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

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Key findings

1. 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.
2. The percent of reads mapped to the reference genome was up to 97.48%.
3. The results showed that 96.84% of the plant single-copy orthologues were complete. Complete single-copy and multi copy genes accounted for 87.36% and 9.48% of the genes, respectively. Taken together, these results indicated that our Dan340 genome assembly presented high quality and coverage.

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

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

59 **Plant material and DNA sequencing**

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

69 were evaluated using a NanoDrop 2000 spectrophotometer (NanoDrop Technologies,
70 Wilmington, DE, USA) and electrophoresis on a 0.8% agarose gel, respectively.
71 PacBio circular consensus sequencing (CCS) libraries were prepared using SMRTbell
72 Express Template Prep Kit 2.0 (Pacific Biosciences Ref. No. 101-685-400), following
73 the manufacturer's protocols, and subsequently sequenced on the PacBio sequel II
74 platform (Pacific Biosciences, Menlo Park, CA, USA). Consensus reads (CCS reads,
75 also termed HiFi reads) were generated using ccs software v.3.0.0
76 (https://github.com/pacificbiosciences/unanimity/) with the parameter '--min-passes 3
77 --min-rq 0.99'. The total CCS yield was 63.54 Gb, with a mean read length of 15.60
78 kb.
79 In addition, one Illumina paired-end sequencing library with an insert size of 350 bp
80 was generated using NEB Next Ultra DNA Library Prep Kit (NEB, USA) following
81 manufacturer's protocol, and subsequently sequenced using an Illumina HiSeq X Ten
82 platform (Illumina, San Diego, CA, USA) in Novogene Bioinformatics Institute,
83 Beijing, China. Approximately 80.66 Gb (~20X) of Illumina sequencing data were
84 obtained.
85 One Hi-C library was constructed using young leaves following the published
86 procedures with certain modifications (Belton et al., 2012). In brief, approximately
87 5-g leaf samples were cut into minute pieces and cross-linked by 4% formaldehyde
88 solution at room temperature in a vacuum for 30 min. Then, the sample was mixed
89 with excess 2.5 M glycine to quench the cross linking reaction for 5 min and then

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

97 **Genome assembly**

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

112 (https://bitbucket.org/mroachawri/purge_haplotigs/overview) was used to remove
113 redundant sequences formed due to heterozygosity.
114 For Hi-C reads, to avoid reads with artificial bias, we removed the following type of
115 reads: (a) Reads with ≥ 10% unidentified nucleotides (N); (b) Reads with > 10 nt
116 aligned to the adapter, allowing ≤ 10% mismatches; (c) Reads with > 50% bases
117 having phred quality < 5. The filtered Hi-C reads were aligned against the contig
118 assemblies with BWA (version 0.7.8). Reads were excluded from subsequent analysis
119 if they did not align within 500 bp of a restriction site or did not uniquely map, and
120 the number of Hi-C read pairs linking each pair of scaffolds is tabulated. ALLHiC
121 v0.8.12 (Zhang et al., 2019) was used in simple diploid mode to scaffold the genome
122 and optimize the ordering and orientation of each clustered group, producing a
123 chromosomal level assembly. Juicebox Assembly Tools v1.9.8
124 (https://github.com/aidenlab/Juicebox) (Durand et al., 2016) was finally used to
125 visualize and manually correct the large-scale inversions and translocations to obtain
126 the final pseudo-chromosomes. The final assembly of Dan340 genome was 2,348.72
127 Mb, including 2,738 contigs and 2,315 scaffolds with a N50 of 41.49 Mb and 215.35
128 Mb (Table 1), respectively. A total of 91.30% sequences was anchored to 10
129 pseudo-chromosomes.
130 **Evaluation of assembly quality**
131 We assessed the quality of the assembly by two independent methods. First, the short
132 reads obtained from the Illumina sequencing data were aligned to the final assembly
133 with BWA version 0.7.8 (http://bio-bwa.sourceforge.net/) (Li and Durbin, 2010). Our

134 result showed that the percent of reads mapped to the reference genome was up to
135 97.48%. Second, the BUSCO (Benchmarking Universal Single-Copy Otheologs,
136 (Simao et al., 2015) database embryophyta_odb10 was used to evaluate the
137 completeness of gene regions, which including 1614 conserved core genes. The
138 results showed that 96.84% of the plant single-copy orthologues were complete.
139 (Figure 1)
140

141 **Conclusions**

142 1. We assembled the chromosome-level genome of maize elite inbred line Dan340
143 based on long CCS reads from the third-generation PacBio Sequel II sequencing
144 platform, with scaffolding informed by chromosomal conformation capture (Hi-C).
145 2. The final assembly of Dan340 genome was 2,348.72 Mb, including 2,738 contigs
146 and 2,315 scaffolds with a N50 of 41.49 Mb and 215.35 Mb, respectively.
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230
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

232
233

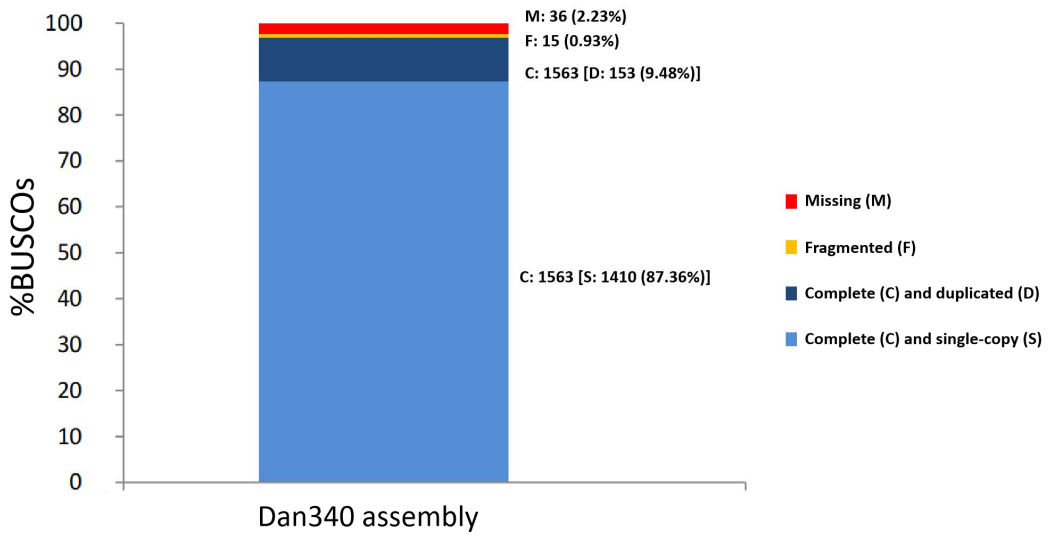

Figure 1 BUSCO Assessment Results (Database: embryophyta_odb10, n: 1614)