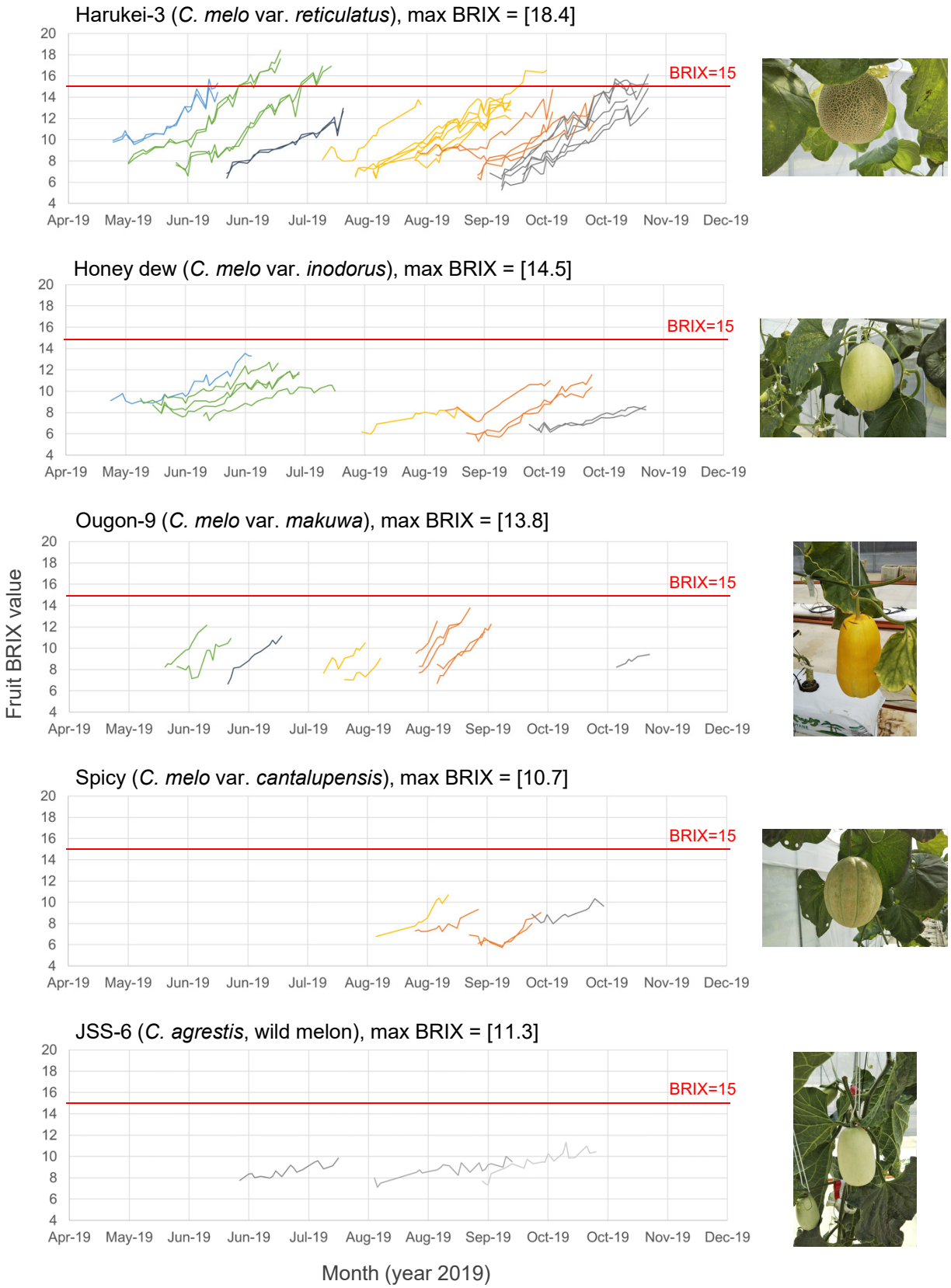
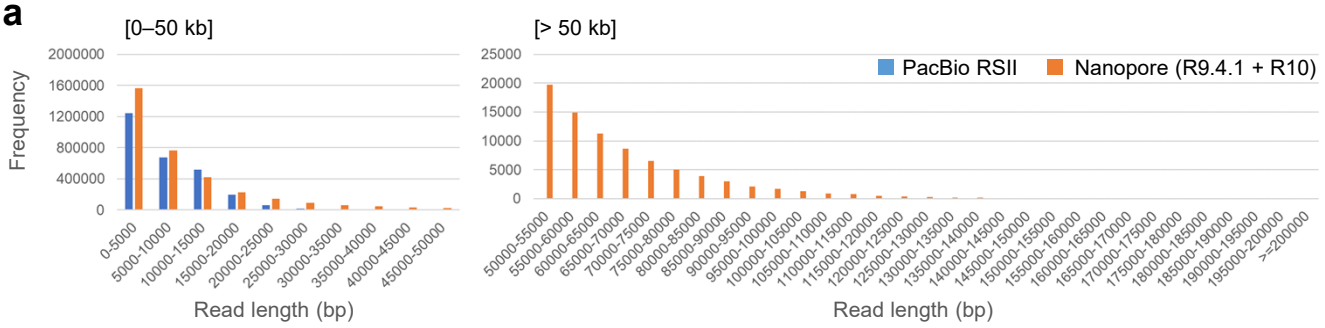



**Supplementary Fig. 1 Seasonal changes in fruit BRIX**
Seasonal changes in fruit BRIX in five melon accessions. Fruit BRIX was continuously analyzed in a non-destructive manner using 'Fruit selector' near-infrared spectrometer (Kubota Co. Ltd., Japan) from April to November in 2019 in the greenhouse at the University of Tsukuba. Line colors distinguish the timing of fruit set from Spring to late Autumn. The melon accessions analyzed were Harukei-3, Honey dew, Ougon-9, Spicy, and JSS-6.

**a**




**b**

**Input data** Oxford Nanopore R9.4.1/R10: 32.6 Gb ($N_{50}$ = 19.0 kb, 73-fold)
PacBio RS II: 19.5 Gb ($N_{50}$ = 11.4 kb, 43-fold)

*Error correction/Contig assembly* → **canu ver.1.8*

| Contig assembly | Num contig | Total (Mb) | Average (kb) | Max (kb) | $N_{50}$ (kb) |
|---|---|---|---|---|---|
| Nanopore R9/R10 | 112 | 368.2 | 3,288 | 23,243 | 8,627 |
| PacBio RSII | 1,381 | 373.1 | 270 | 6,758 | 861 |

**Input data** BioNano: 86 Gb

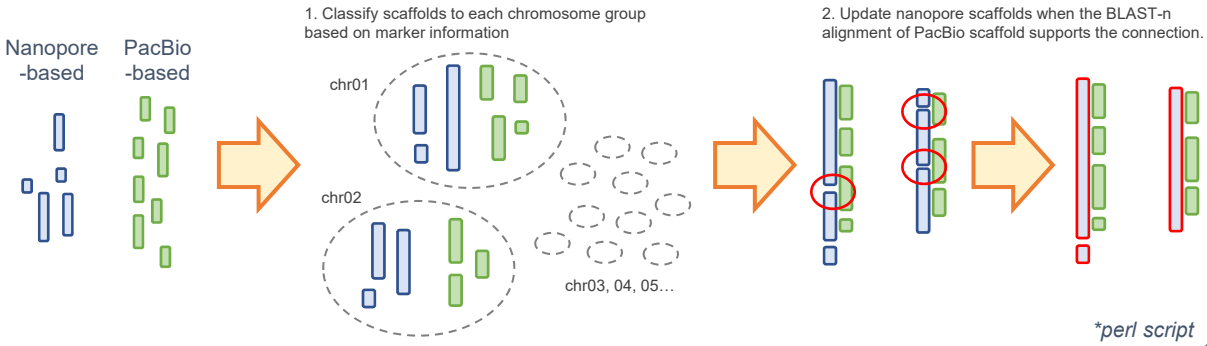
*Optical map assembly* [150kb, 250kb molecule] **irys solve 3.2*

*polishing/error correction* → **bowtie2, pilon* ← **+Illumina PE (37 Gb)** **Input data**

Hybrid scaffolding:
- *hybrid scaffolding* [aggressive mode] → **irysSolve 3.2* ← +BioNano assembly
- *matepair scaffold* [*k*=160, 80, 40, 20, 10, 5] → **SSPACE3* ← **+10 kb, 5 kb Illumina MP (74 Gb)** **Input data**
- *hybrid scaffolding* [strict mode)] → **irys solve 3.2* ← +BioNano assembly

| Scaffold assembly | Num scaffold | Total (Mb) | Average (kb) | Max (kb) | $N_{50}$ (Mb) | Num gap |
|---|---|---|---|---|---|---|
| Nanopore R9/R10 | 80 | 375.4 | 4,692 | 28,978 | 17.5 | 78 |
| PacBio RSII | 460 | 391.7 | 851 | 25,224 | 11.4 | 864 |

Updating nanopore scaffolds using PacBio scaffolds as hints.

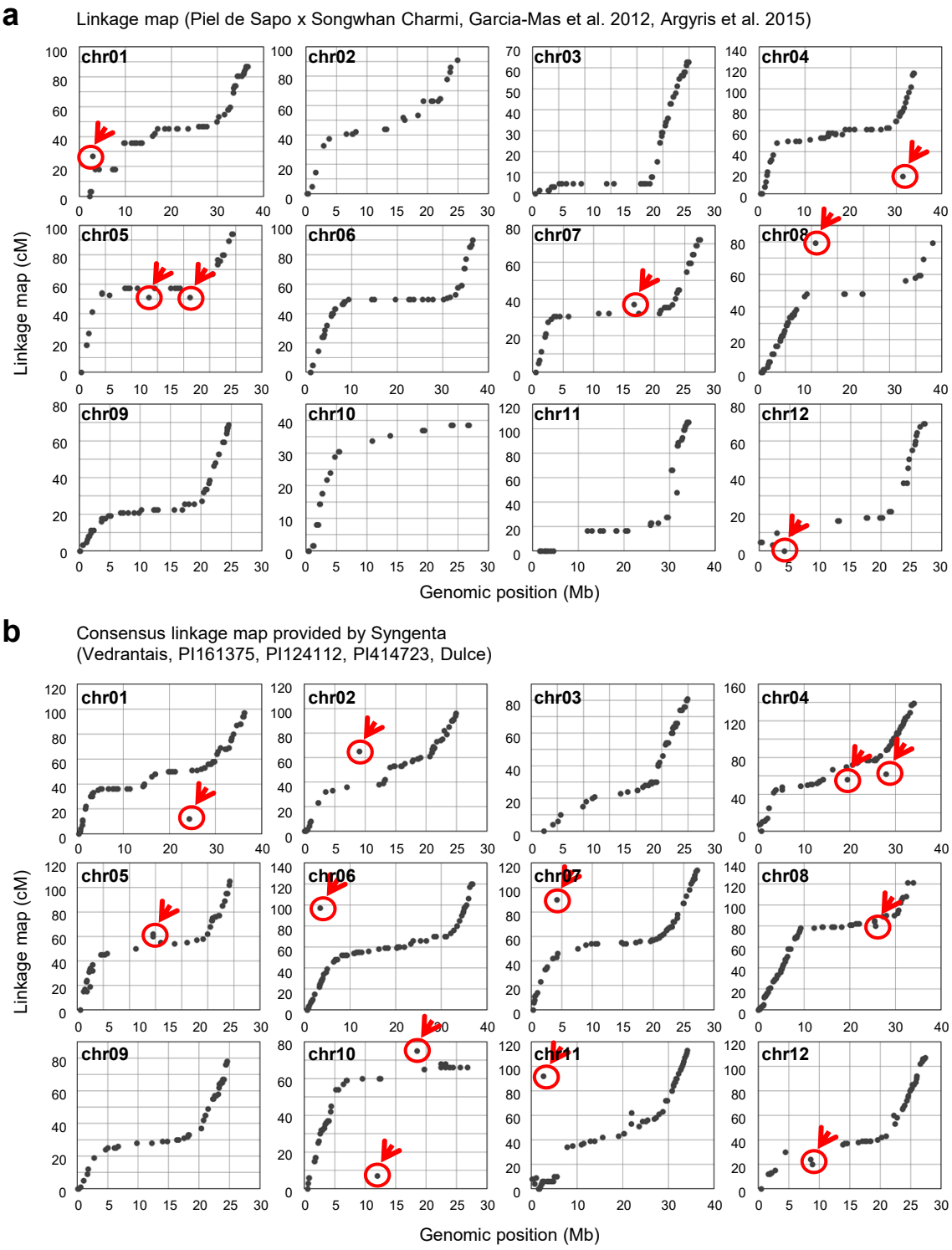



| Scaffold assembly | Num scaffold | Total (Mb) | Average (kb) | Max (kb) | $N_{50}$ (Mb) | Num gap |
|---|---|---|---|---|---|---|
| Integrated scaffold | 66 | 376.0 | 5,697 | 28,978 | 18.9 | 92 |

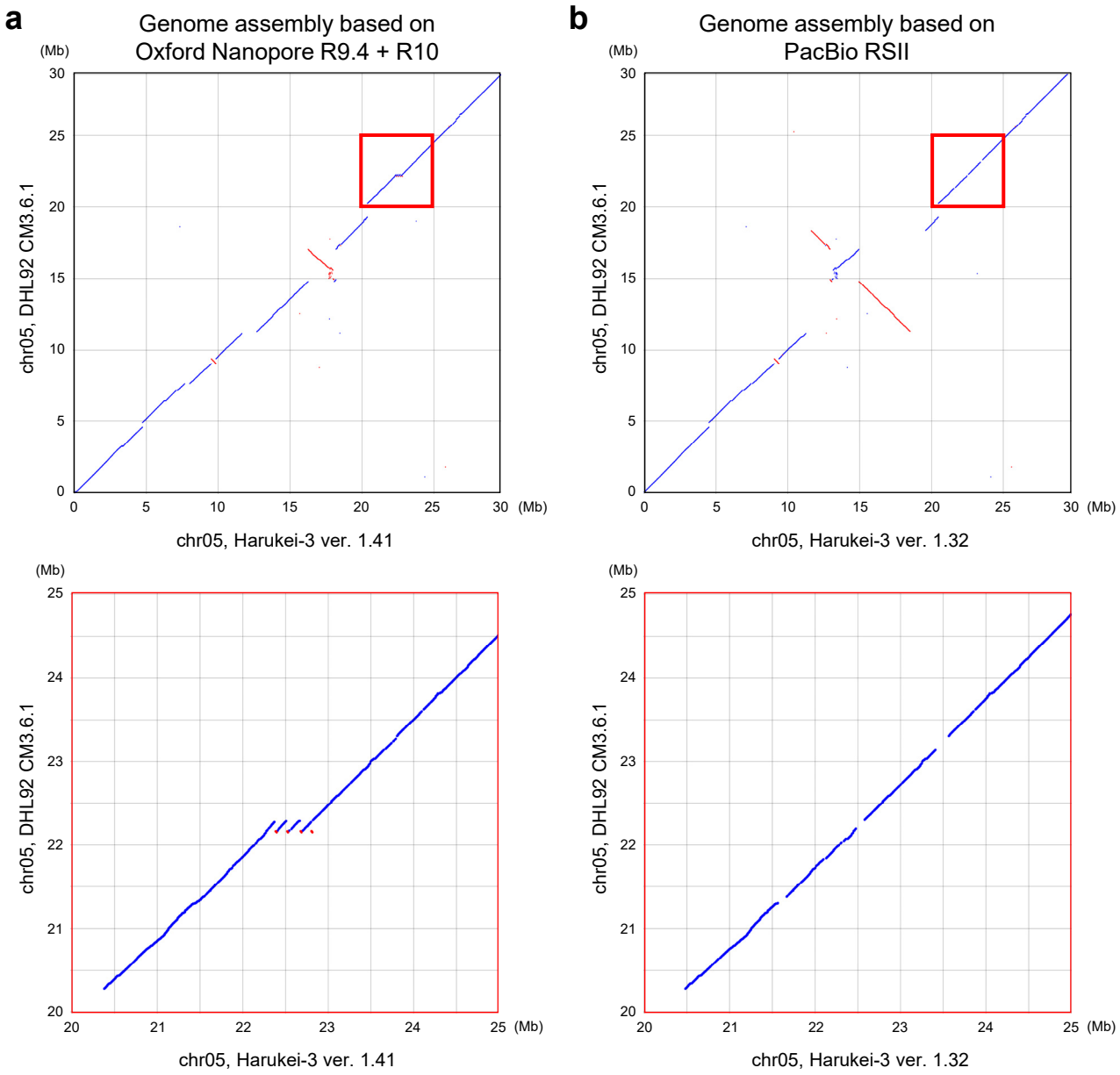
**Anchor scaffold with linkage map* ← Kawazu et al. (2018), Garcia-Mas et al. (2012), Argyris et al. (2015), Consensus map from ICuGI DB

**Pseudomolecule (constructed by 28 scaffolds, 368.5 Mb)**

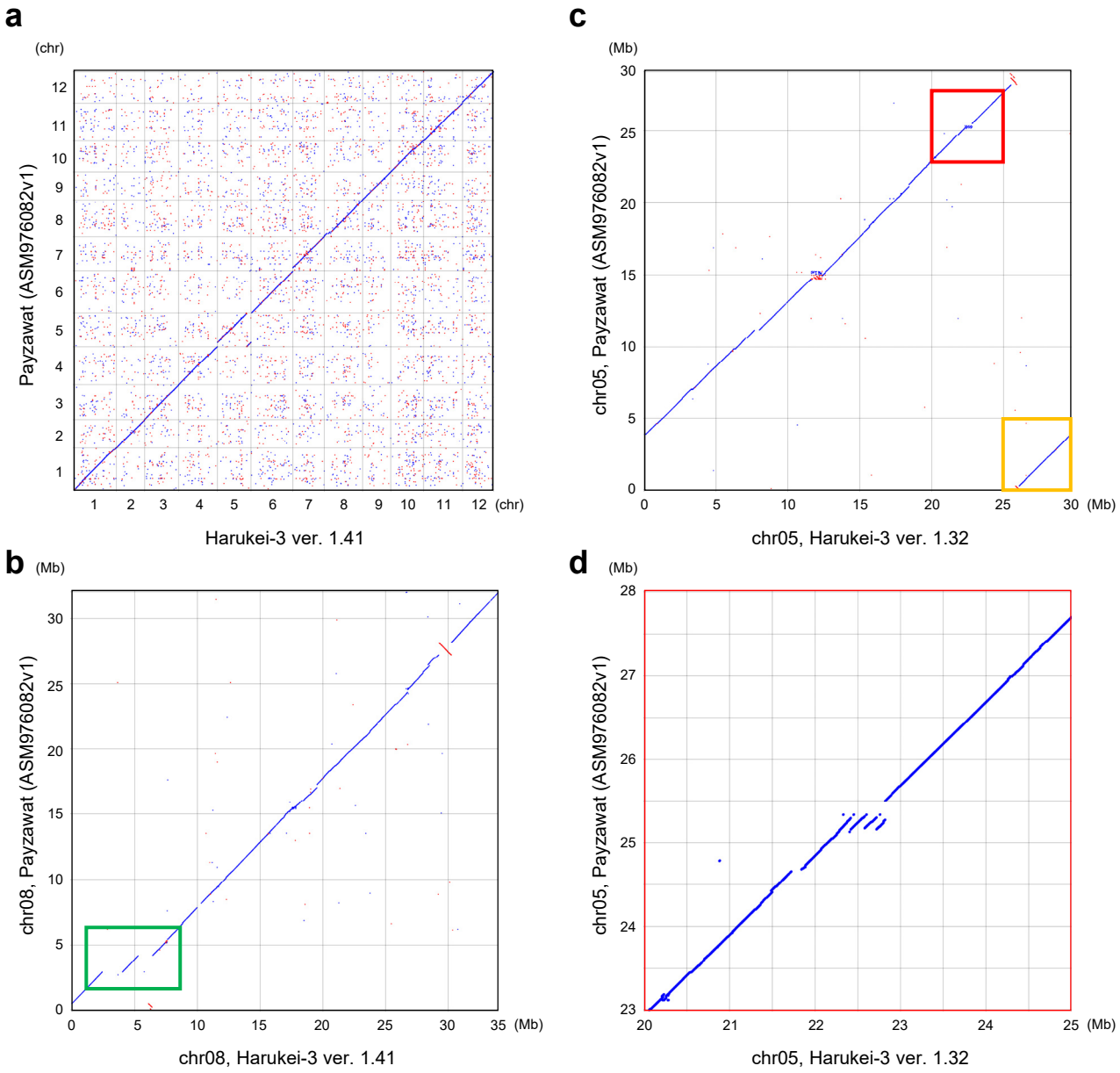
**Supplementary Fig. 2 Procedure of whole genome assembly in Harukei-3**
**a** Histograms of sequenced reads in Oxford Nanopore technology (ONT, R9.4.1 and R10 flow cells) and PacBio RSII. Reads with > 50 kb are present only in ONT reads (right). **b** Procedure of genome assembly. Contigs were separately assembled based on ONT or PacBio reads; then, scaffolds were assembled using BioNano optical map and Illumina mate-pair. ONT-based scaffolds were further updated by using Pacbio-based scaffolds as a hint; both scaffolds were first classified into each chromosome group based on the linkage marker information[1,2,3], then hint scaffolds (Pacbio-based) that connect two different ONT-based scaffolds were identified based on BLAST-n alignment. Finally, the chromosome-scale pseudomolecule was constructed by 28 genomic scaffolds that were anchored and oriented using the linkage map information[1,2,3].




**Supplementary Fig. 3 Comparison of Harukei-3 genome sequence with linkage map information**
**a, b** Comparison of Harukei-3 genome sequence with linkage maps. Linkage maps were derived from a cross between Piel de Sapo and Songwhan Charmi[3] (a) or the consensus genetic map available in Cucurbit Genomics Database[4,5] (b). Red circles and arrows indicate genetic markers in which the physical position does not match the linkage map position.




**Supplementary Fig. 4 Comparison of genome assembly between ONT and PacBio in Harukei-3**
**a, b** Genomic alignments of chromosome 5 between DHL92 and two versions of Harukei-3. The Harukei-3 sequences are ONT-based (a, ver. 1.41) or PacBio RSII-based (b, ver. 1.32). Entire chromosome (top) and the magnified view of the specific region (20–25 Mb) (bottom) are shown. ONT-based Harukei-3 assembly can resolve large genome block duplications of around 22–23 Mb, whereas it is absent in the PacBio-based assembly. Blue and red dots indicate that DNA is aligned in the forward or reverse directions, respectively.




**Supplementary Fig. 5 Genomic alignment between Harukei-3 and Payzawat melon genomes**
**a, b, c** Genomic alignment of entire chromosomes (a), chromosome 8 (b), or chromosome 5 (c) between Harukei-3 (ver. 1.41) and Payzawat[6] (ASM976082v1) genomes. Large genomic blocks that are present in Harukei-3 but not in Payzawat genome are indicated by the green rectangle in b. Inter-chromosomal translocation observed in chromosome 5 between the two genomes is indicated by the yellow rectangle in c. **d** Magnified view of the specific region of chromosome 5 (20–25 Mb in in Harukei-3, 23–28 Mb in Payzawat; shown by the red rectangle in c. Harukei-3 genome can resolve large genomic block duplications around 22–23 Mb, but it is absent or not assembled in the Payzawat genome. Blue and red dots indicate that DNA is aligned in the forward or reverse directions, respectively.

**a**

Combined RNA set1

| Sample | Condition | Bulked replicate | Total RNA (μg) |
|---|---|---|---|
| Seedling cotyledon | 7d after imbibition on filter paper | 2 x 4 seedlings | 20 |
| Seedling root | 7d after imbibition on filter paper | 2 x 4 seedlings | 20 |
| Seedling hypocotyl | 7d after imbibition on filter paper | 2 x 4 seedlings | 20 |
| Germinating seed | 1d imbibition on filter paper | 2 x 4 seedlings | 30 |
| Germinating seed | 3d imbibition on filter paper | 1 x 4 seedlings | 30 |
| Dry seed | - | 1 x >100 seeds | 20 |

Combined RNA set2

| Sample | Condition | Bulked replicate | Total RNA (μg) |
|---|---|---|---|
| Expanded leaves (6th) | Hydroponically-cultivated in the greenhouse | 3 | 15 |
| Expanded leaves (9th) | Hydroponically-cultivated in the greenhouse | 3 | 15 |
| Expanded leaves (12th) | Hydroponically-cultivated in the greenhouse | 3 | 15 |
| Hole-punched leaves | infected with powdery mildew | 8 | 15 |
| Hole-punched leaves | without powdery mildew | 8 | 15 |
| Root | Hydroponically-cultivated in the greenhouse | 4 | 25 |
| Main stem | Hydroponically-cultivated in the greenhouse | 2 | 16 |
| Tendril | Hydroponically-cultivated in the greenhouse | 2 | 6 |

Combined RNA set3

| Sample | Condition | Bulked replicate | Total RNA (μg) |
|---|---|---|---|
| Anther (female) | just after flowering | 4 x >10 flowers | 12.5 |
| Anther (male) | just after flowering | 4 x >10 flowers | 12.5 |
| Ovary (female) | just after flowering | 3 x 4 ovaries | 25 |
| Petal | just after flowering | 4 x >10 flowers | 25 |
| Pistil (female) | just after flowering | 3 x 4 ovaries | 25 |

Combined RNA set4

| Sample | Condition | Bulked replicate | Total RNA (μg) |
|---|---|---|---|
| Fruit peel | DAF8 | 2 | 4 |
| Fruit peel | DAF15 | 2 | 4 |
| Fruit peel | DAF22 | 2 | 4 |
| Fruit peel | DAF29 | 2 | 2.5 |
| Fruit peel | DAF36 | 2 | 2.5 |
| Fruit peel | DAF43 | 2 | 2.5 |
| Fruit peel | DAF50 | 2 | 2.5 |
| Fruit peel | Post harvest, 3weeks | 2 | 2.5 |
| Fruit peel | Post harvest, 1week | 2 | 2.5 |
| Fruit peel | Post harvest, 4weeks | 2 | 2.5 |
| Fruit peel | Post harvest, 2weeks | 2 | 2.5 |
| Fruit | DAF4 | 4 ovaries | 10 |
| Fruit flesh | DAF8 | 2 | 4 |
| Fruit flesh | DAF15 | 2 | 4 |
| Fruit flesh | DAF22 | 2 | 4 |
| Fruit flesh | DAF29 | 2 | 2.5 |
| Fruit flesh | DAF36 | 2 | 2.5 |
| Fruit flesh | DAF43 | 2 | 2.5 |
| Fruit flesh | DAF50 | 2 | 2.5 |
| Fruit flesh | Post harvest, 3weeks | 2 | 2.5 |
| Fruit flesh | Post harvest, 1week | 2 | 2.5 |
| Fruit flesh | Post harvest, 4weeks | 2 | 2.5 |
| Fruit flesh | Post harvest, 2weeks | 2 | 2.5 |

Combined RNA set5

| Sample | Condition | Bulked replicate | Total RNA (μg) |
|---|---|---|---|
| Hole-punched leaves | infected with powdery mildew (2018-Apr) | 4 | 50 |
| Hole-punched leaves | infected with powdery mildew (2018-Oct) | 4 | 50 |

Combined RNA set6

| Sample | Condition | Bulked replicate | Total RNA (μg) |
|---|---|---|---|
| Etiolated seedling | germinted in the dark | >10 seedlings | 100 |

Combined RNA set7

| Sample | Condition | Bulked replicate | Total RNA (μg) |
|---|---|---|---|
| Shoot apex | Hydroponically-cultivated in the greenhouse | 4 | 130 |

**ONT direct RNA-seq (R9.4.1 flow cell)**

1. Combine RNA in each set
2. Poly-A+ purification
3. Nanopore RNA-seq library prep.
4. Sequencing
5. Basecall with guppy

Total 8.2 Gb, 7.6 million reads (five flow cells)

**b**

RNA (hole-punshed leaves)

| Sample | Condition | Bulked replicate | Total RNA (μg) |
|---|---|---|---|
| Harukei-3 (rep.1) | Hydroponically-cultivated in the greenhouse | 4 | >100 |
| Harukei-3 (rep.2) | Hydroponically-cultivated in the greenhouse | 4 | >100 |
| Harukei-3 (rep.3) | Hydroponically-cultivated in the greenhouse | 4 | >100 |
| Harukei-3 (rep.4) | Hydroponically-cultivated in the greenhouse | 4 | >100 |
| Honey dew (rep.1) | Hydroponically-cultivated in the greenhouse | 4 | >100 |
| Honey dew (rep.2) | Hydroponically-cultivated in the greenhouse | 4 | >100 |
| Awamidori (rep.1) | Hydroponically-cultivated in the greenhouse | 4 | >100 |
| Awamidori (rep.2) | Hydroponically-cultivated in the greenhouse | 4 | >100 |
| Ougon-9 (rep.1) | Hydroponically-cultivated in the greenhouse | 4 | >100 |
| Ougon-9 (rep.2) | Hydroponically-cultivated in the greenhouse | 4 | >100 |
| JSS-6 (rep.1) | Hydroponically-cultivated in the greenhouse | 4 | >100 |
| JSS-6 (rep.1) | Hydroponically-cultivated in the greenhouse | 4 | >100 |

**ONT cDNA-seq (R9.4.1 flow cell)**

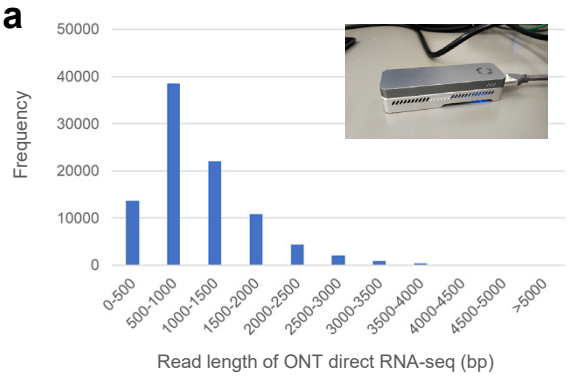
1. Poly-A+ purification
2. Nanopore RNA-seq library prep.
3. Multiplex sequencing
4. Basecall with guppy

Total 8.8 Gb, 11.5 million reads (one flow cell)

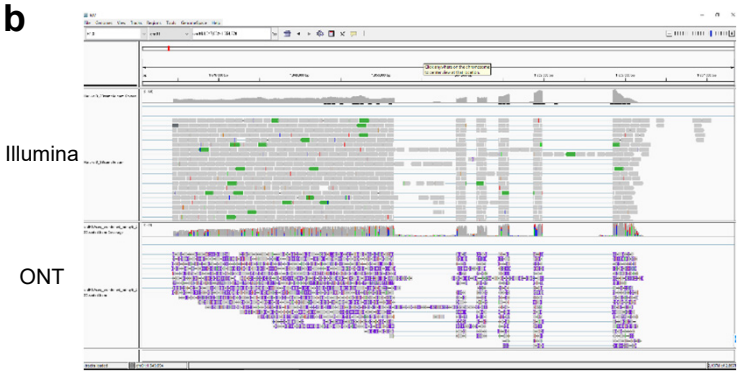
**Supplementary Fig. 6 Summary of RNA samples used for ONT RNA-seq in Harukei-3**

**a, b** Summary of RNA samples used for ONT direct RNA-seq (a) or cDNA RNA-seq (b). Both RNA-seq datasets were used for gene prediction in the Harukei-3 genome. Sequencing strategies are also briefly described on the left.

**a**




**b**




**c**

**Gene prediction based on ONT RNA-seq**

Oxford Nanopore® direct RNA-seq
Eight runs with 5 flow cells (R9.4.1)
Total 8.2 Gb, 7.6 million reads
↓
Alignment to Harukei-3 ver. 1.41 genome
(Minimap2 with parameters '-ax splice -uf -k14')
↓
Prediction of mRNA (exon-intron) structure based on ONT read alignment
(pinfish with different parameters '*c* = 2,3,5,10')
↓
Integrate pinfish results between '*c* = 2,3,5,10' and protein coding DNA sequences from DHL92 CM4.0
↓
ORF search followed by hmmsearch to keep the best possible ORF with known protein domain
(Longest ORF is kept in case of no protein domain)
↓
Transcripts are grouped into gene unit based on BLAST-n/p search and genomic position information
↓
Best-possible ORF are selected again based on hmmsearch and BLAST-p search against protein sequences from 9 plant genomes
↓
**[i] 31,306 protein-coding genes (36,826 transcripts)**
**1,437 non-coding genes**

**Gene prediction using other methods**

AUGUSTUS
Parameter training was first performed with ONT-based annotation obtained in [i] then genes were predicted with AUGUSTUS ver. 3.3.2.

Braker2
Reads of tissue-wide Illumina Hiseq® RNA-seq (45 samples, total 118 Gb, 920 million reads) were first aligned to Harukei-3 ver. 1.41 sequence with hisat2, then genes were predicted with braker2.

Genome threader
Protein sequences of 10 plant genomes were aligned to Harukei-3 ver. 1.41 genome sequence.

StringTie
Transcript annotation was obtained with StringTie based on the HISAT2 read alignment of tissue-wide Illumina Hiseq® RNA-seq (45 samples).

Integrate annotation datasets with Evidence modeler using the following parameter condition

| | | |
|---|---|---|
| TRANSCRIPT | StringTie | 10 |
| ABINITIO_PREDICTION | AUGUSTUS | 8 |
| ABINITIO_PREDICTION | Braker2 | 1 |
| PROTEIN | Genome threader | 1 |

**[ii] 59,613 protein-coding genes (59,613 transcripts)**

*Add 2,524 genes that were present in [ii] but absent in [i]
*Update annotation of 1,023 genes in [i] based on [ii]

Small RNA prediction with cmscan and tRNAscan
(These are not protein-coding genes but described in GFF file)

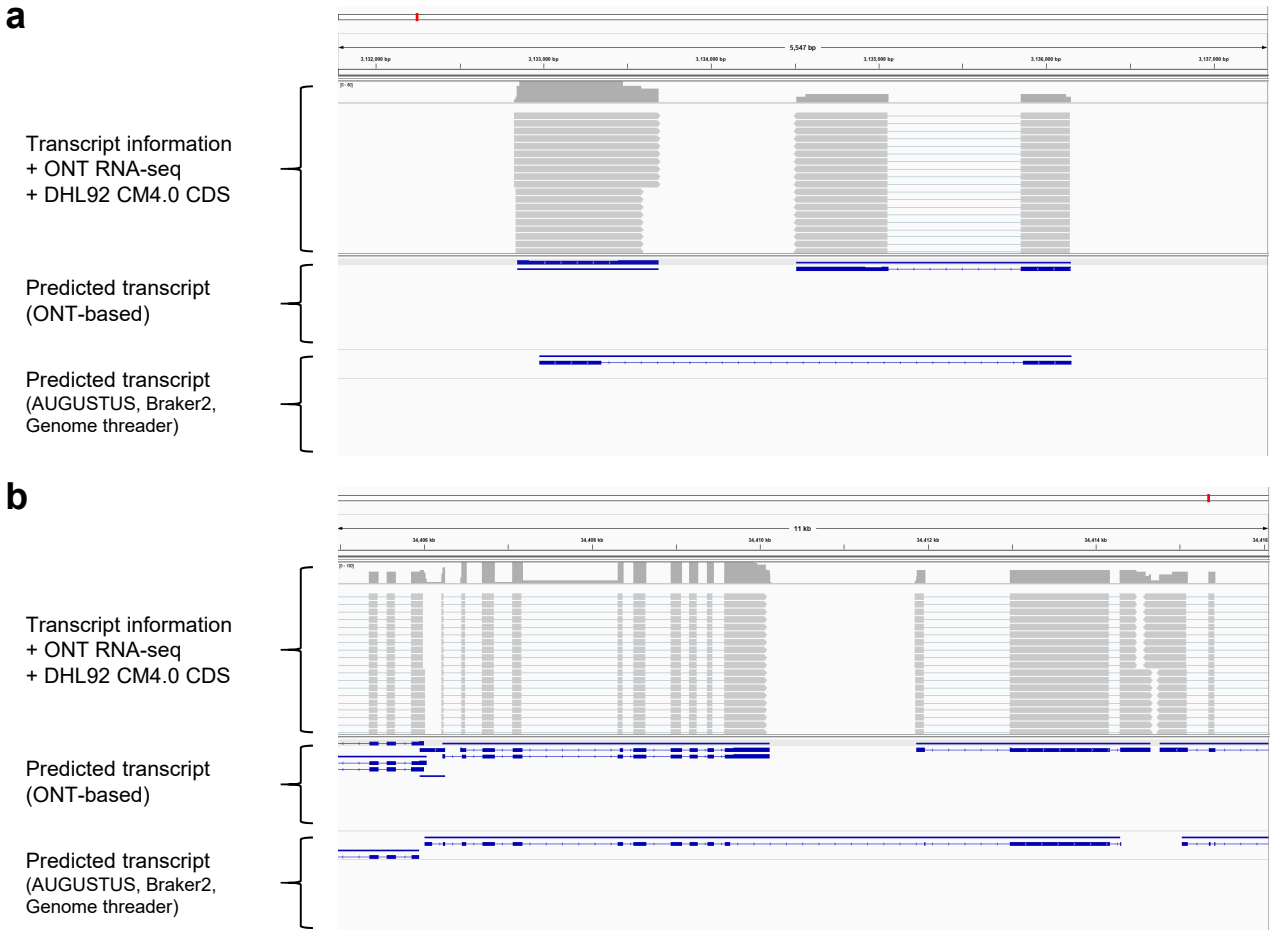
**[iii] 33,829 protein-coding genes (40,363 transcripts)**
**3,425 non-coding genes**

| | Protein BUSCO benchmark (ver3.0) | | |
|---|---|---|---|
| Gene prediction dataset | Complete | Fragmented | Missing |
| Final dataset [iii] | 1,372 (95.3%) | 16 | 52 |
| ONT-based gene dataset [i] | 1,362 (94.6%) | 24 | 55 |
| EVM [ii] | 1,348 (93.6%) | 33 | 59 |

**d**

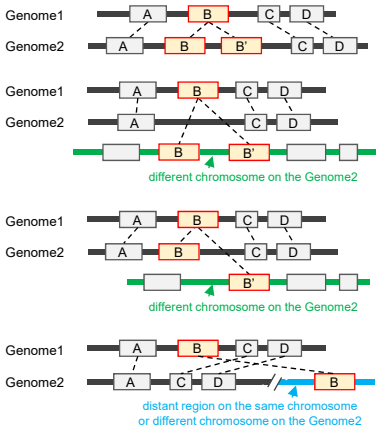
| Genome reference | ONT-based gene prediction | Genome threader |
|---|---|---|
| *Aarabidopsis thaliana* TAIR10 | Yes | Yes |
| *Solanum lycopersicum* iTAG3.0 | Yes | Yes |
| *Cucumis melo* DHL92 CM4.0 | Yes | Yes |
| *Cucumis sativus* Chineese Long ver3.0 | Yes | Yes |
| *Cucumis sativus* PI183967 | Yes | Yes |
| *Cucumis sativus* Gy14 ver1.0 | - | Yes |
| *Citrullus lanatus* 97103 ver1.0 | Yes | Yes |
| *Lagenaria siceraria* USVL1Vr-Ls | Yes | Yes |
| *Cucurbita moschata* Rifu ver1.1 | Yes | Yes |
| *Cucurbita maxima* Rimu ver1.1 | Yes | Yes |

**Supplementary Fig. 7 Summary of ONT-based gene prediction in Harukei-3**
**a** Read-length histogram of ONT direct RNA-seq in Harukei-3. **b** Examples of RNA-seq read alignment. Reads of ONT RNA-seq (bottom) or Illumina RNA-seq (top, control) were aligned to Harukei-3 ver. 1.41 genomic sequence. Although the ONT reads contained many errors as shown by the purple lines, it was enough to predict exon-intron gene structure. **c** Summarized procedure of gene prediction in Harukei-3 ver. 1.41 genome reference. ONT-based method that integrates several software, such as Minimap2, pinfish, BLAST, and HMMER, predicted 31,306 protein-coding genes with a complete BUSCO ver. 3.0 score = 1,362 (94.6%). In parallel, Evidence Modeler (EVM) was used to integrate the results of AUGUSTUS (*ab initio* method), Braker2 (Illumina RNA-seq based method), Genome Threader (protein-based method), and StringTie (transcript annotation based on Illumina RNA-seq). The EVM-based method predicted 59,613 protein-coding genes with a complete BUSCO ver. 3.0 score = 1,348 (93.6%). This EVM-based dataset was used to update the ONT-based dataset: 2,524 genes that were absent in the ONT-based dataset but present in the EVM-based dataset were added while open reading frame (ORF) structures of 1,023 genes in the ONT-based dataset were corrected based on the EVM-based dataset. Finally, a dataset of 33,829 protein-coding genes (40,363 transcripts) were obtained (BUSCO ver. 3.0 score = 1,372 [95.3%]). **d** Summary of the genome dataset used as supporting information in gene prediction analysis.




**Supplementary Fig. 8 Comparison of predicted gene structures between the ONT-based method and EVM-based method**
**a, b** Exon-intron structures of predicted genes are compared between different gene prediction methods. Top: alignment of transcript sequences obtained from ONT RNA-seq or DHL92 CM4.0 genome annotation; middle: structure of genes predicted by the ONT-based method; bottom: structure of genes predicted by EVM coupled with AUGUSTUS, Braker2, and Genome Threader. Please note that the EVM-based method predicted strange introns that were absent in the transcript sequence, whereas the ONT-based method correctly predicted exon-intron gene structure according to the transcript sequence.

**a**




**b**

| Enriched ID (Interproscan ID) | Fisher's exact test | | Information of GO ID | Num gene |
|---|---|---|---|---|
| | *p*-value | *q*-value | | |
| IPR012337 | 6.59E-150 | 2.70E-147 | Ribonuclease H-like domain | 291 |
| IPR001584 | 1.92E-118 | 3.93E-116 | Integrase \| catalytic core | 225 |
| IPR005162 | 9.25E-100 | 1.26E-97 | Retrotransposon gag domain | 155 |
| IPR013242 | 1.04E-98 | 1.06E-96 | Retroviral aspartyl protease | 171 |
| IPR000477 | 3.43E-93 | 2.81E-91 | Reverse transcriptase | 178 |
| IPR021109 | 1.13E-90 | 7.73E-89 | Aspartic peptidase | 170 |
| IPR001878 | 1.38E-85 | 8.06E-84 | Zinc finger \| CCHC-type | 152 |
| IPR004242 | 1.62E-53 | 8.28E-52 | Transposon \| En/Spm-like | 61 |
| IPR023780 | 1.11E-49 | 5.04E-48 | Chromo domain | 113 |
| IPR025312 | 1.27E-40 | 5.18E-39 | Domain of unknown function DUF4216 | 40 |
| IPR025724 | 2.59E-38 | 9.62E-37 | GAG-pre-integrase domain | 59 |
| IPR013103 | 2.08E-36 | 7.09E-35 | Reverse transcriptase \| RNA-dependent DNA polymerase | 79 |
| IPR025452 | 2.44E-30 | 7.68E-29 | Domain of unknown function DUF4218 | 34 |
| IPR008906 | 2.28E-28 | 6.66E-27 | HAT dimerisation domain \| C-terminal | 44 |
| IPR016197 | 5.80E-25 | 1.54E-23 | Chromo domain-like | 51 |
| IPR025525 | 6.01E-25 | 1.54E-23 | Domain of unknown function DUF4413 | 31 |
| IPR003653 | 1.02E-19 | 2.45E-18 | Peptidase C48 \| SUMO/Sentrin/Ubl1 | 32 |
| IPR019557 | 1.96E-16 | 4.32E-15 | Aminotransferase-like \| plant mobile domain | 32 |
| IPR003656 | 2.01E-16 | 4.32E-15 | Zinc finger \| BED-type predicted | 24 |
| IPR015410 | 8.28E-15 | 1.69E-13 | Domain of unknown function DUF1985 | 23 |

**Supplementary Fig. 9 Retrotransposon-related functions are enriched in candidates of copy number or presence/absence polymorphisms between Harukei-3 and DHL92 genomes**

**a** Schematic illustration of the definition of copy number polymorphism (CNP) and presence/absence polymorphism (PAP). Four cases are assumed; genes are tandemly duplicated at the same chromosome region or a different chromosome (top, 2nd from the top). Gene is apparently copied to a distant region of the same chromosome or a different chromosome (3rd from the top). Gene is apparently translocated to a distant region of the same chromosome or a different chromosome (bottom). In total, 1203 genes or protein-coding sequences were applied to these cases (also shown in Fig. 2a). **b** InterPro ID enriched in the 1203 CNP or PAP candidates. ID enrichment analysis was performed using the 'GO enrichment analysis tool' in Melonet-DB (https://melonet-db.dna.affrc.go.jp/ap/got).

**a**

**Genome reference: Harukei-3 ver. 1.41 (var. *reticulatus*)**

| | | | Concordantly aligned | | Discordantly aligned | | One mate aligned | |
|---|---|---|---|---|---|---|---|---|
| Accession | Species, subgroup | Total reads | %aligned | %complete match | %aligned | %complete match | %aligned | %complete match |
| Harukei-3 | *C. melo* var. *reticulatus* | 459,736,006 | 99.18 | 90.68 | 0.37 | 0.33 | 0.08 | 0.03 |
| Honey dew | *C. melo* var. *inodorus* | 54,438,848 | 93.91 | 66.52 | 1.03 | 0.50 | 1.73 | 0.41 |
| Spicy | *C. melo* var. *cantalupensis* | 56,537,156 | 96.68 | 75.53 | 0.30 | 0.13 | 0.94 | 0.27 |
| Manshuu | *C. melo* var. *makuwa* | 55,690,052 | 88.87 | 42.88 | 1.02 | 0.22 | 3.43 | 0.61 |
| Ougon-9 | *C. melo* var. *makuwa* | 64,043,888 | 88.90 | 43.41 | 1.16 | 0.27 | 3.48 | 0.63 |
| JSS-6 | *C. agrestis* (wild melon) | 61,361,440 | 88.98 | 43.20 | 1.06 | 0.22 | 3.44 | 0.61 |

**Genome reference: DHL92 CM3.6.1 (var. *inodorus x conomon-chinensis*)**

| | | | Concordantly aligned | | Discordantly aligned | | One mate aligned | |
|---|---|---|---|---|---|---|---|---|
| Accession | Species, subgroup | Total reads | %aligned | %complete match | %aligned | %complete match | %aligned | %complete match |
| Harukei-3 | *C. melo* var. *reticulatus* | 459,736,006 | 93.40 | 60.69 | 1.31 | 0.53 | 1.59 | 0.38 |
| Honey dew | *C. melo* var. *inodorus* | 54,438,848 | 88.98 | 51.75 | 2.56 | 0.87 | 3.07 | 0.67 |
| Spicy | *C. melo* var. *cantalupensis* | 56,537,156 | 87.84 | 45.95 | 2.46 | 0.68 | 3.48 | 0.71 |
| Manshuu | *C. melo* var. *makuwa* | 55,690,052 | 90.17 | 56.30 | 2.22 | 0.75 | 2.59 | 0.70 |
| Ougon-9 | *C. melo* var. *makuwa* | 64,043,888 | 90.37 | 57.17 | 2.26 | 0.80 | 2.61 | 0.70 |
| JSS-6 | *C. agrestis* (wild melon) | 61,361,440 | 89.70 | 55.52 | 2.45 | 0.84 | 2.76 | 0.75 |

**Genome reference: Payzawat, ASM976082v1 (var. *inodorus*)**

| | | | Concordantly aligned | | Discordantly aligned | | One mate aligned | |
|---|---|---|---|---|---|---|---|---|
| Accession | Species, subgroup | Total reads | %aligned | %complete match | %aligned | %complete match | %aligned | %complete match |
| Harukei-3 | *C. melo* var. *reticulatus* | 459,736,006 | 93.88 | 69.44 | 0.86 | 0.36 | 1.41 | 0.39 |
| Honey dew | *C. melo* var. *inodorus* | 54,438,848 | 90.09 | 63.54 | 1.47 | 0.58 | 2.71 | 0.85 |
| Spicy | *C. melo* var. *cantalupensis* | 56,537,156 | 88.86 | 55.37 | 1.23 | 0.31 | 3.18 | 0.85 |
| Manshuu | *C. melo* var. *makuwa* | 55,690,052 | 84.23 | 36.96 | 1.50 | 0.26 | 4.67 | 0.84 |
| Ougon-9 | *C. melo* var. *makuwa* | 64,043,888 | 84.20 | 37.48 | 1.62 | 0.31 | 4.70 | 0.85 |
| JSS-6 | *C. agrestis* (wild melon) | 61,361,440 | 84.26 | 37.05 | 1.57 | 0.28 | 4.67 | 0.84 |

**b**

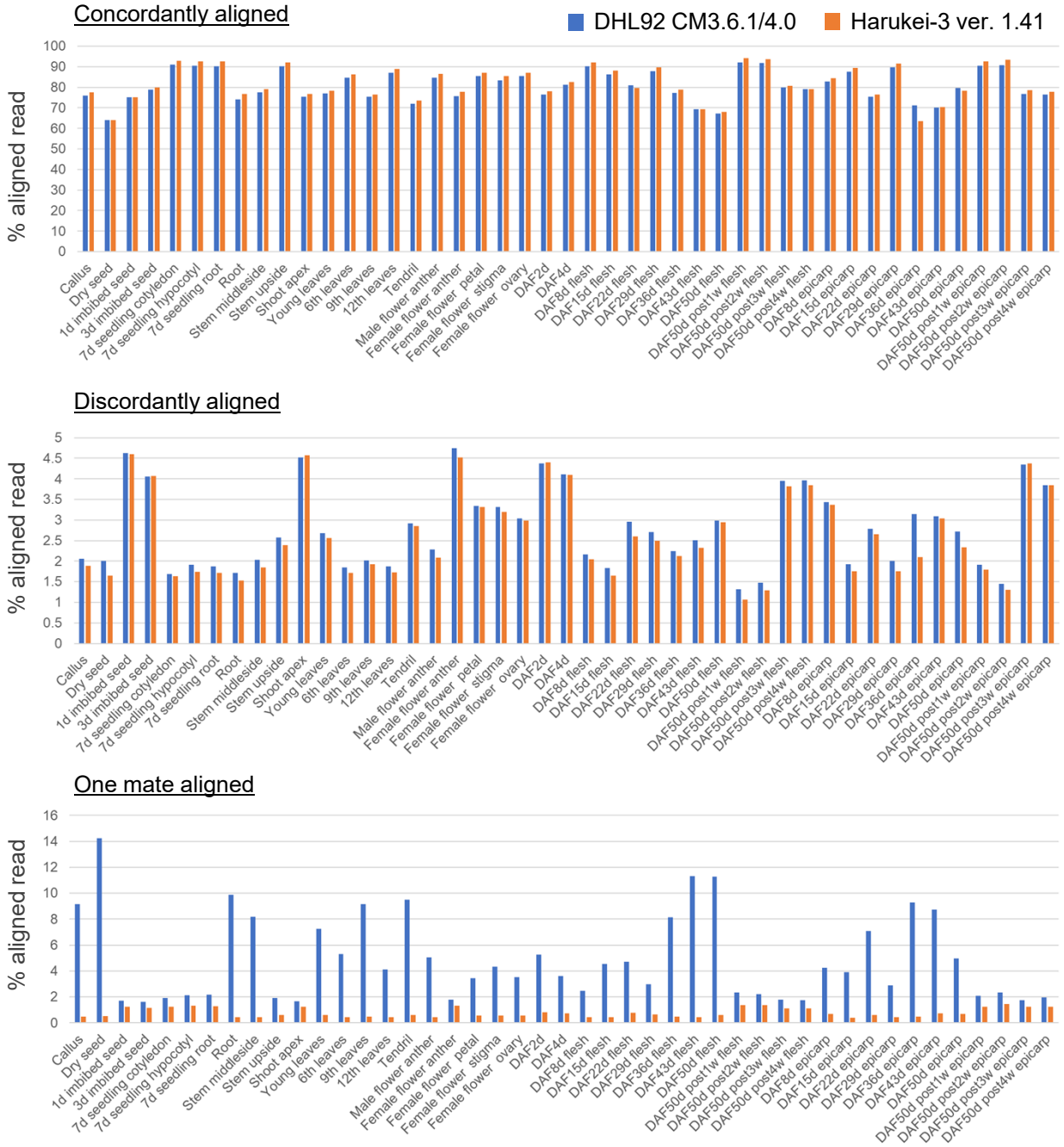
**Genome reference: Harukei-3 ver. 1.41 (var. *reticulatus*)**

| Accession | Intergenic | Synonymous | Non-CDS exon | Intron | Splicing junction | Nonsense | Missense | Mutation at start codon | Mutation at stop codon* |
|---|---|---|---|---|---|---|---|---|---|
| Harukei-3 | 22,588 | 336 | 1,727 | 6,774 | 31 | 145 | 570 | 8 | 41 |
| Honey dew | 748,925 | 18,512 | 33,906 | 211,054 | 579 | 1,313 | 20,743 | 119 | 459 |
| Spicy | 484,305 | 13,176 | 24,174 | 139,551 | 431 | 1,589 | 17,440 | 92 | 298 |
| Manshuu | 1,658,203 | 38,035 | 68,107 | 423,972 | 1,147 | 2,944 | 45,327 | 246 | 849 |
| Ougon-9 | 1,797,255 | 40,739 | 72,799 | 453,835 | 1,256 | 3,025 | 47,783 | 260 | 943 |
| JSS-6 | 1,485,476 | 35,092 | 62,158 | 378,455 | 1,084 | 2,610 | 41,023 | 220 | 780 |

**Genome reference: DHL92 CM3.6.1 + CM4.0 annotation (var. *inodorus x conomon-chinensis*)**

| Accession | Intergenic | Synonymous | Non-CDS exon | Intron | Splicing junction | Nonsense | Missense | Mutation at start codon | Mutation at stop codon* |
|---|---|---|---|---|---|---|---|---|---|
| Harukei-3 | 1,548,547 | 24,062 | 36,825 | 252,908 | 1,025 | 2,621 | 29,119 | 389 | 651 |
| Honey dew | 1,350,119 | 23,728 | 38,207 | 239,690 | 1,003 | 2,537 | 27,229 | 349 | 593 |
| Spicy | 1,616,475 | 29,635 | 46,548 | 297,333 | 1,223 | 3,353 | 35,955 | 421 | 689 |
| Manshuu | 962,558 | 17,714 | 29,345 | 175,515 | 822 | 2,584 | 22,426 | 299 | 531 |
| Ougon-9 | 911,926 | 17,143 | 27,925 | 169,146 | 758 | 2,346 | 21,155 | 299 | 516 |
| JSS-6 | 1,007,793 | 19,160 | 31,793 | 186,004 | 838 | 2,647 | 23,955 | 322 | 564 |

**Supplementary Fig. 10 Evaluation of melon genome references using Illumina short read resequencing**
**a** Comparison of short read alignment ratios between Harukei-3 (ver.1.41), DHL92[1,7] (CM3.6.1 assembly), and Payzawat[6] (ASM976082v1) genomes. Illumina short reads of six melon accession (Harukei-3, Honey Dew, Spicy, Manshuu, Ougon-9, and JSS-6) were aligned to the genome sequences with bowtie2. **b** Comparison of detected mutation type between Harukei-3 (ver. 1.41) and DHL92 (CM3.6.1 assembly + CM4.0 annotation).

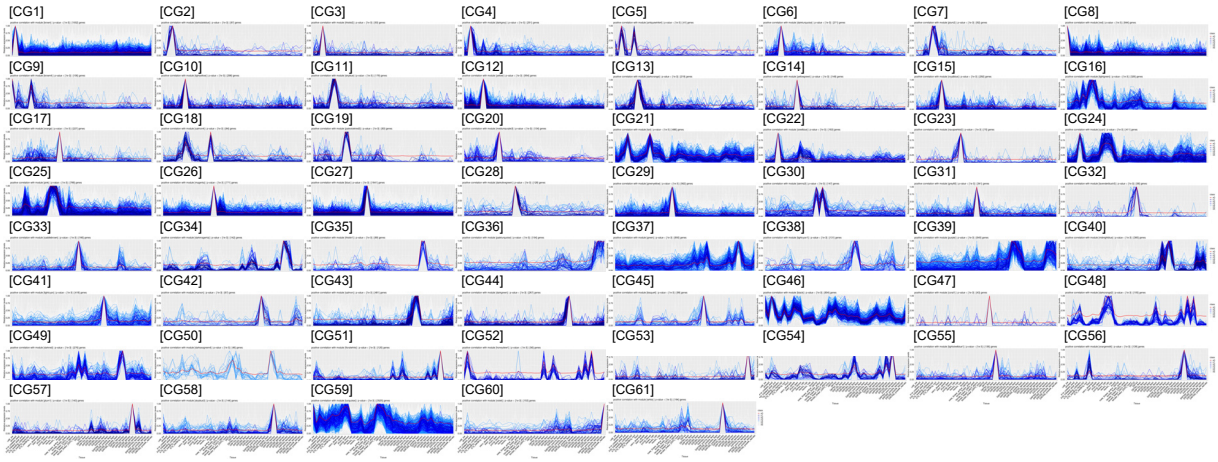



Harukei-3 melon, plant tissues or developmental stages

Number of genes with FPKM > 0.1 in at least one RNA-seq sample

Harukei-3 : **27,687** genes
DHL92 : **16,013** genes

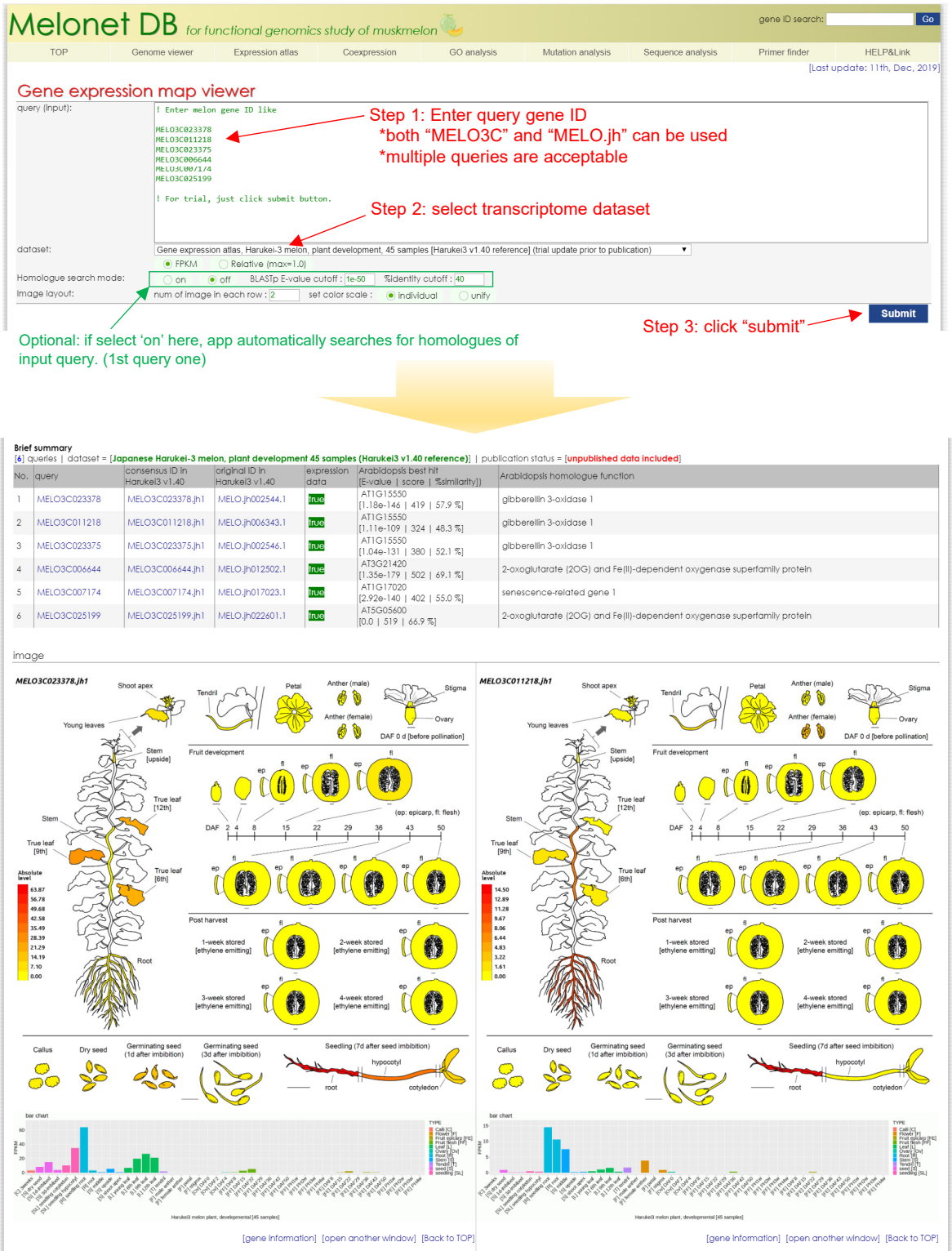
**Supplementary Fig. 11 Comparison of Illumina RNA-seq alignment ratio**
Illumina RNA-seq short reads of 45 tissue-wide samples (also summarized in Fig. 3a) were aligned to Harukei-3 (ver. 1.41) or DHL92 (CM 3.6.1 assembly + CM 4.0 annotation) genomes with HISAT2; then, alignment ratios were compared between the two genomes. Gene expression levels were calculated with StringTie, then, the number of genes with FPKM ≥ 0.1 in at least one sample was calculated.



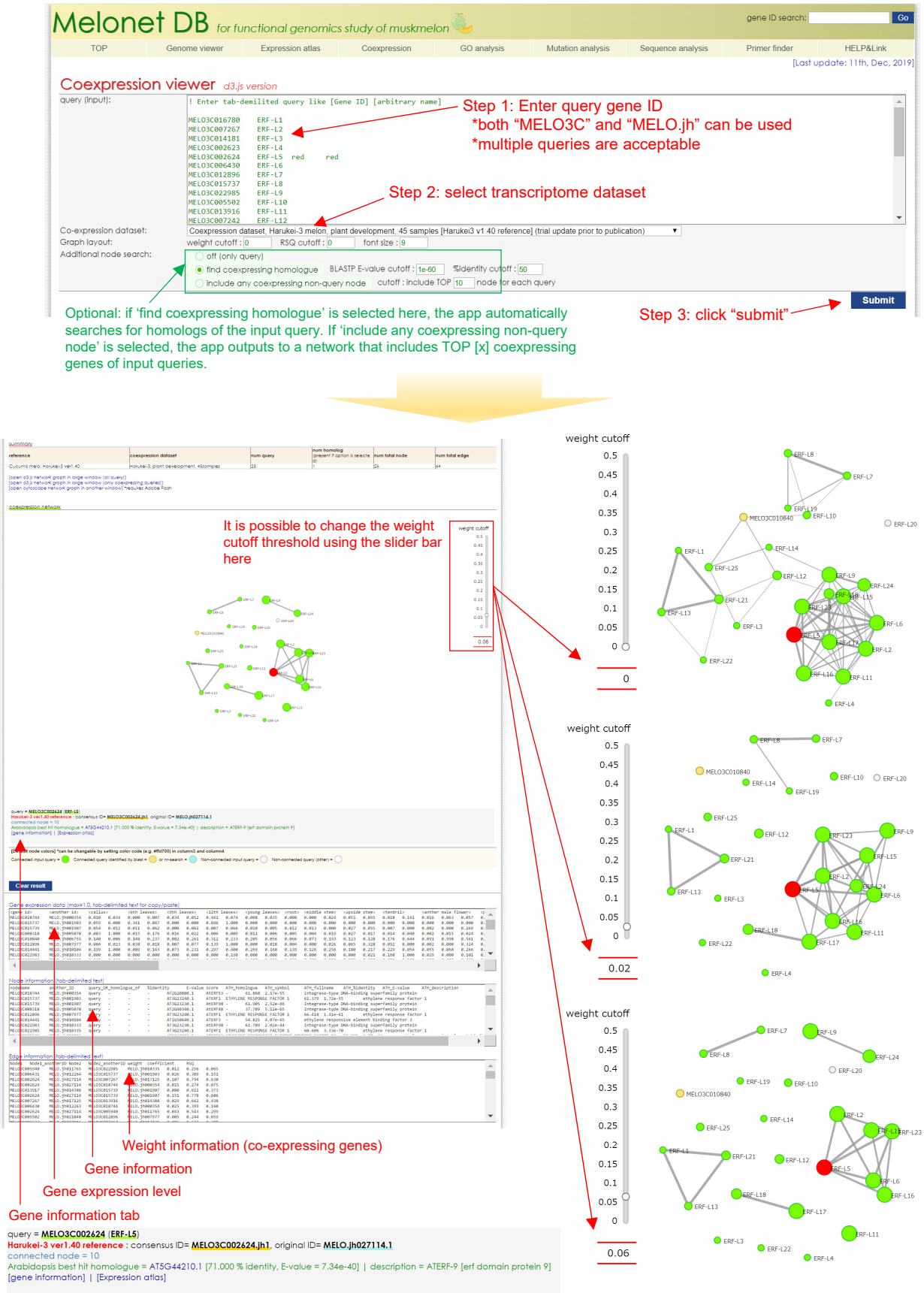

| Group | Tissue specificity | positive | negative |
|---|---|---|---|
| CG1 | Dry seed | 1,552 | 0 |
| CG2 | Imbibed seed | 87 | 0 |
| CG3 | Imbibed seed | 93 | 0 |
| CG4 | Imbibed seed \| callus | 251 | 0 |
| CG5 | Imbibed seed \| root (seedling) \| callus | 41 | 0 |
| CG6 | Hypocotyl (seedling) | 271 | 0 |
| CG7 | Hypocotyl (seedling) \| root (seedling) | 92 | 0 |
| CG8 | Callus | 844 | 0 |
| CG9 | Root (seedling) \| callus \| root \| imbibed seed | 106 | 0 |
| CG10 | Root \| root (seedling) | 298 | 0 |
| CG11 | Root \| root (seedling) | 179 | 0 |
| CG12 | Root \| root (seedling) | 954 | 0 |
| CG13 | Root \| stem \| root (seedling) \| hypocotyl(seedling) | 219 | 0 |
| CG14 | Shoot apex | 148 | 0 |
| CG15 | Stem | 292 | 0 |
| CG16 | Stem \| root \| hypocotyl (seedling) | 326 | 0 |
| CG17 | Tendril | 227 | 0 |
| CG18 | Tendril \| root \| stem \| hypocotyl (seedling) \| root (seedling) | 84 | 0 |
| CG19 | Young leaves \| shoot apex | 83 | 0 |
| CG20 | Young leaves \| shoot apex \| cotyledon (seedling) | 134 | 0 |
| CG21 | Cotyledon (seedling) \| young leaves | 486 | 10 |
| CG22 | Cotyledon (seedling) \| young leaves \| expanded leaves | 163 | 0 |
| CG23 | Expanded leaves \| tendril | 70 | 0 |
| CG24 | Expanded leaves \| young leaves \| cotyledon (seedling) | 411 | 28 |
| CG25 | Expanded leaves \| young leaves \| cotyledon (seedling) \| tendril \| hypocotyl (seedling) | 789 | 18 |
| CG26 | Male anther | 771 | 0 |
| CG27 | Male anther \| female anther | 1,641 | 17 |
| CG28 | Male anther \| female anther \| petal \| stigma | 128 | 3 |
| CG29 | Petal | 562 | 0 |
| CG30 | Petal \| stigma \| female anther \| male anther | 147 | 0 |
| CG31 | Stigma | 341 | 0 |

| Group | Tissue specificity | positive | negative |
|---|---|---|---|
| CG32 | Ovary at DAF0, 2, 4 | 58 | 0 |
| CG33 | Ovary at DAF0, 2, 4 \| fruit epicarp DAF8, 15 | 166 | 0 |
| CG34 | Fruit epicarp around DAF36 to 50 (highest at DAF36) | 142 | 1 |
| CG35 | Fruit epicarp around DAF8 to 15 (highest at DAF15) | 89 | 0 |
| CG36 | Fruit epicarp post harvest 1w-4w (highest at 2w) | 154 | 0 |
| CG37 | Fruit flesh and epicarp around DAF22 to 50 | 850 | 14 |
| CG38 | Fruit flesh and epicarp around DAF36 to 50 (highest at DAF43) | 131 | 1 |
| CG39 | Fruit flesh and epicarp post harvest 1w-4w (highest at 1w) | 645 | 10 |
| CG40 | Fruit flesh and epicarp post harvest 1w-4w (highest at 3w) | 385 | 25 |
| CG41 | Fruit flesh around DAF36 to 50 (highest at DAF50) \| post harvest 1w | 418 | 2 |
| CG42 | Fruit flesh post harvest 1w-4w (highest at 2w) | 67 | 0 |
| CG43 | Fruit flesh post harvest 1w-4w (highest at 3w) | 481 | 1 |
| CG44 | Fruit flesh post harvest 3w-4w (highest at 4w) | 267 | 0 |
| CG45 | - | 99 | 0 |
| CG46 | - | 804 | 7 |
| CG47 | - | 43 | 0 |
| CG48 | - | 105 | 2 |
| CG49 | - | 276 | 0 |
| CG50 | - | 46 | 0 |
| CG51 | - | 120 | 0 |
| CG52 | - | 56 | 0 |
| CG53 | - | 123 | 0 |
| CG54 | - | 63 | 0 |
| CG55 | - | 136 | 0 |
| CG56 | - | 139 | 0 |
| CG57 | - | 143 | 0 |
| CG58 | - | 144 | 0 |
| CG59 | - | 3,525 | 23 |
| CG60 | - | 153 | 0 |
| CG61 | - | 194 | 0 |
| CG62 | - | 0 | 0 |

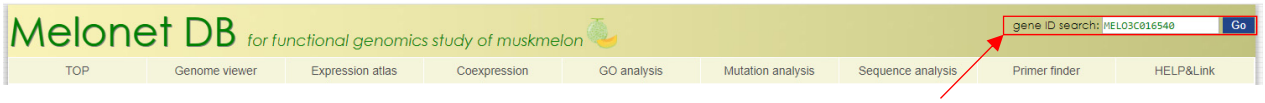
**Supplementary Fig. 12 WGCNA clustering of tissue-wide transcriptome dataset**
WGCNA co-expression clustering was performed based on the tissue-wide transcriptome dataset of Harukei-3 (Fig. 3a). In total, 62 co-expression groups were found. Pink and red rectangles indicate co-expression groups associating with fruit development or post-harvest ripening fruits, respectively.




**Supplementary Fig. 13 Updated version of 'Gene expression map viewer' in the Melonet-DB**
This web application is available at the following URL: https://melonet-db.dna.affrc.go.jp/ap/mvw.




**Supplementary Fig. 14 Updated version of 'Coexpression viewer' in the Melonet-DB**
This web application is available at the following URL: https://melonet-db.dna.affrc.go.jp/ap/mds.



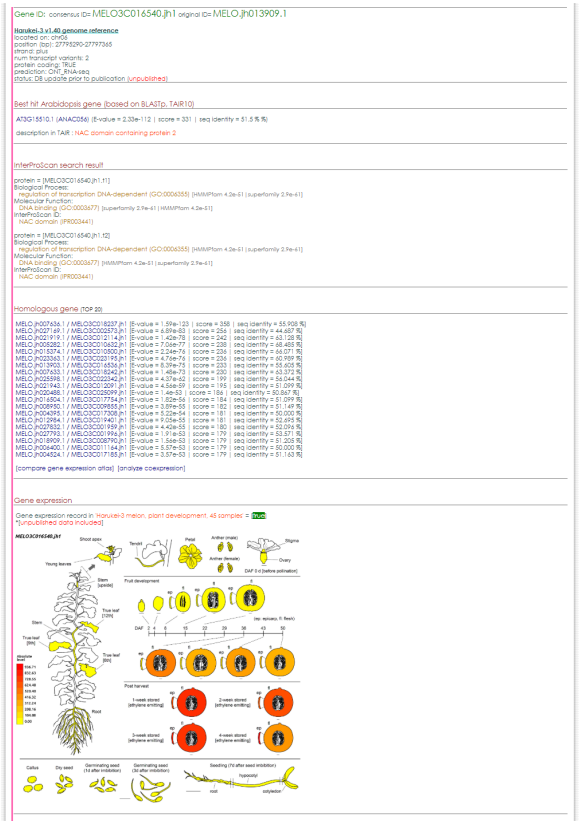

Enter query gene ID and click 'GO'
*both "MELO3C" and "MELO.jh" can be used
*multiple queries are acceptable

summary

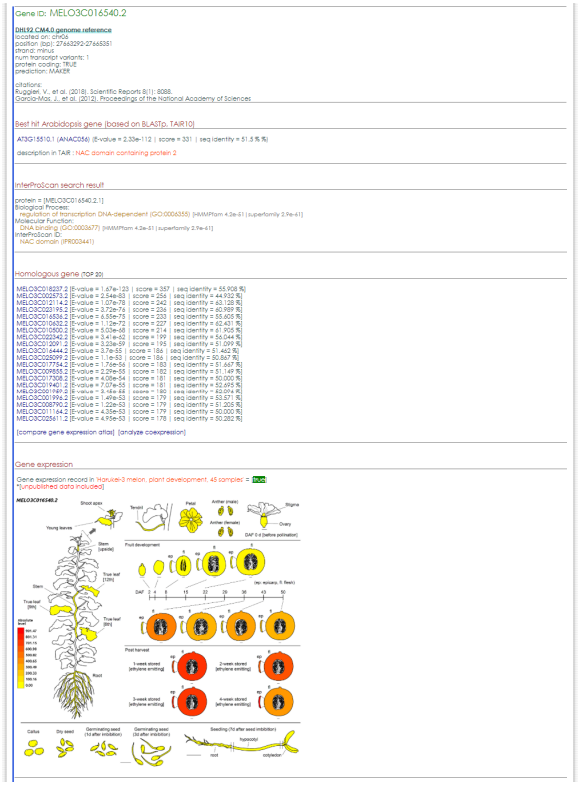
| query | DHL92 CM4.0 reference | Harukei-3 v1.40 reference (original ID) | Harukei-3 v1.40 reference (consensus ID, present if 1to1 ortholog) |
|---|---|---|---|
| MELO3C016540.jh1 | MELO3C016540.2 | MELO.jh013909.1 | MELO3C016540.jh1 |

compare sequences between references: [transcript] [protein]

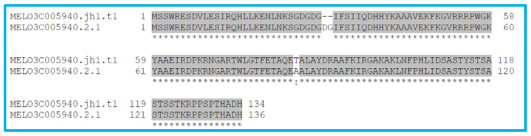
Information based on Harukei-3 reference



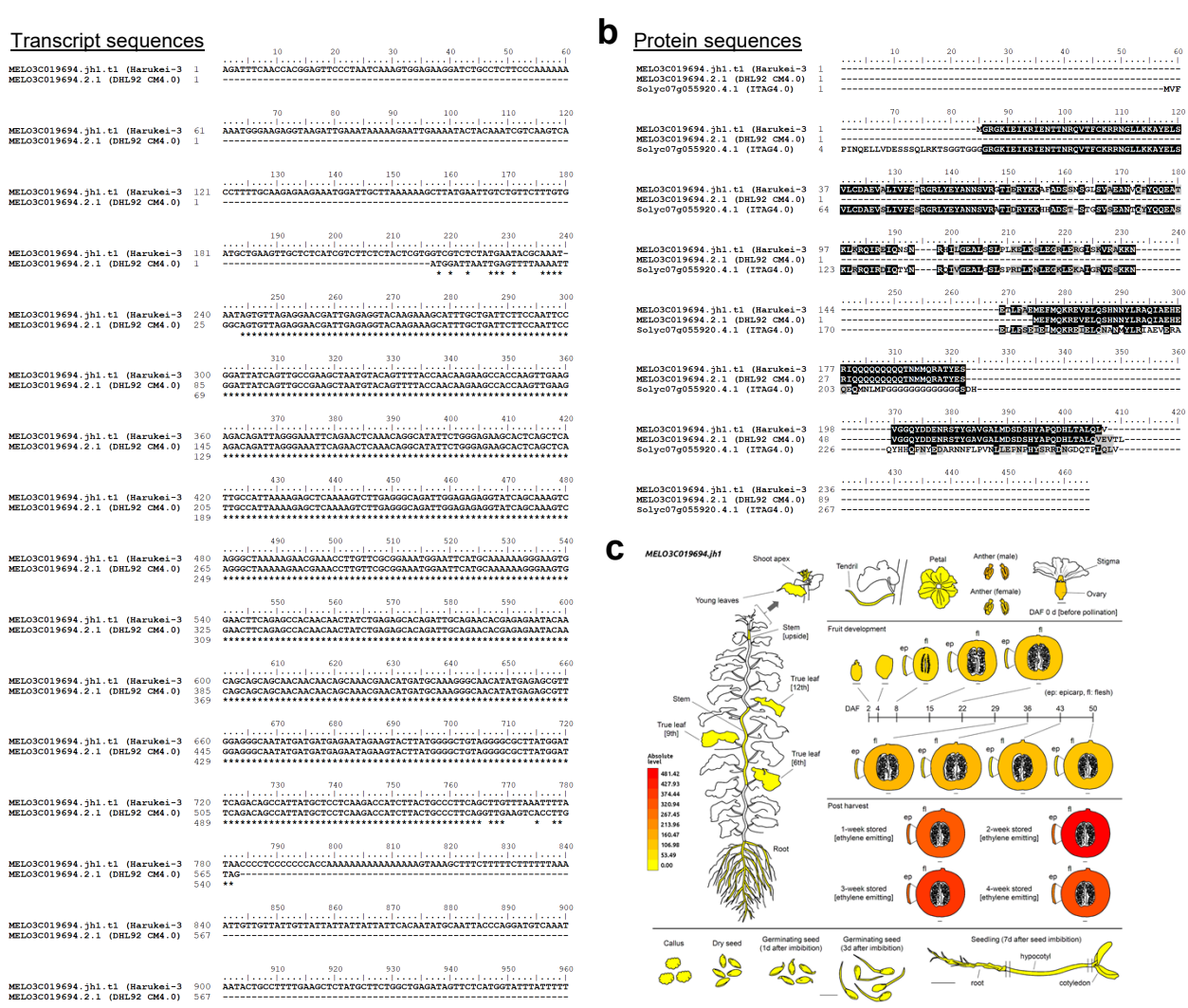
Information based on DHL92 reference



By clicking '[transcript]' or '[protein]' link, it is possible to perform sequence alignment between the two genome references.

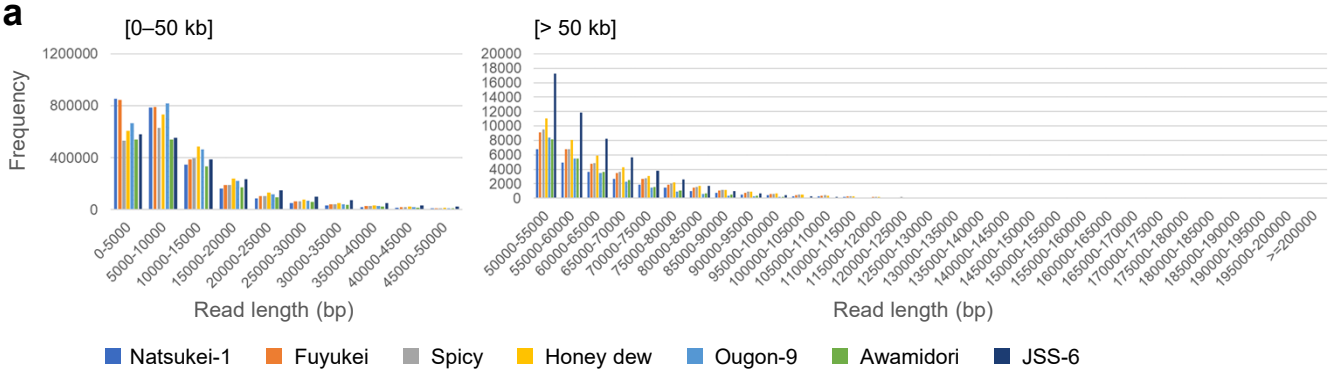

**Supplementary Fig. 15 Gene information search function in the Melonet-DB**
This web application is available in Melonet-DB web site: https://melonet-db.dna.affrc.go.jp/.




**Supplementary Fig. 16 Sequence and gene expression pattern of *MELO3C019694***

**a, b** Comparison of transcript (a) or protein amino acid (b) sequences of an *AGAMOUS*-like gene, *MELO3C019694*, between Harukei-3 (top) and DHL92 (bottom). c Tissue-wide gene expression pattern of *MELO3C019694* in Harukei-3.

**a**




**b**

| Accession | Read count | Total length (bp) | Average (bp) | Max (bp) | $N_{50}$ (bp) |
|---|---|---|---|---|---|
| Natsukei1 | 2,393,835 | 22,828,785,451 | 9,536 | 218,124 | 13,972 |
| Fuyukei | 2,519,061 | 26,060,465,229 | 10,345 | 313,129 | 15,542 |
| Spicy | 2,060,998 | 24,591,127,917 | 11,932 | 286,854 | 16,856 |
| HoneyDew | 2,444,716 | 29,608,876,134 | 12,111 | 244,739 | 16,941 |
| Ougon9 | 2,490,216 | 27,243,224,015 | 10,940 | 204,102 | 15,172 |
| Awamidori | 1,867,169 | 21,287,972,918 | 11,401 | 218,625 | 16,795 |
| JSS6 | 2,248,973 | 30,902,529,697 | 13,741 | 207,635 | 21,374 |

**c**

Canu ver. 1.8 contig assembly

| Accession | Num contig | Total length (Mb) | Average (kb) | Max (kb) | $N_{50}$ (kb) |
|---|---|---|---|---|---|
| Natsukei-1 | 367 | 371.8 | 1,013 | 13,823 | 3,946 |
| Fuyukei | 265 | 371.3 | 1,401 | 20,607 | 4,863 |
| Spicy | 223 | 367.9 | 1,650 | 20,683 | 5,966 |
| HoneyDew | 288 | 373.2 | 1,296 | 15,114 | 5,084 |
| Ougon-9 | 250 | 361.0 | 1,444 | 19,751 | 4,143 |
| Awamidori | 271 | 361.0 | 1,332 | 16,057 | 5,513 |
| JSS6 | 129 | 361.6 | 2,803 | 32,807 | 10,243 |

Corrected contig assembly

| Accession | Num contig | Total length (Mb) | Average (kb) | Max (kb) | $N_{50}$ (kb) |
|---|---|---|---|---|---|
| Natsukei-1 | 462 | 373.7 | 809 | 13,898 | 3,505 |
| Fuyukei | 349 | 373.1 | 1,069 | 18,205 | 4,043 |
| Spicy | 262 | 369.6 | 1,411 | 20,815 | 5,044 |
| HoneyDew | 354 | 374.3 | 1,057 | 11,987 | 4,767 |
| Ougon-9 | 295 | 363.1 | 1,231 | 17,961 | 3,706 |
| Awamidori | 315 | 362.8 | 1,152 | 16,150 | 4,709 |
| JSS6 | 157 | 363.6 | 2,316 | 25,717 | 10,148 |

**Supplementary Fig. 17 Contig assembly in seven melon accessions**
**a** Histograms of sequenced reads in Oxford NanoPore technology (ONT; R9.4.1 flow cell). **b** Read length statistics of ONT reads. **c** Sequence length statistics of the contig assemblies in seven melon accessions. Contigs were initially assembled with Canu ver. 1.8 software based on ONT reads and the resultant contigs were subjected to racon and medaka polishing to correct sequence errors. Then, contigs with chimeric sequences (incorrectly assembled contigs) were identified and split by searching for the positions where the number of aligned ONT reads were less than four. The top panel indicates the value of the sequence length statistics of the Canu ver. 1.8 contig assemblies while the bottom indicates those of the corrected contig assemblies.

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