1 A chromosome-level genome assembly of a maize elite breeding line Dan340

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14 Key findings

- 15 1. The final assembly of Dan340 genome was 2,348.72 Mb, including 2,738 contigs
16 and 2,315 scaffolds with a N50 of 41.49 Mb and 215.35 Mb, respectively.
- 17 2. The percent of reads mapped to the reference genome was up to 97.48%.
- 18 3. The results showed that 96.84% of the plant single-copy orthologues were
19 complete. Complete single-copy and multi copy genes accounted for 87.36% and
20 9.48% of the genes, respectively. Taken together, these results indicated that our
21 Dan340 genome assembly presented high quality and coverage.

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Abstract

The maize cultivar Dan340 is an excellent backbone inbred line of Luda Red Cob Group with several desirable characters, such as disease resistance, lodging resistance, high combining ability, wide adaptability and so on. In this study, we constructed a high-quality chromosome-level reference genome for Dan340 by combining PacBio long HiFi sequencing, Illumina short reads and chromosomal conformational capture (Hi-C) sequencing reads. The final assembly of Dan340 genome was 2,348.72 Mb, including 2,738 contigs and 2,315 scaffolds with a N50 of 41.49 Mb and 215.35 Mb, respectively. The percent of high quality Illumina reads mapped to the reference genome was up to 97.48%. The assembly of this genome will not only facilitate our understanding about intraspecific genome diversity in maize, but also provides a novel resource for maize breeding.

Background

Maize (*Zea mays* ssp. *mays* L.) is one of the most important crops grown worldwide for food, forage, and biofuel, with an annual production of more than 1 billion tons (Yang et al., 2017). With the rapid growth of human population and economic demand, it has been predicted that maize will account for 45% of total cereal demand by the year 2050 (Hubert et al., 2010). In addition, it is also an important model organism for fundamental research in genetics and genomics (Hake and Ross-Ibarra, 2015).

Several reference genome of common maize inbred line in breeding have been released since 2009 (Schnable et al., 2009; Hirsch et al., 2016; Jiao et al., 2017; Sun et

al., 2018; Li et al., 2019). The maize genome exhibits high levels of genetic diversity among different inbred lines (Lu et al., 2015; Yang et al., 2017; Sun et al., 2018). The maize cultivar Dan340 is an excellent backbone inbred line of Luda Red Cob Group with several desirable characters, such as disease resistance, lodging resistance, high combining ability, wide adaptability and so on (Zhang et al., 2018). In the present study, we constructed a high-quality chromosome-level reference genome for Dan340 by combining PacBio long HiFi sequencing, Illumina short reads and chromosomal conformational capture (Hi-C) sequencing reads. The assembly of this genome will not only facilitate our understanding about intraspecific genome diversity in maize, but also provides a novel resource for maize breeding improvement.

Plant material and DNA sequencing

Inbred line Dan340 was selected for genome sequencing and assembly because it is an elite maize cultivar, which plays an important role in maize breeding and genetic research. The plants were grown at 25°C in a greenhouse of Beijing Academy of Agriculture and Forestry Sciences, Beijing, China. Fresh and tender leaves were harvested from the best-growing individual and immediately frozen in liquid nitrogen, followed by preservation at -80 °C in the laboratory prior to DNA extraction. Genomic DNA was extracted from the leaf tissue of a single plant using the DNasecure Plant Kit (Tiangen Biotech Co., Ltd., Beijing, China). To ensure that DNA extracts were useable for all types of genomic libraries, the quality and quantity

were evaluated using a NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and electrophoresis on a 0.8% agarose gel, respectively. PacBio circular consensus sequencing (CCS) libraries were prepared using SMRTbell Express Template Prep Kit 2.0 (Pacific Biosciences Ref. No. 101-685-400), following the manufacturer's protocols, and subsequently sequenced on the PacBio sequel II platform (Pacific Biosciences, Menlo Park, CA, USA). Consensus reads (CCS reads, also termed HiFi reads) were generated using ccs software v.3.0.0 (<https://github.com/pacificbiosciences/unanimity/>) with the parameter '--min-passes 3 --min-rq 0.99'. The total CCS yield was 63.54 Gb, with a mean read length of 15.60 kb.

In addition, one Illumina paired-end sequencing library with an insert size of 350 bp was generated using NEB Next Ultra DNA Library Prep Kit (NEB, USA) following manufacturer's protocol, and subsequently sequenced using an Illumina HiSeq X Ten platform (Illumina, San Diego, CA, USA) in Novogene Bioinformatics Institute, Beijing, China. Approximately 80.66 Gb (~20X) of Illumina sequencing data were obtained.

One Hi-C library was constructed using young leaves following the published procedures with certain modifications (Belton et al., 2012). In brief, approximately 5-g leaf samples were cut into minute pieces and cross-linked by 4% formaldehyde solution at room temperature in a vacuum for 30 min. Then, the sample was mixed with excess 2.5 M glycine to quench the cross linking reaction for 5 min and then

placed on ice for 15 min. The cross-linked DNA was extracted and then digested with MboI restriction enzyme. The sticky ends of the digested fragments were biotinylated and proximity ligated to form ligation junctions that were enriched for and then ultrasonically sheared to a size of 200-600 bp. The biotin-labelled DNA fragments were pulled down and ligated with Illumina paired-end adapters and then amplified by PCR to produce the Hi-C sequencing library. The library was sequenced using an Illumina HiSeq X Ten platform (Illumina, San Diego, CA, USA).

Genome assembly

To obtain a high quality genome assembly of Dan340, we employed both PacBio HiFi sequences and Illumina short-read, with scaffolding informed by chromosomal conformation capture (Hi-C). The assembly was performed in a stepwise fashion. First, de novo assembly of the long CCS reads generated from the PacBio SMRT Sequencing was performed using Hifiasm (Cheng et al., 2021). In brief, we first used all CCS clean reads to do error correction by performing all-vs-all pairwise alignment between them. In this step, haplotype phasing was achieved using heterozygous SNPs and most sequencing errors had been removed. Then, error-corrected reads were used to construct phased assembly graph by determining if one overlap is among the reads coming from different haplotypes. Next, based on the graph topological structures and the phasing relationship among different haplotypes, Hifiasm produced a primary contigs (p-contigs). Then, Pilon (Walker et al., 2014) was used to perform the second round of error correction with short paired-end reads generated from Illumina HiSeq Platforms. Subsequently, the Purge Haplotigs pipeline

(https://bitbucket.org/mroachawri/purge_haplotigs/overview) was used to remove redundant sequences formed due to heterozygosity.

For Hi-C reads, to avoid reads with artificial bias, we removed the following type of reads: (a) Reads with $\geq 10\%$ unidentified nucleotides (N); (b) Reads with > 10 nt aligned to the adapter, allowing $\leq 10\%$ mismatches; (c) Reads with $> 50\%$ bases having phred quality < 5 . The filtered Hi-C reads were aligned against the contig assemblies with BWA (version 0.7.8). Reads were excluded from subsequent analysis if they did not align within 500 bp of a restriction site or did not uniquely map, and the number of Hi-C read pairs linking each pair of scaffolds is tabulated. ALLHiC v0.8.12 (Zhang et al., 2019) was used in simple diploid mode to scaffold the genome and optimize the ordering and orientation of each clustered group, producing a chromosomal level assembly. Juicebox Assembly Tools v1.9.8 (<https://github.com/aidenlab/Juicebox>) (Durand et al., 2016) was finally used to visualize and manually correct the large-scale inversions and translocations to obtain the final pseudo-chromosomes. The final assembly of Dan340 genome was 2,348.72 Mb, including 2,738 contigs and 2,315 scaffolds with a N50 of 41.49 Mb and 215.35 Mb (Table 1), respectively. A total of 91.30% sequences was anchored to 10 pseudo-chromosomes.

Evaluation of assembly quality

We assessed the quality of the assembly by two independent methods. First, the short reads obtained from the Illumina sequencing data were aligned to the final assembly with BWA version 0.7.8 (<http://bio-bwa.sourceforge.net/>) (Li and Durbin, 2010). Our

result showed that the percent of reads mapped to the reference genome was up to 97.48%. Second, the BUSCO (Benchmarking Universal Single-Copy Otheologs, (Simao et al., 2015) database embryophyta_odb10 was used to evaluate the completeness of gene regions, which including 1614 conserved core genes. The results showed that 96.84% of the plant single-copy orthologues were complete. (Figure 1)

Conclusions

1. We assembled the chromosome-level genome of maize elite inbred line Dan340 based on long CCS reads from the third-generation PacBio Sequel II sequencing platform, with scaffolding informed by chromosomal conformation capture (Hi-C).
2. The final assembly of Dan340 genome was 2,348.72 Mb, including 2,738 contigs and 2,315 scaffolds with a N50 of 41.49 Mb and 215.35 Mb, respectively.

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231 **Table 1** Results of genome assembly assisted by Hi-C technology.

	Contig length (bp)	Scaffold length (bp)	Contig number	Scaffold number
Total	2, 348, 678, 871	2, 348, 721, 171	2, 738	2, 315
Max	97, 718, 682	343, 826, 844	–	–
Number >=2000	–	–	2, 738	2, 315
N50	41, 489, 332	215, 353, 106	20	5
N60	32, 900, 726	193, 940, 028	26	6
N70	22, 291, 114	184, 974, 134	35	7
N80	11, 599, 268	165, 218, 414	49	9
N90	3, 788, 017	153, 467, 472	80	10

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Figure 1 BUSCO Assessment Results (Database: embryophyta_odb10, n: 1614)