Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742, USA

*To whom correspondence should be addressed. Tel. +86 13871595425. Fax. +86 27 87282010. Email: ckang@mail.hzau.edu.cn

Edited by Dr. Sachiko Isobe

Received 13 July 2017; Editorial decision 25 August 2017; Accepted 29 August 2017

Abstract

The genome of the wild diploid strawberry species Fragaria vesca, an ideal model system of cultivated strawberry (Fragaria × ananassa, octoploid) and other Rosaceae family crops, was first published in 2011 and followed by a new assembly (Fvb). However, the annotation for Fvb mainly relied on ab initio predictions and included only predicted coding sequences, therefore an improved annotation is highly desirable. Here, a new annotation version named v2.0.a2 was created for the Fvb genome by a pipeline utilizing one PacBio library, 90 Illumina RNA-seq libraries, and 9 small RNA-seq libraries. Altogether, 18,641 genes (55.6% out of 33,538 genes) were augmented with information on the 5' and/or 3' UTRs, 13,168 (39.3%) protein-coding genes were modified or newly identified, and 7,370 genes were found to possess alternative isoforms. In addition, 1,938 long non-coding RNAs, 171 miRNAs, and 51,714 small RNA clusters were integrated into the annotation. This new annotation of F. vesca is substantially improved in both accuracy and integrity of gene predictions, beneficial to the gene functional studies in strawberry and to the comparative genomic analysis of other horticultural crops in Rosaceae family.

Key words: genome annotation, PacBio-RNAseq, Illumina-RNAseq, strawberry, Fragaria vesca

1. Introduction

The diploid strawberry F. vesca, also known as Alpine or woodland strawberry, is the most widely distributed species naturally growing in the northern hemisphere. It also contributed to one set of the octoploid genome of the cultivated strawberry Fragaria × ananassa.¹ F. vesca genome (~240 Mb) is relatively small and its genome sequence was published in 2011.² Several attributes of F. vesca make it a good model species of fruit crops, such as short stature, short life cycle, recurrent flowering, and high efficiency of transformation. To facilitate the gene functional studies in strawberry, we have identified and profiled the expression of protein-coding genes, lncRNAs and miRNAs using extensive transcriptome datasets generated mainly from flower and fruit tissues at different developmental stages with >80 individual libraries in F. vesca.³-⁷ As an emerging model species, F. vesca is playing more and more important roles both in the fields of basic biological research and in horticultural and agricultural research.

The genome of F. vesca was first sequenced from a fourth-generation inbred line of Hawaii⁴ (F. vesca ssp. vesca) solely by
second generation short read technologies, and its genome annotation v1.1 only contains protein-coding genes derived from de novo gene predictions.2 Then, an updated annotation version (v1.1.a2) became available using 50 RNA-seq libraries generated from 25 different fruit tissue types to improve the annotation accuracy of protein-coding genes.4 In the meantime, a refined assembly of the F. vesca reference genome, named Fvb, was generated based on dense linkage maps of the North American diploid F. vesca ssp. bracteata.7 Compared with the original genome FvH4, Fvb features many translocations and inversions. Although Fvb still contains a large number of gaps that could be closed by utilizing long read sequencing technology, it has a much shorter unanchored pseudo-chromosome. The location of FeMYB10, a master regulator of anthocyanin production in strawberry, is moved from Chromosome 6 in FvH4 to Chromosome 1 in Fvb, where it is supposed to be according to genetic studies, illustrating that Fvb is of high quality.10 However, the gene annotation of Fvb (v2.0.a1) is not updated and merely obtained from the realignment of v1.1.

Previously, we analysed the transcriptomes of floral and fruit tissues in F. vesca.4,5 During data analysis and gene functional studies, we noticed that a considerable proportion of genes are mis-annotated. The RNA-seq datasets from F. vesca (90 individual libraries) provide a rich data resource to re-annotate the genome. In addition, the PacBio full-length transcripts obtained from the fruit receptacle in F. vesca are also available,6 which were proven to significantly increase the accuracy of genome annotation without any assembly.11,12

The annotation v2.0.a1 of Fvb contains only the coding regions from translation start site (ATG) to stop codon of protein-coding genes. However, recent studies highlight the importance of short ORFs (open reading frame) in 5' UTRs of fine-tuning gene functions.13 The 3' UTR sequences are also frequently needed for designing experiments. In addition, alternative splicing is an important regulatory mechanism at the post-transcriptional level, and up to 60% multi-exon protein-coding genes in plant genomes possess more than one isoform.7 Besides protein-coding genes, non-coding transcripts are pervasively expressed and constitute an integral part of the transcriptomes, including long non-coding RNAs (lncRNAs) and miRNAs.5,6 Collectively, an updated genome annotation for F. vesca that marries the Fvb assembly with the broader collection of accurately predicted genes, coding and non-coding, is timely and highly desirable.

Here, we optimized the genome annotation pipeline to improve the gene predictions in Fvb. We used a combination of MAKER2, AUGUSTUS, Program to Assemble Spliced Alignments (PASA), and manual curation to annotate protein-coding genes through integrating de novo gene predictions, 90 RNA-seq transcriptome libraries and one PacBio full-length transcript library.14-17 The new annotation named V2.0.a2 not only updates the gene models of 13,168 loci, but also augments alternatively spliced isoforms of 7,370 genes, 5' and 3' UTRs of 18,641 genes, 1,938 lncRNAs, 171 miRNAs, and 51,714 small RNA clusters. Overall, V2.0.a2 provides a much more complete annotation of the F. vesca genome and will better serve gene functional studies in strawberry and other Rosaceae species.
date last accessed) was employed to identify and classify repeats from the genome Fvb, and then RepeatMasker (Refbase version 20160829) was used to generate a masked genome. Plant protein sequences were downloaded from UniProt (http://www.uniprot.org/) (5 September 2017, date last accessed) on 21 January 2017. MAKER2 was run on the masked Fvb with following evidence: Fvb annotation v2.0.a1, the comprehensive transcriptome, UniProt proteins, AUGUSTUS trained models, SNAP trained models, and GeneMark trained models.

2.5. Further improve the genome using Augustus and EVM

To use Augustus, the following evidence were provided: (i) intron hints converted from uniquely mapped RNA-seq reads using the script bet_to_gff.pl bundled with GeneMark; (ii) intron hints converted from PacBio full-length transcripts by GMAP (http://bioinf.uni-greifswald.de/bioinf/wiki/pmwiki.php?n=Augustus.PacBioGMAP (5 September 2017, date last accessed)); (iii) Protein hints generated from mapping UniPro proteins to masked genome by Exonerate; (iv) repeat hints from the RepeatMasker output (http://bioinf.uni-greifswald.de/bioinf/wiki/pmwiki.php?n=Augustus.IncorporateRepeats (5 September 2017, date last accessed)). These hints were fed into Augustus to generate the high confidence gene models.

Next, EVidenceModeler (EVM) was used to combine MAKER2 gene models, Augustus gene models, transcripts from SMRT and Illumina RNA-seq, and UniProt proteins with a nonstochastic weighted value into confident consensus gene models. The weight value for each part is: six for MAKER2 gene models, eight for Augustus gene models, 10 for SMRT transcripts, eight for Illumina transcripts, and three for homologous proteins. Finally, PASA was used to improve the EVM gene models by modifying gene structures and adding UTR annotations and alternatively spliced isoforms.

2.6. Manual curation of new annotation

All the gene models were inspected one by one and manually curated using a plug-in called Apollo in JBrowse Genome Viewer based on the mapped reads from different RNA-seq libraries.

2.7. Alternative splicing analysis

ASTalavista was employed to classify the alternative splicing events in the v2.0.a2 annotation. It groups the events into four major types, namely IR, ES, AA, and AD. The event belonging to each type were extracted from the ASTalavista output by following codes: IR (AS code: 1^2,0), ES (AS code: 1- 2^, 0), AA (AS code: 1-,2-), and AD (AS code: 1^,2^).

2.8. Prediction of lncRNAs

Two approaches were used to identify lncRNAs. The transcripts combined from the assemblies of Illumina RNA-seq and PacBio full-length reads were used for screening lncRNAs. The mono-exonic transcripts were removed to reduce false positives due to transcriptional noise and ambiguous alignment. In the first approach, the transcripts with class_code ‘u’ (unknown intergenic transcript), ‘o’ (generic exonic overlap with a reference transcript), ‘x’ (natural antisense transcript, NAT), and ‘i’ (intronic transcript) were selected. Then, the transcripts with length > 200nt and ORF < 300nt were retained. Next, the left transcripts were blasted against the Swiss-Protein database by BLASTX (2.6.0) with E-values < 1e-3 to remove potential protein-coding transcripts. At last, we used Coding Potential Calculator to calculate the CPC score for each transcript, and only those with a CPC score < 0 were considered as lncRNA candidates. In the second approach, lncRNAs were identified from the same set of transcripts by FEELnc using the specificity threshold 0.97. The transcripts overlapping protein-coding genes in v2.0.a2 and < 200nt in length were eliminated. FEELnc used the ORF coverage (i.e. length of the longest ORF/length of the lncRNA transcript), k-mer frequency, nucleotide frequency, and codon usage to distinguish between miRNAs and lncRNAs. Finally, the consensus transcripts identified by both methods were designated as lncRNAs. These lncRNAs were grouped into two major types (genic and intergenic) and six subtypes based on the localization and the direction of transcription relative to the proximal protein-coding genes by the FEELnc classifier module called FEELncclassifier.

2.9. Alignment of small RNAs

The raw reads from 9 small RNA-seq libraries generated from tissues of F. vesca were used for the identification of small RNAs. First, the 3’ adapters were trimmed off using Cutadapt. Then, the resulting .fasta file was mapped against the Fvb genome using bowtie (version 0.12.8) with following parameters: -q -v 0 -p 30 -S -a -m 50. ShortStack was subsequently employed to identify small RNA clusters, and only the dominant 20- to 24-nt in length and DCL-derived small RNA loci (more possibly related to RNAi) called out by ShortStack were retained. The expression level of each small RNA cluster was normalized using the count mode in ShortStack with default settings.

2.10. Alignment of miRNAs

The stem-loop sequences of 171 miRNAs in F. vesca predicted by a previous report were mapped to the new F. vesca genome assembly Fvb by bowtie2 with default parameters, then the SAM file was converted into the GFF format by an in-house python script and integrated into the v2.0.a2 annotation file.

2.11. Experimental verification of new gene models

Total RNA was isolated from the fruit receptacle of YWSAF7 (the 7th generation inbred line of Yellow Wonder, an accession of F. vesca) using the Plant Total RNA Isolation Kit (Sangon Biotech, Shanghai, China. No. SK8631) following manufacturer’s instructions. cDNA was synthesized from 1 μg total RNA in 20 μl solution using the PrimeScriptTM RT reagent kit (TaKaRa, Shiga, Japan, Cat# RR047A). KOD DNA polymerase (TOYOBO Bio-Tech, Cat# F0934K) was used to amplify the coding regions of selected genes for Sanger sequencing with primers listed in Supplementary Table S5. The sequencing results were aligned with the sequences obtained from new annotations by Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) (5 September 2017, date last accessed).

3. Results and discussion

3.1. Update of gene models of protein-coding genes in Fvb based on SMRT and Illumina RNA-seq datasets

In this study, a new Fvb annotation named version 2.0.a2 (v2.0.a2) was created that includes protein-coding genes, alternatively spliced isoforms of multiexon genes, small RNAs, and lncRNAs. An optimized workflow to annotate protein-coding genes and their isoforms is shown in Figure 1. To take advantage of the extensive RNA-seq datasets, MAKER2, specifically designed for second-generation
genome projects, was first used to generate an initial protein-coding gene annotation.\textsuperscript{15} Input data for MAKER2 include (i) repeats masked genome Fvb, (ii) \textit{ab initio} gene predictors, including SNAP, GeneMark, and Augustus, each trained with high-fidelity gene models, (iii) plant protein sequences downloaded from UniProt databases, (iv) transcripts assembled from a total of 90 Illumina RNA-seq libraries, (v) full length transcripts obtained from one PacBio transcriptome library (Supplementary Table S1). The Illumina RNA-Seq datasets were generated from a series of tissue types in \textit{F. vesca}, including leaf, seedling, root, shoot apical meristem, flower meristem, anther and carpel from flowers at different developmental stages and different fruit tissues at five stages with an average of 30 million and a range from 12 million to 89 million reads per library (see details in Supplementary Table S1). In total, there are 2.5 billion RNA-Seq short reads. The PacBio data was generated from fruit receptacles pooled from small green to mature stages in \textit{F. vesca}, containing 82,360 full-length consensus transcripts derived from 442,601ReadofInserts.

The ambiguity of transcript reconstruction will affect the accuracy of gene annotation when using MAKER2. To avoid assembly errors in gene prediction, it is advantageous to use the transcript information in unassembled mapped reads.\textsuperscript{27} Augustus is a gene finder that can incorporate data from RNA-seq reads (junctions reads) directly into weighted predictions, join exons into a single gene and identify alternative transcription events.\textsuperscript{23} Therefore, Augustus was employed to further improve gene predictions through incorporating the intron hints from RNA-seq reads and protein hints from UniProt databases. Then, the gene structures predicted by both MAKER2 and Augustus were combined into consensus gene models using EVidenceModeler (EVM).\textsuperscript{27} and the gene models generated from EVM were updated by PASA.\textsuperscript{14} At last, the new annotation was inspected across the entire genome in IGV\textsuperscript{18} to evaluate the prediction and select the optimal gene models by comparing with the aligned RNA-seq reads, and about 1,000 (3\%) genes were manually curated in this way.

Annotation v2.0.a2 of the genome Fvb contains a final set of 33,538 protein-coding loci with 50,738 transcripts (Table 1 and Supplementary Table S2). The Locus IDs for 30,012 identical or newly numbered using the same nomenclature as previous annotations (i.e. GeneXXXXX). The Locus IDs in both versions for the newly modified genes are listed in Supplementary Table S3. One improvement of v2.0.a2 is that a total of 18,641 genes were augmented by inclusion of 5' UTRs and/or 3' UTRs, representing nearly 55.6\% of all protein-coding genes (Fig. 2a and b). Moreover, the gene models of 13,168 protein-coding genes were modified and updated (Fig. 2a and c). More specifically, 2,692 genes were derived from splitting longer genes (previously fused genes), and 213 genes were obtained by fusing neighbouring genes (Fig. 2a, d, and e). In addition, 2,031 genes were removed, and 631 genes were added (Fig. 2a). To further evaluate the quality of the annotation, the BUSCO genes\textsuperscript{39} were compared between v2.0.a1 and v2.0.a2. The results show a significant increase of complete BUSCOs from 88.9\% for v2.0.a1 to 95.7\% for v2.0.a2, while the fragmented and missing BUSCOs were reduced in v2.0.a2 (Table 1), indicating a higher quality annotation. These updates will better serve gene structure and functional studies.

3.2. Functional annotation of protein-coding genes

To update the functional annotation of novel and pre-existing protein-coding genes in v2.0.a2, all the protein sequences were blasted against known proteins in the InterPro databases using InterProScan.\textsuperscript{40} In addition, each gene was also blasted against the NCBI non-redundant (nr) protein database to gain the Gene ontology (GO) term by Blast2go.\textsuperscript{41} As a result, a total of 28,798 and 22,106 genes in v2.0.a2 gained functional annotations and GO terms, respectively (Table 1). Especially, there is a great increase in the percentage of genes with GO terms, from 51\% in v2.0.a1 to 66\% in v2.0.a2 (Table 1). For novel genes, 527 of the 631 loci (83.5\%) were assigned with functional annotations.

Transcription factors and regulators and protein kinases usually play crucial roles during signal transduction. In the iTAK pipeline\textsuperscript{42} that we used for the classification of these genes, transcription factors are defined as proteins that regulate the expression of target genes by binding to specific cis-elements in promoter regions, while transcriptional regulators operate indirectly via interaction with the basal transcription apparatus (e.g. transcription factors) or by altering the accessibility of DNA to TFs via chromatin remodelling to regulate
the expression of target genes. As a result, we identified 1,542 transcription factors from 68 families, and 390 transcriptional regulators in the v2.0.a2 annotation by using the iTAK pipeline (Supplementary Table S4). The total number of transcription factors is comparable to that of v2.0.a1, but a few gene families have gained relatively more members, such as the B3 family from 67 to 78, MYB from 112 to 118, and bHLH from 90 to 101 (Supplementary Table S4). There are 1,055 protein kinase encoding genes in v2.0.a2, which are 90 more in the older annotation (Supplementary Table S4).

Disease resistance is an important agricultural trait for strawberry as well as other fruit crops, thus we also used the RGAugury pipeline to analyse R-genes and pattern-recognition receptors (PRRs), collectively called resistance gene analogs (RGAs), in the two annotation versions of Fvb. The RGAs are classified into four major families based on conserved domains and motifs in this pipeline, namely NBS encoding, membrane associated RLP (receptor-like protein) and RLK (receptor-like protein kinase), and TM-CC (transmembrane-coiled-coil). The first three families were further grouped into a few subfamilies. A total of 1,190 RGAs were identified, including 340 NBS encoding genes, 122 RLPs, 590 RLKs, and 138 TM-CCs in v2.0.a2 (Supplementary Table S5). Although the gene number in the NBS encoding family is comparable between the two annotations, the TNL subfamily has much more members (from 17 to 79), while the TX subfamily has fewer members (from 124 to 59) (Supplementary Table S5). The size of the RLP and RLK families doesn’t change much. However, the gene number in the TM-CC family is significantly decreased, from 192 genes in v2.0.a1 to 138 genes in v2.0.a2.

With the advantage of strand-specific RNA-seq reads, we were able to distinguish the sense and antisense transcripts from one locus. One example of such a locus having overlapping transcripts derived from opposite strands is gene02467, which codes for a tRNA ligase. This gene is located in one intron of an extremely long gene called gene02466 that encodes the ATP-binding cassette transporter A1 (Fig. 3a). By contrast, the same locus was previously annotated to
have four genes in v2.0.a1 (Fig. 3a). FvSOC1, the homolog of AtSOC1 (*SUPPRESSOR OF THE OVEREXPRESSION OF CONSTANS*), is a key negative regulator of flowering and a positive regulator of running in *F. vesca*.44 FvSOC1 does not exist in v2.0.a1. However, the modified gene model in v2.0.a2 has exactly the same sequence as the EST of FvSOC1 (accession number in NCBI: JF806634) (Fig. 3b). In addition, some gene models were fine-tuned in v2.0.a2 to have a higher annotation quality. For instance, FvTFL1, another crucial floral repressor in *F. vesca*,45,46 has a new translation start site producing a shorter protein, which becomes consistent with the published sequence in NCBI (accession number: JF806631) (Fig. 3c). A few other mis-annotated genes encoding glutathione S-transferases (GSTs), the red/far-red photoreceptor phytochrome B, and a bHLH transcription factor were PCR amplified, cloned and sequenced to confirm the v2.0.a2 annotation (Supplementary Fig. S1 and Table S6). Taken together, not only were a large proportion of pre-existing genes corrected, but also novel genes with potentially important roles were discovered and incorporated into the new annotation.

3.3. Alternatively spliced genes in v2.0.a2

Alternative splicing (AS) is an important regulatory mechanism at post-transcriptional level and is prevalent in animals as well as plants.47,48 However, the v2.0.a1 annotation does not provide any information about the isoforms alternatively spliced from the premRNAs. The v2.0.a2 annotation takes into account and provides the specific information on the alternatively spliced transcripts predicted by our analysis pipeline (Fig. 1). To increase the prediction accuracy, weakly expressed transcripts in the RNA-seq datasets were removed during the assembly using Stringtie.23 A total of 16,705 alternative transcripts were detected genome-wide from 7,370 multiexon genes, accounting for 21.98% of the 33,538 protein-coding genes. The average transcript number per gene is 1.51. As we used a more stringent filtering strategy, much fewer isoforms were presented in v2.0.a2 than that of our previous study (47,000 isoforms from 13,591 alternatively spliced genes, accounting for 41.6% of all the protein coding genes).7

Among the AS genes, about half of them (46.85%) possess only two isoforms, 23.19% genes possess three isoforms, and 1.28% genes have >10 isoforms (Fig. 4a). 7,370 AS genes showed enriched GO terms in all kinds of metabolic processes (Supplementary Table S7), consistent with our previous studies.7,49 To better characterize alternative splicing (AS) in the new annotation, AStalavista was used to group the AS events into a few major types.30 3,434 events are of alternative 3′ splice sites (A3), 1,351 events are of alternative 5′ splice sites (A5), 4,245 events are due to intron retention (IR), and 1,029 events result from exon skipping (ES) (Fig. 4b). Consistent with other studies,7,49 the percentage of IR (32.39%) is the greatest, while the percentage of ES (7.85%) is the least.

3.4. Non-coding RNAs

Non-coding RNAs are the integral part of the genome. To further improve the annotation of *F. vesca* genome, the loci producing small RNAs and lncRNAs were both integrated into the new annotation v2.0.a2.

3.4.1. Small RNAs

Thus far, most of the small RNAs with known functions are 21–24nt long, such as small interfering RNAs (siRNAs), miRNAs, phasiRNAs, and tasiRNAs. These small RNAs regulate the expression of target genes through transcriptional gene silencing (TGS) or post-transcriptional gene silencing (PTGS). Among them, 171
Figure 4. Features of the alternative splicing events in v2.0.a2. (a) Pie chart showing the percentage of genes with different number of isoforms among the 7,370 alternatively spliced genes. Different colour indicates genes with different number of isoforms per gene. (b) Summary of the different types of alternative splicing events that generate alternative transcripts in v2.0.a2.

Figure 5. Circular representation of genome-wide distribution of the genomic features. Tracks from outer to inner circles indicate: protein-coding (PC) gene models in v2.0.a2, v2.0.a1, lncRNA, miRNA, and smallRNA loci, respectively. Colour density represents gene density. Darker colour indicates a higher density.
LncRNAs are defined as non-coding RNAs with a length >300 nt. The distribution of lncRNAs showed that lncRNAs have fewer exons than the overlapped protein-coding gene; containing, lncRNA is longer than the overlapped protein-coding gene at both ends; nested, lncRNA is shorter than the overlapped protein-coding gene at both ends. Among the 4,042 lncRNA transcripts, the intergenic type contains 1,413 same_strand, 873 convergent, and 786 divergent lncRNA transcripts; the genic type contains 689 overlapping, 74 containing, and 207 nested transcripts (Fig. 6b; Supplementary Fig. S2 and Table S10).

4. Conclusions

The *F. vesca* genome was first released with annotations of merely protein coding genes that were mainly derived from *ab initio* predictions. Then, an updated genome of high quality called Fvb was re-assembled based on genetic linkage mapping with the same annotation. RNA-seq datasets generated from strawberry fruits have been previously utilized for *F. vesca* genome reannotation, however, it was based on limited number and type of tissue samples, short Illumina reads, and was again restricted to protein-coding genes. With the recent advancement in genome sequencing, especially the availability of third generation long read sequencing data, and accumulation of large number of RNA-seq datasets from diverse tissues, creating a new and improved re-annotation for the most recent genome assembly is timely. Here, we developed an optimized pipeline to re-annotate the strawberry Fvb genome taking advantage of PacBio full-length transcripts and an extensive dataset of RNA-seq libraries. This new annotation, v2.0.a2, updated the *F. vesca* gene models and includes new information ranging from 5′ and 3′ UTRs to alternatively spliced transcripts. In addition, non-coding genes, including small RNAs and lncRNAs, were also integrated into this new annotation. To conclude, our new annotation is much more complete than the older annotation and will provide a valuable resource for the strawberry and the *Rosaceae* research community as well as for comparative and functional studies in flowering plants.

5. Data availability

Supplementary Table S2 is the gtf file of the new Fvb annotation v2.0.a2, which is also available in GDR (https://www.rosaceae.org/ (5 September 2017, date last accessed)) and SGR (http://bioinformatics.towson.edu/strawberry/ (5 September 2017, date last accessed)).

Acknowledgements

The authors would like to thank Dr. Guogui Ning for the help on establishing the analysis pipeline. This work was supported by the National Natural Science Foundation of China (31572098 to C.K.) and the Scientific and Technological Self-innovation Foundation of Huazhong Agricultural University (2014RC005 to Z.L. and 2014RC017 to C.K.).
Author contributions
Conceived and designed the experiments: Y.L. and C.K. Analyzed the data: Y.L., W.W., and M.P. Performed the experiments: J.F. and H.L. Wrote the paper: C.K., Y.L., and Z.L.

Supplementary data
Supplementary data are available at DNARES online.

Conflict of interest
None declared.

References
1. Liston, A., Cronn, R. and Ashman, T. L. 2014, Fragaria: a genus with deep historical roots and ripe for evolutionary and ecological insights. Am. J. Bot., 101, 1686–99.
2. Shulaev, V., Sargent, D. J., Crowhurst, R. N., et al. 2011, The genome of woodland strawberry (Fragaria vesca). Nat. Genet., 43, 109–16.
3. Kang, C., Darwish, O., Geret, A., Shahar, R., Alkharouf, N. and Liu, Z. 2013, Genome-scale transcriptomic insights into early-stage fruit development in woodland strawberry Fragaria vesca. Plant Cell, 25, 1960–78.
4. Hollender, C. A., Kang, C., Darwish, O., et al. 2014, Floral transcriptomes in woodland strawberry uncover developing receptacle and anther gene networks. Plant Physiol., 165, 1062–75.
5. Xia, R., Ye, S., Liu, Z., Meyers, B. and Liu, Z. 2015, Novel and recently evolved miRNA clusters regulate expansive F-box gene networks through phased miRNAs in wild diploid strawberry. Plant Physiol., 169, 594–610.
6. Kang, C. and Liu, Z. 2015, Global identification and analysis of long non-coding RNAs in diploid strawberry Fragaria vesca during flower and fruit development. BMC Genomics, 16, 815.
7. Li, Y., Dai, C., Hu, C., Liu, Z. and Kang, C. 2017, Global identification of alternative splicing via comparative analysis of SMRT- and Illumina-based RNA-seq in strawberry. Plant J., 90, 164–76.
8. Darwish, O., Shahar, R., Liu, Z., Slovin, J. P. and Alkharouf, N. W. 2015, Re-annotation of the woodland strawberry (Fragaria vesca) genome. BMC Genomics, 16, 29.
9. Tennenbaum, J. A., Govindaraju, R., Ashman, T. L. and Liston, A. 2014, Evolutionary origins and dynamics of octoploid strawberry genomes revealed by dense targeted capture linkage maps. Genome Biol. Evol., 6, 3295–3113.
10. Hawkins, C., Caruana, J., Schikons, E. and Liu, Z. 2016, Genome-scale DNA variant analysis and functional validation of a SNP underlying yellow fruit color in wild strawberry. Sci. Rep., 6, 29017.
11. Minoche, A. E., Dohm, J. C., Schneider, J., et al. 2015, Exploiting single-molecule transcript sequencing for eukaryotic gene prediction. Genome Biol., 16, 184.
12. Dong, L., Liu, H., Zhang, J., et al. 2015, Single-molecule real-time transcript sequencing facilitates common wheat genome annotation and grain transcriptome research. BMC Genomics, 16, 1039.
13. Xu, G., Yuan, M., Ai, C., et al. 2017, tORF-mediated translation allows engineered plant disease resistance without fitness costs. Nature, 545, 491–4.
14. Haas, B. J., Delcher, A. L., Mount, S. M., et al. 2003, Improving the Arabidopsis genome annotation using maximal transcript alignment assemblies. Nucleic Acids Res., 31, 5654–66.
15. Holt, C. and Yandell, M. 2011, MAKER2: an annotation pipeline and genome-database management tool for second-generation genome projects. BMC Bioinformatics, 12, 491.
16. Stanke, M., Dikshans, M., Baertsch, R. and Haussler, D. 2008, Using native and syntetically mapped cDNA alignments to improve de novo gene finding, Bioinformatics, 24, 637–44.
17. Stanke, M. and Waack, S. 2003, Gene prediction with a hidden Markov model and a new intron submodel. Bioinformatics, 19, l215–25.
18. Toljamo, A., Blande, D., Karenlampi, S. and Kokko, H. 2016, Reprogramming of strawberry (Fragaria vesca) root transcriptome in response to phytophthora cactorum. PLoS One, 11, e0161078.
19. Salmela, L. and Rivals, E. 2014, LoRDEC: accurate and efficient long read error correction. Bioinformatics, 30, 3506–14.
20. Wu, T. D. and Watanabe, C. K. 2005, GMAP: a genomic mapping and alignment program for mRNA and EST sequences. Bioinformatics, 21, 1859–75.
21. Kim, D., Pettea, G., Trapnell, C., Pimentel, H., Kelley, R. and Salzberg, S. L. 2013, TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol., 14, R36.
22. Li, H., Handsaker, B., Wysoker, A., et al. 2009, The Sequence Alignment/Map format and SAMtools. Bioinformatics, 25, 2078–9.
23. Pettea, G., Pettea, G. M., Antonescus, C. M., Chang, T. C., Mendell, J. T. and Salzberg, S. L. 2015, StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. Nat. Biotechnol., 33, 290.
24. Grubb, M. G., Haas, B. J., Yassour, M., et al. 2011, Full-length transcript assembly from RNA-Seq data without a reference genome. Nat. Biotechnol., 29, 644–52.
25. Stanke, M., Schoffmann, O., Morgenstern, B. and Waack, S. 2006, Gene prediction in eukaryotes with a generalized hidden Markov model that uses hints from external sources. BMC Bioinformatics, 7, 62.
26. Slater, G. S. and Birney, E. 2005, Automated generation of heuristics for biological sequence comparison. BMC Bioinformatics, 6, 31.
27. Haas, B. J., Salzberg, S. L., Zhu, W., et al. 2008, Automated eukaryotic gene structure annotation using EvidencedModeler and the program to assemble spliced alignments. Genome Biol., 9, R7.
28. Lee, E., Helt, G. A., Reese, J. T., et al. 2013, Web Apollo: a web-based genomic annotation editing platform. Genome Biol., 14, R83.
29. Stein, L. D., Mungall, C., Shu, S. Q., et al. 2002, The generic genome browser: a building block for a model organism system database. Genome Res., 12, 1599–610.
30. Foissac, S. and Sammeth, M. 2007, ASTALAVISTA: dynamic and flexible analysis of alternative splicing events in custom gene datasets. Nucleic Acids Res., 35, W297–9.
31. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. 1990, Basic local alignment search tool. J. Mol. Biol., 215, 403–10.
32. Kong, L., Zhang, Y., Ye, Z.-Q., et al. 2007, CPC: assess the protein-coding potential of transcripts using sequence features and support vector machine. Nucleic Acids Res., 35, W345–9.
33. Wucher, V., Legeai, F., Hedan, B., et al. 2017, FEELnc: a tool for long non-coding RNA annotation and its application to the dog transcriptome. Nucleic Acids Res., 45, e57.
34. Martin, M. 2011, Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J., 17, 10–22.
35. Langmead, B. 2010, Aligning short sequencing reads with Bowtie.Curr. Protoc. Bioinformatics, doi: 10.1002/0471295953.bi11073.
36. Axtell, M. J. 2013, ShortStack: comprehensive annotation and quantification of small RNA genes. RNA, 19, 740–51.
37. Steiger, T., Abril, J. F., Engström, P. G., et al. 2013, Assessment of transcript reconstruction methods for RNA-seq. Nat. Methods, 10, 1177–84.
38. Robinson, J. T., Thorvaldsdottir, H., Winckler, W., et al. 2011, Integrative genomics viewer. Nat. Biotechnol., 29, 24–26.
39. Smoak, F. A., Waterhouse, R. M., Ioannidis, P., Krieviste, E. and Zdobnov, E. M. 2015, BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics, 31, 3210–2.
40. Jones, P., Binns, D., Chang, H.-Y., et al. 2014, InterProScan 3: genome-scale protein function classification. Bioinformatics, 30, 1236–40.
41. Conesa, A., Götz, S., García-Gómez, J. M., Terol, J., Talón, M. and Robles, M. 2005, Blast2GO: a universal tool for annotation, visualization and analysis of functional genomics research. Bioinformatics, 21, 3674–6.
42. Zhang, Y., Jiao, C., Sun, H., et al. 2016, iTAKE: a program for genome-wide prediction and classification of plant transcription factors, transcriptional regulators, and protein kinases. Mol Plant, 9, 1667–70.
43. Li, P., Quan, X., Jia, G., Xiao, J., Clouter, S. and You, F. M. 2016, RGAugury: a pipeline for genome-wide prediction of resistance gene analogs (RGAs) in plants. BMC Genomics, 17, 852.
44. Mouhu, K., Kurokura, T., Koskela, E. A., Albert, V. A., Elomaa, P. and Hytönen, T. 2013, The *Fragaria vesca* homolog of SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 represses flowering and promotes vegetative growth. *Plant Cell*, 25, 3296–310.

45. Iwata, H., Gaston, A., Remay, A., et al. 2012, The TFL1 homologue KSN is a regulator of continuous flowering in rose and strawberry. *Plant J.*, 69, 116–25.

46. Koskela, E. A., Mouhu, K., Albani, M. C., et al. 2012, Mutation in TERMINAL FLOWER1 reverses the photoperiodic requirement for flowering in the wild strawberry *Fragaria vesca*. *Plant Physiol.*, 159, 1043–54.

47. Lee, Y. and Rio, D. C. 2015, Mechanisms and regulation of alternative pre-mRNA splicing. *Annu. Rev. Biochem.*, 84, 291–323.

48. Reddy, A. S., Marquez, Y., Kalyna, M. and Barta, A. 2013, Complexity of the alternative splicing landscape in plants. *Plant Cell*, 25, 3657–83.

49. Cheng, C. Y., Krishnakumar, V., Chan, A. P., Thibaud-Nissen, F., Schobel, S. and Town, C. D. 2017, Araport11: a complete reannotation of the *Arabidopsis thaliana* reference genome. *Plant J.*, 89, 789–804.

50. Jex, A. R., Nejsum, P., Schwarz, E. M., et al. 2014, Genome and transcriptome of the porcine whipworm *Trichuris suis*. *Nat. Genet.*, 46, 701–6.

51. Coruh, C., Cho, S. H., Shahid, S., Liu, Q., Wierzbicki, A. and Axtell, M. J. 2015, Comprehensive annotation of *Physcomitrella patens* small RNA loci reveals that the heterochromatic short interfering RNA pathway is largely conserved in land plants. *Plant Cell*, 27, 2148–62.