Chromosome-level assembly of *Cucumis sativus cv.* 'Tokiwa' as a reference genome of Japanese cucumber.

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Abstract

Cucumber is one of the most important vegetables in the Japanese market. To facilitate genomics-based breeding, there is a demand for reference genome of Japanese cucumber. However, although cucumber genome is relatively small, its assembly is a challenging issue because of tandem repeats comprising ~30% (~100 Mbp) of the genome. To overcome, we deployed the Oxford nanopore sequencing that produces long reads with N50 length of >30 kbp. With this technology we achieved a chromosome-level assembly of cv 'Tokiwa', a founder line of Japanese cucumber represented with the elongated fruit shape and high-crisp texture. Compared to the existing cucumber genomes, the Tokiwa genome is 20% longer and annotated with 10% more genes. The assembly with nanopore long reads also resolved tandem repeats spanning >100 kbp, demonstrating its strength in overcoming repetitive sequences.

Introduction

Cucumber (*Cucumis sativus* L.) shares the 3rd place in the value production in the Japanese vegetable market, after tomato, and onion (https://www.usdajapan.org/wpusda/wp-content/uploads/2019/03/Japanese-Fresh-Vegetable-Market-Overview-2018_Osaka-ATO_Japan_12-21-2018-1.pdf). The Japanese cultivars of cucumber are represented with a long and straight fruit shape (Shimomura et al. 2016) and a crispy texture (Yoshioka et al., 2009), due to specific needs of the customers. Thus, breeding efforts of cucumber mainly target resistance to diseases including melon yellow spot virus and powdery mildews without spoiling the important traits described above. Heat tolerance has also become an important target because of the global warming. To facilitate breeding of such cultivars, we need to reconstruct a reference genome of Japanese cucumber and implement marker-assisted selection, allele mining via GWAS and prediction models for genomic selection.

However, efforts to sequence cucumber genomes have faced difficulties despite its relatively small size (~350 Mbp) (Huang et al., 2009, Yang et al., 2012, Li et al., 2019). The current reference genome is “Chinese long” inbred line 9930, which was sequenced with single molecule real-time (SMRT) sequencing technology (Li et al., 2019). The 9930 genome consists of 7 pseudomolecules covering 211 Mbp + 15 Mbp unanchored scaffolds, leaving ~120 Mbp sequences missing (Huang et al., 2009). More recently 11 more accessions have also been sequenced with the SMRT sequencing, but the assemblies were only slightly better than the 9930 genome (Li et al., 2022). The difficulty in assembling cucumber genome derives from long arrays of tandemly repeated sequences around centromeric regions (Ganal et al., 1986, Ganal and Hemleben, 1988).

To overcome the difficulties, increasing read length is the simplest solution. We adopted a recently-developed technology by Oxford Nanopore, which can generate ultra long reads spanning more than 100 kbp (Wang et al, 2021). Many plant genomes have already been sequenced with this technology, some achieving the NG50 length of more than 10 Mbp (Dumschott et al., 2020). Thus, we

expect it may improve *de novo* assembly of a cucumber genome.

Here, we sequenced and assembled the whole genome of a Japanese cucumber cultivar, cv. 'Tokiwa' (Fig. 1). 'Tokiwa' was developed from a cross between North China type 'Shimo-shirazu' and South China type 'Shindome' around 1960. Given its Japanese favored fruit morphology and texture and adaptability to both summer and fall cropping system, it has been a founder line of various modern cultivars, which is now called as "Tokiwa group" (Fujieda, 2006). The Nanopore-only assembly generated greatly improved contiguity and coverage compared to the 9930 genome, providing a qualified knowledge-base for Japanese breeders.

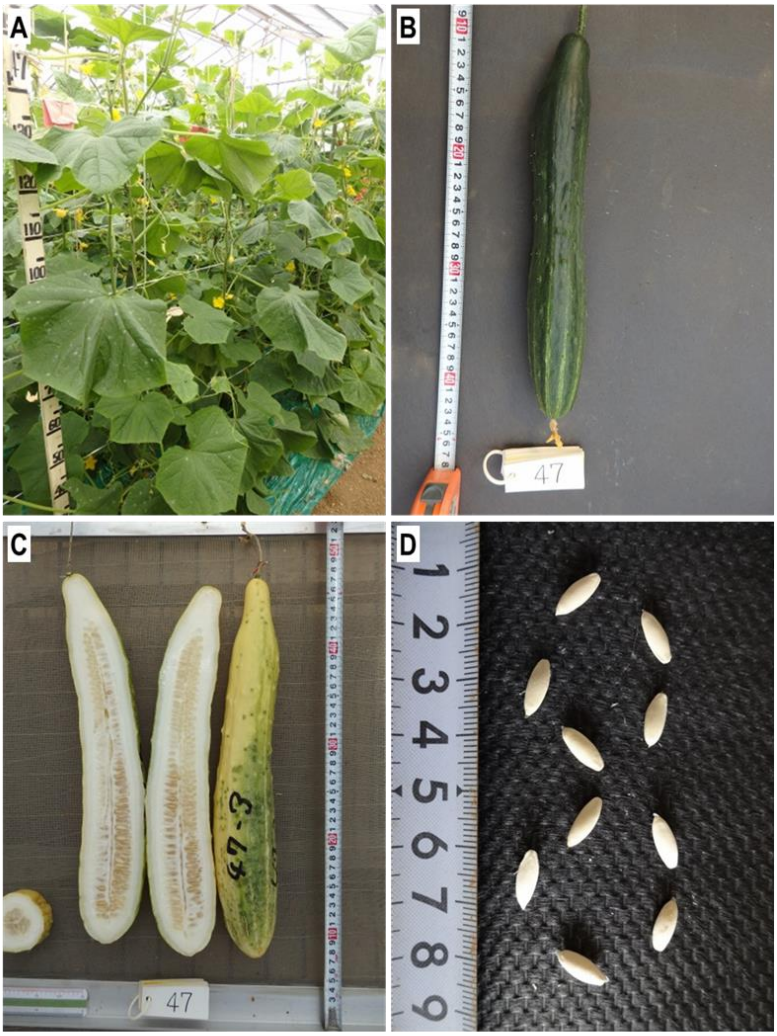

Figure 1. Photographs of *Cucumis sativus* L. cv 'Tokiwa'. a. Plants growing in a greenhouse. b. A green fresh fruit. c. Mature fruites. d. Seeds.

Materials and methods

Plant material and DNA extraction

The cucumber cultivar 'Tokiwa' was provided by National Genebank Program in Research Center of Genetic Resources, NARO. We extracted genomic DNA from 3 g of young leaves using Nucleobond HMW DNA kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). We basically followed the DNA extraction protocol provided by the manufacturer, but we extended the lysis step up to 3 h instead of 30 min. In the precipitation + washing step with isopropanol and 70% (v/v) ethanol, we did not centrifuge but directly transfer the precipitated DNA by pipetting.

We also extracted RNA from the leaves for gene annotation used by NucleoSpin RNA Plant kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany).

Sequence of DNA and RNA

For long-read sequencing, we followed the protocol as described by Naito (2023). First, we size-selected the genomic DNA using Short Read Eliminatior XL kit (Pacific Biosciences of California, Inc., California,USA) to remove the DNA fragments <10 kbp. Second, we put 3 μg of the size-selected DNA for end-repair using NEBNext FFPE DNA Repair Mix (NEW INGLAND BioLabs inc., Massachusetts, USA) and NEBNext Ultra II End Repair/dA-Tailing Module. Third, we ligated the end-repaired DNA with the sequencing adaptor in SQK-LSK109 (Oxford Nanopore Technologies KK, Tokyo, Japan) using NEBNext Quick Ligation Module (NEW INGLAND BioLabs inc., Massachusetts, USA). In each step, we purified DNA using AMPure XP (BECKMAN COULTER inc., California, USA). The prepared library was then loaded to R9.4.1 Flowcell (Oxford Nanopore Technologies KK, Tokyo, Japan) and was run in PromethION24 (Oxford Nanopore Technologies PLC, Oxford, UK) for 10 h. We also obtained short-reads for polishing the draft assembly with Illumina Hiseq 4000, which was provided as a customer service by GeneBay, Inc. (Yokohama, Japan). For

94 scaffolding, we obtained optical mapping data with Bionano Sahyr, provided as a customer service by
95 AS ONE corp. (Osaka, Japan). We also sequenced the RNA with Hiseq4000 (Illumina Inc., San Diego,
96 USA) for gene annotation, provided as a customer service by GeneBay Inc. (Yokohama, Japan). All
97 the data is uploaded to the Sequence Read Archive in NCBI website, with BioSample ID
98 SAMN40100976 in BioProject ID PRJNA1080095.
99
100 *Genome assembly and scaffolding*
101 We also followed the protocol described by Naito (2023) for genome assembly. Fist, we used necat-
102 0.0.1 (Chen et al., 2021) with default parameters except "PREP_OUTPUT_COVERAGE=60" and
103 "CNS_OUTPUT_COVERAGE=40". The draft contigs were then polished twice with racon-1.4.3
104 (Vaser et al., 2017) with options of "-m 8 -x -6 -g -8 -w 500" and medaka-1.0.3
105 (https://github.com/nanoporetech/medaka) with options of "-g -m r941_prom_high_g360". To check
106 misassemblies, we first aligned the polished contigs to the 9930 genome by D-genies (Cabanettes and
107 Klopp 2018) to visualize conflicted sites. We then mapped the long-reads with minimap2-2.14 (Li,
108 2018) and manually checked the conflicted sites. If the mapped reads were discontinuous as presented
109 by Sakai et al. (2015), we broke the contig at the site. The corrected contigs were further polished with
110 Illumina short-reads twice with Hypo-1.0.3 (https://github.com/kensung-lab/hypo) with default
111 parameters. The Hypo-polished contigs were then run with purge_haplotigs-1.1.1 (Roach et al., 2018)
112 to remove low-coverage contigs and those derived from heterozygous regions.
113 After assembling, polishing and filtering the contigs, we obtained optical mapping data
114 provided as a customer service by AS ONE, corp. (Osaka, Japan). High-molecule genomic DNA was
115 extracted from young leaves with the Plant DNA Isolation Kit (Bionano Genomics, San Diego, CA,
116 USA) according to Bionano Prep Plant Tissue DNA Isolation Base Protocol. The DNA was treated
117 with DLE-1 nickase, labelled with DLS DNA Labeling Kit (Bionano Genomics), and run on the

118 Saphyr Genome Mapping Instrument (Bionano Genomics). The obtained Saphyr reads were
119 assembled and then merged with the draft assembly to generate hybrid scaffolds sequences with
120 Bionano Solve (Bionano Genomics) with the default parameters. Tha Bionano data is also available
121 from NCBI website with BioProject ID: PRJNA1098469.

122 Finally, pseudomolecules were reconstructed by anchoring the hybrid scaffolds to the
123 genetic map, which was previously constructed (Sugiyama et al., 2016).

124 To compare the assembly with 9930 genome, we made a whole genome alignment by
125 minimap2 and ran the output with D-genies to generate a dotplot.

126

127 *Assembling organelle genomes*

128 Given there are hundreds of chloroplasts and tens of mitochondria in a single cell, the coverage depth
129 is usually too high for assembling the organellar genomes. Thus, we had to extract and downsample
130 the long reads derived from the organellar genomes.

131 To do so, we first identified the organellar contigs by BLASTing the reference genomes of
132 chloroplast (Pląder et al., 2007) and mitochondrion (Alverson et al., 2011) as query to the draft
133 assembly. Then we mapped the long reads to the draft assembly and recovered the reads that were
134 mapped to the organellar contigs. We then downsampled the recovered reads to ~50x coverage and
135 ran Flye-2.9 (Kolmogorov et al., 2019). The assembled sequences were then polished with long reads
136 with medaka.

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138 *Gene predictions*

139 Briefly, we predicted gene structures using Braker-2.0 (Brůna et al., 2021) using protein sequences
140 and GeMoMa-1.9 (Keilwagen et al., 2018) using our own RNA-seq data and the coding sequences of
141 9930 genome.

First, we softmasked the Tokiwa genome by running RepeatModeler-2.0.5 (https://www.repeatmasker.org/RepeatModeler/) and RepeatMasker-4.0.9 (https://www.repeatmasker.org/RepeatMasker/) with default parameters. Second, we downloaded the sequences of plant proteins from OrthoDB (Kuznetsov et al., 2022) as well as the license for GeneMark-EP+ (Brůna et al., 2020), which was required for running Braker-2.0. Third, we ran braker-2.0 on the softmasked genome with an option of "--softmasking".

Before running GeMoMa, we removed noisy reads from the RNA-seq data. To do so, we *de novo* assembled the RNA-seq data with Trinity-2.13.0 (Grabherr et al., 2011) with default parameters. Then we mapped the RNA-seq reads to the assembled transcript sequences using HISAT-2.2.1 (Kim et al., 2019) and recovered only concordantly mapped reads. The recovered RNA-seq reads were then mapped to Tokiwa genome by HISAT-2.2.1 with an option of "--max-intronlen 15000", which was used for intron prediction by GeMoMa. Then we ran GeMoMa using 9930 genome and gene annotations as reference, with parameters for unstranded RNA-seq data. After running the annotation tools, we merged the outputs of GeMoMa and Braker, by filtering the overlapping annotations. Then we ran EnTAP-0.10.8 (Hart et al., 2020) with default parameters to functionally annotate the predicted genes. The final annotations retained those identified as "Viridiplantae" and "Eukaryotes" for Taxonomy scope.

We also reannotated the 9930 genome and those of the 11 accessions assembled by Li et al. (2022). The 11 genome sequences were downloaded from the website of NCBI datasets. The downloaded genomes were annotated by GeMoMa-1.9 with the predicted gene models of 'Tokiwa' as reference. JCVI MCscan (https://github.com/tanghaibao/jcvi/wiki/MCscan-(Python-version)) was used to predict synteny block between each genome.

The predicted genes were evaluated with eudicots_odb10 (Kriventseva et al., 2018) by BUSCO-5.5 (Manni et al., 2021).

Phylogenetic analysis

To place the Tokiwa genome in a phylogenetic position, we first ran orthofinder-2.5.5 (Emms et al., 2019) on the protein sequences of the 12 accessions, with "-M msa" option. Then we reconstructed a maximum likelihood tree from the ouput mafft file of single copy orthologs by running iqtree-2.2.6 with options of "-m MFP -bb 1000 -alrt 1000 -nt AUTO".

Repeat annotation

To identify sequences derived from transposable elements, we ran EDTA-2.2.2 (Ou et al., 2019) with the predicted coding sequences of 'Tokiwa' in a sensitive mode. To identify tandem repeats, we ran trf-4.0.9 with parameters suggested by the official protocol.

Results

De novo *Assembly of Tokiwa genome*

We have successfully reconstructed the pseudomolecules of the 7 chromosomes in Tokiwa genome, covering 50 Mbp more than the 9930 genome (Table 1, Fig. 2).

Using the Oxford Nanopore sequencer, we obtained 34.2 Gbp of long reads with the N50 length of 33.2 kbp (Supplemental Table 1). The draft assembly with NECAT was ~300 Mbp sequences in 645 contigs, with the N50 length of 12.1 Mbp and the maximum length of 20.1 Mbp (Table 1). Purge_haplotigs identified 260 contigs as repetitive or low-coverage junk and thus retained 385 contigs covering 278.7 Mbp. The following optical mapping scaffolded only 71 contigs but improved the N50 length to 23.4 Mbp and the maximum length to 45.1 Mbp. Finally, we anchored the 14 scaffolds to the genetic linkage map (Sugiyama et al., 2015) and reconstructed the pseudomolecules of the 7 chromosomes (Fig. 2). After removing the scaffolds/contigs derived from organelle genome

and possible contaminations, 23.6 Mbp in 130 scaffolds/contigs remained unplaced. All the seven pseudomolecules contained telomeric repeats in both ends.

We also separately assembled the genome sequences of the chloroplast and mitochondrion. The chloroplast genome was successfully assembled into a single circular genome. However, due to the highly recombining feature of mitochondrion, we could not circularize the mitochondrial genome and ended up with 6 contigs (Supplemental Table 2).

Table 1. The statistics of genome assemblies.

	NECAT	Purge_haplotigs	BioNano	Tokiwa v1.0	9930 v3
Num	645	385	314	7	7
Sum	300,877,148	278,732,282	299,675,636	257,281,677	210,974,704
Min	208	4,095	4,095	27,903,525	22,466,726
Ave	466,476	723,980	954,381	36,754,525	30,139,243
Max	20,967,667	20,967,667	45,117,507	45,117,507	40,877,379
N50	12,103,241	12,256,096	23,376,839	36,771,724	31,913,682
GAP	-	-	20,923,098	15,419,875	36,170
Total ATGC	-	-	278,752,538	241,861,802	210,938,534
Non-placed scaffolds	-	-	-	130	81
Non-placed bps	-	-	-	23,587,426	15,666,262
Protein coding genes				27,905	24,317

High duplication rate in the Tokiwa genome than others

The pseudomolecules of Tokiwa genome reached 257 Mbp, which was >40 Mbp longer than those of the 9930 genome (Fig. 2B). Even though the extended sequences were mostly in centromeric, highly repetitive regions, gene coding sequences were also present (Fig. 2A). Although we used the Tokiwa's gene models to reannotate the 9930 and other genomes for fair comparison, the Tokiwa genome contained ~3,000 more genes than others (Table 2). The BUSCO scores were not greatly different among them, but the predicted gene models in the Tokiwa genome contained ~10 times more

206 duplicated BUSCO genes (Table 2).

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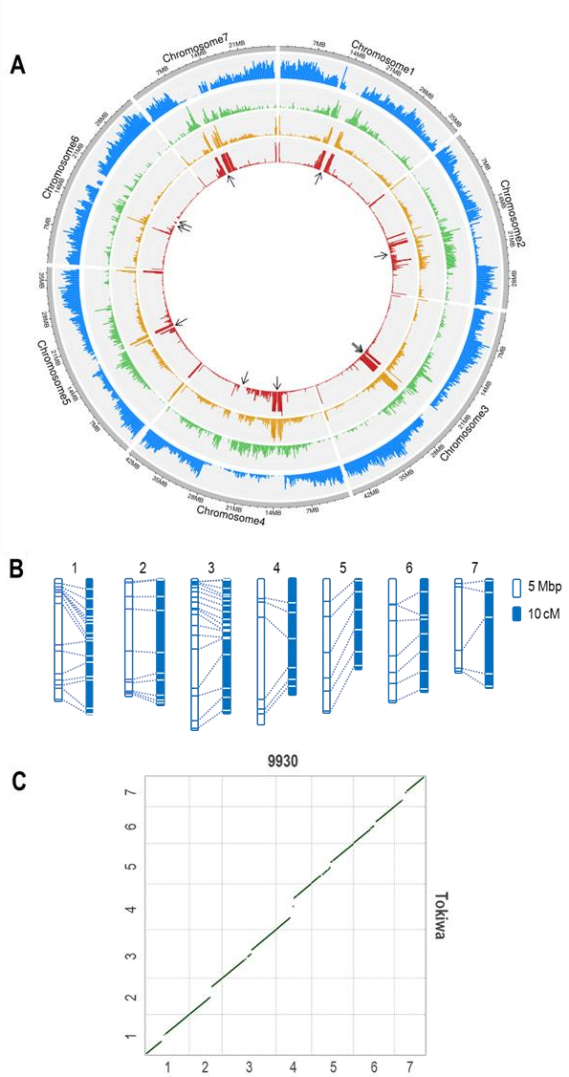

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222 Figure 2. Overview of Taokiwa genome. A. Circos plot indicating densities of, from the outer to inner tracks, protein coding sequences, LTR retrotransposons,

223 DNA transposable elements, and tandem repeats. B. Coliniarity between the physical positions and the genetic linkage map of the Tokiwa genome. White

224 and blue boxes indicate the physical and genetic map, respectively. Dashed lines indicate the corresponding positions. C. Dotplot of a whole genome alignment

225 of 9930 and Tokiwa. *x*- and *y*-axes indicate Chromosome numbers. Cultivar names are indicated above the *x*-axis and right to the *y*-axis.

226 The genome annotation revealed highly conserved synteny between the cucumber genomes

227 including wild accessions, except several inversions in Chromosomes 4, 5, and 7 of Cuc64 and W8,

228 which were also revealed by genome alignment-based analysis (Li et al., 2022). (Fig. 3). However, it

229 also revealed that the genes present in the newly assembled regions of the Tokiwa genomes were

mostly the duplicated copies of those in other regions (Fig. 3). The high rate of duplicated genes was also noted when we extracted single-copy orthologs for phylogenetic analysis. Without the Tokiwa genome, there were 17,619 single-copy orthologs among the 11 genomes. However, with the Tokiwa genome, the number decreased to 13,542. This was because the ~4,000 genes, present as single copy in all the Pacbio-sequenced genomes, had duplicated copies in the Tokiwa genome.

Table 2. BUSCO scores of the Tokiwa other genomes

	Complete		Single		Duplicate		Fragmented		Missing	
	Number	(%)	Number	(%)	Number	(%)	Number	(%)	Number	(%)
Tokiwa	2,281	(98.1)	1,797	(77.3)	484	(20.8)	6	(0.3)	39	(1.6)
9930	2,268	(97.5)	2,217	(95.3)	51	(2.2)	12	(0.5)	46	(2.0)
9110gt	2,250	(96.7)	2,213	(95.1)	37	(1.6)	11	(0.5)	65	(2.8)
Cu2	2,266	(97.4)	2,212	(95.1)	54	(2.3)	10	(0.4)	50	(2.2)
Cuc37	2,262	(97.3)	2,221	(95.5)	41	(1.8)	11	(0.5)	53	(2.2)
Cuc64	2,273	(97.7)	2,231	(95.9)	42	(1.8)	8	(0.9)	45	(2.0)
Cuc80	2,272	(97.7)	2,225	(95.7)	47	(2.0)	8	(0.3)	46	(2.0)
Gy14	2,256	(97.0)	2,212	(95.1)	44	(1.9)	10	(0.4)	60	(2.6)
Hx14	2,251	(96.7)	2,213	(95.1)	38	(1.6)	11	(0.5)	64	(2.8)
Hx117	2,233	(96.0)	2,189	(94.1)	44	(1.9)	15	(0.6)	78	(3.4)
W4	2,267	(97.4)	2,224	(95.6)	43	(1.8)	9	(0.4)	50	(2.2)
W8	2,251	(96.7)	2,206	(94.8)	45	(1.9)	13	(0.6)	62	(2.7)
XMTC	2,259	(97.1)	2,217	(95.3)	42	(1.8)	12	(0.5)	55	(2.4)

With the 13,542 single-copy orthologs, we reconstructed the phylogenetic tree (Fig. 3). Our phylogenetic tree grouped the cultivars (9110gt, 9930, XMTC, Tokiwa, Cu2, Cuc37, Gy14, Cuc80, Hx117 and HX14) and the wild cucumbers (*C. sativus* var. *hardwickii*) (W4, Cuc64 and W8) into monophyletic clades, respectively. ‘Tokiwa’ formed an East-Asian group with the Chinese cultivars 9110gt, 9930, XMTC, and Cu2 (Fig. 3).

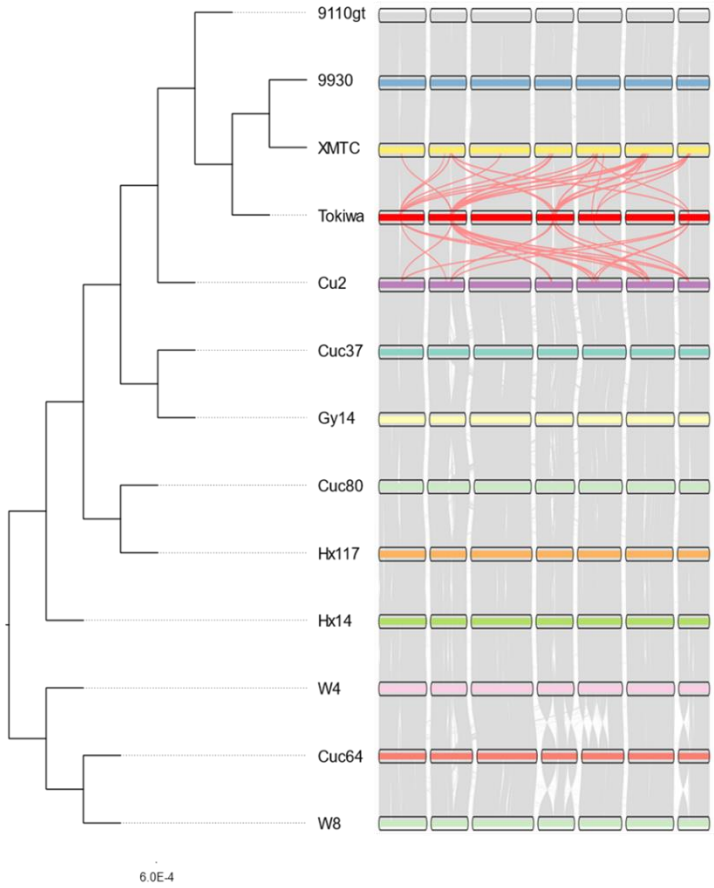

Figure 3. Phylogenetic tree and synteny plot of the 12 chromosome-level genome assemblies. Gray lines indicate synteny blocks between the genomes. Light red lines indicate pseudo synteny blocks between the newly assembled regions of the Tokiwa genome and XMTC or Cu2. Bootstrap values were 100 in all the nodes.

Distribution of transposable elements in Tokiwa genome

Because of the relatively small size of the genome, transposable elements shared only 23.9% of the Tokiwa genome (Supplemental Table 2). Overall, DNA transposons were more abundant than LTR retrotransposons were. The most abundant was Mutator family, comprising nearly 10% (22.8 Mbp) of the Tokiwa genome, followed by Gypsy, Copia, and CACTA.

In the Tokiwa genome, the distribution of LTR retrotransposons and DNA transposons were basically exclusive to each other (Fig. 2A). While the DNA transposons were highly condensed near centromeric and the telomeric regions, the LTR retrotransposons were more broadly distributed across the pericentromeric regions.

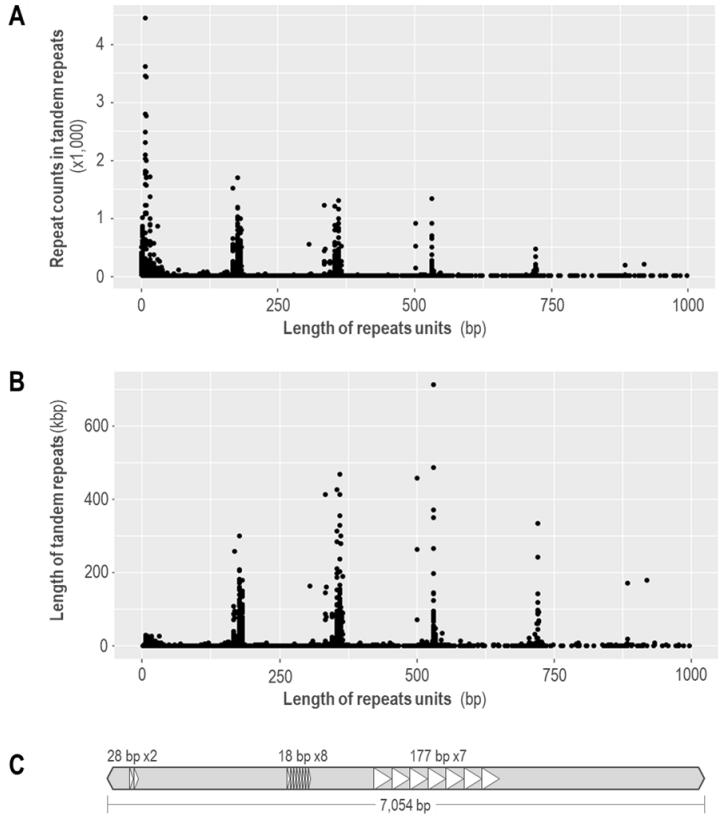

Figure 4. Distribution of unit size, repeat count and total length of the tandem repeats in the Tokiwa genome. A. Scatter plot of unit size (*x*-axis) and repeat count (*y*-axis) of each tandem repeat. B. Scatter plot of unit size (*x*-axis) and the total length of each tandem repeat (*y*-axis). C. Schematic of a Mutator-like element containing tandem repeats. Triangles indicate the units of each tandem repeat.

Tandem repeat in Tokiwa genome

As cucumber genome is peculiar for harboring long stretches of tandem repeats, we also surveyed the Tokiwa genome with tandem repeat finder (Benson 1999). The survey revealed that tandem repeats comprise 35.2 Mbp (12.6%) of the Tokiwa genome.

Although the size of the repeat unit varied, those of ~180 bp, ~360 bp, ~540 bp and ~720 bp had extremely high copy numbers as well as those <10 bp (Fig. 4). The longest stretch of the tandem repeats was that of 712.6 kbp on Chromosome 4, where a unit of 531 bp sequence was tandemly repeated by 1,331 times (Table 3). Other chromosomes also harbored tandem repeats spanning >100 kbp (Table. 3), which could be even longer, as many of them ended with gaps.

292 Interestingly, the long stretches of tandem repeats (>10 kbp) were often overlapped with
293 annotations of Mutator and CACTA transposons (Fig 2). In addition, the Mutator and CACTA
294 elements identified by EDTA often contained the tandem repeats as their internal sequences (Fig. 4C).
295 Namely, the tandem repeats did not cover the full length of the transposable elements. Thus, increase
296 in tandem repeats did not necessarily occur via TE amplifications.
297
298 Table 3. The top 10 longest tandem repeats in the Tokiwa Genome.

Chromosome	Start	End	Unit size	Repeat Count	Length
Chromosome4	12,049,307	12,761,914	531	1331.1	712,608
Chromosome3	25,304,414	25,792,696	531	915.6	488,283
Chromosome5	109,973	578,952	360	1301	468,980
Chromosome1	14,073,064	14,531,408	501	903.2	458,345
Chromosome7	7,938,677	8,366,883	354	1203.4	428,207
Chromosome7	8,006,713	8,377,207	531	693.4	370,495
Chromosome7	27,538,573	27,895,514	360	989.3	356,942
Chromosome7	10,064,253	10,414,004	531	654.1	349,752
Chromosome5	37,052,513	37,392,146	720	463.2	334,450

299
300 **Discussion**
301 Here we present the most contiguous assembly of the cucumber genome, owing to the long reads by
302 Oxford Nanopore. The assembled Tokiwa genome is ~20% longer and contains 10% more gene coding
303 sequences compared to the 9930 genome, or other existing assemblies (Table 2, Figs. 2, 3). The
304 annotation predicted more duplicated genes, less fragmented genes, and much more repeat sequences
305 including tandem repeats. As 'Tokiwa' is a founder line of modern Japanese cultivars, the highly
306 contiguous and accurate genome could facilitate breeding for urgent and long-term demands in
307 Japanese market.
308 Our results have demonstrated the importance of read length to overcome the repetitive
309 sequences in *de novo* assembly. The nanopore sequencer produced long reads with N50 length of 33.2

310 kbp, which was >3 time longer than those used for assembling the 9930 genome (Supplementary Table
311 1). Such long reads have enabled us to resolve and assemble long stretches of tandem repeats,
312 including the one over 700 kbp (Table 3).

313 However, we should also note that we could not assemble centromeric regions without the
314 optical mapping by Bionano. Although the draft assembly already generated contigs containing long
315 stretches of tandem repeats, such contigs could not be directly anchored to the linkage map because
316 there were no genetic markers in such highly repetitive regions. Thus, optical mapping was the only
317 way to place the repeat-rich contigs in centromeric or pericentromeric regions.

318 The newly resolved repeat sequences also give insight into the relationship between the
319 DNA transposons and the tandem repeats. The distribution of the unit size in the tandem repeats
320 indicated that those with high copy numbers are overrepresented with specific unit sizes. This led us
321 to suspect that such tandem repeats could arise from cis-transposition of transposable elements.
322 However, many of the tandem repeats are actually "internal" sequences of transposons. Namely, one
323 single copy of a transposon could be very long, containing a long stretch of tandem repeats within its
324 sequence (Fig. 4). Thus, the tandem repeats could have increased its copy number via other
325 mechanisms than transposition, such as illegitimate recombination or double strand break repair.

326 Although the long reads by Oxford Nanopore greatly facilitated assembling highly repetitive
327 regions, the Tokiwa genome is still far from gapless assembly. Given the estimated size of cucumber
328 genome is ~350 Mbp, there remains ~70 Mbp sequences unassembled. As the Oxford Nanopore have
329 greatly improved the accuracy of raw reads (Zhao et al., 2023), new assemblers optimized for lower
330 error rate would further facilitate resolving the tandem repeats.

331 To conclude, this study presented a *de novo* assembly of Tokiwa genome using long reads
332 of Oxford Nanopore, which could serve as a reference of Japanese cucumber. We hope the Tokiwa
333 genome would be a fundamental knowledge-base for future breeding of Japanese cultivars.

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Author's contributions

KN, NT and **KK** conceived the study.

TS and **CM** performed sequencing and assembly.

CM, **KS**, **YK**, **MS**, **RY** and **KN** fixed misassemblies and performed scaffolding.

TS and **KN** performed genome annotation and data analysis.

TS, KS and **KN** wrote the paper.

All the authors read and approved the final manuscript.

Data availability

All the sequence data were deposited at Biosample IDs SAMN40100976, under Bioproject PRJNA1080095.

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452 **Figure legends**

453 Figure 1. Photographs of *Cucumis sativus* L. cv 'Tokiwa'. a. Plants growing in a greenhouse. b. A
454 green fresh fruit. c. Mature fruites. d. Seeds.

455

456 Figure 2. Overview of Taokiwa genome. A. Circos plot indicating densities of, from the outer to inner
457 tracks, protein coding sequences, LTR retrotransposons, DNA transposable elements, and tandem
458 repeats. B. Coliniarity between the physical positions and the genetic linkage map of the Tokiwa
459 genome. White and blue boxes indicate the physical and genetic map, respectively. Dashed lines
460 indicate the corresponding positions. C. Dotplot of a whole genome alignment of 9930 and Tokiwa.
461 *x*- and *y*-axes indicate Chromosome numbers. Cultivar names are indicated above the *x*-axis and right
462 to the *y*-axis.

463

464 Figure 3. Phylogenetic tree and synteny plot of the 12 chromosome-level genome assemblies. Gray
465 lines indicate synteny blocks between the genomes. Light red lines indicate pseudo synteny blocks
466 between the newly assembled regions of the Tokiwa genome and XMTC or Cu2. Bootstrap values
467 were 100 in all the nodes.

468

469 Figure 4. Distribution of unit size, repeat count and total length of the tandem repeats in the Tokiwa
470 genome. A. Scatter plot of unit size (*x*-axis) and repeat count (*y*-axis) of each tandem repeat. B. Scatter
471 plot of unit size (*x*-axis) and the total length of each tandem repeat (*y*-axis). C. Schematic of a Mutator-
472 like element containing tandem repeats. Triangles indicate the units of each tandem repeat.

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